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Synthetic studies on

α(1,2)-

Mannans

and preparation of a mannosidase inhibitor

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December 1995

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements of the degree of

Master of Science

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Abstract

Attempts to design a novel mannosylation were carried, unsuccessfully, on *in situ* generated 3,4,6-tri-O-benzyl-β-D-mannose-1,2-sulfite, and on 3,4,6-tri-O-benzyl-β-D-mannose-1,2-carbonate, with sequential treatments with bromotrimethylsilane and with glycosyl acceptors in the presence of a base. Trials were then conducted on Lewis acid catalysed mannosylations with the two above compounds, and the use of the in situ generated 3,4,6-tri-O-benzyl-β-D-mannose-1,2-sulfite with a mixture of chlorotrimethylsilane and zinc triflate in glycosylating simple alcohols was successfully demonstrated. However this activation was insufficient to build disaccharides using 1,3,4,6-tetra-O-benzyl-α-D-mannopyranose, methyl-2,3,4-tri-O-benzyl-α-D-glucopyranoside and methyl-2,3,6-tri-O-benzyl-α-D-glucopyranoside as acceptors.

A mannosylating agent, namely 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl trichloacetimidate, designed for the synthesis of 1,2-mannans was prepared, and used to build a benzylated 1,2-mannan trimer.

α-D-Mannopyranosylmethylamine-p-nitrophenyl-triazene, a mannosidase inhibitor was prepared by a slight modification of the literature procedure, for future use, in order to alkylate the active site of certain substrate specific α–mannosidases.

Résumé

La conception d' une nouvelle mannosylation a été tentée, de manière infructueuse, par le traitement sequentiel avec du bromo-trimethylsilane, suivi d' un accepteur glycosyle en milieu basique, du 3,4,6-tri-O-benzyl-β-D-mannose-1,2-sulfite generé in situ et du 3,4,6-tri-O-benzyl-β-D-mannose-1,2-carbonate. Des essais sur des mannosylations par des acides de Lewis, avec les composés précedants furent poursuivis, et l' usage du 3,4,6-tri-O-benzyl-β-D-mannose-1,2-sulfite generé *in situ* avec un mélange de chloro-trimethylsilane et de triflate de zinc, dans la glycosylation d' alcools simples, a été démontré. Cependant cette activation fut insuffisante pour la construction de disaccharides avec le 1,3,4,6-tetra-O-benzyl-α-D-mannopyranose, le methyl-2,3,4-tri-O-benzyl-α-D-glucopyranoside et le methyl-2,3,6-tri-O-benzyl-α-D-glucopyranoside en tant qu' accepteurs.

Un agent mannosylant, nommément le 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl trichloacetimidate, conçu pour la synthèse de 1,2-mannan, a été préparé, et a été utilisé pour la construction d' une trimère benzylée de 1,2-mannan.

Le α-D-Mannopyranosylmethylamine-p-nitrophenyl-triazene, un inhibiteur de mannosidases, a été préparé avec une légère modification de la procédure publiée, dans le but futur d' alkyler le site actif de certaines α-mannosidases specifiques à leur substrat.

Acknowledgements

First and foremost, I would like to thank Dr. T.H. Chan for his guidance, and his advice. In spite of his overwhelming schedule, he always seems to find the right suggestion to his students .

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Last but not least, I would like to thank my family, and especially my father and my mother for supporting and encouraging me during my everlasting studies. Their daily presence and the things they provide are irreplaceable. and their sum would fill up valleys. *Befrahkem !*

List of abbreviations

Ac	Acetyl
Bn	Benzyl
DMF	N,N-Dimethylformamide
HPLC	High pressure liquid chromatography
Hz	Hertz
iPr	Isopropyl
Me	Methyl
TEA	Triethylamine
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Trimethyl silyl

CHAPTER ONE

Natural Oligosaccharides with emphasis to Mannans

I] Natural carbohydrates

1) Introduction

Over the relatively short history of modern organic chemistry, carbohydrates have always been a source of interest. Initially, this interest was purely investigative in nature, as organic chemists were slowly unraveling the molecular components of life. Carbohydrates, the source of energy of living organisms, were then found quite easily and in large amounts[1]. However, as Biology made large progresses towards the understanding of the molecular basis for life, carbohydrates found themselves ignored, as they were thought to be nothing more than structural components of cells and an efficient energy storage method. In the past few decades however a large amount of interest was generated by the fact that biomolecules (proteins, nucleic acids and others) can carry information. It was then not surprising that carbohydrates were discovered as essential information carriers not only for intracellular communication but also as intercellular messengers[2,3]. Such a discovery brought about a renewed interest for carbohydrates as these allow information transfer not only at a cellular but at an organismal level, with the possible biomedical consequences of such a discovery. This explains the renaissance of carbohydrate chemistry.

Oligosaccharides, short oligomers of several glycosidic units, have been found in most of the different sets of natural products. There are only a few types of molecules made solely of carbohydrate units : apart from the scaffolding of cells and the carbohydrates in metabolism, most of the carbohydrates are found as glycoconjugates[4]. These are molecules possessing a carbohydrate moiety and an aglycon moiety. This moiety can be a steroid, an antibiotic, a peptide or any other biomolecule to which the sugar is attached. With the tremendous progresses in methods of isolation and especially in the sequencing of carbohydrates the number of known glycoconjugates has increased enormously[5,6].

How do glycoconjugates carry information? For carbohydrates, there are no complex machineries to read them, like the nucleic acids. Rather the oligosaccharides behave like a contact key: each of them can switch on a pathway, or a cellular response. Indeed, they each have a specific sequence, as well as three-dimensional structure, which can be bound by a protein and hence triggers a response[7,8]. This can be a switch to a cellular response, like a metabolic pathway. Or it can be an intercellular switch, and this later is the main pursuit of modern glycobiology. The ability of a carbohydrate to bind a protein, serves as a docking site for a cell unto another cell. This binding is the probable trigger to a type of signal transduction through the plasma membranes of the cells, which brings about an overall cell response. In an organism, such a mechanism is essential. This is how cells bind other cells[9,10], or bind the extracellular matrix[11], and then know how they are supposed to behave. This allows for cell differentiation[12], and for immune responses[13,14]. This is also the basis for metastasis[15,16,17]. Furthermore, this system allows for bacteria[18,19] to penetrate in the organism. And it serves as a dock for the viruses[20], enabling them to recognize their target cells. Another use of oligosaccharides is in intracellular trafficking[21,22,23]. This is the system which assigns the different proteins to their specific location (organelles, membrane...). In this system, oligosaccharides found on the proteins, which have just been synthesized, allow it to bind on a specific receptor. This receptor has the ability to trigger the formation of a vesicle around the protein-

receptor complex, vesicle which will fuse with the intended organelle, or the plasma membrane.

All the above refers to glycoproteins, but is also valid for other type of glycoconjugates, such as glycolipids, or proteoglycans. An example is the ability of the carbohydrate moiety of certain gangliosides (a type of glycolipid) to promote neurite growth[24].
Other glycoconjugates bring insights in the possible roles of carbohydrates in nature. A good example is the presence of a modified oligosaccharide on the antibiotic, calicheamicin, which is reported to be involved with the DNA binding[25]. Another example is the glycocalix, a 'collar' of carbohydrates around the cell, and whose role is not quite well understood, but which might serve to slow down invaders by diluting their target oligosaccharides in a sea of other carbohydrates[26].

It is therefore this renewed interest in the biology of carbohydrates which has triggered a renewed effort in the total synthesis of oligosaccharides and polysaccharides. This has generated both novel methodologies in the formation of glycosidic bonds and an effort in the improvement of the methods in carbohydrate chemistry.

2) Biosynthesis

a) Glycogen

Glycogen (animal starch) is a polymeric form of glucose, made of linear $\alpha(1,4)$ linked glucose units with $\alpha(1,6)$ linked branch points (figure 1). Every single glycogen molecule contains a single free anomeric position, referred to as the 'reducing end', as opposed to several 'nonreducing ends' [27].

Glycogen biosynthesis is an essential part of energy storage mechanisms. It is taken care of by a set of enzymes. The first step is the transformation of glucose into 6-phosphoglucose (**1**) by hexokinases (at least four types known)[28]. This is followed by the conversion of 6-phosphoglucose to 1-phosphoglucose (**2**) by phosphoglucomutase[29]. This compound is then transformed into uridyldiphospho-glucose (UDP-glucose, **3**) by the UDP-glucose

phosphorylase[29]. The aim of all this is to provide a high-energy substrate for the glycogen synthase.

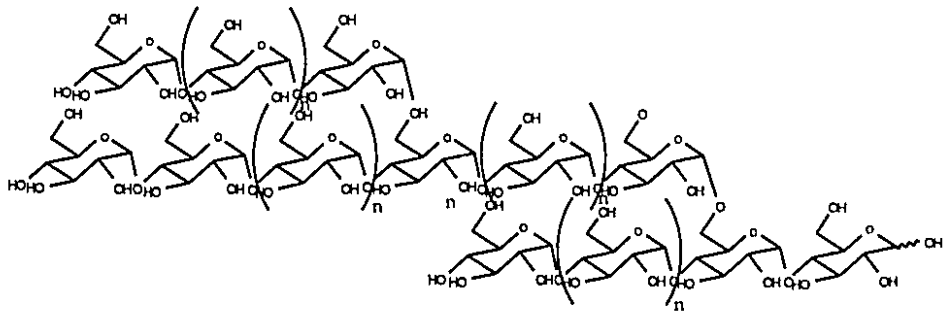
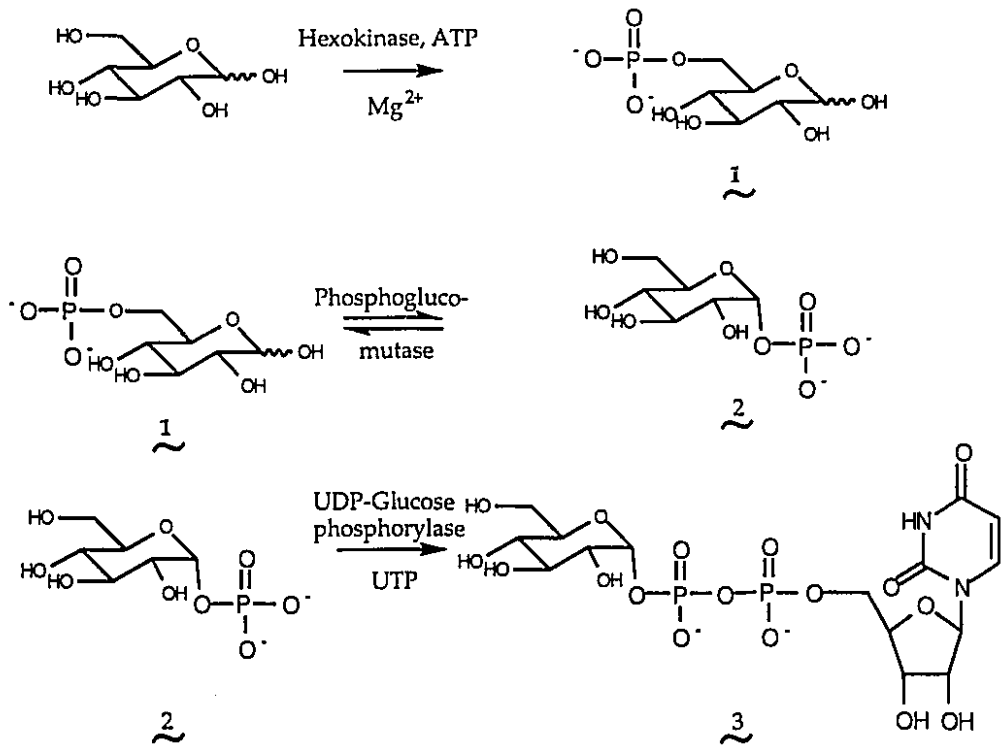


figure 1



This enzyme adds a glucose unit in an α-(1,4) linked fashion to the growing end of the growing glycogen molecule[30].

UDP - glucose + Glycogen (n-residues) ⟶ Glycogen (n+1-residues)

As for the α-(1,6) glycosidic bonds, these are due to a glycogen branching enzyme : amylo (1,4 -> 1,6) transglycosylase[31]. This enzyme cleaves an α-(1,4) glycosidic bond, and forms an α-(1,6) glycosidic bond. This is done on a group of at least seven glucose residues coming from a stretch of at least eleven residues, and the new branch point is formed at least three residues away from other branch points.

Furthermore, it is important to notice that the previous enzymes are the target of control pathways in cells[32].

b) Asparagine-linked oligosaccharides

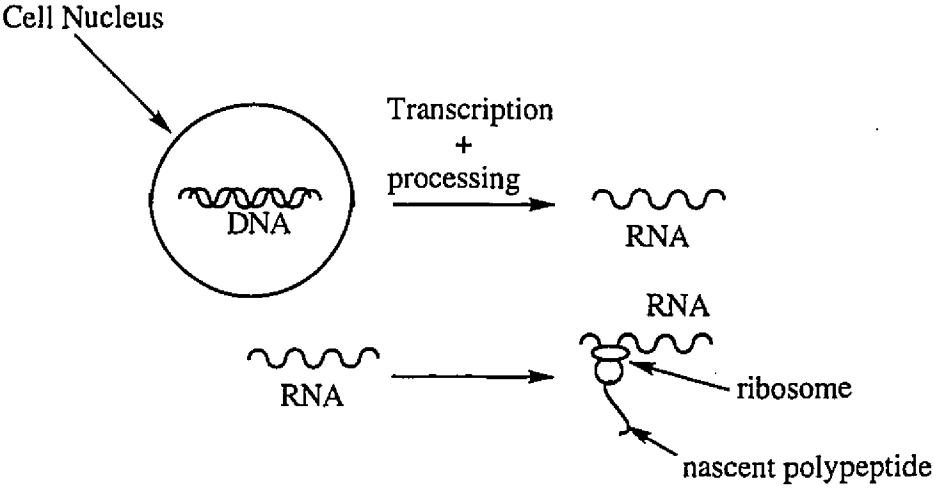


Figure 2

The biosynthesis of proteins generally goes through the following process : the protein's gene is encoded on the deoxyribonucleic acid (DNA) as a stretch of nucleotides. This stretch is translated into a sequence of ribonucleic acid (RNA) by an RNA polymerase and a complex set of other proteins (transcription factors) (figure 2). This RNA is then used as a template to synthesize the protein, thanks to a huge ensemble of proteins and nucleic acids, called a ribosome. This process is called translation. This ribosome reads the RNA strand and translates it in a peptide strand, again with the help of a set of other proteins (translation factors) (figure 2).

When the peptide synthesized is to become an N-glycosylated glycoprotein, the translation is brought to a halt after a stretch of amino acids has been synthesized. The ribosome diffuses to a type of organelles called the endoplasmic reticulum (E.R.). There, the ribosome docks on the organelle, and, through a not so-well defined system, the nascent peptide is translocated inside the organelle, while the ribosome stays outside. As the peptide' s synthesis starts again, it is slowly inserted into the E.R. (figure 3). On this organelle, a lipid linked oligosaccharide is found, the lipid being dolichol phosphate, an isoprenoid lipid (4).

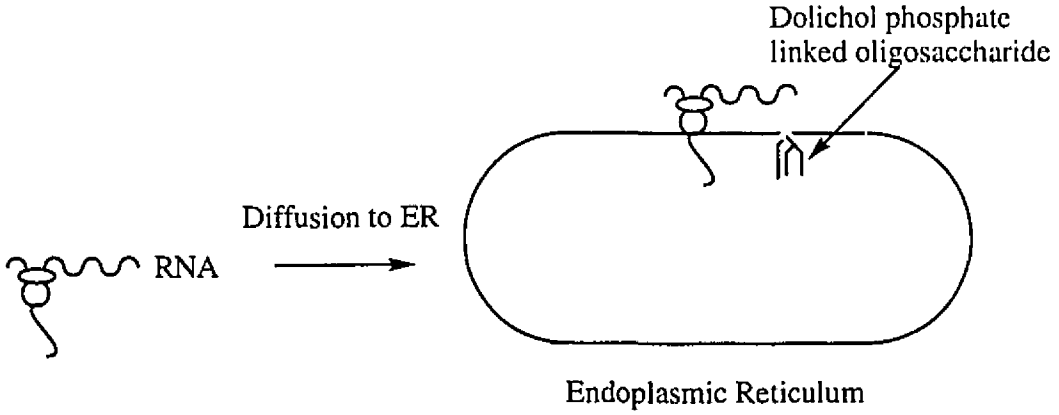
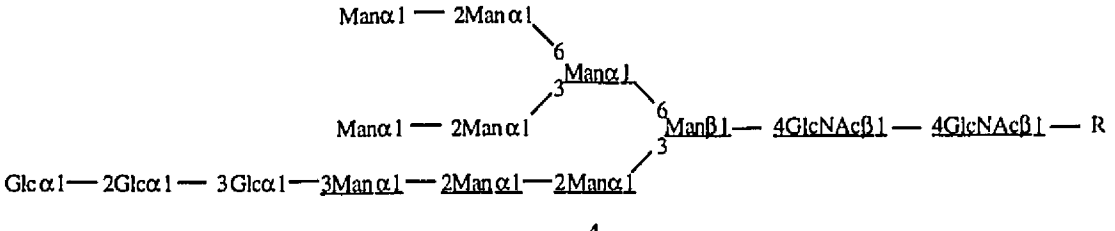


figure 3



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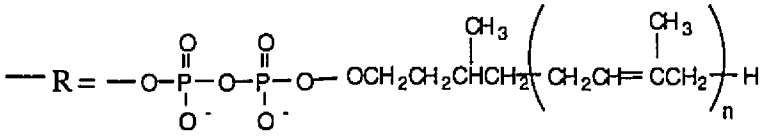
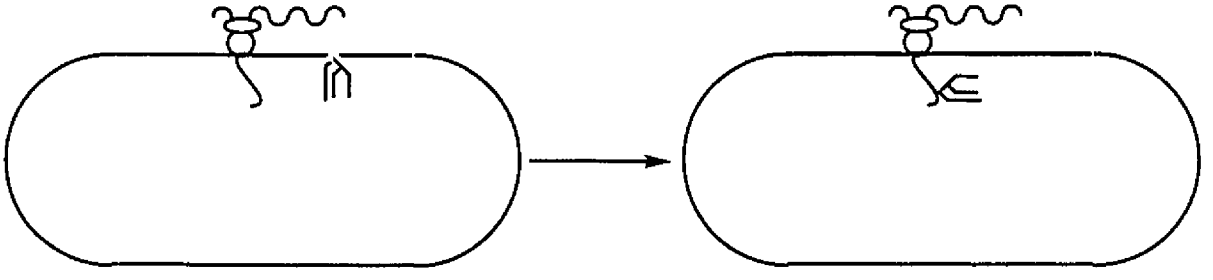


Man = Mannose
Glc = Glucose
GlcNAc = N-acetyl-glucosamine
n=15 to 20

This lipid bound oligosaccharide is probably assembled in the cytoplasmic side of the organelle, and then translocated in the inside (lumen)[33,34]. The biosynthesis of this lipid has been extensively studied, and reviewed[35,36,37]. This compound is biosynthesized using two types of glycosides. The underlined residues come from the sugar-nucleotides, uridyl diphospho-N-acetyl-glucosamine (UDP-GlcNAc) and guanidyl diphospho-mannose (GDP-Mannose), in a manner similar to the UDP-glucose used to synthesized glycogen. The other residues are derived from the lipids dolichol-phospho-mannose (Dol-P-mannose) and dolichol-phospho-glucose (Dol-P-Glucose). The resulting glycolipid is translocated to the lumen and then transfered to the nascent protein, by the enzyme oligosaccharyl transferase[38](figure 4). The transfer is done to a asparagine residue found in the sequence asparagine-X-serine/threonine, where X is any amino acid residue except proline and aspartic acid[39], and it is probable that certain other requirements for the above sequence are also necessary, as not all the arginines found in such a sequence are glycosylated[40].



Endoplasmic Reticulum Endoplasmic Reticulum

figure 4

In the next steps, the oligosaccharide on the protein will be processed by glycosidases and by glycosyltransferases. Simultaneously the protein moves from the endoplasmic reticulum to the Golgi apparatus, a complex set of organelles. In this location, not only will the processing continue, but it becomes selective, so that the resulting oligosaccharide can allow for targeting of the protein to its

subcellular compartment or to the membrane, or simply excretion[41]. Figure 5 represents the processing of membrane proteins, but the same is valid for the secreted and the organelle bound proteins.

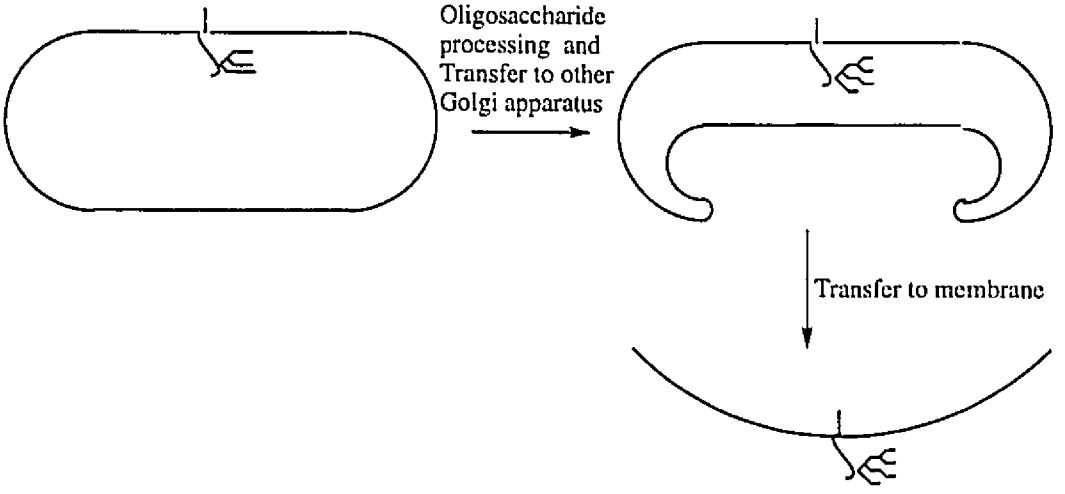


figure 5

c) Other natural oligo- and polysaccharides.

The biosynthesis of both glycogen and N-linked glycoproteins shows the following pattern : the cell uses glycosyl transferases to build oligosaccharides from activated glycosyl precursors, whether they are sugar-nucleotides (UDP-glucose, UDP-GlcNAc, GDP-mannose) or glycolipids (Dol-P-mannose, Dol-P-glucose). This pattern seems to be conserved in the biosynthesis of other carbohydrates in the body. Such is the case of proteoglycans and glycosaminoglycans, large sets of carbohydrates which are part of the extracellular matrix, and which are made of a long protein backbone, on which polysaccharides are grafted[42]. Such is also the cases of the carbohydrate components of the cell envelope of yeasts[43] and of plants[44]. As for other glycoconjuguates, the synthesis seems to be similar, such as for glycolipids[45], even if for certain bacterias, for instance, the machinery can be very different[46].

II] Mannans

Mannans are polymers of mannose which are found in natural sources.

1) Yeast mannans

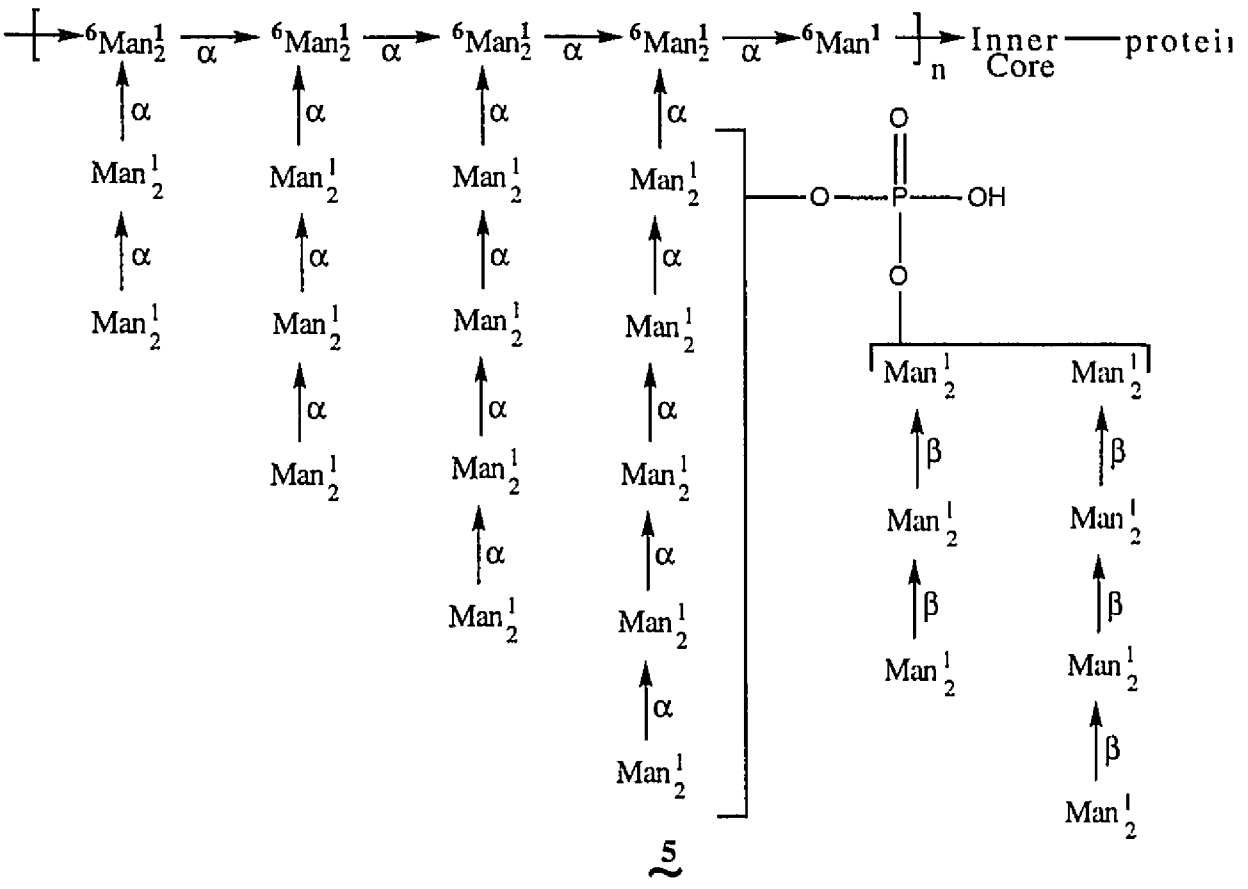
Fungi can take two forms, a multicellular and a unicellular form. The unicellular fungi are refered to as yeasts and this form, being microbial, is sometimes pathogenic. Yeasts have a very complex cell envelope. This is made of several layers. For instance, the cell envelope of the pathogenic yeast *Candida albicans* has five layers (from the plasma membrane to the external medium) : a mannoprotein layer, a β-glucan-chitin layer, a β-glucan layer, another mannoprotein layer, and a fibrillar layer[43].

The mannoprotein (5) layer is made of a protein scaffold, to which the mannan is grafted through two N-Acetyl-glucosamines, on arginines[47,48]. The mannan itself is made of a set of of α-(1,2) linked mannan bound on a central chain on the 6 position[49,50].

The mannan of parasitic yeasts has been shown to be antigenic in nature, although fragments of this mannan are not[51]. But even more interestingly, the mannan of certain yeasts has been shown to be immunoinhibitory[52,53]. Indeed, the mannan seems to affect white blood cells[54]. And this inhibitory effect is also seen on skin cells[55], which may indicate that the mannan is used by the pathogens to penetrate in the organism.

2) Plant cell wall mannans.

Plant cells differ from animal cells in the fact that the plasma membrane is surrounded by a large cell wall, which is made of several organic and inorganic materials, including a large carbohydrate component. Most of this is cellulose (β-(1,4) linked glucan)[56]. However mannans are also found, in three general categories (see figure 6).



Possible structure for the cell wall mannans of *Candida stellatoidea*

The first, and constitutively the most important, are the homopolymeric β-(1,4) linked mannans, which has been found to be the major constituent of several plant[57] and algae[58] cell-walls.

The second are the galactomannans, heteropolymers made of a β-(1,4) linked mannan, on which galactose residues branch themselves through an α–(1,6) or α-(1,2) glycosidic bond.

The third are the glucomannans and the galactoglucomannans, which are similar to the galactomannans except that the β-(1,4) linked mannan is replace by alternating mannose and glucose residues linked in the same fashion[58].

These compounds not only give the plant cell a scaffolding. They seem to be involved in a series of different functions, such as playing the role of natural molecular sieves for the cell, in cell-cell

and cell-matrix interactions or in the defense against microbes[58]. In this last respect, it has been shown that the degradation of the carbohydrates (especially of the galactomannan and the galacto-glucomannan), by the microbial invader, brings about the synthesis and accumulation in the cell of antimicrobial agents[59,60].

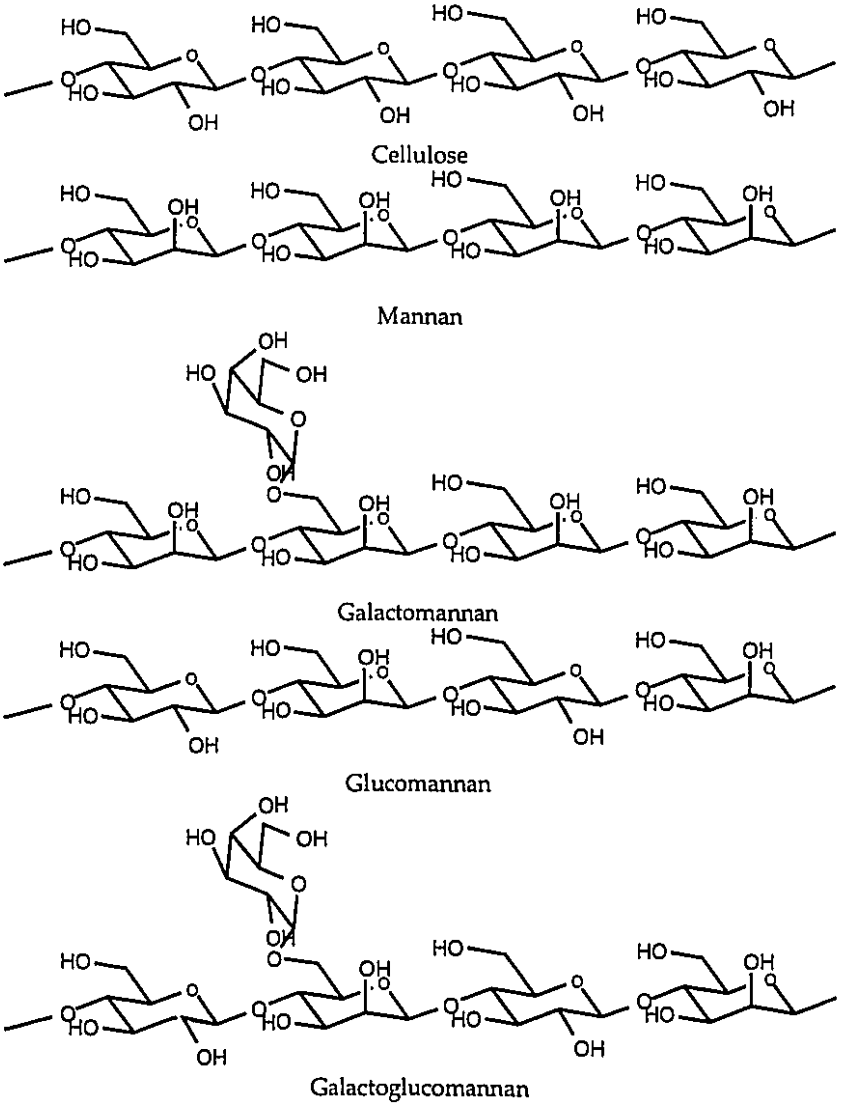
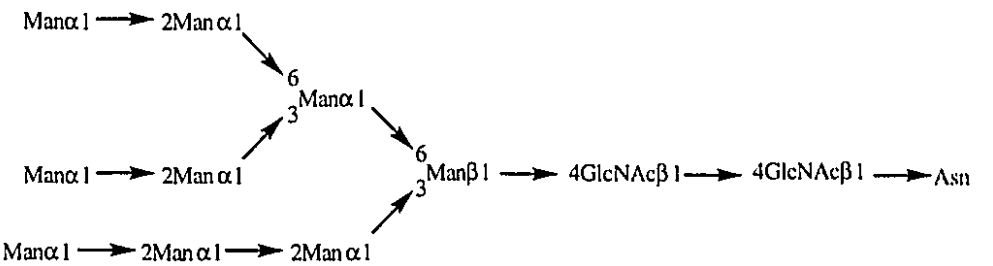


Figure 6

3) High-mannose glycoprotein

These represent particular N-linked glycoproteins for which the processing has given a mannose oligosaccharide (for example 6), in which all of the residues, except the two N-acetyl-glucosamines bound to the arginines, are mannoses.



6

High-mannose type glycoproteins

These are found in normal cells, such as plant cells[61], or in cells which have been treated with mannosidase inhibitors and which lack the ability to process the oligosaccharides properly[62].

4) Biomedical purposes for mannans.

With the recent progress in the chemical synthesis of carbohydrates, the preparation of large quantities of oligosaccharides for biological purposes has become possible. Indeed, the carbohydrates involved in biological processes are available from natural sources only in small amounts, which complicates considerably the studies. In the case of glycosans, another complication lies in the fact that the carbohydrates are polymeric, so that their polydispersity after extraction from natural sources complicates their use even more.

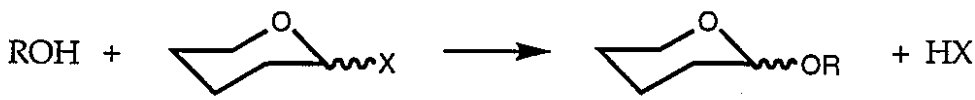
Examples of the use of chemically synthesized glycosans in biology are available. For instance, stepwise chemically synthesized α(1,6)-linked oligomers of glucose have been used to study the immunogenicity of clinical Dextran. A crystal structure of an antibody binding such an oligomer has been obtained[63].

Another example is the use of yeast mannan, which is enzymatically phosphorylated in vitro, by a hexokinase, and bound on alumina. This was used as a stationary phase in the chromatographic isolation of mammalian mannose-binding proteins, which are involved in many cellular and physiological phenomena[64]. In our case, we were interested in the synthesis of α(1,2)-mannans, and, ultimately, of the mannan portion of high-mannose glycoproteins, to be used as a substrate for certain very substrate-specific mannosidases and in the evaluation of the substrate specificity of these enzymes[65].

III] The chemical synthesis of oligosaccharides

1) The mechanism of glycosylation

Glycosylations are the reaction between an activated pyranose ring, the glycosyl donor (**6**), and an alcohol, the glycosyl acceptor.



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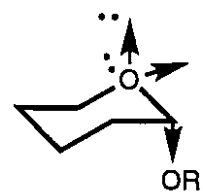
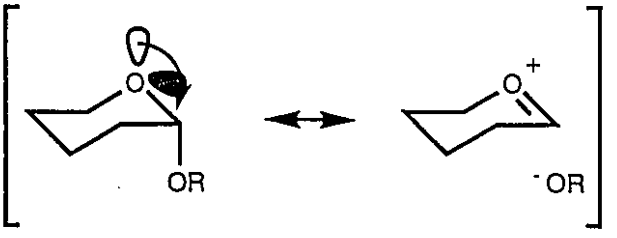
This reaction is governed by the unexpected preference of the pyranose ring for an axial orientation of the electronegative substituent at the anomeric carbon. That is, the α-anomer is preferred to the β-anomer. This phenomenon is called the "anomeric effect"[66], and is due to the destabilizing interaction between the dipole created by the lone pairs on the ring oxygen and the dipole of the carbon-electronegative atom bond in the β-anomer.

β-glycoside

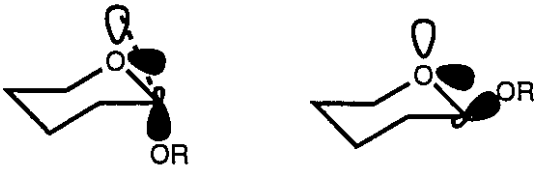


α-glycoside

Another explanation for this effect has been provided by considering the lone pairs of the ring oxygen. The initial explanation was proposed in view of to the fact that, in an α-configuration, one of the lone pairs is positioned trans to the glycosidic bond. This allows to donate an electron, stabilizing the structure by resonance[67]:



This is not possible in the β-configuration, adding stability to the α-anomer. A more modern view of this explanation is that one of the lone pair orbital of the ring oxygen, in the axial configuration, can overlap with the s^* antibonding orbital of the glycosidic bond[68]:



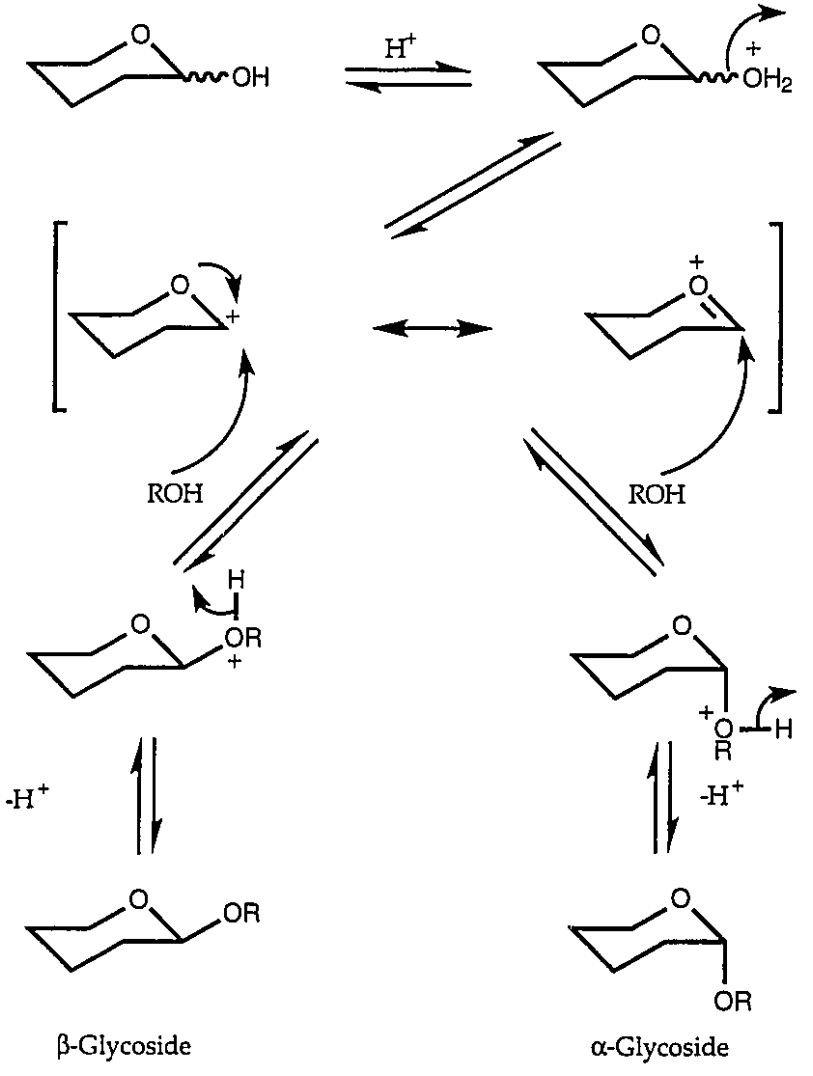
α-glycoside β-glycoside

This effect has been reviewed[69] and it is not necessary to discuss it further, but just to accept its existence, and to have an idea of its magnitude : The equilibrium constant between α-glucose penta-acetate and β-glucose pentaacetate is 5, in 50% acetic acid in acetic anhydride, at 25°C[70]. The thermodynamic result of the anomeric effect is quite moderate.

The studies on the mechanisms of glycosylation are quite rare because of the generally synthetic purpose of the work done on glycosylation. The first insights and the most simple example of glycoside synthesis were given by E. Fisher. His preparation consisted in the acid-catalyzed alcoholysis of monosaccharides[71,72]. Although

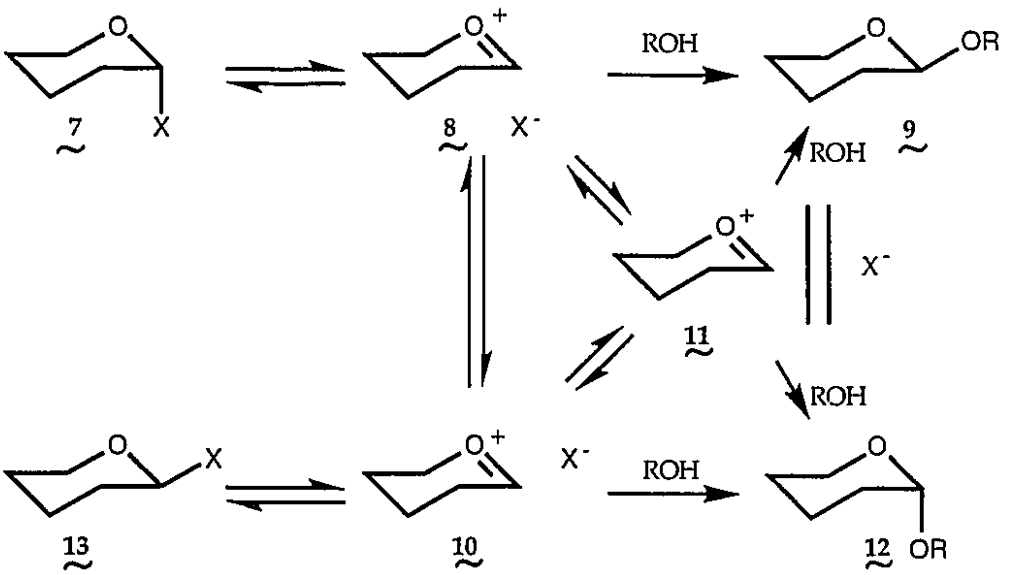
not very efficient, his method allows one to draw a simple mechanism.

As one can see, this reaction involves an equilibrium between the original free sugar and the two anomers of the product glycoside:



From this mechanism, a relatively simple scheme was designed[73], which may explain the stereoselectivity of the glycosylation. As one can see, the presence of the ring oxygen allows the reaction to proceed via an S_N1 mechanism. However, the reaction

somewhat behaves like an S_N2, as the α-anomer of the glycosyl donor tends to give more of the β-glycoside in some instances, than the β-anomer. To explain such a behavior, the existence of a tight ion-pair between the glycosyl cation and the activating group is postulated. Hence the general model is the following. The α-anomer of the glycosyl donor (**7**), is in equilibrium with a tight ion-pair (**8**), which is also in the α configuration. This tight ion-pair can either react to give the β-glycoside (**9**), or equilibrate with the β-anomer of the tight ion-pair (**10**) or with the loose ion pair (**11**). The tight ion-pair **10** can then react to give the α-glycoside.(**12**). The β-anomer of the glycosyl donor (**13**) also gives the tight ion pair **10**. Finally the loose ion pair **11** can give any of the two glycosides, with a preference to the α-anomer, due to the anomeric effect.



This scheme shows that if **11** is formed then the selectivity of the reaction can be largely lost. It is also important to remark that because of the anomeric effect the reaction between the α-anomer of the tight ion-pair (**8**) with the alcohol is presumably much slower than the reaction with the β-anomer (**10**).

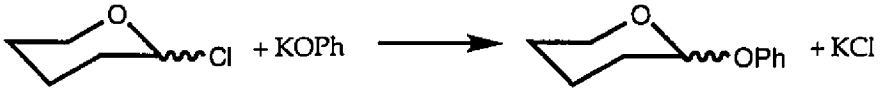
Therefore most of the glycosylation reactions can be rationalized with this scheme. This allows to account, among others, for the solvent effects, the participating groups and the leaving groups.

2) Common glycosylations.

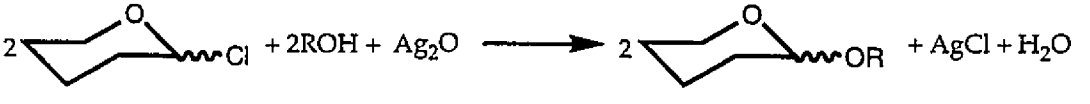
A huge number of glycosylation methods exist, and a vast amount of literature reviews have been published on the subject. The glycosylations presented here do not form an exhaustive list of the existing methods, but just a quick glance at the methods which have been successfully applied to the synthesis of oligosaccharides.

a) The Koenigs-Knorr method.

The typical Fisher method is, as said earlier, not a very practical method, and, from very early on, better glycosylation methods were required. For this reason, around 1880, Michael used glycosyl chlorides and potassium phenoxide to generate the glycoside[74, 75,76]:

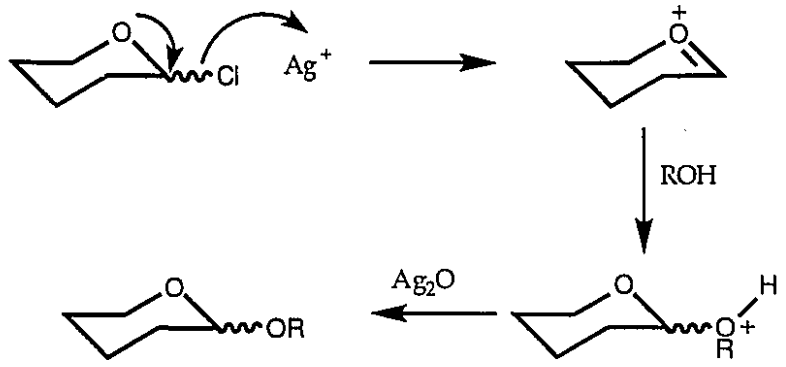


W. Koenigs and E. Knorr decided to improve this strategy by using alcohols as glycosyl acceptors, and to avoid the generation of the hydrogen halide, they used silver oxide as a hydrochloric acid acceptor[77].



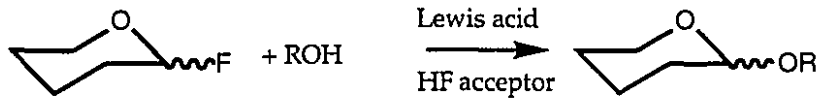
As people studied this reaction further, it became obvious that the silver oxide didn't act only as an HCl acceptor, but as a catalyst, helping to generate the different ion-pairs[78,79]. Furthermore, the result of using silver oxide was the generation of water, which is nocive to the reaction conditions. So it became necessary to use a drying agent, like molecular sieves, or calcium sulfate (Drierite).

Other improvements were brought about by the introduction of glycosyl bromides as glycosyl donors, with the use of mercury salts.



Other catalysts were used, to try to avoid the requirements for toxic and expensive heavy metals, such as tin (IV) and tin (II) salts, and boron trifluoride etherate[80]. But none of these methods were satisfactory, or very general in nature. Other hydrogen halide acceptors were used to allow for the use of catalytic amounts of heavy metal salts, such as tetramethylurea, or *sym* - collidine[80]. But the requirements for these salts were still fairly high.

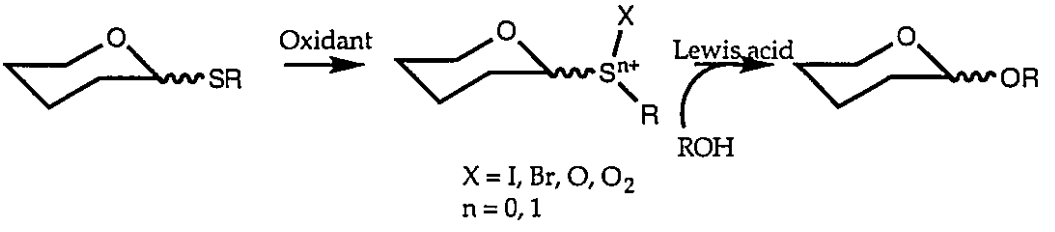
The Koenigs-Knorr method was improved by Mukaiyama, who introduced the glycosyl fluorides as glycosyl donors[81]. These are more stable than the other glycosyl halides, and can be generated quite easily from the thioglycosides, by the addition of (diethylamino)sulfur trifluoride (DAST) and N-bromo-succinimide (NBS)[82]. The fluorides can be activated by a silver perchlorate and stannous chloride mixture[81]. Other Lewis acids were also used without the presence of silver salts, including silicon based Lewis acids[83].



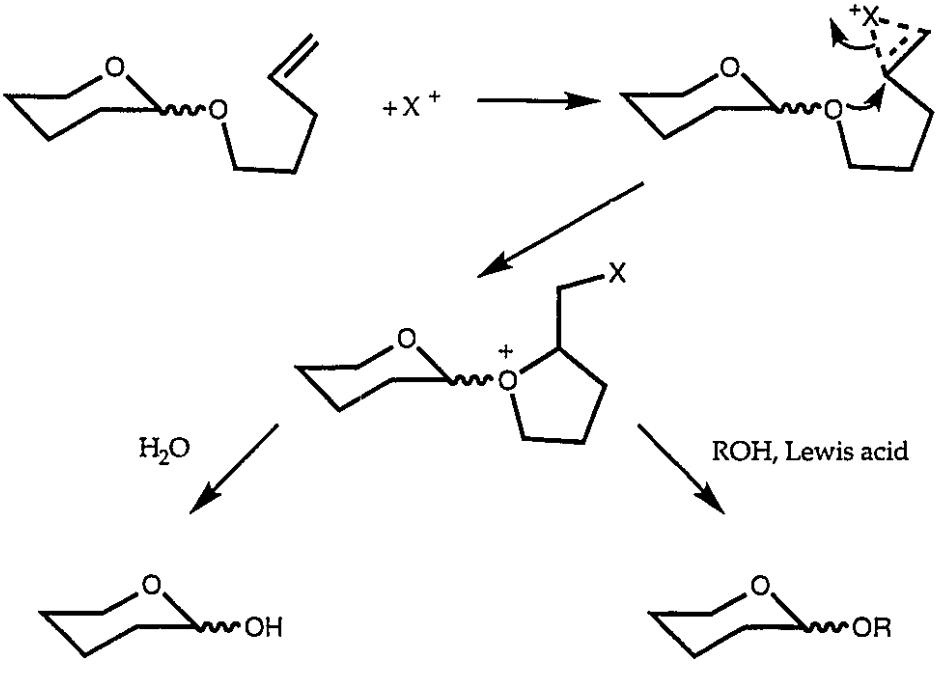
b) Thioglycosides

Thioglycosides are very versatile glycosyl donors. As said previously, the thioglycosides can be used to generate the corresponding glycosyl fluorides[79]. However, they are, themselves, glycosyl donors, in the presence of thiophilic substances, such as mercury (II)[84,85,86], copper (II)[87] and palladium (II)[88] salts. Other

methods use oxidative conditions, hence turning the sulfide into sulfones or sulfoxides, which are better leaving groups. Hence, bromine[89], NBS[90], N-iodo-succinimide (NIS)-trifluoromethanesulfonic (triflic) acid[91,92], and other oxidation methods, including electrochemical[93,94] have all been used.

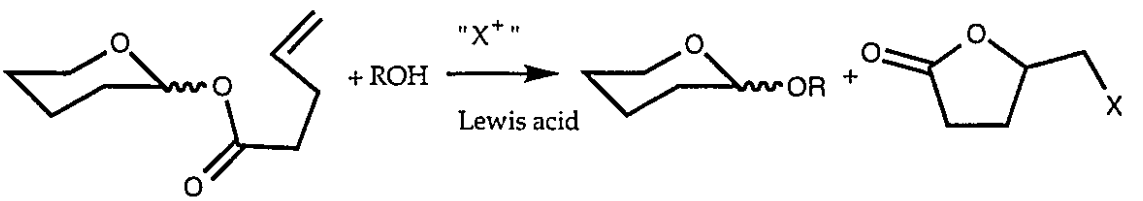


c) The 4-pentenyl alcohol glycosylation



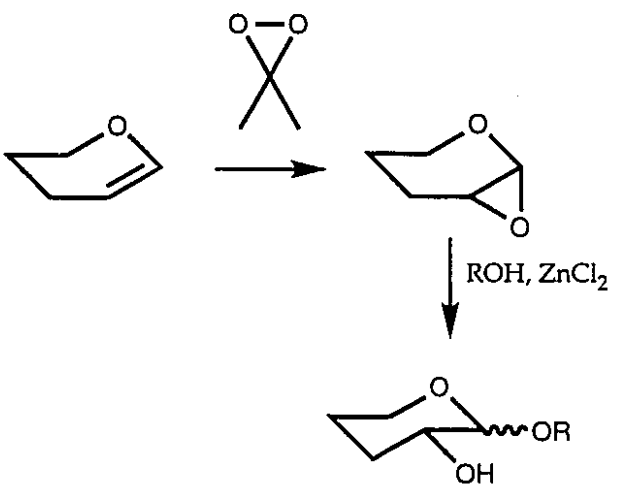
This method was initially developed as a protecting group for the anomeric position : this position is protected by a 4-pentenyl group, which is easily hydrolyzed in the presence of halogens, and other halogen cation generators, such as NBS or NIS, in water[95]. The idea, developed by Fraser-Reid, was to replace water by an alcohol to get the glycoside[96]. This required also a Lewis acid. Common

activators are NIS-triflic acid[97], NIS-triethylsilyl triflate[98], and IDCP[96]. The disadvantage with this method is the fact that putting on the 4-pentenol is already a glycosylation. So a derivative method has been proposed and developed, using the ester of 4-pentenoic acid[99,100].



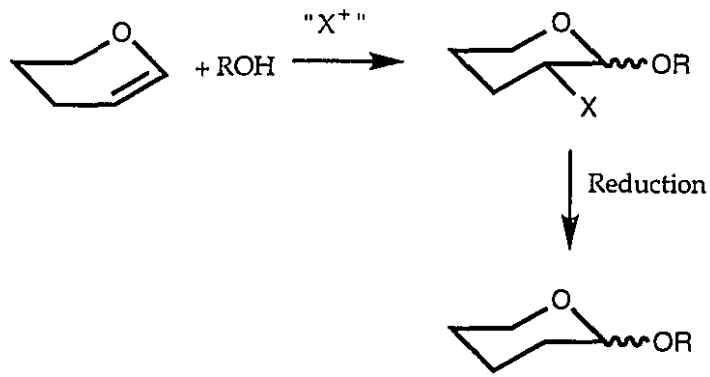
d) 1,2-Anhydrosugars as glycosyl donors

Brigl was the first to introduce the 1,2-anhydrosugars[101]. But they became increasingly popular, when it was discovered that they can be easily generated from the corresponding glycals, by oxidation with dimethyl dioxirane.



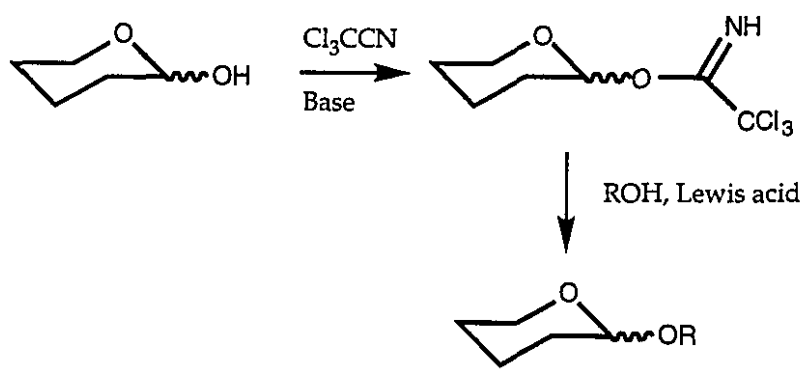
Then it became possible to use these anhydrosugars for the preparation of trans glycoside, by treatment with the glycosyl acceptor and a weak Lewis acid, such as zinc (II) chloride[102,103,104,105,106].

Actually, glycals themselves are used as glycosyl donors, in the preparation of 2-deoxy glycosides. They react with the alcohols, in the presence of an iodine source to give the 2-deoxy-2-iodo glycoside, which can be reductively de-iodinated[107,108,109]. Several other methodologies were based on such a scheme, that is, on the preparation of carbohydrates with a reductively removable group on the second position[110].



e) The trichloroimidate glycosylation

This is probably the most general glycosylation found to date. It was initially proposed by Sinaÿ[111,112] and was developed in its applications by Schmidt[1].



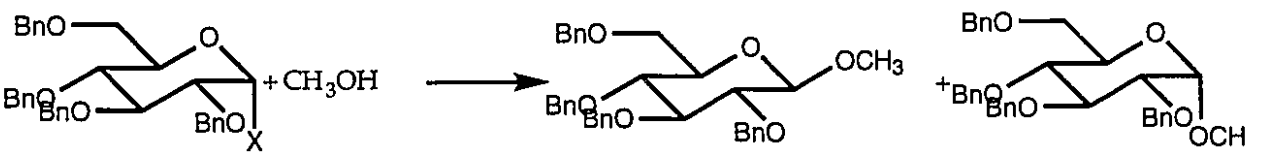
It involves the addition of the free anomeric hydroxy group on a trichloroacetonitrile in the presence of bases. The resulting imidate is fairly stable and can be used to glycosylate even the most

unreactive alcohols, as well as carboxylic acids[113,114], phosphoric acids[115] and others[1].

3) Control of stereochemistry at the anomeric center

As said earlier on, the magnitude of the anomeric effect is limited, so that a synthetic chemist could not always rely on it to build α-glycosides. Furthermore, this effect rendered the preparation of β-glycosides extremely difficult, as even if one is to start with the α-glycosyl donor, the tight ion pair **8** is quite hard to maintain, especially in the polar solvents that one has to use in carbohydrate chemistry. Therefore, it appeared necessary, from very early on, to provide methods to be used in obtaining carbohydrates with high selectivity.

A certain number of observations brought about the initial works. For instance, it was noticed that the methanolysis of 2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl bromide gave 45% of the α-glucoside[116], whereas the same reaction with the corresponding chloride gives only 6% of the same anomer[117]. This is of course quite easy to understand given the scheme for glycosylation, as the tight ion pair of the chloride is much easier to maintain. Another observation is the fact that the amount of α-anomer increases with dilution of the alcohol or with increasing amount of halide salts added in the reaction mixture[116].



X	β	α
Br	55%	45%
Cl	94%	6%

Fréchet and Schuerch[118] conducted studies on the production of increasing amounts of the α-glycosides in the methanolysis of glucopyranosyl bromides, by using different p-substituted benzoates

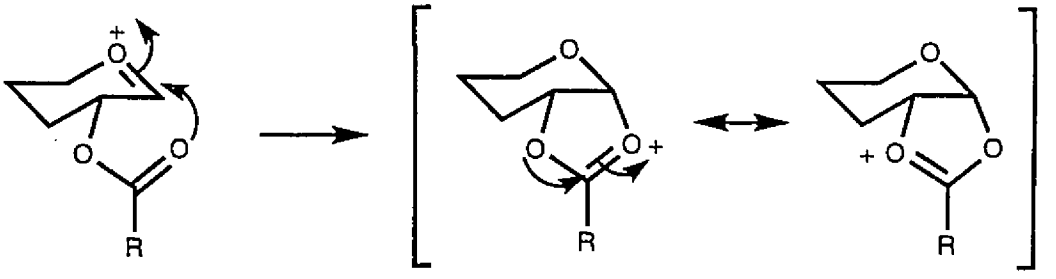
at the 6 position of their glycosyl donors. The idea is that if the substituent is electron withdrawing, then this destabilizes the corresponding glycosyl carbocation, so that the tight ion-pairs would exchange rapidly, and the amount of the β-anomer of the tight ion pair **10** increases. For obvious reasons, this anomer is more unstable than the other anomer and reacts faster to give the α-glycoside. The same way, if the substituent is electron donating, then the stability of the α-anomer of the tight ion-pair increases, promoting the formation of β-glycosides. Hence the methanolysis of the 2,3,4-tri-O-Benzyl-6-O-(p-substituted benzoyl)-α-D-glucopyranosyl bromides followed the pattern set by the Hammett substituent constants. For example, the corresponding 6-p-methoxybenzoyl glycosyl donor gave 84% of the corresponding β−product, whereas the 6-p-nitrobenzoyl one gave 95% of the corresponding α-product.

Fletcher discovered a particularity in these reactions : the presence of a source of the halide in solution allowed a better selectivity in favor of the α-anomer[119,120]. To account for this, Lemieux[121] proposed the following : the α-glycosyl halide generates the glycosyl cation in a tight pair with the halide. This tight pair can either isomerize to the corresponding β-tight pair, or react to give the β-glycoside. The rapidity of the isomerization, relatively to the glycosylation, dictates the stereochemical outcome, all the more that the β-tight pair reacts quicker, because of its higher instability. Therefore the faster it is, the higher the α-glycoside content. Adding halide to the milieu, and hence increasing the propensity to anomerize, favors the formation of the α-glycoside. This technique has been successfully applied to the preparation of orthoesters from the corresponding cis-glycosyl halides[122].

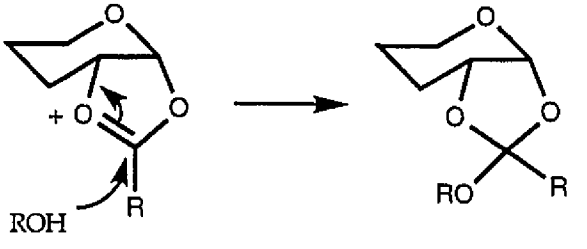
However, all of these methods are highly impractical, as the desired stereochemistry can be obtained only through a large amount of trial and error, each of which requires a novel setup. For this reason, a set of methods were needed, which can predictably give the desired stereochemistry. Two general methods were designed to do so.

The most important and probably the most common method is the neighboring group participation. This method consists of an

intramolecular and reversible nucleophilic attack on the glycosyl carbocation, setting the angle of attack of the incoming glycosyl acceptor. The most well known example of this is the participation of an acyl group at position 2:

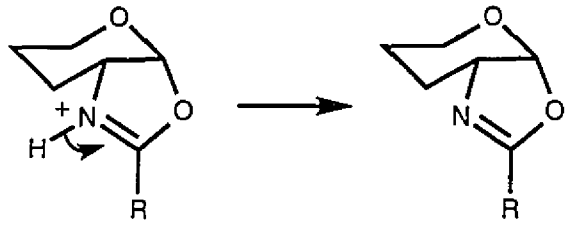


This participation has been noticed as early as in the 1940's, with the synthesis of aryl glycosides with potassium phenoxide by Michael[74,75,76]. This method of controlling the stereochemistry has two major disadvantages. On the one hand, this method results in the formation of orthoesters, by the nucleophilic attack of the alcohol on the ester carbonyl:

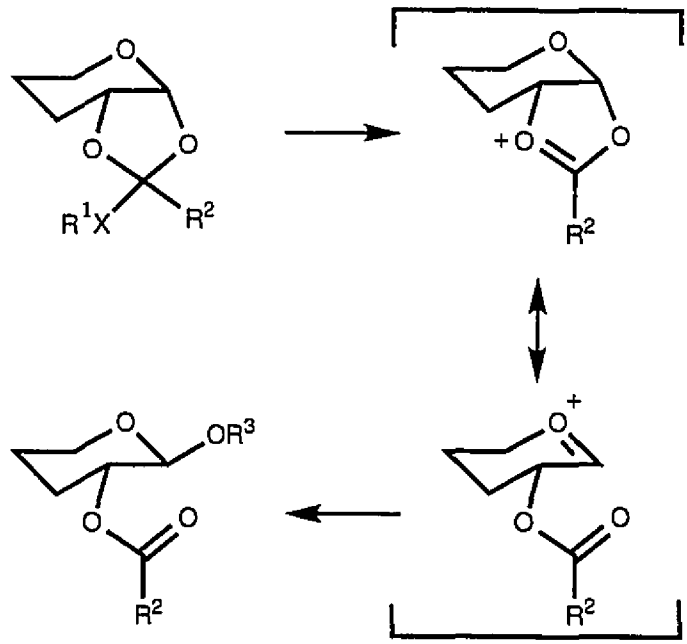


In strong Lewis acidic conditions, these orthoesters are unstable and rearrange to the glycoside. Another way of avoiding these is to use a hindered ester at position 2, such as pivaloates[123], which can still participate in the reaction, but are too hindered to give the orthoester.

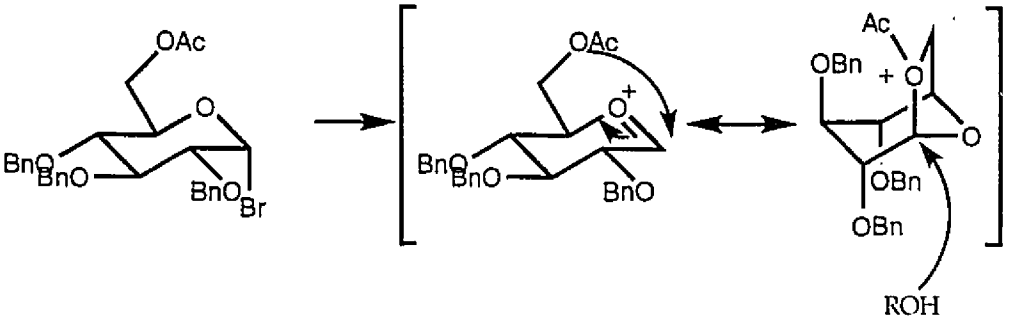
Another problem is with the 2-deoxy-2-acetamido-pyranoses. In these, the participating nitrogen can be deprotonated and give oxazolines. These are quite stable to acids, and hence cannot be used to give good yields of the glycoside. Therefore, it has not been a method of choice in the formation of N-acetyl-glycosides[124].



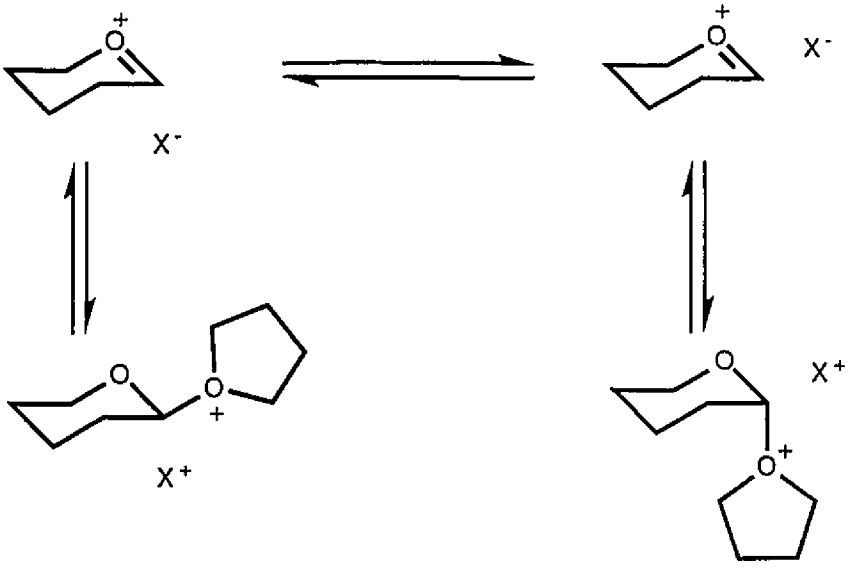
This participation method has been the basis for the development of a glycosylation methodology, where orthoesters of t-butanol[125] and thiophenol[126] are used. These are generally more unstable than other alcohols, and upon specific treatments, with mild acids[125] for the alcohols, NIS and a Lewis acid[127] for the thiophenol, they release the corresponding glycosyl cation, which reacts with the glycosyl donor :



Some other forms of neighboring group participation have already been invoked. For instance the high selectivity of the base-mediated glycosylation of 6-O-acetyl-2,3,4-tri-O-benzyl-α-D-gluco-pyranosyl bromide, in giving the α-glycoside, has been attributed to the participation of the 6 position in the glycosylation[128,129]:



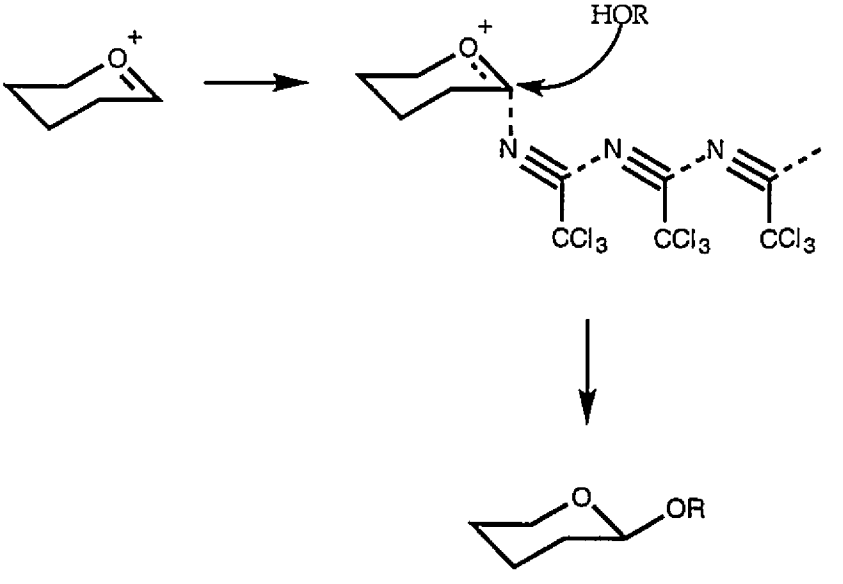
The second form of participation has been developed on the basis that, as said earlier on, the solvent can participate in the glycosylation. Studies have been conducted on the solvent effects. Indeed, as the solvent has increasing donor properties, the equilibrium between the different ion-pairs increases, favoring the α–anomer, by giving time for the β–anomer of the tight ion-pair to be generated. Certain good donor solvents have even been postulated to form an alternative set of ion pairs, together with the corresponding loose ion-pair. For instance, with THF or pyridine, one has the following :



This type of effect can invert, under certain conditions, the expected stereochemistry of the glycoside product. The most important thing to notice is that the heteroatom bonded to the

anomeric carbon is electron deficient and benefits from the reverse anomeric effect, that is the solvent molecule is preferably in an equatorial position[130].

Building on this, Schmidt proposed to use acetonitrile at -30 °C to control the anomeric stereochemistry. His purpose was to slow down the glycosylation, in order to allow for the solvent to act as a partially charged counter-ion to the glycosyl carbocation. This would happen faster in an α stereochemistry, as the solvent's heteroatom is still electron rich, and because of the anomeric effect, forces the glycoside to come in in an equatorial fashion yielding the β-glycoside[131]. This technique has been successfully applied to his trichloroacetimidate methodology:



Although not always applicable, the above mentioned methods have proven quite useful to the carbohydrate chemist, as they have dictated the choice of synthons, especially with respect to protecting groups, in the building of oligosaccharides.

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CHAPTER TWO

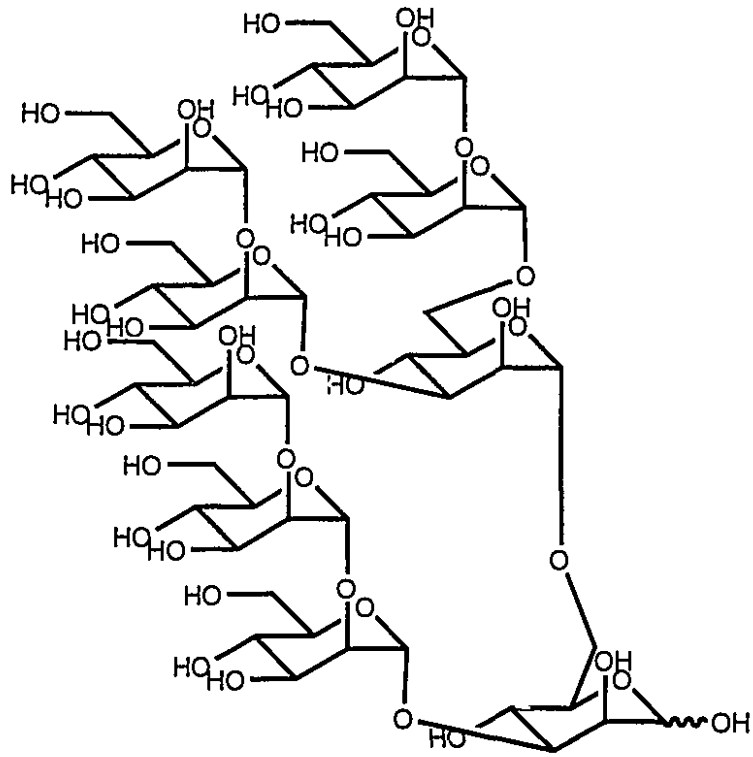
The preparation of a benzylated 1,2-mannan trimer and studies towards the development of a mannosylation reaction

I] Synthetic scope

The aim of the present study was to investigate potential methods in the stepwise synthesis of 1,2-mannans. This investigation is part of our ongoing effort towards the preparation of the mannan component of high-mannose oligosaccharides (1). This preparation has two aims, one being the preparation of large quantities of this mannan, to be used in biological investigations. The second purpose is to demonstrate the ability to use a polymer supported strategy in the synthesis of such oligosaccharides.

1) Solid-phase and polymer-supported strategies in oligosaccharide synthesis

In synthetic terms, biopolymers differ from their artificial counterparts, in the fact that they are made from a set of different monomers, the order of which, in the biopolymer, are extremely essential to the bioactivity. The change of a single unit can virtually annihilate the purpose of this biopolymer. Therefore, the only way to



1

build these molecules chemically, is to do it in a stepwise fashion, adding the units only one, or a few, at a time. The problems encountered in chemical synthesis are that each step comes with its workup, its purifications and its isolation problems, which account for a loss in yield. Especially, as the polymer grows, it appears that the product and its precursor are so close in physical properties, it is extremely hard to obtain pure molecules. Hence it becomes clear that the best strategies are in a " one-pot " fashion : all the units are added before a last larger purification, hence cutting out the losses in yield. However to do this, one has to devise a method to eliminate all reagents except the growing polymer, at the end of each unit addition.

To do so, two strategies have been developed:

- A solid, insoluble support is used on which the growing oligomer is bound. At the end of each reaction, the support is simply filtered out, and used for the next step.
- A soluble polymeric support is used, which can selectively be precipitated, or chromatographed in such a fashion, that all other components of the reaction mixture are removed readily from it.

In both cases, the product biopolymer is cleaved from the polymeric support before a last purification. Therefore a component of the polymer, here refered to as linker, must be present, which can stand all the reaction conditions, but which can be cleaved selectively at the end of the synthesis.

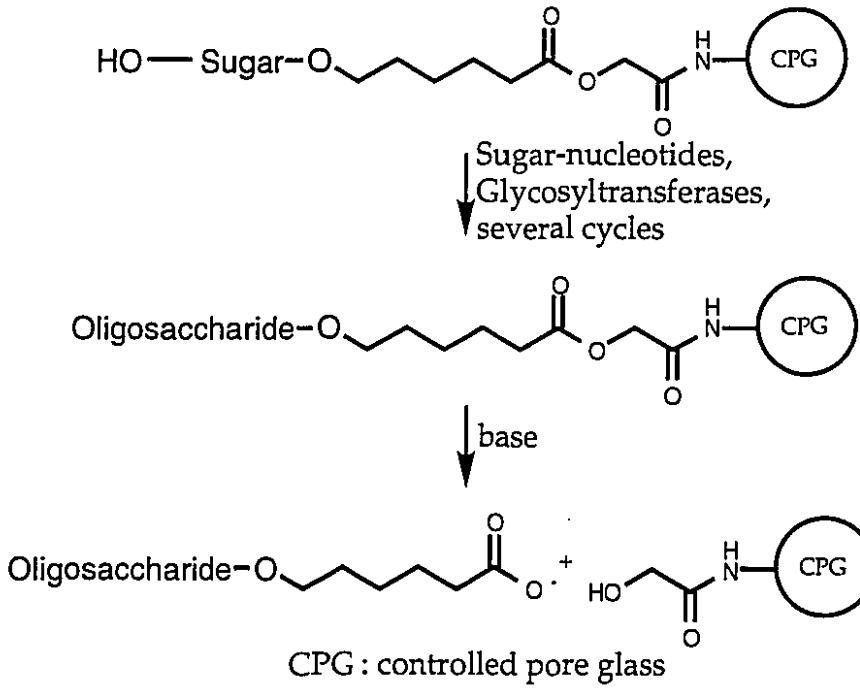
Such strategies have been employed with proteins[1] and nucleic acids[2], with great success. However, when it comes to carbohydrates, the problem is more complicated. Indeed, the proteins and the nucleic acids are linear polymers, in general, and therefore the linker must be stable to only one type of deprotection. Oligosaccharides are however generally branched. Therefore the linker has to be stable to several deprotection conditions. Furthermore, glycosylations are done generally in strongly acidic conditions, and therefore protecting groups have to be acid stable. In other words, they are to be removed in neutral or basic conditions. Hence the search for a suitable linker has been largely complicated.

In this respect, a few linkers have been used, but none of them have proven to be general in their use.

An ester functionality, in conjunction with enzyme-catalyzed glycosylations, has been successfully applied to synthesis of a few olisaccharides (figure 1)[3]. However this method is almost exclusively reserved for enzyme catalyzed glycosylation, as an ester group is too labile to be used in a chemical synthesis.

Another approach is to use a silyl ether as a linker. This was applied to the iterative synthesis of a number of oligosaccharides[4]. However, this sequence involved little protection and deprotection

reactions and therefore its use has been shown to be limited (figure 2)[5].



CPG : controlled pore glass

Figure 1

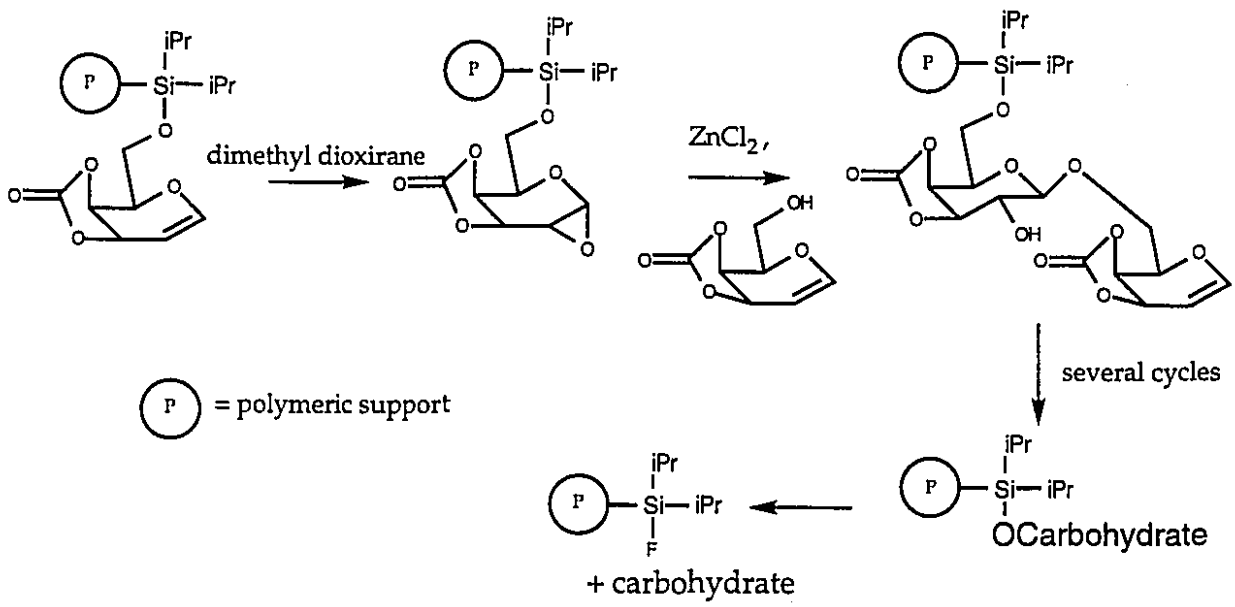


Figure 2

A few linkers were introduced as potentially useful. The hydroxyethyl sulfide group has been investigated. This sulfide can be oxidized to the sulfone by dimethyl dioxirane[6,7,8]. This transformation makes the hydrogens α to the sulfone acidic, and allows for the carbohydrate to be freed by base in an E_{1cb} fashion. This linker was first tested as the benzylthioethyl group, a base stable and base removable protecting group (figure 3). This showed the carbohydrate to be released in virtually quantitative yields[9]. However, this linker was found to be quite unsuccessful in a polymeric support allowing for the release in about 60% yield[10a]. Furthermore a certain number of glycosylation reactions, such as the Koenigs-Knorr method, the thioglycosides, and the 4-pentenyl glycosides, involve oxidants and/or thiophilic substances. The presence of the sulfur atom is therefore not very desirable.

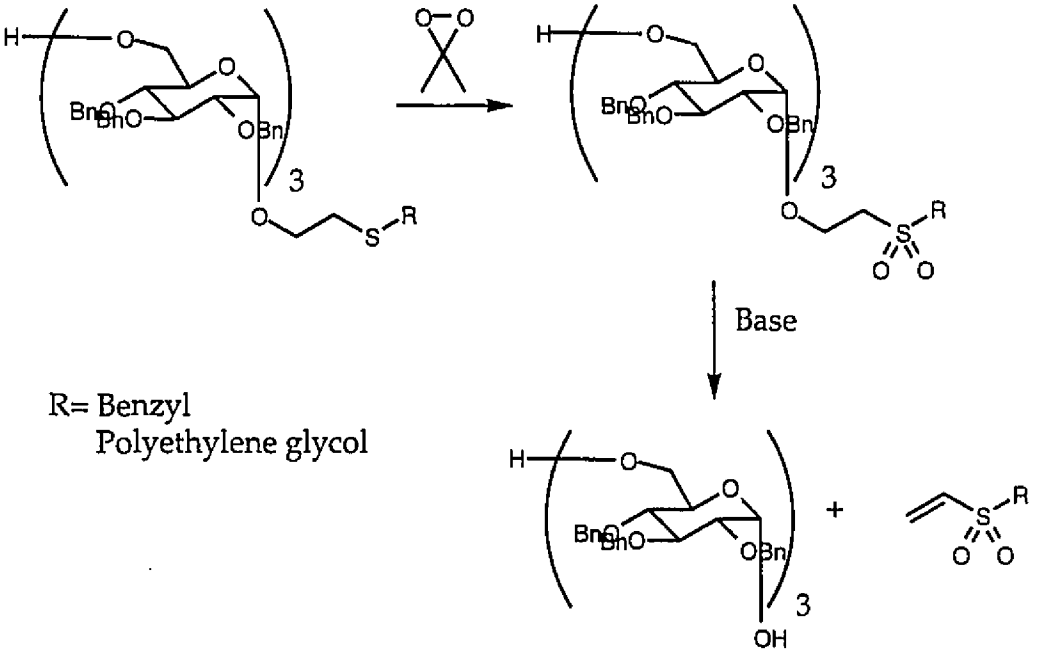


Figure 3

Another method involves polymethoxybenzyl alcohols as linkers. These, especially at the anomeric position, can be cleaved selectively, in the presence of other benzylic protecting groups, by treatment with dichloro-dicyano-quinone (DDQ). These soluble

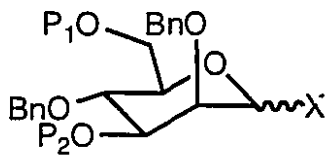
protecting groups are stable in basic conditions, as shown by their use in the synthesis of α (1,6) glucan[10b] in basic conditions (*Vide infra*), but if the acidic conditions of the typical glycosylations are used, they are too easily cleaved[10b].1,4-Benzenedimethanol was used as a linker, one side bound to polyethylene glycol through an ether linkage, and the other side used as glycosyl acceptor. It can be cleaved by an unexplained, but reproducible, selective hydrogenolysis on the polyethylene glycol side, followed by debenzylation[11].

Presently, the use of several modified benzyl alcohols as linkers is being studied.

2) Synthetic strategy

The strategy here is quite simple: two types of carbohydrate monomers are to be prepared. These are chosen to investigate the viability of any potential linker. The linker is chosen to be used as a glycosyl acceptor, since the glycosyl donor can be used in excess, allowing for higher yields.

The two monomers are chosen so as one gives the branch points (2) and one gives the α (1,2) mannans (3).



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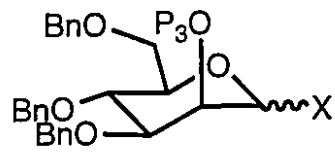

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There has to be three protecting groups, where P_3 can be deprotected in the presence of the others, and P_2 can be cleaved in the presence of P_1.

The aim of the present study is to investigate several possible glycosylations to synthesize the α (1,2) mannan[12] component, that is, to develop possible monomers for 3 .

II] Attempts towards a base mediated mannosylation

The glycosylation reactions presented in the first chapter are promoted by a Lewis acid or a set of Lewis acids. However, the first glycosidation, prepared by Michael, was the displacement of a glycosyl halide by potassium phenoxide, and was therefore in alkaline conditions.

With respect to solid-phase or polymer supported oligosaccharide synthesis, a base-mediated glycosylation is quite attractive. Indeed, the most common protective groups, the esters, are generally removed under basic, nucleophilic or under certain special conditions. This brings about the requirement for a linker which is extremely base stable. Since most glycosidations are carried out in strongly Lewis acidic conditions, the linkers also have to be acid stable. This makes the choice for a linker complicated. However, if the glycosidation is base mediated, then a base stable, but not necessarily acid stable, group can be used as a linker. Furthermore, some of the linkers developed have been shown to be quite successful in base mediated glycosylation[9,10,11].

All this made the choice for a base mediated glycosylation quite attractive. The idea is simple : a glycosyl halide is to be generated, and the halide is to be displaced by the glycosyl acceptor, in the presence of a hindered base.

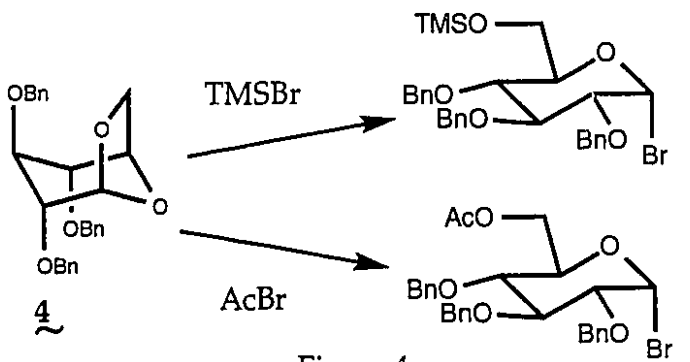


Figure 4

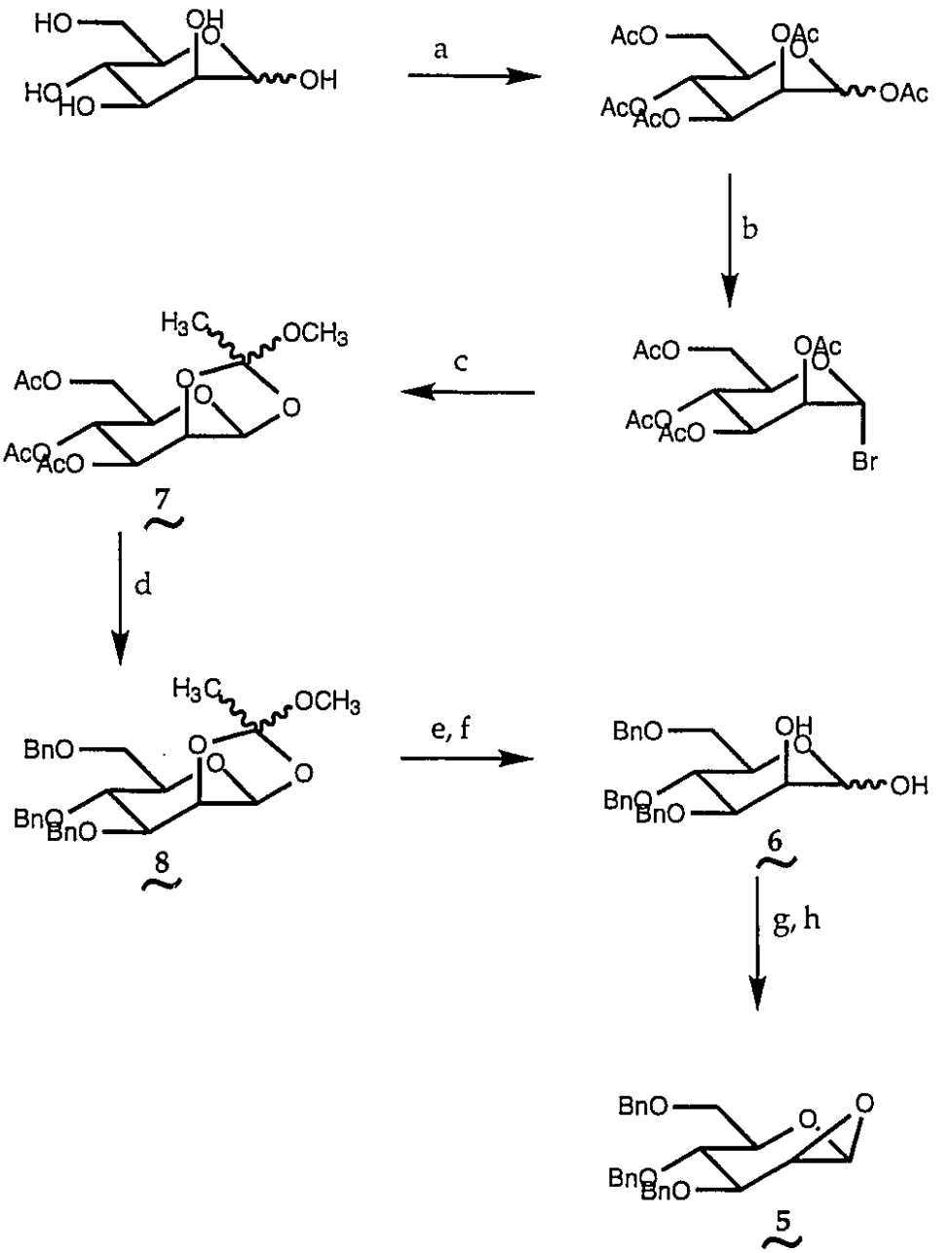
The generation of glycosyl bromides has been done for a long time by treatment of glycosides with a saturated solution of hydrogen bromide in acetic acid. The conditions for such preparations are however quite strong, and, for instance, a benzyl protecting group will be too unstable to stand them.

Bromotrimethylsilane has been known to react with 2-methoxy-tetrahydropyran to give the corresponding 2-tetrahydropyranosyl bromide[13]. But such a reaction has be shown to be ineffective in the generation of glucopyranosyl bromides from fully protected methyl-glucopyranosides[14]. A more strained monomer has to be used. The preparation from tri-O-benzyl-1,6-anhydroglucose (**4**) has been shown to be successful[15] (figure 4). The same anhydro glucose treated with acetyl bromide also gave a glucopyranosyl bromide[9] (figure 4).

These bromides can then be used to glycosylate alcohols in the presence of diisopropylethylamine (Hünig's base). This has been done with typical alcohols as well as with the 6-OH of glucose[15]. Furthermore, this method has been applied to the synthesis of α (1,6) linked glucose trimers[9]. This method has been also applied to tri-O-benzyl-1,6-anhydrogalactose[9], and is being applied to tri-O-benzyl-1,6-anhydromannose[16].

We reasoned that the general scheme for a base mediated glycosylation is the following : an anhydro sugar is to be opened into the bromide by bromo-trimethylsilane or acetyl bromide, and the generated bromide can be used as the glycosyl donor, in the presence of diisopropylethylamine. In our case, the desirable anhydrosugar is tri-O-benzyl-1,2-anhydromannose (**5**). This compound has been prepared from 3,4,6-tri-O-benzyl mannose (**6**)[17]. The adopted synthetic scheme is shown in figure 5[17,18].

In the preparation, mannose was peracetylated, then transformed to the corresponding bromide, in such a manner that neither of these compounds were isolated. Rather the reasonably pure tetra-O-acetyl-mannopyranosyl bromide was used directly to produce the 3,4,6-tri-O-acetyl - mannose 1,2- (methyl orthoacetate)



a : Acetic anhydride, pyridine, 0°C, two days, or Acetic anhydride, perchloric acid, 2 hrs. l HBr in acetic acid, 16 hrs. c : MeOH, $CHCl_3$, 2,6-Lutidine, 24 hrs. d : Benzyl chloride, THF, KOH, 100°C. e : 80% Acetic acid, O/N. f : NaOMe in MeOH, O/N. g : sat. HCl in ether at two days. h : NH_3 in benzene, 0°C.

Figure 5

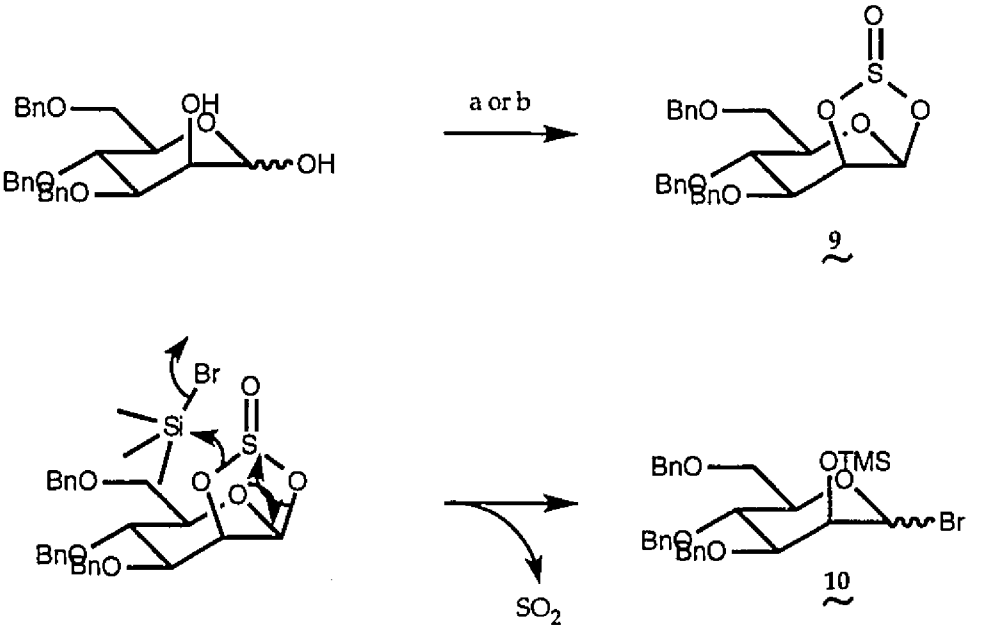
(7). This compound was benzylated with benzyl chloride and potassium hydroxide, but this benzylation gave the product as a mixture with benzyl chloride, benzyl alcohol and benzyl ether. The

suggested workup for this reaction was to distil the benzyl chloride and the benzyl ether, under vacuum at 100°C. In our hands, however, this workup gave a substantial amount of charring. The best method was by the azeotropic removal of these side-products with water. An alternative method was to use sodium methoxide in methanol to deacetylate, and following it by treatment with benzyl bromide and sodium hydride. In this case, the product can be purified by column chromatography in Florisil. Opening of the orthoester in acid followed by deacetylation with a catalytic amount of sodium methoxide in methanol gave the desired 3,4,6-tri-O-benzyl mannose (**6**). A catalytic amount of sodium methoxide had to be used as larger amounts induced the formation of an insoluble material, which is assumed to be the result of repeated Aldol condensations.

The closure of **6** to the epoxide **5** however posed a problem. This closure can be affected in a sequence of chlorinating the anomeric position, followed by an internal cyclization to the epoxide. The 3,4,6-tri-O-benzyl mannopyranosyl chloride seemed to form quite purely, as indicated by N.M.R., but the closure was not achieved in ammonia in an acceptable fashion. The result was indeed a mixture of products, in which the presence of the epoxide was not evident. Attempts to purify the mixture by flash chromatography were totally unsuccessful, except to obtain the starting material. A similar result was obtained when ammonia was replaced with 1,8 - diazabicyclo[5.4.0] undec-7-ene (DBU).

The problems encountered in the formation of the epoxide made us redirect the preparation. A certain number of publications have been made presenting the preparation of glucose-1,2 sulfites[19,20,21,22,23]. These compounds struck us to be nothing more than the epoxide, to which a molecule of sulfur dioxide is added. The idea was therefore the following : one can use the 3,4,6-tri-O-benzyl mannopyranosyl-1,2- sulfite (**9**) as an analog of the epoxide. Treatment with bromotrimethylsilane, or acetyl bromide should yield the desired glycosyl donor (figure 6). The sulfites can easily be obtained from the reaction of the 1 and 2 unprotected sugars either

with thionyl chloride in pyridine[19] or with a freshly prepared solution of thionyl diimidazole ($SO(im)_2$) in THF[23].



a : $SOCl_2$, pyridine. b: $SO(im)_2$, THF

Figure 6

In this case however, the formation of the sulfite was complicated by the fact that, contrary to the glucose and the galactose analogs, this one was in the β configuration. This configuration is not very favorable as the anomeric effect favors the 3,4,6-tri-O-benzyl-α-mannopyranose, so that a reasonable amount of the carbohydrate is lost in other forms (figure 7). These other forms are difficult to assess properly as they are quite unstable. However, from the NMR of the mixture, the starting compound seems to be absent. Therefore, this crude mixture was subjected to bromotrimethylsilane. This resulted in the formation of the two glycosyl bromide anomers (**10**), as shown by NMR. This can be attributed to the fact that the assumed non - cyclic sulfites (**11**),

resulting from the α anomer of the starting compound, can also convert to give the glycosyl bromides (figure 8).

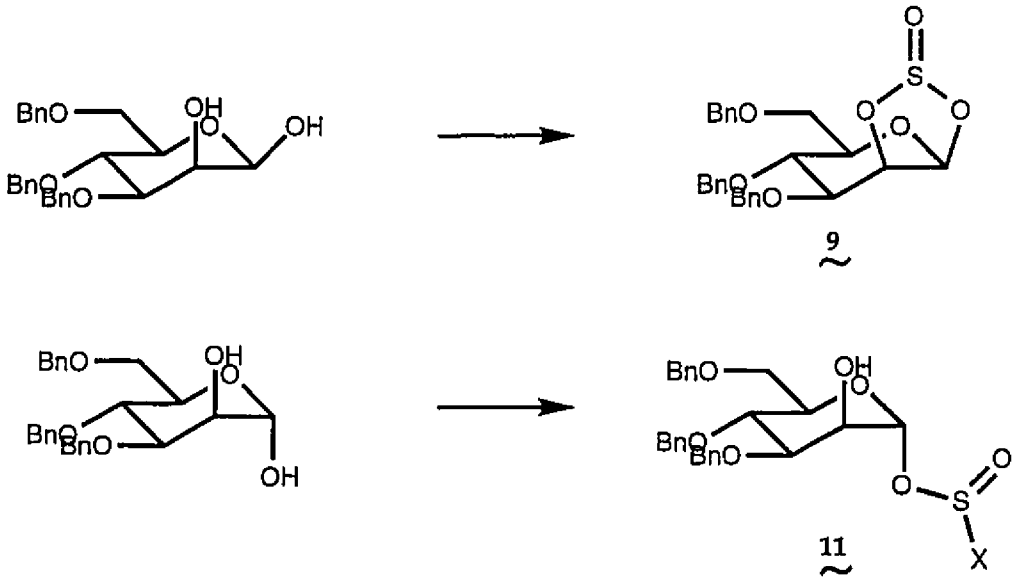


Figure 7

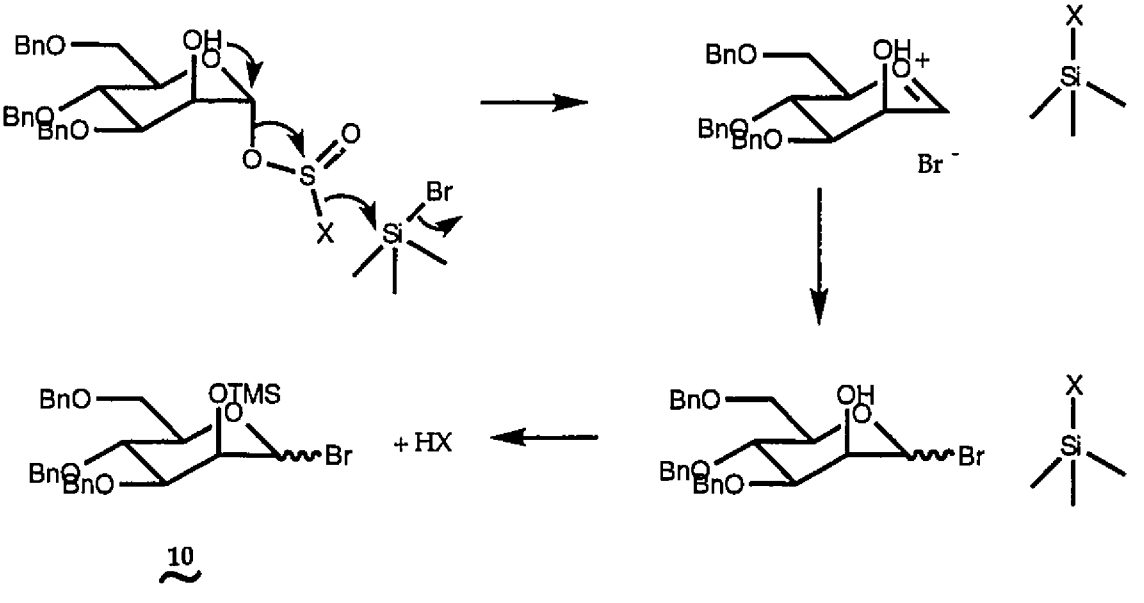
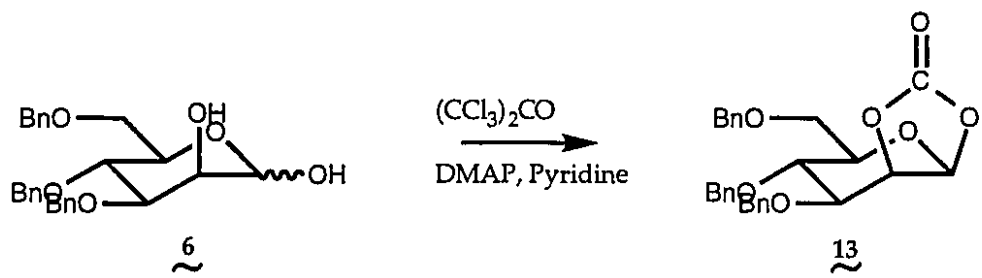


Figure 8

However the resulting bromides were almost not capable of glycosylation in basic conditions. Using isopropanol, a reasonably hindered alcohol, as a test substrate, one was able to obtain the desilylated product (**12**), in yields of about 20%.

To verify the validity of the above set of reactions, it was decided to produce the equivalent 3,4,6-tri-O-benzyl mannopyranosyl-1,2-carbonate (**13**), by the reaction of **6** with triphosgene and 6-dimethylamino-pyridine in pyridine[24]. This compound is similar to the sulfite in the sense that it can also give the glycosyl bromide anomers (**10**), by the release of carbon dioxide. As for the sulfite, a low yield was expected as only the β anomer of **6** reacts to give the desired product. However, this time, the product is stable enough to be isolated and stored. Therefore this compound was obtained pure, but in poor yields. Its reaction with bromotrimethylsilane gave the same spectrum as the reaction with the sulfites (**9** and **11**), validating the hypothesis that the pair of compounds **10** formed. Again, the reaction of this pair with isopropanol and Hünig's base gave a low yield (18%) of the mannoside (**12**).



6 13

Figure 9

The explanation for this low yield is not so obvious, but the fact is that silyl protecting groups are known to improve the nucleophilicity of hydroxy group, due to the low electronegativity of silicon. Therefore, it can be postulated that upon generation of the tight ion pair, the 2- oxygen can nucleophilically bind to the anomeric centre, giving an oxonium ion, which can be desilylated by the

alcohol. The result is the 1,2-anhydro compound (figure 9), which is destroyed either through oligomerization, or by hydrolysis upon workup.

The use of acetyl bromide can not be put forward as an alternative to that of bromotrimethylsilane, as the resulting mannopyranosyl bromides would, in basic conditions, give the orthoester, due to participation of the acetate at position 2.

Therefore this path for glycosylation was set aside.

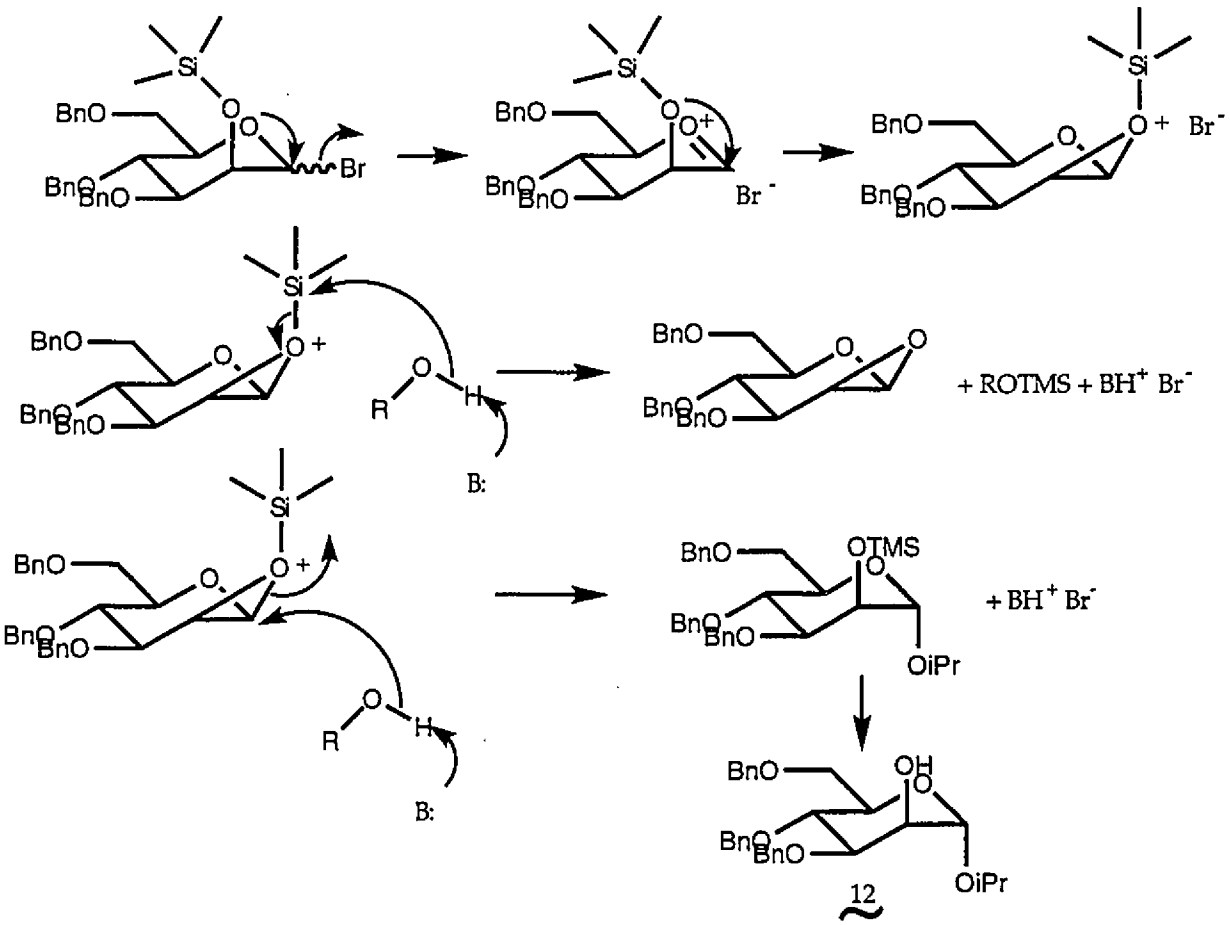
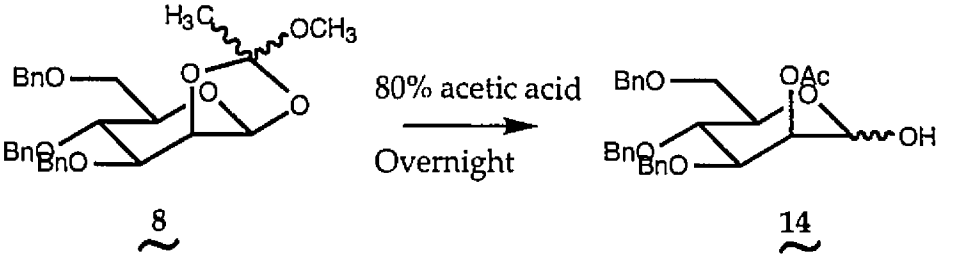


Figure 10

III] Synthesis of mannans using glycosyl imidates

1) Glycosylation of simple alcohols with a glycosyl donor having a free anomeric position

The scheme presented in figure 5 offered the opportunity for another glycosyl donor. Indeed, the opening of the benzylated orthoester (**8**) allows for the preparation of 2-O-acetyl-3,4,6-tri-O-benzyl mannose (**14**) (figure 11).



8 **14**

Figure 11

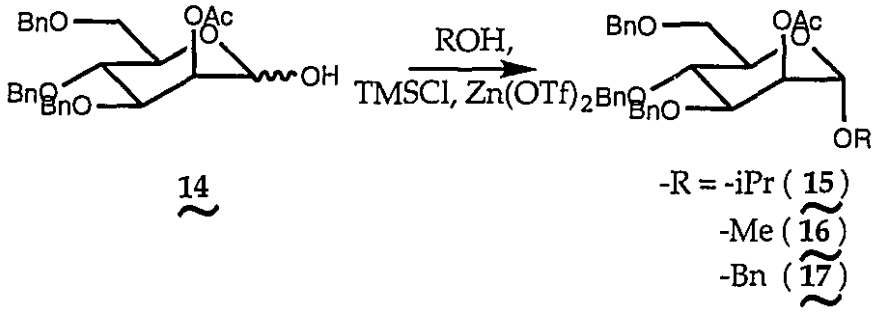
Glycosylations using glycosyl donors with a free anomeric position have been and are being developed[25,26]. These reactions have the advantage of using a stable and easily used carbohydrate, and of requiring no activation reaction.

Activation can be performed either by 1.1 equivalents of trimethylsilyl-trifluoromethanesulfonate (TMS-triflate), or by two equivalents of chlorotrimethylsilane and a catalytic amount of zinc triflate. The latter was used for convenience. The idea is based on the fact that 1-O-silyl glycosides and silyl protected alcohols are used, respectively, as glycosyl donors[27,28] and glycosyl acceptors[29]. The strategy is to generate them *in situ*, from the free anomeric glycosides and the free alcohols, and therefore the solution used has to be fairly dilute to allow time for the reagents to get silylated. This strategy was used on **14** with different alcohols (figure 11), and the reaction gave satisfactory yields with simple alcohols, giving the corresponding glycosides (table 1).

Table 1 : Glycosylations using 11 as the donor

Alcohol	Glycoside	yield
isopropanol	**15**	70%
methanol	**16**	61%
benzyl alcohol	**17**	72%

This reaction gave exclusively the α anomer as a participating group at position 2 was present, and, fortunately, the conditions were acidic enough to avoid the formation of the orthoesters.



14

-R = -iPr (15)
-Me (16)
-Bn (17)

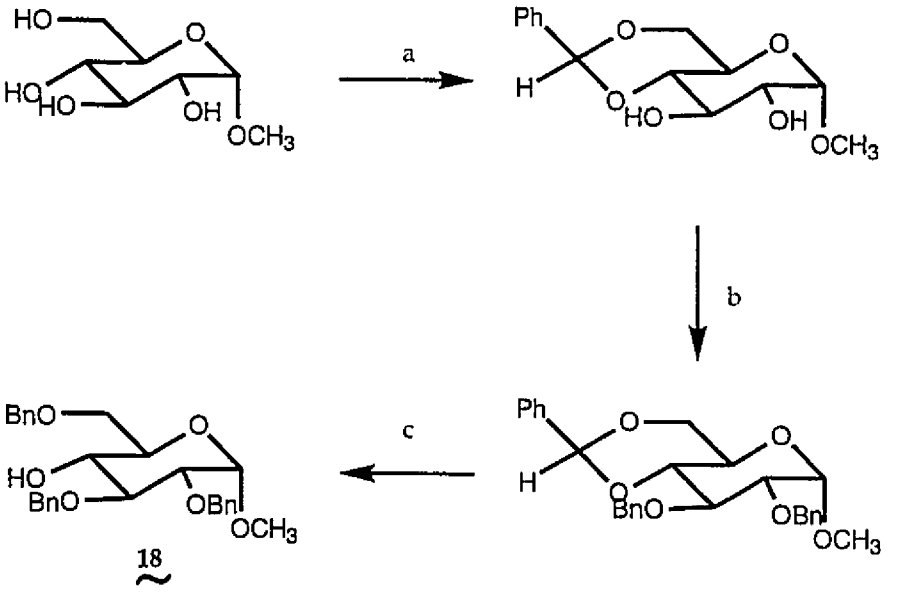
Figure 12

2) Attempts on model carbohydrate functional groups

The next step was to attempt the use of this reaction on a carbohydrate alcohol functionality, which has never been attempted in the literature. One would like this glycosylation method to be as general as possible, as it might be used in syntheses of several kinds of different oligosaccharides. For this purpose, the fourth position of glucose has been indicated to be the test substrate of choice[30].

The chosen glycosyl acceptor, methyl 2,3,6-tri-O-benzyl-glucopyranoside (**18**), is easily prepared by the reduction of the benzylidene protected glucoside[31], with sodium cyanoborohydride, and anhydrous hydrochloric acid in ether[32]. This compound was therefore prepared according to the scheme in figure 13. However, an attempt to glycosylate it using glycosyl donor **14** was unsuccessful, with the chlorotrimethylsilane/zinc triflate activation, even when using a threefold excess of the donor.

An alternate attempt was made on the slightly more reactive position-2 of mannose, in 1,3,4,6-tetra-O-benzyl-α-D-mannopyranose (**19**) (figure 15), obtained by the deacetylation of **17**.



18

a : $PhCH(OMe)_2$, pTsOH, DMF, 1hr. b : BnBr, NaH, THF, overnight.
c : $Na(CN)BH_3$, 1M HCl in ether

Figure 13

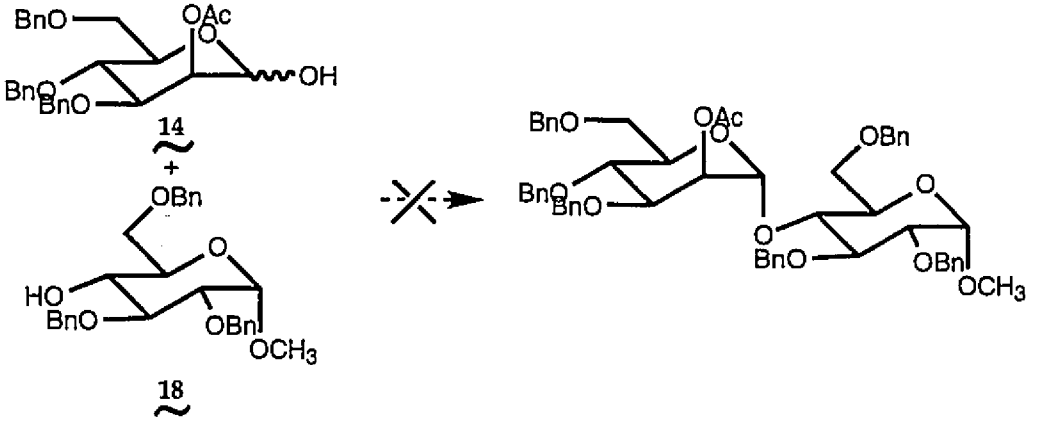
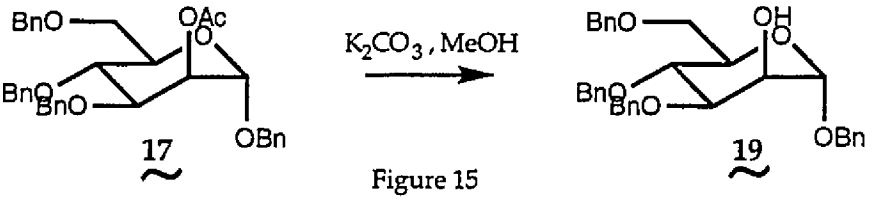


Figure 14

The glycosylation again was unsuccessful. In both cases, the reaction could not be allowed to extend in time, as the donor and the acceptors would degrade, probably by debenzylation under the strongly acidic conditions.

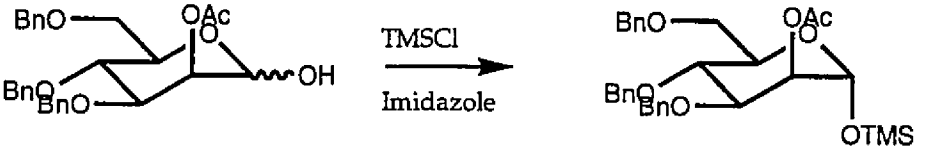
To remedy to this, a possible solution would be to repeat the reaction under less dilute conditions.



17 19

Figure 15

This was attempted on both acceptors **18** and **19** with the glycosyl acceptor **14**, and its 1-O-silylated counterpart (**20**) (figure 16). The idea behind this later, which was brought about by the inability to glycosylate other carbohydrates even with decreased dilution, is to eliminate the need for *in situ* silylation. However this glycosyl donor was also unable to give the desired disaccharide, with the chlorotrimethylsilane/zinc triflate mixture, TMS-triflate or boron trifluoride etherate (the normal activator for 1-O-silyl glycosides)[28].



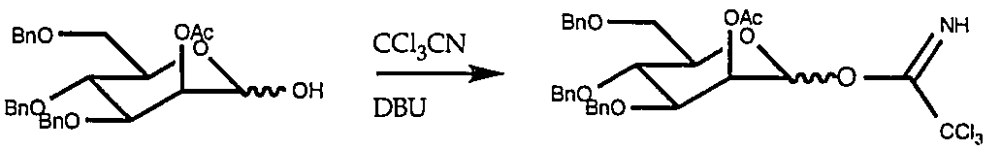
14 20

Figure 16

3) The use of glycosyl imidates

A more reactive glycosyl donor had to be sought. The easiest one to obtain was the trichloroacetimidate, which can be prepared by treatment of the free anomeric sugars with trichloroacetonitrile, in the presence of DBU[33].

The resulting trichloroacetimidate (**21**) (figure 17) has been obtained and since this category of glycosyl donors is well studied in the literature, and, as said earlier (chapter one, section III.2.e), has been shown to glycosylate even the most unreactive acceptors, it wasn't necessary to probe its reactivity any further.



14 21

Figure 17

Hence this donor 21 was used to successfully synthesize the disaccharide 22 (figure 18) in 72% yield with acceptor 19, with boron trifluoride etherate as the activating Lewis acid. The resulting disaccharide was deacetylated quantitatively with potassium carbonate, in methanol, to give the disaccharide 23, which was glycosylated again to give the trisaccharide (24) in 63% yield.

This tends to show that the ideal glycosylation monomer in the quest for the synthesis of mannans is likely to be the imidate 21.

IV] Attempts towards the development of an acid catalyzed mannosylation reaction.

1) The glycosylation of simple alcohols

As said earlier (chapter 1, section III.2.d), the building of oligosaccharides using the glycal strategy, has been quite successful in the last few years. This strategy involves the direct epoxidation of glycals, in the presence of dimethyl dioxirane, to give the corresponding 1,2-anhydrosugar. This epoxide can then be opened, in mildly Lewis acidic conditions, with an alcohol, to give the glycoside. This methodology, however, can be applied only to β-mannosides, and with the requirements for transformations, following the glycosylation. Indeed, glucal is the glycal derived from both glucose and mannose. The epoxidation with dimethyl dioxirane is almost exclusively selective to the 1,2-anhydroglucoses, with which the glycosylation yields β-glucosides. However, it is possible to transform

the β-glucoside to a β-mannoside by oxidation and selective reduction at the C-2 position[34].

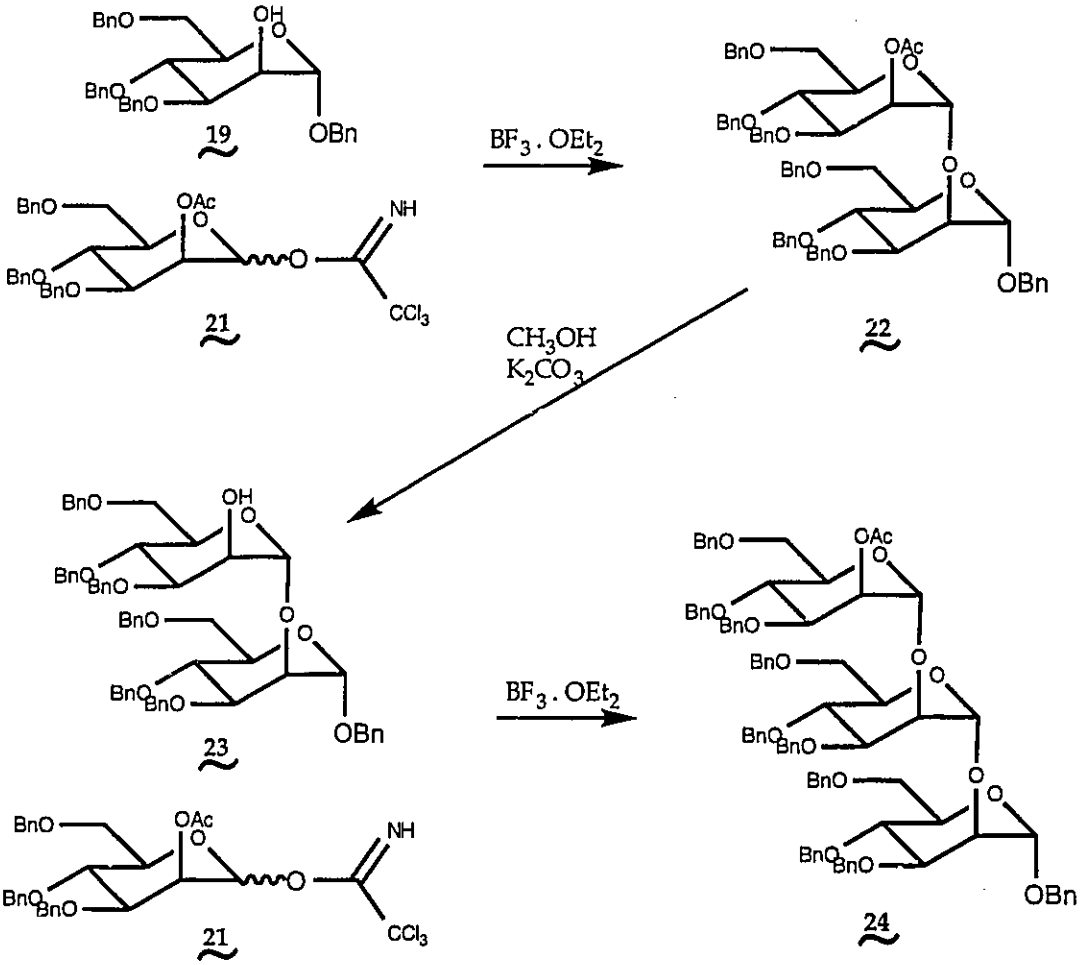


Figure 18

As we were investigating the use of the mannose derived 1,2-sulfite **9** and 1,2-carbonate **13**, it was thought that it would be interesting to assess the ability of these to generate the corresponding α-mannosides, in a similar fashion to that of the glycal strategy. We, therefore, attempted to use the mixture, obtained by reacting 3,4,6-tri-O-benzyl mannose and thionyl diimidazole, in the presence of several Lewis acids, and isopropanol, as a test glycosyl acceptor. Most of these Lewis acids (zinc chloride, zinc triflate, boron trifluoride etherate) gave quite low yields of isopropyl 3,4,6-tri-O-

benzyl mannopyranose (10 to 20%). These yields are quite reproducible. Indeed, the preparation of the cyclic carbonate **13** gave only 26% yield. This serves as a good indication that the mixture, obtained by reacting 3,4,6-tri-O-benzyl mannose and thionyl diimidazole contains roughly 20% of the desired sulfite **9**. This sulfite reacts in the glycosylation to give an apparently low yield as the other components presumably did not react.

It was clear that a perhaps stronger activation, such as the one for 1-OH sugars, could allow for the use of this mixture, in glycosylation. The hope is that the components of the mixture, which are not the sulfite **9**, could also react. The mixture was therefore reacted with chlorotrimethylsilane in the presence of zinc triflate (figure 19). The results are shown in table 2.

This work was repeated with the carbonate **13** without success.

The reason, for this glycosylation to work, is easy to understand with respect to the cyclic sulfite. However, with the other components of the mixture, one has to postulate other mechanisms. There are probably two major components other than the sulfite in the mixture: a sulfite linking two mannoses by their anomeric position (**28**), or, predominantly by far, a free sulfite imidazole salt (**29**). The former can react with the silyl chloride, in the presence of the Lewis acid, to generate a glycosyl cation and a 1-O-silyl mannose (**30**), which serves as a glycosyl donor itself[27,28] (figure 20). The latter probably reacts with the silyl chloride to give a sulfite (**31**), which can react with another equivalent of the silyl chloride, to give the mannopyranosyl cation - the glycosyl donor- and hexamethyldisiloxane (figure 21).

Table 2 : Glycosylation with reaction mixture of sulfite

Alcohol	Mannoside	yield (%)	α/β ratio
isopropanol	**25**	71	93 / 7
methanol	**26**	42	86 / 14
benzyl alcohol	**27**	64	82 / 18

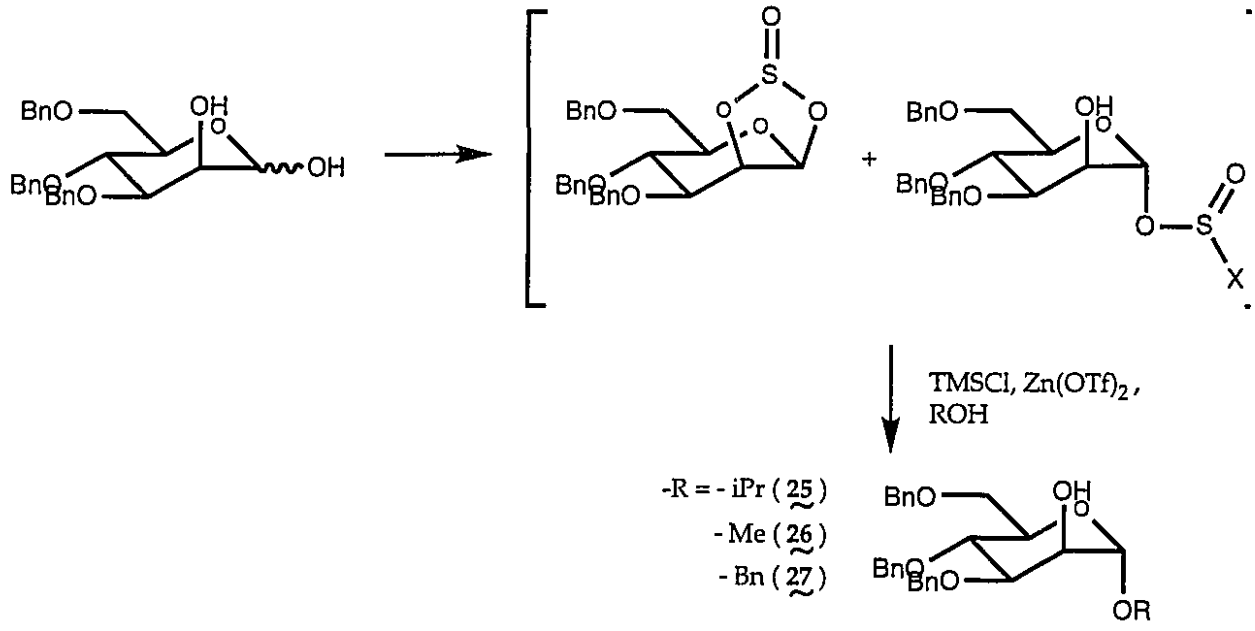


Figure 19

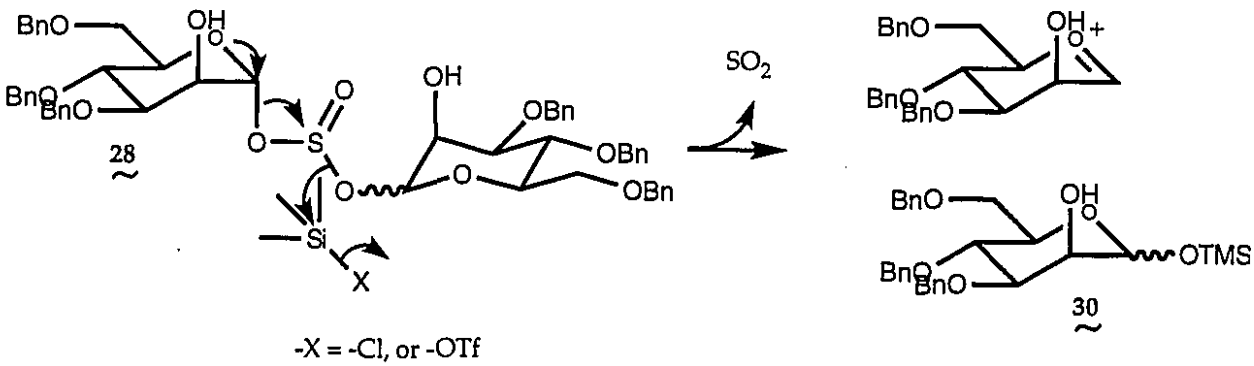


Figure 20

2) Attempts to glycosylate carbohydrates.

The above-mentioned glycosylation procedure had to be assessed for its reactivity. Therefore it was used on three substrate acceptors, with the same donor mixture.

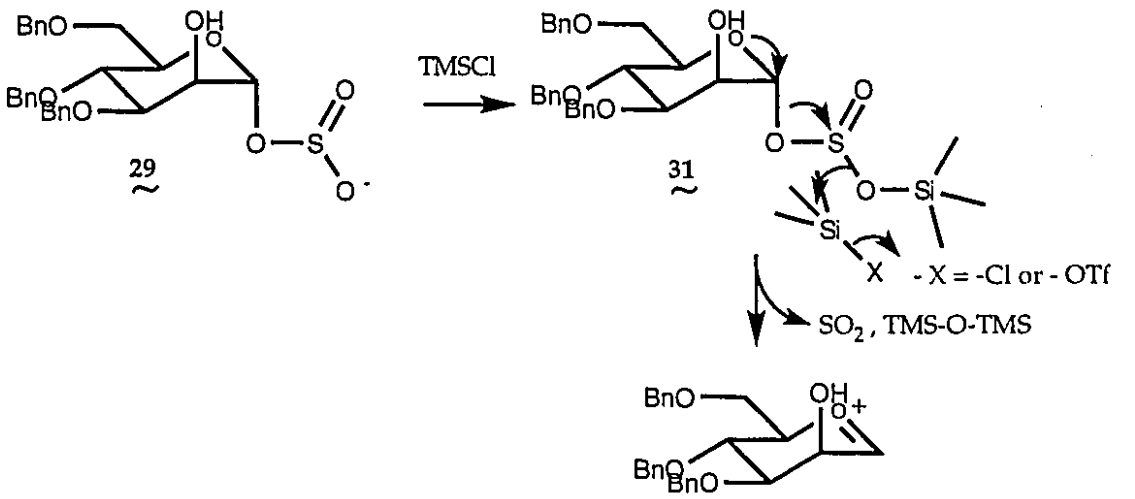


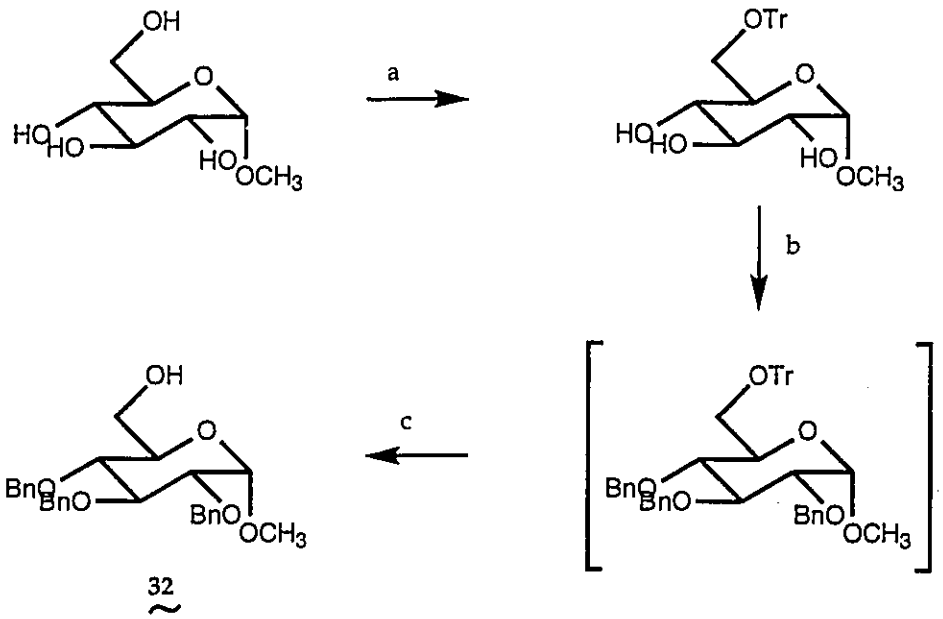
Figure 21

The initially attempted glycosylation was of methyl 2,3,6-tri-O-benzyl-α-D-glucopyranoside (**18**), which had been previously prepared. This substrate was however not glycosylated. Therefore the sterically less hindered 1,3,4,6-tetra-O-benzyl-α-D-manno-pyranoside (**19**) was tried. Again this method was not successful.

A last attempt was made on methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside (**32**). Indeed, the sixth position of hexopyranoses is a more active position than the other. It can, for instance, be protected selectively, in the presence of the other alcohol functional groups. This compound can easily be prepared by the selective tritylation of the 6 position of methyl α-D-glucopyranoside by trityl chloride in pyridine. This reaction is followed by benzylation with benzyl bromide and sodium hydride, which gave an inextricable mixture, but addition of concentrated hydrochloric acid, to a solution of the mixture in acetic acid led to the desired compound (**32**) (figure 22).

The glycosylation was attempted on the acceptor **32**, but again the result was negative. Rather, one seemed in all of the above three cases to obtain, out of a complex mixture, one major component, generally in about 10% yield. To this compound can be assigned the structure **33**. It is probably due to an opening of the 1,2-sulfite, in the presence of chlorotrimethylsilane to give the corresponding

chloride **34** (figure 23). The trimethyl-silyl protected oxygen at C-2 of mannose then is more nucleophilic than the alcohol acceptor, and the result is that it is glycosylated. This is not seen with the simple alcohols as these are nucleophilic enough to react with the glycosyl cation before the chloride ion does so.



32

a : TrCl, Pyridine; b : BnBr, NaH, DMF; c : Acetic Acid, conc. HCl
Figure 22

3) Attempted improvements to the method

The difficulty with the previous mannosylation rested on the fact that the preparation of the 1,2-sulfite posed a problem. The only way to circumvent this is to improve the preparation of this sulfite. As said earlier on, certain results were obtained, with simple Lewis acids (zinc chloride, zinc triflate, boron trifluoride etherate). This and the formation of **33** tend to indicate that the sulfite itself is a potent glycosyl donor, but it is formed in too low a yield.

The requirements for improving the yield are of two categories: either one can provide for a reversible formation of the sulfite, which would lead to the cyclic sulfite, while the acyclic ones are in equilibrium with the starting material, or one can deliver the

anomeric oxygen selectively in a β-fashion. We chose this second possibility, as no reversible sulfite formation is known. The best strategy would be to develop a method in which positions 1 and 2 are tied up, and released only upon reaction.

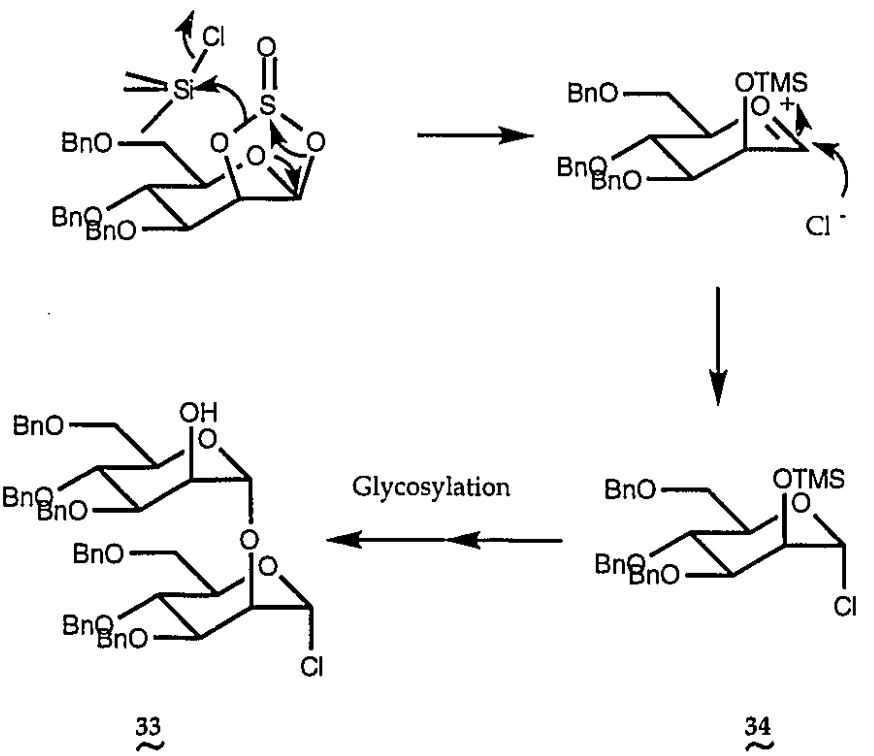


Figure 23

The tying up of these two positions can be achieved using dibutyltin oxide, via the stannylene **35**. This type of compound is well known in the literature[35], although never used in this context. It is formed by the carbohydrate in toluene with 1.5 equivalents of dibutyltin oxide (figure 24). The formation of an oxygen-tin bond makes the oxygen quite nucleophilic, and can be used in alkylation or acylation reactions.

The generated stannylene **35** was not purified, but was directly used in the reaction. The addition of thionyl chloride, in excess or in stochiometric amounts, however never yielded the sulfite. Lowering the temperature to - 78 °C, diluting or using very nonpolar solvents,

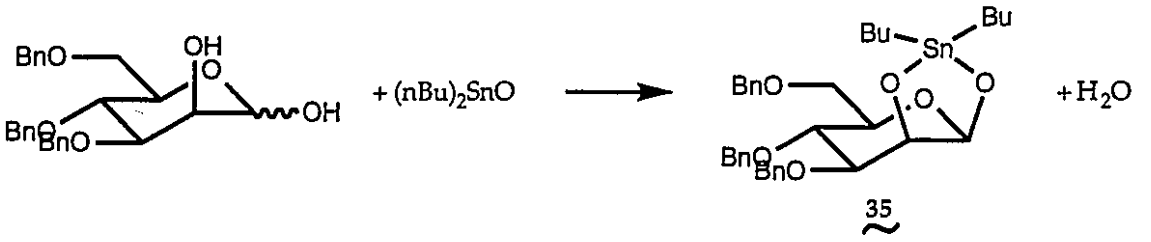


Figure 24

followed by filtration through florisil to eliminate the tin salts, didn't improve the situation. It was therefore postulated that the problem was the following : the addition of thionyl chloride resulted in the formation of dibutyltin chloride, which is a powerful Lewis acid. The problem was that a glycosyl donor was generated and a strong glycosyl acceptor was present in the form of the stannylene sugar. Therefore as soon as the sulfite is generated, it glycosylates the stannylene. Indeed, a structure corresponding to the dimer (**36**) has been obtained from the reaction mixture (figure 25), but in poor yield. Another way of confirming this hypothesis is to put a glycosyl acceptor in the mixture, shortly after adding thionyl chloride. This was done with isopropanol, and the corresponding glycoside **25** (almost exclusively the α anomer) was obtained although in poor yields, together with **36**. As this in itself was a potential glycosylation, an attempt was made to see if the yield of the glycoside could be improved. The reaction temperature was lowered, without any effect other than to change the α/β ratio to 5/1, which is easily understood as the solvent, THF, at this temperature can participate (as shown in chapter one section III.3) to give a change in the anomer content.

The other possibility for the low yields of both **25** and **36** was the fact that the stannylene might react not in an concerted, but in a stepwise fashion, with initial cleavage of the tin-oxygen bond, which would yield a mixture of different sulfites. To verify this hypothesis, it was decided to react the dibutyltin oxide treated carbohydrate with triphosgene, as the product carbonate (**11**) is stable enough to

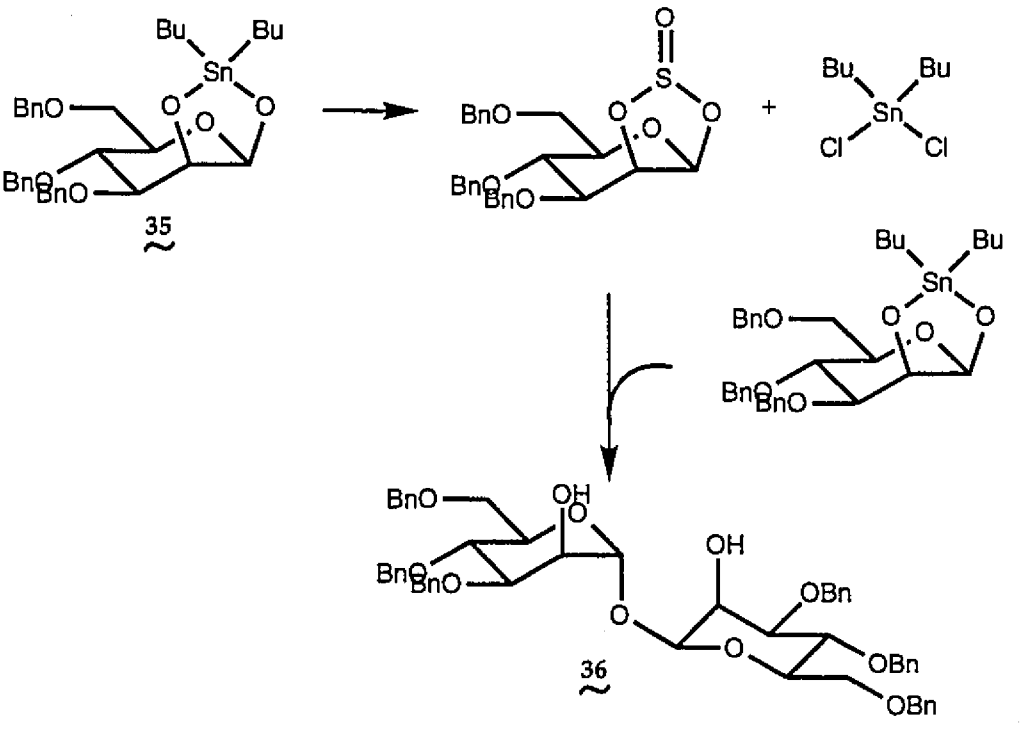


Figure 25

be isolated. However, even in this case, and even upon cooling to -78°C, the carbonate **13** did not form. Rather one again obtained the same yields of the dimer **36**. Such a result was also obtained with 1,1'-carbonyl diimidazole, in which the imidazole base, replacing the halide, was added to counteract the formation of the Lewis acidic tin species. It appears therefore that this methodology is by far too reactive to provide for the building of the sulfite, since even the carbonate cannot stand the conditions.

V] Experimental procedure

General : Chemicals were purchased from Aldrich chemical company and were reagent grade. Anhydrous THF and anhydrous ether were obtained by refluxing with sodium until the addition of benzophenone gave a black color, and distillation; anhydrous dichloromethane, anhydrous DMF and anhydrous acetonitrile by distillation from calcium hydride; anhydrous methanol by refluxing with magnesium and distillation. Thionyl chloride was distilled from 10% (w / w) triphenyl phosphite.

Silica gel chromatography was performed on silica gel 60 (230-400 mesh) and analytical thin layer chromatography was performed on silica gel 60 F plastic back plates and visualised by dipping in a solution of ammonium molybdate (2.5 g) and ceric sulphate (1 g), in concentrated sulphuric acid (10 ml in 90 ml water), and heating with a heat gun. Florisil chromatography was performed using florisil F100 (60-100 mesh).

N.M.R. spectra were recorded on a JEOL CPF-270, a Varian Gemini and a Varian Unity 500 spectrometers, at 200, 270 and 500 MHz for the ^{1}H N.M.R. and 60 and 125 MHz for the ^{13}C N.M.R. using indicated solvent residues as references. Peaks are reported as singlets (s), doublets (d), triplets (t), quartets (q), double doublets (dd), double triplets (dt), and multiplets (m). IR spectra were recorded on an Analect AQS-18 FTIR spectrometer. Optical rotations were measured on a JASCO DIP-140 digital polarimeter. Mass spectra were recorded on a Kratos MS25RFA mass spectrometer. Melting points were measured on a Gallenkamp melting point apparatus and are uncorrected.

1) Preparation of 3,4,6-tri-O-benzyl-mannose

Mannose pentaacetate

To a mixture of 68.5 ml (0.85 moles) of dry pyridine and 46.5 ml (0.5 moles) of acetic anhydride, cooled in an ice-acetone bath, was added 10 g (55.5 mmoles) of mannose. The mixture was stirred until

the mannose dissolved. Then the solution was kept for two days at 0°C. The reaction was then quenched by adding it to 250 ml of ice-water. The resulting solution was extracted with toluene, and the organic layer was washed several times with a saturated sodium hydrogen carbonate solution, and then with water, dried with sodium sulfate and evaporated to a syrup. Most of the remaining pyridine was then azeotropically removed with toluene, and the resulting gum was left overnight under vacuum. This dried gum was used directly in the next step.

1-Bromo- 2,3,4,6-tetra-O-acetyl-α-D-mannopyranose

The above gum was dissolved in 20 ml of dichloromethane, and 20 ml of a solution of HBr (0.1 moles) saturated at 0°C in glacial acetic acid was added. The solution was stirred for twenty-four hours, and then diluted with 20 ml of dichloromethane. The resulting solution was slowly poured in 200 ml of ice-water, and was transfered to a separatory funnel where it was shaken and separated. The organic layer was washed several times with a saturated solution of sodium hydrogen carbonate in water, then with water, dried with sodium sulfate and evaporated to a syrup. The residue was taken up in 100 ml of ether. This solution was decolorized by filtering through successive pads of sodium sulfate and decolorizing charcoal. The two pads were washed with another 50 ml of ether, and the combined filtrates were dried in vacuo, to give a gum used directly in the next step.

3,4,6-Tri-O-acetyl-α-D-mannose 1,2-(methyl orthoacetate) (7)

The syrupy 1-bromo-2,3,4,6-tetra-O-acetyl-α-D-manno-pyranose obtained from 10 g (55.5 mmoles) of mannose was dissolved in 40 ml of chloroform. To this solution was added a mixture of 88 ml of methanol and 11.5 ml (98.7 mmoles) of 2,6-lutidine. The solution was left for twenty-four hours at room temperature, diluted with 100 ml of chloroform and washed with a dilute solution of sodium hydrogen carbonate (typically 3%). The

aqueous layer was extracted with 50 ml more chloroform and the combined organic layers were washed with water, dried with sodium sulfate and decolorized with charcoal. The resulting solution was evaporated in vacuo, and the resulting syrup was crystallized from methanol-water, to give 17.87 g (88.9%) of product contaminated with some lutidine (desirable if the orthoester was to be kept long times).

m.p.: 103-106 °C. $[\alpha]_D^{25} = -21.25^{o} \pm 0.4$ (c=1.36, chloroform). ^{1}H N.M.R. (270 MHz, $CDCl_3$, ppm): 5.47 (d, 1H, *J* = 2.6 Hz, *H-1*), 5.28 (apparent t, 1H, *J* = 9.9 Hz, *H-4*), 5.12 (dd, 1H, *J* = 4.0 Hz, 9.9 Hz, *H-3*), 4.59 (apparent t, 1H, *J* = 4.0 Hz, *H-2*), 4.22 (dd, 1H, *J* = 5.3 Hz, 12.5 Hz, *H-6*), 4.12 (dd, 1H, *J* = 2.6 Hz, 12.5 Hz, *H-6'*), 3.26 (octet, 1H, *J* = 2.6 Hz, 5.3 Hz, 9.9 Hz, *H-5*), 3.26 (s, 3H, *methoxy group*), 2.1, 2.05, 2.03, 2.00 (s, 3H each, *acetates*), 1.72 (s, 2.5H, *orthoacetate exo*), 1.5 (s, 0.4H, *orthoacetate endo*). exo diastereomer is 87% and endo is 13% according to litterature assignment[36]. ^{13}C N.M.R. (125 MHz, $CDCl_3$, ppm) : 169.23, 168.29 (*Acetate carbonyls*), 123.91 (*Orthoester carbon*), 97.12 (*C-1*), 76.56 (*C-2*), 71.39 (*C-5*), 70.68 (*C-3*), 65.62 (*C-4*), 62.48 (*C-6*), 50.23 (*Orthoester methoxy*), 24.97 (*Orhtoester methyl*), 21.47, 21.41, 21.34 (*acetate methyls*).

3,4,6-Tri-O-benzyl-α-D-mannose 1,2-(methyl orthoacetate) (**8**)

3,4,6-tri-O-acetyl-mannose 1,2-(methyl orthoacetate) (5 g,13.8 mmoles) was added to a mixture of 10 ml of anhydrous THF and 28 ml (0.24 moles) of benzyl chloride, in a three-necked round bottom flask, and slowly brought to about 90°C under anhydous conditions. Then heating was discontinued and 10 g (0.18 moles) of KOH were added (good stirring was necessary as the mixture becomes quite viscous), and heating was continued for another 15 hours. Then the reaction flask was cooled to room temperature and water (about 50 ml) was added to dissolved the remaining KOH. The flask was washed with dichloromethane and the dichloromethane was added to the previous aqueous solution in a separatory funnel, and shaken. The organic layer was removed, washed with brine several times and then evaporated to a syrup. Water was added to the syrup and

evaporated in vacuo, and this was repeated several times (to remove the remaining benzyl chloride and benzyl ether) until a solid residue was obtained. This residue was crystallized with ether-hexanes giving 5.3 g (75.7%) of product.

m.p.: 76-80 °C. $[\alpha]_D^{25} = 11.2^{o} \pm 0.8$ (c=1.24, chloroform) ^{1}H N.M.R. (500 MHz, $CDCl_3$, ppm): 7.3 (m, 15H, *benzylic aromatic proton*), 5.33 (d, 1H, *J* = 2.4 Hz, *H-1*), 4.88 (d, 1H, *J* = 10.7 Hz, *benzylic proton*), 4.77 (m, 2H, *benzylic protons*), 4.6 (d, 2H, *J* = 11.2 Hz, *benzylic protons*), 4.53 (d, 1H, *J* = 9.8 Hz, *benzylic proton*), 4.38 (d, 1H, *J* = 4.4 Hz, *H-2*), 3.91 (d, 1H, *J* = 9.5 Hz, *H-6*), 3.71 (m, 3H, *H-3, H-4 and H-5*), 3.41 (m, 1H, *H-6'*), 3.27 (s, 3H, *methoxy group*), 1.72 (s, 2.5H, *Orthoester methyl exo*), 1.48 (s, 0.3 H, *Orthoester methyl endo*). ^{13}C N.M.R. (125 MHz, $CDCl_3$, ppm) : 137.48, 137.09, 127.88, 127.77, 127.67, 127.41, 127.14, 126.89,123.42 (*Benzyl aromatic carbons*), 97.29 (*C-1*), 78.98 (*C-5*), 77.20 (*C-2*), 75.24 (*benzylic carbon*), 74.22 (*C-6 and orthoester carbon*), 73.40 (*Benzylic carbon*), 72.41 (*Benzylic carbon*), 69.08 (*C-3 and C-4*), 50.07 (*methoxy group*), 25.01 (*Orthoester methyl group*).

3,4,6-Tri-O-benzyl-D-mannose (**6**)

To 1.07 g (2.1 mmoles) of 3,4,6-tri-O-benzyl-mannose 1,2-(methyl orthoacetate) was added 12 ml of acetic acid and 8 ml of water. The mixture was stirred for four hours on a steam bath. The solid will slowly dissolve. The solvents were then evaporated in vacuo, and the residual syrup was taken up in dichloromethane, washed with saturated sodium hydrogen carbonate until neutral, and evaporated to a syrup which was deacetylated overnight with catalytic amounts of sodium methoxide in methanol. The methoxide was then neutralized with acetic acid, and the solution was evaporated. Ether was added, and the solution was dried, decolorized with charcoal, evaporated and the syrup was crystallized with ether-hexanes to give 0.61 g (63.6%) of 3,4,6-Tri-O-benzyl-Mannose.

m.p.: 88-91 °C. $[\alpha]_D^{25} = 20.26^{o} \pm 0.6$ (c=1.22, chloroform). ^{1}H N.M.R. (500 MHz, $CDCl_3$, ppm): 7.34 (m, 12H, *benzyl aromatic protons*), 7.10 (m, 3H, *benzyl aromatic protons*), 5.28 (broad s, 1H, *H-*

1), 4.81 (m, 2H, *Benzylic protons*), 4.68 (m, 3H, *benzylic protons and H-1**), 4.58 (m, 2H, *benzylic protons*), 4.50 (m, 3H, *benzylic protons*), 4.02 (m, 2H, *H-2 and H-5*), 3.96 (m, 1H, *H-2**), 3.92 (dd, 1H, 3.4, 9.0 Hz, *H-3*), 3.80 (t, 1H, 9.5 Hz, *H-4**), 3.74 (m, 1H, *H-4*), 3.71 (d, 2H, 3.2 Hz, *H-6 and H-6'*), 3.66 (m, 2H, *H-6* and H-6*'*), 3.57 (m, 1H, *H-3**), 3.39 (m, 1H, *H-5**). ^{13}C N.M.R. (125 MHz, $CDCl_3$, ppm) : 138.15, 138.08, 138.03, 138.00, 137.86, 128.58, 128.55, 128.42, 128.35, 128.26, 128.23, 128.17, 128.12, 128.09, 128.06, 128.03, 127.76, 127.93, 127.87, 127.76, 127.73, 127.70, 127.65 (*benzyl aromatic carbons*), 94.06 (*C-1'**), 93.91 (*C-1*), 81.60 (*C-3'**), 79.65 (*C-3*), 75.08 (*benzylic carbons*), 74.65 (*C-5'**), 74.41 (*C-4*), 73.77 (*C-4**), 73.57 (*benzylic carbons*), 73.44 (*benzylic carbons*), 72.03 (*benzylic carbons*), 71.84 (*benzylic carbons*), 70.98 (*C-5*), 69.24 (*C-6*), 68.80 (*C-6**), 68.69 (*C-2**), 68.42 (*C-2*).

2) Glycosylation using donor with free anomeric position

2-O-Acetyl-3,4,6-tri-O-benzyl-D-mannopyranose (**14**)

To 1.07 g (2.1 mmoles) of 3,4,6-tri-O-benzyl-mannose 1,2-(methyl orthoacetate) was added 16 ml of acetic acid and 4 ml of water. The mixture was stirred overnight, at room temperature. The solid slowly will dissolve. The solvents were then evaporated in vacuo, and the residual syrup was taken up in dichloromethane, washed with saturated sodium hydrogen carbonate until neutral, and evaporated to 1 g (2.093 mmoles) of a yellowish syrup (96%).

$[\alpha]_D^{25}=40.3^o \pm 0.3$ (c=1.15, chloroform). I.R. ($CHCl_3$, cm^{-1}): 980, 1120, 1240, 1372, 1455, 1737, 2870, 2926. ^1H N.M.R. (500 MHz, $CDCl_3$, ppm): 7.31 (m, 15H, *benzyl aromatic protons*), 5.34 (broad s, 1H, *H-2*), 5.17 (broad s, 1H, *H-1*), 4.83 (d, 1H, *J* = 9.52 Hz, *benzylic proton*), 4.67 to 4.42 (broad m, 5H, *benzylic protons*), 4.04 (m, 2H, *H-3 and H-4*), 3.69 (m, 3H, *H-5, H-6 and H-6'*), 2.12 (s, 3H, *acetate methyl*). ^{13}C N.M.R. (125 MHz, $CDCl_3$, ppm) : 170.53 (*acetate carbonyl*), 138.26, 137.92, 137.78, 128.53, 128.45, 128.38, 128.32, 128.18, 128.09, 128.05, 127.92, 127.88, 127.74, 127.64, 126.96 (*benzyl aromatic carbons*), 92.42 (*C-1*), 77.66 (*C-3*), 75.07 (*benzylic*

carbon), 774.49 (*C-5*), 73.39 (*benzylic carbon*), 71.75 (*benzylic carbon*), 71.03 (*C-4*), 69.28 (*C-6*), 69.13 (*C-2*). Mass spectrum (C.I. with NH_3) *m/e* : 510 (17.3, $M+NH_4^+$), 475 (100), 383 (15.9), 295 (72.5), 277 (26.7), 181 (56.6)

Typical glycosylation procedure for simple alcohols

To 100 mg (0.2 mmoles) of 2-O-acetyl-3,4,6-tri-O-benzyl mannose and 22 mg (0.06 mmoles) in 3 ml acetonitrile, was added 5 mmoles of the alcohol and 70 µl of chlorotrimethylsilane (0.55 mmoles). The reaction mixture was stirred at room temperature for the times shown and the solvents were evaporated in vacuo, before the mixture was separated by silica gel column chromatography (Hexanes : Ethyl acetate 3:1)

Mannosides

Isopropyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**15**)

Yield: 70%. $[\alpha]_D^{25}=23.5^o \pm 0.3$ (c=1.55, chloroform). I.R. ($CHCl_3$, cm^{-1}): 1069, 1374, 1704, 1736. ^1H N.M.R. (500 MHz, $CDCl_3$, ppm): 7.1 (m, 15H, *benzyl aromatic protons*), 5.26 (dd, 1H, *J* = 1.8, 3.3 Hz, *H-2*), 4.89 (d, 1H, *J* = 1.8 Hz, *H-1*), 4.78 (d, 1H, *J* = 11.0 Hz, *benzylic proton*), 4.65 (d, 1H, *J* = 11.0 Hz, *benzylic proton*), 4.63 (d, 1H, *J* = 12.0 Hz, *benzylic proton*), 4.47 (d, 1H, *J* = 11.0 Hz, *benzylic proton*), 4.43 (d, 1H, *J* = 12.0 Hz, *benzylic proton*), 4.40 (d, 1H, *J* = 11.0 Hz, *benzylic proton*), 3.94 (dd, 1H, *J* = 3.3, 9.0 Hz, *H-3*), 3.83 (m, 3H, *H-4, H-5, and isopropyl C-H*), 3.75 (dd, 1H, *J* = 4.0, 10.1 Hz, *H-6*), 3.63 (dd, 1H, *J* = 1.5, 10.1 Hz, *H-6'*), 2.08 (s, 3H, *acetate methyl*), 1.12 (d, 3H, *J* = 6.0 Hz, *isopropyl methyl*), 1.07 (d, 3H, *J* = 6.5 Hz, *isopropyl methyl*). ^{13}C N.M.R. (125 MHz, $CDCl_3$, ppm) : 170.59 (*acetate carbonyl*), 138.36, 138.26, 138.07, 128.36, 128.31, 128.28, 128.02, 127.96, 127.76, 127.71, 127.66, 127.63, 127.54 (*benzyl aromatic carbons*), 95.84 (*C-1*), 78.36 (*C-3*), 75.24 (*benzylic carbon*), 74.47 (*C-4*), 73.40 (*benzylic carbon*), 71.75 (*benzylic carbon*), 71.29 (*C-5*), 69.67 (*isopropyl methenyl carbon*), 69.32 (*C-2*), 68.88 (*C-6*), 23.15,

21.36 (*isopropyl methyl carbons*), 21.17 (*acetate methyl carbon*). Mass spectrum (C.I. with NH_3) *m/e* : 552 (3.1, $M+NH_4^+$), 475 (47.7), 383 (28.3), 337 (100), 277 (34.4), 181 (33.5), 127 (23.2)

Methyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**16**)

Yield: 61%. $[\alpha]_D^{25}=29.9^o \pm 0.6$ (c=1.15, chloroform). I.R. ($CHCl_3$, cm^{-1}): 928, 1048, 1079, 1425, 1477, 1522, 1750. ^1H N.M.R. (500 MHz, $CDCl_3$, ppm): 7.25 (m, 15H, *benzyl aromatic protons*), 5.35 (dd, 1H, *J* = 1.5, 3.2 Hz, *H-2*), 4.85 (d, 1H, *J* = 10.5 Hz, *benzylic proton*), 4.73 (d, 1H, *J* = 1.5 Hz, *H-1*), 4.69 (d, 1H, *J* = 11.0 Hz, *benzylic proton*), 4.68 (d, 1H, *J* = 12.0 Hz, *benzylic proton*), 4.52 (d, 2H, *J* = 11.0 Hz, *benzylic protons*), 4.47 (d, 1H, *J* = 10.5 Hz, *benzylic proton*), 3.96 (dd, 1H, *J* = 3.2, 9.0 Hz, *H-3*), 3.87 (t, 1H, *J* = 9.0 Hz, *H-4*), 3.79 (dd, 1H, *J* = 4.4, 10.3 Hz, *H-6*), 3.76 (m, 1H, *H-5*), 3.73 (dd, 1H, *J* = 1.5, 10.3 Hz, *H-6'*), 3.35 (s, 3H, *methoxy protons*), 2.14 (s, 3H, *acetate methyl*). ^{13}C N.M.R. (125 MHz, $CDCl_3$, ppm) : 170.47 (*acetate carbonyl*), 138.40, 138.23, 137.94, 128.37, 128.28, 128.03, 127.80, 127.75, 127.70, 127.61, 127.57, 127.56 (*benzyl aromatic carbons*), 98.76 (*C-1*), 78.14 (*C-3*), 75.10 (*benzylic carbon*), 74.30 (*C-4*), 73.42 (*benzylic carbon*), 71.73 (*benzylic carbon*), 71.24 (*C-5*), 68.90 (*C-6*), 68.64 (*C-2*), 54.91 (*methoxy carbon*), 21.10 (*acetate methyl carbon*). Mass spectrum (C.I. with NH_3) *m/e* : 524 (8.7, $M+NH_4^+$), 475 (52.0), 415 (15.7), 399 (14.0), 383 (10.0), 309 (100), 277 (16.7), 181 (24.7).

2-O-Acetyl-1,3,4,6-tetra-O-benzyl-α-D-mannopyranoside (**17**)

Yield: 72%. $[\alpha]_D^{25}=30.1^o \pm 0.4$ (c=1.32, chloroform). I.R. ($CHCl_3$, cm^{-1}): 981, 1023,1084, 1137, 1370, 1455, 1739. ^1H N.M.R. (500 MHz, $CDCl_3$, ppm): 7.3 (m, 20H, *benzyl aromatic protons*), 5.41 (dd, 1H, *J* = 1.5, 3.4 Hz, *H-2*), 4.93 (d, 1H, *J* = 1.5 Hz, *H-1*), 4.84 (d, 1H, *J* = 10.7 Hz, *benzylic proton*), 4.69 (d, 2H, *J* = 11.7 Hz, *benzylic protons*), 4.68 (d, 1H, *J* = 6.4 Hz, *benzylic proton*), 4.52 (d, 2H, *J* = 11.7 Hz, *benzylic protons*), 4.49 (d, 1H, *J* = 6.4 Hz, *benzylic proton*), 4.47 (d, 1H, *J* = 10.7 Hz, *benzylic proton*), 4.02 (dd, 1H, *J* = 3.4, 8.8 Hz, *H-3*), 3.90 (t, 1H, *J* = 9.5 Hz, *H-4*), 3.84 (m, 1H, *H-5*), 3.80 (dd, 1H, *J* = 4.4, 10.5 Hz,

H-6'), 3.69 (dd, 1H, *J* = 1.5, 10.5 Hz, *H-6'*), 2.13 (s, 3H, *acetate methyl*). ^{13}C N.M.R. (125 MHz, $CDCl_3$, ppm) : 170.43 (*acetate carbonyl*), 138.32, 138.21, 137.93,136.82, 128.42, 128.36, 128.31, 128.14, 128.07, 127.92, 127.90, 127.87, 127.80, 127.77, 127.70, 127.66, 127.62, 127.58 (*benzyl aromatic carbons*), 97.08 (*C-1*), 78.28 (*C-3*), 75.21 (*benzylic carbon*), 74.32 (*C-4*), 73.44 (*benzylic carbon*), 71.83 (*benzylic carbon*), 71.58 (*C-5*), 69.30 (*benzylic carbon*), 68.80 (*C-6*), 68.77 (*C-2*), 21.11 (*acetate methyl carbon*). Mass spectrum (C.I. with NH_3) *m/e* : 600 (4.6, $M+NH_4^+$), 475 (40.5), 385 (100), 277 (28.4), 181 (56.9).

3) Glycosylation with mannopyranosyl imidate

2-O-Acetyl-tri-O-benzyl-α-D-mannopyranosyl-trichloroacetimidate (21)

To 179 mg (0.36 mmoles) of 2-O-acetyl-tri-O-benzyl-D-mannopyranose in 1.5 ml of THF, was added 400 µl (4 mmoles) of trichloroacetonitrile. With stirring, DBU was added dropwise until the mixture turned yellow. The solution then gradually turns dark black (about ten minutes). It was evaporated, and purified by flash chromatography on silica gel, using 2:1 hexanes : ethyl acetate with 5% triethylamine as an eluent. This gives 196 mg (0.31 mmoles) of a yellow syrup (85% yield).

$[\alpha]_D^{25}=33.7^o \pm 0.9$ (c=1.54, chloroform). I.R. ($CHCl_3$, cm^{-1}): 909, 973, 1101,1220, 1674, 1747. ^1H N.M.R. (500 MHz, $CDCl_3$, ppm): 8.67 (s, 1H, *imidate proton*), 7.31 (m, 12H, *benzyl aromatic protons*), 7.21 (m, 3H, *benzyl aromatic protons*), 6.29 (dd, 1H, *J* = 2.0, 4.7 Hz, *H-1*), 5.49 (dd, 1H, *J* = 2.3, 4.7 Hz, *H-2*), 4.87 (d, 1H, *J* = 10.5 Hz, *benzylic proton*), 4.73 (d, 1H, *J* = 11.2 Hz, *benzylic proton*), 4.68 (d, 1H, *J* = 12.2 Hz, *benzylic proton*), 4.57 (d, 1H, *J* = 11.2 Hz, *benzylic proton*), 4.53 (d, 2H, *J* = 11.5 Hz and 12.2 Hz, *benzylic protons*), 4.00 (m, 3H, *H-3, H-4 and H-5*), 3.83 (dd, 1H, *J* = 4.0, 6.1 Hz, *H-6*), 3.70 (dd, 1H, *J* = 4.0, 11.2 Hz, *H-6'*), 2.18 (s, 3H, *acetate protons*). ^{13}C N.M.R. (125 MHz, $CDCl_3$, ppm) : 170.10 (*acetate carbonyl*), 159.93 (*imidate carbon*), 138.16, 138.11, 138.09, 137.47, 128.52, 128.44, 128.41,

128.36, 128.30, 128.28, 128.24, 128.07, 127.91, 127.84, 127.78, 127.68, 127.60 (*benzyl aromatic carbons*), 95.34 (*C-1*), 77.31 (*C-3*), 76.75 (*trichlorocarbon*), 75.43 (*benzylic carbon*), 74.36 (*C-5*), 73.65 (*C-4*), 73.42 (*benzylic carbon*), 72.06 (*benzylic carbon*), 68.35 (*C-6*), 67.31 (*C-2*), 21.00 (*acetate methyl*). Mass spectrum (FAB with m-nitrobenzyl alcohol (NBA)/NaCl matrix) *m/e* : 658 (1.7, $M+Na^+$), 475 (29.1), 225 (30.8), 181 (100), 154 (51.4), 136 (43.8).

Typical procedure for glycosylation with trichloroimidate

The glycosyl acceptor (0.43 mmoles), 2-O-acetyl-tri-O-benzyl-α-D-mannopyranosyl-trichloroimidate (500 mg, 0.79 mmoles), and 100 mg of powdered 4Å molecular sieves were stirred in 2 ml of dichloromethane for 1/2 hours. Then 430 µl of 1 M boron trifluoride etherate in dichloromethane was added, and the mixture was stirred at room temperature for 6 hours. The reaction was quenched by the addition of 1 ml of a saturated sodium bicarbonate solution. The mixture was filtered, separated, the aqueous layer extracted two more times with dichloromethane, and the combined dichloromethane layers were washed with water and finally dried with anhydrous sodium sulfate. The solvent was then evaporated and the mixture was separated by silica gel chromatography using 2:1 hexanes : ethyl acetate as the eluent. This gave 315 mg (0.31 mmoles) of a clear syrup.

1,3,4,6-Tetra-O-benzyl-2-O-(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (22)

Yield : 72%. $[\alpha]_D^{25}=20.9^o \pm 1.0$ (c=0.97, chloroform). I.R. ($CHCl_3$, cm^{-1}): 908, 1078, 1110, 1209, 1218, 1381, 1452, 1602, 1738, 2874, 2978, 2998, 3007. ^1H N.M.R. (500 MHz, $CDCl_3$, ppm): 7.28 (m, 28H, *benzyl aromatic protons*), 7.15 (m, 7H, *benzyl aromatic protons*), 5.54 (broad s, 1H, *benzylic proton*), 5.33 (m, 1H, *benzylic proton*), 5.05 (t, 1H, *J* = 1.8 Hz, *H-1*), 4.94 (t, 1H, *J* = 1.9 Hz, *H-1**), 4.83 (m, 2H, *benzylic protons*), 4.64 (m, 3H, *benzylic protons*), 4.59 (d, 1H, *J* = 11.5 Hz, *benzylic proton*), 4.51 (m, 2H, *benzylic protons*), 4.43 (d, 2H, *J* =

12.2 Hz, *benzylic protons*), 4.41 (d, 1H, J = 8.0 Hz, *benzylic proton*), 4.35 (d, 1H, J = 11.9 Hz, *benzylic proton*), 4.02 (m, 2H, *H-2 and H-2**), 3.95 (m, 2H, *H-3 and H-3**), 3.85 (m, 2H, *two of H-4, H-5, H-4* or H-5**), 3.76 (m, 2H, *two of H-4, H-5, H-4* or H-5**), 3.16 (m, 3H, *three of H-6, H-6', H-6* or H-6*'*), 3.53 (d, 1H, J = 10.7 Hz, *one of H-6, H-6', H-6* or H-6*'*), 2.12 (s, 3H, *acetate methyl*). ^{13}C N.M.R. (125 MHz, $CDCl_3$, ppm) : 170.12 (*acetate carbonyl*), 138. 45, 138.42, 138.31, 138.13, 137. 96, 137.19, 128.68, 128.58, 128.53, 128.50, 128.44, 128. 42, 128.35, 128.30, 128.27, 128.24, 128.17, 128.07, 128.04, 128.01, 127.95, 127.91, 127.85, 127.79, 127.75, 127.72, 127.68, 127.61, 127.57, 127.50, 127.48, 127.42, 127.39 (*benzyl aromatic carbons*), 99.58 (*C-1*), 97.93 (*C-1**), 79.62 (*C-3*), 78.12 (*C-3**), 75.16 (*one of C-4, C-5, C-4* or C-5**), 75.02 (*benzylic carbon*), 74.81 (*benzylic carbon*), 74.59 (*C-2**), 74.21 (*C-2*), 73.36 (*one of C-4, C-5, C-4* or C-5**), 73.27 (*benzylic carbon*), 72.05 (*benzylic carbon*), 72.00 (*benzylic carbon*), 71.90 (*One of C-4, C-5, C-4* or C-5**), 71.70 (*one of C-4, C-5, C-4* or C-5**), 69.16 (*benzylic carbon*), 68.94 (*C-6 or C-6**), 68.74 (*C-6 or C-6**), 68.64 (*benzylic carbon*), 21.12 (*acetate methyl*). Mass spectrum (FAB with m-nitrobenzyl alcohol (NBA)/NaCl matrix) *m/e* : 1037 (2.3, $M+Na^+$), 475 (14.6), 277 (12.1), 181 (100), 154 (59.8), 136 (52.3).

1,3,4,6-Tetra-O-benzyl-2-O-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**23**)

150 mg (0.148 mmoles) of acetylated carbohydrate was deacetylated by reaction with 200 mg potassium carbonate in 2 ml methanol. Ether was added and the precipitate was filtered. The filtrate was evaporated, then the resulting material was taken up with ether and washed with water. The organic layer was dried with anhydrous sodium sulfate, then evaporated to 141 mg (0.145 mmoles) of the product as a syrup (98%.yield).

$[\alpha]_D^{25}=11.4^o \pm 0.5$ (c=0.59, chloroform). I.R. ($CHCl_3$, cm^{-1}): 909, 1060, 1096, 1222, 1602, 2361, 3012. ^1H N.M.R. (500 MHz, $CDCl_3$, ppm): 7.29 (m, 28H, *benzyl aromatic protons*), 7.18 (m, 7H, *benzyl aromatic protons*), 5.13 (broad s, 1H, *H-1*), 4.98 (broad s, 1H, *H-1**), 4.80 (apparent t due to overlapping d, 2H, J =5.9 and 9.5 Hz,

benzylic protons), 4.66 (m, 3H, *benzylic protons*), 4.56 (m, 5H, *benzylic protons*), 4.46 (m, 2H, *benzylic protons*), 4.33 (d, 2H, J = 12.2 Hz, *benzylic protons*), 4.11 (broad s, 1H, *H-2*), 4.06 (broad s, 1H, *H-2**), 3.95 (m, 1H, *H-3**), 3.82 (m, 5H, *H-3, H-4, H-5, H-4* and H-5**), 3.67 (m, 4H, *four of H-6, H-6', H-6* or H-6*'*), 3.55 (d, 1H, J = 10.3 Hz, *one of H-6, H-6', H-6* or H-6*'*). ^{13}C N.M.R. (125 MHz, $CDCl_3$, ppm) : 138.39, 138.36, 138.24, 128.73, 128.55, 128.49, 128.45, 128.43, 128.38, 128.34, 128.29, 128.27, 128.20, 128.17, 128.10, 128.07, 127.96, 127.93, 127.91, 127.88, 127.83, 127.79, 127.77, 127.74, 127.71, 127.69, 127.64, 127.58, 127.55, 127.51, 127.48, 127.45, 127.41, 127.37 (*benzyl aromatic carbons*), 101.09 (*C-1*), 98.13 (*C-1**), 79.97 (*C-3*), 79.70 (*C-3**), 75.13 (*benzylic carbon*), 74.98 (*C-2**), 74.93 (*benzylic carbon*), 74.77 (*C-4, C-5 or C-5**), 74.29 (*C-4**), 73.38 (*benzylic carbon*), 73.27 (*benzylic carbon*), 72..29 (*benzylic carbon*), 72.11 (*C-4, C-5 or C-5**), 72.06 (*benzylic carbon*), 71.46 (*C-4, C-5 or C-5**), 69.23 (*C-6 or C-6**), 69.00 (*C-6 or C-6**), 68.93 (*benzylic carbon*), 68.48 (*C-2*). Mass spectrum (FAB with m-nitrobenzyl alcohol (NBA)/NaCl matrix) *m/e* : 995 (2.7, $M+Na^+$), 433 (5.5), 307 (11.1), 181 (100), 154 (95.2), 136 (81.8).

<u>1,3,4,6-Tetra-O-benzyl-2-O-(3,4,6-tri-O-benzyl-2-O-(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-α-D-mannopyranosyl)-α-D-mannopyranoside (**24**)</u>

Yield : 63%. $[\alpha]_D^{25} = 17.4^o \pm 0.6$ (c=1.98, chloroform). I.R. ($CHCl_3$, cm^{-1}): 909, 982, 1061, 1092, 1205, 1239, 1386, 1456, 1739, 3022. ^{1}H N.M.R. (500 MHz, $CDCl_3$, ppm): 7.28 (m, 40H, *benzyl aromatic protons*), 7.16 (m, 10H, *benzyl aromatic protons*), 5.53 (dd, 1H, J = 2.0 and 3.4 Hz, *H-2** of the non-reducing end*), 5.18 (d, 1H, J = 2.0 Hz, *H-1*), 5.05 (d, 1H, J = 2.0 Hz, *H-1***), 5.01 (d, 1H, J = 2.0 Hz, *H-1**), 4.82 (m, 3H, *benzylic protons*), 4.28 to 4.74 (m, 17H, *benzylic protons*), 3.32 to 4.15 (m, 17H, *pyranose ring protons*), 2.12 (s, *acetate methyl group*). ^{13}C N.M.R. (125 MHz, $CDCl_3$, ppm) : 170.09 (*acetate carbonyl*), 138.57, 138.45, 138.38, 138.34, 138.19, 138.18, 138.09, 138.03, 137.35 (*benzyl aromatic carbons*), 128.87, 128.84, 128.58, 128.54, 128.50, 128.42, 128.37, 128.34, 128.29, 128.26,

128.21, 128.19, 128.13, 128.08, 128.06, 128.04, 128.01, 127.95, 127.91, 127.87, 127.80, 127.78, 127.77, 127.73, 127.69, 127.66, 127.64, 127..60, 127.57, 127.53, 127.50, 127.49, 127.41, 127.39, 127.36, 127.31 (*benzyl aromatic carbons*), 100.67 (*C-1*), 99.36 (*C-1**), 98.21 (*C-1***), 80.33, 79.46, 78.13, 75.12, 75.06, 74.98, 74.96, 74.95, 74.92, 74.85, 74.23, 73.49, 73.46, 73.31, 73.29, 73.24, 73.07, 72.16, 72.10, 72.03, 71.93, 71.85, 69.05, 68.73, 68.70 (*pyranose ring and benzylic carbons*), 21.06 (*acetate methyl*). Mass spectrum (FAB with m-nitrobenzyl alcohol (NBA)/NaCl matrix) *m/e* : 1469 (0.4, M+Na^+), 475 (4.6), 277 (6.4), 271 (6.3), 217 (6.0), 181 (100), 154 (95.2), 136 (10.4).

4) Glycosylation with sulfite mixture

Typical glycosylation with sulfite

To a solution of 38.4 mg (0.56 mmoles) of imidazole in 440 µl of THF cooled in an ice/brine bath, 50 µl (0.69 mmoles) of thionyl chloride was added, and the mixture was stirred for 5 mn. It was then filtered through glass wool onto a ice-cold solution of 50 mg (0.11 mmoles) of 3,4,6-tri-O-benzyl-α-D-mannopyranose in 250 µl THF. The reaction mixture was left to stir and to come to room temperature with the ice bath. After 4 $^1/_2$ hours, the mixture was filtered through a florisil plug, which was washed with ether. The solvents were evaporated in vacuo without an external source of heat. To this mixture and 10 mg (0.03 mmoles) of zinc triflate, was added 1.5 ml of acetonitrile, 0.5 mmoles of the acceptor and 50 µl (0.4 mmoles) of chlorotrimethylsilane. The reaction mixture was stirred for 4 hours, then a few drops of a saturated sodium bicarbonate solution were added, and the mixture is extracted three times with ether. The combined ether extracts were washed with water, dried with anhydrous sodium sulfate, and evaporated in vacuo. The resulting residue was then chromatographed on silica gel with a 2:1 mixture of hexanes and ethyl acetate. to give the product as a clear syrup.

Isopropyl 3,4,6-tri-O-benzyl-α-D-mannopyranoside (25)

Yield: 71%. α/β ratio: 93/7. $[\alpha]_D^{25}$=43.6° ± 2.0 (c=0.47, chloroform). I.R. ($CHCl_3$, cm^{-1}): 909, 982, 1053, 1099, 11315, 1368, 1455, 2337, 2362, 2871, 2930, 2976, 3003, 3036. ^1H N.M.R. (500 MHz, $CDCl_3$, ppm): 7.30 (m, 12H, *benzyl aromatic protons*), 7.19 (m, 3H, *benzyl aromatic protons*), 5.00 (d, 1H, *J* = 2.0 Hz, *H-1*), 4.80 (d, 1H, *J* = 10.7 Hz, *benzylic proton*), 4.70 (d, 1H, *J* = 11.2 Hz, *benzylic proton*), 4.66 (d, 1H, *J* = 11.2 Hz, *benzylic proton*), 4.65 (d, 1H, *J* = 12.2 Hz, *benzylic proton*), 4.51 (d, 1H, *J* = 12.2 Hz, *benzylic proton*), 4.47 (d, 1H, *J* = 10.7 Hz, *benzylic proton*), 3.99 (broad d, 1H, *J* = 2.2 Hz, *H-2*), 3.93 (heptet, 1H, *J* = 6.1 Hz, *isopropyl CH*), 3.89 (d, 1H, *J* = 6.4 Hz, *H-3*), 3.86 (t, 1H, *J* = 8.1 Hz, *H-4*), 3.84 (m, 1H, *H-5*), 3.75 (dd, 1H, *J* = 3.9, 10.8 Hz, *H-6*), 3.67(dd, 1H, *J* = 1.1, 10.8 Hz, *H-6'*), 1.16 (d, 3H, *J* = 6.1 Hz, *isopropyl methyl*), 1.12 (d, 3H, *J* = 6.1 Hz, *isopropyl methyl*). ^{13}C N.M.R. (125 MHz, $CDCl_3$, ppm) : 138.24, 138.00, 128. 45, 128.42, 128.31, 128.26, 127.97, 127.83, 127.80, 127.72, 127.64, 127.49 (*benzyl aromatic carbons*), 97.05 (*C-1*), 80.33 (*isopropyl CH*), 75.16 (*benzylic carbon*), 74.44 (*C-4*), 73.38 (*benzylic carbon*), 71.87 (*benzylic carbon*), 70.93 (*C-3*), 68.91 (*C-6*), 68.87 (*C-5*), 68.80 (*C-2*), 23.16 (*isopropyl methyl*), 21.18 (*isopropyl methyl*). Mass spectrum (C.I. with NH_3) *m/e* : 510 (46.6, $M+NH_4^+$), 450 (35.8), 433 (45.2), 401 (27.1), 341 (73.4), 205 (80.7), 181 (100).

Methyl 3,4,6-tri-O-benzyl-α-D-mannopyranoside (26)

Yield: 42%. α/β ratio: 86/14. $[\alpha]_D^{25}$=50.9° ± 2.2 (c=0.24, chloroform). I.R. ($CHCl_3$, cm^{-1}): 1059, 1103, 1456, 1602, 2363, 2925. ^1H N.M.R. (500 MHz, $CDCl_3$, ppm): 7.28 (m, 12H, *benzyl aromatic protons*), 7.16 (m, 3H, *benzyl aromatic protons*), 4.80 (d, 1H, *J* = 10.7 Hz, *benzylic proton*), 4.78 (d, 1H, *J* = 2.2 Hz, *H-1*), 4.66 (d, 2H, *J* = 3.9 Hz, *benzylic protons*), 4.63 (d, 1H, *J* = 12.1 Hz, *benzylic proton*), 4.52 (d, 1H, *J* = 12.1 Hz, *benzylic proton*), 4.48 (d, 1H, *J* = 10.7 Hz, *benzylic proton*), 3.99 (dd, 1H, *J* = 2.2, 4.7 Hz, *H-2*), 3.83 (t, 1H, *J* = 4.2 Hz, *H-3*), 3.81 (t, 1H, *J* = 8.7 Hz, *H-4*), 3.70 (m, 3H, *H-5, H-6 and H-6'*), 3.34 (s, 3H, *methoxy*). ^{13}C N.M.R. (125 MHz, $CDCl_3$, ppm) : 137.48, 137.09,

127.88, 127.77, 127.67, 127.41, 127.14, 126.89, 123.415 (*benzyl aromatic carbons*), 97.29 (*C-1*), 78.98 (*C-3*), 77.11 (*benzylic carbon*), 75.24 (*C-4*), 74.23 (*benzylic carbon*), 74.22 (*benzylic carbon*), 73.40 (*C-5*), 72.41 (*C-6*), 69.84 (*C-2*), 50.07 (*methoxy group*). Mass spectrum (C.I. with NH_3) *m/e* : 482 (27.6, $M+NH_4^+$), 450 (18.0), 433 (26.1), 373 (84.1), 341 (48.1), 235 (34.6), 181 (100), 159 (32.5).

1,3,4,6-Tetra-O-benzyl-α-D-mannopyranose (**19**), (**27**)

In addition to the procedure, this compound had to be separated from the benzyl alcohol, after the chromatography, by azeotropic removal with water.

Yield: 64%. α/β ratio: 82/18. $[\alpha]_D^{25}=40.7^o \pm 1.0$ (c=0.56, chloroform). I.R. ($CHCl_3$, cm^{-1}): 1057, 1238, 2337, 2361, 2892. ^1H N.M.R. (500 MHz, $CDCl_3$, ppm): 7.30 (m, 15H, *benzyl aromatic protons*), 7.17 (m, 3H, *benzyl aromatic protons*), 4.99 (d, 1H, *J* = 1.7 Hz, *H-1*), 4.81 (d, 1H, *J* = 10.3 Hz, *benzylic proton*), 4.71 (d, 1H, *J* = 12.1 Hz, *benzylic proton*), 4.67 (s, 2H, *benzylic protons*), 4.66 (d, 1H, *J* = 13.4 Hz, *benzylic proton*), 4.54 (d, 1H, *J* = 12.1 Hz, *benzylic proton*), 4.49 (d, 1H, *J* = 11.7 Hz, *benzylic proton*), 4.48 (d, 1H, *J* = 10.3 Hz, *benzylic proton*), 4.06 (d, 1H, *J* = 1.7 Hz, *H-2*), 3.92 (dd, 1H, *J* = 1.7, 8.4 Hz, *H-3*), 3.87 (t, 1H, *J* = 9.5 Hz, *H-4*), 3.85 (m, 1H, *H-5*), 3.76 (dd, 1H, *J* = 4.0, 10.6 Hz, *H-6*), 3.69 (dd, 1H, *J* = 1.5, 10.6 Hz, *H-6'*). ^{13}C N.M.R. (125 MHz, $CDCl_3$, ppm) : 137.52, 128.05, 127.99, 127.89, 127.79, 127.71, 127.57, 127.52, 127.44, 127.34, 127.21, 127.06, 126.94, 98.16 (*C-1*), 80.24 (*C-3*), 75.15 (*benzylic carbon*), 74.36 (*C-4*), 73.50 (*benzylic carbon*), 72.09 (*benzylic carbon*), 71.36 (*C-5*), 69.18 (*C-6*), 69.02 (*benzylic carbon*), 68.53 (*C-2*). Mass spectrum (C.I. with NH_3) *m/e* : 482 (27.6, $M+NH_4^+$), 450 (18.0), 433 (26.1), 373 (84.1), 341 (48.1), 235 (34.6), 181 (100), 159 (32.5).

5) Preparation of Methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside (18)

Methyl 4,6-O-benzylidene-α-D-glucopyranoside

A mixture of 9.7 g (50 mmoles) of methyl α-D-glucopyranoside, 7.5 ml (50 mmoles) of benzaldehyde dimethylacetal and 90 mg (0.52 mmoles) of p-toluenesulfonic acid monohydrate in 40 ml of DMF was put on a rotary evaporator with a solvent trap. The flask was then heated to 100°C for 1 hour, during which the DMF was slowly collected in the trap. A residue was left in the reaction flask. Then 50 ml of water containing 1 g (11.9 mmoles) of sodium bicarbonate was added and the resulting mixture was then heated to 100°C. The resulting cloudy solution was then left to cool. The large mass of crystals obtained on cooling was filtered, dried on a high vacuum pump and kept in a dessicator. The yield was 10.2 g (36 mmoles, 72%).

$[\alpha]_D^{25}=105.7^{o} \pm 0.4$ (c=1.36, chloroform). I.R. ($CHCl_3$, cm^{-1}): 1052, 1087, 1204, 1371, 1374, 1455, 1723, 2337, 2362, 2870. ^1H N.M.R. (500 MHz, $CDCl_3$, ppm): 7.47 (m, 2H, *benzylidene aromatic protons*), 7.31 (m, 3H, *benzylidene aromatic protons*), 5.51 (s, 1H, *benzylidene proton*), 4.77 (d, 1H, J = 3.9 Hz, *H-1*), 4.27 (dd, 1H, J = 4.4, 9.8 Hz, *H-6*), 3.91 (t, 1H, J = 9.3 Hz, *H-3*), 3.79 (dt, 1H, J = 4.4, 9.8 Hz, *H-5*), 3.72 (t, 1H, J = 10.0 Hz, *H-6'*), 3.61 (broad s, 1H, *H-2*), 3.47 (t, 1H, J = 9.5 Hz, *H-4*), 3.44 (s, 3H, *methyl group*). ^{13}C N.M.R. (125 MHz, $CDCl_3$, ppm) : 129.26 (*benzylidene aromatic carbons*), 128.33 (*benzylidene aromatic carbons*), 126.30 (*benzylidene aromatic carbons*), 101.94 (*benzylidene non-aromatic carbon*), 99.73 (*C-1*), 80.883 (*C-4*), 72.88 (*C-2*), 71.81 (*C-3*), 68.92 (*C-6*), 62.35 (*C-5*), 55.58 (*methyl group*). Mass spectrum (C.I. with NH_3) *m/e* : 283 (100, M+1), 251 (7.8), 179 (6.5), 162 (6.3), 145 (5.8), 133 (3.7), 118 (6.1).

Methyl 2,3-di-O-benzyl-4,6-O-benzylidene-α-D-glucopyranoside

To 1.54 g (5.5 mmoles) of methyl 4,6-O-benzylidene-α-D-glucopyranoside and 2 g (50 mmoles) of sodium hydride (60%

dispersion in oil) in 15 ml of anhydrous THF was added dropwise 4 ml (33.6 mmoles) of benzyl bromide. The mixture was left to stir at room temperature for 24 hours. Then the excess sodium hydride was destroyed with anhydrous methanol. The mixture was partitioned between water and dichloromethane. The organic layer was washed with water twice, dried over sodium sulfate, filtered. Then 10 ml of florisil was added and the dichloromethane was evaporated. The florisil was layered over another 50 ml of florisil in a glass sintered funnel, and washed with 150 ml of hexanes, before the product was eluted with 200 ml of ethyl acetate. This solvent was then evaporated and the resulting syrup was crystallized by dissolving in ether, adding petroleum ether and slowly evaporating the ether, giving 1.45 g (3.1 mmoles) of product (57% yield).

$[\alpha]_D^{25}$=-12.3° ± 0.5 (c=1.01, chloroform). I.R. ($CHCl_3$, cm^{-1}): 1036, 1065, 1205, 1230, 1380, 2869, 2929, 2974. ^1H N.M.R. (500 MHz, $CDCl_3$, ppm): 7.47 (m, 3H, *benzyl aromatic protons*), 7.31 (m, 12H, *benzyl aromatic protons*), 5.53 (s, 1H, *benzylidene proton*), 4.90 (d, 1H, *J* = 11.9 Hz, *benzylic proton*), 4.84 (d, 1H, *J* = 8.6 Hz, *benzylic proton*), 4.82 (d, 1H, *J* = 8.6 Hz, *benzylic proton*), 4.68 (d, 1H, *J* = 11.9 Hz, *benzylic proton*), 4.58 (d, 1H, *J* = 3.8 Hz, *H-1*), 4.25 (dd, 1H, *J* = 5.0, 10.1 Hz, *H-6*), 4.03 (t, 1H, *J* = 9.3 Hz, *H-3*), 3.81 (dt, 1H, *J* = 5.0, 10.0 Hz, *H-5*), 3.69 (t, 1H, *J* = 10.5 Hz, *H-6'*), 3.59 (t, 1H, *J* = 9.4 Hz, *H-4*), 3.54 (dd, 1H, *J* = 3.8, 9.3 Hz, *H-2*), 3.39 (s, 3H, *methoxy group*). ^{13}C N.M.R. (125 MHz, $CDCl_3$, ppm) : 138.76, 137.43, 129.66, 129.25, 129.24, 128.93, 128.89, 128.51, 128.44, 128.34, 128.32, 128.29, 128.24, 128.20, 128.11, 128.01, 127.90, 127.56, 126.33, 126.08, 126.04 (*benzyl aromatic carbons*), 101.28 (*benzylidene*), 99.27 (*C-1*), 82.17 (*C-4*), 79.22 (*C-2*), 78.61 (*C-3*), 75.32 (*benzylic carbon*), 73.78 (*benzylic carbon*), 69.08 (*C-6*), 62.33 (*C-5*), 55.34 (*methoxy group*). Mass spectrum (C.I. with NH_3) *m/e* : 463 (13.6, M+1), 371 (56.9), 197 (14.4), 373 (42.8), 181 (12.8), 177 (16.4), 159 (17.5), 149 (17.9), 121 (100).

Methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside (**18**)

To 106 mg (0.23 mmoles) of methyl 2,3-di-O-benzyl-4,6-O-benzylidene-α-D-glucopyranoside, 60 mg of powdered 4Å molecular sieves and 200 mg (3 mmoles) of sodium cyanoborohydride in 3 ml THF, was added, dropwise, a saturated solution of hydrochloric acid in ether. When the resulting bubbling stopped to occur, the mixture was stirred for 20 mn, and as the TLC (with 6:1 hexanes : ethyl acetate as eluent) showed no more starting material, a little water was added, and the mixture filtered. The filtrate was partitioned between water and dichloromethane, and the extraction with dichloromethane was repeated three times. The combined organic layers were dried with anhydrous sodium sulfate, and the solvents were evaporated in vacuo. The residue obtained was chromatographically separated with a 2:1 mixture of hexanes and ethyl acetate. The result is 87.4 mg (0.19 mmoles) of the product as a clear syrup (83% yield). Methyl 2,3,6-tri-O-benzyl-α-D-glucopyranoside (9mg, 0.02 mmoles, 8%) was also obtained.

$[\alpha]_D^{25=}$ 11.0° ± 0.6 (c=2.19, chloroform). I.R. ($CHCl_3$, cm^{-1}): 911, 989, 1055, 1096, 1212, 1222, 1381, 1460, 2255, 2337, 2361, 2869, 2928. ^1H N.M.R. (500 MHz, $CDCl_3$, ppm): 7.31 (m, 15H, *benzyl aromatic protons*), 4.97 (d, 1H, *J* = 11.0 Hz, *benzylic proton*), 4.86 (d, 1H, *J* = 12.0 Hz, *benzylic proton*), 4.82 (d, 1H, *J* = 12.0 Hz, *benzylic proton*), 4.78 (d, 1H, *J* = 12.4 Hz, *benzylic proton*), 4.63 (apparent t, made of two d, 2H, *J* = 12.4, 11.0 Hz, *benzylic protons*), 4.54 (d, 1H, *J* = 3.7 Hz, *H-1*), 3.99 (t, 1H, *J* = 9.3 Hz, *H-3*), 3.74 (double dd, 1H, *J* = 2.7, 5.1, 11.7 Hz, *H-6*), 3.68 (dd, 1H, *J* = 4.0, 7.4 Hz, *H-6'*), 3.63 (m, 1H, *H-5*), 3.49 (overlapping t and dd at 3.50 and 3.48 respectively, d, 2H, *J* = 8.5 Hz and*J* = 3.7, 8.9 Hz respectively, *H-4 and H-2*), 3.34 (s, 3H, *methoxy group*). ^{13}C N.M.R. (125 MHz, $CDCl_3$, ppm) : 138.54, 137.94, 128.31, 128.30, 128.24, 127.96, 127.88, 127.80, 127.76, 127.72, 127.46 (*benzyl aromatic carbons*), 98.01 (*C-1*), 81.78 (*C-3*), 79.79 (*C-2*), 77.20 (*C-4*), 75.59 (*benzylic carbon*), 74.86 (*benzylic carbon*), 73.26 (*benzylic carbon*), 70.46 (*C-5*), 61.71 (*C-6*), 55.02 (*methoxy group*). Mass spectrum (C.I. with NH_3) *m/e* : 482 (27.7, $M+NH_4^+$), 450

(12.1), 433 (22.1), 373 (42.8), 341 (32.2), 253 (55,8), 181 (100), 121 (67.3).

6) Preparation of Methyl 2,3,6-tri-O-benzyl-α-D-glucopyranoside (32)

Methyl 6-O-trityl-α-D-glucopyranoside

2.0 g (7.18 mmoles) of trityl chloride and 1.43 g (7.4 mmoles) of methyl-α-D-glucopyranoside were dissolved in 7 ml of pyridine. The mixture was stirred overnight at room temperature. Chloroform was added and the mixture is filtered through celite. The solvents in the filtrate were evaporated, and then toluene was used to azeotrope pyridine. The resulting syrup was layered on florisil, and eluted with 1:1 hexanes : ethyl acetate to remove the less polar components and, then, with 1:9 methanol : ethyl acetate to obtain a syrup, which was crystallized by taking it up in dichloromethane, adding hexanes and letting the dichloromethane evaporate slowly. This gave 1.85 g (4.23 mmoles) of the product as a white powder (59% yield).

m.p. : 132-136 °C. $[\alpha]_D^{25=}$ 88.3° ± 0.8 (c=2.12, pyridine) (literature values : m.p. : 138-140 °C. $[\alpha]_D^{16=}$ 86.3° (pyridine)[37]). ^{1}H N.M.R. (500 MHz, $CDCl_3$, ppm): 7.41 (m, 3H, *benzyl aromatic protons*), 7.22 (m, 9H, *benzyl aromatic protons*), 7.17 (m, 3H, *benzyl aromatic protons*), 4.69 (d, 1H, J = 4.2 Hz, *H-1*), 3.62 (m, 2H, *H-3 and H-5*), 3.45 (dd, 1H, J = 3.8 and 9.5 Hz, *H-4*), 3.39 (dt, 1H, J = 4.2 and 8.8 Hz, *H-2*), 3.35 (s, 3H, *methoxy group*), 3.34 (dd buried under the previous singlet, 1H, J = 3.4 and 9.9 Hz, *H-6*), 3.27 (dd, 1H, J = 5.5 and 9.9 Hz, *H-6'*). ^{13}C N.M.R. (125 MHz, $CDCl_3$, ppm) : 143.61, 128.50, 127.75, 127.71, 126.93 (*benzyl aromatic carbons*), 98.93 (*C-1*), 86.74 (*trityl benzylic carbon*), 74.41 (*C-3*), 71.95 (*C-4*), 71.42 (*C-2*), 69.91 (*C-5*), 63.77 (*C-6*), 54.98 (*methoxy group*).

Methyl 2,3,6-tri-O-benzyl-α-D-glucopyranoside (32)

To 1 g (2.3 mmoles) of methyl 6-O-trityl-α-D-glucopyranoside and 1.5 g (37.5 mmoles) of sodium hydride (60% dispersion in oil), in

10 ml of THF and 10 ml of DMF, was added 3 ml (25.2 mmoles) of benzyl bromide. The mixture was stirred for two days, then the excess hydride destroyed with methanol, and then the mixture was partitioned between water and chloroform. The aqueous layer was extracted two more times with chloroform, and then the combined organic phases are dried with anhydrous sodium sulfate, and then the solvents were evaporated in vacuo. The resulting residue was taken up in 20 ml of acetic acid, and then concentrated hydrochloric acid was added dropwise, until a yellow color forms. Following this, a yellow precipitate appears. After 1/2 hours, a saturated sodium bicarbonate solution was added dropwise, to bring the pH to about 3. Then the solvents were evaporated in vacuo, and the resulting residue was taken up in chloroform, washed with a saturated sodium bicarbonate solution, then water, and dried with anhydrous sodium sulfate. The solvents are evaporated, and the residue is run through silica gel chromatography with 2:1 hexanes : ethyl acetate as the eluent. This gave 664 mg (1.43 mmoles) of the product as a clear syrup (62% yield).

$[\alpha]_D^{25=}$ 21.3° ± 0.7 (c=2.06, chloroform). I.R. ($CHCl_3$, cm^{-1}): 910, 1026, 1052, 1068, 1090, 1157, 1236, 1247, 1273, 1363, 1455, 1602, 1732, 2337, 2362, 2878, 2932. ^1H N.M.R. (500 MHz, $CDCl_3$, ppm): 7.30 (m, 15H, *benzyl aromatic protons*), 4.99 (d, 1H, *J* = 11.5 Hz, *benzylic proton*), 4.75 (d, 1H, *J* = 12.2 Hz, *benzylic proton*), 4.72 (d, 1H, *J* = 11.5 Hz, *benzylic proton*), 4.64 (d, 1H, *J* = 12.2 Hz, *benzylic proton*), 4.62 (d, 1H, *J* = 3.4 Hz, *H-1*), 4.57 (d, 1H, *J* = 12.2 Hz, *benzylic proton*), 4.52 (d, 1H, *J* = 12.2 Hz, *benzylic proton*), 3.77 (t, 1H, *J* = 9.0 Hz, *H-3*), 3.63 (m, 2H, *H-5 and H-6*), 3.59 (t, 1H, *J* = 9.3 Hz, *H-6'*), 3.52 (dd, 1H, *J* = 3.4, 9.3 Hz, *H-4*), 3.46 (dd, 1H, *J* = 6.9, 14.2 Hz, *H-2*), 3.37 (s, 3H, *methoxy group*). ^{13}C N.M.R. (125 MHz, $CDCl_3$, ppm) : 138.79, 138.04, 137.99, 128.57, 128.46, 128.34, 128.11, 127.98, 127.94, 127.83, 127.60 (*benzyl aromatic carbons*), 98.18 (*C-1*), 81.45 (*C-3*), 79.58 (*C-4*), 75.42 (*Benzylic carbon*), 73.57 (*benzylic carbon*), 73.15 (*benzylic carbon*), 70.70 (*C-6*), 69.87 (*C-5*), 69.46 (*C-2*), 55.23 (*methoxy group*). Mass spectrum (C.I. with NH_3) *m/e* : 482 (20.8, $M+NH_4^+$), 450 (19.7), 373 (44.1), 253 (57.3), 181 (100), 175 (42.7), 121 (55.3)

7) Other mannopyrannosyl donors

1-O-Silyl-2-O-acetyl-tri-O-benzyl-α-D-mannopyranose (**20**)

To 237 mg (0.48 mmoles) of 2-O-acetyl-tri-O-benzyl-D-mannopyranose and 40 mg (0.58 mmoles) of imidazole in 750 µl THF, cooled in an ice bath, was added 200 µl (1.6 mmoles) of chlorotrimethylsilane. The reaction mixture was stirred and left to come to room temperature on its own. After four hours, it was filtered through a florisil plug, which was washed with 5 ml ether. Evaporation of the solvents gave 276 mg (0.48 mmoles) of a clear syrup (quantitative).

$[\alpha]_D^{25}=20.7^o \pm 1.0$ (c=1.46, chloroform). I.R. ($CHCl_3$, cm^{-1}): 403, 909, 982, 1071, 1144, 1249, 1370, 1457, 1740, 2863. ^1H N.M.R. (500 MHz, $CDCl_3$, ppm): 7.3 (m, 12H, *benzyl aromatic protons*), 7.18 (m, 3H, *benzyl aromatic protons*), 5.24 (dd, 1H, *J* = 2.1, 3.3 Hz, *H-2*), 5.17 (d, 1H, *J* = 2.1 Hz, *H-1*), 4..84 (d, 1H, *J* = 10.5 Hz, *benzylic proton*), 4.70 (d, 2H, *J* = 12.0 Hz, *benzylic protons*), 4.54 (d, 1H, *J* = 11.0 Hz, *benzylic proton*), 4.46 (two overlapping d, 2H, *J* = 10.5 and *J* = 12.0 Hz, *benzylic protons*), 4.05 (dd, 1H, *J* = 3.3, 8.9 Hz, *H-3*), 3.93 (t, 1H, *J* = 9.4 Hz, *H-4*), 3.89 (m, 1H, *H-5*), 3.83 (dd, 1H, *J* = 3.7, 10.5 Hz, *H-6*), 3.64 (dd, 1H, *J* = 1.7, 10.5 Hz, *H-6'*), 2.15 (s, 3H, *acetate protons*), 0.14 (s, 9H, *silyl methyl protons*). ^{13}C N.M.R. (125 MHz, $CDCl_3$, ppm) : 170.66 (*acetate carbonyl*), 138.37, 138.02, 128.37, 128.32, 128.26, 128.08, 127.98, 127.82, 127.71, 127.64, 127.53 (*benzyl aromatic carbons*), 92.72 (*C-1*), 77.76 (*C-3*), 75.27 (*benzylic carbon*), 74.37 (*C-5*), 73.44 (*benzylic carbon*), 71.75 (*benzylic carbon*), 71.21 (*C-4*), 70.30 (*C-2*), 68.78 (*C-6*), 21.18 (*acetate methyl*), -0.23 (*silyl methyls*). Mass spectrum (C.I. with NH_3) *m/e* : 583 (3.3, $M+NH_4^+$), 475 (54.9), 383 (31.5), 367 (100), 277 (43.2), 181 (33.9)

3,4,6-Tri-O-benzyl-β-D-mannopyranosyl 1,2-carbonate (**13**)

To 54.4 mg (0.11 mmoles) of 3,4,6-tri-O-benzyl mannose, 50 mg (0.17 mmoles) of triphosgene and 5 mg (0.04 mmoles) of 4-dimethyl-amino-pyridine in 250 ml of THF, cooled in an ice/acetone bath was added 20 ml of pyridine, the mixture was stirred at room temperature for 24 hours, then evaporated and the residue was separated on silica gel in 2:1 ethyl acetate:hexanes to give 15.0 mg of a clear syrup (26% yield).

$[\alpha]_D^{25}=47.5^o \pm 0.7$ (c=0.59, chloroform). I.R. ($CHCl_3$, cm^{-1}): 890, 1005, 1033, 1102, 1144, 1321, 1372, 1456, 1494, 1581, 1653, 1747, 2338, 2361, 2928, 2979. ^1H N.M.R. (500 MHz, $CDCl_3$, ppm): 7.3 (m, 12H, *benzyl aromatic protons*), 7.16 (m, 3H, *benzyl aromatic protons*), 5.96 (s, 1H, *H-1*), 5.33 (s, 1H, *H-2*), 4.84 (d, 1H, *J* = 9.3 Hz, *benzylic proton*), 4.7 (d, 1H, *J* = 12.2 Hz, *benzylic proton*), 4.62 (d, 1H, *J* = 12.2 Hz, *benzylic proton*), 4.60 (d, 1H, *J* = 13.0 Hz, *benzylic proton*), 4.48 (d, 1H, *J* = 13.0 Hz, *benzylic proton*), 4.46 (d, 1H, *J* = 9.3 Hz, *benzylic proton*), 4.22 (d, 1H, *J* = 9.3 Hz, *H-3*), 4.05 (d, 1H, *J* = 7.8 Hz, *H-5*), 3.93 (t, 1H, *J* = 9.8 Hz, *H-4*), 3.77 (dd, 1H, *J* = 3.9, 9.3 Hz, *H-6*), 3.65 (d, 1H, *J* = 11.0 Hz, *H-6'*). ^{13}C N.M.R. (125 MHz, $CDCl_3$, ppm) : 154.03 (*carbonate*), 138.00, 137.68, 137.45, 128.51, 128.42, 128.41, 128.35, 128.28, 128.12, 127.99, 127.94, 127.86, 127.82, 127.80, 127.75, 127.72 (*benzyl aromatic carbons*), 89.87 (*C-1*), 76.61 (*C-3*), 75.45 (*C-2*), 75.14 (*benzylic carbon*), 74.30 (*C-4*), 73.53 (*benzylic carbon*), 73.43 (*benzylic carbon*), 72.22 (*C-5*), 67.83 (*C-6*). Mass spectrum (C.I. with NH_3) *m/e* : 463 (25.4), 449 (47.5, M-CO_2 +NH_4^+), 343 (90.4), 253 (57.2), 235 (47.2), 217 (49.2), 181 (100)

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CHAPTER THREE

Preparation of a Mannosidase inhibitor

I] Glycosidases and their mechanisms

As mentioned previously (chapter 1, section I.2.b), glycosidases play a prime role in the biosynthesis of oligosaccharides and of most cell surface carbohydrates. As such, they are the target of many studies, as well as that of many inhibitors[1].

In this respect, the studies on their mechanisms are of particular usefulness and, hence, they have been carefully studied and certain generalities drawn[2]. Many different glycosidases exist, as these enzymes can be classified as furanose and pyranose cleaving enzymes. We shall consider only the pyranose cleaving glycosidases.

Early studies used the terms "inverting" and "retaining" glycosidases to designate glycosidases which inverted or retained the anomeric configuration of the residue they cleaved. However a more accurate terminology has been introduced with the apparent need to encompass the stereochemistry of the anomeric carbon in the nomenclature by designating it as **e** for equatorial or **a** for axial[3]. Hence, instead of two different categories of pyranose cleaving mannosidases, four were used :

- **e** $\longrightarrow$ **a** or **a** $\longrightarrow$ **e** for the inverting enzymes
- **e** $\longrightarrow$ **e** or **a** $\longrightarrow$ **a** for the retaining enzymes

The mechanisms for these four categories have been studied carefully. The following generalizations can be drawn :

- retaining glycosidases probably involve the opening of the pyran ring and the formation of an oxygen cation which is dealkylated by the presence of water.(figure 1). Such a opening of the pyran ring allows for the subsequent closure with retention of stereochemistry due to the particular arrangement of the general acid and the general base catalysts in the active site.

- inverting glycosidases can go through a similar pathway, that is, with ring opening of the pyranose but this time the closure will be performed with inversion of stereochemistry or without opening the pyran ring in a direct S_N2 type of displacement of the leaving alcohol by water (figure 2). Again the arrangement of the residues in space and the particular environment of the active site will allow water to displace the alkoxide ion.

It appears clearly that the above mechanisms share a certain number of features which allow for the design of inhibitors. One such a feature is the necessity in all cases to have both a general acid and a general base in the active site. The other feature is the necessity to fit, at the very least, one carbohydrate residue in this site. These two features are essential to any prospective inhibitor.

Our interest was in the determination of the position of the active site of two particular mannosidases. These enzymes are involved in the processing of N-linked oligosaccharides. They cleave a specific mannose residue and it has been determined that the Man9GlcNAc oligosaccharide (**1**, figure 3) is a sufficient substrate for these enzymes[4,5,6,7]. Both of them are processing 1,2-mannosidases. These enzymes have been isolated from two different sources : the yeast *Saccharomyces Cerevisiae*[8] and a murine source. While the first one removes a single mannose unit, the mammalian enzyme cleaves three units[9] Studies are in progress to determine the specificity and the active site of the enzymes.

Furthermore the *S. Cerevisiae* processing 1,2-mannosidase has been shown to be an inverting (**e** $\longrightarrow$ **a**) glycosidase by N.M.R. studies[10]

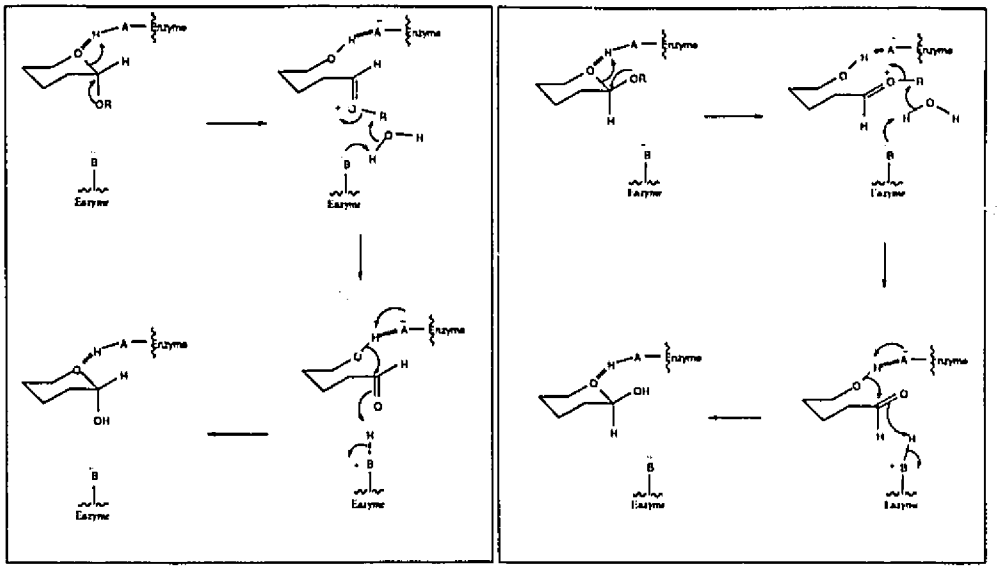


figure 1

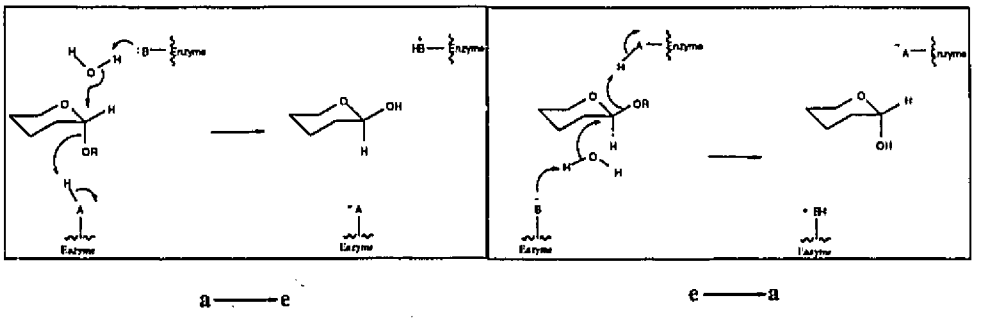


figure 2

The amino acid sequence of the enzyme (a type II transmembrane protein) is being investigated[11] and a soluble mutant has been produced with the same properties as the purified endogenous enzyme[5,8,12], which allows one to obtain large amounts of the enzyme without the trouble created by having to deal with typical membrane proteins (i.e. having to work with microsomes).

Therefore it appears that an irreversible inhibitor that would bind to the active site would easily give us access to the sequence of

this active site as well as to the catalytic residues, and, hence, to the mechanism.

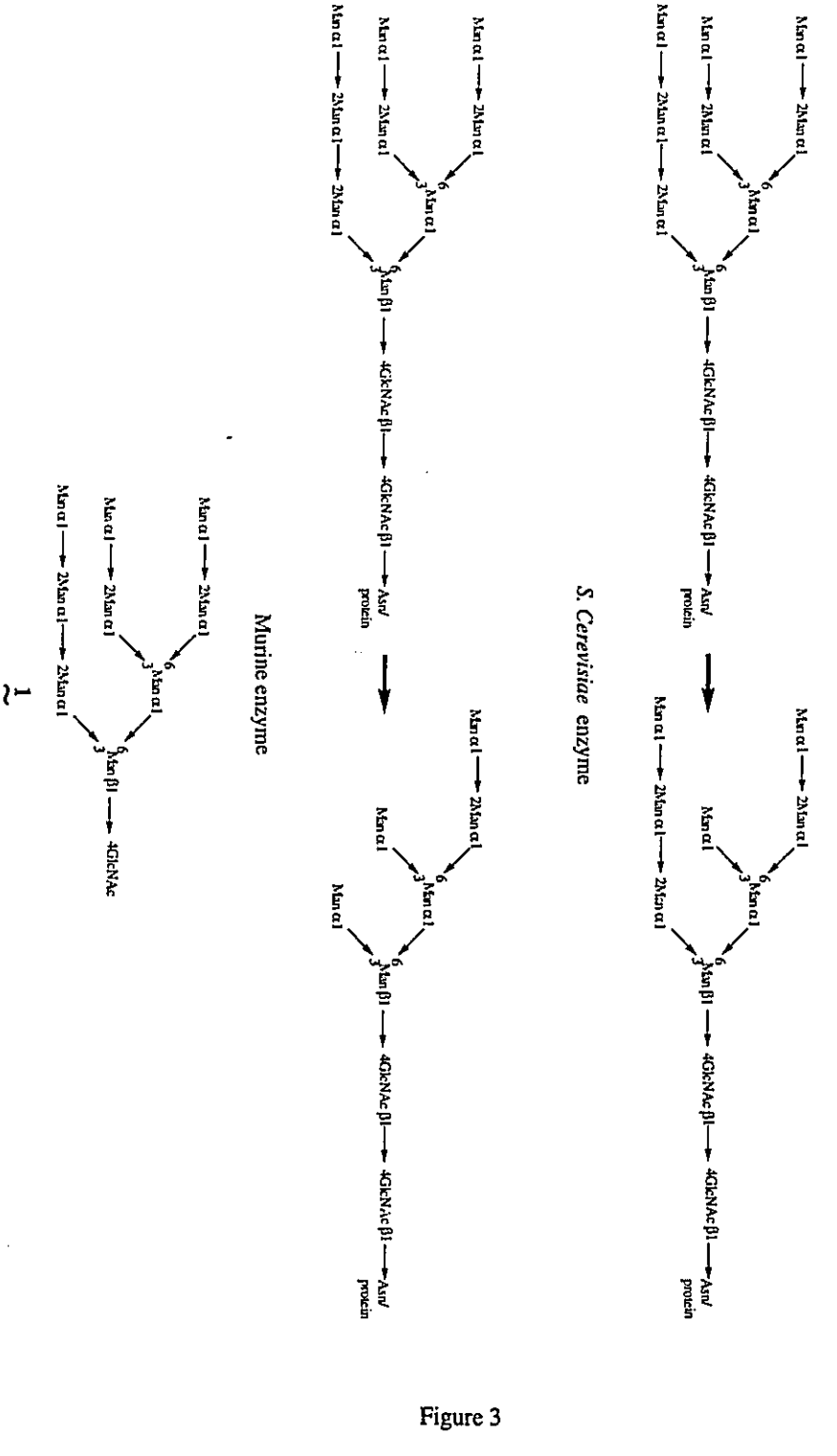
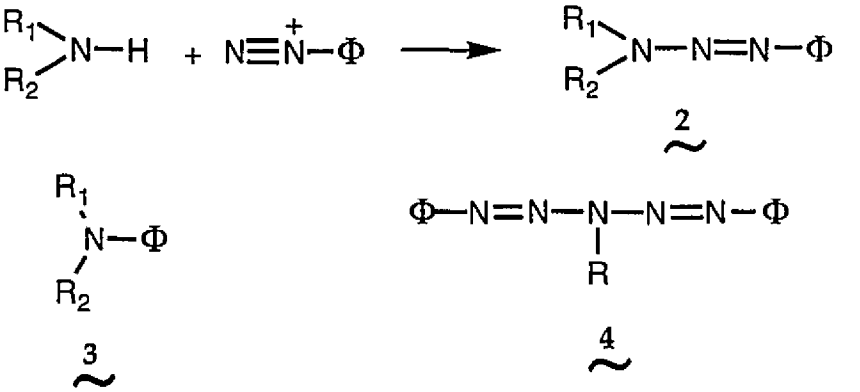


Figure 3

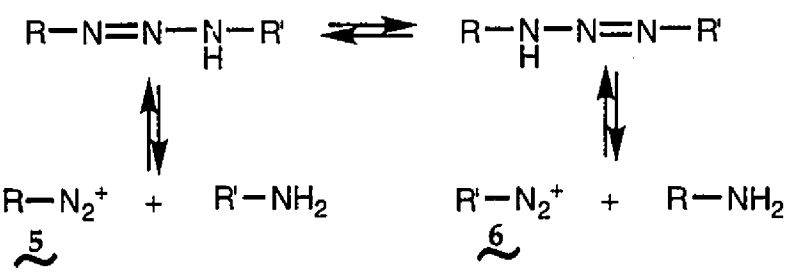
II] Triazenes as alkylating agents

Triazenes are coumpounds of general structure 2, which are generally obtained by the nucleophilic attack of an amine on an aryl diazonium salt [13]. Such a reaction is reversible and is accompanied by two general side products: the amine, 3, due to the nucleophilic attack at the carbon bearing the diazonium unit, and, if the amine used is primary, that is if R_2- is a hydrogen, the pentazine, 4, due to two consecutive nucleophilic attacks[14].

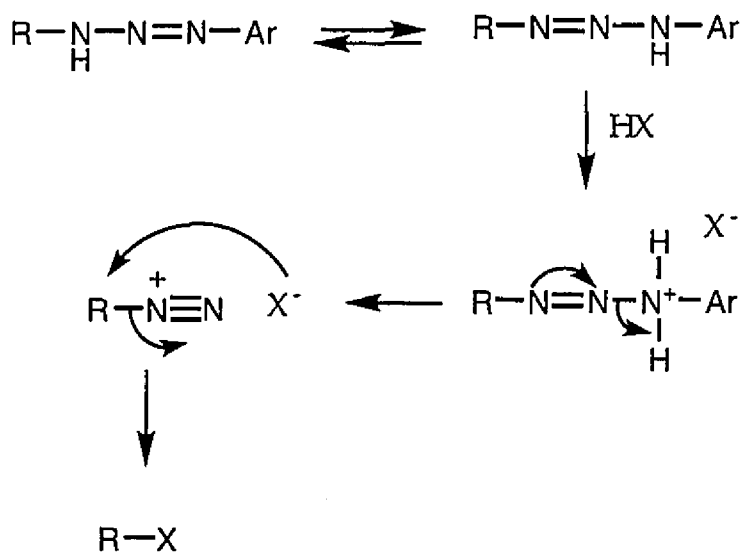


Monoalkyl triazenes (triazenes built with a primary amine) have been shown to tautomerize :

Since the reaction is reversible, and because of this tautomerism, two different diazonium salts can be produced : 5 and 6.



Studies have been conducted on this cleavage of the triazenes. The results show that if both substituents are aromatic, then the cleavage results preferably in the less basic amine and the more stable diazonium. If one substituent is aromatic and the other one is aliphatic, then the cleavage produces predominantly the aromatic amine and the aliphatic alcohol[15] (due to the hydrolysis of the aliphatic diazonium salt[16]). Hence triazenes appeared as a convenient way of deaminating aliphatic amines under mild conditions :



Such a scheme has been used with inorganic acids to produce the alcohol or with dry organic acids to produce the ester[17].

Because of the ease with which triazenes can convert amines to diazonium ions, even in physiological conditions, these compounds have been used as bioactive substances leading to the generation in situ of the active alkylating agents, and hence can be used as enzyme inhibitors or as antitumor drugs[18].

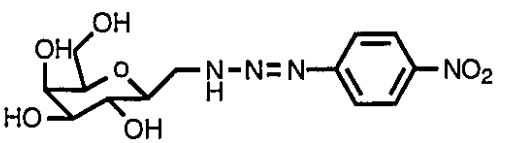
III] Glycosylmethyl-aryl-triazenes as glycosidase inhibitors.

The particular ability of triazenes to generate alkylating agents in situ has made them a prime choice for the synthesis of irreversible

inhibitors, especially in the case of glycosidases. The idea is that an analog of the enzyme substrate, with a triazene moiety, can generate the diazonium in situ and alkylate a nucleophilic residue.

The closest to the natural substrate of any glycosidase is the glycosylmethyl aryl triazenes, and in general the aryl moiety is chosen to be the p-nitrophenyl group. These are chosen and not the glycosyl-p-nitrophenyl triazene because of the highly reactive nature of this latter and its inability to survive in water.

The use of these inhibitors was started with the finding that β-galactopyranosylmethyl p-nitrophenyl triazene (**GalMNT**, 7) can be used to label the β-galactosidase[19]. From then on these have been extensively studied. Particularly, this triazene was shown to alkylate a single residue (methionine 500) in the *Escherichia Coli* β-galactosidase[20].



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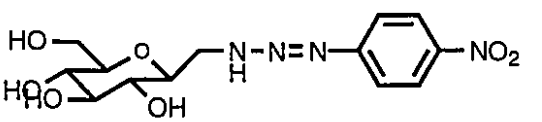

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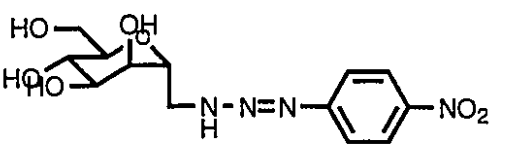


9

Studies have been conducted on the ability of this triazene and of β-glucopyranosylmethyl-p-nitrophenyl triazene (**GlcMNT**, 8) to inhibit lysosomal glycosidases directly on cell cultures, and on the mechanisms of restoration of enzymes levels *in vivo*, that is on the way the damaged enzymes are replaced[21,22]. These inhibitors are also used in research on diseases which affect glycoconjugate biosynthesis[22,23].

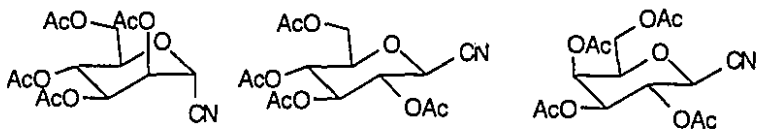
The same approach has been used on α-mannosidases with the corresponding α-mannopyranosylmethyl-p-nitrophenyl triazene (**ManMNT**, **9**)[24,25,26].

As described earlier on, the *S. Cervisiae* processing α-(1,2)-mannosidase is an inverting (e → a) glycosidase[7]. Therefore it appears suited to use the α-mannopyranosylmethyl-p-nitrophenyl triazene. As shown in figure 4, the mechanism of the enzyme should be well suited for this inhibitor provided the active site is not too specific to its substrate. This scheme is of course not meant to show that the general acid catalyst' s conjugated base will be alkylated. In fact, any nucleophilic center can be alkylated, provided it is in the vicinity. It is just intended to show that the minimum requirements for this enzyme's putative mechanism are sufficient for the inhibitor to act.

Hence it is quite interesting to prepare this inhibitor and to test it on specific mannosidases, which it has not been known to inhibit.

IV] Preparation of the inhibitor

The typical method to synthesize triazenes is to use the aliphatic amine and the aromatic diazonium. In this case a commercially available diazonium salt is used: p-nitrobenzenediazonium tetrafluoroborate. The mannopyranosyl-methylamine is therefore the only moiety to be prepared. This amine and the related glucopyranose and galactopyranose are best prepared by the reduction of the corresponding peracetylated cyanides (**10**, **11**, **12**) by lithium aluminum hydride.



10 **11** **12**

The preparation of such cyanides have long been known, by

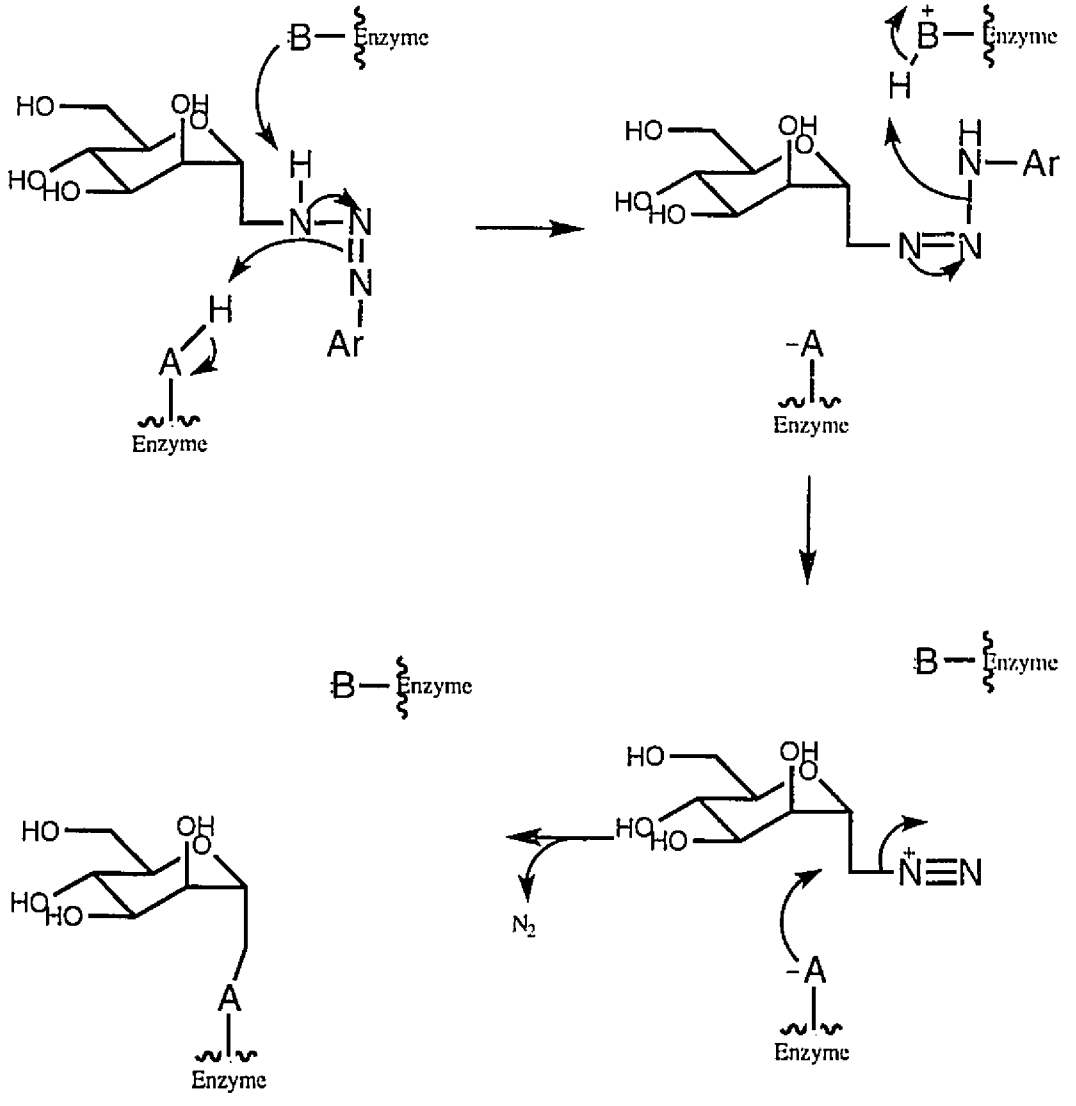
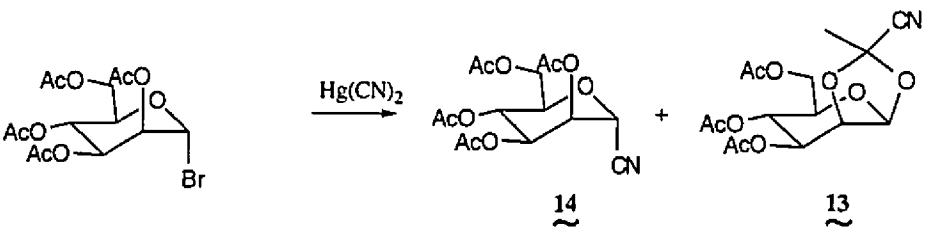
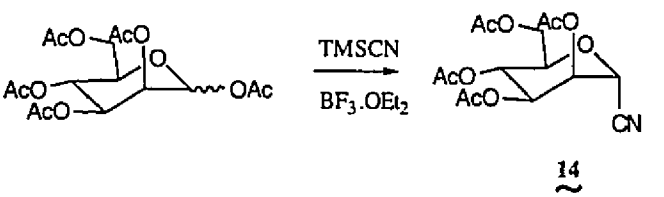


figure 4

using mercury(II) cyanide with the 2,3,4,6-tetra-acetyl-1-bromo-glyco-pyranoses[27]. However this is not a technique of choice for mannose as this yields a product (**13**), similar in concept to the orthoesters, which are so stable for the mannose series. For this reason a second step is required, in which a strong Lewis acid - boron trifluoride etherate is the typical one - opens the five membered ring to yield the cyanide (**14**). A simpler procedure involves the use of

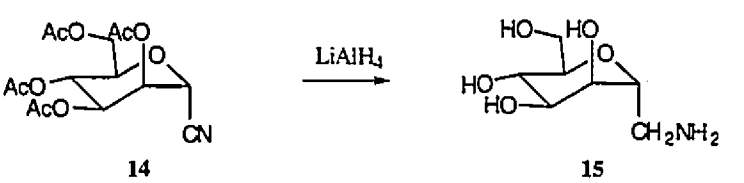
trimethylsilyl cyanide and a catalytic amount of $BF_3.OEt_2$, with the peracetylated sugar[28,29,30]. The purpose for this combination is that the Lewis acid can polarize the bond between silicon and the cyanide carbon sufficiently to virtually allow for cyanide to act as a nucleophile. At the same time, this Lewis acid acts on the pentaacetate to generate the carbocation at the anomeric carbon, which serves as an electrophile. Furthermore, if **13** forms, it will rearrange on presence of boron trifluoride.





The reaction mixture that resulted from this was not however ideal, as the product was found to run very closely with other impurities on chromatography. Ideally, the mixture should be separated on thick layer chromatography. However, very careful flash chromatography on silica gel afforded a reasonably pure product as a syrup.

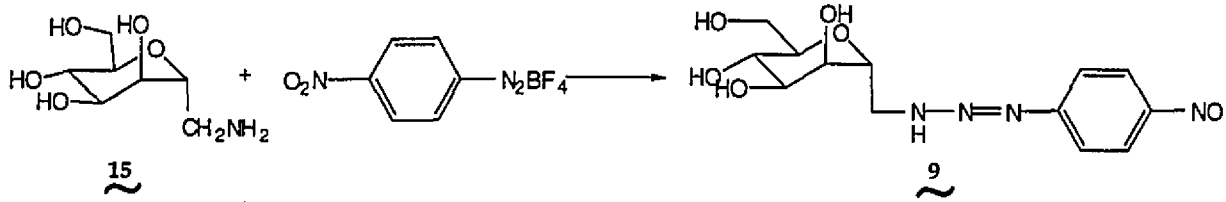
The next step involved the reduction of the cyanide **14** to the amine **15** by lithium aluminum hydride[31].

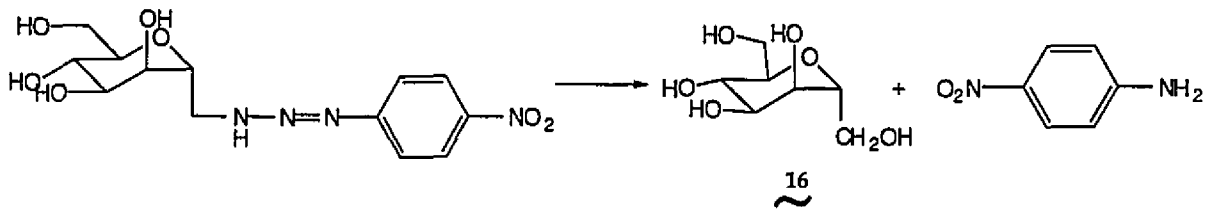


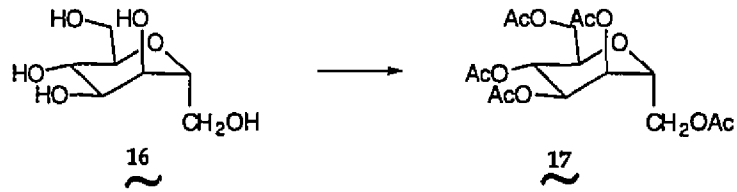
This reaction went very smoothly, but the purification posed a problem, as the product is completely insoluble in water-immiscible organic solvents, and hence cannot be separated from the lithium and aluminum salts by extraction or by standard chromatography. The residue obtained after reaction, and containing both the product and the salts, can be extracted with hot absolute ethanol to remove most salts but, some were always present. Typically, such amines are purified by running them through a cation exchange resin. However, the NMR spectrum, in our case, indicated no presence of other organics, other than this product. Since the next step was to be carried in polar solvents, and since purification of the triazene **9** had never been achieved it seemed that the use of an extremely pure amine was not necessary.

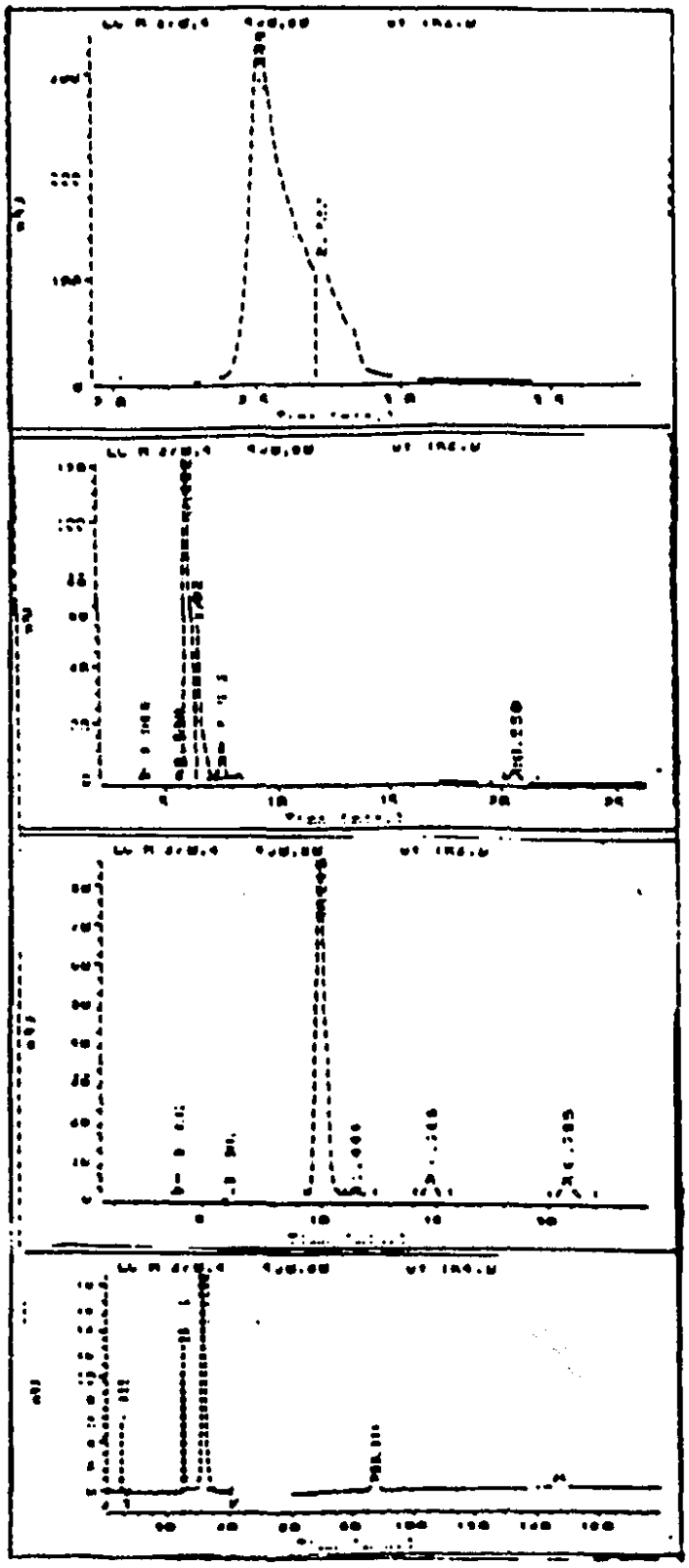
Dissolving the unpurified amine with water and attempting the formation of the triazene was, however, unsuccessful, as the NMR spectra clearly showed the amine not to be the only carbohydrate present after the reaction. Preliminary attempts were made with sodium bicarbonate as a base, and it was thought that the reaction mixture was too alkaline for the diazonium to survive. It was therefore thought that perhaps using two equivalents of the amine, one as a reactant and one as a base, as had been done previously, would be a solution. This second attempt gave the same kind of results. Changing the solvent to dimethylformamide[17], and precipitating the hoped-for product with ether, or methanol did not show more success. It appeared that the mixture after reduction was perhaps too alkaline. Indeed the pH of a solution of the residue in water was close to 13. The pH of this solution was therefore brought to 9 by the addition of hydrochloric acid, and the residue was evaporated. Formation of the triazene was then attempted, and the NMR spectra showed that a certain number of products were formed. As the GalMNT was successfully purified and obtained as a crystalline solid[32], it was thought that, similarly, the ManMNT should be easily obtained. Attempts to purify the product by using silica gel chromatography, using different methanol : diethyl ether eluents and 10% triethylamine to deactivate the column were unsuccessful, as no

carbohydrates were recovered from this. Using florisil as the adsorbent, with isopropanol and ethanol as eluents, purification was also unsuccessful, although some carbohydrates could be seen in NMR spectra. Studying the product mixture on analytical HPLC (HP 1090 series II liquid chromatogram with a Regis ODS II 5 micron spherisorb S50DSII 25cm x 4.6mm high-chrom reversible HPLC column), using a UV detector set at 370nm, resulted in very closely positioned peaks, with varying ratios of methanol : water as eluents (figure 5). Hence, it became apparent that one would not be able to separate the product mixture, as was done with GalMNT (7). It was therefore decided that the formation of the triazene would be assessed by degrading it in acid. This reaction allowed one to prepare the alcohol (16) corresponding to ManMNT. This alcohol was isolated as its peracetylated derivative (17), which hence clearly showed that the triazene was formed, as the only way this alcohol can be produced was if the diazonium was generated.









95 : 5
Methanol : water

70 : 30
Methanol : water

50 : 50
Methanol : water

30 : 70
Methanol : water

Figure 5

V] Experimental procedures

General : Chemicals were purchased from Aldrich chemical company and were reagent grade. Anhydrous THF was obtained by refluxing with sodium until the addition of benzophenone gives a black color, and distillation.

Silica gel chromatography was performed on silica gel 60 (230-400 mesh) and analytical thin layer chromatography was performed on silica gel 60 F plastic back plates and visualised by dipping in a solution of ammonium molybdate (2.5 g) and ceric sulphate (1 g), in concentrated sulphuric acid (10 ml in 90 ml water), and heating with a heat gun.

N.M.R. spectra were recorded on a Varian Gemini and a Varian Unity 500 spectrometers, at 200 and 500 MHz for the ^{1}H N.M.R. and 60 and 125 MHz for the ^{13}C N.M.R. using indicated solvent residues as references. Peaks are reported as singlets (s), doublets (d), triplets (t), quartets (q), double doublets (dd), double triplets (dt), and multiplets (m). IR spectra were recorded on an Analect AQS-18 FTIR spectrometer. Optical rotations were measured on a JASCO DIP-140 digital polarimeter. Mass spectra were recorded on a Kratos MS25RFA mass spectrometer.

Tetra-O-acetyl-α-D-mannopyranosyl cyanide (**14**)

To 5.32 g (13.6 mmoles) of syrupy mannose pentacetate (as prepared before) in 110 ml of nitromethane distilled from calcium hydride was added 5.4 ml of trimethylsilyl cyanide (40.5 mmoles) and 200 µl of boron trifluoride etherate. The mixture was stirred at room temperature for 48 hours and gradually turned dark. Then another portion of 200 µl of boron trifluoride etherate is added and the solution kept stirred for another 24 hours after which a portion of 1 ml of trimethylsilyl cyanide and 200 µl of boron trifluoride was added. The mixture was again stirred overnight and the solvent and excess trimethyl cyanide were evaporated in vacuo. The resulting

syrup was dissolved in chloroform and then washed twice with saturated sodium hydrogen carbonate and twice with water and dried over anhydrous sodium sulfate before the solvent was evaporated on a rotary evaporator and then on a vacuum pump overnight. The resulting residue (4.52 g) is purified by flash chromatography on silica gel with 2:1 ethyl acetate-hexanes (with 1% triethylamine) as an eluent. This gave 2.32 g of tetra-O-acetyl mannopyranosyl cyanide as a slightly colored syrup (50% yield).

$[\alpha]_D^{25}=18.6^o \pm 0.8$ (c=1.83, chloroform). I.R. ($CHCl_3$, cm^{-1}) : 890, 962, 981, 1016, 1057, 1084, 1127, 1191, 1243, 1372, 1754, 2362. ^1H N.M.R. (500 MHz, $CDCl_3$, ppm) : 5.4 (dd, 1H, *J* =2.0, 3.0 Hz, *H -2*), 5.33 (triple d, 1H, *J* =1.0, 3.0, 9.6 Hz, *H -3*), 5.27 (t, 1H, *J* = 9.6 Hz, *H* -4), 4.87 (d, 1H, *J* =2.0 Hz, *H -1*), 4.30 (dd, 1H, *J* =5.5, 12.3 Hz, *H -6*), 4.13 (dd, 1H, *J* = 2.3, 12.3 Hz, *H -6'*), 4.05 (triple d, 1H, *J* =2.3, 5.5, 9.6 Hz, *H -5*), 2.14, 2.08, 2.05 and 2.02 (s, 3H each, *acetate protons*). ^{13}C-N.M.R. (125 MHz, $CDCl_3$, ppm) : 170.45, 169.61, 169.51 and 169.46 (*acetate carbonyls*), 113.39 (*cyanide group*), 74.22 (*C - 5*), 68.85 (*C - 3*), 68.64 (*C - 2*), 65.57 (*C - 1*), 64.99 (*C - 4*), 61.65 (*C - 6*), 20.64, 20.60, 20.51 and 20.48 (*acetate methyls*). Mass spectrum (C.I. with NH_3): 375 (100, $M+NH_4^+$), 331 (14.7), 315 (6.7), 298 (26.4), 135 (6.8).

1-Aminomethyl-α-D-mannopyranose (**15**)

To a suspension of 200 mg of Lithium aluminium hydride in 2.5 ml of THF was added dropwise a solution of 186 mg of tetra-O-acetyl mannopyranosyl cyanide in 11 ml of THF. The solution was brought to reflux for 1 hour. Then it was cooled to room temperature and absolute ethanol was added slowly to quench the remaining hydride. This was followed by the addition of a little celite (previously washed with ethanol, water and 5 N ammonium hydroxide), water and 5 N ammonium hydroxide. The mixture was filtered through celite and the filtrate was evaporated in vacuo to give the product together with lithium and aluminum salts. This residue was extracted with 6 ml of boiling absolute ethanol and the boiling ethanol is filtered to remove any solids. This was repeated six times. The clear ethanolic

solution was then evaporated to give a residue which was used directly in the next step.

^{1}H N.M.R. (500 MHz, D_2O, ppm): 3.6 to 3.8 (m, 2H, *H-1 and H-6*), 3.5 to 3.6 (m, 2H, *H-2 and H-6'*), 3.4 to 3.5 (m, 2H, *H-3 and H-4*), 3.35 (m, 1H, *H-5*), 2.79 (dd, 1H, *J* = 10.8 and 12.4 Hz, *methenyl proton*), 2.50 (dd, 1H, *J* = 2.8 and 12.4 Hz, *methenyl proton*) ^{13}C-N.M.R. (60 MHz, D_2O, referenced on methanol at 49 ppm, ppm): 78.94, 74.29, 71.02, 69.56, 67.55, 61.33, 38.72 .

α-D-Mannopyranosylmethylamine p-nitrophenyl triazene (**9**)

The previous residue was taken up in 20 ml of water and the pH was brought to 9 by the addition of concentrated (70%) hydrochloric acid and, if needed, 2 M sodium hydoxide. The aqueous solution turned cloudy. It was evaporated in vacuo. 88 mg of sodium bicarbonate was added and the mixture is dissolved in 3.5 ml of water. The solution was cooled to 0°C and 120 mg of p-nitrobenzenediazonium tetrafluoroborate.was added. The solution was stirred for half an hour at 0°C. 8.5 ml of cold water was added and the mixture was filtered. The filtrate was washed with 12 ml of cold water-saturated ether, and this process was done three times. The aqueous layer was then extracted with 4.5 ml of cold saturated n-butanol. This process was done three times, and the combined butanol extracts were evaporated without external source of heat on a high-vacuum pump. This yielded a residue in which the triazene content was estimated by the absorbance at 362nm (ε=22000)[24,25,26].

(2,3,4,6-O-Acetyl-α-D-mannopyranosyl)-methyl Acetate (**17**)

The previously obtained residue was stirred in 6 ml acetic acid for three hours, then the solvent was evaporated, and the resulting mixture was taken up in 6 ml pyridine and 4 ml acetic anhydride, and stirred at room temperature for three days. The solvents were then evaporated and the mixture was run carefully through a silica gel chromatographic column to isolate the residue with R_f=.0.46 (1:1 ethyl acetate : hexanes). Starting with 105 mg (0.29 mmoles) of

tetra-O-acetyl-α-D-mannopyranosyl cyanide, one was able to isolate 16 mg (0.039 mmoles) of the reasonnably pure product (13 % yield). $[\alpha]_D^{25}=9.4^o \pm 1.8$ (c=0.28, chloroform). I.R. ($CHCl_3$, cm^{-1}): 1121, 1236, 1371, 1560, 1602, 1652, 1701, 1747, 2337, 2361. ^1H N.M.R. (500 MHz, $CDCl_3$, ppm) : 5.27 (s, 1H, *H -2*), 5.04 (s, 1H, *H -3*), 4.59 (t, 1H, *J* = 9.8 Hz, *H -5*), 4.19 (d, 1H, *J* = 4.9 Hz, *H -1*), 4.08 (d, 2H, *J* = 12.5 Hz, *H -6* and *H- 6'*), 4.03 (s, 1H, *H -4*), 3.66 (s, 2H, *methenyl protons*), 2.09 (s, 6H, *acetate methyls*), 2.07 (s, 9H, *acetate methyls*). ^{13}C - N.M.R. (150 MHz, $CDCl_3$, ppm): 169.55, 168.51, 168.45, 168.32 (*acetate carbonyls*), 72.90 (*C-4*), 71.38 (*C-1*), 67.92 (*C-3*), 67.65 (*C-2*), 61.42 (*C-5*), 61.21 (*C-6*), 42.71 (*methenyl carbon*), 21.45 (*acetate methyls*), 21.35 (*acetate methyls*). Mass spectrum (C.I. with NH_3): 345 (100, M - acetate), 331 (12.1), 285 (24.2), 225 (58.9), 211 (54.6), 183 (75.3), 169 (63.5), 145 (41.5), 123 (62.7)

VI] References

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