AN EXCURSION INTO THE CHEMISTRY OF *N*- AND *C*-GLYCOSIDES OF D-GALACTURONIC ACID

DISSERTATION

zur

Erlangung des akademischen Grades

doctor rerum naturalium (Dr. rer. nat.)

der Mathematisch-Naturwissenschaftlichen Fakultät

der Universität Rostock

vorgelegt von

Gnuni Karapetyan

geb. am 08.09.1981

Rostock, April 2008

[*urn:nbn:de:gbv:28-diss2008-0045-2*]

Dekan: Prof. Dr. Dieter Neßelmann

Gutachter der Dissertation:

1. Prof. Dr. Christian Vogel

2. Prof. Dr. Udo Kragl

Termin der mündlichen Prüfung: 21.05.2008

1. Prüfer: Prof. Dr. Christian Vogel (Hauptfach: Organische Chemie)

2. Prüfer: PD. Dr. Birgit Tiefenbach (Nebenfach: Toxikologie)

Termin des wissenschaftlichen Kolloqiums: 03.06.2008

If you want to build a ship don't herd people together to collect wood and don't assign them tasks and work, but rather teach them to long for the endless immensity of the sea.

Antoine-Marie-Roger de Saint-Exupery

Preface

The presented work in this dissertation was carried out from July 2005 till November 2007 at the Institute of Chemistry at the University of Rostock.

I would like to thank my supervisor Prof. Dr. *Christian Vogel*, for his continuous support and insightful comments on my work. I have highly benefited from his scientific and personal experience.

Prof. Dr. *Otto Holst* (Research Center Borstel, Leibniz Center for Medicine and Biosciences, Germany) is greatly acknowledged for the excellent scientific and personal support that I received during my stay in Borstel. The special thanks to all my colleges and friends from Research Center Borstel for creating brilliant working atmosphere and for providing me with the effective training. It was my great pleasure to be a part of international club "Ausländerbande-Borstel".

I would like to express my gratitude to Prof. Dr. *Aida Avetisyan* (Faculty of Chemistry, Yerevan State University, Armenia) for her kind support.

Herbstreith & Fox and especially Dr. Hans-Ulrich Endress is acknowledged for financial support. The analytical measurements in presented work were performed by analytical staff of the Institute of Chemistry and Leibniz Institute for Catalysis at the University of Rostock. All colleagues are gratefully acknowledged for their excellent cooperation. Among others my special thanks to Prof. *Helmut Reinke* for X-ray studies, Dr. *Roland Ruth* and Dr. *Christina Fischer* for MS measurements, Dr. *Dirk Michalik*, *Brigitt Schimanski* for NMR measurements, Dr. *Ursula Bentrup*, Dr. *Annegret Tillak* and *Angela Niemann* for IR measurements. Furthermore, I thank group members of Prof. Christian Vogel for a good teamwork in the lab. I highly appreciate the hours of very interesting and motivating discussions I had with Dr. Anahit Pews-Davtyan and Dr. *Leonid Kononov*. My very special thanks to Dr. *Man Kin Tse*. This manuscript benefited greatly from his meticulous corrections. Last but not least, I deeply indebted to my father, who regardless of the distance has always been next to me, to *Arevik*, my wife, for her unconditional understanding and patience during this thesis journey. A big thank you to all my friends spread all over the world. I am lucky and happy that you all exist. You all give me a spiritual power for science and for life.

Gnuni Karapetyan

April 2008, Rostock

TABLE OF CONTENTS

ABBREVIATIONS

1-HOBT	1-hydroxybenzotriazole
abs.	absolute
Ac	acetyl
All	allyl
aq	aqueous
ATR	attenuated total reflection
Bz	benzoyl
Bn	benzyl
br.	broud
calcd	calculated
CI	chemical ionization
COSY	correlated spectroscopy
CP	citrus pectin
dd	doublet of doublets
ddd	doublet of doublet of doublets
dddd	doublet of doublet of doublet of doublets
dec.	decomposition
DEPT	distortionless enhancement by polarisation transfer
Dha	3-deoxy-D-lyxo-2-heptulosaric acid
DIPEA	diisopropylethylamine
DM	degree of methyl esterification
DMAP	*N*,*N*'-dimethyl-4-aminopyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dq	doublet of quartets
dt	doublet of triplets
dRG	dimmer of rhamnogalacturonan
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EI	electron impact
ELISA	enzyme linked immuno sorbent assay
ESI	electrospray
Et	ethyl
FAB	fast atom bombardment
Fig.	figure
GAA	glycosylamino acid
GalA	galacturonic acid
Gal*p*A	galactopyranuronic aicd
HG	homogalacturonan
HMBC	heteronuclear multiple bond correlation
HMPT	hexamethylphosphoramide
HPLC	high performance liquid chromatography
HRMS	high-resolution mass spectroscopy
HSQC	heteronuclear single quantum coherence
Hz	hertz
IR	Infrared spectroscopy
Kdo	2-keto-3-deoxy-D-manno-octulosonic acid
LC-MS	coupled liquid chromatography-mass spectroscopy

lit.	literature
m	multiplet
mAb	monoclonal antibody
MCP	modified citrus pectin
Me	methyl
MHz	megahertz
mp	melting point
MPLC	medium pressure liquid chromatography
MS	Mass spectroscopy
MS 4Å	molecular siev 4 angstrem
m/z	mass to charge ratio
ND	not determined
NMR	Nuclear magnetic resonance (spectroscopy)
NOESY	nuclear overhauser effect spectroscopy
pD	*pondus deutorii*
PG	polygalacturonase
pH	*pondus hydrogenii*
Ph	phenyl
PME	pectin methyl esterase
q	quartet
ref.	referenced
R	organic moiety, rest
R_f	retention factor
RG	rhamnogalacturonan
RP	reversed phase
r.t.	room temperature
s	singlet
SAA	sugar amino acid
t	triplet
TEMPO	2,2,6,6-tetramethylpiperidine-1-oxyl
THF	tetrahydrofuran
TLC	thin layer chromatography
Trt	trityl
XGA	xylogalacturonan
$[\alpha]$	optical rotation
δ	chemical shift

1. INTRODUCTION

Science is about opening your eyes to what's going on in nature, at all levels - molecular, biological, the engineering level - and appreciating and having fun with that. In particular, sugars are mind-blowing. We haven't even begun to understand the complexity. Chemical biology and chemical glycobiology have just been the place, where the most incredible stories happen, the most incredible mechanisms emerge. Instinctively, we're much happier with simple codes - taking DNA, and reading it out, to give you an exact protein structure. The concept of the genome is like a recipe. Sugars are almost the opposite, mediating subtle and specific events. The way that sugars do things is not lock and key, it's dynamic, it's flexible, it fluctuates - it's complexity, both in a molecular sense and in a systems sense, a mathematical sense. Sugars are nature's fuzzy logic, and that's what's fascinating.[1]

B. J. Davis

Pectin is a very complex biopolymer and an important constituent of plant cell wall. Pectin fragments, among others, contain mainly D-galacturonic acid moieties. Hence, there is a raising interest toward a synthesis of pectin fragments of defined structures containing labelling moieties to elucidate the biological role of pectin in plants as well as in dietary fiber in human nutrition. In the present work the synthesis of D-galacturonic acid *N*- and *C*-glycosides as suitable markers and anchors was described.

1.1. THE PLANT CELL WALL

Plant cells are surrounded by a cell wall which is a structurally complex and highly varied domain consisting of polysaccharides as the most abundant component as well as proteins and sometimes lignin.[2] The polysaccharides of the wall are usually divided in cellulose, hemicellulose and pectin. Pectin is the most abundant class of macromolecule within this matrix[3,4] and, in addition, it is also abundant in the middle lamellae between primary cell walls where it functions in regulating intercellular adhesion (**Fig. 1**). Pectin occurs in all cells and it is generally thought to account for about one third of all primary cell wall macromolecules, although lower levels occur in primary cell walls of certain families belonging to the Poales.[5,6] When a cell grows, the bonds between existing wall polysaccharides are broken and, as the wall expands, newly synthesized wall polysaccharides are inserted between the existing one.[7] This process undoubtedly involves the breaking and formation of numerous covalent and non-covalent bonds.

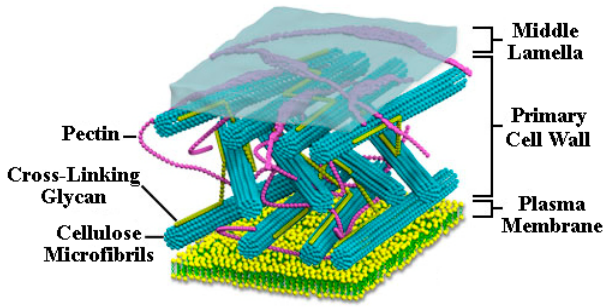


Figure. 1. The structure of plant cell wall[8]

In this way, cells can elongate their length without weakening the wall. Furthermore, the fine structures of some of the primary cell wall polysaccharides such as pectin undergo changes as the cells pass through different stages of development.

1.2. PECTIC POLYSACCHARIDES

1.2.1. COMPOSITION AND STRUCTURE

Pectins are a group of heterogeneous polysaccharides that contain mainly D-galacturonic acid (D-GalA), L-rhamnose, xylose, arabinose and galactose. The relative abundance and structural details of these polysaccharides varies with sources and conditions of extraction, location and many other environmental factors. The extracted pectin can be fractionated into homogalacturonan (**HG**), rhamnogalacturonan-I (**RG-I**), rhamnogalacturonan-II (**RG-II**) and xylogalacturonan (**XGA**)[9] (**Fig. 2**). Apparently, these polysaccharide domains are covalently linked to each other to form a massively large pectic network throughout the primary cell wall matrix and middle lamellae (**Fig. 3a**), but it has been very difficult to get a clear picture of how the different pectic polysaccharides are connected and several models exist.[10]

HG is a linear chain of 1,4-linked α-D-galactopyranuronic acid [→4)-α-D-Gal*p*A-(1→] residues (called *smooth region*) that are often methylesterified.[11,12] The pattern of methyl-esterification has an important impact for the physical properties of the calcium-pectin gels with respect to the stiffness and strength of the gels formed[13] (**Fig. 3c**).

RG-I (called *hairy region*) acidic pectic domain consists of a backbone of disaccharide [→4)-α-D-Gal*p*A-α-(1→2)-α-L-Rha*p*-(1→] as the basic repeating unit. The proportion of branched rhamnosyl residues generally varies from approximately 20–80% depending on the source of the polysaccharide. The rhamnose residues are often substituted with side chains of galactan, arabinan or arabinogalactan I. However the side chains are abundant and heterogeneous.

Common structural features of the side chains include polymeric (1→4)-β-D-galactopyranosyl (galactan) and (1→5)-α-L-arabinofuranosyl (arabinan) residues.

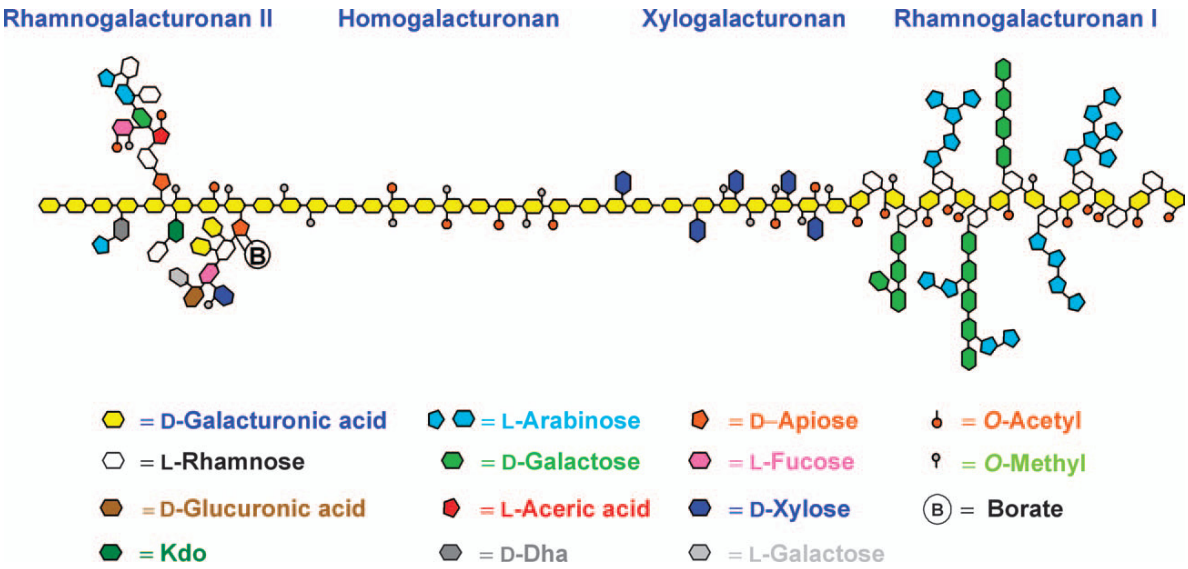


Figure. 2. Schematic structure of pectin

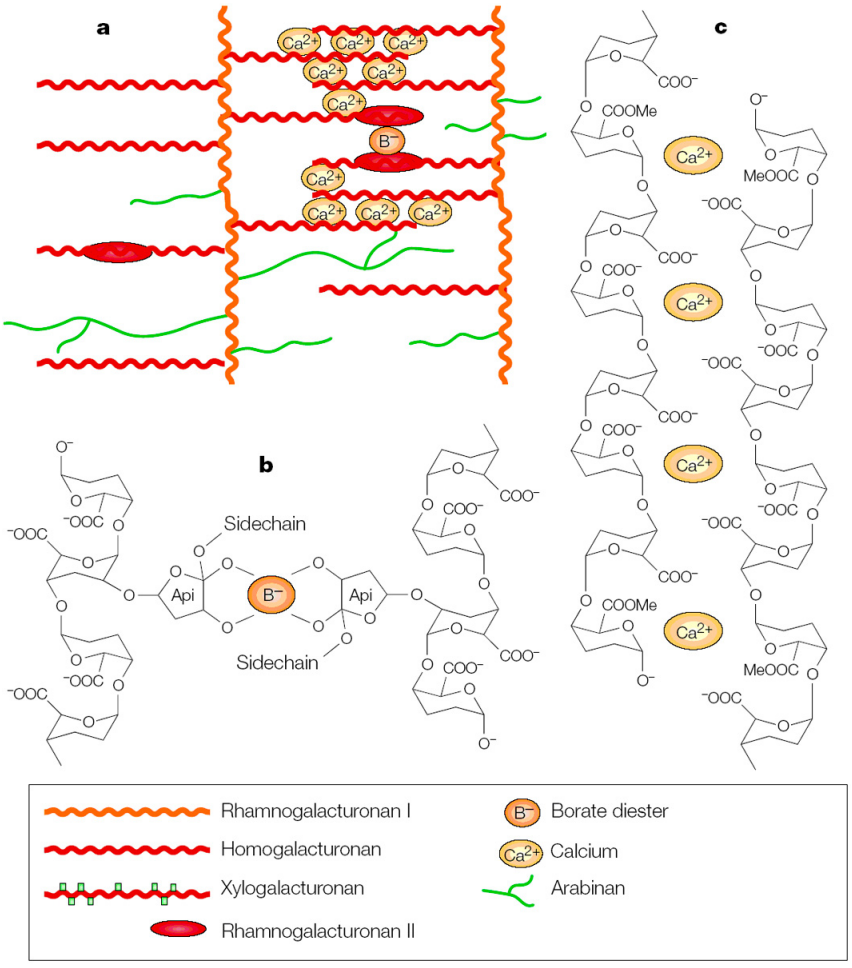


Figure 3. a) covalently crosslinked pectic domains, b) **RG-II** dimmer formation as borate esters, c) calcium crosslinked **HG** fragments (the picture is taken from [7])

RG-II is a very complex polysaccharide but its structure appears to be remarkably conserved in all vascular plants.[14,15] The name **RG-II** is misleading because its backbone is not rhamnogalacturonan but a short stretch of **HG** substituted with four different side chains. **RG-II** consists of at least 12 different monosaccharides in more than 20 different linkages. The residues in **RG-II** include some monosaccharides that are rarely found in other polysaccharides: D-apiose, 3-C-carboxy-5-deoxy-L-xylose (L-aceric acid), 2-O-methyl-L-fucose, 2-O-methyl-D-xylose, L-galactose, 3-deoxy-D-lyxo-2-heptulosaric acid (Dha) and 2-keto-3-deoxy-D-manno-octulosonic acid (Kdo). **RG-II** exists predominantly as dimers that are covalently cross-linked by borate diesters[16] (**Fig. 3b**).

In the case of polysaccharides, less is known about their secondary and tertiary level of structural organization than for the other families of biological macromolecules. In particular, on account of their size and its highly conserved nature, **RG-II** is a good target for the analysis of secondary structure. However, the complexity of its monosaccharide composition makes the elucidation of its conformational parameters a formidable task. Recent studies have addressed this problem by NMR spectroscopy[17] and molecular modelling,[18] although a complete 3-dimensional model has not yet been proposed.

1.2.2. THE BIOLOGICAL ACTIVITIES

A number of different biological activities of pectin fragments have been reported.[19] The development of isolation, purification and characterization methods during the last 25 years made it possible to investigate a scientific basis for traditional use of pectic polysaccharides isolated from medicinal plants as immunostimulatory, antitumor, antimutagen or anti-inflammatory agents.[20] *Acanthus ebracteatus* is a plant traditionally used for various ailments, amongst those skin diseases in Thai traditional medicine. The stem of the plant was shown to contain neutral and acidic pectic polysaccharides with effect in the complement system that was quite high compared to the used normal standard.[21] The roots of Sino-Japanese medicinal herb *Bupleurum falcatum* have been utilized for the treatment of chronic hepatitis, nephritic syndrome and autoimmune diseases.[22] The polysaccharide fraction responsible for the anti-ulcer and mitogenic effect Bupleuran 2IIc consist ~70% 1,4-linked α-D-GalA, of which ~30% are methyl esterified. Bupleuran 2IIc also contains ramified or hairy regions consisting of **RG** core having neutral side chains of mainly galactose and arabinose units. Rare sugars as KDO, apiose and aceric acid are also present in minor quantities.[19] Leaves of different cabbage

species are used as wound healing remedies in traditional medicine. Studying the structure-activity relationship of different cabbage varieties on the complement-fixing activity, it was investigated that this supposed wound healing activity is associated with the presence of immunomodulating water soluble pectin.[23]

It is necessary to mention that biological activities of pectins are usually determined using heterogeneous mixtures of oligosaccharides which were obtained either by partial acidic hydrolysis of plant cell walls, or by treatment of the walls with pectic-degrading enzymes. Thus, the tested samples contain both active and inactive molecules.

1.2.3. PECTIN IN HUMAN DIET. NATURAL CATION EXCHANGER

Pectins are important in the human diet and health since they are a major component of dietary fiber. The ability of pectins to bind cations is due to the anionic character of non-methyl esterified GalA residues. This property is well established and has been widely exploited within the food industry for the preparation of jams and jellies.[24] In addition, pectin has also been employed as a thickener, water binder and stabilizer in beverages and dairy products. In contrast to low methylated pectins, pectins with a degree of methyl esterification higher than 40% are able to form gells under specific conditions: the pH has to be adjusted to bellow 3.5 and large quantities of a cosolute (typically sucrose, >55 wt %) have to be added.[24] The addition of pectic polysaccharides to the human diet has been shown to reduce the uptake of toxic metals, lanthanides and actinides.[25] It was found that borate esterified **RG-II** dimers (**dRG-II-B**) (Fig. 3b) selectively bind Pb^{2+}, Ba^{2+}, Sr^{2+}, La^{3+}, Eu^{3+} Ce^{3+}, Pr^{3+}, Nd^{3+} by complexation.[26] Thus, due to the higher degree of selectivity for cations, the **dRG-II-B** has potential applications as a food additive for the removal of toxic cations. Recently, a clinical trial provided the first evidence that oral administration of modified citrus pectin (MCP molecular weight 15400; DM 3.8%, **RG-II** content 10%) increases significantly the urinary excretion of toxic metals in subjects with a 'normal' body load of metals.[27] However, pectins also bind physiologically important cations (Ca^{2+}, Cu^{2+}, Mg^{2+} or Zn^{2+}) and elevated consumption of pectins may result in a decrease in the availability of essential minerals.

1.2.4. PECTIN IN CANCER RESEARCH

The role of pectin fragments in cancer prevention and progression is an area of increasing clinical and scientific interest.[28] Owing to diverse carbohydrate constituents, pectin fragments

show anticancer activities against various cancer cells. The carbohydrate moieties of glycoproteins or glycolipids present on the cell membrane are recognized by lectins. Lectins are able to detect subtle differences between complex carbohydrate structures. Typically, lectins and their complimentary carbohydrates are located on the surfaces of opposing cells, which can be of the same type or different types (**Fig. 4a**). Due to their affinity to immune cell lectins, the complimentary carbohydrates of cancer cells bind to them and so deactivate immune cells to escape recognition by the immune cells as they migrate through the body. In blood vessels, tumour cells use their complimentary carbohydrates to form emboli (cell aggregates), which protect them in the hostile host environment.[28] According to existing model, pectin fragments composed of various sugar moieties can selectively bind to cancer cells and so block the recognition site of cancer cell lectins. Thus, pectin fragments compete with the natural ligands of tumor cell surface galectins (**Fig. 4b**) possibly preventing the formation of tumor cell emboli as well as inhibiting the interaction of cancer cells with the endothelium of the target organ.

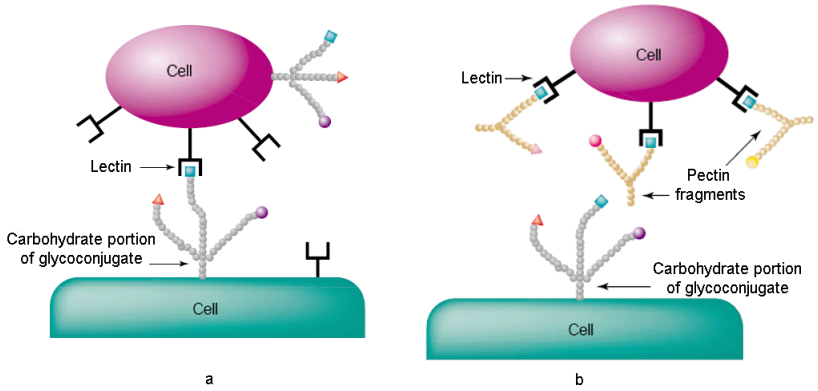


Figure 4.[28] a) cell-cell interactions mediated by lectins, b) a model of how a pectin fragments could interfere with the cell–cell recognition mediated by lectins

Previous research has shown that pectin can suppress colonic tumour incidence in rats[29] and inhibit cancer cell metastasis in mice[30] and rats.[31] Furthermore, when injected intravenously in mice, relatively large commercial pectin increased homotypic cell–cell aggregation and metastasis to the lung while pH-modified, relatively small pectin inhibited lung metastasis,[30] demonstrating a differential response depending upon the type of pectin used. Oral administration of a pH-modified citrus pectin (CP) significantly reduced metastasis of rat prostate adenocarcinoma MATLyLu to the lung.[32] It is noteworthy that those anti-metastatic effects of pectins occurred in the absence of cell toxicity.[33] Recently, the first evidence was presented that specific structural characteristics of pectin are responsible for inducing apoptosis in human prostate cancer cells.[34]

1.3. OBJECTIVES

Various pectin fragments containing galacturonic acid were synthesized to elucidate the biological role of pectin in cell wall to construct the structure-biological activity relationship. Since D-galacturonic acid is the most common constituent in pectin, studies of its chemistry is essential from multilateral point of view. In this context, the synthesis and structural characterisation of *N*- and *C*-glycosides of GalA is a very attractive and important task.

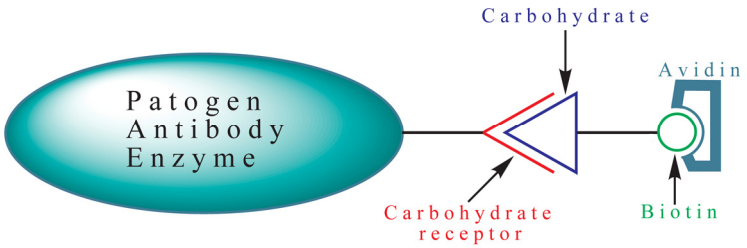


Figure 5. A model for attaching proteins to the cell surface by Bertozzi and Bednarski[35]

As a part of a program directed toward the design and synthesis of pectin fragments suitable for biochemical assays[36] of biotinylated galacturonate mimetics as markers and anchors preparative scale synthesis are required. Due to the high affinity to avidin, biotin has been widely used for labelling various types of molecules, in particular, carbohydrates, antibodies and enzymes (**Figure 5**).[35-42] Therefore, the following targets were defined:

- Introduction of an azido group at the anomeric centre of a D-galacopyranuronic acid and the synthesis of a library of partially protected galactopyranuronate azides building blocks
- Derivatization of the azido group and biotinylation
- Synthesis and characterization of a library of partially protected D-galacturonic acide *C*-glycosides
- Derivatization of the *C*-glycosidic residue and subsequent biotinylation.

2. RESULTS & DISCUSSION

2.1. SYNTHESIS OF D-GALACTOPYRANOSYL URONATE N-GLYCOSIDES

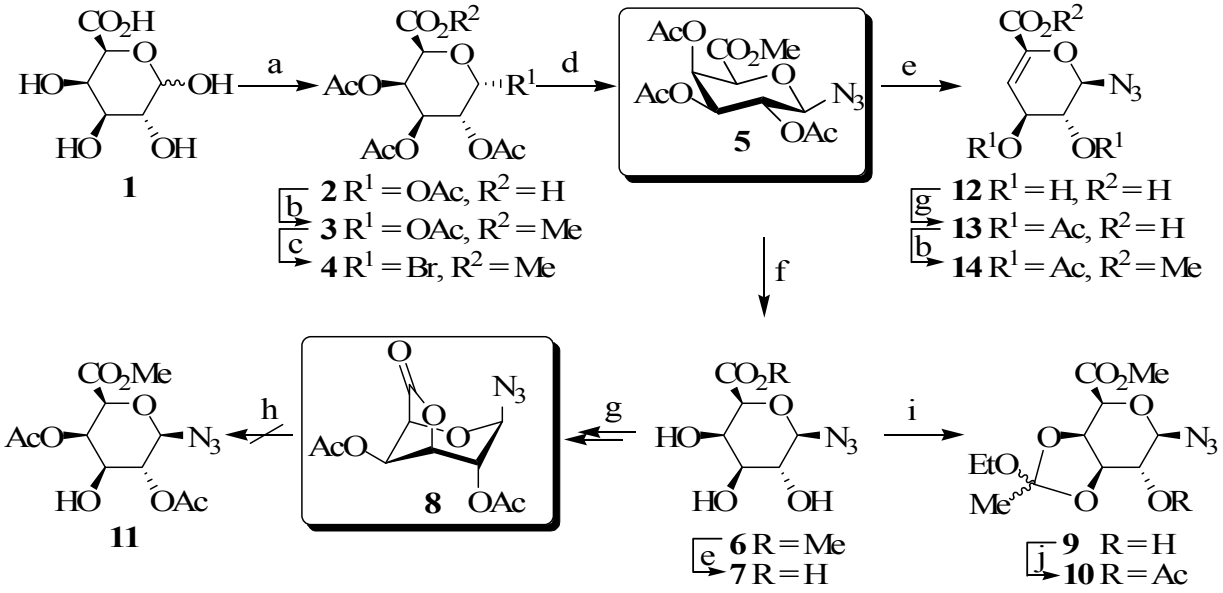
2.1.1. SYNTHESIS OF DIFFERENTLY PROTECTED D-GALACTOPYRANOSYL URONATE AZIDES AS GLYCOSAMINO ACID PRECURSORS

Glycosamino acids (GAAs) are molecules that combine the structural features of simple amino acids and carbohydrates.[43] They are highly substituted polyfunctional building blocks which can be used for synthesis of compound libraries by means of combinatorial synthesis. GAAs are classified into five groups (A_0-A_4) depending on the position of the amino acid moiety on the carbohydrate residue.[44] The incorporation of an amine and a carboxylic acid function into cyclic carbohydrate skeleton results in sugar amino acids (SAAs)[45] of so called A_0 type. An example of such a SAA precursors are uronic acid azides which bear masked amino group. As configurationally stable groups, azides are suitable starting materials for the introduction of various nitrogen-containing functionalities, such as amines, amides, ureas, carbodiimides, and others.[46] Moreover, due to the dipole character of azido group they can function both as nucleophiles and electrophiles, and readily undergo dipolar cycloadditions. Especially, after the foundation of "click chemistry"[47,48] the glycosyl azides got additional importance as starting materials for 1,3-dipolar cycloaddition to obtain the corresponding triazolylglycosides. Very recently it has been shown that glycosyl azides are versatile donors in trans-glycosylation reactions with various glycosidases.[49,50] In this context, the synthesis of galactopyranuronate azides as GAA precursors is an attractive and important task in preparative carbohydrate chemistry. In this chapter the preparation of (*O*-acyl and *O*-alkyl) 1-azido D-galacturonic acid derivatives with different protecting strategies suitable as *N*-glycosidic building blocks are described.

Several methods for the synthesis of 1-azido sugars are known.[46] Two general strategies exist for the synthesis of the corresponding 1-azido glycopyranosyluronic acids.[51] The first strategy includes the introduction of an azido group at the anomeric centre of protected neutral sugar molecules. After deprotection, the primary hydroxyl group of the free sugar is selectively oxidized to provide the corresponding 1-azido glycopyranuronates. For instance, Györgydeák and Thiem demonstrated the feasibility of this strategy on a number of monosaccharides.[51] Thus, methyl 2,3,4-tri-*O*-acetyl-β-D-galactopyranosyluronate azide **5** was synthesized in 64% overall yield starting from the corresponding β-D-galactopyranosyl azide.[51] In the course of

this pathway the free uronic acid azide **7** was transformated into the acetylated methylester **5** in order to simplify chromatographic purification. Later, Ying and Gervay-Hague[52] presented a protocol for desalting the unprotected uronate azides and purifying them on reversed-phase HPLC. This protocol principally makes possible the isolation of uronic acid azides, although only in small scale.

Alternatively, esters of per-*O*-acetylated uronic acids can be directly converted to glycosyl azides. Thus, azido compound **5** was obtained from the corresponding bromide in 41% yield by treating **4** with sodium azide in HMPT.[51] Starting from *methyl 1,2,3,4-tetra-O-acetyl-α-D-galactopyranuronate* **3**, the application of trimethylsilyl azide in CH_2Cl_2 as the azidation reagent increased the yield of target compound **5** to 53%.[51] An appreciable improvement of this pathway was achieved by the reaction of commercially available tetramethylguanidinium azide[53-55] with bromide **4** yielding compound **5** in 92%. The key intermediate **4** was prepared from *D-galacturonic acid* (**1**) by an improved procedure developed in our group including acetylation (**a**),[56] esterification of uronic acid (**b**),[57] and activation as a glycosyl bromide (**c**)[58] (**Scheme 1**).



Scheme 1. Improved synthesis of galacturonate azide **5** and consecutive reactions. (a) Ac_2O, $HClO_4$, 4 h, 5 °C; (b) ethereal CH_2N_2; (c) 33% HBr in acetic acid; (d) tetramethylguanidinium azide, anhydrous CH_3NO_2, 5 h, r.t.; (e) 0.3 M LiOH in MeOH/H_2O/THF, 2.5 h, 0 °C; (f) 0.28 M methanolic HCl, 24 h, r.t.; (g) Ac_2O, 3 h, 85 °C; (h)[59,60] MeOH (2 mol eq.), THF, MS 4Å, 10 h, 65 °C; (i) $CH_3C(OEt)_3$, *p*-toluenesulfonic acid, 14 h, r.t.; (j) Ac_2O, anhydrous pyridine 24 h, r.t.

Comparing the analytical data of **5** with those from the literature,[51] an excellent agreement was found for the value of optical rotation, for the allocation of ^{1}H and ^{13}C NMR signals, and for elemental analysis. But the uncorrected melting point of crystalline **5** was not in agreement with the published data.[51] Even after several crystallisation attempts from ethanol or a mixture of ethyl acetate-heptane, a melting point of 99–100 °C was determined whereas a melting point of 163 °C was reported in literature.[51] Moreover, the X-ray diffraction studies of single crystal of compound **5** (**Figure 6**, for crystal data see **Appendix**) confirmed clearly the β-configuration of the azido group at the anomeric centre and the $^{4}C_{1}$ conformation of pyranose ring which were already assigned for solution state from NMR data.

In order to create a library of azidouronates, derivative **5** was at first deacetylated with 0.28 M hydrochloric acid in anhydrous methanol to give compound **6** in 93% yield (**Scheme 1**). After treatment of **6** with lithium hydroxide at 0 °C in a solvent mixture of methanol-water-tetrahydrofuran uronic acid azide **7**[52] was obtained in 75% yield.

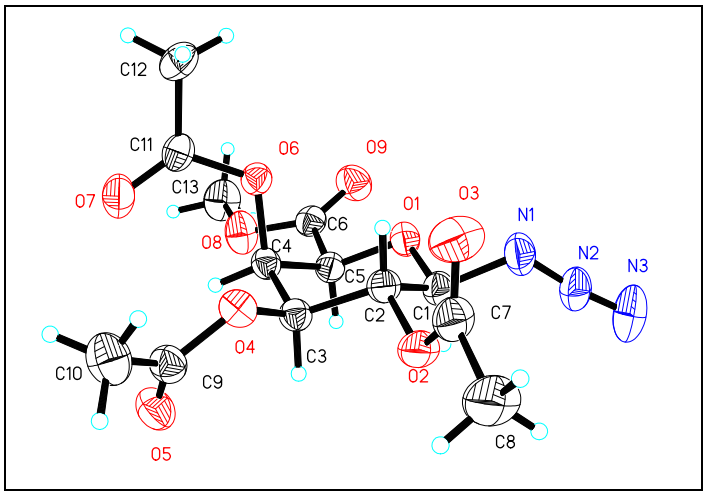


Figure 6. An ORTEP diagram of compound **5** with 30% probability for the thermal ellipsoids.

Two step ester bond cleavage in *galacto*-configurated compound **5** was necessary to avoid β-elimination. Using one step deprotection procedure the unsaturated azide **12** was formed in considerable amounts besides compound **7** in a ratio of 7 : 10*. It should be mentioned that in the case of the corresponding *gluco*– configurated uronate, the complete deprotection can be achieved under the same reaction conditions without a risk of β-elimination.[61] The unsaturated compound **12** was structurally characterized after acetylation (**g**) and treatment with diazomethane (**b**) (**Scheme 1**). In the ^{1}H NMR spectra the formation of the double bond

* The ratio was determined from ^{1}H NMR spectra.

caused noticeable shift of proton signal H-4. In comparison to **5**, the proton signals H-4 of **13** and **14** appear at lower field (δ 5.96 and δ 6.21) due to deshielding by the double bond (**Table 1**). Furthermore, the formation of the double bond is accompanied by conformational changes of the pyranose ring. Basically, Δ^4-uronates could adopt both $^1H_2$ and $^2H_1$ conformations.[62] The H-1 and H-4 signals in ^{1}H NMR spectra of **13** and **14** appear as clearly resolved double doublets (dd) due to the long-range 4-bond coupling with H-3 and H-2 ($^4J_{1,3}$ and $^4J_{2,4}$), respectively (**Table 1**). The signals for H-2 and H-3 are split to doublets of double doublets (ddd) because of the long-range coupling too. Such long-range couplings (0.9-1.2 Hz) indicate a planar W-arrangement of the involved protons. Together with the values of the coupling constants $^3J_{1,2}$, $^3J_{2,3}$ and $^3J_{3,4}$ (2.8–4.6 Hz) which are characteristic for a quasi-equatorial orientation of all ring protons, we suggested that the pyranose rings of **13** and **14** adopt mainly a $^1H_2$ conformation.[62]

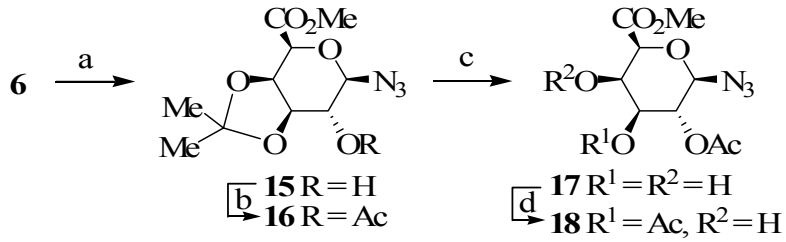
	^{1}H NMR chemical shifts δ of ring protons and *J* coupling constants (Hz)				
	H-1 $^3J_{1,2}$	H-2 $^3J_{2,3}$	H-3 $^3J_{3,4}$	H-4	$^4J_{1,3}$ $^4J_{2,4}$
5	4.66 d 8.5	5.17 dd 10.4	5.07 dd 3.3	5.72 dd 1.5	
13	5.59 dd 4.6	4.97 ddd 3.7	5.27 ddd 4.3	5.96 dd	0.9 0.9
14	5.57 dd 3.7	5.01 ddd 2.8	5.21 ddd 4.6	6.21 dd	1.2 1.2
22	5.64 dd 3.7	3.65 ddd 3.1	4.10 ddd 4.6	6.27 dd	1.2 1.2
23	5.37 dd 5.8	3.67 ddd 4.8	4.12 ddd 3.7	6.22 dd	0.6 0.6

Table 1. ^{1}H NMR chemical shifts and coupling constants of Δ^4-uronates

In pursuing our program we were interested in partially acetylated uronate azides. At first the fully protected 6,3-lactone **8** was obtained in 68% yield by heating compound **7** in acetic anhydride at 85 °C for 3 h (**Scheme 1**). The formation of the lactone ring caused a conformational flip of the pyranose ring from $^4C_1$ to $^1C_4$. In ^{1}H NMR spectra the value of coupling constant of lactone **8** ($^3J_{2,3}$ = 1.2 Hz) indicated the conformational change into $^1C_4$, while the coupling constant $^3J_{2,3}$ 8–10 Hz is typical for the diaxial orientation of H-2 and H-3 in $^4C_1$ *galacto*-configurated ring. Unfortunately, our attempts to provide the partially protected azide **11** by selective alcoholysis of the lactone ring using conditions applied for corresponding *gluco*-configurated 6,3-lactone[59,60] failed due to the distinctly different

reactivity of **8** compared to its *gluco*-analog.[63] An alternative route to compound **11** is based on the conversion of uronate **6** into the the cyclic orthoesters **9**. Cyclic orthoesters are widely used as temporary protecting group, owing to their stability under basic conditions. It has been often shown that the regioselective ring opening of orthoesters under mild acidic conditions proceeds stereoelectronically controlled.[64,65] Indeed, after acetylation (**j**) and subsequently treatment with aqueous acetic acid, the orthoester ring opening of **10** occurred regioselectively and provided 2,4-di-*O*-acetate **11** as the only product in 74% overall yield (**Scheme 1**).

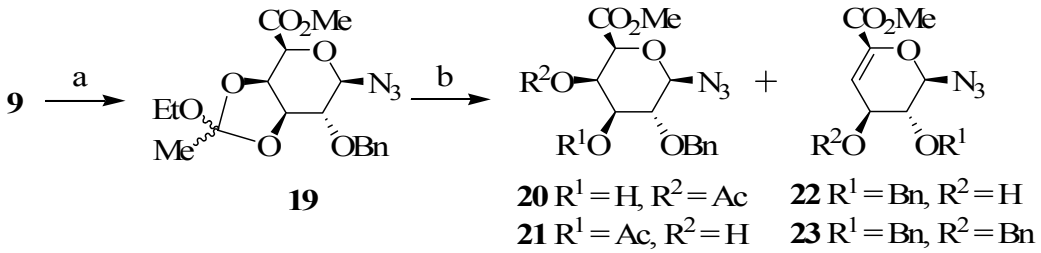
To determine the position of acetyl groups at partially acetylated galacturonate azides by NMR spectroscopy, derivatives **11**, **17** and **18** were synthesized (**Scheme 1** and **2**). Thus, treatment of triol **6** with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid gave compound **15** (94%) which was acetylated to furnish fully protected derivative **16** in quantitative yield. Removal of the isopropylidene group with 90% aq trifluoroacetic acid provided **17** in 92% yield (**Scheme 2**).



Scheme 2. Synthesis of partially acetylated galacturonate azides. (a) $(CH_3)_2C(OCH_3)_2$, anhydrous acetone, *p*-toluenesulfonic acid, 20 h, r.t.; (b) Ac_2O, anhydrous pyridine 20 h, r.t.; (c) 90% aq trifluoroacetic acid, MeOH/$CHCl_3$; (d) acetyl chloride in anhydrous benzene (1.02 mol equiv.), anhydrous pyridine, -35 °C – +12 °C, 15 h.

Subsequent low temperature acetylation of compound **17** gave 2,4-di-*O*-acetate **11** (21%), 2,3-di-*O*-acetate **18** (30%), triacetate **5** (6%) and starting material **17** (20%). In ^{1}H NMR spectra the removal of acetyl group at position *C*-3 (**11**) caused an upfield chemical shift of H-3 compared to **5**. Thus, the signal at δ 5.07 was assigned as H-3 of **5**, while the signal for H-3 of **11** was assigned at higher energy field δ 3.91. In the same way the signal at δ 5.72 was assigned as H-4 of **5**, while the signal for H-4 of **18** was assigned at higher energy field δ 4.47. For compound **17** the both signals for H-3 and H-4 appeared at comparably higher energy field at δ 3.80 and 4.22, respectively.

In contrast to the introduction of acyl protecting groups, alkylation of uronates, especially galacturonates, still poses a challenge in synthetic carbohydrate chemistry. In order to introduce non-participating benzyl protecting group at *C*-2 position of galacturonate azides, silver oxide mediated benzylation was examined.[66] Thus, the reaction of the orthoester **9** with benzyl bromide and freshly prepared silver oxide was performed in order to synthesize compound **19** (**Scheme 3**). TLC monitoring of the benzylation indicated several side products. After removal of silver salts and the solvent, the residue was treated with aqueous acetic acid. Besides acetates **20** and **21** (ratio ~ 1 : 1, total yield 35%), the unsaturated 2-*O*-benzyl **22** (14%) and 2,3-di-*O*-benzyl derivatives **23** (8%) were also isolated as side products and structurally characterized. Generally, the hydrolysis of cyclic orthoesters fused to six-member rings occurs regioselectively and gives almost exclusively to the hydroxyester, in which the ester function and the hydroxyl group are at axial and equatorial positions respectively.[65] The formation of both acetates **20** and **21** could be a result of either a lack of stereoelectronic control or acyl migration. In ^{1}H NMR spectra the signal corresponding to H-4 of 4-*O*-acetyl derivative **20** (δ 5.59) was shifted to lower energy field compared to H-4 of diol **26** (δ 4.27). Analogously, the signal of H-3 of 3-*O*-acetyl derivative **21** was shifted to δ 4.93 compared to H-3 (δ 3.67) of **26**.

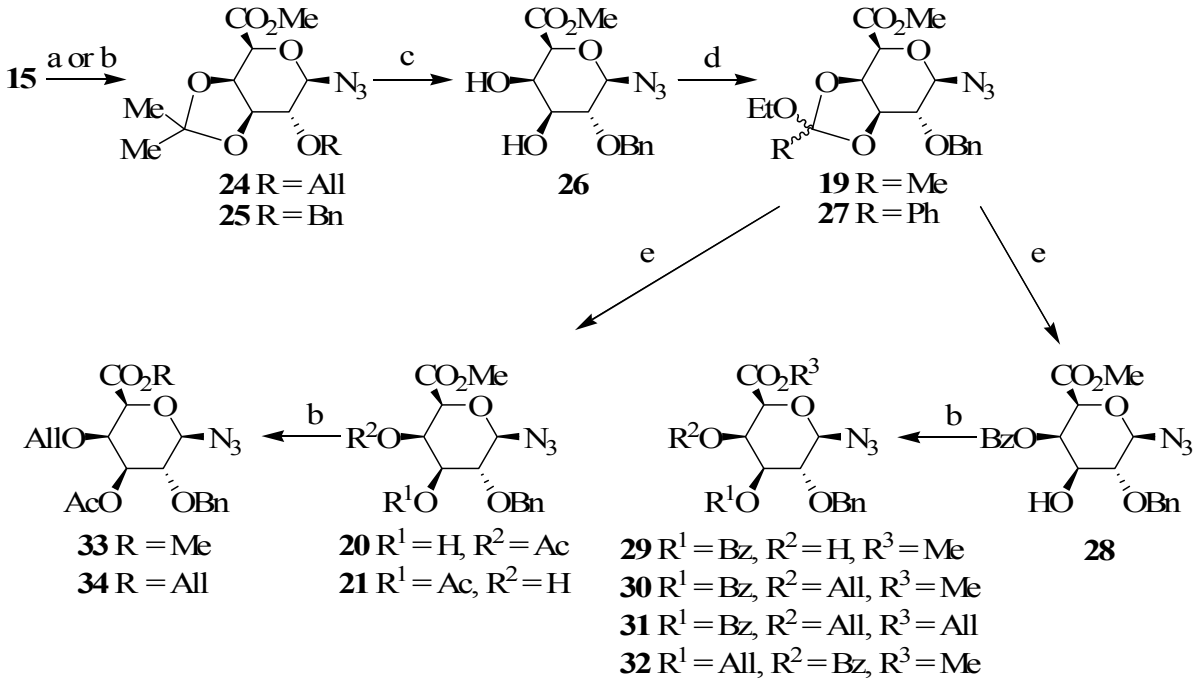


Scheme 3. Alkylation and subsequent hydrolysis of orthoesters **9**. (a) benzyl bromide, freshly prepared Ag_2O, anhydrous benzene, r.t.; (b) 80% aq acetic acid, 1 h.

The isolation of Δ^4-uronates **22** and **23** is an evidence of β-elimination. It seems, that under mild basic condition the orthoacetate worked as a leaving group from the C-4 of sugar ring. In the ^{1}H NMR spectra proton signals H-1 and H-4 of compounds **22** and **23** appear as well resolved double doublets (**Table 1**) caused again by long-range couplings with H-3 and H-2 ($^4J_{1,3}$, $^4J_{2,4}$ 0.6–1.2 Hz). The signals for H-2 and H-3 are split to ddd, due to vicinal and long-range couplings. The vicinal coupling constants of 2-*O*-benzyl substituted **22** ($^3J_{1,2}$ 3.7 Hz, $^3J_{2,3}$ 3.1 Hz, $^3J_{3,4}$ 4.6 Hz) are characteristically for quasi-equatorial orientation of all ring protons leading to the proposal that **22** adopts a $^1H_2$ conformation. In a case of 2,3-di-*O*-

benzyl derivative **23** the value $^{3}J_{1,2}$ 5.8 Hz is considerably higher than expected for coupling between the equatorial and the quasi-equatorial protons. This fact, together with the presence of long-range coupling indicates that the conformational equilibrium of **23** is considerably shifted from $^{1}H_{2}$ to the $^{2}H_{1}$ as a result of the protecting groups pattern.[62]

In order to overcome the β-elimination, an alternative pathway for the synthesis of 2-*O*-alkyl-3,4-orthoesters was designed (**Scheme 4**). Hence, alkylation of compound **15** was attained by the procedure of Kováč using allyl bromide or benzyl bromide and freshly prepared silver oxide[66] to yield the corresponding 2-*O*-allyl and 2-*O*-benzyl derivatives **24** (65%) and **25** (75%). Obviously, the application of 3,4-*O*-isopropylidene group suppressed the formation of unsaturated Δ^{4} uronate derivatives. After deisopropylidenation of **25**, the corresponding diol **26** was transformed into the cyclic orthoesters **19**. Again, the orthoacetate ring opening under mild acidic conditions did not proceed regioselectively. Consequently, a mixture of the 4-*O*-acetate **20** and the 3-*O*-acetate **21** was obtained in a total yield of 97%, but in a ratio varying between 4 : 1 and 1 : 1.



Scheme 4. Synthesis of partially alkylated galacturonate azided. (a) benzyl bromide, freshly prepared Ag_2O, anhydrous benzene, r.t.; (b) allyl bromide, freshly prepared Ag_2O, anhydrous benzene, r.t.; (c) 90% aq trifluoroacetic acid, MeOH/$CHCl_3$; (d) $CH_3C(OEt)_3$, or $PhC(OEt)_3$, *p*-toluenesulfonic acid; (e) 80% aq acetic acid, ~1 h, r.t.

It is known that the benzoyl group generally shows a quite lower tendency of migration than acetyl group.[67] In comparison to orthoacetates **19**, the ring opening of orthobenzoate **27** was

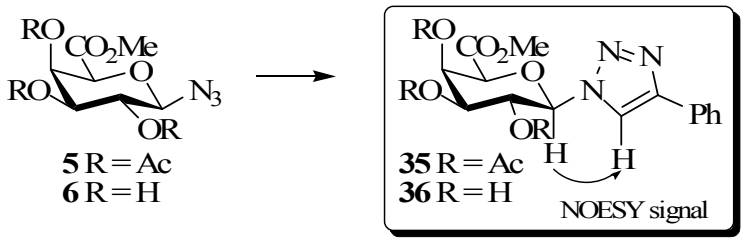
regioselective and provided the 4-*O*-benzoate **28** in 75% yield (**Scheme 4**). Compound **28** can be used as a versatile key intermediate to build up more complex SAA precursors.

It is known that in contrast to acyl protecting groups, bearing electron withdrawing effect, benzyl or allyl ethers increase the reactivity of anomeric centre and generally do not possess an anchimeric effect. Unfortunately, silver oxide mediated allylation of the 4-*O*-benzoate **28** with allyl bromide gave a complex mixture. Separation by HPLC provided as main product the 3-*O*-benzoate **29** (39%), followed by the corresponding 4-*O*-allyl ether **30** (18%) and allyl ester **31** (9%). The desired 3-*O*-allyl-4-*O*-benzoyl derivative **32** was obtained in poor 15% yield. From these results it could be concluded that silver oxide catalyzed not only the allylation reaction but also benzoyl group migration.[68] Considering this fact, the allylation of a mixture of partially acetylated compounds **20** and **21** (molar ratio ~ 1 : 1) was performed under such conditions that the course of the reaction mixture was followed by TLC until the 4-*O*-allyl derivative **33** was formed as main product. After chromatographic purification compound **33** was obtained in 65% yield, and allyl ester **34** was isolated as side product in only 10% yield.

2.1.2. DERIVATISATION OF AZIDO GROUP

The azido group as precursor has enormous potential in natural products synthesis. Glycosyl azides are versatile materials for preparation of biological active glycoconjugates,[69] amides,[70] glycosyl ureas[71] and many other classes. Moreover, they were used for the synthesis of glycoconjugate markers by coupling of glycosylamines to a fluorescent carboxylic dye or to fluorescent amino acid.[72] On the other hand after foundation of "click chemistry" [47,48,73] the glycosyl azides got additional importance as starting materials for 1,3-dipolar cycloaddition to synthesize corresponding triazolylglycoside libraries. These triazoles are potential glycosidase inhibitors,[74] which are useful anti-viral, anti-proliferative, and anti-diabetic agents.[75] Moreover, they were synthesized for their potential antimicrobial[76] and anticancer[77] activities.

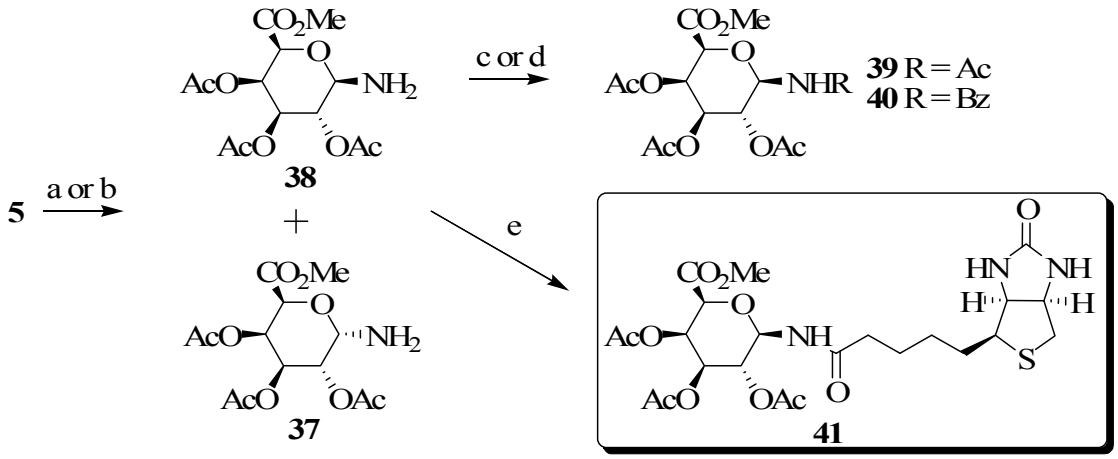
In order to examine the chemical behaviour of galacturonate azides, 1,3-dipolar cycloaddition of compound **5** with phenylacetylene under conditions[78] of "click chemistry" was performed (**Scheme 5**). The phenyltriazole **35** was obtained in 70% yield. However, in the case of the deacetylated azide **6**, the corresponding phenyltriazole **36** was isolated in only 32% yield.



Scheme 5. Synthesis of triazolyl uronates.

The regioselectivity of the addition was established by NOESY experiments. For both compounds **35** and **36** a correlation of H-1 of the pyranose ring with the proton of triazole was detected which proved the strictly regioselective course of the cycloaddition.

Next, the azido group of **5** was reduced. When the reduction was carried out under Staudinger conditions, an anomeric mixture of the amines **37** and **38** was obtained whose α : β ratio varied between 1 : 6 to 1 : 3 (**Scheme 6**). It is well known that the anomerization of 1-amino glycopyranosyl derivatives in contrast to corresponding 1-azido sugars[79,80] is still a problematic task in carbohydrate chemistry.[81]



Scheme 6. Reduction of the azido group and following reactions. (a) Ph_3P, THF/H_2O, r.t – +65 °C, 12 h; (b) Pd^0/C, H_2, ethyl acetate – methanol, 3 h, r.t.; (c) Ac_2O, pyridine, 17 h, -15 °C – r.t.; (d) benzoyl chloride, pyridine, 10 h, r.t.; (e) D-(+)-biotin, DIPEA, EDC, 1-HOBT, DMF, 54 h, 0 °C – r.t.

In the ^{1}H NMR spectra of **37** the α-configuration of the amino group in was confirmed by a coupling constant of $^3J_{1,2}$ 3.7 Hz while the β-anomer **38** possess a value of $^3J_{1,2}$ 7.6 Hz. During crystallization experiment we received crystals that contained both the α- and β-anomers in a ratio of 0.115 : 0.885 (**Figure 7**).

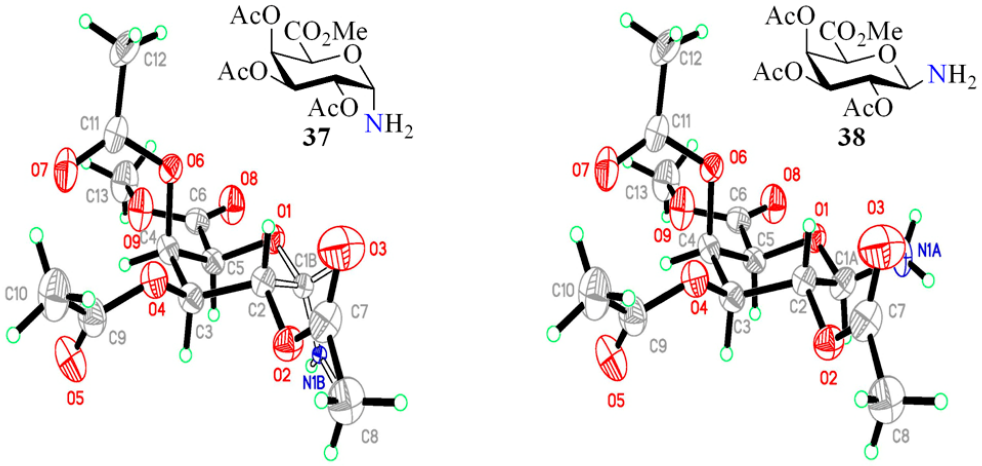


Figure 7. An ORTEP diagram of **37** and **38** from mixed crystal (with 30% probability for the thermal ellipsoids).

Fortunately, we obtained also single crystals of pure **38** and the X-ray measurement confirms the $^4C_1$ conformation of the pyranose ring and the β-configuration at the anomeric centre (**Figure 8**).

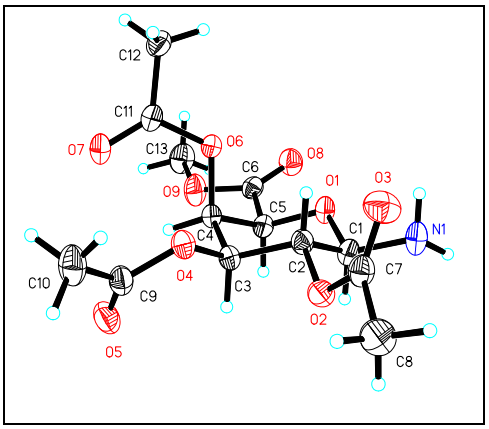
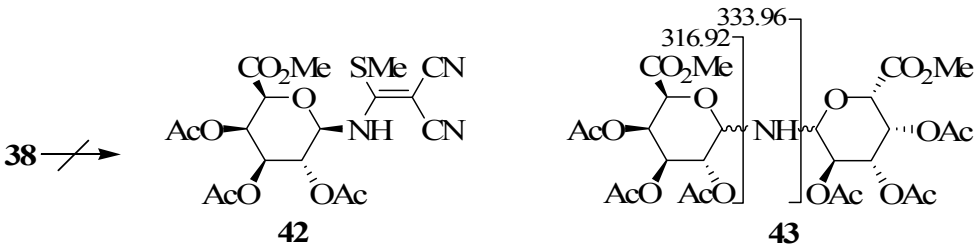


Figure 8. An ORTEP diagram of **38** from a pure crystal with 50% probability for the thermal ellipsoids.

In contrast, palladium catalyzed hydrogenation provided only β-derivative **38** in quantitative yield. To proof the reactivity of the anomeric amino group, compound **38** was acetylated and benzoylated to give the corresponding *N*-acetyl derivative **39** and *N*-benzoyl derivative **40** nearly in 60% yields (**Scheme 7**). In a model reaction, amine **38** was then coupled with D-(+)-biotin in the presence of EDC/1-HOBT leading to the biotin anchored galacturonate **41** in 36% yield. The TLC monitoring of the reaction was practically not possibly even by using reversed phase TLC plates. Due to the hydrophilic character of biotinyl residue, compound **41** was not soluble in a number of organic solvents *e.g.* ethyl acetate, chloroform, methanol, pyridine *etc*. Only dimethyl sulfoxide was a suitable solvent for analytical investigations.

It is known, that aliphatic and aromatic amines can be condensed with ketene dithioacetals derived from malononitrile to provide building blocks which are suitable intermediates for the synthesis of a variety of heterocyclic compounds.[82-84] Among others, several sugar moieties containing an amino group were chosen as starting materials for the synthesis of carbohydrate derivatives which are linked *via* nitrogen to heterocycles.[82-84] To explore the preparative scope of amine **38** a reaction with 2-cyano-3,3-bis(methylthio)acrylonitrile was performed in order to prepare **42** (**Scheme 7**).



Scheme 7. Trial to condensate anime **41** with ketene dithioacetals.

However, at room temperature no reaction occurred and at elevated temperature instead of desired compound **42** formation of α-β- and β-β connected *bis(methyl 2,3,4-tri-O-acetyl-D-galactopyranuronosyl uronate) amines* **43** were observed. Several attempts to separate both isomers by preparative HPLC were unsuccessful. The proof of the structure of **43** was provided by ^{1}H NMR and ^{13}C NMR, IR spectroscopy as well as by MS. As expected, the LC-MS gave two peaks indicating the presence of both isomers. The ESI mass spectra of these peaks were identical and a protonated molecular ion of *m/z* 649.96 [M+H^{+}] was detected in agreement with a dimeric structure. Two ionic fragments with *m/z* 316.92 [Mono+H^{+}] and *m/z* 333.96 [MonoNH+H^{+}] were also detected (**Scheme 7**). The fact that glycosyl amines can be converted into bis(glycosyl)amines under coupling conditions by elimination of ammonia is known from the literature.[85,86]

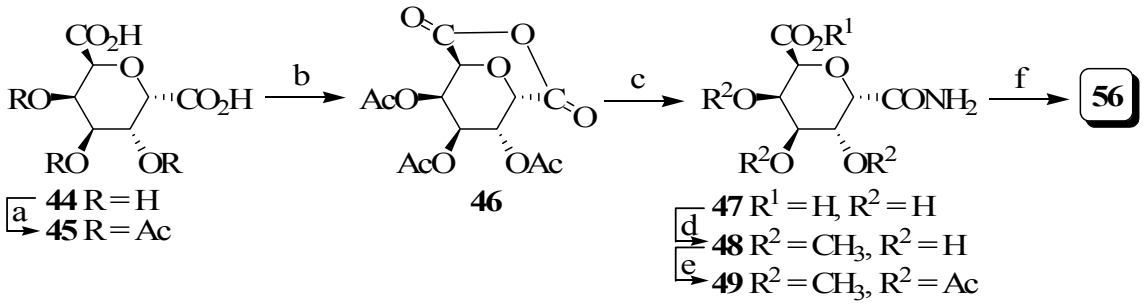
In summary, the preparation of *methyl 2,3,4-tri-O-acetyl-β-D-galactopyranosyluronate azide* (**5**) was appreciably improved by using commercially available tetramethylguanidinium azide. Several protection group manipulations were described and a library of fully and partially acylated and alkylated galacturonate azides was furnished. Some of these compounds were obtained by lucrative pathways and can be used as sugar amino acid (SAA) building blocks. It was shown that the peracetylated uronate azide **5** gave the triazolyl glycoside **35** in 70% yield by 1,3-cycloaddition under "click chemistry" conditions. In comparison to Staudinger reduction of **5**, the reduction with hydrogen catalyzed with palladium gave exclusively the *β*-anomer *i.e. β-D-galactopyranosyluronate amine* **38** in quantitative yield. A highlight of this chapter was the coupling of amine **38** with biotin to furnish *N-(methyl 2,3,4-tri-O-acetyl-β-D-galactopyranosyluronate) biotinylamide* (**41**). As a part of this program, directed toward the design and synthesis of biotinylated galacturonates as important tools for biochemical assays, *N-(methyl 2,3,4-tri-O-acetyl-β-D-galactopyranosyluronate) biotinylamide* (**41**) was synthesised on a model reaction from *methyl (2,3,4-tri-O-acetyl-β-D-galactopyranosyluronate amine* **38** in preparative scale in 36% yield. This reaction conditions can be used for biotinylation of corresponding functionalized pectin fragments as suitable anchored carbohydrate ligands.

2.2. D-GALACTOPYRANOSYLURONATE C-GLYCOSIDES[†]

C-Glycosides are carbohydrate analogs wherein glycosidic oxygen is replaced by a carbon atom. *C*-Glycosyl derivatives received considerable attention in carbohydrate and natural product chemistry, because: (i) there are numerous naturally occurring *C*-glycosides with important biological or pharmacological activities; (ii) these compounds can be considered as hydrolytically stable counterparts of *O*- and *N*-glycosides, and therefore suitable to mimic biologically important carbohydrate derivatives; (iii) the use of *C*-glycosides as intermediates is also of great importance in syntheses of complex structures of natural origins.[87,88] In this chapter the synthesis of D-galactopyranosyluronate *C*-glycosides is presented.

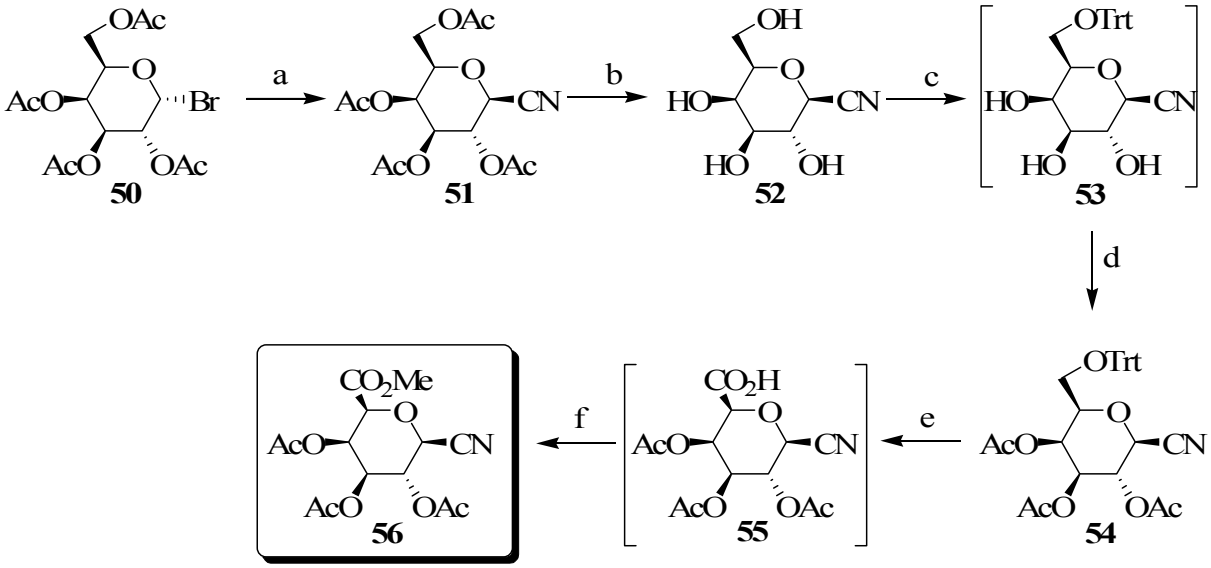
2.2.1. SYNTHESIS OF β-D-GALACTOPYRANOSYLURONATE CYANIDE (56)

Glycosyl cyanides are anomerically functionalized one-carbon-extended C-glycosyl compounds that have been utilized for the preparation of carbohydrate analogs of different biochemical interests.[89] The direct synthesis of peracetylated 1-cyanoaldoses from acylglycosyl bromides is well described,[90] but in the case of uronate cyanides, no preparative pathway is known.[91] Although Fuchs and Lehman reported the synthesis of *methyl (2,3,4-tri-O-acetyl-β-D-galactopyranosyl cyanide) uronate* **56** in 24% overall yield in six step procedure but they started from commercially not available dicarboxyl acid **44** (**Scheme 8**). Additionally, a part from optical rotation and IR spectra no analytical data were given.



Scheme 8. Synthesis of galcturonate cyanide **56** by Fuchs and Lehmann.[91] (a) Ac_2O, H_2SO_4, 30 min, 110 °C; (b) $POCl_3$, 15 min, reflux; (c) methanolic ammonia, 7 h, 0 °C and then Amberlite IR-120 (H^+); (d) ethereal diazomethane (e) Ac_2O, anhydrous pyridine; (f) *p*-toluenesulfonyl chloride, pyridine, 4 h, 80-85 °C.

[†]. To overcome misunderstandings during conformational analysis, the pyranose ring of *C*-glycosides are numbered analogously to *O*-glycosides. Though, it is known that special nomenclature for the *C*-glycosides is afforded.



Scheme 9. Synthesis of galcturonate cyanide **56**. (a) mercuric cyanide, anhydrous CH_3NO_2, 48 h, r.t.; (b) CH_3ONa, in methanol, 30 min, r.t.; (c) Ph_3CCl, DMAP, anhydrous pyridine, 15 h, r.t; (d) Ac_2O, anhydrous pyridine; (e) CrO_3 in 3.5 M H_2SO_4, acetone/dichloromethane, 7 h, 0 °C – r.t.; (f) ethereal CH_2N_2 or CH_3I, Bu_4NBr, $NaHCO_3$, dichloromethane-water , 20 h, r.t.

Alternatively, using a traditional route[57] uronate **56** was synthesized in 37% overall yield (**Scheme 9**). Starting from acetobromgalactose **50**[92] the peracetylated β-D-galactopyranosyl-cyanide **51** was prepared in 68% yield by a method described by Myers and Lee.[90] Comparing the ^{13}C NMR ($CDCl_3$, 62.9 MHz) data our assignment differs slightly from the literature.[90] By application of HSQC $^{1}H,^{13}C$ correlation spectra C-1 of pyranose ring was assigned at δ 66.8, C-4 at δ 66.7, and C-2 at δ 66.0, whereas Myers and Lee published the following order: δ 66.1 (C-1), and δ 66.8 (C-2, C-4).[90]

X-Ray structure of compound **51** was already described by Foces-Foces *et al*.[93] However, we performed the data collection of **51** at – 100 °C (Crystal data see Appendix) and so more precise data were obtained for the structure (**Figure 9**).

In the next step, Zemplén deacetylation of compound **51** at room temperature afforded **52** in 91% yield. Low-temperature conditions were not necessary. Additionally, our NMR data for **52** were more precise than those of the literature.[94] Surprisingly, TEMPO oxidation of primary hydroxyl group of unprotected derivative **52** according to the procedure described by Ying and Gervay-Hague[52] did not give the desired unprotected uronate cyanide and the starting material was fully recovered.

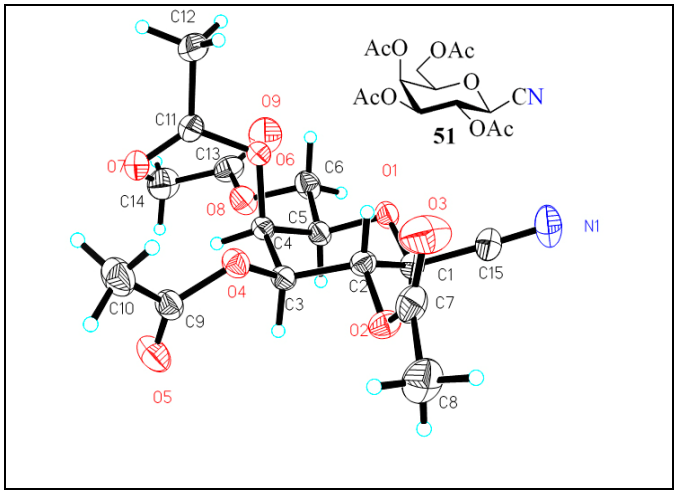


Figure 9. An ORTEP diagram of compound **51** with 50% probability for the thermal ellipsoids.

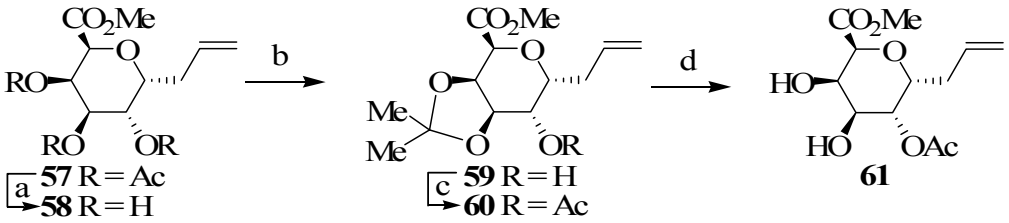
Alternatively, successive one-pot procedure including tritylation (**c**) and acetylation (**d**)[95,96] provided fully protected derivative **54** *via* **53** in excellent yield (94%). Subsequent Jones oxidation of **54** led to **55** which was not isolated, but immediately esterified under phase transfer conditions to give the peracetylated cyanouronate **56** as crystalline solid in 59% yield.

Unfortunately, all our attempts to transform the bromide **4** directly into the corresponding cyanide **56** using the method of Myers and Lee[90] failed. We only observed complex reaction mixtures on TLC plate and the desired compound **56** was only formed in traces. Likewise, comparable complex mixtures were obtained by Kochetkov *et al.*[97] by the reaction of **4** either with NaCN in acetonitrile or AgCN in refluxing xylene. In this context, the pathway for the synthesis of uronate **56** illustrated in **Scheme 9** is up today the best preparative route.

2.2.2. SYNTHESIS OF O-ACYL PROTECTED C-ALLYL α-D-GALACTOPYRANURONATES

Generally, the strategy of our syntheses is oriented towards the chemistry of uronate derivatives and their application using glycuronic acid as precursors. In the case of uronate cyanide **56** we did not found suitable reaction conditions to realize this strategy. Therefore, we focused our interest on *C*-allyl uronates[98] which were obtained by highly stereoselective radical allylation[99] directly from acetobromgalactopyranutonate **4** by photolytic irradiation.[100] In future investigations we plan to incorporate *C*-allyl uronates in synthetic pectin fragments either as anchor or as labelling moiety. It is well known that galacturonate moieties in pectins are partially acetylated. Therefore, an appropriate library of partially acetylated *C*-allyl uronates was furnished.

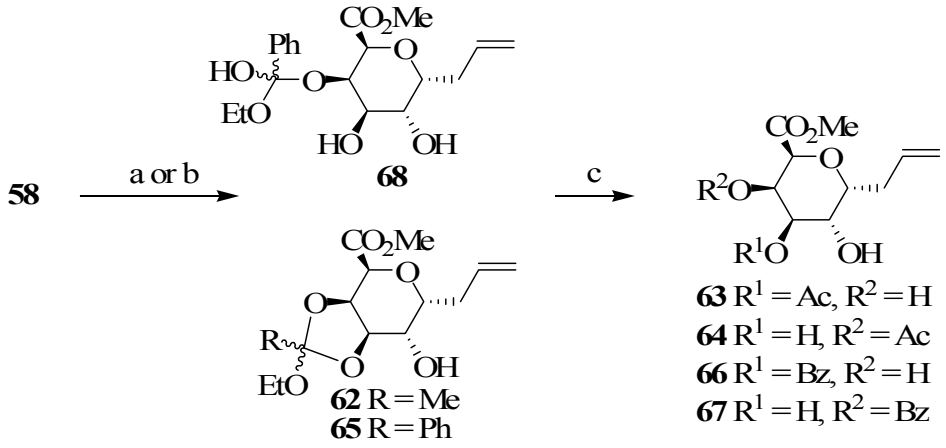
At first, the acetyl protecting groups of fully acetylated **57** were removed with 0.28 M methanolic HCl that gave compound **58** in 93% yield (**Scheme 10**). In order to get a set of monoacetyl derivatives, compound **58** was treated with 2,2-dimethoxypropane to provide 3,4-*O*-isopropylidene derivative **59**[98] in 93% yield. Subsequent acetylation of **59** gave the fully protected **60** in 94% yield. The removal of isopropylidene group by treatment with 90% aq. trifluoroacetic acid furnished the 2-*O*-acyl uronate **61** in 82% yield. Thus, the 2-*O*-acetyl derivative **61** was obtained in overall 67% yield starting from **57**.



Scheme 10. Synthesis of the 2-*O*-acetyl derivative **61**. (a) 0.28 M methanolic HCl, 24 h, r.t; (b) $(CH_3)_2C(OCH_3)_2$, anhydrous acetone, *p*-toluenesulfonic acid, 5 h, r.t.; (c) Ac_2O, anhydrous pyridine 20 h, 0 °C – r.t.; (d) 90% aq trifluoroacetic acid, MeOH/$CHCl_3$ 1 h, r.t.

Next, to get the 4-*O*-acetyl derivative **64**, cyclic orthoester strategy was chosen[64,100,101] (**Scheme 11**). The reaction of **58** with triethyl orthoacetate, catalyzed by camphorsulfonic acid, provided a mixture of *exo*-*endo* diastereomers **62** (**Scheme 11**) which was used for the next step without further characterization. Generally, the hydrolysis of cyclic orthoesters

fused to six-membered rings give almost exclusively that hydroxyester in which the ester function is axial and the hydroxyl group equatorial.[65] However, the hydrolysis of orthacetates **62** with aq acetic acid gave a mixture of 3-*O*-acetyl and 4-*O*-acetyl derivatives **63** and **64** in a ratio ~1 : 1[‡] and in 72% yield over two steps. The analytical samples were purified by preparative HPLC. The position of free hydroxyl groups in **63** and **64** were determined by comparison of ^{1}H NMR spectra with fully acetylated derivative **57** (**Table 2**). Thus, the proton signals of H-2 and H-4 of 3-*O*-acetyl **63** appear at δ 3.94 and 4.44, respectively, whereas the signals of H-2, H-3 (δ 5.19–5.23) and H-4 (δ 5.57) of **57** are shifted to lower energy field due the deshielding effect of acetyl substituents. Analogously, the proton signals H-2 and H-3 of 4-*O*-acetate **64** appear at δ 3.97 and 4.02.



Schema 11. Synthesis of mono-*O*-acyl protected *C*-allyl α-D-galacturonates. (a) $CH_3C(OEt)_3$, *p*-toluenesulfonic acid, 14 h, r.t.; (b) $PhC(OEt)_3$, *p*-toluenesulfonic acid, anhydrous dichloromethane, 17 h, r.t.; (c) 90% aq acetic acid, r.t.

To minimize the risk of migration, the orthoester procedure was applied using triethyl orthobenzoate. The cyclic orthoester **65** was obtained as a mixture of diastereomers in 82% yield. The orthoester cleavage of **65** again gave a mixture of 3-*O*- and 4-*O*-benzoyl substituted **66** and **67** in an equal ratio in overall 98% yield. But in this case separation of pure samples of both compounds by HPLC was not possible. Therefore, the NMR data were obtained by enriched fractions of each compound. Thus, based on experimental results, it was shown that either 3,4-*O*-(1-ethoxyethylidene) nor 3,4-*O*-(1-ethoxybenzylidene) orthoester ring opening proceeds not regioselectively.

Curiously, besides cyclic orthoesters **65** (TLC, R_f 0.25) the acyclic hemiorthoester **68** (TLC, R_f 0.34) was observed as a side product and isolated even by flash chromatography. In the

[‡] The ratios of isomers were determined from ^{1}H NMR.

^{1}H NMR spectra of **68**, among others, five aromatic protons and protons of an ethoxy group were detected. The well resolved signals of both methylene protons (doublet of quartet-s at δ 3.96 and 3.88) indicated their non equivalent surrounding caused by a new asymmetric substituted *C*-atom (**Figure 10**). In ^{13}C NMR spectra the signal at δ 121.7 was assigned to this chiral quaternary carbon atom. Obviously, only one of both possible diastereomers was formed. Complete assignment of structure was obtained from ^{1}H, ^{1}H correlation and from ^{1}H, ^{13}C HMBC experiments. Thus, the ^{1}H, ^{13}C - *heteronuclear multi bond correlation* (HMBC) experiment (not shown) revealed that the aromatic ring and the ethoxy group are attached to a quaternary carbon atom which itself is connected to *O*-4. Furthermore, the LC-MS indicated a molecular ion peak for $C_{19}H_{26}O_8$ at *m/z* 382, which underlined the proposed structure. Surprisingly, the comparison of the vicinal coupling constants of **68** (**Table 2**, **Figure 10**) with those of published in literature[103] indicated that the pyranose ring adopts a skew conformation $^{0}S_{2}$.[104]

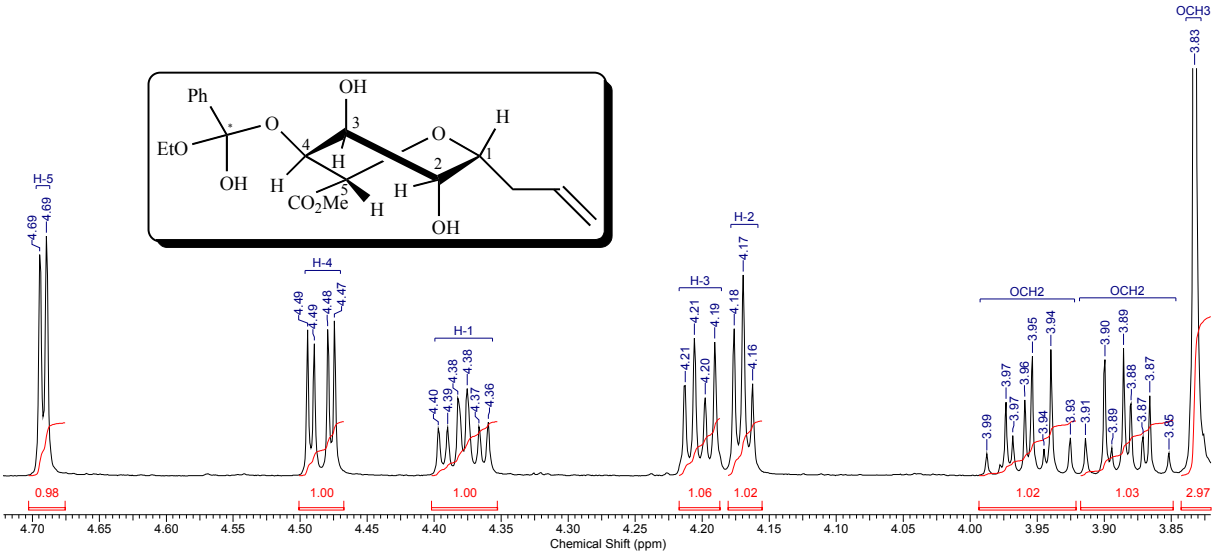
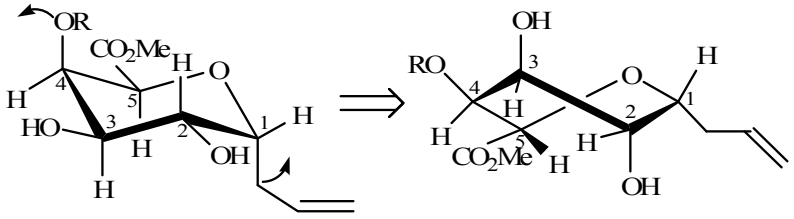


Figure 10. A part of ^{1}H NMR spectra of **68** measured at 500 MHz.

The unusual conformational change of a simple *galacto*-configurated pyranose ring can be explained by the endeavour of both axial substituents to adopt a thermodynamically more stable equatorial orientation (shown by arrows in **Figure 11a**). As a result the rigid chair configuration ($^{4}C_{1}$) flipped to a quite more flexible skew form ($^{0}S_{2}$) in which both substituents at *C*-1 and *C*-4 reach an isoclinic orientation (**Figure 11b**). In addition, the amorphous hemiorthoester **68** was stable in solid state whereas the NMR data observation of an acid free solution revealed the complete formation of both monobenzoates **66** and **67** in a ratio of 1 : 1 at room temperature during ~ 30 days. In our best knowledge, this is the first time that such a type of hemiorthoester was isolated and characterized.



a) $^4C_1$ conformation **b)** $^0S_2$ conformation

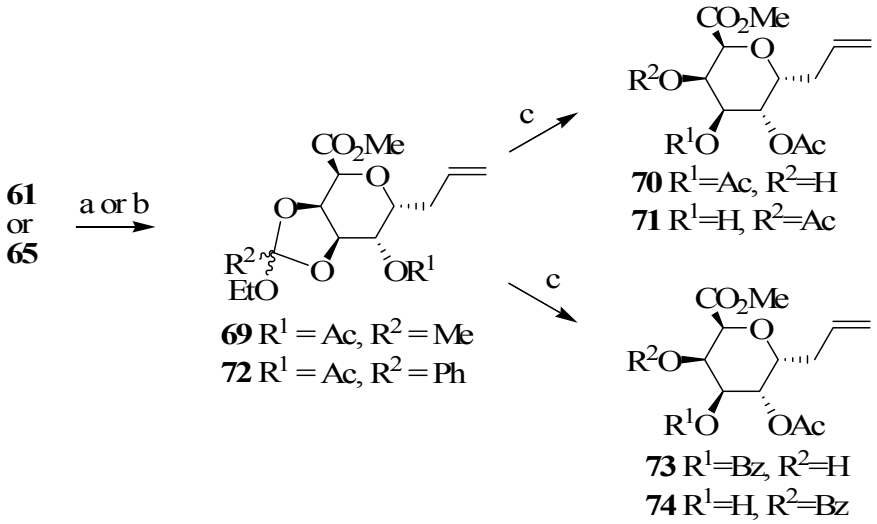
Figure 11. $^4C_1$ and $^0S_2$ conformations of pyranose ring of *C*-allyl uronates.

	^{1}H NMR chemical shifts δ of ring protons and ^3J coupling constants (Hz)				
	1 ($^3J_{1,2}$)	2 ($^3J_{2,3}$)	3 ($^3J_{3,4}$)	4 ($^3J_{4,5}$)	5
3[105]	6.50 d 1.8	*5.38 m* ND	*5.38 m* 2.6	*5.81 d* 1.7	4.75 d
57[98]	4.53 ddd 3.8	*5.23-5.19 m* ND	*5.23-5.19 m* 2.5	*5.57 dd* 4.1	4.56 d
59	4.19 ddd 2.4	3.86 dd 3.0	4.35 dd 7.3	4.63 dd 2.3	4.68 d
61	4.35 ddd 2.6	*4.94 dd* 5.2	3.96 dd 2.4	4.20 dd 5.6	4.42 d
63	4.12 ddd 3.1	3.94 dd 6.0	*5.06 dd* 2.8	4.44 dd 5.2	4.45 d
64	4.39 ddd 3.7	3.97 dd 7.5	4.02 dd 3.0	*5.43 dd* 4.0	4.52 d
66	4.17 ddd 2.8	4.05 dd 5.3	*5.35 ddd* 1.6 (1.6[§])	4.60-4.56 m 5.3	4.60-4.56 m
67	4.45 ddd 4.4	4.06 dd 7.7	4.14 dd 3.2	*5.72 dd* 3.8	4.61 d
68	4.38 ddd 3.5	4.17 t 3.5	4.20 dd 7.6	4.48 dd 2.5	4.69 d
70	4.25 ddd 2.8	*5.11-5.06 ND* 5.5	*5.17 dd* 2.7	4.36 dd 5.5	4.49 d
71	4.52 ddd 3.0	*5.02 dd* 5.8	4.06 dd 3.1	*5.31 dd* 5.4	4.63 d
73	4.35 ddd 2.6	*5.25 dd* 5.4	*5.39 dd* 2.8	4.50 br 5.6	4.59 d
74	4.60 ddd 3.0	*5.13-5.05 ND* ND	4.20 dd 3.0	*5.62 dd* 5.4	4.72 d
75[106]	*6.34 d* 3.8	*5.19 dd* 7.2	*4.40 dd* 6.0	*4.59 dd* 3.0	4.74 d
76	4.08 ddd 1.7	3.75 dd 2.7	*4.32 dd* 7.6	*4.69 dd* 2.1	4.50 d

Table 2. ^{1}H NMR chemical shifts and vicinal H - H coupling constants of ring protons of partial acyl derivatives. The chemical shifts of substituted positions are shown in italic.

[§] $^4J_{3,5}$ coupling constant

In order to get di-*O*-acetylated *C*-allyl galactopyranuronates, the monoacetyl derivative **61** was treated with triethyl orthoacetate in the presence of *p*-toluenesulfonic acid to furnish the fully protected derivative **69** in 95% yield (**Scheme 12**). As expected, the orthoester cleavage of **69** by treatment with 90% acetic acid gave a mixture of 2,3-di-*O*-acetyl **70** and 2,4-di-*O*-acetyl **71** in a ratio ~1 : 1, in overall 91% yield. After chromatographic separation by preparative HPLC the pure compounds were isolated and fully characterized. In ^{1}H NMR spectra the presence of an acetyl group at position *C*-3 (**70**) caused a downfield chemical shift compared to **61** (**Table 2**). Thus, the signal at δ 5.17 was assigned as H-3 of **70**, while the signal for H-3 of **61** was assigned at higher energy field δ 3.96. In the same way the signal at δ 5.31 was assigned as H-4 of **71**, while the signal for H-4 of **61** was assigned at higher energy field δ 4.20.



Schema 12. Synthesis of di-*O*-acyl protected *C*-allyl α-D-galacturonates. (a) $CH_3C(OEt)_3$, *p*-toluenesulfonic acid, 14 h, r.t.; (b) Ac_2O, anhydrous pyridine 24 h, 0 °C – r.t.; (c) 90% aq acetic acid, r.t.

Single crystals of **71** were obtained and the X-ray diffraction studies showed (**Figure 11**) that in the solid state the pyranose ring adopts a $^4C_1$ conformation (puckering parameters in Attachment). However, the torsional angle between H5-C5-C4-O4 was measured to be 164.19° which shows that the diaxial orientation of O4 and H5 is slightly distorted and the six-membered ring is flatter than for usual $^4C_1$ conformation.

To exclude acyl migration, the orthoester ring opening, of the corresponding orthobenzoate **72** was integrated into our investigation. The acetylation of **65** furnished **72** in 92% yield

(**Scheme 12**). The subsequent orthoester ring opening provided the 2-*O*-acetyl-3-*O*-benzoyl derivative **73** and 2-*O*-acetyl-4-*O*-benzoyl derivative **74** in a ratio 1 : 1 in 96% yield. This experiment indicated that migration is not the reason for loss of regioselectivity during cyclic orthoester ring opening. After separation by preparative HPLC the pure compounds were isolated and fully characterized. The position of benzoyl group in **73** and **74** was indicated by comparison of the ^{1}H NMR spectra with those of **61** (**Table 2**). The benzoyl group at position *C*-3 in ^{1}H NMR spectra of **73** caused a downfield chemical shift of H-3 (δ 5.39) compared to **61** (δ 3.96). Analogously, the signal assigned as H-4 of **74** was detected at δ 5.62, while the signal for H-3 of **61** was recorded at higher energy field δ 4.20.

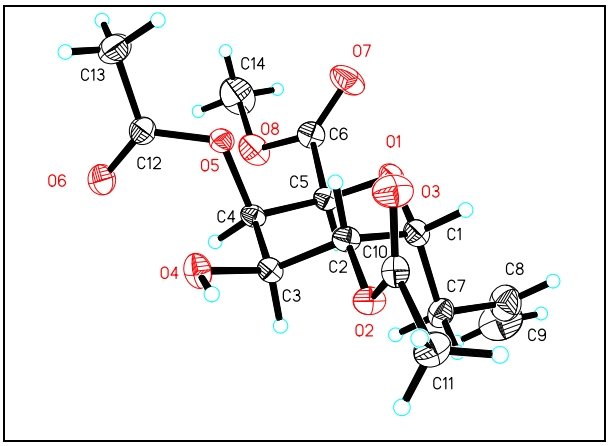


Figure 11. An ORTEP diagram of compound **71** with 50% probability for the thermal ellipsoids.

Thus, based on above obtained experimental results, it was concluded that neither orthoester type nor the acetylation pattern of position *O*-2 do not influence on the cyclic orthoester ring opening. In all discussed cases the orthoester cleavage was not selective and nearly equal ratios of both possible hydroxester derivatives were obtained.

Generally, King and Allbut have shown that the hydrolysis of orthoesters fused to anchored six-membered rings give almost exclusively that hydroxylester in which the ester function is *axial* (and the hydroxyl group *equatorial*).[65] The authors discussed two possible boundary structures that are responsible for stereoselective hydrolysis (**Figure 12 I** and **II**). They suggested that in order to achieve maximum stabilization in tetrahedral intermediate transition state, one free electron pair on five-membered cycle constituent oxygen must be nearly *anti*-periplanar to the leaving group. As shown in **Figure 12** (**I**), the *exo* lone-pair electrons of the *axial* oxygen can comparatively readily become *anti*-periplanar to the *equatorial* oxygen (marked green, see also the Newman projection **a**). Similarly as indicated in **II** the *endo* lone-

pair electrons of the *equatorial* oxygen can also be so arranged (marked green, see also the Newman projection **d**). However, the *endo* substituent (marked blue) moves very close to the nearest *axial* hydrogen (marked blue) of the pyranose ring. The authors expect that all species in which the *endo* function is either an aryl, alkyl, alkoxyl, or hydroxyl group, the interaction of the *endo* group with the neighbouring *axial* hydrogen would make the transition state derived from **II** of much higher energy than the one derived from **I**. Thus, referring to lowest energy of **I**, the orthoester hydrolysis route derived from **I** was favourable that leads to the *axial* ester-*equatoria*l alcohol which was confirmed in most experimental cases.

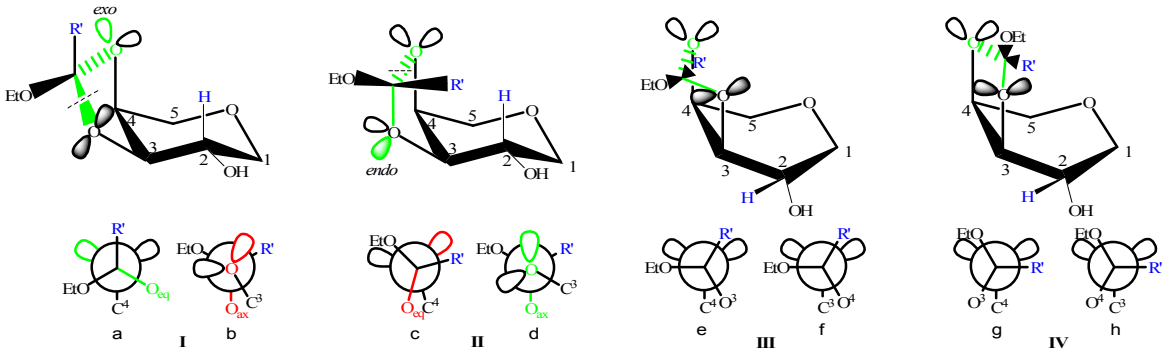
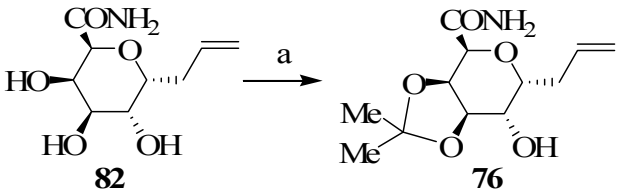


Figure 12. I, II - two possible boundary structures for orthoesters fused to $^4C_1$ chair anchored six-membered rings. III, IV - two possible structures for orthoesters fused to $^0S_2$ six-membered rings.

In order to find an explanation for the loss of regioselectivity during cyclic orthoester cleavage, the isopropylidene ring was chosen as a model for 3,4-cyclic orthoester fused to pyranose ring. The conformation of the condensed ring systems in compounds **75**,[106] **59**,[98] and **76** were compared (**Figure 13**). Compound **76** was synthesized by isopropylidenation of **82** (for the synthesis of **82** see **Chapter 2.2.2**) 91% yield (**Scheme 13**).



Scheme 13. Synthesis of **76**. (a) $(CH_3)_2C(OCH_3)_2$, anhydrous acetone, *p*-toluenesulfonic acid, 20 h, r.t.

As shown earlier, the introduction of an isopropylidene ring at *C*-3/*C*-4 of 1-*O*-acetyl uronate **3** had no significant influence on the conformation of pyranose ring in **75** (see the X-ray structures in **Figure 13**). Both *O*-glycosides **3**[98] and **75**[106] adopted a $^4C_1$ conformation either in solution or solid states.

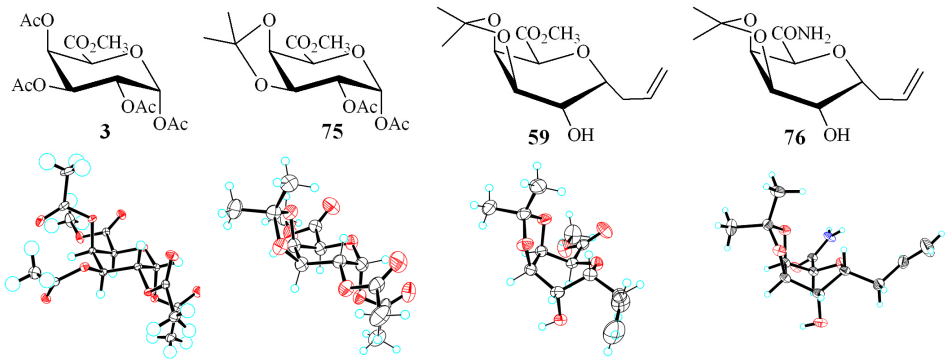


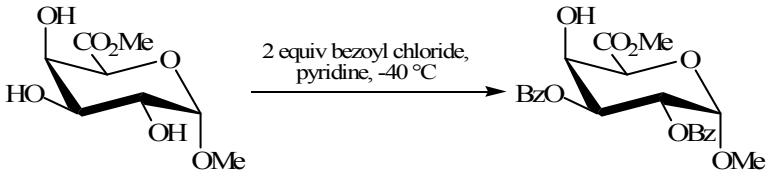
Figure 13. The comparison of conformational changes of *O*- and *C*-glycosides based on X-ray diffraction studies.

In the ^{1}H NMR spectra of compounds **3** and **75** the vicinal coupling constant $^{3}J_{2,3}$ determined in a region 7–10 Hz (**Table 2**) underlined this affiliation. The X-ray studies confirmed this fact for solid state (**Figure 13**). In contrast, the skew form of **59** and **76** in solution state were evident from coupling constants $^{3}J_{2,3}$ 3.0 Hz and 2.7 Hz, as well as $^{3}J_{3,4}$ 7.3 Hz and 7.6 Hz, respectively. These both *C*-glycosides maintain the skew conformation $^{0}S_{2}$ in solid state as elucidated by X-ray diffraction studies. Thus, five membered dioxolan ring fused to a pyranose ring at *C*-3/*C*-4 can causes a conformational flip from the chair to the skew form ($^{0}S_{2}$). Obviously, the skew form $^{0}S_{2}$ posses no conformation in which the substituent of an orthoester structure can have a significant influence on the regioselectivity of the ring opening neither by stereoellectronic effects nor by steric hindrances (**Figure 12 III** and **IV**).

In summary, in order to synthesize a library of partially acetylated *C*-allyl galactopyranuronates, cyclic orthoester method was applied. Surprisingly, in contrast to *O*-glycosides, the orthoester ring opening of *C*-glycosides occurred with a complete loss of regioselectivity for both orthoacetate and orthobenzoate ring systems. Nevertheless, a library of partially acetylated *C*-allyl α-D-galacturonates was created and the derivatives were fully characterized. Based on NMR data and X-ray analysis of model structures it was suggested that the lack of selectivity is caused by conformational changes of the pyranose ring. Furthermore, for the first time a labile acyclic hemi orthoester **68** was isolated and fully characterized.

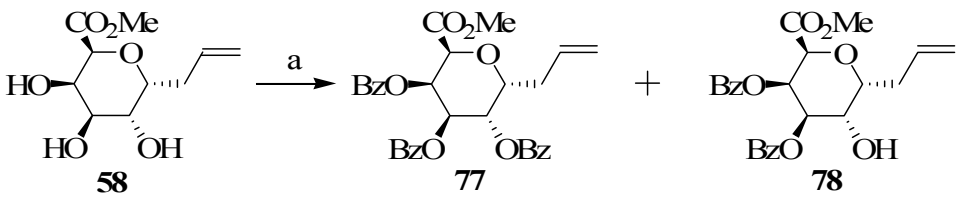
2.2.3. C-ALLYL α-D-GALACTOPYRANURONATES AND THEIR TEMPERATURE CONTROLLED BENZOYLATION

Besides theoretical interest, the relative reactivity of hydroxyl groups in sugar molecules and their analogs gives also predictable practical application in preparative carbohydrate chemistry. The reactivity of hydroxyl groups is directly related to the conformation of pyranose ring. In the previous chapter it was shown that the absence of the anomeric effect in α-D-galactopyranuronate *C*-glycosides can lead to substantial conformational changes. In dynamic state this conformational change of pyran ring can affect the relative reactivity of all hydroxyl groups, which can be investigated *e.g.* by low-temperature acylation. It has been shown that due to its axial orientation, the OH group at *C*-4 position of methyl α-D-galactopyranoside[107] and methyl (methyl α-D-galactopyranosid) uronate[108] undergo acylation less rapidly than equatorial OH groups (**Scheme 14**). In that case, the reactivity differences of hydroxyl groups allow temperature controlled regioselective benzoylation even in preparative scale. Thus, the comparison of the relative reactivity of hydroxyl groups of α-D-galactopyranuronate *C*-glycosides is an interesting and attractive task.



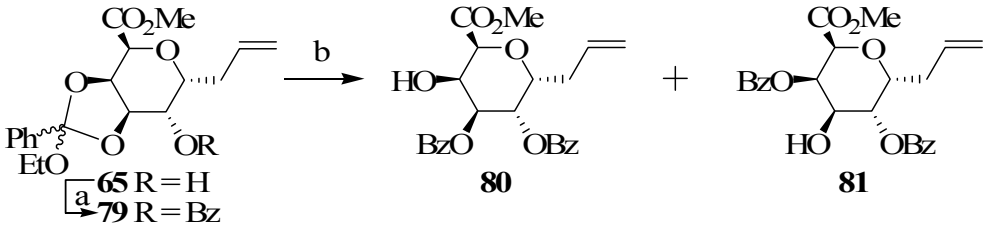
Scheme 14. Temperature controlled selective benzoylation of methyl (methyl α-D-galactopyranosid)uronate[108]

Benzoylation of **58** with two equivalents of benzoyl chloride in anhydrous pyridine at -38 °C provided fully benzoylated derivative **77** and the partially benzoylated derivative **78** in 43% and 35% yields, respectively (**Scheme 15**).



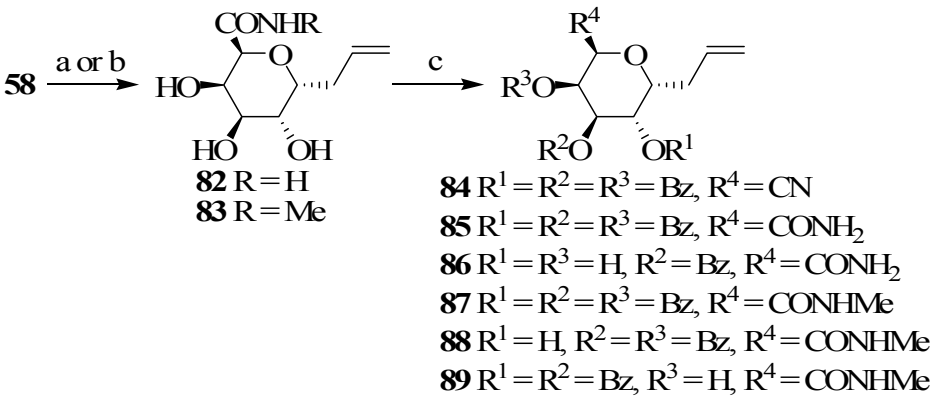
Scheme 15. Temperature controlled dimolar benzoylation of *C*-allyl α-D-galactopyranuronate **58**. (a) 2 equivalent benzoyl chloride, anhydrous pyridine, -38 °C

In addition, both constitutional isomers **80** and **81** were synthesized using the orthoester procedure (**Scheme 16**). Thus, the benzoylation of **65** with benzoyl chloride in pyridine yielded **79**, which was treated without further purification with 90% aqueous acetic acid.
As expected (see **Chapter 2.2.1**), orthoester ring opening of **79** gave 2,3-di-*O*-benzoyl **80** and 2,4-di-*O*-benzoyl **81** in a ratio of ~1 : 1 in 72% overall yield over two steps.



Scheme 16. Synthesis of di-*O*-benzoylated **80** and **81** by orthoester method. (a) benzoyl chloride, anhydrous pyridine, 14 h, 0 °C – r.t.; (b) 90% aq acetic acid, r.t.

The position of free OH group in partially benzoylated **78**, **80**, and **81** was determined by comparison of ^{1}H NMR spectra with those of fully benzoylated **77** (**Table 3**). Due to the deshielding effect of *O*-benzoyl substituent, the proton signal of neighbouring CH group of pyran ring shifts to lower energy field. In particular, a ^{1}H-^{1}H COSY experiment assigned the signal at δ 5.61 as H-2 of tri-*O*-benzoate **77**, while the H-2 signal for **78** was assigned at δ 4.21. This indicates that the unsubstituted hydroxyl group in the di-*O*-benzoylated derivative is connected to C-2 of pyran ring. In this way, the H-3 and H-4 signals of perbenzoylated **77** were detected at δ 5.85 and δ 5.93, respectively, whereas the H-3 of **81** was assigned at δ 4.35, and the H-4 of **80** at δ 4.61.
In order to identify the influence of the methoxycarbonyl group on the relative reactivity of the hydroxyl groups in *C*-allyl α-galactopyranuronates, the corresponding amides **82** and **83** were synthesized by aminolysis of **58** with methanolic ammonia or ethanolic methylamine, respectively (**Scheme 17**). The amides **82** and **83** were obtained in quantitative yields. The dimolar benzoylation of **82** and **83** was carried out under the same conditions as described for **58**. Benzoylation of amido derivative **82** gave corresponding 2,3,4-tri-*O*-benzoyl **85** in 29% and 3-*O*-benzoyl **86** in 33% yields respectively. The position of the benzoyl group in **86** was determined by comparison of ^{1}H NMR spectra with perbenzoate **85** (**Table 3**) as described above. Thus, in contrast to the downfield shift signals of H-2 (δ 5.81) and H-4 (δ 6.29) of **85**, the allocation of corresponding proton signals (H-2 at δ 4.19 and H-4 at δ 4.62) of **86** indicated that the hydroxyl groups in monobenzoylated derivative **86** are connected to C-2 and C-4.



Scheme 17. Synthesis of *C*-allyl α-D-galactopyranuronamides **82** and **83** and the temperature controlled dimolar benzoylation. (a) methanolic ammonia, 16 h, r.t.; (b) ethanolic methylamine 15 min., r.t.; (c) 2 equivalent benzoyl chloride, anhydrous pyridine, -38 °C.

Besides benzoylation also the nitrile **84** was isolated in 9% yield as a result of water elimination from the amido group. In ^{13}C NMR spectra the signal at δ 116.1 ppm was assigned to the nitrile group. The IR spectrum of **84** (**Figure 14**, marked red) did not show the expected absorption band for a nitrile group. However, in raman experiment (**Figure 14,** marked blue) a sharp adsorption signal at 2241 cm^{-1} characteristic for nitrile group was detected.

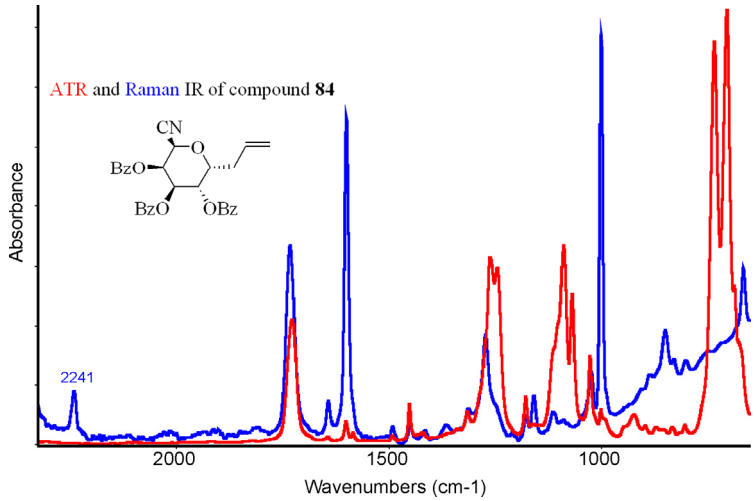


Figure 14. Raman and ATR spectra of **84** (Raman-spectrum was measured with 532 nm laser; FTIR-spectrum was measured with Diamant-ATR).

In the case of benzoylation of *N*-methyl amido derivative **83** 2,3,4-tri-*O*-benzoyl **87**, 3,4-di-*O*-benzoyl **88** and 2,3-di-*O*-benzoyl **89** were isolated in 43%, 15% and 18% yields respectively.

Single crystals of **88** were obtained and the X-ray diffraction studies provided the information that the pyranose ring of the 3,4-di-*O*-benzoate adopted $^4C_1$ conformation (**Figure 15** , for

crystal data see **Appendix**). In ^{1}H NMR spectra of **88** the coupling constant $^{3}J_{2,3}$ 9.4 Hz (typical for diaxial orientation of H-2 and H-3 protons) assigned the same conformation in solution (**Table 3**). Moreover, it was found that the crystal lattice of **88** contains chains of hydrogen bridges from the OH at C-2 of pyranose ring via the carbonyl of benzoyl substituent at O-3 of the neighbouring molecule (**Figure 16**). Furthermore, hydrogen bridges exist between NH and carbonyl groups of amido group of neighbouring molecules.

	^{1}H NMR chemical shifts δ of ring protons and ^{3}J coupling constants (Hz)				
	1 ($^{3}J_{1,2}$)	2 ($^{3}J_{2,3}$)	3 ($^{3}J_{3,4}$)	4 ($^{3}J_{4,5}$)	5
77	5.00 ddd 3.3	*5.61 dd* 6.5	*5.85 dd* 3.1	*5.93 dd* 5.2	4.96 d
78	4.73 ddd 3.5	4.21 dd 6.6	*5.59* dd 3.2	*5.92 dd* 5.2	4.84 d
80	4.48 ddd 2.5	*5.49 dd* 5.3	*5.55 dd* 2.6	4.61 m 5.8	4.66 d
81	4.76 ddd 3.0	*5.33 dd* 5.7	4.35 dd 3.0	*5.76 dd* 5.5	4.82 d
85	4.76 ddd 5.3	*5.81 dd* 10.3	*5.86 dd* 3.1	*6.29 dd* 1.9	4.57 d
86	4.08 3.4	4.19 dd 6.5	*5.27 dd* 3.2	4.62 dd 4.8	4.39 d
87	4.75 ddd 5.7	*5.79 dd* 10.5	*5.88 dd* 3.2	*6.29 dd* 1.8	4.54 d
88	4.47–4.39	4.47–4.39 9.4	*5.42 dd* 3.4	*6.18 dd* 1.9	4.47–4.39
89	4.31 ddd 3.6	*5.65 dd* 7.0	*5.54 dd* 3.1	4.69 dd 4.6	4.45 d

Table 3. ^{1}H NMR chemical shifts and H – H coupling constants of ring protons of benzoylated derivatives. The chemical shifts of substituted positions are shown in italic.

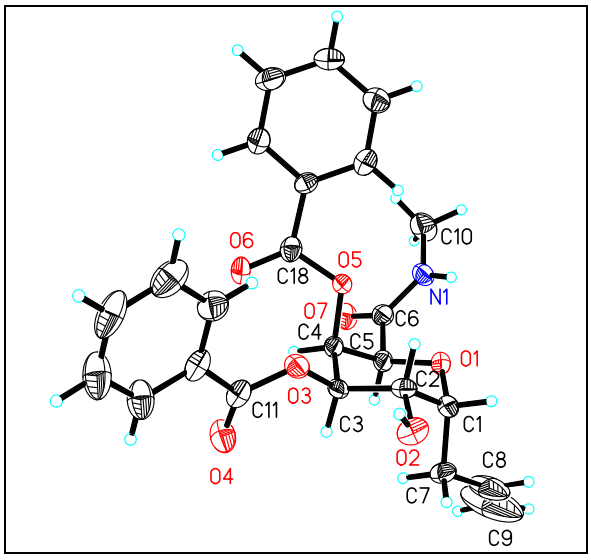


Figure 15. An ORTEP diagram of compound **88** with 50% probability for the thermal ellipsoids.

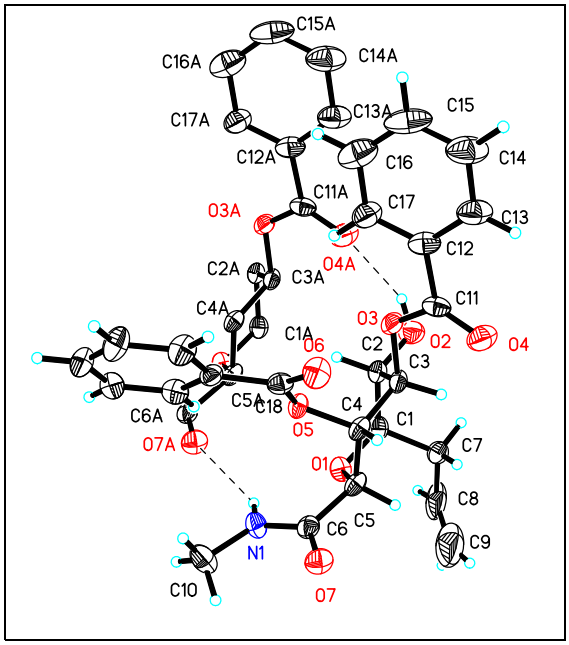
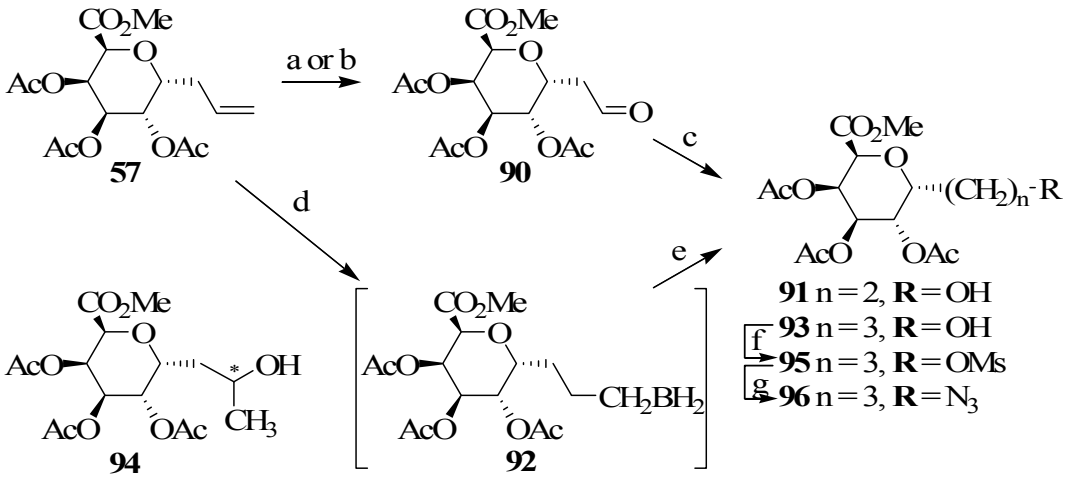


Figure 16. Crystal lattice of **88** (in the figure only a part of the second molecule is shown)

In conclusion, the temperature controlled partial benzoylation of *C*-allyl α-galactopyranuronates **58**, **82**, and **83** indicates that the difference in the relative reactivity of the hydroxyl groups are quite small compared to *O*-glycosides. This fact is evident by the formation of high amounts of perbenzoylated products **77**, **85**, and **87**. Furthermore, the formation of 3,4-di-*O*-benzoyl derivatives **78** and **88** as well as the 3-*O*-monobenzoate **89** indicates that the hydroxyl group at position *C*-3 is the most reactive one, whereas the relative reactivity differences between hydroxyl groups at *C*-2 and *C*-4 positions seems to be negligible.

2.2.4. DERIVATISATION OF C-ALLYL GROUP

Sugar amino acids (SAAs) bearing both amino and carboxylic groups on furan or pyran backbones[109] are versatile building blocks for the synthesis of glycomimetic libraries.[110] But, as shown in **Chapter 2.1.2**, an amino function directly connected to the anomeric centre is less nucleophilic then aliphatic amines. Therefore, we were interested in the preparation of SAAs based on galacturonates in which the amino function is linked *via* a spacer to the pyranose ring. As described earlier,[98] an effective pathway for the synthesis of *C*-glycosyl analog **57** starting from D-galacturonic acid was developed. Noteworthy, only one purification step by column chromatography was necessary in this route and the desired compound **57** was obtained in 50% overall yield. Pursuing the programme toward the synthesis of SAA building blocks, two pathways were explored to introduce an amino function at the end of a carbon chain spacer (**Scheme 18**).

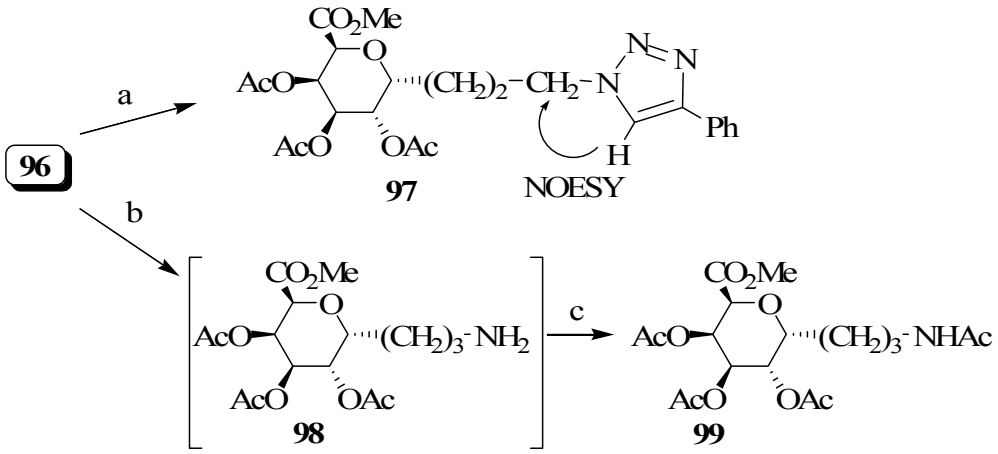


Scheme 18. Two possible pathways for derivatization of the C=C double bond of compound **57**. (a) OsO_4, dioxan-water, 1 h, r.t., then $NaIO_4$, 16 h, r.t; (b) O_3, anhydrous dichloromethane, 2 h, -78 °C, then Ph_3P, 4 h, r.t.; (c) $NaBH_4$, anhydrous dichloromethane-methanol, 4 h, -78 °C – -2 °C, then $NaBH_4$, 1.5 h, 5 °C; (d) BH_3·THF, anhydrous THF, 30 min, 0 °C; (e) phosphate buffer (pH 7.0), 0 °C, then 30% aq H_2O_2, 5 h, r.t.; (f) methanesulfonyl chloride, anhydrous dichloromethane, triethylamine, 7 h, r.t.; (f) NaN_3, anhydrous DMF, 18-crown-6, 55 h, r.t.

In the first route osonolysis of the double bond in **57** or alternatively oxidation by osmium tetroxide provided the aldehyde **90** in 66% and 68%, respectively. Subsequent reduction of **90** furnished the corresponding alcohol **91** in 43% yield. In the second route, the hydroboration of **57** with borane-tetrahydrofuran complex provided **92** and following treatment with hydrogen peroxide gave the primary alcohol **93** in 42% yield. As side products a mixture of diastereomeric secondary alcohols **94** was isolated in 7% yield and characterized by NMR

spectroscopy. In ^{1}H and ^{13}C NMR spectra of **94** signals were doubled indicating the presence of both diastereomers. In ^{1}H NMR spectra two doublets at 1.20 ppm were recorded which were assigned as methyl protons attached to diastereomeric centre.

Comparing both pathways the second one is more convenient, because the alcohol **93** was obtained in an one-pot procedure in 42% yields whereas alcohol **91** was accessible only in 29% overall yield. Therefore, the further experiments were performed using alcohol **93**. Mesylation of the free hydroxyl group of **93** furnished compound **95** quantitatively (**Scheme 18**). Subsequent substitution of methanesulfonyl group by azide furnished **96** in 82% yield.
In a preliminary experiment, Cu (I) catalyzed cycloaddition of azido derivative **96** with phenyl acetylene gave the triazole **97** in excellent yield (87%) (**Scheme 19**). The regioselectivity of the cycloaddition was established by NOESY experiment in which a typical correlation of methylene protons of the alkyl chain with the proton of triazole was detected (shown by arrow).

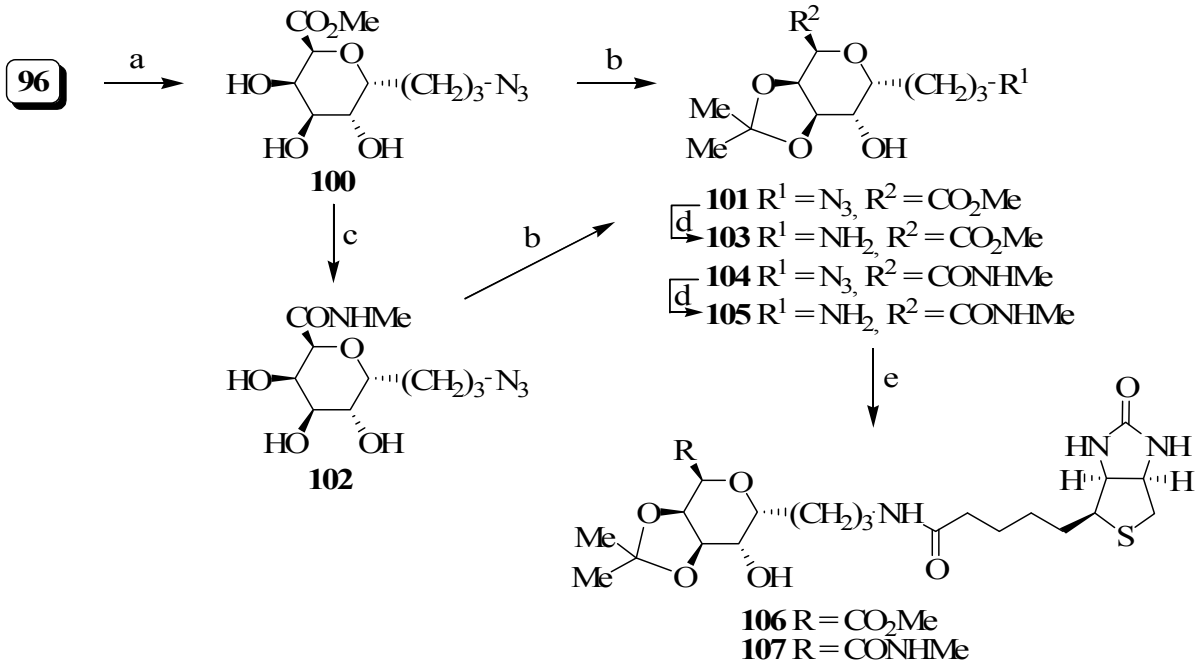


Scheme 19. Further functionalization of azido group of compound **96**. (a) Copper (II) sulfate x 5 H_2O, L-(+)-ascorbic acid, H_2O-DMF, 48 h, 75 °C; (b) Pd^0/C, H_2, anhydrous ethyl acetate – methanol, 6 h, r.t.; (c) Ac_2O, anhydrous pyridine, 17 h, -15 °C – r.t.

Next, the azido group of compounds **96** was reduced by hydrogen catalyzed with palladium on charcoal. The desired amine **98** could not be separated from the obtained complex reaction mixture by chromatographic procedures. Using the Staudinger procedure for reduction a similarly complex mixture was observed. Noteworthy, the amine **38** (**Chapter 2.1.2, Scheme 6**) was stable in the presence of *O*-acetyl protecting groups in contrast to **98**. This fact underlines the noticeable reactivity differences of amino groups in these cases. We suppose that the amino group of **98** attacks the ester bonds which provides a palette of acetyl migration

products. To get a homogeneous product, the reaction mixture was immediately treated after reduction with acetic anhydride in anhydrous pyridine. The fully acetylated derivative **99** was isolated in disappointing 14% yield.

To overcome the problems connected with the acetyl protecting groups, they were removed by 1% methanolic HCl **(100)** and subsequently replaced by an isopropylidene group to obtain **101** (**Scheme 20**). The treatment of **100** with ethanolic methylamine provided compound **102** in quantitative yield. The isopropylidenation was performed also with the *N*-methylamide **102** to furnish **104** in 78% yield. Compound **104** possess the advantage over **101** of being free of ester linkages. Subsequent reduction of the azido group in **101** and in **104** under a hydrogen atmosphere and in the presence of palladium on charcoal provided **103** and **105**. These intermediates were biotinylated in DMF with D-(+)-biotin in the presence of diisopropylethylamine (DIPEA) and EDC. The TLC monitoring of the reactions was practically not possibly even by using reversed phase TLC plates. Therefore, the reaction mixtures were worked up after 24 h.



Scheme 20. Synthesis of hydrolytically stable byotinylated α-D-galactopyranuronate *C*-glycosyl analogs **106** and **107**. (a) 0.28 M methanolic HCl, 24 h, r.t.; (b) $(CH_3)_2C(OCH_3)_2$, anhydrous acetone, *p*-toluenesulfonic acid, 12 h, r.t.; (c) ethanolic methylamine 2 h, r.t.; (d) Pd^0/C, H_2, anhydrous ethyl acetate – methanol, 5 h, r.t.; (e) D-(+)-biotin, DIPEA, EDC, 1-HOBT, DMF, 24 h, 0 °C – r.t.

After purification by preparative reversed phase HPLC biotinylamido compounds **106** and **107** were obtained in 60% and 65%, respectively. Biotinylated compounds **106** and **107** were not soluble in a number of organic solvents *e.g.* ethyl acetate, chloroform, methanol, pyridine *etc*. Only dimethyl sulfoxide was a suitable solvent for analytical investigations.

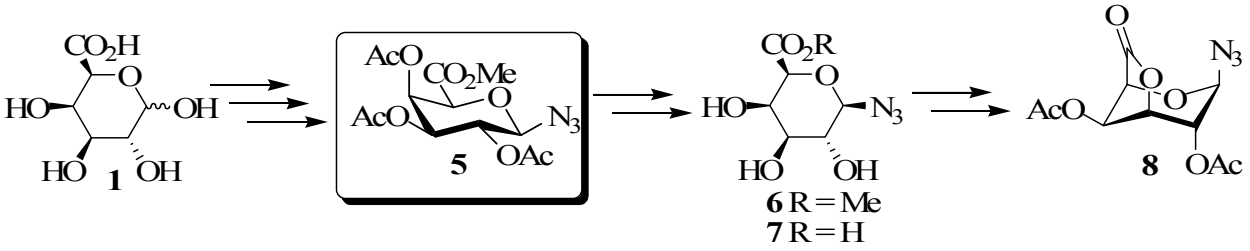
In conclusion, two possible pathways for derivatization of the C=C double bond of the *C*-allyl moiety of compound **57** were compared. As a result SAA stable precursor **96** was synthesized in preparative scale. After displacement of acetyl groups by isopropylidene protecting group the reduction of the azido function in **101** and in **104** were performed. The biotinylation of the amino derivatives **103** and **105** furnished hydrolytically stable byotinylated α-D-galactopyranuronate *C*-glycosyl analogs **106** and **107** in preparative scale as markers and anchors of oligosaccharides.

2. SUMMARY

Pectin is a very complex biopolymer and an important constituent of plant cell wall. Pectin fragments, among others, contain mainly D-galacturonic acid moieties. Hence, there is a raising interest toward a synthesis of pectin fragments of defined structures containing labelling moieties to elucidate the biological role of pectin in plants as well as in dietary fiber in human nutrition. In the present work the synthesis of D-galacturonic acid *N*- and *C*-glycosides as suitable markers and anchors was described.

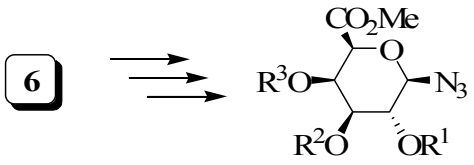
D-GALACTOPYRANOSYLURONATE *N*-GLYCOSIDES

Starting from D-galacturonic acid an improved procedure for the preparation of *methyl 2,3,4-tri-O-acetyl-β-D-galactopyranosyluronate azide* (**5**) in multi-gram scale as a key intermediate was furnished.



Several protecting strategies were described in order to create a library of fully and partially acylated and alkylated galacturonate azides. For the complete deprotection of **5**, a two step protocol was required to avoid the well known β-elimination of galacturonates. The desired compound **7** was obtained in 70% overall yield. The lactonization of **7** gave 6,3-lactone **8** in 68% yield.

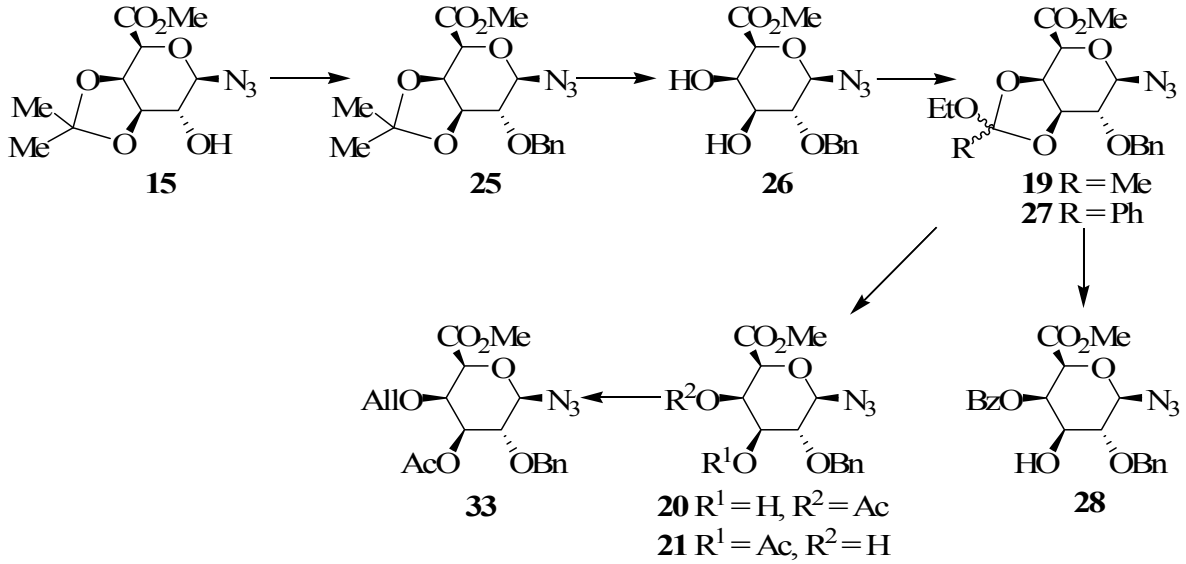
For NMR investigations partially acetylated galacturonate azide derivatives **11**, **17** and **18** were synthesized. Isopropylidenation of **6** followed by acetylation and subsequent removal of the isopropylidene group furnished the target 2-*O*-acetyl derivative **17** in 93% overall yield.



11 $R^1 = R^3 = Ac$, $R^2 = H$
17 $R^1 = Ac$, $R^2 = R^3 = H$
18 $R^1 = R^2 = Ac$, $R^3 = H$

Temperature controlled acetylation of **17** gave the 2,4-di-*O*-acetyl **11** and the 2,3-di-*O*-acetyl compound **18** in 21% and 30% yields, respectively. Alternatively, the 2,4-di-*O*-acetyl uronate azide **11** was obtained starting from **6** by application of orthoester method. The target compound **11** was then obtained in 93% yield.

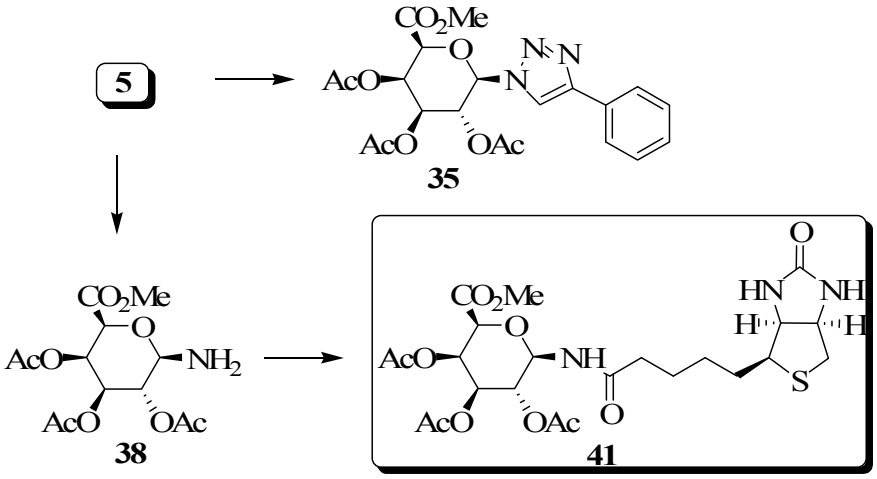
To get partially alkylated building blocks, the 3,4-isopropylidene derivative **15** was benzylated to yield **25** in 75%. After removal of isopropylidene group the desired 2-*O*-benzyl derivative **26** was obtained in 90% yield. Again the orthoester method was applied to provide exclusively the compound **28** in 75% yield *via* orthobenzoate **27**. In contrast, the orthoacetate ring opening of **19** was not regioselective and gave a mixture of **20** and **21** in overall 95% yield.



Exploiting the acetyl migration under the conditions of allylation catalyzed by silver oxide the mixture of 3-*O*-acetyl and 4-*O*-acetyl derivatives **20** and **21** was converted to compound **33** in 65% yield.

To prove the azido group as a suitable anchor "click chemistry" was applied. The triazolyl glycosides **35** was obtained in 70% yield by 1,3-cycloaddition of peracetylated uronate azide **5** with phenylacetylene.

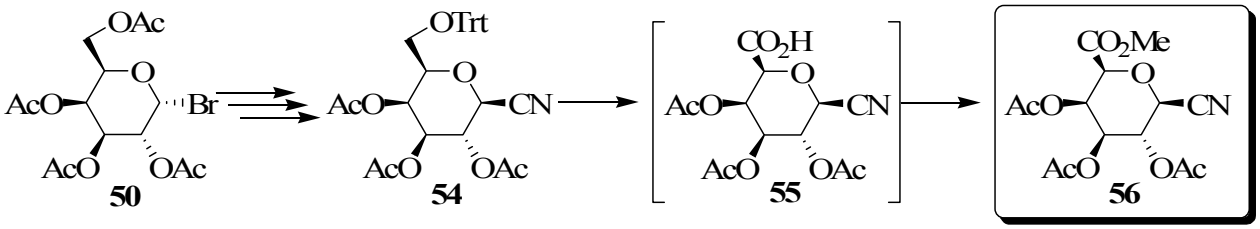
The palladium catalyzed reduction of **5** under a hydrogen atmosphere furnished exclusively the β-anomer **38** in quantitative yield.



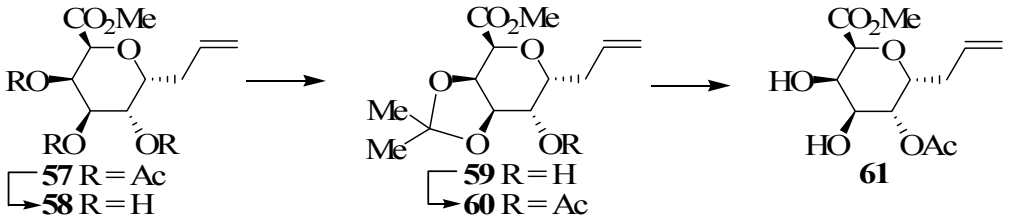
Biotinylation of compound **38** provided *N-(methyl 2,3,4-tri-O-acetyl-β-D-galactopyranosyluronate) biotinylamide* (**41**) in 36% yield. Compound **41** can be considered as a suitable intermediate in biological assays.

D-GALACTOPYRANOSYLURONATE *C*-GLYCOSIDES

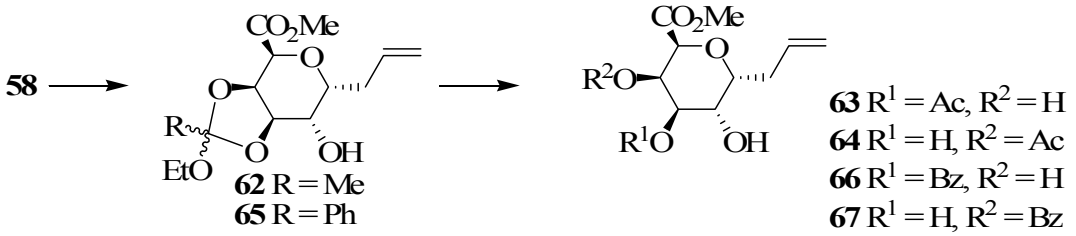
Starting from acetobromgalactose **50** an efficient pathway for synthesis of the cyanide **56** in five steps is described. In spite of the crucial Jones oxidation of **54** the peracetylated cyanouronate **56** was afforded in 37% overall yield. This pathway is up today the best preparative route for the synthesis of uronate **56**.



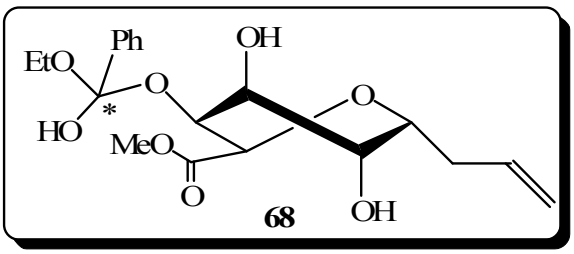
Considering the fact that galacturonic acid moieties in pectin occur partially acetylated, a library of corresponding *C*-allyl uronates was furnished. At first, the acetyl protecting groups of fully acetylated **57** were removed with 1% methanolic HCl to give compound **58** in 93% yield. In order to get a set of monoacetyl derivatives, compound **61** was converted to the 3,4-*O*-isopropylidene derivative **59** in 93% yield. Subsequent acetylation of **59** gave the fully protected **60** in 94% yield. The removal of isopropylidene group provided the 2-*O*-acyl uronate **61** in 82% yield.



To get the 4-*O*-acetyl derivative **64**, cyclic orthoester strategy was chosen. The reaction of **58** with triethyl orthoacetate provided **62**. Surprisingly, the ring opening of the diastereomersic orthocetates **62** with aq acetic acid was not regioselective and gave a mixture of 3-*O*-acetyl and 4-*O*-acetyl derivatives **63** and **64** in a ratio ~1:1 and in 72% yield.

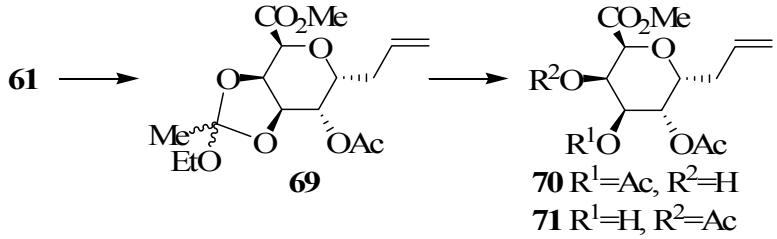


To minimize the tendency of migration of acetyl groups the experiments were repeated with the corresponding orthobenzoate **65**. Again a mixture of 3-*O*- and 4-*O*-benzoyl substituted **66** and **67** in a ratio 1:1 was observed in overall 98% yield. Careful control of the course of the orthobenzoylation allowed the isolation of the acyclic hemiorthoester **68** for the first time. Surprisingly, the pyranose ring of compound **68** adopts a skew conformation ${}^{0}S_{2}$.

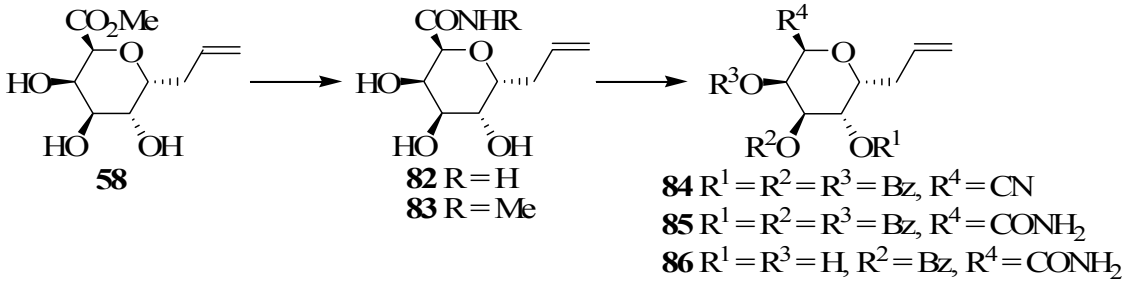


Based on stereoelectronic effects described by King and Allbutt, for the first time a new model was suggested for the orthoester ring opening to explain the loss of regioselectivity. We suppose that the lack of selectivity is caused by conformational changes of the pyranose ring as indicated in ^{1}H NMR investigations and confirmed by X-ray diffraction studies.

In spite of the loss of regioselectivity of the orthoester procedure the deacetylated derivatives **70** and **71** were obtained in overall 87% yield.

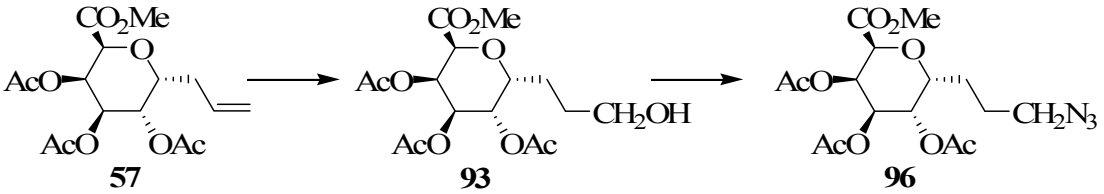


The relative reactivity of hydroxyl groups in *C*-allyl α-galactopyranuronamide **82** has been established. The temperature controlled dimolar benzoylation resulted in the formation of perbenzoylated product **85** and the 2,4-di-*O*-benzoyl compound **86**. This indicated that the difference in reactivity of the hydroxyl groups are smaller than those in *O*-glycosides. As a side product, the nitrile **84** was isolated. The corresponding methylester **58** and *N*-methylamide **83** gave comparable results concerning reactivity differences of hydroxyl groups.

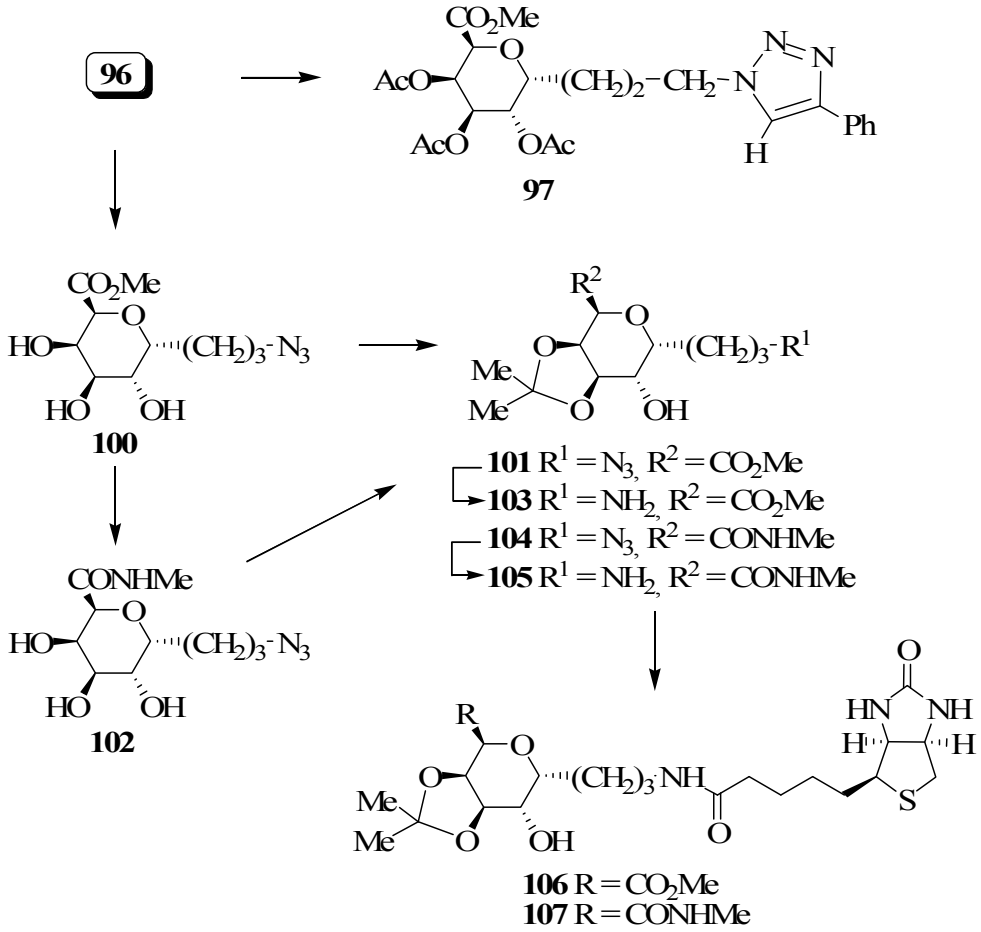


Furthermore, the formation of the 3,4-di-*O*-benzoyl derivative **89** indicates that the hydroxyl group at position *C*-3 is the most reactive one, whereas the reactivity difference between hydroxyl groups at *C*-2 and *C*-4 positions seems to be negligible.

For the derivatization of the *C*-allyl moiety of compound **57** two pathways were performed. As a sugar amino acid stable precursor, **96** was synthesized using the procedure borhydration-oxydation *via* **93** in preparative scale.



1,3-Dipolar cycloaddition of compound **96** with phenylacetylene under conditions of "click chemistry" gave the triazole **97** in excellent yield (87%).



The deacetylation of compound **96** gave the ester **100** in 89% yield. The amide **102** was obtained in quantitative yield by treatment of **100** with ethanolic methylamine. Both compounds **100** and **102** were isopropylidenated to yield **101** and **104** in 99% and 78%, respectively. Following reduction of the azido group in **101** and in **104** provided **103** and **105**, respectively. These intermediates were biotinylated to furnish hydrolytically stable *C*-glycosyl galacturonates **106** and **107** in 60% and 65% yields, respectively. Thus, the compounds **106** and **107** were synthesized in preparative scale and represent suitable markers and/or anchors for the synthesis of labelled pectin fragments.

4. EXPERIMENTAL SECTION

4.1. GENERAL

4.1.1. Analytics

Melting points were determined with a Boetius micro-heating plate BHMK 05 (Rapido, Dresden) and are uncorrected. Optical rotations were measured for solutions in a 2-cm cell with an automatic polarimeter "GYROMAT" (Dr. Kernchen Co.). ^{1}H NMR spectra (250.13 MHz, 300.13 MHz and 500 MHz) and ^{13}C NMR spectra (62.9 MHz, 75.5 MHz and 125.8 MHz) were recorded on Bruker instruments AVANCE 250, ARX 300, and AVANCE 500, with $CDCl_3$, MeOH-*d* and DMSO-d_6 as solvents. The calibration of spectra was carried out on solvent signals ($CDCl_3$: δ ^{1}H = 7.25, δ ^{13}C = 77.00; DMSO-d_6: δ ^{1}H = 2.49, δ ^{13}C = 39.50; MeOH-d_4: δ ^{1}H = 3.30, δ ^{13}C = 49.00). The ^{1}H and ^{13}C NMR signals were assigned by DEPT and two–dimensional ^{1}H,^{1}H COSY, ^{1}H,^{1}H NOESY and ^{1}H,^{13}C correlation spectra (HMBC and HSQC). The mass spectra were recorded on an AMD 402/3 spectrometer (AMD Intectra GmbH). The LC-MS (ESI) analysis was performed on a LTQ Thermo Finnigan spectrometer. Elemental analysis was performed on a Leco CHNS–932 instrument**.** For the X–ray structure determination an X8Apex system with CCD area detector was used (λ = 0.71073 Å, graphite monochromator). The structures were solved with direct methods (Bruker-SHELXTL). The refinement calculations were done by the full-matrix least-squares method of Bruker SHELXTL, Vers.5.10, Copyright 1997, Bruker Analytical X-ray Systems. All non-hydrogen atoms were refined anisotropically. The hydrogen atoms were put into theoretical positions and refined using the riding model. CCDC 641609–641611 contain the supplementary crystallographic data for this paper. Via www.ccdc.cam.ac.uk/conts/retrieving.html these data can be obtained free of charge (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

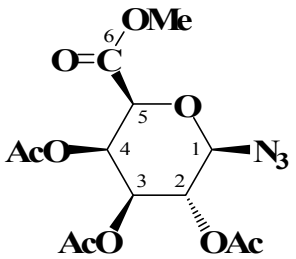
4.1.2. Reagents and materials

All washing solutions were cooled to ~5 °C. The $NaHCO_3$ and NaCl solutions were saturated. All reactions were monitored by thin-layer chromatography (TLC, Silica Gel 60, F_{254}, Merck KGaA). The followings eluent systems (v/v) were used: (A_1) 1 : 4, (A_2) 1 : 3, (A_3) 1 : 2, (A_4) 1 : 1, (A_5) 2 : 1, (A_6) 3 : 1, (A_7) 4 : 1, (A_8) 5 : 1, (A_9) 3 : 2 petrol ether-ethyl acetate; (B_1) 5 : 1 toluene-ethyl acetate; (C_1) 1 : 1, (C_2) 5 : 3, (C_3) 3 : 1, (C_4) 4 : 1, (C_5) 5 : 1, (C_6) 9 : 1, (C_7) 10 : 1, (C_8) 20 : 1 chloroform-methanol; (D_1) 1 : 20 : 0.1 ethyl acetate-toluene-acetic acid; (E_1) 10 : 1 ethyl acetate-methanol. The spots were made visible by spraying with ethanolic 10%

H_2SO_4 solution and charring them for 1–2 min with a heat gun. Detection of UV active derivatives was effected by UV fluorescence. Preparative flash chromatography, MPLC and HPLC were performed by elution from columns of slurry–packed Silica Gel 60 (Merck, 63–200 μm, 40–63 μm) and Nucleosil 100–7 (Knauer, 7.0 μm) respectively. Preparative reversed-phase HPLC was performed using Nucleosil 100-C_{18} (Knauer, 7.0 μm) column eluting with 25% aqueous acetonitrile. All solvents and reagents were purified and dried according to standard procedures[111]. Light petrol ether (40–60 °C) was used as solvent for t.l.c. and column chromatography. After classical work up of the reaction mixtures, the organic layers as a rule, were dried over cotton, and then concentrated under reduced pressure (rotary evaporator).

4.2. SYNTHESIS OF DIFFERENTLY PROTECTED D-GALACTOPYRANOSYL URONATE AZIDES

4.3.1. Methyl 2,3,4-tri-*O*-acetyl-β-D-galactopyranosyluronate azide (5)[51]



Tetramethylguanidinium azide (1.80 g, 11.17 mmol) was added to a solution of methyl 2,3,4-tri-*O*-acetyl-α-D-galactopyranosyluronate bromide **4**[58] (2.95 g, 7.45 mmol) in anhydrous acetonitrile (40 mL). The homogeneous solution was stirred at room temperature under an argon atmosphere for 5 h (TLC, eluent B_1). Diethyl ether (200 mL) was added, and the solution was stirred for a few minutes. The formed precipitate was filtered off, and washed with diethyl ether. The combined filtrate and washings were concentrated and the residue was purified by column chromatography (eluent ethyl acetate gradient 20→60% in petrol ether) to obtain azido compound **5**.

Yield: 2.45 g, 92%, colourless crystals

Melting point: 99–101 °C (from chloroform–petrol ether); mp 99–101 °C (from ethanol); lit[51]. mp 163 °C (from ethanol)

$[\alpha]_D^{21}$ +9.5 (*c* 3.5, chloroform), lit[51]. $[\alpha]_D^{21}$ +7 (*c* 3.1, chloroform)

R_f 0.46 (eluent A_4)

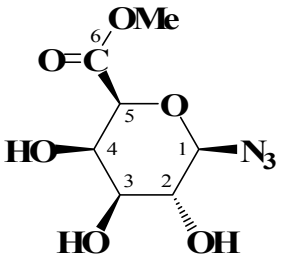
IR (KBr), ν 2121.0 cm^{-1} (N_3)

^{1}H NMR (CDCl$_3$, 250.13 MHz): δ 5.72 (dd, 1H, $^3J_{4,5}$ 1.5 Hz, H-4), 5.17 (dd, 1H, $^3J_{2,3}$ 10.4 Hz, H-2), 5.07 (dd, 1H, $^3J_{3,4}$ 3.3 Hz, H-3), 4.66 (d, 1H, $^3J_{1,2}$ 8.5 Hz, H-1), 4.38 (d, 1H, H-5), 3.76 (s, 3H, OCH_3), 2.11, 2.07, 1.98 (3s, 9H, 3 x CH_3CO)

^{13}C NMR (CDCl$_3$, 62.9 MHz): δ 169.9, 169.7, 169.2 (3 x CH_3CO), 165.8 (C-6), 88.3 (C-1), 73.9 (C-5), 70.3 (C-3), 67.9 (C-4), 67.6 (C-2), 52.8 (OCH_3), 20.6, 20.4, 20.4 (3 x CH_3CO)

$C_{13}H_{17}N_3O_9$ (359.10)	calcd:	C 43.46	H 4.77	N 11.70
	found:	C 43.61	H 4.82	N 11.52

4.3.2. Methyl β-D-galactopyranosyluronate azide (6)



Peracetylated azide **5** (11.75 g, 32.72 mmol) was added to a stirred solution of methanolic hydrogen chloride (0.28 M, prepared by adding of 14.6 mL acetyl chloride to 360 mL ice-cold anhydrous methanol), and the mixture was kept for 24 h at room temperature under an argon atmosphere (TLC, eluent C_6). The reaction mixture was neutralized with $PbCO_3$–$Pb(OH)_2$ (64.1 g). After stirring for 2 h, the lead salts were filtered off [it is of advantage to use Glass Microfiber filter (GF/A, Whatman, Cat. No. 1820042)], washed with methanol, and the filtrate and washings were combined and concentrated. The residue was applied to a column of silica gel (eluent C_6) to provide **6**.

Yield: 7.1 g, 93%, amorphous solid

$[\alpha]_D^{21}$ –50.4 (*c* 1.2, methanol)

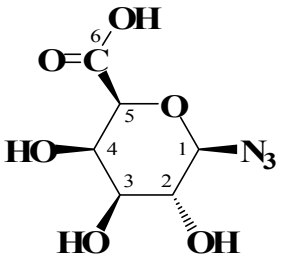
R_f 0.20 (eluent C_6)

^{1}H NMR (CD$_3$OD; 250.13 MHz): δ 4.48 (d, 1H, $^3J_{1,2}$ 7.9, H-1), 4.34 (d, 1H, H-5), 4.16 (dd, 1H, $^3J_{4,5}$ 1.2 Hz, H-4), 3.78 (s, 3H, OC*H*$_3$), 3.58–3.46 (m, 2H, $^3J_{3,4}$ 3.1 Hz, H-2, H-3)

^{13}C NMR (CD$_3$OD, 62.9 MHz): δ 169.9 (C-6), 92.0 (C-1), 77.0 (C-5), 74.0 (C-3), 71.0 (C-2), 70.9 (C-4), 52.7 (OCH_3)

$C_7H_{11}N_3O_6$ (233.18)	calcd:	C 36.06	H 4.75	N 18.02
	found:	C 36.29	H 4.72	N 18.11

4.3.3. β-D-Galactopyranuronic acid 1-azide (7)[52]



Uronate **6** (240 mg, 1.03 mmol) was suspended in a solution of lithium hydroxide (0.3 M, methanol-water-tetrahydrofuran 5:4:1, 20 mL) at 0 °C and stirred for 2.5 h at that temperature (TLC, eluent C_1). The reaction mixture was then diluted with water, and the pH was adjusted to 2 with Amberlite IR–120 (H^+) resin. After removal of the resin by filtration, the filtrate was concentrated to dryness to give analytically pure compound **7**.

Yield: 170 mg, 75%, amorphous solid

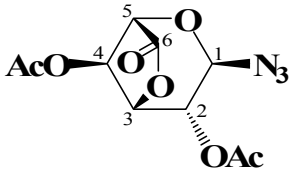
$[\alpha]_D^{22}$ –34 (*c* 1.8, methanol); lit[52] $[\alpha]_D^{22}$ –31.9 (*c* 0.8, water)

^{1}H NMR (D_2O, 250.13 MHz): δ 4.71 (d, 1H, $^3J_{1,2}$ 8.6 Hz, H-1), 4.46 (d, 1H, H-5), 4.30 (dd, 1H, $^3J_{4,5}$ 1.2 Hz, H-4), 3.76 (dd, 1H, $^3J_{3,4}$ 3.4 Hz, H-3), 3.52 (dd, 1H, $^3J_{2,3}$ 9.8 Hz, H-2)

^{13}C NMR (D_2O, 62.9 MHz): δ 172.3 (C-6), 90.9 (C-1), 76.3 (C-5), 72.9 (C-3), 70.3 (C-2), 70.1 (C-4)

$C_6H_9N_3O_6$ (219.15)	calcd:	C 32.88	H 4.14	N 19.07
	found:	C 32.92	H 4.17	N 18.59

4.3.4. 2,4-Di-*O*-acetyl-β-D-galactopyranurono-6,3-lactone 1-azide (8)



Uronic acid **7** (128 mg, 0.58 mmol) was suspended in acetic anhydride (6 mL) and heated at 85 °C under an argon atmosphere for 3 h (TLC, eluent A_4). After concentration of the reaction mixture, the residue was repeated coevaporated with toluene (5x) and then purified by HPLC (eluent A_4) to provide compound **13**.

Yield: 113 mg, 68%, colourless syrup

$[\alpha]_D^{21}$ –293 (*c* 1.0, chloroform)

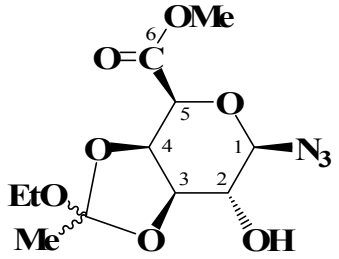
R_f 0.16 (eluent A_4)

^{1}H NMR ($CDCl_3$, 250.13 MHz): δ 5.38 (s, 1H, H-1), 5.35 (d, 1H, $^3J_{2,3}$ 1.2 Hz, H-2), 5.08 (d, 1H, H-5), 4.88 (dd, 1H, $^3J_{4,5}$ 4.6 Hz, H-4), 4.22 (t, 1H, $^3J_{3,4}$ 1.5 Hz, H-3), 2.17, 2.10 (2s, 6H, 2 x CH_3CO)

^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 170.8, 169.4, 169.0 (3 x CO), 88.2 (C-1), 77.5 (C-4), 71.0 (C-2), 70.5 (C-3), 70.1 (C-5), 20.5, 20.5 (2 x CH$_3$CO)

$C_{10}H_{11}N_3O_7$ (285.21)	calcd:	C 42.11	H 3.89	N 14.37
	found:	C 42.31	H 3.82	N 14.96

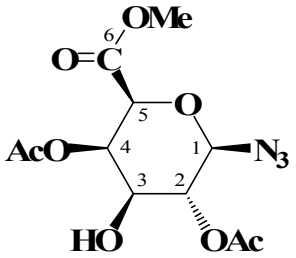
4.3.5. Methyl 3,4-*O*-(1-ethoxyethylidene)-β-D-galactopyranosyluronate azide (9)



Compound **6** (1.01 g, 4.33 mmol) and *p*-toluenesulfonic acid (7.5 mg) were dried together by threefold coevaporation with toluene. Triethyl orthoacetate (5.8 mL, 31. 7 mmol) was then added and the suspension was stirred for 14 h at ambient temperature under an argon atmosphere (TLC, eluent A_3). After adding of triethylamine (2.6 mL), the reaction mixture was diluted with diethyl ether (75 mL). The organic layer was washed with ice-water (3 x 30 mL), dried, and concentrated. The residue was purified by MPLC (eluent ethyl acetate gradient 2→50% in petrol ether) to provide a syrupy mixture of *exo*/*endo* diastereomers **9.**

Yield: 1.21 g, 92%, colourless syrup

4.3.6. Methyl 2,4-di-*O*-acetyl-β-D-galactopyranosyluronate azide (11)



The mixture of diastereomers **9** (840 mg, 2.77 mmol) was dissolved in abs pyridine (8.8 mL) and acetic anhydride (2.2 mL), and the reaction mixture was kept for 24 h at room temperature under an argon atmosphere (TLC, eluent A_3). After dilution with chloroform (25 mL), the solution was poured into ice-water (80 mL). The organic phase was then separated, and the aqueous layer was extracted with chloroform (3 x 40 mL). The combined

organic layers were washed with aq. $NaHCO_3$ (2 x 50 mL), ice-water (50 mL), dried, and concentrated. After repeated coevaporation with toluene–heptane–ethanol (5:1:1, 4 x 50 mL), the residue was dried in high vacuum to gave compound **10** which was used without further characterization. After dissolving of **10** in 80% aq. AcOH (28 mL), the reaction mixture was kept for 30 min at room temperature (TLC, eluent A_3) and then concentrated. Repeated coevaporation of the residue with toluene (5x) followed by chromatographical purification (eluent ethyl acetate gradient 5→60% in petrol ether) afforded derivative **11**.

Yield: 704 mg, 80%, colourless crystals

Melting point: 119–121 °C (from petrol ether–ethyl acetate)

$[\alpha]_D^{21}$ +2.1 (*c* 1.0, chloroform)

$\mathbf{R}_f$ 0.29 (eluent A_3)

IR (Nujol); ν 3433 (OH), 2128.0 cm^{-1} (N_3)

^{1}H NMR ($CDCl_3$, 250.13 MHz): δ 5.59 (dd, 1H, $^3J_{4,5}$ 1.2 Hz, H-4), 4.96 (dd, 1H, $^3J_{2,3}$ 9.9 Hz, H-2), 4.65 (d, 1H, $^3J_{1,2}$ 8.9 Hz, H-1), 4.30 (d, 1H, H-5), 3.91 (dd, 1H, $^3J_{3,4}$ 3.7, H-3), 3.76 (s, 3H, OCH_3), 2.75 (br s, 1H, OH), 2.14, 2.14 (2s, 6H, 2 x CH_3CO)

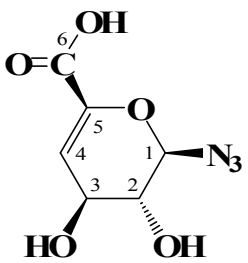
^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 170.7, 170.5, 166.4 (2 x CH_3CO, C-6), 88.2 (C-1), 74.1 (C-5), 71.2, 70.9, 70.4 (C-2, C-3, C-4), 52.8 (OCH_3), 20.8, 20.6 (2 x CH_3CO)

$C_{11}H_{15}N_3O_8$ (317.25)	calcd:	C 41.64	H 4.77	N 13.25
	found:	C 41.90	H 4.64	N 13.11

4.3.7. Saponification of uronate 5

Uronate **5** (335 mg, 0.93 mmol) was suspended in a solution of lithium hydroxide (0.3 M, methanol-water-tetrahydrofuran 5:4:1, 20 mL) at 0 °C and stirred for 2 h at that temperature (TLC, eluent C_1). The reaction mixture was then diluted with water, and the pH was adjusted to 2 with Amberlite IR–120 (H^+) resin. After removal of the resin by filtration, the filtrate was concentrated to dryness to give a mixture of **7** and the unsaturated compound **12** as colourless syrup in a molar ratio of 7:10 (determined from ^{1}H NMR spectra).

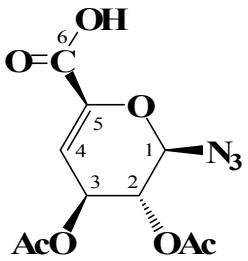
4.3.8. 1,4-Dideoxy-α-L-*threo*-hex-4-enopyranuronic acid 1-azide (12)



^{1}H NMR (D_2O, 250.13 MHz): 6.06 (dd, 1H, H-4), 5.44 (dd, 1H, $^3J_{1,2}$ 6.3 Hz, $^4J_{1,3}$ 0.8 Hz, H-1), 4.21 (ddd, 1H, $^3J_{3,4}$ 3.7 Hz, H-3), 3.73–3.68 (m, 1H, $^3J_{2,3}$ 4.9 Hz, $^4J_{2,4}$ 0.9 Hz, H-2)

^{13}C NMR (D_2O, 62.9 MHz): δ 166.6 (C-6), 145.8 (C-5), 111.9 (C-4), 88.9 (C-1), 70.2 (C-2), 66.7 (C-3)

4.3.9. 2,3-Di-*O*-acetyl-1,4-dideoxy-α-L-*threo*-hex-4-enopyranuronic acid 1-azide (13)



The mixture of compounds **7** and **12** was suspended in acetic anhydride (9 mL) and heated at 85 °C under an argon atmosphere for 3 h (TLC, eluent A_4). After concentration of the reaction mixture, the residue was repeated coevaporated with toluene (5x). Separation by flash chromatography (eluent A_3, then eluent C_3) provided 3,6-lactone **8** (71 mg, 27% from **5**) and compound **13** (46 mg, 18%).

Yield: 46 mg, 18%, colourless syrup

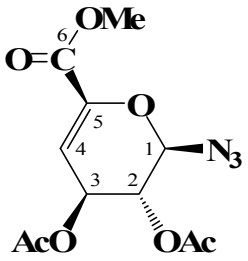
$[\alpha]_D^{23}$ –52.4 (*c* 1.02, methanol)

^{1}H NMR (CD_3OD, 250.13 MHz): δ 5.96 (dd, 1H, H-4), 5.59 (dd, 1H, $^3J_{1,2}$ 4.6 Hz, $^4J_{1,3}$ 0.9 Hz, H-1), 5.27 (ddd, 1H, $^3J_{3,4}$ 4.3 Hz, H-3), 4.97 (ddd, 1H, $^3J_{2,3}$ 3.7 Hz, $^4J_{2,4}$ 0.9 Hz, H-2), 2.07, 2.05 (2s, 6H, 2 x CH_3CO)

^{13}C NMR (CD_3OD, 62.9 MHz): δ 171.8, 171.0 (2 x CH_3CO), 168.9 (C-6), 148.7 (C-5), 104.5 (C-4), 86.4 (C-1), 69.7 (C-2), 67.2 (C-3), 20.8, 20.6 (2 x CH_3CO)

$C_{10}H_{11}N_3O_7$ (285.21)	calcd:	C 42.11	H 3.89	N 14.37
	found:	C 41.95	H 3.99	N 14.58

4.3.10. Methyl 2,3-di-*O*-acetyl-1,4-dideoxy-α-L-*threo*-hex-4-enopyranosyluronate azide (14)



Compound **13** (40 mg, 0.14 mmol) was dissolved in a minimum amount of chloroform and treated with an ethereal diazomethane solution. After 30 min the excess of diazomethane was destroyed by addition of acetic acid. After concentration of the reaction mixture, the residue was coevaporated with toluene (3x) and purified by HPLC (eluent A_4) to provide **14**.

Yield: 41 mg, 98%

$[\alpha]_D$ –69.3 (*c* 2.1, dichloromethane) lit.[69]

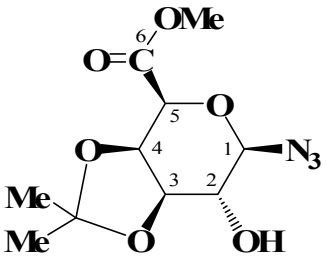
R_f 0.43 (eluent A_4)

^{1}H NMR ($CDCl_3$, 250.13 MHz): δ 6.21 (dd, 1H, H-4), 5.57 (dd, 1H, $^3J_{1,2}$ 3.7 Hz, $^4J_{1,2}$ 1.2 Hz, H-1), 5.21 (ddd, 1H, $^3J_{3,4}$ 4.6 Hz, H-3), 5.01 (ddd, 1H, $^3J_{2,3}$ 2.8 Hz, $^4J_{2,4}$ 1.2 Hz, H-2), 3.83 (s, 3H, OCH_3), 2.09, 2.08 (2s, 6H, 2 x CH_3CO)

^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 169.8, 169.2, 161.5 (2 x CH_3*C*O, C-6), 142.7 (C-5), 107.4 (C-4), 84.9 (C-1), 67.8 (C-2), 64.0 (C-3), 52.8 (OCH_3), 20.7, 20.6 (2 x *C*H_3CO)

$C_{11}H_{13}N_3O_7$ (299.24)	calcd:	C 44.15	H 4.38	N 14.04
	found:	C 44.00	H 4.25	N 13.94

4.3.11. Methyl 3,4-*O*-isopropylidene-β-D-galactopyranosyluronate azide (15)



p-Toluenesulfonic acid monohydrate (326 mg) was added to the suspension of compound **6** (1.90 g, 8.15 mmol) in 2,2-dimethoxypropane (16.3 mL) and dry acetone (65 mL), and the reaction mixture was stirred for 20 h at ambient temperature under an argon atmosphere (TLC, eluent A_3). The mixture was then passed through a layer of alkaline aluminia (3 x 3 cm), the alkaline aluminia was washed with acetone, and the filtrate and washings were combined. After removal of the solvent, the residue was purified by flash chromatography on silica gel (eluent ethyl acetate gradient 0→33% in petrol ether) to provide compound **15**.

Yield: 2.10 g, 94%, colourless syrup

$[\alpha]_D^{23}$ –41.3 (*c* 1.3, chloroform)

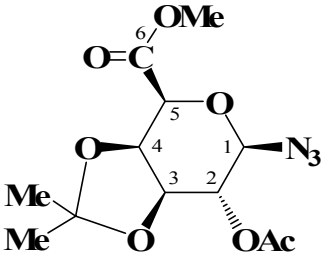
R_f 0.25 (eluent A_3)

^{1}H NMR (CDCl$_3$, 300.13 MHz): δ 4.55 (d, 1H, $^3J_{1,2}$ 8.4 Hz, H-1), 4.52–4.49 (m, 2H, H-4, H-5), 4.18–4.13 (m, 1H, H-3), 3.84 (s, 3H, OCH_3), 3.57 (ddd, $^3J_{2,3}$ 6.5 Hz, 1H, H-2), 2.65 (br d, 1H, $^3J_{H,OH}$ 2.5 Hz, OH), 1.50, 1.34 [2s, 6H, $(CH_3)_2C$]

^{13}C NMR (CDCl$_3$, 75.5 MHz): δ 166.9 (C-6), 110.9 [$(CH_3)_2C$], 89.8 (C-1), 77.8 (C-3), 73.5, 73.5 (C-4, C-5), 72.1 (C-2), 52.7 (OCH_3), 27.7, 26.1 [$(CH_3)_2C$]

$C_{10}H_{15}N_3O_6$ (273.24)	calcd:	C 43.96	H 5.53	N 15.38
	found:	C 44.10	H 5.61	N 15.47

4.3.12. Methyl 2-*O*-acetyl-3,4-*O*-isopropylidene-β-D-galactopyranosyluronate azide (16)



Acetic anhydride (1.5 mL) was added to a solution of compound **15** (240 mg, 0.88 mmol) in abs. pyridine (4.5 mL) at 4 °C and the reaction mixture was stirred at ambient temperature under an argon atmosphere. After 20 h (TLC, eluent A_3) ethanol (0.6 mL) was added at 0 °C and stirring was continued for 30 min. The reaction mixture was concentrated and traces of pyridine were removed by coevaporation with repeated addition of toluene. The residue was purified by flash chromatography (eluent ethyl acetate gradient 5→60% in petrol ether) to furnish compound **16**.

Yield: 271 mg, 98%, colourless crystals

Melting point: 116–118 °C (ethyl acetate–petrol ether)

$[\alpha]_D^{22}$ –17.4 (*c* 1.1, chloroform)

R_f 0.49 (eluent A_3)

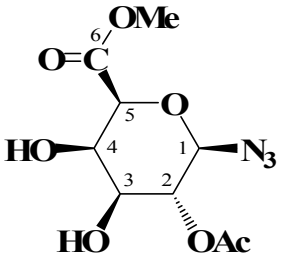
^{1}H NMR (CDCl$_3$, 250.13 MHz): δ 4.99 (dd, 1H, $^3J_{2,3}$ 6.4, H-2), 4.59 (d, 1H, $^3J_{1,2}$ 7.3 Hz, H-1), 4.54 (dd, 1H, $^3J_{4,5}$ 2.4, H-4), 4.47 (d, 1H, H-5), 4.28 (dd, 1H, $^3J_{3,4}$ 5.6 Hz, H-3), 3.84 (s, 3H, OCH_3), 2.11 (s, 3H, CH_3CO), 1.54, 133 [2s, 6H, $(CH_3)_2C$]

^{13}C NMR (CDCl$_3$, 75.5 MHz): δ 169.2, 166.8 (2 x CH_3CO, C-6), 111.3 [$(CH_3)_2C$], 87.5 (C-1), 75.2 (C-3), 73.4 (C-5), 73.3 (C-4), 70.8 (C-2), 52.7 (OCH_3), 27.1, 25.9 [$(CH_3)_2C$], 20.8 (CH_3CO)

$C_{12}H_{17}N_3O_7$ (315.28)	calcd:	C 45.71	H 5.43	N 13.33

found: C 45.92 H 5.54 N 13.31

4.3.13. Methyl 2-*O*-acetyl-β-D-galactopyranosyluronate azide (17)



90% aq. trifluoroacetic acid (7.5 mL) was added to a solution of isopropylidene derivative **16** (247 mg, 0.78 mmol) in chloroform (4 mL) and methanol (4 mL). The reaction mixture was kept for 10 min at ambient temperature, diluted with toluene (30 mL), evaporated and coevaporated with repeated addition of toluene (3 x 30 mL). The residue was purified by flash chromatography (eluent A_3) to provide **17**.

Yield: 198 mg, 92%, colourless solid

$[\alpha]_D^{22}$ –63.6 (*c* 1.65, methanol)

R_f 0.13 (eluent A_3)

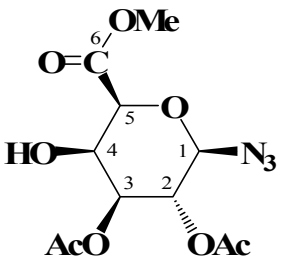
^{1}H NMR (CD$_3$OD, 250.13 MHz): δ 5.01 (dd, 1H, $^3J_{2,3}$ 10.1 Hz, H-2), 4.62 (d, 1H, $^3J_{1,2}$ 8.9 Hz, H-1), 4.45 (d, 1H, H-5), 4.22 (dd, 1H, $^3J_{4,5}$ 1.8 Hz, H-4), 3.80 (dd, 1H, $^3J_{3,4}$ 3.0 Hz, H-3), 3.77 (s, 3H, OCH_3), 2.10 (s, 3H, CH_3CO)

^{13}C NMR (CD$_3$OD, 62.9 MHz): δ 171.79, 169.79 (2 x CH_3CO, C-6), 89.20 (C-1), 77.20 (C-5), 72.22 (C-3), 72.15 (C-2), 71.17 (C-4), 52.81 (OCH_3), 20.83 (CH_3CO)

$C_{12}H_{17}N_3O_7$ (315.28) calcd: C 39.28 H 4.76 N 15.27

found: C 39.25 H 4.69 N 15.20

4.3.14. Methyl 2,3-di-*O*-acetyl-β-D-galactopyranosyluronate azide (18)



Acetyl chloride (93 µL) in abs benzene (1 mL) was added to a solution of compound **17** (350 mg, 1.27 mmol) in abs pyridine (4.6 mL) at –35 °C during 20 min. The reaction mixture was kept for 1 h at -35 °C and then for 15 h at 12 °C under an argon atmosphere. After concentration, the residue was coevaporated with repeated addition of toluene in order to

remove traces of pyridine. Purification by flash chromatography (eluent ethyl acetate gradient 33→66% in petrol ether) and by HPLC (eluent A_4) provided **5** (29 mg, 6%), **11** (84 mg, 21%), **17** (69 mg, 20%), and desired compound **18** (120 mg, 30%).

Yield: 120 mg, 30%, colourless syrup

$[\alpha]_D^{22}$ –1.1 (*c* 1.0, chloroform)

R_f 0.43 (eluent A_3)

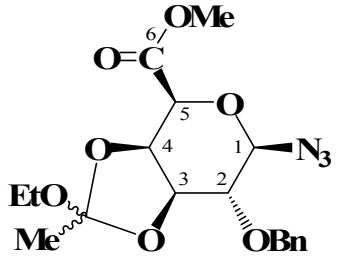
^{1}H NMR ($CDCl_3$, 250.13 MHz): δ 5.24 (dd, 1H, $^3J_{2,3}$ 10.2 Hz, H-2), 5.00 (dd, 1H, $^3J_{3,4}$ 3.3 Hz, H-3), 4.63 (d, 1H, $^3J_{1,2}$ 8.7 Hz, H-1), 4.47 (m, 1H, $^3J_{4,5}$ 1.3 Hz, H-4), 4.30 (d, 1H, H-5), 3.82 (s, 3H, OCH_3), 2.87 (br s, 1H, OH), 2.09, 2.07 (2s, 6H, 2 x CH_3CO)

^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 170.07, 169.40, 167.12 (2 x CH_3CO, C-6), 88.27 (C-1), 75.44 (C-5), 72.42 (C-3), 68.00 (C-4), 67.92 (C-2), 52.90 (OCH_3), 20.71, 20.61 (2 x CH_3CO)

$C_{11}H_{15}N_3O_8$ (317.25) calcd: C 41.64 H 4.77 N 13.25

found: C 41.60 H 4.74 N 13.36

4.3.15. Methyl 2-*O*-benzyl-3,4-*O*-(1-ethoxyethylidene)-β-D-galactopyranosyluronate azide (19)



Compound **26** (2.30 g, 7.12 mmol) and *p*-toluenesulfonic acid (13 mg) were dried together by threefold coevaporation with toluene. Triethyl orthoacetate (11.4 mL, 62. 2 mmol) was then added and the suspension was stirred for 14 h at ambient temperature under an argon atmosphere (TLC, eluent A_3). After adding of triethylamine (4.5 mL), the reaction mixture was diluted with chloroform (200 mL). The organic layer was washed with ice-water (3 x 70 mL), dried, and concentrated. The residue was purified by MPLC (eluent ethyl acetate gradient 2→33% in petrol ether) to provide **19** (2.74 g, 98%) as a colourless foam which was used in the next step without further characterization.

4.3.16. Orthoester cleavage of compound 19

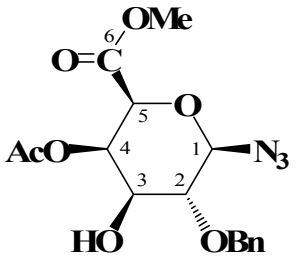
Acetic acid (80%, 60 mL) was added to compound **19** (2.47 g, 6.28 mmol) and the reaction mixture was kept for 45 min at ambient temperature (TLC, eluent A_5). The mixture was then diluted with toluene (50 mL), concentrated, and the residue was coevaporated with repeated

addition of toluene. Purification by MPLC (eluent ethyl acetat gradient 2→33% in petrol ether) gave a mixture of **20** and **21** (2.24 g, 97%). The ratio of **20**/**21** varied between 4:1 and 1:1 and separation of pure samples of both compounds by HPLC was not possible. Therefore, the NMR data were obtained by enriched fractions of each compound.

4.3.17. Benzylation and ortoester cleavage of 9

Benzyl bromide (1.25 mL, 10.5 mmol) and freshly prepared silver oxide (1.76 g, 7.6 mmol) were added to a solution of compound **9** (771 mg, 2.54 mmol) in anhydrous benzene (5 mL), and the mixture was shaken at room temperature under an argon atmosphere for 23 h (TLC, eluent A_5). The silver salts were filtered off and washed with chloroform. The combined filtrate and washings were concentrated and the residue was then treated with 80% aq. acetic acid (25 mL) at room temperature for 1 h (TLC, eluent A_5). The solution was diluted with toluene (20 mL), concentrated, and the residue was coevaporated with repeated addition of toluene. Purification by MPLC (eluent ethyl acetate gradient 0→16% in petrol ether) provided **22** (105 mg, 14%), **23** (81 mg, 8%) and a mixture of compounds **20** and **21** (325 mg, 35%).

4.3.18. Methyl 4-*O*-acetyl-2-*O*-benzyl-β-D-galactopyranosyluronate azide (20)

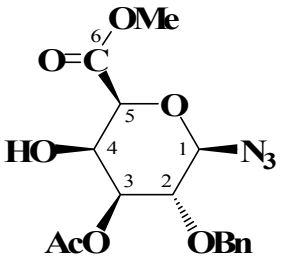


R_f 0.25 (eluent A_5)

^{1}H NMR (CDCl$_3$, 250.13 MHz): δ 7.38–7.27 (m, 5H, C_6H_5), 5.59 (dd, 1H, $^3J_{4,5}$ 1.2 Hz, H-4), 4.91, 4.70 (2d, 2H, ^2J 11.0 Hz, OCH_2C$_6$H$_5$), 4.69 (d, 1H, $^3J_{1,2}$ 8.5 Hz, H-1), 4.26 (d, 1H, H-5), 3.85 (dd, 1H, $^3J_{3,4}$ 3.4 Hz, H-3), 3.75 (s, 3H, OCH$_3$), 3.47 (dd, 1H, $^3J_{2,3}$ 9.5 Hz, H-2), 2.49 (br s, 1H, OH), 2.09 (s, 3H, CH$_3$CO)

^{13}C NMR (CDCl$_3$, 62.9 MHz): δ 170.0, 166.6 (CH$_3$*C*O, C-6), 137.5–128.0 (6 signals, C$_6$H$_5$), 90.3 (C-1), 78.0 (C-2), 75.2 (O*C*H$_2$C$_6$H$_5$), 74.1 (C-5), 71.9 (C-3), 70.2 (C-4), 52.8 (OCH$_3$), 20.6 (*C*H$_3$CO)

4.2.19. Methyl 3-*O*-acetyl-2-*O*-benzyl-β-D-galactopyranosyluronate azide (21)

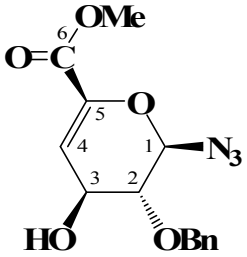


R_f 0.25 (eluent A_5)

^{1}H NMR (CDCl$_3$, 250.13 MHz): δ 7.38–7.27 (m, 5H, C_6H_5), 4.93 (dd, 1H, $^3J_{3,4}$ 3.4 Hz, H-3), 4.83 (d, 1H, ^2J 11.3 Hz, OCH_2C$_6$H$_5$), 4.68 (d, 1H, $^3J_{1,2}$ 8.6 Hz, H-1), 4.63 (d, 1H, ^2J 11.3 Hz, OCH_2C$_6$H$_5$), 4.42 (dd, 1H, $^3J_{4,5}$ 1.2 Hz, H-4), 4.24 (d, 1H, H-5), 3.81 (s, 3H, OCH$_3$), 3.67 (dd, 1H, $^3J_{2,3}$ 9.8 Hz, H-2), 2.49 (br s, 1H, OH), 2.03 (s, 3H, CH$_3$CO)

^{13}C NMR (CDCl$_3$, 62.9 MHz): δ 169.9, 167.4 (CH$_3$*C*O, C-6), 137.5–127.0 (6 signals, C_6H_5), 90.5 (C-1), 78.0 (C-2), 75.4 (C-5), 75.2 (O*C*H$_2$C$_6$H$_5$), 75.2 (C-3), 68.2 (C-4), 52.8 (OCH$_3$), 20.8 (*C*H$_3$CO)

4.2.20. Methyl 2-*O*-benzyl-1,4-dideoxy-α-L-*threo*-hex-4-enopyranosyluronate azide (22)



Yield: 105 mg, 14%, colourless syrup

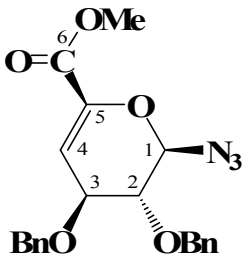
R_f 0.36 (eluent A_5)

^{1}H NMR (CDCl$_3$, 250.13 MHz): δ 7.40–7.27 (m, 5H, C_6H_5), 6.27 (dd, 1H, H-4), 5.64 (dd, 1H, $^3J_{1,2}$ 3.7 Hz, $^4J_{1,3}$ 1.2 Hz, H-1), 4.70, 4.64 (2d, 2H, ^2J 11.9 Hz, OCH_2C$_6$H$_5$), 4.10 (ddd, 1H, $^3J_{3,4}$ 4.6 Hz, H-3), 3.81 (s, 3H, OCH$_3$), 3.65 (ddd, 1H, $^3J_{2,3}$ 3.1 Hz, $^4J_{2,4}$ 1.2 Hz, H-2), 2.48 (br s, 1H, OH)

^{13}C NMR (CDCl$_3$, 62.9 MHz): δ 162.0 (C-6), 140.4 (C-5), 136.9, 128.6, 128.2, 127.8 (C_6H_5, 2 signals are isochronic), 111.9 (C-4), 86.4 (C-1), 75.4 (C-2), 72.5 (O*C*H$_2$C$_6$H$_5$), 63.4 (C-3), 52.5 (COO*C*H$_3$)

$C_{14}H_{15}N_3O_5$ (305.29)	calcd:	C 55.08	H 4.95	N 13.76
	found:	C 55.15	H 4.74	N 13.55

4.2.21. Methyl 2,3-di-*O*-benzyl-1,4-dideoxy-α-L-*threo*-hex-4-enopyranosyluronate (23)



Yield: 81 mg, 8%, colourless syrup

$[\alpha]_D^{21}$ –24.2 (*c* 1.4, chloroform)

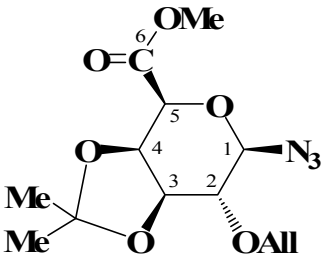
R_f 0.27 (eluent A_8)

^{1}H NMR ($CDCl_3$, 250.13 MHz): δ δ 7.40–7.27 (m, 10H, 2 x C_6H_5), 6.22 (dd, 1H, H-4), 5.37 (dd, 1H, $^3J_{1,2}$ 5.8 Hz, $^4J_{1,3}$ 0.6 Hz, H-1), 4.72, 4.67 (2d, 2H, ^2J 11.3 Hz, O$CH_2C_6H_5$), 4.65, 4.59 (2d, 4H, ^2J 11.9 Hz, O$CH_2C_6H_5$), 4.12 (ddd, 1H, $^3J_{3,4}$ 3.7 Hz, H-3), 3.82 (s, 3H, OCH_3), 3.67 (ddd, 1H, $^3J_{2,3}$ 4.8 Hz, $^4J_{2,4}$ 0.6 Hz, H-2)

^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 161.9 (C-6), 141.8 (C-5), 137.4, 137.1, 128.5, 128.2, 128.0, 127.9 (2 x C_6H_5, 4 signals are isochronic), 109.9 (C-4), 87.6 (C-1), 75.5 (C-2), 73.5 (O$CH_2C_6H_5$), 72.1 (C-3), 71.4 (O$CH_2C_6H_5$), 52.6 (CH_3CO)

$C_{21}H_{21}N_3O_5$ (395.41)	calcd:	C 63.79	H 5.35	N 10.63
	found:	C 63.88	H 5.19	N 10.48

4.3.22. Methyl (2-*O*-allyl-3,4-isopropylidene-β-D-galactopyranosyl azide) uronate (24)



Allyl bromide (0.37 mL, 4.2 mmol) and silver oxide (710 mg, 3.06 mmol) were added to a solution of **15** (279 mg, 1.02 mmol) in dry benzene (2 mL), and the mixture was shaken at room temperature for 8h (TLC, eluent A_3). The mixture was filtered off; the solid was washed with chloroform, and the combined filtrate and washings were concentrated. The syrupy product was purified by MPLC (eluent ethyl acetate gradient 2→50% in petrol ether) to get **24**.

Yield: 208 mg, 65%, colourless syrup

$[\alpha]_D^{24}$ -36.3 (*c* 1.1, chloroform)

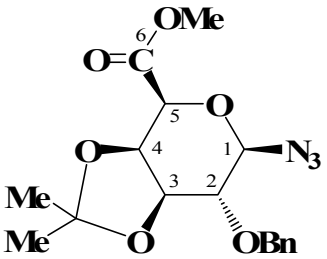
R_f 0.54 (eluent A_3)

^{1}HNMR ($CDCl_3$, 250.13 MHz): δ 5.97–5.81 (m, 1H, OCH_2CHCH_2), 5.33–5.18 (2m, 2H, OCH_2CHCH_2), 4.61, (d, 1H, $^3J_{1,2}$ 7.0 Hz, H-1), 4.51 (dd, 1H, $^3J_{4,5}$ 2.4, H-4), 4.45 (d, 1H, H-5), 4.27 (dd, 1H, $^3J_{3,4}$ 5.8, H-3), 4.23-4.18 (m, 2H, OCH_2CHCH_2), 3.83 (s, 3H, $COOCH_3$), 3.43 (dd, 1H, $^3J_{2,3}$ 6.1 Hz, H-2), 1.50, 1.34 [2s, 6H, $(CH_3)_2C$]

^{13}CNMR ($CDCl_3$, 75.5 MHz): δ 167.13 (C-6), 133.94 (OCH_2CHCH_2), 118.10 (OCH_2CHCH_2), 110.78 [$(CH_3)_2C$], 88.92 (C-1), 77.33 (C-2), 77.13, 73.41, 73.31 (C-3, C-4, C-5), 72.26 (OCH_2CHCH_2), 52.61 (CH_3COO), 27.27, 25.92 [$(CH_3)_2C$]

$C_{13}H_{19}N_3O_6$ (313.31)	calcd:	C 49.84	H 6.11	N 13.41
	found:	C 50.01	H 6.23	N 13.18

4.3.23. Methyl 2-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyluronate azide (25)



Benzyl bromide (5.2 mL, 32.9 mmol) and silver oxide (7.3 g, 31.5 mmol) were added to a solution of compound **15** (2.90 g, 10.62 mmol) in dry benzene (22 mL), and the reaction mixture was shaken at room temperature for 15 h under an argon atmosphere (TLC, eluent A_6). The silver salts were filtered off and washed with chloroform. The combined filtrate and washings were concentrated. The crude product was purified by MPLC (eluent ethyl acetate gradient 2→25% in petrol ether) to yield crystalline **25**.

Yield: 2.90 g, 75%, colourless crystals

Melting point: 59–61 °C (from petrol ether–ethyl acetate)

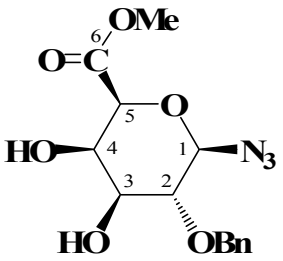
$[\alpha]_D^{24}$ –15.8 (*c* 1.5, chloroform)

R_f 0.26 (eluent A_6)

^{1}H NMR ($CDCl_3$, 250.13 MHz): δ 7.37–7.26 (m, 5H, C_6H_5), 4.74 (d, 1H, $^3J_{1,2}$ 5.5 Hz, H-1), 4.66, 4.64 (2d, 2H, ^2J 11.6, $OCH_2C_6H_5$), 4.51 (dd, 1H, $^3J_{4,5}$ 2.4 Hz, H-4), 4.46 (d, 1H, H-5), 4.31 (t, 1H, $^3J_{3,4}$ 5.8 Hz, H-3), 3.82 (s, 3H, OCH_3), 3.47 (dd, 1H, $^3J_{2,3}$ 5.8 Hz, H-2), 1.41, 1.33 (2s, 6H, $[CH_3]_2C$)

$C_{17}H_{21}N_3O_6$ (363.36)	calcd:	C 56.19	H 5.83	N 11.56
	found:	C 56.49	H 5.87	N 11.31

4.3.24. Methyl 2-*O*-benzyl-β-D-galactopyranosyluronate azide (26)



The isopropylidene group of compound **25** (2.91 g, 8.01 mmol) was removed as described for the synthesis of compound **17** to furnish **26**.

Yield: 2.33 g, 90%, colourless crystals

Melting point: 107–108 °C (benzene)

$[\alpha]_D^{25}$ –17.1 (*c* 1.4, chloroform)

R_f 0.26 (eluent A_3)

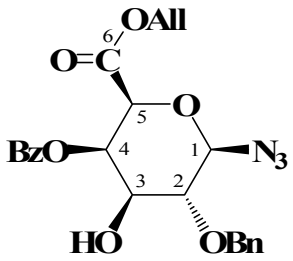
IR (Nujol); ν 3512 and 3398 (OH), 2130 cm^{-1} (N_3)

^{1}H NMR ($CDCl_3$, 250.13 MHz): δ 7.37–7.30 (m, 5H, C_6H_5), 4.91, 4.69 (2d, 2H, ^2J 11.3, $OCH_2C_6H_5$), 4.61 (d, 1H, $^3J_{1,2}$ 8.6 Hz, H-1), 4.27 (dd, 1H, $^3J_{4,5}$ 1.5 Hz, H-4), 4.17 (d, 1H, H-5), 3.82 (s, 3H, OCH_3), 3.67 (dd, 1H, $^3J_{3,4}$ 3.4 Hz, H-3), 3.47 (dd, 1H, $^3J_{2,3}$ 9.5 Hz, H-2), 2.55 (br s, 2H, OH)

^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 167.9 (C-6), 137.6, 128.7, 128.2 (C_6H_5, 2 signals are isochronic) 90.1 (C-1), 77.9 (C-2), 75.4 (C-5), 75.0 ($OCH_2C_6H_5$), 72.8 (C-3), 69.4 (C-4), 52.8 (OCH_3)

$C_{14}H_{17}N_3O_6$ (323.30)	calcd:	C 52.01	H 5.30	N 13.00
	found:	C 52.28	H 5.24	N 12.91

4.3.25. Methyl 4-*O*-benzoyl-2-*O*-benzyl-β-D-galactopyranosyluronate azide (28)



Triethyl orthobenzoate (1.2 mL, 5.14 mmol) was added to a solution of compound **26** (151 mg, 0.47 mmol) and *p*-toluenesulfonic acid monohydrate (7 mg) in abs. benzene

(5.5 mL). The solution was then stirred for 2 h at ambient temperature under an argon atmosphere to afford the diastereomeric mixture of 3,4-orthobenzoates **27** (TLC, eluent A_3) which wasn't further characterized. Aq. 80% acetic acid (12 mL) was then added to the reaction mixture, and, after 1.5 h later (TLC, eluent A_5), the solution was poured into ice-water (70 mL). The aqueous phase was extracted with chloroform (3 x 20 mL) and the combined extracts were washed with water (20 mL), $NaHCO_3$ (2 x 20 mL), water (2 x 20 mL), dried, and concentrated. The residue was purified by column chromatography (eluent ethyl acetate gradient 2→33% in petrol ether) to afford compound **28**.

Yield: 150 mg, 75%, colourless syrup

$[\alpha]_D^{22}$ –6.0 (*c* 1.36, chloroform)

$\mathbf{R}_f$ 0.43 (eluent A_5)

^{1}H NMR ($CDCl_3$, 250.13 MHz): δ 8.04–7.97, 7.61–7.54, 7.46–7.26 (3m, 10H, C_6H_5), 5.80 (dd, 1H, $^3J_{4,5}$ 1.4 Hz, H-4), 4.90, 4.74 (2d, 2H, ^2J 11.0 Hz, $OCH_2C_6H_5$), 4.71 (d, 1H, $^3J_{1,2}$ 8.6 Hz, H-1), 4.32 (d, 1H, H-5), 3.94 (dd, 1H, $^3J_{3,4}$ 3.4 Hz, H-3), 3.68 (s, 3H, OCH_3), 3.55 (dd, 1H, $^3J_{2,3}$ 9.5 Hz, H-2), 2.77 (br s, 1H, OH)

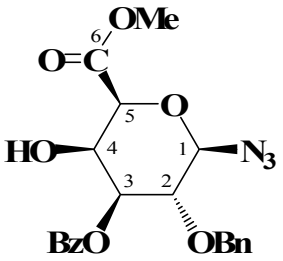
^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 166.6, 166.1 (CH_3CO, C-6), 137.5, 133.5, 130.0, 128.9, 128.5, 128.5, 128.2, 128.1 (2 x C_6H_5, four signals are isochronic), 90.2 (C-1), 78.0 (C-2), 75.2 ($OCH_2C_6H_5$), 74.2 (C-5), 72.1 (C-3), 71.1 (C-4), 52.7 (OCH_3)

$C_{21}H_{21}N_3O_7$ (427.41)	calcd:	C 59.01	H 4.95	N 9.83
	found:	C 59.10	H 4.87	N 9.64

4.3.26. Allylation of compound 28

Allyl bromide (0.7 mL, 8.1 mmol) and silver oxide (1.23 g, 5.3 mmol) were added to a solution of compound **28** (750 mg, 1.76 mmol) in dry benzene (4.5 mL), and the mixture was shaken at room temperature for 9 h under an argon atmosphere (TLC, eluent A_7). The silver salts were filtered off and washed with chloroform. The combined filtrate and washings were concentrated. The residue was purified by flesh chromatography and then by HPLC (eluent A_1, eluent A_7) to yield compounds **32** (122 mg, 15%). **31** (293 mg, 39%), **32** (147 mg, 18%), and **33** (123 mg, 9%).

4.3.27. Methyl 3-*O*-benzoyl-2-*O*-benzyl-β-D-galactopyranosyluronate azide (29)



Yield: 293 mg, 39%, colourless syrup

$[\alpha]_D^{21}$ +85.8 (*c* 0.9, chloroform)

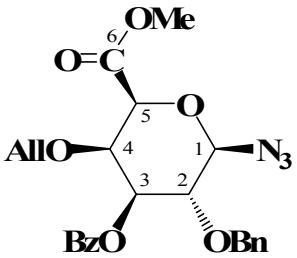
R_f 0.25 (eluent A_5)

^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 8.01–7.97, 7.62–7.56, 7.47–7.41, 7.20–7.16 (4m, 10H, 2 x C_6H_5), 5.20 (dd, 1H, $^3J_{3,4}$ 3.2 Hz, H-3), 4.82 (d, 1H, ^2J 11.1 Hz, OC$H_2C_6H_5$), 4.80 (d, 1H, $^3J_{1,2}$ 8.4 Hz, H-1), 4.69 (d, 1H, ^2J 11.1 Hz, OC$H_2C_6H_5$), 4.56 (m, 1H, $^3J_{4,5}$ 1.3 Hz, H-4), 4.33 (d, 1H, H-5), 3.86 (dd, 1H, $^3J_{2,3}$ 9.9 Hz, H-2), 3.81 (s, 3H, OCH_3), 2.46 (br.d, 1H, $^3J_{HOCH}$ 5.2 Hz, OH)

^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 167.4, 165.5 (CH_3CO, C-6), 137.2, 133.6, 129.8 129.2, 128.5, 128.4, 128.2, 127.9 (2 x C_6H_5, four signals are isochronic), 90.6 (C-1), 75.3 (C-5), 75.2 (C-2), 75.1 ($OCH_2C_6H_5$), 74.7 (C-3), 68.3 (C-4), 52.8 (OCH_3)

$C_{21}H_{21}N_3O_7$ (427.41)	calcd:	C 59.01	H 4.95	N 9.83
	found:	C 59.16	H 4.85	N 9.71

4.3.28. Methyl 4-*O*-allyl-3-*O*-benzoyl-2-*O*-benzyl-β-D-galactopyranosyluronate azide (30)



Yield: 147 mg, 18%, colourless syrup

$[\alpha]_D^{22}$ +60.1 (*c* 1.15, chloroform)

R_f 0.21 (eluent A_7)

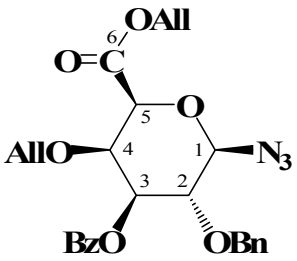
^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 8.03–7.99, 7.64–7.58, 7.49–7.43 (3m, 5H, $OCOC_6H_5$), 7.18 (m, 5H, $OCH_2C_6H_5$), 5.70 (m, 1H, $OCH_2CH{=}CH_2$), 5.21 (dd, 1H, $^3J_{3,4}$ 3.1 Hz, H-3), 5.11–4.99 (2m, 2H, $OCH_2CH{=}CH_2$), 4.83 (d, 1H, ^2J 11.1 Hz, OC$H_2C_6H_5$), 4.78 (d, 1H, $^3J_{1,2}$

8.4 Hz, H-1), 4.69 (d, 1H, OC*H*$_2$C$_6$H$_5$), 4.37 (dd, 1H, $^3J_{4,5}$ 1.3 Hz, H-4), 4.31 (d, 1H, H-5), 4.11–3.93 (2m, 2H, OC*H*$_2$CH=CH$_2$), 3.90 (dd, 1H, $^3J_{2,3}$ 10.1 Hz, H-2), 3.80 (s, 3H, OCH$_3$)

^{13}C NMR (CDCl$_3$, 75.5 MHz): δ 167.3, 165.5 (CH$_3$*C*O, C-6), 137.3, 133.5, 129.8, 128.6, 128.3, 128.1, 127.8 (2 x *C*$_6$H$_5$, five signals are isochronic), 133.9 (OCH$_2$*C*H=CH$_2$), 117.7 (OCH$_2$CH=*C*H$_2$), 90.4 (C-1), 75.7 (C-2), 75.3 (C-5), 75.2 (C-4), 75.1 (O*C*H$_2$C$_6$H$_5$), 75.0 (C-3), 74.2 (O*C*H$_2$CH=CH$_2$), 52.6 (OCH$_3$)

C$_{24}$H$_{25}$N$_3$O$_7$ (467.47)	calcd:	C 61.66	H 5.39	N 8.99
	found:	C 61.59	H 5.28	N 8.87

4.2.29. Allyl 4-*O*-allyl-3-*O*-benzoyl-2-*O*-benzyl-β-D-galactopyranosyluronate azide (31)



Yield: 80 mg, 9%, colourless syrup

[α]$_D^{22}$ +54.5 (*c* 2.02, chloroform)

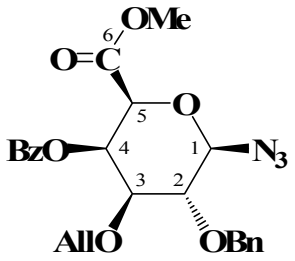
R$_f$ 0.28 (eluent *A*$_7$)

^{1}H NMR (CDCl$_3$, 300.13 MHz): δ 8.03–7.99, 7.64–7.58, 7.49–7.43 (3m, 5H, OCOC$_6$H$_5$), 7.18 (m, 5H, OCH$_2$C$_6$*H*$_5$), 5.93 (m, 1H, CO$_2$CH$_2$C*H*=CH$_2$), 5.71 (m, 1H, OCH$_2$C*H*=CH$_2$), 5.40–5.25 (2m, 2H, CO$_2$CH$_2$CH=C*H*$_2$), 5.22 (dd, 1H, $^3J_{3,4}$ 3.2 Hz, H-3), 5.11–4.98 (2m, 2H, OCH$_2$CH=C*H*$_2$), 4.83 (d, 1H, ^2J 11.1 Hz, OC*H*$_2$C$_6$H$_5$), 4.78 (d, 1H, $^3J_{1,2}$ 8.6 Hz, H-1), 4.71–4.67 (m, 3H, OC*H*$_2$C$_6$H$_5$, CO$_2$C*H*$_2$CH=CH$_2$), 4.38 (dd, 1H, $^3J_{4,5}$ 1.3 Hz, H-4), 4.32 (d, 1H, H-5), 4.11–3.95 (2m, 2H, OC*H*$_2$CH=CH$_2$), 3.91 (dd, 1H, $^3J_{2,3}$ 10.1 Hz, H-2)

^{13}C NMR (CDCl$_3$, 75.5 MHz): δ 166.5, 165.6 (CH$_3$*C*O, C-6), 137.3, 133.5, 129.7, 129.2, 128.6, 128.3, 128.1, 127.8 (C$_6$H$_5$, four signals are isochronic), 133.9 (OCH$_2$*C*H=CH$_2$), 131.1 (CO$_2$CH$_2$*C*H=CH$_2$), 119.6 (CO$_2$CH$_2$CH=*C*H$_2$), 117.7 (OCH$_2$CH=*C*H$_2$), 90.3 (C-1), 75.7 (C-2), 75.2 (C-5), 75.1 (C-4, O*C*H$_2$C$_6$H$_5$ one signal is isochronic), 75.0 (C-3), 74.2 (O*C*H$_2$CH=CH$_2$), 66.4 (CO$_2$*C*H$_2$CH=CH$_2$)

C$_{26}$H$_{27}$N$_3$O$_7$ (493.51)	calcd:	C 63.28	H 5.51	N 8.51
	found:	C 63.25	H 5.63	N 8.45

4.2.30. Methyl 3-*O*-allyl-4-*O*-benzoyl-2-*O*-benzyl-β-D-galactopyranosyluronate azide (32)



Yield: 122 mg, 15%, colourless syrup

$[\alpha]_D^{22}$ +29.0 (*c* 1.3, chloroform)

R_f 0.19 (eluent A_7)

^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 8.06–7.98, 7.62–7.55, 7.47–7.42, 7.39–7.26 (4m, 10H, 2 x C_6H_5), 5.96 (dd, 1H, $^3J_{4,5}$ 1.3 Hz, H-4), 5.94–5.80 (m, 1H, $OCH_2CH{=}CH_2$), 5.39–5.14 (m, 2H, $OCH_2CH{=}CH_2$), 4.84–4.77 (m, 2H, $OCH_2C_6H_5$), 4.72 (d, 1H, $^3J_{1,2}$ 8.4 Hz, H-1), 4.35 (d, 1H, H-5), 4.34–4.25, 4.14–4.07 (2m, 2H, $OCH_2CH{=}CH_2$), 3.71 (dd, 1H, $^3J_{3,4}$ 3.2 Hz, H-3), 3.70 (s, 3H, OCH_3), 3.62 (dd, 1H, $^3J_{2,3}$ 9.4 Hz, H-2)

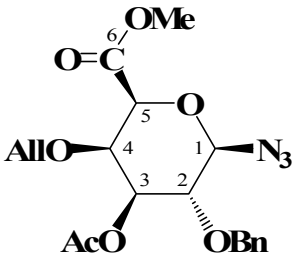
^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 166.6, 165.2 (CH_3CO, C-6), 137.7 ($OCH_2CH{=}CH_2$), 134.1 134.0, 133.3, 130.0, 129.3, 128.5, 128.4, 128.2, 127.9 (2 x C_6H_5, four signals are isochronic), 117.9 ($OCH_2CH{=}CH_2$), 90.4 (C-1), 78.9 (C-3), 77.4 (C-2), 75.7 ($OCH_2C_6H_5$), 74.4 (C-5), 71.2 ($OCH_2CH{=}CH_2$), 68.1 (C-4), 52.8 (OCH_3)

$C_{24}H_{25}N_3O_7$ (467.47)	calcd:	C 61.66	H 5.39	N 8.99
	found:	C 61.57	H 5.38	N 8.82

4.3.31. Allylation of the mixture of compounds 20 and 21

Allyl bromide (2.15 mL, 25.47 mmol) and silver oxide (3.6 g, 15.5 mmol) were added to a mixture of derivatives **20** and **21** (1.55 g, 3.82 mmol) in dry benzene (8.4 mL) and the mixture was shaken at room temperature for 9 h under an argon atmosphere (TLC, eluent A_5). The silver salts were filtered off and washed with chloroform. The combined filtrate and washings were concentrated and the syrupy residue was purified by MPLC (eluent ethyl acetate gradient 2→30% in petrol ether) to yield compounds **33** (1.03 g, 65%) and **34** (164 mg, 10%).

4.3.32. Methyl 3-*O*-acetyl-4-*O*-allyl-2-*O*-benzyl-β-D-galactopyranosyluronate azide (33)



Yield: 1.03 g, 65%, colourless syrup

$[\alpha]_D^{24}$ –15.8 (*c* 1.5, chloroform)

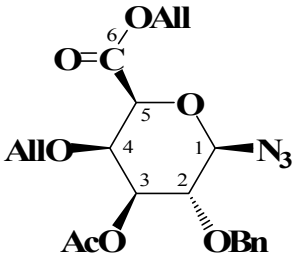
R_f 0.51 (eluent A_5)

^{1}H NMR ($CDCl_3$, 250.13 MHz): δ 7.38–7.25 (m, 5H, C_6H_5), 5.75 (m, 1H, $OCH_2CH{=}CH_2$), 5.21–5.11 (m, 2H, $OCH_2CH{=}CH_2$), 4.91 (dd, 1H, $^3J_{3,4}$ 2.9 Hz, H-3), 4.84 (d, 1H, ^2J 11.3 Hz, $OCH_2C_6H_5$), 4.69 (d, 1H, $^3J_{1,2}$ 8.6 Hz, H-1), 4.63 (d, 1H, ^2J 11.3 Hz, $OCH_2C_6H_5$), 4.24–4.22 (m, 2H, H-4, H-5), 4.02 (m, 2H, $OCH_2CH{=}CH_2$), 3.79 (s, 3H, OCH_3), 3.72 (dd, 1H, $^3J_{2,3}$ 10.1 Hz, H-2), 2.02 (s, 3H, CH_3CO)

^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 170.1, 167.4 (CH_3CO, C-6), 137.7, 128.4, 127.9, 127.8 (C_6H_5, two signals are isochronic), 134.0 ($OCH_2CH{=}CH_2$), 117.7 ($OCH_2CH{=}CH_2$), 90.3 (C-1), 75.9 (C-2), 75.2, 75.2, 74.5 (C-3, C-4, C-5), 75.2 ($OCH_2C_6H_5$), 74.2 ($OCH_2CH{=}CH_2$), 52.6 (OCH_3), 20.9 (CH_3CO)

$C_{19}H_{23}N_3O_7$ (405.40)	calcd:	C 56.29	H 5.72	N 10.37
	found:	C 56.41	H 5.81	N 10.21

4.3.33. Allyl 3-*O*-acetyl-4-*O*-allyl-2-*O*-benzyl-β-D-galactopyranosyluronate azide uronate (34)



Yield: 164 mg, 10%, colourless syrup

R_f 0.60 (eluent A_5)

^{1}H NMR ($CDCl_3$, 250.13 MHz): δ 7.37–7.27 (m, 5H, C_6H_5), 6.00–5.69 (m, 2H, 2 x $OCH_2CH{=}CH_2$), 5.40–5.10 (m, 4H, 2 x $OCH_2CH{=}CH_2$), 4.91 (dd, 1H, $^3J_{3,4}$ 3.1 Hz, H-3), 4.84, (d, 1H, ^2J 11.3 Hz, $OCH_2C_6H_5$), 4.71–4.66 (m, 3H, $^3J_{1,2}$ 8.6 Hz, H-1, H-4, H-5), 4.63 (d,

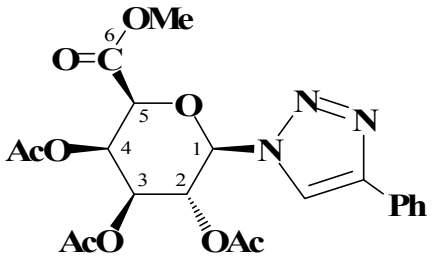
1H, OC*H*$_2$C$_6$H$_5$), 4.25–4.22 (m, 2H, OC*H*$_2$CH=CH$_2$), 4.11–3.95 (m, 2H, COOC*H*$_2$CH=CH$_2$), 3.73 (dd, 1H, $^3J_{2,3}$ 10.1 Hz, H-2), 2.02 (s, 3H, CH$_3$CO)

^{13}C NMR (CDCl$_3$, 62.9 MHz): δ 170.1, 166.5 (CH$_3$*C*O, C-6), 134.0 (OCH$_2$C*H*=CH$_2$), 131.1 (COOCH$_2$*C*H=CH$_2$), 137.7, 127.9, 127.8, 127.8 (OCH$_2$*C*$_6$H$_5$, two signals are isochronic), 119.6 (COOCH$_2$CH=*C*H$_2$) 117.6 (OCH$_2$CH=*C*H$_2$), 90.3 (C-1), 75.9 (C-2), 75.2, 75.2, 74.5, (C-3, C-4, C-5), 75.2 (O*C*H$_2$C$_6$H$_5$), 74.2 (O*C*H$_2$CH=CH$_2$), 66.4 (COO*C*H$_2$CH=CH$_2$), 20.8 (*C*H$_3$CO)

$C_{21}H_{25}N_3O_7$ (431.44)	calcd:	C 58.46	H 5.84	N 9.74
	found:	C 58.61	H 5.91	N 9.85

4.3. DERIVATISATION OF AZIDO GROUP

4.3.1. 1-(Methyl 2,3,4-tri-*O*-acetyl-β-D-galactopyranosyluronate)-4-phenyl-1,2,3-triazole (35)



Copper (II) sulfate x·5 H$_2$O (5 mg, 20 μmol), L-(+)-ascorbic acid (38 mg, 0.22 mmol) and phenylacetylene (115 μL, 1.05 mmol) were added to a solution of azide **5** (370 mg, 1.03 mmol) in water (6 mL) and the mixture was heated at 75 °C for 21 h (TLC, eluent A_1). After cooling to 4 °C, the obtained precipitate was filtered off and washed with ice-water. The crude product was purified by flash chromatography on silica gel (eluent A_4) to provide triazoleglycoside **35**.

Yield: 335 mg, 70%, amorphous solid

Melting point: 150–151 °C

$[\alpha]_D^{23}$ –20.6 (*c* 1.1, chloroform)

R_f 0.54 (eluent A_1)

^{1}H NMR (CDCl$_3$; 250.13 MHz): δ 8.13 (s, 1H, NCH=C), 7.86–7.82, 7.45–7.29 (2m, 5H, C$_6$H$_5$), 5.96 (d, 1H, $^3J_{1,2}$ 9.2 Hz, H-1), 5.87 (dd, 1H, $^3J_{4,5}$ 1.2 Hz, H-4), 5.66 (dd, 1H, $^3J_{2,3}$ 10.4 Hz, H-2), 5.34 (dd, 1H, $^3J_{3,4}$ 3.4 Hz, H-3), 4.67 (d, 1H, H-5), 3.74 (s, 3H, OCH$_3$), 2.18, 2.01, 1.88 (3s, 9H, 3 x CH$_3$CO)

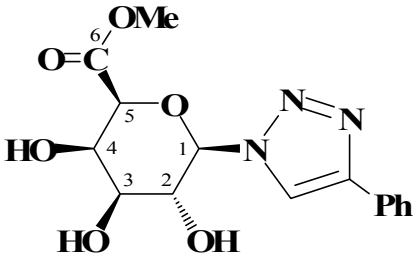
^{13}C NMR (CDCl$_3$, 75.5 MHz): δ 169.7, 169.5, 169.1, 165.4 (3 x CH$_3$*C*O, C-6), 148.5 (–HC=*C*), 129.9, 128.8, 128.5, 125.9 (C$_6$H$_5$, two signals are isochronic), 118.1 (–H*C*=C), 86.0

(C-1), 74.8 (C-5), 70.5 (C-3), 68.0 (C-4), 67.4 (C-2), 52.9 (OCH_3), 20.5, 20.4, 20.2 (3 x CH_3CO)

MS (EI), *m/z* 461.2 $[M]^+$ (6.62 %).

$C_{21}H_{23}N_3O_9$ (461.42)	calcd:	C 54.66	H 5.02	N 9.11
	found:	C 54.81	H 5.06	N 9.04

4.3.2. 1-(Methyl β-D-galactopyranosyluronate)-4-phenyl-1,2,3-triazole (36)



Copper (II) sulfate x·$5H_2O$ (12 mg, 48 μmol), L-(+)-ascorbic acid (124 mg, 0.70 mmol) and phenylacetylene (378 μL, 3.44 mmol) were added to a solution of azide **6** (789 mg, 3.38 mmol) in water (19.5 mL) and the mixture was heated at 75 °C for 26 h. After cooling to 4 °C, the obtained precipitate was filtered off, washed with ice-water, and dried at 80 °C to gave analytical pure triazoleglycoside **36**.

Yield: 361 mg, 32%, amorphous solid

Melting point: >250 °C

$[\alpha]_D^{21}$ –67.7 (*c* 1.1, abs. pyridine)

^{1}H NMR (DMSO–d_6; 250.13 MHz): δ 8.82 (s, 1H, CH=C), 7.94–7.91, 7.49–7.31 (2m, 5H, C_6H_5), 5.64 (d, 1H, $^3J_{1,2}$ 9.2 Hz, H-1), 5.42 (d, 1H, $^3J_{HOCH}$ 5.8 Hz, OH), 5.28 (d, 1H, $^3J_{HOCH}$ 5.2 Hz, OH), 5.19 (d, 1H, $^3J_{HOCH}$ 5.5 Hz, OH), 4.70 (d, 1H, H-5), 4.19–4.07 (m, 2H, $^3J_{4,5}$ 1.8 Hz, H-2, H-4), 3.74-3.65 (m, H-3), 3.64 (s, 3H, OCH_3)

^{13}C NMR (DMSO-d_6; 75.5 MHz): δ 168.2 (C-6), 146.4 (–HC=*C*), 130.5, 128.9, 128.0, 125.2 (C_6H_5, two signals are isochronic), 120.4 (–H*C*=C), 87.5 (C-1), 76.3 (C-5), 72.9 (C-3), 70.0 (C-4), 68.5 (C-2), 51.7 (OCH_3)

MS (CI, isobutane): *m/z* 336 $[M+H]^+$

$C_{15}H_{17}N_3O_6$ (335.31)	calcd:	C 53.73	H 5.11	N 12.53
	found:	C 53.84	H 5.18	N 12.50

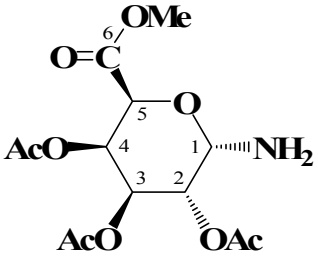
4.3.3. Reduction of azide 5

Via Staudinger procedure. – Triphenylphosphane (1.96 g, 7.49 mmol) was added to a stirred solution of azide **5** (2.69 g, 7.49 mmol) in tetrahydrofuran (100 mL) and water (1.2 mL).

After stirring for 2 h at room temperature the reaction mixture was heated at 65 °C for 10 h (TLC, eluent A_4 and eluent A_2). Brine (9 mL) was then added to the reaction mixture at room temperature, and the organic phase was separated. After extraction of the aqueous layer with diethyl ether (2 x 90 mL), the combined organic solutions were dried, and concentrated. The residue was applied to column chromatography (eluent ethyl acetate gradient 5→66% in petrol ether) to provide the amines **37** (R_f 0.38, eluent A_2) and **38** (R_f 0.12, eluent A_2) in a total yield of 55% (1.37 g). The α:β ratio of several attempts vary between 1:6 to 1:3.

Via H_2/Pd/C reduction: – 10% Palladium on charcoal (80 mg) was added to a solution of compound **5** (390 mg, 1.09 mmol) in ethyl acetate–methanol (36 mL, 1:1) and the reaction mixture was stirred for 3 h (TLC, eluent A_2) under a hydrogen atmosphere (1 bar) at ambient temperature and then filtered through Celite. The filtrate was concentrated to dryness to provide compound **38** (358 mg, 99%) as a foam which was pure enough for further reactions. An analytical sample was obtained by MPLC purification (eluent ethyl acetate gradient 30→60% in petrol ether).

4.3.4. Methyl 2,3,4-tri-*O*-acetyl-α-D-galactopyranosyluronate amine (37)



Yield: Colourless syrup

[α] $^{21}_{D}$ +88.6 (*c* 1.2, chloroform)

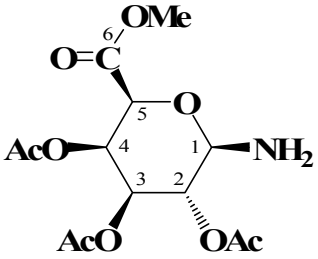
R_f 0.38 (eluent A_2)

^{1}H NMR ($CDCl_3$, 250.13 MHz): δ 5.74 (dd, 1H, $^3J_{4,5}$ 1.5 Hz, H-4), 5.58 (d, 1H, $^3J_{1,2}$ 3.7 Hz, H-1), 5.47 (dd, 1H, $^3J_{3,4}$ 3.4 Hz, H-3), 5.17 (dd, 1H, $^3J_{3,2}$ 10.7 Hz, H-2), 4.81 (d, 1H, H-5), 3.68 (s, 3H, OCH_3), 2.07, 2.01, 1.96 (3s, 9H, C*H*$_3$CO)

^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 170.3, 170.0, 168.1, 166.7 (3 x CH_3CO, C-6), 90.8 (C-1), 69.1 (C-4), 68.2 (C-5), 67.7 (C-2), 66.9 (C-3), 52.8 (OCH_3), 20.8, 20.8, 20.6 (3 x *C*H_3CO)

$C_{13}H_{19}NO_9$ (333.29)	calcd:	C 46.85	H 5.75	N 4.20
	found:	C 47.00	H 5.82	N 4.11

4.3.5. Methyl 2,3,4-tri-*O*-acetyl-β-D-galactopyranosyluronate amine (38)



Yield: 358 mg, 99% via Pd/C, colourless crystals

Melting point: 107 °C (dec., ethyl acetate–petrol ether)

IR (Nujol); ν 3434 and 3367 cm^{-1} (NH_2)

$[\alpha]_D^{21}$ +53.8 (*c* 1.0, chloroform)

R_f 0.12 (eluent A_3)

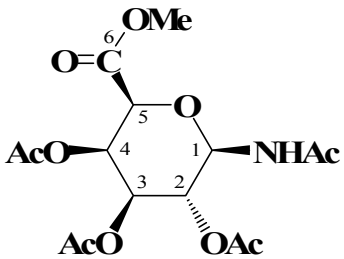
^{1}H NMR ($CDCl_3$, 250.13 MHz): δ 5.71 (dd, 1H, $^3J_{4,5}$ 1.5 Hz, H-4), 5.10–5.07 (m, 2H, $^3J_{3,4}$ 3.1 Hz, H-2, H-3), 4.31 (d, 1H, H-5), 4.17 (d, 1H, $^3J_{1,2}$ 7.6 Hz, H-1), 3.70 (s, 3H, OCH_3), 2.06, 2.03, 1.95 (3s, 9H, 3 x CH_3CO)

^{1}H NMR (DMSO-d6, 250.13 MHz): δ 5.50 (dd, 1H, $^3J_{4,5}$ 1.2 Hz, H-4), 5.20 (dd, 1H, $^3J_{3,4}$ 3.7 Hz, H-3), 4.80 (dd, 1H, $^3J_{2,3}$ 10.1 Hz, H-2), 4.76 (d, 1H, H-5), 4.26 (br, 1H, $^3J_{1,2}$ 9.2 Hz, H-1), 3.62 (s, 3H, OCH_3), 2.63 (br s, 2H, NH_2), 2.03, 1.99, 1.90 (3s, 9H, 3 x CH_3CO)

^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 170.3, 170.0, 169.8, 167,1 (3 x CH_3CO, C-6), 85.5 (C-1), 73.1 (C-5), 70.8, 69.1 (C-2, C-3), 68.7 (C-4), 52.8 (OCH_3), 20.8, 20.6, 20.6 (3 x CH_3CO)

$C_{13}H_{19}NO_9$ (333.29)	calcd:	C 46.85	H 5.75	N 4.20
	found:	C 46.92	H 5.81	N 4.08

4.3.6. *N*-(Methyl 2,3,4-tri-*O*-acetyl-β-D-galactopyranosyluronate) acetamide (39)



Acetic anhydride (0.5 mL) was added to a solution of amine **38** (70 mg, 0.21 mmol) in abs. pyridine (1 mL) at –15 ˚C. After stirring for 17 h at ambient temperature under an argon atmosphere (TLC, eluent A_2), methanol (2 mL) was added, and stirring of the reaction mixture was continued for additional 1 h. Ice water (5 mL) was added and the aqueous phase was extracted with chloroform (4 x 1.5 mL). The combined organic layers were dried and

concentrated. The residue was purified by flash chromatography (eluent ethyl acetate gradient 20→60% in petrol ether) to furnish the N-acetyl derivative **39**.

Yield: 48 mg, 60%, colourless foam

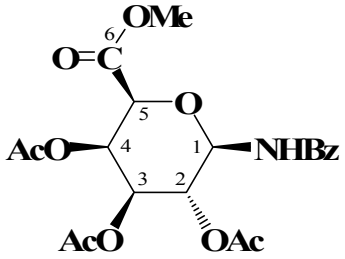
R_f 0.43 (eluent A_2)

^{1}H NMR ($CDCl_3$, 250.13 MHz): δ 7.19 (d, 1H, ^3J 9.5, NH), 5.76 (dd, 1H, $^3J_{4,5}$ 1.5 Hz, H-4), 5.31 (t, 1H, $^3J_{1,2}$ 9.5 Hz H-1), 5.20–5.09 (m, 2H, $^3J_{3,4}$ 3.4 Hz, H-2, H-3), 4.50 (d, 1H, H-5), 3.71 (s, 3H, OCH_3), 2.13 (s, 3H, CH_3CONH), 2.00, 1.96, 1.92 (3s, 9H, 3 x CH_3CO)

^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 171.3, 170.1, 169.8, 169.5, 166.7 (4 x CH_3CO, C-6), 78.0 (C-1), 73.7 (C-5), 70.9 (C-3), 68.4 (C-4), 67.5 (C-2), 52.8 (OCH_3), 23.0 (CH_3CONH), 20.6, 20.5, 20.4 (3 x CH_3CO)

$C_{15}H_{21}NO_{10}$ (375.33)	calcd:	C 48.00	H 5.64	N 3.73
	found:	C 48.05	H 5.69	N 3.80

4.3.7. *N*-(Methyl 2,3,4-tri-*O*-acetyl-β-D-galactopyranosyluronate) benzamide (40)



Benzoyl chloride (0.53 mL) was added to a solution of amine **38** (252 mg, 0.756 mmol) in abs. pyridine (0.73 mL) at room temperature under an argon atmosphere. After 10 h (TLC, eluent A_2) methanol (0.2 mL) was added and the stirring was continued for 1 h. The reaction mixture was then concentrated and traces of pyridine were removed by evaporation with repeated addition of toluene. The residue was dissolved in a mixture of chloroform–petrol ether (1:2, 4.5 mL) and the organic layer was washed with ice water (2 x 2 mL), aq. $NaHCO_3$ (2 x 2 mL) and ice water (2 x 2 mL), dried and concentrated. The crude product was purified by flash chromatography (eluent ethyl acetate gradient 20→60% in petrol ether) to provide N-benzoyl derivative **40**.

Yield: 200 mg, 60%, colourless crystals

Melting point: 204–206 °C

$[\alpha]_D^{23}$ +48.1 (*c* 1.36, chloroform)

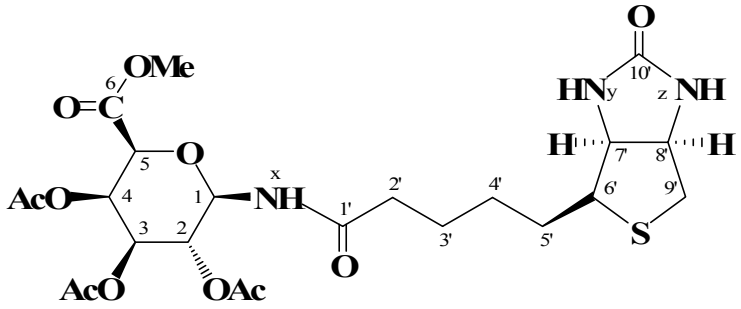
R_f 0.47 (eluent A_2)

^{1}H NMR (CDCl$_3$, 250.13 MHz): δ 7.78–7.74, 7.57–7.41 (2m, 5H, C_6H_5), 7.15 (d, 1H, ^3J 9.2 Hz, NH), 5.78 (dd, 1H, $^3J_{4,5}$ 1.5 Hz, H-4), 5.47 (t, 1H, $^3J_{1,2}$ 8.9 Hz, H-1), 5.32–5.26 (m, 2H, $^3J_{3,4}$ 3.1 Hz, H-2, H-3), 4.53 (d, 1H, H-5), 3.17 (s, 3H, OCH_3), 2.12, 2.04, 2.03 (3s, 9H, 3 x CH_3CO)

^{13}C NMR (CDCl$_3$, 75.5 MHz): δ 171.6, 169.6, 169.6, 167.1, 166.5 (3 x CH_3CO, C_6H_5CO, C-6), 132.6, 132.4, 128.7, 127.2 (C_6H_5, 2 signals are isochronic), 78.9 (C-1), 73.9 (C-5), 70.4 (C-3), 68.6 (C-4), 67.9 (C-2), 52.8 (OCH_3), 20.8, 20.6, 20.5 (3 x CH_3CO)

$C_{20}H_{23}NO_{10}$ (437.13)	calcd:	C 54.92	H 5.30	N 3.20
	found:	C 55.01	H 5.35	N 3.24

4.3.8. *N*-(Methyl 2,3,4-tri-*O*-acetyl-β-D-galactopyranosyluronate) biotinylamide (41)



A solution of amine **38** (46 mg, 0.138 mmol) in abs. *N,N*-dimethylformamide (1.2 mL) was added to a solution of D-(+)-biotin (39 mg, 0.16 mmol, purchased from Merck) and diisopropylethylamine (DIPEA) (40 μL) in abs. *N,N*-dimethylformamide (2.5 mL) at ambient temperature under an argon atmosphere. Then the mixture was cooled to 0 °C and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC) (31 mg, 0.16 mmol) and *N*-hydroxybenzotiazole (1-HOBT) (43 mg, 0.32 mmol) were added. The reaction mixture was stirred at 0 °C for 2.5 h followed by stirring at rt for 51 h. The eluent was then removed *in vacuo* and the residue was purified by flash chromatography (eluent methanol gradient 0→33% in ethyl acetate) and then by reversed-phase HPLC on a C_{18} silica gel (eluent 25% acetonitrile in water) to yield biotinylamid **41**.

Yield: 30 mg, 36%, amorphous solid

$[\alpha]_D^{21}$ +73.2 (*c* 1.1, dimethyl sulfoxide)

^{1}H NMR (DMSO-d$_6$, 500 MHz): δ 8.77 (d, 1H, $^3J_{NH,H\text{-}1}$ 9.8 Hz, NH_x), 6.39 (br, 1H, NH_y), 6.33 (br, 1H, NH_z), 5.51 (dd, 1H, $^3J_{4,5}$ 1.5 Hz, H-4), 5.37 (t, 1H, $^3J_{1,2}$ 9.4 Hz, H-1), 5.32 (dd, 1H, $^3J_{3,4}$ 3.6 Hz, H-3), 5.00 (dd, $^3J_{2,3}$ 10.2 Hz, 1H, H-2), 4.95 (d, 1H, H-5), 4.29 (m, 1H, $^3J_{8',9'a}$ 5.1 Hz, H-8'), 4.12 (ddd, 1H, $^3J_{7',NHy}$ 1.7 Hz, $^3J_{7',8'}$ 7.6 Hz, H-7') 3.62 (s, 3H, OCH_3),

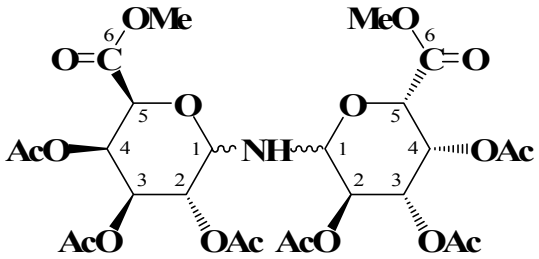
3.08 (ddd, 1H, $^{3}J_{6',7'}$ 4.6 Hz, H-6'), 2.80 (dd, 1H, $^{3}J_{9'a,9'b}$ 12.5 Hz, H-9'a), 2.57 (d, 1H, H-8'b), 2.13-2.06 (m, 1H, H-2'a, H H-2'b), 2.03, 1.95, 1.90 (3s, 9H, CH_3CO), 1.63–1.56 (m, 1H, $^{3}J_{5'a,6'}$ 6.0 Hz, H-5'a), 1.53–1.40 (m, 3H, $^{3}J_{5'b,6'}$ 8.5 Hz, H-3'a, H-3'b, H-5'b), 1.33–1.21 (m, 2H, H-4'a, H-4'b)

^{13}C NMR (DMSO-d$_6$, 125.7 MHz): δ 172.8 (C-1'), 169.5, 169.4, 169.1 (3 x CH_3CO), 166.9 (C-6), 162.7 (C-10'), 76.7 (C-1), 72.8 (C-5), 70.6 (C-3), 68.6 (C-4), 67.9 (C-2), 61.03 (C-7'), 59.2 (C-8'), 55.3 (C-6'), 52.2 (OCH_3), 39.8 (C-9'), 35.0 (C-2'), 28.0 (C-4', C-5'), 25.0 (C-3'), 20.35, 20.34, 20.28 (3 x CH_3CO)

MS (EI), *m/z* 559 $[M]^+$

HRMS (EI), calcd for $C_{23}H_{33}O_{11}N_3S$ (M^+) 559.18303. Found 559.183116

4.3.9. Bis (methyl 2,3,4-tri-*O*-acetyl-D-galactopyranosyluronate) amine (43)



2-cyano-3,3-bis(methylthio)acrylonitrile (1.37 mmol) was added to a solution of amine **38** (455 mg, 1.37 mmol) in abs. ethanol (6 mL) at room temperature under an argon atmosphere and the reaction mixture was heated at 70 ˚C. The After 24 h the eluent was evaporated under reduced pressure and the residue purified by flash chromatography (eluent ethyl acetate gradient 20→50% in petrol ether) to yield to a mixture of bis-glycosyl amines **43**.

Yield: 187 mg, 42%, colourless powder

R_f 0.44 (eluent A_2)

^{1}H NMR (CDCl$_3$, 250.13 MHz): δ 5.69–5.62 (m, H-4 from *αβ*, *ββ*), 5.29–5.23 (overlapped m, H-1, H-3 from *α*β), 5.11–4.94 (m, H-2 from *αβ* and *ββ*, H-3 from α*β*, *ββ*), 4.26–4.16 (m, H-5 from *αβ* and *ββ*, H-1 from α*β* and *ββ*), 3.65, 3.65, 3.62 (3 x OCH_3), 3.25–3.18 (m, NH), 3.13 (dd, $^{2}J_{NH}$ 8.9 Hz, 4.6 Hz, NH), 2.11, 2.05, 2.05, 2.03, 2.01, 1.98, 1.96, 1.94, 1.93 1.93 (CH_3CO)

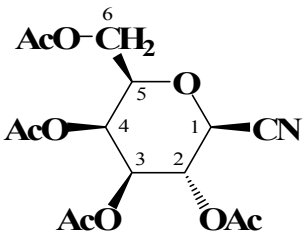
^{13}C NMR (CDCl$_3$, 75.5 MHz): δ 171.4, 170.7, 169.9, 169.8, 169.8, 169.8, 169.8, 169.8, 169.7, 167.9, 166.7, 166.5 (12 x CO), 87.9 (C-1β), 86.7 (C-1β), 82.6 (C-1α), 73.0, 72.9, 72.9 (C-5), 70.7–67.4 (C-2, C-3, C-4 from *αβ*, *ββ* moieties), 52.5, 52.4, 52.34 (3 x OCH_3), 20.9–20.5 (12 x CH_3CO)

MS (ESI), *m/z* 649.96 $[M+H]^+$, *m/z* 672.26 $[M+Na]^+$, *m/z* 333.96 $[M\text{-}GalA]^+$, *m/z* 316.92 $[M\text{-}GalANH]^+$

$C_{26}H_{35}NO_{18}$ (649.55)	calcd:	C 48.08	H 5.43	N 2.16
	found:	C 47.96	H 5.48	N 2.03

4.4. SYNTHESIS OF β-D-GALACTOPYRANOSYRURONATE CYANIDE (56)

4.4.1. 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl cyanide (51)



Melting point: 169–170 °C (from chloroform–petrol ether)[90.] mp 167–168 °C (from chloroform–diethyl ether)

$[\alpha]_D^{22}$ +70 (*c* 2.9, water)

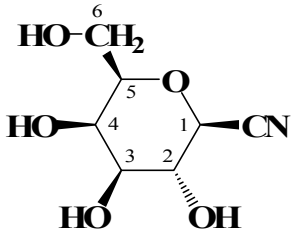
R_f 0.40 (eluent A_4)

^{1}H NMR ($CDCl_3$, 250.13 MHz): δ 5.52 (dd, 1H, $^3J_{2,3}$ 10.1 Hz, H-2), 5.42 (dd, 1H, $^3J_{4,5}$ 1.2 Hz, H-4), 4.99 (dd, 1H, $^3J_{3,4}$ 3.4 Hz, H-3), 4.27 (d, 1H, $^3J_{1,2}$ 10.4 Hz, H-1), 4.13–4.08 (m, 2H, H-6a, H-6b), 3.92 (ddd, 1H, $^3J_{5,6a}$ 5.8 Hz, $^3J_{5,6b}$ 6.1 Hz, H-5), 2.17, 2.11, 2.05, 1.99 (4s, 12H, 4 x C*H*$_3$CO)

^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 170.3, 169.9, 169.8, 168.7 (4 x CH_3*C*O), 114.3 (CN), 75.4 (C-5), 70.8 (C-3), 66.8 (C-1), 66.7 (C-4), 66.0 (C-2), 61.2 (C-6), 20.6, 20.5, 20.4, 20.4 (4 x *C*H$_3$CO)

$C_{15}H_{19}NO_9$ (357.11)	calcd:	C 50.42	H 5.36	N 3.92
	found:	C 50.48	H 5.39	N 4.01

4.4.2. β-D-Galactopyranosyl cyanide (52)



A methanolic sodium methoxide solution (0.6 M, 128 µL) was added to a suspension of peracetylated galactopyranosyl cyanide **51** (1.70 g, 4.76 mmol) in dry methanol (14 mL).

After stirring for 30 min at ambient temperature under an argon atmosphere (TLC, eluent C_2), the reaction mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered, and concentrated. The residue was purified by column chromatography (eluent C_4) to yield compound **53**.

Yield: 820 mg, 91%, colourless crystals

Melting point: 115–116 °C (2-propanol); lit.[112] mp 115–116 °C

$[\alpha]_D^{22}$ +70 (*c* 2.9, water); lit.[112] $[\alpha]_D^{20}$ +68.2 (*c* 0.88, water)

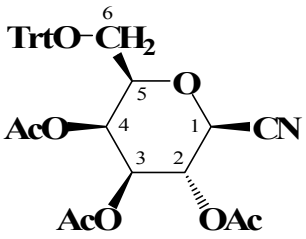
R_f 0.38 (eluent C_2)

^{1}H NMR (CD_3OD, 250.13 MHz): δ 4.09 (d, 1H, $^3J_{1,2}$ 10.1 Hz, H-1), 3.86 (dd, 1H, $^3J_{4,5}$ 1.2 Hz, H-4), 3.82 (dd, 1H, $^3J_{2,3}$ 9.3 Hz, H-2), 3.75 (1H, dd, $^3J_{5,6a}$ 7.0 Hz, H-6a), 3.66 (dd, 1H, $^2J_{6a,6b}$ 11.6 Hz, H-6b), 3.55 (ddd, 1H, $^2J_{5,6b}$ 4.9 Hz, H-5), 3.42 (dd, 1H, $^3J_{3,4}$ 3.2 Hz, H-3)

^{13}C NMR (CD_3OD, 62.9 MHz): δ 118.43 (CN), 81.53 (C-5), 75.34 (C-3), 70.55 (C-1), 70.26 (C-4), 70.03 (C-2), 62.51 (C-6)

$C_7H_{11}NO_5$ (189.17)	calcd:	C 44.45	H 5.86	N 7.40
	found:	C 44.40	H 5.81	N 7.34

4.4.3. 2,3,4-Tri-*O*-acetyl-6-*O*-trityl-β-D-galactopyranosyl cyanide (54)



N,*N*'-Dimethyl-4-aminopyridine (108 mg, 0.88 mmol) and triphenylmethyl chloride (2.47 g, 8.86 mmol) was added to a solution of compound **52** (931 mg, 4.92 mmol) in dry pyridine (8.4 mL). After stirring for 15 h at ambient temperature under an argon atmosphere (TLC, eluent C_3), a mixture of acetic anhydride (7.9 mL) and pyridine (23.7 mL) was added at 0 °C. The solution was then stirred for further 20 h at room temperature under an argon atmosphere (TLC, eluent B_1). After cooling to 0 °C, ethanol (16.5 mL) was added dropwise to decompose the excess of acetic anhydride. After 1 h at that temperature, the solution was poured into ice-water (150 mL). The aqueous layer was extracted with chloroform (3 x 50 mL), and the combined organic layers were diluted with heptane (300 mL). The organic layer was then washed successively with ice-water (100 mL), cold aq. 15% $NaHSO_4$ (3 x 100 mL) and water (2 x 100 mL), dried and evaporated. The crude material was purified by MPLC (eluent ethylacetate gradient 0→33% in petrol ether) to yield trityl derivative **54**.

Yield: 2.57 g, 94%, colourless syrup

$[\alpha]_D^{22}$ –14.2 (*c* 1.8, chloroform)

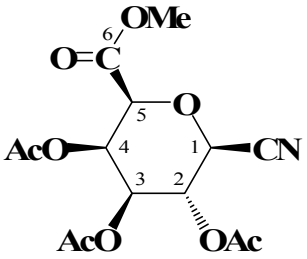
R_f 0.40 (eluent B_1)

^{1}H NMR ($CDCl_3$, 250.13 MHz): δ 7.42–7.24 (m, 15H, 3 x C_6H_5), 5.65 (dd, 1H, $^3J_{4,5}$ 1.2 Hz, H-4), 5.50 (dd, 1H, $^3J_{2,3}$ 10.4 Hz, H-2), 5.06 (dd, 1H, $^3J_{3,4}$ 3.2 Hz, H-3), 4.22 (d, 1H, $^3J_{1,2}$ 10.1 Hz, H-1), 3.79 (ddd, 1H, $^3J_{5,6a}$ 5.5 Hz, $^3J_{5,6b}$ 8.1 Hz, H-5), 3.44 (dd, 1H, $^2J_{6a,6b}$ 9.1 Hz, H-6a), 3.11 (dd, 1H, H6-b), 2.12, 2.03, 1.95 (3s, 9H, 3 x CH_3CO)

^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 169.7, 169.5, 168.7 (3 x CH_3CO), 143.0, 128.4, 127.8, 127.2 [$(C_6H_5)_3C$], 114.4 (CN), 87.0 [$(C_6H_5)_3C$], 76.7 (C-5), 71.0 (C-3), 66.8 (C-4), 66.7 (C-1), 66.2 (C-2), 60.4 (C-6), 20.4, 20.3, 20.3 (3 x CH_3CO)

$C_{32}H_{31}NO_8$ (557.59)	calcd:	C 68.93	H 5.60	N 2.51
	found:	C 68.92	H 5.55	N 2.48

4.4.4. Methyl 2,3,4-tri-*O*-acetyl-β-D-galactopyranosyluronate cyanide (56)



A solution of chromium (VI) oxide (1.02 g, 10.2 mmol) in aq. 3.5 M H_2SO_4 (4.1 mL) was added dropwise during 15 min to a solution of compound **54** (1.14 g, 2.04 mmol) in acetone (6.5 mL) and dichloromethane at 0 °C. After stirring for 30 min at that temperature, the chilling was terminated and the mixture was stirred for additional 6.5 h at ambient temperature. Ethanol (3.5 mL) was then added at 0 °C, and, after 30 min, the solid separated was filtered off, thoroughly washed with acetone, and the combined organic phases were concentrated to 50 mL. $NaHCO_3$ (1.64 g, 19.5 mmol) was added to the concentrated solution in small portions, and the suspension evaporated. For esterification of the carboxylic group, the residue was portioned between dichloromethane (12 mL) and water (8 mL), Bu_4NBr (653 mg) and MeI (1.06 mL) were added, and the suspension was stirred vigorously for 20 h at ambient temperature. Water (12 mL) was then added, the phases were separated, and the aqueous phase was extracted with chloroform (3 x 10 mL), dried and evaporated. The ammonium salts were precipitated from dichloromethane–diethyl ether. The mother liquid was evaporated and processed by column chromatography (eluent ethyl acetate gradient 1→13% in toluene) to yield uronate **56**.

Yield: 415 mg, 59%, colourless crystals

Melting point: 122–123 °C (from chloroform–petrol ether)

$[\alpha]_D^{22}$ +60.7 (*c* 1.14, chloroform); lit.[91] $[\alpha]_D^{25}$ +61 (*c* 1,0 chloroform)

R_f 0.16 (eluent B_1)

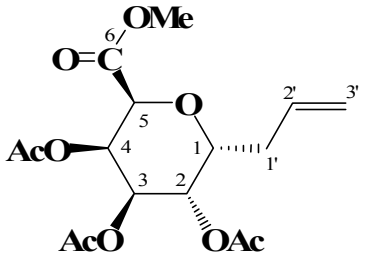
^{1}H NMR ($CDCl_3$, 250.13 MHz): δ 5.75 (dd, 1H, $^3J_{4,5}$ 1.2 Hz, H-4), 5.57 (t, 1H, $^3J_{2,3}$ 10.1 Hz, H-2), 5.06 (dd, 1H, $^3J_{3,4}$ 3.4 Hz, H-3), 4.31 (d, 1H, $^3J_{1,2}$ 10.1 Hz, H-1), 4.28 (d, 1H, H-5), 3.77 (s, 3H, OCH_3), 2.14, 2.12, 2.01 (3s, 9H, 3 x CH_3CO)

^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 169.7, 169.5, 168.6 (3 x CH_3*C*O), 165.2 (C-6), 113.8 (CN), 76.2 (C-5), 70.5 (C-3), 67.8 (C-4), 66.7 (C-1), 65.8 (C-2), 53.0 (OCH_3), 20.5, 20.5, 20.4 (3 x *C*H_3CO)

$C_{14}H_{17}NO_9$ (343.29)	calcd:	C 48.98	H 4.99	N 4.08
	found:	C 49.05	H 5.15	N 4.28

4.5. SYNTHESIS OF O-ACYL PROTECTED C-ALLYL α-D-GALACTOPYRAN-URONATES

4.5.1. Methyl 2,3,4-tri-*O*-acetyl-1-deoxy-1-(prop-2-enyl)-α-D-galactopyranuronate (57)[98]



Hexabutyldistannane (10.7 mL, 21.4 mmol) was added to a solution of methyl 2,3,4-tri-*O*-acetyl-α-D-galactopyranosyluronate bromide **4** (5.6 g, 14.2 mmol) and allylphenylsulphone (6.3 mL, 42.6 mmol) in dry benzene (78 mL). The reaction mixture was degassed by sonification for 30 min at ambient temperature under an argon atmosphere and irradiated then with a mercury lamp (150W) in an ultra violet reactor system (Heraeus) at rt followed by TLC (eluent A_3). The reaction mixture was then washed with water (2 x 40 mL), cold sat aq $NaHCO_3$ (2 x 40 mL), water (3 x 40 mL), dried and evaporated. The residue was purified by MPLC (eluent ethyl acetate gradient 0→33% in petrol ether) to provide **57**.

Yield: 3.0 g, 59%, colourless syrup

$[\alpha]_D^{23}$ +102 (*c* 1.0, chloroform)

R_f 0.40 (eluent A_4)

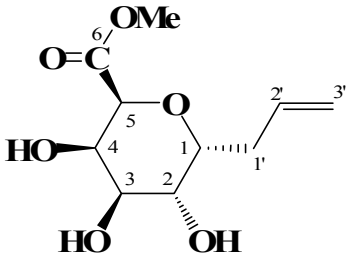
^{1}H NMR (CDCl$_3$, 500 MHz): δ 5.80 (1H, $^3J_{2',3'trans}$ 17.1 Hz, $^3J_{2',3'cis}$ 10.1 Hz, $^3J_{1'a,2'}$ = $^3J_{1'b,2'}$ 6.7 Hz, H-2'), 5.57 (dd, 1H, $^3J_{4,5}$ 4.1 Hz, H-4), 5.23–5.19 (m, 2H, $^3J_{3,4}$ 2.5 Hz, H-2, H-3), 5.15–5.06 (m, 2H, H-3'a, H-3'b), 4.56 (d, 1H, H-5), 4.53 (ddd, 1H, ^3J 3.8 Hz, ^3J 5.4 Hz, ^3J 9.2 Hz, H-1), 3.71 (s, 3H, OCH$_3$), 2.50–2.20 (m, 2H, H-1'a, H-1'b), 2.00, 2.06 (2s, 9H, 3 x CH$_3$CO)

^{13}C NMR (CDCl$_3$, 125.8 MHz): δ 169.7, 169.7, 169.5 (3 x CO), 168.5 (C-6), 133.3 (C-2'), 117.7 (C-3'), 71.8 (C-1), 70.2 (C-5), 68.7, 68.2 (C-2, C-3), 67.8 (C-4), 52.1 (OCH$_3$), 32.1 (C-1'), 20.6, 20.5, 20.5 (3 x *C*H$_3$CO)

MS-70ev: *m/z* 359 [M]$^+$, *m/z* 317 [M-allyl]$^+$, *m/z* 299 [M-OAc]$^+$

$C_{16}H_{22}O_9$ (358.34)	calcd:	C 53.63	H 6.19
	found:	C 53.81	H 6.27

4.5.2. Methyl 1-deoxy-1-(prop-2-enyl)-α-D-galactopyranuronate 58[98]



Methanolic 1% HCl (1340 mL) was added to a compound **57** (11.3 g, 31.54 mmol) with stirring and the mixture kept for 24 h under argon atmosphere at room temperature. The reaction mixture was neutralized with $PbCO_3 \cdot Pb(OH)_2$ (94 g). After stirring for 2 h, the lead salts were filtered off [it is advantage to use Glass Microfiber filter (GF/A, Whatman, Cat. No. 1820042)], washed with methanol, and the filtrate and washings were combined and concentrated. The residue was applied to a column of silica gel (eluent C_6) to provide **58**.

Yield: 6.81 g, 93%, an amorphous solid

$[\alpha]_D^{21}$ +62 (*c* 1.0, methanol)

R_f 0.23 (eluent C_6)

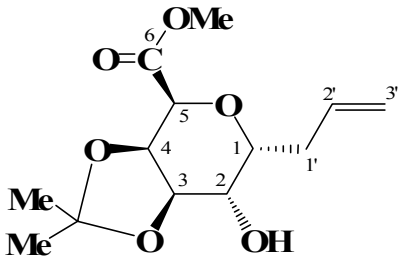
^{1}H NMR (CD$_3$OD; 250.13 MHz): δ5.85 (dddd, 1H, $^3J_{2',3'trans}$ 17.2 Hz, $^3J_{2',3'cis}$ 10.1 Hz, $^3J_{1'a,2'}$ = $^3J_{1'b,2'}$ 6.9 Hz, H-2'), 5.11 (dq, 1H, $^4J_{1'a,3'trans}$ = $^4J_{1'b,3'trans}$ = 1.6 Hz, H-3'trans), 5.02 (m, 1H, H-3'cis), 4.35 (d, 1H, H-5), 4.19–4.12 (m, 2H, $^3J_{1,2}$ 4.6 Hz, $^3J_{4,5}$ 3.5 Hz, H-1, H-4), 3.85 (dd, 1H, $^3J_{2,3}$ 8.0 Hz, H-2), 3.77–3.73 (m, 1H, $^3J_{3,4}$ 3.2 Hz, H-3 overlapped with OCH$_3$), 3.74 (s, 3H, OCH$_3$), 2.43–2.35 (m, 2H, H-1'a, H-1'b)

^{13}C NMR (DMSO-d_6, 125.8 MHz): δ 170.9 (C-6), 136.3 (C-2'), 116.7 (C-3'), 73.8 (C-1), 71.8 (C-5), 70.3, 68.6 (C-2, C-3), 69.2 (C-4), 51.7 (OCH$_3$), 30.7 (C-1')

MS (FAB): *m/z* 232 $[M]^+$

$C_{10}H_{16}O_5$ (232.23)	calcd:	C 51.72	H 6.94
	found:	C 51.51	H 6.89

4.5.3. Methyl 1-deoxy-3,4-*O*-isopropylidene-1-(prop-2-enyl)-α-D-galactopyranuronate (59)[98]



p-Toluenesulfonic acid monohydrate (63 mg) was added to the suspension of compound **58** (365 mg, 1.57 mmol) in 2,2-dimethoxypropane (3.3 mL) and dry acetone (13 mL), and the reaction mixture was stirred for 5 h at ambient temperature under an argon atmosphere (TLC, eluent A_4). The mixture was then passed through a layer of alkaline aluminia (2 x 3 cm), the alkaline aluminia was washed with chloroform, and the filtrate and washings were combined. After removal of the solvent, the residue was purified by flash chromatography on silica gel (eluent ethyl acetate gradient 25→50% in petrol ether) to provide compound **59**.

Yield: 399 mg, 93%, colourless crystals

Melting point: 108–110°C (heptane-ethyl acetate)

$[\alpha]_D^{23}$ +12 (*c* 1.0, chloroform)

R_f 0.14 (eluent A_5)

IR (KBr), 3432 (OH), 1753.6 cm^{-1} (CO)

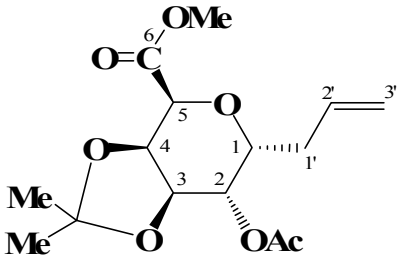
^{1}H NMR ($CDCl_3$, 250.13 MHz): δ 5.85 (dddd, 1H, $^3J_{2',3'trans}$ 17.1 Hz, $^3J_{2',3'\ cis}$ 10.3 Hz, $^3J_{1'a,2'}$ 7.5 Hz, $^3J_{1'b,2'}$ 6.8 Hz, H-2'), 5.17 (dq, 1H, $^4J_{1'a,3'trans}$ 1.5 Hz, $^4J_{1'b,3'trans}$ 1.9 Hz, H-3'trans), 5.10 (dqt, 1H, $^4J_{1'a,3'cis}$ 1.0 Hz, $^4J_{1'b,3'cis}$ 1.1 Hz, H-3'cis), 4.68 (d, 1H, H-5), 4.63 (dd, $^3J_{4,5}$ 2.3 Hz, H-4), 4.35 (dd, 1H, $^3J_{3,4}$ 7.3 Hz, H-3), 4.19 (ddd, 1H, $^3J_{1,2}$ 2.4 Hz, $^3J_{1,1'a}$ 6.7 Hz, $^3J_{1,1'b}$ 7.8 Hz, H-1), 3.86 (dd, 1H, $^3J_{2,3}$ 3.0 Hz, H-2), 3.79 (s, 3H, OCH_3), 2.50–2.42 (m, 2H, H-1'a, H-1'b), 2.08 (br, OH), 1.46, 1.32 (2s, 6H, $[CH_3]_2C$)

^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 170.0 (C-6), 133.9 (C-2'), 117.9 (C-3'), 110.1 ($[CH_3]_2C$), 73.9 (C-3), 73.1 (C-4), 70.7 (C-1), 70.3 (C-5), 67.9 (C-2), 52.31 (OCH_3), 35.31 (C-1'), 26.45, 24.42 ($[CH_3]_2C$)

MS-70ev: *m/z* 272 $[M]^+$

$C_{13}H_{20}O_6$ (272.29)	calcd:	C 57.34	H 7.40
	found:	C 57.60	H 7.55

4.5.4. Methyl 2-*O*-acetyl-1-deoxy-3,4-*O*-isopropylidene-1-(prop-2-enyl)-α-D-galactopyranuronate (60)



Acetic anhydride (2.4 mL) was added to a solution of compound **59** (379 mg, 1.39 mmol) in abs. pyridine (7.2 mL) at 4 °C and the reaction mixture was stirred at ambient temperature under an argon atmosphere. After 20 h (TLC, eluent A_5) ethanol (1.5 mL) was added at 0 °C and stirring was continued for 30 min. The reaction mixture was concentrated and traces of pyridine were removed by coevaporation with repeated addition of toluene. The residue was purified by flash chromatography (eluent ethyl acetate gradient 20→25% in petrol ether) to furnish compound **60**.

Yield: 412 mg, 94%, colourless syrup

$[\alpha]_D^{22}$ +22.7 (*c* 1.0, chloroform)

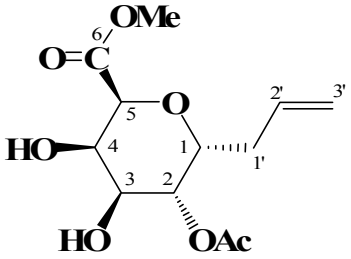
R_f 0.34 (eluent A_5)

^{1}H NMR ($CDCl_3$, 250.13 MHz): δ 5.72 (dddd, 1H, $^3J_{2',3'trans}$ 17.3 Hz, $^3J_{2',3'cis}$ 9.8 Hz, $^3J_{1'a,2'}$ 7.6 Hz, $^3J_{1'b,2'}$ 6.5 Hz, H-2'), 5.10–5.00 (m, 2H, H-3'a, H-3'b overlapped with H-2), 5.01 (dd, 1H, overlapped with H-3'a,b, $^3J_{2,3}$ 2.8, H-2), 4.60 (dd, $^3J_{4,5}$ 2.1 Hz, H-4), 4.54 (d, 1H, H-5), 4.31 (dd, 1H, $^3J_{3,4}$ 7.4 Hz, H-3 overlapped with H-1), 4.28 (ddd, 1H, $^3J_{1,2}$ 2.7 Hz, $^3J_{1,1'a}$ 6.8 Hz, $^3J_{1,1'b}$ 8.7 Hz, H-1), 3.79 (s, 3H, OCH_3), 2.48–2.36, 2.29–2.16 (2m, 2H, H-1'a, H-1'b), 2.09 (s, 3H, CH_3CO), 1.47, 1.29 (2s, 6H, $[CH_3]_2C$)

^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 169.5, 169.4 (2 x CO), 132.8 (C-2'), 118.0 (C-3'), 110.5 ($[CH_3]_2C$), 73.1 (C-4), 71.7 (C-3), 70.4 (C-5), 69.9 (C-1), 68.8 (C-2), 52.4 (OCH_3), 35.2 (C-1'), 26.3, 24.4 ($[CH_3]_2C$), 20.8 (CH_3CO)

$C_{15}H_{22}O_7$ (314.33)	calcd:	C 57.32	H 7.05
	found:	C 57.40	H 7.12

4.5.5. Methyl 2-*O*-acetyl-1-deoxy-1-(prop-2-enyl)-α-D-galactopyranuronate (61)



90% aq. trifluoroacetic acid (16 mL) was added to a solution of isopropylidene derivative **60** (364 mg, 1.16 mmol) in chloroform (7 mL) and methanol (7 mL). The reaction mixture was kept for 1 h at ambient temperature, diluted with toluene (30 mL), evaporated and coevaporated with repeated addition of toluene (3 x 30 mL). The residue was purified by flash chromatography (eluent ethyl acetate gradient 50→60% in petrol ether) to provide **61**.

Yield: 260 mg, 82%, colourless crystals

Melting point: 114–115°C (petrol ether-ethyl acetate)

$[\alpha]_D^{22}$ +67.9 (*c* 1.3, chloroform)

R_f 0.11 (eluent A_4)

^{1}H NMR ($CDCl_3$, 250.13 MHz): δ 5.79 (dddd, 1H, $^3J_{2',3'trans}$ 17.0 Hz, $^3J_{2',3'cis}$ 10.3 Hz, $^3J_{1'a,2'} = {}^3J_{1'b,2'}$ 6.8 Hz, H-2'), 5.15–5.04 (m, 2H, H-3'a, H-3'b), 4.94 (dd, 1H, $^3J_{2,3}$ 5.2 Hz, H-2), 4.42 (d, 1H, H-5), 4.35 (ddd, 1H, $^3J_{1,2}$ 2.6 Hz, $^3J_{1,1'a}$ 5.8 Hz, $^3J_{1,1'b}$ 8.4 Hz, H-1), 4.20 (dd, $^3J_{4,5}$ 5.6 Hz, H-4), 3.96 (dd, 1H, $^3J_{3,4}$ 2.4 Hz, H-3), 3.80 (s, 3H, OCH_3), 2.44–2.29, 2.28–2.14 (2m, 2H, H-1'a, H-1'b), 2.10 (s, 3H, CH_3CO)

^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 172.1, 170.6 (2 x CO), 133.5 (C-2'), 117.5 (C-3'), 72.4 (C-2), 71.4 (C-5), 69.3 (C-1), 68.8 (C-3), 67.6 (C-4), 52.5 (OCH_3), 33.7 (C-1'), 20.9 (CH_3CO)

$C_{12}H_{18}O_7$ (274.27) calcd: C 52.55 H 6.62
found: C 52.63 H 6.71

4.5.6. General procedure for the preparation of cyclic ethoxyethylidene orthoesters

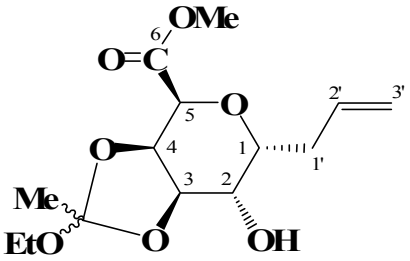
p-Toluenesulfonic acid monohydrate (2 mg/mmol) was added to a suspension of C-glycoside (1 mmol) in triethyl orthoacetate (1.6 mL [8.7 mmol]/mmol) and the reaction mixture was stirred for 14 h at ambient temperature under an argon atmosphere (TLC control). After adding of triethylamine (0.6 mL/mmol), the reaction mixture was diluted with chloroform (30 mL/mmol). The organic layer was washed with ice-water (3 x 10 mL/mmol), dried, and concentrated. The residue was purified by flash chromatography (eluent ethyl acetate gradient

in petrol ether containing 1.5% [v/v] triethylamine) to provide a syrupy mixture of *exo*/*endo* diastereomers which was used without further characterization**.**

4.5.7. General procedure for the cleavage of cyclic orthoesters

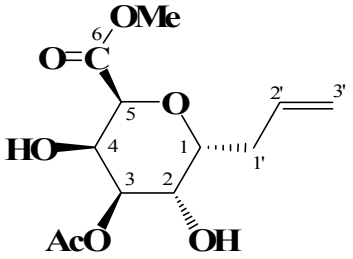
The diastereomeric mixture of 3,4-orthoesters (1mmol) was treated with aq. 90% acetic acid (7 mL/mmol). The reaction mixture was kept for 30 min at room temperature (TLC control) and then concentrated. Repeated coevaporation of the residue with toluene (5x) followed by chromatographic purification (eluent ethyl acetate gradient 5→60% in petrol ether) afforded a mixture of 3-OAc and 4-OAc derivatives. For analytical purposes the mixture was purified by HPLC using petrol ether ethyl acetate as eluent.

4.5.8. Methyl 1-deoxy-3,4-*O*-(1-ethoxyethylidene)-1-(prop-2-enyl)-α-D-galactopyranuronate (62)



To a solution of **58** (233 mg, 1 mmol) in abs. dichloromethane (6.2 mL) camphorsulfonic acid (20 mg) and triethyl orthoacetate (5 x 0.19 mL, total 5 mmol) and the reaction mixture was stirred for 3 h at ambient temperature under an argon atmosphere (TLC, eluent A_3). After adding of triethylamine (3.1 mL), the reaction mixture was diluted with chloroform (30 mL), washed with water (3 x 10 mL), dried, and concentrated. The crude product was dissolved in 90% aq. acetic acid (5 mL) and stirred for 2 h at ambient temperature. The mixture was then diluted with toluene (10 mL), concentrated, and the residue was coevaporated with repeated addition of toluene. Purification by flash chromatography (eluent ethyl acetate gradient 14→40% in petrol ether) gave a mixture of **63** and **64** (197 mg, 72% over two steps) in a ratio of 1:1 (NMR). The analytical sample of **63** was obtained by HPLC purification (eluent A_9).

4.5.9. Methyl 3-*O*-acetyl-1-deoxy-1-(prop-2-enyl)-α-D-galactopyranuronate (63)



Yield: 98 mg, 36%, colourless thick syrup

$[\alpha]_D^{22}$ +54.6 (*c* 1.4, chloroform)

R_f 0.13 (eluent A_9)

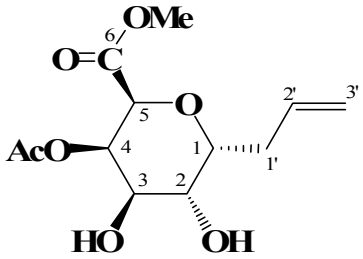
^{1}H NMR (CDCl$_3$, 500.13 MHz): δ 5.84 (dddd, 1H, $^3J_{2',3'trans}$ 17.0 Hz, $^3J_{2',3'cis}$ 10.1 Hz, $^3J_{1'a,2'}$ = $^3J_{1'b,2'}$ 6.9 Hz, H-2'), 5.16(dq, 1H, $^4J_{1'a,3'trans}$ = $^4J_{1'b,3'trans}$ = $^2J_{3'trans,3'cis}$ 1.7 Hz, H-3'trans), 5.09 (m, 1H, H-3'cis), 5.06 (dd, 1H, $^3J_{3,4}$ 2.8 Hz, H-3), 4.45 (d, 1H, H-5), 4.44 (dd, $^3J_{4,5}$ 5.2 Hz, H-4), 4.12 (ddd, 1H, $^3J_{1,2}$ 3.1 Hz, $^3J_{1,1'a}$ 5.7 Hz, $^3J_{1,1'b}$ 8.8 Hz, H-1), 3.94 (dd, 1H, $^3J_{2,3}$ 6.0 Hz, H-2), 3.81 (s, 3H, OCH_3), 3.55 (br, OH), 2.48–2.41, 2.39–2.33 (2m, 2H, H-1'a, H-1'b), 2.06 (s, 3H, CH_3CO)

^{13}C NMR (CDCl$_3$, 125.8 MHz): δ 171.4 (C-6), 170.1 (O*C*OCH_3), 133.9 (C-2'), 117.5 (C-3'), 73.3 (C-3), 72.1 (C-1), 71.5 (C-5), 67.6 (C-2), 66.0 (C-4), 52.3 (OCH_3), 32.8 (C-1'), 20.8 (*C*H_3CO)

$C_{12}H_{18}O_7$ (274.27)	calcd:	C 52.55	H 6.62
	found:	C 52.62	H 6.71

MS (CI, isobutane): *m/z* 275 $[M+H]^+$

4.5.10. Methyl 4-*O*-acetyl-1-deoxy-1-(prop-2-enyl)-α-D-galactopyranuronate (64)



Yield: 98 mg, 36%

R_f 0.13 (eluent A_9)

^{1}H NMR (CDCl$_3$, 250.13 MHz): δ 5.84 (dddd, 1H, $^3J_{2',3'trans}$ 17.0 Hz, $^3J_{2',3'cis}$ 10.2 Hz, $^3J_{1'a,2'}$ = $^3J_{1'b,2'}$ 6.8 Hz, H-2'), 5.43 (dd, $^3J_{4,5}$ 4.0 Hz, H-4), 5.15 (dq, 1H, $^4J_{1'a,3'trans}$ = $^4J_{1'b,3'trans}$ = $^2J_{3'trans,3'cis}$ 1.7 Hz, H-3'trans), 5.11–5.06 (m, 1H, H-3'cis), 4.52 (d, 1H, H-5), 4.39 (ddd, 1H, $^3J_{1,2}$ 3.7 Hz, $^3J_{1,1'a}$ 5.9 Hz, $^3J_{1,1'b}$ 8.8 Hz, H-1), 4.02 (dd, 1H, $^3J_{3,4}$ 3.0 Hz, H-3), 3.97 (dd, 1H,

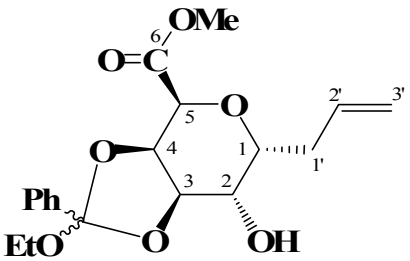
$^3J_{2,3}$ 7.5 Hz, H-2), 3.74 (s, 3H, OCH_3), 2.63 (br, 2H, 2 x OH), 2.46–2.36 (m, 2H, H-1'a, H-1'b), 2.10 (s, 3H, CH_3CO)

^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 170.7, 169.5 (2 x CO), 134.0 (C-2'), 117.5 (C-3'), 73.0 (C-1), 70.0 (C-5), 69.9 (C-4), 69.40 (C-2), 69.36 (C-3), 52.5 (OCH_3), 31.2 (C-1'), 20.8 (*C*H_3CO)

$C_{12}H_{18}O_7$ (274.27)	calcd:	C 52.55	H 6.62
	found:	C 52.64	H 6.71

MS (CI, isobutane): *m/z* 275 $[M+H]^+$

4.5.11. Methyl 1-deoxy-3,4-O-(1-ethoxybenzylidene)-1-(prop-2-enyl)-α-D-galactopyranuronate (65)



Compound **58** (195 mg, 0.84 mmol) and p-toluenesulfonic acid (10 mg) were dried together by threefold coevaporation with toluene. The mixture was suspesed in dry dichloromethan (5 ml) and freshly distilled triethyl orthobenzoate (0.9 mL, 4mmol) was added. The reaction mixture was stirred for 17 h at ambient temperature under an argon atmosphere (TLC, eluent A_5). After adding of triethylamine (2.5 mL), the reaction mixture was diluted with chloroform (40 mL), the organic layer was washed with ice-water (3 x 10 mL), dried, and concentrated. The residue was purified by flash chromatography (eluent ethyl acetate gradient 20→25% in petrol ether containing 1.5% [v/v] triethylamine) to provide hemi orthoester **68** and syrupy mixture of *exo*/*endo* diastereomers (2:9 from NMR) **65** (251 mg, 82%) which was used without further characterization.

Yield: 251 mg, 82%, colourless syrup

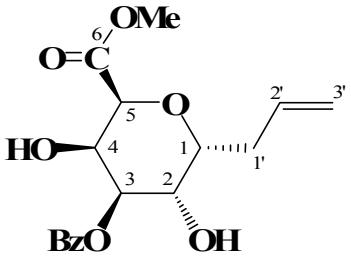
R_f 0.25 (eluent A_5)

4.5.12. Orthoester cleavage of compound 65

Acetic acid (90%, 2.8 mL) was added to compound **65** (144 mg, 0.39 mmol) and the reaction mixture was kept for 45 min at ambient temperature (TLC, eluent A_5). The mixture was then diluted with toluene (10 mL), concentrated, and the residue was coevaporated with repeated

addition of toluene. Purification by HPLC (eluent A_5) gave a mixture of **66** and **67** (130 mg, 98%). The ratio of **66** / **67** was 1:1 (NMR). Separation of pure samples of both compounds by HPLC was not possible. Therefore, the NMR data were obtained by enriched fractions of each compound.

4.5.13. Methyl 3-O-benzoyl-1-deoxy-1-(prop-2-enyl)-α-D-galactopyranuronate (66)



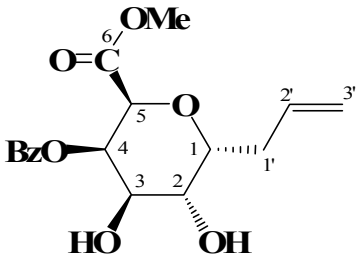
R_f 0.28 (eluent A_4)

^{1}H NMR (CDCl$_3$, 250.13 MHz): δ 8.04–7.94, 7.61–7.53, 7.48–7.39 (3m, 5H, C_6H_5), 5.84 (dddd, 1H, $^3J_{2',3'trans}$ 17.0 Hz, $^3J_{2',3'cis}$ 10.2 Hz, $^3J_{1'a,2'} = {}^3J_{1'b,2'}$ 6.8 Hz, H-2'), 5.35 (ddd, 1H, $^4J = {}^3J_{3,4}$ 1.6 Hz, H-3), 5.21–5.06 (m, 2H, H-3'a, H-3'b), 4.60–4.56 (m, 2H, $^3J_{4,5}$ 5.3 Hz, H-4, H-5), 4.17 (ddd, 1H, $^3J_{1,2}$ 2.8 Hz, $^3J_{1,1'a}$ 5.7 Hz, $^3J_{1,1'b}$ 8.3 Hz, H-1), 4.05 (dd, $^3J_{2,3}$ 5.3 Hz, H-2), 3.80 (s, 3H, OCH_3), 2.60–2.31 (m, 2H, H-1'a, H-1'b)

^{13}C NMR (CDCl$_3$, 75.5 MHz): δ 171.9 (C-6), 166.0 (O*C*OC_6H_5), 133.91 (C-2'), 117.5 (C-3'), 133.6, 129.8, 129.2, 128.5 (two signals are isochronic C_6H_5), 73.8 (C-3), 71.8 (C-5), 71.6 (C-1), 68.0 (C-2), 65.8 (C-4), 52.4 (OCH_3), 31.0 (C-1')

MS (CI, isobutane): *m/z* 337 $[M+H]^+$

4.5.14. Methyl 4-*O*-benzoyl-1-deoxy-1-(prop-2-enyl)-α-D-galactopyranuronate (67)



R_f 0.28 (eluent A_4)

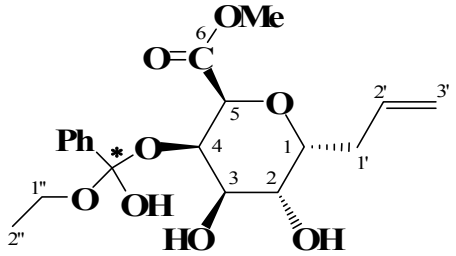
^{1}H NMR (CDCl$_3$, 250.13 MHz): δ 8.04–7.94, 7.61–7.53, 7.48–7.39 (3m, 5H, C_6H_5), 5.87 (dddd, 1H, $^3J_{2',3'trans}$ 17.0 Hz, $^3J_{2',3'cis}$ 10.2 Hz, $^3J_{1'a,2'} = {}^3J_{1'b,2'}$ 6.8 Hz, H-2'), 5.72 (dd, $^3J_{4,5}$

3.8 Hz, H-4), 5.21–5.06 (m, 2H, H-3'a, H-3'b), 4.61 (d, 1H, H-5), 4.45 (ddd, 1H, 1H, $^3J_{1,2}$ 4.4 Hz, $^3J_{1,1'a}$ 5.6 Hz, $^3J_{1,1'b}$9.0 Hz, H-1), 4.14 (dd, 1H, $^3J_{3,4}$ 3.2 Hz, H-3), 4.06 (dd, 1H, $^3J_{2,3}$ 7.7 Hz, H-2), 3.66 (s, 3H, OCH_3), 2.60–2.31 (m, 2H, H-1'a, H-1'b)

^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 169.5 (C-6), 166.3 (O*C*OC_6H_5), 134.6 (C-2'), 117.5 (C-3), 133.5, 129.9, 129.2, 128.5 (two signals are isochronic C_6H_5), 73.4 (C-1), 70.7 (C-4), 70.3 (C-5), 69.6 (C-3), 69.5 (C-2), 52.5 (OCH_3), 33.5 (C-1')

MS (CI, isobutane): *m/z* 337 $[M+H]^+$

4.5.15. Methyl 1-deoxy-4-(ethoxy[hydroxy][phenyl]methoxy)-1-(prop-2-enyl)-α-D-galactopyranuronate (68)



Yield: 34 mg, 11%, amorphous powder

R_f 0.34 (eluent A_5)

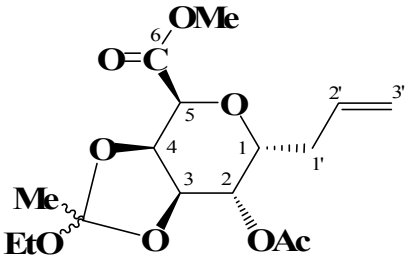
^{1}H NMR ($CDCl_3$, 500.13 MHz): δ 7.52–7.49, 7.37–7.32 (2m, 5H, C_6H_5), 5.85 (dddd, 1H, $^3J_{2',3'trans}$ 17.2 Hz, $^3J_{2',3'cis}$ 10.2 Hz, $^3J_{1'a,2'}$ = $^3J_{1'b,2'}$ 7.1 Hz, H-2'), 5.21 (dq, 1H, $^4J_{1'a,3'trans}$ 1.4 Hz, $^4J_{1'b,3'trans}$ 1.6 Hz, H-3'trans), 5.12 (dqt, 1H, $^4J_{1'a,3'cis}$ = $^4J_{1'b,3'cis}$ 1.0 Hz, H-3'cis), 4.69 (d, 1H, H-5), 4.48 (dd, 1H, $^3J_{4,5}$ 2.5 Hz, H-4), 4.38 (ddd, 1H, $^3J_{1,2}$ 3.5 Hz, $^3J_{1,1'a}$ = $^3J_{1,1'b}$ 7.6 Hz, H-1), 4.20 (dd, 1H, $^3J_{3,4}$ 7.6 Hz, H-3), 4.17 (t, $^3J_{1,2}$ = $^3J_{2,3}$ 3.5 Hz, H-2), 3.96 (dq, $^2J_{1''a,1''b}$ 9.8 Hz, $^3J_{1''a,2''}$ 7.1 Hz, H-1''a), 3.88 (dq, $^2J_{1''a,1''b}$ 9.8 Hz, $^3J_{1''b,2''}$ 7.1 Hz, H-1''b), 3.83 (s, 3H, OCH_3), 2.56–2.52 (m, 2H, H-1'a, H-1'b), 1.20 (t, 3H, H-2''a, H-2''b)

^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 169.4 (C-6), 133.7 (C-2'), 118.0 (C-3'), 138.9, 129.0, 128.2, 125.8 (two signals are isochronic C_6H_5), 121.7 (C_6H_5[OEt]*C*), 72.7 (C-4), 72.5 (C-3), 72.2 (C-1), 70.7 (C-5), 66.4 (C-2), 58.7 (C-1''), 52.2 (OCH_3), 34.7 (C-1'), 15.2 (C-2'')

The MS was determined by LC-MS (using APCI method, positive ion mode, scan range 100.0–2000.0 amu) performed on LCQ Advantage Instrument (ThermoFinnigan) applying following HPLC-gradient program: methanol–water–0.1% aq. formic acid 2 : 7 : 1→methanol–water–0.1% aq. formic acid 8 : 1 : 1 (15 min)→methanol–water–0.1% aq. formic acid 8 : 1 : 1 (25 min).

MS: *m/z* 382 $[M]^+$, *m/z* 319 $[M-(H_2O+EtO^-)]^+$

4.5.16. Methyl 2-*O*-acetyl-1-deoxy-3,4-(1-ethoxyethylidene)-1-(prop-2-enyl)-α-D-galactopyranuronate (69)

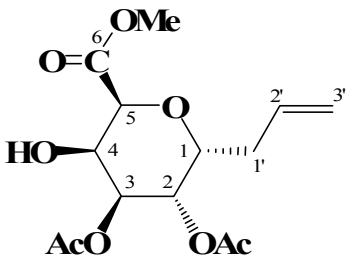


p-Toluenesulfonic acid monohydrate (2 mg) was added to a suspension of compound **61** (200 mg, 0.73 mmol) in triethyl orthoacetate (1.2 mL, 5.5 mmol) and the suspension was stirred for 18 h at ambient temperature under an argon atmosphere (TLC, eluent A_4). After adding of triethylamine (0.5 mL), the reaction mixture was diluted with chloroform (50 mL). The organic layer was washed with ice-water (3 x 10 mL), dried, and concentrated. The residue was purified by flash chromatography (eluent ethyl acetate gradient 20→33% in petrol ether in the presence of 1.5% triethylamine) to provide a syrupy mixture of *exo*/*endo* diastereomers **69** (240 mg, 95%), which was used in the next step without further characterization.

4.5.17. Orthoester cleavage of compound 69

Acetic acid (90%, 3.5 mL) was added to compound **69** (183 mg, 0.53 mmol) and the reaction mixture was kept for 15 min at ambient temperature (TLC, eluent A_4). The mixture was then diluted with toluene (10 mL), concentrated, and the residue was coevaporated with repeated addition of toluene that provide a mixture of **70** and **71** (total 152 mg, 91%) in a ratio 1:1 (NMR). Analytical samples were obtained by by HPLC purification (eluent A_9).

4.5.18. Methyl 2,3-di-*O*-acetyl-1-deoxy-1-(prop-2-enyl)-α-D-galactopyranuronate (70)



Melting point: 136–138°C (petrol ether-ethyl acetate)

$[\alpha]_D^{22}$ +78.7 (*c* 1.2, chloroform)

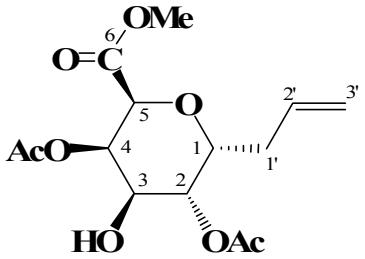
R_f 0.25 (eluent A_4)

^{1}H NMR (CDCl$_3$, 250.13 MHz): δ 5.80 (dddd, 1H, $^3J_{2',3'trans}$ 17.0 Hz, $^3J_{2',3'cis}$ 10.2 Hz, $^3J_{1'a,2'}$ = $^3J_{1'b,2'}$ 6.8 Hz, H-2'), 5.17 (dd, 1H, $^3J_{3,4}$ 2.7 Hz, H-3 overlapped with H-3'a), 5.16–5.16, 5.11–5.06 (2m, 3H, $^3J_{2,3}$ 5.5 Hz, H-3'a, H-3'b, H-2), 4.49 (d, 1H, H-5), 4.36 (dd, $^3J_{4,5}$ 5.5 Hz, H-4), 4.25 (ddd, 1H, $^3J_{1,2}$ 2.8 Hz, $^3J_{1,1'a}$ 5.5 Hz, $^3J_{1,1'b}$ 8.6 Hz, H-1), 3.83 (s, 3H, OCH$_3$), 2.41–2.28, 2.27–2.15 (2m, 2H, H-1'a, H-1'b), 2.10, 2.06 (2s, 6H, 2 x CH$_3$CO)

^{13}C NMR (CDCl$_3$, 75.5 MHz): δ 171.4, 169.5, 169.1 (3 x CO), 133.4 (C-2'), 117.6 (C-3'), 71.6 (C-5), 70.3 (C-1), 70.2 (C-3), 69.1 (C-2), 66.3 (C-4), 52.4 (OCH$_3$), 33.6 (C-1'), 20.7, 20.7 (2 x *C*H$_3$CO)

$C_{14}H_{20}O_8$ (316.30)	calcd:	C 53.16	H 6.37
	found:	C 53.22	H 6.47

4.5.19. Methyl 2,4-di-*O*-acetyl-1-deoxy-1-(prop-2-enyl)-α-D-galactopyranuronate (71)



Melting point: 98–101°C (petrol ether-ethyl acetate)

$[\alpha]_D^{22}$ +125.1 (*c* 1.6, chloroform)

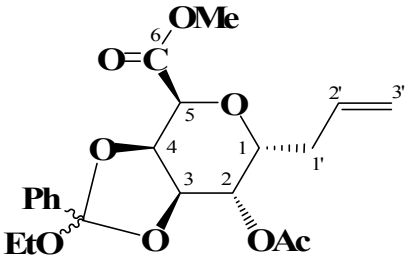
R_f 0.25 (eluent A_4)

^{1}H NMR (CDCl$_3$, 250.13 MHz): δ 5.79 (dddd, 1H, $^3J_{2',3'trans}$ 17.0 Hz, $^3J_{2',3'cis}$ 10.2 Hz, $^3J_{1'a,2'}$ = $^3J_{1'b,2'}$ 6.8 Hz, H-2'), 5.31 (dd, $^3J_{4,5}$ 5.4 Hz, H-4), 5.15–5.13, 5.10–5.04 (2m, 2H, H-3'a, H-3'b), 5.02 (dd, 1H, $^3J_{2,3}$ 5.8 Hz, H-2), 4.63 (d, 1H, H-5), 4.52 (ddd, 1H, $^3J_{1,2}$ 3.0 Hz, $^3J_{1,1'a}$ 5.8 Hz, $^3J_{1,1'b}$ 8.6 Hz, H-1), 4.06 (dd, 1H, $^3J_{3,4}$ 3.1 Hz, H-3), 3.74 (s, 3H, OCH$_3$), 2.45–2.32, 2.30–2.18 (2m, 2H, H-1'a, H-1'b), 2.11, 2.11 (2s, 6H, 2 x CH$_3$CO)

^{13}C NMR (CDCl$_3$, 75.5 MHz): δ 170.5, 170.2, 170.1 (3 x CO), 133.4 (C-2'), 117.6 (C-3'), 71.9 (C-2), 70.0 (C-5), 69.8 (C-1), 68.9 (C-4), 67.3 (C-3), 52.3 (OCH$_3$), 33.3 (C-1'), 20.8, 20.7 (2 x *C*H$_3$CO)

$C_{14}H_{20}O_8$ (316.30)	calcd:	C 53.15	H 6.37
	found:	C 53.21	H 6.51

4.5.20. Methyl 2-*O*-acetyl-1-deoxy-3,4-*O*-(1-ethoxybenzylidene)-1-(prop-2-enyl)-α-D-galactopyranuronate (72)



The mixture of diastereomers **65** (170 mg, 0.47 mmol) was dissolved in abs pyridine (3.2 mL) and acetic anhydride (0.8 mL), and the reaction mixture was kept for 24 h at room temperature under an argon atmosphere (TLC, eluent A_5). After dilution with chloroform (20 mL), the solution was poured into ice-water (10 mL). The organic phase was then separated, and the aqueous layer was extracted with chloroform (3 x 10 mL). The combined organic layers were washed with aq. $NaHCO_3$ (2 x 10 mL), ice-water (10 mL), dried, and concentrated. After repeated coevaporation with toluene (3 x 10 mL), the residue was concentrated and purified by flash chromatography (eluent ethyl acetate gradient 15→20% in petrol ether containing 1.5% [v/v] triethylamine) to provide **72** as a mixture of *exo*/*endo* diastereomers which was used without further characterization**.**

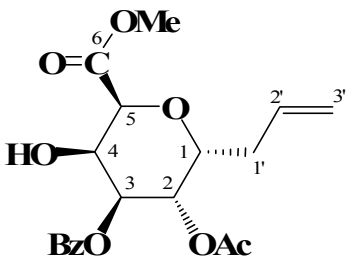
Yield: 178 mg, 93%, colourless syrup

R_f 0.24 (eluent A_7)

4.5.21. Orthoester cleavage of compound 72

Orthoester cleavage of **72** was analog to **69**. Reagents: **72** (145 mg, 0.36 mmol); 90% aq. acetic acid (10 mL). Purification by flash chromatography (eluent ethyl acetate gradient 14→33% in petrol ether) gave a mixture of **73** and **74** (132 mg, 97%) in a ratio of 1:1 (NMR). Separation by HPLC (eluent A_6) gave analytically pure derivatives **73** and **74**.

4.5.22. Methyl 2-*O*-acetyl-3-*O*-benzoyl-1-deoxy-1-(prop-2-enyl)-α-D-galactopyranuronate (73)



Yield: 66 mg, 48%, colourless thick syrup

$[\alpha]_D^{22}$ +125.5 (*c* 1.2, chloroform)

R_f 0.21 (eluent A_5)

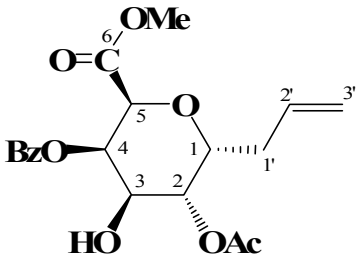
^{1}H NMR ($CDCl_3$, 250.13 MHz): δ 7.99–7.94, 7.62–7.55, 7.49–7.41 (3m, 5H, C_6H_5), 5.82 (dddd, 1H, $^3J_{2',3'trans}$ 17.0 Hz, $^3J_{2',3'cis}$ 10.2 Hz, $^3J_{1'a,2'} = {}^3J_{1'b,2'}$ 6.8 Hz, H-2'), 5.39 (dd, $^3J_{3,4}$ 2.8 Hz, H-3), 5.25 (dd, 1H, $^3J_{2,3}$ 5.4 Hz, H-2), 5.18–5.16, 5.11–5.06 (2m, 2H, H-3'a, H-3'b), 4.59 (d, 1H, H-5), 4.50 (br, 1H, $^3J_{4,5}$ 5.6 Hz, H-4), 4.35 (ddd, 1H, $^3J_{1,2}$ 2.6 Hz, $^3J_{1,1'a}$ 5.2 Hz, $^3J_{1,1'b}$ 8.6 Hz, H-1), 3.77 (s, 3H, OCH_3), 3.60 (br, 1H, OH), 2.45–2.32, 2.30–2.18 (2m, 2H, H-1'a, H-1'b), 2.12 (s, 3H, CH_3CO)

^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 171.4 (C-6), 169.5 (O*C*OCH_3), 165.2 (O*C*OC_6H_5), 133.4 (C-2'), 117.7 (C-3'), 133.7, 129.8, 129.0, 128.5 (two signals are isochronic C_6H_5), 71.8 (C-5), 70.8 (C-3), 70.3 (C-1), 69.1 (C-2), 66.3 (C-4), 52.5 (OCH_3), 33.8 (C-1'), 20.7 (*C*H_3CO)

$C_{19}H_{22}O_8$ (378.37) calcd: C 60.31 H 5.86

found: C 60.50 H 5.91

4.5.23. Methyl 2-*O*-acetyl-4-*O*-benzoyl-1-deoxy-1-(prop-2-enyl)-α-D-galactopyranuronate (74)



Yield: 66 mg, 48%, colourless thick syrup

$[\alpha]_D^{22}$ +104.1 (*c* 0.90, chloroform)

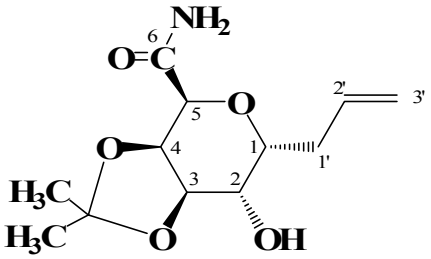
R_f 0.27 (eluent A_5)

^{1}H NMR ($CDCl_3$, 250.13 MHz): δ 8.05–7.99, 7.61–7.54, 7.49–7.39 (3m, 5H, C_6H_5), 5.83 (dddd, 1H, $^3J_{2',3'trans}$ 17.0 Hz, $^3J_{2',3'cis}$ 10.1 Hz, $^3J_{1'a,2'} = {}^3J_{1'b,2'}$ 6.8 Hz, H-2'), 5.62 (dd, 1H, $^3J_{4,5}$ 5.4 Hz, H-4), 5.18–5.16, 5.13–5.05 (m, 3H, $^3J_{2,3}$ 5.8 Hz, H-3a', H-3b', H-2), 4.72 (d, 1H, H-5), 4.60 (ddd, 1H, $^3J_{1,2}$ 3.0 Hz, $^3J_{1,1'a}$ 5.7 Hz, $^3J_{1,1'b}$ 8.6 Hz, H-1), 4.20 (dd, 1H, $^3J_{3,4}$ 3.0 Hz, H-3), 3.67 (s, 3H, OCH_3), 3.14 (br, 1H, OH), 2.49–2.36, 2.35–2.21 (2m, 2H, H-1'a, H-1'b), 2.13 (s, 3H, CH_3CO)

^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 170.5 (O*C*OCH_3), 170.2 (C-6), 165.7 (O*C*OC_6H_5), 133.4 (C-2'), 117.7 (C-3'), 133.6, 129.8, 129.1, 128.5 (two signals are isochronic C_6H_5), 72.1 (C-2), 70.6 (C-5), 79.0 (C-1), 69.3 (C-4), 67.6 (C-3), 52.4 (OCH_3), 33.4 (C-1'), 20.9 (*C*H_3CO)

$C_{19}H_{22}O_8$ (378.37)	calcd:	C 60.31	H 5.86
	found:	C 60.43	H 5.94

4.5.24. 2,3, 1-deoxy-3,4-*O*-isopropylidene-1-(prop-2-enyl)-α-D-galactopyranuronamide (76)



p-Toluenesulfonic acid monohydrate (23 mg) was added to the suspension of compound **82** (201 mg, 0.925 mmol) in 2,2-dimethoxypropane (1.2 mL) and dry acetone (4.7 mL), and the reaction mixture was stirred for 20 h at ambient temperature under an argon atmosphere (TLC, eluent A_3). The mixture was then passed through a layer of alkaline aluminia (2 x 3 cm), the alkaline aluminia was washed with acetone, and the filtrate and washings were combined. After removal of the solvent, the residue was purified by flash chromatography on silica gel (eluent ethyl acetate gradient 33→80% in petrol ether) to provide compound **76**.

Yield: 216 mg, 91%, colourless syrup

Melting point: 80–82 °C (ethyl acetate–petrol ether)

$[\alpha]_D^{23}$ +1.9 (*c* 1.3, chloroform)

R_f 0.11 (eluent A_3)

^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 6.73 (br d, 1H, $^2J_{NHa,NHb}$ 3.1 Hz, NHa), 6.02 (br d, 1H, NH_b), 5.83 (dddd, 1H, $^3J_{2',3'trans}$ 17.2 Hz, $^3J_{2',3'cis}$ 10.3 Hz, $^3J_{1'a,2'} = {}^3J_{1'b,2'}$ 7.1 Hz, H-2'), 5.15 (dq, 1H, $^3J_{2',3'trans}$ 17.2 Hz, $^4J_{1'a,3'trans} = {}^4J_{1'b,3'trans} = {}^2J_{3'a,3'b}$ 1.5 Hz, H-3'trnas), 5.08 (m, 1H, H-3'cis), 4.69 (dd, 1H, $^3J_{4,5}$ 2.1 Hz, H-4), 4.50 (d, 1H, H-5), 4.32 (dd, 1H, $^3J_{3,4}$ 7.6 Hz, H-3), 4.08 (ddd, 1H, $^3J_{1,2}$ 1.7 Hz, $^3J_{1,1'a}$ 6.4 Hz, $^3J_{1,1'b}$ 7.5 Hz, H-1), 3.75 (dd, $^3J_{2,3}$ 2.7 Hz, H-2), 3.05 (br s, OH), 2.48–2.27 (m, 2H, H-1'a, H-1'b), 1.44, 1.31 (2s, 6H, $[CH_3]_2C$)

^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 173.0 (C-6), 134.1 (C-2'), 117.7 (C-3'), 109.6 ($[CH_3]_2C$), 73.9 (C-3), 72.6 (C-4), 70.6, 70.6 (C-1, C-5), 68.3 (C-2), 35.9 (C-1'), 26.5, 24.2 ($[CH_3]_2C$)

$C_{12}H_{19}NO_5$ (257.28)	calcd:	C 56.02	H 5.44	N 5.44
	found:	C 56.10	H 5.61	N 5.67

4.6. O-BENZOYL PROTECTED C-ALLYL α-D-GALACTOPYRANURONATES: THE TEMPERATURE CONTROLLED BENZOYLATION

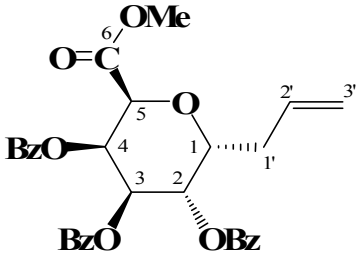
4.6.1. General procedure of dimolar benzoylations

Benzoyl chloride (2 molar equiv.) was added dropwise over 30 min to a solution of corresponding C–glycosyl derivative (2 molar equiv.) in abs. pyridine at –38 °C under argon atmosthere. The reaction mixture was kept at –38 °C for 2 h and then let to reach 12 °C during overnight, finally decomposed with ethanol. The reaction mixture was concentrated and the traces of pyridine were removed by evaporation with repeated addition of toluene. The residue was purified by flash chromatography and if necessary by HPLC.

4.6.2. Benzoylation of 58

Reagents: Benzoyl chloride (500 μL, 4.3 mmol); C–glycoside **58** (500 mg, 2.15 mmol); pyridine (4.8 mL). Purification was performed by flesh chromatography (eluent ethyl acetate gradient 12→17% in petrol ether). Products: tri-O-benzoyl derivative **77** (500 mg, 43%); di-O-benzoyl derivative **78** (335 mg, 35%).

4.6.3. Methyl 2,3,4-tri-*O*-benzoyl-1-deoxy-1-(prop-2-enyl)-α-D-galactopyranuronate (77)



Yield: 500 mg, 43%, colourless foam

$[\alpha]_D^{23}$ +153.4 (*c* 1.01, chloroform)

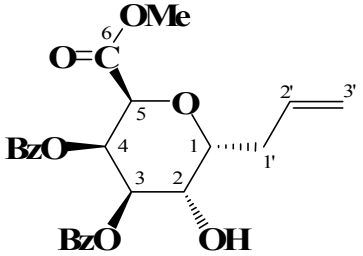
R_f 0.53 (eluent A_5)

^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 8.10–7.91, 7.61–7.35 (2m, 15H, 3 x C_6H_5), 5.98–5.86 (dddd, 1H, $^3J_{2',3'trans}$ 17.1 Hz, $^3J_{2',3'cis}$ 10.3 Hz, $^3J_{1'a,2'} = {}^3J_{1'b,2'}$ 6.8 Hz, H-2'), 5.93 (dd, 1H, $^3J_{4,5}$ 5.2 Hz, H-4 overlapped with H-2'), 5.85 (dd, 1H, $^3J_{3,4}$ 3.1 Hz, H-3), 5.61 (dd, $^3J_{2,3}$ 6.5 Hz, H-2), 5.17 (dq, 1H, $^3J_{2',3'trans}$ 17.1 Hz, $^4J_{1'a,3'trans} = {}^4J_{1'b,3'trans} = {}^2J_{3'a,3'b}$ 1.6 Hz, H-3'trnas), 5.13-5.08 (2m, 1H, H-3'cis), 5.00 (ddd, 1H, $^3J_{1,2}$ 3.3 Hz, $^3J_{1,1'b}$ 4.9 Hz, $^3J_{1,1'a}$ 9.2 Hz, H-1), 4.96 (d, 1H, H-5), 3.52 (s, 3H, OCH_3), 2.66–2.54, 2.49–2.39 (2m, 2H, H-1'a, H-1'b)

^{13}C NMR (CDCl$_3$, 75.5 MHz): δ 169.4, 165.4, 165.3, 165.1 (4 x CO), 133.2 (C-2'), 117.9 (C-3'), 133.6, 133.5, 133.4, 129.92, 129.88, 129.77, 129.0, 128.6, 128.5 (nine signals are isochronic 3 x C_6H_5), 71.0 (C-1), 70.1 (C-5), 69.7 (C-2), 68.2 (C-3), 68.1 (C-4), 52.1 (OCH_3), 33.3 (C-1')

$C_{31}H_{28}O_9$ (544.55)	calcd:	C 68.37	H 5.18
	found:	C 68.51	H 5.31

4.6.4. Methyl 3,4-di-*O*-benzoyl-1-deoxy-1-(prop-2-enyl)-α-D-galactopyranuronate (78)



Yield: 335 mg, 35%, colourless foam

$[\alpha]_D^{23}$ +85.6 (*c* 0.90, chloroform)

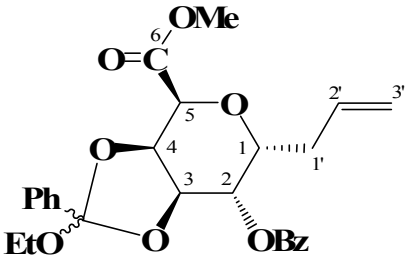
R_f 0.43 (eluent A_5)

^{1}H NMR (CDCl$_3$, 300.13 MHz): δ 7.99–7.9, 7.60–7.51, 7.45–7.35 (3m, 10H, 2 x C_6H_5), 5.93 (dddd, 1H, $^3J_{2',3'trans}$ 17.2 Hz, $^3J_{2',3'cis}$ 10.1 Hz, $^3J_{1'a,2'} = {}^3J_{1'b,2'}$ 6.9 Hz, H-2' overlapped with H-4), 5.92 (dd, 1H, $^3J_{4,5}$ 5.2 Hz, H-4 overlapped with H-2'), 5.59 (dd, 1H, $^3J_{3,4}$ 6.8 Hz, H-3), 5.24 (dq, 1H, $^4J_{1'a,3'trans} = {}^4J_{1'b,3'trans} = {}^2J_{3'a,3'b}$ 1.6 Hz, H-3'trnas), 5.16–5.11 (2m, 1H, H-3'cis), 4.84 (d, 1H, H-5), 4.73 (ddd, 1H, $^3J_{1,2}$ 3.5 Hz, $^3J_{1,1'a}$ 5.8 Hz, $^3J_{1,1'b}$ 8.9 Hz, H-1), 4.21 (dd, $^3J_{2,3}$ 6.6 Hz, H-2), 3.53 (s, 3H, OCH_3), 2.64-2.46 (m, 2H, H-2'a, H-1'b)

^{13}C NMR (CDCl$_3$, 75.5 MHz): δ 169.4, 165.9, 165.4 (3 x CO), 133.9 (C-2'), 117.7 (C-3'), 133.5, 133.4, 129.9, 129.7, 128.5, 128.5 (six signals are isochronic 2 x C_6H_5), 72.6 (C-1), 71.4 (C-3), 70.1 (C-5), 68.2 (C-2), 67.9 (C-4), 52.1 (OCH_3), 32.4 (C-1')

$C_{24}H_{24}O_8$ (440.44)	calcd:	C 65.45	H 5.49
	found:	C 65.48	H 5.66

4.6.5. Methyl 2-*O*-benzoyl-1-deoxy-3,4-*O*-(1-ethoxybenzylidene)-1-(prop-2-enyl)-α-D-galactopyranuronate (79)

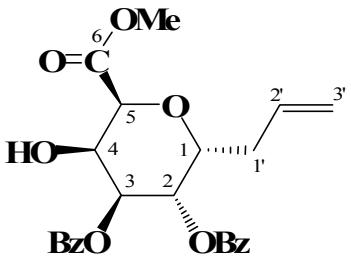


Benzoyl chloride (147 µL, 1.26 mmol) was added to a solution of **65** (230 mg, 0.63 mmol) in abs. pyridine (450 µL) at room temperature under an argon atmosphere. After 14 h (TLC, eluent A_7) methanol (2 mL) was added at 0°C and the stirring was continued at room temperature for 1 h. The reaction mixture was then concentrated and traces of pyridine were removed by evaporation with repeated addition of toluene. The residue was dissolved in chloroform (20 mL) and the organic layer was washed with ice water (1 x 10 mL), aq. $NaHCO_3$ (2 x 5 mL) and ice water (2 x 10 mL), dried and concentrated. The crude product **79** was used for the next step without further purification.

4.6.6. Orthoester cleavage of compound 79

Reagents: Crude product from **79**; 90% aq. acetic acid (5 mL). Purification by flash chromatography (eluent ethyl acetate gradient 14→20% in petrol ether) gave a mixture of **80** and **81** (198 mg, 72% over two steps). The ratio of **80** / **81** was 1:1 (NMR). Separation of pure samples of both compounds by HPLC (eluent A_7) gave analytically pure derivatives **80** and **81**.

4.6.7. Methyl 2,3-di-*O*-benzoyl-1-deoxy-1-(prop-2-enyl)-α-D-galactopyranuronate (80)



Yield: 99 mg, 36%, colourless foam

[α] $^{22}_{D}$ +69.5 (*c* 1.6, chloroform)

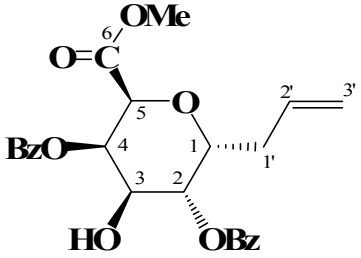
R_f 0.25 (eluent A_7)

^{1}H NMR (CDCl$_3$, 250.13 MHz): δ 8.11–7.96, 7.63–7.54, 7.50–7.41 (3m, 10H, 2 x C_6H_5), 5.84 (dddd, 1H, $^3J_{2',3'trans}$ 17.0 Hz, $^3J_{2',3'cis}$ 10.3 Hz, $^3J_{1'a,2'} = {}^3J_{1'b,2'}$ 6.8 Hz, H-2'), 5.55 (dd, $^3J_{3,4}$ 2.6 Hz, H-3), 5.49 (dd, 1H, $^3J_{2,3}$ 5.3 Hz, H-2), 5.15–5.13, 5.09–5.03 (2m, 2H, H-3'a, H-3'b), 4.66 (d, 1H, H-5), 4.61 (m, 1H, $^3J_{4,5}$ 5.8 Hz, H-4), 4.48 (ddd, 1H, $^3J_{1,2}$ 2.5 Hz, $^3J_{1,1'a}$ 5.3 Hz, $^3J_{1,1'b}$ 8.7 Hz, H-1), 3.80 (s, 3H, OCH_3), 2.56–2.41, 2.39–2.26 (m, 2H, H-1'a, H-1'b)

^{13}C NMR (CDCl$_3$, 75.5 MHz): δ 171.5 (C-6), 165.3, 165.1 (2 x O*C*OC_6H_5), 133.3 (C-2'), 117.8 (C-3'), 133.6, 133.5, 129.9, 129.9, 129.1, 129.1, 128.5, 128.55 (two signals are isochronic 2 x C_6H_5), 71.8 (C-5), 70.9 (C-3), 70.5 (C-1), 69.7 (C-2), 66.6 (C-4), 52.5 (OCH_3), 33.9 (C-1')

$C_{24}H_{24}O_8$ (440.44)	calcd:	C 65.45	H 5.49
	found:	C 65.48	H 5.66

4.6.8. Methyl 2,4-di-*O*-benzoyl-1-deoxy-1-(prop-2-enyl)-α-D-galactopyranuronate (81)



Yield: 99 mg, 36%, colourless foam

$[\alpha]_D^{23}$ +113.7 (*c* 1.2, chloroform)

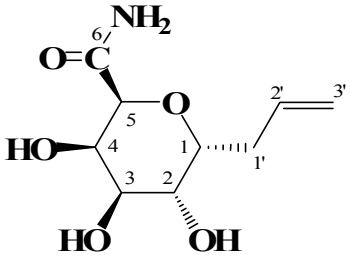
R_f 0.42 (eluent A_7)

^{1}H NMR (CDCl$_3$, 250.13 MHz): δ 8.10–8.01, 7.61–7.52, 7.48–7.38 (3m, 10H, 2 x C_6H_5), 5.85 (dddd, 1H, $^3J_{2',3'trans}$ 17.0 Hz, $^3J_{2',3'cis}$ 10.2 Hz, $^3J_{1'a,2'} = {}^3J_{1'b,2'}$ 6.8 Hz, H-2'), 5.67 (dd, 1H, $^3J_{4,5}$ 5.5 Hz, H-4), 5.33 (dd, 1H, $^3J_{2,3}$ 5.7 Hz, H-2), 5.15–5.04 (m, 2H, H-3'a, H-3'b), 4.82 (d, 1H, H-5), 4.76 (ddd, 1H, $^3J_{1,2}$ 3.0 Hz, $^3J_{1,1'a}$ 5.7 Hz, $^3J_{1,1'b}$ 8.6 Hz, H-1), 4.35 (dd, $^3J_{3,4}$ 3.0 Hz, H-3), 3.68 (s, 3H, OCH_3), 2.59–2.46, 2.43–2.31 (2m, 2H, H-1'a, H-1'b)

^{13}C NMR (CDCl$_3$, 75.5 MHz): δ 170.2 (C-6), 166.0, 165.7 (2 x O*C*OC_6H_5), 133.5 (C-2'), 117.7 (C-3'), 133.4, 133.3, 129.81, 129.76, 129.2, 129.1, 128.5, 128.4 (four signals are isochronic 2 x C_6H_5), 72.7 (C-2), 70.4 (C-5), 70.1 (C-1), 69.4 (C-4), 67.6 (C-3), 52.3 (OCH_3), 33.6 (C-1')

$C_{24}H_{24}O_8$ (440.44)	calcd:	C 65.45	H 5.49
	found:	C 65.54	H 5.60

4.6.9. 1-deoxy-1-(prop-2-enyl)-α-D-galactopyranuronamide (82)



Methanolic ammonia (35 mL, 2 M) was added to a compound **58** (3.29 g, 14.17 mmol) under argon atmosphere, and the reaction mixture kept for 16 h at rt without stirring. The methanolic ammonia was evaporated under reduced pressure and the residue was purified by MPLC (eluent methanol gradient 5→20% in chloroform) to furnish **82**.

Yield: 3.01 g, 98%, colourless crystals

Melting point: 164–166 °C

$[\alpha]_D^{25}$ +62.2 (*c* 1.8, pyridine)

R_f 0.04 (eluent C_5)

IR (Nujol); ν 3458 and 3229 cm^{-1} (NH_2, OH)

^{1}H NMR (DMSO-d_6, 250.13 MHz): δ 7.20, 6.88 (2s, 2H, NH_a, NH_b), 5.77 (dddd, 1H, $^3J_{2',3'trans}$ 17.0 Hz, $^3J_{2',3'cis}$ 10.1 Hz, $^3J_{1'a,2'} = {}^3J_{1'b,2'}$ 6.9 Hz, H-2'), 5.11–4.96 (4m, 2H, H-3'a, H-3'b), 4.86 (d, 1H, $^3J_{OH,CH}$ 4.8 Hz, OH-2), 4.82 (d, 1H, $^3J_{OH,CH}$ 5.4 Hz, OH-3), 4.77 (d, 1H, $^3J_{OH,CH}$ 5.2 Hz, OH-4), 3.98–3.89 (m, 3H, H-1, H-4, H-5), 3.70 (ddd, 1H, $^3J_{OH,CH}$ 4.8 Hz, $^3J_{1,2}$ 5.3 Hz, $^3J_{2,3}$ 8.4 Hz, H-2), 3.52 (ddd, 1H, $^3J_{OH,CH}$ 5.4 Hz, $^3J_{3,4}$ 3.1 Hz, H-3), 2.45–2.19 (m, 2H, H-1'a, H-1'b)

^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 172.3 (C-6), 136.1 (C-2'), 116.4 (C-3'), 74.5, 72.0 (C-1, C-5) 70.0 (C-3), 68.6 (C-4), 68.0 (C-2), 29.6 (C-1')

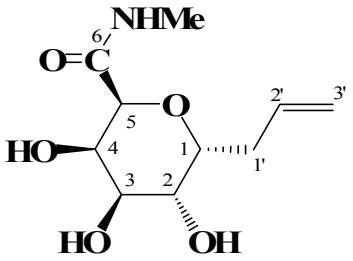
MS (ESI), *m/z* 217.8 $[M+H]^+$

$C_9H_{15}NO_5$ (217.22)	calcd:	C 49.76	H 6.96	N 6.45
	found:	C 49.42	H 6.96	N 6.20

4.6.10. Benzoylation of 82

Reagents: Benzoyl chloride (628 μL, 5.4 mmol); C–glycoside **82** (587 mg, 2.7 mmol); pyridine (6 mL). Purification was performed by flesh chromatography (eluent ethyl acetate gradient 10→80% in petrol ether) and then by HPLC (eluent B_3). Products: tri-O-benzoyl cyano derivative **84** (130 mg, 9%); tri-O-benzoyl amido derivative **85** (400 mg, 28%); mono-O-benzoyl derivative **86** (283 mg, 33%).

4.6.11. N-Methyl 1-deoxy-1-(prop-2-enyl)-α-D-galactopyranuronamide (83)



Ethanolic methylamine (6.5 mL, 33% wt) was added to a compound **58** (853 mg, 3.67 mmol) under argon atmosphere at rt. After 15 min, the solvent was evaporated *in vacuo* followed by evaporation of toluene from the residue (x 5) to furnish analytical pure **83** in quantitative yield as amorphous white solid.

Yield: 849 mg, quantitative yield, colourless crystals

Melting point: 222 °C (dec.)

$[\alpha]_D^{25}$ +65.7 (*c* 1.14, pyridine)

R_f 0.27 (eluent C_5)

IR (Nujol); ν 3443, 3402, 34346 cm^{-1} (NH_2, OH)

IR (KBr); ν 3065, 2971, 2952, 2922, 2899 (CH_2) cm^{-1}

^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.44 (q, 1H, $^3J_{NH,CH3}$ 4.7 Hz, N*H*CH_3), 5.78 (dddd, 1H, $^3J_{2',3'trans}$ 17.0 Hz, $^3J_{2',3'cis}$ 10.1 Hz, $^3J_{1'a,2'} = {}^3J_{1'b,2'}$ 6.9 Hz, H-2'), 5.11–4.98 (4m, 2H, H-3'a, H-3'b), 4.87 (d, 1H, $^3J_{OH,CH}$ 4.7 Hz, OH-2), 4.81 (d, 1H, $^3J_{OH,CH}$ 4.1 Hz, OH-3), 4.71 (d, 1H, $^3J_{OH,CH}$ 4.4 Hz, OH-4), 3.99–3.94 (m, 3H, $^3J_{1,2}$ 4.7 Hz, H-1, H-4, H-5), 3.74 (ddd, 1H, $^3J_{2,3}$ 8.9 Hz, $^3J_{1,2}={}^3J_{OH,CH}$=4.7, H-2), 3.55 (m, 1H, H-3), 2.60 (d, 3H, NHCH_3), 2.44–2.38, 2.32–2.26 (2m, 2H, H-1'a, H-1'b)

^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 169.9 (C-6), 136.2 (C-2'), 116.4 (C-3'), 74.9 (C-1), 72.2 (C-5), 69.8 (C-3), 68.8 (C-4), 67.7 (C-2), 29.2 (C-1'), 25.3 ($NHCH_3$)

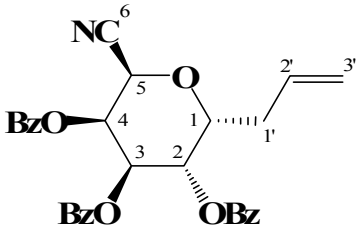
MS (CI, isobutane): *m/z* 232 $[M+H]^+$

$C_{10}H_{17}NO_5$ (231.25)	calcd:	C 51.94	H 7.41	N 6.06
	found:	C 51.99	H 7.46	N 5.83

4.6.12. Benzoylation of 83

Reagents: Benzoyl chloride (500 μL, 4.30 mmol); C–glycoside **83** (497 mg, 2.15 mmol); pyridine (4.8 mL). Purification was performed by flesh chromatography (eluent ethyl acetate gradient 16→66% in petrol ether) and then by HPLC (eluent A_4 and then A_3). Products: tri-O-benzoyl derivative **87** (500 mg, 43%), di-O-benzoyl derivative **88** (138 mg, 15%), di-O-benzoyl derivative **89** (172 mg, 18%).

4.6.13. 2,3,4-tri-*O*-benzoyl-1-deoxy-1-(prop-2-enyl)-α-D-galactopyranurononitrile (84)



Yield: 130 mg, 9%, colourless syrup

[α] $^{23}_{D}$ +83.6 (*c* 1.97, chloroform)

R$_f$ 0.72 (eluent A_4)

IR (Raman); ν 2241 cm^{-1} (CN)

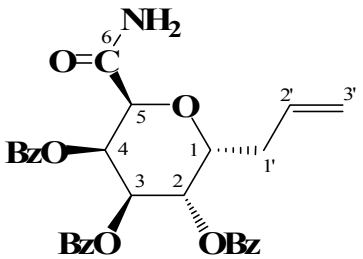
^{1}H NMR (CDCl$_3$, 300.13 MHz): δ 8.32–8.28, 8.15–8.11, 7.92–7.88, 7.67–7.61, 7.56–7.48, 7.38–7.32 (6m, 15H, 3 x C_6H_5), 5.89 (t, 1H, $^3J_{3,4}$ = $^3J_{2,3}$ = 3.7 Hz, H-3), 5.83 (dddd, 1H, $^3J_{2',3'trans}$ 17.6 Hz, $^3J_{2',3'cis}$ 9.8 Hz, $^3J_{1'a,2'}$ = $^3J_{1'b,2'}$ 6.8 Hz, H-2' overlapped with H-3), 5.66 (dd, 1H, $^3J_{4,5}$ 6.4 Hz, H-4), 5.45 (dd, 1H, $^3J_{2,3}$ 3.7 Hz, H-2), 5.29 (d, H-5), 5.16–5.09 (m, 2H, H-3'a, H-3'b), 4.53 (ddd, 1H, $^3J_{1,2}$ 1.1 Hz, $^3J_{1,1a'}$ 5.9 Hz, $^3J_{1,1'b}$ 7.5 Hz, H-1), 2.55–2.44, 2.44–2.34 (m, 2H, H-1'a, H-1'b)

^{13}C NMR (CDCl$_3$, 75.5 MHz): δ 165.01, 164.97, 164.8 (3 x CO), 132.2 (C-2'), 118.7 (C-3'), 133.92, 133.87, 133.83, 130.5, 130.0, 129.9, 128.70, 128.67, 128.59, 128.57 (eight signals are isochronic 3 x C_6H_5), 116.1 (CN), 71.4 (C-1), 69.0 (C-2), 66.3 (C-3), 64.7 (C-4), 63.3 (C-5), 34.5 (C-1')

MS (EI), *m/z* 511 [M]$^+$

$C_{30}H_{25}NO_7$ (511.52)	calcd:	C 70.44	H 4.93	N 2.74
	found:	C 70.28	H 5.18	N 2.58

4.6.14. 2,3,4-tri-*O*-benzoyl-1-deoxy-1-(prop-2-enyl)-α-D-galactopyranuronamide (85)



Yield: 400 mg, 28%, colourless foam

[α] $^{23}_{D}$ +205.2 (*c* 0.71, chloroform)

R$_f$ 0.33 (eluent A_4)

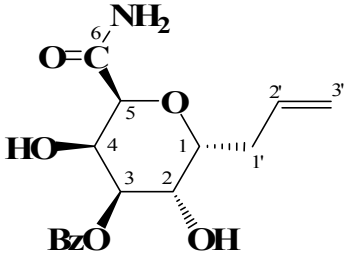
^{1}H NMR (CDCl$_3$, 300.13 MHz): δ 8.03–7.99, 7.96–7.92, 7.82–7.77, 7.60–7.22 (4m, 15H, 3 x C_6H_5), 6.59 (br d, 1H, $^2J_{NHa,NHb}$ 3.1 Hz, NHa), 6.29 (dd, 1H, $^3J_{4,5}$ 1.9 Hz, H-4), 5.86 (dd, 1H, $^3J_{3,4}$ 3.1 Hz, H-3), 5.81 (dd, 1H, $^3J_{2,3}$ 10.3 Hz, H-2), 5.84–5.74 (m, 2H, H-2', NHb), 5.23–5.11 (4m, 2H, H-3'a, H-3'b), 4.76 (ddd, 1H, $^3J_{1,2}$ 5.3 Hz, $^3J_{1,1a'}$ 4.0 Hz, $^3J_{1,1'b}$ 11.2 Hz, H-1), 4.57 (d, H-5), 2.80–2.68, 2.55–2.45 (2m, 2H, H-1'a, H-1'b)

^{13}C NMR (CDCl$_3$, 75.5 MHz): δ 169.4, 165.6, 165.5, 164.9 (4 x CO), 132.8 (C-2'), 118.5 (C-3'), 133.6, 133.3, 133.2, 129.9, 129.8, 129.7, 129.4, 129.0, 128.8, 128.5, 128.5, 128.2 (six signals are isochronic 3 x C_6H_5), 73.5 (C-1), 70.7 (C-5), 69.2 (C-4), 68.9 (C-2), 68.6 (C-3), 30.0 (C-1')

MS (EI), *m/z* 529 $[M]^+$

$C_{30}H_{27}NO_8$ (529.54)	calcd:	C 68.04	H 5.14	N 2.65
	found:	C 67.85	H 5.20	N 2.43

4.6.15. 3-*O*-benzoyl-1-deoxy-1-(prop-2-enyl)-α-D-galactopyranuronamide (86)



Yield: 283 mg, 33%, colourless crystals

Melting point: 177–178 °C (chlorogorm/petrol ether)

$[\alpha]_D^{23}$ +139.2 (*c* 1.1, methanol)

R_f 0.26 (eluent A_1)

^{1}H NMR (CDCl$_3$, 300.13 MHz): δ 8.06–7.98, 7.58–7.52, 7.45–7.38 (3m, 5H, C_6H_5), 6.69, 6.55 (2 x br s, 2H, NH_2), 5.83 (dddd, 1H, $^3J_{2',3'trans}$ 17.0 Hz, $^3J_{2',3'cis}$ 10.2 Hz, $^3J_{1'a,2'} = {}^3J_{1'b,2'}$ 7.1 Hz, H-2'), 5.27 (dd, 1H, $^3J_{3,4}$ 3.2 Hz, H-3), 5.21–5.08 (m, 2H, H-3'a, H-3'b), 4.62 (dd, 1H, $^3J_{4,5}$ 4.8 Hz, H-4), 4.39 (d, 1H, H-5), 4.19 (dd, 1H, $^3J_{2,3}$ 6.5 Hz, H-2), 4.08 (ddd, 1H, $^3J_{1,2}$ 3.4 Hz, $^3J_{1,1'a}$ 5.7 Hz, $^3J_{1,1'b}$ 9.5 Hz, H-1), 2.55–2.33 (2m, H-1'a, H-1'b)

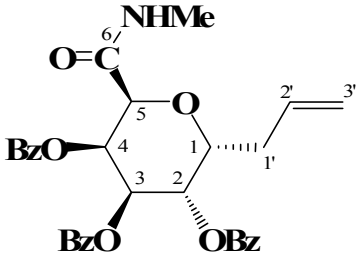
^{13}C NMR (MeOH-*d*$_4$, 75.5 MHz): δ 174.8, 167.7 (2 x CO), 136.2 (C-2'), 117.5 (C-3'), 134.3, 131.4, 130.9, 129.5 (two signals are isochronic C_6H_5), 76.8 (C-1), 75.4 (C-3), 72.9 (C-5), 68.4 (C-4), 67.1 (C-2), 30.7 (C-1')

MS (EI), *m/z* 321 $[M]^+$ (0.29 %)

$C_{16}H_{19}NO_6$ (321.33)	calcd:	C 59.81	H 5.96	N 4.36

found: C 59.99 H 5.84 N 4.49

4.6.16. N-Methyl 2,3,4-tri-*O*-benzoyl-1-deoxy-1-(prop-2-enyl)-α-D-galactopyranuronamide (87)



Yield: 500 mg, 43%, colourless syrup

$[\alpha]_D^{22}$ +195.2 (*c* 0.97, chloroform)

R_f 0.37 (eluent A_5)

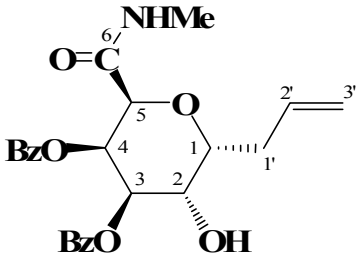
^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 8.01–7.91, 7.81–7.77, 7.61–7.34, 7.27–7.21 (4m, 15H, 3 x C_6H_5), 6.64 (q, 1H, $^2J_{NH,CH3}$ 5.0 Hz, N*H*CH_3), 6.29 (dd, 1H, $^3J_{4,5}$ 1.8 Hz, H-4), 5.88 (dd, 1H, $^3J_{3,4}$ 3.2 Hz, H-3), 5.84–5.72 (m, 1H, H-2', overlapped with H-2), 5.79 (dd, 1H, $^3J_{2,3}$ 10.5 Hz, H-2), 5.22–5.10 (3m, 2H, H-3'a, H-3'b), 4.75 (ddd, 1H, $^3J_{1,2}$ 5.7 Hz, $^3J_{1,1a'}$ 4.2 Hz, $^3J_{1,1'b}$ 11.3 Hz, H-1), 4.54 (d, 1H, H-5), 2.76 (d, 3H, NHCH_3), 2.79–2.68, 2.54–2.45 (2m, 2H, H-1'a, H-2'b)

^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 167.2, 165.6, 165.5, 164.8 (4 x CO), 132.8 (C-2'), 118.4 (C-3'), 133.5, 133.2, 133.1, 129.8, 129.7, 129.7, 129.5, 129.1, 128.8, 128.5, 128.4, 128.2 (six signals are isochronic 3 x C_6H_5), 73.5 (C-1), 70.8 (C-5), 69.5 (C-4), 70.0 (C-2), 68.5 (C-3), 29.9 (C-1'), 25.8 ($NHCH_3$)

$C_{31}H_{29}NO_8$ (543.56) calcd: C 68.50 H 5.38 N 2.58

found: C 68.25 H 5.51 N 2.42

4.6.17. N-Methyl 3,4-di-*O*-benzoyl-1-deoxy-1-(prop-2-enyl)-α-D-galactopyranuronamide (88)



Yield: 138 mg, 15%, colourless crystals

Melting point: 205–206 °C (ethyl acetate/petrol ether)

$[\alpha]_D^{22}$ +119.9 (*c* 1.13, chloroform)

R_f 0.26 (eluent A_5)

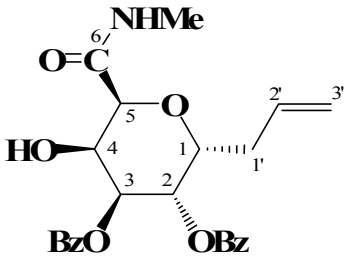
^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 7.98–7.94, 7.87–7.83, 7.59–7.38, 7.34–7.27 (3m, 10H, 2 x C_6H_5), 6.64 (q, 1H, $^3J_{NH,CH3}$ 5.0 Hz, N*H*CH_3), 6.18 (dd, 1H, $^3J_{4,5}$ 1.9 Hz, H-4), 5.84 (dddd, 1H, $^3J_{2',3'trans}$ 17.0 Hz, $^3J_{2',3'cis}$ 10.2 Hz, $^3J_{1'a,2'}$ 7.5 Hz, $^3J_{1'b,2'}$ 6.2 Hz, H-2'), 5.42 (dd, 1H, $^3J_{3,4}$ 3.4 Hz, H-3), 5.22–5.11 (3m, 2H, H-3'a, H-3'b), 4.47–4.39 (m, 3H, $^3J_{2,3}$ 9.4 Hz, H-1, H-2, H-5), 2.79 (br, 1H, OH), 2.71 (d, 3H, NHC*H*$_3$), 2.65–2.47 (m, 2H, H-1'a, H-1'b)

^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 167.6, 166.6, 164.9 (3 x CO), 133.9 (C-2'), 117.9 (C-3'), 133.3, 133.2, 129.9, 129.73, 129.68, 129.2, 128.4, 128.3 (four signals are isochronic 2 x C_6H_5), 72.5 (C-3), 76.1, 70.8, 67.4 (C-1, C-2, C-5), 69.4 (C-4), 28.9 (C-1'), 25.7 ($NHCH_3$)

MS (CI, isobutane): *m/z* 440 $[M+H]^+$

$C_{24}H_{25}NO_7$ (439.46)	calcd:	C 65.59	H 5.73	N 3.19
	found:	C 65.64	H 5.95	N 3.03

4.6.18. N-Methyl 3,4-di-*O*-benzoyl-1-deoxy-1-(prop-2-enyl)-α-D-galactopyranosyluronamide (89)



Yield: 172 mg, 18%, colourless crystals

Melting point: 100–101 °C

$[\alpha]_D^{22}$ +129.5 (*c* 0.68, chloroform)

R_f 0.06 (eluent A_5)

^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 8.06–8.00, 7.59–7.52, 7.46–7.38 (3m, 10H, 2 x C_6H_5), 6.66 (q, 1H, $^3J_{NH,CH3}$ 5.0 Hz, N*H*CH_3), 5.81 (dddd, 1H, 1H, $^3J_{2',3'trans}$ 16.8 Hz, $^3J_{2',3'cis}$ 10.4 Hz, $^3J_{1'a,2'}$ 7.4 Hz, $^3J_{1'b,2'}$ 6.6 Hz, H-2'), 5.65 (dd, 1H, $^3J_{2,3}$ 7.0 Hz, H-2), 5.54 (dd, 1H, $^3J_{3,4}$ 3.1 Hz, H-3), 5.18-5.11 (m, 2H, H-3'a, H-3'b), 4.69 (dd, 1H, $^3J_{4,5}$ 4.6 Hz, H-4), 4.45 (d, 1H, H-5), 4.31 (ddd, 1H, $^3J_{1,2}$ 3.6 Hz, $^3J_{1,1'a}$ 4.3 Hz, $^3J_{1,1'b}$ 10.1 Hz, H-1), 2.89 (d, 1H, NHC*H*$_3$), 2.54-2.42, 2.38-2.28 (2m, 2H, H-1'a, H-1'b)

^{13}C NMR (CDCl$_3$, 75.5 MHz): δ 170.9, 165.6, 165.3 (3 x CO), 133.5 (C-2'), 118.7 (C-3'), 133.4, 133.39, 129.9, 129.8, 129.3, 129.1, 128.5, 128.4 (four signals are isochronic 2 x C_6H_5), 71.8 (C-1), 71.5 (C-5), 70.8 (C-3), 69.5 (C-2), 67.1 (C-4), 32.6 (C-1'), 25.7 ($NHCH_3$)

MS (CI, isobutane): *m/z* 440 $[M+H]^+$

$C_{24}H_{25}NO_7$ (439.46)	calcd:	C 65.59	H 5.73	N 3.19
	found:	C 65.70	H 5.81	N 3.00

4.7. DERIVATISATION OF C-ALLYL GROUP

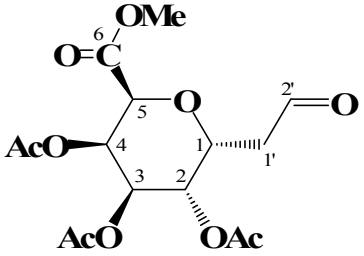
4.7.1. Oxydation of compound 57 *via* ozonolysis

Compound **57** (1.5 g, 4.19 mmol) was dissolved in dry CH_2Cl_2 (40 mL), and was treated with O_3 at -78˚C until the solution became light blue in colour (~2 h). Subsequently, PPh_3 (8.38 mmol) was added and the reaction mixture was allowed to warm up to room temperature, and stirred for 4 h. The reaction mixture was concentrated and the residue was purified by column chromatography (eluent ethyl acetate gradient 10→50% in petrol ether) to obtain compound **90** (995 mg, 66%).

4.7.2. Oxydation of compound 57 *via* osmium tetroxide/periodate

A stock solution of OsO_4 was prepared dissolving OsO_4 (1 g, 0.4 mmol) in 100 mL dioxan, and stored at 4°C. Before using, the solution was let to reach room temperature and was shaken. The solution OsO_4/dioxan (0.37 mL) was then added to a solution of compound **57** (1.3 g, 3.63 mmol) in dioxan (58 mL) and water (5.8 mL). The reaction mixture was kept for 1 h at room temperature. Sodium periodate (2.32 g, 10.8 mmol) was then added, and the reaction mixture was stirred for additional 16 h. The formed precipitate was filtered off, and washed with ethyl acetate. The combined filtrate and washings were concentrated and the residue was purified by column chromatography (eluent ethyl acetate gradient 10→50% in petrol ether) to obtain compound **90** (889 mg, 68%).

4.7.3. Methyl 2,3,4-tri-*O*-acetyl-1-deoxy-1-(2'-oxoethyl)-α-D-galactopyranuronate (90)



Yield: 889 mg, 68%, colourless syrup

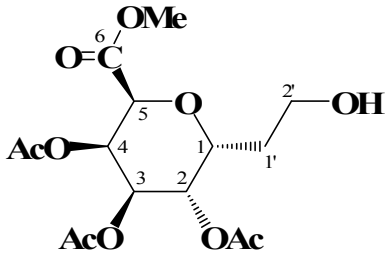
R_f 0.21 (eluent A_4)

^{1}H NMR (CDCl$_3$, 250.13 MHz): δ 9.78 (dd, H-2'), 5.52 (dd, 1H, $^3J_{4,5}$ 4.8 Hz, H-4), 5.25 (dd, 1H, $^3J_{2,3}$ 7.1 Hz, H-2), 5.17 (dd, 1H, $^3J_{3,4}$ 2.8 Hz, H-3), 5.09 (ddd, 1H, $^3J_{1,2}$ 3.9 Hz, $^3J_{1,1'a}$ 6.0 Hz, $^3J_{1,1'b}$ 7.4 Hz, H-1), 4.62 (d, 1H, H-5), 3.73 (s, 3H, OCH$_3$), 2.64–2.60 (m, 2H, $^3J_{1'a,2'}$ 1.8 Hz, $^3J_{1'b,2'}$ 2.4 Hz, H-1'a, H-1'b), 2.07, 2.06, 2.02 (3s, 9H, 3 x CH$_3$CO)

^{13}CNMR (CDCl$_3$, 62.9 MHz): δ 198.9 (C-2'), 169.7, 169.6, 169.5, 168.4 (4 x CO), 70.9, 68.7, 68.2, 67.7, 67.0 (C-1, C-2, C-3, C-4, C-5), 52.3 (OCH$_3$), 42.6 (C-1'), 20.6, 20.6, 20.6 (3 x *C*H$_3$CO)

$C_{15}H_{20}O_{10}$ (360.31)	calcd:	C 50.00	H 5.59
	found:	C 50.07	H 5.61

4.7.4. Methyl 2,3,4-tri-*O*-acetyl-1-deoxy-1-(2'-hydroxyethyl)-α-D-galactopyranuronate (91)



$NaBH_4$ (745 mg, 19.6 mmol) was added to a solution of aldehyde **90** (505 mg, 1.4 mmol) in abs. dichloromethane and methanol (1 : 2 v/v, 99 mL) under argon atmosphere at –78 °C. The reaction mixture was allowed to attain –2 °C over a period of 4 h, and an additional portion of $NaBH_4$ (532 mg, 14 mmol) was added. The mixture was stirred for additional 1.5 h at 5–10 °C, neutralized with acetic acid (6 mL) and concentrated to dryness. The residue was purified by flash chromatography (eluent A_4) to furnish compound **91**.

Yield: 220 mg, 43%, colourless syrup

$[\alpha]_D^{22}$ +59.2 (*c* 0.8, chloroform)

R_f 0.13 (eluent A_4)

^{1}H NMR (CDCl$_3$, 250.13 MHz): δ 5.66 (dd, 1H, $^3J_{4,5}$ 5.4 Hz, H-4), 5.17 (dd, 1H, $^3J_{2,3}$ 6.6 Hz, H-2), 5.14 (dd, 1H, $^3J_{3,4}$ 2.6 Hz, H-3), 4.71 (d, 1H, Hz, H-5), 4.42 (ddd, 1H, $^3J_{1,2}$ 3.4 Hz, $^3J_{1,1'a}$ 3.5 Hz, $^3J_{1,1'b}$ 10.8 Hz, H-1), 3.91 – 3.72 (m, 2H, H-2'a, H-2'b), 3.74 (s, 3H, OCH$_3$), 2.07, 2.06, 2.01 (3s, 9H, 3 x CH$_3$CO), 1.81–1.58 (m, 2H, H-1'a, H-1'b)

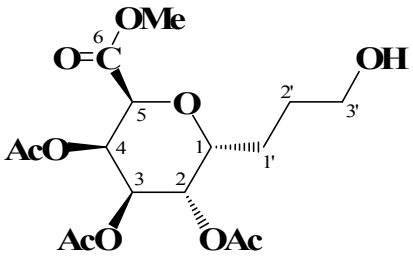
^{13}CNMR (CDCl$_3$, 75.5 MHz): δ 170.0, 169.7, 169.7, 169.6 (4 x CO), 71.7 (C-5), 70.6 (C-1), 70.4 (C-2), 69.6 (C-3), 67.0 (C-4), 59.1 (C-2'), 52.5 (OCH$_3$), 30.2 (C-2'), 30.6 (C-1'), 20.7, 20.6, 20.6 (3 x *C*H$_3$CO)

$C_{15}H_{22}O_{10}$ (362.33)	calcd:	C 49.72	H 6.12
	found:	C 49.99	H 6.21

4.7.5. Hydroboration-oxydation of 57 with borane tetrahydrofuran complex

Borane tetrahydrofuran complex solution in tetrahydrofuran (4 mL, 1 M) was added to a solution of compound **57** (459 mg, 1.38 mmol) in abs THF (30 mL) at ~0–4 °C for 5 min. After stirring for 1 h under an argon atmosphere at that temperature (TLC, eluent A_4) phosphate buffer (50 mL, pH 7.0) and H_2O_2 (10 mL) were successively added at ~0–4 °C and the reaction mixture was stirred overnight at room temperature (TLC, eluent A_3). The reaction mixture was then extracted with chloroform (3 x 30 mL), and the combined organic phase was dried and evaporated. The residue was purified by MPLC (eluent ethyl acetate gradient 0→66% in petrol ether) to yield **93** (220 mg, 42%) and **94** (34 mg, 7%).

4.7.6. Methyl 2,3,4-tri-*O*-acetyl-1-deoxy-1-(3'-hydroxypropyl)-α-D-galactopyranuronate (93)



Yield: 220 mg, 42%, colourless syrup

$[\alpha]_D^{23}$ +104.1 (*c* 1.3, chloroform)

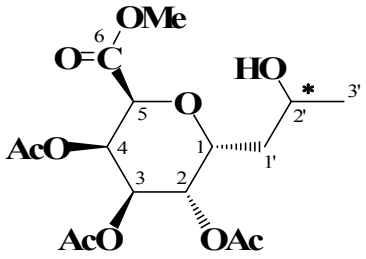
R_f 0.18 (eluent A_3)

^{1}HNMR (CDCl$_3$, 250.13 MHz): δ 5.46 (dd, 1H, $^3J_{4,5}$ 3.7 Hz, H-4), 5.11 (dd, 1H, $^3J_{2,3}$ 8.3 Hz, H-2 overlapped with H-3), 5.07 (dd, 1H, $^3J_{3,4}$ 2.7 Hz, H-3 overlapped with H-2), 4.48 (d, 1H, H-5), 4.30 ("dt", 1H, $^3J_{1,2}$ 3.3 Hz, $^3J_{1,1'a}$ 3.8 Hz, $^3J_{1,1'b}$ 10.4 Hz, H-1), 3.60 (s, 3H, OCH_3), 3.52 (t, 2H, *J* 5.7 Hz, H-3'a, H-3'b), 2.70 (br s, 1H, OH), 1.94, 1.87 (2s, 9H, 3 x CH_3CO), 1.70-1.38 (m, 4H, H-1'a, H-1'b, H-2'a, H-2'b)

^{13}CNMR (CDCl$_3$, 62.9 MHz): δ 169.7, 169.6, 169.5, 168.3 (4 x CO), 72.2 (C-1), 69.5 (C-5), 68.4 (C-2), 67.9 (C-3), 67.8 (C-4), 61.5 (C-3'), 52.0 (OCH_3), 28.1 (C-1'), 22.6 (C-2'), 20.4, 20.2, 20.2 (3 x *C*H_3CO)

$C_{16}H_{24}O_{10}$ (376.36)	calcd:	C 51.06	H 6.43
	found:	C 51.00	H 6.49

4.7.7. Methyl 2,3,4-tri-*O*-acetyl-1deoxy-1-(2'-hydroxypropyl)-α-D-galactopyranuronate (94)



Yield: 34 mg, 7%, colourless syrup ()

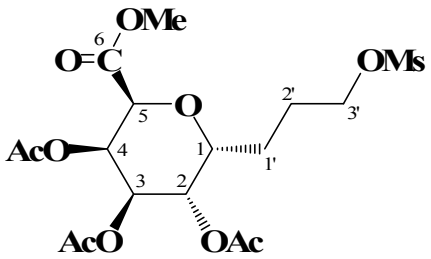
R_f 0.34 (eluent A_3)

^{1}HNMR (CDCl$_3$, 500.13 MHz): δ 5.64 (dd, 1H, $^3J_{4,5}$ 5.9 Hz, H-4A), 5.62 (dd, 1H, $^3J_{4,5}$ 5.7 H, H-4B), 5.16 (dd, 1H, $^3J_{2,3}$ 6.9 Hz, H-2A), 5.13–5.10 (m, 3H, $^3J_{3,4}$ 2.7 Hz [A], $^3J_{3,4}$ 1.9 Hz [B], H-2B, H-3A, H-3B), 4.71 (d, 1H, H-5A overlapped with H-5B), 4.70 (d, 1H, H-5B overlapped with H-5A), 4.59 (m, 1H, $^3J_{1,2}$ 3.8 Hz, H-1A), 4.57 (m, 1H, H-1B), 4.13–4.05 (m, 2H, H-2'$^*_A$, H-2'$^*_B$), 3.731 (s, 3H, OCH$_3$A), 3.727 (s, 3H, OCH$_3$B), 2.82 (br, OH), 2.08, 2.06, 2.05, 2.00, 2.00 (5s, 18H, 6 x CH$_3$CO), 1.74 (ddd, 1H, ^3J 8.8 Hz, ^3J 10.8 Hz, ^2J 14.8 Hz, H-1'a[A]), 1.66 (ddd, 1H, ^3J 2.8 Hz, ^3J 11.5 Hz, ^2J 14.2 Hz, H-1'a[B]), 1.47 (ddd, 1H, ^3J 2.1 Hz, ^3J 3.4 Hz, ^2J 14.8 Hz, H-1'b[A]), 1.35 (ddd, 1H, ^3J 2.8 Hz, ^3J 10.3 Hz, ^2J 14.2 Hz, H-1'b[B]); 1.20, 120 (2 x overlapped d, 6H, $^3J_{H2',H3'}$ 6.3 Hz, H-3' A, H.3' B)

^{13}CNMR (CDCl$_3$, 125.8 MHz): δ 170.1, 170.0, 169.9, 169.6, 169.6, 168.9 (two signals are isochronic, 8 x CO), 73.3 (C-1A), 71.7 (C-5B), 71.3 (C-5A), 70.6 (C-2B), 69.9 (C-2A), 69.7 (C-3B), 69.4 (C-1B), 69.1 (C-3A), 67.3 (C-2'*A), 66.9 (C-4A), 66.9 (C-4B), 63.2 (C-2'*B), 52.4 (OCH$_3$B), 52.4 (OCH$_3$A), 37.4 (C-1'B), 36.5 (C-1'A), 23.2 (C-3'A), 22.8 (C-3'B), 20.7, 20.6, 20.6, 20.6 (6 x *C*H$_3$CO)

$C_{16}H_{24}O_{10}$ (376.36)	calcd:	C 51.06	H 6.43
	found:	C 51.14	H 6.59

4.7.8. Methyl 2,3,4-tri-*O*-acetyl-1-deoxy-1-(3'-[mesyloxy]propyl)-α-D-galactopyranuronate (95)



Methanesulfonyl chloride (0.42 mL, 5.43 mmol) was added dropwise to a solution of **93** (1.01 g, 2.68 mmol) in abs. dichloromethane (45 mL) and triethylamine (3.7 mL) at room temperature. After stirring for 7 h at ambient temperature under an argon atmosphere (TLC, eluent A_3) the reaction mixture was diluted with chloroform (300 mL), washed with satd aq $NaHCO_3$ (2 x 100 mL) and brine (100 mL), dried and evaporated. The crude material was purified by flash chromatography on silica gel (eluent A_4) to get compound **95.**

Yield: 1.20 g, 98%, colourless syrup

$[\alpha]_D^{22}$ +90.1 (*c* 1.8, chloroform)

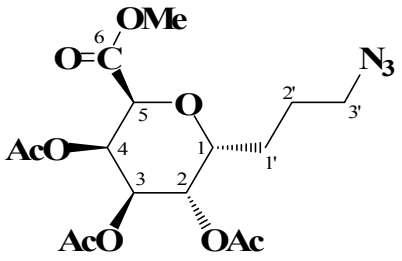
R_f 0.42 (eluent A_3)

^{1}HNMR ($CDCl_3$; 300.13 MHz): δ 5.57 (dd, 1H, $^3J_{4,5}$ 4.6 Hz, H-4), 5.19 (dd, 1H, $^3J_{2,3}$ 7.4 Hz, H-2 overlapped with H-3), 5.16 (dd, 1H, $^3J_{3,4}$ 2.7 Hz, H-3 overlapped with H-2), 4.59 (d, 1H, H-5), 4.45 (dt, 1H, $^3J_{1,2} = {}^3J_{1,1a'} = 3.2$ Hz, $^3J_{1,1'b}$ 10.6 Hz, H-1), 4.38–4.24 (m, 2H, H-3'a, H-3'b), 3.72 (s, 3H, OCH_3), 3.00 (s, 3H, SO_2CH_3), 2.09, 2.06, 2.01 (3s, 9H, 3 x CH_3CO), 2.00–1.80 (m, 2H, H-2'a, H-2'b), 1.76–1.65, 1.61–1.49 (2m, 2H, H-1'a, H-1'b)

^{13}CNMR ($CDCl_3$, 75.5 MHz): δ 169.8, 169.7, 169.6, 168.6 (4 x CO), 71.6 (C-1), 70.4 (C-5), 69.28 (C-3'), 69.25 (C-2), 68.4 (C-3), 67.4 (C-4), 52.2 (OCH_3), 37.3 (SO_2CH_3), 25.4 (C-2'), 23.5 (C-1'), 20.7, 20.6, 20.7 (3 x *C*H_3CO)

$C_{17}H_{26}O_{12}S$ (454.45)	calcd:	C 44.93	H 5.77	S 7.06
	found:	C 45.06	H 5.80	S 7.29

4.7.9. Methyl 2,3,4-tri-*O*-acetyl-1-deoxy-1-(3'-azidopropyl)-α-D-galactopyranuronate (96)



Sodium azide (2.11 g, 32.5 mmol) was added to a solution of **95** (1.20 g, 2.64 mmol) in abs. DMF (25 mL) and 18-crown-6 (731 mg, 2.77 mmol). After stirring for 55 h at ambient temperature under argon (TLC, eluent A_3) the reaction mixture was diluted with ethyl acetate (200 mL), washed with water (2 x 200 mL), dried and evaporated. The residue was purified by flash chromatography on silica gel (eluent ethyl acetate gradient 16→25% in petrol ether) to get azido derivative **96**.

Yield: 878 mg, 82%, colourless syrup

$[\alpha]_D^{22}$ +104.4 (*c* 1.13, chloroform)

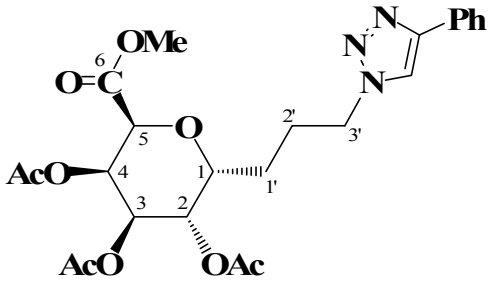
R_f 0.16 (eluent A_6)

^{1}HNMR ($CDCl_3$; 300.13 MHz): δ 5.59 (dd, 1H, $^3J_{4,5}$ 4.2 Hz, H-4), 5.22 (dd, 1H, $^3J_{2,3}$ 7.8 Hz, H-2), 5.17 (dd, 1H, $^3J_{3,4}$ 2.9 Hz, H-3), 4.57 (d, 1H, H-5), 4.43 (ddd, 1H, $^3J_{1,2}$ 4.0 Hz, $^3J_{1,1a'}$ 3.3 Hz, $^3J_{1,1'b}$ 9.7 Hz, H-1), 3.73 (s, 3H, OCH_3), 3.37 (t, 2H, $^3J_{2',3'}$ 6.3 Hz, H-3'a, H-3'b), 2.09, 2.07, 2.01 (3s, 9H, 3 x CH_3CO), 1.84–1.46 (m, 4H, H-1'a, H-1'b, H-2'a, H-2'b)

^{13}CNMR ($CDCl_3$, 75.5 MHz): δ 169.8, 169.7, 169.6, 168.5 (4 x CO), 71.9 (C-1), 70.2 (C-5), 69.1 (C-2), 68.4 (C-3), 67.7 (C-4), 52.2 (OCH_3), 50.9 (C-3'), 25.1 (C-1'), 24.3 (C-2'), 20.7, 20.6, 20.6 (3 x *C*H_3CO)

$C_{17}H_{26}N_3O_9$ (401.37)	calcd:	C 47.88	H 5.78	N 10.47
	found:	C 48.04	H 5.96	N 10.38

4.7.10. Methyl 2,3,4-tri-*O*-acetyl-1-deoxy-1-(3' 4-phenyl-1,2,3-triazolylopropyl)-α-D-galactopyranuronate (97)



Copper (II) sulfate x·5 H_2O (4 mg, 20 μmol), L-(+)-ascorbic acid (28 mg, 0.16 mmol) and phenylacetylene (90 μL, 0.82 mmol) were added to a solution of azide **96** (300 mg, 0.75 mmol) in water (4.5 mL) and DMF (1 mL). The reaction mixture was heated at 75 °C for 48 h (TLC, eluent A_3). The solvents were removed under high vacuum and the residue was purified by flash chromatography on silica gel (eluent A_3) to provide **97**.

Yield: 330 mg, 87%, amorphous solid

$[\alpha]_D^{23}$ +83.1 (*c* 1.6, chloroform)

R_f 0.34 (eluent A_3)

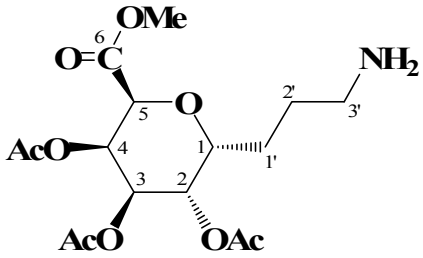
^{1}H NMR ($CDCl_3$; 300.13 MHz): δ 7.83–7.78, 7.44–7.38, 7.34–7.28 (3m, 6H, NCH=C, C_6H_5), 5.57 (dd, 1H, $^3J_{4,5}$ 4.8 Hz, H-4), 5.18–5.12 (m, 2H, $^3J_{2,3}$ 7.3 Hz, $^3J_{3,4}$ 2.3 Hz, H-2, H-3), 4.60 (d, 1H, H-5), 4.56–4.42 (m, 3H, H-1, H-3'a, H-3'b) 3.72 (s, 3H, OCH_3), 2.23–2.06 (m, 2H, H-2'a, H-2'b), 2.06, 2.05, 2.01 (3s, 9H, 3 x CH_3CO), 1.75–1.62, 1.52–1.40 (2m, 2H, H-1'a, H-1'b)

^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 169.9, 169.7, 169.5 (3 x CH_3CO), 168.8 (C-6), 147.8 (br, HC=*C*), 130.6, 128.8, 128.1, 125.7 (C_6H_5, two signals are isochronic), 119.6 (–H*C*=C), 71.7 (C-1), 70.6 (C-5), 69.4, 68.5 (C-2, C-3), 67.3 (C-4), 52.2 (OCH_3), 49.6 (C-3'), 26.6 (C-1'), 24.4 (C-2'), 20.7, 20.6, 20.6 (3 x CH_3CO)

MS (EI), *m/z* 503 $[M]^+$ (5.98 %).

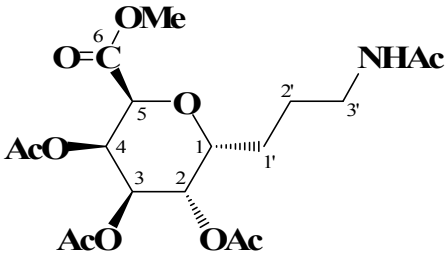
$C_{24}H_{29}N_3O_9$ (503.50)	calcd:	C 57.25	H 5.81	N 8.35
	found:	C 57.32	H 5.96	N 8.28

4.7.11. Methyl 2,3,4-tri-*O*-acetyl-1-deoxy-1-(3'-aminopropyl)-α-D-galactopyranuronate (98)



10% Palladium on charcoal (18 mg) was added to a solution of compound **96** (110 mg, 0.274 mmol) in abs. ethyl acetate–methanol (9 mL, 1:1) under an argon atmosphere and the reaction mixture was stirred for 6 h (TLC, eluent A_6) under a hydrogen atmosphere at ambient temperature and then filtered through Celite. The filtrate was concentrated to dryness to provide compound **98** as s colourless glassy syrup. The purification was not possible since the compound might be not stable. Further reactions were performed with crude product.

4.7.12. Methyl 2,3,4-tri-*O*-acetyl-1-deoxy-1-(3'-acetamidopropyl)-α-D-galactopyranuronate (99)



Acetic anhydride (0.5 mL) was added to a solution of fresh prepared crude amine **98** (100 mg, 0.266 mmol) in abs. pyridine (1 mL) at –15 ˚C. After stirring for 17 h at ambient temperature under an argon atmosphere (TLC, eluent E_1), methanol (2 mL) was added, and stirring of the reaction mixture was continued for additional 1 h. After concentration of the reaction mixture, the residue was repeated coevaporated with toluene (3 x) and then purified by flash

chromatography on silica gel (eluent methanol gradient 9→17% in ethyl acetate) and then by HPLC to provide compound **99**.

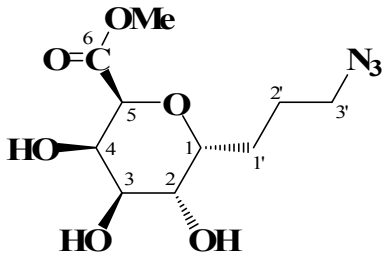
Yield: 16 mg, 14%, colourless foam

$[\alpha]_D^{23}$ +81.5 (*c* 1.1, chloroform)

^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 5.76 (br, 1H, NH), 5.57 (dd, 1H, $^3J_{4,5}$ 4.3 Hz, H-4), 5.19 (dd, 1H, $^3J_{2,3}$ 7.6 Hz, H-2 overlapped with H-3), 5.15 (dd, 1H, 1H, $^3J_{3,4}$ 2.5 Hz, H-3 overlapped with H-2), 4.20 (d, 1H, H-5), 4.42 ("dt", 1H, $^3J_{1,2}$ =$^3J_{1,1'a}$ = 3.4 Hz, $^3J_{1,1'b}$ 9.9 Hz, H-1), 3.72 (s, 3H, OCH_3), 3.27 ("ddd", 1H, $^3J_{3',NH}$ 5.8 Hz, $^3J_{3',2'a}$.6.5 Hz, $^3J_{3',2'b}$ 7.3 Hz, H-3'a, H-3'b), 2.07, 2.06, 2.00 (3s, 9H, 3 x CH_3CO), 1.94 (s, 3H, C*H*$_3$CONH), 1.78–1.35 (m, 4H, H-1'a, H-1'b, H-2'a, H-2'b)

^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 170.1, 169.8, 169.7, 169.6, 168.7 (5 x CO), 72.0 (C-1), 70.2 (C-5), 69.1 (C-2), 68.4 (C-3), 67.6 (C-4), 52.2 (OCH_3), 39.2 (C-3'), 25.6, 24.9 (C-1', C-2'), 23.2 (*C*H_3CONH), 20.7, 20.6, 20.6 (3 x *C*H_3CO)

4.7.13. Methyl 1-deoxy-1-(3'-azidopropyl)-α-D-galactopyranuronate (100)



Methanolic hydrogen chloride (prepared by adding of 560 μmL acetyl chloride to 27 mL ice-cold dry methanol) was added to peracetylated azide **96** (308 mg, 0.767 mmol), and the reaction mixture was kept for 24 h at room temperature under an argon atmosphere (TLC, eluent C_7). The reaction mixture was neutralized with methanolic ammonia (7 N, 1.2 mL). After stirring for 15 min, the ammonium salts were filtered off, washed with methanol, and the filtrate and washings were combined and concentrated. The residue was applied to a column of silica gel (eluent methanol gradient 4→9% in chloroform) to provide **100**.

Yield: 189 mg, 89%, colourless crystals

Melting point: 110–111 °C (from methanol)

$[\alpha]_D^{23}$ +71.1 (*c* 0.84, methanol)

R_f 0.16 (eluent C_7)

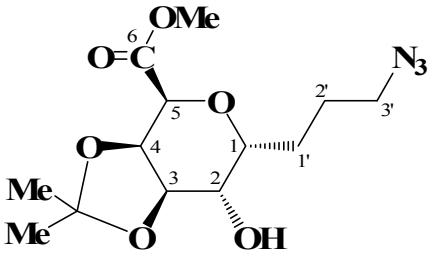
^{1}HNMR (CD_3OD, 500.13 MHz): δ 4.34 (d, $^3J_{4,5}$ 3.8 Hz, H-5), 4.18 ("t", 1H, $^3J_{3,4}$ 3.0 Hz, H-4), 4.10 ("dt", 1H, $^3J_{1,2}$ 4.4 Hz, $^3J_{1,1'a}$ 3.8 Hz, $^3J_{1,1'b}$ 9.9 Hz, H-1), 3.81 (dd, 1H, $^3J_{2,3}$ 7.9 Hz,

H-2), 3.75 (s, 3H, OCH_3), 3.74 (dd, 1H, $^3J_{3,4}$ 3.2 Hz, H-3), 3.36 (t, 2H, ^3J 6.3 Hz, H-3'a, H-3'b), 1.77–1.61 (m, 4H, H-1'a, H-1'b, H-2'a, H-2'b)

^{13}CNMR (CD_3OD, 75.47 MHz): δ 172.6 (C-6), 75.2 (C-1), 73.1 (C-5), 71.9 (C-3), 70.7 (C-2), 70.4 (C-4), 52.4 (OCH_3), 52.2 (C-3'), 26.6, 24.3 (C-1', C-2')

$C_{10}H_{17}N_3O_6$ (275.26)	calcd:	C 43.63	H 6.23	N 15.27
	found:	C 43.67	H 6.41	N 15.19

4.7.14. Methyl 1-deoxy-3,4-*O*-isopropylidene-1-(3'-azidopropyl)-α-D-galactopyranuronate (101)



p-Toluenesulfonic acid monohydrate (9 mg) was added to the solution of compound **100** (65 mg, 0.236 mmol) in 2,2-dimethoxypropane (472 μmL) and dry acetone (2 mL) and the reaction mixture was stirred for 12 h at rt (TLC, eluent A_3). The mixture was then passed through a layer of alkaline aluminia, the alkaline aluminia was washed with acetone, and the filtrate and washings were combined. The solvent was removed and the residue was purified by flash chromatography on silica gel (eluent ethyl acetate gradient 10→66% in petrol ether) to provide compound **101**.

Yield: 74 mg, 99%, colourless syrup

$[\alpha]_D^{23}$ +7.3 (*c* 0.99, chloroform)

R_f 0.40 (eluent A_3)

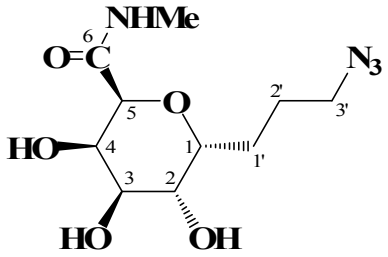
^{1}HNMR ($CDCl_3$, 250.13 MHz): δ 4.65–4.60 (m, 2H, H-4, H-5), 4.34 (ddd, 1H, $^3J_{3,4}$ 8.2 Hz, *J* 1.2 Hz, H-3), 4.10 (ddd, 1H, $^3J_{1,2}$ 2.5 Hz, $^3J_{1,1'a}$ 5.1 Hz, $^3J_{1,1'b}$ 7.6 Hz, H-1), 3.80 (ddd, 1H, $^3J_{2,3}$ 3.2 Hz, *J* 0.6 Hz, H-2 overlapped with OCH_3), 3.79 (s, 3H, OCH_3), 3.39–3.33 (m, 2H, H-3'a, H-3'b), 1.96 (br s, 1H, OH), 1.86–1.55 (4H, H-1'a, H-1'b, H-2'a, H-2'b), 1.47, 1.32 (2s, 6H, $[CH_3]_2C$)

^{13}CNMR ($CDCl_3$, 250.13 MHz): δ 169.9 (C-6), 110.0 ($[CH_3]_2C$), 74.4 (C-3), 72.9 (C-4), 71.0 (C-1), 70.1 (C-5), 68.6 (C-2), 52.3 (OCH_3), 51.4 (C-3'), 27.5, 25.1 (C-1', C-2'), 26.6, 24.5 ($[CH_3]_2C$)

$C_{13}H_{21}N_3O_6$ (315.32)	calcd:	C 49.52	H 6.71	N 13.33

found: C 49.61 H 6.79 N 13.06

4.7.15. N-Methyl 1-deoxy-1-(3'-azidopropyl)-α-D-galactopyranuronamide (102)



33% ethanolic methylamine (1 mL) was added to a solution of ester **100** (121 mg, 0.44 mmol) in abs. methanol (3 mL), and the reaction mixture was stired at ambient temperature. After 2 h (TLC, eluent C_7) the solvents were removed under high vacuum to provide analytically pure methylamido derivative **102**.

Yield: 120 mg, 100%, colourless crystals

Melting point: 168–169 °C (from methanol)

$[\alpha]_D^{24}$ +38.5 (*c* 0.85, methanol)

$\mathbf{R}_f$ 0.07 (eluent C_7)

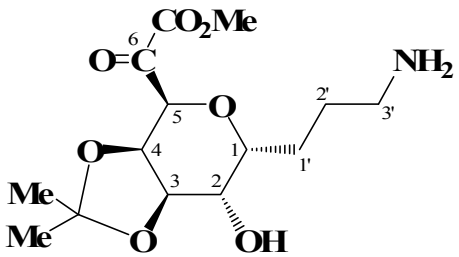
^{1}HNMR (CD_3OD, 300.13 MHz): δ 4.18 (dd, 1H, $^3J_{4,5}$ 2.1 Hz, H-4), 4.10–4.03 (m, 2H, $^3J_{1,2}$ 5.8 Hz, H-1, H-5), 3.94 (dd, 1H, $^3J_{2,3}$ 9.4 Hz, H-2), 3.67 (dd, 1H, $^3J_{3,4}$ 3.3 Hz, H-3), 3.35 (t, 2H, ^3J 6.3 Hz, H-3'a, H-3'b), 2.77 (s, 3H, NHCH_3), 1.77–1.54 (m, 4H, H-1'a, H-1'b, H-2'a, H-2'b)

^{13}CNMR (CD_3OD, 75.47 MHz): δ 172.6 (C-6), 77.4 (C-1), 73.3 (C-5), 71.5 (C-3), 71.0 (C-4), 69.4 (C-2), 52.3 (C-3'), 26.6, 22.3 (C-1', C-2'), 26.0 (CH_3NH)

MS (CI, isobutane): *m/z* 275 $[M+H]^+$.

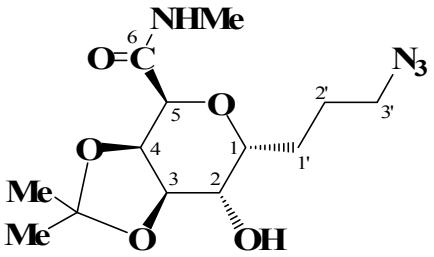
$C_{10}H_{18}N_4O_5$ (274.27)	calcd:	C 43.79	H 6.61	N 20.43
	found:	C 43.99	H 6.84	N 20.31

4.7.16. N-Methyl 1-deoxy-3,4-*O*-isopropylidene-1-(3'-aminopropyl)-α-D-galactopyranuronamide (103)



10% Palladium on charcoal (10 mg) was added to a solution of compound **101** (49 mg, 0.155 mmol) in abs. ethyl acetate–methanol (5 mL, 1:1) under an argon atmosphere and the reaction mixture was stirred for 5 h (TLC, eluent C_7) under a hydrogen atmosphere at ambient temperature and then filtered through Celite. The filtrate was concentrated to dryness to provide compound **103** as a colourless syrup which was used for the next step without further purification and characterization.

4.7.17. N-Methyl 1-deoxy-3,4-*O*-isopropylidene-1-(3'-azidopropyl)-α-D-galactopyranuronamide (104)



p-Toluenesulfonic acid monohydrate (12 mg) was added to the solution of compound **102** (88 mg, 0.32 mmol) in 2,2-dimethoxypropane (640 μmL) and dry acetone (2.7 mL) and the reaction mixture was stirred for 15 h at rt (TLC, eluent C_7). The mixture was then passed through a layer of alkaline aluminia, the alkaline aluminia was washed with acetone, and the filtrate and washings were combined. The solvent was removed and the residue was purified by flash chromatography on silica gel (eluent ethyl acetate gradient 75→80% in petrol ether) to provide compound **104**.

Yield: 78 mg, 78%, colourless syrup

$[\alpha]_D^{22}$ –5.6 (*c* 1.0, chloroform)

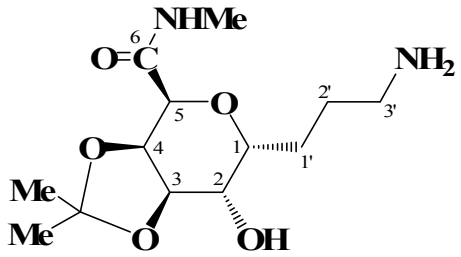
$\mathbf{R}_f$ 0.40 (eluent C_7)

^{1}HNMR ($CDCl_3$, 500.13 MHz): δ 6.71 (q, 1H, $^3J_{NH,CH3}$ 5.0 Hz, NH), 4.70 (dd, 1H, $^3J_{4,5}$ 1.9 Hz, H-4), 4.49 (d, 1H, H-5), 4.32 (dd, 1H, $^3J_{3,4}$ 7.6 Hz, H-3), 4.01 (ddd, 1H, $^3J_{1,2}$ 1.9 Hz, $^3J_{1,1'a}$ 5.2 Hz, $^3J_{1,1'b}$ 8.3 Hz, H-1), 3.71 ("t", 1H, $^3J_{2,3}$ 2.8 Hz, H-2), 3.37–3.28 (m, 2H, H-3'a, H-3'b), 2.89 (br, 1H, OH), 2.84 (d, 3H, NHCH_3), 1.81–1.55 (m, 4H, H-1'a, H-1'b, H-2'a, H-2'b), 1.43, 1.31 (2s, 6H, $[CH_3]_2C$)

^{13}CNMR ($CDCl_3$, 75.47 MHz): δ 170.6 (C-6), 109.6 ($[CH_3]_2C$), 73.9 (C-3), 72.7 (C-4), 70.7 (C-1), 70.5 (C-5), 68.6 (C-2), 51.4 (C-3'), 28.6, 25.1 (C-1', C-2'), 26.5, 24.1 ($[CH_3]_2C$), 25.7 (CH_3NH)

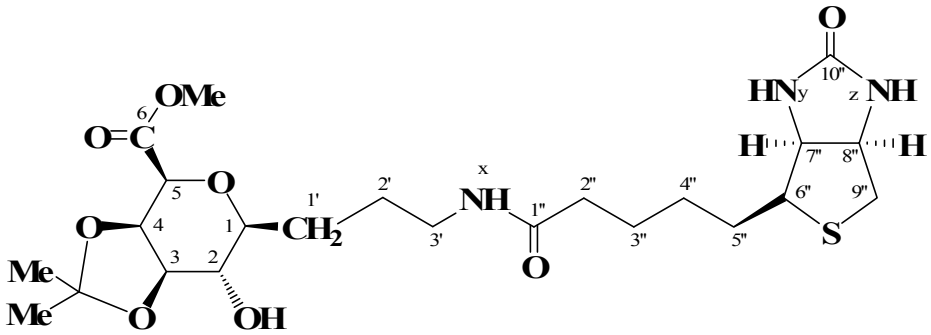
MS (CI, isobutane): *m/z* 315 $[M+H]^+$

4.7.18. N-Methyl 1-deoxy-3,4-*O*-isopropylidene-1-(3'-aminopropyl)-α-D-galactopyranuronamide (105)



10% Palladium on charcoal (10 mg) was added to a solution of compound **104** (51 mg, 0.162 mmol) in abs. ethyl acetate–methanol (5 mL, 1:1) under an argon atmosphere and the reaction mixture was stirred for 5 h (TLC, eluent C_7) under a hydrogen atmosphere at ambient temperature and then filtered through Celite. The filtrate was concentrated to dryness to provide compound **105** as a colourless syrup which was used for the next step without further purification and characterization.

4.7.19. Methyl 1-deoxy-3,4-*O*-isopropylidene-1-(3'-biotinylamidopropyl)-α-D-galactopyranuronate (106)



A solution of crude amine **103** in abs. *N,N*-dimethylformamide (1.3 mL) was added to a solution of D-(+)-biotin (41 mg, 0.166 mmol) and diisopropylethylamine (DIPEA) (60 μL) in abs. *N,N*-dimethylformamide (1.6 mL) at ambient temperature under an argon atmosphere. Then the mixture was cooled to 0 °C and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC) (32 mg, 0.166 mmol) and *N*-hydroxybenzotiazole (1-HOBT) (45 mg, 0.33 mmol) were added. The reaction mixture was stirred at 0 °C for 2.5 h followed by stirring at rt for 24 h. The solvent was then removed *in vacuo* and the residue was purified by by reversed-phase HPLC on a C_{18} silica gel (eluent 25% acetonitrile in water) to yield biotinylamide **106**.

Yield: 48 mg, 60% over two steps, white amorphous solid

$[\alpha]_D^{22}$ +45.3 (*c* 1.5, dimethyl sulfoxide)

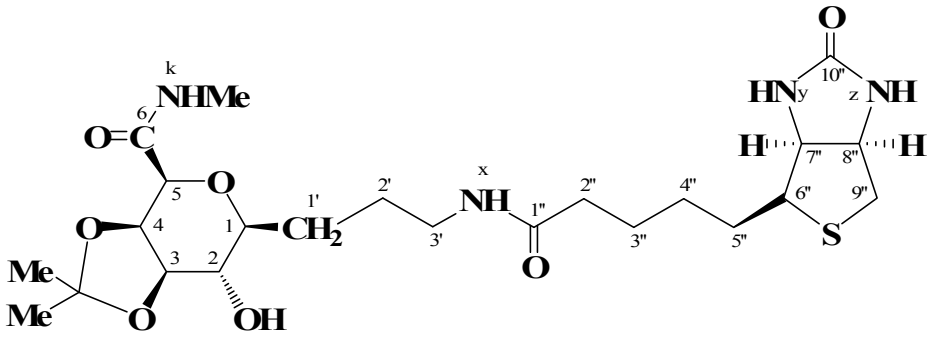
^{1}H NMR (DMSO-d$_6$, 500 MHz): δ 7.74 (t, 1H, $^3J_{NH,H}$ 5.6 Hz, NH_X), 6.40 (br, 1H, NH_Y), 6.34 (br, 1H, NH_Z), 5.38 (br, OH), 4.53 (dd, 1H, $^3J_{4,5}$ 2.2 Hz, H-4), 4.51 (d, 1H, H-5), 4.29 (m, 1H, $^3J_{8'',9''a}$ 5.0 Hz, H-8''), 4.25 (dd, 1H, $^3J_{3,4}$ 7.3 Hz, H-3), 4.12 (ddd, 1H, $^3J_{7'',NHy}$ 2.2 Hz, $^3J_{7'',8''}$ 7.7 Hz, H-7''), 3.79 (ddd, 1H, $^3J_{1,2}$ 2.2 Hz, $^3J_{1,1'a}$ 4.7 Hz, $^3J_{1,1'b}$ 8.2 Hz, H-1), 3.64 (s, 3H, OCH_3), 3.55 ("t", 1H, $^3J_{2,3}$ 3.2 Hz, H-2), 3.09 (ddd, 1H, $^3J_{6'',7''}$ 4.7 Hz, H-6''), 3.05–2.99 (m, 2H, H-3'a, H-3'b), 2.81 (dd, 1H, $^3J_{9''a,9''b}$ 12.3 Hz, H-9''a), 2.56 (d, 1H, H-9''b), 2.03 (t, 2H, $^3J_{2'',3''}$ 7.3 Hz, H-2''a, H-2''b), 1.64–1.22 (m, 10H, $^3J_{5''a,6''}$ 8.4 Hz, $^3J_{5''b,6''}$ 6.1 Hz, H-1'a, H-1'b, H-2'a, H-2'b, H-3''a, H-3''b, H-4''a, H-4''b, H-5''a, H-5''b), 1.34, 1.23 (2 x s, 6H, $[CH_3]_2C$)

^{13}C NMR (DMSO-d$_6$, 125.7 MHz): δ 171.8 (C-1''), 169.6 (C-6), 162.7 (C-10''), 108.9 ($[CH_3]_2C$), 73.8 (C-3), 73.0 (C-4), 70.8 (C-1), 69.2 (C-5), 66.7 (C-2), 61.0 (C-7''), 59.2 (C-8''), 55.4 (C-6''), 51.6 (OCH_3), 39.8 (C-9''), 38.5 (C-3' rotamer), 38.3 (C-3' rotamer), 35.2 (C-2'' rotamer), 35.2 (C-2''rotamer), 28.2, 28.0, 27.8, 25.3, 25.3 (C-1', C-2', C-3'', C-4'', C-5''), 26.4,24.4 ($[CH_3]_2C$)

MS (EI), *m/z* 515.2 $[M]^+$

HRMS (EI), calcd for $C_{23}H_{37}O_8N_3S$ (M^+) 515.22959. Found 515.229547

4.7.20. N-Methyl 1-deoxy-3,4-*O*-isopropylidene-1-(3'-biotinylamidopropyl)-α-D-galactopyranuronamide (107)



A solution of amine **105** in abs. *N,N*-dimethylformamide (1.4 mL) was added to a solution of D-(+)-biotin (43 mg, 0.173 mmol) and diisopropylethylamine (DIPEA) (100 μL) in abs. *N,N*-dimethylformamide (1.7 mL) at ambient temperature under an argon atmosphere. Then the mixture was cooled to 0 °C and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC) (33 mg, 0.173 mmol) and *N*-hydroxybenzotiazole (1-HOBT) (47 mg, 0.35 mmol) were added. The reaction mixture was stirred at 0 °C for 2.5 h followed by stirring at rt for 24 h. The solvent was then removed *in vacuo* and the residue was purified by by reversed-phase HPLC on a C_{18} silica gel (eluent 25% acetonitrile in water) to yield biotinylamide **107**.

Yield: 54 mg, 65% over two steps, white amorphous solid

$[\alpha]_D^{23}$ +43.9 (*c* 1.7, dimethyl sulfoxide)

^{1}H NMR (DMSO-d$_6$, 500 MHz): δ 7.77 (t, 1H, $^3J_{NH,H}$ 5.5 Hz, NH_X), 7.48 (q, 1H, $^3J_{NH,CH3}$ 4.6, NH_K), 6.39 (br, 1H, NH_Y), 6.34 (br, 1H, NH_Z), 5.31 (d, 1H, $^3J_{OH,H-2}$ 4.7 Hz, OH), 4.54 (dd, 1H, $^3J_{4,5}$ 1.9 Hz, H-4), 4.29 (dddd, 1H, $^3J_{H,NH}$ 1.1 Hz, $^3J_{8'',9''a}$ 5.0 Hz, H-8''), 4.22 (d, 1H, H-5), 4.19 (dd, 1H, $^3J_{3,4}$ 7.6 Hz, H-3), 4.11 (ddd, 1H, $^3J_{7'',NHy}$ 1.7 Hz, $^3J_{7'',8''}$ 7.7 Hz, H-7''), 3.83 (ddd, 1H, $^3J_{1,2}$ 1.9 Hz, $^3J_{1,1'a}$ 5.4 Hz, $^3J_{1,1'b}$ 8.2 Hz, H-1), 3.46 (dd, $^3J_{2,3}$ 2.7 Hz, 1H, H-2), 3.09 (ddd, 1H, $^3J_{6'',7''}$ 4.5 Hz, H-6''), 3.02 (m, 2H, H-3'a, H-3'b overlapped with H-6''), 2.81 (dd, 1H, $^3J_{9''a,9''b}$ 12.3 Hz, H-9''a), 2.62 (s, 3H, NHCH_3), 2.57 (d, 1H, H-9''b), 2.04 (t, 2H, $^3J_{2'',3''}$ 7.4 Hz, H-2''a, H-2''b), 1.64–1.21 (3m, 10H, $^3J_{5''a,6''}$ 8.5 Hz, $^3J_{5''b,6''}$ 6.0 Hz, H-1'a, H-1'b, H-2'a, H-2'b, H-3''a, H-3''b, H-4''a, H-4''b, H-5''a, H-5''b), 1.34, 1.23 [2s, 6H, (CH_3)$_2$C]

^{13}C NMR (DMSO-d$_6$, 125.7 MHz): δ 171.8 (C-1''), 169.3 (C-6), 162.7 (C-10''), 108.3 ([CH$_3$]$_2$*C*), 73.8 (C-3), 72.5 (C-4), 70.5 (C-1), 69.9 (C-5), 67.5 (C-2), 61.0 (C-7''), 59.2 (C-8''), 55.4 (C-6''), 39.8 (C-9''), 38.4 (C-3' rotamer), 38.3 (C-3' rotamer), 35.2 (C-2'' rotamer), 35.2 (C-2''rotamer), 28.2, 28.1, 28.0, 25.3, 25.2 (C-1', C-2', C-3'', C-4'', C-5''), 25.4, 25.3 (CH$_3$NH rotamers), 26.6, 24.2 ([*C*H$_3$]$_2$C)

MS (EI), *m/z* 514 $[M]^+$

5. REFERENCES

1. Davis, B. G. *Chem. Biol.*, **2007**, *2*, Interview: Sugar solutions
2. Bacic, A.; Harris, P. J.; Stone, B. A. *The Biochemistry of Plants*, **1988**, *14*, 297–371.
3. Willats, W. G. T.; McCartney, L.; Mackie, W.; Knox, J. P. *Plant Mol. Biol.*, **2001**, *47*, 9–27.
4. Ridley, B. L.; O'Neill, M. A.; Mohnen, D. *Phytochemistry*, **2001**, *57*, 929–967.
5. Mohnen, D. *Comprehensive Natural Products Chemistry*, **1999**, *3*, 497–527.
6. Smith, B. G.; Harris, P. J. *Biochem. Syst. Ecol.*, **1999**, *27*, 33–53.
7. Cosgrove, D. J. *Nat. Rev. Mol. Cell Bio.*, **2005**, *6(11)*, 850–861.
8. http://micro.magnet.fsu.edu/cells/plants/cellwall.html
9. O'Neill, M. A.; Albersheim, P.; Darvill, A. G. *Methods in Plant Biochemistry*, **1990**, *2*, 415–441.
10. Vincken, J. P.; Schols, H. A.; Oomen, R. J.; McCann, M. C; Ulvskov, P.; Voragen, A. G.; Visser, R. G. *Plant Physiol.*, 2003, *132*, 1781–1789.
11. McNeil, M.; Darvill, A. G.; Fry, S. C.; Albersheim, P. *Annu. Rev. Biochem.*, **1984**, *53*, 625–663.
12. Schols, H., A.; Voragen, A. G., J. *Progress in Biotechnology*, **1996**, *14*, 3–19.
13. Willats, W. G. T.; Orfila, C.; Limberg, G.; Buchholt, H. C.; Van Alebeek, G. J. W. M.; Voragen, A. G. J.; Marcus, S. E.; Christensen, T. M. I. E.; Mikkelsen, J. D.; Murray, B. S.; Knox, J. P. *J. Biol. Chem.*, **2001**, *276(22)*, 19404–19413.
14. Matsunaga, T.; Ishii, T.; Matsumoto, S.; Higuchi, M.; Darvill, A.; Albersheim, P.; O'Neill, M. A. *Plant Physiol.*, **2004**, *134*, 339–351.
15. O'Neill, M. A.; Ishii, T.; Albersheim, P.; Darvill, A. G. *Annu. Rev. Plant Biol.*, **2004**, *55*, 109–139.
16. Ishii, T.; Matsunaga, T.; Pellerin, P.; O'Neil, M. A.; Darvill, A.; Albersheim, P. *J. Biol. Chem.*, **1999**, *274(19)*, 13098–13104.
17. du Penhoat, C. H.; Gey, C.; Pellerin, P.; Pérez, S. *J. Biomol. NMR*, **1999**, *14*, 253–271.
18. Pérez, S.; Mazeau, K.; du Penhoat, C. H. *Plant Physiol. Biochem.*, **2000**, *38*, 37–55.
19. Paulsen, B. S.; Barsett, H. *Adv. Polym. Sci.*, **2005**, *186*, 69–101.
20. Yamada, H. *Progress in Biotechnology*, **1996**, *14*, 173–190.
21. Hokputsa, S.; Harding, S. E.; Inngjerdingen, K.; Jumel, K.; Michaelsen, T. E.; Heinze, T.; Koschella, A.; Paulsen, B. S. *Carbohydr. Res.*, **2004**, *339*, 753–762.

22. Yamada, H.; Kiyohara, H.; Matsumoto, T. *Advances in Pectin and Pectinase Research*, **2003**, 481–490.
23. Samuelsen, A. B.; Westereng, B.; Yousif, O.; Holtekjolen, A. K.; Michaelsen, T. E.; Knutsen, S. H. *Biomacromolecules*, **2007**, *8(2)*, 644–649.
24. Thakur, B. R.; Singh, R. K.; Handa, A. K. *Crit. Rev. Food Sci. Nutr*., **1997**, *37*, 47–73.
25. Schlemmer, U. *Z. Lebensm. Unters Forsch*, **1986**, *183*, 339–343.
26. Pellerin, P.; O'Neill, M. A. *Analusis*, **1998**, *26(6)*, M32–M36.
27. Eliaz, I.; Hotchkiss, A. T.; Fishman, M. L.; Rode, D. *Phytothe. Res.*, **2006**, *20*, 859–864.
28. Nangia-Makker, P.; Conklin, J.; Hogan, V.; Raz, A. *TRENDS Mol. Med.*, **2002**, *8(4)*, 187–192.
29. Heitman, D., W.; Hardman, W. E.; Cameron, I. L. *Carcinogenesis*, **1992**, *13*, 815–818.
30. Platt, D.; Raz, A. *J Natl. Cancer Inst.*, **1992**, *84*, 438–442.
31. Nangia-Makker, P.; Hogan, V.; Honjo, Y.; Baccarini, S.; Tait, L.; Bresalier, R.; Raz, A. *J Natl. Cancer Inst.*, **2002**, *94*, 1854–1862.
32. Pienta, K. J.; Naik, H.; Akhtar, A.; Yamazaki, K.; Replogle, T. S.; Lehr, J.; Donat, T. L.; Tait, L.; Hogan, V.; Raz, A. *J Natl. Cancer Inst.*, **1995**, *87*, 348–353.
33. Inohara, H.; Raz, A. *Glycoconjugate J*., **1994**, *11*, 527–532.
34. Jackson, C. L.; Dreaden, T. M.; Theobald, L. K.; Tran, N. M.; Beal, T. L.; Eid, M.; Gao, M. Y.; Shirley, R. B.; Stoffel, M. T.; Kumar, M. V.; Mohnen, D. *Glycobiology*, **2007**, *17(8)*, 805–819.
35. Bertozzi, C.; Bednarski, M. *Carbohydr Res.*, **1992**, *223*, 243–253.
36. Gervay, J.; McReynolds, K. D. *Curr. Med. Chem.*, **1999**, *6*, 129–153.
37. Bertozzi, C. R.; Bednarski, M. D. *J. Am. Chem. Soc.*, **1992**, *114*, 2242–2245.
38. Bertozzi, C. R.; Bednarski, M. D. *J. Am. Chem. Soc.*, **1992**, *114*, 5543–5546.
39. Wilchel, M.; Bayer, E. A. *Anal. Biochem.*, **1988**, *171*, 1–32.
40. Brattauer, C. L. *Methods Mol. Biol.*, **1999**, *115*, 203–214.
41. Green, N. M. *Biochem. J.*, **1963**, *89*, 585–591.
42. Green, N. M. *Protein Chem.*, **1975**, *29*, 85–133.
43. McDevitt, J. P.; Lansbury, P. T., Jr. *J. Am. Chem. Soc.*, **1996**, *118*, 3818–3828.
44. Schweizer, F. *Angew. Chem. Int. Ed.*, **2002**, *41*, 230–253.
45. Risseeuw, M. D. P.; Overhand, M.; Fleet, G. W. J.; Simone, M. *Tetrahedron: Asymmetry*, **2007**, 2001–2010.
46. Györgydeák, Z.; Thiem, J. *Adv. Carbohydr. Chem. Biochem.*, **2006,** *60*, 103–182.

47. Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.*, **2001**, *40*, 2004–2021.
48. Wilkinson, B. L.; Bornaghi, L. F.; Poulsen, S. A.; Houston, T. A. *Tetrahedron*, **2006**, *62*, 8115–8125 and the references cited there.
49. Fialova, P.; Carmona, A. T.; Robina, I.; Ettrich, R.; Sedmera, P.; Prikrylova, V.; Petraskova-Husakova, L.; Kren, V. *Tetrahedron Lett.*, **2005**, *46*, 8715–8718.
50. Bojarova, P.; Petraskova, L.; Ferrandi, E. E.; Monti, D.; Pelantova, H.; Kuzma, M.; Simerska, P.; Kren, V. *Adv. Synth. Catal.*, **2007**, *349*, 1514–1520.
51. Györgydeák, Z.; Thiem, J. *Carbohydr. Res.*, **1995,** *268*, 85–92.
52. Ying, L.; Gervay-Hague, J. *Carbohydr. Res.*, **2003**, *338*, 835–841.
53. Papa, A. J. *J. Org. Chem.* **1966**, *31*, 1426–1430.
54. Li, C.; Arasappan, A.; Fuchs, P. L. *Tetrahedron Lett.*, **1993**, *34(22),* 3535–3538.
55. Li, C.; Shih, T. L.; Jeong, J. U.; Arasappan. A.; Fuchs, P. L. *Tetrahedron Lett.* **1994**, *35*, 2645–2646.
56. Vogel, C.; Jeschke, S.; Kramer, S.; Ott, A. J. *Liebigs Ann./Recueil*, **1997**, *4*, 737–743.
57. Kramer, S.; Nolting, B.; Ott, A. J.; Vphel, C. *J. Carbohydr. Chem.*, **2000**, *19(7)*, 891–921.
58. Vogel, C.; Steffan, W.; Boye, H.; Kristen, H.; Betaneli, V.; Ott, A. Y.; Kochetkov, N. *Carbohydr. Res.*, **1992**, *237*, 131–144.
59. Tosin, M.; Gouin, S. G.; Murphy, P. V. *Org. Lett.*, **2005**, *7*, 211–214.
60. Tosin, M. Murphy, P. V. *J. Org. Chem.*, **2005**, *70*, 4107–4117.
61. Tosin, M.; O'Brien, C.; Fitzpatrick, G. M.; Mueller-Bunz, H.; Glass, W. K.; Murphy, P. V. *J. Org. Chem.*, **2005**, *70(10),* 4096–4106.
62. Bazin, H. G.; Capila, I.; Lindhardt, R. J. *Carbohydr. Res.*, **1998**, *309*, 135–144.
63. Chernyak, A. Ya.; Kononov, L. O.; Antonov, K. V. *Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya*, **1988**, *7*, 1660–1667.
64. Deslongchamps, P.; Chenevert, R.; Taillefer, R. J.; Moreau, C.; Saunders, J. K. *Can. J. Chem.*, **1975**, *53*, 1601–1616.
65. King, J. F.; Allbut, A. D. *Can. J. Chem.*, **1970**, *48*, 1754–1769.
66. Kováč, P. *Carbohydr. Res.*, **1972**, *22*, 464–466.
67. Kocienski, P. J. *Protecting Groups*, 1st ed., Georg Thieme Verlag, 3th edition **2005**.
68. Deng, S.; Cheng-Wei T. C. *Synlett*, **2006**, 756–760.
69. Pitt, N.; Duane, R. M.; O'Brien, A.; Bradley, H.; Wilson, S. J.; O'Boyle, K. M.; Murphy, P. V. *Carbohydr. Res.*, **2004**, *339*, 1873–1887.

70. Czifrak, K.; Hadady, Z.; Docsa, T.; Gergely, P.; Schmidt, J.; Wessjohann, L.; Somsak, L. *Carbohydr. Res.*, **2006**, *341*, 947–956.
71. Bianchi, A.; Ferrario, D.; Bernardi, A. *Carbohydr. Res.*, **2006**, *341*, 1438–1446.
72. Esteves, A. P.; Rodrigues, L. M.; Silva, M. E.; Gupta, S.; Oliveira-Campos, A. M. F.; Machalicky, O.; Mendonca, A. J. *Tetrahedron*, **2005**, *61*, 8625–8632.
73. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.*, **2002**, *41*, 2596–2599.
74. Rossi, L. L.; Basu, A. *Bioorg. & Med. Chem. Lett.*, **2005**, *15*, 3596–3599.
75. Asano, N. *Glycobiology*, **2003**, *13*, 93R–104R.
76. Khalil, N. S. A. M. *Carbohyd. Res.*, **2006**, *341*, 2187–2199.
77. Al-Masoudi, N. A.; Al-Soud, Y. A. *Tetrahedron Lett.*, **2002**, *43*, 4021–4022.
78. Akula, R. A.; Temelkoff, D. P.; Artis, N. D.; Norris, P. *Heterocycles*, **2004**, *63(12)*, 2719–2725.
79. Bräse, S.; Gil, C.; Knepper, K.; Zimmermann, V. *Angew. Chem. Int. Ed.*, **2005**, 5288–5240.
80. Boullanger, P.; Maunier, V.; Lafont, D. *Carbohydr. Res.*, **2000**, *324*, 97–106.
81. Damkaci, F.; DeShong, P. *J. Am. Chem. Soc.*, **2003**, *125*, 4408–4409.
82. Gries, P.; Kristen, H.; Vogel, C. *Carbohydr. Res.*, **1987**, *167*, 87–92.
83. Yokoyama, M.; Kumata, K.; Yamada, N.; Noro, H.; Sudo, Y. *J. Chem. Soc. Perkin Trans. I*, **1988**, 2309–2313.
84. Hanessian, S.; Pernett, A. G. *Adv. Carbohydr. Chem. Biochem.*, **1976**, *33*, 111–188.
85. Hirano, S.; Iwaki, H.; Ishigami, M. *Carbohydr. Res.*, **1979**, *70*, 169–171.
86. Linek, K.; Alföldi, J. *Carbohydr. Res.*, **1987**, *164*, 195–205.
87. Levy, D. E.; Fügedi, P. *The Organic Chemisstry of Sugars*, **2006**, *7*, 269–348.
88. Levy, D., E.; Tang, C. *The Chemistry of C-Glycosides*, 1st ed., Pergamon, Tetrahedron Organic Chemistry Series, Volume 13, **1995**.
89. Levy, D. E; Tang, C. *The Chemistry of C-glycosides*, 1st ed., Pergamon, **1995**, Chapt. 2.
90. Myers, R. W.; Lee, Y. C. *Carbohydr. Res.*, **1984**, *132*, 61–82.
91. Fuchs, E. F.; Lehmann, J. *Carbohydr. Res.*, **1975**, *45*, 135–141.
92. Betaneli, V. I.; Ovchinnikov, M. V.; Backinowsky, L. V.; Kochetkov, N. K. *Carbohydr. Res.*, **1980,** *84*, 211–224.
93. Foces-Foces, C.; Cano, F. H.; Garcia-Blanco, S. *Acta Crystallographica*, **1976**, *B32*, 964–966.

94. Somsak, L. *Carbohydr. Res.*, **1996,** *286*, 167–171.

95. Dmitriev, B. A.; Kocharova, N. A.; Kochetkov, N. K. *Bioorganicheskaya Khimiya*, **1982**, *8(9)*, 1234–41.

96. Steffan, W.; Vogel, C.; Kristen, H. *Carbohydr. Res.*, **1990**, *204*, 109–120.

97. Litvak, M. M.; Betaneli, V. I.; Backinowsky, L. V.; Kochetkov, N. K. *Bioorg. Khim.*, **1982,** *8*, 1133–1142.

98. Vogel, C.; Farouk, M.; Michalik, M.; Reinke, H.; Jarosz, S. *Pol. J. Chem.*, **2005**, *79(2)*, 251–265.

99. Giese, B. *Angew. Chem. Int. Edit.*, **1989**, *101(8)*, 969–1146.

100. Ponten, F.; Magnusson, G. *J. Org. Chem.*, **1996**, *61(21)*, 7463–7466.

101. Deslongchamps. P.; Lessard, J.; Nadeau, Y. *Can. J. Chem.*, **1985**, *63*, 2485–2492.

102. Capon, B.; Lee, Y. C. *J. Org. Chem.*, **1991**, *56*, 4428–4435.

103. Roslund, M. U.; Klika, K. D.; Lehtila, R. L.; Tähtinen, P.; Sillanpää, R.; Leino. R. *J. Org. Chem.*, **2004**, *69*, 18–25.

104. According to IUPAC nomenclature. McNaught, A. D. *Pure Appl. Chem.*, **1996**, *68*, 1919–2008.

105. Vogel, C.; Boye, H.; Kristen, H. *J. Prakt. Chem.*, **1990**, *332*, 28–36

106. Farouk, M. *Ph.D. Thesis*, University of Rostock, **2005**.

107. Williams, J. M.; Richardson, A. C. *Tetrahedron*, **1967,** *23*, 1369–1378.

108. Gill, P. L.; Horner, M. W.; Hough, L.; Richardson, A. C. *Carbohydr. Res.*, **1971**, *17*, 213–215.

109. Xie, J. *J. Org. Chem.*, **2002**, 3411–3418.

110. Nicolaou, K. C.; Flörke, H.; Egan, M. G.; Barth, T.; Estevez, V. A. *Tetrahedron Lett.*, **1995**, *36*, 1775–1778.

111. Perrin, D. D.; Amarego, W. L. F. *Purification of Laboratory Chemicals*, 3rd Ed.; Pergamon Press: Oxford, **1988**. Purification methods

112. BeMiller, J. N.; Yadav, M. P.; Klalabokis, V. N.; Myers, R. W. *Carbohydr. Res.* **1990**, *200*, 111–126.

6. *APPENDIX*

CRYSTAL DATA AND STRUCTURE REFINEMENT

Methyl 1,2,3,4-tetra-*O*-acetyl-α-D-galactopyranuronate (3)[98]

Empirical formula	$C_{15}H_{20}O_{11}$	
Formula weight	376.31	
Temperature	293(2) K	
Wavelength	1.54184 Å	
Crystal system	Monoclinic	
Space group	$P2_1$	
Unit cell dimensions	a = 8.9363(10) Å	α= 90°.
	b = 9.8422(10) Å	β= 95.069(10)°.
	c = 10.7823(10) Å	γ = 90°.
Volume	944.63(17) $Å^3$	
Z	2	
Density (calculated)	1.323 Mg/m^3	
Absorption coefficient	0.996 mm^{-1}	
F(000)	396	
Crystal size	0.65 x 0.20 x 0.06 mm^3	
Θ range for data collection	4.12 to 59.96°.	
Index ranges	-9≤h≤10, -10≤k≤11, -12≤l≤12	
Reflections collected	2761	
Independent reflections	2653 [R(int) = 0.0174]	
Completeness to Θ = 59.96°	99.9 %	
Absorption correction	None	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2653 / 1 / 236	
Goodness-of-fit on F^2	1.034	
Final R indices [I>2σ(I)]	R1 = 0.0367, wR2 = 0.1015	
R indices (all data)	R1 = 0.0409, wR2 = 0.1052	
Absolute structure parameter	0.0(2)	
Extinction coefficient	0.0096(11)	
Largest diff. peak and hole	0.198 and -0.153 $e.Å^{-3}$	
Puckering parameter:	Q: 0.546 (2) Å, Θ 1.5 (2)°, Φ 322.0 (10)°	

Methyl 2,3,4-tri-*O*-acetyl-β-D-galactopyranosyluronate azide (5)

Empirical formula	$C_{13}H_{17}N_3O_9$	
Formula weight	359.30	
Temperature	293(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group (H.-M.)	$P2_12_12_1$	
Space group (Hall)	P 2ac 2ab	
Unit cell dimensions	a = 7.8603(16) Å	α= 90°.
	b = 11.659(2) Å	β= 90°.
	c = 19.028(4) Å	γ = 90°.
Volume	1743.8(6) $Å^3$	
Z	4	
Density (calculated)	1.369 Mg/m^3	
Absorption coefficient	0.117 mm^{-1}	
F(000)	752	
Crystal size	1.00 x 0.87 x 0.25 mm^3	
Θ range for data collection	2.05 to 27.50°.	
Index ranges	-10≥h≥10, -15≥k≥15, -24≥l≥24	
Reflections collected	45926	
Independent reflections	4003 [R(int) = 0.0255]	
Completeness to Θ = 27.50°	99.8 %	
Absorption correction	None	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4003 / 0 / 229	
Goodness-of-fit on F^2	1.059	
Final R indices [I>2σ(I)]	R1 = 0.0364, wR2 = 0.0978	
R indices (all data)	R1 = 0.0406, wR2 = 0.1049	
Absolute structure parameter	0.2(8)	
Largest diff. peak and hole	0.189 and -0.229 $e.Å^{-3}$	
Puckering parameter:	Q: 0.586 (1) Å, Θ 2.1 (1)°, Φ 7 (4)°	

Methyl 2,3,4-tri-*O*-acetyl-α-D-galactopyranosyluronate amine (37) and **methyl 2,3,4-tri-*O*-acetyl-β-D-galactopyranosyluronate amine (38)** mixed crystall

Empirical formula	$C_{13}H_{19}NO_9$	
Formula weight	333.29	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group (H.-M.)	$P2_1$	
Space group (Hall)	P 2yb	
Unit cell dimensions	a = 8.7214(15) Å	α= 90°.
	b = 7.9624(14) Å	β= 95.830(8)°.
	c = 11.325(2) Å	γ = 90°.
Volume	782.4(2) $Å^3$	
Z	2	
Density (calculated)	1.415 Mg/m^3	
Absorption coefficient	0.121 mm^{-1}	
F(000)	352	
Crystal size	0.74 x 0.02 x 0.02 mm^3	
Θ range for data collection	2.81 to 27.50°.	
Index ranges	-11≤h≤11, -10≤k≤10, -14≤l≤14	
Reflections collected	16140	
Independent reflections	3596 [R(int) = 0.0356]	
Completeness to Θ = 27.50°	99.7 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9976 and 0.9158	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3596 / 0 / 217	
Goodness-of-fit on F^2	1.074	
Final R indices [I>2σ(I)]	R1 = 0.0370, wR2 = 0.1076	
R indices (all data)	R1 = 0.0397, wR2 = 0.1104	
Absolute structure parameter	0.2(8)	
Largest diff. peak and hole	0.299 and -0.305 $e.Å^{-3}$	
Puckering parameter:	Q: 0.569 (9) Å, Θ 6.9 (9)°, Φ 310 (8)°	

Methyl 2,3,4-tri-*O*-acetyl-β-D-galactopyranosyluronate amine (38)

Empirical formula	$C_{13}H_{19}NO_9$	
Formula weight	333.29	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group (H.-M.)	$P2_1$	
Space group (Hall)	P 2yb	
Unit cell dimensions	a = 9.0487(4) Å	α= 90°.
	b = 7.7246(3) Å	β= 95.055(2)°.
	c = 11.3898(5) Å	γ = 90°.
Volume	793.02(6) $Å^3$	
Z	2	
Density (calculated)	1.396 Mg/m^3	
Absorption coefficient	0.119 mm^{-1}	
F(000)	352	
Crystal size	0.59 x 0.34 x 0.15 mm^3	
Θ range for data collection	2.76 to 27.98°.	
Index ranges	-11≤h≤11, -10≤k≤10, -15≤l≤ 15	
Reflections collected	24335	
Independent reflections	3788 [R(int) = 0.0280]	
Completeness to Θ = 27.98°	99.3 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9823 and 0.9329	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3788 / 1 / 220	
Goodness-of-fit on F^2	1.042	
Final R indices [I>2σ(I)]	R1 = 0.0277, wR2 = 0.0768	
R indices (all data)	R1 = 0.0285, wR2 = 0.0776	
Absolute structure parameter	0.4(5)	
Largest diff. peak and hole	0.233 and -0.181 $e.Å^{-3}$	
Puckering parameter:	Q: 0.569 (9) Å, Θ 6.9 (9)°, Φ 310 (8)°	

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl cyanide (50)

Empirical formula	$C_{15}H_{19}NO_9$	
Formula weight	357.31	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group (H.-M.)	$P2_1$	
Space group (Hall)	P 2yb	
Unit cell dimensions	a = 9.0658(2) Å	α= 90°.
	b = 8.5972(2) Å	β= 103.4370(10)°.
	c = 11.5404(3) Å	γ = 90°.
Volume	874.84(4) $Å^3$	
Z	2	
Density (calculated)	1.356 Mg/m^3	
Absorption coefficient	0.113 mm^{-1}	
F(000)	376	
Crystal size	0.85 x 0.50 x 0.25 mm^3	
Θ range for data collection	2.31 to 27.50°.	
Index ranges	-8≤h≤11, -11≤k≤11, -14≤l≤14	
Reflections collected	25648	
Independent reflections	3983 [R(int) = 0.0238]	
Completeness to Θ = 27.50°	99.6 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9722 and 0.9097	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3983 / 1 / 230	
Goodness-of-fit on F^2	1.051	
Final R indices [I>2σ(I)]	R1 = 0.0263, wR2 = 0.0717	
R indices (all data)	R1 = 0.0267, wR2 = 0.0723	
Absolute structure parameter	0.1(5)	
Largest diff. peak and hole	0.164 and -0.161 $e.Å^{-3}$	
Puckering parameter:	Q: 0.583 (1) Å, Θ 5.9 (1)°, Φ 6.5 (11)°	

Methyl 1-deoxy-3,4-*O*-isopropylidene-1-(prop-2-enyl)-α-D-galactopyranuronate (59)[106]

Empirical formula	$C_{13}H_{20}O_6$	
Formula weight	272.29	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	$P2_1$	
Unit cell dimensions	a = 5.4679(2) Å	α= 90°.
	b = 7.8710(3) Å	β= 92.2870(10)°.
	c = 16.5303(5) Å	γ = 90°.
Volume	710.86(4) $Å^3$	
Z	2	
Density (calculated)	1.272 Mg/m^3	
Absorption coefficient	0.100 mm^{-1}	
F(000)	292	
Crystal size	0.43 x 0.27 x 0.05 mm^3	
Θ range for data collection	3.70 to 25.00°.	
Index ranges	-6≤h≤6, -9≤k≤9, -19≤l≤19	
Reflections collected	13367	
Independent reflections	2495 [R(int) = 0.0317]	
Completeness to Θ = 25.00°	99.6 %	
Max. and min. transmission	0.9950 and 0.9581	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2495 / 1 / 176	
Goodness-of-fit on F^2	1.034	
Final R indices [I>2σ(I)]	R1 = 0.0304, wR2 = 0.0700	
R indices (all data)	R1 = 0.0374, wR2 = 0.0731	
Absolute structure parameter	1.0(9)	
Largest diff. peak and hole	0.178 and -0.127 $e.Å^{-3}$	
Puckering parameter:	Q: 0.698 (2) Å, Θ 93.1 (2)°, Φ 286.7 (13)°	

Methyl 2,4-di-*O*-acetyl-1-deoxy-1-(prop-2-enyl)-α-D-galactopyranuronate (71)

Empirical formula	$C_{14}H_{20}O_8$	
Formula weight	316.30	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group (H.-M.)	$P2_12_12_1$	
Space group (Hall)	P 2ac 2ab	
Unit cell dimensions	a = 5.39220(10) Å	α= 90°.
	b = 8.7789(2) Å	β= 90°.
	c = 33.0667(9) Å	γ = 90°.
Volume	1565.30(6) $Å^3$	
Z	4	
Density (calculated)	1.342 Mg/m^3	
Absorption coefficient	0.111 mm^{-1}	
F(000)	672	
Crystal size	0.46 x 0.15 x 0.09 mm^3	
Θ range for data collection	2.40 to 30.00°.	
Index ranges	-7≤h≤7, -11≤k≤12, -44≤l≤46	
Reflections collected	18290	
Independent reflections	4586 [R(int) = 0.0299]	
Completeness to Θ = 30.00°	100.0 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9897 and 0.9508	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4586 / 0 / 206	
Goodness-of-fit on F^2	1.036	
Final R indices [I>2σ(I)]	R1 = 0.0408, wR2 = 0.0926	
R indices (all data)	R1 = 0.0515, wR2 = 0.0986	
Absolute structure parameter	0.4(8)	
Largest diff. peak and hole	0.267 and -0.233 $e.Å^{-3}$	
Puckering parameter:	Q: 0.562 (1) Å, Θ 12.0 (1)°, Φ 78.7 (6)°	

Methyl 1,2-di-*O*-acetyl-3,4-*O*-isopropylidene-α-D-galactopyranuronate (75)[106]

Empirical formula	$C_{14}H_{20}O_9$	
Formula weight	332.30	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group (H.-M.)	P1	
Space group (Hall)	P 1	
Unit cell dimensions	a = 5.4179(3) Å	α= 83.995(2)°.
	b = 8.4186(4) Å	β= 86.469(2)°.
	c = 8.7460(4) Å	γ = 82.427(2)°.
Volume	392.80(3) $Å^3$	
Z	1	
Density (calculated)	1.405 Mg/m^3	
Absorption coefficient	0.119 mm^{-1}	
F(000)	176	
Crystal size	0.36 x 0.33 x 0.16 mm^3	
Θ range for data collection	3.22 to 25.00°.	
Index ranges	-6≤h≤6, -10≤k≤10, -10≤l≤10	
Reflections collected	8850	
Independent reflections	2589 [R(int) = 0.0334]	
Completeness to Θ = 25.00°	97.5 %	
Absorption correction	None	
Max. and min. transmission	0.9813 and 0.9585	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2589 / 3 / 208	
Goodness-of-fit on F^2	1.070	
Final R indices [I>2σ(I)]	R1 = 0.0499, wR2 = 0.1295	
R indices (all data)	R1 = 0.0540, wR2 = 0.1350	
Absolute structure parameter	0.8(12)	
Largest diff. peak and hole	0.383 and -0.465 $e.Å^{-3}$	
Puckering parameter:	Q: 0.510 (3) Å, Θ 13.4 (3)°, Φ 15.2 (15)°	

N-Methyl 3,4-di-*O*-benzoyl-1-deoxy-1-(prop-2-enyl)-α-D-galactopyranosyl-uronamide (89)

Empirical formula	$C_{24}H_{25}NO_7$	
Formula weight	439.45	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group (H.-M.)	$P2_12_12_1$	
Space group (Hall)	P 2ac 2ab	
Unit cell dimensions	a = 5.1476(3) Å	α= 90°.
	b = 19.2564(10) Å	β= 90°.
	c = 22.1214(12) Å	γ = 90°.
Volume	2192.8(2) $Å^3$	
Z	4	
Density (calculated)	1.331 Mg/m^3	
Absorption coefficient	0.098 mm^{-1}	
F(000)	928	
Crystal size	0.25 x 0.19 x 0.06 mm^3	
Θ range for data collection	2.80 to 21.56°.	
Index ranges	-5≤h≤5, -19≤k≤19, -22≤l≤22	
Reflections collected	10378	
Independent reflections	2522 [R(int) = 0.0475]	
Completeness to Θ = 21.56°	99.0 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9941 and 0.9758	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2522 / 0 / 291	
Goodness-of-fit on F^2	1.031	
Final R indices [I>2σ(I)]	R1 = 0.0410, wR2 = 0.0873	
R indices (all data)	R1 = 0.0559, wR2 = 0.0943	
Absolute structure parameter	0.9(16)	
Largest diff. peak and hole	0.250 and -0.262 $e.Å^{-3}$	
Puckering parameter:	Q: 0.560 (4) Å, Θ 6.8 (4)°, Φ 96 (3)°	

ERKLÄRUNG

Ich versichere hiermit an Eides statt, dass ich die vorliegende Arbeit selbstständig angefertigt und ohne fremde Hilfe verfasst habe, keine außer den von mir angegebenen Hilfsmitteln und Quellen dazu verwendet habe und die den benutzten Werken inhaltlich und wörtlich entnommenen Stellen als solche kenntlich gemacht habe.

Rostock, 04.04.2008

Gnuni Karapetyan

PERSONAL INFORMATION

Date of Birth:	08/09/1981
Place of Birth:	Yerevan, Armenia
Gender:	Male
Marital Status:	Married

EDUCATION

12/2007 – 11/2007 12/2006 – 11/2006	Scientific co-worker at the **Chair of Physics of New Materials**, Institute of Physics, **University of Rostock**, Germany
06/2005 – 01/2005	Guest scientist at **Research Center Borstel**, Leibniz Center for Medicine and Biosciences, Division of Structural Biochemistry, **Germany**
up today – 10/2004	Ph. D. thesis at the Department of Chemistry, Institute for Chemistry, **University Rostock** (**Germany**),"Some contributions to the chemistry of nitrogen bearing uronic acids" under supervision of Prof. Dr. rer. nat. Christian Vogel
07/2004	Ph. D. student under supervision of Prof. Dr. Aida A. Avetisyan, Organic Chemistry Division, **Yerevan State University, Armenia**.
31/05/2004	By the resolution of The State Examination Commission awarded "**The Master's Degree of Chemistry in The Field of Chemistry"**
04/2003 – 10/2002 04/2004 – 10/2003	Master thesis external at the **University of Rostock, Germany**, Department of Organic Chemistry (in collaboration with **Yerevan State University, Armenia)**: "Some investigations in the synthesis of branched pectin fragments"; supervisor: Prof. Dr. rer. nat. Christian Vogel; **qualification: Master of Chemistry; final result: 5** (maximum: 5)
06/2004 – 07/2002	Master studies of Chemistry in the Department of Chemistry, **Yerevan State University, Armenia**. Major subject: **Organic Chemistry**
29/05/2002	State Examination for Bachelor Degree of Chemistry; By the resolution of The State Examination Commission awarded "**The Bachelor's Degree of Chemistry in The Field of Chemistry"; final result: Diploma With Honour**
04/2002 – 10/2001	Bachelor thesis external at the **University of Rostock, Germany**, Department of Organic Chemistry (in collaboration with **Yerevan State University, Armenia**): "Synthesis of L-Rhamnose derivatives as suitable acceptors for glycosylation"; supervisor: Prof. Dr. rer. nat. Christian Vogel; **final result: 5** (maximum: 5)
06/2002 – 08/1998	Bachelor studies of Chemistry, Department of Chemistry, **Yerevan State University, Armenia**
08/1998	Admission to the Department of Chemistry, **Yerevan State University, Armenia**
06/1998 – 09/1987	Secondary school of Dzoraghbyur; **The Yerevan High School after the Knights and Daughters of Vartan, Yerevan, Armenia** Advanced-level courses: Chemistry, Mathematics, English; qualification: **"Red Certificate"**

Workshops:

- 14th European Carbohydrate Symposium – Eurocarb 14. Lübeck, Germany 2007
- 2nd Baltic Meeting on Microbial Carbohydrates 2006. Rostock, Germany 2006
- Glycostructures in Biosystems XIV – Glycochemistry: Synthesis, Biology and Application. Hamburg, Germany 2005
- The Carbohydrate Workshop. Borstel, Germany 2004
- The Carbohydrate Workshop. Güstrow, Germany 2003
- The Carbohydrate Workshop. Borstel, Germany 2002

Publications:

- Nemati, N.; Karapetyan, G.; Nolting, B.; Endress, H., U.; Vogel, C. "Synthesis of rhamnogalacturonan I fragments by a modular design principle" Carbohydr. Res. (*in print*)
- Otterstein, E.; Karapetyan, G., Nicula, R.; Stir, M.; Schick, C.; Burkel, E. „Sol-gel synthesis and characterisation of fine-grained ceramics in the alumina-titania system" Thermochimica Acta, **2008**, 468, 10-14
- Karapetyan G.; Reinke H.; Endreß, H.-U.; Avetisyan, A.; Vogel, Ch. "Synthesis of *C*- and *N*-glycosides of D-galacturonic acid as suitable building blocks". Abstract Book of 14th European Carbohydrate Symposium **2006**, 245, PO-057 (poster presentation)
- Otterstein, E.; Karapetyan, G.; Nicula, R.; Stir, M.; Schick, C.; Burkel, E. „Synthesis of nanopowder precursors for β-Ti_2AlO_5 ceramics by cogelification of metal alkoxides" Euromat 07, **2007**, (poster presentation)
- Karapetyan, G., A. "Synthesis of derivatives of L-rhamnose as suitable acceptors for glycosylation" Chemical Journal of Armenia (Hayastani Kimiakan Handes), **2007**, 60(1), 52-60
- Karapetyan, G.; Vogel, C. "Synthesis of special C- and N- glycosides of galacturonic acid" 2nd Baltic Meeting on Mycrobial Carbohydrates 2006, Rostock, Germany, October 2006
- Holst, O.; Karapetyan, G.; Kaczynski, Z.; Iacobellis, N., S.; Evidente, A. „The Structures of the Exopolysaccharide and the O-Antigen from *Burkholderia gladioli* pv. *agaricicola*, the Causative Agent of Soft Rot Disease of the Edible Mushroom *A. bitorquis*" XXIIIrd International Carbohydrate Symposium (ICS2006), Whistler, Canada, July 2006
- Karapetyan, G.; Kaczynski, Z.; Iacobellis, S. A.; Evidente, A.; Holst, O. "The structure of the O-lipopolysaccharide from *Burkholderia gladioli* pv. *agaricicola*" Carbohydr. Res., **2006**, 341, 930-934
- Kaczynski, Z.; Karapetyan, G.; Evidente, A.; Iacobellis, N., S.; Holst, O. "The structure of a putative exopolysaccharide of *Burkholderia gladioli* pv. *agaricicola*" Carbohydr. Res., **2006**, 341, 285-288
- Karapetyan, G.; Pews-Davtyan, A.; Avetisyan, A.; Vogel, Ch. "The synthesizes of D-galactopyranosyl-β-(1→4)-L-rhamnopyranosides as building blocks for branched pectin fragments". Abstract Book of The Carbohydrate Workshop. Borstel 2004: P 12 (poster presentation)

AFFILIATION/AWARD

12/2007	DAAD Prize for Excellent Performences of International Students (Germany)
09/2007	GlaxoSmithKline grant for participation in 14th European Carbohydrate Symposium
05/2007	Member of German Chemical Society (GDCh-Germany)
10/2006	Member of Organization Committee of "2nd Baltic Meeting on Microbial Carbohydrates" (Rostock, Germany)
05/2002	"Diploma With Honour" for Bachelor's Degree (YSU)
06/2001	"The Best Student of Yerevan State University" in 2001 (YSU)
09/1999 – 10/2001	Students' representative in Scientific Commission of Chemical Department (YSU)