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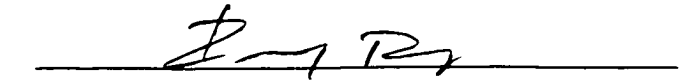

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Application of olefin metathesis reaction towards the synthesis of oligosaccharide mimics : A novel strategy in chemical glycobiology

Romyr Dominique

Thesis submitted to the School of Graduate Studies and Research
in partial fulfillment for the degree of Doctor of Philosophy
in Organic Chemistry

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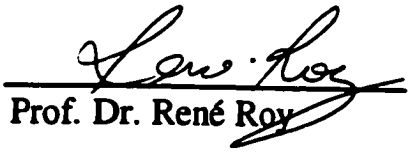

Prof. Dr. René Roy

ABSTRACT

Glycobiology has gained a lot of interest in the scientific community in the past few years. It is now well established that the oligosaccharides on cell surfaces are involved in key recognition processes. However, the determination of oligosaccharide sequence on a specific glycoconjugate and their chemical synthesis are still far from routine. Herein, we report a new strategy in chemical glycobiology making use of olefin metathesis reaction as a powerful chemical tool towards the synthesis of oligosaccharide mimics. This strategy allows the synthesis of multivalent molecules that could help to understand and control events mediated by oligosaccharides. A glycomimetic approach to the branched trimannoside 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside will also be described.

The synthesis of carbohydrate homodimers was accomplished by self metathesis of *O*- and *C*- alkenyl glycosides using 10 mol% of Grubbs' catalyst bis (tricyclohexylphosphine) benzylidene ruthenium (IV) dichloride, $[(PCy_3)_2RuCl_2{=}CHPh]$. The reaction is high yielding, compatible with different protecting groups and the length of the linker can be easily changed. A mixture of stereoisomers was obtained in which the *E* isomer was major. Once deprotected, these homodimers can be viewed as chemical inducers of dimerization therefore the hypothesis that they can induce signal transduction was suggested.

Extension of *O*- and *C*- alkenyl glycosides was also achieved by cross-metathesis reaction using Grubbs' catalyst. Extended alkenyl glycosides with reactive functional groups were obtained in high yield and good stereoselectivity. The utilization of 2-4 equiv. of alkenes and 20 mol % of catalyst were sufficient to favor the formation of the cross-metathesis products. A novel C-linked (1-6)-pseudodisaccharide could be synthesized using this procedure.

A sequence of olefin metathesis and Sharpless asymmetric dihydroxylation reactions were used to create a novel class of glycoclusters having varied structural and stereochemical environments. Well-defined tri- and tetra-valent mannoside-based glycoclusters were thus prepared.

Another application of olefin metathesis reaction was found in the synthesis of a trimannoside ligand toward human Mannose-binding protein which plays an important

role in the innate immunity. To further understand its role, the design of high affinity ligands was attempted. Preliminary biological results showed improved inhibitory potentials.

Finally, two generation of glycomimetics of 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside, the natural ligand recognized by legume lectin, concanavalin A were synthesized. Thus, highly substituted C-aryl mannoside, as well as O, N, C-linked 1,2,3-triazole mannosides were prepared.

ACKNOWLEDGMENTS

It is good to have an end to this journey. In the five years that resulted in obtaining my Ph. D. in organic chemistry, I can say that I enjoyed this adventure full of ups and downs. Periods where all reactions are working "like a charm" as Mary would have said, or periods where nothing is working, no reaction or decomposition of starting material. The most important is to always try to stay in the good periods, thus you'll have an enjoyable adventure.

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I want to thank also Philippe and Bertrand Ferrus, Verdi Boyer for their friendship. I will remember those places like Platinum Palace, Atomic, China White and Suite 400. Barbara Flores for her love, patience and understanding during the last year. Thank you so much.

Finally, I must thank God for looking after me during these five years. Granting me with the strength and discipline required for accomplishing my thesis.

"For my parents Jerome and Myrtha Dominique"

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Ac	acetate
Ac_2O	acetic anhydride
AcOH	acetic acid
Ar	aryl
b	broad
Bn	benzyl
Boc	tert-butoxycarbonyl
bs	broad singlet
tBu	tert-butyl
cat	catalytic
CBz	carbobenzyloxy
CH_2Cl_2	dichloromethane
CI	chemical ionization
CSA	camphor sulphonic acid
COSY	shift correlation spectroscopy
d	doublet
DABCO	1,4-diazabicyclo[2.2.2]octane
dd	doublet of doublet
ddd	doublet of doublet of doublet
DEPT	distortionless enhanced polarization *trans*fer
DIPEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	N,N-dimethylformamide
DMS	dimethylsufide
equiv	equivalent(s)
ES	electrospray
Et_3N	triethylamime
EtOAc	ethyl acetate
EtOH	ethanol
FAB	fast atom bombardment

Gal	galactose
GlcNAc	N-acetylglucosamine
h	hour(s)
HMQC	heteronuclear multiple quantum coherence
Hz	Hertz
HRMS	high resolution mass spectra
IC_{50}	concentration required for 50% inhibition
Lit.	literature
m	multiplet
M	molar
M+	parent molecular ion
Man	mannose
MeOH	methanol
Me	methyl
MHz	megaHertz
min	minute(s)
mmol	millimole(s)
mp	melting point
MS	mass spectrometry
MW	molecular weight
m/z	mass to charge ratio
NMM	4-methylmorpholine
NMR	nuclear magnetic resonance
Ph	phenyl
pTSA	para-toluene sulphonic acid
ppm	parts per million
rt	room temperature
s	singlet
Tr	trityl
THF	tetrahydrofuran
TLC	thin layer chromatography
TMSOTf	trimethylsilylmethyl trifluoromethanesulfonate

CHAPTER 1: INTRODUCTION

1.1. Glycobiology

Glycobiology, a rapid emerging area of biochemistry, has become a popular topic in recent years. First, introduced in 1988 by Raymond Dwek, an Oxford University biochemist, the term glycobiology refers to the science studying the biological roles of oligosaccharides which exist mainly in covalent association with proteins or lipids (glycoproteins and glycolipids also called glycoconjugates).[1]

Now that the human genome has been decoded, glycobiology will gain even more interest. Proteomic is the next logical step beyond genomics, however the final products of the genome are by far not simple proteins but modified ones. Their most common and versatile modification is the attachment of carbohydrates, which results in the formation of glycoproteins with precise biological activities (see Figure 1.1.1). The systematic identification and characterization of all the carbohydrate chains of glycoproteins, with the aid of the information derived from the genome, will be a major part of "glycomics".[2]

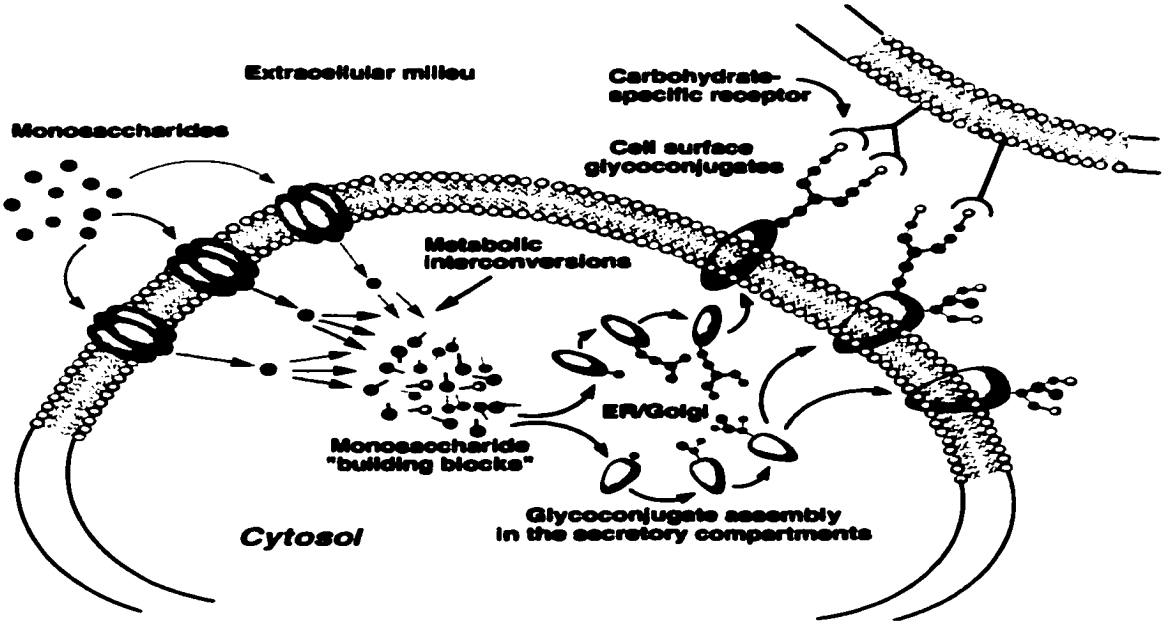

Figure 1.1.1. Glycoproteins biosynthesis.[3]

The information encoded by oligosaccharides branched with proteins and lipids are by far larger than the one found in peptides and oligonucleotides. In contrast to the nucleotides and the amino acids, which can interconnect in only one way like boxcars in a train, the monosaccharide units in oligosaccharides can attach to one another at multiple points and with different anomeric configurations of the glycosidic linkages, giving them a multitude of three-dimensional shapes. Therefore, two identical monosaccharides can join to form 11 different disaccharides, whereas two molecules of single amino acids or nucleotides can form only one dipeptide or one dinucleotide, respectively. More impressively, four different monosaccharides can form up to 35560 distinct tetrasaccharides compared to only 24 tetrameric structures created with the same number of amino acids or nucleotides.[4] Moreover, further structural diversification may occur by covalent attachment of sulfate, phosphate, and acetyl groups. Thus, oligosaccharides are superb effective carriers of information considering their enormous potential for structural diversity. It was unlikely that Nature would ignore this resource, and it is now quite well established that she did not.

1.2. Biological roles of oligosaccharides

Until the late 1960s, carbohydrates were thought to serve only as energy sources, as structural materials, and considered unimportant as carrier of genetic informations. In the 1970s it became well established that almost all cells carry carbohydrates on their surfaces in the form of glycoproteins, glycolipids, and polysaccharides. Nearly all cells are coated with a material consisting of carbohydrate-rich molecules called the glycocalix. (see Figure 1.2.1).

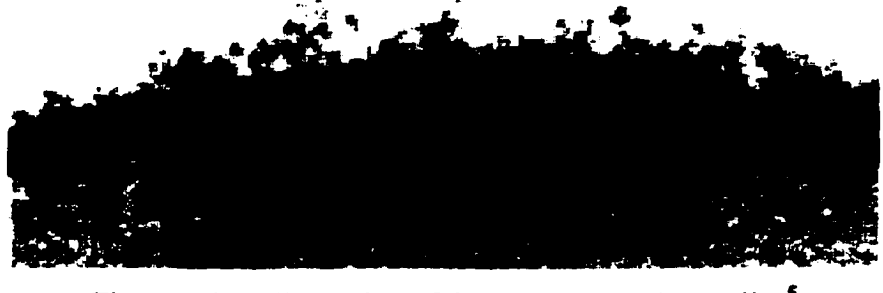

Figure 1.2.1. Illustration of the erythrocyte glycocalix.[5]

Only during the last two decades, the idea that carbohydrates were involved in cellular recognition was accepted by the scientific community. It is now known that carbohydrates are key elements in various molecular recognition processes.[6] The biological roles of oligosaccharides appear to span the spectrum from those that are trivial, to those that are crucial for the development, growth, communication, function or survival of an organism.

1.2.1. Structural, protective and stabilizing roles of oligosaccharides

Some oligosaccharides, such as those of the proteoglycans and the collagens, are important in the physical maintenance of tissue structure, integrity and porosity.[7] The oligosaccharides decorating proteins on the cell surface can also serve to protect the polypeptide chain from recognition by proteases or antibodies. For example, the fibronectins showed an increased susceptibility to proteases after alteration of their oligosaccharide chains.[8] Another well-accepted function of the oligosaccharide units of glycoproteins is that they are involved in the initiation of correct polypeptide folding in the rough endoplasmic reticulum (ER), and in the subsequent maintenance of protein solubility and conformation. Thus, many proteins that are incorrectly glycosylated fail to fold properly and fail to exit the ER, and are consequently degraded.[9-11]

1.2.2. Oligosaccharides as specific receptors for viruses, bacteria and toxins

The infection of cells by pathogens such as bacteria, viruses and toxins depends also on carbohydrate recognition. Certain oligosaccharides act as highly specific receptors for a variety of pathogens. For example, the initial steps in HIV (human immunodeficiency virus) infection include binding of the HIV surface envelope glycoprotein gp120 to CD4.[12-13] The assembly of this complex ultimately facilitates virus-cell or cell-cell fusion. It is the same story for the influenza viruses, where the virus binds to erythrocytes and other cells by recognizing *N*-acetylneuraminic acid, one of the sialic acids, present on the cell surface to initiate an infection[14]. There are many other examples in which the oligosaccharides on the cell surface are behaving in this "traitorous" manner, allowing pathogens to start an infection. [15]

1.2.3. The "tuning" function of oligosaccharides

There are several examples in which glycosylation can substantially modulate the interaction of peptides with their cognate ligands or receptors. Some cell surface receptors for growth factors appear to acquire their binding functions in a glycosylation-dependant manner. Thus, the acquisition of function of a newly synthesized receptor may be delayed until it is well on its way to the cell surface. This might limit unwanted early interactions with a growth factor synthesized in the same cell. An example of this function is found with the human hormone β chorionic gonadotrophin (β-HCG), in which a deglycosylation causes a failure to transmit a signal via stimulation of adenylate cyclase.[16]

1.2.4. Intracellular and intercellular trafficking functions of oligosaccharides

The best understood example of intracellular trafficking is the role of mannose-6-phosphate residues in targeting newly synthesized lysosomal enzymes from the Golgi apparatus to their final destination, the lysosomes.[17,18] The human disease state in which phosphorylation is deficient (I-cell disease and pseudo-Hurler polydystrophy) is characterized by a failure of lysosomal enzyme targeting in several cell types.[19] This provides conclusive evidence for the importance of the oligosaccharide modification in mediating this important pathway.

Recognition of oligosaccharides sequences by certain receptors can result in removal of the glycoconjugate or even the whole cell from the circulation.[20] There have been several examples of the action of such intercellular trafficking receptors. In mammalian plasma proteins, exposed terminal β-Gal residues are recognized by the asialoglycoprotein receptor causing the clearance from circulation.[21] The cancer cells leave the circulation and establish new tumors by metastasis, a process regulated by a sugar binding protein known as galectin.[22]

1.2.5. Hormonal actions of oligosaccharides

It is now recognized that free oligosaccharides can themselves have biological effects, thus acting like hormones. For example, free heparin fragments released by certain types of cells have effects on other cell types, like inhibition of cell growth, acting perhaps via binding and internalization.[23]

1.2.6. Role of oligosaccharides in cell-cell recognition

Since all cells are covered with a dense coating of sugars, it has been predicted that oligosaccharides must be critical determinants of cell-cell interactions. This is the case during the inflammation process, where the selectins, a group of carbohydrate-binding proteins, residing in cell membranes mediate the initial recognition of immunologically important events. Such as lymphocyte routing and neutrophil and monocyte recruitments to the injury site.[24-28] There is also substantial evidence that fertilization is mediated by carbohydrate recognition. The binding of sperm to eggs is regulated by carbohydrate moieties of the zona pellucida (ZP) and carbohydrate binding proteins of the sperm surface.[29]

1.2.7. Role of oligosaccharides in the immune system

Almost all of the key molecules involved in antigen recognition and the orchestration of the subsequent events are glycoproteins. The humoral immunity, which is most effective against bacterial infections and the extracellular phases of viral infections, is mediated by an enormously diverse collection of related glycoproteins known as antibodies or immunoglobulins.[30] On the other hand, tumor cells are using sugar groups on their surface to slip past the immune system. Thus, transformed tumor cells hide from normal immune surveillance by displaying glycoproteins and glycolipids therefore continuing their growth.[31]

In fact, carbohydrates are performing an astonishing range of jobs that are essential to many processes involved in important biological phenomenon. The ability to control

these events with selective molecule inhibitors offers enormous potential for the study of biology. It also provides a better understanding of molecular mechanisms underlying cell recognition and great promise for the development of carbohydrate-based therapeutics. So, how could we control these events ?

1.3. Chemical glycobiology

Chemical glycobiology, which is using chemical tools to understand the myriad biological functions of oligosaccharides, represent an attractive strategy to control those events mainly mediated by carbohydrate-protein interactions. Carbohydrate-binding proteins, excluding enzymes and antibodies are known as lectins which are widely distributed in nature, including plants, animals and microorganisms. Binding mono- and oligosaccharides reversibly and with high affinity. The lectins have been tremendously useful as tools for the study of carbohydrate-protein interaction, contributing great insight into the binding specificity of those interactions.[32] Unfortunately, the interactions of individual saccharide groups with lectins are of low affinity and the dissociation constants (K_D) are in the milli- to micro-molar range.[33] This is largely due to the shallow, solvent-exposed nature of the lectin binding sites, which makes few direct ligand contacts. To overcome these weak binding interactions, many research groups followed Nature's approach to form binding with high affinity by multivalent interactions. Many reviews currently exists on this subject.[34]

1.3.1. Multivalent interactions and the glycoside cluster effect

Nature effects tight binding by multivalency which can be achieved by the oligosaccharides covering the cell surface.[35] The oligosaccharides serve as multivalent ligands for specific receptors as the lectins, giving rise to high binding affinity and specificity. In order to mimic, the arrangement of the oligosaccharides present on the cell surface, several families of multivalent neoglycoconjugates have been synthesized since the determination of oligosaccharide sequence on a specific glycoconjugate and their

chemical synthesis are still far from routine. Thus, chemists are in a position to supply enough materials for biological studies.

The first synthetic oligovalent glycoligands or glycoclusters which showed enhanced affinities for their receptors were introduced by Lee (Figure 1.3.1). Using tris(hydroxymethyl)aminomethane as scaffold to create a molecule bearing three galactose residues, Lee first observed a binding enhancement caused by multivalency, a phenomenon that he called "glycoside cluster effect".[36-37]

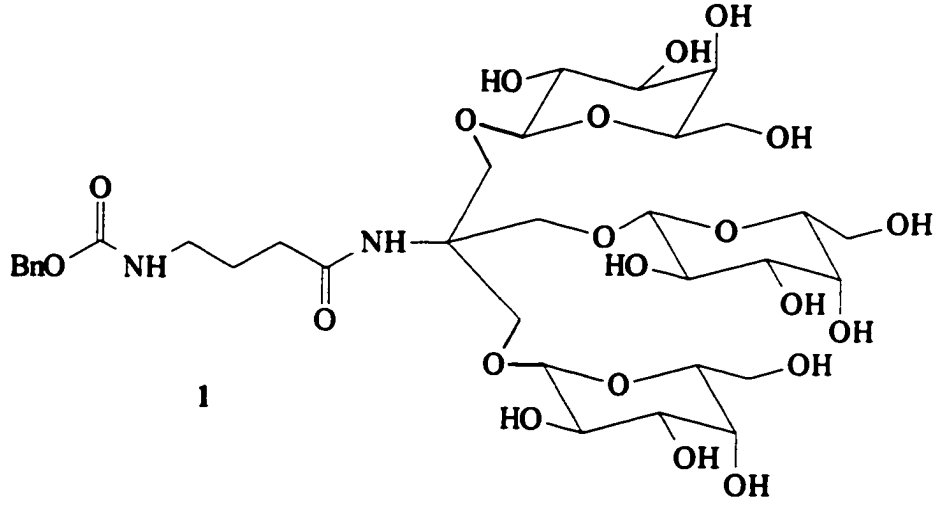

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Figure 1.3.1. The first synthetic glycocluster.

Since then, a large variety of multivalent neoglycoconjugates have been designed and prepared to understand cell-surface interactions. Glycoclusters having scaffolds giving access to tri-, tetra-, penta- and hexa-meric structures have been synthesized revealing the creativity involved in the design of neoglycoconjugates. Core molecules such as tris(2-aminoethyl) amine **5**,[38] benzene ring **2**,[39] pentaerythrytol derivatives **3**,[40] macrocycle,[41] cyclodextrin,[42] calixarene,[43] and also modified glucose derivatives[44] (Figure 1.3.2.) have been successfully functionalized with carbohydrates showing an increased binding

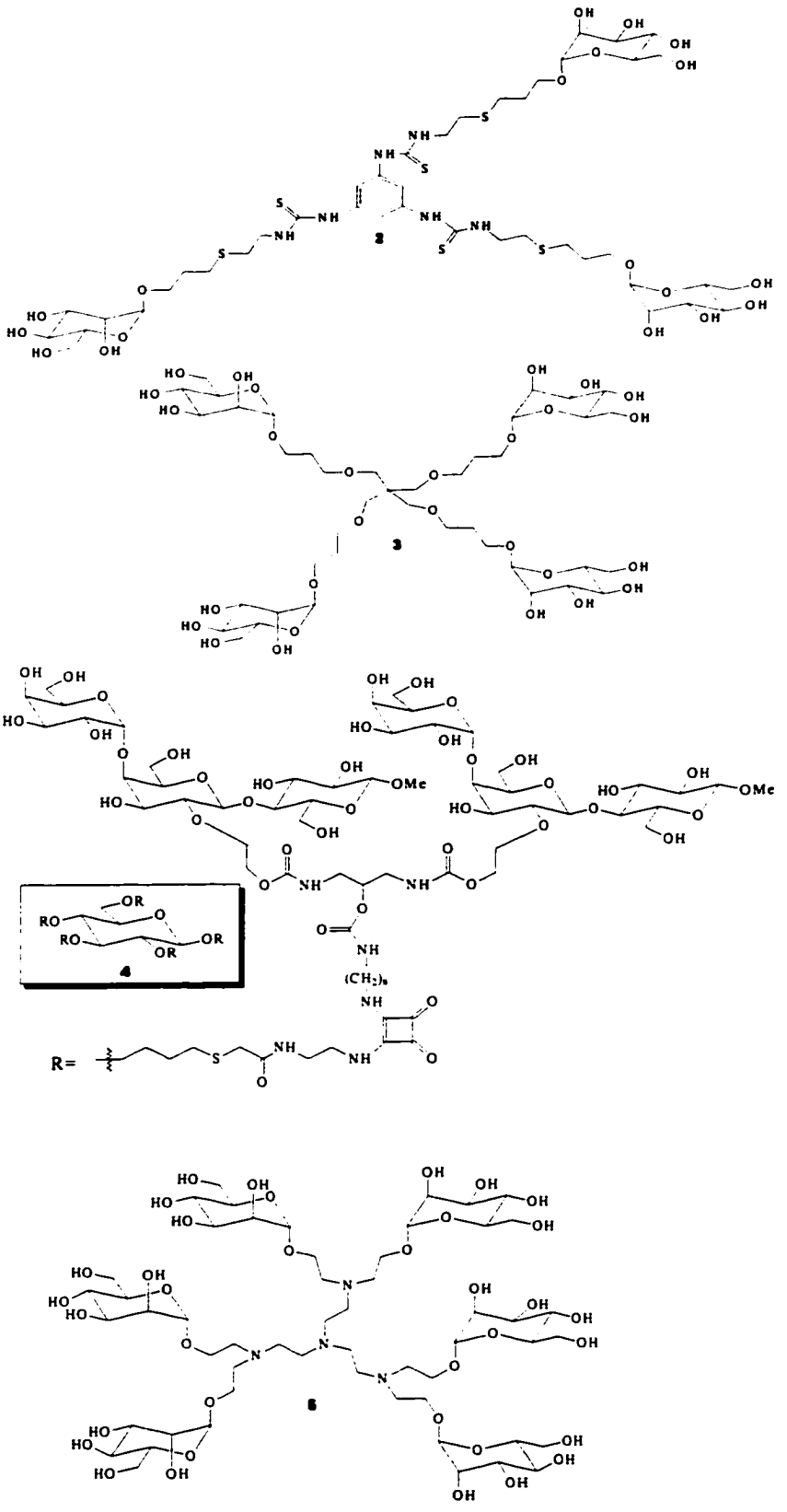
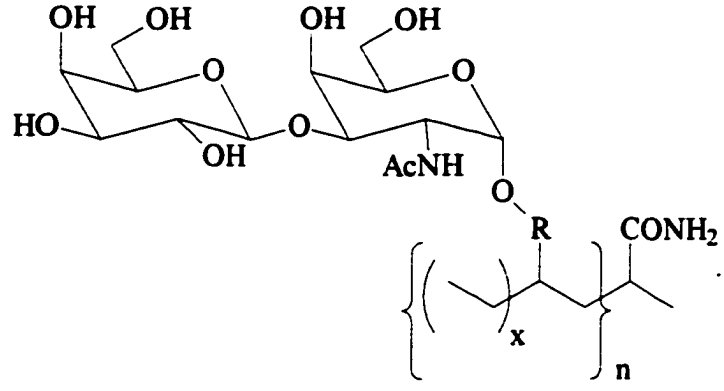


Figure 1.3.2. Examples of glycoclusters.

affinity in the nanomolar range in some cases. As one example, multivalent carbohydrate ligand **4** (named STARFISH) synthesized by Bundle *et al.* showed subnanomolar inhibitory activity against Shiga-like toxins.[45]

To allow the synthesis of larger multivalent carbohydrate ligands, many different backbones have been utilized, including liposomes, polymers, and telomers.[46] Recently, chemistry professor Wong and coworkers at Scripps Research institute created a sialic acid liposome and a lysoganglioside poly-l-glutamic acid conjugate as multivalent picomolar inhibitors of the flu virus hemagglutinin.[47] Roy and coworkers utilized the Thomsen-Friedenreich carbohydrate antigen (T-Ag), a cancer-related marker, to synthesize T-Ag-containing glycopolymers.[48] In related pharmaceutical applications, these glycopolymers have been employed in high-throughput screening toward the development of a solid-phase glycosyltransferase assay for drug discovery research.[49]



6 R = CH_2
7 R = $(CH_2)_3SCH_2CH_2NHC(O)$

Figure 1.3.3. T-Ag-containing glycopolymers.

In addition, T-Ag glycodendrimers were also prepared and their binding properties against a mouse monoclonal IgG antibody were evaluated.[50] Glycodendrimers represent another class of multivalent neoglycoconjugates. They are chemically well defined, tree-like molecules with covalently attached carbohydrate residues. They represent the improved version of glycoclusters and glycopolymers since their valencies and size can be easily adjusted. The first glycodendrimer synthesis in which up to 2, 4, 8 and 16 sialic

acid residues were attached to the periphery of a multi-branched L-lysine core was reported by Roy and coworkers in 1993.[51]

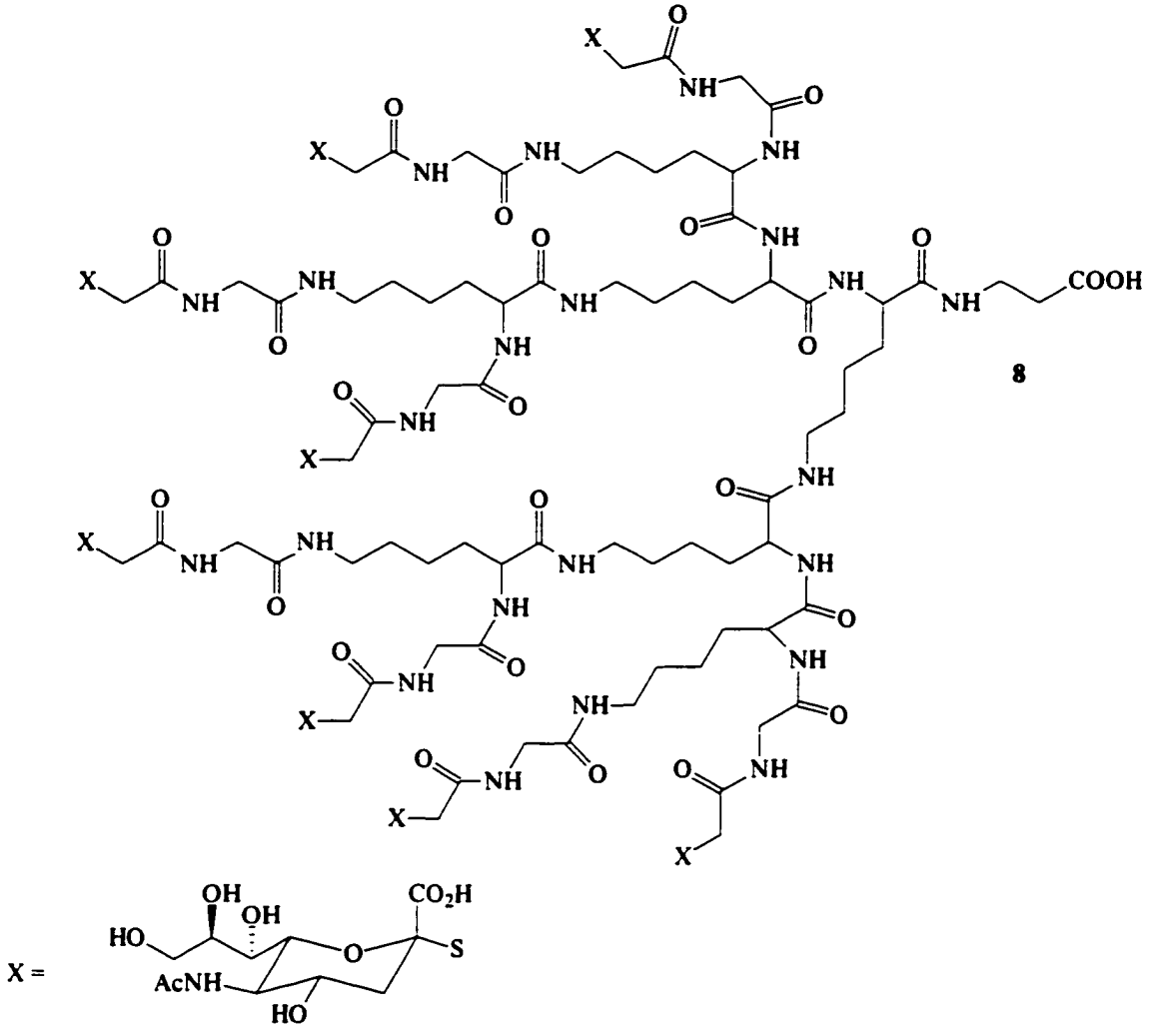


Figure 1.3.4. First glycodendrimer.

In the past few years, many other types of glycodendrimers have appeared in the literature using a variety of scaffold such as polyamidoamine (PAMAM),[52] polypropylene amine **10**,[53] gallic acid **9**,[54] carbosilane,[55] aminobis propylamine,[56] and benzene ring[57] . Very high valent ligands have been constructed in this fashion. Okada *et al.* reported incorporation of up to 1000 GlcNAc residues on a PAMAM dendrimer.[58]

The multivalent neoglycoconjugates have enormously contributed to the development of glycobiology. They are more easily accessible than the natural material which is heteregeneous and often also scarce. Moreover, the strategy used for their synthesis usually allows a lot of variations concerning scaffold symmetries, linker lengths and spacer properties, as well as the systematic variation of their size and sugar valencies. Finally, the addition of functional groups, pharmacophores and biolabels is facilitated and could lead to a better understanding underlying carbohydrate-protein interactions.

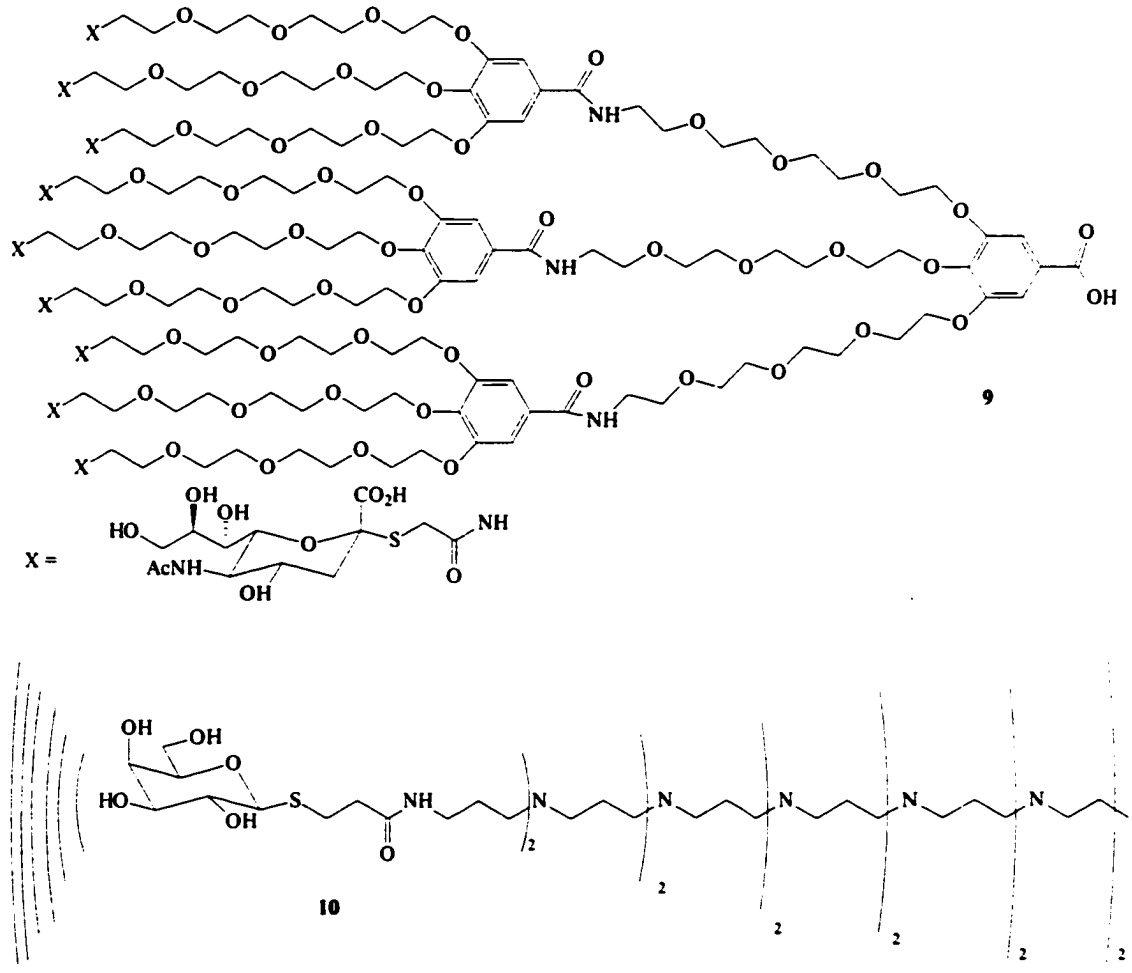


Figure 1.3.5. Examples of glycodendrimers.

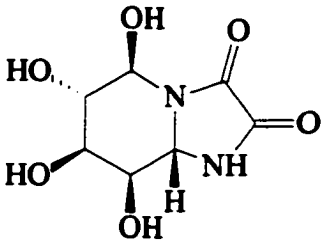
Although, multivalent interactions were gaining most of the attention, a small group of researchers were trying to understand more about monovalent interaction between a carbohydrate derivative and the specific receptor opening the field of glycomimetics.

1.3.2. Glycomimetics

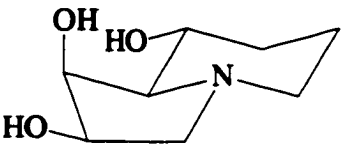
Glycomimetics based molecules represent another option to overcome the low binding affinity of carbohydrates with proteins. Blocking the formation or the function of oligosaccharides present on the cell surface represents a strategy of choice to study the biological roles played by those oligosaccharides. This approach refers to the design of small molecules that contain the essential functional groups to resemble the active conformation of a carbohydrate associated with important signaling and recognition events.[59] Glycomimetics offer a large number of advantages over their parent structures as therapeutics agents. They can be designed in such a way that they are more stable towards endogenous degradative enzymes, possess improved bioavailability, reduced clearance rates and have higher affinity and selectivity for their cognate receptors.

The rationale design of glycomimetics agents is mainly based on the key interactions involved in carbohydrate-protein recognition or the different group found in the active site of the enzyme (in the case of glycosidase) which can be observed by examination of the X-ray structure. Once the key interactions are identified, the removal of unnecessary functional groups and addition of other functional groups can be done to improve the affinity. For example, the incorporation of a hydrophobic group has the potential to substantially increase the binding affinity if there is a complementary hydrophobic site in the receptor. In the same way, a charged group can add favorable interactions if a complementary group exists in the binding site. Another strategy toward the construction of glycomimetics is to abandon the glycosidic scaffold. Instead, a new, non carbohydrate framework is built and the required functional groups are attached so as to have the same orientation in space as they do in the parent structure. For example, the replacement of the endocyclic oxygen atom by a nitrogen or a carbon atom leads to a different class of molecules called azasugars[60] and carbasugars, respectively. Those modifications frequently used nowadays have generated several potent inhibitors. Two general types of inhibitors have been explored: those that block the oligosaccharide biosynthesis and those that interfere with oligosaccharide recognition. Oligosaccharide biosynthesis in eukaryotic organisms require glycosyltransferases to form glycosidic bonds and

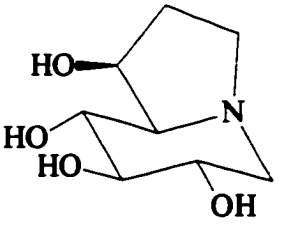
glycosidases to hydrolyze them. Both enzymes can be inhibited by selective inhibitors (Figure 1.3.6).[61]



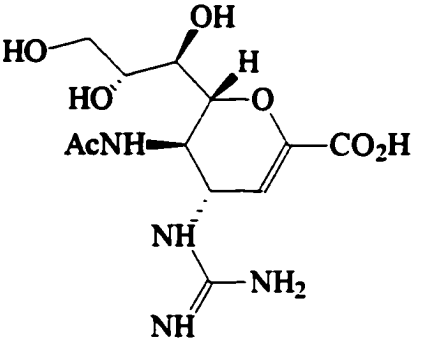
11 Kifunensine
α- Mannosidase I



12 Swainsonine
Lysosomal mannosidase



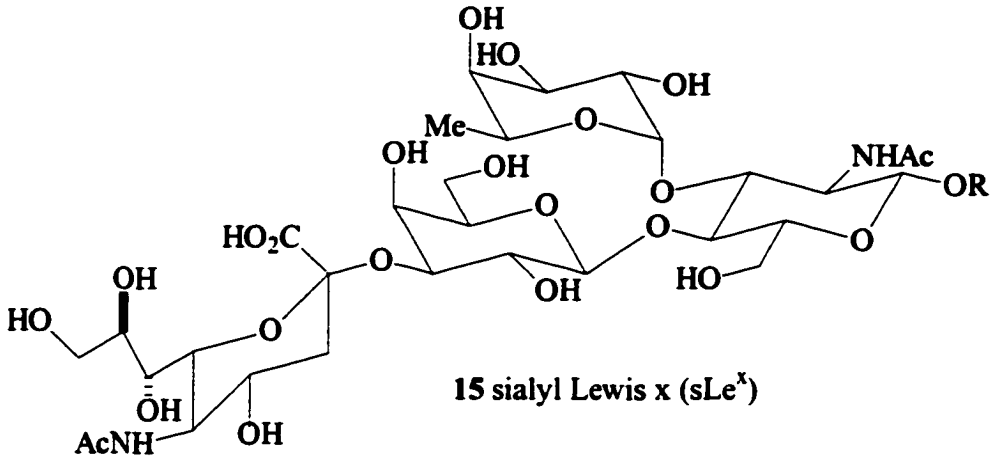
13 Castanospermine
α-Glucosidase II



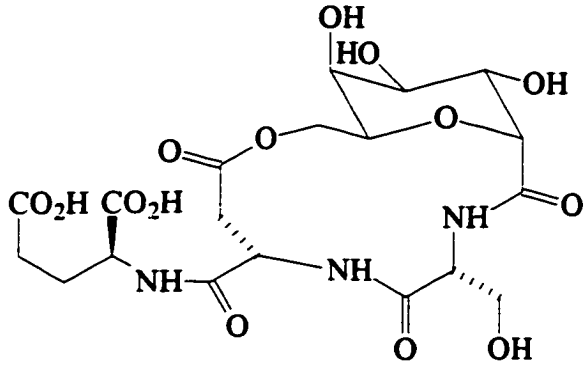
14 GG167 (Glaxo Wellcome)
Influenza A neuraminidase

Figure 1.3.6. Representative inhibitors of glycosidases and glycosyltransferases.

Much work has been done to interfere with oligosaccharide recognition, mainly with sialyl Lewis X **(15)** which is an oligosaccharide recognized by the selectins (type of lectins) involved in the inflammation processes. The syntheses of Sialyl Lewis X mimetics have played a very important role in defining the structure-activity relationship revealing the critical contacts for selectin binding, information that has guided the generation of more potent inhibitors.[62] For example, a macrocyclic Sialyl Lewis X analog **(16)** showed a dramatic enhancement (about 1000) relative to that of sLeX.[63]



15 sialyl Lewis x (sLe^x)



16

Figure 1.3.7. sLe^x structure and sLe^x mimetic.

1.3.3. Metabolic engineering of cell surface oligosaccharides

The most native context in which carbohydrate-protein interactions can be studied, is, of course, on the surface cells. Unfortunately, controlling the cell surface expression of defined oligosaccharide epitopes through genetic manipulations is virtually impossible. Consequently, several groups have adopted chemical approaches to engineer the cell surface oligosaccharides, in such a way that the cell will bear unnatural monosaccharides.[64] One of the most interesting example of this strategy was reported by the group of Bertozzi. It involved the metabolic introduction of orthogonal functional groups like an azide into cell surface oligosaccharides, followed by their covalent

reaction (Staudinger) with appropriately functionalized synthetic carbohydrates.[65] The presentation of an azide on cell surface provides an orthogonal mean of chemically remodeling glycoproteins within their native biological context. (Figure 1.3.8.)[66]

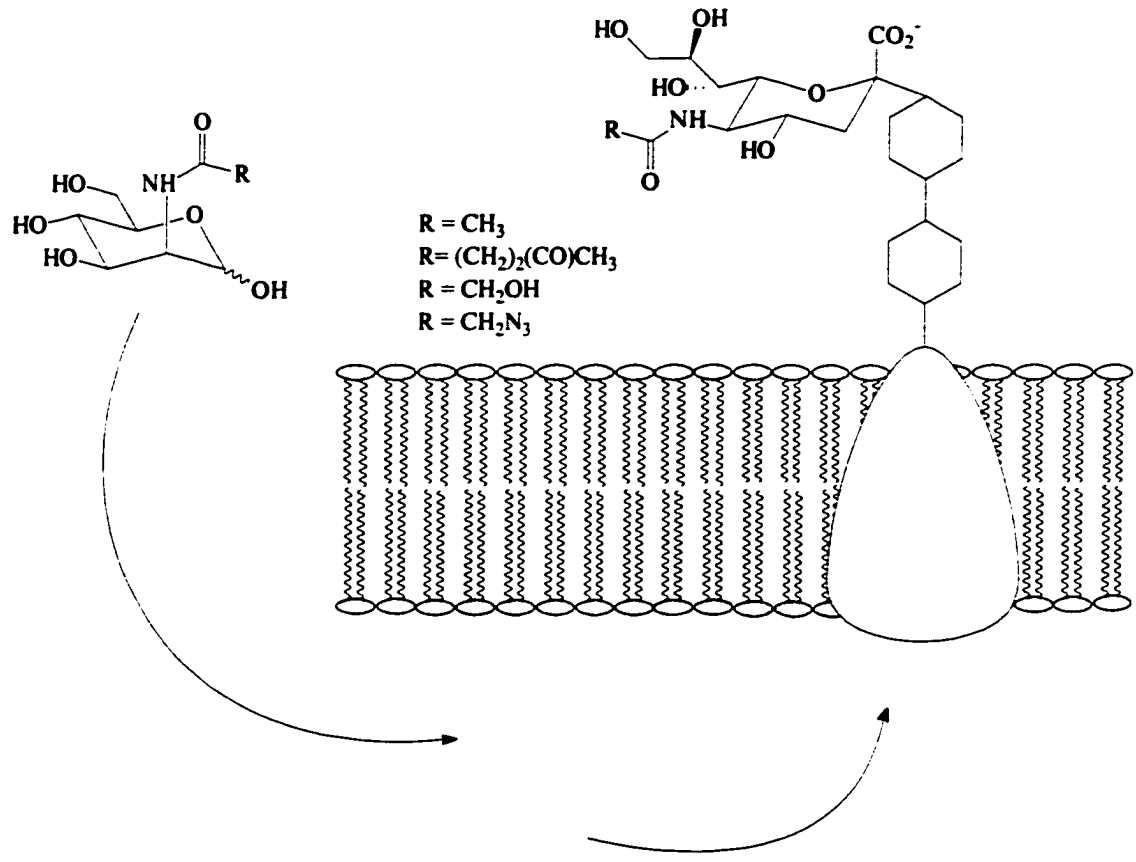


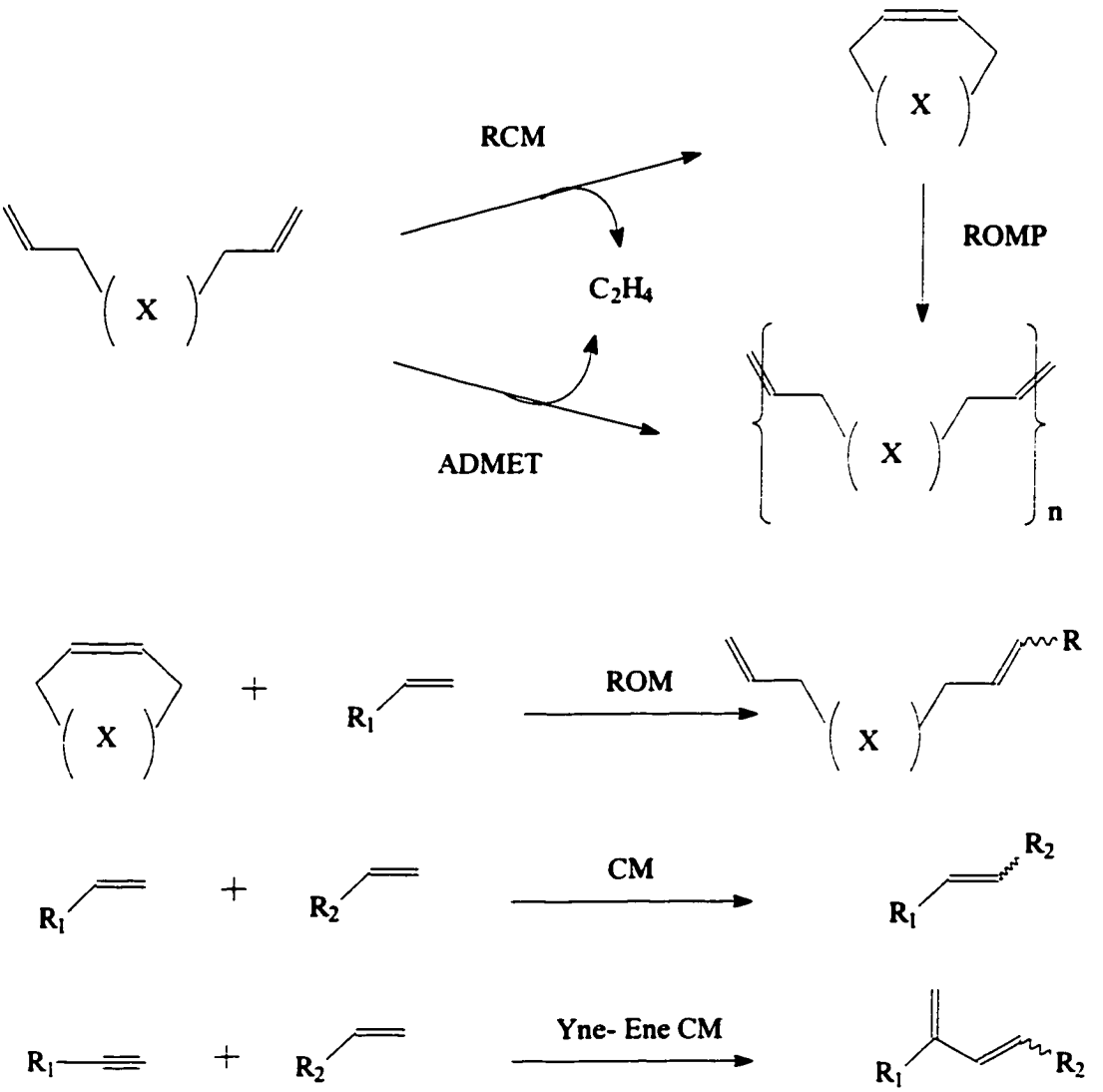
Figure 1.3.8. Biosynthetic engineering of unnatural sialic acids on cell surface glycoconjugates.

Chemical glycobiology have given great insight into the myriad biological functions of oligosaccharides. However, there is still a lot of work to be done for a complete understanding of the way the cell works. In this thesis dissertation, we are using the multivalent approach to understand carbohydrate-protein interactions, mainly with the synthesis of glycoclusters. To do so, we wanted to use the olefin metathesis reaction which is offering many advantages over previous startegies. We have also used the glycomimetic approach in order to determine the essential groups involved in ligand recognition in a model system.

However, before getting farther in the results obtained during our study, a small introduction about the olefin metathesis reaction is necessary to understand the usefulness of this reaction, not only in glycobiology but also in organic chemistry.

1.4. The olefin metathesis reaction

The olefin metathesis reaction is one of the very few fundamentally novel organic transformations that profoundly shaped organic synthesis during the last decade in such a way that olefin metathesis is one of the key tools in every organic chemist's toolbox nowadays.[67]

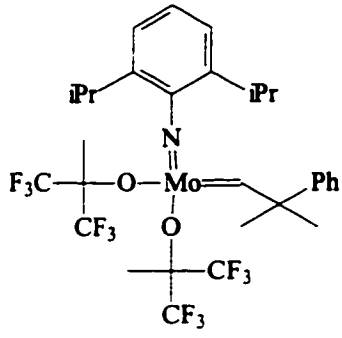


Scheme 1.4.1. Different applications of olefin metathesis reactions.

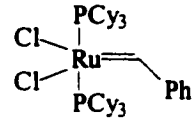
Although, discovered in the mid-1950s, the olefin metathesis reaction, a metal-catalyzed exchange of alkylidene moieties between alkenes, started to gain popularity among the scientific community only in the early 1990's. The development of well-defined catalysts with remarkable tolerance to functional groups have largely contributed to this explosion in popularity representing an impressive lesson on the chemist's ability to innovate in finding new applications (Scheme 1.4.1)

1.4.1. Catalysts

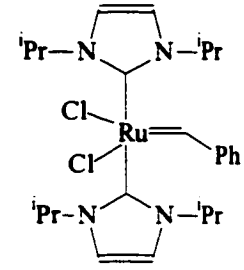
The scope of the olefin metathesis reaction has grown significantly after the advent of highly active transition metal catalysts (Figure 1.4.1).



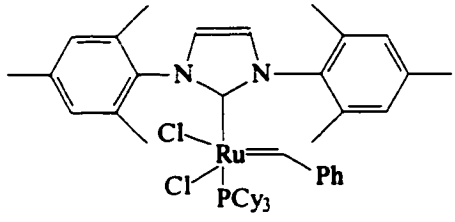
17
SCHROCK



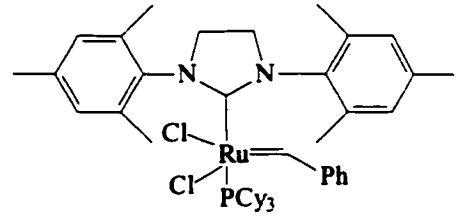
18
GRUBBS
(1st generation)



19
HERRMANN



20
GRUBBS



21
GRUBBS
(2nd generation)

Figure 1.4.1. Catalysts used in olefin metathesis.

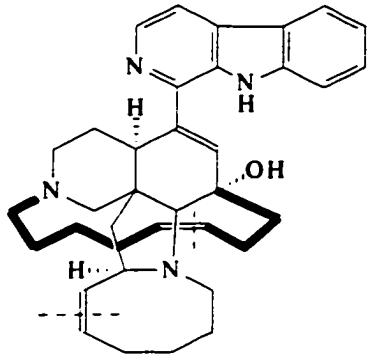
Among the plethora of metathesis pre-catalysts described in the literature, Schrock's molybdenum carbene **17** [68] , 2,6-Diisopropylphenylimidoneophylidene molybdenum (VI)

bis(hexafluoro-t-butoxide), played a prominent role and set the standard in this field. The most impressive feature is its high activity which allows it to react with both terminal and internal olefins. However, this catalyst based on an early transition metal, the molybdenum, is limited by the high oxophilicity of the metal center, which makes it extremely sensitive to oxygen and moisture, therefore it must be handled in rigorously dried solvents using Schlenk techniques. Aside from these inconveniences, early metal catalysts are more fundamentally less tolerant towards functional groups. Those disadvantages found with the Schrock's catalyst were solved with the first generation of Grubbs' catalyst, Bis(tricyclohexylphosphine)benzylidene ruthenium (IV) dichloride, compound **18**. Although, less reactive than the Schrock's catalyst, the Grubbs' catalyst **18**,[69] ruthenium based (late transition metal) have the great advantage to be more tolerant towards many types of functional groups and less sensitive towards humidity and oxygen. Thus, catalyst **18** meet a crucial criterion for application in organic synthesis, the possibility to work with the Grubbs' catalyst without a glove-box just launched its utilization and contributes largely to its nomination as Fluka's "Reagent of the year" in 1998. However, catalyst development did not stop at this stage. In the same year, Herrmann and co-workers introduced imidazolinylidene (*N*- heterocyclic carbene) complexes such as **19**,[70] Bis[1,3-di(isopropyl)imidazolyl-2-ylidene][benzylidene] ruthenium (IV) dichloride which showed a similar activity profile but an improved stability to high temperature compared to **18**. When only one of the PCy_3 unit was replaced by an imidazolinylidene unit, an highly active ruthenium carbene catalyst **20**, Tricyclohexylphosphine[1,3-bis(2,4,6-trimethylphenyl)imidazolyl-2-ylidene][benzylidene] ruthenium (IV) dichloride was formed.[71] Finally, the discovery by Grubbs *et al* that a catalyst containing an *N*-heterocyclic carbene with a saturated backbone lead to the generation of novel catalyst **21** (2nd generation of Grubbs's catalyst),[72] Tricyclohexylphosphine[1,3-bis(2,4,6-trimethylphenyl)4,5-dihydroimidazolyl-2-ylidene] [benzylidene] ruthenium (IV) dichloride. This new generation combined the activity found in Schrock's catalyst and the functional group tolerance found in the first generation. The replacement of one of the phosphine ligand by an *N*-heterocyclic carbene with a saturated backbone greatly enhance the activity of the catalyst as demonstrated by the formation of tetrasubstituted double bonds. This increased metathesis activity is

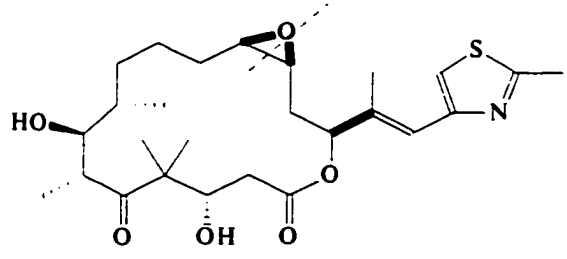
explained by the interesting properties of *N*-heterocyclic carbene which are stronger σ donors and much less labile. As a result, the more strongly electron-donating carbene ligand might enhance the dissociation of the more labile trans phosphine from the metal center. Then, by virtue of its steric bulk and electron-donating properties, the same ligand should more effectively stabilize the electron-deficient intermediates and promote olefin metathesis. Compounds **17-21** have to be regarded as precatalysts. The catalytically active species are the corresponding methylidene complexes.

1.4.2. Mechanism and fundamental types of applications

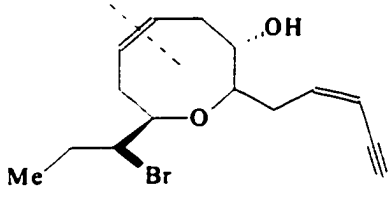
Of the different types of olefin metathesis, the ring closing metathesis (RCM) has found the widest use in the synthesis of medium to large ring systems.[73] It is one of the most popular synthetic methods at the present time. Perhaps one of the most impressive indicators, is the number of natural product synthesized using this reaction as one of the key steps.



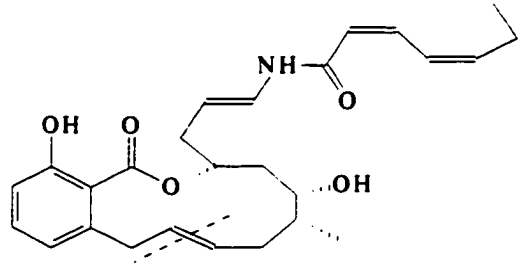
22
Manzamine A



23
Epothilone A



24
Prelaureatin

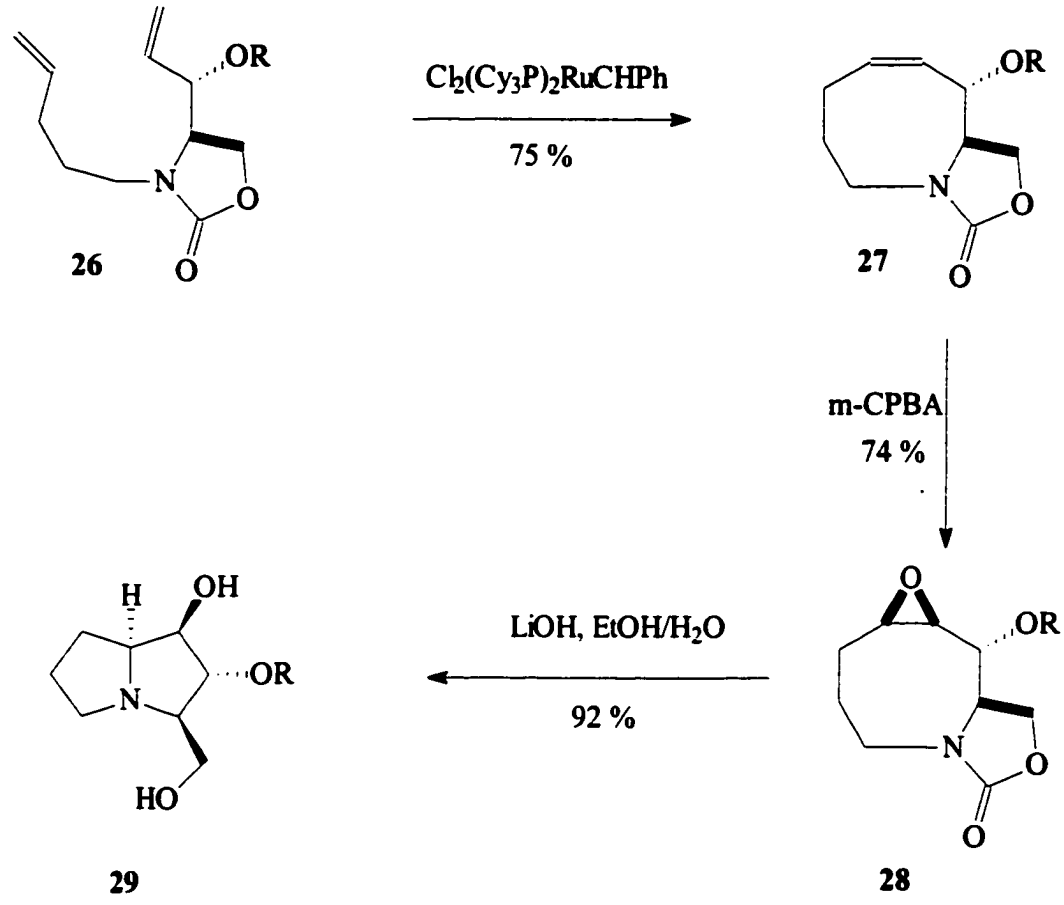


25
Salicylihalamide A

Figure 1.4.2. Examples of natural products synthesized by RCM.

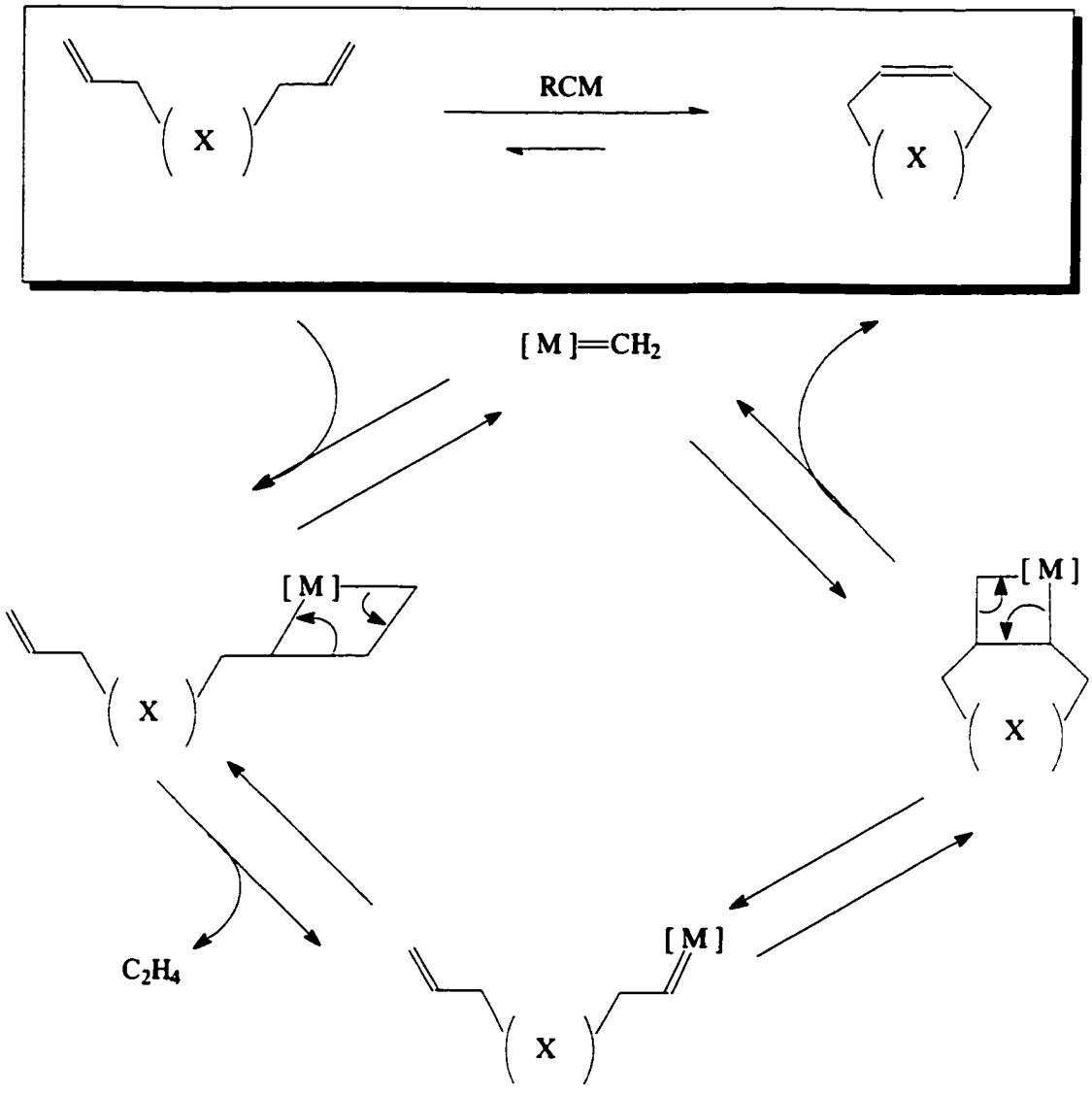
Some impressive examples have been reported in the literature (Figure 1.4.2.) where the RCM was used to construct complex molecules such as Manzamine A,[74] Epothilone A,[75] Prelaureatin[76] and Salicylihalamide A.[77]

The RCM protocol found also many applications towards the synthesis of polyhydroxylated pyrrolizidine alkaloids. White and co-workers reported an elegant strategy giving access to this class of compounds by a sequence of Ring-closing metathesis affording an azacyclooctene followed by epoxidation and transannular cyclization for the obtention of the bicyclic framework of a pyrrolizidine[78] (Scheme 1.4.2.).



Scheme 1.4.2. Synthesis of polyhydroxylated pyrrolizidine alkaloids.

The generally accepted mechanism of the RCM reaction (Chauvin mechanism)[79] proceeds by a sequence of [2+2]cycloaddition/cycloreversions involving alkenes, metal carbenes, and metallacyclobutane intermediates as shown in Scheme 1.4.3. It is noteworthy to mention that all individual steps are in equilibrium. Therefore, the formation of the cyclic product, which is in fact a mixture of *Z* and *E* stereoisomers, is mainly driven by the removal of ethylene gas.

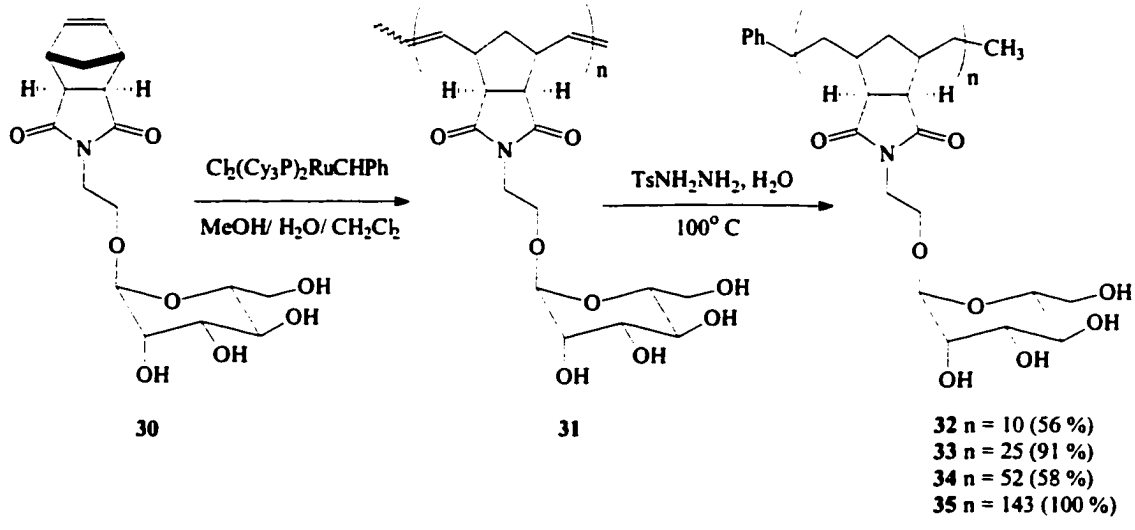


Scheme 1.4.3. Catalytic cycle of RCM.

The ring opening metathesis polymerization (ROMP) has found also many applications in polymer synthese.[80] Strained cyclic olefins are used as monomers, since, due to the

bond strain, the double bond react highly selectively with the catalyst and enhance the polymerization. A key advantage of ROMP is that the polymerization can be living, that is, elongation can proceed more rapidly than termination of chain transfer. Kiessling *et al* reported an interesting application of the ROMP to generate collections of biologically active glycopolymers in which the number of repeating units within a set is systemically varied.[81] The oligomerization of **30** was effected with ruthenium carbene **18**, with increasing monomer-to catalyst ratios employed to produce polymers of increasing length (Scheme 1.4.4.). The ability of these novel glycopolymers to interfere with cell agglutination mediated by the carbohydrate-binding protein concanavalin A was evaluated and was consistent with a glycoside cluster effect.

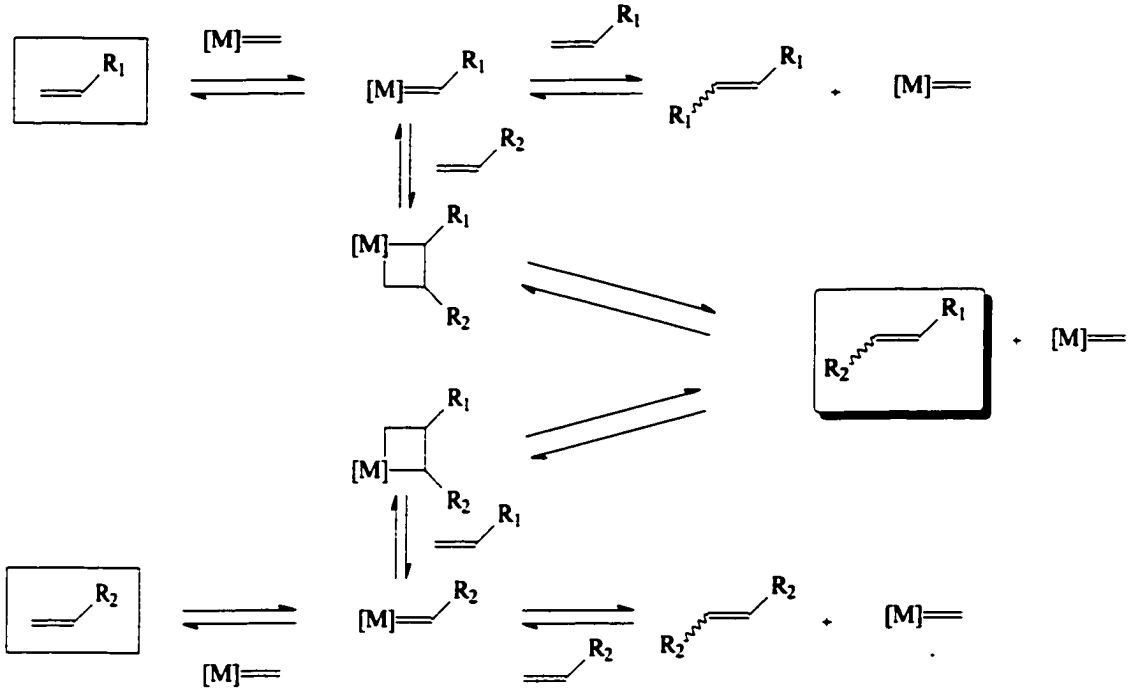
A relatively new polymerization variant is the acyclic diene metathesis (ADMET) in which terminal dienes are used as starting materials.[82]



Scheme 1.4.4. Synthesis of glycopolymers by ROMP.

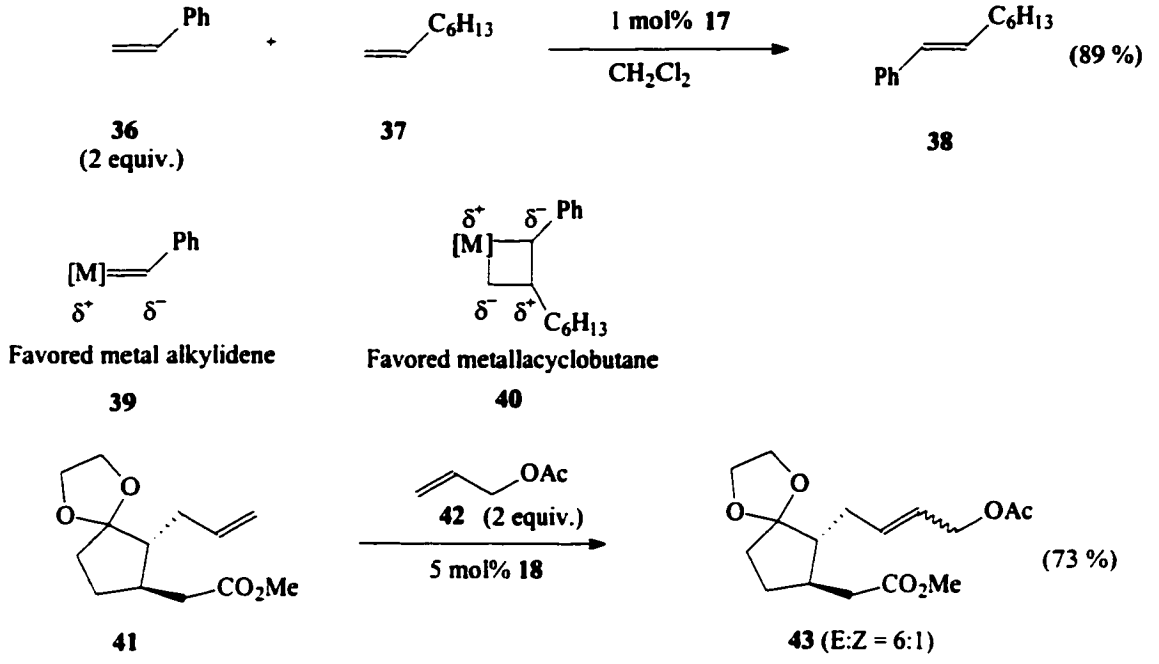
The volume of work reported in the area of RCM have overshadowed that reported for olefin cross-metathesis (CM). This unique method for the intermolecular formation of carbon-carbon double bonds represent an underdeveloped area of metathesis chemistry. Addressing issues of product selectivity and stereoselectivity are central to the utility of

CM which is entropically more challenging than RCM. Generally, the reaction between two different olefins can afford three disubstituted products, each of them as a mixture of *cis* and *trans* isomers, so a total of 6 products can be formed (Scheme 1.4.5.) Therefore, prerequisites for a successful application of this reaction are the suppression of the formation of undesired self metathesis products and the control of the configuration at the newly formed double bond.



Scheme 1.4.5. Catalytic cycle of CM.

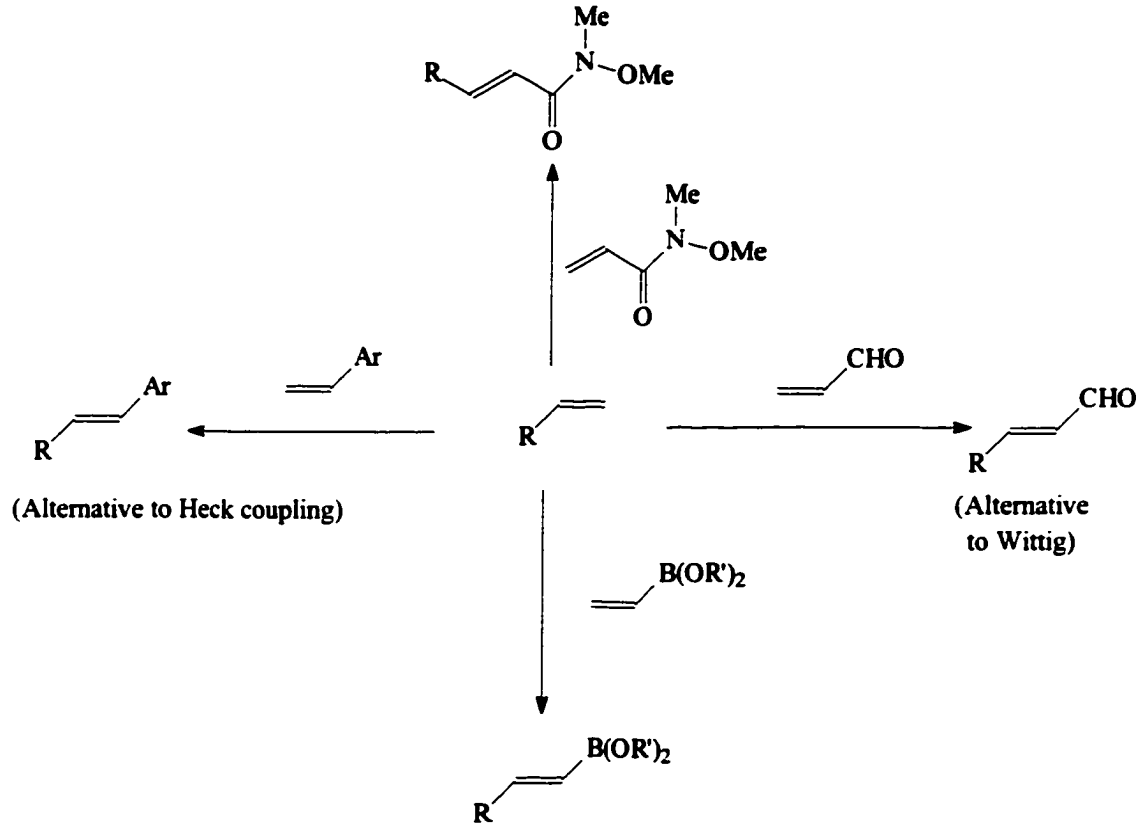
Concerning the selectivity, some progress were made to favor the formation of the desired cross-metathesis product in recent years. For example, Crowe *et al.* have shown that small, alkyl-substituted olefins undergo chemo- and stereo- selective cross-metathesis with π-substituted terminal olefins such as styrene or acrylonitrile in the presence of the Schrock-metal carbene.[83] The Crowe group argues that the extended π-system allows styrene to serve as a good alkylidene donor but not as a nucleophile, therefore favoring the formation of metal alkylidene **39**. Whereas, the alkyl-substituted olefin, being more nucleophilic, prefers to react with metal alkylidene to generate metallacyclobutane **40**. This can then fragment to form a methylene complex, and the desired cross metathesis product **38** (Scheme 1.4.6.).



Scheme 1.4.6. Selective CM reactions.

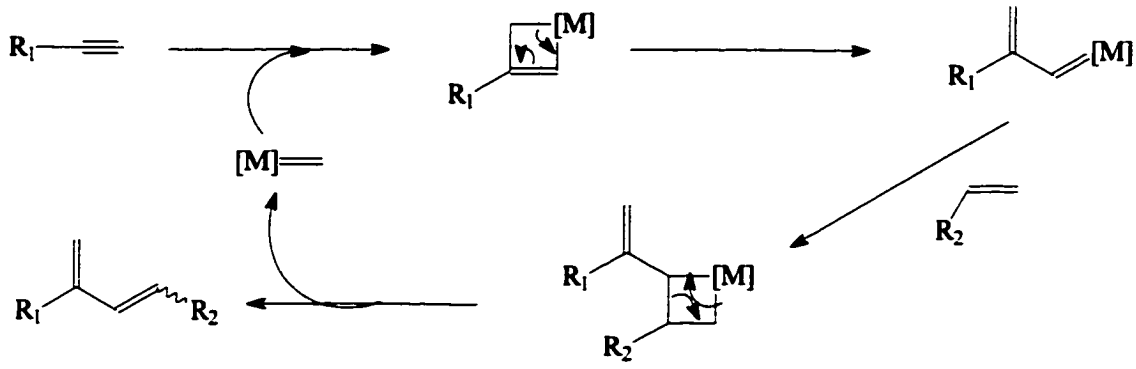
Blechert *et al.* have shown that sterically hindered terminal olefins do not undergo self-metathesis and can be selectively functionalized.[84] In this way, various derivatives of jasmonic acid **41** could be prepared in good yields (Scheme 1.4.6).

After all those developments in the area of CM and the development of highly active catalysts, several nice applications appeared in the literature recently.[85] The simplicity and power of CM as an intermolecular carbon-carbon bond forming reaction could make this reaction be considered as an appealing alternative to the usual ways to elongate carbon chains Wittig homologation, Heck reaction, etc (see Scheme 1.4.7).



Scheme 1.4.7. New applications of CM with catalyst **21**.

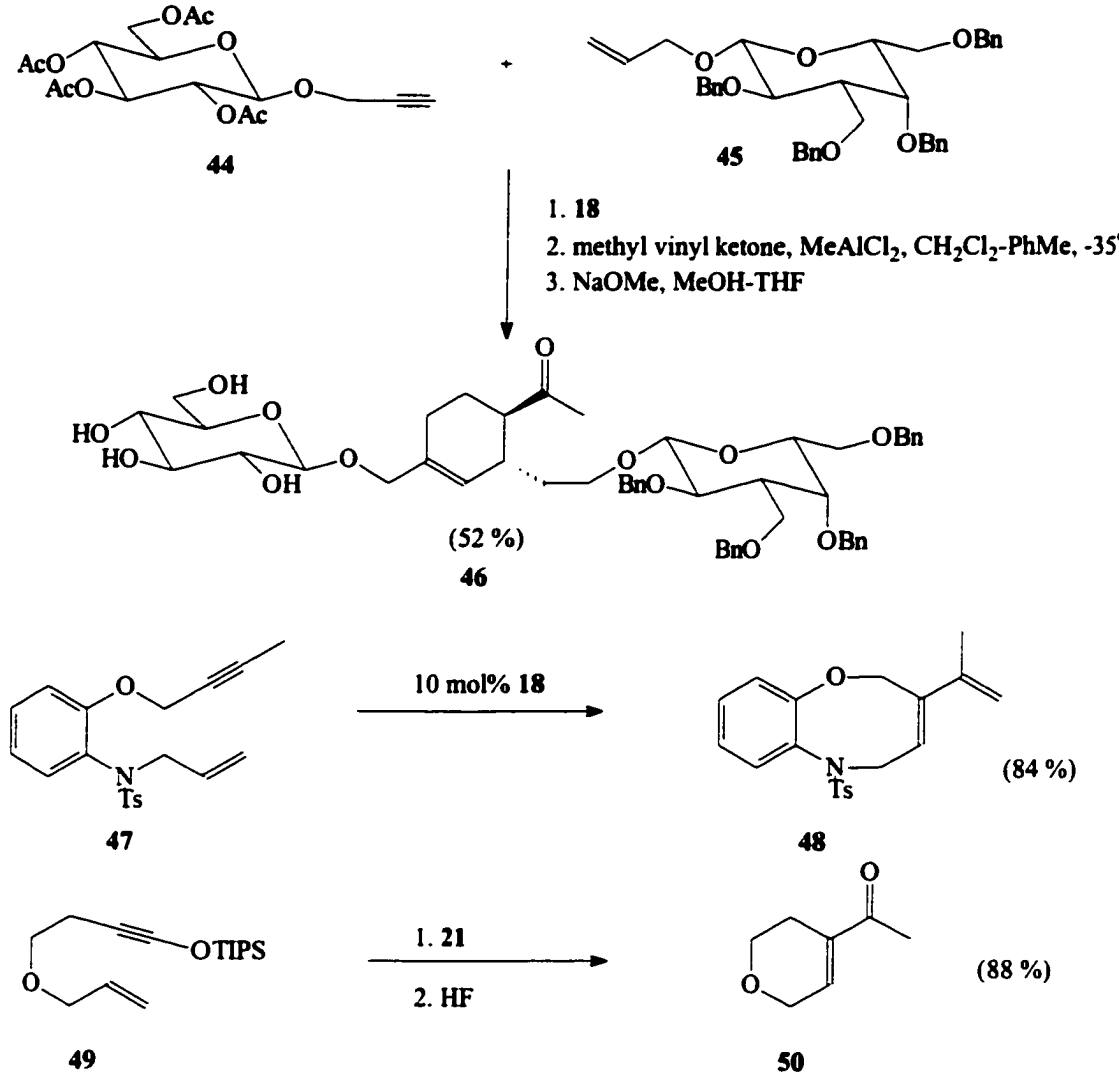
The selective cross-metathesis between monosubstituted acetylene and terminal olefins is called yne-ene cross metathesis and gives access to 1,3-disubstituted dienes. The reaction pathway of this reaction is given in scheme 1.4.8. The reaction starts at the triple bond via a cycloaddition leading to the formation of a ruthenacyclobutene, followed by a subsequent cycloreversion giving rise to vinylidene complex. Finally a selective cross-metathesis with the olefin affords the 1,3-diene releasing the active catalytic species.



Scheme 1.4.8. Mechanism of yne-ene CM.

The yne-ene CM reaction has been applied toward the synthesis of pseudo-oligosaccharides such as **46** by Blechert et al.[86] (Scheme 1.4.9.) The approach shown is very flexible and allows different sugars to be coupled at different positions.

In addition, another application was found in the synthesis of medium-sized rings.Various eight- and nine- membered ring compounds were synthesized using an intramolecular yne-ene CM reaction. Treatment of enyne **47** with 10 mol % of **18** afforded regioselectively benzocyclooctene **48** in high yield. The formation of an eight-membered ring over a nine-membered ring was confirmed by X-ray analysis. Mori *et al*[87] observed that the presence of the two heteroatoms in **47** were essential for the success of the cyclization.

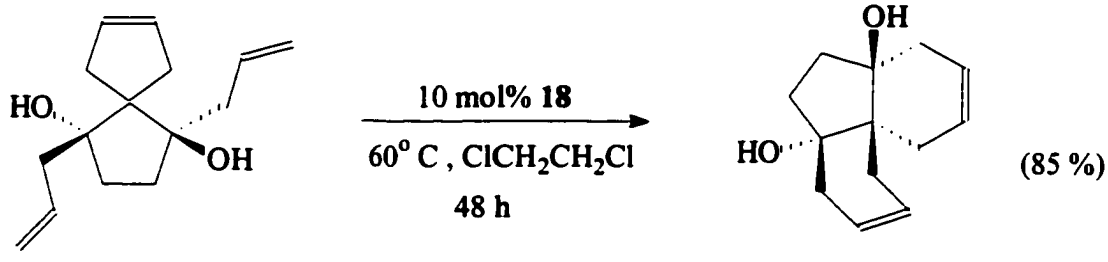


Scheme 1.4.9. Application of yne-ene CM reaction.

Among a range of synthetically useful transformations the yne-ene metathesis is particularly noteworthy for the ability to assemble two carbon-carbon bonds in a single step in such a way to obtain butadiene derivatives. Kozmin and coworkers used the interesting feature of this reaction to develop a novel methodology giving access to highly functionalized cyclic enones **50** by using activated yne-ene precursors such as siloxyalkynes **49**.[88] This methodology provides a powerful alternative for the preparation

of cyclic enones avoiding the regioselectivity problem associated with intramolecular aldol condensation.

Tandem or domino reactions can shorten synthetic routes significantly and are very interesting for synthetic strategies and methods. Sequences of multiple subsequent olefin metathesis reactions are also known.[89] Harrity *et al* reported the formation of a variety of angularly fused tricycles by a tandem RCM reaction.[90] Treatment of spirocycle **51** with 10 mol% of **18** smoothly afforded tricycle **52** in 85 % yield via a sequence of ring opening metathesis and two ring closing metathesis (Scheme 1.4.10.).



51 **52**

Scheme 1.4.10. Example of tandem RCM reaction.

Olefin metathesis reaction has just emerged as a powerful tool for the synthetic organic chemists. Its utilization in chemical glycobiology directly stemmed from this dissertation and is fully discussed in Chapter 2 – 5. In chapter 6, an approach to the synthesis of 3,6-di-O-(α-D-mannopyranosyl)-α-D mannopyranoside mimetics will be shown.

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Chapter 2. Synthesis of carbohydrate homodimers

2.1. Homodimerization : A general mechanism for signal transduction

In the past decade, ligand-induced receptor dimerization has evolved as a general mechanism for signal transduction.[1] This process by which extracellular signaling substances are transmitted via a receptor to the interior of the cell via intracellular signal cascades, mediate the first step in cell communication.[2] The reaction triggered inside the cell by these signals can then result to growth, differentiation, and death of the cell (Figure 2.1.1).

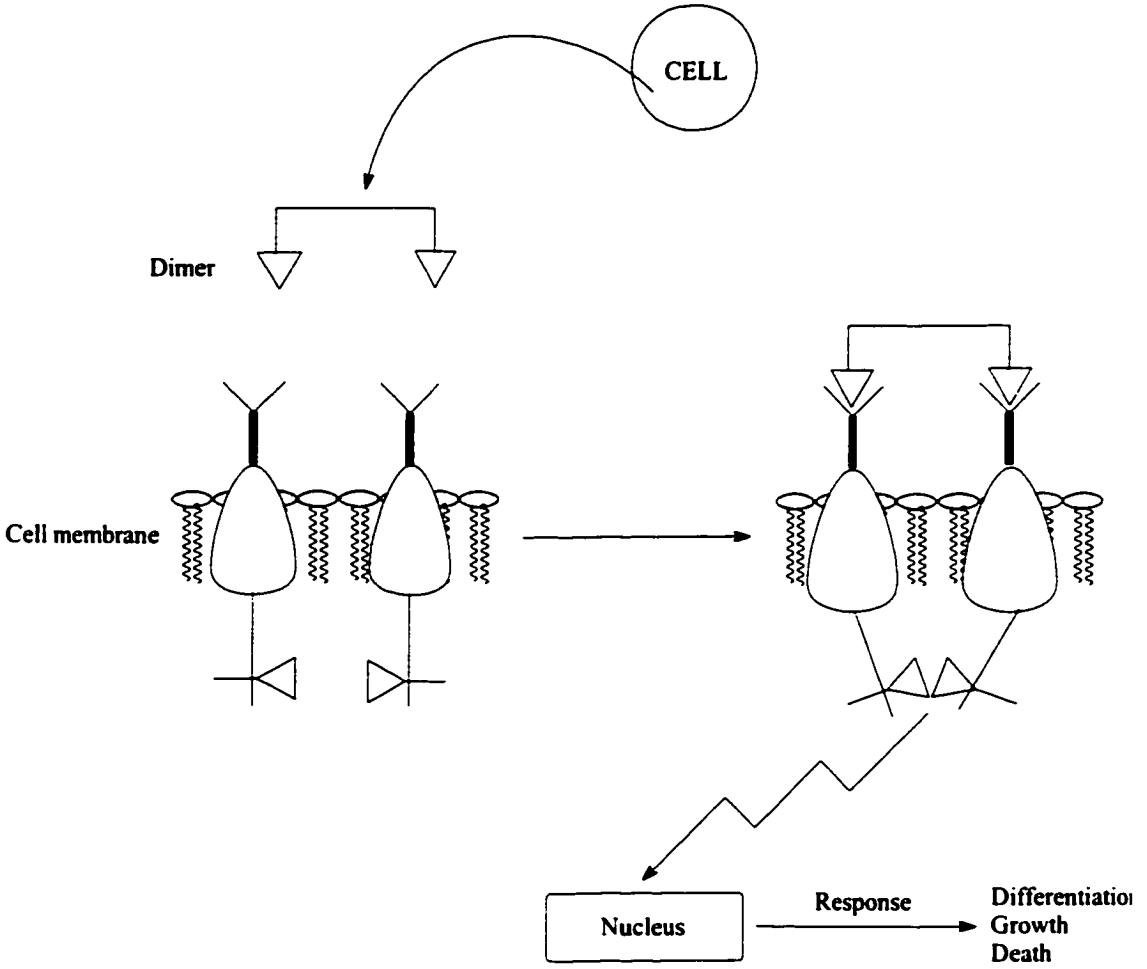


Figure 2.1.1. Signal transduction mediated by a bivalent molecule.

Many signal transduction pathways are initiated by the binding of bivalent ligands to their receptors on the cell surface. Thus, defaults in the transmission of the proper signals are responsible for diseases such as cancer, diabetes, and disorders of the immune system. Therefore, bivalency became an effective strategy in drug discovery.[3] Moreover, several dimeric natural product-like inhibitors have been designed and synthesized, including FK506,[4] Vancomycin,[5] Phorbol[6] and Vitamin D3.[7] These compounds have been coined by Schreiber and Crabtree as chemical inducers of dimerization. The rationale for employing bivalent ligands stems from the possibility that dimeric structures may be capable of bridging independent recognition sites on the receptor resulting in a thermodynamically more favorable binding interaction than the monovalent binding of two molecules.

Many examples have been reported in the literature where bivalent molecules have interesting biological properties (Figure 2.1.2)[8].

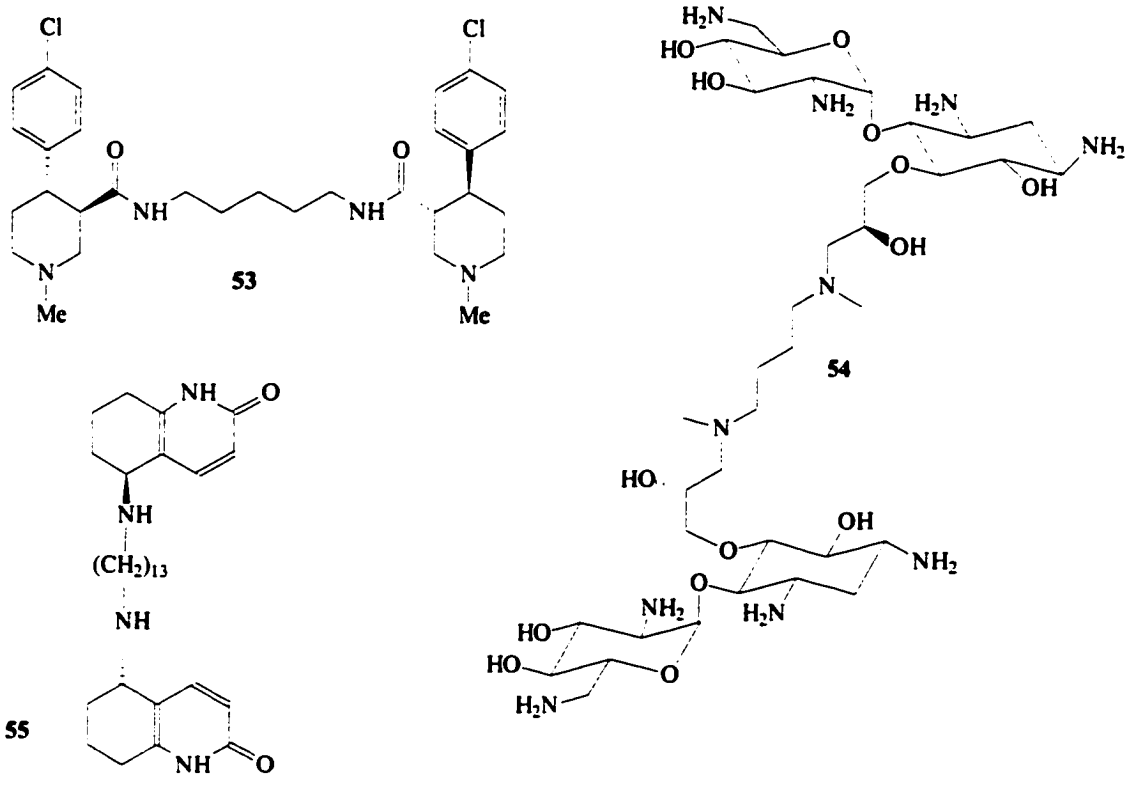
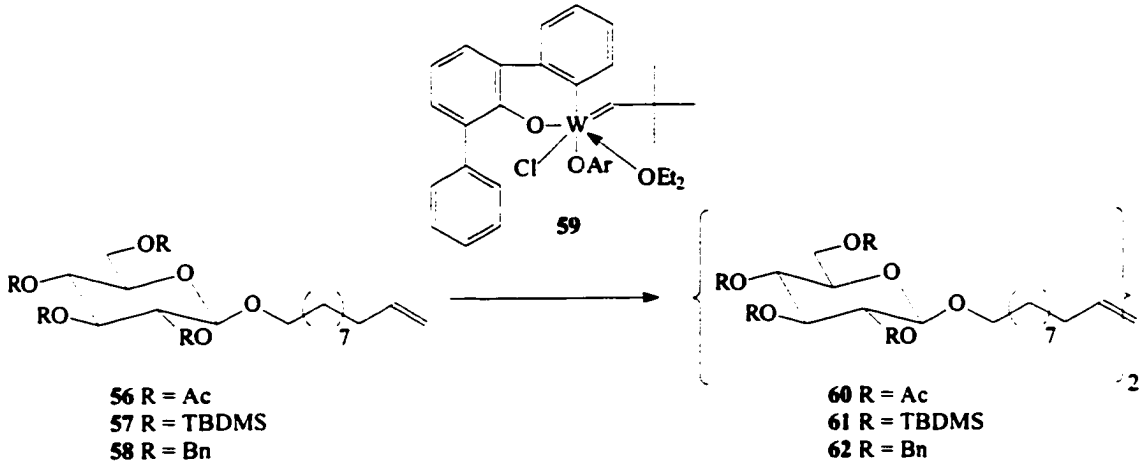


Figure 2.1.2. Examples of dimers with interesting biological properties.

For example, in order to combat the growing problem of antibiotic resistance[9], Chi-Huey Wong and coworkers has created a novel type of antibacterial agent. The aminoglycoside neamine dimer **54** can kill bacteria and at the same time inhibit the enzymes that cause resistance. It was found to be active against six different types of bacteria, including an aminoglycoside resistant clinical isolate.[10]

Therefore, a rapid and efficient method allowing to synthesize carbohydrate homodimers would be a valuable tool in order to evaluate their biological activities.
The conventional way to synthetise carbohydrate homodimers is making use of a double glycosidation approach in which one may encounter some problems such as obtaining monoglycosylated compounds or / and a mixture of diastereoisomers at the anomeric position. The olefin metathesis reaction represents an appealing alternative that will circumvent those problems since it is known to form homodimer during his catalytic cycle. The advantage of this method is that it is possible to obtain complete stereo-control at the anomeric center. Moreover, by the olefin metathesis reaction it is now possible to generate the C-allyl version of a homodimer which could not be obtained by conventional methods.

Before this thesis, the only example of carbohydrate homodimerization was reported by Descotes *et al.* using a generation of chloro-aryloxide complexes of tungsten to form unsaturated bolaamphiphiles which are compounds consisting of two hydrophilic heads joined together by a long hydrophobic spacer.[11] (scheme 2.1.1)



Scheme 2.1.1. Synthesis of carbohydrate-based bolaforms by Descotes.[11]

During the past few years this group of compounds have met growing interest because of their unusual properties as surfactant and high ability to form monolayer membranes. They are also used for studying mechanisms of membrane processes, as well as some enzymatic reactions, and have been investigated as liquid crystals.

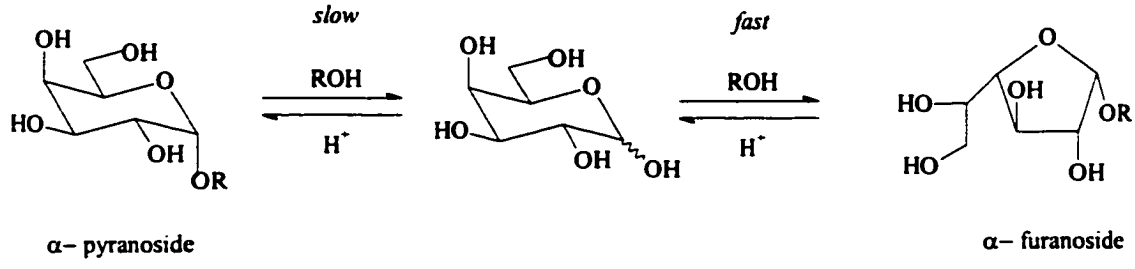
The catalyst **59** developed in their laboratory was found to be active for pentenyl and undecenyl glucosides with different protecting groups such as acetyl esters or silyl ether, whereas benzyl ether protected glucosides were deactivating both the catalyst and resulted in lower yield, even when a higher catalyst/substrate ratio was employed. They found also that allyl glucoside was not giving any metathesis product. They rationalized their results by proposing that the catalysts were deactivated by competitive coordination of the ether oxygen atoms to the metallocarbene which could not occur in the case of silylated substrate because the TBDMS group was large enough to inhibit this kind of complexation with the also sterically hindered tungsten.

In this chapter, we report the synthesis of some biologically important carbohydrate homodimers using Grubb's catalyst starting from O- and C-allyl as well as O- pentenyl glycosides with a variety of protecting groups.

2.2. Results and Discussion

2.2.1. Synthesis of alkenyl and pentenyl glycosides

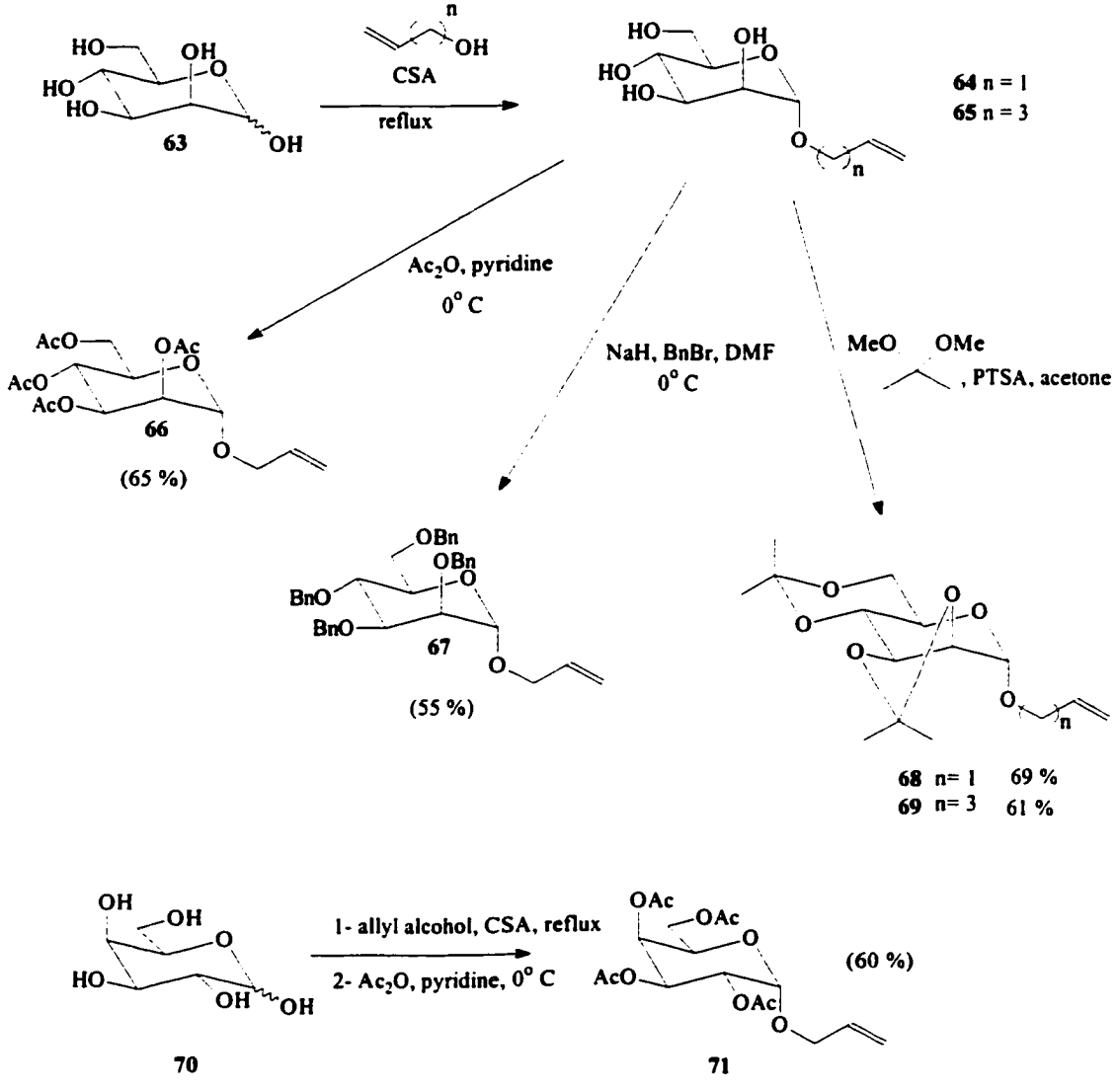
The synthesis of allyl α-D-mannopyranoside was prepared by using the classical Fisher synthesis which is a valuable method for the direct formation of glycosides from fully unprotected carbohydrates and simple alcohols, including methanol, benzyl alcohol, or allyl alcohol.[12]



Scheme 2.2.1. The classical Fisher synthesis.

The reaction requires the presence of strong acids, such as hydrogen chloride, sulphonic acids or a strong acidic ion exchange resin. Initially the reaction produces the kinetically favoured furanosides whereas longer reaction times yield the pyranosides as the thermodynamically preferred products with an α configuration as major component of the reaction mixture[13] (Scheme 2.2.1).

Thus, treatment of D-mannose with allyl alcohol as solvent in the presence of a catalytic amount of CSA afforded the desired allyl α-D mannopyranoside **64** as the major product. The crude mixture was then treated with acetic anhydride and pyridine (1:1) at 0° C to gives allyl 2,3,4,6- tetra-O-acetyl-α-D-mannopyranoside **66** in 65 % yield.

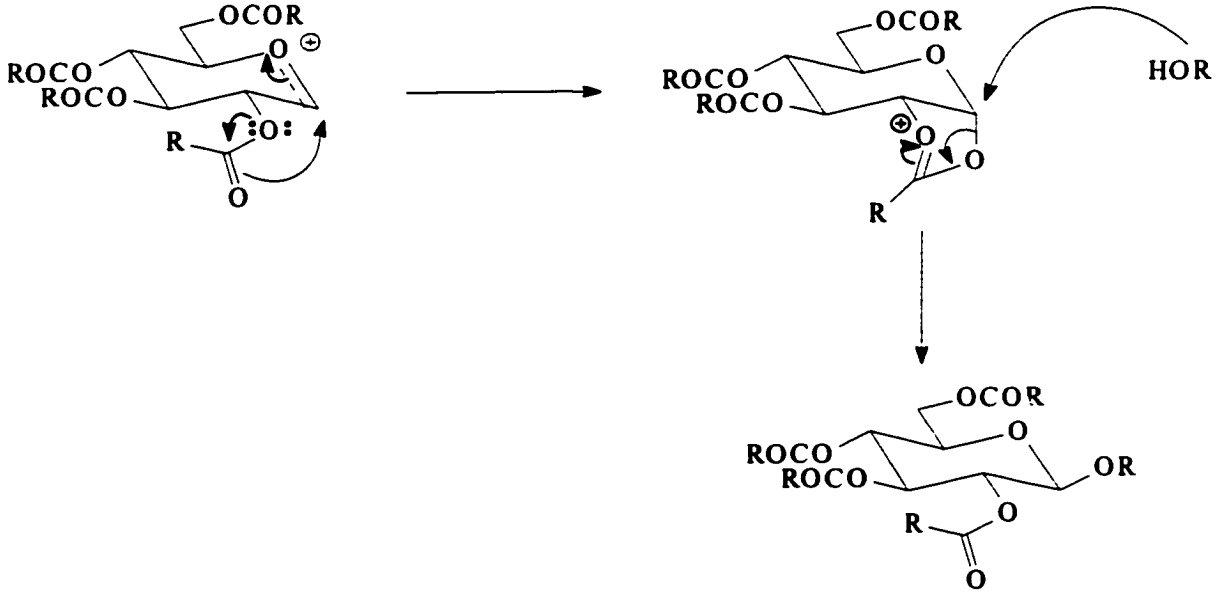


Scheme 2.2.2. Synthesis of alkenyl α- glycosides.

To obtain allyl 2,3,4,6-tetra-O-benzyl-α-D-mannopyranoside **67**, the crude mixture was treated with sodium hydride and benzyl bromide at 0° Celsius, and for allyl 2,3:4,6-O-diisopropylidene-α-D-mannopyranoside **68**, the crude mixture was treated with 2,2-dimethoxypropane in acetone with a catalytic amount of PTSA at room temperature. In the same way, pentenyl 2,3:4,6-O-diisopropylidene-α-D-mannopyranoside **65** was prepared in 61% but during the Fisher glycosidation, pentenyl alcohol was used (Scheme 2.2.2.).

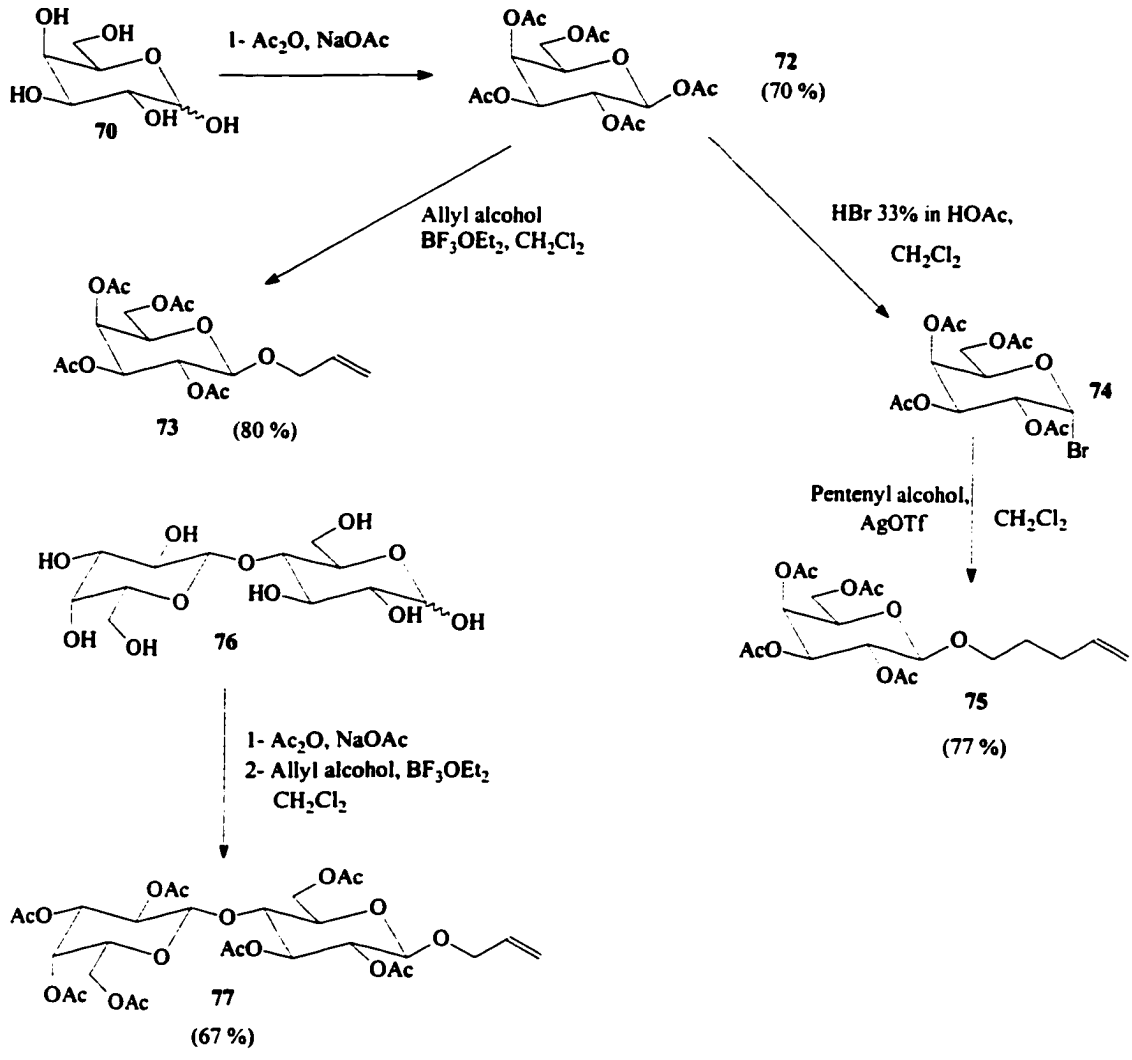
The allyl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside **71** was also prepared in 60% yield using a similar procedure starting from D-galactose .

To obtain the requisite β linkage, we could not use the Fisher glycosidation procedure. It is known that the presence of an ester functionality at C-2 directs the formation of 1,2-*trans* linked O-glycosides found in β-glycosides. Esters groups are known to be neighboring group active and participate in the reaction by forming an acyloxonium ion from the initially produced oxycarbonium ion. Nucleophilic opening of the ring at C-1 can only occur as a *trans* cleavage leading to the β-glycosides[13] (Scheme 2.2.3).



Scheme 2.2.3. Neighboring group participation.

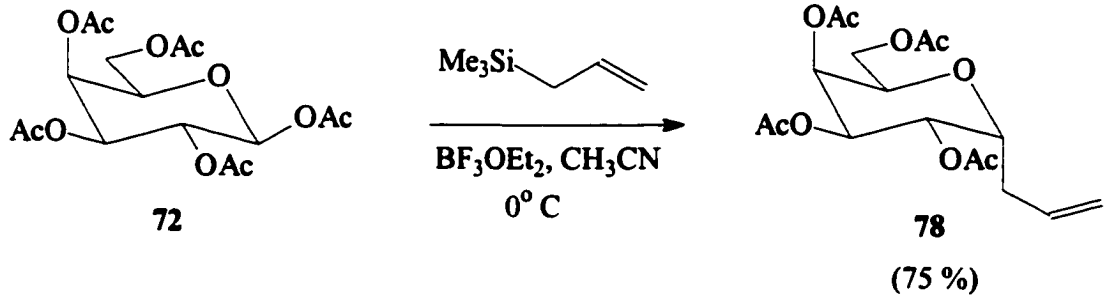
Therefore to form allyl 2,3,4,6-tetra-O-acetyl- β -D-galactopyranoside **73**, we have to start from 1,2,3,4,6- penta O-acetyl-β-D galactose pentaacetate **72** prepared by treating D-galactose with acetic anhydride and sodium acetate which favors the formation of the β anomer. The pentaacetate derivative was then reacted with allyl alcohol in the presence of boron trifluoride dietherate in dichloromethane to afford compound **73** in 80% yield. Under the same conditions, starting from lactose **76**, derivative **77** was prepared in 67% yield.



Scheme 2.2.4. Synthesis of alkenyl β-glycosides.

The desired β-linkage could also be obtained via the Koenigs-Knorr glycosidation method which used glycosyl bromides and chlorides as donors for the glycosidation reaction.[14] In this fashion, galactose pentaacetate **72** was converted to the peracetylated galactosyl bromide **74** by treatment with 33 % HBr in acetic acid. The crude mixture after work-up was then subjected to the Koenigs-Knorr procedure using AgOTf and pentenyl alcohol to afford 4-pentenyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside **75** in 77 % overall yield.

C-glycosides which are stereochemical isosteres for traditional O-glycosides wherein the anomeric oxygen is replaced with a carbon substituent have received considerable attention during the past few years. They are found as fragments in the structures of many natural products and they are stable mimics for natural O-glycosides. Consequently, methods for the synthesis of C-glycosides have been extensively studied.[15] The α- C-allyl derivative was prepared by reacting galactose pentaacetate with allyl trimethylsilane and boron trifluoride dietherate in acetonitrile to afford the C-allyl derivative **78** in a ratio (4:1) α/β anomer which could be purified by several recrystallization. The solvent plays a major role concerning the stereochemical outcome of this reaction. It was observed that replacing acetonitrile by dichloroethane would give an equal mixture of the 2 diastereoisomers.[16]



Scheme 2.2.5. Synthesis of C-alkenyl α-glycoside.

2.2.2 Olefin self-metathesis.

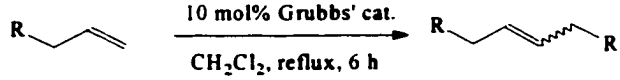
Treatment of allyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside **66** with 10 mol% of Grubbs' catalyst **18** in refluxing dichloromethane (0.14M) under a nitrogen atmosphere resulted in the clean formation of homodimer **79** in 93 % yield as a mixture of unseparable *E* and *Z* stereoisomers in 3:1 molar ratio. The only other by-products isolated from these sequential [2+2] cycloaddition and cycloreversion equilibria were the recovered starting material along with trace amount of cross-metathesis obtained from the initially released styrene. The ^{1}H-NMR spectra of **79** clearly showed the disappearance of the characteristic H-2' multiplet at δ 5.96-5.76 ppm found in the starting material **66**. Instead a triplet was found at δ 5.79 and 5.74 ppm in a 3:1 ratio confirming the homodimerization of **66**. The assignment of the major product as the *trans* streoisomer was possible via inspection of the ^{13}C-NMR and DEPT spectra. It is generally accepted that the carbon α to a double bond is more shielded in the *Z* isomer than in the *E* isomer due to the γ effect.[17] Therefore, this empirical relationship allowed us to assign the weak signal at δ 63.3 ppm to C-1' of the *Z* isomer and the signal at δ 67.4 ppm to C-1' of the *E* isomer.

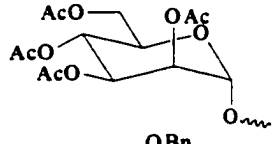
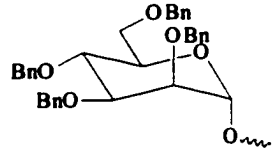
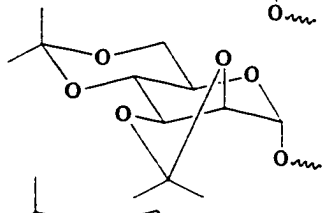
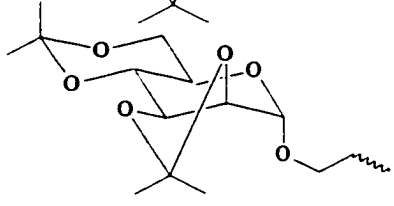
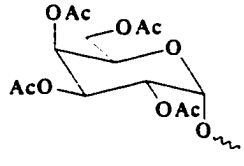
This assignment was reinsured when we performed the reaction with allyl 2,3,4,6- tetra-O-benzyl-α-D-mannopyranoside **67**. In this case, the homodimerization gave a separable mixture of *E* and *Z* stereoisomers in a 5:1 ratio. The major *trans* stereoisomer **80a** showed a signal for C-1' at δ 67.0 ppm whereas the *cis* isomer **80b** showed it at δ 63.0 ppm (see in appendix).

To further explore the scope and generality of this method, the self metathesis reaction was performed on α-mannosides having acetonides as protecting groups and a longer spacer chain. The self metathesis of allyl 2,3:4,6-O-diisopropylidene-α-D-mannopyranoside **68** under the same conditions gave homodimer **81** in 96 % yield as a separable mixture of *E* and *Z* stereoisomers in 5:1 molar ratio. For pentenyl 2,3,4,6-O-diisopropylidene-α-D-mannopyranoside **65**, the homodimer obtained in a 5:1 molar ratio in favor of the *E* isomer. In these two last cases, only the *trans* stereoisomer was isolated. By changing the sugar for galactose, slightly higher yields were obtained but similar

stereoselectivities. Homodimer **83** was obtained in 92 % yield as an unseparable mixture of *E* and *Z* stereoisomers in 5:1 molar ratio. Table 2.2.1 summarizes the results of the self-metathesis reaction with alkenyl α-glycosides. In order to compare the catalytic activity of the Grubbs' catalyst to that of the Schrock's catalyst **17**, the reaction performed under Schlenk conditions, provided dimer **83** in 80 % yield. In both cases the yields were more or less the same so it can be state therefore that for the self metathesis, both catalysts had similar activities however the Grubbs' catalyst was much easier to handle.

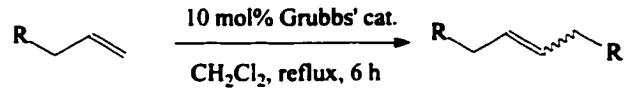
Table 2.2.1. Olefin self-metathesis of alkenyl α-glycosides.

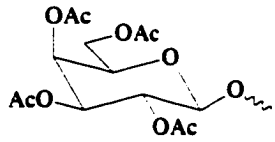
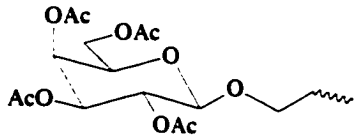
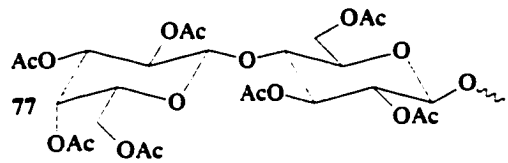


Entry		R	Yield (%)	ratio (E/Z)
1	**66**		93	**79** (3/1)
2	**67**		77	**80** (5/1)
3	**68**		96	**81** (5/1)
4	**69**		93	**82** (5/1)
5	**71**		92	**83** (5/1)

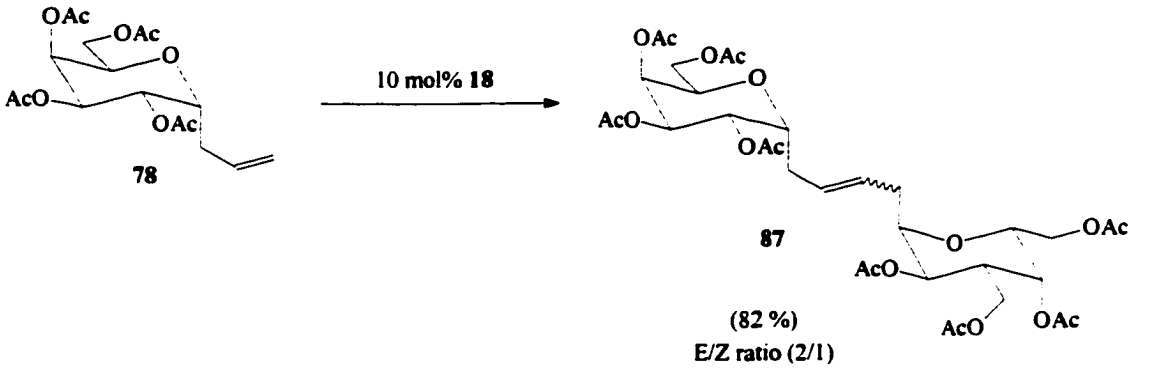
Similarly, olefin metathesis of the corresponding β-anomers allyl **73** and pentenyl **75** peracetylated derivatives provided homodimer **84** and **85** in 95% and 85% yields as unseparable mixture of *E* and *Z* isomers in a 4:1 and 5:1 ratios, respectively. It seems that the use of acetates as protecting groups did not allow the separation of the two stereoisomers obtained for mannose and galactose derivatives. However, the homodimerization of allyl octaacetyl lactopyranoside **77** afforded 89% of **86** as a separable mixture of the two stereoisomers in a 4:1 ratio in favor of the *trans* isomer. Table 2.2.2 summarizes the results with alkenyl β- glycosides.

Table 2.2.2. Olefin self-metathesis of alkenyl β-glycosides.



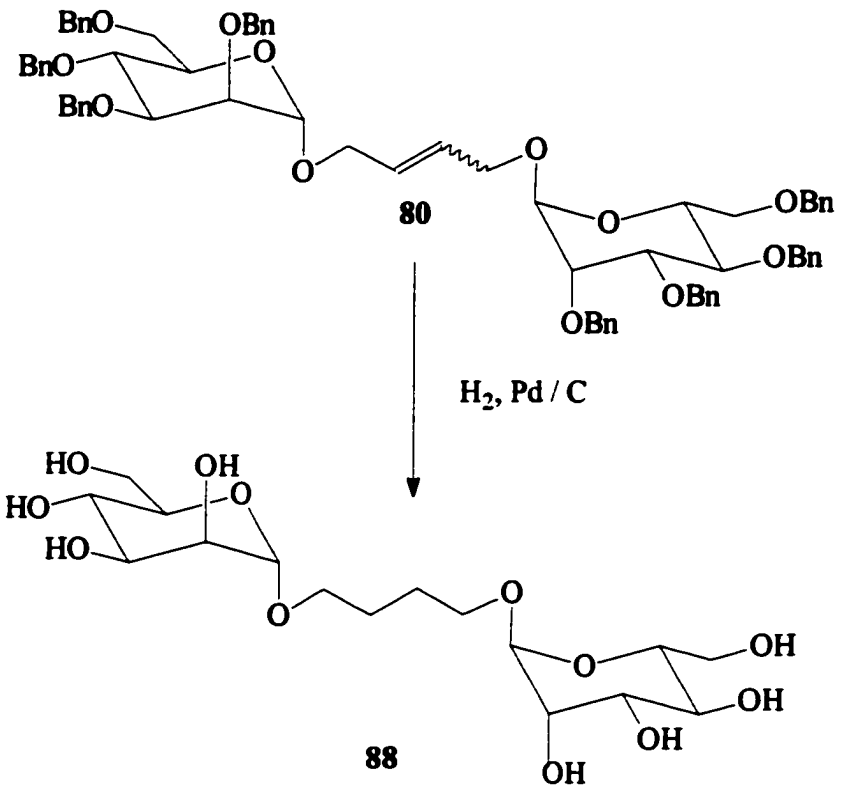
Entry		R	Yield (%)	ratio (E/Z)
2	**73**		95	**84** (4/1)
2	**75**		85	**85** (5/1)
3	**77**		89	**86** (4/1)

In view of the interesting properties associated with C-glycosides, the homodimerization of 3-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-1-propene **78** was envisaged. The reaction gave homodimer **87** in 82% yield as a mixture of unseparable stereoisomers in a poor (*E/Z*) 2:1 ratio. The replacement of an oxygen by a carbon substituent is having a non-negligeable influence on the stereochemical outcome of the reaction. This is probably due to the increased rigidity found in C-glycosides.



Scheme 2.2.6. Homodimerization of **78**.

To evaluate the cross-linking properties of the carbohydrate homodimers, removal of the protecting groups was necessary. To this end, homodimer **80** was treated with 10% palladium/carbon under an hydrogen atmosphere to afford unprotected homodimer **88** in quantitative yield (Scheme 2.2.7.). Then its relative binding properties was compared to monomeric methyl α-D-mannopyranoside by an inhibition of hemagglutination assay. Rabbit erythrocytes were allowed to hemagglutinate in the presence of tetrameric plant lectin Concanavalin A according to a published procedure.[18] In this assay, the minimum mannoside concentration necessary to inhibit the hemagglutination of the rabbit erythrocytes by the lectins was determined. The inhibitory properties of homodimer **88** (450 μM) was improved by seven times compared to methyl α-D-mannopyranoside (3100 μM).



Scheme 2.2.7. Hydrogenation of **80**.

2.3. Conclusions

In summary, olefin self metathesis with readily available Grubbs's catalyst was applied toward the synthesis of polyfunctionalized carbohydrate homodimers for the preparation of potential cross-linkers of biological interest in signal transduction. The reaction is general, high yielding, gives good stereoselectivity in favor of the *E* isomer in most of the cases and is compatible with the usual carbohydrate protecting groups. We have also shown that by varying the protecting groups, it was possible to separate by column chromatography the two stereoisomers. In the case where it was not possible to separate them, reduction of the double bond provided one product. This powerful methodology allowed to attach sugar residues in a very efficient manner and the formation of carbohydrate C-linked homodimers.

It is noteworthy to mention that after publication[19] of this work other groups also used the same strategy to form carbohydrate[20] and non-carbohydrate homodimers[7,21] with interesting biological applications showing the broad application of this methodology.

2.4. Experimental Methods

2.4.1. General Methods

^{1}H NMR and ^{13}C NMR spectra were obtained on Bruker AMX500, Bruker Avance 300 or Varian Gemini 200 instruments at 500, 300 and 200 MHz for protons and 125.7, 75.4 and 50.3 MHz for carbons, respectively. Proton chemical shifts (δ) are relative to internal standard deuterated chloroform ($CDCl_3$) at δ 7.24 ppm and to HOD at 4.76 ppm. Repeated exchange of protons for deuterium with D_2O and lyophylization for unprotected carbohydrates to simplify proton spectra was performed. Carbon chemical shifts are given relative to internal standard $CDCl_3$ at δ 77.0 ppm. Abbreviations used to record NMR multiplicities were reported using singlet (s), broad singlet (bs), doublet (d), doublet of doublets (dd), doublet of doublet of doublets (ddd), triplet (t), quartet (q) and multiplet (m). Special analyses were performed by the first order approximations and were based on shift correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), and 1- and 2-dimensional distortionless enhancement by polarization transfer (DEPT) experiments.

Mass spectra (FAB, EI, CI) were recorded on a Kratos Concept II instrument. Xenon and cesium were used as the neutral carrier atoms and in FAB-MS experiments. Electron Spray Ionization (ESI) was recorded on a Micromass Qualtro LC having a capillary voltage of 3.5 - 4.5 kv and methanol was used as solvent.

Melting points were determined on a Gallenkamp apparatus and are uncorrected.

Optical rotation ($[\alpha]_D$) values were determined using a Perkin-Elmer model 241 set at the sodium D line (589 nm) and were run at room temperature.

Reactions were followed by thin-layer chromatography using Kieselgel 60 F_{254} precoated 0.25 mm thick aluminium or glass backed plates. The reagents used for compound detection include ammonium molybdate (2.5% w/v) in 10% (v/v) aqueous sulfuric acid, iodine, ninhydrin (0.4% w/v) in aqueous pyridine (4% v/v) or potassium

permanganate (1% w/v) with sodium carbonate (2% w/v) in water or short wave UV light.

Purifications were performed by gravity or flash chromatography on silica gel 60 (230 – 400 mesh, E. Merck No 9385). Solvent were reagent grade and evaporated under reduced pressure using a Büchi rotary evaporator connected to a water aspirator, air aspirator, or air vacuum.

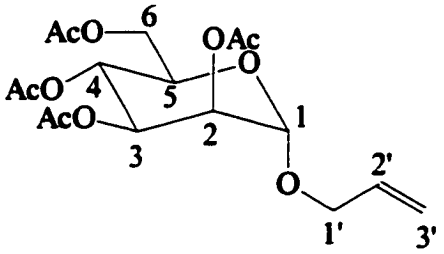
Purifications were also performed via preparative scale size exclusion chromatography. Columns were connected to a Pharmacia Peristaltic Pump P3 and eluted with distilled water. Waters Differential Refractometer R401 or R403 apparatus was used for detection and recorded on a linear 1200 or 2000 chart recorder. Fractions were collected using LKB 2112 Redirac or Pharmacia Model 5051 fraction collectors.

Lyophilization was carried out on a VIRTIS-24 freeze dryer.

All reactions, unless stated otherwise were carried out in oven dried flasks under an nitrogen atmosphere. All chemical and solvents used in experiments were of reagent grade. Further purifications were performed, when necessary, following published procedures.[21]

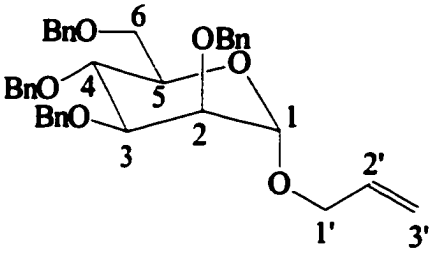
Allyl α-D-mannopyranoside (64)[22]

A suspension of D-mannose (10.00 g, 55.55 mmol), allyl alcohol (100 mL) and camphor sulphonic acid (1.00 g, 4.3 mmol) in a 250-ml round bottomed flask was stirred and heated under reflux (100^0 C) for 24 h. The resulting clear yellow solution was neutralized with Et_3N (1mL) and then evaporated with toluene under reduced pressure. Co-evaporation with toluene was repeated until the last traces of allyl alcohol were removed. The crude allyl α-D-mannopyranoside **64** obtained as a light brown syrup (12.00 g) was used for subsequent reactions without further purification;



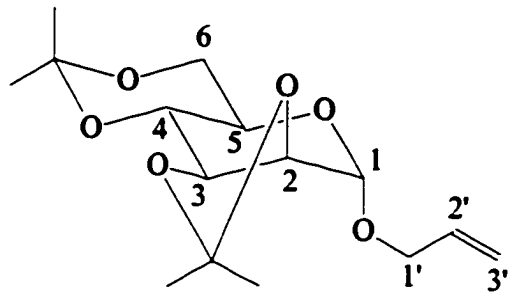
Allyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (66)[23]

The crude allyl α-D-mannopyranoside **64** (6.00 g, 27.78 mmol) was dissolved in pyridine (25 mL). The solution was cooled at 0° C and acetic anhydride (25 mL, 265 mmol) was added. The reaction mixture was stirred and allowed to warm to room temperature. The progress of the reaction was followed by TLC (hexane/ethyl acetate 2/1) until complete disappearance of the starting material (6 h). Methanol (25 mL) was then added at 0° C to neutralize excess of acetic anhydride and stirred for 10 minutes. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed several times with a 10% HCl solution (25 mL) until the complete removal of pyridine from the organic phase. The organic layer was dried over Na_2SO_4, evaporated under reduced pressure and purified via flash chromatography on silica gel using a gradient of hexanes/ethyl acetate 5/1 to 2/1 as eluent. Title compound **66** was isolated as a white solid in 65% yield (7.13 g, 18.37 mmol); m.p: 53-54 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 5.96-5.76 (m, 1H, H-2'), 5.37-5.30 (m, 2H, H-3'a, H-3'b), 5.25-5.18 (m, 3H, H-2, H-3, H-4), 4.84 (s, 1H, H-1), 4.30-4.35 (m, 5H, H-1'a, H-1'b, H-5, H-6a, H-6b), 2.12, 2.07, 2.01, 1.96 (4s, 12H, OAc); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 170.6, 170.0, 169.8, 169.7 (C=O), 132.8 (C-2'), 118.4 (C-3'), 96.5 (C-1), 69.5 (C-2), 69.0 (C-3), 68.6 (C-5), 68.4 (C-1'), 66.1 (C-4), 62.4 (C-6), 20.8, 20.6 (Me); CI-MS, m/z= 389, $[M+1]^+$ calcd for $C_{17}H_{24}O_{10}$.



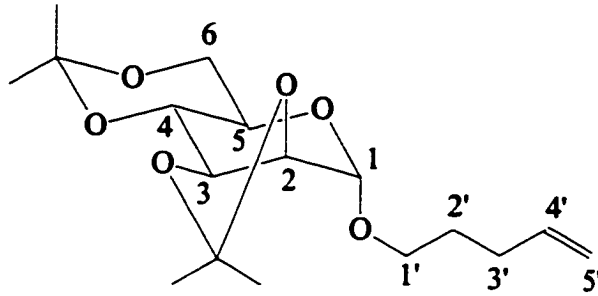
Allyl 2,3,4,6-tetra-O-benzyl-α-D-mannopyranoside (67)

Benzyl bromide (20 mL, 168.68 mmol) was added to a solution of the crude allyl -α-D-mannopyranoside **64** (6.00 g, 27.78mmol) dissolved in 350 mL of DMF cooled to 0° C under N_2 atmosphere. Sodium hydride (6.75 g, 168.68 mmol) pre-washed with hexane to remove the oil, was added slowly. The reaction mixture was stirred and allowed to warm to room temperature for 16 h. The progress of the reaction was monitored by TLC (hexane/ethyl acetate 5/1). Methanol (25 mL) was then added at 0° C to neutralize excess of sodium hydride and stirred for 10 min. The reaction mixture was diluted with ether (200 mL) and washed several times with water until the complete removal of DMF from the organic phases. The organic layer was dried over Na_2SO_4, evaporated under reduced pressure and purified via flash chromatography on silica gel using a gradient of hexanes and hexanes/ethyl acetate 10/1 to 5/1 as eluent. Title compound **67** was isolated as an oil in 55% yield (8.86 g, 15.27 mmol). ^{1}H NMR ($CDCl_3$, 500 MHz): δ(ppm) 7.38-7.15 (m, 20H, Ph), 5.90-5.82 (m, 1H, H-2'), 5.22 (dq, 1H, H-3'a), 5.15 (dq, 1H, H-3'a), 4.93 (d, J=1.9 Hz, 1H, H-1), 4.90-4.51 (m, 8H, CH_2Ph), 4.18-4.14 (m, 1H, H-1'a), 4.00-3.91 (m, 3H), 3.82-3.72 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ(ppm) 138.9, 138.8, 138.7, 138.7 (Ph), 134.0 (C-2'), 128.5, 128.5, 128.5, 128.2, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6 (Ph), 117.4 (C-3'), 97.3 (C-1), 80.5, 75.3 (CH_2), 75.2, 75.0, 75.6 (CH_2), 72.8 (CH_2), 72.4 (CH_2), 72.1, 69.5 (C-6), 68.0 (C-1'); ESI-MS, m/z= 598.2, $[M+NH_4]^+$ calcd for $C_{37}H_{40}O_6$ +NH_4.



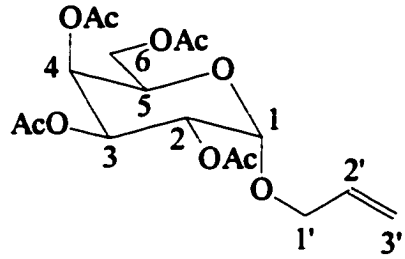
Allyl 2,3:4,6-di-O-isopropylidene-α-D-mannopyranoside (68)[24]

2,2-Dimethoxypropane (30 mL, 244 mmol) and toluene-p-sulphonic acid monohydrate (400 mg, 2.10 mmol) was added to a suspension of the crude allyl -α-D-mannopyranoside **64** (6.00 g, 27.78 mmol) in acetone (60 mL). The reaction mixture was stirred for 6h at rt and monitored by TLC (hexane/ethyl acetate 5/1). Et_3N (2 mL) was added and the solvent was evaporated under reduced pressure. The resulting crude extract was diluted with ether and washed with saturated aqueous $NaHCO_3$. The organic layer was dried over Na_2SO_4, evaporated under reduced pressure and purified via flash chromatography on silica gel using a gradient of hexane/ethyl acetate 20/1 to 5/1 as eluent. The title compound **68** was isolated as a white solid in 69% yield (5.75 g, 19.17 mmol); m.p.: 58-60 °C. Lit.[24] m.p. 59° C.; ^1H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.89-5.80 (m, 1H, H-2'), 5.27-5.15 (m, 2H, H-3'a, H-3'b), 5.01 (s, 1H, H-1), 4.15-4.09 (m, 3H, H-1'a, H-3, H-4), 3.93 (ddd, J=1.3, 4.3, 12.8 Hz, 1H, H-1'b), 3.84-3.80 (m, 1H, H-6a), 3.72-3.67 (m, 2H, H-2, H-6b), 3.57-3.52 (m, 1H, H-5), 1.50, 1.46, 1.37, 1.30 (4s, 12H, Me); ^{13}C NMR ($CDCl_3$, 125 MHz): δ(ppm) 133.4 (C-2'), 117.8 (C-3'), 109.3, 99.6 (MeCMe), 99.6 (C-1), 76.0, 74.8 (C-3, C-4), 72.7 (C-2), 68.1 (C-1'), 62.0 (C-6), 61.3 (C-5), 29.0, 28.1, 26.0, 18.7 (Me); ESI-MS, m/z= 301.0, $[M+1]^+$ calcd for $C_{15}H_{24}O_6$.



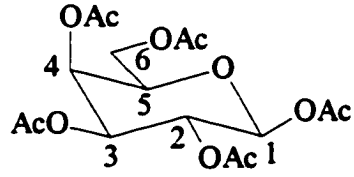
4-Pentenyl 2,3:4,6-di-O-isopropylidene-α-D-mannopyranoside (69)[25]

D-Mannose (1.00 g, 5.55 mmol) was added to a solution of 4-penten-1-ol (5 mL, 48.41 mmol) containing camphor sulphonic acid (50 mg, 0.22 mmol). The reaction mixture was stirred and heated at 100° C for 24 h. The bulk of the pentenol was distilled under vacuum and saved for future glycosidations. The remaining mixture was basified with Et_3N (5 drops), poured into water, and extracted with CH_2Cl_2. the aqueous phase was evaporated and dried under vacuum. Whitout further purification, acetone (10 mL) was added to the crude 4-pentenyl α-D-mannopyranoside **65**. To the resulting suspension, 2,2-dimethoxypropane (5 mL, 40.66 mmol) and toluene-*p*-sulphonic acid monohydrate (100 mg, 0.53 mmol) was added and stirred for 5h at room temperature. The reaction mixture was monitored by TLC (hexane/ethyl acetate 5/1). Et_3N (5 drops) was added and the solvent was evaporated under reduced pressure. The resulting crude extract was diluted with ether and washed with a saturated aqueous solution $NaHCO_3$. The organic layer was dried over Na_2SO_4, evaporated under reduced pressure and purified by a flash chromatography on silica gel using a gradient of hexane/ethyl acetate 10/1 to 7/1 as eluent. The title compound **69** was isolated as a white oil in 61% yield (1.11 g, 3.38 mmol); $[\alpha]_D$ +4.6° (c= 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz): δ(ppm) 5.81-5.72 (m, 1H, H-4'), 5.02-4.92 (m, 3H, H-1, H-5'a, H-5'b), 4.13-4.09 (m, 2H, H-3, H-4), 3.82 (dd, J= 5.6, 10.9 Hz, 1H, H-6a), 3.73-3.63 (m, 3H, H-2, H-6b, H-1'a), 3.53 (ddd, J= 5.2, 10.2 Hz, 1H, H-5), 3.40-3.36 (m, 1H, H-2, H-1'b), 2.11-2.07 (m, 2H, H-3'), 2.00-1.62 (m, 2H, H-2'), 1.51, 1.48, 1.39, 1.31 (4s, 12H, Me); ^{13}C NMR ($CDCl_3$, 125 MHz): δ(ppm) 137.8 (C-4'), 115.0 (C-5'), 109.3, 99.6 (MeCMe), 97.8 (C-1), 76.1, 74.9 (C-3, C-4), 72.7 (C-2), 67.0 (C-1'), 62.0 (C-6), 61.3 (C-5), 30.1 (C-3'), 29.0 (Me), 28.5 (C-2'), 28.1, 26.1, 18.7 (Me). CI-MS, m/z= 329, $[M+1]^+$ calcd for $C_{17}H_{28}O_6$.



Allyl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside (71)[26]

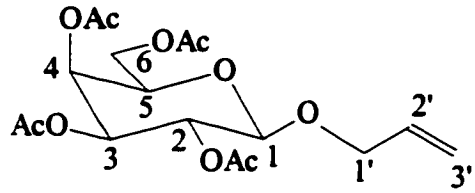
Following the methodology as described for **66**, title compound **71** was isolated as a white solid in 60% yield (6.47 g, 16.67 mmol); m.p. 81-83°. Li.[26] 82-83° C; 1H- and 13C-NMR spectral data agree with reported values; ^{1}H NMR ($CDCl_3$, 500 MHz): δ(ppm) 5.89-5.81 (m, 1H, H-2'), 5.43 (dd, J= 1.2, 3.4 Hz, 1H, H-4), 5.37-5.34 (m, 1H, H-3), 5.30-5.26 (m, 1H, H-3'a), 5.23-5.16 (m, 1H, H-3'b), 5.13-5.10 (m, 2H, H-1, H-2), 4.21 (ddd, J=1.1, 5.8, 6.4 Hz, 1H, H-5), 4.18-4.14 (m, 1H, H-1'a), 4.06 (d, J= 6.4 Hz, 2H, H-6a, H-6b), 4.02, 3.97 (m, 1H, H-1'b), 2.11, 2.05, 2.02, 1.96 (4s, 12H, OAc); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 170.3, 170.3, 170.1, 169.9 (C=O), 133.2 (C-2'), 117.9 (C-3'), 95.3 (C-1), 68.7 (C-1'), 68.1 (C-4), 68.0 (C-2), 67.5 (C-3), 66.3 (C-5), 61.7 (C-6), 20.7, 20.6, 20.6, 20.5 (Me); FAB-MS calcd. For $C_{17}H_{24}O_{10}$: 388.37; found 389.16 (M^+ + 1).



1,2,3,4,6-penta-O-acetyl-β-D-galactopyranoside (72)[27]

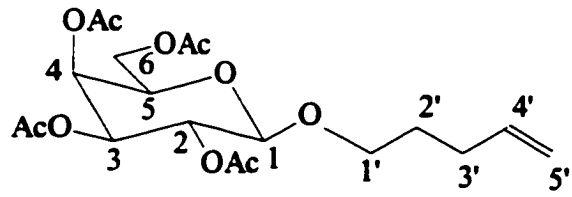
To a suspension of anhydrous sodium acetate (10.00 g, 122 mmol) in acetic anhydride (140 mL) heated at reflux was slowly added D-galactose (20.00 g, 0.111 mmol) over a period of 30 min. The reaction mixture was vigorously stirred during the addition to prevent an accumulation of solid sugar on the bottom of the flask. After complete dissolution of the material, the clear yellow reaction mixture was cooled to room temperature and stirred for 6 h. The progress of the reaction was followed by TLC

(hexane/ethyl acetate 2/1) until complete disappearance of the starting material. The reaction mixture was then poured with stirring onto 1 Lof cracked ice. After standing overnight with vigorous stirring, the crystalline material was filtered with suction and washed with cold water. The crude product was purified by recrystallization using hot ethanol 99% to give **72** as a white solid in 70 % yield (30.3 g, 77.69 mmol); m.p.: 140-141 °C. Lit.[26] m.p. 142 ° C.; ^{1}H NMR ($CDCl_3$, 200 MHz): δ(ppm) 5.64 (d, J= 8.3 Hz, 1H, H-1), 5.36 (d, J= 3.3 Hz, 1H, H-4), 5.25 (dd, J= 8.3, 10.4 Hz, 1H, H-2), 5.02 (dd, J= 3.3, 10.4 Hz, 1H, H-3), 4.12-3.99 (m, 3H, H-5, H-6a, H-6b), 2.11, 2.07, 1.99, 1.98, 1.94 (5s, 15H, OAc); Physical data in agreement with previously reported values.



Allyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (73)[28]

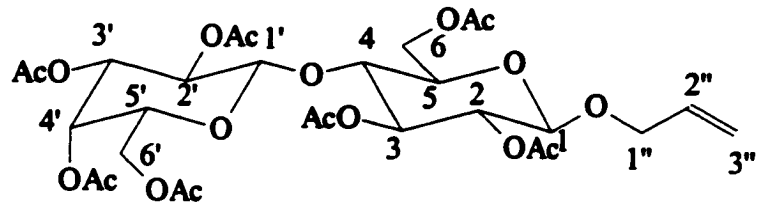
Boron trifluoride etherate (3.25 mL, 25.65 mmol) was added to a solution of peracetylated galactose (2.00 g, 5.13 mmol) and allyl alcohol (0.7 mL, 10.26 mmol) in dry CH_2Cl_2 (40 mL). The reaction mixture was stirred for 6h at room temperature and monitored by TLC (hexane/ethyl acetate 1/1) until the starting material was consumed. The reaction mixture was then washed with 100 mL of aqueous saturated $NaHCO_3$. Drying (Na_2SO_4) and evaporation of the organic phase gave a crude residue that was purified by flash chromatography on silica gel using a gradient of hexanes/ethyl acetate 3/1 to 1/1 as eluent. Title compound **73** was isolated as a white solid in 80% yield (1.59 g, 4.10 mmol); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.72-5.60 (m, 1H, H-2'), 5.19 (d, J=3.3 Hz, 1H, H-4), 5.13-4.96 (m, 3H, H-2, H-3'a, H-3'b), 4.85 (dd, J= 3.3 Hz, 10.3 Hz, 1H, H-3), 4.37 (d, J= 7.8 Hz, 1H, H-1), 4.21-4.11 (m, 1H, H-1'a), 3.99-3.87 (t, J= 6.2 Hz, 1H, H-5), 1.95, 1.86, 1.84, 1.68 (4s, 12H, OAc); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 170.7, 170.6, 170.4, 169.7 (C=O), 133.9 (C-2'), 117.8 (C-3'), 100.5 (C-1), 71.3 (C-3), 71.0 (C-5), 70.3 (C-1'), 69.3 (C-2), 67.6 (C-4), 61.8 (C-6), 21.1, 21.0, 20.9 (Me); Physical data in agreement with reported values.



4-Pentenyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (75)[25]

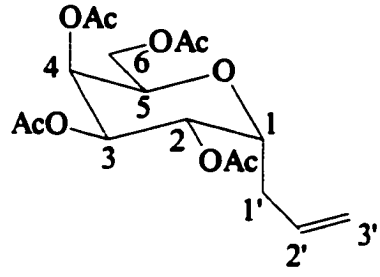
A solution of peracetylated galactose **72** (1.00 g, 2.56 mmol) in 4 mL of 30 wt. % HBr in glacial acetic acid was stirred for 1h. The reaction mixture was monitored by TLC (hexane/ethyl acetate 2/1) and diluted with $CHCl_3$ (30 mL) and washed with saturated aqueous $NaHCO_3$. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to give galactosyl bromide **74** as a white foam which was dried under vacuum before its utilization in the glycosidation.

Silver triflate was evaporated with toluene and then dried under vacuum. 4-penten-1-ol (0.6 ml, 5.81 mmol) was added to a mixture of the dried silver triflate (0.79 g, 3.07 mmol) and 0.9 g of powdered activated 4 Å molecular sieves in dry CH_2Cl_2 (10 mL) at 0° C. The galactosyl bromide **74** dissolved in dry CH_2Cl_2 (5 mL), was added gradually. The progress of the reaction was monitored by TLC (hexane/ethyl acetate 2/1) and judged complete after 1h. The reaction mixture was then quenched with saturated aqueous $NaHCO_3$, diluted with CH_2Cl_2 and filtered. The filtrate was washed with saturated aqueous $NaHCO_3$. The organic layer was dried over Na_2SO_4, evaporated under reduced pressure and purified via flash chromatography on silica gel using a gradient of hexane/ethyl acetate 5/1 to 1/1 as eluent. Title compound **75** was isolated as a white oil in 77 % yield (816 mg, 1.96 mmol); ^1H NMR ($CDCl_3$, 500 MHz): δ(ppm) 5.76-5.67 (m, 1H, h-4'), 5.33 (dd, J= 0.9, 3.3 Hz, 1H, H-4), 5.18 (dd, J= 7.9, 10.5 Hz, 1H, H-2), 5.01-4.89 (m, 3H, H-3, H-5'a, H-5'b), 4.40 (d, J= 7.9 Hz, 1H, H-1), 4.10 (dd, J= 5.0, 6.8 Hz, 2H, H-6a, H-6b), 3.89-3.80 (m, 2H, H-5, H-1'a), 3.48-3.42 (m, 1H, H-1'b), 2.10 (s, 3H, OAc), 2.06-2.02 (m, 2H, H-3'), 2.01, 2.00, 1.94 (3s, 9H, OAc), 1.75-1.61 (m, 2H, H-2'); ^{13}C NMR ($CDCl_3$, 125 MHz): δ(ppm) 171.0, 170.9, 170.8, 170.0 (C=O), 138.4 (C-4'), 115.7 (C-5'), 101.9 (C-1), 71.5 (C-3), 71.1 (C-5), 70.0 (C-1'), 69.5 (C-2), 67.6 (C-4), 61.9 (C-6), 30.4 (C-2'), 29.1 (C-3'), 21.3, 21.3, 21.2 (Me); Physical data in agreement with reported values.



Allyl 2,2',3,3',4',6,6'-Hepta-O-acetyl-α-D-lactopyranoside (77)

Following the methodology described for **73**, title compound **77** was isolated in 67% yield (6.47 g, 16.67 mmol); ^{1}H NMR ($CDCl_3$, 200 MHz): 5.83-5.65 (m, 1H, H-2''), 5.26-4.78 (m, 7H), 4.45-4.36 (m, 3H), 4.26-4.16 (m, 1H), 4.09-3.92 (m, 4H), 3.84-3.68 (m, 2H), 3.54-3.48 (m, 1H), 2.06, 2.03, 1.99, 1.95, 1.95, 1.87 (6s, 21H, OAc); ^{13}C NMR ($CDCl_3$, 125 MHz): δ(ppm) 170.2, 170.1, 169.9, 169.7, 169.5, 169.0 (C=O), 133.2 (C-2''), 117.5 (C-3''), 100.9, 99.2 (C-1, C-1'), 76.2, 72.7, 72.5, 71.5, 70.8, 70.5, 69.9, 69.0, 66.5, 61.9, 60.7, 20.7, 20.7, 20.5, 20.4; ESI-MS, m/z= 715.0, $[M+K]^+$ calcd for $C_{29}H_{40}O_{18}$+ K.



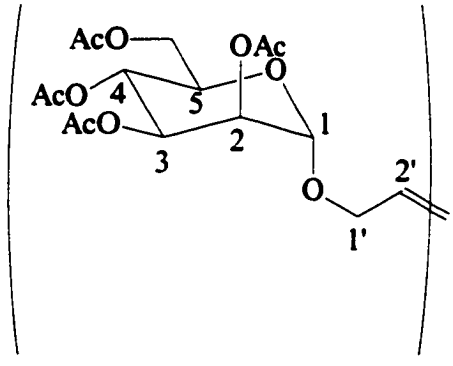
3-(2,3,4,6-Tetra-O-acetyl-α-D-galactopyranosyl)-1-propene (78)[16]

Peracetylated galactose **72** (2.00 g, 5.13 mmol) was dissolved in 25 mL of acetonitrile. Then, allyltrimethylsilane (2.44 mL, 15.39 mmol) and boron trifluoride etherate (3.16 ml, 25.65mmol) were added successively under an atmosphere of nitrogen. The reaction was stirred at 0° C and allowed to warm to room temperature. The reaction was monitored by TLC (hexane/ethyl acetate 2/1) and after consumption of the starting material, the reaction mixture was poured into a saturated aqueous $NaHCO_3$ solution. The product was extracted 3 times with CH_2Cl_2. The organic layer was dried over Na_2SO_4, evaporated under reduced pressure and purified via flash chromatography on silica gel using a gradient of hexane/ethyl acetate 5/1 to 2/1 as eluent Title compound **78** was

isolated as a solid in 77% yield (816 mg, 1.96 mmol). The product was contaminated with a small amount of the β anomer. Recrystallization with a mixture of hexanes and ether afforded the pure α anomer; m.p. 39-42 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.75-5.67 (m, 1H, H-2'), 5.37 (dd, J= 2.1, 3.2 Hz, 1H, H-4), 5.22 (dd, J= 4.8, 9.3 Hz, 1H, H-2), 5.17 (dd, J= 3.2, 9.3 Hz, 1H, H-3), 5.10-5.01 (m, 2H, H-3'a, H-3'b), 4.25 (ddd, J= 4.8, 4.8, 10.1 Hz, 1H, H-1), 1.18-4.13 (m, 1H, H-6a), 4.08-4.02 (m, 2H, H-5, H-6b), 2.42 (ddd, J= 7.7, 9.3, 16.4 Hz, 1H, H-1'a), 2.27-2.20 (m, 1H, H-1'b), 2.07, 2.02, 1.99, 1.98 (4s, 12H, OAc); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 170.4, 170.0, 169.8, 169.7 (C=O), 133.3 (C-2'), 117.6 (C-3'), 71.4 (C-1), 68.2 (C-2, C-3), 67.9 (C-5), 67.5 (C-4), 61.4 (C-6), 30.9 (C-1'), 20.7, 20.6, 20.5 (Me); Physical data in agreement with reported values.

General procedure for olefin self-metathesis reaction.

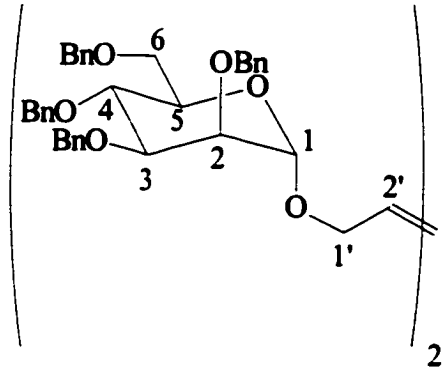
To a solution of alkenyl glycoside (0.14 mmol) in dry CH_2Cl_2 (1 mL) and under nitrogen atmosphere was added Grubbs' catalyst (10 mg, 0.014 mmol). The resulting purple colored solution was stirred and allowed to reflux for 6h, to give a black solution which was directly purified by flash chromatography.



(E,Z) Di-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyloxy)-1,4-but-2-ene (79).

Homodimer **79** was purified by flash chromatography on silica gel using hexanes/ethyl acetate as eluent (2/1) and obtained as syrup of unseparated E/Z stereoisomers (3:1) in 93% yield ; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.79 (t, J= 3.0 Hz, 2H, H-2'E), 5.74 (t, J= 4.2 Hz, 2H, H-2'Z), 5.33 (dd, J=3.5, 10.0 Hz, 2H, H-3), 5.27

(dd, J=3.7, 10.0 Hz, 2H, H-3), 5.27 (dd, J=3.7, 10.0 Hz, 2H, H-4), 5.23 (dd, J=1.8, 3.5 Hz, 2H, H-2), 4.83 (d, J= 1.6 Hz, 2H, H-1), 4.29-4.26 (m, 2H, H-6a), 4.20-4.18 (m, 2H, H-1'a), 4.10-4.07 (m, 2H, H-6b), 4.02-3.97 (m, 4H, H-5, H-1'b), 2.13, 2.09, 2.02, 1.97 (4s, 24H, OAc); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 170.6, 170.0, 169.8, 169.7 (C=O), 128.8 (C-2'Z), 128.6 (C-2'E), 96.9 (C-1Z), 96.8 (C-1E), 69.6 (C-2), 69.1 (C-3), 68.6 (C-5), 67.4 (C-1'E), 61.2 (C-4), 63.3 (C-1'Z), 62.5 (C-6), 20.8, 20.7, 20.7, 20.6 (Me). FAB-MS calcd. For $C_{32}H_{44}O_{20}$: 748.68; found 749.2 (M^+ + 1).



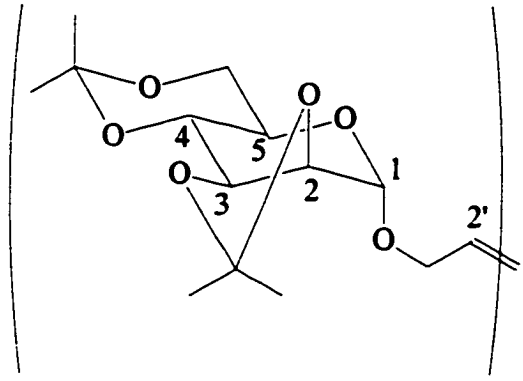
(E) Di-O-(2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyloxy)-1,4-but-2-ene (80a)

Homodimer **80** was obtained in 77% in a 3/1 (*E/Z*) ratio and was purified by flash chromatography on silica gel using hexanes/ethyl acetate as eluent (5/1) and obtained as pure E isomer. ^1H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.39-7.15 (m, 20H, Ph), 5.72 (t, J= 2.8 Hz, 1H, H-2'), 4.90-4.49 (m, 9H, H-1, 4 x CH_2), 4.15-4.12 (m, 1H, H-1'a), 4.01-3.97 (m, 1H, H-1'b), 3.94-3.89 (m, 2H), 3.82-3.81 (m, 1H), 3.79-3.71 (m, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 138.8, 138.7, 138.6, 138.6, 128.7, 128.5, 128.5, 128.2, 128.0, 128.0, 127.8, 127.7, 127.7 (Ph), 97.5 (C-1), 80.5, 75.4 (CH_2), 75.2, 74.9, 73.6 (CH_2), 72.9 (CH_2), 72.4 (CH_2), 72.2, 69.5 (C-6), 67.0 (C-1'); ESI-MS, m/z= 1171.2, $[M+K]^+$ calcd for $C_{72}H_{76}O_{12}$+ K.

(Z) Di-O-(2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyloxy)-1,4-but-2-ene (80b)

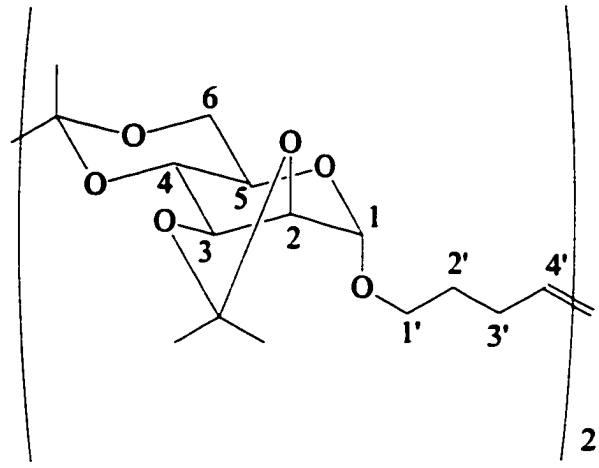
Homodimer **80b** was purified by flash chromatography on silica gel using hexanes/ethyl acetate as eluent (5/1) and obtained as pure *Z* isomer. ^1H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.38-7.15 (m, 20H, Ph), 5.60 (t, J= 4.1 Hz, 1H, H-2'), 4.87-4.50 (m, 9H, H-1, 4 x CH_2), 4.16 (dd, J= 3.4, 12.5 Hz, H-1'a), 4.02-3.97 (m, 2H), 3.88 (dd, J= 2.9,

9.4 Hz, 1H), 3.78-3.68 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 138.8, 138.7, 138.6, 138.6, 129.0, 128.5, 128.5, 128.2, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6 (Ph), 97.6 (C-1), 80.5, 75.3 (CH_2), 75.2, 75.0, 73.6 (CH_2), 72.9 (CH_2), 72.4 (CH_2), 72.3, 69.5 (C-6), 63.0 (C-1'); ESI-MS, m/z= 1171.2, $[M+K]^+$ calcd for $C_{72}H_{76}O_{12}$+ K.



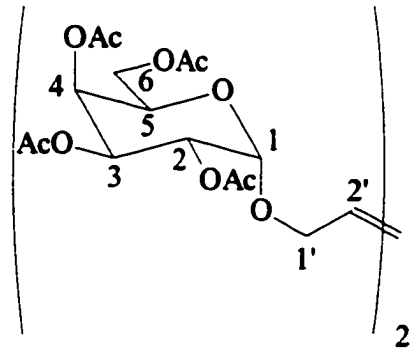
(E) Di-O-(2,3:4,6-di-O-isopropylidene-α-D-mannopyranosyloxy)-1,4-but-2-ene (81)

Homodimer **81** was obtained in 96% yield in a 5/1 (*E/Z*) ratio and was purified by flash chromatography on silica gel using hexanes/ethyl acetate as eluent (5/1). Only the E isomer was isolated. ^1H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.77 (t, J= 2.9 Hz, 2H, H-2'), 5.02 (s, 2H, H-1), 4.16-4.05 (m, 6H, H-1'a, H-3, H-4), 3.97-3.94 (m, 2H, H-1'b), 3.83 (dd, J= 5.7, 10.8 Hz, 2H, H-6'a), 3.74-3.68 (m, 4H, H-2, H-6b), 3.57-3.52 (m, 2H, H-5), 1.50, 1.47, 1.38, 1.31 (4s, 12H, Me); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 128.8 (C-2'), 109.4. 99.6 (MeCMe), 97.1 (C-1), 76.0, 74.8, (C-3, C-4), 72.7 (C-2), 66.9 (C-1'), 62.0 (C-6), 61.4 (C-5), 29.0, 28.1, 26.1, 18.7 (Me); ESI-MS, m/z= 590.2, $[M+NH_4]^+$ calcd for $C_{28}H_{44}O_{12}$+ NH_4.



(E) Di-O-(2,3:4,6-di-O-isopropylidene-α-D-mannopyranosyloxy)-1,8-oct-4-ene (82)

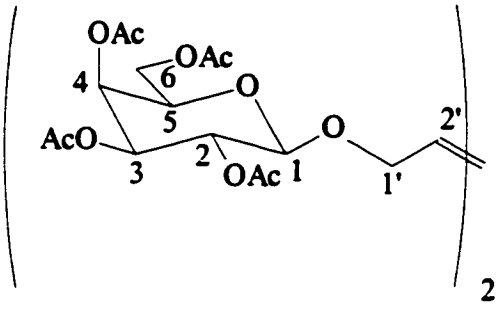
Homodimer **82** was obtained in 93% yield in a 5/1 (*E/Z*) ratio and was purified by flash chromatography on silica gel using hexane/ethyl acetate (5/1) as eluent. Only the E isomer was isolated. ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.39 (t, J= 3.3 Hz, 2H, H-4'), 4.96 (s, 2H, H-1), 4.14-4.08 (m, 4H, H-3, H-4), 3.83 (dd, J= 5.5, 10.7 Hz, 2H, H-6a), 3.74-3.62 (m, 6H, H-2, H-6b, H-1'a), 3.53 (ddd, J= 5.5, 10.2 Hz, 2H, H-5), 3.40-3.35 (m, 2H, H-1'b), 2.11-2.01 (m, 4H, H-3'), 1.65-1.56 (m, 4H, H-2'), 1.51, 1.48, 1.38, 1.32 (4s, 24H, Me); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 129.9 (C-4'), 109.3, 99.6 (MeCMe), 97.7 (C-1), 76.1, 74.9 (C-3, C-4), 72.7 (C-2), 67.1 (C-1'), 62.0 (C-6), 61.2 (C-5), 29.1 (C-2'), 29.0 (Me), 28.9 (C-3'), 28.1, 28.1, 18.7 (Me); CI-MS, m/z= 629, $[M+1]^+$ calcd for $C_{32}H_{52}O_{12}$.



(E,Z) Di-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyloxy)-1,4-but-2-ene (83)

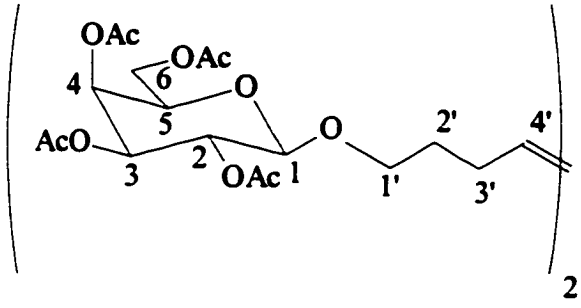
Homodimer **83** was obtained in 92% yield in a 5/1 (*E/Z*) and was purified by flash chromatography on silica gel using hexanes/ethyl acetate as eluent (2/1) and obtained as

syrup of unseparated *E/Z* stereoisomers. ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.78 (t, J=2.8 Hz, 2H, H-2'E), 5.71 (t, J= 3.9 Hz, 2H, H-2'Z), 5.43 (dd, J= 1.3, 3.4 Hz, 2H, H-4), 5.36-5.30 (m, 2H, H-3), 5.12-5.07 (m, 4H, H-1, H-2), 4.22-4.16 (m, 4H, H-5, H-1'a), 4.07 (d, 4H, J=3.9 Hz, H-6a, H-6b), 4.01-3.98 (m, 2H, H-1'b), 2.11, 2.06, 2.02, 1.95 (4s, 24H, OAc); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 170.3, 170.3, 170.1, 169.9 (C=O), 128.8 (C-2'Z), 128.5 (C-2'E), 95.6 (C-1Z), 95.5 (C-1E), 68.1 (C-4), 68.0 (C-3), 67.5 (C-1'E), 67.5 (C-2), 66.3 (C-5), 63.5 (C-1'Z), 61.6 (C-6), 20.7, 20.6, 20.6 (Me). FAB-MS calcd. For $C_{32}H_{44}O_{20}$: 748.68; found 749.2 (M^+ + 1).



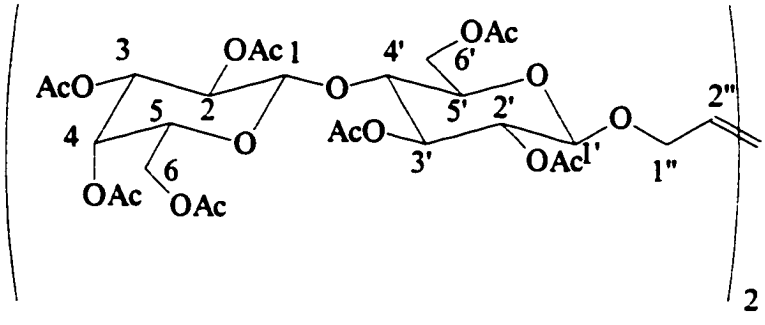
(E,Z) Di-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyloxy)-1,4-but-2-ene (84)

Homodimer **84** was purified by flash chromatography on silica gel using hexanes/ethyl acetate as eluent (2/1) and obtained as syrup of unseparated E/Z stereoisomers (4:1) in 95% yield; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.67 (t, J=2.8 Hz, 2H, H-2'E), 5.62 (t, J= 4.1 Hz, 2H, H-2'Z), 5.32 (dd, J= 1.0, 3.4 Hz, 2H, H-4), 5.14 (dd, J= 8.9, 10.5 Hz, 2H, H-2), 4.96 (dd, J= 3.4, 10.5 Hz, 2H, H-3), 4.44 (d, J= 8.0 Hz, 2H, H-1), 4.30-4.27 (m, 2H, H-1'a), 4.15-4.00 (m, 6H, H-1'a, H-6a, H-6b), 3.85 (t, J= 6.4 Hz, 2H, H-5), 2.07, 1.99, 1.97, 1.90 (4s, 24H, OAc); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 170.2, 170.1, 169.9, 169.3 (C=O), 128.6 (C-2'Z), 128.2 (C-2'E), 100.1 (C-1), 70.7 (C-3), 70.5 (C-5), 68.7 (C-1'E), 68.7 (C-2), 66.9 (C-4), 64.9 (C-1'Z), 61.1 (C-6), 20.6, 20.5, 20.5, 20.4 (Me); FAB-MS calcd. For $C_{32}H_{44}O_{20}$: 748.68; found 749.2 (M^+ + 1).



(E,Z) Di-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyloxy)-1,8-oct-4-ene (85)

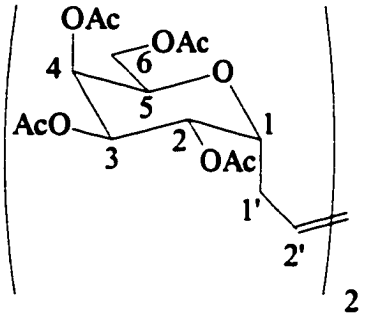
Homodimer **85** was purified by flash chromatography on silica gel using hexanes/ethyl acetate as eluent (2/1) and obtained as syrup of unseparated *E/Z* stereoisomers (5:1) in 85% yield; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.34-5.33 (m, 4H, H-4, H-4'), 5.15 (dd, J= 7.9, 10.5 Hz, 2H, H-2), 4.96 (dd, J= 3.5, 10.5 Hz, 2H, H-3) 4.40 (d, J= 7.9 Hz, 2H, H-1), 4.15-4.05 (m, 4H, H-6a, H-6b), 3.87-3.82 (m, 4H, H-5, H-1'a), 3.45-3.42 (m, 2H, H-1'b), 2.09, 2.00, 1.99, (3s, 18H, OAc), 1.99-1.98 (m, 4H, H-3'), 1.92 (s, 6H, OAc), 1.65-1.58 (m, 4H, H-2'); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 170.3, 170.2, 170.1, 169.2 (C=O), 129.9 (C-4'E), 129.4 (C-4'Z), 101.2 (C-1), 70.9 (C-3), 70.5 (C-5), 69.4 (C-1'), 68.9 (C-2), 67.0 (C-4), 61.2 (C-6), 29.4 (C-2'Z), 29.2 (C-2'E), 28.5 (C-3'E), 23.3 (C-3'Z), 20.7, 20.6, 20.5 (Me); FAB-MS calcd. For $C_{36}H_{52}O_{20}$: 804.79; found 805.25 (M^+ + 1).



(E) Di-O-(2,2',3,3',4',6,6'-Hepta-O-acetyl-α-D-lactopyranosyloxy)-1,4-but-2-ene (86)

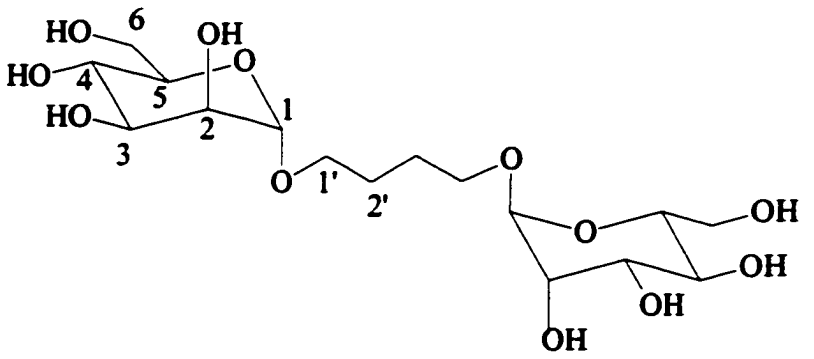
Homodimer **86** was isolated in 89% yield in a 4/1 (*E/Z*) ratio and was purified by flash chromatography on silica gel using hexanes/ethyl acetate as eluent (1/1).Only the E isomer was isolated; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.60 (t, J=3.3 Hz, 2H, H-2''), 5.30 (dd, J= 0.8, 2.7 Hz, 2H, H-4), 5.14 (t, J= 9.2 Hz, 2H, H-3'), 5.05 (dd, J=7.8, 10.1 Hz, 2H, H-2), 4.93 (dd, J= 3.4, 10.1 Hz, 2H, H-3), 4.87 (dd, J=7.0, 9.2 Hz, 2H, H-2'), 4.47-

4.44 (m, 6H, H-6'a, H-1, H-1'), 4.26-4.23 (m, 2H, H-1''a), 4.12-3.97 (m, 8H, H-6'b, H-6a, H-6b, H-1''b), 3.82 (t, J= 6.6 Hz, 2H, H-5), 3.75 (t, J= 9.6 Hz, 2H, H-4'), 3.55-3.53 (m, 2H, H-5'), 2.11, 2.09, 2.02, 2.01, 2.00, 1.93 (6s, 48H, OAc); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 170.2, 170.1, 170.0, 169.7, 169.5, 169.0 (C=O), 128.3 (C-2''), 101.0, 99.5 (C-1, C-1'), 76.1 (C-4'), 72.7 (C-3'), 72.6 (C-5'), 71.6 (C-2'), 70.9 (C-3), 70.6 (C-4'), 69.1 (C-2), 68.8 (C-1''), 66.6 (C-4), 61.9 (C-6'),20.9, 20.8, 20.7, 20.6, 20.5, 20.4 (Me); FAB-MS calcd. For $C_{56}H_{76}O_{34}$: 1325.18; found 1326.65 (M^+ + 1).



(E,Z) Di-C-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-1,4-but-2-ene (87)

Homodimer **87** was purified by flash chromatography on silica gel using hexanes/ethyl acetate as eluent (2/1) and obtained as syrup of unseparated *E/Z* stereoisomers (2:1) in 82% yield; ^1H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.48 (t, J= 4.5 Hz, 2H, H-2'E), 5.45 (t, J= 3.7 Hz, 2H, H-2'Z), 5.36 (t, J= 3,4 Hz, 2H, H-4), 5.18 (dd, J= 4.6, 9.2 Hz, 2H, H-2), 5.15 (dd, J= 3.2, 9.2 Hz, 2H, H-3), 4.22-4.17 (m, 4H, H-1, H-6a), 4.08-4.01 (m, 4H, H-5, H-6b), 2.38-2.35 (m, 2H, H-1'a), 2.22-2.16 (m, 2H, H-1'b), 2.07, 2.03, 2.01, 1.99 (4s, 24H, OAc); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 170.4, 170.0, 169.8, 169.7 (C=O), 128.0 (C-2'E), 126.7 (C-2'Z), 71.3 (C-1), 68.2 (C-2, C-3), 67.8 (C-5), 67.2 (C-4), 60.9 (C-6), 29.8 (C-1'E), 24.8 (C-1'Z), 20.7, 20.7, 20.6, 20.6 (Me); FAB-MS calcd. For $C_{32}H_{44}O_{18}$: 716.68; found 717.2 (M^+ + 1).



1,4-Bis(α-D-mannopyranosyloxy)butane (88)

To a solution of homodimer **80** (50 mg, 0.044 mmol) was added 10% palladium/ carbon (10 mg) and the reaction mixture was stirred under a hydrogen atmosphere at room temperature for 3h. The catalyst was filtered off over celite and the filtrate concentrated to afford compound **88** in quantitative yield (18 mg, 0.044 mmol) as a syrup. $[\alpha]_D$= +76 (c=1, in water); ^{1}H NMR (D_2O, 200 MHz): δ (ppm) 4.82 (s, 2H, H-1), 3.88-3.47 (m, 16H, H-1', H-2, H-3, H-4, H-5, H-6), 1.65 (bs, 4H, H-2'); ^{13}C NMR (D_2O , 125 MHz): δ (ppm) 101.1 (C-1), 74.2, 72.1, 71.7, 68.9, 68.2 (C-1', C-2, C-3, C-4, C-5, 62.4 (C-6), 26.9 (C-2'); HRMS calcd for [M+1] $C_{16}H_{30}O_{12}$: 415.1816; found 415.1800.

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Chapter 3. Synthesis of extended alkenyl glycosides.

3.1. Introduction

The extension of carbon skeletons by the construction of carbon-carbon bonds represents one of the most important areas in synthetic organic chemistry. Ruthenium-catalyzed cross-metathesis reaction is especially valuable in this regard and offer a convenient route to extend alkenyl glycosides with interesting functional groups which could be further transformed into useful neoglycoconjugates including proteins, dendrimers, and polymers (figure 3.1.1.).

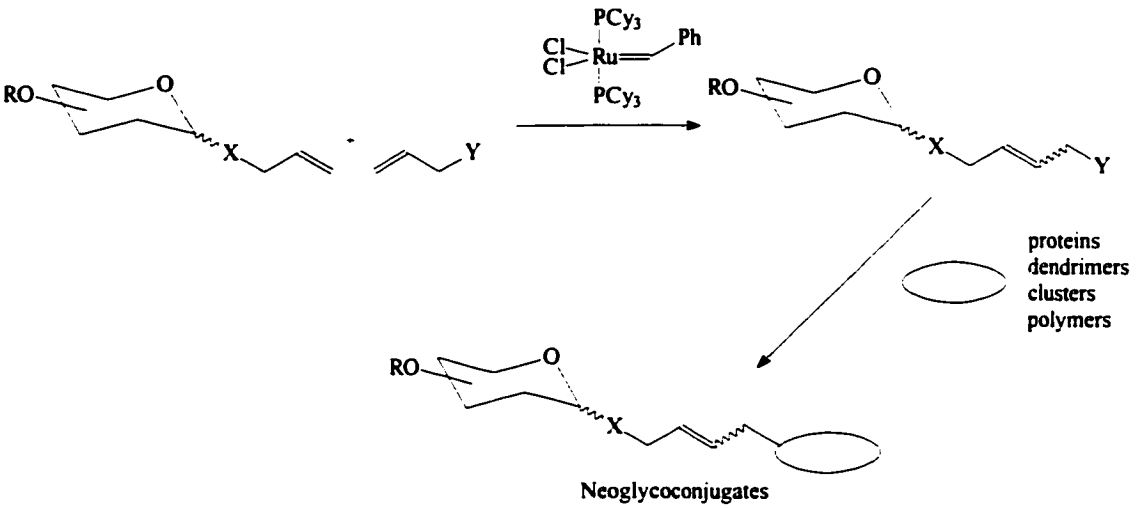
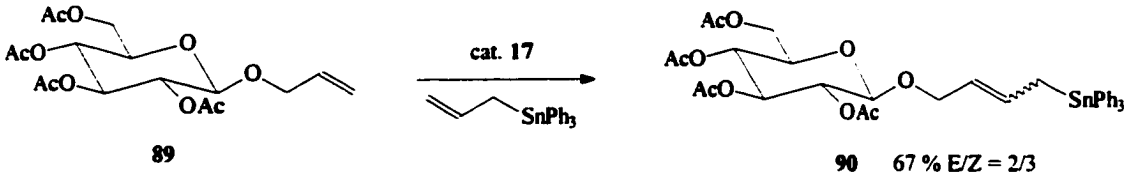


Figure 3.1.1. Extension of alkenyl glycosides.

The cross metathesis reaction represents a unique method for the formation of carbon-carbon double bonds but has not yet found widespread application in organic synthesis. This is largely due to the complex mixture which results from non-selective cross metathesis reaction. Generally, this reaction proceeds to yield three unique products: one desired heterodimeric product and two undesired homodimeric products, each as a mixture of olefin stereoisomers. For a successful application of this type of reaction, the reaction mixture should be composed predominantly of the desired

heterodimeric product and offer a certain control on the configuration of the newly formed double bond (i.e. the *E/Z* ratio).

The first example of selective CM reaction applied to carbohydrate substrates was reported by Blechert *et al.* where he showed that with a two fold excess of allyltriphenylstannane, the allylstannanes were prepared in reasonable yields using Schrock's catalyst[1] (Scheme 3.1.1).



Scheme 3.1.1. First example of selective cross-metathesis reaction with carbohydrates

Herein, we report the selective CM reaction catalyzed by Grubbs' catalyst of O- and C-allyl glycoside derivatives and its application toward novel C-linked pseudodisaccharides. A novel procedure for the deprotection of allyl ethers will also be described.

3.2. Results and Discussion

3.2.1. Selective cross-metathesis reaction with *O*-alkenyl glycosides

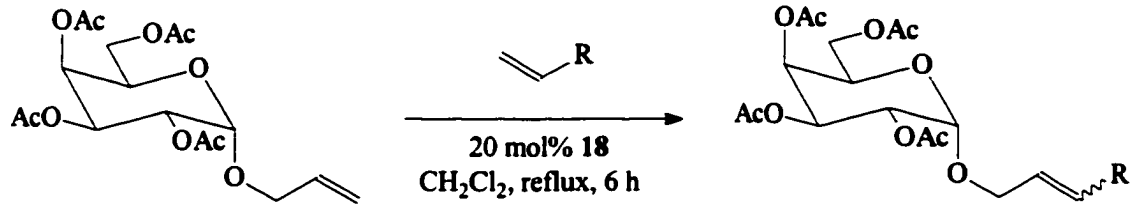
In order to obtain glycosides having extended spacers and functionality in the aglycon moiety, the selective cross metathesis reaction were performed with different alkenes bearing interesting functional groups. To determine the best conditions suitable for a selective cross-methathesis reaction, we chose to use allyltrimethylsilane which is known to offer good selectivity for the cross-metathesis product due to the pronounced nucleophilic character of the silane (β effect of silicon). Moreover allylsilanes hold tremendous utility as reagents and reactions intermediates in organic synthesis.[2] Thus, the cross-metathesis reaction of **71** (0.06 M solution of dry CH_2Cl_2) with 2 equiv. of

allyltrimethylsilane and 20 mol% of Grubbs' catalyst under reflux conditions gave the desired substituted allylsilane derivative **91** in 94% yield (based on allyl glycoside) as a mixture of *trans* and *cis* stereoisomers in a ratio of 4:1 which were assigned on the basis of ^{1}H NMR spectrum. The chemical shift of the α carbon to the double bond in the *Z* isomer appeared ca. 5 ppm upfield from that of the *E* isomer due to the γ effect. Those conditions were the best found, however they were not the first ones tried. Attempts to vary the catalyst load or the concentration of the reaction mixture showed that these were critical factors for the selective cross-metathesis reaction. Therefore, when the reaction was repeated with 10 mol% of catalyst, the yield decreased to 50 %. The remaining starting material was recovered along with 10% of homodimer **83** resulting from self-metathesis of **71**. The use of 0.13 M solution of **71** in CH_2Cl_2 gave also a lower yield of cross-metathesis product due to competitive homodimer formation. To further explore the scope and generality of this method, this reaction was performed with a variety of alkenes.Table 3.2.1 is summarizing the results.

The reaction of **71** with methyl 4-pentenoate under the optimal conditions gave 67% of the cross-metathesis product **92** with modest *trans* selectivity (ca. 2:1) while treatment with allyloxy-tert-butyldimethylsilane gave derivative **93** with high *trans* selectivity (ca. 95:5) without diminishing the overall yield (69% as unseparable isomers). Once the reaction conditions were optimized for aliphatic alkenes, the selective cross-metathesis was directed toward aromatic alkenes such as styrene and 4-acetoxystyrene. The reaction between **71** and 2 equiv. of styrene gave 60% of the desired heterodimeric product **94** but the use of 4 equiv. of styrene was found to be more efficient and gave 80% with excellent trans-stereoselectivity (97:3). In a similar manner, treatment of **71** with 4 equiv.. of 4-acetoxystyrene gave 75% of the desired product **95**. In both cases, the pure *E* isomers were isolated. Although 4-acetoxystyrene contains an electron-withdrawing group, the yield and the stereoselectivity were found to be similar to that of styrene, implying that inductive effects does not play a significant role in this case. This high *trans*-stereoselectivity observed with bulky substituents or styrene derivatives has been previously observed.[3]

To investigate the influence of symmetrically disubstituted olefin in cross-metathesis, we prepared *cis*-2-butene-1,4-diol diacetate. The reaction proceeded smoothly and produced 70 % of the cross-metathesis product **96** in a 5:1 *E/Z* ratio of unseparable isomers.

Table 3.2.1: Isolated yields and *E/Z* ratios of ruthenium catalyzed cross-metathesis of **71** with different alkenes.



71 **91-98**

Entry	R	Equiv.	Reaction Conditions	Product	Yield	Ratio
1	CH_2SiMe_3	2	A	**91**	94	4:1
2	$CH_2CH_2CO_2Me$	2	A	**92**	67	2:1
3	CH_2OTBS	2	A	**93**	69	95:5
4	Ph	2	A	**94**	60	90:10
5	Ph	4	A	**94**	80	97:3
6	p-AcOPh	4	A	**95**	75	95:5
7	$(=CHCH_2OAc)_2$	2	A	**96**	70	5:1
8	CH_2NHBoc	2	A	**97**	30	4:1
9	CH_2NHBoc	2	B	**97**	57	4:1
10	CH_2NHCbz	2	A	**98**	39	4:1
11	CH_2NHCbz	2	B	**98**	65	4:1

Method A: CH_2Cl_2, reflux, 6h; **method B:** CH_2Cl_2; rt, 15 h

To extend alkenyl glycosides with amine containing substrates, the reaction was accomplished with allylamine and **71**, no cross-metathesis product was formed. The presence of the free amino group apparently poisoned the Grubbs' catalyst. This problem was, however easily overcome by using Boc-protected allylamine. The metathesis

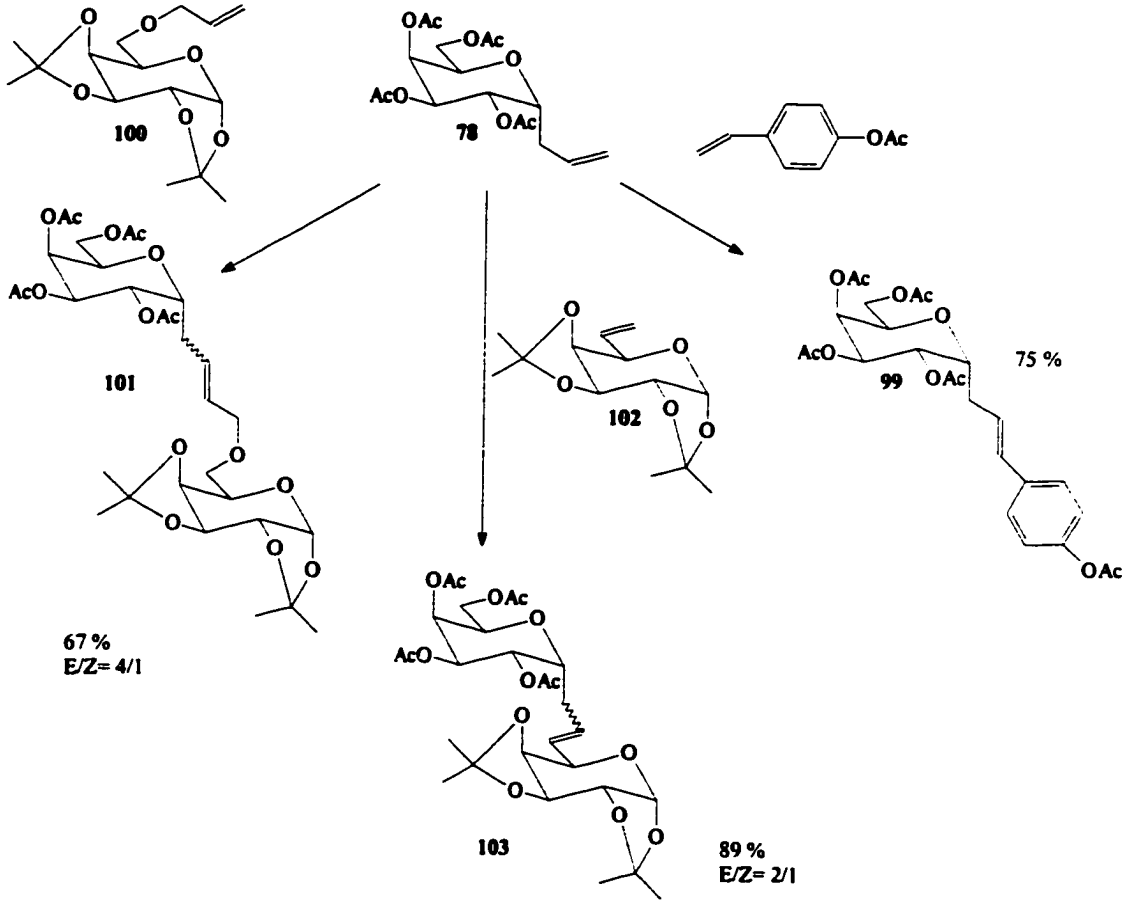
product provided a direct route to the corresponding protected allylamine. Thus, treatment of 0.06 M solution of **71** in dry CH_2Cl_2 with 2 equiv. of N-(tert-butoxycarbonyl)allylamine[4] at 40° C gave **97** in a disappointing 30% yield. Nevertheless, the yield could be improved by using 0.13 M solution of **71** and reached 57 % when the reaction was carried out at room temperature. This is probably because the amide group deactivates the ruthenium catalyst at higher temperature. The *E/Z* ratio was found to be 4:1 and the pure *E* isomer could be isolated. No improvement was observed when the catalyst was added incrementally (4 times 5%). In a similar manner, when 2 equiv. of N-(benzyloxycarbonyl)allylamine was treated with **71** (0.13 M CH_2Cl_2 solution), compound **98** was obtained in 65% yield with a similar *E/Z* ratio.

The extension of O-alkenyl glycoside by this method offers an interesting alternative to the classical glycosidation procedure. We could also obtain the same derivative by performing the cross-metathesis reaction between two alkenes and then do a glycosidation. This is why this methodology was extended to C-alkenyl glycoside where in this case other alternative would necessitate several extra steps. In all cases mentioned above, 10-20% of self-metathesis starting materials that can be easily recovered and recycled in the cross-metathesis step were formed.

3.2.2. Selective cross-metathesis reaction with C-alkenyl glycoside

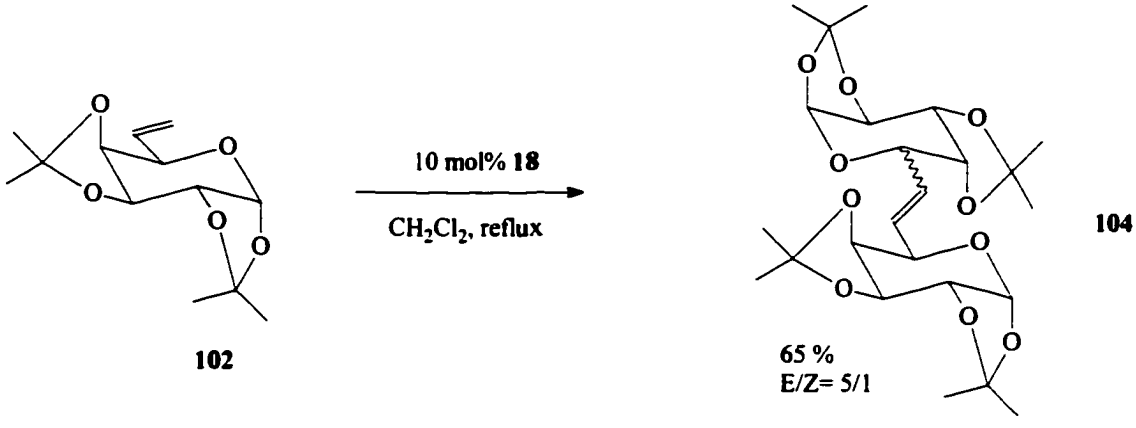
The cross-metathesis reaction between **78** (0.06 M solution in dry dichloromethane) and 2 equiv. of 4-acetoxystryrene led to the formation of the aryl derivative **99** in 50% yield. The yield could be further improved to 75% by using 4 equiv. of 4-acetoxystyrene (Scheme 3.2.1). Compound **99** was obtained as a single *E* isomer together with 19% of homodimer **87** (based on C-glycoside). It seems that the replacement of oxygen for a carbon gives more rigidity to the metallacyclobutane intermediate which is less free to rotate and ensure the formation of a single product. When treatment of the C-galactoside **78** was extended to 6-O-allyl-1,2:3,4-di-O-isopropylidene-α-D-galactopyranoside, the reaction provided **101** in 67% yield (*E/Z* 4:1) and 14% of homodimer **87** was isolated accordingly. Another potentially powerful application of this methodology was to prepare C-linked pseudodisaccharide that could

be used as glycomimetic precursors. For that, the other partner of the cross-metathesis reaction was prepared from 1,2:3,4-di-O-isopropylidene-α-D-galactopyranose by sequential oxidation using Swern conditions and Wittig homologation to afford 6,7-dideoxy-1,2:3,4-di-O-isopropylidene-α-D-galacto-hept-6-enopyranose **102**.[5] The metathesis reaction between **78** and 2 equiv. of the vinyl derivative **102** led to the formation of 89% the cross product **103**. Each stereoisomer was obtained pure after silica gel column chromatography where the *E* isomer was predominant (*E/Z* 2:1). The ^{1}H NMR of the two stereoisomers showed significant differences (see appendix).



Scheme 3.2.1. CM reaction of C-allyl galactoside derivative **78** catalyzed by **18**.

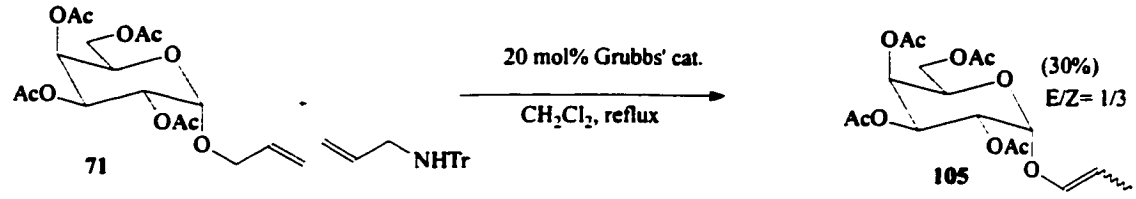
For control purposes, alkene **102** was separately homodimerized under the optimal conditions for the self metathesis. Homodimer **104** was obtained in 65% yield in a ratio *E/Z* 5:1 separable by column chromatography (Scheme 3.2.2.). A competitive experiment was also performed to compare the relative reactivity **78** of and **102** under the homodimerization conditions. Preliminary results indicated that, despite being apparently more hindered, alkene **102** was reacting a little faster than **78**. Presumably, this observation may result from a decreased reactivity of the catalyst originating from a better coordinating ability of the acetate groups of **78**. In some ways, this may represent another example of "armed" and "disarmed" reactivity.[6]



Scheme 3.2.2. Homodimerization of **102**.

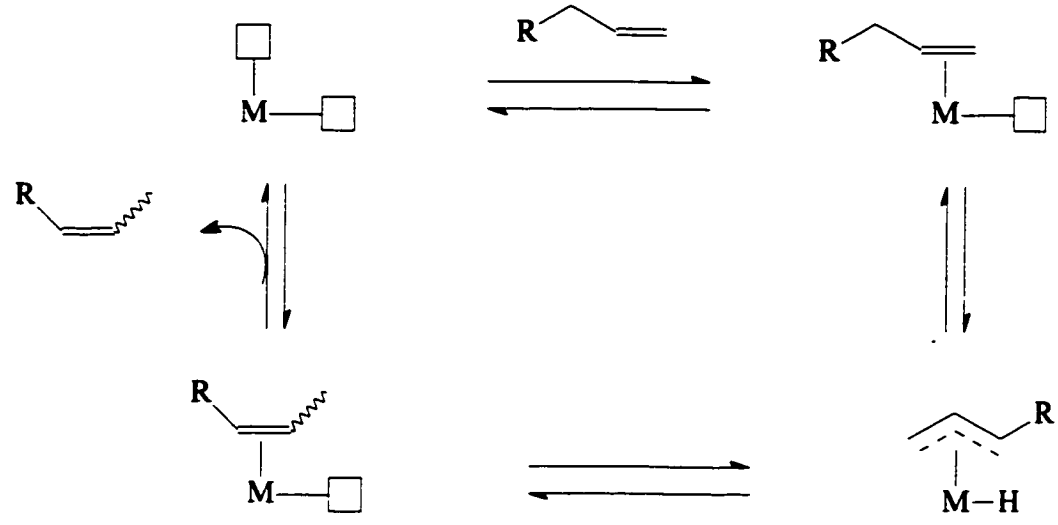
3.2.3. New procedure for the deprotection of allyl ethers

During our studies on the cross-metathesis reaction of O-alkenyl glycosides like allyl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside **71** and various alkenes with Grubbs' catalyst, an interesting side product arose from the reaction mixture when N-allyltritylamine was used. An unexpected product was isolated in 30% yield instead of the anticipated cross-metathesis compound. Furthermore, neither sugar nor N-allyltritylamine dimers could be detected. Careful inspection of the ^{1}H NMR (see appendix) and MS spectra revealed the formation of the isomerized product **105** where the terminal double bond became an internal double bond, in another word a 1,3-migration of hydrogen substituent on the alkene was observed (Scheme 3.2.3.).



Scheme 3.2.3. Isomerization of **71** catalyzed by **18**.

The mechanism shown in scheme 3.2.3 rationalize the formation of the isomerized product. The allyl mechanism is adopted by the metal fragments that have two $2e^-$ vacant sites. In this mechanism the C-H bond at the activated allylic position of the alkene undergoes an oxidative addition to the metal. The product is an η^3–allyl hydride which only needs a reductive elimination to give the isomerized alkene.[7]



Scheme 3.2.4. Allyl mechanism of alkene isomerization.

For a better understanding of this reaction, we decided to explore further this reaction. In this view, we thought that the presence of a base in the reaction mixture could help the isomerization, thus different bases were added to observe their influence on the formation of the isomerized product. Treatment of **71** with DABCO in the presence of Grubbs' catalyst afforded only 4% of the isomerized product **105** with 40% of homodimer **83**. Similar results were obtained when triethylamine was used as base. Neither isomerization

nor metathesis products were detected when diethylamine or pyridine were used. The isomerized product were obtained with the highest yield when **71** was treated with DIPEA in the presence of Grubbs' catalyst. However, all the isomerization yields of those reactions were still low in the absence of N-allyltritylamine. When both **71** and 1 equiv. of DIPEA were simultaneously used in the reaction, the isomerization yield was improved dramatically to 80% and no self-metathesis product could be detected.

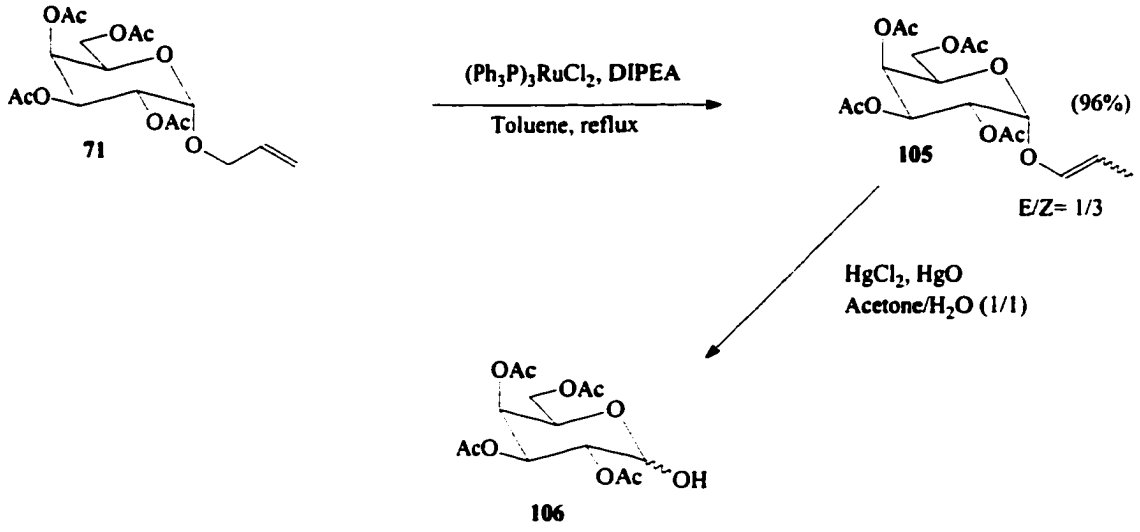
Bearing in mind the above observations, alternative bases which could provide both basicity and olefinic functionality were explored. To this end, several N-allylated amine derivatives were used to test the reaction. Several tertiary amines with increasing chain length also failed to give the isomerization product. These results showed that the strong steric hindrance of N-allyltritylamine had an important influence on the isomerization process as well as its basicity.

More detailled studies showed that the isomerization of allyl ethers to propenyl ethers proceeded smoothly in the presence of 10 mol % of Grubbs' catalyst, 2 equiv. of N-allyltritylamine and 1 equiv. of DIPEA in toluene, heated at reflux. Interestingly, those conditions were suppressing the formation of metathesis products, probably due to the bulky trityl groups which prevents the formation of the metallocyclobutane intermediate.

Although the Grubbs' catalyst could be used for this isomerization process, it is not really designed for this type of reaction. This is why, in search for a better catalytic system we turned ourselves toward the commercially available dichlorotris(triphenylphosphine)ruthenium(II)[$(PPh_3)_3RuCl_2$].

Treatment of allyl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside **71** with 10 mol% of catalyst in presence of 2 equiv. of DIPEA in refluxing toluene afforded the desired isomerized product in 96% yield.

Once the yields and the method optimized, the idea that this reaction could be used for the selective deprotection of the anomeric hydroxyl group came up since it is known that allyl ethers can be used as protecting groups and their deprotection involved initial isomerization of allyl to prop-1-enyl ethers.[8] Therefore, the labile prop-1-enyl group in the isomerized products were hydrolyzed using $HgCl_2$-HgO in aqueous acetone[9] (Scheme 3.2.5).



Scheme 3.2.5. Deprotection of allyl ether.

The whole procedure could be simplified as a two-step one-pot reaction: after isomerization of allyl ether, toluene and DIPEA were removed under reduced pressure and the resulting residue was then subjected to 0.5 equiv. of HgO and 1.0 equiv. of $HgCl_2$ to liberate the anomeric hydroxyl group. This method provides a new route towards the deprotection of O-allyl glycosides which has the big advantage to be performed under mild conditions.

3.3. Conclusions

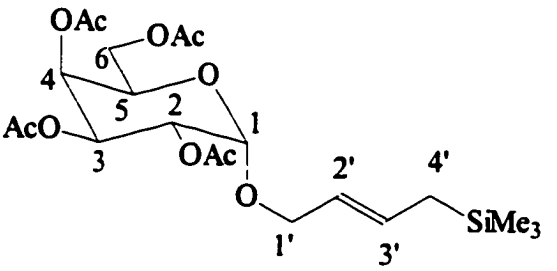
In conclusion, we have demonstrated the potential use of Grubbs' ruthenium benzylidene catalyzed cross-metathesis reaction for the introduction of functional groups on the aglycon moiety of O- and C-alkenyl glycosides.[10] It is a powerful synthetic method that allowed to prepare extended alkenyl glycosides bearing alcohols, acids, amines and aryl groups which could be easily further transformed into useful neoglycoconjugates. The main advantage of the cross-metathesis reaction for the preparation of extended alkenyl glycosides is its operational simplicity moreover, the reaction proceeded under very mild and neutral conditions.

We have also reported the utility of this reaction towards the synthesis of biologically interesting glycomimetic precursors like pseudodisaccharides where the two saccharide

units are linked with carbon atoms. This example, was to our knowledge the first example in the literature where the cross-metathesis reaction was used to synthesize such molecules. After publication of this work, other examples were reported using similar approaches.[11-12] Finally, a novel procedure was developed for the deprotection of allyl ethers involving an isomerization reaction which can be catalyzed by the Grubbs' catalyst.[13]

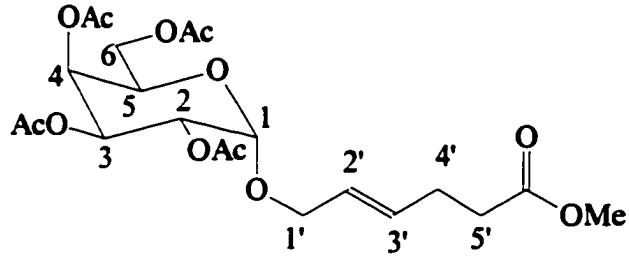
3.4. Experimental methods

General procedure for cross-metathesis reaction: To a 0.06 M solution of allyl glycoside **71** (50 mg, 0.129 mmol) in dry dichloromethane (2 mL), the appropriate alkene and Grubbs' catalyst (20 mol%) were added and the reaction mixture was heated at reflux under nitrogen for 6 h. The solution was concentrated under reduced pressure and the residue was purified by silica gel column chromatography using ethyl acetate and hexane as eluent.



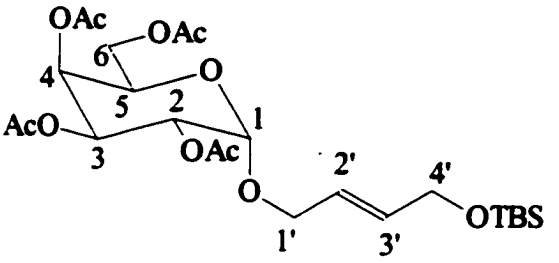
4-Trimethylsilanylbut-2-ene-1-yl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside (91). Compound **91** was purified by silica gel column chromatography using ethyl acetate and hexanes as eluent (2\1) and obtained in 94% yield as a syrup of unseparated *E/Z* stereoisomers (4:1); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.65 (ddd, J=8.2, 9.3, 15.2 Hz, 1H, H-3'), 5.45 (dd, J=1.4, 3.4 Hz, 1H, H-4), 5.35 (m, 2H, H-2', H-3), 5.13 (dd, J=3.7, 8.2 Hz, 1H, H-2), 5.12 (d, 1H, J=3.7 Hz, H-1), 4.22 (t, J=6.7 Hz, 1H, H-5), 4.10 (m, 3H, H-6a, H-6b, H-1'a), 3.93 (dd, J=7.5, 11.9 Hz, 1H, H-1'b), 2.12, 2.05. 2.03, 2.00 (4s, 12H, OAc), 1.48 (d, 2H, J=8.2 Hz, H-4'a, H-4'b), 0.00 (s, 9H, Me); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 170.4, 170.3, 170.2, 170.0 (CO), 133.4 (C-3' trans), 131.3 (C-3' cis), 122.9 (C-2' trans), 121.8 (C-2' cis), 94.5 (C-1), 68.7 (C-1' trans), 68.2 (C-2),

68.1 (C-4), 67.7 (C-3), 66.2 (C-5), 63.4 (C-1'cis), 61.8 (C-6), 23.0 (C-4' trans), 20.8, 20.7, 20.6 (Me), 19.2 (C-4' cis), -1.9 (Me). Anal. Calcd. for $C_{21}H_{34}O_{10}Si$: C, 53.15; H, 7.22. Found: C, 52.99; H, 7.17.



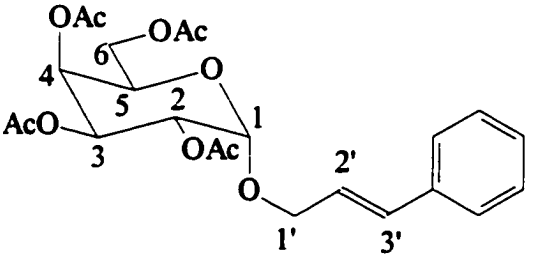
Methyl 6-(2, 3, 4, 6-tetra-O-acetyl-α-D-galactopyranosyloxy)-hex-4-enoate (92).

Compound **92** was isolated by column chromatography using ethyl acetate and hexane (1\1) in 67% yield as a thick syrup of unseparated *E/Z* stereoisomers (2:1); ^1H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.70 (m, 1H, H-3'), 5.55 (m, 1H, H-2'), 5.40 (t, J=3.4 Hz, 1H, H-4), 5.31 (dd, J=3.4, 8.7 Hz, 1H, H-3), 5.10 (m, 2H, H-1, H-2), 4.25-3.90 (m, 5H, H-1'a, H-1'b, H-5, H-6a, H-6b), 3.64, 3.63 (2s, 3H, OMe), 2.35 (m, 4H, H-4', H-5'), 2.09, 2.04, 2.01, 1.94 (4s, 12H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 173.1, 170.3, 170.2, 170.1 (CO), 133.3 (C-3' trans), 132.5 (C-3' cis), 126.0 (C-2' trans), 125.8 (C-2' cis), 95.1 (C-1), 68.1 (C-1' trans), 68.1 (C-4), 68.0 (C-2), 67.5 (C-3), 66.2 (C-5), 63.1 (C-1' cis), 61.6 (C-6), 51.5 (OMe), 33.6 (C-5'), 27.4 (C-4' *trans*), 22.9 (C-4'cis), 20.7, 20.6, 20.5 (Me). Anal. Calcd. for $C_{21}H_{30}O_{12}$: C, 53.16; H, 6.37. Found: C, 52.98; H, 6.49.



4-*tert*-Butyldimethylsilyloxybut-2-ene-1-yl 2,3,4,6-tetra-O-acetyl-αgalactopyranoside (93).

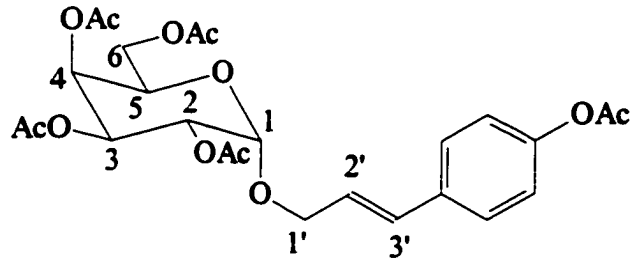
Column chromatography was performed with ethyl acetate and hexane (2\1) as eluent to obtain compound syrupy **93** in 69% yield as an unseparated *E/Z* stereoisomeric mixture (95:5); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.79 (dt, J=4.1, 15.5 Hz, 1H, H-2'), 5.70 (dt, J=4.5, 15.5 Hz, 1H, H-3'), 5.41 (dd, J=1.3, 3.3 Hz, 1H, H-4), 5.33 (dd, J=3.3, 10.8 Hz, 1H, H-3), 5.12-5.08 (m, 2H, H-1, H-2), 4.21-4.14 (m, 4H, H-1'a, H-4'a, H-4'b, H-5), 4.06 (dd, J=3.3, 6.4 Hz, 2H, H-6a, H-6b), 4.00-3.95 (m, 1H, H-1'b), 2.10, 2.03, 2.01, 1.94 (4s, 12H, OAc), 0.87 (s, 9H), 0.03 (s, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 170.3, 170.3 170.2, 169.9 (CO), 133.6 (C-2'), 124.4 (C-3'), 95.1 (C-1), 68.1 (C-4), 68.0 (C-2), 67.8 (C-1' trans), 67.6 (C-3), 66.3(C-5), 63.7 (C-1' cis), 62.8 (C-4' trans), 59.4 (C-4' cis), 61.6 (C-6), 25.9, 20.7, 20.6, 20.6, -5.3 (Me); MS (FAB) m/z: 533.31 (M+1) calcd for $C_{24}H_{40}O_{11}Si$.



3-Phenylprop-2-ene-1-yl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside (94).

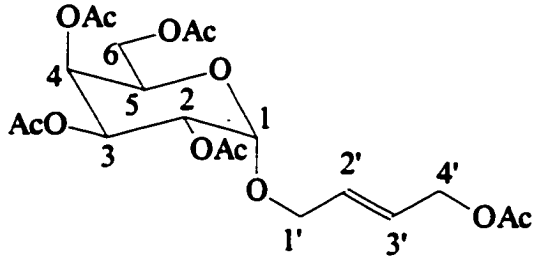
After silica gel column chromatography with ethyl acetate and toluene as eluent (1\4), compound **94** was isolated in 80% yield as a thick syrup of *E/Z* mixture (97:3); The *E*-isomer: ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.38-7.15 (m, 5H, aromatic), 6.58 (d, J=15.9 Hz, 1H, H-3'), 6.21 (ddd, J=5.8, 6.6, 15.9Hz, 1H, H-2'), 5.45 (dd, J=1.4, 3.4 Hz, 1H, H-4), 5.38 (dd, J=3.4, 10.8 Hz, 1H, H-3), 5.19 (d, J=3.4 Hz, 1H, H-1), 5.14 (dd,

J=3.4, 10.8 Hz, 1H, H-2) 4.33 (ddd, J=1.5, 5.7, 12.8 Hz, 1H, H-1'a), 4.27 (t, J=6.5 Hz, 1H, H-5), 4.17 (ddd, J=1.5, 6.6, 12.8 Hz, 1H, H-1'b) 4.08 (d, J=6.5 Hz, 2H, H-6a, H-6b), 2.10, 2.05, 2.01, 2.00 (4s, 12H, OAc); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 170.3, 170.3, 170.2, 169.9 (CO), 136.3, 133.4, 128.6 (Aromatic), 127.9 (C-3'), 126.5 (Aromatic), 124.3 (C-2'), 95.4 (C-1), 68.6 (C-1'), 68.1 (C-4), 68.1 (C-2), 67.6 (C-3), 66.4 (C-5), 61.7 (C-6), 20.7, 20.6, 20.6, 20.6 (Me). Anal. Calcd. for $C_{23}H_{28}O_{10}$: C, 59.48; H, 6.08. Found: C, 59.38; H, 5.99.



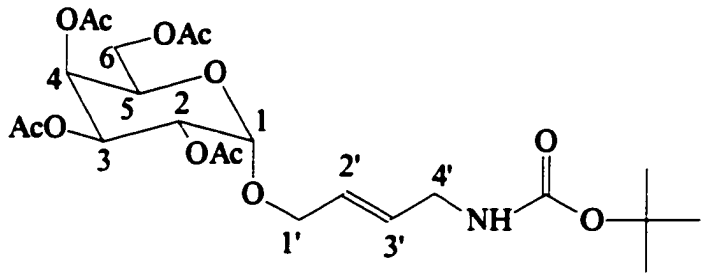
3-*p*-Acetoxyphenylprop-2-ene-1-yl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside (95).

Purification of syrupy *E/Z* mixture (95:5) **95** obtained in 75% yield was performed by silica gel column chromatography using ethyl acetate and hexanes (1\1) as eluent. The E-isomer was separated from the mixture. For *E*-isomer, $[\alpha]^{20}_D = +115^0$ (*c* 4, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.36, 7.01 (2d, J=8.5 Hz, 4H, aromatic), 6.58 (d, J=15.9 Hz, 1H, H-3'), 6.16 (ddd, J=5.8, 6.5, 15.9 Hz, 1H, H-2'), 5.44 (dd, J=1.2, 3.3 Hz, 1H, H-4), 5.35 (dd, J=3.3, 10.8 Hz, 1H, H-3), 5.18 (d, J=3.7 Hz, 1H, H-1), 5.12 (dd, J=3.7, 10.8 Hz, 1H, H-2), 4.31(ddd, J=1.5, 5.8, 12.9 Hz, 1H, H-1'a), 4.25 (ddd, J=1.2, 6.5 Hz, 1H, H-5), 4.15 (ddd, J=1.5, 6.4, 12.9 Hz, 1H, H-1'b), 4.09 (d, J=6.5 Hz, 2H, H-6a, H-6b), 2.25, 2.11, 2.04, 2.01,1.95 (5s, 15H, OAc); ^{13}C NMR ($CDCl_3$, 125 MHz): δ(ppm) 170.3, 170.3, 170.1, 169.9, 169.3 (CO), 150.3, 134.1, 132.3, 127.4, 124.6, 121.7 (aromatic and olefinic), 95.4 (C-1), 68.4 (C-1'), 68.1 (C-4), 68.0 (C-2), 67.6 (C-3), 66.4 (C-5), 61.7 (C-6), 21.0, 20.7, 20.6, 20.6 (Me). MS (FAB) *m/z*: 561.15 ($M+K^+$) calcd for $C_{25}H_{30}O_{12}$+ K.



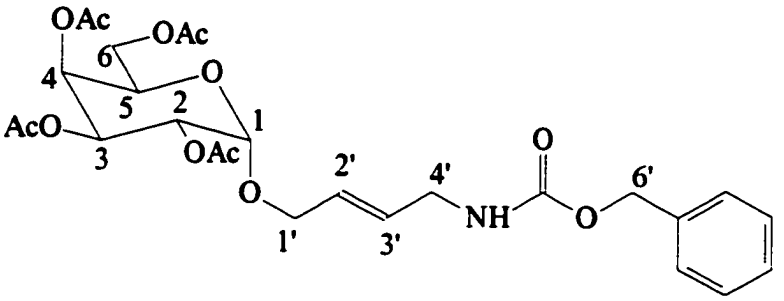
4-Acetoxybut-2-ene-1-yl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside (96).

Purification of **96** obtained in 70% yield, as an unseparated mixture of *E/Z* stereoisomers (5:1), was performed by silica gel column chromatography (ethyl acetate:hexanes=1:1); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.82-5.68 (m, 2H, H-2', H-3'), 5.39 (dd, J=2.0, 3.4 Hz, 1H, H-4), 5.31 (dd, J=3.4, 9.1 Hz, 1H, H-3), 5.07 (m, 2H, H-1, H-2), 4.57 (m, 2H, H-4'cis), 4.52 (d, J=4.4 Hz, 2H, H-4'trans), 4.25-4.05 (m, 2H, H-5, H-1'a), 4.03 (d, J=6.7 Hz, 2H, H-6a, H-6b), 3.98 (dd, J=5.1, 13.5 Hz, 1H, H-1'b), 2.08, 2.03, 2.03, 2.00 (4s, 15H, OAc); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 170.5, 170.2, 170.2, 170.1, 169.9 (CO), 129.2, 127.4 (C-2',C-3'), 95.5 (C-1), 68.0 (C-4), 67.9 (C-2), 67.5 (C-3), 67.4 (C-1'trans), 66.4 (C-5), 63.9 (C-4'trans), 63.2 (C-1'cis), 61.7 (C-6), 59.8 (C-4' cis), 20.8, 20.7, 20.6. 20.5, 20.5 (Me). Anal. Calcd. for $C_{20}H_{28}O_{12}$: C, 52.17; H, 6.13. Found: C, 51.79; H, 6.07.



4-(*tert*-Butoxycarbonylamino) but-2-ene-1-yl 2,3,4,6-tetra-O-acetyl-galactopyranoside (97).

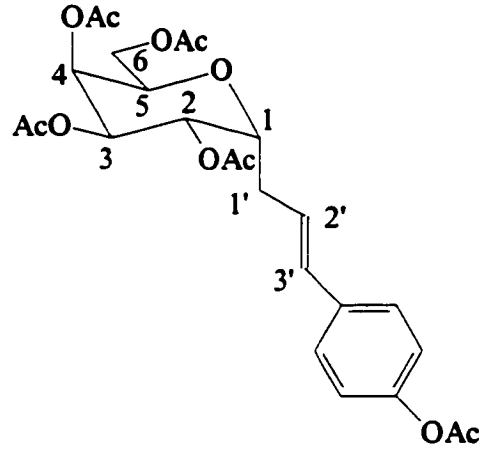
Compound **97** obtained in 57% yield, as a syrupy mixture of *E/Z* stereoisomers (4:1), was isolated by silica gel column chromatography using ethyl acetate and hexanes (3\1). The *E*-isomer was separated from the isomeric mixture. $[\alpha]^{20}_{D}$ = + 66^{0} (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.71 (dt, J=5.4, 15.5 Hz, 1H, H-3'), 5.62 (dt, J=5.4, 15.5 Hz, 1H, H-2'), 5.41 (dd, J=1.4, 3.3 Hz, 1H, H-4), 5.32 (dd, J=3.3, 10.1 Hz, 1H, H-3), 5.10 (m, 2H, H-1, H-2), 4.70 (bs, 1H, NH), 4.24-3.90 (m, 5H, H-5, H-6a, H-6b, H-1'a, H-1'b), 3.70 (bs, 2H, H-4'a, H-4'b), 2.10, 2.04, 2.02, 1.95 (4s, 12H, OAc); ^{13}C NMR ($CDCl_3$, 125 MHz): δ(ppm) 170.4, 170.3, 170.3, 169.9 (CO), 155.7 (CO), 131.1, 126.4 (C-2', C-3'), 95.4 (C-1), 68.1 (C-4), 68.0 (C-2), 67.9 (C-1'), 67.5 (C-3), 66.2 (C-5), 61.6 (C-6), 41.8 (C-4'), 28.3, 20.7, 20.6, 20.6, 20.5 (Me). HRMS (FAB): Calcd. for $C_{23}H_{35}NO_{12}$(K+): 556.1796; Found: 556.1783 ($M+K^+$).



4-(Benzyloxycarbonylamino) but-2-ene-1-yl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside (98).

Silica gel column chromatography was performed using ethyl acetate and hexanes (3\1) to give **98** in 65% yield as a thick syrup of an isomeric *E/Z* mixture (4:1); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.35-7.27 (m, 5H, aromatic), 5.73 (bdt, J=5.4, 15.6 Hz, 1H,

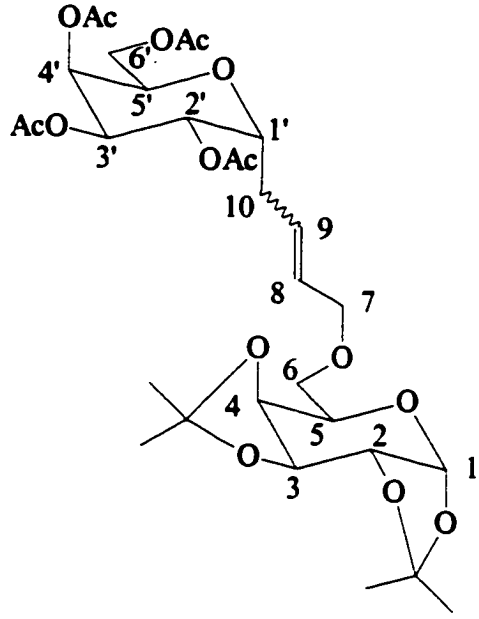
H-3'), 5.65 (bdt, J=5.4, 15.6 Hz, 1H, H-2'), 5.41 (dd, J=1.3,3.4 Hz, 1H, H-4), 5.32 (dd, J=3.5, 10.2 Hz, 1H, H-3), 5.13-5.08 (m, 4H, H-1, H-2, H-6'a, H-6'b), 4.95 (bs, 1H, NH), 4.19 (bt, J=6.6 Hz, 1H, H-5), 4.14-3.96 (m, 4 H, H-1'a, H-1'b, H-6a, H-6b), 3.81 (bt, J=5.2 Hz, 2H, H-4'), 2.11, 2.04, 2.01, 1.96 (4s, 12H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ(ppm) 170.4, 170.4, 170.2, 169.9, 156.2 (CO), 136.5, 130.6, 128.5, 126.9 (ArH, =CHs), 95.4 (C-1), 68.1 (C-4), 68.0 (C-2), 67.8 (C-1' trans), 67.5 (C-3), 66.8 (C-6'), 66.2 (C-5), 62.9 (C-1'cis), 61.5 (C-6), 42.3 (C-4'), 20.8, 20.7, 20.6. Anal. Calcd. for $C_{26}H_{33}NO_{12}$: C, 56.62; H, 6.03; N, 2.54. Found: C, 56.79; H, 6.14; N, 2.48.



Cross-metathesis of 78 with 4-Acetoxystyrene . (99)

Compound **99** was isolated in 75% yield as a syrup by silica gel column chromatography using ethyl acetate and hexanes (1\1) as eluent; ^1H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.32,7.01 (2d, J= 8.5 Hz, aromatic), 6.41 (d, J=15.8 Hz, 1H, H-3'), 6.05 (dt, J=7.3, 15.8 , 1H, H-2'), 5.41 (t, J=3.2 Hz, 1H, H-4), 5.26 (dd, J=4.7, 9.1 Hz, 1H, H-2), 5.18 (dd, J=3.2, 9.1 Hz, 1H, H-3), 4.35 (ddd, J=4.7, 10.2 Hz, 1H, H-1), 4.21 (dd, J=7.8, 11.3 Hz, 1H, H-6a), 4.11 (m, 1H, H-5), 4.04 (dd, J=4.7, 11.4 Hz, 1H, H-6b), 2.61-2.58 (m, 1H, H-1'a), 2.43-2.38 (m, 1H, H-1'b), 2.25, 2.09, 2.04, 2.01, 1.85 (5s, 15 H,

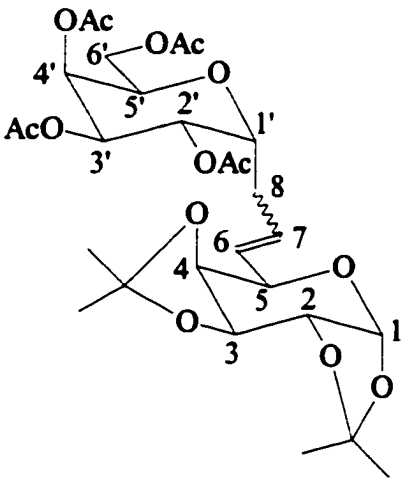
OAc); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 170.6, 170.0, 169.8, 169.8, 169.3 (CO), 149.8, 134.9, 131.6, 126.9, 125.1, 121.6 (aromatic and olefinic), 71.3 (C-1), 68.5 (C-2), 68.4 (C-5), 67.8 (C-3), 67.5 (C-4), 61.4 (C-6), 30.3 (C-1'), 21.0, 20.7, 20.7, 20.6, 20.5 (Me). MS (FAB) m/z: 545.21 ($M + K^+$) calcd for $C_{25}H_{30}O_{11}$+ K.



Cross metathesis of (78) with 6-O-allyl 1,2:3,4-Di-O-isopropylidene-α-D-galactopyranose . (101)

Compound **101** (*E/Z* mixture, 4:1) was isolated by silica gel column chromatography using ethyl acetate and hexanes (1\1) as eluent and obtained in 67% yield; ^1H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.70-5.60 (m, 2H, H-8, H-9), 5.49 (d, J=5.0 Hz, 1H, H-1), 5.37 (t, J=2.9 Hz, 1H, H-4'), 5.21 (dd, J=1.3, 9.2 Hz, 1H, H-3'), 5.16 (dd, J=4.3, 9.2 Hz, 1H, H-2'), 4.55 (dd, J=2.4, 7.9 Hz, 1H, H-3), 4.27-4.15 (m, 4H, H-1', H-2, H-4, H-6'a), 4.08-4.03 (m, 2H, H-5', H-6'b), 3.97-3.90 (m, 3H, H-5, H-7), 3.60 (dd, J=5.7, 10.1 Hz, 1H, H-6a), 3.50 (dd, J=4.8, 10.1 Hz, 1H, H-6b), 2.53-2.39 (m, 1H, H-10a), 2.27-2.20 (m, 1H, H-10b), 2.07, 2.03, 2.01, 1.99 (4s, 12H, OAc), 1.49, 1.40, 1.30, 1.29 (4s, 12H, Me); ^{13}C NMR ($CDCl_3$, 125 MHz): δ(ppm) 170.4, 169.9, 169.8, 169.7 (CO), 129.7, 128.2 (C-8, C-9), 109.1, 108.4 ($\underline{C}Me_2$), 96.3 (C-1), 71.6 (C-7 *trans*), 71.4 (C-4), 71.2 (C-1'), 70.6 (C-3), 70.5 (C-2), 68.8 (C-6), 68.3 (C-5'), 68.2 (C-3'), 67.8 (C-2'), 67.4 (C-4'), 66.8 (C-7 *cis*), 66.8 (C-5), 61.2 (C-6'), 29.4 (C-10 *trans*), 26.0, 25.9,

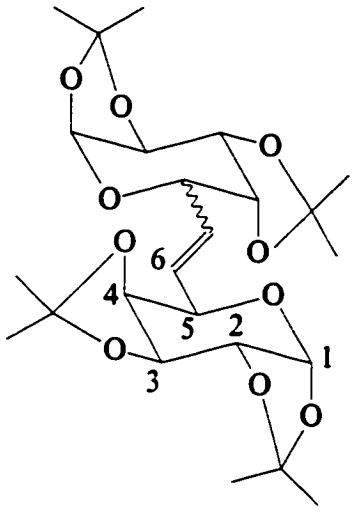
25.0 (C-10 *cis*), 24.8, 24.4, 20.7, 20.6, 20.6 (Me). Anal. Calcd. for $C_{30}H_{44}O_{15}$. C, 55.89; H, 6.88. Found: C, 55.86; H, 6.67.



Cross-metathesis of 78 with 6,7-dideoxy 1,2:3,4-di-O-isopropylidene-α-D-galacto-hept-6-enopyranose. (103)

Compound **103** was purified by silica gel column chromatography with ethyl acetate and hexane (3\1) and *E/Z* stereoisomers were separated in 2:1 ratio in 89% yield. *Z*-isomer: White solid, mp 50-51^{0}C; $[\alpha]^{20}_D$ = - 6^0 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.81 (bt, J=9.5 Hz, 1H, H-6), 5.61 (ddd, J=9.5, 10.5 Hz, 1H, H-7), 5.48 (d, J=5.2 Hz, 1H, H-1), 5.37 (dd, J=1.6, 3.3 Hz, 1H, H-4'), 5.29 (dd, J=5.4, 9.9 Hz, 1H, H-2'), 5.21 (dd, J=3.3, 9.9 Hz, 1H, H-3'), 4.58 (dd, J=2.2, 7.9 Hz, 1H, H-3), 4.47 (bd, J=8.1 Hz, 1H, H-5), 4.35 (ddd, J=4.5, 11.2 Hz, 1H, H-1'), 4.26 (dd, J=2.3, 5.2 Hz, 1H, H-2), 4.20-4.14 (m, 2H, H-5', H-6'a), 4.11 (dd, J=1.7, 7.9 Hz, 1H, H-4), 3.96 (dd, J=6.4, 10.4 Hz, 1H, H-6'b), 2.81-2.74 (m, 1H, H-8a), 2.20-2.17 (m, 1H, H-8b), 2.09, 2.01, 1.99, 1.97 (4s, 12H, OAc), 1.61, 1.42, 1.33, 1.31 (4s, 12H, Me); ^{13}C NMR ($CDCl_3$, 125 MHz): δ(ppm) 170.5, 170.1, 169.8 (CO), 130.5 (C-7), 127.1(C-6), 109.1, 108.3 (CMe_2), 96.6 (C-1), 72.7 (C-4), 71.9 (C-1), 70.9 (C-3), 70.0(C-2), 68.3 (C-4'), 68.2 (C-2'), 67.9 (C-3',C-5'), 63.1 (C-5), 61.1 (C-6), 25.9, 25.8, 25.0 (C-8), 24.7, 24.2, 20.8, 20.7, 20.6, 20.6 (Me). HRMS (FAB): Calcd. for $C_{28}H_{41}O_{14}$: 601.2496; Found: 601.2524 (M+1).

E–Isomer: White solid, mp 65^{0}C; $[\alpha]^{20}_D$ = - 1^0 (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.68-5.67 (m, 2H, H-6, H-7), 5.48 (d, J=5.0 Hz, 1H, H-1), 5.38 (d, J=2.2 Hz, 1H, H-4'), 5.21 (dd, J=5.1, 9.4 Hz, 1H, H-2'), 5.15 (dd, J=3.3, 9.4 Hz, 1H, H-3'), 4.55 (dd, J=2.4, 7.8 Hz, 1H, H-3), 4.26-4.24 (m, 2H, H-1', H-5), 4.20 (d, J=2.7 Hz, 1H, H-2), 4.14-4.10 (m, 2H, H-4, H-6'a), 4.07-4.03 (m, 2H, H-5', H-6'b), 2.47-2.42 (m, 1H, H-8a), 2.37-2.28 (m, 1H, H-8b), 2.08, 2.02, 2.00, 1.98 (4s, 12H, OAc), 1.48, 1.41, 1.29, 1.28 (4s, 12H, Me); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 170.4, 170.0, 169.9, 169.8 (CO), 128.9, 128.7 (C-6, C-7), 109.1, 108.3 (CMe_2), 96.3 (C-1), 73.6 (C-4), 71.5 (C-1'), 70.8 (C-3), 70.3 (C-5), 68.7 (C-2) , 68.2 (C-2'), 68.0 (C-3'), 67.9 (C-5'), 67.4 (C-4'), 61.1 (C-6'), 29.7 (C-8), 26.1, 25.9, 24.8, 24.3, 20.7, 20.6, 20.6, 20.6 (Me). Anal. Calcd. for $C_{28}H_{40}O_{14}$: C, 55.99; H, 6.71. Found: C, 56.21; H, 6.52.



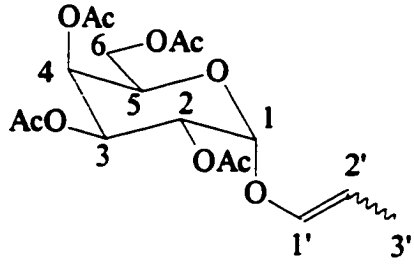
Homodimer of 102. (104)

Using the conditions described in chapter 1, dimerization of **102** gave **104** as pure *E* and *Z* isomers (65%) which were separated by silica gel column chromatography (ethyl acetate : hexane- 1:2) in 5:1 ratio.

Z-Isomer: Thick syrup, $[\alpha]_{20}$= - 110° (*c* 1, CHCl3); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.71 (dd,J=1.4, 4.4 Hz, 1H, H-6), 5.52 (d, J=5.0 Hz, 1H, H-1), 4.64 (dd, J=1.9, 4.4 Hz, 1H, H-5), 4.54 (dd, J=2.4, 7.9 Hz, 1H, H-3), 4.29 (dd, J=1.9, 7.9 Hz, 1H, H-4), 4.25 (dd, J=2.4, 5.0 Hz, 1H, H-2), 1.51, 1.44, 1.31, 1.30 (4s, 12H); ^{13}C ($CDCl_3$, 125 MHz): δ (ppm)

128.4 (C-6), 109.1, 108.3 (CMe_2), 96.3 (C-1), 73.2 (C-4), 70.6 (C-3), 70.2 (C-2), 65.3 (C-5), 26.2, 25.9, 24.8, 24.2 (Me). MS (FAB) m/z: 485.26 (M + 1) calcd for $C_{24}H_{37}O_{10}$.

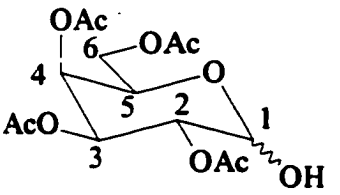
E-Isomer: White solid, mp 127^{0}C; $[\alpha]^{20}_D$ = - 166^0 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.86 (dd, J=1.9, 4.0 Hz, 1H, H-6), 5.50 (d, J=5.0 Hz, 1H, H-1), 4.55 (dd, J=2.4, 7.9 Hz,1H, H-3), 4.29 (ddd, J=1.9, 4.0, 1H, H-5), 4.25 (dd, J=2.4, 5.0 Hz, 1H, H-2), 4.19 (dd, J=1.9, 7.9 Hz, 1H, H-4), 1.49, 1.43, 1.30, 1.29 (4s, 12H); ^{13}C ($CDCl_3$) δ 129.5 (C-6), 109.1, 108.4 (CMe_2), 96.3 (C-1), 73.5 (C-4), 70.8 (C-3), 70.4 (C-2), 68.7 (C-5), 26.1, 25.9, 24.9, 24.3 (Me). HRMS (FAB): Calcd. for $C_{24}H_{37}O_{10}$: 485.2387; Found: 485.2333 (M+1).



Prop-1-enyl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside (105).

To a solution of allyl 2,3,4,6-tetra-O-acetyl-α- D-galactopyranoside **71** (120 mg, 0.30 mmol) in toluene (6 mL), dichloro tris (triphenylphosphine) ruthenium II (30mg, 0.03 mmol) and DIPEA (105 μL, 0.6 mmol) were added under a nitrogen atmosphere. The resulting black solution was stirred under reflux conditions for 2 h. Toluene and DIPEA were removed under reduced pressure and the residue was purified purified via flash chromatography on silica gel using a gradient of hexanes/ethyl acetate 3/1 to 1/1 as eluent. **104** was isolated in 96% yield as a syrup (111 mg, 0.29 mmol) in E/Z = 1/3; ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 6.11 (dq, J=1.7, 12.3 Hz, 1H, H-1'E), 6.02 (dq, J=1.7, 6.2 Hz, 1H, H-1'Z), 5.47 (dd, J= 1.2, 3.4 Hz, 1H, H-4), 5.39 (dd, J=3.4, 7.7 Hz, 1H, H-3), 5.27 (d, J= 3.8 Hz, 1H, H-1), 5.15 (dd, J= 3.8, 7.7 Hz, 1H, H-2), 4.62 (dq, J= 6.9, 13.1 Hz, 1H, H-2'), 4.22-4.18 (m, 1H, H-5), 4.11 (dd, J= 7.5, 11.2 Hz, 1H, H-6a), 4.02 (dd, J= 6.7, 11.2 Hz, 1H, H-6b), 2.12, 2.06, 2.00, 1.98 (4s, 12H, OAc), 1.60 (dd, J=1.7, 6.9 Hz, 3H, H-3'Z), 1.53 (dd, J=1.7, 7.9 Hz, 3H, H-3'E); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 170.4, 170.3, 170.1, 170.0 (CO), 142.4 (C-1'E), 141.3 (C-1'Z), 105.7 (C-2'), 96.0 (C-1Z), 95.4 (C-1E), 67.9 (C-4), 67.7 (C-2), 67.6 (C-3), 66.9 (C-5), 61.4 (C-6), 20.7, 20.6,

20.6, 20.6 (Me), 12.3 (C-3'E), 9.3 (C-3'Z); HRMS (FAB), cacld. For $C_{17}H_{24}O_{10}K$ ($M+K^+$): 427.1007, found 427.1456.



2,3,4,6-Tetra-O-acetyl-α,β-D-galactopyranose (106).

Compound **105** (40 mg, 0.10 mmol) was dissolved in 2 mL of acetone:water (1:1), then mercuric oxide (11 mg, 0.05 mmol) and mercuric chloride (47 mg, 0.1 mmol) were added. The reaction mixture was stirred for 2 h at room temperature, monitored by TLC (hexane/ethyl acetate 1/1). The orange solution was washed with saturated aqueous KI solution and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, evaporated under reduced pressure and purified via flash chromatography on silica gel using a gradient of hexane/ethyl acetate 3/1 to 1/2 as eluent. Title compound **106** was isolated in 92% yield (32 mg, 0.092 mmol); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.47 (t, J= 3.4 Hz, 1H, H-1 α), 5.43 (dd, J=1.7, 3.3 Hz, 1H, H-4), 5.36 (dd, J= 3.3, 10.9 Hz, 1H, H-3), 5.10 (dd, J= 3.4, 10.9 Hz, 1H, H-2), 4.67 (t, J=7.5 Hz, 1H, H-1 β), 4.44 (t, J= 6.6 Hz, 1H, H-5), 4.12-3.91 (m, 2H, H-6a, H-6b), 3.68 (bs, 1H, α-OH), 2.92 (bs, 1H, β-OH), 2.11, 2.06, 2.01, 1.95 (4s, 12H, OAc); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 170.6, 170.4, 170.3, 170.1 (CO), 95.9 (C-1β), 90.6 (C-1α), 68.3 (C-4), 68.2 (C-2), 67.2 (C-3), 66.1 (C-5), 61.8 (C-6), 20.8, 20.6, 20.6, 20.6 (Me); Physical data are in agreement with reported values.

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Chapter 4. Stereoselective synthesis of glycoclusters

4.1. Introduction

From the positive results obtained with the olefin metathesis reaction, the application of this methodology to the synthesis of glycoclusters was envisaged. The chemical synthesis of high affinity ligands for protein receptors (lectins, selectins and antibodies) that are able to compete or even surpass naturally occuring oligosaccharides present on the cell surface are necessary for the design of potent carbohydrate therapeutics. However, the structural requirements for optimal binding, the "glycocode" are not known in most cases; therefore, it has to be determined in each individual cases for rational design of high affinity ligands.

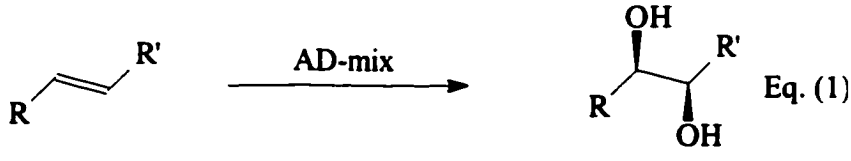
In this chapter, we report the reiterative synthesis of a novel class of glycoclusters using olefin metathesis and Sharpless asymmetric dihydroxylation reactions as key steps to generate glycoclusters of varied geometry.

This approach, which provides an unique access to a new class of oligosaccharide mimics, has the advantage to generate glycoclusters with varied structural and stereochemical environments to help determining the "glycocode". Moreover, this process offers control on chirality, spacer length and valency, features that are not obtained in other methods such as ring-opening metathesis polymerization.

4.2. Results and discussion

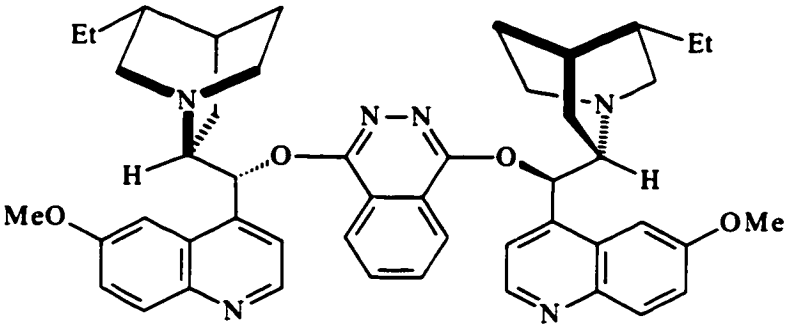
4.2.1. The Sharpless asymmetric dihydroxylation reaction

The osmium catalyzed asymmetric dihydroxylation (Sharpless AD) has developed into one of the most important tools for the enantioselective functionalization of olefins (Eq.1).[1]



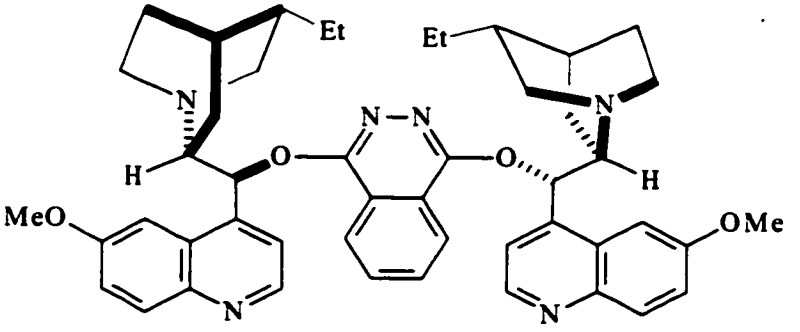
Eq. (1)

Several groups have proposed different catalysts based on diamino compounds but the development of quinine-derived ligands led to considerable improvements. The cinchona alkaloids dihydroquinidine (DHQD) and dihydroquinine (DHQ) are now mainly used in the last generation of AD catalysts. The main characteristics of these catalysts is the presence of two copies of a chiral alkaloid, linked by aromatic bridges (Figure 4.2.1.). The phthalazine (PHAL) and diphenylpyrimidine (PYR) cores have led to considerable increase in both the enantioselectivity and the scope of the reaction. These two classes of ligands have superseded the previous chlorobenzoate (CLB), phenanthryl ether (PHN) and 4-methyl-2-quinolyl (MEQ) ligands (Figure 4.2.2.).



$(DHQ)_2$-PHAL

Ligand used in AD-mix α

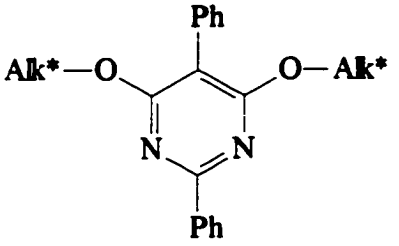


$(DHQD)_2$-PHAL

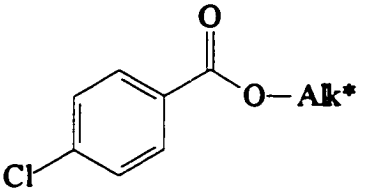
Ligand used in AD-mix β

Figure 4.2.1. The most common chiral ligands used in Sharpless AD.

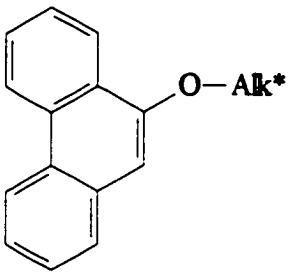
AD reactions are usually performed with commercially available AD-mixtures, consisting of 0.4 mol% $K_2[OsO_2(OH)_4]$, 1 mol% $(DHQD)_2PHAL$ (AD-mix β) or $(DHQ)_2PHAL$ (AD-mix α), 3 equiv. of $K_3[Fe(CN)_6]$ and 3 equiv. of K_2CO_3. In the case of internal olefins the addition of at least stoiechiometric amount of a hydrolysis aid such as Et_4NOAc[2] or $MeSO_2NH_2$[3] is required to obtain reasonable reaction rates, due to hydrolysis problems of sterically hindered osmate esters.



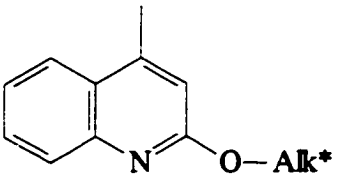
Diphenylpyrimidine
(PYR)



Chlorobenzoate
(CLB)



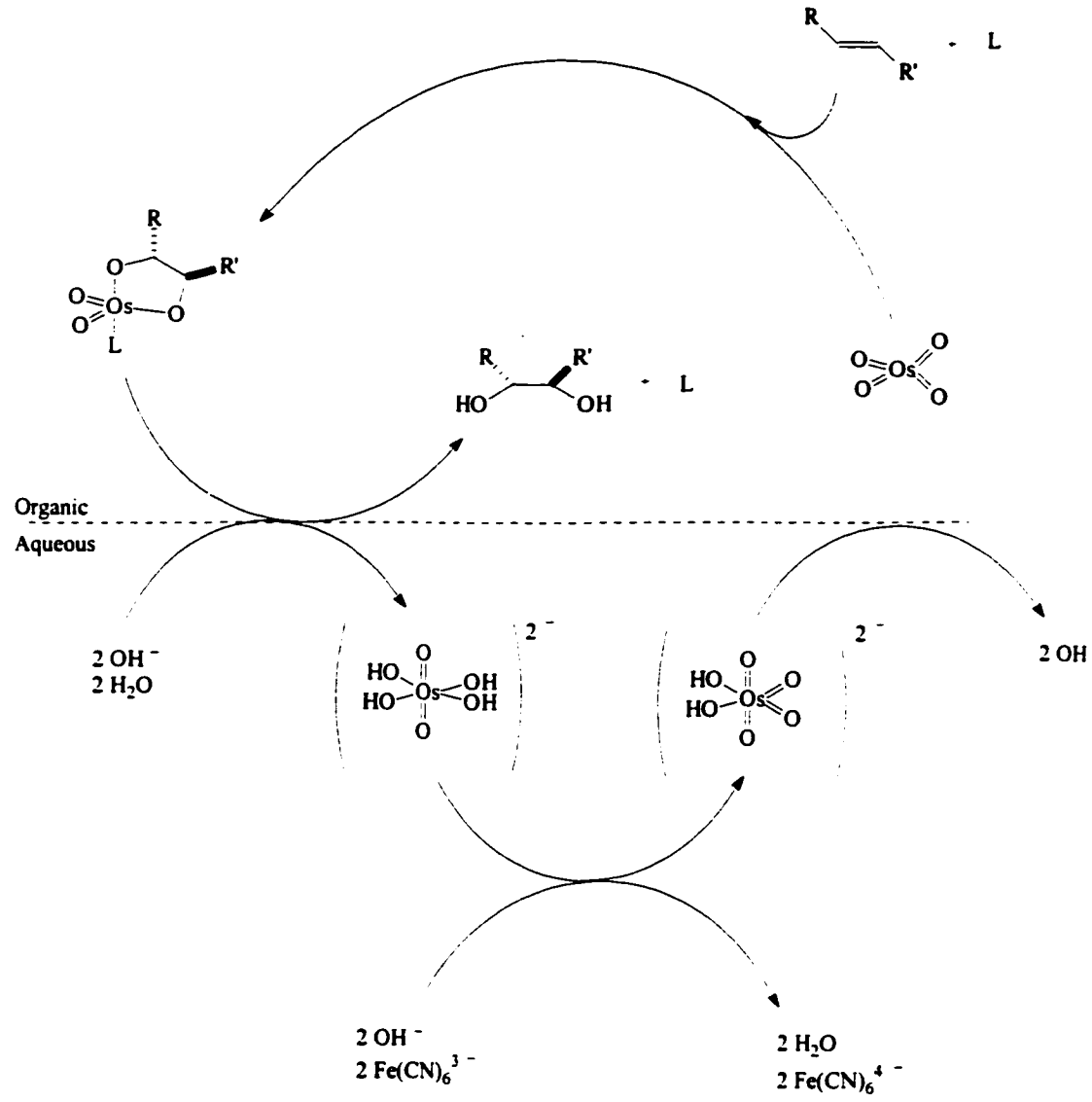
Phenantryl ether
(PHN)



4-methyl-2-quinolyl ether
(MEQ)

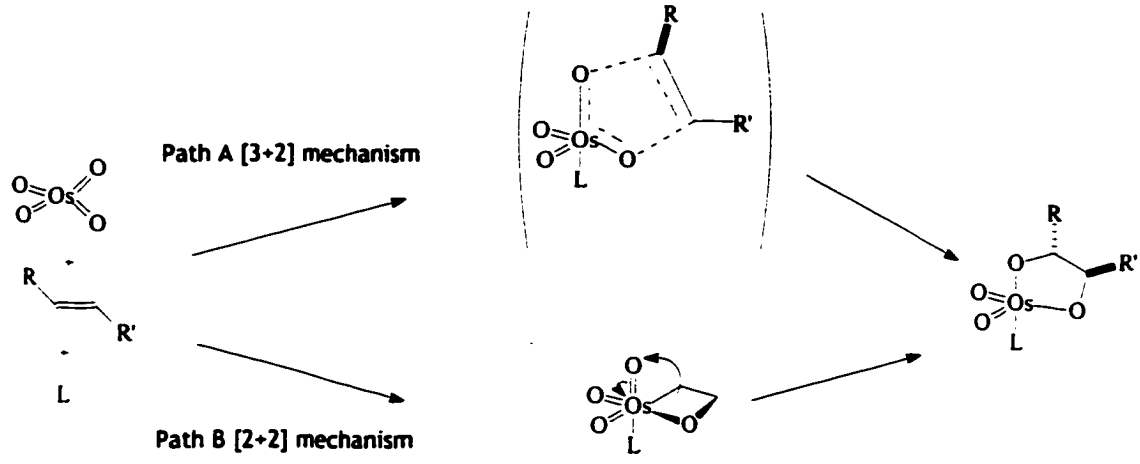
Figure 4.2.2. Other ligands used in Sharpless AD (Alk*= DHQD or DHQ).

The catalytic cycle of the AD reaction with $K_3Fe(CN)_6$ as the cooxidant is shown in Scheme 4.2.1.The reaction is performed under two-phase condition with $K_3Fe(CN)_6$ as the stoiechiometric reoxidant. The osmylation is taking place in the organic layer, the resulting osmium (VI) monoglycolate ester undergoes hydrolysis, releasing diol and ligand in the organic layer and Os(VI) in the aqueous layer before its reoxidation can occur.



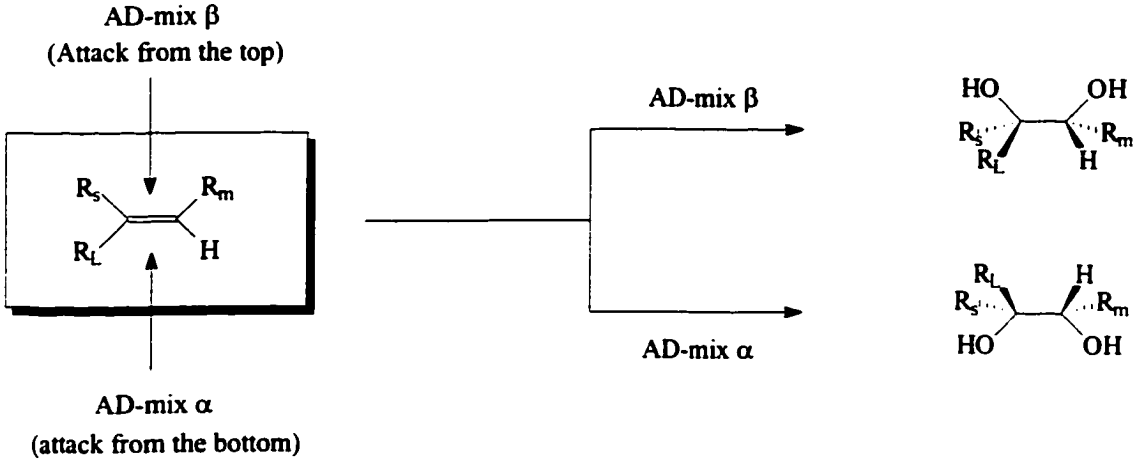
Scheme 4.2.1. Catalytic cycle of the AD reaction with $K_3Fe(CN)_6$ as the cooxidant.

The AD reaction has been the center of extensive mechanistic investigations and two different mechanisms have been suggested: a concerted [3+2] pathway (path A) and a stepwise reaction which is initiated by a [2+2] like addition (path B) as shown in Scheme 4.2.2. The long-standing controversy about the mechanism of the OsO_4 addition to olefins has been recently settled with the help of quantum chemical calculations which are in agreement with the [3+2] mechanism.[4]



Scheme 4.2.2. Mechanisms suggested for the AD reaction.

On the other hand, models have been proposed to explain the stereochemical outcome of the AD reaction. On the basis of experimental results, Sharpless suggested the AD mnemonic,[3] which can predict the sense of the AD and whether AD-mix α or β should be used to prepare the desired enantiomer (Scheme 4.2.3.). The enantiofacial selectivity depends on the ligand used, for dihydroquinidine derivatives (AD-mix β) the olefin attack from the top face whereas for dihydroquinine derivatives (AD-mix α), the bottom face is favored.



Scheme 4.2.3. AD mnemonic.

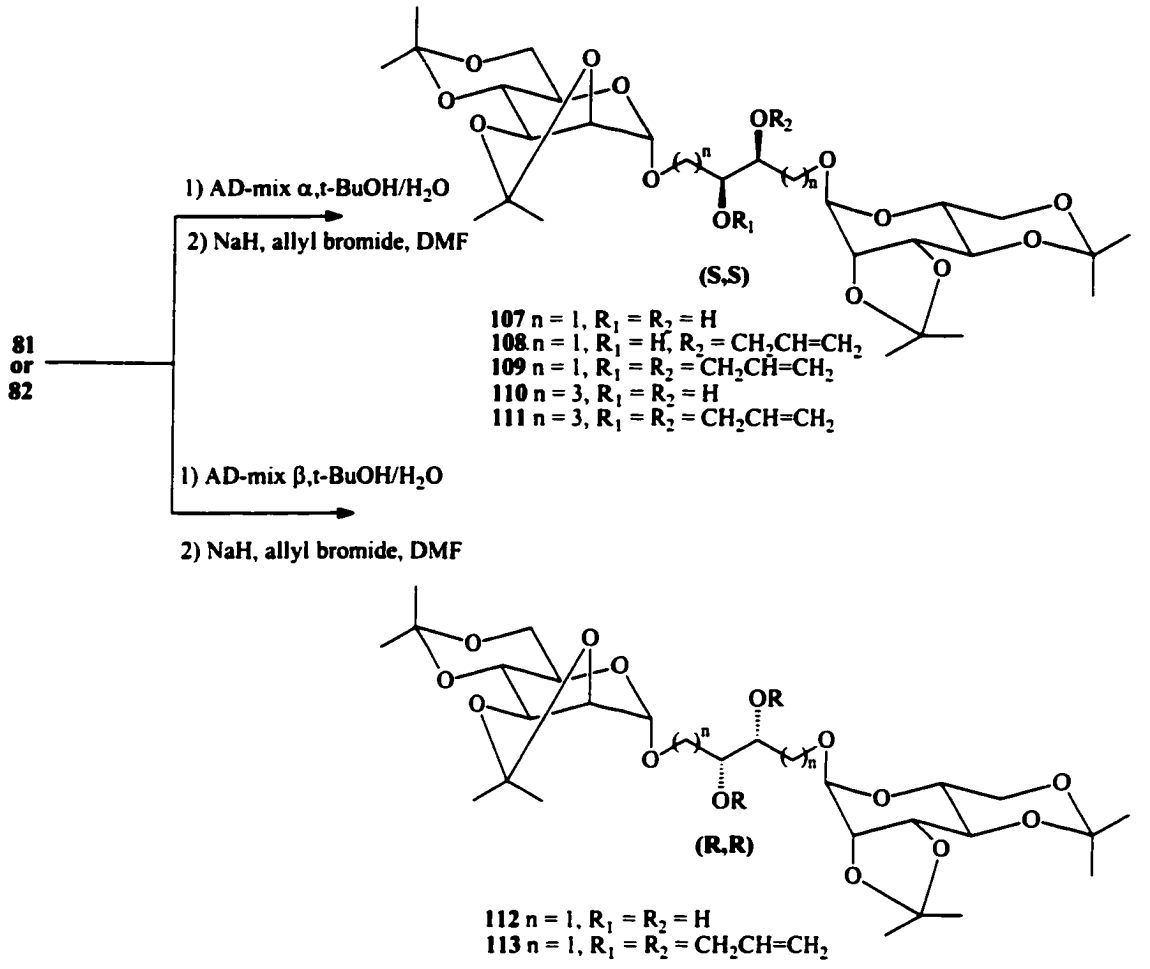
The AD reaction represents therefore a powerful method for the enantioselective synthesis of diols from prochiral olefins and can be used in a synthetic scheme toward the synthesis of glycoclusters.

4.2.2. Synthesis of glycoclusters

Treatment of homodimer **81** with a catalytic amount of osmium tetroxide and stoichiometric amount of N-methylmorpholine-N-oxide[5] afforded the dihydroxylated products **107** and **112** in a poor 1:1 ratio. The reaction showed no diastereoselectivity indicating a lack of asymmetric induction from the sugar moiety. Based on those results, we decided to use the Sharpless asymmetric dihydroxylation reaction making use of chiral ligands AD-mix α and AD-mix β. Dihydroxylation of **81** under the AD reaction condition with AD-mix α afforded compound **107** in 88%. The diastereoselectivity excess of the reaction could be determined on compound **109** after bis-allylation of the resulting diol with an excess of allyl bromide. Compound **109** was obtained in 92 % yield and showed a satisfactory 9:1 ratio in favor of the (S,S) in agreement with the mnemonic model developed by Sharpless. The same sequence was applied to obtain **113** in similar yields using AD-mix β. It is interesting to point out that compounds **109** and **113** are diastereoisomers so they should show differences in their NMR spectra. Surprisingly, no big differences could be observed by comparison of their ^{1}H-NMR (500 MHz) spectra, however the anomeric proton showed significant difference which revealed a distinctive signal for both of the diastereoisomers. This difference led us to the determination of the ratio of the reaction.

The outcome of the diastereoselectivity was confirmed by treating compound **107** with AcOH / H_2SO_4 to release the aglycon part[6] which was then benzoylated using benzoyl chloride and Et_3N to provide L-threitol tetrabenzoate for which the optical rotation compared favorably with known value.[7]

In the same manner, treatment of homodimer **82** under the Sharpless AD and bis-allylation conditions afforded diol **110** and bis-allyl derivatives **111** in 85% and 82% yields respectively. It was also possible to introduce only one allyl group by using 1 equiv.

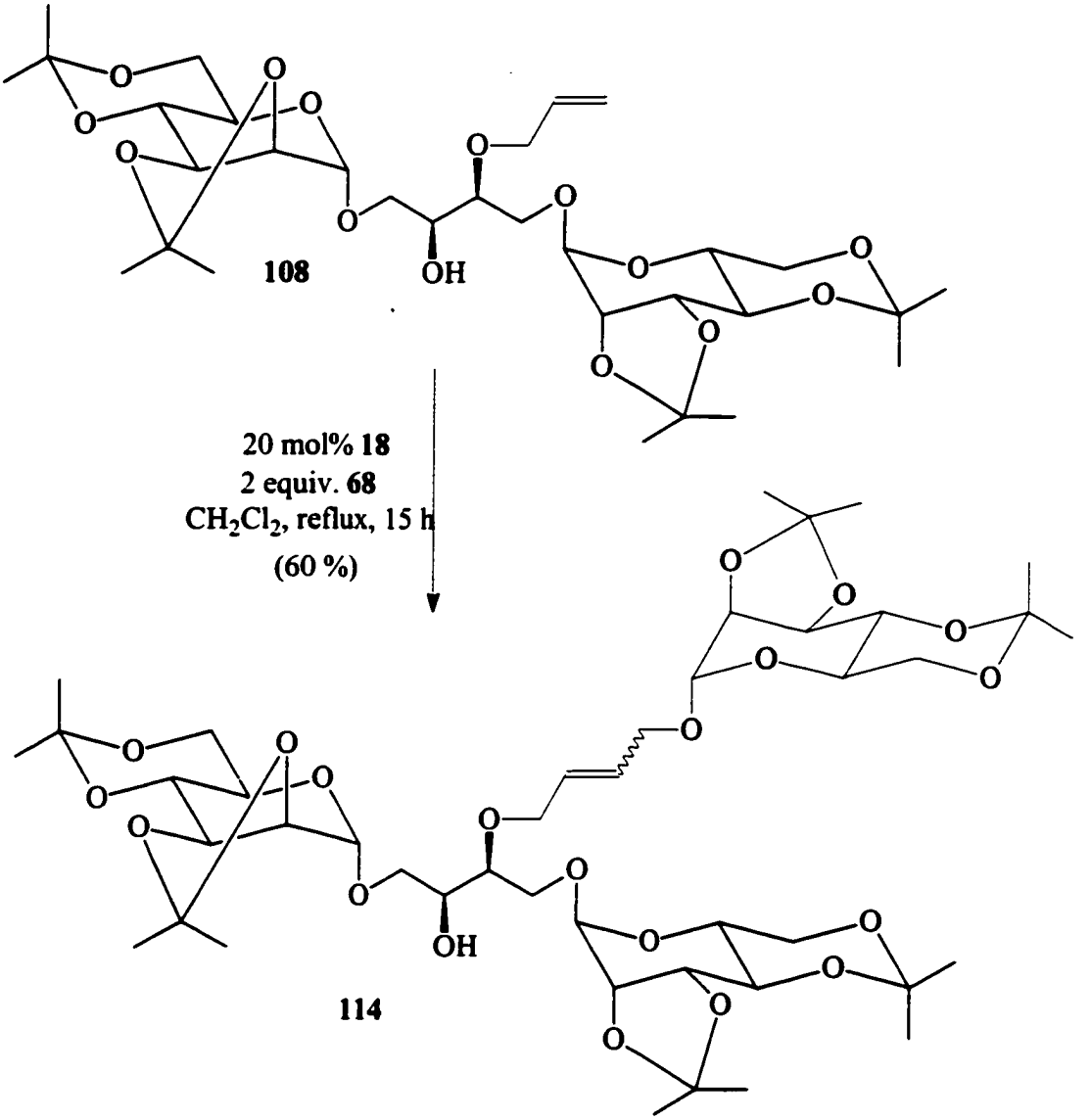


Scheme 4.2.4. AD reaction and subsequent allylation.

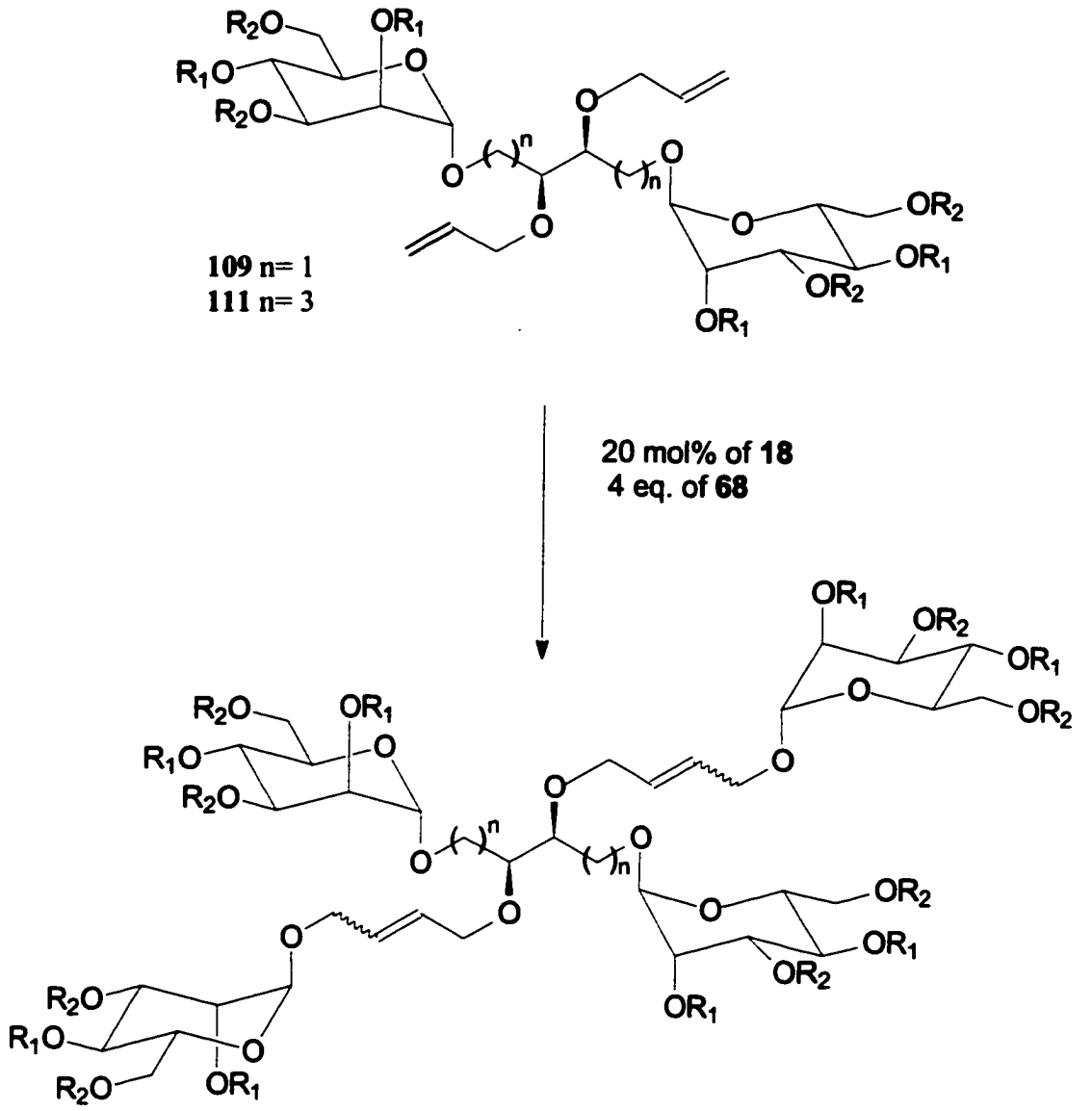
of allyl bromide to provide mono-allylated compound **108** in 68% yield (Scheme 4.2.4.), thus leaving an intact hydroxyl group that could serve as a handle for further heterobifunctional modifications. The remaining allyl group could be used to do a cross-metathesis reaction with allyl 2,3:4,6-di-O-isopropylidene-α-D-mannopyranoside **68** to afford a trimeric glycocluster in 60% yield (scheme 4.2.5).

With the bis-allylated compound in hand, we were set for a double cross-metathesis reaction that could give us access to a tetrameric glycocluster. There are few examples in the literature where the Grubbs' catalyst was used in multiple cross-metathesis reactions due to the complexity that can arise from the reaction. Our major concern was that ring-closing metathesis (RCM) would compete against the desired cross-metathesis reaction.

However it was anticipated that with an excess of glycoside **68** it could be possible to direct the reaction toward the cross-metathesis product between **109** and **68** . Indeed, the use of 4 equiv. of **68** in CH_2Cl_2 containing 20 mol% of the Grubbs' catalyst lead, after purification to 32 % of the cross-metathesis product **115** along with 35% of RCM product **117** (Scheme 4.2.6.).



Scheme 4.2.5. Trivalent glycocluster.

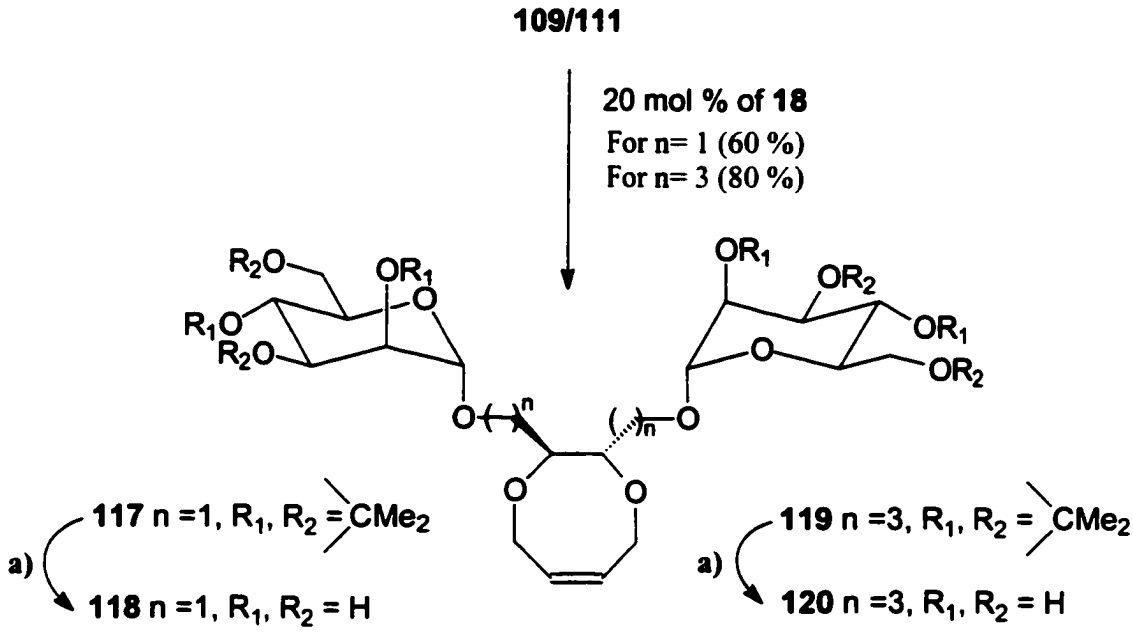


Scheme 4.2.6. Tetravalent glycoclusters.

In the double cross-metathesis for compound **111**, the RCM product was even more favored and under the same conditions, the reaction gave 60 % of RCM product **119** and 30 % of tetramer **116** (see in appendix).

As an attempt to reduce the undesired RCM product in the double cross-metathesis reaction, we envisaged to perform the reaction in a more concentrated media. Thus, when the reaction was done in a 0.04 M CH_2Cl_2 solution, the cross metathesis product became the major product while cutting by half the amount of RCM product.

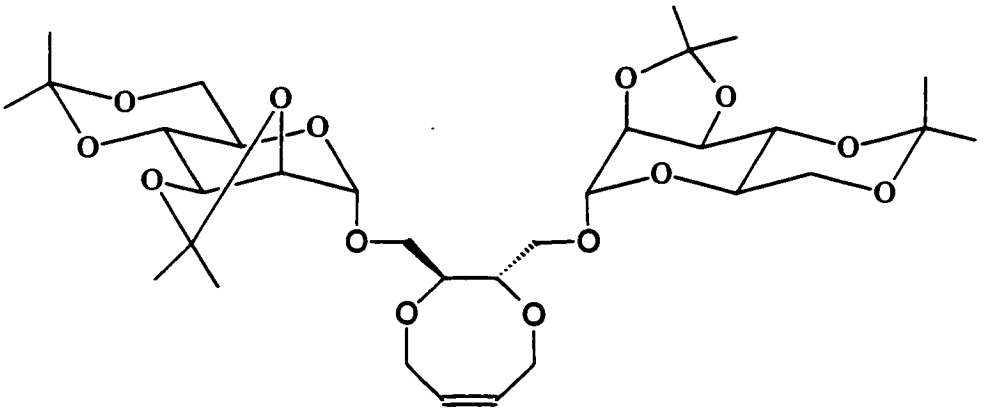
Encouraged by those results, we anticipated that a tandem ring-opening (ROM) and cross-metathesis (CM) reaction on the RCM product **117** could afford the desired cross-metathesis product in the presence of allyl glycoside **68**. To this end, treatment of **109** or **111** with 20 mol% of Grubbs' catalyst in refluxing dichloromethane afforded the eight–membered ring RCM product in 60 and 80 % yield (Scheme 4.2.7). In each case, only one steroisomer could be observed by NMR. The same reactions were used to obtain the other diastereoisomer containing the eight-membered ring derivative **121** from **113**.



a) 60 % aq. AcOH, 80° C, 1h, quant.

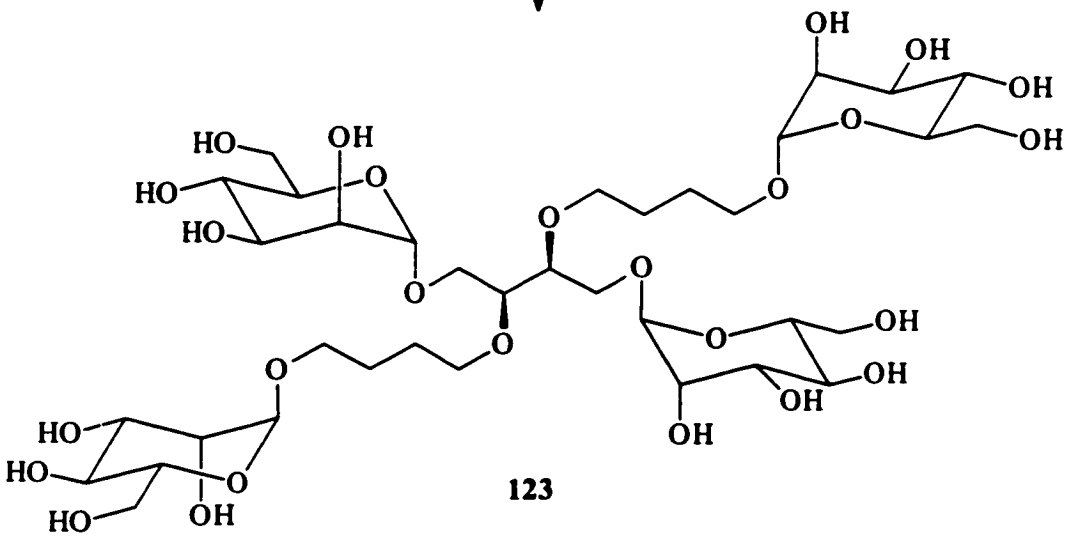
Scheme 4.2.7. Formation of an eight-membered ring derivative by RCM.

The removal of the acetonides was accomplished in quantitative yields using 60% aq. AcOH (80 ° C, 1h). At this stage, we were set for the tandem reaction, thus treatment of the RCM product **117** with allyl glycoside **68**, gratifyingly produced 48 % of the desired tetramer **115** along with 15 % of unreacted RCM product.



117

1. 20 mol% of **18**
   4 eq. of **68** (48 %)
2. 10% Pd/ C, H_2, MeOH, quant.
3. 60 % aq. AcOH, 80° C, 1h, quant.



123

Scheme 4.2.8. Tandem ROM/CM reactions.

Finally, the tetrameric glycocluster **123** was obtained after hydrogenation of the double bond and removal of the acetonides as described above.

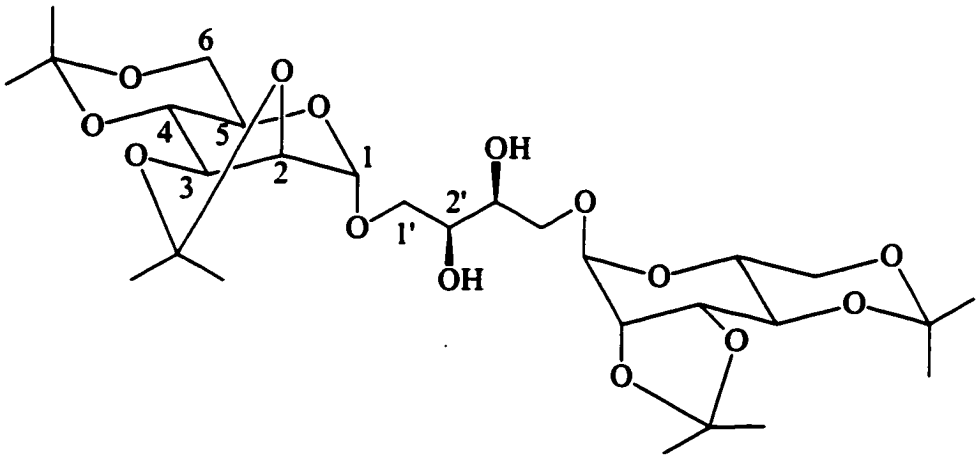
4.3. Conclusions

In conclusion, an asymmetric synthesis of tetrameric glycocluster was accomplished using olefin metathesis and Sharpless asymmetric dihydroxylation sequence. Our approach has the advantage of being highy flexible and provides access to glycoclusters of varied structural and stereochemical environments.[8] The testing of these products are underway.

4.4. Experimental methods

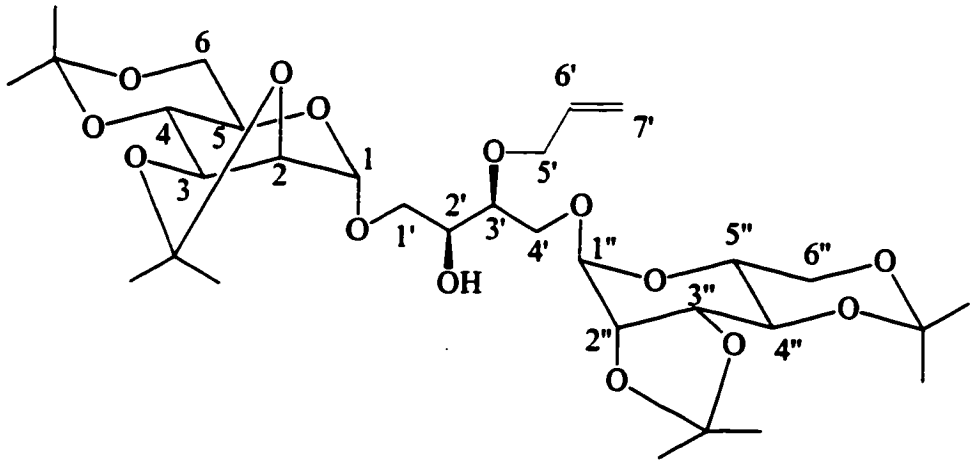
General procedure for Asymmetric dihydroxylation reaction with AD-mix α and β:

1 mmol of homodimer was dissolved in 10 mL of tert-butyl alcohol/water (1:1) and stirred at 0° C. 1.4 g of AD-mix α or AD-mix β then 2 mmol of methanesulfonamide were added to the reaction mixture. The heterogeneous slurry was stirred at 0° C for 6 h and allowed to warm to room temperature overnight. The reaction was monitored by TLC and after consumption of the starting material (12 h), solid sodium sulfite (1.5 g) was added and stirred for an additional 10 min. CH_2Cl_2 (10 mL) was added to the reaction mixture, and after separation of the layers, the aqueous phase was further extracted with CH_2Cl_2 (3 x 5 mL). The combined organic exctracts were dried over Na_2SO_4, evaporated under reduced pressure and purified via flash chromatography on silica gel using a gradient of hexanes/ethyl acetate.



(S,S)-(2,3-Dihydroxybutyl) di-O-(2,3:4,6-di-O-isopropylidene)-α-D mannopyranoside (107).

Following the general procedure for asymmetric dihydroxylation reaction using AD-mix α, compound **107** was purified via flash chromatography on silica gel using a gradient of hexanes and hexanes/ethyl acetate 2/1 to 1/2 as eluent and obtained in 88% yield; $[\alpha]^{20}_{D}$ = + 34.6^{0} (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.03 (s, 2H, H-1), 4.18 (d, J= 5.7 Hz, 2H, H-2), 4.13 (dd, J= 5.7, 7.8 Hz, 2H, H-3), 3.92-3.71 (m, 10H, H-4, H-6a, H-6b, H-1'a, H-2'), 3.62-3.51 (m, 4H, H-5, H-1'b), 2.95 (bs, 2H, OH), 1.52, 1.49, 1.40, 1.33 (4s, 12H, Me; ^{13}C NMR ($CDCl_3$, 125 MHz): δ(ppm) 109.6, 99.7 (MeCMe), 98.4 (C-1), 75.9 (C-2), 74.8 (C-3), 72.5 (C-4), 69.9 (C-2'), 69.2 (C-1'), 62.0 (C-6), 61.7 (C-5), 29.0, 28.1, 26.1 18.7 (Me); ESI-MS, m/z= 624.2, $[M+NH_4]^+$ calcd for $C_{28}H_{46}O_{14}$+ NH_4.



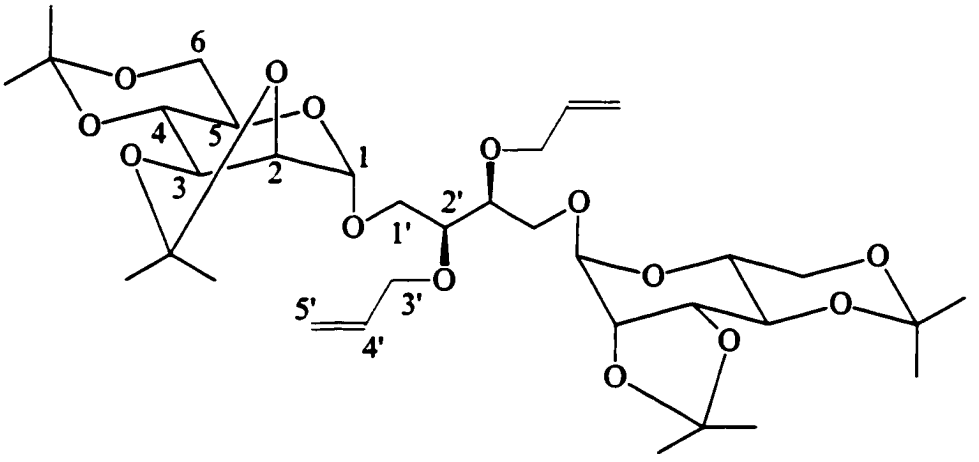
(S,S)-(2-Allyloxy-3-hydroxybutyl) di-O-(2,3:4,6-di-O-isopropylidene)-α-D-manno-pyranoside(108)

Allyl bromide (21 μL, 0.248 mmol) was added to a solution of **107** (100 mg, 0.165 mmol) dissolved in 5 mL of DMF cooled to 0° C under N_2 atmosphere. Sodium hydride, 60 % dispersion in mineral oil (10 mg, 0.25 mmol) pre-washed with hexane to remove the oil, was added slowly. The reaction mixture was stirred and allowed to warm to room temperature for 6 h. The progress of the reaction was monitored by TLC (hexane/ethyl acetate 1/1). Methanol (2 mL) was then added at 0° C to neutralize excess of sodium hydride and stirred for 10 min. The reaction mixture was diluted with ether (10 mL) and washed several times with water until the complete removal of DMF from the organic phases. The organic layer was dried over Na_2SO_4, evaporated under reduced pressure and purified via flash chromatography on silica gel using a gradient of hexane and hexane/ethyl acetate 2/1 to 1/2 as eluent Title compound **108** was isolated in 68% yield (72 mg, 0.111 mmol); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.93-5.82 (m, 1H, H-6'), 5.28-5.18 (m, 2H, H-7'a, H-7'b), 5.04, 5.01 (2s, 2H, H-1, H-1''), 4.20-4.12 (m, 5H, H-2, H-2'', H-3, H-3'', H-5'a), 4.06-4.02 (m, 1H, H-5'b), 3.88-3.83 (m, 4H, H-6a, H-6''a, H-2', H-1'a), 3.76-3.71 (m, 5H, H-4, H-4'', H-6b, H-6''b, H-5'a), 3.58-3.47 (m, 5H, H-5, H-5'', H-1'b, H-4'b, H-3'), 2.60 (bs, 1H, OH), 2.14, 1.53, 1.49, 1.40, 1.40, 1.33 (6s, 24H, Me); ^{13}C NMR ($CDCl_3$, 125 MHz): δ(ppm) 134.3 (C-6'), 117.6 (C-7'), 109.5, 99.7 (MeCMe), 98.4, 98.1 (C-1, C-1''), 76.9 (C-3'), 75.9 (C-2, C-2''), 74.8 (C-3, C-3''), 72.6 (C-4, C-4''), 72.1 (C-5'), 70.1 (C-2'), 66.8, 67.8 (C-4', C-1'), 62.0 (C-6, C-6''), 61.6 (C-

5, C-5''), 29.0, 28.1, 26.1, 18.7 (Me); ESI-MS, m/z= 664.2, $[M+NH_4]^+$ calcd for $C_{31}H_{50}O_{14}+NH_4$.

General procedure for bis-allylation of diol:

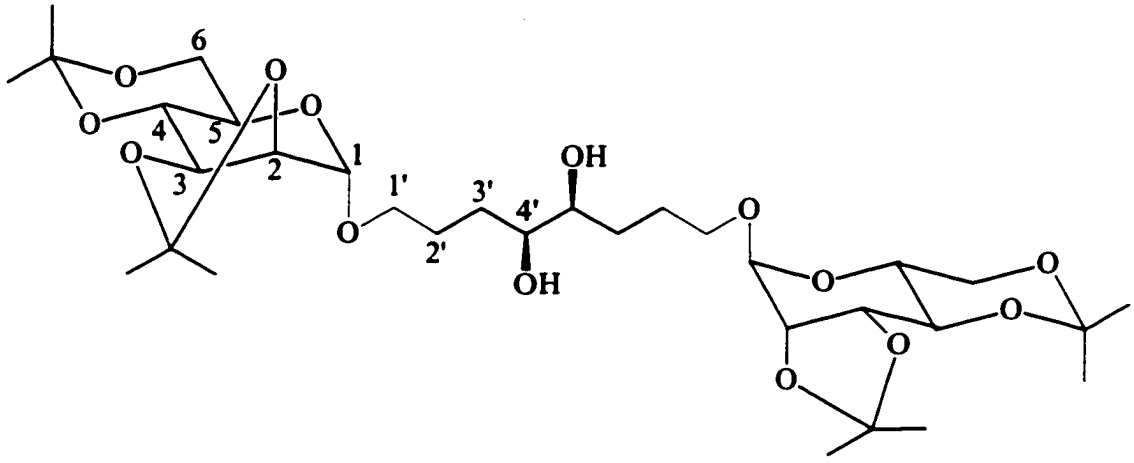
Allyl bromide (85 μL, 0.99 mmol) was added to a solution of **107** (100 mg, 0.165 mmol) dissolved in 5 mL of DMF cooled to 0° C under N_2 atmosphere. Sodium hydride, 60% dispersion in mineral oil (40 mg, 0.99 mmol) pre-washed with hexane to remove the oil, was added slowly. The reaction mixture was stirred and allowed to warm to room temperature for 6 h. The progress of the reaction was monitored by TLC (hexane/ethyl acetate 1/1). Methanol (2 mL) was then added at 0° C to neutralize excess of sodium hydride and stirred for 10 min. The reaction mixture was diluted with ether (10 mL) and washed several times with water until the complete removal of DMF from the organic phases. The organic layer was dried over Na_2SO_4, evaporated under reduced pressure and purified via flash chromatography on silica gel using a gradient of hexane and hexanes/ethyl acetate.



(S,S)-(2,3-Diallyloxybutyl)di-O-(2,3:4,6-di-O-isopropylidene)-α-D-mannopyranoside (109).

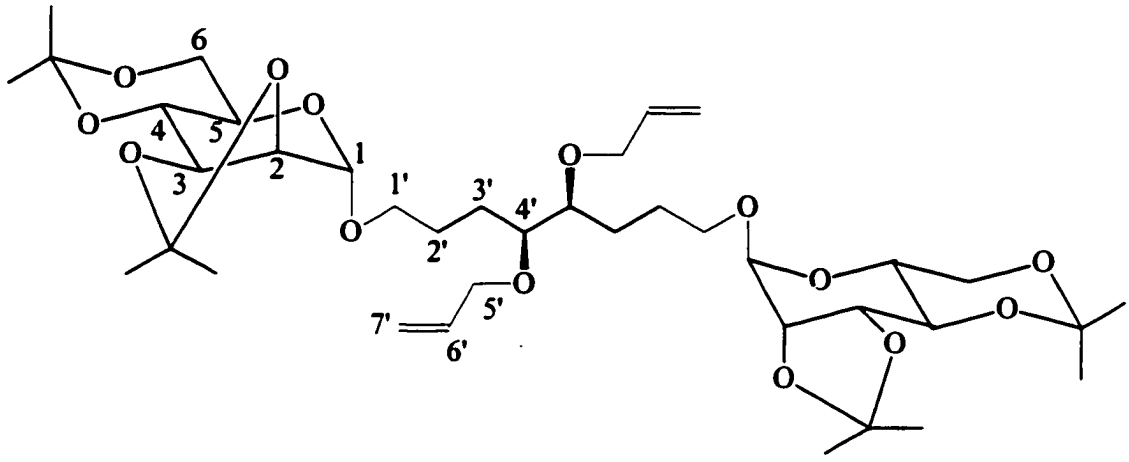
Compound **109** was purified via flash chromatography on silica gel using a gradient of hexanes and hexanes/ethyl acetate 2/1 to 1/2 as eluent and obtained in 92% yield; $[\alpha]^{20}_D = +10.4^0$ (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.92-5.85 (m, 2H, H-4'), 5.28-5.24 (m, 2H, H-5'a), 5.19-5.16 (m, 2H, H-5'b), 5.01 (s, 2H, H-1), 4.17-4.10 (m, 6H, H-2, H-3, H-3'a), 4.07-4.03 (m, 2H, H-3'b), 3.88-3.83 (m, 4H, H-4, H-6a),

4.75-3.70 (m, 4H, H-1'a, H-6b), 3.65-3.61 (m, 2H, H-2'), 3.55 (ddd, J=5.6, 10.2 Hz, 2H, H-5), dd, J=6.2, 10.4 Hz, 2H, H-1'b), 1.53, 1.49, 1.40, 1.33 (4s, 12H, Me); ^{13}C NMR ($CDCl_3$, 125 MHz): δ(ppm) 135.1 (C-4'), 117.7 (C-5'), 109.9, 100.1 (MeCMe), 98.6 (C-1), 77.0 (C-2), 76.4 (C-3), 75.2 (C-4), 73.0 (C-2'), 72.6 (C-1'), 67.4 (C-3'), 62.4 (C-6), 61.8 (C-5), 29.4, 28.6, 26.5, 19.1(Me); ESI-MS, m/z= 704.1, $[M+NH_4]^+$ for $C_{34}H_{54}O_{14}$+ NH_4.



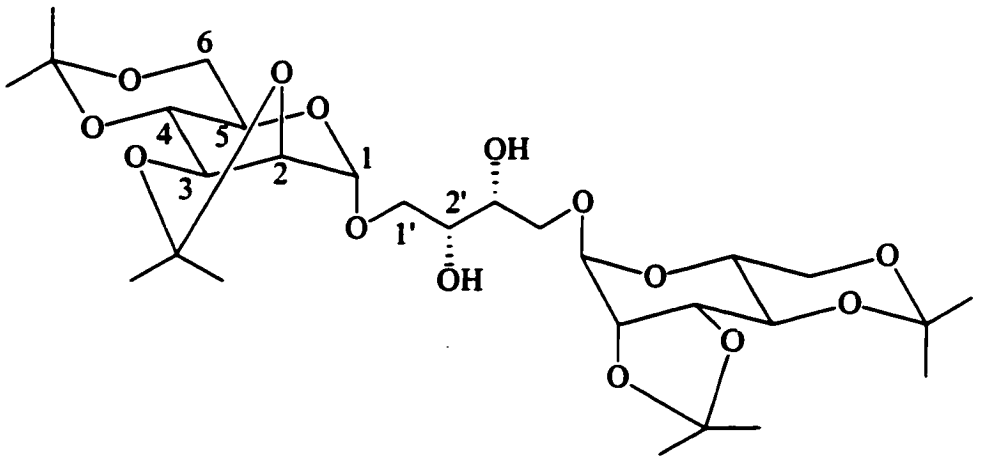
(S,S)-(4,5-Dihydroxyoctyl)di-O-(2,3:4,6-di-O-isopropylidene)-α-D mannopyranoside (110).

Following the general procedure for asymmetric dihydroxylation reaction with AD-mix α, compound **110** was purified via flash chromatography on silica gel using a gradient of hexanes and hexanes/ethyl acetate 2/1 to 1/2 as eluent and obtained in 85% yield; ^1H NMR ($CDCl_3$, 500 MHz): δ (ppm) 4.98 (s, 2H, H-1), 4.14-4.10 (m, 4H, H-3, H-4), 3.84 (dd, J= 5.6, 10.7 Hz, 2H, H-6a), 3.74-3.69 (m, 6H, H-2, H-6b, H-1'a), 3.52 (ddd, J= 5.6, 10.2 Hz, 2H, H-5), 3.45-3.41 (m, 2H, H-1'b), 2.35 (bs, 2H, OH), 1.80-1.56 (m, 4H, H-2', H-3'), 1.52, 1.48, 1.39, 1.32 (4s, 12H, Me); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 109.4, 99.7 (MeCMe), 97.9 (C-1), 76.1, 74.8 (C-3, C-4), 74.1 (C-4'), 72.7 (C-2), 67.8 (C-1'), 62.0 (C-6), 61.4 (C-5), 30.5 (C-3'), 29.0, 28.1, 27.1 (Me), 25.8 (C-2'), 18.7 (Me); CI-MS, m/z= 663, $[M+1]^+$ for $C_{32}H_{54}O_{14}$.



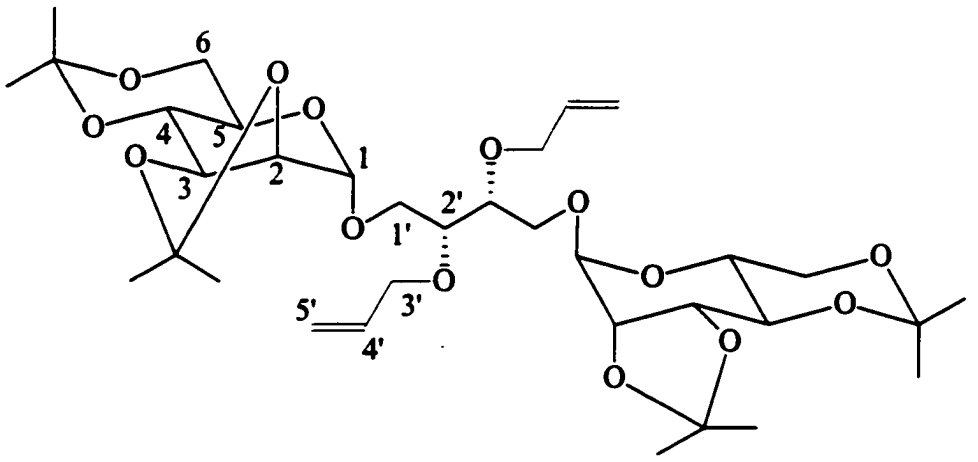
(S,S)-(4,5-Diallyloxyoctyl)di-O-(2,3:4,6-di-O-isopropylidene)-α-D mannopyranoside (111).

Following the general procedure for the bis-allylation of diol, compound **111** was purified via flash chromatography on silica gel using a gradient of hexanes and hexanes/ethyl acetate 2/1 to 1/2 as eluent and obtained in 82% yield; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.93-5.85 (m, 2H, H-6'), 5.23 (dq, J=1.6, 3.3, 17.3 Hz, 2H, H-7'a), 5.14 (dq, J= 1.3, 3.0, 10.4 Hz, 2H, H-7'b), 4.98 (s, 2H, H-1), 4.15-4.06 (m, 6H, H-3, H-4, H-5'a), 4.01-3.97 (m, 2H, H-5'b), 3.84 (dd, J= 5.5, 6.7 Hz, 2H, H-6a), 3.75-3.64 (m, 6H, H-2, H-6b, H-1'a), 3.57-3.52 (m, 2H, H-5), 3.43-3.33 (m, 4H, H-1'b, H-4'), 1.78-1.73 (m, 2H, H-2'a), 1.63-1.54 (m, 4H, H-2'b, H-3'a), 1.52, 1.49 (2s, 12H, Me), 1.45-1.42 (m, 2H, H-3'b), 1.40, 1.33 (2s, 12H, Me); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 135.2 (C-6'), 116.8 (C-7'), 109.3, 99.6 (MeCMe), 97.8 (C-1'), 79.7 (C-4'), 76.1, 74.9 (C-3, C-4), 72.8 (C-2), 71.8 (C-5'), 67.9 (C-1'), 62.1 (C-6), 61.3 (C-5), 29.0, 28.1 (Me), 26.5 (C-3'), 26.1 (Me), 26.1 (C-2'), 18.7 (Me); ESI-MS, m/z= 760.2, $[M+NH_4]^+$ for $C_{38}H_{62}O_{14}$+ NH_4.



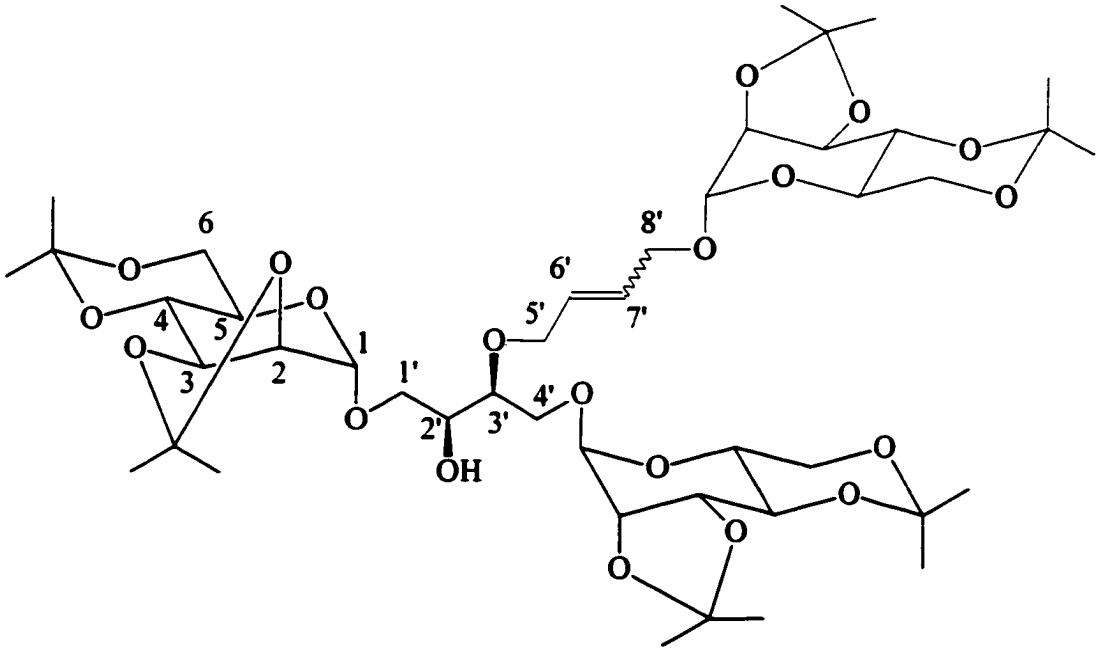
(R,R)-(2,3-Dihydroxybutyl)di-O-(2,3:4,6-di-O-isopropylidene)-α-D mannopyranoside (112).

Following the general procedure for asymmetric dihydroxylation reaction with AD-mix β, compound **112** was purified via flash chromatography on silica gel using a gradient of hexane and hexane/ethyl acetate 2/1 to 1/2 as eluent and obtained in 88% yield; $[\alpha]^{20}_{D}$ = + 10.7^{0} (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.02 (s, 2H, H-1), 4.17 (d, J= 5.7 Hz, 2H, H-2), 4.12 (dd, J= 5.7, 7.8 Hz, 2H, H-3), 3.85 (dd, J=5.6, 10.8 Hz, 2H, H-6a), 3.80 (t, J=4.3 Hz, 2H, H-2'), 3.76-3.70 (m, 6H, H-4, H-6b, H-1'a), 3.59 (dd, J=4.3, 10.3 Hz, 2H, H-1'b), 3.52 (ddd, J= 5.6, 10.2 Hz, 2H, H-5), 2.78 (bs, 2H, OH), 1.51, 1.48, 1.39, 1.32 (4s, 12H, Me); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 109.5, 99.7 (MeCMe), 98.4 (C-1), 75.9 (C-2), 74.8 (C-3), 72.5 (C-4), 69.8 (C-2'), 69.4 (C-1'), 61.9 (C-6), 61.8 (C-5), 29.0, 28.1, 26.1, 18.7 (Me); ESI-MS, m/z= 624.2, $[M+NH_4]^+$ for $C_{28}H_{46}O_{14}$+ NH_4.



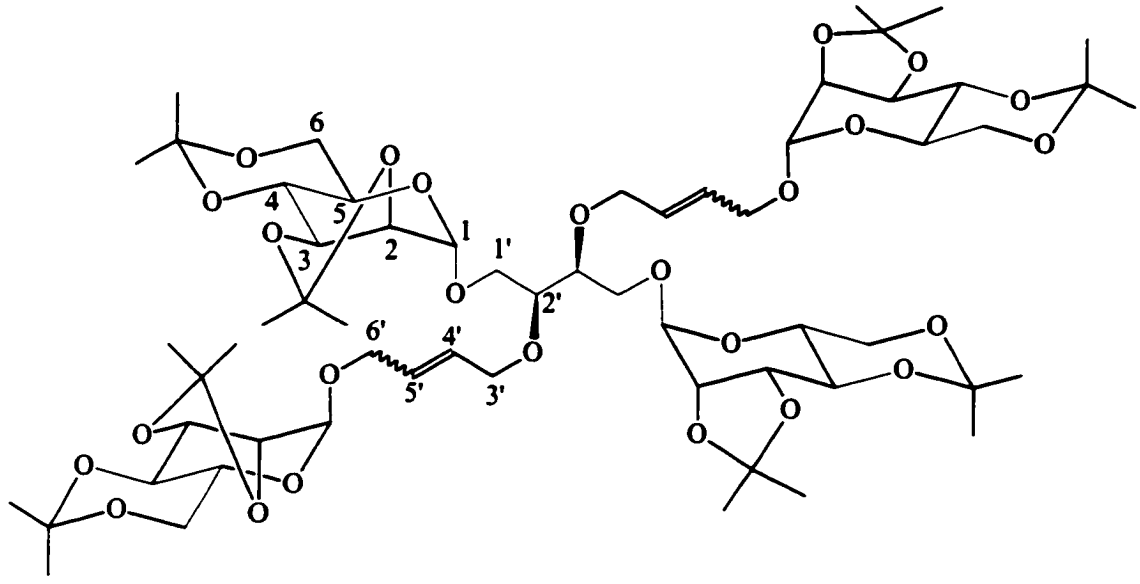
(R,R)-(2,3-Diallyloxybutyl)di-O-(2,3:4,6-di-O-isopropylidene-α-D-mannopyranoside(113).

Compound **113** was purified via flash chromatography on silica gel using a gradient of hexanes and hexanes/ethyl acetate 2/1 to 1/2 as eluent and obtained in 92% yield; $[\alpha]^{20}_{D}$ = + 12.8^{0} (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.92-5.85 (m, 2H, H-4'), 5.27-5.22 (m, 2H, H-5'a), 5.18-5.15 (m, 2H, H-5'b), 5.00 (s, 2H, H-1), 4.17-4.10 (m, 6H, H-2, H-3, H-3'a), 4.06-4.01 (m, 2H, H-3'b), 3.86 (dd, J=5.7, 10.8 Hz, 2H, H-6a), 3.77-3.70 (m, 6H, H-4, H-6b, H-1'a), 3.63-3.59 (m, 4H, H-2', H-1'b), 3.53 (ddd, J= 5.6, 10.2 Hz, 2H, H-5), 1.53, 1.49, 1.40, 1.34 (4s, 12H, Me); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 135.1 (C-4'), 117.9 (C-5'), 109.8, 100.1 (MeCMe), 98.6 (C-1), 77.1 (C-2), 76.3 (C-3), 75.2 (C-4), 73.0 (C-2'), 72.7 (C-1'), 66.9 (C-3'), 62.3 (C-6), 61.9 (C-5), 29.4, 28.5, 26.5, 19.1 (Me); ESI-MS, m/z= 704.1, $[M+NH_4]^+$ for $C_{34}H_{54}O_{14}$+ NH_4.



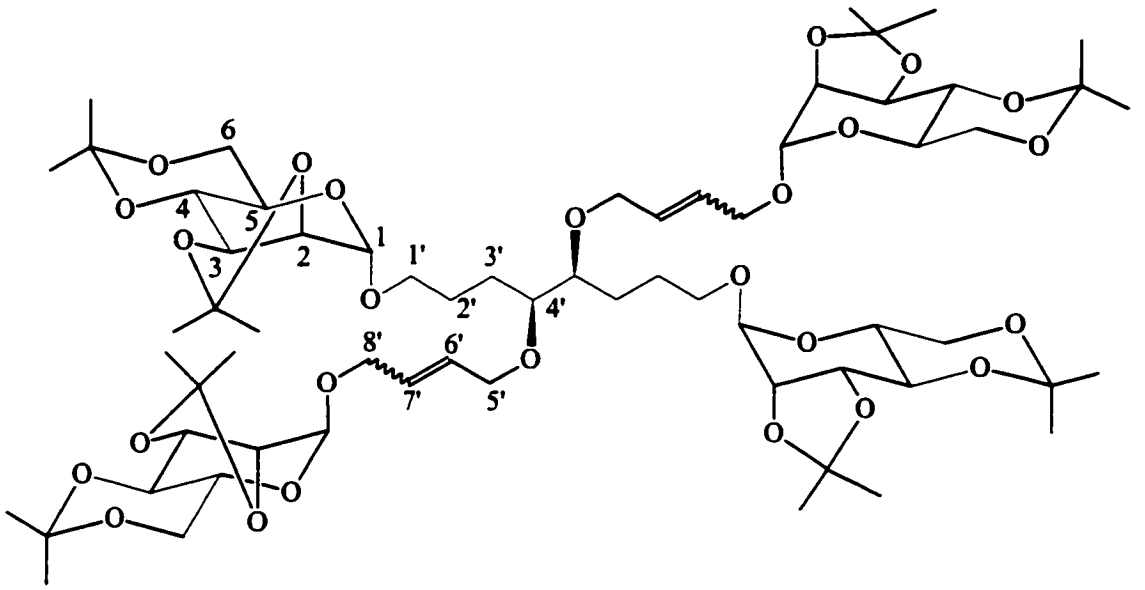
Trivalent glycocluster(114).

To a solution of mono-allyl derivative **108** (82 mg, 0.127 mmol) in dry dichloromethane (2 mL), allyl 2,3:4,6-di-O-isopropylidene-α-D-mannopyranoside **68** (78 mg, 0.26 mmol) and Grubbs' catalyst **18** (21 mg, 0.026 mmol) were added and the reaction mixture was heated at reflux under nitrogen for 15 h. The solution was concentrated under reduced pressure and the residue was purified by silica gel column chromatography using a gradient of hexanes and hexanes/ethyl acetate 3/1 to 1/2 as eluent. The title compound **114** was isolated in 60% yield (70 mg, 0.076 mmol); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.81-5.77 (m, 2H, H-6', H-7'), 5.04, 5.03, 5.01 (3s, 3H, H-1), 4.23-3.97 (m, 10H, H-2, H-3, H-5'a, H-5'b, H-8'a, H-8'b), 3.91-3.83 (m, 5H, H-6a, H-1'a, H-2'), 3.77-3.70 (m, 7H, H-4, H-6b, H-4'a), 3.59-3.46 (m, 6H, H-5, H-1'b, H-4'b, H-3'), 2.33 (bs, 1H, OH), 1.52, 1.49, 1.40, 1.34, 1.33, 1.33 (7s, 36H, Me); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 129.7, 128.4 (C-6', C-7'), 109.5, 109.4, 99.7 (MeCMe), 98.4, 98.1, 97.2 (C-1), 77.4 (C-3'), 76.0, 75.9 (C-2), 74.8 (C-3), 72.7, 72.6, 72.5 (C-4), 71.0 (C-5'), 70.1 (C-2'), 68.8 (C-4'), 67.0 (C-1'), 66.9 (C-8'), 61.9 (C-6), 61.6, 61.5, 61.4 (C-5), 29.0, 28.1, 26.1, 18.7 (Me); ESI-MS, m/z= 936.3, $[M+NH_4]^+$ for $C_{44}H_{70}O_{20}$+ NH_4.



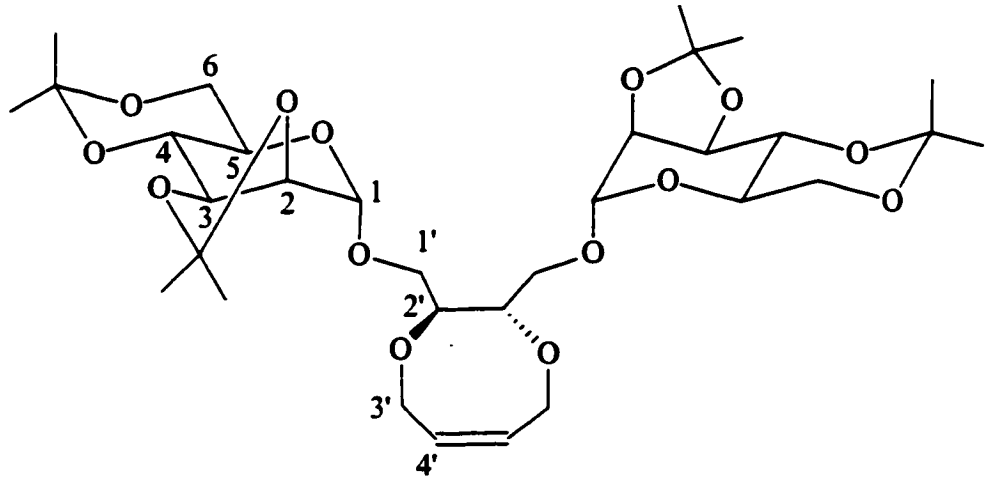
Tetravalent glycocluster with butyl group as spacer (115).

To a solution of bis-allyl derivative **109** (57 mg, 0.083 mmol) in dry dichloromethane (2 mL), allyl 2,3:4,6-di-O-isopropylidene-α-D-mannopyranoside **68** (100 mg, 0.33 mmol) and Grubbs' catalyst (14 mg, 0.017 mmol) were added and the reaction mixture was heated at reflux under nitrogen for 15 h. The solution was concentrated under reduced pressure and the residue was purified by silica gel column chromatography using a gradient of hexanes and hexanes/ethyl acetate 5/1 to 1/2 as eluent. The title compound **115** was isolated in 40% yield (41 mg, 0.033 mmol); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.80-5.78 (m, 4H, H-4', H-5'), 5.02, 5.01 (2s, 4H, H-1), 4.18-3.94 (m, 16H, H-2, H-3, H-3', H-5'), 3.86-3.82 (m, 6H, H-6a, H-1'a), 3.74-3.69 (m, 8H, H-6b, H-4), 3.63-3.51 (m, 8H, H-5, H-1'b, H-2'), 1.52, 1.51, 1.48, 1.39, 1.33, 1.33, 1.32 (7s, 48H, Me); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 130.6, 128.2 (C-4', C-5'), 109.9, 109.8, 100.1, 100.0 (MeCMe), 98.5, 97.4 (C-1), 78.1 (C-2'), 76.4, 76.3 (C-2), 75.2 (C-3), 73.1, 73.0 (C-4), 71.5 (C-3'), 67.5, 67.4 (C-1', C-5'), 62.4 (C-6), 61.9, 61.7 (C-5), 29.4, 28.6, 26.6, 26.5, 19.1 (Me); ESI-MS, m/z= 1248.1, $[M+NH_4]^+$ for $C_{60}H_{94}O_{26}+NH_4$.



Tetravalent glycocluster with octyl group as spacer (116).

To a solution of bis-allyl derivative **111** (78 mg, 0.105 mmol) in dry dichloromethane (3 mL), allyl 2,3:4,6-di-O-isopropylidene-α-D-mannopyranoside **68** (126 mg, 0.42 mmol) and Grubbs' catalyst (17 mg, 0.021 mmol) were added and the reaction mixture was heated at reflux under nitrogen for 17 h. The solution was concentrated under reduced pressure and the residue was purified by silica gel column chromatography using a gradient of hexanes and hexanes/ethyl acetate 5/1 to 1/1 as eluent. The title compound **116** was isolated in 30% yield (40 mg, 0.031 mmol); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.86-5.77 (m, 4H, H-6', H-7'), 5.06, 5.01 (2s, 4H, H-1), 4.21-4.10 (m, 12H, H-3, H-4, H-5'a, H-8'a), 4.07-3.98 (m, 4H, H-5'b, H-8'b), 3.89-3.78 (m, 4H, H-6a), 3.78-3.68 (m, 10H, H-2, H-6b, H-1'a), 3.61-3.54 (m, 4H, H-5), 3.46-3.42 (m, 2H, H-1'b), 3.35 (d, J=8.1 Hz, 2H, H-4'), 1.81-1.77 (m, 2H, H-2'a), 1.67-1.58 (m, 6H, H-2'b, H-3'), 1.55, 1.52, 1.43, 1.35 (4s, 48H, Me); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 131.0, 127.8 (C-6', C-7'), 109.6, 99.9 (MeCMe), 98.1, 97.3 (C-1), 80.6 (C-4'), 76.4, 76.3 (C-4), 75.1, 75.1 (C-3), 73.0, 73.0 (C-2), 71.0 (C-5'), 68.1 (C-1'), 67.4 (C-8'), 62.3, 62.3 (C-6), 61.6, 61.6 (C-5), 29.3, 28.4 (Me), 27.0 (C-3'), 26.4 (Me), 26.3 (C-2'), 19.0 (Me).

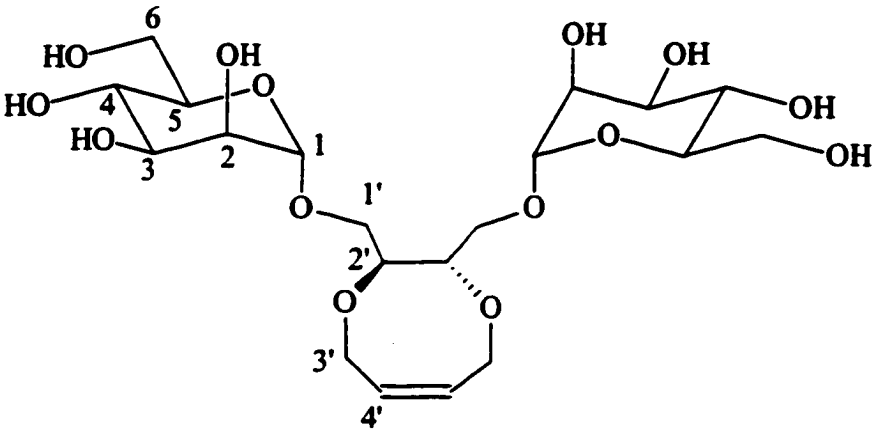


(S,S)-RCM product with butyl group as spacer (117).

To a solution of bis-allyl derivative **109** (38 mg, 0.055 mmol) in dry dichloromethane (5 mL), Grubbs' catalyst (10 mg, 0.011 mmol) was added and the reaction mixture was heated at reflux under nitrogen for 11 h. The solution was concentrated under reduced pressure and the residue was purified by silica gel column chromatography using a gradient of hexanes and hexanes/ethyl acetate 3/1 to 1/1 as eluent. The title compound **117** was isolated in 60% yield (22 mg, 0.033 mmol); $[\alpha]^{20}_{D}$ = + 7.7^{0} (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.70 (t, J= 2.7 Hz, 1H, H-4'), 4.99 (s, 2H, H-1), 4.45-4.30 (m, 4H, H-3'a, H-3'b), 4.20-4.15 (m, 4H, H-2, H-3), 3.87-3.69 (m, 8H, H-4, H-6a, H-6b, H-1'a), 3.62-3.42 (m, 6H, H-5, H-1'b, H-2'), 1.52, 1.49, 1.40, 1.33 (4s, 12H, Me); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 129.7 (C-4'), 100.9, 100.0 (MeCMe), 98.7 (C-1), 81.4 (C-2'), 76.3 (C-2), 75.2 (C-3), 73.0 (C-4), 69.5 (C-3'), 68.5 (C-1'), 62.4 (C-6), 61.8 (C-5), 29.4, 28.5, 26.5, 19.1 (Me).; ESI-MS, m/z= 676.0, $[M+NH_4]^+$ for $C_{32}H_{50}O_{14}$+ NH_4.

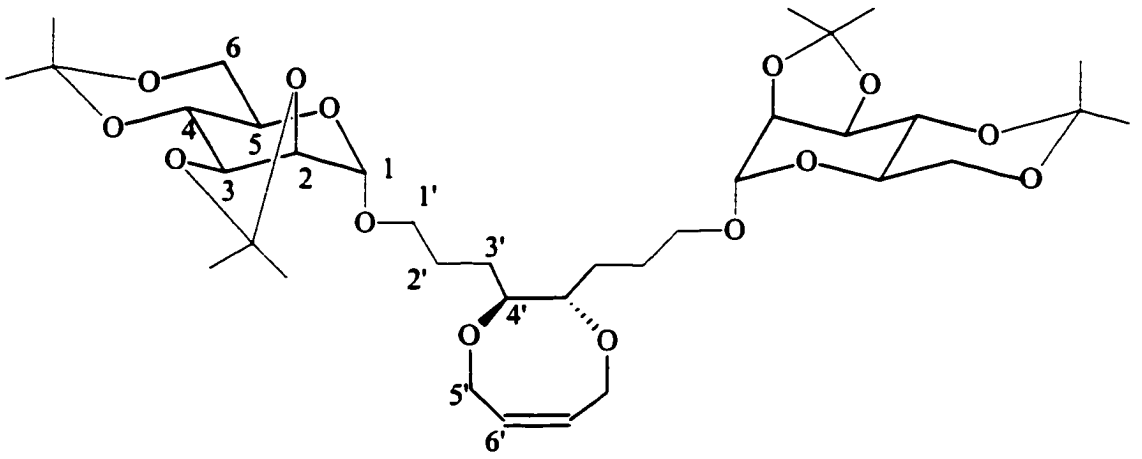
General procedure for the removal of acetonide groups:

0.05 mmol of glycoside was dissolved in 60 % aqueous AcOH (4 mL) and heated at 80° C for 1h. The reaction mixture was then diluted with toluene (4 mL) and evaporated under reduced pressure to afford the resulting deprotected glycoside.



Fully deprotected (S,S)-RCM product with butyl group as spacer (118).

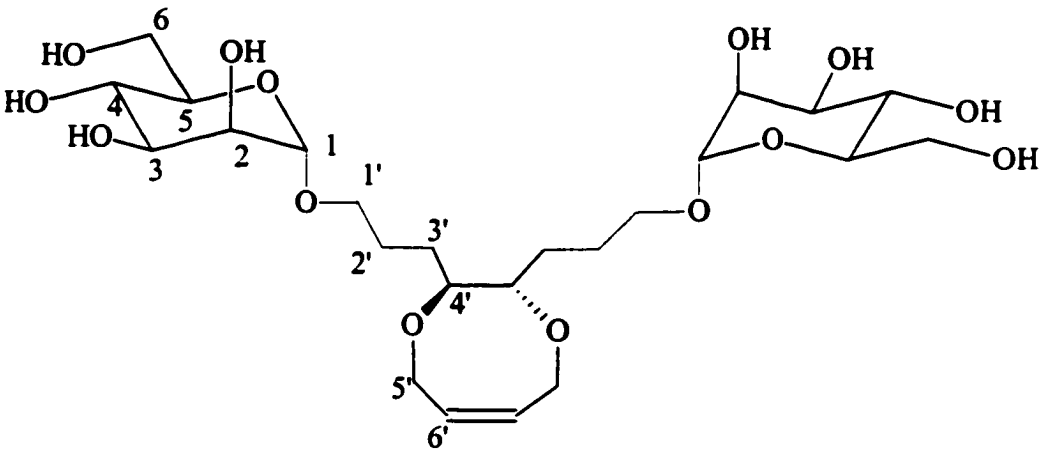
^{1}H NMR (D_2O, 500 MHz): δ (ppm) 5.66 (bs, 2h, H-4'), 4.71 (d, J= 1.7 Hz, 2H, H-1), 4.34 (d, J=16.8 Hz, 2H, H-3'a), 4.23 (d, J= 16.4 Hz, 2H, H-3'b), 3.83 (dd, J=1.7, 3.3 Hz, 2H, H-2), 3.80-3.36 (m, 16H, 2H-3, 2H-4, 2H-5, 4 H-6, 4H-1', 2H-2'); ESI-MS, m/z= 572.2, $[M+NH_4]^+$ for $C_{24}H_{42}O_{14}$+ NH_4.



(S,S)-RCM product with octyl group as spacer (119).

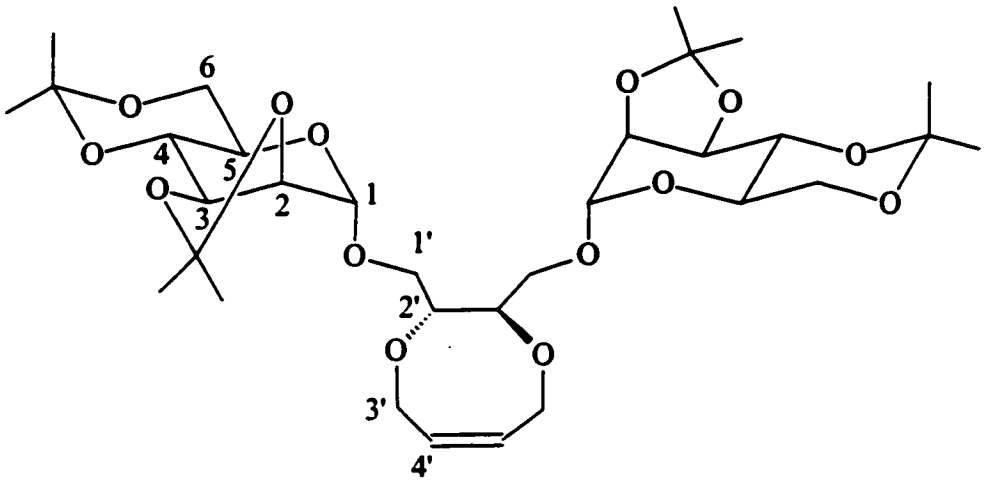
To a solution of bis-allyl derivative **111** (64 mg, 0.087 mmol) in dry dichloromethane (9 mL), Grubbs' catalyst (15 mg, 0.017 mmol) was added and the reaction mixture was heated at reflux under nitrogen for 11 h. The solution was concentrated under reduced pressure and the residue was purified by silica gel column chromatography using a gradient of hexanes and hexanes/ethyl acetate 5/1 to 1/1 as eluent. The title compound **119** was isolated in 80% yield (50 mg, 0.070 mmol); $[\alpha]^{20}_D$ = -3.8^0 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.70 (bs, 2H, H-6'), 5.00 (s, 2H, H-1), 4.45 (dd, J= 2.6, 14.3 Hz, 2H, H-5'a), 4.26 (dd, J= 2.6, 15.5 Hz, 2H, H-5'b),

4.16-4.12 (m, 4H, H-3, H-4), 3.85 (dd, J= 5.7, 10.8 Hz, 2H, H-6a), 3.77-3.66 (m, 6H, H-2, H-6b, H-1'a), 3.55 (ddd, J= 5.6, 10.1 Hz, 2H, H-5), 3.45-3.41 (m, 2H, H-1'b), 3.13 (t, J= 7.9 Hz, 2H, h-4'), 1.86-1.79 (m, 2H, H-2'a), 1.70-1.60 (m, 2H, H-2'b), 1.54, 1.51 (2s, 12H, Me), 1.47-1.44 (m, 2H, H-3'a), 1.42 (s, 6H, Me), 1.38-1.30 (m, 2h, H-3'b), 1.34 (s, 6H, Me); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 129.3 (C-6'), 109.3, 99.6 (MeCMe), 97.8 (C-1), 85.6 (C-4'), 76.1 (C-4), 74.9 (C-3), 72.7 (C-2), 70.1 (C-5'), 67.8 (C-1'), 62.0 (C-6), 61.3 (C-5), 29.1 (C-3'), 29.0, 28.1, 26.1 (Me), 25.6 (C-2''), 18.7 (Me); ESI-MS, m/z= 732.1, $[M+NH_4]^+$ for $C_{36}H_{58}O_{14}$+ NH_4.



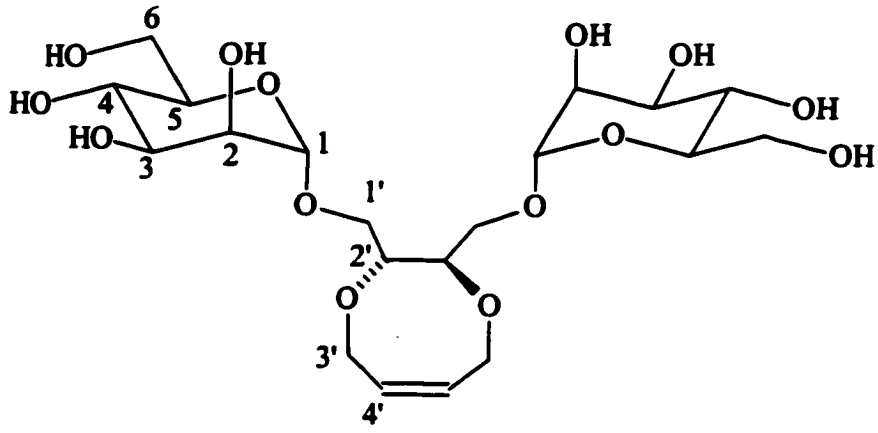
Fully deprotected (S,S)-RCM product with octyl group as spacer (120).

^1H NMR (D_2O, 500 MHz): δ (ppm) 5.83 (s, 2H, H-6'), 4.93 (s, 2h, H-1), 4.58 (d, J= 17.0 Hz, 2H, H-5'a), 4.36 (d, J=15.2 hz, 2H, H-5'b), 4.01-3.94 (m, 4H, H-3, H-6a), 3.90-3.81 (m, 6H, H-4, H-6b, H-1'a), 3.79-3.61 (m, 6H, H-2, H-5, H-1'b), 3.57-3.52 (m, 2H, H-4'), 1.89-1.76 (m, 6H, H-2', H-3'a), 1.48-1.42 (m, 2H, H-3'b); ^{13}C NMR (D_2O, 125 MHz): δ (ppm) 128.4 (C-6'), 99.2 (C-1), 85.4 (C-4'), 72.3 (C-2), 70.2 (C-4), 69.6 (C-3), 69.6 (C-5'), 67.2 (C-1'), 66.3 (C-5), 60.5 (C-6), 27.9 (C-3'), 24.2 (C-2'); ESI-MS, m/z= 572.2, $[M+NH_4]^+$ for $C_{24}H_{42}O_{14}$+ NH_4.



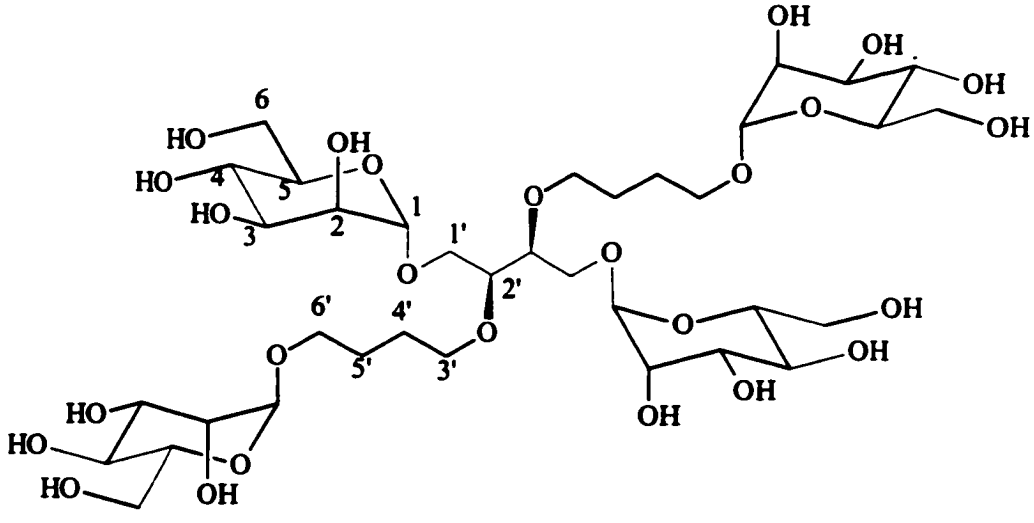
(R,R)-RCM product with butyl group as spacer (121).

To a solution of bis-allyl derivative **113** (75 mg, 0.109 mmol) in dry dichloromethane (13 mL), Grubbs' catalyst (20 mg, 0.022 mmol) was added and the reaction mixture was heated at reflux under nitrogen for 11 h. The solution was concentrated under reduced pressure and the residue was purified by silica gel column chromatography using a gradient of hexane and hexane/ethyl acetate 3/1 to 1/1 as eluent. The title compound **121** was isolated in 65% yield (46 mg, 0.070 mmol); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 5.70 (t, J=3.7 Hz, 2H, H-4'), 5.02 (s, 2H, H-1), 4.47-4.29 (m, 4H, H-3'a, H-3'b), 4.22 (d, J= 5.6 Hz, 1H, H-2), 4.18-4.04 (m, 2H, H-6a), 3.86 (dd, J=5.7, 10.8 hz, 2H, H-6b), 3.75-3.61 (m, 10H, H-3, H-4, H-1'a, H-1'b, H-2'), 3.57-3.43 (m, 2H, H-5), 1.51, 1.49, 1.40, 1.32 (4s, 12H, Me); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 129.7 (C-4'), 109.8, 100.1 (MeCMe), 98.7 (C-1), 81.1 (C-2'), 76.3 (C-2), 75.1 (C-3), 72.9 (C-4), 69.7 (C-3'), 68.8 (C-2'), 62.3 (C-6), 62.2 (C-5), 29.4, 28.5, 26.5, 19.1 (Me); ESI-MS, m/z= 676.0, $[M+NH_4]^+$ for $C_{32}H_{50}O_{14}$+ NH_4.



Fully deprotected (R,R)-RCM product with butyl group as spacer (122).

$[\alpha]^{20}_D$ = +46.4^0 (*c* 1, H_2O); ^{1}H NMR (D_2O, 500 MHz): δ (ppm) 5.85 (t, J= 2.1 Hz, 2H, H-4'), 4.92 (d, J= 1.2 Hz, 2H, H-1), 4.58-4.55 (m, 2H, H-3'a), 4.45-4.41 (m, 2H, H-3'b), 4.05 (dd, J= 1.2, 3.3 Hz, 2H, H-2), 3.93 (dd, J= 2.1, 12.3 Hz, 2H, H-6a), 3.87-3.77 (m, 10H, H-3, H-6b, H-1'a, H-1'b, H-2'), 3.71 (t, J= 9.8 Hz, 2H, H-4), 3.67-3.62 (m, 2H, H-5); ^{13}C NMR (D_2O , 125 MHz): δ (ppm) 128.4 (C-4'), 99.7 (C-1), 80.2 (C-2'), 72.7 (C-5), 70.0 (C-3), 69.4 (C-2), 68.9 (C-3'), 67.3 (C-1'), 66.1 (C-4), 60.4 (C-6); ESI-MS, m/z= 516.1, $[M+NH_4]^+$ for $C_{20}H_{34}O_{14}$+ NH_4.



Fully deprotected Tetravalent glycocluster with butyl group as spacer (123).

To a solution of the RCM product **117** (30 mg, 0.04 mmol) in dry dichloromethane (2 mL), allyl 2,3:4,6-di-O-isopropylidene-α-D-mannopyranoside **68** (48 mg, 0.16 mmol) and Grubbs' catalyst (10 mg, 0.08 mmol) were added and the reaction

mixture was heated at reflux under nitrogen for 17 h. The solution was concentrated under reduced pressure and the residue was purified by silica gel column chromatography using a gradient of hexanes and hexanse/ethyl acetate 5/1 to 1/1 as eluent. The tetravalent glycocuster **115** was isolated in 48 % yield (24 mg, 0.020 mmol).

To a solution of **115** (24 mg, 0.020 mmol) was added 10% palladium/carbon (2 mg) and the reaction mixture was stirred under a hydrogen atmosphere at room temperature for 1h. The catalyst was filtered off over celite and the filtrate concentrated to afford compound the hydrogenated compound which was treated under the conditions described above to remove the acetonide groups. Coumpound **123** was then further purified using a biogel P-4 column using H_2O as eluent.; ^1H NMR (D_2O, 300 MHz): δ (ppm) 4.71 (s, 4H, H-1), 3.83-3.41 (m, 38H, 4H-2, 4H-3, 4H-4, 4H-5, 8H-6, 4H-1', 2H-2', 4H-3', 4H-6'), 1.54 (bs, 8H, 4H-4', 4H-5'); ESI-MS, m/z= 932.2, $[M+NH_4]^+$ for $C_{36}H_{66}O_{26}+ NH_4$.

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Chapter 5. Design and synthesis of a trimannoside ligand toward the human Mannose-Binding Protein (hMBP).

5.1. Human Mannose-Binding Protein

The human mannose-binding protein (hMBP) is a member of the collectin family of molecules that play a role in first line host defence.[1-3] Found in the serum of many mammalian species,[4-8] this protein mediates immunoglobin-independent defensive reactions against pathogens. Its action occurs within minutes of exposure to an infectious agent providing immediate defense during the 1-3 days lag period required for induction of specific antibodies.[9] The mannose-binding proteins function by recognizing oligomannose on the cell surface of various bacteria and viruses. They bind and neutralize them by complement-mediated cell lysis or facilitate their recognition by phagocytes (figure 5.1.1).

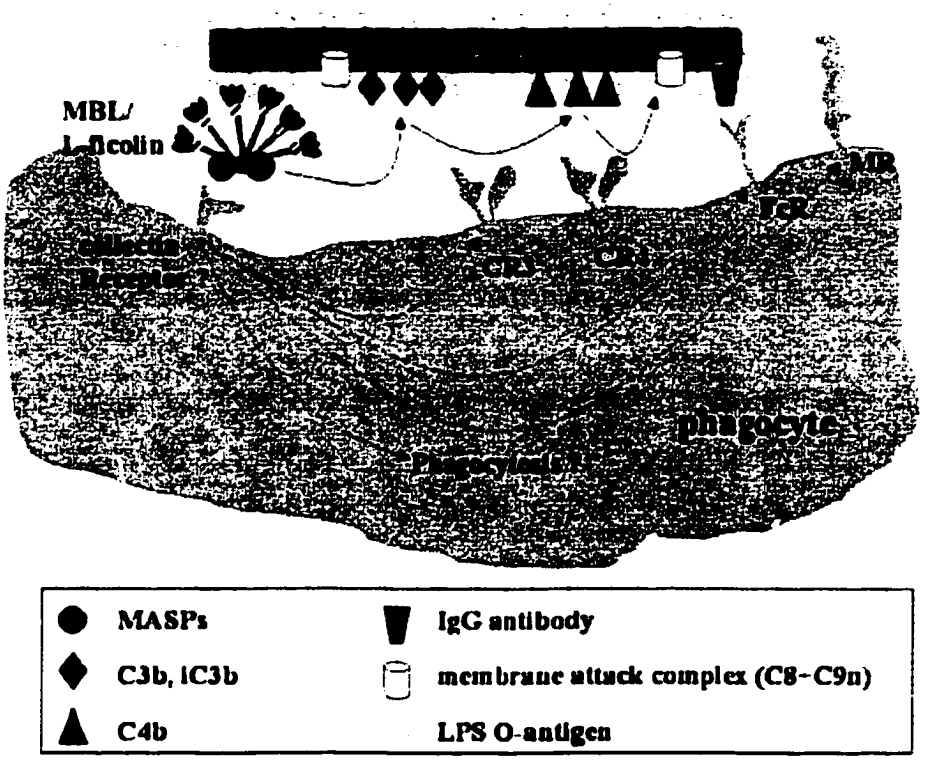


Figure 5.1.1. MBP mediates microbial killing and clearance through complement activation and phagocytosis. [10]

Upon exposure to pathogens, the concentration of MBP in the serum increase.[11] Patients with reduced levels of MBP, due to a point mutation in the MBP gene, show a propensity for repeated, severe bacterial infections.[12] MPB's also act as inhibitors of human immunodeficiency virus and influenza virus, presumably by binding to the high mannose carbohydrates of the viral envelope glycoproteins and blocking attachment to the host cell. All those observations are clearly showing the importance of the MBP in the innate immune system.

The structure of the hMBP consits of 18 identical subunits arranged as a hexamer of trimers, adopting a bouquet-like structure (Figure 5.1.2.).[13-15] Each subunits chain consists of four distinct regions. This includes a cysteine-rich region, a collagen-like region, a neck region and a carbohydrate recognition domain (CRD) that is calcium-dependent. The oligomerization is possible through the disulfide bonds in the cysteine-rich region and due to the characteristic properties of the collagen-like domain and the neck region, it tends to form a trimer. The clustering of three CRDs in close proximity significantly increases the affinity for small sugar clusters. The impact of further clustering can ensure that these molecules only bind with high affinity to dense sugar arrays like the one found on the surface of bacteria and viruses.

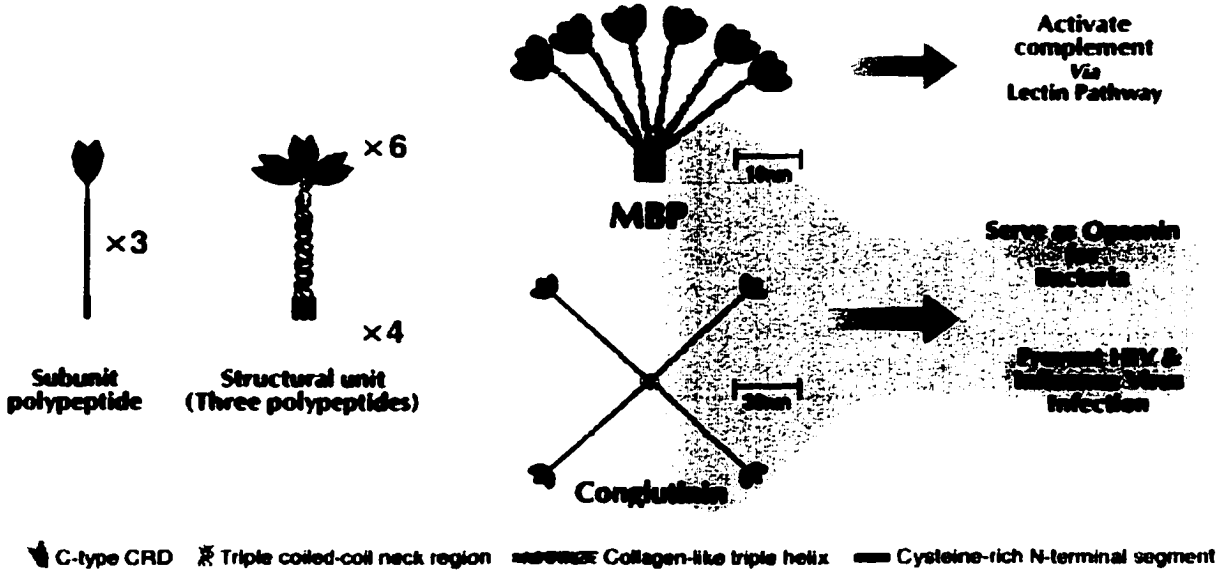


figure 5.1.2. Structure and biological roles of hMPB.[13]

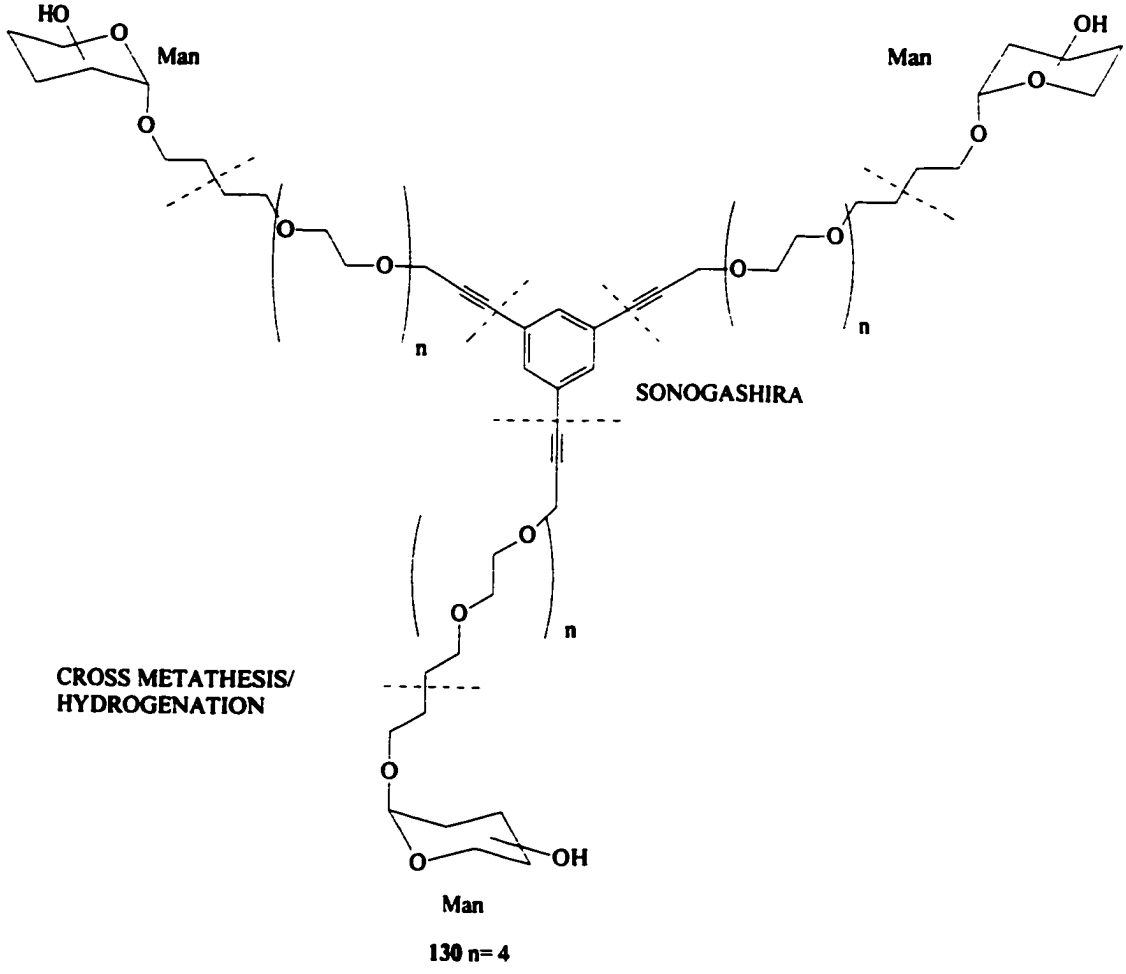
The hMBP forms oligomeric bouquets or cruciform structures of trimeric subunits each composed of three polypeptide chains coiled up in a collagenous triple-helical arrangement. This trimerization domain orients each carbohydrate recognition domain (CRD) so that the ligands binding sites are 45 A° apart and 26 A° from the center of the trimer (Figure 5.1.3.). Thus, simultaneous ligation of three binding sites could not be achieved by a single molecule but rather elongated branched oligosaccharides as found in the cell walls of yeast, certain gram negative and gram positive organisms, and the envelope glycoprotein of the human immunodeficiency virus, all of which have been shown to bind to MBP. This suggests that the CRDs sites are ideally configured for multivalent interactions, thereby increasing the binding affinity of hMBP to pathogens.

Figure 5.1.3. Crystal structure of hMBP. [16]

5.2. Results and Discussion

5.2.1. Design of the trimannoside ligand

In order to synthesize a molecule that would occupy the three CRDs sites of a hMBP trimer simultaneously, in such a way that would mimic the vast arrays of carbohydrates that decorate the cell walls of pathogens, we had to take in consideration two important factors: a central core with three arms and a long spacer that would separate the mannose residues from the central core by approximately 26 Å.



Scheme 5.2.1. Design of trimannoside ligand toward hMBP.

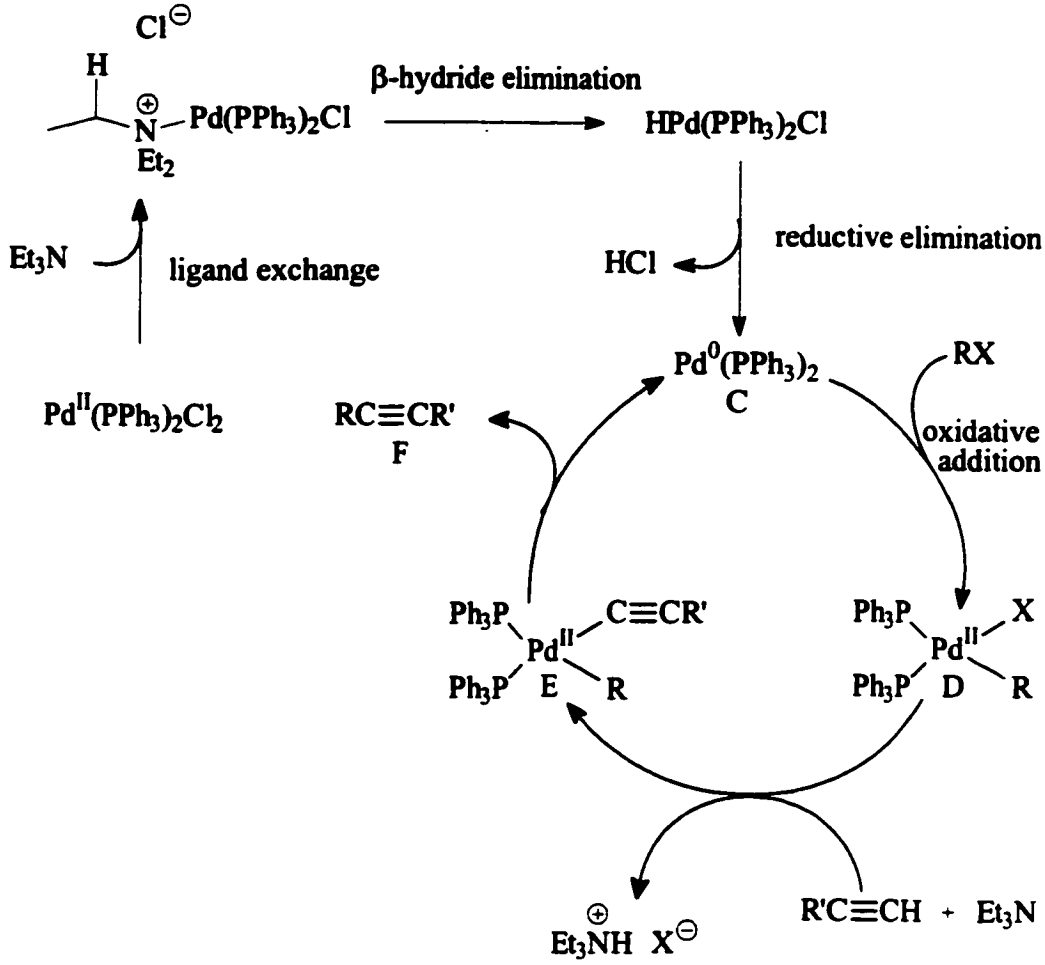
For this purpose, oligoethylene glycol as spacer was considered to elongate each of the arms since it is available at different length and have access to extended mannoside residues by cross-metathesis reaction. For the central core, 1,3,5-triiodobenzene was an ideal candidate to obtain the trimannoside glycocluster by an efficient triple cross-coupling Sonogashira reaction (Scheme 5.2.1).

5.2.2. The Sonogashira coupling reaction[17]

The Sonogashira coupling reaction is a valuable tool in organic synthesis and has been applied toward the synthesis of several natural products.[18] In this reaction, a terminal alkyne is coupled with aryl or vinyl halides in the presence of catalytic amounts of a palladium (0) complex, a base, usually diethylamine and copper (I) iodide as co-catalyst. The palladium(0) complex can be generated in situ, by reduction of a palladium (II) complex with Et_3N or PPh_3 (Scheme 5.2.2.).

In the catalytic cycle for the Sonogashira reaction the palladium(0) is the active catalyst, once formed, the highly coordinatively unsaturated 14-electron palladium(0) complex C participates in an oxidative addition reaction with the aryl or vinyl halide to give the 16-electron palladium(II) complex D which is alkynylated to furnish an aryl- or vinylalkynyl palladium(II) complex E. Finally, a terminating reductive elimination step reveals the coupling product F and regenerates the active palladium(0) catalyst.

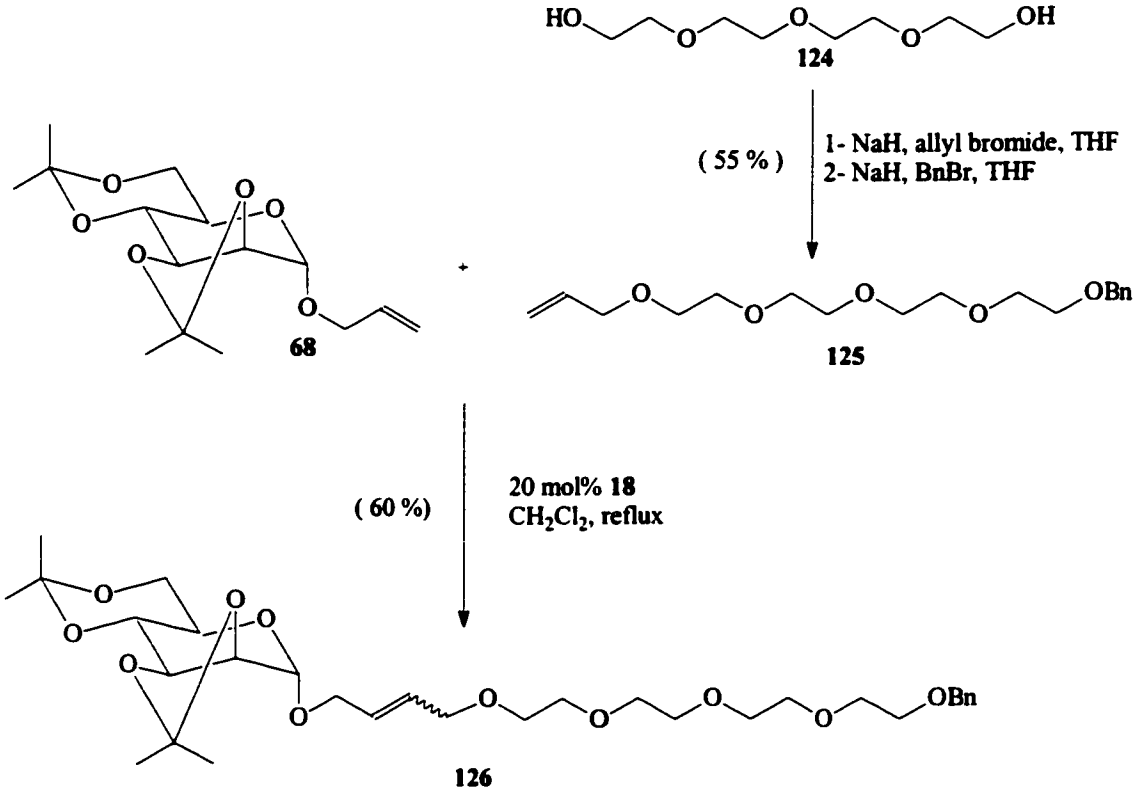
In the literature, it has been shown that it was possible to perform multiple Sonogashira coupling reactions, we therefore wanted to apply this reaction toward the synthesis of the trimannoside ligand.



Scheme 5.2.2. Catalytic cycle for the Sonogashira reaction.

As mentioned above, extension of a mannoside residue was required in order to obtain the 26Å length necessary to reach the three binding sites in the human mannose binding protein. The methodology developed in Chapter 3 was therefore ideal for obtaining an extended mannoside residue. Thus, preparation of allyl, benzyl ether tetraethylene glycol **125** was achieved by treating tetraethylene glycol **124** with 1.5 equiv. of allyl bromide followed by 2 equiv. of benzyl bromide in presence of sodium hydride in THF. In this way, **125** could be obtained in 55 % yield. When DMF was used instead of THF, yield dropped to 35% due to loss of the product in the aqueous phase during the extraction process.

The structure of compound **125** was easily confirmed by ^{1}H-NMR spectroscopy revealing the presence of the characteristic two terminal alkene protons at δ 5.03-5.20 ppm and the two benzylic protons at δ 4.44 ppm.

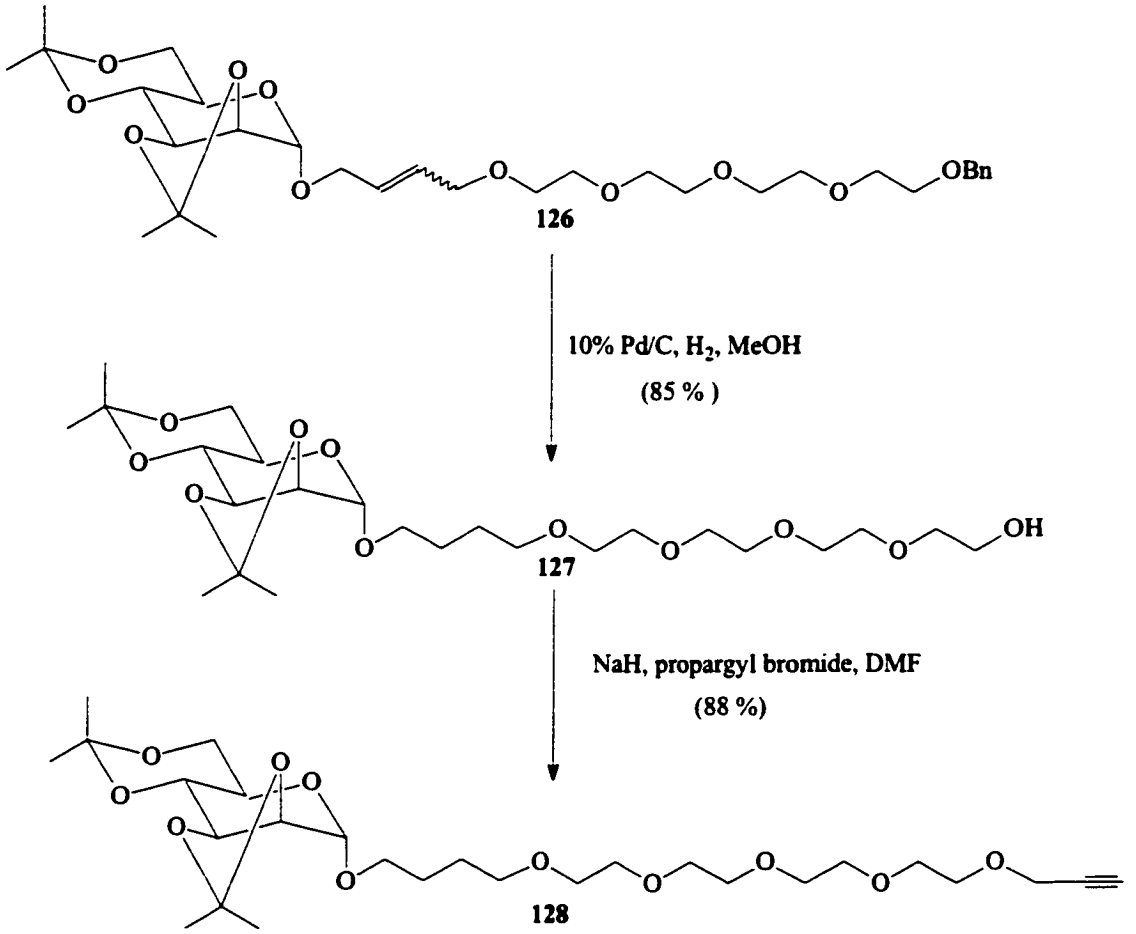


Scheme 5.2.3. Synthesis of extended mannoside **126**.

Cross-metathesis reaction between **68** and 2 equiv. of **125** under the conditions described

in Chapter 3 afforded extended mannoside **126** in 60 % yield as a mixture of unseparable stereoisomers in a 6:1 ratio in favor of *E* isomer (Scheme 5.2.2). The ratio was determined by ^{1}H-NMR (500 MHz) spectroscopy which showed a difference in chemical shift for H-1 of 0.01 ppm between the two stereoisomers. Homodimer **81** as well as homodimer of **125** were formed during the reaction in minor amount.

COSY experiments on extended mannoside **126** clearly indicated the presence of tetraethylene glycol spacer. The olefinic protons H-2' and H-3' at δ 5.72-5.83 ppm correlated with the methylene groups H-1' and H-4' respectively.



Scheme 5.2.4. Synthesis of extended mannoside **128**.

Compound **126** was then transformed into a single product by hydrogenation of the double bond which also removed the benzyl protecting group to give free mannoside

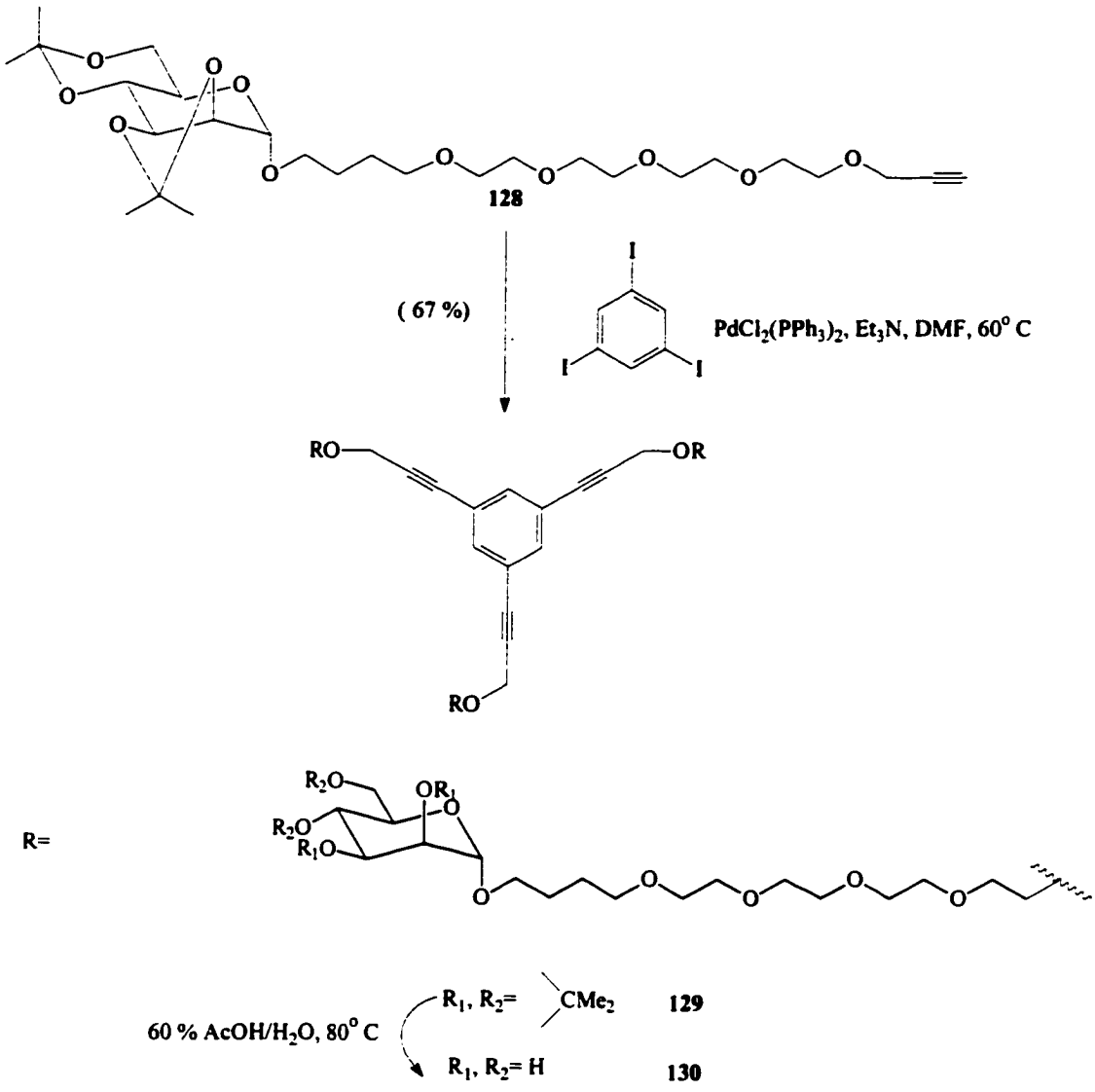
alcohol derivative **127** in 85% yield. The chemical shift corresponding to the hydroxyl group appeared as a broad singulet at δ 2.82 ppm. Moreover, the presence of a new multiplet upfield at δ 1.59-1.62 ppm and the disappearance of olefinic protons confirmed structure **127**.

The next step was to introduce the propynyl functionality for the final Sonogashira reaction. Therefore, treatment of **127** with an excess of sodium hydride and propargyl bromide in DMF afforded terminal alkyne **128** in 88% yield. The alkynyl proton showed a distinctive triplet at δ 2.39 ppm in its ^{1}H-NMR spectra and a peak at δ 80.0 ppm corresponding to the sp hybrized terminal carbon in the ^{13}C-NMR spectra. The Sonogashira coupling reaction which was performed with 1,3,5-triiodobenzene and 3.3 equiv. of **128** in a solution of Et_3N and DMF (1:1) heated at 60° C using dichlorobis(triphenylphosphine)palladium(II) 5 mol%. In this way, protected trimannoside ligand **129** separated from the central core by 26 Å was prepared in 67% yield. The ^{1}H-NMR spectra showed a clear singlet integrating for three protons at δ 7.40 ppm assigned to the magnetically equivalent aromatic hydrogens.

Finally, removal of the acetonides groups was accomplished by treatment of **129** with 60% aqueous AcOH (80° C, 1 h). The fully deprotected trimannoside ligand (see appendix) was further purified by size exclusion column using Bio-gel P4 column and water as eluent and sent for testing. Preliminary results for the inhibitory potential of **130** were promising showing IC_{50} value in the nM range. More extensive biological tests are currently underway.

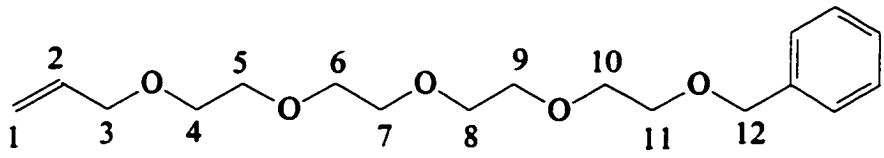
5.3. Conclusions

An efficient synthesis of trimannoside ligand **130** toward human Mannose–binding protein has been shown. This protein found in the serum plays key roles in innate immunity. To further understand these roles, high affinity ligands were required. It is anticipated that the binding of trimannoside ligand **130** to mannose-binding protein could stimulate the production of macrophages in order to stop an infection.



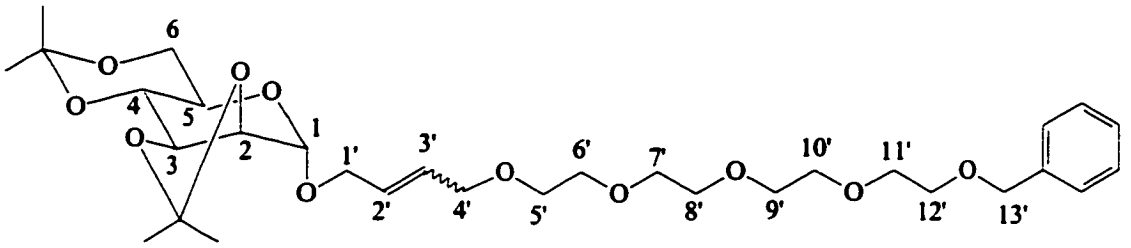
Scheme 5.2.5. Synthesis of trimannoside ligand.

5.4. Experimental methods



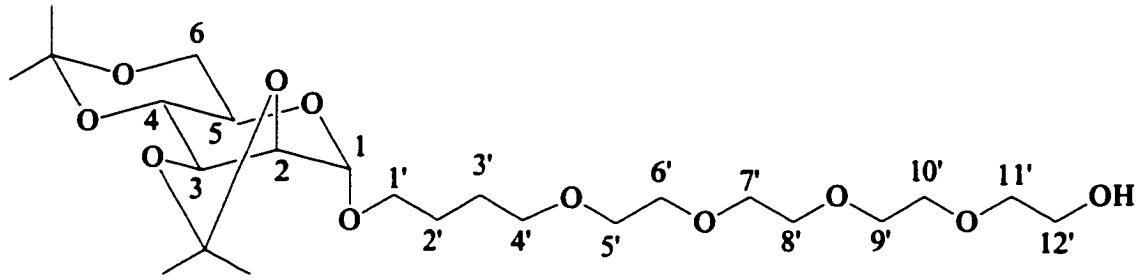
1-Allyloxy-11-benzyloxy-3,6,9-trioxaundecane (125).

Sodium hydride as a 60% dispersion in mineral oil (1.60 g, 39.71 mmol) was added to a solution of tetraethylene glycol (7.00 g, 36.1 mmol) and allyl bromide in THF (100 mL) cooled at 0° C. The reaction mixture was stirred and allowed to warm to room temperature. The progress of the reaction was monitored by TLC (hexanes/ethyl acetate 1/3) and starting material was consumed after 5 h. The reaction mixture was again cooled to 0° C and benzyl bromide (6.5 mL, 54.15 mmol) followed by sodium hydride, 60% dispersion in oil (2.20 g, 54.15 mmol) were added. The reaction was stirred and allowed to warm to room temperature. After 3 h, the reaction was completed and MeOH (20 mL) was added and stirred for an additional 10 min. The crude product was concentrated under reduced pressure and purified by flash chromatography using a gradient of hexanes, ethyl acetate and ethyl acetate/ methanol 5/1 as eluent. Title compound **125** was isolated in 55% yield as a clear oil (6.43 g, 19.84 mmol); ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.23-7.12 (m, 5H, Ph), 5.86-5.73 (m, 1H, H-2), 5.20-5.03 (m, 2H, H-1), 4.44 (s, 2H, H-12), 3.91-3.88 (m, 2H, H-3), 3.57-3.45 (m, 16H, H-4 to H-11); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 138.6, 135.1, 128.6, 128.0, 127.9, 117.3, 73.4, 72.4, 70.9, 70.9, 69.7



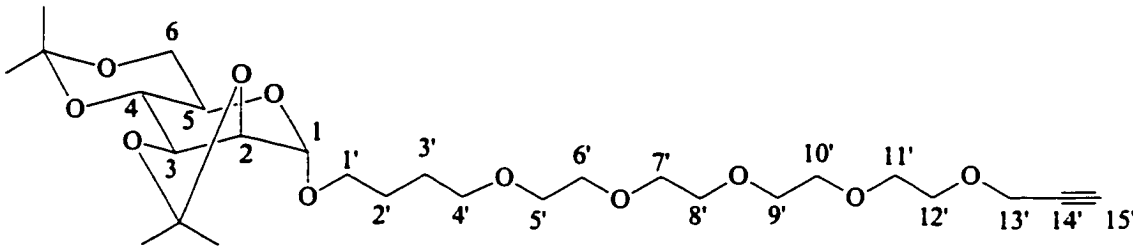
Cross-metathesis product 126.

To a 0.06 M solution of allyl glycoside **68** (300 mg, 1 mmol) in dry dichloromethane (17 mL), allyl ether, benzyl ether **125** (648 mg, 2mmol) and Grubbs' catalyst (165 mg, 0.2 mmol) were added and the reaction mixture was heated at reflux under nitrogen for 6 h. The solution was concentrated under reduced pressure and the residue was purified by silica gel column chromatography using a gradient of hexanes ethyl acetate 5/1 to 1/4. Title compound **126** was isolated in 60% yield (355 mg, 0.60 mmol); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.32-7.23 (m, 5H, Ph), 5.83-5.72 (m, 2H, H-2', H-3'), 5.02 (s, 1H, H-1), 4.53 (s, 2H, H-13'), 4.15-4.10 (m, 3H, H-2, H-3, H-1'a), 4.00-3.93 (m, 3H, H-4'a, H-4'b, H-1'b), 3.83 (dd, J= 5.6, 10.8 Hz, 1H, H-6a), 3.74-3.67 (m, 2H, H-4, H-6b), 3.65-3.53 (m, 17H, H-5, H-5' to H-12'), 1.51, 1.48, 1.39, 1.31 (4s, 12H, Me); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 138.3 (Ph), 130.4 (C-2'), 128.3, 127.8 (Ph), 127.7 (C-3'), 127.5 (Ph), 109.4, 99.6 (MeCMe), 96.9 (C-1), 76.1 (C-2), 74.8 (C-3), 73.2 (C-13'), 72.7 (C-4), 70.9 (C-4'), 70.6, 69.6, 69.4 (C-5' to C-12'), 67.1 (C-1'), 62.0 (C-6), 61.4 (C-5), 29.0, 28.1, 26.1, 18.7 (Me); ESI-MS, m/z= 614.2, $[M+NH_4]^+$ for $C_{31}H_{48}O_{11}+NH_4$.



Hydrogenated compound 127.

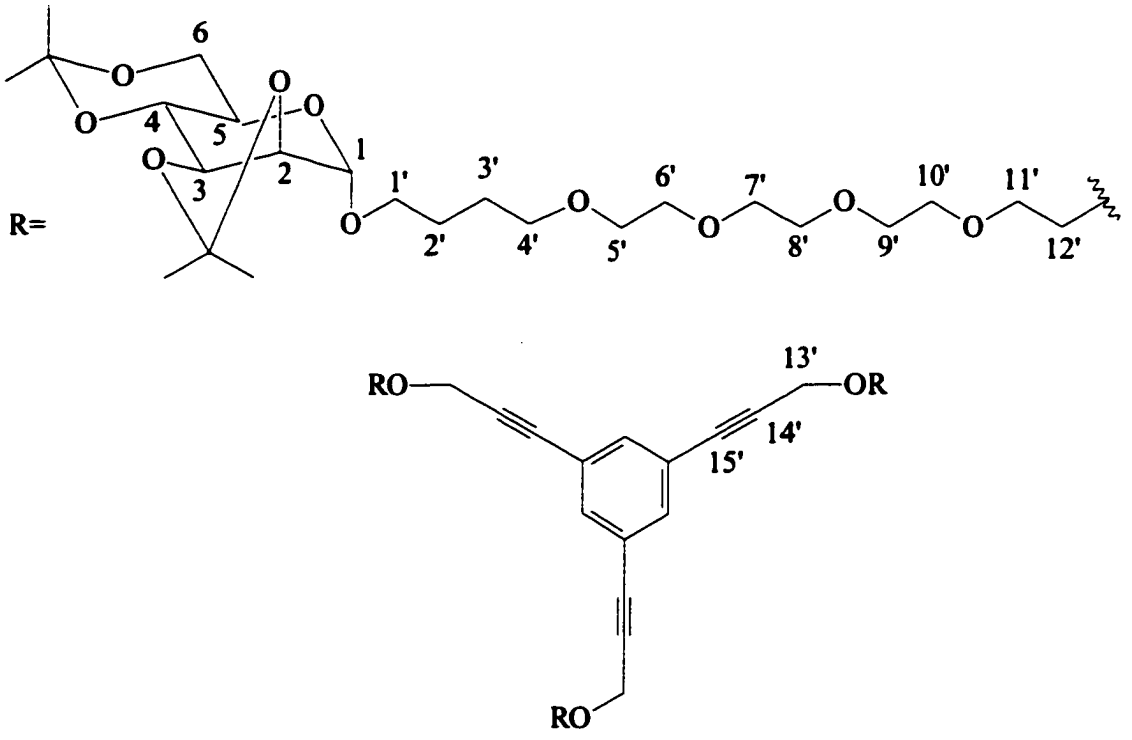
To a solution of **126** (295 mg, 0.49 mmol) in MeOH (10 mL) was added 10% palladium/carbon (60 mg) and the reaction mixture was stirred under a hydrogen atmosphere at room temperature for 2 h. The catalyst was filtered off over celite and the filtrate concentrated to afford compound **127** in 85% yield (212 mg, 0.42 mmol) as a syrup. $[\alpha]_D$= +5.0 (c=1, in $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz): δ (ppm) 4.94 (s, 1H, H-1), 4.10 (d, J=5.6 Hz, 1H, H-2), 4.07 (dd, J= 5.6, 8.7 Hz, 1H, H-3), 3.80 (dd, J= 5.6, 10.7 Hz, 1H, H-6a), 3.71-3.35 (m, 23H, H-4, H-5, H-6b, H-1', H-4', H-5' to H-12'), 2.82 (bs, 1H, OH), 1.62-1.59 (m, 4H, H-2', H-3'), 1.49, 1.46, 1.37, 1.29 (4s, 12H, Me); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 109.3, 99.6 (MeCMe), 97.7 (C-1), 76.1 (C-2), 74.8 (C-3), 72.7 (C-4), 72.5 (C-4'), 70.9, 70.5, 70.3, 70.1 (C-5' to C-11'), 67.5 (C-1'), 62.0 (C-6), 61.6 (C-12'), 61.2 (C-5), 29.0, 28.1 (Me), 26.3, 26.1 (C-2'. C-3'), 26.1, 18.7 (Me); CI-MS, m/z= 509, $[M+1]^+$ for $C_{24}H_{44}O_{11}$.



Propargylated compound 128.

Sodium hydride as a 60% dispersion in mineral oil (15 mg, 0.36 mmol) was added to a solution of **127** (120 mg, 0.24 mmol) and propargyl bromide, 80 wt% solution in toluene (47 μL, 0.36 mmol) in DMF (10 mL) cooled to 0° C. The resulting black solution

was stirred and allowed to warm to room temperature. The progress of the reaction was monitored by TLC (hexane/ethylacetate 1/3). When starting material was completely consumed, MeOH (2 mL) was added and the reaction mixture was stirred for 10 min. The reaction mixture was then diluted with ether and washed with water. The organic layer was dried over Na_2SO_4, evaporated under reduced pressure and purified via flash chromatography on silica gel using a gradient of hexane/ethyl acetate 1/1 to 1/3 as eluent. Title compound **128** was obtained in 88% yield (115 mg, 0.21 mmol); $[\alpha]_D$= +2.5 (c=1, in $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz): δ (ppm) 4.94 (s, 1H, H-1), 4.15 (d, J= 2.4 Hz, 2H, H-13'), 4.12-4.05 (m, 2H, H-2, H-3), 3.81 (dd, J= 5.8, 10.8 Hz, 1H, H-6a), 3.73-3.34 (m, 23H, H-4, H-5, H-6b, H-5' to H-12', H-1', H-4'), 2.39 (t, J= 2.4 Hz, 1H, H-15'), 1.61-1.56 (m, 4H, H-2', H-3'), 1.49, 1.46, 1.37, 1.30 (4s, 12H, Me); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 109.7, 100.0 (MeCMe), 98.1 (C-1), 80.0 (C-14'), 76.5 (C-2), 75.2 (C-15'), 74.9 (C-3), 73.1 (C-4), 71.3 (C-4'), 70.9, 70.7, 70.5, 69.4 (C-5' to C-12'), 67.9 (C-1'), 62.4 (C-6), 61.6 (C-5), 58.7 (C-13'), 29.4, 28.6, 26.7 (Me), 26.5, 26.5 (C-2', C-3'), 19.1 (Me); ESI-MS, m/z= 564.3, $[M+NH_4]^+$ for $C_{27}H_{46}O_{11}+NH_4$.



Fully protected trimannoside ligand 129.

To a solution of compound **128** (42.4 mg, 0.078 mmol) in 4 mL of DMF-Et_3N (1:1) was added 1,3,5-triiodobenzene (10 mg, 0.022 mmol) and $Pd(PPh_3)_2Cl_2$ (3.0 mg, 0.0043 mmol). The solution was stirred under nitrogen atmosphere at 60° C for 6 h. The progress of the reaction was monitored by TLC (ethyl acetate). The reaction mixture was evaporated under reduced pressure and purified by flash chromatography on silica gel using a gradient of hexanes/ethyl acetate 1/1 to ethyl acetate. Title compound **129** was isolated in 67% yield (25 mg, 0.015 mmol); ^1H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.40 (s, 3H, Ph), 4.97 (s, 3H, H-1), 4.38 (s, 6H, H-13'), 4.15-4.08 (m, 6H, H-2, H-3), 3.83 (dd, J= 5.7, 10.7 Hz, 3H, H-6a), 3.76-3.38 (m, 69H, H-4, H-5, H-6b, H-1', H-4', H-5' to H-12'), 1.63-1.61 (m, 12H, H-2', H-3'), 1.52, 1.49, 1.40, 1.32 (4s, 36H, Me); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 134.5, 123.2 (aromatic), 109.3, 100.5 (MeCMe), 97.7 (C-1), 85.1, 84.5 (acetylenic), 76.1 (C-2), 74.8 (C-3), 72.7 (C-4), 70.9 (C-4'), 70.5, 70.1, 69.2 (C-5' to C-12'), 67.5 (C-1'), 62.0 (C-6), 61.0 (C-5), 59.0 (C-13'), 29.0, 28.2, 26.8 (Me), 26.3, 26.1 (C-2', C-3'), 18.7; ESI-MS, m/z= 1728.4, $[M+NH_4]^+$ for $C_{87}H_{138}O_{33}+NH_4$.

Fully deprotected trimannoside ligand 130.

A solution of **129** (23 mg, 0.013 mmol) in 60 % aqueous AcOH (2 mL) was heated at 80° C and stirred for 1 h. The reaction mixture was then diluted with toluene (2 mL) and evaporated under reduced pressure to afford the deprotected trimannoside ligand. The title compound was further purified by size exclusion chromatography on Biogel-P4 column using water as eluent ; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.61 (s, 3H, Ph), 4.88 (s, 3H, H-1), 4.53 (s, 6H, H-13'), 3.97-3.55 (m, 78H, H-2, H-3, H-4, H-5, H-6, H-1', H-4', H-5' to H-12'), 1.66 (bs, 12H, H-2', H-3'); ESI-MS, m/z= 1509.0, $[M+K]^+$ for $C_{69}H_{114}O_{33}$+K.

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Chapter 6. Glycomimetics to branched trimannoside

6.1. Introduction

In this last chapter, a glycomimetic approach was used to generate high affinity ligands to carbohydrate-binding proteins known as lectins. Unlike, the multivalency approach where an increase in binding affinity is found due to a cluster glycoside effect (multiple binding), the glycomimetic way is capitalizing in a single binding site by improving the interactions made by essential functional groups to resemble the active conformation of the natural ligand.

Lectins are found in most organisms, ranging from viruses and bacteria to plants and animals.[1] The biological functions associated with the carbohydrate binding activities of these protein families are diverse however their major function appears to be in cell recognition. They represent a heteregenous group of oligomeric proteins that vary widely in size, structure, molecular organization, as well as the constitution of their combining sites. Concanavalin A (Con A) was chosen as model for our glycomimetic approach since it represents one of the most extensively studied lectins.[2-4] The binding site of Con A has been mapped by X-ray crystallography on several occasions and has been shown to be rather shallow, composed of a number of threonine, tyrosine, aspartate as well as asparagines and main-chain amide groups.[5-7] These amino acid residues are forming a network of hydrogen bonds to the most complementary ligand for Con A, the branched trimannoside unit (3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside). This natural ligand, found in the trimannoside core of N-linked glycoproteins, constitutes the minimum carbohydrate epitope that completely fills the sugar binding site. However, the majority of the hydrogen bonds in the binding site are formed with the nonreducing, peripheral mannoside moieties of the branched trimannoside, rather than with the central mannose group as shown in Figure 6.1.1. Thus, designing a glycomimetic to the branched trimannoside where the two terminal saccharide moieties would be replaced was envisaged.

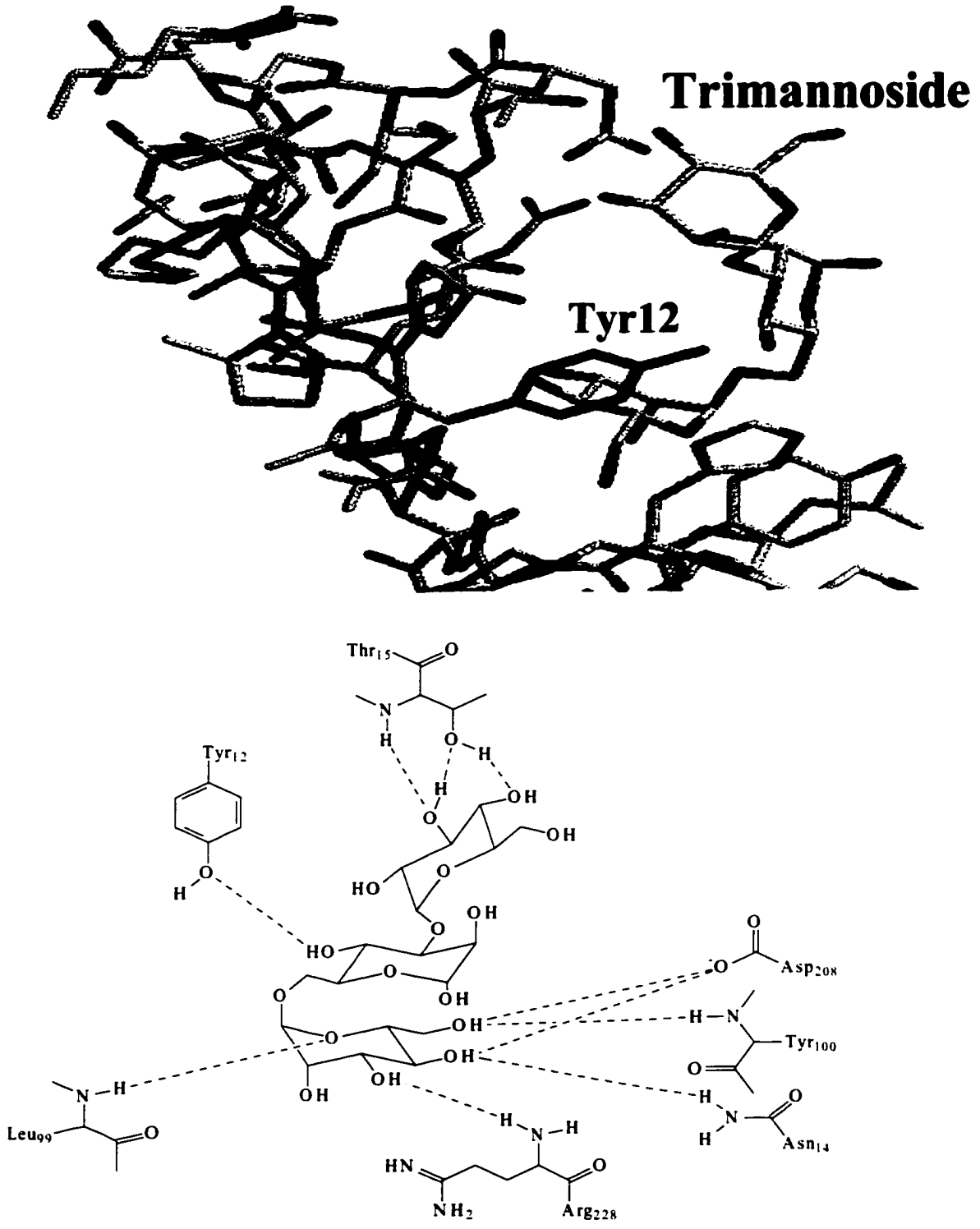


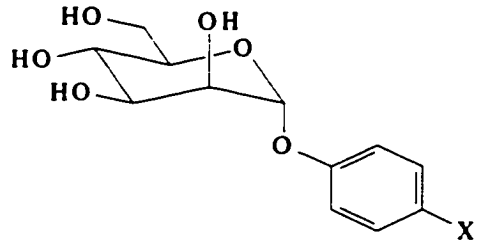
Figure 6.1.1. X-ray structure of the extended combining site of Con A with branched trimannoside ligand (top) and its schematic representation.[6]

6.2. Design of glycomimetics

Our rational design toward glycomimetics to branched trimannoside was based on the fact that para-substituted phenyl α-mannopyranosides bind strongly to Con A. Furthermore, a study conducted by Loontiens and coworkers showed that aryl mannosides with para-substituted electron donating groups had higher binding affinity to Con A than aryl mannosides with para-substituted electron withdrawing groups.[8] As shown in table 6.2.1, p-alkoxy derivatives bind 3-4 times better than the p-nitro derivative.

Table 6.2.1. IC_{50} values for para-substituted phenyl α-D- mannopyranosides toward Con A.

Para-subsituent X	**IC_{50} X 10^{-5} M**
Methoxy	3.498
Ethoxy	2.841
Methyl	5.618
Ethyl	4.552
tert-Butyl	3.275
Hydrogen	10.79
Chloro	6.440
Bromo	5.953
Acetyl	8.751
Nitro	12.05



Based on these results it was suggested that π–π charge complex formation between the tyrosine residues in Con A's binding site and the phenyl ring in the mannopyranoside could account for the increased binding affinity. Incorporation of a hydrophobic moieties was therefore necessary to complement the hydrophobic site found in Con A in order to create high affinity ligand. We were also interested in designing a mimetic that would have improved properties with regard to stability since many natural saccharides are rapidly degraded by digestive, plasma, and cellular glycosidases. Taking into consideration all the information, two generations of glycomimetics were designed: C-aryl mannoside derivatives **131** and **132** and O-, N- and C- linked 1,2,3-triazole mannosides **133-138**.

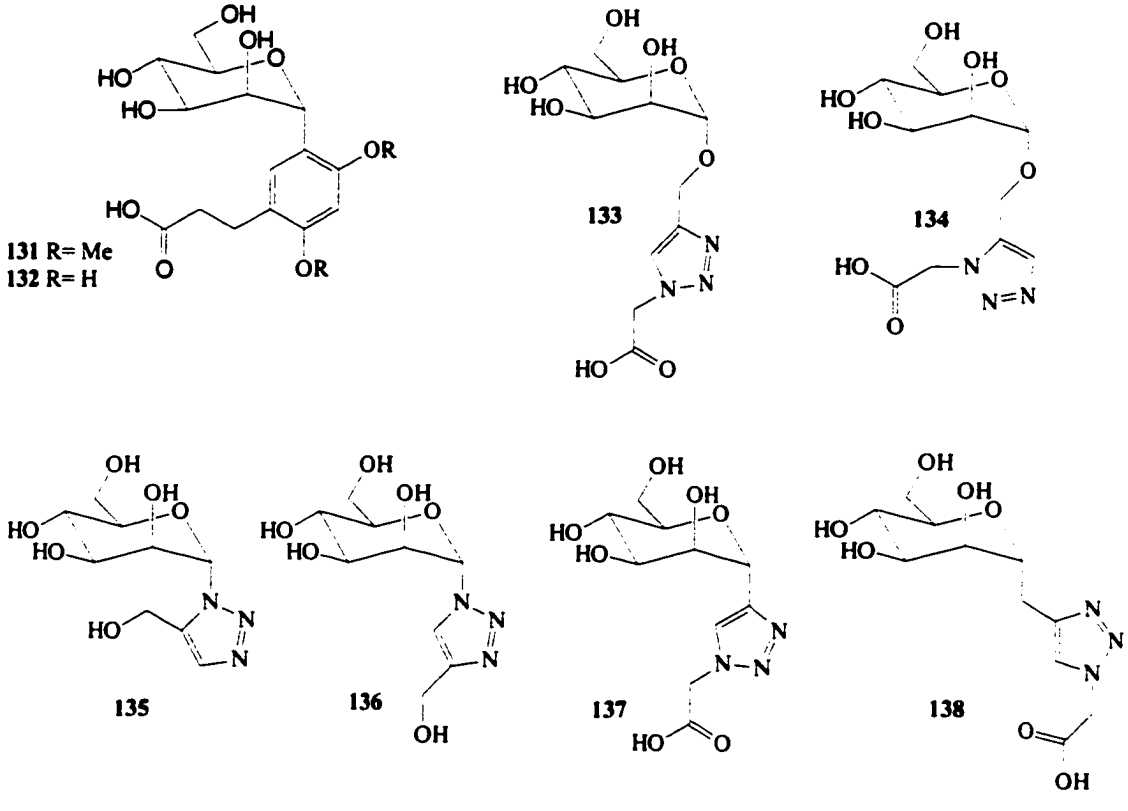


Figure 6.2.1. Glycomimetics to branched trimannoside.

Superimposition of C-aryl mannoside **131** with the bound conformation of the trimannoside using Cache computational software was accomplished and good overlap between the two structures could be obtained by adjustment of dihedral angles of glycomimetic **131** as shown in figure 6.2.2.

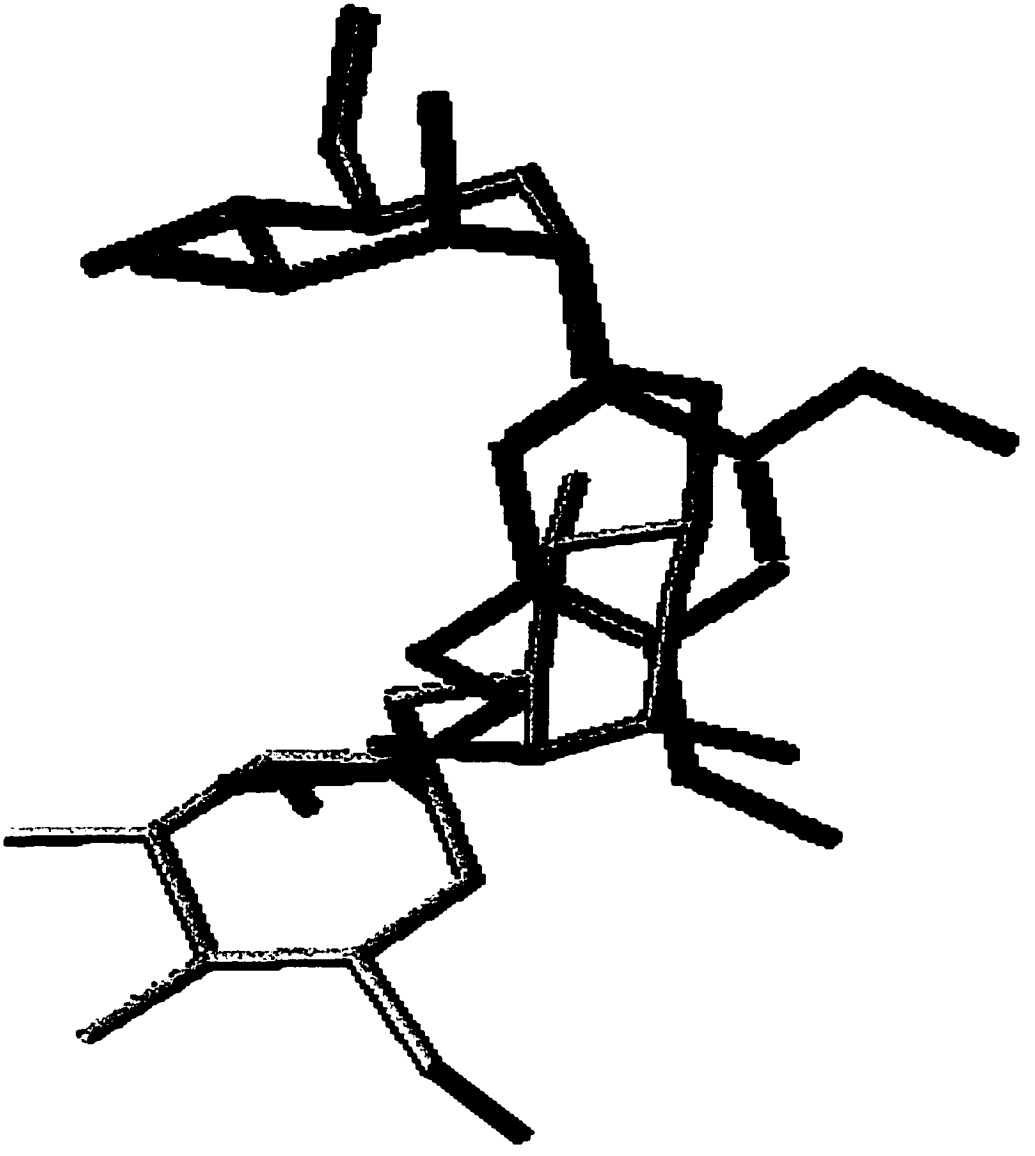
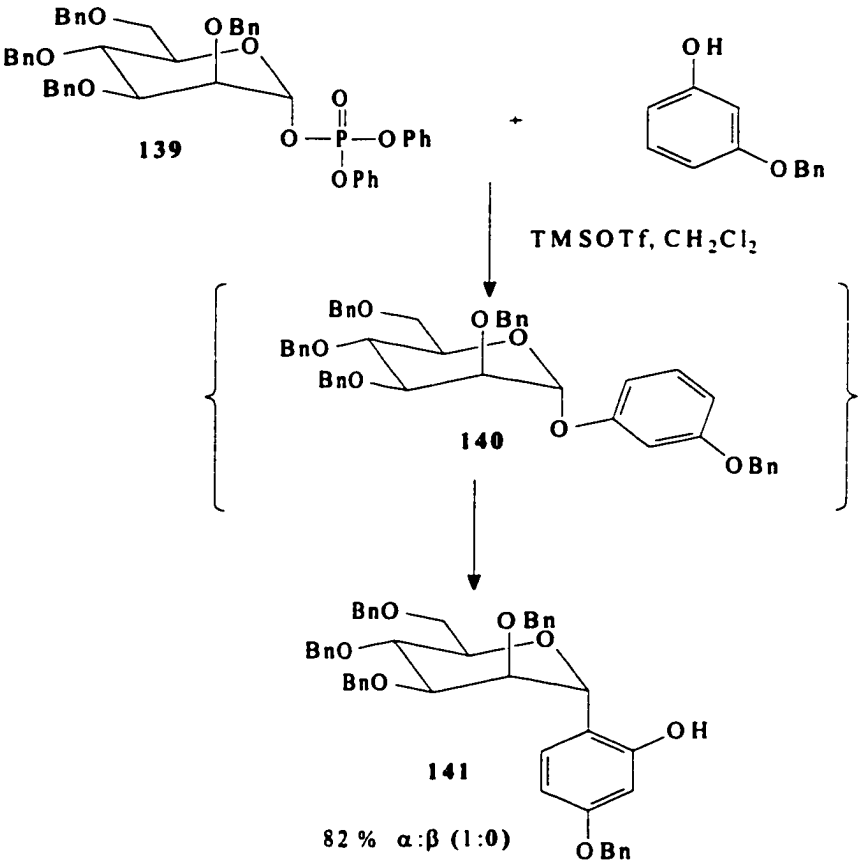


Figure 6.2.2. Superimposition of glycomimetic **131** (blue) with the bound conformation of branched trimannoside (yellow).

6.3. Results and discussion

6.3.1. First generation of glycomimetics: *C*-Aryl mannosides

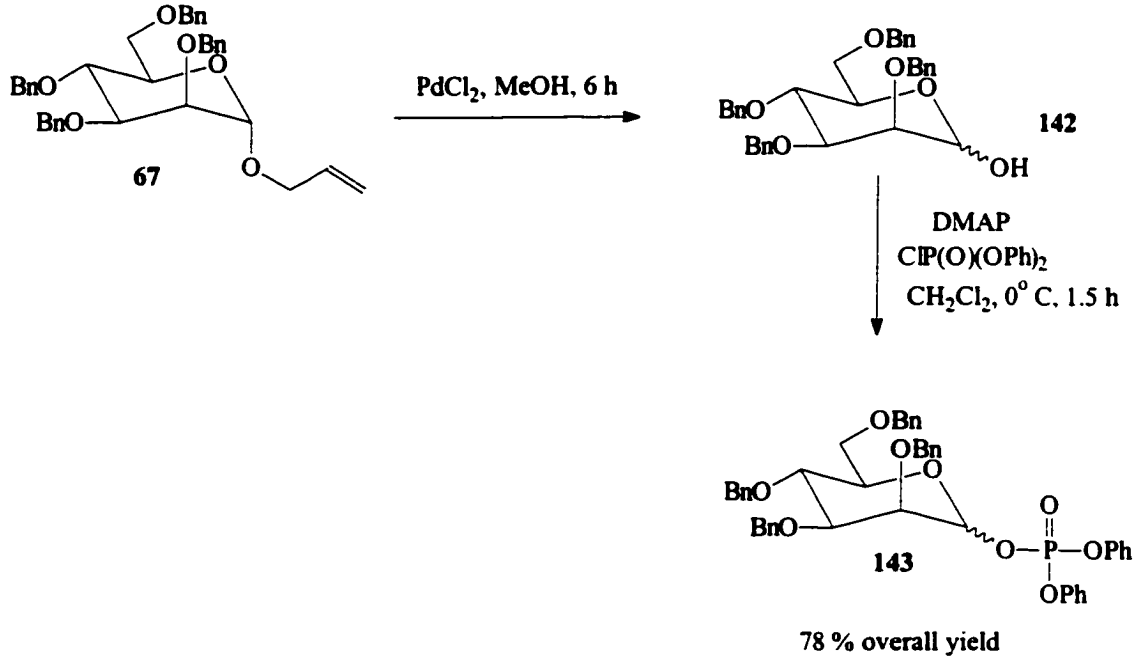
C- Aryl glycosides are a relatively small group of naturally occurring compounds with unique chemical and biological properties.[9] Several different approaches for the synthesis of this class of compounds have been explored. Recently, Seeberger and coworkers developed an indirect route to form a C-aryl glycosidic linkage. In their methodology, glycosyl phosphates treated with aromatic phenols in the presence of Lewis acids were involved in a Fries-like O-to C-rearrangement to afford C- aryl glycosides in good yields.[10] This reaction was proposed to proceed through the initial formation of an O-glycoside which rearranges to a C- glycoside under the reaction conditions.



Scheme 6.3.1. Fries-like O- to C- rearrangement.

They reported the synthesis of α-C-mannosides of 3-benzyloxyphenol in excellent yield and with absolute stereocontrol (Scheme 6.3.1.). This reaction was therefore well suited to accomplish the synthesis of our first generation of glycomimetics.

The required mannosyl phosphate **143** was prepared in two steps starting from allyl 2,3,4,6-tetra-O-benzyl-α-D-mannopyranoside **67**. The first step involved a mild palladium mediated deprotection of *O*-allyl ether[11] followed by phosphorylation of the resulting hemiketal using diphenyl chlorophosphate and 4-*N,N*-dimethylaminopyridine.[12] This sequence afforded the known diphenyl (tetra-O-benzyl-α-D-mannopyranosyl) phosphate **143** in 78% overall yield (Scheme 6.3.2). Derivative **143** appeared to be very sensitive to acidic conditions and column chromatography resulted in partial hydrolysis of the phosphate moiety. Therefore, the mannosyl phosphate was used as crude mixture for the arylation step.



Scheme 6.3.2. Preparation of mannosyl phosphate **143**.

Unfortunetaly, the yields reported by Seeberger could not be reproduced in our hands, giving a poor 45% yield for C-aryl **141.** Therefore, by using a Friedel-Crafts methodology, the desired C-aryl mannosides could be obtained if electron-rich aromatic rings were used as nucleophiles. There are many precedents of this reaction with various glycosyl donors,[13] however to our knowledge no reports have been made yet using glycosyl phosphates. There were no reasons why the reaction could not proceed since glycosyl phosphates are known to be very reactive in glycosylation reactions. Thus, treatment of mannosyl phosphate **143** with 1.5 equiv. of 1,3-dimethoxybenzene and 1.5 equiv. of boron trifluoride etherate and molecular sieves gave 2,4-dimethoxy-1-(2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl)benzene **144** in 65% yield.

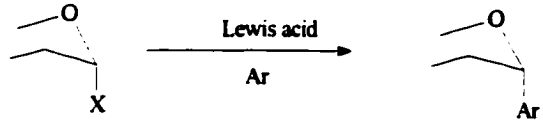
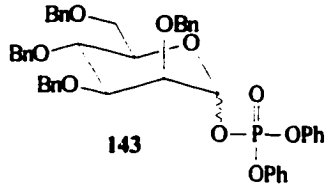
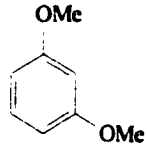
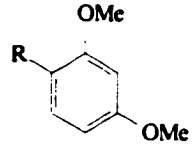
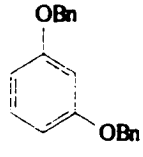
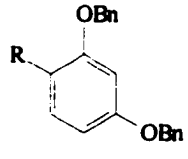
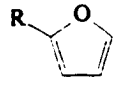
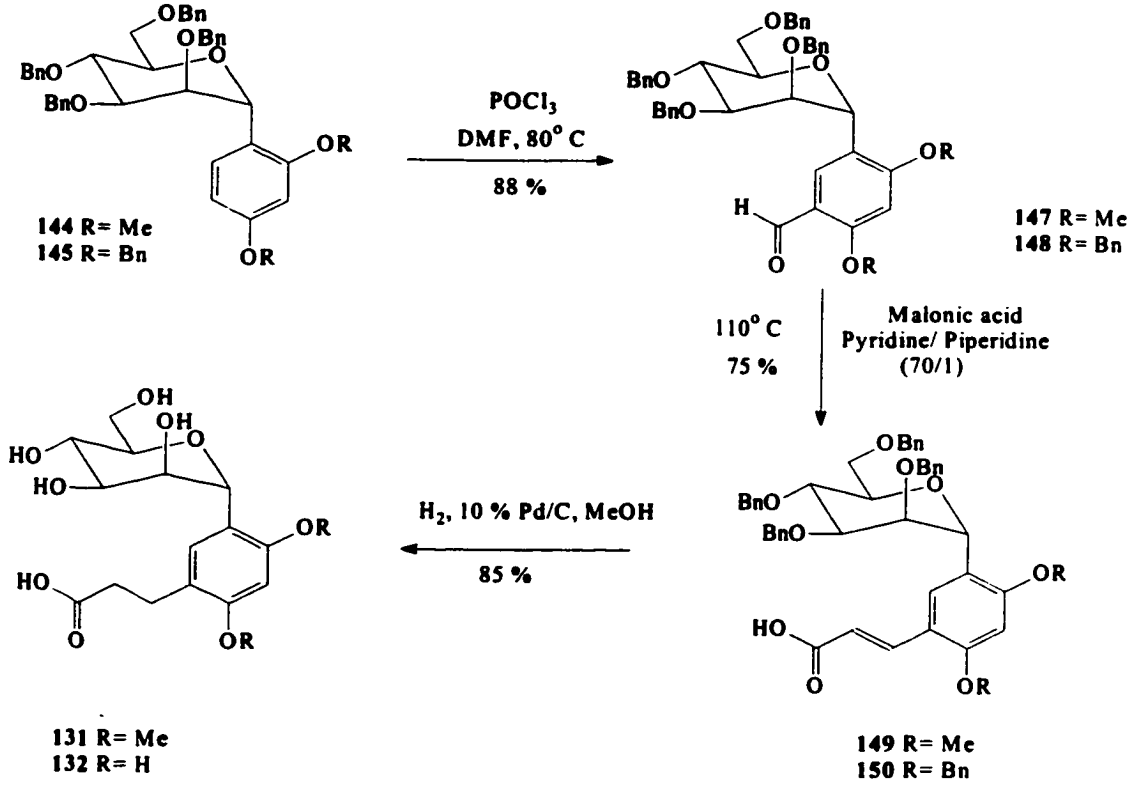


Table 6.3.1. Friedel-Crafts methodology to afford C-aryl glycosides.

Donor	Acceptor	C-glycoside product	Yield (%)
 **143**		 **144**	65
		 **145**	60
		 **146**	70

The substitution pattern on the aromatic ring was confirmed by the ^{1}H-NMR spectrum of **144** which clearly showed each of the aromatic protons attached to the dimethoxy phenyl group in which one of them showed a strong ortho coupling constant (J= 8.5 Hz). To further extend the scope of this reaction, the same conditions were applied between mannosyl phosphate **143** with various nucleophiles such as 1,3-dibenzyloxybenzene and furan. In this way, 2,4-dibenzyloxy-1-(2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl) benzene **145** and 2-(2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl)furan **146** were obtained in 60% and 70% yields, respectively. Table 6.3.1 summarizes the results.

Functionalization of the benzene ring could be accomplished by treating the C-aryl mannosides **144** and **145** under Vilsmeier-Haack conditions to provide the formyl derivatives **147** and **148** in 88% yields.[14] Then a Knoevenagel-Doebber reaction afforded the α,β-unsaturated acid **149** and **150** in 75% yields.[15] Finally, simple hydrogenation gave glycomimetics **131** and **132** (Scheme 6.3.3).

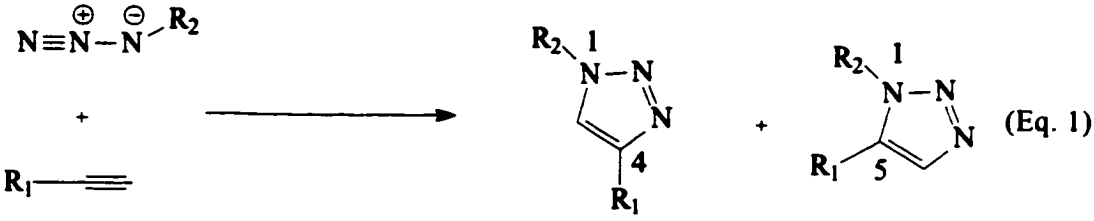


Scheme 6.3.3. Synthesis of glycomimetics **131** and **132.**

6.3.2. Second generation of glycomimetics: *O-*, *N-*, *C-* triazole mannosides.

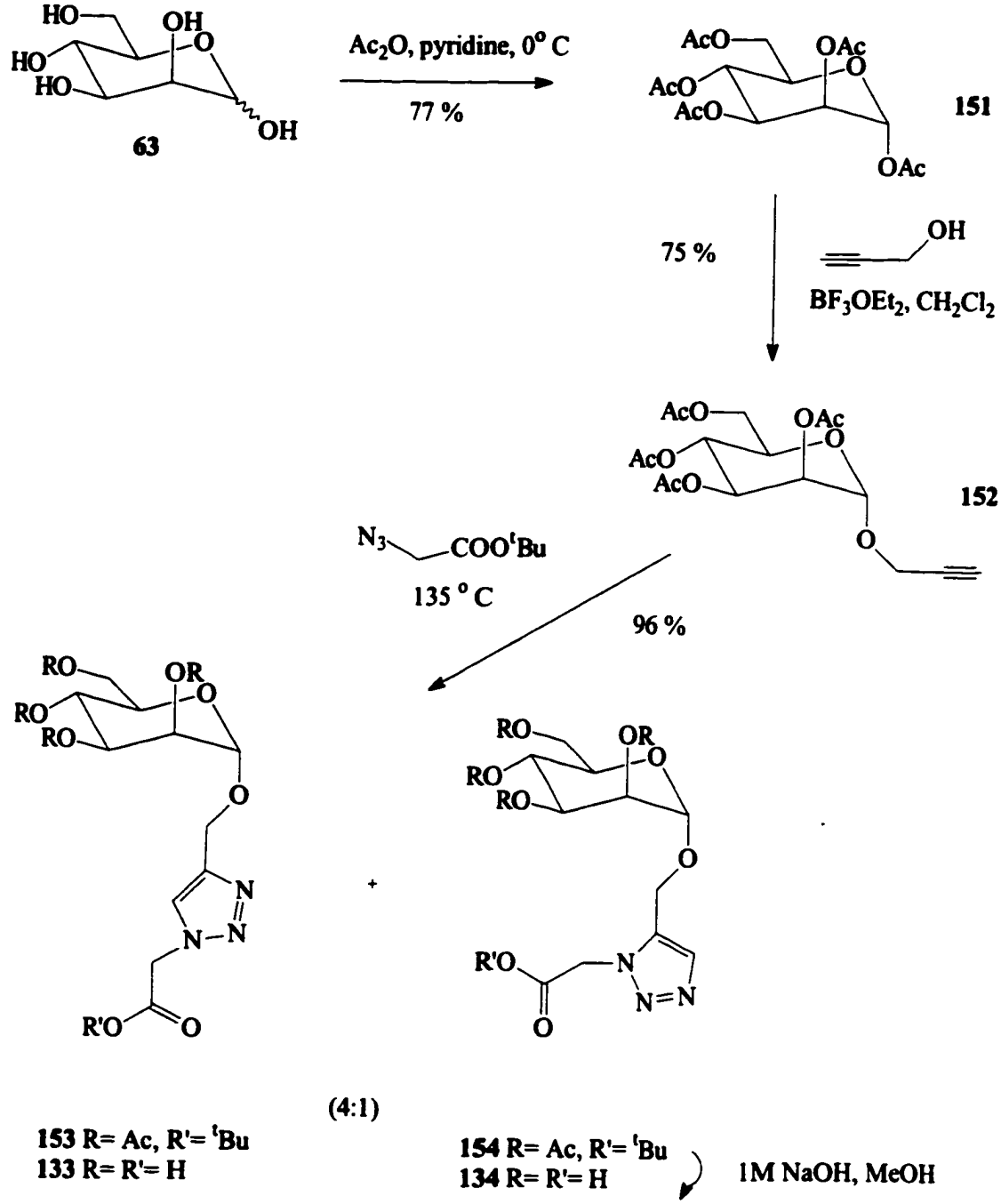
1,2,3-Triazole derivatives are very interesting components in terms of biological activity.[16-17] They could also forms $\pi-\pi$ charge complex with the tyrosine residues in Con A's binding site, therefore it will be relevant to see if an increase in binding affinity could be observed with triazole containing mannosides.

Huisgen 1,3-dipolar cycloadditions between azides and alkynes provide a fast access to an enormous variety of five-membered rings triazoles.[18] The cycloaddition requires elevated temperature and, usually results in a mixture of the 1,4 and 1,5 regioisomers. (Eq. 1).



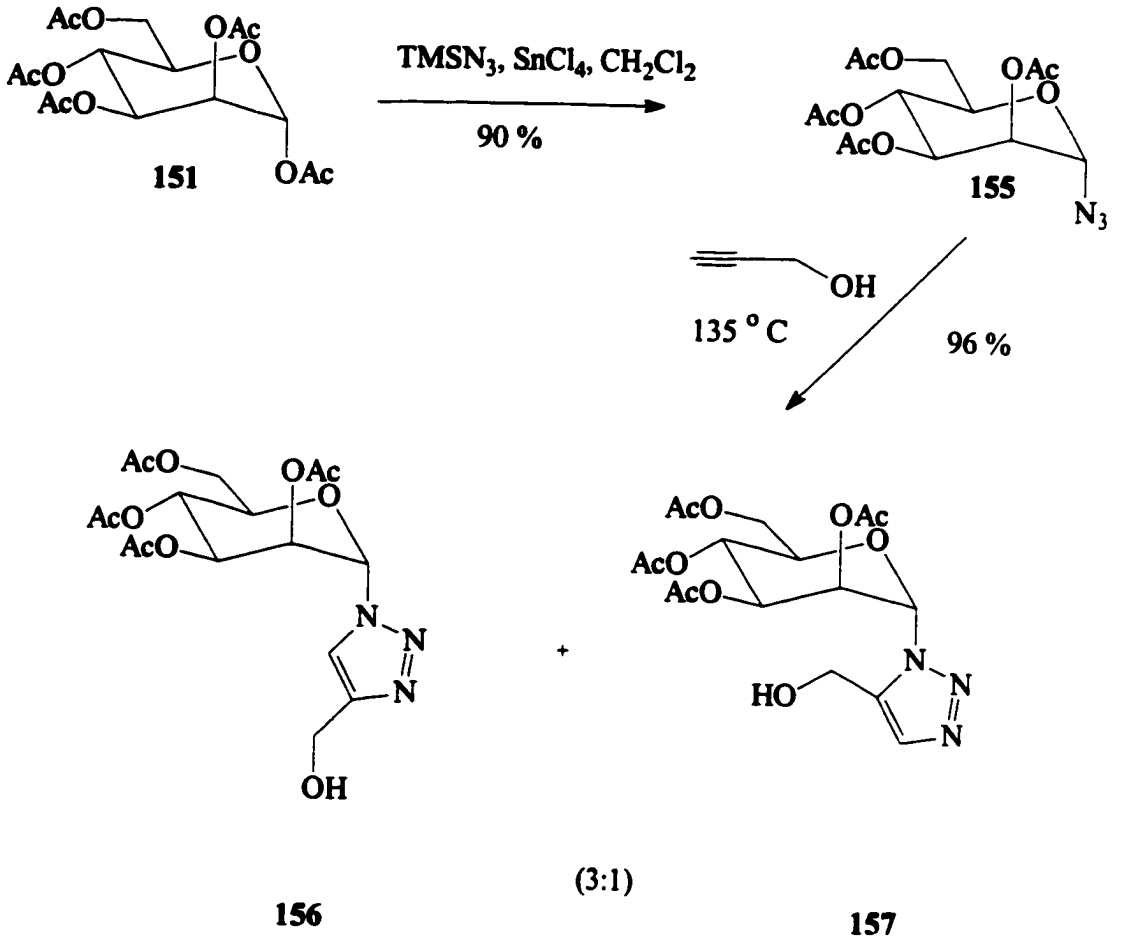
(Eq. 1)

The idea of generating two analogs in one reaction was attractive and therefore we envisaged to try the reaction first with readily available 2-propynyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside **152** and azido tert-butyl acetate.[19] The cycloaddition was performed in toluene in a seal tube and heated at 135° C to afford regioisomers 1,4 and 1,5 in 96% yields in a 4:1 ratio after 10 h. The regioisomers could be separated by flash chromatography. The 4 or 5 substitution of compounds **153** and **154** was determined by ^{1}H- NMR on the basis of the differences in chemical shifts of the H-3' aromatic protons. It is known that this proton is appearing more downfield for the 1,4 regioisomers.[20] Thus, H-3' aromatic proton showed a singlet at δ 7.68 ppm for **153** compared to δ 7.64 ppm for **154**. The triazole derivatives **153** and **154** were then deprotected using a 1M NaOH solution in MeOH (Scheme 6.3.4).



Scheme 6.3.4. Synthesis of *O*-linked triazole mannosides.

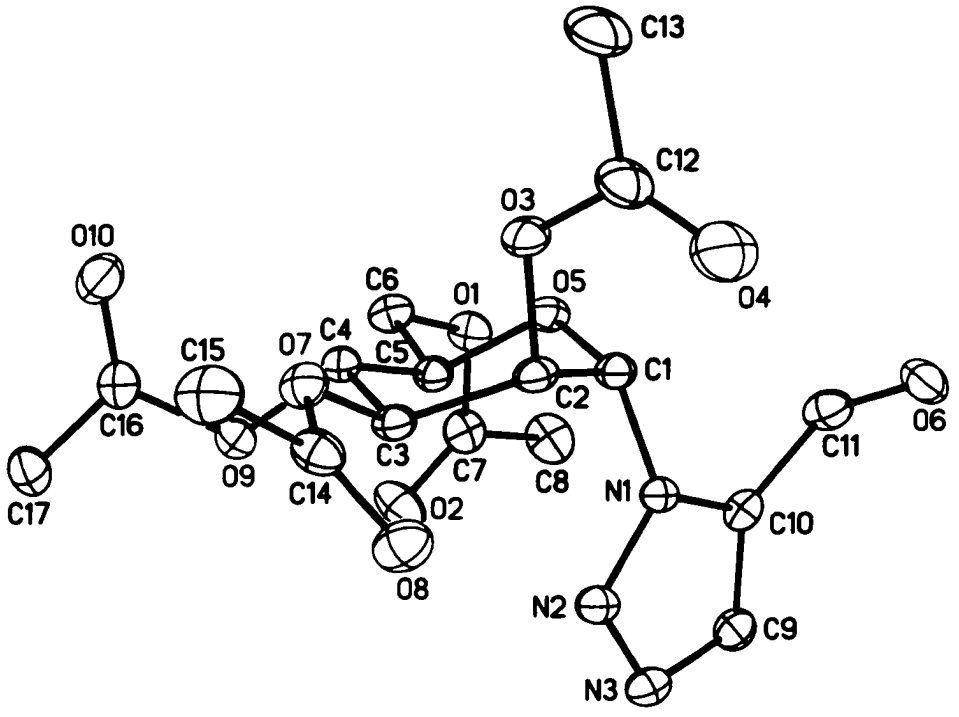
Encouraged with these results, the cycloaddition with readily available 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl azide **155**[29] and propargyl alcohol was envisaged. Under the same conditions decribed above, the reaction afforded a mixture of regioisomers **156** and **157** in 96 % yield in a 3:1 ratio (Scheme 6.3.5.). The regioisomers could be separated by column chromatography.



Scheme 6.3.5. Synthesis of N-linked triazoles mannosides.

The 4 or 5 substitution was also confirmed by the difference in chemical shift for the aromatic H-1' which in this case was more important Δδ 0.16 ppm for regioisomers **156** and **157** compared to Δδ 0.04 ppm for **153** and **154.** Furthermore, this empirical rule was

confirmed by the X-ray structure of derivative **157** which clearly showed the 1,5 subtitution (figure 6.3.1.).



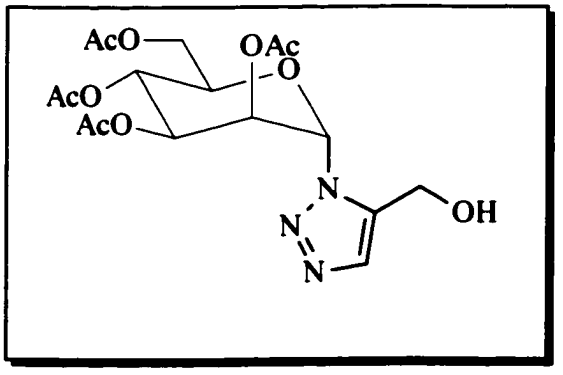
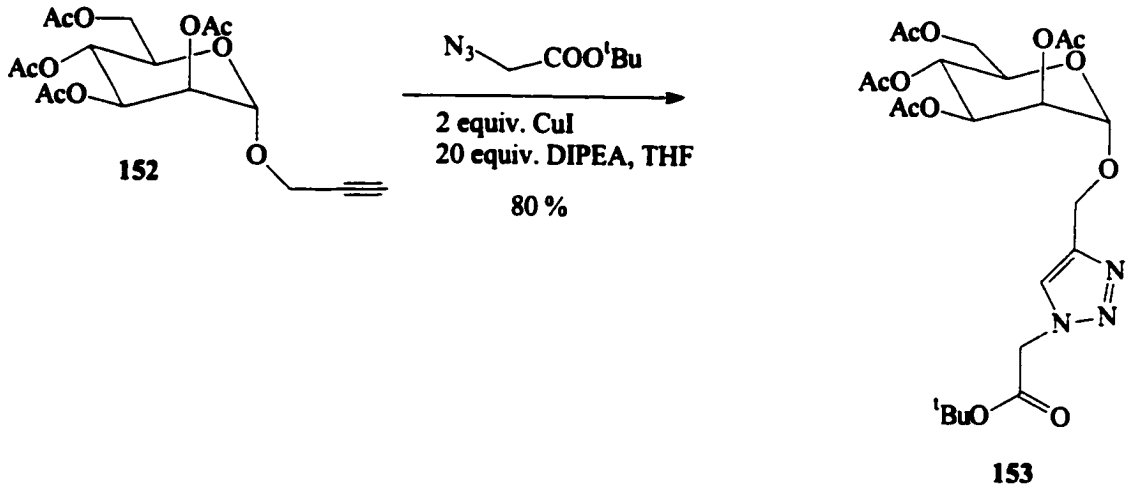


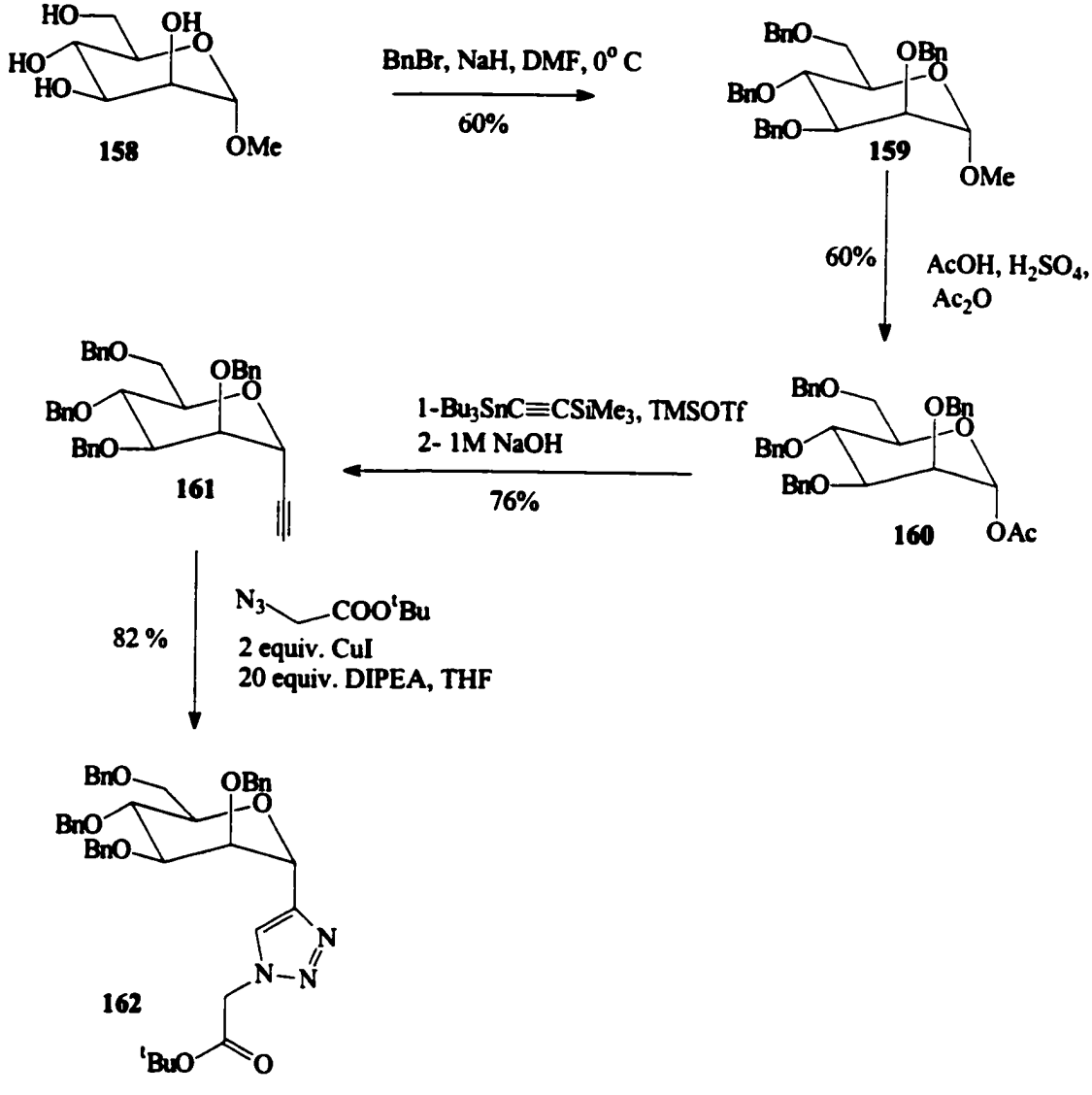
Figure 6.3.1. X-ray structure of **157**.

The Huisgen cycloaddition under thermal conditions provides access to both regioisomers. We were fortunate to be able to separate them by column chromatography and to perform the reaction in relatively large scale since the materials were readily available. However, in some cases, the starting materials are very precious and formation of a single product would be preferred. To control this 1,4 versus 1,5-regioselectivity problem, Meldal and coworkers, recently discovered a novel regiospecific copper (I)-catalyzed 1,3 dipolar cycloaddition.[21] This reaction gave exclusively the 1,4-disubstituted 1,2,3-triazole when a terminal alkyne was treated with 2 equiv. of azide, 2 equiv. of CuI and 20 eq. of DIPEA in THF. In this fashion, triazole **153** was formed exclusively in 80% yield after 2 h (scheme 6.3.6). Triazole **156** was formed only in minor amounts after 24 h under the same conditions, probably due to inductive effect of the anomeric position.



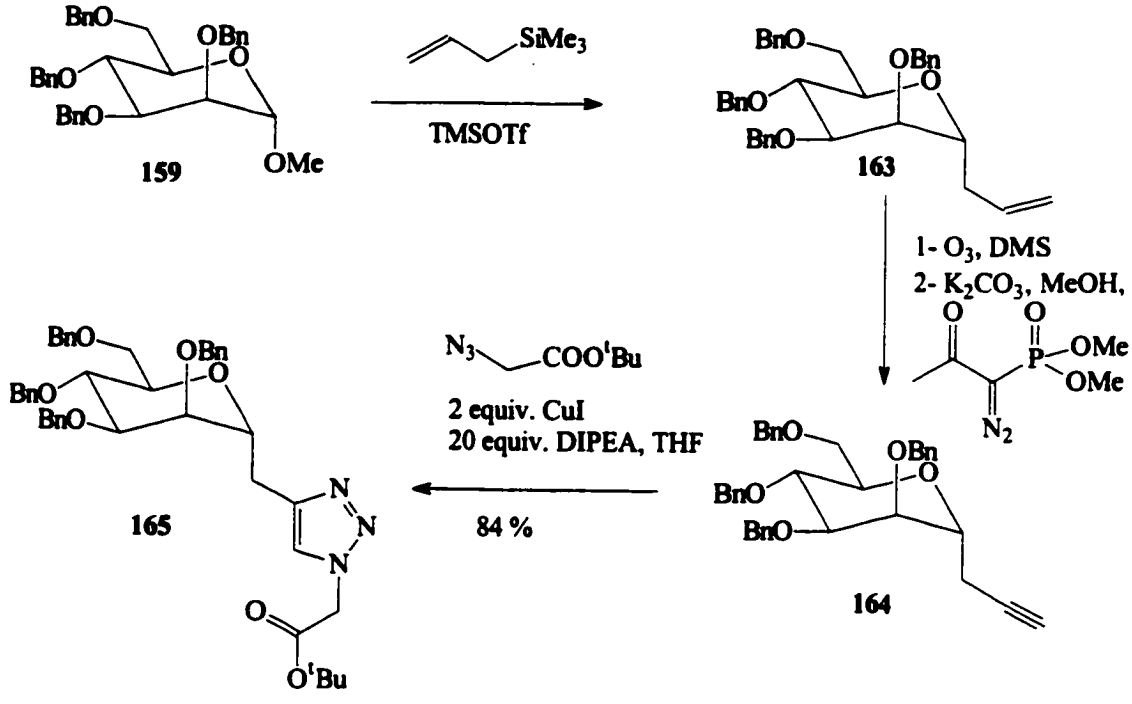
Scheme 6.3.6. Formation of **153** under Meldal conditions.

From those results, the synthesis of C-linked triazole mannosides were considered. The cycloaddition of ethynyl α-C-mannoside **161** and propynyl α-C-mannoside **164** with azido *tert*-butyl acetate under Meldal conditions was envisaged. The known compound **161** was prepared following the procedures developed by Isobe and coworkers.[22] Under the Cu(I)-catalyzed conditions, cycloadduct **162** was formed from **161** regiospecifically in 82% yield (Scheme 6.3.7).



Scheme 6.3.7. Synthesis of C-linked triazole mannoside **162**.

On the other hand, compound **164** was prepared from the C- allyl α-D-mannoside **163** by ozonolysis followed by homologation of the additional carbon using Ohira's modification of the Gilbert-Seyferth conditions[23] in 72% yield. The cycloaddition produced smoothly cycloadduct **165** in 84% yield (Scheme 6.3.8.).

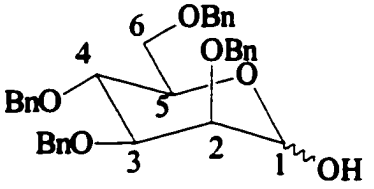


Scheme 6.3.8. Synthesis of C-linked triazole mannoside **165.**

6.4. Conclusions

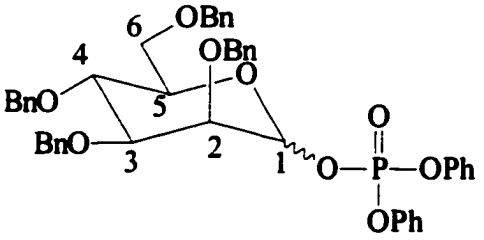
Various glycomimetics to 3,6-branched trimannoside have been synthesized. These glycomimetics could also be extended to other carbohydrate-binding proteins similar to Con A such as *Dioclea grandiflora* lectin (DGL) and some bacterial-surface lectins. It will be interesting to see if our glycomimetics could bind selectively to only one type of lectins. The preliminary biological tests to verify the affinity for Con A and DGL revealed that derivative **131** and **132** were not good inhibitors, whereas **133** and **134** were having inhibitory potential similar to that of methyl α-D-mannopyranoside. The other derivatives were not tested yet.

6.5. Experimental methods



2,3,4,6-Tetra-O-benzyl-D-mannopyranose (142).[24]

A mixture of **67** (4.00 g, 6.90 mmol), and $PdCl_2$ (200 mg, 1.13 mmol) in methanol (100 mL) was stirred vigorously for 8 h at room temperature. The progress of the reaction was monitored by TLC (hexane/ethyl acetate 3/1). The reaction mixture was filtered through celite. The filtrate was concentrated under reduced pressure and purified via flash chromatography on silica gel using a gradient of hexanes/ ethyl acetate 5/1 to 3/1. Title compound **142** was obtained in 92 % yield (3.43 g, 6.35 mmol); ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 7.39-7.16 (m, 20H, Ph), 5.26 (d, J= 1.4 Hz, 1H, H-1), 4.95-4.48 (m, 8H, 4xCH_2Ph), 4.20-3.61 (m, 6H, H-2, H-3, H-4, H-5, H-6a, H-6b); ^{13}C NMR ($CDCl_3$, 50 MHz): δ (ppm) 139.0, 138.9, 138.8, 138.3, 129.0, 128.9, 128.8, 128.8, 128.7, 128.5, 128.4, 128.3, 128.1, 128.1, 128.0, 93.0 (C-1), 80.0, 75.5, 75.3, 75.2, 73.6, 73.0, 72.5, 71.9, 70.0 (C-6); ESI-MS, m/z= 558.2, $[M+NH_4]^+$ calcd for $C_{34}H_{36}O_6$+NH_4.



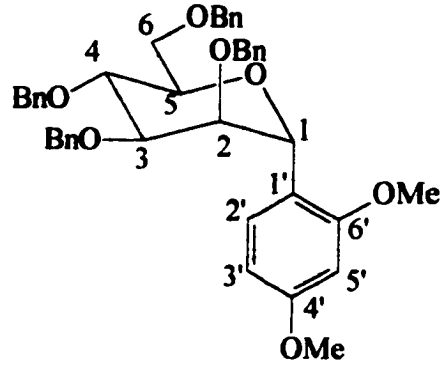
Diphenyl (2,3,4,6-tetra-O-benzyl-D-mannopyranosyl) phosphate (143).[25]

A solution of **142** (300 mg, 0.55 mmol) in CH_2Cl_2 (10 mL) containing DMAP (149 mg, 1.22 mmol) was cooled at 0° C. Diphenyl chlorophosphate (255 μL, 1.22 mmol) was added slowly and the reaction was stirred for 1 h. The progress of the reaction was monitored by TLC (hexanes/ethyl acetate 2/1). The reaction mixture was washed with HCl 10%. The organic layer was then washed with a saturated aqueous $NaHCO_3$ solution. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The

crude **143** was dried under vacuum and used without further purification; ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 7.47-7.17 (m, 30H, Ph), 6.11 (dd, J= 1.9, 6.4 Hz, 1H, H-1), 4.98-4.52 (m, 8H, 4xCH_2Ph), 4.23 (t, J= 9.3 Hz, 1H, H-4), 3.99-3.59 (m, 5H, H-2, H-3, H-5, H-6a, H-6b); ^{13}C NMR ($CDCl_3$, 50 MHz): δ (ppm) 138.2, 137.5, 129.8, 128.4, 128.3, 128.0, 127.8, 127.7, 127.6, 125.5, 120.1, 120.0, 97.5 (C-1), 78.8, 75.2, 74.2, 73.8, 73.4, 72.7, 72.2, 68.3.

General procedure for the formation of C-aryl mannosides:

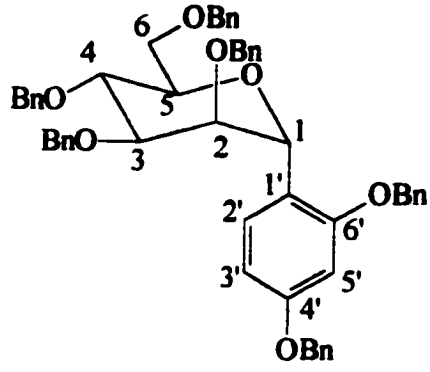
A mixture of the crude phosphate **143** (0.55 mmol) and powdered 4 Å molecular sieves in anhydrous CH_2Cl_2 was cooled at 0° C and stirred for 15 minutes. Aromatic nucleophiles (1.21 mmol) and boron trifluoride etherate (153 μL, 1.21 mmol) were then added. The reaction was monitored by TLC (hexane/ethyl acetate 3/1) and after consumption of the starting material (1 h), the reaction mixture was poured into a saturated aqueous $NaHCO_3$ solution. The organic layer was dried over Na_2SO_4, evaporated under reduced pressure and purified via flash chromatography on silica gel using a gradient of hexane/ethyl acetate.



2,4-Dimethoxy-1-(2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl)benzene (144).

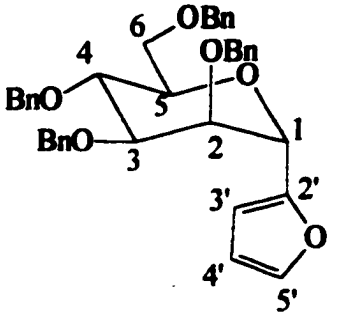
Title compound **144** was obtained in 65% yield; $[\alpha]_D$ +27.1° (c= 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.53 (d, J= 8.5 Hz, 1H, H-2'), 7.40-6.96 (m, 20H, Ph), 6.53 (dd, J= 2.4, 8.5 Hz, 1H, H-3'), 6.35 (d, J= 2.4 Hz, 1H, H-5'), 4.95 (d, J= 10.8 Hz, 1H, CH_2Ph), 4.76 (d, J= 12.2 Hz, 1H, CH_2Ph), 4.71 (s, 2H, CH_2Ph), 4.69 (s, 1H, H-1), 4.63-4.22 (m, 4H, 2xCH_2Ph), 4.06-4.03 (m, 2H, H-2, H-4), 3.89-3.79 (m, 6H, H-3, H-6a,

H-6b, OMe), 3.67 (s, 3H, OMe), 3.64-3.61 (m, 1H, H-5); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 163.3, 156.3 (C-4', C-6'), 139.0, 138.9, 129.3 (C-2'), 128.5, 128.5, 128.2, 128.2, 128.1, 127.8, 127.7, 127.7, 127.6, 127.2, 120.0 (C-1'), 104.5 (C-3'), 98.1 (C-5'), 85.0 (C-3), 80.3 (C-5), 75.5 (C-2), 75.3 (CH_2), 75.1 (C-1), 74.8 (C-4), 74.4 (CH_2), 73.7(CH_2), 72.3 (CH_2), 70.1 (C-6), 55.6 (OMe), 55.3 (OMe); CI-MS, m/z= 660, $[M]^+$ calcd for $C_{42}H_{44}O_7$.



2,4-Dibenzyloxy-1-(2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl)benzene (145).

Title compound **145** was obtained in 60% yield; $[\alpha]_D$ +34.7° (c= 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.54 (d, J= 8.5 Hz, 1H, H-2'), 7.46-7.08 (m, 30H, Ph), 6.63 (dd, J= 2.3, 8.5 Hz, 1H, H-3'), 6.50 (d, J= 2.3 Hz, 1H, H-5'), 5.11-4.90 (m, 6H, 3xCH_2Ph), 4.70 (s, 1H, H-1), 4.62-4.29 (m, 6H, 3xCH_2Ph), 4.13 (d, J= 2.8 Hz, 1H, H-2), 3.99 (t, J= 9.5 Hz, 1H, H-4), 3.86-3.80 (m, 2H, H-6a, H-6b), 3.67 (dd, J= 2.8, 9.5 Hz, 1H, H-3), 3.61-3.58 (m, 1H, H-5) ; ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 159.4, 155.5 (C-4', C-6'), 139.0, 138.9, 138.7, 137.4, 137.2, 129.1 (C-5'), 128.8, 128.8, 128.6, 128.5, 128.3, 128.2, 128.2, 128.1, 128.1, 127.8, 127.7, 127.7, 127.6, 127.5, 127.3, 120.7 (C-1'), 106.1 (C-3'), 100.2 (C-5'), 85.2 (C-3), 80.4 (C-5), 75.3 (CH_2), 75.3 (C-4), 75.1 (C-1), 74.0 (CH_2), 73.9 (C-2), 73.7 (CH_2), 71.9 (CH_2), 70.5 (CH_2), 70.2 (C-6), 70.1 (CH_2); ESI-MS, m/z= 851.3, $[M+K]^+$ calcd for $C_{54}H_{52}O_7$+K.

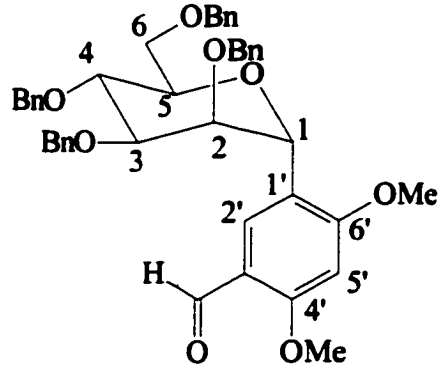


2-(2,3,4,6-Tetra-O-benzyl-α-D-mannopyranosyl) furan (146).[26]

Title compound **146** was obtained in 70% yield; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.38-7.13 (m, 21H, Ph, H-5'), 6.29 (dd, J= 1.8, 3.3 Hz, 1H, H-4'), 6.10 (d, J= 3.3 Hz, 1H, H-3'), 5.11 (d, J= 3.1 Hz, 1H, H-1), 4.81-4.49 (m, 8H, 4xCH_2Ph), 4.15 (t, J= 3.2 Hz, 1H, H-2), 4.02-3.97 (m, 1H, H-4), 3.83 (dd, J= 3.2, 8.4 Hz, 1H, H-3), 3.80-3.71 (m, 3H, H-5, H-6a, H-6b); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 151.4 (C-2'), 142.6 (C-5'), 138.8, 138.7, 138.5, 128.7, 128.7, 128.4, 128.4, 128.3, 128.2, 128.1, 128.0, 128.0, 127.9, 110.8 (C-4'), 109.3 (C-3'), 79.4 (C-3), 75.3 (C-4), 75.1 (C-5), 75.0 (C-2), 73.7 (CH_2), 72.6 (CH_2), 72.4 (CH_2), 70.8 (C-1), 69.5 (C-6); CI-MS, m/z= 591, $[M]^+$ calcd for $C_{38}H_{38}O_6$.

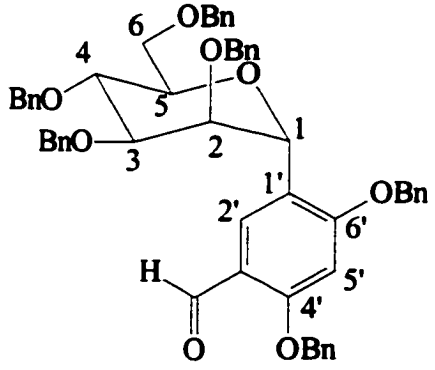
General procedure for the Vilsmeier-Haack reaction:

To a solution of C-aryl mannoside (0.3 mmol) dissolved in DMF (2 mL) was added 5 mL of a solution of $POCl_3$/ DMF (v/v:1/4). The reaction mixture was heated at 90° C and stirred for 4 h. The reaction was monitored by TLC (hexane/ethyl acetate 2/1). The reaction mixture was washed with ether and water. The organic layer was dried over Na_2SO_4, evaporated under reduced pressure and purified via flash chromatography on silica gel using a gradient of hexanes/ethyl acetate 2/1 to 1/2.



2,4-Dimethoxy-5-formyl-1-(2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl)benzene (147).

Title compound **147** was obtained in 88% yield; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 10.26 (s, 1H, CHO), 8.09 (s, 1H, H-2'), 7.40-6.87 (m, 20H, Ph), 6.19 (s, 1H, H-5'), 4.90-4.22 (m, 9H, H-1, 4xCH_2Ph), 3.97-3.91 (m, 5H, H-2, H-4, OMe), 3.81-3.59 (m, 7H, H-3, H-5, H-6a, H-6b, OMe); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 188.5 (CHO), 163.0, 161.3, 138.9, 138.9, 138.8, 138.6, 131.1, 128.6, 128.5, 128.4, 128.3, 128.1, 127.9, 127.8, 127.8, 127.6, 127.2, 120.4, 118.8, 93.9 (C-5'), 85.1 (C-3), 80.5 (C-5), 75.7 (C-2), 75.4 (CH_2), 74.5 (C-4), 74.3 (CH_2), 74.3 (C-1), 73.6 (CH_2), 72.7 (CH_2), 70.0 (C-6), 56.0 (OMe), 55.7 (OMe); ESI-MS, m/z= 706.2, $[M+ NH_4]^+$ calcd for $C_{43}H_{44}O_8$+NH_4.



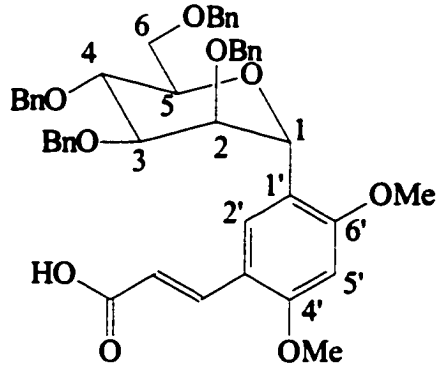
2,4-Dibenzyloxy-5-formyl-1-(2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl)benzene (148).

Title compound **148** was obtained in 88% yield; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 10.41 (s, 1H, CHO), 8.16 (s, 1H, H-2'), 7.46-6.89 (m, 30H, Ph), 6.35 (s, 1H, H-

5'), 5.19-4.29 (m, 13H, H-1, 6xCH_2Ph), 4.05-4.03 (m, 1H, H-2), 3.96 (t, J= 9.5 Hz, 1H, H-4), 3.82-3.78 (m, 2H, H-6a, H-6b), 3.65 (dd, J= 2.6, 9.5 Hz, 1H, H-3), 3.61-3.56 (m, 1H, H-5); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 188.3 (CHO), 162.1, 160.3, 138.9, 138.8, 138.6, 138.6, 138.5, 138.4, 136.4, 136.0, 130.3 (C-2'), 129.0, 129.0, 128.6, 128.5, 128.5, 128.2, 128.1, 128.0, 127.8, 127.6, 127.5, 127.4, 121.2, 119.4, 96.7 (C-5'), 85.2 (C-3), 80.5 (C-5), 75.4 (CH_2), 75.4 (C-4), 74.6 (C-1), 73.9 (CH2), 73.6 (CH_2), 73.3 (C-2), 72.2 (CH_2), 71.0 (CH_2), 70.5 (CH_2), 70.0 (C-6); ESI-MS, m/z= 879.1, $[M+K]^+$ calcd for $C_{55}H_{52}O_8$+K.

General procedure for the Knoevenagel-Doebber reaction:

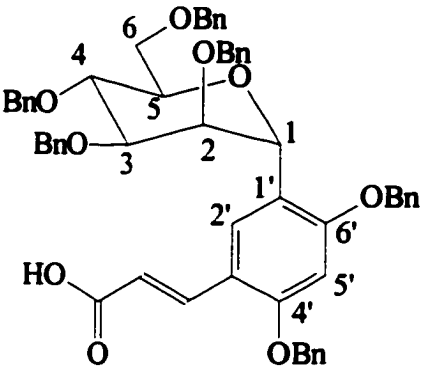
A suspension of formyl derivative (0.15 mmol), malonic acid (0.6 mmol) and pyridine-piperidine (70:1 v/v, 6 mL) was heated at reflux for 24 h. After cooling, the reaction mixture was diluted with CH_2Cl_2 and washed with HCl 10%. The organic layer was dried over Na_2SO_4, evaporated under reduced pressure and purified via flash chromatography on silica gel using a gradient of hexanes/ethyl acetate 3/1 to 1/2.



***Trans*- (2,4-Dimethoxy-1-(2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl) cinnamic acid (149).**

Title compound **149** was obtained in 75% yield; ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.06 (d, J= 16.0 Hz, 1H, H olefinic), 7.78 (s, 1H, H-2'), 7.73-6.86 (m, 20H, Ph), 6.42 (d, J= 16.0 Hz, 1H, H olefinic), 6.20 (s, 1H, H-5'), 4.92-4.18 (m, 9H, H-1, 4xCH_2Ph), 4.05-3.60 (m, 12H, H-2, H-3, H-4, H-5, H-6a, H-6b, 2 OMe); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 173.1 (COOH), 159.6, 158.5, 142.4 (C olefinic), 138.9, 138.8,

138.8, 138.5, 129.9, 128.9, 128.8, 128.8, 128.7, 128.6, 128.5, 128.3, 128.1, 128.0, 127.9, 127.5, 119.9 (C olefinic), 115.8, 115.3, 94.2 (C-5'), 85.2 (C-3), 80.4 (C-5), 75.7 (C-2), 75.6 (CH_2), 74.7 (C-4), 74.6 (CH2), 74.4 (C-1), 73.7 (CH_2), 72.7 (CH_2), 70.0 (C-6), 56.0 (OMe), 55.7 (OMe); ESI-MS, m/z= 748.2, $[M+ NH_4]^+$ calcd for $C_{45}H_{46}O_9$. $+NH_4$.

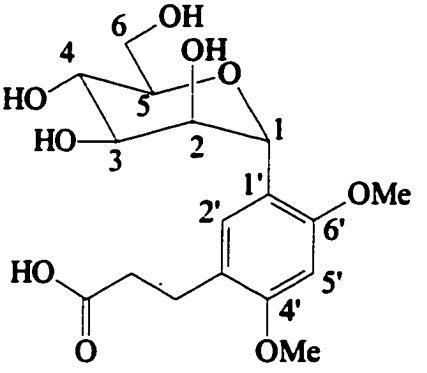


***Trans*- (2,4-Dibenzyloxy-1-(2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl) cinnamic acid (150).**

Title compound **150** was obtained in 75% yield; ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.18 (d, J= 16.0 Hz, 1H, H olefinic), 7.84 (s, 1H, H-2'), 7.45-6.80 (m, 30H, Ph), 6.50 (d, J= 16.0 Hz, 1H, H olefinic), 6.32 (s, 1H, H-5'), 5.15-4.26 (m, 13H, H-1, 6xCH_2Ph), 4.08-4.00 (m, 1H, H-2), 3.94 (t, J= 9.4 Hz, 1H, H-4), 3.84-3.75 (m, 2H, H-6a, H-6b), 3.68-3.60 (m, 2H, H-3, H-5); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 172.8 (COOH), 158.3, 157.4, 142.2 (C olefinic), 138.8, 138.7, 138.7, 138.5, 136.9, 136.6, 129.2, 129.1, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 127.9, 127.6, 127.6, 120.7 (C olefinic), 116.7, 115.8, 97.5 (C-5'), 85.3 (C-3), 80.4 (C-5), 75.6 (CH_2), 75.5 (C-4), 74.7 (C-1), 74.1 (CH_2), 73.7 (C-2), 73.3 (CH_2), 72.2 (CH_2), 71.1 (CH_2), 70.4 (CH_2), 70.0 (C-6); ESI-MS, m/z= 900.3, $[M+ NH_4]^+$ calcd for $C_{57}H_{42}O_9+NH_4$.

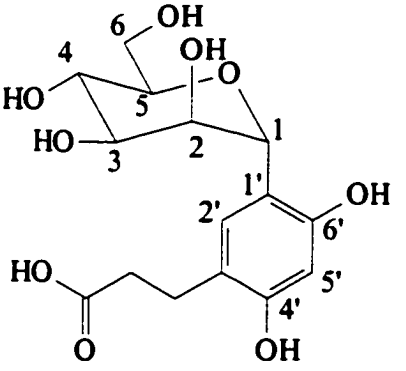
General procedure for the deprotection of C-aryl mannosides:

To a solution of C-aryl mannoside (0.1 mmol) was added 10% palladium/carbon (10 mg) and the reaction mixture was stirred under a hydrogen atmosphere at room temperature for 6 h. The catalyst was filtered off over celite and the filtrate concentrated to afford fully deprotected C-aryl mannoside.



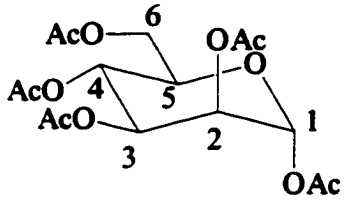
2,4-Dimethoxy-1-(α-D-mannopyranosyl) cinnamic acid (131).

Title compound **131** was obtained in 85% yield; ^{1}H NMR (D_2O), 300 MHz): δ (ppm) 7.15 (s, 1H, H-2'), 6.58 (s, 1H, H-5'), 4.77 (s, 1H, H-1), 3.90 (d, J= 3.1 Hz, 1H, H-2), 3.83-3.32 (m, 11H, H-3, H-4, H-5, H-6a, H-6b, 2 OMe), 2.76-2.71 (m, 2H, methylene), 2.50-2.45 (m, 2H, methylene); ^{13}C NMR (D_2O, 75 MHz): δ (ppm) 178.7 (COOH), 157.8, 155.4, 129.0, 120.4, 118.3, 96.6 (C-5'), 80.6, 74.6, 74.5, 71.0, 67.3, 61.6, 56.2,56.0, 34.8, 25.2.



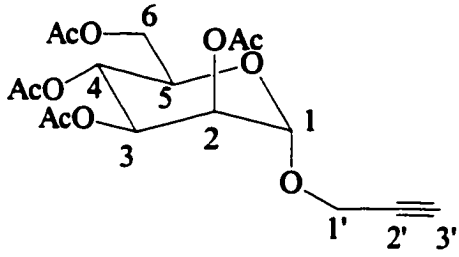
2,4-Dihydroxy-1-(α-D-mannopyranosyl) cinnamic acid (132).

Title compound **132** was obtained in 85% yield; ^{1}H NMR (D_2O, 300 MHz): δ (ppm) 7.16 (s, 1H, H-2'), 6.40 (s, 1H, H-5'), 4.93 (s, 1H, H-1), 4.06-3.48 (m, 6H, H-2, H-3, H-4, H-5, H-6a, H-6b), 2.80-2.77 (m, 2H, methylene), 2.61-2.57 (m, 2H, methylene).



1,2,3,4,6-Penta-O-acetyl-α-D-mannopyranose (151).[27]

D-mannose (5 g, 27.8 mmol) was added to a mixture of acetic anhydride (40 mL, 0.42 mmol) and pyridine (40 mL) cooled to 0° C. The reaction mixture was stirred and allowed to warm to room temperature. The progress of the reaction was monitored by TLC (hexanes/ethyl acetate 1/1). Upon completion of the reaction, the reaction mixture was diluted with CH_2Cl_2 and washed several times with HCl 10%. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The resulting oil was crystallized under a vacuum pump and recrystallized with EtOH 99% to afford a white solid in 77% yield (8.4 g, 21.5 mmol); m.p. 63-65° C (lit. m.p. 64° C); ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 6.04 (d, J= 1.9 Hz, 1H, H-1), 5.33-5.29 (m, 2H, H-3, H-4), 5.23-5.19 (m, 1H, H-2) 4.20 (dd, J= 5.8, 12.7 Hz, 1H, H-6a), 4.08-4.03 (m, 2H, H-5, H-6b); ^{13}C NMR ($CDCl_3$, 50 MHz): δ (ppm) 170.6, 169.9, 169.7, 169.5, 168.0 (C=O), 90.5 (C-1), 70.5, 68.6, 68.2, 65.4, 62.0 (C-6), 20.8, 20.7, 20.6, 20.6, 20.5 (Me).



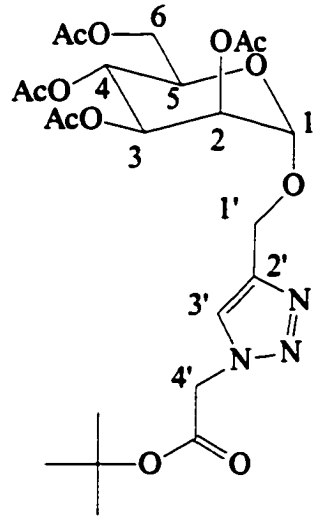
2-Propynyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (152).[28]

Boron trifluoride etherate (10.0 mL, 76.9 mmol) was added to a solution of peracetylated mannose **151** (6.00 g, 15.38 mmol) and propargyl alcohol alcohol (3.0 mL, 46.14 mmol) in dry CH_2Cl_2 (120 mL). The reaction mixture was stirred for 12 h at room temperature and monitored by TLC(hexanes/ethyl acetate 1/1) until the starting material had been consumed. The reaction mixture was then washed with 100 ml of aqueous

saturated $NaHCO_3$. Drying (Na_2SO_4) and evaporation of the organic phase gave a crude residue that was purified by flash chromatography on silica gel using a gradient of hexanes/ethyl acetate 3/1 to 1/1 as eluent. Title compound **73** was isolated in 75% yield as a white solid (4.45 g, 11.53 mmol); m.p. 100° C; $[\alpha]_D$ +56° (c= 2.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 5.31 (dd, J= 3.4, 10.0 Hz, 1H, H-3), 5.26 (t, J= 10.0 Hz, 1H, H-4), 5.23 (dd, J= 1.7, 3.4 Hz, 1H, H-2), 4.99 (d, J= 1.7 Hz, 1H, H-1), 4.24 (dd, J= 5.2, 12.2 Hz, 1H, H-6a), 4.24-4.22 (m, 2H, H-1'a, H-1'b), 4.07-4.00 (m, 2H, H-5, H-6b), 2.44 (t, J= 2.4 Hz, 1H, H-3'), 2.12, 2.07, 2.01, 1.96 (4s, 12H, OAc); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 170.5, 169.8, 169.7, 169.6 (C=O), 96.2 (C-1), 77.9 (C-2'), 75.0 (C-3'), 70.6, 69.3, 68.9, 67.9, 62.3 (C-6), 54.9 (C-1'), 20.8, 20.7, 20.6, 20.6 (Me); MS (FAB): m/z= 387.1264, $[M+1]^+$ calcd for $C_{17}H_{22}O_{10}$.

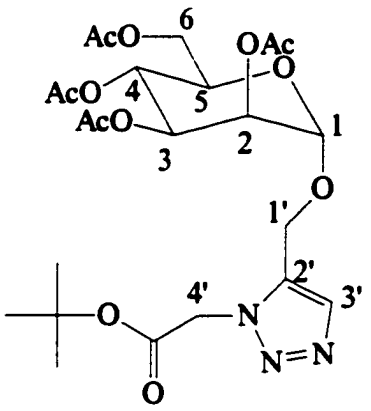
Cycloaddition between 152 and azido *tert*-butyl acetate.

In a sealed tube, a mixture of **152** (112 mg, 0.29 mmol), azido *tert*-butyl acetate (50 mg, 0.32 mmol), and toluene (1 mL) was stirred and heated at 135° C for 12 h. Then the solution was purified by flash chromatography on silica gel using a gradient of hexanes/ethyl acetate 2/1 to 1/1 as eluent to afford regioisomers 1,4 **(153)** and 1,5 **(154)** in a 4:1 ratio in 96% yield (152 mg, 0.28 mmol).

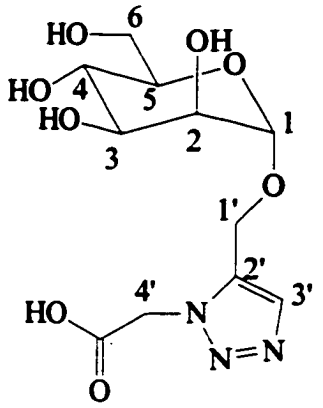


Cycloadduct 153. ^1H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.68 (s, 1H, H-3'), 5.28-5.22 (m, 2H, H-3, H-4), 5.19 (dd, J= 1.8, 3.1 Hz, 1H, H-2), 5.03 (s, 2H, H-4'), 4.91 (d, J= 1.8 Hz, 1H, H-1), 4.81 (d, J= 12.4 Hz, 1H, H-1'a), 4.46 (d, J= 12.4 Hz, 1H, H-1'b), 4.22 (dd,

J= 5.1, 12.2 Hz, 1H, H-6a), 4.04 (dd, J= 2.4, 12.2 hz, 1H, H-6b), 4.02-3.99 (m, 1H, H-5), 2.09, 2.06, 1.98, 1.92 (4s, 12H, OAc), 1.44 (s, 9H, *t*-Bu); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 170.8, 170.1, 169.9, 169.8, 165.3 (C=O), 144.1 (C-2'), 124.5 (C-3'), 97.2 (C-1), 84.1 ($\underline{C}Me_3$), 69.7 (C-2), 69.3 (C-5), 69.0 (C-4), 66.4 (C-3), 62.6 (C-6), 61.3 (C-1'), 51.7 (C-4'), 28.1, 20.9, 20.9, 20.8, 20.8 (Me); ESI-MS, m/z= 544.1, $[M+1]^+$ calcd for $C_{23}H_{33}N_3O_{12}$.

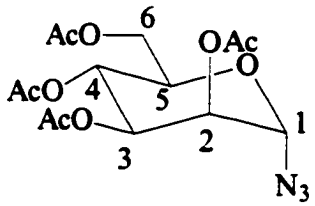


Cycloadduct 154. ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.64 (s, 1H, H-3'), 5.22-5.19 (m, 2H, H-3, H-4), 5.16 (dd, J= 1.7, 2.9 Hz, 1H, H-2), 5.08 (d, J= 5.0 Hz, 2H, H-4'), 4.91 (d, J= 1.8 Hz, 1H, H-1), 4.81 (d, J= 12.4 Hz, 1H, H-1'a), 4.46 (d, J= 12.4 Hz, 1H, H-1'b), 4.75-4.65 (m, 3H, H-1, H-1'a, H-1'b), 4.20 (dd, J= 5.4, 12.3 Hz, 1H, H-6a), 4.04 (dd, J= 2.3, 12.3 Hz, 1H, H-6b), 3.86-3.83 (m, 1H, H-5), 2.08, 2.07, 1.99, 1.93 (4s, 12H, OAc), 1.44 (s, 9H, *t*-Bu); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 170.6, 169.9, 169.9, 169.7, 165.5 (C=O), 134.8 (C-3'), 132.6 (C-2'), 96.8 (C-1), 84.2 (CMe_3), 69.5 (C-5), 69.2 (C-2), 69.0 (C-4), 66.1 (C-3), 62.6 (C-6), 57.5 (C-1'), 50.4 (C-4'), 28.1, 20.9, 20.8, 20.7, 20.7 (Me); ESI-MS, m/z= 566.1, $[M+Na]^+$ calcd for $C_{23}H_{33}N_3O_{12}$+Na.



Fully deprotected cycloadduct 154 (134).

A solution of the cycloadduct **154** (70 mg, 0.13 mmol) dissolved in 1 M NaOH solution of MeOH (5 mL) was stirred at room temperature for 3 h. The solution was then treated with Amberlite IR-120 cation exchange resin and the filtrate evaporated under reduced pressure to afford title compound **134** in quantitative yield (40 mg, 0.13 mmol); ^{1}H NMR (D_2O, 300 MHz): δ (ppm) 7.75 (s, 1H, H-3'), 5.22 (s, 2H, H-4'), 4.75-4.72 (m, 3H, H-1, H-1'a, H-1'b), 3.77-3.39 (m, 6H, H-2, H-3, H-4, H-5, H-6a, H-6b); ^{13}C NMR (D_2O, 75 MHz): δ (ppm) 170.9 (COOH), 135.3 (C-3'), 133.2 (C-2'), 99.7 (C-1), 73.5, 70.7, 70.0, 66.8, 61.1 (C-6), 57.2 (C-1'), 50.2 (C-4'); ESI-MS, m/z= 342.1, $[M+ Na]^+$ calcd for $C_{11}H_{17}N_3O_8$+Na.



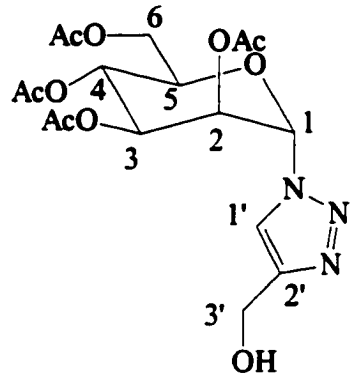
2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl azide (155).[29]

Stannic tetrachloride (0.5 mL, 4.27 mmol) was added to a solution of D-mannopyranose pentaacetate (2.00 g, 5.13 mmol) and trimethylsilyl azide (0.75 mL, 5.64 mmol) in CH_2Cl_2 (45 mL). After 6 h, the solution was washed successively with water, aqueous saturated $NaHCO_3$, and water. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to give title compound in 90% yield (1.43 g, 3.83 mmol); ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 5.30 (d, J= 1.8 Hz, 1H, H-1), 5.21-5.10 (m,

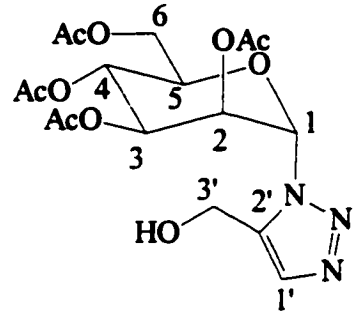
2H, H-3, H-4), 5.05-5.03 (m, 1H, H-2), 4.20 (dd, J= 5.8, 12.7 Hz, 1H, H-6a), 4.08-4.03 (m, 2H, H-5, H-6b), 2.04, 2.00, 1.95, 1.88 (4s, 12H, OAc); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 170.9, 170.1, 170.0, 169.9 (C=O), 87.7 (C-1), 70.9 (C-5), 69.4 (C-2), 68.5 (C-3), 65.8 (C-4), 62.4 (C-6), 21.1, 21.0, 20.9, 20.8 (Me);

4- and 5-(hydroxymethyl)-1-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-1,2,3-triazole (156 and 157).

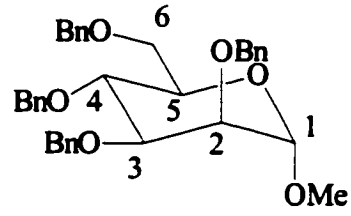
In a sealed tube, a mixture of **155** (100 mg, 0.27 mmol), propargyl alcohol (17 mg, 0.30 mmol), and toluene (1 mL) was stirred and heated at 135° C for 12 h. Then the solution was purified by flash chromatography on silica gel using a gradient of hexanes/ethyl acetate 1/1 to 1/5 as eluent to afford regioisomers 1,4 **(156)** and 1,5 **(157)** in a 3:1 ratio in 96% yield (111 mg, 0.26 mmol).



Cycloadduct 156. ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.75 (s, 1H, H-1'), 5.97 (d, J= 1.7 Hz, 1H, H-1), 5.85-5.80 (m, 2H, H-3, H-4), 5.34-5.27 (m, 1H, H-2), 4.72 (s, 2H, H-3'), 4.27 (dd, J= 5.2, 12.6 Hz, 1H, H-6a), 3.95 (dd, J= 2.6, 12.6 Hz, 1H, H-6b), 3.84-3.79 (m, 1H, H-5), 3.47 (bs, 1H, OH), 2.11, 2.01, 1.99, 1.96 (4s, 12H, OAc); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 170.9, 170.1, 170.1, 170.0 (C=O), 148.9 (C-2'), 122.9 (C-1'), 84.0 (C-1), 72.3 (C-5), 69.3 (C-3), 68.5 (C-4), 66.1 (C-2), 61.9 (C-6), 56.4 (C-3'), 21.1, 21.0, 21.0, 20.9 (Me); CI-MS, m/z= 430, $[M+1]^+$ calcd for $C_{17}H_{23}N_3O_{10}$.



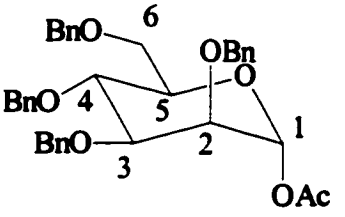
Cycloadduct 157. ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.59 (s, 1H, H-1'), 6.16 (s, 1H, H-1), 6.01 (dd, J= 3.6, 9.6 Hz, 1H, H-3), 5.89-5.87 (m, 1H, H-2), 5.34 (t, J= 9.6 Hz, 1H, H-4), 4.79 (d, J= 4.0 Hz, 1H, H-3'a), 4.73 (d, J= 4.0 Hz, 1H, H-3'b), 4.18 (dd, J= 5.5, 12.4 Hz, 1H, H-6a), 4.01-3.97 (m, 1H, H-6b), 3.71-3.67 (m, 1H, H-5), 3.14 (bs, 1H, OH), 2.16, 2.01, 1.97 (3s, 12H, OAc); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 170.9, 170.2, 170.1, 170.1 (C=O), 138.1 (C-2'), 133.8 (C-1'), 82.9 (C-1), 77.7 (C-5), 69.5 (C-3), 68.7 (C-2), 66.3 (C-4), 62.3 (C-6), 53.2 (C-3'), 21.1, 21.0, 21.0, 20.9 (Me); CI-MS, m/z= 430, $[M+H]^+$ calcd for $C_{17}H_{23}N_3O_{10}$.



Methyl 2,3,4,6-tetra-O-benzyl-α-D-mannopyranoside (159).[30]

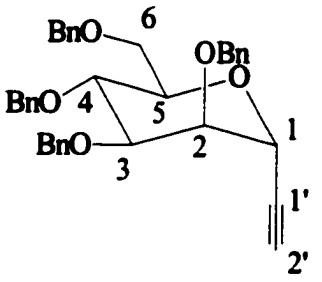
Benzyl bromide (6 mL, 50.44mmol) was added to a solution of methyl α-D-mannopyranoside **64** (1.2 g, 6.18 mmol) dissolved in 30 mL of DMF cooled to 0° C under N_2 atmosphere. Sodium hydride (2.02 g, 50.44 mmol, 60% mineral oil dispersion) was added slowly. The reaction mixture was stirred and allowed to warm to room temperature for 16 h. The progress of the reaction was monitored by TLC (hexanes/ethyl acetate 5/1). Methanol (10 mL) was then added at 0° C to neutralize the excess of sodium hydride and the solution was stirred for 10 min. The reaction mixture was diluted with ether (30 mL) and washed several times with water until the complete removal of DMF from the organic phases. The organic layer was dried over Na_2SO_4, evaporated under

reduced pressure and purified via flash chromatography on silica gel using a gradient of hexanes and ethyl acetate as eluent Title compound **159** was isolated as an oil in 60% yield (2.05 g, 3.71 mmol); ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.38-7.18 (m, 20H, Ph), 4.92-4.46 (m, 9H, H-1, 4xCH_2Ph), 4.14-3.75 (m, 6H, H-2, H-3, H-4, H-5, H-6a, H-6b), 3.34 (s, 3H, OMe); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 138.9, 138.9, 138.8, 138.7, 128.7, 128.3, 128.2, 128.2, 128.0, 127.9, 99.3 (C-1), 80.6, 75.5, 75.3, 74.9, 73.8, 73.0, 72.5, 72.1, 69.7 (C-6), 55.1 (OMe); ESI-MS, m/z= 572.2, $[M+ NH_4]^+$ calcd for $C_{35}H_{38}O_6$+NH_4.



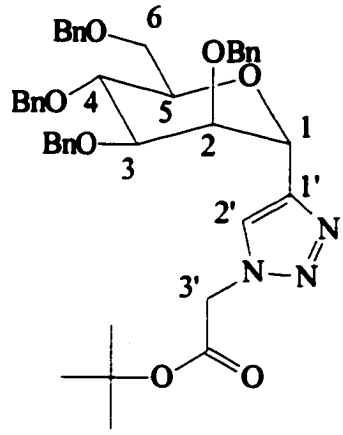
1-O-Acetyl-2,3,4,6-tetra-O-benzyl-α-D-mannopyranose (160).[31]

To a solution of **159** (300 mg, 0.54 mmol) in acetic anhydride (4.5 mL) and AcOH (7.5 mL) was added a mixture of AcOH (3 mL) and conc. H_2SO_4 (6 μL). The reaction mixture was stirred at room temperature for 80 min. The progress of the reaction was monitored by TLC (hexane/ethyl acetate 1/1). The reaction mixture was poured into ice-water containing solid $NaHCO_3$ and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, evaporated under reduced pressure and purified via flash chromatography on silica gel using hexane and hexane/ethyl acetate 1/1 as eluent Title compound **159** was isolated in 60% yield as an oil (188 mg, 0.32 mmol); ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 7.41-7.15 (m, 20H, Ph), 6.22 (d, J= 2.0 Hz, 1H, H-1), 5.01-4.52 (m, 8H, 4xCH_2Ph), 4.08 (t, J= 9.5 Hz, 1H, H-4), 3.88-3.81 (m, 5H, H-2. H-3, H-5, H-6a, H-6b), 2.06 (s, 3H, OAc).



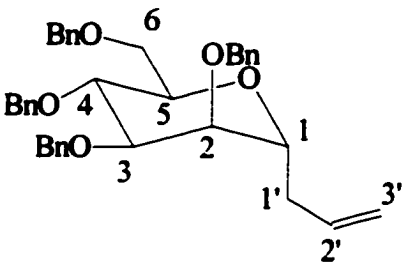
2,3,4,6-Tetra-O-benzyl-α-D-mannopyranosyl acetylene (161).[32]

A mixture of acetate **160** (500 mg, 0.86 mmol), tributylstannyl(trimethylsilyl) acetylene (935 mg, 2.58 mmol), activated 4-Å powdered molecular sieves (500 mg), and dry CH_2Cl_2 (5 mL) was stirred at room temperature for 15 min, and then trimethylsilyl triflate (0.3 mL, 1.71 mmol) was added dropwise. The reaction mixture was stirred for an additional 1 h. The progress of the reaction was monitored by TLC (hexanes/ethyl acetate 1/1). The reaction mixture was diluted with Et_3N (0.5 mL) and CH_2Cl_2 (15 mL), filtered through a pad of celite. The filtrate was evaporated under reduced pressure and purified by column chromatography on silica gel using hexane/ethyl acetate 4/1 as eluent to afford silylated product. The removal of the trimethylsilyl group was accomplished as follow. A solution of silylated product (0.86 mmol) in 5:1 MeOH-CH_2Cl_2 (28 mL), was treated at room temperature for 1 h with 1 M NaOH (1.4 mL), neutralized with 1 M HCl, concentrated and purified by chromatography on silica gel using hexane/ethyl acetate 2/1. Title compound **161** was obtained as a syrup in 76% yield (358 mg, 0.65 mmol); ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.46-7.12 (m, 20H, Ph), 4.94-4.54 (m, 9H, H-1, 4xCH_2Ph), 4.11-4.01 (m, 3H, H-2, H-3, H-4), 3.98-3.75 (m, 3H, H-5, H-6a, H-6b), 2.53 (d, J= 2.4 Hz, 1H, H-2'); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 138.3, 138.2, 138.1, 128.3, 128.3, 128.2, 128.0, 127.8, 127.8, 127.7, 127.6, 127.6, 127.4, 80.1, 78.5, 77.5, 76.6, 76.0, 75.2, 74.6, 73.3, 72.0, 71.7, 69.0, 65.9.



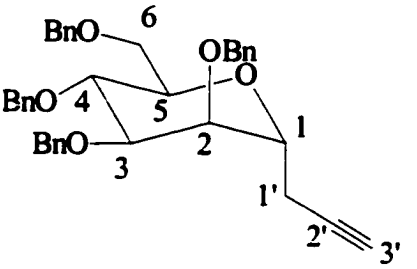
Cu(I) catalyzed cycloaddition between 161 and azido *tert*-butyl acetate.

Cycloadduct 162. To a magnetically stirred solution of acetylenic sugar **161** (230 mg, 0.42 mmol) and azido *tert*-butyl acetate (70 mg, 0.84 mmol) in THF (6 mL) was added CuI (160 mg, 0.84 mmol). Then DIPEA (1.4 mL, 8.4 mmol) was added and the solution was kept at room temperature until TLC (ether/hexane 2/1) showed complete disappearance of the starting material (1 h). The reaction mixture was then evaporated under reduced pressure and ethyl acetate was added. The solution was filtered through a pad of Celite and washed with HCl 10%. The organic layer was dried over Na_2SO_4, evaporated under reduced pressure and purified via flash chromatography on silica gel using ether/hexanes 2/1 as eluent. The Title compound **162** was isolated in 82% yield (243 mg, 0.34 mmol); $[\alpha]_D$ +30° (c 1, chloroform); ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.57 (s, 1H, H-2'), 7.44-7.12 (m, 20H, Ph), 5.33 (d, J= 2.3 Hz, 1H, H-1), 5.08-4.51 (m, 11H, H-2, H-3', 4xCH_2Ph), 4.03-3.91 (m, 2H, H-3, H-4), 3.76-3.65 (m, 3H, H-5, H-6a, H-6b), 1.47 (s, 9H, *t*-Bu); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 164.9 (C=O), 145.3 (C-1'), 138.4, 138.3, 128.2, 128.2, 128.2, 127.8, 127.8, 127.8, 127.7, 127.5, 127.4, 124.0 (C-2'), 83.7 (CMe_3), 79.7, 76.6, 74.9, 74.7 (CH_2), 74.2, 73.2 (CH_2), 72.3 (CH_2), 71.8 (CH_2), 70.6, 69.6 (C-6), 51.4 (C-3'), 27.9 (Me); ESI-MS, m/z= 706.2, $[M+ 1]^+$ calcd for $C_{42}H_{47}N_3O_7$.



3-(2,3,4,6-Tetra-O-benzyl-α-D-mannopyranosyl)propene (163).[33]

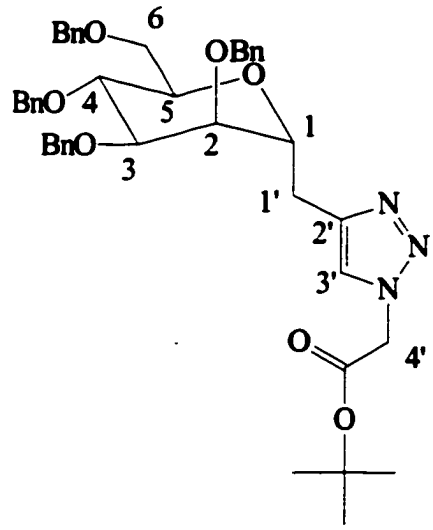
To a solution of **159** (3.00 g, 5.42 mmol) in CH_3CN (12 mL) was added allyltrimethyl silane (1.8 mL, 11.4 mmol) at 0° C under nitrogen. To the mixture was added TMSOTf (0.5 mL, 2.71 mmol), and the reaction was allowed to warm to room temperature and stirred overnight. The reaction mixture was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$. The organic layer was dried over Na_2SO_4, evaporated under reduced pressure and purified via flash chromatography on silica gel using hexanes/ethyl acetate 3/1 as eluent. The title compound **163** was isolated in 70% yield (2.14 g, 3.79 mmol); ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.39-7.18 (m, 20H, Ph), 5.81-5.67 (m, 1H, H-2'), 5.03-4.98 (m, 2H, H-3'a, H-3'b), 4.70-4.49 (m, 8H, 4xCH_2Ph), 4.07-4.01 (m, 1H, H-1), 3.88-3.61 (m, 6H, H-2, H-3, H-4, H-5, H-6a, H-6b), 2.38-2.31 (m, 2H, H-1'a, H-1'b); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 138.3, 138.2, 138.1, 134.2 (C-2'), 128.3, 128.3, 128.3, 128.0, 128.0, 127.9, 127.7, 127.7, 127.6, 127.5, 117.2 (C-3'), 76.8, 75.0, 74.8, 73.9, 73.6, 73.2, 72.3, 72.0, 71.4, 69.0 (C-6), 34.6 (C-1').



3-(2,3,4,6-Tetra-O-benzyl-α-D-mannopyranosyl)propyne (164).

A solution of compound **163** (1.38 g, 2.4 mmol) in MeOH (100 mL) was cooled to -78° C. The solution was degassed by bubbling dry nitrogen. Ozone was then passed

through the solution under vigorous stirring. After 30 min, TLC (ether/hexane 2/1) showed disappearance of the starting material. Dry nitrogen was passed through the cold solution in order to remove excess ozone. Dimethyl sulfide (1.5 mL, 20 mol) was added. After 30 min the cold bath was removed and the solution was allowed to warm up to room temperature. After 1h, the solution was evaporated. The resulting crude material was dissolved in MeOH (100 mL) and K_2CO_3 (0.676 g, 4.89 mmol) was added. The solution was cooled at 0° C. Dimethyl (1-azoacetonyl)phosphonate (Ohira's reagent (0.7 g, 3.64 mmol) dissolved in 10 mL of MeOH was added and the reaction mixture kept overnight at room temperature. The solution was then evaporated, ether (200 mL) was added and the solution washed with water. The organic layer was dried over Na_2SO_4, evaporated and purified via flash chromatography on silica gel using hexanes/ether 1/1 as eluent. The title compound **164** was isolated in 72% yield (0.98 g, 1.74 mmol); $[\alpha]_D$ +34° (c 1, chloroform); ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.45-7.18 (m, 20H, Ph), 4.89-4.49 (m, 8H, 4xCH_2Ph), 4.20-4.14 (m, 1H, H-1), 4.00-3.73 (m, 6H, H-2, H-3, H-4, H-5, H-6a, H-6b), 2.67-2.50 (m, 2H, H-1'a, H-1'b), 2.03 (t, J= 2.6 Hz, 1H, H-3'); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 138.2, 138.1, 138.0, 137.9, 128.4, 128.3, 128.2, 128.0, 127.8, 127.8, 127.6, 127.4, 127.4, 80.2, 75.6, 74.4, 74.2, 74.2, 73.2, 73.0, 72.0, 71.5, 70.5, 70.3, 68.6, 20.9 (C-1'); ESI-MS, m/z= 580.3, $[M+ NH_4]^+$ calcd for $C_{37}H_{38}O_5$+NH_4.



Cu(I) catalyzed cycloaddition between 164 and azido *tert*-butyl acetate.

Cycloadduct 165. Following the same procedure described for the formation of cycloadduct **162**, title compound **165** was obtained in 84% yield; $[\alpha]_D$ -4° (c 1, chloroform); ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.66 (s, 1H, H-3'), 7.32-7.21 (m, 20H, Ph), 4.74-4.49 (m, 10H, H-4', 4xCH_2Ph), 4.29-4.23 (m, 1H, H-1), 3.99-3.69 (m, 6H, H-2, H-3, H-4, H-5, H-6a, H-6b), 3.16-2.97 (m, 2H, H-1'a, H-1'b), 1.44 (s, 9H, *t*-Bu); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 165.2 (C=O), 144.2 (C-2'), 138.2, 138.0, 138.0, 137.9, 128.3, 128.2, 128.0, 127.8, 127.7, 127.6, 127.6, 127.5, 124.2 (C-3'), 83.2 (CMe_3), 76.7, 75.2, 75.1, 73.6, 73.4, 73.1, 72.1, 71.6, 71.5, 69.4, 51.0 (C-4'), 27.8 (Me), 26.5 (C-1'); ESI-MS, m/z= 720.2, $[M+1]^+$ calcd for $C_{43}H_{49}N_3O_7$.

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Claims to Original Research

1 Olefin metathesis with Grubbs catalyst (first generation) was applied for the first time in chemical glycobiology.

2 A new method has been developed for the synthesis of carbohydrate homodimers with potential role in signal transduction. The method is simple, very efficient and could be applied to *O*- as well to *C*- alkenyl glycopyranosides.

3 Extension of O- and C-alkenyl glycopyranosides was accomplished by selective cross-metathesis in good to excellent yields. These derivatives represent interesting precursors for the synthesis of neoglycoconjugates.

4 A novel C-linked (1-6)-pseudodisaccharide was synthesized via cross-metathesis reaction.

5 A new procedure for the deprotection of O-allyl ether was developed. A two-step one-pot reaction involving isomerization and cleavage of the resulting prop-1-enyl linkage.

6 A sequence of olefin metathesis and Sharpless asymmetric dihydroxylation reactions were used to create a novel class of glycoclusters having varied structural and stereochemical environments.

7 The design and synthesis of the first synthetic ligand toward Human Mannose-binding Protein which plays an important role in the innate immunity was accomplished.

8 Glycomimetics of 3,6-di-*O*-(α-D-mannopyranosyl)α-D-mannopyranoside, the natural ligand rcognized by Con A were synthesized.

9 Synthesis of C-aryl α-D-mannosides was prepared using Friedel-Crafts methodology on mannosyl phosphate. Their relative inhibitory effect on the binding of Con A to yeast mannan was evaluated and showed lower inhibitory effect than aryl α-D-mannopyranosides. Thus, indicating that an additional carbon between the sugar part and the aryl group is necessary for high affinity glycomimetics.

10 Synthesis of O-, N- and C- linked 1,2,3-triazole α-D-mannosides were prepared by 1,3 cyloaddition. The relative binding inhibitory effect of the O-linked 1,2,3-triazole α-D-mannosides on the binding of Con A was evaluated and showed inhibitory effect similar to methyl-α-D-mannopyranoside.

11 Combining glycomimetics and multivalent approaches would lead to very potent high affinity ligands.

Publications

1 R. Dominique, S.K. Das, B. Liu, J. Nahra, B. Schmor, Z. Gan, R. Roy, **Ruthenium carbenoids as catalysts for olefin metathesis of ω-alkenyl glycosides,** *Methods in Enzymology, in press*

2 R. Dominique, R. Roy, **Stereoselective synthesis of glycoclusters using an olefin metathesis and Sharpless dihydroxylation sequence;** *Tetrahedron Lett.* **2002**, 43, 395-398.

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5 R. Roy, S. K. Das, R. Dominique, M. C. Trono, F. Hernandez-Mateo, F. Santoyo-Gonzalez, **Transition-metal catalyzed neoglycoconjugates synthesis** ; *Pure & Applied Chem.* **1999**, 71, 565-571.

6 S. K. Das, R. Dominique, C. Smith, J. Nahra, R. Roy, **Novel carbohydrate homodimers by olefin metathesis reactions of alkenyl glycosides;** *Carbohydr. Letters*, **1999**, 3, 361-368.

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8 R. Dominique, S. K. Das , R. Roy, **Alkenyl O- and C- glycopyranoside homodimerization by olefin metathesis reaction**; *Chem.Comm.*, **1998**, 2437-2438.

Conference Proceedings

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2 Romyr Dominique, Rene Roy. "Stereoselective synthesis of neoglycoconjugates". **84e Congrès et exposition de la SCC** (26-30 May 2001, Montreal)

3 Romyr Dominique, Rene Roy. "Stereoselective synthesis of neoglycoconjugates". **OCCI** (1-2 May 2001 Université d'Ottawa).

4 René Roy, Bingcan Liu, Romyr Dominique, Sanjoy Kumar Das. Transition metal transformations toward the synthesis of Molecular asterisks containing galactosylated ligands involved in xenotransplantation". **Treizièmes entretiens du centre Jacques Cartier** (4-6 October 2000 Université de Montréal).

5 Romyr Dominique, René Roy. " Synthèse de Néoglycoconjugués". **ACFAS** (11-15 May 2000 Universite de Montreal).

6 Romyr Dominique, René Roy. "Synthesis of neoglycoconjugates". **OCCI** (1-2 May 2000 Université Carleton).

7 René Roy, Sanjoy Kumar Das, Romyr Dominique, M.Corazon Trono, Fernando Hernandez-Mateo and Francisco Santoyo-Gonzalez. "Synthesis of sugar dimers using transition metal catalyzed reactions". **Gordon Research conferences** (21-25 June 1999 New Hampshire)

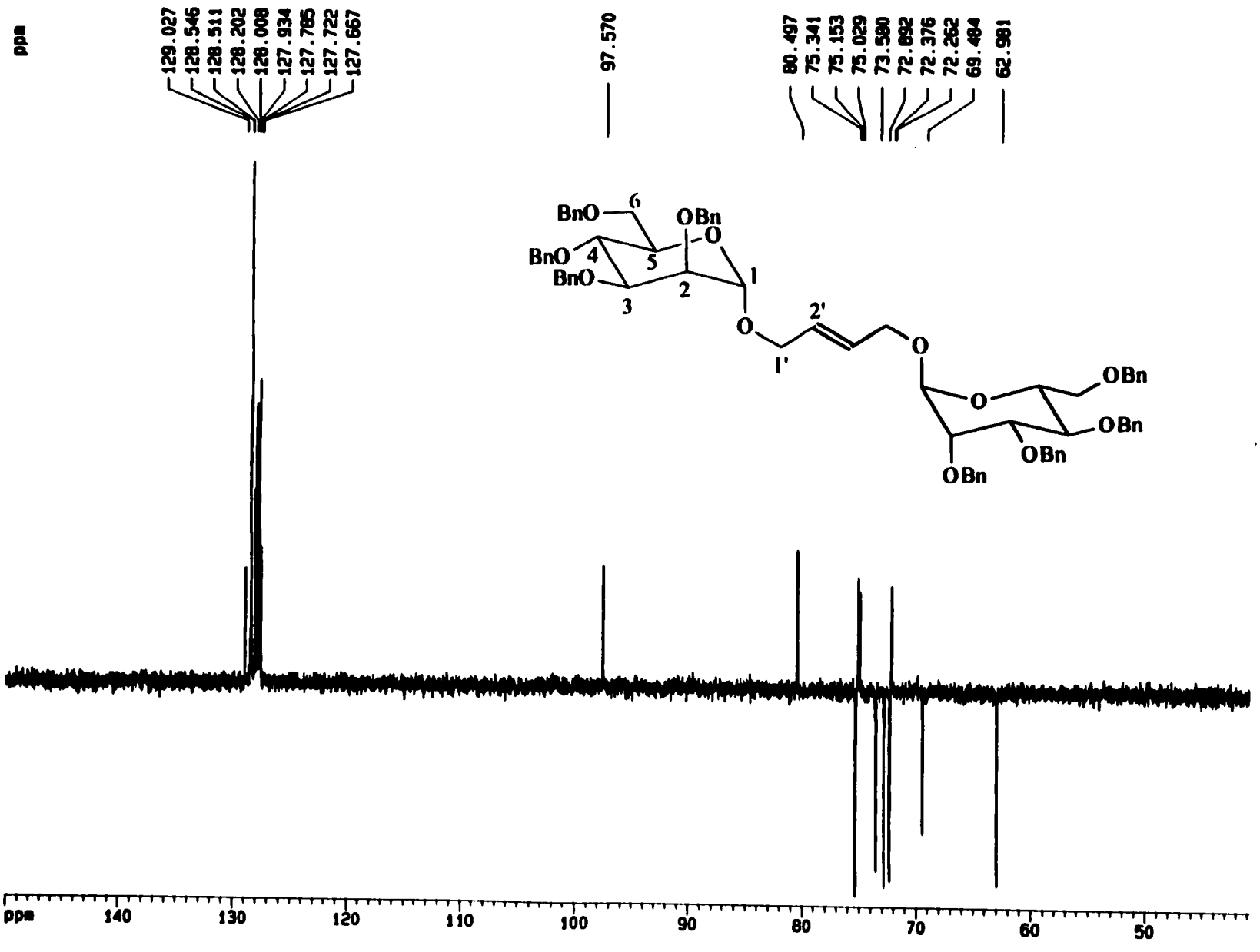
8 Romyr Dominique, Sanjoy Kumar Das, René Roy. "Formation de liens C-C sur des glycosides via la réaction de métathèse des alcènes.". **ACFAS** (10-14 May 1999 University of Ottawa).

9 Sanjoy Kumar Das, Romyr Dominique, René Roy. "Transition metal-catalyzed synthesis of neoglycoconjugates". **Symposium in synthetic organic** (14-16 April 1999 University of Montreal)

10 René Roy, Sanjoy Kumar Das, Romyr Dominique, M.Corazon Trono, Fernando Hernandez-Mateo and Francisco Santoyo-Gonzalez. "Transition-metal catalyzed neoglycoconjugates synthesis". **3rd International Congress of the cuban chemical society** (1-4 December 1998, Havana, Cuba).

"The organic chemist is more than a logician and strategist: he is an explorer strongly influenced to speculate, to imagine, and even to create".
E. J. Corey

APPENDIX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BnO
BnO
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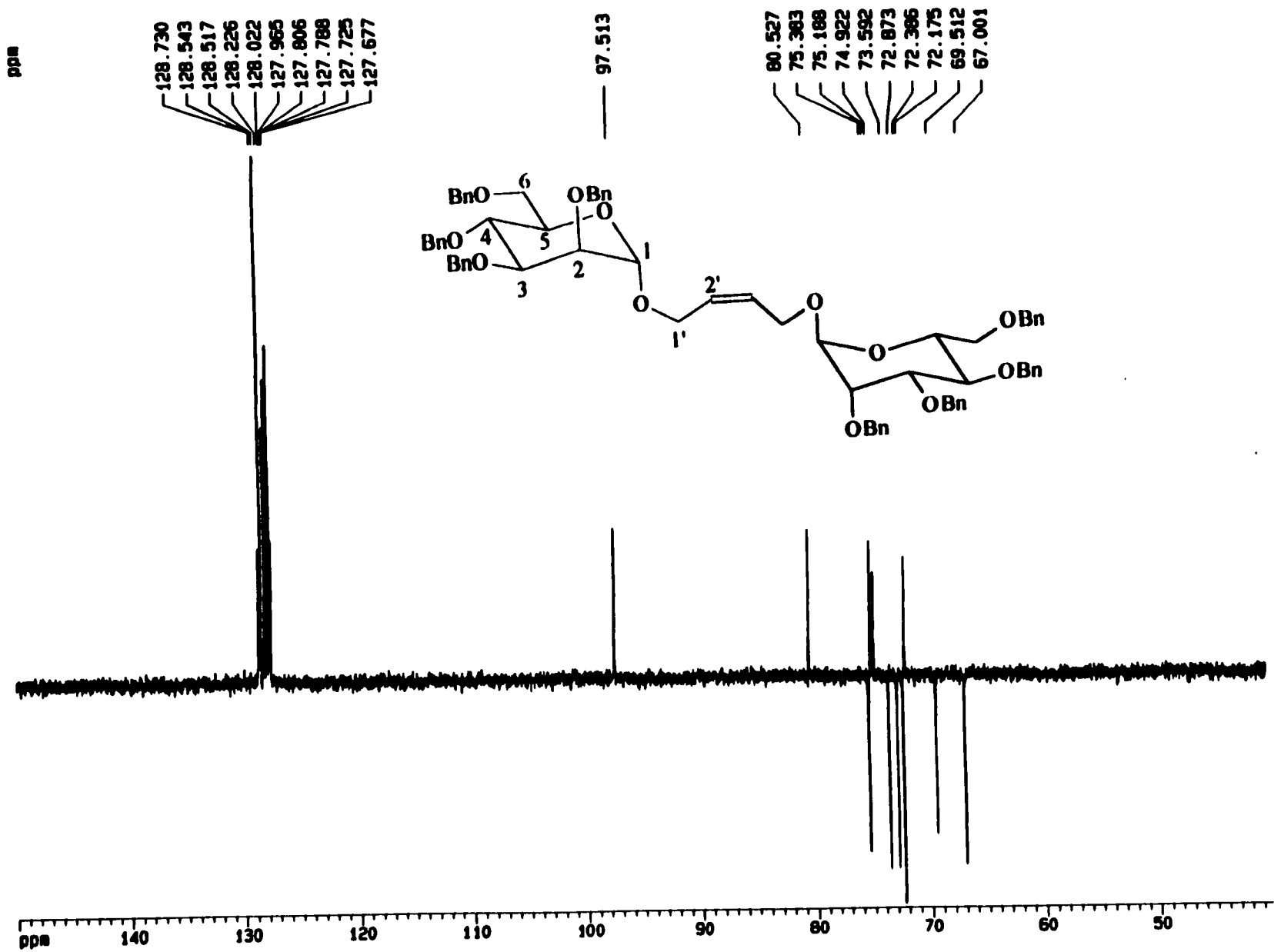
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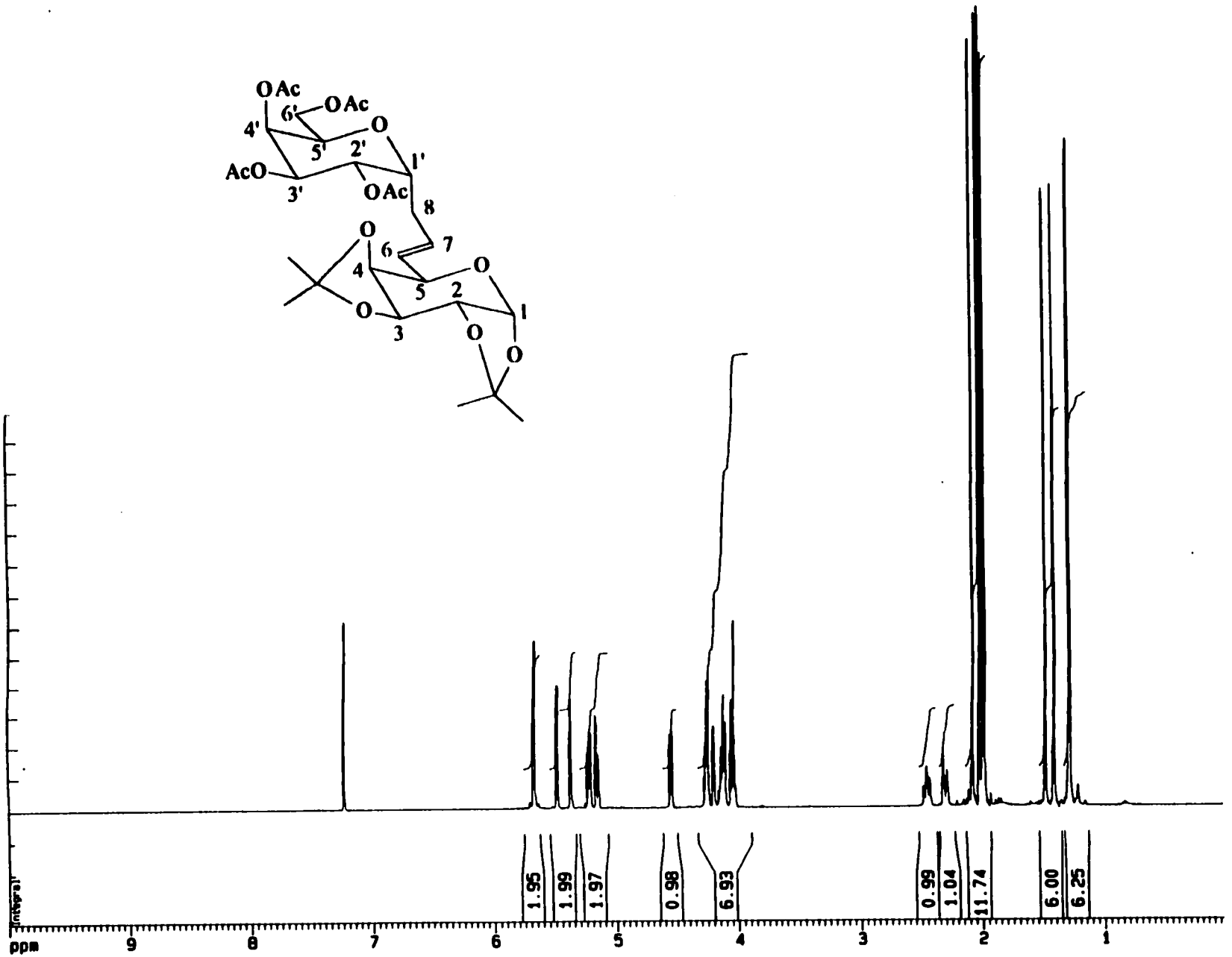
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5
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O
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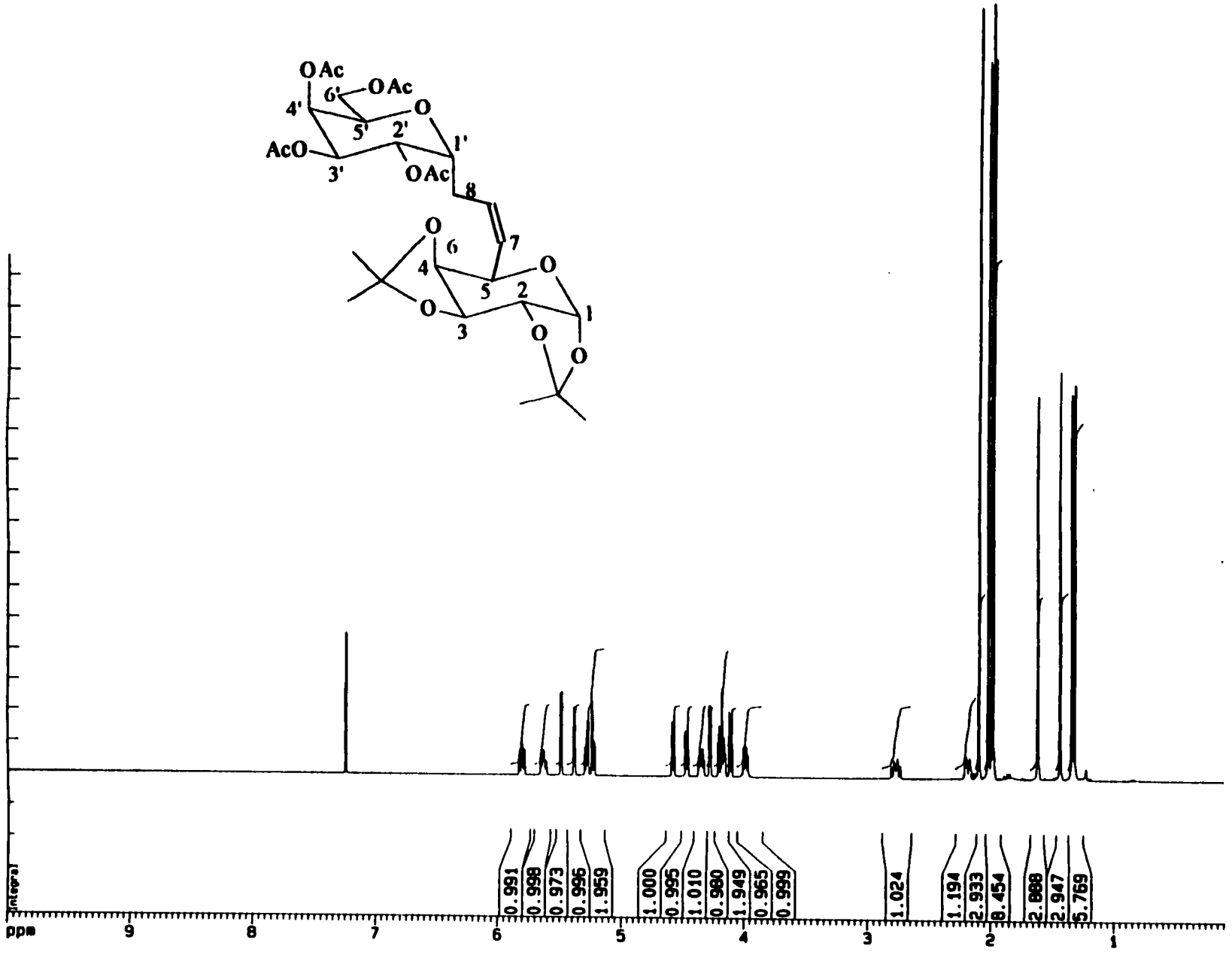
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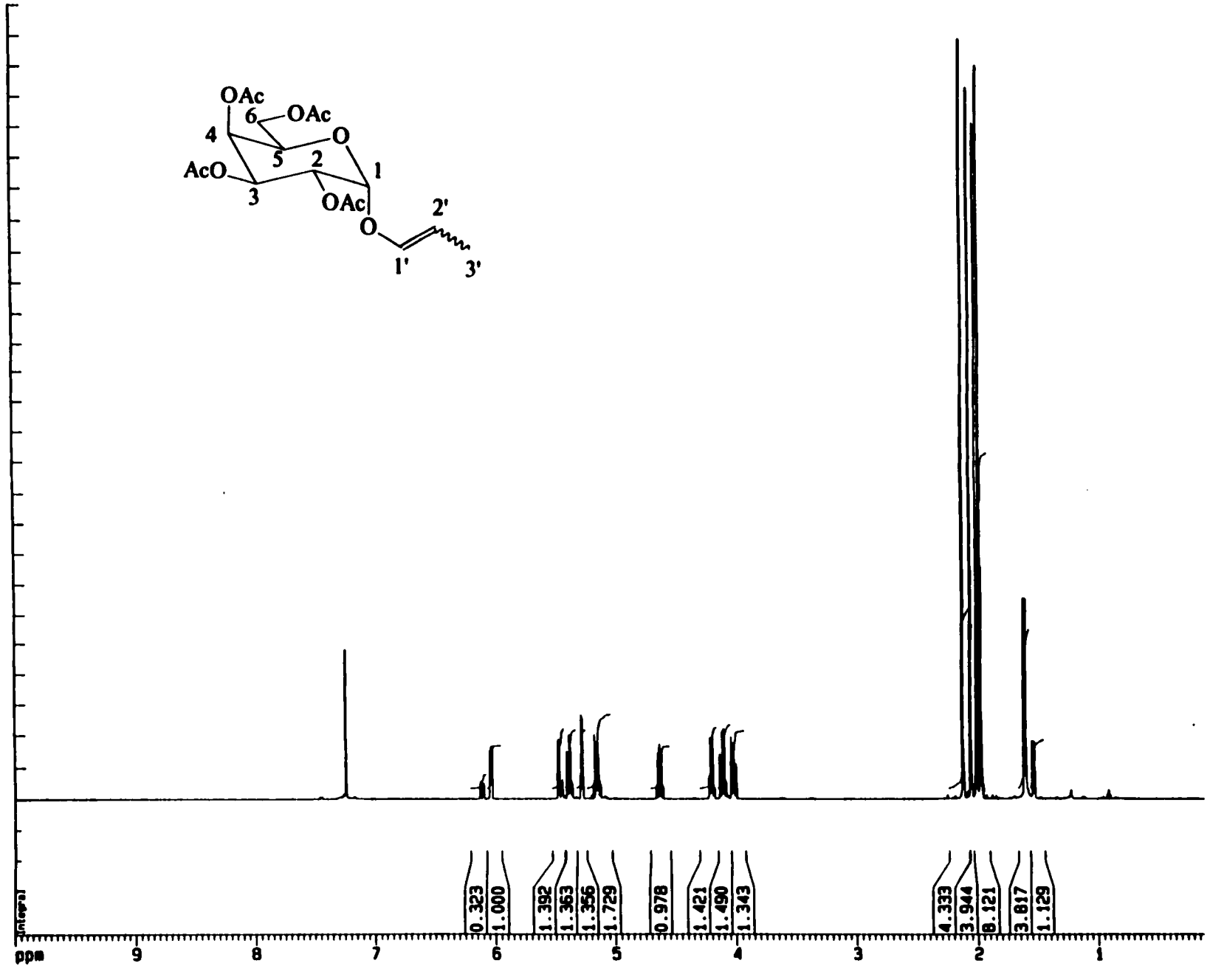
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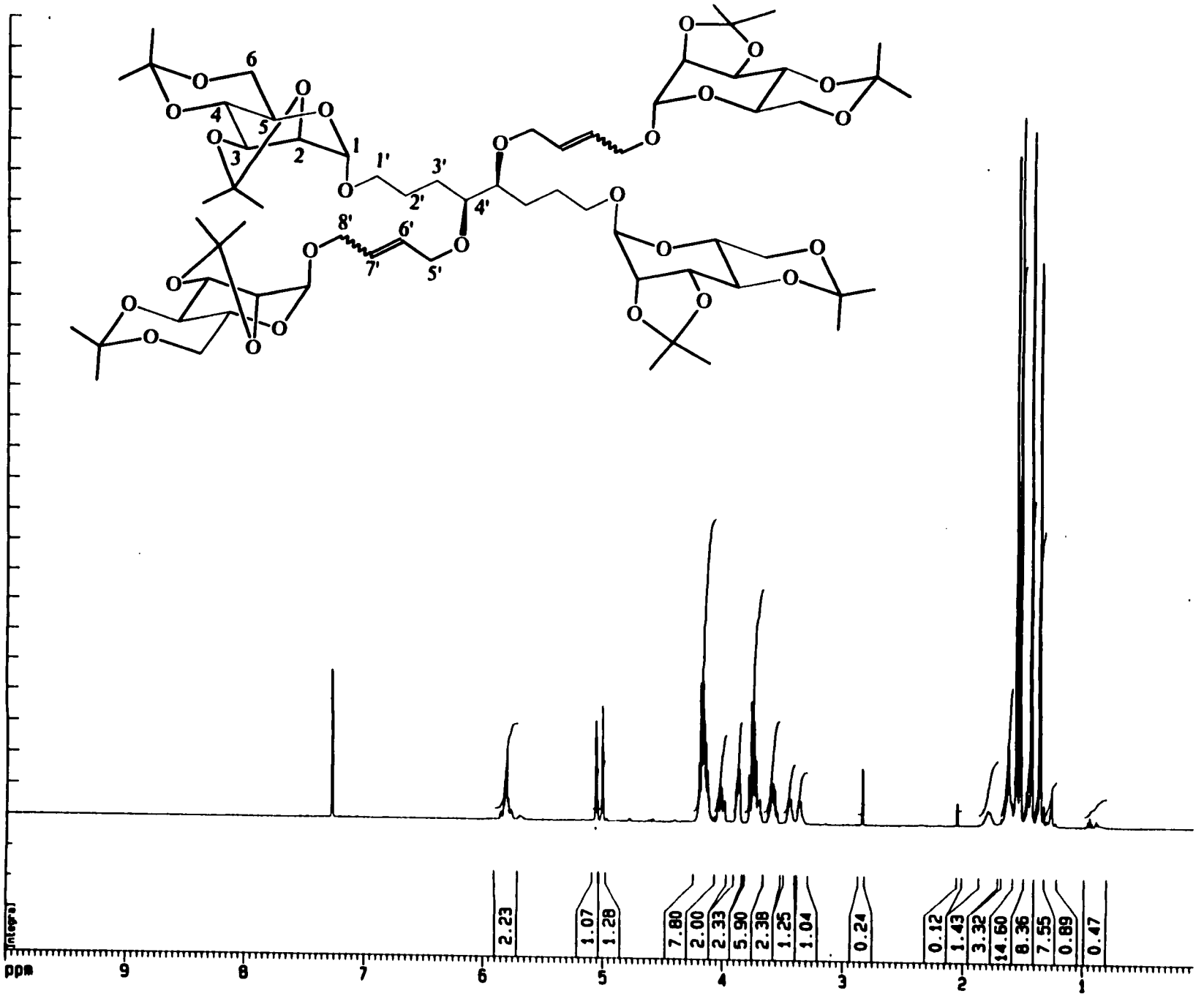
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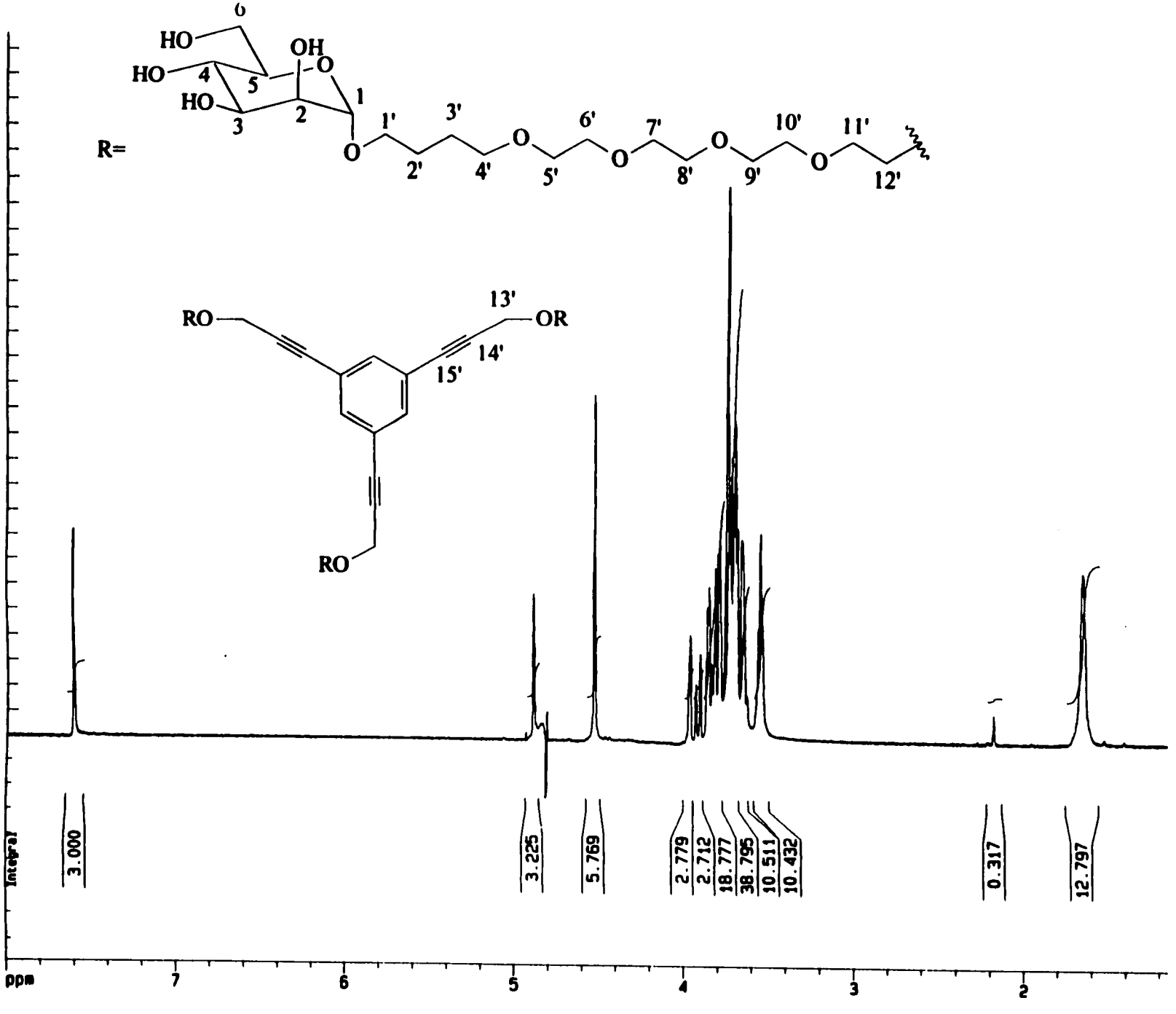
3
2
1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10'
11'
12'
RO
OR
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15'
Integral
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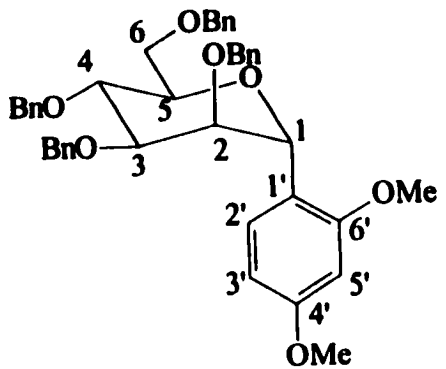
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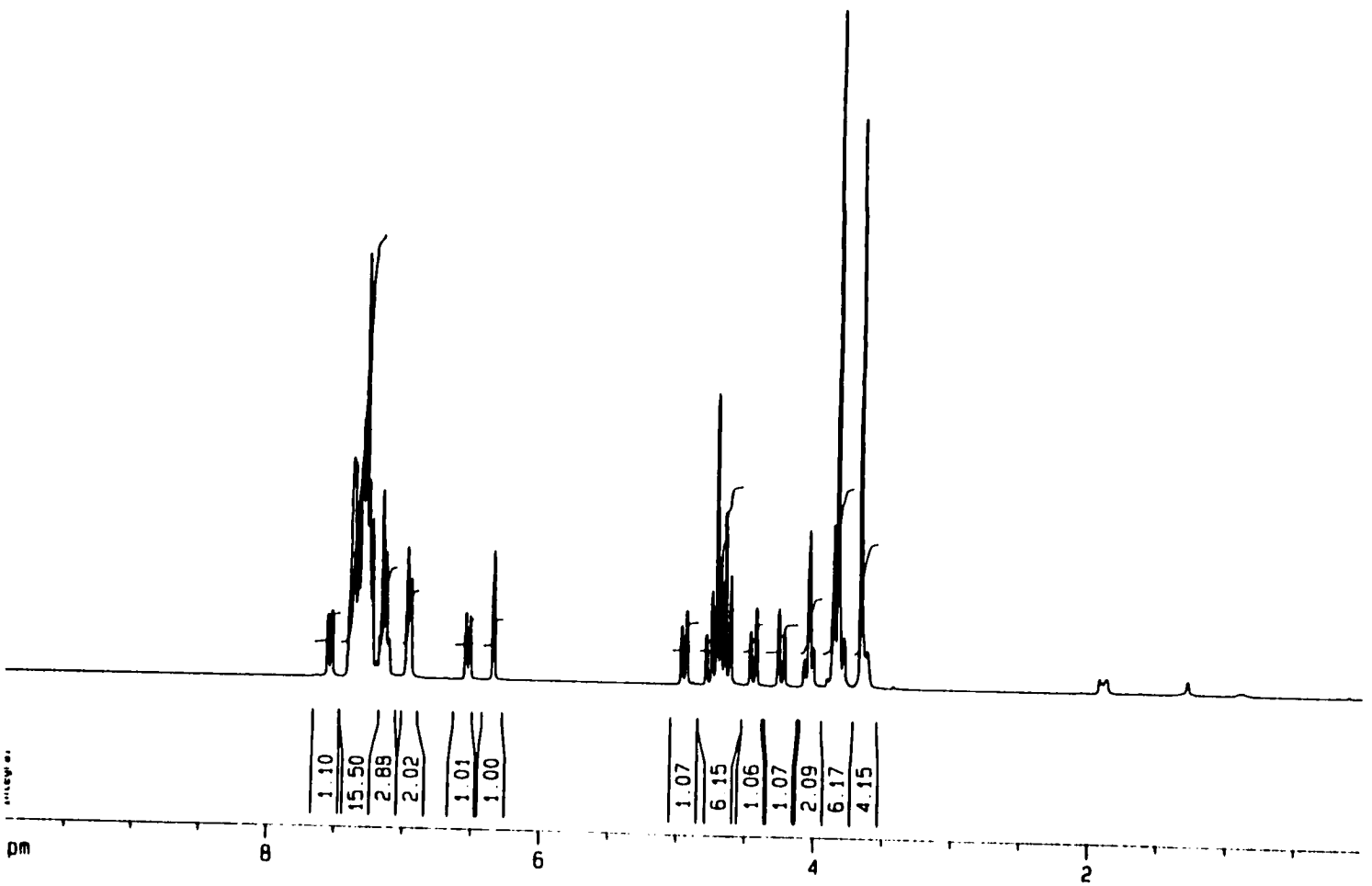
4'
OMe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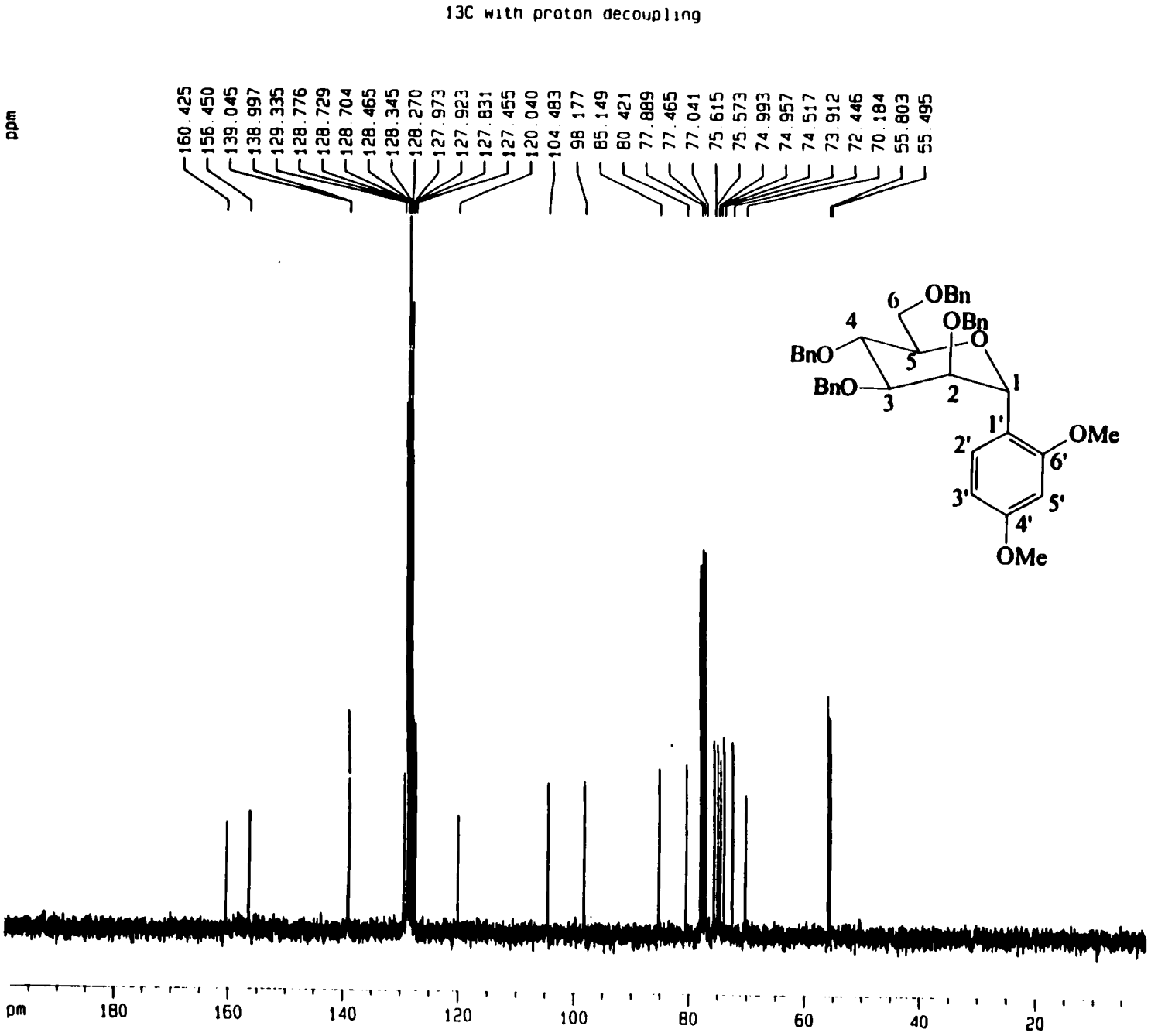
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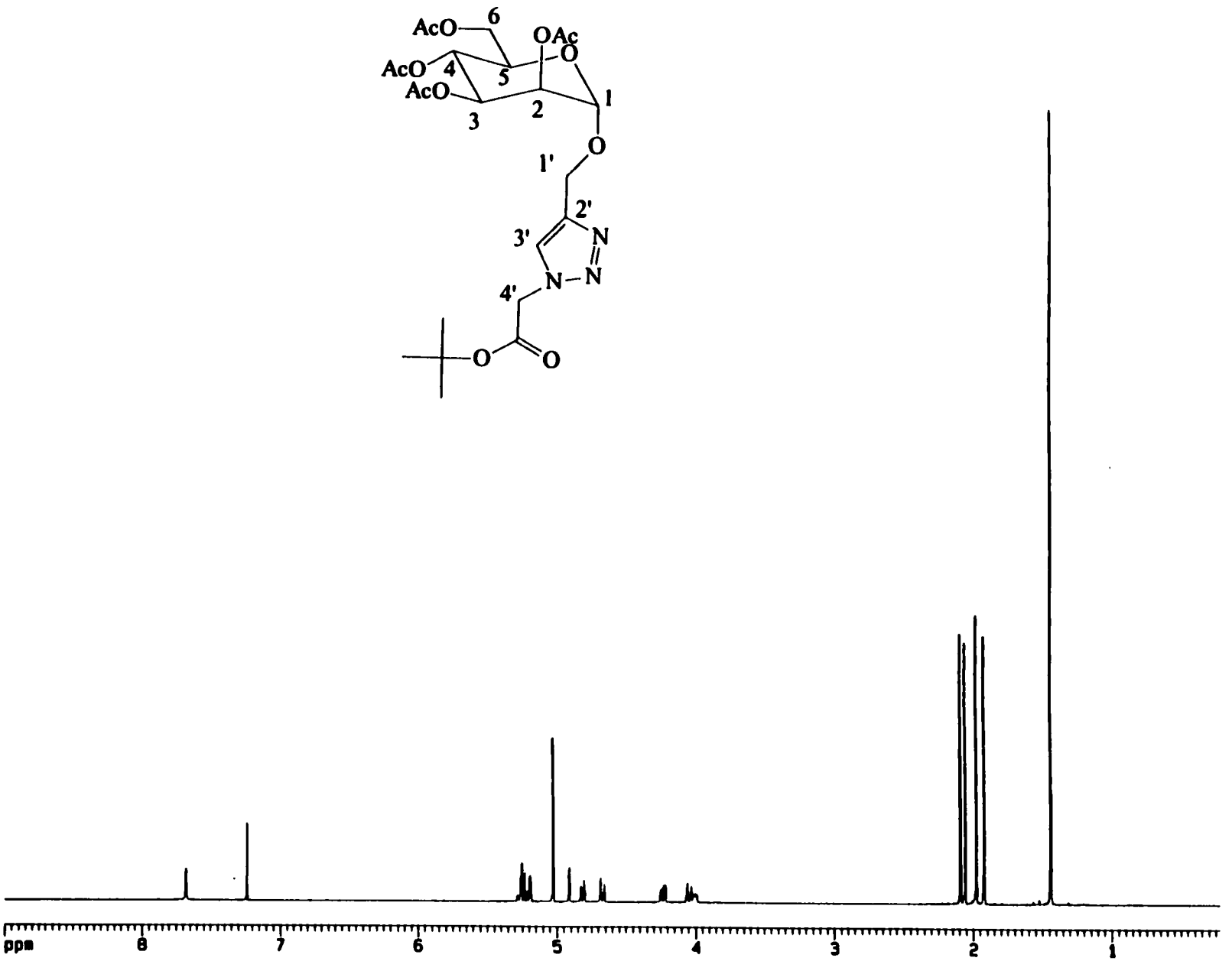
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O
O
ppm
8
7
6
5
4
3
2
1

Structural determination of **157.**

A suitable crystal was selected, mounted on a thin, glass fiber using paraffin oil and cooled to the data collection temperature. Data were collected on a Bruker AXS SMART 1k CCD diffractometer using 0.3° ω-scans at 0, 90, and 180° in ϕ. Initial unit-cell parameters were determined from 60 data frames collected at different sections of the Ewald sphere. Semi-empirical absorption corrections based on equivalent reflections were applied (SADABS, Bruker AXS, Madison, WI, 2000).

The systematic absences in the data and unit-cell parameters were uniquely consistent with the reported space group. The structure was solved by direct methods, completed with difference Fourier syntheses and refined with full-matrix least-squares procedures based on F^2. The absolute structure parameter was ambiguous because of large experimental error and the absolute structure was assigned based on non-crystallographic information. All hydrogen atoms were treated as idealized contributions. All scattering factors are contained in the SHELXTL 6.12 program library (Sheldrick, G. M., Bruker AXS, Madison, WI, 2001).

Table 1. Crystal data and structure refinement for rr2002.

Identification code	rr2002
Empirical formula	C17 H23 N3 O10
Formula weight	429.38
Temperature	203(2) K
Wavelength	0.71073 A
Crystal system, space group	Orthorhombic, P2(1)2(1)2(1)
Unit cell dimensions	a = 8.476(2) A alpha = 90 deg. b = 9.530(3) A beta = 90 deg. c = 27.048(7) A gamma = 90 deg.
Volume	2184.8(10) A^3
Z, Calculated density	4, 1.305 Mg/m^3
Absorption coefficient	0.109 mm^-1
F(000)	904
Crystal size	0.16 x 0.10 x 0.08 mm
Theta range for data collection	1.51 to 28.89 deg.
Limiting indices	-11<=h<=11, -12<=k<=12, -35<=l<=36
Reflections collected / unique	18904 / 5242 [R(int) = 0.0480]
Completeness to theta = 28.89	93.4 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9914 and 0.9828
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	5242 / 0 / 271
Goodness-of-fit on F^2	1.016
Final R indices [I>2sigma(I)]	R1 = 0.0554, wR2 = 0.1157

R indices (all data)	R1 = 0.1002, wR2 = 0.1337
Absolute structure parameter	-0.2(13)
Largest diff. peak and hole	0.346 and -0.328 e.A^-3

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (A^2 x 10^3) for rr2002. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
C(1)	3571(3)	6765(3)	8734(1)	30(1)
C(2)	4392(3)	5466(3)	8949(1)	29(1)
C(3)	3544(3)	4089(3)	8822(1)	30(1)
C(4)	1759(3)	4199(3)	8873(1)	30(1)
C(5)	1149(3)	5527(3)	8622(1)	30(1)
C(6)	-603(3)	5753(3)	8702(1)	37(1)
C(7)	-1393(4)	6793(3)	7943(1)	40(1)
C(8)	-1876(4)	8142(4)	7694(1)	54(1)
C(9)	3742(4)	7834(3)	7469(1)	36(1)
C(10)	3576(3)	8201(3)	7953(1)	31(1)
C(11)	3311(4)	9579(3)	8213(1)	39(1)
C(12)	5471(4)	6275(4)	9714(1)	47(1)
C(13)	5186(5)	6317(4)	10258(1)	59(1)
C(14)	5556(4)	2507(3)	9104(1)	37(1)
C(15)	5912(4)	1413(3)	9488(1)	51(1)
C(16)	216(3)	2061(3)	8878(1)	38(1)
C(17)	-522(4)	1011(3)	8541(1)	43(1)
O(1)	-1160(2)	6966(2)	8434(1)	39(1)
O(2)	-1178(3)	5681(2)	7735(1)	57(1)
O(3)	4281(2)	5614(2)	9481(1)	36(1)
O(4)	6547(3)	6808(4)	9490(1)	87(1)
O(5)	1928(2)	6743(2)	8826(1)	31(1)
O(6)	4725(3)	10083(2)	8426(1)	47(1)
O(7)	4035(2)	2971(2)	9153(1)	36(1)
O(8)	6427(3)	2945(2)	8789(1)	47(1)
O(9)	1063(2)	3023(2)	8615(1)	36(1)
O(10)	85(3)	2122(3)	9318(1)	62(1)
N(1)	3874(3)	6980(2)	8204(1)	29(1)
N(2)	4187(3)	5905(2)	7888(1)	37(1)
N(3)	4114(3)	6435(3)	7438(1)	39(1)

Table 3. Bond lengths [A] and angles [deg] for rr2002.

C(1)-O(5)	1.415(3)
C(1)-N(1)	1.470(3)
C(1)-C(2)	1.535(4)
C(2)-O(3)	1.449(3)
C(2)-C(3)	1.535(4)
C(3)-O(7)	1.451(3)
C(3)-C(4)	1.523(4)
C(4)-O(9)	1.446(3)
C(4)-C(5)	1.527(4)
C(5)-O(5)	1.444(3)
C(5)-C(6)	1.517(4)
C(6)-O(1)	1.444(3)
C(7)-O(2)	1.214(4)
C(7)-O(1)	1.353(4)
C(7)-C(8)	1.508(4)
C(9)-C(10)	1.362(4)
C(9)-N(3)	1.372(4)
C(10)-N(1)	1.371(3)
C(10)-C(11)	1.507(4)
C(11)-O(6)	1.414(4)
C(12)-O(4)	1.207(4)
C(12)-O(3)	1.346(4)
C(12)-C(13)	1.491(4)
C(14)-O(8)	1.202(4)
C(14)-O(7)	1.369(4)
C(14)-C(15)	1.502(4)
C(16)-O(10)	1.197(4)
C(16)-O(9)	1.365(3)
C(16)-C(17)	1.492(4)
N(1)-N(2)	1.360(3)
N(2)-N(3)	1.321(3)

O(5)-C(1)-N(1)	110.3(2)
O(5)-C(1)-C(2)	111.5(2)
N(1)-C(1)-C(2)	113.7(2)
O(3)-C(2)-C(1)	105.6(2)
O(3)-C(2)-C(3)	106.0(2)
C(1)-C(2)-C(3)	113.1(2)
O(7)-C(3)-C(4)	106.2(2)
O(7)-C(3)-C(2)	110.8(2)
C(4)-C(3)-C(2)	112.7(2)
O(9)-C(4)-C(3)	107.9(2)
O(9)-C(4)-C(5)	106.8(2)
C(3)-C(4)-C(5)	110.6(2)
O(5)-C(5)-C(6)	106.1(2)
O(5)-C(5)-C(4)	109.9(2)
C(6)-C(5)-C(4)	112.6(2)
O(1)-C(6)-C(5)	111.2(2)
O(2)-C(7)-O(1)	122.6(3)
O(2)-C(7)-C(8)	125.4(3)
O(1)-C(7)-C(8)	111.9(3)
C(10)-C(9)-N(3)	109.4(2)

C(9)-C(10)-N(1)	103.8(2)
C(9)-C(10)-C(11)	133.5(3)
N(1)-C(10)-C(11)	122.4(2)
O(6)-C(11)-C(10)	111.1(2)
O(4)-C(12)-O(3)	121.8(3)
O(4)-C(12)-C(13)	127.3(3)
O(3)-C(12)-C(13)	110.8(3)
O(8)-C(14)-O(7)	122.3(3)
O(8)-C(14)-C(15)	127.4(3)
O(7)-C(14)-C(15)	110.3(3)
O(10)-C(16)-O(9)	122.4(3)
O(10)-C(16)-C(17)	127.0(3)
O(9)-C(16)-C(17)	110.6(2)
C(7)-O(1)-C(6)	116.3(2)
C(12)-O(3)-C(2)	117.6(2)
C(1)-O(5)-C(5)	113.2(2)
C(14)-O(7)-C(3)	116.6(2)
C(16)-O(9)-C(4)	118.8(2)
N(2)-N(1)-C(10)	111.4(2)
N(2)-N(1)-C(1)	122.8(2)
C(10)-N(1)-C(1)	124.7(2)
N(3)-N(2)-N(1)	106.4(2)
N(2)-N(3)-C(9)	109.0(2)

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters (A^2 x 10^3) for rr2002.
The anisotropic displacement factor exponent takes the form:
-2 pi^2 [h^2 a*^2 U11 + ... + 2 h k a* b* U12]

	U11	U22	U33	U23	U13	U12
C(1)	30(2)	35(2)	25(1)	-4(1)	2(1)	-3(1)
C(2)	30(1)	36(1)	19(1)	-1(1)	1(1)	-1(1)
C(3)	35(2)	32(1)	22(1)	1(1)	3(1)	3(1)
C(4)	35(2)	32(2)	22(1)	-1(1)	1(1)	-2(1)
C(5)	32(1)	33(1)	24(1)	-1(1)	0(1)	-2(1)
C(6)	35(2)	44(2)	32(2)	2(1)	3(1)	5(1)
C(7)	32(2)	44(2)	44(2)	0(2)	-8(1)	1(1)
C(8)	56(2)	49(2)	56(2)	3(2)	-19(2)	8(2)
C(9)	45(2)	36(2)	28(1)	8(1)	-1(1)	0(1)
C(10)	26(1)	35(2)	31(1)	8(1)	-1(1)	1(1)
C(11)	45(2)	37(2)	36(2)	5(1)	8(1)	11(1)
C(12)	45(2)	57(2)	39(2)	-11(2)	-9(2)	-5(2)
C(13)	78(3)	66(2)	34(2)	-13(2)	-15(2)	-4(2)
C(14)	41(2)	37(2)	32(2)	-4(1)	-6(1)	7(1)
C(15)	57(2)	52(2)	43(2)	7(2)	-5(2)	18(2)
C(16)	36(2)	34(2)	43(2)	2(1)	-2(1)	-2(1)
C(17)	39(2)	36(2)	54(2)	-5(1)	0(1)	-2(1)
O(1)	38(1)	40(1)	38(1)	-2(1)	-2(1)	8(1)
O(2)	75(2)	47(1)	49(1)	-14(1)	-22(1)	9(1)
O(3)	39(1)	45(1)	22(1)	0(1)	-3(1)	-7(1)
O(4)	64(2)	129(3)	67(2)	-35(2)	9(2)	-45(2)

O(5)	30(1)	34(1)	29(1)	-5(1)	4(1)	3(1)
O(6)	71(2)	33(1)	35(1)	-6(1)	-6(1)	-4(1)
O(7)	41(1)	35(1)	32(1)	6(1)	1(1)	6(1)
O(8)	43(1)	52(1)	46(1)	4(1)	9(1)	11(1)
O(9)	42(1)	34(1)	31(1)	-4(1)	-1(1)	-6(1)
O(10)	85(2)	64(2)	38(1)	4(1)	3(1)	-33(2)
N(1)	34(1)	27(1)	25(1)	-1(1)	4(1)	0(1)
N(2)	48(2)	34(1)	30(1)	-2(1)	7(1)	0(1)
N(3)	48(2)	42(1)	28(1)	2(1)	7(1)	-2(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (A^2 x 10^3) for rr2002.

	x	y	z	U(eq)
H(1A)	4004	7592	8909	36
H(2A)	5509	5429	8843	34
H(3A)	3805	3821	8478	35
H(4A)	1450	4187	9226	36
H(5A)	1368	5474	8263	36
H(6A)	-1183	4920	8592	44
H(6B)	-808	5883	9056	44
H(8A)	-2031	7976	7344	80
H(8B)	-1056	8840	7740	80
H(8C)	-2852	8479	7839	80
H(9A)	3621	8444	7199	44
H(11A)	2909	10271	7976	47
H(11B)	2515	9456	8472	47
H(13A)	6054	6797	10419	89
H(13B)	5107	5367	10384	89
H(13C)	4210	6816	10324	89
H(15A)	6991	1093	9449	76
H(15B)	5198	625	9447	76
H(15C)	5776	1812	9815	76
H(17A)	-1119	337	8733	65
H(17B)	295	527	8356	65
H(17C)	-1224	1487	8312	65
H(6C)	4553	10841	8567	70

Table 6. Torsion angles [deg] for rr2002.

O(5)-C(1)-C(2)-O(3)	-67.9(3)
N(1)-C(1)-C(2)-O(3)	166.6(2)
O(5)-C(1)-C(2)-C(3)	47.5(3)
N(1)-C(1)-C(2)-C(3)	-78.0(3)
O(3)-C(2)-C(3)-O(7)	-46.4(3)
C(1)-C(2)-C(3)-O(7)	-161.7(2)
O(3)-C(2)-C(3)-C(4)	72.5(3)
C(1)-C(2)-C(3)-C(4)	-42.8(3)
O(7)-C(3)-C(4)-O(9)	-74.8(2)
C(2)-C(3)-C(4)-O(9)	163.7(2)
O(7)-C(3)-C(4)-C(5)	168.72(19)
C(2)-C(3)-C(4)-C(5)	47.2(3)
O(9)-C(4)-C(5)-O(5)	-173.8(2)
C(3)-C(4)-C(5)-O(5)	-56.7(3)
O(9)-C(4)-C(5)-C(6)	68.0(3)
C(3)-C(4)-C(5)-C(6)	-174.8(2)
O(5)-C(5)-C(6)-O(1)	63.4(3)
C(4)-C(5)-C(6)-O(1)	-176.3(2)
N(3)-C(9)-C(10)-N(1)	0.5(3)
N(3)-C(9)-C(10)-C(11)	174.2(3)
C(9)-C(10)-C(11)-O(6)	-102.2(4)
N(1)-C(10)-C(11)-O(6)	70.5(3)
O(2)-C(7)-O(1)-C(6)	1.3(4)
C(8)-C(7)-O(1)-C(6)	-176.5(3)
C(5)-C(6)-O(1)-C(7)	77.8(3)
O(4)-C(12)-O(3)-C(2)	4.7(5)
C(13)-C(12)-O(3)-C(2)	-179.1(3)
C(1)-C(2)-O(3)-C(12)	-91.1(3)
C(3)-C(2)-O(3)-C(12)	148.7(2)
N(1)-C(1)-O(5)-C(5)	68.3(3)
C(2)-C(1)-O(5)-C(5)	-59.1(3)
C(6)-C(5)-O(5)-C(1)	-173.7(2)
C(4)-C(5)-O(5)-C(1)	64.3(3)
O(8)-C(14)-O(7)-C(3)	-3.6(4)
C(15)-C(14)-O(7)-C(3)	176.9(2)
C(4)-C(3)-O(7)-C(14)	168.5(2)
C(2)-C(3)-O(7)-C(14)	-68.8(3)
O(10)-C(16)-O(9)-C(4)	-3.3(4)
C(17)-C(16)-O(9)-C(4)	174.8(2)
C(3)-C(4)-O(9)-C(16)	116.8(3)
C(5)-C(4)-O(9)-C(16)	-124.3(2)
C(9)-C(10)-N(1)-N(2)	-0.9(3)
C(11)-C(10)-N(1)-N(2)	-175.5(2)
C(9)-C(10)-N(1)-C(1)	-168.6(2)
C(11)-C(10)-N(1)-C(1)	16.8(4)
O(5)-C(1)-N(1)-N(2)	-97.6(3)
C(2)-C(1)-N(1)-N(2)	28.6(3)
O(5)-C(1)-N(1)-C(10)	68.8(3)
C(2)-C(1)-N(1)-C(10)	-165.0(2)
C(10)-N(1)-N(2)-N(3)	0.9(3)
C(1)-N(1)-N(2)-N(3)	168.9(2)

N(1)-N(2)-N(3)-C(9)	-0.6(3)
C(10)-C(9)-N(3)-N(2)	0.0(3)