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THE UNIVERSITY OF ALBERTA

THE SYNTHESES AND CONFORMATIONAL PROPERTIES OF THE DIASTEREOISOMERIC METHYL 6-*C*-METHYL-α-ISOMALTOSIDES

by

Nghia Le

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMISTRY

EDMONTON, ALBERTA

SPRING, 1990

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "The syntheses and conformational properties of the diastereoisomeric methyl 6-C-methyl-α-isomaltosides" submitted by Nghia Le in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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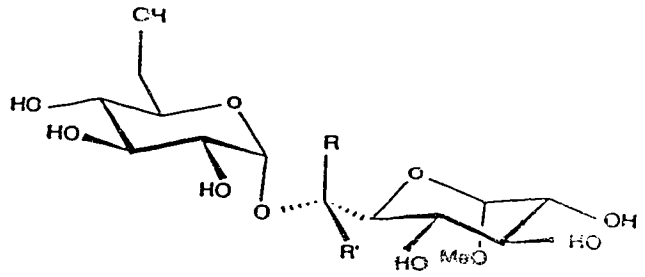
To my family,

ABSTRACT

The disaccharide methyl α-isomaltoside (methyl α-D-glucopyranosyl (1→6)-α-D-glucopyranoside, structure **1**) is conformationally labile in aqueous solution due to the flexible α-(1→6) linkage. However, the rotation around the α-(1→6) linkage is restricted when the protons at C-6 of methyl α-isomaltoside were selectively substituted by methyl groups to give the conformationally well-defined diastereoisomeric disaccharides; namely, methyl 6-*R*-methyl-α-isomaltoside (structure **2**) and methyl 6-*S*-methyl-α-isomaltoside (structure **3**).



1, R=R'=H **2**, R=Me, R'=H **3**, R=H, R'=Me

The conformational preference of the diastereoisomers (**2** and **3**) in D_2O were investigated by nuclear magnetic resonance spectroscopy including the determination of nuclear Overhauser enhancements.

The kinetics for the hydrolysis of the interunit glucosidic bond of **1** and the diastereoisomeric disaccharides (**2** and **3**) were examined when catalyzed by the enzyme amyloglucosidase type II. It was found that the rate of hydrolysis of methyl 6-*R*-methyl-α-isomaltoside (**2**) was faster than that of methyl α-isomaltoside (**1**) which in turn was faster than that of methyl 6-*S*-methyl-α-isomaltoside (**3**). The results are in agreement with expectations based on the preferred conformations.

ACKNOWLEDGEMENTS

I wish to acknowledge my deepest gratitude to Professor R. U. Lemieux, my research supervisor, for his support, guidance, encouragement and patience during the course of this work and for his interest and assistance in the preparation of this thesis. I am also grateful for his advises, not only about Chemistry but also about Life, for which I can look upon as guiding lights for many years to come.

I would also like to thank Dr. O. Hindsgaul for his guidance, invaluable ideas and encouragements, Dr. U. Spohr for her help and discussions in the synthesis of the compounds, Dr. M. Palcic for her help and useful disscussions in the enzyme work, Mr. L. Steele for the work on enzyme analysis, Professor K. Bock provided the enzyme Amyloglucosidase type II and computer software to analyze the enzyme hydrolysis data, Dr. H. Beierbeck for the help with the HSEA calculations and Mr. R. Mendez in the preparation of the starting materials.

I would also like to thank the Department of Chemistry of The University of Alberta for financial support and good education.

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I. INTRODUCTION

1. Overview

The disaccharide isomaltose (α-D-glucopyranosyl (1→6)-D-glucopyranose, Figure 1) exists as a building unit in the polysaccharides amylopectin, glycogen and dextran.[1] The compound is therefore a building unit of extremely important and abundant biological products and as such must be involved in a wide variety of biological processes—enzymatic and immunological.[2-7]

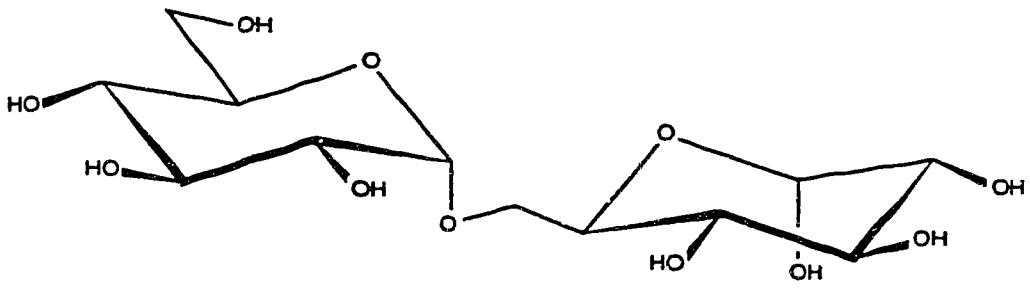


Figure 1. α-Isomaltose (6-*O*-α-D-glucopyranosyl-α-D-glucopyranose).

However, in contrast to the isomeric maltose which has a well defined conformational preference,[8] this is not the case for isomaltose. Because the non-reducing unit of isomaltose is linked to a primary carbon, there exists only very low barriers to rotation about the C-5 to C-6 bond. On the other hand, the exo-anomeric effect[9-12] is expected to substantially restrict rotation about the C-1′ to O-6 glucosidic bond. Therefore, because of its conformational flexibility, isomaltose could be expected to become involved in biochemical transformations in a variety of energetically nearly equivalent conformations

depending on the demands of the combining sites. This investigation was aimed at restricting the conformational population of methyl α-isomaltoside through appropriate structural modification and to test the effect of the set conformational preference on the biological transformation.

The notations used in this thesis for labelling the atoms of the glucose units and torsion angles of isomaltose are shown in Figure 2.

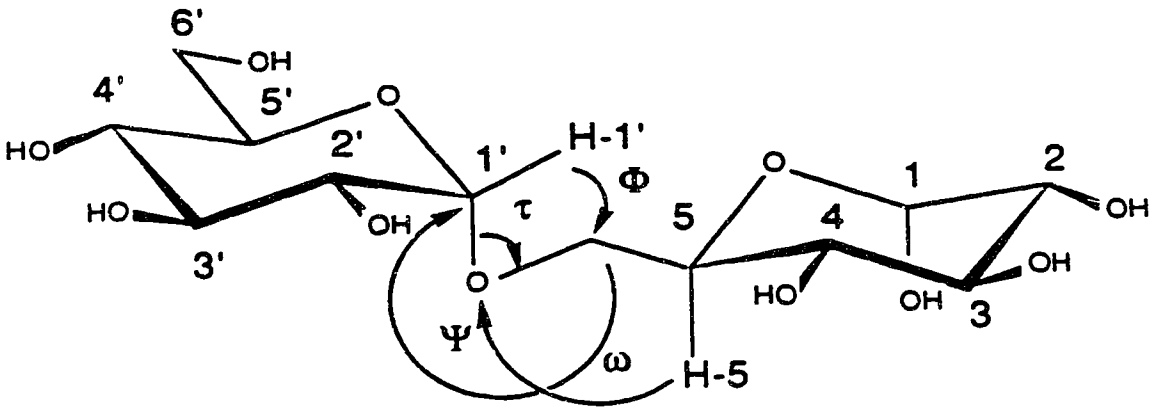


Figure 2. Designations for torsion angles of α-isomaltose or 6-*O*-(α-D-glucopyranosyl)-α-D-glucopyranose.

The primed numbers with the atoms indicate the non-reducing unit. The notations for the torsion angles (positive or negative depending on the screw pattern) are Φ for the torsion angle defined by H-1′ and C-6 for the linkage H-1′—C-1′—O-1′—C-6, Ψ is that defined by C-1′ and C-5 for the linkage C-1′—O-1′—C-6—C-5 and ω is that defined by O-1′ and H-5 in O-1′—C-6—C-5—H-5. The symbol τ is used to represent the valence angle for the glucosidic bond C-1′—O-1′—C-6. These presentations are in accord with IUPAC recommendations.[13,14]

The three staggered orientations for the O-6 are identified by reference to O-5. The generally accepted notations **gt**, **gg** and **tg** are shown in Figure 3 where **g** is for gauche and **t** is for trans.[15]

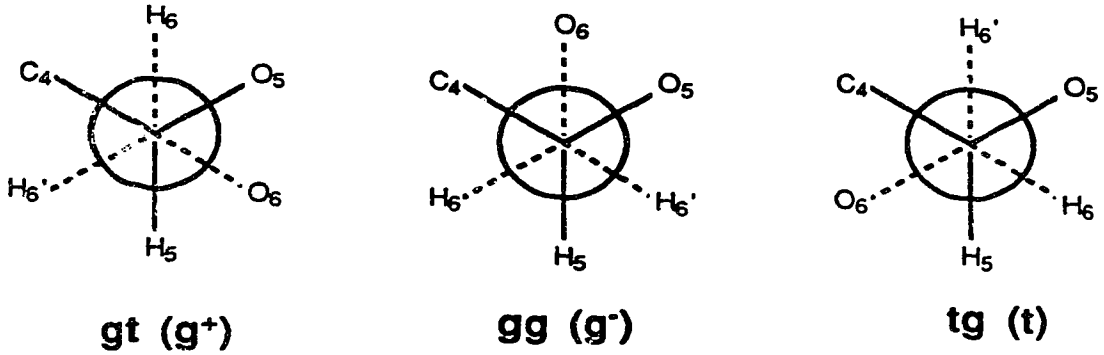


Figure 3. The three staggered orientations: **gt** (g^+), **gg** (g^-) and **tg** (**t**) for the O-6 of α-isomaltose

This nomenclature requires remembering that the O-5 to O-6 relationship be placed first. However, the three orientations are most readily identified by simply indicating the numerical sign of the gauche torsion angle defined by the two oxygen atoms and, consequently, in this thesis, the three staggered orientations will be simply identified as g^+, g^- and **t**, respectively.

The conformations of carbohydrates in crystalline states can normally be obtained by single crystal X-ray crystallography. However, X-ray crystal structures have not been reported for isomaltose or its glycosides, most likely because suitable crystals have not been obtained. The structure of dextran (a polysaccharide consisting of glucopyranose units linked α-(1→6)), obtained by X-ray diffraction of the powder[16], was estimated to have the torsion angles $\Phi = -63.7^{\circ}$, $\Psi = 292.0^{\circ}$ and $\omega = 52.2^{\circ}$. Theoretical estimations[17-21] predicted that because of the α-(1→6) linkage, isomaltose could exist in many conforma-

tions of comparable energies in D_2O solution. The hard sphere (H.S.) theoretical calculations[17,18] estimated that isomaltose would have the torsion angles $\Phi = -60^{o}$, $\Psi = 80—280^{o}$ and $\omega = 40—160^{o}$ and $270—300^{o}$. Hard Sphere Exoanomeric (HSEA) calculations[19] gave the torsion angles $\Phi = -50^{o}$, $\Psi = 190^{o}$ and $\omega = 180^{o}$ for the minimum energy conformation. It can be seen that the X-ray diffraction conformation of dextran is in the general range of the theoretical estimations.[17-21] The above torsion angles estimated by X-ray and theoretical calculations are summarized in Table 1.

Table 1. X-ray and H.S. calculated Φ, Ψ and ω torsion angles of dextran and isomaltose.

Torsion Angles	Solid Structure of Dextran[16]	H.S. calculations[17,18] of isomaltose
$\Phi^{C\text{-}6,H\text{-}1'}$	-63.7^{o}	-60^{o}
$\Psi^{C\text{-}5,C\text{-}1'}$	292.0^{o}	$80^{o} - 280^{o}$
$\omega^{H\text{-}5,O\text{-}1'}$	52.2^{o}	$40^{o} - 160^{o}$
		$270^{o} - 300^{o}$

It should be noted that the solid state conformations of carbohydrate molecules need not be the same as those in aqueous solution since the crystal lattice forces may pack the molecules in conformations that are in only very low abundance in the dissolved state. An example of this was shown by Lemieux and Alvarado[8] for the case of maltose, where the preferred conformation of maltose differs from that in the crystal both in aqueous solution and solution in DMSO-d_6. In fact, the conformer in DMSO-d_6 differs substantially from that in water.[8]

Conformational analysis of isomaltose in aqueous solution is important particularly for the studies of substrate recognition such as in the hydrolysis of isomaltose catalyzed by the enzymes[7] or for noncovalent binding by antibodies. An example of this can be illustrated with dextrans. Kabat and co-workers[3] have shown that the monoclonal antibodies to dextrans have a binding site that can accept an α-(1→6) linked linear chain

of up to seven glucose units. Furthermore, it was also shown that the monoclonal antibodies are specific for the α-(1→6) linkage.[3] It is expected that the antibodies recognize the isomalto-oligosaccharides in a specific conformation for binding, but this conformation is not known.

A number of attempts to achieve conformational analysis of carbohydrate molecules in solution have been made including interpretation of optical rotation data[22] and NMR spectroscopy.[23-25] Rees[22] suggested that optical rotation data could be used to estimate the Φ and Ψ torsion angles of the glycosides and obtained some good agreement between the calculated and the observed linkage rotation data but it was shown by Alvarado[8] that these agreements were fortuitous and could be highly misleading.

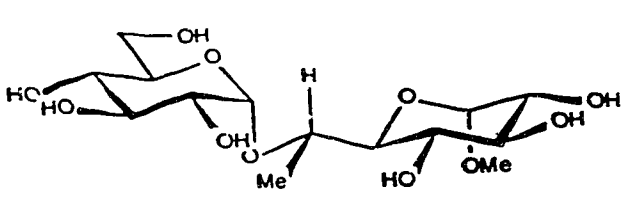
It is generally agreed that the best available method for conformational analysis of carbohydrate molecules in solution is NMR spectroscopy.[23-25] The NMR parameters useful for conformational analysis are proton–proton coupling constants, chemical shifts[23-24] and nuclear Overhauser enhancements (n.O.e.).[25] Since the ^{1}H-NMR spectrum of isomaltose in D_2O is complex because of overlapping of two sets of signals the model compounds methyl α- and/or methyl β-isomaltosides, which provide simpler ^{1}H-NMR spectra for analysis, were used.

There can be no doubt that the $^4C_1$ conformation is well maintained by the two pyranose rings of methyl isomaltoside. Thus, the main point of interest to this thesis is the determination of the preferred orientations at the C-6 of the disaccharide derivatives. The preferred orientations for the O-6 of the reducing units of methyl isomaltoside have been studied by ^{1}H-NMR spectroscopy.[26,27] Bock and Pedersen[26] concluded that methyl β-isomaltoside existed with the $\mathbf{g}^-$ conformation for O-5/O-6 in D_2O to the extend of 65% by graphical analysis[28-29] of the coupling constants of the 6-(*S*)-deuterium labelled isomaltose derivative.[30,31] This result was in agreement with the data by Ohrui *et al.*[27]

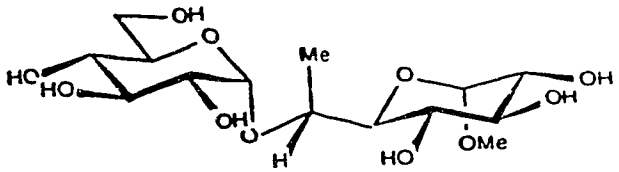
which were obtained from the similar 6-(*R*)-and 6-(*S*)- deuterium labelled methyl α- and methyl β-isomaltoside derivatives. Ohrui's data[27] were analyzed with the empirical relationships derived by Wu *et al.*[32] for coupling constants and it was found that the $\mathbf{g}^-$ conformer was favoured to the extend of 70%. In both cases the analysis of the coupling constants indicated that the $\mathbf{g}^+$ conformation population was about 30-35% and that the **t** conformation was not appreciably present. The ^{1}H-NMR data obtained by Bock *et al.*[26] and Ohrui *et al.*[27] were not in agreement with previously published results.[33,34]

2. Objective of this study

The main synthetic goal of this research was to prepare the 6-*S*- and 6-*R*-methyl derivatives of methyl α-isomaltoside (**40** and **43**, Figure 4) in order to restrict the population of conformers arising from rotation about the C-5 to C-6 bond (changes in the ω torsion angle). Hopefully, the substitution would lead, in each case, to a large abundance of one conformer that would retain biolog al activity. Indeed, application of HSEA calculations to anticipate the conformational equilibria for these 6-*C*-methyl compounds suggested that the substitution by the methyl group may be useful to achieve conformationally well-defined models for methyl α-isomaltoside. This strategy was shown to be successful by Lemieux *et al.*[35-36] with the trisaccharides β-LacNac (1→6)-7-deoxy-D-*glycero*-D-*galacto*-heptopyranose and β-LacNac (1→6)-7-deoxy-L-*glycero*-D-*galacto*-heptopyranose.



40



43

Figure 4. Structure of methyl 6-*S*-methyl α-isomaltoside (**40**) and methyl 6-*R*-methyl-α-isomaltoside (**43**).

II. DISCUSSION OF THE SYNTHESES

The first synthetic goal was to achieve an ample supply of the appropriately blocked diastereoisomeric 6-*C*-methyl derivatives of methyl α-D-glucopyranoside (**1**); namely, compounds **9** and **10** of Scheme 6 (p. 27). These compounds would then be used to synthesize the diastereoisomeric disaccharides **40** and **43** of Schemes 19 (p. 54) and 20 (p. 59), respectively and which represent the 6-*C*-methyl derivatives of methyl α-isomaltoside.

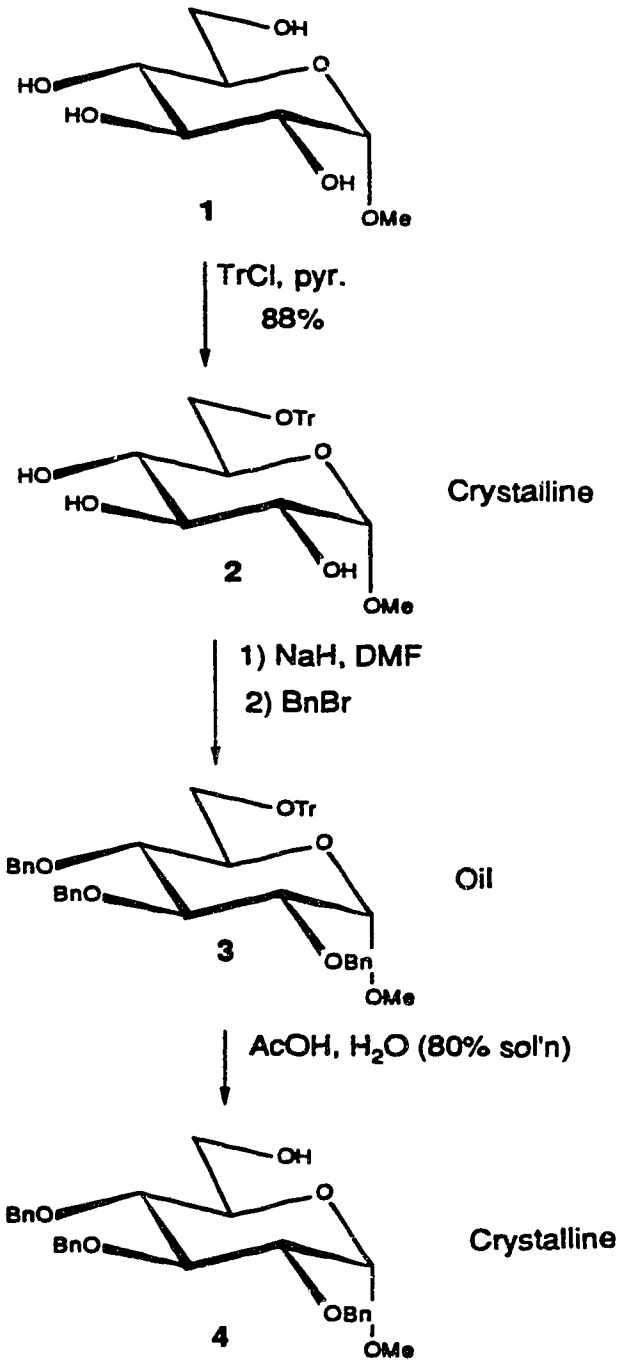
The general approach was to oxidize methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**4**, Schemes 1 and 2, p. 9 and 11 respectively) to the aldehyde **7** (Scheme 3, p. 13) and then to react **7** with methyl Grignard reagent to produce the 6-*C*-methyl derivatives of **4**; namely **9** and **10** (Scheme 6). Compounds **9** and **10** would then be reacted with 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl bromide (**31**) under halide-ion catalyzed conditions[37] to establish the inter-sugar α-glycosidic linkage as indicated in Schemes 19 and 20 (p. 54 and 59 respectively) for compound **38** and **41**. Deblocking would then produce the desired 6-*C*-methyl derivatives of methyl α-isomaltoside (**39** and **43**). Although quite simple in concept, the overall synthesis required considerable study to overcome unexpected problems.

1. Synthesis of 6-C-methyl derivatives (15 and 21) of methyl α-D-glucopyranoside (1)

1.1. Synthesis of methyl 7-deoxy-β-L-glycero-D-glucoheptapyranoside (**15**)

Initially, the approach described in Scheme 1, which starts from the 6-*O*-trityl ether of methyl α-D-glucopyranoside,[38] was used to prepare **4**. According to established procedures, methyl α-D-glucopyranoside (**1**) was first reacted with trityl chloride in pyridine[38,39] to give the methyl 6-*O*-trityl-α-D-glucopyranoside (**2**) (88% yield) which was *O*-benzylated with benzyl bromide and sodium hydride in dimethyl formamide[40] to give the methyl

Scheme 1


OH
O
HO
HO
OH
OMe
1
TrCl, pyr.
88%
OTr
O
HO
HO
OH
OMe
2
Crystalline
1) NaH, DMF
2) BnBr
OTr
O
BnO
BnO
OBn
OMe
3
Oil
AcOH, H2O (80% sol'n)
OH
O
BnO
BnO
OBn
OMe
4
Crystalline

2,3,4-tri-*O*-benzyl-6-*O*-trityl-α-D-glucopyranoside (**3**) as an oil in 85% yield. Removal of the trityl blocking group by cleavage with 80% aqueous acetic acid solution[41] produced a mixture of trityl alcohol and the desired product **4**. It turned out that **4** could not be obtained by selective crystallization or selective solvent extraction. Column chromatography over silica gel proved successful but was not attractive for large scale preparation. Accordingly another method was developed.

The method for the synthesis of **4** from methyl α-D-glucopyranoside (**1**) that is outlined in Scheme 2 also followed published procedures. The starting material was the well-known 4,6-*O*-benzylidene derivative of **1** (**5**)*[42-44] which was *O*-benzylated[40] to provide the intermediate methyl 2,3-*O*-benzyl-4,6-benzylidene-α-D-glucopyranoside (**6**). Reductive ring cleavage of the 4,6-*O*-benzylidene group using lithium aluminum hydride in the presence of aluminum trichloride to provide the acid catalysis,[45] proceeded with preferential cleavage at the primary carbon of the cyclic acetal and provided **4**. Selective crystallization[46,47] from ether/n-hexane resulted in an average yield of 80% for **4**.

1.1.1. Preparation of methyl 2,3,4-tri-O-benzyl-α-D-gluco-1,6-dialdehydopyranoside (7)

It proved desirable to achieve oxidation of the alcohol **4** to the aldehyde **7** cleanly and in high yield under anhydrous condition since **7** could not be purified by column chromatography without drastically reducing the yield. Although the reasons for this were not established, the main loss in yield may have arisen from β-elimination of the 4-benzyloxy group or by autooxidation. Furthermore, any exposure to water was found to lead to the highly stable aldehydrol (hydrate) formation. A number of different oxidation agents[48,58] such as pyridinium dichromate (PDC),[48,51] pyridinium dichlorochromate

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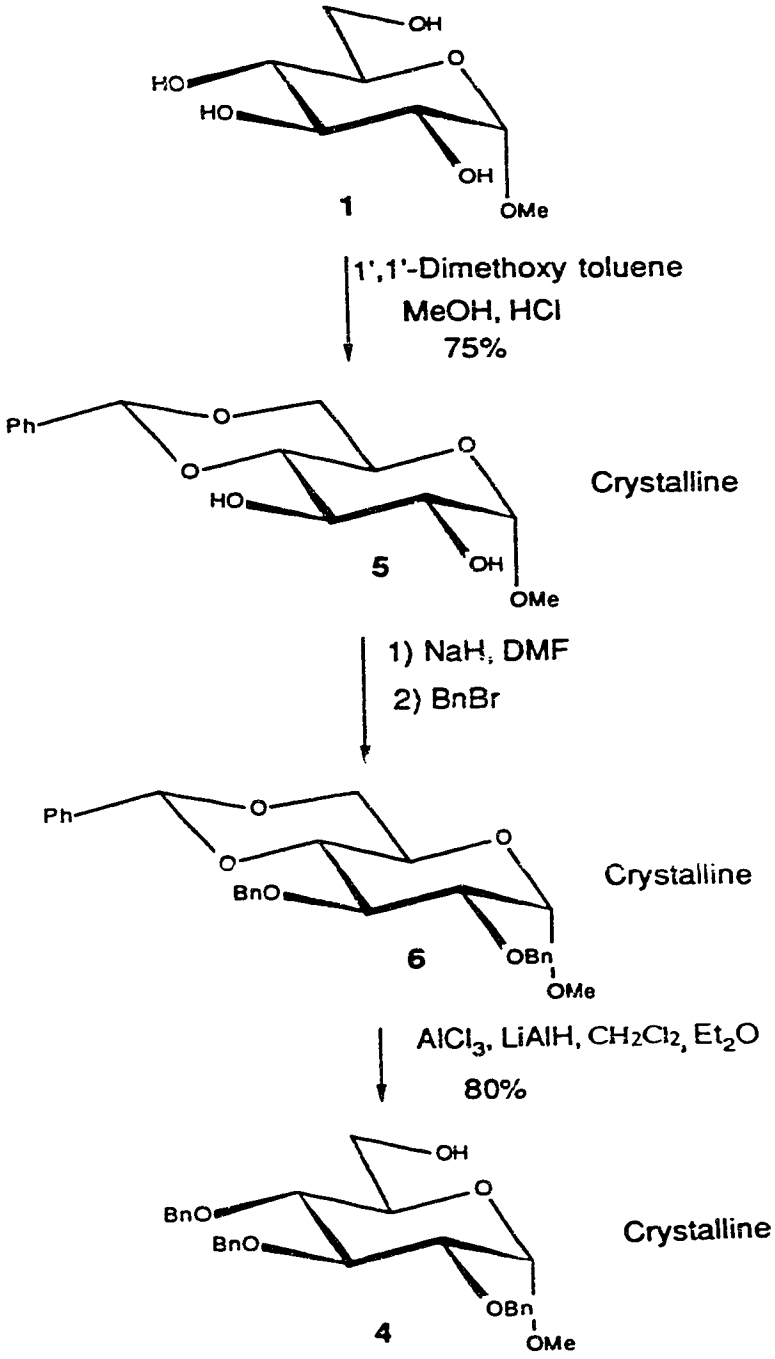
* Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**5**) was prepared by R. Mendez in our laboratory according to references 42 and 43.

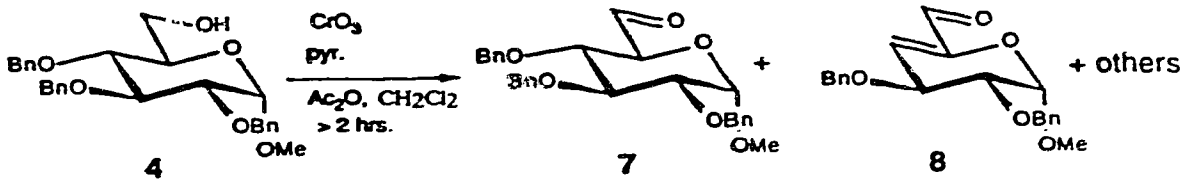
Scheme 2


OH
O
HO
HO
OH
OMe
1
1',1'-Dimethoxy toluene
MeOH, HCl
75%
Ph
O
O
O
HO
OH
OMe
5
Crystalline
1) NaH, DMF
2) BnBr
Ph
O
O
O
BnO
OBn
OMe
6
Crystalline
AlCl3, LiAlH, CH2Cl2, Et2O
80%
OH
O
BnO
BnO
OBn
OMe
4
Crystalline

(PCC),[52,53] N,N'-dicyclohexylcarbodiimide and dimethyl sulfoxide,[54] and chromium trioxide and acetic anhydride[55-57] were attempted but in our hands these agents did not oxidize the alcohol **4** to the aldehyde **7** cleanly and in high yield.

At the beginning of these experiments for the oxidation of **4** to **7**, chromium trioxide (4 eq.) and pyridine (8 eq.) in dichloromethane followed by the addition of acetic anhydride[56,57] was used (Method I, Scheme 3). These conditions provided a mixture after 2 hrs in which the aldehyde **7** was present in about 50 to 60% yield. This conclusion could be reached on the basis of the ^{1}H-NMR by integration of the OMe singlets of the mixture at $\delta = -3.4$ ppm as displayed in Figure 5(a). If the reaction time was longer than 2 hrs, more of the aldehyde **7** underwent β-elimination to produce unsaturated aldehyde **8** (Scheme 3). The isolated yield of **8** varied from 5 to 15%. Also as the research progressed, it became evident that the addition of the alcohol **4** to the aldehyde **7** to form an hemiacetal had to be minimized. Therefore, the best approach to the preparation of **7** would involve the shortest possible existence of the highly sensitive compound which was formed basically as an intermediate in the overall reaction under the conditions used. It was therefore considered that optimum conditions would involve a brief reaction time, simple and fast work-up to yield a product of sufficient purity to be used without further purification in the next step which involves the Grignard reaction. The solution to the problem proved to be quite straight forward. The alcohol (**4**) in dichloromethane was added all at once at room temperature to an excess of chromium trioxide (12 eq.) in pyridine (24 eq.) and dichloromethane[58] (Method II, Scheme 3). After only 10 min., the reaction was near complete as judged by ^{1}H-NMR and t.l.c. The work up for this reaction was extremely simple since it involved only filtration and evaporation of the solvent. The product (90% pure by ^{1}H-NMR integration) was obtained in 75% yield. The procedure was suitable for large scale preparation. Furthermore, the crude aldehyde **7** obtained was sufficiently pure to be used directly in the Grignard reaction. The ^{1}H-NMR and ^{13}C-NMR

Scheme 3



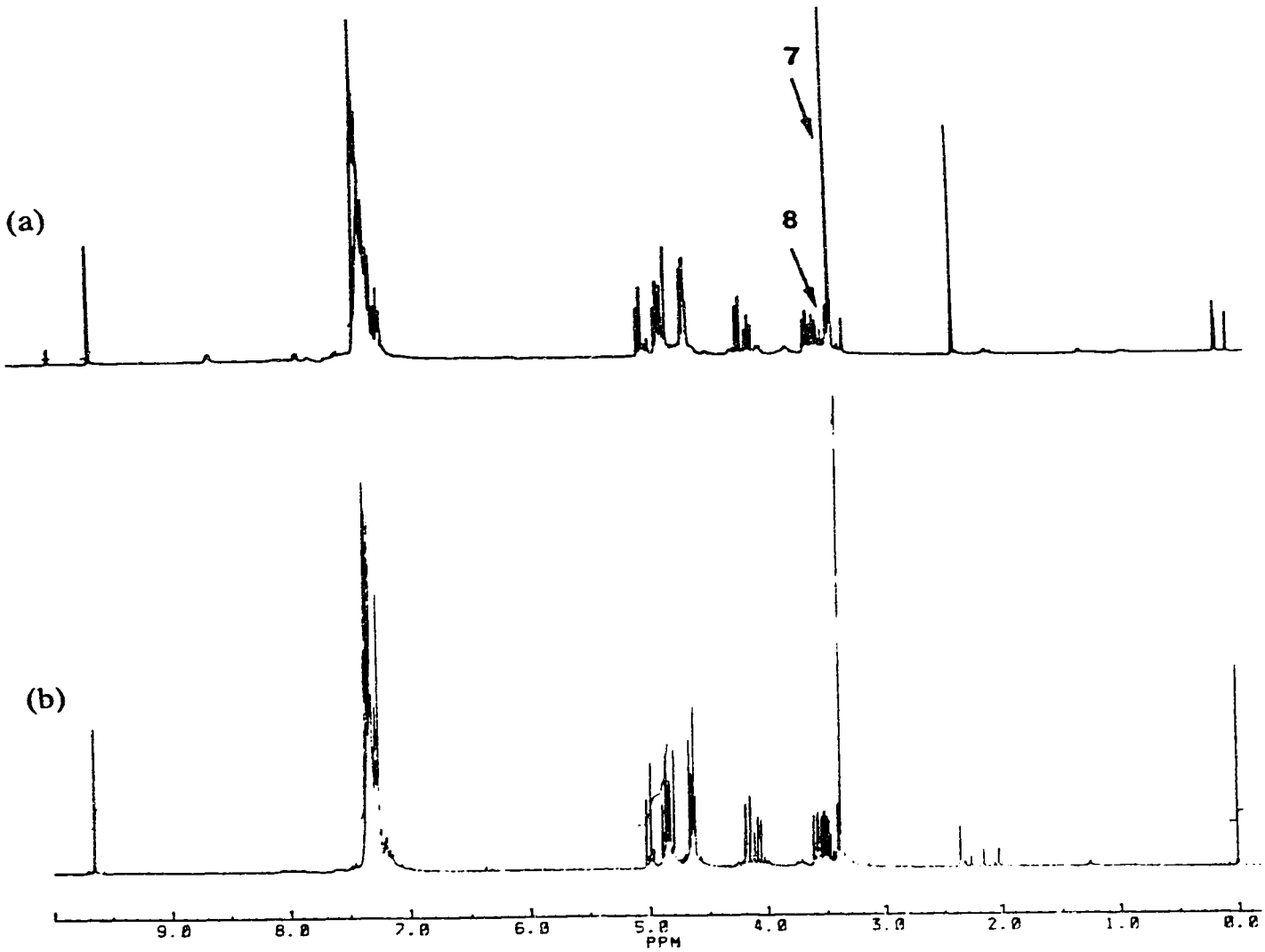


Figure 5. ^{1}H-NMR spectrum (see Table 2 and 3 for assignments) of the crude oxidation product mixture (a) obtained by oxidation in CH_2Cl_2 for 3 hrs with a 4-fold molar excess of the CrO_3.2pyridine complex (about 60% of 7). (b) obtained by oxidation using only CH_2Cl_2 as solvent for 10 min. with 12-fold molar excess of CrO_3.2pyridine complex (about 90% pure of 7) .

Table 2. ^{1}H-NMR chemical shifts (ppm) and coupling constants (in brackets, Hz) of the monosaccharide and disaccharide derivatives.

Comp.	OCH_3	H-1	H-2	H-3	H-4	H-5	H-6	C-6 – CH_3	CHO	Ar-H	Ph-CH_2	para-H	ortho-H
7	3.38	—	3.50 (3.5) (9.5)	4.08 (9.5) (9.0)	3.57 (9.0) (10.0)	4.17 (10.0)			9.65	7.40-7.10	5.05-4.50		
9	3.34	4.58 (3.5)	3.50 (3.5) (9.5)	3.99 (9.5) (9.0)	3.62 (9.0) (10.0)	3.42 (10.0) (1.5)	4.05 (1.5) (6.5)	1.25 (6.5)		7.40-7.20	5.01-4.63		
10	3.31	4.50 (3.5)	3.43 (3.5) (9.5)	3.95 (9.5) (9.0)	3.35 (9.0) (10.0)	3.55 (10.0) (4.5)	3.88 — —	1.05 (6.5)		7.34-7.16	4.98-4.53		
11	3.36	4.56 (4.0)	3.50 (4.0) (9.5)	4.05 (9.5) (8.5)	3.61 (8.5) (10.0)	3.55 (10.0)	—	1.22 1.19		7.40-7.20	5.10-4.50		
14	3.42	—	3.60 (3.5) (10.0)	4.05 (10.0) (9.0)	3.42 — —	3.71 (10.0) (1.5)	5.62 (1.5) (6.5)	1.48 (6.5)		7.45-7.15	5.45-5.05	9.21 (2.0)	9.16 (2.0)
15	3.38	– –	3.55 (3.5) (10.0)	3.62 (10.0) (9.0)	3.46 (9.0) (10.0)	3.38 (10.0) (1.5)	4.16 (1.5) (6.5)	1.29 (6.5)					

Table 2 (cont.). ^{1}H-NMR chemical shifts (ppm) and coupling constants (in brackets, Hz) of the monosaccharide and disaccharide derivatives.

Comp.	OCH_3	H-1	H-2	H-3	H-4	H-5	H-6	C-6-CH_3	Ar-H	Ph-CH_2	SO_2CH_3	$OCOCH_3$
17	3.41	4.93 (3.5)	4.87 (3.5) (10.0)	5.57 (10.0) (9.0)	3.91 (9.0) (10.0)	4.15 (10.0) (6.0)	4.50 (6.0) (7.0)	1.48 (7.0)	7.50-7.25	5.80*		2.05,2.15
18	3.36	—	—	—	—	4.02 — —	5.32 (1.0) (6.5)	1.53 (6.5)	7.40-7.25	5.06-4.62	3.04	
19	3.38	4.64 (4.0)	3.52 (4.0) (9.5)	4.04 (9.5) (9.0)	3.42 (9.0) (10.0)	3.93 (10.0) (2.0)	5.47 (2.0) (7.0)	1.18 (7.0)	8.10-7.20	5.03-4.65		
20	3.46	4.78 (4.0)	3.66 (4.0) (10.0)	4.08 — (8.5)	3.60 (8.5) (10.0)	3.75 (10.0) (1.5)	5.63 (1.5) (6.5)	1.48 (6.5)	8.10-7.20	5.15-4.47		
21	3.44	—	3.58 (4.0) (10.0)	3.68 (10.0) (9.0)	3.38 (9.0) (10.0)	3.71 (10.0) (2.5)	4.20 (2.5) (6.0)	1.25 (6.0)				
27	—	6.31 (3.5)	3.67 (3.5) (9.5)	3.98 (9.5) —	3.57 (9.0) (10.0)	3.93 (10.0) —	4.26 (4.0,2.0) (−12)		7.40-7.25	5.03-4.53		2.02,2.16

*Benzylidene group.

Table 2 (cont.). ^{1}H-NMR chemical shifts (ppm) and coupling constants (in brackets, Hz) of various disaccharide derivatives.

Comp.	OMe	H-1	H-1'	H-2	H-2'	H-3	H-3'	H-4	H-4'	H-5	H-5'	H-6	H-6'	C-6-CH_3	$COOCH_3$	Ar-H	Ph-CH_2
38	3.38	—	5.09 (3.5)	—	—	—	—	3.77 (9.5) (9.0)	3.46 (9.0) (10.0)	3.56 (9.0) (2.0)	—		4.09 (2.0) 4.15 (4.0)	1.33 (6.0)	1.92	7.40-7.20	5.02-4.49
39	3.36	—	5.03 (3.5)	—	3.46 (3.5) (9.5)	3.98 (9.5) (10.0)	4.01 (9.5) (9.0)	—	—	—	—	—	4.32 (1.5) (6.5)	1.32 (6.5)		7.40-7.10	5.01-4.57
40	3.43	—	5.09 (3.5)	—	3.56 (3.5) (9.5)	—	3.69 — —	—	3.43 — —	3.64 — —	—	—	4.28 — (6.5)	1.28 (6.5)			
41	3.29	4.67 (3.5)	5.00 (3.5)	—	3.41 (3.5) (9.5)	—	—	—	—	3.78 (0.5) (10.0)	—	4.27 (4.5) 4.21 (2.5)	—	1.16 (6.5)	1.99	7.40-7.20	5.03-4.51
42	3.29	—	4.98 (3.5)	3.41 (3.5) (9.5)	3.46 (3.5) (9.5)	4.04 (9.0)	3.99 (9.0)	—	—	—	—	—	—	1.13 (6.5)		7.40-7.20	4.98-4.53
43	3.44	4.80 (4.0)	5.07 (3.5)	3.57 (4.0) (9.5)	3.55 (3.5) (9.5)	3.67 (9.5) (9.0)	3.73 (9.5) (10.0)	3.39 (9.0) (10.5)	3.41 — —	3.83 (10.5) (2.5)		3.88 (5.0) 3.76 (4.0)	4.17 (2.5) (6.5)	1.29 (6.5)			

Table 3. ^{13}C-NMR chemical shifts (ppm) of monosaccharide and disaccharide derivatives.

Comp.	O<u>C</u>H$_3$	C-1	C-2—C-5	C-6	C-7	Ar. Carbons	Ph-<u>C</u>	<u>C</u>=O	CO$_2$<u>C</u>H$_3$	OSO$_2$<u>C</u>H$_3$
7	55.77	98.41	75.32—73.60			138.43—125.31	87.76—77.82	197.45		
9	55.01	98.15	75.70—72.83	65.05	20.15	138.79—127.57	82.17—77.72			
10	55.16	97.64	75.75—72.31	68.37	17.81	138.61—127.73	82.38—80.01			
11	55.26	97.74	75.62—73.28	72.01	27.06, 25.10	138.47—126.44	82.74—80.05			
14	56.30	99.01	83.23—71.80		17.35	162.81—123.38	76.86—74.53			
15	54.89	99.24	73.49—64.26	69.74	18.72					
17	55.34	97.59	72.74—69.66	64.45	11.28	137.40—126.24	94.07		20.65, 20.79	
18	55.33	98.26	82.30—72.38		17.73	138.54—127.74	75.74—75.00			39.54
19	54.99	97.80	82.44—70.12		13.57	136.66—127.75	75.92—73.41	165.76		
20	55.15	98.07	75.97—68.26		16.34	138.50—127.74	82.36—77.77	165.90		
21	54.96	99.22	73.37—66.34		15.05					

Table 3 (cont.). ^{13}C-NMR chemical shifts (ppm) of disaccharide derivatives.

Comp.	O<u>C</u>H$_3$	C-1′	C-1	C-2′—C-5′, C-2—C-6	C-6′	C-7	Ar. Carbons	Ph-<u>C</u>H$_2$	<u>C</u>=O	CO<u>C</u>H$_3$
38	55.09	97.90	95.20	82.56—69.19	63.24	16.31	138.81—127.39	75.72—73.00	170.75	20.01
39	55.09	97.88	95.39	82.54—71.31	61.72	16.40	138.84—127.37	75.60—73.13		
40	55.67	100.03	95.56	74.49—68.50	61.17	14.76				
41	55.13	97.76		82.61—69.08	63.27	16.55	138.85—127.68	75.61—74.72	170.74	20.84
42	55.09	97.89	95.37	82.53—71.32	61.73	16.38	137.90—124.38	75.69—70.48		
43	56.09	100.13	97.98	73.89—71.48	61.40	15.12				

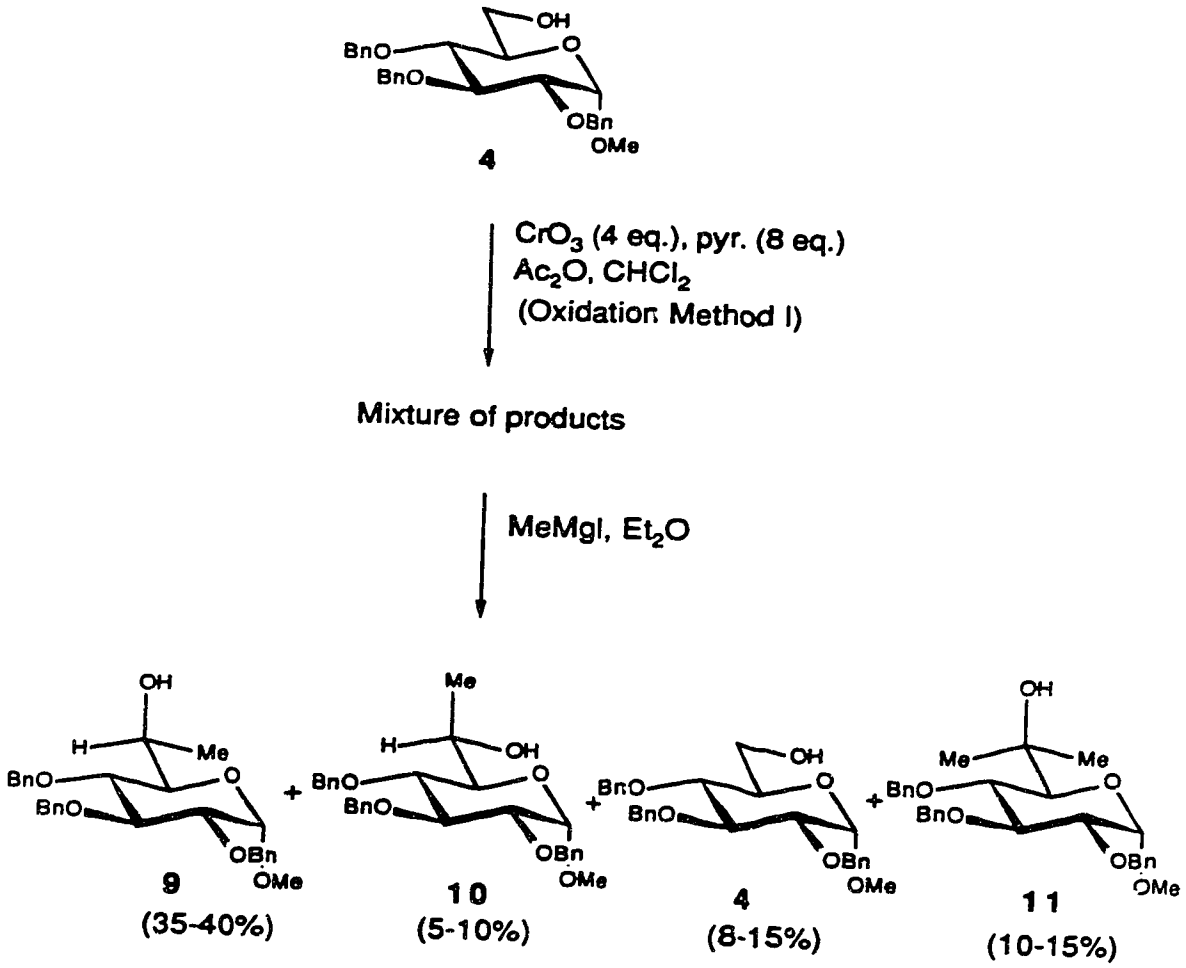
assignments are tabulated in Table 2 and 3 (p. 14 and 17, respectively). The ^{1}H-NMR spectrum is reproduced in Figure 5(b) where the singlet at δ=9.6 ppm is the aldehyde signal. The purity of the crude mixture was assessed by integration of the OMe singlets. As shown in Figure 5(b) the more intense singlet at δ=3.38 ppm is the OMe signal of compound **7** and the smaller singlet at δ=3.42 ppm is the OMe of an impurity (probably **8**). The ratio of the intensities of these two signals indicated the yield of compound **7** to be about 90%.

*1.1.2. The diastereomeric 6-C-methyl derivatives of methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside (**9** and **10**)*

Since it was impractical to purify the aldehyde **7** by column chromatography, the crude aldehyde mixture was reacted directly with methylmagnesium iodide.

Initially, the Grignard reagent was added to the crude preparation of the aldehyde **7** obtained from Method I (Scheme 3). T.l.c. of the product mixture was complex, however, 3 or 4 possible products could be seen of which one spot was identified as the starting alcohol **4** by co-spotting (solvent system 3:2 n-hexane/ethyl acetate). Column chromatography with silica gel gave four identifiable products (Scheme 4): the expected simple addition products 6-*S*-methyl (**9**) (35—40%) and 6-*R*-methyl (**10**) (5—10%) derivatives, some of the starting alcohol (**4**) (8—15%) and a product which proved to be the dimethyl derivative (**11**) (10—15%). The protons and ^{13}C assignments for **9**, **10** and **11** are summarized in Table 2 and 3 (p. 14 and 17, respectively). The ^{1}H-NMR spectra of **9** and **10** are reproduced in Figure 6 and 7, respectively. It can be observed that the H-6 (δ=3.9 ppm), H-4 (δ=3.4 ppm), H-5 (δ=3.5 ppm) and C-6—CH_3 (δ=1.1 ppm) signals of **10** were shifted significantly compared to those in **9**. The significance of these chemical shifts will be discussed in Part III. The high field portion (δ=1.0—1.50 ppm) of the ^{1}H-NMR spectrum at 300 MHz of the crude product from the Grignard reaction is reproduced in

Scheme 4


OH
BnO
BnO
O
OBn
OMe
4
CrO_3 (4 eq.), pyr. (8 eq.)
Ac_2O, $CHCl_2$
(Oxidation Method I)
Mixture of products
MeMgI, Et_2O
OH
H
Me
BnO
BnO
O
OBn
OMe
9
(35-40%)
Me
H
OH
BnO
BnO
O
OBn
OMe
10
(5-10%)
OH
BnO
BnO
O
OBn
OMe
4
(8-15%)
OH
Me
Me
BnO
BnO
O
OBn
OMe
11
(10-15%)

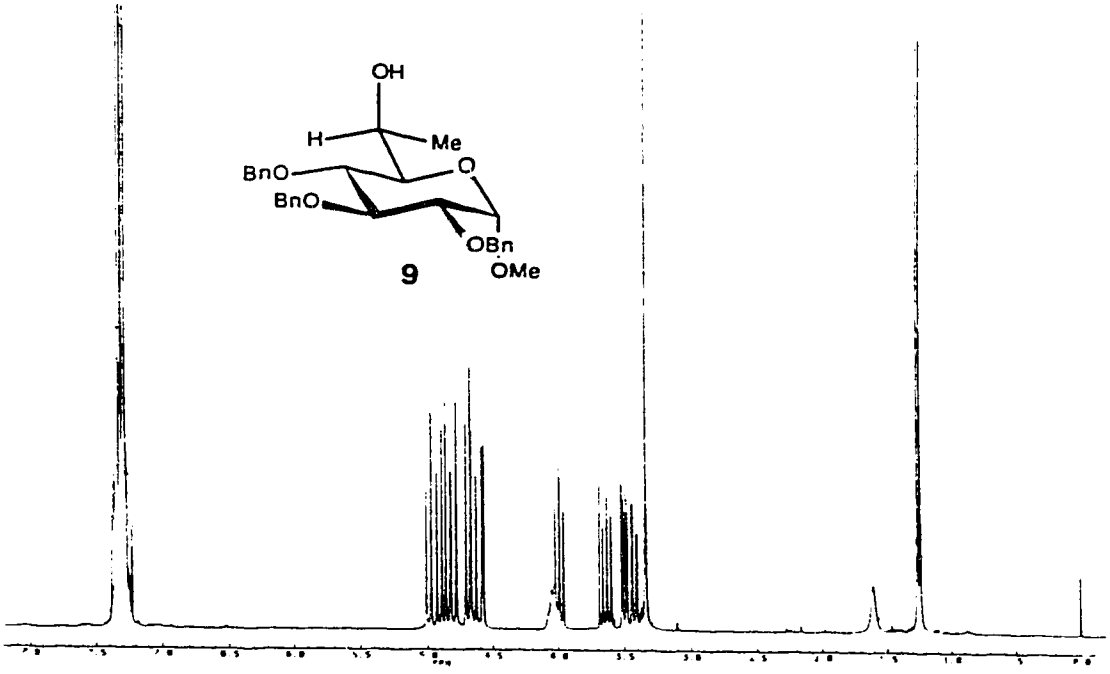


Figure 6. ^{1}H-NMR spectrum of methyl 2,3,4-tri-*O*-benzyl-7-deoxy-β-L-glycero-D-glucoheptapyranoside (**9**) (see Table 2 for assignments).

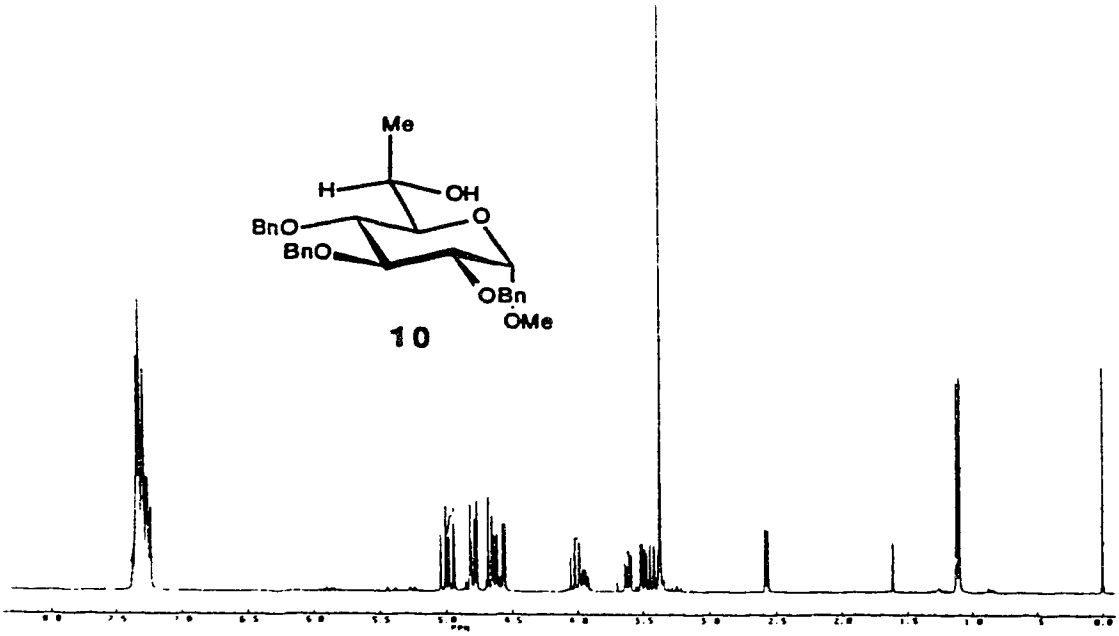


Figure 7. ^{1}H-NMR spectrum of methyl 2,3,4-tri-*O*-benzyl-7-deoxy-α-D-glycero-D-glucoheptapyranoside (**10**) (see Table 2 for assignments).

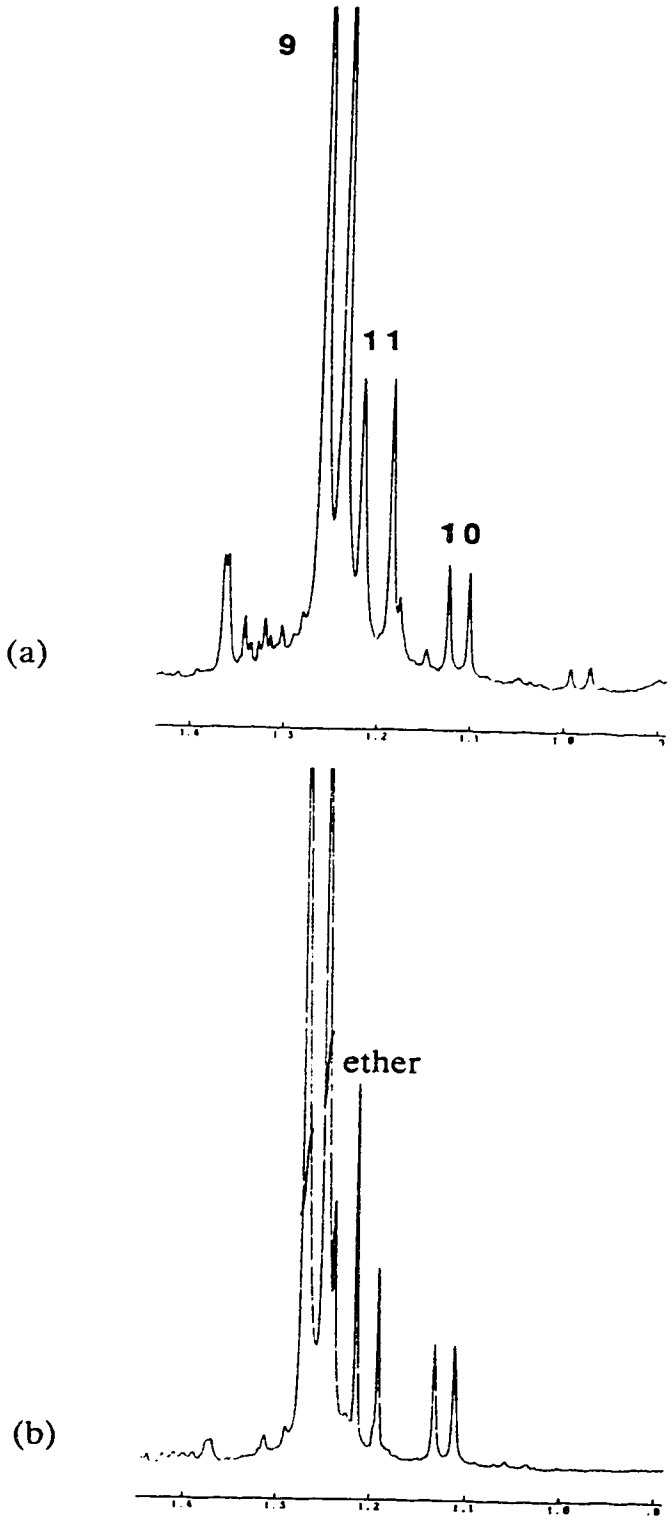


Figure 8. The high field region of the ^{1}H-NMR spectra of the crude product from the Grignard reaction. (a) From the reaction of the Grignard reagent with the crude aldehyde **7** obtained from oxidation method I. (b) From the reaction of the Grignard reagent with the crude aldehyde **7** obtained from oxidation method II.

Figure 8 (top trace) and the signals of the products **9**, **10**, and **11** are assigned based on the individual spectra of **9**, **10** and **11** (spectra of **11** will be discussed subsequently). The signals for 6-*C*-methyl groups of **9** and **10** could be expected to be doublets with spacings of near 6.5 Hz because of coupling with the geminal H-6 atom. Indeed when the spectra were measured at 360 MHz the spacings (6.5 Hz) for the signals centered at δ=1.11 and 1.25 ppm did not change and, therefore, likely arose from the desired products **9** and **10**. Details for the assignments of 6-*R* and 6-*S* configurations will be presented later. The signals at δ=1.18 and 1.22 ppm appeared to arise from two different methyl groups because the spacing changed with the change in applied field; i.e 10.0 Hz at 300 MHz and 11.0 Hz at 360 MHz. It proved that these signals in fact arose from two methyl groups on quaternary type carbon in the same compound, the structure of which was proven to be that displayed for **11** in Scheme 4.

The ^{1}H-NMR and ^{13}C-NMR spectra of **11** are reproduced in Figure 9 and 10, respectively. The proton signals in the ^{1}H-NMR were assigned by double resonance technique and the assignments were as shown in Table 2 (p. 14). For compound **11**, it is expected that the H-5 (δ=3.55 ppm) proton signal would appear as a doublet because it can only couple with H-4 and in the ^{13}C-NMR spectrum two signals would be detected at high field for the two different methyl groups at C-6. The H-6 proton is not present and H-5 at δ=3.55 ppm can be seen as a doublet with the $J_{5,4}$=9.0 Hz. Integration of the two equal intensity singlet signals at δ=1.18 and 1.22 ppm showed that six protons were present, indicating that each singlet contains three protons. In the ^{13}C-NMR (Figure 10, Table 3), the two distinctive methyl groups at C-6 were evidenced at δ=27.06 and 25.10 ppm. The low intensity signal at δ=72.01 ppm was assigned to C-6 because it is a quarternary carbon and therefore would take longer to relax. Based on the ^{1}H-NMR and ^{13}C-NMR spectra, compound **11** was concluded to have the structure shown in Scheme 4.

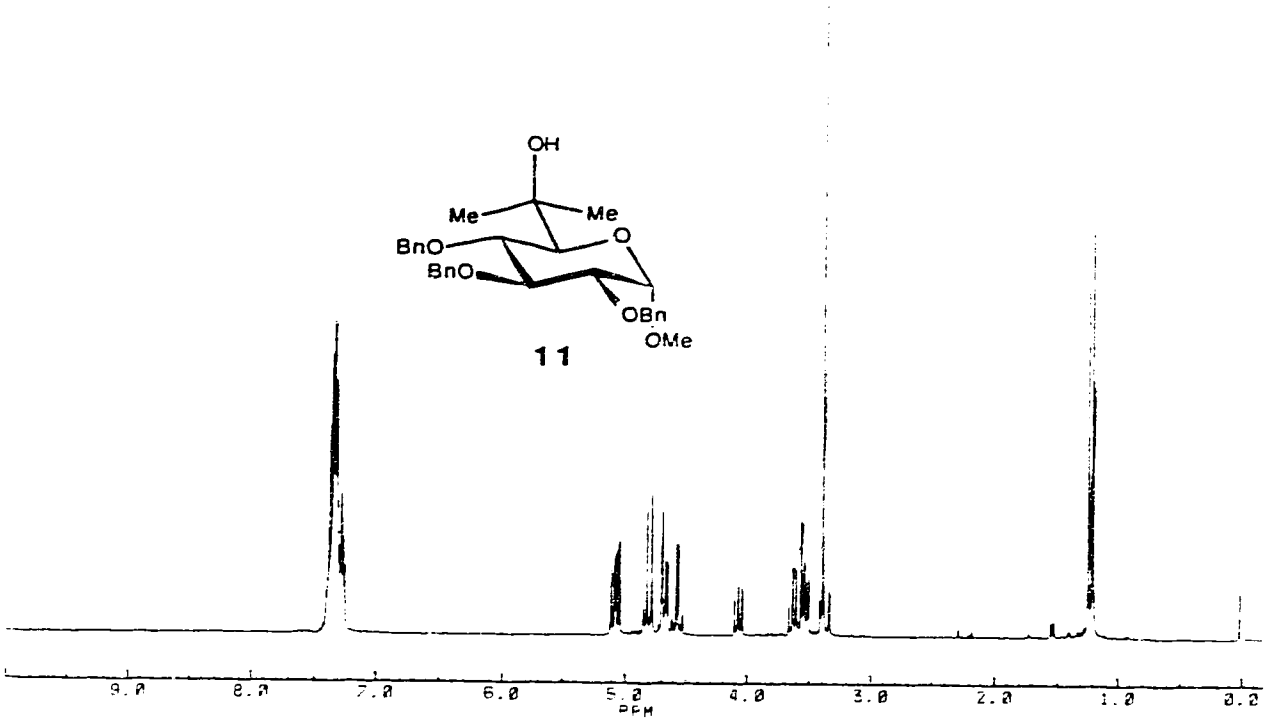


Figure 9. ^{1}H-NMR spectrum of methyl 6,6′-dimethyl-α-D-glucopyranoside (**11**)

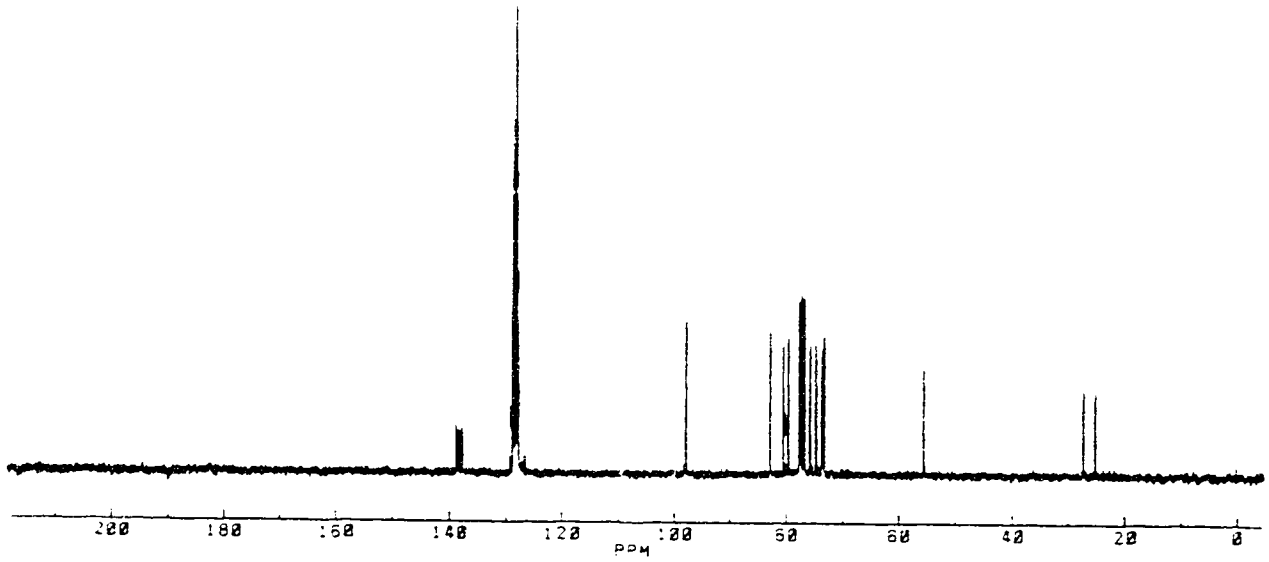
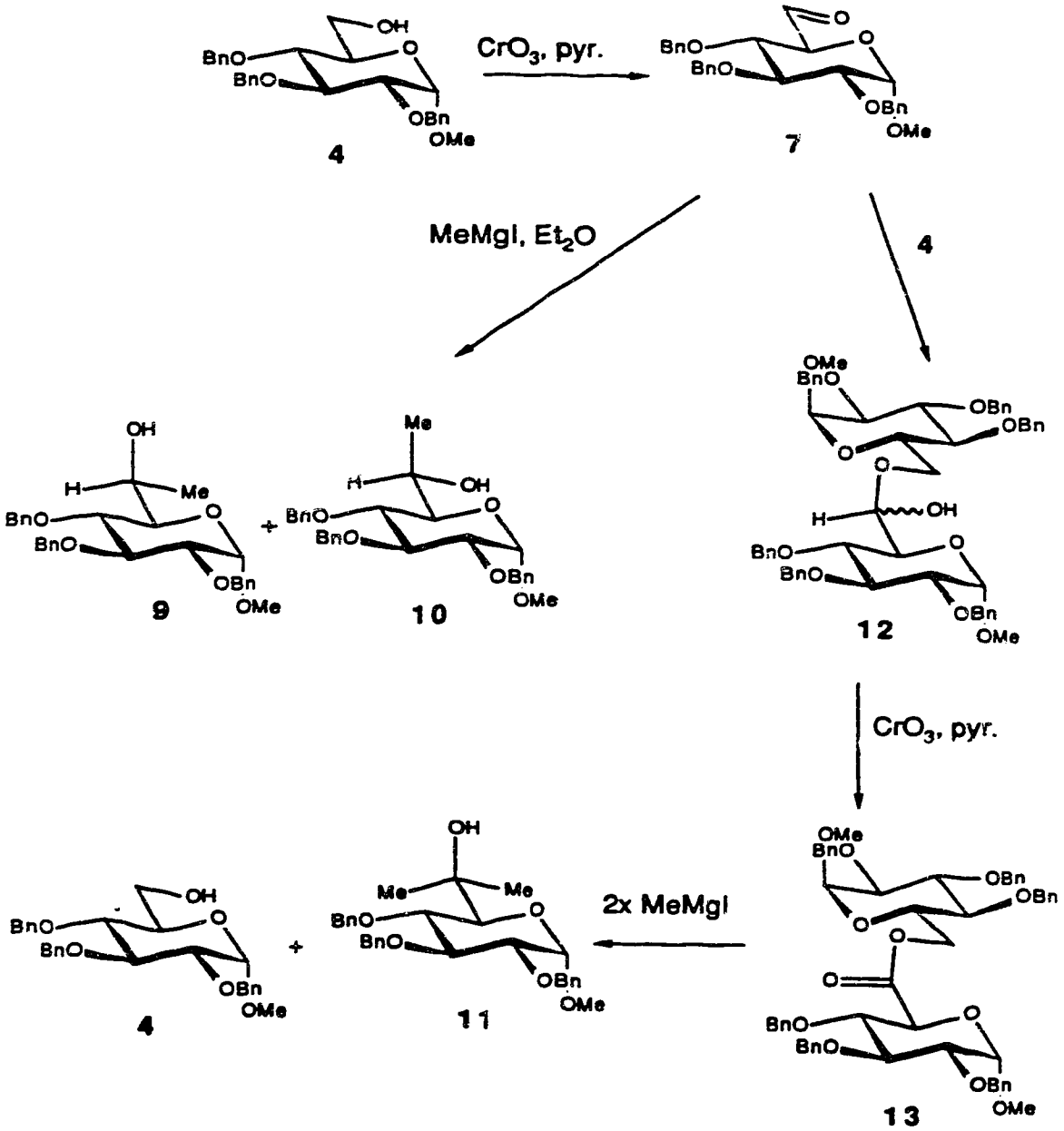


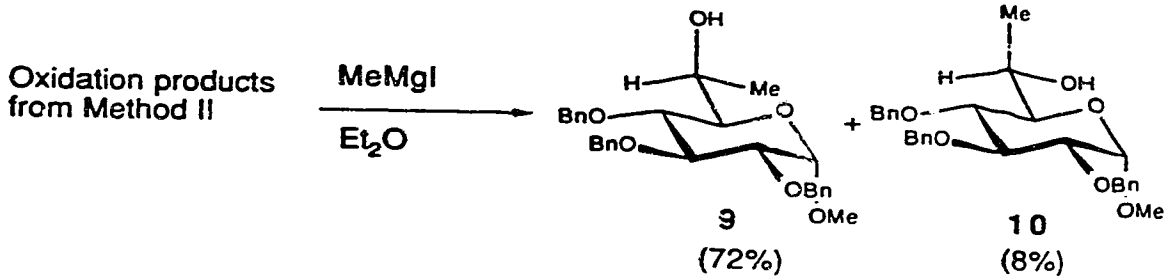
Figure 10. ^{13}C-NMR spectrum of methyl 6,6′-dimethyl-α-D-glucopyranoside (**11**)

Scheme 5

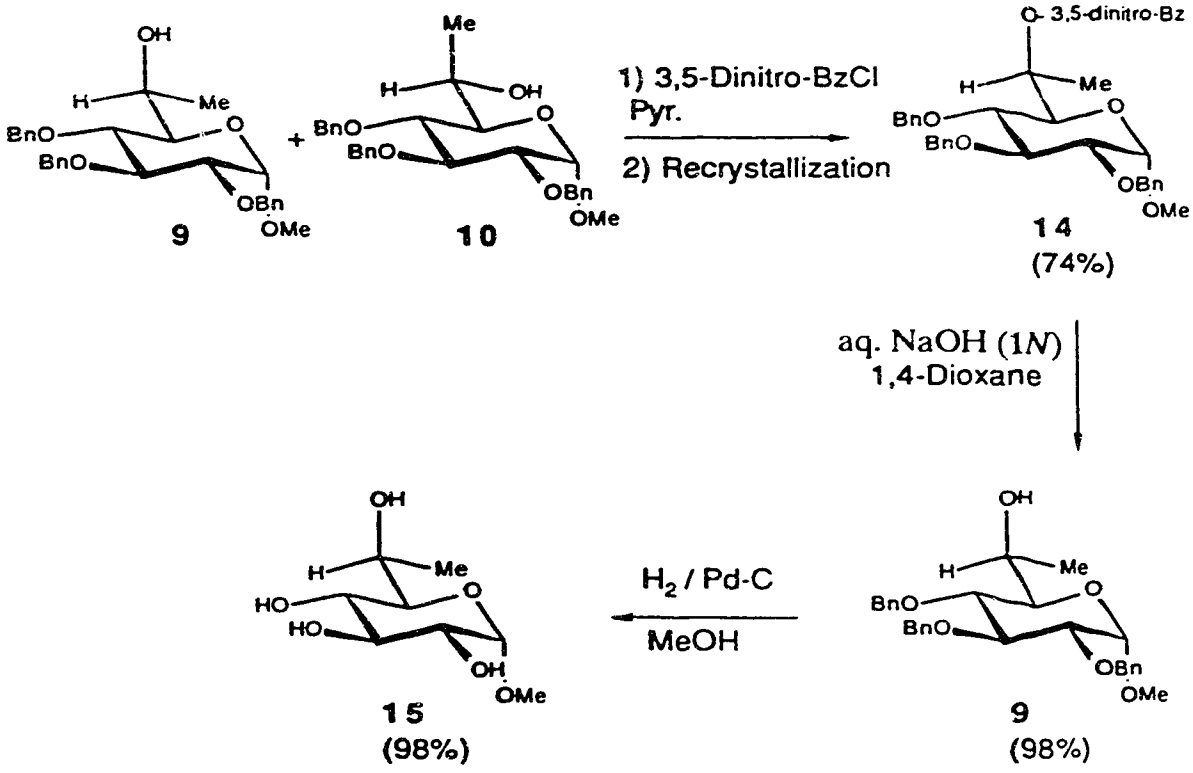


The formation of the dimethyl derivative (**11**) and the regeneration of the starting alcohol (**4**) in near equal ratio to each other suggested, as indicated in Scheme 5, that, the alcohol (**4**) forms an hemi-acetal (**12**) with the formed aldehyde (**7**) which could be oxidized by chromate reagent to give an ester (**13**). Therefore, the crude oxidation product mixture would contain both the aldehyde (**7**) and the ester (**13**) along with other mino. products such as **8**. On this basis, reactions of the crude aldehyde with methyl magnesium iodide would give rise to not only the desired 6-*S*-methyl and the 6-*R*-methyl derivatives (**9** and **10** respectively) but also to tertiary alcohol **11** and the alcohol **4** (Scheme 5). Therefore, to minimize the yield of **11**, formation of the hemiacetal had to be minimized. It was possible that this could be accomplished should the rate of oxidation be substantially greater than the rate of hemiacetal formation. Thus, the concentration of the alcohol would always be low. Indeed, reaction of the crude aldehyde (**7**) obtained from the oxidation method II (Scheme 3, p. 13) which involved fast addition of the alcohol (**4**) to excess chromium trioxide (12 eq.) and pyridine (24 eq.) with methyl magnesium iodide in ether gave only two identifiable products; i.e. **9** and **10**. The crude yield of the reaction was about 80%. Column chromatography using silica gel (solvent system 3:2 n-hexane/EtOAc) gave the 6-*S*-methyl derivative (**9**) and 6-*R*-methyl derivative (**10**) in 72% and 8% yield respectively (Scheme 6). ^{1}H-NMR spectra are identical to that in Figure 6 and 7. The high field portion of ^{1}H-NMR spectrum (δ=1.0—1.5 ppm) of the product mixture was reproduced in Figure 8 (lower trace, p. 22) for comparison purposes. As shown, the C-6—CH_3 doublet signals of **9** and **10** are identical in chemical shifts in both spectra and there is no evidence for the formation of **11** in the lower spectrum since the signals for the C-methyl groups at δ=1.22 and 1.18 ppm are not present. The ratio of **9** and **10** was usually greater than 10:1 as evidenced by the relative intensities of the signals for the 6-*C*-methyl groups. The preferential formation of **9** can be rationalized by Cram's rule[59] and will be discussed subsequently.

Scheme 6



Scheme 7



1.1.3. Preparation of methyl 7-deoxy-β-L-glycero-D-glucoheptapyranoside (**15**)

The reaction sequences in the preparation of **15** are summarized in Scheme 7. Although the 6-*S*-methyl derivative (**9**) could be obtained by column chromatography, as was indicated in section 1.1.2., this procedure was not suitable for large scale preparation. Therefore, a search was made to achieve purification by selective crystallization of an appropriate derivative. The acetyl, benzoyl, 4-nitrobenzoyl and 3,5-dinitrobenzoyl derivatives were prepared for this purpose. Although each derivative was brought to a high state of purity by chromatography on silica gel, only the 3,5-dinitrobenzoyl derivative crystallized. Thus, this derivative (**14**) could be purified by crystallization.

The crude Grignard product mixture (**9** and **10**) obtained (Scheme 6) was reacted with 3,5-dinitrobenzoyl chloride in pyridine and the product mixture was crystallized from hot methanol to give **14** in 74% yield. The ^{1}H-NMR spectrum of **14** is shown in Figure 11 and the assignments are shown in Table 2 (p. 14). The presence of the 3,5-dinitrobenzoyl group at O-6 can be deduced from the deshielding of H-6 (doublet of quartet) which was observed at δ=5.62 ppm (H-6 of **9** was observed at δ=4.05 ppm) and the characteristic 3,5-dinitrobenzoyl aromatic protons were observed at δ=9.21 as a triplet and at δ=9.16 ppm as a doublet.

Removal of the 3,5-dinitrobenzoyl blocking group was accomplished by saponification using a 1*N* aqueous sodium hydroxide solution in 1,4-dioxane to the desired product **9**, which has an identical ^{1}H-NMR spectrum as in Figure 6, in 98% yield. In the ^{1}H-NMR spectrum, the down field doublet and triplet signals which belong to the aromatic ring protons of the 3,5-dinitrobenzoyl group have now disappeared. The H-6 and the C-6—CH_3 protons also shifted up field ($\Delta\delta$= 1.57 and 0.23 ppm, respectively).

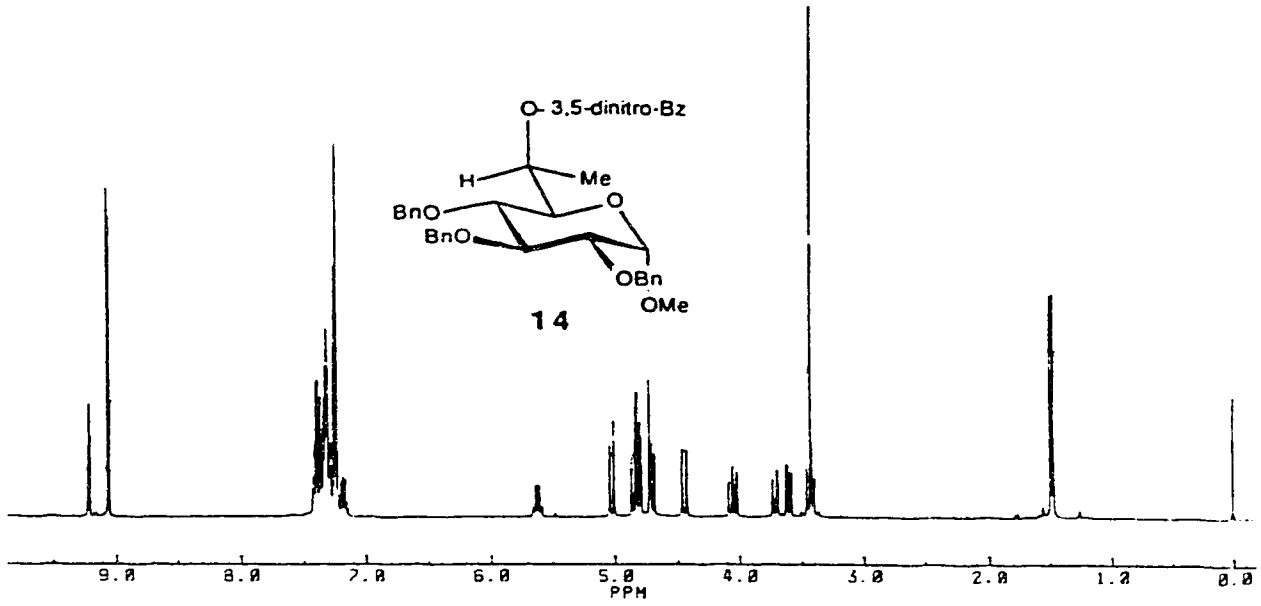


Figure 11. ^{1}H-NMR spectrum of methyl 6-*O*-(3,5-dinitrobenzoyl)-2,3,4-tri-*O*-benzyl-7-deoxy-*β*-L-*glycero*-D-glucoheptapyranoside (**14**).

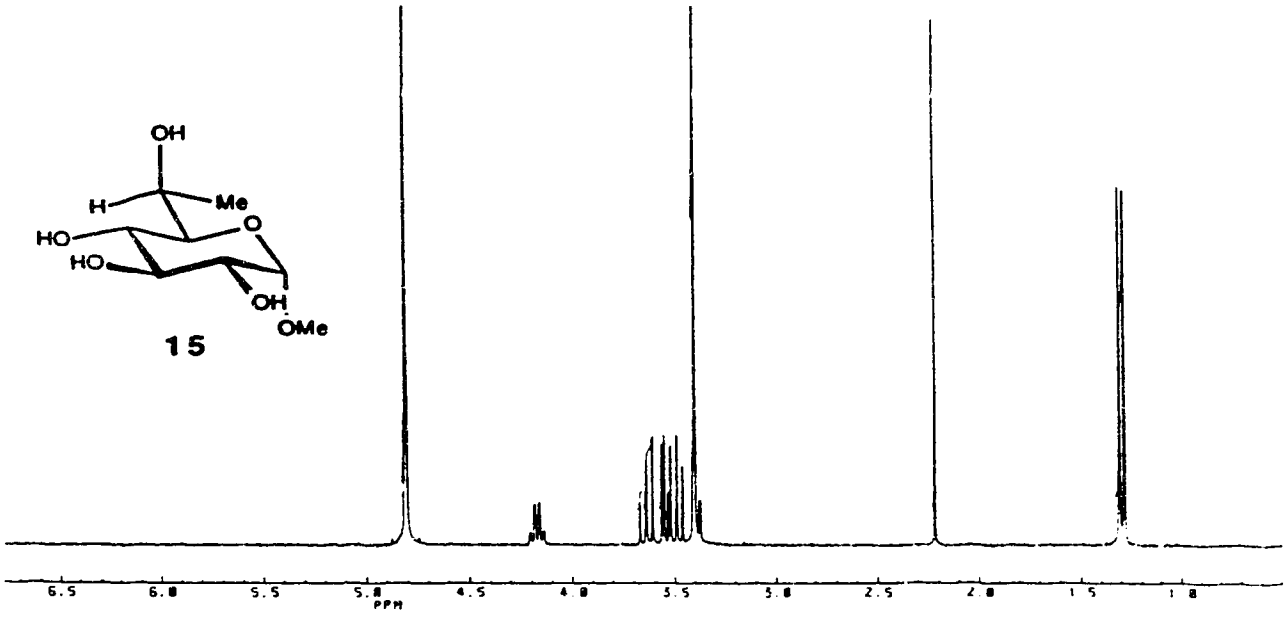


Figure 12. ^{1}H-NMR spectrum of methyl 7-deoxy-*β*-L-*glycero*-D-glucoheptapyranoside (**15**).

The benzyl groups were then removed by catalytic hydrogenolysis using palladium-on-carbon and hydrogen at 100 lb. in^{-2} in methanol to give the deblocked compound **15** in 98% yield. The structure of **15** was confirmed by the ^{1}H-NMR spectrum (Figure 12, Table 2) which shows that the signals for benzyl groups, observed at δ=5.01—4.63 ppm for **9**, are not present.

1.1.4. The theoretical basis for the proof of the configuration of the chiral C-6 of **9**

Since the absolute configurations of the chiral centers related to the D-glucopyranose portion of **9** were known, the absolute configuration of the new chiral center at position C-6 could be established by relating its configuration to one or more of the other asymmetric centers. This was accomplished first by restricting the rotation about the C-5 to C-6 bond by preparing the 4,6-*O*-benzylidene derivative (**17**) under the reaction conditions described in Scheme 8. Thus, as seen in the formula for **17** (Figure 13), the methyl group becomes fixed in a very different environment than in its diastereoisomer (**24**).

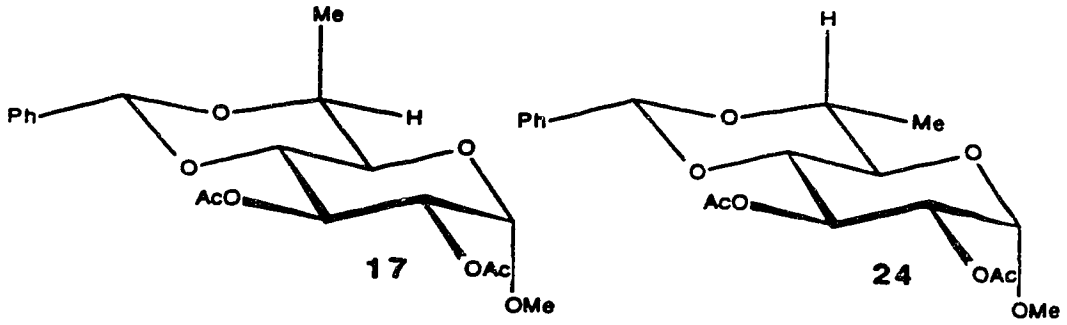


Figure 13. Formulas for methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-7-deoxy-β-L-*glycero*-D-glucoheptapyranoside (**17**) and methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-7-deoxy-α-D-*glycero*-D-glucoheptapyranoside (**24**).

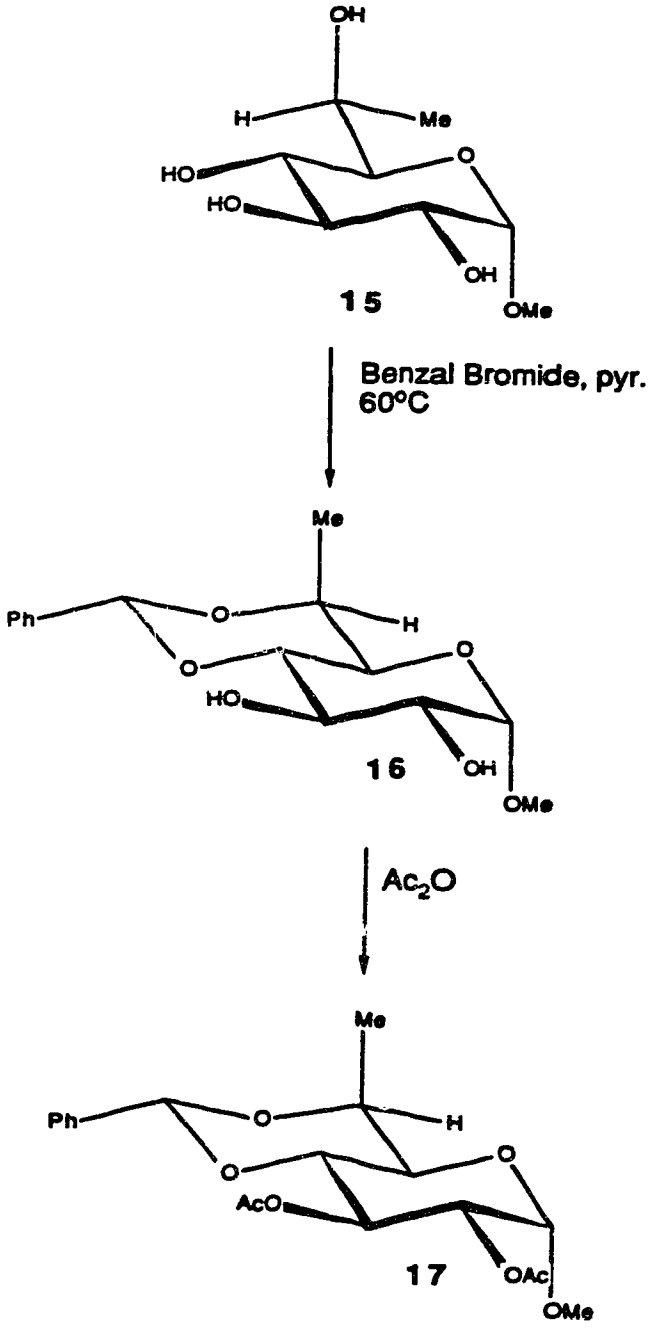
The absolute configuration of **17** could be established both by examination of $J_{5,6}$ and by nuclear Overhauser enhancement caused by saturation of the methyl group hydrogens. Based on a Karplus-type[60] relationship for proton-proton spin coupling and also the experimental parameters derived by Altona *et al.*,[61,62] the gauche coupling constant $J_{5,6}$ of **17** was estimated to be 5.4 Hz.

The measurement of nuclear Overhauser enhancement (n.O.e.)[22] is the most powerful and widely used tool in conformational analysis of complex molecules in solution. In this technique, a proton is saturated and the relative contributions of neighbouring protons receiving relaxation contribution are measured. The n.O.e. observed in a given proton signal is related to the distance between itself and the saturated proton and is therefore useful in molecular conformational analysis. It should be noted that the steady state n.O.e. does not provide absolute distances between protons but only the relative contributions of internuclear dipole-dipole relaxation. For compound **17**, saturation of the C-6—CH_3 signal should cause n.O.e. enhancement of the benzylidene proton, H-6 and H-4.

1.1.4.1. The fixing of a rigid conformation by preparing **17**

Reaction of **15** with benzal bromide in pyridine upon heating (60°C) produced the 4,6-*O*-benzylidene derivative (**16**) which was not isolated but was reacted further with acetic anhydride to give the acetate **17** in 51% yield (Scheme 8). The ^{1}H-NMR spectrum of **17** is reproduced in Figure 14 (bottom trace). The protons were assigned as shown in Table 2 (p. 15) using double resonance and 2D-NMR techniques. In the spectrum of **17**, the singlet signal which appears at δ=5.8 ppm is characteristic of an α-hydrogen of a benzylidene group and therefore, was assigned as H-7. The signal at δ=4.15 ppm was assigned as H-5 and the observed coupling $J_{5,6}$ was 6.0 Hz and is in agreement as was discussed above with the estimated value 5.4 Hz.[61,62] The ^{13}C-NMR data of **17** is shown in Table 3 (p. 17) and the spectrum is reproduced in Figure 15. The signal which appears

Scheme 8


OH
H
Me
HO
O
HO
OH
OMe
15
Benzal Bromide, pyr.
60°C
Me
Ph
O
H
O
O
HO
OH
OMe
16
Ac2O
Me
Ph
O
H
O
O
AcO
OAc
OMe
17

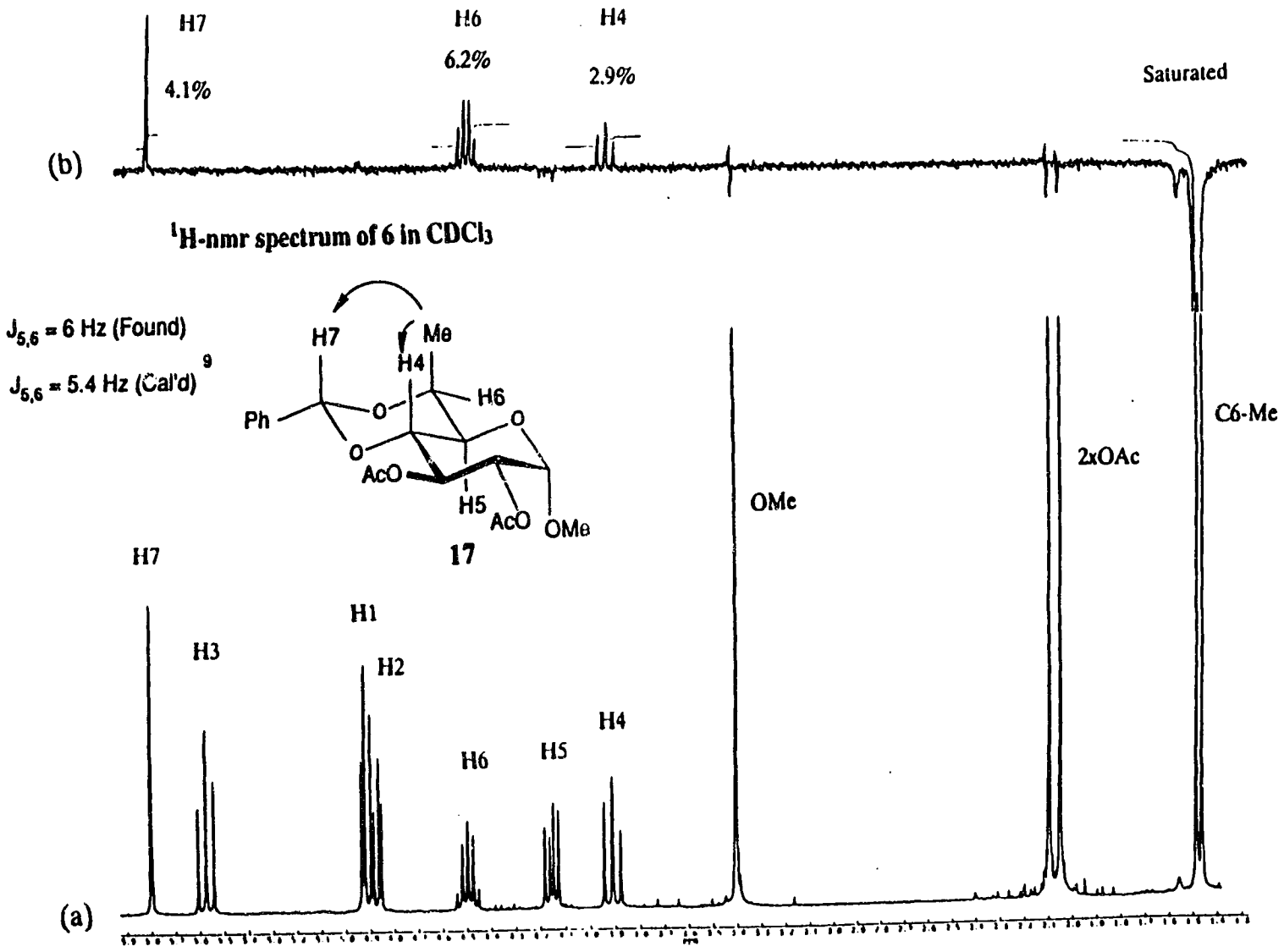


Figure 14. (a) ^{1}H-NMR spectrum of methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-7-deoxy-β-L-*glycero*-D-glucoheptapyranoside (**17**). (b) N.O.e. experiment involving **17**.

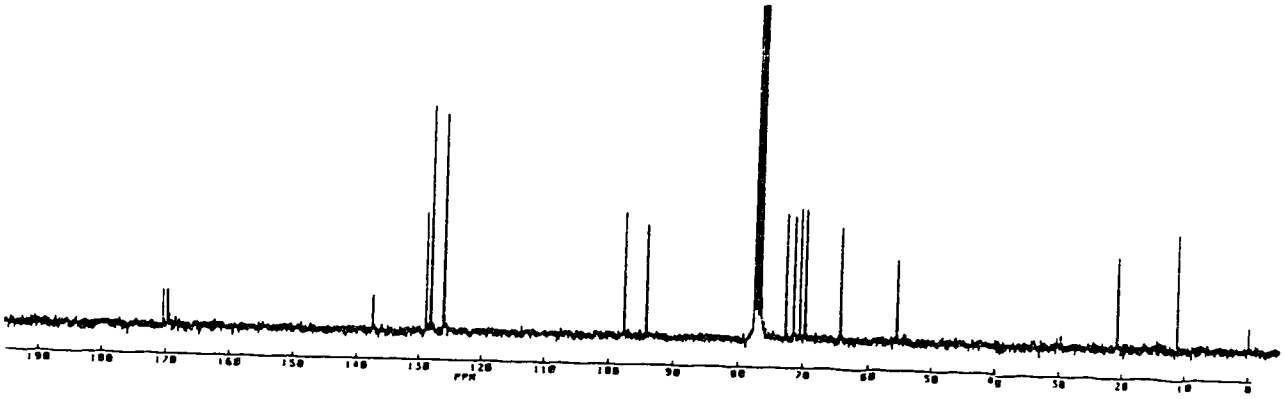


Figure 15. ^{13}C-NMR spectrum of methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-7deoxy-β-L-*glycero*-D-glucoheptapyranoside (**17**) (see Table 3).

at δ=94.07 ppm is typicaily that of a benzylidene α-carbon (Ph-C-). Therefore, based on the ^{1}H-NMR and ^{13}C-NMR spectra, the structure of compound **17** is as that shown in Figure 13.

1.1.4.2. NMR experiments involving **17**.

Additional evidence for the structure of **17** was obtained from nuclear Overhauser enhancement difference spectroscopy and is reproduced in Figure 14 (top trace). Irradiation of the C-6 methyl doublet of **17** at δ=1.48 ppm caused 2.4% and 4.1% enhancements of H-6 and the benzylidene hydrogen. The pattern of the n.O.e. indicated that the methyl group at C-6 of compound **17** is arranged cis-axially to H-4 and the benzylidene hydrogen. Based on the coupling $J_{5,6}$ and the n.O.e. pattern, it was concluded that the configuration at the C-6 center is *L*- for compound **17** and therefore *L*- for compounds **15** and **9**.

1.1.5. Rationalization of the stereoselectivity displayed by the Grignard reaction

As mentioned above, the reaction of the Grignard reagent methylmagnesium iodide with the aldehyde (**7**) obtained from the oxidation method II gave predominantly (about 10:1) the 6-*S*-methyl compound (**9**) (Scheme 4). This result can be rationalized by Cram's rule[59] which requires that reaction occur with the aldehyde group of **7** be aligned in the plane of the C-5 to O-5 bond. Complexation with magnesium ion as indicated in Scheme 9 would provide this alignment. The methyl carbanion would then be expected to preferentially approach the carbonyl center at C-6 from the less sterically hindered side to produce the 6-*S*-methyl derivative (**9**). Attack from the C-4 side would yield the minor product, i.e., 6-*R*-methyl derivative (**10**).

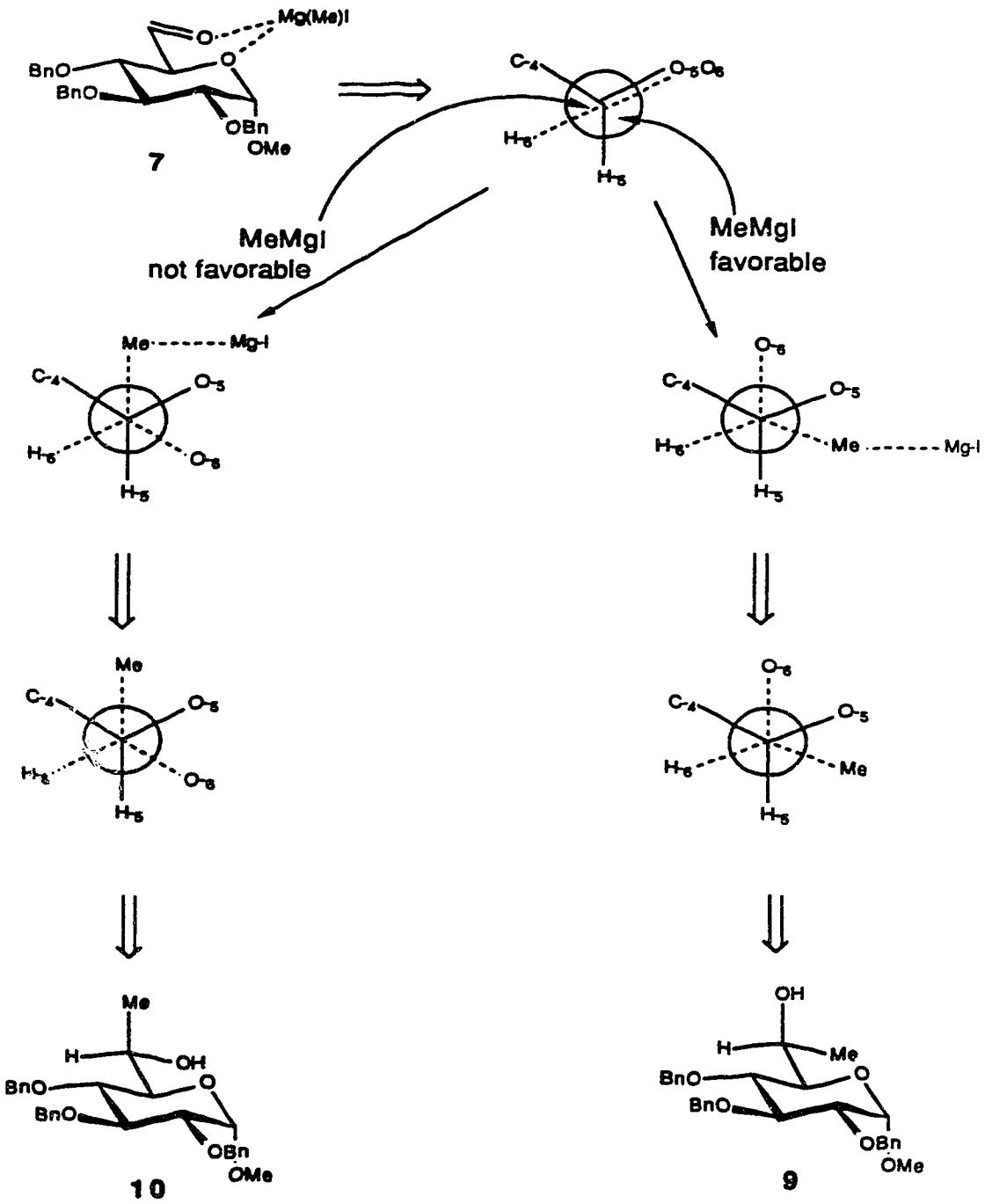
*1.2. Synthesis of methyl 7-deoxy-α-D-glycero-D-glucoheptapyranoside (**21**)*

The 6-*R*-methyl (**10**) was a very minor product (~ 10%) of the Grignard reaction and, therefore, this direct procedure was impractical for large scale preparation. Consequently it was decided to prepare **10** from the pure isomer **9** which could be readily prepared in substantial quantity by way of the crystalline 3,5-dinitrobenzoyl derivative (**14**) (Scheme 7, p. 27).

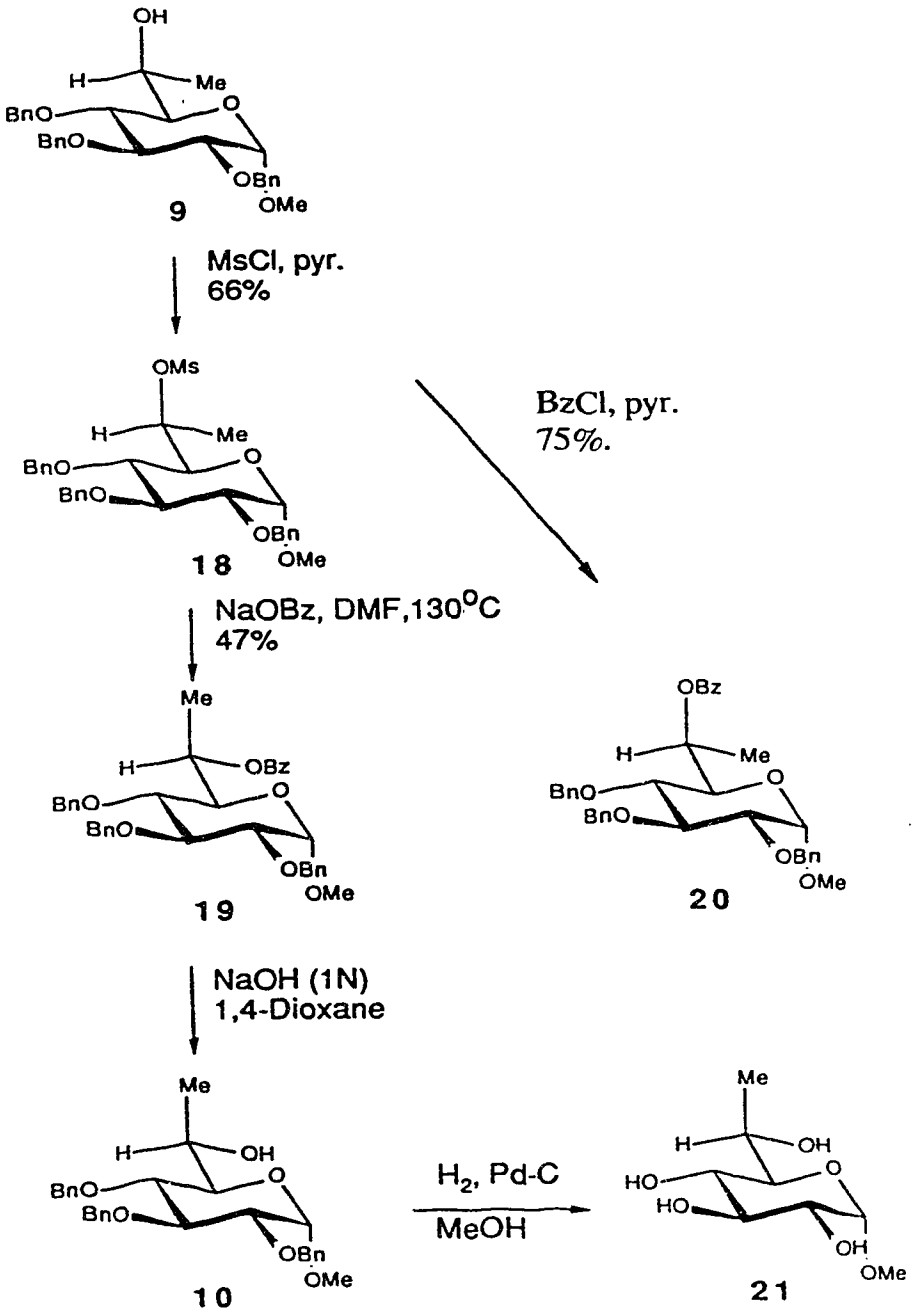
*1.2.1. Synthesis of methyl 6-O-benzoyl-2,3,4-tri-O-benzyl-7-deoxy-α-D-glycero-D-glucoheptapyranoside (**19**) via the mesylate derivative (**18**)*

The reaction sequence in the preparation of **19** is as shown in Scheme 10. Reaction of the 6-*S*-methyl (L-*glycero*) compound (**9**) with methanesulfonyl chloride in pyridine gave the mesylate derivative (**18**) in 66% yield after purification by column chromatography. The ^{1}H-NMR and ^{13}C-NMR assignments are shown in Tables 2 and 3 (p. 15 and 17), respectively. The ^{1}H-NMR spectrum of **18** is reproduced in Figure 16. As shown, the signal at δ=5.32 ppm was assigned as H-6 by double resonance technique. This assignment

Scheme 9


Mg(Me)I
BnO
BnO
OBn
OMe
7
MeMgI
not favorable
MeMgI
favorable
Me
Mg-I
C-4
O-5
O-6
H-6
H-5
10
9
OH
H
Me

Scheme 10


OH
H
Me
BnO
O
BnO
OBn
OMe
9
MsCl, pyr.
66%
OMs
18
BzCl, pyr.
75%.
NaOBz, DMF,130°C
47%
OBz
19
20
NaOH (1N)
1,4-Dioxane
10
H2, Pd-C
MeOH
HO
21

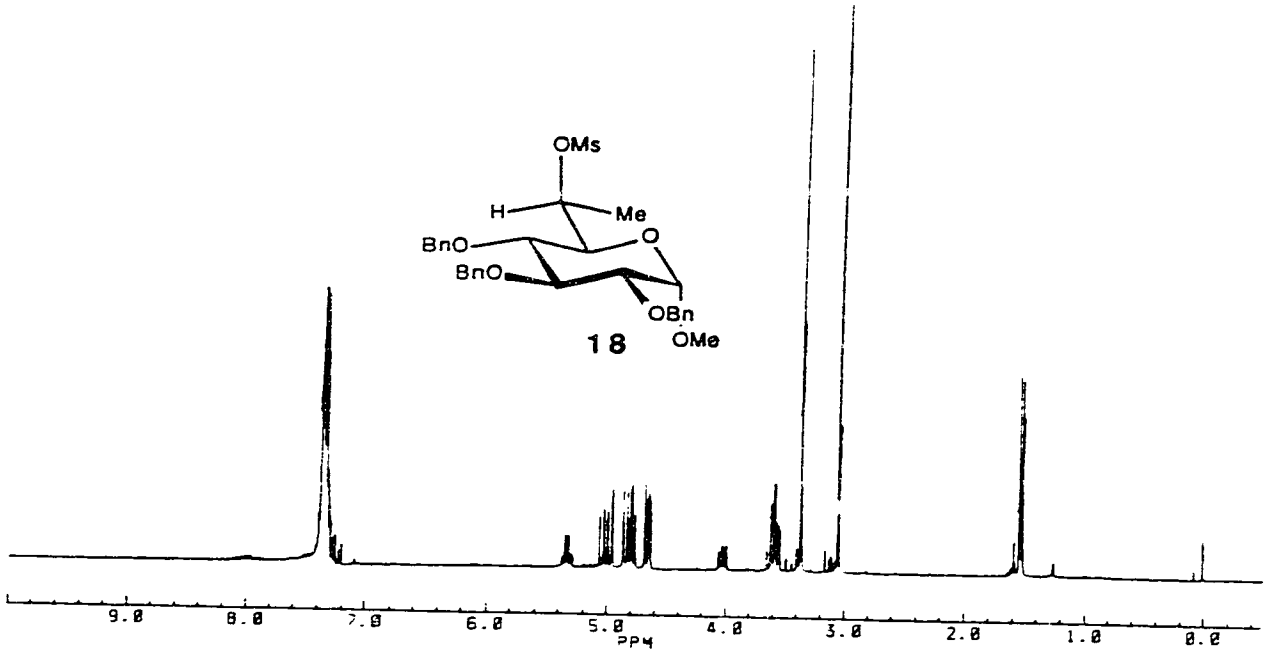


Figure 16. ^{1}H-NMR spectrum of the methyl 2,3,4-tri-*O*-benzyl-7-deoxy-6-*O*-methanesulfonyl-β-L-*glycero*-D-glucoheptapyranoside (**18**) (see Table 2).

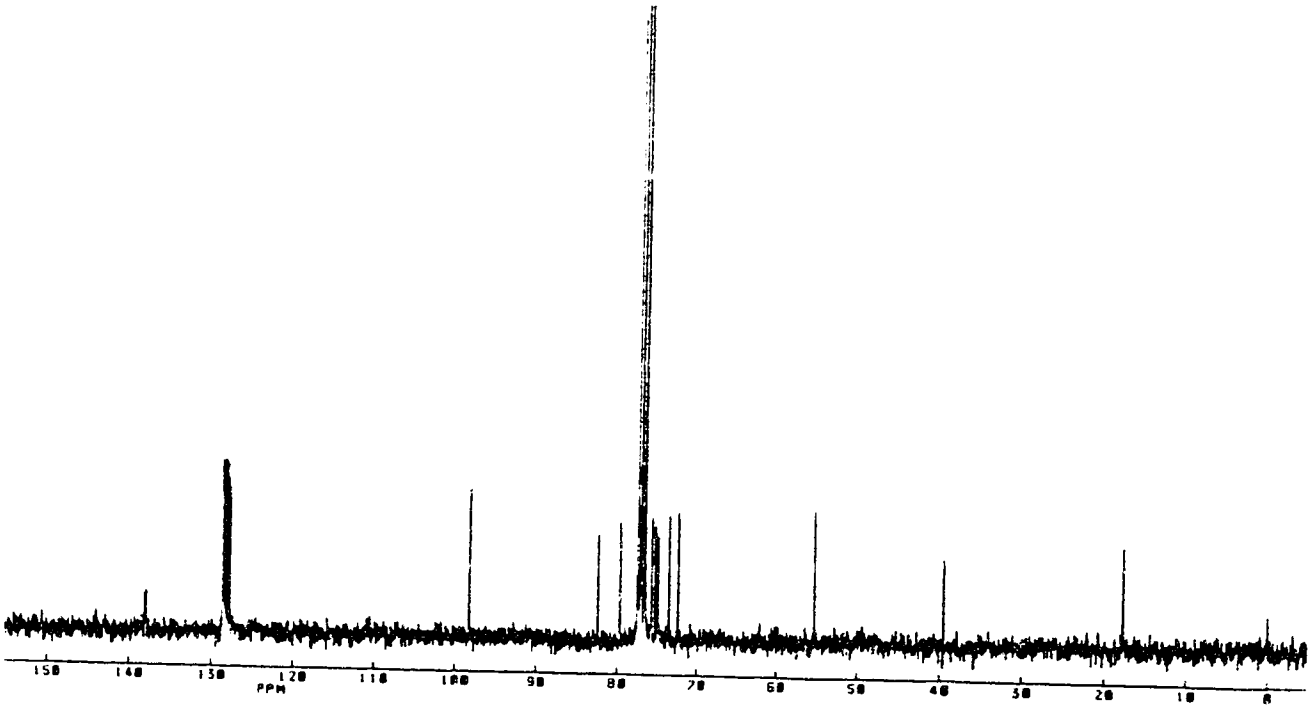


Figure 17. ^{13}C-NMR spectrum of methyl 2,3,4-tri-*O*-benzyl-7-deoxy-6-*O*-methanesulfonyl-β-L-*glycero*-D-glucoheptapyranoside (**18**) (see Table 3).

is consistent with the fact that a mesylate group should deshield H-6 to lower field as compared to the chemical shift of H-6 of the 6-*S*-methyl derivative (**9**) (δ=4.05 ppm). The singlet signal appearing at δ=3.04 ppm was assigned as the methyl group of the mesylate. The doublet signal at δ= 1.53 was assigned as the methyl group at C-6. The ^{13}C-NMR of **18** is reproduced in Figure 17. The assignments are summarized in Table 3 (p. 17). The signal at δ=39.54 ppm was assigned as the carbon of the mesylate methyl group and the signal at δ=17.73 ppm was assigned as C-7. The ^{1}H-NMR and ^{13}C-NMR spectra were consistent with the structure of **18** as projected in Scheme 10.

The mesylate derivative **18** was then reacted with sodium benzoate in dimethyl formamide at 130°C to give the C-6 inverted D-benzoate (**19**) in 47% yield. The t.l.c. of the crude reaction product showed that at least 3 compounds were present and the two main products have very close R_f values. Normal column chromatography using silica gel could not separate the reaction mixture to give pure **19**. The D-benzoate (**19**) was finally purified using HPLC (solvent 10:1 n-hexane / acetone).

The ^{1}H-NMR spectrum of the D-benzoate (**19**) is reproduced in Figure 18 (top trace). For comparison, the methyl 6-*S*-methyl-α-D-glucopyranoside (**9**) was converted to the 6-*O*-benzoyl derivative (**20**) by reaction with benzoyl chloride in pyridine. The ^{1}H-NMR spectrum of **20** is also reproduced in Figure 18 (bottom trace). The chemical shifts of C-6—CH_3, H-4 and H-5 of the inverted benzoate (**19**) were found at δ=1.18, 3.42 and 3.93 ppm respectively whereas in **20** they were found at δ= 1.52, 3.60 and 3.75, respectively (Table 2, p. 16). Because the ^{1}H-NMR spectra of **19** and **20** are different, it is concluded that compound **19** and **20** are the sought after epimers at the C-6 center.

1.2.2. Synthesis of methyl 6-O-benzoyl-2,3,4-tri-O-benzyl-7-deoxy-α-D-glycero-D-glucopyranoside (**19**) *via the tosylate derivative.*

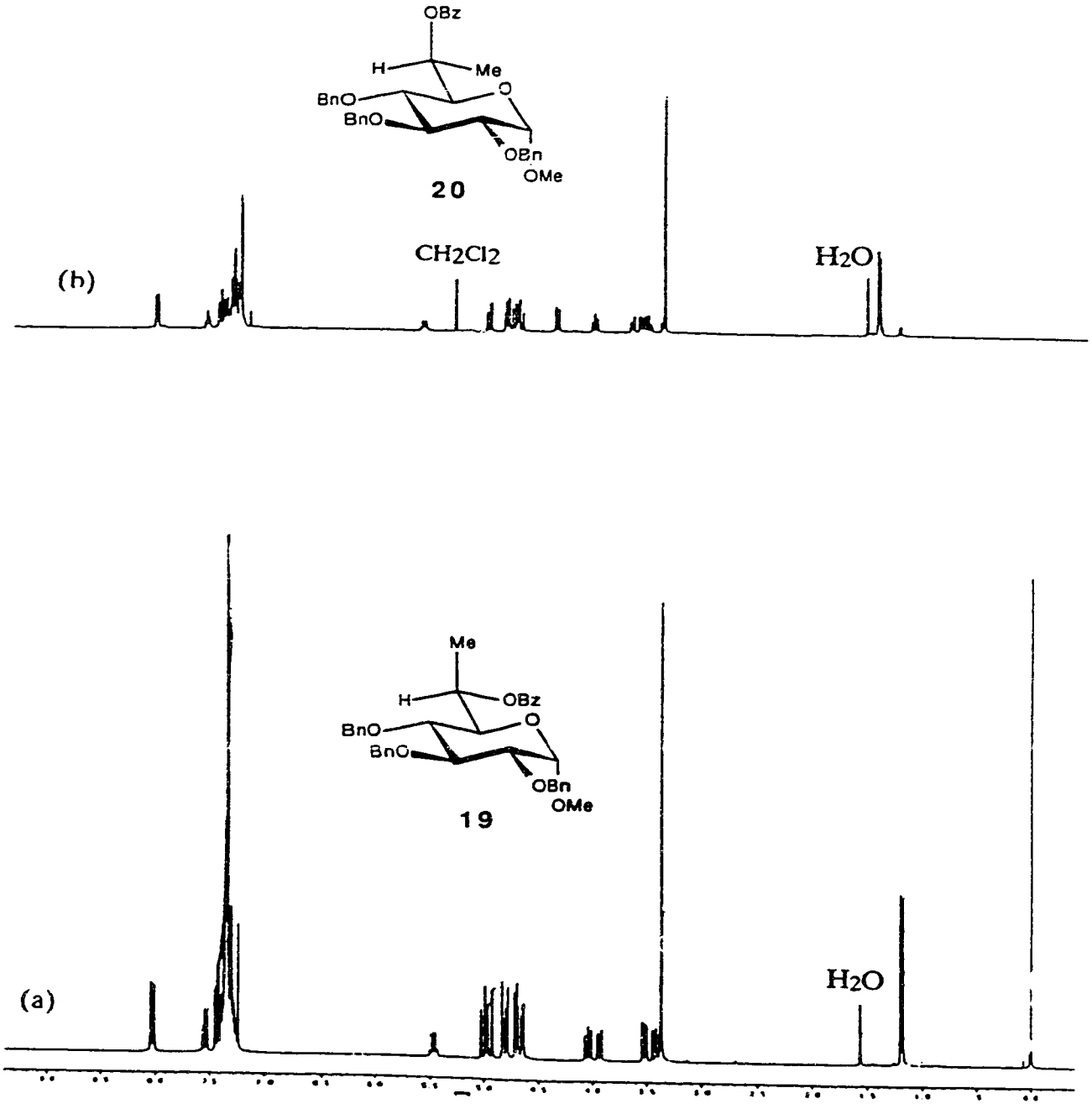


Figure 18. ^{1}H-NMR spectra of (a) methyl 6-*O*-benzoyl-2,3,4-tri-*O*-benzyl-7-deoxy-α-D-*glycero*-D-glucoheptapyranoside (**19**). (b) methyl 6-*O*-benzoyl-2,3,4-tri-*O*-benzyl-7-deoxy-β-L-*glycero*-D-glucoheptapyranoside (**20**).

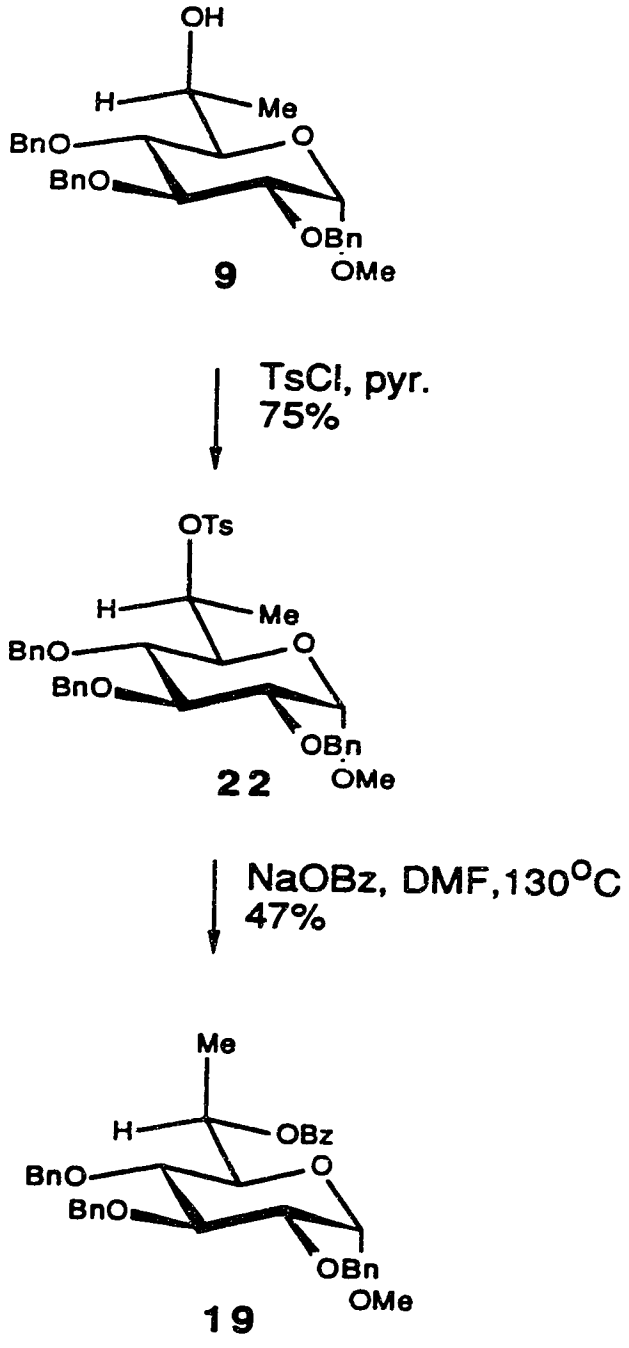
The procedure which was previously developed by Dr. Devlin from this laboratory,[63] provides the intermediate tosylate (**22**) in crystalline form, a product which is easier to handle than the syrupy intermediate mesylate (**20**). Consequently, this procedure (Scheme 11) was used in the latter period of the research to prepare methyl 6-*R*-*C*-methyl-α-D-glucopyranoside (**10**). Compound **9** was reacted with toluenesulfonyl chloride in pyridine to give the tosylate derivative **22** which could be crystallized from n-hexane and ether in 75% yield. The ^{1}H-NMR and ^{13}C-NMR spectra of **22** was identical to that obtained previously.[63] The tosylate derivative **22** was then converted to the D-benzoate derivative (**19**) according to the conditions described above (section 1.2.1) in 70% yield. The ^{1}H-NMR and ^{13}C-NMR spectra of this D-benzoate was identical to that for **19**.

1.2.3. Synthesis of methyl 7-deoxy-α-D-glycero-D-glucoheptapyranoside (**21**)

The benzoate (**19**, Scheme 10) was saponified using 1*N* sodium hydroxide in aqueous dioxane[64] to produce compound **10** which had already been isolated as a Grignard reaction product (Scheme 6, p. 27). The ^{1}H-NMR spectrum was identical to that for **10** (Figure 7, p. 21).

The benzyl groups of **10** were then removed by catalytic hydrogenation with hydrogen and palladium on carbon to produce the desired deblocked compound **21** in 98% yield (Scheme 10, p. 37). The ^{1}H-NMR spectrum of **21** is compared to that of **15** in Figure 19. It is observed that, for **21**, H-4 (δ=3.30 ppm) and C-6—CH_3 (δ=1.18 ppm) are shifted upfield, and H-5 (δ=3.65 ppm) is shifted down field (Table 2, p. 15) compared to H-4, C-6—CH_3 and H-5 (δ=3.50, 1.30 and 3.40 ppm, respectively, Table 2, p. 14) of **15**. The chemical shifts pattern observed here for **21** and **15** is similar to that of **10** and **9** discussed previously. The significance of the ^{1}H-NMR chemical shifts in the spectra of **21** and **15** will be discussed in conformational analysis section (Part III). Based on the differences in

Scheme 11


OH
H
Me
BnO
O
BnO
OBn
OMe
9
TsCl, pyr.
75%
OTs
H
Me
BnO
O
BnO
OBn
OMe
22
NaOBz, DMF, 130°C
47%
Me
H
OBz
BnO
O
BnO
OBn
OMe
19

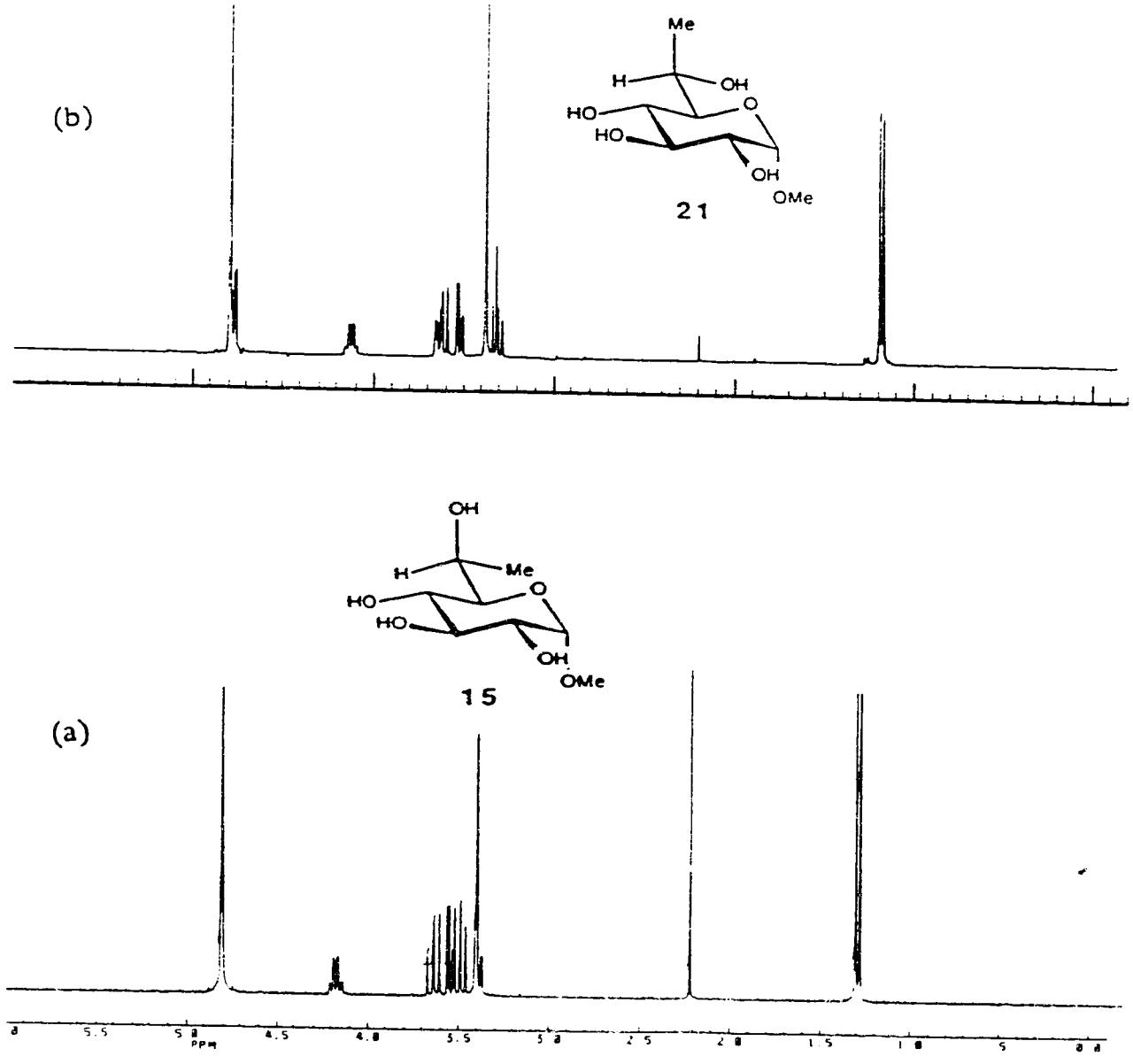


Figure 19. ^{1}H-NMR spectra of methyl 7-deoxy-α-D-*glycero*-D-glucoheptapyranoside (**21**, a) and methyl 7-deoxy-β-L-*glycero*-D-glucoheptapyranoside (**15**, b).

the ^{1}H-NMR spectra and the nature of the reaction involved, it was deduced that compounds **15** and **21** are epimers at the C-6 center.

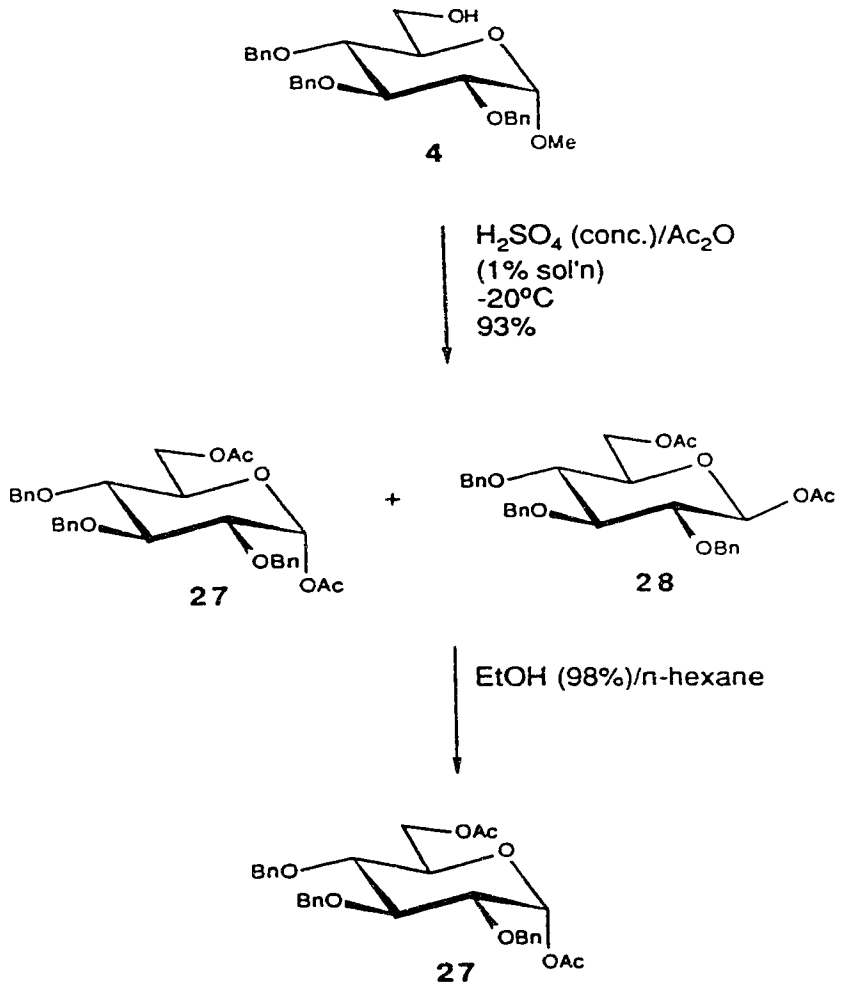
2. Synthesis of the diastereoisomeric methyl 6-C-methyl-α-isomaltosides

The synthetic goal was to synthesize α-(1 → 6) linked oligosaccharides with the reducing units derived from 6-*C*-methyl-α-D-glucopyranose (Schemes 19 and 20, p. 54 and 59 respectively). The glucosyl bromide derivative **31** was synthesized from the 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranose (**27**), an important intermediate in the synthesis of the disaccharide derivatives. Compound **27** had been synthesized from methyl 2,3,4,6-tetra-*O*-benzyl-α-D- glucopyranoside (**25**)[65] and the 2,3,4-tri-*O*-benzyl levoglucosan,[66] it was strategically useful to synthesize **27** from **4** because the procedure could be applied for the preparation of the glucosyl bromides from compounds **9** and **10**.

2.1. Synthesis of the 1,6-di-O-acetyl-2,3,4-tri-O-benzyl-α-D-glucopyranose (27)

The synthesis of the compound **27** is as outlined in Scheme 12. The alcohol **4** was acetolyzed with 1% solution of concentrated sulfuric acid in acetic anhydride[63-65] at − 20°C (dry ice/acetone)[63] to give cleanly after work up, a mixture of α- and β- diacetates **27** and **28** in 93% combined yield. The α-diacetate (**27**) was obtained in pure form by selective crystallization from 98% ethanol and n-hexane. The ^{1}H-NMR assignments of **27** are summarized in Table 2 (p. 16). The spectrum of the mixture (**27** and **28**) and that of **27** are reproduced in Figure 20 and 21. The ratio of **27** to **28** was determined by ^{1}H-NMR relative intensities of the acetate signals at δ=2.14 and 2.08 ppm and was found to be 3:1 (Figure 20). In Figures 20 and 21, it can be seen that the OMe signal which were present in the starting alcohol (**4**) at δ=3.35 ppm were no longer detected. The H-1 and H-6 signals

Scheme 12


OH
O
BnO
BnO
OBn
OMe
4
H_2SO_4 (conc.)/Ac_2O
(1% sol'n)
-20°C
93%
OAc
O
BnO
BnO
OBn
OAc
27
+
OAc
O
BnO
BnO
OAc
OBn
28
EtOH (98%)/n-hexane
OAc
O
BnO
BnO
OBn
OAc
27

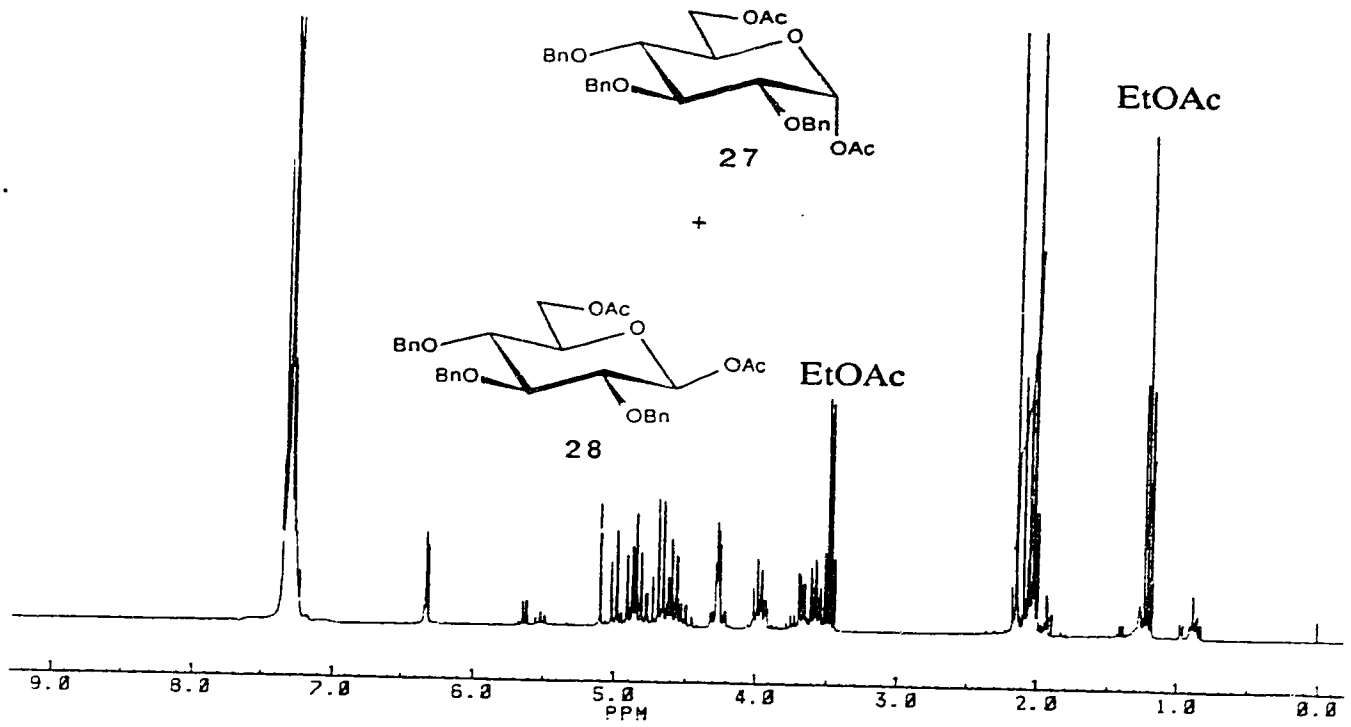


Figure 20. ^{1}H-NMR spectrum of the 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-D-glucopyranose (**27** and **28**) (see Table 2).

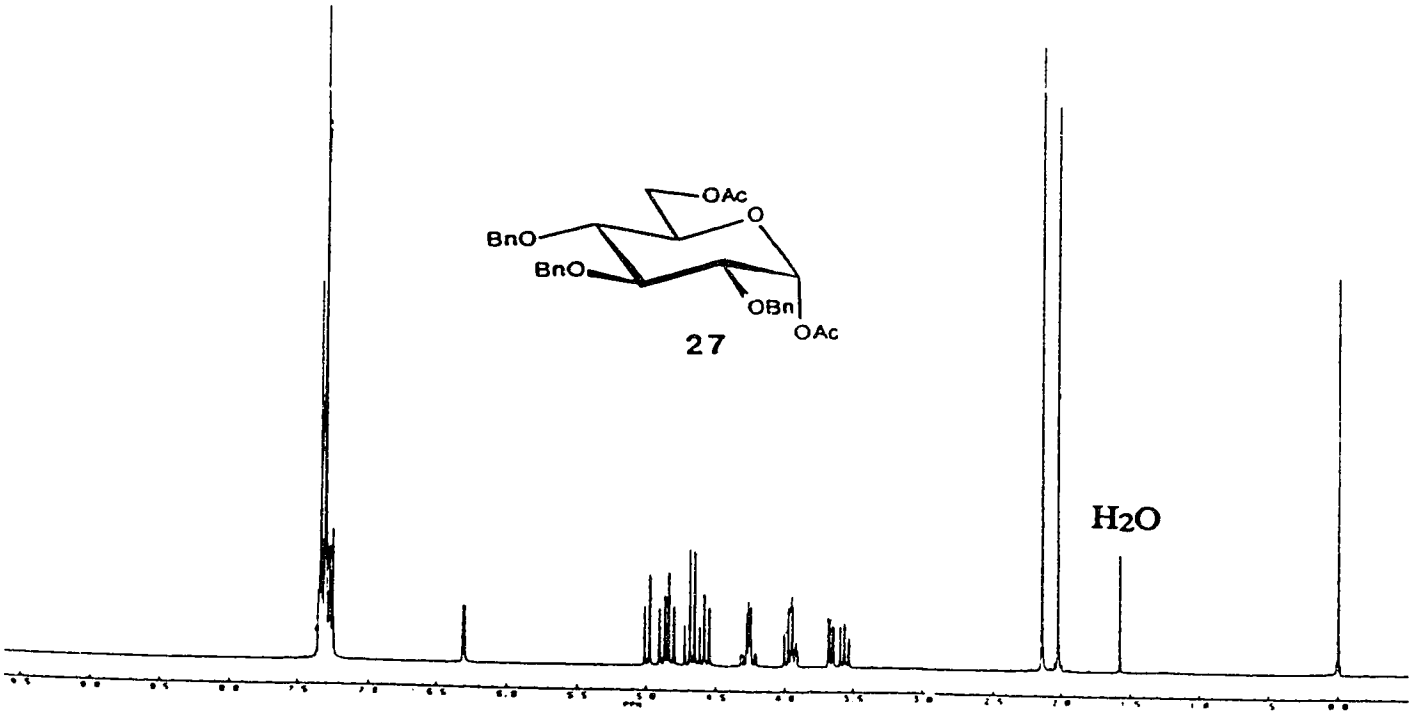
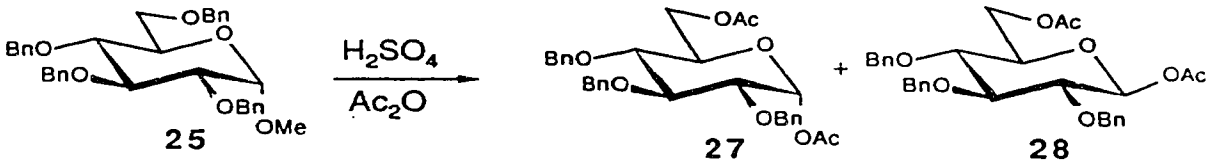
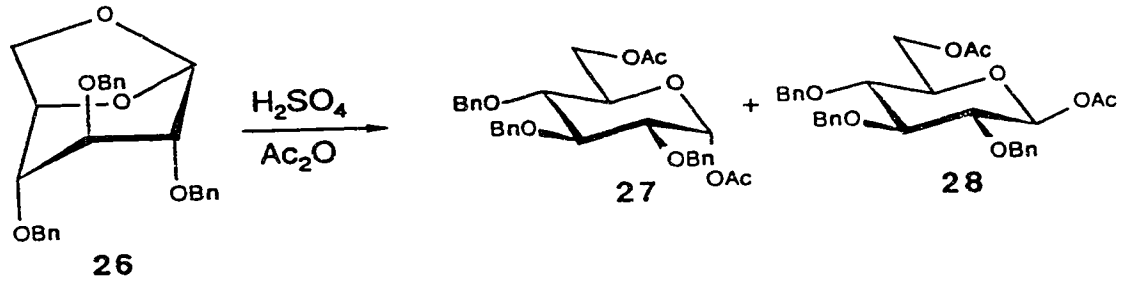


Figure 21. ^{1}H-NMR spectrum of 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranose (**27**) (see Table 2).

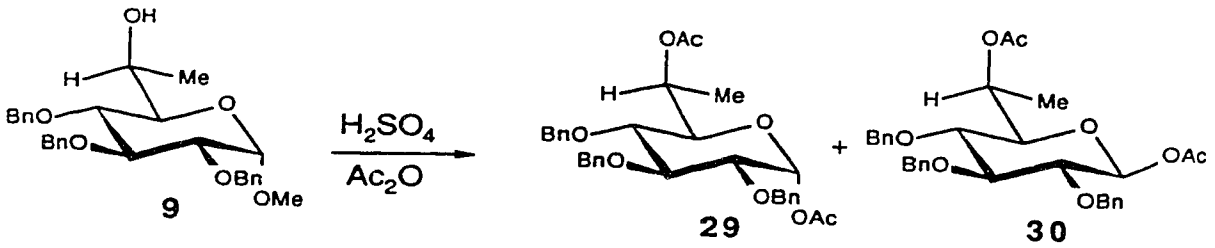
Scheme 13



Scheme 14



Scheme 15



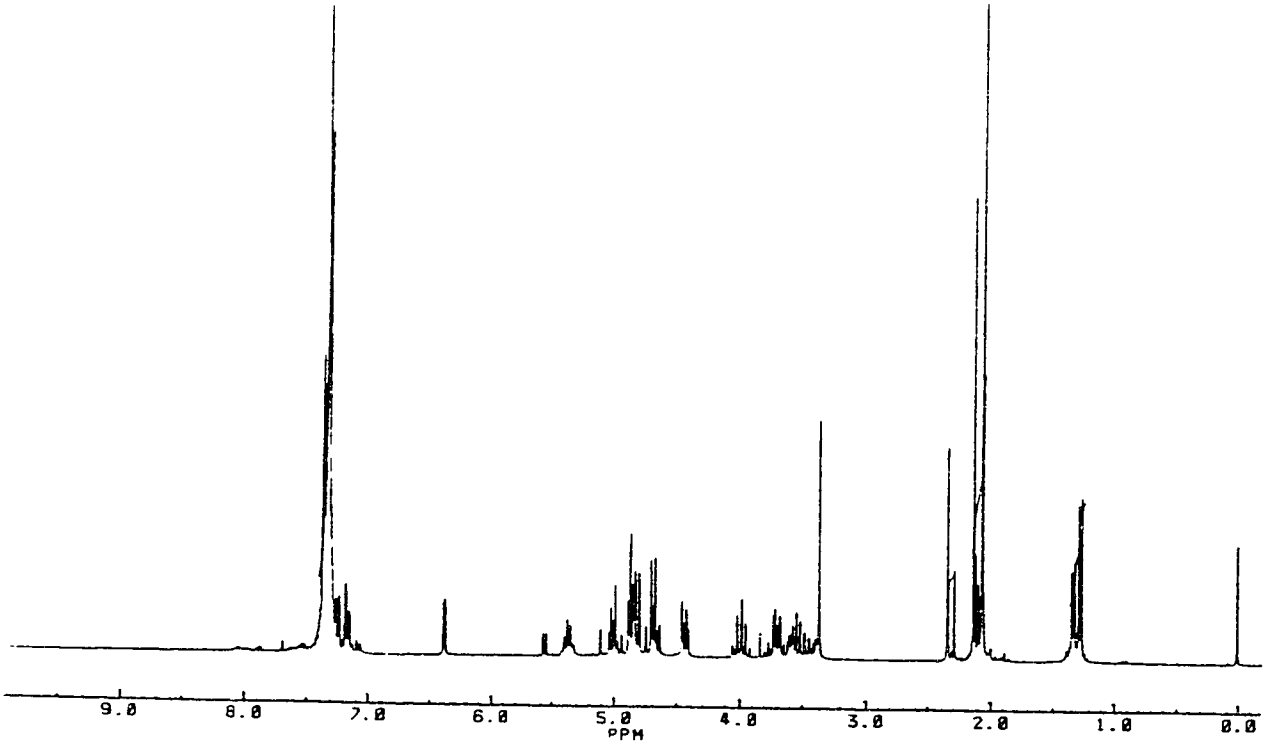


Figure 22. ^{1}H-NMR spectra of 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-7-deoxy-L-*glycero*-D-glucoheptapyranose (**29** and **30**) (see Table 2).

were shown to be deshielded from δ=4.6 and 3.8 ppm respectively in **4** to δ=6.3 and 4.3 ppm in **27** which confirmed the presence of the acetate groups at O-1 and O-6.

The diacetates **27** and **28** could also be obtained by acetolysis of methyl 2,3,4,6-tetra-benzyl-α-D-glucopyranoside[65] and of 2,3,4-tri-*O*-benzyl-levoglucosan (**26**) according to the procedure established by Zemplen *et al.*[66] (Scheme 13 and 14, respectively). Reaction of compound **26** with 10% concentrate sulfuric acid in glacial acetic anhydride also gave a mixture of **27** and **28** in ratio 3:1. The ^{1}H-NMR spectra of the mixture was identical to that in Figure 20. The α-diacetate was also obtained from the mixture by selective crystallization procedure as above and the ^{1}H-NMR of the α-diacetate was identical to that for **27**.

The acetolysis procedure described above in the synthesis of the α- and β-diacetates (**27** and **28**) was also applied to compound **9** (Scheme 15). The reaction gave a mixture of the α- and β-diacetates (**29** and **30**). The ^{1}H-NMR spectrum of the mixture is reproduced in Figure 22. The integration of the OAc signals intensities at δ=2.0 and 2.4 ppm gave the ratio of α/β of 2:1. The H-6, H-1 signals were also shifted downfield for **29** and **30** compared to those of **9** (Table 2, p. 14 and 16). The OMe signal was also not detected. Therefore the acetolysis reaction involving **9** also gives the expected products, α- and β- diacetates (**29** and **30**). The acetolysis reaction was not attempted for compound **10** but it is expected that similar behavior will be observed.

2.2. Synthesis of the 6-O-acetyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyl bromide (**31**).

Although the α-diacetate (**27**) could be selectively crystallized out of the α/β mixture, this was not necessary for the preparation of the bromide **31**. The α- and β-diacetate mixture (**27** and **28**) in dichloromethane was reacted with anhydrous hydrogen bromide gas to give crude α-bromide **31** in about 80% yield. Due to the instability of glucosyl

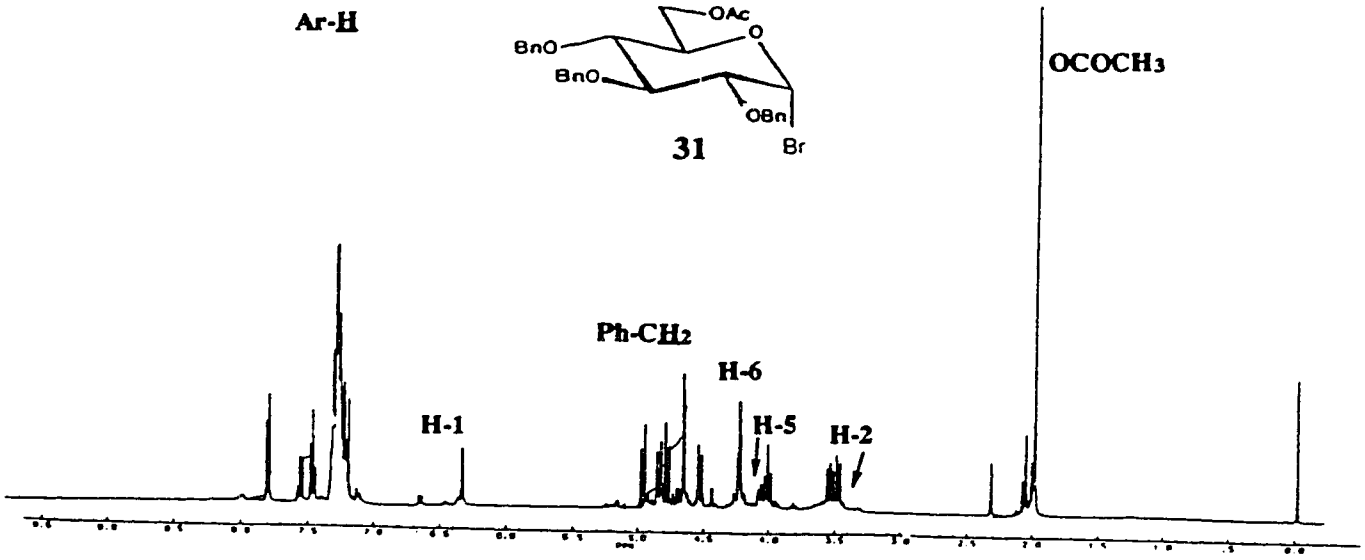


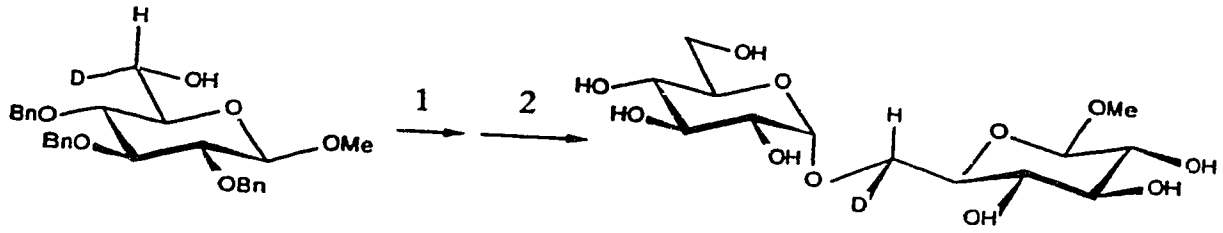
Figure 23. ^{1}H-NMR spectrum of the crude 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl bromide (**31**).

bromides in general, **31** was not isolated but was reacted immediately in the glycosylation reaction. The crude ^{1}H-NMR spectrum of **31** and the assignments are shown in Figure 23 It can be seen that the three benzyl groups still remained in **31**. The H-1 of **31** was observed at δ=6.4 ppm and together with the disappearance of the OAc signal at δ=2.15 ppm confirmed the presence of the bromide substituent at C-1. As expected, the $J_{1,2}$ was 3.5 Hz. The H-6 signal is still present at δ=4.30 ppm which confirms that the OAc substituent is still present at C-6.

2.3. Synthesis of methyl α-isomaltoside (37)

The most recent applications of the halide-ion catalyzed glycosylation method[37] in the synthesis of α-linked isomaltoside derivatives were published by K. Bock *et al.*[26] (Scheme 16) and H. Ohrui *et al.*[27] (Scheme 17). K. Bock *et al.*[26] prepared the 6-(*S*)-deuterium labelled methyl β-isomaltoside (**32**) in 36% yield by halide-ion catalyzed glycosylation reaction involving tetra-*O*-benzyl-α-D-glucopyranosyl bromide and methyl 6-(*S*)-2,3,4-tri-O-benzyl-6-^{2}H$_1$-β-D-glucopyranoside[27] according to Scheme 17. The deuterium-labelled

Scheme 16

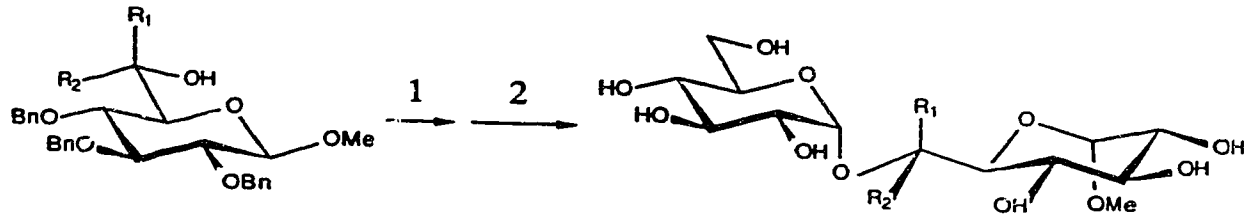


32 (36%)

1- Tetra-*O*-benzyl-α-D-glucopyranosyl bromide[37]

2- Pd/C and Ac_2O/MeOH

Scheme 17



33 R_1= H, R_2= D

34 R_1= D, R_2= H

1- Tetra-*O*-benzyl-α-D-glucopyranosyl bromide[37]

2- Pd/C

aglycone was prepared according to the route published by Ohrui *et al.*[27,30,31] Deblocking was accomplished by hydrogenation with Pd/C in acetic acid—methanol to give the desired product in 92% yield. Ohrui *et al.*[27] synthesized the disaccharide derivatives from the methyl 6-(*S*)-$^{2}H_{1}$- or the methyl 6-(*R*)-$^{2}H_{1}$-α-D-glucopyranoside (**33** and **34**)[30,31] as the glucosyl acceptor and the glucosyl halide derivatives as the donor, however, the deblocking procedures and the yields of the steps involved were not reported (Scheme 17).

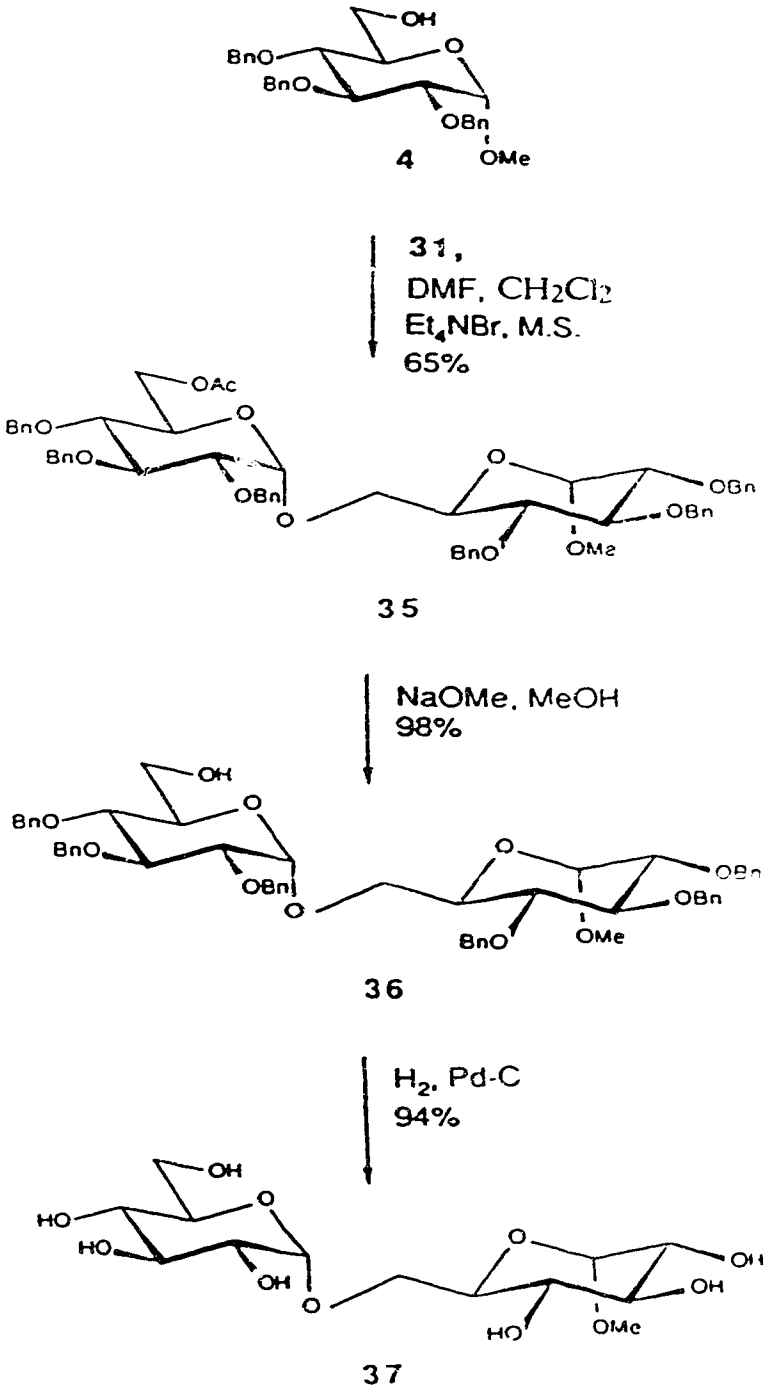
The synthesis of methyl α-isomaltoside (**37**) was undertaken because it is needed as a standard in the conformational analysis of the diastereoisomeric methyl 6-*C*-methyl-α-isomaltoside (**40** and **43**) and in the enzyme studies.

The reaction sequences are shown in Scheme 18. Reaction of the alcohol (**4**) with the α-bromide (**31**) under the halide-ion catalyzed reaction conditions gave the disaccharide derivative **35** in 65% yield. ^{1}H-NMR and ^{13}C-NMR data were in agreement with that published previously.[26,27,67] Removal of the acetate blocking group with sodium methoxide in dry methanol gave the alcohol **36** in 98% yield. Compound **36** was reacted with hydrogen and Pd/C in dry methanol to give the deblocked methyl α-isomaltoside (**37**) in 94% yield. The structure of compound **37** was assigned on the basis of previously published data.[26,27,67]

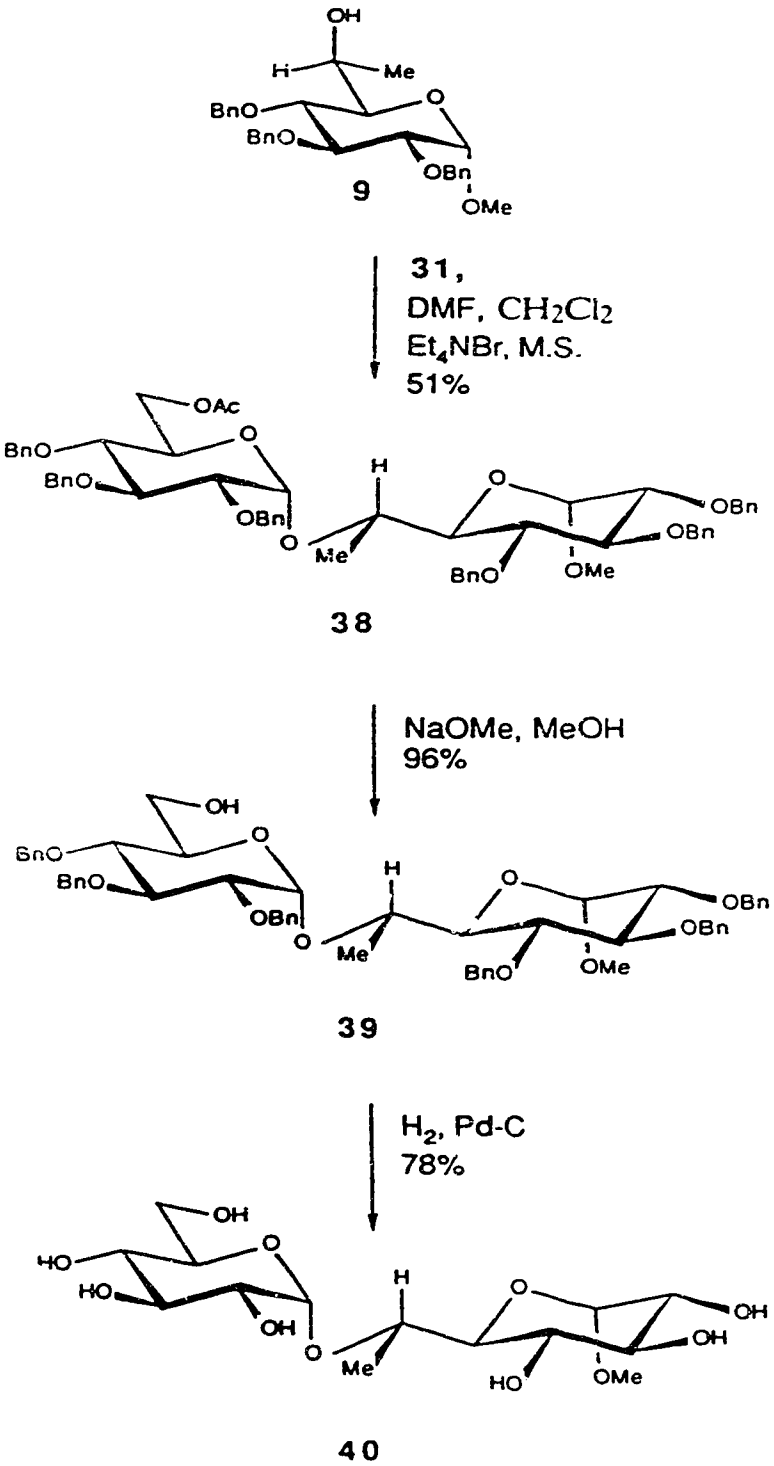
*2.4. Synthesis of methyl 6-S-methyl-α-isomaltoside (**40**)*

The synthesis of **40** is summarized in Scheme 19. Reaction of the methyl 6-*S*-methyl-α-D-glucopyranoside (**9**) with the α-bromide (**31**) according to the halide ion catalyzed reaction conditions[37] gave the derivative (**38**) in 51% yield. Compound **38** was characterized by ^{1}H-NMR (Figure 24, Table 2) and ^{13}C-NMR (Figure 25, Table 3). The doublet signal at δ=5.09 ppm was assigned as the H-1′ by 2D-COSY NMR technique and the α-linkage of **38** was established from the coupling constant $J_{1,2}$=3.5 Hz. The H-6′ signals

Scheme 18


OH
BnO
BnO
O
OBn
OMe
4
31,
DMF, CH_2Cl_2
Et_4NBr, M.S.
65%
OAc
OBn
35
NaOMe, MeOH
98%
36
H_2, Pd-C
94%
HO
37

Scheme 19


OH
H
Me
BnO
BnO
O
OBn
OMe
9
31,
DMF, CH_2Cl_2
Et_4NBr, M.S.
51%
OAc
BnO
BnO
OBn
O
H
O
OBn
OBn
Me
BnO
OMe
38
NaOMe, MeOH
96%
OH
BnO
BnO
OBn
O
H
O
OBn
OBn
Me
BnO
OMe
39
H_2, Pd-C
78%
OH
HO
HO
OH
O
H
O
OH
OH
Me
HO
OMe
40

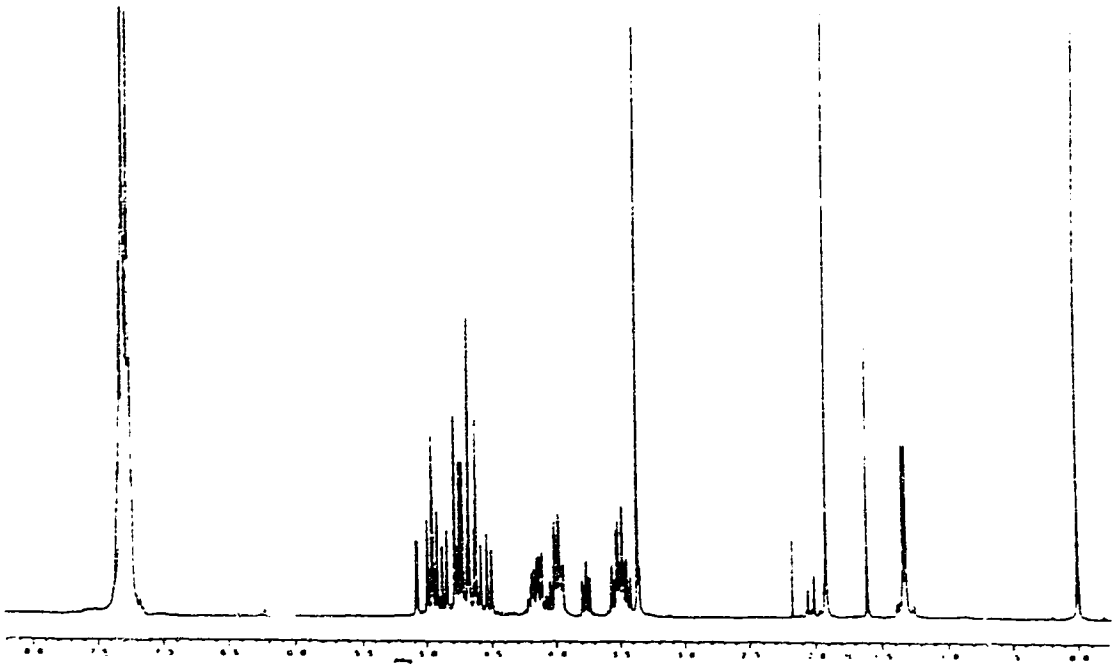


Figure 24. ^{1}H-NMR spectrum of methyl 6′-*O*-acetyl-2,2′,3,3′,4,4′-hexa-*O*-benzyl-6-*S*-methyl-α-isomaltoside (**38**) (see Table 2).

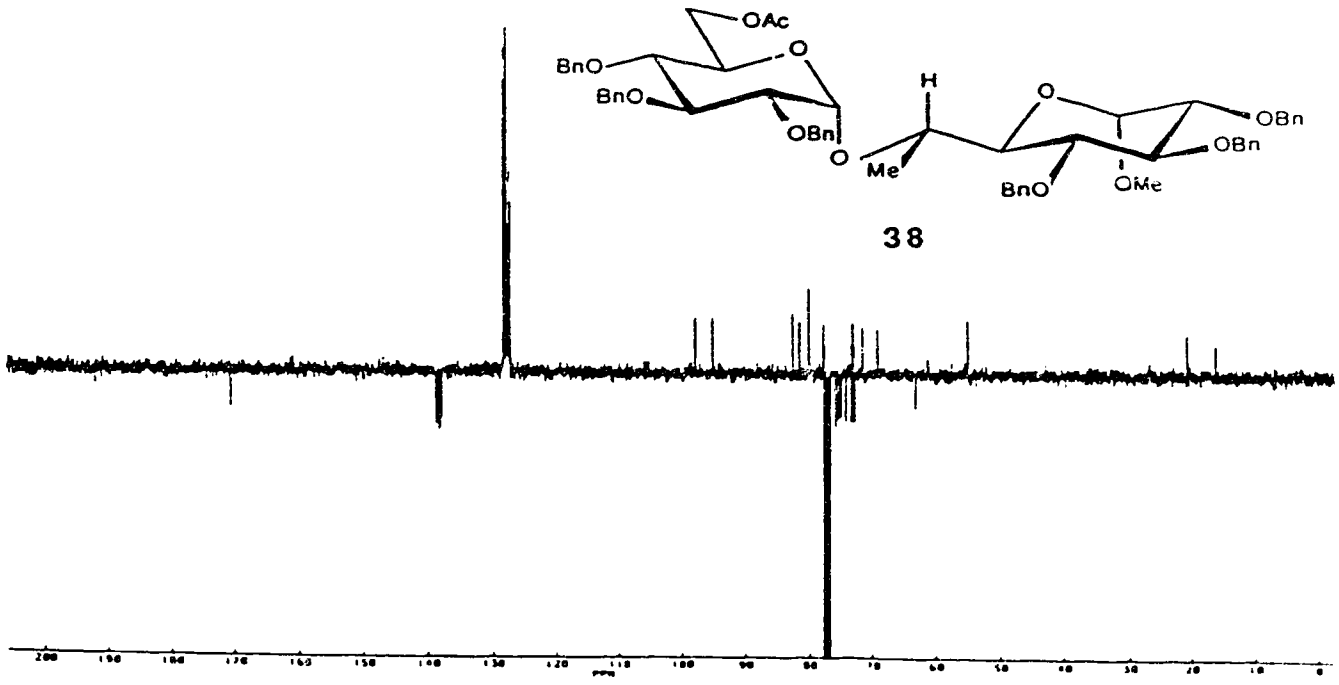


Figure 25. ^{13}C-NMR APT spectrum of methyl 6′-*O*-acetyl-2,2′,3,3′,4,4′-hexa-*O*-benzyl-6-*S*-methyl-α-isomaltoside (**38**). The C-H and CH_3 signals are above the base line and the C- and C-H_2 signals are below the base line (see Table 3).

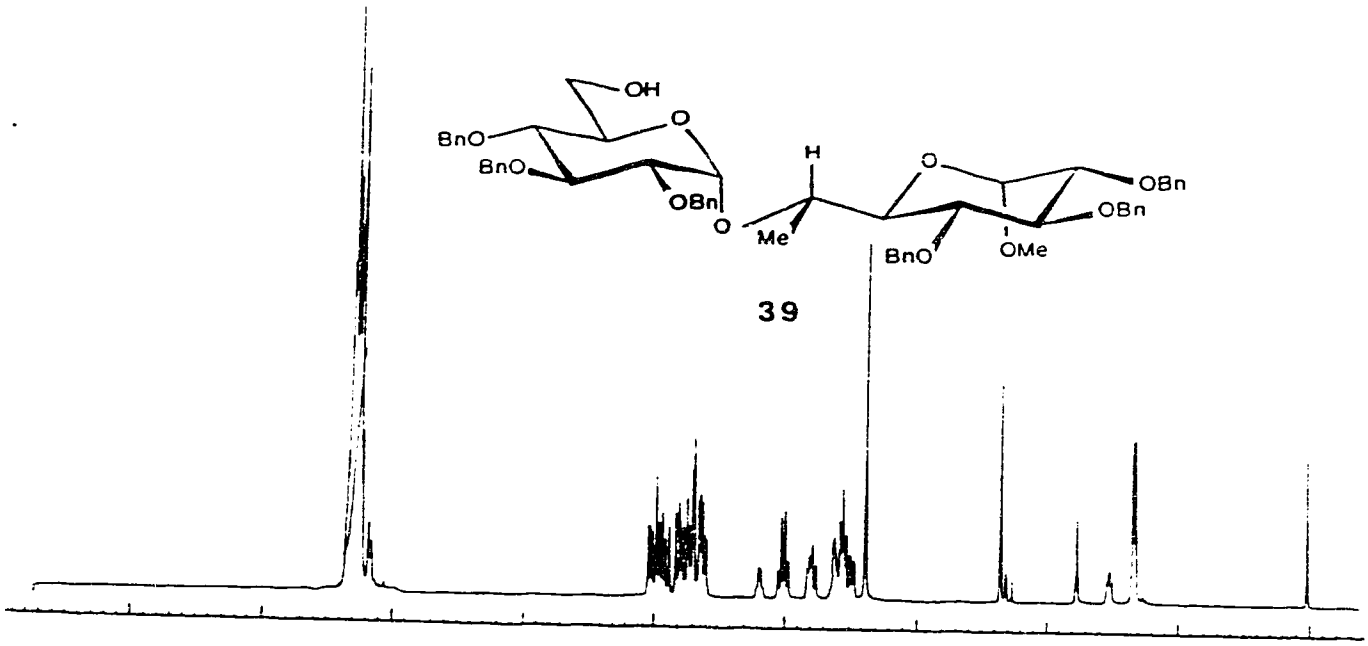


Figure 26. ^{1}H-NMR spectrum of methyl 2,2',3,3',4,4'-hexa-*O*-benzyl-6-*S*-methyl-α-isomaltoside (**39**) (see Table 2).

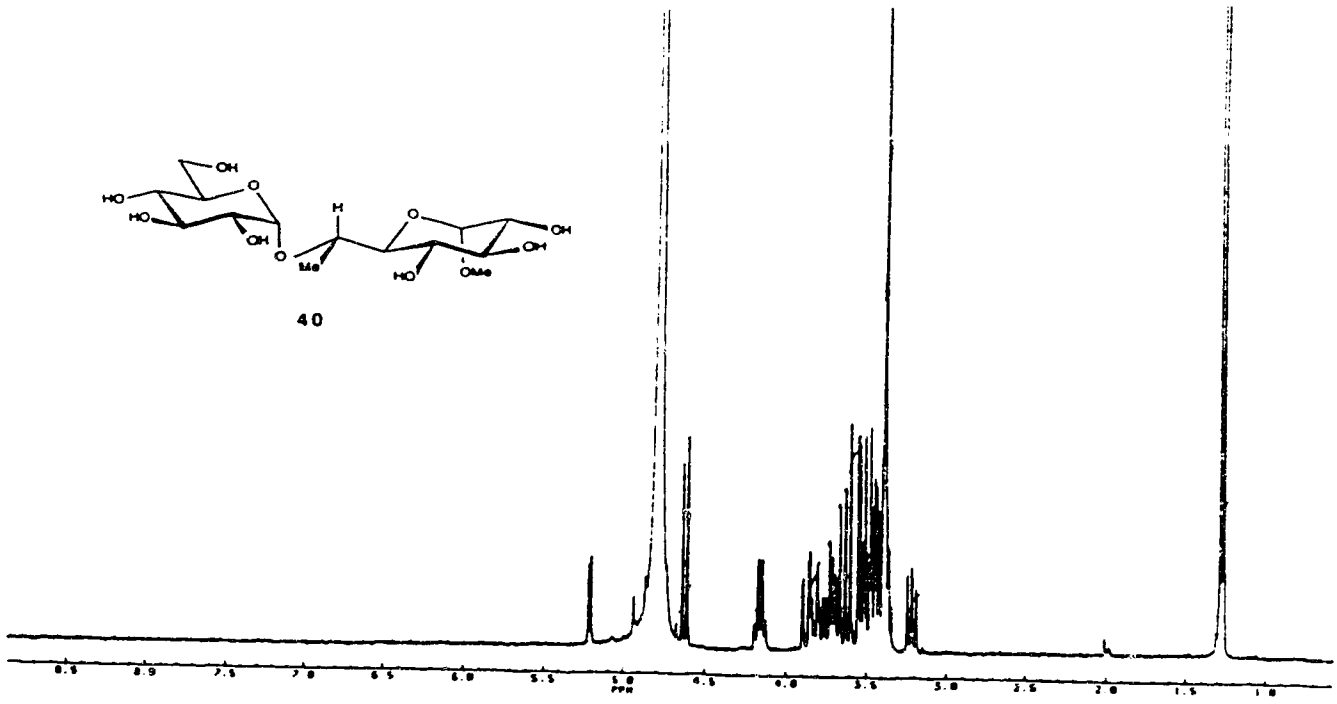


Figure 27. ^{1}H-NMR spectrum of methyl 6-*S*-methyl-α-isomaltoside (**40**) (see Table 2)

were found at δ=4.26—4.06 ppm which are similar in chemical shift to the H-6 signal of **9** hence confirming that an OAc substituent was still present at C-6. The C-6—CH_3 signal was detected at δ=1.33 ppm as a doublet with the $J_{5,6}$=6.5 Hz. In the ^{13}C-NMR spectrum (Figure 25), there were two signals at δ=97.90 and 95.20 ppm which were assigned (Table 3, p. 18) as the C-1′ and C-1 which confirm that compound **38** is a disaccharide. The signal at δ=170.75 ppm was characteristic of the carbonyl carbon of the OAc substituent at C-6′. The high field signal at δ=16.31 ppm was assigned as C-7. Therefore, based on the ^{1}H-NMR and ^{13}C-NMR spectra, the disaccharide derivative (**38**) was concluded to be as shown in Scheme 19.

Deacetylation of **38** with sodium methoxide in dry methanol produced the alcohol derivative (**39**) in 96% yield. The ^{1}H-NMR spectrum of **39** is reproduced in Figure 26 (see Table 2 for assignments). Disappearance of the OAc singlet signal at δ=1.92 ppm and the up field shift of H-6′ from δ=4.26—4.06 ppm to δ=3.66—3.40 ppm in the ^{1}H-NMR spectrum of **39** confirmed that the OAc substituent was removed in **39**.

Removal of the benzyl blocking groups by catalytic hydrogenation using hydrogen and palladium-on-carbon gave the desired disaccharide derivative (**40**) in 78% yield. The ^{1}H-NMR spectrum in D_2O is shown in Figure 27 and the assignments are shown in Table 2 (p. 16) where it can be observed that the benzyl groups signals were no longer present at δ=5.01—4.57 ppm.

2.5 Synthesis of methyl 6-R-methyl-α-isomaltoside (43)

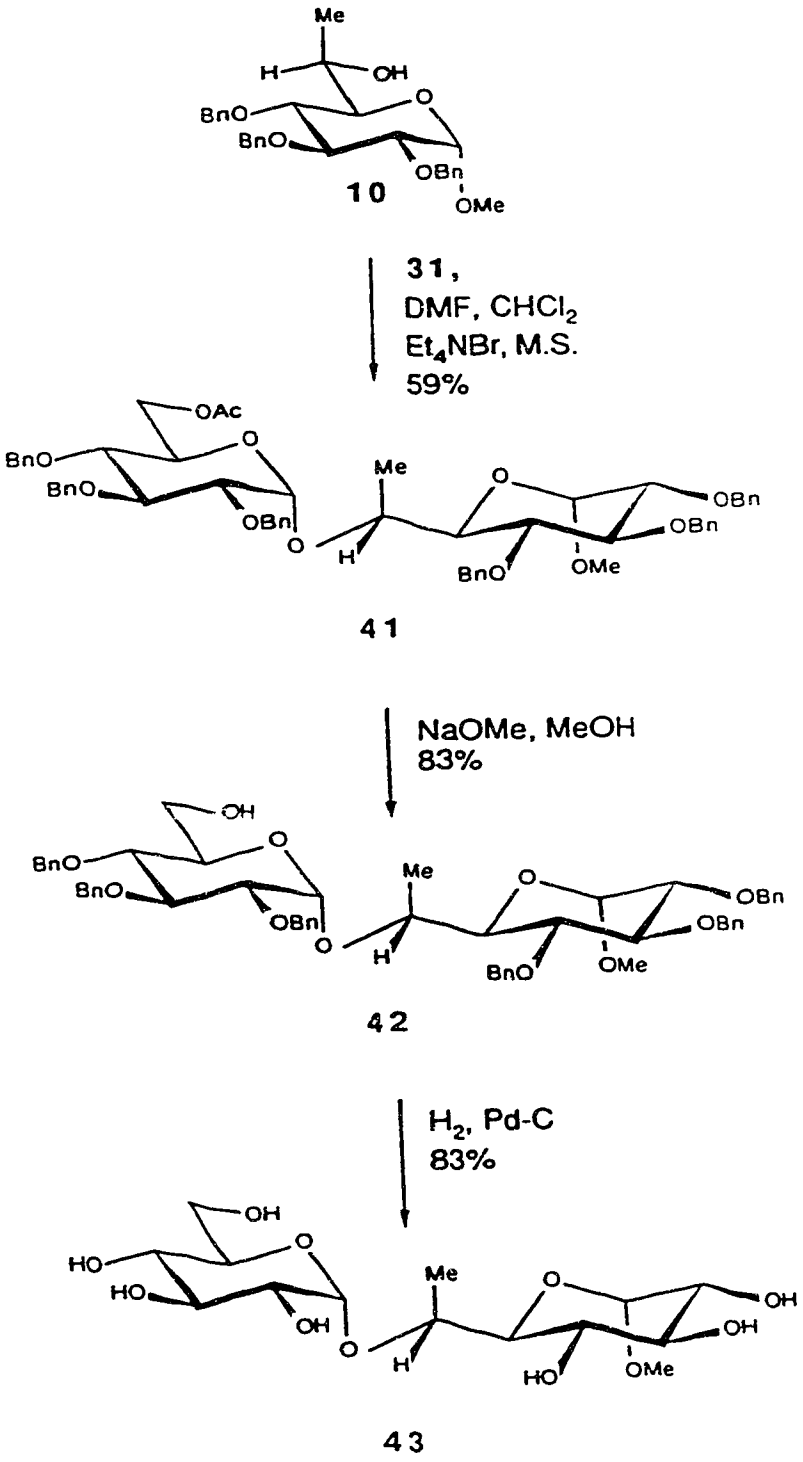
The synthesis of the disaccharide derivative (**43**) followed the same procedure as in the synthesis of **40** and is summarized in Scheme 20. The methyl 6-*R*-methyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**10**) was reacted with the α-bromide (**31**) under the halide-ion catalyzed reaction conditions[37] to give the disaccharide derivative **41** in 59% yield. The ^{1}H-NMR and ^{13}C-NMR assignments are summarized in Table 2 and 3 (p. 16 and 18, respectively). The spectra of **41** are reproduced in Figure 28 and 29. In the ^{1}H-NMR

spectrum (Figure 28), the H-1′ doublet signal was observed at δ=5.00 ppm and the $J_{1',2'}$ was also 3.5 Hz which established the α-linkage of the disaccharide. The signals at δ=4.27 and 4.21 ppm in the ^{1}H-NMR was assigned as the two signals of H-6′ and in the ^{13}C-NMR spectrum (Figure 29), the signal at δ=170.74 ppm was assigned as the carbonyl carbon of the OAc substituent at C-6′. Both of these signals confirmed the presence of the OAc substituent at C-6′. The signal at δ=1.16 ppm was assigned to the C-6—$\underline{CH_3}$ group and the corresponding ^{13}C-NMR signal for C-7 was detected at δ=16.55 ppm. Thus, the structure of **41** as shown in Scheme 20 was supported by both the ^{1}H-NMR and ^{13}C-NMR spectra.

Deacetylation of compound **41** using sodium methoxide in dry methanol gave the disaccharide **42** in 83% yield. The ^{1}H-NMR spectrum showed the disappearance of the signal at δ=1.99 ppm (Figure 30, Table 2) and the upfield shift of H-6′ from δ=4.27—4.21 ppm to δ= ~ 3.7 ppm.

Removal of the benzyl blocking groups in **42** was achieved with hydrogen and Pd/C and the deblocked disaccharide derivative (**43**) was obtained in 83% yield. The ^{1}H-NMR spectrum in D_2O was reproduced in Figure 31 (see Table 2 for assignments). The disappearance of the benzyl groups was evidenced by the disappearance of the signals in the ^{1}H-NMR spectrum in the aromatic region (δ=7.14—7.20 ppm) and the Ph-CH_2 region (δ=4.98—4.53 ppm).

Scheme 20


Me
H
OH
BnO
BnO
O
OBn
OMe
10
31,
DMF, CHCl2
Et4NBr, M.S.
59%
OAc
41
NaOMe, MeOH
83%
42
H2, Pd-C
83%
HO
43

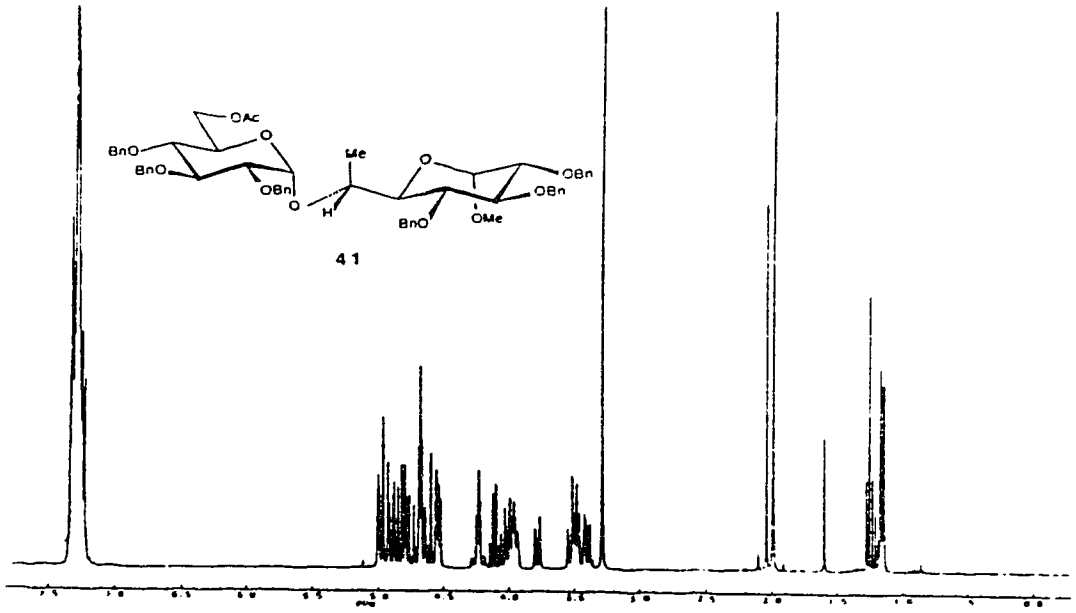


Figure 28. ^{1}H-NMR spectrum of methyl 6′-*O*-acetyl-2,2′,3,3′,4,4′-hexa-*O*-benzyl-6-*R*-methyl-α-isomaltoside (**41**) (see Table 2).

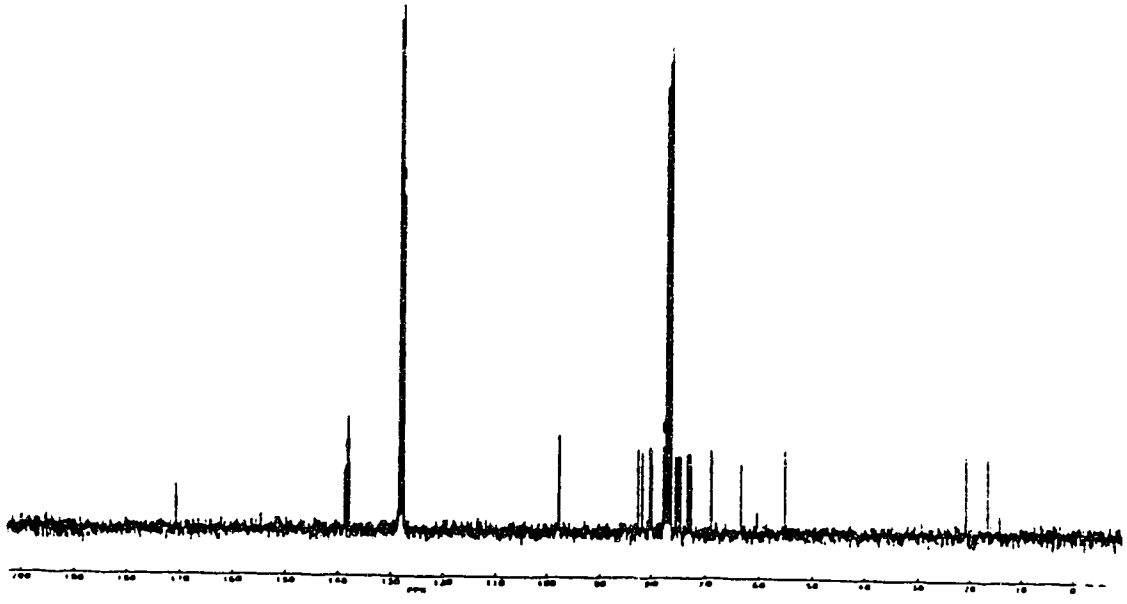


Figure 29. ^{13}C-NMR spectrum of methyl 6′-*O*-acetyl-2,2′,3,3′,4,4′-hexa-*O*-benzyl-6-*R*-methyl-α-isomaltoside (**41**) (see Table 3).

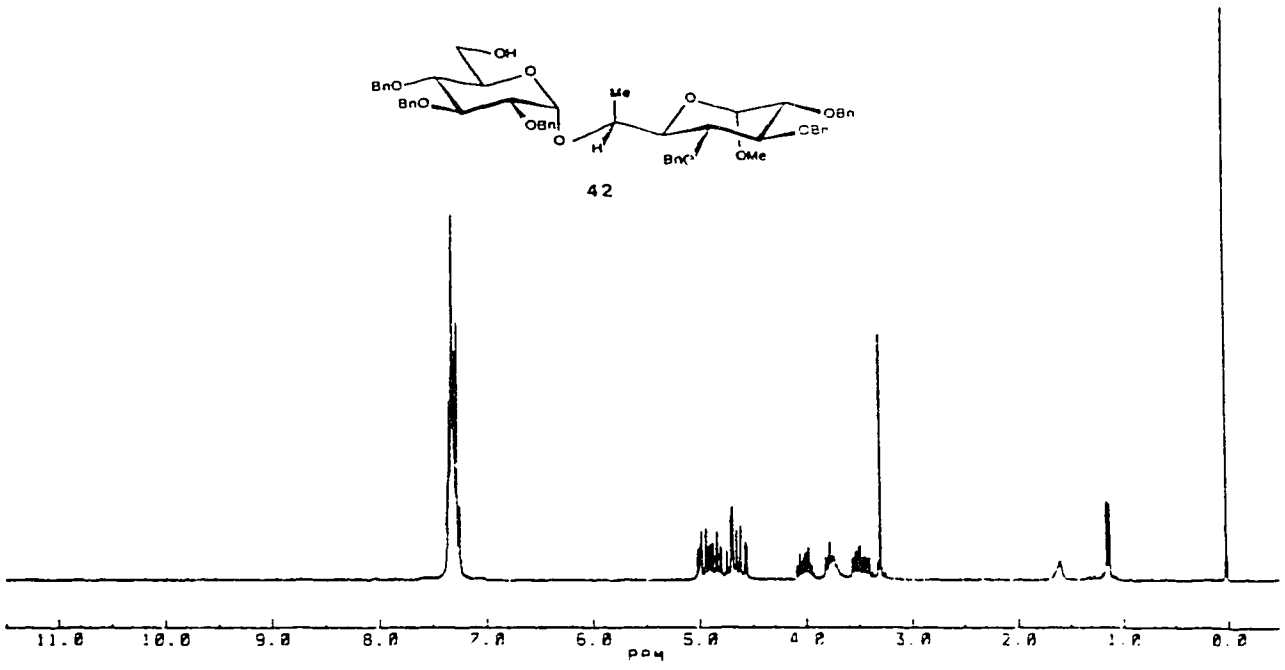


Figure 30. ^{1}H-NMR spectrum of methyl 2,2',3,3',4,4'-hexa-*O*-benzyl-6-*R*-methyl-α-isomaltoside (**42**) (see Table 2).

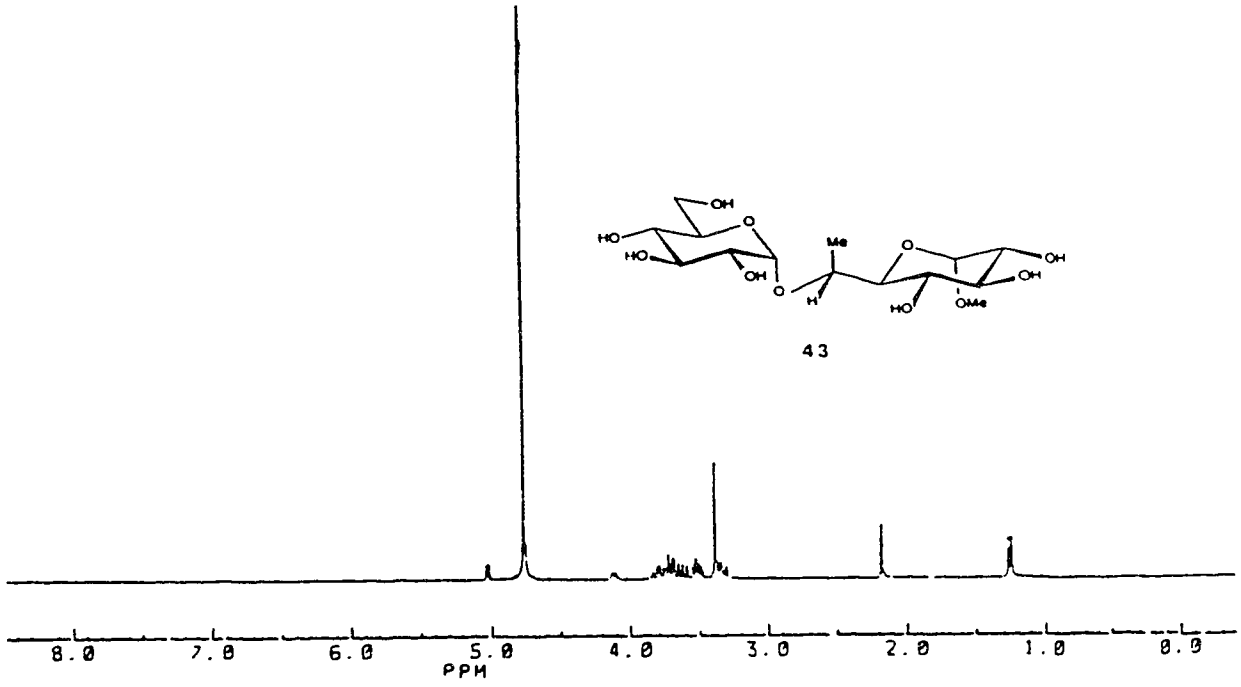


Figure 31. ^{1}H-NMR spectrum of methyl 6-*R*-methyl-α-isomaltoside (**43**) (see Table 2).

III. CONFORMATIONAL ANALYSIS

1. Conformational analysis of the diastereoisomeric methyl 6-S-methyl-α-D-glucopyranoside (15) and methyl 6-R-methyl-α-D-glucopyranoside (21)[69]

1.1. Chemical shifts and coupling constants

The analysis of the pyranose rings for the two diastereomeric compounds (**15** and **21**) was based on the coupling constants of the respective ring protons which are summarized in Table 4.

Table 4. Coupling constants of the ring protons of the diastereoisomeric methyl 6-*S*-methyl-α-D-glucopyranoside (**15**) and methyl 6-*R*-methyl-α-D-glucopyranoside (**21**).

Compounds	$J_{1,2}$ (Hz)	$J_{2,3}$ (Hz)	$J_{3,4}$ (Hz)	$J_{4,5}$ (Hz)
15	3.5	10.0	9.0	10.0
21	4.0	10.0	9.0	10.0

The small coupling constants $J_{1,2}$=3.5 and 4.0 Hz established the α-linkage of the OMe aglycon. The $J_{2,3}$, $J_{3,4}$ and $J_{4,5}$ coupling constants were 10.0, 9.0 and 10.0 Hz for both compounds (**15** and **21**) which are typical of anti-periplanar coupling constant. Therefore, there is no doubt that the $^4C_1$ is well maintained by the two pyranose rings. The observed ^{1}H-NMR chemical shifts of the methyl α-D-glucopyranoside (**1**)[77] and the diastereoisomers **15** and **21** in D_2O are presented in Table 5 where it is seen that the chemical shifts of H-1, H-2, H-3 and OCH_3 differ only slightly for the three compounds **1**, **15**, and **21** ($\Delta\delta \leq 0.05$ ppm) and, therefore, it can be concluded that these protons are in similar chemical environments. The H-6's of **15** and **21** (δ=4.05 and 4.04 ppm, respectively) are shifted downfield considerably compared to the chemical shifts of both H-6's of **1**. The reason for this downfield shift of H-6 of **15** may be due to the O-4 if the

H-6 and O-4 are syn-axially oriented.[71] If this is the case then O-6 of **15** would be syn-axially oriented with H-4 and O-6 of **21** would not. Consequently, H-4 of **15** would be deshielded compared to H-4 of **21**. This proved to be true as shown in Table 5. The H-4 of **15** is found at δ=3.46 ppm and the H-4 of **21** is found at δ=3.38 ppm ($\Delta\delta$=0.06 ppm). The H-5 of **15** is at δ=3.38 ppm and is considerably upfield in comparison to H-5 of **21** (δ=3.71 ppm). The H-5 downfield shift of **21** can be attributed to the nearby oxygen at C-6.

Table 5. ^{1}H-NMR chemical shifts (ppm) of **1**, **15** and **21** in D_2O at 295°K (reference acetone δ=2.12 ppm).[77]

Compounds	H-1	H-2	H-3	H-4	H-5	H-6	C-6–CH_3	OCH_3
1	4.70	3.46	3.56	3.29	3.54	3.77 3.66	–	3.31
15	—	3.55	3.62	3.46	3.38	4.16	1.29	3.38
21	—	3.58	3.68	3.38	3.71	4.20	1.22	3.44

Based on the chemical shifts of H-4, H-5 and H-6 of **15** compared to that of **21**, the methyl 6-*S*-methyl-α-D-glucopyranoside (**15**) was concluded to prefer the $\mathbf{g}^-$ conformation and the methyl 6-R-methyl-α-D-glucopyranoside (**21**) the $\mathbf{g}^+$ conformation. The preferred conformations $\mathbf{g}^-$ of **15** and $\mathbf{g}^+$ of **21** are shown in Figure 32.

The coupling constant $J_{5,6}$ of **15** and $J_{5,6}$ of **21** were found to be 1.4 Hz and 2.6 Hz respectively. The $J_{5,6}$ coupling constants can be estimated using Altona's parameters.[61,62] For **15** the estimated $J_{5,6}$ was 1.5 Hz and for **21**, 3.8 Hz. The estimated coupling constants $J_{5,6}$ are in agreement with the observed coupling constants. These small coupling constants

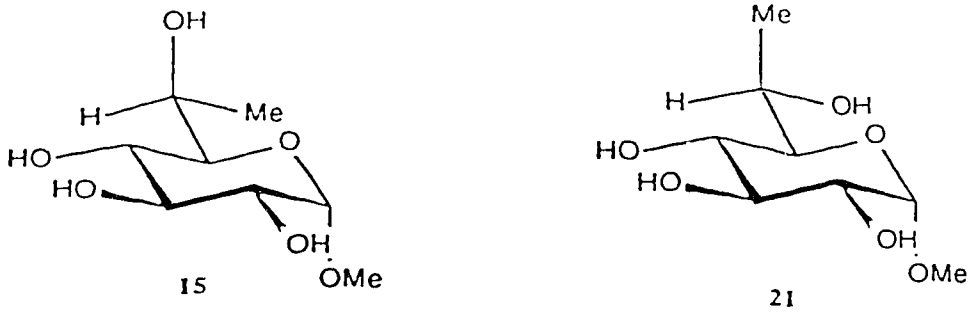


Figure 32. The preferred conformations of methyl 6-*S*-methyl-α-D-glucopyranoside (**15**) and methyl 6-*R*-methyl-α-D-glucopyranoside (**21**)

indicate that H-5 and H-6 are gauche to each other for both **15** and **21**. The observed gauche couplings are in support of the conclusions derived from chemical shifts above for **15** and **21**.

The chemical shifts and coupling constants of compounds **15** and **21** were also studied at higher temperatures. The results are shown in Table 6 where it is seen that the chemical shifts of H-4, H-5, H-6 and $J_{5,6}$ of **15** and **21** did not vary significantly in the temperature range 295°C to 355°C and is apparent that the $\mathbf{g}^-$ and $\mathbf{g}^+$ conformations of methyl 6-*S*-methyl-α-D-glucopyranoside (**15**) and methyl 6-*R*-methyl-α-D-glucopyranoside (**21**), are maintained.

At T=310°K and T=325°K, the variation of the chemical shifts were less than 0.02 ppm and the coupling constants varied less than 0.3 Hz. At T=340°K (the H-6 signal was overlapped with the HOD signal at T=355°K), the chemical H-6 shifts of **15** was found at δ=4.157 ppm and for **21** at δ=4.121 ppm ($\Delta\delta$=0.02 and 0.04 ppm compared to chemical shifts at T=295°K). At T=355°K, the H-4 chemical shifts of **15** was observed at δ=3.525 ppm and for **21** at δ=3.388 ppm ($\Delta\delta$=0.03 and 0.05 ppm compared to chemical shifts at T=295°K). The H-5 of **15** was observed at δ=3.394 ppm and for **21** at δ=3.613 ppm ($\Delta\delta$=0.00 and 0.06 ppm compared to chemical shifts at T=295°K). The coupling constant $J_{5,6}$ for **15** and **21** were observed to be 1.9 Hz and 3.4 Hz respectively ($\Delta J_{5,6}$=0.5 and 0.7

Hz respectively in comparison to the $J_{5,6}$ of **15** and **21** at T=295°K).

Table 6. Chemical shifts (ppm) and coupling constants (in brackets, Hz) of methyl 6-*S*-methyl-α-D-glucopyranoside (**15**) and methyl 6-*R*-methyl-α-D-glucopyranoside (**21**) in D_2O (reference acetone δ=2.225 ppm*) at various temperatures.

Temp.(°K)	Comp.	H-1	H-2	H-3	H-4	H-5	H-6	C6-Me
295	**15**	—	3.548	3.643	3.495	3.394	4.177	1.297
			(3.6)	(9.6)	(8.6)	—	(1.4)	(6.6)
			(9.5)	(8.6)	(9.7)	(1.4)	(6.4)	
	21	—	3.551	3.646	3.340	3.668	4.156	1.216
			(3.8)	(9.6)	(8.7)	(10.0)	(2.6)	(6.4)
			(9.6)	(8.8)	(10.0)	(2.7)	(6.4)	
310	**15**	4.815	3.549	3.647	3.502	—	4.170	1.296
		(3.7)	(3.7)	(9.5)	(9.0)	—	(1.4)	(6.4)
			(9.5)	(8.8)	(9.5)	—	(6.5)	
	21	4.799	3.549	3.649	3.353	3.653	4.147	1.220
		(3.7)	(3.7)	(9.4)	(8.8)	(10.0)	(2.9)	(6.5)
			(9.5)	(8.6)	(9.8)	(2.9)	(6.5)	
325	**15**	4.815	3.550	3.652	3.509	3.390	4.162	1.295
		(3.7)	(3.7)	(9.5)	(8.8)	(9.7)	(1.7)	(6.5)
			(9.5)	(8.9)	(9.9)	(1.7)	(6.5)	
	21	4.797	3.547	3.654	3.364	3.636	4.131	1.225
		(3.8)	(3.7)	—	(9.0)	—	(3.1)	(6.5)
			(9.5)		(9.8)		(6.5)	
340	**15**	4.817	3.551	3.659	3.518	3.392	4.157	1.297
		(3.7)	(3.7)	(9.5)	(8.6)	—	(1.8)	(6.5)
			(9.5)	(8.6)	(9.7)	(1.8)	(6.4)	
	21	4.798	3.546	3.661	3.375	3.624	4.121	1.231
		(3.8)	(3.7)	(9.6)	(8.7)	(9.9)	(3.2)	(6.4)
			(9.6)	(8.7)	(9.8)	(3.1)	(6.5)	
355	**15**	4.823	3.553	3.665	3.525	3.394	—	1.297
		(3.8)	(3.8)	(9.6)	(9.0)	(9.8)		(6.6)
			(9.6)	(8.8)	(9.8)	(1.9)		
	21	4.798	3.546	3.669	3.388	3.613	—	1.238
		(3.7)	(3.7)	(9.5)	(8.8)	(9.9)		(6.4)
			(9.5)	(8.7)	(9.8)	(3.4)		(6.4)

* The chemical shift reference used by Bock.[23]

1.2. Nuclear Overhauser enhancements

The conformations of the monosaccharides **15** and **21** were further supported by n.O.e experiments. The results of the n.O.e. studies of **15** and **21** are summarized in Table 7.

Saturation of the C-6—CH_3 signal of **15** caused 4.2% and 6.3% enhancements of H-5 and H-6 which indicated that C-6—CH_3 is near to H-5 and H-6 and no other proton. On the other hand, saturation of H-6 signal of **15** caused 6.8%, 11.7% and 7.8% enhancements of H-4, H-5 and C-6—CH_3 respectively. These n.O.e. results require the $\mathbf{g}^-$ conformational assignment of **15**.

Table 7. N.O.e. results for the diastereoisomeric methyl 6-*C*-methyl-α-D-glucopyranosides (**15** and **21**).

Compound	Signal saturated	Signal enhanced (%)			
		H-4	H-5	H-6	C-6–CH_3
15	C-6–CH_3	—	4.2	6.3	—
	H-6	6.8	11.7	—	7.8
21	C-6–CH_3	5.5	0.9	5.9	—
	H-6	3.9	9.1	—	5.2

For compound **21**, irradiation of C-6—CH_3 signal caused 5.5%, 0.9% and 5.9% enhancements of H-4, H-5 and H-6, respectively, indicating that the C-6—CH_3 group is on the average much closer in space to H-4 than to H-5. The 0.9% enhancement of H-5 may have arisen from n.O.e. transfer of another proton such as H-6 because if the conformation of compound **21** is $\mathbf{g}^+$ then the C-6—CH_3 is anti-periplanar in arrangement to H-5, hence too far to produce n.O.e enhancement on H-5. Irradiation of the H-6 signal caused 3.9%, 9.1% and 5.2% enhancements of H-4, H-5 and C-6—CH_3, respectively. This pattern of enhancements is similar to the n.O.e. of H-4, H-5 and C-6—CH_3, when H-6 of

compound **15** is irradiated and indicated that both the H-6's of **15** and **21** are in similar spatial environment with H-4 and H-5. This pattern of n.O.e requires the $\mathbf{g}^+$ conformation of **21**.

1.3. Conformational analyses based on nonbonded interactions

The main point of the following discussion is the orientation for the O-6 of the diastereoisomeric 6-*C*-methyl monosaccharides.

Assuming that the staggered orientation for the C-5/C-6 bond will be preferred, the population of the rotamers $\mathbf{g}^+$, $\mathbf{g}^-$ and **t** (Figure 3, Part I) can be roughly estimated using the known energy of interactions[70] of the various substituents on the C-5 and C-6 bonds. The energies of interactions used are:[70] O//H=0.45 Kcal, O//O=1.5 Kcal, O//Me=2.5 Kcal, Me//H=0.9 Kcal, O/Me=0.45 Kcal. The notation // denotes the syn-axial interaction and / denotes the gauche interactions. The energies of interactions were roughly estimated for the $\mathbf{g}^+$, $\mathbf{g}^-$ and **t** rotamers by rotation of C-5 to C-6 bond of the three monosaccharides: namely, methyl α-D-glucopyranoside (**1**), methyl 6-*S*-methyl-α-D-glucopyranoside (**15**) and methyl 6-*R*-methyl α-D-glucopyranoside (**21**) and are summarized in Table 8.

Conformational analysis based on the energies of interaction predict that conformation $\mathbf{g}^+$ signals was favored for the methyl α-D-glucopyranoside (**1**) and the 6-*R*-methyl-α-D-glucopyranoside (**21**) to the extend of 67% and 75% respectively whereas the conformation $\mathbf{g}^-$ was favored for the methyl 6-*S*-methyl-α-D-glucopyranoside (**15**) to the extend of 86%.

Table 8. The estimated energies of interactions (Kcal/mol) of the rotamers at C-5/C-6 bond and rotamer population (in brackets) at 296°K.

Compounds	Interaction Free Energies, Kcal/mol		
	g^+	g^-	t
1	−0.35 (67%)	0.1 (30%)	1.5 (3%)
15	1.0 (12%)	0.55 (86%)	1.7 (2%)
21	1.0 (75%)	2.15 (10%)	1.95 (15%)

1.4. Energies of interaction using HSEA

The energy of interactions upon rotation of the C-5/C-6 bond for compounds **15** and **21** were also estimated by HSEA calculations. The HSEA method[19] is based on the procedure used to estimate conformational preferences of polypeptides.[72] The method uses a Buckingham equation which was modified by Kitaigorodsky[73] for the non-bonded interactions (Equation 1).

$$E=3.5(-0.03/Z^6 + 8.5\times10^3 \exp(-13Z))\ \text{Kcalmol}^{-1} \quad (1)$$

Where $Z=r_{ij}/r_o$, r_{ij}=internuclear distance between the two interacting atoms

r_o=equilibrium internuclear distance between the two interacting atoms

The value r_o is $1.11(r_i + r_j)$ where r_i and r_j are the van der Waals radii for the atoms involved in the interaction. An exoanomeric term[9] which was obtained from ab-initio calculation for dimethoxymethane[74] is added to the sum of the non-bonded energies. The equations expressing the α- and β-exoanomeric effects are shown in Equation 2 and 3 respectively.

$$E_{exo,\alpha}=1.58(1-\cos\Phi^{O-5})-0.74(1-\cos2\Phi^{O-5})-0.70(1-\cos3\Phi^{O-5})+1.72 \quad (2)$$

$$E_{exo,\beta}=2.61(1-\cos\Phi^{O-5})-1.21(1-\cos2\Phi^{O-5})-1.18(1-\cos3\Phi^{O-5})+2.86 \quad (3)$$

Where, $E_{xo,\alpha}$= exo-anomeric effect energy (Kcal mol^{-1}) for the α-anomer.

$E_{exo,\beta}$= exo-anomeric effect energy (Kcal mol^{-1}) for the β-anomer.

HSEA method has predicted the preferred conformation of a variety of oligosaccharides including the human blood group B trisaccharide (α-L-Fuc (1→2)[α-D-Gal (1→3)]-β-D-Gal-OMe). The predicted conformation of the B blood group determinant has been verified by NMR[10] and X-ray crystallography.[75] An advantage in the HSEA method is the short computer time required for the calculations. The method has a disadvantage because it can not anticipate the interaction energies between strongly solvated groups such as hydroxyl groups.[36] This disadvantage will be evident in the following discussion when dealing with the monosaccharides **15** and **21**.

For the calculations, the methyl groups at C-6 of compounds **15** and **21** were constructed from the neutron diffraction coordinates of methyl α-D-glucopyranoside[76] using the program TRANSF.OBJ by extending the appropriate C-H bond to a distance of 1.536Å for a C-C bond. Then the proton of the extended bond was replaced with a methyl group. The energy of interactions were estimated using the program GENT.OBJ which rotated the ω angle (O-1′—C-6—C-5—H-5) from $\omega=0^{o}$ to 350^{o} in 5^{o} or 10^{o} steps. These resulting energies were then plotted as a function of ω (Figure 33 and 34) and the rotamers energies were determined for the diastereoisomers (**15** and **21**). It should be noted that if the proton of the extended bond was replaced by a carbon and the hydrogens attached to this carbon were generated to form the methyl group using the program TRANSF.OBJ, then this methyl group will not rotate with the molecule as a whole and therefore this procedure was avoided in all calculations.

In Figure 33, the three rotamers corresponding to the staggered conformations of the C-5/C-6 bond of compound **15** are shown. According to the estimated energies, the favored conformation was $\mathbf{g}^{-}$ at E=0.96 Kcal. The least favored rotamer was the **t** conformation

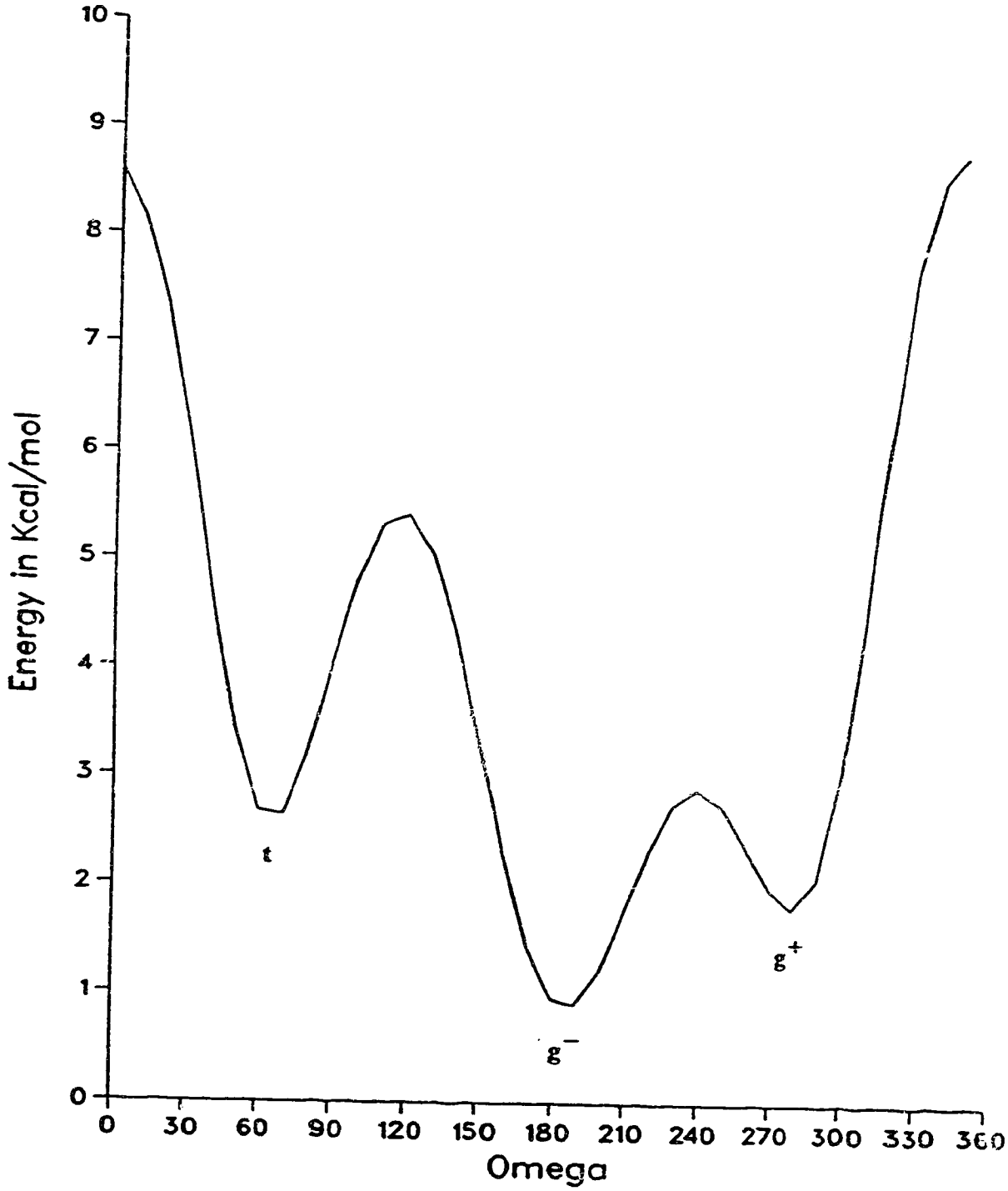


Figure 33. The energy of interaction at C-6 as a function of ω for the methyl 6-*S*-methyl-α-D-glucopyranoside (**15**) estimated by HSEA calculations.

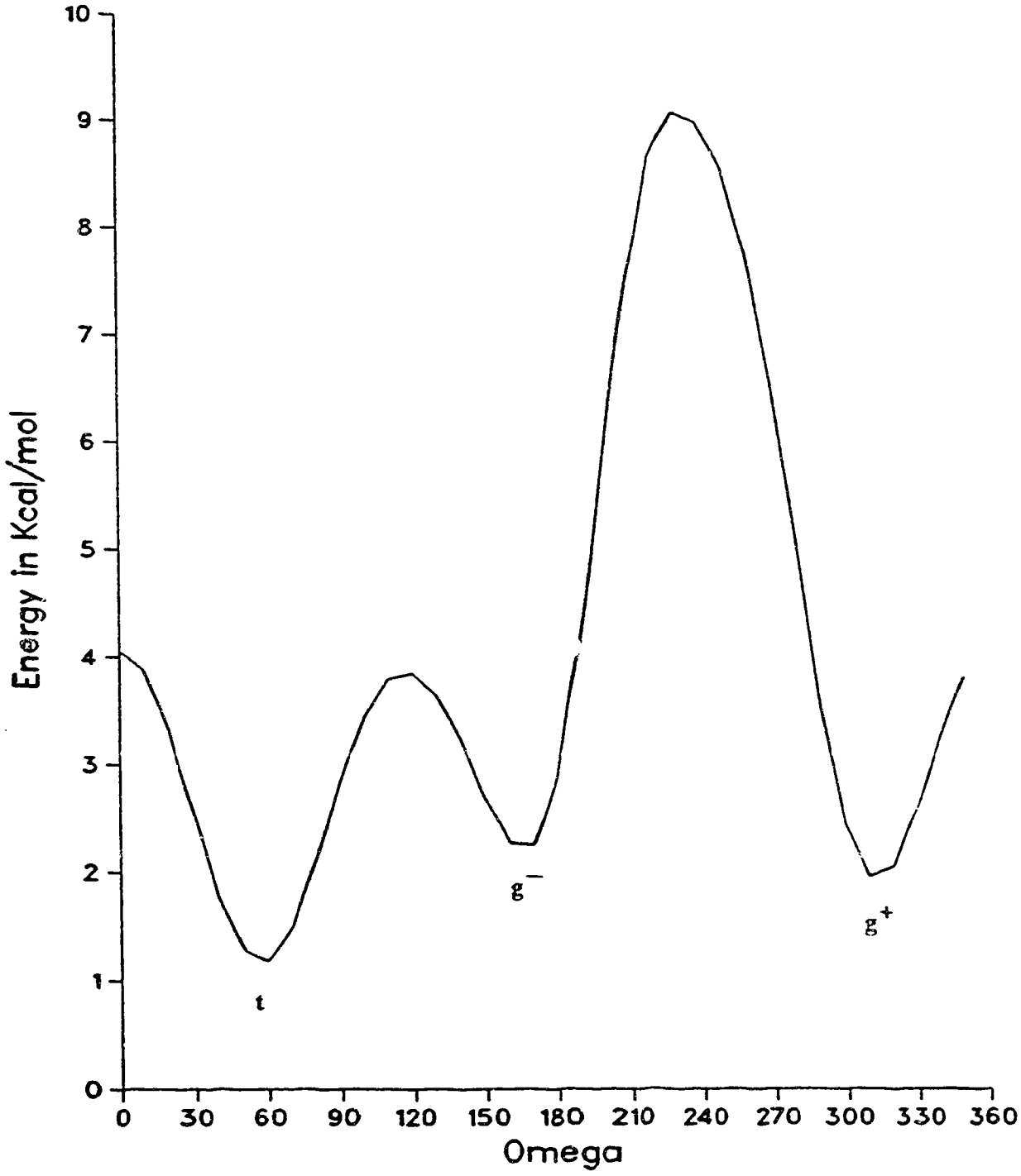


Figure 34. The energy of interaction at C-6 as a funtion of ω for the methyl 6-*R*-methyl-α-D-glucopyranoside (**21**) estimated by HSEA calculations.

with an energy of 2.68 Kcal. The $\mathbf{g}^+$ conformation had an energy of 1.80 Kcal. This trend is in agreement with that obtained from the simple estimations (Section 1.3) above but the energies were higher.

In Figure 34, the favored conformation for compound **21** is shown to be the **t** rotamer with the estimated energy of 1.12 Kcal. The $\mathbf{g}^+$ rotamer has an energy of 1.94 Kcal and the **g**– rotamer has an energy of 2.2 Kcal. The corrected energies gave the **t** rotamer an energy of 1.12 Kcal, $\mathbf{g}^+$ rotamer an energy of 1.14 Kcal and $\mathbf{g}^-$ rotamer an energy of 1.4 Kcal. These results were not in agreement with that obtained by simple estimations (Table 8).

In summary, the corrected HSEA energy for the rotamer $\mathbf{g}^+$ (1.14 Kcal) was in good agreement with that obtained by simple estimation (1.0 Kcal), however the HSEA energies for the rotamer $\mathbf{g}^-$ and **t** were not in agreement with that obtained by simple estimations based in emperical non-bonded interaction free energies.

The discrepancies between the simple estimations and the HSEA calculations are probably due to the oxygen parameters used in the HSEA program.[36] The hydroxyl groups of the free glucoside would be expected to be hydrogen bonded to the water molecule in aqueous solution, therefore would be more bulky. The effective radii of the hydroxyl groups would in effect be larger than the anticipated radii of the hydroxyl groups from van der Waals radii, hence would lead to greater steric interactions in aqueous solution compared to non H-bonded solvent. The radii parameter for the hydroxyl groups that HSEA program employed was derived from van der Waals radii therefore the estimated energies did not reflect the aqueous solution condition. Hence further examinations of the hydroxyl parameters employed by HSEA are necessary so that the results could be properly interpreted.

1.5. Conclusions

The conformational analysis based on rough calculations of the energies of interactions predicts that the conformer $\mathbf{g}^+$ will be favored for the methyl α-D-glucopyranoside (**1**) and the methyl 6-*R*-methyl-α-D-glucopyranoside (**21**) whereas the conformer $\mathbf{g}^-$ will be favored for the methyl 6-*S*-methyl-α-D-glucopyranoside (**15**). The $\mathbf{g}^-$ rotamer population of **15** was estimated to be about 86% and the $\mathbf{g}^+$ rotamer population of **21** was about 75% in aqueous solution. HSEA calculations predicted similar result for compound **15** but not for **21**. The experimental results based on chemical shifts, coupling constants and n.O.e support the conclusion derived from the rotational populations of energies of interactions. The preferred rotamers $\mathbf{g}^-$ and $\mathbf{g}^+$ of **15** and **21** respectively are also retained at higher temperatures up to 60°C increment.

2. Conformational analysis of the diastereoisomeric methyl 6-C-methyl-α-isomaltosides (40 and 43)

2.1. Chemical shifts and coupling constants.

The ^{1}H-NMR chemical shifts and coupling constants of the methyl α- and methyl β-isomaltosides were reported to be similar to the spectra of isomaltose.[26] The conformational analysis of the preferred rotamer at C-6′ of the non-reducing unit is difficult because of overcrowding of signals. Therefore, the hydroxymethyl group at C-6′ was assumed to have both $\mathbf{g}^+$ and $\mathbf{g}^-$ rotamers but the population of each rotamer cannot be determined. It was assumed that the C-6′ hydroxymethyl group has a similar population of conformers as in the methyl α-D-glucopyranoside (**1**) (see conformational analysis of the monosaccharides, Part III, Section 1) which consists of 67% for $\mathbf{g}^+$ and 30% for $\mathbf{g}^-$.

The chemical shifts and coupling constants of the H-5 and H-6's for the non-reducing unit of the methyl α-isomaltosides and the diastereoisomeric methyl 6-*C*-methyl-α-isomaltosides are summarized in Table 9.

Table 9. Chemical shifts (ppm) and coupling constants (Hz) for H-5, H-6*R* and H-6*S* of the reducing glucopyranose unit of the methyl α-isomaltoside and the diastereoisomeric methyl 6-*C*-methyl-α-isomaltosides (**40** and **43**).

Compounds	H-5(ppm)	H-6*R*(ppm)	H-6*S*(ppm)	$J_{5,6R}$(Hz)	$J_{5,6S}$(Hz)
Methyl β-isomaltoside	3.65	3.99	3.77	4.3	1.8
Methyl 6-*S*-6-^{2}H$_1$-β-isomaltoside	3.66	3.99	—	4.3	1.8
Methyl α-isomaltoside (**37**)	—	4.01	3.73	4.3	1.8
Methyl 6-*S*-methyl-α-isomaltoside (**40**)	3.60	4.28	—	—	—
Methyl 6-*R*-methyl-α-isomaltoside (**43**)	3.83	—	4.17	2.5	—

The chemical shifts and coupling constants of H-5, H-6*R* and H-6*S*, reported by Bock

and Pedersen,[26] for the unlabelled compound methyl β-isomaltoside and that of the labelled compound methyl 6-(*S*)-6-$^2H_1$-β-isomaltoside were in agreement with Ohrui's data[27] but were not in agreement with earlier reported values by Rao *et al.*[33] and DeBruyn *et al.*[34]

The H-6*R* and H-6*S* protons of the methyl 6-*S*-methyl-α-isomaltoside (**40**) and methyl 6-*R*-methyl-α-isomaltoside (**43**) were shifted down field compared to the H-6*R* and H-6*S* protons of the methyl α-isomaltoside ($\Delta\delta$=0.27 and 0.44 ppm respectively) and methyl β-isomaltoside ($\Delta\delta$=0.29 and 0.40 ppm, respectively). This trend was also observed for the monosaccharides; namely, methyl 6-*S*-methyl-α-D-glucopyranoside (**15**) and methyl 6-*R*-methyl-α-D-glucopyranoside (**21**) in which the H-6*R* and H-6*S* protons were shifted $\Delta\delta$=0.28 and 0.21 ppm respectively downfield. Similar conclusions can then be made that the H-6*R* and H-6*S* of the diastereoisomeric methyl 6-*C*-methyl-α-isomaltosides (**40** and **43**) were in similar environment and may be deshielded by the hydroxyl at C-4 if they are syn-axially oriented.

Bock and Pedersen,[26] using graphical analysis,[28] concluded from the coupling constants $J_{5,6}$ that isomaltose existed in the $\mathbf{g}^-$ conformer to the extend of 65% along with 35% of the **g**+ conformer. The conclusion was in agreement with that of Ohrui *et al.*[27] where $\mathbf{g}^-$ conformer population was calculated to be about 70% and the $\mathbf{g}^+$ conformer population was about 29%. Therefore the $\mathbf{g}^-$ conformer was concluded to be favored for the methyl α-isomaltoside as was predicted by HSEA calculations.

Both the coupling constants $J_{5,6}$ of **40** and **43** are observed to be small (<0.5 and 2.5 Hz respectively). The calculated coupling constants using Altona's parameters[61,62] gave $J_{5,6}$=1.4 Hz for **40** and $J_{5,6}$=3.8 Hz. The $J_{5,6}$ values indicate that H-5 and H-6 are gauche to each other.

The deshielding of H-6*R* and H-6*S* and the coupling constants of **40** and **43** confirm the g^- and g^+ rotamers to be favored in the reducing glucopyranose unit of the corresponding disaccharides.

2.2. Nuclear Overhauser enhancements

The conformations of the diastereoisomeric methyl 6-*C*-methyl-α-isomaltosides (**40** and **43**) were examined using n.O.e. experiments. The signal chosen for saturation in both compounds were C-6—CH_3, H-6 and H-1′ because these signals were not in the vicinity of other signals and therefore saturation would not cause partial collapse of adjacent signals. The n.O.e results are shown in Table 10.

Table 10. N.O.e. results for the methyl 6-*S*-methyl-α-isomaltoside (**40**) and methyl 6-*R*-methyl-α-isomaltoside (**43**).

Compounds	Signal saturated	Signal Enhanced (%)						
		H-1′	H-2′	H-4	H-5	H-6	C-6–CH_3	OCH_3
40	C-6–CH_3	3.5	—	—	2.6	3.2	—	1.4
	H-6	6.6	—	9.7		—	6.2	—
	H-1′	—	9.5	—	—	2.4	4.8	—
43	C-6–CH_3	—	—	3.2	0.8	3.7	—	—
	H-6	10.8	—	3.5	3.8	—	7.4	—
	H-1′	—	15.2	—	7.6	9.9	—	—

The n.O.e. values (%) of the disaccharide derivatives (**40** and **43**) are generally lower than that of the monosaccharide derivatives (**15** and **21**). This is because the disaccharide derivatives have more protons closer in space to the saturated signals, therefore the

relaxation of the saturated signal should be faster, resulting in lower n.O.e values.

Saturation of the C-6—CH_3 in the methyl 6-*S*-methyl-α-isomaltoside (**40**) caused enhancements of 3.5%, 2.6%, 3.2% and 1.4% of H-1′, H-5, H-6 and OCH_3. Saturation of H-6 caused enhancements of 6.6%, 6.2% of H-1′ and C-6—CH_3 and 9.6% combined enhancements of H-4 and H-5. Besides the reduction in n.O.e. values and the n.O.e. enhancement of H-1′, this pattern of n.O.e. was similar to the n.O.e. pattern of methyl 6-*S*-methyl-α-D-glucopyranoside (**15**).

Saturation of the C-6—CH_3 in the methyl 6-*R*-methyl-α-isomaltoside (**43**) caused enhancements of 3.2%, 0.8% and 3.7% of H-4, H-5 and H-6. Saturation of H-6 caused enhancements of 3.5%, 3.8%, 7.4% and 10.8% of H-4, H-5, C-6—CH_3 and H-1′. This n.O.e. pattern observed upon saturation of C-6—CH_3 and H-6 is similar to that observed for the methyl 6-*R*-methyl-α-D-glucopyranoside (**21**).

The similarity in the n.O.e. patterns of the diastereoisomeric disaccharides (**40** and **43**) to that of the diastereoisomeric monosaccharides (**15** and **21**) suggested that the conformations at the C-6 center of the reducing glucopyranosyl unit of the disaccharide derivatives (**40** and **43**) are similar to the conformations at the C-6 center of the monosaccharide derivatives (**15** and **21**).

It is of interest to discuss the n.O.e. enhancement of H-1′ of the non-reducing glucopyranosyl unit in both compounds **40** and **43**. In **40**, saturation of C-6—CH_3 signal caused enhancement of 3.5% and 1.4% of H-1′ and OCH_3. Saturation of H-1′ signal caused enhancements of 9.5%, 2.4% and 4.8% of H-2', H-6 and C-6—CH_3. Saturation of H-6 signal caused 6.6% enhancement of H-1′. The H-1′, besides being near in space to H-2′, is also close in space with H-6 and C-6—CH_3. Based on the minimum energy conformation calculated by HSEA (Figure 33), the H-1′ must be located in between the

H-6 and C-6—CH_3 group and if the conformation at the C-6 center of the reducing unit of the disaccharide **40** is the same as in the monosaccharide **15** then H-5 would be too far away and it is expected that no n.O.e. would be observed for H-5 when H-1′ is saturated.

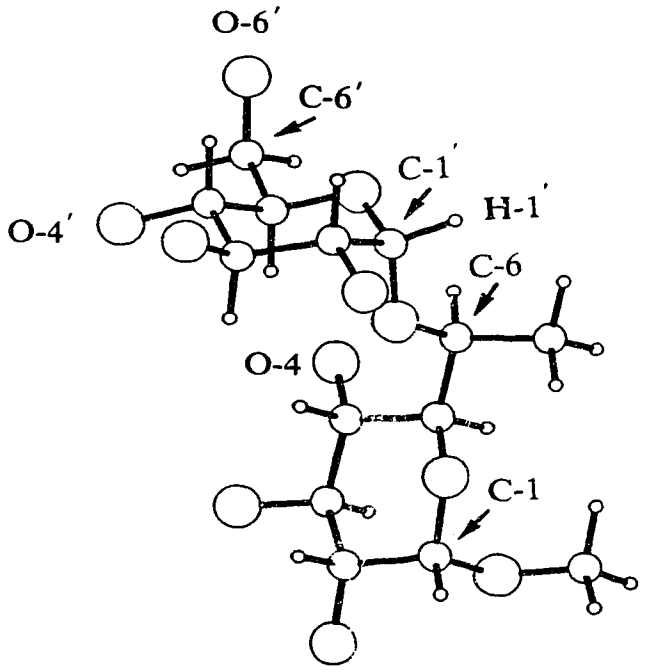


Figure 35. Minimum energy conformation of methyl 6-*S*-methyl-α-isomaltoside (**40**) calculated by HSEA.

This is found to be correct experimentally (Table 10).

In **43**, saturation of H-6 caused enhancement of 10.8% of H-1′. Saturation of H-1′ caused enhancements of 15.2%, 7.6% and 9.9% of H-2′, H-5 and H-6. Therefore the H-1′ is near in space with H-2′, H-5 and H-6. Also, based on minimum energy conformation calculated by HSEA (Figure 36), in order to cause n.O.e enhancement for H-5 and H-6 when H-1′ is saturated, H-1′ must be located between these two protons. And if the conformation at the C-6 center of the disaccharide derivative **43** is the same as in the monosaccharide derivative **21** then the C-6—CH_3 group would be located trans to H-5 hence trans to H-1′. Therefore no n.O.e is expected for C-6—CH_3 when H-1′ is saturated.

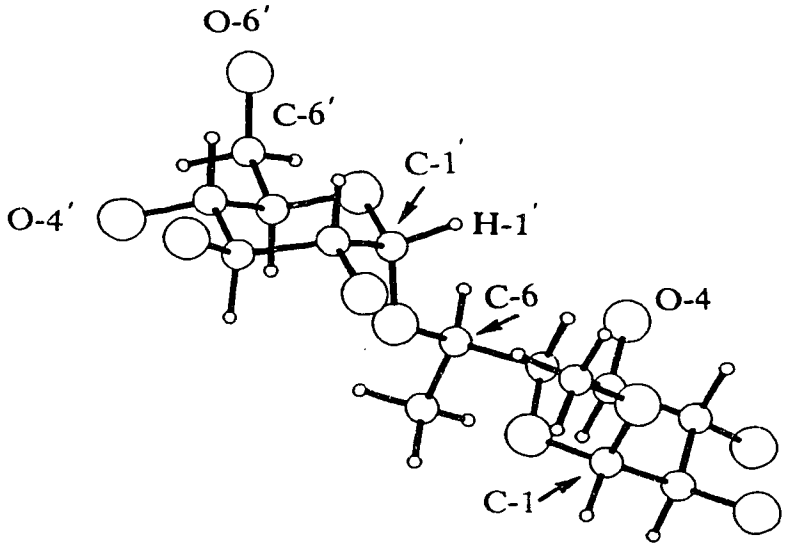


Figure 36. Minimum energy conformation of methyl 6-*R*-methyl-α-isomaltoside (**43**) calculated by HSEA.

This is confirmed experimentally (Table 10).

Therefore the n.O.e. patterns, the chemical shifts and the coupling constants require the conformations at the C-6 of the diastereoisomeric methyl 6-*C*-methyl-α-isomaltosides to be $\mathbf{g}^-$ for **40** and $\mathbf{g}^+$ for **43**. In other words the conformation at C-6 of the diastereomeric monosaccharides are retained in the reducing glucopyranosyl units of the diastereomeric disaccharides.

2.3. HSEA calculations

For HSEA calculations, the coordinates of methyl α-isomaltoside (**37**) and the diastereoisomeric methyl 6-*C*-methyl-α-isomaltosides were built from the neutron diffrac-

tion data of methyl α-D-glucopyranoside.[76] The methyl groups were generated at C-6 and O-1 using the TRANSF.OBJ program. The ω' angle of the non-reducing unit was set at 180^{o} or 300^{o} as required and the ω angle of the reducing unit was rotated in 5^{o} or 10^{o} step using the GENT.OBJ program. HSEA calculations were performed for the methyl α-isomaltoside, the methyl 6-*S*-methyl-α-isomaltoside (**40**) and the methyl 6-*R*-methyl-α-isomaltoside (**43**) with the torsion angles ω' and ω set at the predetermined values mentioned above and the torsion angles Φ and Ψ were varied until the minimum energies were obtained. The results are shown in Table 11.

Table 11. HSEA calculations results of the methyl α-isomaltoside (**37**) and the diastereomeric 6-C-methyl α-isomaltosides (**40** and **43**).

Compounds	**Torsional Angles, ^{o}**					Internuclear Distances, A	
	ω'	Φ	Ψ	ω	**E** (Kcal/mol)	O-4–O-4′	O-4–O-6′
37	180	−50	195	180	−2.29	5.08	5.30
	300	−45	240	60	−1.90	4.51	4.90
	300	−50	190	180	−2.45	5.16	3.53
	300	−50	200	300	−1.74	8.25	6.41
40	180	−50	195	180	−1.46	5.10	5.30
	300	−50	180	300	−0.28	5.35	4.02
43	180	−50	90	300	−1.26	8.17	7.10
	300	−50	90	300	−1.81	8.17	5.68

Recalculation of the methyl α-maltoside in conformation obtained previously[8] where $\Phi/\Psi = -25^{o}/-15^{o}$ with $\omega' = 180^{o}$ and $\omega = 300^{o}$ gave an energy minimum of 1.35 Kcal. Because of the polar hydroxyl groups OH-3, OH-4′ and OH-6′ were involved in the hydrolyses catalyzed by the enzyme Amyloglucosidase Type II, the distances between these groups were also calculated for methyl α-maltoside and will be discussed in Section

IV.

HSEA calculations predicts that methyl α-isomaltoside (**37**) prefers the conformation with the torsion angles $\omega'/\Phi/\Psi/\omega = 300^{o}/-50^{o}/190^{o}/180^{o}$; methyl 6-*S*-methyl-$\alpha$-isomaltoside (**40**) prefers the conformation with the torsion angles $\omega'/\Phi/\Psi/\omega = 180^{o}/-50^{o}/195^{o}/180^{o}$ and methyl 6-*R*-methyl-α-isomaltoside (**43**) prefers the conformation with the torsion angles $\omega'/\Phi/\Psi/\omega = 180^{o}/-50^{o}/90^{o}/300^{o}$. The minimum energy conformations of **40** and **43** were plotted and reproduced in Figure 35 and 36 (p. 78 and 79, respectively).

The torsion angles $\omega = 180^{o}$ and 300^{o} for **40** and **43** correspond to the $\mathbf{g}^{-}$ and $\mathbf{g}^{+}$ conformers respectively for the C-5/C-6 bond in the reducing unit. The HSEA calculations therefore predicts that the conformation at the C-6 of the reducing unit will be retained for the methyl 6-*S*-methyl-α-isomaltoside (**40**) and methyl 6-*R*-methyl-α-isomaltoside (**43**).

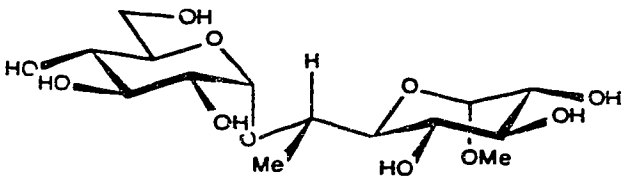
2.4. Conclusions

From conformational analysis of the monosaccharide derivatives, it was concluded that at the C-6 center, methyl 6-*S*-methyl-α-D-glucopyranoside (**15**) preferred the $\mathbf{g}^{-}$ rotamer and methyl 6-*R*-methyl-α-D-glucopyranoside (**21**) preferred the $\mathbf{g}^{+}$ rotamer. The conformations are also favored even at higher temperatures. It was expected that these conformations would also be favored in the disaccharide derivatives. The conformations at the C-6 center of the diastereoisomeric methyl 6-*S*-methyl-α-isomaltoside (**40**) and methyl 6-*R*-methyl-α-isomaltoside (**43**) were determined to be mainly $\mathbf{g}^{-}$ and $\mathbf{g}^{+}$ respectively.

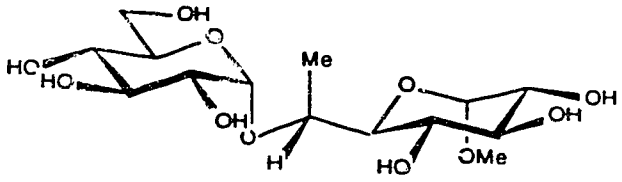
IV. ENZYME STUDIES

1. The Enzymes

Two enzymes Isomaltase and Amyloglucosidase Type II were used in kinetic studies of the hydrolysis of the methyl α-isomaltoside (**37**) and its C-6 methyl derivatives; namely methyl 6-*S*-methyl-α-isomaltoside (**40**) and methyl 6-*R*-methyl-α-isomaltoside derivatives (**43**).



40



43

The enzyme Isomaltase (EC 3.2.1.10)[78-83] is found in the bean, potato,[81] autolyzed brewer's yeast[82] and muscle.[83] This enzyme is used especially to cleave the α-D-(1→6) linkage and therefore plays an important role in the depolymerization of amylopectins and glycogens. Because of this specificity, the enzyme Isomaltase was selected to be used in the hydrolysis studies.

The enzyme Amyloglucosidase (EC 3.2.1.3),[84-87] which is produced by the microorganism *Aspergillus niger*, consists of two forms: Amyloglucosidase Type I (AMG I) and Amyloglucosidase Type II (AMG II).[88,89] Both enzymes are reported to be glycoproteins containing D-mannose, D-glucose and D-galactose.[90] Although the enzymes have been characterized and the amino acid sequence has been determined,[26,87,90-100] the enzymes have not crystallized.

The AMG enzymes are used in the processing of starch because of their abilities to catalyze hydrolytic cleavage of both the α-D-(1→4) linkage between glucopyranose units such as is present in maltose and α-D-(1→6) linkage between glucopyranose units such as is present in isomaltose. Both enzymes are stable up to 60°C with little loss of activity for about 30 min., but are rapidly inactivated at higher temperature. The enzymes exhibit nearly identical pH profiles with starch as the substrate with the pH optimum at ~4.5. Both enzymes hydrolyze the α-D-(1→4) linkage approximately 40 times faster than the α-D-(1→6) linkage.[90]

Our attention was turned to the enzyme AMG II primarily because Bock and coworkers[26,97-100] had made a systematic study of the effects of substitutional changes on maltose on the kinetics of the hydrolysis in which the reactions were followed using ^{1}H-NMR techniques.[100] They found that the AMG II active site requires accommodation of both the D-glucopyranose units of maltose since the enzyme hydrolyzes methyl β-maltoside but not methyl β-D-glucopyranoside.

In the hydrolysis of the various derivatives catalyzed by AMG II, it was shown[99] that the enzyme can accept a wide range of modifications as well as larger substrates. The competitive experiments showed that inhibitions in the reaction of the methyl β-maltoside were observed with the 6-chloro- or 6-fluoro- derivatives. It was concluded that the 6-chloro- or 6-fluoro- derivatives were bound very tightly to the active site in order to inhibit the reactions.[99] Based on qualitative data for hydrolysis,[97] catalyzed by AMG II, of the mono- and several dideoxy derivatives of methyl β-maltoside, Bock *et al.*[97,99] concluded that three hydroxyl groups, OH-3 in the reducing glucopyranose unit, OH-4′ and OH-6′ in the non-reducing glucopyranose unit, are essential for AMG II catalyzed hydrolysis. These findings are depicted in Figure 37(a). Evidence for this conclusion was further supported when the pentadeoxy derivative (Figure 37(b)), in which only the key polar groups were present (OH-3, OH-4′ and OH-6′), was found to be a substrate for the

enzyme AMG II. The HSEA calculated minimum energy conformation of methyl α-maltoside,[101,103] which is supported by ^{1}H-NMR and ^{13}C-NMR data, presents hydrophobic area defined by H-2', H-4', H-1' and O-5' of the non-reducing unit and H-4 as well as the hydroxymethyl group of the reducing unit which is expected to possess substantial conformational rigidity. Both the three key hydroxyl groups and the hydrophobic area were regarded as essential for AMG II hydrolysis by Bock *et al.*[99] and are in support of the hydrated polar-group gate theory proposed by Lemieux.[104]

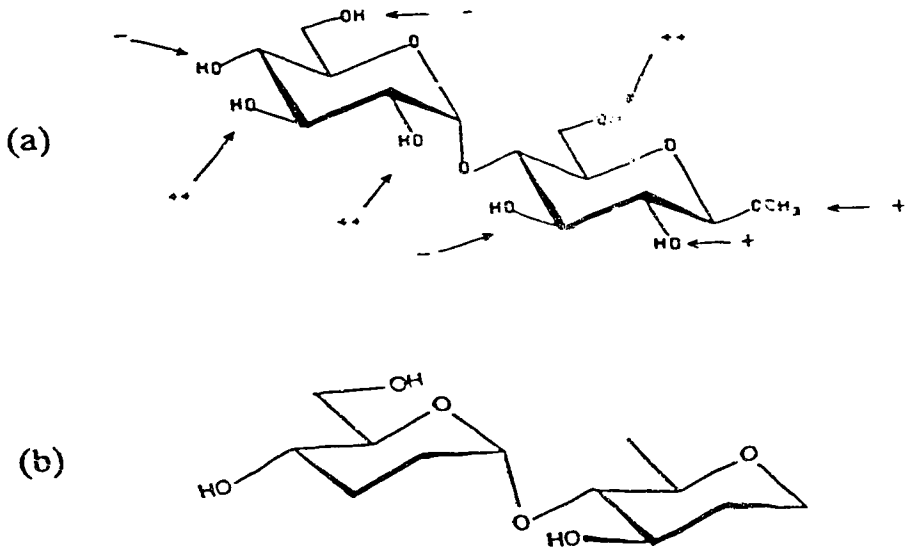


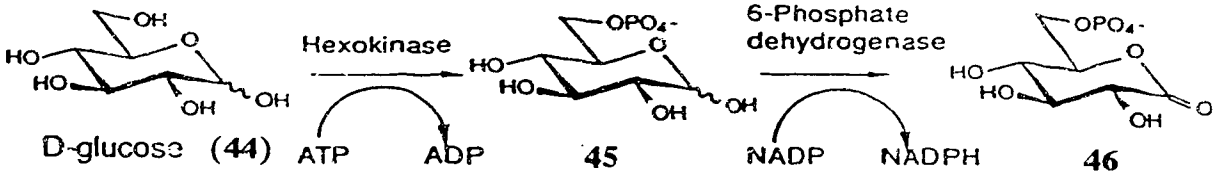
Figure 37. (a) Substrate specificity for Amyloglucosidase II (AMG II)[99] for different deoxy derivatives of maltose. + indicates substrate activity. – indicates no substrate activity and + + indicates compounds which are hydrolyzed faster than methyl β-maltoside. (b) Pentadeoxy derivative of maltose which preserves the key polar hydroxyl groups for hydrolysis catalyzed by AMG II.

2. Enzyme Kinetic Studies

2.1. Preliminary Studies

A collaboration was established with Dr. Monica Palcic of the Department of Food Science, University of Alberta for a kinetic study of the hydrolysis of **37**, **40** and **43**. Initially, it was thought that the most suitable method was to follow the reaction progress by using uv-vis spectroscopy.

The uv-vis spectroscopy technique involved the coupling method with the product(s) of the reaction. In the hydrolysis of methyl α-isomaltoside and the methyl 6-*C*-methyl-α-isomaltoside derivatives catalyzed by AMG II, the possible products of the hydrolysis reactions are D-glucopyranose and the 6-*C*-methyl-α-D-glucopyranose derivatives. The method employed for the detection of free glucopyranose (**44**)[105] released in the enzyme hydrolysis of the dissaccharides is shown in Scheme 21.



Scheme 21. Outline of the colorimetric coupled enzyme method for the determination of D-glucose.

The coupled-enzyme detects free glucopyranose by first a phosphorylation of the 6-hydroxyl by ATP in the presence of Hexokinase to produce D-glucopyranose-6-phosphate (**45**). This phosphate is then converted to the lactone (**46**) by 6-Phosphate Dehydrogenase in the presence of NADP (Scheme 21) which is detected due to the color change at $\lambda = 340$ nm as the result of the formation of the NADPH.

The monosaccharides D-glucopyranose (**44**), 6-*S*-methyl-D-glucopyranose (**47**) and 6-*R*-methyl-D-glucopyranose (**48**)[106] were then tested with Hexokinase and 6-Phosphate Dehydrogenase in the presence of ATP and NADP[105] and the results are shown in Table 12.

Table 12. Reaction of the monosaccharides derivatives with Hexokinase and 6-Phosphate Dehydrogenase in the presence of ATP and NADP.

Compounds	Reaction	Rel. Vel.
D-glucopyranose (**44**)	+	2.0
6-*S*-methyl-D-glucopyranose (**47**)[106]	-	–
6-*R*-methyl-D-glucopyranose (**48**)[106]	+	1.0

In contrast with D-glucopyranose (**44**) and 6-*R*-methyl-D-glucopyranose (**48**), the 6-*S*-methyl-D-glucopyranose (**47**) did not react. As discussed above in connection with Scheme 22, 6-Phosphate Dehydrogenase does not dehydrogenate D-glucopyranose. Instead the glucose must first be phosphorylated at the 6-OH position to produce **45**. Therefore it is possible the 6-*S*-methyl-D-glucopyranose resists phosphorylation at the 6-OH position. Conformational analyses presented in the previous section showed that both α-D-glucopyranose and 6-*R*-methyl-D-glucopyranose (**44** and **48**, respectively) prefer the $\mathbf{g}^+$ conformation for the orientation of the 6-OH whereas the $\mathbf{g}^-$ is preferred for the 6-*S*-methyl-D-glucopyranose (**47**) (see Figure 38).

Since it can be expected that the enzyme will phosphorylate the 6-hydroxyl of the glucopyranose in a specific conformation the reason for the high resistance to phosphorylation exhibit by the *S*-compound (**47**) can be attributed to the low abundance of the $\mathbf{g}^+$ conformer. However, it is possible that the 6-*C*-methyl group also contributes to the inactivity by hindering the formation of the enzyme-substrate complex.

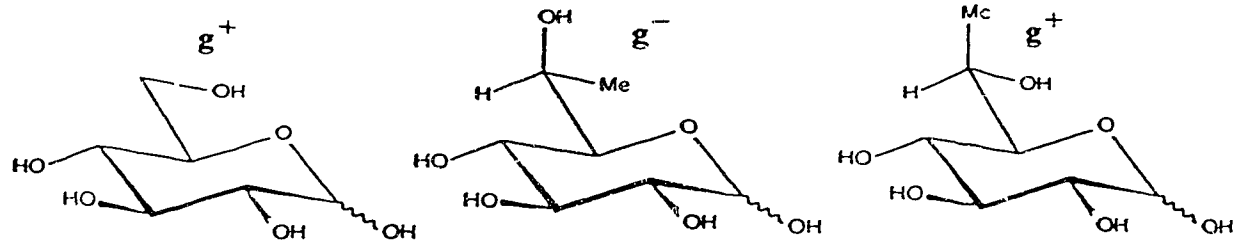


Figure 38. The preferred conformations of D-glucopyranose (**44**), 6-*S*-methyl-D-glucopyranose (**47**) and 6-*R*-methyl-D-glucopyranose (**48**)

In the hydrolyses reactions of the disaccharide derivatives, the various monosaccharides were used for standardization before the disaccharides were tested. Isomaltase and Amyloglucosidase Type II were used and the results of the preliminary studies are summarized in Table 13, 14 (the data are summarized in the Appendix).

The results presented in Table 13 and 14 were obtained with only one assay at fixed concentration (100 mM) per substrate. The rates (ΔOD min^{-1}) were only rough approximations because the objective was to see if a trend existed in the hydrolysis catalyzed by the enzyme that warranted further investigation.

Table 13. Hydrolysis catalyzed by Isomaltase at 37°C and pH=6.8.*

Compounds	Isomaltase hydrolysis	Rel. Rate (ΔOD min^{-1})
Phenyl α-D-glucopyranoside	+	39
Methyl α-D-glucopyranoside (**1**)	+	2
Methyl 6-*S*-methyl-α-D-glucopyranoside (**15**)	–	–
Methyl 6-*R*-methyl-α-D-glucopyranoside (**21**)	v. slight	–
Methyl α-isomaltoside (**37**)	+	1
Methyl 6-*S*-methyl-α-isomaltoside (**40**)	v. slight	–
Methyl 6-*R*-methyl-α-isomaltoside (**43**)	–	–

*(+) sign indicates that a reaction took place, a (—) sign indicates no reaction. Rel. Rate was measured in unit OD min^{-1}. Rel. Rate is the rate relative to compound **37**.

Table 14. Hydrolysis catalyzed by AMG II at 37°C and pH=4.3.*

Compounds	AMG Hydrolysis	Rel. Rate
Methyl α-D-glucopyranoside (**1**)	–	–
Methyl 6-*S*-methyl-α-D-glucopyranoside (**15**)	v. slight	1.4
Methyl 6-*R*-methyl-α-D-glucopyranoside (**21**)	v. slight	1
Isomaltose	+	67.5
Methyl α-isomaltoside (**37**)	+	81.3
Methyl 6-*S*-methyl-α-isomaltoside (**40**)	+	31.3
Methyl 6-*R*-methyl-α-isomaltoside (**43**)	+	112.5

*(+) sign indicates a reaction took place and a (–) sign indicates no reaction. Rel. Rate was measured in unit OD min^{-1}

As seen in Table 13, phenyl α-D-glucopyranoside, a known substrate for Isomaltase, is hydrolyzed 39 times faster than methyl α-isomaltoside (**37**) and about 20 times faster than methyl α-D-glucopyranoside (**1**). The monosaccharides **15** and **21** and the disaccharides **40** and **43** were shown not to be substrates for Isomaltase since there were no detectable or very slight rates in the hydrolysis.

In the case of hydrolysis catalyzed by AMG II (Table 14), methyl 6-*S*-methyl-α-D-glucopyranoside (**15**) was hydrolyzed 1.4 times faster than the methyl 6-*R*-methyl-α-D-glucopyranoside (**21**) and methyl α-D-glucopyranoside was not a substrate. It is seen that the disaccharides **40** and **43** proved to be substrates for this enzyme. The fact that the *R*-disaccharide **43** was superior to **37** as a substrate whereas **40** was less effective set the basis for this investigation.

2.2 The kinetic studies of the hydrolysis of methyl α-isomaltoside and 6-C-methyl- derivatives catalyzed by AMG II.

It was desirable to examine the hydrolysis of the methyl α-isomaltoside (**37**) and the methyl 6-*C*-methyl-α-isomaltoside (**40** and **43**) catalyzed by AMG II by the uv-vis spectroscopy. Unfortunately, the optimum pH required for the hydrolysis catalyzed by AMG II is 4.3 which is too acidic for the Hexokinase/6-Phosphate Dehydrogenase coupling assay (optimum pH=6.8). Furthermore, substrates which were synthesized in limited quantity may be required for further investigations. Since it was shown by Bock *et. al.*[26,97-101,106] that the progress of the hydrolysis of methyl β-maltoside and derivatives catalyzed by AMG II could be followed by ^{1}H-NMR spectroscopy, it was decided that further enzyme kinetic investigations would involved the use of ^{1}H-NMR spectroscopy. The method allows the observation of the disappearance of the disaccharide and the formation of the two monosaccharides simultaneously as a function of time. In the hydrolysis of methyl α-isomaltoside (**37**), it was possible to observe the decreasing signals of H-1′ at δ=4.95 ppm and H-1 at δ=4.85 ppm of the disaccharide and the increasing signals of H-1 of the products; namely, β-D-glucopyranose at δ=4.70 ppm and methyl α-D-glucopyranoside at δ=4.83 ppm simultaneously. For the methyl 6-*S*-methyl-α-isomaltoside (**40**), the disappearance of the broad quartet H-6 at δ=4.28 ppm of **40** and the appearance of the doublet of quartets H-6 of the methyl 6-*S*-methyl-α-D-glucopyranoside (**15**) at δ=4.05 ppm were also followed. For the methyl 6-*R*-methyl-α-isomaltoside (**43**), the disappearance of the doublet methyl signal at C-6 of compound **43** (δ=1.29 ppm) and the appearance of the doublet methyl signal of H-6 of methyl 6-*R*-methyl-α-D-glucopyranoside (**21**) at δ=1.10 ppm was also followed.

Representative spectra of the progress of the hydrolysis of the disaccharides; namely, methyl α-isomaltoside (**37**), methyl 6-*S*-methyl-α-isomaltoside (**40**) and methyl 6-*R*-methyl-α-isomaltoside (**43**) are reproduced in Figure 39, 40, and 41. Integrations of signals allowed

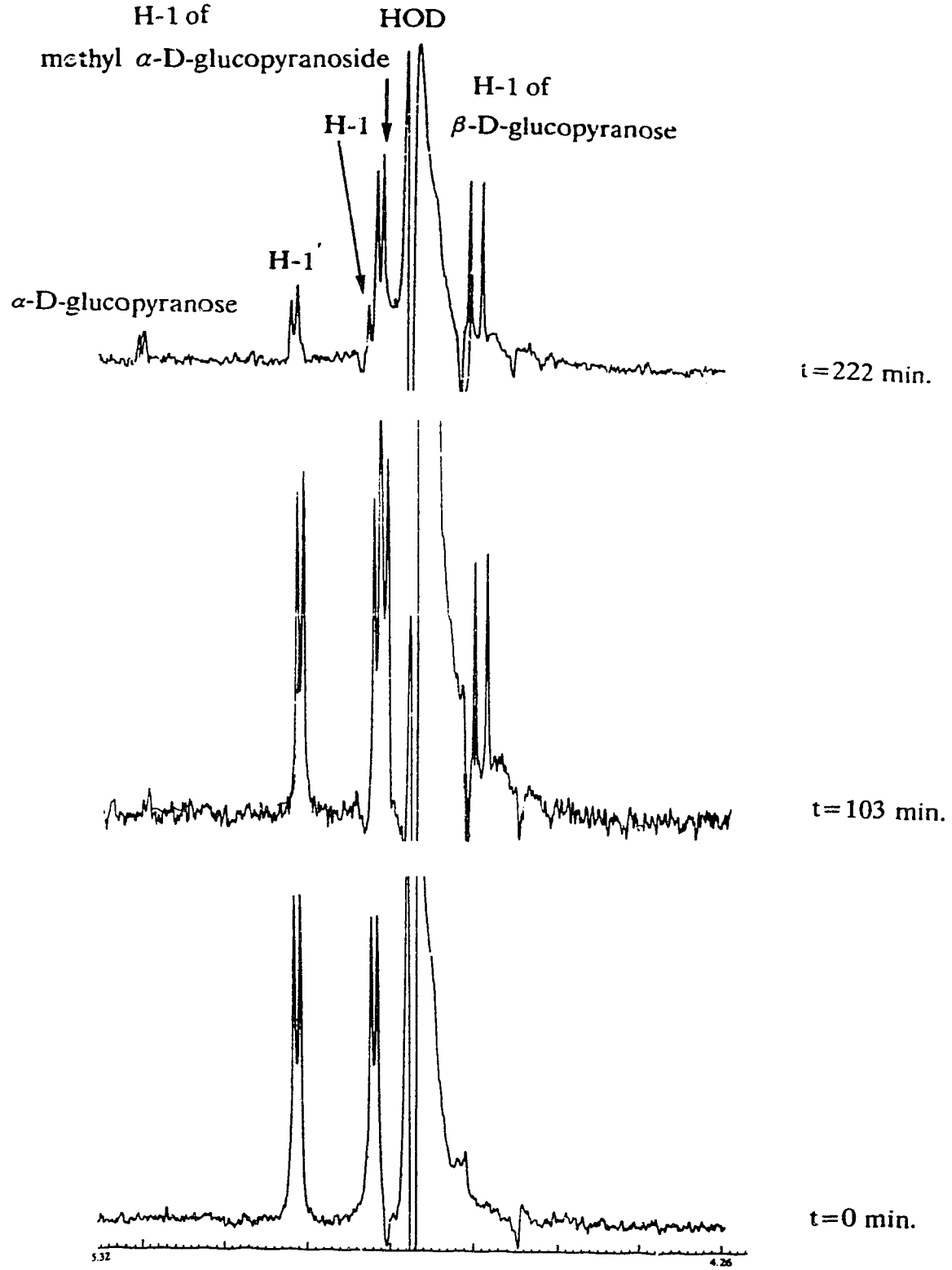


Figure 39. Reaction progress of the hydrolysis of methyl α-isomaltoside (**37**) catalyzed by AMG II with time.

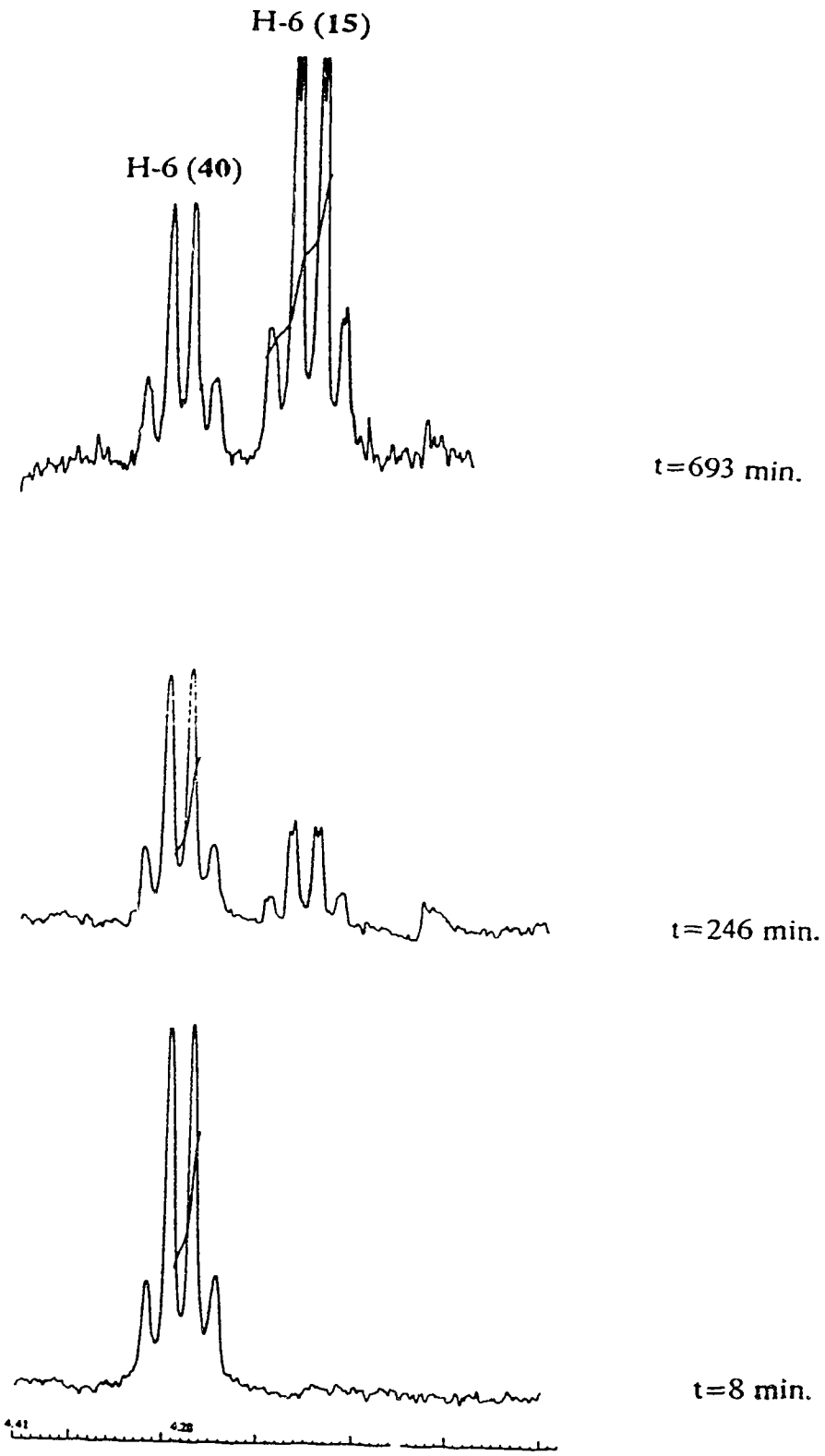


Figure 40. Reaction progress of the hydrolysis of methyl 6-S-methyl-α-isomaltoside (**40**) catalyzed by AMG II with time.

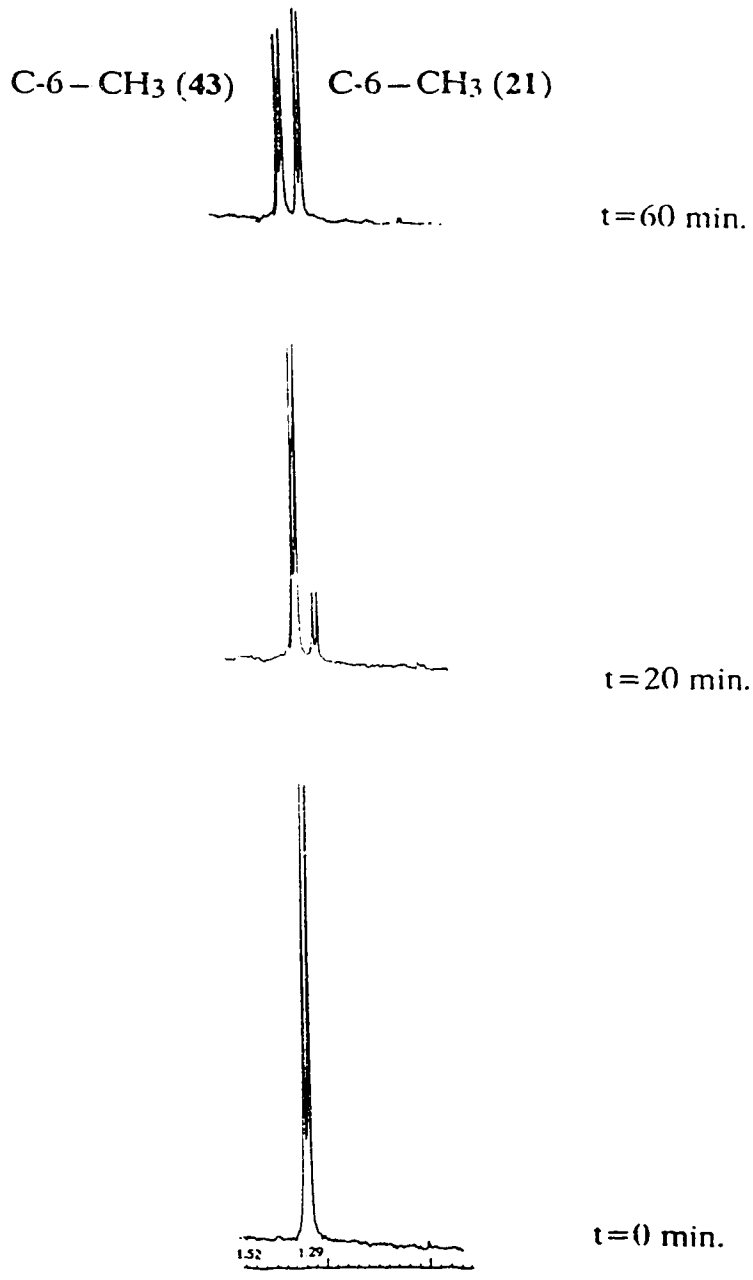


Figure 41. Reaction progress of the hydrolysis of methyl 6-R-methyl-α-isomaltoside (**43**) catalyzed by AMG II with time.

Table 15. Kinetic parameters for the disaccharide derivatives in the hydrolysis catalyzed by AMG II.

Substrate	Conc. (mM)	V_0 (mM/min)	$T_{1/2}$ (min)	V_{max} (mM/min)	K_{cat} (s^{-1})	K_m (mM)	K_{cat}/K_m ($M^{-1} s^{-1}$)
Methyl α-maltoside	9.26	1.6	3	1.6	2.6	—	—
Methyl β-maltoside	9.26	0.613	8.1	0.613	0.9	—	—
Methyl β-maltoside	18.52	0.727	14	0.727	1.2	—	—
Methyl 6-*S*-methyl-α-isomaltoside (**40**)	9.26	0.012	500	0.040	0.84	35	2.8
Methyl 6-*S*-methyl-α-isomaltoside (**40**)	18.52	0.027	575	—	—	—	—
Methyl α-isomaltoside (**37**)	9.26	0.033	161	0.043	0.069	2.9	23.8
Methyl α-isomaltoside (**37**)	18.52	0.04	247	0.04	0.064	2.2×10^{-4}	—
Methyl 6-*R*-methyl-α-isomaltoside (**43**)	9.26	0.081	63	0.083	0.13	0.22	600
Methyl 6-*R*-methyl-α-isomaltoside (**43**)	18.52	0.068	148	0.0688	0.11	0.22	500

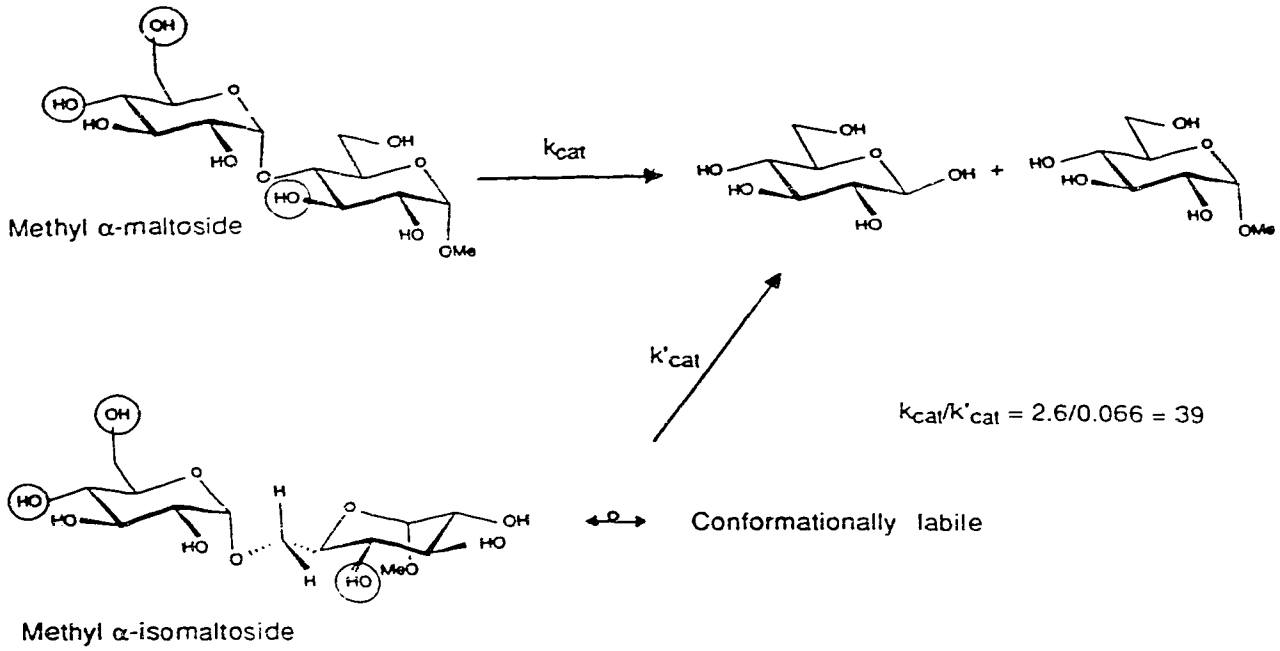


Figure 42. Methyl α-maltoside and methyl α-isomaltoside hydrolysis catalyzed by AMG II.

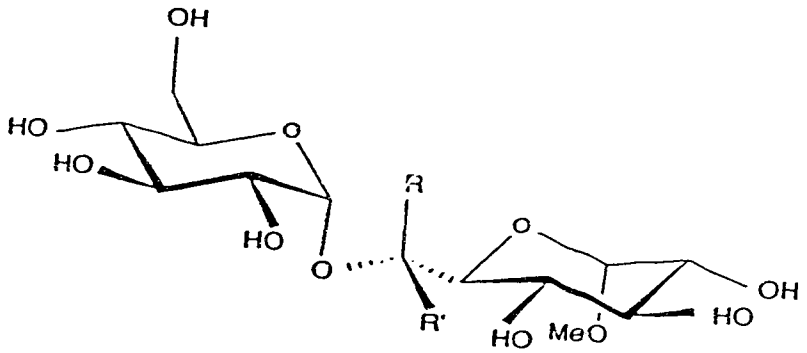
the construction of the progress curves (conc. vs. time). The data were analyzed using the REGGRAFA program[101] developed by Professor Bock and coworker on the personal computer. The results were reproduced in the experimental section. The parameters estimated from the progress curves are reproduced in Table 15.

The enzyme AMG II catalyzed hydrolysis of the disaccharide derivatives are irreversible[90] and, as mentioned above, gave two monosaccharides as products in which one was the methyl α-D-glucopyranoside or the C-6 methyl substituted derivatives and the other was the β-D-glucopyranose (Figure 42). The β-D-glucopyranose would then anomerized to the α-D-glucopyranose which could be observed by ^{1}H-NMR spectroscopy.

The general expression for a reaction catalyzed by an enzyme is:

$$E + S \rightleftharpoons [ES] \xrightarrow{k_{cat}} E + P$$

Table 16. Kinetic data of the hydrolysis catalyzed by AMG II of methyl α-isomaltoside (**37**) and the methyl 6-*C*-methyl isomaltoside derivatives (**40** and **43**).



Substituents at C-6			Population of the	Kinetic Data		Relative	$t_{1/2}$, min.
R	R′	Configuration	Key Conformer	k_{cat},sec^{-1}	Km, mM	$K_{Assoc.}$	9.26 mM
H	H	—	medium	0.069	2.9	12	160
Me	H	R	high	0.12	0.22	155	60
H	Me	S	low	0.84	35	1	500

The rate constant k_{cat} is the sum of all the rate constants of all subsequent steps after the formation of the enzyme-substrate complex ([ES]).

In the hydrolysis catalyzed by AMG II for methyl α-maltoside and methyl α-isomaltoside (Figure 42), the overall rates of hydrolysis were assumed to be k_{cat} for methyl α-maltoside and k'_{cat} for methyl α-isomaltoside. The overall rate ratio k_{cat}/k'_{cat} is about 39 and, therefore AMG II catalyzes the hydrolysis of methyl α-maltoside 39 times faster than methyl α-isomaltoside. This overall rate ratio is similar to the ratio for maltose and isomaltose. Because methyl α-isomaltoside is a poor substrate in comparision to methyl α-maltoside, it was assumed that the dissociation constant K_S for the [ES] complex is the same as the Michaelis-Menten constant K_M. This assumption may not be valid for all cases involved but can be made for poor substrates.[108]

Examination of Table 15 shows that the hydrolysis catalyzed by AMG II are in the decreasing order: methyl α-maltoside > methyl 6-*R*-methyl-α-isomaltoside (**40**) > methyl α-isomaltoside (**37**) > methyl 6-*S*-methyl-α-isomaltoside (**43**) and in agreement with the data reported in Table 14. The slightly faster rate of hydrolysis shown for methyl α-maltoside as compared to its β-anomer may not be real since reactions were too fast to be followed with precision. The kinetic data for the hydrolysis of methyl α-isomaltoside (**37**) and its epimeric 6-*C*-methyl derivatives (**40** and **43**) are presented in Table 16 along with comments regarding the key conformer to be discussed below.

The k_{cat} of methyl α-isomaltoside (**37**) is of the same order of magnitude as the k_{cat} found for methyl 6-*S*-methyl-α-isomaltoside (**40**) and approximately half of that found for methyl 6-*R*-methyl-α-isomaltoside (**43**). The variation in k_{cat} is minor (2 fold) compared to the differences in K_m, the major factor in determining the relative rates of reaction. The K_m values can be assumed to be a useful measure of the association constants $K_{Assoc.}$. The $K_{Assoc.}$ of methyl α-isomaltoside is about 12 times the $K_{Assoc.}$ of methyl 6-*S*-methyl-α-isomaltoside (**40**). On this basis, methyl 6-*R*-methyl-α-isomaltoside (**43**) is bound 13 times more strongly by the enzyme than is methyl α-isomaltoside and about 155 times more than is methyl 6-*S*-methyl-α-isomaltoside. It is seen that the stronger the binding the faster the hydrolysis (lower $T_{1/2}$).

2.3 The Key Hydroxyl Groups.

As discussed above with reference to Figure 37(a), Bock *et al.*,[26,97-101] showed that OH-6', OH-4' of the non-reducing unit and OH-3 of the reducing unit are necessary for the hydrolysis of methyl β-maltoside by the enzyme AMG II. This phenomenon was reminiscent of the findings by Lemieux and coworkers in their studies of the binding of oligosaccharides by lectins and antibodies[109] where they observed in each case that the

binding was dependent on the presence of a cluster of two or three hydroxyl groups termed the key polar grouping. It was shown by Lemieux *et al.*[8] that the α-(1 → 4) linkage in maltose are conformationally well defined by the torsion angles $\Phi^{H\text{-}1',C\text{-}4}/\Psi^{C\text{-}1',H\text{-}4} = -25^{o}/-15^{o}$. Therefore the key polar groups OH-4′ and OH-3 should be energetically well presented at a fixed separation in aqueous solution. As discussed above, the other key polar group OH-6′ is conformationally labile because of freedom of rotation about the C-5 to C-6 bond. Thus, the C-5/C-6′ fragment can readily adopt either the $\mathbf{g}^{-}$ or the $\mathbf{g}^{+}$ conformation (Figure 43) and it is not clear at present which conformer is required by the enzyme AMG II. For the present discussion, the $\mathbf{g}^{-}$ conformation at C-6′ is arbitrarily assumed.

The enzyme AMG II is expected to enter into a polar interaction with the key polar groups (Figure 37(a)) of methyl α-maltoside. In view of the conformational rigidity possessed by methyl α-maltoside,[8] the disposition of these hydroxyl groups can be expected to be near that presented by methyl α-maltoside in the minimum energy conformer which as seen in Fig. 44 is expected to be near 7.1 Å and 7.3 Å for the distance between O-4′ – O-3 and O′-6– O-3, respectively. On the basis of the key polar group theory, it is expected that the hydrolysis of the methyl α-isomaltoside (**37**) and its derivatives (**40** and **43**) will have key polar groups in similar disposition in space.

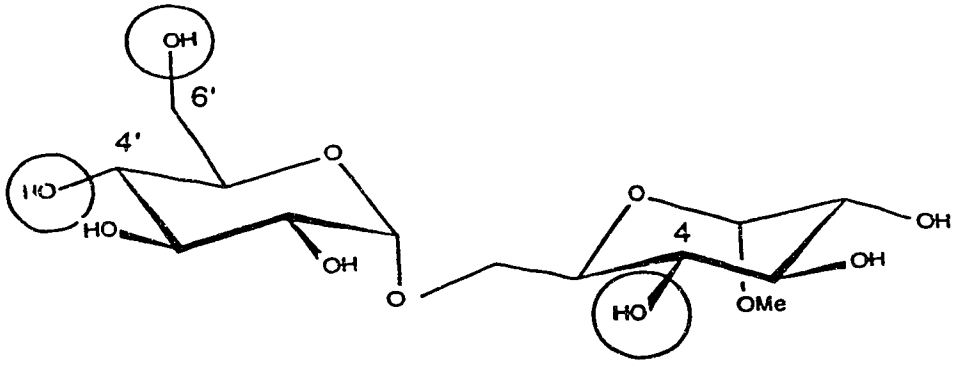


Figure 43. The OH-4, OH-4′ and OH-6′ (circled) groups of methyl α-isomaltoside which are expected to form the key polar grouping.

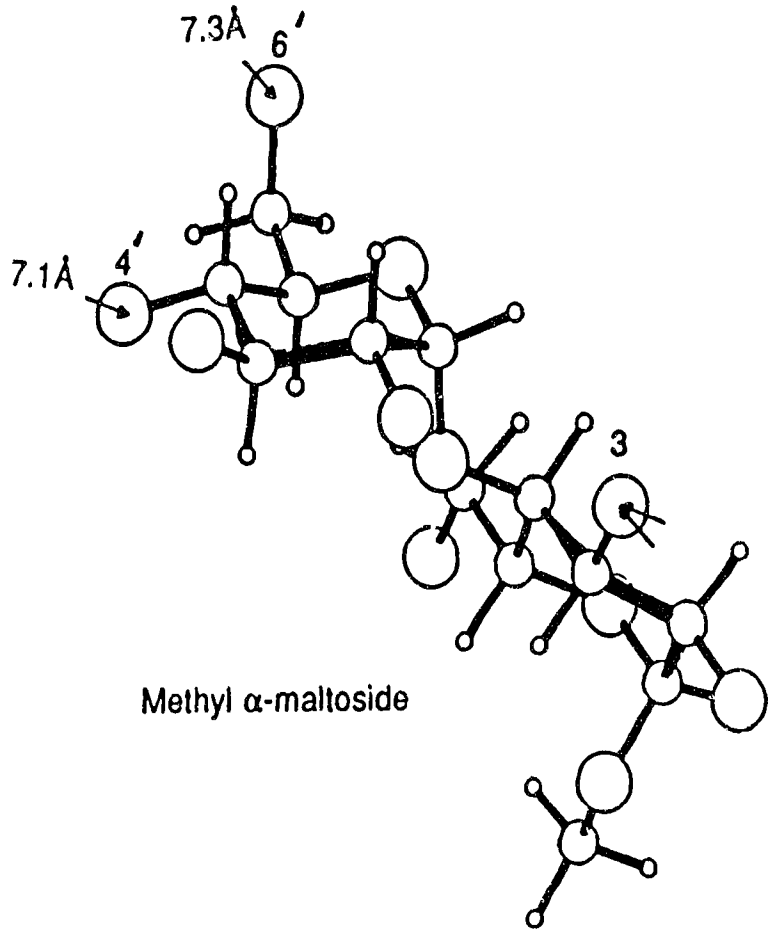
In the methyl α-isomaltoside and its derivatives series, the two key polar hydroxyl groups of the non-reducing unit will still be OH-6′ and OH-4′ but the key polar hydroxyl group of the reducing unit could not be OH-3 because it would be one bond too far away. The OH-4 would be most likely.

The key conformers with similar distances as the OH-3/OH-4′ and OH-3/OH-6′ of methyl α-maltoside were then searched for methyl α-isomaltoside (**37**), methyl 6-*R*-methyl-α-isomaltoside (**43**) and methyl 6-*S*-methyl-α-isomaltoside (**40**) using HSEA calculations. The variations in the estimated distances of the key conformers in the methyl α-isomaltoside and the 6-*C*-methyl derivatives compared to the key conformer of methyl α-maltoside are not more than 0.4 Å. The results are summarized in Table 17 and the key conformers were computer drawn in Figures 44 and 45.

According to HSEA calculations (see Table 17), the minimum energy conformation of methyl α-isomaltoside has the torsion angles $\omega' = 180^{o}$, $\Phi = -50^{o}$, $\Psi = 190^{o}$ and $\omega = 180^{o}$. The torsion angle $\omega = 180^{o}$ at C-6 corresponds to the $\mathbf{g}^{-}$ conformation and is in agreement with the experimental data obtained by Bock *et al.*[26] The key conformer with similar internuclear distances O-4 – O-4′ = 6.9 Å and O-4 – O-6′ = 7.1 Å (Table 17) has the torsion angles $\omega' = 180^{o}$, $\Phi = -50^{o}$, $\Psi = 120^{o}$ and $\omega = 50^{o}$. The key conformer of methyl α-isomaltoside is 1.49 Kcal mol^{-1} higher in energy than its minimum energy conformer and, therefore, can be expected on this basis to be a poorer substrate than methyl α-maltoside for which the key conformer is energetically very close to the preferred conformation. The key conformer for methyl 6-*R*-methyl-α-isomaltoside (**43**) with the same torsion angles ($\omega'/\Phi/\Psi/\alpha = 180^{o}/-50^{o}/120^{o}/50^{o}$) is only 0.31 Kcal mol^{-1} higher than its ^{1}H-NMR conformer. In contrast, the key conformer for methyl 6-*S*-methyl-α-isomaltoside (**40**) has the torsion angles $\omega' = 180^{o}$, $\Phi = -60^{o}$, $\Phi = 160^{o}$ and $\omega = 50^{o}$ with internuclear distances O-4 – O-4′ = 7.0 Å, O-4 – O-6′ = 7.7 Å and is 3.78 Kcal mol^{-1} higher in energy than its ^{1}H-NMR conformer (Figure 45).

Table 17. HSEA calculations for ^{1}H-NMR and Key conformers of methyl α-maltoside, methyl α-isomaltoside (**37**) and methyl 6-*C*-methyl-α-isomaltosides (**40** and **43**)

Compounds	Conformer	Torsion Angles, ^{0}	Energy	Internuclear Distances, Å	
		$\omega'/\Phi/\Psi/\omega$	Kcal/mol	O-4—O-4′	O-4—O-6′
Methyl α-isomaltoside (**37**)	NMR	180/−50/190/180	−2.45	5.2	3.5
	KEY	180/−50/120/180	−0.96	6.9	7.1
Methyl 6-*R*-methyl-α-isomaltoside (**43**)	NMR	180/−50/90/300	−1.26	8.2	7.1
	KEY	180/−50/120/50	−0.85	6.9	7.1
Methyl 6-*S*-methyl-α-isomaltoside (**40**)	NMR	180/−50/195/180	−1.46	5.1	5.3
	KEY	180/−60/160/50	2.32	7.0	7.7
Methyl α-maltoside	NMR	180/−25/−15/300	1.35	7.1 (O-3−O-4′)	7.3 (O-3−O-6′)



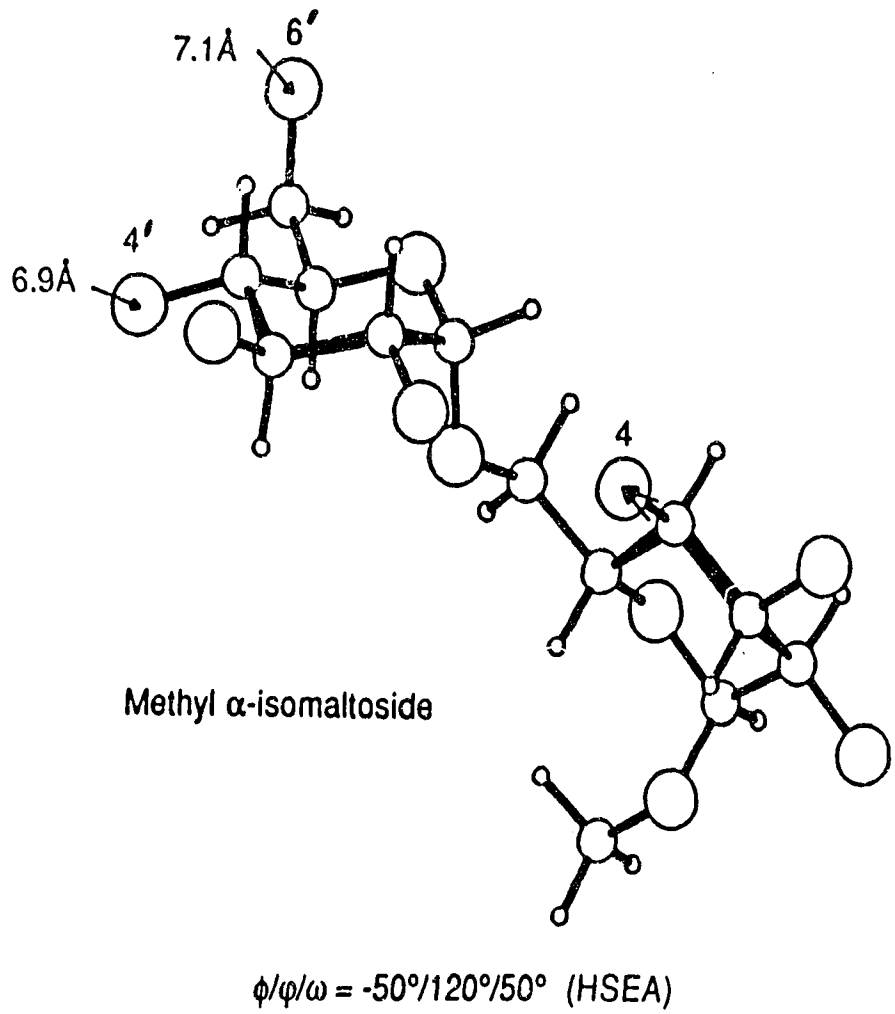
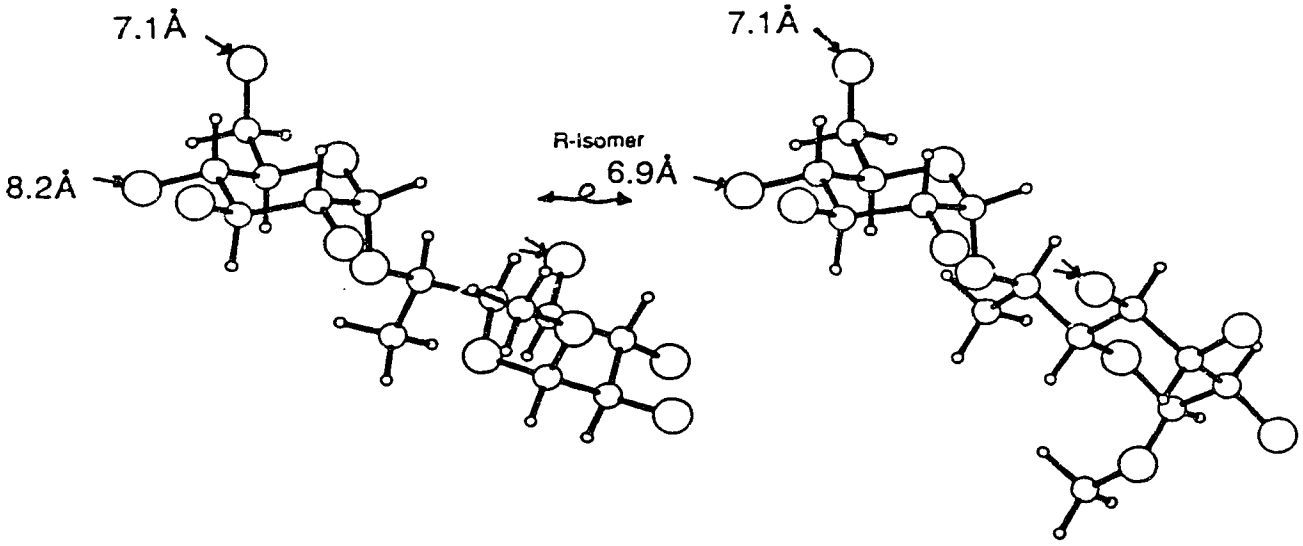
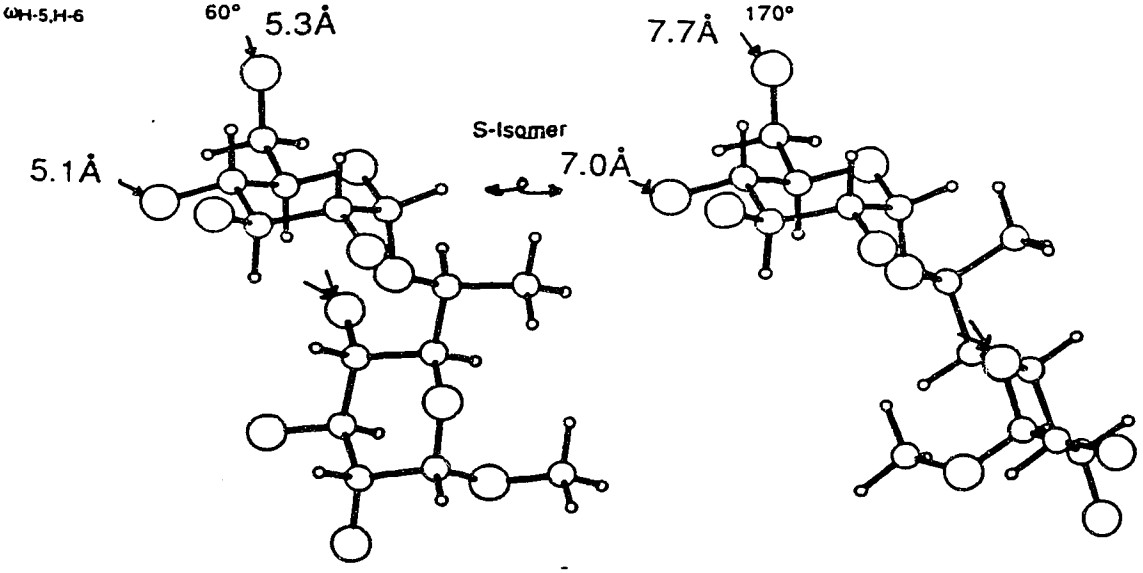


Figure 44. The presentation of the key polar group.



NMR Conformer ($J_{H-5,H-6}$ = 2.5 Hz)		Key Conformer
φ/φ/ω	-50°/90°/-60°	-50°/120°/50°
E, kcal/mole	-1.26	-0.85
$\omega_{H-5,H-6}$	60°	170°



NMR Conformer ($J_{H-5,H-6}$ < 0.5 Hz)		Key Conformer
φ/φ/ω	-50°/195°/180°,	-60°/160°/50°
E, kcal/mole	-1.46	2.32
$\omega_{H-5,H-6}$	60°	-70°

Figure 45. Conformational analysis of the diastereoisomeric methyl 6-*C*-methyl-α-isomaltosides (**43** and **40**).

It is possible now to rationalize partially why the enzyme AMG II catalyzed the hydrolysis of methyl α-maltoside, methyl α-isomaltoside and 6-*C*-methyl derivatives (**40** and **43**). Since enzymes are expected to be substrate specific, a change in linkage such as α-(1→4) in methyl α-maltoside to α-(1→6) in methyl α-isomaltoside will result in a change in conformation of the disaccharide which then becomes a very different substrate. However, AMG II can still catalyze the hydrolysis of the α-(1→6) linkage of methyl α-isomaltoside because the latter can adopt a conformation similar to that of methyl α-maltoside where the key polar hydroxyl groups are in similar spatial arrangement.

The rate of hydrolysis by AMG II was about 40 times faster for methyl α-maltoside compared to methyl α-isomaltoside. Although the methyl α-isomaltoside can adopt the appropriate conformation for hydrolysis, this key conformer is only one of several possible conformers of comparable energies because of the flexibility of the O-5/O-6 fragment of the reducing unit. In other words the population of the key conformer that can be recognized by the enzyme AMG II is low and the organization of the molecule for reaction must present a barrier to reaction. Furthermore, because methyl α-isomaltoside is an α-(1→6) linkage, the nonpolar surface of the disaccharide would be different compared to the α-(1→4) linkage of methyl α-maltoside, the methyl α-isomaltoside may not fit the binding site as well as in the case of methyl α-maltoside which would also result in the reduction of rate. The effect of the distance variations (0.4 Å) in the key polar hydroxyl groups on the rate of hydrolysis is not known at present but it is expected that changes in distance would probably decrease the rate.

Based on the $T_{1/2}$ at 10 mM concentration (see Table 16), methyl 6-*R*-methyl-α-isomaltoside (**43**) hydrolyzed about 2.6 times faster than methyl α-isomaltoside (**37**) which in turn is hydrolyzed about 3.1 times faster than methyl 6-*S*-methyl-α-isomaltoside (**40**). If for hydrolysis, the isomaltoside derivatives must imitate the conformation of methyl α-maltoside in terms of the internuclear distances of the three key hydroxyl groups, then

methyl 6-*R*-methyl-α-isomaltoside (**43**) must have higher population of the proper key conformer than methyl α-isomaltoside. On the other hand, methyl 6-*S*-methyl-α-isomaltoside (**40**) must have lower population of the proper key conformer than has methyl α-isomaltoside. The differences in energies (Kcal mol^{-1}) as estimated by HSEA calculations (Table 17) for adopting the key conformations were 0.41 for methyl 6-*R*-methyl-α-isomaltoside (**43**), 1.49 for methyl α-isomaltoside and 3.78 for methyl 6-*S*-methyl-α-isomaltoside. Using Boltzman's equation for population distribution (Equation 4), it is possible to estimate that the population of the key conformer is 33% for methyl 6-*R*-methyl-α-isomaltoside (**43**), 8% for methyl α-isomaltoside and 0.2% for methyl 6-*S*-methyl-α-isomaltoside. These population estimates reflect the trend for the hydrolysis catalyzed by the enzyme AMG II.

$$\frac{N1}{N2}=e^{-\Delta E/RT} \qquad (4)$$

where $N1$= population of the ^{1}H-NMR conformer

$N2$= population of the key conformer

R= gas constant

T= temperature ^oK

V. SUMMARY

1. Methyl 6-*R*-C-methyl-α-D-glucopyranoside (**21**) was synthesized and found to prefer the $^4C_1 - g^+$ conformation in aqueous solutions.

2. Hydrolysis of **21**, provided 6-*R*-methyl-D-glucose (**48**) which was dehydrogenated to aldonic lactone by the enzyme system of Hexokinase and 6-Phosphate Dehydrogenase.

3. Methyl 6-*S*-C-methyl-α-D-glucopyranoside (**15**) was synthesized and found to prefer the $^4C_1 - g^-$ conformation in aqueous solution.

4. Hydrolysis of **15**, provided 6-*S*-methyl-D-glucose (**47**) which was not dehydrogenated to aldonic lactone by the enzyme system of Hexokinase and 6-Phosphate Dehydrogenase.

5. In the temperature range 295°C to 355°C, compounds **21** and **15** still retain their preferred conformations; i.e. g^+ and g^- respectively.

6. Methyl 6-*R*-methyl-α-isomaltoside (**43**) was synthesized and found to prefer g^+ conformation at the C-6 center of the reducing glucose unit in aqueous solution.

7. Hydrolysis catalyzed by the enzyme Amyloglucosidase Type II of **43** was observed to be about 3 times faster than methyl α-isomaltoside (**37**).

8. Methyl 6-*S*-methyl-α-isomaltoside (**40**) was synthesized and found to prefer g^- conformation at the C-6 center of the reducing glucose unit in aqueous solution.

9. Hydrolysis catalyzed by the enzyme Amyloglucosidase Type II of **40** was observed to be about 3 times slower than methyl α-isomaltoside (**37**).

VI. EXPERIMENTAL

1. General methods

Nuclear magnetic resonance spectra were obtained at 297°K in D_2O or $CDCl_3$ on Bruker instruments operating at 300 or 400 MHz when provided by the Spectral Service Laboratory under the supervision of Dr. T. Nakashima and by the writer when at 360 MHz.. Acetone was used as internal reference (δ=2.225 ppm) for D_2O and tetramethylsilane (TMS) was used for $CDCl_3$ in proton spectra. Dioxane was used as internal reference for ^{13}C-spectra. Assignments were carried out using either the double resonance technique or the 2D-COSY technique. Infra-red spectra were obtained on the Nicolet 7199 FT-IR. Melting points were determined on the Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter at 589 nm in a 1 dm cell at room temperature (23± 1°C). Elemental analyses were performed by the departmental Analytical Service Laboratory under the supervision of Mr. R. Swindlehurst. Thin layer chromatography (t.l.c.) was run with precoated silica gel 60-F254 plates (E. Merck, Darmstadt) and developed by quenching of flourescence and/or charring with 5%-10% sulfuric-ethanol solution.

Dry dichloromethane and pyridine were obtained by distilling over calcium hydride and were used immediately. Chromium trioxide was obtained from Aldrich and was dried over P_2O_5 and under vacuum for at least 48 hrs. Other solvents and reagents used were reagent grade and if better purity was needed, standard procedures[110] were followed. Anhydrous solution transfers were done under nitrogen atmosphere using standard syringe techniques.[111]

N.O.e. experiments

Solutions in the NMR tubes were previously degassed by bubbling dry nitrogen through for 3-5 minutes. The D_2O used was purchased from Aldrich (99.9% D, low in paramagnetic impurities). The steady state nuclear Overhauser enhancements were obtained after 512 scans normally.

HSEA calculations

The atomic coordinates were obtained from neutron diffraction studies of methyl α-D-glucopyranoside crystal structure.[76] Modification of the methyl α-D-glucopyranoside structure at C-6 were achieved with the program TRANSF.OBJ using the distance 1.54Å for C-C bond. Rotation of the ω torsion angle was done with the program GENT.OBJ. Molecular minimum energy calculation was estimated using the HDSP3.OBJ program. Molecular conformation was plotted using the coordinates of the minimum energy conformation generated by the HDSP3.OBJ program. The programs TRANSF.OBJ, GENT.OBJ and HDSP3.OBJ were available from the University of Alberta Computing Library.

2. Syntheses

Methyl 2,3,4,-tri-O-benzyl-α-D-gluco-1,6-dialdo-hexopyranoside (7)

Dried chromium trioxide (25.9 g, 259 mmol) was added to a solution of pyridine (40.9 g, 517 mmol) in dichloromethane (400 mL). The mixture was mechanically stirred at room temperature for 30 min.. A solution of methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**4**) (10.0 g, 21.6 mmol) in dichloromethane (22 mL) was added rapidly to the oxidation mixture and a black tar precipitated immediately. The resulting mixture was stirred at room temperature for 10 min., decanted and the black tarry precipitate was washed with ether several times. The combined ether solutions were filtered through a pad of silica gel-G to give a colorless filtrate which was dried with anhydrous magnesium sulfate. Ether was removed to give a slightly yellow oily residue which was coevaporated with toluene (2 x 200 mL) to remove residual pyridine. The oily residue was dried under vacuum overnight to give **7** (6.94 g, 70%). Proton NMR of this crude residue showed that it contained ~90% of the desired compound **3**. $[\alpha]_D$=8.2° (c 1.0, $CHCl_3$) lit.[47] 13.6°.

^{1}H-NMR ($CDCl_3$): δ 9.65 (s, 1H, H-6), 7.40—7.10 (m, 15H, Ar-H), 5.05—4.50 (m, 7H, 3 x Ph-CH_2 and H-1), 4.17 (d, 1H, H-5, $J_{4,5}$= 10.0 Hz), 4.08 (dd, 1H, H-3, $J_{3,4}$=9.0 Hz, $J_{2,3}$=9.5 Hz), 3.57 (dd, 1H, H-4, $J_{3,4}$=9.0 Hz, $J_{4,5}$=10.0 Hz), 3.50 (dd, 1H, H-2, $J_{1,2}$=3.5 Hz, $J_{2,3}$=9.5 Hz), 3.38 (s, 3H, OCH_3); ^{13}C-NMR ($CDCl_3$): δ 197.45 (C̲HO); 138.43—125.31 (Ar. Carbons); 98.41 (C-1); 87.76, 79.30, 77.82 (3 x Ph-C̲H_2); 75.32, 75.11, 74.22, 73.60 (C-2—C-5); 55.77 (OC̲H_3); IR ν_{max} (cm^{-1}) 1741.133 (CHO).

Methyl 2,3,4-tri-O-benzyl-7-deoxy-β-L-glycero-D-glucoheptapyranoside (9)

A solution of methyl iodide (4.26 g, 30.04 mmol) in anhydrous ether (20 mL) was added dropwise under nitrogen to a mixture of magnesium (0.73 g, 30.04 mmol) and ether (30 mL). After warming for a few minutes, the mixture turned cloudy and began to boil. Addition of methyl iodide solution was such that boiling was maintained. After the addition

of methyl iodide was complete, the resulting Grignard mixture was left stirring for 1 hr. to ensure complete formation of the reagent. A solution of compound **7** (6.94 g, 15.02 mmol) in ether (30 mL) was then added dropwise and the reaction mixture was left stirring at room temperature for 2 hrs. The reaction was quenched with the dropwise addition of saturated ammonium chloride (10 mL) and the reaction mixture solidified. Distilled water (50 mL) was added to dissolve the solid. The aqueous layer was extracted with ether several times (total volume 150 mL) and the combined ether solutions were washed with water (2 x 100 mL) and dried over anhydrous magnesium sulfate. Removal of solvent gave a yellow crude oil (5.96 g) in which the major product was **9** by t.l.c. (R_f=0.54, solvent 3:2 n-hexane:EtOAc).

(i) Methyl 2,3,4,-tri-O-benzyl-7-deoxy-6-O-(3,5-dinitrobenzoyl)-β-L-glycero-D-glucoheptapyranoside **(14)**

The crude Grignard product (0.587 g, 1.2 mmol) was dissolved in pyridine (10 mL) and 3,5-dinitrobenzoyl chloride (0.513 g, 2.25 mmol) was added along with a few crystals of dimethylaminopyridine (DMAP). The reaction mixture was stirred at room temperature until analysis by t.l.c. (R_f S.M.=0.58, R_f Prod.=0.85, solvent 3:2 n-hexane:EtOAc) showed no remaining starting material. Water was then added dropwise with stirring for 5 min. The aqueous layer was extracted with chloroform (75 mL). The chloroform layer was separated, washed with water (2 x 75 mL) and dried over anhydrous magnesium sulfate. The solvent was then evaporated to give a yellow residue which was recrystallized from hot methanol to give **14** (0.612 g, 74%); mp 68-70°C; $[\alpha]_D$=52.2° (c 0.85, $CHCl_3$). ^{1}H-NMR ($CHCl_3$): δ 9.21 (t, 1H, para-H, $J_{ortho,para}$=2.0 Hz), 9.16 (d, 2H, ortho-H, $J_{ortho,para}$=2.0 Hz), 7.45—7.15 (m, 15, Ar-H), 5.62 (dq, 1H, H-6, $J_{5,6}$=1.5 Hz, $J_{6,7}$=6.5 Hz), 5.45—5.05 (3 x dd and d, 7H, 3 x Ph-CH_2 and H-1), 4.05 (dd, 1H, H-3, $J_{2,3}$=10.0 Hz, $J_{3,4}$=9.0 Hz), 3.71 (dd, 1H, H-5, $J_{5,6}$=1.5 Hz, $J_{4,5}$=10.0 Hz), 3.60 (dd, 1H, H-2, $J_{1,2}$=3.5

Hz, $J_{2,3}$=10.0 Hz), 3.42 (t, 4H, H-4 and OCH_3), 1.48 (d, 3H, C_6-CH_3, $J_{6,7}$=6.5 Hz); ^{13}C-NMR ($CDCl_3$): δ 228.13 (C=O); 162.81—123.38 (Ar. Carbons); 99.01 (C-1); 83.23, 81.11, 77.69, 72.51, 71.80 (C-2—C6); 76.86, 75.88, 74.53 (3 x Ph-CH_2); 56.30 (OCH_3); 17.35 (C-7).

Anal. calc. for $C_{36}H_{36}N_2O_{11}$•½MeOH:	C	63.66	H 5.56	N 4.07
found:	C	63.49	H 5.24	N 4.27

(ii) Methyl 2,3,4-tri-O-benzyl-7-deoxy-β-L-glycero-D-glucoheptapyranoside (9) from **14**

Compound **14** (1.00 g, 1.49 mmol) was dissolved in dioxane (50 mL) and 1N sodium hydroxide solution (20 mL) added and stirred at room temperature for 1 hr. The reaction was followed by t.l.c. (R_f **14**=0.85, R_f **9**=0.57, solvent 3:2 n-hexane:EtOAc). When the reaction was completed (after 1 hr.), dichloromethane (250 mL) was added to the reaction mixture and stirred for 5 min.. Then the reaction mixture was filtered over a pad of silica gel-G which was then washed with water (2 x 100 mL) and dried over anhydrous magnesium sulfate. The solvent was then evaporated, the residue dried under vacuum to give solid **9** (0.692 g, 97%); mp 82-84°C; $[\alpha]_D$=26.9° (c 1.0, $CHCl_3$).

^{1}H-NMR ($CDCl_3$): δ 7.40—7.20 (m, 15H, Ar-H), 5.01—4.63 (3 x dd, 6H, Ph-CH_2), 4.58 (d, 1H, H-1, $J_{1,2}$=3.5 Hz), 4.05 (dq, 1H, H-6, $J_{5,6}$=1.5 Hz, $J_{6,7}$=6.5 Hz), 3.99 (dd, 1H, H-3, $J_{3,4}$=9.0 Hz, $J_{2,3}$=9.5 Hz), 3.62 (dd, 1H, H-4, $J_{4,5}$=10.0 Hz, $J_{3,4}$=9.0 Hz), 3.50 (dd, 1H, H-2, $J_{2,3}$=9.5 Hz, $J_{1,2}$=3.5 Hz), 3.42 (dd, 1H, H-5, $J_{5,6}$=1.5 Hz, $J_{4,5}$=10.0 Hz), 3.34 (s, 3H, OCH_3), 1.25 (d, 3H, C_6-CH_3, $J_{6,7}$=6.5 Hz); ^{13}C-NMR ($CDCl_3$): δ 138.79—127.57 (Ar. Carbons); 98.15 (C-1); 82.17, 79.93, 77.72 (3 x Ph-CH_2); 75.70, 75.11, 73.42, 72.83 (C-2—C-5); 65.05 (C-6); 55.01 (OCH_3); 20.15 (C-7).

Anal. calc. for $C_{29}H_{34}O_6$:	C	72.78	H 7.16	
found:	C	72.94	H 6.82	

Methyl 7-deoxy-β-L-glycero-D-glucoheptapyranoside (15)

Compound **9** (200 mg, 0.42 mmol) was dissolved in methanol (25 mL). Then 5% palladium-on-charcoal (200 mg) was added in small portions. The mixture was shaken with hydrogen (100 lb. in^{-2}) at room temperature overnight. The reaction mixture was then filtered over celite (washed with methanol). The solvent was evaporated to give **15** (85 mg, 98%) as a solid which could be recrystallized from n-hexane / 98% ethanol to give needle like crystals. Mp 136-137°C, $[\alpha]_D$=150° (c .23, H_2O). ^{1}H-NMR (D_2O): δ 4.68 (d, 1H, H-1, $J_{1,2}$=3.5 Hz), 4.05 (dq, 1H, H-6, $J_{5,6}$=1.5 Hz, $J_{6,7}$=6.5 Hz), 3.51 (dd, 1H, H-3, $J_{2,3}$=10.0 Hz, $J_{3,4}$=9.0 Hz), 3.42 (dd, 1H, H-2, $J_{1,2}$=3.5 Hz, $J_{2,3}$=10.0 Hz), 3.36 (dd, 1H, H-4, $J_{3,4}$=9.0 Hz, $J_{4,5}$=10.0 Hz), 3.27 (s, 3H, OCH_3), 3.26 (dd, 1H, H-5, $J_{5,6}$=1.5 Hz, $J_{4,5}$=10.0 Hz), 1.16 (d, 3H, C_7H_3, $J_{6,7}$=6.5 Hz). ^{13}C-NMR (D_2O): δ 99.24 (C-1); 73.49, 73.41 (C-3, C-5); 71.31 (C-2); 69.74 (C-6); 64.26 (C-4); 54.89 ($O\underline{C}H_3$); 18.72 (C-7).

Anal. calc. for $C_8H_{16}O_6$:	C	46.15	H	7.75
found:	C	46.04	H	7.46

Methyl 2,3-di-O-acetyl-4,6-benzylidene-7-deoxy-β-L-glycero-D-glucoheptapyranoside (17)

A solution of benzal bromide (112 mg, 0.40 mmol) in pyridine (1 mL) was added to a solution of compound **15** (16 mg, 0.77 mmol) in pyridine (15 mL) and the mixture was refluxed at 125°C for 2 hrs. with stirring. Acetic anhydride (3 mL) was then added to the cooled reaction mixture and the solution was stirred for 48 hrs. at room temperature. The reaction mixture was then poured into water (75 mL) and the aqueous layer was extracted with chloroform (50 mL). The chloroform layer was washed with water (50 mL), saturated sodium bicarbonate solution (50 mL), water (50 mL) and dried over anhydrous magnesium sulfate. Chloroform was evaporated and the residue was coevaporated with toluene (2 x 15 mL). Preparative t.l.c. of the residue (R_f=0.66, solvent 3:2 n-hexane / EtOAc) gave **17** (15 mg, 51%) as a solid. Mp 132-134°C; $[\alpha]_D$=50.2° (c 0.31, $CHCl_3$).

^{1}H-NMR ($CDCl_3$): δ 7.50—7.25 (m, 5H, Ar-H), 5.80 (s, 1H, Ph-CH), 5.57 (dd, 1H, H-3, $J_{3,4}$=9.0 Hz, $J_{2,3}$=10.0 Hz), 4.93 (d, 1H, H-1, $J_{1,2}$=3.5 Hz), 4.87 (dd, 1H, H-2, $J_{2,3}$=10.0 Hz, $J_{1,2}$=3.5 Hz), 4.5 (dq, 1H, H-6, $J_{5,6}$=6.0 Hz, $J_{6,7}$=7.0 Hz), 4.15 (dd, 1H, H-5, $J_{4,5}$=10.0 Hz, $J_{5,6}$=6.0 Hz), 3.91 (dd, 1H, H-4, $J_{3,4}$=9.0 Hz, $J_{4,5}$=10.0 Hz), 3.41 (s, 3H, $OCOCH_3$), 1.48 (d, 3H, C_7H_3, $J_{6,7}$=7.0 Hz). ^{13}C-NMR ($CDCl_3$): δ 170.48, 169.76 (2 x C=O); 137.40, 128.92, 128.22, 126.24 (Ar. Carbons); 97.59 (C-1), 94.07 (Ph-CH); 72.74, 71.50, 70.49, 69.66 (C-2—C-5); 64.45 (C-6); 55.34 (OCH_3); 20.65, 20.79 (2 x $COCH_3$), 11.28 (C-7).

Anal. cal. for $C_{19}H_{24}O_8$:	C	59.99	H	6.36
found:	C	59.55	H	6.06

Methyl 6-O-methanesulfonyl-2,3,4-tri-O-benzyl-7-deoxy-β-L-glycero-D-glucoheptapyranoside (**18**)

Methanesulfonyl chloride (87 mg, 0.76 mmol) was added to a solution of compound **9** (135 mg, 0.282 mmol) in pyridine (5 mL) and the reaction mixture was stirred at room temperature for 2 hrs. T.l.c. analysis indicated that the reaction was completed after 2 hrs. and virtually one product had formed (R_f **9**=0.58, R_f **18**=0.67, solvent 3:1 toluene / acetone). The reaction mixture was then poured into a mixture of ice / water (50 mL) and the aqueous layer was extracted with dichloromethane (50 mL). The dichloromethane layer was washed with cold water (2 x 50 mL) and dried over anhydrous magnesium sulfate. The solvent was then evaporated, the residue was coevaporated with toluene (2 x 25 mL) and chromatographed (solvent 6:1 toluene/acetone) to give **18** (104 mg, 66%) as an oil. $[\alpha]_D$=9.3° (c 1.22, $CHCl_3$).

^{1}H-NMR ($CDCl_3$): δ 7.40—7.25 (m, 15H, Ar-H), 5.32 (dq, 1H, H-6, $J_{5,6}$=1.0 Hz, $J_{6,7}$=6.5 Hz), 5.06—4.62 (m, 7H, 3 x Ph-CH_2 and H-1), 4.02 (m, 1H, H-5), 3.65—3.53 (m, 3H, H-3, H-4 and H-2), 3.36 (s, 3H, OCH_3), 3.04 (s, 3H, SO_2CH_3), 1.53 (d, 3H, C_6-CH_3,

$J_{6,7}$=6.5 Hz). ^{13}C-NMR ($CDCl_3$): δ 138.54—127.74 (Ar. Carbons); 98.26 (C-1); 82.30, 79.71, 77.17, 73.55, 72.38 (C-2—C6); 75.74, 75.31, 75.00 (3 x Ph-$\underline{C}H_2$); 55.33 (O$\underline{C}H_3$); 39.54 ($OSO_2\underline{C}H_3$); 17.73 (C-7).

Anal. calc. for $C_{30}H_{36}O_8S$:	C 64.73	H 6.52	S 5.76
found:	C 64.85	H 6.55	S 5.52

Methyl 6-O-benzoyl-2,3,4-tri-O-benzyl-7-deoxy-α-D-glycero-D-glucoheptapyranoside (**19**)

Crude mesylate **18** (0.60 g, 1.08 mmol) was dissolved in dry N,N-dimethyl formamide (DMF) (5mL), anhydrous sodium benzoate (1.01 g, 7.0 mmol) was added and the reaction mixture was heated at 120°C and stirred for 17 hrs.. The cooled reaction mixture was then dissolved in dichloromethane (50 mL), washed with water (6 x 75 mL) and dried over anhydrous magnesium sulfate. The solvent was evaporated and the residue was chromatographed on HPLC (solvent 10:1 n-hexane / acetone) to give **19** (0.294 g, 47%) as a syrup. $[\alpha]_D$=9.8° (c 1.04, $CHCl_3$).

^{1}H-NMR ($CDCl_3$): δ 8.40—7.20 (m, 20H, Ar-H), 5.47 (dq, 1H, H-6, $J_{5,6}$=2.0 Hz, $J_{6,7}$=7.0 Hz), 5.03—4.65 (m, 6H, 3 x Ph-CH_2), 4.64 (d, 1H, H-1, $J_{1,2}$=4.0 Hz), 4.04 (dd, 1H, H-3, $J_{2,3}$=9.5 Hz, $J_{3,4}$=9.0 Hz), 3.93 (dd, 1H, H-5, $J_{5,6}$=2.0 Hz, $J_{5,4}$=10.0 Hz), 3.52 (dd, 1H, H-2, $J_{1,2}$=4.0 Hz, $J_{2,3}$=9.5 Hz), 3.42 (dd, 1H, H-4, $J_{3,4}$=9.0 Hz, $J_{4,5}$=10.0 Hz), 3.38 (s, 3H, OCH_3), 1.18 (d, 3H, C_6-CH_3, $J_{6,7}$=7.0 Hz). ^{13}C-NMR ($CDCl_3$): δ 165.76 ($\underline{C}$=O); 136.66—127.75 (Ar. Carbons); 97.80 (C-1); 82.44, 80.16, 77.82, 71.39, 70.12 (C-2—C-6); 75.92, 74.62, 73.41 (3 x Ph-$\underline{C}H_2$), 54.99 (O$\underline{C}H_3$); 13.57 (C-7).

Anal. calc. for $C_{36}H_{38}O_7$:	C 74.20	H 6.57	
found:	C 74.33	H 6.55	

Methyl 2,3,4-tri-O-benzyl-7-deoxy-α-D-glycero-D-glucoheptapyranoside **(10)**

Compound **19** (137 mg, 0.24 mmol) was dissolved in dioxane (10 mL) and 1N sodium hydroxide solution (6 mL) was added. The reaction mixture was stirred at 60°C and t.l.c. analysis showed that the reaction was over after 48 hrs. (R_f **19**=0.85, R_f **10**=0.43, solvent 3:2 n-hexane:EtOAc). The cooled mixture was then diluted with dichloromethane (50 mL) and filtered over a pad of silica gel-G. The dichloromethane solution was washed with water (2 x 50 mL), saturated sodium bicarbonate solution (50 mL), water (50 mL) and dried over anhydrous magnesium sulfate. The solvent was then removed and the residue solidified slowly under vacuum to give **10** (80 mg, 71%). Mp 89-90°C, $[\alpha]_D$=28° (c 0.4, $CHCl_3$).

^{1}H-NMR ($CDCl_3$): δ 7.34—7.16 (m, 15H, Ar-H), 4.98—4.53 (m, 6H, 3 x Ph-CH_2), 4.50 (d, 1H, H-1, $J_{1,2}$=3.5 Hz), 3.95 (dd, 1H, H-3, $J_{2,3}$=9.5 Hz, $J_{3,4}$=9.0 Hz), 3.88 (m, 1H, H-6), 3.55 (dd, 1H, H-5, $J_{4,5}$=10.0 Hz, $J_{5,6}$=4.5 Hz), 3.43 (dd, 1H, H-2, $J_{1,2}$=3.5 Hz, $J_{2,3}$=9.5 Hz), 3.35 (dd, 1H, H-4, $J_{3,4}$=9.0 Hz, $J_{4,5}$=10.0 Hz), 3.31 (s, 3H, OCH_3), 2.49 (d, 1H, OH, $J_{6,OH}$=6.0 Hz), 1.05 (d, 3H, C_6-CH_3, $J_{6,7}$=6.5 Hz). ^{13}C-NMR ($CDCl_3$): δ 138.61—127.73 (Ar. Carbons); 97.64 (C-1); 82.38, 80.29, 80.01 (3 x Ph-$\underline{C}H_2$); 75.75, 74.81, 73.37, 72.31 (C-2—C-5); 68.37 (C-6); 55.16 ($O\underline{C}H_3$), 17.81 (C-7).

Anal. calc. for $C_{29}H_{34}O_6$: C 72.78 H 7.16

found: C 72.81 H 7.07

Methyl 7-deoxy-α-D-glycero-D-glucoheptapyranoside **(21)**

To a solution of **10** (70 mg, 0.15 mmol) in methanol (12 mL), 5% palladium-on-charcoal (74 mg) was added in small portions. The mixture was shaken with hydrogen (100 lb. in^{-2}) at room temperature for 6 hrs. The reaction mixture was then filtered over celite (washed with methanol). The solvent was evaporated and the residue was coevaporated

with toluene (2 x 25 mL) and dried under vacuum. The residue solidified under vacuum to give **21** (30 mg, 98%). Mp 135-136°C, $[\alpha]_D = 147.8^\circ$ (c 1.0, H_2O).

^{1}H-NMR (D_2O): δ 4.68 (d, 1H, H-1, $J_{1,2}$=4.0 Hz), 4.04 (dq, 1H, H-6, $J_{5,6}$=2.5 Hz, $J_{6,7}$=6.0 Hz), 3.56 (dd, 1H, H-5, $J_{5,6}$=2.5 Hz, $J_{4,5}$=10.0 Hz), 3.53 (dd, 1H, H-3, $J_{3,4}$=9.0 Hz, $J_{2,3}$=10.0 Hz), 3.44 (dd, 1H, H-2, $J_{1,2}$=4.0 Hz, $J_{2,3}$=10.0 Hz), 3.23 (dd, 1H, H-4, $J_{4,5}$= 10.0 Hz, $J_{3,4}$=9.0 Hz), 3.29 (s, 3H, OCH_3), 1.10 (d, 3H, C_6-CH_3, $J_{6,7}$=6.0 Hz). ^{13}C-NMR (D_2O): δ 99.22 (C-1); 73.37, 73.32 (C-2 and C-5); 71.27, 70.93 (C-3 and C-6); 66.34 (C-4); 54.96 ($O\underline{C}H_3$), 15.05 (C-7).

Anal. calc. for $C_8H_{16}O_6$:	C	46.15	H	7.75
found:	C	45.99	H	7.50

Methyl 6'-O-acetyl-2,2',3,3',4,4'-hexa-O-benzyl-7-deoxy-6-O-(α-D-glucopyranosyl)-β-L-glycero-D-glucoheptapyranoside (**38**)

Methyl 2,3,4-tri-*O*-benzyl-7-deoxy-β-L-*glycero*-D-glucoheptapyranoside (**9**) (100 mg, 0.209 mmol) was dissolved in dichloromethane (10 mL). Then tetraethylamonium bromide (76 mg), dried molecular sieves (BDH, 4 A, 1 g) and dried DMF (1.4 mL) was added and the mixture was bubbled with nitrogen to reduce the volume. Then freshly prepared 6-O-acetyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyl bromide (**31**) (2 eq.) in dichloromethane (6 mL) was added and the mixture was stirred at room temperature under nitrogen. The reaction progress was followed by t.l.c. until completed (R_f **38**=0.69, R_f **24**=0.55, solvent 3:2 n-hexane / ethyl acetate). After the reaction was over, the partially dry mixture was dissolved in dichloromethane (25 mL), filtered to remove undissolved materials, washed with water (2x50 mL), saturated sodium bicarbonate solution (2x50 mL), water (2x50 mL) and dried over magnesium sulfate. Dichloromethane was evaporated and the residue was chromatographed with silica gel (solvent 4:1 n-hexane /

ethyl acetate) to give the desired disaccharide **38** as an oil which was dried under vacuum with heating (101 mg, 51%). $[\alpha]_D=0.54^o$ (c 1.06, $CDCl_3$).

^{1}H-NMR ($CDCl_3$): δ 7.40—7.20 (m, 30H, Ar-H), 5.09 (d, 1H, H-1′, $J_{1',2'}=3.5$ Hz), 5.02—4.49 (m, 7H, 3 x Ph-CH_2 and H-1), 4.26—4.06 (m, 3H, 2 x H-6′ and H-6), 4.06—3.94 (q and m, 3H, H-3′, H-3 and H-5′), 3.77 (dd, 1H, H-4, $J_{3,4}=9.5$ Hz, $J_{4,5}=9.0$ Hz), 3.56 (dq, 1H, H-5, $J_{4,5}=9.0$ Hz, $J_{5,6}=2.0$ Hz), 3.56—3.47 (m, 2H, H-2′ and H-2), 3.46 (dd, 1H, H-4′, $J_{3',4'}=9.0$ Hz, $J_{4',5'}=10.0$ Hz), 3.38 (s, 3H, OCH_3), 1.92 (s, 3H, CH_3COO-), 1.33 (d, 3H, C_6-CH_3, $J_{6,7}=6.0$ Hz). ^{13}C-NMR ($CDCl_3$): δ 170.75 (C=O); 138.81 (t-Carbons of Ph.); 128.47—127.39 (Ph. Carbons); 97.90 (C-1′); 95.20 (C-1); 75.72, 75.53, 75.08, 74.32, 73.40, 73.00 (6 x Ph-CH_2); 82.56, 81.60, 80.19 (3 x C); 77.73, 77.48, 73.22, 71.69, 69.19 (9 x C: C-2, C-2′, C-3, C-3′, C-4, C-4′, C-5, C-5′, C-6); 63.24 (C-6′), 55.09 (OCH_3), 20.01 ($COOCH_3$), 16.31 (C-7)

Anal. calc. for $C_{57}H_{64}O_{12}$:	C 73.09	H 6.77
found:	C 72.84	H 6.85

Methyl 2,2′,3,3′,4,4′-hexa-O-benzyl-7-deoxy-6-O-(α-D-glucopyranosyl)-β-L-glycero-D-glucoheptapyranoside (**39**)

Methyl 6′-*O*-acetyl-2,2′,3,3′,4,4′-hexa-*O*-benzyl-7-deoxy-6-*O*-(α-D-glucopyranosyl)-β-L-*glycero*-D-glucoheptapyranoside (**38**) (293 mg, 0.307 mmol) was dissolved in dry methanol (10 mL). A catalytic amount of sodium methoxide was added to the solution and the solution was stirred at room temperature overnight. The reaction progress was followed by t.l.c. (R_f **39**=0.49, R_f **38**=0.72, solvent 3:2 n-hexane / ethyl acetate). When the reaction was completed, resin (IRC-50-H^+, washed with distilled water and methanol) was added and stirred until the mixture turned acidic (pH paper). Then the mixture was filtered and the solvent was evaporated to give a foamy product **39** which was dried under

vacuum (269 mg, 96%). $[\alpha]_D$=47.6° (c 1.99, $CHCl_3$).

1H-NMR ($CDCl_3$): δ 7.40—7.10 (m, 30H, Ph-H), 5.03 (d, 1H, H-1′, $J_{1',2'}$=3.5 Hz), 5.01—4.57 (m, 13H, 6 x Ph-CH_2 and H-1), 4.32 (dq, 1H, H-6, $J_{5,6}$=1.5 Hz, $J_{6,7}$=6.5 Hz), 4.01 (dd, 1H, H-3′, $J_{2',3'}$=9.5 Hz, $J_{3',4'}$=9.0 Hz), 3.98 (dd, 1H, H-3, $J_{2,3}$=9.5 Hz, $J_{3,4}$=10.0 Hz), 3.84—3.72 (m, 2H, H-5′ and H-4), 3.66—3.49 (m, 5H, 2 x H-6, H-5, H-4′ and H-2), 3.46 (dd, 1H, H-2′, $J_{1',2'}$=3.5 Hz, $J_{2',3'}$=9.5 Hz), 3.36 (s, 3H, OCH_3), 1.32 (d, 3H, C_6-CH_3, $J_{6,7}$=6.5 Hz). ^{13}C-NMR ($CDCl_3$): δ 138.84—138.27 (t-Ph. Carbons); 128.46—127.37 (Ph. Carbons); 97.88 (C-1′); 95.39 (C-1); 82.54, 81.52, 80.19, 80.14, 77.64, 73.13, 71.84, 71.31 (9 Carbons: C-2, C-2′, C-3, C-3′, C-4, C-4′, C-5, C-5′, C-6); 61.72 (C-6′); 55.09 (OCH_3); 16.40 (C-7).

Anal. calc. for $C_{56}H_{62}O_{11}$:	C	73.80	H	6.86
found:	C	73.20	H	6.95

Methyl 7-deoxy-6-O-(α-D-glucopyranosyl)-β-L-glycero-D-glucoheptapyranoside (**40**)

Methyl 2,2′,3,3′,4,4′-hexa-*O*-benzyl-7-deoxy-6-*O*-(α-D-glucopyranosyl)-β-L-*glycero*-D-glucopyranoside (**39**) (126 mg, 0.138 mmol) was dissolved in dry methanol (12.5 mL). Then 5% palladium-on-charcoal (126 mg) was added in small portions to the solution and the mixture was shaken under hydrogen (100 lb. in^{-2}) at room temperature. The reaction progress was followed by t.l.c. (solvent 4:1 dichloromethane / methanol). When the reaction was completed the mixture was filtered and the filtrate was evaporated to give a yellowish solid residue. The crude residue was chromatographed (Iatrobeads, solvent 4:1 dichloromethane / methanol) to give colorless crystalline **40** (40 mg, 78%). Mp 127-128°C, $[\alpha]_D$=175.3° (c 0.26, D_2O).

^{1}H-NMR (D_2O): δ 5.09 (d, 1H, H-1′, $J_{1',2'}$=3.5 Hz), 4.28 (broaden q, 1H, H-6), 3.69 (t, 1H, H-3′), 3.64 (broad d, 1H, H-5), 3.56 (dd, 1H, H-2′, $J_{1',2'}$=3.5 Hz, $J_{2',3'}$=9.5 Hz),

3.89—3.52 (m, 10H, H-5′, 2 x H-6′, H-3, H-3′, H-4, H-5, H-5′, H-2, H-2′), 3.43 (t, 4H, OCH_3 and H-4′). ^{13}C-NMR (D_2O): δ 100.03 (C-1′); 95.56 (C-1); 74.49, 74.15, 73.68, 73.04, 72.25, 71.99, 70.24, 68.50 (9 Carbons: C-2, C-2′, C-3, C-3′, C-4, C-4′, C-5, C-5′, C-6); 61.17 (C-6′), 55.67 (OCH_3), 14.76 (C-7).

Anal. calc. for $C_{14}H_{25}O_{11}$: C 45.53 H 6.82

found: C 42.41 H 6.61

Methyl 6′-O-acetyl-2,2′,3,3′,4,4′-hexa-O-benzyl-7-deoxy-6-O-(α-D-glucopyranosyl)-α-D-glycero-D-glucoheptapyranoside (**41**)

Compound **10** (500 mg, 1.04 mmol) was dissolved in dichloromethane (5 mL). Tetraethyl ammonium bromide (420 mg), dry molecular sieves (BDH 4A, 5 g) and DMF (1 mL) was added to the solution and the mixture was placed under nitrogen atmosphere. Then 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl bromide (**31**, 2 eq.) in dichloromethane (5 mL) was added and the mixture was stirred at room temperature under nitrogen. The reaction progress was followed by t.l.c. (R_f **41**=0.59, R_f **10**=0.26solvent system 3:2 n-hexane / ethyl acetate). When the reaction was completed, dichloromethane (100 mL) was added and the mixture was filtered. The dichloromethane solution was washed with saturated sodium carbonate solution (100 mL), water (4 x 100 mL) and dried over magnesium sulfate. The solvent was then evaporated to give an oily residue which was chromatographed with silica gel (solvent 4:1 n-hexane / ethyl acetate) to give the desired product **41** (587 mg, 59%). $[\alpha]_D$=78.2° (c 1.06, $CDCl_3$).

^{1}H-NMR ($CDCl_3$): δ 7.40—7.20 (m, 30, Ph-H), 5.00 (d, 1H, H-1′, $J_{1',2'}$=3.5 Hz), 4.67 (d, 1H, H-1, $J_{1,2}$=3.5 Hz), 5.03—4.51 (m, 12H, 6 x Ph-CH_2), 4.27 (dd, 1H, H-6′, $J_{5',6'}$=4.5 Hz, $J_{6'a,6'b}$=– 11.5 Hz), 4.21 (dd, 1H, H-6′b, $J_{5',6'b}$=2.5 Hz, $J_{6'b,6'a}$=– 11.5 Hz), 4.08—3.91 (m, 4H, H-3, H-3′, H-6, H-5′), 3.78 (dd, 1H, H-5, $J_{5,6}$=0.5 Hz, $J_{4,5}$=10.0 Hz),

3.57—3.43 (m, 3H, H-4, H-4', H-2), 3.41 (dd, 1H, H-2', $J_{1',2'}$=3.5 Hz, $J_{2',3'}$=9.5 Hz), 3.29 (s, 3H, OCH_3), 1.99 (s, 3H, $OCOCH_3$), 1.16 (d, 3H, C_7H_3, $J_{6,7}$=6.5 Hz). ^{13}C-NMR ($CDCl_3$): δ 170.74 (C=O), 138.85—137.97 (t-Ph. Carbons); 97.76 (C-1' and C-1); 82.61, 81.76, 80.25, 80.03, 77.94, 77.64, 72.83, 72.57, 69.08 (9 Carbons: C-2, C-2', C-3, C-3', C-4, C-4', C-5, C-5', C-6), 63.27 (C-6), 55.13 (OCH_3); 20.84 ($OCOCH_3$), 16.55 (C-7)

Anal. calc. for $C_{58}H_{64}O_{12}$: C 73.09 H 6.77

found: C 73.46 H 6.61

Methyl 2,2',3,3',4,4'-hexa-O-benzyl-7-deoxy-6-O-(α-D-glucopyranosyl)-α-D-glycero-D-glucopyranoside (**42**)

Methyl 6'-*O*-acetyl-2,2',3,3',4,4'-hexa-*O*-benzyl-7-deoxy-6-*O*-(α-D-glucopyranosyl)-α-D-*glycero*-D-glucoheptapyranoside (**41**) (440 mg, 0.462 mmol) was dissolved in dry methanol (20 mL). A catalytic amount of sodium methoxide was added to the solution and the solution was stirred at room temperature overnight. The reaction progress was followed by t.l.c. (solvent system 3:2 n-hexane:ethyl acetate, R_f **42**=0.42). When the reaction was over, resin (IRC-50-H, washed with water and methanol) was added and stirred until the mixture turned acidic (pH paper). Then the mixture was filtered and the solvent was evaporated to give an oily residue which was dried under vacuum overnight to give **42** (347 mg, 83%). $[\alpha]_D$=65.2° (c .34, H_2O).

^{1}H-NMR ($CDCl_3$): δ 7.40—7.20 (m, 30H, Ph-H), 5.00 (d, 1H, H-1', $J_{1',2'}$=4.5 Hz), 4.98—4.53 (m, 13H, 6 x Ph-CH_2 and H-1), 4.08—3.91 (m, 3H, H-3, H-3' and H-6), 3.81—3.67 (m, 4H, H-5, H-5' and 2 x H-6'), 3.56—3.37 (m, 4H, H-4, H-4', H-2 and H-2'), 3.29 (s, 3H, OCH_3), 1.13 (d, 3H, C_6-CH_3, $J_{6,7}$=6.5 Hz). ^{13}C-NMR ($CDCl_3$): δ 138.85—138.27 (t-Ph. Carbons); 129.07—125.33 (Ph. Carbons); 97.89 (C-1'); 95.37 (C-1); 82.53, 81.52, 80.19, 77.64, 77.57, 73.14, 71.43, 71.32 (9 Carbons: C-2, C-2', C-3, C-3', C-4, C-4', C-5, C-5', C-6); 61.73 (C-6'); 55.09 (OCH_3), 16.38 (C-7).

Anal. calc. for $C_{56}H_{62}O_{11}$: C 73.82 H 6.86

found: C 73.23 H 7.23

Methyl 7-deoxy-6-O-(α-D-glucopyranosyl)-α-D-glycero-D-glucoheptapyranoside (**43**)

Compound **42** (247 mg, 0.27 mmol) was first dissolved in dry methanol (25 mL). Then 5% palladium-on-charcoal (254 mg) was added in small portions to the solution and the mixture was shaken under hydrogen (100 lb. in^{-2}) at room temperature and the reaction progress was followed by t.l.c. (solvent 4:1 dichloromethane / methanol). When the reaction was over, the mixture was filtered and the filtrate was evaporated to give a yellowish solid residue. The crude residue was chromatographed (Iatrobeads, solvent 4:1 dichloromethane / methanol) to give colorless crystalline **43** (80 mg, 80%). Mp 231-232°C. $[\alpha]_D = 228.1^o$ (c .43, H_2O).

^{1}H-NMR (D_2O): δ 4.80 (d, 1H, H-1, $J_{1,2}$=4.0 Hz), 3.57 (dd, 1H, H-2, $J_{1,2}$=4.0 Hz, $J_{2,3}$=9.5 Hz), 3.67 (dd, 1H, H-3, $J_{2,3}$=9.5 Hz, $J_{3,4}$=9.0 Hz), 3.39 (dd, 1H, H-4, $J_{4,5}$=10.5 Hz, $J_{4,3}$=9.0 Hz), 3.83 (dd, 1H, H-5, $J_{4,5}$=10.5 Hz, $J_{5,6}$=2.5 Hz), 4.17 (dq, 1H, H-6, $J_{5,6}$=2.5 Hz, $J_{6,7}$=6.5 Hz), 3.44 (s, 3H, OCH_3), 1.29 (d, 3H, C_6-CH_3, $J_{6,7}$=6.5 Hz), 5.07 (d, 1H, H-1′, $J_{1',2'}$=3.5 Hz), 3.55 (dd, 1H, H-2′, $J_{1',2'}$=3.5 Hz, $J_{2',3'}$=9.5 Hz), 3.73 (dd, 1H, H-3′, $J_{2',3'}$=9.5 Hz, $J_{3',4'}$=10.0 Hz), 3.41 (dd, 1H, H-4′), 3.88 (dd, 1H, H-6′a, $J_{6'a,6'b}$= – 14.5 Hz, $J_{6'a,5'}$=5.0 Hz), 3.76 (dd, 1H, H-6′b, $J_{6'a,6'b}$= – 14.5 Hz, $J_{6'b,5'}$=4.0 Hz). ^{13}C-NMR (D_2O): δ 100.13 (C-1′); 97.98 (C-1); 73.89, 73.73, 73.37, 72.98, 72.02, 71.89, 71.80, 71.48 (C-2—C5 and C-2′—C5′); 70.51 (C-6′), 56.09 (OCH_3), 15.12 (C-7).

Anal. calc. for $C_{14}O_{11}H_{25}$: C 45.53 H 6.82

found: C 45.18 H 6.97

3. Enzyme hydrolysis experiments

All enzyme hydrolysis experiments were performed on the Cary model 214 and the Bruker WM-360 spectrometer operating at 360 MHz at preset temperatures. The buffers used were potassium phosphate buffer at pH=6.8, M.E.S. buffer at pH=6.5, sodium acetate–acetic acid in D_2O at pD=4.3 sodium acetate-d_3–acetic acid-d_4 in D_2O at pD=4.3. The enzyme hydrolysis data were analyzed by Dr. M. Palcic and Mr. L. Steele in the Food Science Department on the PC using computer programs developed by Professor K.Bock and co-workers from the Department of Organic Chemistry, the Technical University of Denmark. The enzyme Isomaltase, ATP and NADP were purchased from Aldrich. The enzymes Hexokinase and 6-Phosphate Dehydrogenase were provided by Dr. M. Palcic. AMG type II was a gift from Professor K. Bock.

The method, as recommended by Bock,[85] is the application of high resolution ^{1}H-NMR spectroscopy to monitor substrate and product time depencencies in progress curve enzyme kinetics.[112-116] This method has an advantage since kinetic parameters can be estimated in a single run therefore saving both time and substrate material. The disadvantages are: (i) in general, mechanistic properties cannot be inferred directly from a single progress curve, and (ii) errors in the estimated parameters may be significant, depending on the experimental conditions and the method of data analysis.

The data were obtained as follows. The ^{1}H-NMR spectrum of the substrate in buffered solution was taken as standard at t=0. Then the enzyme in buffered solution was added and the reaction progress was followed by recording ^{1}H-NMR spectra at predetermined times until the reaction was over. The time dependent spectra were plotted and the concentration of the substrate and the product(s) were obtained from the integrations of the appropriate peaks. The concentration of substrate vs. time progress curve was constructed and analyzed on IBM personal computers using a non-linear least squares

fitting program package REGGRAFA obtained from Professor Bock. The time dependent concentration data are summarized in the next section.

The progress curve analysis[85] is based on the integrated and reparameterized Michaelis-Menten equation (Equation 5).

$$t = (\chi\,|y_o - y'|/v_o)\,A\,[\chi + \ln(1-\chi)] \qquad (5)$$

Where $\chi = (y_o - y)/(y_o - y')$

$A = (2v_o t_{1/2} - |y_o - y'|)/(v_o(1 - \ln 4))$

v_o = initial rate

$t_{1/2}$ = half time ($y(t_{1/2}) = 1/2[y_o - y']$

y_o = initial concentration

y' = final concentraion

The χ is the degree of progressed reaction and it followed from the definition above that χ always be in the interval $0 < \chi < 1$.

Equation 5 is then rearranged to Equation 6 to calculate the values of concentration (dependent variable) as a function of time (independent variable).

$$y = (y' + (y_o - y'))\exp[((v_o t - \chi\,|y_o - y'|)/Av_o) - \chi] \qquad (6)$$

The equation 6 is solved by computer by the bisection method.

All experiments with methyl α-isomaltoside (**37**), methyl 6-*S*-methyl-α-isomaltoside (**40**) and methyl 6-*R*-methyl-α-isomaltoside (**43**) were performed under exactly the same conditions.

VII. APPENDIX

Table 18. The time dependent concentration data for methyl α-isomaltoside (**37**) in the course of hydrolysis catalyzed by AMG II.

Time (min.)	**Conc.** (mM)
0.00	18.52
9.18	18.31
23.83	17.46
47.47	16.15
71.30	15.96
95.03	15.41
118.77	14.09
142.50	12.89
166.23	12.85
189.97	11.91
213.70	9.69
237.34	9.57
261.17	8.28
284.90	7.39
308.63	6.85

Table 19. The time dependent concentration data for methyl α-isomaltoside (**37**) in the course of hydrolysis catalyzed by AMG II.

Time (min.)	**Conc.** (mM)
0.00	9.26
31.33	8.38
55.13	7.37
78.93	7.10
102.73	5.94
126.53	5.78
150.33	5.03
174.13	4.39
197.93	3.56
221.73	3.02
245.53	2.80
269.33	2.28
293.13	1.76
352.63	1.03
412.13	0.75
471.63	0.19

Table 20. The time dependent concentration data for methyl 6-*S*-methyl-α-isomaltoside (**40**) in the course of hydrolysis catalyzed by AMG II.

Time (min.)	**Conc.** (mM)
0.00	9.26
8.20	8.67
47.93	8.71
97.60	8.03
147.27	7.45
196.93	7.29
246.60	6.18
296.27	5.46
345.93	5.55
395.27	5.40
444.93	4.82
494.60	4.61
544.27	4.61
593.93	4.17
643.60	3.88
693.27	3.73
742.93	3.33
792.60	3.12
843.27	2.95
892.93	2.76
894.93	2.69
934.66	2.56
984.33	2.34
1092.00	2.31
1105.60	2.15
1145.35	1.97
1195.00	1.92
1244.70	1.83
1294.35	1.41
1344.00	1.56
1443.00	1.56
1483.00	1.37
1727.00	1.07
1816.00	0.89
1916.00	0.93
2015.00	0.70
2114.00	0.67
2213.00	0.84
2313.00	0.59
2412.00	0.59
2512.00	0.41
2612.00	0.30

Table 21. The time dependent concentration data for methyl 6-*S*-methyl-α-isomaltoside (**40**) in the course of hydrolysis catalyzed by AMG II.

Time (min.)	**Conc.** (mM)
0.00	18.52
57.00	17.28
118.42	15.89
179.83	15.09
241.25	13.80
302.67	12.96
364.08	11.93
425.50	11.00
468.42	10.28
548.33	9.52
609.75	9.09
780.17	7.28
829.58	6.83

Table 22. The time dependent concentration data for methyl 6-*R*-methyl-α-isomaltoside (**43**) in the course of hydrolysis catalyzed by AMG II.

Time (min.)	**Conc.** (mM)
0.00	18.52
8.33	17.96
20.33	17.31
32.33	16.80
44.33	16.07
56.33	15.26
68.33	14.61
80.33	13.85
92.33	13.70
104.33	11.96
116.33	11.37
128.33	10.37
140.33	9.69
147.38	9.61
159.38	8.57
171.38	8.50
183.38	7.44
195.38	6.59
207.38	5.41
219.38	4.98
231.38	4.07
243.38	3.50
255.38	2.41
267.38	1.89
279.38	1.39
291.38	0.91
315.38	0.00

Table 23. The time dependent concentration data for methyl 6-*R*-methyl-α-isomaltoside (**43**) in the course of hydrolysis catalyzed by AMG II.

Time (min.)	**Conc.** (mM)
0.00	9.26
6.50	8.65
20.28	7.93
30.25	6.97
40.25	6.34
60.25	4.27
70.25	3.97
80.25	3.19
90.25	2.56
100.25	1.80
110.25	1.15
120.25	0.55
130.25	0

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tion data of methyl α-D-glucopyranoside.[76] The methyl groups were generated at C-6 and O-1 using the TRANSF.OBJ program. The ω' angle of the non-reducing unit was set at 180^{o} or 300^{o} as required and the ω angle of the reducing unit was rotated in 5^{o} or 10^{o} step using the GENT.OBJ program. HSEA calculations were performed for the methyl α-isomaltoside, the methyl 6-*S*-methyl-α-isomaltoside (**40**) and the methyl 6-*R*-methyl-α-isomaltoside (**43**) with the torsion angles ω' and ω set at the predetermined values mentioned above and the torsion angles Φ and Ψ were varied until the minimum energies were obtained. The results are shown in Table 11.

Table 11. HSEA calculations results of the methyl α-isomaltoside (**37**) and the diastereomeric 6-C-methyl α-isomaltosides (**40** and **43**).

Compounds	Torsional Angles, ^{o}					Internuclear Distances, A	
	ω'	Φ	Ψ	ω	**E** (Kcal/mol)	O-4–O-4′	O-4–O-6′
37	180	–50	195	180	–2.29	5.08	5.30
	300	–45	240	60	–1.90	4.51	4.90
	300	–50	190	180	–2.45	5.16	3.53
	300	–50	200	300	–1.74	8.25	6.41
40	180	–50	195	180	–1.46	5.10	5.30
	300	–50	180	300	–0.28	5.35	4.02
43	180	–50	90	300	–1.26	8.17	7.10
	300	–50	90	300	–1.81	8.17	5.68

Recalculation of the methyl α-maltoside in conformation obtained previously[8] where $\Phi/\Psi = -25^{o}/-15^{o}$ with $\omega' = 180^{o}$ and $\omega = 300^{o}$ gave an energy minimum of 1.35 Kcal. Because of the polar hydroxyl groups OH-3, OH-4′ and OH-6′ were involved in the hydrolyses catalyzed by the enzyme Amyloglucosidase Type II, the distances between these groups were also calculated for methyl α-maltoside and will be discussed in Section

IV.

HSEA calculations predicts that methyl α-isomaltoside (**37**) prefers the conformation with the torsion angles $\omega'/\Phi/\Psi/\omega = 300^{o}/-50^{o}/190^{o}/180^{o}$; methyl 6-*S*-methyl-$\alpha$-isomaltoside (**40**) prefers the conformation with the torsion angles $\omega'/\Phi/\Psi/\omega = 180^{o}/-50^{o}/195^{o}/180^{o}$ and methyl 6-*R*-methyl-α-isomaltoside (**43**) prefers the conformation with the torsion angles $\omega'/\Phi/\Psi/\omega = 180^{o}/-50^{o}/90^{o}/300^{o}$. The minimum energy conformations of **40** and **43** were plotted and reproduced in Figure 35 and 36 (p. 78 and 79, respectively).

The torsion angles $\omega = 180^{o}$ and 300^{o} for **40** and **43** correspond to the $\mathbf{g}^{-}$ and $\mathbf{g}^{+}$ conformers respectively for the C-5/C-6 bond in the reducing unit. The HSEA calculations therefore predicts that the conformation at the C-6 of the reducing unit will be retained for the methyl 6-*S*-methyl-α-isomaltoside (**40**) and methyl 6-*R*-methyl-α-isomaltoside (**43**).

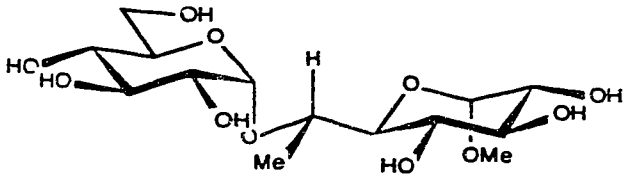
2.4. Conclusions

From conformational analysis of the monosaccharide derivatives, it was concluded that at the C-6 center, methyl 6-*S*-methyl-α-D-glucopyranoside (**15**) preferred the $\mathbf{g}^{-}$ rotamer and methyl 6-*R*-methyl-α-D-glucopyranoside (**21**) preferred the $\mathbf{g}^{+}$ rotamer. The conformations are also favored even at higher temperatures. It was expected that these conformations would also be favored in the disaccharide derivatives. The conformations at the C-6 center of the diastereoisomeric methyl 6-*S*-methyl-α-isomaltoside (**40**) and methyl 6-*R*-methyl-α-isomaltoside (**43**) were determined to be mainly $\mathbf{g}^{-}$ and $\mathbf{g}^{+}$ respectively.

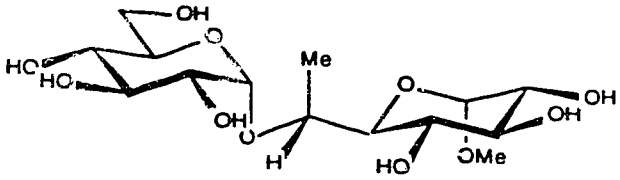
IV. ENZYME STUDIES

1. The Enzymes

Two enzymes Isomaltase and Amyloglucosidase Type II were used in kinetic studies of the hydrolysis of the methyl α-isomaltoside (**37**) and its C-6 methyl derivatives; namely methyl 6-*S*-methyl-α-isomaltoside (**40**) and methyl 6-*R*-methyl-α-isomaltoside derivatives (**43**).



40



43

The enzyme Isomaltase (EC 3.2.1.10)[78-83] is found in the bean, potato,[81] autolyzed brewer's yeast[82] and muscle.[83] This enzyme is used especially to cleave the α-D-(1→6) linkage and therefore plays an important role in the depolymerization of amylopectins and glycogens. Because of this specificity, the enzyme Isomaltase was selected to be used in the hydrolysis studies.

The enzyme Amyloglucosidase (EC 3.2.1.3),[84-87] which is produced by the microorganism *Aspergillus niger*, consists of two forms: Amyloglucosidase Type I (AMG I) and Amyloglucosidase Type II (AMG II).[88,89] Both enzymes are reported to be glycoproteins containing D-mannose, D-glucose and D-galactose.[90] Although the enzymes have been characterized and the amino acid sequence has been determined,[26,87,90-100] the enzymes have not crystallized.

The AMG enzymes are used in the processing of starch because of their abilities to catalyze hydrolytic cleavage of both the α-D-(1→4) linkage between glucopyranose units such as is present in maltose and α-D-(1→6) linkage between glucopyranose units such as is present in isomaltose. Both enzymes are stable up to 60°C with little loss of activity for about 30 min., but are rapidly inactivated at higher temperature. The enzymes exhibit nearly identical pH profiles with starch as the substrate with the pH optimum at ~4.5. Both enzymes hydrolyze the α-D-(1→4) linkage approximately 40 times faster than the α-D-(1→6) linkage.[90]

Our attention was turned to the enzyme AMG II primarily because Bock and coworkers[26,97-100] had made a systematic study of the effects of substitutional changes on maltose on the kinetics of the hydrolysis in which the reactions were followed using ^{1}H-NMR techniques.[100] They found that the AMG II active site requires accommodation of both the D-glucopyranose units of maltose since the enzyme hydrolyzes methyl β-maltoside but not methyl β-D-glucopyranoside.

In the hydrolysis of the various derivatives catalyzed by AMG II, it was shown[99] that the enzyme can accept a wide range of modifications as well as larger substrates. The competitive experiments showed that inhibitions in the reaction of the methyl β-maltoside were observed with the 6-chloro- or 6-fluoro- derivatives. It was concluded that the 6-chloro- or 6-fluoro- derivatives were bound very tightly to the active site in order to inhibit the reactions.[99] Based on qualitative data for hydrolysis,[97] catalyzed by AMG II, of the mono- and several dideoxy derivatives of methyl β-maltoside, Bock *et al.*[97,99] concluded that three hydroxyl groups, OH-3 in the reducing glucopyranose unit, OH-4′ and OH-6′ in the non-reducing glucopyranose unit, are essential for AMG II catalyzed hydrolysis. These findings are depicted in Figure 37(a). Evidence for this conclusion was further supported when the pentadeoxy derivative (Figure 37(b)), in which only the key polar groups were present (OH-3, OH-4′ and OH-6′), was found to be a substrate for the

enzyme AMG II. The HSEA calculated minimum energy conformation of methyl α-maltoside,[101,103] which is supported by ^{1}H-NMR and ^{13}C-NMR data, presents hydrophobic area defined by H-2', H-4', H-1' and O-5' of the non-reducing unit and H-4 as well as the hydroxymethyl group of the reducing unit which is expected to possess substantial conformational rigidity. Both the three key hydroxyl groups and the hydrophobic area were regarded as essential for AMG II hydrolysis by Bock *et al.*[99] and are in support of the hydrated polar-group gate theory proposed by Lemieux.[104]

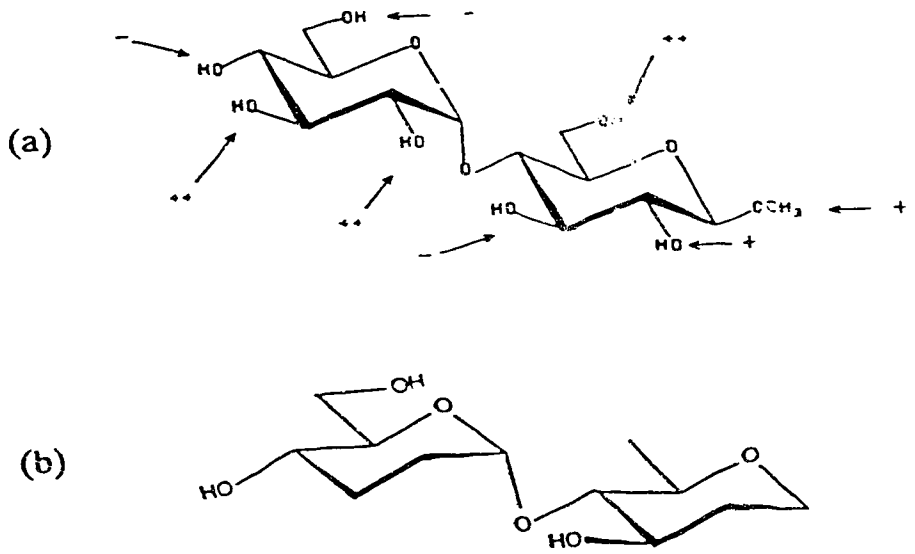


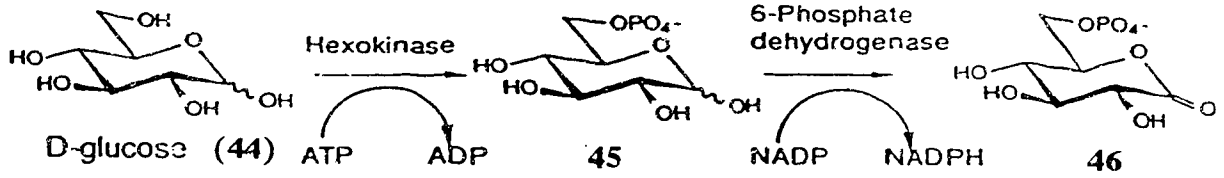
Figure 37. (a) Substrate specificity for Amyloglucosidase II (AMG II)[99] for different deoxy derivatives of maltose. + indicates substrate activity. – indicates no substrate activity and + + indicates compounds which are hydrolyzed faster than methyl β-maltoside. (b) Pentadeoxy derivative of maltose which preserves the key polar hydroxyl groups for hydrolysis catalyzed by AMG II.

2. Enzyme Kinetic Studies

2.1. Preliminary Studies

A collaboration was established with Dr. Monica Palcic of the Department of Food Science, University of Alberta for a kinetic study of the hydrolysis of **37**, **40** and **43**. Initially, it was thought that the most suitable method was to follow the reaction progress by using uv-vis spectroscopy.

The uv-vis spectroscopy technique involved the coupling method with the product(s) of the reaction. In the hydrolysis of methyl α-isomaltoside and the methyl 6-*C*-methyl-α-isomaltoside derivatives catalyzed by AMG II, the possible products of the hydrolysis reactions are D-glucopyranose and the 6-*C*-methyl-α-D-glucopyranose derivatives. The method employed for the detection of free glucopyranose (**44**)[105] released in the enzyme hydrolysis of the dissaccharides is shown in Scheme 21.



Scheme 21. Outline of the colorimetric coupled enzyme method for the determination of D-glucose.

The coupled-enzyme detects free glucopyranose by first a phosphorylation of the 6-hydroxyl by ATP in the presence of Hexokinase to produce D-glucopyranose-6-phosphate (**45**). This phosphate is then converted to the lactone (**46**) by 6-Phosphate Dehydrogenase in the presence of NADP (Scheme 21) which is detected due to the color change at $\lambda = 340$ nm as the result of the formation of the NADPH.

The monosaccharides D-glucopyranose (**44**), 6-*S*-methyl-D-glucopyranose (**47**) and 6-*R*-methyl-D-glucopyranose (**48**)[106] were then tested with Hexokinase and 6-Phosphate Dehydrogenase in the presence of ATP and NADP[105] and the results are shown in Table 12.

Table 12. Reaction of the monosaccharides derivatives with Hexokinase and 6-Phosphate Dehydrogenase in the presence of ATP and NADP.

Compounds	Reaction	Rel. Vel.
D-glucopyranose (**44**)	+	2.0
6-*S*-methyl-D-glucopyranose (**47**)[106]	-	–
6-*R*-methyl-D-glucopyranose (**48**)[106]	+	1.0

In contrast with D-glucopyranose (**44**) and 6-*R*-methyl-D-glucopyranose (**48**), the 6-*S*-methyl-D-glucopyranose (**47**) did not react. As discussed above in connection with Scheme 22, 6-Phosphate Dehydrogenase does not dehydrogenate D-glucopyranose. Instead the glucose must first be phosphorylated at the 6-OH position to produce **45**. Therefore it is possible the 6-*S*-methyl-D-glucopyranose resists phosphorylation at the 6-OH position. Conformational analyses presented in the previous section showed that both α-D-glucopyranose and 6-*R*-methyl-D-glucopyranose (**44** and **48**, respectively) prefer the $\mathbf{g}^+$ conformation for the orientation of the 6-OH whereas the $\mathbf{g}^-$ is preferred for the 6-*S*-methyl-D-glucopyranose (**47**) (see Figure 38).

Since it can be expected that the enzyme will phosphorylate the 6-hydroxyl of the glucopyranose in a specific conformation the reason for the high resistance to phosphorylation exhibit by the *S*-compound (**47**) can be attributed to the low abundance of the $\mathbf{g}^+$ conformer. However, it is possible that the 6-*C*-methyl group also contributes to the inactivity by hindering the formation of the enzyme-substrate complex.

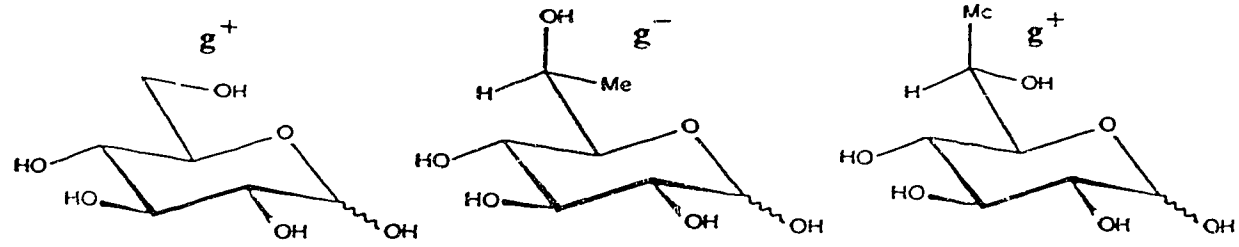


Figure 38. The preferred conformations of D-glucopyranose (**44**), 6-*S*-methyl-D-glucopyranose (**47**) and 6-*R*-methyl-D-glucopyranose (**48**)

In the hydrolyses reactions of the disaccharide derivatives, the various monosaccharides were used for standardization before the disaccharides were tested. Isomaltase and Amyloglucosidase Type II were used and the results of the preliminary studies are summarized in Table 13, 14 (the data are summarized in the Appendix).

The results presented in Table 13 and 14 were obtained with only one assay at fixed concentration (100 mM) per substrate. The rates (ΔOD min^{-1}) were only rough approximations because the objective was to see if a trend existed in the hydrolysis catalyzed by the enzyme that warranted further investigation.

Table 13. Hydrolysis catalyzed by Isomaltase at 37°C and pH=6.8.*

Compounds	Isomaltase hydrolysis	Rel. Rate (ΔOD min^{-1})
Phenyl α-D-glucopyranoside	+	39
Methyl α-D-glucopyranoside (**1**)	+	2
Methyl 6-*S*-methyl-α-D-glucopyranoside (**15**)	–	–
Methyl 6-*R*-methyl-α-D-glucopyranoside (**21**)	v. slight	–
Methyl α-isomaltoside (**37**)	+	1
Methyl 6-*S*-methyl-α-isomaltoside (**40**)	v. slight	–
Methyl 6-*R*-methyl-α-isomaltoside (**43**)	–	–

*(+) sign indicates that a reaction took place, a (—) sign indicates no reaction. Rel. Rate was measured in unit OD min^{-1}. Rel. Rate is the rate relative to compound **37**.

Table 14. Hydrolysis catalyzed by AMG II at 37°C and pH=4.3.*

Compounds	AMG Hydrolysis	Rel. Rate
Methyl α-D-glucopyranoside (**1**)	–	–
Methyl 6-*S*-methyl-α-D-glucopyranoside (**15**)	v. slight	1.4
Methyl 6-*R*-methyl-α-D-glucopyranoside (**21**)	v. slight	1
Isomaltose	+	67.5
Methyl α-isomaltoside (**37**)	+	81.3
Methyl 6-*S*-methyl-α-isomaltoside (**40**)	+	31.3
Methyl 6-*R*-methyl-α-isomaltoside (**43**)	+	112.5

*(+) sign indicates a reaction took place and a (–) sign indicates no reaction. Rel. Rate was measured in unit OD min^{-1}

As seen in Table 13, phenyl α-D-glucopyranoside, a known substrate for Isomaltase, is hydrolyzed 39 times faster than methyl α-isomaltoside (**37**) and about 20 times faster than methyl α-D-glucopyranoside (**1**). The monosaccharides **15** and **21** and the disaccharides **40** and **43** were shown not to be substrates for Isomaltase since there were no detectable or very slight rates in the hydrolysis.

In the case of hydrolysis catalyzed by AMG II (Table 14), methyl 6-*S*-methyl-α-D-glucopyranoside (**15**) was hydrolyzed 1.4 times faster than the methyl 6-*R*-methyl-α-D-glucopyranoside (**21**) and methyl α-D-glucopyranoside was not a substrate. It is seen that the disaccharides **40** and **43** proved to be substrates for this enzyme. The fact that the *R*-disaccharide **43** was superior to **37** as a substrate whereas **40** was less effective set the basis for this investigation.

2.2. The kinetic studies of the hydrolysis of methyl α-isomaltoside and 6-C-methyl- derivatives catalyzed by AMG II.

It was desirable to examine the hydrolysis of the methyl α-isomaltoside (**37**) and the methyl 6-*C*-methyl-α-isomaltoside (**40** and **43**) catalyzed by AMG II by the uv-vis spectroscopy. Unfortunately, the optimum pH required for the hydrolysis catalyzed by AMG II is 4.3 which is too acidic for the Hexokinase/6-Phosphate Dehydrogenase coupling assay (optimum pH=6.8). Furthermore, substrates which were synthesized in limited quantity may be required for further investigations. Since it was shown by Bock *et. al.*[26,97-101,106] that the progress of the hydrolysis of methyl β-maltoside and derivatives catalyzed by AMG II could be followed by ^{1}H-NMR spectroscopy, it was decided that further enzyme kinetic investigations would involved the use of ^{1}H-NMR spectroscopy. The method allows the observation of the disappearance of the disaccharide and the formation of the two monosaccharides simultaneously as a function of time. In the hydrolysis of methyl α-isomaltoside (**37**), it was possible to observe the decreasing signals of H-1′ at δ=4.95 ppm and H-1 at δ=4.85 ppm of the disaccharide and the increasing signals of H-1 of the products; namely, β-D-glucopyranose at δ=4.70 ppm and methyl α-D-glucopyranoside at δ=4.83 ppm simultaneously. For the methyl 6-*S*-methyl-α-isomaltoside (**40**), the disappearance of the broad quartet H-6 at δ=4.28 ppm of **40** and the appearance of the doublet of quartets H-6 of the methyl 6-*S*-methyl-α-D-glucopyranoside (**15**) at δ=4.05 ppm were also followed. For the methyl 6-*R*-methyl-α-isomaltoside (**43**), the disappearance of the doublet methyl signal at C-6 of compound **43** (δ=1.29 ppm) and the appearance of the doublet methyl signal of H-6 of methyl 6-*R*-methyl-α-D-glucopyranoside (**21**) at δ=1.10 ppm was also followed.

Representative spectra of the progress of the hydrolysis of the disaccharides; namely, methyl α-isomaltoside (**37**), methyl 6-*S*-methyl-α-isomaltoside (**40**) and methyl 6-*R*-methyl-α-isomaltoside (**43**) are reproduced in Figure 39, 40, and 41. Integrations of signals allowed

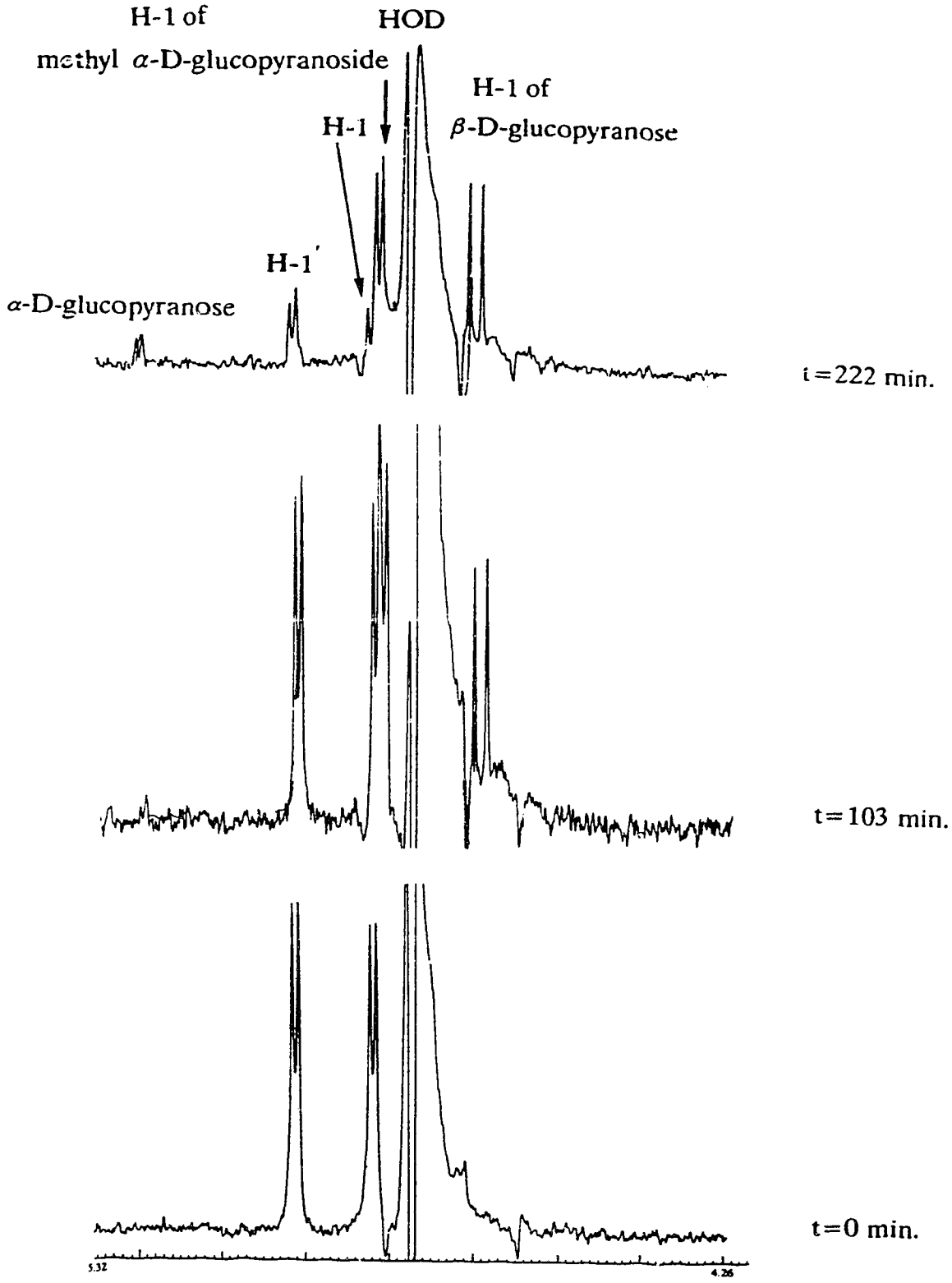


Figure 39. Reaction progress of the hydrolysis of methyl α-isomaltoside (**37**) catalyzed by AMG II with time.

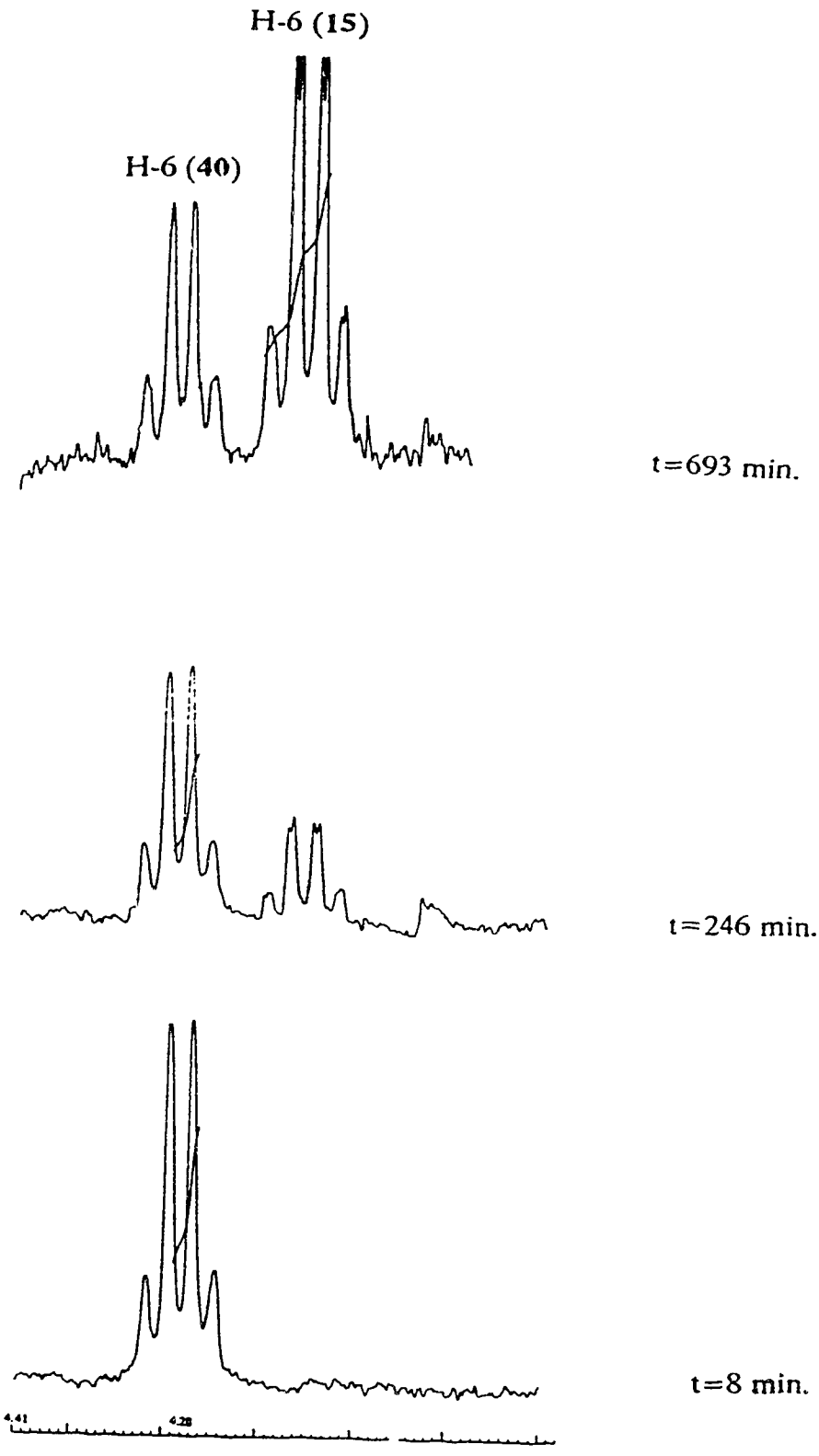


Figure 40. Reaction progress of the hydrolysis of methyl 6-S-methyl-α-isomaltoside (**40**) catalyzed by AMG II with time.

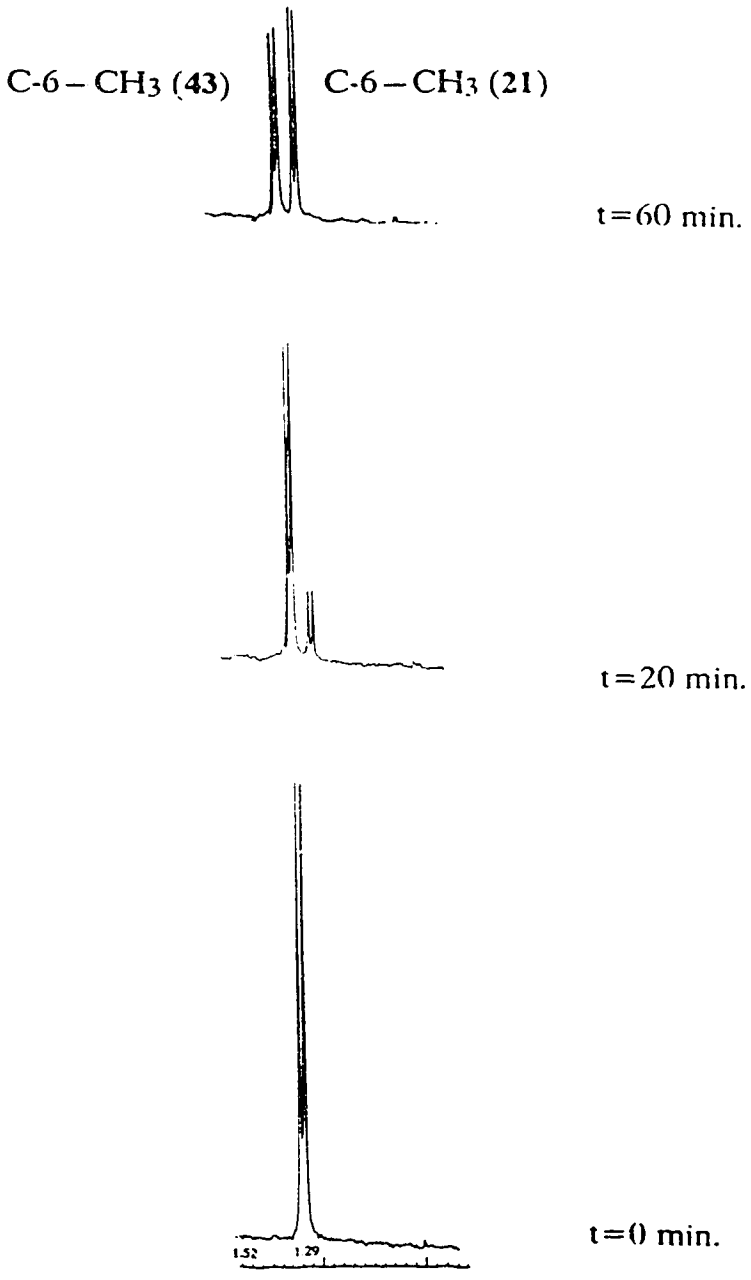


Figure 41. Reaction progress of the hydrolysis of methyl 6-R-methyl-α-isomaltoside (**43**) catalyzed by AMG II with time.

Table 15. Kinetic parameters for the disaccharide derivatives in the hydrolysis catalyzed by AMG II.

Substrate	Conc. (mM)	V_0 (mM/min)	$T_{1/2}$ (min)	V_{max} (mM/min)	K_{cat} (s^{-1})	K_m (mM)	K_{cat}/K_m ($M^{-1}s^{-1}$)
Methyl α-maltoside	9.26	1.6	3	1.6	2.6	—	—
Methyl β-maltoside	9.26	0.613	8.1	0.613	0.9	—	—
Methyl β-maltoside	18.52	0.727	14	0.727	1.2	—	—
Methyl 6-*S*-methyl-α-isomaltoside (**40**)	9.26	0.012	500	0.040	0.84	35	2.8
Methyl 6-*S*-methyl-α-isomaltoside (**40**)	18.52	0.027	575	—	—	—	—
Methyl α-isomaltoside (**37**)	9.26	0.033	161	0.043	0.069	2.9	23.8
Methyl α-isomaltoside (**37**)	18.52	0.04	247	0.04	0.064	2.2×10^{-4}	—
Methyl 6-*R*-methyl-α-isomaltoside (**43**)	9.26	0.081	63	0.083	0.13	0.22	600
Methyl 6-*R*-methyl-α-isomaltoside (**43**)	18.52	0.068	148	0.0688	0.11	0.22	500

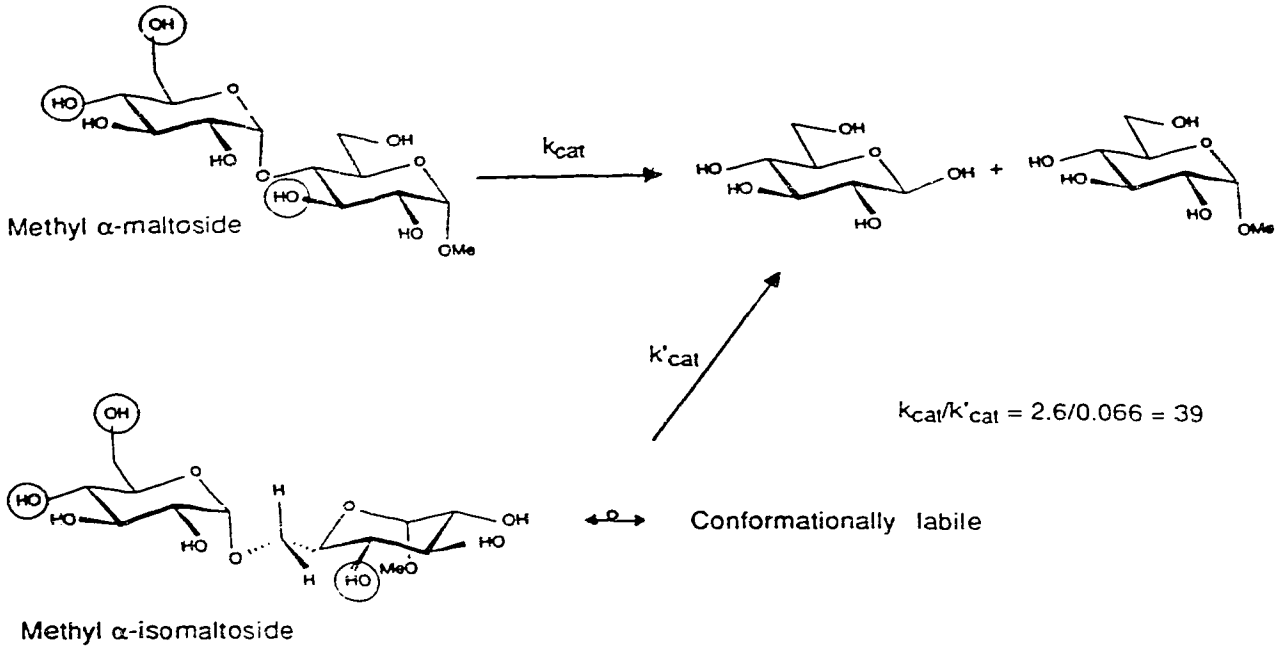


Figure 42. Methyl α-maltoside and methyl α-isomaltoside hydrolysis catalyzed by AMG II.

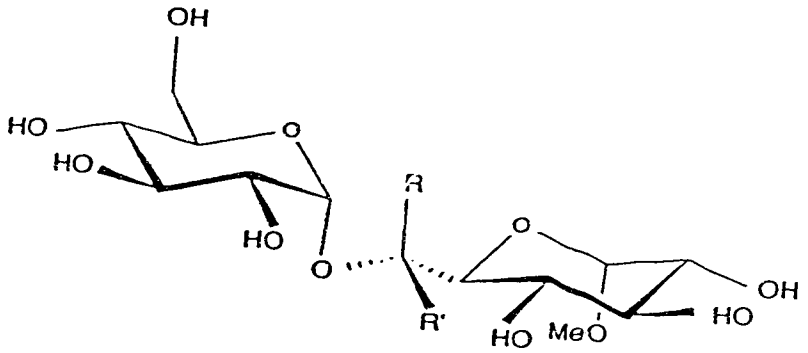
the construction of the progress curves (conc. vs. time). The data were analyzed using the REGGRAFA program[101] developed by Professor Bock and coworker on the personal computer. The results were reproduced in the experimental section. The parameters estimated from the progress curves are reproduced in Table 15.

The enzyme AMG II catalyzed hydrolysis of the disaccharide derivatives are irreversible[90] and, as mentioned above, gave two monosaccharides as products in which one was the methyl α-D-glucopyranoside or the C-6 methyl substituted derivatives and the other was the β-D-glucopyranose (Figure 42). The β-D-glucopyranose would then anomerized to the α-D-glucopyranose which could be observed by ^{1}H-NMR spectroscopy.

The general expression for a reaction catalyzed by an enzyme is:

$$E + S \rightleftharpoons [ES] \xrightarrow{k_{cat}} E + P$$

Table 16. Kinetic data of the hydrolysis catalyzed by AMG II of methyl α-isomaltoside (**37**) and the methyl 6-*C*-methyl isomaltoside derivatives (**40** and **43**).



Substituents at C-6			Population of the	Kinetic Data		Relative	$t_{1/2}$, min.
R	R'	Configuration	Key Conformer	k_{cat},sec^{-1}	Km, mM	$K_{Assoc.}$	9.26 mM
H	H	—	medium	0.069	2.9	12	160
Me	H	R	high	0.12	0.22	155	60
H	Me	S	low	0.84	35	1	500

The rate constant k_{cat} is the sum of all the rate constants of all subsequent steps after the formation of the enzyme-substrate complex ([ES]).

In the hydrolysis catalyzed by AMG II for methyl α-maltoside and methyl α-isomaltoside (Figure 42), the overall rates of hydrolysis were assumed to be k_{cat} for methyl α-maltoside and k'_{cat} for methyl α-isomaltoside. The overall rate ratio k_{cat}/k'_{cat} is about 39 and, therefore AMG II catalyzes the hydrolysis of methyl α-maltoside 39 times faster than methyl α-isomaltoside. This overall rate ratio is similar to the ratio for maltose and isomaltose. Because methyl α-isomaltoside is a poor substrate in comparision to methyl α-maltoside, it was assumed that the dissociation constant K_S for the [ES] complex is the same as the Michaelis-Menten constant K_M. This assumption may not be valid for all cases involved but can be made for poor substrates.[108]

Examination of Table 15 shows that the hydrolysis catalyzed by AMG II are in the decreasing order: methyl α-maltoside > methyl 6-*R*-methyl-α-isomaltoside (**40**) > methyl α-isomaltoside (**37**) > methyl 6-*S*-methyl-α-isomaltoside (**43**) and in agreement with the data reported in Table 14. The slightly faster rate of hydrolysis shown for methyl α-maltoside as compared to its β-anomer may not be real since reactions were too fast to be followed with precision. The kinetic data for the hydrolysis of methyl α-isomaltoside (**37**) and its epimeric 6-*C*-methyl derivatives (**40** and **43**) are presented in Table 16 along with comments regarding the key conformer to be discussed below.

The k_{cat} of methyl α-isomaltoside (**37**) is of the same order of magnitude as the k_{cat} found for methyl 6-*S*-methyl-α-isomaltoside (**40**) and approximately half of that found for methyl 6-*R*-methyl-α-isomaltoside (**43**). The variation in k_{cat} is minor (2 fold) compared to the differences in K_m, the major factor in determining the relative rates of reaction. The K_m values can be assumed to be a useful measure of the association constants $K_{Assoc.}$. The $K_{Assoc.}$ of methyl α-isomaltoside is about 12 times the $K_{Assoc.}$ of methyl 6-*S*-methyl-α-isomaltoside (**40**). On this basis, methyl 6-*R*-methyl-α-isomaltoside (**43**) is bound 13 times more strongly by the enzyme than is methyl α-isomaltoside and about 155 times more than is methyl 6-*S*-methyl-α-isomaltoside. It is seen that the stronger the binding the faster the hydrolysis (lower $T_{1/2}$).

2.3 The Key Hydroxyl Groups.

As discussed above with reference to Figure 37(a), Bock *et al.*,[26,97-101] showed that OH-6', OH-4' of the non-reducing unit and OH-3 of the reducing unit are necessary for the hydrolysis of methyl β-maltoside by the enzyme AMG II. This phenomenon was reminiscent of the findings by Lemieux and coworkers in their studies of the binding of oligosaccharides by lectins and antibodies[109] where they observed in each case that the

binding was dependent on the presence of a cluster of two or three hydroxyl groups termed the key polar grouping. It was shown by Lemieux *et al.*[8] that the α-(1 → 4) linkage in maltose are conformationally well defined by the torsion angles $\Phi^{H\text{-}1',C\text{-}4}/\Psi^{C\text{-}1',H\text{-}4} = -25^{o}/-15^{o}$. Therefore the key polar groups OH-4′ and OH-3 should be energetically well presented at a fixed separation in aqueous solution. As discussed above, the other key polar group OH-6′ is conformationally labile because of freedom of rotation about the C-5 to C-6 bond. Thus, the C-5/C-6′ fragment can readily adopt either the $\mathbf{g}^{-}$ or the $\mathbf{g}^{+}$ conformation (Figure 43) and it is not clear at present which conformer is required by the enzyme AMG II. For the present discussion, the $\mathbf{g}^{-}$ conformation at C-6′ is arbitrarily assumed.

The enzyme AMG II is expected to enter into a polar interaction with the key polar groups (Figure 37(a)) of methyl α-maltoside. In view of the conformational rigidity possessed by methyl α-maltoside,[8] the disposition of these hydroxyl groups can be expected to be near that presented by methyl α-maltoside in the minimum energy conformer which as seen in Fig. 44 is expected to be near 7.1 Å and 7.3 Å for the distance between O-4′ – O-3 and O′-6– O-3, respectively. On the basis of the key polar group theory, it is expected that the hydrolysis of the methyl α-isomaltoside (**37**) and its derivatives (**40** and **43**) will have key polar groups in similar disposition in space.

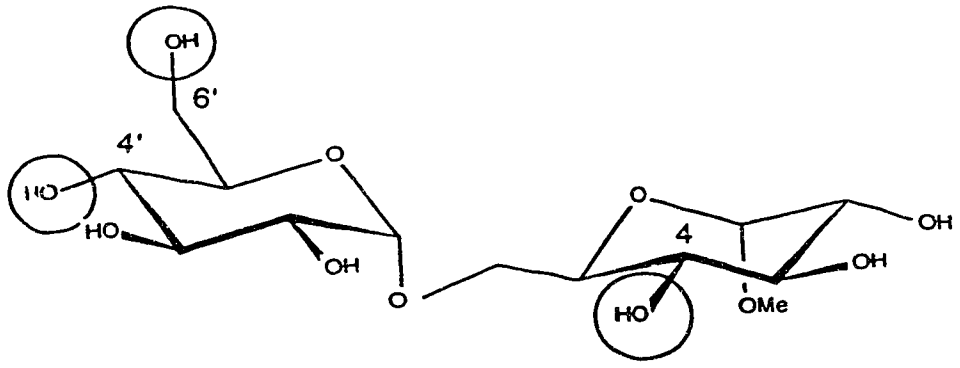


Figure 43. The OH-4, OH-4′ and OH-6′ (circled) groups of methyl α-isomaltoside which are expected to form the key polar grouping.

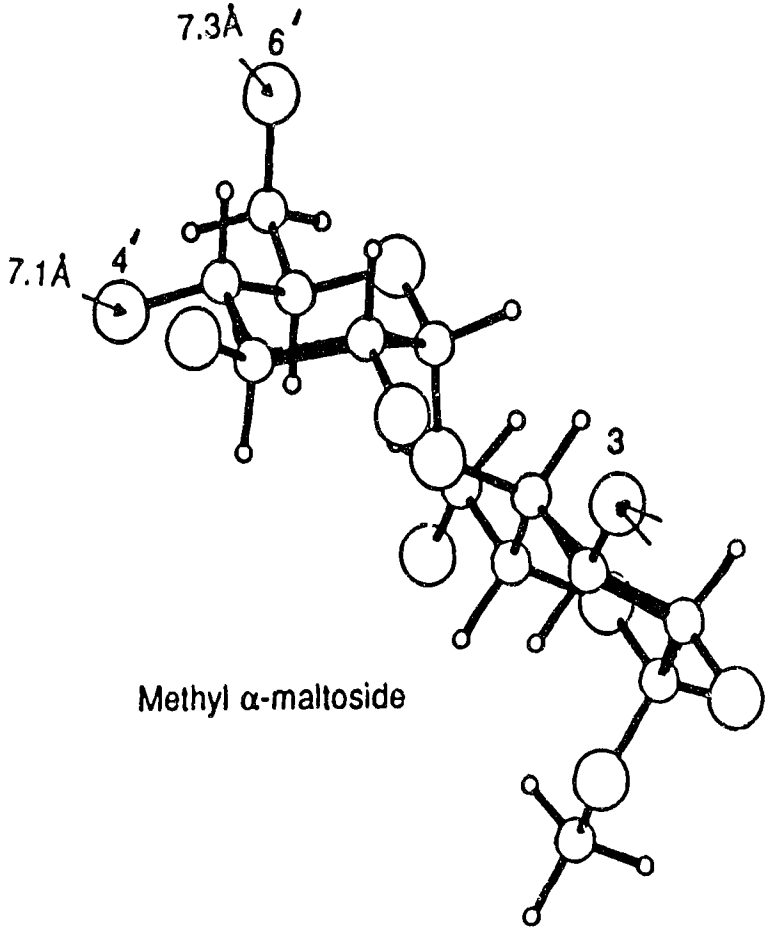
In the methyl α-isomaltoside and its derivatives series, the two key polar hydroxyl groups of the non-reducing unit will still be OH-6′ and OH-4′ but the key polar hydroxyl group of the reducing unit could not be OH-3 because it would be one bond too far away. The OH-4 would be most likely.

The key conformers with similar distances as the OH-3/OH-4′ and OH-3/OH-6′ of methyl α-maltoside were then searched for methyl α-isomaltoside (**37**), methyl 6-*R*-methyl-α-isomaltoside (**43**) and methyl 6-*S*-methyl-α-isomaltoside (**40**) using HSEA calculations. The variations in the estimated distances of the key conformers in the methyl α-isomaltoside and the 6-*C*-methyl derivatives compared to the key conformer of methyl α-maltoside are not more than 0.4 Å. The results are summarized in Table 17 and the key conformers were computer drawn in Figures 44 and 45.

According to HSEA calculations (see Table 17), the minimum energy conformation of methyl α-isomaltoside has the torsion angles $\omega' = 180^{o}$, $\Phi = -50^{o}$, $\Psi = 190^{o}$ and $\omega = 180^{o}$. The torsion angle $\omega = 180^{o}$ at C-6 corresponds to the $\mathbf{g}^{-}$ conformation and is in agreement with the experimental data obtained by Bock *et al.*[26] The key conformer with similar internuclear distances O-4 – O-4′ = 6.9 Å and O-4 – O-6′ = 7.1 Å (Table 17) has the torsion angles $\omega' = 180^{o}$, $\Phi = -50^{o}$, $\Psi = 120^{o}$ and $\omega = 50^{o}$. The key conformer of methyl α-isomaltoside is 1.49 Kcal mol^{-1} higher in energy than its minimum energy conformer and, therefore, can be expected on this basis to be a poorer substrate than methyl α-maltoside for which the key conformer is energetically very close to the preferred conformation. The key conformer for methyl 6-*R*-methyl-α-isomaltoside (**43**) with the same torsion angles ($\omega'/\Phi/\Psi/\alpha = 180^{o}/-50^{o}/120^{o}/50^{o}$) is only 0.31 Kcal mol^{-1} higher than its ^{1}H-NMR conformer. In contrast, the key conformer for methyl 6-*S*-methyl-α-isomaltoside (**40**) has the torsion angles $\omega' = 180^{o}$, $\Phi = -60^{o}$, $\Phi = 160^{o}$ and $\omega = 50^{o}$ with internuclear distances O-4 – O-4′ = 7.0 Å, O-4 – O-6′ = 7.7 Å and is 3.78 Kcal mol^{-1} higher in energy than its ^{1}H-NMR conformer (Figure 45).

Table 17. HSEA calculations for ^{1}H-NMR and Key conformers of methyl α-maltoside, methyl α-isomaltoside (**37**) and methyl 6-*C*-methyl-α-isomaltosides (**40** and **43**)

Compounds	Conformer	Torsion Angles, ^{0}	Energy	Internuclear Distances, Å	
		$\omega'/\Phi/\Psi/\omega$	Kcal/mol	O-4—O-4'	O-4—O-6'
Methyl α-isomaltoside (**37**)	NMR	180/−50/190/180	−2.45	5.2	3.5
	KEY	180/−50/120/180	−0.96	6.9	7.1
Methyl 6-*R*-methyl-α-isomaltoside (**43**)	NMR	180/−50/90/300	−1.26	8.2	7.1
	KEY	180/−50/120/50	−0.85	6.9	7.1
Methyl 6-S-methyl-α-isomaltoside (***40***)	*NMR*	*180/−50/195/180*	−1.46	5.1	5.3
	KEY	180/−60/160/50	2.32	7.0	7.7
Methyl α-maltoside	NMR	180/−25/−15/300	1.35	7.1 (O-3−O-4')	7.3 (O-3−O-6')



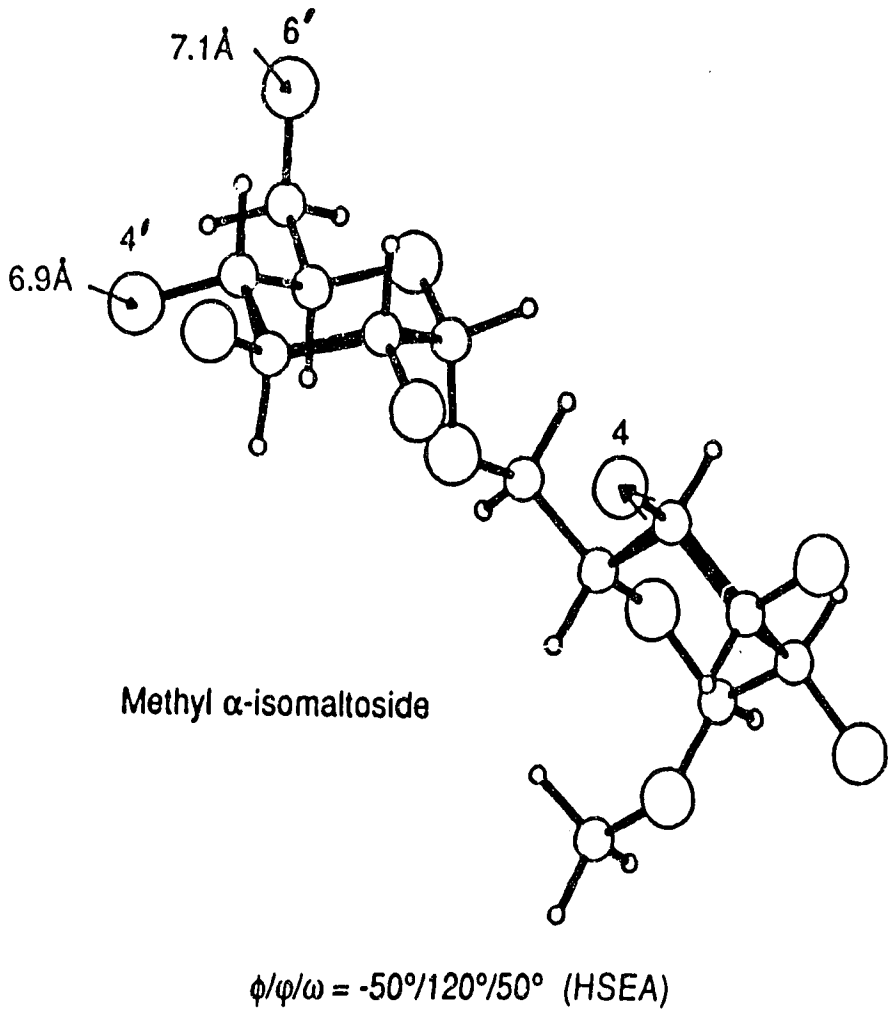
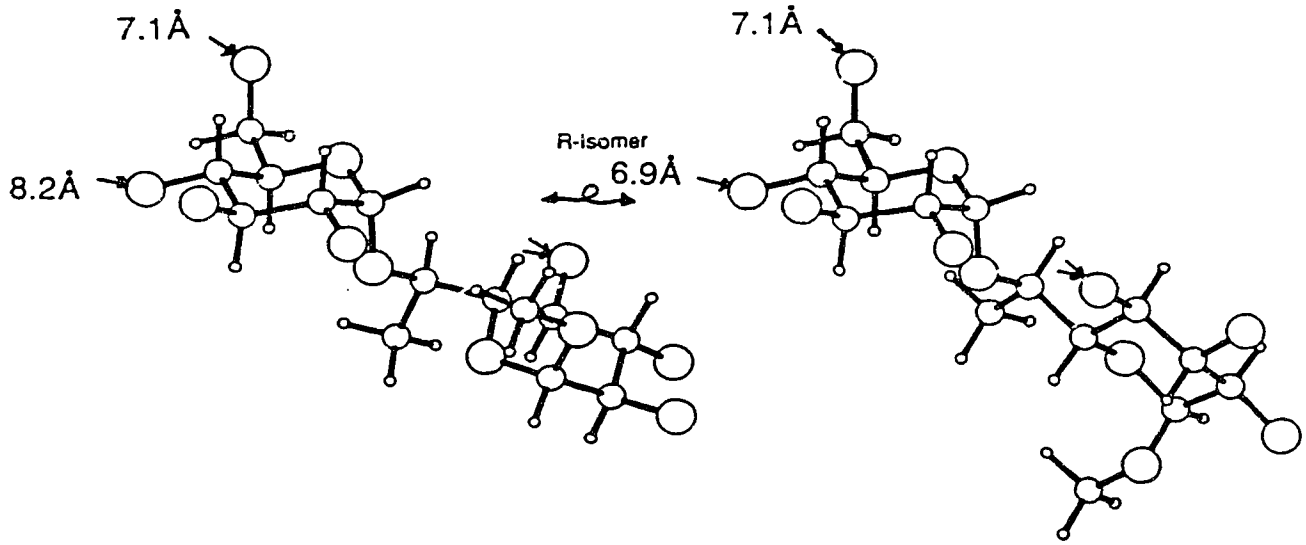
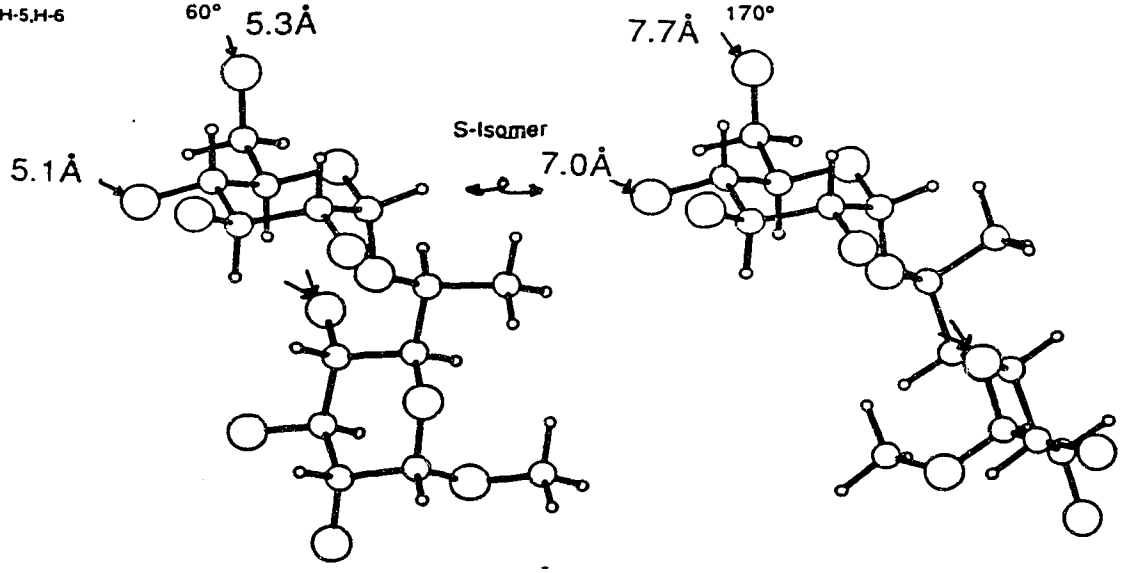


Figure 44. The presentation of the key polar group.



	NMR Conformer ($J_{H-5,H-6}$ = 2.5 Hz)	Key Conformer
ϕ/φ/ω	-50°/90°/-60°	-50°/120°/50°
E, kcal/mole	-1.26	-0.85
$\omega_{H-5,H-6}$	60°	170°



	NMR Conformer ($J_{H-5,H-6}$ < 0.5 Hz)	Key Conformer
ϕ/φ/ω	-50°/195°/180°,	-60°/160°/50°
E, kcal/mole	-1.46	2.32
$\omega_{H-5,H-6}$	60°	-70°

Figure 45. Conformational analysis of the diastereoisomeric methyl 6-*C*-methyl-α-isomaltosides (**43** and **40**).

It is possible now to rationalize partially why the enzyme AMG II catalyzed the hydrolysis of methyl α-maltoside, methyl α-isomaltoside and 6-*C*-methyl derivatives (**40** and **43**). Since enzymes are expected to be substrate specific, a change in linkage such as α-(1→4) in methyl α-maltoside to α-(1→6) in methyl α-isomaltoside will result in a change in conformation of the disaccharide which then becomes a very different substrate. However, AMG II can still catalyze the hydrolysis of the α-(1→6) linkage of methyl α-isomaltoside because the latter can adopt a conformation similar to that of methyl α-maltoside where the key polar hydroxyl groups are in similar spatial arrangement.

The rate of hydrolysis by AMG II was about 40 times faster for methyl α-maltoside compared to methyl α-isomaltoside. Although the methyl α-isomaltoside can adopt the appropriate conformation for hydrolysis, this key conformer is only one of several possible conformers of comparable energies because of the flexibility of the O-5/O-6 fragment of the reducing unit. In other words the population of the key conformer that can be recognized by the enzyme AMG II is low and the organization of the molecule for reaction must present a barrier to reaction. Furthermore, because methyl α-isomaltoside is an α-(1→6) linkage, the nonpolar surface of the disaccharide would be different compared to the α-(1→4) linkage of methyl α-maltoside, the methyl α-isomaltoside may not fit the binding site as well as in the case of methyl α-maltoside which would also result in the reduction of rate. The effect of the distance variations (0.4 Å) in the key polar hydroxyl groups on the rate of hydrolysis is not known at present but it is expected that changes in distance would probably decrease the rate.

Based on the $T_{1/2}$ at 10 mM concentration (see Table 16), methyl 6-*R*-methyl-α-isomaltoside (**43**) hydrolyzed about 2.6 times faster than methyl α-isomaltoside (**37**) which in turn is hydrolyzed about 3.1 times faster than methyl 6-*S*-methyl-α-isomaltoside (**40**). If for hydrolysis, the isomaltoside derivatives must imitate the conformation of methyl α-maltoside in terms of the internuclear distances of the three key hydroxyl groups, then

methyl 6-*R*-methyl-α-isomaltoside (**43**) must have higher population of the proper key conformer than methyl α-isomaltoside. On the other hand, methyl 6-*S*-methyl-α-isomaltoside (**40**) must have lower population of the proper key conformer than has methyl α-isomaltoside. The differences in energies (Kcal mol^{-1}) as estimated by HSEA calculations (Table 17) for adopting the key conformations were 0.41 for methyl 6-*R*-methyl-α-isomaltoside (**43**), 1.49 for methyl α-isomaltoside and 3.78 for methyl 6-*S*-methyl-α-isomaltoside. Using Boltzman's equation for population distribution (Equation 4), it is possible to estimate that the population of the key conformer is 33% for methyl 6-*R*-methyl-α-isomaltoside (**43**), 8% for methyl α-isomaltoside and 0.2% for methyl 6-*S*-methyl-α-isomaltoside. These population estimates reflect the trend for the hydrolysis catalyzed by the enzyme AMG II.

$$\frac{N1}{N2} = e^{-\Delta E/RT} \qquad (4)$$

where $N1$= population of the ^{1}H-NMR conformer

$N2$= population of the key conformer

R= gas constant

T= temperature ^oK

V. SUMMARY

1. Methyl 6-*R*-C-methyl-α-D-glucopyranoside (**21**) was synthesized and found to prefer the $^4C_1 - \mathbf{g}^+$ conformation in aqueous solutions.

2. Hydrolysis of **21**, provided 6-*R*-methyl-D-glucose (**48**) which was dehydrogenated to aldonic lactone by the enzyme system of Hexokinase and 6-Phosphate Dehydrogenase.

3. Methyl 6-*S*-C-methyl-α-D-glucopyranoside (**15**) was synthesized and found to prefer the $^4C_1 - \mathbf{g}^-$ conformation in aqueous solution.

4. Hydrolysis of **15**, provided 6-*S*-methyl-D-glucose (**47**) which was not dehydrogenated to aldonic lactone by the enzyme system of Hexokinase and 6-Phosphate Dehydrogenase.

5. In the temperature range 295°C to 355°C, compounds **21** and **15** still retain their preferred conformations; i.e. $\mathbf{g}^+$ and $\mathbf{g}^-$ respectively.

6. Methyl 6-*R*-methyl-α-isomaltoside (**43**) was synthesized and found to prefer $\mathbf{g}^+$ conformation at the C-6 center of the reducing glucose unit in aqueous solution.

7. Hydrolysis catalyzed by the enzyme Amyloglucosidase Type II of **43** was observed to be about 3 times faster than methyl α-isomaltoside (**37**).

8. Methyl 6-*S*-methyl-α-isomaltoside (**40**) was synthesized and found to prefer $\mathbf{g}^-$ conformation at the C-6 center of the reducing glucose unit in aqueous solution.

9. Hydrolysis catalyzed by the enzyme Amyloglucosidase Type II of **40** was observed to be about 3 times slower than methyl α-isomaltoside (**37**).

VI. EXPERIMENTAL

1. General methods

Nuclear magnetic resonance spectra were obtained at 297°K in D_2O or $CDCl_3$ on Bruker instruments operating at 300 or 400 MHz when provided by the Spectral Service Laboratory under the supervision of Dr. T. Nakashima and by the writer when at 360 MHz.. Acetone was used as internal reference (δ=2.225 ppm) for D_2O and tetramethyl-silane (TMS) was used for $CDCl_3$ in proton spectra. Dioxane was used as internal reference for ^{13}C-spectra. Assignments were carried out using either the double resonance technique or the 2D-COSY technique. Infra-red spectra were obtained on the Nicolet 7199 FT-IR. Melting points were determined on the Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter at 589 nm in a 1 dm cell at room temperature (23± 1°C). Elemental analyses were performed by the departmental Analytical Service Laboratory under the supervision of Mr. R. Swindlehurst. Thin layer chromatography (t.l.c.) was run with precoated silica gel 60-F254 plates (E. Merck, Darmstadt) and developed by quenching of flourescence and/or charring with 5%-10% sulfuric-ethanol solution.

Dry dichloromethane and pyridine were obtained by distilling over calcium hydride and were used immediately. Chromium trioxide was obtained from Aldrich and was dried over P_2O_5 and under vacuum for at least 48 hrs. Other solvents and reagents used were reagent grade and if better purity was needed, standard procedures[110] were followed. Anhydrous solution transfers were done under nitrogen atmosphere using standard syringe techniques.[111]

N.O.e. experiments

Solutions in the NMR tubes were previously degassed by bubbling dry nitrogen through for 3-5 minutes. The D_2O used was purchased from Aldrich (99.9% D, low in paramagnetic impurities). The steady state nuclear Overhauser enhancements were obtained after 512 scans normally.

HSEA calculations

The atomic coordinates were obtained from neutron diffraction studies of methyl α-D-glucopyranoside crystal structure.[76] Modification of the methyl α-D-glucopyranoside structure at C-6 were achieved with the program TRANSF.OBJ using the distance 1.54Å for C-C bond. Rotation of the ω torsion angle was done with the program GENT.OBJ. Molecular minimum energy calculation was estimated using the HDSP3.OBJ program. Molecular conformation was plotted using the coordinates of the minimum energy conformation generated by the HDSP3.OBJ program. The programs TRANSF.OBJ, GENT.OBJ and HDSP3.OBJ were available from the University of Alberta Computing Library.

2. Syntheses

Methyl 2,3,4,-tri-O-benzyl-α-D-gluco-1,6-dialdo-hexopyranoside (7)

Dried chromium trioxide (25.9 g, 259 mmol) was added to a solution of pyridine (40.9 g, 517 mmol) in dichloromethane (400 mL). The mixture was mechanically stirred at room temperature for 30 min.. A solution of methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**4**) (10.0 g, 21.6 mmol) in dichloromethane (22 mL) was added rapidly to the oxidation mixture and a black tar precipitated immediately. The resulting mixture was stirred at room temperature for 10 min., decanted and the black tarry precipitate was washed with ether several times. The combined ether solutions were filtered through a pad of silica gel-G to give a colorless filtrate which was dried with anhydrous magnesium sulfate. Ether was removed to give a slightly yellow oily residue which was coevaporated with toluene (2 x 200 mL) to remove residual pyridine. The oily residue was dried under vacuum overnight to give **7** (6.94 g, 70%). Proton NMR of this crude residue showed that it contained ~90% of the desired compound **3**. $[\alpha]_D$=8.2° (c 1.0, $CHCl_3$) lit.[47] 13.6°.

^{1}H-NMR ($CDCl_3$): δ 9.65 (s, 1H, H-6), 7.40—7.10 (m, 15H, Ar-H), 5.05—4.50 (m, 7H, 3 x Ph-CH_2 and H-1), 4.17 (d, 1H, H-5, $J_{4,5}$= 10.0 Hz), 4.08 (dd, 1H, H-3, $J_{3,4}$=9.0 Hz, $J_{2,3}$=9.5 Hz), 3.57 (dd, 1H, H-4, $J_{3,4}$=9.0 Hz, $J_{4,5}$=10.0 Hz), 3.50 (dd, 1H, H-2, $J_{1,2}$=3.5 Hz, $J_{2,3}$=9.5 Hz), 3.38 (s, 3H, OCH_3); ^{13}C-NMR ($CDCl_3$): δ 197.45 (<u>C</u>HO); 138.43—125.31 (Ar. Carbons); 98.41 (C-1); 87.76, 79.30, 77.82 (3 x Ph-<u>C</u>H_2); 75.32, 75.11, 74.22, 73.60 (C-2—C-5); 55.77 (O<u>C</u>H_3); IR ν_{max} (cm^{-1}) 1741.133 (CHO).

Methyl 2,3,4-tri-O-benzyl-7-deoxy-β-L-glycero-D-glucoheptapyranoside (9)

A solution of methyl iodide (4.26 g, 30.04 mmol) in anhydrous ether (20 mL) was added dropwise under nitrogen to a mixture of magnesium (0.73 g, 30.04 mmol) and ether (30 mL). After warming for a few minutes, the mixture turned cloudy and began to boil. Addition of methyl iodide solution was such that boiling was maintained. After the addition

of methyl iodide was complete, the resulting Grignard mixture was left stirring for 1 hr. to ensure complete formation of the reagent. A solution of compound **7** (6.94 g, 15.02 mmol) in ether (30 mL) was then added dropwise and the reaction mixture was left stirring at room temperature for 2 hrs. The reaction was quenched with the dropwise addition of saturated ammonium chloride (10 mL) and the reaction mixture solidified. Distilled water (50 mL) was added to dissolve the solid. The aqueous layer was extracted with ether several times (total volume 150 mL) and the combined ether solutions were washed with water (2 x 100 mL) and dried over anhydrous magnesium sulfate. Removal of solvent gave a yellow crude oil (5.96 g) in which the major product was **9** by t.l.c. (R_f=0.54, solvent 3:2 n-hexane:EtOAc).

(i) Methyl 2,3,4,-tri-O-benzyl-7-deoxy-6-O-(3,5-dinitrobenzoyl)-β-L-glycero-D-glucoheptapyranoside ***(14)***

The crude Grignard product (0.587 g, 1.2 mmol) was dissolved in pyridine (10 mL) and 3,5-dinitrobenzoyl chloride (0.513 g, 2.25 mmol) was added along with a few crystals of dimethylaminopyridine (DMAP). The reaction mixture was stirred at room temperature until analysis by t.l.c. (R_f S.M.=0.58, R_f Prod.=0.85, solvent 3:2 n-hexane:EtOAc) showed no remaining starting material. Water was then added dropwise with stirring for 5 min. The aqueous layer was extracted with chloroform (75 mL). The chloroform layer was separated, washed with water (2 x 75 mL) and dried over anhydrous magnesium sulfate. The solvent was then evaporated to give a yellow residue which was recrystallized from hot methanol to give **14** (0.612 g, 74%); mp 68-70°C; $[\alpha]_D$=52.2° (c 0.85, $CHCl_3$). ^{1}H-NMR ($CHCl_3$): δ 9.21 (t, 1H, para-H, $J_{ortho,para}$=2.0 Hz), 9.16 (d, 2H, ortho-H, $J_{ortho,para}$=2.0 Hz), 7.45—7.15 (m, 15, Ar-H), 5.62 (dq, 1H, H-6, $J_{5,6}$=1.5 Hz, $J_{6,7}$=6.5 Hz), 5.45—5.05 (3 x dd and d, 7H, 3 x Ph-CH_2 and H-1), 4.05 (dd, 1H, H-3, $J_{2,3}$=10.0 Hz, $J_{3,4}$=9.0 Hz), 3.71 (dd, 1H, H-5, $J_{5,6}$=1.5 Hz, $J_{4,5}$=10.0 Hz), 3.60 (dd, 1H, H-2, $J_{1,2}$=3.5

Hz, $J_{2,3}$=10.0 Hz), 3.42 (t, 4H, H-4 and OCH_3), 1.48 (d, 3H, C_6-CH_3, $J_{6,7}$=6.5 Hz); ^{13}C-NMR ($CDCl_3$): δ 228.13 (C=O); 162.81—123.38 (Ar. Carbons); 99.01 (C-1); 83.23, 81.11, 77.69, 72.51, 71.80 (C-2—C6); 76.86, 75.88, 74.53 (3 x Ph-CH_2); 56.30 (OCH_3); 17.35 (C-7).

Anal. calc. for $C_{36}H_{36}N_2O_{11}\bullet\frac{1}{2}MeOH$: C 63.66 H 5.56 N 4.07

found: C 63.49 H 5.24 N 4.27

(ii) Methyl 2,3,4-tri-O-benzyl-7-deoxy-β-L-glycero-D-glucoheptapyranoside (9) from **14**

Compound **14** (1.00 g, 1.49 mmol) was dissolved in dioxane (50 mL) and 1N sodium hydroxide solution (20 mL) added and stirred at room temperature for 1 hr. The reaction was followed by t.l.c. (R_f **14**=0.85, R_f **9**=0.57, solvent 3:2 n-hexane:EtOAc). When the reaction was completed (after 1 hr.), dichloromethane (250 mL) was added to the reaction mixture and stirred for 5 min.. Then the reaction mixture was filtered over a pad of silica gel-G which was then washed with water (2 x 100 mL) and dried over anhydrous magnesium sulfate. The solvent was then evaporated, the residue dried under vacuum to give solid **9** (0.692 g, 97%); mp 82-84°C; $[\alpha]_D$=26.9° (c 1.0, $CHCl_3$).

^{1}H-NMR ($CDCl_3$): δ 7.40—7.20 (m, 15H, Ar-H), 5.01—4.63 (3 x dd, 6H, Ph-CH_2), 4.58 (d, 1H, H-1, $J_{1,2}$=3.5 Hz), 4.05 (dq, 1H, H-6, $J_{5,6}$=1.5 Hz, $J_{6,7}$=6.5 Hz), 3.99 (dd, 1H, H-3, $J_{3,4}$=9.0 Hz, $J_{2,3}$=9.5 Hz), 3.62 (dd, 1H, H-4, $J_{4,5}$=10.0 Hz, $J_{3,4}$=9.0 Hz), 3.50 (dd, 1H, H-2, $J_{2,3}$=9.5 Hz, $J_{1,2}$=3.5 Hz), 3.42 (dd, 1H, H-5, $J_{5,6}$=1.5 Hz, $J_{4,5}$=10.0 Hz), 3.34 (s, 3H, OCH_3), 1.25 (d, 3H, C_6-CH_3, $J_{6,7}$=6.5 Hz); ^{13}C-NMR ($CDCl_3$): δ 138.79—127.57 (Ar. Carbons); 98.15 (C-1); 82.17, 79.93, 77.72 (3 x Ph-CH_2); 75.70, 75.11, 73.42, 72.83 (C-2—C-5); 65.05 (C-6); 55.01 (OCH_3); 20.15 (C-7).

Anal. calc. for $C_{29}H_{34}O_6$: C 72.78 H 7.16

found: C 72.94 H 6.82

Methyl 7-deoxy-β-L-glycero-D-glucoheptapyranoside (15)

Compound **9** (200 mg, 0.42 mmol) was dissolved in methanol (25 mL). Then 5% palladium-on-charcoal (200 mg) was added in small portions. The mixture was shaken with hydrogen (100 lb. in^{-2}) at room temperature overnight. The reaction mixture was then filtered over celite (washed with methanol). The solvent was evaporated to give **15** (85 mg, 98%) as a solid which could be recrystallized from n-hexane / 98% ethanol to give needle like crystals. Mp 136-137°C, $[\alpha]_D$=150° (c .23, H_2O). ^{1}H-NMR (D_2O): δ 4.68 (d, 1H, H-1, $J_{1,2}$=3.5 Hz), 4.05 (dq, 1H, H-6, $J_{5,6}$=1.5 Hz, $J_{6,7}$=6.5 Hz), 3.51 (dd, 1H, H-3, $J_{2,3}$=10.0 Hz, $J_{3,4}$=9.0 Hz), 3.42 (dd, 1H, H-2, $J_{1,2}$=3.5 Hz, $J_{2,3}$=10.0 Hz), 3.36 (dd, 1H, H-4, $J_{3,4}$=9.0 Hz, $J_{4,5}$=10.0 Hz), 3.27 (s, 3H, OCH_3), 3.26 (dd, 1H, H-5, $J_{5,6}$=1.5 Hz, $J_{4,5}$=10.0 Hz), 1.16 (d, 3H, C_7H_3, $J_{6,7}$=6.5 Hz). ^{13}C-NMR (D_2O): δ 99.24 (C-1); 73.49, 73.41 (C-3, C-5); 71.31 (C-2); 69.74 (C-6); 64.26 (C-4); 54.89 ($O\underline{C}H_3$); 18.72 (C-7).

Anal. calc. for $C_8H_{16}O_6$: C 46.15 H 7.75

found: C 46.04 H 7.46

Methyl 2,3-di-O-acetyl-4,6-benzylidene-7-deoxy-β-L-glycero-D-glucoheptapyranoside (17)

A solution of benzal bromide (112 mg, 0.40 mmol) in pyridine (1 mL) was added to a solution of compound **15** (16 mg, 0.77 mmol) in pyridine (15 mL) and the mixture was refluxed at 125°C for 2 hrs. with stirring. Acetic anhydride (3 mL) was then added to the cooled reaction mixture and the solution was stirred for 48 hrs. at room temperature. The reaction mixture was then poured into water (75 mL) and the aqueous layer was extracted with chloroform (50 mL). The chloroform layer was washed with water (50 mL), saturated sodium bicarbonate solution (50 mL), water (50 mL) and dried over anhydrous magnesium sulfate. Chloroform was evaporated and the residue was coevaporated with toluene (2 x 15 mL). Preparative t.l.c. of the residue (R_f=0.66, solvent 3:2 n-hexane / EtOAc) gave **17** (15 mg, 51%) as a solid. Mp 132-134°C; $[\alpha]_D$=50.2° (c 0.31, $CHCl_3$).

^{1}H-NMR ($CDCl_3$): δ 7.50—7.25 (m, 5H, Ar-H), 5.80 (s, 1H, Ph-CH), 5.57 (dd, 1H, H-3, $J_{3,4}$=9.0 Hz, $J_{2,3}$=10.0 Hz), 4.93 (d, 1H, H-1, $J_{1,2}$=3.5 Hz), 4.87 (dd, 1H, H-2, $J_{2,3}$=10.0 Hz, $J_{1,2}$=3.5 Hz), 4.5 (dq, 1H, H-6, $J_{5,6}$=6.0 Hz, $J_{6,7}$=7.0 Hz), 4.15 (dd, 1H, H-5, $J_{4,5}$=10.0 Hz, $J_{5,6}$=6.0 Hz), 3.91 (dd, 1H, H-4, $J_{3,4}$=9.0 Hz, $J_{4,5}$=10.0 Hz), 3.41 (s, 3H, $OCOCH_3$), 1.48 (d, 3H, C_7H_3, $J_{6,7}$=7.0 Hz). ^{13}C-NMR ($CDCl_3$): δ 170.48, 169.76 (2 x C=O); 137.40, 128.92, 128.22, 126.24 (Ar. Carbons); 97.59 (C-1), 94.07 (Ph-CH); 72.74, 71.50, 70.49, 69.66 (C-2—C-5); 64.45 (C-6); 55.34 (OCH_3); 20.65, 20.79 (2 x $COCH_3$), 11.28 (C-7).

Anal. cal. for $C_{19}H_{24}O_8$:	C	59.99	H	6.36
found:	C	59.55	H	6.06

Methyl 6-O-methanesulfonyl-2,3,4-tri-O-benzyl-7-deoxy-β-L-glycero-D-glucoheptapyranoside (**18**)

Methanesulfonyl chloride (87 mg, 0.76 mmol) was added to a solution of compound **9** (135 mg, 0.282 mmol) in pyridine (5 mL) and the reaction mixture was stirred at room temperature for 2 hrs. T.l.c. analysis indicated that the reaction was completed after 2 hrs. and virtually one product had formed (R_f **9**=0.58, R_f **18**=0.67, solvent 3:1 toluene / acetone). The reaction mixture was then poured into a mixture of ice / water (50 mL) and the aqueous layer was extracted with dichloromethane (50 mL). The dichloromethane layer was washed with cold water (2 x 50 mL) and dried over anhydrous magnesium sulfate. The solvent was then evaporated, the residue was coevaporated with toluene (2 x 25 mL) and chromatographed (solvent 6:1 toluene/acetone) to give **18** (104 mg, 66%) as an oil. $[\alpha]_D$=9.3° (c 1.22, $CHCl_3$).

^{1}H-NMR ($CDCl_3$): δ 7.40—7.25 (m, 15H, Ar-H), 5.32 (dq, 1H, H-6, $J_{5,6}$=1.0 Hz, $J_{6,7}$=6.5 Hz), 5.06—4.62 (m, 7H, 3 x Ph-CH_2 and H-1), 4.02 (m, 1H, H-5), 3.65—3.53 (m, 3H, H-3, H-4 and H-2), 3.36 (s, 3H, OCH_3), 3.04 (s, 3H, SO_2CH_3), 1.53 (d, 3H, C_6-CH_3,

$J_{6,7}$=6.5 Hz). ^{13}C-NMR ($CDCl_3$): δ 138.54—127.74 (Ar. Carbons); 98.26 (C-1); 82.30, 79.71, 77.17, 73.55, 72.38 (C-2—C6); 75.74, 75.31, 75.00 (3 x Ph-$\underline{C}H_2$); 55.33 (O$\underline{C}H_3$); 39.54 ($OSO_2\underline{C}H_3$); 17.73 (C-7).

Anal. calc. for $C_{30}H_{36}O_8S$:	C 64.73	H 6.52	S 5.76
found:	C 64.85	H 6.55	S 5.52

Methyl 6-O-benzoyl-2,3,4-tri-O-benzyl-7-deoxy-α-D-glycero-D-glucoheptapyranoside (**19**)

Crude mesylate **18** (0.60 g, 1.08 mmol) was dissolved in dry N,N-dimethyl formamide (DMF) (5mL), anhydrous sodium benzoate (1.01 g, 7.0 mmol) was added and the reaction mixture was heated at 120°C and stirred for 17 hrs.. The cooled reaction mixture was then dissolved in dichloromethane (50 mL), washed with water (6 x 75 mL) and dried over anhydrous magnesium sulfate. The solvent was evaporated and the residue was chromatographed on HPLC (solvent 10:1 n-hexane / acetone) to give **19** (0.294 g, 47%) as a syrup. $[\alpha]_D$=9.8° (c 1.04, $CHCl_3$).

^{1}H-NMR ($CDCl_3$): δ 8.40—7.20 (m, 20H, Ar-H), 5.47 (dq, 1H, H-6, $J_{5,6}$=2.0 Hz, $J_{6,7}$=7.0 Hz), 5.03—4.65 (m, 6H, 3 x Ph-CH_2), 4.64 (d, 1H, H-1, $J_{1,2}$=4.0 Hz), 4.04 (dd, 1H, H-3, $J_{2,3}$=9.5 Hz, $J_{3,4}$=9.0 Hz), 3.93 (dd, 1H, H-5, $J_{5,5}$=2.0 Hz, $J_{5,4}$=10.0 Hz), 3.52 (dd, 1H, H-2, $J_{1,2}$=4.0 Hz, $J_{2,3}$=9.5 Hz), 3.42 (dd, 1H, H-4, $J_{3,4}$=9.0 Hz, $J_{4,5}$=10.0 Hz), 3.38 (s, 3H, OCH_3), 1.18 (d, 3H, C_6-CH_3, $J_{6,7}$=7.0 Hz). ^{13}C-NMR ($CDCl_3$): δ 165.76 ($\underline{C}$=O); 136.66—127.75 (Ar. Carbons); 97.80 (C-1); 82.44, 80.16, 77.82, 71.39, 70.12 (C-2—C-6); 75.92, 74.62, 73.41 (3 x Ph-$\underline{C}H_2$), 54.99 (O$\underline{C}H_3$); 13.57 (C-7).

Anal. calc. for $C_{36}H_{38}O_7$:	C 74.20	H 6.57	
found:	C 74.33	H 6.55	

Methyl 2,3,4-tri-O-benzyl-7-deoxy-α-D-glycero-D-glucoheptapyranoside **(10)**

Compound **19** (137 mg, 0.24 mmol) was dissolved in dioxane (10 mL) and 1N sodium hydroxide solution (6 mL) was added. The reaction mixture was stirred at 60°C and t.l.c. analysis showed that the reaction was over after 48 hrs. (R_f **19**=0.85, R_f **10**=0.43, solvent 3:2 n-hexane:EtOAc). The cooled mixture was then diluted with dichloromethane (50 mL) and filtered over a pad of silica gel-G. The dichloromethane solution was washed with water (2 x 50 mL), saturated sodium bicarbonate solution (50 mL), water (50 mL) and dried over anhydrous magnesium sulfate. The solvent was then removed and the residue solidified slowly under vacuum to give **10** (80 mg, 71%). Mp 89-90°C, $[\alpha]_D$=28° (c 0.4, $CHCl_3$).

^{1}H-NMR ($CDCl_3$): δ 7.34—7.16 (m, 15H, Ar-H), 4.98—4.53 (m, 6H, 3 x Ph-CH_2), 4.50 (d, 1H, H-1, $J_{1,2}$=3.5 Hz), 3.95 (dd, 1H, H-3, $J_{2,3}$=9.5 Hz, $J_{3,4}$=9.0 Hz), 3.88 (m, 1H, H-6), 3.55 (dd, 1H, H-5, $J_{4,5}$=10.0 Hz, $J_{5,6}$=4.5 Hz), 3.43 (dd, 1H, H-2, $J_{1,2}$=3.5 Hz, $J_{2,3}$=9.5 Hz), 3.35 (dd, 1H, H-4, $J_{3,4}$=9.0 Hz, $J_{4,5}$=10.0 Hz), 3.31 (s, 3H, OCH_3), 2.49 (d, 1H, OH, $J_{6,OH}$=6.0 Hz), 1.05 (d, 3H, C_6-CH_3, $J_{6,7}$=6.5 Hz). ^{13}C-NMR ($CDCl_3$): δ 138.61—127.73 (Ar. Carbons); 97.64 (C-1); 82.38, 80.29, 80.01 (3 x Ph-$\underline{C}H_2$); 75.75, 74.81, 73.37, 72.31 (C-2—C-5); 68.37 (C-6); 55.16 ($O\underline{C}H_3$), 17.81 (C-7).

Anal. calc. for $C_{29}H_{34}O_6$: C 72.78 H 7.16

found: C 72.81 H 7.07

Methyl 7-deoxy-α-D-glycero-D-glucoheptapyranoside **(21)**

To a solution of **10** (70 mg, 0.15 mmol) in methanol (12 mL), 5% palladium-on-charcoal (74 mg) was added in small portions. The mixture was shaken with hydrogen (100 lb. in^{-2}) at room temperature for 6 hrs. The reaction mixture was then filtered over celite (washed with methanol). The solvent was evaporated and the residue was coevaporated

with toluene (2 x 25 mL) and dried under vacuum. The residue solidified under vacuum to give **21** (30 mg, 98%). Mp 135-136°C, $[\alpha]_D = 147.8^o$ (c 1.0, H_2O).

^{1}H-NMR (D_2O): δ 4.68 (d, 1H, H-1, $J_{1,2}$=4.0 Hz), 4.04 (dq, 1H, H-6, $J_{5,6}$=2.5 Hz, $J_{6,7}$=6.0 Hz), 3.56 (dd, 1H, H-5, $J_{5,6}$=2.5 Hz, $J_{4,5}$=10.0 Hz), 3.53 (dd, 1H, H-3, $J_{3,4}$=9.0 Hz, $J_{2,3}$=10.0 Hz), 3.44 (dd, 1H, H-2, $J_{1,2}$=4.0 Hz, $J_{2,3}$=10.0 Hz), 3.23 (dd, 1H, H-4, $J_{4,5}$= 10.0 Hz, $J_{3,4}$=9.0 Hz), 3.29 (s, 3H, OCH_3), 1.10 (d, 3H, C_6-CH_3, $J_{6,7}$=6.0 Hz). ^{13}C-NMR (D_2O): δ 99.22 (C-1); 73.37, 73.32 (C-2 and C-5); 71.27, 70.93 (C-3 and C-6); 66.34 (C-4); 54.96 ($O\underline{C}H_3$), 15.05 (C-7).

Anal. calc. for $C_8H_{16}O_6$:	C	46.15	H	7.75
found:	C	45.99	H	7.50

Methyl 6'-O-acetyl-2,2',3,3',4,4'-hexa-O-benzyl-7-deoxy-6-O-(α-D-glucopyranosyl)-β-L-glycero-D-glucoheptapyranoside (**38**)

Methyl 2,3,4-tri-*O*-benzyl-7-deoxy-β-L-*glycero*-D-glucoheptapyranoside (**9**) (100 mg, 0.209 mmol) was dissolved in dichloromethane (10 mL). Then tetraethylamonium bromide (76 mg), dried molecular sieves (BDH, 4 A, 1 g) and dried DMF (1.4 mL) was added and the mixture was bubbled with nitrogen to reduce the volume. Then freshly prepared 6-O-acetyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyl bromide (**31**) (2 eq.) in dichloromethane (6 mL) was added and the mixture was stirred at room temperature under nitrogen. The reaction progress was followed by t.l.c. until completed (R_f **38**=0.69, R_f **24**=0.55, solvent 3:2 n-hexane / ethyl acetate). After the reaction was over, the partially dry mixture was dissolved in dichloromethane (25 mL), filtered to remove undissolved materials, washed with water (2x50 mL), saturated sodium bicarbonate solution (2x50 mL), water (2x50 mL) and dried over magnesium sulfate. Dichloromethane was evaporated and the residue was chromatographed with silica gel (solvent 4:1 n-hexane /

ethyl acetate) to give the desired disaccharide **38** as an oil which was dried under vacuum with heating (101 mg, 51%). $[\alpha]_D=0.54^o$ (c 1.06, $CDCl_3$).

^{1}H-NMR ($CDCl_3$): δ 7.40—7.20 (m, 30H, Ar-H), 5.09 (d, 1H, H-1′, $J_{1',2'}=3.5$ Hz), 5.02—4.49 (m, 7H, 3 x Ph-CH_2 and H-1), 4.26—4.06 (m, 3H, 2 x H-6′ and H-6), 4.06—3.94 (q and m, 3H, H-3′, H-3 and H-5′), 3.77 (dd, 1H, H-4, $J_{3,4}=9.5$ Hz, $J_{4,5}=9.0$ Hz), 3.56 (dq, 1H, H-5, $J_{4,5}=9.0$ Hz, $J_{5,6}=2.0$ Hz), 3.56—3.47 (m, 2H, H-2′ and H-2), 3.46 (dd, 1H, H-4′, $J_{3',4'}=9.0$ Hz, $J_{4',5'}=10.0$ Hz), 3.38 (s, 3H, OCH_3), 1.92 (s, 3H, CH_3COO-), 1.33 (d, 3H, C_6-CH_3, $J_{6,7}=6.0$ Hz). ^{13}C-NMR ($CDCl_3$): δ 170.75 (C=O); 138.81 (t-Carbons of Ph.); 128.47—127.39 (Ph. Carbons); 97.90 (C-1′); 95.20 (C-1); 75.72, 75.53, 75.08, 74.32, 73.40, 73.00 (6 x Ph-CH_2); 82.56, 81.60, 80.19 (3 x C); 77.73, 77.48, 73.22, 71.69, 69.19 (9 x C: C-2, C-2′, C-3, C-3′, C-4, C-4′, C-5, C-5′, C-6); 63.24 (C-6′), 55.09 (OCH_3), 20.01 ($COOCH_3$), 16.31 (C-7)

Anal. calc. for $C_{57}H_{64}O_{12}$:	C 73.09	H 6.77
found:	C 72.84	H 6.85

Methyl 2,2′,3,3′,4,4′-hexa-O-benzyl-7-deoxy-6-O-(α-D-glucopyranosyl)-β-L-glycero-D-glucoheptapyranoside (**39**)

Methyl 6′-*O*-acetyl-2,2′,3,3′,4,4′-hexa-*O*-benzyl-7-deoxy-6-*O*-(α-D-glucopyranosyl)-β-L-*glycero*-D-glucoheptapyranoside (**38**) (293 mg, 0.307 mmol) was dissolved in dry methanol (10 mL). A catalytic amount of sodium methoxide was added to the solution and the solution was stirred at room temperature overnight. The reaction progress was followed by t.l.c. (R_f **39**=0.49, R_f **38**=0.72, solvent 3:2 n-hexane / ethyl acetate). When the reaction was completed, resin (IRC-50-H^+, washed with distilled water and methanol) was added and stirred until the mixture turned acidic (pH paper). Then the mixture was filtered and the solvent was evaporated to give a foamy product **39** which was dried under

vacuum (269 mg, 96%). $[\alpha]_D=47.6^o$ (c 1.99, $CHCl_3$).

1H-NMR ($CDCl_3$): δ 7.40—7.10 (m, 30H, Ph-H), 5.03 (d, 1H, H-1', $J_{1',2'}=3.5$ Hz), 5.01—4.57 (m, 13H, 6 x Ph-CH_2 and H-1), 4.32 (dq, 1H, H-6, $J_{5,6}=1.5$ Hz, $J_{6,7}=6.5$ Hz), 4.01 (dd, 1H, H-3', $J_{2',3'}=9.5$ Hz, $J_{3',4'}=9.0$ Hz), 3.98 (dd, 1H, H-3, $J_{2,3}=9.5$ Hz, $J_{3,4}=10.0$ Hz), 3.84—3.72 (m, 2H, H-5' and H-4), 3.66—3.49 (m, 5H, 2 x H-6, H-5, H-4' and H-2), 3.46 (dd, 1H, H-2', $J_{1',2'}=3.5$ Hz, $J_{2',3'}=9.5$ Hz), 3.36 (s, 3H, OCH_3), 1.32 (d, 3H, C_6-CH_3, $J_{6,7}=6.5$ Hz). ^{13}C-NMR ($CDCl_3$): δ 138.84—138.27 (t-Ph. Carbons); 128.46—127.37 (Ph. Carbons); 97.88 (C-1'); 95.39 (C-1); 82.54, 81.52, 80.19, 80.14, 77.64, 73.13, 71.84, 71.31 (9 Carbons: C-2, C-2', C-3, C-3', C-4, C-4', C-5, C-5', C-6); 61.72 (C-6'); 55.09 (OCH_3); 16.40 (C-7).

Anal. calc. for $C_{56}H_{62}O_{11}$:	C	73.80	H	6.86
found:	C	73.20	H	6.95

Methyl 7-deoxy-6-O-(α-D-glucopyranosyl)-β-L-glycero-D-glucoheptapyranoside (**40**)

Methyl 2,2',3,3',4,4'-hexa-*O*-benzyl-7-deoxy-6-*O*-(α-D-glucopyranosyl)-β-L-*glycero*-D-glucopyranoside (**39**) (126 mg, 0.138 mmol) was dissolved in dry methanol (12.5 mL). Then 5% palladium-on-charcoal (126 mg) was added in small portions to the solution and the mixture was shaken under hydrogen (100 lb. in^{-2}) at room temperature. The reaction progress was followed by t.l.c. (solvent 4:1 dichloromethane / methanol). When the reaction was completed the mixture was filtered and the filtrate was evaporated to give a yellowish solid residue. The crude residue was chromatographed (Iatrobeads, solvent 4:1 dichloromethane / methanol) to give colorless crystalline **40** (40 mg, 78%). Mp 127-128°C, $[\alpha]_D=175.3^o$ (c 0.26, D_2O).

^{1}H-NMR (D_2O): δ 5.09 (d, 1H, H-1', $J_{1',2'}=3.5$ Hz), 4.28 (broaden q, 1H, H-6), 3.69 (t, 1H, H-3'), 3.64 (broad d, 1H, H-5), 3.56 (dd, 1H, H-2', $J_{1',2'}=3.5$ Hz, $J_{2',3'}=9.5$ Hz),

3.89—3.52 (m, 10H, H-5′, 2 x H-6′, H-3, H-3′, H-4, H-5, H-5′, H-2, H-2′), 3.43 (t, 4H, OCH_3 and H-4′). ^{13}C-NMR (D_2O): δ 100.03 (C-1′); 95.56 (C-1); 74.49, 74.15, 73.68, 73.04, 72.25, 71.99, 70.24, 68.50 (9 Carbons: C-2, C-2′, C-3, C-3′, C-4, C-4′, C-5, C-5′, C-6); 61.17 (C-6′), 55.67 ($O\underline{C}H_3$), 14.76 (C-7).

Anal. calc. for $C_{14}H_{25}O_{11}$:	C	45.53	H	6.82
found:	C	42.41	H	6.61

Methyl 6′-O-acetyl-2,2′,3,3′,4,4′-hexa-O-benzyl-7-deoxy-6-O-(α-D-glucopyranosyl)-α-D-glycero-D-glucoheptapyranoside (**41**)

Compound **10** (500 mg, 1.04 mmol) was dissolved in dichloromethane (5 mL). Tetraethyl ammonium bromide (420 mg), dry molecular sieves (BDH 4A, 5 g) and DMF (1 mL) was added to the solution and the mixture was placed under nitrogen atmosphere. Then 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl bromide (**31**, 2 eq.) in dichloromethane (5 mL) was added and the mixture was stirred at room temperature under nitrogen. The reaction progress was followed by t.l.c. (R_f **41**=0.59, R_f **10**=0.26solvent system 3:2 n-hexane / ethyl acetate). When the reaction was completed, dichloromethane (100 mL) was added and the mixture was filtered. The dichloromethane solution was washed with saturated sodium carbonate solution (100 mL), water (4 x 100 mL) and dried over magnesium sulfate. The solvent was then evaporated to give an oily residue which was chromatographed with silica gel (solvent 4:1 n-hexane / ethyl acetate) to give the desired product **41** (587 mg, 59%). $[\alpha]_D$=78.2° (c 1.06, $CDCl_3$).

^{1}H-NMR ($CDCl_3$): δ 7.40—7.20 (m, 30, Ph-H), 5.00 (d, 1H, H-1′, $J_{1',2'}$=3.5 Hz), 4.67 (d, 1H, H-1, $J_{1,2}$=3.5 Hz), 5.03—4.51 (m, 12H, 6 x Ph-CH_2), 4.27 (dd, 1H, H-6′, $J_{5',6'}$=4.5 Hz, $J_{6'a,6'b}$=−11.5 Hz), 4.21 (dd, 1H, H-6′b, $J_{5',6'b}$=2.5 Hz, $J_{6'b,6'a}$=−11.5 Hz), 4.08—3.91 (m, 4H, H-3, H-3′, H-6, H-5′), 3.78 (dd, 1H, H-5, $J_{5,6}$=0.5 Hz, $J_{4,5}$=10.0 Hz),

3.57—3.43 (m, 3H, H-4, H-4', H-2), 3.41 (dd, 1H, H-2', $J_{1',2'}$=3.5 Hz, $J_{2',3'}$=9.5 Hz), 3.29 (s, 3H, OCH_3), 1.99 (s, 3H, $OCOCH_3$), 1.16 (d, 3H, C_7H_3, $J_{6,7}$=6.5 Hz). ^{13}C-NMR ($CDCl_3$): δ 170.74 (C=O), 138.85—137.97 (t-Ph. Carbons); 97.76 (C-1' and C-1); 82.61, 81.76, 80.25, 80.03, 77.94, 77.64, 72.83, 72.57, 69.08 (9 Carbons: C-2, C-2', C-3, C-3', C-4, C-4', C-5, C-5', C-6), 63.27 (C-6), 55.13 (OCH_3); 20.84 ($OCOCH_3$), 16.55 (C-7)

Anal. calc. for $C_{58}H_{64}O_{12}$: C 73.09 H 6.77

found: C 73.46 H 6.61

Methyl 2,2',3,3',4,4'-hexa-O-benzyl-7-deoxy-6-O-(α-D-glucopyranosyl)-α-D-glycero-D-glucopyranoside (**42**)

Methyl 6'-*O*-acetyl-2,2',3,3',4,4'-hexa-*O*-benzyl-7-deoxy-6-*O*-(α-D-glucopyranosyl)-α-D-*glycero*-D-glucoheptapyranoside (**41**) (440 mg, 0.462 mmol) was dissolved in dry methanol (20 mL). A catalytic amount of sodium methoxide was added to the solution and the solution was stirred at room temperature overnight. The reaction progress was followed by t.l.c. (solvent system 3:2 n-hexane:ethyl acetate, R_f **42**=0.42). When the reaction was over, resin (IRC-50-H, washed with water and methanol) was added and stirred until the mixture turned acidic (pH paper). Then the mixture was filtered and the solvent was evaporated to give an oily residue which was dried under vacuum overnight to give **42** (347 mg, 83%). $[\alpha]_D$=65.2° (c .34, H_2O).

^{1}H-NMR ($CDCl_3$): δ 7.40—7.20 (m, 30H, Ph-H), 5.00 (d, 1H, H-1', $J_{1',2'}$=4.5 Hz), 4.98—4.53 (m, 13H, 6 x Ph-CH_2 and H-1), 4.08—3.91 (m, 3H, H-3, H-3' and H-6), 3.81—3.67 (m, 4H, H-5, H-5' and 2 x H-6'), 3.56—3.37 (m, 4H, H-4, H-4', H-2 and H-2'), 3.29 (s, 3H, OCH_3), 1.13 (d, 3H, C_6-CH_3, $J_{6,7}$=6.5 Hz). ^{13}C-NMR ($CDCl_3$): δ 138.85—138.27 (t-Ph. Carbons); 129.07—125.33 (Ph. Carbons); 97.89 (C-1'); 95.37 (C-1); 82.53, 81.52, 80.19, 77.64, 77.57, 73.14, 71.43, 71.32 (9 Carbons: C-2, C-2', C-3, C-3', C-4, C-4', C-5, C-5', C-6); 61.73 (C-6'); 55.09 (OCH_3), 16.38 (C-7).

Anal. calc. for $C_{56}H_{62}O_{11}$: C 73.82 H 6.86

found: C 73.23 H 7.23

Methyl 7-deoxy-6-O-(α-D-glucopyranosyl)-α-D-glycero-D-glucoheptapyranoside (**43**)

Compound **42** (247 mg, 0.27 mmol) was first dissolved in dry methanol (25 mL). Then 5% palladium-on-charcoal (254 mg) was added in small portions to the solution and the mixture was shaken under hydrogen (100 lb. in^{-2}) at room temperature and the reaction progress was followed by t.l.c. (solvent 4:1 dichloromethane / methanol). When the reaction was over, the mixture was filtered and the filtrate was evaporated to give a yellowish solid residue. The crude residue was chromatographed (Iatrobeads, solvent 4:1 dichloromethane / methanol) to give colorless crystalline **43** (80 mg, 80%). Mp 231-232°C. $[\alpha]_D$=228.1° (c .43, H_2O).

^{1}H-NMR (D_2O): δ 4.80 (d, 1H, H-1, $J_{1,2}$=4.0 Hz), 3.57 (dd, 1H, H-2, $J_{1,2}$=4.0 Hz, $J_{2,3}$=9.5 Hz), 3.67 (dd, 1H, H-3, $J_{2,3}$=9.5 Hz, $J_{3,4}$=9.0 Hz), 3.39 (dd, 1H, H-4, $J_{4,5}$=10.5 Hz, $J_{4,3}$=9.0 Hz), 3.83 (dd, 1H, H-5, $J_{4,5}$=10.5 Hz, $J_{5,6}$=2.5 Hz), 4.17 (dq, 1H, H-6, $J_{5,6}$=2.5 Hz, $J_{6,7}$=6.5 Hz), 3.44 (s, 3H, OCH_3), 1.29 (d, 3H, C_6-CH_3, $J_{6,7}$=6.5 Hz), 5.07 (d, 1H, H-1′, $J_{1',2'}$=3.5 Hz), 3.55 (dd, 1H, H-2′, $J_{1',2'}$=3.5 Hz, $J_{2',3'}$=9.5 Hz), 3.73 (dd, 1H, H-3′, $J_{2',3'}$=9.5 Hz, $J_{3',4'}$=10.0 Hz), 3.41 (dd, 1H, H-4′), 3.88 (dd, 1H, H-6′a, $J_{6'a,6'b}$=– 14.5 Hz, $J_{6'a,5'}$=5.0 Hz), 3.76 (dd, 1H, H-6′b, $J_{6'a,6'b}$=– 14.5 Hz, $J_{6'b,5'}$=4.0 Hz). ^{13}C-NMR (D_2O): δ 100.13 (C-1′); 97.98 (C-1); 73.89, 73.73, 73.37, 72.98, 72.02, 71.89, 71.80, 71.48 (C-2—C5 and C-2′—C5′); 70.51 (C-6′), 56.09 (OCH_3), 15.12 (C-7).

Anal. calc. for $C_{14}O_{11}H_{25}$: C 45.53 H 6.82

found: C 45.18 H 6.97

3. Enzyme hydrolysis experiments

All enzyme hydrolysis experiments were performed on the Cary model 214 and the Bruker WM-360 spectrometer operating at 360 MHz at preset temperatures. The buffers used were potassium phosphate buffer at pH=6.8, M.E.S. buffer at pH=6.5, sodium acetate–acetic acid in D_2O at pD=4.3 sodium acetate-d_3–acetic acid-d_4 in D_2O at pD=4.3. The enzyme hydrolysis data were analyzed by Dr. M. Palcic and Mr. L. Steele in the Food Science Department on the PC using computer programs developed by Professor K.Bock and co-workers from the Department of Organic Chemistry, the Technical University of Denmark. The enzyme Isomaltase, ATP and NADP were purchased from Aldrich. The enzymes Hexokinase and 6-Phosphate Dehydrogenase were provided by Dr. M. Palcic. AMG type II was a gift from Professor K. Bock.

The method, as recommended by Bock,[85] is the application of high resolution ^{1}H-NMR spectroscopy to monitor substrate and product time depencencies in progress curve enzyme kinetics.[112-116] This method has an advantage since kinetic parameters can be estimated in a single run therefore saving both time and substrate material. The disadvantages are: (i) in general, mechanistic properties cannot be inferred directly from a single progress curve, and (ii) errors in the estimated parameters may be significant, depending on the experimental conditions and the method of data analysis.

The data were obtained as follows. The ^{1}H-NMR spectrum of the substrate in buffered solution was taken as standard at t=0. Then the enzyme in buffered solution was added and the reaction progress was followed by recording ^{1}H-NMR spectra at predetermined times until the reaction was over. The time dependent spectra were plotted and the concentration of the substrate and the product(s) were obtained from the integrations of the appropriate peaks. The concentration of substrate vs. time progress curve was constructed and analyzed on IBM personal computers using a non-linear least squares

fitting program package REGGRAFA obtained from Professor Bock. The time dependent concentration data are summarized in the next section.

The progress curve analysis[85] is based on the integrated and reparameterized Michaelis-Menten equation (Equation 5).

$$t = (\chi\,|y_o - y'|/v_o)\,A\,[\chi + \ln(1-\chi)] \qquad (5)$$

Where $\chi = (y_o - y)/(y_o - y')$

$A = (2v_o t_{1/2} - |y_o - y'|)/(v_o(1 - \ln 4))$

v_o = initial rate

$t_{1/2}$ = half time ($y(t_{1/2}) = 1/2[y_o - y']$

y_o = initial concentration

y' = final concentraion

The χ is the degree of progressed reaction and it followed from the definition above that χ always be in the interval $0 < \chi < 1$.

Equation 5 is then rearranged to Equation 6 to calculate the values of concentration (dependent variable) as a function of time (independent variable).

$$y = (y' + (y_o - y'))\exp[((v_o t - \chi\,|y_o - y'|)/Av_o) - \chi] \qquad (6)$$

The equation 6 is solved by computer by the bisection method.

All experiments with methyl α-isomaltoside (**37**), methyl 6-*S*-methyl-α-isomaltoside (**40**) and methyl 6-*R*-methyl-α-isomaltoside (**43**) were performed under exactly the same conditions.

VII. APPENDIX

Table 18. The time dependent concentration data for methyl α-isomaltoside (**37**) in the course of hydrolysis catalyzed by AMG II.

Time (min.)	**Conc.** (mM)
0.00	18.52
9.18	18.31
23.83	17.46
47.47	16.15
71.30	15.96
95.03	15.41
118.77	14.09
142.50	12.89
166.23	12.85
189.97	11.91
213.70	9.69
237.34	9.57
261.17	8.28
284.90	7.39
308.63	6.85

Table 19. The time dependent concentration data for methyl α-isomaltoside (**37**) in the course of hydrolysis catalyzed by AMG II.

Time (min.)	**Conc.** (mM)
0.00	9.26
31.33	8.38
55.13	7.37
78.93	7.10
102.73	5.94
126.53	5.78
150.33	5.03
174.13	4.39
197.93	3.56
221.73	3.02
245.53	2.80
269.33	2.28
293.13	1.76
352.63	1.03
412.13	0.75
471.63	0.19

Table 20. The time dependent concentration data for methyl 6-*S*-methyl-α-isomaltoside (**40**) in the course of hydrolysis catalyzed by AMG II.

Time (min.)	**Conc.** (mM)
0.00	9.26
8.20	8.67
47.93	8.71
97.60	8.03
147.27	7.45
196.93	7.29
246.60	6.18
296.27	5.46
345.93	5.55
395.27	5.40
444.93	4.82
494.60	4.61
544.27	4.61
593.93	4.17
643.60	3.88
693.27	3.73
742.93	3.33
792.60	3.12
843.27	2.95
892.93	2.76
894.93	2.69
934.66	2.56
984.33	2.34
1092.00	2.31
1105.60	2.15
1145.35	1.97
1195.00	1.92
1244.70	1.83
1294.35	1.41
1344.00	1.56
1443.00	1.56
1483.00	1.37
1727.00	1.07
1816.00	0.89
1916.00	0.93
2015.00	0.70
2114.00	0.67
2213.00	0.84
2313.00	0.59
2412.00	0.59
2512.00	0.41
2612.00	0.30

Table 21. The time dependent concentration data for methyl 6-*S*-methyl-α-isomaltoside (**40**) in the course of hydrolysis catalyzed by AMG II.

Time (min.)	**Conc.** (mM)
0.00	18.52
57.00	17.28
118.42	15.89
179.83	15.09
241.25	13.80
302.67	12.96
364.08	11.93
425.50	11.00
468.42	10.28
548.33	9.52
609.75	9.09
780.17	7.28
829.58	6.83

Table 22. The time dependent concentration data for methyl 6-*R*-methyl-α-isomaltoside (**43**) in the course of hydrolysis catalyzed by AMG II.

Time (min.)	**Conc.** (mM)
0.00	18.52
8.33	17.96
20.33	17.31
32.33	16.80
44.33	16.07
56.33	15.26
68.33	14.61
80.33	13.85
92.33	13.70
104.33	11.96
116.33	11.37
128.33	10.37
140.33	9.69
147.38	9.61
159.38	8.57
171.38	8.50
183.38	7.44
195.38	6.59
207.38	5.41
219.38	4.98
231.38	4.07
243.38	3.50
255.38	2.41
267.38	1.89
279.38	1.39

Table 23. The time dependent concentration data for methyl 6-*R*-methyl-α-isomaltoside (**43**) in the course of hydrolysis catalyzed by AMG II.

Time (min.)	**Conc.** (mM)
0.00	9.26
6.50	8.65
20.28	7.93
30.25	6.97
40.25	6.34
60.25	4.27
70.25	3.97
80.25	3.19
90.25	2.56
100.25	1.80
110.25	1.15
120.25	0.55
130.25	0

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tion data of methyl α-D-glucopyranoside.[76] The methyl groups were generated at C-6 and O-1 using the TRANSF.OBJ program. The ω' angle of the non-reducing unit was set at 180^{o} or 300^{o} as required and the ω angle of the reducing unit was rotated in 5^{o} or 10^{o} step using the GENT.OBJ program. HSEA calculations were performed for the methyl α-isomaltoside, the methyl 6-*S*-methyl-α-isomaltoside (**40**) and the methyl 6-*R*-methyl-α-isomaltoside (**43**) with the torsion angles ω' and ω set at the predetermined values mentioned above and the torsion angles Φ and Ψ were varied until the minimum energies were obtained. The results are shown in Table 11.

Table 11. HSEA calculations results of the methyl α-isomaltoside (**37**) and the diastereomeric 6-C-methyl α-isomaltosides (**40** and **43**).

Compounds	Torsional Angles, ^{o}					Internuclear Distances, A	
	ω'	Φ	Ψ	ω	**E** (Kcal/mol)	O-4–O-4′	O-4–O-6′
37	180	−50	195	180	−2.29	5.08	5.30
	300	−45	240	60	−1.90	4.51	4.90
	300	−50	190	180	−2.45	5.16	3.53
	300	−50	200	300	−1.74	8.25	6.41
40	180	−50	195	180	−1.46	5.10	5.30
	300	−50	180	300	−0.28	5.35	4.02
43	180	−50	90	300	−1.26	8.17	7.10
	300	−50	90	300	−1.81	8.17	5.68

Recalculation of the methyl α-maltoside in conformation obtained previously[8] where $\Phi/\Psi = -25^{o}/-15^{o}$ with $\omega' = 180^{o}$ and $\omega = 300^{o}$ gave an energy minimum of 1.35 Kcal. Because of the polar hydroxyl groups OH-3, OH-4′ and OH-6′ were involved in the hydrolyses catalyzed by the enzyme Amyloglucosidase Type II, the distances between these groups were also calculated for methyl α-maltoside and will be discussed in Section

IV.

HSEA calculations predicts that methyl α-isomaltoside (**37**) prefers the conformation with the torsion angles $\omega'/\Phi/\Psi/\omega = 300^{o}/-50^{o}/190^{o}/180^{o}$; methyl 6-*S*-methyl-$\alpha$-isomaltoside (**40**) prefers the conformation with the torsion angles $\omega'/\Phi/\Psi/\omega = 180^{o}/-50^{o}/195^{o}/180^{o}$ and methyl 6-*R*-methyl-α-isomaltoside (**43**) prefers the conformation with the torsion angles $\omega'/\Phi/\Psi/\omega = 180^{o}/-50^{o}/90^{o}/300^{o}$. The minimum energy conformations of **40** and **43** were plotted and reproduced in Figure 35 and 36 (p. 78 and 79, respectively).

The torsion angles $\omega = 180^{o}$ and 300^{o} for **40** and **43** correspond to the $\mathbf{g}^{-}$ and $\mathbf{g}^{+}$ conformers respectively for the C-5/C-6 bond in the reducing unit. The HSEA calculations therefore predicts that the conformation at the C-6 of the reducing unit will be retained for the methyl 6-*S*-methyl-α-isomaltoside (**40**) and methyl 6-*R*-methyl-α-isomaltoside (**43**).

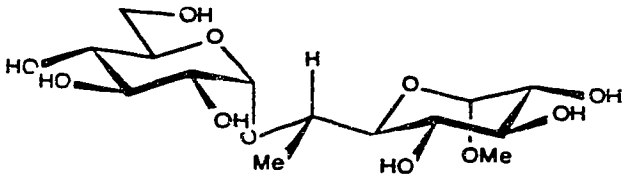
2.4. Conclusions

From conformational analysis of the monosaccharide derivatives, it was concluded that at the C-6 center, methyl 6-*S*-methyl-α-D-glucopyranoside (**15**) preferred the $\mathbf{g}^{-}$ rotamer and methyl 6-*R*-methyl-α-D-glucopyranoside (**21**) preferred the $\mathbf{g}^{+}$ rotamer. The conformations are also favored even at higher temperatures. It was expected that these conformations would also be favored in the disaccharide derivatives. The conformations at the C-6 center of the diastereoisomeric methyl 6-*S*-methyl-α-isomaltoside (**40**) and methyl 6-*R*-methyl-α-isomaltoside (**43**) were determined to be mainly $\mathbf{g}^{-}$ and $\mathbf{g}^{+}$ respectively.

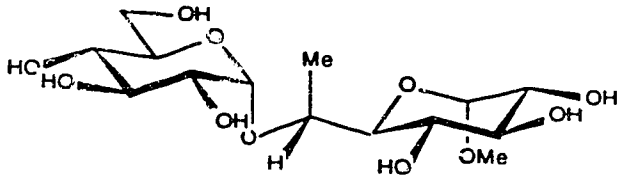
IV. ENZYME STUDIES

1. The Enzymes

Two enzymes Isomaltase and Amyloglucosidase Type II were used in kinetic studies of the hydrolysis of the methyl α-isomaltoside (**37**) and its C-6 methyl derivatives; namely methyl 6-*S*-methyl-α-isomaltoside (**40**) and methyl 6-*R*-methyl-α-isomaltoside derivatives (**43**).



40



43

The enzyme Isomaltase (EC 3.2.1.10)[78-83] is found in the bean, potato,[81] autolyzed brewer's yeast[82] and muscle.[83] This enzyme is used especially to cleave the α-D-(1→6) linkage and therefore plays an important role in the depolymerization of amylopectins and glycogens. Because of this specificity, the enzyme Isomaltase was selected to be used in the hydrolysis studies.

The enzyme Amyloglucosidase (EC 3.2.1.3),[84-87] which is produced by the microorganism *Aspergillus niger*, consists of two forms: Amyloglucosidase Type I (AMG I) and Amyloglucosidase Type II (AMG II).[88,89] Both enzymes are reported to be glycoproteins containing D-mannose, D-glucose and D-galactose.[90] Although the enzymes have been characterized and the amino acid sequence has been determined,[26,87,90-100] the enzymes have not crystallized.

The AMG enzymes are used in the processing of starch because of their abilities to catalyze hydrolytic cleavage of both the α-D-(1→4) linkage between glucopyranose units such as is present in maltose and α-D-(1→6) linkage between glucopyranose units such as is present in isomaltose. Both enzymes are stable up to 60°C with little loss of activity for about 30 min., but are rapidly inactivated at higher temperature. The enzymes exhibit nearly identical pH profiles with starch as the substrate with the pH optimum at ~4.5. Both enzymes hydrolyze the α-D-(1→4) linkage approximately 40 times faster than the α-D-(1→6) linkage.[90]

Our attention was turned to the enzyme AMG II primarily because Bock and coworkers[26,97-100] had made a systematic study of the effects of substitutional changes on maltose on the kinetics of the hydrolysis in which the reactions were followed using ^{1}H-NMR techniques.[100] They found that the AMG II active site requires accommodation of both the D-glucopyranose units of maltose since the enzyme hydrolyzes methyl β-maltoside but not methyl β-D-glucopyranoside.

In the hydrolysis of the various derivatives catalyzed by AMG II, it was shown[99] that the enzyme can accept a wide range of modifications as well as larger substrates. The competitive experiments showed that inhibitions in the reaction of the methyl β-maltoside were observed with the 6-chloro- or 6-fluoro- derivatives. It was concluded that the 6-chloro- or 6-fluoro- derivatives were bound very tightly to the active site in order to inhibit the reactions.[99] Based on qualitative data for hydrolysis,[97] catalyzed by AMG II, of the mono- and several dideoxy derivatives of methyl β-maltoside, Bock *et al.*[97,99] concluded that three hydroxyl groups, OH-3 in the reducing glucopyranose unit, OH-4′ and OH-6′ in the non-reducing glucopyranose unit, are essential for AMG II catalyzed hydrolysis. These findings are depicted in Figure 37(a). Evidence for this conclusion was further supported when the pentadeoxy derivative (Figure 37(b)), in which only the key polar groups were present (OH-3, OH-4′ and OH-6′), was found to be a substrate for the

enzyme AMG II. The HSEA calculated minimum energy conformation of methyl α-maltoside,[101,103] which is supported by ^{1}H-NMR and ^{13}C-NMR data, presents hydrophobic area defined by H-2', H-4', H-1' and O-5' of the non-reducing unit and H-4 as well as the hydroxymethyl group of the reducing unit which is expected to possess substantial conformational rigidity. Both the three key hydroxyl groups and the hydrophobic area were regarded as essential for AMG II hydrolysis by Bock *et al.*[99] and are in support of the hydrated polar-group gate theory proposed by Lemieux.[104]

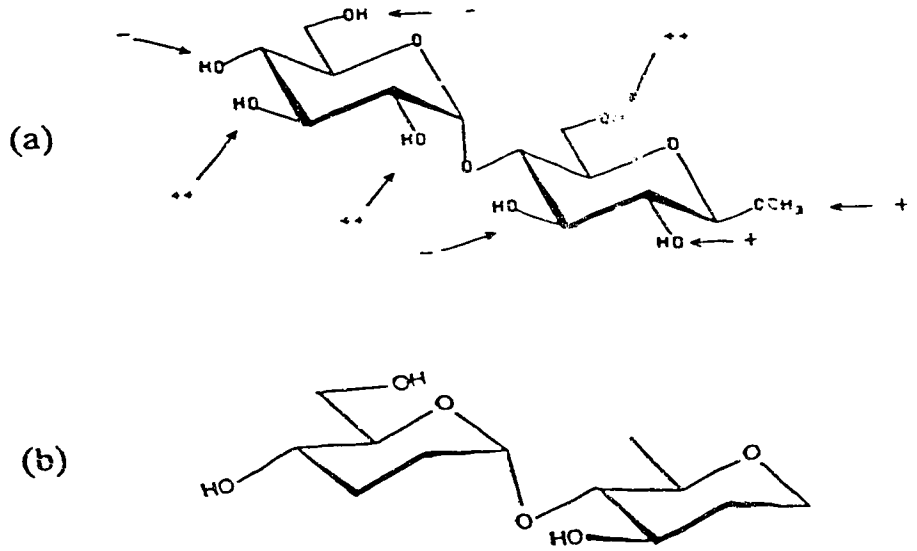


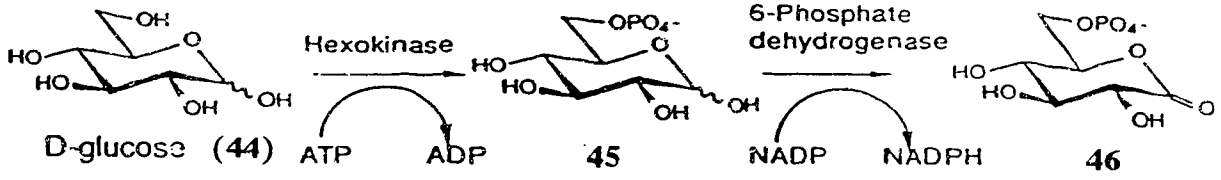
Figure 37. (a) Substrate specificity for Amyloglucosidase II (AMG II)[99] for different deoxy derivatives of maltose. + indicates substrate activity. – indicates no substrate activity and + + indicates compounds which are hydrolyzed faster than methyl β-maltoside. (b) Pentadeoxy derivative of maltose which preserves the key polar hydroxyl groups for hydrolysis catalyzed by AMG II.

2. Enzyme Kinetic Studies

2.1. Preliminary Studies

A collaboration was established with Dr. Monica Palcic of the Department of Food Science, University of Alberta for a kinetic study of the hydrolysis of **37**, **40** and **43**. Initially, it was thought that the most suitable method was to follow the reaction progress by using uv-vis spectroscopy.

The uv-vis spectroscopy technique involved the coupling method with the product(s) of the reaction. In the hydrolysis of methyl α-isomaltoside and the methyl 6-*C*-methyl-α-isomaltoside derivatives catalyzed by AMG II, the possible products of the hydrolysis reactions are D-glucopyranose and the 6-*C*-methyl-α-D-glucopyranose derivatives. The method employed for the detection of free glucopyranose (**44**)[105] released in the enzyme hydrolysis of the dissaccharides is shown in Scheme 21.



Scheme 21. Outline of the colorimetric coupled enzyme method for the determination of D-glucose.

The coupled-enzyme detects free glucopyranose by first a phosphorylation of the 6-hydroxyl by ATP in the presence of Hexokinase to produce D-glucopyranose-6-phosphate (**45**). This phosphate is then converted to the lactone (**46**) by 6-Phosphate Dehydrogenase in the presence of NADP (Scheme 21) which is detected due to the color change at $\lambda = 340$ nm as the result of the formation of the NADPH.

The monosaccharides D-glucopyranose (**44**), 6-*S*-methyl-D-glucopyranose (**47**) and 6-*R*-methyl-D-glucopyranose (**48**)[106] were then tested with Hexokinase and 6-Phosphate Dehydrogenase in the presence of ATP and NADP[105] and the results are shown in Table 12.

Table 12. Reaction of the monosaccharides derivatives with Hexokinase and 6-Phosphate Dehydrogenase in the presence of ATP and NADP.

Compounds	Reaction	Rel. Vel.
D-glucopyranose (**44**)	+	2.0
6-*S*-methyl-D-glucopyranose (**47**)[106]	-	–
6-*R*-methyl-D-glucopyranose (**48**)[106]	+	1.0

In contrast with D-glucopyranose (**44**) and 6-*R*-methyl-D-glucopyranose (**48**), the 6-*S*-methyl-D-glucopyranose (**47**) did not react. As discussed above in connection with Scheme 22, 6-Phosphate Dehydrogenase does not dehydrogenate D-glucopyranose. Instead the glucose must first be phosphorylated at the 6-OH position to produce **45**. Therefore it is possible the 6-*S*-methyl-D-glucopyranose resists phosphorylation at the 6-OH position. Conformational analyses presented in the previous section showed that both α-D-glucopyranose and 6-*R*-methyl-D-glucopyranose (**44** and **48**, respectively) prefer the $\mathbf{g}^+$ conformation for the orientation of the 6-OH whereas the $\mathbf{g}^-$ is preferred for the 6-*S*-methyl-D-glucopyranose (**47**) (see Figure 38).

Since it can be expected that the enzyme will phosphorylate the 6-hydroxyl of the glucopyranose in a specific conformation the reason for the high resistance to phosphorylation exhibit by the *S*-compound (**47**) can be attributed to the low abundance of the $\mathbf{g}^+$ conformer. However, it is possible that the 6-*C*-methyl group also contributes to the inactivity by hindering the formation of the enzyme-substrate complex.

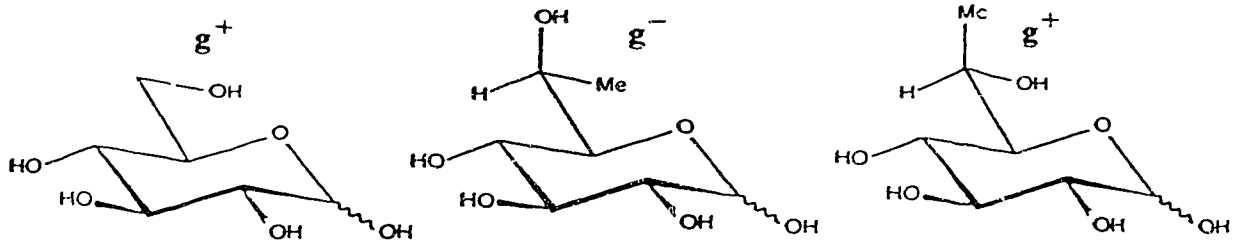


Figure 38. The preferred conformations of D-glucopyranose (**44**), 6-*S*-methyl-D-glucopyranose (**47**) and 6-*R*-methyl-D-glucopyranose (**48**)

In the hydrolyses reactions of the disaccharide derivatives, the various monosaccharides were used for standardization before the disaccharides were tested. Isomaltase and Amyloglucosidase Type II were used and the results of the preliminary studies are summarized in Table 13, 14 (the data are summarized in the Appendix).

The results presented in Table 13 and 14 were obtained with only one assay at fixed concentration (100 mM) per substrate. The rates (ΔOD min^{-1}) were only rough approximations because the objective was to see if a trend existed in the hydrolysis catalyzed by the enzyme that warranted further investigation.

Table 13. Hydrolysis catalyzed by Isomaltase at 37°C and pH=6.8.*

Compounds	Isomaltase hydrolysis	Rel. Rate (ΔOD min^{-1})
Phenyl α-D-glucopyranoside	+	39
Methyl α-D-glucopyranoside (**1**)	+	2
Methyl 6-*S*-methyl-α-D-glucopyranoside (**15**)	–	–
Methyl 6-*R*-methyl-α-D-glucopyranoside (**21**)	v. slight	–
Methyl α-isomaltoside (**37**)	+	1
Methyl 6-*S*-methyl-α-isomaltoside (**40**)	v. slight	–
Methyl 6-*R*-methyl-α-isomaltoside (**43**)	–	–

*(+) sign indicates that a reaction took place, a (—) sign indicates no reaction. Rel. Rate was measured in unit OD min^{-1}. Rel. Rate is the rate relative to compound **37**.

Table 14. Hydrolysis catalyzed by AMG II at 37°C and pH=4.3.*

Compounds	AMG Hydrolysis	Rel. Rate
Methyl α-D-glucopyranoside (**1**)	–	–
Methyl 6-*S*-methyl-α-D-glucopyranoside (**15**)	v. slight	1.4
Methyl 6-*R*-methyl-α-D-glucopyranoside (**21**)	v. slight	1
Isomaltose	+	67.5
Methyl α-isomaltoside (**37**)	+	81.3
Methyl 6-*S*-methyl-α-isomaltoside (**40**)	+	31.3
Methyl 6-*R*-methyl-α-isomaltoside (**43**)	+	112.5

*(+) sign indicates a reaction took place and a (–) sign indicates no reaction. Rel. Rate was measured in unit OD min^{-1}

As seen in Table 13, phenyl α-D-glucopyranoside, a known substrate for Isomaltase, is hydrolyzed 39 times faster than methyl α-isomaltoside (**37**) and about 20 times faster than methyl α-D-glucopyranoside (**1**). The monosaccharides **15** and **21** and the disaccharides **40** and **43** were shown not to be substrates for Isomaltase since there were no detectable or very slight rates in the hydrolysis.

In the case of hydrolysis catalyzed by AMG II (Table 14), methyl 6-*S*-methyl-α-D-glucopyranoside (**15**) was hydrolyzed 1.4 times faster than the methyl 6-*R*-methyl-α-D-glucopyranoside (**21**) and methyl α-D-glucopyranoside was not a substrate. It is seen that the disaccharides **40** and **43** proved to be substrates for this enzyme. The fact that the *R*-disaccharide **43** was superior to **37** as a substrate whereas **40** was less effective set the basis for this investigation.

2.2. The kinetic studies of the hydrolysis of methyl α-isomaltoside and 6-C-methyl-derivatives catalyzed by AMG II.

It was desirable to examine the hydrolysis of the methyl α-isomaltoside (**37**) and the methyl 6-*C*-methyl-α-isomaltoside (**40** and **43**) catalyzed by AMG II by the uv-vis spectroscopy. Unfortunately, the optimum pH required for the hydrolysis catalyzed by AMG II is 4.3 which is too acidic for the Hexokinase/6-Phosphate Dehydrogenase coupling assay (optimum pH=6.8). Furthermore, substrates which were synthesized in limited quantity may be required for further investigations. Since it was shown by Bock *et. al.*[26,97-101,106] that the progress of the hydrolysis of methyl β-maltoside and derivatives catalyzed by AMG II could be followed by ^{1}H-NMR spectroscopy, it was decided that further enzyme kinetic investigations would involved the use of ^{1}H-NMR spectroscopy. The method allows the observation of the disappearance of the disaccharide and the formation of the two monosaccharides simultaneously as a function of time. In the hydrolysis of methyl α-isomaltoside (**37**), it was possible to observe the decreasing signals of H-1′ at δ=4.95 ppm and H-1 at δ=4.85 ppm of the disaccharide and the increasing signals of H-1 of the products; namely, β-D-glucopyranose at δ=4.70 ppm and methyl α-D-glucopyranoside at δ=4.83 ppm simultaneously. For the methyl 6-*S*-methyl-α-isomaltoside (**40**), the disappearance of the broad quartet H-6 at δ=4.28 ppm of **40** and the appearance of the doublet of quartets H-6 of the methyl 6-*S*-methyl-α-D-glucopyranoside (**15**) at δ=4.05 ppm were also followed. For the methyl 6-*R*-methyl-α-isomaltoside (**43**), the disappearance of the doublet methyl signal at C-6 of compound **43** (δ=1.29 ppm) and the appearance of the doublet methyl signal of H-6 of methyl 6-*R*-methyl-α-D-glucopyranoside (**21**) at δ=1.10 ppm was also followed.

Representative spectra of the progress of the hydrolysis of the disaccharides; namely, methyl α-isomaltoside (**37**), methyl 6-*S*-methyl-α-isomaltoside (**40**) and methyl 6-*R*-methyl-α-isomaltoside (**43**) are reproduced in Figure 39, 40, and 41. Integrations of signals allowed

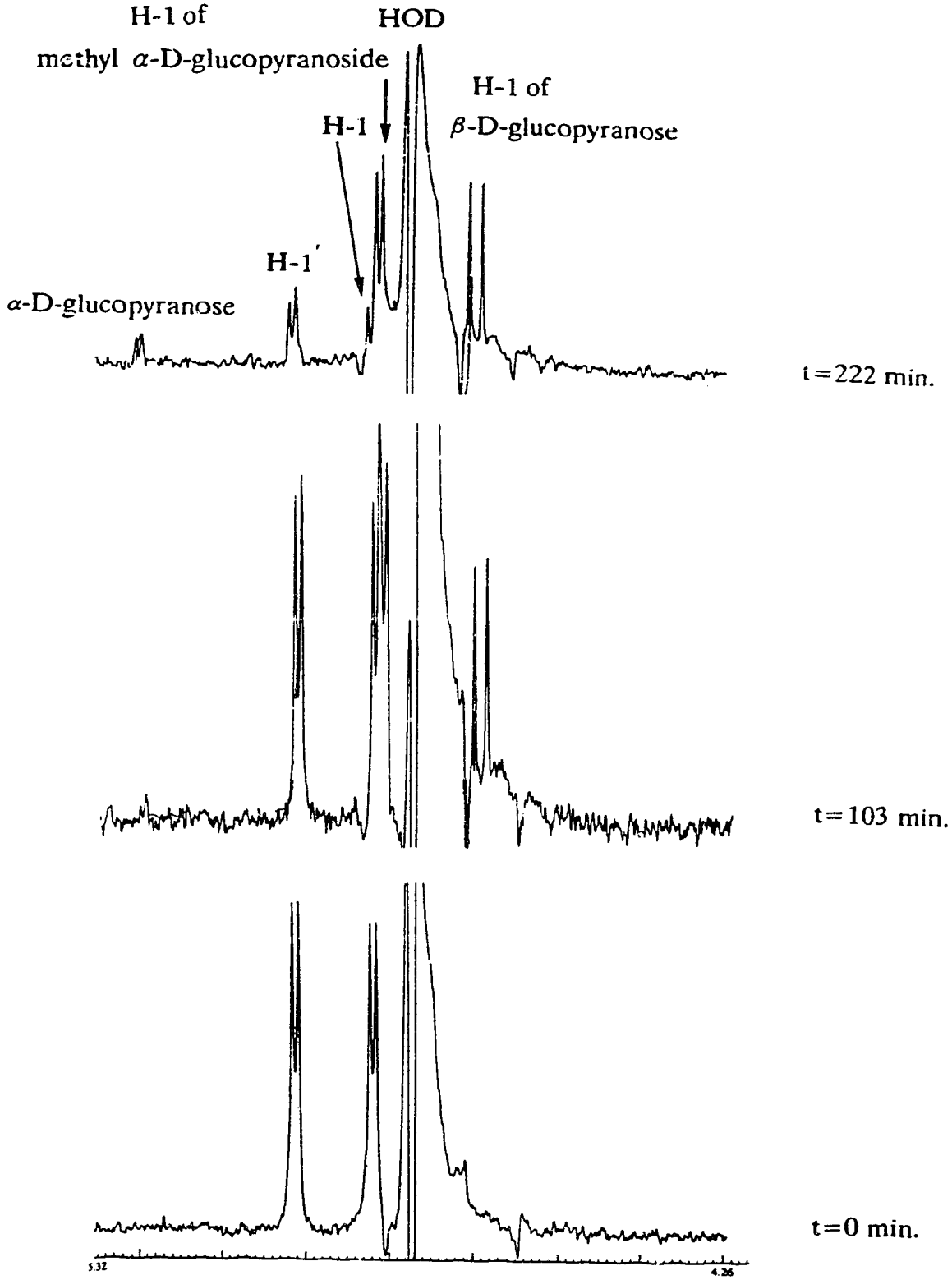


Figure 39. Reaction progress of the hydrolysis of methyl α-isomaltoside (**37**) catalyzed by AMG II with time.

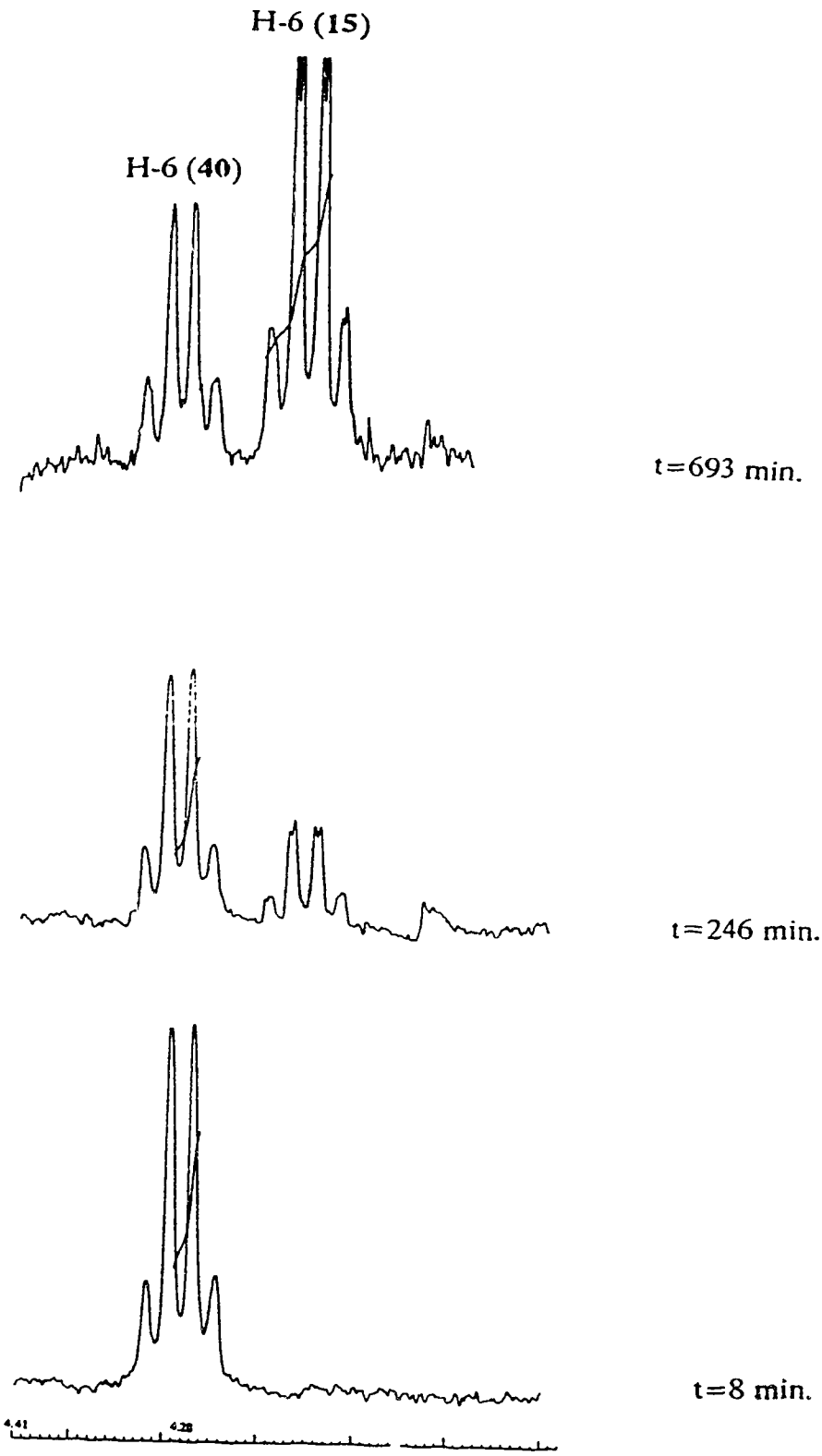


Figure 40. Reaction progress of the hydrolysis of methyl 6-S-methyl-α-isomaltoside (**40**) catalyzed by AMG II with time.

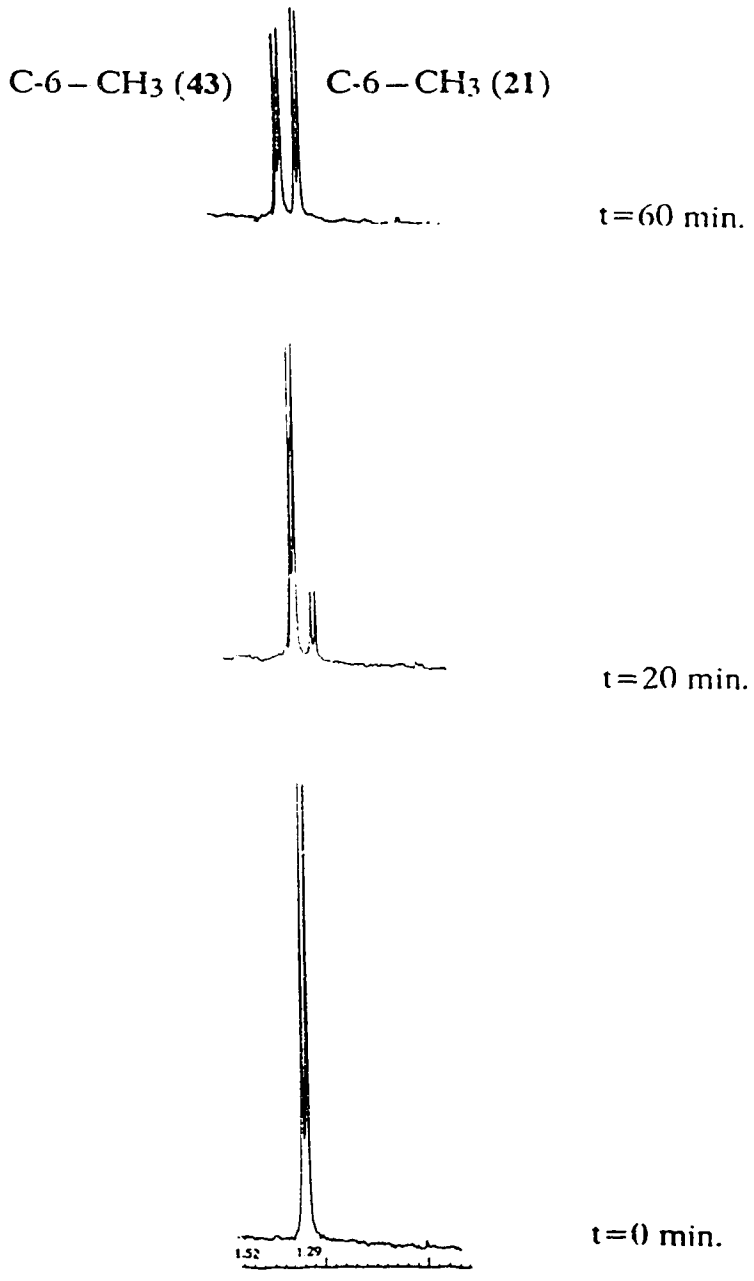


Figure 41. Reaction progress of the hydrolysis of methyl 6-R-methyl-α-isomaltoside (**43**) catalyzed by AMG II with time.

Table 15. Kinetic parameters for the disaccharide derivatives in the hydrolysis catalyzed by AMG II.

Substrate	Conc. (mM)	V_0 (mM/min)	$T_{1/2}$ (min)	V_{max} (mM/min)	K_{cat} (s^{-1})	K_m (mM)	K_{cat}/K_m ($M^{-1} s^{-1}$)
Methyl α-maltoside	9.26	1.6	3	1.6	2.6	—	—
Methyl β-maltoside	9.26	0.613	8.1	0.613	0.9	—	—
Methyl β-maltoside	18.52	0.727	14	0.727	1.2	—	—
Methyl 6-*S*-methyl-α-isomaltoside (**40**)	9.26	0.012	500	0.040	0.84	35	2.8
Methyl 6-*S*-methyl-α-isomaltoside (**40**)	18.52	0.027	575	—	—	—	—
Methyl α-isomaltoside (**37**)	9.26	0.033	161	0.043	0.069	2.9	23.8
Methyl α-isomaltoside (**37**)	18.52	0.04	247	0.04	0.064	2.2×10^{-4}	—
Methyl 6-*R*-methyl-α-isomaltoside (**43**)	9.26	0.081	63	0.083	0.13	0.22	600
Methyl 6-*R*-methyl-α-isomaltoside (**43**)	18.52	0.068	148	0.0688	0.11	0.22	500

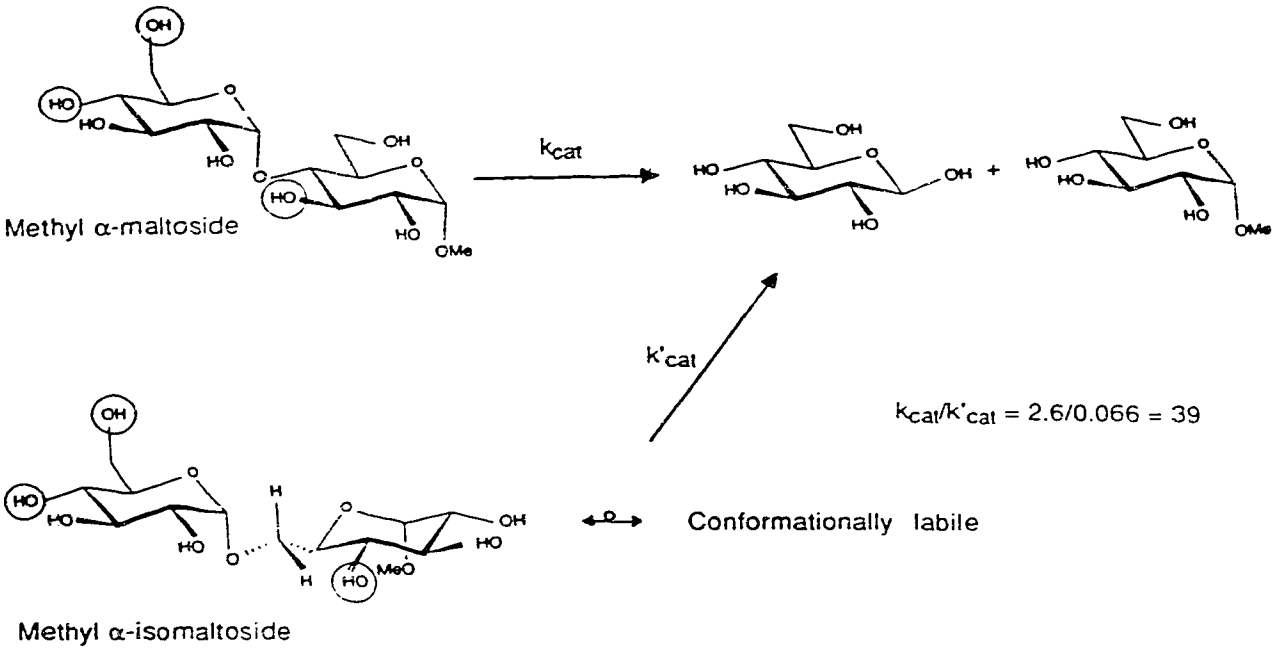


Figure 42. Methyl α-maltoside and methyl α-isomaltoside hydrolysis catalyzed by AMG II.

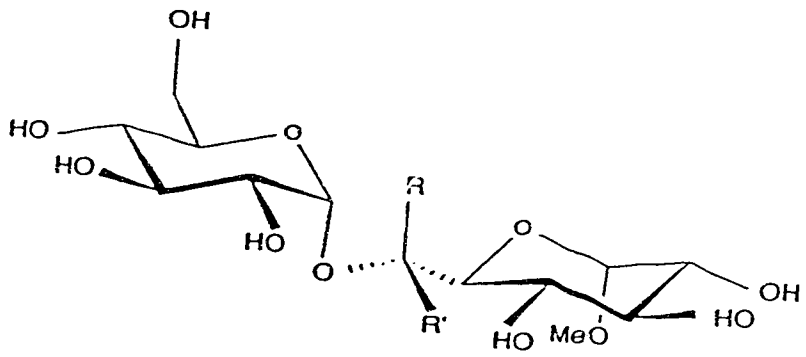
the construction of the progress curves (conc. vs. time). The data were analyzed using the REGGRAFA program[101] developed by Professor Bock and coworker on the personal computer. The results were reproduced in the experimental section. The parameters estimated from the progress curves are reproduced in Table 15.

The enzyme AMG II catalyzed hydrolysis of the disaccharide derivatives are irreversible[90] and, as mentioned above, gave two monosaccharides as products in which one was the methyl α-D-glucopyranoside or the C-6 methyl substituted derivatives and the other was the β-D-glucopyranose (Figure 42). The β-D-glucopyranose would then anomerized to the α-D-glucopyranose which could be observed by ^{1}H-NMR spectroscopy.

The general expression for a reaction catalyzed by an enzyme is:

$$E + S \rightleftharpoons [ES] \xrightarrow{k_{cat}} E + P$$

Table 16. Kinetic data of the hydrolysis catalyzed by AMG II of methyl α-isomaltoside (**37**) and the methyl 6-*C*-methyl isomaltoside derivatives (**40** and **43**).



Substituents at C-6			Population of the	Kinetic Data		Relative	$t_{1/2}$, min.
R	R'	Configuration	Key Conformer	k_{cat},sec^{-1}	Km, mM	$K_{Assoc.}$	9.26 mM
H	H	—	medium	0.069	2.9	12	160
Me	H	R	high	0.12	0.22	155	60
H	Me	S	low	0.84	35	1	500

The rate constant k_{cat} is the sum of all the rate constants of all subsequent steps after the formation of the enzyme-substrate complex ([ES]).

In the hydrolysis catalyzed by AMG II for methyl α-maltoside and methyl α-isomaltoside (Figure 42), the overall rates of hydrolysis were assumed to be k_{cat} for methyl α-maltoside and k'_{cat} for methyl α-isomaltoside. The overall rate ratio k_{cat}/k'_{cat} is about 39 and, therefore AMG II catalyzes the hydrolysis of methyl α-maltoside 39 times faster than methyl α-isomaltoside. This overall rate ratio is similar to the ratio for maltose and isomaltose. Because methyl α-isomaltoside is a poor substrate in comparision to methyl α-maltoside, it was assumed that the dissociation constant K_S for the [ES] complex is the same as the Michaelis-Menten constant K_M. This assumption may not be valid for all cases involved but can be made for poor substrates.[108]

Examination of Table 15 shows that the hydrolysis catalyzed by AMG II are in the decreasing order: methyl α-maltoside > methyl 6-*R*-methyl-α-isomaltoside (**40**) > methyl α-isomaltoside (**37**) > methyl 6-*S*-methyl-α-isomaltoside (**43**) and in agreement with the data reported in Table 14. The slightly faster rate of hydrolysis shown for methyl α-maltoside as compared to its β-anomer may not be real since reactions were too fast to be followed with precision. The kinetic data for the hydrolysis of methyl α-isomaltoside (**37**) and its epimeric 6-*C*-methyl derivatives (**40** and **43**) are presented in Table 16 along with comments regarding the key conformer to be discussed below.

The k_{cat} of methyl α-isomaltoside (**37**) is of the same order of magnitude as the k_{cat} found for methyl 6-*S*-methyl-α-isomaltoside (**40**) and approximately half of that found for methyl 6-*R*-methyl-α-isomaltoside (**43**). The variation in k_{cat} is minor (2 fold) compared to the differences in K_m, the major factor in determining the relative rates of reaction. The K_m values can be assumed to be a useful measure of the association constants $K_{Assoc.}$. The $K_{Assoc.}$ of methyl α-isomaltoside is about 12 times the $K_{Assoc.}$ of methyl 6-*S*-methyl-α-isomaltoside (**40**). On this basis, methyl 6-*R*-methyl-α-isomaltoside (**43**) is bound 13 times more strongly by the enzyme than is methyl α-isomaltoside and about 155 times more than is methyl 6-*S*-methyl-α-isomaltoside. It is seen that the stronger the binding the faster the hydrolysis (lower $T_{1/2}$).

2.3 The Key Hydroxyl Groups.

As discussed above with reference to Figure 37(a), Bock *et al.*,[26,97-101] showed that OH-6', OH-4' of the non-reducing unit and OH-3 of the reducing unit are necessary for the hydrolysis of methyl β-maltoside by the enzyme AMG II. This phenomenon was reminiscent of the findings by Lemieux and coworkers in their studies of the binding of oligosaccharides by lectins and antibodies[109] where they observed in each case that the

binding was dependent on the presence of a cluster of two or three hydroxyl groups termed the key polar grouping. It was shown by Lemieux *et al.*[8] that the α-(1 → 4) linkage in maltose are conformationally well defined by the torsion angles $\Phi^{H\text{-}1',C\text{-}4}/\Psi^{C\text{-}1',H\text{-}4} = -25^{o}/-15^{o}$. Therefore the key polar groups OH-4′ and OH-3 should be energetically well presented at a fixed separation in aqueous solution. As discussed above, the other key polar group OH-6′ is conformationally labile because of freedom of rotation about the C-5 to C-6 bond. Thus, the C-5/C-6′ fragment can readily adopt either the $\mathbf{g}^{-}$ or the $\mathbf{g}^{+}$ conformation (Figure 43) and it is not clear at present which conformer is required by the enzyme AMG II. For the present discussion, the $\mathbf{g}^{-}$ conformation at C-6′ is arbitrarily assumed.

The enzyme AMG II is expected to enter into a polar interaction with the key polar groups (Figure 37(a)) of methyl α-maltoside. In view of the conformational rigidity possessed by methyl α-maltoside,[8] the disposition of these hydroxyl groups can be expected to be near that presented by methyl α-maltoside in the minimum energy conformer which as seen in Fig. 44 is expected to be near 7.1 Å and 7.3 Å for the distance between O-4′ – O-3 and O′-6– O-3, respectively. On the basis of the key polar group theory, it is expected that the hydrolysis of the methyl α-isomaltoside (**37**) and its derivatives (**40** and **43**) will have key polar groups in similar disposition in space.

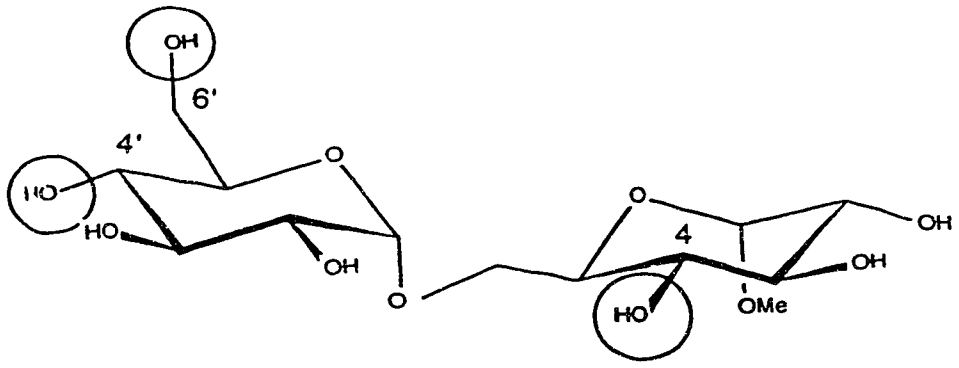


Figure 43. The OH-4, OH-4′ and OH-6′ (circled) groups of methyl α-isomaltoside which are expected to form the key polar grouping.

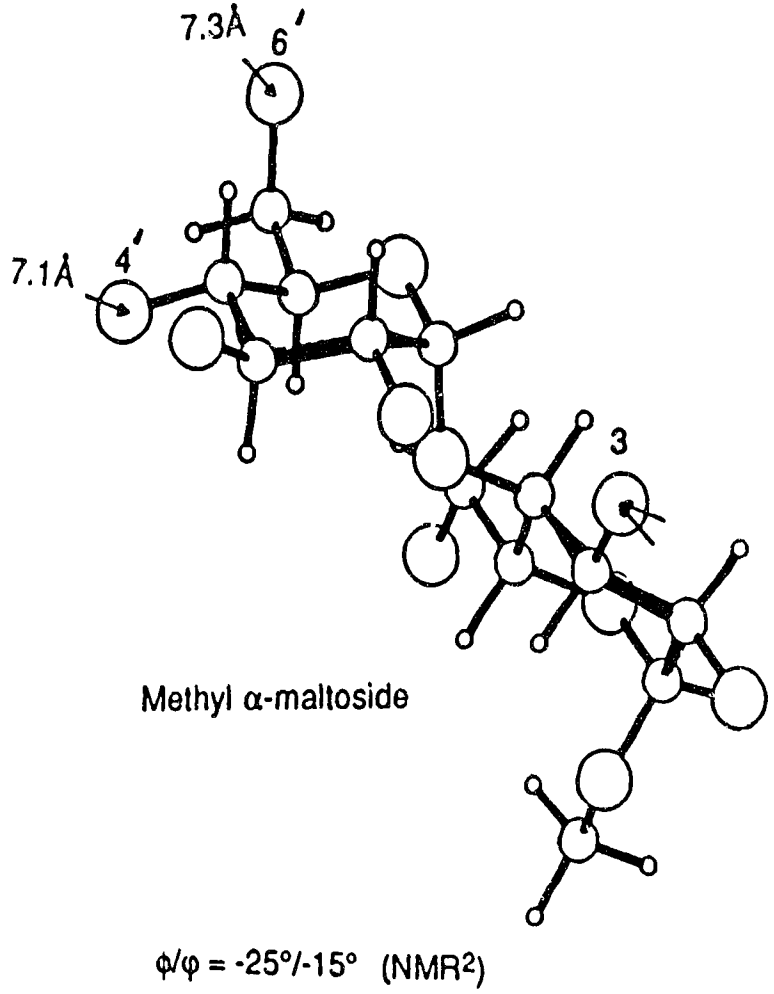
In the methyl α-isomaltoside and its derivatives series, the two key polar hydroxyl groups of the non-reducing unit will still be OH-6′ and OH-4′ but the key polar hydroxyl group of the reducing unit could not be OH-3 because it would be one bond too far away. The OH-4 would be most likely.

The key conformers with similar distances as the OH-3/OH-4′ and OH-3/OH-6′ of methyl α-maltoside were then searched for methyl α-isomaltoside (**37**), methyl 6-*R*-methyl-α-isomaltoside (**43**) and methyl 6-*S*-methyl-α-isomaltoside (**40**) using HSEA calculations. The variations in the estimated distances of the key conformers in the methyl α-isomaltoside and the 6-*C*-methyl derivatives compared to the key conformer of methyl α-maltoside are not more than 0.4 Å. The results are summarized in Table 17 and the key conformers were computer drawn in Figures 44 and 45.

According to HSEA calculations (see Table 17), the minimum energy conformation of methyl α-isomaltoside has the torsion angles $\omega' = 180^{o}$, $\Phi = -50^{o}$, $\Psi = 190^{o}$ and $\omega = 180^{o}$. The torsion angle $\omega = 180^{o}$ at C-6 corresponds to the $\mathbf{g}^{-}$ conformation and is in agreement with the experimental data obtained by Bock *et al.*[26] The key conformer with similar internuclear distances O-4– O-4′ =6.9 Å and O-4– O-6′ =7.1 Å (Table 17) has the torsion angles $\omega' = 180^{o}$, $\Phi = -50^{o}$, $\Psi = 120^{o}$ and $\omega = 50^{o}$. The key conformer of methyl α-isomaltoside is 1.49 Kcal mol^{-1} higher in energy than its minimum energy conformer and, therefore, can be expected on this basis to be a poorer substrate than methyl α-maltoside for which the key conformer is energetically very close to the preferred conformation. The key conformer for methyl 6-*R*-methyl-α-isomaltoside (**43**) with the same torsion angles ($\omega'/\Phi/\Psi/\alpha = 180^{o}/-50^{o}/120^{o}/50^{o}$) is only 0.31 Kcal mol^{-1} higher than its ^{1}H-NMR conformer. In contrast, the key conformer for methyl 6-*S*-methyl-α-isomaltoside (**40**) has the torsion angles $\omega' = 180^{o}$, $\Phi = -60^{o}$, $\Phi = 160^{o}$ and $\omega = 50^{o}$ with internuclear distances O-4–O-4′ =7.0 Å, O-4– O-6′ =7.7 Å and is 3.78 Kcal mol^{-1} higher in energy than its ^{1}H-NMR conformer (Figure 45).

Table 17. HSEA calculations for ^{1}H-NMR and Key conformers of methyl α-maltoside, methyl α-isomaltoside (**37**) and methyl 6-*C*-methyl-α-isomaltosides (**40** and **43**)

Compounds	Conformer	Torsion Angles, ^{0}	Energy	Internuclear Distances, Å	
		$\omega'/\Phi/\Psi/\omega$	Kcal/mol	O-4—O-4′	O-4—O-6′
Methyl α-isomaltoside (**37**)	NMR	180/−50/190/180	−2.45	5.2	3.5
	KEY	180/−50/120/180	−0.96	6.9	7.1
Methyl 6-*R*-methyl-α-isomaltoside (**43**)	NMR	180/−50/90/300	−1.26	8.2	7.1
	KEY	180/−50/120/50	−0.85	6.9	7.1
*Methyl 6-S-methyl-α-isomaltoside (**40**)*	*NMR*	*180/−50/195/180*	−1.46	5.1	5.3
	KEY	180/−60/160/50	2.32	7.0	7.7
Methyl α-maltoside	NMR	180/−25/−15/300	1.35	7.1 (O-3−O-4′)	7.3 (O-3−O-6′)



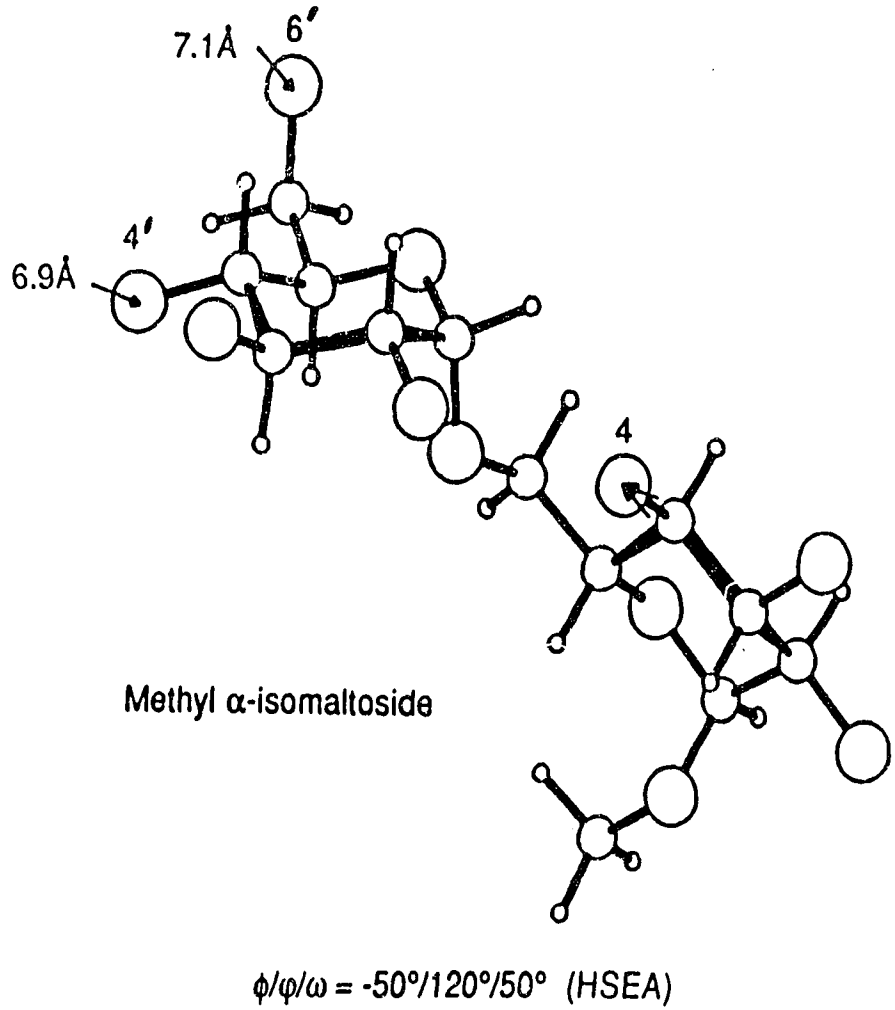
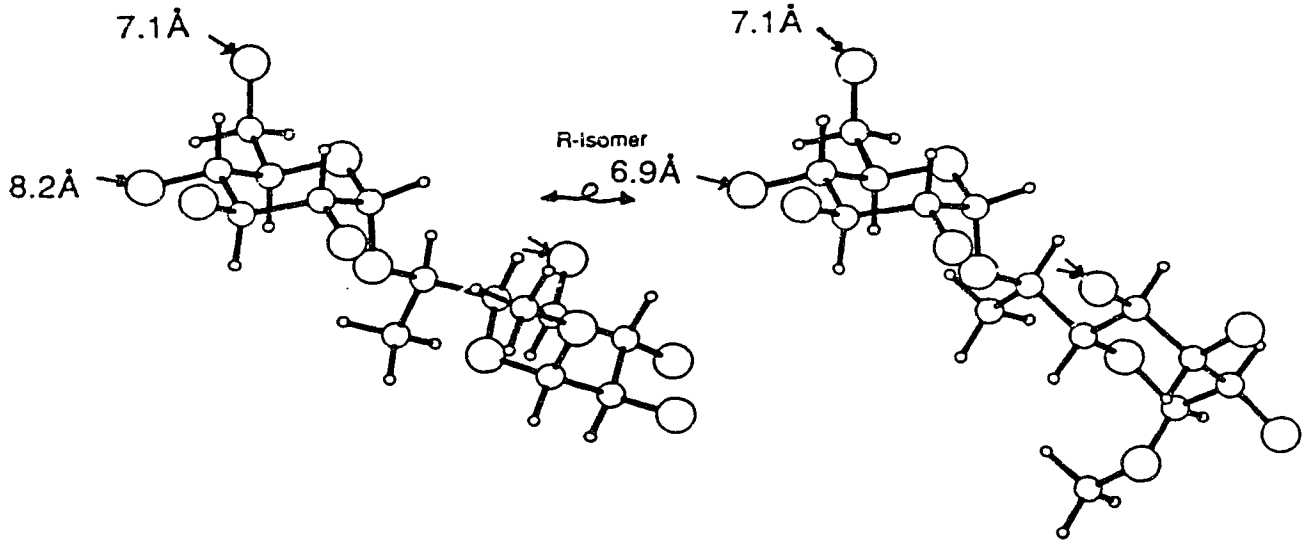
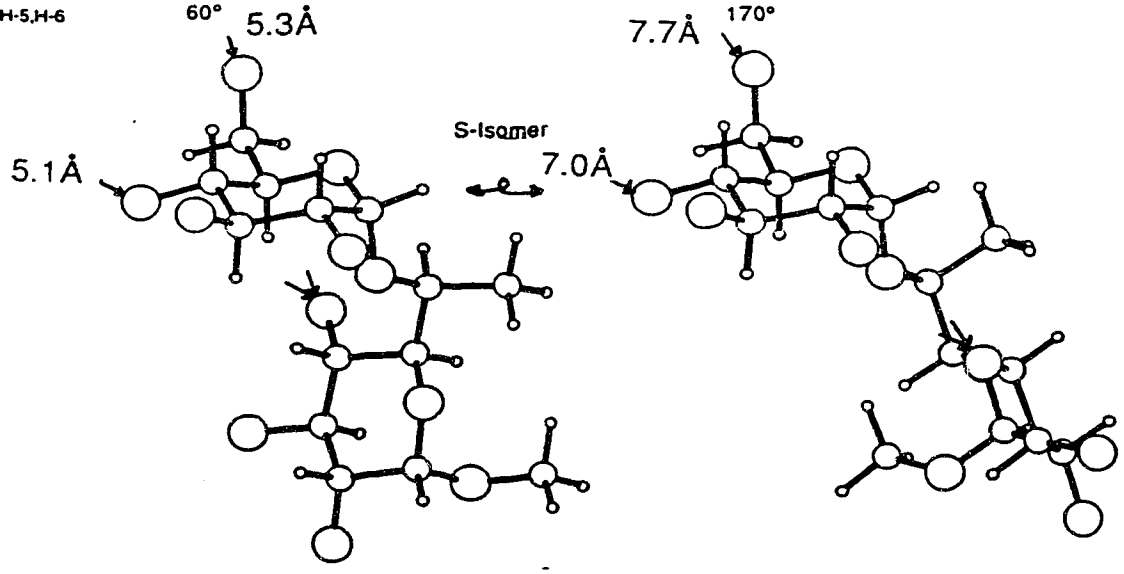


Figure 44. The presentation of the key polar group.



	NMR Conformer ($J_{H-5,H-6}$ = 2.5 Hz)	Key Conformer
ϕ/φ/ω	-50°/90°/-60°	-50°/120°/50°
E, kcal/mole	-1.26	-0.85
$\omega_{H-5,H-6}$	60°	170°



	NMR Conformer ($J_{H-5,H-6}$ < 0.5 Hz)	Key Conformer
ϕ/φ/ω	-50°/195°/180°,	-60°/160°/50°
E, kcal/mole	-1.46	2.32
$\omega_{H-5,H-6}$	60°	-70°

Figure 45. Conformational analysis of the diastereoisomeric methyl 6-*C*-methyl-α-isomaltosides (**43** and **40**).

It is possible now to rationalize partially why the enzyme AMG II catalyzed the hydrolysis of methyl α-maltoside, methyl α-isomaltoside and 6-*C*-methyl derivatives (**40** and **43**). Since enzymes are expected to be substrate specific, a change in linkage such as α-(1→4) in methyl α-maltoside to α-(1→6) in methyl α-isomaltoside will result in a change in conformation of the disaccharide which then becomes a very different substrate. However, AMG II can still catalyze the hydrolysis of the α-(1→6) linkage of methyl α-isomaltoside because the latter can adopt a conformation similar to that of methyl α-maltoside where the key polar hydroxyl groups are in similar spatial arrangement.

The rate of hydrolysis by AMG II was about 40 times faster for methyl α-maltoside compared to methyl α-isomaltoside. Although the methyl α-isomaltoside can adopt the appropriate conformation for hydrolysis, this key conformer is only one of several possible conformers of comparable energies because of the flexibility of the O-5/O-6 fragment of the reducing unit. In other words the population of the key conformer that can be recognized by the enzyme AMG II is low and the organization of the molecule for reaction must present a barrier to reaction. Furthermore, because methyl α-isomaltoside is an α-(1→6) linkage, the nonpolar surface of the disaccharide would be different compared to the α-(1→4) linkage of methyl α-maltoside, the methyl α-isomaltoside may not fit the binding site as well as in the case of methyl α-maltoside which would also result in the reduction of rate. The effect of the distance variations (0.4 Å) in the key polar hydroxyl groups on the rate of hydrolysis is not known at present but it is expected that changes in distance would probably decrease the rate.

Based on the $T_{1/2}$ at 10 mM concentration (see Table 16), methyl 6-*R*-methyl-α-isomaltoside (**43**) hydrolyzed about 2.6 times faster than methyl α-isomaltoside (**37**) which in turn is hydrolyzed about 3.1 times faster than methyl 6-*S*-methyl-α-isomaltoside (**40**). If for hydrolysis, the isomaltoside derivatives must imitate the conformation of methyl α-maltoside in terms of the internuclear distances of the three key hydroxyl groups, then

methyl 6-*R*-methyl-α-isomaltoside (**43**) must have higher population of the proper key conformer than methyl α-isomaltoside. On the other hand, methyl 6-*S*-methyl-α-isomaltoside (**40**) must have lower population of the proper key conformer than has methyl α-isomaltoside. The differences in energies (Kcal mol^{-1}) as estimated by HSEA calculations (Table 17) for adopting the key conformations were 0.41 for methyl 6-*R*-methyl-α-isomaltoside (**43**), 1.49 for methyl α-isomaltoside and 3.78 for methyl 6-*S*-methyl-α-isomaltoside. Using Boltzman's equation for population distribution (Equation 4), it is possible to estimate that the population of the key conformer is 33% for methyl 6-*R*-methyl-α-isomaltoside (**43**), 8% for methyl α-isomaltoside and 0.2% for methyl 6-*S*-methyl-α-isomaltoside. These population estimates reflect the trend for the hydrolysis catalyzed by the enzyme AMG II.

$$\frac{N1}{N2} = e^{-\Delta E/RT} \qquad (4)$$

where $N1$= population of the ^{1}H-NMR conformer

$N2$= population of the key conformer

R= gas constant

T= temperature ^oK

V. SUMMARY

1. Methyl 6-*R*-C-methyl-α-D-glucopyranoside (**21**) was synthesized and found to prefer the $^4C_1 - \mathbf{g}^+$ conformation in aqueous solutions.

2. Hydrolysis of **21**, provided 6-*R*-methyl-D-glucose (**48**) which was dehydrogenated to aldonic lactone by the enzyme system of Hexokinase and 6-Phosphate Dehydrogenase.

3. Methyl 6-*S*-C-methyl-α-D-glucopyranoside (**15**) was synthesized and found to prefer the $^4C_1 - \mathbf{g}^-$ conformation in aqueous solution.

4. Hydrolysis of **15**, provided 6-*S*-methyl-D-glucose (**47**) which was not dehydrogenated to aldonic lactone by the enzyme system of Hexokinase and 6-Phosphate Dehydrogenase.

5. In the temperature range 295°C to 355°C, compounds **21** and **15** still retain their preferred conformations; i.e. $\mathbf{g}^+$ and $\mathbf{g}^-$ respectively.

6. Methyl 6-*R*-methyl-α-isomaltoside (**43**) was synthesized and found to prefer $\mathbf{g}^+$ conformation at the C-6 center of the reducing glucose unit in aqueous solution.

7. Hydrolysis catalyzed by the enzyme Amyloglucosidase Type II of **43** was observed to be about 3 times faster than methyl α-isomaltoside (**37**).

8. Methyl 6-*S*-methyl-α-isomaltoside (**40**) was synthesized and found to prefer $\mathbf{g}^-$ conformation at the C-6 center of the reducing glucose unit in aqueous solution.

9. Hydrolysis catalyzed by the enzyme Amyloglucosidase Type II of **40** was observed to be about 3 times slower than methyl α-isomaltoside (**37**).

VI. EXPERIMENTAL

1. General methods

Nuclear magnetic resonance spectra were obtained at 297°K in D_2O or $CDCl_3$ on Bruker instruments operating at 300 or 400 MHz when provided by the Spectral Service Laboratory under the supervision of Dr. T. Nakashima and by the writer when at 360 MHz.. Acetone was used as internal reference (δ=2.225 ppm) for D_2O and tetramethylsilane (TMS) was used for $CDCl_3$ in proton spectra. Dioxane was used as internal reference for ^{13}C-spectra. Assignments were carried out using either the double resonance technique or the 2D-COSY technique. Infra-red spectra were obtained on the Nicolet 7199 FT-IR. Melting points were determined on the Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter at 589 nm in a 1 dm cell at room temperature (23± 1°C). Elemental analyses were performed by the departmental Analytical Service Laboratory under the supervision of Mr. R. Swindlehurst. Thin layer chromatography (t.l.c.) was run with precoated silica gel 60-F254 plates (E. Merck, Darmstadt) and developed by quenching of flourescence and/or charring with 5%-10% sulfuric-ethanol solution.

Dry dichloromethane and pyridine were obtained by distilling over calcium hydride and were used immediately. Chromium trioxide was obtained from Aldrich and was dried over P_2O_5 and under vacuum for at least 48 hrs. Other solvents and reagents used were reagent grade and if better purity was needed, standard procedures[110] were followed. Anhydrous solution transfers were done under nitrogen atmosphere using standard syringe techniques.[111]

N.O.e. experiments

Solutions in the NMR tubes were previously degassed by bubbling dry nitrogen through for 3-5 minutes. The D_2O used was purchased from Aldrich (99.9% D, low in paramagnetic impurities). The steady state nuclear Overhauser enhancements were obtained after 512 scans normally.

HSEA calculations

The atomic coordinates were obtained from neutron diffraction studies of methyl α-D-glucopyranoside crystal structure.[76] Modification of the methyl α-D-glucopyranoside structure at C-6 were achieved with the program TRANSF.OBJ using the distance 1.54Å for C-C bond. Rotation of the ω torsion angle was done with the program GENT.OBJ. Molecular minimum energy calculation was estimated using the HDSP3.OBJ program. Molecular conformation was plotted using the coordinates of the minimum energy conformation generated by the HDSP3.OBJ program. The programs TRANSF.OBJ, GENT.OBJ and HDSP3.OBJ were available from the University of Alberta Computing Library.

2. Syntheses

Methyl 2,3,4,-tri-O-benzyl-α-D-gluco-1,6-dialdo-hexopyranoside (7)

Dried chromium trioxide (25.9 g, 259 mmol) was added to a solution of pyridine (40.9 g, 517 mmol) in dichloromethane (400 mL). The mixture was mechanically stirred at room temperature for 30 min.. A solution of methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**4**) (10.0 g, 21.6 mmol) in dichloromethane (22 mL) was added rapidly to the oxidation mixture and a black tar precipitated immediately. The resulting mixture was stirred at room temperature for 10 min., decanted and the black tarry precipitate was washed with ether several times. The combined ether solutions were filtered through a pad of silica gel-G to give a colorless filtrate which was dried with anhydrous magnesium sulfate. Ether was removed to give a slightly yellow oily residue which was coevaporated with toluene (2 x 200 mL) to remove residual pyridine. The oily residue was dried under vacuum overnight to give **7** (6.94 g, 70%). Proton NMR of this crude residue showed that it contained ~90% of the desired compound **3**. $[\alpha]_D$=8.2° (c 1.0, $CHCl_3$) lit.[47] 13.6°.

^{1}H-NMR ($CDCl_3$): δ 9.65 (s, 1H, H-6), 7.40—7.10 (m, 15H, Ar-H), 5.05—4.50 (m, 7H, 3 x Ph-CH_2 and H-1), 4.17 (d, 1H, H-5, $J_{4,5}$=10.0 Hz), 4.08 (dd, 1H, H-3, $J_{3,4}$=9.0 Hz, $J_{2,3}$=9.5 Hz), 3.57 (dd, 1H, H-4, $J_{3,4}$=9.0 Hz, $J_{4,5}$=10.0 Hz), 3.50 (dd, 1H, H-2, $J_{1,2}$=3.5 Hz, $J_{2,3}$=9.5 Hz), 3.38 (s, 3H, OCH_3); ^{13}C-NMR ($CDCl_3$): δ 197.45 (<u>C</u>HO); 138.43—125.31 (Ar. Carbons); 98.41 (C-1); 87.76, 79.30, 77.82 (3 x Ph-<u>C</u>H_2); 75.32, 75.11, 74.22, 73.60 (C-2—C-5); 55.77 (O<u>C</u>H_3); IR ν_{max} (cm^{-1}) 1741.133 (CHO).

Methyl 2,3,4-tri-O-benzyl-7-deoxy-β-L-glycero-D-glucoheptapyranoside (9)

A solution of methyl iodide (4.26 g, 30.04 mmol) in anhydrous ether (20 mL) was added dropwise under nitrogen to a mixture of magnesium (0.73 g, 30.04 mmol) and ether (30 mL). After warming for a few minutes, the mixture turned cloudy and began to boil. Addition of methyl iodide solution was such that boiling was maintained. After the addition

of methyl iodide was complete, the resulting Grignard mixture was left stirring for 1 hr. to ensure complete formation of the reagent. A solution of compound **7** (6.94 g, 15.02 mmol) in ether (30 mL) was then added dropwise and the reaction mixture was left stirring at room temperature for 2 hrs. The reaction was quenched with the dropwise addition of saturated ammonium chloride (10 mL) and the reaction mixture solidified. Distilled water (50 mL) was added to dissolve the solid. The aqueous layer was extracted with ether several times (total volume 150 mL) and the combined ether solutions were washed with water (2 x 100 mL) and dried over anhydrous magnesium sulfate. Removal of solvent gave a yellow crude oil (5.96 g) in which the major product was **9** by t.l.c. (R_f=0.54, solvent 3:2 n-hexane:EtOAc).

(i) Methyl 2,3,4,-tri-O-benzyl-7-deoxy-6-O-(3,5-dinitrobenzoyl)-β-L-glycero-D-glucoheptapyranoside ***(14)***

The crude Grignard product (0.587 g, 1.2 mmol) was dissolved in pyridine (10 mL) and 3,5-dinitrobenzoyl chloride (0.513 g, 2.25 mmol) was added along with a few crystals of dimethylaminopyridine (DMAP). The reaction mixture was stirred at room temperature until analysis by t.l.c. (R_f S.M.=0.58, R_f Prod.=0.85, solvent 3:2 n-hexane:EtOAc) showed no remaining starting material. Water was then added dropwise with stirring for 5 min. The aqueous layer was extracted with chloroform (75 mL). The chloroform layer was separated, washed with water (2 x 75 mL) and dried over anhydrous magnesium sulfate. The solvent was then evaporated to give a yellow residue which was recrystallized from hot methanol to give **14** (0.612 g, 74%); mp 68-70°C; $[\alpha]_D$=52.2° (c 0.85, $CHCl_3$). ^{1}H-NMR ($CHCl_3$): δ 9.21 (t, 1H, para-H, $J_{ortho,para}$=2.0 Hz), 9.16 (d, 2H, ortho-H, $J_{ortho,para}$=2.0 Hz), 7.45—7.15 (m, 15, Ar-H), 5.62 (dq, 1H, H-6, $J_{5,6}$=1.5 Hz, $J_{6,7}$=6.5 Hz), 5.45—5.05 (3 x dd and d, 7H, 3 x Ph-CH_2 and H-1), 4.05 (dd, 1H, H-3, $J_{2,3}$=10.0 Hz, $J_{3,4}$=9.0 Hz), 3.71 (dd, 1H, H-5, $J_{5,6}$=1.5 Hz, $J_{4,5}$=10.0 Hz), 3.60 (dd, 1H, H-2, $J_{1,2}$=3.5

Hz, $J_{2,3}$=10.0 Hz), 3.42 (t, 4H, H-4 and OCH_3), 1.48 (d, 3H, C_6-CH_3, $J_{6,7}$=6.5 Hz); ^{13}C-NMR ($CDCl_3$): δ 228.13 (C=O); 162.81—123.38 (Ar. Carbons); 99.01 (C-1); 83.23, 81.11, 77.69, 72.51, 71.80 (C-2—C6); 76.86, 75.88, 74.53 (3 x Ph-CH_2); 56.30 (OCH_3); 17.35 (C-7).

Anal. calc. for $C_{36}H_{36}N_2O_{11}\bullet\frac{1}{2}MeOH$:	C	63.66	H 5.56	N 4.07
found:	C	63.49	H 5.24	N 4.27

(ii) Methyl 2,3,4-tri-O-benzyl-7-deoxy-β-L-glycero-D-glucoheptapyranoside (9) from **14**

Compound **14** (1.00 g, 1.49 mmol) was dissolved in dioxane (50 mL) and 1N sodium hydroxide solution (20 mL) added and stirred at room temperature for 1 hr. The reaction was followed by t.l.c. (R_f **14**=0.85, R_f **9**=0.57, solvent 3:2 n-hexane:EtOAc). When the reaction was completed (after 1 hr.), dichloromethane (250 mL) was added to the reaction mixture and stirred for 5 min.. Then the reaction mixture was filtered over a pad of silica gel-G which was then washed with water (2 x 100 mL) and dried over anhydrous magnesium sulfate. The solvent was then evaporated, the residue dried under vacuum to give solid **9** (0.692 g, 97%); mp 82-84°C; $[\alpha]_D$=26.9° (c 1.0, $CHCl_3$).

^{1}H-NMR ($CDCl_3$): δ 7.40—7.20 (m, 15H, Ar-H), 5.01—4.63 (3 x dd, 6H, Ph-CH_2), 4.58 (d, 1H, H-1, $J_{1,2}$=3.5 Hz), 4.05 (dq, 1H, H-6, $J_{5,6}$=1.5 Hz, $J_{6,7}$=6.5 Hz), 3.99 (dd, 1H, H-3, $J_{3,4}$=9.0 Hz, $J_{2,3}$=9.5 Hz), 3.62 (dd, 1H, H-4, $J_{4,5}$=10.0 Hz, $J_{3,4}$=9.0 Hz), 3.50 (dd, 1H, H-2, $J_{2,3}$=9.5 Hz, $J_{1,2}$=3.5 Hz), 3.42 (dd, 1H, H-5, $J_{5,6}$=1.5 Hz, $J_{4,5}$=10.0 Hz), 3.34 (s, 3H, OCH_3), 1.25 (d, 3H, C_6-CH_3, $J_{6,7}$=6.5 Hz); ^{13}C-NMR ($CDCl_3$): δ 138.79—127.57 (Ar. Carbons); 98.15 (C-1); 82.17, 79.93, 77.72 (3 x Ph-CH_2); 75.70, 75.11, 73.42, 72.83 (C-2—C-5); 65.05 (C-6); 55.01 (OCH_3); 20.15 (C-7).

Anal. calc. for $C_{29}H_{34}O_6$:	C	72.78	H 7.16	
found:	C	72.94	H 6.82	

Methyl 7-deoxy-β-L-glycero-D-glucoheptapyranoside (15)

Compound **9** (200 mg, 0.42 mmol) was dissolved in methanol (25 mL). Then 5% palladium-on-charcoal (200 mg) was added in small portions. The mixture was shaken with hydrogen (100 lb. in^{-2}) at room temperature overnight. The reaction mixture was then filtered over celite (washed with methanol). The solvent was evaporated to give **15** (85 mg, 98%) as a solid which could be recrystallized from n-hexane / 98% ethanol to give needle like crystals. Mp 136-137°C, $[\alpha]_D$=150° (c .23, H_2O). ^{1}H-NMR (D_2O): δ 4.68 (d, 1H, H-1, $J_{1,2}$=3.5 Hz), 4.05 (dq, 1H, H-6, $J_{5,6}$=1.5 Hz, $J_{6,7}$=6.5 Hz), 3.51 (dd, 1H, H-3, $J_{2,3}$=10.0 Hz, $J_{3,4}$=9.0 Hz), 3.42 (dd, 1H, H-2, $J_{1,2}$=3.5 Hz, $J_{2,3}$=10.0 Hz), 3.36 (dd, 1H, H-4, $J_{3,4}$=9.0 Hz, $J_{4,5}$=10.0 Hz), 3.27 (s, 3H, OCH_3), 3.26 (dd, 1H, H-5, $J_{5,6}$=1.5 Hz, $J_{4,5}$=10.0 Hz), 1.16 (d, 3H, C_7H_3, $J_{6,7}$=6.5 Hz). ^{13}C-NMR (D_2O): δ 99.24 (C-1); 73.49, 73.41 (C-3, C-5); 71.31 (C-2); 69.74 (C-6); 64.26 (C-4); 54.89 ($O\underline{C}H_3$); 18.72 (C-7).

Anal. calc. for $C_8H_{16}O_6$: C 46.15 H 7.75

found: C 46.04 H 7.46

Methyl 2,3-di-O-acetyl-4,6-benzylidene-7-deoxy-β-L-glycero-D-glucoheptapyranoside (17)

A solution of benzal bromide (112 mg, 0.40 mmol) in pyridine (1 mL) was added to a solution of compound **15** (16 mg, 0.77 mmol) in pyridine (15 mL) and the mixture was refluxed at 125°C for 2 hrs. with stirring. Acetic anhydride (3 mL) was then added to the cooled reaction mixture and the solution was stirred for 48 hrs. at room temperature. The reaction mixture was then poured into water (75 mL) and the aqueous layer was extracted with chloroform (50 mL). The chloroform layer was washed with water (50 mL), saturated sodium bicarbonate solution (50 mL), water (50 mL) and dried over anhydrous magnesium sulfate. Chloroform was evaporated and the residue was coevaporated with toluene (2 x 15 mL). Preparative t.l.c. of the residue (R_f=0.66, solvent 3:2 n-hexane / EtOAc) gave **17** (15 mg, 51%) as a solid. Mp 132-134°C; $[\alpha]_D$=50.2° (c 0.31, $CHCl_3$).

^{1}H-NMR ($CDCl_3$): δ 7.50—7.25 (m, 5H, Ar-H), 5.80 (s, 1H, Ph-CH), 5.57 (dd, 1H, H-3, $J_{3,4}$=9.0 Hz, $J_{2,3}$=10.0 Hz), 4.93 (d, 1H, H-1, $J_{1,2}$=3.5 Hz), 4.87 (dd, 1H, H-2, $J_{2,3}$=10.0 Hz, $J_{1,2}$=3.5 Hz), 4.5 (dq, 1H, H-6, $J_{5,6}$=6.0 Hz, $J_{6,7}$=7.0 Hz), 4.15 (dd, 1H, H-5, $J_{4,5}$=10.0 Hz, $J_{5,6}$=6.0 Hz), 3.91 (dd, 1H, H-4, $J_{3,4}$=9.0 Hz, $J_{4,5}$=10.0 Hz), 3.41 (s, 3H, $OCOCH_3$), 1.48 (d, 3H, C_7H_3, $J_{6,7}$=7.0 Hz). ^{13}C-NMR ($CDCl_3$): δ 170.48, 169.76 (2 x C=O); 137.40, 128.92, 128.22, 126.24 (Ar. Carbons); 97.59 (C-1), 94.07 (Ph-CH); 72.74, 71.50, 70.49, 69.66 (C-2—C-5); 64.45 (C-6); 55.34 (OCH_3); 20.65, 20.79 (2 x $COCH_3$), 11.28 (C-7).

Anal. cal. for $C_{19}H_{24}O_8$:	C	59.99	H	6.36
found:	C	59.55	H	6.06

Methyl 6-O-methanesulfonyl-2,3,4-tri-O-benzyl-7-deoxy-β-L-glycero-D-glucoheptapyranoside (**18**)

Methanesulfonyl chloride (87 mg, 0.76 mmol) was added to a solution of compound **9** (135 mg, 0.282 mmol) in pyridine (5 mL) and the reaction mixture was stirred at room temperature for 2 hrs. T.l.c. analysis indicated that the reaction was completed after 2 hrs. and virtually one product had formed (R_f **9**=0.58, R_f **18**=0.67, solvent 3:1 toluene / acetone). The reaction mixture was then poured into a mixture of ice / water (50 mL) and the aqueous layer was extracted with dichloromethane (50 mL). The dichloromethane layer was washed with cold water (2 x 50 mL) and dried over anhydrous magnesium sulfate. The solvent was then evaporated, the residue was coevaporated with toluene (2 x 25 mL) and chromatographed (solvent 6:1 toluene/acetone) to give **18** (104 mg, 66%) as an oil. $[\alpha]_D$=9.3° (c 1.22, $CHCl_3$).

^{1}H-NMR ($CDCl_3$): δ 7.40—7.25 (m, 15H, Ar-H), 5.32 (dq, 1H, H-6, $J_{5,6}$=1.0 Hz, $J_{6,7}$=6.5 Hz), 5.06—4.62 (m, 7H, 3 x Ph-CH_2 and H-1), 4.02 (m, 1H, H-5), 3.65—3.53 (m, 3H, H-3, H-4 and H-2), 3.36 (s, 3H, OCH_3), 3.04 (s, 3H, SO_2CH_3), 1.53 (d, 3H, C_6-CH_3,

$J_{6,7}$=6.5 Hz). ^{13}C-NMR ($CDCl_3$): δ 138.54—127.74 (Ar. Carbons); 98.26 (C-1); 82.30, 79.71, 77.17, 73.55, 72.38 (C-2—C6); 75.74, 75.31, 75.00 (3 x Ph-$\underline{C}H_2$); 55.33 (O$\underline{C}H_3$); 39.54 ($OSO_2\underline{C}H_3$); 17.73 (C-7).

Anal. calc. for $C_{30}H_{36}O_8S$:	C 64.73	H 6.52	S 5.76
found:	C 64.85	H 6.55	S 5.52

Methyl 6-O-benzoyl-2,3,4-tri-O-benzyl-7-deoxy-α-D-glycero-D-glucoheptapyranoside (**19**)

Crude mesylate **18** (0.60 g, 1.08 mmol) was dissolved in dry N,N-dimethyl formamide (DMF) (5mL), anhydrous sodium benzoate (1.01 g, 7.0 mmol) was added and the reaction mixture was heated at 120°C and stirred for 17 hrs.. The cooled reaction mixture was then dissolved in dichloromethane (50 mL), washed with water (6 x 75 mL) and dried over anhydrous magnesium sulfate. The solvent was evaporated and the residue was chromatographed on HPLC (solvent 10:1 n-hexane / acetone) to give **19** (0.294 g, 47%) as a syrup. $[\alpha]_D$=9.8° (c 1.04, $CHCl_3$).

^{1}H-NMR ($CDCl_3$): δ 8.40—7.20 (m, 20H, Ar-H), 5.47 (dq, 1H, H-6, $J_{5,6}$=2.0 Hz, $J_{6,7}$=7.0 Hz), 5.03—4.65 (m, 6H, 3 x Ph-CH_2), 4.64 (d, 1H, H-1, $J_{1,2}$=4.0 Hz), 4.04 (dd, 1H, H-3, $J_{2,3}$=9.5 Hz, $J_{3,4}$=9.0 Hz), 3.93 (dd, 1H, H-5, $J_{5,5}$=2.0 Hz, $J_{5,4}$=10.0 Hz), 3.52 (dd, 1H, H-2, $J_{1,2}$=4.0 Hz, $J_{2,3}$=9.5 Hz), 3.42 (dd, 1H, H-4, $J_{3,4}$=9.0 Hz, $J_{4,5}$=10.0 Hz), 3.38 (s, 3H, OCH_3), 1.18 (d, 3H, C_6-CH_3, $J_{6,7}$=7.0 Hz). ^{13}C-NMR ($CDCl_3$): δ 165.76 ($\underline{C}$=O); 136.66—127.75 (Ar. Carbons); 97.80 (C-1); 82.44, 80.16, 77.82, 71.39, 70.12 (C-2—C-6); 75.92, 74.62, 73.41 (3 x Ph-$\underline{C}H_2$), 54.99 (O$\underline{C}H_3$); 13.57 (C-7).

Anal. calc. for $C_{36}H_{38}O_7$:	C 74.20	H 6.57	
found:	C 74.33	H 6.55	

Methyl 2,3,4-tri-O-benzyl-7-deoxy-α-D-glycero-D-glucoheptapyranoside (**10**)

Compound **19** (137 mg, 0.24 mmol) was dissolved in dioxane (10 mL) and 1N sodium hydroxide solution (6 mL) was added. The reaction mixture was stirred at 60°C and t.l.c. analysis showed that the reaction was over after 48 hrs. (R_f **19**=0.85, R_f **10**=0.43, solvent 3:2 n-hexane:EtOAc). The cooled mixture was then diluted with dichloromethane (50 mL) and filtered over a pad of silica gel-G. The dichloromethane solution was washed with water (2 x 50 mL), saturated sodium bicarbonate solution (50 mL), water (50 mL) and dried over anhydrous magnesium sulfate. The solvent was then removed and the residue solidified slowly under vacuum to give **10** (80 mg, 71%). Mp 89-90°C, $[\alpha]_D$=28° (c 0.4, $CHCl_3$).

^{1}H-NMR ($CDCl_3$): δ 7.34—7.16 (m, 15H, Ar-H), 4.98—4.53 (m, 6H, 3 x Ph-CH_2), 4.50 (d, 1H, H-1, $J_{1,2}$=3.5 Hz), 3.95 (dd, 1H, H-3, $J_{2,3}$=9.5 Hz, $J_{3,4}$=9.0 Hz), 3.88 (m, 1H, H-6), 3.55 (dd, 1H, H-5, $J_{4,5}$=10.0 Hz, $J_{5,6}$=4.5 Hz), 3.43 (dd, 1H, H-2, $J_{1,2}$=3.5 Hz, $J_{2,3}$=9.5 Hz), 3.35 (dd, 1H, H-4, $J_{3,4}$=9.0 Hz, $J_{4,5}$=10.0 Hz), 3.31 (s, 3H, OCH_3), 2.49 (d, 1H, OH, $J_{6,OH}$=6.0 Hz), 1.05 (d, 3H, C_6-CH_3, $J_{6,7}$=6.5 Hz). ^{13}C-NMR ($CDCl_3$): δ 138.61—127.73 (Ar. Carbons); 97.64 (C-1); 82.38, 80.29, 80.01 (3 x Ph-$\underline{C}H_2$); 75.75, 74.81, 73.37, 72.31 (C-2—C-5); 68.37 (C-6); 55.16 ($O\underline{C}H_3$), 17.81 (C-7).

Anal. calc. for $C_{29}H_{34}O_6$:	C 72.78	H 7.16
found:	C 72.81	H 7.07

Methyl 7-deoxy-α-D-glycero-D-glucoheptapyranoside (**21**)

To a solution of **10** (70 mg, 0.15 mmol) in methanol (12 mL), 5% palladium-on-charcoal (74 mg) was added in small portions. The mixture was shaken with hydrogen (100 lb. in^{-2}) at room temperature for 6 hrs. The reaction mixture was then filtered over celite (washed with methanol). The solvent was evaporated and the residue was coevaporated

with toluene (2 x 25 mL) and dried under vacuum. The residue solidified under vacuum to give **21** (30 mg, 98%). Mp 135-136°C, $[\alpha]_D = 147.8^o$ (c 1.0, H_2O).

^{1}H-NMR (D_2O): δ 4.68 (d, 1H, H-1, $J_{1,2}$=4.0 Hz), 4.04 (dq, 1H, H-6, $J_{5,6}$=2.5 Hz, $J_{6,7}$=6.0 Hz), 3.56 (dd, 1H, H-5, $J_{5,6}$=2.5 Hz, $J_{4,5}$=10.0 Hz), 3.53 (dd, 1H, H-3, $J_{3,4}$=9.0 Hz, $J_{2,3}$=10.0 Hz), 3.44 (dd, 1H, H-2, $J_{1,2}$=4.0 Hz, $J_{2,3}$=10.0 Hz), 3.23 (dd, 1H, H-4, $J_{4,5}$= 10.0 Hz, $J_{3,4}$=9.0 Hz), 3.29 (s, 3H, OCH_3), 1.10 (d, 3H, C_6-CH_3, $J_{6,7}$=6.0 Hz). ^{13}C-NMR (D_2O): δ 99.22 (C-1); 73.37, 73.32 (C-2 and C-5); 71.27, 70.93 (C-3 and C-6); 66.34 (C-4); 54.96 ($O\underline{C}H_3$), 15.05 (C-7).

Anal. calc. for $C_8H_{16}O_6$:	C	46.15	H	7.75
found:	C	45.99	H	7.50

Methyl 6'-O-acetyl-2,2',3,3',4,4'-hexa-O-benzyl-7-deoxy-6-O-(α-D-glucopyranosyl)-β-L-glycero-D-glucoheptapyranoside (**38**)

Methyl 2,3,4-tri-*O*-benzyl-7-deoxy-β-L-*glycero*-D-glucoheptapyranoside (**9**) (100 mg, 0.209 mmol) was dissolved in dichloromethane (10 mL). Then tetraethylamonium bromide (76 mg), dried molecular sieves (BDH, 4 A, 1 g) and dried DMF (1.4 mL) was added and the mixture was bubbled with nitrogen to reduce the volume. Then freshly prepared 6-O-acetyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyl bromide (**31**) (2 eq.) in dichloromethane (6 mL) was added and the mixture was stirred at room temperature under nitrogen. The reaction progress was followed by t.l.c. until completed (R_f **38**=0.69, R_f **24**=0.55, solvent 3:2 n-hexane / ethyl acetate). After the reaction was over, the partially dry mixture was dissolved in dichloromethane (25 mL), filtered to remove undissolved materials, washed with water (2x50 mL), saturated sodium bicarbonate solution (2x50 mL), water (2x50 mL) and dried over magnesium sulfate. Dichloromethane was evaporated and the residue was chromatographed with silica gel (solvent 4:1 n-hexane /

ethyl acetate) to give the desired disaccharide **38** as an oil which was dried under vacuum with heating (101 mg, 51%). $[\alpha]_D=0.54^o$ (c 1.06, $CDCl_3$).

^{1}H-NMR ($CDCl_3$): δ 7.40—7.20 (m, 30H, Ar-H), 5.09 (d, 1H, H-1', $J_{1',2'}=3.5$ Hz), 5.02—4.49 (m, 7H, 3 x Ph-CH_2 and H-1), 4.26—4.06 (m, 3H, 2 x H-6' and H-6), 4.06—3.94 (q and m, 3H, H-3', H-3 and H-5'), 3.77 (dd, 1H, H-4, $J_{3,4}=9.5$ Hz, $J_{4,5}=9.0$ Hz), 3.56 (dq, 1H, H-5, $J_{4,5}=9.0$ Hz, $J_{5,6}=2.0$ Hz), 3.56—3.47 (m, 2H, H-2' and H-2), 3.46 (dd, 1H, H-4', $J_{3',4'}=9.0$ Hz, $J_{4',5'}=10.0$ Hz), 3.38 (s, 3H, OCH_3), 1.92 (s, 3H, CH_3COO-), 1.33 (d, 3H, C_6-CH_3, $J_{6,7}=6.0$ Hz). ^{13}C-NMR ($CDCl_3$): δ 170.75 (C=O); 138.81 (t-Carbons of Ph.); 128.47—127.39 (Ph. Carbons); 97.90 (C-1'); 95.20 (C-1); 75.72, 75.53, 75.08, 74.32, 73.40, 73.00 (6 x Ph-CH_2); 82.56, 81.60, 80.19 (3 x C); 77.73, 77.48, 73.22, 71.69, 69.19 (9 x C: C-2, C-2', C-3, C-3', C-4, C-4', C-5, C-5', C-6); 63.24 (C-6'), 55.09 (OCH_3), 20.01 ($COOCH_3$), 16.31 (C-7)

Anal. calc. for $C_{57}H_{64}O_{12}$:	C 73.09	H 6.77
found:	C 72.84	H 6.85

Methyl 2,2',3,3',4,4'-hexa-O-benzyl-7-deoxy-6-O-(α-D-glucopyranosyl)-β-L-glycero-D-glucoheptapyranoside (**39**)

Methyl 6'-*O*-acetyl-2,2',3,3',4,4'-hexa-*O*-benzyl-7-deoxy-6-*O*-(α-D-glucopyranosyl)-β-L-*glycero*-D-glucoheptapyranoside (**38**) (293 mg, 0.307 mmol) was dissolved in dry methanol (10 mL). A catalytic amount of sodium methoxide was added to the solution and the solution was stirred at room temperature overnight. The reaction progress was followed by t.l.c. (R_f **39**=0.49, R_f **38**=0.72, solvent 3:2 n-hexane / ethyl acetate). When the reaction was completed, resin (IRC-50-H^+, washed with distilled water and methanol) was added and stirred until the mixture turned acidic (pH paper). Then the mixture was filtered and the solvent was evaporated to give a foamy product **39** which was dried under

vacuum (269 mg, 96%). $[\alpha]_D = 47.6^o$ (c 1.99, $CHCl_3$).

1H-NMR ($CDCl_3$): δ 7.40—7.10 (m, 30H, Ph-H), 5.03 (d, 1H, H-1′, $J_{1',2'} = 3.5$ Hz), 5.01—4.57 (m, 13H, 6 x Ph-CH_2 and H-1), 4.32 (dq, 1H, H-6, $J_{5,6} = 1.5$ Hz, $J_{6,7} = 6.5$ Hz), 4.01 (dd, 1H, H-3′, $J_{2',3'} = 9.5$ Hz, $J_{3',4'} = 9.0$ Hz), 3.98 (dd, 1H, H-3, $J_{2,3} = 9.5$ Hz, $J_{3,4} = 10.0$ Hz), 3.84—3.72 (m, 2H, H-5′ and H-4), 3.66—3.49 (m, 5H, 2 x H-6, H-5, H-4′ and H-2), 3.46 (dd, 1H, H-2′, $J_{1',2'} = 3.5$ Hz, $J_{2',3'} = 9.5$ Hz), 3.36 (s, 3H, OCH_3), 1.32 (d, 3H, C_6-CH_3, $J_{6,7} = 6.5$ Hz). ^{13}C-NMR ($CDCl_3$): δ 138.84—138.27 (t-Ph. Carbons); 128.46—127.37 (Ph. Carbons); 97.88 (C-1′); 95.39 (C-1); 82.54, 81.52, 80.19, 80.14, 77.64, 73.13, 71.84, 71.31 (9 Carbons: C-2, C-2′, C-3, C-3′, C-4, C-4′, C-5, C-5′, C-6); 61.72 (C-6′); 55.09 (OCH_3); 16.40 (C-7).

Anal. calc. for $C_{56}H_{62}O_{11}$:	C	73.80	H	6.86
found:	C	73.20	H	6.95

Methyl 7-deoxy-6-O-(α-D-glucopyranosyl)-β-L-glycero-D-glucoheptapyranoside (**40**)

Methyl 2,2′,3,3′,4,4′-hexa-*O*-benzyl-7-deoxy-6-*O*-(α-D-glucopyranosyl)-β-L-*glycero*-D-glucopyranoside (**39**) (126 mg, 0.138 mmol) was dissolved in dry methanol (12.5 mL). Then 5% palladium-on-charcoal (126 mg) was added in small portions to the solution and the mixture was shaken under hydrogen (100 lb. in^{-2}) at room temperature. The reaction progress was followed by t.l.c. (solvent 4:1 dichloromethane / methanol). When the reaction was completed the mixture was filtered and the filtrate was evaporated to give a yellowish solid residue. The crude residue was chromatographed (Iatrobeads, solvent 4:1 dichloromethane / methanol) to give colorless crystalline **40** (40 mg, 78%). Mp 127-128°C, $[\alpha]_D = 175.3^o$ (c 0.26, D_2O).

^{1}H-NMR (D_2O): δ 5.09 (d, 1H, H-1′, $J_{1',2'} = 3.5$ Hz), 4.28 (broaden q, 1H, H-6), 3.69 (t, 1H, H-3′), 3.64 (broad d, 1H, H-5), 3.56 (dd, 1H, H-2′, $J_{1',2'} = 3.5$ Hz, $J_{2',3'} = 9.5$ Hz),

3.89—3.52 (m, 10H, H-5′, 2 x H-6′, H-3, H-3′, H-4, H-5, H-5′, H-2, H-2′), 3.43 (t, 4H, OCH_3 and H-4′). ^{13}C-NMR (D_2O): δ 100.03 (C-1′); 95.56 (C-1); 74.49, 74.15, 73.68, 73.04, 72.25, 71.99, 70.24, 68.50 (9 Carbons: C-2, C-2′, C-3, C-3′, C-4, C-4′, C-5, C-5′, C-6); 61.17 (C-6′), 55.67 ($O\underline{C}H_3$), 14.76 (C-7).

Anal. calc. for $C_{14}H_{25}O_{11}$:	C	45.53	H	6.82
found:	C	42.41	H	6.61

Methyl 6′-O-acetyl-2,2′,3,3′,4,4′-hexa-O-benzyl-7-deoxy-6-O-(α-D-glucopyranosyl)-α-D-glycero-D-glucoheptapyranoside (**41**)

Compound **10** (500 mg, 1.04 mmol) was dissolved in dichloromethane (5 mL). Tetraethyl ammonium bromide (420 mg), dry molecular sieves (BDH 4A, 5 g) and DMF (1 mL) was added to the solution and the mixture was placed under nitrogen atmosphere. Then 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl bromide (**31**, 2 eq.) in dichloromethane (5 mL) was added and the mixture was stirred at room temperature under nitrogen. The reaction progress was followed by t.l.c. (R_f **41**=0.59, R_f **10**=0.26solvent system 3:2 n-hexane / ethyl acetate). When the reaction was completed, dichloromethane (100 mL) was added and the mixture was filtered. The dichloromethane solution was washed with saturated sodium carbonate solution (100 mL), water (4 x 100 mL) and dried over magnesium sulfate. The solvent was then evaporated to give an oily residue which was chromatographed with silica gel (solvent 4:1 n-hexane / ethyl acetate) to give the desired product **41** (587 mg, 59%). $[\alpha]_D$=78.2° (c 1.06, $CDCl_3$).

^{1}H-NMR ($CDCl_3$): δ 7.40—7.20 (m, 30, Ph-H), 5.00 (d, 1H, H-1′, $J_{1',2'}$=3.5 Hz), 4.67 (d, 1H, H-1, $J_{1,2}$=3.5 Hz), 5.03—4.51 (m, 12H, 6 x Ph-CH_2), 4.27 (dd, 1H, H-6′, $J_{5',6'}$=4.5 Hz, $J_{6'a,6'b}$=– 11.5 Hz), 4.21 (dd, 1H, H-6′b, $J_{5',6'b}$=2.5 Hz, $J_{6'b,6'a}$=– 11.5 Hz), 4.08—3.91 (m, 4H, H-3, H-3′, H-6, H-5′), 3.78 (dd, 1H, H-5, $J_{5,6}$=0.5 Hz, $J_{4,5}$=10.0 Hz),

3.57—3.43 (m, 3H, H-4, H-4', H-2), 3.41 (dd, 1H, H-2', $J_{1',2'}$=3.5 Hz, $J_{2',3'}$=9.5 Hz), 3.29 (s, 3H, OCH_3), 1.99 (s, 3H, $OCOCH_3$), 1.16 (d, 3H, C_7H_3, $J_{6,7}$=6.5 Hz). ^{13}C-NMR ($CDCl_3$): δ 170.74 (C=O), 138.85—137.97 (t-Ph. Carbons); 97.76 (C-1' and C-1); 82.61, 81.76, 80.25, 80.03, 77.94, 77.64, 72.83, 72.57, 69.08 (9 Carbons: C-2, C-2', C-3, C-3', C-4, C-4', C-5, C-5', C-6), 63.27 (C-6), 55.13 (OCH_3); 20.84 ($OCOCH_3$), 16.55 (C-7)

Anal. calc. for $C_{58}H_{64}O_{12}$: C 73.09 H 6.77

found: C 73.46 H 6.61

Methyl 2,2',3,3',4,4'-hexa-O-benzyl-7-deoxy-6-O-(α-D-glucopyranosyl)-α-D-glycero-D-glucopyranoside (**42**)

Methyl 6'-*O*-acetyl-2,2',3,3',4,4'-hexa-*O*-benzyl-7-deoxy-6-*O*-(α-D-glucopyranosyl)-α-D-*glycero*-D-glucoheptapyranoside (**41**) (440 mg, 0.462 mmol) was dissolved in dry methanol (20 mL). A catalytic amount of sodium methoxide was added to the solution and the solution was stirred at room temperature overnight. The reaction progress was followed by t.l.c. (solvent system 3:2 n-hexane:ethyl acetate, R_f **42**=0.42). When the reaction was over, resin (IRC-50-H, washed with water and methanol) was added and stirred until the mixture turned acidic (pH paper). Then the mixture was filtered and the solvent was evaporated to give an oily residue which was dried under vacuum overnight to give **42** (347 mg, 83%). $[\alpha]_D$=65.2° (c .34, H_2O).

^{1}H-NMR ($CDCl_3$): δ 7.40—7.20 (m, 30H, Ph-H), 5.00 (d, 1H, H-1', $J_{1',2'}$=4.5 Hz), 4.98—4.53 (m, 13H, 6 x Ph-CH_2 and H-1), 4.08—3.91 (m, 3H, H-3, H-3' and H-6), 3.81—3.67 (m, 4H, H-5, H-5' and 2 x H-6'), 3.56—3.37 (m, 4H, H-4, H-4', H-2 and H-2'), 3.29 (s, 3H, OCH_3), 1.13 (d, 3H, C_6-CH_3, $J_{6,7}$=6.5 Hz). ^{13}C-NMR ($CDCl_3$): δ 138.85—138.27 (t-Ph. Carbons); 129.07—125.33 (Ph. Carbons); 97.89 (C-1'); 95.37 (C-1); 82.53, 81.52, 80.19, 77.64, 77.57, 73.14, 71.43, 71.32 (9 Carbons: C-2, C-2', C-3, C-3', C-4, C-4', C-5, C-5', C-6); 61.73 (C-6'); 55.09 (OCH_3), 16.38 (C-7).

Anal. calc. for $C_{56}H_{62}O_{11}$: C 73.82 H 6.86

found: C 73.23 H 7.23

*Methyl 7-deoxy-6-O-(α-D-glucopyranosyl)-α-D-glycero-D-glucoheptapyranoside (**43**)*

Compound **42** (247 mg, 0.27 mmol) was first dissolved in dry methanol (25 mL). Then 5% palladium-on-charcoal (254 mg) was added in small portions to the solution and the mixture was shaken under hydrogen (100 lb. in^{-2}) at room temperature and the reaction progress was followed by t.l.c. (solvent 4:1 dichloromethane / methanol). When the reaction was over, the mixture was filtered and the filtrate was evaporated to give a yellowish solid residue. The crude residue was chromatographed (Iatrobeads, solvent 4:1 dichloromethane / methanol) to give colorless crystalline **43** (80 mg, 80%). Mp 231-232°C. $[\alpha]_D = 228.1^\circ$ (c .43, H_2O).

^{1}H-NMR (D_2O): δ 4.80 (d, 1H, H-1, $J_{1,2}$=4.0 Hz), 3.57 (dd, 1H, H-2, $J_{1,2}$=4.0 Hz, $J_{2,3}$=9.5 Hz), 3.67 (dd, 1H, H-3, $J_{2,3}$=9.5 Hz, $J_{3,4}$=9.0 Hz), 3.39 (dd, 1H, H-4, $J_{4,5}$=10.5 Hz, $J_{4,3}$=9.0 Hz), 3.83 (dd, 1H, H-5, $J_{4,5}$=10.5 Hz, $J_{5,6}$=2.5 Hz), 4.17 (dq, 1H, H-6, $J_{5,6}$=2.5 Hz, $J_{6,7}$=6.5 Hz), 3.44 (s, 3H, OCH_3), 1.29 (d, 3H, C_6-CH_3, $J_{6,7}$=6.5 Hz), 5.07 (d, 1H, H-1′, $J_{1',2'}$=3.5 Hz), 3.55 (dd, 1H, H-2′, $J_{1',2'}$=3.5 Hz, $J_{2',3'}$=9.5 Hz), 3.73 (dd, 1H, H-3′, $J_{2',3'}$=9.5 Hz, $J_{3',4'}$=10.0 Hz), 3.41 (dd, 1H, H-4′), 3.88 (dd, 1H, H-6′a, $J_{6'a,6'b}$= – 14.5 Hz, $J_{6'a,5'}$=5.0 Hz), 3.76 (dd, 1H, H-6′b, $J_{6'a,6'b}$= – 14.5 Hz, $J_{6'b,5'}$=4.0 Hz). ^{13}C-NMR (D_2O): δ 100.13 (C-1′); 97.98 (C-1); 73.89, 73.73, 73.37, 72.98, 72.02, 71.89, 71.80, 71.48 (C-2—C5 and C-2′—C5′); 70.51 (C-6′), 56.09 (OCH_3), 15.12 (C-7).

Anal. calc. for $C_{14}O_{11}H_{25}$: C 45.53 H 6.82

found: C 45.18 H 6.97

3. Enzyme hydrolysis experiments

All enzyme hydrolysis experiments were performed on the Cary model 214 and the Bruker WM-360 spectrometer operating at 360 MHz at preset temperatures. The buffers used were potassium phosphate buffer at pH=6.8, M.E.S. buffer at pH=6.5, sodium acetate–acetic acid in D_2O at pD=4.3 sodium acetate-d_3–acetic acid-d_4 in D_2O at pD=4.3. The enzyme hydrolysis data were analyzed by Dr. M. Palcic and Mr. L. Steele in the Food Science Department on the PC using computer programs developed by Professor K.Bock and co-workers from the Department of Organic Chemistry, the Technical University of Denmark. The enzyme Isomaltase, ATP and NADP were purchased from Aldrich. The enzymes Hexokinase and 6-Phosphate Dehydrogenase were provided by Dr. M. Palcic. AMG type II was a gift from Professor K. Bock.

The method, as recommended by Bock,[85] is the application of high resolution ^{1}H-NMR spectroscopy to monitor substrate and product time depencencies in progress curve enzyme kinetics.[112-116] This method has an advantage since kinetic parameters can be estimated in a single run therefore saving both time and substrate material. The disadvantages are: (i) in general, mechanistic properties cannot be inferred directly from a single progress curve, and (ii) errors in the estimated parameters may be significant, depending on the experimental conditions and the method of data analysis.

The data were obtained as follows. The ^{1}H-NMR spectrum of the substrate in buffered solution was taken as standard at t=0. Then the enzyme in buffered solution was added and the reaction progress was followed by recording ^{1}H-NMR spectra at predetermined times until the reaction was over. The time dependent spectra were plotted and the concentration of the substrate and the product(s) were obtained from the integrations of the appropriate peaks. The concentration of substrate vs. time progress curve was constructed and analyzed on IBM personal computers using a non-linear least squares

fitting program package REGGRAFA obtained from Professor Bock. The time dependent concentration data are summarized in the next section.

The progress curve analysis[85] is based on the integrated and reparameterized Michaelis-Menten equation (Equation 5).

$$t = (\chi\,|y_o - y'|/v_o)\,A\,[\chi + \ln(1-\chi)] \qquad (5)$$

Where $\chi = (y_o - y)/(y_o - y')$

$A = (2v_o t_{1/2} - |y_o - y'|)/(v_o(1 - \ln 4))$

v_o = initial rate

$t_{1/2}$ = half time ($y(t_{1/2}) = 1/2[y_o - y']$

y_o = initial concentration

y' = final concentraion

The χ is the degree of progressed reaction and it followed from the definition above that χ always be in the interval $0 < \chi < 1$.

Equation 5 is then rearranged to Equation 6 to calculate the values of concentration (dependent variable) as a function of time (independent variable).

$$y = (y' + (y_o - y'))\exp[((v_o t - \chi\,|y_o - y'|)/Av_o) - \chi] \qquad (6)$$

The equation 6 is solved by computer by the bisection method.

All experiments with methyl α-isomaltoside (**37**), methyl 6-*S*-methyl-α-isomaltoside (**40**) and methyl 6-*R*-methyl-α-isomaltoside (**43**) were performed under exactly the same conditions.

VII. APPENDIX

Table 18. The time dependent concentration data for methyl α-isomaltoside (**37**) in the course of hydrolysis catalyzed by AMG II.

Time (min.)	**Conc.** (mM)
0.00	18.52
9.18	18.31
23.83	17.46
47.47	16.15
71.30	15.96
95.03	15.41
118.77	14.09
142.50	12.89
166.23	12.85
189.97	11.91
213.70	9.69
237.34	9.57
261.17	8.28
284.90	7.39
308.63	6.85

Table 19. The time dependent concentration data for methyl α-isomaltoside (**37**) in the course of hydrolysis catalyzed by AMG II.

Time (min.)	**Conc.** (mM)
0.00	9.26
31.33	8.38
55.13	7.37
78.93	7.10
102.73	5.94
126.53	5.78
150.33	5.03
174.13	4.39
197.93	3.56
221.73	3.02
245.53	2.80
269.33	2.28
293.13	1.76
352.63	1.03
412.13	0.75
471.63	0.19

Table 20. The time dependent concentration data for methyl 6-*S*-methyl-α-isomaltoside (**40**) in the course of hydrolysis catalyzed by AMG II.

Time (min.)	**Conc.** (mM)
0.00	9.26
8.20	8.67
47.93	8.71
97.60	8.03
147.27	7.45
196.93	7.29
246.60	6.18
296.27	5.46
345.93	5.55
395.27	5.40
444.93	4.82
494.60	4.61
544.27	4.61
593.93	4.17
643.60	3.88
693.27	3.73
742.93	3.33
792.60	3.12
843.27	2.95
892.93	2.76
894.93	2.60

Table 21. The time dependent concentration data for methyl 6-*S*-methyl-α-isomaltoside (**40**) in the course of hydrolysis catalyzed by AMG II.

Time (min.)	**Conc.** (mM)
0.00	18.52
57.00	17.28
118.42	15.89
179.83	15.09
241.25	13.80
302.67	12.96
364.08	11.93
425.50	11.00
468.42	10.28
548.33	9.52
609.75	9.09
780.17	7.28
829.58	6.83

Table 22. The time dependent concentration data for methyl 6-*R*-methyl-α-isomaltoside (**43**) in the course of hydrolysis catalyzed by AMG II.

Time (min.)	**Conc.** (mM)
0.00	18.52
8.33	17.96
20.33	17.31
32.33	16.80
44.33	16.07
56.33	15.26
68.33	14.61
80.33	13.85
92.33	13.70
104.33	11.96
116.33	11.37
128.33	10.37
140.33	9.69
147.38	9.61
159.38	8.57
171.38	8.50
183.38	7.44
195.38	6.59
207.38	5.41
219.38	4.98
231.38	4.07
243.38	3.50
255.38	2.41
267.38	1.89
279.38	1.39
291.38	0.91
315.38	0.00

Table 23. The time dependent concentration data for methyl 6-*R*-methyl-α-isomaltoside (**43**) in the course of hydrolysis catalyzed by AMG II.

Time (min.)	**Conc.** (mM)
0.00	9.26
6.50	8.65
20.28	7.93
30.25	6.97
40.25	6.34
60.25	4.27
70.25	3.97
80.25	3.19
90.25	2.56
100.25	1.80
110.25	1.15
120.25	0.55
130.25	0

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