Université de Montréal

Synthesis and Antitumor Activity of Aryl Glycosides (Glycomers)

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A été évalué par un jury composé des personnes suivantes :

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Dr. Stephen Hanessian	Directeur de recherche

To my Mother

my husband, Xiaochun

and my daughter, Nancy

&

In the memory of my father

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Abbreviations

$[\alpha]_D$	Specific rotation
Ac	Acetyl
Asn	Asparagine
Asp	Aspartic acid
BCA	Bicinchroninic acid
Bn	Benzyl
Bu	Butyl
Bz	Benzoyl
d	Doublet
δ	Chemical shift in ppm
DAPI	4',6'-diamidino-2-phenylindolyhydrochloride
DMAP	4-Dimethylaminopyridine
DMEM	Dubecco Modified Eagle Medium
DMF	N,N-Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
ECM	Extracellular matrix
ELISA	Enzyme linked ImmunoSorbent assay
Et	Ethyl
EtOAc	Ethyl acetate
Ether	Diethyl ether
eq	Equivalent

fdd	Fine doublet of doublets
FCS	Fetal calf serum
Gal	Galactose
GlcNAc	N-acetyl Glucose
h	Hour (s)
HCEC	Human brain-derived endothelial cells
Hex	Hexane
HRMS	High resolution mass spectrum
^{3}HT	Tritiated thymidine
Hz	Hertz
IC_{50}	Value calculated from dose-response curves as the concentration inducing 50% of growth inhibition
IR	Infrared spectroscopy
in vacuo	under vacuum
m	Multiplet
μ	Micro 10^{-6}
μL	Microliter
μM	Micromolar
Me	Methyl
mg	Milligram
min	Minute
ml	Milliliter
mmol	Millimole

mp	Melting point
MS	Mass spectrum
MTT	(3,4,5-dimethylthiazol-yl)-2,5-diphenyl tetrazolium
NMO	4-Methylmorpholine *N*-oxide
NMR	Nuclear magnetic resonance
Ph	Phenyl
Pr	Propyl
ppm	Parts per million
Pyr	Pyridine
s	Singlet
SD	Standard deviations
SDS	Sodium dodecyl sulfate
t	Triplet
TFA	Trifluoroacetic acid
m-TFMBSO$_2$Cl	*m*-(Trifluoromethyl)benzenesulfonyl chloride
Ser	Serine
SRIF	Somatostatin
THF	Tetrahydrofuran
Thr	Threonine
TLC	Thin layer chromatography

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Abstract

The object of this study was to synthesize a functionalized glycomer library with diversified substituents at five different positions in a pyranose ring, and with different anomeric configurations and different orientations at C-4. Through the systematic modification of each of the side chains, a series of compounds were synthesized and their effects on proliferation (^{3}H-thymidine incorporation), adhesiveness and survival (MTT reduction), and apoptosis (nucleosome-histone fragmentation) were evaluated in human glioblastoma cells in culture. The essential structural elements required for the antiproliferative activity toward glioblastoma cancer cells were identified, and biological results showed that these molecules represent interesting new potential agents to control glioblastoma progression.

Keywords : glioblastoma, DNA, apoptosis, arylglycoside

Résumé

Le sujet principal du travail de recherche présenté dans ce mémoire est la synthèse d'une banque de composés de la famille des glycomères. Différentes substitutions aux cinq positions fonctionalisables du cycle à six membres ont été effectuées. Aussi, l'effet de l'orientation de certains groupements a été étudié par la synthèse de composés dont l'orientation des groupements a été variée aux positions 1 et 4 du cycle à six membres.

La modification systématique de chacun des 5 substituants du cycle à six a permis de faire une étude de "structure-activité" (communément appelée S.A.R., acronyme anglais de Structure Activity Relationship).

L'étude s'est basée sur les effets de prolifération des tumeurs de type glioblastomique (insertion de ^{3}H-thymidine), l'adhésion et la survie de telles cellules (réduction de MTT), et leur apoptose (fragmentation des liens nucléosome-histone).

Les éléments structuraux essentiels à une activité antiprolifération contre les cellules cancéreuses gliobastomiques ont été identifiés et de bonnes activités biologiques rapportées par des tests de croissance de tumeurs ont révélé des propriétés intéressantes dans l'optique de développer des nouvelles méthodes de contrôle des tumeurs de type glioblastomique.

Mots clés : glioblastome, DNA, apoptose, arylglycoside

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Chapter One

Carbohydrates as drugs and potential therapeutics

1-1. Introduction

Carbohydrates are found throughout the body and are involved in many biological events but, unlike proteins and nucleic acids, they have remained relatively underutilized as a source of therapeutic agents. They have been long considered as merely a source of energy or integral parts of intracellular matrices, and the biological impact of this class of biopolymers is underestimated. In addition, the inherent complexity of oligosaccarides and the lack of adequate analytical and synthetic tools made the localization and functional characterization extremely difficult. Carbohydrate synthesis has been considered as involving mostly carbon-oxygen bond formation. However, in practice, synthesis with carbohydrates requires several steps, and purification and characterization can be tedious. These and other factors have hampered the widespread utilization of carbohydrates as drug candidates.

Carbohydrates play diverse and crucial roles in a wide range of biological processes, such as cell-growth regulation, intercellular recognition, cell adhesion, intracellular targeting, immunological response, cancer cell metastasis, inflammation, fertilization and viral infections.[1] Biomolecules containing carbohydrates, such as proteoglycans, glycolipids and glycoproteins, are found on all cell surfaces and they can interact with invading microorganisms, adjacent cells, various proteins and other molecules.[2] The saccharide portion in a glycoprotein is often as important as the protein itself, and glycosylation can have many effects on the function, structure, physical properties and targeting of a protein, for example.[3] The information-carrying potential of oligosaccharides is far greater than that of proteins and nucleic acids of equivalent molecular weight.[3] These unique properties can evoke new concepts and technologies in therapeutics of carbohydrates, and a new science known as glycobiology is currently a most dynamic field. Carbohydrates are now in the forefront of contemporary biological-medicinal research.

The enormous structural variability in oligosaccharides is not only caused by the sequence of sugar units, but also by their anomeric α or β-configuration and positions of inter-residue linkages (i.e. 2-OH, 3-OH, 4-OH or 6-OH of a hexopyranose for example). For instance, four different monosaccharides can be arranged to make 35,560 unique

tetrasaccharides, whereas four different amino acids can make only 24 different tetrapeptides with only a single linear linkage of amide bonds between proteins. In spite of the complexity of carbohydrates, progress in analytical, synthetic and enzymatic technologies[4] has been spectacular. X-ray crystallography, high quality mass spectra with electrospray technology, multidimensional NMR techniques and computer-aided modeling of oligosaccharides are all providing useful tools for the study of carbohydrate structures. The glycosylation methods and protective group strategies have been greatly improved recently in the synthesis of biologically active oligosaccharides. With respect to glycosylation methodology, the Koenigs-Knorr metal-mediated glycosylation has been supplemented with more efficient reactions,[5] such as those using trichloroacetimidate,[6] fluoride,[7] pentenyl[8], and 3-methoxy-2-pyridone (MOP)[9] activation. Hanessian and coworkers developed stereocontrolled glycosylation with unprotected glycosyl donors in which minimal or no hydroxyl protection is needed[10]. Wang and coworkers[11] developed a programmable method for the synthesis of the Globo H hexasaccharide using the anomeric reactivity-based one–pot strategy without the need for intermediate work-up and purification and tedious protecting group manipulation. Those recent technologies make the large-scale production of carbohydrates as drug candidates not as difficult as it was two decades ago.

The recent trends in the search for novel carbohydrate therapeutics have focused on the Structure-Activity Relationships (SARs)[12] and combinatorial chemistry[13] whereby "chemical libraries"[14] are prepared as potential sources of new leads for carbohydrate drug discovery.

1-2 Molecular features of glycoconjugates

The majority of carbohydrates present in cells are covalently attached to proteins or lipids forming the so-called glycoproteins and glycolipids, which are major components of the outer surface of mammalian cells[3].

1-2-1 Glycoproteins

Glycoproteins are very important in biological processes, such as fertilization,

immune defense, cell growth, cell-cell adhesion, degradation of blood clots, and inflammation. Proteins link with an oligosaccharide via the δ-amino group of asparagines (*N*-linked glycosides) or through an α-linkage to the side-chain of the hydroxyl group of serine or threonine (*O*-linked glycosides) (Figure 1).[3]

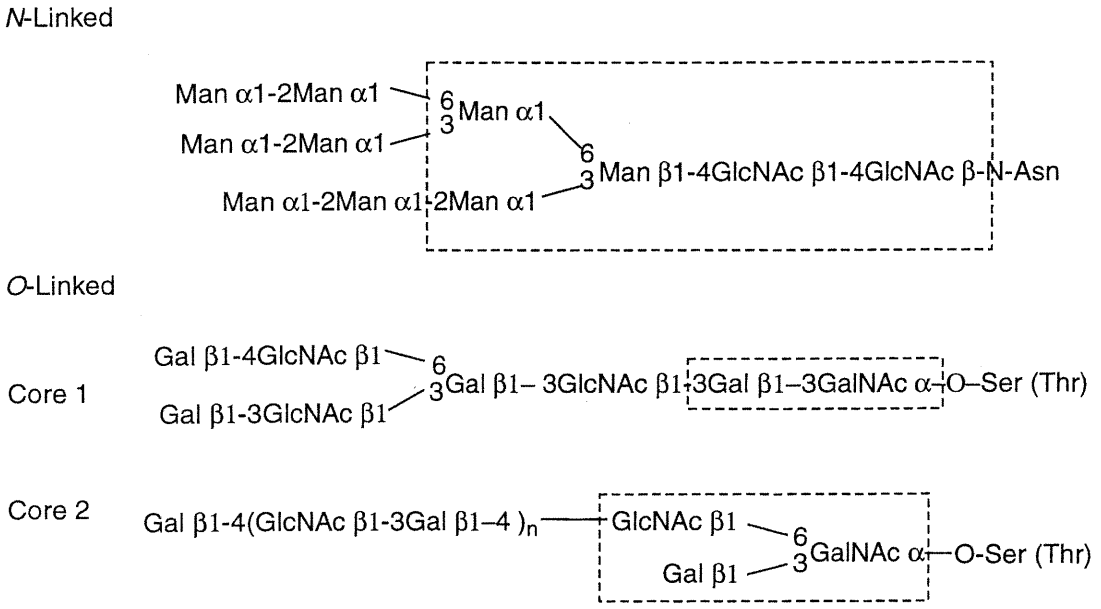


Figure 1 *N*-Linked and *O*-linked glycoproteins

Depending on the global and local protein conformation and the availability of the glycosylation-processing enzymes for the particular cell type, protein glycosylation is protein-specific, site-specific, and tissue/cell-specific.[3,15] Various pathological conditions are associated with altered glycosylations, which result in alterations in the cell surface oligosaacharides. Abnormal glycosylation is diagnostic of a number of disease states including rheumatoid arthritis and cancer.[16] The sugar chains, connected to proteins by glycosylation, occupy a large molecular volume in a glycoprotein, and are able to shield functionally important areas of proteins, thus, modulating the interactions of glycoconjugates with other molecules. They are involved in cell-cell adhesion and communication.[2b] They allow proper protein folding and stabilization of tertiary structure, and binding to carbohydrates may function as the onset of signal transduction.[2b] For example, metastasis is a process mediated by carbohydrate binding through cell

adhesion and detachment events (Figure 2).[17] These events may be modified by carbohydrate or carbohydrate-like molecules that mimic the sugars involved in the interaction, hence a therapeutic potential.[18]

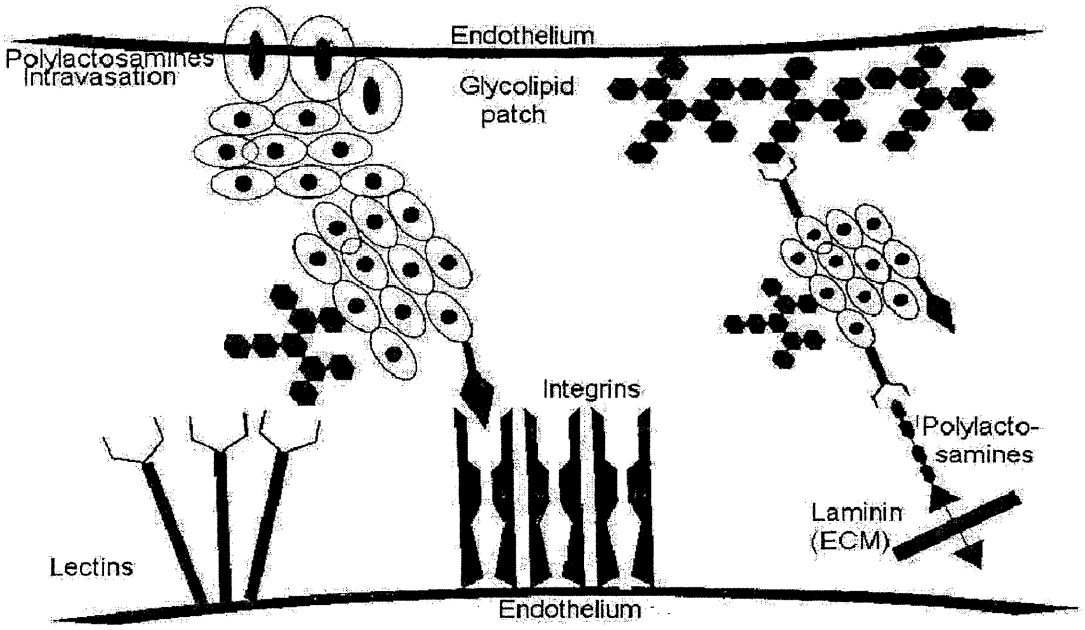


Figure 2 Selected carbohydrate-mediated binding interactions of tumor cells with other cells and the ECM (adapted from ref. 17a). *(Schematic of tumor cells during movement through the bloodstream and invasion of distant tissue. Sugar chains (sialyl-Lewis^x) at one cell surface may bind with lectins at another surface such as endothelial cells or other tumor cells. Recognition of peptide sequences by integrins (cell adhesion molecules) can strengthen these interactions, with which tumor clump together as emboli and interact with the endothelial cell surface causing intravasation. ECM (extracellular matrix) proteins such as laminin bind with lectins on tumor cells through polylactosamine chains and encourage the degradation of the matrix or dissemination and promote tumor cell invasion.)*

As many of these biological processes require the interaction of proteins with saccharides, an understanding of the features and factors associated with these interactions at a molecular level is of prime importance.

One of the important factors is H-bonding, which plays major roles such as providing stability, conferring specificity, and controlling dynamics[19]. X-ray crystallographic studies showed that hydrogen bonds, which are formed by the hydroxyl

groups and ring oxygen atoms in carbohydrates, are mainly responsible for specificity and affinity in protein-carbohydrate interactions.[19, 20] Because they are stable enough to significantly provide the requisite ligand-binding affinity (stability), but of sufficiently low strength to permit rapid ligand dissociation (dynamics), thereby, allowing active protein transport to take place. Compared to the non-directional Van der Waals forces, H-bonds are strong and highly directional, which contribute to specificity. Hydroxyl groups, the main moiety found in carbohydrates, are involved in H-bonding. Most likely, the carbohydrate recognition is through the complementary, convergent arrangement of hydrogen bond, and /or acceptor functionalities. An example of the hydrogen bonding in glucose binding protein as revealed by X-ray is shown in Figure 3.

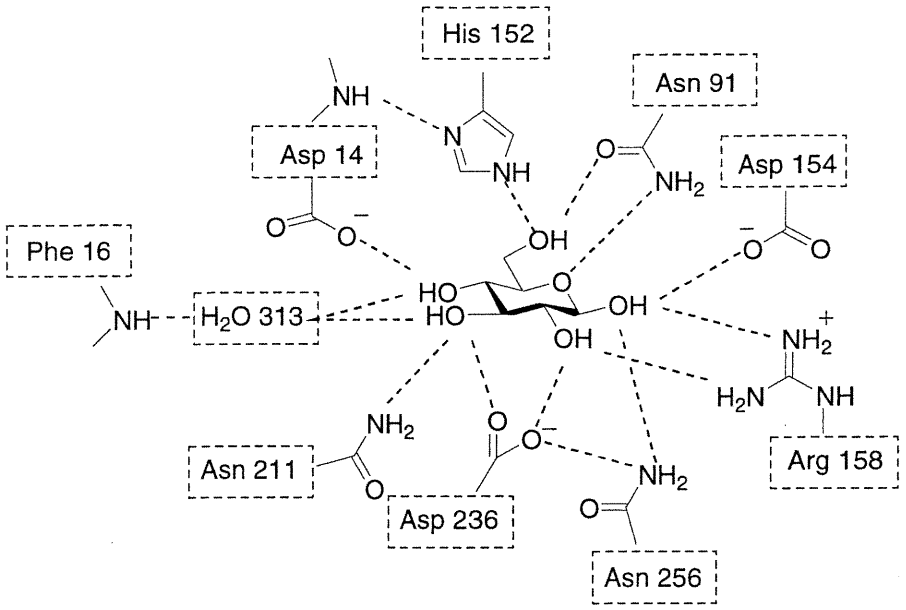


Figure 3 Schematic diagrams of hydrogen bonds in the complex of D-glucose-binding protein with D-glucose (adapted form ref. 20b)

Hydrophobic packing is another feature that contributes to protein-carbohydrate interactions.[19] The hydrophobic patch in a sugar is formed by the aliphatic methine protons and carbons that are part of the framework. The locations of the aromatic residues are consistent with the presence of hydrophobic patches especially in

pyranosides that can interact with aromatic residues in proteins and other hydrophobic regions such as aliphatic chains. For instance, D-glucose has a hydrophobic patch on the β-face[21] composed of C3, C5, and C6 and a minor patch on the α-face composed of C2 and C4. NMR studies[19] revealed that chemical shifts of specific protons in a sugar residue vary due to magnetic fields produced by aromatic ring currents. These protons are found near the aromatic rings in the crystal structure of lectin-sugar complexes. Aliphatic residues adjacent to hydrophobic patches of sugars could achieve similar effects as the aromatic rings do.

Hydrogen-bonding and hydrophobic packing enhance Van der Waals forces, which is a significant feature of protein-carbohydrate interactions. They enable the polar residues and aromatic residues, respectively, to come within Van der Waals distances of the bound sugar substrates. In addition, recognition of charged groups on sugars and association with metals are also important features in carbohydrate-protein interactions.[20] For example, the carboxylate moiety of sialic acid is associated with the guanidino group of an Asp residue in influenza neuraminidase,[22] and the legume lectins using Ca^{2+} and Mn^{2+} to stabilize the binding site.[23]

There are several conditions that could weaken or obliterate the protein-carbohydrate interaction, such as fewer numbers of H-bonds and Van der Waals contacts. Water molecules that may compete with the sugar hydroxyls and be H-bonded to the bound saccharide. These factors are likely to decrease the affinity of a protein-carbohydrate interaction.

Unlike many protein-protein interactions, the inherent low affinity of carbohydrate ligands and protein receptors is a potential problem in developing effective drugs based on carbohydrates. The study of lectins,[24] which are responsible for cell-surface sugar recognition in bacteria, animals, and plants, showed that lectins typically exist *in vivo* as oligomeric species rather than monomeric structures. This phenomenon allows Nature to take advantage of the multiple and simultaneous molecular contacts to overcome weak individual interactions. Polyvalent interactions can be collectively much stronger than corresponding monovalent interactions, and they can provide a number of characteristics that the monovalent interaction do not. This suggests a concept of multivalency,[25] in that

carbohydrate-binding proteins and their ligands at the cell surface are clustered together. Accordingly, the affinity is increased by sometimes as much as several orders of magnitude. Multivalency has been applied with some success to drug development by the synthesis of hub-and spoke systems (STARFISH/finger systems)[26a,b], dendrimers[26c,d], and polymers[25a]. Lee[25b] *et al* developed a theoretical model for calculating binding enhencement due to multivelency.

1-2-2 Glycolipids

The glycolipids found on the cell surface also play very important roles in cell-cell interactions and in the immune response to tumor cells[27]. The most prevalent glycolipids in mammalian tissues are the glycosphingolipids (GSLs), which are characterized by an oligosaccharide chain O-linked to the 1-position hydroxyl of ceramide, a lipid with two long hydrophobic chains that anchor the GSL to the cell membrane. GSLs containing one sialic acid residue are classified as gangliosides, which are ubiquitous in the plasma membranes of mammalian cells, but are found in much higher concentration in neural tissues. They are responsible for promoting neural repair, neutrophil cell adhesion, and pathogen receptors.

1-3 Oligosaccharides as drugs

With the increasing knowledge about the key roles carbohydrates play in the body, medicinal chemists have turned their attention to carbohydrates as potential drug candidates. Some of the approaches involve the chemical modification of existing compounds such as aminoglycosides, natural carbohydrates (sialic acid, etc.), mimetics of the parent carbohydrate whose biological function has been known, and screening carbohydrate derivatives against various biological targets.

1-3-1 Natural products as drugs

The earliest reported use of carbohydrate-containing drugs can be traced back to 1600 BC, when the squill bulb was found to be a cardiotonic glycoside in ancient Egyptian medicinal prescriptions.[1c] In 1785 Withering reported on the use of foxglove as a cardiotonic glycoside, and in 1869 Nativelle purified its different components,

particularly digoxin.[1c]

Heparin, which was found accidentally in 1916 by a medical student, J. McLean, at Johns Hopkins in Baltimore, has been used in clinic since 1937 in the prevention and treatment of venous thrombosis (the clotting of blood in veins). Although commercial heparin is of animal origin, it has been clearly established that it is also present in humans. Chemically, heparin is a glycosaminoglycan consisting of repeating disaccharide units, all of which contain D-glucosamine and a uronic acid, either as D-glucuronic acid or L-iduronic acid. Heparin has attracted considerable attention because of its anticoagulant properties,[28] which are mediated by the serine protease inhibitor antithrombin Ⅲ (AT Ⅲ), a 432-amino-acid protein that is an endogenous inhibitor of blood coagulation. Binding of heparin to AT Ⅲ induces a conformational change of the protein which considerably affects the rate of inhibition of some blood coagulation

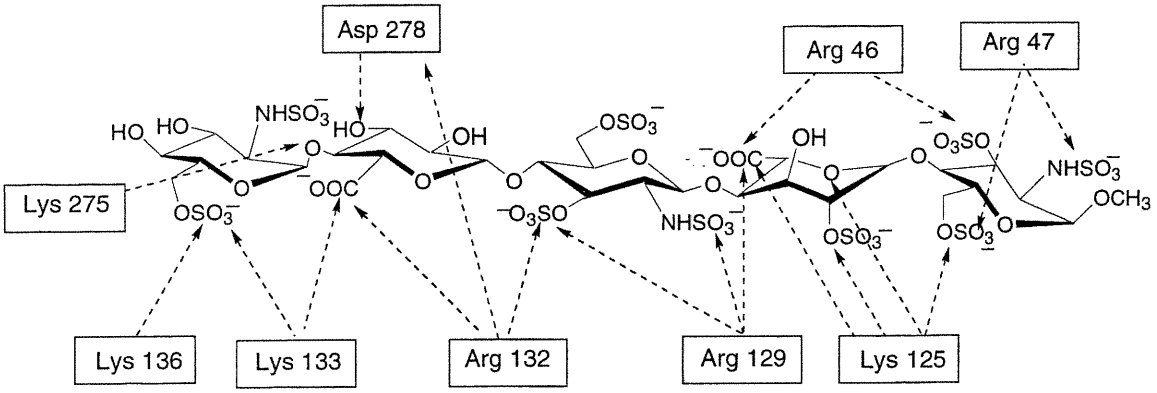


Figure 4 Schematic diagram of the hydrogen-bonding network in an energy-minimized model of the complex of AT Ⅲ and pentasaccharide (adapted from ref.28b)

factors, particularly thrombin and factor Xa. It was discovered that a specific pentasaccharide sequence of 6-SO_3-GlcNAc α4 GlcA β4 3,6-$(SO_3)_2$-GlcNSO_3 α4 2-SO_3-IdoA α4 6-SO_3-GlcNSO_3 in heparin is responsible for binding to AT Ⅲ (Figure 4).[28b] This low molecular weight heparin (LMWH) binds to AT Ⅲ and induces specific inhibition of blood coagulation factor Xa, but it has little activity against thrombin. The biological properties of heparin are modified not only by the size of the molecule but also by the position of sulfation and the stereochemistry of the carboxylate group. SARs

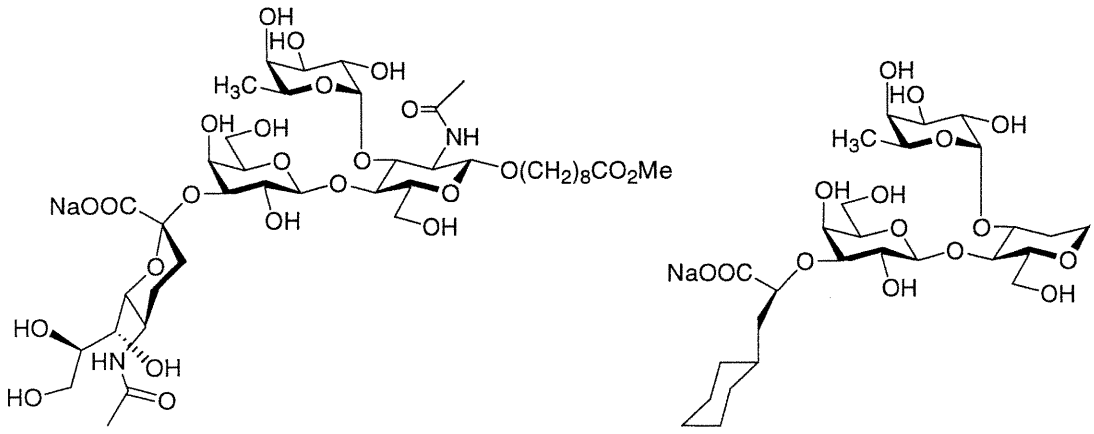
studies[29] indicated that, high-affinity binding is particularly dependent on the arrangement of *O*-sulfate groups. Removal of either the non-reducing terminal sulfate (from GluNAc) or the 3-sulfate on the central residue in the pentasaccharide reduces AT III-binding affinity ~1000-fold. Numerous analogs of this lead pentasaccharide have been prepared and are currently being evaluated for their antithrombotic effect.[30]

1-3-2 Carbohydrate mimetics

Considerable interest has been focused on carbohydrate mimetics,[18, 31] that is, small molecules that contain the essential functional groups (often with additional hydrophobic or charged groups) to resemble the active conformation of the parent structures. This approach could overcome the fundamental concern of the low affinity of binding between protein-carbohydrate interactions. However, the glycosidic linkages are subject to degradation by digestive glycosidases or cleaved by receptors in the liver, which makes bioavailability a problem. Due to the similar oligosaccharide sequences on various glycoproteins associated with both normal and disease states, the use of the exact endogenous oligosaccharide ligand structure as inhibitors for a target receptor may lead to unwanted side-effects. Thus, the goals of the mimetics are to increase the affinity, bioavailability, functional selectivity, have a reasonable lifetime, and be inexpensive to make. The most recent mimetic designs are developed through the application of both mechanism-based and structure-based approaches to rational drug design.[18]

Sialyl Lewis^x, which is a terminal tetrasaccharide of glycolipids displayed on the surface of certain cancer cells as well as that of white blood cells, was recently found to play an important role in inflammatory response,[31] such as rheumatoid arthritis, asthma, and in cancer cell metastasis. It has been suggested that sialyl Lewis^x or a close analogue may be an excellent antimetastatic agent since early 1990's.[32] A large variety of sialyl Lewis ^{x} analogues have been designed and synthesized [4b, 18] based on the known NMR structure of the tetrasaccharide bound to E-[31] and P-selectins,[33] the crystal structure of the lectin and EGF-like domains of E-selectin, and the knowledge of the important structural and functional groups for selectin binding. For E-selectin recognition, three hydroxyl groups on fucose, 4- and 6-OH groups of galactose and the carboxylate moiety of sialic acid are all necessary. GlcNAc appears to contribute no groups explicitly necessary for

binding, and has been replaced with a variety of bifunctional linkers. Replacement of the sugars with aromatic or other hydrophobic groups (in hope of improving the hydrophobic interactions) has resulted in compounds with higher affinity for E-selectin than sialyl



Sialyl Lewis
IC_{50}(E-Sel) =1mM

Mimetic
IC_{50}(E-Sel) = 36 μM

Figure 5 Sialyl Lewis^x and its mimetic

Lewis^x.[18] Among all the carbohydrate-type mimetics that have been made, the one that has the best affinity for E-selectin, with 30-fold improved activity compared to Sialyl Lewis^x, contains D-galactose and L-fucose to provide all the essential hydroxyl groups required for E-selectin binding.[34] Neuraminic acid and *N*-Acetyl-D-glucose units were replaced by *S*-cyclohexyllactic acid and 1,5-anhydro-1,2-dideoxy-D-glucitol, respectively (Figure 5). The bioactive conformation of this mimetic has been studied by NMR and molecular modeling studies.[34c] The design and synthesis of the mimetics is an ongoing area of research. Hanessian and coworkers have contributed to the synthetic strategies in devising a total synthesis of the Novartis mimetic utilizing MOP glycoside technology.[35]

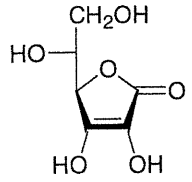
1-4 Monosaccharides as drug candidates

Monosaccharides encompass high structural diversity with various types of biological activity such as antiflammatory, antidiabetic, anticonvulsant, antibiotic, as well

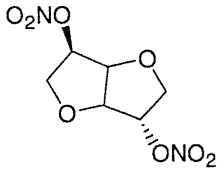
as antiviral. The most important leads have been of natural origin.

1-4-1 Miscellaneous structures

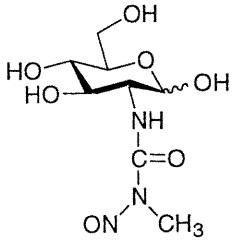
Perhaps the most recognizable monosaccharide for human use is Ascorbic acid, the so-called vitamin C, whose biological role is well-known and defined.[36] Isosorbide dinitrite (Varscardin) is a representative of polyhydroxy alcohols, and its esters are often used in the treatment of angina pectoris attacks. Two examples of the important



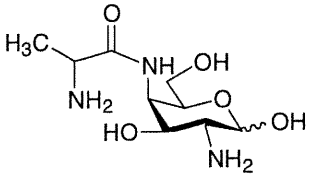
Ascorbic Acid
(Vitamin C)



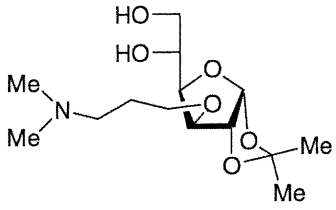
isosorbide Dinitrate
(Vascardin)



Streptozotocin



Prumycin



Therafectin

Figure 6 Derivatives of monosaccharides

aminosugars are Streptozotocin and Prumycin.[1d] Streptozotocin, a derivative of glucosamine, is clinically used only against malignant cancers, such as insulinomas and Hodgkins' disease, owing to its specific toxicity to the B-cells of the islets of Langerhans. Prumycin, a derivative of galactosamine, is less active than Streptozotocin in treatment of cancer. Another therapeutic drug for treatment of rheumatoid arthritis is Therafectin[1d] (Figure 6).

Monosaccharides containing a guanidine moiety are also of biological importance. For example, the biguanides in Figure 7 are potential antidiabetic drugs.[1d] They contain a

glucose scaffold with a C-6 biguanidine group and the hydroxyls are substituted with alkyl moieties.

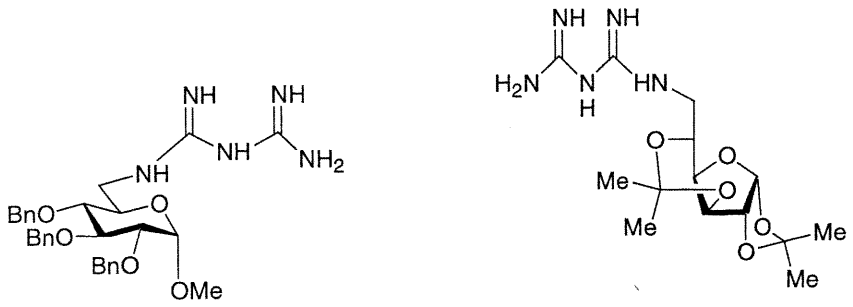
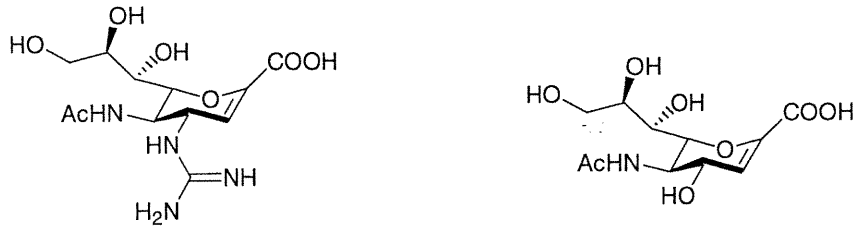


Figure 7 Monosaccharides containing a biguanide functionality

The sialic acid analogue, 2-deoxy-2, 3-didehydro-*N*-acetylneuraminic acid (DANA) has been known as a potent and selective inhibitor of influenza neuraminidase for many



Zanamivir DANA

Figure 8 Sialic acid analogues

years.[37] Recently, a sialic acid analogue, 2,4-dideoxy-2,3-didehydro-4-guanidino-*N*-acetylneuraminic acid (Zanamivir), has been approved by the FDA for treatment of influenza A and B viruses (Figure 8).[38]

To improve the stability of the saccharides, the *C*-linked and *S*-linked saccharides[39] are employed to replace the customary *O*-linked counterparts. For example, the *C*-glycoside PP-55B is an excellent inhibitor of the mammalian Glc-P-Dol synthase, a membrane-associated glycosyltransferase.[40] Auranofin, a *S*-glycoside, is used clinically for the treatment of chronic rheumatoid conditions, such as rheumatoid arthritis.[1d] It is the first orally effective derivative of gold to be marketed. Lincomycin and Clindamycin are two important antibiotics in the lincosaminide family (Figure 9). [1d]

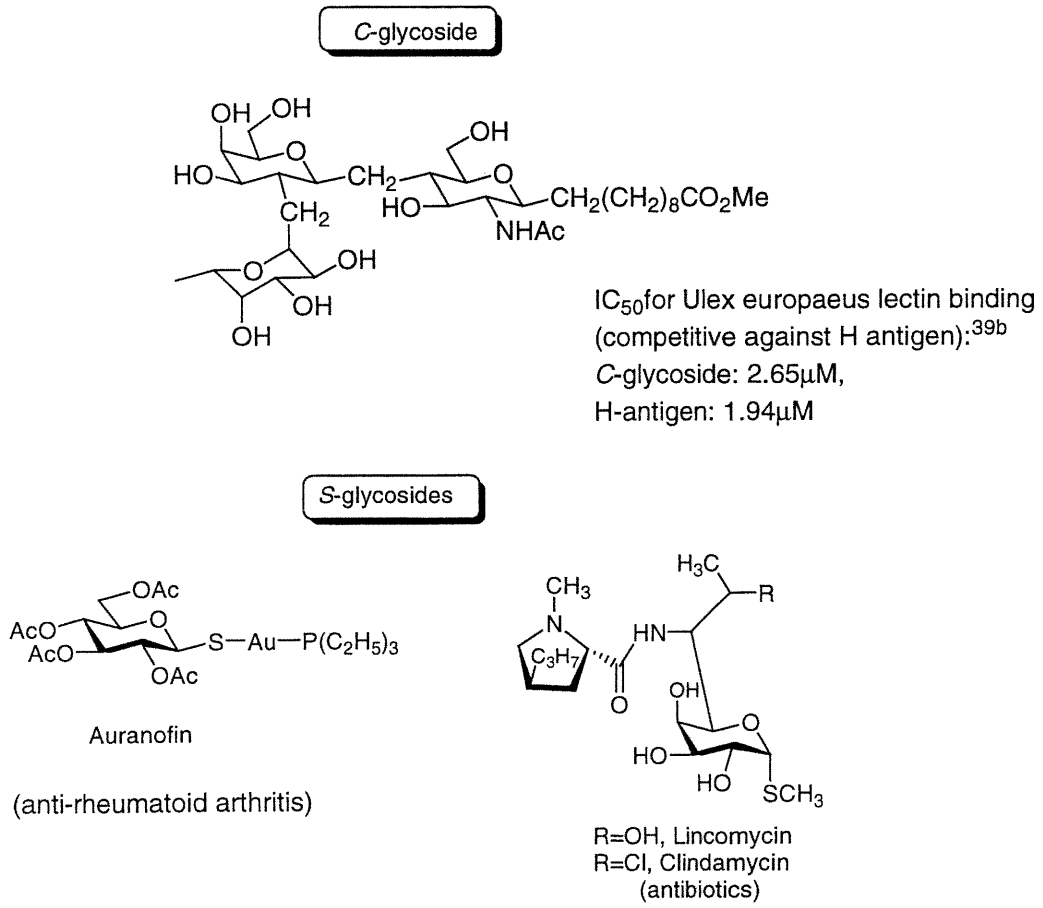


Figure 9 *C*- and *S*-Glycosides as drug candidates

1-4-2 Carbohydrates as scaffolds

A scaffold is defined as a core motif or structure upon which a series of potential pharmacophores can be appended to simulate the spatial topological and functional requirements for a bioactive conformation between a drug and biological receptor.

One of the original utilizations of a sugar as a scaffold was by Hanessian and coworkers.[41] They devised megacaloric nutrients that provide D-glucose and malonate as a result of hydrolysis by serum esterases. Although this strategy did not address a direct interaction with an active site, it nevertheless demonstrated the idea of sugar as spacers and clustered entities.

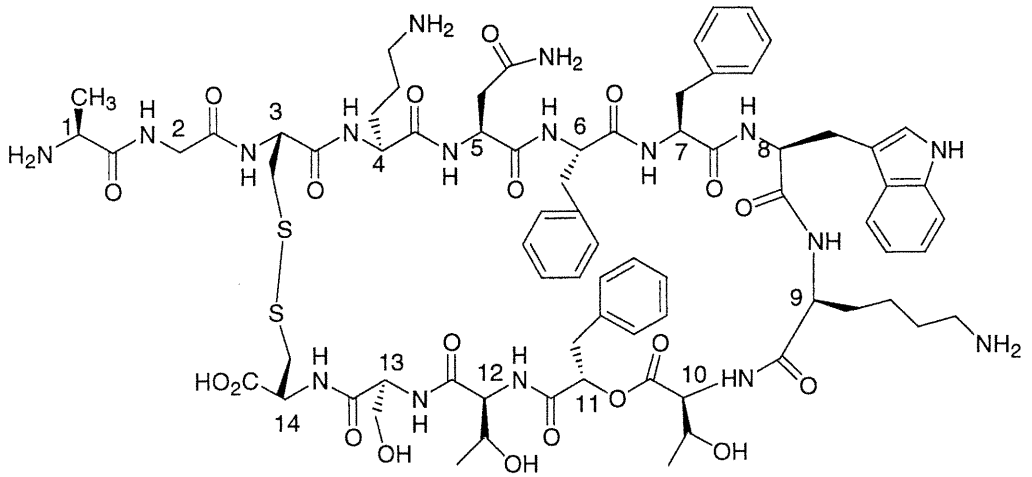
Recently, medicinal chemists have resolved to identify potential therapeutics

through a combinatorial approach.[13] This strategy leads to large chemical libraries by synthetic and repetitive covalent connection of a set of different "building blocks". Carbohydrates as scaffolds[42] have also been used to generate chemical libraries as potential leads for drug discovery. The basic protocol follows these steps 1) random screening in order to find a "hit" in the absence of any structure information; 2) once a hit is found, a more restricted library is constructed by chemical modification; 3) a lead compound is identified and SARs (Structure Activity Relationship) is done to optimize biological potency.

Aside from the potential therapeutic value of carbohydrates themselves, their inherent structural diversity renders carbohydrates as potential candidates as mimetics of other classes of compounds, such as peptides. Due to their poor bioavailability and the lability of amide bonds towards proteolytic enzymes, peptides are not considered ideal drug candidates.[43] Peptidomimetics, which contain a reduced number of amide bonds, can increase biological half-lives and have sometimes enhanced potency, but they can also have serious limitations. Their bioavailability remains a problem because of the presence of residual peptide bonds, and a given replacement of an amide bond at a certain position of a peptide may not be permissible at the corresponding position in another series. Thus, novel scaffolds devoid of formal peptide-like amide bonds have attracted an ever-increasing attention of medicinal chemists.

Hirschmann[44] *et al* employed β-D-glucose as novel non-peptide scaffold in the design of mimetics of the tetradecapeptide hormone Somatostatin (SRIF, Figure 10), which inhibits the release of several hormones, including the growth hormone (GH). SRIF contains a β-turn involving the tetrapeptide sequence Phe-Trp-Lys-Thr, wherein the same orientation of constrained side chains as the bioactive conformation of SRIF makes the tetrapeptide retain the ability to elicit SRIF-like biological effect. The well defined pyranose ring of glucose can potentially mimic the constrained tetrapeptide sequence and position the required side chains in an equatorial disposition around the ring, hence mimic SRIF-like topology. With the aid of molecular modeling and SARs studies of SRIF and its peptide mimetics,[13] Hirschmann and coworkers designed a lead compound **1** with the proposal that the substituents at C-2, C-1, and C-6 in the tribenzyl glycoside **1**

provide appropriately positioned replacements for the critical Phe-Trp-Lys side chains respectively, and that the benzyl group at C-4 be able to replace the hydrophobic region defined by Asn[5] and Thr[12] of SRIF and provide the favorable hydrophobic interaction with the SRIF receptor.



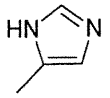
SRIF

Mimetics of SRIF:

1. R = Ph, R' = OBn

2. R = Imidazole, R' = H

imidazole =



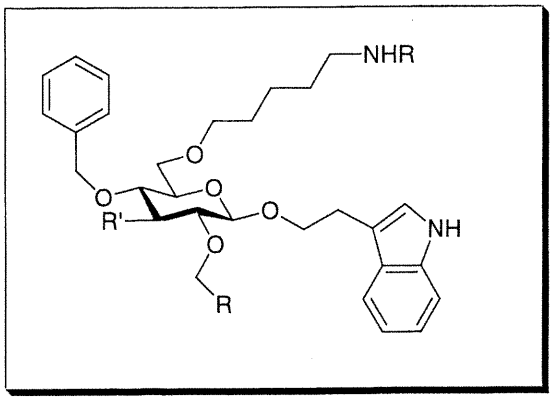


Figure 10 Non-peptide mimetics of Somatostatin agonist

Through the systematic deletion and modification of each of the side chains in β-D-glucopyranose, a series of compounds were synthesized and biologically screened. The best affinity to SRIF receptor using ^{125}I-Tyr-SRIF as the radioligand was compound **2** (1.9 μM) in this protocol.

Chapter Two

Functionalized glycomers as antitumor agents

2-1. Definition of glycomer

A glycomer is an analog of an original carbohydrate scaffold (ex. D-glucopyranose) in which every position is systematically modified to provide a set of functional groups at will.

2-2. Functionalized Glycomers

Hanessian and coworkers[45] designed and synthesized compounds (type A, B and C) shown in Figure 11 as mimetics of the YVNV tetrapeptide,[46] which is a ligand of high affinity toward Grb2-SH2. They deployed a series of aryl β-D-glucosides (glycomers) as non-peptidic scaffolds with diverse functionalities to probe their binding affinities (Figure 11). They employed the *p*- iodophenyl aglycone simply based on the presence of the

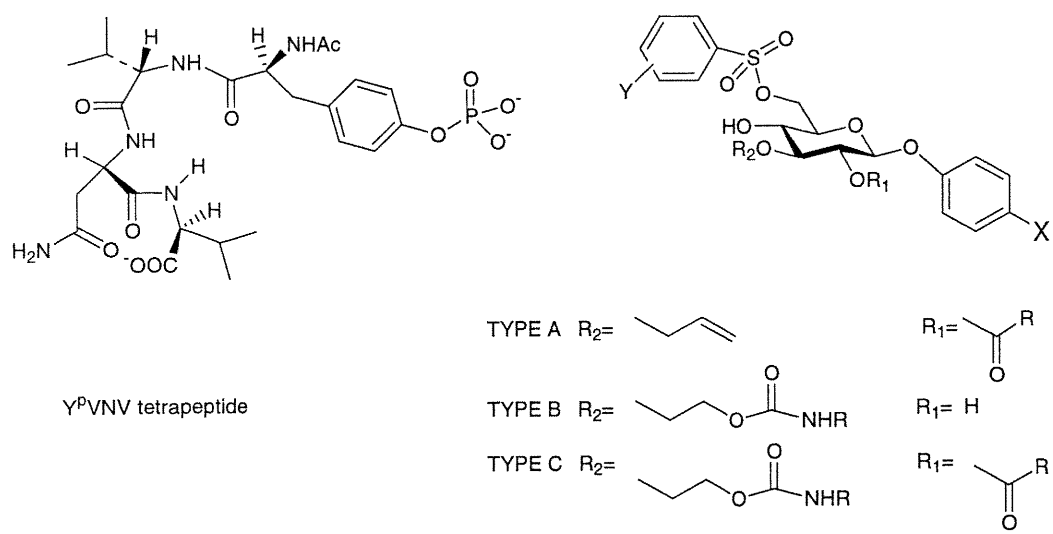
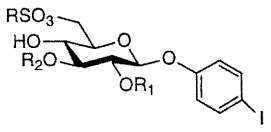
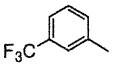
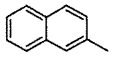
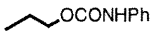
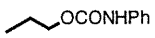


Figure 11 Y^{P}VNV and its mimetics

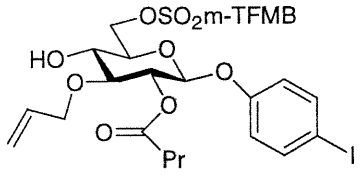
tyrosine unit in Y^{P}VNV and on the opportunities offered by cross-coupling reactions in subsequent transformations. The results for binding to Grb2-SH2 were disappointing. Fortunately, these glycosides were also tested for their cytotoxicity against A-431 human epidermoid and HT-29 colon carcinoma cell lines with encouraging results (Table 1).

Recently, we found that iodophenyl 3-allyl-2-butyryl-6-(*m*-trifluoromethyl)benzene sulfonyl-*β*-D-glucopyranose **3** (Figure 12), showed interesting antiproliferative activity

Table 1. Cytotoxicity of glycomers against A-431 and HT-29 cell lines[45]

Entry	Structure			A-431 (IC_{50} μM)	HT-29 (IC_{50} μM)
			 =mTFMB	 =Naph	
1	R=mTFMB	R_1=EtCO	R_2=allyl	2.17	4.65
2	R=mTFMB	R_1=PrCO	R_2=allyl	2.60	4.00
3	R=mTFMB	R_1=iPrCO	R_2=allyl	3.30	4.20
4	R=mTFMB	R_1=PhCO	R_2=allyl	7.00	7.50
5	R=mTFMB	R_1=$PhCH_2CO$	R_2=allyl	5.15	4.95
6	R=mTFMB	R_1=$Ph(CH_2)_2CO$	R_2=allyl	2.00	3.00
7	R=2-naphthyl	R_1=H	R_2= 	5.10	3.67
8	R=mTFMB	R_1=$Ph(CH_2)_2CO$	R_2= 	4.52	7.15

toward LN18 and LN308 glioblastoma cell lines. Based on this hit, we proceeded to prepare analogs (glycomers) in which each substituent was systematically varied in order



3

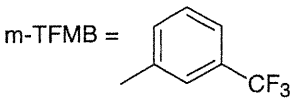
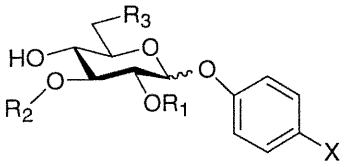


Figure 12 Lead compound from a glycomer library

to determine a minimum functional and stereochemical requirements for activity. We selected the D-glucopyranose and D-galactopyranose scaffolds and modified the substituents as shown in Figure 13. The *p*-substituent of anomeric aryl group was changed from H to Br, I, and OMe. To probe any orientational effect, we synthesized *p*-iodophenyl α-D-glucopyransides to explore the importance of anomeric configuration.

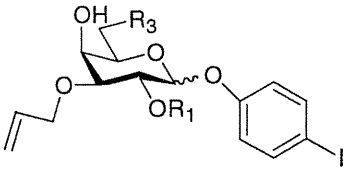
The relevance of the stereochemistry at C-4, if any, was achieved by exploiting D-galactopyranose as a scaffold (Figure 13). Variations at C-6 included arylsufonate esters, aromatic ethers, esters and sulfonamides. Our goal is to identify the essential structural elements required for the antiprolifertive activity toward the glioblastoma cancer cells with various structural modifications and to optimize the therapeutic index of these compounds. Since the original hit came from different cancer cell lines(A-431 and H-29), but fortunately revealed antitumor activity, we chose to maintain the structural type with its substituents and to systematically modify them rather than to initiate a new series. A major problem in this area is the lack of information for the rational modification of a lead compound. Thus, the challenge at hand was not only to uncover new antitumor activity against glioblastomas, but also to probe the mechanism of action.

glucose-based scaffold



X = H, Br, I, OCH_3
R_1=H, MeCO, EtCO, PrCO, i-PrCO
R_2=Allyl, Me, Pr
R_3=OSO_2m-TFMB,$NHSO_2$m-TFMB, OBn, OBz,

galactose-based scaffold



R_1=Pr, i-Pr
R_3=OSO_2m-TFMB, $NHSO_2$m-TFMB

Figure 13 Compounds with glucose- and galactose-based scaffolds

A feature of the glycomer library is that they all contain more than two functional groups such as sulfonate, sulfonamide, alcohol, ester, and an aromatic ring. We hope that the presence of these functional groups offer opportunities for hydrogen-bonding that are essential for specific drug interaction with its targets. Furthermore the aromatic ring, sulfonate, sulfonamide or benzyl ether, and allyl group may contribute to hydrophobic interactions. Although the molecular diversity in the glycomer library is far from being complete, the unique feature of the carbohydrate-based library with both regiochemical and stereochemical diversities presents an excellent way of building a SAR.

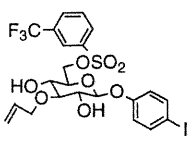
2-3. Glioblastoma

Human malignant gliomas, the most common of primary brain tumors, are among the most malignant and most intensely vascularized solid tumors. Glioblastoma multiforme (GBM) is the most malignant glioma characterized by rapid growth, intense angiogenesis, vascular malformations and poor survival rate. Notwithstanding the significant achievements in neuroimaging and neurosurgical techniques, the average survival time of a patient with GBM has rarely improved over the past 50 years and average about 12-18 months.[47] The recurrence rate is virtually 100% even with aggressive surgical treatment. Glioblastomas are brain cancers with a very poor prognosis, mainly due to two reasons in addition to their proliferative potential. First, glioblastoma tumor cells as well as the cerebral vasculature forming the blood-brain barrier, express multidrug resistance systems and are resistant to chemotherapeutic agents and to radiotherapy[48], precluding conventional treatments. Second, while glioblastomas rarely metastasize outside of the brain, they have a very high migratory potential in the central nervous system[49] precluding complete surgical resection. Thus the development of drugs able to control the growth of glioblastoma cells, without inducing drug resistance, would be of benefit to brain cancer treatment. Disaccharide or tetrasaccharide derivatives able to control glioblastoma cell growth may be efficient compounds of potential therapeutic interest[50]. However simpler functionalized glycoside derivatives may be advantageous in biological systems, displaying increased stability and potential to be transported through physiological barriers and cell membranes.

2-4 Glycomer library

We synthesized a library of functionalized glycomers as shown in the following pages, and obtained preliminary biological testing results toward glioblastoma LN18 and LNZ 308 cancer cells with very promising results.

Library of Glycomers(1)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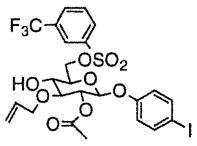
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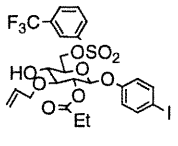
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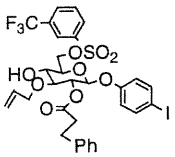
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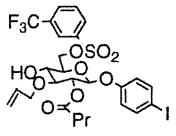
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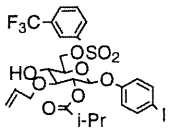
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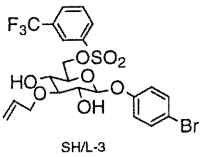
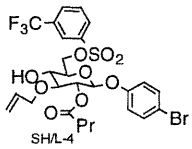

SH/L-3


SH/L-23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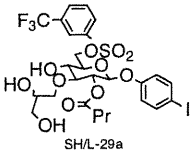
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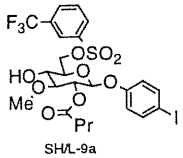
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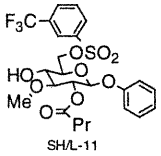
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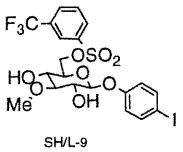
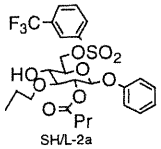

SH/L-9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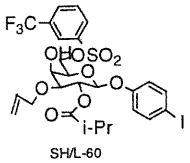
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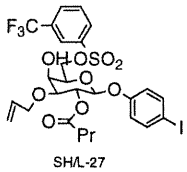
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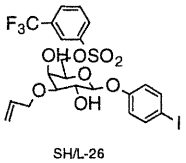
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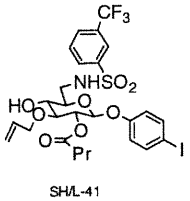
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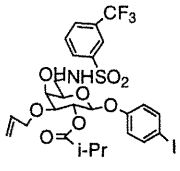
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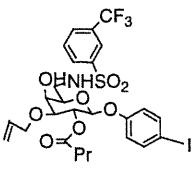
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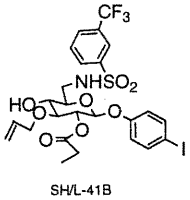
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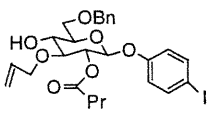
** The number of compounds is used for the biological test*

Library of Glycomers(2)



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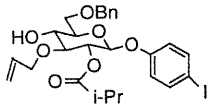

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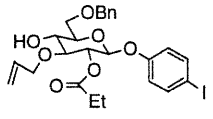
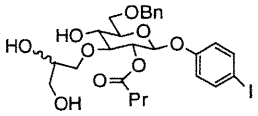
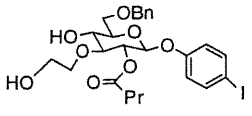


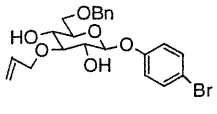
SH/L-002B



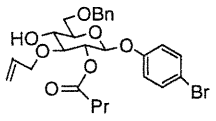
SH/L-29



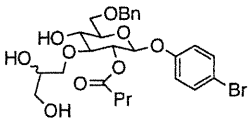
SH/L-30



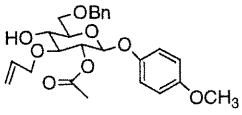
SH/L-5



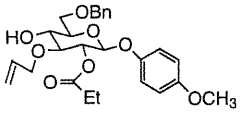
SH/L-6



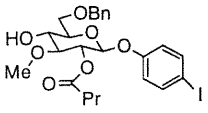
SH/L-6a



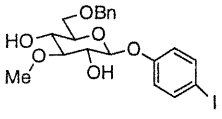
SH/L-71



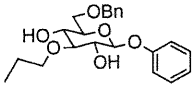
SH/L-70



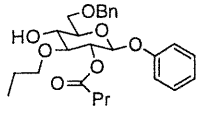
SH/L-8



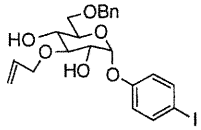
SH/L-7



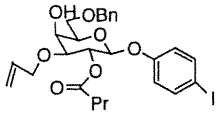
SH/L-31



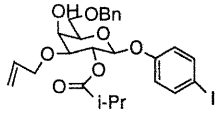
SH/L-31a



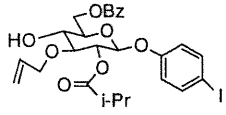
SH/L-20



SH/L-62



SH/L-63



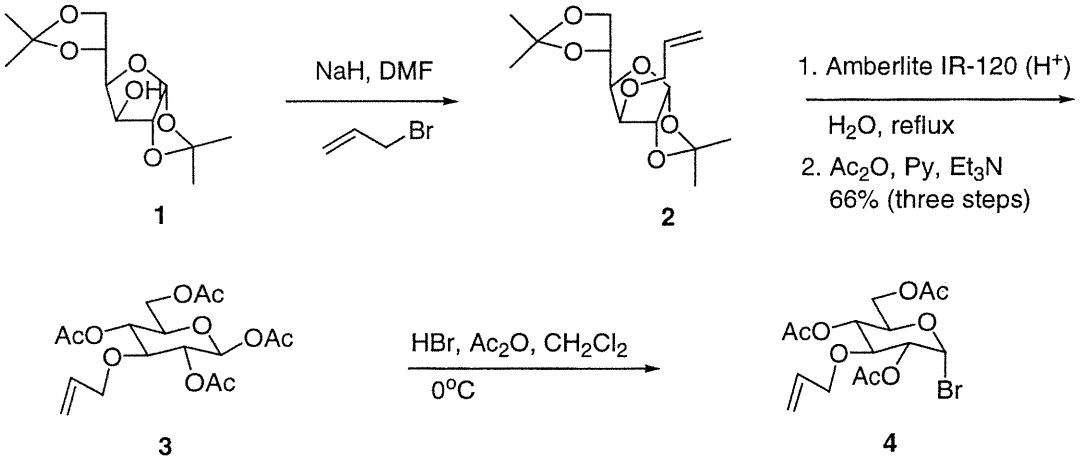
SH/L-008

Chapter Three

Synthesis of functionalized glycomers

4-1 Synthesis of glycomers using D-glucopyranose as scaffold

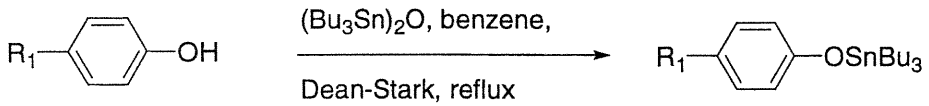
The synthesis of glucose-based *O*-aryl substituted glycosides started from the commercially available compound 1,2:5,6-diisopropylidene-D-glucofuranose **1**[51], which can be prepared from D-glucose in one step. Allylation of the free hydroxyl group in compound **1** gave an intermediate **2**[52], which was rearranged in acid condition to form a six-membered ring[53] and then acetylated to afford 3-*O*-allyl-1,2,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosides **3** in quantitative yield. Bromination of **3** had to be done carefully at 0°C to avoid the by-product of bromination at the allylic double bond[54] (scheme 1).



Scheme 1 Synthesis of 3-allyl-2, 4, 6-tri-*O*-acetyl-*β*-D-glucopyranosyl bromide

Due to the low nucleophilicity of the phenoxide oxygen atoms, the glycosidation between bromide **4** and aromatic alcohols couldn't be readily achieved. The tributyl stannyl phenoxides **5a**, **5b** and **5c**[55] obtained by treating with bis[tri-n-butyltin]oxide and 4-iodophenol, 4-bromophenol and 4-methoxyphenol, respectively (Scheme 2), were employed to enhance the nucleophilicity of the phenoxide oxygen atoms.[56]

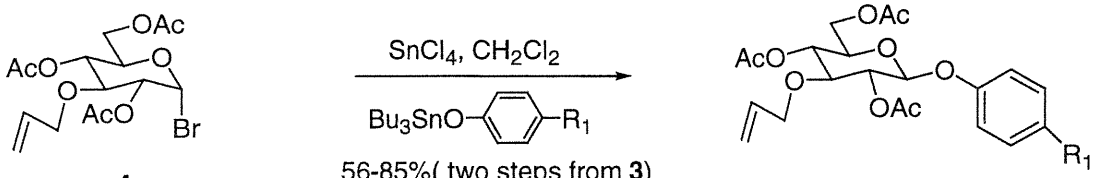
After the removal of the solvent, **5a**, **5b** and **5c** were used immediately in the glycosidation with the bromide **4** in presence of Lewis acid[56] to provide aryl β-D-glucopyranosides **6a, 6b** and **6c** (Scheme 3). In addition to *p*-iodophenyl, *p*-bromophenyl,



5a. R_1 = I
5b. R_1 = Br
5c. R_1 = OCH_3

Scheme 2 Synthesis of tributylstannyl phenoxides

and *p*-methoxyphenyl glycosides, we also prepared a series of compounds without any substituent on the aromatic ring (see later).



4

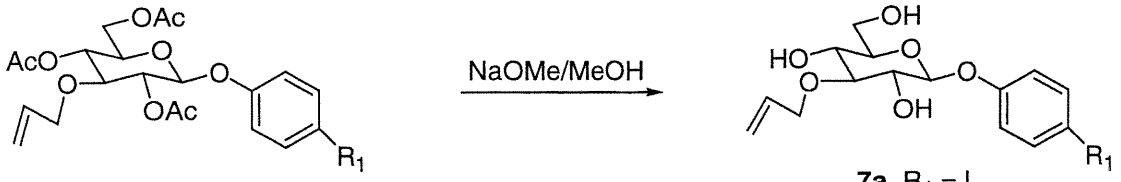
56-85%(two steps from **3**)

5a. R_1 = I
5b. R_1 = Br
5c. R_1 = OCH_3

6a. R_1 = I (α:β < 1:10)
6b. R_1 = Br (α: trace)
6c. R_1 = OCH_3 (α:β=1:5)

Scheme 3 Synthesis of aryl *β*-D-glucopyranosides

The major products **6a, 6b** and **6c** obtained by glycosylation were then subject to hydrolysis to afford the triols **7a, 7b** and **7c** in quantitative yields (Scheme 4).

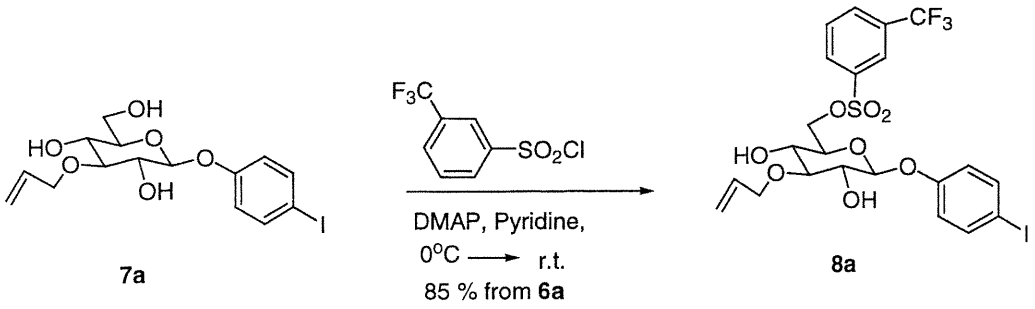


6a. R_1 = I
6b. R_1 = Br
6c. R_1 = OCH_3

7a. R_1 = I
7b. R_1 = Br
7c. R_1 = OCH_3

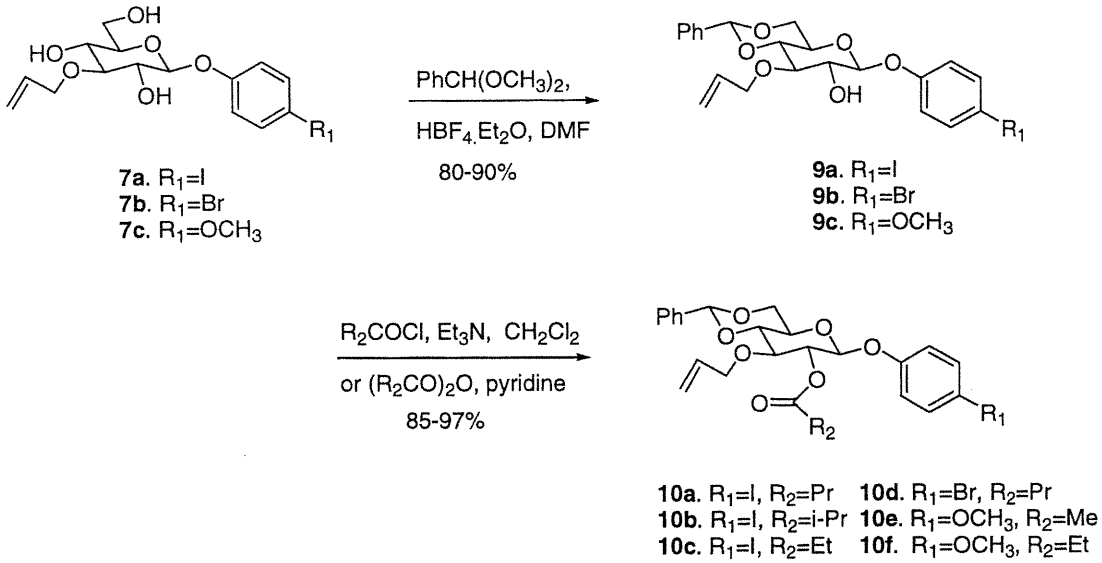
Scheme 4 Deprotection of aryl tri-*O*-acetyl-*β*-D-glucopyranosides

It was then time to introduce functional groups containing sulfonyl and carbonyl moieties at C-6 and C-2 positions, respectively. First, an effort was made to introduce a 3-(trifluoromethyl)benzenesulfonyl group on the primary alcohol of compound **7a**. The sulfonylation product **8a** was obtained in good yield under the condition[57] described in Scheme 5.



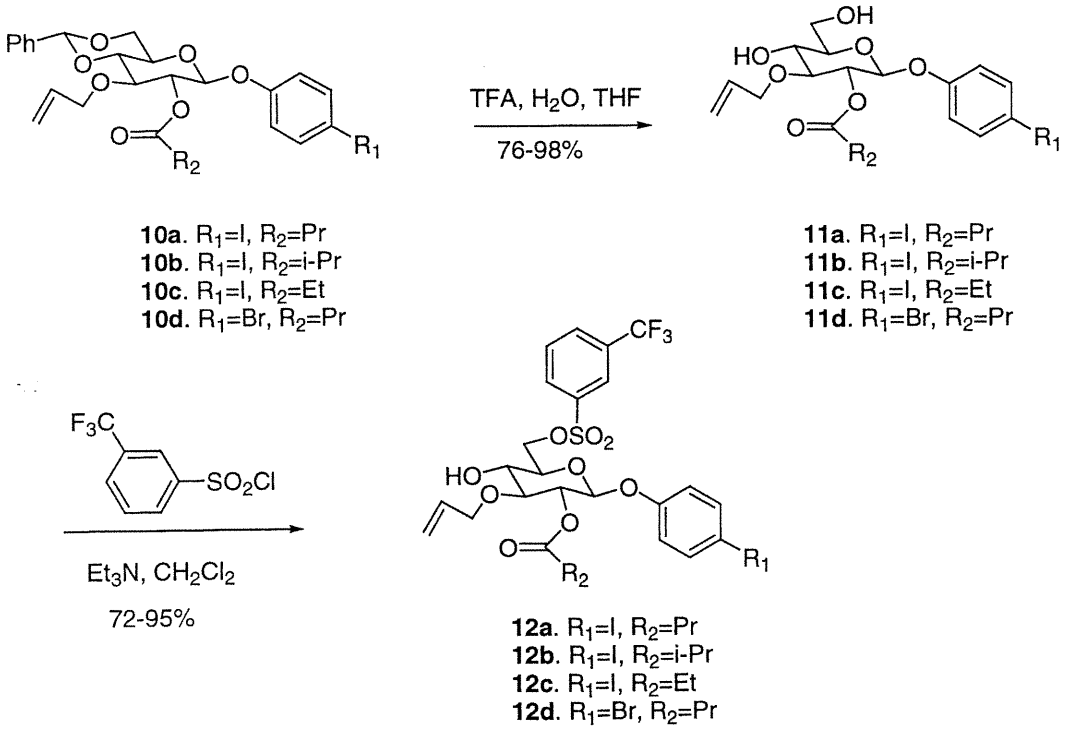
Scheme 5 Synthesis of 4-iodophenyl 3-*O*-allyl-6-*O*-(*m*-trifluoromethyl) benzenesulfonyl-*β*-D-glucopyranoside

Then we tried to introduce an ester selectively at C-2 of compound **8a**. Unfortunately, the chemoselectivity was not encouraging. When **8a** was reacted with



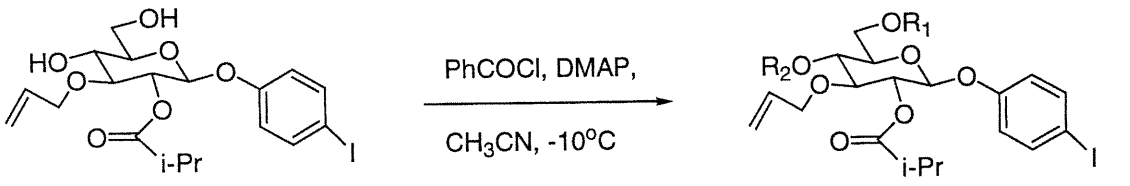
Scheme 6 Synthesis of aryl 4, 6-*O*-benzylidene-*β*-D-glucopyranosides

butyryl chloride and Et_3N at low temperature, the products were a mixture of 2-, 4-, and 2,4-di-butyryl esters of **8a**. This problem can be simply avoided by protecting the 4,6-diol of **7a, 7b** and **7c** as a benzylidene acetal[58] (**9a-9c**) before esterification to afford **10a-10f** (Scheme 6). Then the benzylidene acetal rings were opened with TFA to free the diols **11a-11d**[59], and the 3-(trifluoromethyl)benzenesulfonyl group was selectively introduced on the primary alcohol with 3-(trifluoromethyl)benzenesulfonyl chloride to afford **12a-12d** (Scheme 7).



Scheme 7 Synthesis of aryl 2-*O*-acyl-6-*O*-(*m*-trifluoromethyl)benzenesulfonyl-β-D-glucopyranosides

Acylation of **11b** with benzyoyl chloride and DMAP got the desired product **13a**[60] and only a small amount of **13b** (13%) (Scheme 8).

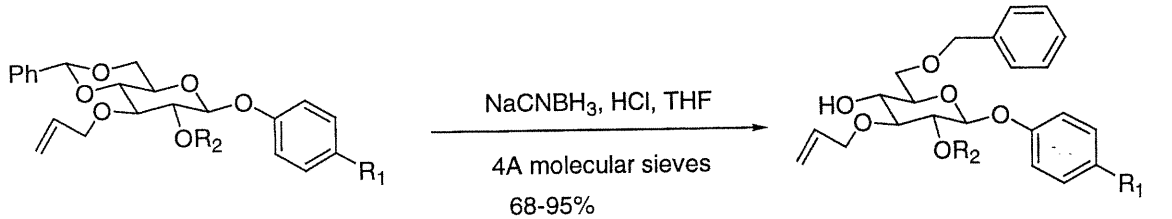


11b

13a. R_1=Bz, R_2=H (65%)
13b. R_1=Bz, R_2=Bz (13%)

Scheme 8 Synthesis of aryl 6-*O*-benzoyl-*β*-D-glucopyranosides

The benzylidene ring can be opened selectively with sodium cyanoborohydride and hydrogen chloride in THF to give benzyl ether at C-6 selectively[61] (**14a-15f**) (Scheme 9).

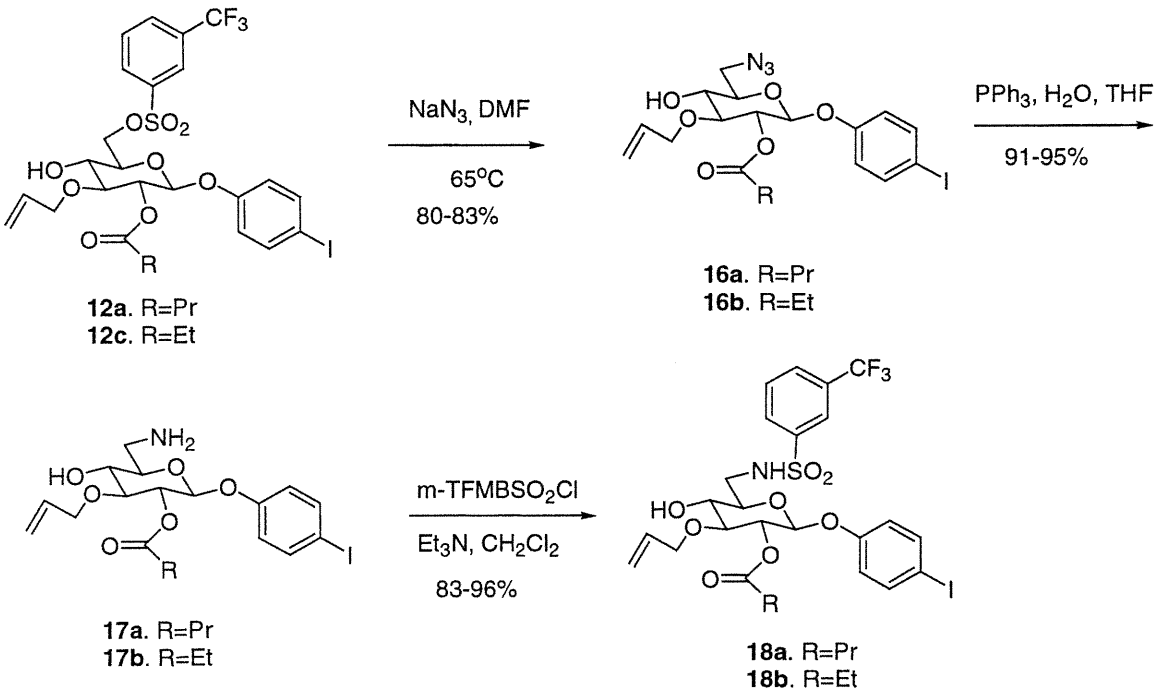


9b. R_1=Br, R_2=H
10a. R_1=I, R_2=PrCO
10b. R_1=I, R_2=i-PrCO
10c. R_1=I, R_2=EtCO
10d. R_1=Br, R_2=PrCO
10e. R_1=OCH_3, R_2=Ac
10f. R_1=OCH_3, R_2=EtCO

14b. R_1=Br, R_2=H
15a. R_1=I, R_2=PrCO
15b. R_1=I, R_2=i-PrCO
15c. R_1=I, R_2=EtCO
15d. R_1=Br, R_2=PrCO
15e. R_1=OCH_3, R_2=Ac
15f. R_1=OCH_3, R_2=EtCO

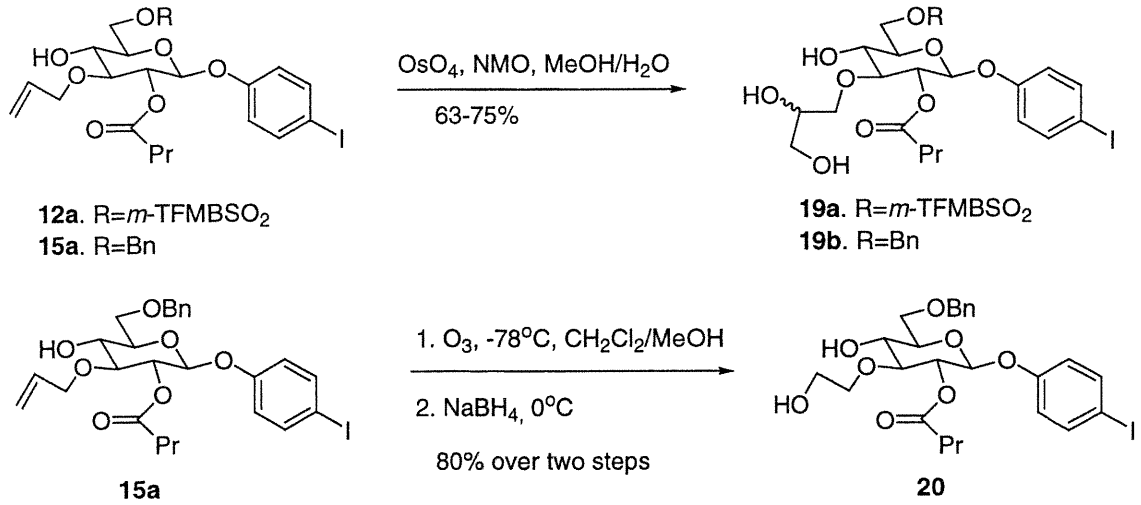
Scheme 9 Synthesis of aryl 6-*O*-benzyl-*β*-D-glucopyranosides

In order to obtain compounds with a *C*-sulfonamide instead of a *C*-sulfonate, the *m*-(trifluoromethyl)benzenesulfonate group of **12a, 12b** were converted to an azide by treatment with sodium azide in DMF at an ambient temperature[62]. The azides **16a** and **16b** were then reduced with triphenylphosphine and water in THF to produce amines **17a** and **17b**[63], which were converted to the *m*-(trifluoromethyl)benzenesulfonamides **18a** and **18b** upon treatment with *m*-(trifluoromethyl)benzenesulfonyl chloride (Scheme 10).



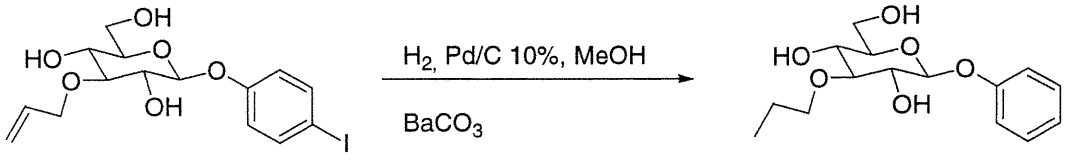
Scheme 10 Synthesis of aryl 6-deoxy-6-(*m*-trifluoromethyl)benzenesulfonamide-*β*-D-glucopyranosides

Because of the low solubility of these glycomers in aqueous solution or DMSO-water, we decided to introduce one or two more hydrophilic groups on these molecules.



Scheme 11 Dihydroxylation and ozonolysis of allylic double bonds

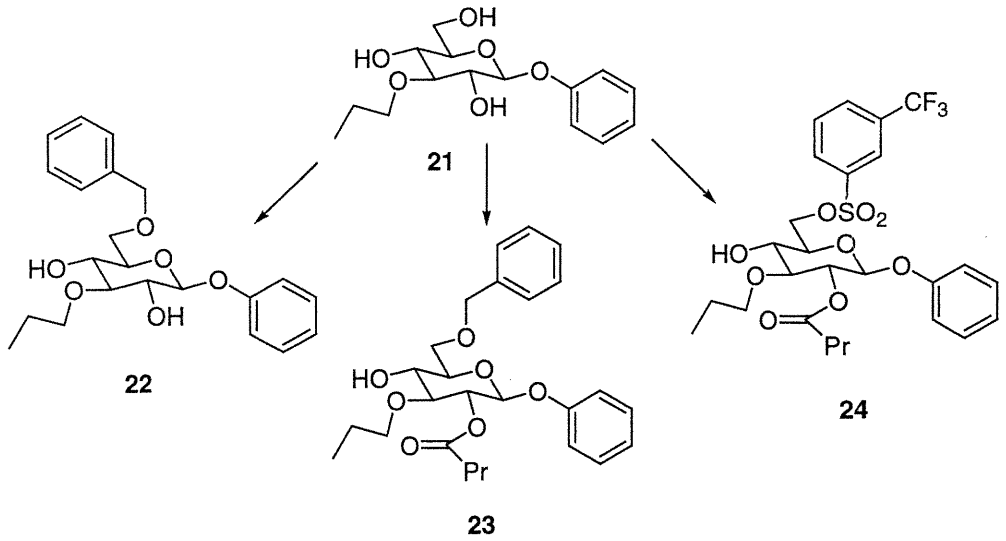
The allylic double bonds of compounds **12a** and **15a** were dihydroxylated[64] to afford diols **19a** and **19b**, respectively. The double bond of **15a** was also cleaved by ozonolysis to form an aldehyde, which was reduced *in situ* to form the primary alcohol **20**[60] (Scheme 11).



7a **21**

Scheme 12 Hydrogenation of 4-iodophenyl 3-*O*-allyl-*β*-D-glucopyranosides

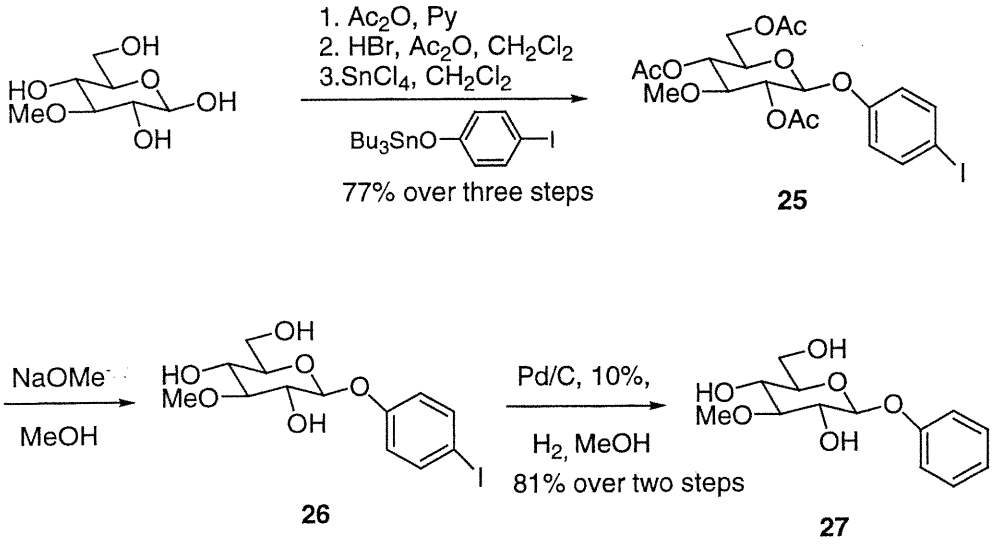
As mentioned above, we hydrogenated compound **7a** to remove the iodo group on the aromatic ring. When the reaction was done without $BaCO_3$, the allyl group was cleaved probably by isomerization to a 2-propenyl group followed by acid (HI) catalyzed cleavage.[65] In presence of the base, the hydrogen iodide, which was produced during the hydrogenation, was neutralized and the allyl group was reduced as expected to give **21** (Scheme 12).



Scheme 13 Derivatives of compound **21**

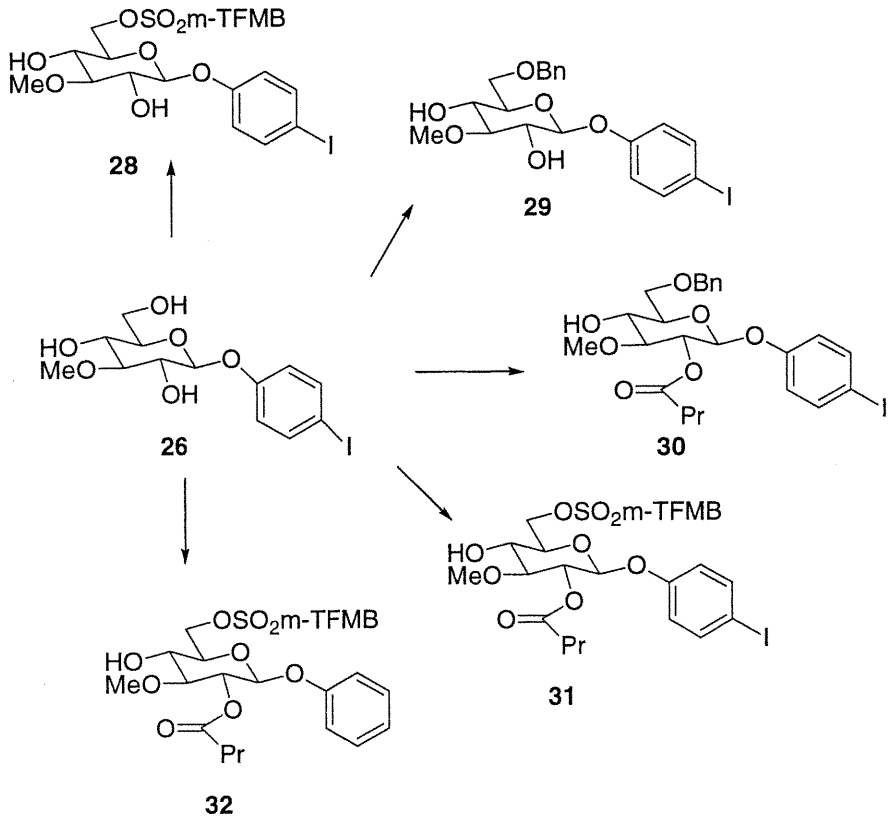
Starting with **21**, we used the same transformations as those shown for **14b, 15a,** and **12a** to get **22**, **23**, and **24**, respectively (Scheme 13).

Another series of compounds with a methyl group in 3-position were synthesized by using the commercially available 3-*O*-methyl-D-glucopyranose. Using the same chemistry as we described before, we prepared the aryl glycosides **25** from 3-*O*-methyl-D-glucopyranose by acetylation, bromination, and glycosidation, followed by deprotection to afford **26** (Scheme 14).



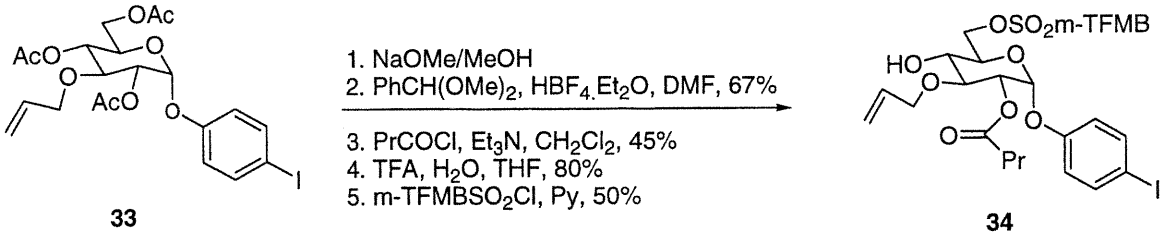
Scheme 14 Synthesis of aryl 3-*O*-methyl-*β*-D-glucopyranosides

Compound **26** was then sulfonylated with *m*-(trifluoromethyl)benzenesulfonyl chloride to afford **28**. Protection as a benzylidene acetal with bezaldehyde and zinc chloride[66], followed by reductive opening furnished the benzyl ether **29**. Protection as a benzylidene acetal, esterfication at C-2 followed by selective opening of the benzylidene ring afforded **30**. On the other hand, cleavage with TFA to free the diol and *m*-(trifluoromethyl)benzenesulfonylation afforded the sulfonate **31**. For compound **32**, **26** was hydrogenated **to** remove the iodo group on the aromatic ring forming **27**, which was converted to **32** using the same chemistry as for **31** (Scheme 15).



Scheme 15 Derivatives of compound **26**

In order to probe the orientation of the anomeric substituent, we also synthesized the aryl glycoside with the α-configuration. From compound **33**, which is a minor product of glycosylation with **4** and **5a** in Scheme 3, we introduced a benzylidene acetal to protect the 4,6-diol. Conversion to the butyryl ester at the 2-position[67], cleavage of the benzylidene ring with TFA in H_2O/THF to free the diol, followed by sulfonylation with *m*-(trifluoromethyl) benzenesulfonyl chloride at the 6-position gave **34** (Scheme 16).

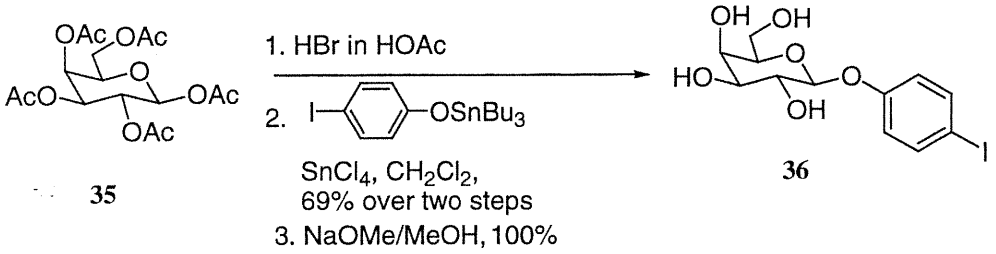


Scheme 16 Synthesis of 4-iodophenyl 3-*O*-allyl-2-*O*-butyryl-6-*O*-(*m*-trifluoromethyl) benzenesulfonyl α-D-glucopyranoside(**34**)

4-2 Synthesis of glycomers using D-galactopyranose as scaffold

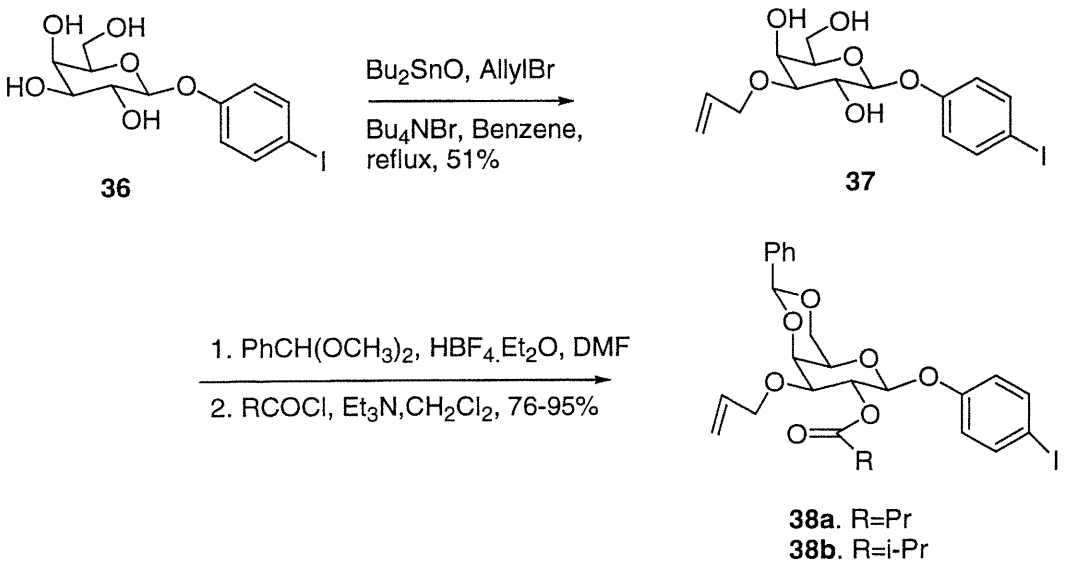
Up to this point we synthesized a series of aryl D-glycopyranosides with modifications at the anomeric configuration, on the substituents on the anomatic ring, and on the functional groups at 2-, 3-, and 6- positions. We then focused on the synthesis of the D-galacto analogues for comparison of antitumor activities.

The synthesis commenced from the commercially available D-galactose pentaacetate **35**, which was brominated with HBr (30 wt % in ether) and catalytic amount of acetic anhydride in CH_2Cl_2, followed by a glycosylation with tributyl stannyl 4-iodophenoxide (**5a**) in presence of a Lewis acid. The tri-*O*-acetyl arylglycoside was deprotected to afford **36** (Scheme 17).



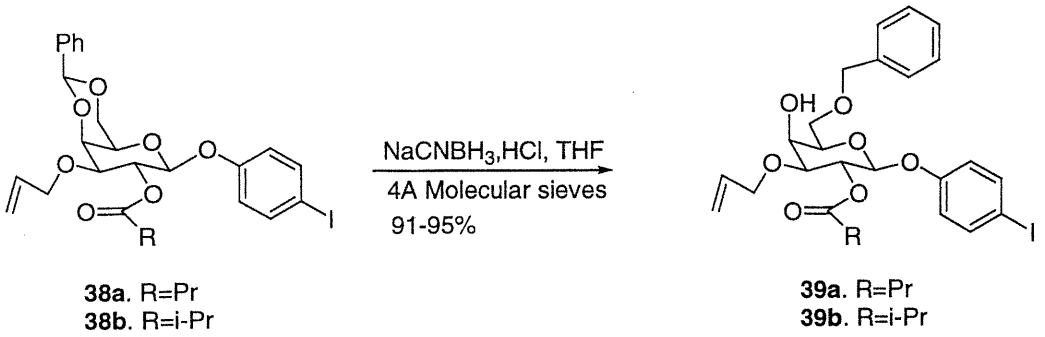
Scheme 17 Synthesis of 4-iodophenyl β-D-galactopyranoside

Allylation of **36** with allyl bromide, dibutyltin oxide, and tetrabutyl ammonium bromide in benzene under reflux afforded 4-iodophenyl 3-*O*-allyl-β-D-galactopyranoside **37** (51%) as a major product and two minor products, 4-iodophenyl 2,3-di-*O*-allyl-β-D-galactopyranoside (21%), and 4-iodophenyl 3,6-di-*O*-allyl-β-D-galactopyranoside (5%)[68]. The 4,6-diol in **37** was protected with benzaldehyde dimethyl acetal and tetrafluoroboric acid in DMF followed by esterification at C-2 with acyl chlorides to afford **38a** and **38b** (Scheme 18).



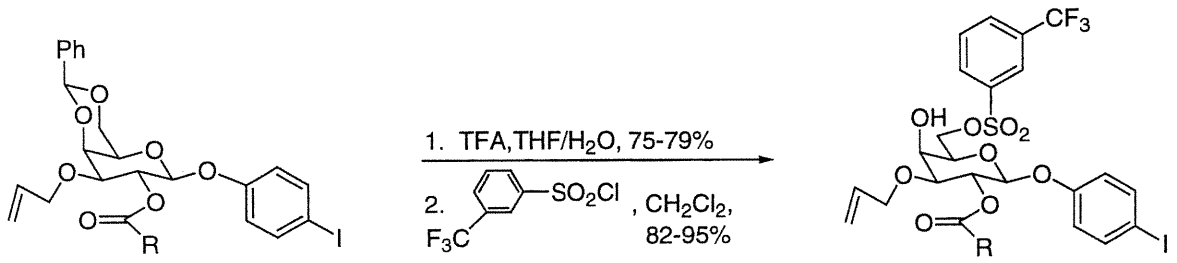
Scheme 18 Synthesis of 4-iodophenyl 4, 6-*O*-benzylidene-*β*-D-galactopyranosides

The benzylidene acetal rings in compounds **38a** and **38b** were then selectively opened by sodium cyanoborohydride and HCl (1M in ether) in THF to form benzyl ether at C-6 as in **39a** and **39b** (Scheme 19).



Scheme 19 Synthesis of 4-iodophenyl 6-*O*-benzyl-*β*-D-galactopyranosides

We also opened the benzylidene acetal of **38a** and **38b** with TFA in H_2O/THF to free the 4,6-diol and introduced a 3-(trifluoromethyl)benzenesulfonyl group at C-6 with 3-(trifluoromethyl)benzenesulfonyl chloride and Et_3N in CH_2Cl_2 to obtain **40a** and **40b** (Scheme 20)

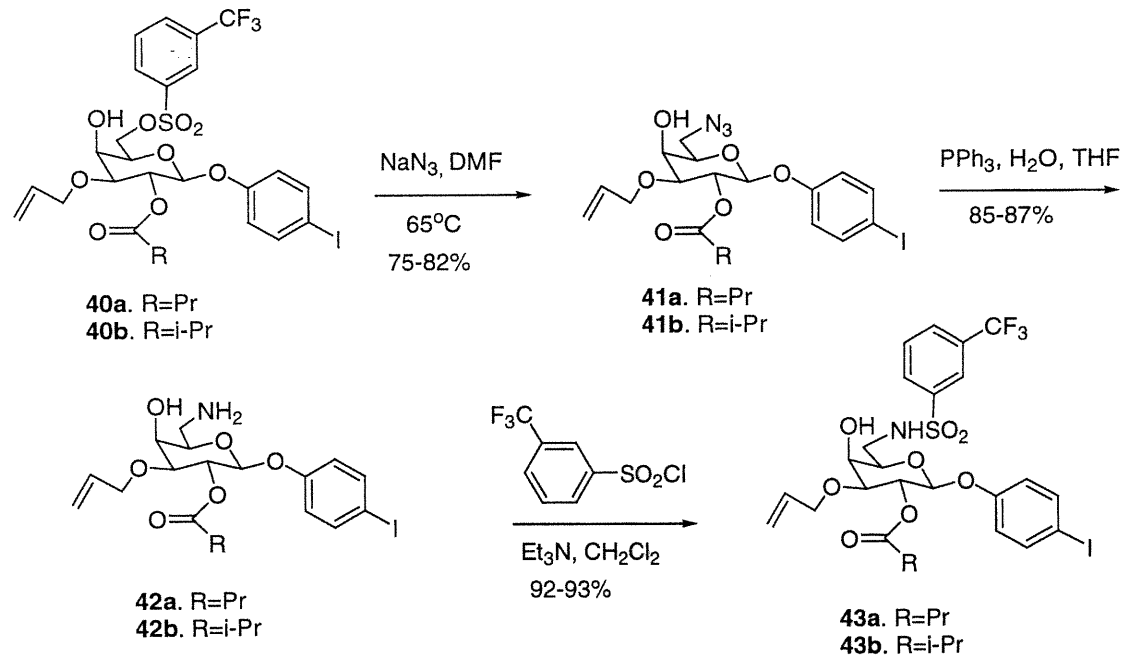


38a. R=Pr
38b. R=i-Pr

40a. R=Pr
40b. R=i-Pr

Scheme 20 Synthesis of 4-iodophenyl 2-*O*-acyl-6-*O*-(*m*-trifluoromethyl) benzenesulfonyl-*β*-D-glucopyranosides

The sulfonate groups in **40a** and **40b** were replaced by sodium azide in DMF to form azido compounds **41a** and **41b**, which were reduced to the amines **42a** and **42b** with PPh_3 and water in THF, followed by sulfonation with *m*-(trifluoromethyl)benzenesulfonyl chloride and Et_3N in CH_2Cl_2 to form sulfonamides **43a** and **43b** (Scheme 21).



40a. R=Pr
40b. R=i-Pr

41a. R=Pr
41b. R=i-Pr

42a. R=Pr
42b. R=i-Pr

43a. R=Pr
43b. R=i-Pr

Scheme 21 Synthesis of 4-iodophenyl 3-*O*-allyl-2-*O*-butyryl-6-*deoxy*-6-(*m*-trifluoromethyl)benzenesulfonamide-*β*-D-galactopyranosides

Chapter Four

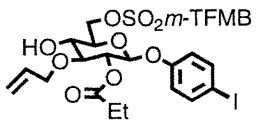
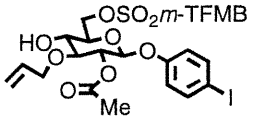
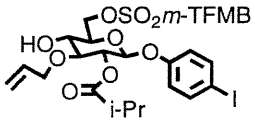
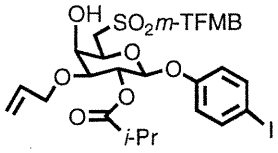
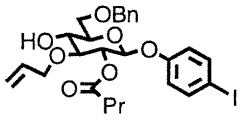
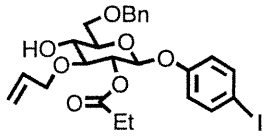
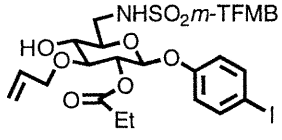
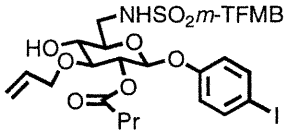
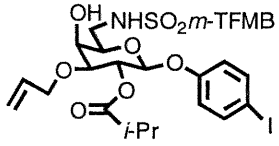
Activity of glycomers toward glioblastoma cell lines

(The experiments were performed by Dr. Lucienne Juillerat, Institute of Pathology, Bugnon 27, CH 1011 Lausanne, Switzerland)

4-1. The antiproliferative activity of glycomers toward glioblastoma

A series of compounds described above were tested toward human glioblastoma cell lines LN 18 and LNZ 308[69]. Their effects on proliferation were evaluated by MTT[70] (3,4,5-dimethylthiazol-yl)-2,5-diphenyl tetrazolium) in human glioblastoma cells in culture. The results are shown in Tables 2-4.

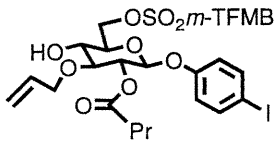
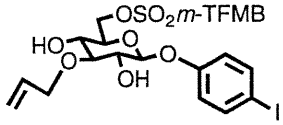
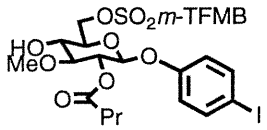
Table 2 Glycomers with $IC_{50} < 20$ μM

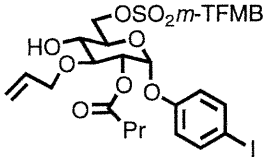
	 **12c**	 **12f**	 **12b**
LN 18	*7* μM	*12.5* μM	*12.5* μM
LNZ308	*11.5* μM	*13* μM	*8.5* μM
	 **40b**	 **15a**	 **15c**
LN 18	*18.5* μM	*7.5* μM	*11.5* μM
LNZ 308	*18* μM	*11.5* μM	*17.5* μM
	 **18b**	 **18a**	 **43b**
LN 18	*11.5* μM	*6.5* μM	*12.5* μM
LNZ 308	*18.5* μM	*17.5* μM	*7.5* μM

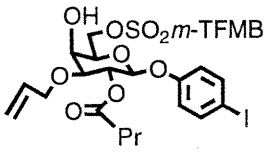
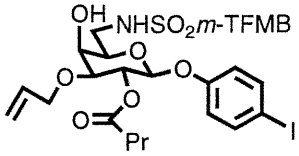
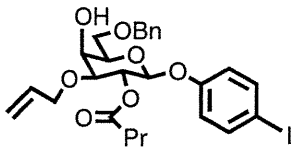
Cells were grown for 24 h with FCS (Fetal calf serum), and then deprived of FCS for 24 h. Glycomers were added to cells for 24 h and MTT assay was performed. The % of growth inhibition was calculated as the ratio of MTT reduction (absorbance at 540 nm) of treated to control cells and IC_{50} was

calculated from dose-response curves as the concentration inducing 50% of growth inhibition.

Table 3 Glycomers with IC_{50} 20~45 μM

	 **12a**	 **8a**	 **31**
LN 18	*43* μM	21 μM	*26* μM
LNZ 308	*41* μM	19 μM	*22* μM

 **34**	
LN 18	*25* μM
LNZ 308	*42* μM

	 **40a**	 **43a**	 **39a**
LN 18	*40* μM	25 μM	*20* μM
LNZ 308	*29* μM	26 μM	*37* μM

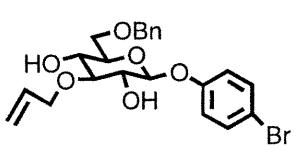
In terms of the cell growth inhibition, the compounds with iodo substituents are more active than those with the bromo, methoxy and without substituents on the aromatic rings. As shown in Table 2, all the compounds with the IC_{50} less than 20μM have an iodo substituent on the aromatic ring.

The chain length at C-2 ester has also an effect on the activity. The longer chain with 3-phenyl-propionic ester (compound **12e,** Table 4) is inactive compared with

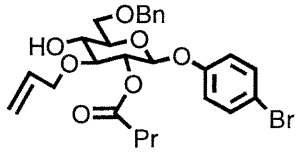
compound with shorter chain (for example **12b**, **12c** and **12f**, Table 2), possibly reflecting substrate preference of cellular esterases. It is of interest that cleavage of the C-2 ester did not result in loss of activity within the same series (compare **12a** and **8a**, Table 3).

With one exception (compound **31**, Table 3), methyl ethers at C-3 have lower activity than that of the compounds with allyl ether at C-3. Increasing the polarity of the side-chain at C-3 by hydroxylation of the allyl group or by an oxidation-reduction sequence to afford a primary alcohol (ex. **19b** and **20**, Table 4) resulted in loss of activity.

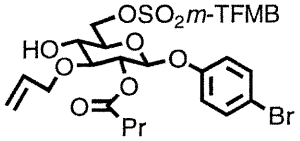
Table 4 Glycomers with $IC_{50} > 50$ μM



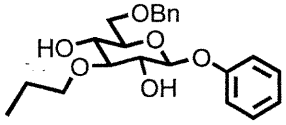
14b



15d



12d



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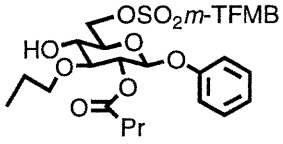

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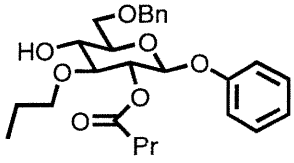

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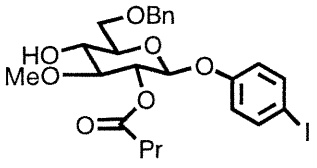

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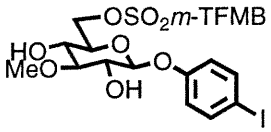
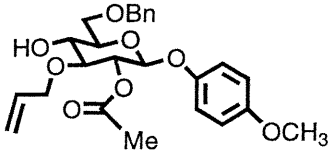
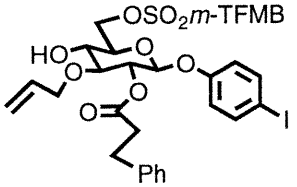


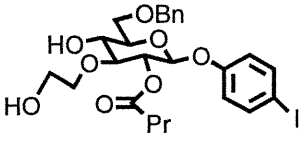
28



15e



12e



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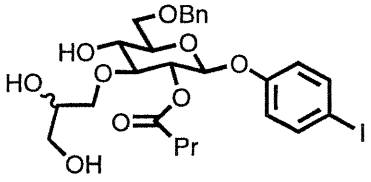
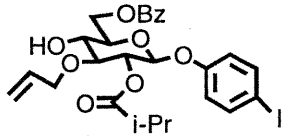


19b

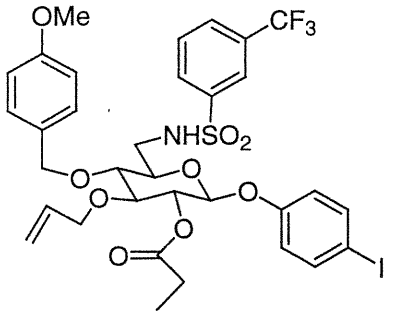


13a

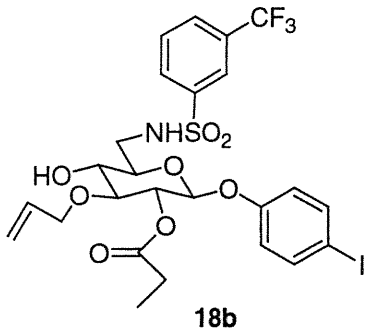
Initially, the active glycomers contained a C-6 sulfonate group. In order to eliminate the possibility of alkylation by displacement of the sulfonate by a nucleophile in a protein such as a thio group, we prepared more stable groups. Substituents such as benzyl ether were equally active (compare **40a** and **39a**, Table 3). This result indicated that the alkylating effect of a sulfonate is unlikely to be important in inhibition of growth of glioblastoma cells, and indicated the versatility of substituents at C-6. Curiously, a benzoate ester was not efficacious (compound **13a**, Table 4), possibly reflecting hydrolysis by cellular esterases. The promising antiproliferative activity of C-6 sulfonamides and C-6 benzyl ethers was noteworthy, since as mentioned above, these glycomers are probably chemically more stable in a biological medium, except for the action of the esterases.

Within the same series, the anomeric configuration is not critical for optional activity (compare **12a** and **34**, Table 3).

The configuration at C-4 has no apparent effect on the activity as evidenced by D-gluco and D-galacto glycomers within the same series (**12a**, **40a**, Table 3). In a separate study, we prepared a *p*-methoxy benzyl ether at C-4 corresponding to **18b**, and found it to be inactive, thus delineating the importance of the free hydroxyl group at C-4 in this glycomer (scheme 22).



LN 18 > 50 μM
LNZ 308 > 50 μM



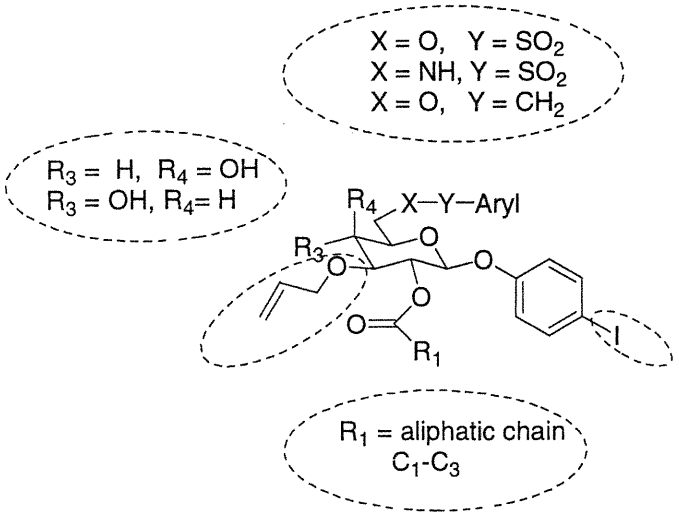
18b

LN 18 11.5 μM
LNZ 308 18.5 μM

Scheme 22 The influence of the substituent at C-4

Considering an "optimal" substitution pattern represented by the glycomers with IC_{50} < 20μM (Table 2) on both glioblastoma cell lines, it appears that the

absence of one or more functional groups significantly diminishes the antiproliferative activity (Table 4). Scheme 23 depicts the structural and functional requirements of an "active" glycomer.



Scheme 23 The optimal glycomers

4-2. Inhibition of DNA synthesis, protein synthesis and induction of apoptosis of glioblastoma cells by glycomers

Having demonstrated the growth inhibitory activity of some glycomers in glioblastoma cells, we turned our attention to study the process in more detail. In an effort to elucidate the biological pathways involved in the antiproliferative action of these glycomers, we studied the growth inhibition of glioblastoma through inhibition of DNA synthesis (^{3}H-thymidine incorporation, Figure 14A), adhesiveness and survival (MTT reduction, Figure 14B), protein content (Figure 14C), and apoptosis (nucleosome-histone fragmentation, Table 5), as a decreased cell number may result either from increased cell death or decreased proliferation or both.

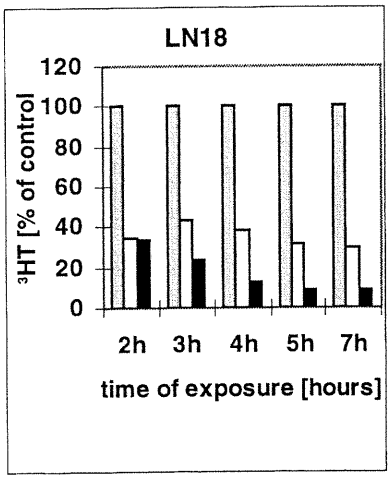
Figure 14 Evaluation of antiproliferative effects of glycomers by ^{3}HT incorporation,

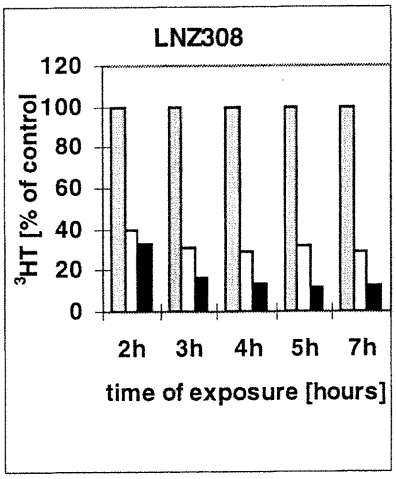
MTT reduction, and protein content

Test: Cells were left to adhere for 24 h in the presence of serum (FCS), and then incubated for another 24 h in the absence of FCS. Medium was changed and glycomers were added to cells at increasing concentrations (0-20 μM) in the absence of FCS for 2 h - 24 h. Thymidine incorporation and MTT assay were performed for the two last hours of incubation, and protein content was determined at the end of the incubation period.

(A): Thymidine incorporation[71]

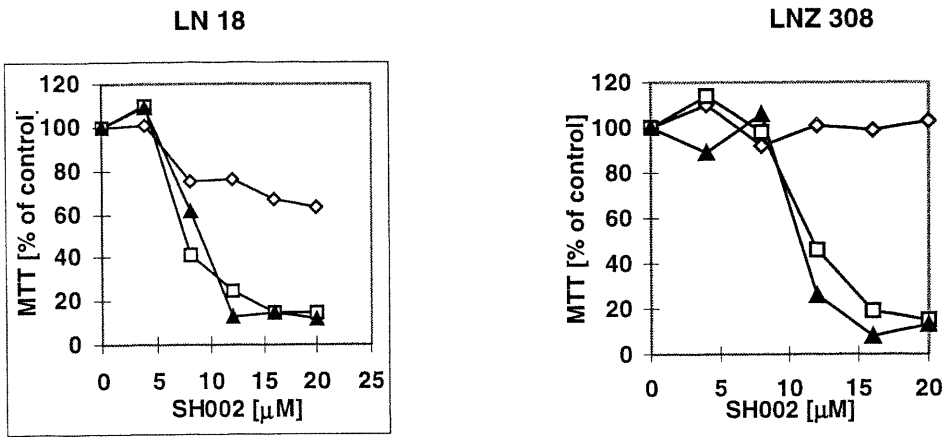
Tritiated thymidine incorporation (^{3}HT) was used to ascertain DNA synthesis. Following treatment, cells were exposed to 0.8 μCi/ml [^{3}H]-thymidine (Amersham Pharmacia, Dübendorf, Switzerland) for 2h - 3h and washed to remove unincorporated ^{3}HT, and radioactivity was quantitated in a beta-counter (LKB) after precipitation with 10% trichloracetic acid and solubilization in 0.1% SDS - 0.1 N NaOH and 5 ml Optiphase scintillation cocktail (Wallac, Fisher Chemicals, England). Grey bars: no ***12c****(SH002); white bars:* ***12c****(SH002) 4 μM; black bars:* ***12c****(SH002) 12 μM.*





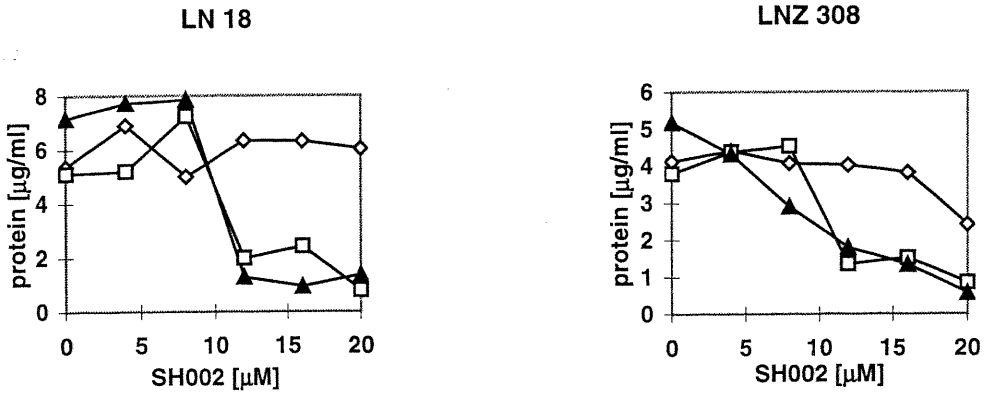
(B): MTT[70] assay.

MTT ((3,4,5-dimethylthiazol-yl)-2,5-diphenyl tetrazolium, Sigma), an indicator of mitochondrial function, was used to quantify the number of metabolically active cells. Following treatment, cells were exposed to 0.25 mg/ml MTT in DMEM (Dubecco Modified Eagle Medium) medium for 2h, supernatant was aspirated and the precipitated formazan was dissolved in 0.1 N HCl in isopropanol and quantified at 540 nm. ✧: 8 h exposure to ***12c****(SH002); ❑: 24 h exposure ; ▲: 48 h exposure.*



(C): Protein content

Protein content was determined with the BCA (bicinchroninic acid) protein assay kit (Pierce, Socochim, Switzerland) and bovine serum albumin as standard. After washing, the cell layer is dissolved in 0.1% Triton in PBS (Phosphate Buffered Saline) and protein quantitated in an aliquot. ✧ *: 8 h exposure to* ***12c*** *(SH002);* ❑: 24 h exposure; ▲: *48 h exposure.*



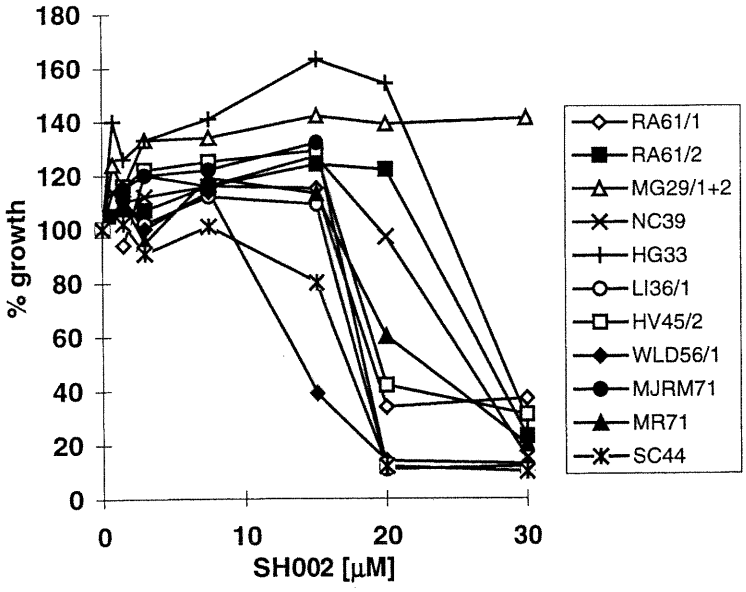
The thymidine incorporation showed that inhibition of DNA synthesis was already observed 2 hours after addition of **12c** (SH002) and increased only slightly (at 4 µM) with longer exposure time (Figure 14A). After exposure to **12c** (SH002) at 12 µM for 5h, the DNA synthesis was inhibited up to 90% in both cell lines. To observe a decrease in the number of metabolically active cells, using the MTT assay and the quantification of protein content in the culture wells, exposure to **12c** (SH002) for periods longer than 8 hours was necessary, with a maximal effect after 24 h, which did not further increase by incubating the cells for 48 h. The metabolically active cell survival was

almost completely inhibited with **12c** (SH002) at ca 16 μM on the LNZ 308 cell line after 48h (Figure 14B), for example. The protein content was reduced to 1 μg/ml with **12c** (SH002) at ca 12 μM on the LN 18 cell line after 48h, comparing to an average of 6 μg/ml of the control without adding the glycomer (Figure 14C). Exposure to SH002 as short as 5 min was already efficient in inhibiting thymidine incorporation and the effect was maximal after 60 min exposure of glioblastoma cells (not shown). Thus the primary effect of these glycomers appears to inhibit DNA synthesis in glioblastoma cells and the efficacy and rapidity of inhibition is depending on the chemical modifications.

In order to evaluate the effects of glycomers on non-tumoral human cells, we determined their effects in primary cultures of human fibroblasts (11 different preparations) and human brain-derived endothelial cells (HCEC cell lines)[72].

Figure 15 Growth inhibition of primary human fibroblasts by a glycomer

Cells were grown in the presence of FCS, and then incubated for 24 h in the absence of FCS. Medium was changed and SH002 glycomer was added to cells at increasing concentrations in the absence of FCS for 24 h. MTT assay was performed during the last 2 hours of incubation. Each curve represents culture from a different surgical specimen.



Encouragingly, human brain-derived endothelial cells (HCEC) (not shown) were less sensitive to inhibition of DNA synthesis by this compound. Similarly growth and adherence of primary normal human fibroblasts (Figure 15) were inhibited at slightly higher concentrations of **12c** (SH002) (IC_{50} 20-25 μM) than glioblastoma cells. Thus, some selectivity towards tumor cells versus normal cells has also been obtained.

We then studied whether growth inhibition of glioblastoma cells by the glycomers can also be achieved through induction of apoptosis. **14b**(SH/L-5) and **22**(SH/L-31) (Table 4) did not induce apoptosis, when growth inhibition and loss of adhesivity was not observed, while in the presence of the most efficient growth inhibitors **12c**(SH002) and **15a**(SH007) glycomers (Table 2), nucleosome fragmentation was observed. However, **13a**(SH008, Table 4), which did not inhibit growth and cell adherence could induce apoptosis. Thus inhibition of cell growth and apoptosis induction by some functionalized glycomers could be dissociated. The glycomer **12c**(SH002, Table 2) was studied on a shorter time-course (4-8 h) of exposure before cell detachment was observed but at the time of cell rounding. The study showed that **12c** was able to induce apoptosis as determined by chromatin condensation (using the fluorescent nuclear marker 4',6'-diamidino-2-phenylindolylhydrochloride,DAPI, not shown), or as quantitated by nucleosome fragmentation(Table 5). These results show that functionalized glycomers are able to inhibit DNA synthesis and induce apoptosis in human glioblastoma cells. Thus, this new class of molecules represents potentially interesting tools to achieve specific effects in human tumor cells in order to control brain tumor progression.

Table 5 Induction of apoptosis[70] by selected glycomers.

Apoptosis, a programmed cell death, was determined in cells in culture by evaluation of nuclear fragmentation. The quantitative determination of nuclear fragmentation by measuring the level of DNA-histone fragments (nucleosomes) in culture wells (Cell Death ELISA^{plus}, Boehringer Roche). Cells were grown for 24 h with FCS, deprived of FCS for 24 h, and then glycomers were added to cells for 24 h at 25μM concentration, unless otherwise stated. Quantification of nucleosome fragments was performed at the end of the incubation. Apoptosis index[73] (amount of nucleosme fragments in treated versus control cells) was defined as 1.00 for untreated cell culture wells under identical culture conditions than the treated cells. [a] *: Initial concentration 25μM, precipitation of the compound in the culture medium starting*

after 15 min of incubation;[b]*: initial concentration 50 μM, precipitation of the compound in the culture medium starting after 15 min of incubation;* [c]*: 16 μM, 8h exposure; ND: not determined.*

Compound	*LN18*	*LNZ308*
12a (SH003)	0.44	0.41
12b (SH005)	1.10	1.09
15a (SH007)[a]	1.60	2.27
13a (SH008)[b]	2.81	4.64
22 (SH/L-31)	1.29	ND
14b (SH/L-5)	1.19	ND
12c (SH002)[c]	3.82	8.97

4-3. Conclusion

In conclusion, we have developed and evaluated a series of new molecules, functionalized glycomers, and shown specific effects on growth inhibition and apoptosis induction of these molecules toward human glioblastoma cells in culture. The studies of systematic substitution at selected positions of the aryl glycopyranoside backbone indicate that the most important group for the biological activity is the 4-iodobenzyl at position C-1, while substitutions at positions C-2, C-3 and C-6 exert fine-tuning of the effects. The first event induced by exposure of glioblastoma cells to functionalized glycomers is an inhibition of DNA synthesis, ultimately resulting in loss of adhesive properties and induction of apoptosis. These molecules thus represent interesting new potentials agents to control glioblastoma progression. However the exact mechanisms of action, the effect of serum components and the passage through the cerebral vasculature of these functionalized glycomers need to be analyzed in more detail in order to optimize their chemical structures and cell selectivity.

Chapter Five

Experimental Part

5-1 General experimental notes

All yields reported are isolated yields except where indicated. The stereoselectivity was measured by ^{1}H-NMR analysis.

Melting points (mp) were measured on a Fisher-Johns melting point apparatus. All melting points are uncorrected.

Spectra of nuclear magnetic resonance of proton (^{1}H-NMR) and Carbon-13 (^{13}C-NMR) were recorded on a Varian VXR-300 (300 MHz), a Bruker AMX-300 (300 MHz) or a Bruker AMX-400 (400 MHz) spectrometer in a deuterated solvent as indicated with $CHCl_3$ (H, δ = 7.27 ppm; C, δ = 77.23 ppm) or CD_3OD (H, δ = 4.87, 3.31 ppm; C, δ = 49.15 ppm) as the internal reference. Chemical shifts (δ) and coupling constants (J) are expressed in ppm (part per million) and Hz (Hertz), respectively. The abbreviations used for the description of the peaks are as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad; f, fine; dd, doublet of doublets; dt, doublet of triplets. DEPT experiments were performed routinely, methyl (CH_3) and methyne (CH) give positive signals, methylene (CH_2) gives a negative signal (-), and carbon without hydrogen gives no signal (0). **^{13}C-NMR** and DEPT data are listed together. All chemical shifts are measured from the center of the resolved peaks; the unresolved multiplet and broad peaks are normally indicated as a range.

Low resolution mass spectra (MS) and high resolution mass spectra (HRMS) were determined on a VG Micro Mass 1212 and a Kratos MS-50 TCTA mass spectrometer, respectively, with desorption chemical ionization (CI), or fast atom bombardment (FAB).

Infrared spectra (IR) were recorded on a Perkin-Elmer 781 or Paragon 1000 infrared spectrophotometer with a KBr pellet or in a chloroform solution with a sodium chloride cell.

Optical rotations ($[\alpha]_D$) were measured at the sodium line with a Perkin-Elmer 241 polarimeter at ambient temperature.

All original data are available from Professor Stephen Hanessian, Université de Montréal.

Chromatography

Flash chromatography was carried out according to the procedure of Still[74] using silica gel 60 (0.040-0.063 mm, 230-400 mesh ASTA) (E. Merck).

Thin layer chromatography (TLC) was performed using commercial precoated glass-backed Silica Gel 60 F254 plates with a layer thickness of 250 μm (E. Merck). This technique was used to follow the course of reactions, to determine the suitable solvent system for flash chromatography, and to check fractions of flash chromatography. A mixture of ethyl acetate-hexane on v/v basis, as indicated, was used as eluant.

TLC visualization

UV light

UV254 lamp was used to view TLC plates with UV light active compounds.

Stain solution

The TLC plate was dipped into the stain solution and heated to develop the colored spots.

(A) *Molybdate-Ceric solution*

This solution was prepared by dissolving 50 g of ammonium molybdate (VI) tetrahydrate, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ and 20 g of ammonium cerium (IV) sulfate dihydrate, $(NH_4)_4Ce(SO_4)\cdot 2H_2O$ in a solution of 1800 ml of distilled water and 200 ml of concentrated sulfuric acid.

(B) *Ninhydrin solution*

This solution was prepared by dissolving 2 g of ninhydrin dihydrate in a solution of 600 ml of butanol and 18 ml of acetic acid. This solution was used with nitrogen-containing compounds.

(C) *$KMnO_4$ solution*

A 10% aqueous solution of $KMnO_4$ was used with olefin-containing compounds.

Solvents

Hexane, ethyl acetate and dichloromethane were distilled to remove any non-volatile material for chromatography and general use.

Anhydrous solvents were dried and distilled over suitable drying agents as listed below:

Solvent	Drying agent
THF	potassium/benzophenone
dichloromethane	calcium hydride
toluene	calcium hydride
triethylamine	calcium hydride
acetone	calcium chloride

Reagents

All reagents were purchased from Aldrich, Sigma or Lancaster, and were used without further purification, except where indicated. All commercially unavailable reagents were prepared following the procedures listed below or known procedures.

Anhydrous $ZnCl_2$

Zinc chloride was heated with flame molten and smoked for 15 min, and then cooled down in a desiccator under vacuum. The anhydrous zinc chloride was grinded into powder before using.

Anhydrous Reaction Conditions

All anhydrous reactions were carried out under an atmosphere of dry nitrogen. The glass vessels, luer lock syringes, needles, and stirring bars were oven-dried at 110-140°C or flame-dried with a propane torch, and cooled to room temperature under a current of dry nitrogen. Micro-syringes were dried under vacuum using an oil pump at room temperature for at least 2 hours prior to use.

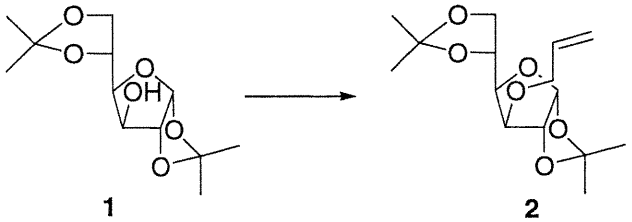
Temperature Control

The temperatures expressed in the reaction schemes and in the text of the experimental part are the temperatures of the outside of reaction vessels except where indicated.

-78°C	acetone-dry ice bath
-10°C (internal)	salt-ice-water bath
0°C	ice-water bath
room temperature	ambient temperature without any control

5-2 Experimental notes

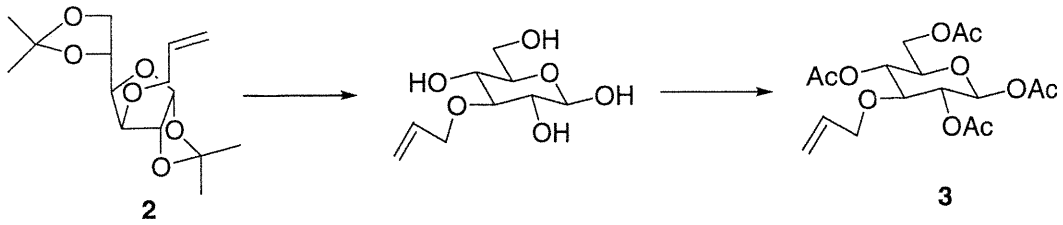
3-*O*-Allyl-1,2:5,6-di-*O*-isopropylidene-D-glucofuranose (2)



1 2

A mixture of **1**(1.0 g, 3.84 mmol) and NaH (60% in mineral oil, 0.2 g, 5.0 mmol) in DMF (30 ml) was stirred for 30 min at room temperature. Allyl bromide (excess) was then added and the reaction mixture was stirred overnight. The reaction mixture was concentrated *in vacuo*, and the crude was used in the next step without further purification.

3-*O*-Allyl-1, 2, 4, 6-tetra-*O*-acetyl-*β*-D-glucopyranoside (3)



2 3

The crude compound **2** and Amberlite IR-200 (H^+) ion-exchange resin (2 g) in water (100 ml) were heated overnight at 90° C. The resin was filtered, and the solution concentrated *in vacuo*. The residue was dissolved in MeOH, and toluene was added and concentrated again. The resulting crude product of 3-allyl glucose was then dissolved in a mixture of Ac_2O (50 ml), pyridine (50 ml), and Et_3N (1.5 ml). The reaction was completed after 4h,

and the mixture was concentrated. The crude was crystallized from hexane-ethyl acetate to yield **3** as colorless crystals (4.94 g, 66% in three steps from **1**).

m.p. +108°C

$[\alpha]_D$ -2.2 (c 0.75, $CHCl_3$)

^{1}H-NMR (300 Hz, $CDCl_3$): δ (ppm) =5.79-5.66(ddd, 1H, vinyl-H), 5.59(d, 1H, $J_{1,2}$=8.2Hz, 1-H), 5.19-5.02(m, 4H, 2-H, 4-H and vinyl-Hs), 4.18(dd, 1H, $J_{5,6}$=4.9Hz and $J_{6'6}$=12.5Hz, 6-H), 4.06-4.02(m, 3H, 6'-H and allyl-Hs), 3.71-3.66(m, 1H, 5-H), 3.60(t, 1H, J =9.3Hz, 3-H)

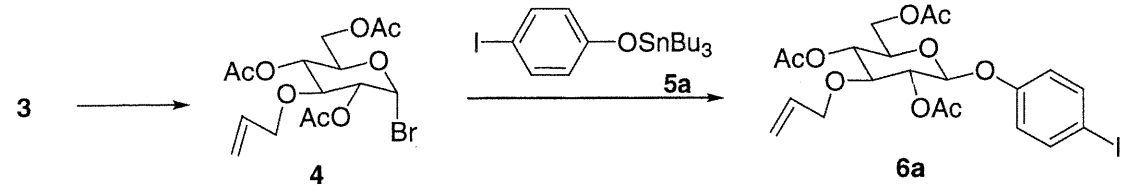
^{13}C-NMR (300Hz, $CDCl_3$): δ (ppm)= 171.18(0), 169.67(0), 169.49(0), 134.43(0), 117.69(-), 92.36, 80.08, 73.56(-), 73.39, 71.87, 69.37, 62.20(-), 21.31, 21.25, 21.22, 21.18

MS: (FAB, *m/z*) 387.1(m-1)

HRMS: $C_{17}H_{24}O_{10;}$ Calcd: 388.13695; Found: 388.1356

IR: 1741.0(br), 1370.1, 1222.5(br), 1063.6(br), 910.3 cm^{-1}

4-Iodophenyl 2,4,6-tri-*O*-acetyl-3-*O*-allyl-*β*-D-glucopyranoside (6a)



To a solution of compound **3** (2.38 g, 6.13 mmol) in dichloromethane (20 ml) at 0° C, was added dropwise HBr (30 wt %in acetic acid, 14 ml). After stirring for 20 min at 0° C, the reaction mixture was diluted with CH_2Cl_2, washed with cold water twice, cold $NaHCO_3$ saturated aqueous solution and cold water again, dried over Na_2SO_4, and concentrated. The crude 3-*O*-ally-2,4,6-tri-*O*-acetyl glucopyranosyl bromide **4** was dissolved in dry CH_2Cl_2 and used in the next step without further purification. 4-iodophenol (1.35 g, 6.13 mmol) and bis-[tri-n-butyltin]oxide (1.56 ml) were refluxed (100° C) in benzene (250 ml)

with a Dean-Stark apparatus. After 1h, the solvent was removed *in vacuo* and the crude tributyl stannyl 4-iodophenoxide **5a** was dissolved in anhydrous dichloromethane for the next step. To a solution of crude **4** and crude **5a** in CH_2Cl_2 (30 ml), $SnCl_4$ (1 M in CH_2Cl_2, 6.13 ml) was added dropwise at 0° C (bath). After stirring 30 min at 0° C, the reaction mixture was allowed to warm to room temperature, and stirred for 4h. The reaction was quenched with dilute $NaHCO_3$ and ether, and the organic layer washed with water, dried over Na_2SO_4, and concentrated. The crude was purified by flash chromatography (ethylacetate-hexane 1:2) to yield **6a** (2.84, 85 %) as a white solid.

$[\alpha]_D$ -22.0 (c 1.065, CH_3Cl)

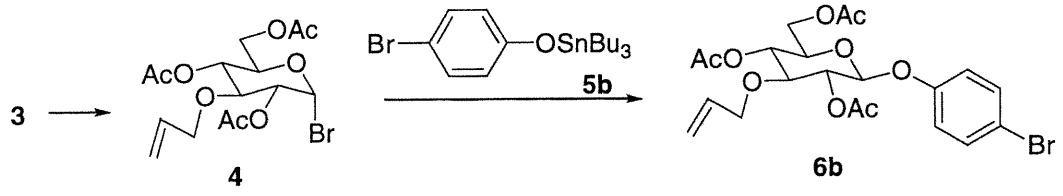
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) =7.59-7.55(m, 2H), 6.77-6.74(m, 2H), 5.82-5.75(m 1H, vinyl-H), 5.26-5.10(m, 4H, 2-H, 4-H and vinyl-Hs), 4.94(d, 1H, $J_{1,2}$=7.8Hz, 1-H), 4.22(dd, $J_{5,6}$=5.8Hz and $J_{6',6}$=12.3Hz, 6-H), 4.14(dd, 1H, $J_{5,6'}$=2.7Hz, 6'-H), 4.11-4.09(fm, 2H, allyl-Hs), 3.77-3.73(m, 1H, 5-H), 3.68(t, $J_{3,4}$=$J_{2,3}$=9.3Hz, 3-H), 2.10(s, 6H), 2.07(s, 3H)

^{13}C-NMR (300Hz, $CDCl_3$): δ (ppm)=170.99(0), 169.68(0), 169.46(0), 157.23(0), 138.77, 134.46, 119.55, 117.63(-), 99.49, 86.29(0), 79.90, 73.36(-), 72.74, 72.52, 69.77, 62.65(-), 21.46, 21.26, 21.13

MS: (FAB, *m/z*) 548.0

IR: 1758.3, 1727.8, 1487.9, 1374.9, 1265.5, 1229.0 (br), 1057.1 cm^{-1}

4-Bromophenyl 2, 4, 6-*O*-acetyl-3-*O*-allyl-*β*-D-glucopyranoside (6b)



Prepared as described for **6a**.Yield: 1.64 g, 83%, colorless crystals.

m.p. 97-98°C.

$[\alpha]_D$ -16.8 (c1.18, $CHCl_3$)

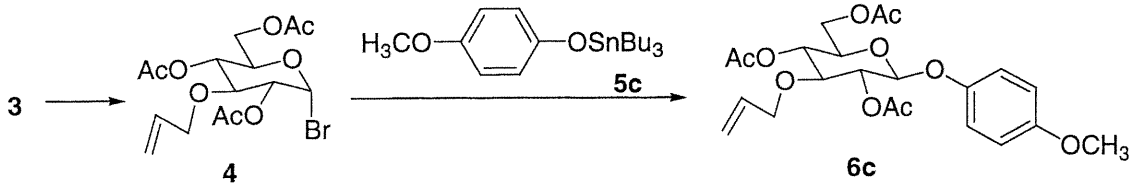
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) =7.37(ddd, 2H), 6.86(ddd, 2H), 5.82-5.75(m 1H, vinyl-H), 5.26-5.10(m, 4H, 2-H, 4-H and vinyl-Hs), 4.93(d, 1H, $J_{1,2}$=7.8Hz, 1-H), 4.22(dd, $J_{5,6}$=5.4Hz and $J_{6'6}$=12.3Hz, 6-H), 4.15(dd, 1-H, $J_{5,6'}$=2.7Hz, 6'-H), 4.11-4.09(fm, 2H, allyl-Hs), 3.77-3.72(m, 1H, 5-H), 3.68(t, $J_{3,4}$=$J_{2,3}$=9.2Hz, 3-H), 2.10(s, 6H), 2.07(s, 3H)

^{13}C-NMR (300Hz, $CDCl_3$): δ (ppm)=171.05(0), 169.70(0), 169.49(0), 156.46(0), 134.46, 132.81, 119.13, 117.68(-), 116.03(0), 99.66, 79.91, 73.33(-), 72.77, 72.54, 69.76, 62.67(-), 21.29, 21.26, 21.15

MS: (FAB, *m/z*) 500.1

HRMS: $C_{21}H_{25}BrO_9$; Calcd: 500.06820; Found: 500.071289

4-Methoxyphenyl 2,4,6-tri-*O*-acetyl-3-*O*-allyl-*β*-D-glucopyranoside (6c)



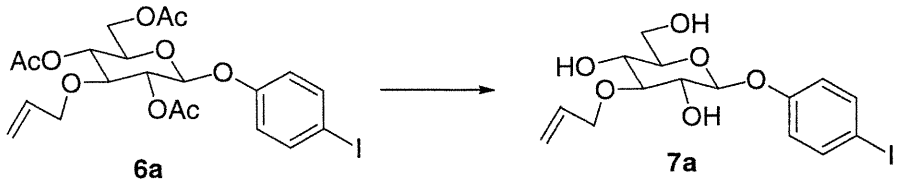
Prepared as described for **6a** to yield **6c** (1.5g, 56 % two steps from **3**) as a white solid.

^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 6.91(d, 2H), 6.78(d, 2H), 5.82-5.72(m, 1H, vinyl-H), 5.22-5.07(m, 4H, 2-H, 4-H, and vinyl-Hs), 4.84(d, 1H, $J_{1,2}$=7.9Hz, 1-H), 4.21(dd, 1H, allyl-H), 4.14-4.07(m, 3H, allyl-H, 6-H and 6'-H), 3.73(s, 3H, OCH_3), 3.71-3.67(m, 1H, 5-H), 3.65(t, 1H, $J_{2,3}$=$J_{3,4}$=9.3Hz, 3-H), 2.09(s, 3H, $COCH_3$), 2.07(s, 3H, $COCH_3$), 2.04(s, 3H, $COCH_3$)

^{13}C-NMR (300Hz, $CDCl_3$): δ (ppm): 171.04(0), 169.72(0), 169.53(0), 155.96(0), 151.49(0), 134.55, 118.87, 117.48(-), 114.84, 100.82, 80.08, 73.33(-), 72.71, 72.51, 69.88, 62.68(-), 55.99, 21.29, 21.23, 21.13

MS: (FAB, *m/z*) 452.2

4-Iodophenyl 3-*O*-allyl-*β*-D-glucopyranoside (7a)

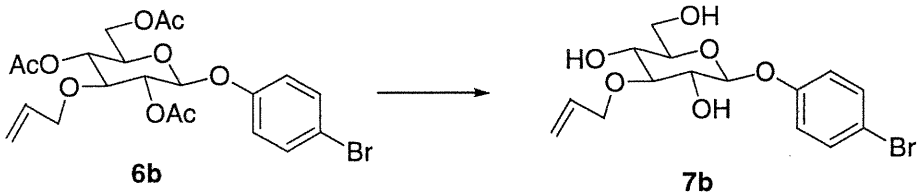


To a solution of **6a** (1.0 mmol) in dried MeOH (0.14M), was added a catalytic amount of NaOMe (pH~9). After TLC (CH_2Cl_2-MeOH 5:1) indicated that the starting material was consumed completely, the reaction was stirred for an additional 2h. The solution was neutralized with Amberlite IR-200 (H^+) ion-exchange resin, filtered, and the filtrate was evaporated to a yellowish residue that was normally used in the next step without further purification.

^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) =7.59(dt, 2H), 6.91(dt, 2H), 6.08-5.98(m 1H, vinyl-H), 5.30(ddt, 1H, vinyl-H), 5.14(ddt, 1H,, vinyl-H), 4.88(d, 1H, $J_{1,2}$=7.8Hz, 1-H), 4.44-4.34(m, 2H, ally-Hs), 3.88(dd, $J_{5,6}$=2.0Hz and $J_{6',6}$=12.1Hz, 6-H), 3.69(dd, 1H, $J_{5,6'}$=5.3Hz, 6'-H), 3.52(dd, $J_{2,3}$=9.1Hz, 2-H), 3.47-3.44(m, 2H), 3.36-3.31(m, 2H)

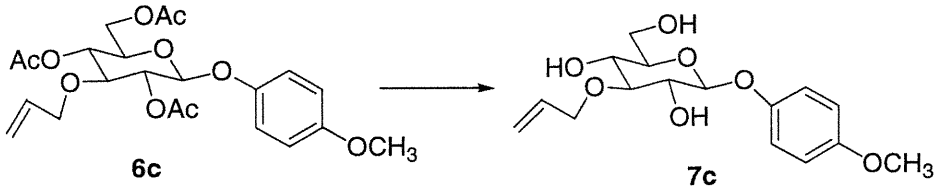
^{13}C-NMR (400Hz, $CDCl_3$): δ (ppm)=159.210), 139.57, 137.11, 120.31, 116.91(-), 102.29, 85.84, 85.51(0), 78.20, 75.33(-), 75.02, 71.13, 62.53(-)

4-Bromophenyl 3-*O*-allyl-*β*-D-glucopyranoside (7b)



Prepared as described for **7a.** Crude **7b** as a yellowish solid was used directly in the next step without further purification.

4-Methoxyphenyl 3-*O*-allyl-*β*-D-glucopyranoside (7c)



Prepared as described for **7a.** Crude as a yellowish solid could be used directly in the next step without further purification

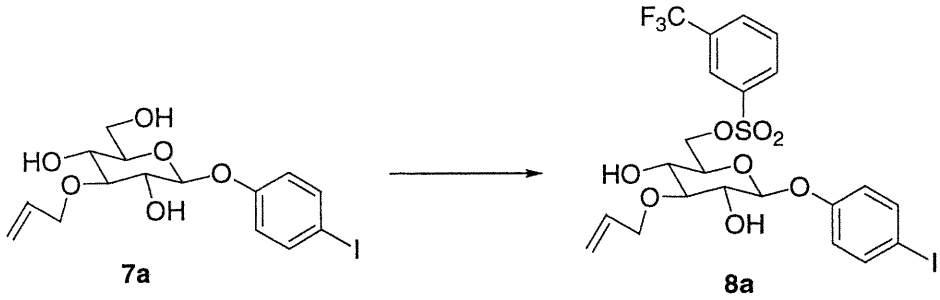
$[\alpha]_D$ -35.5 (c 0.55, MeOH)

^{1}H-NMR (400 Hz, CD_3OD): δ (ppm) = 7.06(dd, 2H0, 6.82(dd, 2H), 6.08-5.98(m, 1H, vinyl-H), 5.30(dd, 1H, vinyl-H), 5.06(dd, 1H, vinyl-H), 4.77(d, 1H, $J_{1,2}$=7.9Hz, 1-H), 4.44-4.34(m, 2H, allyl-Hs), 3.88(dd, 1H, 6-H), 3.78-3.68(m, 4H, OCH_3, and 6'-H), 3.52-3.42(m, 2H, 2-H and 4-H), 3.41-3.25(m, 1H, 5-H),

^{13}C-NMR (300z, $CDCl_3$): δ (ppm) =157.03(0), 153.62(0), 137.38, 119.60, 117.19(-), 115.83, 103. 83, 86.17, 78.35, 75.58(-), 75.41, 71.47, 62.85(-), 56.43

MS: (FAB, *m/z*) 326.1

4-Iodophenyl 3-*O*-allyl-6-*O*-(*m*-trifluoromethyl)benzenesulfonyl-*β*-D-glucopyranoside (8a)



To a solution of **7a** (42 mg, 0.1 mmol) in dry pyridine at 0° C, 3-(trifluoromethyl)benzene sulfonyl chloride (1.2 eq) was added dropwise. The reaction was stirred for 1h at 0° C, and then allowed to warm to room temperature. After TLC indicated the completion of the reaction, the solvent was removed *in vacuo*, the residue dissolved in CH_2Cl_2, washed with water, 1N HCl, and water again, the organic layer separated and concentrated. The

crude was purified by chromatography (EtOAc/Hex 3:5) to yield **8a** (53mg, 85 %) as a white solid (foam).

$[\alpha]_D$ -33.3 (c1.5, CH_3Cl)

^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) =8.14(s, 1H), 8.01(d, 1H), 7.81(d, 1H), 7.57-7.52(m, 3H), 6.73-6.69(m, 2H), 6.00-5.91(m, 1H, vinyl-H), 5.32(dd, 1H, vinyl-H), 5.23(dd, 1H, vinyl-H), 4.77(d, 1H, $J_{1,2}$=7.7Hz, 1-H), 4.49-4.25(m, 4H, 6-H, 6'-H and allyl-Hs), 3.67-3.62(m, 2H, 2-H and 5-H), 3.54(dt, 1H, $J_{3,4}$=$J_{4,5}$=8.9Hz, $J_{4,\ OH}$=2.8Hz, 4-H), 3.78(t, 1H, $J_{2,3}$=8.9Hz, 3-H), 2.65(d, 1H, 2-OH), 2.52(d, 1H, 4-OH)

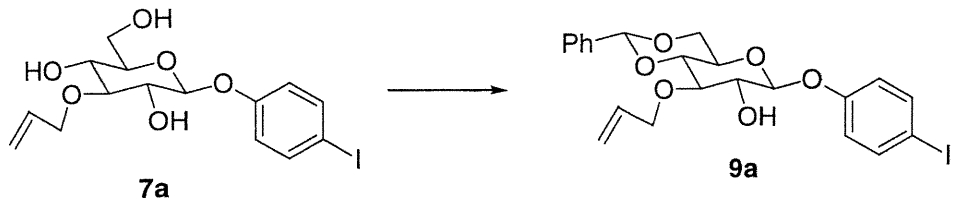
^{13}C-NMR (300Hz, $CDCl_3$): δ (ppm) =156.46(0), 138.27, 136.76(0), 134.50, 130.96, 130.36(0), 130.33, 124.88(0), 124.84, 118.79, 117.68(-), 100.36, 85.67(0), 82.84, 73.81(-), 73.64, 73.16, 69.00(-), 68.72

MS: (FAB, *m/z*) 630.0

HRMS: $C_{22}H_{22}F_3IO_8S$; Calcd: 630.00322; Found: 630.00387

IR: 3571.6, 3422.0(br), 1485.5, 1327.0, 1239.3, 1187.3, 1139.5, 1108.6, 1075.5, 1056.7cm^{-1}

4-Iodophenyl 3-*O*-allyl-4, 6-*O*-benzylidene-β-D-glucopyranoside (9a)



To a solution of **7a** (422 mg, 1.0 mmol) in DMF (18 ml) were added benzaldehyde dimethyl acetal (2.0 mmol, 2 eq) and tetrafluoric acid (54% in ether, 2.1 ml, 1.5 eq). The reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure. The residue was dissolved in $CHCl_3$, filtered with a short block of silica gel, washed with excess of $CHCl_3$ and then concentrated. The crude was purified by chromatography (Hex/EtOAc 1:5) to yield **9a** (443 mg, 87 %) as a white solid.

$[\alpha]_D$ -11.7 (c 0.75, $CHCl_3$)

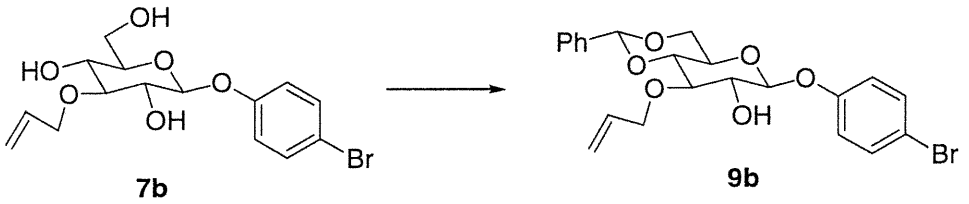
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) =7.60(dd, 2H), 7.50-7.48(m, 2H), 7.40-7.26(m, 3H), 6.83(dd, 2H), 6.02-5.92(m, 1H, vinyl-H), 5.57(s, 1H, CHPh), 5.32(dq, 1H, vinyl-H), 5.22(dq, 1H, vinyl-H), 5.00(d, 1H, $J_{1,2}$=7.7Hz, 1-H), 4.48(dq, 1H, allyl-H), 4.36(dd, 1H, $J_{5,6}$=5.0Hz, $J_{6,6'}$=10.5Hz, 6-H), 4.29(dq, 1H, allyl-H), 3.84-3.64(m, 4H, 2-H, 3-H, 4-H and 6'-H), 3.59-3.55(m, 1H, 5-H), 2.68(d, 1H, 2-OH)

^{13}C-NMR (300Hz, $CDCl_3$): δ (ppm) =157.10(0), 138.88, 137.44(0), 135.03, 129.52, 128.73, 126.39, 119.70, 118.13(-), 101.72, 101.43, 86.40(0), 81.46, 80.32, 74.16(-), 74.10, 68.95(-), 67.00

MS: (FAB, *m/z*) 511.0 (m+1)

HRMS: $C_{22}H_{24}O_6I$, Calcd: 511.06177; Found: 511.05980

4-Bromophenyl 3-*O*-allyl-4, 6-*O*-benzylidene-β-D-glucopyranoside (9b)



Prepared as described for **9a** to yield **9b** (140 mg, 88% two steps from **6b**) as a white solid.

$[\alpha]_D$ -2.6 (c 0.33, $CHCl_3$)

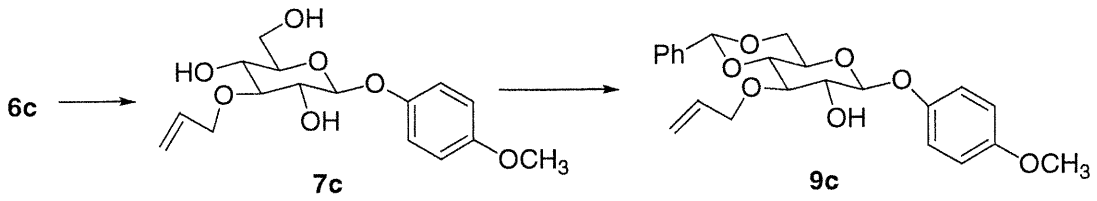
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 7.51-7.48(m, 2H), 7.43-7.38(m, 5H), 6.95(ddd, 2H), 6.01-5.93(m, 1H, vinyl-H), 5.58(s, 1H, CHPh), 5.34(dq, 1H, vinyl-H), 5.22(dd, 1H, vinyl-H), 5.00(d, 1H, $J_{1,2}$=7.7Hz, 1-H), 4.50-4.45(m, 1H, allyl-H), 4.37(dd, 1H, $J_{5,6}$ =4.9Hz, $J_{6,6'}$=10.4Hz, 6-H), 4.33-4.28(m, 1H, allyl-H), 3.84-3.65(m, 4H), 3.58-3.55(m, 1H, 5-H),

^{13}C-NMR (400Hz, $CDCl_3$): δ (ppm) = 156.14(0), 137.26(0), 134.87, 132.69, 129.29, 128.50, 126.19, 119.10, 117.86(-), 115.88(0), 101.52, 101.48, 81.26, 80.15. 73.94, 73.92(-), 68.75(-), 66.79

MS: (FAB, *m/z*) 463

HRMS: $C_{22}H_{24}O_6{}^{79}Br$ Calcd: 463.07562; Found: 463.07380

4-Methoxyphenyl 3-*O*-allyl-4,6-*O*-benzylidene- *β*-D-glucopyranoside (9c)



Prepared as described for **9a** to yield **9c** (210 mg, 80% two steps from **6c**) as a white solid.

[α]$_D$ -29.0 (c 0.755, $CHCl_3$)

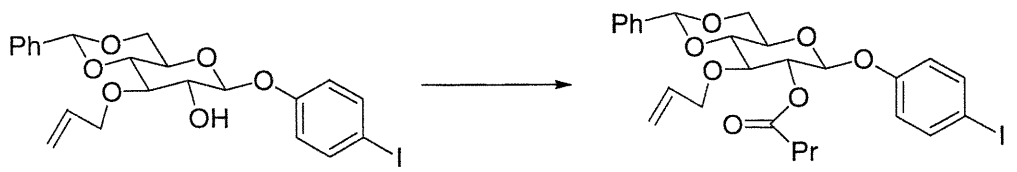
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 7.50(dd, 2H), 7.42-7.37(m, 3H), 7.02(dd, 2H), 6.84(dd, 2H), 6.03-5.93(m, 1H, vinyl-H), 5.57(s, 1H, CHPh), 5.33(dd, 1H, vinyl-H), 5.21(dd, vinyl-H), 4.92(d, 1H, $J_{1,2}$=7.7Hz, 1-H), 4.50-4.45(m, 1H, allyl-H), 4.36(dd, 1H, $J_{5,6}$=5.1Hz, $J_{6,6'}$=10.1Hz, 6-H), 4.34-4.27(m, 1H, allyl-H), 3.85-3.65(m, 7H), 3.55-3.49(m, 1H, 5-H), 2.80(d, 1H, 2-OH)

^{13}C-NMR (300Hz, $CDCl_3$): δ (ppm) = 156.06(0), 151.31(0), 137.57(0), 135.20, 129.44, 128.68,126.40, 119.17, 117.93(-), 114.98, 102.94, 101.67, 81.58, 80.37. 74.29, 74.09(-), 69.06(-), 66.90, 56.04

MS: (FAB, *m/z*) 415.2(m+1)

HRMS: $C_{23}H_{27}O_7$; Calcd: 415.17568; Found: 415.17876

4-Iodophenyl 2-*O*-butyryl-3-*O*-allyl-4, 6-*O*-benzylidene-*β*-D-glucopyranoside (10a)



9a **10a**

To a solution of **9a** (510 mg, 1.0 mmol) in CH_2Cl_2, Et_3N (1.2 mmol) and butyryl chloride (1.2 mmol) was added. The reaction mixture was stirred overnight at room temperature, then Et_3N (0.5 mmol) and MeOH (0.5mmol) was added and stirred for 1h. The reaction mixture was diluted with CH_2Cl_2, washed with water, 1N HCl, and water, dried over Na_2SO_4, and concentrated. The crude was chromatographed (Hex/EtOAc 3:1) to yield **10a** (522mg, 90%) as a white solid.

$[\alpha]_D$ -11.6 (c 0.58, $CHCl_3$)

^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm)= 7.58(dd, 2H), 7.49(dd, 2H), 7.41-7.36(m, 3H), 6.76(dd, 2H), 5.90-5.77(m, 1H, vinyl-H), 5.59(s, 1H, CHPh), 5.29-5.22(m, 2H, 2-H and vinyl-H), 5.15(fdq, 1H, vinyl-H), 5.05(d, 1H, $J_{1,2}$=7.8Hz, 1-H), 4.40-4.34(m, 2H, 6-H and allyl-H), 4.14(dq, 1H, allyl-H), 3.86-3.73(m, 3H, 3-H, 4-H and 6'-H), 3.62-3.54(m, 1H, 5-H), 2.33(t, 2H), 1.75-1.61(dt, 2H), 0.95(t, 3H)

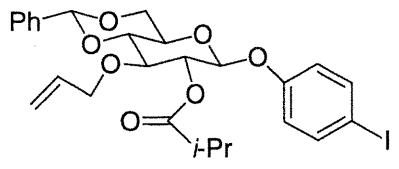
^{13}C-NMR (400Hz, $CDCl_3$):): δ (ppm)= 172.08(0), 156.99(0), 138.66, 137.21(0), 134.72, 129.29, 128.49, 126.18, 119.29, 117.16(-), 101.49, 99.88, 86.09(0), 81.24, 78.60, 73.47(-), 72.38, 68.73(-), 66.70, 36.32(-), 18.67(-), 13.83

MS: (FAB, *m/z*) 581.0(m+1)

HRMS: $C_{26}H_{30}O_7I$; Calcd: 581.10364; Found: 581.10140

IR (film) 1746.2, 1483.9, 1383.9, 1242.0, 1098.5, 1078.2 cm^{-1}

4-Iodophenyl 3-*O*-allyl-4,6-*O*-benzylidene-2-*O*-*iso*butyryl-*β*-D-glucopyranoside (10b)



Prepared as described for **10a** to yield **10b** (165 mg, 87 %) as colorless crystals.

m.p. 179°C

$[\alpha]_D$ -11.4 (c 0.175, $CHCl_3$)

^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm)= 7.61-7.57(fm, 2H), 7.52-7.48(fm, 2H), 7.41-7.38(fm, 3H), 6.78-6.75(fm, 2H), 5.94-5.80(m, 1H, vinyl-H), 5.60(s, 1H, CHPh), 5.29-5.22(m, 2H, 2-H and vinyl-H), 5.16(dd, 1H, vinyl-H), 5.05(d, 1H, $J_{1,2}$=7.9Hz, 1-H), 4.43-4.36(m, 2H, 6-H and allyl-H), 4.20-4.10(m, 1H, allyl-H), 3.88-3.76(m, 3H, 6-H, 4-H, and 3-H), 3.65-3.52(m, 1H, 5-H), 2.64-2.52(m, 1H), 1.20-1.60(m, 6H)

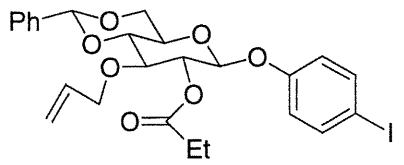
^{13}C-NMR (400Hz, $CDCl_3$): δ (ppm)=175.84(0), 157.31(0), 138.92, 137.42(0), 134.89, 129.54, 128.75, 126.41, 119.49, 117.42(-), 101.74, 100.29, 86.36(0), 81.51, 78.89, 73.81(-), 72.51, 68.99(-), 66.97, 34.49, 19.53, 19.36

MS: (FAB, *m/z*) 581.0(m+1)

HRMS: $C_{26}H_{30}O_7I$; Calcd: 581.10364; Found: 581.10765

IR: 1745.5, 1485.0; 1236.6, 1081.4 cm^{-1}

4-Iodophenyl 3-*O*-allyl-4, 6-*O*-benzylidine-2-*O*-propionyl-*β*-D-glucopyranoside (10c)



Prepared as described for **10a** to yield **10c** (267 mg, 96 %) as a white solid.

$[\alpha]_D$ = -8.7(c 0.67, $CHCl_3$)

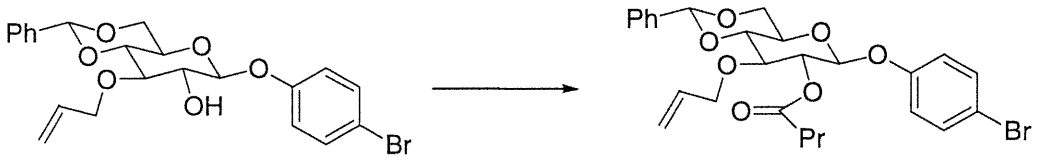
^{1}H-NMR (300 Hz, $CDCl_3$): δ (ppm) = 7.55(d, 2H), 7.47-7.44(m, 2H), 7.38-7.34(m, 3H), 6.74(d, 2H), 5.87-5.74(m, 1H, vinyl-H), 5.55(s, 1H, CHPh), 5.27-5.23(m, 2H, 2-H and vinyl-H), 5.12(dd, 1H, vinyl-H), 5.02(d, 1H, $J_{1,2}$=7.8Hz, 1-H), 4.38-4.32(m, 2H, 6-H and allyl-H), 4.17-4.08(dd, 1H, allyl-H), 3.84-3.68(m, 3H, 6-H, 4-H and 3-H), 3.59-3.51(m, 1H, 5-H), 2.33(q, 2H), 1.14(t, 3H)

^{13}C-NMR: (300 Hz, $CDCl_3$): δ (ppm) =173.22(0), 157.26(0), 138.92, 137.42(0), 134.96, 129.54, 128.74, 126.42, 119.59, 117.40(-), 101.73, 100.17, 86.38(0), 81.44, 78.86, 73.74(-), 72.74, 68.97(-), 66.95, 28.04(-), 9.69

MS: (FAB) *m/z* 566.1

IR: 1746.0, 1483.3, 1381.9, 1242.3, 1185.1, 1077.1 cm^{-1}

4-Bromophenyl 3-*O*-allyl-4, 6-*O*-benzylidene-2-*O*-butyryl-*β*-D-glucopyranoside (10d)



Prepared as described for **10a** to yield **10d** (129 mg, 95 %) as a white solid.

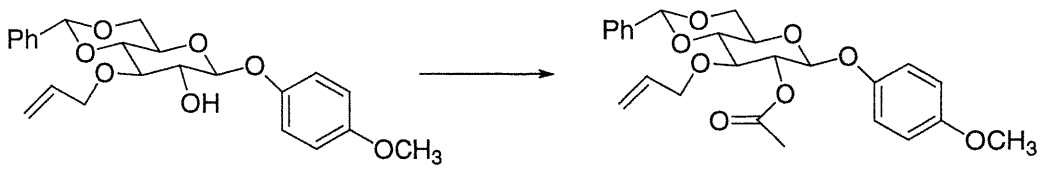
$[\alpha]_D$ -19.4 (0.7, $CHCl_3$)

^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm)= 7.58(dd, 2H), 7.49(dd, 2H), 7.41-7.36(m, 3H), 6.76(dd, 2H), 5.90-5.77(m, 1H, vinyl-H), 5.59(s, 1H, CHPh), 5.29-5.22(m, 2H, 2-H and vinyl-H), 5.15(fdq, 1H, vinyl-H), 5.05(d, 1H, $J_{1,2}$=7.8Hz, 1-H), 4.40-4.34(m, 2H, 6-H and allyl-H), 4.14(dq, 1H, allyl-H), 3.86-3.73(m, 3H, 3-H, 4-H and 6'-H), 3.62-3.54(m, 1H, 5-H), 2.33(t, 2H), 1.75-1.61(dt, 2H), 0.95(t, 3H)

^{13}C-NMR (400Hz, $CDCl_3$): δ (ppm)= 172.08(0), 156.99(0), 138.66, 137.21(0), 134.72, 129.29, 128.49, 126.18, 119.29, 117.16(-), 101.49, 99.88, 86.09(0), 81.24, 78.60, 73.47(-), 72.38, 68.73(-), 66.70, 36.32(-), 18.67(-), 13.83

MS: (FAB, *m/z*) 532.1

4-Methoxyphenyl 2-*O*-acetyl-3-*O*-allyl-4,6-*O*-benzylidene-*β*-D-glucopyranoside (10e)



9c **10e**

Prepared as described for **10a** to yield **10e** (87 mg, 90 %) as a white solid.

$[\alpha]_D$ -15.7 (c 1.105, $CHCl_3$)

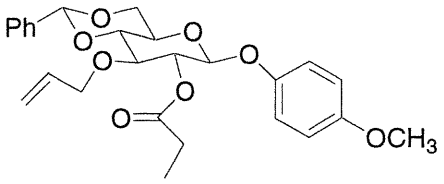
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 7.52-7.50(m, 2H), 7.41-7.39(m, 3H), 6.96(d, 2H), 6.84(d, 2H), 5.91-5.84(m, 1H, vinyl-H), 5.60(s, 1H, CHPh), 5.30-5.16(m, 3H, 2-H and vinyl-Hs), 5.00(d, 1H, $J_{1,2}$=7.9Hz, 1-H), 4.42-4.39(m, 2H, 6-H and allyl-H), 4.20-4.15(m, 1H, allyl-H), 3.89-3.73(m, 6H, 4-H, 6'-H, OCH_3 and 3-H), 3.59-3.53(m, 1H, 5-H), 2.14(s, 3H, $COCH_3$)

^{13}C-NMR (300z, $CDCl_3$): δ (ppm) = 169.70(0), 156.04(0), 151.48(0), 137.48(0), 135.03, 129.45, 128.68, 126.38, 118.95, 117.27(-), 114.96, 101.66, 101.44, 81.54, 78.93, 73.64(-), 73.14, 69.03(-), 66.83, 56.05, 21.28

MS: (FAB, *m/z*) 457.2(m+1)

HRMS: $C_{25}H_{29}O_8$; Calcd: 457.18625; Found: 457.18965

4-Methoxyphenyl 3-*O*-allyl-4,6-*O*-benzylidene-2-*O*-propionyl-β-D-glucopyranoside (10f)



Prepared as described for **10a** to yield **10f** (67 mg, 97 %) as a white solid.

$[\alpha]_D$ -17.6 (c 0.34, $CHCl_3$)

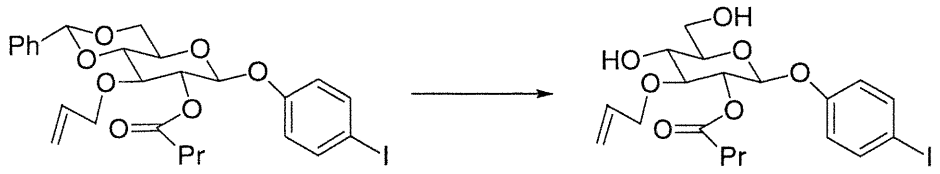
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 7.51-7.49(m, 2H), 7.41-7.38(m, 3H), 6.95(d, 2H), 6.83(d, 2H), 5.90-5.81(m, 1H, vinyl-H), 5.60(s, 1H, CHPh), 5.28-5.24(m, 2H, 2-H, and vinyl-H), 5.16(dd, 1H, vinyl-H), 4.98(d, 1H, $J_{1,2}$=7.9Hz, 1-H), 4.41-4.37(m, 2H, 6-H and allyl-H), 4.17-4.13(m, 1H, allyl-H), 3.88-3.72(m, 6H, 4-H, 6'-H, OCH_3 and 3-H), 3.58-3.52(m, 1H, 5-H), 2.39(q, 2H), 1.20(t, 3H)

^{13}C-NMR (300z, $CDCl_3$): δ (ppm) = 173.18(0), 156.01(0), 151.52(0), 137.49(0), 135.02, 129.44, 128.68, 126.38, 118.93, 117.24(-), 114.95, 101.66, 101.53, 81.54, 78.98, 73.65(-), 72.94, 69.04(-), 66.84, 56.04, 28.04(-), 9.67

MS: (FAB, *m/z*) 469.2(m-1)

HRMS: $C_{26}H_{29}O_8$; Calcd: 469.18724; Found: 469.19675

4-Iodophenyl 3-*O*-allyl-2-*O*-butyryl-*β*-D-glucopyranoside (11a)



10a **11a**

The reaction mixture of **10a** (140 mg, 0.24 mmol) and TFA (2.4 ml) in water/THF (5/20 ml) was refluxed at 85° C for 2h, and then the reaction was controlled by TLC (Hex/EtOAc 1:1). The crude was transferred to a separatory funnel and diluted with CH_2Cl_2, washed with $NaHCO_3$ saturated solution, aqueous phase extracted with CH_2Cl_2, organic phase combined, washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by chromatography (Hex/EtOAc, 2:1) to yield **11a** (100 mg, 98 %) as a white solid (foam).

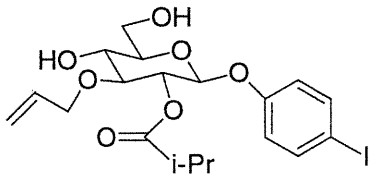
$[\alpha]_D$ -20.2 (c 0.575, $CHCl_3$)

^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm)=7.55(d, 2H), 6.71(d, 2H), 5.90-5.84(m, 1H, vinyl-H), 5.28-5.14(m, 3H, vinyl-Hs and 2-H), 4.96(d, 1H, $J_{1,2}$=7.9Hz, 1-H), 4.21-4.20(fm, allyl-Hs), 3.92(dd, 1H, $J_{5,6}$=3.2Hz, $J_{6,6'}$=12.1Hz, 6-H), 3.82(dd, 1H, $J_{5,6'}$=4.8Hz, 6'-H), 3.75(t, 1H, $J_{4,5}$=$J_{3,4}$=9.3Hz, 4-H), 3.54-3.48(m, 2H, 3-H and 5-H), 2.30(t, 2H), 1.65(tq, 2H), 0.93(t, 3H)

^{13}C-NMR (400Hz, $CDCl_3$): δ (ppm)= 172.24(0), 157.04(0), 138.66, 134.67, 118.94, 117.69(-), 99.25, 85.77(0), 82.24, 75.90, 73.63(-), 72.43, 70.14, 62.35(-), 36.40(-), 18.61(-), 13.82

MS: (FAB, *m/z*) 493.1(m+1), 515.1(m+23)

4-Iodophenyl 3-*O*-allyl-2-*O*-*iso*butyryl-*β*-D-glucopyranoside (11b)



Prepared as described for **11a** to yield **11b** (154 mg, 88 %) as a white solid.

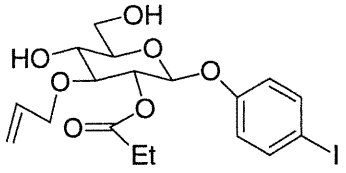
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 7.60-7.55(fm, 2H), 6.74-6.70(fm, 2H), 5.91-5.82(m, 1H, vinyl-H), 5.31-5.15(m, 3H, 2-H and vinyl-Hs), 4.97(d, 1H, $J_{1,2}$=7.9Hz, 1-H), 4.23-4.20(m, 2H, allyl-Hs), 3.96(dd, 1H, 6-H), 3.87-3.75(m, 2H, 6'-H and 4-H), 3.58-3.51(m, 2H, 3-H and 5-H), 2.60-2.53(m, 1H), 1.19-1.15(m, 6H)

^{13}C-NMR (400z, $CDCl_3$): δ (ppm) = 175.76(0), 157.11(0), 138.68, 134.62, 118.89, 117.73(-), 99.44, 85.82(0), 82.24, 75.89, 73.69(-), 72.42, 70.10, 62.33(-), 34.33, 19.35, 18.98

MS: (FAB, *m/z*) 493.1(m+1), 515.1(m+23)

HRMS: $C_{19}H_{26}O_7I$, Calcd: 493.07233; Found: 493.07220

4-Iodophenyl 3-*O*-allyl-2-*O*-propionyl-*β*-D-glucopyranoside (11c)



Prepared as described for **11a** to yield **11c** (250 mg, 82 %) as a white solid.

$[\alpha]_D$ -12.7(c 1.05, $CHCl_3$)

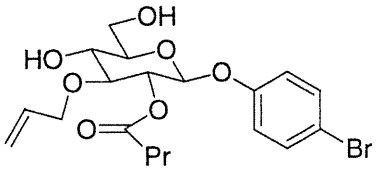
^{1}H-NMR (300 Hz, $CDCl_3$): δ (ppm) = 7.53(d, 2H), 6.70(d, 2H), 5.94-5.78(m, 1H, vinyl-H), 5.27-5.12(m, 3H, 2-H and vinyl-Hs), 4.94(d, 1H, $J_{1,2}$=7.9Hz, 1-H), 4.17(d, 2H, allyl-Hs), 3.95-3.88(m, 1H, 6-H), 3.84-3.70(m, 2H, 6-H and 4-H), 3.53-3.46(m, 2H, 5-H and 3-H), 2.83(d, 1H, 4-OH), 2.32(q, 2H), 2.15(t, 1H, 6-OH), 1.13(t, 3H)

^{13}C-NMR (300z, $CDCl_3$): δ (ppm)=173.32(0), 157.31(0), 138.93, 134.83, 119.22, 118.05(-), 99.58, 86.10(-), 82.55, 76.09, 73.92(-), 72.83, 70.35, 62.66(-), 28.13(-), 9.65

MS: (FAB, *m/z*) 478.1

IR: 3354.1, 1748.4, and 1485.2cm^{-1}

4-Bromophenyl 2-*O*-butyryl-3-*O*-allyl-*β*-D-glucopyranoside (11d)



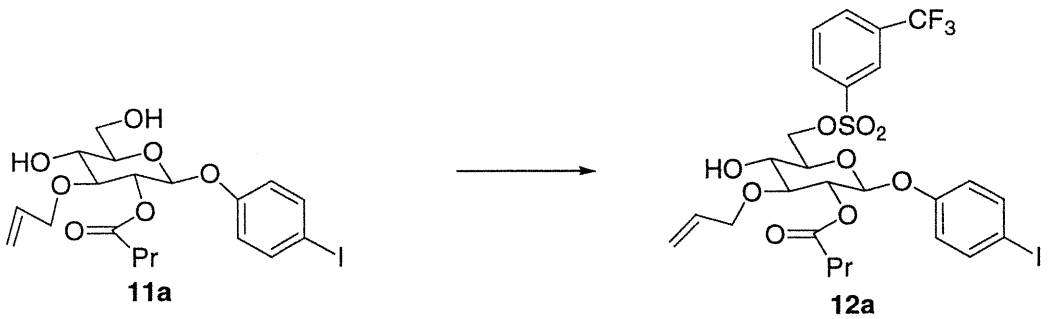
Prepared as described for **11a** to yield **11d** (98 mg, 76 %) as a white solid.

^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm)=7.55(d, 2H), 6.71(d, 2H), 5.90-5.84(m, 1H, vinyl-H), 5.28-5.14(m, 3H, vinyl-Hs and 2-H), 4.96(d, 1H, $J_{1,2}$=7.9Hz, 1-H), 4.21-4.20(fm, allyl-Hs), 3.92(dd, 1H, $J_{5,6}$=3.2Hz, $J_{6,6'}$=12.1Hz, 6-H), 3.82(dd, 1H, $J_{5,6'}$=4.8Hz, 6'-H), 3.75(t, 1H, $J_{4,5}$=$J_{3,4}$=9.3Hz, 4-H), 3.54-3.48(m, 2H, 3-H and 5-H), 2.30(t, 2H), 1.65(tq, 2H), 0.93(t, 3H)

^{13}C-NMR (400Hz, $CDCl_3$): δ (ppm)= 172.24(0), 157.04(0), 138.66, 134.67, 118.94, 117.69(-), 99.25, 85.77(0), 82.24, 75.90, 73.63(-), 72.43, 70.14, 62.35(-), 36.40(-), 18.61(-), 13.82

MS: (FAB, *m/z*) 444.1

4-Iodophenyl 3-*O*-allyl-2-*O*-butyryl-6-*O*-(*m*-trifluoromethyl)benzenesulfonyl-*β*-D-glucopyranoside (12a)



To a solution of **11a** (0.14 mmol) in pyridine (2.0 ml) containing catalytic amount of DMAP (0.01mmol), 3-(trifluoromethyl)benzenesulfonyl chloride (27 µl, 1.2 eq) was added at 0° C and stirred for 30 min. Then the reaction mixture was allowed to warm to room temperature and stirred for 2h. The solvent was removed *in vacuo*, the residue dissolved in CH_2Cl_2, washed with water, 1N HCl, and water again, the organic layer separated and concentrated. The crude was purified by chromatography (Hex/EtOAC 5:2) to yield **12a** (92 mg, 94 %) as a white solid (foam).

$[\alpha]_D$ -28.8 (c 0.7, $CHCl_3$)

^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) =8.15(dd, 1H, J=0.6Hz and 1.2Hz), 8.02(dd, 1H, *J*=8.5Hz and 0.5Hz), 7.82(dd, 1H, *J*=7.9Hz and 0.5Hz), 7.58-7.53(m, 3H), 6.66-6.63(m, 2H), 5.92-5.82(m, 1H, vinyl-H), 5.31-5.20(m, 2H, vinyl-Hs), 5.12(dd, $J_{1,2}$=7.9Hz and $J_{2,3}$=9.4Hz, 2-H), 4.87(d, 1H, 1-H), 4.49(dd, 1H, $J_{5,6}$=1.5Hz and $J_{6,6'}$=11.0Hz, 6-H), 4.36(dd, 1H, $J_{6',5}$=5.2Hz, 6'-H), 4.22-4.17(m, 2H, allyl-Hs), 3.72-3.61(m, 2H, 4-H and 5-H), 3.49 (dd, 1H, $J_{3,4}$=9.3Hz, 3-H), 2.31(t, 2H, *J*=7.3Hz), 1.70-1.63(m, 2H), 0.94(t, 3H, *J*=7.3Hz)

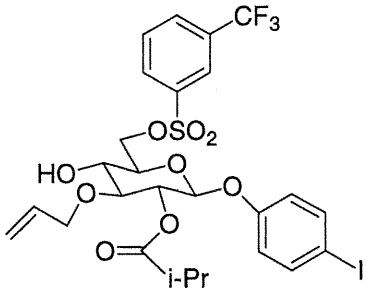
^{13}C-NMR (400Hz, $CDCl_3$): δ (ppm) =171.71(0), 156.50(0), 138.31, 137.00(0), 134.08, 131.02, 130.42, 129.95, 124.87, 118.70, 117.74(-), 98.86, 85.73(0), 81.75, 73.36(-), 73.32, 71.88, 69.01, 68.93(-), 36.06(-), 18.28(-), 13.50

MS: (FAB, *m/z*) 701.2(m+1)

HRMS: $C_{26}H_{29}O_9ISF_3$, Calcd: 701.05292; Found: 701.05570

IR (neat/NaCl): 1743.6cm^{-1}

4-Iodophenyl 3-*O*-allyl-2-*O*-*iso*butyryl-6-*O*-(*m*-trifluoromethyl)benzenesulfonyl-β-D-glucopyranoside (12b)



Prepared as described for **12a** to yield **12b** (36 mg, 90 %) as a white solid.

$[\alpha]_D$ -17.4 (c 0.5, $CHCl_3$)

^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 8.15(s, 1H), 8.03(d, 1H), 7.82(d, 1H), 7.58-7.51(m, 3H), 6.66-6.62(fm, 2H), 5.92-5.82(m, 1H, vinyl-H), 5.30-5.18(m, 2H, vinyl-Hs), 5.10(dd, 1H, $J_{2,3}$=9.2Hz, 2-H), 4.86(d, 1H, $J_{1,2}$=7.9Hz, 1-H), 4.49(dd, $J_{5,6}$=1.3Hz, $J_{6,6'}$=10.9Hz, 6-H), 4.37(dd, 1H, $J_{5,6'}$=5.1Hz, 6'-H), 4.21-4.18(m, 2H, allyl-Hs), 3.74-3.62(m, 2H, 5-H and 4-H), 3.51(t, 1H, J=9.2Hz, 3-H), 2.62-2.49(m, 1H), 1.18-0.86(m, 6H)

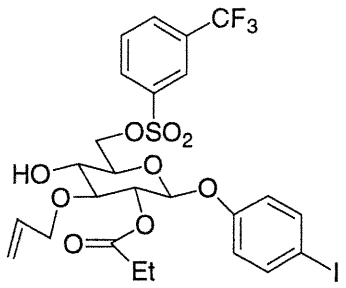
^{13}C-NMR (300z, $CDCl_3$): δ (ppm) =175.31(0), 156.68(0), 136.32, 136.78, 134.13, 131.05, 130.46, 130.42, 129.99, 124.88, 124.84, 118.64, 117.64(-), 98.98, 85.60(0), 81.74, 73.46(-), 73.34, 71.88, 69.09(-), 34.00, 19.01, 18.64

MS: (FAB, *m/z*) 701.2(m+1)

HRMS: $C_{26}H_{29}O_9ISF_3$, Calcd: 701.05292; Found: 701.05765

IR (neat/NaCl): 1742.4, 1485.0, 1370.9, 1327.5, 1236.1, 1185.5, 1136.7, and 1074.8cm^{-1}.

4-Iodophenyl 3-*O*-allyl-2-*O*-propionyl-6-*O*-(*m*-trifluoromethyl)benzenesulfonyl-*β*-D-glucopyranoside (12c)



Prepared as described for **12a** to yield **12c** (200 mg, 95 %) as a white solid.

$[\alpha]_D$ -24.9 (c 0.515, $CHCl_3$)

^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 8.14(s, 1H), 8.02(d, 1H), 7.81(d, 1H), 7.57-7.52(m, 3H), 6.64(d, 2H), 5.91-5.81(m, 1H, vinyl-H), 5.30-5.18(m, 2H, vinyls-Hs), 5.10(dd, 1H, $J_{2,3}$=9.0Hz, 2-H), 4.87(d, 1H, $J_{1,2}$=7.9Hz, 1-H), 4.48(dd, $J_{5,6}$=1.2

$J_{6,6'}$=11.3Hz, 6-H), 4.36(dd, 1H, $J_{5,6'}$=4.9Hz, 6'-H), 4.22-4.17(m, 2H, allyl-Hs), 3.74-3.64(m, 2H, 4-H and 5-H), 3.49(t, *J*=9.3Hz, 3-H), 2.99(s, 1H), 2.34(q, 2H), 1.15(t, 3H)

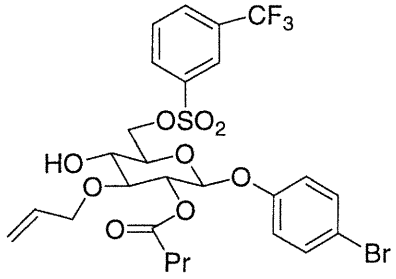
^{13}C-NMR (300z, $CDCl_3$): δ (ppm)=173.27(0), 157.15(0), 138.84 137.20(0), 134.66, 131.59, 131.04, 131.0, 130.54, 125.44, 125.39, 119.26, 118.23(-), 99.37, 86.18(0), 82.25, 74.05(-), 73.84, 72.54, 69.57(-), 69.52, 28.09(-), 9.61

MS: (FAB, *m/z*) 686.0 $[M]^+$

HRMS: $C_{25}H_{26}F_3IO_9S$ calcd 686.02944; found 686.02488

IR: 3514.2, 3077.6, 2982.6, 2883.4, 1745.1, 1611.1, 1586.7, 1484.5, 1369.6, 1327.0, 1236.4, 1184.6, 1136.6, and 1074.2 cm^{-1}

4-Bromophenyl 3-*O*-allyl-2-*O*-butyryl-6-*O*-(*m*-trifluoromethyl)benzenesulfonyl-β-D-glucopyranoside (12d)



Prepared as described for **12a** to yield **12d** (15 mg, 72 %) as a white solid.

$[\alpha]_D$ -35.8 (c 0.7, $CHCl_3$)

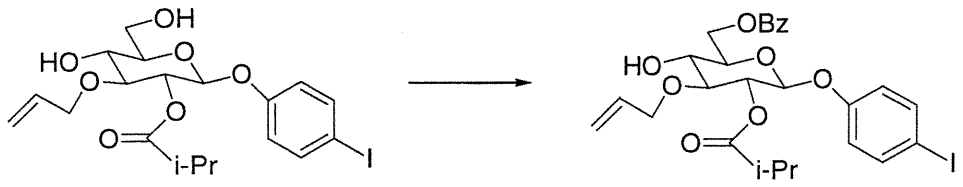
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 8.15(s, 1H), 8.02(d, 1H, *J*= 7.9Hz), 7.82(dd, 1H, *J*=0.7Hz and 7.9Hz), 7.56(dd, 1H, *J*=7.9Hz), 7.36-7.33(m, 2H), 6.77-6.74(m, 2H), 5.90-5.83(m, 1H, vinyl-H), 5.30-5.19(m, 2H, vinyl-H), 5.11(dd, 1H, $J_{1,2}$=7.8Hz, $J_{2,3}$=9.4Hz, 2-H), 4.86(d, 1H, 1-H), 4.48(dd, 1H, $J_{5,6}$=1.5Hz, $J_{6,6'}$=11.0Hz, 6-H), 4.36(dd, $J_{6',5}$=5.2Hz, 6'-H), 4.21-4.16(m, 2H, allyl-H), 3.68-3.62(m, 2H, 4-H and 5-H), 3.49(dd, 1H, $J_{3,4}$=9.4Hz, 3-H), 2.31(t, 2H, *J*=7.3Hz), 1.70-1.63(m, 2H), 0.94(t, 3H)

^{13}C-NMR (400Hz, $CDCl_3$): δ (ppm) =172.08(0), 156.09(0), 137.07(0), 134.38, 132.63, 131.34, 130.74, 130.27, 125.18, 118.54, 118.09(-), 115.70(0), 99.28, 82.04, 73.71, 73.61, 72.18, 69.28, 69.26(-), 36.37(-), 18.60(-), 13.82

MS: (FAB, *m/z*) 653.1, 678.0 (m+23)

HRMS: $C_{26}H_{29}O_9F_3S\ ^{79}Br$, Calcd: 653.06677; Found: 653.06470

4-iodophenyl 3-*O*-allyl-6-*O*-benzoyl-2-*O*-butyryl-*β*-D-glucopyranoside (13a)



11b **13a**

To a solution of **11b** (50 mg, 0.1 mmol), and catalytic DMAP (0.01mmol) in CH_3CN (3 ml) at -10^oC, 5% excess of benzoyl chloride was added and the reaction mixture was stirred at -10^oC for 1.5h. The reaction mixture was diluted with CH_2Cl_2, extracted with 1N HCl, saturated $NaHCO_3$ and brine, dried over Na_2SO4, filtered and the solution concentrated. The crude was purified by chromatography (Hex/EtOAc 3:1) to yield **13a** (40 mg, 65%) as a white solid.

$[\alpha]_D$ -50.9 (c 1.0, $CHCl_3$)

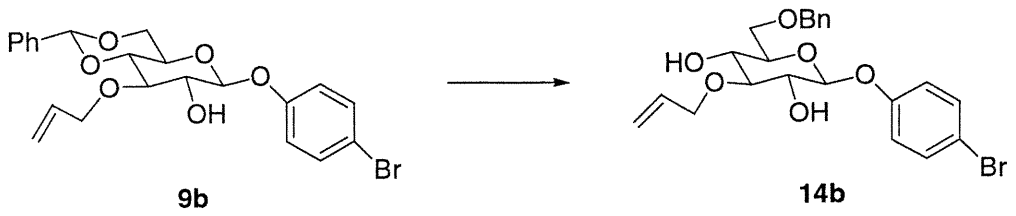
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 7.98-7.94(fm, 2H), 7.54(t, 1H), 7.43-7.38(m, 4H), 6.70-6.66(fm, 2H), 5.88-5.76(m, 1H, vinyl-H), 5.25-5.11(m, 3H, 2-H and vinyl-Hs), 4.49(d, 1H, $J_{1,2}$=7.9Hz, 1-H), 4.59(d, 2H, 6-H and 6'-H), 4.21-4.12(m, 2H, allyl-Hs), 3.74-3.64(m, 2H, 4-H and 5-H), 3.49(t, 1H, *J*=9.2Hz, 3-H), 2.90(s, 1H, OH), 2.57-2.46(m, 1H), 1.16-1.09(m, 6H)

^{13}C-NMR (300z, $CDCl_3$): δ (ppm)=175.66(0), 167.05(0), 157.14(0), 138.56, 134.56, 133.67, 130.03, 129.65(0), 128.69, 119.23, 117.81(-), 99.53, 85.85(0), 82.16, 74.42(-), 73.84, 72.36, 70.16, 63.64(-), 34.34, 19.36, 19.02

MS: 597.2(m+1)

HRMS: $C_{26}H_{30}IO_8$, Calcd: 597.108555, Found: 597.11768

4-Bromophenyl 3-*O*-allyl-6-*O*-benzyl-*β*-D-glucopyranoside (14b)



9b **14b**

To a solution of **9b** (23 mg, 0.05 mmol) and Sodium cyanoborohydride (37 mg, 12 eq) in THF (6 ml) containing molecular sieves, 1N HCl was added at room temperature until the evolution of gas ceased. The mixture was diluted with CH_2Cl_2 and water, filtered over celite. The filtrate was extracted with CH_2Cl_2, washed with water and $NaHCO_3$ saturated solution, dried over Na_2SO_4, and concentrated. The yellow syrup was applied to a flash chromatography (hexane-ethyl acetate 1:1) to yield **14b** (20mg, 87%) as a white solid.

$[\alpha]_D$ -8.8 (c 0.39, $CHCl_3$)

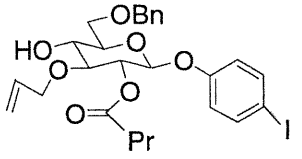
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 7.41-7.30(m, 7H), 6.95(ddd, 2H), 6.03-5.93(m, 1H, vinyl-H), 5.38-5.31(dq 1H, vinyl-H), 5.26-5.21(dq, 1H vinyl-H), 4.85(d, 1H, $J_{1,2}$=7.7Hz, 1-H), 4.58(AB, 2H, *J*=11.9Hz and *J*=17.6Hz, $\underline{CH_2}$Ph), 4.52-4.37(m, 2H, allyl-Hs), 3.85-3.62(m, 5H), 3.43(t, 1H, J=9.2Hz, 3-H),

^{13}C-NMR (400Hz, $CDCl_3$): δ (ppm) = 155.96(0), 137.51(0), 134.76, 132.25, 130.76,128.66, 128.31, 127.71,127.54, 118.59, 117.40(-), 115.21(0), 100.72, 83.30, 74.38, 73.74(-), 73.55(-), 73.54, 71.11, 69.86(-)

MS: (FAB, *m/z*) 464.1

HRMS: $C_{22}H_{25}BrO_6$; Calcd: 464.08345; Found: 464.08957

4-Iodophenyl 2-*O*-butyryl-3-*O*-allyl-6-*O*-benzyl-*β*-D-glucopyranoside (15a)



Prepared as described for **14b** to yield **15a** (35mg, 95 %) as a white solid.

$[\alpha]_D$ -26.2 (c 0.45, $CHCl_3$)

^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 7.56-7.53(m, 2H), 7.36-7.27(m, 5H), 6.78-6.76(m, 2H), 5.96-5.82(m, 1H, vinyl-H), 5.30-5.17(m, 3H, 2-H and vinyl-Hs), 4.92(d, 1H, $J_{1,2}$=7.9Hz, 1-H), 4.58(AB, 2H, *J*=11.9Hz and *J*=20.4Hz, $\underline{CH_2}$Ph), 4.30-4.27(m, 2H, allyl-Hs), 3.87-3.73(m, 3H, 6, 6'-H and 4-H), 3.71-3.60(m, 1H, 5-H), 3.53(t, 1H, *J*=9.2Hz, 3-H), 2.66-2.52(m, 1H), 1.20-1.16(m, 6H)

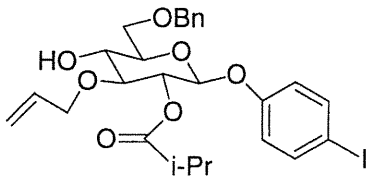
^{13}C-NMR (400z, $CDCl_3$): δ (ppm) = 172.60(0), 157.12(0), 138.61, 137.68(0), 134.58, 128.73, 128.17, 127.98, 119.26, 117.90(-), 99.40, 85.86(0), 82.18, 74.67, 73.98(-), 73.66(-), 72.41, 71.65, 70.10(-). 36.44(-), 18.63(-), 13.84

MS: (FAB, *m/z*) 363.2 (M-OPhI)

HRMS: $C_{25}H_{29}IO_7$ calcd: 582.11145, found: 582.13276

IR: 1744.8, 1484.8, 1235.2, 1185.8, 1101.5, 1070.7, and 1054.7cm^{-1}

4-Iodophenyl 2-*O*-*iso*butyryl-3-*O*-allyl-6-*O*-benzyl-*β*-D-glucopyranoside (15b)



Prepared as described for **14b** to yield **15b** (26mg, 87 %) as a white solid.

$[\alpha]_D$ -27.5 (c 1.0, $CHCl_3$)

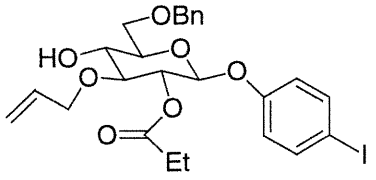
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 7.55-7.52(m, 2H), 7.34-7.25(m, 5H), 6.76-6.74(m, 2H), 5.94-5.82(m, 1H, vinyl-H), 5.29-5.16(m, 3H, 2-H and vinyl-Hs), 4.93(d, 1H, $J_{1,2}$=7.9Hz, 1-H), 4.56(AB, 2H, *J*=11.9Hz and *J*=19.8Hz, $\underline{CH_2}$Ph), 4.28-4.26(m, 2H, allyl-Hs), 3.84-3.74(m, 3H, 6, 6'-H and 4-H), 3.65-3.61(m, 1H, 5-H), 3.52(t, 1H, J=9.2Hz, 3-H), 2.32(t, 2H), 1.65(dt, 2H), 0.94(t, 3H)

^{13}C-NMR (400z, $CDCl_3$): δ (ppm) = 175.68(0), 157.28(0), 138.58, 138.83(0), 134.71, 128.68, 128.08, 127.92, 119.22, 117.54(-), 99.57, 85.73(0), 82.30, 74.81(-), 73.95(-), 73.61, 72.32, 71.62, 70.20(-), 34.34, 19.36, 19.01

MS: (FAB, *m/z*) 582.1, 363.1 (M-OPhI)

HRMS: $C_{26}H_{31}IO_7$, Calcd: 582.11145 Found: 582.23875

4-Iodophenyl 3-*O*-allyl-6-*O*-benzyl-2-*O*-propionyl-β-D-glucopyranoside (15c)



Prepared as described for **14b** to yield **15c** (47mg, 89 %) as a white solid.

$[\alpha]_D$ -11.1 (c 0.54, $CHCl_3$)

^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 7.48(d, 2H), 7.29-7.21(m, 5H), 6.70(d, 2H), 5.87-5.78(m, 1H, vinyl-H), 5.24-5.11(m, 3H, 2-H and vinyl-Hs), 4.86(d, 1H, $J_{1,2}$=7.9Hz, 1-H), 4.51(AB, 2H, *J*=11.9Hz and *J*=16.4Hz, $\underline{CH_2}$Ph), 4.17-4.15(fm, 2H, allyl-Hs), 3.80-3.66(m, 3H, 6, 6'-H and 4-H), 3.60-3.54(m, 1H, 5-H), 3.45(t, 1H, *J*=9.2Hz, 3-H), 2.76(d, 1H, 4-OH), 2.30(q, 2H), 1.12(t, 3H)

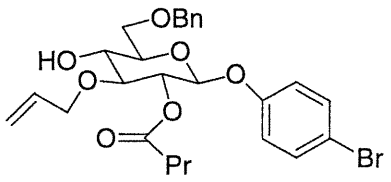
^{13}C-NMR (300z, $CDCl_3$): δ (ppm) = 173.27(0), 157.44(0), 138.78, 138.02(0), 134.93, 128.90, 128.30, 128.14, 119.52, 117.79(-), 99.66, 85.98(0), 82.50, 74.96, 74.16(-), 73.83(-), 72.69, 71.83, 70.44(-), 53.86, 28.11(-), 9.64

MS: (FAB, *m/z*) 568.1

HRMS: $C_{25}H_{29}IO_7$, Calcd 568.09580: Found 568.10087

IR (neat/NaCl): 1745.9, 1484.3, 1235.9, 1174.4, 1073.8 cm^{-1}

4-Bromophenyl 3-*O*-allyl-6-*O*-benzyl-2-*O*-butyryl-*β*-D-glucopyranoside (15d)



Prepared as described for **14b** to yield **15d** (28mg, 78 %) as a white solid.

$[\alpha]_D$ -24.1 (c 0.27, $CHCl_3$)

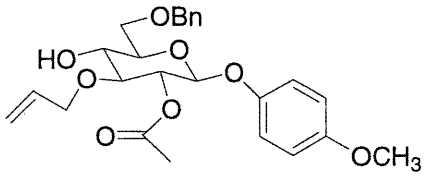
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 7.37-7.28(m, 7H), 6.86(ddd, 2H), 5.93-5.83(m, 1H, vinyl-H), 5.28(dq, 1H, vinyl-H), 5.23-5.13(m, 2H, vinyl-H and 2-H), 4.92(d, 1H, $J_{1,2}$=7.9Hz, 1-H), 4.57(AB, 2H, *J*=11.9Hz and *J*=17,6Hz, $\underline{CH_2}$Ph), 4.26-4 17(m, 2H, allyl-Hs), 3.83(dd, 1H, $J_{5,6}$=4.1Hz and $J_{6,6'}$=10.4Hz, 6-H), 3.79-3.74(m, 2H, 6'-H and 4-H), 3.65-3.61(m, 1H, 5-H), 3.51(t, 1H, *J*=9.2Hz, 3-H), 2.78(fd, 1H, 4-OH) 2.32(t, 2H), 1.65(dt, 2H), 0,92(t, 3H)

^{13}C-NMR (400z, $CDCl_3$): δ (ppm) = 172.20(0), 156.42(0), 137.83(0), 134.73, 132.59, 130.76, 128.70, 128.10,127.94, 118.81, 117.61(-), 115.48(0), 99.56, 82.30, 74.75, 73.97(-), 73.58(-), 72.37, 71.67, 70.24(-), 36.45(-), 18.66(-), 13.86

MS: (FAB, *m/z*) 534.1

HRMS: $C_{26}H_{31}BrO_7$; Calcd: 534.12532; Found: 534.12876

4-Methoxyphenyl 2-*O*-acetyl-3-*O*-allyl-6-*O*-benzyl- *β*-Dglucopyranoside (15e)



Prepared as described for **14b** to yield **15e** (23mg, 76 %) as a white solid.

$[\alpha]_D$ -22.9 (c 1.11, $CHCl_3$)

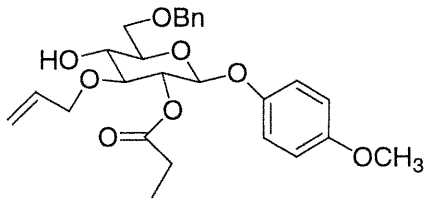
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 7.40-7.30(m, 5H), 6.96(d, 2H), 6.80(d, 2H), 5.96-5.88(m, 1H, vinyl-H), 5.28(dd, 1H, vinyl-H), 5.20-5.14(m, 2H, vinyl-H and 2-H), 4.86(d, 1H, $J_{1,2}$=7.9Hz, 1-H), 4.64-4.56(AB, 2H, *J*=11.9Hz and *J*=17.6Hz, $\underline{CH_2}$Ph), 4.30-4 18(m, 2H, allyl-Hs), 3.88-3.72(m, 6H, 6-H, 6'-H, 4-H and OCH_3), 3.63-3.58(m, 1H, 5-H), 3.51(t, 1H, *J*=9.2Hz, 3-H), 2.87(fd, 1H, 4-OH) 2.13(s, 3H)

^{13}C-NMR (300z, $CDCl_3$): δ (ppm) = 169.86(0), 155.81(0), 151.70(0), 138.09(0), 135.01, 128.86, 128.23,128.13, 118.83, 117.71(-), 114.88, 100.88, 82.54, 74.71, 74.14(-), 73.77(-), 73.03, 72.09, 70.61(-), 56.04, 21.41

MS: (FAB, *m/z*) 458.3

HRMS: $C_{25}H_{30}O_8$; Calcd: 458.19407; Found: 458.19543

4-Methoxyphenyl 3-*O*-allyl-6-*O*-benzyl- 2-*O*-propionyl-*β*-D-glucopyranoside (15f)



Prepared as described for **14b** to yield **15f** (18 mg, 68 %) as a white solid.

$[\alpha]_D$ = -18.7 (c 0.865, $CHCl_3$)

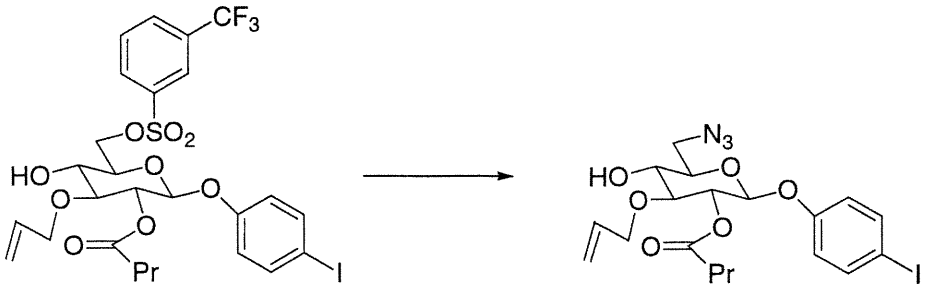
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 7.35-7.28(m, 5H), 6.95(d, 2H), 6.79(d, 2H), 5.94-5.84(m, 1H, vinyl-H), 5.30-5.17(m, 3H, 2-H and vinyl-Hs), 4.85(d, 1H, $J_{1,2}$=7.9Hz, 1-H), 4.64-4.56(AB, 2H, *J*=11.9Hz and 18.2Hz, $\underline{CH_2}$Ph), 4.27-4.18(m, 2H, allyl-Hs), 3.86-3.72(m, 6H, 6-H, 6'-H, 4-H and OCH_3), 3.63-3.60(m, 1H, 5-H), 3.51(t, 1H, *J*=9.2Hz, 3-H), 2.83(fd, 1H, 4-OH) 2.39(q, 2H), 1.19(t, 3H)

^{13}C-NMR (300z, $CDCl_3$): δ (ppm)=173.26(0), 155.79(0), 151.76(0), 138.11(0), 135.02, 128.86, 128.22, 128.13, 118.81, 117.65(-), 114.87, 101.99, 82.60, 74.71, 74.14(-), 73.73(-), 72.85, 72.09, 70.65(-), 56.03, 28.14(-), 9.64

MS: (FAB, *m/z*) 472.3

HRMS: $C_{26}H_{32}O_8$, Calcd: 472.20972; Found: 472.20888

4-Iodophenyl 3-*O*-allyl-6-azido-2-*O*-butyryl-6-deoxy-*β*-D-glucopyranoside (16a)



12a **16a**

The mixture of **12a** (100 mg, 0.14 mmol) and NaN_3 (56 mg, 0.86 mmol) in DMF (1.4 ml) was stirred for 1h at 60-65° C and then the solvent was removed *in vacuo*. The residue was partitioned between CH_2Cl_2 and water, the aqueous phase was extracted with excess of CH_2Cl_2, the combined organic layer dried over Na_2SO_4 and concentrated. The crude product was purified by flash column chromatography (hexane-ethyl acetate 5:2) to yield **16a** (60 mg, 83%) as a white solid.

$[\alpha]_D$ -81.8 (c 0.5, $CHCl_3$)

^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm)=7.57(dd, 2H), 6.76(dd, 2H), 5.92-5.84(m, 1H, vinyl-H), 5.31-5.20(m, 3H, 2-H and vinyl-Hs), 4.93(d, 1H, $J_{1,2}$=7.8Hz, 1-H), 4.26-4.12(m, 2H, allyl-Hs), 3.68-3.46(m, 5H, 3-H, 6-H, 6'-H, 4-H and 5-H), 2.33(t, 2H), 1.67(dt, 2H), 0.95(t, 3H)

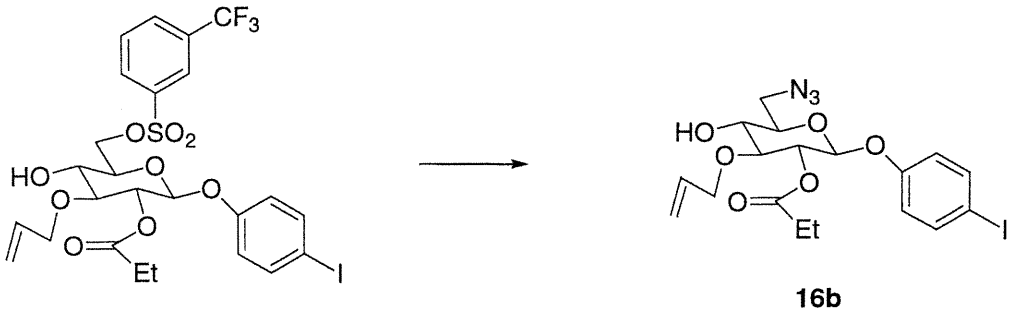
^{13}C-NMR (400Hz, $CDCl_3$): δ (ppm)=172.24(0), 157.26(0), 138.88, 134.65, 119.65, 118.32(-), 99.81, 85.98(0), 82.48, 75.47, 73.83(-), 72.67, 70.78, 51.91(-), 36.61(-), 18.82(-), 14.05

MS: (FAB, *m/z*) 516.1(m-1), 540.2 (m+23)

HRMS: $C_{19}H_{25}O_6IN_3$, Calcd: 518.07880; Found: 518.07960

IR: 3486.2, 2967.9, 2933.2, 2876.4, 2102.6, 1745.2, 1484.5, 1280.5, 1235.8, 1174.5, 1076.5, 1056.2, and 1005.3 cm^{-1}

4-Iodophenyl 3-*O*-allyl-6-azido-6-deoxy-2-*O*-propionyl-β-D-glucopyranoside (16b)



12c **16b**

Prepared as described for **12a** to yield **16b** (58mg, 80 %) as a white solid.

$[\alpha]_D$ -52.3 (c 0.325, $CHCl_3$)

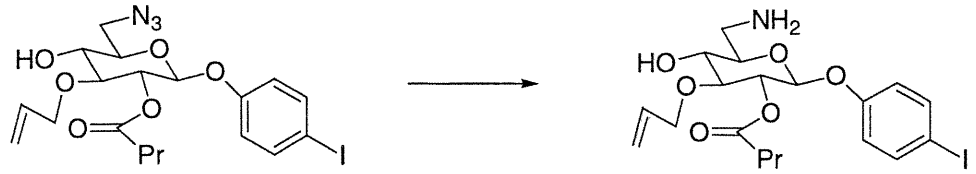
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) =7.61-7.58(fm, 2H), 6.79-6.77(fm, 2H), 5.94-5.84(m, 1H, vinyl-H), 5.32-5.21(m, 3H, 2-H, and vinyl-Hs), 4.94(d, 1H, $J_{1,2}$=7.8Hz, 1-H), 4.27-4.13(m, 2H, allyl-Hs), 3.70-3.62(m, 3H, 4-H, 6-H and 6'-H), 3.53-3.49(m, 2H, 3-H and 5-H), 2.65(d, 1H, OH), 2.36(q, 2H), 1.19(t, 3H)

^{13}C-NMR (300z, $CDCl_3$): δ (ppm)=173.24(0), 157.31(0), 138.90, 134.65, 119.72, 118.31(-), 99.91, 86.47(0), 82.52, 75.49, 73.91(-), 72.83, 70.78, 51.91(-), 28.14(-), 9.65

MS (FAB, *m/z*) 502.1 (m-1)

IR: 2102.5, 1743.9 cm^{-1}

4-Iodophenyl 3-*O*-allyl-6-amino-2-*O*-butyryl-6-deoxy-β-D-glucopyranoside (17a)



16a **17a**

A mixture of **16a** (57 mg, 0.11mmol) and triphenylphosphine (32mg, 0.12 mmol) in THF (0.5 ml) containing water (2 μl) was stirred at room temperature for *ca* 20h, and then concentrated *in vacuo*. The residue was purified by flash column chromatography (CH_2Cl_2-MeOH-NH_4OH 9:1:0.25, lower layer used as eluant) to yield **17a** (49mg, 91%) as a white solid.

$[\alpha]_D$ =-22.4 (c 0.42, $CHCl_3$)

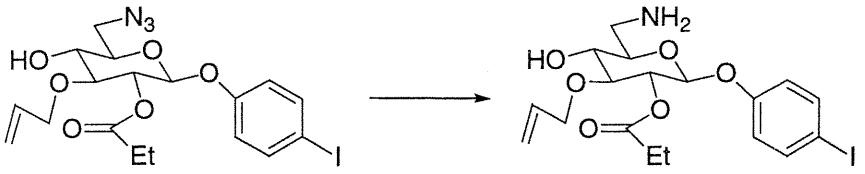
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm)=7.56(dd, 2H), 6.73(dd, 2H), 5.94-5.83(m, 1H, vinyl-H), 5.30-5.15(m, 3H, vinyl-Hs and 2-H), 4.94(d, 1H, $J_{1,2}$=7.9Hz, 1-H), 4.26-4.20(m, 2H, allyl-Hs), 3.70(t, 1H, $J_{3,4}$=$J_{4,5}$=9.2Hz, 4-H), 3.51(t, 1H, $J_{2,3}$=9.2Hz, 3-H), 3.47-3.40(m, 1H, 5-H), 3.09-3.07(fm, 2H, 6-H and 6'-H), 2.31(t, 2H), 1.66(dt, 2H), 0.94(t, 3H)

^{13}C-NMR (400Hz, $CDCl_3$): δ (ppm)=171.92(0), 156.76(0), 138.26, 134.48, 118.63, 117.11(-), 98.87, 85.31(0), 81.87, 75.34, 73.22(-), 72.55, 71.98, 43.73(-), 36.08(-), 18.30(-), 13.51

MS: (FAB, *m/z*) 492.0(m+1)

HRMS: $C_{19}H_{27}O_6NI$, Calcd: 492.08832; Found: 492.08680

4-Iodophenyl 3-*O*-allyl-6-amino-6-deoxy-2-*O*-propionyl-β-D-glucopyranoside (17b)



16b **17b**

Prepared as described for **17a** to yield **17b** (256 mg, 95 %) as a white solid.

$[\alpha]_D$ -16.9 (c 1.075, $CHCl_3$)

^{1}H-NMR (400 Hz, $CDCl_3$): *δ* (ppm) =7.59-7.55(fm, 2H), 6.75-6.72(fm, 2H), 5.92-5.84(m, 1H, vinyl-H), 5.30-5.25(m, 1H), 5.20-5.17(m, 2H, vinyl-H and 2-H), 4.95(d, 1H, $J_{1,2}$=7.9Hz, 1-H), 4.29-4.18(m, 2H, allyl-Hs), 3.72(t, 1H, $J_{3,4}$=$J_{4,5}$=9.2Hz, 4-H), 3.52(t,

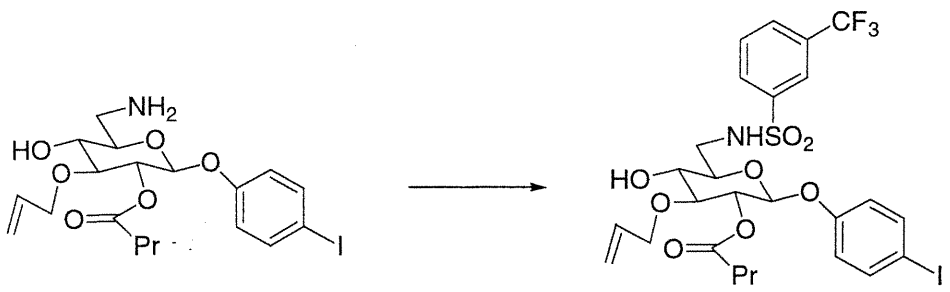
1H, $J_{2,3}$=$J_{3,4}$=9.2Hz, 3-H), 3.45-3.41(m, 1H, 5-H), 3.12-3.09(m, 2H, 6-H and 6'-H), 2.35(q, 2H), 1.16(t, 3H)

^{13}C-NMR (300z, $CDCl_3$): δ (ppm)=173.40(0), 157.38(0), 138.86, 135.07, 119.29, 117.66(-), 99.53, 85.96(0), 82.46, 75.91, 73.88(-), 72.74, 43.99(-), 28.13(-), 9.67

MS: (FAB, *m/z*) 477.1

IR: 3368.9, 2981.6, 1743.6, and 1465.2 cm^{-1}

4-Iodophenyl 3-*O*-allyl-2-*O*-butyryl-6-deoxy-6-(*m*-trifluoromethyl)benzenesulfon amide-β-D-glucopyranoside (18a)



17a **18a**

To a solution of **17a** (27 mg, 0.06 mmol) and Et_3N (0.009 ml) in CH_2Cl_2 (1 ml), 3-(trifluoromethyl)benzenesulfonyl chloride (0.011 ml) was added at room temperature. After stirring overnight at room temperature, MeOH (0.01 ml) and Et_3N (0.01 ml) were added and stirred for 1h. The reaction mixture was diluted with CH_2Cl_2, washed with water, 1N HCl, and water again, dried over Na_2SO4 and concentrated. The crude was purified by chromatography to yield **18a** (32 mg, 83 %) as a white solid (foam).

[α]$_D$ -21.8 (c 1.305, $CHCl_3$)

^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm)=8.13(s, 1H), 8.03(d, 1H), 7.85(d, 1H), 7.65(t, 1H), 7.65(t, 1H), 7.56(d, 2H), 6.66(d, 2H), 5.92-6.82(m, 1H, vinyl-H), 5.29-5.09(m, 4H, 2-H, vinyl-Hs and NH), 4.94(d, 1H, $J_{1,2}$=7.9Hz, 1-H), 4.21(fm, 2H, allyl-Hs), 3.73-3.68(m, 1H, 6-H), 3.58-3.52(m, 1H, 6'-H), 3.52(t, 1H, $J_{2,3}$=$J_{3,4}$=9.2Hz, 3-H), 3,43-3,30(m, 2H, 4-H and 5-H), 2.90(d, 1H, 4-OH), 2.30(t, 2H), 1.64(dt, 2H), 0.94(t, 3H)

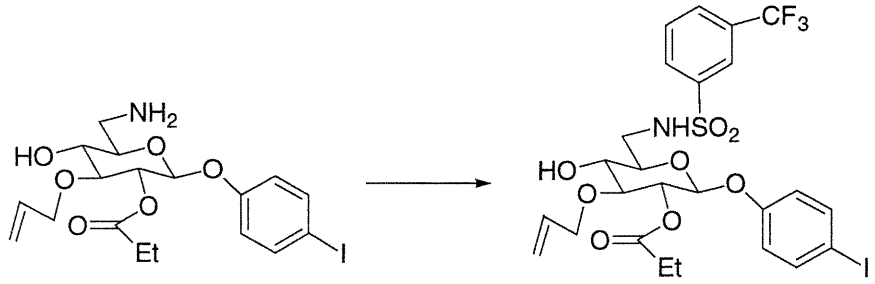
^{13}C-NMR (400Hz, $CDCl_3$): δ (ppm) =171.83(0), 156.47(0), 141.04(0), 138.39, 134.18, 130.00, 129.91, 129.35, 123.87, 118.45, 117.50(-), 98.76, 85.59(0), 81.22, 74.07, 73.38(-), 71.92, 70.14, 43.59(-), 36.01(-), 18.25(-), 13.51

MS: (FAB, *m/z*) 722.0 (m+23)

HRMS: $C_{26}H_{29}O_8NSF_3INa$, Calcd: 722.05084; Found: 722.04890

IR (neat/NaCl): 1744.7, 1485.5, 1327.2, 1235.2, 1164.0, 1072.1 cm^{-1}

4-Iodophenyl 3-*O*-allyl-6-deoxy-2-*O*-propionyl-6-*O*-(*m*-trifluoromethyl)benzene sulfonamide- *β*-D-glucopyranoside (18b)



17b **18b**

Prepared as described for **18a** to yield **18b** (158 mg, 96 %) as a white solid.

$[\alpha]_D$ -33.0 (c 0.385, $CHCl_3$)

^{1}H-NMR (400 Hz, $CDCl_3$): *δ* (ppm) = 8.10(s, 1H), 8.01(d, 1H), 7.80(d, 1H), 7.61(t, 1H), 7.52(dd, 2H), 6.66(dd, 2H), 5.90-5.81(m, 1H, vinyl-H), 5.54(t, 1H, NH), 5.28-5.09(m, 3H, vinyls-Hs and 2-H), 4.92(d, 1H, $J_{1,2}$=8.0Hz, 1-H), 4.26-4.16(m, 2H, allyl-Hs), 3.72(dt, 1H, 4-H), 3.60-3.47(m, 3H, 3-H, 5-H, and OH), 3.38-3.35(m, 2H, 6-H and 6'-H), 2.33(q, 2H), 1.13(t, 3H)

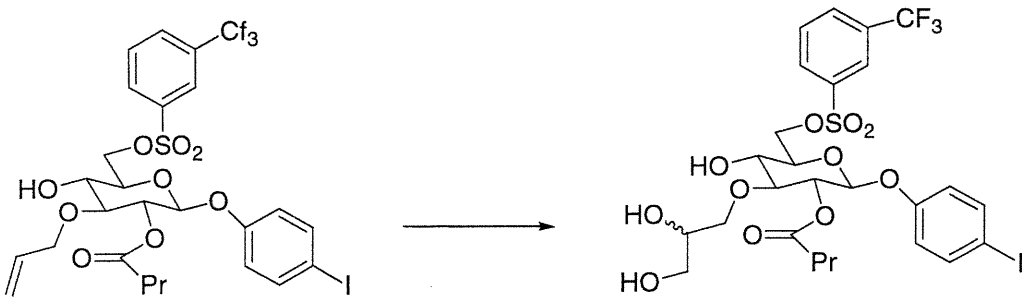
^{13}C-NMR (300z, $CDCl_3$): δ (ppm)=173.57(0), 157.16(0), 141.53(0), 138.94, 134.91, 130.65, 130.53, 129.90, 129.86, 127.91, 119.25, 117.92(-), 99.51, 86.24(0), 81.86, 74.72, 74.09(-), 72.67, 70.65, 44.06(-), 28.10(-), 9.63

MS: (FAB, *m/z*) 707.0 (m+23)

HRMS calcd for $C_{25}H_{26}F_3INO_8SNa$ 707.02736; found 707.02877

IR: 3503.0, 3295.0, 1743.7, 1484.3, 1327.1, 1236.4, 1164.3, 1134.1, and 1072.1 cm^{-1}

4-Iodophenyl 2-*O*-butyryl-3-*O*-(2,3-dihydroxy-propyl)-6-*O*-(*m*-trifluoromethyl) benzenesulfonyl-*β*-D-glucopyranoside(1 :1) (19a)



12a **19a**

To a suspension of **12a** (14 mg) and NMO (2.8 mg) in acetone/water (1:1, 0.5 ml), catalytic amount of OsO_4 was added and stirred at room temperature till TLC indicating the completion of the reaction. The mixture was extracted with EtOAc and the organic phase was dried (Na_2SO_4) and concentrated. The crude was purified by chromatography (hexane-ethyl acetate 1:1) to yield **19a** (9.3mg, 63%) as a white solid

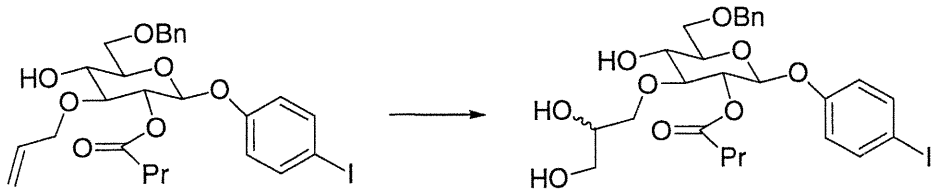
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 8.13(s, 1H), 8.01(d, 1H), 7.18(m, 3H), 6.61(d, 2H), 5.10-5.03(m, 1H), 4.88(d, 0.5H, $J_{1,2}$=8.0Hz, 1-H), 4.87(d, 0.5H, $J_{1,2}$=7.9Hz, 1'-H), 4.45(m, 2H), 3.88-3.69(m, 5H), 3.59-3.49(m, 2H), 2.32(t, 2H), 1.67-1.59(m, 2H), 0.91(t, 3H)

^{13}C-NMR (300z, $CDCl_3$): δ (ppm) =172.50(0), 156.81(0), 138.61, 136.97(0), 131.38, 130.72, 130.28, 125.17, 118.91, 98.80, 85.90(0), 84.42, 84.07, 73.69, 72.28, 71.74, 71.11, 69.47(-), 69.14, 63.65(-), 63.48(-), 36.37(-), 18.66(-), 13.77

MS: (FAB, *m/z*) 757.1(m+23)

HRMS: $C_{26}H_{30}O_{11}SF_3INa$, Calcd: 757.04034; Found: 757.04360

4-Iodophenyl 2-*O*-butyryl-3-*O*-(2,3-dihydroxy-propyl) –6-*O*-benzyl-*β*-D-gluco pyranoside (19b)



15a **19b**

To a solution of **15a** (13 mg), NMO (3.4 mg) in *i*-propanol/acetone/water(1:1:1, 6 ml), catalytic amount of OsO_4 was added and stirred at room temperature till TLC indicating the completion of the reaction. Sodium sulfite was added and stirred for 30 mins. The mixture was extracted with EtOAc and the organic phase was dried (Na_2SO_4) and concentrated. The crude was purified by chromatography (hexane-ethyl acetate 1:1) to yield **19b** (10 mg, 75%) as a white solid

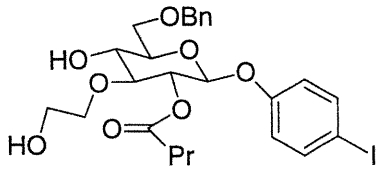
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 7.53(d, 2H), 7.36-7.26(m, 5H), 6.74(d, 2H), 5.15(t, 1H, J=8.3Hz, 2-H), 4.91(d, 1H, *J*=7.9Hz, 1-H), 4.57(AB, 2H, *J*=1.5Hz, and 7.4Hz, $PhCH_2$), 3.87-3.49(m, 11H), 2.30(t, 2H), 1.63(dt, 2H), 0.91(t, 3H)

^{13}C-NMR (300z, $CDCl_3$): δ (ppm) = 172.66(0), 156.28(0), 138.84, 129.03, 128.27, 119.46, 99.46, 85.85(0), 84.81, 84.30, 77.65, 74.32(-), 74.16(-), 74.00, 72.57, 71.00, 70.79(-), 64.10(-), 64.00(-), 36.62(-), 18.90(-), 14.05

MS (FAB, *m/z*) 639.1(m+23)

HRMS: $C_{26}H_{33}O_9INa$, Calcd: 639.10669, Found: 639.10480

4-Iodophenyl 6-*O*-benzyl-2-*O*-butyryl-3-*O*-(2-hydroxy-ethyl)-*β*-D-glucopyranoside (20)



To a solution of **15a** (26 mg, 0.04mmol) in $MeOH/CH_2Cl_2$ (1:1, 5 ml) at -78°C, O_3 was bubbled until the solution turned to be light blue (TLC), then argon was bubbled through

until colorless (1h), $NaBH_4$ (2 mg) was then added at 0° C and stirred for 1h. The mixture was concentrated, and the residue coevaporated with MeOH couple of times. The crude was purified by flash chromatography (hexane-ethyl acetate 1:1) to afford **20** (18 mg, 80%) as a white solid.

$[\alpha]_D$ -21.8 (c 0.445, $CHCl_3$)

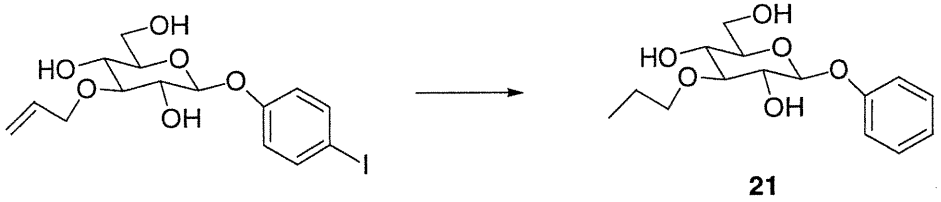
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) =7.57-7.55(fm, 2H), 7.37-7.31(m, 5H), 6.78-6.75(fm, 2H), 5.18(dd, 1H, $J_{2,3}$=9.6Hz, 2-H), 4.94(d, 1H, $J_{1,2}$=7.7Hz, 1-H), 4.60(AB, J=11.9Hz, and 17.6Hz, CH_2Ph), 4.07(s, 1H, OH), 3.92-3.71(m, 7H), 3.68-3.64(m, 1H, 5-H), 3.56(t, 1H, J=9.2Hz, 3-H), 3.02(s, 1H, OH), 2.35(t, 2H), 1.72-1.66(m, 2H), 0.96(t, 3H)

^{13}C-NMR (300z, $CDCl_3$): δ (ppm) =172.69(0), 157.30(0), 138.79, 137.78(0), 128.94, 128.40, 128.17, 119.42, 99.44, 88.92(0), 84.18, 74.50, 74.43(-), 74.23(-), 72.69, 72.39, 70.71(-), 62.92(-), 36.59(-), 18.88(-), 14.02

MS: (FAB, *m/z*) 586.1

HRMS: $C_{25}H_{31}IO_8$, Calced: 586.10637; Found: 586.23008

Phenyl 3-*O*-propyl-*β*-D-glucopyranoside (21)



7a **21**

To a solution of **7a** (33mg) in MeOH (2 ml), $BaCO_3$ (15 mg) and Pd/C (10% wt, 30 mg) was added. The hydrogen in balloon was put through and stirred for 2 days at room temperature. The mixture was filtered and evaporated. The red residue was precipitated from hexane-ethyl acetate to afford a white solid.

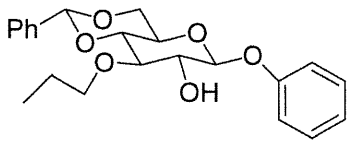
^{1}H-NMR (400 Hz, MeOD): δ (ppm) = 7.29-7.24(m, 2H) 7.07(dt, 2H), 6.99(dt, 1H), 4.88(d, $J_{1,2}$= 7.7Hz, 1-H), 3.90-3.72(m, 3H), 3.70-3.64(dd, 1H), 3.50(dd, $J_{2,3}$= 9.2Hz, 2-H), 3.44-3.42(fm, 2H), 3.26(t, 1H, 3-H), 1.64(dt, 2H), 0.95(t, 3H)

^{13}C-NMR (400z, MeOD): δ (ppm)= 159.35(0), 130.56, 123.51, 117.89, 102.51, 86.46, 78.22, 76.22(-), 75.08, 71.22, 62.61(-), 24.65(-), 11.01

MS: (FAB, *m/z*) 298.1

HRMS: $C_{15}H_{22}O_6$, Calcd: 298.141639; Found: 298.142828

Phenyl 4,6-*O*-benzylidene-3-*O*-propyl-*β*-D-glucopyranoside



Prepared as described for **9a** to yield the title compound (98 mg, 67 %) as a white solid.

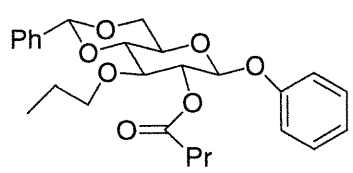
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm)= 7.51(dd, 2H), 7.40-7.30(m, 5H), 7.10-7.05(m, 3H), 5.59(s, 1H, CHPh), 5.05(d, 1H, $J_{1,2}$=7.9Hz, 1-H), 4.38(dd, 1H, *J*= 5.0Hz and 10.5Hz, 6-H), 3.94-3.88(m, 1H), 3.87-3.78(m, 2H), 3.75-3.68(m, 2H), 3.63-3.56(m, 2H), 1.67(dt, 2H), 0.95(t, 3H)

^{13}C-NMR (400z, $CDCl_3$): δ (ppm)= 157.10(0), 137.39(0), 129.78, 129.18, 128.45, 126.16, 123.34, 117.19, 101.47, 101.40, 81.29, 81.04, 75.04(-), 74.12, 68.83(-), 66.81, 23.56(-), 10.68

MS: (FAB, *m/z*) 387.2(m+1)

HRMS: $C_{22}H_{27}O_6$, Calcd: 387.18076; Found: 387.18190

Phenyl 4,6-*O*-benzylidene-2-*O*-butylryl-3-*O*-propyl-*β*-D-glucopyranoside



Prepared as described for **10a** to yield the title compound (67 mg, 78 %) as a white solid.

$[\alpha]_D$ -46.8 (c 0.38, $CHCl_3$)

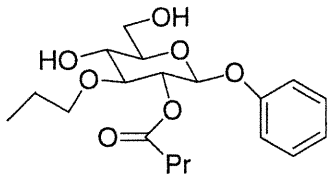
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 7.51-7.49(m, 2H), 7.40-7.38(m, 3H), 7.32-7.28(m, 2H), 7.06(t, 1H), 7.00(dd, 2H), 5.60(s, 1H, CHPh), 5.28(dd, 1H, $J_{2,3}$= 9.2Hz, 2-H), 5.09(d, 1H, $J_{1,2}$= 7.9Hz, 1-H), 4.40(dd, 1H, *J*=5.0Hz, and 10.5Hz, 6-H), 3.88-3.77(m, 3H), 3.67(t, 1H, *J* =9.2Hz, 3-H), 3.59-3.49(m, 2H), 2.34(t, 2H), 1.72-1.59(dt, 2H), 1.60-1.53(dt, 2H), 0.96(t, 3H), 0.90(t, 3H)

^{13}C-NMR (400z, $CDCl_3$): δ (ppm)=171.79(0), 156.90(0), 137.00(0), 129.42, 128.86, 128.12, 125.83, 122.93, 116.67, 101.07, 99.79, 80.89, 79.28, 74.34(-), 72.30, 68.48(-), 66.41, 36.00(-), 23.14(-), 18.30(-), 13.48, 10.35

MS (FAB, *m/z*): 457.2(m+1)

HRMS: $C_{26}H_{33}O_7$, Calcd: 457.22263; Found: 457.22370

Phenyl 2-*O*-butyryl-3-*O*-propyl-*β*-D-glucopyranoside



Prepared as described for **11a** to yield the title compound (35 mg, 87 %) as a white solid.

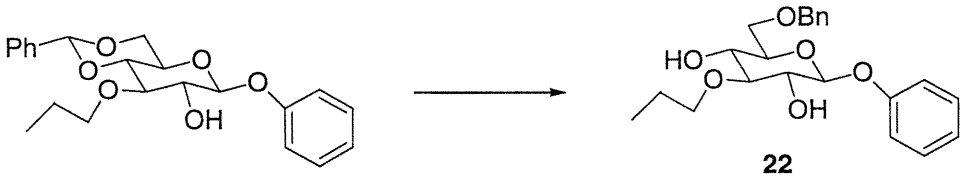
$[\alpha]_D$ -35.5 (c 0.75, $CHCl_3$)

^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 7.31-7.26(m, 2H), 7.04(t, 1H), 6.95(dd, 2H), 5.19(dd, 1H, $J_{2,3}$= 9.4Hz, 2-H), 5.02(d, 1H, $J_{1,2}$=7.9Hz, 1-H), 3.95(dd, 1H, $J_{5,6}$= 3.3Hz, $J_{6,6'}$=12.0Hz, 6-H), 3.83(dd, 1H, $J_{5,6'}$=5.1Hz, 6'-H), 3.75(t, 1H, *J*=9.4Hz, 4-H), 3.56-3.60(m, 2H), 3.57-3.53(m, 1H, 5-H), 3.47(t, 1H, *J*=9.4Hz, 3-H), 2.78(s, 1H, OH), 2.32(t, 2H), 1.67(dt, 2H), 1.58(dt, 2H), 0.95(t, 3H), 0.90(t, 3H)

^{13}C-NMR (400z, $CDCl_3$): δ (ppm)=171.93(0), 156.88(0), 129.45, 122.74, 116.29, 99.10, 82.47, 75.44, 74.10(-), 72.23, 69.85, 62.19(-), 36.07(-), 23.27(-), 18.24(-), 13.47, 10.31

MS: (FAB, *m/z*) 368.2

Phenyl 6-*O*-benzyl-3-*O*-propyl-*β*-D-glucopyranoside (22)



22

Prepared as described for **14b** to yield the title compound **22** (23 mg, 76 %) as a white solid.

$[\alpha]_D$ -38.2 (c 0.5, $CHCl_3$)

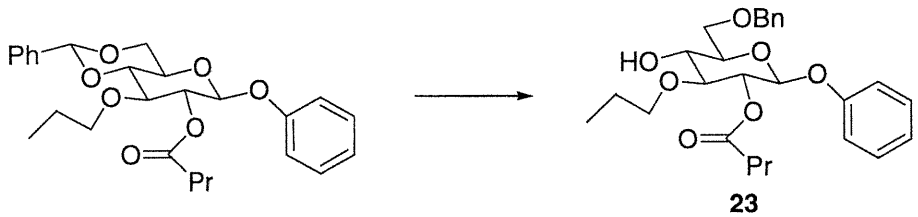
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) =7.33-7.26(m, 7H), 7.08-7.04(m, 3H), 4.90(d, 1H, $J_{1,2}$=7.8Hz, 1-H), 4.58(AB, 2H, *J*=2.0Hz, 15.0Hz, CH_2Ph), 3.90-3.82(m, 2H), 3.77-3.72(m, 3H), 3.67-3.62(m, 2H), 3.36(t, 1H, *J*=8.9Hz, 3-H), 2.82(s, 1H, OH), 2.57(s, 1H, OH), 1.66(dt, 2H), 0.96(t, 3H)

^{13}C-NMR (400z, $CDCl_3$): δ (ppm)=157.31(0), 138.00(0), 129.73, 128.63, 127.97, 127.89, 123.10, 117.10, 101.15, 84.22, 74.99, 74.76(-), 74.01, 73.89(-), 71.52, 70.39(-), 23.71(-), 10.69

MS: (FAB, *m/z*) 388.2

HRMS: $C_{22}H_{28}O_6$, Calced: 388.18859; Found: 388.20064

Phenyl 6-*O*-benzyl-2-*O*-butyryl-3-*O*-propyl-*β*-D-glucopyranoside (23)



23

Prepared as described for **14b** to yield the title compound **23** (24 mg, 89 %) as a white solid.

$[\alpha]_D$ -37.1 (c 0.56, $CHCl_3$)

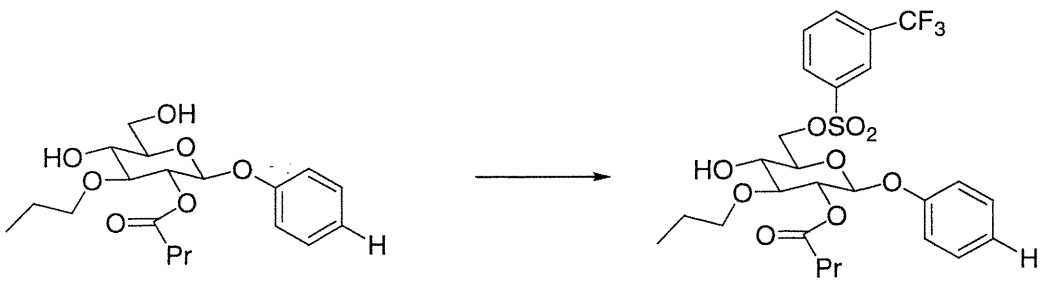
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) =7.34-7.25(m, 7H), 7.04-6.98(m, 3H), 5.22(dd, 1H, $J_{2,3}$=9.5Hz, 2-H), 4.96(d, 1H, $J_{1,2}$=8.0Hz, 1-H), 4.59(AB, 2H, *J*=1.9Hz, 15.9Hz, $PhCH_2$), 3.85(dd, 1H, *J*=4.1Hz, 10.5Hz, 6-H), 3.80-3.73(m, 2H), 3.69-3.62(m, 2H), 3.60-3.57(m, 1H, 5-H), 3.46(t, 1H, *J*=9.5Hz, 3-H)

^{13}C-NMR (400z, $CDCl_3$): δ (ppm)=172.31(0), 157.45(0), 137.93(0), 129.69, 128.66, 128.02, 127.94, 123.00, 116.98, 99.66, 82.84, 74.74, 74.45(-), 73.97(-), 72.49, 71.64, 70.37(-), 36.46(-), 23.64(-), 18.64(-), 13.86, 10.68

MS: (FAB, *m/z*) 459.1(m+1)

HRMS: $C_{26}H_{35}O_7$, Calcd: 459.23829; Found: 459.24563

Phenyl 2-*O*-butyryl-3-*O*-propyl-6-*O*-(*m*-trifluoromethyl)benzenesulfonyl-β-D-gluco pyranoside (24)



24

Prepared as described for **12a** to yield the title compound **24** (15 mg, 74 %) as a white solid.

$[\alpha]_D$ = -39.5 (c 0.615, $CHCl_3$)

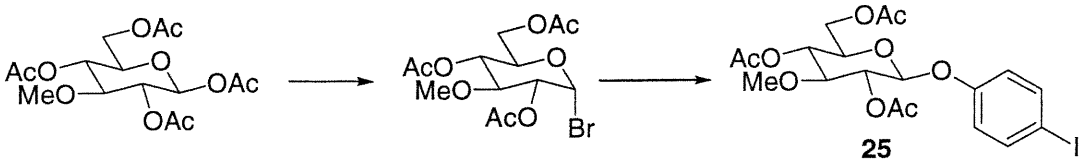
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) =8.15(s, 1H), 8.01(d, 1H), 7.77(d, 1H), 7.49(t, 1H), 7.28-7.24(m, 2H), 7.05(t, 1H), 6.86(dt, 2H), 5.11(dd, 1H, $J_{2,3}$=9.5Hz, 2-H), 4.90(d, 1H, $J_{1,2}$=7.9Hz, 1-H), 4.50(dd, 1H, $J_{5,6}$ =1.9Hz, and 11.0Hz, 6-H), 4.35(dd, 1H, $J_{5,6'}$=5.3Hz, 6'-H), 3.70-3.58(m, 4H), 3.42(dd, 1H, $J_{3,4}$=8.7Hz, 3-H), 2.30(t, 2H), 1.66(dt, 2H), 1.56(dt, 2H), 0.94(t, 3H), 0.90(t, 3H)

^{13}C-NMR (400z, $CDCl_3$): δ (ppm)=172.12(0), 157.13(0), 137.06(0), 131.42, 130.67(0), 130.66, 130.24, 129.73, 125.19, 123.19, 116.70, 99.32, 82.57, 74.57(-), 73.49, 72.27, 69.40(-), 69.31, 36.39(-), 23.61(-), 18.58(-), 13.83, 10.65

MS: (FAB, *m/z*) 575.4(m-1), 599.3(m+23)

HRMS: $C_{26}H_{31}F_3O_9S$, Calcd: 576.16409; Found: 576.17098

4-Iodophenyl 2,4,6-tri-*O*-acetyl-3-*O*-methyl-*β*-D-glucopyranoside (25)



Prepared as described for **6a** to yield the title compound **25** (995 mg, 74% in three steps) as a white solid.

$[\alpha]_D$ -17.4(c 1.115, $CHCl_3$)

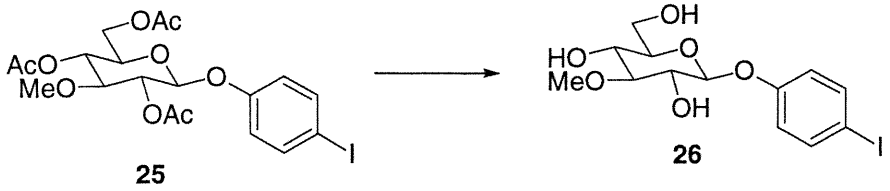
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) =7.58-7.55(m, 2H), 6.76-6.74(m, 2H), 5.23(dd 1H, *J*=9.3Hz, 2-H), 5.11(t, 1H, *J*=9.5Hz, 4-H), 4.94(d, 1H, *J* =7.7Hz, 1-H), 4.22(dd, 1H, *J*=5.8Hz, *J*=12.3Hz, 6-H), 4.14(dd, 1H, *J*=2.6Hz, 6'-H), 3.77-3.72(ddd, 1H, 5-H), 3.56(t, 1H, *J*=9.3Hz, 3-H), 3.44(s, 3H, OCH_3), 2.11, 2.10 and 2.06(s, 3H, OAc)

^{13}C-NMR (400Hz, $CDCl_3$): δ (ppm)=170.78(0), 169.54(0), 169.34(0), 157.02(0), 138.58, 119.34, 99.31, 86.09(0), 81.29, 72.53, 71.80, 68.99, 62.43(-), 59.13, 21.02, 20.91

MS: (FAB, *m/z*) 521.0(m-1), 545.0(m+23)

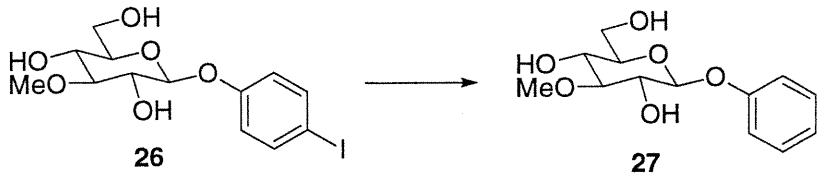
HRMS: $C_{19}H_{22}O_9I$, Calcd: 521.03088; Found: 521.02890

4-iodophenyl 3-*O*-methyl-*β*-D-glucopyranoside (26)



Prepared as described for **7a.** Crude was used directly in the next step without further purification.

Phenyl 3-*O*-methyl-*β*-D-glucopyranoside (27)

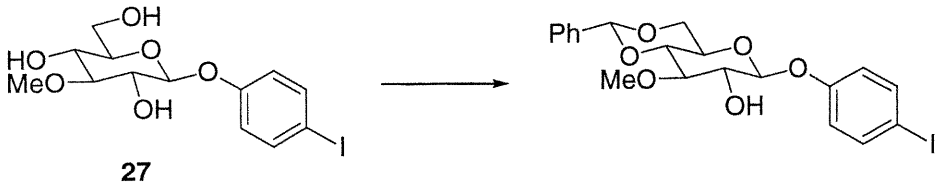


Prepared as described for **21** to afford **27** (170, 81%) as a white solid.

^{1}H-NMR (400 Hz, MeOD): δ (ppm) =7.26(dd, 2H), 7.07(dd, 2H), 7.00(dt, 1H), 4.89(d, 1H, *J*=7.4Hz, 1-H), 3.87(dd, 1H, *J*=2.0Hz, and 11.9Hz, 4-H), 3.70-3.66(m, 4H), 3.50(dd, 1H, *J*=7.9Hz and 11.9Hz, 2-H), 3.43(m, 2H), 3.18(t, 1H, 3-H)

^{13}C-NMR (400Hz, $CDCl_3$): δ (ppm)=159.57(0), 130.81, 123.77, 118.13, 102.65, 88.17, 78.37, 75.18, 71.31, 62.76(-), 61.60

4-Iodophenyl 4,6-*O*-benzylidene-3-*O*-methyl-*β*-D-glucopyranoside



The compound **27** (170 mg, 0.63 mmol) was dissolved in warm benzaldehyde (50 ml), powered anhydrous zinc chloride (40mg) was added, and the mixture was stirred vigorously overnight. The reaction mixture was filtered over celite, diluted with CH_2Cl_2, washed with water, and concentrated. The residue was dissolved in $CHCl_3$, filtered with a short block of silica gel, washed with excess of $CHCl_3$ and then concentrated. The crude was purified by chromatography (Hex/EtOAc 1:5) to yield the title compound (190 mg, 85 %) as a white solid.

$[\alpha]_D$ -27.3(0.315, $CHCl_3$)

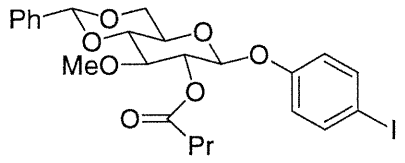
^{1}H-NMR (300 Hz, $CDCl_3$): δ (ppm) =7.56(d, 2H), 7.48-7.45(m, 2H), 7.38-7.34(m, 3H), 6.80(d, 2H), 5.55(s, 1H, CHPh), 4.97(d, 2H, *J* =7.7Hz, 1-H), 4.33(dd, 1H, *J*=4.9Hz, and *J*=10.5Hz, 6-H), 3.81-3.65(m, 6H, 4-H, 6'-H, 2-H, and OCH_3), 3.58-3.45(m, 2H, 5-H, and 3-H), 2.63(d, 1H, 2-OH)

^{13}C-NMR (300Hz, $CDCl_3$): δ (ppm)=157.08(0), 138.88, 137.41(0), 129.51, 128.70, 126.41, 119.68, 101.74, 101.43, 86.39(0), 82.57, 81.56, 74.13, 68.95(-), 66.95, 61.53,

MS: (FAB, *m/z*) 485.0(m+1)

HRMS: $C_{20}H_{22}O_6I$, Calcd: 485.04611; Found: 485.04550

4-Iodophenyl 4,6-*O*-benzylidene-2-*O*-butyryl-3-*O*-methyl-*β*-D-glucopyranoside



Prepared as described for **10a** to yield the title compound (75 mg, 87 %) as a white solid.

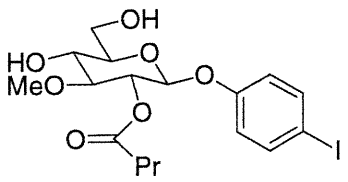
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) =7.60-7.58(m, 2H), 7.51-7.48(m, 2H), 7.40-7.27(m, 3H), 6.78-6.75(m, 2H), 5.59(s, 1H, CHPh), 5.23(dd, *J*=7.8Hz, and *J*=8.8Hz, 2-H), 5.05(d, 1H, *J* =7.8Hz, 1-H), 4.39(dd, 1H, *J*=4.8Hz, and *J*=12.4Hz, 6-H), 3.86-3.78(m, 2H, 4-H, and 6-H), 3.62-3.56(m, 5H, 5-H, 3-H, and OCH_3), 2.34(dq, 2H), 1.68(dt, 2H), 0.95(t, 3H)

^{13}C-NMR (400Hz, $CDCl_3$): δ (ppm)=172.41(0), 157.16(0), 138.87, 137.36(0), 129.52, 128.70, 126.42, 119.47, 101.74, 99.99, 86.28(0), 81.32, 81.04, 72.56, 68.96(-), 66.85, 60.88, 36.56(-), 18.95(-), 13.92

MS: (FAB, *m/z*) 555.1(m+1)

HRMS: $C_{24}H_{28}O_7I$, Calcd: 555.08795; Found: 555.08960

4-Iodophenyl 2-*O*-butyryl-3-*O*-methyl-*β*-D-glucopyranoside



Prepared as described for **11a** to yield the title compound (40 mg, 91 %) as a white solid.

$[\alpha]_D$ = -22.5(c 0.57, $CHCl_3$)

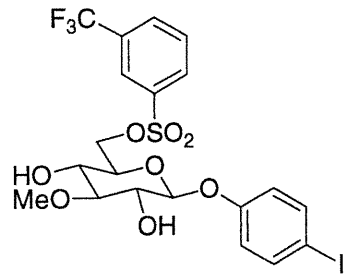
^{1}H-NMR (300 Hz, $CDCl_3$): δ (ppm) =7.60-7.53(m, 2H), 6.75-6.70(m, 2H), 5.17(dd, 1H, $J_{2,3}$=9.5Hz, 2-H), 4.98(d, 1H, $J_{1,2}$=7.9Hz, 1-H), 3.98-3.92(m, 1H, 6-H), 3.86-3.80(m, 1H, 6'-H), 3.78-3.72(dt, 1H, $J_{4,OH}$=2.9Hz, $J_{3,4}$=9.5Hz, 4-H), 3.55(s, 3H, OCH_3), 3.58-3.51(m, 1H, 5-H), 3.40(t, 1H, 3-H), 2.82(d, 1H, 4-OH), 2.34(t, 2H), 2.14(t, 1H, 6-OH), 1.68(m, 2H), 0.95(t, 2H)

^{13}C-NMR (400Hz, $CDCl_3$): δ (ppm)=171.96(0), 156.65(0), 138.34, 118.55, 99.87, 85.46(0), 83.71, 75.44, 71.98, 69.49, 62.08(-), 36.07(-), 18.30(-), 13.43

MS: (FAB, *m/z*) 467.1(m+1)

HRMS: $C_{17}H_{24}O_7I$, Calcd: 467.05667; Found: 467.05530

4-Iodophenyl 3-*O*-methyl-6-*O*-(*m*-trifluoromethyl)benzenesulfonyl-*β*-D-glucopyranoside (28)



Prepared as described for **8a** to yield the title compound **28** (35 mg, 75 %) as a white solid.

$[\alpha]_D$ -57.1(c 1.16, $CHCl_3$)

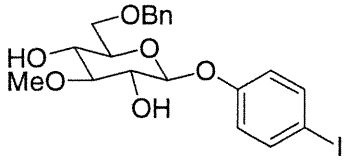
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) =8.14(s, 1H), 8.02(d, 1H), 7.82(d, 1H), 7.61-7.52(m, 3H), 6.74-6.71(fm, 2H), 4.77(d, 1H, $J_{1,2}$=7.8Hz, 1-H), 4.46(dd, 1H, $J_{5,6}$=2.1Hz, and $J_{6,6'}$=11.1Hz, 6-H), 4.34(dd, 1H, $J_{5,6'}$=5.5Hz, 6'-H), 3.76-3.66(m, 6H), 3.55-3.49(m, 1H, 5-H), 3.23(t, 1H, *J*=9.1Hz, 3-H), 2.54(d, 1H, OH), 2.40(d, 1H, OH)

^{13}C-NMR (300Hz, $CDCl_3$): δ (ppm)=156.99(0), 138.83, 137.27(0), 132.46(0), 131.51, 130.94, 130.40, 125.43, 125.38, 119.55, 119.33, 100.91, 86.24, 85.32, 74.11, 73.72, 69.52(-), 69.24, 61.54

MS: (FAB, *m/z*) 547.1(m-1)

HRMS: $C_{20}H_{20}F_3IO_8S$, Calcd: 603.98757; Found: 604.09873

4-Iodophenyl 6-*O*-benzyl-3-*O*-methyl-*β*-D-glucopyranoside (29)



Prepared as described for **14b** to yield the title compound **29** (23 mg, 83 %) as a white solid.

$[\alpha]_D$ -41.1(c 1.06, $CHCl_3$)

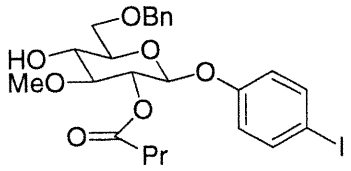
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) =7.58-7.54(m, 2H), 7.36-7.27(m, 5H), 6.85-6.81(m, 2H), 4.85(d 1H, *J*=7.8Hz, 1-H), 4.57(AB, 2H, *J*=11.9Hz, and *J*=18.6Hz, CH_2Ph), 3.81(d, 1H, *J*=4.0Hz, and *J*=10.4Hz, 6-H) 3.75-3.60(m, 7H, 2-H, 4-H, 6'-H, 5-H, and OCH_3), 3.27(t, 1H, *J*=9.0Hz, 3-H), 2.84(d, 1H, *J*=1.8Hz, OH), 2.54(d, 1H, *J*=2.1Hz, OH)

^{13}C-NMR (300Hz, $CDCl_3$): δ (ppm)=157.24(0), 138.80, 137.99(0), 128.89, 128.30, 128.12, 119.56, 101.07, 86.06(0), 85.67, 74.79, 74.11(-), 74.02, 71.77, 70.43(-), 61.37

MS: (FAB, *m/z*) 485.0(m-1)

HRMS: $C_{20}H_{23}IO_6$, Calcd: 486.05394; Found: 486.06754

4-Iodophenyl 6-*O*-benzyl-2-*O*-butyryl-3-*O*-methyl-*β*-D-glucopyranoside (30)



Prepared as described for **14b** to yield the title compound **30** (21 mg, 75 %) as a white solid.

$[\alpha]_D$ -32.4(c 0.67, $CHCl_3$)

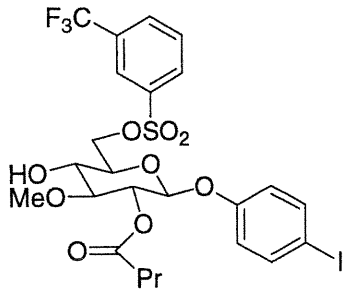
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) =7.56-7.52(m, 2H), 7.36-7.26(m, 5H), 6.77-6.74(m, 2H), 5.21-5.17(dd, 1H, *J*=7.9Hz, and *J*=9.6Hz, 2-H), 4.92(d, 1H, *J*=7.9Hz, 1-H), 4.57(AB, *J*=11.9Hz, and *J*=18.6HZ, CH_2Ph), 3.83(dd, 1H, *J*=4.1Hz, and *J*=10.4Hz, 6-H), 3.77-3.72(m, 2H, 4-H, and 6'-H), 3.66-3.61(m, 1H, 5-H), 3.55(s, 3H, OCH_3), 3.85(t, 1H, *J*=9.2Hz, 3-H)

^{13}C-NMR (400Hz, $CDCl_3$): δ (ppm)=171.96(0), 156.81(0), 138.22, 137.44(0), 128.35, 127.76, 127.58, 126.99, 118.87, 98.98, 85.38(0), 83.67, 74.32, 73.60(-), 71.85, 70.96, 69.82(-), 59.85, 36.10(-), 18.34(-), 13.44

MS: (FAB, *m/z*) 555.1(m-1), 579.3(m+23)

HRMS: $C_{24}H_{29}INaO7$, Calced: 579.08557, Found: 579.09234

4-Iodophenyl 2-*O*-butyryl-3-*O*-methyl-6-*O*-(*m*-trifluoromethyl)benzenesulfonyl-*β*-D-glucopyranoside (31)



Prepared as described for **12a** to yield the title compound **31** (15 mg, 93 %) as a white solid.

$[\alpha]_D$ -35.8(c 0.36, $CHCl_3$)

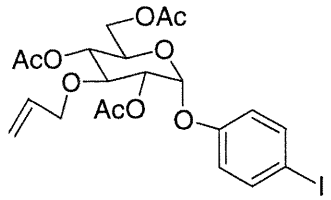
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) =8.14(fd, 1H), 8.01(fdd, 1H), 7.82(fdd, 1H), 7.57-7.52(m, 3H), 6.66-6.62(m, 2H), 5.10(dd, 1H, $J_{2,3}$=9.2Hz, 2-H), 4.86(d, 1H, $J_{1,2}$=7.8Hz, 1-H), 4.78(dd, 1H, $J_{5,6}$=1.8Hz, and $J_{6,6'}$=11.0Hz, 6-H), 4.34(dd, 1H, $J_{5,6'}$=5.4Hz, 6'-H), 3.70-3.60(m, 2H, 4-H, and 5-H), 3.53(s, 3H, OCH_3), 3.36(t, 1H, *J*=9.0Hz, 3-H), 2.69(d, 1H, *J*=3.1Hz, 4-OH), 2.31(t, 2H), 1.71-1.62(m, 2H), 0.96(t, 3H)

^{13}C-NMR (300Hz, $CDCl_3$): δ (ppm)=172.37(0), 157.05(0), 138.81, 137.20(0), 132.46(0), 131.55, 131.00, 130.47, 125.42, 125.37, 119.14, 99.26, 86.12(0), 84.01, 73.73, 72.23, 69.43, 69.23, 60.58(-), 36.57(-), 18.82(-), 13.98

MS: (FAB, *m/z*) 547.1(m-1)

HRMS: $C_{24}H_{26}F_3IO_9S$ Calced: 673.02161, Found: 673.02398

4-Iodophenyl 2,4,6-tri-*O*-acetyl-3-*O*-allyl-α-D-glucopyranoside (33)



By-product from **6a**.Yield <10%, white solid.

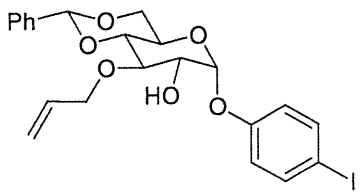
$[\alpha]_D$ +119 (c 0.45, $CHCl_3$)

^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 7.59-7.57(m, 2H), 6.87-6.84(m, 2H), 5.85-5.81(m 1H, vinyl-H), 5.68(d, 1H, $J_{1,2}$=3.7Hz, 1-H), 530-5.08(m, 3H, 4-H and vinyl-Hs), 4.94(dd, 1-H, $J_{2,3}$=10.0Hz, 2-H), 4.27-4.11(m, 4H, 6-H, 6'-H and allyl-Hs), 4.03(t, 1H, 3-H), 3.98-3.94(m, 1H, 5-H), 2.10(s, 6H), 2.07(s, 3H)

^{13}C-NMR (300Hz, $CDCl_3$): δ (ppm) = 171.04(0), 170.48(0), 169.79(0), 156.42(0), 138.88, 134.80, 119.37, 117.25(-), 94.93, 86.06(0), 77.09, 74.28(-), 73.05, 69.82, 68.98, 62.25(-), 21.24, 21.09

MS: (FAB, *m/z*) 548.05

4-Iodophenyl 3-*O*-allyl-4, 6-*O*-benzylidene-α-D-glucopyranoside



Prepared as described for **9a** to yield the title compound (87 mg, 67 %) as a white solid.

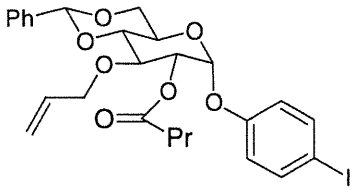
$[\alpha]_D$ +178.7(c 0.445, $CDCl_3$)

^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 7.62-7.58(m, 2H), 7.49-7.46(m, 2H), 7.40-7.35(m, 3H), 6.89-6.86(m, 2H), 6.04-5.94(m, 1H, vinyl-H), 5.58(d, 1H, *J*=3.8Hz, 1-H), 5.56(s, 1H, PhCH), 5.34(ddt, 1H, *J*= 1.6Hz, 1.6Hz, and 17.2Hz, vinyl-H), 5.22(ddt, 1H, *J*=1.2Hz, 1.6HZ, and 12.6Hz, vinyl-H), 4.50(ddt, 1H, *J*=1.4Hz, 5.5Hz, 12.6Hz, allyl-H), 4.30(ddt, 1H, *J*= 1.3Hz, 6.1Hz, 12.6Hz, allyl-H), 4.21(dd, 1H, *J*=4.9Hz, and 10.2Hz, 6-H), 3.97-3.90(m, 2H), 3.88-3.85(m, 1H, 5-H), 3.73(t, 1H, *J*=10.3Hz), 3.67(t, *J*=9.4Hz, 1H), 2.46(d, 1H, OH)

^{13}C-NMR (300z, $CDCl_3$): δ (ppm) = 155.94(0), 138.36, 136.98(0), 134.64, 128.88, 128.11, 125.83, 118.95, 117.36(-), 101.17, 97.19, 85.49(0), 81.63, 78.01, 73.68(-), 71.75, 68.57(-), 63.44

MS (FAB, *m/z*) 511.1(m+1)

4-Iodophenyl 3-*O*-allyl-2-*O*-butylryl-4,6-*O*-benzylidene-α-D-glucopyranoside



Prepared as described for **10a** to yield the title compound (97mg, 45 %) as a white solid.

$[\alpha]_D$ +146.1(c 1.475, $CDCl_3$)

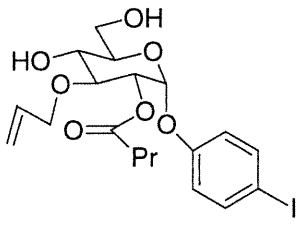
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm)= 7.60-7.57(fm, 2H), 7.50-7.48(fm, 2H), 7.40-7.33(m, 3H), 6.80(dd, 2H), 5.97-5.87(m, 1H, vinyl-H), 5.70(d, 1H, $J_{1,2}$=3.8Hz, 1-H), 5.59(s, 1H, CHPh), 5.29(ddt, 1H, vinyl-H), 5.17(ddt, 1H, vinyl-H), 4.97(dd, 1H, $J_{2,3}$=9.7Hz, 2-H), 4.40(ddt, 1H, allyl-H) 4.27-4.19(m, 2H), 4.12(t, 1H, *J*=9.5Hz), 3.99-3.93(m, 1H), 3.76-3.71(m, 2H), 2.37(t, 2H), 1.66(tq, 2H), 0.94(t, 3H)

^{13}C-NMR (400Hz, $CDCl_3$):): δ (ppm)= 172.23(0), 156.38(0), 138.70, 137.32(0), 135.01, 129.22, 128.44, 126.21, 119.17, 116.99(-), 101.57, 95.44, 85.72(0), 81.90, 75.63, 73.97(-), 72.49, 68.85(-), 63.52, 36.26(-), 18.59(-), 13.80

MS: (FAB, *m/z*) 581.2(m+1)

HRMS: $C_{26}H_{30}O_7I$, Calcd: 581.10364; Found: 581.10560

4-Iodophenyl 3-*O*-allyl-2-*O*-butylryl-α-D-glucopyranoside



Prepared as described for **11a** to yield the title compound (56 mg, 80 %) as colorless syrup.

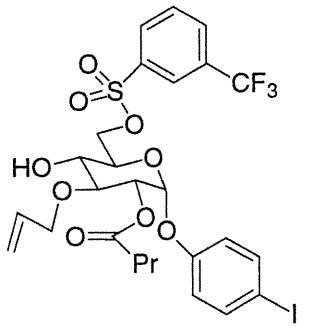
$[\alpha]_D$ +125 (c 1.2, $CHCl_3$)

^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm)=7.58(dt, 2H), 6.82(dt, 2H), 5.99-5.89(m, 1H, vinyl-H), 5.67(d, 1H, $J_{1,2}$=3.8Hz, 1-H), 5.32(ddt, 1H, vinyl-H), 5.21(ddt, 1H, vinyl-H), 4.84(dd, 1H, $J_{2,3}$=9.5Hz, 2-H), 4.35(ddt, 1H, ally-H), 4.27(ddt, 1H, allyl-H), 3.97-3.92(m, 1H), 3.80-3.76(m, 4H), 2.34(t, 2H), 1.64(tq, 2H), 0.92(t, 3H).

^{13}C-NMR (400Hz, $CDCl_3$): δ (ppm)= 173.21(0), 156.48(0), 138.70, 134.90, 119.17, 117.48(-), 95.10, 85.67(0), 79.19, 74.26, 73.13(-), 72.12, 70.13, 62.10(-), 36.34(-), 18.55(-), 13.80

MS: (FAB, *m/z*) 492.1

4-Iodophenyl 3-*O*-allyl-2-*O*-butyryl-6-*O*-(*m*-trifluoromethyl)benzenesulfonyl-*α*-D-glucopyranoside (34).



Prepared as described for **12a** to yield the title compound **34** (18 mg, 50 %) as a white solid.

$[\alpha]_D$ +68.3 (c 0.12, $CDCl_3$);

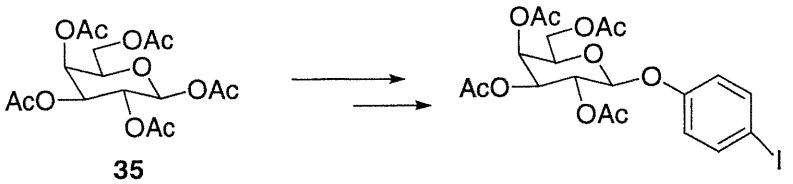
^{1}H NMR (400 Hz, $CDCl_3$) δ 8.18(s, 1H), 8.07(d, 1H), 7.93(d, 1H), 7.72(t, 1H), 7.54(dd, 2H), 6.73(dd, 2H), 5.98-5.87(m, 1H, vinyl-H), 5.52(d, 1H, $J_{1,2}$ = 3.6Hz, 1-Hα), 5.31(dd, 1H, vinyl-H), 5.22(dd, 1H, vinyl-H), 4.75(dd, 1H, $J_{2,3}$ = 9.9Hz, 2-H), 4.42(dd, $J_{5,6}$ = 3.3Hz, $J_{5,6'}$ = 11.2Hz, 6-H), 4.37-4.30(m, 2H), 4.23(dd, 1H, J = 5.9Hz, J = 12.7Hz), 3.91-3.83(m, 2H), 3.65(t, 1H, J = 9.1Hz, 3-H), 2.70-2.59(broad, 1H, OH), 2.32(t, 2H), 1.62(tq, 2H), 0.92(t, 3H)

^{13}C NMR (400 Hz, $CDCl_3$) δ 173.17, 156.51, 138.91, 138.00, 134.87, 131.54, 130.96, 130.49, 125.43, 119.23, 117.89, 95.25, 85.06, 79.13, 74.51, 72.95, 70.25, 69.43, 69.25, 36.48, 18.73, 13.97;

MS (FAB, *m/z*) 700.0

HRMS: $C_{26}H_{28}F_3IO_9S$, Calcd: 700.04509; Found: 700.05943

4-Iodophenyl tetra-*O*-acetyl-*β*-D-galactopyranoside



35

To a solution of β-D-galactose pentaacetate **35** (1.63 g, 4.2 mmol) in dichloromethane (10 ml), acetic anhydride (catalytic) and HBr (30% wt. in acetic acid, 7 ml), were added carefully at room temperature. The reaction mixture was stirred 2h, then diluted with CH_2Cl_2 (30 ml), washed with cold water (50ml) twice, $NaHCO_3$ saturated aqueous solution (50 ml) and water (50 ml), dried over Na_2SO_4 and concentrated. The crude was used in the next step without further purification. Tin (IV) chloride (1M in CH_2Cl_2, 4.2 ml) was added dropwise to a solution of tetra-*O*-acetyl-α-D-galactopyranosyl bromide (1.73 mg, 4.2mmol) and tributylstannyl-4-iodophenoxide (**5c**, 4.2mmol, prepared as described in **6a**) in CH_2Cl_2 (10ml) at 0° C. The purple solution was stirred 5min at 0° C, then was allowed to warm up to room temperature and stirred for 1h. The reaction was quenched with dilute $NaHCO_3$. The organic layer was collected and washed with water, dried over Na_2SO_4 and concentrated. The crude was purified by chromatography (ethyl acetate-hexane 1:2), to yield the title compound (1.6g, 69%) as a white solid.

$[\alpha]_D$ +8.5 (c 1.0,$CHCl_3$)

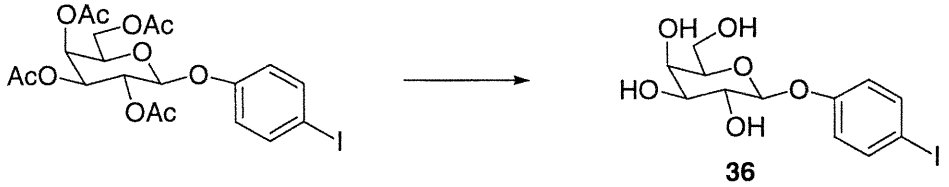
^{1}H-NMR (400Hz, $CDCl_3$): δ (ppm) = 7.60(d, 2H, *J*=9.0Hz), 6.79(d, 2H, *J*=9.0Hz), 5.51-5.47(m, 2H, 2-H, 4-H), 5.12(dd, 1H, $J_{2,3}$=9.8Hz, $J_{3,4}$=3.4Hz, 3-H), 5.02(d, 1H, $J_{1,2}$=7.9Hz, 1-H), 4.24-4.07 (m, 3H, 6-H, 6'-H, 5-H)

^{13}C-NMR (300Hz, $CDCl_3$): δ (ppm) = 170.73(0), 170.60(0), 170.49(0), 169.73(0) 157.11(0), 138.85, 119.56, 99.84, 86.53(0), 71.49, 71.10, 66.88, 67.17, 61.73(-), 21.43, 21.13, 21.08, 20.99

MS: (FAB, *m/z*) 573.0(m+23), 549.0(m-1)

HRMS: $C_{20}H_{22}O_{10}I$, Calcd: 549.02576; Found: 549.02740

4-Iodophenyl β-D-galactopyranoside (36)



36

To a solution of 4-iodophenyl tetra-*O*-acetyl-*β*-D-galactopyranoside (1.50 g, 2.7 mmol) in dry MeOH (18 ml), was added catalytic amount of NaOMe (pH 9). After stirring for 4h at room temperature, the base was neutralized with Amberlite IR-120 (H^+) ion exchange resin, the suspension filtered and the filtrate concentrated to dryness. The crude was used in the next step without further purification.

$[\alpha]_D$ -39.1(c 1.0, MeOH)

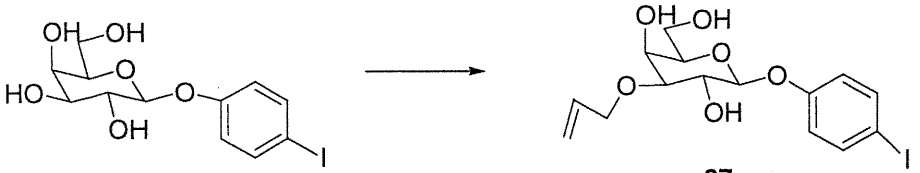
^{1}H-NMR (400Hz, CD_3OD): δ (ppm)=7.60(dd, 2H), 6.94(dd, 2H), 4.85(d, 1H, $J_{1,2}$= 7.7Hz, 1-H), 3.91(dd, 1H, $J_{3,4}$=3.4Hz, 4-H), 3.82-3.69(m, 4H), 3.59(dd, 1H, $J_{2,3}$=9.7Hz, 3-H)

^{13}C-NMR (300Hz, CD_3OD): δ (ppm)=159.56(0), 139.79, 120.60, 103.18, 85.66(0), 77.44, 75.18, 72.56, 70.58, 62.80(-)

MS: 383.0(m+1), 382.0(*m/z*)

HRMS: $C_{12}H_{16}O_6I$, Calcd: 382.99918; Found: 383.00070

4-Iodophenyl 3-*O*-allyl-*β*-D-galactopyranoside (37)



36 **37**

A mixture of **36** (crude, *ca* 2.5 mmol) and dibuytltin oxide (0.64g) in benzene (50 ml) was refluxed at 100°C for 20h with azeotropic removal of water using Dean-Stark apparatus. The solution was evaporated to half-volume and cooled. Allyl bromide (1.3 ml) and tetrabutylammonium bromide (810 mg, 2.5 mmol) were added and the resulting mixture was refluxed under argon at 80° C for 36h. The solvent was removed under reduced pressure and the residue portioned between ether (3 x 50 ml) and 20 ml portion of 5% NaHCO3. The organic layer was concentrated, and the residue dissolved in MeOH, filtered and dried over Na_2SO_4 to afford **37** (530 mg, 51%)as white solids.

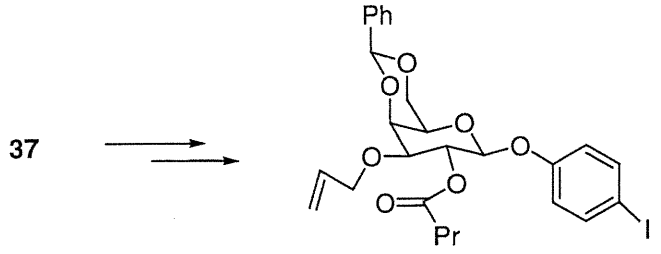
$[\alpha]_D$ -7.8 (c 0.9, MeOH)

^{1}H-NMR (400Hz, CD_3OD): δ (ppm)=7.59(dd, 2H), 6.93(dd, 2H), 6.08-5.97(m, 1H, vinyl-H), 5.39-5.34(m, 1H, vinyl-H), 5.21-5.18(m, 1H, vinyl-H), 4.87(d, 1H, $J_{1,2}$= 7.7Hz, 1-H), 4.34-4.18(m, 2H, allyl-Hs), 4.09(dd, 1H, $J_{4,5}$=2.8Hz, 4-H), 3.89(dd, 1H, $J_{2,3}$=9.7Hz, 2-H), 3.80-3.66(m, 3H, 5-H, 6-H, and 6'-H), 3.43(dd, 1H, $J_{3,4}$=3.3Hz,3-H)

^{13}C-NMR (300Hz, CD_3OD): δ (ppm)=159.52(0), 139.80, 136.87, 121.09,120.63, 117.88(-), 103.12, 85.70(0), 82.40, 77.27, 72.20(-), 71.67, 67.32, 62.78(-)

MS: (FAB, *m/z*) 422.1, 443.2 (m+23)

4-Iodophenyl 3-*O*-allyl-2-*O*-butyryl-4, 6-*O*-benzylidene-*β*-D-galactopyranoside (38a)



38a

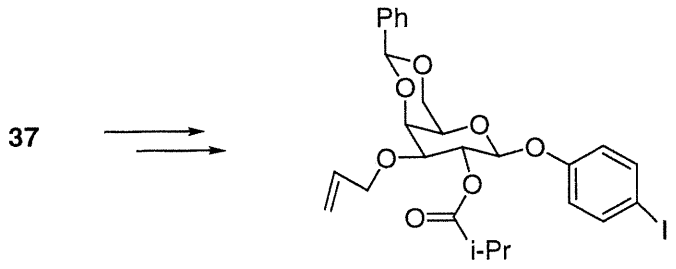
Prepared as described for **10a** to yield the title compound **38a** (180 mg, 76 %) as a white solid.

$[\alpha]_D$ -10.4 (c 1.0, $CHCl_3$)

^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 7.56-7.53(m, 4H), 7.37-7.34(m, 3H), 6.78(dd, 2H) 5.94-5.83(m, 1H, vinyl-H), 5.63-5.57(m, 2H, 2-H and CHPh), 5.32-5.19(m, 2H, vinyl-Hs), 4.99(d, 1H, $J_{1,2}$=8.0Hz 1-H), 4.36-4.34(m, 2H, 4-H and 6-H), 4.22-4.10(m, 3H, $H_{6'}$ and allyl-Hs), 3.67(dd, 1H, $J_{2,3}$ =10.1Hz and $J_{3,4}$=3.4Hz, 3-H), 3.57-3.55(m, 1H, 5-H), 2.31(t, 2H), 1.69-1.63(m, 2H), 0.94(t, 3H)

^{13}C-NMR (400Hz, $CDCl_3$): δ (ppm) =172.36(0), 157.54(0), 138.65, 137.80(0), 134.97, 129.46, 128.57, 126.87, 119.79, 117.92(-), 101.62, 100.10, 85.90(0), 77.37, 73.54, 70.99(-), 69.85, 69.38(-), 67.26, 36.63(-), 18.96(-), 14.04

4-Iodophenyl 3-*O*-allyl-4,6-*O*-benzylidene-2-*O*-isobutyryl-*β*-D-galactopyranoside (38b)



Prepared as described for **10a** to yield the title compound **38b** (160 mg, 95 %) as a white solid.

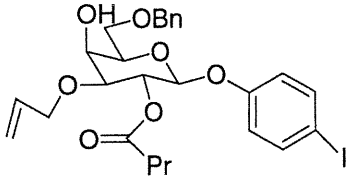
$[\alpha]_D$ -9.1 (c 0.515, $CHCl_3$)

^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 7.57-7.55(m, 4H), 7.34-7.28(m, 3H), 6.80(dd, 2H) 5.94-5.87(m, 1H, vinyl-H), 5.62-5.57(m, 2H, 2-H and CHPh), 5.33-5.19(m, 2H, vinyl-Hs), 5.01(d, 1H, $J_{1,2}$=8.0Hz 1-H), 4.37-4.34(m, 2H, 4-H and 6-H), 4.22-4.11(m, 3H, H_6, and allyl-Hs), 3.70(dd, 1H, $J_{2,3}$ =10.1Hz and $J_{3,4}$=3.4Hz, 3-H), 3.57(fm, 1H, 5-H), 2.60-2.56(m,1H), 1.21-1.17(m, 6H)

^{13}C-NMR (300Hz, $CDCl_3$): δ (ppm) =175.85(0), 157.63(0), 138.67, 137.81(0), 134.95, 129.42, 128.55, 126.84, 119.74, 117.89(-), 101.56, 100.24, 85.90(0), 77.43, 73.57, 71.05(-), 69.76, 69.39(-), 67.28, 34.53, 19.57, 19.37

MS: (FAB, *m/z*) 579.1(m-1), 603.1(m+23)

4-Iodophenyl 3-*O*-allyl-6-*O*-benzyl-2-*O*-butyryl-*β*-D-galactopyranoside (39a)



Prepared as described for **14b** to yield the title compound **39b** (25 mg, 91 %) as a white solid.

$[\alpha]_D$ +5.0 (c 1.105, CH_3Cl)

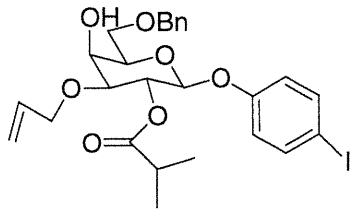
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 7.53(d, 2H), 7.36-7.31(m, 5H), 6.79(d, 2H), 5.93-5.80(m, 1H, vinyl-H), 5.44(dd, 1H, $J_{2,3}$=9.7Hz, 2-H), 5.32-5.20(m, 2H, vinyl-Hs), 4.90(d, 1H, $J_{1,2}$=8.0Hz, 1-H), 4.58(s, 2H, CH_2PH), 4.21-4.15(m, 1H, allyl-H), 4.11(d, 1H, $J_{4,5}$=0, 4-H), 4.07-4.01(m, 1H, allyl-H), 3.89-3.74(m, 3H, 5-H, 6-H and 6'-H), 3.55(dd, 1H, $J_{3,4}$=3.4Hz, 3-H), 2.32(t, 2H), 1.70-1.63(m, 2H), 0.95(t, 3H)

^{13}C-NMR (300Hz, $CDCl_3$): δ (ppm) =172.54(0), 157.43(0), 138.71, 138.24, 134.25, 128.87, 128.25, 128.20, 119.56, 118.45(-), 99.70, 85.81(0), 78.93, 74.45, 74.23(-), 71.25, 70.32, 69.45(-), 66.60, 36.62(-), 18.92(-), 14.01

MS: (FAB *m/z*) 582.1

HRMS: $C_{26}H_{31}IO_7$, Calcd: 582.11145; Found 582.12876

4-Iodophenyl 3-*O*-allyl-6-*O*-benzyl-2-*O*-*iso*butyryl-β-D-galactopyranoside (39b)



Prepared as described for **10a** to yield the title compound **39b** (34 mg, 95 %) as a white solid.

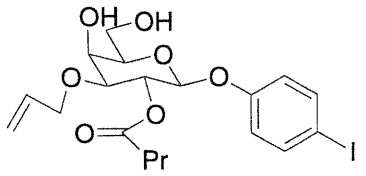
$[\alpha]_D$ +1.1 (c 1.135, CH_3Cl)

^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 7.55-7.53(m, 2H), 7.37-7.32(m, 5H), 6.81-6.79(m, 2H), 5.95-5.84(m, 1H, vinyl-H), 5.44(dd, 1H, $J_{2,3}$=9.8Hz, 2-H), 5.32-5.22(m, 2H, vinyl-Hs), 4.91(d, 1H, $J_{1,2}$=8.1Hz, 1-H), 4.60(s, 2H, CH_2PH), 4.22-4.16(m, 1H, allyl-H), 4.14(fdd, 1H, 4-H), 4.10-4.04(m, 1H, allyl-H), 3.92-3.78(m, 3H, 5-H, 6-H,and 6'-H), 3.57(dd, 1H, $J_{3,4}$=3.4Hz, 3-H), 2.60-2.57(m, 2H, 4-OH and $\underline{CH}(CH_3)_2$), 1.21-1.17(m, 6H)

^{13}C-NMR (300Hz, $CDCl_3$): δ (ppm) =176.04, 157.53(0), 138.73, 138.25(0), 134.24, 128.87, 128.26, 128.21, 119.54, 118.43(-), 99.88, 85.84(0), 78.96, 74.47, 74.24(-), 74.32(-), 70.25, 69.46(-), 66.63, 34.52, 19.53, 19.33

MS: (FAB, *m/z*) 581.1(m-1), 621.1 (m+23)

4-Iodophenyl 3-*O*-allyl-2-*O*-butyryl-*β*-D-galactopyranoside



Prepared as described for **11a** to yield the title compound (78 mg, 79%) as a white solid.

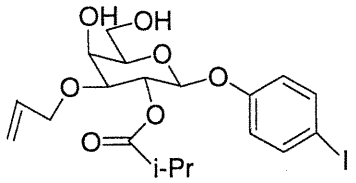
$[\alpha]_D$ +3.1 (c 1.0, $CHCl_3$)

^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 7.55(dd, 2H), 6.74(dd, 2H), 5.90-5.80(m, 1H, vinyl-H), 5.43(dd, 1H, $J_{2,3}$=9.8Hz, 2-H), 5.31-5.21(m, 2H, vinyl-Hs), 4.94(d, 1H, $J_{1,2}$=8.1Hz 1-H), 4.20-4.12(m, 2H, allyl-H and 4-H), 4.07-4.00(m, 2H, allyl-H and 6-H), 3.90-3.84(m,1H, 6'-H), 3.69-3.66 (m, 1H, 5-H), 3.56(dd, 1H, $J_{3,4}$=3.3Hz, 3-H), 2.90(s, 1H, 4-OH), 2.47-2.42(m, 1H, 6-OH), 2.31(t, 2H), 1.68-1.63(m, 2H), 0.94(t, 3H)

^{13}C-NMR (300Hz, $CDCl_3$): δ (ppm) =172.67(0), 157.32(0), 138.81, 134.24, 119.28, 118.55(-), 99.58, .85.87(0), 78.83, 75.24, 71.29(-), 70.24, 66.85, 62.49(-), 36.62(-), 18.94(-), 14.03

MS: (FAB, *m/z*) 515.0(m+23)

4-Iodophenyl 3-*O*-allyl-2-*O*-*iso*butyryl-*β*-D-galactopyranoside



Prepared as described for **11a** to yield the title compound (45 mg, 75 %) as a white solid.

$[\alpha]_D$ +1.1 (c 1.055, $CHCl_3$)

^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 7.56(dd, H), 6.76(dd, 2H), 5.90-5.79(m, 1H, vinyl-H), 5.42(dd, 1H, $J_{2,3}$=9.8Hz, 2-H), 5.32-5.22(m, 2H, vinyl-Hs), 4.95(d, 1H, $J_{1,2}$=8.1Hz, 1-H), 4.20-4.13(m, 2H, allyl-H and 4-H), 4.08-4.00(m, 2H, allyl-H and 6-H), 3.91-

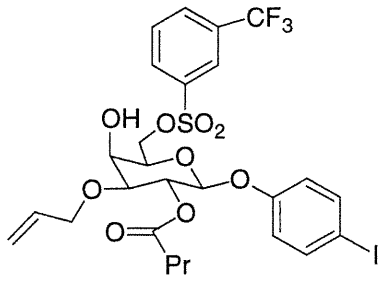
3.85(m,1H, 6'-H), 3.71-3.67 (m, 1H, 5-H), 3.59(dd, 1H, $J_{3,4}$=3.3Hz, 3-H), 2.72(s, 1H, 4-OH), 2.60-2.53(m, 1H), 2.18-2.15(dd, 1H, 6-OH), 1.17(m, 6H)

^{13}C-NMR (300Hz, $CDCl_3$): δ (ppm) =176.09(0), 157.40(0), 138.82, 134.16, 119.23, 118.59(-), 99.73, .85.87(0), 78.84, 75.25, 71.40(-), 70.14, 66.95, 62,59(-), 34.52, 19.52, 19.33

MS: (FAB, *m/z*) 493.1(m+1), 515.0(m+23)

HRMS: $C_{19}H_{26}O_7I$, Calcd: 493.07233; Found: 493.07030

4-Iodophenyl 3-*O*-allyl-2-*O*-butyryl-6-*O*-(*m*-trifluoromethyl)benzenesulfonyl-*β*-D-galactopyranoside (40a)



Prepared as described for **12a** to yield the title compound **40a** (96 mg, 82 %) as a white solid.

$[\alpha]_D$ -33.3 (c 1.0, $CHCl_3$)

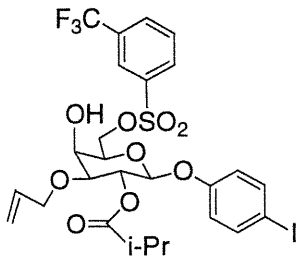
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 8.15(s, 1H), 8.04(d, 1H, J= 7.9Hz), 7.88(d, 1H, *J*=7.9Hz), 7.61(t, 1H, *J*=7.9Hz), 7.54(dd, 2H, *J*=2.1Hz and 6.9Hz), 6.70(dd, 2H, *J*=2.1Hz and 6.9Hz), 5.87-5.80(m, 1H, vinyl-H), 5.35(dd, 1H, $J_{1,2}$=8.1Hz and $J_{2,3}$=9.8Hz, 2-H), 5.31-5.22(m, 2H, vinyl-H), 4.88(d, 1H, 1-H), 4.42-4.32(m, 2H, 6-H and 6'-H), 4.19-4.01(m, 3H, allyl-H and 4-H), 3.95-3.92(m, 1H, 5-H), 3.57(dd, 1H, $J_{3,4}$=3.4Hz, 3-H), 2.52(s, 1H, OH), 2.30(t, 2H, J=7.3), 1.68-1.61(m, 2H), 0.93(t, 3H)

^{13}C-NMR (400Hz, $CDCl_3$): δ (ppm) =171.93(0), 156.57(0), 138.23, 136.56(0), 133.37, 130.98, 130.56, 130.03, 124.84, 118.74, 118.33(-), 99.78, 85.53(0), 77.72, 72.15, 71.02(-), 69.25, 68.86(-), 65.53, 35.99(-), 18.33(-), 13.43

MS: (FAB, *m/z*) 698.9(m-1), 722.9 (m+23), 481.1 (m - OPhI)

HRMS: $C_{26}H_{28}F_3SIO_9Na$, Calcd: 723.03485; Found: 723.03310

4-Iodophenyl 3-*O*-allyl-2-*O*-isobutyryl-6-*O*-(*m*-trifluoromethyl)benzenesulfonyl-*β*-D galactopyranoside (40b)



Prepared as described for **12a** to yield the title compound **40b** (88 mg, 95 %) as a white solid.

$[\alpha]_D$ -31.5 (c 0.355, $CHCl_3$)

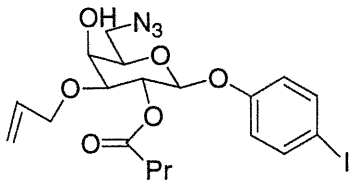
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 8.17(s, 1H, Ar), 8.05(d, 1H, Ar), 7.89(d, 1H, Ar), 7.62(t, 1H, Ar), 7.55(m, 2H, Ar), 6.70(d, 2H, Ar), 5.88-5.78(m, 1H, vinyl-H), 5.35(dd, 1H, $J_{1,2}$=8.1Hz and $J_{2,3}$=9.8Hz, 2-H), 5.37-5.31(m, 2H, vinyl-H), 4.88(d, 1H, 1-H), 4.44-4.34 (m, 2H, 6-H and 6'-H), 4.19-4.02 (m, 3H, allyl-H and 4-H), 3.96-3.93 (m, 1H, 5-H), 3.59(dd, 1H, $J_{3,4}$=3.4Hz, 3-H), 2.64-2.52(m, 1H), 2.50(s,1H, 4-OH),1.16(m, 6H)

^{13}C-NMR (400Hz, $CDCl_3$): δ (ppm)=175.98(0), 157.20(0), 138.80, 137.10(0), 133.88, 132.22(0), 131.54,131.15, 130.58, 125.42, 119.24, 118.94(-), 99.49, 86.11(0), 78.28, 72.69, 71.65(-), 69.71, 69.38(-), 66.10, 34..48, 19.50, 19.29

MS: (FAB, *m/z*) 699.0(m-1), 723.0(m+23)

HRMS: $C_{26}H_{28}F_3SIO_9Na$, Calcd: 723.03485; Found: 723.03310

4-Iodophenyl 3-*O*-allyl-6-azido-2-*O*-butyryl-6-deoxy-*β*-D-galactopyranoside (41a)



Prepared as described for **16a** to yield the title compound **41a** (75 mg, 82 %) as a white solid.

$[\alpha]_D$ -41.4 (c1.175, $CHCl_3$)

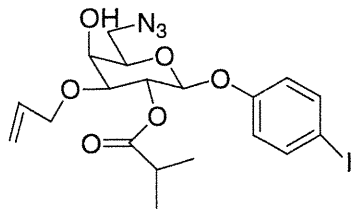
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) =7.57(dd, 2H, Ar), 6.78(dd, 2H, Ar), 5.90-5.82(m, 1H, vinyl-H), 5.42(dd, 1H, $J_{1,2}$=8.1Hz and $J_{2,3}$=9.7Hz, 2-H), 5.32-5.22(m, 2H, vinyl-H), 4.90(d, 1H, 1-H), 4.20-4.03(m, 3H, allyl-H and 4-H), 3.76(dd, 1H, $J_{5,6}$=7.9Hz and $J_{6,6'}$=12.4Hz, 6-H), 3.70-3.67(m, 1H, 5-H), 3.57(dd, 1H, $J_{3,4}$=3.4Hz, 3-H), 3.47(dd, 1H, $J_{5,6'}$=4.4Hz, 6'-H), 2.61(s, 1H, 4-OH), 2.31(t, 2H), 1.70-1.63(m, 2H), 0.95(t, 3H)

^{13}C-NMR (300Hz, $CDCl_3$): δ (ppm)=172.52(0), 157.30(0), 138.79, 134.09, 119.63, 118.69(-), 99.83, .86.21(0), 78.61, 74.33, 71.46(-), 70.04, 66.74, 51.37(-), 36.59(-), 18.91(-), 14.01

MS: (FAB, *m/z*) 516.1(m-1), 540.1(m+23)

IR: 2966.9, 2935.3, 2876.6, 2104.4, 1743.8, 1484.7, 1004.4, and 912.0 cm^{-1}

4-Iodophenyl 3-*O*-allyl-6-azido-6-deoxy-2-*O*-isobutyryl-β-D-galactopyranoside (41b)



Prepared as described for **16a** to yield the title compound **41b** (67 mg, 75 %) as a white solid.

$[\alpha]_D$ = -47.3 (c 0.825, $CHCl_3$)

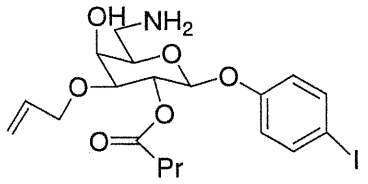
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) =7.57(dd, 2H), 6.77(dd, 2H), 5.89-5.82(m, 1H, vinyl-H), 5.40(dd, 1H, and $J_{2,3}$=9.8Hz, 2-H), 5.31-5.22(m, 2H, vinyl-Hs), 4.90(d, 1H, $J_{1,2}$=8.1Hz 1-H), 4.16-4.03(m, 3H, allyl-Hs and 4-H), 3.77(dd, 1H, $J_{5,6}$=7.9Hz and $J_{6,6'}$=12.4Hz, 6-H), 3.72-3.68(m, 1H, 5-H), 3.58(dd, 1H, $J_{3,4}$=3.4Hz, 3-H), 3.47(dd, 1H, $J_{5,6'}$=4.4Hz, 6'-H), 2.62-2.52(m,1H), 1.19-1.16(m,6H).

^{13}C-NMR (300Hz, $CDCl_3$): δ (ppm)=175.95(0), 157.44(0), 138.83, 134.11, 119.66, 118.60(-), 100.08, 86.21(0), 78.74, 74.37, 71.56(-), 70.08, 66.85, 51.41(-), 34.53(-), 19.50, 19.31

MS: (FAB, *m/z*) 540.2(m+23)

IR: 2966.9, 2935.3, 2876.6, 2104.4, 1743.8, 1484.7, 1004.4,912.0

4-iodophenyl 3-*O*-allyl-6-amino-2-*O*-butyryl-6-deoxy-β-D-galactopyranoside (42a)



Prepared as described for **17a** to yield the title compound **42a** (45 mg, 87 %) as a white solid.

$[\alpha]_D$ +2.8 (c 0.76, $CHCl_3$)

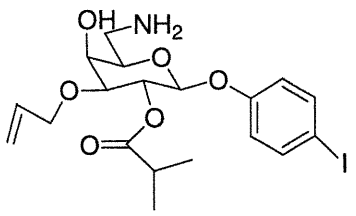
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) =7.55(dd, 2H,), 6.76(dd, 2H,), 5.94-5.84(m, 1H, vinyl-H), 5.48(dd, 1H, $J_{2,3}$=9.8Hz, 2-H), 5.30-5.19(m, 2H, vinyl-Hs), 4.95(d, 1H, $J_{1,2}$=8.1Hz 1-H), 4.20-4.06(m, 3H, allyl-Hs and 4-H), 3.56-3.51(m, 2H, 3-H and 5-H), 3.18-3.11(m, 2H, 6-H and 6'-H), 2.30(t, 2H), 1.68-1.63(m, 2H), 0.93(t, 3H)

^{13}C-NMR (300Hz, $CDCl_3$): δ(ppm)=172.50(0), 157.44(0), 138.74, 134.56, 119.30, 118.12(-), 99.75, .85.66(0), 79.09, 75.33, 71.08(-), 70.28, 68.33, 43.37(-), 36.64(-), 18.93(-), 14.00

MS: (FAB, *m/z*) 492.0(m+1)

HRMS: $C_{19}H_{27}O_6NI$, Calcd: 492.08853; Found: 492.08930 cm^{-1}

4-Iodophenyl 3-*O*-allyl-6-amino-2-*O*-isobutyryl-6-deoxy-β-D-galactopyranoside (42b)



Prepared as described for **17a** to yield the title compound **42b** (32 mg, 85 %) as a white solid.

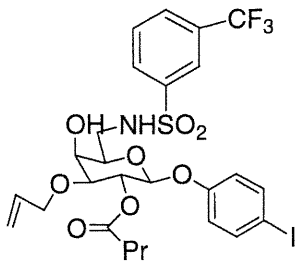
$[\alpha]_D$ +1.2 (c 0.895, $CHCl_3$)

^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) =7.55(dd, 2H,), 6.76(dd, 2H,), 5.91-5.82(m, 1H, vinyl-H), 5.46(dd, 1H, $J_{2,3}$=9.8Hz, 2-H), 5.30-5.19(m, 2H, vinyl-Hs), 4.94(d, 1H, $J_{1,2}$=8.0Hz 1-H), 4.21-4.02(m, 3H, allyl-Hs and 4-H), 3.57-3.52(m, 2H, 3-H and 5-H), 3.22-3.10(m, 2H, 6-H and 6'-H), 2.59-2.52(m, 1H), 1.15(m, 6H),

^{13}C-NMR (300Hz, $CDCl_3$): δ (ppm)=176.04(0), 157.52(0), 138.76, 134.53, 119.26, 118.11(-), 99.90, .85.68(0), 79.15, 75.45, 71.13(-), 70.21, 68.23, 43.30(-), 34.52(-), 19.52, 19.35

MS: (FAB, *m/z*) 492.0(m+1)

4-Iodophenyl 3-*O*-allyl-6-deoxy-2-*O*-butyryl-6-(*m*-trifluoromethyl)benzenesulfonyl amide-β-Dgalactopyranoside (43a)



Prepared as described for **18a** to yield the title compound **43a** (35 mg, 93 %) as a white solid.

$[\alpha]_D$ -19.0 (c 1.225, $CHCl_3$)

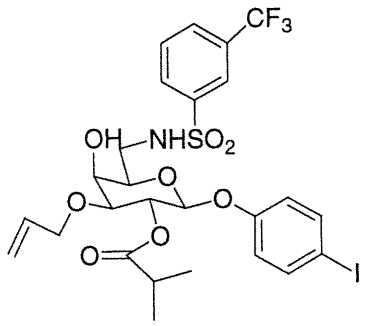
^{1}H-NMR (400 Hz, $CDCl_3$): δ (ppm) = 8.12(s, 1H), 8.02(d, 1H,), 7.80(d, 1H), 7.63-7.57(m, 3H), 6.71(d, 2H), 5.90-5.80(m, 1H, vinyl-H), 5.36(dd, 1H, $J_{2,3}$=9.7Hz, 2-H), 5.31-5.18(m, 3H, vinyl-Hs and 6-NH), 4.92(d, 1H, $J_{1,2}$=8.1Hz 1-H), 4.20-4.04(m, 3H, allyl-Hs and 4-H), 3.84-3.82(m, 1H, 5-H), 3.57(dd, 1H, $J_{3,4}$=3.4Hz, 3-H), 3.40-3.37(m, 2H, 6-H and 6'-H), 2.68(s, 1H, 4-OH), 2.30(t, 2H), 1.69-1.62(m, 2H), 0.94(t, 3H)

^{13}C-NMR (400Hz, $CDCl_3$): δ (ppm) =172.64(0), 157.04(0), 141.44(0) 138.91, 133.97, 132.41(0), 131.96, 130.55, 130.42, 129.84, 124.45, 124.40, 118.99, 118.86(-), 99.17, 85.96(0), 78.42, 73.68, 71.52(-), 69.87, 66.84, 43.89(-) 36.56(-), 18.89(-), 13.98

MS: 722.0(m+23)

HRMS: $C_{26}H_{29}F_3SIO_8NaN$, Calcd: 722.05084; Found: 722.04820

4-Iodophenyl 3-*O*-allyl-6-deoxy-2-*O*-isobutyryl-6-(*m*-trifluoromethyl)benzene sulfonamide-β-D-galactopyranoside (43b)



Prepared as described for **18a** to yield the title compound **43b** (34 mg, 92 %) as a white solid.

$[\alpha]_D$ -22.6 (c 0.97, $CHCl_3$)

^{1}H-NMR (300 Hz, $CDCl_3$): δ (ppm) = 8.02(s, 1H), 7.94(dd, 1H,), 7.72(dd, 1H), 7.58-7.46(m, 3H), 6.65(d, 2H), 5.82-5.70(m, 1H, vinyl-H), 5.30-5.14(m, 4H, 2-H, vinyl-Hs and 6-NH), 4.84(d, 1H, $J_{1,2}$=8.1Hz 1-H), 4.14-3.92(m, 3H, allyl-Hs and 4-H), 3.80-3.75(1H, 5-H), 3.50(dd, 1H, $J_{2,3}$=9.7Hz and $J_{3,4}$=3.4Hz, 3-H), 3.33-3.28(m, 2H, 6-H and 6'-H), 2.68(s, 1H, 4-OH), 2.55-2.43(m, 1H), 1.12-1.08(m, 6H)

^{13}C-NMR (30Hz, $CDCl_3$): δ (ppm) =176.17(0), 157.13(0), 141.42(0) 138.92, 133.96, 132.39(0), 130.55, 130.43, 129.89, 125.34, 124.45, 124.40, 118.97, 118.83(-), 99.37, 85.99(0), 78.45, 73.69, 71.57(-), 69.83, 66.86, 43.88(-) 34.49, 19.49, 19.29

MS: 697.9(m-1), 721.9(m+23)

HRMS: $C_{26}H_{28}F_3SIO_9Na$, Calcd: 723.03485; Found: 723.03310

5-3 Biological approaches

Cells

The human LN18 and LNZ308 glioblastoma cell lines were developed from surgical specimens of human glioblastoma (a kind gift of AC. Diserens and N. de Tribolet, Neurosurgery Division, CHUV, Lausanne). Human brain-derived endothelial cells (HCEC cells) were a kind gift of Dr. D. Stanimirovic, Ottawa, Canada. Human primary urogenital fibroblasts were cultured from surgical biopsies performed during delivery or reconstructive surgery in women, according to a protocol approved by the Ethics Commitee of the CHUV in Lausanne, using the explant technique. All cells were grown in DMEM medium (Gibco-BRL, Basel, Switzerland) containing 4.5 g/l glucose, 10% fetal calf serum (FCS) and antibiotics.

Cell culture and treatments

The cell culture reagents were from Life Technologies or Gibco (both in Basel, Switzerland). The human cell lines were routinely maintained in DMEM supplemented with 10% FCS and detached from the plate using trypsin-EDTA. Cells were seeded in 48-well plates (Costar) as monolayers for 24 h in DMEM-10% FCS to half confluence and, unless otherwise stated, incubated 24 h in DMEM without FCS, then exposed for the time indicated to the glycomers under investigation. Then either thymidine incorporation to evaluate DNA synthesis, or protein content and MTT reduction to evaluate cell viability or nucleosome-DNA fragments to quantitate apotosis, were performed. Experiments were done in triplicate and means and standard deviations (SD) were calculated.

Thymidine incorporation

Thymidine incorporation was used to ascertain DNA synthesis. Following treatment, cells were exposed to 0.8 μCi/ml [^{3}H]-thymidine (Amersham Pharmacia, Dübendorf, Switzerland) for 2h - 3h and radioactivity was quantitated in a beta-counter (LKB) after precipitation with 10% trichloracetic acid and solubilization in 0.1% SDS - 0.1 N NaOH and 5 ml Optiphase scintillation cocktail (Wallac, Fisher Chemicals, England).

Evaluation of cell proliferation by MTT

MTT ((3,4,5-dimethylthiazol-yl)-2,5-diphenyl tetrazolium); Sigma, Buchs, Switzerland) was used to quantify the number of metabolically active cells. Following treatment, cells were exposed to 0.25 mg/ml MTT in DMEM medium for 2h, supernatant was aspirated and the precipitated formazan was dissolved in 0.1 N HCl in isopropanol and quantified at 540 nm.

Apoptosis determination and quantification

Apoptosis was determined in cells in culture by evaluation of nuclear chromatin condensation using the fluorescent nuclear marker 4',6'-diamidino-2-phenylindolylhydrochloride, (DAPI, Boehringer Roche). Cells were grown on histological sides and exposed to glycomers according to the same protocols than cells grown in culture wells. Following fixation in methanol/acetic acid (3: 1) slides were exposed to 1 μg/ml DAPI for 30 min at 37°C, then mounted in 20% glycerol in PBS and examined and photographed under a fluorescent microscope (360/500 nm, excitation/emission wavenlength, respectively). Alternatively, following buffered paraformaldehy fixation, Giemsa and hematoxylin-eosin staining to vizualize nuclei were performed according to standard procedures.

Apoptosis was quantified using the Cell Death Detection ELISA^{PLUS} (Roche, Rotkreuz, Switzerland), a photometric enzyme-linked immunoassay for quantitative *in vitro* determination of cytoplasmic histone-associated-DNA-fragments (mono- and oligo-nucleosomes), a marker of DNA breakdown and laddering, which is considered as a landmark of apoptosis. Determinations were performed according to the supplier's instructions. Using this method, the increase in absorbance at 405 nm is proportional to apoptosis. The enrichment in apoptotic cell proportion (apoptosis index) was calculated as the ratio of absorbance of treated cells/absorbance of untreated cells as previously described [73].

Protein concentration

Protein content was determined with the BCA protein assay kit (Pierce, Socochim, Switzerland) and bovine serum albumin as standard.

Chapter Six

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Appendix

Growth Inhibition Table

LN18

uM	SH001	SH002	SH003	SH004	SH005	SH007	SH008	SH/L-1	SH/L-002I	SH/L-2a	SH/L-4
0	100	100	100	100	100	100	100	100	100	100	100
0.7				117							
1.5	126			125				134	115		
2											
3	117			139				125	107		
4		110									
5			95		101	128	114			96	97
7.5	123	41		120				158	71		
10.0			105		74	75	123			96	105
12.5		25									
15	94	15		12				90	29		
20	96	15		10							
25			109		42	13	142			95	100
30	85			10				16	20		
40								8	10		
50			29		24	12	142			106	103
60	40			5				7	7		
100			24				13	156		94	89

LN308

uM	SH001	SH002	SH003	SH004	SH005	SH007	SH008	SH/L-1	SH/L-002I	SH/L-2a	SH/L-4
0	100	100	100	100	100	100	100	100	100	100	100
0.7				128							
1.5	108			125				155	103		
2											
3	120			145				148	107		
4		114									
5			94		55	108	122			103	102
7.5	113	98		118				163	114		
10.0			89		63	66	129			93	102
12.5		46									
15	109	19		20				138	88		
20	114	15		12				35	16		
25			100		47	16	132			96	100
30	87			11				10	11		
40											
50			21		40	14	134			107	98
60	72			5				10	7		
100			19			14	113			97	85

SH/L-5	SH/L-6	SH/L-6a	SH/L-8	SH/L-9	SH/L-9a	SH/L-23	SH/L-26	SH/L-27	SH/L-29a	SH/L-30	SH/L-31
100	100	100	100	100	100	100	100	100	100	100	100
				138			130				
			101		118			92		88	
			118	181	120		97	96		120	
93	100	112							97		90
			115	154	131	116	118	87		113	
69	103	120							108		91
			116		133			112		137	
				164		86	109				
			142	193	101		36	88		175	
77	73	96							79		87
			120	129	19	40	14	69		99	
						24					
57	103	36							12		28
			120	48	7	13	11	24		83	
	100	19							11		

SH/L-31a	SH/L-41	SH/L-41B	SH/L-52	SH/L-60	SH/L-61	SH/L-62	SH/L-64	SH/L-71	
100	100	100	100	100	100	100	100	100	0 uM
	128	112	145	128	128	111	111	129	0.7 uM 1.5 uM
	117	120	164	117	88	132	105	116	2 3
94									4 5
98	31	115	26	136	126	134	79	160	7.5 10.0
									12.5
	12	30	19	85	110	64	37	147	15
91	10	7	17	33	76	54	31	180	20
	9	6	10	18	30	57	25	179	25 30
85									40
	5	5	5	16	16	59	16	179	50 60
50									100

SH/L-5	SH/L-6	SH/L-6a	SH/L-8	SH/L-9	SH/L-9a	SH/L-23	SH/L-26	SH/L-27	SH/L-29a	SH/L-30	SH/L-31
100	100	100	100	100	100	100	100	100	100	100	100
				146			108				
			85		103			81		78	
			106	182	99		102	103		91	
85	100	105							105		90
			88	138	108	113	120	79		83	
81	98	109							106		86
			109		125			118		94	
				154		135	98				
			111	188	56		99	75		113	90
80	88	91							93		
			125	149	20	70	22	47		122	
						47					
57	98	19							20		19
			122	37	6	21	11	15		80	
	98	12							8		

SH/L-31a	SH/L-41	SH/L-41B	SH/L-52	SH/L-60	SH/L-61	SH/L-62	SH/L-64	SH/L-71	
100	100	100	100	100	100	100	100	100	0 uM
	126	87	89	118	103	108	99	102	0.7 uM 1.5 uM
	122	100	97	111	91	106	95	121	2 3
97									4 5
107	100	93	75	120	104	112	48	112	7.5 10.0
									12.5
	95	97	21	88	81	106	22	136	15
102	14	27	13	37	66	112	15	125	20
	5	10	9	17	41	58	11	97	25 30
112									40
98	5	3	5	15	14	32	12	121	50 60
									100