MONASH UNIVERSITY
THESIS ACCEPTED IN SATISFACTION OF THE
REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
ON..... 14 June 2005.....
.....[redacted].....
Sec. Research Graduate School Committee

Synthesis of Sialyl Mimetics

as

Biological Probes

A thesis submitted for the Degree of

DOCTOR OF PHILOSOPHY

by

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November 2004

Statement of Originality

I declare that this thesis has not been submitted in any form for another degree or diploma at any university. The material discussed in this thesis is my own work, and information derived from the literature or unpublished work of others has been acknowledged in the text and a list of references provided. This thesis is less than one hundred thousand words in length, excluding tables and references.

Tho Van Phan

Acknowledgments

I would like to thank my supervisors Professor Mark von Itstein and Dr Gaik Kok for their assistance and advice during the course of my work. I would also like to thank Professor Peter Scammells for his presence and assistance in the absence of Mark.

My sincere appreciation goes to Dr Samia Abo, Dr Carolyn Trower for their efforts to perform biological assays, Dr Ben Tehan for his help and advice on computer modelling, and Mr Stuart Thompson for NMR and mass spectral data. My gratitude also goes to all the staff and students of the Department of Medicinal Chemistry.

I wish to express my deepest gratitude to my long-time friends Ms Milica Radovanovic, Dr Roland Chung and Mr Brendan Mackey for their friendship, assistance and encouragement.

Finally, I must thank my family for support and encouragement.

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Abbreviations

Ac	acetyl
Anal.	analytical
app	apparent
aq	aqueous
Ar	aryl
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
Bz	benzoyl
br	broad
C	Celsius
calcd.	calculated
conc.	concentrated
COSY	correlation spectroscopy
CSA	camphorsulfonic acid
d	doublet
dd	doublet of doublets
ddd	doublet of doublets of doublets
DIAD	diisopropyl azodicarboxylate
DMAP	4-(dimethylamino)-pyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
EC	enzyme commision
ESI	electronspray ionization

Et	ethyl
FAB	fast atom bombardment
h	hour(s)
Hex	hexane
HMQC	heteronuclear multiple quantum correlation
HRMS	high resolution mass spectroscopy
Hz	hertz
IR	infrared
KDN	3-deoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid
KDO	3-deoxy-D-*manno*-octulosononic acid
K_i	dissociation constant of inhibitor
lit.	literature
LRMS	low resolution mass spectroscopy
m	multiplet
Me	methyl
min	minute(s)
mp	melting point
m/z	mass to charge ratio
Neu5Ac	5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulosonic acid or *N*-acetylneuraminic acid
Neu5Ac2en	5-acetamido-2,6-anhydro-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-non-2-enoic acid or 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid
NMR	nuclear magnetic resonance
PCC	pyridinium chlorochromate
PDC	pyridinium dichromate

Ph	phenyl
ppm	parts per million
q	quartet
R_f	retention factor (in chromatography)
rt	room temperature
s	singlet
satd.	saturated
t	triplet
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
Tf_2O	trifluoromethanesulfonic anhydride
THF	tetrahydrofuran
TLC	thin layer chromatography
$TMSN_3$	trimethylsilyl azide
TPP	triphenylphosphine
Ts	*p*-toluenesulfonyl
wt/vol	weight for volume
w/w	weight for weight

Abstract

Sialic acids are widely distributed in many living systems. Sialic acids and their associated enzymes are involved in a large number of biologically important processes. Influenza virus sialidase has been suggested to play a pivotal role in viral infection. This thesis focuses on the investigation of the synthesis of sialyl mimetics as biological probes towards the development of potential inhibitors for influenza virus sialidase.

Five chapters comprise this thesis. The first chapter includes an introduction to sialic acids and fructose along with a brief overview of sialyl mimetics developed over recent years as influenza sialidase inhibitors.

Chapter 2 describes the synthesis of fructofuranose derivatives, which were used as frameworks to synthesize the target compounds that are presented in Chapter 3. Two approaches towards the synthesis of 4-azido-4-deoxy-fructofuranosidonic esters were investigated. Successful synthesis of these esters without extensive synthetic manipulations were achieved. Examination of ^{1}H and ^{13}C NMR properties of the fructofuranose derivatives synthesized is also included in Chapter 2.

The synthesis of C-6 modified fructofuranosidonic acids and derivatives are presented in Chapter 3. The successful inc rporation of an *N*-acetyl functionality at the 6-position of fructofuranose esters led to the target compound methyl 6-acetamido-4-amino-4,6-dideoxy-β-D-fructofuranosidonic acid. The synthesis of carbon-carbon chain-extended C-6 modified fructofuranosidonic esters employing the Wittig reaction was investigated. Fructofuranosidonic acid that incorporated a nitro functionality on the side chain was synthesized. The biological activity of these target compounds against influenza sialidase is also presented in Chapter 3.

In Chapter 4 a report on an alternative synthesis of the known furanose-based sialidase inhibitors, 6-acetamido-3,6-dideoxy-D-*glycero*-D-*allo*-2-nonulofuranosonic acid and 6-acetamido-3,6-dideoxy-D-*glycero*-D-*altro*-2-nonulofuranosonic acid, is presented. Synthesis of the 4,6-diacetamido analogues was also investigated.

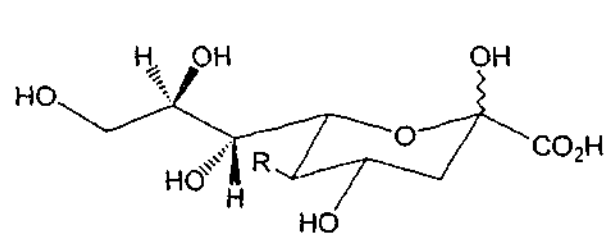
The final chapter, Chapter 5, brings together full experimental details in support of the information presented in Chapters 2 to 4.

CHAPTER 1

Introduction

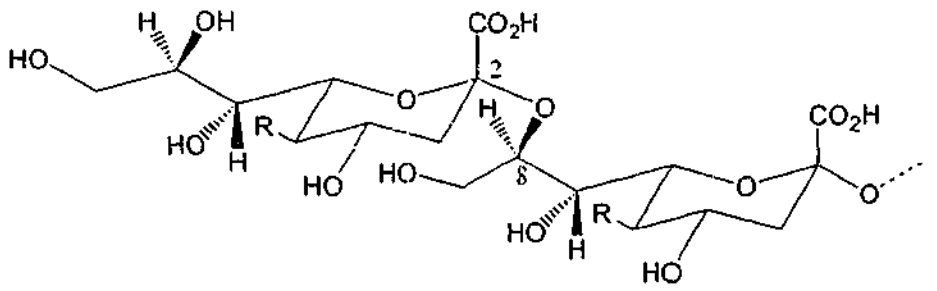
1.1 Sialic acids

Sialic acids are a family of carbohydrates comprising more than 40 naturally occurring nonulosonic acids[1-4]. They are commonly found as derivatives of 5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (Neu, **1**). The most widespread form is the *N*-acetylated derivative 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (Neu5Ac, **2**)[4,5]. Less common than Neu5Ac is the *N*-glycolyl derivative 5-hydroxyacetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (Neu5Gc, **3**). 3-deoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid[1,6] or KDN (**4**), isolated in 1986, is a deaminated form of sialic acids with a hydroxy group at C-5.



1 R = NH_2 (Neu)
2 R = $NHCOCH_3$ (Neu5Ac)
3 R = $NHCOCH_2OH$ (Neu5Gc)
4 R = OH (KDN)

Sialic acids are widely distributed[1-4] in all vertebrates and in some invertebrates. They are also detected in several species of bacteria and viruses. They rarely occur as free molecules. The majority of sialic acids are found in α-glycosidically linked form, as the terminal residue or an internal unit of a glycan chain. They are predominantly attached to a neighbouring hexose through α(2→3) and α(2→6) linkages, or to another sialic acid through α(2→8) linkage[1,2,7] as shown in structure **5**.



5 R = NHAc

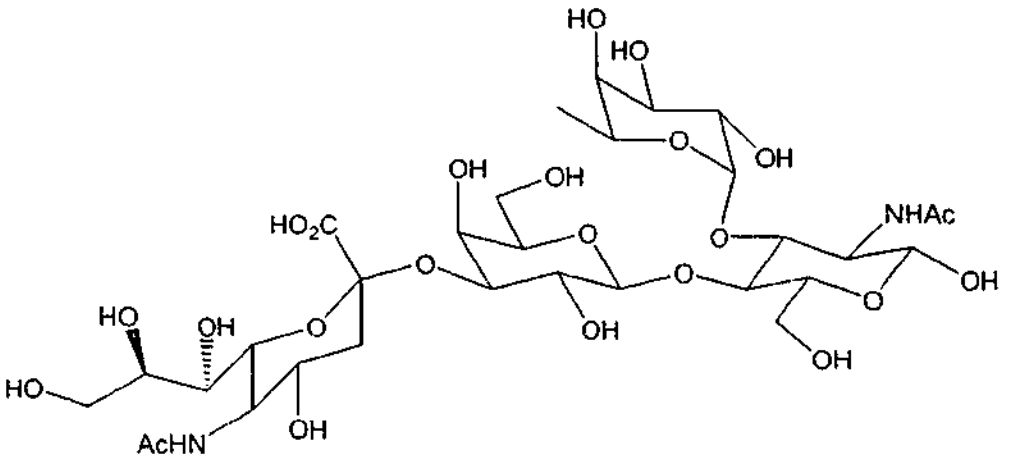
1.1.1 Biological functions of sialic acids

As a constituent of sialoglycoconjugates, sialic acids, given their structural diversity and position within glycoconjugates, play important roles in the regulation of many biological processes[2,8]. Located at the termini of numerous cell-suface oligosaccharides, the main function of sialic acids is to participate in cellular and molecular recognition phenomena.

Sialic acids, negatively charged at physiological pH, are involved in the binding, transport of positively charged molecules and in attraction and repulsion phenomena between cells and molecules[2,8]. Sialic acids can function as a protective shield for the subterminal part of the molecules or cells[8].

Sialic acids play significant roles as a component of receptors in cell to cell interactions, binding of toxins and in the pathogenesis of microorganisms. The action of the Influenza virus in adhesion and infection of cells has been extensively studied[2,4]. Understanding these biological activities together with structural information from the X-ray crystallographic study of influenza virus sialidase provided the basis for the development of anti-influenza drugs[9,10,11].

An important group of sialic acid-recognizing biomolecules are the selectins which play a pivotal role in the adhesion of white blood cells to endothelia, facilitating the extravasation of the leukocytes to the site of injury[4]. Selectins are located on endothelial cells and their interactions with sialyloligossaccharides such as sialyl Lewis x (**6**) are believed to be involved in the inflammation process and tumour metastasis[4]. Significant effort is currently devoted to the development of biological probes or inhibitors of selectins[12,13-32].



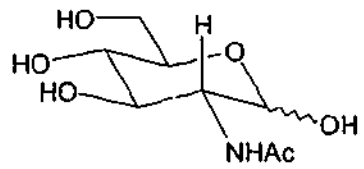
sialyl Lewis x (**6**)

1.1.2 Synthesis of sialic acids

A substantial amount of literature has been published on the synthesis of both natural and unnatural sialic acids[33-53]. These syntheses can be broadly divided in two categories, chemical synthesis and enzymic synthesis. The latter includes chemoenzymatic method which involves chemical modification before or after enzymatic synthesis.

1.1.2.1 Chemical synthesis of sialic acids

In 1958, Comforth *et al.*[36] reported the first chemical synthesis of Neu5Ac (**2**) based on the condensation of *N*-acetylglucosamine (**7**) with oxalacetic acid (**8**) under basic conditions to give **2** in *ca.* 2% yield. Since that time a number of modifications have been made to the synthetic method to achieve better yields.



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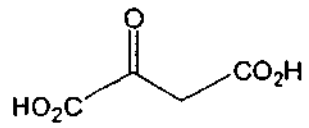

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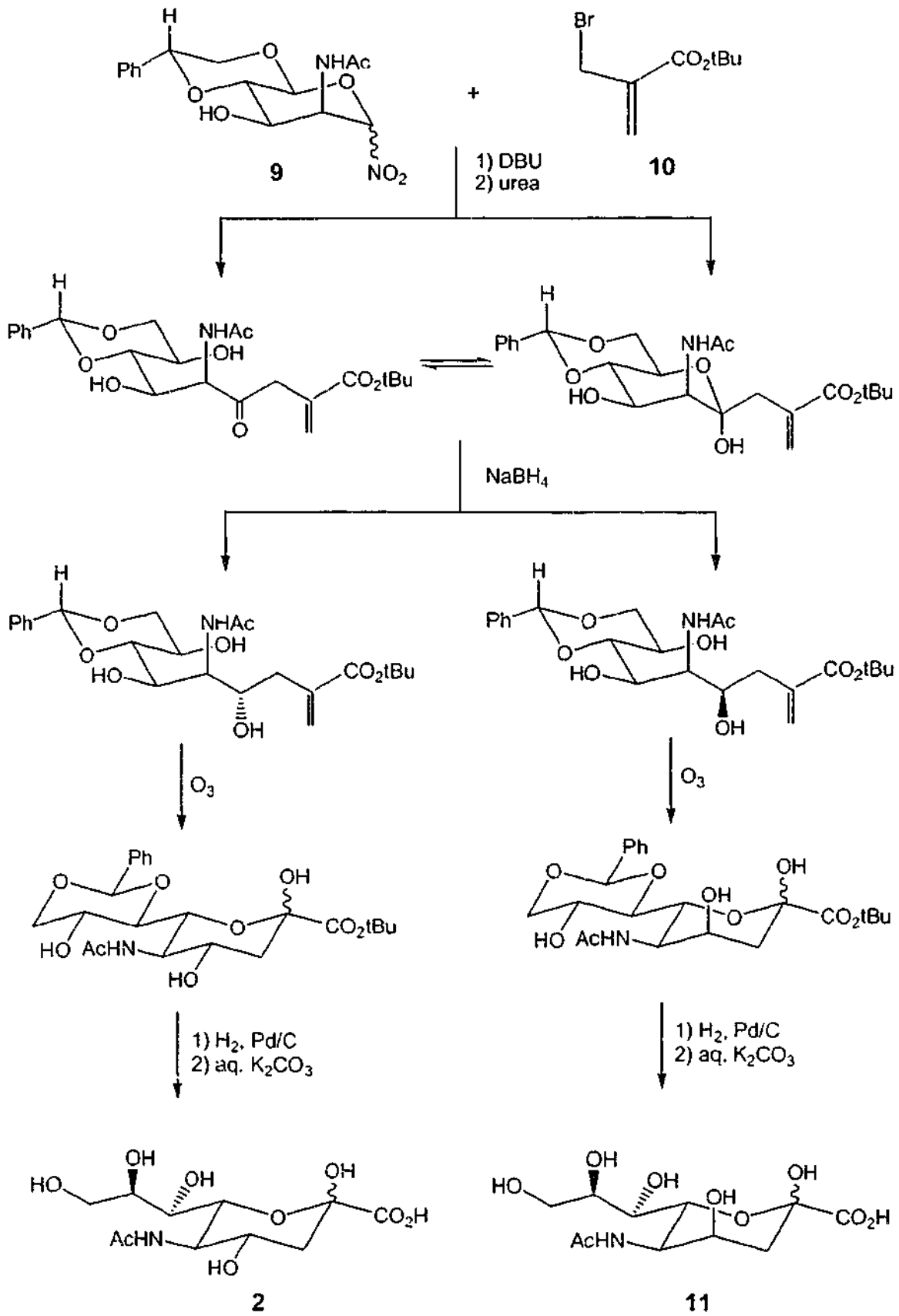
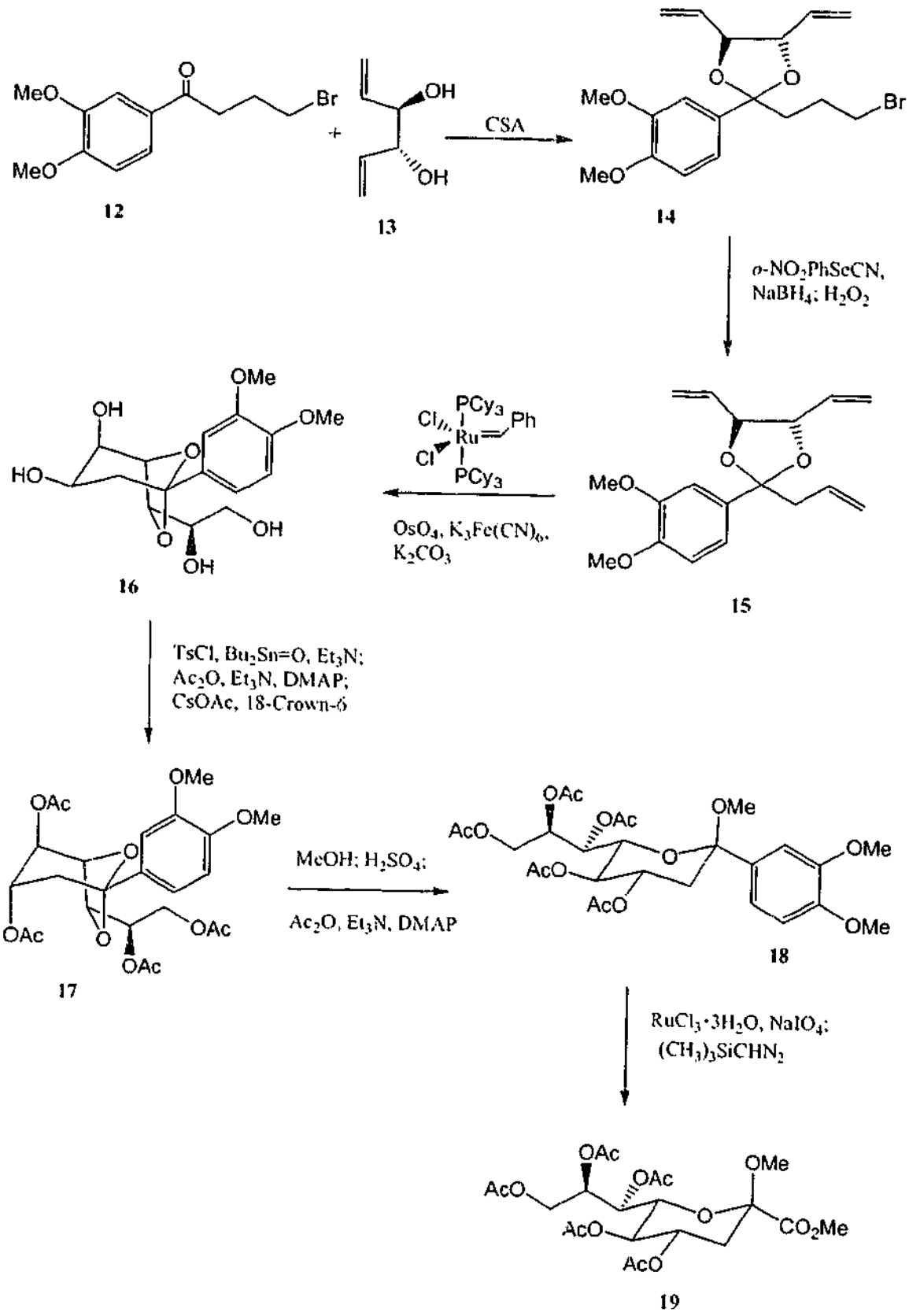
Baumberger and Vasella[38], in 1986, described the synthesis of Neu5Ac (**2**) and its 4-epimer (**11**), based on the Michael addition of the partially protected 2-acetamido-1,2-dideoxy-1-nitro-D-mannose (**9**) to *tert*-butyl 2-(bromomethyl)acrylate (**10**), followed by a β-elimination as the key transformation (Scheme 1.1).


9
10
1) DBU
2) urea
NaBH4
O3
O3
1) H2, Pd/C
2) aq. K2CO3
1) H2, Pd/C
2) aq. K2CO3
2
11

Scheme 1.1

Recently, tin, zinc, and indium have been utilized to effect the addition of allylic halides to carbohydrates, to synthesize higher carbon sugars including Neu5Ac (**2**), KDN (**4**), and derivatives[42,43,61,62,75]. These metal mediated allylation reactions could be carried out using unprotected sugars in aqueous media.

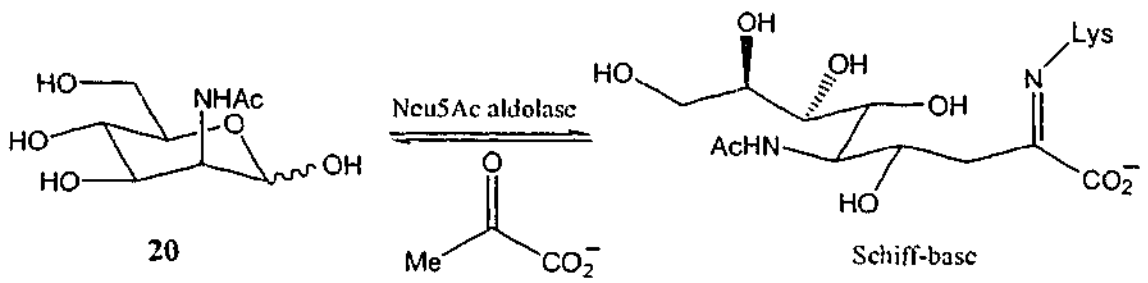
Synthesis of sialic acids, particularly Neu5Ac (**2**) and KDN (**4**), from non-carbohydrate sources has been of significant interest in recent years[60-63]. The first total synthesis of Neu5Ac (**2**) from non-carbohydrate precursors was reported by Danishefsky *et al.*[60] in 1988. More recently, Burke and Voight[63] reported an efficient total synthesis of KDN, which involved the formation of triene **15,** via the preparation of ketal **14** from **12** and **13**. Ring-closing metathesis of **15** succeeded by dihydroxylation gave the tetraol **16**. Partial tosylation of **16**, followed by peracetylation, and subsequent treatment with cesium acetate provided the inverted tertaacetate **17**. Exposure of **17** to acidic methanol gave **18,** which was treated with RuO_4 to unmask the carboxylic acid functionality to give the protected KDN (**19**) in good overall yield (Scheme 1.2).

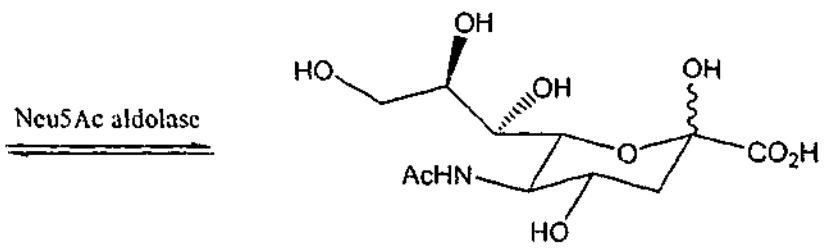

MeO
MeO
Br
O
OH
OH
CSA
12
13
14
o-NO2PhSeCN,
NaBH4; H2O2
15
PCy3
Cl
Ru
Ph
Cl
PCy3
OsO4, K3Fe(CN)6,
K2CO3
OMe
OH
HO
OH
OH
16
TsCl, Bu2Sn=O, Et3N;
Ac2O, Et3N, DMAP;
CsOAc, 18-Crown-6
OAc
17
MeOH; H2SO4;
Ac2O, Et3N, DMAP
AcO
18
RuCl3·3H2O, NaIO4;
(CH3)3SiCHN2
CO2Me
19

Scheme 1.2

1.1.2.2 Enzymatic and chemoenzymatic synthesis of sialic acids

Enzymatic synthesis of sialic acids offers a high degree of stereoselectivity without the need for protection and deprotection chemistry. However, the success of enzymatic synthesis relies on the enzyme substrate specificity. The enzymatic synthesis of the sialic acid Neu5Ac (**2**) involved the employment of Neu5Ac aldolase (*N*-acetylneuraminate pyruvate lyase, EC 4.1.3.3) to catalyse the aldol condensation between *N*-acetyl-D-mannosamine (ManNAc, **20**) and pyruvate (Scheme 1.3)[72,73]. The equilibrium can be shifted in favour of the aldol product by using an excess of pyruvate[64].



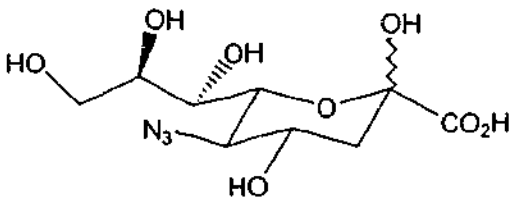


Neu5Ac (**2**)

Scheme 1.3

Chemoenzymatic synthesis of sialic acids involves chemical modifications of the substrates before and/or modification of the products after enzymatic synthesis[47-50,69,70]. For example, 5-azido-5-deoxy-KDN (**21**) was synthesized enzymatically from 2-azido-

2-deoxy-D-mannose (**22**)[45,47,71], and substrate **22** was prepared chemically from D-glucose[71].



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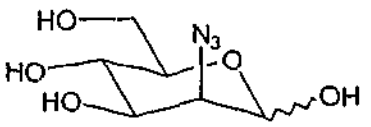
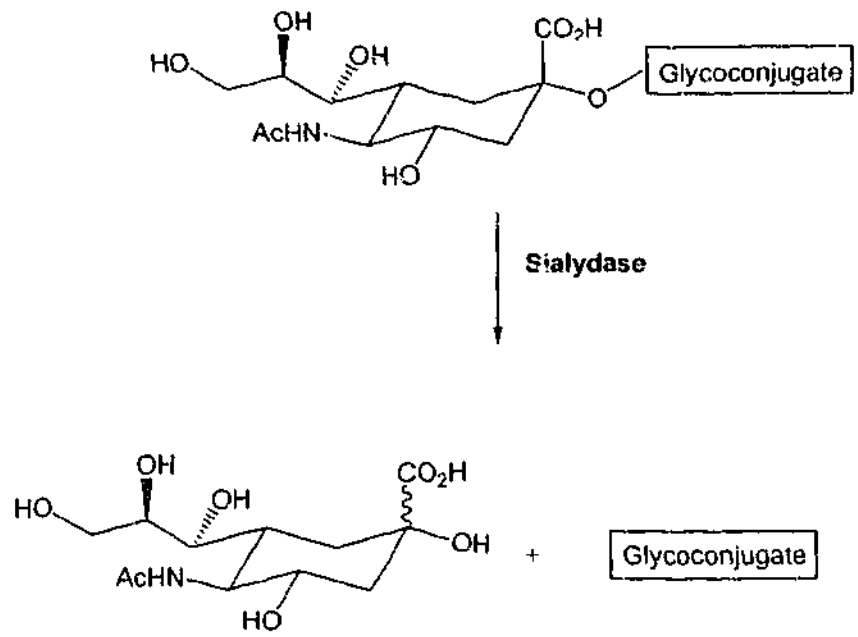


22

Certain modifications of the substrate are tolerated by the enzyme. Synthesis using C-2, C-4, C-6 modified ManNAc derivatives have been successful[65-67]. Attempts to prepare Neu5Ac derivatives using C-3 modified ManNAc were unsuccessful as these ManNAc derivatives were not accepted as substrates for Neu5Ac aldolase[65,68].

1.2 Sialidases

Sialidases are widely distributed in a diverse range of species from viruses and bacteria to mammalians[8,76,77]. They play an important role in sialic acid metabolism which involves a number of key enzymes including synthases, tranferases, sialidases and lyases[4,74]. Sialidases are responsible for the cleavage of the glycosidic linkage between the terminal sialic acid and the glycoconjugate[8,33,74] (Scheme 1.4).



Scheme 1.4 Sialidase catalyzed cleavage of an *O*-link Neu5Ac glycoconjugate.

Little is known about mammalian sialidases[8] due to their membrane-bound nature and the problem of tissue fractionation. As the key enzymes of sialic acid catabolism, sialidases are involved in a number of biological activities including antigenic expression, protein degradation, receptor recognition and masking[4,78]. Viral sialidases are considered to play a key role in viral infection. They may facilitate the transport of viral particles to and from the site of infection and assist the elution of progeny viruses from the host cells[74]. In bacteria, sialidases not only play a pivotal role in pathogenesis, they may also have a nutritional function as well[4].

Sialidase substrate specificities range from relatively non-selective enzymes to those cleaving particular glycosidic linkages[8,74]. With the exception of the β-glycosidic CMP-Neu5Ac, naturally occurring sialic acids are found in the α-glycosidic configuration. It is this α-configuration that is recognised by most sialidases[8]. CMP-Neu5Ac, thus, was not a substrate for sialidases.

Sialidases are intrinsic membrane glycoproteins of influenza A and B viruses. Influenza B virus contains a single type of sialidase[79]. Influenza A virus sialidase is classified into nine subtypes (N1-N9). The three-dimensional structure of some viral sialidases have been studied, providing vital information about the catalytic site of the enzymes[80-83]. Influenza virus sialidase is a tetramer of four identical subunits. Each monomer comprises six four-stranded antiparallel β-sheets arranged as if on the blades of a propeller (Figure 1.1)[9]. The catalytic site is located close to the centre of a sixfold pseudo-symmetry axis, which passes through the centre of the monomer and relates the six β-sheets to each other.

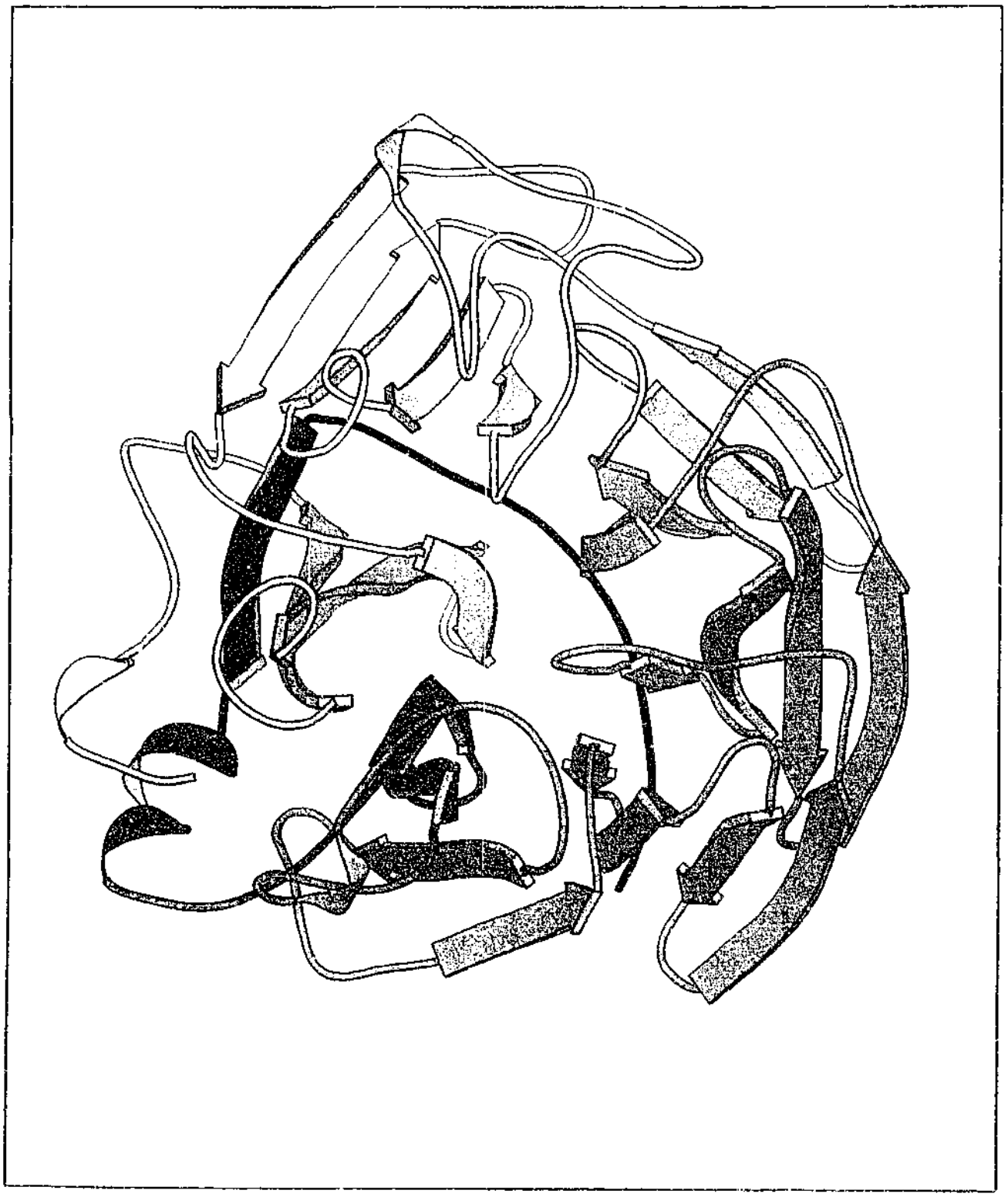
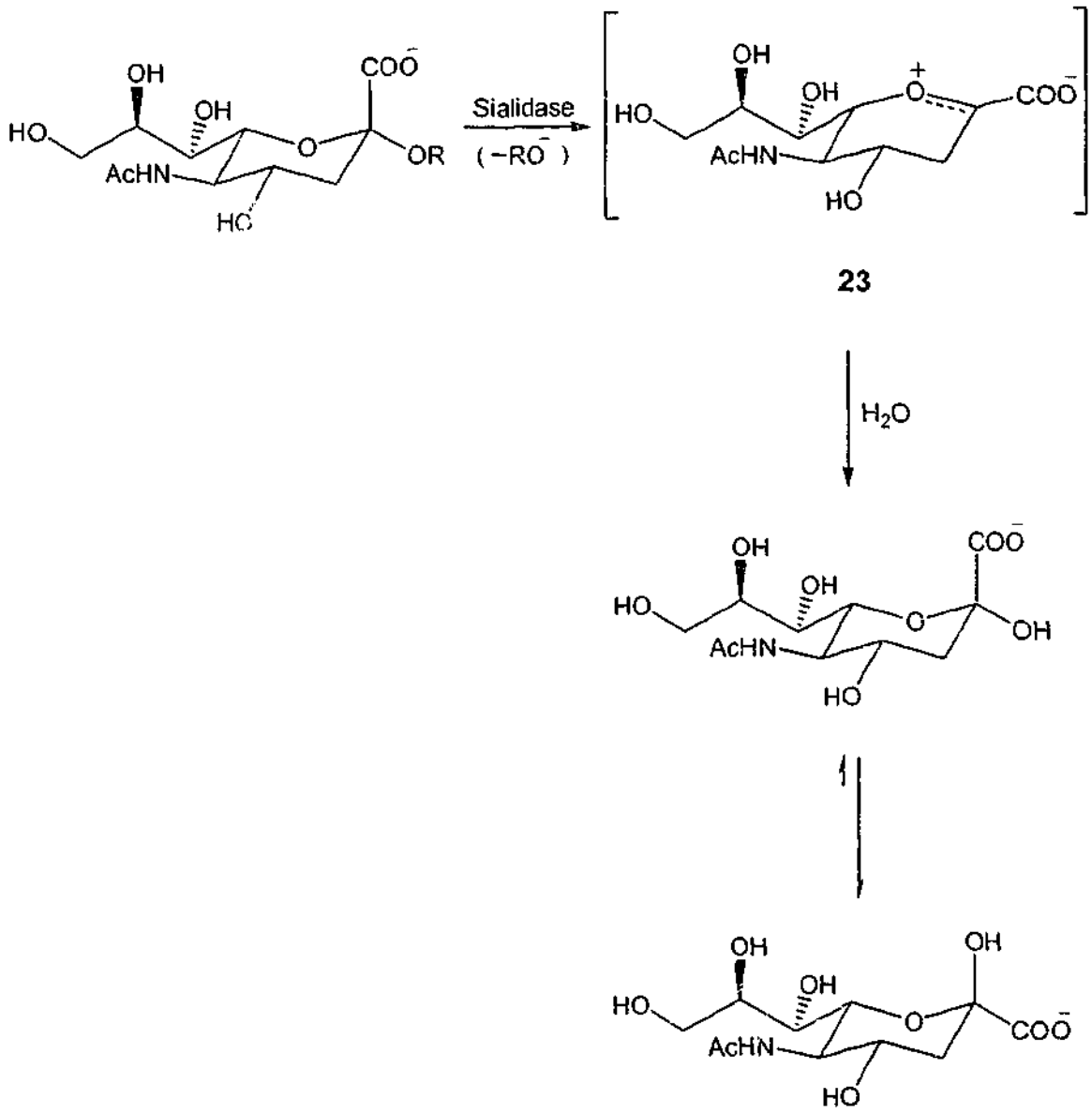


Figure 1.1 Molscript[86] diagram showing the secondary structure of influenza virus sialidase monomer.

Studies on the action of influenza virus sialidase[84,85,89,90] indicated that the catalysis reaction proceeds via the oxocarbonium intermediate **23**. The proposed transition state for the influenza virus sialidase mechanism involved in the cleavage of terminal *N*-acetylneuraminic acid residues (Scheme 1.5).



Scheme 1.5 Proposed transition state for the sialidase mechanism.

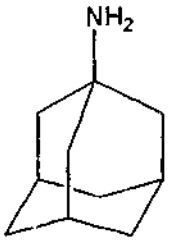
1.3 Sialylmimetics as inhibitors of influenza virus sialidase.

1.3.1 Prelude

Influenza virus is the major cause of acute respiratory illness in terms of morbidity and mortality. Influenza is considered to be responsible for the deaths of

about 20 million people in 1918-1919. Since then, fortunately, less serious pandemics have occurred and are the result of introduction of a new viral subtype as a consequence of the ability of the virus to modify its surface antigens[92]. The symptoms of influenza include fever at the onset, usually associated with severe headache, cough, myalgia, sore throat and malaise.

Prior to the advent of the new range of sialidase-inhibiting anti-influenza compounds, treatments of influenza relied on amantadine (**24**) and its analogue rimantadine (**25**), which have severe limitations. These compounds are ineffective against influenza virus B, cause adverse side effects and prompt the rapid emergence of mutated virus resistant to the compounds[95]. Vaccine strategies, which involves employing a cocktail of several attenuated forms of the virus, is limited by the highly variable mutation of the virus[93,94].



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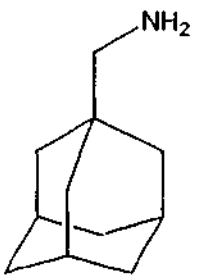

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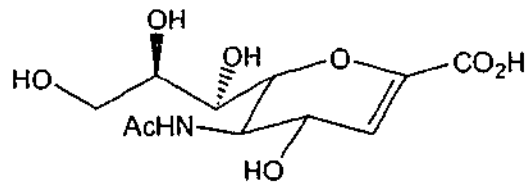
Over recent years, there has been a trend to design compounds that are mimics of carbohydrates, especially with regard to recognition events in biological systems. The term carbohydrate mimetic can be considered as any carbohydrate derivative or other compound that has structural and functional features essential for interaction with a given biomolecule[12,97-99].

A significant amount of effort has been devoted to the development of sialyl mimetics as inhibitors of influenza virus sialidase[9,99]. Influenza virus sialidase, a glycohydrolase, catalyzes the cleavage of sialic acids from the host-cell surface glycoproteins, promoting the release of aggregated viral progeny from the infected cell[81,82]. The ability to interfere with the active site of this enzyme should hinder the proliferation of the virus thus enabling the host's immune system to target the virus more effectively.

1.3.2 Sialic acid-based mimetics

One of the earliest identified sialidase inhibitors was 2-deoxy-2,3-didehydro-D-*N*-acetylneuraminic acid (Neu5Ac2en) (**26**), which exhibited a potent inhibition[87,104]against influenza virus sialidase (K_i = 4x10^{-6} M). Biochemical studies[88] suggested that Neu5Ac2en is a transition state analogue binding to the active site of influenza sialidase[89,91]. Neu5Ac2en, however, showed nonselective inhibition against viral, bacterial and mammalian sialidases and was initially found not effective *in vivo*[96].



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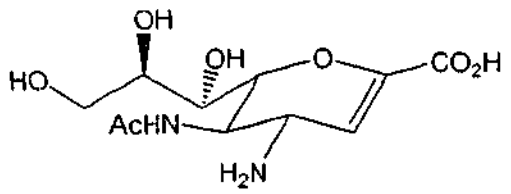
Structurally modified derivatives of Neu5Ac2en (**26**) provide the possibility of achieving better level of inhibition than Neu5Ac2en itself with selectivity for a particular sialidase. Successful Neu5Ac2en-based sialidase inhibitors[9] require the double bond in the ring between C-2 and C-3, and the following functional groups: the

carboxyl group at C-2, a positively charged group at C-4, the *N*-acetyl group at C-5, and the glycerol side chain at C-6.

The introduction of an amino group in place of the hydroxy group at C-4 of **26** gave 4-amino-4-deoxy-Neu5Ac2en (**27**)[11] and resulted in an approximately two orders of magnitude better inhibition of influenza virus sialidase than Neu5Ac2en. Zanamavir or the 4-guanidino-substituted Neu5Ac2en (**28**)[11] is an even more potent influenza virus sialidase inhibitor with an inhibition constant (K_i) of $2x10^{-10}$ M. Zanamavir (**28**), marketed as Relenza™, is delivered directly to the lungs, the major source of influenza infection, by an inhaler. **28** is the first anti-influenza drug that was designed based on the proposed transition state in the mechanism of action of influenza virus sialidase.



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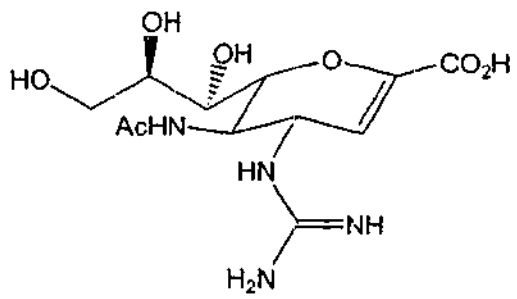

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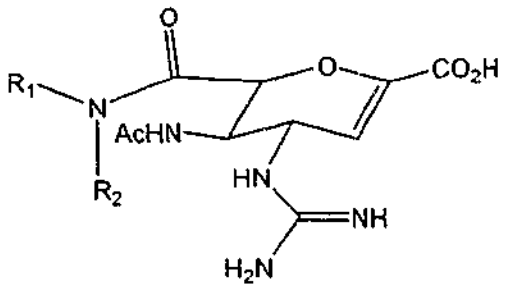
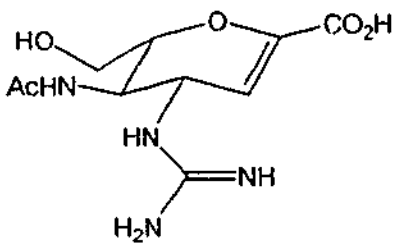
A number of Zanamavir analogues have been synthesized and tested for their influenza virus sialidase inhibitory potential. Compounds such as the carboxamide derivatives (**29**)[100], the truncated derivative (**30**)[101], the guanidino modified derivatives (**31**)[92] and the 5-trifluoroacetamide derivative (**32**)[102] have been prepared. All these analogues of Zanamavir failed to improve on the inhibition of influenza virus sialidase.



29 R_1/R_2 = alkyl, aryl



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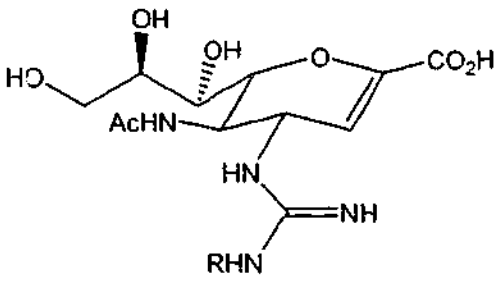
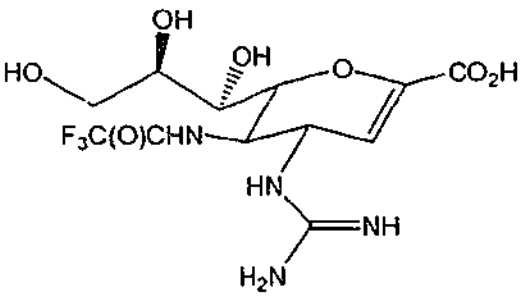


31 R = Me, OH, NH_2, CO_2Et



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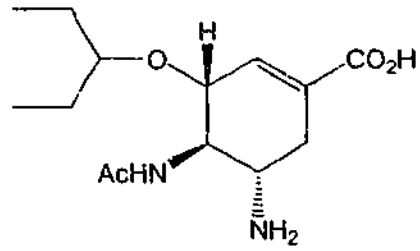
1.3.3 Cyclohexene-based mimetics

The search for anti-influenza agents with improved physiochemical properties has led to the development of non-carbohydrate compounds based on the simple cyclohexene template that mimics the ring conformation of the transition state analogue **23** (Scheme 1.4). There has been a significant number of cyclohexene-based derivatives reported in the literature[10,54-58,92,103] with inhibition data of both influenza virus A and B sialidase. In 1997, Kim *et al.*[10] reported the design and synthesis of 4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid (**33**), which showed inhibition of influenza virus sialidase comparable to Zanamavir (**28**). Compound **33** is currently marketed in the form of its orally active ethyl ester prodrug as Tamiflu™.



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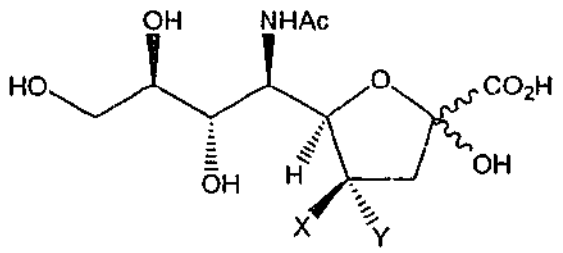
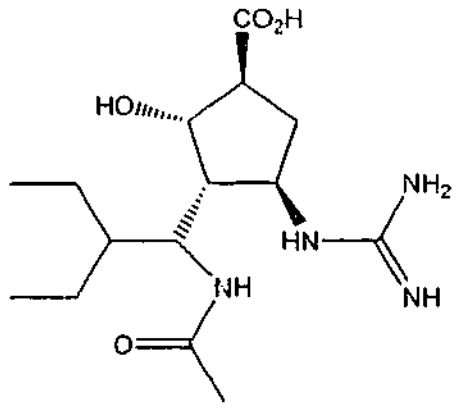
1.3.4 Five-membered ring-based mimetics

In 1992, Yamamoto *et al.*[106] reported five-membered ring sialidase inhibitors, **34** and **35**, based on a substituted furanose derivative (*vide infra*). These compounds were shown to possess comparable influenza virus sialidase inhibitory activity to Neu5Ac2en (**26**). Recently, Peramivir, the five-membered carbocyclic compound (**36**)[107] has been reported to exhibit exellent sialidase activity that is comparable to both Zanamavir (**28**) and **33** against a number of influenza virus strains. **36** was also shown to have very good *in vivo* inhibitory activity in a mouse model[108] and had undergone clinical trials. However, findings from the Phase III clinical trials revealed no statistically significant difference in the primary efficacy end point between groups treated with Peramivir and groups treated with placebo. The development of Peramivir for the treatment of influenza, therefore, was discontinued[109].



34 (X = H, Y = OH)
35 (X = OH, Y = H)



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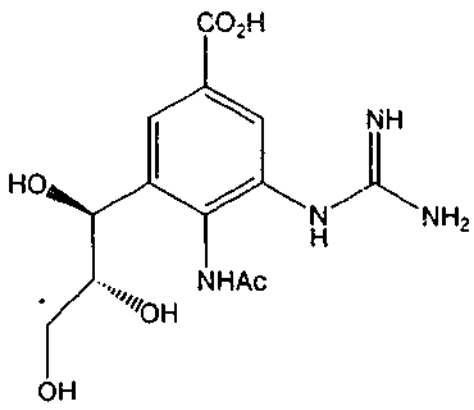
1.3.5 Benzoic acid-based mimetics.

Benzoic acid has also been used as the template for mimetics of Neu5Ac2en-based sialidase inhibitors. Several factors were considered in the choice of the benzoic acid template, including the simple chemistry involved and the bioavailability of compounds that may arise from the benzoic acid[9]. No inhibition was observed for the benzoic acid-based compound **37**[110]. However, compound **38**, without the glycerol side chain present in its structure, exhibited inhibition equivalent to that observed for Neu5Ac2en (**26**)[115]. No compounds from this family of inhibitors were considered for clinical trials.



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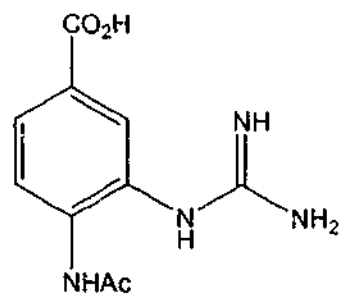

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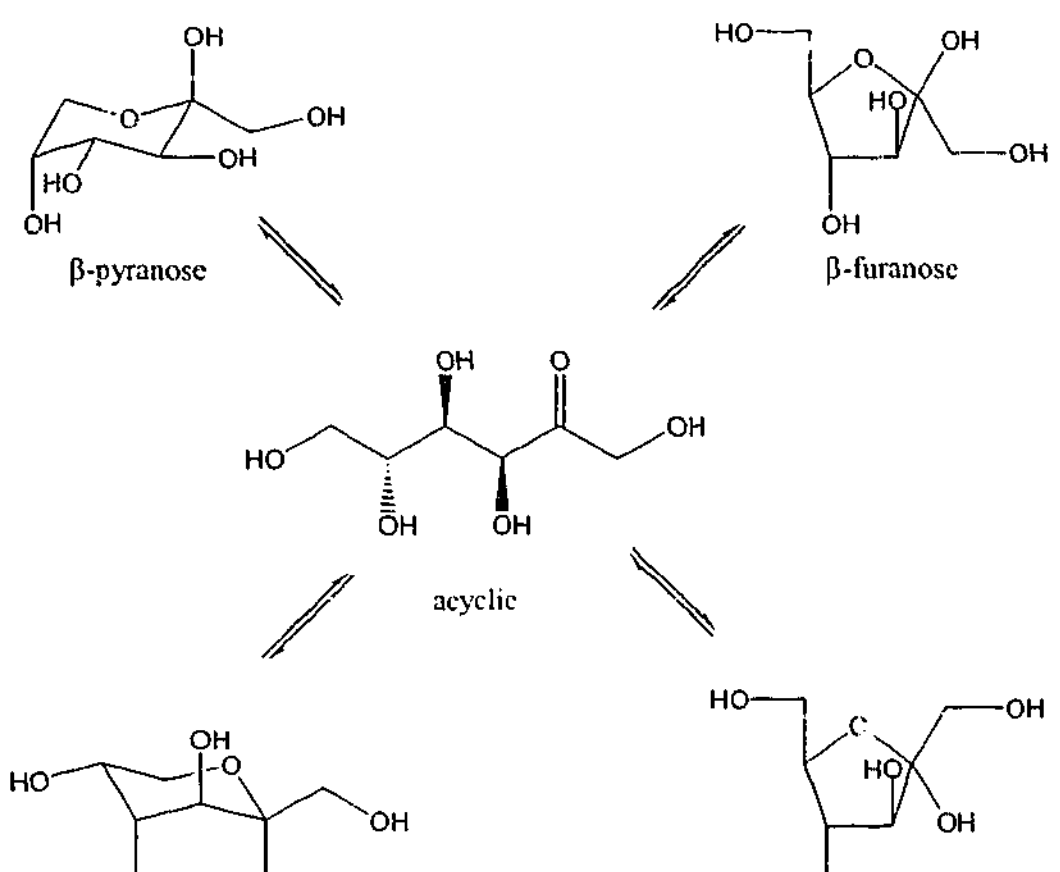
1.4 Fructose

Some preliminary molecular modelling studies indicated that fructose in its furanose form may provide an appropriate framework for Neu5Ac2en mimetics[167].

The name fructose derives from the Latin word *fructus*, which means fruit. Having a sweetness eight times that of sucrose[111], D-Fructose, originally known as fruit sugar, occurs abundantly in nature. In the free state, it is found in fruits and honey[112-114] (34.8% by weight in honey). In combination, D-Fructose is present, invariably as D-fructofuranose, in the disaccharide sucrose[139], in oligosaccharides[116] such as melezitose, gentianose, raffinose, stachyose, verbascose, and in many polysaccharides[117]. L-Fructose does not occur naturally[116].

As a ketose monosaccharide, D-Fructose crytallizes in the β-pyranoid form[118]. In solution, D-Fructose is present mainly in four tautomers (Scheme 1.6): α-pyranose, β-pyranose, α-furanose, and β-furanose. The composition of the tautomers depends on the solvent and the temperature[119,120] (Table 1). The acyclic keto-form is negligible in solution.



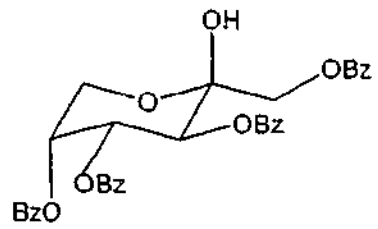
Scheme 1.6

Table 1.1 Equilibrium composition in % of D-fructose tautomers in water, dimethyl sulfoxide and pyridine[120]

Solvent	Temperature (°C)	β-pyranose	α-pyranose	β-furanose	α-furanose
Water	0	80	2	15	3
	25	73	2	20	5
	50	64	3	25	8
	70	56	4	30	10
Me_2SO	20	32	3	46	19
	25	27	4	48	20
	50	21	4	51	24
Pyridine	0	60	4	27	9
	20	54	5	30	11
	60	42	6	36	15

Simple derivatizations of D-Fructose such as glycosidations, acylations and alkylations usually yield mixtures of tautomers. The outcome of these derivatizations[121] are determined by the reaction parameters such as the composition of tautomers during the reaction and the different hydroxy group reactivities within the various tautomeric forms and the steric bulk of the reagents. An exemplary derivatization is the well studied benzoylation of D-Fructose in pyridine. The gradual addition of D-fructose to a cooled (-10 °C) mixture of pyridine, chloroform and benzoyl chloride followed by warming to ambient temperature resulted in the formation[122] of the β-pyranoid

tetrabenzoate (**39**) in an isolable yield of 50%. When the benzoylation is performed at ambient temperature, a mixture of products[123], the β-pyranoid tetrabenzoate **39**, the acyclic pentabenzoate **40**, and the α-furanoid tetrabenzoate **41**, is formed in yields of up to 20% only. The benzoylation at 60-70 C° gives an entirely different product composition[124]. The major component, tetrabenzoate **41** is isolated in yields of up to 60% with the pentabenzoate **42** and the acyclic **40** as minor products. If D-fructose in dichloromethane-pyridine (7:1) is exposed to benzoyl chloride at ambient temperature the α-furanoid tetrabenzoate **41** is obtained in 70% yield, whereas the same reaction in 1:1 dichloromethane-pyridine affords 80% of the α-furanoid pentabenzoate **42**[125].



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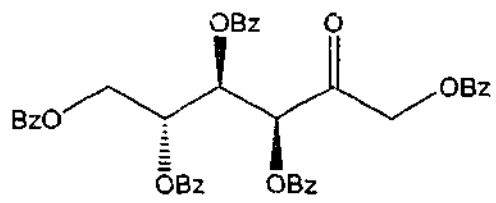

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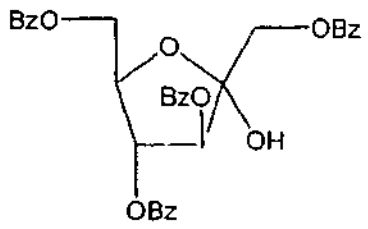

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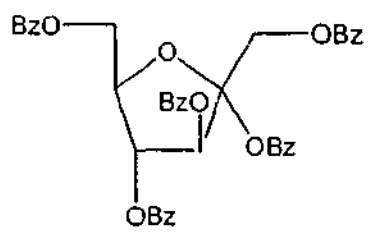


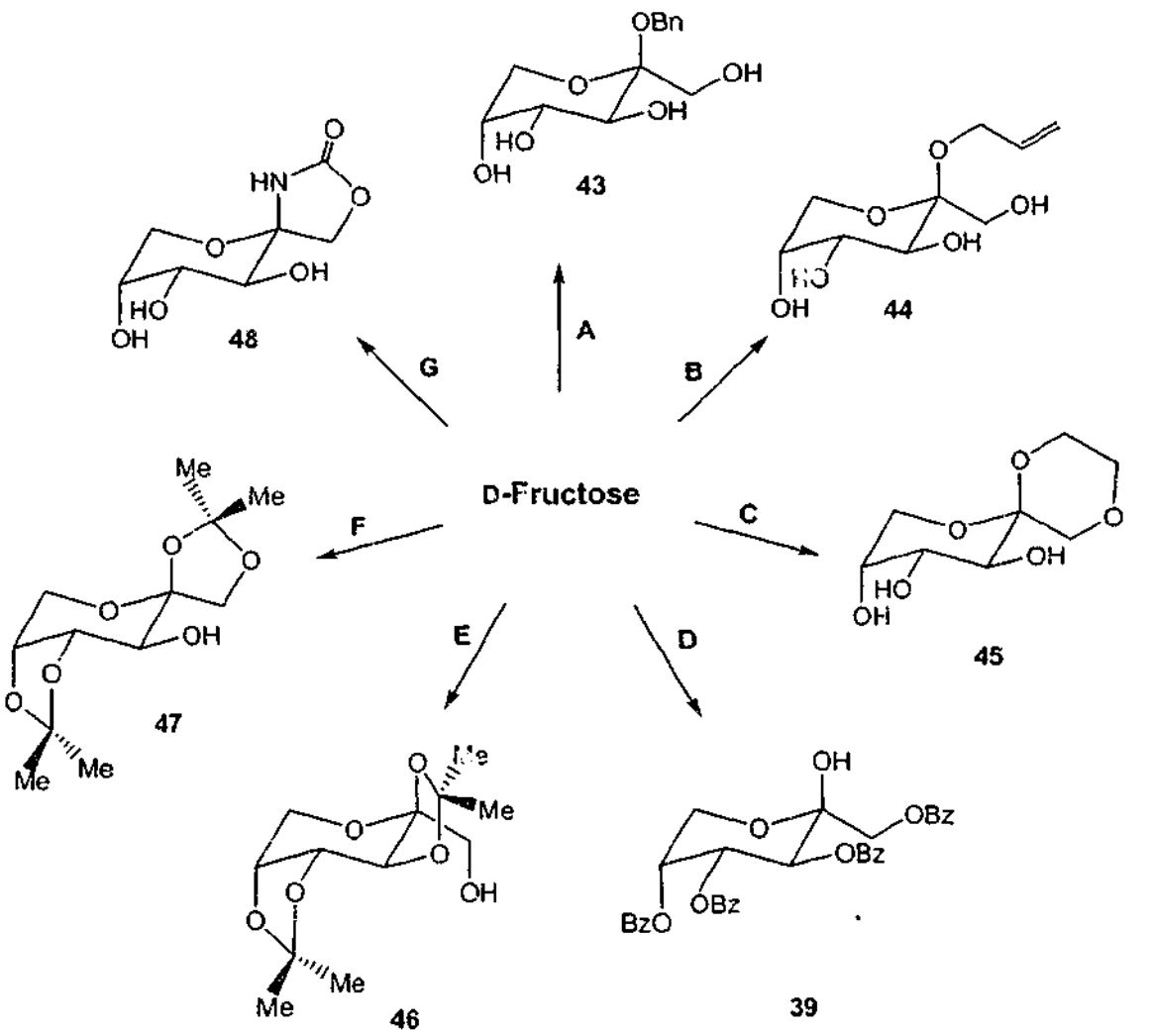
42

As demonstrated in the benzoylation of D-fructose, it is vitally important in utilising the entry reactions to meet preparative criteria for exploiting the chemistry of fructose towards products of a certain template.

1.4.1 Pyranoid fructose derivatives

Scheme 1.7 compiles entry reactions that provide a variety of pyranoid fructose derivatives. The glycosidation of D-fructose using benzyl alcohol/HCl provides the

benzyl β-D-fructopyranoside **43**, isolated by crystallization, in 30% yield[126]. Due to the ease of isolation, **43** has been used as starting material for the preparation of various 4-aminohexuloses[127,128]. Of significant importance are the two diacetone-fructopyranoses **46**[129,130] and **47**[131], as only one hydroxyl group is unprotected, hence, available for further modifications. Most notably, the *spiro*-glycoside **45**[132] and the tetrabenzoate **39**[122] can be prepared in high yields.

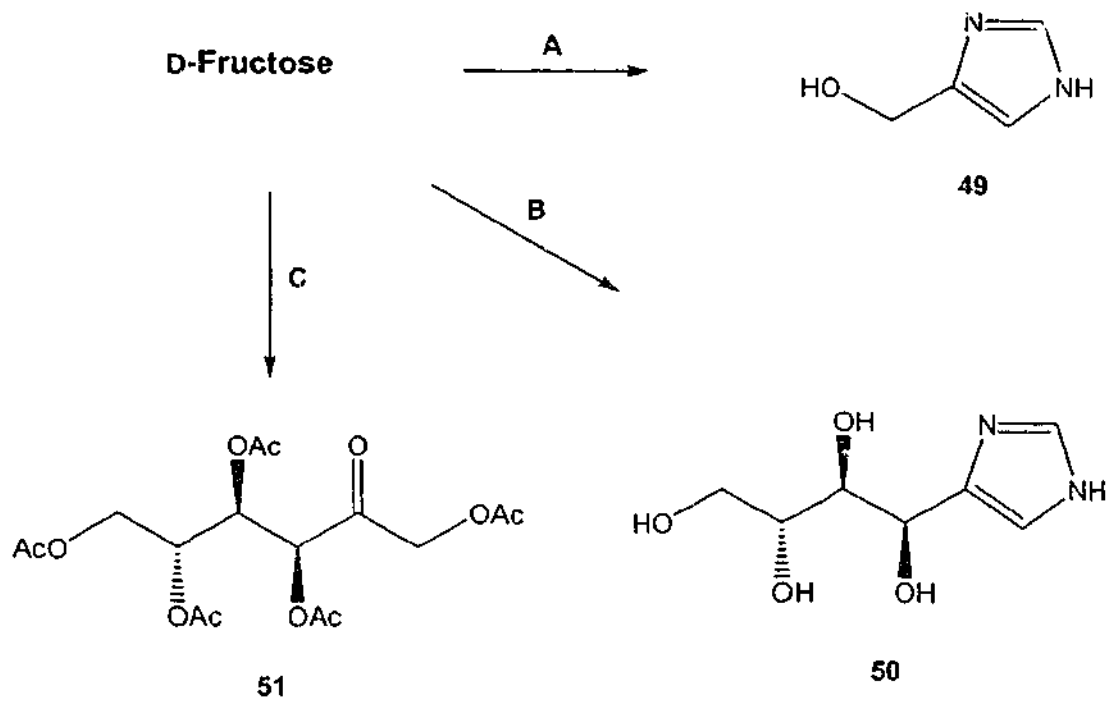


A BnOH / HCl, 30% [ref. 126]
B allyl alcohol / AcCl, 49% [ref. 133]
C 2-chloroethanol / HCl, then NaOMe / MeOH, 74% [ref. 132]
D BzCl / pyridine, -10°C, 80% [ref. 122]
E acetone / 5% H_2SO_4, 80% [ref. 129, 130]
F acetone / cat. H_2SO_4, 45% [ref. 131]
G KOCN, buffer, 31% [ref. 123]

Scheme 1.7 Entry reactions from D-fructose to derivatives in pyranoid form.

1.4.2 Acyclic derivatives of D-fructose

The few entry reactions from fructose to acyclic derivatives are compiled in Scheme 1.8. Cu(II)-promoted reaction of D-fructose with formaldehyde and ammonia provides the 4-hydroxymethylimidazole **49** in 60% yield[162]. Treating D-fructose with formamidinium acetate in liquid ammonia at 75°C in a pressure bottle gives **50,** which can be isolated from the resulting mixture with **49,** in 38% yield[163]. The pentaacetate **51** crystallizes on work up of the acetic anhydride / $ZnCl_2$ acylation of D-fructose[164].

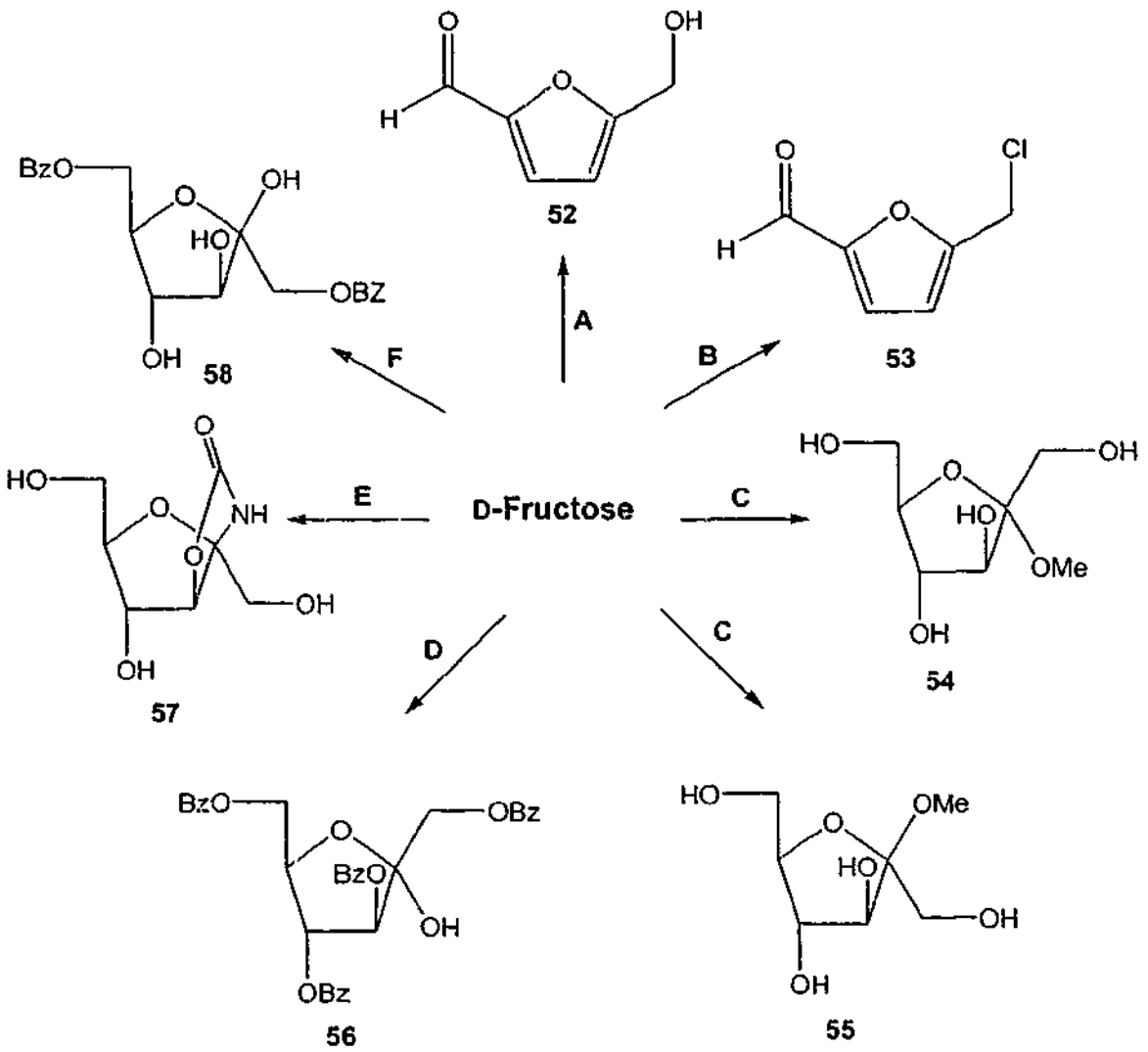


A CH_2O / aq. NH_3 / $CuCO_3{\cdot}Cu(OH)_2$, 2h, 100°C, 60% [ref. 162]
B $H_2NCH{=}NH{\cdot}HOAc$ / liq. NH_3, reflux, 38% [ref. 163]
C Ac_2O / $ZnCl_2$, 5h, 0°C, 2h, 50°C, 48% [ref. 164]

Scheme 1.8 Acyclic derivatives of D-fructose

1.4.3 Furanoid fructose derivatives

The entry reactions that provide simple fructose derivatives fixed in furanoid form are compiled in Scheme 1.9. Of these furanoid products, 5-hydroxy-methylfurfural (HMF) (**52**) is of high industrial potential[134,161], readily accessible by acid-induced elimination of three molecules of water[135] from D-fructose. HMF has been used to prepare a wide range of products[140-145] as intermediate chemicals, with the potential for the manufacture of useful industrial materials, such as polyesters[146-149] and polyamides[150-152]. The acid-catalyzed methanolysis of D-fructose, which leads to an anomeric mixture of methyl furanosides and pyranosides, has been significantly investigated[153-160,169]. The composition of the fructoside mixture depends on the reaction parameters, particularly the amount of acid used. In 1973, Bethell and Ferrier[158] conducted methanolysis of D-fructose using a ^{14}C-based analytical method. Exposure of fructose to 0.1% methanolic HCl generated β-frutopyranoside as the major product, whereas for the reaction using 0.01% methanolic HCl, the anomeric methyl fructofuranosides were the predominant major components in the fructoside mixture.

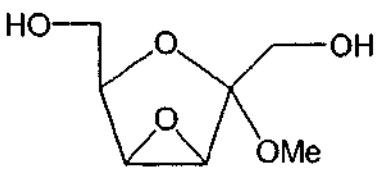


A H^+, 90% [ref 134,135]
B HCl / toluene, 60-85° C, 65% [ref. 136]
C MeOH / HCl, **54** : 43%, **55** : 41% [ref. 180]
D BzCl / pyridine, 60° C, 60% [ref. 137]
E KOCN / buffer, 32% [ref. 123]
F HCN / NH_3, then BzCl / pyridine, finally Ag_2CO_3 / EtOAc / reflux, 60% [ref. 138]

Scheme 1.9 Entry reactions from D-fructose to derivatives in furanoid form.

A considerable amount of effort has been devoted to the modification of fructofuranosides, particularly that of methyl α- and β-D-fructofuranosides[125,160,165-168,180,197]. In 1980, Guthrie *et al.*[176] reported the preparation of methyl 3,4-anhydro-α- and β-D-tagatofuranosides (**59** and **60**) by treatment methyl α- and β-D-fructofuranosides (**54** and **55**), respectively, with triphenylphosphine and diethyl azodicarboxylate in DMF. The oxirans **59** and **60** are potential intermediates for the

synthesis of 4-modified fructofuranose derivatives since the opening of the epoxide ring of **59** and of **60** by nucleophiles occurs favourably[173,174] at C-4 (*vide infra*).



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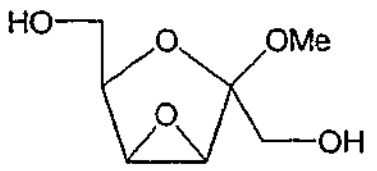
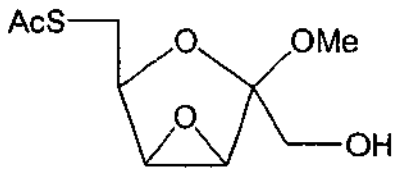


60

The Mitsunobu reaction (*vide infra*) has been employed to achieve fructose derivative products with high regioselectivity. Thus, 6-thio compound **61** was prepared[178] from methyl 3,4-anhydro-β-D-tagatofuranosides (**60**) without protection of the 1-hydroxyl group.



61

1.5 Thesis overview

The aim of this project was to investigate the synthesis of five-membered ring sialyl mimetics as biological probes of sialidases. D-Fructose, an inexpensive sugar, providing readily accessible furanoid compounds, was utilised as starting material in the synthesis of novel furanose analogues. These compounds were achieved through manipulation and functionalization of the methyl fructofuranosides **54** and **55**. Chapter Two discusses the preparation of a series of functionalized fructofuranose derivatives via protection, deprotection and Mitsunobu chemistry. Chapter Three describes the synthesis of C-6 modified fructofuranosidonic acids and derivatives. The deprotected

compounds were evaluated with respect to biological activity against influenza sialidase.

It was also our intention to investigate the new synthesis of the known furanose-based sialidase inhibitors[106] **34** and **35,** starting from diacetone-D-glucose, with a view to improving the overall yields (Chapter Four). Synthesis of analogues of **34** and **35** was also investigated. The final chapter, Chapter Five, depicts full experimental details of the compounds made throughout Chapters Two to Four.

CHAPTER 2

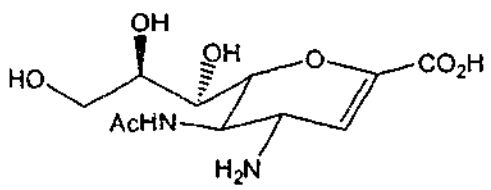
Synthesis of Fructofuranosidonic Ester Derivatives from D-Fructose

2.1 Introduction

As mentioned in chapter 1 (*vide supra*)[167], the furanose form of fructose may provide the appropriate template for the development of sialyl mimetics. The aim of this work was to design and synthesize fructofuranoside ester analogues which incorporate the essential features of potent sialidase inhibitors[9,11,99].

In choosing the target compounds, it is appropriate to take into consideration the spatial relationship of various functionalities of the furanose to that of the Neu5Ac2en template. As the carboxylic acid functionality of fructofuranosidonic acids on C-2 of the furanose template is analogous to that on C-2 of the Neu5Ac2en template, C-3, C-4 and C-5 of the furanose template are considered to correspond to C-3, C-4 and C-5 of the six-membered ring counterpart, respectively.

One important functionality is the amino group, the presence of which in 4-amino-4-deoxy-Neu5Ac2en (**27**) results in a hundred-fold decrease in the K_i value[104] from $4x10^{-6}$ M for Neu5Ac2en (**26**) to $4x10^{-8}$ M for 4-amino-4-deoxy-Neu5Ac2en (**27**).



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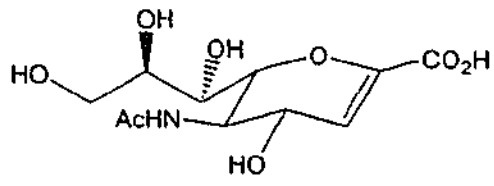
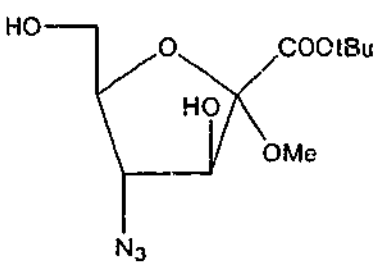
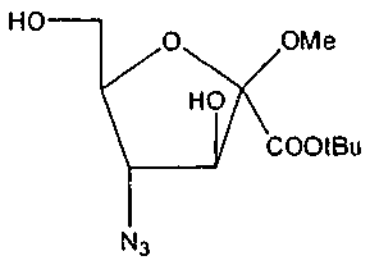


26

The incorporation of an amino group into fructofuranoside esters, therefore, would be of great interest to us and C-4 was the position of choice. Thus, in our work, we chose the 4-azido-4-deoxy-fructofuranoside esters **62** and **63** as our immediate target compounds. The azido group, a masked amino functionality, would be reduced to the amino group at some appropriate stage of the synthetic sequences of the final target compounds.

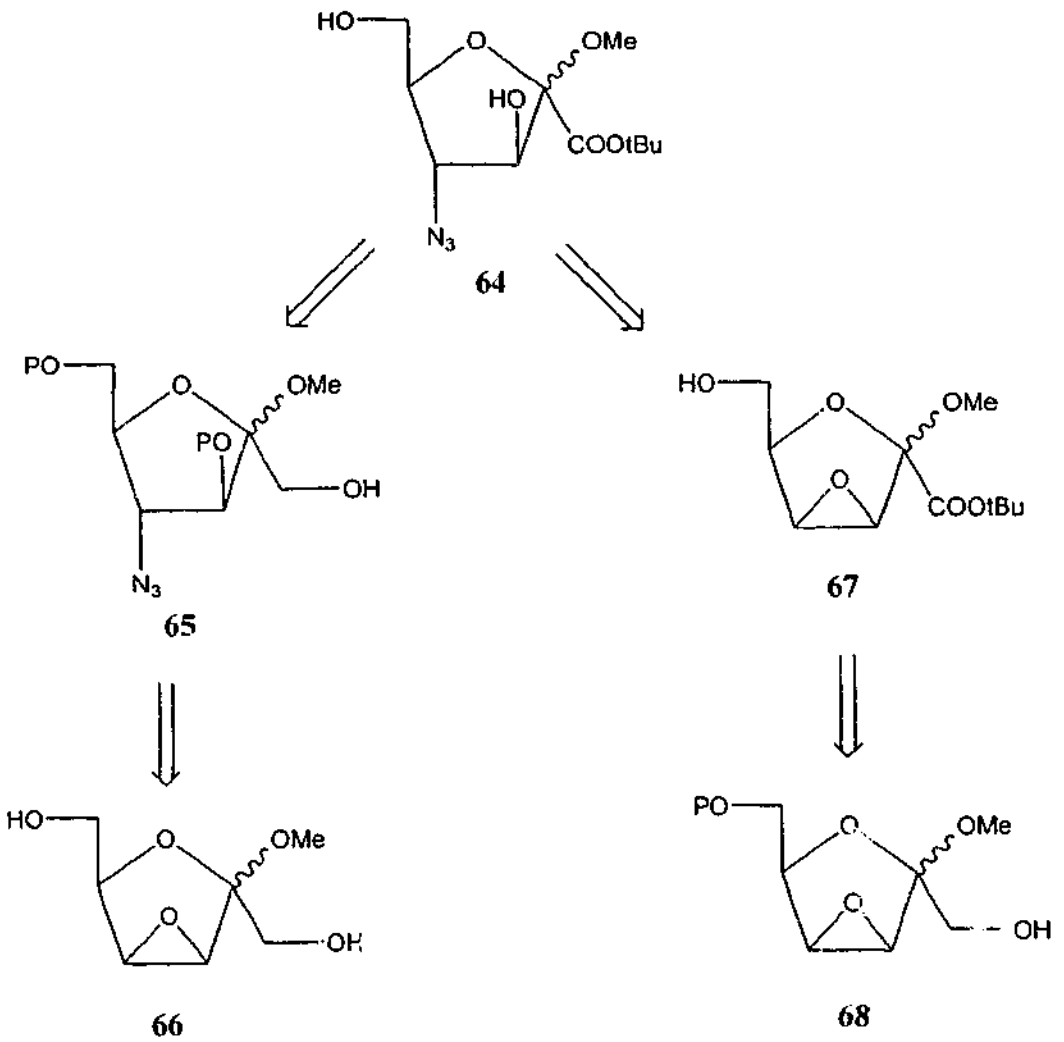


62



63

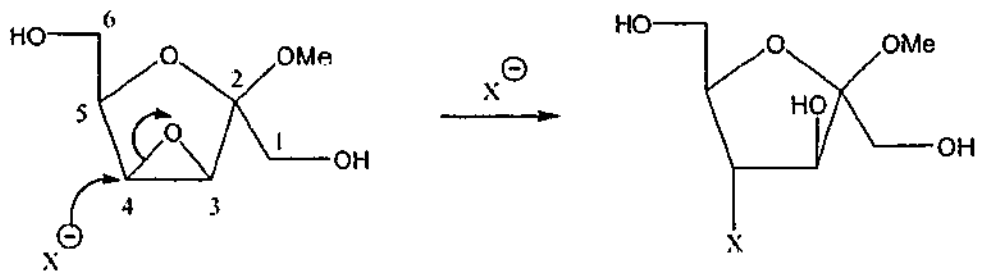
Two possible pathways were envisaged towards the synthesis of these esters, as depicted in retrosynthetic terms in Scheme 2.1A. The first involved epoxide-ring opening of the oxiran **66** to insert the azido group, subsequent protection and deprotection of the hydroxyl groups would expose the 1-hydroxyl group for oxidation to give **64**. In the second approach the introduction of the azido functionality was carried out after the oxidation step. Since epoxides are susceptible to oxidative cleavage under various oxidation conditions[222-227], the first pathway was the approach of choice to prepare 4-azido-fructofuranoside esters.



P = Protecting Group

Scheme 2.1A

The epoxide-ring opening of methyl 3,4-anhydro-α- and β-tagatofuranosides (**59** and **60**) is expected to proceed selectively[173,174] at C-4 (Scheme 2.1B). The analogous methyl 2,3-anhydro-β-D-pentofuranosides react almost exclusively[255] at C-3 (the analogous position to C-4 in **59** and **60**). The neopentyl-type nature of C-3 is an additional factor that would be expected to drive the opening towards C-4. Guthrie *et al.*[173] have successfully carried out epoxide-ring opening of **60** employing either basic or acidic reagents. Martin *et al.*[174], in 1989, reported the use of sodium phenyl selenide to prepare 4-seleno-D-fructose derivatives from α- and β- forms of 3,4-anhydro furanosides.



60 **69**

Scheme 2.1B

The introduction of a functional group at C-4 to form a product such as **69** reveals the need for protecting group manipulations before functionalisation at a certain position could be carried out.

Mitsunobu chemistry has been extensively studied and employed as a versatile and powerful tool in organic synthesis, particularly in carbohydrate chemistry, and a variety of products have been achieved under conditions which are known to be mild and selective[173,177,185-189,207-214]. Mitsunobu conditions were used by Jenkins *et al.*[177] to perform regioselective thioacetylation of the fructose unit of sucrose in the presence of three secondary hydroxy groups. Norton and von Itzstein[171] have successfully used Mitsunobu-like conditions to selectively activate the 6-hydroxy group of **60** in the presence of an oxiran. Methyl 1-*O*-acetyl-6-*S*-acetyl-3,4-anhydro-6-thio-β-D-tagatofuranoside (**70**) was obtained in 56% yield after acetylation of the product of this regioselective thioacetylation reaction.

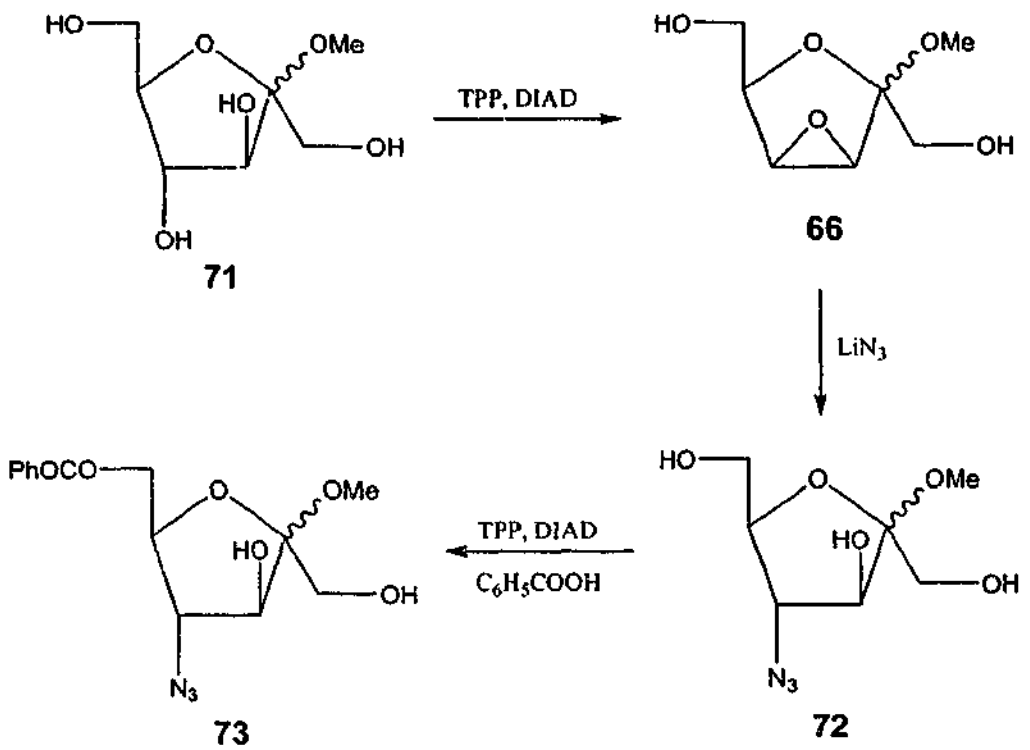
60 **70**

Reagents and conditions: (i) $P(Ph)_2C_6H_4$-*p*-NMe_2, DIAD, CH_3COSH, THF; (ii) Ac_2O, pyridine.

2.2 Synthesis of 4-azido-4-deoxy-6-*O*-benzoyl-fructofuranosides

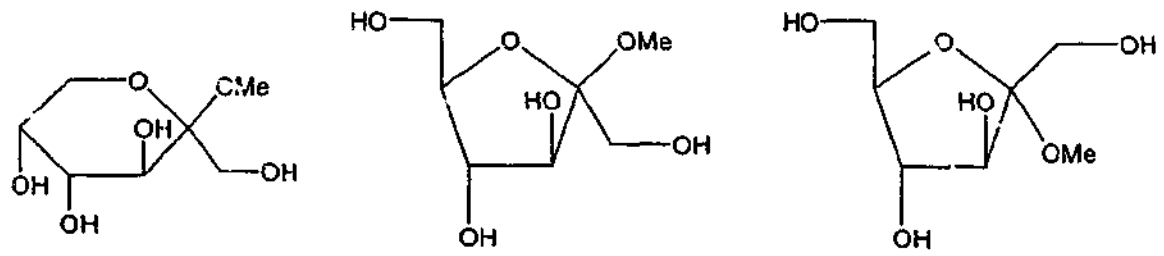
Our initial objective was to prepare fructofuranosides with an azido group, a masked amino functionality, at C-4 and a hydroxyl protecting group on C-6 to provide the intermediate derivatives for further manipulations.

The synthesis of methyl 4-azido-4-deoxy-6-*O*-benzoyl-fructofuranoside **73** was envisioned to involve the preparation of the 4-azido sugars **72** by epoxide-ring opening of the anhydro sugar **66** using lithium azide. Subsequent selective protection of the 6-hydroxyl group as a benzoate empioying Mitsunobu conditions would then give the desired fructofuranoside **73** (Scheme 2-2).

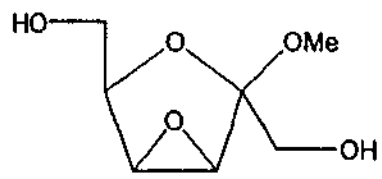


Scheme 2.2

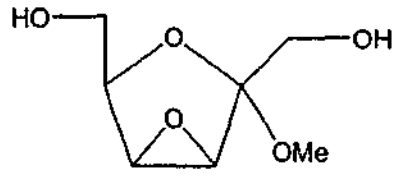
Methyl fructosides[175] were prepared from the treatment of D-fructose with methanol and acidic resin. Purification of the fructoside mixture through a column of basic resin afforded methyl β-D-fructopyranoside (**74**), methyl β-D-fructofuranoside (**55**) and methyl α-D-fructofuranoside (**54**) in yields of 9%, 48% and 38% respectively. The ^{13}C NMR of each glycoside was consistent with that reported in the literature[179].



Treatment of methyl β-D-fructofuranoside (**55**) with TPP and DIAD in DMF under conditions similar to those reported by Guthrie and co-workers[176] gave methyl 3,4-anhydro-β-D-tagatofuranoside (**60**) in 89% yield. Likewise, methyl 3,4-anhydro-α-D-tagatofuranoside (**59**) was prepared in high yield from methyl α-D-fructofuranoside (**54**) using the same method. ^{13}C NMR spectra of the anhydro sugars **60** and **59** were assigned by comparison with the literature[176].

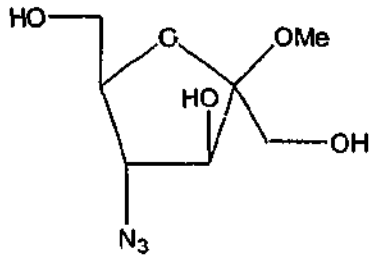


60



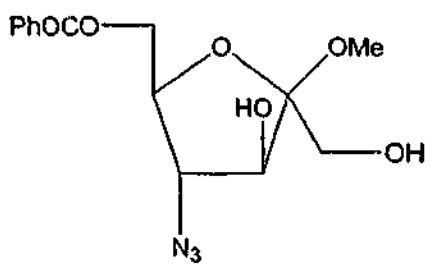
59

Reaction of methyl 3,4-anhydro-β-D-tagatofuranoside (**60**) with lithium azide in DMF in the presence of acidic resin at ca. 100°C gave methyl 4-azido-4-deoxy-β-D-fructofuranoside (**75**), after purification, in 68% yield. It is worth noting that without the use of acidic resin the reaction of **60** with lithium azide was very sluggish, often not proceeding to completion. That the epoxide-ring opening proceeds favourbly[173,174] at C-4 and the presence of a strong infra-red absorption at 2110 cm^{-1} confirmed the introduction of an azido group at C-4. The ^{1}H NMR spectrum revealed a downfield shift for H-3 from δ 3.75 ppm in **60** to δ 4.19 ppm in **75** resulting from the deshielding effect of the hydroxy group on H-3. Further evidence for the formation of **75** was obtained from the ^{13}C NMR spectrum. The downfield shifts of C-3 and C-4 from below δ 58 ppm in **60** to δ 76.5 and 66.5 ppm, respectively, in **75** were consistent with the presence of a hydroxyl at C-3 and an azido functionality at C-4. Electrospray mass spectrum recorded a molecular ion plus sodium at *m/z* 242.



75

Treatment of **75** with benzoic acid under Mitsunobu conditions (TPP-DIAD in DMF) resulted in the formation of the regioselective product methyl 4-azido-6-*O*-benzoyl-4-deoxy-β-D-fructofuranoside (**76**). The infra-red spectrum exhibited a strong absorption at 1736 cm^{-1} indicative of a carbonyl functionality. The ^{1}H NMR spectrum showed the presence of five aryl protons with resonances of three multiplets at δ 7.44, 7.57 and 8.07 ppm. The ^{13}C NMR also supported the incorporation of a benzoate group by the presence of four signals for the aromatic carbons at δ 128.4-133.3 ppm and a carbonyl resonance at δ 166.2 ppm. The ESI high resolution mass spectrum showed a molecular ion plus $^{+}NH_4$ peak at *m/z* 341.14605 which is in agreement with the calculated value of 341.14611 for $C_{14}H_{21}N_4O_6$ $[M+NH_4]$.

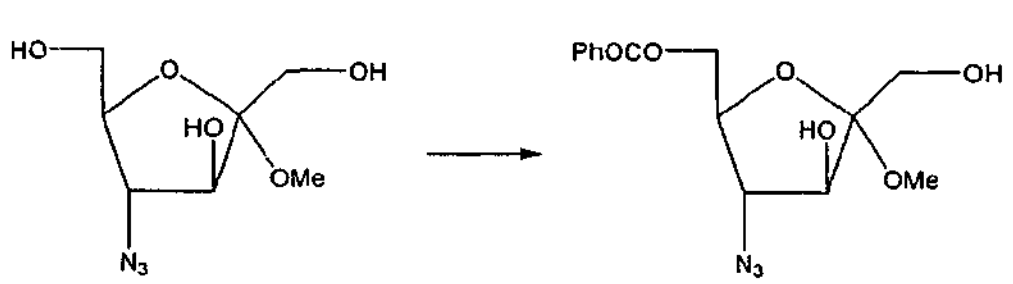


76

Having successfully prepared **76**, the synthesis of methyl 4-azido-6-*O*-benzoyl-4-deoxy-α-D-fructofuranoside (**78**) was expected to follow the same methodology. Thus, exposure of methyl 3,4-anhydro-α-D-tagatofuranoside (**59**) to lithium azide and

acidic resin in DMF at ca. 100°C gave methyl 4-azido-4-deoxy-α-D-fructofuranoside (**77**) in 66% yield. The infra-red spectrum clearly indicated the presence of an azido group with a strong absorption at 2112 cm^{-1}. As was observed for **75** and **60** ^{13}C NMR spectral differences between **77** and **59** were consistent with the introduction of an azide group at C-4.

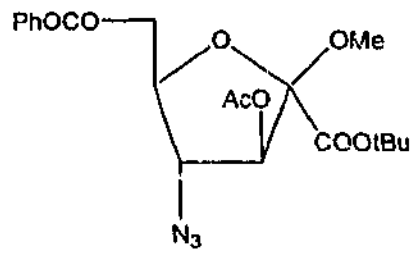
Treatment of **77** with TPP, DIAD and benzoic acid in DMF afforded **78** in 81% yield. Spectral analysis of **78** supported the formation of the desired product. The ^{1}H NMR spectrum recorded five aromatic protons at δ 7.52, 7.64 and 8.12 ppm. In the ^{13}C NMR spectrum the benzoate group was observed by the presence of four signals for the aromatic carbons at δ 128.4-133.3 ppm and a carbonyl resonance at δ 166.4 ppm. Accurate mass determination confirmed the molecular formula for **78**.



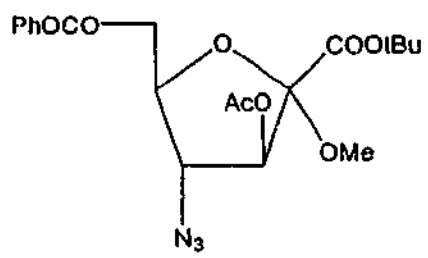
77 **78**

2.3 Synthesis of 4-azido-4-deoxy-β-fructofuranosidonic ester

With the success in introducing the azido functionality at C-4 and selective protection of the C-6 hydroxyl group, we then set out to investigate the synthesis of the 4-azido esters **79** and **80**.

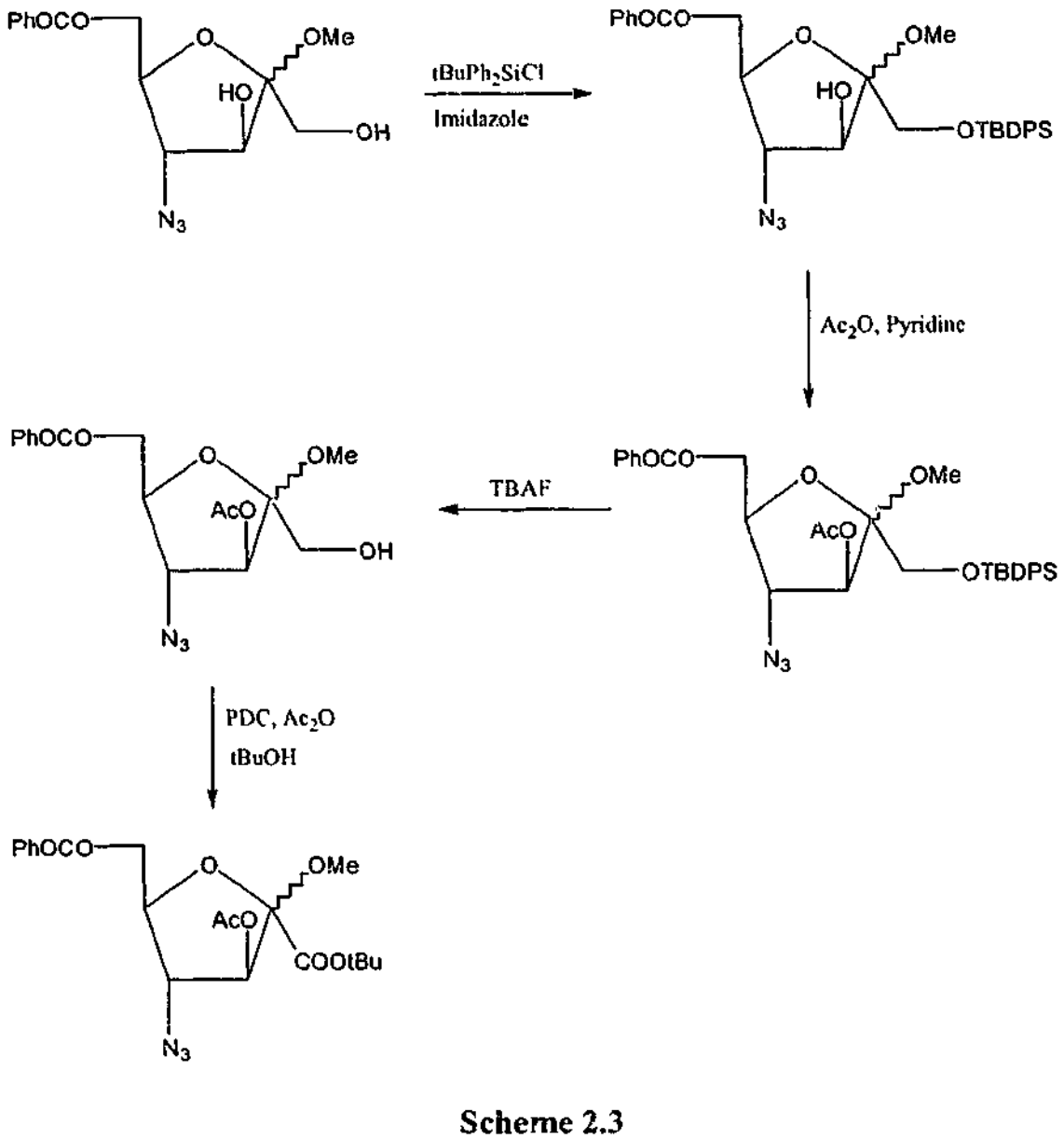


79



80

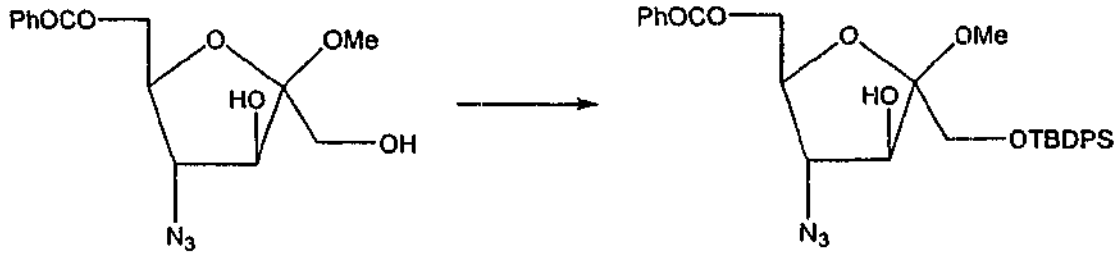
The synthesis of these esters was envisaged to involve the protection of the primary hydroxyl group on C-1 and the secondary hydroxyl group on C-3 with different protecting groups which upon deprotection would expose the C-1 hydroxyl group for oxidative-esterification to give the desired esters as outlined in scheme 2.3. Protection of the C-1 primary hydroxyl group of **76** and **78**, apparently, should be undertaken before protection of the C-3 secondary hydroxyl group. Hydroxyl group protection is a widely performed task in organic synthesis, numerous methods and a large number of reagents are available for the exercise[180,218-221]. The protecting groups are formed and removed under a wide variety of conditions. As for our purpose, the appropriate method of protection should not only be able to selectively protect the primary hydroxyl group but also, upon removal of the protecting group, the conditions employed should be compatible with other existing functional groups. Thus, *tert*-butyldiphenylsilyl ether protecting group[180] was the group of choice for the protection of C-1 hydroxyl group for two reasons. Firstly, primary hydroxyl groups are silylated preferentially[181] to secondary, and secondly, tetrabutylammonium fluoride, which was used to cleave the silyl protecting group, has shown to be compatible with the benzoate group[284].



Scheme 2.3

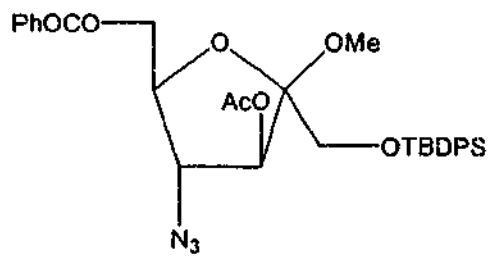
Accordingly, selective silylation[180,181] of the C-1 hydroxyl of methyl 4-azido-6-*O*-benzoyl-4-deoxy-β-D-fructofuranoside (**76**) was effected by exposure of **76** to *tert*-butyldiphenylsilyl chloride and imidazole in DMF for 18 hours at room temperature. The product, methyl 4-azido-6-*O*-benzoyl-1-*O*-*tert*-butyldiphenylsilyl-4-deoxy-β-D-fructofuranoside (**81**) was isolated in exellent yield (91%). The presence of the TBDPS group was observed in both ^{1}H NMR and ^{13}C NMR spectra. A singlet of nine protons at δ 1.07 ppm was assigned for the *tert*-butyl group. A total of fifteen aromatic protons were observed at δ 7.32-8.10 ppm of which ten were due to the TBDPS group. In the

^{13}C NMR spectrum resonances for the *tert*-butyl group were observed at δ 19.4 ppm for the quaternary carbon, and δ 27.0 ppm for three methyl carbons. High resolution ESI mass spectrometry showed a molecular ion plus $^{+}NH_4$ peak at *m/z* 579.26438 which was consistent with the molecular formula for **81**.



76 **81**

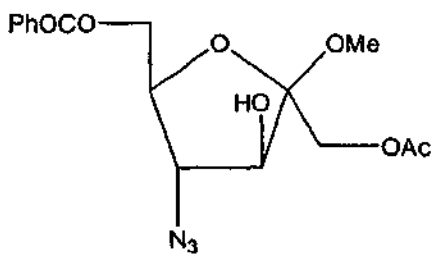
Acetylation of **81** with acetic anhydride and pyridine gave methyl 3-*O*-acetyl-4-azido-6-*O*-benzoyl-1-*O*-*tert*-butyldiphenylsilyl-4-deoxy-β-D-fructofuranoside (**82**) in 94% yield. A three proton singlet at δ 2.18 ppm was evident of the presence of an acetate group. Accurate mass determination confirmed the formula for **82**.



82

Desilylation of **82** by treatment with tetrabutylammonium fluoride in THF for 4 hours gave methyl 1-*O*-acetyl-4-azido-6-*O*-benzoyl-4-deoxy-β-D-fructofuranoside (**83**) and methyl 4-azido-6-*O*-benzoyl-4-deoxy-β-D-fructofuranoside (**76**). Compound **83** is formed as a result of acetate migration from C-3 to C-1, the latter being the minor

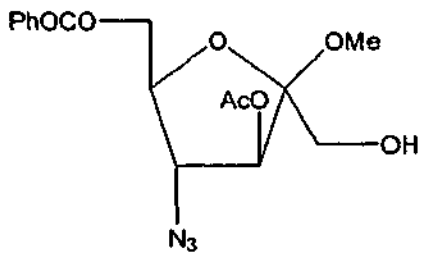
product forming from the loss of the acetyl group in the course of the desilylation reaction. Examination of the ^{1}H NMR spectrum of **83** revealed the disappearance of the resonances corresponding to the TBDPS group. A methyl proton resonance at δ 2.14 ppm indicated the presence of an acetate. The significant upfield shift of H-3, from δ 5.84 ppm in **82** to δ 4.25 ppm in **83**, and the downfield shift of the C-1 methylene protons, from δ 3.72 and 3.76 ppm in **82** to δ 4.20 and 4.36 ppm in **83**, were evidence of the migration of the acetate group from C-3 to C-1. That the minor product was methyl 4-azido-6-*O*-benzoyl-4-deoxy-β-D-fructofuranoside (**76**) was confirmed by spectral data which are identical to that of the material prepared earlier (*vide supra*).



83

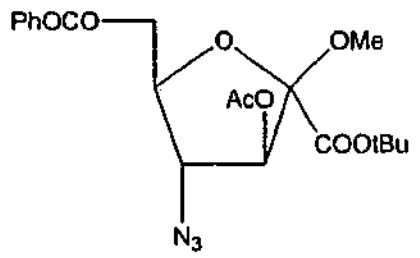
It has been reported[182] that tetrabutylammonium fluoride, being quite basic, may cause side reactions with base-sensitive substrates. It was felt that acetate migration could be avoided if the desilylation reaction was carried out under neutral conditions. Thus, a solution of **82** and TBAF in THF was adjusted to pH 7 with acetic acid before leaving to stir at room temperature until no starting material remained (16 hours). TLC examination of the reaction mixture indicated one product (**84**) formed which was isolated, after purification, in 72% yield. Removal of the TBDPS group was confirmed as the resonances assigned to the TBDPS group were absent in the ^{1}H and ^{13}C NMR spectra of **84**. Proton resonances of the methyl group (δ 2.21 ppm) and H-3 (δ [illegible] ppm) were evident and supported the notion that the acetate at C-3 remained intact.

High resolution ESI mass spectrometry confirmed the molecular formula for methyl 3-*O*-acetyl-4-azido-6-*O*-benzoyl-4-deoxy-β-D-fructofuran · ide (**84**). It is worth noting that the desilylation using TBAF under neutral conditions took longer (16 h) than that carried out under normal (i.e. basic) conditions (1-5 h)[180].



84

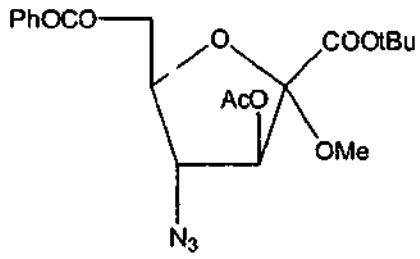
Having successfully deprotected **82** we then proceeded to convert **84** to the ester **79** using a method developed by Corey *et al.*[183] with some modification. Pyridinium dichromate was used in place of a freshly prepared chromium trioxide-pyridine complex which we found inconvenient to handle due to the hygroscopic nature of chromium trioxide resulting in inconsistent yields. Thus, exposure of **84** to PDC, acetic anhydride and *tert*-butyl alcohol in DMF-CH_2Cl_2 afforded *tert*-butyl (methyl 3-*O*-acetyl-4-azido-6-*O*-benzoyl-4-deoxy-β-D-fructofuranosid)onate (**79**) in 85% yield after purification. ^1H and ^{13}C NMR spectra of **79** revealed the disappearance of the resonances assigned to the 1-methylene group in **84**. The presence of a *tert*-butyl group was observed as a nine proton singlet at δ 1.50 ppm correlating to carbon resonances at δ 27.7 ppm for three methyl and δ 83.3 ppm for the quaternary carbon. Resonances for the ester carbonyl were observed at 165.2 ppm. High resolution ESI mass spectrum confirmed the molecular formula for **79**.



79

Given the success with the synthesis of the β-ester **79** from **76**, we then proceeded to prepare the α-ester **80** employing the same methodology. Exposure of **78** to *tert*-butyldiphenylsilyl chloride and imidazole in DMF for 18 hours afforded methyl 4-azido-6-*O*-benzoyl-1-*O*-*tert*-butyldiphenylsilyl-4-deoxy-α-D-fructofuranoside (**85**) in 89% yield. Resonances assigned to the TBDPS group similar to those observed for **81** were evident in the ^{1}H and ^{13}C NMR spectra of **85**. Electrospray mass spectrum confirmed the formation of **85**.

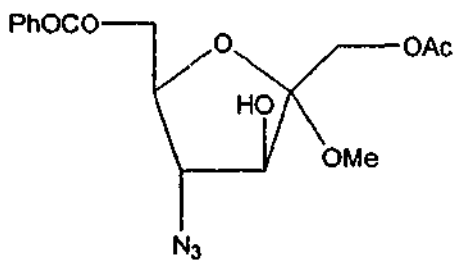
Acetylation of **85** (Ac_2O/pyridine) gave **86** in 96% yield. The presence of an acetate was observed as a three proton singlet at δ 1.93 ppm. A downfield shift of H-3 from δ 4.41 ppm to δ 5.32 ppm was evident that acetylation of the C-3 hydroxyl group had occured. Accurate mass determination confirmed the formula for **86**.



80

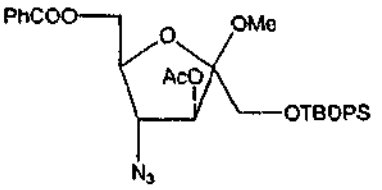
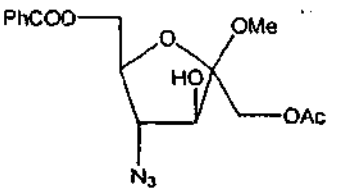
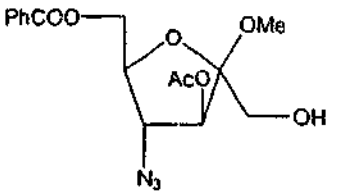
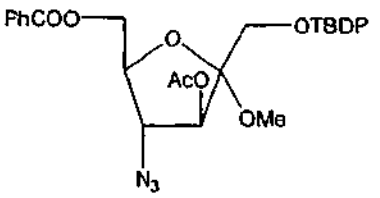
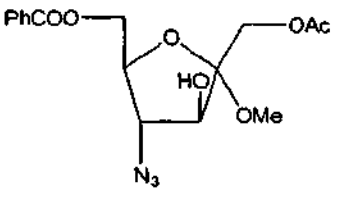
85 **86**

Given that desilylation of **82** was successfully carried out at neutral pH, **86** was thus exposed to TBAF in THF at pH 7. To our disappointment, no desired product was formed. Instead, two products were isolated, the major one **87** formed from the migration of the acetate (C-3 to C-1) and the minor product **78** as the result of a loss of the acetyl group. When pH of the reaction mixture was lowered (i.e. pH 6-6.5) starting material remained unreacted after stirring for 18 hours. Spectral data of **78** was identical to that of the material prepared earlier (*vide supra*). Similar to **83** (Table 2.1) the ^{1}H NMR spectrum of **87** revealed the resonance of the acetate methyl protons at δ 2.12 ppm. An upfield shift was observed for H-3 from δ 5.32 ppm in **86** to δ 4.07 ppm in **87**. Also observed was a downfield shift of the C-1 methylene protons from δ 3.82 and 3.88 ppm in **86** to δ 4.23 and 4.42 ppm in **87** consistent with the presence of an acetate at C-1. Assignment of the ^{1}H NMR spectrum was confirmed by ^{1}H-^{1}H COSY NMR experiment.



87

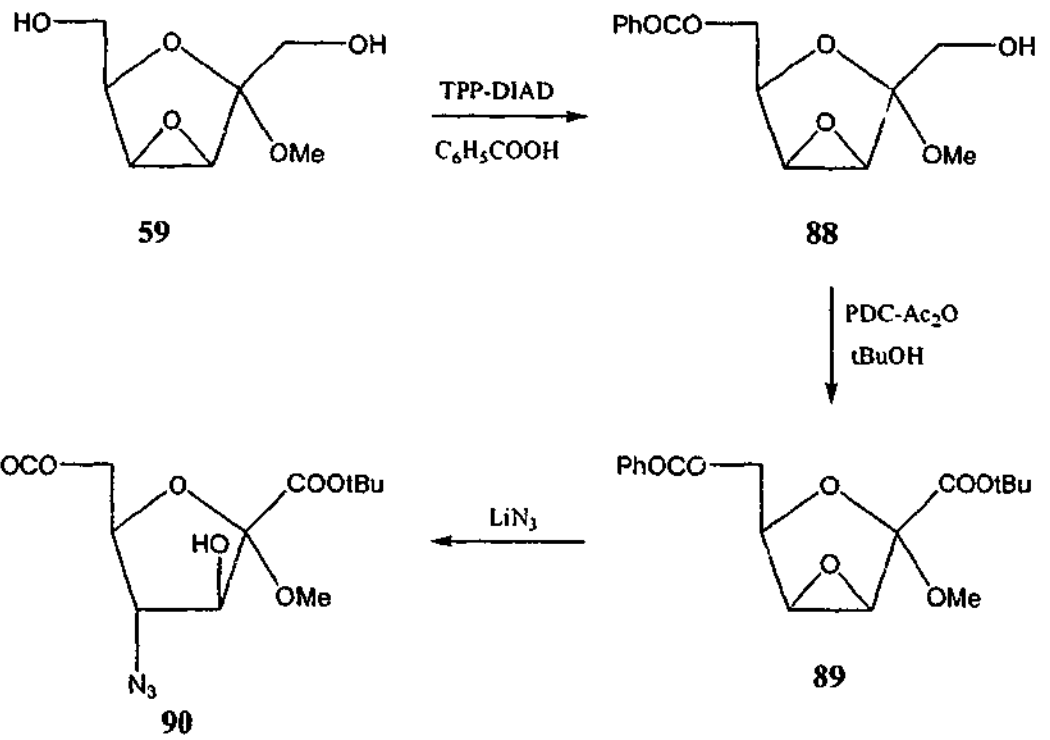
Table 2.1 ^{1}H resonances of H-1, H-1′ and H-3 for compounds **82**, **83**, **84**, **86** and **87** in ppm.

		H-1	H-1′	H-3
82		3.72	3.76	5.84
83		4.20	4.36	4.25
84		3.64	3.77	5.22
86		3.82	3.88	5.32
87		4.23	4.42	4.07

With the failure of the attempts to prepare the α-ester **80**, it was reasoned that the stereofacial proximity between C-1 and the C-3 acetate in the α-derivative **86** was a major problem causing acetate migration which prevented a successful desilylation. It was, therefore, decided to investigate an alternative approach for the preparation of an α-ester as described in the following section.

2.4 Synthesis of 3,4-anhydro-fructofuranosidonic esters

As has been demonstrated in Scheme 2.2 and Scheme 2.3, the introduction of the azido group at C-4 exposed another hydroxyl at C-3 together with the existing hydroxyls at C-1 and C-6. As a consequence, a number of protection and deprotection steps were involved which proved troublesome in the desilylation of the α-fructoside **86**. In the alternative approach Mitsunobu conditions[185] were employed to regioselectively benzoylate the anhydro sugar **59** at C-6. Subsequent oxidative esterification of the 6-*O*-benzoyl anhydro sugar **88** followed by epoxide-ring opening of the ester **89** with lithium azide should give the desired ester **90**.



59 **88** **89** **90**

Scheme 2.4

Exposure of methyl 3,4-anhydro-α-D-tagatofuranoside (**59**) to TPP, DIAD and benzoic acid for 16 hours afforded the regioselectively protected 6-*O*-benzoyl derivative

88 in 82% yield. The ^{1}H NMR spectrum of **88** indicated the presence of five aromatic protons at δ 7.38, 7.51 and 7.99 ppm. In the ^{13}C NMR spectrum the aromatic carbons resonated at δ 128.0, 128.3, 129.6 and 133.1 ppm with the carbonyl group observed at δ 166.1 ppm. The mass spectrum gave a molecular ion plus proton peak at *m/z* 281. Microanalysis provided C, 60.30; H, 5.88% which is in agreement with calculated figures (C, 59.99; H, 5.75%) for $C_{14}H_{16}O_6$.

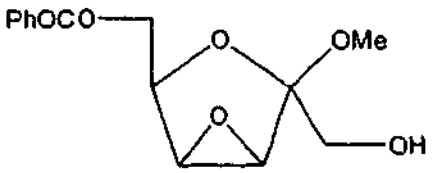
The next step involved the oxidative-esterification of **88**. Thus, treatment of **88** with PDC, acetic anhydride and *tert*-butyl alcohol in DMF-CH_2Cl_2 (1:4) resulted in the formation of the ester **89** in 81% yield. The initial concern that the epoxide ring might not be able to withstand the oxidative-esterification conditions was unfounded as spectral data confirmed the structure of the desired ester **89**. The disappearance of the 1-methylene group was observed in both the ^{1}H and ^{13}C NMR spectra. A nine proton singlet at δ 1.54 ppm was assigned to the *tert*-butyl group. In the ^{13}C NMR spectrum the *tert*-butyl ester group resonances were observed at δ 27.9 ppm for three methyl carbons, at δ 82.9 ppm for quaternary carbon and at one of the two carbonyl peaks δ 164.5 and 166.0 ppm (the other one for the carbonyl of the benzoate group). That the epoxide ring of **89** remained intact was confirmed by examination of the resonances of H-3, H-4, C-3 and C-4 which were similar to those in **88**. The ESI mass spectrum showed a molecular ion plus $^{+}NH_4$ peak at *m/z* 368. Microanalysis provided C, 61.84; H, 6.40%, which is in agreement with calculated figures (C, 61.71; H, 6.33%) for $C_{18}H_{22}O_7$.

Epoxide-ring opening of **89** to form **90** proceeded readily using similar conditions (LiN_3, acidic resin, DMF) to those for **60** and **59** except that the reaction took less time to go to completion (16 hours compared to 50 hours for **60** and **59**). The

presence of an azido group was clearly observed in the infra-red spectrum of **90** with a strong absorption at 2112 cm^{-1}. The ^1H NMR spectrum revealed a downfield shift for H-3 from δ 3.87 ppm in **89** to δ 4.35 ppm in **90** consistent with the deshielding effect of the 3-hydroxyl group on H-3. Further evidence was from ^{13}C NMR spectrum of **90** which showed resonances of C-3 at δ 78.3 ppm and C-4 at δ 66.9 ppm, compared to those in **89** at δ 58.6 and 54.3 ppm respectively. Accurate mass determination confirmed the molecular formula for **90**.

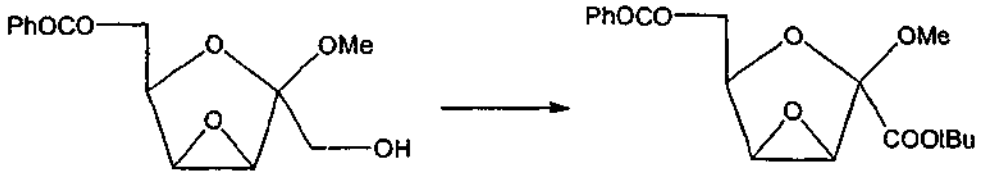
The synthesis of the 4-azido ester **90** from methyl 3,4-anhydro-α-D-tagatofuranoside (**59**) required only three steps and was very efficient compared to the six steps required from methyl 3,4-anhydro-β-D-tagatofuranoside (**59**) to the ester **79**. It was, therefore, quite attractive to employ the methodology to prepare the β-ester derivative.

A Mitsunobu reaction of **60** using TPP, DIAD and benzoic acid afforded the regioselective product methyl 3,4-anhydro-6-*O*-benzoyl-β-D-tagatofuranoside (**91**) in 84% yield. The ^1H NMR spectrum of **91** revealed the presence of five aromatic protons at δ 7.44, 7.55 and 8.06 ppm. The ^{13}C NMR exhibited the aromatic carbons at δ 128.4-133.2 ppm and the carbonyl carbon at δ 166.3 ppm. Electrospray mass spectrum showed a molecular ion plus sodium peak at *m/z* 303. Microanalysis supported the molecular formula of $C_{14}H_{16}O_6$.



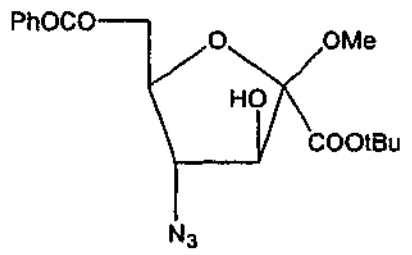
91

Exposure of **91** to PDC, acetic anhydride and *tert*-butyl alcohol in DMF-CH_2Cl_2 (1:4) for 16 hours furnished the ester **92** in 86% yield after purification. Examination of ^1H and ^{13}C NMR spectra revealed the disappearance of the C-1 methylene group in **92**. Resonances for the ester *tert*-butyl group were observed as a nine proton singlet at δ 1.54 ppm. In the ^{13}C NMR spectrum the resonances for the *tert*-butyl ester group were assigned as follows: three methyl carbons at δ 28.1 ppm, the quaternary carbon at δ 83.3 ppm and the carbonyl carbon at either δ 166.1 or 166.5 ppm (the other one for the benzoate carbonyl). Low resolution mass spectrum showed a molecular ion plus $^+NH_4$ peak at *m/z* 368. Microanalysis determination confirmed the molecular formula of $C_{18}H_{22}O_7$ for **92**.

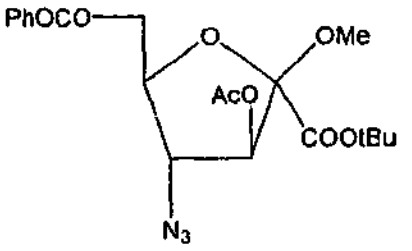


91 **92**

Reaction of **92** with lithium azide and acidic resin in DMF at *ca.* 95°C for 16 hours gave **93** in 85% yield. The infra-red spectrum exhibited a strong absorption at 2106 cm^{-1} indicating the presence of an azide group. ^1H NMR spectrum of **93** showed the deshielding effect of the C-3 hydroxy group on H-3 which was observed at δ 4.24 ppm compared to δ 3.83 ppm for H-3 in **92**. The ^{13}C NMR spectrum of **93** revealed the downfield shifts of C-3 and C-4 which resonated at δ 77.4 and 66.0 ppm, respectively, compared to δ 57.0 and 54.4 ppm, respectively, in **92**. Accurate mass determination confirmed the formula for **93**. Acetylation of **93** under standard conditions (acetic anhydride, pyridine) gave **79**.



93

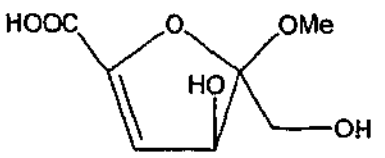


79

2.5 Methyl (methyl β-D-*glycero*-hex-2-en-5-ulofuranosid)onate (94) and its acid derivative (95)

2.5.1 Preliminary molecular modelling

From the outset of this work, we have focussed on the syntheses of furanosidonic acids with an amino functionality at 4-position. However, we thought that it would be of value to investigate the synthesis of an analogue with an α,β-unsaturated carboxylic acid moiety that was present in many potent influenza sialidase inhibitors[9] including Zanamavir (**28**)[11] and GS4071 (**33**)[10,58]. To this end, compound **95** was chosen as the target for its viable and simple synthetic route (*vide infra*).

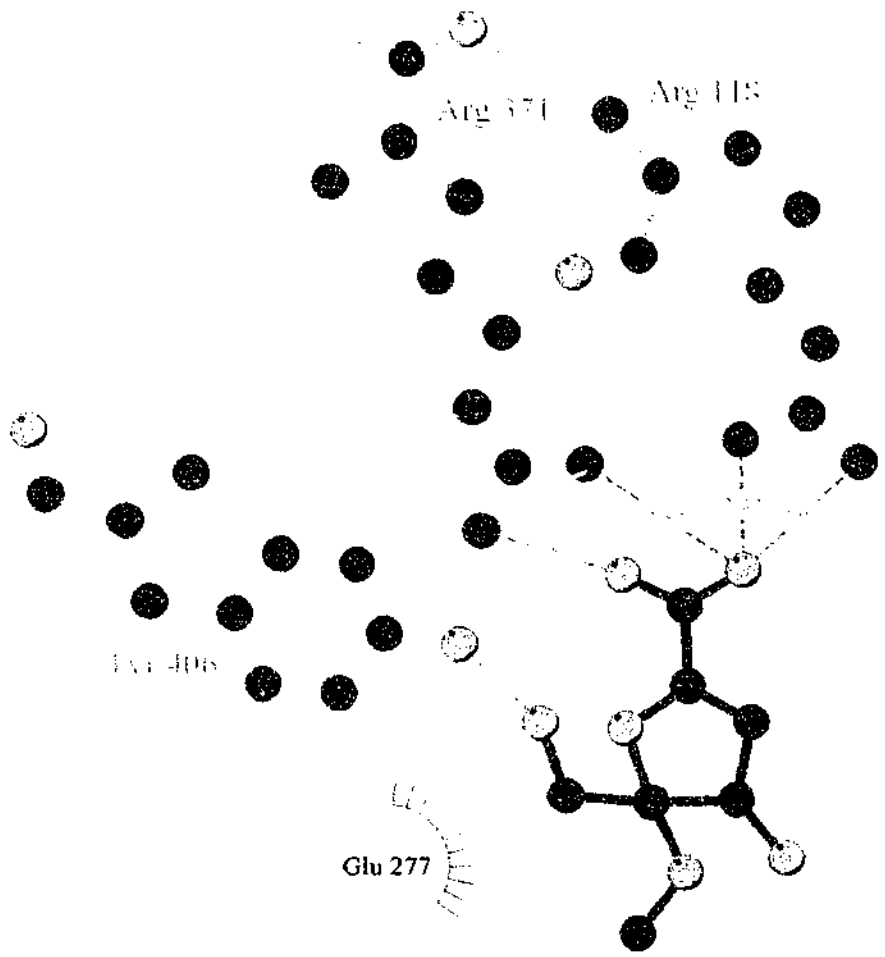


95

Before synthesis work was carried out, we decided to undertake a preliminary molecular modelling study to investigate the effect of **95** on the influenza virus sialidase active site. The **95** structure was constructed with Sybyl[204] on a Silicon Graphics work station and minimized using MMFF94s Force Field and MMFF94 charges[205]. An

additional minimization was undertaken using Kollman All Atom Force Field and Kollman charges[206] when compound **95** was placed in the catalytic site.

Preliminary results from the modelling of **95** in the active site indicated that the carboxylic acid moiety of **95** was stabilized via hydrogen bonds with the guanidine groups of Arg 371 and Arg 118. Hydrogen bond was also observed between the primary hydroxyl group of **95** and the hydroxyl group of Tyr 406. There were hydrophobic interactions between **95** and the residues Tyr 406 and Glu 277. It was possible, from visual examination, that the delocalized π electrons of the α,β-unsaturated carboxylic acid moiety of **95** were stabilized by the aromatic π electrons of Tyr 406 (Figure 2.1).



Key

Ligand bond

Non-ligand bond

Hydrogen bond and its length

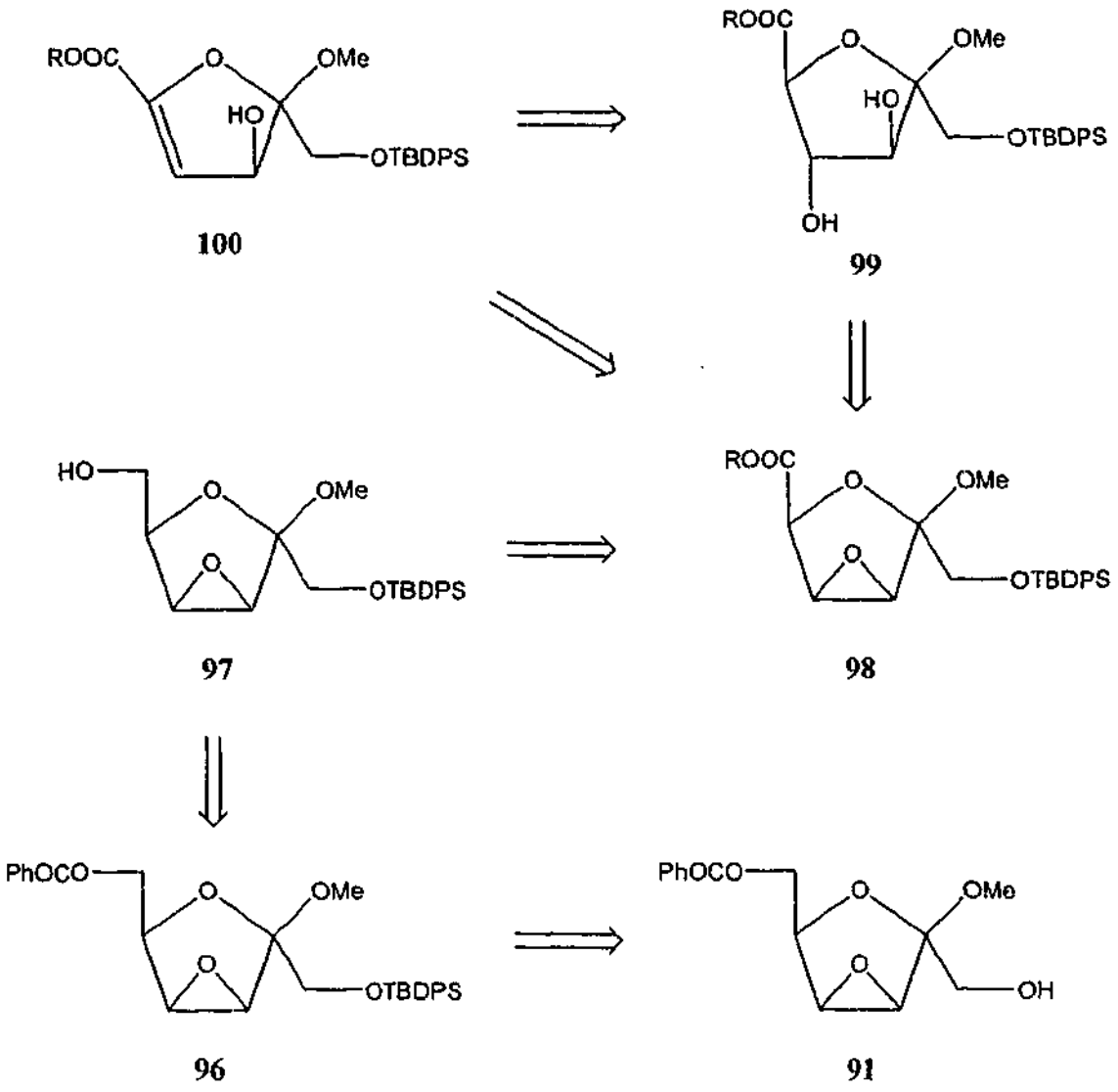
His 53 — Non-ligand residues involved in hydrophobic contact(s)

Corresponding atoms involved in hydrophobic contact(s)

Figure 2.1 Compound **95** in the influenza virus sialidase active site.

2.5.2 Syntheses of methyl (methyl β-D-*glycero*-hex-2-en-5-ulofuranosid)onate (94) and methyl β-D-*glycero*-hex-2-en-5-ulofuranosidonic acid (95)

Examination of the target compound **95** suggested that there were two approaches towards the synthesis of the α,β-unsaturated ester **100**, the precursor of **95**. In the first approach, ester **100** could be formed from the dehydration of the 3,4-dihydroxy ester **99** by treatment with aluminium ethoxide or diethoxyaluminium chloride, then with lithium diisopropylamide[190]. Compound **99** could be readily prepared by the hydrolysis of the anhydro derivative **98**. Alternatively, in the second approach, isomerization of the epoxide **98** to the allylic alcohol **100** could be effected using various reagents[191-201]. The retrosynthetic sequence towards **100** relied upon the successful preparation of **98**. The best candidate as a subject of a successful oxidation to form **101** would be **97** which had a protecting group for the C-1 hydroxyl group and a free hydroxyl group on C-6 available for the oxidant to attack. **97** could be prepared in two steps, protection and deprotection, from the 6-*O*-benzoyl-anhydro sugar **91** which we had in stock. The synthesis of **100** is depicted in retrosynthetic terms in Scheme 2.5.



Scheme 2.5

Protection of the C-1 hydroxyl group of **91** was achieved by exposure of the starting material to *tert*-butyldiphenylsilyl chloride and imidazole in DMF for 18 hours at room temperature. After work up and purification, the product **96** was obtained in 88% yield. Similar to **81** and **85**, spectral features assigned to the TBDPS group were observed in the ^{1}H and ^{13}C NMR spectra of **96**. Accurate mass determination confirmed the formula for **96**.

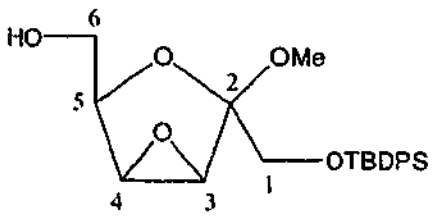
Treatment of **96** with sodium methoxide in methanol gave the 6-hydroxy-anhydro sugar **97** in excellent yield (92%). ^{1}H NMR spectrum of **97** showed ten protons

in the aromatic region compared to fifteen aromatic protons in that of **96**. In the ^{13}C NMR spectrum, resonance assigned to the benzoate carbonyl group in **96** was not observed in **97**. The ESI mass spectrum gave a molecular ion plus $^{+}NH_4$ peak at *m/z* 432 for **97**.

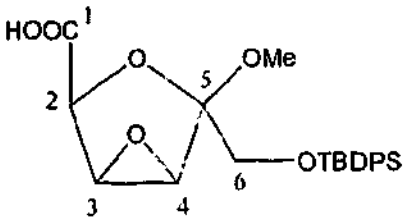
Prior to the use of PDC in place of a freshly prepared chromium trioxide-pyridine complex for the conversion of an alcohol to *tert*-butyl ester, we had experienced inconsistent yields following the method of Corey *et al.*[183]. Therefore, we wanted to explore the Shyrock and Zimmerman method[184] of oxidation of a primary hydroxyl group in the presence of an epoxide ring. The acid obtained could then be converted to the methyl ester quantitatively by reaction with diazomethane[168,169], or alternatively, the esterification could be conveniently effected under Mitsunobu conditions[185].

Thus, exposure of **97** to Pt black and bubbling oxygen at pH 8 for 4 hours at 80 C° afforded the acid **101** in 81% yield after purification. The disappearance of the 1-methylene group was observed in both ^{1}H and ^{13}C NMR spectra of **101**. Resonance at δ 175.7 ppm in the ^{13}C NMR spectrum was assigned to the acid carbonyl group. Accurate mass determination supported the molecular formula for **101**.

It should be noted that the formation of **101** has introduced a different carbon numbering to that of the starting material as shown on the structures below.

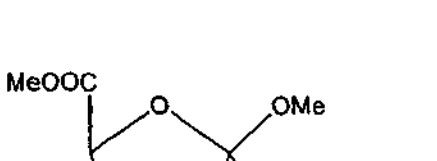


97

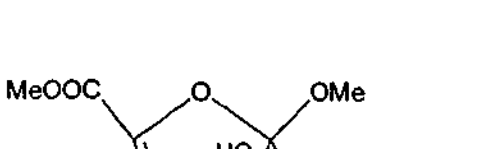


101

Due to the availability of TPP and DIAD, we decided to try the esterification under Mitsunobu conditions[185] on a small scale. Thus, **101** was exposed to TPP, DIAD and anhydrous methanol for 36 hours at room temperature. To our delight, we isolated from the reaction mixture not the expected anhydro ester **103**, rather the α,β-unsaturated ester **102**. Methylation of the carboxyl group was evident by the presence of the methyl protons at δ 3.80 ppm. Further inspection of the ^{1}H NMR spectrum of **102** revealed the absence of H-2 which resonated at δ 3.79 ppm for **101**, and the downfield shift of H-3 from δ 4.09 ppm in **101** to δ 5.99 ppm in **102**, thus consistent with the formation of a double bond between C-2 and C-3. ^{13}C NMR spectrum provided further evidence with the resonances of the olefinic C-2 and C-3 at δ 147.2 and 113.7 ppm, respectively (Table 2.2). Accurate mass determination confirmed the molecular formula for **102**.

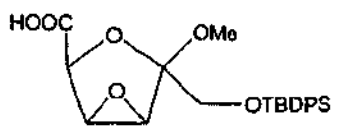
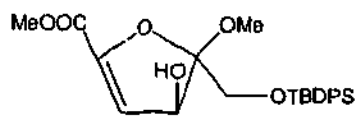


103

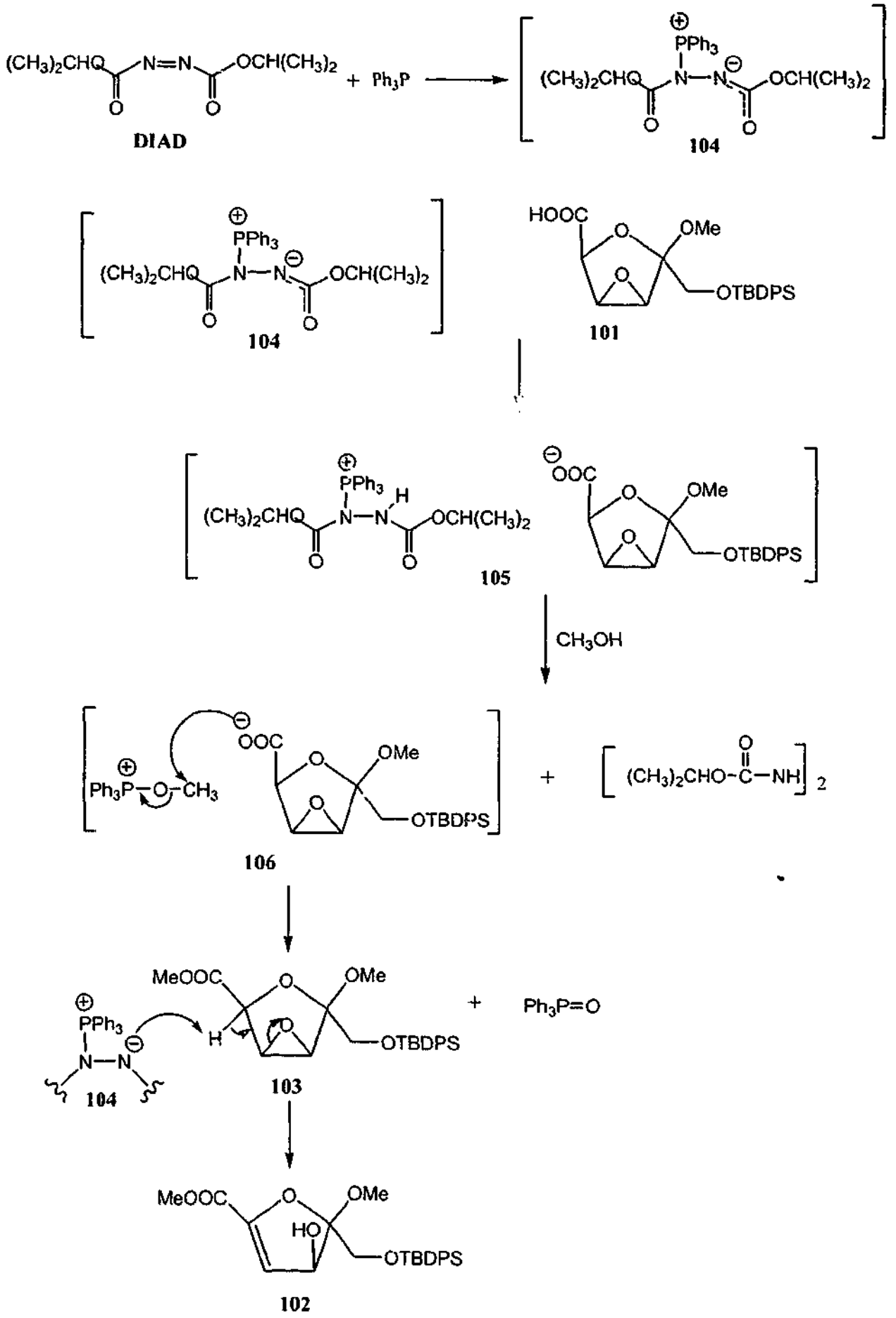


102

Table 2.2 H-2, H-3, H-4, H-6, H-6′, C-1, C-2, C-3, C-4, C-5, C-6 resonances for **101** and **102** in ppm.

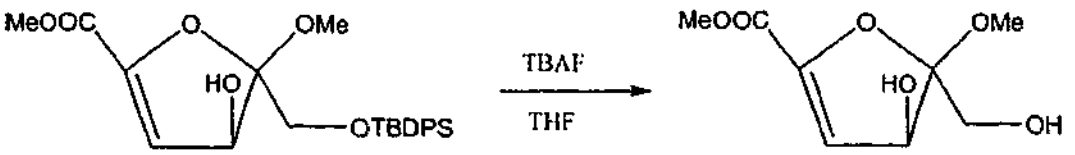
	 **101**	 **102**
H-2	3.79	–
H-3	4.09	5.99
H-4	4.59	5.14
H-6	3.67	3.75
H-6′	3.75	3.94
C-1	175.7	161.9
C-2	58.0	147.2
C-3	58.0	113.7
C-4	58.0	75.2
C-5	107.5	107.8
C-6	68.3	62.5

A search of the literature revealed no precedent examples of the formation of an α,β-unsaturated ester from a β,δ-anhydro acid or ester using Mitsunobu conditions. Examination of the reaction conditions and mechanism of the Mitsunobu reaction suggested that the α,β-unsaturated ester **102** was formed via the methyl ester **103** following the generally accepted Mitsunobu reaction mechanism[185-189,207-211] as shown in Scheme 2.6. The quaternary phosphonium salt **104** formed from the addition of TPP and DIAD was protonated by the acid **101** to give **105** which then reacted with methanol to form the methoxy phosphonium salt **106**. A S_N2 type displacement of **106** occurred to give the methyl ester **103** and triphenylphosphine oxide. The quaternary salt **104** then removed the acidic proton H-2 from **103** to form a double bond between C-2 and C-3. This step is analogous to an E2 elimination reaction except that the leaving group was the epoxide-ring oxygen which upon elimination and ring opening resulted in the creation of the C-4 hydroxyl group. Thus, the net result was the formation of methyl (methyl 6-*O*-*tert*-butyldiphenylsilyl-β-D-*glycero*-hex-2-en-5-ulofuranosid)onate (**102**).

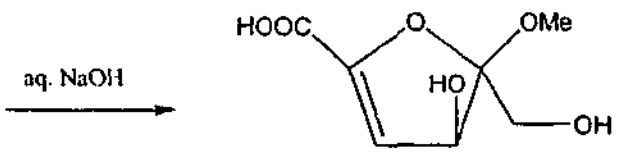

(CH3)2CHO
N=N
OCH(CH3)2
Ph3P
PPh3
DIAD
104
HOOC
OMe
OTBDPS
101
105
CH3OH
OOC
Ph3P-O-CH3
106
+
(CH3)2CHO-C-NH 2
MeOOC
HO
103
Ph3P=O
102

Scheme 2.6

Having synthesized the ester **102**, we then proceeded to the next steps which involved desilylation followed by hydrolysis of the ester to give the target compound **95**. Thus, exposure of **102** to tetrabutylammonium fluoride in THF afforded **94** in 82% yield after purification. The disappearance of the TBDPS group was observed in both ^{1}H and ^{13}C NMR. Accurate mass determination supported the molecular formula for **94**. Hydrolysis of the methyl ester was effected by treatment of **94** with aqueous sodium hydroxide to give **95** as a white amorphous mass.



102 **94**



95

2.6 NMR spectroscopy

There has been significant interest in the ^{1}H and ^{13}C NMR properties of D-fructofuranosides and related compounds including 3,4-oxiran derivatives[202,203]. Therefore, it was of interest for us to undertake an examination of these properties of fructose derivatives we have synthesized with a view to comparing our results, where appropriate, with those reported in the literature[202,203].

2.6.1 ^{1}H NMR spectroscopic properties of methyl 4-azido-4-deoxy-fructofuranoside derivatives

The chemical shift of the α- and β-anomers of 4-azido furanosides are shown in Tables 2.3 and 2.4. In most cases, the spectra showed good signal resolution, however, the spectra of **77** and **75** displayed overwhelming signal overlaps that prohibited a complete analysis. For both anomers, H-6 and H-6′ of the 6-benzoate derivatives were observed as two doublet of doublets centred at approximately 4.47 and 4.55 ppm. With the exception of **87** where H-3 was slightly more shielded than H-5, for both anomers, H-3 was located further downfield than H-4 and H-5. The H-5 signal was observed to be deshielded compared to that of H-4 in the α-forms. Whereas, in the β-compounds the chemical shift of H-5 was equivalent (in **76**, **81** and **83**) or lower (in **75** and **82**) than that of H-4.

Guthrie *et al.*[202] reported that the ring protons (H-3, H-4, H-5) of the methyl β-D-fructofuranoside peracetates were downfield compared with the corresponding α-anomers except for methyl 3,4-di-*O*-acetyl-1,6-dideoxy-1,6-difluoro-β-D-fructofuranoside and its corresponding α-anomer, which the reported chemical shifts[202] revealed H-5 of the β-anomers upfield to that of the α-anomer, at δ 3.90 and δ 3.94 ppm, respectively.

This is not the case here, however, whiist H-4 of β-anomers was observed downfield to that of the α-forms, the opposite was noted for H-5 in most cases but one, whereas H-3 did not follow any particular trend.

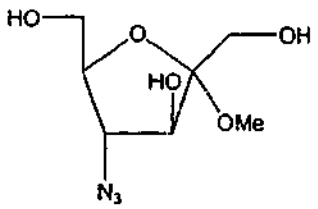
Table 2.3 ^{1}H NMR chemical shift data for

methyl 4-azido-4-deoxy-α-D-fructofuranoside derivatives in ppm.

(OAc, OCOPh, TBDPS signals omitted)

Compound	H-1	H-1′	H-3	H-4	H-5	H-6	H-6′	OCH_3
77	3.64*	3.64*	4.08	3.64*	3.78	3.64*	3.64*	3.19
78	3.83	3.83	4.31	3.83	4.15	4.55	4.60	3.27
85	3.88	3.95	4.41	3.83	4.17	4.49	4.59	3.09
86	3.82	3.88	5.32	3.80	4.14	4.48	4.52	3.20
87	4.23	4.42	4.07	3.81	4.16	4.49	4.52	3.34

* Approximate



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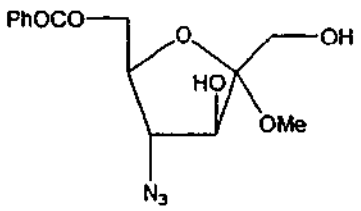

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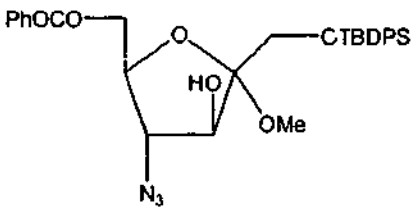

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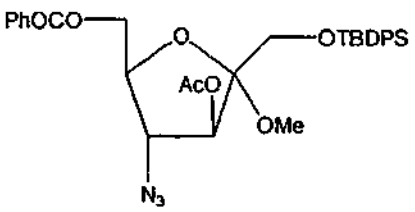

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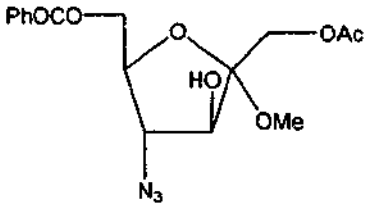


87

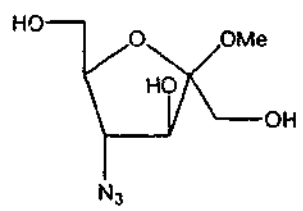
Table 2.4 ^{1}H NMR chemical shift data for

methyl 4-azido-4-deoxy-β-D-fructofuranoside derivatives in ppm.

(OAc, OCOPh, TBDPS signals omitted)

Compound	H-1	H-1′	H-3	H-4	H-5	H-6	H-6′	OCH_3
75	3.60*	3.60*	4.19	3.95	3.60*	3.60*	3.60*	3.29
76	3.70	3.76	4.29	4.05	4.05	4.41	4.53	3.31
81	3.72	3.77	4.39	4.03	4.03	4.39	4.52	3.20
82	3.72	3.76	5.84	4.26	4.19	4.50	4.59	3.23
83	4.20	4.36	4.25	4.08	4.08	4.43	4.[illegible]	3.39

* Approximate



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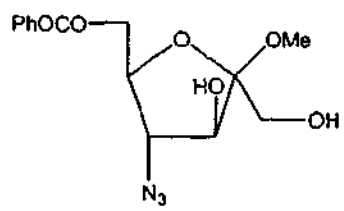

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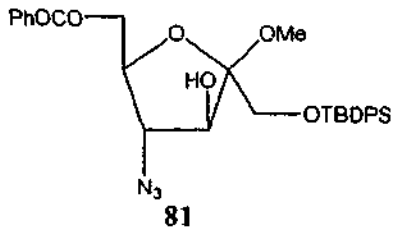

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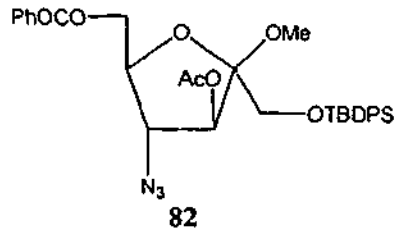

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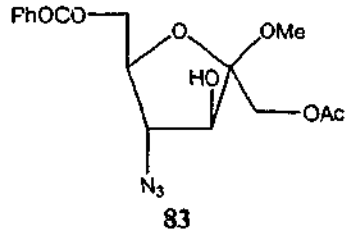


83

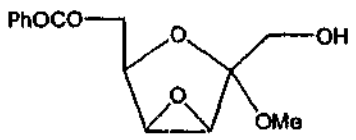
2.6.2 ^{1}H NMR spectroscopic properties of oxiran derivatives

The chemical shifts of the oxiran derivatives are shown in Table 2.5. In most cases, H-5, H-6 and H-6′ were observed as an ABX system, however in **91** the methylene protons of C-6 were not resolved and appeared as an apparent doublet. For both anomers, H-5 was located further downfield than H-3 and H-4, with H-3 slightly more deshielded than H-4 (except for **92** where both H-3 and H-4 resonated at 3.83 ppm). These observations are consistent with that reported by Guthrie *et al*[202].

Table 2.5 ^{1}H NMR chemical shift data for oxiran derivatives in ppm.

(C_6H_5 and $C(CH_3)_3$ signals omitted)

Compound	H-1	H-1′	H-3	H-4	H-5	H-6	H-6′	OCH_3
88	3.65	3.80	3.80	3.71	4.34	4.40	4.47	3.31
91	3.59	3.68	3.80	3.78	4.28	4.56	4.56	3.54
89	–	–	3.87	3.84	4.38	4.54	4.64	3.38
92	–	–	3.83	3.83	4.38	4.57	4.62	3.54



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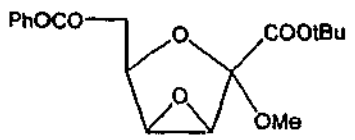

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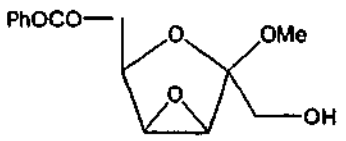

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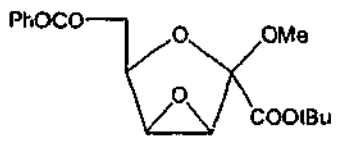


92

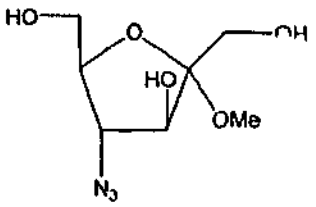
2.6.3 ^{13}C NMR spectroscopic properties of methyl 4-azido-4-deoxy-fructofuranoside derivatives

The chemical shifts for anomeric pairs of methyl 4-azido-4-deoxy-fructofuranoside derivatives are shown in Table 2.6. The ^{13}C NMR spectra of methyl α- and β-D-fructofuranoside derivatives have been studied by Guthrie *et al.*[203]. These authors noted that the C-1 and C-6 signals in the α-anomers were located further upfield than those in the β-anomers, whilst C-2, C-3, C-4 and C-5 signals were more deshielded in the α-forms than they were in the β-compounds. Comparison of the anomeric pairs of methyl 4-azido-fructofuranoside derivatives revealed similar conclusions. Guthrie *et al.*[203] also pointed out that the chemical shifts for the secondary carbons were in the order: C-4< C-3< C-5. Assignments of C-4, C-3 and C-5 were carried out accordingly. However, in the cases of **77** and **76** definite assignments for C-3 and C-5 were not undertaken as the differences in chemical shifts, Δδ, were only 0.1-0.3 ppm.

Table 2.6 ^{13}C NMR chemical shift data for anomeric pairs of methyl 4-azido-4-deoxy-fructofuranoside derivatives in ppm. (COPh, $COCH_3$ and TBDPS signals omitted)

Compound	C-1	C-2	C-3	C-4	C-5	C-6	OCH_3
77	59.6	107.8	80*	67.0	80*	61.2	48.0
75	60.1	103.8	76.5	66.5	79.7	62.6	48.4
78	59.7	107.5	78.1	68.3	82.2	62.8	49.0
76	60.3	103.4	77*	66.2	77*	64.2	49.2
85	64.2	108.0	78.8	68.7	83.2	61.5	48.6
81	64.4	103.7	77.6	66.9	78.2	62.0	49.5
86	64.7	109.4	81.2	68.4	82.1	60.3	49.1
82	65.7	105.4	77.5	65.4	78.5	65.1	50.5

* Approximate



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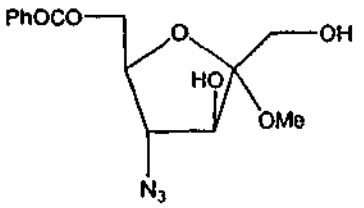

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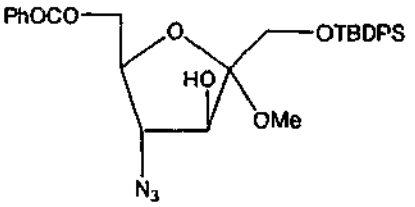

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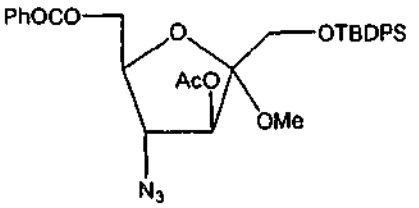

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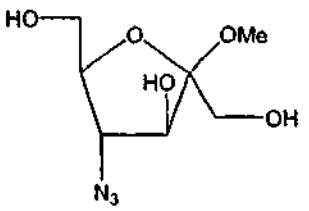

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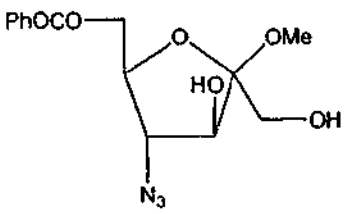

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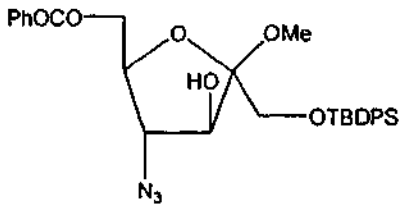

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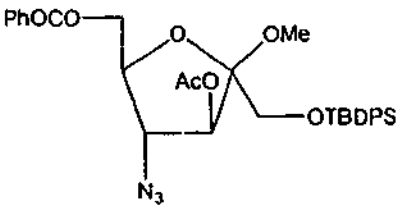


82

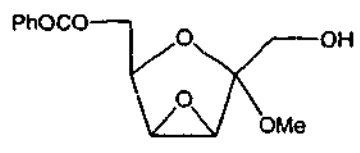
2.6.4 ^{13}C NMR spectroscopic properties of oxiran derivatives

The chemical shifts for oxiran derivatives are shown in Table 2.7. The ^{13}C NMR spectra of oxiran derivatives showed similar general features to those observed for methyl 4-azido-4-deoxy-fructofuranoside derivatives. Significant chemical shift differences between C-5 signal and those of C-4 and C-3 were noticed.

Table 2.7 ^{13}C NMR chemical shift data for oxiran derivatives in ppm.

Compound	C-1	C-2	C-3	C-4	C-5	C-6	OMe
88	62.9	105.2	57.3	55.3	75.8	60.1	49.5
91	65.2	104.9	56.5	54.1	74.3	63.0	52.5
89	165*	103.2	58.6	54.4	75.4	62.4	51.4
92	166.5	103.0	57.0	54.4	75.0	62.4	53.3

* Approximate



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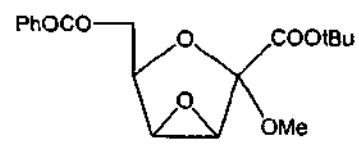

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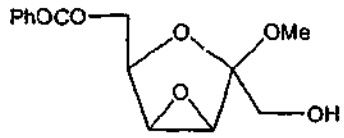

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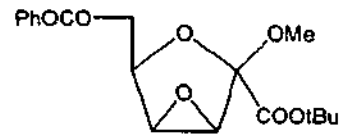


92

2.7 Conclusion

In summary, we have successfully synthesized both α and β anomers of 4-azido-4-deoxy-fructofuranosidonic esters. The investigation into these syntheses has shown that epoxide-ring opening of the anhydro sugars in the early stage of the process was either inefficient or problematic. We have demonstrated that the oxidative esterification of a primary hydroxyl group in the presence of an epoxide bridge using a modified Corey method[183] proceeded in good yields for fructofuranoside derivatives. Thus, the pivotal compounds, 3,4-anhydro esters **89** and **92,** were efficiently prepared in four

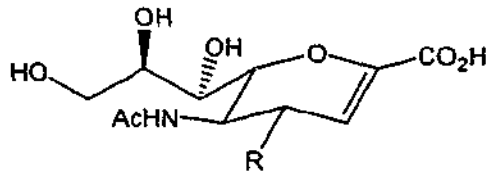
steps from D-fructose. Furthermore, the synthesis of the α,β-unsaturated ester **102** was achieved without extensive synthetic manipulations.

CHAPTER 3

Synthesis of C-6 Modified Fructofuranosidonic Acids and Derivatives

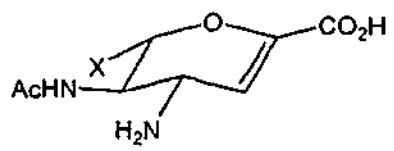
3.1 Introduction

The glycerol side chain at C-6 of 4-deoxy-4-guanidino-Neu5Ac2en (**28**) and 4-amino-4-deoxy-Neu5Ac2en (**27**) plays an important role in the activity of these influenza virus sialidase inhibitors[9] (*vide supra*). A number of Neu5Ac2en-based analogues with modified glycerol side chain have been synthesized[101,222]. Compounds such as the truncated analogues[101] **107, 108, 30** and **109** exhibited decreases in inhibitory activity with decreasing C-6 chain length. Complete removal of the stereochemically demanding glycerol side chain resulted in the loss of activity[101].



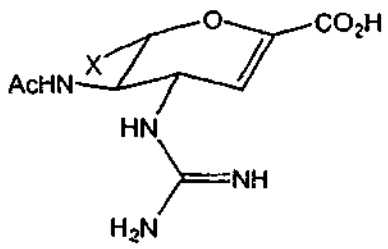
27 R = NH$_2$

28 R = NHC(NH)NH$_2$



107 X = CH_2OH

108 X = $CH(OH)CH_2OH$

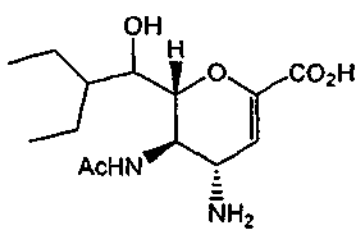


30 X = CH_2OH

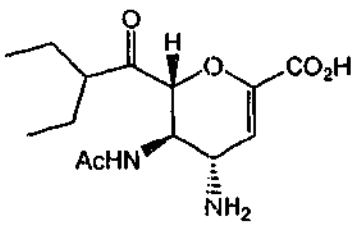
109 X = $CH(OH)CH_2OH$

Attempts to replace the glycerol side chain resulted in the development of a number of analogues[9] with varying degrees of inhibition against influenza sialidase, such as **110**[229]

with a branched hydroxy-substituted alkyl chain and its corresponding oxidized ketone form **111**[229], or **112**[230] with an isopropyl group as the aglycon unit. Most notably are the cyclohexene-based analogue **33**[10] and the five-membered ring analogue **36**[107]. **33** with a 1-ethylpropoxyl group, and **36** with an acetamino-substituted alkyl chain exhibited inhibition of influenza virus sialidase comparable to Zanamavir (**28**).



110



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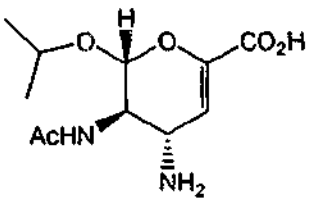

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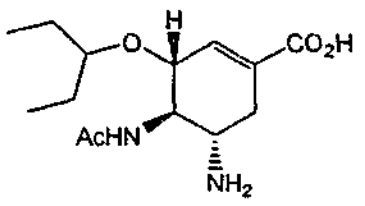

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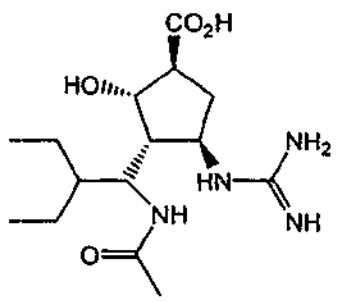


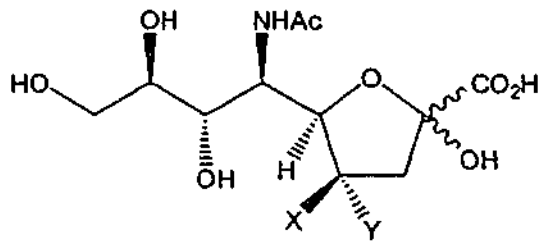
36

To gain insight into the role of the side chain on the furanose template, we sought to prepare a number of C-6 modified fructofuranosidonic acids and derivatives.

3.2 Synthesis of methyl 6-acetamido-4-amino-4,6-dideoxy-β-D-*arabino*-hex-2-ulofuranosidonic acid (125)

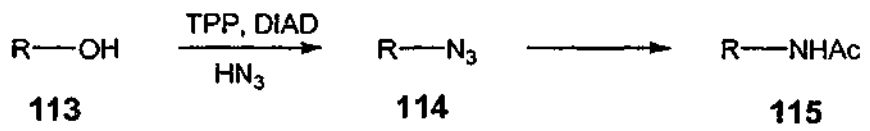
Having successfully prepared furanose esters with an azido functionality at C-4 from D-fructose, we then set out to synthesize C-6 modified 4-amino ester and acid derivatives. As discussed in section 1.2.3, successful Neu5Ac2en-based sialidase inhibitors appear to require a number of functional groups including an *N*-acetyl group. Therefore, it was

desirable to incorporate an *N*-acetyl functionality at the 6-position of fructofuranose esters comparable to that found in the five-membered ring sialidase inhibitors[106] **34** and **35** (*vide infra*).



34 (X = H, Y = OH)
35 (X = OH, Y = H)

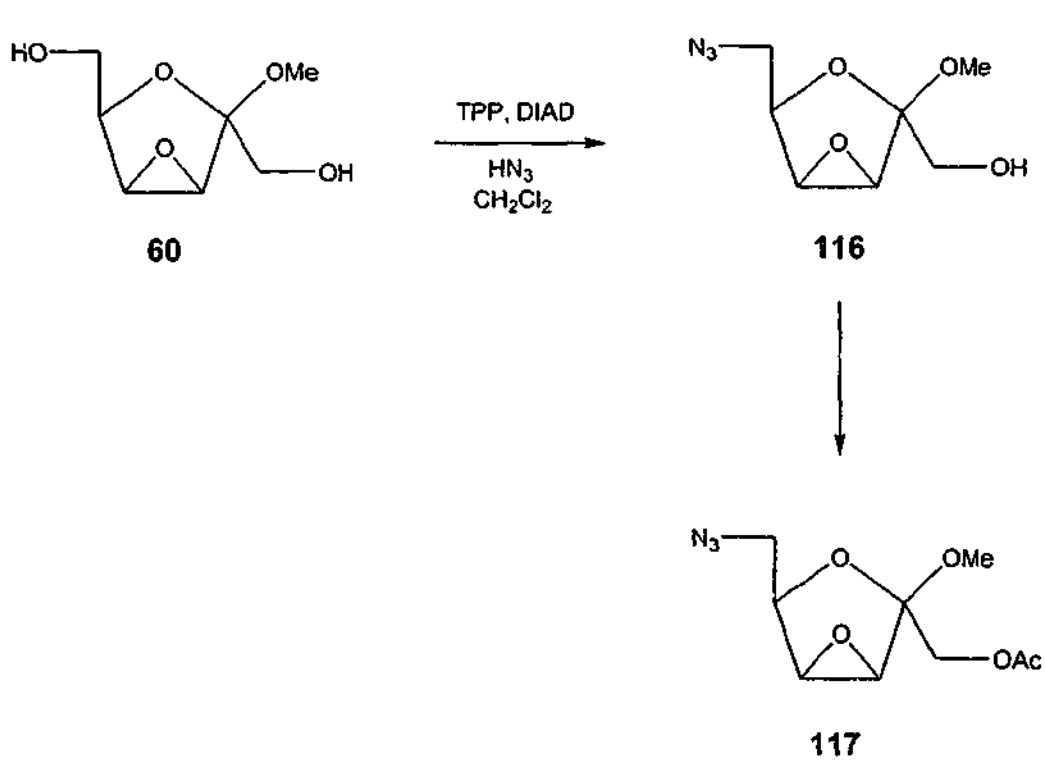
The conversion of an alcohol such as **113** to the *N*-acetyl compound **115** was thought to be achievable by reaction of **113** with hydrazoic acid under Mitsunobu conditions[185] to give the 6-azido compound **114**, which can be then transformed into **115** under standard conditions.



113 **114** **115**

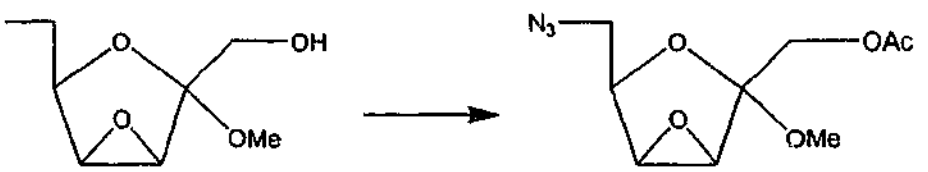
In Chapter 2 we have described the selective benzoylation of the anhydro sugars **59** and **60** employing Mitsunobu conditions[185] to provide exclusively the 6-benzoate compounds **88** and **91**, respectively. It was envisaged that using Mitsunobu conditions that these anhydro sugars upon treatment with hydrazoic acid would also selectively react at the 6-position without affecting the 1-hydroxyl group.

Accordingly, treatment of methyl 3,4-anhydro-β-D-tagatofuranoside (**60**) with TPP, DIAD and hydrazoic acid gave the 6-azido compound **116** in 66% yield. Formation of **116** was supported by mass spectrometry, which showed a molecular ion plus $^{+}NH_4$ peak at *m/z* 219, in 54% abundance. The IR spectrum recorded a strong absorption at 2130 cm^{-1} confirming the presence of the azido functionality. In the ^1H NMR spectrum, the methylene protons were observed at δ 3.49 and 3.56 ppm, as the result of the upfield shifts of 0.36 and 0.29 ppm, respectively, from those of the starting material. ESI mass spectrometry supported the molecular formula for **116**. In order to unambiguously determine that the azido group was at the 6-position, **116** was acetylated to provide **117**. ^1H NMR analysis revealed that the 1-methylene protons, which resonated at approximately δ 3.6- 3.7 ppm in **60** and **116**, have shifted downfield to δ 4.05 and 4.28 ppm in **117**, confirming the position of the acetate group at C-1.



60 **116** **117**

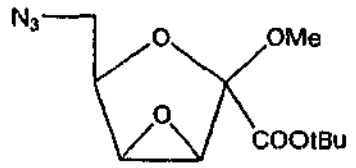
Similar to the β-epoxide **60**, Mitsunobu reaction of the α-epoxide **59** with hydrazoic acid gave exclusively the 6-azido compound **118** in 69% yield. The presence of the 6-azido group was supported by IR, ^{1}H NMR spectroscopy and also by mass spectroscopy. Acetylation of **118** to give **119** further confirmed the structure of **118**.



118 **119**

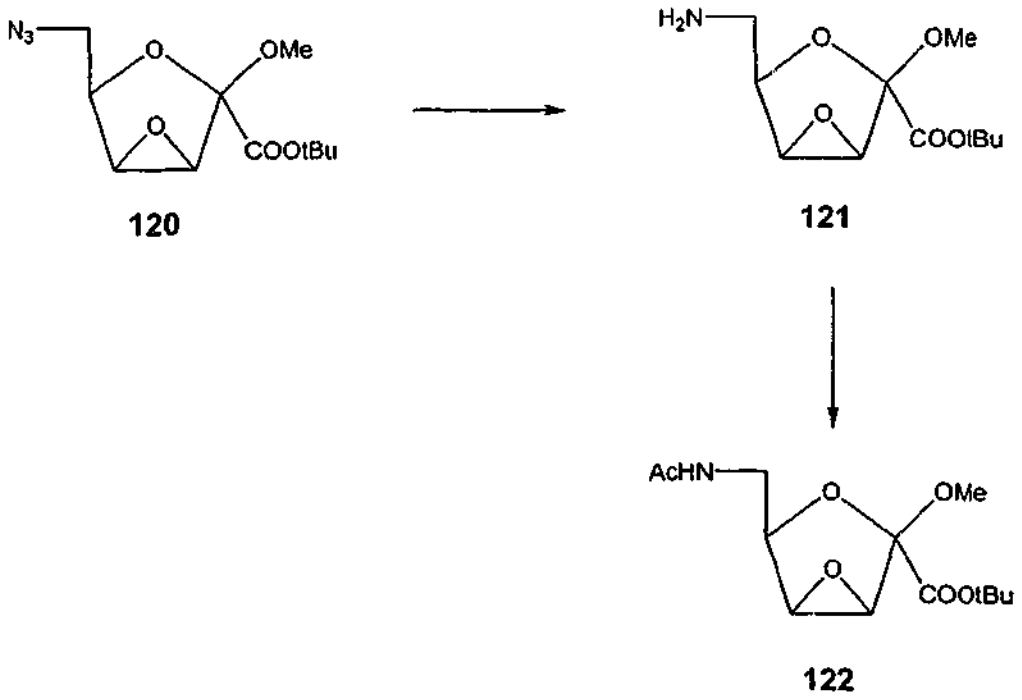
Having successfully prepared the 6-azido anhydro compounds, we then proceeded to synthesize the 4-azido-6-acetamido esters. It was envisaged that the synthesis of these esters would be similar to that of the 4-azido-6-*O*-benzoyl esters described in Chapter 2.

Thus, oxidative-esterification of **116** using PDC, acetic anhydride and *tert*-butyl alcohol in DMF-CH_2Cl_2 (1:4) gave the *tert*-butyl ester **120**. Inspection of the ^{1}H and ^{13}C NMR spectra revealed the disappearance of the 1-methylene group in **120**. Resonances of the ester *tert*-butyl group were observed as a nine proton singlet at δ 1.50 ppm. In the ^{13}C NMR spectrum, the resonances at δ 28.2 ppm for three methyl carbons, at δ 83.7 ppm for the quaternary carbon, and at δ 166.7 ppm for the carbonyl carbon, were assigned to the *tert*-butyl ester group. High resolution mass spectrometry confirmed the molecular formula for **120**.



120

Hydrogenation of the 6-azido compound **120** gave the amine **121**. Without further purification, the amine was subjected to acetylation using acetic anhydride and pyridine, affording after column chromatography the 6-acetamido compound **122** in 83% yield from **120**. ^{1}H NMR analysis revealed the resonances of the *N*-acetyl methyl group as a three proton singlet at δ 2.01 ppm. In the ^{13}C NMR spectrum, resonances for the *N*-acetyl carbons were observed at δ 23.0 ppm for the methyl carbon, and at either δ 166.4 or 170.8 ppm for the carbonyl carbon, with the other being for the ester carbonyl. ESI high resolution mass spectroscopy showed a protonated molecular ion peak at *m/z* 288.14427 confirming the molecular formula of $C_{13}H_{21}NO_6$ for **122**.

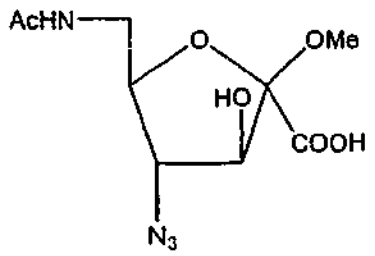


120 **121** **122**

Exposure of **122** to lithium azide and Dowex-50X-400 (H^+) resin in DMF for 16 hours at *ca.* 95°C resulted in the epoxide-ring opening to give the 4-azido-6-acetamido ester **123** in 80% yield. The IR spectrum recorded a strong absorption at 2132 cm^{-1} confirming the presence of an azido group. That the epoxide-ring opening proceeds favourably[173,174] at C-4, due to the neopentyl-type nature of C-3 (*vide supra*), was supported by ^1H NMR analysis, which revealed the downfield shift of H-3 from δ 3.82 ppm in **122** to δ 4.16 ppm in **123**, as a result of the presence of the 3-hydroxyl group. High resolution mass spectrometry confirmed the molecular formula for **123**.

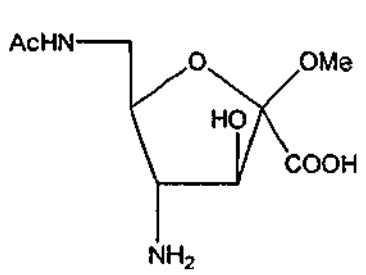
122 **123**

Hydrolysis[233] of the ester **123** was performed by stirring a solution of **123** in dichloromethane with trifluoroacetic acid for 4 hours at room temperature, to provide the acid **124** as a white amorphous mass. A protonated molecular peak in the mass spectrum at *m/z* 275, in 46% abundance, indicated the hydrolysis had occurred. ^1H and ^{13}C NMR analysis revealed the disappearance of the *tert*-butyl group, thus confirming the formation of **124**.

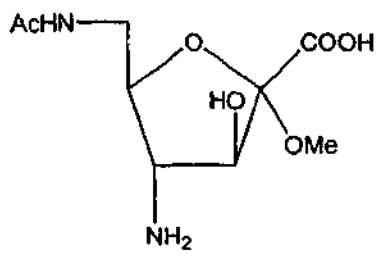


124

Reduction of the azide **124** under hydrogenation conditions gave the amine **125** as a white amorphous mass. Formation of **125** was supported by ^{1}H and ^{13}C NMR spectroscopy, and also by mass spectroscopy. Synthesis of the α-anomer **126** was envisaged to follow that of the β-amine **125**, however, as a result of time it was discontinued.



125



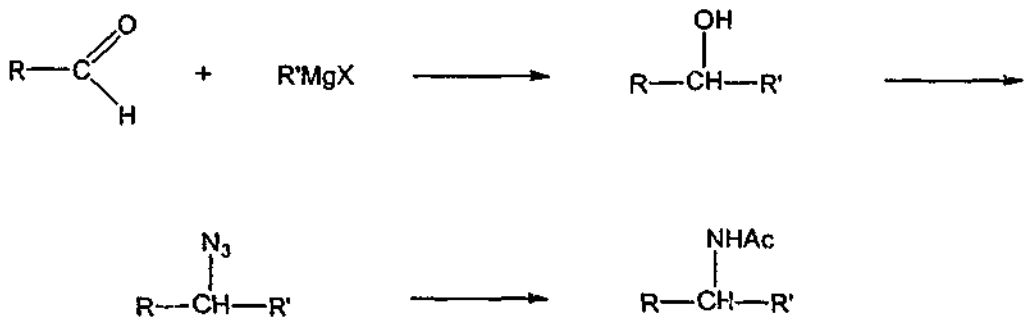
126

3.3 Synthesis of 6-acetamido-fructofuranosidonic acids and derivatives

As mentioned earlier, the *N*-acetyl group at C-5 and the side chain at C-6 of Neu5Ac2en (**26**) and sialic acid-based inhibitors play important roles in inhibitory activity[9] (*vide supra*). Having successfully prepared the 6-acetamido-4-amino acid **125**, we thought that carbon-carbon chain-extended C-6 modified esters and acids would be better mimetics of Neu5Ac2en (**26**) and its derivatives. Thus, we decided, firstly, to synthesize compounds with an *N*-acetyl functional group at the 6-position on the extended chain.

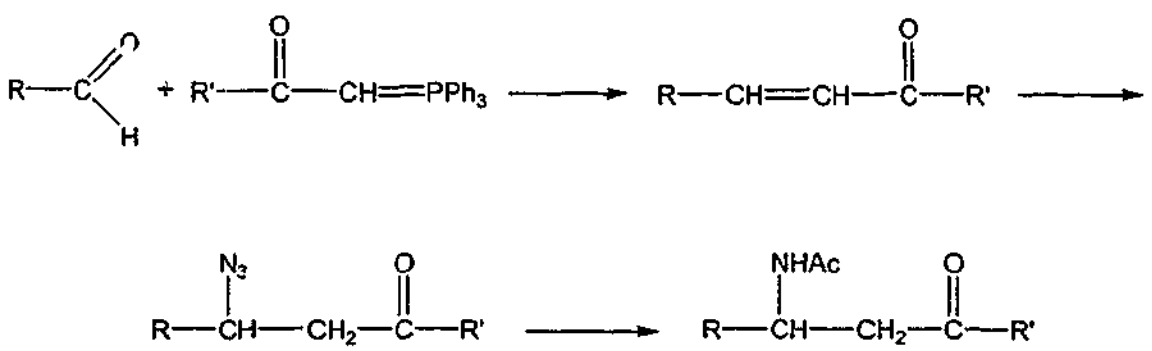
3.3.1 Formation of 4,5-unsaturated esters

The formation of carbon-carbon bonds followed by the introduction of an *N*-acetyl functionality can be achieved by a number of approaches, and numerous reagents are available that will lead to various carbon-carbon chain-extended products[169-171]. One approach is the use of an organometallic reagent in the presence of an aldehyde[169-171], the alcohol product formed can then be converted to an azide[185], the precursor of an *N*-acetyl compound (Scheme 3.1).



Scheme 3.1

Another approach is the use of a Wittig reaction[169-171] to form an α,β-unsaturated carbonyl compound from an aldehyde, followed by a Michael addition reaction to give an azide[276-279], which is then converted to an *N*-acetyl compound. (Scheme 3.2).

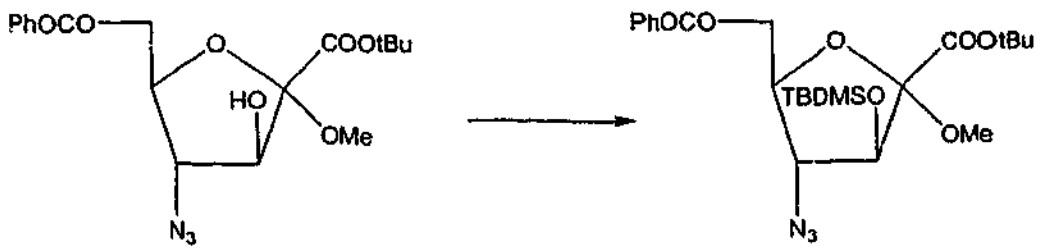


Scheme 3.2

As we were interested in the formation of an extended side chain with a carbonyl functionality besides the *N*-acetyl group, we decided to investigate the approach employing the Wittig reaction.

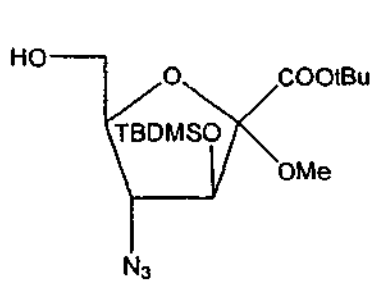
Before the carbon-carbon bond extension work on C-6 was carried out, it was necessary to protect the 3-hydroxyl group followed by removal of the benzoate protecting group on C-6. Towards this end, we decided to protect the 3-hydroxyl functionality using the *tert*-butyldimethylsilyl ether, as this protecting group is quite stable to basic hydrolysis and a variety of reagents[180], including those that we intended to employ for the C-6 modified chain extension work.

Thus, treatment of *tert*-butyl (methyl 4-azido-6-*O*-benzoyl-4-deoxy-α-D-fructofuranosid)onate (**90**) with *tert*-butyldimethylsilyl chloride and imidazole in DMF at 32°C for 18 hours gave the 3-silyl ether compound **127**, after column chromatography, in 90% yield. The presence of the TBDMS group was evident by analysis of ^{1}H and ^{13}C NMR spectra. Two singlets of a total of six protons at δ 0.14 and 0.16 ppm corresponded to two Si-methyl groups, and a nine proton singlet at δ 0.90 ppm was assigned for the Si-*tert*-butyl group. In the ^{13}C NMR spectrum, resonances for the TBDMS carbons were observed at δ -5.1 and -4.6 ppm for the two Si-methyl carbons, δ 18.0 ppm for the quaternary carbon and δ 25.7 ppm for the three methyl carbons of the Si-*tert*-butyl group. High resolution ESI mass spectrometry showed a molecular ion plus $^{+}NH_4$ peak at *m/z* 525.27571 confirming the molecular formula for **127**.



90 **127**

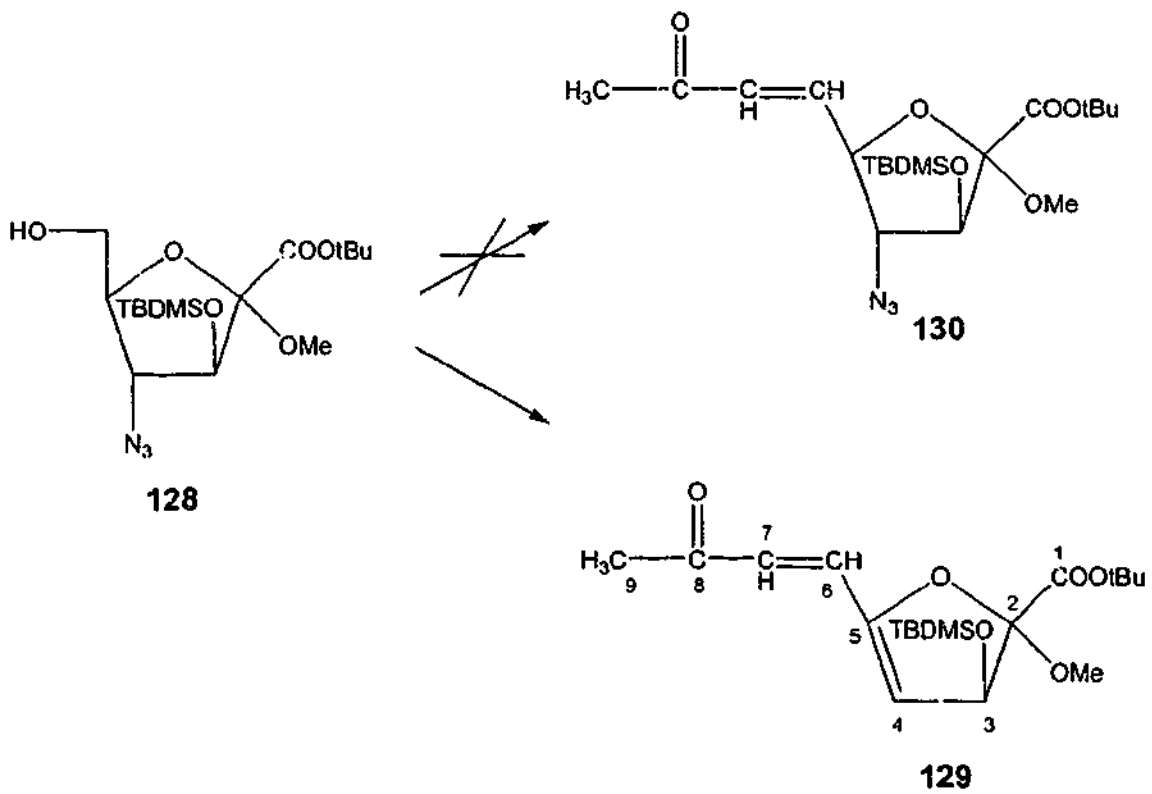
Removal of the 6-benzoate group of **127** was effected by treatment with sodium methoxide in methanol providing **128** in 92% yield. Loss of the benzoate group was confirmed as the resonances assigned to this group were absent in the ^{1}H and ^{13}C NMR spectra of **128**. The ESI high resolution mass spectrum recorded a molecular ion plus $^{+}NH_4$ peak at *m/z* 421.24594 which was consistent with the molecular formula of $C_{17}H_{33}N_3O_6Si$ for **128**.



128

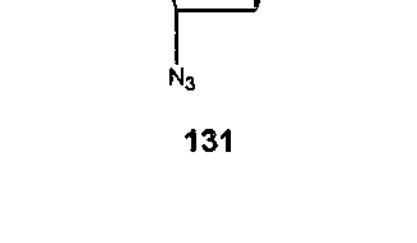
Having successfully prepared the 6-hydroxy compound **128**, we then focussed our attention on the conversion of this compound to an aldehyde, which will be the substrate in the subsequent Wittig reaction. Thus, conversion of the 6-primary hydroxyl group of **128** to the aldehyde functionality was effected by the exposure of **128** to oxalyl chloride and dimethyl sulfoxide in dichloromethane[231]. After workup, the aldehyde was used in the next step without further purification. Treatment of this aldehyde with 1-triphenylphosphoranylidene-2-propanone in toluene for 16 hours gave the unstable product

129, which decomposed quickly in solution. Analysis of the ^{1}H NMR spectrum of this compound showed the incorporation of the ketone moiety of the Wittig reagent at δ 6.94 and 6.63 ppm for the olefinic protons H-6 and H-7, respectively, and δ 2.31 ppm for three protons at C-9. The coupling constant between H-6 and H-7 was determined to be 15.9 Hz, which indicated that the C-6, C-7 double bond assumed the *trans* configuration[275]. However, further inspection of the spectrum revealed that H-4 resonated further downfield as a doublet at δ 5.43 ppm, from δ 3.99 ppm in **128**, and no resonance for H-5 was observed. It was concluded that **129** was not the desired product **130**, most likely, it resulted from the elimination of HN_3 in the process (Scheme 3.3). As **129** was very unstable and quickly decomposed, other characterization with ^{13}C NMR spectroscopy, mass spectrometry or IR were not carried out.

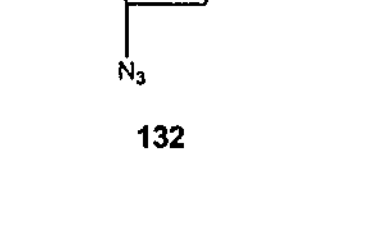


Scheme 3.3

Given the disappointing result of the chain extension work for the α-ester, we then attempted to perform the carbon-carbon bond extension reactions using the β-ester. Thus, exposure of *tert*-butyl (methyl 4-azido-6-*O*-benzoyl-4-deoxy-β-D-fructofuranosid)onate (**93**) to *tert*-butyldimethylsilyl chloride and imidazole in DMF at 32°C for 18 hours afforded the 3-silyl ether compound **131** in 87% yield, as a syrup. The presence of the TBDMS group was confirmed by ^{1}H and ^{13}C NMR spectroscopy and also by mass spectroscopy. Treatment of **131** with sodium methoxide in methanol gave the 6-hydroxy compound **132** in excellent yield (95%).



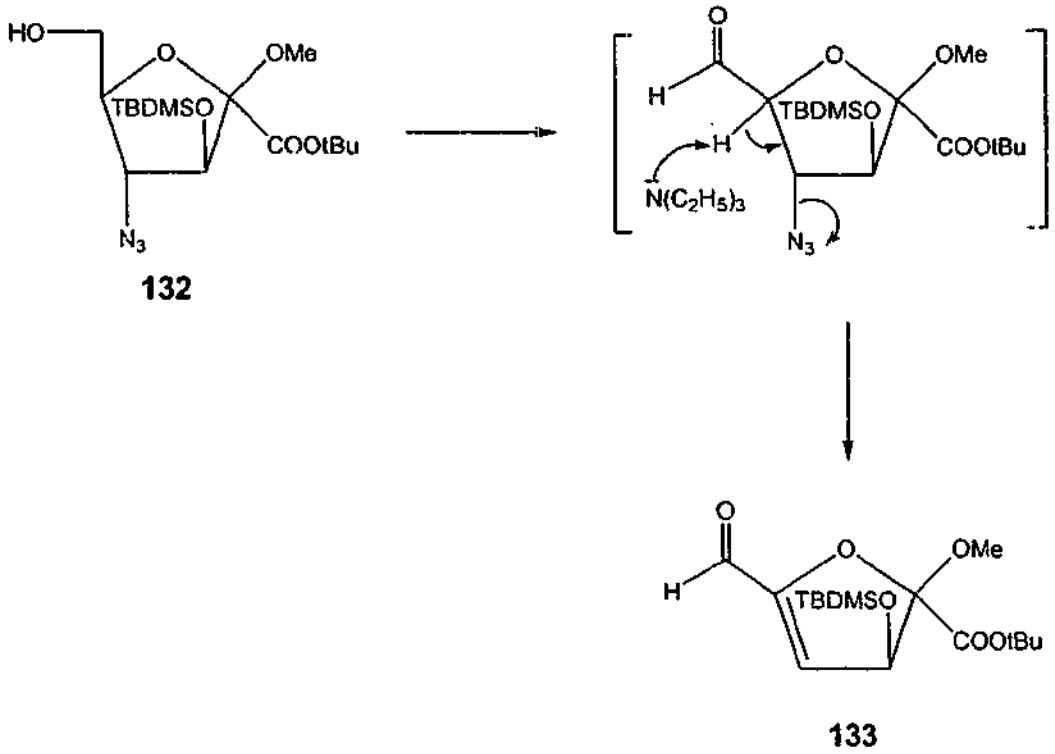
131



132

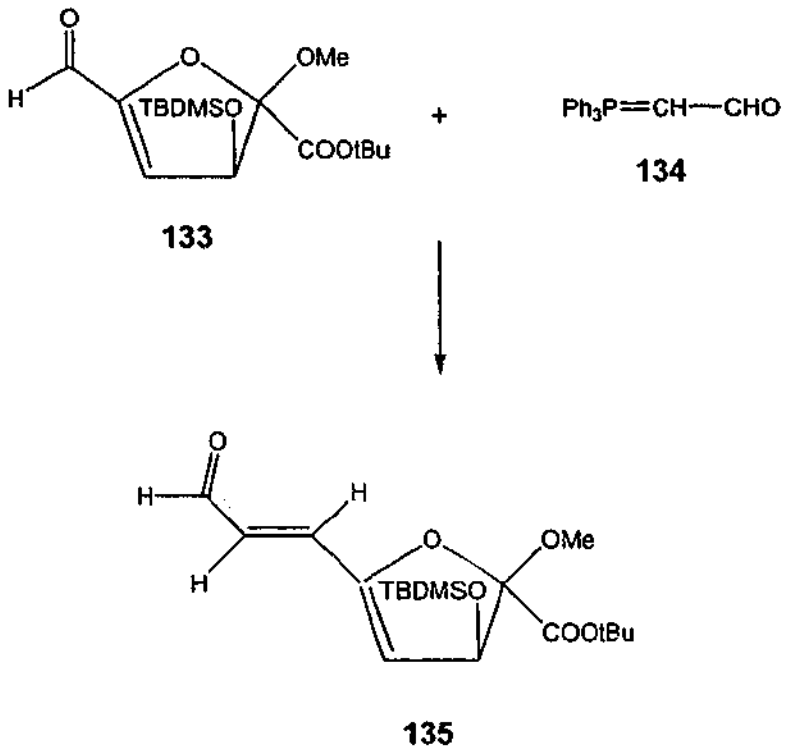
Having prepared the alcohol **132**, we then proceeded to the oxidation step. Thus, **132** was exposed to oxalyl chloride and dimethyl sulfoxide in dichloromethane at *ca.* -60°C for 45 minutes, followed by treatment with triethyl amine[231]. Workup of the reaction mixture gave a residue, which was chromatographed on a short silica column giving a syrup. ^{1}H NMR analysis of this material (**133**) showed a one proton singlet at δ 9.50 ppm, which was characteristic of the aldehyde proton[275]. However, the fact that H-4 resonated as a doublet at δ 5.96 ppm, further downfield compared to δ 3.90 ppm of H-4 in the starting material, and no resonance for H-5 was observed, indicated that a β-elimination of the 4-

azido group had occurred, most likely, subsequent to the formation of the aldehyde functionality. The proposed mechanism of the elimination step is shown in Scheme 3.4.



Scheme 3.4

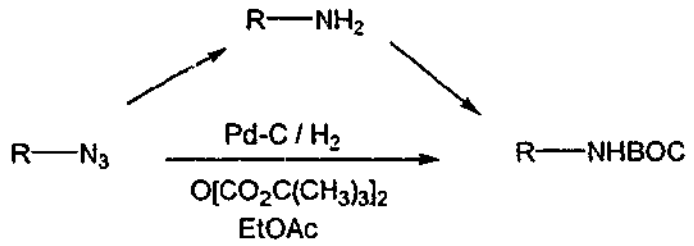
Reaction of the aldehyde **133** with (triphenylphosphoranylidene)acetaldehyde (**134**) in toluene gave the Wittig adduct **135**, as an unstable compound. Examination of the ^1H NMR confirmed the presence of the double bond between C-4 and C-5, which was evident by a one proton doublet for H-4 at δ 5.80 ppm and the absence of the resonance for H-5. The presence of the aldehyde functionality of the adduct was supported by the resonance of the aldehyde proton as a doublet at δ 9.50 ppm. That the C-6, C-7 double bond adopted the *trans* configuration[275] was confirmed by the resonances of H-6 and H-7 at δ 7.27 and 6.45 ppm, respectively, with a mutual coupling constant of 15.6 Hz.



3.3.2 Synthesis of methyl 5-(1-acetamino-3-oxobutyl)-4-amino-4-deoxy-α-D-*arabino*-pent-2-ulofuranosidonic acid (145)

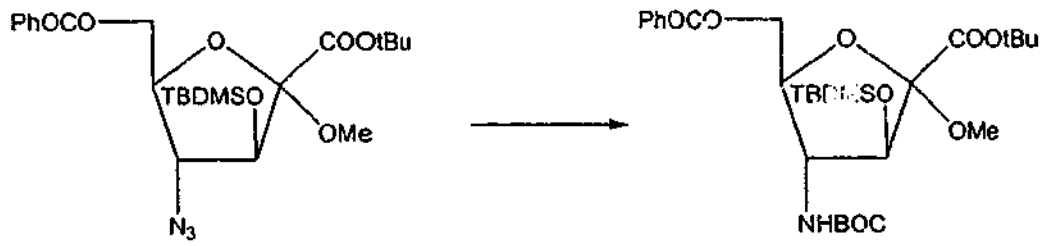
Given the problem of the elimination of the 4-azido group caused by the formation of an aldehyde functionality to create a conjugated α,β-unsaturated carbonyl compound, it was considered prudent to reduce the 4-azido group prior to forming the aldehyde functionality at C-5. Thus, *tert*-butyl carbamate (BOC) was selected as the amine protecting group for two reasons. Firstly, it is inert under basic conditions, and secondly, the BOC group can subsequently be hydrolyzed together with the *tert*-butyl ester under acidic conditions (*i.e.* trifluoroacetic acid).

Conversion of an azido functionality to the protected amine is carried out in two steps, with, firstly, the reduction of the azide to an amine, followed by conversion of the amine to a *tert*-butyl carbamate compound. In 1989, Saito *et al.*[232] reported a convenient one pot reaction for such a transformation, employing palladium on charcoal and di-*tert*-butyl dicarbonate in a hydrogen atmosphere (Scheme 3.5).



Scheme 3.5

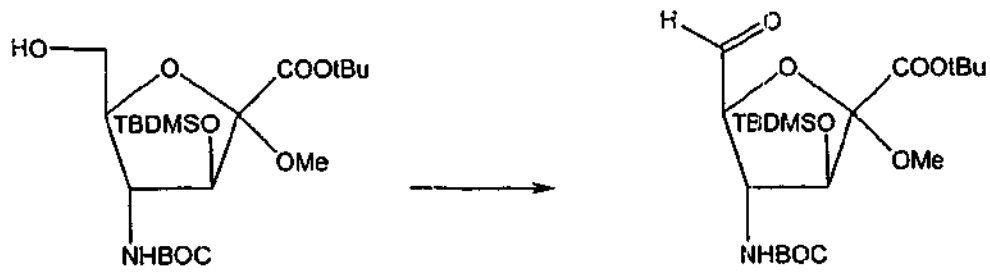
Accordingly, exposure of **127** to Pd/C (10%) and di-*tert*-butyl dicarbonate in ethyl acetate, in a hydrogen atmosphere for 20 hours, provided the 4-*N*-(*tert*-butoxycarbonyl) compound **136** in 93% yield. A protonated molecular ion peak at *m/z* 582 in 81% abundance indicated the formation of **136**. The presence of the BOC group was observed in both ^{1}H and ^{13}C NMR spectra. High resolution mass spectrometry confirmed the molecular formula for **136**.



Having prepared the *N*-protected compound **136**, we then proceeded to hydrolyze the 6-benzoate group prior to the oxidation of the 6-hydroxyl group to an aldehyde functionality for the carbon-carbon bond forming Wittig reaction.

Treatment of **136** with sodium methoxide in methanol gave the 6-hydroxy compound **137** in 97% yield. Oxidation of **137** using oxalyl chloride and dimethyl sulfoxide in dichloromethane at *ca.* -60°C provided the aldehyde **138**. Inspection of the ^{1}H

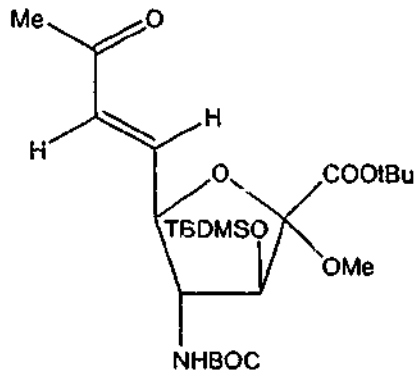
NMR spectrum of **138** revealed a one proton signal at δ 9.71 ppm, confirming the presence of an aldehyde functionality. H-3 and H-4 were observed to resonate as a two proton multiplet at δ 4.32 ppm, and the presence of H-5 was confirmed by a one proton multiplet at δ 4.11 ppm. A broad doublet at δ 5.32 ppm was assigned to the nitrogen-proton.



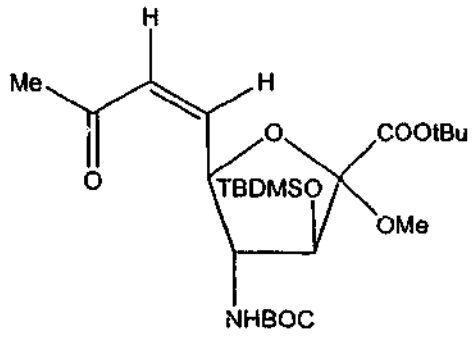
137 **138**

The Wittig reaction of **138** with 1-triphenylphosphoranylidene-2-propanone was carried out in toluene, at room temperature, giving two geometrical isomers, which were separated by column chromatography. The fast moving component was the minor product **139**, the *Z* isomer, and the major product **140**, the slow moving component, was the *E* isomer, in 14% and 46% yields, respectively, from **137**. It is worth noting that the *Z* isomer isomerized to the *E* isomer after being left in solution for 24 hours. Low resolution mass spectrometry of the major product **140** revealed the incorporation of the methyl ketone moiety with the presence of a molecular ion plus $^{+}NH_4$ peak at *m/z* 533, in 100% abundance. ESI high resolution mass spectrometry confirmed the molecular formula for **140**. In the ^{1}H NMR spectrum, the 9-methyl protons were observed at δ 2.26 ppm. Resonances for the olefinic protons H-6 and H-7 were at δ 6.87 and 6.45 ppm, respectively, with a coupling constant[275] calculated for these protons of 16.0 Hz, which confirmed the *E* configuration.

That the minor product **139** adopted the *Z* configuration was supported by the examination of the ^{1}H NMR spectrum, which revealed the presence of the olefinic protons H-6 and H-7 at δ 6.28 and 6.19 ppm, respectively, with a mutual coupling constant[275] of 11.5 Hz.



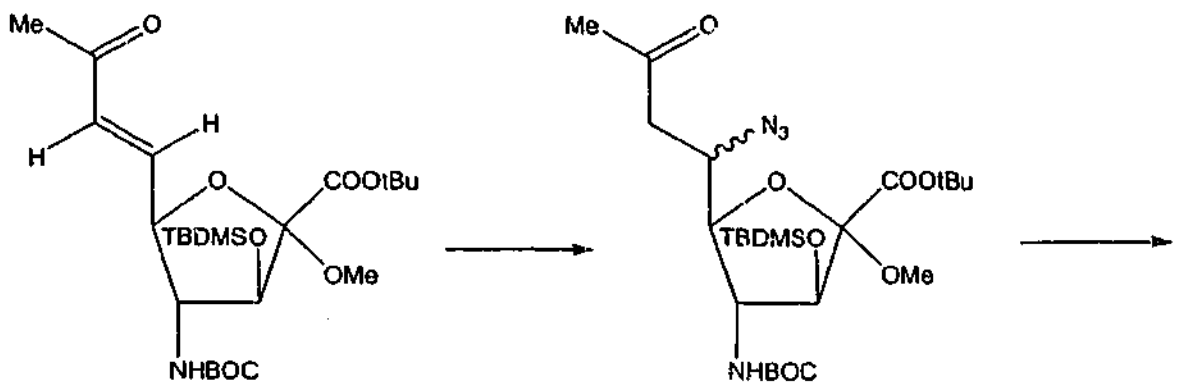
140



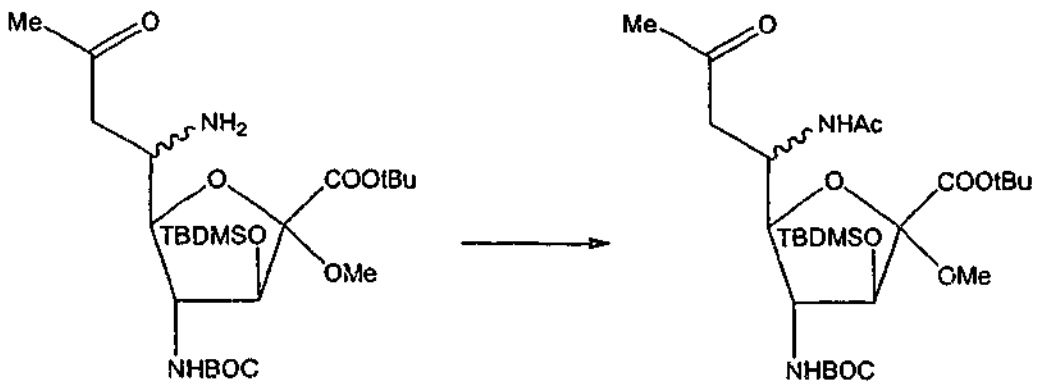
139

Having successfully synthesized the ketone **139** and **140**, we then focussed on the introduction of an *N*-acetyl group at the 6-position. It was envisaged that an azido group could be first introduced at C-6 employing Michael reaction[276-279], followed by conversion to the acetamido functionality (*vide infra*).

Thus, exposure of **140** to trimethylsilyl azide[278] in the presence of a catalytic amount of Bu_4NF in CH_2Cl_2 at -20°C for four days gave the azide **141**. Without further purification, this azide was hydrogenated in the presence of 10% Pd/C. The crude amine **142** obtained was then acetylated using acetic anhydride and pyridine, affording the 6-acetamido compound **143** in an overall yield of 59% for the three steps involved.



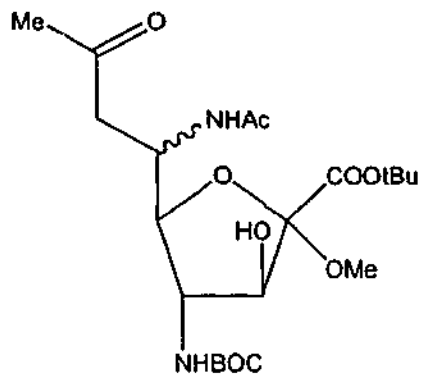
140 **141**



142 **143**

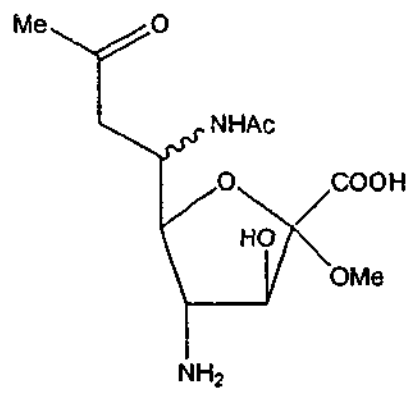
Having synthesized the 6-acetamido compound **143**, we then proceeded to the next steps which involved hydrolysis of the *tert*-butyl ester, and the removal of protecting groups for the 3-hydroxy and the 4-amino functionalities. The TBDMS group can be cleaved using tetrabutylammonium fluoride[183], and hydrolysis of the *tert*-butyl ester[233] and the *tert*-butyl carbamate[234] group can be effected by treatment with trifluoroacetic acid. Notwithstanding the order of deprotection, the same end product should be obtained.

Accordingly, exposure of **143** to tetrabutylammonium fluoride in THF for 2 hours at room temperature gave the 3-hydroxy compound **144**, in 93% yield. ^{1}H and ^{13}C NMR spectroscopy confirmed the disappearance of the TBDMS group. ESI high resolution mass spectrum supported the molecular formula for **144**.



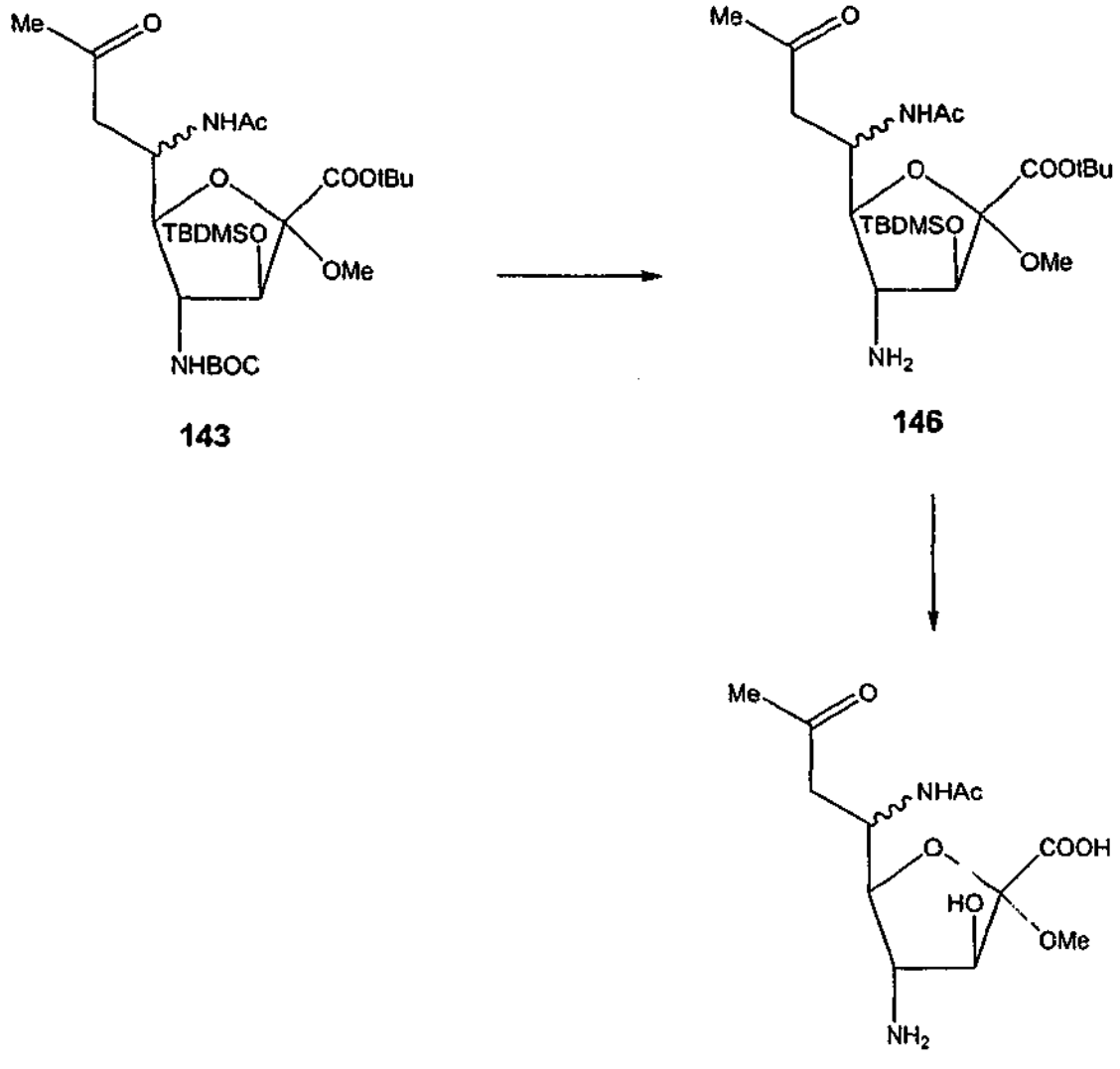
144

Treatment of **144** with trifluoroacetic acid in dichloromethane provided the 4-amino acid **145**, as an amorphous mass. Formation of **145** was supported by ^{1}H and ^{13}C NMR spectroscopy and also by mass spectroscopy.



145

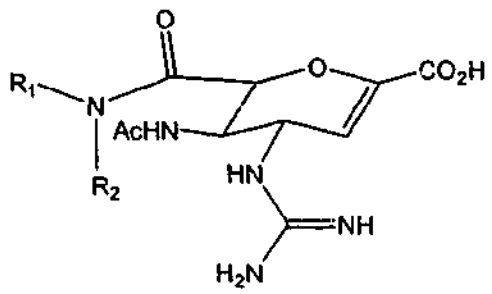
Alternatively, hydrolysis of the *tert*-butyl ester and the *tert*-butyl carbamate group can be carried out first by treatment of **143** with trifluoroacetic acid to give the acid **146**. Desilylation of **146** using tetrabutylammonium fluoride provided the target compound **145**. The overall yields for the two steps are similar for both routes.



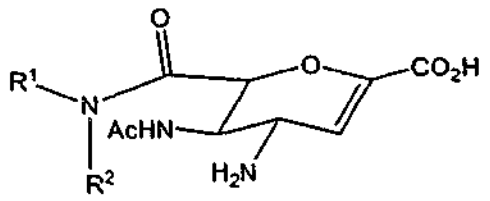
143 146 145

3.4 Synthesis of methyl 4-amino-4-deoxy-5-(1-hydroxy-2-nitroethyl)-α-D-*arabino*-pent-2-ulofuranosidonic acid (150)

The incorporation of nitrogen containing functionalities into the side chain of sialidase inhibitors has been reported[9,100]. Compounds **29** and **147** having a carboxamide functionality on the side chain have exhibited potent influenza sialidase inhibitory activity[9,100].



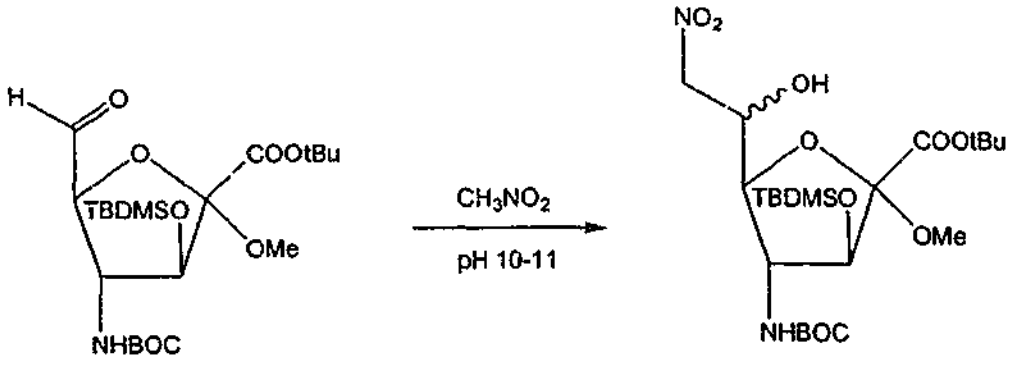
29 R_1/R_2 = alkyl, aryl



147 R_1/R_2 = alkyl, aryl

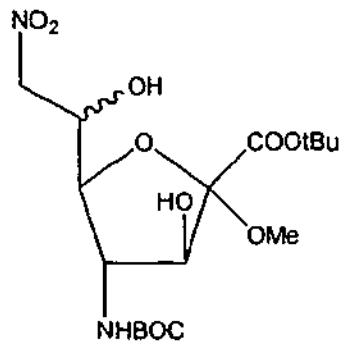
To date, there are no reports in the literature concerning the nitro functionality on the side chain of sialidase inhibitors. Therefore, the synthesis of a fructofuranosidonic acid with a nitro functional group on the side chain was of interest to us.

Thus, treatment of the aldehyde **138** with nitromethane at pH 10-11 for 5 hours at room temperature gave the 6-hydroxy-7-nitro compound **148**, as an inseparable diastereomeric mixture, in 62% yield. Formation of **148** was supported by mass spectrometry, which showed a molecular ion plus $^{+}NH_4$ peak at *m/z* 554 in 5% abundance. In the ^{1}H NMR spectrum, H-6, H-7 and H-7' were observed to resonate at δ 4.55-4.75 ppm. In the ^{13}C NMR spectrum, resonances of C-6 and C-7 were at δ 68.5 and 78.1 ppm, respectively. High resolution mass spectrometry confirmed the molecular formula for **148**.



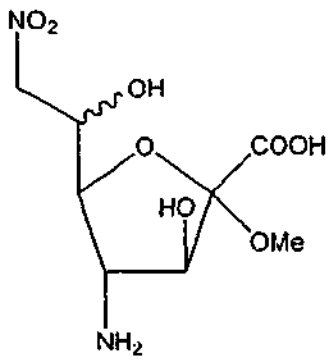
138 **148**

Exposure of **148** to tetrabutylammonium fluoride in THF for 90 minutes at room temperature gave the 3-hydroxy compound **149** in 89% yield. ^{1}H NMR analysis revealed the disappearance of the TBDMS group indicating that desilylation had occurred. High resolution mass spectrometry confirmed the molecular formula for **149**.



149

Treatment of **149** with trifluoroacetic acid in dichloromethane for 16 hours at room temperature gave the acid **150**, as a white amorphous mass. Formation of **150** was supported by ^{1}H NMR analysis. High resolution mass spectroscopy confirmed the molecular formula for **150**.

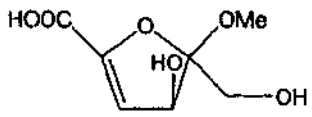
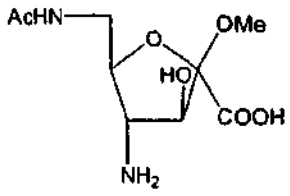
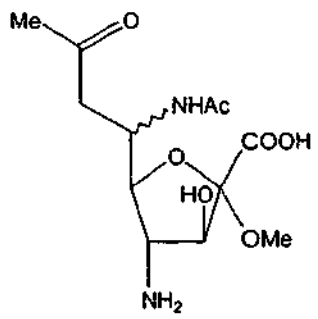
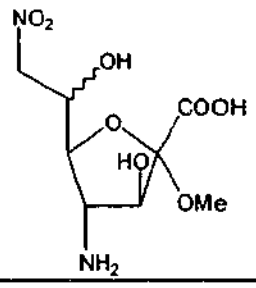


150

3.5 Biological evaluation of fructofuranosidonic acids

The biological activity of the previously synthesized acids **95**, **125**, **145**, and **150** against Influenza virus sialidase (N9) was investigated. Inhibition assays[104,288] were performed using 4-methylumbelliferyl *N*-acetyl-α-D-neuraminic acid as the substrate. The activity of the sialidase was measured by the release of 4-methylumbelliferone, which was detected fluorometrically. The results in Table 3.1 showed that none of the compounds tested displayed significant inhibitory activity against the enzyme at 1mM concentration of compounds. The 6-acetamido compound **125** exhibited a low level of inhibition (10%). No inhibition was observed for **145** and **150**. It is interesting to note that the α,β-unsaturated acid **95** showed activity against N9 sialidase, albeit at a low level of inhibition.

Table 3.1 Inhibition of influenza virus sialidase (N9)[a].

Compound		% Inhibition[b,c]
95		10
125		11
145		NI
150		NI

(a) These assays were performed by Samia Abo and Carolyn Trower[172].
(b) % inhibition at 1 mM.
(c) "NI" no inhibition.

3.6 Conclusion

We have successfully prepared the 6-acetamido-4-amino acid **125** from the anhydro sugar **59**. Although some difficulties were initially encountered, the syntheses of the C-6 chain-extended compounds **145** and **150** were achieved from the corresponding ester **127**.

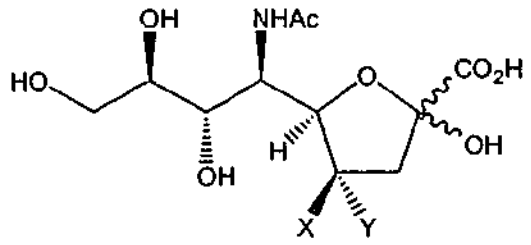
However, none of the target compounds displayed significant inhibition against the influenza virus sialidase. This observation suggests perhaps that more modifications at C-6 or at other carbon centres of the fructofuranoses should be undertaken in order to gain a better insight into the behaviour of fructofuranose derivatives towards influenza virus sialidase.

CHAPTER 4

An Alternative Synthesis of Furanose-Based Sialidase Inhibitors

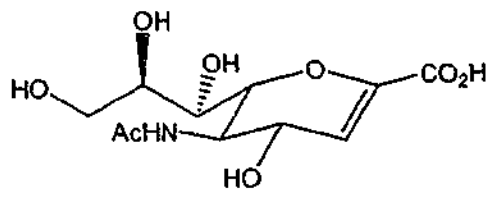
4.1 Introduction

In 1992 Yamamoto *et al.*[106] reported the synthesis of five-membered ring analogues (**34** and **35**) of *N*-acetylneuraminic acid, which were found to inhibit influenza virus sialidase with a potency comparable to that of Neu5Ac2en (**26**). The sialic acid derivative Neu5Ac2en (**26**) is a potent inhibitor[104,235] of influenza sialidase *in vitro* with a K_i value of $4x10^{-6}$ M.



34 (X = H, Y = OH)

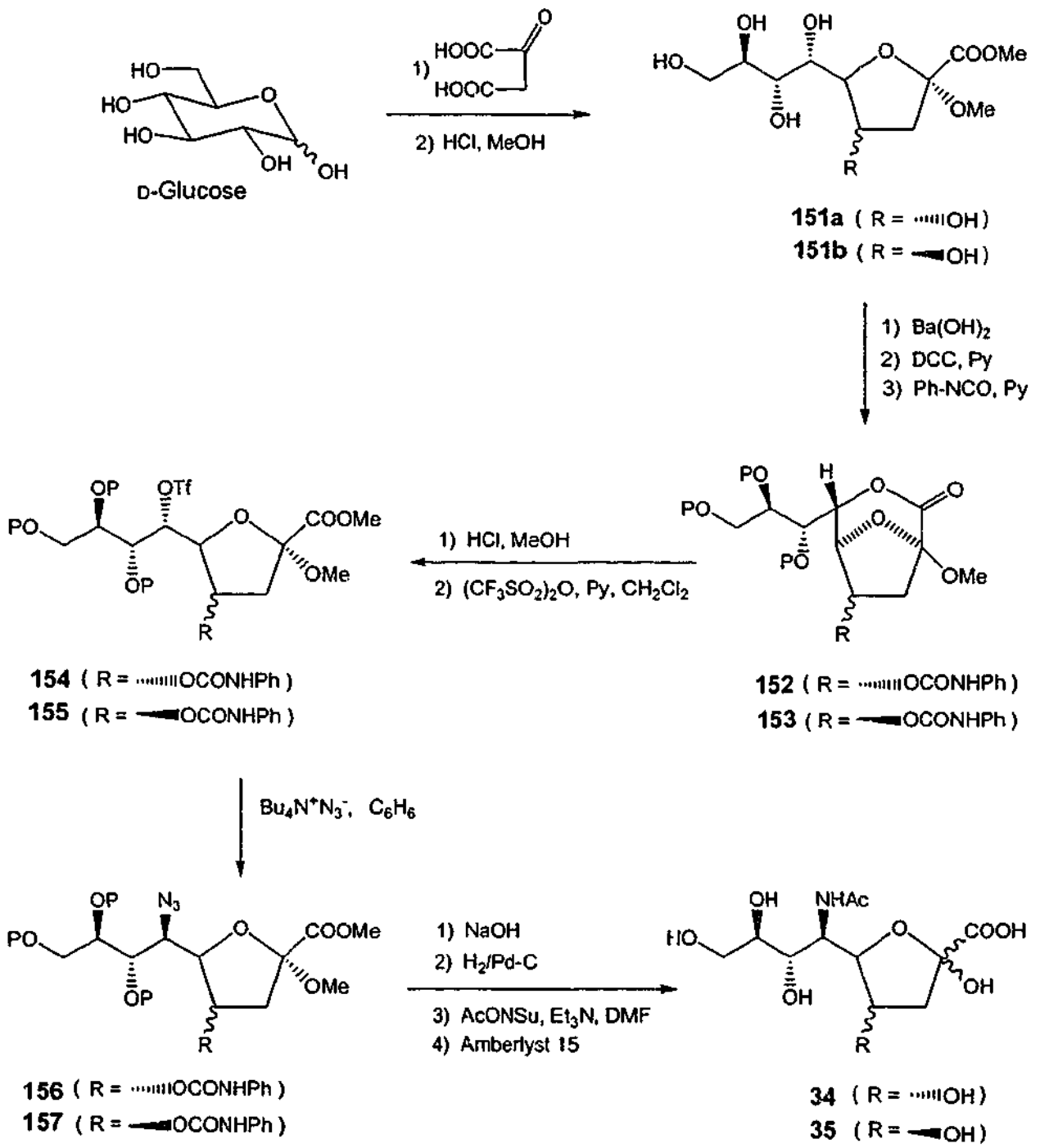
35 (X = OH, Y = H)



26

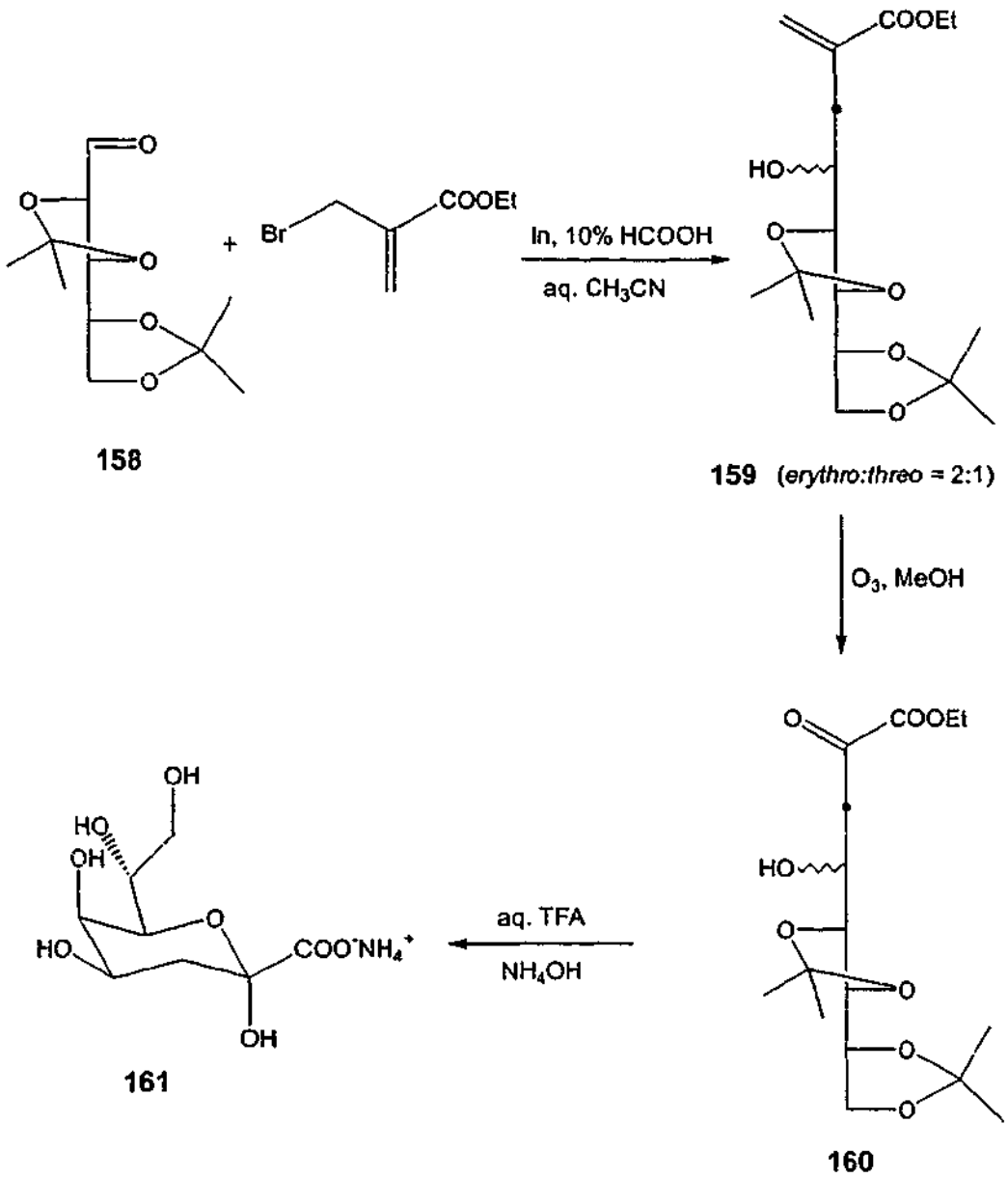
The synthesis of **34**, shown in Scheme 4.1, involved the aldol condensation of D-glucose with oxalacetic acid, followed by treatment with HCl in methanol to give a mixture of five isomers, including **151a** and **151b,** which were separated by HPLC. **151a** was converted to the key intermediates 6-azido derivative **156** via the triflate compound **154**. Compound **35** was prepared from **151b** following the same route as that for **34**. The preparation of **34** and **35** from D-glucose involved twelve steps with poor overall yields[106,236] of 1.2 and 0.4%, respectively. It was, therefore, of interest to us to investigate an alternative synthesis of **34** and **35** with a view to improving the overall yield.

Furthermore, synthesis of analogues of **34** and **35** with an acetamido functionality at C-4 was also attractive to us as these compounds are potentially useful biological probes and this would provide further insight into the role of different functional groups at C-4.



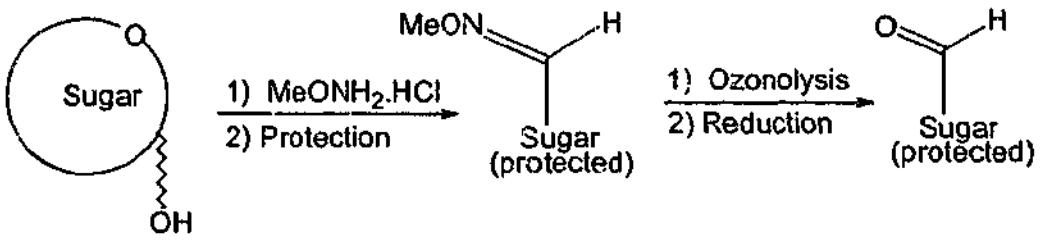
Scheme 4.1

In 1980, Luche and Damiano[243] reported the coupling reactions of non-sugar carbonyl compounds with allylic halides in the presence of zinc or tin metal in good to excellent yields. Vasella[244,245], Whitesides[75,246,247,249], Chan[250-252], and others[43,61,253] have utilized these metal-mediated reactions to prepare a wide range of carbohydrate compounds. Of particular interest were the synthesis of sialic acids and analogues[43,61,75,249-252], exemplified by the synthesis[249] of KDO shown in Scheme 4.2. The synthetic sequence involved the formation of **159** from the reaction of ethyl α-(bromomethyl)acrylate with 2,3:4,5-di-*O*-isopropylidene-D-arabinose (**158**) in the presence of indium metal. Ozonolysis of the major component (*erythro* compound) provided the keto ester **160**. Hydrolysis of the ester and acetonides followed by neutralization with aqueous ammonia gave the ammonium salt of KDO (**161**).



Scheme 4.2

Weitz and Bednarski[254], in 1989, reported an efficient method for the preparation of acyclic sugar aldehydes based on the ozonolysis of methyloxime-protected aldoses. The acyclic sugar oximes, readily accessible from carbohydrates by treatment with methoxylamine hydrochloride, can be protected, and the oxime ozonolised to generate acyclic aldehydo sugars (Scheme 4.3).

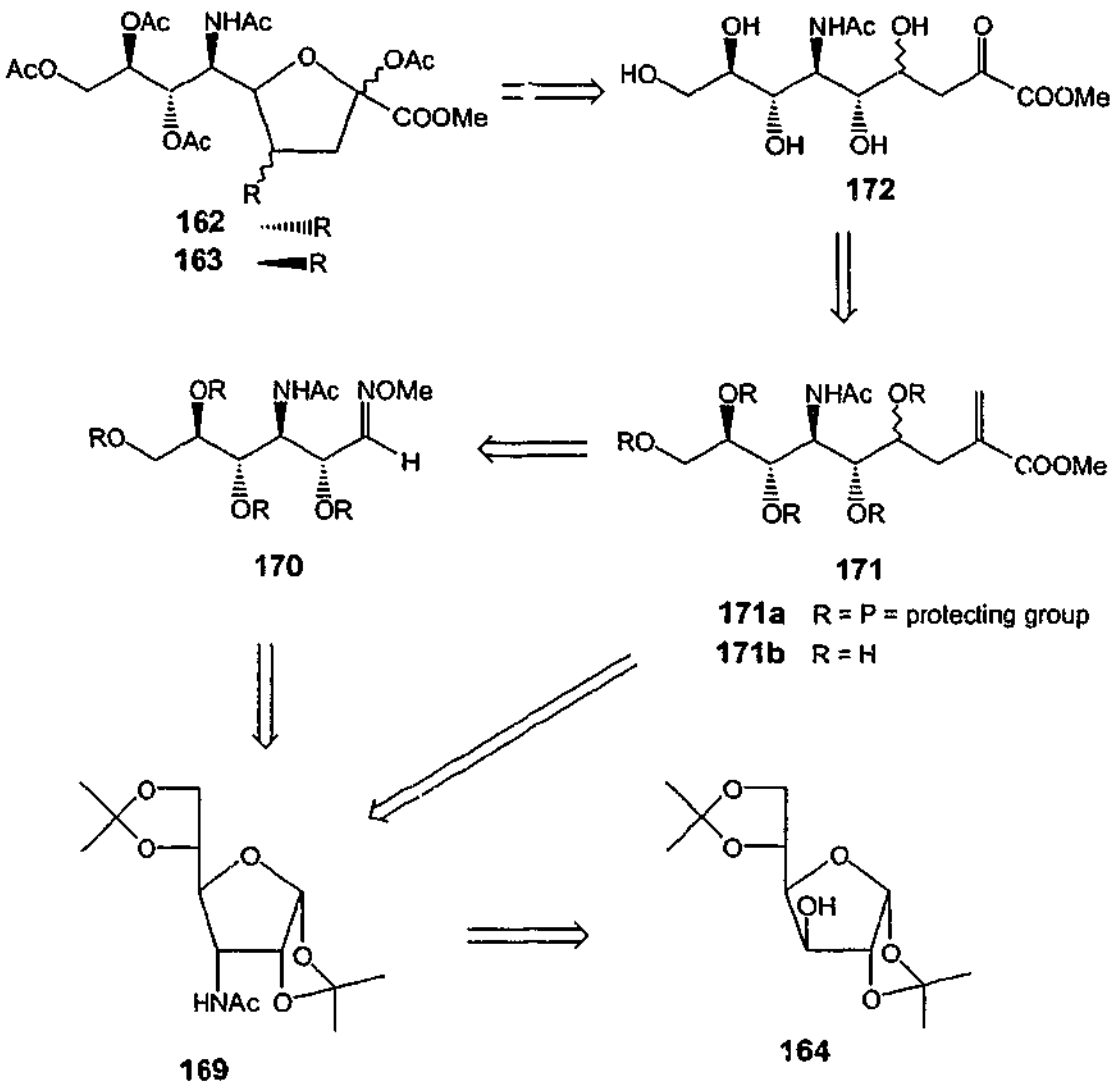


Scheme 4.3

We thought that application of the above-mentioned methodologies would enable us to approach the synthesis of **34** and **35** following a synthetic route different from that of Shiba *et al.*[106].

4.2 Synthesis of methyl 6-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,6-dideoxy-D-*glycero*-D-*allo*-2-nonulofuranosonate (162) and methyl 6-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,6-dideoxy-D-*glycero*-D-*altro*-2-nonulofuranosonate (163)

Our strategy towards the synthesis of **162** and **163** is shown in retrosynthetic terms in Scheme 4.4. The furanose compound **162/163** would be formed from cyclization of the ketoester **172**, which could be produced via the ozonolysis of **171**. The synthetic scheme relies upon the successful construction of the key compound **171**. The protected form **171a** could be prepared from the allylation of the aldehydo product of ozonolysis of the oxime **170**. **170** would be derived from diacetone-D-glucose **164** via the 3-acetamido derivative **169**. Alternatively, the unprotected form **171b** could be produced from allylation of the deprotected product of **169**.

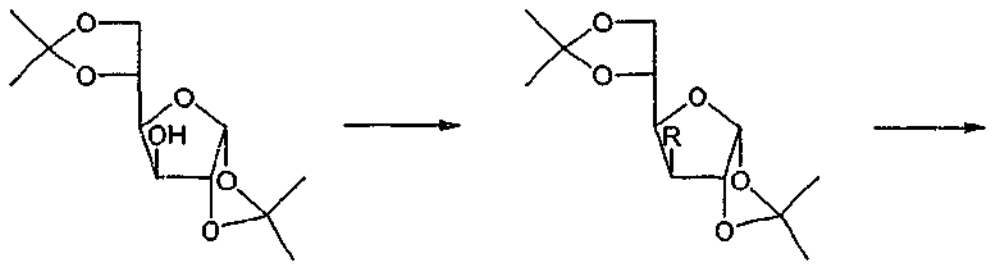


Scheme 4.4

4.2.1 Preparation of 3-acetamido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*allo*furanose (169)

The 3-acetamido compound (**169**) can be readily accessed from diacetone-D-glucose (**164**) in four steps (Scheme 4.5). The key compound in this synthesis is the 3-azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*allo*furanose (**166**). Nayak and Whistler[258], in 1969, reported the preparation of **166**, in 53% yield, from treatment of the 3-*O*-tosyl compound (**165a**) with sodium azide in DMF and water at 115°C for 15 days. However, if hexamethylphosphoramide was used as the solvent, the reaction time was only 18 hours[259],

but the yield of **166** reduced to 42%. A number of early reports[239,260-269] describing the reactions of trifluoromethanesulfonate derivatives of carbohydrates presented the employment of these triflate esters as a simple method for activating hydroxyl groups for a wide variety of reactions. The markedly enhanced reactivity of secondary triflates as compared to tosylates was exploited in the preparation of the 3-azido compound **166** by Baer and Gan[240].



164 **165a** R = OTs **165b** R = OTf

167 **166** **168** **169**

Scheme 4.5

Thus, in our work, **166** was prepared following the synthetic route used by these authors with modifications of the reaction conditions. Exposure of **164** in dichloromethane-pyridine to triflic anhydride at -15°C for an hour gave the triflate ester[257] **165b**. Formation of the triflate **165b** was confirmed by melting point (68-70°C), and ^{1}H NMR spectrum was in good agreement with published literature[257]. The crude triflate ester **165b** was used in the next step without purification.

Treatment of **165b** with tetrabutylammonium azide[256] in benzene at room temperature furnished the 3-azido compound **166** in 62.5% yield with the elimination product **167** in 17.2% yield. Spectral data for **166** and **167** were consistent with those recorded in the literature[237,240].

Hydrogenation of the 3-azido compound **166** under atmospheric pressure in the presence of 10% Pd/C gave 3-amino-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*allo*furanose (**168**) in excellent yield (97%). Structural confirmation was supported by the presence of the protonated molecule (M+H, *m/z* 260) in both low and high resolution mass spectra. ^{1}H NMR spectrum was in agreement with that in the literature[240].

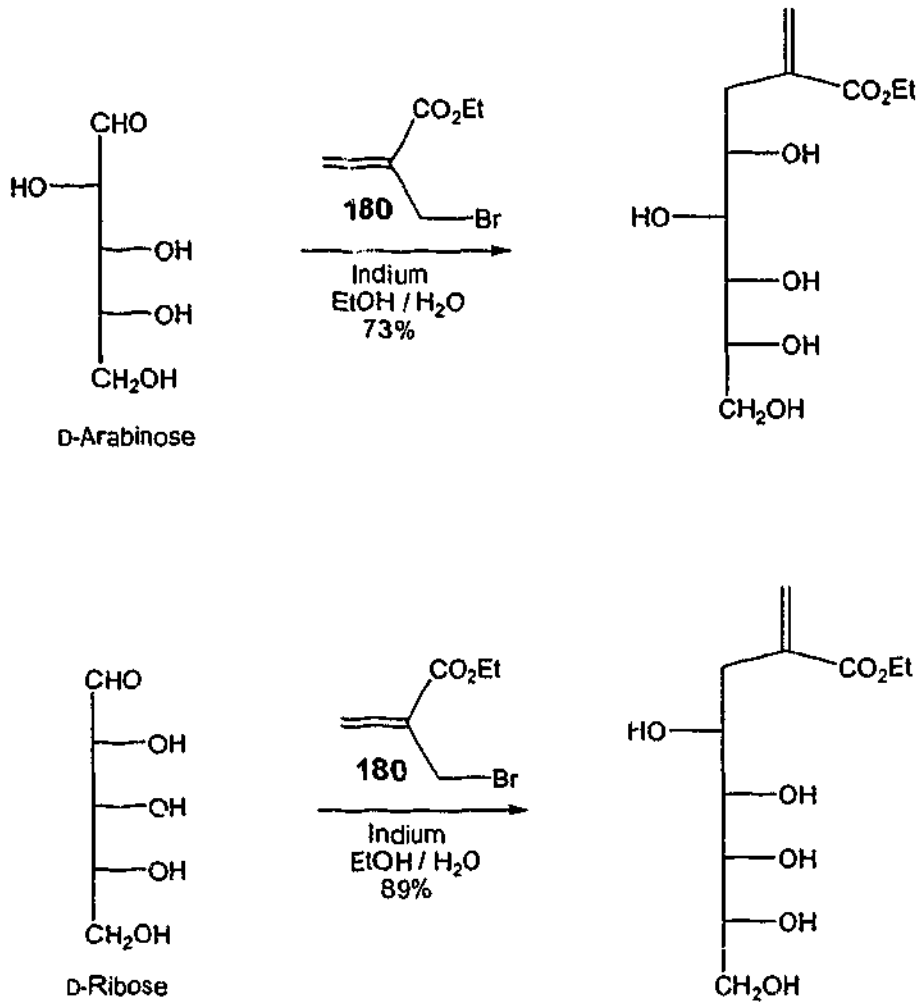
Acetylation of the 3-amino compound **168** was effected using acetic anhydride and pyridine. The product obtained after column chromatography was an amorphous solid, in 90% yield. Conversion to the 3-acetamido compound **169** was indicated by the low resolution mass spectrum that showed the protonated molecular peak at *m/z* 302, in 45% abundance. IR, ^{1}H and ^{13}C NMR spectral data were consistent with those recorded in the literature[240].

4.2.2 Preparation of the homoallylic alcohols

4.2.2.1 Allylation of the deprotected sugar

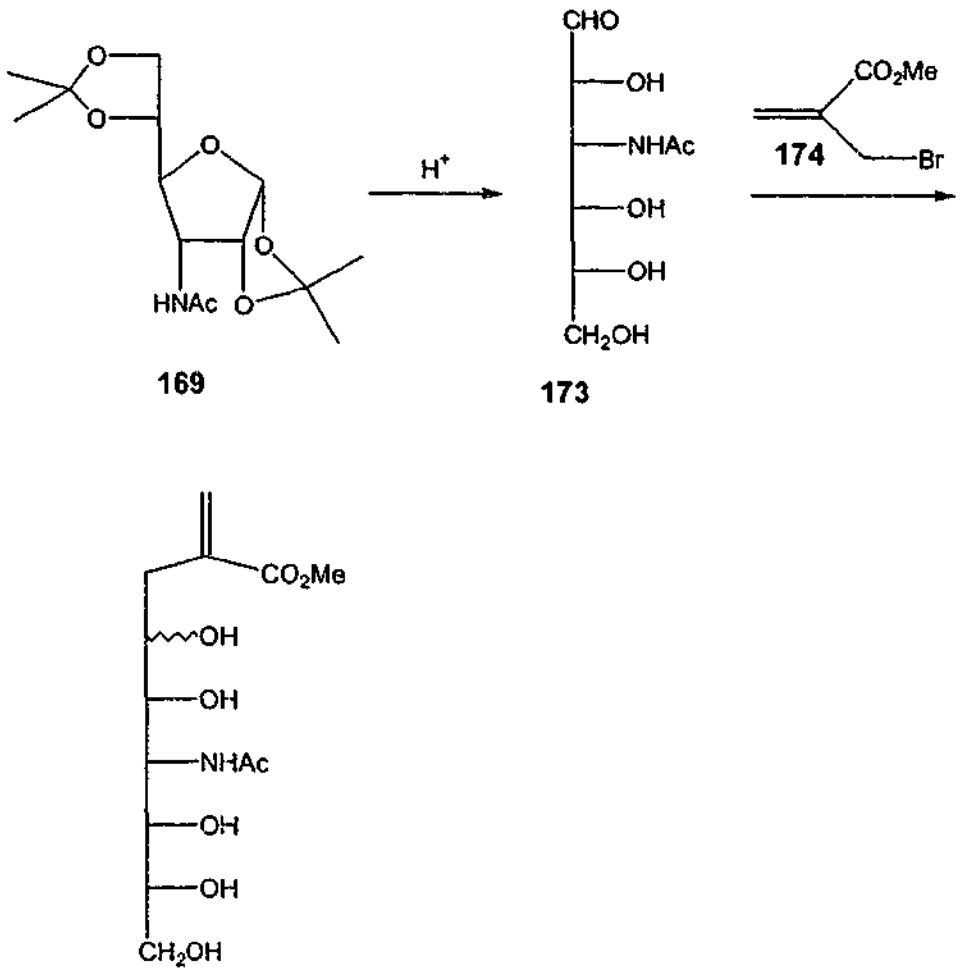
As mentioned previously, the ester **171** could be constructed by following two different routes (Scheme 4.4). In the first approach, **171** could be prepared via the acyclic sugar oxime **170** which requires protection prior to the preparation of **171**, and subsequent removal of the protecting groups towards the formation of the target compounds **162** and **163**. However, in the second approach, the ester **171** could possibly be prepared directly from the unprotected sugar without the need to convert the sugar to the acyclic aldehydo form.

There are a number of reports in the literature describing the metal-mediated addition of allyl groups to the carbonyl moieties of unprotected carbohydrates[75,246,247,250,251,253]. For instance, Whitesides *et al.*[247] have described the allylation of D-arabinose and D-ribose using ethyl 2-(bromomethyl)acrylate (**180**) in the presence of indium (Scheme 4.6).



Scheme 4.6

The second approach was attractive to us as it required a lesser number of steps. In this manner, synthesis of **171b** would involve the coupling reaction between **173** and methyl 2-(bromomethyl)acrylate (**174**) as illustrated in Scheme 4.7.



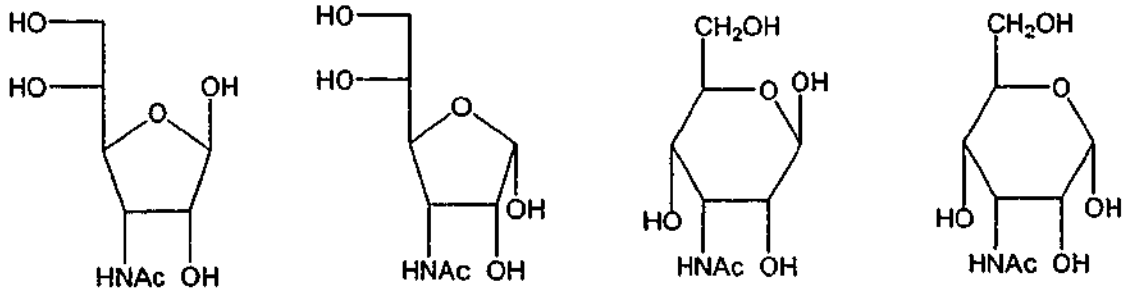
169 **173**

171b

Scheme 4.7

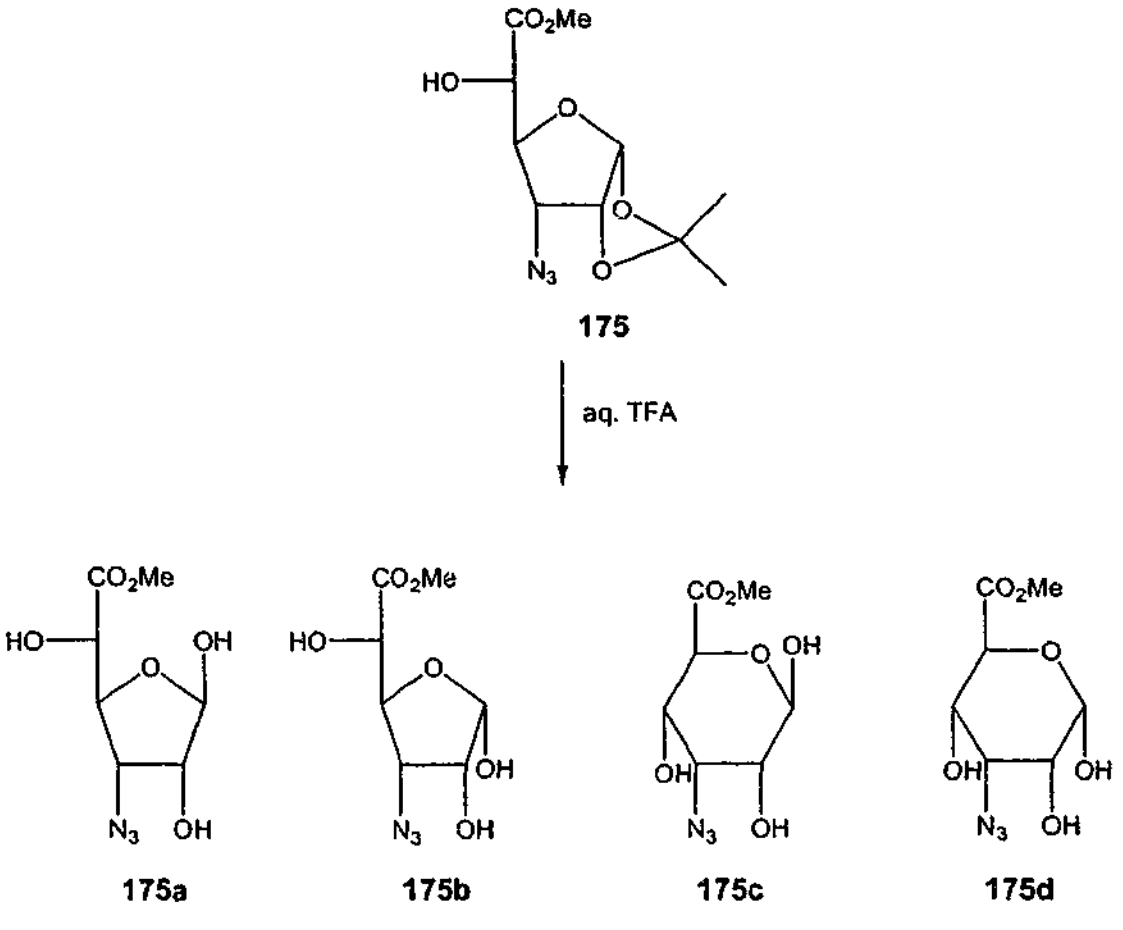
The deprotection of **169** was effected by treatment with Amberlite IR-120 (H^+) resin in water at 50°C. The reaction was completed after 48 hours by TLC analysis, which showed a single spot at R_f 0.10 (EtOAc/MeOH, 6:1). The resin was filtered off, followed by solvent removal and column chromatography of the residue, which gave the deprotected sugar as a hygroscopic mass. The conversion was supported by inspection of the low resolution mass spectrum, which exhibited the protonated molecular peak (M+H) at *m/z* 222. High resolution mass spectrometry confirmed the molecular formula of $C_8H_{15}NO_6$. Examination of ^1H and ^{13}C NMR spectra, in D_2O, indicated that the deprotected sugar

existed in four different tautomers. In the ^{1}H NMR spectrum, there were four singlets at δ 2.01, 2.04, 2.05 and 2.06 ppm representing four *N*-acetyl methyl groups. More telling were the four distinct H-1 protons, appearing as three doublets at δ 4.80, 5.15 and 5.37 ppm, and one singlet at δ 5.24 ppm, with a ratio of intensities of 28:31:16:25, respectively. Coupling constants of the four signals were calculated as $J_{1,2}$ 8.4, 3.0, 3.9 and 0 Hz corresponding to H-1 resonances for β-pyranose **173c**, α-pyranose **173d**, α-furanose **173b**, and β-furanose **173a**, respectively. The ^{13}C NMR showed distinguishable resonances for the four isomeric *N*-acetyl groups with four methyl carbon peaks at δ 21.8, 21.9, 22.1, 22.6 ppm and four distinct carbonyl signals at δ 173.9, 174.1, 175.4 and 175.8 ppm.



173a **173b** **173c** **173d**

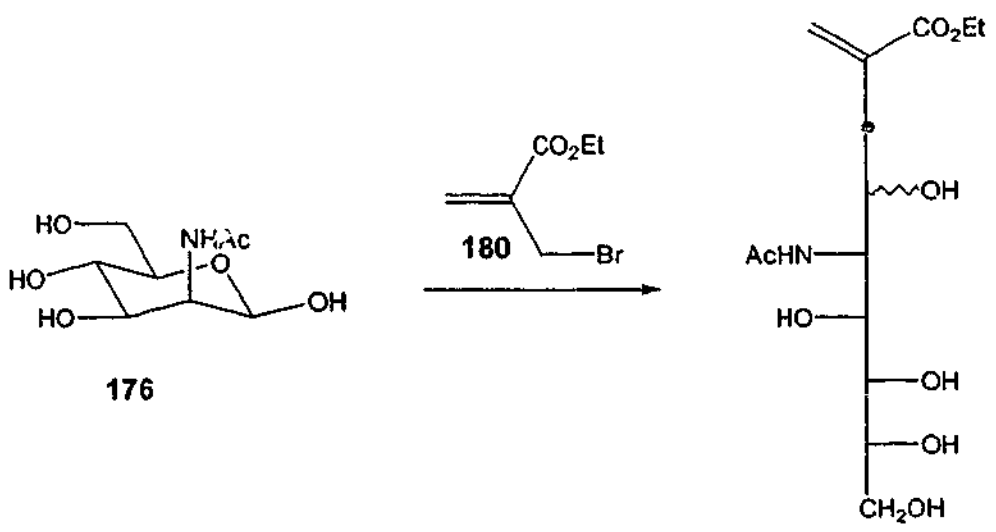
That the deprotected sugar was present in four tautomeric forms, **173a**, **173b**, **173c** and **173d**, in water was not unexpected, as the removal of the isopropylidene group of methyl 3-azido-3-deoxy-1,2-*O*-isopropylidene-α-D-*allo*furanuronate (**175**) was reported[270] to give an isomeric mixture of 3-azido sugar that existed in four tautomeric forms in water (Scheme 4.8). ^{1}H NMR assignments for **173a**, **173b**, **173c** and **173d**, thus, were carried out by comparison with the literature[270].



Scheme 4.8

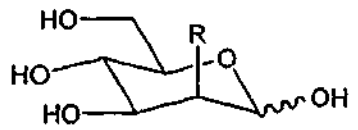
Having prepared the deprotected sugar 3-acetamido-3-deoxy-D-allose (**173**), we then began an investigation into the allylation of this sugar. Thus, following the procedure reported by Chan and Li[250,274], **173** was exposed to methyl 2(-bromomethyl)acrylate (**174**) in water, in the presence of indium metal. TLC analysis after 24 hours revealed that the starting material remained unchanged. The use of aqueous ethanol instead of water did not make any difference to the result.

Gordon and Whitesides[75] observed that the allylation reaction of *N*-acetyl-β-D-mannosamine (**176**) in a mixture of ethanol and 0.1 N HCl resulted in the formation of the product (**177**) that, otherwise, would not have formed if only aqueous ethanol was used.

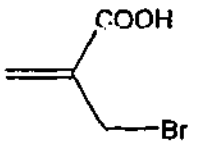


Accordingly, **173** was exposed to methyl 2-(bromomethyl)acrylate (**174**) at 40°C for 24 hours in a mixture of ethanol and 0.1 N HCl (6:1), in the presence of indium. TLC examination of the reaction mixture revealed a complex mixture of products. Attempts of chromatographic separation of the products proved difficult. ^{1}H NMR spectroscopy of the complex mixture indicated an incorporation of the methylene and methylidene protons that exists in **174**, and it was estimated (by ^{1}H NMR intergration) that the conversion rate was less than 10%. Acetylation of the product mixture did not improve the separation of the products. Similarly, attempts to vary the reaction conditions such as temperature, reaction time, ratio of ethanol and 0.1 N HCl mixture, and the use of tin or zinc instead of indium, also gave an inseparable complex mixture of products.

In 1995, Chan and Lee[251] successfully coupled 2-(bromomethyl)acrylic acid (**178**) with D-mannose (**179**), and with *N*-acetyl-D-mannosamine (**20**), and the products were utilised in the synthesis of KDN (**4**) and Neu5Ac (**2**).



179 R = OH
20 R = NHAc



178

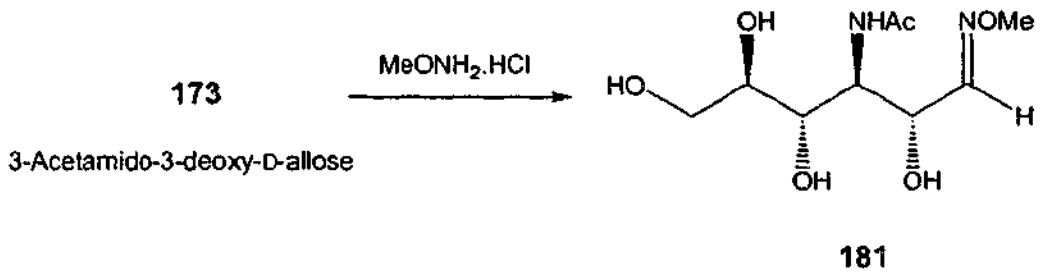
Therefore, we decided to investigate the allylation reaction using 2-(bromomethyl)acrylic acid. (**178**) Unfortunately, treatment of the deprotected sugar **173** with **178** in aqueous ethanol and 0.1 N HCl gave a complex mixture of products of similar mobility on TLC, in a variety of solvent systems, and all attempts to purify the mixture were unsuccessful. Further investigation of this reaction pathway was not continued.

4.2.2.2 Allylation of the acyclic sugar aldehyde

4.2.2.2.1 3-Acetamido-3-deoxy-D-allose methyloxime (181)

Given the unsuccessful attempts to prepare the allylation product **171b** or its acid derivative using unprotected sugar, we decided to explore the alternative approach, in which the sugar is converted to acyclic sugar aldehyde via the formation of an acyclic sugar oxime (*vide supra*). The aldehyde would be readily allylated under metal-mediated conditions to give the desired homoallylic alcohol.

Thus, treatment of the isomeric mixture **173** with methoxylamine hydrochloride in pyridine gave the acyclic sugar 3-acetamido-3-deoxy-D-allose methyloxime (**181**), as an amorphous white solid, in 77% yield.



181

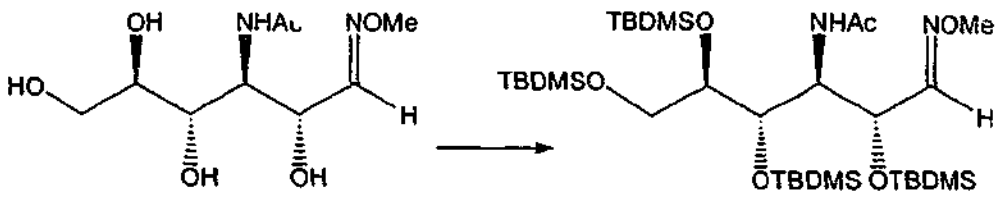
^{1}H NMR analysis and ^{1}H- ^{1}H COSY NMR experiment revealed that the methyloxime existed as a mixture of *E* and *Z* geometrical isomers, which showed two anomeric protons at 7.46 for the *E* oxime, and 6.81ppm for the *Z* oxime, with intensity ratio of 10 to 1, respectively. The assignments were based on observations[271-273] that the *E* oxime proton characteristically appears downfield of its *Z* counterpart.

4.2.2.2.2 Preparation of methyl 6-acetamido-5,7,8,9-tetra-*O-tert*-butyldimethylsilyl-2,3,6-trideoxy-2-methylidene-D-*glycero*-D-*allo*-nononate (185) and methyl 6-acetamido-5,7,8,9-tetra-*O-tert*-butyldimethylsilyl-2,3,6-trideoxy-2-methylidene-D-*glycero*-D-*altro*-nononate (184)

Having successfully synthesized the methyloxime **181**, we then proceeded to investigate the preparation of the acrylate ester derivatives **185** and **184**. As previously discussed, acyclic sugar oximes can be protected, and the oxime ozonolised to generate

acyclic aldehydo sugars, which undergo metal-mediated allylation to give acrylate ester derivatives.

Exposure of **181** to *tert*-butyldimethylsilyl chloride and imidazole for 3 days at 40°C gave **182**, in 86% yield. The presence of four *tert*-butyldimethylsilyl groups was observed in both ^{1}H NMR and ^{13}C NMR spectra. Six singlets of twenty-four protons at δ 0.01-0.11 ppm represented the Si-dimethyl groups, and five singlets of thirty-six protons at δ 0.87-0.93 ppm were assigned for the Si-*tert*-butyl groups. In the ^{13}C NMR spectrum, resonances for the Si-dimethyl groups were observed at δ -5.4 to -4.2 ppm, and resonances for the Si-*tert*-butyl groups were detected at δ 17.9-18.4 ppm for the quaternary carbons, and δ 25.6-26.0 ppm for the methyl carbons. High resolution ESI mass spectrometry recorded a protonated molecular ion peak at *m/z* 707.47205, which was consistent with the molecular formula for **182**.

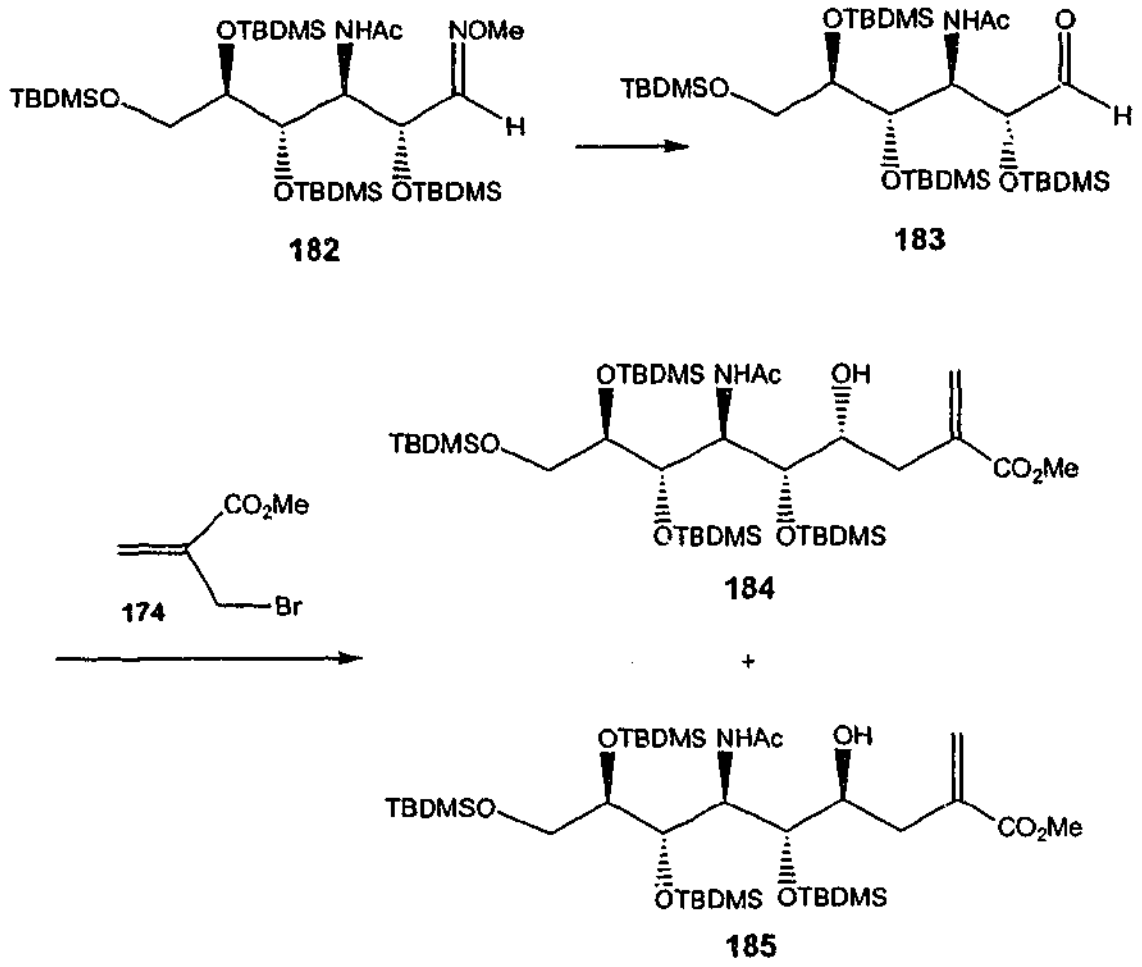


181 **182**

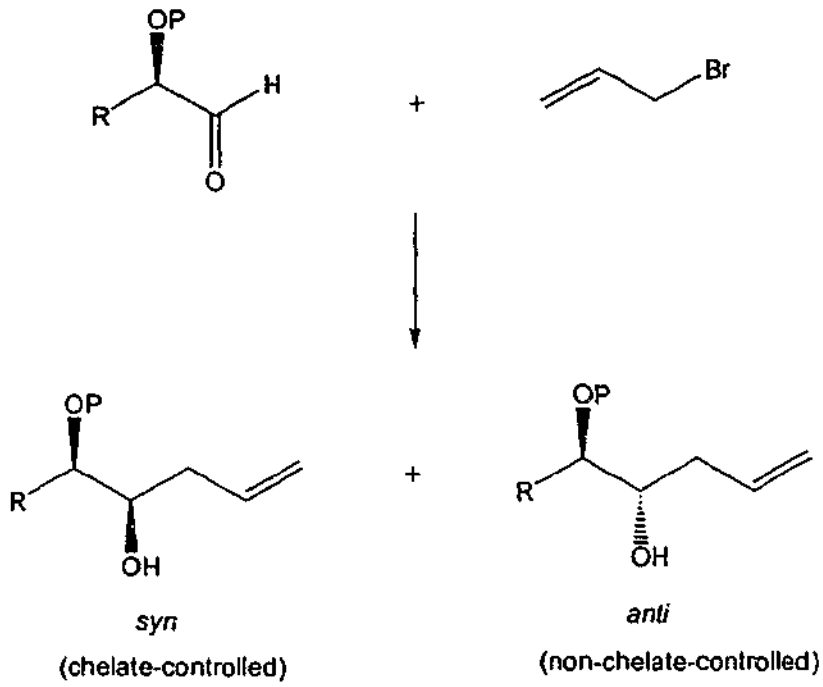
Ozonolysis of methyloxime **182** in dichloromethane at -78°C provided the aldehyde **183**. Without purification, the crude aldehyde **183** was reacted with methyl 2-(bromomethyl)acrylate (**174**) in the presence of activated zinc powder. TLC analysis revealed two products, which were separated by column chromatography to give the fast

moving product **184**, in 9% yield, and the second product **185**, in 42% yield. Mass spectrometry in the low and high resolution modes supported the molecular formula of $C_{37}H_{79}NO_8Si_4$ for **184** and **185**. ^1H and ^{13}C NMR spectrometric analysis further confirmed the formation of the allylation products. Examination of the ^1H NMR spectrum of **184** revealed the presence of the 3-methylene protons at δ 2.30 and 2.67 ppm, the methylidene protons at δ 5.65 and 6.26 ppm, and the carboxylate methyl protons at δ 3.76 ppm. In the ^{13}C NMR spectrum of **184**, resonances for the 3-methylene carbon, the ester methyl carbon and the methylidene carbon were at δ 37.3, 51.9 and 126.8 ppm, respectively. Similar features were also observed in the ^1H and ^{13}C NMR spectra of **185**.

That methyl 6-acetamido-5,7,8,9-tetra-*O*-*tert*-butyldimethylsilyl-2,3,6-trideoxy-2-methylidene-D-*glycero*-D-*allo*-nononate (**185**) was the major product and methyl 6-acetamido-5,7,8,9-tetra-*O*-*tert*-butyldimethylsilyl-2,3,6-trideoxy-2-methylidene-D-*glycero*-D-*altro*-nononate (**184**) was the minor component, could not be determined at this stage. Only after conversion of these products to the target compounds **162** and **163** were their structures confirmed.



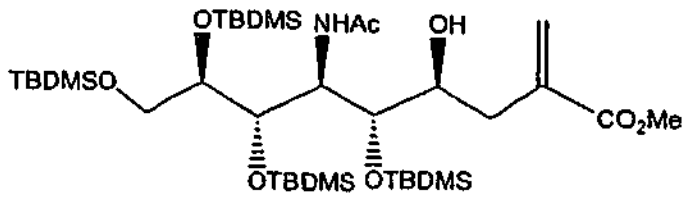
The diastereoselectivity of the allylation of the aldehyde **183**, which favoured the formation of the *anti* isomer (*anti* isomer/*syn* isomer, 4.7:1), is in agreement with results reported by Schmid[282] and others[43,61,241,242,249]. Schmid[282] observed that allylation of protected polyhydroxy aldehydes led to products of *anti* selectivity. However, the same allylation reaction performed on the deprotected aldehydes gave *syn* isomer as the major products, which Chan[250-252] and others have also observed[75,246,247,253]. In 1996, Paquette and Mitzel[283] conducted a detailed comparative study of the indium-promoted allylation of chiral α- and β-oxygenated aldehydes. The results of this study confirmed that when chelate control is operational, as in the case of unprotected aldehyde, the major allylation product is the *syn* diastereomer. For non-chelate-controlled behaviour, as in the case of protected aldehyde, the *anti* product is favoured (Scheme 4.9)



Scheme 4.9

4.2.3 Methyl 6-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,6-dideoxy-D-*glycero*-D-*allo*-2-nonulofuranosonate (162)

Conversion of the ester **185** to the target compound **162** was envisaged to involve the formation of the keto ester **186**, followed by cyclization upon deprotection of the TBDMS protection groups. Acetylation of the cyclized compound would provide **162** (Scheme 4.10).



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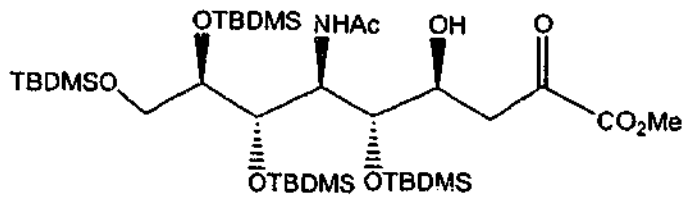

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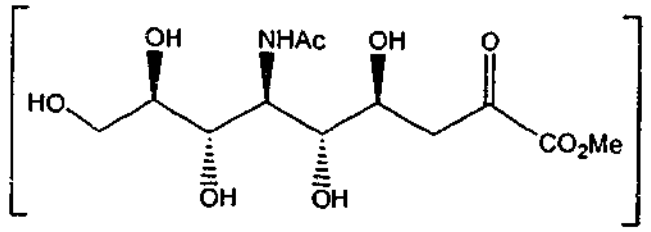

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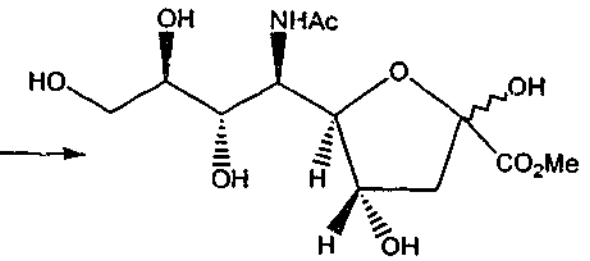

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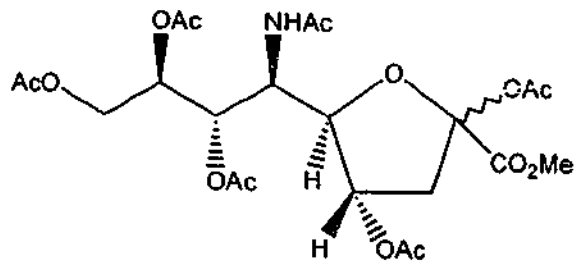


162

Scheme 4.10

Ozonolysis of **185** in dichloromethane at -78°C for one hour provided the α-keto ester **186** in near quantitative yield (98%). ^{1}H NMR spectroscopy analysis revealed the

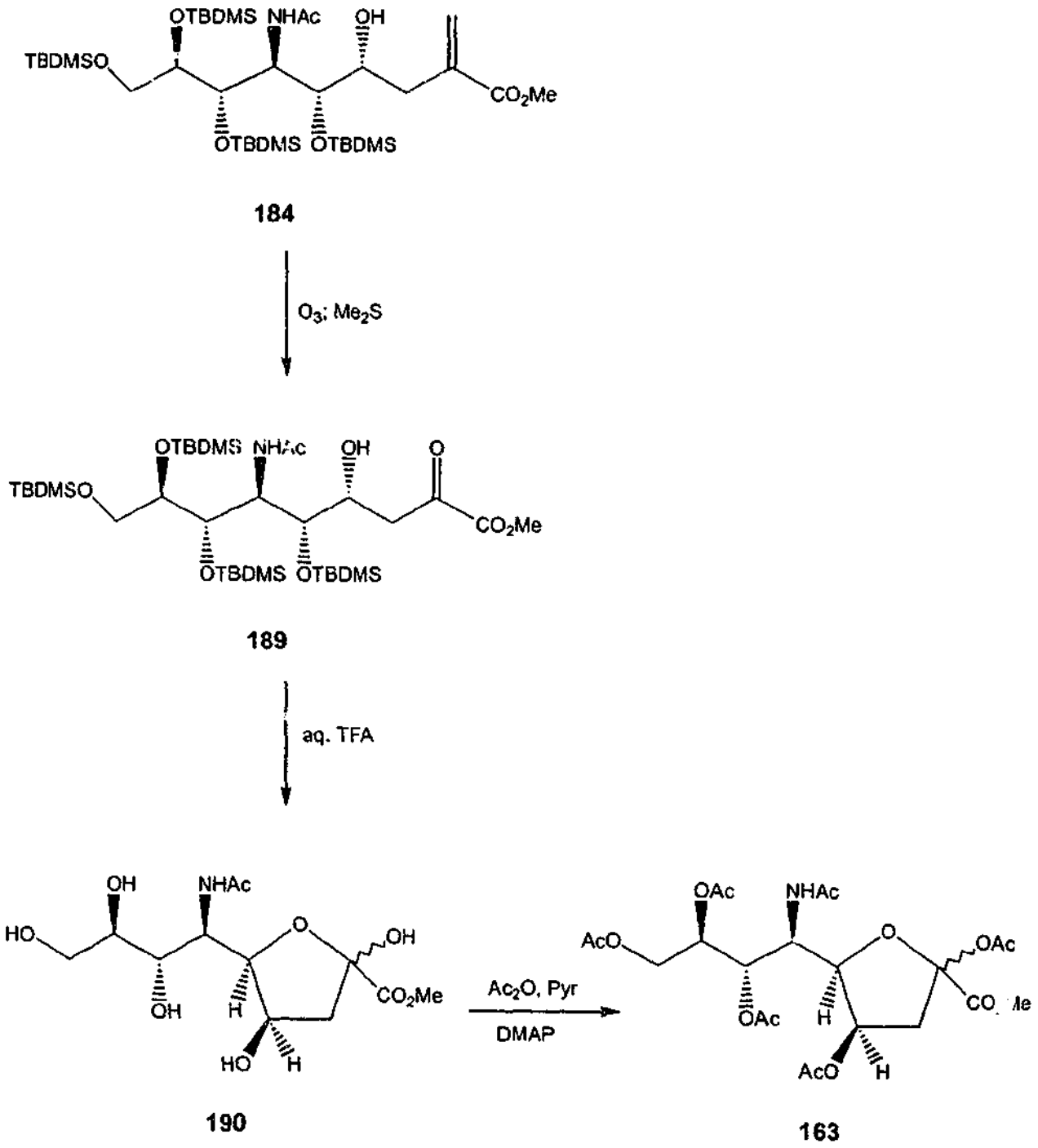
disappearance of the methylidene protons that were present at δ 5.72 and 6.25 ppm in the spectrum of the starting material **185**. High resolution ESI mass spectrometry recorded a protonated molecular ion peak at *m/z* 780.47621, confirming the molecular formula for **186**.

Deprotection of the TBDMS groups was effected by treatment of the α-keto ester **186** with aqueous trifluoroacetic acid. Upon treatment with acidic resin (Dowex 50WX8) the intermediate **187** is able to cyclize to **188**, as subsequent acetylation gave the target compound methyl 6-acetamido-2,4,7,8,9-*O*-acetyl-3,6-dideoxy-D-*glycero*-D-*allo*-2-nonulofuranosonate (**162**), in 43% yield (based on **186**). **162** existed as an inseparable mixture of α and β anomers, as indicated by ^{1}H and ^{13}C NMR spectral data. Structural confirmation was supported by the low resolution mass spectrum, which displayed a protonated molecular ion peak at *m/z* 534. High resolution mass spectrometry confirmed the molecular formula for **162**. The ^{1}H NMR spectrum was in agreement with that reported in the literature[106].

4.2.4 Methyl 6-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,6-dideoxy-D-*glycero*-D-*altro*-2-nonulofuranosonate (163)

The synthesis of **163** was envisioned to be similar to that of **162**, via the formation of an α-keto ester (Scheme 4.11). Thus, exposure of **184** to ozone at -78°C for one hour in dichloromethane, followed by reductive workup gave the α-keto ester **189**, in 98% yield. Subsequent deprotection of the TBDMS groups followed by treatment with acidic resin afforded the cyclized compound **190**. Acetylation of **190** provided the target compound **163**, in 41% yield, as an inseparable mixture of α and β anomers. High resolution mass spectrometry, which showed a molecular ion plus $^{+}NH_4$ peak at *m/z* 551.20626, confirmed

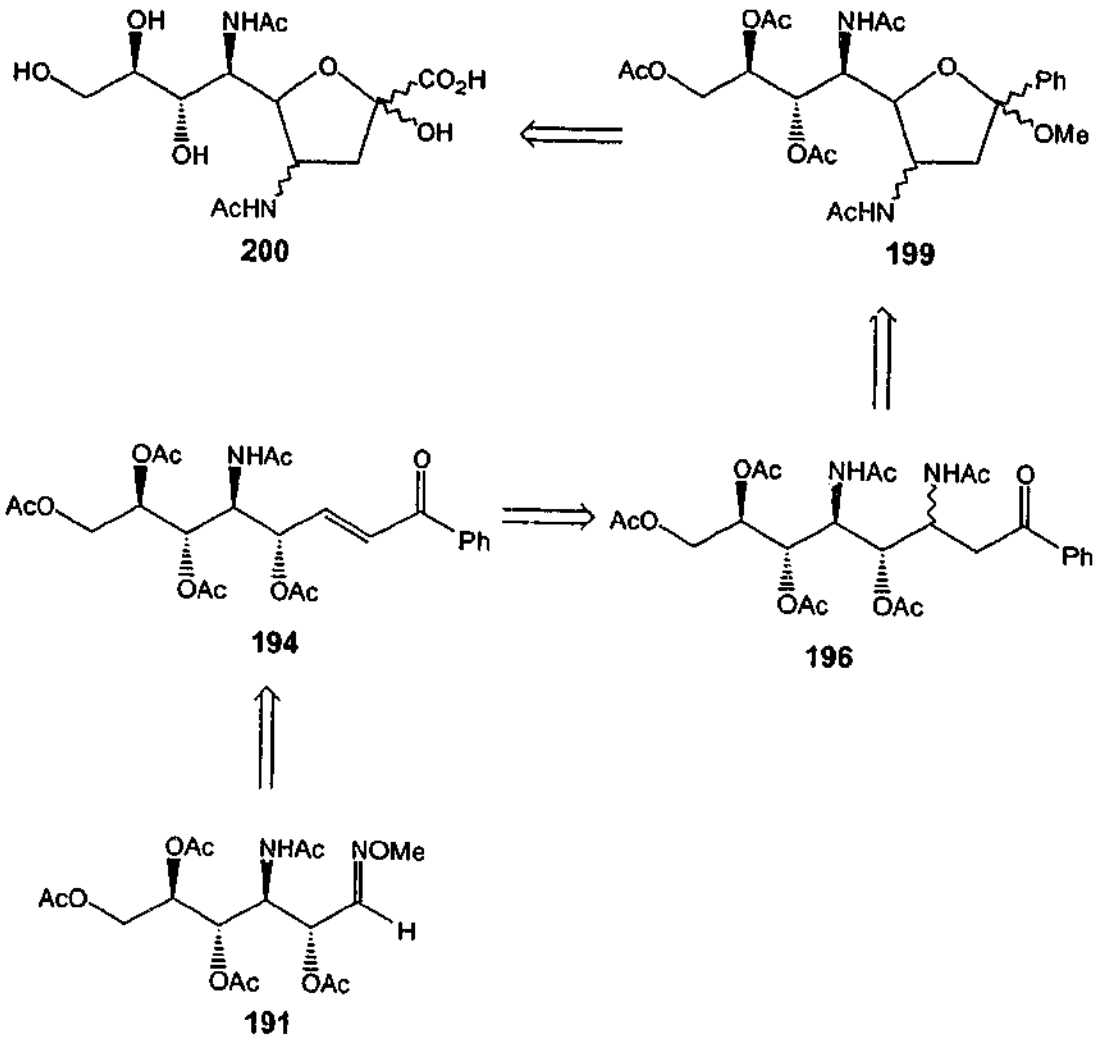
the molecular formula for **163**. The ^{1}H NMR spectrum was in agreement with that reported in the literature[106].



Scheme 4.11

4.3 Approaches to the synthesis of 4,6-diacetamido-3,4,6-trideoxy-D-*glycero*-D-*allo/altro*-2-nonulofuranosonic acid (200)

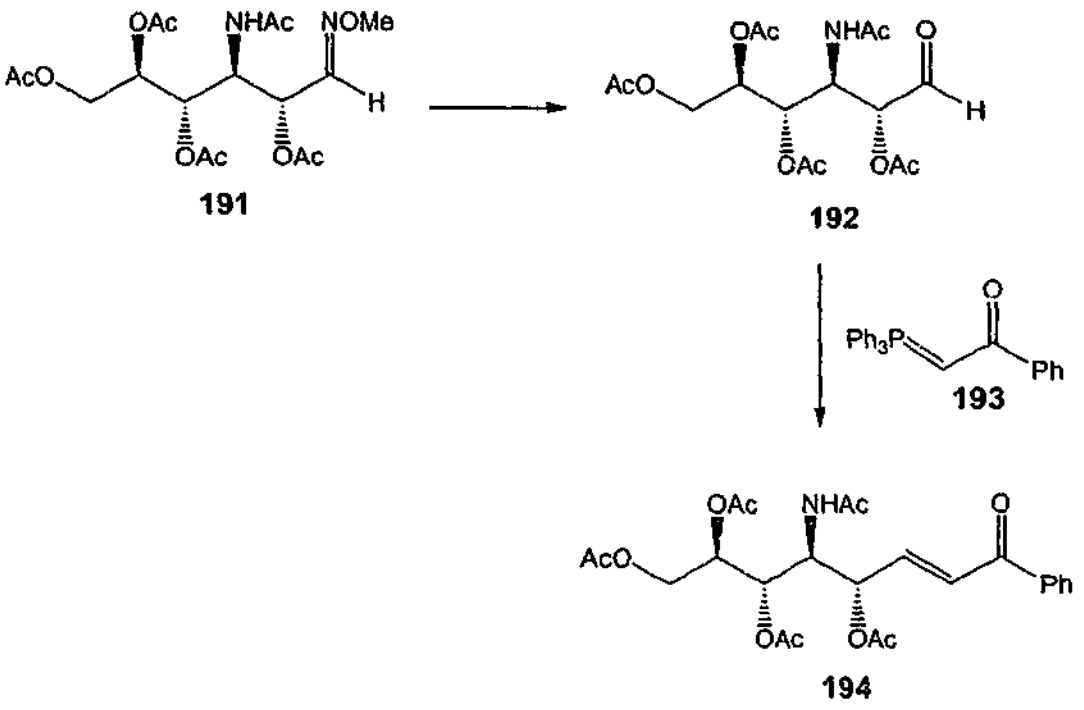
Having successfully synthesized **162** and **163**, we then decided to investigate the synthesis of C-4 modified derivatives, initially with the intention of preparing 4,6-diacetamido-3,4,6-trideoxy-D-*glycero*-D-*allo/altro*-2-nonulofuranosonic acid (**200**). The retrosynthesis of **200** is shown in Scheme 4.12. The α,β-unsaturated ketone **194** is accessible from **191**, and **194** would give rise to **196**. Cyclization of the deprotected form of **196** would lead to **199**. Formation of the target compound **200** would involve conversion of the phenyl group of **199** to the carboxylic acid functionality under catalytic oxidation conditions[281].



Scheme 4.12

4.3.1 (2*E*)-5-Acetamido-4,6,7,8-tetra-*O*-acetyl-2,3,5-trideoxy-1-*C*-phenyl-D-*allo*-oct-2-enose (194)

The preparation of **194** was thought achievable by ozonolysis of the oxime **191** to generate the aldehyde **192**, followed by Wittig reaction between **192** and benzoylmethylene triphenylphosphorane (**193**) (Scheme 4.13).



Scheme 4.13

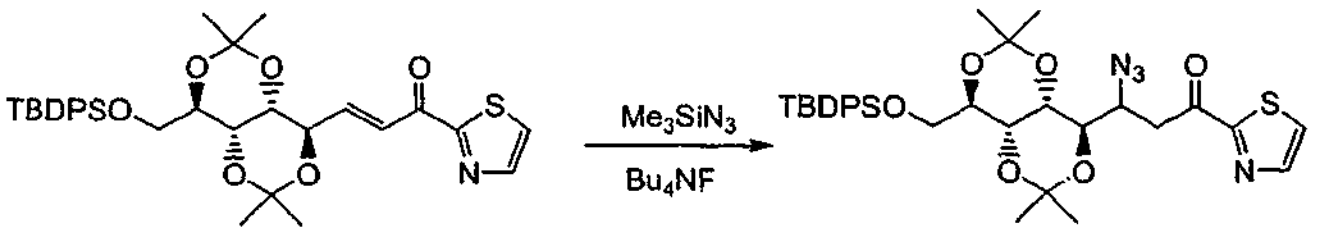
The methyloxime **191** can be prepared, either by acetylation of the unprotected oxime **181**, or preferentially by treatmemt of the isomeric mixture **173** with methoxylamine hydrochloride in pyridine for 48 hours, followed by the addition of acetic anhydride and DMAP with stirring for a further 18 hours. Synthesis of **191** was, therefore, carried out employing the latter method. After purification by column chromatography, **191** was obtained in 73% yield. Different from the unprotected oxime, which existed almost exclusively in the *E* form (*E*/*Z* = 10:1), ^{1}H NMR analysis indicated that the ratio of *E* to *Z* for **191** was only 3 to 1. However, once subjected to ozonolysis both forms would give the same aldehyde product, regardless of the *E*/*Z* ratio.

Ozonolysis of **191** in dichloromethane at -78°C, followed by reductive workup gave the aldehyde **192**. Without purification, the crude aldehyde was reacted with benzoylmethylene triphenylphosphorane (**193**) in dichloromethane for 20 hours at ambient

temperature. TLC analysis revealed one charring spot with R_f of 0.63 in ethyl acetate. Purification by chromatography provided exclusively the *trans* isomer **194** in 70% yield. Low resolution mass spectrometry displayed a protonated molecular ion peak at *m/z* 492, in 100% abundance, supporting the formation of **194**. High resolution mass spectrometry confirmed the molecular formula of $C_{24}H_{29}NO_{10}$ for **194**. Analysis of the ^1H NMR spectrum revealed the resonances for H-2 and H-3 occurred at δ 7.03 and 6.90 ppm, respectively, with a mutual coupling constant of 15.6 Hz, which is indicative of *trans*-olefinic coupling[275].

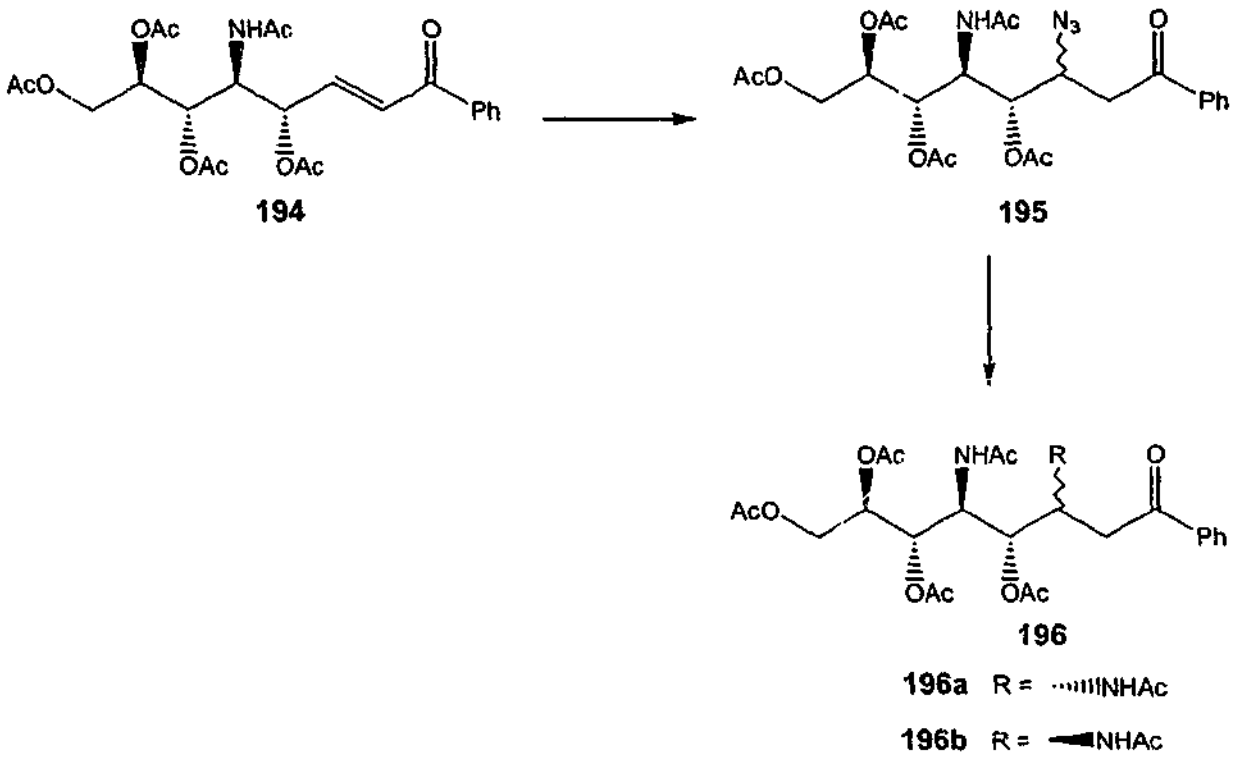
4.3.2 3,5-Diacetamido-4,6,7,8-tetra-*O*-acetyl-2,3,5-trideoxy-1-*C*-phenyl-D-*glycero*-D-*altro*/*allo*-oct-1-ulose (196)

Having successfully prepared (2*E*)-5-Acetamido-4,6,7,8-tetra-*O*-acetyl-2,3,5-trideoxy-1-*C*-phenyl-D-*allo*-oct-2-enose (**194**), we then concentrated on the preparation of the diacetamido compound (**196**). Several literature methods[276-279] have described the synthesis of β-azido carbonyl compounds from α,β-unsaturated carbonyl compounds. Dondoni *et al.*[278], in 1994, reported the formation of the β-azido ketone **203** in 94% yield, from treatment of **202** with trimethylsilyl azide in the presence of Bu_4NF.



202 **203**

We felt that the synthesis of **192** would be achievable by β-azidonation of **194** according to Dondoni *et al.*[278] method, followed by reductive acylation of the azido group to provide the *N*-acetyl derivative. Thus, exposure of the α,β-unsaturated ketone **194** to trimethylsilyl azide and a catalytic amount of Bu_4NF in dichloromethane at -20°C for 4 days gave the β-azido **195** as indicated by TLC with a single spot (R_f 0.29, CH_2Cl_2/MeOH, 10:1). Attempts to purify this compound by column chromatography were unsuccessful due to considerable decomposition. Thus, the crude product was treated with thiolacetic acid[238] for 4 hours at room temperature giving the diacetamido compound **196** in 42% yield. **196** was also obtained from hydrogenation of **195**, followed by acetylation using acetic anhydride and pyridine, but the yield was only 32% for the two steps. Structural confirmation was supported by the protonated molecular ion peak at *m/z* 551 in the ESI low resolution mass spectrum. High resolution mass spectrometry confirmed the molecular formula for **196**. The presence of two *N*-acetyl groups was confirmed by the presence of the two resonances at δ 6.11 and 6.53 ppm in the ^1H NMR spectrum. Analysis of both the ^1H and ^{13}C NMR spectra revealed that only one diastereoisomer was isolated. However, these spectral data were insufficient to determine conclusively whether the isolated product was the *syn*-isomer (3,5-diacetamido-4,6,7,8-tetra-*O*-acetyl-2,3,5-trideoxy-1-*C*-phenyl-D-*glycero*-D-*altro*-oct-1-ulose, **196a**) or the *anti*-isomer (3,5-diacetamido-4,6,7,8-tetra-*O*-acetyl-2,3,5-trideoxy-1-*C*-phenyl-D-*glycero*-D-*allo*-oct-1-ulose, **196b**).

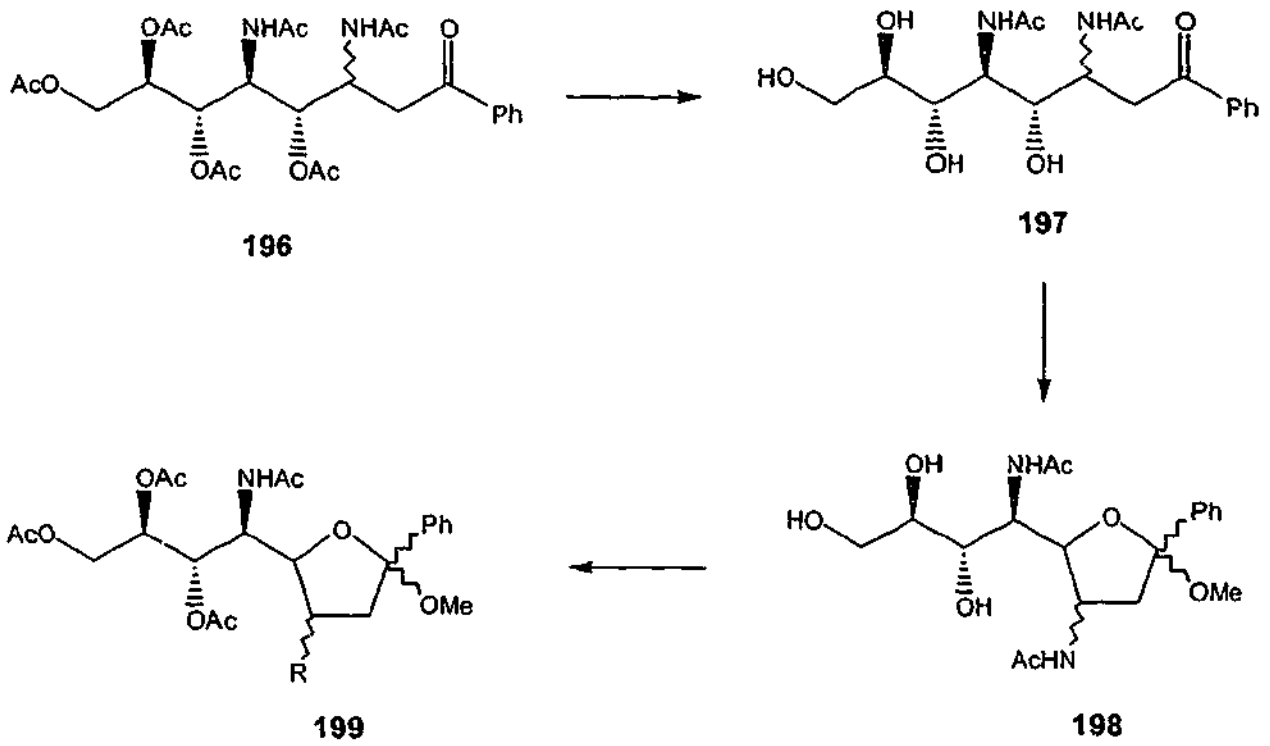


4.3.3 Methyl 3,5-diacetamido-6,7,8-tri-*O*-acetyl-2,3,5-trideoxy-1-phenyl-D-*glycero*-D-*altro/allo*-octofuranoside (199)

With our success in introducing *N*-acetyl functionality at C-3 of **194** to form the diacetamido **196**, we then proceeded to prepare the furanose compound **199**.

Deacetylation of **196** using sodium methoxide in methanol, followed by treatment of the crude product with Dowex 50WX8 (H^+) resin in anhydrous methanol gave **198**. Care had to be taken in working up the reaction, as hydrolysis of **198** to **197** was observed after removal of methanol. Thus, pyridine was added to the solution before solvent removal. Treatment of crude **198** with acetic anhydride and DMAP in pyridine provided **199** in 73% yield from **196**, as a mixture of α and β anomers. Formation of **199** was supported by low resolution mass spectrum, which displayed a protonated molecular ion peak at *m/z* 523 in 50% abundance. Inspection of the ^{1}H NMR spectrum revealed the presence of the OMe

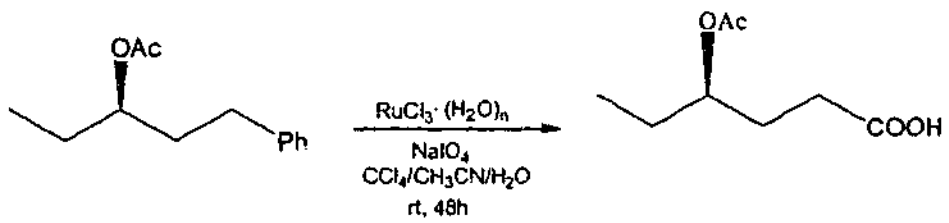
group attached to the anomeric carbon as a singlet at δ 2.99 ppm. High resolution ESI mass spectrometry confirmed the molecular formula for **199**. As discussed in section 4.3.2, the exact stereochemistry of the acetamido group at C-3 of **196** could not be determined at that stage. However, we thought that formation of the furanose compound **199** would allow us to unequivocally assign the stereochemistry of C-3. Unfortunately, ^{1}H NMR spectral analysis revealed overwhelming signal overlaps for H-3 and H-4 preventing the calculation of coupling constants.



4.3.4 Attempted unmasking of the carboxylic acid functionality

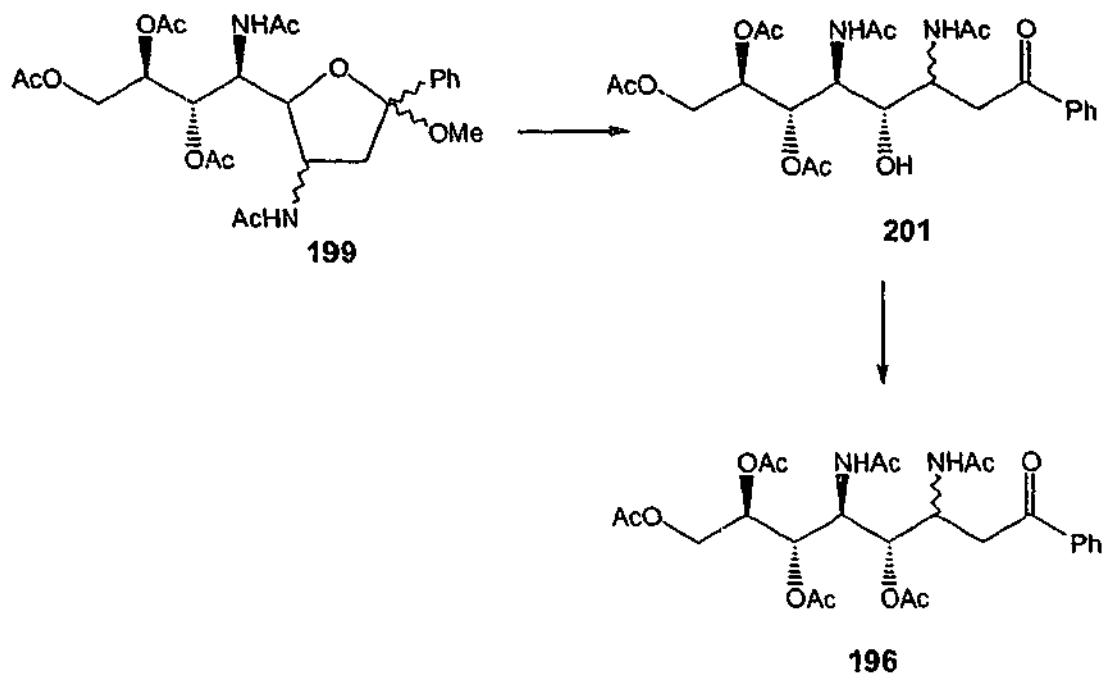
4.3.4.1 Oxidation of methyl 3,5-diacetamido-6,7,8-tri-*O*-acetyl-2,3,5-trideoxy-1-phenyl-D-*glycero*-D-*altro/allo*-octofuranoside (199)

Having successfully synthesized the furanose **199**, we then set out to carry out the unmasking of the carboxylic acid functionality. Ruthenium tetraoxide has been employed by Carlsen *et al.*[281] to oxidize phenyl groups to carboxylic acids in good to excellent yields, as illustrated in Scheme 4.14.



Scheme 4.14

Thus, **199** was oxidized according to the method of Carlsen *et al.*[281]. Accordingly, **199** was exposed to ruthenium trichloride hydrate and sodium metaperiodate in carbon tetrachloride, acetonitrile and water for 48 hours at room temperature. Disappointingly, the aromatic ring remained intact under these conditions while the furanose ring was opened by hydrolysis to give **201**, which was converted to **196** by treatment with acetic anhydride and pyridine.



4.3.4.2 Oxidation of 3,5-diacetamido-4,6,7,8-tetra-*O*-acetyl-2,3,5-trideoxy-1-*C*-phenyl-D-*glycero*-D-*altro*/*allo*-oct-1-ulose (196)

Given the problem of ring opening of the furanose **199** under ruthenium tetraoxide-catalyzed oxidation conditions, it was thought that the unmasking of the carboxylic functionality should be carried out on the acyclic sugar prior to the cyclization step. Thus, **196** was exposed to ruthenium trichloride hydrate and sodium metaperiodate in carbon tetrachloride, acetonitrile and water. To our disappointment, starting material remained unreacted after 3 days. Heating the reaction under reflux resulted in decomposition of **196**. Further investigation of the synthesis of 4,6-diacetamido-3,4,6-trideoxy-D-*glycero*-D-*allo*/*altro*-2-nonulofuranosonic acid (**200**) was abandoned.

4.4 Conclusion

Although some difficulties were encountered, we have successfully synthesized the furanose isomers **162** and **163** employing different synthetic pathway from that of

Yamamoto *et al.*[106], in overall yields of 5.2% and 1.1%, respectively. The final deprotection steps (i.e. deacetylation and hydrolysis of methyl ester), though not carried out, can be effected in high to quantitative yields[180]. These results represented a significant improvement over that of Yamamoto *et al.*[106] (1.2% and 0.4%). Despite some lack of success in the oxidation of the furanose compound **199** to a furanosonic acid, we envisage that careful consideration on the choice of the carboxyl surrogate should lead to the successful unmasking of the carboxylic acid functionality.

CHAPTER 5

Experimental

5.1 General methods

Melting points were determined on a Gallenkemp MFB-595 apparatus and are uncorrected. Optical rotations were measured on a Jasco DIP-370 polarimeter. Infra-red spectra were recorded using a Hitachi 270-30 infra-red spectrometer as KBr discs or between NaCl windows.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-300 spectrometer. Chemical shifts (δ) are expressed in parts per million (ppm) with either CD_3COCD_3 (2.07 ppm), $CDCl_3$ (7.27 ppm), D_2O (4.70 ppm), CD_3OD (3.35 ppm) or d_6 DMSO (2.50 ppm) as the internal reference. Coupling values (J) are given in Hertz (Hz). In the assignment of NMR spectra, the abbreviations app, br, s, d, t, q and m have the usual meanings. In order to confirm assignments 2-D NMR experiments were performed using the ^{1}H-^{1}H COSY experiment for proton-proton interactions and the ^{1}H-^{13}C HMQC experiment for proton-carbon interactions.

Electrospray ionisation (ESI) mass spectra were recorded on a Micromass Platform II. Fast atom bombardment (FAB) mass spectra were recorded on a JEOL JMS-DX-300 using a 1:1 thioglycerol-glycerol matrix. High resolution mass spectra (HRMS) were recorded using a Bruker Bio-Apex II spectrometer in ESI mode.

Column Chromatography was performed using Merck Kieselgel 60 (230-400 mesh) unless otherwise indicated. Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} pre-coated aluminium plates and visualised using 5% H_2SO_4 in EtOH followed by heating to 200°C.

Elemental analyses were carried out by the microanalysis service of the Deparment of Chemistry, University of Queensland.

All organic solvents were distilled prior to use or were of analytical grade. Dried solvents were distilled according to literature methods[287].

5.2 General procedures

General procedure 1: Conversion of primary alcohol to carboxylic *tert*-butyl ester.

The carbohydrate (2.20 mmol) was added to a stirring solution of pyridinium dichromate (3.30 g, 8.80 mmol) in DMF-CH_2Cl_2 (25 ml, 1:4). Acetic anhydride (1.66 ml, 17.6 mmol) was then added to the mixture followed by *tert*-butyl alcohol (6.30 ml, 66.0 mmol) and the reaction left to stirred at rt for 18 h. Ethanol (2 ml) was added and the mixture stirred for an additional 15 min. The mixture was then passed through a short silica column and eluted with ethyl acetate. Removal of solvent under reduced pressure and column chromatography of the residue gave the *tert*-butyl ester in good yield.

General procedure 2: Acetylation

A solution of the carbohydrate (0.35 mmol) in pyridine (8 ml) at 0°C was treated with acetic anhydride (6 ml). The reaction was warmed to rt and left to stir for 16 h. Concentration of the reaction mixture under reduced pressure gave a residue which was re-evaporated with toluene. The organic phase was extracted into EtOAc (60 ml), washed with water (2x10 ml), dried (Na_2SO_4) and concentrated. The residue was then chromatographed on a silica column.

General procedure 3: Debenzoylation

To a solution of the carbohydrate (0.20 mmol) in anhydrous MeOH (6 ml) was added NaOMe in MeOH (0.50 ml of a 0.1 M solution) and the mixture stirred at rt under N_2. The reaction was followed by TLC, generally complete in two hours. It was then neutralised with Dowex 50WX8-400 (H^+) ion-exchange resin. The resin was filtered off and washed with MeOH (3 x 3 ml). Removal of solvent followed by chromatography of the residue gave the deprotected compound in good to excellent yields.

General procedure 4: Oxidation of primary alcohol to aldehyde

Dimethyl sulfoxide (0.130 ml, 1.68 mmol) in CH_2Cl_2 (1 ml) was added to a solution of oxalyl chloride (76µl, 0.84 mmol) in CH_2Cl_2 (5 ml) at *ca.* -60°C. The mixture was stirred for 2 min and the carbohydrate (0.50 mmol) in CH_2Cl_2 (5 ml) was added dropwise. Stirring was continued for an additional 45 min. Triethylamine (415µl, 3.0 mmol) was added and the reaction mixture stirred for 5 min then allowed to warm to rt. CH_2Cl_2 (20 ml) was added and the mixture washed with water (3 x 5 ml), dried (Na_2SO_4), and concentrated giving the aldehyde which was used in the next step without further purification.

5.3 Synthesis of 4-azido-4-deoxy-fructofuranosidonic esters

α/β-Methyl fructosides (54), (55) and (74)

To a solution of D-fructose (10.0 g, 55.51 mmol) in anhydrous methanol (350 ml) was added Amberlite IR-120 (H^+) ion-exchange resin (2.50 g). The resulting mixture was left to stir at rt for 18 h. The resin was filtered off, washed with methanol (200 ml)

and the filtrate concentrated. The residue was chromatographed (eluent: water) on a column of Amberlite IRA-400 (OH^-) ion-exchange resin giving methyl β-D-fructopyranoside (**74**) (0.97 g, 9%), methyl β-D-fructofuranoside (**55**) (5.17 g, 48%) and methyl α-D-fructofuranoside (**54**) (4.10 g, 38%).

74:

^{13}C NMR (D_2O) δ 49.4 (OCH_3), 61.9 (C-1), 64.7 (C-6), 69.4 (C-3), 70.0 (C-5), 70.6 (C-4), 101.3 (C-2).

55:

^{13}C NMR (D_2O) δ 49.8 (OCH_3), 60.7 (C-1), 63.6 (C-6), 76.0 (C-4), 77.8 (C-3), 82.1 (C-5), 104.6 (C-2).

54:

^{13}C NMR (D_2O) δ 49.2 (OCH_3), 58.9 (C-1), 62.2 (C-6), 78.2 (C-4), 81.1 (C-3), 84.1 (C-5), 109.1 (C-2).

Assignment of ^{13}C NMR spectra was made by comparison with the literature [179].

Methyl 3,4-anhydro-β-D-tagatofuranoside (60)

A solution of methyl β-D-fructofuranoside (**55**) (2.30 g, 11.9 mmol) and TPP (7.76 g, 29.6 mmol) in DMF (30 ml) at 0°C was treated with DIAD (5.83 ml, 29.6 mmol). The reaction was warmed to rt and left to stir for 16 h. The solvent was removed under reduced pressure and the residue chromatographed (CH_2Cl_2/acetone, 3:1, R_f 0.30) to give **60** (1.86 g, 89%) as a syrup.

^1H NMR ($CDCl_3$) δ 2.73 (s, 3H, OCH_3), 3.56 (d, 1H, $J_{1,1'}$ 11.7, H-1), 3.65 (d, 1H, $J_{1',1}$ 11.7, H-1'), 3.71 (d, 1H, $J_{4,3}$ 2.7 , H-4), 3.75 (d, 1H, $J_{3,4}$ 2.7, H-3), 3.85 (d, 2H, $J_{6,5}$ 5.7, H-6), 4.08 (t, 1H, $J_{5,6}$ 5.7, H-5).

^{13}C NMR (CD_3COCD_3) δ 52.2 (OCH_3), 55.2, 57.5 (C-3, C-4), 61.5 (C-6), 65.7 (C-1), 78.2 (C-5), 105.9 (C-2).

^{1}H and ^{13}C NMR assignments were in agreement with the literature[176,202].

Methyl 3,4-anhydro-α-D-tagatofuranoside (59)

To a chilled solution of methyl α-D-fructofuranoside (**54**) (1.86 g, 9.62 mmol) and TPP (6.28 g, 23.9 mmol) in DMF (24 ml) was added DIAD (4.71 ml, 23.9 mmol). The reaction was warmed to rt and left to stir for 16 h. Concentration of the reaction mixture under reduced pressure gave a residue which was chromatographed (CH_2Cl_2/acetone, 3:1, R_f 0.30) on a silica column giving **59** (1.47 g, 87%) as a syrup.

^{1}H NMR ($CDCl_3$) δ 2.93 (s, br ,1H, OH), 3.33 (s, 3H, OCH_3), 3.62 (d, 1H, $J_{1,1'}$ 11.7, H-1), 3.76 (m, 5H, H-1', H-3, H-4, H-6, H-6'), 4.15(t, 1H, $J_{5,6} = J_{5,6'}$ 5.5, H-5).

^{13}C NMR ($CDCl_3$) δ 49.5 (O*Me*), 55.3, 56.9 (C-3, C-4), 60.0, 61.5 (C-1, C-6), 78.1 (C-5), 105.0 (C-2).

^{13}C NMR assignments were by comparison with the literature[176]

Methyl 4-azido-4-deoxy-β-D-fructofuranoside (75)

To a solution of **60** (840 mg, 4.77mmol) in dry DMF (25 ml) was added lithium azide (1.29 g, 26.3 mmol) and dry Amberlite IR-120 (H^+) ion exchange resin (1.75 g). The resulting mixture was stirred at *ca.*100°C for 50 h under N_2. The resin was filtered off, washed with DMF (15 ml) and the filtrate concentrated to give a residue which was partitioned between ethyl acetate (100 ml) and water (10 ml). The organic layer was dried (Na_2SO_4), concentrated and chromatographed (EtOAc, R_f 0.33) to afford **75** (709 mg, 68%) as a colourless oil.

IR (NaCl) ν_{max} 3352, 2981, 2110 (N_3), 1276, 1135, 1062 cm^{-1}.

^1H NMR (CD_3COCD_3-D_2O) δ 3.29 (s, 3H, OCH_3), 3.48- 3.71 (m, 5H, H-1, H-1', H-5, H-6, H-6'), 3.95 (app t, 1H, $J_{4,3} = J_{4,5}$ 8.3, H-4), 4.19 (d, 1H, $J_{3,4}$ 8.3, H-3).

^{13}C NMR (CD_3COCD_3) δ 48.4 (OCH_3), 60.1(C-1), 62.6 (C-6), 66.5 (C-4), 76.5 (C-3), 79.7 (C-5), 103.8 (C-2).

LRMS (ESI) *m/z* 242 $[M+Na]^+$ (17%), 201 (100), 165 (18), 133(23), 108 (36).

^1H and ^{13}C NMR assignments were confirmed using ^1H-^1H COSY and ^1H-^{13}C HMQC NMR spectroscopy.

Methyl 4-azido-6-*O*-benzoyl-4-deoxy-β-D-fructofuranoside (76)

To a chilled solution of TPP (320 mg, 1.22 mmol) in DMF (2 ml) was added compound **75** (150 mg, 0.68 mmol). The resulting mixture was then treated with DIAD (0.24 ml, 1.22 mmol) followed by the addition of benzoic acid (83 mg, 0.68 mmol). The reaction was warmed to rt and left to stir under N_2 for 16 h. Concentration followed by chromatography (EtOAc/hexane, 1:1, R_f 0.52 in EtOAc) gave **76** (187 mg, 85%) as a colourless oil.

IR (NaCl) ν_{max} 3479, 3048, 2972, 2141 (N_3), 1736, 1462, 1281, 1069 cm^{-1}.

^1H NMR ($CDCl_3$) δ 3.32 (s, 3H, OCH_3), 3.70 (d, 1H, $J_{1,1'}$ 12.0, H-1), 3.76 (d, 1H, $J_{1',1}$ 12.0, H-1'), 4.05 (m, 2H, H-4/H-5), 4.29 (m, 1H, H-3), 4.41 (dd, 1H, $J_{6,5}$ 4.3, $J_{6,6'}$ 12.0, H-6), 4.53 (dd, 1H, $J_{6',5}$ 3.5, $J_{6'6}$ 12.0, H-6'), 7.44 (m, 2H, Ar-H-3/Ar-H-5), 7.57(m, 1H, Ar-H-4), 8.07 (m, 2H, Ar-H-2/Ar-H-6).

^{13}C NMR ($CDCl_3$) δ 49.2 (OCH_3), 60.3 (C-1), 64.2 (C-6), 66.2 (C-4), 77.4, 77.7 (C-3, C5), 103.4 (C-2), 128.4, 129.4, 129.6, 133.3 (Ph), 166.2 (CO).

LRMS (ESI) *m/z* 346 $[M+Na]^+$ (100%), 293 (10), 292 (38), 202 (11), 161 (16).

HRMS (ESI) calcd for $C_{14}H_{21}N_4O_6$ $[M+NH_4]^+$ 341.14611. Found *m/z* 341.14605.

^{1}H and ^{13}C NMR assignments were confirmed using ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMQC NMR spectroscopy.

Methyl 4-azido-4-deoxy-α-D-fructofuranoside (77)

To a solution of **59** (560 mg, 3.18 mmol) in dry DMF (15 ml) was added lithium azide (860 mg, 17.5 mmol) and dry Amberlite IR-120 (H^+) ion-exchange resin (1.20g). The resulting mixture was stirred at *ca.*100°C for 50 h under N_2. The resin was filtered off, washed with DMF (10 ml) and the filtrate concentrated. The residue was partitioned between ethyl acetate (80 ml) and water (8 ml). The organic layer was dried (Na_2SO_4), concentrated and chromatographed (EtOAc, R_f 0.41) affording **77** (462 mg, 66%) as a syrup.

IR (NaCl) $\underline{\nu}_{max}$ 3487, 2936, 2112 (N_3), 1267, 1059 cm^{-1}.

^{1}H NMR (CD_3COCD_3-D_2O) δ 3.19 (s, 3H, OCH_3), 3.56-3.71 (m, 6H, H-1, H-1', H-4, H-6, H-6'), 3.78 (m, 1H, H-5), 4.08 (d, 1H, $J_{3,4}$ 4.1, H-3).

^{13}C NMR (CD_3COCD_3) δ 48.0 (OCH_3), 59.6 (C-1), 61.2 (C-6), 67.0 (C- 4), 80.4, 80.5 (C-3, C-5), 107.8 (C-2).

LRMS (ESI) *m/z* 242 $[M+Na]^+$ (18%), 201 (58), 173 (22), 133 (17), 95 (31), 77 (100).

^{1}H NMR assignments were confirmed using ^{1}H-^{1}H COSY NMR spectroscopy.

Methyl 4-azido-6-*O*-benzoyl-4-deoxy-α-D- fructofuranoside (78)

To a chilled solution of TPP (320 mg, 1.22 mmol) in DMF (2 ml) was added compound **77** (150 mg, 0.68 mmol). The resulting mixture was treated sequentially with DIAD (0.24 ml, 1.22 mmol) and benzoic acid (83 mg, 0.68 mmol). The reaction was

warmed to rt and left to stir for 16 h. Concentration under reduced pressure followed by chromatography (EtOAc/hexane, 1:1, R_f 0.28) afforded **78** (179 mg, 81%) as a syrup.

IR (NaCl) ν_{max} 3473, 2948, 2896, 2108, 1721, 1452, 1275, 1066 cm^{-1}.

^1H NMR (CD_3OD) δ 3.36 (s, 3H, OCH_3), 3.71 (d, 1H, $J_{1,1'}$ 12.1, H-1), 3.76 (d, 1H, $J_{1',1}$ 12.1, H-1'), 3.83 (dd, 1H, $J_{4,3}$ 4.1, $J_{4,5}$ 7.0, H-4), 4.16 (m, 1H, H-5), 4.23 (d, 1H, $J_{3,4}$ 4.1, H-3), 4.52 (dd, 1H, $J_{6,5}$ 5.3, $J_{6,6'}$ 11.8, H-6), 4.56 (dd, 1H, $J_{6',5}$ 4.4, $J_{6',6}$ 11.8, H-6'), 7.52 (m, 2H, Ar-H-3/Ar-H-5), 7.64 (m, 1H, Ar-H-4), 8.12 (m, 2H, Ar-H-2/Ar-H-6).

^{13}C NMR ($CDCl_3$) δ 49.0 (OCH_3), 59.7 (C-1), 62.8 (C-6), 68.3 (C-4), 78.1 (C-3), 82.2 (C-5), 107.5 (C-2), 128.4, 129.4, 129.6, 133.3 (Ph), 166.4 (CO).

LRMS (ESI) *m/z* 346 $[M+Na]^+$ (91%), 303 (24), 293 (16), 292 (100), 249 (39).

HRMS (ESI) calcd for $C_{14}H_{21}N_4O_6$ $[M+NH_4]$ 341.14611. Found *m/z* 341.14542.

^1H NMR assignments were confirmed using ^1H-H^1 COSY NMR spectroscopy.

^1H NMR spectrum of **78** in $CDCl_3$ which was not well resolved, is included for comparison with the spectrum of the β-anomer **76** in section 2.6.1.

^1H NMR ($CDCl_3$) δ 2.71 (br, 1H, OH), 3.31 (s, 3H, OCH_3), 3.83 (m, 3H, H-1, H-1', H-4), 4.15 (m, 1H, H-5), 4.31 (br, 1H, H-3), 4.55 (dd, 1H, $J_{6,5}$ 3.9, $J_{6,6'}$ 12.1, H-6), 4.60 (dd, 1H, $J_{6',5}$ 4.8, $J_{6',6}$ 12.1, H-6'), 7.45 (m, 2H, Ar-H-3/Ar-H-5), 7.58 (m, 1H, Ar-H-4), 8.06 (m, 2H, Ar-H-2/Ar -H-6).

Methyl 4-azido-6-*O*-benzoyl-1-*O*-*tert*-butyldiphenylsilyl-4-deoxy-β-D-fructofuranoside (81)

A solution of **76** (450 mg, 1.39 mmol) in DMF (1 ml) was treated with *tert*-butyldiphenylsilyl chloride (459 mg, 1.67 mmol) and imidazole (236 mg, 3.47 mmol). The reaction was left to stir for 18 h at rt. Concentration of the reaction mixture under reduced pressure gave a residue, which was partitioned between EtOAc (60 ml) and

water (6 ml). The organic layer was dried (Na_2SO_4) and concentrated. Chromatography of the residue (EtOAc/hexane, 1:6, R_f 0.23) gave the title compound **81** (711 mg, 91%) as a colourless syrup.

IR (KBr) ν_{max} 3549, 3051, 2949, 2112 (N_3), 1728, 1273, 1117, 1062 cm^{-1}.

^1H NMR ($CDCl_3$) δ 1.07 (s, 9H, $SiC(CH_3)_3$), 2.71 (d, 1H, $J_{OH,3}$ 8.8, OH), 3.20 (s, 3H, OCH_3), 3.72 (d, 1H, $J_{1,1'}$ 11.0, H-1), 3.77 (d, 1H, $J_{1',1}$ 11.0, H-1'), 4.03 (m, 2H, H-4/H-5), 4.39 (m, 2H, $J_{6,5}$ 4.2, $J_{6,6'}$ 12.0, H-3/H-6), 4.52 (dd, 1H, $J_{6',5}$ 3.2, $J_{6',6}$ 12.0, H-6'), 7.32-8.10 (m, 15H, 3x Ph).

^{13}C NMR ($CDCl_3$) δ 19.4 ($SiC(CH_3)_3$), 27.0 ($SiC(CH_3)_3$), 49.5 (OCH_3), 62.0 (C-6), 64.4 (C-1), 66.9 (C-4), 77.6 (C-3), 78.2 (C-5), 103.7 (C-2), 128.0, 128.6, 129.9, 130.0, 132.9, 133.4, 135.7, 135.8 (3 x Ph), 166.4 (CO).

LRMS (ESI) 579 *m/z* $[M+NH_4]^+$ (100%), 547 (27), 530 (48), 311 (10), 165 (16).

HRMS (ESI) calcd for $C_{30}H_{39}N_4O_6Si$ $[M+NH_4]$ 579.26389. Found *m/z* 579.26438.

^1H NMR assignments were confirmed using ^1H-^1H COSY NMR spectroscopy.

Methyl 3-*O*-acetyl-4-azido-6-*O*-benzoyl-1-*O*-*tert*-butyldiphenylsilyl-4-deoxy-β-D-fructofuranoside (82).

Acetylation of compound **81** (110 mg) was carried out according to General Procedure 2. The crude residue was chromatographed (EtOAc /hexane, 1:3, R_f 0.56) to give the title compound **82** (111 mg, 94%) as an oil.

IR (NaCl) ν_{max} 3090, 2951, 2108, 1751, 1725, 1428, 1271, 1226, 1111, 1110 cm^{-1}.

^1H NMR ($CDCl_3$) δ 1.09 (s, 9H, $SiC(CH_3)_3$), 2.18 (s, 3H, $OCOCH_3$), 3.23 (s, 3H, OCH_3), 3.72 (d, 1H, $J_{1,1'}$ 11.1, H-1), 3.76 (d, 1H, $J_{1',1}$ 11.1, H-1'), 4.19 (m, 1H, H-5), 4.26 (app t, 1H, $J_{4,3} = J_{4,5}$ 8.7, H-4), 4.50 (dd, 1H, $J_{6,5}$ 4.6, $J_{6,6'}$ 12.1, H-6), 4.59 (dd, 1H, $J_{6',5}$ 3.7, $J_{6',6}$ 12.1, H-6'), 5.84 (d, 1H, $J_{3,4}$ 8.7, H-3), 7.35-8.1 (m, 15H, 3 x Ph).

^{13}C NMR (CD_3OD) 20.0 ($SiC(CH_3)_3$), 20.6 ($OCOCH_3$), 27.2 ($SiC(CH_3)_3$), 50.5 (OCH_3), 65.1 (C-6), 65.4 (C-4), 65.7 (C-1), 77.5 (C-3), 78.5 (C-5), 105.4 (C-2), 128.7, 129.6, 130.6, 130.8, 130.9, 131.4, 133.8, 133.9, 134.6, 135.3, 136.6, 136.7, (3 x Ph), 167.4 (OCOPh), 171.3 (OCOMe).

LRMS (ESI) *m/z* 621 $[M+NH_4]^+$ (100%), 573(20), 572 (53), 353 (10), 179 (19), 159 (17), 115 (22).

HRMS (ESI) calcd for $C_{32}H_{41}N_4O_7Si$ $[M+NH_4]^+$ 621.27445. Found *m/z* 621.27498.

Methyl 1-*O*-acetyl-4-azido-6-*O*-benzoyl-4-deoxy-β-D-fructofuranoside (83).

To a solution of **82** (72 mg, 0.12 mmol) in anhydrous THF (4 ml) was added tetrabutylammonium fluoride (0.35 ml, 0.35 mmol, 1.0 M solution in THF). The resulting mixture was stirred at rt for 4 h until no starting material remained (TLC, EtOAc/hexane, 1:3). The reaction was concentrated and chromatographed (EtOAc/hexane, 1:1) giving two products, **83** (R_f 0.62,16 mg, 37%) and **76** (R_f 0.20, 7 mg, 16%,).

83:

^1H NMR ($CDCl_3$) δ 2.14 (s, 3H, OCO*Me*), 3.39 (s, 3H, O*Me*), 4.08 (m, 2H, H-4/ H-5), 4.20 (d, 1H, $J_{1,1'}$ 12.1, H-1), 4.25 (m, br, 1H, H-3), 4.36 (d, 1H, $J_{1',1}$ 12.1, H-1'), 4.43 (dd, 1H, $J_{6,5}$ 4.1, $J_{6,6'}$ 12.0, H-6), 4.55(dd, 1H, $J_{6',5}$ 3.5, $J_{6',6}$ 12.0, H-6'), 7.51 (m, 2H, Ar-H-3/Ar-H-5), 7.63 (m, 1H, Ar-H-4), 8.10 (m, 2H, Ar -H-2/Ar-H-6).

^1H NMR assignments were confirmed using ^1H-^1H COSY NMR spectroscopy.

Spectral data of **76** was identical to that of the material prepared earlier.

Methyl 3-*O*-acetyl-4-azido-6-*O*-benzoyl-4-deoxy-β-D-fructofuranoside (84).

To a solution of **82** (80 mg, 0.13 mmol) in anhydrous THF (4 ml) was added TBAF (0.4 ml, 0.4 mmol, 1.0 M solution in THF). The resulting mixture was adjusted to pH 7 with acetic acid. The reaction was then left to stir at rt for 16 h, concentrated *in vacuo* and chromatographed (EtOAc/hexane, 1:2, R_f 0.18) affording the title compound **84** (35 mg, 72%) as a colourless syrup.

IR (NaCl) ν_{max} 3510, 2947, 2110, 1724, 1450, 1271, 1232, 1066 cm^{-1}.

^1H NMR ($CDCl_3$) δ 2.21 (s, 3H, $OCOCH_3$), 3.31(s, 3H, OCH_3), 3.64 (d, 1H, $J_{1,1'}$ 12.5, H-1) 3.77 (d , 1H, $J_{1',1}$ 12.5, H-1'), 4.15 (m, 1H, H-5), 4.40 (dd, 1H, $J_{4,3} = J_{4,5}$ 8,4, H-4), 4.47 (dd, 1H, $J_{6,5}$ 4.9, $J_{6,6'}$ 12.0, H-6), 4.53 (dd, 1H, $J_{6',5}$ 4.6, $J_{6',6}$ 12.0, H-6'), 5.22 (d, 1H, $J_{3,4}$ 8.4, H-3) 7.45 (m, 2H, Ar-H-3/Ar-H-5), 7.58 (m, 1H, Ar-H-4), 8.08 (m, 2H, Ar-H-2/Ar-H-6).

^{13}C NMR ($CDCl_3$) δ 20.8 ($OCOCH_3$), 49.4 (OCH_3), 61.67 (C-6), 63.8 (C-4), 64.1 (C-1), 77.0, 77.2 (C-3/C-5), 104.1 (C-2), 128.4, 129.4, 129.7, 133.3 (Ph), 166.1 (OCOPh), 171.2 (OCOMe).

LRMS (ESI) *m/z* 383 $[M+NH_4]^+$ (23%), 335 (17), 334 (100), 242 (13), 169 (27).

HRMS (ESI) calcd for $C_{16}H_{23}N_4O_7$ $[M+NH_4]^+$ 383.15668. Found *m/z* 383.15588.

^1H NMR assignments were confirmed using ^1H-^1H COSY NMR spectroscopy.

tert-Butyl (methyl 3-_O_-acetyl-4-azido-6-_O_-benzoyl-4-deoxy-β-D-fructofuranosid) onate (79)

{ _tert_-Butyl (methyl 3-_O_-acetyl-4-azido-6-_O_-benzoyl-4-deoxy-β-D-_arabino_-hex-2-ulofuranosid)onate }

Methyl 3-_O_-acetyl-4-azido-6-_O_-benzoyl-4-deoxy-β-D-fructofuranoside (**84**) (60 mg) was converted to ester **79** according to General Procedure 1. Chromatography of the crude residue (EtOAc/hexane, 1:3, R_f 0.46) gave **79** (63 mg, 85%).

IR (NaCl) ν_{max} 2982, 2111, 1749, 1719, 1269, 1224, 1087 cm^{-1}.

^1H NMR ($CDCl_3$) δ 1.50 (s, 9H, $C(CH_3)_3$), 2.18 (s, 3H, $OCOCH_3$), 3.38 (s ,3H, OCH_3), 4.25 (m, 1H, H-5), 4.34 (dd, 1H, $J_{4,3} = J_{4,5}$ 8.6, H-4), 4.50 (dd, 1H, $J_{6,5}$ 4.0, $J_{6,6'}$ 12.2, H-6), 4.56 (dd, 1H, $J_{6',5}$ 4.2, $J_{6',6}$ 12.2, H-6'), 5.42 (d, 1H, $H_{3,4}$ 8.6, H-3), 7.47 (m, 2H, Ar-H-3/Ar-H-5), 7.59 (m, 1H, Ar-H-4), 8.08 (m, 1H, Ar-H-2 / Ar-H-6).

^{13}C NMR ($CDCl_3$) δ 20.5 ($OCOCH_3$), 27.7 ($C(CH_3)_3$), 51.3 (OCH_3), 63.0 (C-4), 63.4 (C-6), 77.4, 77.5 (C-3, C-5), 83.3 ($C(CH_3)_3$), 100.5 (C-2), 128.4, 129.3, 129.7, 133.3 (Ph), 165.2 (COOtBu), 166.0 (OCOPh), 169.3 (OCOMe).

LRMS (ESI) *m/z* 453 $[M+NH_4]^+$ (78%), 381 (20), 380 (100), 349 (11), 348 (52).

HRMS (ESI) calcd for $C_{20}H_{29}N_4O_8$ $[M+NH_4]^+$ 453.19854. Found *m/z* 453.19732.

^1H NMR assignments were confirmed using ^1H-^1H COSY NMR spectroscopy.

Methyl 4-azido-6-_O_-benzoyl-1-_O_-_tert_-butyldiphenylsilyl-4-deoxy-α-D-fructofuranoside (85).

To a solution of **78** (484 mg, 1.50 mmol) in DMF (1 ml) was added *tert*-butyldiphenylsilyl chloride (495 mg, 1.80 mmol) and imidazole (254 mg, 3.74 mmol). The mixture was stirred at rt for 18 h, concentrated and the residue partitioned between

EtOAc (60 ml) and water (6 ml). The organic layer was dried (Na_2SO_4) and concentrated. Chromatography of the residue (EtOAc/hexane, 1:6, R_f 0.22) afforded compound **85** (748 mg, 89%) as a oil.

IR (NaCl) ν_{max} 3500, 3070, 2948, 2115, 1738, 1281, 1120 cm^{-1}.

^1H NMR ($CDCl_3$) δ 1.10 (s, 9H, $SiC(CH_3)_3$), 3.09 (s, 3H, OCH_3), 3.56 (d, 1H, $J_{OH,3}$ 3.7, OH), 3.83 (dd, 1H, $J_{4,3}$ 3.3, $J_{4,5}$ 6.8, H-4), 3.88 (d, 1H, $J_{1,1'}$ 11.3, H-1), 3.95 (d, 1H, $J_{1',1}$ 11.3, H-1'), 4.17 (m, 1H, H-5), 4.41 (app s, br, 1H, H-3), 4.49 (dd, 1H, $J_{6,5}$ 5.4, $J_{6,6'}$ 11.9, H-6), 4.59 (dd, 1H, $J_{6',5}$ 4.2, $J_{6',6}$ 11.9, H-6'), 7.42-8.07 (m, 15H, 3 x Ph).

^{13}C NMR ($CDCl_3$) δ 19.20 ($SiC(CH_3)_3$), 28.9 ($SiC(CH_3)_3$), 48.6 (OCH_3), 61.5 (C-6), 64.2 (C-1), 68.7 (C-4), 78.8 (C-3), 83.2 (C-5), 108.0 (C-2), 128.7, 128.0, 128.5, 129.6, 129.8, 130.1, 130.2, 132.2, 133.2, 135.6, 135.7 (3 x Ph), 166.2 (CO).

LRMS (ESI) *m/z*584 $[M+Na]^+$ (14%), 579 $[M+NH_4]^+$ (27), 531 (34), 530 (94), 371 (14), 343 (17), 241 (21), 227 (42), 223 (100).

HRMS (ESI) calcd for $C_{30}H_{39}N_4O_6Si$ $[M+NH_4]^+$ 579.26389. Found *m/z* 579.26808.

Methyl 3-*O*-acetyl-4-azido-6-*O*-benzoyl-1-*O*-*tert*-butyldiphenylsilyl-4-deoxy-α-D-fructofuranoside (86).

Compound **85** (120 mg) was acetylated according to General Procedure 2. Chromatography of the crude residue (EtOAc/hexane, 1:3, R_f 0.53) on silica column gave **86** (124 mg, 96%) as a syrup.

^1H NMR ($CDCl_3$) δ 1.09 (s, 9H, $SiC(CH_3)_3$), 1.93 (s, 3H, $OCOCH_3$), 3.20 (s, 3H, OCH_3), 3.80 (dd, 1H, $J_{4,3}$ 1.7, $J_{4,5}$ 5.8, H-4), 3.82 (d, 1H, $J_{1,1'}$ 11.3, H-1), 3.88 (d, 1H, $J_{1',1}$ 11.3, H-1'), 4.14 (m, 1H, H-5), 4.48 (dd, 1H, $J_{6,5}$ 4.6, $J_{6,6'}$ 11.9, H-6), 4.52 (dd, 1H, $J_{6',5}$ 4.5, $J_{6',6}$ 11.9, H-6'), 5.32 (d, 1H, $J_{3,4}$ 1.7, H-3), 7.40-8.05 (m, 15H, 3 x Ph).

^{13}C NMR (CD_3OD) δ 19.9, (SiC(CH_3)$_3$), 20.8 (OCOCH_3), 27.1 (SiC(CH_3)$_3$), 49.1 (OCH_3), 60.3 (C-6), 64.7 (C-1), 68.4 (C-4), 81.2 (C-3), 82.1 (C-5), 109.4 (C-2), 128.7, 129.4, 130.5, 130.9, 131.0, 134.3, 136.5, 136.7 (3 x Ph), 167.3 (OCOPh), 170.8 (OCOMe).

LRMS (ESI) *m/z* 621 $[M+NH_4]^+$ (19%), 334 (32), 328 (16), 237 (19), 197 (34), 196 (100), 195 (67), 182 (38).

HRSM (ESI) Calcd for $C_{32}H_{41}N_4O_7Si$ $[N+NH_4]^+$ 621.27445. Found *m/z* 621.27354.

Methyl 1-*O*-acetyl-4-azido-6-*O*-benzoyl-4-deoxy-α-D-fructofuranoside (87).

A solution of **86** (306 mg, 0.51 mmol) in anhydrous THF (15 ml) was treated with TBAF (1.53 ml, 1.53 mmol, 1.0 M solution in THF) and adjusted to pH 7 using acetic acid. The reaction mixture was stirred at rt for 8 h until no starting material remained (TLC, EtOAc/hexane, 1:2). Concentration of the reaction mixture, followed by chromatography (EtOAc/hexane, 1:2) of the residue gave two products, **87** (91 mg, 49%) and **78** (12 mg, 6.5%).

87:

^{1}H NMR ($CDCl_3$) δ 2.12 (s, 3H, $OCOCH_3$), 3.34 (s, 3H, OCH_3), 3.76 (app s, br, 1H, OH), 3.81 (dd, 1H, $J_{4,3}$ 1.8, $J_{4,5}$ 5.2, H-4), 4.07 (app s, br; after D_2O exchange: d, 1H, $J_{3,4}$ 1.8, H-3) 4.16 (m, 1H, H-5), 4.23 (d, 1H, $J_{1,1'}$ 12.7, H-1), 4.42 (d, 1H, $J_{1',1}$ 12.7, H-1'), 4.49 (dd, 1H, $J_{6,5}$ 4.8, $J_{6,6'}$ 12.0, H-6), 4.52 (dd, 1H, $J_{6',5}$ 4.9, $J_{6',6}$ 12.0, H-6'), 7.45 (m, 2H, Ar-H-3/Ar-H-5), 7.56 (m, 1H, Ar-H-4), 8.07 (m, 2H, Ar-H-2/Ar-H-6).

^{13}C NMR ($CDCl_3$) δ 20.6 (OCOCH_3), 48.8 (OCH_3), 58.8 (C-1), 64.0 (C-6), 68.2 (C-4), 78.9 (C-3), 80.6 (C-5), 107.4 (C-2), 128.4, 129.5, 129.7, 133.2 (Ph), 166.1 (OCOPh), 171.7 (OCOMe).

LRMS (ESI) *m/z* 383 $[M+NH_4]^+$ (20%), 335 (18), 334 (100), 291 (19), 196 (17), 183 (12).

HRMS (ESI) calcd for $C_{16}H_{23}N_4O_7$ $[M+NH_4]^+$ 383.15668. Found *m/z* 383.15585.

^1H and ^{13}C NMR assignments were confirmed using ^1H-^1H COSY and ^1H-^{13}C HMQC NMR spectroscopy.

Spectral data of **78** was identical to that of the material prepared earlier.

Methyl 3,4-anhydro-6-*O*-benzoyl-α-D-tagatofuranoside (88)

{ Methyl 3,4-anhydro-6-*O*-benzoyl-α-D-*lyxo*-hex-2-ulofuranoside }

DIAD (1.07 ml, 5.46 mmol) was added dropwise to chilled solution of TPP (1.43 g, 5.46 mmol) in DMF (6 ml) and the mixture stirred for 20 min at 0°C. Compound **59** (480 mg, 2.73 mmol) in DMF (1.5 ml) was added, followed by benzoic acid (0.333 mg, 2.73 mmol). The reaction was warmed to rt and left to stir for 16 h at rt. The mixture was concentrated and the residue chromatographed (EtOAc/hexane, 1:2, R_f 0.16) to give **88** (626 mg, 82%) as a syrup which crystallized on standing.

$[\alpha]_D$ +25.7° (*c* 2.12, $CHCl_3$).

^1H NMR ($CDCl_3$) δ 3.31 (s, 3H, OCH_3), 3.65(d, 1H, $J_{1,1'}$ 11.7, H-1), 3.71 (app d, 1H, $J_{4,3}$ 2.8, H-4), 3.80 (m, 2H, H-1', H-3), 4.34 (m, 1H, H-5), 4.40 (dd, 1H, $J_{6,5}$ 5.7, $J_{6,6'}$ 11.1, H-6), 4.47 (dd, 1H, $J_{6',5}$ 5.9, $J_{6',6}$ 11.1, H-6'), 7.38 (m, 2H, Ar-H-3, Ar-H-5), 7.51 (m, 1H, Ar-H-4), 7.99 (m, 2H, Ar-H-2, Ar-H-6).

^{13}C NMR ($CDCl_3$) δ 49.5 (OCH_3), 55.3 (C-4), 57.3 (C-3), 60.1 (C-6), 62.9 (C-1), 75.8 (C-5), 105.2 (C-2), 128.0, 128.3, 129.6, 133.1 (Ph), 166.1 (OCOPh).

LRMS (FAB) *m/z* 281$[M+H]^+$ (70%), 263 (17), 250 (100), 249 (100), 231 (30), 189 (26), 163 (40).

Anal. Calcd. for $C_{14}H_{16}O_6$: C, 59.99; H, 5.75. Found C, 60.30; H, 5.88%.

tert-Butyl (methyl 3,4-anhydro-6-_O_-benzoyl-α-D-tagatofuranosid)onate (89)

{ _tert_-Butyl (methyl 3,4-anhydro-6-_O_-benzoyl-α-D-_lyxo_-hex-2-ulofuranosid)onate }

Compound **88** (550 mg, 1.96 mmol) was converted to ester **89** according to General Procedure 1. The crude residue was chromatographed (EtOAc/hexane, 1:4, R_f 0.28) to give **89** (557 mg, 81%) as a syrup.

$[\alpha]_D$ +19.6° (*c* 1.41, $CHCl_3$).

^{1}H NMR ($CDCl_3$) δ 1.54 (s, 9H, $C(CH_3)_3$), 3.38 (s, 3H, OCH_3), 3.82 (app d, 1H, $J_{4,3}$ 2.7, H-4), 3.87 (d, 1H, $J_{3,4}$ 2.7, H-3), 4.38 (app t, 1H, H-5), 4.54 (dd, 1H, $J_{6,5}$ 6.4, $J_{6,6'}$ 11.3, H-6), 4.64 (dd, 1H, $J_{6',5}$ 5.3, $J_{6',6}$ 11.3, H-6'), 7.45 (m, 2H, Ar-H-3, Ar-H-5), 7.57 (m, 1H, Ar-H-4), 8.07 (m, 2H, Ar-H-2, Ar-H-6).

^{13}C NMR ($CDCl_3$) δ 27.9 ($C(CH_3)_3$), 51.4 (OCH_3), 54.4 (C-4), 58.6 (C-3), 62.4 (C-6), 75.4 (C-5), 82.9 ($C(CH_3)_3$), 103.2 (C-2), 128.3, 129.7, 129.8, 133,1 (Ph), 164.5, 166.0 (OCOPh, COOtBu).

LRMS (ESI) *m/z* 368 $[M+NH_4]^+$ (38%), 296 (15), 295 (100), 263 (11).

Anal. Calcd for $C_{18}H_{22}O_7$: C, 61.71; H, 6.33. Found C, 61.84; H, 6.40%.

^{1}H NMR assignments were confirmed using ^{1}H-^{1}H COSY NMR spectroscopy.

tert-Butyl (methyl 4-azido-6-_O_-benzoyl-4-deoxy-α-D-fructofuranosid)onate (90)

{ _tert_-Butyl (methyl 4-azido-6-_O_-benzoyl-4-deoxy-α-D-_arabino_-hex-2-ulofuranosid) onate }

Compound **89** (180 mg, 0.51 mmol) in DMF (6 ml), lithium azide (150 mg, 3.06 mmol) and dry Dowex-50WX8-400 (H^+) ion-exchange resin (370 mg) were stirred at *ca.* 95°C for 16 h under N_2. The resin was filtered off and washed with DMF (6 ml). The filtrate was concentrated under reduced pressure and the residue obtained

partitioned between EtOAc (40 ml) and water (10 ml). The organic layer was dried (Na_2SO_4) and concentrated. Chromatography (EtOAc/hexane, 1:4, R_f 0.23) of the residue gave **90** (179 mg, 89%) as an oil.

IR (NaCl) ν_{max} 3495, 3006, 2112 (N_3), 1733, 1461, 1280 cm^{-1}.

^1H NMR ($CDCl_3$) 1.51(s, 9H, $C(CH_3)_3$), 3.36 (s, 3H, OCH_3), 3.89 (dd, 1H, $J_{4,3}$ 4.2, $J_{4,5}$ 7.0, H-4), H-5 (m, 1H, H-5), 4.35 (d, 1H, $J_{3,4}$4.2, H-3), 4.55 (dd, 1H, $J_{6,5}$ 4.6, $J_{6,6'}$12.0, H-6), 4.63 (dd, 1H, $J_{6',5}$ 4.3, $J_{6',6}$ 12.0, H-6'), 7.45 (m, 2H, Ar-H-3, Ar-H-5), 7.56 (m, 1H, Ar-H-4), 8.07 (m, 2H, Ar-H-2, Ar-H-6).

^{13}C NMR ($CDCl_3$) δ 27.8 ($C(CH_3)_3$), 51.5 (OCH_3), 63.6 (C-6), 66.9 (C-4), 78.3 (C-3), 82.8 (C-5), 83.7 ($C(CH_3)_3$), 107.4 (C-2), 128.3, 129.5, 129.7, 133.2 (Ph), 166.0, 166.2 (OCOPh, COOtBu).

LRMS (ESI) *m/z* 411 $[M+NH_4]^+$ (24%), 339 (18), 338 (100), 306 (22), 196 (17).

HRMS (ESI) calcd for $C_{18}H_{27}N_4O_7$ $[M+NH_4]^+$ 411.18798, found *m/z* 411.18720.

Methyl 3,4-anhydro-6-*O*-benzoyl-β-D-tagatofuranoside (91)

{ Methyl 3,4-anhydro-6-*O*-benzoyl-β-D-*lyxo*-hex-2-ulofuranoside }

A chilled solution of TPP (1.07 g, 4.08 mmol) in DMF (5 ml) was treated with DIAD (0.80 ml, 4.08 mmol). The resulting mixture was stirred for 20 min at 0°C. Compound **60** (359 mg, 2.04 mmol) in DMF (1 ml) was added, followed by benzoic acid (249 mg, 2.04 mmol). The reaction mixture was warmed to rt, then stirred for a further 16 h. Concentration of the reaction mixture and column chromatography the residue (EtOAc/hexane, 1:2, R_f 0.16) furnished **91** (480 mg, 84%) as a colourless oil which crystallized upon standing.

$[\alpha]_D$ -8.9°C (*c* 1.64, $CHCl_3$)

^{1}H NMR ($CDCl_3$) δ 3.54 (s, 3H, OCH_3), 3.59 (d, 1H, $J_{1,1'}$ 11.7, H-1), 3.68 (d, 1H, $J_{1',1}$ 11.7, H-1'), 3.78 (dd, 1H, $J_{4,3}$ 2.9, $J_{4,5}$ 0.9, H-4), 3.80 (d, 1H, $J_{3,4}$ 2.9, H-3), 4.28 (ddd, 1H, $J_{5,4}$ 0.9 , $J_{5,6} = J_{5,6'}$ 5.8 , H-5), 4.56 (app d, 2H, H-6, H-6'), 7.44 (m, 2H, Ar-H-3, Ar-H-5), 7.55 (m, 1H, Ar-H-4), 8.06 (m, 2H, ArH-2, Ar-H-6).

^{13}C NMR ($CDCl_3$) δ 52.5 (OCH_3), 54.1 (C-4), 56.5 (C-3), 63.0 (C-6), 65.2 (C-1), 74.3 (C-5), 104.9 (C-2), 128.4, 129.7, 129.8, 133.2 (Ph), 166.3 (OCOPh).

LMRS (ESI) *m/z* 303 $[M+Na]^+$ (17%), 250 (15), 249 (100), 191(12), 177 (14).

Anal. calcd for $C_{14}H_{16}O_6$: C, 59.99; H, 5.75%. Found C, 60.25; H, 5.76%.

^{1}H and ^{13}C NMR assignments were confirmed using ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMQC NMR spectroscopy.

tert-Butyl (methyl 3,4-anhydro-6-*O*-benzoyl-β-D-tagatofuranosid)onate (92)

{ _tert_-Butyl (methyl 3,4-anhydro-6-*O*-benzoyl-β-D-*lyxo*-hex-2-ulofuranosid)onate }

Methyl 3,4-anhydro-6-*O*-benzoyl-β-D-tagatofuranoside (91) (465 mg, 1.66 mmol) was converted to ester **92** following General Procedure 1. Chromatography of the residue (EtAOc/hexane, 1:4, R_f 0.29) gave **92** (501 mg, 86%) as an oil.

$[\alpha]_D$ -39.2° (*c* 9.4, $CHCl_3$).

^{1}H NMR ($CDCl_3$) δ 1.54 (s, 9H , $C(CH_3)_3$), 3.54 (s, 3H. OCH_3), 3.83 (m, 2H, H-3, H-4), 4.38 (m, 1H, H-5), 4.57 (dd, 1H, $J_{6,5}$ 6.0, $J_{6,6'}$ 11.3, H-6), 4.62 (dd, 1H, $J_{6',5}$ 5.8, $J_{6',6}$ 11.3, H-6'), 7.45 (m, 2H, Ar-H-3, Ar-H-5), 7.58 (m, 1H, Ar-H-4), 8.07 (m, 2H, Ar-H-2, Ar-H-6).

^{13}C NMR ($CDCl_3$) δ 28.1 ($C(CH_3)_3$), 53.3 (OCH_3), 54.4 (C-4), 57.0 (C-3), 62.4 (C-6), 75.0 (C-5), 83.3 ($C(CH_3)_3$), 103.0 (C-2), 128.3, 129.6, 133.1 (Ph), 166.1, 166.5 (OCOPh, COOtBu).

LRMS (ESI) *m/z* 368 $[M+NH_4]^+$ (64%), 296 (16), 295 (100), 263 (31).

Anal. calcd for $C_{18}H_{22}O_7$: C, 61.71; H, 6.33. Found C, 62.18; H, 6 .43%.

tert-Butyl (methyl 4-azido-6-*O*-benzoyl-4-deoxy-β-D-fructofuranosid)onate (93)

{ _tert_-Butyl (methyl 4-azido-6-*O*-benzoyl-4-deoxy-β-D-*arabino*-hex-2-ulofuranosid) onate }

To a solution of **92** (140 mg, 0.40 mmol) in DMF (5 ml) was added lithium azide (117 mg, 2.39 mmol) and dry Dowex-50WX8-400 (H^+) ion-exchange resin (290 mg). The mixture was left to stir at *ca.* 95°C for 16 h under N_2. The resin was filtered off and washed with DMF (5 ml). The filtrate was concentrated under reduced pressure and the residue obtained partitioned between EtOAc (30 ml) and water (5 ml). The organic layer was dried (Na_2SO_4), concentrated and chromatographed (EtOAc/hexane, 1:4, R_f 0.23) to give **93** (134 mg, 85%) as an oil.

IR (NaCl) ν_{max} 3422, 2984, 2106 (N_3), 1720, 1700, 1453, 1265 cm^{-1}.

^1H NMR ($CDCl_3$) δ 1.52 (s, 9H, $C(CH_3)_3$), 3.39 (s ,3H, OCH_3), 4.04 (app t, 1H, $J_{4,3}$ = $J_{4,5}$ 8.1, H-4), 4.18 (m, 1H,H-5), 4.24 (d, 1H, $J_{3,4}$ 8.1, H-3), 4.43 (dd, 1H, $J_{6,5}$ 4.2, $J_{6,6'}$ 12.0, H-6), 4.54 (dd, 1H, $J_{6',5}$ 4.0, $J_{6',6}$12.0, H-6'), 7.46 (m, 2H, Ar-H-3, Ar-H-5), 7.59 (m, 1H, Ar-H-4), 8.07 (m, 2H, Ar-H-2, Ar-H-6).

^{13}C NMR ($CDCl_3$) δ 27.9 ($C(CH_3)_3$), 51.51 (OCH_3), 63.6 (C-6), 66.0 (C-4), 77.4 (C-3), 78.2 (C-5), 83.6 ($OC(CH_3)_3$), 101.3 (C-2), 128.4, 129.5, 129.7, 133.4 (Ph), 165.9, 166.1 (*O*COPh, *C*OOtBu).

LRMS (ESI) *m/z* 416$[M+Na]^+$ (24%), 410 (19), 411 (100), 362 (28), 338 (60), 306 (68).

HRMS (ESI) calcd for $C_{18}H_{23}N_3O_7Na$ $[M+Na]^+$ 416.14327. Found *m/z* 416.14339.

^1H NMR assignments were confirmed using ^1H-^1H COSY NMR spectroscopy.

5.4 Synthesis of methyl β-D-*glycero*-hex-2-en-5-ulofuranosidonic acid (95)

Methyl 3,4-anhydro-6-*O*-benzoyl-1-*O*-*tert*-butyldiphenylsilyl-β-D-tagatofuranoside (96)

{ Methyl 3,4-anhydro-6-*O*-benzoyl-1-*O*-*tert*-butyldiphenylsilyl-β-D-*lyxo*-hex-2-ulofuranoside }

To a solution of **91** (596 mg, 2.13 mmol) in DMF was added *tert*-butyldiphenylsilyl chloride (704 mg, 2.56 mmol) and imidazole (362 mg, 5.32 mmol). The mixture was stirred for 18 h at rt, concentrated and the residue partitioned between EtOAc (100 ml) and water (10 ml). The organic layer was dried (Na_2SO_4) and concentrated. Chromatography of the residue (EtOAc/hexane, 1:5, R_f 0.50) afforded **96** (970 mg, 88%) as a syrup.

^{1}H NMR ($CDCl_3$) δ 1.08 (s, 9H, $C(CH_3)_3$), 3.48 (s, 3H, OCH_3), 3.76 (m, 4H, H-1, H-1', H-3, H-4), 4.48 (m, 1H, H-5), 4.56 (app d, 2H, H-6, H-6'), 7.36-8.09 (m, 15H, 3 x Ph).

^{13}C NMR ($CDCl_3$) δ 19.2 ($SiC(CH_3)_3$), 26.8 ($SiC(CH_3)_3$), 52.6 (OCH_3), 55.1, 57.3 (C-3, C-4), 63.6 (C-6), 67.8 (C-1), 75.3 (C-5), 105.6 (C-2), 127.9, 128.0, 128.6, 129.8, 130.0, 130.1, 130.2, 133.3, 135.0, 135.4, 135.7, 135.8 (3 x Ph). Resonance for C=O was not observed in the ^{13}C NMR spectrum.

LRMS (ESI) *m/z* 541 $[M+Na]^+$ (12%), 365 (13), 234 (19), 233 (94), 191 (18), 115 (19), 105 (100).

HRMS (ESI) calcd for $C_{30}H_{38}NO_6Si$ $[M+NH_4]^+$ 536.24684, found *m/z* 536.24535.

^{13}C NMR assignments were confirmed using ^{1}H-^{13}C HMQC NMR spectroscopy.

Methyl 3,4-anhydro-1-*O-tert*-butyldiphenylsilyl-β-D-tagatofuranoside (97)

{ Methyl 3,4-anhydro-1-*O-tert*-butyldiphenylsilyl-β-D-*lyxo*-hex-2-ulofuranoside }

Compound **96** (456 mg, 0.88 mmol) was debenzoylated according to General Procedure 3. Chromatography of the residue (EtOAc/hexane, 1:2, R_f 0.36) gave **97** (335 mg, 92%) as a syrup.

^{1}H NMR ($CDCl_3$) δ 1.08 (s, 9H, $C(CH_3)_3$), 3.46 (s, 3H, OCH_3), 3.73 (m, 4H, H-1, H-1', H-3, H-4), 3.85 (dd, 1H, $J_{6,5}$ 5.1, $J_{6,6'}$ 11.1, H-6), 3.90 (dd, 1H, $J_{6',5}$ 6.0, $J_{6',6}$ 11.1, H-6'), 4.26 (app t, 1H, H-5), 7.36-7.68 (m, 10H, 2 x Ph).

^{13}C NMR ($CDCl_3$) δ 19.1 ($C(CH_3)_3$), 26.8 ($C(CH_3)_3$), 52.2 (OCH_3), 54.9, 56.9 (C-3, C-4), 62.2 (C-6), 67.3 (C-1), 77.5 (C-5), 105.1 (C-2), 127.7, 129.8, 129.9, 132.6, 132.7, 135.4, 135.5 (2 x Ph).

LRMS (ESI) *m/z* 432 $[M+NH_4]^+$ (62%), 365 (13), 336 (28), 335 (100).

HRMS (ESI) calcd for $C_{23}H_{34}NO_5Si$ $[M+NH_4]^+$ 433.22063. Found *m/z* 432.21818.

Methyl 3,4-anhydro-6-*O-tert*-butyldiphenylsilyl-β-D-*arabino*-hex-5-ulofuranosidonic acid (101)

To a solution of **97** (210 mg, 0.51 mmol) in isopropanol/water (3:1, 32 ml) was added Pt black (300 mg, freshly prepared from the hydrogen reduction of PtO_2). The mixture was adjusted to pH 8 by saturated aq $NaHCO_3$ solution. The reaction mixture was stirred vigorously for 4 h at 80°C whilst O_2 was bubbled through. The catalyst was filtered off and washed with water (3x30 ml). Concentration of the filtrate gave a residue which was column chromatographed (EtOAc/hexane, 5:1, R_f 0.18) to give the title compound **101** (176 mg, 81%) as a gum.

^{1}H NMR (CD_3OD) δ 1.06 (s, 9H, $C(CH_3)_3$), 3.50 (s, 3H, OCH_3), 3.67 (d, 1H, $J_{6,6'}$ 10.6, H-6), 3.75 (d, 1H, $J_{6',6}$ 10.6, H-6'), 3.79 (dd, 1H, $J_{2,3}$ 2.7, H-2), 4.09 (br, 1H, H-3), 4.59 (s, br, 1H, H-4), 7.37-7.71 (m, 10H, 2 x Ph).

^{13}C NMR (CD_3OD) δ 20.1 ($SiC(CH_3)_3$), 27.5 ($C(CH_3)_3$), 53.8 (OCH_3), 57.8, 57.9, 58.0 (C-2, C-3, C-4), 68.3 (C-6), 107.5 (C-5), 129.1, 131.2, 131.3, 133.9, 134.1, 136.8, 136.9 (2 x Ph), 175.7 (COOH).

LRMS (ESI) *m/z* 446 $[M+NH_4]^+$ (100%), 414 (11), 397 (13), 291 (21), 249 (17).

HRMS (ESI) calcd for $C_{23}H_{32}NO_6Si$ $[M+NH_4]^+$ 446.19989. Found *m/z* 446.19996.

Methyl (methyl 6-*O*-*tert*-butyldiphenylsilyl-β-D-*glycero*-hex-2-en-5-ulofuranosid) onate (102)

TPP (146 mg, 0.56 mmol) in DMF and anhydrous MeOH (85 µl, 2.10 mmol) (1 ml) were cooled to 0°C. The mixture was treated with DIAD (110 µl, 0.56 mmol) followed by the addition of **101** (60 mg, 0.14 mmol). The resulting mixture was brought to rt and left to stir for 36 h. Solvent removal and chromatography of the residue (EtOAc/hexane, 1:4, R_f 0.24) gave **102** (47 mg, 76%) as a gum.

^{1}H NMR ($CDCl_3$) δ 1.04 (s, 9H, $C(CH_3)_3$), 2.86 (d, 1H, $J_{OH,4}$ 7.5, OH), 3.41 (s, 3H, OCH_3), 3.75 (d, 1H, $J_{6,6'}$ 11.1, H-6), 3.80 (s, 3H, $COOCH_3$), 3.94 (d, 1H, $J_{6',6}$ 11.1, H-6'), 5.14 (dd, 1H, $J_{4,3}$ 2.4, $J_{4,OH}$ 7.5, H-4), 5.99 (d, 1H, $J_{3,4}$ 2.4, H-3), 7.37-7.68 (m, 10H,2 x Ph).

^{13}C NMR ($CDCl_3$) δ 19.2 ($SiC(CH_3)_3$), 26.6 ($SiC(CH_3)_3$), 51.4, 52.2 (OCH_3, $COOCH_3$), 62.5 (C-6), 75.2 (C-4), 107.8 (C-5), 113.7 (C-3), 127.7, 129.8, 132.6, 132.7, 135.5, 135.6 (2 x Ph), 147.2 (C-2), 161.9 (COOMe).

LRMS (ESI) *m/z* 465 $[M+Na]^+$ (86%), 460 $[M+NH_4]^+$ (100), 425 (30), 333 (19), 255 (23), 233 (20).

HRMS (ESI) calcd for $C_{24}H_{34}NO_6Si$ $[M+NH_4]^+$ 460.21554. Found *m/z* 460.21458.

Methyl (methyl β-D-*glycero*-hex-2-en-5-ulofuranosid)onate (94)

To a solution of **102** (45 mg, 0.10 mmol) in THF (6 ml) was added tetrabutylammonium fluoride (300 µl, 0.30 mmol, 1.0 M in THF). The mixture was stirred at rt for 45 min and concentrated *in vacuo*. Column chromatography of the residue (EtOAc/hexane, 2:1, R_f 0.18) afforded **94** (17 mg, 82%) as a white amorphous mass.

^{1}H NMR (CD_3OD) δ 3.49 (s, 3H, OCH_3), 3.60 (d, 1H, $J_{6,6'}$ 12.0, H-6), 3.71 (d, 1H, $J_{6',6}$ 12.0, H-6'), 3.81 (s, 3H, $COOCH_3$), 5.01 (d, 1H, $J_{4,3}$ 2.5, H-4), 5.95 (d, 1H, $J_{3,4}$ 2.5, H-3).

^{13}C NMR (CD_3OD) δ 52.3, 52.8 (OCH_3, COO CH_3), 63.7 (C-6), 75.2 (C-4), 110.7 (C-5), 114.8 (C-3), 148.5 (C-2), 162.2 (COOMe).

LRMS (ESI) *m/z* 205 $[M+H]^+$ (20%), 165 (100), 149 (46).

HRMS (ESI) calcd for $C_8H_{12}O_6Na$ $[M+Na]^+$ 227.05316. Found *m/z* 227.05267.

Methyl β-D-*glycero*-hex-2-en-5-ulofuranosidonic acid (95)

A solution of **94** (10 mg) in MeOH (0.5 ml) was treated with aq NaOH (0.3 M) to pH 12. The resulting mixture was stirred at rt for 4 h. Water (3 ml) was added and the solution was neutralized with Dowex-50WX8-400 (H^+) ion-exchange resin. The resin was filtered off, washed with water (3 ml) and the filtrate concentrated. The residue obtained was redissolved in water and lyophilized affording **95** (8 mg, 86%) as a white amorphous mass.

^{1}H NMR (D_2O) δ 3.46 (s, 3H, OCH_3), 3.60 (d, 1H, $J_{6,6'}$ 12.4, H-6), 3.77 (d, 1H, $J_{6',6}$ 12.4, H-6'), 4.89 (app s, 1H, H-4), 5.70 (app s, 1H, H-3).

LRMS (ESI) *m/z* 191 $[M+H]^+$ (13%), 190 (69), 151 (53), 149 (100), 121 (83).

5.5 Synthesis of C-6 modified fructofuranosidonic acids and derivatives

Methyl 3,4-anhydro-6-azido-6-deoxy-β-D-tagatofuranoside (116)

{ Methyl 3,4-anhydro-6-azido-6-deoxy-β-D-*lyxo*-hex-2-ulofuranoside }

A chilled solution of TPP (2.77 g, 10.57 mmol) in DMF (12 ml) was treated with DIAD (2.07 ml, 10.57 mmol) and the resulting mixture stirred for 20 min at 0°C. To this mixture was added compound **60** (930 mg, 5.28 mmol), followed by HN_3 (30 ml, 54 mmol, 1.8 M in CH_2Cl_2, prepared according to literature procedure[286]). The reaction was warmed to rt, then left to stir for 3 days. Concentration of the reaction mixture followed by column chromatography of the residue (EtOAc/hexane, 1:1, R_f 0.27) afforded **116** (701 mg, 66%) as a syrup. Acetylation of **116** according to General Procedure 2 provided **117**.

116:

IR (NaCl) ν_{max} 3469, 2998, 2130 (N_3), 1480, 1261, 1117, 1062 cm^{-1}.

^1H NMR ($CDCl_3$) δ 3.49 (dd, 1H, $J_{6,5}$ 6.0, $J_{6,6'}$ 12.3, H-6), 3.51 (s, 3H, OCH_3), 3.56 (dd, 1H, $J_{6',5}$ 7.4, $J_{6',6}$ 12.3, H-6'), 3.57 (d, 1H, br, $J_{1,1'}$ 11.9, H-1), 3.68 (d, 1H, $J_{1',1}$ 11.9, H-1'), 3.69 (app d, 1H, H-4), 3.80 (d, 1H, $J_{3,4}$ 3.0, H-3), 4.07 (m, 1H, H-5).

^{13}C NMR ($CDCl_3$) δ 50.7 (C-6), 52.4 (OCH_3), 54.0 (C-4), 56.7 (C-3), 64.9 (C-1), 75.2 (C-5), 105.0 (C-2).

LRMS (ESI) *m/z* 219 $[M+NH_4]^+$ (54%), 183 (36), 166 (100), 149 (61).

HRMS (ESI) calcd for $C_7H_{11}N_3O_4Na$ $[M+Na]^+$ 224.06472. Found *m/z* 224.06445.

^1H NMR assignments were confirmed using ^1H-^1H COSY NMR spectroscopy.

117:

^{1}H NMR ($CDCl_3$) δ 2.11 (s, 3H, $OCOCH_3$), 3.47 (dd, 1H, $J_{6,5}$ 6.6, $J_{6,6'}$ 12.3, H-6),3.50 (s, 3H, OCH_3), 3.58 (dd, 1H, $J_{6,5}$ 6.6, $J_{6,6'}$ 12.3, H-6'), 3.70 (app s, 2H, H-3, H-4), 4.05 (d, 1H, $J_{1,1'}$ 12.0, H-1), 4.08 (m, 1H, H-5), 4.28 (d, 1H, $J_{1',1}$ 12.0, H-1').

LRMS (ESI) *m/z* 266 $[M+Na]^+$ (51%), 261 $[M+NH_4]^+$ (12), 212 (100), 97 (62).

Methyl 3,4-anhydro-6-azido-6-deoxy-α-D-tagatofuranoside (118)

{ Methyl 3,4-anhydro-6-azido-6-deoxy-α-D-*lyxo*-hex-2-ulofuranoside }

To a chilled solution of TTP (2.09 g, 7.96 mmol) in DMF (9 ml) was added DIAD (1.56 ml, 7.96 mmol) and the resulting mixture stirred for 20 min at 0°C. To this mixture was added compound **59** (701 mg, 3.98 mmol) followed by HN_3 (23 ml, 41.4 mmol, 1.8 M in CH_2Cl_2, prepared according to literature procedure[286]). The reaction was warmed to rt and left to stir for 3 days. Concentration of the reaction mixture followed by column chromatography of the residue (EtOAc/hexane, 1:1, R_f 0.30) afforded the title compound **118** (552 mg, 69%) as a syrup. Acetylation of **118** according to General Procedure 2 provided **119**.

118:

IR (NaCl) ν_{max} 3485, 2961, 2131 (N_3), 1468, 1279, 1140, 1050 cm^{-1}.

^{1}H NMR ($CDCl_3$) δ 3.39 (s, 3H, OCH_3), 3.48 (app d, 2H, H-6, H-6'), 3.69 (d, 1H, $J_{1,1'}$ 11.8, H-1), 3.80 (app s, 2H, H-3, H-4), 3.85 (d, 1H, $J_{1',1}$ 11.8, H-1'), 4.23 (app t, $J_{5,6}$ = $J_{5,6'}$ 6.2).

^{13}C NMR ($CDCl_3$) δ 49.4 (OCH_3), 50.5 (C-6), 55.2 (C-4), 57.2 (C-3), 59.6 (C-1), 76.2 (C-5), 105.3 (C-2).

LRMS (ESI) *m/z* 224 $[M+Na]^+$ (35%), 219 $[M+NH_4]^+$ (50), 159 (38), 121 (39), 97 (82), 83 (33).

HRMS (ESI) calcd for $C_7H_{11}N_3O_4Na$ $[M+Na]^+$ 224.06472. Found *m/z* 224.06420.

119:

^1H NMR ($CDCl_3$) δ 2.14 (s, 3H, $OCOCH_3$), 3.34 (s, 3H, OCH_3), 3.45 (dd, 1H, $J_{6,5}$ 6.0, $J_{6,6'}$ 12.4, H-6), 3.50 (dd, 1H, $J_{6',5}$ 6.6, $J_{6',6}$ 12.4, H-6'), 3.73 (app d, 1H, $J_{4,3}$ 2.7, H-4), 3.77 (d, 1H, $J_{3,4}$ 2.7, H-3), 3.98 (d, 1H, $J_{1,1'}$ 12.0, H-1), 4.20 (app t, 1H, H-5), 4.51 (d, 1H, $J_{1,1'}$ 12.0, H-1').

LRMS (ESI) 261 $[M+NH_4]^+$ (19%), 212 (88), 97 (22).

tert-Butyl (methyl 3,4-anhydro-6-azido-6-deoxy-β-D-tagatofuranosid)onate (120)

{ _tert_-Butyl (methyl 3,4-anhydro-6-azido-6-deoxy-β-D-_lyxo_-hex-2-ulofuranosid) onate }

Compound **116** (458 mg, 2.28 mmol) was converted to *tert*-butyl ester **120** following General Procedure 1. Column chromatography of the crude residue (EtOAc/hexane, 1:4, R_f 0.45) afforded the title compound **120** (488 mg, 79%) as a syrup.

IR (NaCl) ν_{max} 2996, 2102, 1748 (C=O), 1460, 1290, 1161 cm^{-1}.

H NMR ($CDCl_3$) δ 1.50 (s, 9H, $C(CH_3)_3$), 3.49 (dd, 1H, $J_{6,5}$ 6.7, $J_{6,6'}$ 12.4, H-6), 3.50 (s, 3H, OCH_3), 3.57 (dd, 1H, $J_{6',5}$ 6.2, $J_{6',6}$12.4, H-6'), 3.71 (app d, 1H, $J_{4,3}$ 2.8, H-4), 3.80 (d, 1H, $J_{3,4}$ 2.8, H-3), 4.16 (app t, 1H, H-5).

^{13}C NMR ($CDCl_3$) δ 28.2 ($C(CH_3)_3$), 50.5 (C-6), 53.7 (OCH_3), 54.7 (C-4), 57.6 (C-3), 76.4(C-5), 83.7($C(Me)_3$), 103.5 (C-2), 166.7 (CO).

LRMS (ESI) *m/z* 289 $[M+NH_4]^+$ (88%), 244 (59), 233 (100), 216 (48), 200 (52).

HRMS (ESI) calcd for $C_{11}H_{21}N_4O_5$ $[M+NH_4]^+$ 289.15120. Found *m/z* 289.14972.

^{1}H NMR assignments were confirmed using ^{1}H-^{1}H COSY NMR spectroscopy.

tert-Butyl (methyl 6-acetamido-3,4-anhydro-6-deoxy-β-D-tagatofurasid)onate (122)

{ _tert_-Butyl (methyl 6-acetamido-3,4-anhydro-6-deoxy-β-D-_lyxo_-hex-2-ulofuranosid) onate }

To a solution of **120** (177 mg, 0.65 mmol) in ethyl acetate (15 ml) was added 10% Pd/C (85 mg) and the mixture was hydrogenated for 2 h at rt. The catalyst was filtered off and washed (EtOAc, 2 x 5 ml). Concentration of the filtrate and washings gave the crude amine which was acetylated according to General Procedure 2. Chromatography of the residue (EtOAc, R_f 0.50) afforded **122** (156 mg, 83%) as a syrup.

^{1}H NMR ($CDCl_3$) δ 1.50 (s, 9H, $C(CH_3)_3$), 2.01 (s, 3H, $NCOCH_3$), 3.50 (m, 4H, H-6, OCH_3), 3.70 (app d, $J_{4,3}$ 2.9, H-4), 3.82 (m, 2H, H-3, H-6'), 4.16 (app q, $J_{5,6}$ 6.8, $J_{5,6'}$ 3.6, H-5), 5.97 (br, 1H, NH).

^{13}C NMR ($CDCl_3$) δ 23.0 ($NCOCH_3$), 27.9 ($C(CH_3)_3$), 40.0 (C-6), 53.4 (OCH_3), 54.7 (C-4), 56.5 (C-3), 75.8 (C-5), 83.6 ($C(CH_3)_3$), 103.1 (C-2), 166.4, 170.8 (COOtBu, NCOMe).

LRMS (ESI) *m/z* 305 $[M+NH_4]^+$ (23%), 288 $[M+H]^+$ (48), 232 (100).

HRMS (ESI) calcd for $C_{13}H_{22}NO_6$ $[M+H]^+$ 288.14471. Found *m/z* 288.14427.

^{1}H NMR assignments were confirmed using ^{1}H-^{1}H COSY NMR spectroscopy.

tert-Butyl (methyl 6-acetamido-4-azido-4,6-dideoxy-β-D-fructofuranosid)onate (123)

{ _tert_-Butyl (methyl 6-acetamido-4-azido-4,6-dideoxy-β-D-_arabino_-hex-2-ulofuranosid)onate }

To a solution of **122** (80 mg, 0.28 mmol) in DMF (10 ml) was added lithium azide (80 mg, 1.63 mmol) and dry Dowex-50WX8-400 (H^+) (160 mg) ion-exchange resin. The mixture was stirred under N_2 at *ca.* 95°C for 16 h. The resin was filtered off, washed with DMF (6 ml) and the filtrate concentrated. The residue obtained was partitioned between ethyl acetate (60 ml) and water (10 ml). The organic layer was dried (Na_2SO_4), concentrated and chromatographed (EtOAc, R_f 0.32) to give **123** (74 mg, 80%) as a syrup.

IR (NaCl) ν_{max} 3340, 2895, 2132 (N_3), 1756, 1738, 1655, 1560, 1280 cm^{-1}.

^1H NMR ($CDCl_3$) δ 1.52 (s, 9H, $C(CH_3)_3$), 2.03 (s, 3H, $NCOCH_3$), 3.46 (m, 4H, H-6, OCH_3), 3.63 (m, 1H, H-6'), 3.79 (app t, 1H, $J_{4,3} = J_{4,5}$ 8.1, H-4), 3.93 (m, 1H, H-5), 4.16 (d , $J_{3,4}$ 8.1, H-3), 5.77 (br, 1H, NH).

^{13}C NMR ($CDCl_3$) δ 23.0 ($NCOCH_3$), 27.8 ($C(CH_3)_3$), 42.0 (C-6), 51.4 (OCH_3), 66.8 (C-4), 78.9, 79.5 (C-3, C-5), 83.5 ($C(CH_3)_3$), 101.5 (C-2), 166.0, 170.5 (COOtBu, NCOMe).

LRMS (ESI) *m/z* 353 $[M+Na]^+$ (13%), 348 $[M+NH_4]^+$ (21), 331 $[M+H]^+$ (53), 299 (14), 275 (100), 243 (14).

HRMS (ESI) calcd for $C_{13}H_{26}N_5O_6$ $[M+NH_4]^+$ 348.18831. Found *m/z* 348.18787.

^1H NMR assignments were confirmed using ^1H-^1H COSY NMR spectroscopy.

Methyl 6-acetamido-4-azido-4,6-dideoxy-β-D-fructofuranosidonic acid (124)

{ Methyl 6-acetamido-4-azido-4,6-dideoxy-β-D-*arabino*-hex-2-ulofuranosidonic acid}

To a solution of **123** (35 mg, 0.13 mmol) in CH_2Cl_2 (3 ml) was added trifluoroacetic acid (0.60 ml) and the resulting mixture stirred for 4 h at rt. Concentration of the reaction mixture followed by chromatography of the residue (EtOAc/MeOH, 4:1, R_f 0.51 in EtOAc/MeOH/0.1% HCl, 5:4:1) gave **124** (25 mg, 86%) as a syrup.

^{1}H NMR (CD_3OD) δ 2.07 (s, 3H, $NCOCH_3$), 3.47 (m, 6H, H-4, H-6, H-6', OCH_3), 3.86 (m, 1H, H-5), 4.10 (d, 1H, $J_{3,4}$ 8.1, H-3).

LRMS (ESI) *m/z* 275 $[M+H]^+$ (46%), 257 (12), 243 (100).

^{1}H NMR assignments were confirmed using ^{1}H-^{1}H COSY NMR spectroscopy.

Methyl 6-acetamido-4-amino-4,6-dideoxy-β-D-fructofuranosidonic acid (125)

{ Methyl 6-acetamido-4-amino-4,6-dideoxy-β-D-*arabino*-hex-2-ulofuranosidonic acid }

To a solution of **124** (25 mg, 0.091 mmol) in methanol (5 ml) was added 10% Pd/C (6 mg) and the mixture hydrogenated for 4 h at rt. The catalyst was filtered off and washed with water (10 ml). Concentration of the filtrate gave a residue which was redissolved in water (1 ml) and lyophilized to give **125** (18 mg, 80%) as a white amorphous mass.

^{1}H NMR (D_2O) δ 2.00 (s, 3H,$NCOCH_3$), 3.29 (s, 3H, OCH_3), 3.39 (m, 3H, H-4, H-6, H-6'), 3.56 (m, 1H, H-5), 4.20 (d, 1H, $J_{3,4}$ 8.7, H-3).

^{13}C NMR (D_2O) δ 21.8 ($NCOCH_3$), 42.2 (C-6), 50.8 (OCH_3), 56.9 (C-4), 76.5, 76.7 (C-3, C-5), 103.6 (C-2), 169.1 (COOH), 174.7 (NCOMe).

LRMS (ESI) *m/z* 249 $[M+H]^+$ (100%), 217 (96), 195 (43).

HRMS (ESI) calcd for $C_9H_{17}N_2O_6$ $[M+H]^+$ 249.10866. Found *m/z* 249.10803.

tert-Butyl (methyl 4-azido-6-*O*-benzoyl-3-*O*-_tert_-butyldimethylsilyl-4-deoxy-α-D-fructofuranosid)onate (127)

{ _tert_-Butyl (methyl 4-azido-6-*O*-benzoyl-3-*O*-_tert_-butyldimethylsilyl-4-deoxy-α-D-_arabino_-hex-2-ulofuranosid)onate }

Compound **90** (260 mg, 0.66 mmol) in DMF (1 ml), *tert*-butyldimethylsilyl chloride (175 mg, 1.16 mmol) and imidazole (166 mg, 2.44 mmol) were stirred for 18 h at 32°C. The reaction mixture was concentrated and the residue redissolved in EtOAc (40 ml), washed with water (5 ml), dried (Na_2SO_4) and concentrated. Chromatography of the residue (EtOAc/hexane, 1:5, R_f 0.65) furnished **127** (302 mg, 90%) as a syrup.

IR (NaCl) ν_{max} 2951 , 2108 , 1732 , 1754 , 1459 , 1275 cm^{-1}.

^{1}H NMR ($CDCl_3$) δ 0.14, 0.16 (2 x s, 6H, $Si(CH_3)_2$), 0.90 (s, 9H, $SiC(CH_3)_3$), 1.51 (s, 9H, $OC(CH_3)_3$), 3.35 (s, 3H, OCH_3), 3.78 (dd, 1H, $J_{4,3}$ 5.1, $J_{4,5}$ 7.2, H-4), 4.22 (m, 2H, H-3, H-5), 4.56 (dd, 1H, $J_{6,5}$ 5.1, $J_{6,6'}$ 11.6, H-6), 4.61 (dd, 1H, $J_{6',5}$ 5.4, $J_{6'6}$ 11.6, H-6'), 7.45 (m, 2H, Ar-H-3, Ar-H-5), 7.58 (m, 1H, Ar-H-4), 8.09 (m, 2H, Ar-H-2, Ar-H-6).

^{13}C NMR ($CDCl_3$) δ -5.1, -4.6 ($Si(CH_3)_2$), 18.0 ($SiC(CH_3)_3$), 25.7 ($SiC(CH_3)_3$), 28.1 ($OC(CH_3)_3$), 51.5 (OCH_3), 64.6 (C-6), 68.5 (C-4), 78.1 (C-3), 82.7 ($OC(CH_3)_3$), 83.2 (C-5), 107.3 (C-2), 128.4, 129.8 , 133.2 (Ph), 165.4,166.2 (OCOPh, COOtBu).

LRMS (ESI) *m/z* 525 $[M+NH_4]^+$ (100%), 453 (19), 452 (67), 420 (31).

HRMS (ESI) calcd for $C_{24}H_{41}N_4O_7Si$ $[M+NH_4]^+$ 525.27445. Found *m/z* 525.27571.

^{1}H NMR assignments were confirmed using ^{1}H-^{1}H COSY NMR spectroscopy.

tert-Butyl (methyl 4-azido-3-*O*-_tert_-butyldimethylsilyl-4-deoxy-α-D-fructofuranosid)onate (128)

{ _tert_-Butyl (methyl 4-azido-3-*O*-_tert_-butyldimethylsilyl-4-deoxy-α-D-_arabino_-hex-2-ulofuranosid)onate }

Compound **127** (295 mg, 0.58 mmol) was debenzoylated following General Procedure 3. Chromatography (EtOAc/hexane, 1:4, R_f 0.24) of the crude residue afforded **128** (216 mg, 92%) as a colourless oil.

^{1}H NMR ($CDCl_3$) δ 0.15, 0.17 (2 x 3, 6H, $Si(CH_3)_2$), 0.91 (s, 9H, $SiC(CH_3)_3$), 1.53 (s, 9H, $OC(CH_3)_3$) , 3.33 (s, 3H, OCH_3), 3.70 (app d, 1H, $J_{6,6'}$ 12.0, H-6), 3.99 (m, 3H, H-4, H-5, H-6'), 4.22 (d, 1H, $J_{3,4}$ 5.7, H-3).

^{13}C NMR ($CDCl_3$) δ -5.1, -4.6 ($Si(CH_3)_2$), 18.0 ($SiC(CH_3)_3$), 25.7 ($SiC(CH_3)_3$), 28.1 ($OC(CH_3)_3$) , 51.7 (OCH_3), 61.1 (C-6), 65.1 (C-4), 80.6 (C-3), 83.3 ($OC(CH_3)_3$), 83.6 (C-5) ,106.9 (C-2), 166.7 (COOtBu).

LRMS (ESI) *m/z* 421 $[M+NH_4]^+$ (100%), 349(18), 348 (77), 316 (40).

HRMS (ESI) calcd for $C_{17}H_{37}N_4O_6Si$ $[M+NH_4]^+$ 421.24824. Found *m/z* 421.24594.

tert-Butyl {methyl 3-*O*-_tert_-butyldimethylsilyl-4-deoxy-5-[(*E*)-3-oxo-1-butenyl]-α-L-_glycero_-pent-4-en-2-ulofuranosid}onate (129)

Compound **128** (195 mg, 0.48 mmol) was oxidized following General Procedure 4 to give an aldehyde (167 mg) which was dissolved in dry toluene (8 ml). To this solution was added 1-triphenylphosphoranylidene-2-propanone (153 mg, 0.48mmol) and the mixture stirred for 16 h at rt. Concentration followed by chromatography of the residue (EtOAc/hexane, 1:5, R_f 0.43) on a short silica column gave **129** (117 mg, 61%) as a syrup.

^{1}H NMR ($CDCl_3$) δ 0.16, 6H, $Si(CH_3)_2$), 0.89 (s, 9H, $SiC(CH_3)_3$), 1.54 (s, 9H,$OC(CH_3)_3$), 2.31 (s, 3H, H-9), 3.41 (s, 3H, OCH_3), 4.79 (d, 1H, $J_{3,4}$ 2.7, H-3), 5.43 (d, 1H, $J_{4,3}$ 2.7, H-4), 6.63 (d, 1H, $J_{7,6}$ 15.9, H-7), 6.94 (d, 1H, $J_{6,7}$ 15.9, H-6).

tert-Butyl (methyl 4-azido-6-_O_-benzoyl-3-_O-tert_-butyldimethylsilyl-4-deoxy-β-D-fructofuranosid)onate (131)

{ _tert_-Butyl (methyl 4-azido-6-_O_-benzoyl-3-_O-tert_-butyldimethylsilyl-4-deoxy-β-D-_arabino_-hex-2-ulofuranosid)onate }

Compound **93** (280 mg, 0.71 mmol) in DMF (1 ml), *tert*-butyldimethylsilyl chloride (188 mg, 1.25 mmol) and imidazole (178 mg, 2.61 mmol) were stirred for 18 h at 32°C. The reaction mixture was concentrated and the residue redissolved in EtOAc (40 ml), washed with water (5 ml), dried (Na_2SO_4) and concentrated, Chromatography of the residue (EtOAc/hexane, 1:5, R_f 0.66) gave **131** (314 mg, 87%) as a syrup.

IR (NaCl) ν_{max} 2962 , 2105, 1737 , 1459 , 1271 cm^{-1}.

^{1}H NMR ($CDCl_3$) δ 0.12, 0.14 (2 x s, 6H, $Si(CH_3)_2$), 0.91 (s, 9H , $SiC(CH_3)_3$), 1.49 (s, 9H, $OC(CH_3)_3$), 3.41 (s, 3H, OCH_3), 3.96 (app t, 1H, $J_{4,3} = J_{4,5}$ 8.1, H-4), 4.13 (m, 1H, H-5), 4.36 (d, 1H, $J_{3,4}$ 8.1, H-3), 4.49 (app d, 2H, $J_{6,5} = J_{6',5}$ 4.3, H-6, H-6'), 7.44 (m, 2H, Ar-H-3, Ar-H-5), 7.56 (m, 1H, Ar-H-4), 8.05 (m, 2H, Ar-H-2, Ar-H-6).

^{13}C NMR ($CDCl_3$) δ -4.9, -4.6 ($Si(CH_3)_2$), 17.9 ($SiC(CH_3)_3$), 25.6 ($SiC(CH_3)_3$), 28.1 ($OC(CH_3)_3$), 51.3 (OCH_3), 64.0 (C-6), 67.2 (C-4), 76.9 (C-3), 80.0 (C-5), 82.9 ($OC(CH_3)_3$), 102.1 (C-2), 128.3, 129.5, 129.7, 133.2 (Ph), 166.1, 167.0 (OCOPh, COOtBu).

LRMS (ESI) *m/z* 525 $[M+NH_4]^+$ (100%) 508 $[M+H]^+$ (48), 476 (30), 452 (62), 421 (21), 420 (72).

HRMS (ESI) calcd for $C_{24}H_{41}N_4O_7Si$ $[M+NH_4]^+$ 525.27445. Found *m/z* 525.27284.

^{1}H NMR assignments were confirmed using ^{1}H-^{1}H COSY NMR spectroscopy.

tert-Butyl (methyl 4-azido-3-_O-tert_-butyldimethylsilyl-4-deoxy-β-D-fructofuranosid) onate (132)

{ _tert_-Butyl (methyl 4-azido-3-_O-tert_-butyldimethylsilyl-4-deoxy-β-D-_arabino_-hex-2-ulofuranosid)onate }

Compound **131** (236 mg, 0.465mmol) was debenzoylated according to General Procedure 3. Chromatography (EtOAc/hexane, 4:1, R_f 0.18) of the crude residue gave **132** (178 mg, 95%) as a syrup.

IR (NaCl) ν_{max} 3526, 2937 , 2110 , 1739 , 1256 cm^{-1}.

^{1}H NMR ($CDCl_3$) δ 0.11, 0.15 (2 x s, 6H, $Si(CH_3)_2$), 0.91 (s, 9H, $SiC(CH_3)_3$), 1.50 (s, 9H, $OC(CH_3)_3$), 3.46 (s, 3H, OCH_3), 3.70 (dd, 1H, $J_{6,5}$ 3.9, $J_{6,6'}$ 11.7, H-6), 3.90 (m, 3H, H-4, H-5, H-6'), 4.36 (d, 1H, $J_{3,4}$ 7.5, H-3).

^{13}C NMR ($CDCl_3$) δ -5.07, -4.69 ($Si(CH_3)_2$), 17.9 ($SiC(CH_3)_3$), 25.6 ($SiC(CH_3)_3$), 27.9 ($OC(CH_3)_3$), 51.8 (OCH_3), 62.3 (C-6), 65.9 (C-4), 79.7, 79.9 (C-3, C-5), 83.0 ($OC(CH_3)_3$), 102.2 (C-2), 167.0 (COOtBu).

LRMS (ESI) *m/z* 421 $[M+NH_4]^+$ (84%), 349 (11), 348 (48), 317 (22), 316 (100).

HRMS (ESI) calcd for $C_{17}H_{37}N_4O_6Si$ $[M+NH_4]^+$ 421.24824. Found *m/z* 421.24848.

tert-Butyl {methyl 3-_O-tert_-butyldimethylsilyl-4-deoxy-5-[(_E_)-3-oxo-1-propenyl]-β-L-_glycero_-pent-4-en-2-ulofuranosid}onate (135)

Compound **132** (120 mg, 0.30 mmol) was oxidized according to General Procedure 4 to give the aldehyde **133** (106 mg, R_f 0.37 in EtOAc/hexane, 1:5), which was dissolved in dry toluene (5 ml). (Triphenylphosphoranylidene)acetaldehyde (91 mg,

0.30 mmol) was added and the mixture stirred for 16 h at rt. Concentration of the reaction mixture followed by chromatography of the residue (EtOAc/hexane, 1:5, R_f 0.36) on a short silica column gave **135** (73 mg, 64%) as a syrup.

133:

^{1}H NMR ($CDCl_3$) δ 0.11 (s, 6H, $Si(CH_3)_2$), 0.87 (s, 9H, $SiC(CH_3)_3$), 1.55 (s, 9H, $OC(CH_3)_3$), 3.55 (s, 3H, OCH_3), 5.22 (d, 1H, $J_{3,4}$ 2.7, H-3), 5.96 (d, 1H, $J_{4,3}$ 2.7, H-4), 9.50 (s, 1H, H-6).

135:

^{1}H NMR (CD_3COCD_3) δ 0.21 (s, 6H, $Si(CH_3)_2$), 0.96 (s, 9H, $SiC(CH_3)_3$), 1.59 (s, 9H, $OC(CH_3)_3$), 3.57 (s, 3H, OCH_3). 5.21 (d , 1H, $J_{3,4}$ 2.7, H-3), 5.80 (d, 1H, $J_{4,3}$ 2.7, H-4), 6.45 (dd, 1H, $J_{7,6}$ 15.6, $J_{7,8}$ 7.8, H-7), 7.26 (d, 1H, $J_{6,7}$ 15.6, H-6), 9.74 (d, 1H, $J_{8,7}$ 7.8, H-8).

tert-Butyl (methyl 6-_O_-benzoyl-4-_N_-_tert_-butoxycarbonyl-3-_O_-_tert_-butyldimethylsilyl-4-deoxy-α-D-fructofuranosid)onate (136)

{ _tert_-Butyl (methyl 6-_O_-benzoyl-4-_N_-_tert_-butoxycarbonyl-3-_O_-_tert_-butyldimethylsilyl -4-deoxy-α-D-_arabino_-hex-2-ulofuranosid)onate }

A suspension of 10% Pd/C (25 mg) in EtOAc (2 ml) was stirred under H_2 until the uptake of hydrogen ceased. Di-_tert_-butyl dicarbonate (77 mg, 0.35 mmol) and **127** (120 mg, 0.24 mmol) were added, and the resulting mixture was stirred under hydrogen at rt for 20 h. The reaction mixture was filtered through celite and the filtrate concentrated. Chromatography of the residue (EtOAc/hexane, 1:6, R_f 0.37) gave **136** (128 mg, 93%) as a syrup.

^{1}H NMR ($CDCl_3$) δ 0.13, 0.17 (2 x s, 6H, $Si(CH_3)_2$) , 0.91 (s, 9H, $SiC(CH_3)_3$), 1.43 (s, 9H, $NCOOC(CH_3)_3$), 1.52 (s, 9H, $COOC(CH_3)_3$), 3.33 (s, 3H, OCH_3), 4.17 (m, 3H, H-3, H-4, H-5), 4.45 (dd, br, 1H, $J_{6,5}$ 5.7, $J_{6,6'}$ 11.0, H-6), 4.63 (dd, br, 1H, $J_{6',5}$ 5.8, $J_{6',6}$ 11.0, H-6'), 5.10 (d, br, $J_{NH,4}$ 8.7, NH), 7.40 (m, 2H, Ar-H-3, Ar-H-5), 7.53 (m, 1H, Ar-H-4), 8.08 (m, 2H, Ar-H-2, Ar-H-6).

^{13}C NMR ($CDCl_3$) δ -4.96, -4.85 ($Si(CH_3)_2$), 17.8 ($SiC(CH_3)_3$), 25.6 ($SiC(CH_3)_3$), 28.0, 28.2 ($COOC(CH_3)_3$, $NCOOC(CH_3)_3$), 50.7(OCH_3), 59.4 (C-4), 65.3 (C-6), 79.6 ($NCOOC(CH_3)_3$), 82.5 ($COOC(CH_3)_3$, 82.2, 83.0 (C-3, C-5), 108.8 (C-2), 128.1, 129.8, 129.9, 132.8 (Ph), 154.4 ($NCOOC(CH_3)_3$), 164.8, 166.1 (COOtBu, OCOPh).

LRMS (ESI) *m/z* 582 $[M+H]^+$ (81%), 550 (15), 527 (31), 527 (95), 494 (41), 470 (41), 439 (28), 438 (100).

HRMS (ESI) calcd for $C_{29}H_{48}NO_9Si$ $[M+H]^+$ 582.30984. Found *m/z* 582.30710.

tert-Butyl (methyl 4-_N-tert_-butoxycarbonyl-3-_O-tert_-butyldimethylsilyl-4-deoxy-α-D-fructofuranosid)onate (137)

{ _tert_-Butyl (methyl 4-_N-tert_-butoxycarbonyl-3-_O-tert_-butyldimethylsilyl-4-deoxy-α-D-_arabino_-hex-2-ulofuranosid)onate }

Compound **136** (406 mg, 0.70 mmol) was debenzoylated according to General Procedure 3. Chromatography of the crude residue (EtOAc/hexane, 1:3, R_f 0.19) gave **137** (324 mg, 97%) as a syrup.

^{1}H NMR ($CDCl_3$) δ 0.12, 0.16 (2 x s, 6H, $Si(CH_3)_2$), 0.90 (s, 9H, $SiC(CH_3)_3$), 1.45 (s, 9H, $NCOOC(CH_3)_3$), 1.53 (s, 9H, $COOC(CH_3)_3$), 3.30 (s, 3H, OCH_3), 3.78 (dd, br, 1H, $J_{6,5}$ 4.5, $J_{6,6'}$ 11.7, H-6), 3.85 (dd, br, 1H, $J_{6',5}$ 3.6, $J_{6',6}$ 11.7, H-6'), 3.95 (m, 1H, H-5), 4.02 (ddd, 1H, $J_{4,3}$ 1.8, $J_{4,5}$ 3.9, $J_{4,NH}$ 9.5, H-4), 4.14 (d, 1H, $J_{3,4}$ 1.8, H-3), 5.05 (d, br, 1H, $J_{NH,4}$ 9.5, NH).

^{13}C NMR ($CDCl_3$) δ -4.9 ($Si(CH_3)_2$), 17.8 ($SiC(CH_3)_3$), 25.6 ($SiC(CH_3)_3$), 28.0 , 28.2 ($COOC(CH_3)_3$), $NCOOC(CH_3)_3$), 50.6(OCH_3), 58.7 (C-4), 62.8 (C-6), 79.9 ($NCOOC(CH_3)_3$), 82.1 (C-3), 82.8 ($COOC(CH_3)_3$), 85.8 (C-5), 108.2 (C-2), 154.9 ($NCOOC(CH_3)_3$), 165.1 (COOtBu).

LRMS (ESI) *m/z* 495 $[M+NH_4]^+$ (61%), 452 (42), 422 (77), 390 (31), 366 (84), 334 (100).

HRMS (ESI) calcd for $C_{22}H_{47}N_2O_8Si$ $[M+NH_4]^+$ 495.31017. Found *m/z* 495.30992.

^{1}H NMR assignments were confirmed using ^{1}H-^{1}H COSY NMR spectroscopy.

tert-Butyl {methyl 4-_N-tert_-butoxycarbonyl-3-_O-tert_-butyldimethylsilyl-4-deoxy-5-[(_Z_)-3-oxo-1-butenyl]-α-D-_arabino_-pent-2-ulofuranosid}onate (139)

and

tert-Butyl {methyl 4-_N-tert_-butoxycarbonyl-3-_O-tert_-butyldimethylsilyl-4-deoxy-5-[(_E_)-3-oxo-1-butenyl]-α-D-_arabino_-pent-2-ulofuranosid}onate (140)

Compound **137** (272 mg, 0.57 mmol) was oxidized according to General Procedure 4. The aldehyde, *tert*-Butyl (methyl 4-*N*-*tert*-butoxycarbonyl-3-*O*-*tert*-butyldimethylsilyl-4-deoxy-5-formyl-α-D-*arabino*-pent-2-ulofuranosid)onate **(138)** (204 mg, R_f 0.22 in EtOAc/ hexane, 2:5), obtained was dissolved in dry toluene (6 ml). 1-Triphenylphosphoranylidene-2-propanone (204 mg, 0.64 mmol) was added and the mixture stirred for 20 h at rt. Solvent removal and chromatography of the residue (gradient from EtOAc/hexane, 1:8, to EtOAc/hexane, 1:4) gave **139** (42 mg, 14%, R_f 0.31 in EtOAc/hexane, 2:5) and **140** (136 mg, 46%, R_f 0.24 in EtOAc/ hexane, 2:5). The yields of **139** and **140** were calculated based on **137**.

138:

^{1}H NMR ($CDCl_3$) δ 0.10, 0.13 (2 x s, 6H, $Si(CH_3)_2$), 0.83 (s, 9H, $SiC(CH_3)_3$), 1.45 (s, 9H, $NCOOC(CH_3)_3$), 1.54 (s, 9H, $COOC(CH_3)_3$), 3.37 (s, 3H, OCH_3), 4.11 (m, 1H, H-5), 4.32 (m, br, 2H, H-3, H-4), 5.32 (d, br, 1H, $J_{NH,4}$ 9.8, NH), 9.71 (app s, 1H, H-6).

139:

^{1}H NMR ($CDCl_3$) δ 0.09, 0.12 (2 x s, 6H, $Si(CH_3)_2$), 0.84 (s, 9H , $SiC(CH_3)_3$), 1.44 (s, 9H, $NCOOC(CH_3)_3$), 1.54 (s, 9H, $COOC(CH_3)_3$), 2.22 (s, 3H, H-9), 3.32 (s, 3H, OCH_3), 4.02 (app d, 1H, $J_{4,NH}$ 9.6, H-4), 4.10 (d,1H, $J_{3,4}$ 1.8, H-3), 4.54 (m, 1H, H-5), 5.23 (d, br, 1H, $J_{NH,4}$ 9.6, NH), 6.19 (d, 1H, $J_{7,6}$ 11.5, H-7), 6.28 (dd, 1H, $J_{6,5}$ 7.5, $J_{6,7}$ 11.5, H-6).

140:

^{1}H NMR ($CDCl_3$) δ 0.09, 0.12 (2 x s, 6H, $Si(CH_3)_2$), 0.85 (s, 9H, $SiC(CH_3)_3$), 1.45 (s, 9H, $NCOOC(CH_3)_3$), 1.55 (s, 9H, $COOC(CH_3)_3$), 2.26 (s, 3H, H-9), 3.33 (s, 3H, OCH_3), 4.04 (app d, 1H, $J_{4,NH}$ 8.9, H-4), 4.13 (app s, 1H, H-3), 4.54 (m, 1H, H-5), 5.27 (d, br, $J_{NH,4}$ 8.9, NH), 6.45 (d, 1H, $J_{7,6}$ 16.0, H-7), 6.87 (dd, 1H, $J_{6,5}$ 4.8, $J_{6,7}$ 16.0, H-6).

^{13}C NMR ($CDCl_3$) δ -5.0 ($Si(CH_3)_2$), 17.6 ($SiC(CH_3)_3$), 25.5 ($SiC(CH_3)_3$), 27.1 (C-9), 28.0, 28.2 ($COOC(CH_3)_3$, $NCOOC(CH_3)_3$), 50.8 (OCH_3), 61.9 (C-4), 80.0 ($NCOOC(CH_3)_3$), 81.7 (C-5), 82.9 ($COOC(CH_3)_3$), 85.5 (C-3), 109.4 (C-2), 130.2 (C-6), 143.6 (C-7), 154.6 (NCOOtBu), 164.8 (COOtBu), 198.1 (CH_3COC=C).

LRMS (ESI) *m/z* 533 $[M+NH_4]^+$ (100%), 460 (30), 405 (12), 404 (12), 372 (31), 360 (22).

HRMS (ESI) calcd for $C_{25}H_{49}N_2O_8Si$ $[M+NH_4]^+$ 533.32582. Found *m/z* 533.32631.

^{1}H NMR assignments were confirmed using ^{1}H-^{1}H COSY NMR spectroscopy.

***tert*-Butyl [methyl 5-(1-acetamino-3-oxobutyl)-4-*N*-*tert*-butoxycarbonyl-3-*O*-*tert*-butyldimethylsilyl-4-deoxy-α-D- *arabino*-pent-2-ulofuranosid]onate (143)**

To a solution of **140** (114 mg, 0.22 mmol) in CH_2Cl_2 (5 ml) at -20°C was added azidotrimethylsilane (74 µl, 0.55 mmol) and tetrabutylammonim fluoride (43 µl, 0.043 mmol, 1.0 M in THF). The reaction was left for 4 days at -20°C. The mixture was concentrated under reduced pressure and the residue redissolved in toluene-methanol (6 ml, 2:1). To this solution was added 10% Pd/C (30 mg) and the mixture hydrogenated at rt for 18 h. The reaction mixture was filtered through celite, washed with methanol (2 x 10 ml) and concentrated to give the crude amine **142**. This amine was acetylated according to General Procedure 2. Column chromatography of the residue (EtOAc/hexane, 1:1, R_f 0.16) gave **143** (76 mg, 60%) as an amorphous mass.

^{1}H NMR ($CDCl_3$) (other diastereomer chemical shift given in parentheses where visible): δ 0.10, 0.12 (0.17) (2 x s, 6H, $Si(CH_3)_2$), 0.89 (s, 9H, $SiC(CH_3)_3$), 1.42 (s, 9H, $NCOOC(CH_3)_3$), 1.51 (s, 9H, $COOC(CH_3)_3$), 1.97 (s, 3H, $NCOCH_3$), 2.16 (2.18) (s, 3H, H-9), 2.82 (2.66) (m, 1H, H-7), 3.19 (2.92) (dd, 1H, $J_{7',6}$ 4.8, $J_{7',7}$ 17.5, H-7'), 3.25 (s, 3H, OCH_3), 3.85-4.15 (m, 3H, H-3, H-4, H-5), 4.38 (4.67) (m, 1H, H-6), 5.08 (4.93) (d, br , 1H, $J_{NH,4}$ 10.2, NHCOOtBu), 6.60 (6.93) (d, br, 1H, $J_{NH,6}$ 8.4 , NHCOMe).

^{13}C NMR ($CDCl_3$) (other diastereomer chemical shift given in parentheses where visible): δ -4.9 ($Si(CH_3)_2$), 17.7 ($SiC(CH_3)_3$), 23.1 (23.3) ($NCOCH_3$), 25.7 ($SiC(CH_3)_3$), 28.0 , 28.2 ($COOC(CH_3)_3$), $NCOOC(CH_3)_3$), 30.2 (29.8) (C-9), 43.6 (44.5) (C-7), 48.5 (45.9) (C-6), 50.5 (50.8) (OCH_3), 58.9 (58.4) (C-4), 80.1 ($NCOOC(Me)_3$), 81.9 (82.4) (C-3), 82.7 ($COOC(Me)_3$), 85.7 (84.6) (C-5), 108.5 (107.4) (C-2), 154.5 (NCOOtBu), 164.8, 165.7 (CCOOtBu, NHCOMe). Resonance for C-8 were not observed in the ^{13}C NMR spectrum.

LRMS (ESI) *m/z* 575 $[M+H]^+$ (100%), 520 (19), 519 (89), 487 (10), 463 (21).

HRMS (ESI) calcd for $C_{27}H_{51}N_2O_9Si$ $[M+H]^+$ 575.33639. Found *m/z* 575.33383.

^1H NMR assignments were confirmed using ^1H-^1H COSY NMR spectroscopy.

tert-Butyl [methyl 5-(1-acetamino-3-oxobutyl)-4-_N-tert_-butoxycarbonyl-4-deoxy-α-D-_arabino_-pent-2-ulofuranosid]onate (144)

A solution of **143** (63 mg, 0.11 mmol) in THF (7 ml) was treated with tetrabutylammonium fluoride (0.33 ml, 0.33 mmol, 1.0 M in THF) and the resulting mixture stirred for 2 h at rt. Concentration of the reaction mixture followed by chromatography of the residue (EtOAc, R_f 0.29) gave **144** (47 mg, 93%) as a syrup.

^1H NMR ($CDCl_3$) (other diastereomer chemical shift given in parentheses where visible): δ 1.42 (s, 9H, $NCOOC(CH_3)_3$), 1.51 (s, 9H, $COOC(CH_3)_3$), 2.03 (2.04) (s, 3H, $NCOCH_3$), 2.19 (s, 3H, H-9), 2.81 (2.78) (m, 1H, H-7), 3.30 (m, 4H, OCH_3, H-7'), 3.20-3.39 (m, 3H, H-3, H-4, H-5), 4.41 (4.52) (m, 1H, H-6), 5.26 (5.12) (d, br, 1H, $J_{NH,4}$ 9.6, $NHCOOC(Me)_3$), 7.38 (d, br, 1H, NHCOMe).

^{13}C NMR ($CDCl_3$) (other diastereomer chemical shift given in parentheses where visible): δ 23.1 (23.01) ($NCOCH_3$), 27.9 , 28.3 ($CCOOC(CH_3)_3$, $NCOOC(CH_3)_3$), 30.5 (30.1) (C-9), 43.1 (44.8) (C-7), 48.7 (46.3) (C-6), 50.9 (51.3) (OCH_3), 58.6 (58.8) (C-4), 80.0 (80.6) ($NCOOC(Me)_3$), 80.9 (82.4) (C-3), 82.9 (83.3) ($COOC(Me)_3$), 86.5 (C-5), 108.74 (C-2), 155.4 (NCOOtBu), 165.2, 171.4 (COOtBu, NHCOMe). Resonance for C-8 were not observed in the ^{13}C NMR spectrum.

LRMS (ESI) 461$[M+H]^+$ (28%), 405 (25), 350 (22), 349 (81), 317 (42), 273 (24), 197 (77), 169 (35), 154 (25), 132 (100).

HRMS (ESI) calcd for $C_{21}H_{37}N_2O_9$ $[M+H]^+$ 461.24991. Found *m/z* 461.24985.

^1H NMR assignments were confirmed using ^1H-^1H COSY NMR spectroscopy.

Methyl 5-(1-acetamino-3-oxobutyl)-4-amino-3-*O*-*tert*-butyldimethylsilyl-4-deoxy-α-D-*arabino*-pent-2-ulofuranosidonic acid (146)

To a solution of **143** (50 mg, 0.087 mmol) in CH_2Cl_2 (3 ml) was added trifluoroacetic acid (1 ml) and the resulting mixture stirred for 4 h at rt. The mixture was concentrated under reduced pressure and the residue chromatographed (EtOAc/MeOH, 3:1) to give **146** (32 mg, 89%, R_f 0.33 in EtOAc/MeOH/0.1% HCl, 6:4:1) as a gum.

^{1}H NMR (D_2O) (other diastereomer chemical shift given in parentheses where visible): δ 0.071, 0.085 (0.118) (2 x s, 6H, $Si(CH_3)_2$), 0.81, (0.84) (s, 9H, $SiC(CH_3)_3$), 1.94 (1.96) (s, 3H, $NCOCH_3$), 2.18 (2.19) (s, 3H, H-9), 2.99 (m, 2H, H-7), 3.17 (3.21) (s, 3H, OCH_3), 3.55 (app d, 1H, $J_{4,5}$ 3.9, H-4), 4,10 (m, 1H, H-5), 4.36 (4.29) (app s, 1H, H-3), 4.56 (m, 1H, H-6).

LRMS (ESI) *m/z* 419 $[M+H]^+$ (100%), 388 (21), 387 (40).

^{1}H NMR assignments were confirmed using ^{1}H-^{1}H COSY NMR spectroscopy.

Methyl 5-(1-acetamino-3-oxobutyl)-4-amino-4-deoxy-α-D- *arabino*-pent-2-ulofuranosidonic acid (145)

To a solution of compound **144** (35 mg, 0.076 mmol) in CH_2Cl_2 (2 ml) was added trifluoroacetic acid (1 ml) and the mixture stirred for 12 h at rt. The reaction mixture was concentrated under reduced pressure and the residue redissolved in water (6 ml). This solution was adjusted to pH 7 with Amberlite IRA-400 (OH^-) resin. The resin was filtered off, washed with water (3 x 4 ml) and the filtrate lyophilized to give **145** (19 mg, 82%, R_f 0.18 in EtOAc/MeOH/0.2%HCl, 5:4:1) as a white amorphous mass.

Alternatively: A solution of **146** (29 mg, 0.069 mmol) in THF (5 ml) was treated with tetrabutylammonium fluoride (0.21 ml, 0.21mmol, 1.0 M in THF) and the mixture stirred for 90 min at rt. The reaction mixture was concentrated and the residue chromatographed on a short silica column (EtOAc/isopropanol/H_2O, 3:2:1) to give **145** (18 mg, 85%), identical in all respects to the material described aboved.

^{1}H NMR (D_2O) (other diastereomer chemical shift given in parentheses where visible): δ 1.91 (1.89) (s, 3H, $NCOCH_3$), 2.15 (2.16) (s, 3H, H-9), 2.84 (2.95) (dd, 1H, $J_{7,6}$ 9.0, $J_{7,7'}$ 17.7, H-7), 3.04 (dd, 1H, $J_{7',6}$ 3.8, $J_{7',7}$ 17.7, H-7'), 3.19 (3.17) (s, 3H, OCH_3), 3.53 (3.30) (app d, 1H, $J_{4,5}$ 3.3, H-4), 4.11 (m, 1H, H-5), 4.46 (4.30) (app s, 1H, H-3), 4.49 (4.61) (m, 1H, H-6).

LRMS (ESI) *m/z* 305 $[M+H]^+$ (100%), 287 (31), 273 (32), 228 (45).

HRMS (ESI) calcd for $C_{12}H_{21}N_2O_7$ $[M+H]^+$ 305.13488. Found *m/z* 305.13436.

^{1}H NMR assignments were confirmed using ^{1}H-^{1}H COSY NMR spectroscopy.

tert-Butyl [methyl 4-_N_-_tert_-butoxycarbonyl-3-_O_-_tert_-butyldimethylsilyl-4-deoxy-5-(1-hydroxy-2-nitroethyl)-α-D-_arabino_-pent-2-ulofuranosid]onate (148)

Compound **137** (57 mg, 12 mmol) was oxidized according to General Procedure 4. The aldehyde (**138**) obtained was dissolved in nitromethane (5 ml) and this solution was adjusted to pH 10-11 with NaOMe. The resulting mixture was stirred for 5 h at rt, then neutralized with Dowex-50WX8-400 (H^+) ion-exchange resin. The resin was filtered off, washed with methanol (3 x 5 ml). Solvent removal followed by chromatography (EtOAc/hexane, 1:3, R_f 0.16) afforded **148** (40 mg, 62%) as a syrup.

^{1}H NMR ($CDCl_3$) (other diastereomer chemical shift given in parentheses where visible): δ 0.11, 0.15 (0.10, 0.14) (2 x s, 6H, $Si(CH_3)_2$), 0.89 (s, 9H, $SiC(CH_3)_3$), 1.44 (s, 9H, $NCOOC(CH_3)_3$), 1.52 (s, 9H, $COOC(CH_3)_3$), 3.28 (3.29) (s, 3H, OCH_3), 3.80 (m,

br, 1H, H-5), 4.06 (m, 2H, H-3, H-5), 4.55-4.75 (m, 3H, H-6, H-7, H-7'), 5.14 (5.04) (d, br, 1H, $J_{NH,4}$ 9.1, NH).

^{13}C NMR ($CDCl_3$) (other diastereomer chemical shift given in parentheses where visible): δ -5.0 ($Si(CH_3)_2$), 17.9 ($SiC(CH_3)_3$), 25.5 ($SiC(CH_3)_3$), 28.0, 28.2 ($COOC(CH_3)_3$), $NCOOC(CH_3)_3$), 51.0 (50.8) (OCH_3), 59.6 (58.4) (C-4), 68.5 (69.4) (C-6), 78.1 (C-7), 80.4 ($NCOOC(CH_3)_3$), 81.9 (81.5) (C-3), 83.5 (83.1) ($COOC(CH_3)_3$), 86.2 (84.6) (C-5), 108.2 (C-2), 155.5 (NCOOtBu), 164.6 (COOtBu).

LRMS (ESI) *m/z* 554 $[M+NH_4]^+$ (88%), 537 $[M+H]^+$ (35), 482 (17), 481(56), 426 (22), 425(100), 394 (19), 393 (80).

HRMS (ESI) calcd for $C_{23}H_{48}N_3O_{10}Si$ $[M+NH_4]^+$ 554.31090. Found *m/z* 554.31177.

^1H NMR assignments were confirmed using ^1H-^1H COSY NMR spectroscopy.

tert-Butyl [methyl 4-_N-tert_-butoxycarbonyl-4-deoxy-5-(1-hydroxy-2-nitroethyl)-α-D-_arabino_-pent-2-ulofuranosid]onate (149)

Compound **148** (40 mg, 0.075 mmol) in THF (5 ml) and tetrabutylammonium flucride (0.22 ml, 0.22 mmol, 1.0 M in THF) were stirred for 90 min at rt. The reaction mixture was concentrated and the residue chromatographed (EtOAc/hexane, 1:1, R_f 0.51) on silica column to afford **149** (28 mg, 89%) as a syrup.

^1H NMR ($CDCl_3$) (other diastereomer chemical shift given in parentheses where visible): δ 1.42 (s, 9H, $NCOOC(CH_3)_3$), 1.51 (s, 9H, $COOC(CH_3)_3$), 3.30 (3.31) (s, 3H, OCH_3), 3.93 (m, br,1H, H-5), 4.07 (m, 2H, H-3, H-4), 4.67 (m, 3H, H-6, H-7, H-7'), 5.29 (5.31) (d, br, 1H, $J_{NH,4}$ 8.3, NH).

LRMS (ESI) *m/z* 423 $[M+H]^+$ (52%), 368 (12), 367 (69), 312 (13), 311 (100).

HRMS (ESI) calcd for $C_{17}H_{31}N_2O_{10}$ $[M+H]^+$ 423.19787. Found *m/z* 423.19719.

^1H NMR assignments were confirmed using ^1H-^1H COSY NMR spectroscopy.

Methyl 4-amino-4-deoxy-5-(1-hydroxy-2-nitroethyl)-α-D- *arabino*-pent-2-ulofuranosidonic acid (150)

Trifluoroacetic acid (1 ml) was added to a solution of **149** (22 mg, 0.052 mmol) in CH_2Cl_2 (2 ml) and the resulting mixture stirred for 12 h at rt. The reaction mixture was concentrated and the residue redissolved in water (4 ml). This solution was neutralized with Amberlite IRA-400 (OH^-) resin. The resin was filtered off, washed with water (3 x 3 ml) and the filtrate lyophilized to give **150** (11 mg, 79%, R_f 0.19 in EtOAc/MeOH/0.2%HCl, 5:4:1) as a white amorphous mass.

^1H NMR (D_2O) (other diastereomer chemical shift given in parentheses where visible): δ 3.25 (3.23) (s, 3H, OCH_3), 3.74 (m, br, 1H, H-5), 4.13 (4.24) (m, br, 1H, H-4), 4.33 (4.51) (m, br,1H, H-3), 4.74-4.98 (m, 3H, H-6, H-7, H-7').

LRMS (ESI) *m/z* 289 $[M+Na]^+$ (46%), 267 $[M+H]^+$ (87), 235 (19), 217 (17), 186 (57).

HRMS (ESI) calcd for $C_8H_{15}N_2O_8$ $[M+H]^+$ 276.08284. Found *m/z* 267.08203.

^1H NMR assignments were confirmed using ^1H-^1H COSY NMR spectroscopy.

5.6 Synthesis of methyl 6-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,6-dideoxy-D-*glycero*-D-*allo*-2-nonulofuranosonate (162) and methyl 6-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,6-dideoxy-D-*glycero*-D-*altro*-2-nonulofuranosonate (163)

3-Azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*allo*furanose (166)

Triflic anhydride (7.70 ml, 45.77 mmol) was added dropwise to a solution of 1,2:5,6-di-*O*-isopropylidence-α-D-glucofuranose (diacetone-D-glucose) (**164**) (10.0 g, 38.46 mmol) in dry pyridine (8.0 ml, 98.9 mmol) and dry CH_2Cl_2 (100 ml) at -15°C under N_2. After stirring for 1 h at -15°C the reaction mixture was quenched with satd. aq. $NaHCO_3$ (15 ml). The organic layer was separated and washed with H_2O (2 x 10

ml), satd. aq. $CuSO_4$ (2 x 15 ml), H_2O (10 ml) and dried (Na_2SO_4). Solvent removal under reduced pressure gave the triflate ester **165b** as a pale yellow solid. The crude solid was then dissolved in benzene (100 ml), treated with tetrabutylammonium azide (prepared according to literature procedure[256]) (13.1 g, 46.1 mmol), and the mixture stirred at rt for 48 h. After dilution with EtOAc (100 ml), the reaction mixture was washed with water (2 x 20 ml), dried (Na_2SO_4) and the solvents removed *in vacuo*. The residue was chromatographed on silica with EtOAc/hexane (1:3) as the eluent. The first compound eluted from the column was **167** (R_f 0.56, 1.60 g, 17.2% from **164**) which was recrystallized from hexane to give colourless crystals. mp 50-51°C (lit.[267,268] mp 50-51°C and 50°C).

^{1}H NMR ($CDCl_3$) δ 1.39, 1.44 (2 x s, 6H, $C(CH_3)_2$), 1.46 (s, 6H, $C(CH_3)_2$), 3.96 (dd, 1H, $J_{6,5}$ 5.7, $J_{6,6'}$ 8.4, H-6), 4.14 (dd, 1H, $J_{6',5}$ 6.9, $J_{6',6}$ 8.4, H-6'), 4.58 (m, 1H, $J_{5,6}$ 6.9, $J_{5,6'}$ 5.7, H-5), 5.25 (d, 1H, $J_{3,2}$ 1.4, H-3), 5.30 (dd, 1H, $J_{2,1}$ 5.1, $J_{2,3}$ 1.4, H-2), 6.07 (d, 1H, $J_{1,2}$ 5.1, H-1). The ^{1}H NMR spectrum is consistent with that reported in the literature[237].

Further elution gave the title compound **166** (R_f 0.34, 6.85 g, 62.5% from **164**), an oil which solidified on standing.

IR (KBr) ν_{max} 3000, 2100 (N_3), 1398, 1380, 1262, 1203, 1162 cm^{-1}.

^{1}H NMR ($CDCl_3$) δ 1.36, 1.38, 1.48, 1.58 (4 x s, 12H, 2 x $C(CH_3)_2$), 3.52 (dd, 1H, $J_{3,2}$ 4.8, $J_{3,4}$ 9.0, H-3), 3.97-4.22 (m, 4H, H-4, H-5, H-6, H-6'), 4.73 (dd, 1H, $J_{2,1}$ 3.6, $J_{2,3}$ 4.8, H-2), 5.78 (d, 1H, $J_{1,2}$ 3.6, H-1).

^{13}C NMR ($CDCl_3$) δ 24.9, 26.1, 26.3, 26.4 (2x$C(CH_3)_2$), 62.7 (C-5), 66.6 (C-6), 75.7 (C-3), 77.9 (C-2), 80.4 (C-4), 103.8 (C-1), 109.9, 113.0 (2x$C(CH_3)_2$).

The ^{1}H NMR spetrum is in agreement with that reported in the literature[240].

In order to characterize the triflate ester **165b**, a small amount of the crude material was recrystallized in hexane to afford white crystals, m.p 68-70°C (lit[257].m.p.70°C).

^{1}H NMR ($CDCl_3$) δ 1.34 (s, 6H, $C(CH_3)_2$), 1.44, 1.52 (2 x s , 6H, $C(CH_3)_2$), 3.89-4.40 (m, 4H, H-4, H-5, H-6, H-6'), 4.85 (d, 1H, $J_{2,1}$ 4.0, H-2), 5.35 (s, br, H-3), 6,09 (d, 1H, $J_{2,1}$ 4.0, H-1).

The ^{1}H NMR spectrum is in agreement with that reported in the literature[257].

3-Amino-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*allo*furanose (168)

The azide **166** (5.0 g, 17.54 mmol) in MeOH (250 ml) was hydrogenated in the presence of 10% Pd/C (500 mg) under atmospheric pressure for 16 h. The catalyst was filtered off, washed with MeOH (3 x 50 ml) and the filtrate concentrated. Chromatography (EtOAc, R_f 0.20) of the residue on a short silica column gave the amine **168** as an off-white solid (4.40 g, 96.8 %).

^{1}H NMR (CD_3OD) δ 1.33, 1.34, 1.42, 1.52 (4 x s, 12H, 2 x $C(CH_3)_2$), 3.02 (br, 1H, H-3), 3.68 (dd, 1H, $J_{4,3}$ 9.0, $J_{4,5}$ 6.0, H-4), 3.88-4.18 (m, 3H, H-5, H-6, H-6'), 4.59 (dd, 1H, $J_{2,1}$ 3.6, $J_{2,3}$ 4.2, H-2), 5.75 (d, 1H, $J_{1,2}$ 3.6, H-1).

^{13}C NMR ($CDCl_3$) δ 24.0, 25.1, 25.3, 25.5 (2 x $C(CH_3)_2$), 57.4 (C-3), 66.1 (C-6), 75.8 (C-5), 80.8, 81.1(C-2, C-4), 104.3 (C-1), 109.3, 112.0 (2 x $C(CH_3)_2$).

LRMS (ESI) *m/z* 260 $[M+H]^+$ (100%), 243 (22), 202 (33), 160 (8).

HRMS (ESI) calcd for $C_{12}H_{22}NO_5$ $[M+H]^+$ 260.14979. Found *m/z* 260.14915.

The ^{1}H NMR spectrum was confirmed by comparison with the literature[240].

3-Acetamido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*allo*furanose (169)

The amine **168** (4.40 g, 16.99 mmol) in pyridine (10 ml) was treated with acetic anhydride (10 ml), and the mixture was stirred at rt for 1 h. The reaction was concentrated under reduced pressure, and the residue re-evaporated with toluene. The organic phase was extracted into EtOAc (150 ml), washed with H_2O (2 x 10 ml), dried (Na_2SO_4) and concentrated *in vacuo*. Column chromatography of the residue (EtOAc, R_f 0.60) gave **169** as an amorphous solid (4.60 g, 90.0%).

$[\alpha]_D$ +69.6° (*c* 1.42, $CHCl_3$).

IR (KBr) ν_{max} 3350, 3000, 1680, 1540, 1380, 1262, 1220, 1160 cm^{-1}.

^1H NMR ($CDCl_3$) δ 1.34 (s, 6H, $C(CH_3)_2$), 1.44, 1.56 (2 x s, 6H, $C(CH_3)_2$), 2.02 (s, 3H, $NCOCH_3$), 3.88 (dd, 1H, $J_{4,3}$ 9.7, $J_{4,5}$ 4.3, H-4), 3.95 (dd, 1H, $J_{6,5}$ 6.2, $J_{6,6'}$ 8.2, H-6), 4.10 (dd, 1H, $J_{6',5}$ 6.6, $J_{6',6}$ 8.2, H-6'), 4.22 (m, 2H, H-3, H-5), 4.61 (dd, 1H, $J_{2,1}$ 3.7, $J_{2,3}$ 4.3, H-2), 5.82 (d, 1H, $J_{1,2}$ 3.7, H-1).

^{13}C NMR ($CDCl_3$) δ 23.1 ($NCOCH_3$), 25.2, 26.3, 26.5 (2 x $C(CH_3)_2$), 53.3 (C-3), 65.1 (C-6), 75.6, 78.6, 78.9 (C-2, C-4, C-5), 104.1 (C-1), 109.5, 112.4 (2 x $C(CH_3)_2$), 169.8 (CO).

LRMS (ESI) *m/z* 302 $[M+H]^+$ (45%), 244 (100).

HRMS (ESI) calcd for $C_{14}H_{23}NO_6Na$ $[M+Na]^+$ 324.14231. Found *m/z* 324.14264.

The ^1H NMR, ^{13}C NMR and IR spectra are consistant with those reported in the literature[240].

3-Acetamido-3-deoxy-D-allose (173a, 173b, 173c, 173d)

Amberlite IR-120 (H^+) ion-exchange resin (washed with H_2O prior to use) (1.5 ml) was added to a suspension of **169** (4.50 g, 14.95 mmol) in H_2O (80 ml). The

mixture was stirred at 50°C for 48 h, after which time tlc analysis (EtOAc/MeOH, 6:1) showed a single spot with R_f 0.10. The resin was filtered off and washed with H_2O (2 x 30 ml). Concentration of the filtrate followed by chromatography of the residue on a short silica column (EtOAc/MeOH, 6:1, R_f 0.10) gave a hygroscopic mixture of 4 isomers of 3-acetamido-3-deoxy-D-allose (**173a, 173b, 173c, 173d**) (2.68 g, 81%).

The ^{1}H NMR (D_2O) spectrum displayed H-1 signals with intensity percentages of *ca.* 28%, 31%, 25%, 16% at δ 4.80 (d, *J* 8.4), 5.15 (d, *J* 3.0), 5.24 (s) and 5.37 (d, *J* 3.9) for **173c**, **173d**, **173a** and **173b**, respectively, and four singlets at 2.01, 2.04, 2.05 and 2.06 (NCOCH_3). All the remaining signals for the 4 isomers were ill-resolved in the δ 3.50-4.60 region.

^{13}C NMR (D_2O) δ 21.8, 21.9, 22.1, 22.6 ($NCOCH_3$), 50.0, 52.1, 52.5(C-3), 60.6, 61.1, 62.1, 62.3 (C-6), 65.3, 65.4, 65.8, 67.5, 69.7, 70.2, 71.9, 73.1, 74.4, 74.5, 80.2, 81.2 (C-2, C-4, C-5), 92.0, 93.5, 96.1, 101.2 (C-1), 173.9, 174.1, 175.4, 175.8 (CO).

LRMS (ESI) *m/z* 222 $[M+H]^+$ (32%), 204 (48), 190 (40), 186 (38), 185 (23), 147 (57), 145 (100).

HRMS (ESI) calcd for $C_8H_{16}NO_6$ $[M+H]^+$ 222.09776. Found *m/z* 222.09708.

Attempted allylation of 3-acetamido-3-deoxy-D-allose (173a, 173b, 173c, 173d)

To a solution of 3-acetamido-3-deoxy-D-allose (**173a, 173b, 173c, 173d**) (250 mg, 1.13 mmol) in water (20 ml) was added indium (778 mg, 6.78 mmol) and methyl 2-(bromomethyl)acrylate (1.21 g, 6.78 mmol). The mixture was stirred for 24 hours at rt. TLC examination revealed starting material remained unchanged. ^{1}H NMR spectrum of the material obtained after workup and chomatography was identical in all respects to unreacted starting material. No reaction was observed when aqueous ethanol (20%) was used instead of water. The use of EtOH/0.1N HCl (6:1, 14 ml) as solvent resulted in no

reaction after stirring for 16 hours at rt. Increasing the temperature to 40°C for 24 hours led to a complex mixture of products. Attempted separation of the mixture by chromatography was unsuccessful. The use of tin or zinc instead of indium gave similar results.

In another attempt, 2-(bromomethyl)acrylic acid (1.03 g, 6.24 mmol) and indium (716 mg, 6.24 mmol) were added to a solution of 3-acetamido-3-deoxy-D-allose **(173a, 173b, 173c, 173d)** (230 mg, 1.04 mmol) in EtOH/0.1N HCl (6:1, 14 ml). After stirring for 16 hours at rt, TLC analysis of the reaction revealed a complex mixture of products, which could not be separated by chromatography.

3-Acetamido-3-deoxy-D-allose methyloxime (181)

To an isomeric mixture of 3-acetamido-3-deoxy-D-allose (**173a**, **173b**, **173c**, **173d**) (1.26 g, 5.70 mmol) in pyridine (36 ml) was added methoxylamine hydrochloride (1.48 g, 17.72 mmol). The reaction mixture was stirred at rt for 48 h. Solvent removal followed by chromatography of the residue (EtOAc/MeOH, 4:1, R_f 0.26) gave an (*E/Z*) isomeric mixture of **181** as an amorphous white solid (1.10 g, 77%). The ratio of *E/Z* isomers, by ^{1}H NMR), is *ca.* 10:1, respectively.

IR (KBr) ν_{max} 3425, 3300, 1650, 1563, 1220, 1040 cm^{-1}.

^{1}H NMR (D_2O): *E* isomer (*Z* isomer chemical shift given in parentheses where visible) δ 1.99 (s, 3H, $NCOCH_3$), 3.56-3.76 (m, 4H, H-4, H-5, H-6, H-6'), 3.82 (s, 3H, OCH_3), 4.27 (m, 1H, H-3), 4.52 (5.02) (m, 1H, H-2) 7.46 (6.81) (d, 1H, $J_{1,2}$ 6.3, H-1).

^{13}C NMR (D_2O): *E* isomer (*Z* isomer chemical shift given in parentheses where visible) δ 21.9 (NCOCH3), 53.7 (53.2) (C-3), 61.4 (NOCH3), 62.0 (C-6), 68.3, 71.1, 72.0 (C-2, C-4, C-5), 151.0 (152.1) (C-1), 174.0 (173.8) (CO).

LRMS (ESI) *m/z* 273 $[M+Na]^+$ (53%), 133 (45), 129 (30), 111 (50), 89 (58), 87 (100).

HRMS (ESI) calcd for $C_9H_{19}N_2O_6$ $[M+H]^+$ 251.12431. Found *m/z* 251.12376.

^{1}HNMR assignments were confirmed using ^{1}H-^{1}H COSY NMR spectroscopy.

(*E*)-3-Acetamido-2,4,5,6-tetra-*O*-*tert*-butyldimethylsilyl-3-deoxy-D-allose methyloxime (182)

A solution of **181** (600 mg, 2.40 mmol) in DMF (6 ml) was treated with *tert*-butyldimethylsilyl chloride (2.40 g, 15.92 mmol) and imidazole (1.22 g, 17.92 mmol). The resulting mixture was left to stir for 3 days at 400°C. Concentration of the reaction mixture *in vacuo* and column chromatography of the residue (Hex/EtOAc, 6:1, R_f 0.40) gave **182** as a colourless syrup (1.45 g, 86%).

^{1}H NMR ($CDCl_3$) δ 0.01, 0.06, 0.07, 0.08, 0.09, 0.11 (6 x s, 24H, 4 x $Si(CH_3)_2$), 0.87, 0.88, 0.89, 0.91, 0.93 (5 x s, 36H, 4 x $SiC(CH_3)_3$), 1.92 (s, 3H, $NCOCH_3$), 3.51 (m, 1H, H-6), 3.81 (s, 3H, OCH_3) 3,85-3.92 (m, 2H, H-5, H-6'), 4.08 (dd, 1H, $J_{4,3}$ 5.4, $J_{4,5}$ 1.5, H-4), 4.33 (m, 1H, H-3), 4.53 (dd, 1H, $J_{2,1}$=$J_{2,3}$ 6.3, H-2), 6.65 (d, 1H, $J_{NH,3}$ 9.3, NH), 7.29 (d, $J_{1,2}$ 6.3, H-1).

^{13}C NMR ($CDCl_3$) δ -5.4, -5.2, -5.1, -4.7, -4.5, -4.4 -4.3, -4.2 (4 x $Si(CH_3)_2$), 17.9, 18.1, 18.4 (4 x $SiC(CH_3)_3$), 23.3 ($NCOCH_3$), 25.6, 25.8, 25.9, 26.0 (4 x $SiC(CH_3)_3$), 55.9 (C-3), 61.4 (NOCH3), 63.9 (C-6), 70.0, 73.5, 76.0 (C-2, C-4, C-5), 149.9 (C-1), 169.3 (CO).

LRMS (ESI) *m/z* 707 $[M+H]^+$ (72%), 577 (19), 576 (42), 575 (100), 433 (20), 411 (21).

HRMS (ESI) calcd for $C_{33}H_{75}N_2O_6Si_4$ $[M+H]^+$ 707.47022. Found *m/z* 707.47205.

^{1}H NMR assignments were confirmed using ^{1}H-^{1}H COSY NMR spectroscopy.

Methyl 6-acetamido-5,7,8,9-tetra-*O-tert*-butyldimethylsilyl-2,3,6-trideoxy-2-methylidene-D-*glycero*-D-*allo*-nononate (185)

and

Methyl 6-acetamido-5,7,8,9-tetra-*O-tert*-butyldimethylsilyl-2,3,6-trideoxy-2-methylidene-D-*glycero*-D-*altro*-nononate (184)

Ozone was bubbled through a solution of **182** (810 mg, 1.15 mmol) in anhydrous CH_2Cl_2 (200 ml) at -78°C for 1 h. The mixture was left for 16 h at -78°C, after which the reaction was purged with nitrogen for 10 min. Dimethyl sulfide (0.42 ml, 5.74 mmol) was added and the mixture warmed to rt. The mixture was stirred for a further 60 min at rt then washed with satd aq NaCl/$NaHCO_3$ (1:1) (2 x 20 ml), dried (Na_2SO_4) and concentrated under reduced pressure to give the aldehyde **183** as a syrup (660 mg, R_f 0.4 in Hex/EtOAc, 6:1). The crude aldehyde **183** was dissolved in anhydrous THF (5 ml). The resulting solution was treated with methyl 2-(bromomethyl)acrylate (0.207 ml, 1.72 mmol), activated zinc powder (144 mg) and satd aq ammonium chloride (9 ml) at O°C. After stirring for 90 min at rt the mixture was extracted with Et_2O (2 x 60 ml) and the organic layer washed with satd aq NaCl/$NaHCO_3$ (1:1) (2 x 10 ml), dried (Na_2SO_4) and concentrated *in vacuo*. Column chromatography of the residue (Hex/EtOAc, 6:1) gave two products , **184** (80 mg, 9%, R_f 0.29) and **185** (374 mg, 42% R_f 0.18) as amorphous masses.

184:

^{1}H NMR ($CDCl_3$) δ 0.06, 0.08, 0.09, 0.15 (4 x s, 24H, 4 x $Si(CH_3)_2$), 0.09 (s, 36H, 4 x $SiC(CH_3)_3$), 1.90 (s, 3H, $NCOCH_3$), 2.30 (dd, 1H, $J_{3,3'}$ 14.3, $J_{3,4}$ 2.1, H-3), 2.55 (d, 1H, $J_{OH,4}$ 9.4, OH), 2.67 (dd, 1H, $J_{3',3}$ 14.3, $J_{3',4}$ 11.0, H-3'), 3.57 (dd, 1H $J_{9,8}$ 7.0, $J_{9,9'}$ 10.2, H-9), 3.76 (s, 3H, OCH_3), 3.79-3.91 (m, 3H, H-7, H-8, H-9'), 4.10-4.19 (m, 2H, H-5, H-

6), 4.28 (m, 1H, H-4), 5.65 (app s, 1H, $=CH_2$), 6.26 (app s, 1H, $=CH_2$), 7.24 (d, 1H, $J_{NH,6}$ 8.3, NH).

^{13}C NMR ($CDCl_3$) δ -5.6, -5.5, -5.4, -4.8, -4.7, -4.4, -4.3, -3.6 (4 x $Si(CH_3)_2$), 17.9, 18.1, 18.2, 18.4 (4 x $SiC(CH_3)_3$), 23.2 ($NCOCH_3$), 25.7, 25.8, 25.9, 26.0 (4 x $SiC(CH_3)_3$), 37.3 (C-3), 51.9 (OCH_3), 55.7 (C-6), 64.8 (C-9), 70.8 (C-5), 72.0 (C-4), 76.9, 77.0 (C-7, C-8), 126.8 ($=CH_2$), 137.6 (C-2), 168.1, 169.3 (COOMe, NHCOMe).

LRMS (ESI) *m/z* 778 $[M+H]^+$ (34%), 648 (25), 647 (64), 646 (100), 515 (16), 514 (34), 382 (30), 352 (43).

HRMS (ESI) calcd for $C_{37}H_{80}NO_8Si_4$ $[M+H]^+$ 778.49610. Found *m/z* 778.49691.

^1H and ^{13}C NMR assignments were confirmed using ^1H-^1H COSY and ^1H-^{13}C HMQC NMR spectroscopy.

185:

^1H NMR ($CDCl_3$) δ 0.09, 0.11, 0.12, 0.14, 0.15 (5 x s, 24H, 4 x $Si(CH_3)_2$), 0.90 (s, 36H, 4 x $SiC(CH_3)_3$), 1.96 (s, 3H, $NCOCH_3$), 2.29 (dd, 1H, $J_{3,3'}$ 14.1, $J_{3,4}$ 9.8, H-3), 2.79 (app d, 1H, H-3'), 3.27 (d, 1H, $J_{OH,4}$ 4.3, OH), 3,55 (dd, 1H, $J_{9,8}$ 7.8, $J_{9,9'}$ 11.7, H-9), 3.62 (m, 1H, H-4), 3.77 (s, 3H, OCH_3), 3.79-3.88 (m, 2H, H-8, H-9'), 4.05 (m, 2H, H-5, H-7), 4.23 (m, 1H, H-6) 5.72 (app s, 1H , $=CH_2$), 6.25 (app s, 1H, $=CH_2$), 6.51 (d, 1H, $J_{NH,6}$ 8.9, NH).

^{13}C NMR ($CDCl_3$) δ -5.3, -5.2, -4.8, -4.4, -4,3, -4.2, -4.0, -3.7 (4 x $Si(CH_3)_2$), 18.2, 18.3, 18.6 (4 x $SiC(CH_3)_3$), 23.4 ($NCOCH_3$), 25.8, 26.0, 26.2, 26.3 (4 x $SiC(CH_3)_3$), 37.2 (C-3), 52.2 (OCH_3), 56.1 (C-6), 64.4 (C-9), 73.7, 74.1 (C-4, C-5), 76.3 (C-7, C-8), 128.1 ($=CH_2$), 138.1 (C-2), 169.1,169.4 (COOMe, NHCOMe).

LRMS (ESI) *m/z* 778 $[M+H]^+$ (39%), 648 (28), 647 (49), 646 (100), 516 (11), 515 (15), 514 (56), 382 (48).

HRMS (ESI) calcd for $C_{37}H_{80}NO_8Si_4$ $[M+H]^+$ 778.49610, found *m/z* 778.49487.

^{1}H and ^{13}C NMR assignments were confirmed using ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMQC NMR spectroscopy.

Methyl 6-acetamido-5,7,8,9-tetra-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-D-*glycero*-D-*allo*-2-nonulosonate (186)

Ozone was bubbled through a solution of **185** (56 mg, 0.072 mmol) in CH_2Cl_2 (100 ml) at -78°C for 1 h. The reaction was purged with nitrogen for 5 min. Dimethyl sulfide (0.026 ml, 0.35 mmol) was added and the mixture stirred at rt for 2 h. The mixture was then washed with satd aq NaCl/$NaHCO_3$ (1:1) (2 x 5 ml), dried (Na_2SO_4), and evaporated *in vacuo* to give the keto ester **186** as an amorphous mass (55 mg, 98%, R_f 0.16 in Hex/EtOAc, 5:1). This material was used in subsequent step without further purification.

^{1}H NMR ($CDCl_3$) δ 0.10, 0.11, 0.12, 0.13, 0.14 (5 x s, 24H, 4 x $Si(CH_3)_2$), 0.89, 0.90, 0.91, 0.92, 0.93, 0.94, 0.95 (7 x s, 36H, 4 x $SiC(CH_3)_3$), 1.99 (s, 3H, $NCOCH_3$), 2.96 (dd, 1H, $J_{3,3'}$ 17.3, $J_{3,4}$ 8.41, H-3), 3.20 (dd, 1H, $J_{3',3}$ 17.3, $J_{3',4}$ 3.96, H-3'), 3.56 (dd, 1H, $J_{9,8}$ 4.64, $J_{9,9'}$ 9.26, H-9), 3.82 (m, 5H, H-8, H-9', OCH_3), 4.21 (m, 4H, H-4, H-5, H-6, H-7), 6.29 (d, 1H, $J_{NH,6}$ 7.6, NH).

LRMS (ESI) *m/z* 780 $[M+H]^+$ (100%), 749 (39), 650 (18), 649 (35), 648 (71), 616 (38), 517 (23), 516 (62).

HRMS (ESI) calcd for $C_{36}H_{78}NO_9Si_4$ $[M+H]^+$ 780.47537. Found *m/z* 780.47621.

Methyl 6-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,6-dideoxy-D-*glycero*-D-*allo*-2-nonulofuranosonate (162)

Crude **186** (55 mg, 0.071mmol), trifluoroacetic acid (4 ml) and H_2O (1 ml) were stirred for 18 h at rt. Upon completion, by TLC (R_f 0.06, EtOAc/MeOH/0.1 M HCl,

3:2:1), the reaction was concentrated and the residue redissolved in anhydrous MeOH (10 ml). Dried Dowex 50WX8 (H^+) ion-exchange resin (60 mg) was added and the mixture stirred for 18 h at rt. The resin was filtered off and washed with MeOH (2 x 10 ml). Pyridine (1 ml) was added to the filtrate, and the mixture concentrated to furnish **188** as an amorphous mass. Without further purification, crude **188** was acetylated for 20 h at rt using pyridine (6 ml), Ac_2O (4.5 ml) and DMAP (cat). Concentration of the reaction mixture gave a residue which was partitioned between EtOAc (30 ml) and H_2O (4 ml). The organic phase was dried (Na_2SO4), concentrated *in vacuo* and the residue chromatographed (EtOAc, R_f 0.34) to afford **162** (16 mg, 43%) as an anomeric mixture (2.2:1 by ^1H NMR).

^1H NMR ($CDCl_3$) (other anomer chemical shift given in parentheses where visible)

δ 2.05, 2.07, 2.08, 2.09, 2.11, 2.14 (6 x s, 18H, $NCOCH_3$, 5 x $OCOCH_3$), 2.50 (2.60) (app d, 1H, $J_{3,3'}$ 15.2, H-3), 2.91 (2.79) (dd, 1H, $J_{3',4}$ 7.0, $J_{3',3}$ 15.2, H-3'), 3.81 (3.79) (s, 3H, OCH_3), 4.16 (4.10) (dd, 1H, $J_{9,8}$ 5.7, $J_{9,9'}$ 12.3, H-9), 4.36 (m, 3H, H-5, H-6, H-9'), 5.25 (m, 3H, H-4, H-7, H-8), 6.51 (6.43) (d, 1H, $J_{NH,6}$ 9.0, NH).

^{13}C NMR ($CDCl_3$) (other anomer chemical shift given in parentheses where visible) δ 19.7, 19.8, 20.0 (5 x $OCOCH_3$), 22.3 ($NCOCH_3$), 38.4 (40.1)(C-3), 49.0 (49.5) (C-6), 52.4 (52.1) (OCH_3), 60.9 (61.5) (C-9), 69.2, 70.7, 73.5 (69.0, 70.6, 72.5) (C-4, C-7, C-8), 86.1 (85.4) (C-5), 104.8 (103.8) (C-2), 165.3 (166.6) (COOMe), 168.7, 169.3, 169.4, 169.8, 170.0) (5 x $OCOCH_3/NCOCH_3$).

LRMS (ESI) *m/z* 534 $[M+H]^+$ (13%), 502 (12), 474 (45), 415 (21), 414 (100).

HRMS (ESI) calcd for $C_{22}H_{31}NO_{14}$ $[M+NH_4]^+$ 551.20883. Found *m/z* 551.20910.

The ^1H NMR spectrum was in agreement with that reported in the literature[106].

^1H NMR and ^{13}C NMR assignments were confirmed using ^1H-^1H COSY and ^1H-^{13}C HMQC NMR spectroscopy.

Methyl 6-acetamido-5,7,8,9-tetra-*O-tert*-butyldimethylsilyl-3,6-dideoxy-D-*glycero*-D-*altro*-2-nonulosonate (189)

Ozone was bubbled through a solution of **184** (68 mg, 0.089 mmol) in CH_2Cl_2 (120 ml) at -78°C for 1 h. The reaction was purged with nitrogen for 5 min. Dimethyl sulfide (0.032 ml, 0.44 mmol) was added and the mixture stirred for 2 h at rt. The mixture was then washed with satd. aq. NaCl/$NaHCO_3$ (1:1) (2 x 6 ml), dried (Na_2SO_4), and concentrated *in vacuo* to give the keto ester **189** as an amorphous mass (67 mg, 98%, R_f 0.28 in Hex/EtOAc, 5:1). This material was used in subsequent step without further purification.

^{1}H NMR ($CDCl_3$) δ 0.07, 0.08, 0.09, 0.11 (4 x s, 24H, 4 x $Si(CH_3)_2$), 0.87, 0.90, 0.92, 0.93 (4 x s, 36H, 4 x $SiC(CH_3)_3$), 1.91 (s, 3H, $NCOCH_3$), 2.69 (dd, 1H, $J_{3,3'}$ 16.2, $J_{3,4}$<1, H-3), 3.29 (dd, 1H, $J_{3',3}$ 16.2, $J_{3',4}$ 10.5, H-3'), 3.57 (dd, 1H, $J_{9,8}$ 6.5, $J_{9,9'}$ 10.2, H-9), 3.71 (dd, 1H, $J_{9',8}$ 5.5, $J_{9',9}$ 10.2, H-9'), 3.86 (m, 4H, H-8, OCH_3), 4.02 (app d, 1H, $J_{7,6}$ 5.1, H-7), 4.15 (app d, 1H, $J_{5,6}$ 3.9, H-5), 4.22 (m, 1H, H-6), 4.82 (m, 1H, H-4), 7.30 (d, 1H, $J_{NH,6}$ 8.4, NH).

LRMS (ESI) *m/z* 780 $[M+H]^+$ (89%), 748 (29), 666 (30), 649(50), 648 (100), 616 (32), 534 (21), 516 (42).

HRMS (ESI) calcd for $C_{36}H_{78}NO_9Si_4$ $[M+H]^+$ 780.47537. Found *m/z* 780.47459.

^{1}H NMR assignments were confirmed using ^{1}H-^{1}H COSY NMR spectroscopy.

Methyl 6-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,6-dideoxy-D-*glycero*-D-*altro*-2-nonulofuranosonate (163)

Crude **189** (67 mg, 0.086 mmol), trifluoroacetic acid (6 ml) and H_2O (1.5 ml) were stirred for 18 h at rt. Upon completion, by TLC (R_f 0.08, EtOAc/MeOH/0.1HCl, 3:2:1), the reaction was concentrated and the residue redissolved in anhydrous MeOH

(12 ml). Dried Dowex 50WX8 (H^+) ion-exchange resin (75 mg) was added and the mixture stirred for 18 h at rt. The resin was filtered off and washed with MeOH (2 x 15 ml). Pyridine (1 ml) was added to the filtrate, and the mixture concentrated to give **190** as an amorphous mass. Without further purification, crude **190** was acetylated at rt for 20 h using pyridine (7.5 ml), Ac_2O (5.5 ml) and DMAP (cat). Concentration of the reaction mixture gave a residue which was partitioned between EtOAc (40 ml) and water (5 ml). The organic phase was dried (Na_2SO_4) and concentrated *in vacuo*. Column chromatography (EtOAc, R_f 0.39) of the residue afforded **163** (19 mg, 41%), as an anomeric mixture (2.3:1 by ^{1}H NMR).

^{1}H NMR ($CDCl_3$) (other anomer chemical shift given in parentheses where visible)

δ 1.97, 2.06, 2.07, 2.08, 2.09 (5 x s, 18H, $NCOCH_3$, 5 x $OCOCH_3$), 2.64 (2.42, 2.69) (m, 2H, H-3, H-3'), 3.80 (3.77) (s, 3H, OCH_3), 4.19 (4.22) (m, 1H, H-9), 4.28 (4.15) (dd, 1H, $J_{5,4}$ 3.6, $J_{5,6}$ 8.7, H-5), 4.48 (4.43) (m, 1H, H-9'), 4.75 (4.77) (m, 1H, H-6), 5.28 (5.15, 5.30) (m, 2H, H7, H8), 5.47 (5.45) (m, 1H, H-4), 6.08 (6.05) (d, 1H, $J_{NH,6}$ 9.9, NH).

^{13}C NMR ($CDCl_3$) (other anomer chemical shift given in parentheses where visible)

δ 20.8 (5 x $OCOCH_3$), 23.2 ($NCOCH_3$), 43.3 (41.8) (C-3), 47.3 (48.2) (C-6), 52.5 (OCH_3), 62.4 (C-9), 70.3,72.0, 72.8 (70.9, 72.3, 73.2) (C-4, C-7, C-8), 81.9 (83.3) (C-5), 104.4 (104.0) (C-2), 166.8 (COOMe), 169.3, 169.5, 169.8, 170.0, 170.1 (169.4, 169.7, 170.3, 171.0) (5 x $OCOCH_3$, $NCOCH_3$).

LMRS (ESI) *m/z* 534 $[M+H]^+$ (42%), 506 (28), 475 (23), 474 (100), 432 (10), 330 (22), 258 (18).

HRMS (ESI) calcd for $C_{22}H_{31}NO_{14}$ $[M+NH_4]^+$ 551.20883. Found *m/z* 551.20626.

The ^{1}H NMR spectrum was in agreement with that reported in the literature[106].

^{1}H NMR and ^{13}C NMR assignments were confirmed using ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMQC NMR spectroscopy.

5. 7 Approaches towards the synthesis of 4,6-diacetamido-3,4,6-trideoxy-D-*glycero*-D-*allo/altro*-2-nonulofuranosonic acid (200)

3-Acetamido-2,4,5,6-tetra-*O*-acetyl-3-deoxy-D-allose methyloxime (191)

To an isomeric mixture of 3-Acetamido-3-deoxy-D-allose (**173a**, **173b**, **173c**, **173d**) (3.50 g, 15.83 mmol) in pyridine (100 ml) was added methoxylamine hydrochloride (4.10 g, 49.10 mmol). The resulting mixture was left to stir for 48 h at rt, after which it was treated with acetic anhydride (32 ml, 339 mmol) and DMAP (30 mg, 0.25 mmol). The reaction was then stirred for a further 18 h. The mixture was concentrated under reduced pressure and the residue re-evaporated with toluene (2 x 20 ml). The organic phase was extracted into ethyl acetate (60 ml), washed with H_2O (2 x 10 ml), satd. aq. $NaHCO_3$ (10 ml), dried (Na_2SO_4) and concentrated under reduced pressure, column chromatography of the residue (EtOAc/Hex, 1:1, R_f 0.60 in EtOAc) afforded an (*E*/*Z*) isomeric mixture of the methyloxime **191** as an amorphous solid (4.84 g, 73%). The ratio of *E*/*Z* isomers (by ^{1}H NMR).is *ca.* 3:1, respectively.

$[\alpha]_D$ +58.9° (*c* 1.28, $CHCl_3$).

IR (KBr) ν_{max} 3301, 1760, 1675, 1575, 1382, 1240, 1050 cm^{-1}.

^{1}H NMR ($CDCl_3$): *E* isomer (*Z* isomer chemical shift given in parentheses where visible) δ 2.04, 2.06, 2.08, 2.09, 2.10 (5 x s, 15H, $NCOCH_3$, 4 x $OCOCH_3$), 3.85 (3.91) (s, 3H, OCH_3), 4.18 (4.15) (dd, 1H, $J_{6,5}$ 6.6, $J_{6,6'}$ 12.3, H-6), 4.38 (4.40) (dd, 1H, $J_{6',5}$ 2.5, $J_{6',6}$ 12.3, H-6'), 4.76 (m, 1H, H-3), 5.13-5.21 (m, 2H, H-4, H-5), 5.36 (5.85) (dd, 1H, $J_{2,3}$ 5.5, H-2), 6.35 (d, 1H, $J_{NH,3}$ 9.9, NH), 7.25 (6.52) (d, 1H, $J_{1,2}$ 6.0, H-1).

^{13}C NMR ($CDCl_3$): *E* isomer (*Z* isomer chemical shift given in parentheses where visible) δ 20.5, 20.6, 20.7 (4 x $OCOCH_3$), 23.1(23.6) ($NCOCH_3$), 49.4 (49.6) (C-3), 61.6 (61.9) (C-6), 62.0 (62.4) ($NOCH_3$), 69.8, 70.3, 71.2 (67.8 ,70.2 ,70.9) (C-2, C-4, C-5), 144.8 (146.4) (C-1), 169.6, 169.7, 169.9, 170.3, 170.6 (4 x $OCOCH_3$, $NCOCH_3$).

LRMS (FAB) *m/z* 419 $[M+H]^+$ (100%), 403 (10), 389 (11), 377 (23), 361 (25), 360 (81), 359 (100), 345 (26).

HRMS (FAB) calcd for $C_{17}H_{27}N_2O_{10}$ $[M+H]^+$ 419.16657. Found *m/z* 419.16684.

^1H NMR assignments were confirmed using ^1H-^1H COSY NMR spectroscopy.

(2*E*)-5-Acetamido-4,6,7,8-tetra-*O*-acetyl-2,3,5-trideoxy-1-*C*-phenyl-D-*allo*-oct-2-enose (194)

Ozone was bubbled through a solution of **191** (2.10 g, 5.02 mmol) in anhydrous CH_2Cl_2 (200 ml) at -78°C for 1 h. The reaction was left standing for 16 h at -78°C. The mixture was then purged with nitrogen for 10 min. Dimethyl sulfide (1.83 ml, 25.0 mmol) was added and the resulting mixture warmed to rt. After stirring for 1 h at rt the mixture was washed with satd aq NaCl/$NaHCO_3$ (1:1) (2 x 40 ml), dried (Na_2SO_4) and concentrated under vacuum to give the crude aldehyde **192** as a syrup (1.68 g, R_f 0.38 in EtOAc). The crude aldehyde was redissolved in anhydrous CH_2Cl_2 (40 ml). Benzoylmethylene triphenylphosphorane (prepared according to literature procedure[285]) (1.96 g, 5.16 mmol) was added and the resulting mixture stirred for 20 h at rt. Concentration of the reaction mixture under reduced pressure and chromatography of the residue (EtOAc, R_f 0.63) afforded, exclusively, the *trans* isomer **194** as an amorphous mass (1.72 g, 70% from **191**).

$[\alpha]_D$ +52.38° (*c* 3.90, $CHCl_3$).

^{1}H NMR ($CHCl_3$) δ 2.05, 2.06, 2.10, 2.16 (4 x s , 15H, $NCOCH_3$, 4 x $OCOCH_3$), 4.17 (dd, 1H, $J_{8,7}$ 6.6, $J_{8,8'}$ 12.3, H-8), 4.41 (dd, 1H, $J_{8',7}$ 2.8, $J_{8',8}$ 12.3, H-8'), 4.77 (m, 1H, H-5), 5.25 (m, 2H, H-6, H-7), 5.59 (dd, 1H, $J_{4,5}$ 4.5, H-4), 6.31 (d, 1H, $J_{NH,5}$ 9.9, NH), 6.90 (dd, 1H, $J_{3,2}$ 15.6, $J_{3,4}$ 5.1, H-3). 7.03 (d, 1H, $J_{2,3}$ 15.6, H-2), 7.47-7.95 (m, 5H, Ph).

^{13}C NMR ($CDCl_3$) δ 20.5, 20.6, 20.7 (4 x $OCOCH_3$), 23.1($NCOCH_3$), 50.8 (C-5), 61.7 (C-8), 70.4, 70.7, 72.5 (C-4, C-6, C-7), 127.1, 128.5, 128.6, 133.8, 137.0, 140.7 (Ph), 169.7, 169.9, 170.0, 170.4, 170.7 (4 x $OCOCH_3$, $NCOCH_3$), 189.6 (COPh).

LRMS (ESI) *m/z* 492 $[M+H]^+$ (100%), 381 (25), 373 (10), 279 (12).

HRMS (ESI) calcd for $C_{24}H_{30}NO_{10}$ $[M+H]^+$ 492.18696. Found *m/z* 492.18685.

^{1}H NMR assignments were confirmed using ^{1}H-^{1}H COSY NMR spectroscopy.

3,5-Diacetamido-4,6,7,8-tetra-*O*-acetyl-2,3,5-trideoxy-1-*C*-phenyl-D-*glycero*-D-*altro*/*allo*-oct-1-ulose (196)

To a solution of **194** (1.50 g, 3.05 mmol) in anhydrous CH_2Cl_2 (60 ml) at -20°C was added azidotrimethylsilane (0.68 ml, 5.13 mmol) and tetrabutyllammonium fluoride (1.0 M in THF, 0.33 ml, 0.33 mmol). The resulting mixture was left standing at -20°C for 4 days. The mixture was concentrated under reduced pressure and the residue treated with thiolacetic acid (985 mg, 12.94 mmol). After stirring for 4 h at rt the reaction mixture was concentrated *in vacuo* and the residue chromatographed on a silica column (CH_2Cl_2/MeOH, 10:1, R_f 0.29) to give **196** as an amorphous mass (0.71 g, 42%).

^{1}H NMR ($CDCl_3$) δ 1.98, 1.99, 2.05,2.10, 2.12, 2.18 (6 x s, 18H, 2 x $NCOCH_3$/4 x $OCOCH_3$), 3.13 (dd, 1H, $J_{2,2'}$ 17.7, $J_{2,3}$ 8.3, H-2), 3.27 (dd, 1H, $J_{2',2}$ 17.7, $J_{2',3}$ 4.5, H-2'), 4.12 (dd, 1H, $J_{8,7}$ 6.0, $J_{8,8'}$ 12.3, H-8), 4.34 (dd, 1H, $J_{8',7}$ 2.7, $J_{8',8}$12.3, H-8'), 4.60 (m, 1H, H-5), 4.75 (m, 1H, H-3), 5.04 (dd, 1H, $J_{6,5}$ 3.7, $J_{6,7}$ 7.6, H-6), 5.17 (dd, 1H, $J_{4,3}$ 2.7,

$J_{4,5}$ 7.8, H-4), 5.32 (m, 1H, H-7), 6.11 (d, 1H, $J_{HN\text{-}3,3}$ 8,7, HN-3), 6.53 (d, 1H, $J_{HN\text{-}5,5}$ 9.9, HN-5), 7.33-7.89 (m, 5H, Ph).

^{13}C NMR ($CDCl_3$) δ 20.8, 20.9, 20.0, 21.1 (4 x $OCOCH_3$), 23.3 (2 x $NCOCH_3$), 40.4 (C-2), 46.1 (C-3), 49.0 (C-5), 62.4 (C-8), 70.0, 72.1, 72.8 (C-4, C-6, C-7), 128.1, 128.8, 133.6 (Ph), 136.6 (*Cipso*), 170.4, 170.6, 170.8, 171.1 (4 x $OCOCH_3$, 2 x $NCOCH_3$), 197.9 (COPh).

LRMS (ESI) *m/z* 551 $[M+H]^+$ (15%), 513 (17), 491 (14), 453 (12), 313 (17), 271 (25), 228 (16), 211 (100).

HRMS (ESI) calcd for $C_{26}H_{35}N_2O_{11}$ $[M+H]^+$ 551.22409. Found *m/z* 551.22163.

^1H NMR assignments were confirmed using ^1H-^1H COSY NMR spectroscopy

Alternatively: a solution of **194** (1.50 g, 3.05 mmol) in anhydrous CH_2Cl_2 (60 ml) at -20°C was treated with azidotrimethylsilane (0.68 ml, 5.13 mmol) and tetrabutylammonium fluoride (1.0 M in THF, 0.33 ml, 0.33 mmol). After leaving for 4 days at -20°C, the reaction mixture was concentrated under reduced pressure and the residue redissolved in MeOH/toluene (1:2, 60 ml). Pd/C (10%, 400 mg) was added and the mixture hydrogenated for 16 h. Insoluble solid was filtered off and washed with MeOH (2 x 20 ml). The filtrate was concentrated to give a syrup which was treated with pyridine (4 ml) and acetic anhydride (4 ml). After stirring for 2 h at rt, the reaction mixture was concentrated and the residue chromatographed on a silica column (CH_2Cl_2/MeOH, 10:1, R_f 0.29) to furnish **196** as an amorphous mass (0.54 g, 32%), identical in all respects to the material described above.

Methyl 3,5-diacetamido-6,7,8-tri-*O*-acetyl-2,3,5-trideoxy-1-phenyl-D-*glycero*-D-*altro/allo*-octofuranoside (199)

A solution of **196** (235 mg, 0.43 mmol) in anhydrous MeOH (25 ml) was treated with NaOMe in MeOH (0.04 M, 5 ml, 0.20 mmol). After stirring for 3 h at rt, the reaction mixture was neutralized with Dowex 50WX8 (H^+) ion-exchange resin. The resin was filtered off and washed with MeOH (2 x 20 ml). Concentration of the filtrate gave an amorphous white solid (150 mg) (R_f 0.18, EtOAc/MeOH, 2:1), which was redissolved in anhydrous methanol (70 ml). Dried Dowex 50WX8 (H^+) resin (150 mg) was added, and the mixture stirred for 16 h at rt. Pyridine (5 ml) was added to reaction mixture followed by filtration to remove the resin. Concentration of the filtrate under reduced pressure gave **198** (R_f 0.44, EtOAc/MeOH, 2:1). Crude **198** was then redissolved in pyridine (15 ml). Acetic anhydride (10 ml) and DMAP (cat.) were added and the mixture was stirred for 18 h at rt. The reaction mixture was concentrated *in vacuo* and the residue re-evaporated with toluene (10 ml). The organic phase was extracted into EtOAc (60 ml), washed with H_2O (2 x 10 ml), satd. aq. $NaHCO_3$ (10 ml), dried and concentrated under reduced pressure. Column chromatography of the residue (EtOAc/MeOH, 10:1, R_f 0.36) afforded **199** as an amorphous mass, (162 mg, 73% from **196**), as a mixture of diastereoisomers (2.5:1 by ^1H NMR).

^1H NMR ($CDCl_3$) (other diastereomer chemical shift given in parentheses where visible) δ 1.84, 1.97, 2.06, 2.12, 2.16 (1.96, 2.03, 2.07, 2.08, 2.14) (5 x s, 15H, 2 x $NCOCH_3$, 3 x $OCOCH_3$), 1.92 (dd, 1H, $J_{2,2'}$ 14.1, $J_{2,3}$ 3.6, H-2), 2.75 (dd, 1H, $J_{2',2}$ 14.1, $J_{2',3}$ 7.6, H-2'), 2.99 (3.17) (s, 3H, OCH_3), 4.16 (m, 2H, H-4, H-6), 4.45-4.68 (m, 2H, H-5, H-7), 4.87 (m, 1H, H-3), 5.32-5.58 (m, 2H, H-8, H-8'), 5.73 (6.71) (d, 1H, $J_{HN\text{-}3,3}$ 9.9, HN-3), 6.18 (6.07) (d, 1H, $J_{HN\text{-}5,5}$ 10.2 , HN-5), 7.30-7.54 (m, 5H, Ph).

^{13}C NMR ($CDCl_3$) (other diastereomer chemical shift given in parentheses where visible) δ 20.7, 20.9, 21.1 (3 x $OCOCH_3$), 23.2 (23.6) (2 x $NCOCH_3$), 48.4 (46.9) (C-2) 48.2, 50.0, 50.5 (C-3, C-5, OCH_3), 63.1 (63.7) (C-8), 70.1, 73.7 (70.2, 73.9) (C-6, C-7), 77.6 (82.2) (C-4), 108.4 (110.5) (C-1), 125.9, 126.1, 128.4, 128.5 (Ph), 139.9 (139.2) (C*ipso*), 169.7, 170.0, 170.3, 170.6 (3 x $OCOCH_3$/2 x $NCOCH_3$).

LRMS (ESI) *m/z* 523 $[M+H]^+$ (50%), 492 (29), 491 (100), 449 (18), 432 (30), 373 (23).

HRMS (ESI) calcd for $C_{25}H_{35}N_2O_{10}$ $[M+H]^+$ 523.22917. Found *m/z* 523.22907.

^1H NMR assignments were confirmed using ^1H-^1H COSY NMR spectroscopy.

Attempted oxidation of methyl 3,5-diacetamido-6,7,8-tri-*O*-acetyl-2,3,5-trideoxy-1-phenyl-D-*glycero*-D-*allo/altro*-octofuranoside (199)

To a solution of **199** (45 mg, 0.086 mmol) in $CCl_4/CH_3CN/H_2O$ (1:1:1, 3 ml) was added ruthenium trichloride hydrate (0.5 mg, 2.2 mmol) and $NaIO_4$ (75 mg, 0.35 mmol). The resulting mixture was stirred for 48 hours at rt. $CHCl_3$ (10 ml) was added, and the mixture stirred for a further 5 minutes. The organic layer was separated, dried (Na_2SO_4) and concentrated. Chromatography (EtOAc/MeOH, 10:1, R_f 0.31) gave 3,5-diacetamido-6,7,8-tri-*O*-acetyl-2,3,5-trideoxy-1-*C*-phenyl-D-*glycero*-D-*allo/altro*-oct-1-ulose **(201)** (31 mg, 71%) as a syrup.

^1H NMR ($CDCl_3$) δ 1.95, 2.00, 2.05, 2.09 (4 x s, 15H, 2 x $NCOCH_3$, 3 x $OCOCH_3$), 3.45 (app. d, 2H, H-2), 3.75 (m, 1H, H-4), 4.09-4.43 (m, 4H, H-3, H-5, H-8, H-8'), 5.04 (s, br, 1H, OH), 5.29 (dd, 1H, $J_{6,5}$ 3.7, $J_{6,7}$ 5.7, H-6), 5.41 (m, 1H, H-7), 6.60 (d, 1H, $J_{HN\text{-}3,3}$ 7.8, HN-3), 6.76 (d, 1H, $J_{HN\text{-}5,5}$ 9.2, HN-5), 7.30-7.99 (m, 5H, Ph).

^1H NMR assignments were confirmed using ^1H-^1H COSY NMR spectroscopy.

Acetylation of **201** (Ac_2O/pyridine) gave **196**, identical in all respects to the material reported previously.

Attempted oxidation of 3,5-diacetamido-4,6,7,8-tetra-*O*-acetyl-2,3,5-trideoxy-1-*C*-phenyl-D-*glycero*-D-*allo/altro*-oct-1-ulose (196)

To a solution of **196** (55 mg, 0.10 mmol) in $CCl_4/CH_3CN/H_2O$ (1:1:1, 3 ml) was added ruthenium trichloride hydrate (0.6 mg, 2.6 mmol) and $NaIO_4$ (86 mg, 0.40 mmol). The resulting mixture was stirred for 3 days at rt. ^1H NMR spectroscopy of the material obtained after workup was identical in all respects to the starting material. Heating the reaction mixture under reflux for 2 hours led to decomposition of the starting material into a complex mixture of products which were not identified.

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