SYNTHESES RELATED TO AZASUGARS

by

SHIMIAO WANG

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ABSTRACT

The best known biological activity of azasugars is that of inhibition of glycosidases and carbohydrate-processing enzymes. In connection with a project concerned with the synthesis and biological evaluation of monosaccharide analogues for studying the effects on the biosynthesis of glycosaminoglycans, an azasugar, 2-acetamido-1,2,5-trideoxy-1,5-imino-D-glucitol (**40**), and its derivatives became the aim of the present synthetic studies. In addition to solution-phase syntheses, the first (to our knowledge) application of solid-phase chemistry for the synthesis of azasugars, namely 1-deoxynojirimycin analogues, was explored.

Compound **40** was synthesized by a ten-step procedure starting from the readily available 2-acetamido-2-deoxy-D-glucose (**41**). By a sequence of *O*-allylation, *O*-benzylation, and *O*-deallylation, compound **41** was first transformed into 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose (**42**), which, upon Swern oxidation, gave 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucono-1,5-lactone (**43**) in very good yield. Ammonolysis of the lactone **43** opened the pyranoid ring rendering 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-gluconamide (**44**). Subsequent oxidation of the C-5 hydroxyl group of **44** by the Jones reagent led to the reformation of the ring through a process of intramolecular nucleophilic attack by the amide-nitrogen onto the nascent carbonyl, thus providing a pair of epimers, namely 2-acetamido-5-amino-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucono-1,5-lactam (**52**) and 2-acetamido-5-amino-3,4,6-tri-*O*-benzyl-2-deoxy-L-idono-1,5-lactam (**53**) in a ratio of 8:1, respectively. Reductive dehydroxylation of the hydroxylactams **52** and **53** led to the exclusive formation of 2-acetamido-5-amino-3,4,6-

tri-*O*-benzyl-2,5-dideoxy-D-glucono-1,5-lactam (**45**). While debenzylation of **45** by catalytic hydrogenolysis gave 2-acetamido-5-amino-2,5-dideoxy-D-glucono-1,5-lactam (**46**), reduction of **45** using sodium borohydride yielded 2-acetamido-3,4,6-tri-*O*-benzyl-1,2,5-trideoxy-1,5-imino-D-glucitol (**54**), which led to **40** by hydrogenolysis.

Compounds **40**, **46**, and their corresponding acetylated analogues **60** and **59**, showed interesting results in the studies of their effects on the biosynthesis of glycosaminoglycans.

An azasugar compound, namely 1,5-dideoxy-*N*-ethyl-1,5-imino-D-glucuronamide (**68**), was successfully synthesized in an overall yield of 25% from solid-phase synthetic studies. In the approach employed, 2,3:4,6-di-*O*-isopropylidene-α-L-*xylo*-hexulosonic acid (**61**) was coupled to the Merrifield chloromethylated styrene resin, the 4,6-*O*-isopropylidene group was selectively removed and, subsequently, the primary hydroxyl group was chemoselectively tosylated, and the tosyloxy group was replaced by azide. The direct cleavage of the substrate from the Merrifield resin was achieved using ethylamine to give 6-azido-6-deoxy-*N*-ethyl-2,3-*O*-isopropylidene-α-L-*xylo*-hexulosonamide (**66**). Removal of the 2,3-*O*-isopropylidene protecting group of **66** followed by direct catalytic hydrogenation gave compound **68** without any purification in between.

ACKNOWLEDGEMENTS

I owe a deep debt of gratitude to my supervisor, Professor Walter A. Szarek, for his guidance, encouragement, and financial support throughout the course of this work. His scientific thinking, dedication towards research, and kindness to others have undoubtedly exemplified for me the proper conduct of a research scientist. I am truly grateful to have him as my supervisor.

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To my wife Ye and our baby girl Amber.....

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LIST OF ABBREVIATIONS

anhydr	anhydrous
aq	aqueous
Bn	benzyl
Calcd	calculated
CI	chemical ionization
^{13}C NMR	carbon-13 nuclear magnetic resonance spectroscopy
DIBAH	diisobutylaluminium hydride
DMAP	4-(*N*,*N*-dimethyl)aminopyridine
DMF	*N*,*N*-dimethylformamide
EI	electron ionization
equiv	equivalent
ES	electrospray
EtOAc	ethyl acetate
Et_2O	diethyl ether
EtOH	ethanol
FAB	fast atom bombardment
GAG	glycosaminoglycan
Gal	D-galactose
GlcA	D-glucuronic acid
GlcN	D-glucosamine
GlcNAc	2-acetamido-2-deoxy-D-glucose

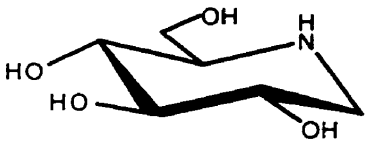
h	hour
H–H COSY	proton–proton correlated spectroscopy
HMBC	heteronuclear multiple-bond correlation
HMQC	heteronuclear multiple-quantum correlation
^{1}H NMR	proton nuclear magnetic resonance spectroscopy
HRMS	high-resolution mass spectroscopy
IR	infrared spectroscopy
*J*mod	coupling constant modulation
lit	literature
LRMS	low-resolution mass spectroscopy
MeOH	methanol
mL	milliliter
mp	melting point
NMR	nuclear magnetic resonance spectroscopy
Pd/C	palladium-on-carbon
psig	pounds per square inch gauge
py	pyridine
rt	room temperature
satd	saturated
THF	tetrahydrofuran
TLC	thin-layer chromatography
TOCSY	total correlation spectroscopy
UDP	uridine diphosphate

Chapter 1. Introduction

The work described in this thesis is concerned primarily with synthetic studies related to azasugars, both in solution and on a solid support. The best known biological activity of azasugars is that of inhibition of glycosidases and carbohydrate-processing enzymes. In the present work, the compounds derived from the solution-phase synthetic studies were submitted for evaluation of their effects on the biosynthesis of glycosaminoglycans, an area of much current interest in this laboratory. As an introduction to the work, some aspects of the biological activity of azasugars and a summary of some results from this laboratory on the inhibition of the biosynthesis of glycosaminoglycans are presented. Also, an overview of the synthesis of azasugars is given.

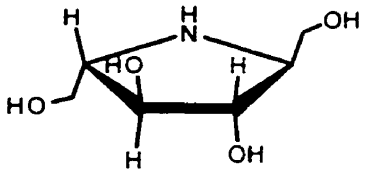
1.1. Classification of azasugars

Azasugars are analogues of monosaccharides in which the ring oxygen has been replaced by nitrogen. Many of them occur naturally and have been isolated primarily from plants and microorganisms[1]. They can be classified into polyhydroxylated piperidines, pyrrolidines, indolizidines, and pyrrolizidines in which they correspond to sugars in the pyranose, furanose, and fused piperidine and pyrrolidine forms, respectively. 1-Deoxynojirimycin (**1**), the 2,5-bis(hydroxymethyl)-3,4-dihydroxypyrrolidine **2**, castanospermine (**3**), and australine (**4**) are common examples of each category.



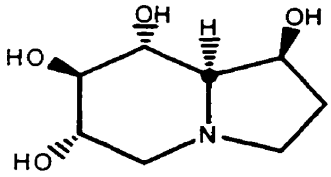
1-Deoxynojirimycin. DNJ
1,5-Dideoxy-1,5-imino-D-glucitol

1



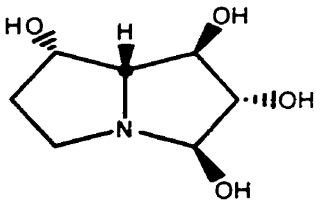
2,5-Bis(hydroxymethyl)-
3,4-dihydroxypyrrolidine

2



Castanospermine

3



Australine

4

1.2. Azasugars as inhibitors of carbohydrate-processing enzymes

1.2.1. Structural basis of the inhibitory ability

Azasugars, as *"nitrogen–in–the–ring"* sugar analogues, can be predicted to possess activity against glycosidases that are specific for glycosides of the parent sugars in many cases[2]. For example, 1-deoxynojirimycin (**1**) and 1-deoxymannojirimycin each bear a striking resemblance to its corresponding monosaccharide and may be regarded as simple azasugar analogues of D-glucose and D-mannose, respectively, in which the pyranose oxygen is replaced by a basic nitrogen atom. In the five-membered series, the 2,5-bis(hydroxymethyl)-3,4-dihydroxypyrrolidine **2** represents an azasugar analogue of β-L-fructofuranose. The polyhydroxylated indolizidine alkaloid castanospermine (**3**)

can be seen to resemble D-glucopyranose. Thus, the structural basis of the inhibition of glycosidases by these compounds is obvious in that they most closely resemble the substrates of the glycosidases. The substitution of the ring oxygen by nitrogen renders the compounds metabolically inert, but does not prevent their recognition by glycosidases and other carbohydrate-recognizing proteins[3].

1.2.2. Biochemical and clinical aspects

Glycosidases and glycosyltransferases are two families of enzymes, which catalyze glycosyl-group transfer reactions in living system[4]. These processes involve cleavage of the glycoside bond linking a sugar's anomeric carbon with an oligo- or poly-saccharide or a nucleoside diphosphate group. The liberated glycosyl group may then be transferred to water by glycosidases or to some nucleophilic acceptor by transferases. Cells constantly recycle certain metabolic intermediates and adjust concentrations of key components according to need. For this reason, the enzymes that catalyze glycosyl-group transfer reactions are vital to the normal growth and development of all cells. Defects or genetic deficiencies of such enzymes are associated with several well-known inherited disorders of carbohydrate metabolism.

Generally, the glycosidases and glycosyltransferases are instrumental in the processing of various oligosaccharide-containing glycoproteins and glycolipids[5]. The profound impact of these enzymes on life processes has made them desirable targets for inhibition. Azasugar compounds are an important family of inhibitors, primarily for the *N*-linked oligosaccharide-processing enzymes[6]. Nevertheless, the formation and

degradation processes of the *O*-linked oligosaccharide glycoconjugates are also of interest for inhibition. Glycoprocessing inhibitors have already been used to treat diabetes and other metabolic disorders[7], and have been implicated in the blocking of microbial infection[8] and metastasis[9].

1.2.3. Insight into inhibition mechanisms

A classic mechanism[10,4(a)] of the enzymatic hydrolysis is shown in **Figure 1**. It involves the following features. (i) A catalytically active carboxylic acid function in the active site protonates the glycoside. (ii) A carboxylate group of the enzyme positioned

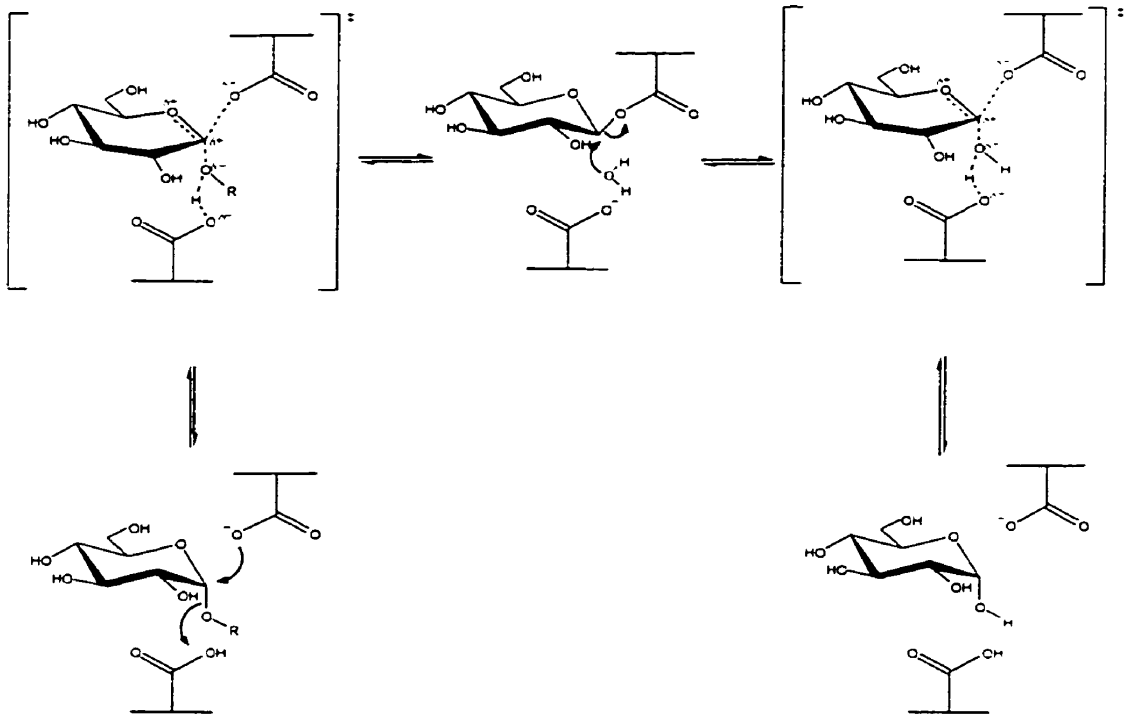


Figure 1. Mechanism of enzyme-catalyzed glycoside hydrolysis

at the opposite side of the sugar ring stabilizes the cyclic oxocarbenium-like transition state by formation of a covalent bond and inversion of the configuration at the anomeric carbon. (iii) The covalently bound glycosyl-enzyme intermediate can be formed from both directions by way of an oxocarbenium-like transition state. (iv) In the hydrolysis step, attack of water at the anomeric center releases the glycoside. (v) Various non-covalent interactions provide most of the rate enhancement.

In theory, the best inhibitors should have features similar to those of the glycosyl cation and match the electronic and steric requirements necessary to bind tightly in the enzyme active site[11]. Indeed this situation pertains with 1-deoxynojirimycin analogues in some aspects, which can mimic the charge and the positioning of the hydroxyl groups on the glycosyl cation[12]. At physiological pH, when these inhibitors bind to the active site of a glucosidase, they interact with the base residues normally involved in the

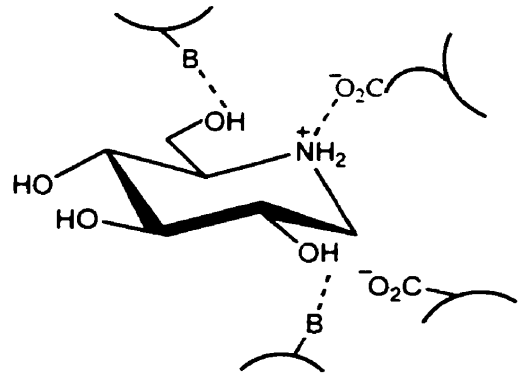


Figure 2. Binding of nitrogen-containing inhibitors in the glycosidase enzyme active site

hydrolysis of a glucoside[11] by forming an ion pair between a protonated inhibitor and an anionic group in the active site (see **Figure 2**). The protonated azasugar will resemble

the glycosyl cation postulated to be formed during the action of glycosidases. The hydroxyl groups on the ring interact favorably with the hydrophilic region in the active site of the enzyme, and, as expected, when hydroxyl groups are removed from the azasugar, binding diminishes[3].

1.3. Inhibition of the biosynthesis of glycosaminoglycans

1.3.1. Background

Glycosaminoglycans (GAGs) are long, unbranched polysaccharide chains composed of repeating disaccharide subunits in which one of the two sugars is either *N*-acetylglucosamine or *N*-acetylgalactosamine while another sugar is either a hexuronic acid residue (D-glucuronic acid or L-iduronic acid) or a D-galactose residue[13]. GAGs are highly negatively charged because of the presence of carboxyl or sulfate groups on many of the sugar residues. GAGs are usually bound in extracellular space in multicellular organisms.

Most GAGs are linked to a core protein as lateral extensions, forming a proteoglycan with a highly extended, brush-like structure. The linkage between the GAGs and the core protein within the proteoglycan is mediated by a specific tetrasaccharide unit that is linked on one side to the repeating disaccharide unit of the GAG and on the other side to a serine hydroxyl group of the core protein (see **Figure 3**). The conserved tetrasaccharide sequence is D-GlcA – D-Gal – D-Gal – D-Xyl.

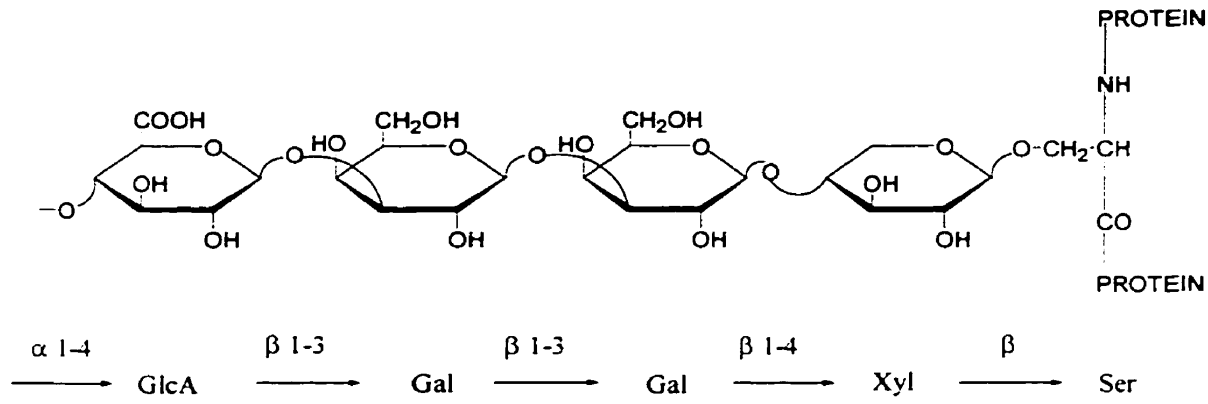


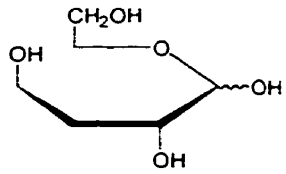
Figure 3. The specific linkage connecting a GAG chain to its core protein

The family of glycosaminoglycan glycopolymers includes hyaluronic acid, which is thought to promote a wide range of adhesion phenomena[14], as well as heparin and heparan sulfate, which are potent anticoagulants and also implicated in the control of cell growth, transformation, and differentiation in mammalian organisms[15].

1.3.2. Design of inhibitors of the biosynthesis of glycosaminoglycans

As depicted in **Section 1.3.1**, GAGs are linked by a specific tetrasaccharide (D-glucuronic acid – D-galactose – D-galactose – D-xylose) to its protein core by a xylose – serine bond[16]. The D-glucuronic acid unit is coupled to the D-galactose in a β-(1→3) linkage, as are the two D-galactose units. The goal was to develop monosaccharide derivatives which would affect GAG synthesis but not interfere cell protein synthesis[17]. To be so, the compounds must have the potential either to be incorporated into the growing GAG chain preventing further elongation of the polysaccharide or to serve as an inhibitor of naturally occurring sugar metabolites interfering the elongation of the

polysaccharide. To this end, 3-deoxy-D-*xylo*-hexose (3-deoxy-D-galactose, **5**) and methyl (methyl 4-chloro-4-deoxy-β-D-galactopyranosid)uronate (**6**) were designed and synthesized. Their effects on GAG synthesis were examined by using hepatocytes in tissue culture.



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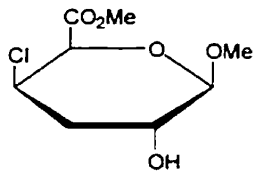

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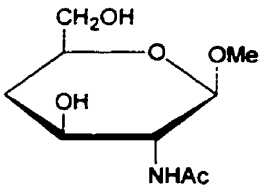
At 10mM and 20mM, 3-deoxy-D-galactose (**5**) reduced [^{3}H]glucosamine and $^{35}SO_4$ incorporation into hepatocyte cellular GAGs to, respectively, 75% and 60% of the control cells. This inhibition of GAG synthesis occurred without any effect on hepatocyte protein synthesis, indicating that 3-deoxy-D-galactose's effect on GAG synthesis is not mediated through an inhibition of proteoglycan core protein synthesis. Furthermore, GAGs in the presence of 20mM of the analogue were significantly reduced in size, 17 kDa vs. 66 kDa in untreated cells. These results reflect either inhibited cellular GAG chain elongation or altered GAG chain degradation.

Compound **6** exhibited a concentration-dependent inhibition of both hepatocyte cellular GAG and protein synthesis. At concentrations of 5, 10 and 20mM, compound **6** inhibited GAG and protein synthesis by 20, 65 and 90%, respectively. Exogenous uridine was able to restore partially the inhibition of protein synthesis, but was unable to reverse the effect of compound **6** on GAG synthesis. These results show that part of the effect of compound **6** on GAG synthesis is not mediated by an inhibition of proteoglycan core protein synthesis. GAGs in the presence of compound **6** are only half

as large as those in the absence of this compound (33 and 66 kDa, respectively). These results again may reflect either impaired cellular GAG chain elongation or altered GAG chain degradation.

Driven by the same thought, Berkin *et al.*[18] have synthesized 4-deoxy analogues of 2-acetamido-2-deoxy-D-glucose, for example methyl 2-acetamido-2,4-dideoxy-β-D-*xylo*-hexopyranoside (**7**). The biological evaluation showed indeed an enhanced



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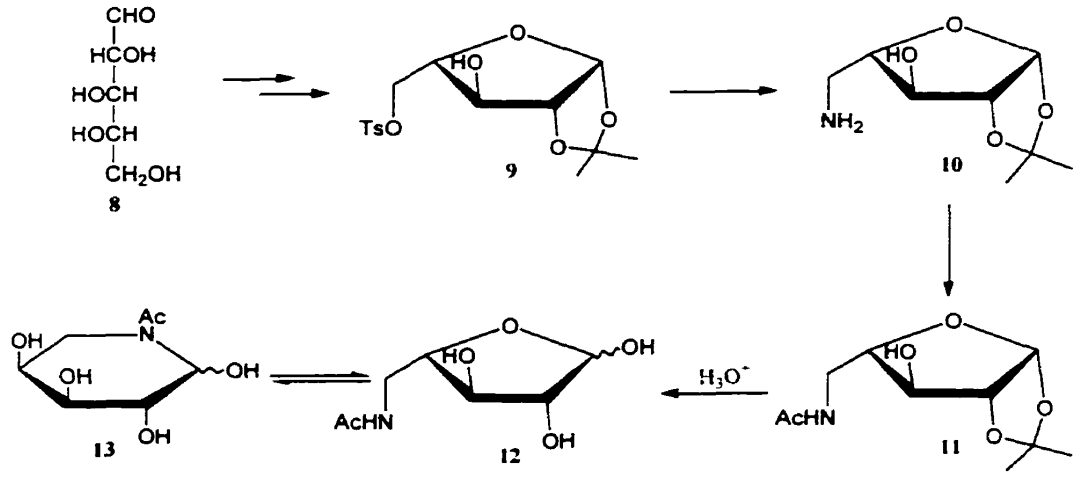
inhibition of [^{3}H]GlcN and [^{35}S]SO_4 incorporation into GAG chains compared to compound **5** at the same conditions. However, the inhibition of protein synthesis, as in the case of compound **6**, was also observed. Their results suggested also that the C-6 substituent is important for the biological activity.

1.4. Chemical synthesis of azasugars

Ever since many antibiotics were found to contain amino sugars[19] as building components in the 1940s, the synthesis of amino sugar derivatives has been of interest to organic chemists. Although a number of azasugars do occur naturally in plants and microorganisms, the extraction and purification are time consuming and costly. Furthermore, some sources are scarce and difficult to obtain. The break-through in the synthesis of azasugars did not occur until the 1960s.

The first synthesis of a compound of this type was achieved by Jones and Turner[20] in 1962; the compound was 5-acetamido-5-deoxy-L-arabinopyranose (**13**). The method that they used is shown in **Scheme 1**.

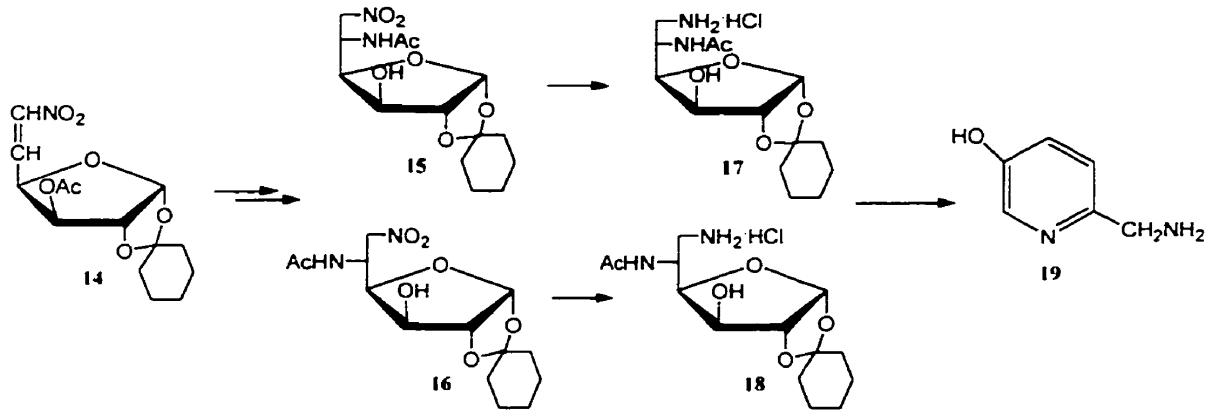


Scheme 1. Synthesis of 5-acetamido-5-deoxy-L-arabinopyranose (**13**)

1,2-*O*-Isopropylidene-5-*O*-*p*-tolylsulfonyl-L-arabinose (**9**), which was prepared from L-arabinose (**8**), was treated with methanolic ammonia. The resulting amino sugar **10** was selectively acetylated to afford 5-acetamido-5-deoxy-1,2-*O*-isopropylidene-L-arabinofuranose (**11**), which, upon partial acid-catalyzed hydrolysis, rendered an equilibrium mixture of 5-acetamido-5-deoxy-L-arabinofuranose (**12**) and 5-acetamido-5-deoxy-L-arabinopyranose (**13**). Although the equilibrium between **12** and **13** can be established from either pure **12** or **13** under acidic conditions, by addition of a drop of ammonia solution, or by heating, both compounds are stable in neutral aqueous solution at room temperature and no transformation into each other was observed. Consistent

with these observations was the isolation of **12** and **13** in pure form by chromatography on cellulose.

Interestingly, at essentially the same time as the Jones and Turner paper about this nitrogen-in-a-ring sugar appeared in the *Journal of the Chemical Society*. Paulsen[21] reported in *Angewandte Chemie* the preparation of 5-amino sugars and their transformation into pyridine derivatives. The nitro olefin **14** easily added on ammonia and, by synchronous acyl transfer, a mixture of the 5-acetamido-6-nitrosugars **15** and **16**



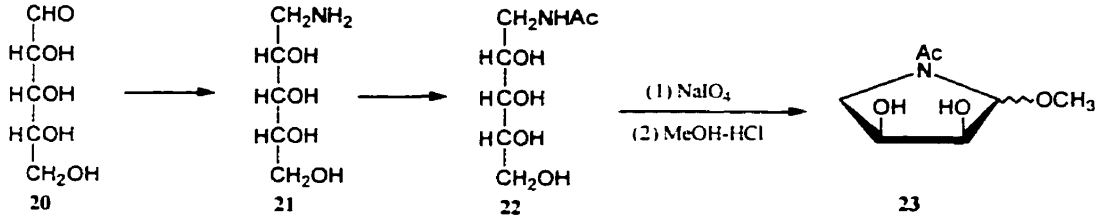
Scheme 2. Preparation of 5-amino sugars and their transformation into pyridine derivatives

was formed (see **Scheme 2**). Hydrogenation of **15** and **16** gave a mixture of amines which was isolated as a mixture of the hydrochloride salts **17** and **18**; mild acid-catalyzed hydrolysis of the mixture gave 2-aminomethyl-5-hydroxypyridine (**19**). The author believed that mild hydrolysis of **17** and **18** first yielded the free sugars by elimination of the cyclohexylidene groups; the sugars invariably reacted further by cleavage of the acetamido groups and, in each case, subsequent spontaneous elimination of three molecules of water and aromatisation to give **19**. In this process, an interaction

of the carbonyl group with the amino group at C-5 must have taken place. However, the intermediate products, which were assumed to be nitrogen-in-the-ring compounds, could not be isolated. Apparently, the opportunity of the preparation of the first azasugar compound slipped away from his hands.

Following Jones and Turner's work, the endeavor in azasugar synthesis has by no means dwindled, a development which can be seen from the synthesis of 5-acetamido-5-deoxy-D-xylopyranose independently by Paulsen[22], Jones and Szarek[23], and Hanessian and Haskell[24]. Although their synthetic routes are somewhat different, they all utilized the same parent sugar, namely D-xylose, as the starting material.

The advent of the first example of a sugar having nitrogen in a five-membered ring came in 1963 by the synthesis of methyl 4-acetamido-4-deoxy-L-erythrofuranoside (**23**) by Szarek and Jones[25] (**Scheme 3**).



Scheme 3. Synthesis of methyl 4-acetamido-4-deoxy-L-erythrofuranoside (**23**)

1-Amino-1-deoxy-D-ribitol (**21**) was prepared from D-ribose (**20**) by reductive amination, and *N*-acetylation of **21** using aqueous acetic anhydride gave 1-acetamido-1-deoxy-D-ribitol (**22**). The ingenious part of this concise synthesis was to take advantage of the fact that a limited quantity of sodium metaperiodate preferentially attacks a glycol system involving a primary hydroxyl group, so that the formation of a nitrogen-

containing furanose ring (see **23**) was made possible by ring closure of the nascent terminal aldehyde group onto the nitrogen atom of the amide group.

In the synthesis of six-membered azasugars and their derivatives, the major challenge is to establish five adjacent stereogenic centers. Because of the close structural resemblance between azasugars and their parent sugars, a classical way of synthesizing azasugars has been the use of monosaccharides as starting materials. Stereogenic centers at C-2, C-3, and C-4 can be pre-established by selecting, for example, a pyranose sugar, which contains the required stereochemistry at the corresponding positions (**Figure 4**). Hence, the introduction of two C–N bonds is

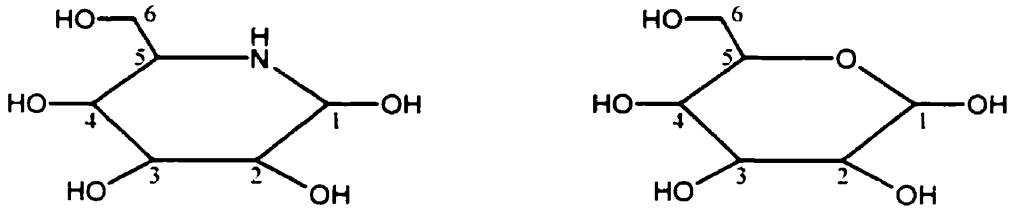
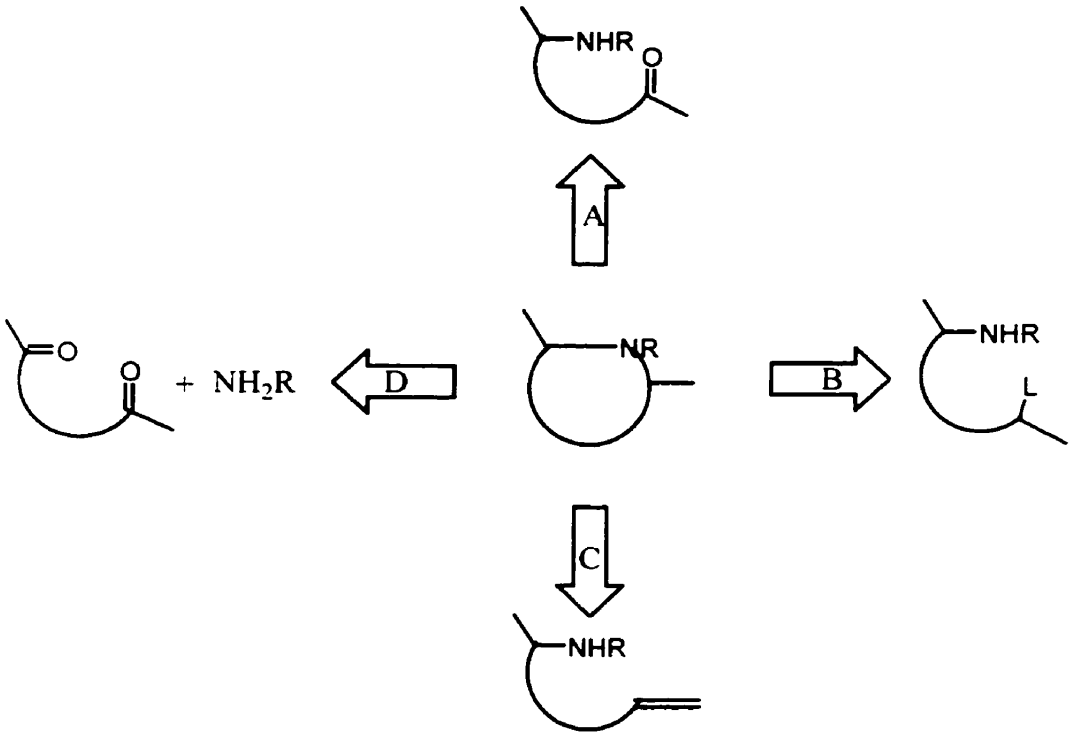


Figure 4.The structural comparison between an azasugar and its parent monosaccharide

usually considered to be crucial in the total synthesis of azasugars. For producing a six-membered azasugar, a nitrogen link is introduced between either C-1 and C-5 or C-2 and C-6 of a corresponding parent sugar. The first synthesis of 5-acetamido-5-deoxy-L-arabinopyranose (**13**) (**Scheme 1**) is an example of this type of approach. Accordingly, formation of pyrrolidines involves the introduction of a nitrogen link between either C-1 and C-4 or C-2 and C-5 of a corresponding sugar. This route is exemplified in the first synthesis of the five-membered azasugar, methyl 4-acetamido-4-deoxy-L-erythrofuranoside (**23**) (**Scheme 3**).

The basic strategy for the synthesis of azasugars involves two aspects: (i) stereoselective introduction of a masked amino function at a selected position with an appropriate functionalization at another suitable position in the parent sugar molecule, and (ii) subsequent cyclization to form the azasugar ring through the activation of the masked amino function.

The common methods for the cyclization , as illustrated in **Scheme 4**, can be summarized as the following: single reductive amination involving an intramolecular reaction of a carbonyl group and an amino group (method A), intramolecular



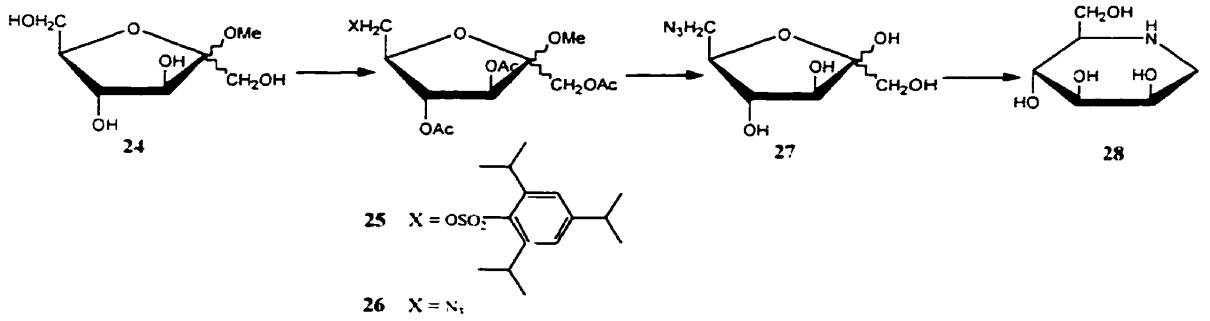
Scheme 4. Common cyclization methods for the formation of azasugars

nucleophilic substitution of a good leaving group by an amine (method B), organomercury-mediated cyclization by intramolecular nucleophilic addition of amine

to a double bond (method C), and a double reductive amination (method D). Examples of each of these methods are shown below.

A. Single reductive amination

Single reductive amination is a commonly used cyclization method to synthesize nitrogen-in-the-ring sugars. Azido group is first introduced into a sugar by nucleophilic substitution. The group can then be easily reduced to the primary amine, and a Schiff base will be formed by intramolecular condensation of the primary amine with the carbonyl group. Under appropriate reaction conditions, the Schiff base can either be hydrogenated or be offered a hydride by a hydride donor; thus, the nitrogen-in-the-ring sugar compound will be formed. Furneaux *et al.*[26] have used this method as an alternative way to synthesize 1-deoxymannojirimycin (1,5-dideoxy-1,5-imino-D-mannitol) (**28**) in only five steps from an anomeric mixture of methyl D-fructofuranosides (**24**) (**Scheme 5**).



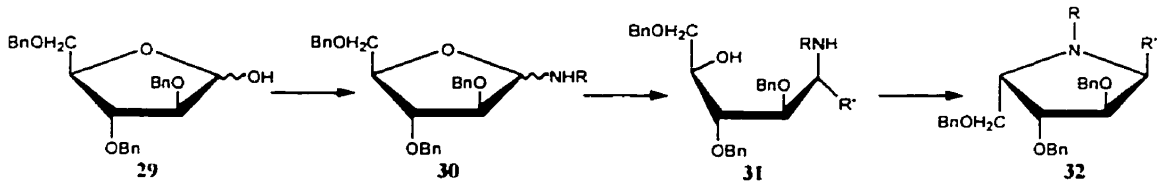
Scheme 5. Application of single reductive amination in the synthesis of 1,5-dideoxy-1,5-imino-D-mannitol (**28**)

The mixture **24** was treated in dry pyridine with 2,4,6-triisopropylbenzenesulfonyl chloride (1.1 equiv), followed by an excess of acetic

anhydride, to give the 6-sulfonate esters **25** with only traces of the corresponding 1,6-disulfonates. An azide displacement (NaN_3, DMSO, 80 °C) on this product **25** then afforded the methyl 1,3,4-tri-*O*-acetyl-6-azido-6-deoxy-α,β-D-fructofuranosides (**26**). Zemplén deacetylation (NaOMe–MeOH) of **26**, followed by acid-catalyzed hydrolysis (50% aq CF_3COOH), afforded 6-azido-6-deoxy-D-fructose (**27**). Hydrogenation of this material over palladium-on-carbon gave 1-deoxymannojirimycin (**28**) which was isolated as its crystalline hydrochloride salt in 25% overall yield.

B. Intramolecular nucleophilic substitution

Because of its strongly nucleophilic nature, an amino group in a sugar will undergo an intramolecular nucleophilic displacement when a good leaving group is introduced into the sugar chain at an appropriate position, thereby accomplishing the cyclization to give the sugar having nitrogen in the ring. Based on this approach, Nicotra and coworkers[27] developed a procedure which can be used on starting sugars by employing different primary amines and Grignard reagents to obtain a variety of azasugars (**Scheme 6**).



Scheme 6. Application of intramolecular nucleophilic substitution in the synthesis of azasugars

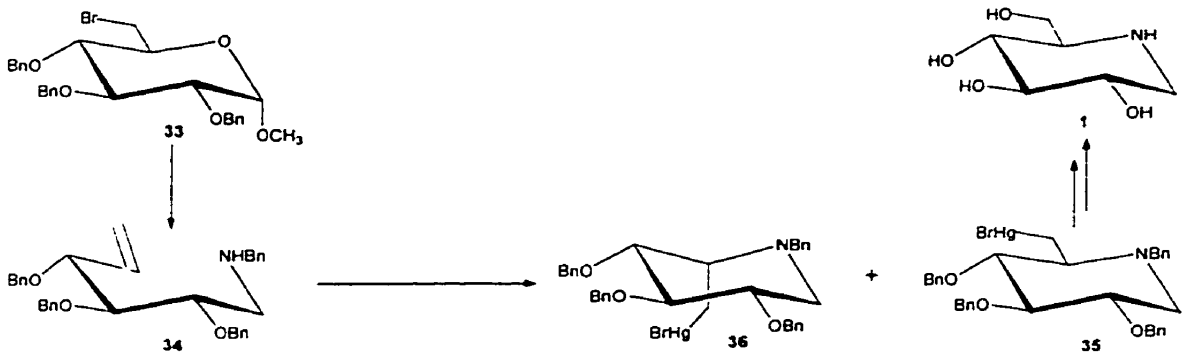
2,3,5-Tri-*O*-benzyl-D-arabinofuranose (**29**), dissolved in dry CH_2Cl_2, was treated with an excess of the primary amine at room temperature to afford the glycosylamine

30. Compound **30** was directly submitted to the subsequent reaction with a Grignard reagent in dry THF, under N_2 atmosphere, affording stereoselectively the product **31**. Treatment of the amino alcohol **31** with trifluoromethanesulfonic anhydride (1.5 equiv) at room temperature afforded the cyclization product **32**. Interestingly, the possible sulfonamide was not formed. This observation opened the way to an easy formation of azasugars following the sequence of a) glycosylamine formation, b) Grignard reaction, and c) cyclization with Tf_2O. This procedure is easily extended to include pyranose rings.

C. Organomercury-mediated cyclization

The addition reactions of an alkene are usually initiated by interaction of a proton with the alkene, a process which causes nucleophilic attack on the double bond. The role of the initial electrophile can be played by metal cations as well. Ganem (see ref. 28) devised an enantioselective synthetic route to azasugars from readily available chiral monosaccharides, using a mercury salt to catalyze the intramolecular nucleophilic addition of an amine to a double bond. The general approach is illustrated in **Scheme 7** for the case of D-glucose.

A one-pot, reductive ring-opening of a pyranose sugar and subsequent reductive amination were achieved by heating the tri-*O*-benzyl-6-bromo-6-deoxypyranoside **33** in the presence of acid-washed zinc dust in propanol–water (19:1) containing benzylamine and $NaBH_3CN$ to afford the amino alkene **34** in 91% overall yield. When this key intermediate was treated with mercuric trifluoroacetate in anhydr THF, a 3:2 mixture of bromomercurials **35** and **36** was isolated in good yield after ligand exchange (LiBr–THF). After chromatographic separation, the major cyclization product **35** could be

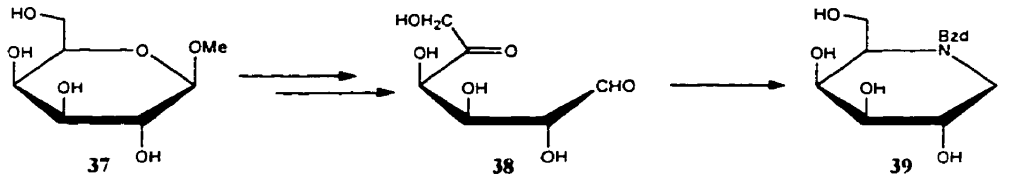


Scheme 7. Organomercury-mediated cyclization in the synthesis of 1-deoxynojirimycin (**1**)

transformed into 1-deoxynojirimycin (**1**) by treatment with $NaBH_4$–DMF–O_2 and subsequent hydrogenolytic deprotection.

D. Double reductive amination

This method, different from those mentioned above, offers a simultaneous introduction of the two C–N bonds into a sugar ring, subject to an easy preparation of a dicarbonyl staring material.



Scheme 8. Application of double reductive amination in the synthesis of 1-deoxy-D-galactostatin derivatives

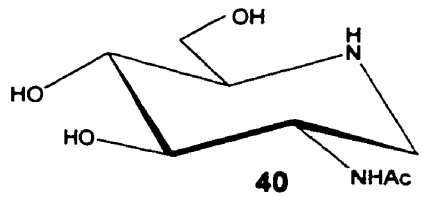
Catelani and coworkers[29] have applied this method to a diastereoselective approach to 1-deoxy-D-galactostatin derivatives (**Scheme 8**). The unprotected L-*arabino*-hexos-5-ulose (**38**), easily obtained from methyl β-D-galactopyranoside (**37**),

was treated with $NaBH_3CN$ and benzhydrylamine to give the product **39** in moderate yield, thus offering an attractive approach to 1-deoxy-D-galactostatin derivatives.

A multitude of synthetic approaches to hydroxylated pyrrolidine, piperidine, pyrrolizidine, and indolizidine derivatives have been reported (see ref. 30), and many new variations have been developed, such as the organomercurial route to azasugars reviewed by Ganem[28] and ring-closing metathesis-mediated synthesis reviewed by Pandit *et al.*[31]. Other developments, such as syntheses from non-carbohydrate sources reviewed by Hudlicky *et al.*[32] and from enzymatic pathways as exemplified by the work of Wong[6], have provided alternative choices of the synthesis of azasugars.

1.5. Research objectives

A primary objective of the present study was to provide further candidate compounds for studying the effects of monosaccharide analogues on the biosynthesis of glycosaminoglycans. On the basis of previous results obtained in this laboratory, particularly those of Berkin *et al.*[18] described in **Section 1.3.2**, it was considered worthwhile to synthesize the azasugar, 2-acetamido-1,2,5-trideoxy-1,5-imino-D-glucitol (**40**), and some related analogues.



40

In addition to these solution-phase syntheses, the synthesis of an azasugar, namely 1-deoxynojirimycin (**1**), on a solid support has been investigated. There is much current interest in solid-phase synthesis in organic and medicinal chemistry, particularly since the advent of combinatorial chemistry as an efficient and rapid approach towards drug discovery. To our knowledge, this work represents the first investigation of solid-phase chemistry for the synthesis of azasugars.

Chapter 2. Results and discussion

As indicated in **Section 1.5** there were two main research objectives in the present study. These were the provision of further candidate compounds for studying the effects of monosaccharide analogues on the biosynthesis of glycosaminoglycans and an investigation of solid-phase chemistry for the synthesis of azasugars. These two endeavors are discussed in this chapter in sections entitled "Solution-phase synthesis" and "Preliminary studies on solid-phase synthesis", respectively. Finally, the results obtained from a biological evaluation of compounds obtained from the solution-phase syntheses are discussed.

2.1 Solution-phase synthesis

In the light of the information discussed in **Chapter 1**, one might expect that compound **40**, 2-acetamido-1,2,5-trideoxy-1,5-imino-D-glucitol, and its analogues would behave as inhibitors towards the biosynthesis of glycosaminoglycans. Compound **40** possesses not only the necessary structural features of compound **7** (see **1.3.2**), namely the C-6 functionality and the 2-acetamido group, but also resembles closely *N*-acetyl-D-glucosamine, which is one of the major constituents of the GAG chain. Compound **40** belongs, of course, to the very important family of inhibitors, namely azasugars, of *N*- or *O*-linked oligosaccharide processing enzymes[33]: the removal of the anomeric hydroxyl group to form 1-deoxy derivatives enhances the metabolic stability and does not destroy inhibitory properties[3].

Compound **40** has been synthesized from D-glucose[34], from methyl α-D-mannopyranoside[35], from *N*-acetyl-D-glucosamine[36], and from sucrose[37]. Other

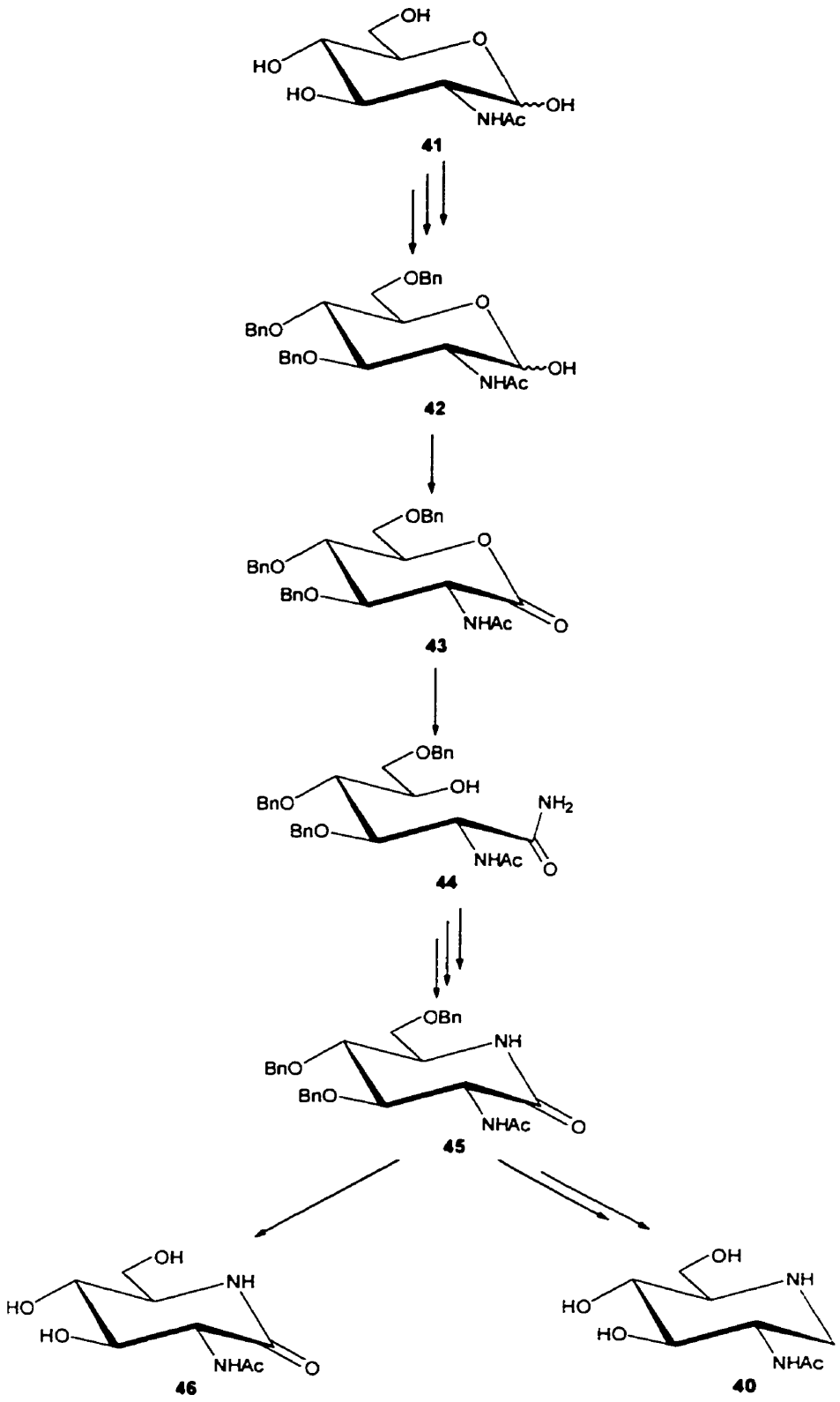
approaches have taken advantage of 1-deoxynojirimycin (**1**) as the starting material[38]. A synthesis of compound **40** has also been achieved with the aid of an aldolase-catalyzed key step[12(a)].

Our route to compound **40** and its analogues is also from the readily available chiral monosaccharide, *N*-acetyl-D-glucosamine (2-acetamido-2-deoxy-D-glucose) (**41**), but by a different synthetic approach. The strategy hinged on cleavage of the pyranoid ring by ammonolysis after an oxidation had been performed at C-1. The corresponding azasugar analogues were formed with retention of the critical stereocenters. The general synthetic approach is outlined in **Scheme 9**. In this section the results of the synthetic studies are presented and discussed under headings for the target compounds of synthetic steps.

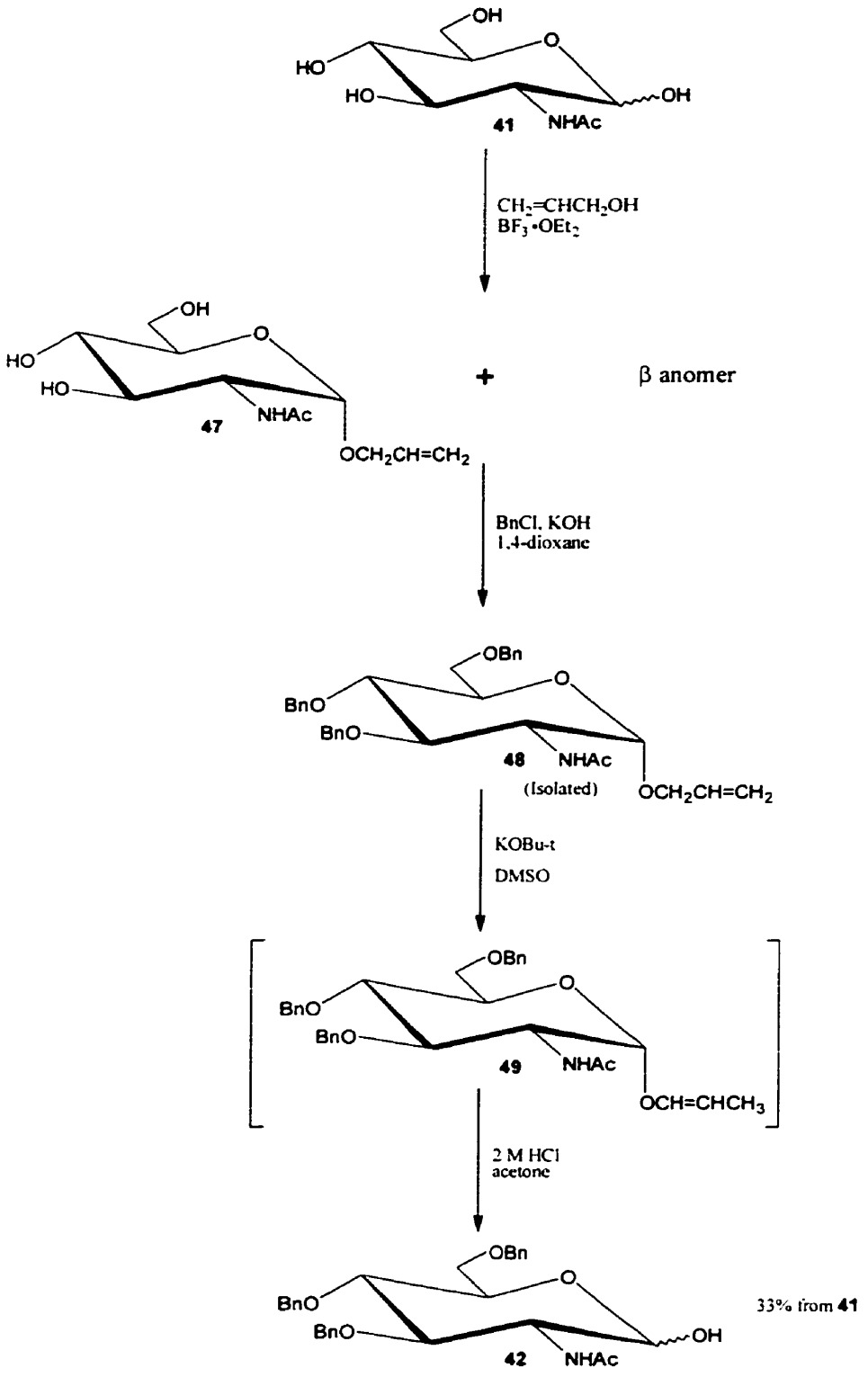
2.1.1. Synthesis of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose (42)

2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose (**42**) is a very valuable intermediate compound in the synthetic plan. It was synthesized in four steps[39] from 2-acetamido-2-deoxy-D-glucose (**41**) by *O*-allylation, *O*-benzylation, and *O*-deallylation in an overall yield of 33%. The procedure employed, as illustrated in **Scheme 10**, is a slightly modified procedure of Warren *et al.*[40] and is convenient for a multigram-scale synthesis.

Allyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**47**) was made by the direct glycosidation of allyl alcohol with 2-acetamido-2-deoxy-D-glucopyranose (**41**) catalyzed by boron trifluoride etherate[41]. An 8:1 mixture of **47** and its β anomer was



Scheme 9. The general synthetic approach towards **40**

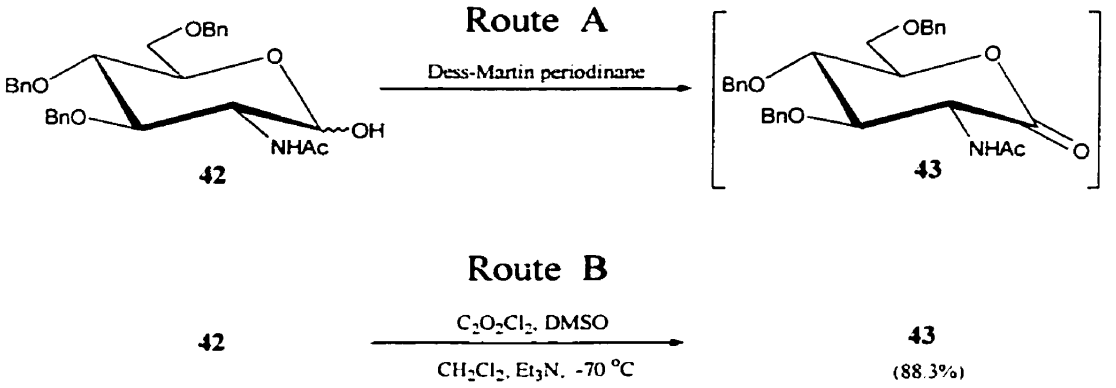


Scheme 10. Synthesis of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose (**42**)

obtained. The mixture was not resolved since the temporary protecting allyl group was to be removed after a perbenzylation. Thus, treatment of the mixture with benzyl chloride and powdered KOH in 1,4-dioxane afforded, after fractionation of the product, a compound whose ^{1}H NMR spectrum was consistent with the structure of allyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**48**). The processing procedure employed was that described by Hoffmann *et al.*[39]. The sample of **48**, obtained from the fractionation by flash column chromatography on silica gel, was treated in DMSO with potassium *tert*-butoxide to effect the isomerization[42] of the allyl group. The product, presumably 1-propenyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**49**), was subjected to acid-catalyzed hydrolysis with hydrochloric acid in acetone to effect removal of the 1-propenyl group. The resultant product, after recrystallization, afforded a compound whose ^{1}H-NMR spectrum was consistent with the structure of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose (**42**) and whose physical constants were in agreement with those reported in the literature (see Experimental) for **42**. In the above synthetic route for compound **42**, the concept of "temporary" and "persistent" protecting groups[43] was employed. The temporary group was the allyl group, which was stable to the benzylation conditions but readily removable by dilute acid after rearrangement with potassium *tert*-butoxide in DMSO[44]. The persistent groups were the benzyl groups, which sustained all of the chemical transformations that follow until their encountering of hydrogenolysis conditions.

2.1.2. Synthesis of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucono-1,5-lactone (43)

The next step in the general synthetic approach towards the target **40** involved oxidation of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose (**42**) to give the lactone **43**. Our first attempt, as shown by Route A in **Scheme 11**, involved treatment of **42** with the Dess-Martin periodinane reagent (see ref. 45). This route was claimed by Granier and Vasella[46] to give the lactone product **43** in a nearly quantitative yield. However, in experiments performed in the present study, it was found that the reaction took a longer time and required more additions of the Dess-Martin periodinane reagent, as evidenced by the consumption of the starting material **42** in TLC, than described by Granier and Vasella. Furthermore, TLC revealed the formation of many by-products, which migrated very close to compound **43**, and hence a separation by column chromatography was difficult. Therefore, an alternative approach (route B), namely

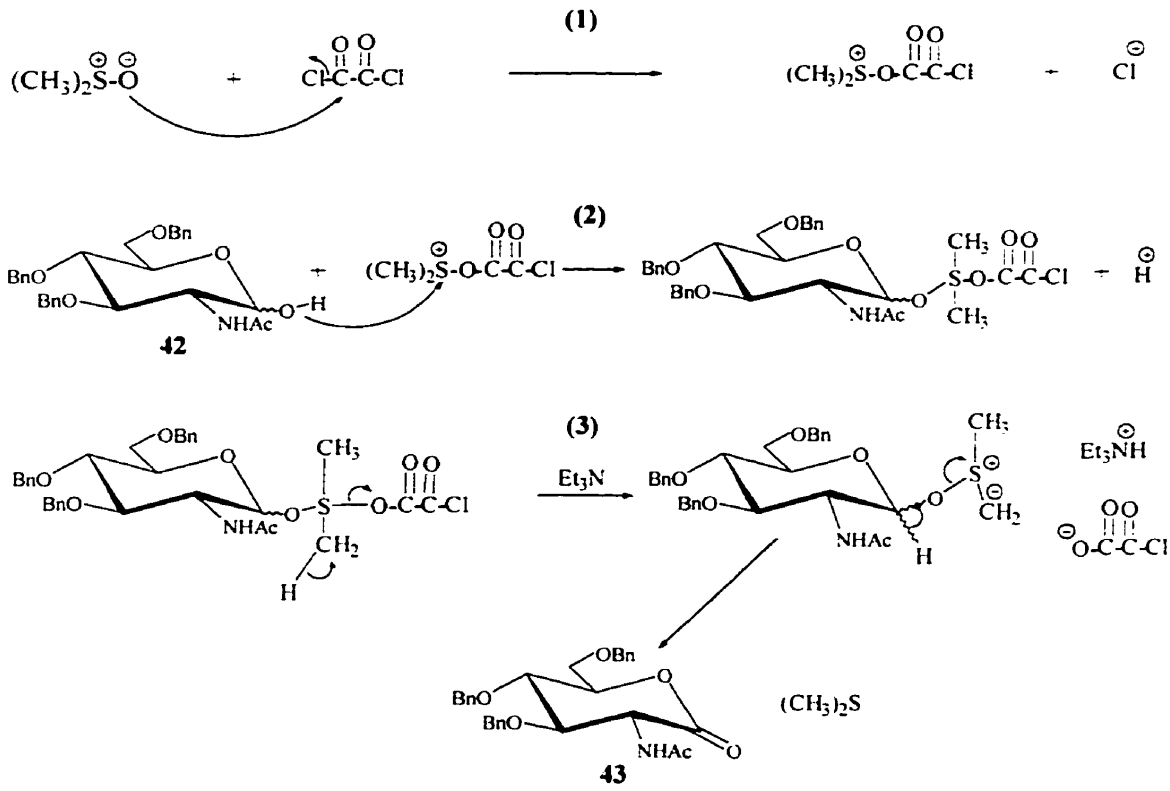


Scheme 11. Synthesis of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucono-1,5-lactone (**43**)

Swern oxidation, was adopted (**Scheme 11**). Thus, compound **42** was treated with a mixture of DMSO and oxalyl chloride at –70 °C, and with a subsequent addition of

triethylamine, to yield the lactone **43** in 88.3% yield. ^{1}H NMR spectroscopy of the oxidation product showed clearly the disappearance of the signal at δ 4.92 ppm (assigned to H-1 of compound **42**), and ^{13}C NMR spectroscopy showed the expected down-field shift of the C-1 signal at δ 90.98 in the spectrum of the hemiacetal **42** to δ 168.82 in the spectrum of the lactone.

The putative mechanism[47] of the Swern oxidation involves (1) activation of

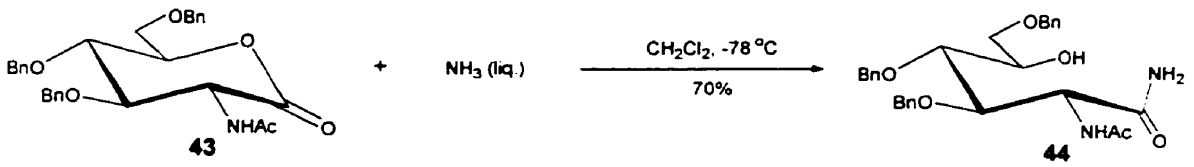


Scheme 12. The mechanism of the Swern oxidation

DMSO, which was accomplished by oxalyl chloride to form a sulfoxonium species by electrophilic attack at the sulfoxide oxygen, (2) addition of the substrate alcohol, and (3) departure of the sulfoxide oxygen (**Scheme 12**).

2.1.3. Ammonolysis of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucono-1,5-lactone (43)

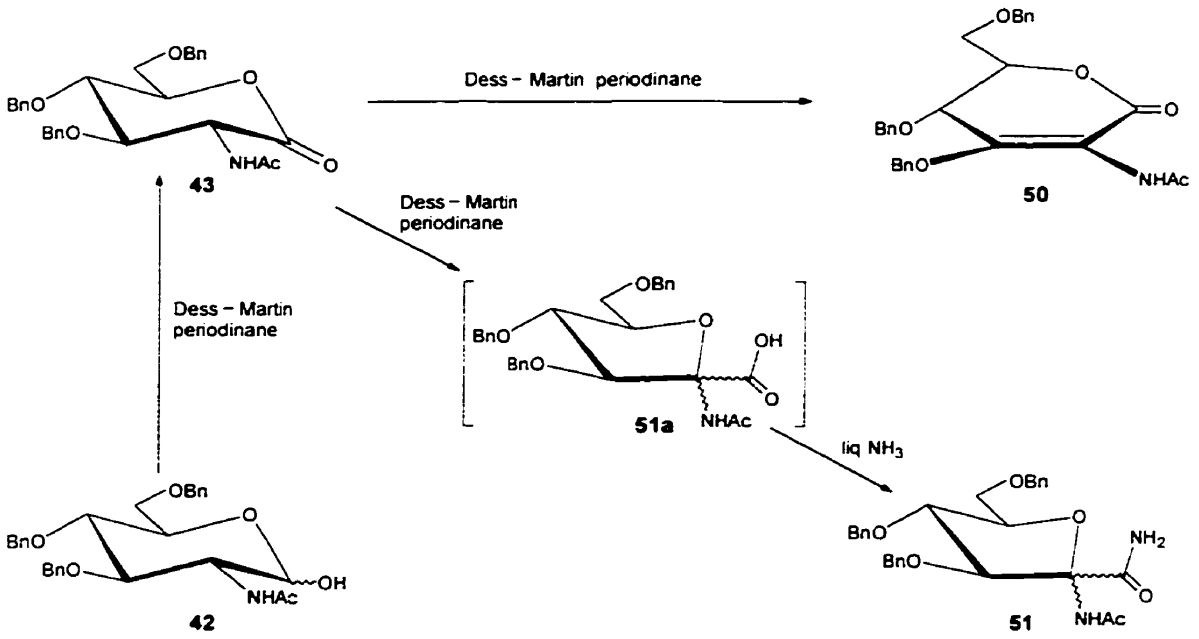
As seen in the general synthetic approach towards the target **40** (**Scheme 9**), a key step is the opening of the pyranoid ring in the lactone **43** to give 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-gluconamide (**44**). The hydroxy amide **44** was obtained simply by treatment of the lactone **43** with liquid ammonia at –78 °C as described by Granier and Vasella[46] and as depicted in **Scheme 13**. This ammonolysis reaction proceeded



Scheme 13. Synthesis of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-gluconamide (**44**)

well to give **44** (in 70% yield) if the sample of the lactone **43** employed was that obtained by a Swern oxidation of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose (**42**). However, if the crude product obtained by treatment of **42** with the Dess-Martin periodinane reagent was employed in the ammonolysis reaction, then, in addition to the gluconamide **44**, two side-products were isolated by gradient column chromatography. On the basis of NMR spectral (^{1}H, ^{13}C–*J*mod, H–H COSY, H–C HMQC, and H–C HMBC) and mass spectrometric data the two side-products were assigned the structures of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-*erythro*-hex-2-enopyrano-1,5-lactone (**50**) and 1-acetamido-1,4-anhydro-2,3,5-tri-*O*-benzyl-1-carboxamido-D-arabinitol (**51**) (see **Scheme 14**). In the ^{1}H NMR spectrum of **50**, the

observation of a value of 2.7 Hz for $J_{4,5}$ was indicative of a twist conformation for the molecule. In a ^{13}C–*J*mod experiment, the signals of C-4 and C-5 were observed at 69.86 and 75.97, respectively, whereas the signals for C-2 and C-3 were observed at considerably lower fields (δ 105.86 and 159.07, respectively). The ^{1}H NMR spectrum of **51** clearly showed signals at δ 4.15 as an apparent triplet having a splitting of 5.5 Hz attributable to H-3, at δ 4.22 as an apparent quartet attributable to H-4, and at δ 4.32 as a doublet having a splitting of 5.1 Hz attributable to H-2. These data are consistent with



Scheme 14. Explanation of the formation of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-*erythro*-hex-2-enopyrano-1,5-lactone (**50**) and 1-acetamido-1,4-anhydro-2,3,5-tri-*O*-benzyl-1-carboxamido-D-arabinitol (**51**).

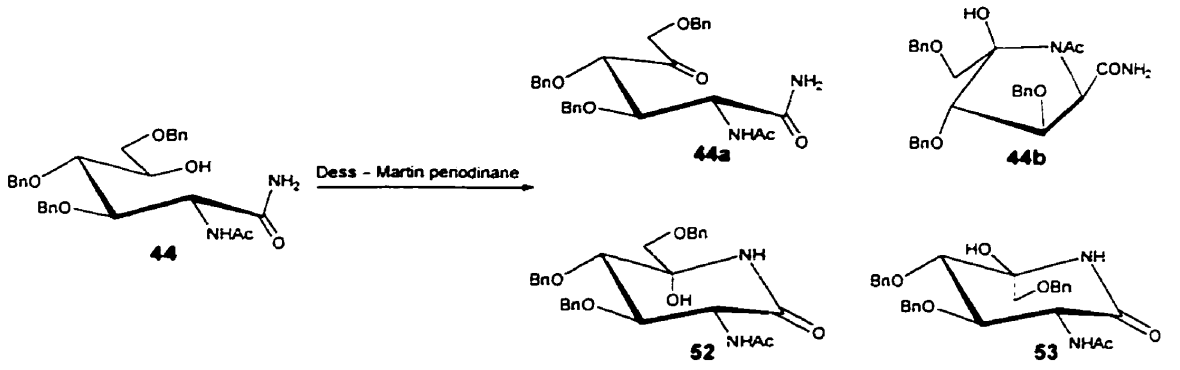
an envelope conformation for **51** as shown in **Scheme 14**. In the ^{13}C–*J*mod NMR spectrum, only three signals were observed in the region commonly attributed to sugar-ring carbons; these signals were observed at δ 80.53, 82.47, and 86.62 attributable to C-

4, C-3, and C-2, respectively. An outline of the process for the formation of **50** and **51** is shown in **Scheme 14**. In the case of **51**, the initially formed compound is postulated to be **51a**, which, under the ammonolysis conditions, could be converted into the amide **51**.

2.1.4. Conversion of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-gluconamide (44) into 2-acetamido-5-amino-3,4,6-tri-*O*-benzyl-2,5-dideoxy-D-glucono-1,5-lactam (45)

The ammonolysis process discussed above effected cleavage of the pyranoid ring in the lactone **43** to afford the acyclic amide **44**. The next stage in the general synthetic approach (**Scheme 9**) gave rise to the first azasugar derivative in the approach, namely the lactam **45**. The process in this stage was envisioned to involve oxidation of the hydroxyl group at C-5 in **44** to afford the corresponding 5-ketoamide, which upon intramolecular cyclization would give a 5-hydroxylactam (or a mixture of the two C-5 epimers); dehydroxylation at C-5 would furnish the desired 2-acetamido-5-amino-3,4,6-tri-*O*-benzyl-2,5-dideoxy-D-glucono-1,5-lactam (**45**) (and/or its C-5 epimer). According to Granier and Vasella[46], treatment of the acyclic amide **44** with the Dess-Martin periodinane reagent (see **Scheme 15**) was claimed to afford quantitatively a mixture of the 5-ketoamide **44a**, the pyrrolidinecarboxamide **44b**, and the 5-hydroxylactams **52/53** in the ratio of 44:28:16:12, respectively. Granier and Vasella claimed also that **44a** was converted into **52/53** in a ratio of 83:17, respectively, on treatment with toluene–AcOH, and also that **44b** was converted into the mixture

44b/52/53 in a ratio of 10:69:21, respectively, on treatment with $BF_3 \bullet OEt_2$ at 5 °C for 24 h. However, in the present study these results claimed by Granier and Vasella could

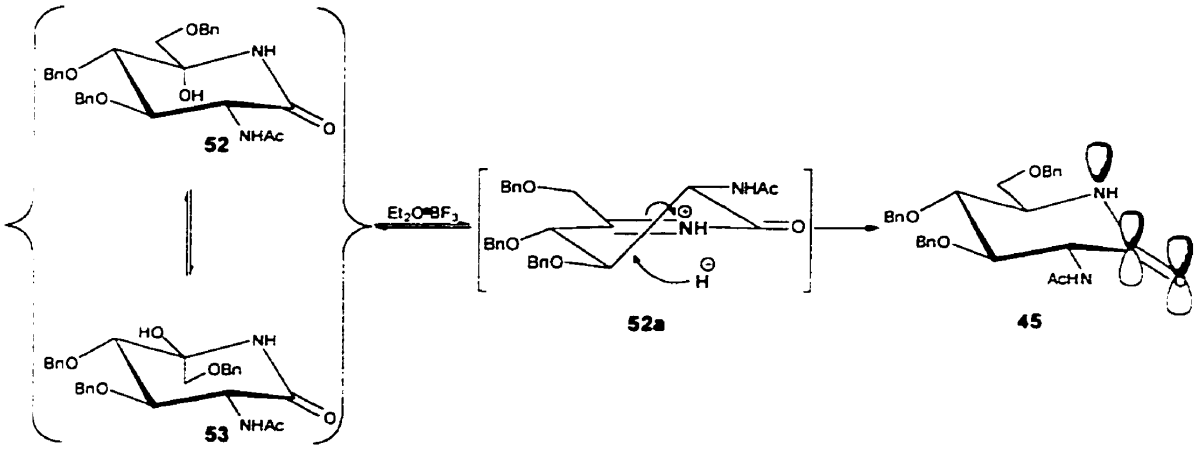


Scheme 15. Synthesis of 2-acetamido-5-amino-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucono-1,5-lactam (**52**) and 2-acetamido-5-amino-3,4,6-tri-*O*-benzyl-2-deoxy-L-idono-1,5-lactam (**53**) according to Granier and Vasella[46]

not be reproduced. Indeed, TLC revealed the formation of numerous products and no discrete compound could be isolated by column chromatography. Consequently, in the present study the applicability of the Jones reagent[48] was investigated. This approach proved to be successful, and the 5-hydroxylactams **52** and **53** were isolated in a ratio of 8:1, respectively, by column chromatography. The assignment of structures to **52** and **53** was based on NMR spectral data (^{1}H, ^{13}C–*J*mod, H–H COSY, H–C HMQC, and H–C HMBC). In the case of **52** the signals for H-2 and H-4 were observed at higher fields (δ 4.02 and 3.83, respectively) than the signals for the corresponding protons in **53** (δ 4.70 and 3.93), whereas, the signal for H-3 in the spectrum of **52** was observed at lower field (δ 4.25) than that for H-3 in the spectrum of **53** (δ 3.97). No fuller characterization of **52** and **53** was deemed to be necessary, since, as seen below, even a mixture of **52** and **53** could be converted in good yield to the desired lactam **45**.

Reductive dehydroxylation at C-5 to afford lactam **45** could be effected using Et_3SiH and $BF_3{\bullet}OEt_2$[49]. Thus, treatment of either **52** or a mixture of **52** and **53** with Et_3SiH and $BF_3{\bullet}OEt_2$ in CH_2Cl_2–MeCN (1:1) led to the formation of a single lactam, **45**; purification by gradient flash column chromatography rendered the product as white crystals in a yield of 73%.

The formation of the single lactam **45** can be rationalized by considering the relevant stereoelectronic factors which operate in the transition state of the reaction (**Scheme 16**). Granier and Vasella[46] suggested that there is a rapid equilibrium between the two epimeric hydroxylactams under acidic conditions, and Overkleeft *et al.*[50], in an



Scheme 16. Synthesis of 2-acetamido-5-amino-3,4,6-tri-*O*-benzyl-2,5-dideoxy-D-glucono-1,5-lactam (**45**)

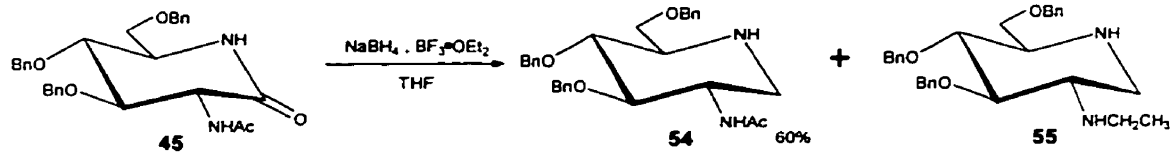
analogous study of perbenzylated hydroxylactams, suggested that either epimer can undergo an acid-catalyzed dehydration process. Thus, the acyliminium ion **52a** is presumed to be formed initially from the substrate mixture. Only when the hydride approaches the α-face of the ring does the configuration of the developing nitrogen

electron-pair generated in this way allow the most effective overlap with the orbitals of the lactam carbonyl; in this situation, the substituents on the sugar ring assume equatorial orientations. In contrast, a β-attack at the iminium carbon would require that, when the ideal overlap between the developing nitrogen lone-pair and the carbonyl orbitals is attained, the transition state bears the three substituents (C-2 acetamido, C-3 and C-4 benzyloxy groups) in the axial orientations. Thus, steric factors in the transition states of the two pathways favor the approach of the hydride on the α-face, and hence to afford the lactam **45**.

Hydrogenolysis of the tri-*O*-benzylated lactam **45** was readily achieved to afford 2-acetamido-5-amino-2,5-dideoxy-D-glucono-1,5-lactam (**46**) in 93.3% yield.

2.1.5. Conversion of 2-acetamido-5-amino-3,4,6-tri-*O*-benzyl-2,5-dideoxy-D-glucono-1,5-lactam (45) into 2-acetamido-1,2,5-trideoxy-1,5-imino-D-glucitol (40)

The final steps in the synthesis of the target, 2-acetamido-1,2,5-trideoxy-1,5-imino-D-glucitol (**40**), involved reductive deoxygenation of the lactam **45** to afford the corresponding 1-deoxy derivative, followed by hydrogenolytic debenzylation. The reductive deoxygenation was achieved by treatment of the lactam **45** with $NaBH_4$ and $BF_3 \bullet OEt_2$[51]. The expected product, namely 2-acetamido-3,4,6-tri-*O*-benzyl-1,2,5-trideoxy-1,5-imino-D-glucitol (**54**), was isolated as a white solid in 60% yield; another compound, which migrated at a slower rate in TLC, was isolated as a brown oil and was assigned the structure of 3,4,6-tri-*O*-benzyl-2-ethylamino-1,2,5-trideoxy-1,5-imino-D-glucitol (**55**) (see **Scheme 17**).

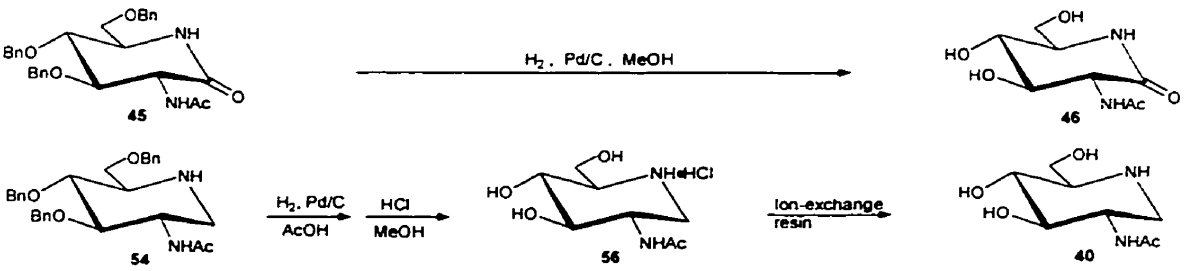


Scheme 17. Synthesis of 2-acetamido-3,4,6-tri-*O*-benzyl-1,2,5-trideoxy-1,5-imino-D-glucitol (**54**)

The ^{1}H NMR spectrum of **54** showed two doublet-of-doublets at δ 2.28 ppm and δ 3.32 ppm attributable to the two H-1 protons having a germinal coupling constant of ~ 13 Hz. The signal of H-5 was observed as a multiplet at a higher field (δ 2.75) than the corresponding multiplet (δ 3.67) in the spectrum of the lactam **45**. In the ^{13}C–*J*mod spectrum, the C-1 signal was observed at a very much higher field (δ 47.9) than the corresponding signal of the lactam **45**. The $J_{H,H}$ values for **54** ($J_{1a,2}$ 10.0, $J_{1b,2}$ 4.8, $J_{2,3}$ 9.6, $J_{3,4}$ 8.8, $J_{4,5}$ 8.8 Hz) are in agreement with a $^{4}C_{1}$ conformation.

The ^{1}H NMR spectrum of **55** did not show the typical resonance of an acetamido group as in **54** (NAc: singlet, 1.74 ppm). Instead, the spectrum of **55** showed signals attributable to an ethylamino group (CH_3: triplet, *J* 7.0 Hz, 1.05 ppm; CH_2: 2 multiplets at δ 2.52 and 2.70). The multiplet attributable to H-2 of **55** was observed at a higher field (δ 2.63) than the corresponding multiplet (δ 3.78) in the spectrum of **54**. The $J_{H,H}$ values for **55** ($J_{1a,2}$ 10.8, $J_{1b,2}$ 4.5, $J_{2,3} = J_{3,4} = J_{4,5} = 9.0$ Hz) are similar to those of **54** and clearly indicate the $^{4}C_{1}$ ring conformation for **55**. In an HMQC NMR experiment the two methylene protons of the ethylamino group at δ 2.52 and 2.70 were indeed shown to correlate to one methylene carbon at δ 42.1.

Although, as indicated in **Section 2.1.5**, the hydrogenolytic debenzylation of the lactam **45** could be readily achieved to afford **46** (see **Scheme 18**), difficulty was experienced in applying the same conditions (H_2, Pd/C, MeOH) for debenzylation of the corresponding 1-deoxy derivative **54**. In a conversion of 2,3,4,6-tetra-*O*-benzyl-1-deoxynojirimycin into 1-deoxynojirimycin (**1**) Ermert and Vasella[52] used AcOH instead of MeOH as the solvent in the hydrogenolysis. When this modification was introduced in the case of the reaction with **54**, debenzylation did indeed occur readily to give a product which was isolated as the hydrochloride salt (**56**) of 2-acetamido-1,2,5-trideoxy-1,5-imino-D-glucitol (**40**). The free base **40** was liberated by treatment of the



Scheme 18. Synthesis of 2-acetamido-5-amino-2,5-dideoxy-D-glucono-1,5-lactam (**46**) and 2-acetamido-1,2,5-trideoxy-1,5-imino-D-glucitol (**40**)

salt with Amberlite IR-120 (H^+ form). The physical constants obtained for the target material **40** were in agreement with literature values[36(a), 36(b)], and the NMR data were identical to those reported[36(b)] for **40**.

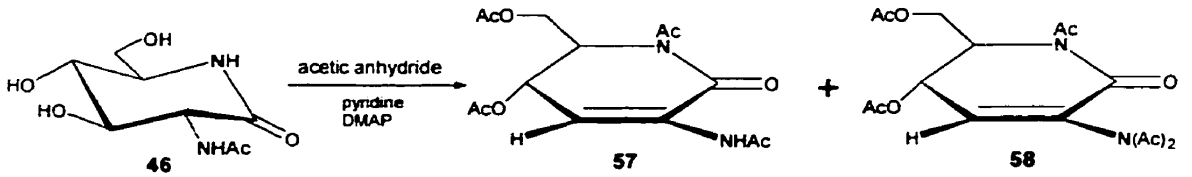
2.1.6. Acetylation of 2-acetamido-5-amino-2,5-dideoxy-D-glucono-1,5-lactam (46) and 2-acetamido-1,2,5-trideoxy-1,5-imino-D-glucitol (40)

In an earlier study in this laboratory[18], it was shown that the acetylated compounds, methyl 2-acetamido-3,6-di-*O*-acetyl-2,4-dideoxy-β-D-*xylo*-hexopyranoside and 2-acetamido-1,3,6-tri-*O*-acetyl-2,4-dideoxy-α,β-D-*xylo*-hexopyranoses, showed a greater inhibition of [^{3}H]GlcN and [^{14}C]Leu incorporation at 1mM compared with their non-acetylated counterparts, but were toxic to hepatocytes at concentrations of 10 and 20 mM. As indicated in **Section 1.5** a primary objective of the present study was to provide further candidate compounds for studying the effects of monosaccharide analogues on the biosynthesis of glycosaminoglycans. In view of the results obtained in this laboratory[18] with the acetylated compounds, it was deemed worthwhile to provide acetylated derivatives of compounds prepared in the present study, in particular of 2-acetamido-5-amino-2,5-dideoxy-D-glucono-1,5-lactam (**46**) and 2-acetamido-1,2,5-trideoxy-1,5-imino-D-glucitol (**40**).

In the initial attempt to synthesize the tri-*O*-acetylated derivative of **46**, namely 2-acetamido-3,4,6-tri-*O*-acetyl-5-amino-2,5-dideoxy-D-glucono-1,5-lactam (**59**), a catalytic amount of DMAP[53] was added to a solution of compound **46** in pyridine containing an excess of acetic anhydride. However, instead of the desired product, **59**, there were isolated two olefinic compounds, namely 2,5-diacetamido-4,6-di-*O*-acetyl-2,3,5-trideoxy-D-*erythro*-hex-2-enopyrano-1,5-lactam (**57**) as a colorless oil and 5-acetamido-4,6-di-*O*-acetyl-2-(*N*,*N*-diacetyl)amino-2,3,5-trideoxy-D-*erythro*-hex-2-enopyrano-1,5-lactam (**58**) as a white solid in 48% and 22% yields, respectively (**Scheme 19**).

The ^{1}H NMR spectrum of **57** showed four singlets at δ 1.93, 1.98, 2.11, and 2.54, corresponding in intensity to twelve protons, indicative of the presence of four

acetyl groups. Also, signals were observed in the region δ 4.00 – 5.60, corresponding in intensity to four protons, and were attributed to 2 H-6, H-5, and H-4. The signal for H-3 was observed at low field (δ 7.42) in accordance with a β-proton of an α,β-unsaturated carbonyl system. The signals for H-3 and H-4 were each observed as a



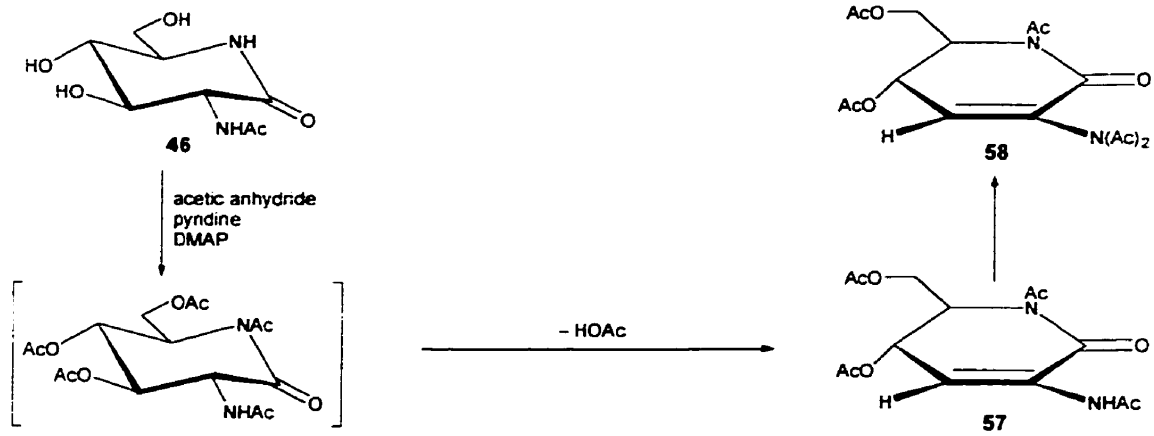
Scheme 19. Formation of 2,5-diacetamido-4,6-di-*O*-acetyl-2,3,5-trideoxy-D-*erythro*-hex-2-enopyrano-1,5-lactam (**57**) and 5-acetamido-4,6-di-*O*-acetyl-2-(*N*,*N*-diacetyl)amino-2,3,5-trideoxy-D-*erythro*-hex-2-enopyrano-1,5-lactam (**58**)

doublet having a splitting of 6.6 Hz. No coupling was observed between H-5 and H-4, a result consistent with a half-chair conformation.

In the case of the ^{1}H NMR spectrum of **58** there were signals at high fields, corresponding in intensity to fifteen protons, indicative of the presence of five acetyl groups. Also, signals were observed in the region δ 4.20 – 5.45, corresponding in intensity to four protons, and were attributed to 2 H-6, H-5, and H-4. The signal for H-3 was observed at low field (δ 6.78). In contrast to the case of compound **57**, H-4 was observed as a doublet-of-doublets having $J_{3,4}$ = 6.3 Hz and $J_{4,5}$ = 1.7 Hz. H-3 was also observed as a doublet-of-doublets having $J_{3,5}$ = 1.4 Hz, a result attributed to long-range W-type coupling of H-3 and H-5.

The structures assigned to compounds **57** and **58** were confirmed also by mass spectrometric data.

The particular conditions employed for the attempted acetylation of **46** were clearly too basic. A possible sequence of reactions for the formation of compounds **57** and **58** is shown in **Scheme 20**.



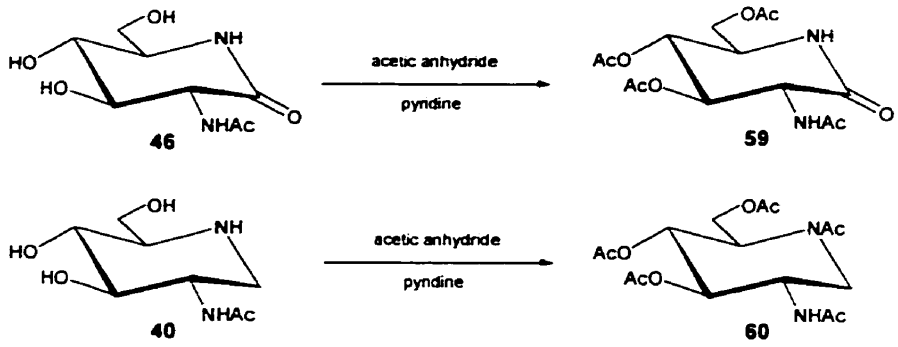
Scheme 20. Possible sequence for the formation of 2,5-diacetamido-4,6-di-*O*-acetyl-2,3,5-trideoxy-D-*erythro*-hex-2-enopyrano-1,5-lactam (**57**) and 5-acetamido-4,6-di-*O*-acetyl-2-(*N*,*N*-diacetyl)amino-2,3,5-trideoxy-D-*erythro*-hex-2-enopyrano-1,5-lactam (**58**)

The acetylation of **46** could be achieved successfully to give 2-acetamido-3,4,6-tri-*O*-acetyl-5-amino-2,5-dideoxy-D-glucono-1,5-lactam (**59**) if **46** were treated simply with acetic anhydride in pyridine without the inclusion of DMAP. Subjecting 2-acetamido-1,2,5-trideoxy-1,5-imino-D-glucitol (**40**) to these conditions afforded 2-acetamido-3,4,6-tri-*O*-acetyl-1,2,5-trideoxy-1,5-(*N*-acetyl)imino-D-glucitol (**60**) (see **Scheme 21**).

The ^{1}H NMR spectrum of **59** showed four singlets in the range δ 1.9 – 2.1, corresponding in intensity to twelve protons, indicating the presence of four acetyl groups. The observation of each of the signals for H-2, H-3, and H-4 as an apparent

triplet having a splitting of 9 – 10 Hz was indicative of the $^4C_1$ conformation for **59** in solution.

In the case of compound **60**, when CD_3OD or $CDCl_3$ was used as the solvent for obtaining the ^1H NMR spectrum, the peaks were broadened to such an extent that no useful information could be retrieved from the spectra. D_2O was a better solvent in that



Scheme 21. Synthesis of 2-acetamido-3,4,6-tri-*O*-acetyl-5-amino-2,5-dideoxy-D-glucono-1,5-lactam (**59**) and 2-acetamido-3,4,6-tri-*O*-acetyl-1,2,5-trideoxy-1,5-(*N*-acetyl)imino-D-glucitol (**60**)

most of the structural information could be obtained using various NMR experiments. However, peak-broadening was still present; each of the signals for H-2, H-3, H-4, and H-5 appeared as a broad singlet. In addition to the peak-broadening, the ^1H NMR spectrum of **60** was unusually complex and it was suggested that either the compound possesses internal hindered rotation or the substance may be a mixture of two compounds in a 2:1 ratio. Evidence for the former suggestion was obtained by measuring an ^1H NMR spectrum at an elevated temperature (348 K); the spectrum was much simplified, especially in the high-field region where the previously observed two sets of acetyl peaks merged into one set of four singlet-peaks corresponding in intensity to five acetyl groups.

Further evidence for the existence of hindered internal rotation in **60** was obtained by performing a TOCSY NMR experiment. In a TOCSY experiment[54] the magnetization of one spin is transferred beyond directly coupled spins, thus enabling the observation of correlations among nuclei that are not directly coupled but that are within the same spin system. In the case of **60**, the two sets of signals in the TOCSY spectrum did indeed appear to be coupled. This result indicated that the two sets of signals in ^{1}H NMR spectrum originated from two isomeric structures of one compound, structures caused by the slow rotation about the hindered "partial double bond" of the acetyl group on the ring-nitrogen at room temperature. This structural feature arises from resonance conjugation between the p-orbital on nitrogen and the p-orbital of the π-electron system resulting in two dipolar structures. The effect of hindered internal rotation was observed also in the ^{1}H NMR spectrum of compound **57**, but to a much less extent.

The structures assigned to compounds **59** and **60** were confirmed also by mass spectrometric analysis.

From the solution-phase synthetic studies described above in **Section 2.1** azasugar derivatives **40**, **46**, **57**, **58**, **59**, and **60** were submitted for evaluation of their effects on the biosynthesis of glycosaminoglycans. The results obtained from this biological evaluation are discussed in **Section 2.3**.

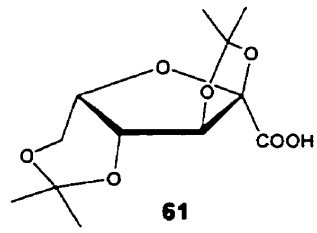
2.2. Preliminary studies on solid-phase synthesis

Polymer-supported synthesis has been used mainly in peptide, oligonucleotide, and oligosaccharide synthesis. An example of the synthesis of pyrrolidine derivatives was provided by Gallop and coworkers[55] based on 1,3-dipolar cycloaddition reactions between resin-bound azomethine ylides and electron-poor olefins as reactive dipolarophiles. The solid-support used in that work was acid-labile Sasrin resin preloaded with Fmoc-protected amino acids.

In this laboratory a new methodology in azasugar synthesis was explored, specifically aimed at 1-deoxynojirimycin (**1**), by coupling a sugar substrate onto a functionalized cross-linked polystyrene resin {Merrifield resin (1% divinylbenzene)}. This resin shows a more pronounced swelling-capacity in aprotic solvents since it contains a low degree of crosslinking[56]. The advantages of using resin-supported reactions are mainly the following. (i) After the reaction proceeds to completion, only filtration and washing of the polymer are required to process the reaction mixture. These operations lend themselves to automation, and hence, the procedure has an obvious industrial attraction. (ii) Since cross-linked polymers are insoluble and non-volatile, they are non-toxic and odorless. These features make the procedure environmentally more acceptable.

The substrate chosen in the present work was the commercially available L-sorbose derivative, 2,3:4,6-di-*O*-isopropylidene-α-L-*xylo*-hexulosonic acid (**61**). The coupling of the substrate to the Merrifield resin was accomplished by converting the substrate into its cesium-salt form, which was then treated with the Merrifield resin in

anhydrous DMF at a higher temperature to form an ester bond as the linkage between the substrate and the resin. Described below are the results obtained from a preliminary



61

exploratory study directed towards the synthesis of an azasugar analogue.

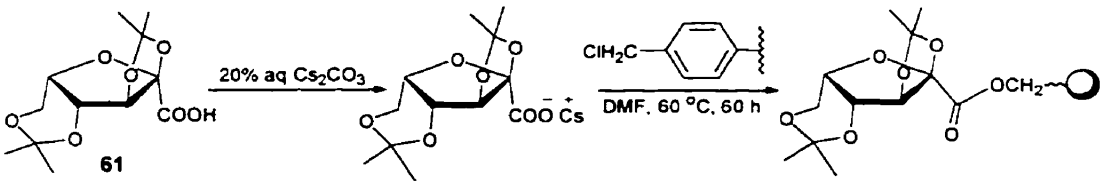
2.2.1. Coupling of 2,3:4,6-di-*O*-isopropylidene-α-L-*xylo*-hexulosonic acid (61) to Merrifield resin

Any type of solid-phase synthesis always involves firstly the attachment of a suitably protected starting compound to a chosen resin. To choose the resin, three factors should be considered: (i) it must be chemically and physically inert to the reagents used in the synthesis yet appropriately functionalized to allow anchoring of the substrate; (ii) its physical properties must allow any excess of reagents to be quickly and efficiently removed at the end of each reaction; and (iii) it must not cause by virtue of its presence significant inhibition of the chemical reactions needed for the functional group transformations. The resin chosen in this preliminary study on solid-phase synthesis was the Merrifield chloromethylated styrene (1% divinylbenzene copolymer) having a loading capacity of 1.5 mequiv g^{-1}. The already existing functional group on

the resin, which is, in effect, an "insoluble benzyl chloride", allowed the attachment of the substrate **61**.

In order that the linkage between **61** and the resin can be formed easily, the carboxylic acid **61** was converted into its cesium salt by titrating the solution of **61** in MeOH–H_2O (10:1) to pH 7.0 with a 20% aq solution of cesium carbonate[57]. Then an S_N2 reaction between the cesium salt of **61** and the resin resulted in the formation of an ester linkage to the resin (**Scheme 22**).

The use of a cesium salt is related to the requirement for aprotic solvents. As shown in **Scheme 22**, the alkylation reaction is fastest when the carboxylate oxygen has



Scheme 22. Coupling of 2,3:4,6-di-*O*-isopropylidene-α-L-*xylo*-hexulosonic acid (**61**) to Merrifield resin

as much ionic character as possible, because a carboxylate oxygen having a full negative charge is more nucleophilic and therefore reacts much more rapidly in S_N2 reactions than a covalent carboxylate oxygen. Cesium is the most electropositive and has the largest atomic radius amongst the readily available alkali metals, hence its carboxylate salts have more ionic character and dissolve in DMF better than lithium, sodium, or potassium salts, and hence are the most reactive in S_N2 reactions.

The solvents used for the reactions of solid-phase synthesis should have the capability to solvate the Merrifield resin, i.e., they should interact well with the groups

of the resin. Because the polystyrene-based Merrifield resin is essentially a polymeric ethylbenzene, it is mostly a hydrocarbon. Some aprotic solvents, such as DMF, THF, and CH_2Cl_2, were found empirically to solvate the Merrifield resin adequately. When such solvents are added to a Merrifield resin, a visually perceptible swelling of the resin bed occurs, which is caused by incorporation of the solvent into the resin. In contrast, a well-swelled resin bed shrinks when solvents such as MeOH and H_2O are added. This feature was also exploited in the filtration step at the end of each reaction by using aprotic and protic solvents alternatively.

The extent of the coupling of **61** to Merrifield resin was inspected mainly by three methods, namely gravimetrical analysis, infrared spectroscopy, and chemical cleavage. The reaction was allowed to be repeated until a satisfactory yield was achieved.

Gravimetrical analysis is based on the calculation of the mole percentage of the functional groups on the Merrifield resin which participated in the coupling reaction.

The infrared spectroscopic method involves recording IR spectra using the same amount of resin sample at different times of the reaction and inspecting the change in intensity of absorption of a characteristic group.

The carbonyl group, which was introduced into the resin polymer by the coupling of **61** to the Merrifield resin, should have strong absorption in the 1750–1735 cm^{-1} region[54]. By comparison of the IR spectra using 2-mg resin samples at reaction times of 0 h, 18 h, 36 h, and 64 h (**Figure 5**), the growth in intensity of the carbonyl band at 1745 cm^{-1} was easily discerned. The halt of its growth after about 60 h of reaction time symbolized a completion of the coupling reaction.

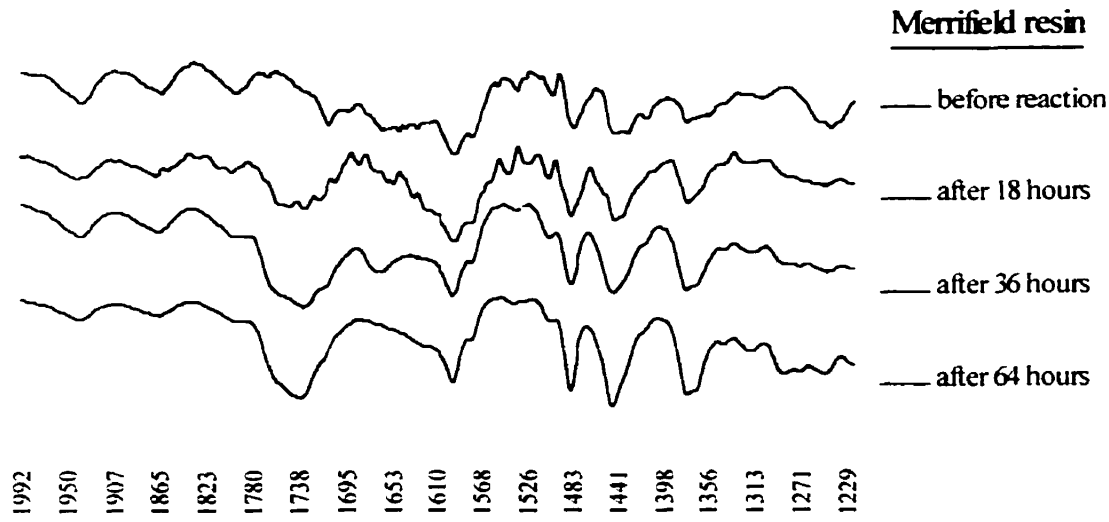
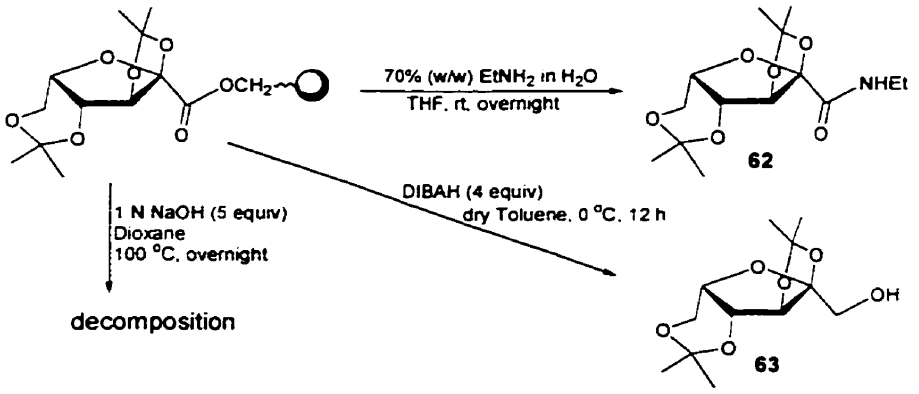


Figure 5. Time-course infrared spectra of the coupling reaction

The chemical cleavage method, which utilizes chemical reactions to cleave the compound from the Merrifield resin after each transformation step, is the most reliable method amongst the three inspection methods. An ester linkage, the type of linkage used in the case of **61**, is cleavable by the use of acids, bases including amines, and some hydride donors. Some reagents known from the literature are HF[57], TFA[58], NaOH[59], NaOMe[60], $EtNH_2$[61], diisobutylaluminum hydride[62], and $LiBH_4$[59].

Some cleavage reactions that have been explored in the present study are illustrated in **Scheme 23**. Treating the resin-ester with ethylamine (70% ethylamine in water) and THF (1:1) at room temperature with overnight shaking gave compound **62** quantitatively. Treating the resin-ester with 4 equiv DIBAH in toluene at 0 °C for 12 h yielded compound **63** in a moderate yield. The structures of the expected products, **62** and **63**, were confirmed by NMR spectral data. The reaction conditions for treating the

resin-ester with 1 N NaOH (5 equiv) in dioxane at 100 °C with overnight stirring seemed too harsh to render the expected compound **61**; thus, only decomposition of the



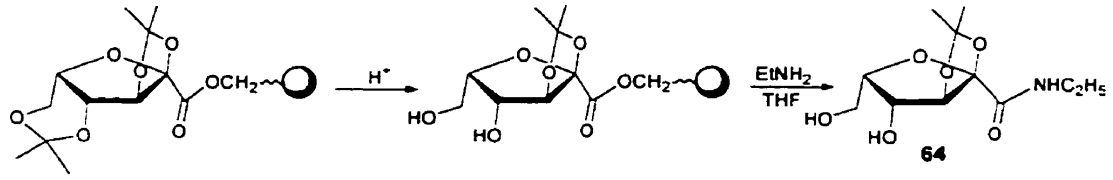
Scheme 23. Some cleavage reactions

starting material was observed. Based on these experiments, ethylamine was chosen as the cleaving reagent in subsequent reactions; in addition to its effective nucleophilic ability to cleave the ester-linker bond, its low boiling point is advantageous, since the reagent can be removed easily by evaporation. The final yield of **62** was 91%, a result in accordance with that obtained by gravimetrical analysis. Hence, 91% of the functional groups on the Merrifield resin were coupled with compound **61**.

2.2.2. Selective removal of the 4,6-*O*-isopropylidene group from the resin-bound 61

To free the primary hydroxyl group in the resin-ester is a key step for the subsequent functional group transformations. In a related solution-phase synthesis,

Szarek *et al.*[63] selectively removed the 4,6-*O*-isopropylidene group in a 2,3:4,6-di-*O*-isopropylidene-α-L-*xylo*-hexulose derivative using 60% aqueous acetic acid. In the present study, in order to remove the 4,6-*O*-isopropylidene protecting group in the resin-ester, a series of conditions were examined: 60% (v/v) aqueous acetic acid at 60 °C, 60% (v/v) acetic acid in dioxane at 60 °C, 1 N HCl in dioxane (1:1) at room temperature[64], 2 N HCl in dioxane (1:1) at room temperature, and 3 N HCl in dioxane (1:1) at room temperature. An inspection of the effectiveness of these conditions was performed by treating the resins with ethylamine to cleave the product from the resin. The successful removal of the 4,6-*O*-isopropylidene group was manifested by the observation of the presence of only one component in TLC which was assigned the structure of *N*-ethyl-2,3-*O*-isopropylidene-α-L-*xylo*-hexulosonamide (**64**, **Scheme 24**) on the basis of NMR spectral and mass spectrometric data. The conditions employed

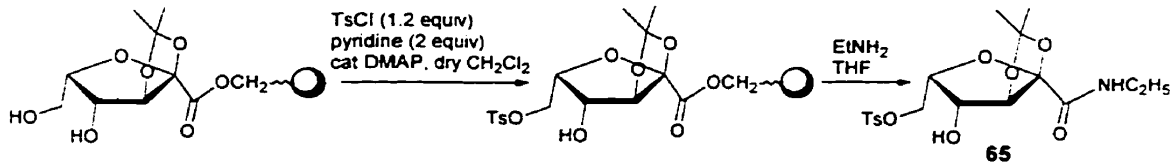


Scheme 24. Selective removal of the 4,6-*O*-isopropylidene protecting group on the resin

for a large-scale reaction involved treatment with 3 N HCl in dioxane (1:1) with shaking at rt for 30 h.

2.2.3. Functional group transformations on the resin

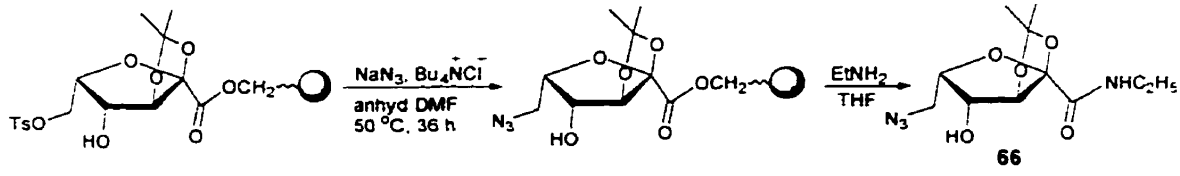
The next stage towards the solid-phase synthesis of 1-deoxynojirimycin involved conversion of the primary hydroxyl group in the resin-diol obtained above into a tosylate, followed by replacement of the tosyloxy group by an azido group. The chemoselective tosylation of the primary hydroxyl group was accomplished by swelling the resin-diol in dry CH_2Cl_2 and then treating it with *p*-toluenesulfonyl chloride in dry pyridine and a catalytic amount of DMAP (**Scheme 25**). This process is analogous to a related solution-phase procedure[63], but a longer reaction time was employed in the present work. However, after a total reaction time of 64 h, the ^1H NMR spectrum of the product, which was obtained after cleavage from an aliquot of the resin using ethylamine, indicated that it was a 3.6:1 mixture of the desired *N*-ethyl-2,3-*O*-isopropylidene-6-*O*-*p*-tolylsulfonyl-α-L-*xylo*-hexulosonamide (**65**) and the diol **64**.



Scheme 25. Tosylation of the primary hydroxyl group on the resin

respectively. This mixture was subjected to preparative thin-layer chromatography, and a homogeneous sample of **65** was isolated whose ^1H and ^{13}C NMR spectra, IR spectrum, and mass spectrometric data were consistent with the assigned structure. Because the reaction time (64 h) was already long, there was concern that a more-prolonged treatment with *p*-toluenesulfonyl chloride might lead to an unwanted tosylation at C-4. Consequently, it was decided that, in this preliminary study, the

product resin obtained in this step would be utilized in the next step. Thus, the product resin was swelled in anhydrous DMF, and an excess of NaN_3 and a catalytic amount of tetra-*n*-butylammonium chloride were added. The reaction mixture was shaken at 50 °C for 36 h (**Scheme 26**).



Scheme 26. Azide substitution on the resin

Interestingly, the IR spectra of the resin from the reaction showed two distinct azide absorption bands increasing in intensity at 2020 cm^{-1} and 2099 cm^{-1}. At the end of the reaction period, a cleavage reaction was performed and the recovered resin was

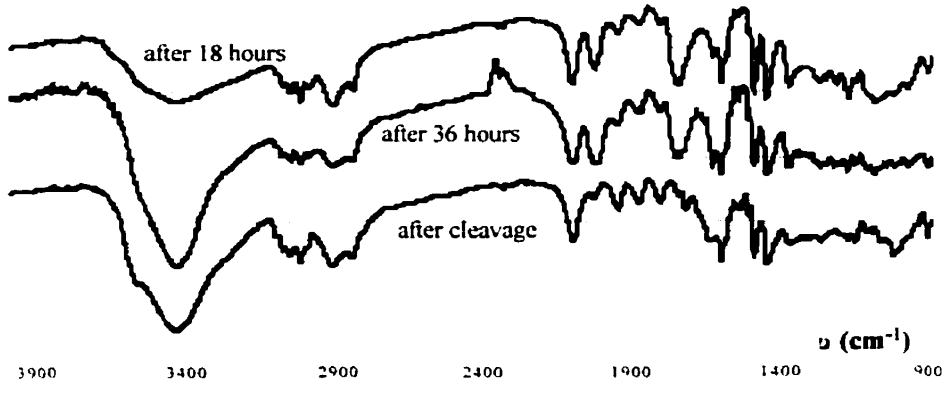
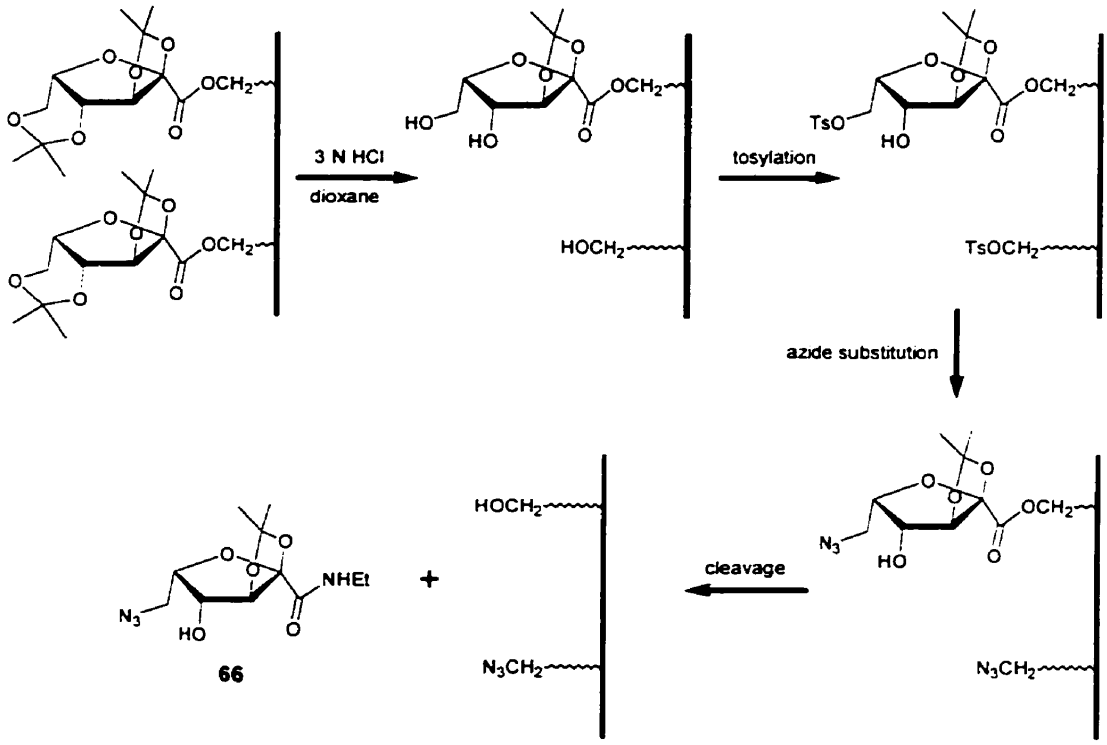


Figure 6. Infrared spectroscopic examination of the azide substitution reaction

analyzed again by IR spectroscopy. The band at 2020 cm^{-1} disappeared, but the absorption band at 2099 cm^{-1} remained (see **Figure 6**). This observation suggested that azide groups were introduced not only into the resin-bound carbohydrate substrate but also into the resin itself. The genesis of the latter reaction might be a liberation from the resin of some of the ester-linked derivatives during the selective hydrolysis reaction using 3 N HCl (see **Scheme 27**). This premature cleavage produced free hydroxyl groups on the resin, which, subsequently, were tosylated by *p*-toluenesulfonyl chloride.



Scheme 27. A possible explanation for the observation of two azide absorption bands in the infrared spectrum of the reaction resin

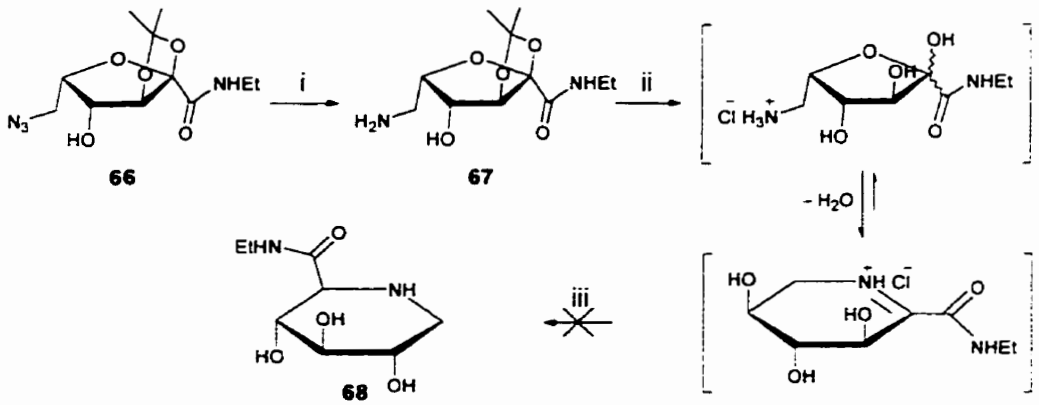
Thus, when the resin was treated with NaN_3, the nucleophilic azide group replaced the tolylsulfonyloxy groups from both the carbohydrate moiety and the resin, and hence

accounting for the observation of two azide absorption bands before the ethylamine cleavage and one azide absorption band after the cleavage. The fast growth of the absorption band at 2099 cm^{-1} in the first 18 h of the reaction and a catch-up growth of the absorption band at 2020 cm^{-1} in the second 18 h, as seen in **Figure 6**, indicated that the tolylsulfonyloxy group on the resin was replaced by azide much faster than that on the carbohydrate moiety, a difference presumably reflective of the reactivity difference between a benzyl and an alkyl group. The premature cleavage would also be a major factor which accounted for the decrease in the overall yield of final product.

2.2.4. Formation of nitrogen-in-the-ring sugar analogues

In the present study, the first attempt to convert the product obtained from the work described in **Section 2.2.3**, namely 6-azido-6-deoxy-*N*-ethyl-2,3-*O*-isopropylidene-α-L-*xylo*-hexulosonamide (**66**), followed the sequence of reactions employed in a related study[63]: (i) reduction of the azido group of compound **66**: (ii) removal of the 2,3-*O*-isopropylidene protecting group to induce a ring rearrangement: (iii) reduction of the cyclic Schiff-base intermediate formed in (ii) using $NaBH_3CN$ (see **Scheme 28**). Thus, catalytic hydrogenation of compound **66** over 10% palladium-on-charcoal in methanol gave 6-amino-6-deoxy-*N*-ethyl-2,3-*O*-isopropylidene-α-L-*xylo*-hexulosonamide (**67**), whose structure was confirmed by NMR spectroscopy and by low- and high-resolution mass spectrometric analysis. Compound **67** then was treated with 2 N HCl in aqueous THF at reflux temperature for 8 h. The residue, obtained by evaporation of the reaction mixture, was treated with an excess of $NaBH_3CN$ in MeOH.

However, the product obtained was not the desired **68** but appeared to be (^{1}H NMR and

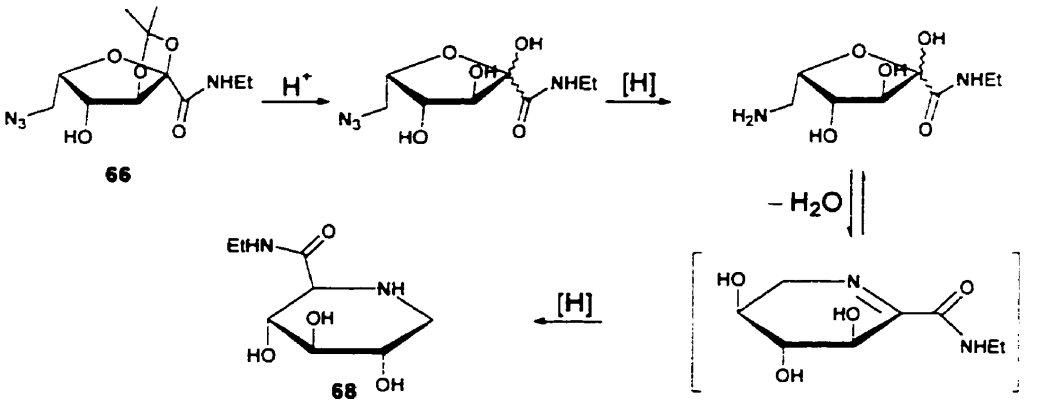


Scheme 28. First attempted formation of 1,5-dideoxy-*N*-ethyl-1,5-imino-D-glucuronamide (**68**)

COSY NMR) a four-proton, three-carbon fragment. Apparently the reaction conditions employed in the acid-catalyzed hydrolysis step were too harsh and caused decomposition of **67**.

Di *et al.*[65] have reported the transformation of an intermediate compound, which is structurally similar to **66**, into 1,5,6-trideoxy-6-fluoro-1,5-imino-D-glucitol. These workers first removed the 2,3-*O*-isopropylidene protecting group and then reduced the azido group by means of catalytic hydrogenation, thus enabling the ring rearrangement to occur to insert the nitrogen in the ring. The approach of Di *et al.* was employed in the present study for the formation of 1,5-dideoxy-*N*-ethyl-1,5-imino-D-glucuronamide (**68**) (see **Scheme 29**). Thus, compound **66** was treated with 2 N HCl in aqueous THF at 50 °C until its total consumption was indicated in TLC. The solvent was then removed under reduced pressure to render a brown residue, which was subsequently subjected to hydrogenation over 10% palladium-on-charcoal in methanol. A Schiff-

base intermediate, formed by way of ring rearrangement, was also reduced under the hydrogenation conditions. Chromatographic fractionation of the crude product under an ammonia atmosphere gave compound **68**. The structure of **68** was assigned on the basis of its NMR spectral and mass spectrometric data. The large coupling constants (9~10 Hz) observed for the signals of the ring protons indicated a $^4C_1$ conformation.

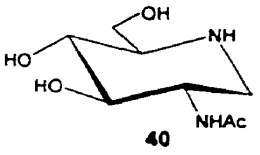


Scheme 29. Second approach to the formation of 1,5-dideoxy-*N*-ethyl-1,5-imino-D-glucuronamide (**68**)

The work described in **Section 2.2** represents only a preliminary investigation aimed at employing solid-phase synthesis for the preparation of azasugar analogues. Nevertheless, the results obtained are encouraging, such that the exploitation of the approach is deemed to be worthwhile.

2.3. Biological evaluation

Azasugar compounds **40**, **46**, **57**, **58**, **59**, and **60**, obtained from the solution-phase syntheses as described in **Section 3.2**, were evaluated to see their effects on the biosynthesis of glycosaminoglycans. These evaluations were performed by Dr. Ali Berkin, and the results are presented only in outline form. The structures of these compounds are shown again as an aid in the following discussion.



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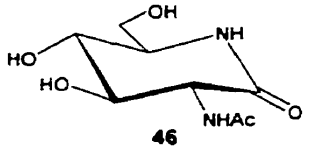

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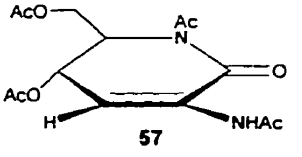

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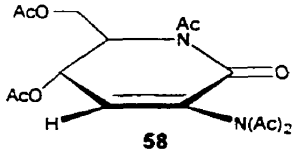

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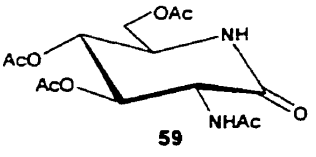

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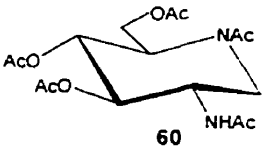

60

The biological evaluation was performed as an *in vitro* assay for cellular GAG and protein synthesis. The method[66] was based on the use of radiolabelled D-[^{3}H]glucosamine, [^{35}S]sulfate, and L-[^{14}C]leucine, and of hepatocyte tissue cultures obtained from experimental mice liver through some biochemical purification procedures. By incubating the hepatocyte cultures with D-[^{3}H]GlcN and [^{35}S]SO_4 for 24 h in the absence (control) or presence of an azasugar candidate at varying concentrations, the effect of the sugar compound was determined by the reduction or increase of D-[^{3}H]GlcN and [^{35}S]SO_4 in isolated GAG chains. Similarly, by applying L-[^{14}C]leucine to the hepatocyte cultures with or without the candidate compound, the

effect on protein synthesis was observed through the reduction or increase of [^{14}C]Leu in isolated glycoconjugates.

The results of the GAG inhibition studies revealed that compound **40** demonstrated an increased incorporation of both [^{3}H]GlcN and [^{35}S]SO_4 into isolated GAGs. The greatest level of GAG stimulation was observed at 0.01 mM by an increase in [^{3}H]GlcN and [^{35}S]SO_4 incorporation to ~140% of the control cells (**Figure 7A**). Interestingly, with increasing concentration of compound **40**, the stimulating effect of

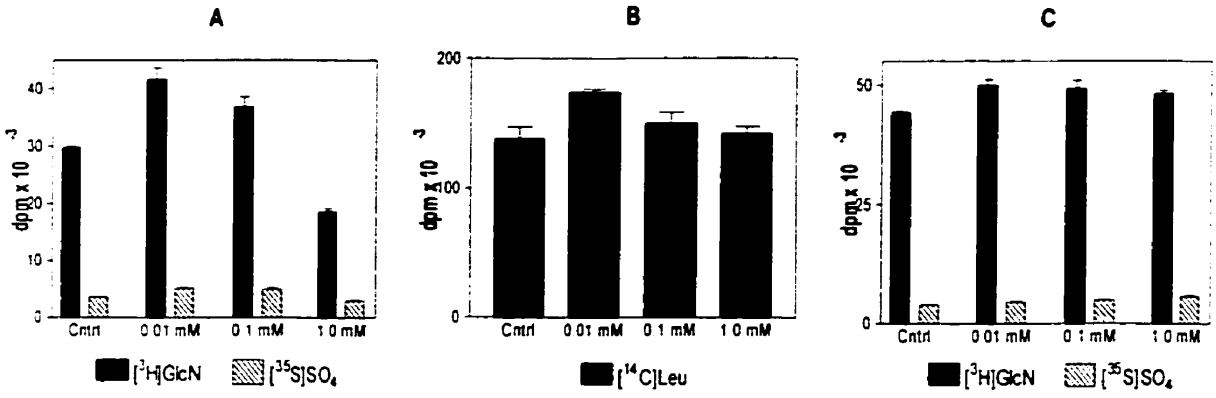

Figure 7. Effect of increasing concentration of compounds **40** and **60** on hepatocyte cellular GAG and on total protein synthesis. Hepatocyte cultures were incubated with D-[^{3}H]glucosamine and [^{35}S]sulfate for 24 h in the absence (control) and presence of compound **40** (panel **A**) and **60** (panel **C**) at 0.01, 0.1, and 1.0 mM. Hepatocyte cultures were incubated with L-[^{14}C]leucine for 24 h in the presence of compound **40** (panel **B**) at 0.01, 0.1, and 1.0 mM. The values represent the mean ± S. D. of triplicate cultures. Statistical analyses using an unpaired *t*-test revealed that in panel **A**, control vs. 0.01 mM, $P < 0.01$; control vs. 0.1 mM, $P < 0.01$; control vs. 1.0 mM, $P < 0.01$. In panel **B**, control vs. 0.01 mM, $P < 0.01$; control vs. 0.1 mM or 1.0 mM, not significant. In panel **C**, control vs. 0.01 mM, $P < 0.01$; control vs. 0.1 mM, $P < 0.01$; control vs. 1.0 mM, $P < 0.01$.

this compound seemed to diminish. At 1.0 mM, an inhibition of incorporation of both radiolabelled compounds to 75% of the control cells was observed. An evaluation of the effect of compound **40** on total protein synthesis indicated a similar stimulatory

effect at 0.01 mM to 125% of control, a result which was not observed at 1.0 mM (**Figure 7B**).

The acetylated analogue of **40**, namely compound **60**, was evaluated for its effect on hepatocyte GAG synthesis at concentrations of 0.01, 0.1, and 1.0 mM (**Figure 7C**). The results showed that the incorporation of both [^{3}H]GlcN and [^{35}S]SO_4 into isolated GAGs was increased by ~112% of the control cells at these concentrations.

Compounds **46** and **59** differ by the presence of acetyl groups at positions 3, 4, and 6 in the latter compound. Within hepatocytes, compound **59** will be transformed into compound **46**, presumably by the action of lysosomal esterases. Both compounds **46** and **59**, indeed, showed similar trends when evaluated for their effects on hepatocyte cellular GAG synthesis at different concentrations (**Figure 8A** and **8B**). Both of them

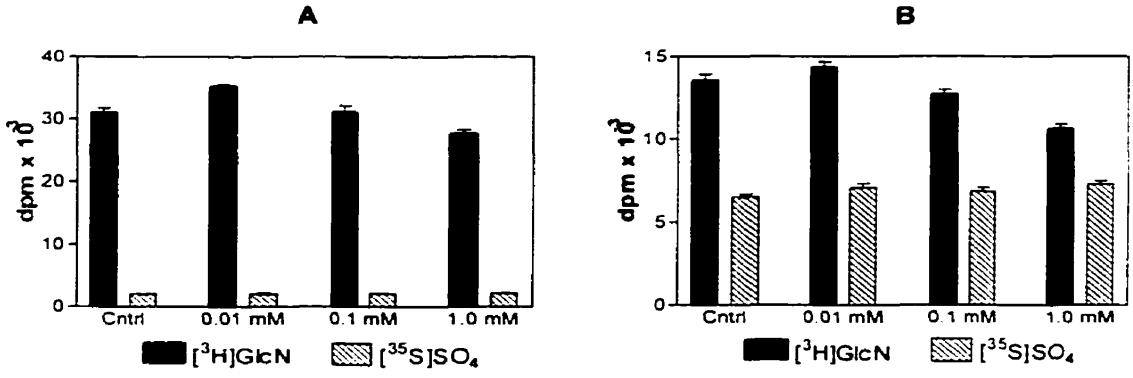

Figure 8. Effect of increasing concentration of compounds **46** and **59** on hepatocyte cellular GAG synthesis. Hepatocyte cultures were incubated with D-[^{3}H]glucosamine and [^{35}S]sulfate for 24 h in the absence (control) and presence of compound **46** (panel **A**) and **59** (panel **B**) at 0.01, 0.1, and 1.0 mM. The values represent the mean ± S. D. of triplicate cultures. Statistical analyses using an unpaired *t*-test revealed that in panel **A**, control vs. 0.01 mM, $P < 0.01$ ([^{3}HGlcN only); control vs. 0.1 mM, not significant; control vs. 1.0 mM, $P < 0.05$. In panel **B**, control vs. 0.01 mM, $P < 0.05$; control vs. 0.1 mM, $P < 0.05$ ([^{3}HGlcN only); control vs. 1.0 mM, $P < 0.01$.

demonstrated a slight inhibition of incorporation of [^{3}H]GlcN at 1.0 mM, but not of [^{35}S]SO_4, into isolated GAGs.

Compounds **57** and **58** differ from each other in the number of acetyl groups on the C-2 amino group, and, as seen in **Figure 9A** and **9B**, demonstrated no significant effects on GAG synthesis up to 1.0 mM.

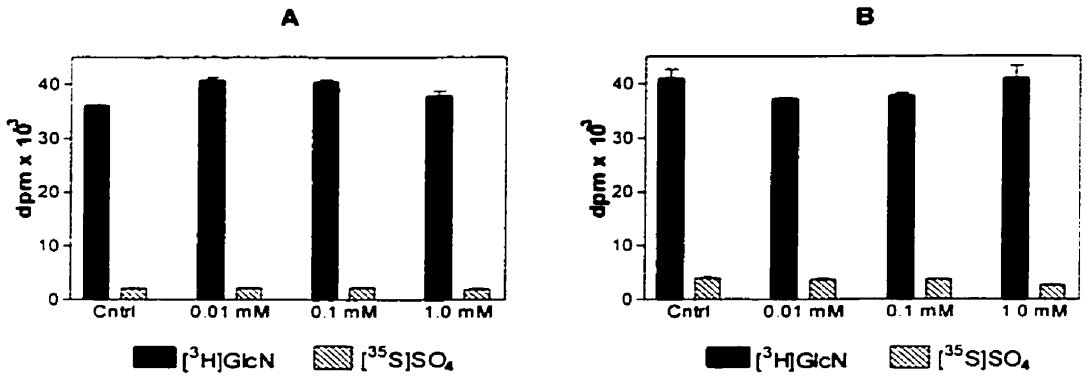

Figure 9. Effect of increasing concentration of compounds **57** and **58** on hepatocyte cellular GAG synthesis. Hepatocyte cultures were incubated with D-[^{3}H]glucosamine and [^{35}S]sulfate for 24 h in the absence (control) and presence of compound **57** (panel **A**) and **58** (panel **B**) at 0.01, 0.1, and 1.0 mM. The values represent the mean ± S. D. of triplicate cultures. Statistical analyses using an unpaired *t*-test revealed that in panel **A**, control vs. 0.01 mM, $P < 0.05$; control vs. 0.1 mM, $P < 0.05$; control vs. 1.0 mM, $P < 0.05$ ([^{3}HGlcN only). In panel **B**, control vs. 0.01 mM, $P < 0.05$ ([^{3}HGlcN only); control vs. 0.1 mM, $P < 0.05$ mM ([^{3}HGlcN only); control vs. 1.0 mM, $P < 0.01$ ([^{35}S]SO_4 only).

All of the compounds mentioned above were contemplated possibly to act as inhibitors of the pathway leading to GAG biosynthesis. The lack of an anomeric hydroxyl group would prevent their being directly incorporated into GAG chains.

Compound **40** demonstrated significant inhibitory effect on GAG synthesis at 1.0 mM without any effects observed on protein synthesis. The inhibitory effect of **40** may be a result of inhibition of the enzymatic pathway for the conversion of GlcN into

its UDP-analogue. However, the stimulatory effects of **40** on GAG and protein synthesis at its low concentrations, namely 0.01 mM and 0.1 mM, is confusing. One possible explanation is that compound **40**, known to act as an inhibitor of *N*-acetylglucosaminidases of various sources[37], may exert inhibitory effects on glycosidases when it enters the cell, thus resulting in the inhibition of proteoglycan degradation. As a result, the proteoglycans may be accumulating on the cell-surface, leading to increased levels of [^{3}H]GlcN, [^{35}S]SO_4, and [^{14}C]Leu.

The selective inhibition of incorporation of [^{3}H]GlcN into isolated GAGs by compounds **46** and **59** at 1.0 mM suggested that both compounds presumably inhibit the enzymatic pathway that converts [^{3}H]GlcN into a UDP-sugar. The inhibitory effects may be a result of the possibility of compounds **46** and **59** undergoing enzymatic transformation to form the GlcNAc-6-PO_4 analogue, but not the GlcNAc-1-PO_4 analogue. Accumulation of the GlcNAc-6-PO_4 analogue could be responsible for the reduction of [^{3}H]GlcN incorporation into GAG chains, but would not affect [^{35}S]SO_4 incorporation into the GAG chains.

The insignificant effects on GAG synthesis shown by compounds **57** and **58** may simply be reflective of a lack of structural resemblance between **57**/**58** and the substrate of the targeted enzyme.

The initial results obtained in the present study on the effects of azasugar compounds on glycoconjugate biosynthesis do indeed suggest that the examination of additional candidates of this nature should be pursued.

Chapter 3. Experimental

3.1. General methods

Melting points were determined on a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 241 polarimeter for solutions in a 1-dm cell at room temperature.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance–300 spectrometer at 300.1 and 75.5 MHz, respectively, or a Bruker Avance–400 spectrometer at 400.1 and 100.6 MHz, respectively, or a Bruker Avance–500 spectrometer at 500.1 and 125.8 MHz, respectively. The signals owing to residual protons in the deuterated solvents were used as internal standards.

Two-dimensional NMR techniques were used in the assignments for all of the chemical compounds obtained throughout this synthetic work, namely COSY (correlated spectroscopy), HMQC (heteronuclear multiple-quantum correlation), and HMBC (heteronuclear multiple-bond correlation). COSY provides the proton–proton correlation through a scalar coupling. HMQC provides correlation between protons and their directly attached carbon. HMBC provides the correlation between protons and carbon through two or three bonds.

Infrared (IR) spectra were recorded on a Bomem MB-series FTIR spectrophotometer.

Analytical thin-layer chromatography (TLC) was performed using glass plates precoated with EM Science Silica Gel 60 F_{254}. The developed plates were examined

under UV light, then sprayed with ceric sulfate solution, and heated at 400 °C. Flash chromatography was performed using EM Science Silica Gel 60 (230–400 mesh). Ion-exchange chromatography was performed on Amberlite IR-120(H^+) resin.

Low- and high-resolution mass spectra were recorded at Queen's University and University of Ottawa, respectively, in the mode of CI, EI, ES, or FAB.

Catalytic hydrogenolyses were performed in a Parr stainless steel bench-top pressure reactor equipped with an electrical motor drive and pressure controller (maximum 60 psig) at room temperature.

The reagents and solvents were commercial grade and were used as supplied. All non-aqueous reactions were performed under an argon atmosphere. Dry solvents, such as THF, pyridine, and CH_2Cl_2, were freshly distilled.

The resin used as the solid support in synthesis is the Merrifield resin with 1% DVB having a 1.5 mmol functionality per gram.

3.2. Synthesis

*2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-*D*-glucopyranose* (**42**).—A suspension of 2-acetamido-2-deoxy-D-glucose (**41**, 37.5 g, 170 mmol), freshly distilled allyl alcohol (500 mL), and $BF_3{\cdot}Et_2O$ (4.90 mL) was heated at reflux temperature for 2–3 h. The hot reaction mixture was filtered, the filtrate was evaporated, and the residue was dried *in vacuo* overnight to give a slightly yellow foam whose ^1H NMR spectrum indicated that it consisted of allyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**47**) and its β anomer in a ratio of 8:1, respectively.

Compound **47**: R_f 0.56 (4:1 MeCN–H_2O); ^1H NMR (CD_3OD): δ 1.89 (s, 3 H, NAc), 3.26 (t, 1 H, J 9.0 Hz, H-4), 3.48 (m, 1 H, H-5), 3.55–3.61 (m, 2 H, H-3, H-6b), 3.71 (dd, 1 H, $J_{6a,5}$ 2.3, J_{gem} 11.8 Hz, H-6a), 3.80 (dd, 1 H, $J_{2,1}$ 3.6, $J_{2,3}$ 10.7 Hz, H-2), 3.88 (ddt, 1 H, J 12.5, 6.3, 1.2 Hz, allyl -CH_a-), 4.09 (ddt, 1 H, J 12.5, 5.1, 1.5 Hz, allyl -CH_b-), 4.72 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.03–5.25 (m, 2 H, allyl =CH_2), 5.82 (m, 1 H, allyl -C*H*=); ^{13}C NMR (CD_3OD): δ 22.4 (NCO*C*H_3), 55.5 (C-2), 62.7 (C-6), 69.1 (allyl -*C*H_2-), 72.1 (C-4), 72.3 (C-3), 73.9 (C-5), 97.6 (C-1), 117.5 (allyl =*C*H_2), 135.4 (allyl -*C*H=), 173.8 (NC=O).

The crude compound **47** obtained above was suspended in dry 1,4-dioxane (750 mL), and benzyl chloride (150 mL) and powdered KOH (112.5 g) were added. The mixture was heated at reflux temperature for 4 h. After it was cooled, the mixture was filtered through a cotton plug under reduced pressure to remove the excess of solid KOH. The filtrate was evaporated and the orange residue was partitioned between $CHCl_3$–H_2O; the organic phase was dried (Na_2SO_4), and the solvent was removed under

vacuum. The oily residue was then co-evaporated with *p*-xylene several times to remove the excess of benzyl chloride and any side-products derived from benzyl chloride. Fractionation by flash chromatography on silica gel (1:4 EtOAc–hexane) gave a white solid whose ^{1}H NMR spectrum was consistent with the structure of allyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**48**) (66.3 g, 73.4% from **41**): R_f 0.21 (1:1 EtOAc–hexane); ^{1}H NMR ($CDCl_3$): δ 1.88 (s, 3 H, NAc), 3.68–3.88 (m, 5 H, H-3, H-4, H-5, 2 H-6), 3.98 (ddt, 1 H, J 12.9, 6.3, 1.2 Hz, allyl –CH_a-), 4.18 (ddt, 1 H, J 12.9, 5.1, 1.5 Hz, allyl –CH_b-), 4.32 (app dt, J 9.9, $J_{2,1}$ 3.6 Hz, H-2), 4.50–4.91 (m, 7 H, 3 benzyl CH_2, H-1), 5.18–5.31 (m, 2 H, allyl =CH_2), 5.37 (d, 1 H, $J_{NH,2}$ 9.4 Hz, NH), 5.89 (m, 1 H, allyl -C*H*=), 7.22–7.40 (m, 15 H, aromatic); ^{13}C NMR ($CDCl_3$): δ 23.4 (NCOCH3), 52.4 (C-2), 68.0 (allyl -*C*H_2-), 68.5 (C-6), 71.0 (C-5), 73.4, 74.8, 75.0 (3 benzyl *C*H_2), 78.4 (C-4), 80.3 (C-3), 96.9 (C-1), 117.5 (allyl =*C*H_2), 127.6–128.4 (aromatic), 133.6 (allyl -*C*H=), 138.0, 138.4 (aromatic), 169.6 (NC=O).

To a solution of the solid material (63.7 g, 0.12 mol) obtained above in anhydrous DMSO (225 mL) was added potassium *tert*-butoxide (22.5 g, 200 mmol) at room temperature; the solution turned dark brown immediately. The mixture was stirred for 3 h at 100 °C. TLC (1:1 hexane–EtOAc) showed the presence of a new component having R_f 0.36. The solution was cooled and diluted with H_2O (1000 mL), and the dark-brown precipitate was removed by filtration. The precipitate was dissolved in acetone (450 mL), and the solution was treated with 2 M HCl (175 mL) and heated at reflux temperature for 30 min. H_2O (1000 mL) was added and the pale-brown precipitate was removed by filtration. The precipitate was then dissolved in hot methanol and the solution was treated with activated carbon. The resulting mixture was

filtered, and the filtrate was evaporated. Recrystallization (3 ×) of the product from MeOH afforded 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose (**42**) as white needles (27.4 g, 33% from **41**): mp 221–223 °C, lit.[67] 218–219 °C, lit.[68] 218 °C: $[\alpha]_D$ +75.4° (c 1.0, $CHCl_3$), lit.[68] +71.3° (c 1.08, $CHCl_3$); ^{1}H NMR (DMSO-d_6): δ 1.85 (s, 3 H, NAc), 3.45 (t, 1 H, *J* 9.4 Hz, H-3), 3.61 (d, 1 H, *J* 9.8 Hz, H-6a), 3.65 (dd, 1 H, *J* 9.8, *J* 4.8 Hz, H-6b), 3.75 (t, 1 H, *J* 10 Hz, H-4), 3.86–3.97 (m, 2 H, H-2, H-5), 4.43–4.77 (m, 6 H, 3 benzyl C*H*$_2$), 4.92 (dd, 1 H, *J* 3.74, *J* 3.61 Hz, H-1), 6.78 (d, 1 H, *J* 4.01 Hz, OH), 7.15–7.40 (m, 15 H, aromatic), 7.99 (d, 1 H, *J* 9.10 Hz, NH); ^{13}C NMR (DMSO-d_6): δ 22.58 (NCO*C*H$_3$), 53.19 (C-2), 69.07 (C-6), 69.70 (C-3), 72.26, 73.94 (3 benzyl *C*H$_2$), 78.53 (C-4), 79.80 (C-5), 90.98 (C-1), 127.29–128.20 (aromatic), 138.25, 138.32, 138.80 (aromatic), 169.20 (C=O); LRMS (CI): 492.3 $[M + H]^+$.

*Treatment of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-*D*-glucopyranose* (**42**) *with Dess–Martin periodinane.*—A solution of compound **42** (1 equiv, 104 mg, 203 mmol) in freshly distilled methylene chloride (40 mL) was treated with Dess–Martin periodinane (2 equiv, 195 mg, 406 mmol), and stirred for 30 min. The reaction mixture was treated again with Dess–Martin periodinane (0.5 equiv, 45 mg, 102 mmol) and stirred for another 20 min or longer depending on the progress of the reaction as monitored by TLC. To the reaction mixture then were added dropwise a satd aq solution of $NaHCO_3$ and a satd aq solution of Na_2SO_3, alternatively, to make the reaction system pH-neutral and to effect reduction of the excess of periodinane. The resulting solution was stirred for 1 h. The aqueous phase was extracted with CH_2Cl_2 (3

×). The combined organic phases were dried ($MgSO_4$) and evaporated to give only a crude sample of compound **43** (107 mg) (see below).

*Synthesis of 2-acetamido-3,4,6-tri-O-benzyl-2-deoxy-*D*-glucono-1,5-lactone* (**43**) *by way of Swern oxidation.*—At –70 ^{0}C, into a solution of freshly distilled oxalyl chloride (2 equiv, 0.50 mL, 5.70 mmol) and freshly dried methylene chloride (15 mL) was added dropwise a solution of DMSO (5 equiv, 0.9 mL, 12.74 mmol) in methylene chloride (12 mL). The resulting solution was stirred for 50 min and was treated dropwise with a solution of compound **42** (1 equiv, 1.225 g, 2.50 mmol) in 25:4 CH_2Cl_2–DMSO (20 mL). The reaction mixture was stirred for 1 h at –70 ^{0}C, warmed to –40 ^{0}C within 1 h, cooled to –70 ^{0}C, and treated dropwise with freshly dried Et_3N (5 equiv, 1.81 mL, 13.00 mmol). The mixture was stirred at –70 ^{0}C for another hour, then warmed to rt, and washed with H_2O (3 × 15 mL). After the organic layer was dried ($MgSO_4$), filtered, and evaporated, the brown oily residue was treated with Et_2O (25 mL) and a few drops of hexane. The formation of a precipitate was observed immediately. Removal of the precipitate by filtration and drying *in vacuo* gave compound **43** in a white solid form (1.077 g, 88.3%): R_f 0.66 (EtOAc); mp 147–149 °C, lit.[69] 141–142 °C; $[\alpha]_D$ + 109.9° (c 0.31, $CHCl_3$), lit.[69] +123.3° (c 0.94, $CHCl_3$): ^{1}H NMR ($CDCl_3$): δ 1.86 (s, 3 H, NAc), 3.77 (dd, 1 H, $J_{6a,5}$ 2.4, J_{gem} 11.1 Hz, H-6a), 3.81 (dd, 1 H, $J_{6b,5}$ 3.0, J_{gem} 11.1 Hz, H-6b), 3.98–4.08 (m, 3 H, H-2, H-3, H-4), 4.47 (m, 1 H, H-5), 4.51 (d, 1 H, J_{gem} 12.0 Hz, PhCH_aO–C-6), 4.61 (d, 1 H, J_{gem} 12.0 Hz, PhCH_bO–C-6), 4.66 (d, 1 H, J_{gem} 11.7 Hz, PhCH_aO–C-4), 4.68 (d, 1 H, J_{gem} 10.8 Hz, PhCH_aO–C-3), 4.83 (d, 1 H, J_{gem} 12.0 Hz, PhCH_bO–C-4), 4.84 (d, 1 H, J_{gem} 10.8 Hz,

PhCH_bO–C-3), 6.45 (d, 1 H, $J_{NH,2}$ 5.93 Hz, NH), 7.22–7.40 (m, 15 H, aromatic); ^{13}C NMR ($CDCl_3$): δ 22.54 (NCOCH_3), 55.48 (C-2), 67.73 (C-6), 73.54 (PhCH_2O–C-6), 74.53, 74.58 (2 benzyl CH_2), 76.01 (C-3), 78.48 (C-5), 79.58 (C-4), 127.83–128.49 (aromatic), 137.48, 137.55, 137.90 (aromatic), 168.82 (C-1), 170.51 (NC=O); LRMS (CI): 490.2 $[M + H]^+$.

*Ammonolysis of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-*D*-glucono-1,5-lactone* (**43**).—The procedure employed for the ammonolysis of the lactone **43** was that of Granier and Vasella[46]. Ammonia (10 mL) was condensed in a three-neck reaction flask which was fitted with a cold-finger cooling trap on the top and kept in a cooling bath at a temperature of –78 °C. To the condensed ammonia was added a solution of the lactone **43** (2.1 g, 4.29 mmol) in dry CH_2Cl_2 (40 mL) dropwise using a cannula. The cooling bath was removed and the solution was kept at a reflux state for 30 min. After removal of the cooling trap and evaporation of NH_3 and CH_2Cl_2 , the oily product was purified by flash chromatography on silica gel (1:5 acetone–CH_2Cl_2) to give a homogeneous (TLC) compound as a white foam whose ^{1}H-NMR spectrum was consistent with the structure of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-gluconamide (**44**)[46] (1.52 g, 70%): R_f 0.30 (1:1 acetone–CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 1.97 (s, 3 H, NAc), 3.32 (br s, 1 H, OH), 3.60–3.80 (m, 3 H, H-4, 2 H-6), 4.00 (m, 1 H, H-5), 4.42 (dd, *J* 2.7, 4.8 Hz, H-3), 4.48–4.65 (m, 4 H, 2 benzyl CH_2), 4.70–4.80 (m, 3 H, H-2, benzyl CH_2), 5.86 (br s, 1 H, NH), 6.56 (br s, 1 H, NH), 6.87 (d, 1 H, $J_{NH,2}$ 6.9 Hz, NHAc), 7.20–7.45 (m, 15 H, aromatic); ^{13}C NMR ($CDCl_3$): δ 23.6 (NCOCH_3), 53.7 (C-

2), 71.1 (C-5), 71.5 (C-6), 73.9, 74.7, 75.1 (3 benzyl CH_2), 79.2, 79.7 (C-3, C-4), 128.2–129.0 (aromatic), 138.0, 138.2, 138.4 (aromatic), 171.4, 173.4 (2 C=O).

If the sample of the lactone **43** employed in the ammonolysis reaction was the crude sample obtained by treatment of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose (**42**) with Dess–Martin periodinane (see above), then, in addition to the gluconamide **44**, two side-products were isolated by gradient column chromatography (acetone–CH_2Cl_2, from 1:9 to 1:1). On the basis of NMR spectral and mass spectrometric data the two side-products were assigned the structures of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-*erythro*-hex-2-enopyrano-1,5-lactone (**50**) and 1-acetamido-1,4-anhydro-2,3,5-tri-*O*-benzyl-1-carboxamido-D-arabinitol (**51**)*.

Compound **50**: R_f 0.66 (EtOAc); ^{1}H NMR ($CDCl_3$): δ 2.13 (s, 3 H, NAc), 3.66 (dd~br t, 1 H, $J_{6a,5}$ 7.5 Hz, H-6a), 3.73 (dd, 1 H, J_{gem} 9.9, $J_{6b,5}$ 4.6 Hz, H-6b), 4.41 (d, 1 H, J_{gem} 12.0 Hz, PhCH_aO–C-6), 4.46 (d, 1 H, J_{gem} 12.0 Hz, PhCH_bO–C-6), 4.59 (d, 1 H, $J_{4,5}$ 2.7 Hz, H-4), 4.63 (d, 1 H, J_{gem} 11.6 Hz, PhCH_aO–C-4), 4.68 (d, 1 H, J_{gem} 11.6 Hz, PhCH_bO–C-4), 4.77 (m, 1 H, H-5), 5.06 (d, 1 H, J_{gem} 12.0 Hz, PhCH_aO–C-3), 5.15 (d, 1 H, J_{gem} 12.0 Hz, PhCH_bO–C-3), 7.25–7.39 (m, 15 H, aromatic), 7.54 (br s, 1 H, NH); ^{13}C NMR ($CDCl_3$): δ 22.76 (NCOCH_3), 68.04 (C-6), 69.86 (C-4), 71.08 (PhCH_2O–C-4), 71.41 (PhCH_2O–C-3), 73.15 (PhCH_2O–C-6), 75.97 (C-5), 105.86 (C-2), 127.19–128.35 (aromatic), 135.52, 136.37, 137.07 (aromatic), 159.07 (C-3), 163.59, 169.34 (2 C=O); LRMS (CI): 488.4 $[M + H]^+$.

Compound **51**: R_f 0.39 (1:1 acetone–CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 1.90 (s, 3 H, NAc), 3.61 (app d, 2 H, J_{gem} 11.1, $J_{5,4}$ 4.5 Hz, 2 H-5), 4.15 (dd~t, 1 H, J 5.5 Hz, H-

* In one experiment the relative proportions of **44**, **50**, and **51** were approximately 3:1:3, respectively.

3), 4.22 (dt~q, 1 H, $J_{4,5}$ 4.8, $J_{4,3}$ 5.4 Hz, H-4), 4.32 (d, 1 H, $J_{2,3}$ 5.1 Hz, H-2), 4.53 (d, 1 H, J_{gem} 11.7 Hz, PhCH_aO–C-3), 4.54 (s, 2 H, PhCH_2O–C-5), 4.59 (d, 1 H, J_{gem} 11.7 Hz, PhCH_bO–C-3), 4.65 (d, 1 H, J_{gem} 11.4 Hz, PhCH_aO–C-2), 4.92 (d, 1 H, J_{gem} 11.4 Hz, PhCH_bO–C-2), 5.95 (br s, 1 H, NH), 6.85 (s, 1 H, N*H*Ac), 7.04 (br s, 1 H, NH), 7.20–7.48 (m, 15 H, aromatic); ^{13}C NMR ($CDCl_3$): δ 23.22 (NCO*C*H_3), 69.45 (C-5), 72.14 (Ph*C*H_2O–C-3), 73.10 (Ph*C*H_2O–C-2), 73.27 (Ph*C*H_2O–C-5), 80.53 (C-4), 82.47 (C-3), 86.62 (C-2), 88.41 (C-1), 127.48–129.72 (aromatic), 136.75, 137.36, 137.84 (aromatic), 169.93 (Me*C*ONH-), 172.77 ($CONH_2$); LRMS (CI): 505.3 $[M + H]^+$.

*Oxidation of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-*D*-gluconamide* (**44**) *by the Jones reagent.*—A solution of compound **44** (739 mg, 1.46 mmol) in acetone (70 mL) was treated at 0 °C with the Jones reagent (1.48 mL) {prepared by dissolving chromic trioxide (26.72 g) in concd sulfuric acid (23 mL), and dilution with water to a volume of 100 mL}. The reaction mixture was stirred at 0 °C for 1 h and at rt for 4 h: the reaction was quenched then with ice–satd aq Na_2CO_3 solution. The mixture was filtered through a cotton plug under reduced pressure, and the residue was washed several times with acetone. The filtrate was evaporated, the residue was dissolved in water, and the solution was extracted three times with CH_2Cl_2. The organic layers were combined, dried (Na_2SO_4), and concentrated. The product was fractionated by flash chromatography on silica gel (1:3 acetone–CH_2Cl_2) to give compounds **52** and **53** as white solids in a ratio of 8:1 (combined yield: 295 mg, 40%).

Compound **52**: R_f 0.35 (1:1 acetone–CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 1.83 (s, 3 H, NAc), 3.43 (s, 2 H, 2 H-6), 3.83 (d, 1 H, $J_{4,3}$ 9.6 Hz, H-4), 4.02 (t, 1 H, *J* 7.8 Hz, H-

2), 4.25 (t, 1 H, J 9.2 Hz, H-3), 4.44 (d, 1 H, J_{gem} 12 Hz, $PhCH_aO$–C-6), 4.54 (d, 1 H, J_{gem} 12 Hz, $PhCH_bO$–C-6), 4.64 (d, 1 H, J_{gem} 11.1 Hz, $PhCH_aO$–C-4), 4.70 (d, 1 H, J_{gem} 11.7 Hz, $PhCH_aO$–C-3), 4.82 (d, 1 H, J_{gem} 11.7 Hz, $PhCH_bO$–C-3), 4.85 (br. s, 1 H, OH), 4.91 (d, 1 H, J_{gem} 11.1 Hz, $PhCH_bO$–C-4), 6.96 (br s, 1 H, NH–C-5), 7.09 (m, 1 H, HNAc), 7.20–7.40 (m, 15 H, aromatic); ^{13}C NMR ($CDCl_3$): δ 22.6 ($NCOCH_3$), 56.3 (C-2), 71.8 (C-6), 73.5 ($PhCH_2O$–C-6), 74.7 ($PhCH_2O$–C-3), 75.2 ($PhCH_2O$–C-4), 78.0 (C-3), 78.5 (C-4), 82.1 (C-5), 127.8–128.4 (aromatic), 137.2, 137.4, 138.0 (aromatic), 170.0 (C-1), 171.4 (NC=O).

Compound **53**: R_f 0.24 (1:1 acetone–CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 1.83 (s, 3 H, NAc), 3.61 (d, 1 H, J_{gem} 9.3 Hz, H-6a), 3.76 (d, 1 H, J_{gem} 9.3 Hz, H-6b), 3.93 (t, 1 H, J 6.0 Hz, H-4), 3.97 (t, 1 H, J 5.7 Hz, H-3), 4.50–4.85 (m, 7 H, H-2, 3 benzyl CH_2), 5.10 (br s, 1 H, OH), 6.23 (a, 1 H, J 8.1 Hz, NHAc), 7.06 (br s, 1 H, NH–C-5), 7.15–7.50 (m, 15 H, aromatic); ^{13}C NMR ($CDCl_3$): δ 22.8 ($NCOCH_3$), 52.6 (C-2), 71.6 (C-6), 73.1, 73.8, 74.0 (3 CH_2Ph), 77.2 (C-3), 78.8 (C-4), 84.2 (C-5), 127.4–128.4 (aromatic), 137.2, 137.2, 137.4 (3 tertiary aromatic), 168.6, 170.4 (2 C=O).

*2-Acetamido-5-amino-3,4,6-tri-*O*-benzyl-2,5-dideoxy-*D*-glucono-1,5-lactam* (**45**).—A solution of a sample of compound **52** obtained above (1 equiv, 1.3 g, 2.58 mmol) in freshly prepared CH_2Cl_2–MeCN 1:1 (50 mL) was treated dropwise at –50 °C for 20 min with a solution of $BF_3{\cdot}Et_2O$ (5 equiv, 1.61 mL, 12.9 mmol) and Et_3SiH (3 equiv, 1.24 mL, 7.74 mmol) in CH_2Cl_2–MeCN 1:1 (40 mL). The solution was warmed to –5 °C within a 30-min period, then cooled to –40 °C, poured into ice–satd aq Na_2CO_3 solution (35 mL), and the mixture was stirred for 30 min. The solution mixture was

evaporated under reduced pressure. To the residue were added CH_2Cl_2 (50 mL) and H_2O (50 mL). The organic layer was separated and the aq layer was extracted twice with CH_2Cl_2 (2 × 50 mL). The combined organic extracts were dried (Na_2SO_4), the solids were removed by filtration through Celite 521 (Aldrich), and the filtrate was concentrated. The residue was submitted to gradient flash column chromatography (EtOAc–hexane, from 9:1 to 19:1) to give compound **45** (0.92 g, 73%) as white crystals: R_f 0.18 (19:1 EtOAc–hexane); mp 153–155 °C, lit.[46] 150 °C; $[\alpha]_D$ +115.3° (c 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.89 (s, 3 H, NAc), 3.37 (dt, 1 H, *J* 2.1, 8.4 Hz, H-6a), 3.61 (dd, 1 H, *J* 2.4, 8.7 Hz, H-6b), 3.67 (m, 2 H, H-4, H-5), 4.06 (t, 1 H, *J* 9.0 Hz, H-3), 4.11 (t, 1 H, *J* 9.0 Hz, H-2), 4.46 (dd, 2 H, *J* 3.0, 12.0 Hz, PhCH_2OC-6), 4.58 (d, 1 H, *J* 11.1 Hz, PhCH_aOC-4), 4.70 (d, 1 H, *J* 11.7 Hz, PhCH_aOC-3), 4.84 (t, 2 H, *J* 11.1 Hz, PhCH_bOC-4), PhCH_bOC-3), 6.45 (s, 1 H, -NH-), 6.67 (d, 1 H, *J* 7.2 Hz, N*H*Ac), 7.21–7.38 (m, 15 H, aromatic); ^{13}C NMR ($CDCl_3$): δ 22.98 (NCO*C*H$_3$), 54.28 (C-5), 54.93 (C-2), 70.22 (C-6), 73.30 (Ph*C*H$_2$OC-6), 74.51, 74.60 (Ph*C*H$_2$OC-4, Ph*C*H$_2$OC-3), 77.34 (C-4), 79.79 (C-3), 127.81–128.49 (aromatic), 137.45–137.96 (tertiary aromatic), 168.87 (C-1), 170.50 (NH*C*OMe); LRMS (CI): 489.3 $[M + H]^+$; HRMS (FAB): Calcd. for $C_{29}H_{33}N_2O_5$: 489.2390; Found: 489.2432.

2-Acetamido-5-amino-2,5-dideoxy-D-*glucono-1,5-lactam* (**46**).—To a solution of compound **45** (1.03 g, 2.11 mmol) in MeOH (40 mL) was added the catalyst 10% Pd/C (1.25 g), and the mixture was subjected to a hydrogen pressure in a Parr apparatus (60 psig) at rt for 3 days. The catalyst was removed by filtration through Celite (Aldrich), and the filtrate was concentrated under reduced pressure to afford **46** as a

white solid (430 mg, 93.3%): R_f 0.34 (1:1 EtOAc–MeOH); mp 207–208 °C, lit.[46] 219 °C; $[\alpha]_D$ +101.7° (c 0.63, MeOH–H_2O 2:1); ^1H NMR (D_2O): δ 1.96 (s, 3 H, NAc), 3.27 (m, 1 H, H-5), 3.60–3.67 (m, 2 H, H-4, H-6a), 3.72 (dd, 1 H, $J_{6,5}$ 2.8, J_{gem} 12.1 Hz, H-6b), 3.80 (t, 1 H, J 10.0 Hz, H-3), 4.03 (d, 1 H, $J_{2,3}$ 10.1 Hz, H-2); ^{13}C NMR (D_2O): δ 22.23 ($NHCOCH_3$), 55.26 (C-2), 56.84 (C-5), 60.51 (C-6), 68.40 (C-4), 71.40 (C-3), 171.43, 174.95 (C-1, NCOMe); LRMS (CI): 219.1 $[M + H]^+$; HRMS (FAB): Calcd. for $C_8H_{15}N_2O_5$: 219.0981; Found: 219.0924.

*2-Acetamido-3,4,6-tri-*O*-benzyl-1,2,5-trideoxy-1,5-imino-*D*-glucitol* (**54**).—To a solution of compound **45** (1 equiv, 1.03 g, 2.10 mmol) in freshly distilled THF (100 mL) was added dropwise $BF_3{\cdot}Et_2O$ (5 equiv, 1.4 mL, 10.50 mmol). The solution was heated for 15 min at 50 °C with stirring, cooled to 4 °C, and treated with $NaBH_4$ (10 equiv, 0.81 g, 21.0 mmol). The reaction mixture was stirred for 20 min at the same temperature. Then the stirred mixture was treated with ice–CH_2Cl_2, and the temperature of the mixture was allowed to rise to rt. After the organic solvents were evaporated under reduced pressure, and the aq solution was extracted three times with CH_2Cl_2, the organic extracts were combined and concentrated to give a syrupy residue which was dissolved in MeOH (80 mL). The resulting solution was treated with concd HCl (2 mL), evaporated under reduced pressure, and this operation was repeated twice. The residue was then dissolved in CH_2Cl_2, the solution was treated with ice–H_2O, and a 7% aq KOH solution was added to adjust the pH to 10. The separated aq layer was extracted with CH_2Cl_2 (2 × 50 mL) and the combined organic phase was dried ($MgSO_4$), and evaporated. Recrystallization of the residue from EtOAc–hexane gave

compound **54** as white solid (306 mg). The mother liquor was concentrated and purified by gradient flash column chromatography (EtOAc–MeOH, from 50:1 to 5:1) to give a further quantity of compound **54** (292 mg; total yield 60%): R_f 0.50 (5:1 EtOAc–MeOH); mp 167–170 °C; $[\alpha]_D$ +68.0° (c 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.74 (s, 3 H, NAc), 2.28 (dd, 1 H, $J_{1a,2}$ 10.0, J_{gem} 12.8 Hz, H-1a), 2.75 (m, 1 H, H-5), 3.32 (dd, 1 H, $J_{1b,2}$ 4.8, J_{gem} 13.2 Hz, H-1b), 3.35 (dd, 1 H, J 8.4, 9.6 Hz, H-3), 3.52 (t, 1 H, J 8.8 Hz, H-4), 3.62 (dd, 1 H, $J_{6a,5}$ 2.8, J_{gem} 9.2 Hz, H-6a), 3.68 (dd, 1 H, $J_{6b,5}$ 4.8, J_{gem} 8.8 Hz, H-6b), 3.78 (m, 1 H, H-2), 4.46 (d, 1 H, J_{gem} 11.6 Hz, $PhCH_aOC$-6), 4.51 (d, 1 H, J_{gem} 12.0 Hz, $PhCH_bOC$-6), 4.56 (d, 1 H, J_{gem} 11.2 Hz, $PhCH_aOC$-4), 4.65 (d, 1 H, J_{gem} 11.6 Hz, $PhCH_aOC$-3), 4.83 (d, 1 H, J_{gem} 10.8 Hz, $PhCH_bOC$-4), 4.87 (d, 1 H, J_{gem} 12.0 Hz, $PhCH_bOC$-3), 5.05 (br. d, 1 H, J 6.8 Hz, NHAc), 7.22–7.44 (m, 15 H, aromatic); ^{13}C NMR ($CDCl_3$): δ 23.31 ($NCOCH_3$), 47.90 (C-1), 52.10 (C-2), 59.55 (C-5), 69.32 (C-6), 73.34 ($PhCH_2C$-6), 74.02 ($PhCH_2C$-3), 74.83 ($PhCH_2C$-4), 80.40 (C-4), 82.53 (C-3), 127.74–128.72 (aromatic), 137.94, 138.07, 138.39 (3 tertiary aromatic), 169.98 (NC=O); LRMS (CI): 475.3 $[M + H]^+$; HRMS (FAB): Calcd. for $C_{29}H_{35}N_2O_4$: 475.2597; Found: 475.2582.

Also isolated by column chromatography was a brown oil which was assigned the structure of 3,4,6-tri-*O*-benzyl-2-ethylamino-1,2,5-trideoxy-1,5-imino-D-glucitol (**55**)[†]: R_f 0.10 (5:1 EtOAc–MeOH); ^{1}H NMR ($CDCl_3$): δ 1.05 (t, 3 H, J 7.0 Hz, CH_3), 1.91 (br s, 2 H, 2 NH), 2.39 (dd, 1 H, $J_{1a,2}$ 10.8, J_{gem} 12.0 Hz, H-1a), 2.48–2.57 (m, 1 H, ethylamino -CH_a-), 2.58–2.65 (m, 1 H, H-2), 2.65–2.75 (m, 1 H, ethylamino -CH_b-), 2.80 (overlapping dddd, 1 H, H-5), 3.25 (dd, 1 H, $J_{1b,2}$ 4.5, J_{gem} 12.3 Hz, H-1b), 3.40

[†] In one experiment the relative proportions of **54** and **55** were approximately 1:1.

(app t, 1 H, J 9.0 Hz, H-3), 3.48 (app t, 1 H, J 9.0 Hz, H-4), 3.60 (dd, 1 H, $J_{6a,5}$ 5.8, J_{gem} 9.0 Hz, H-6a), 3.71 (dd, 1 H, $J_{6b,5}$ 2.4, J_{gem} 9.0 Hz, H-6b), 4.49 (d, 1 H, J_{gem} 12.0 Hz, PhCH_aO–C-6), 4.52 (d, 1 H, J_{gem} 12.0 Hz, PhCH_bO–C-6), 4.57 (d, 1 H, J_{gem} 10.8 Hz, PhCH_aO–C-4), 4.71 (d, 1 H, J_{gem} 11.4 Hz, PhCH_aO–C-3), 4.87 (d, 1 H, J_{gem} 10.8 Hz, PhCH_bO–C-4), 5.01 (d, 1 H, J_{gem} 11.4 Hz, PhCH_bO–C-3), 7.22–7.45 (m, 15 H, aromatic); ^{13}C NMR ($CDCl_3$): δ 15.36 (CH_3), 42.07 (ethylamino CH_2), 48.81 (C-1), 60.20 (C-5), 61.16 (C-2), 70.17 (C-6), 73.36 (PhCH_2O–C-6), 74.89 (PhCH_2O–C-4), 75.35 (PhCH_2O–C-3), 81.29 (C-4), 86.40 (C-3), 127.70–128.56 (aromatic), 137.92, 138.11, 138.48 (aromatic); LRMS (CI): 461.4 $[M + H]^+$.

2-Acetamido-1,2,5-trideoxy-1,5-imino-D-*glucitol* (**40**).—To a solution of compound **54** (0.38 g, 0.81 mmol) in AcOH (20 mL) was added 10% Pd/C (500 mg) and the mixture was subjected to a hydrogen pressure (60 psig) at rt for 6 days. The mixture was filtered through Celite 521 (Aldrich), the filtrate was coevaporated with toluene, and then was concentrated under vacuum to give a clear syrup (0.29 g): R_f 0.27 (NH_3 in 1:1 MeOH–$CHCl_3$). The syrup was diluted with MeOH (10 mL) and centrifuged: the supernatant was filtered, and the pellet was washed twice with MeOH and subjected to centrifugation. The combined filtrates were evaporated and the clear oily residue was dried under vacuum. The resultant product was subjected to flash chromatography on silica gel (NH_3 in 1:2 MeOH–$CHCl_3$). The hydrochloride salt, 2-acetamido-1,2,5-trideoxy-1,5-imino-D-glucitol hydrochloride (**56**) (0.16 g, 81%), was prepared by repeated (5 ×) evaporation of the product in MeOH containing concd aq HCl solution and finally drying under vacuum: ^{1}H NMR (D_2O): δ 1.79 (s, 3 H, NAc),

2.78 (t, 1 H, J 12.5 Hz, H-1a), 3.02 (m, 1 H, H-5), 3.26 (dd, 1 H, $J_{1b,2}$ 4.8, J_{gem} 12.7 Hz, H-1b), 3.41 (t, 1 H, J 9.2 Hz, H-3), 3.45 (t, 1 H, J 9.1 Hz, H-4), 3.65 (dd, 1 H, $J_{6a,5}$ 4.9, J_{gem} 12.9 Hz, H-6a), 3.72 (dd, 1 H, $J_{6b,5}$ 3.1, J_{gem} 12.9 Hz, H-6b), 3.85 (m, 1 H, H-2); ^{13}C NMR (D_2O): δ 22.29 ($NCOCH_3$), 44.32 (C-1), 48.32 (C-2), 57.83 (C-6), 60.09 (C-5), 68.57 (C-4), 73.59 (C-3), 174.82 (NC=O).

A solution of the hydrochloride salt **56** obtained above (155 mg, 0.64 mmol) in H_2O (1 mL) was applied onto a column of Amberlite IR-120 (H^+ form) ion-exchange resin (50 mL); elution with H_2O (100 mL) and then with 5% (w/w) NH_3 in H_2O (600 mL) gave a white solid (131 mg, 100%) that was dissolved in H_2O; the solution was lyophilized to give **40**: mp 228–229 °C, lit.[36(a)] 224 °C, lit.[36(b)] 221 °C, lit.[37] 203 °C; $[\alpha]_D$ 17.1° (c 0.07, H_2O), lit.[36(a)] 14.8° (c 1.00, H_2O), lit.[36(b)] 13.3° (c 1.00, H_2O); ^{1}H NMR (D_2O): δ 1.89 (s, 3 H, NAc), 2.31 (dd, 1 H, $J_{1a,2}$ 11.5, J_{gem} 12.5 Hz, H-1a), 2.43 (m, 1 H, H-5), 2.94 (dd, 1 H, $J_{1b,2}$ 4.9, J_{gem} 12.7 Hz, H-1b), 3.18 (t, 1 H, J 9.2 Hz, H-4), 3.28 (t, 1 H, J 9.5 Hz, H-3), 3.54 (dd, 1 H, $J_{6a,5}$ 6.0, J_{gem} 11.7 Hz, H-6a), 3.62 (m, 1 H, H-2), 3.71 (dd, 1 H, $J_{6b,5}$ 3.0, J_{gem} 11.7 Hz, H-6b); ^{13}C NMR (D_2O): δ 22.35 ($NCOCH_3$), 47.26 (C-1), 52.48 (C-2), 60.75 (C-5), 61.53 (C-6), 72.26 (C-4), 76.14 (C-3), 174.67 (NC=O); LRMS (ES): 205.15 $[M + H]^+$; HRMS (FAB): Calcd. for $C_8H_{17}N_2O_4$: 205.1188; Found: 205.1175.

*2,5-Diacetamido-4,6-di-*O*-acetyl-2,3,5-trideoxy-*D-erythro*-hex-2-enopyrano-1,5-lactam* (**57**) *and 5-acetamido-4,6-di-*O*-acetyl-2-(*N,N*-diacetyl)amino-2,3,5-trideoxy-*D-erythro*-hex-2-enopyrano-1,5-lactam* (**58**).—To a solution of compound **46** (380 mg, 1.73 mmol) in pyridine (40 mL) and acetic anhydride (30 mL) was added a catalytic

amount of 4-(*N*,*N*-dimethyl)aminopyridine (DMAP). The reaction solution was stirred overnight for 14 h at rt. The solution was concentrated and the residue was coevaporated with toluene under reduced pressure. Flash chromatography on silica gel (4:1 toluene–EtOAc) afforded compound **57** as a colorless oil (269 mg, 48%) and compound **58** as a white solid (137 mg, 22%).

Compound **57**: R_f 0.23 (2:1 toluene–EtOAc); $[\alpha]_D$ +204° (c 0.2, $CHCl_3$); ν_{max} (Film): 3340 (NH), 2954 (CH), 1743, 1708, and 1683 (C=O), 1515 (NH), 1398 and 1372 (CH_3), 1224 {C(=O)–O}, and 1045 cm^{-1} (O–C); ^1H NMR ($CDCl_3$): δ 1.93 (s, 3 H, C*H*$_3$COO–C-6), 1.98 (s, 3 H, C*H*$_3$COO–C-4), 2.11 (s, 3 H, C*H*$_3$COO–C-2), 2.54 (s, 3 H, C*H*$_3$COO–C-5), 4.02 (dd, 1 H, $J_{6a,5}$ 6.8, J_{gem} 11.5 Hz, H-6a), 4.17 (dd, 1 H, $J_{6b,5}$ 5.0, J_{gem} 11.5 Hz, H-6b), 4.95 (app t, 1 H, J 5.3 Hz, H-5), 5.51 (d, 1 H, $J_{4,3}$ 6.6 Hz, H-4), 7.42 (d, 1 H, $J_{3,4}$ 6.6 Hz, H-3), 8.10 (s, 1 H, NH); ^{13}C NMR ($CDCl_3$): δ 20.37 (*C*H$_3$COO–C-6), 20.73 (*C*H$_3$COO–C-4), 24.52 (*C*H$_3$COO–C-2), 27.26 (*C*H$_3$COO–C-5), 53.76 (C-5), 62.46 (C-6), 63.95 (C-4), 113.82 (C-3), 130.79 (C-2), 161.21 (C-1), 168.94 (CH$_3$*C*ONH–C-2), 169.50 (CH$_3$*C*OO–C-4), 170.10 (CH$_3$*C*OO–C-6), 172.43 (CH$_3$*C*ON–C-5); LRMS (EI): 326.2 $[M]^+$; HRMS(EI): Calcd. for $C_{14}H_{18}N_2O_7$: 326.1114; Found: 326.1092.

Compound **58**: R_f 0.32 (2:1 toluene–EtOAc); mp 116–118 °C; $[\alpha]_D$ +155.1° (c 0.205, $CHCl_3$); ν_{max} (KBr): 3020, 2990, and 2947 (CH), 1765, 1741, 1725, 1712, 1693, and 1673 (C=O), 1418 (C–N), 1370 (CH_3), 1249 and 1215 {C(=O)–O}, and 1089, 1040, and 1018 cm^{-1} (O–C); ^1H NMR ($CDCl_3$): δ 2.03, 2.04 (2 s, 6 H, C*H*$_3$COO–C-6, C*H*$_3$COO–C-4), 2.29 (s, 6 H, 2 C*H*$_3$CON–C-2), 2.57 (s, 3 H, C*H*$_3$CON–C-5), 4.20 (dd, 1 H, $J_{6a,5}$ 6.0, J_{gem} 11.4 Hz, H-6a), 4.24 (dd, 1 H, $J_{6b,5}$ 8.1, J_{gem} 11.4 Hz, H-6b), 5.15

(ddt, 1 H, $J_{5,6}$ 7.0 Hz, H-5), 5.40 (dd, 1 H, $J_{4,5}$ 1.7, $J_{4,3}$ 6.3 Hz, H-4), 6.78 (dd, 1 H, $J_{3,5}$ 1.4, $J_{3,4}$ 6.3 Hz, H-3); ^{13}C NMR ($CDCl_3$): δ 20.39, 20.59 (CH_3COO–C-6, CH_3COO–C-4), 25.62, 25.62 (2 CH_3CON–C-2), 27.56 (CH_3CON–C-5), 53.99 (C-5), 61.44 (C-6), 63.42 (C-4), 134.43 (C-3), 137.62 (C-2), 160.18 (C-1), 169.64, 170.22 (CH_3COO–C-6, CH_3COO–C-4), 171.80, 172.43 (3 NC=O); LRMS (CI): 369.3 $[M + H]^+$; HRMS (FAB): Calcd. for $C_{16}H_{21}N_2O_8$: 369.1298; Found: 369.1972.

*2-Acetamido-3,4,6-tri-*O*-acetyl-5-amino-2,5-dideoxy-*D*-glucono-1,5-lactam* (**59**).—Compound **46** (65 mg, 0.30 mmol) was treated with acetic anhydride (7 mL) in pyridine (10 mL) at 0 °C, and the reaction mixture was stirred overnight at rt. The solution was concentrated, and the residue was coevaporated with toluene under reduced pressure to give a solid which was subjected to gradient flash column chromatography on silica gel (EtOAc to MeOH–EtOAc 1:9) to yield compound **59** as a white solid (77 mg, 75%): R_f 0.30 (1:9 MeOH–EtOAc); mp 172–174 °C; $[\alpha]_D$ +136.9° (c 0.065, MeOH); ν_{max} (KBr): 3355 and 3127 (NH), 2977 (CH), 1748, 1701, and 1652 (C=O), 1536 (NH), 1377 (CH_3), 1237 {C(=O)–O}, and 1050 cm^{-1} (O–C); ^{1}H NMR ($CDCl_3$): δ 1.95 (s, 3 H, CH_3CONH–C-2), 1.99 (s, 3 H, CH_3COO–C-3), 2.02 (s, 3 H, CH_3COO–C-4), 2.05 (s, 3 H, CH_3COO–C-6), 3.77 (m, 1 H, H-5), 4.03 (dd, 1 H, $J_{6a,5}$ 5.7, J_{gem} 11.8 Hz, H-6a), 4.19 (dd, 1 H, $J_{6b,5}$ 2.7, J_{gem} 11.7 Hz, H-6b), 4.29 (app t, 1 H, J 9.0 Hz, H-2), 5.22 (t, 1 H, J 9.4 Hz, H-4), 5.44 (t, 1 H, J 10.0 Hz, H-3), 6.89 (d, 1 H, J 8.1 Hz, N*H*Ac), 7.02 (s, 1 H, NH); ^{13}C NMR ($CDCl_3$): δ 20.48, 20.60, 20.60 (3 acetyl CH_3), 22.59 (acetamido CH_3), 52.48 (C-5), 53.22 (C-2), 62.82 (C-6), 68.00 (C-4),

70.96 (C-3), 168.93 (C-1), 169.54 (CH_3COO–C-4), 170.08 (CH_3COO–C-3), 170.68 (CH_3COO–C-6), 171.29 (CH_3CONH–C-2); LRMS (CI): 345.3 $[M + H]^+$.

*2-Acetamido-3,4,6-tri-*O*-acetyl-1,2,5-trideoxy-1,5-*(N*-acetyl)imino-*D*-glucitol* (**60**).—Compound **40** (80 mg, 0.39 mmol) was treated with acetic anhydride (9 mL) in pyridine (12 mL) at 0 °C, and reaction mixture was stirred overnight at rt. The solution was concentrated, and the residue was coevaporated with toluene under reduced pressure to give a syrup which was subjected to gradient flash column chromatography on silica gel (1:100 to 5:100, MeOH–EtOAc) to afford compound **60** (128 mg, 88%) as a clear syrup. The product was identified as two isomers in a ratio of ~ 2:1 by ^{1}H NMR spectroscopy (including TOCSY). R_f 0.28 (1:9 MeOH–EtOAc); $[\alpha]_D$ −10.8° (c 0.53, MeOH); ν_{max} (Film): 3400 (NH), 2924 (CH), 1745 and 1651 (C=O), 1529 (NH), 1431 (C–N), 1372 (CH_3), 1226 {C(=O)–O}, and 1043 cm^{-1} (O–C); ^{1}H NMR (D_2O): δ 1.97, 1.98, 2.05, 2.08 (4 s, 18 H, 5 CH_3, 2 CH'_3), 1.93, 2.03, 2.13 (3 s, 4.5 H, 3 CH'_3), 3.27 (d, 0.5 H, J 14.1 Hz, H'-1a), 3.74 (dd, 1 H, $J_{1a,2}$ 2.2, J_{gem} 15.0 Hz, H-1a), 3.82 (d, 1 H, J_{gem} 15.0 Hz, H-1b), 4.07 (app s, 0.5 H, H'-2), 4.11 (app s, 1 H, H-2), 4.26 (app dd, 1.5 H, $J_{6a,5}$ 5.2, J_{gem} 11.6 Hz, H-6a, H'-6a), 4.35 (d, 0.5 H, J 14.5 Hz, H'-1b), 4.44 (app t, 1.5 H, J 10.2 Hz, H-6b, H'-5), 4.62 (t, 0.5 H, 10.4 Hz, H'-6b), 4.84 (m, 1 H, H-3), 4.88 (m, 1.5 H, H-5, H'-3), 4.99 (br s, 1.5 H, H-4, H'-4); ^{13}C NMR (D_2O): δ 20.41–22.24 (5 CH_3, 5 $C'H_3$), 36.59 (C'-1), 42.16 (C-1), 46.03 (C'-2), 46.54 (C-2), 51.33 (C-5), 56.33 (C'-5), 60.74 (C-6), 60.76 (C'-6), 67.14 (C'-4), 67.42 (C-4, C'-3), 68.02 (C-3), 171.90–175.40 (5 C=O, 5 C'=O); LRMS (CI): 373.2 $[M + H]^+$.

3.3. Preliminary studies on solid-phase synthesis

3.3.1. *Coupling of 2,3:4,6-di-*O*-isopropylidene-α-*L-xylo-*hexulosonic acid* (**61**) *to Merrifield resin.*—Compound **61** (8.76 g, 30 mmol) was dissolved in MeOH (150 mL) and water (15 mL) was added. The resulting solution was titrated to pH 7.0 with a 20% aq solution of cesium carbonate. The mixture was evaporated to dryness. To the residue was added anhydrous DMF (75 mL) and the resulting solution was evaporated to dryness at 45 °C. A second portion of anhydrous DMF (75 mL) was added, the resulting solution was again evaporated to dryness at 45 °C, and the residue was dried under vacuum overnight. The Merrifield resin (10 g, 15 mmol[‡]) was swelled in anhydrous DMF (60 mL) and to this solution mixture was added the dry cesium salt of the carboxylic acid in anhydrous DMF (150 mL). The mixture was shaken then at 60 °C for 60 h. The resin was removed by filtration and washed sequentially with DMF (3 × 300 mL), 50% (v/v) aq DMF (3 × 300 mL), 50% (v/v) aq methanol (3 × 300 mL), methanol (3 × 300 mL), and Et_2O (3 × 300 mL). The resin was dried under vacuum to a constant weight. The attachment of compound **61** to the Merrifield resin was 91% based on both gravimetrical analysis and chemical cleavage method (see below).

Cleavage of the product from the Merrifield resin using ethylamine.—A sample (105 mg) of the resin bound with compound **61** was swelled in anhydrous THF (2 mL), and to this mixture was added ethylamine (70% in H_2O) (2 mL, 24.76 mmol). The resulting solution mixture was shaken overnight at rt. The resin was collected by filtration, washed sequentially with H_2O, H_2O–MeOH, MeOH, THF, CH_2Cl_2, and Et_2O,

[‡] The number of mol was based on the chlorine substitution of the Merrifield resin.

and then dried under vacuum; the weight of recovered resin was 74 mg. The combined filtrate and washings were concentrated to give presumably *N*-ethyl-2,3:4,6-di-*O*-isopropylidene-α-L-*xylo*-hexulosonamide (**62**) (43 mg, 91% for the coupling): R_f 0.65 (EtOAc): ^{1}H NMR ($CDCl_3$): δ 1.16 (t, 3 H, *J* 7.3 Hz, $NHCH_2CH_3$), 1.29, 1.41 (2 s, 6 H, 2 isopropylidene-CH_3), 1.51, 1.54 (2 s, 6 H, 2 isopropylidene-CH_3), 3.32 (m, 2 H, $NHCH_2CH_3$), 4.08 (br s, 2 H, 2 H-6), 4.16 (m, 1 H, H-5), 4.29 (d, 1 H, $J_{4,5}$ 2.0 Hz, H-4), 4.56 (s, 1 H, H-3), 7.10 (br s, 1 H, NH); ^{13}C NMR ($CDCl_3$): δ 14.64 ($NHCH_2CH_3$), 18.47 (isopropylidene-CH_3), 25.64, 27.07 (2 isopropylidene-CH_3), 29.06 (isopropylidene-CH_3), 34.46 ($NHCH_2CH_3$), 60.10 (C-6), 72.40 (C-4), 73.42 (C-5), 87.86 (C-3), 97.49 (isopropylidene-*C*), 110.60 (C-2), 114.52 (isopropylidene-*C*), 167.07 (C=O).

Cleavage of the product from the Merrifield resin using diisobutylaluminum hydride.—To a mixture of a sample (98 mg) of the resin bound with compound **61** in dry toluene (15 mL) was added DIBAL–H (Aldrich, 1.0 M solution in CH_2Cl_2)(0.60 mL, 4 equiv) at 0 °C under an argon atmosphere. The mixture was stirred at that temperature for 12 h and H_2O (10 mL) was added. The resulting solid and resin were removed by filtration and washed with CH_2Cl_2. The weight of retrieved resin was 82 mg. The filtrate was diluted with H_2O and extracted with CH_2Cl_2. The combined organic layer was dried (Na_2SO_4) and the extract was evaporated to give a crude sample of presumably 2,3:4,6-di-*O*-isopropylidene-α-L-sorbose (**63**) (16 mg, 47%): ^{1}H NMR ($CDCl_3$): δ 1.36, 1.42, 1.49 (3 s, 12 H, 4 CH_3), 2.19 (app t, 1 H, $J_{1,OH}$ 6.2 Hz, OH), 3.78 (dd, 1 H, $J_{1a,OH}$ 5.0, J_{gem} 11.8 Hz, H-1a), 3.86 (dd, 1 H, $J_{1b,OH}$ 6.3, J_{gem} 11.8 Hz, H-1b), 4.05 (app t, 2 H, 2 H-6), 4.09 (ddd~q, 1 H, $J_{5,4}$ 2.0, $J_{5,6a}$ 2.1, $J_{5,6b}$ 1.8 Hz, H-5), 4.31 (d, 1

H, $J_{4,5}$ 2.0 Hz, H-4), 4.46 (s, 1 H, H-3); ^{13}C NMR ($CDCl_3$): δ 18.56, 26.47, 27.32, 28.92 (4 CH_3), 60.27 (C-6), 63.67 (C-1), 72.24 (C-5), 73.28 (C-4), 84.96 (C-3), 97.55, 111.96, 114.27 (3 ketal *C*).

3.3.2. *Selective removal of the 4,6-O-isopropylidene group from the resin-bound* **61**.—The resin (12.0 g) bound with **61** was suspended in a mixture of dioxane and 3 N hydrochloric acid (1:1, 300 mL). The mixture was shaken for 30 h at rt. The resin was removed by filtration and washed sequentially six times with water, once with acetone, three times with ethanol, and three times with dry ether to give presumably the resin-diol, which was dried under vacuum to a constant weight (11.2 g).

Cleavage of the product from the Merrifield resin using ethylamine.—A sample (95 mg) of the resin-diol was swelled in THF (2.5 mL) and the resulting mixture was treated with ethylamine (70% in H_2O) (2.5 mL). The mixture was then shaken overnight at rt. The resin was collected by filtration, washed sequentially with H_2O, H_2O–MeOH (1:1), MeOH, THF, CH_2Cl_2, and Et_2O, and then dried under vacuum. The weight of the retrieved resin was 65 mg. The combined filtrate and washings were concentrated to give a crude sample of *N*-ethyl-2,3-*O*-isopropylidene-α-L-*xylo*-hexulosonamide (**64**) (36 mg); R_f 0.38 (EtOAc); $[\alpha]_D$ +12° (c 0.05, MeOH); ^{1}H NMR ($CDCl_3$): δ 1.16 (t, 3 H, *J* 7.3 Hz, $NHCH_2CH_3$), 1.40, 1.52 (2 s, 6 H, 2 Me), 3.31 (m, 2 H, $NHCH_2CH_3$), 3.95 (d, 2 H, $J_{5,6}$ 5.1 Hz, 2 H-6), 4.28 (app d, 1 H, $J_{4,5}$ 2.8 Hz, H-4), 4.41 (m, 1 H, H-5), 4.52 (s, 1 H, H-3), 6.94 (br s, 1 H, NH); ^{13}C NMR ($CDCl_3$): δ 14.40 ($NHCH_2CH_3$), 25.84, 27.07 (2 Me), 34.53 ($NHCH_2CH_3$), 60.95 (C-6), 75.14 (C-

4), 83.59 (C-5), 87.67 (C-3), 109.92 (C-2), 114.48 (isopropylidene-ketal *C*), 168.56 (C-1). LRMS (CI): 262 $[M + H]^+$.

3.3.3. *Selective tosylation of the primary hydroxyl group of the resin-diol.*—A sample (11.2 g) of the resin-diol obtained from the last step was swelled in dry CH_2Cl_2 (100 mL), and dry pyridine (2.5 mL, 30.9 mmol) was added. The temperature of the resulting mixture was decreased to –60 °C and TsCl (3.5 g, 18.2 mmol) and DMAP (150 mg, 1.2 mmol) were added. The mixture was then shaken at rt for 64 h. The resin was removed by filtration and washed sequentially with ice–water (3 × 300 mL), DMF (2 × 300 mL), 50% (v/v) aq DMF (2 × 300 mL), MeOH (3 × 300 mL), CH_2Cl_2 (3 × 300 mL), and Et_2O (2 × 300 mL). The resin was dried under vacuum to a constant weight.

Cleavage of the product from the Merrifield resin using ethylamine.—A sample (76 mg) of the resin product obtained above was swelled in THF (2.5 mL) and the resulting mixture was treated with ethylamine (70% in H_2O) (2.0 mL). The mixture was shaken overnight at rt, filtered, and the resin was washed sequentially with H_2O, H_2O–MeOH (1:1), MeOH, THF, CH_2Cl_2, and Et_2O. The combined filtrate and washings were concentrated under reduced pressure and the residue was subjected to preparative thin-layer chromatography on silica gel (1:1 EtOAc–hexane) to yield *N*-ethyl-2,3-*O*-isopropylidene-6-*O*-*p*-tolylsulfonyl-α-L-*xylo*-hexulosonamide (**65**) as a homogeneous, colorless oil (22 mg, 51.2% from the resin-bound **61**): R_f 0.26 (1:1 EtOAc–hexane); $[\alpha]_D$ + 65.6° (c 1.0, $CHCl_3$); ν_{max} (KBr): 3377 (NH, OH), 2985 and 2936 (CH), 1665 (C=O), and 1366 cm^{-1} (SO_2); ^1H NMR ($CDCl_3$): δ 1.16 (t, 3 H, *J* 7.3

Hz, $NHCH_2CH_3$), 1.36, 1.51 (2 s, 6 H, 2 Me), 2.42 (s, 3 H, $PhCH_3$), 3.30 (m, 2 H, $NHCH_2$), 4.18 (app dd, 2 H, J_{gem} 10.7, $J_{6a,5}$ 7.0 Hz, H-6a, H-4), 4.34 (dd, 1 H, J_{gem} 10.7, $J_{6b,5}$ 4.6 Hz, H-6b), 4.45 (s, 1 H, H-3), 4.50 (m, 1 H, H-5), 4.72 (br d, 1 H, *J* 10.3 Hz, OH), 6.72 (br t, 1 H, NH), 7.31 (app d, 2 H, *J* 8.3 Hz, aromatic), 7.78 (app d, 2 H, *J* 8.4 Hz, aromatic); ^{13}C NMR ($CDCl_3$): δ 14.37 ($NHCH_2CH_3$), 21.60 ($PhCH_3$), 25.78, 27.02 (2 Me), 34.54 ($NHCH_2$), 68.07 (C-6), 74.11 (C-4), 81.91 (C-5), 86.59 (C-3), 109.97 (C-2), 114.94 (isopropylidene-ketal *C*), 128.07, 129.85 (4 aromatic *C*H), 132.65 (*tert* aromatic *C*-SO_2-), 144.91 (*tert* aromatic *C*-CH_3), 168.34 (C-1); LRMS (CI): 416.28 [M + H]$^+$.

3.3.4. *Reaction with sodium azide.*—A sample (12.3 g) of the resin obtained from the last step was swelled in anhydrous DMF (150 mL), and to this mixture were added sodium azide (7.11 g, 110 mmol) and tetra-*n*-butylammonium chloride (0.61 g, 2.2 mmol). The resulting mixture was shaken at 50 °C for 36 h. The resin was removed by filtration and washed sequentially with cold water (3 × 300 mL), 50% (v/v) aq DMF (2 × 300 mL), DMF (3 × 300 mL), 50% (v/v) aq methanol (2 × 300 mL), methanol (3 × 300 mL), CH_2Cl_2 (2 × 300 mL), and Et_2O (2 × 300 mL). The resin was dried under vacuum to a constant weight.

Cleavage of the product from the Merrifield resin using ethylamine.—A sample (900 mg) of the resin product was swelled in THF (30 mL) and the resulting mixture was treated with ethylamine (70% in H_2O) (30 mL). The mixture was shaken for 24 h at rt. The resin was collected by filtration, washed sequentially with MeOH, MeOH–

H_2O (1:1), THF, MeOH, CH_2Cl_2, MeOH, and Et_2O, and then dried under vacuum. The weight of the retrieved resin was 785.3 mg. The combined filtrate and washings were concentrated to give a brown oil (291 mg), which was subjected to gradient flash column chromatography (EtOAc–hexane, from 1:10 to 1:3) to give 6-azido-6-deoxy-*N*-ethyl-2,3-*O*-isopropylidene-α-L-*xylo*-hexulosonamide (**66**) as a colorless oil (102 mg, 29% from the resin-bound **61**); R_f 0.39 (1:1 EtOAc–hexane); ν_{max} (Film): 2103 cm^{-1} (N_3); ^1H NMR ($CDCl_3$): δ 1.18 (t, 3 H, *J* 7.2 Hz, $NHCH_2CH_3$), 1.38, 1.54 (2 s, 6 H, 2 Me), 3.32 (m, 2 H, $NHCH_2$), 3.56 (dd, 1 H, J_{gem} 12.9, $J_{6a,5}$ 7.1 Hz, H-6a), 3.59 (dd, 1 H, J_{gem} 12.9, $J_{6b,5}$ 5.7 Hz, H-6b), 4.17 (br s, 1 H, H-4), 4.40 (ddd, 1 H, $J_{5,4}$ 2.4 Hz, H-5), 4.50 (s, 1 H, H-3), 6.74 (br s, 1 H, NH); ^{13}C NMR ($CDCl_3$): δ 14.40 ($NHCH_2CH_3$), 25.77, 27.05 (2 Me), 34.55 ($NHCH_2$), 49.70 (C-6), 74.25 (C-4), 82.89 (C-5), 86.77 (C-3), 109.82 (C-2), 114.79 (isopropylidene-ketal *C*), 168.50 (C-1).

3.3.5. *Conversion of 6-azido-6-deoxy*-N-*ethyl-2,3*-O-*isopropylidene-α*-L-xylo-*hexulosonamide* (**66**) *into the corresponding 6-amino derivative* **67**.—To a solution of compound **66** (93 mg, 0.325 mmol) in MeOH (7 mL) was added 10% Pd/C (40 mg). The mixture was subjected to a hydrogen pressure (50 psig) at rt for 1 h. The mixture was then filtered through Celite 521 (Aldrich) and the filtrate was concentrated under vacuum to give compound **67** as a colorless syrup (85 mg, 100%): R_f 0.32 (5:1 $CHCl_3$–NH_3 in MeOH); $[\alpha]_D$ –8.9° (c 0.19, MeOH); ^1H NMR ($CDCl_3$): δ 1.09 (t, 3 H, *J* 7.3 Hz, $NHCH_2CH_3$), 1.36, 1.46 (2 s, 6 H, 2 Me), 3.01–3.19 (br d, 2 H, 2 H-6), 3.25 (m, 2 H, $NHCH_2$), 3.30–3.50 (br s, 2 H, NH_2), 4.17 (s, 1 H, H-4), 4.22 (m, 1 H, H-5), 4.48 (s, 1 H, H-3), 6.90 (t, 1 H, *J* 5.6 Hz, N*H*Et); ^{13}C NMR ($CDCl_3$): δ 14.38 ($NHCH_2CH_3$),

25.64, 26.89 (2 Me), 34.31 ($NHCH_2CH_3$), 40.42 (C-6), 75.45(C-4), 83.04 (C-5), 87.79 (C-3), 109.95 (C-2), 113.86 (isopropylidene-ketal *C*), 168.12 (C-1); LRMS (CI): 261.2 $[M + H]^+$; HRMS (FAB): Calcd. for $C_{11}H_{21}N_2O_5$: 261.1451; Found: 261.1396.

3.3.6. *Conversion of 6-azido-6-deoxy*-N-*ethyl-2,3*-O-*isopropylidene-α*-L-xylo-*hexulosonamide* (**66**) *into 1,5-dideoxy*-N-*ethyl-1,5-imino*-D-*glucuronamide* (**68**).— Compound **66** (45 mg, 0.16 mmol) was dissolved in a solution of THF (5.0 mL), H_2O (0.6 mL), and concd HCl (1.2 mL). The solution was heated at 50 °C for 6 h until TLC indicated the consumption of the starting compound **66**. The solvent was removed under reduced pressure and the oily residue was subjected to a Kugel Rohr distillation at 50 °C. The brown residue was dissolved in MeOH (7 mL), and one drop of Et_3N and 10% Pd/C (30 mg) were added to the solution. The resulting solution mixture was subjected to a hydrogen pressure (50 psig) at rt for 24 h. The mixture was filtered through Celite 521 (Aldrich), and the filtrate was concentrated under reduced pressure to give a syrup, which was purified first by a gradient flash column chromatography (NH_3 in MeOH–EtOAc, from 1:5 to 1:2) and then by a preparative thin-layer chromatography (1:1 NH_3 in MeOH–EtOAc) on silica gel. Lyophilization rendered compound **68** as a white solid (28 mg, 87%): R_f 0.21 (1:1 NH_3 in MeOH–EtOAc); mp 181–183 °C; $[\alpha]_D$ +42° (c 0.1, H_2O); ^1H NMR (H_2O): δ 0.99 (t, 3 H, *J* 7.2 Hz, Me), 2.32 (app t, 1 H, *J* 11.8 Hz, H-1a), 2.91 (d, 1 H, $J_{5,4}$ 9.6 Hz, H-5), 2.99 (dd, 1 H, J_{gem} 13.1, $J_{1b,2}$ 5.0 Hz, H-1b), 3.11 (q, 2 H, 7.2 Hz, CH_2Me), 3.22 (t, 1 H, *J* 9.0 Hz, H-3), 3.32 (t, 1 H, , *J* 9.4 Hz, H-4), 3.41 (m, 1 H, H-2); ^{13}C NMR (D_2O): δ 13.77 (Me), 34.81 (CH_2Me), 48.55 (C-1), 63.87 (C-5), 71.22 (C-2), 72.94(C-4), 77.97 (C-3), 172.17

(C-6); LRMS (ES): 205.1 $[M + H]^+$; HRMS (FAB): Calcd. for $C_8H_{17}N_2O_4$: 205.1188; Found: 205.1201.

Chapter 4. Conclusions

A facile approach to the synthesis of 2-acetamido-1,2,5-trideoxy-1,5-imino-D-glucitol (2-acetamido-1,2-dideoxynojirimycin) (**40**), as well as some of its derivatives, was established starting from the readily available 2-acetamido-2-deoxy-D-glucose (*N*-acetyl-D-glucosamine) (**41**) in a ten-step procedure. This approach could be utilized, as a general strategy, for the synthesis of a number of six- and five-membered cyclic 1-deoxy azasugars and their derivatives.

Some azasugar compounds, namely **40**, **46**, **59**, and **60**, derived from the solution-phase synthesis, showed interesting results in the studies of their effects on the biosynthesis of glycosaminoglycans. Compound **40** demonstrated a stimulatory effect on hepatocyte GAG biosynthesis at concentrations of 0.01 mM and 0.1 mM and an inhibitory effect on hepatocyte GAG biosynthesis at a concentration of 1.0 mM. Compound **60**, an acetylated analogue of **40**, showed a moderate stimulatory effect on hepatocyte GAG biosynthesis at all three concentrations of 0.01, 0.1, and 1.0 mM. Compounds **46** and **59**, instead, showed slight inhibition of incorporation of [^{3}H]GlcN at 1.0 mM, but not of [^{35}S]SO_4, into isolated GAGs. Future studies may involve reevaluating compound **40** at lower concentrations (0.01 μM, 0.1 μM, and 1.0 μM) to elucidate further details of its function upon the GAG biosynthesis.

Another study was an exploration of the synthesis of 1-deoxynojirimycin analogues on a solid support. A novel azasugar compound, namely 1,5-dideoxy-1,5-imino-D-glucuronamide (**68**), was expeditiously synthesized. The successfully established methodology can be utilized to generate a library of 1-deoxynojirimycin analogues, and also should be applicable to other 2-ketoses to create corresponding azasugar analogues.

The new compound, **68**, generated in the present study, might be an inhibitor of enzymes involved in the degradation or processing of oligosaccharides. Future research may involve the biological evaluation of its effects on the biosynthesis of glycosaminoglycans.

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