3-Azido-tetrahydrofuran carboxylates as scaffolds for oligomers o β-amino-THF carboxylic acids

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Abstract - 3-Azido-tetrahydrofuran carboxylates as scaffolds for oligomers of β-amino-THF carboxylic acids

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This thesis describes investigations into the synthesis and secondary structural properties of novel carbohydrate-derived peptidomimetics.

The syntheses from diacetone-**D**-glucose of five diastereomeric 3-azido-tetrahydrofuran carboxylates are described. These highly functionalised β-amino acid equivalents are accessed *via* the diacetone-3-azido-3-deoxy sugars and 3-azido-3-deoxy-1,4-lactones. An acid-catalysed rearrangement results in the formation of the tetrahydrofuran ring with the protected amine functionality already in place in a position β- to the carboxylate group.

Attempts to synthesise "carbopeptoids" *via* various oligomerisation strategies, including three novel approaches to oligomerisation, are delineated. The first approach attempted involves an iterative addition of a bicyclic lactone, which acts as the activated acid component, to the substrate containing an amine functionality. A new solid-phase oligomerisation strategy, whereby the coupling reagent is tethered to the polystyrene support and the substrates remain in solution, is described. The third attempted novel oligomerisation strategy involves activation by microwave irradiation, in the absence of coupling reagents. The conventional coupling using a carbodiimide coupling reagent proves to be a highly efficient means to access oligomers up to six residues in length, and is utilised for the synthesis of the homooligomers of four diastereomeric carbohydrate-derived β-amino acids.

The prospect that the carbopeptoids may emulate the helical conformations reported for closely related 2-aminocyclopentanecarboxylic acid oligomers is investigated. Different methods of spectroscopic analysis of the tetrameric and hexameric oligomers are discussed. Circular dichroism spectroscopy provides a rapid diagnostic tool for the investigation of peptide bond orientations and forms the basis for a postulation on a possible stabilised hydrogen-bonding secondary structural conformation in two tetrameric species. Several nuclear magnetic resonance spectroscopic experiments are performed, enabling the assignment of each signal in the proton spectrum for a hexameric oligomer. However the resulting data does not provide sufficient evidence for a well-defined secondary structural motif, and so the potential conformational preference postulated on the basis of circular dichroism spectroscopy is not confirmed.

"A sense of reason,
A sense of aim,
Created this,
Created pain."

unbelievable truth

Acknowledgements

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Firstly, Prof. George W. J. Fleet. No praise is too high to describe his enthusiasm and guidance for the duration of my project.

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The final mention is for my favourite girlfriend Helen, whose unconditional love and support over the last couple of years I couldn't have managed without.

Contents

Abbreviations

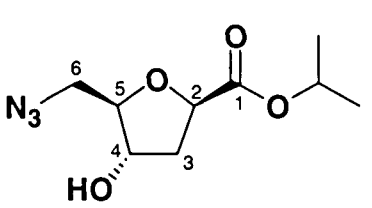
The following abbreviations are used in this thesis:

a-	apparent
Ac	acetyl
Ala	alanine
APCI	atmospheric pressure chemical ionisation
aq	aqueous
Arg	arginine
Asp	aspartic acid
9-BBN	9-bora-bicyclo[3.3.1]nonane
Bn	benzyl
Boc	^{t}butoxycarbonyl
br	broad
c	concentration
CD	circular dichroism
CI	chemical ionisation
COSY	correlation spectroscopy
CSA	camphorsulphonic acid
d	doublet
DCC	1,3-dicyclohexylcarbodiimide
DCM	dichloromethane
dd	double doublet
ddd	double double doublet
DEPT	distortionless enhancement by polarisation
DIC	1,3-diisopropylcarbodiimide
DIPEA	*N,N*-diisopropylethylamine
DMAP	4-(*N,N*-dimethylamino)-pyridine
DMF	*N,N*-dimethylformamide
2,2-DMP	2,2-dimethoxypropane
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
DQF	double quantum filtered
dt	double triplet
EDCI	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
Et	ethyl
Gly	glycine
HATU	*O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate
HIV	human immunodeficiency virus
HOBt	1-hydroxybenzotriazole
HMBC	heteronuclear multiple bond correlation
HPLC	high precision liquid chromatography
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum correlation
Imz	imidazylate
^{i}Pr	isopropyl

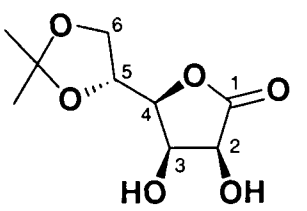
IR	infrared
LCMS	liquid chromatography / mass spectrometry
Leu	leucine
lit.	literature
Lys	lysine
m	multiplet
Me	methyl
m.p.	melting point
NMM	*N*-methylmorpholine
NMP	1-methylpyrrolidin-2-one
NMR	nuclear magnetic resonance
n.O.e.	nuclear Overhauser effect
PCC	pyridinium chlorochromate
PDC	pyridinium dichromate
Pd/C	palladium on activated carbon
Ph	phenyl
Phe	phenylalanine
ppm	parts *per* million
Pro	proline
Pt/C	platinum on activated carbon
q	quartet
R	alkyl
R_f	retention factor
ROESY	rotating frame n.O.e. spectroscopy
r.t.	room temperature
s	singlet
sept	septet
t	triplet
ΔT	heat
TBDMS	*tert*-butyldimethylsilyl
TBTU	*O*-(benzotriazol-1-yl) *N,N,N',N'*-tetramethyl uronium tetrafluoroborate
^{t}Bu	*tert*-butyl
TDE-SO_2	2,2,2-trifluoro-1,1-diphenylethanesulphonate
Tf	triflic
TFA	trifluoroacetic acid
TFE	2,2,2-trifluoroethanol
THF	tetrahydrofuran
Thr	threonine
t.l.c.	thin layer chromatography
TOCSY	total correlation spectroscopy
tosylate	*p*-toluenesulphonate
triflate	trifluoromethanesulfonate
triflation	trifluoromethanesulphonation
triflic	trifluoromethanesulphonic
Trp	tryptophan
Tr-ROESY	transverse rotating frame n.O.e. spectroscopy
Ts	tosyl
Tyr	tyrosine
UV	ultraviolet
Val	valine

A note concerning nomenclature

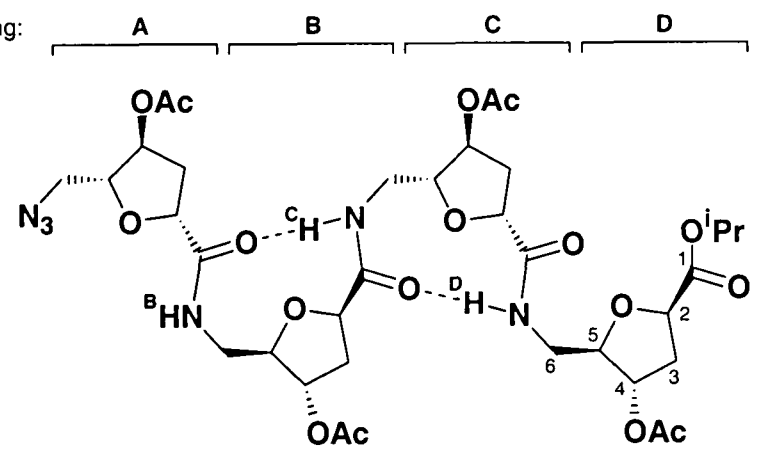
The spectroscopic data of compounds is assigned based on a numbering scheme derived from the systematic naming of materials according to IUPAC recommendations on carboydrate nomenclature[Φ]. Examples are given below:



Isopropyl 2,5-anhydro-6-azido-
3,6-dideoxy-D-*ribo*-hexonate



5,6-*O*-Isopropylidene-D-mannono-
1,4-lactone



Isopropyl 4-*O*-acetoxy-6-amino-2,5-anhydro-3,6-dideoxy-6-*N*-
(4-*O*-acetoxy-6-amino-2,5-anhydro-3,6-dideoxy-6-*N*-
(4-*O*-acetoxy-6-amino-2,5-anhydro-3,6-dideoxy-6-*N*-
(4-*O*-acetoxy-6-amino-2,5-anhydro-6-azido-3,6-dideoxy-D-*ribo*-hexonyl)-
D-*ribo*-hexonyl)-D-*ribo*-hexonyl)-D-*ribo*-hexonate

Φ A.D. McNaught, *Pure Appl. Chem.*, **1996**, *68*, 1919.

Chapter 1: Introduction

1.1 Peptidomimetics

The dramatic developments that have succeeded the sequencing of the human genome are centred on our understanding of how and why proteins fold in the way that they do.[1] The interpretation of the sequence data will reveal the structure and function of many unknown proteins and unascertained protein interactions, generating myriad new medicinal targets. In fact, the bias of biotechnological research has moved toward the human 'proteome,' the full gamut of proteins made by human body cells and tissues.[2]

Although it is recognised to be an oversimplified description of enzyme-receptor interaction, the 'lock and key' idea represents the importance of the conformation of a peptide or protein in determining its biological activity.[3] Proteins fold into well-defined structures in aqueous solution and peptides adopt unique structures while binding to the appropriate receptors.[4] The interface with the active site relies on the distinct three-dimensional structure of the peptide or protein, which is essentially independent of the specific linear sequence of amino acids but is contingent on the hydrophilic or -phobic nature of the residues[5] and solvation effects, as well as the backbone conformational preferences.[6] Many diseases are caused by the misfolding of proteins, but for the majority of proteins only the amino acid sequences are known and not their three-dimensional structures.[7]

Although there is great interest in the elucidation of the underlying principles, our ultimate aim is sufficient understanding of protein structure that we can design sequences of natural and synthetic amino acids that fold according to prediction.[7] Control over oligomer and polymer folding would present the possibility of introducing tailor-made functions into existing or purpose-built proteins, to mimic the conformation and thence the biological functions of natural proteins, such as enzyme inhibition and receptor activation.[3]

Current knowledge stems largely from analysis by crystallography or nuclear magnetic resonance (NMR) spectroscopy of naturally occurring proteins,[7] in which the adoption of complex tertiary folds and quaternary structure depends on the formation of a limited number of recognised secondary structural elements,[8] i.e. the α-helix, the β-sheet, turns and loops[9] (see **Chapter 1.2**).

Despite all the information that has been obtained from the study of natural proteins, their complexities mean that an understanding on a basic level of the principles that govern structure and function has not yet been achieved. Every amino acid residue may have several functions governing the folding and interactions of proteins, and, taking into consideration the possibility for amino acid variation resulting from DNA mutation, some of these amino acid functions are no longer obvious.[7]

Hence there is a necessity for the design and synthesis of new peptides which can be analysed in fundamental detail, in order to formulate and understand their structural elements,[7] as well as the rational design of unnatural peptides with a predetermined structure.[10] A combination of these approaches will permit chemists to develop an understanding of the relationship between the repetitive monomer properties and the conformational structure at the polymer level.[11]

1.2 Secondary structure in α-peptides

There are two principal periodic structures observed in peptides consisting of α-amino acids, the α-helix and the β-sheet.[12] In many circumstances, the β-sheet is comprised of β-strands connected by β-turns.

1.2.1 The α-helix

The 'ideal' α-helix is a right-handed helix in which 13-membered hydrogen bonded rings are formed in the 'backward' direction (C-terminus to N-terminus) by interaction of the NH of residue *i* with CO of residue *i - 4*, with 3.6 residues per turn,[13] **Figure 1.1**.

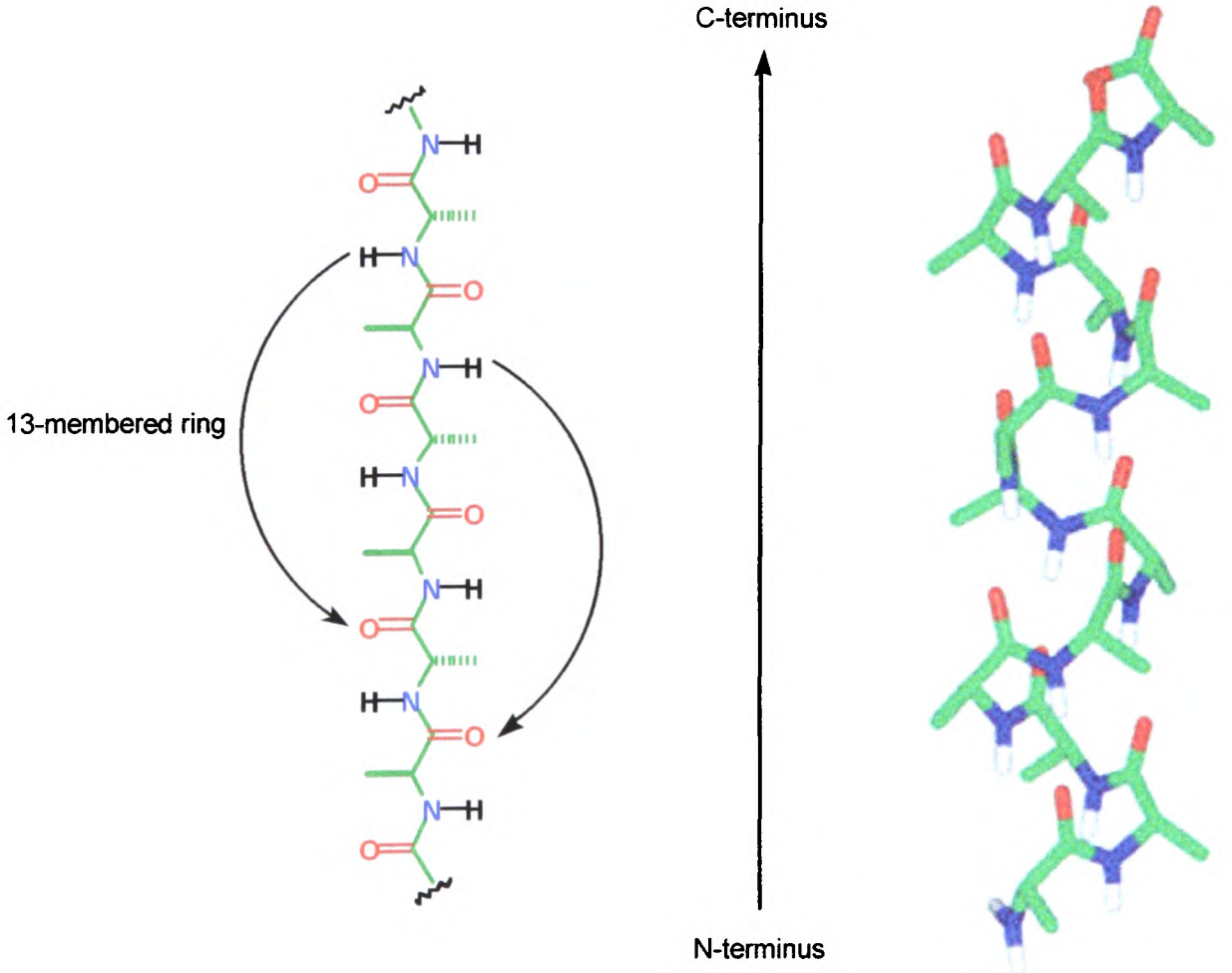


Figure 1.1. The structure of the α-helix. The molecule used to represent the structure is polyalanine. The 3-D representation is reproduced from ref.[14] The non-amide hydrogen atoms have been omitted for clarity.

In reality, however, there is widespread variation from the ideal and hence many actual structures are observed,[14] ranging from the tightly coiled 3_{10}-helix (containing 3 residues per turn and stabilised by 10-membered hydrogen bonded rings) toward the wider π-helix.[13]

1.2.2 The β-sheet: β-strands and turns

β-Strands are peptide chains in which the backbone is fully extended, with no long-range interactions or folding. The β-pleated sheet is formed by the hydrogen bonding between different β-strands, with the side chains above and below the plane of the sheet,[15] **Figure 1.2**. Where the different strands are orientated in the same direction with respect to their C/N termini a parallel β-sheet is formed, and strands which run in the opposite direction to each other create antiparallel β-sheets. Structural regions commonly comprise two to five parallel or antiparallel β-strands.

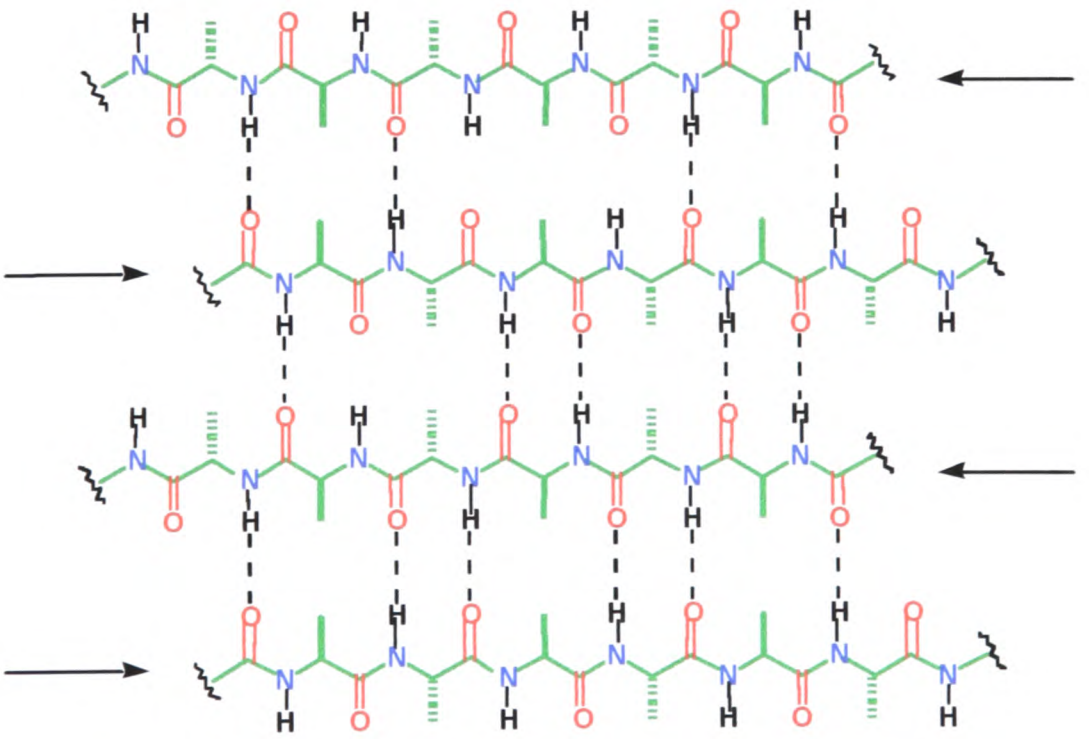


Figure 1.2. The structure of the antiparallel β-sheet. The molecule used to represent the structure is polyalanine.

The β-turn (or β-hairpin) enables the peptide chain to reverse direction in a compact space, **Figure 1.3**. The amide hydrogen of residue *i* is hydrogen bonded to the carbonyl group *i* - *3*, thus forming a 10-membered hydrogen bonded ring.

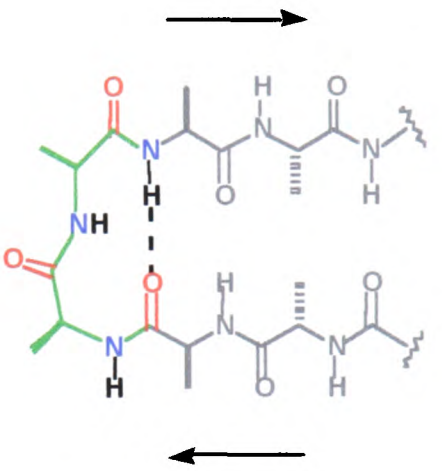


Figure 1.3. The structure of the β-turn. The molecule used to represent the structure is polyalanine.

A more compact change in direction is offered by the γ-turn,[16] which consists of one residue as compared with the two residues of the β-turn, **Figure 1.4**. However, the γ-turn is not seen as commonly as the β-turn due to its constricted nature.[13]

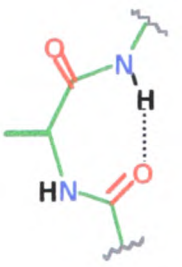


Figure 1.4. The structure of the γ-turn. The molecule used to represent the structure is polyalanine.

1.3 β-Peptides and β-peptide foldamers

Although they play an important role in many biological processes, the use of α-peptides as drugs is limited by several disadvantages. Their susceptibility to hydrolysis by proteases is one factor,[17] while bioavailability is limited by poor oral solubility and transport *in vivo*. Another problem is the high flexibility of linear α-peptide sequences that leads to low population of the desired bioactive conformation, which in turn causes a decrease in the activity and selectivity of the peptides.[10]

The desire to find alternatives to α-amino acids which mimic natural proteins by invoking compact, well-ordered conformations in their polymers has driven the search into the realms of β- and γ-amino acids and their homo- and heterooligomers. Modification of the backbone structure has the potential to restrict rotation and in doing so decrease the number of possible conformers, while side chain derivatisation may induce improved bioavailability. There has been extensive study into β-peptides, the oligomers of β-amino acids, particularly inspired by the work of Samuel Gellman[11,14,18-30] and Dieter Seebach.[31-47] There has been more limited research into γ-amino acid oligomers by both Hanessian[48,49] and Seebach.[50,51]

1.3.1 β-Peptides

It was postulated in 1994 that the desired folding behaviour would be most likely if hydrogen bonds between an amide group and its closest neighbour along the peptide backbone were unfavourable.[18] Previous work had established that this criterion applies to α-peptides,[52-56] and IR and NMR studies showed that nearest-neighbour hydrogen bonding is also unfavourable for derivatives of β-alanine, whereas the γ-amino butyric acid template is conducive to nearest-neighbour hydrogen bonding.[18] This suggested that β-amino acid polymers would be more

promising as 'foldamers,'[11] and the majority of the work in this field has since been directed towards β-peptides.

It has been discovered that the hydrolytic stability of peptides can be increased by the substitution of proteinogenic amino acids by β-amino acids.[31,34,41] For example, the first of Seebach's hexapeptides, which will be discussed later (see **Chapter 1.3.3**) was found to be stable against the peptidase pepsin.[31,34]

Somewhat more surprising, considering the extra rotational possibility of the central C^{α}-C^{β} bond, is that β-peptides are also capable of the formation of secondary structural characteristics analogous to those found in natural peptides and proteins.[11] The higher flexibility afforded by the additional freely-rotating C^{α}-C^{β} bond (see **Figure 1.5, p8**) may be offset by the fact that the conformational flexibility may be restricted by the extra side chain at C^{β} as compared to the α-peptide counterpart. In fact, β-peptides have been found to form much more stable secondary structures than their α-peptidic counterparts.[19,31,32] It has since been shown that γ-amino acids can also stabilise well-defined secondary structures in their oligopeptides.[48-51]

The incorporation of an additional carbon atom between the carboxy and amino groups means that β-amino acids are intrinsically subject to a far greater constitutional and conformational variability than α-amino acids, and this results in a larger number of potential stable secondary structures in β-peptides than are possible in α-peptides.[36] The numerous structural data obtained for β-peptides that have been obtained, along with evidence that β-amino acids can be incorporated into sequences of biologically active peptides without impairing the binding properties,[57,58] illustrate the high potential of β-amino acids for protein and peptide mimicry design.

1.3.2 Secondary structure in β-peptides

Although there is a far greater number of possible conformations arising from the additional degree of torsional freedom inherent in β-amino acids, the picture is simplified by the fact that the θ torsion angle (convention of Balaram) is restricted to either ±60° (*gauche* conformation) or 180° (*trans* conformation)[14] as shown in **Figure 1.5**.

To allow helical or turn structures to take place, β-peptides require a *gauche* conformation about the θ torsion angle defined by the C^{α}-C^{β} bond. A *trans* conformation causes an extended peptide without folding, in which sheet structures are prevalent.[4]

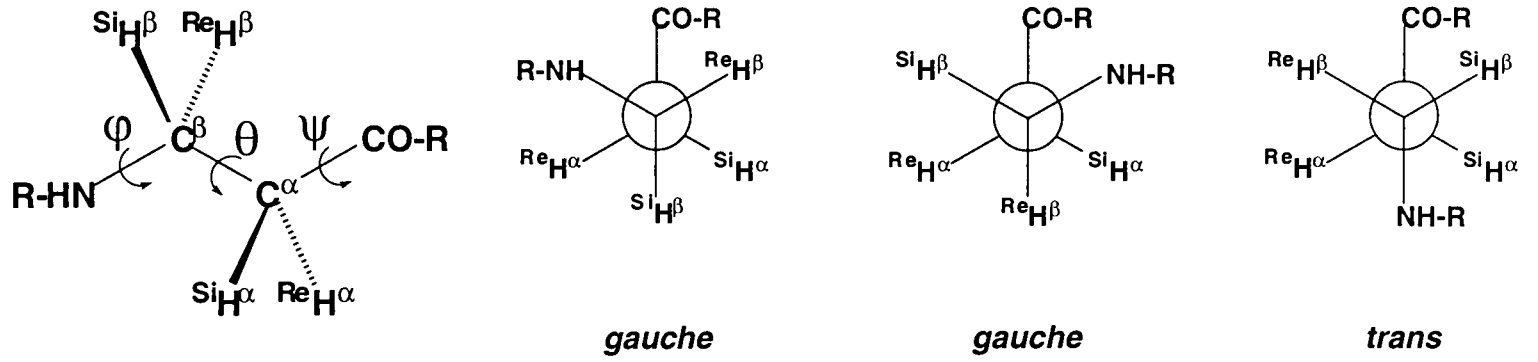


Figure 1.5

An unsubstituted residue such as β-alanine is conformationally flexible in solution and may adopt *gauche* or *trans* conformations,[59] though it has been shown to stabilise γ-,[60] β-[61] and α-turns[62,63] when inserted into cyclic tri-, tetra- and pentapeptides respectively. There have been many experimental studies,[21,23,37,39,64] as well as research based upon molecular mechanics and dynamics[19,20,29,33,38,45,46,65-67] and molecular orbital calculations[17,68,69] to determine the effects of substituents on the conformations of β-peptides.

The presence of any alkyl group at C^{α} or C^{β} will favour a *gauche* conformation about the C^{α}-C^{β} bond, and this conformation is even more favourable in a α,β-disubstituted amino acid where the substituents are both *Re* or both *Si* (*anti* according to the aldol convention).[39] When the acid and amine are *trans* substituents in a carbocyclic ring they are further constrained and the *gauche* conformation is even more strongly promoted.[19,20,22,24,25,27,30]

Conversely, the presence of *syn* substituents (one *Re* and one *Si*) tends towards a *trans* conformation about the C^{α}-C^{β} bond and sheet-like structures are favoured.[21,43]

Disubstituted α-amino acids such as α-aminoisobutyric acid tend to induce helical conformations in α-peptides.[35,70] However, α,α- or β,β-disubstituted β-peptides tend only to form reverse turns.[40,44]

Early research into the structures of β-peptides was centred on those derived from aspartic acid, the only proteinogenic β-amino acid. Interpretation of the data for poly(α-isobutyl-L-aspartate) proved not to be straightforward, and both helical structures and sheet conformations were proposed. [71-75]

1.3.3 β-Peptide foldamers: 14-Helical structures

The greater conformational variability of β-peptides means that instead of the one helix that is commonly seen amongst α-peptides, there is the possibility of formation of one of several different helices. The first of these to be observed was based upon 14-membered hydrogen bonded rings, **Figure 1.6, p10**. The interaction is orientated in the 'forward' (N- to C-terminus) direction relative to the helix, the amide proton of residue *i* forming a hydrogen bond with the carbonyl of residue *i* + *2*, with approximately 3 residues per turn.

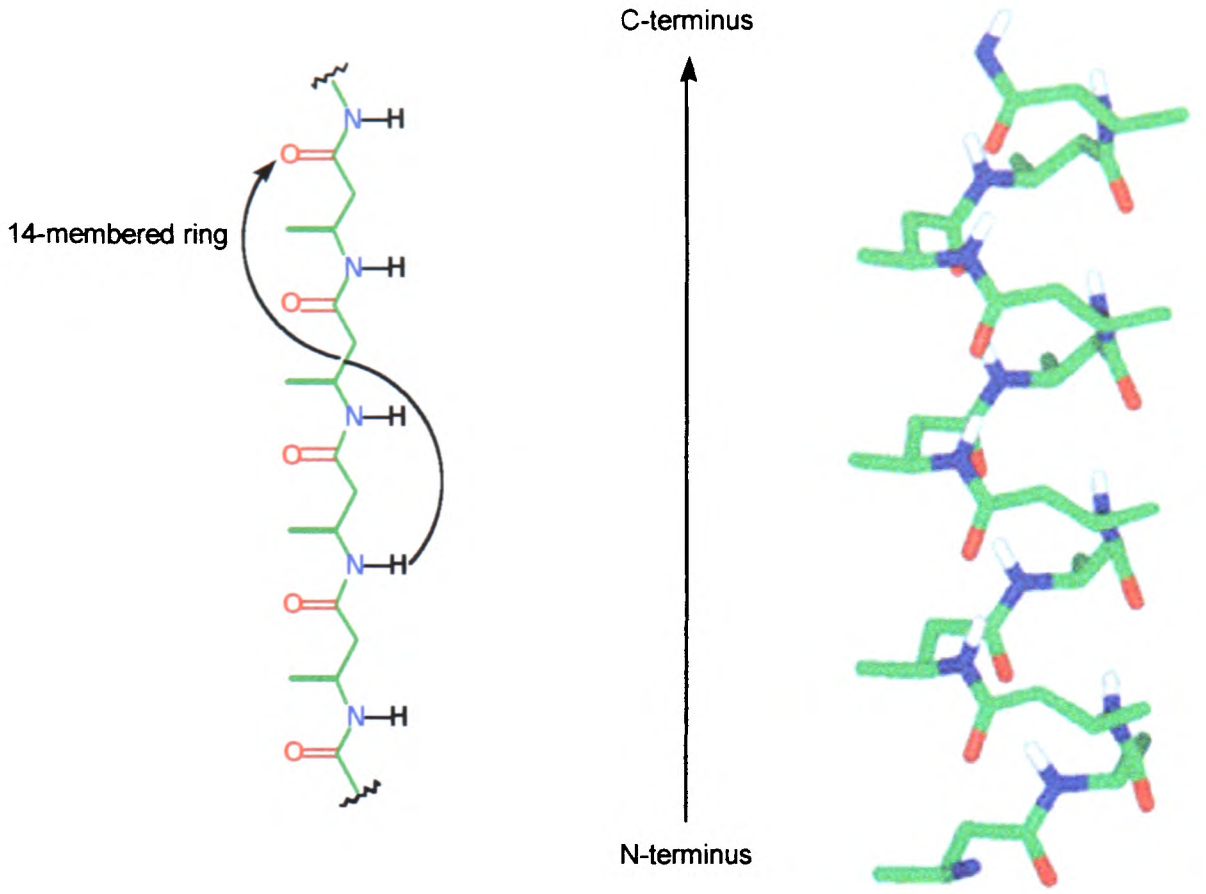
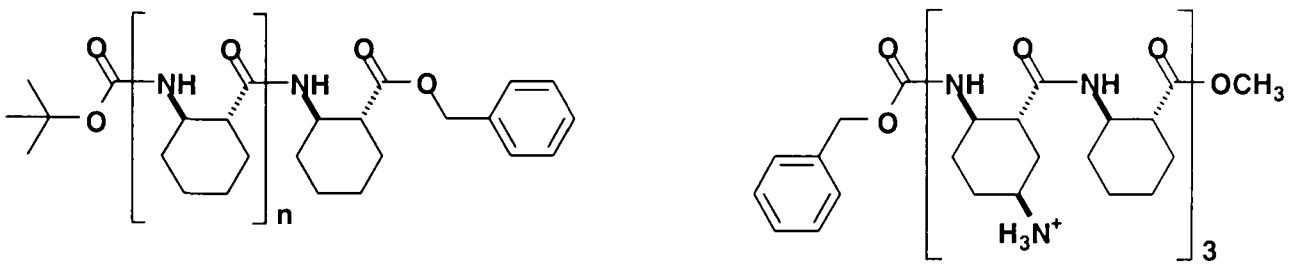


Figure 1.6. The structure of the 14-helix. The molecule used to represent the structure is poly-β-homoalanine. The 3-D representation is reproduced from ref.[14] The non-amide hydrogen atoms have been omitted for clarity.

As mentioned previously, the constraint of a small carbocycle provides substantial rigidity, and a *trans* relationship, which would impose a *gauche* conformation, could result in the formation of helical structures. On the basis of extensive computational studies, incorporating different sizes of both *trans*- and *cis*-substituted ring into six types of helix, Gellman predicted in 1996 that the most likely compounds to display conformational stability, specifically in the framework of a 14-helix, were the oligomers **1** of *trans*-2-aminocyclohexanecarboxylic acid,[19] **Figure 1.7**, **p11**. The hexamer (n = 5) was indeed found to adopt a 14-helical conformation in organic solution and the solid state, as evidenced by NMR and x-ray crystallography respectively.[19,25]

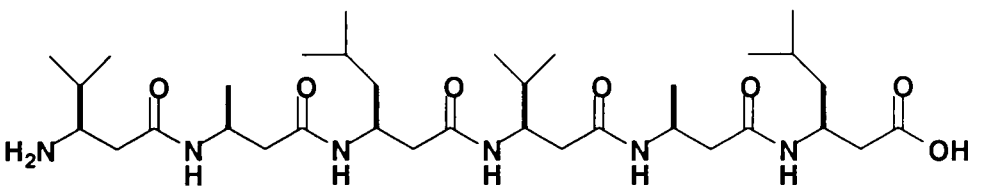


1; n = 0,1,2,3,5 **2**

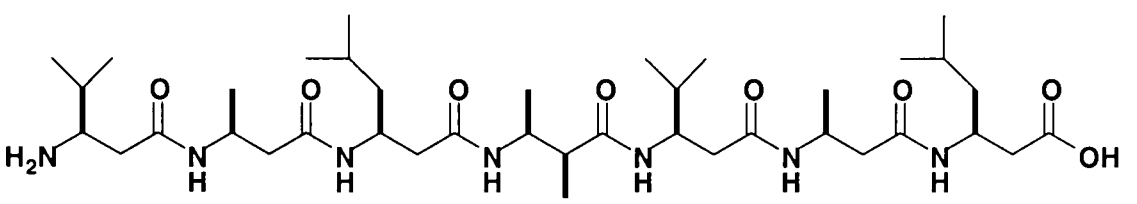
Figure 1.7

Biological interest largely depends on solubility properties. In order to investigate the stability of the 14-helix in aqueous media, a new molecule **2** was synthesised which was designed to have a charge of +3 at pH7 or less.[24] The 14-helical structure was found to be maintained in water. The importance of the conformational constraint of the carbocycles to the stabilisation of the helical structure in water was demonstrated by replacement of cyclohexane-based residues by acyclic equivalents, when circular dichroism (CD) measurements (see **Chapter 4.2**) showed a reversion to a random coil.[24]

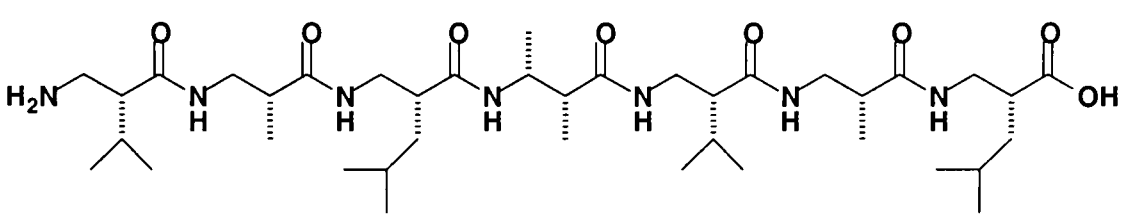
In the same year, Seebach found that linear open-chain β-peptides can also form the 14-helix in solution.[31,32,39] As discussed previously (see **Chapter 1.3.2**) the necessary *gauche* conformation is promoted by the presence of any alkyl group at C^{α} or C^{β}, and even more so in an α,β-*anti*-disubstituted amino acid. Examples of β-substituted **3-4**,[31,32] α-substituted **5**[39] and α,β-*anti*-disubstituted **6**[39] β-peptides, **Figure 1.8, p12**, were shown by NMR and CD studies to adopt a regular 14-helix in pyridine and methanol.



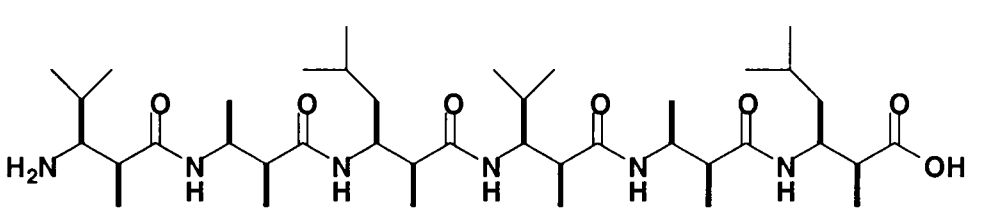
3; TFA salt



4; TFA salt



5; TFA salt



6; TFA salt

Figure 1.8

The β-substituted oligomers were extended to twenty-four residues using a solid phase coupling scheme, but the conformational structure has not been confirmed.[47]

1.3.4 β-Peptide foldamers: 12-Helical structures

Having established the existence of the 14-helix in β-amino acid oligomers containing a cyclohexane ring, Gellman carried out molecular dynamics calculations for the cyclopentane equivalent **7**, **Figure 1.10**, **p13**, and found inherent preferences for different conformations.[29] The cyclohexane ring promotes a torsional angle θ of around ±55°, which specifically stabilises the 14-helix, whereas the smaller cyclopentane ring constrains θ to values approaching 90°. This constraint causes the most favourable conformation to be a 12-helix, a different helical form

containing 12-membered hydrogen bonded rings, the amide proton of residue *i* interacting with the carbonyl oxygen of residue *i* - *3*, **Figure 1.9**. This is orientated in the same 'backward' direction as the α-helix, and turns approximately every 2.5 residues.

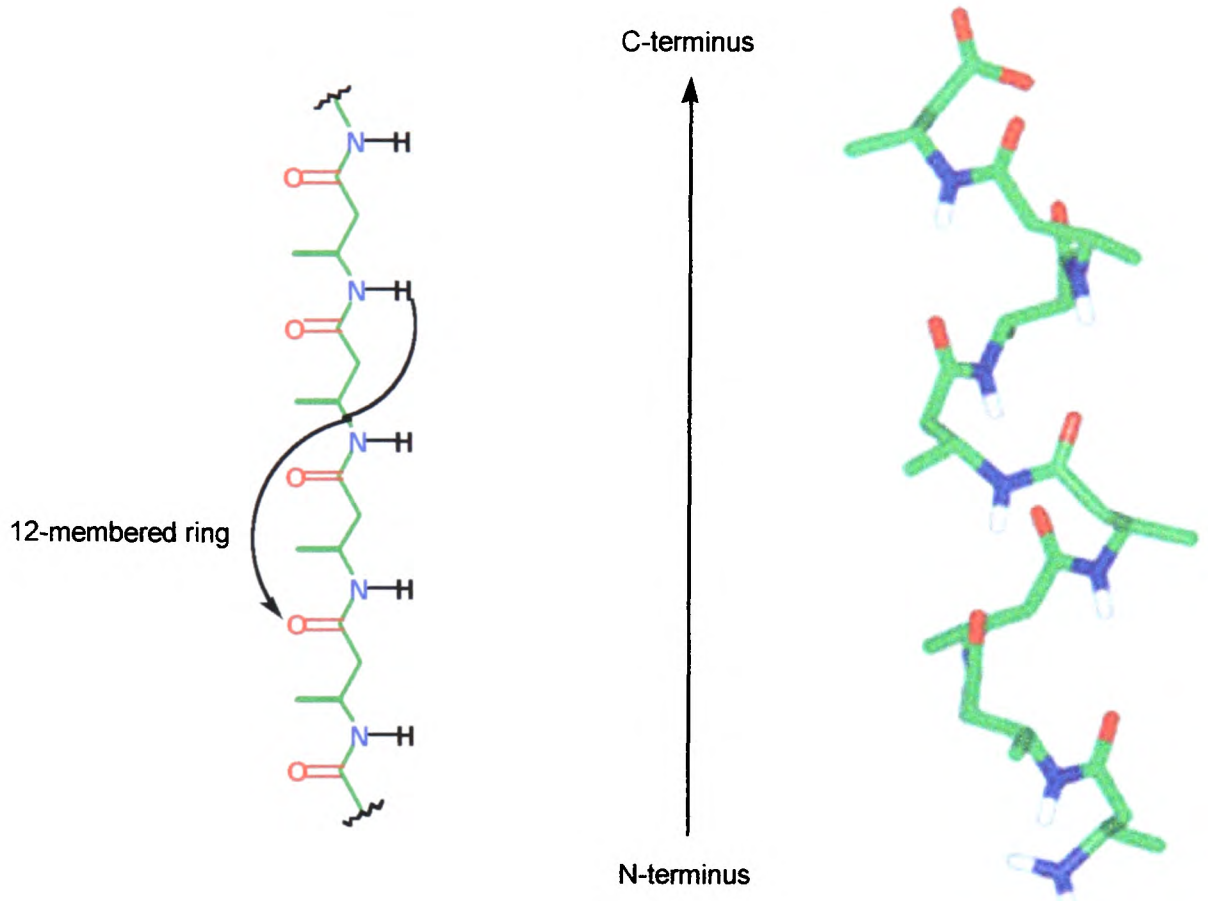
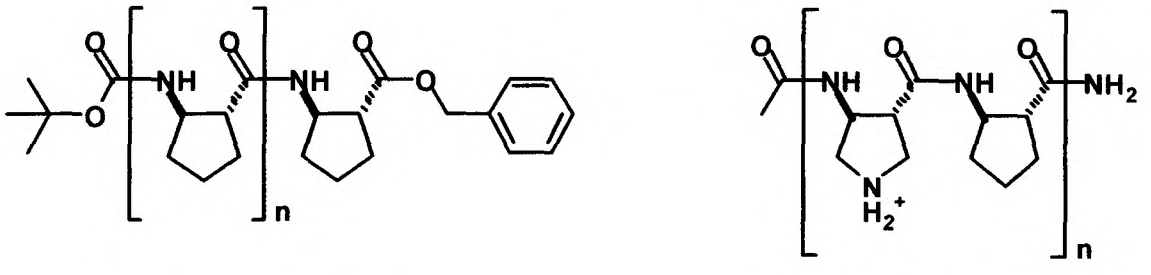


Figure 1.9. The structure of the 12-helix. The molecule used to represent the structure is poly-β-homoalanine. The 3-D representation is reproduced from ref.[14] The non-amide hydrogen atoms have been omitted for clarity.



7; n = 0,1,2,3,5 **8**; n = 2, 3, 4; TFA salt

Figure 1.10

Synthesis of oligomers **7** provided confirmation of the 12-helix, with the hexamer (n = 5) adopting this structure in organic solution as well as in solid state.[20,22,26,28] In order to probe for

12-helical folding in water, the analogous oligomers **8** were prepared containing *trans*-3-aminopyrrolidine-4-carboxylic acid residues.[30] CD and NMR studies indicated that molecules with as few as four residues (n = 2) show substantial population of the 12-helical conformation in water.

1.3.5 β-Peptide foldamers: 10/12-Helical structures

β-Peptides with alternating α- and β-monosubstituted residues, or those with alternating (S)-α / (R)-α or (S)-β / (R)-β substitution patterns, may adopt a 10/12-helical conformation. The structure is an intertwined network of 10- and 12-membered hydrogen bonded rings, the 12-membered rings (NH_i to CO_{i-3}) orientated in the backward direction relative to the helix and the 10-membered rings (NH_{i-1} to CO_i) nearly perpendicular to the helical axis, **Figure 1.11**.

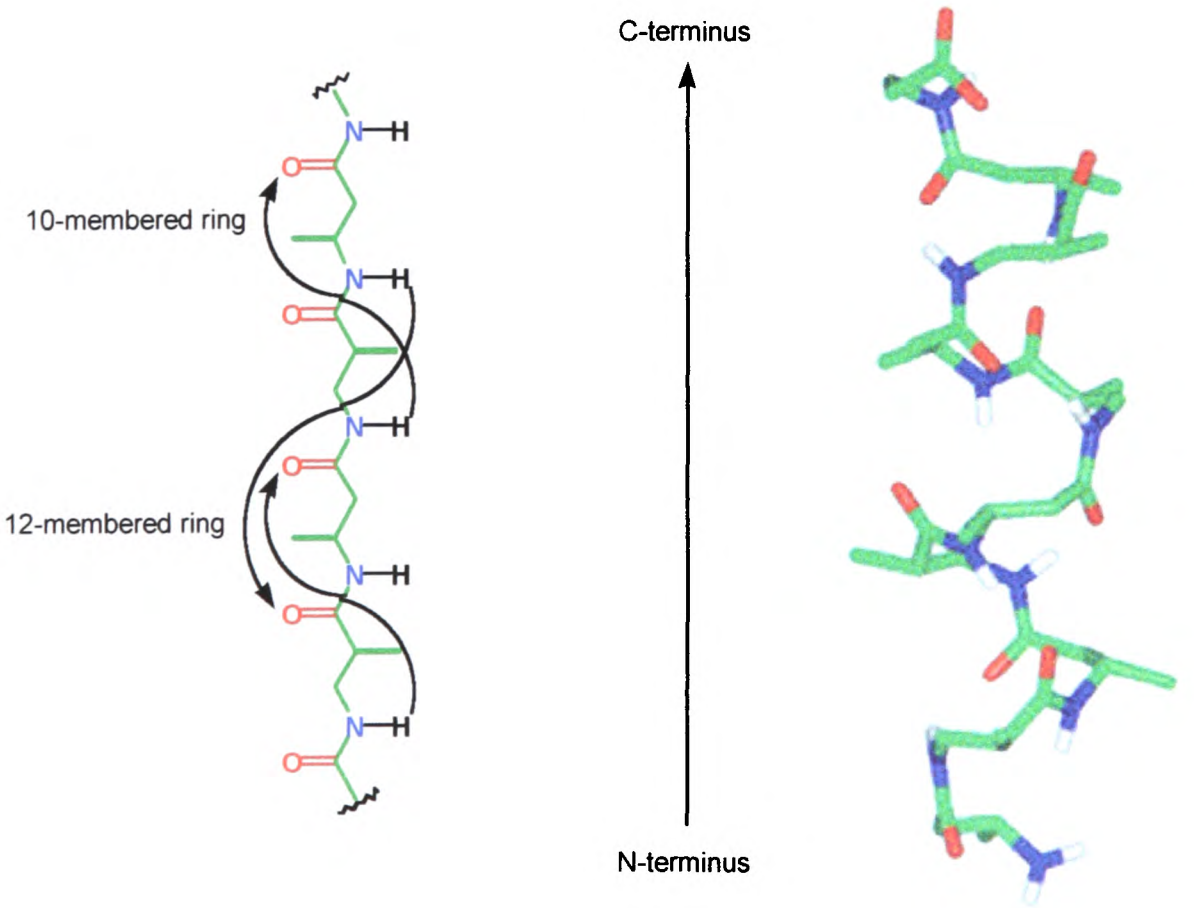
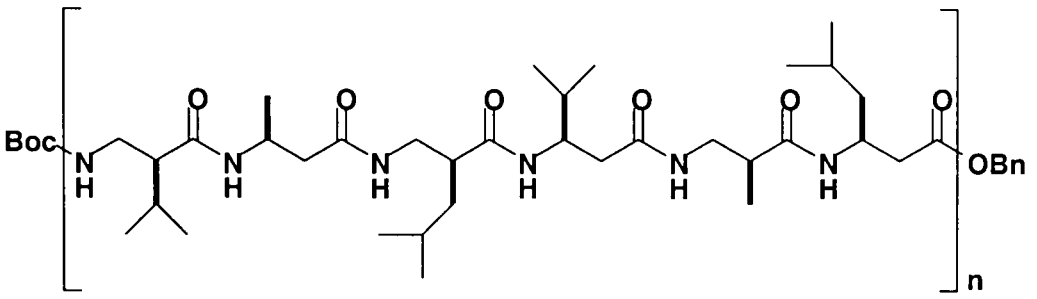


Figure 1.11. The structure of the 10/12-helix. The molecule used to represent the structure is poly-β-homoalanine. The 3-D representation is reproduced from ref.[14] The non-amide hydrogen atoms have been omitted for clarity.

This helix has been shown to be adopted by the repeating (S)-α / (S)-β β-peptide **9**,[37,39] **Figure 1.12**.

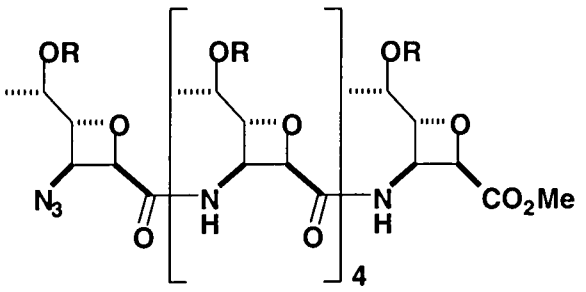


9; n = 1, 2

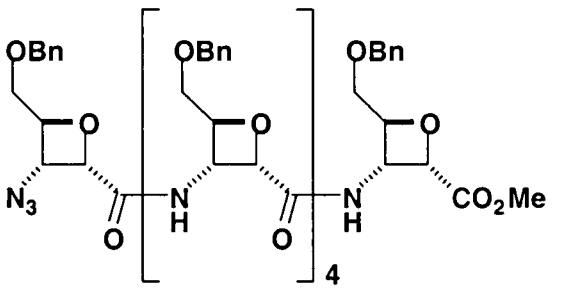
Figure 1.12

1.3.6 β-Peptide foldamers: Other helical structures

Recent work by co-workers involved the synthesis of three hexameric oligomers **10-12** of β-amino acids which are incorporated into an oxetane ring,[76] **Figure 1.13**.



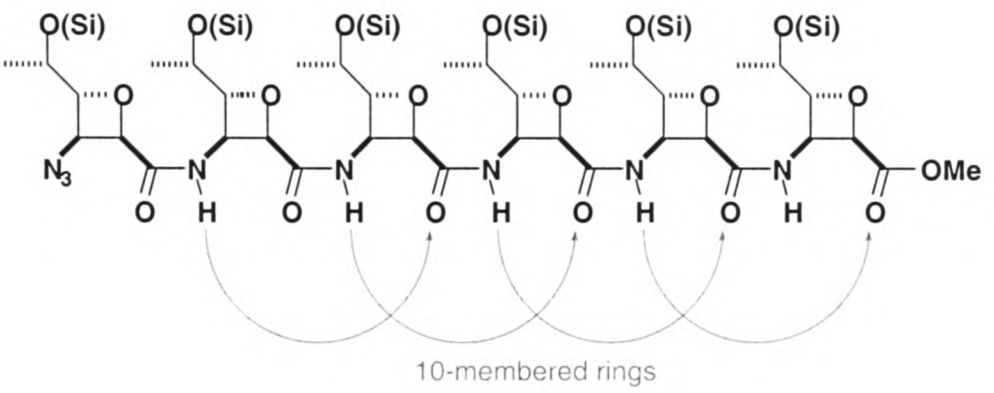
10; R = TBDMS
11; R = Bn



12

Figure 1.13

In contrast to the *trans* β-amino acids in five- and six-membered rings detailed above, there is substantial evidence that helical structures are formed where the amine and carboxylic acid components are *cis*.[77] The proposed structure is helix containing a 10-membered hydrogen bonded ring, the amide proton of residue *i* interacting with the carbonyl group of residue *i* + *1*, **Figure 1.14**.

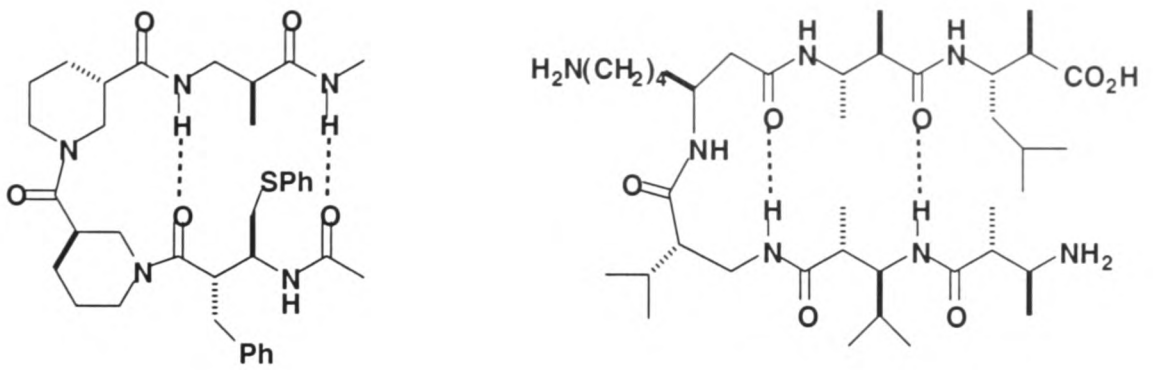


10; (Si) = TBDMS

Figure 1.14

1.3.7 β-Peptide foldamers: Sheets, reverse turns and strands

In principle, β-peptides are able to form two types of sheet structure, one with a *trans* C^{α}-C^{β} torsion angle where all backbone carbonyl groups are orientated in the same direction, and the other with a *gauche* C^{α}-C^{β} torsion angle where the backbone carbonyl groups alternate in direction along each strand, akin to the β-sheet formed by α-peptides.[21] The presence of *syn*-α,β-disubstituted β-amino acids, which favour a C^{α}-C^{β} torsion angle of 180°, leads to the formation of a *trans* sheet such as hexapeptide **14**,[43] **Figure 1.15, p17**. However, oligomers of residues which promote a *gauche* θ torsion angle tend to adopt helical conformations rather than *gauche* sheets. Nevertheless, the potential for formation of more than one sheet structure again highlights the greater conformational diversity of β-peptides relative to their α-peptide counterparts.[14]

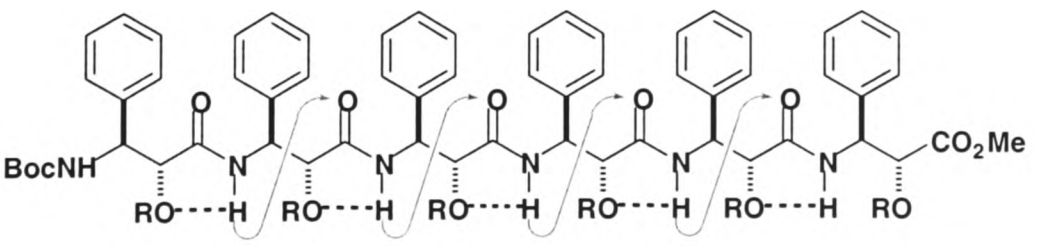


13 **14**

Figure 1.15

This is mirrored by the propensity of β-peptides to form more than one reverse turn, while α-peptides only commonly form the β-turn. The designed β-peptides **13** and **14** illustrate the two types of reverse turn. Where the turn-forming residues consist of a dipeptide composed of two nipecotic acid residues, as in **13**, the ring formed by adoption of the hairpin conformation is 12-membered,[23] whereas in **14** the use of an α-substitued residue followed by a β-substituted residue results in a 10-membered hydrogen bonded ring.[43]

The oligomer of *tert*-butyldimethylsilyl-phenylisoserine **15** adopts a twisted strand conformation in chloroform.[78] Hydrogen bonds are formed not between amides of different strands, but between same-residue amide and neighbouring side-chain functionalities of a single strand, **Figure 1.16**. The β-amino acid residues adopt a *gauche* conformation.



15; R = TBDMS

Figure 1.16

Finally, short oligomers of 1-(aminomethyl)cyclopropanecarboxylic acid display a propensity for the formation of 8-membered hydrogen bonded rings.[42] The repeating structure formed has been described as a 'pleated ribbon'.

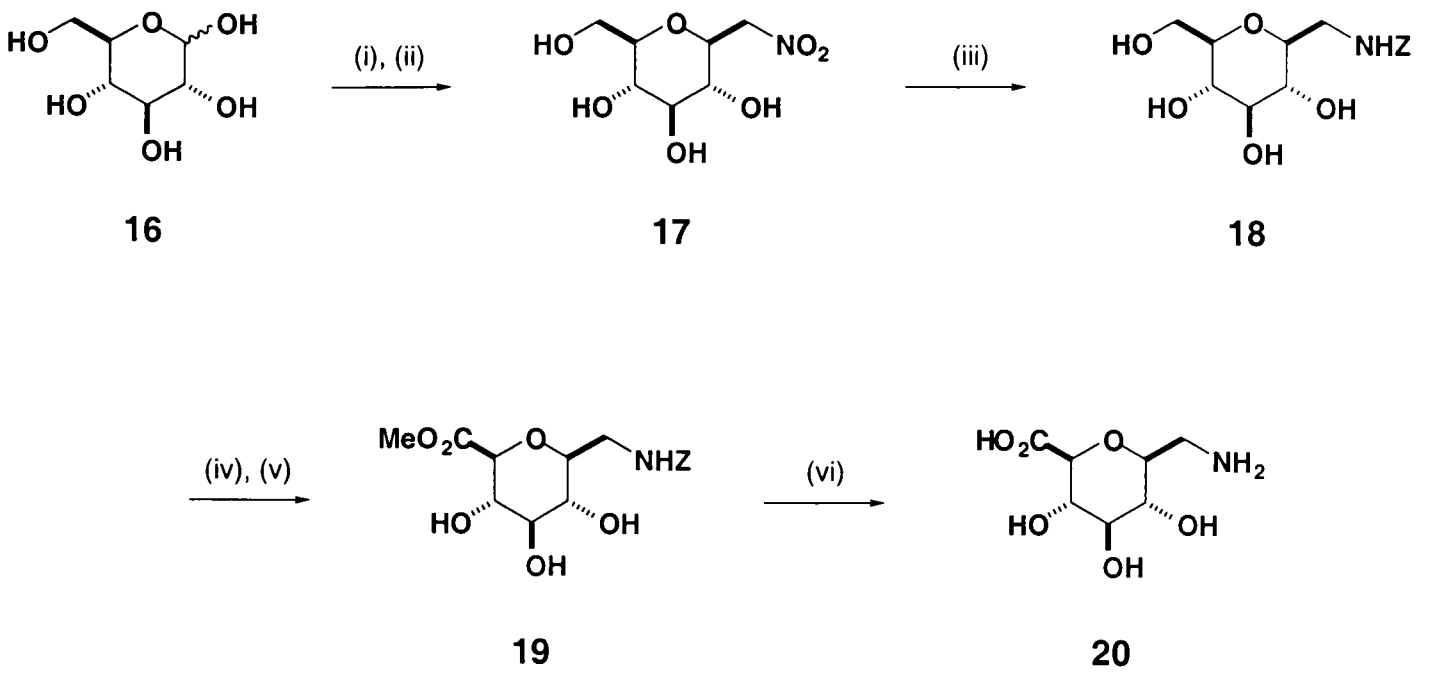
1.4 Glycosamino acids and carbopeptoids

Carbohydrates have many important biological functions, not all of which are fully understood at this time. The functions that have been expounded include energy storage, transport, modulation of protein function, intercellular adhesion, signal transduction, malignant transformation and viral and bacterial cell-surface recognition.[79]

1.4.1 Glycosamino acids

The incorporation of an amino acid moiety into a carbohydrate unit generates a glycosamino acid. These hybrid structures of carbohydrates and amino acids are desirable as building blocks for homo- and heterooligomers, due to their structural diversity and their potential for hydroxyl group derivatisation. They can be used to produce potential glycomimetics and peptidomimetics, and substitution for amino acids within peptides generates the prospect to diversify the functions of the peptides.

Where the carboxylic acid and amine components are incorporated directly into a sugar-derived pyranoid or furanoid ring, the resulting glycosamino acid may be described as a sugar amino acid. The first example in which a sugar amino acid was employed as a peptidomimetic in a peptide chain was as a dipeptide isostere which mimics a β-turn.[80] The δ-amino acid was synthesised in four steps from glucose **16**, **Scheme 1.1**, **p19**.



Reagents and Conditions: (i) CH_3NO_2, NaOMe; (ii) H^+, H_2O, ΔT; (iii) H_2, Pd/C then ZCl (Z = benzyloxycarbonyl); (iv) O_2, Pt/C; (v) DCC, DMAP, MeOH; (vi) NaOH, H_2, Pd/C

Scheme 1.1

Derivative **20** was inserted into cyclic peptide analogues and a natural linear peptide, in order to determine the effect on the conformation of the peptides.[80] For example, in the known somatostatin analogue **21** the cyclising -Pro-Phe- residues were replaced by **20** to form cyclic peptide **22**, **Figure 1.17**, **p20**. The β-turn that is seen in the known compound was reproduced, though the normally planar hexapeptide was twisted due to the interaction of the ring oxygen with the neighbouring carbonyl group. A comparison of the biological activities for the inhibition of growth hormone showed that while somatostatin analogue **21** is highly potent with an IC_{50} of 0.002 μM, synthetic peptide **22** is only 75 times less active.[80] Replacement of the -Gly-Gly- unit in naturally occurring linear leucine enkephalin **23** caused the formation of a β-turn-like structure in the analogue **24**, and also had the effect of eliminating its biological activity.[80]

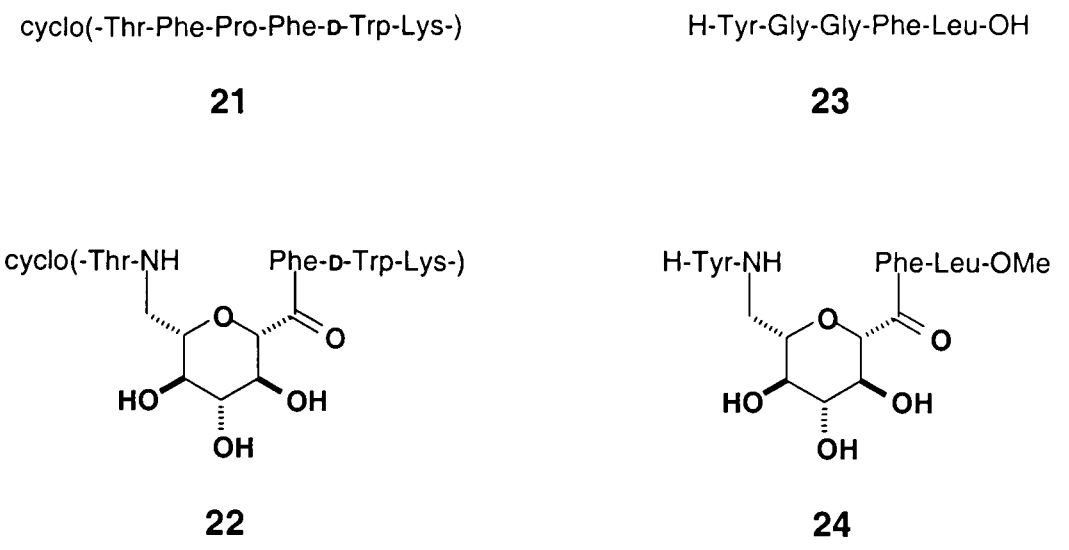
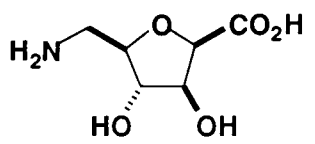


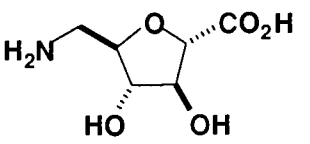
Figure 1.17

δ-Glycosamino acid **20** was attached to the *C*-terminal of leucine enkephalin **23** and methionine enkephalin, the former glycopeptide being a potent and δ-receptor selective opioid agonist.[81] Incorporation of the *O*-benzylated form of **20** as a β-turn mimic to replace the **D**-Phe-Val- fragment of potential antitumour drug cyclo(-Arg-Gly-Asp-**D**-Phe-*N*(Me)Val-) also showed impressive biological capabilities.[82]

Analogous furanoid sugar δ-amino acids **25**, **26** and **27**, **Figure 1.18**, **p21**, have also been integrated into peptide chains as turn mimetics.[83,84] When the 2,5-*cis* stereoisomer **25** was included as a replacement for the -Gly-Gly- dipeptide of leucine enkephalin **23** the resulting peptidomimetic **28** displayed similar analgesic activity to that of the natural peptide,[83] however the insertion of 2,5-*trans* isomer **27** into the same peptide sequence caused reduced activity[84] while the interposition of **26** resulted in no significant activity.[83]



25



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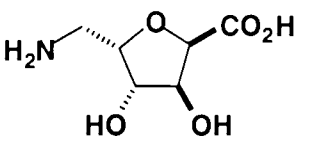
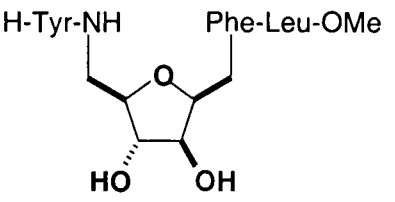


27

H-Tyr-Gly-Gly-Phe-Leu-OH

23



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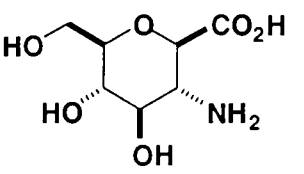


29

Figure 1.18

Finally, the β-sugar amino acid **29** meets the geometric requirements to form a γ-turn-type structure, as proven by the synthesis and CD and NMR analysis of cyclo(-**29**-Ala-**D**-Pro-Ala-Ala-).[85]

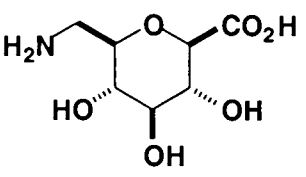
This research showed that glycosamino acids can substitute for constrained α-amino acids without the loss of biological activity, and can mimic the secondary structure seen in natural peptides. Furthermore, peptide side chains may be simulated by the functionalisation of the sugar hydroxyl groups.

1.4.2 Carbopeptoids

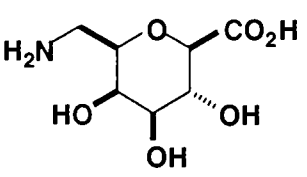
The homooligomers of sugar amino acids were first proposed by Lehmann and Fuchs in 1975[86] when they synthesised the two epimeric monomer units **30** and **31**, **Figure 1.19**, **p22**, later termed 'carbopeptoids' when re-proposed by Nicolaou in 1995,[87] and named 'glycotides' by McDevitt and Lansbury in 1996.[88]

Oligosaccharides, *O*-linked carbohydrate oligomers, are biologically important due to their involvement in cellular recognition, immune response and inflammation.[89] Nicolaou envisioned

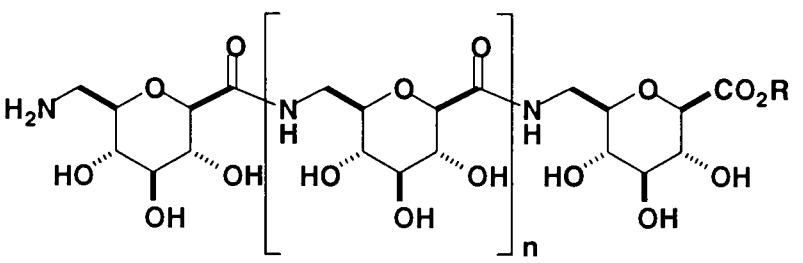
several advantages of glycosamino acid oligomers such as **32** over oligosaccharides, these being the ease of formation, possibility of library generation, choice of solution or solid-phase synthesis and polyfunctionality.[87] Another advantage is the resistance to glycosidases. Amide bonded disaccharides have been known since 1976,[90] though carbopeptoids were not synthesised until later.



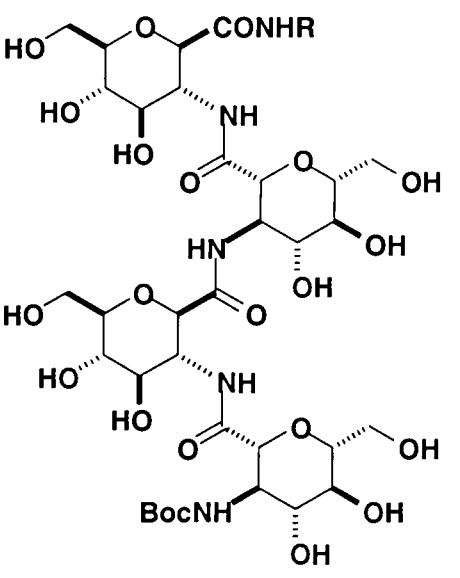
30



31



32



33; R = $CH(CH_2Ph)CO_2CH_3$

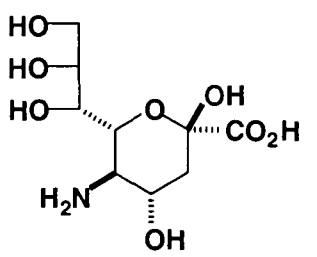
Figure 1.19

The first carbopeptoid synthesis was reported in 1996 by Ichikawa *et al.*,[91] when glucose provided the starting material for formation of the tetrameric β-amino acid oligomer **33**. The *O*-sulphate derivatives of this compound were found to show a strong inhibition against HIV infection to CD4 cell.[91] The *O*-sulphate derivative of **32** was later shown by the same group to have biological activity, in the inhibition of HIV infection of MT2 cells.[92] Additionally, a mixed peptide containing an alternating sequence of glycosamino acid and β-amino acid (aspartic acid) residues demonstrated potent inhibition of lung tumour cell invasion.[93]

Wessel and co-workers used a solid phase approach to synthesise a tetrameric carbopeptoid based upon pyranoid rings.[94] McDevitt and Lansbury synthesised twelve glycosamino acid monomer units from a selection of sugars, and compiled a small library of homo- and heterooligomers.[88]

1.5 Carbopeptoid foldamers

Similarly to α- and β-amino acids, sugar amino acids may induce secondary structures in their homooligomers.[79] Tetramers and longer sequences of the sialyl amino acid **34**, **Figure 1.20**, were shown to display stable secondary structures in water, though the precise conformations have not been elucidated.[95,96]



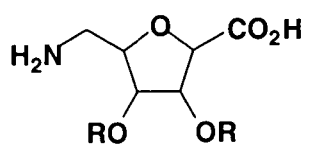
34

Figure 1.20

Since then the emphasis of carbopeptoid foldamer research has been towards amino acids based on tetrahydrofuran (THF) and oxetane rings.[76,77,97-105]

1.5.1 Tetrahydrofuran templated carbopeptoid foldamers

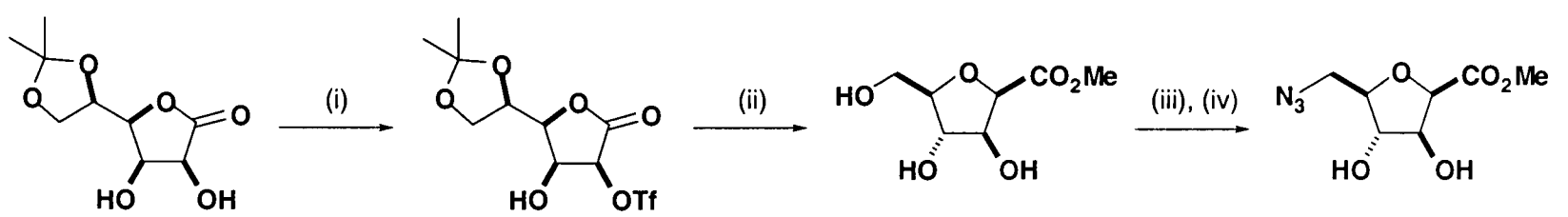
There has been extensive investigation into the structures of the oligomers of sugar-derived δ-glycosamino acids **35** which are based upon a THF template,[97-105] **Figure 1.21**. Co-workers have synthesised monomer units with **D**-*gluco*,[97] **D**-*manno*,[100] **D**-*talo*,[101] **D**-[102] and **L**-*allo*[101] and **D**-*galacto*[104] stereochemistry and constructed each of their homooligomers.



35

Figure 1.21

The first of these 5-aminomethyl-THF-2-carboxylates **39** was synthesised in 62% yield from D-mannose.[97] The key transformation is the acid-catalysed ring rearrangement of lactone **36** to form THF carboxylate **38**, which proceeds by intramolecular displacement of hydroxy triflate **37** with inversion at C-2,[97,106-108] **Scheme 1.2**. This reaction will be covered in more detail in the following chapter (see **Scheme 2.3**, **Chapter 2.1.2**).

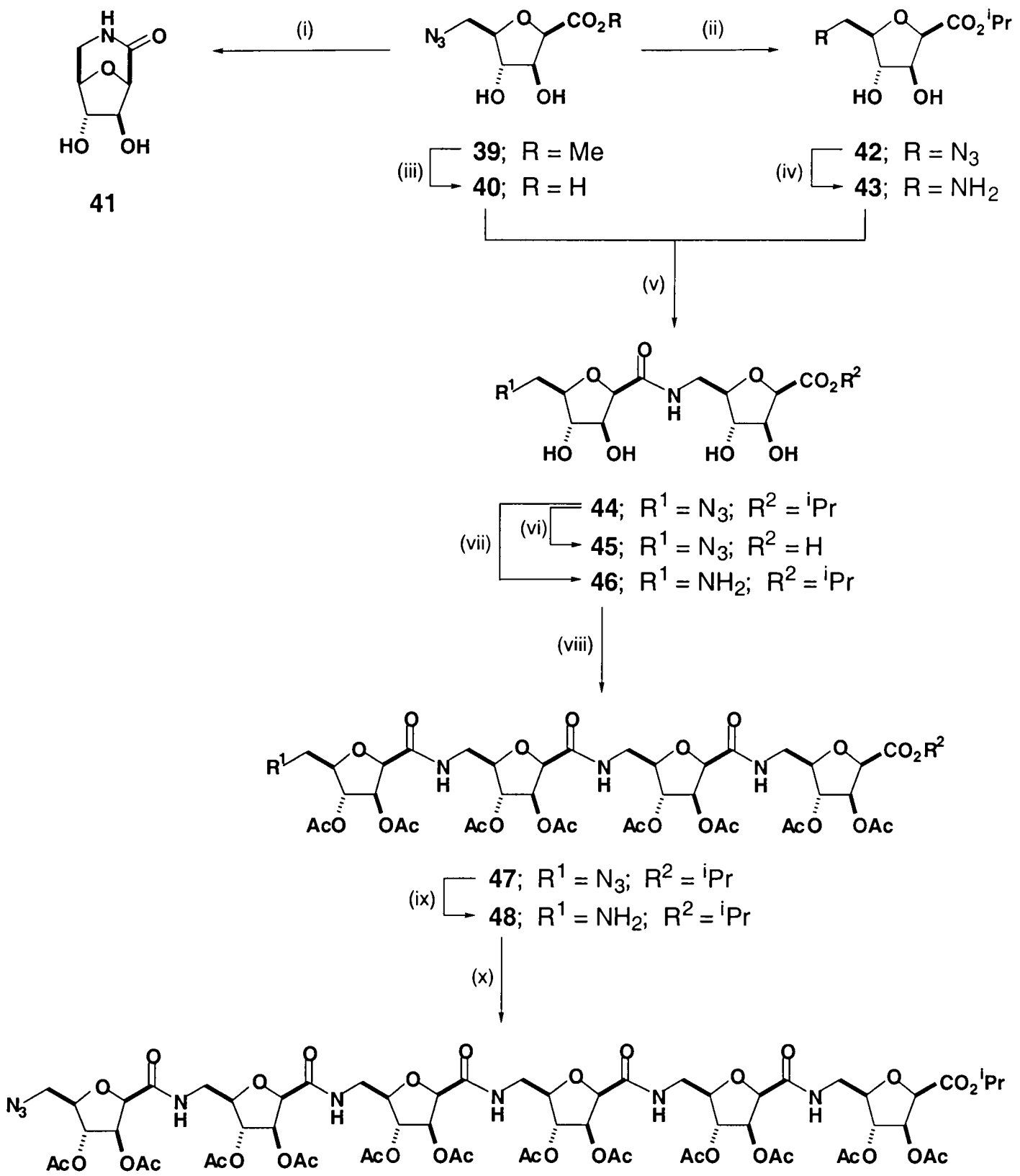


36; D-*manno* **37** **38** **39**; D-*gluco*

Reagents and Conditions: (i) Tf_2O, pyridine, DCM; (ii) MeOH, HCl; (iii) TsCl, pyridine; (iv) NaN_3, DMF

Scheme 1.2

The oligomers of azido ester **39** were formed using well-established peptide bond forming methodology.[97] Hydrolysis of **39** by aqueous sodium hydroxide gave carboxylic acid **40**, **Scheme 1.3, p25**. The methyl ester was converted to the more hindered isopropyl ester **42** before reduction of the azide functionality, in order to avoid both intermolecular condensations[100] and the intramolecular cyclisation[102,105] which leads to bicyclic lactam **41**.[97] Hydrogenation of azide **42** catalysed by palladium on activated carbon in isopropanol gave amine **43**. The coupling of **40** and **43** was achieved by using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBt) in DMF (see **Figure 3.2**, Chapter **3.1.3**) to give dimer **44** in 74% yield. Protection of the secondary hydroxyl groups was found to be unnecessary during the coupling procedure.[97] Iteration of this procedure with dimer **44** yielded the tetrameric **47** (55% from **44**) and hexameric **49** (68% from **47**) species, which were peracetylated for ease of isolation.



41

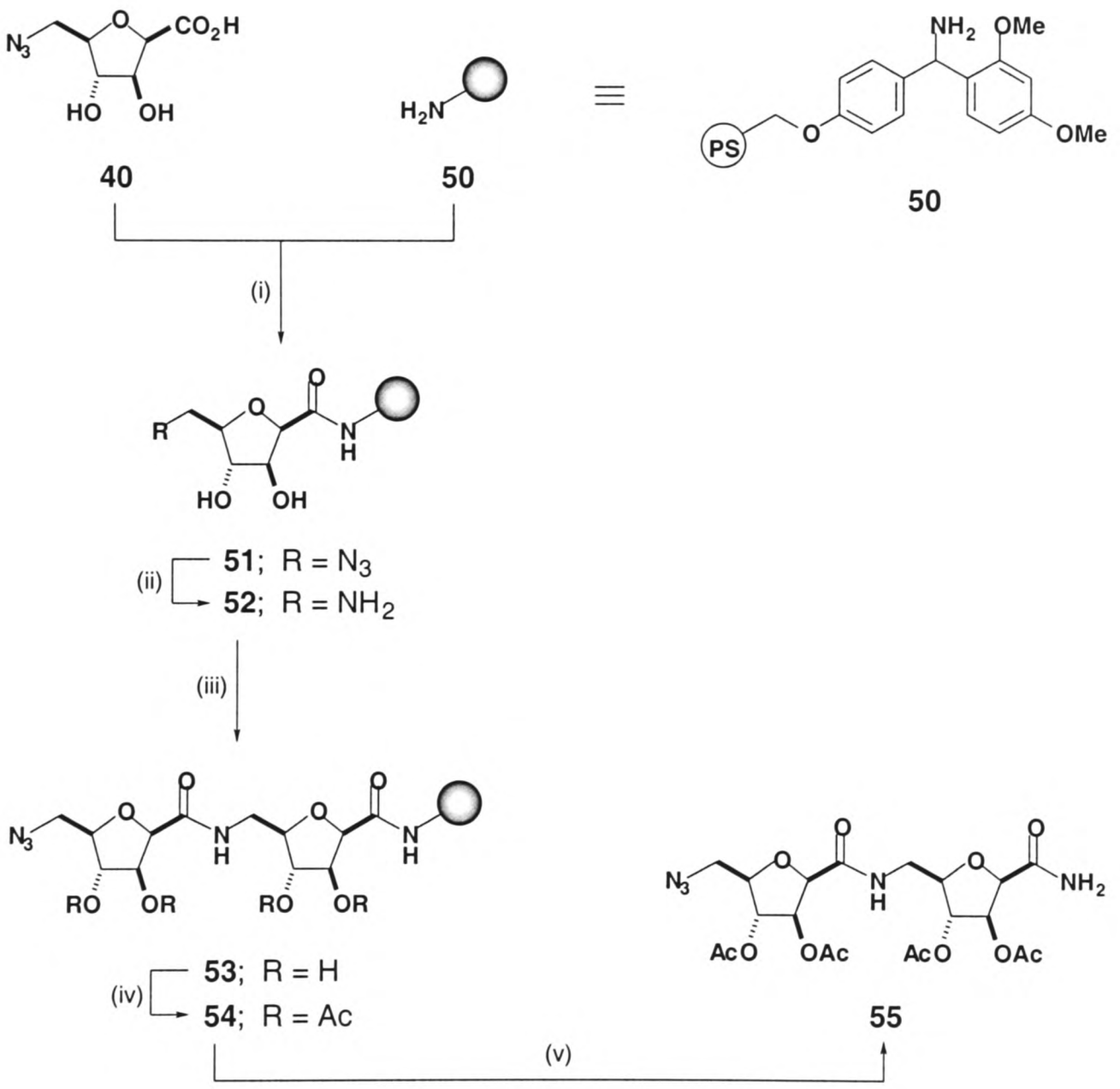
49

Reagents and Conditions: (i) H_2, Pd/C, MeOH; (ii) K_2CO_3, ^iPrOH; (iii) NaOH, H_2O, dioxane then Amberlite IR120 (H^+), H_2O; (iv) H_2, Pd/C, ^iPrOH; (v) EDCI, HOBt, DIPEA, DMF; (vi) NaOH, H_2O, dioxane then Amberlite IR120 (H^+), H_2O; (vii) H_2, Pd/C, ^iPrOH; (viii) **45** (1 eq), EDCI, HOBt, DIPEA, DMF then Ac_2O, pyridine; (ix) H_2, Pd/C, ^iPrOH; (x) **45** (1 eq), EDCI, HOBt, DIPEA, DMF then Ac_2O, pyridine

Scheme 1.3

As one of the advantages envisaged by Nicolaou (see **Chapter 1.4.2**) the compatibility with solid-phase coupling generates the possibility of the synthesis of libraries of compounds that may be used for high-throughput screening. Derivative **39** was found to be suitable for coupling in solid phase.[99]

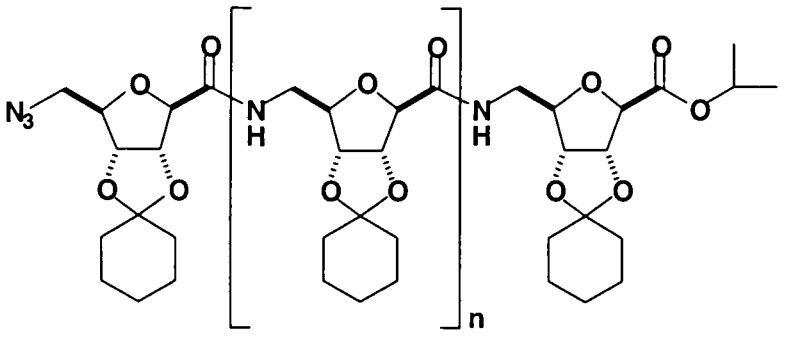
The support used was polystyrene bead and amine linker, **50**. The carboxyl moiety of the first monomer was attached to the linker *via* an amide bond, and each residue of the oligomer coupled iteratively using diisopropylcarbodiimide (DIC) and HOBt in DMF, **Scheme 1.4**. Cleavage of the oligomer from the resin was achieved by treatment with 50% TFA and DCM. In this way the dimer **55** (30% from **40**) and trimer were synthesised in excellent purity.[99]



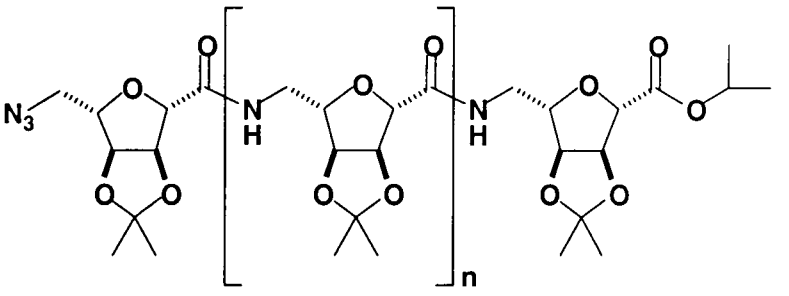
Reagents and Conditions: (i) DIC, HOBt, DMF; (ii) DTT, DIPEA, DMF, 50°C; (iii) **40**, DIC, HOBt, DMF; (iv) Ac_2O, pyridine; (v) TFA, DCM

Scheme 1.4

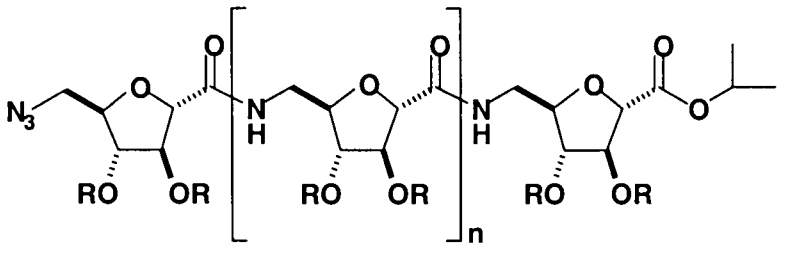
Over the following two years five more monomers were synthesised from sugar lactones by various approaches[100,101,104] and the oligomers **56-68**, **Figure 1.22**, **p27**, were constructed using the methodology described in **Scheme 1.3**, **p25**.[100,102-105]



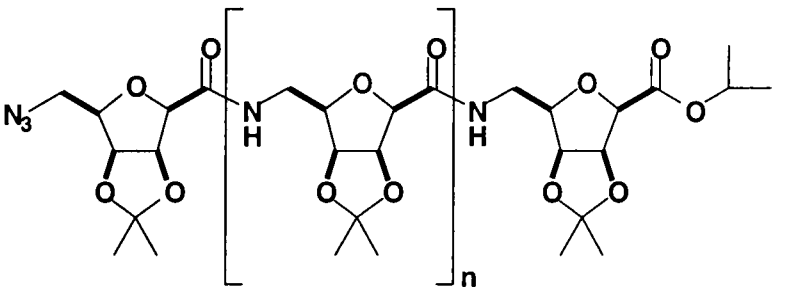
56; n = 0
57; n = 2
58; n = 6
} **D**-*allo*



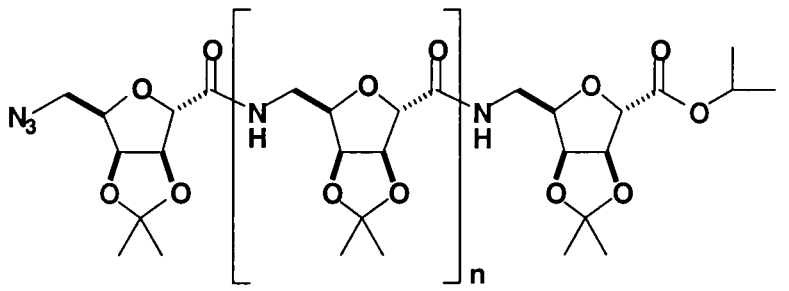
59; n = 0
60; n = 2
} **L**-*allo*



61; n = 0, R = H
62; n = 2, R = Ac
} **D**-*manno*



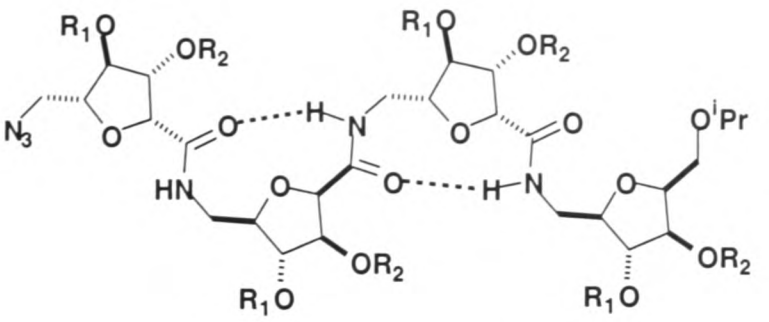
63; n = 0
64; n = 2
65; n = 4
} **D**-*galacto*



66; n = 0
67; n = 2
68; n = 6
} **D**-*talo*

Figure 1.22

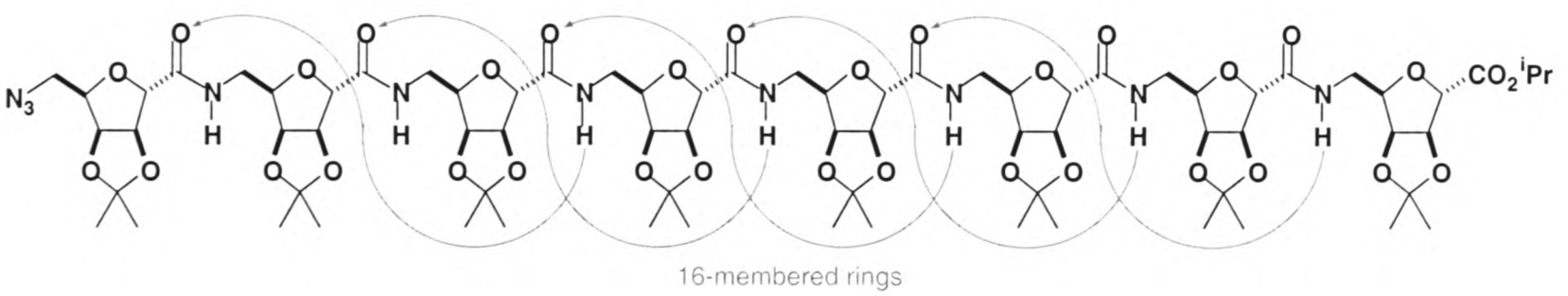
Analysis by NMR spectroscopy showed evidence of well-defined secondary structures in solution for both the tetramer **47** and hexamer **49** of glycosamino acid with **D**-*gluco* stereochemistry.[98] The novel conformation observed was assigned as a 'repeating β-turn,' **Figure 1.23, p28**. This structure was also adopted by the **D**- and **L**-*allo* oligomers **56-60**.[103,105] It is interesting to note that the repeating β-turn is the most favourable conformation for each of the compounds where the amine and carboxylic acid are in a *cis* relationship across the glycosidic oxygen of the THF ring.



47; $R_1 = R_2 = Ac$
57; $R_1,R_2 = C_6H_{10}$

Figure 1.23

In the oligomers of the 2,5-*trans* amino acids different conformations are observed.[100,103,104] While the **D**-*manno* tetramer **62**[100] and **D**-*galacto* tetramer **64** and hexamer **65**[104] demonstrate no long range order in solution, the tetramer **67** and octamer **68** with **D**-*talo* stereochemistry were shown by NMR studies to display a preference for a left-handed helix stabilised by 16-membered inter-residue hydrogen bonded rings,[103] **Figure 1.24**.

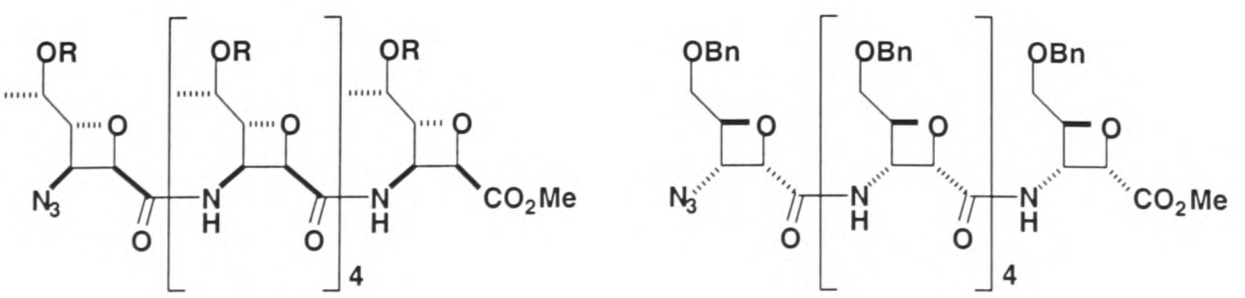


68

Figure 1.24

1.5.2 Oxetane templated carbopeptoid foldamers

The novel 10-helical conformation adopted by hexameric β-peptides **10-12**,[76,77] **Figure 1.25**, has been discussed previously (see **Chapter 1.3.6**).



10; R = TBDMS
11; R = Bn

12

Figure 1.25

1.6 Conclusion

The search for protein mimetics has led to investigations into compounds with controlled secondary structure, with a view to being able to replicate the conformations and emulate the activity of the natural products.

β-Peptides are useful peptidomimetics due to their stability towards peptidases and their propensity to form stable secondary structures, including many conformations that can not be adopted by their natural counterparts.

Glycosamino acids are capable of inducing secondary structures in their oligomers, which are stable to glycosidases. Additionally, the potential for hydroxyl derivatisation of glycosamino acid residues and their incorporation into peptides leads to the possibility of modification of the properties of the peptides, such as increased lipophilicity which could render them more likely to permeate cell membranes.

Integrating β-peptides and glycosamino acids to give β-glycosamino acids could give compounds which combine the advantages of both individual compound types. The following chapters describe the synthesis of a family of β-glycosamino acid monomers and the synthesis and conformational analysis of their homooligomers.

1.7 References

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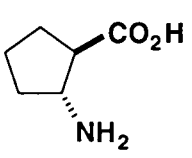
Chapter 2: Synthesis of monomers

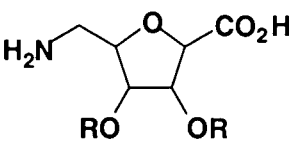
2.1 Introduction

2.1.1 Glycosamino acid targets

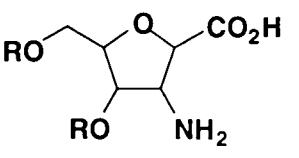
As discussed in the previous chapter (see **Chapter 1.3.4**) it has been discovered that β-peptides are more likely to form stable secondary structures than their α-peptide counterparts. Gellman demonstrated that the oligomers of *trans*-2-aminocyclopentanecarboxylic acid **69**, **Figure 2.1**, form stable 12-helices in solution.[1] The rigidity provided by the cyclopentane ring is believed to play a part in the tendency of these peptides to adopt recognisable secondary structures in oligomers containing as few as six residues.



69



35



70

Figure 2.1

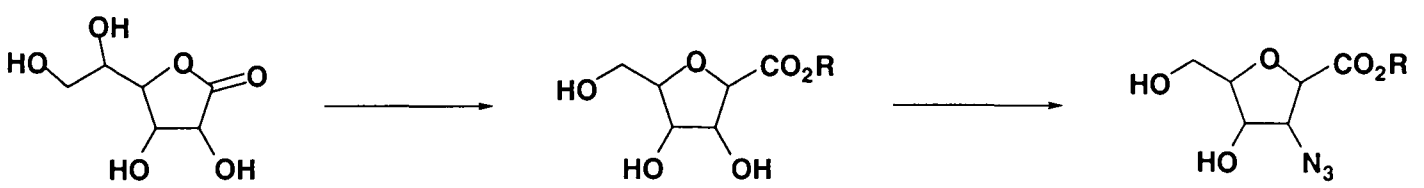
The inclusion of further functionality allows derivatisation to introduce specific properties, including the possibility of lipophilic, hydrophilic and other side-chains, as well as the inherent opportunity for more intramolecular interactions. There has been extensive research into the structures of glycosamino acids and their oligomers.[2]

The synthesis of several THF templated δ-glycosamino acids **35** and their oligomers has been carried out by co-workers[3-11] (see **Chapter 1.5.1**). These compounds have demonstrated secondary structure in the form of a helix[9] and repeating β-turn.[4,9]

However, the corresponding β-glycosamino acids **70**, which have the same configuration as Gellman's compound **69**, had not previously been made. Therefore it was decided to attempt to synthesise this new series of THF β-amino acids, in order to investigate the structural properties of their oligomers and the effect of the extra functionality relative to **69**. The synthesis of libraries of compounds with stereochemical diversity should enable the prediction of the type of secondary structure that a suggested compound would adopt. This chapter deals with the synthesis of the monomer units **70**.

2.1.2 Strategy – introduction of nitrogen after formation of ring

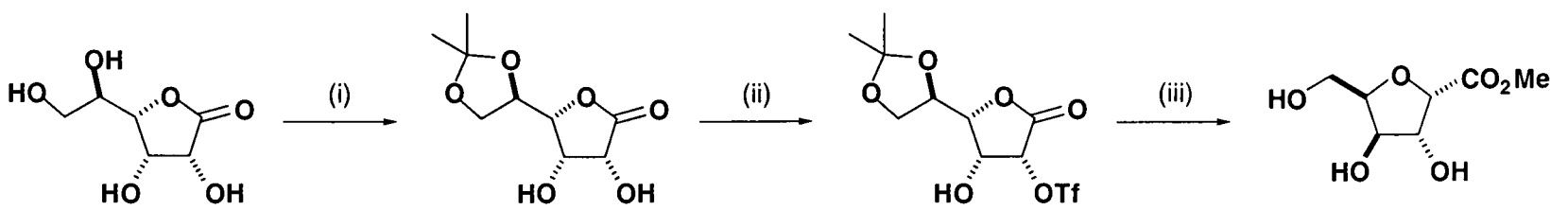
Our initial approach to the THF β-amino acids was synthesis of THF carboxylates **72**, a strategy already employed in the group toward synthesis of δ-glycosamino acids **35**[3,12-14] (see **Scheme 1.2**, **Chapter 1.5.1**). Selective introduction of a nitrogen functionality at the C-3 position would then give the protected β-amino acid targets **73**, **Scheme 2.1**.



71 **72** **73**

Scheme 2.1

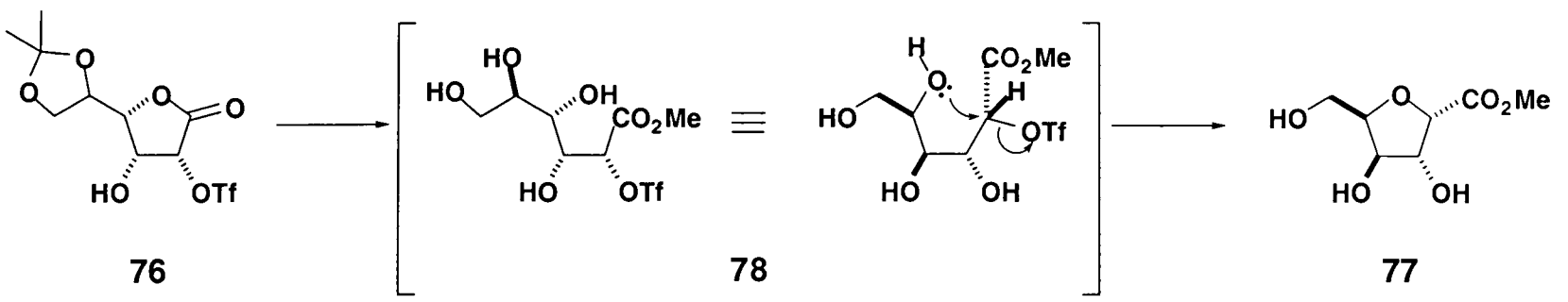
Preliminary investigation was conducted by a co-worker on the D-*ido* configured THF **77**.[15] Commercially available D-gulono-1,4-lactone **74** was converted to the 5,6-monoacetonide **75** by treatment with 2-methoxypropene and *p*-toluenesulphonic acid in DMF, **Scheme 2.2**. Selective trifluoromethanesulphonation (triflation) of the C-2 hydroxyl group was performed *via* low temperature treatment of the acetonide with trifluoromethanesulphonic (triflic) anhydride, and subsequent stirring of triflate **76** in acidic methanol gave THF carboxylate **77**.



Reagents and Conditions: (i) 2-methoxypropene, *p*-TsOH, DMF; (ii) Tf_2O, pyridine, THF, –40°C; (iii) MeOH, HCl

Scheme 2.2

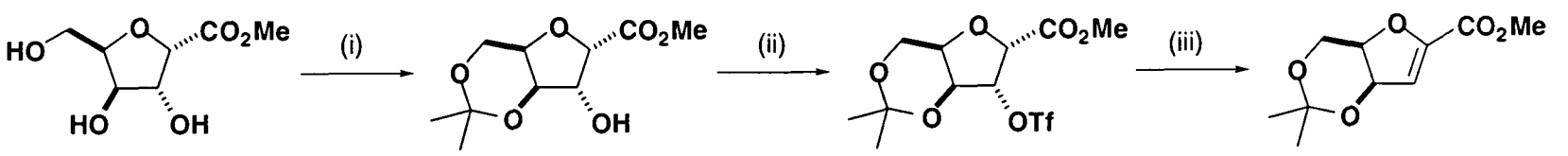
The acidic methanol effects the removal of the acetonide protecting group and simultaneously opens the lactone, to give the transient open chain hydroxy triflate **78**, **Scheme 2.3**. Ring closure proceeds by intramolecular SN2 displacement of the sulphonate group by the C-5 hydroxyl group, and no epoxide, oxetane or tetrahydropyran products, relating to ring closure by the C-3, C-4 or C-6 hydroxyl groups respectively, were observed.



Reagents and Conditions: MeOH, HCl

Scheme 2.3

The 4,6-diol segment of the THF carboxylate **77** was protected as the acetonide **79** to allow selective manipulation of the C-3 hydroxyl group. This conversion was carried out using 2,2-dimethoxypropane and camphorsulphonic acid (CSA) in acetone, **Scheme 2.4**. Treatment of the acetonide **79** with triflic anhydride in the presence of pyridine afforded the unstable triflate **80**, the presence of which was confirmed by analysis using ^{1}H and ^{13}C NMR spectroscopy of the crude material. Further reaction of triflate **80** with sodium azide in DMF gave a single product which was identified as the elimination product **81** on the basis of its crude NMR and mass spectrometry data.



77; D-*ido* **79** **80** **81**

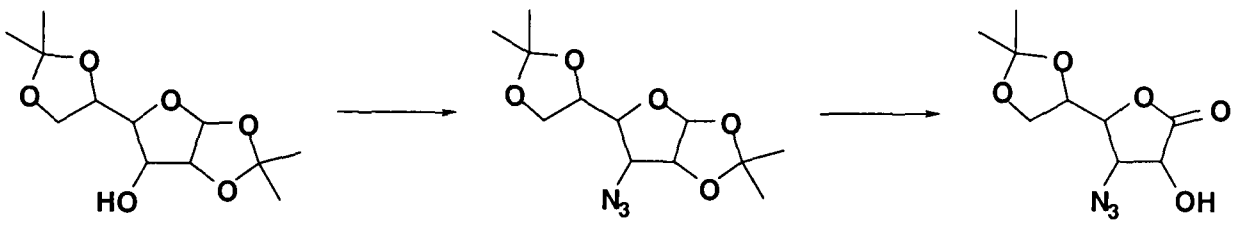
Reagents and Conditions: (i) 2,2-DMP, CSA, acetone; (ii) Tf_2O, pyridine, DCM, –30°C; (iii) NaN_3, DMF

Scheme 2.4

Further attempts were made to introduce nitrogen substituents at C-3 of the THF carboxylate **79**, both by azide displacement of alternative sulphonate leaving groups, and by conjugate addition of nitrogen nucleophiles into the α,β-unsaturated ester **81**, but these proved to be unsuccessful.[16] It was subsequently discovered that there is extensive literature precedent for the elimination of C-3 sulphonate [17-20] and other leaving groups[21-24] in carboxylate compounds related to **77**, and so a change in synthetic approach was required.

2.1.3 Revised strategy – introduction of nitrogen before formation of ring

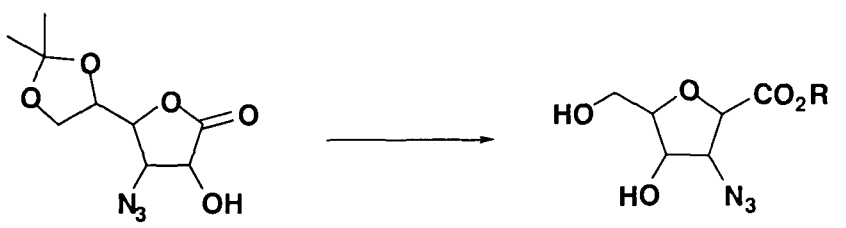
An alternative strategy was envisaged, where the nitrogen functionality is introduced into the carbohydrate skeleton prior to formation of the THF ring. In this scheme, azide displacement of a C-3 sulphonate leaving group in a 1,2:5,6-di-*O*-isopropylidene protected hexose **82** would give the protected 3-azide **83**, **Scheme 2.5**. Deprotection by acid-catalysed removal of the acetonide groups, and oxidation at the C-1 position would give aldonic acid or lactone products, and it was hoped that acetonide protection of these would give the monoacetonide of the γ-lactone, **84**.



82 **83** **84**

Scheme 2.5

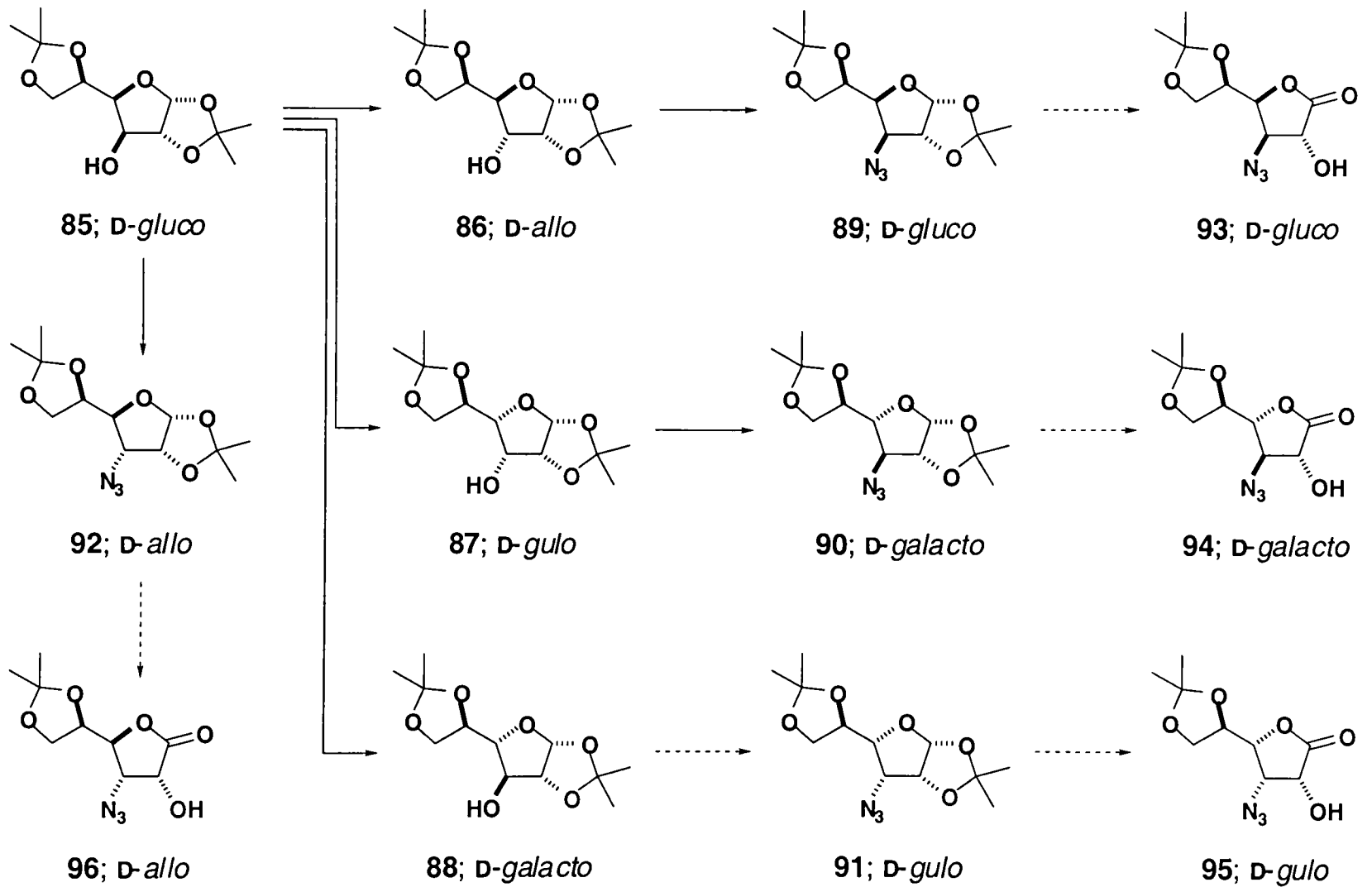
Triflation of **84** at C-2 followed by acid-catalysed rearrangement as detailed earlier (see **Scheme 2.3**, **Chapter 2.1.2**) would give the desired β-azido ester **73**, with the nitrogen functionality already in place at the C-3 position, **Scheme 2.6**.



84 **73**

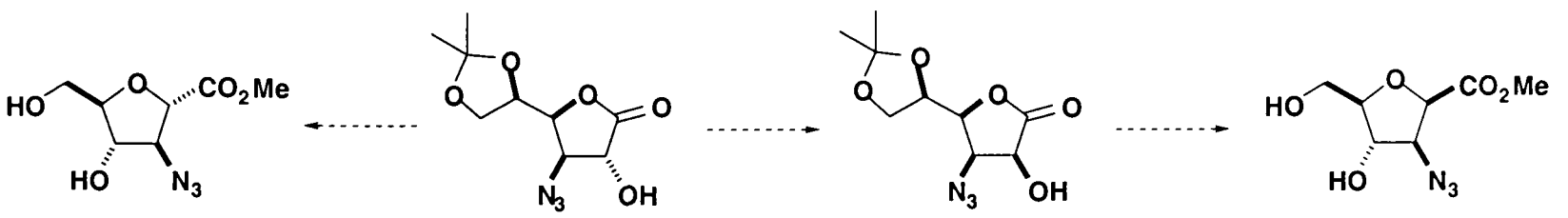
Scheme 2.6

The most readily available 1,2:5,6-di-*O*-isopropylidene protected hexose is diacetone-D-glucose **85**, so this was selected as the starting material. There are established literature precedents for conversion of **85** to the D-*allo* **86**,[25] D-*gulo* **87**[26,27] and D-*galacto* **88**[27,28] diacetonides, **Scheme 2.7**. The introduction of an azide group into the D-*gluco* **85**[29-33], D-*allo* **86**[25] and D-*gulo* **87**[34] diacetonides by nucleophilic displacement of the triflate derivatives is also known. Using the method described above it should therefore be possible to access the four diastereomeric 3-azido γ-lactones **93-96**.

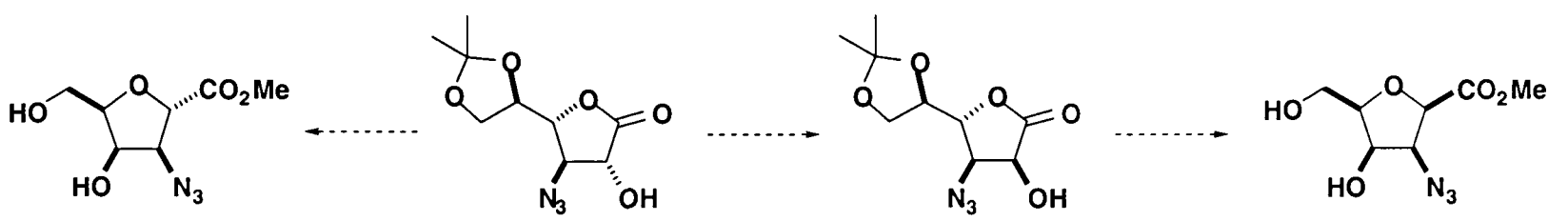


Scheme 2.7

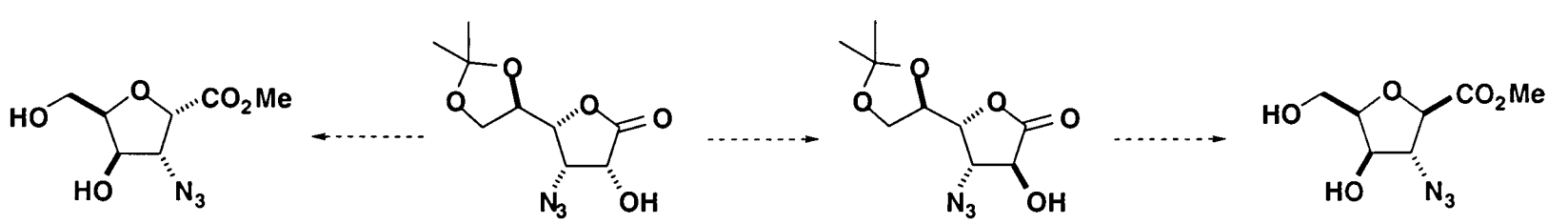
These γ-lactones are the intermediate compounds to the four diastereomeric THF carboxylates with the D-*manno* **97**, D-*talo* **98**, D-*ido* **99** and D-*altro* **100** configurations, **Scheme 2.8**, **p44**. Furthermore, inversion of configuration of the C-2 hydroxyl group in lactones **93-96**, followed by triflation and acid catalysed rearrangement should allow access to the four THF carboxylates **105-108**, which differ from **97-100** by the configuration at C-2.



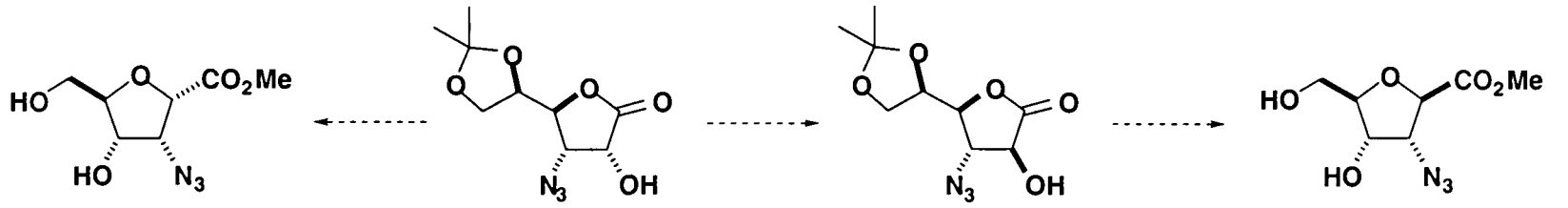
97; D-*manno* **93**; D-*gluco* **101**; D-*manno* **105**; D-*gluco*



98; D-*talo* **94**; D-*galacto* **102**; D-*talo* **106**; D-*galacto*



99; D-*ido* **95**; D-*gulo* **103**; D-*ido* **107**; D-*gulo*



100; D-*altro* **96**; D-*allo* **104**; D-*altro* **108**; D-*allo*

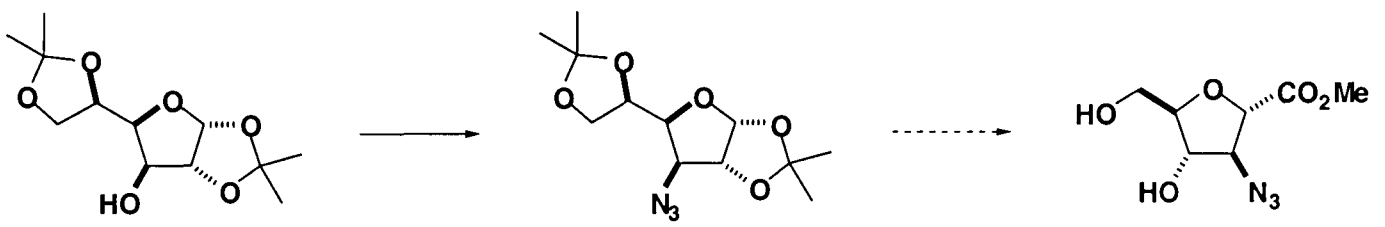
Scheme 2.8

Each of the 2,5-*trans*-β-azido esters **97-100** were initially selected as targets, in order to make a comparison between amino acids in which the amine and carboxylate are *trans* and those in which they are *cis*. This research commenced with the 2,3-*trans*-β-azido esters **97** and **98**. Concurrent with these studies, investigations into the synthesis of the 2,3-*cis*-β-azido esters **99** and **100** were undertaken by a co-worker.[35] The C-2 inverted THF carboxylates **106**, **107** and **108** were subsequently targeted, to probe the effect of the relative configuration at C-5 on the conformation of the oligomers.

2.2 Results and discussion

2.2.1 *Manno* series – synthesis of azide

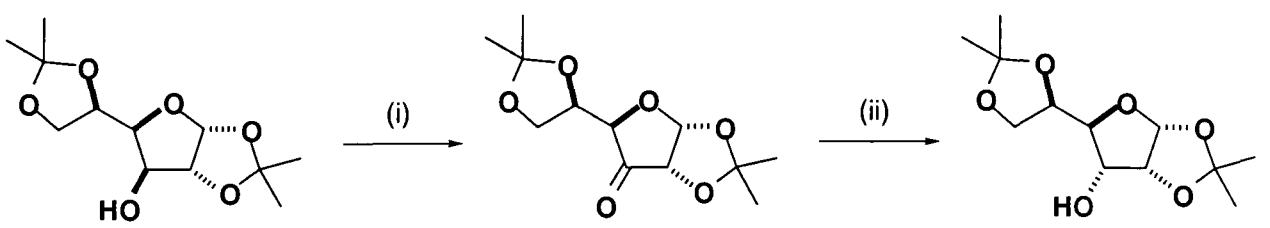
The approach to D-*manno* THF carboxylate **97** involved synthesis of D-*gluco* azide **89**, **Scheme 2.9**.



85; D-*gluco* **89**; D-*gluco* **97**; D-*manno*

Scheme 2.9

The established scheme for introduction of an azide into glucose with overall retention of configuration involves a double inversion at C-3.[25] In accordance with the procedure for conversion to its epimer **86**, diacetone-D-glucose **85** was subjected to oxidation by pyridinium chlorochromate in DCM, in the presence of 3Å molecular sieves, **Scheme 2.10**. The resulting ketone **109** was reduced with sodium borohydride in a mixture of ethanol and water to afford diacetonide **86** in 96% yield, an improvement on the reported yield of 84%. The structure of **86** was confirmed by comparison with the literature data. The 1,2-*O*-isopropylidene group provides sufficient steric hindrance to prevent the reversion to the starting material; the hydride ion is presented from the convex face to give only the allose **86**.

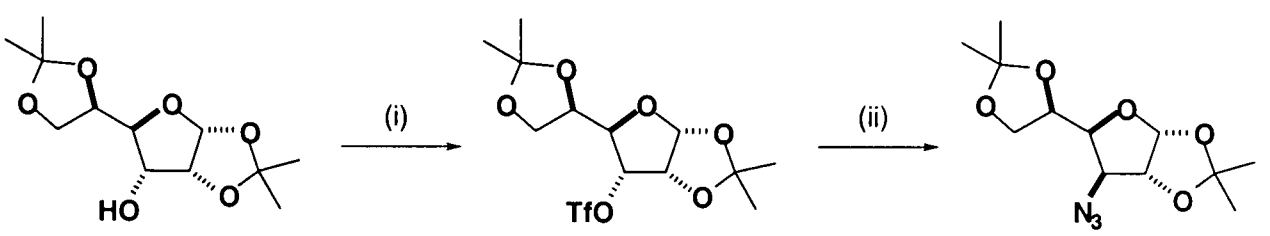


85; D-*gluco* **109** **86**; D-*allo*

Reagents and Conditions: (i) PCC, 3Å mol sieves, DCM; (ii) $NaBH_4$, EtOH, H_2O, 0°C-r.t.

Scheme 2.10

The C-3 hydroxyl group in diacetone allose **86** was activated by treatment with triflic anhydride in pyridine and DCM at –30°C, and the triflate group subjected to nucleophilic displacement by sodium azide in DMF to generate desired azide **89**, **Scheme 2.11**.[25]



86; D-*allo* **110** **89**; D-*gluco*

Reagents and Conditions: (i) Tf_2O, pyridine, DCM, -30°C; (ii) NaN_3, DMF

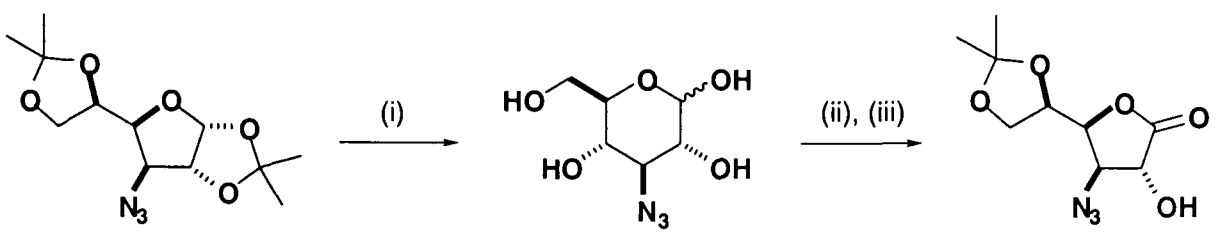
Scheme 2.11

Comparison with the literature data[25,36] shows that this reaction proceeds as expected with inversion of configuration at C-3 to give azide **89** in 95% yield. The four reaction steps from **85** to **89** result in overall retention of D-*gluco* stereochemistry.

2.2.2 *Manno* series – synthesis of THF

Removal of the acetonide protecting groups was effected by stirring azide **89** in 50% aqueous trifluoroacetic acid to give azido sugar **111**, **Scheme 2.12**, **p47**. Oxidation at the anomeric position of aldoses is commonly achieved by treatment with bromine water.[37] The presence of acid inhibits the reaction rate, therefore a buffer is used to remove the hydrogen bromide that is formed during the course of the reaction. Accordingly, the pyranose **111** was treated with bromine water in the presence of excess barium carbonate until thin layer chromatography indicated the formation of a major product. The reaction mixture was filtered through Celite® to remove any excess inorganic impurities, and the product was dried thoroughly at reduced pressure for a minimum of 24 hours. Acetonide protection of a suspension of the residue in dry

acetone was carried out by treatment with camphorsulphonic acid to give the 5,6-monoacetonide **93**.

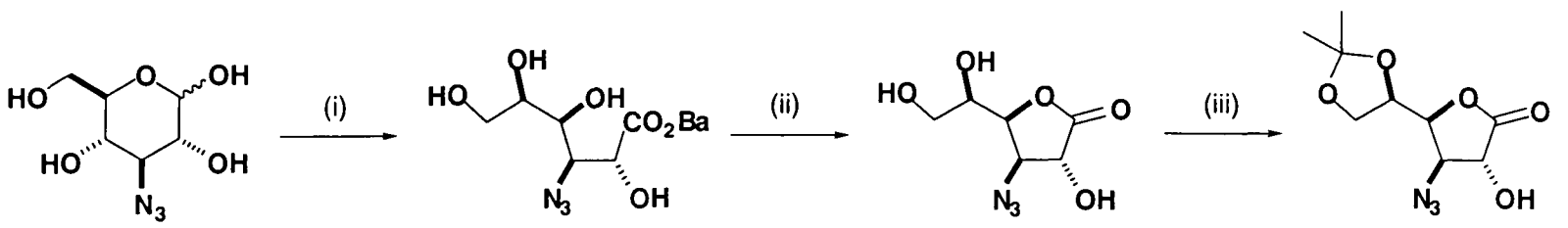


89; D-*gluco* **111** **93**; D-*gluco*

Reagents and Conditions: (i) TFA, H_2O; (ii) Br_2, $BaCO_3$, H_2O, 0°C-r.t.; (iii) CSA, acetone, 50°C

Scheme 2.12

By this method lactone **93** was isolated in 20-40% over three steps. Iteration of this procedure established that the residue of the oxidation reaction was insoluble in acetone, believed to be due to the fact that the aldonic acid exists as the open-chain barium carboxylate salt **112**, and this insolubility was thought to be a factor for the poor yields. Therefore an adaptation of the procedure was devised where intermediate **112** was first subjected to ion exchange by aqueous elution through a column of Amberlite IR-120 (H^+) resin, then desiccated as before, **Scheme 2.13**. Dissolution of the resultant orange foam **113** in acetone was facile, and the yield of **93** increased to 79%, while the reaction time for the acetonide protection was shortened.

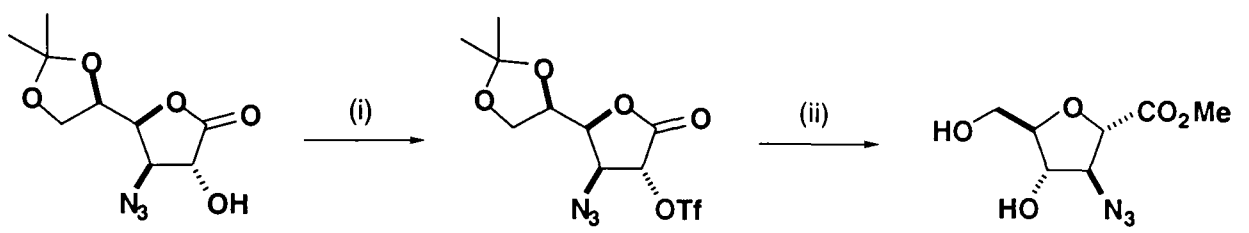


111 **112** **113** **93**

Reagents and Conditions: (i) Br_2, $BaCO_3$, H_2O, 0°C-r.t.; (ii) Amberlite IR-120 (H^+), H_2O; (iii) CSA, acetone, 50°C

Scheme 2.13

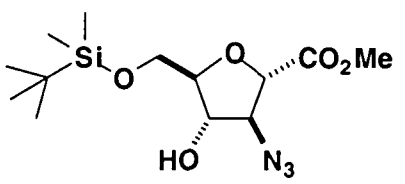
Treatment of lactone **93** with triflic anhydride in pyridine and DCM at 0°C gave the unstable C-2 triflate **114**, which was ring-opened with acidic methanol, **Scheme 2.14**. Ring closure proceeds by intramolecular SN2 attack of the C-5 hydroxyl group on the activated C-2 position to give the THF carboxylate **97**. This sequence proceeds in the same manner as the formation of methyl 2,5-anhydro-D-idonate **77** as previously discussed (see **Scheme 2.3**, **Chapter 2.1.2**) but with the nitrogen functionality already in place at C-3. No oxetane or tetrahydropyran products, resulting from intramolecular attack by the C-4 or C-6 hydroxyls respectively, were observed by thin layer chromatography. The stereochemistry of azido ester **97** was unequivocally confirmed by x-ray crystallographic analysis of the C-6 *tert*-butyldimethylsilyl ether **115**, **Figure 2.2** (see **Figure 3.8**, **Chapter 3.2.2**).



93; D-*gluco* **114** **97**; D-*manno*

Reagents and Conditions: (i) Tf_2O, pyridine, DCM, 0°C; (ii) MeOH, HCl, 0°C-r.t.

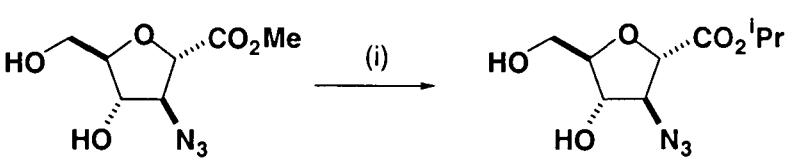
Scheme 2.14



115

Figure 2.2

Attempts to purify ester **97** by flash column chromatography were unsuccessful. The product co-runs with one or more impurities which were found to be inorganic triflate salts on the basis of a CF_3 quartet in the ^{13}C NMR spectrum. Instead, the contaminated material was subjected to transesterification in acidic isopropanol to give isopropyl ester **116**, **Scheme 2.15**, which was desirable for oligomerisation use (see **Chapter 3.2**). Ester **116** was isolated in 62% yield, in three steps from lactone **93**.



97; D-*manno* **116**; D-*manno*

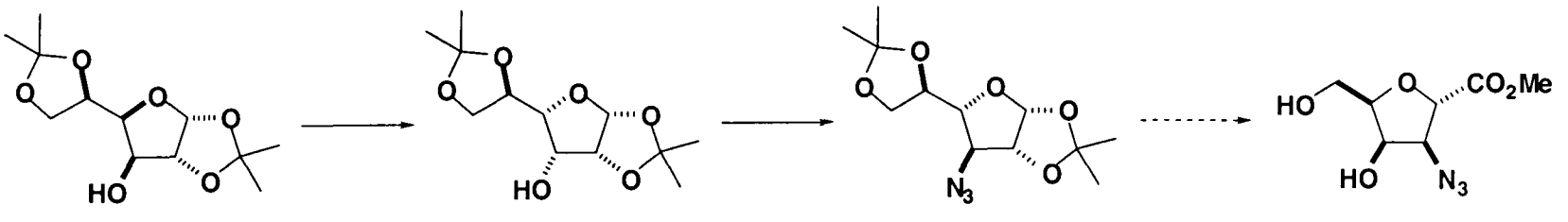
Reagents and Conditions: (i) ^{i}PrOH, HCl, 80°C

Scheme 2.15

A small amount of unreacted methyl ester **97** was also isolated and characterised from the reaction mixture.

2.2.3 *Talo* series – synthesis of azide

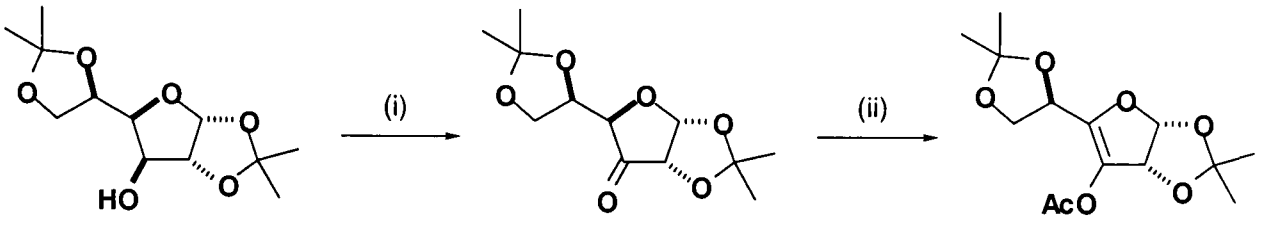
Synthesis of the D-*talo*-azido ester **98** requires the 3-azido sugar with D-*galacto* stereochemistry, **90**, **Scheme 2.16**. The conversions of diacetone-D-glucose to diacetone-D-gulose **87**,[26,27] and of **87** to **90**[34] have been reported.



85; D-*gluco* **87**; D-*gulo* **90**; D-*galacto* **98**; D-*talo*

Scheme 2.16

Accordingly, diacetone-D-glucose **85** was oxidised at C-3 by pyridinium dichromate and acetic anhydride in DCM, and the enol acetate **117** formed in 77% by further treatment of the ketone **109** with acetic anhydride in the presence of pyridine,[26] **Scheme 2.17**.

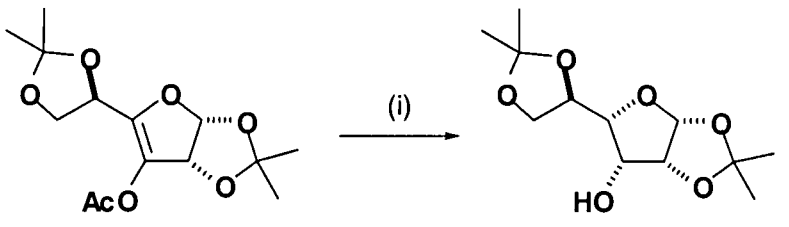


85; D-*gluco* **109** **117**

Reagents and Conditions: (i) PDC, Ac_2O, DCM, 70°C; (ii) Ac_2O, pyridine, 70°C

Scheme 2.17

Enol ester **117** was reduced with activated palladium on carbon under hydrogen, and deacetylated by basic methanolysis to give D-*gulo* sugar **87** in 63% yield, **Scheme 2.18**. The literature reduction is conducted at –15°C to –10°C to ensure stereoselectivity,[26] however the same reagents used at room temperature resulted in the major formation of D-*gulo* isomer **87**. Hydrogenation from the more hindered face would result in the formation of starting material **85**, but none of this diastereomer was isolated.

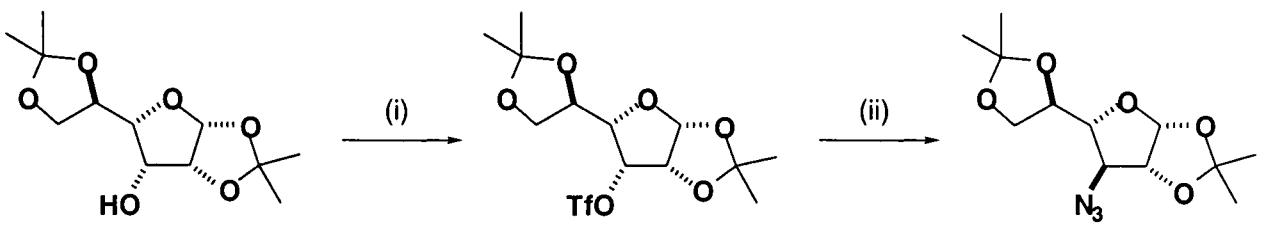


117 **87**; D-*gulo*

Reagents and Conditions: (i) Pd/C, H_2, MeOH then K_2CO_3, MeOH

Scheme 2.18

As with the allose **86** (see **Scheme 2.11**, **Chapter 2.2.1**), the C-3 hydroxyl of **87** was activated by triflation, and subjected to nucleophilic substitution with sodium azide, **Scheme 2.19**.[34]



87; D-*gulo* **118** **90**; D-*galacto*

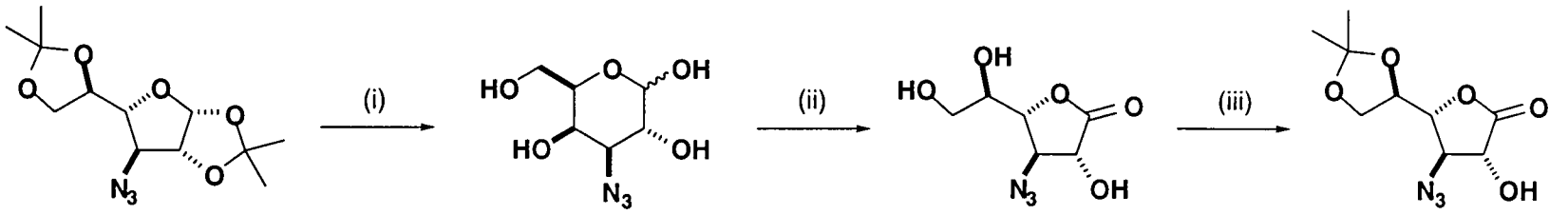
Reagents and Conditions: (i) Tf_2O, pyridine, DCM, 0°C; (ii) NaN_3, DMF

Scheme 2.19

No product relating to the elimination of triflic acid is seen, and azide **90** is obtained in almost quantitative yields. Again, this reaction proceeds with inversion of stereochemistry at the reaction centre, and this was confirmed by NMR spectroscopic analysis. Marked n.O.e. correlations between the C-1, C-2, C-3 and C-4 protons of triflate **118** indicated that they are all on the same side of the ring, while similar analysis of azide **90** showed no correlation between the protons at C-1 and C-3.

2.2.4 *Talo* series – synthesis of THF

Diacetonide **90** was deprotected in 50% aqueous trifluoroacetic acid to give pyranose **119**, **Scheme 2.20**. A co-worker claimed that higher yields for the bromine oxidation step were achieved when the reaction mixture was not buffered, though reaction rates were substantially decreased from hours to days.[38] This finding supports the claim that the absence of barium ion improves the yield for the acetonation reaction (see **Chapter 2.2.2**). Azido sugar **119** was therefore treated with bromine water, without the addition of buffer. The oxidation was complete after stirring in a darkened reaction vessel for four to seven days, after which time desiccation for extended periods at reduced pressure gave lactone **120** directly as an orange foam. Dissolution in acetone and heating in the presence of camphorsulphonic acid gave protected lactone **94** in 70% from azide **90**.

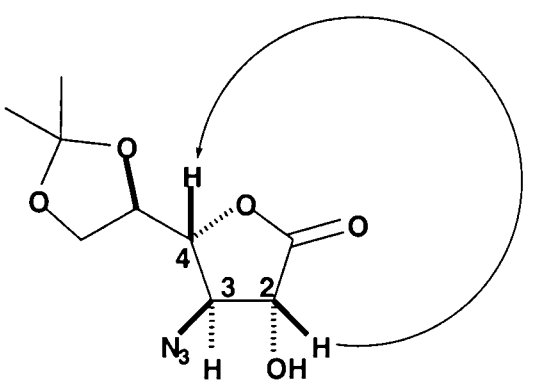


90; D-*galacto* **119** **120** **94**; D-*galacto*

Reagents and Conditions: (i) TFA, H_2O; (ii) Br_2, H_2O, 0°C-r.t.; (iii) CSA, acetone, 50°C

Scheme 2.20

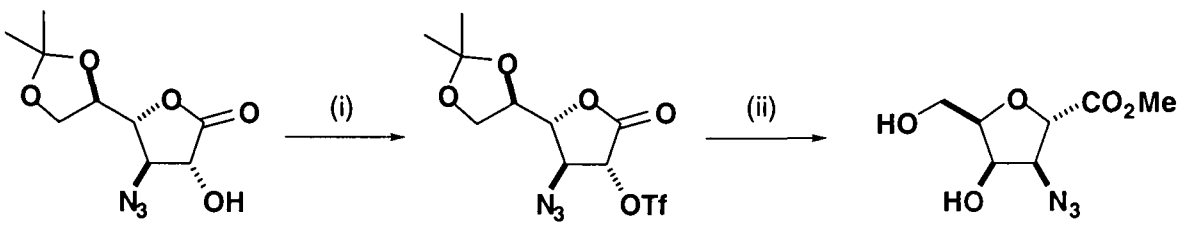
The stereochemistry of **94** was confirmed by NMR spectroscopic analysis – a strong n.O.e. correlation between the protons at C-2 and C-4 was observed, **Figure 2.3**.



94

Figure 2.3

The THF ring was formed as discussed previously (see **Scheme 2.3, Chapter 2.1.2**). Triflation of lactone **94** gave triflate **121**, and direct addition of the reaction mixture to acidic methanol at low temperature gave THF **98** in 61% yield, **Scheme 2.21**. The lesser polarity of the D-*talo* ester compared to the D-*manno* diastereomer, which had to be converted to the isopropyl ester for purification (see **Scheme 2.15, Chapter 2.2.2**) meant that purification of methyl ester **98** was possible by flash column chromatography.

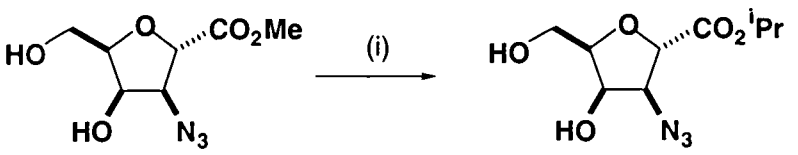


94; D-*galacto* **121** **98**; D-*talo*

Reagents and Conditions: (i) Tf_2O, pyridine, DCM, -10°C; (ii) MeOH, HCl, -10°C-r.t.

Scheme 2.21

The isopropyl ester was required for oligomerisation use (see **Chapter 3.2.6**) and so methyl ester **98** was treated with acidic isopropanol to give isopropyl ester **122** in 85-90% yield, **Scheme 2.22**.



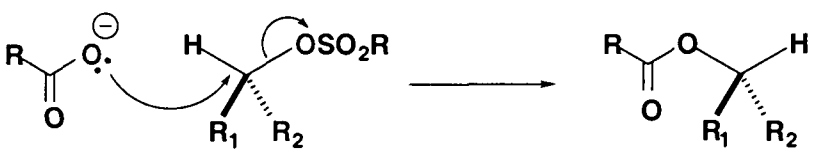
98; D-*talo* **122**; D-*talo*

Reagents and Conditions: (i) ^{i}PrOH, HCl

Scheme 2.22

2.2.5 *Galacto* series – inversion at C-2

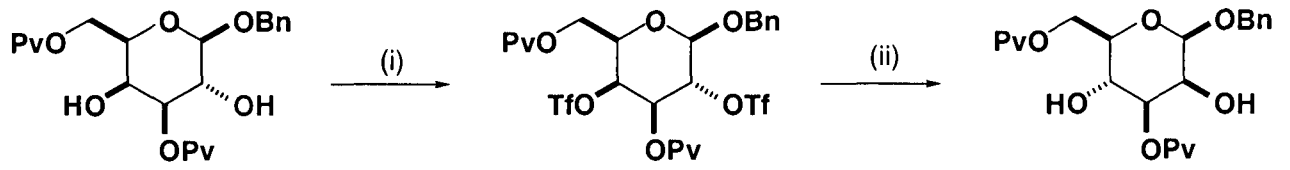
In order to access the 2,5-*cis*-β-azido esters **105-108**, it was intended to invert the stereochemistry at the C-2 hydroxyl group of the γ-lactones **93-96** (see **Scheme 2.8**, **Chapter 2.1.3**). The normal choice of reaction for this transformation is either the Mitsunobu reaction[39] or displacement of a sulphonate leaving group by an oxygen nucleophile, in particular by a carboxylate. The use of caesium propionate as the nucleophile has been reported,[40] and the procedure has been adapted to use the acetate[41,42] as well as caesium benzoate[43] and camphanate,[44] including the displacement of several different sulphonate leaving groups,[45] **Scheme 2.23**.



Scheme 2.23

However, lactone carbonyl groups are highly sensitive to nucleophilic or basic attack. Removal of the propionate or acetate esters can involve extreme conditions such as 5% aqueous potassium hydroxide under reflux,[40] and under these conditions lactone ring opening and elimination reactions are prevalent. Therefore the inversion of a hydroxyl that is α- to a lactone carbonyl requires the use of a carboxylate that can be removed by transesterification under milder conditions.

Trifluoroacetate is an ideal nucleophile in these circumstances, though in the hands of this group the use of the sodium salt in DMF gave low yields of the inverted products.[46] It was reported that in these circumstances a suitable alternative to caesium acetate and sodium trifluoroacetate is commercially available caesium trifluoroacetate, when used in the displacement of a triflate,[46] though this method had been used only once, **Scheme 2.24**.[47] The trifluoroacetic ester can be readily exchanged by treatment with methanol in the presence of potassium carbonate or sodium hydrogencarbonate without affecting the lactone carbonyl, and indeed more reactive trifluoroacetate esters are hydrolysed *in situ* by any water in the solvent or reactants without the need for a separate transesterification step.

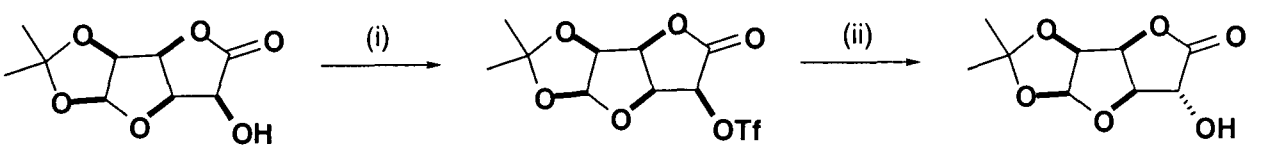


123; D-*galacto*; Pv = pivaloyl **124** **125**; D-*manno*

Reagents and Conditions: (i) Tf_2O, pyridine, DCM, 0°C-r.t.; (ii) CF_3CO_2Cs, 18-crown-6, toluene, DMF, 80°C then MeOH, $NaHCO_3$

Scheme 2.24

The displacement of a triflate by caesium trifluoroacetate has since been used in the inversion of hydroxyl groups that are α- to lactone carbonyls, **Scheme 2.25**.[48] By this method D-*manno* acetonide **126** gave inversion product **128** in 56% yield.

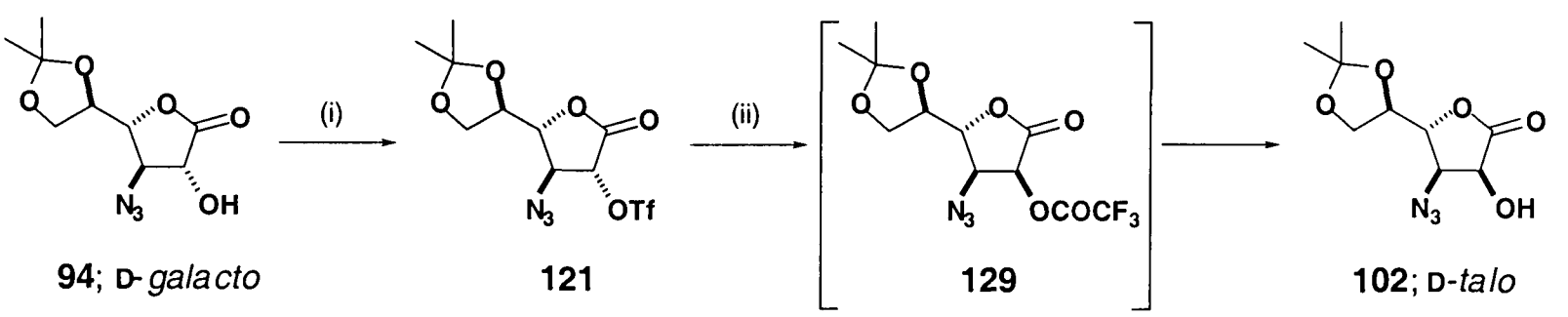


126 **127** **128**

Reagents and Conditions: (i) Tf_2O, pyridine, DCM, 0°C; (ii) CF_3CO_2Cs, butanone then MeOH

Scheme 2.25

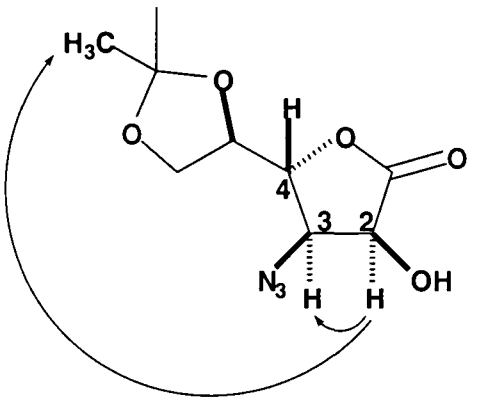
Accordingly, lactone **94** was triflated with triflic anhydride as for the intramolecular rearrangement reaction (see **Scheme 2.21, Chapter 2.2.4**). The triflate was not isolated but diluted with butanone at low temperature and treated with caesium trifluoroacetate, **Scheme 2.26**. After 24 hours at room temperature, evaporation of the solvent and purification gave the inverted alcohol **102** directly in 67% yield.



Reagents and Conditions: (i) Tf_2O, pyridine, DCM, 0°C; (ii) CF_3CO_2Cs, butanone, 0°C-r.t.

Scheme 2.26

The structure of **102** was confirmed by NMR spectroscopic analysis. There was a marked n.O.e. correlation between the C-2 proton and that at C-3, as well as one of the acetonide methyl groups, but no correlation with the proton at C-4 was observed, **Figure 2.4**.

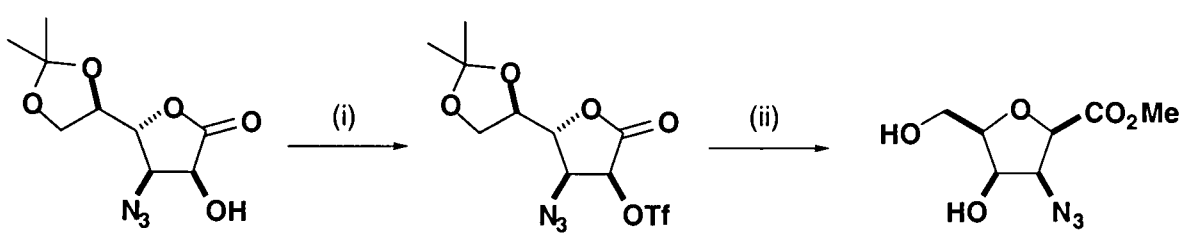


102

Figure 2.4

2.2.6 *Galacto* series – synthesis of THF

Activation of the free hydroxyl group with triflic anhydride and rearrangement in acidic methanol was effected as discussed previously (see **Scheme 2.3**, **Chapter 2.1.2**) to give the all *cis* THF azido ester **106** in 41%, **Scheme 2.27**.



102; D-*talo* **130** **106**; D-*galacto*

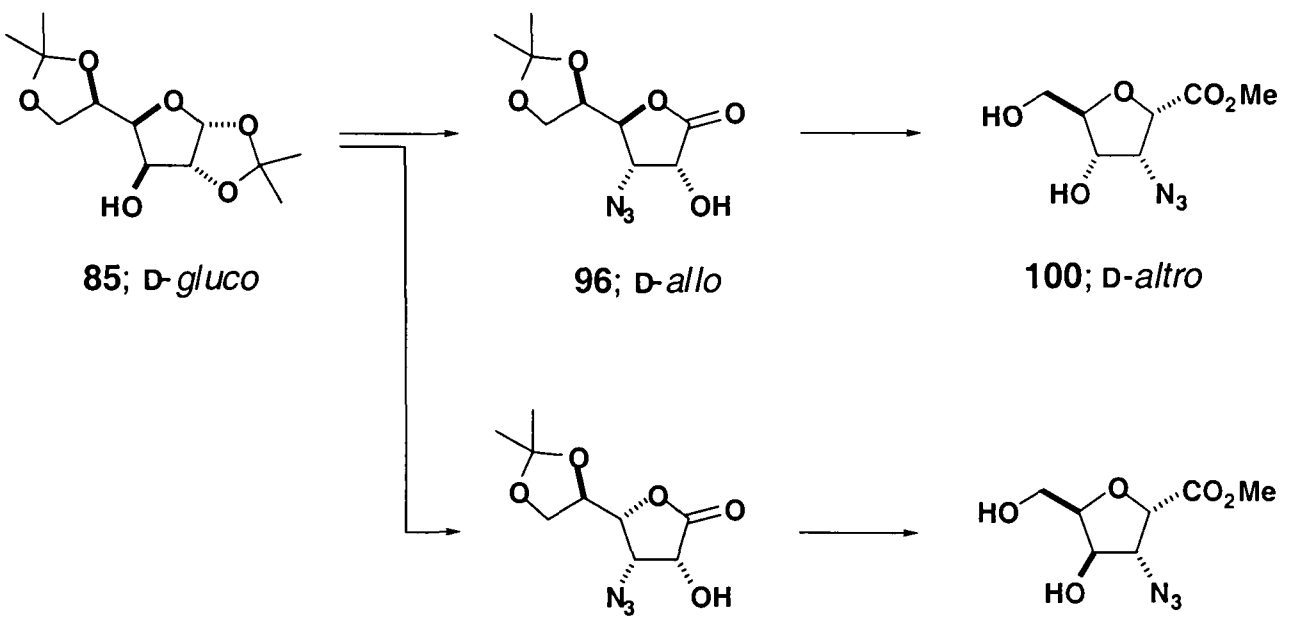
Reagents and Conditions: (i) Tf_2O, pyridine, DCM, 0°C; (ii) MeOH, HCl, 0°C-r.t.

Scheme 2.27

The low yield of this reaction can probably be ascribed to the crowded nature of the product, the relative stereochemistry of which was confirmed by NMR spectroscopic analysis. The strong n.O.e. correlations between each of the ring protons indicate that the substituents are all *cis* to each other.

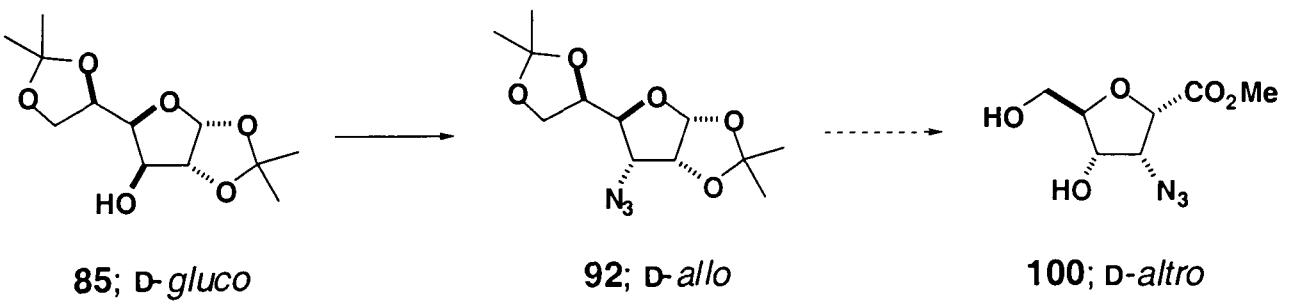
2.2.7 *Allo* series – synthesis of lactone

En route to the two THF carboxylates with D-*ido* **99** and D-*altro* **100** configuration, a co-worker synthesised lactones **95** and **96**, **Scheme 2.28**.[35] However, the lactone intermediates were also to be used in the synthesis of C-2 epimers **107** and **108** (see **Scheme 2.8**, **Chapter 2.1.3**).



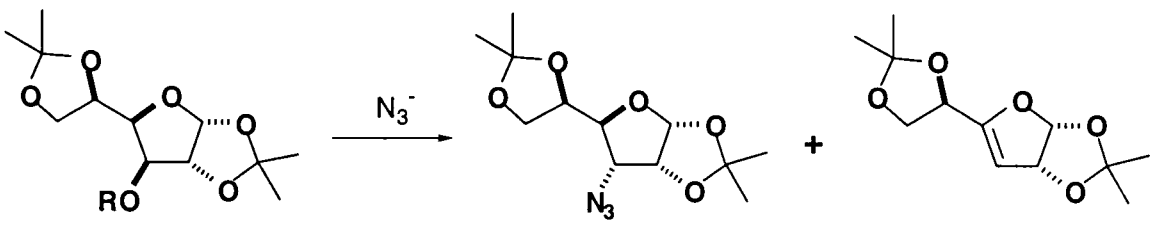
Scheme 2.28

The first of these schemes involved direct introduction of an azide ion into glucose, **Scheme 2.29**.



Scheme 2.29

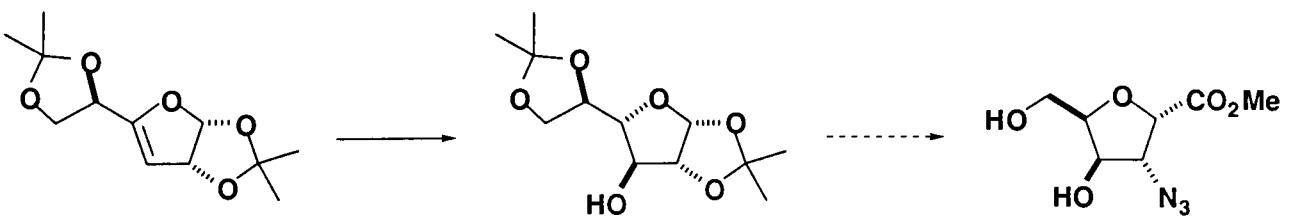
However, the reaction of an azide with diacetone glucose triflate does not result solely in the substitution with inversion that is seen with each of the other triflates (see **Schemes 2.11**, **2.19** and **2.38**, **Chapters 2.2.1**, **2.2.3** and **2.2.9**). Instead, a significant amount of elimination product **135** is always formed along with the azide **92**, **Scheme 2.30**. The best reported yields of azide were 62%, by displacement of triflate[31] or imidazylate (Imz),[33] 53% where the leaving group was the tosylate[29] or 43% *via* the 2,2,2-trifluoro-1,1-diphenylethanesulphonate (TDE-SO_2) under less aggressive conditions.[30]



	92; D-*allo*	**135**
131; R = Tf	62%	-
132; R = Imz	62%	-
133; R = Ts	53%	12%
134; R = TDE-SO_2	43%	40%

Scheme 2.30

However, a route from the elimination product **135** to D-*galacto* sugar **88** *via* hydroboration has also been reported,[49] **Scheme 2.31**. Therefore it was decided to proceed with the synthesis of D-*altro*-azido ester **100**, and use the side-product of this reaction as the starting point for the synthesis of the final THF with D-*ido* stereochemistry **99**.[35]

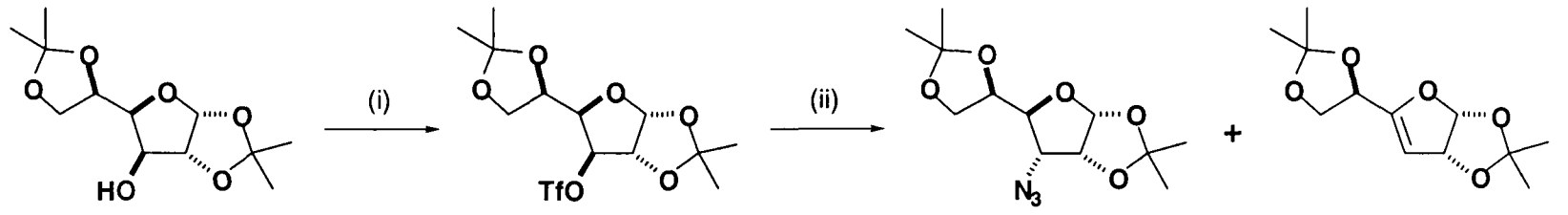


135 **88**; D-*galacto* **99**; D-*ido*

Scheme 2.31

Although the yield of azide is somewhat compromised, the treatment of diacetone glucose triflate **131** with sodium azide at 50°C is reported to give the highest overall yield (96%) of **92** and **135** in a 1 : 1 mixture.[32] This procedure was first followed by a co-worker, who reported that

activation of the C-3 hydroxyl functionality by stirring with triflic anhydride and pyridine at low temperature, and reaction with sodium azide in DMF at 50°C gave **92** and **135** in equal yields of 45%.[35] In a variation of the reaction conditions, the second step was conducted at room temperature over two days and was found to result in a slightly higher yield of substitution product **92** relative to elimination product **135**, **Scheme 2.32**.

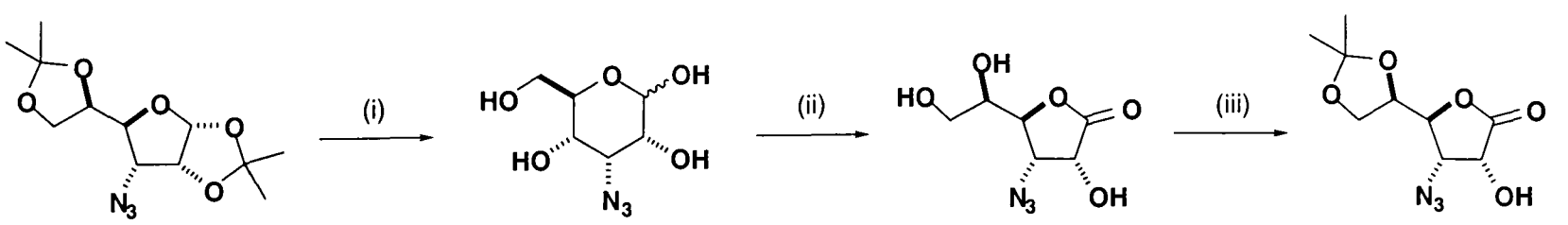


85; D-*gluco* **131** **92**; D-*allo* 50% **135** 40%

Reagents and Conditions: (i) Tf_2O, pyridine, DCM, -15°C; (ii) NaN_3, DMF

Scheme 2.32

Oxidation of the anomeric carbon was achieved as discussed earlier (see **Scheme 2.12**, **Chapter 2.2.2**). The acetonide protecting groups were removed by stirring diacetonide **92** in trifluoroacetic acid and water, and oxidation achieved in bromine water buffered with barium carbonate, **Scheme 2.33**. It was found that removal of the barium ion was not necessary for the acetonation of this isomer to proceed in good yield.[50] Therefore the residue of the oxidation step was suspended in acetone in the presence of camphorsulphonic acid at room temperature, giving lactone **96** in an overall yield of 68% from diacetonide **92**.



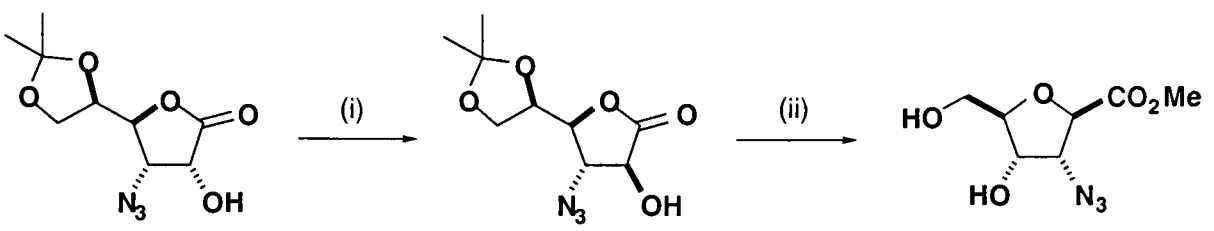
92; D-*allo* **136** **137** **96**; D-*allo*

Reagents and Conditions: (i) TFA, H_2O; (ii) Br_2, $BaCO_3$, H_2O, 0°C-r.t.; (iii) CSA, acetone

Scheme 2.33

2.2.8 *Allo* series – synthesis of THF

The transformation of the lactone **96** to the THF carboxylate **108** was achieved using the same methodology as previously described (see **Chapters 2.2.5-2.2.6**). Compound **96** was converted to its triflate ester and inverted by caesium trifluoroacetate in butanone to give inversion product **104** in 90% yield, **Scheme 2.34**. The triflate was formed and treatment with acidic methanol gave azido ester **108** in 69% yield.

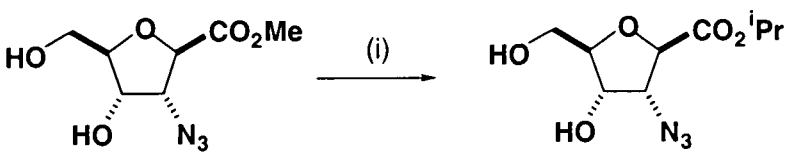


96; D-*allo* **104**; D-*altro* **108**; D-*allo*

Reagents and Conditions: (i) Tf_2O, pyridine, DCM, -30°C then CF_3CO_2Cs, butanone, -30°C-r.t.; (ii) Tf_2O, pyridine, DCM, -30°C then MeOH, HCl, -30°C-r.t.

Scheme 2.34

The isopropyl ester was again necessary for use in the synthesis of the oligomers (see **Chapter 3.2.7**) and so subjection of methyl ester **108** to transesterification conditions afforded isopropyl ester **138** in 53% yield, **Scheme 2.35**.



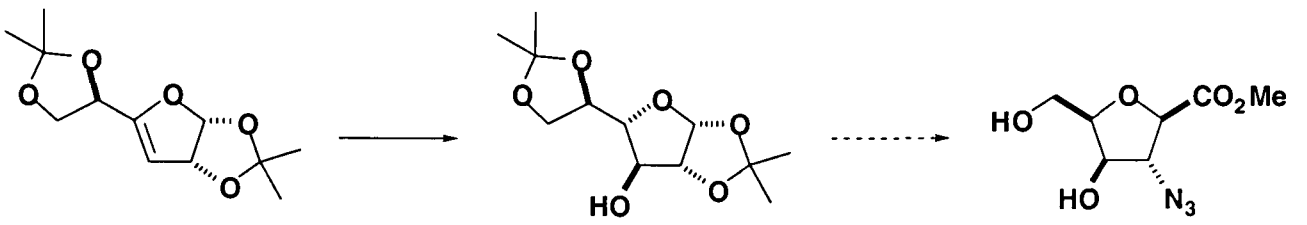
108; D-*allo* **138**; D-*allo*

Reagents and Conditions: (i) ^{i}PrOH, HCl

Scheme 2.35

2.2.9 *Gulo* series – synthesis of lactone

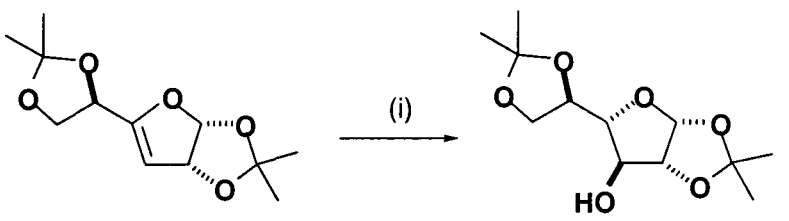
An intermediate in the synthesis of D-*gulo* THF carboxylate **107** is diacetone-D-galactose **88**, **Scheme 2.36**. Diacetonide **88** can be produced from **135**, which is a major by-product in the synthesis of D-*altro* and D-*allo* THF carboxylates (see **Chapter 2.2.7**).



135 **88**; D-*galacto* **107**; D-*gulo*

Scheme 2.36

The established precedent for conversion of unsaturated compound **135** to D-*galacto* sugar **88** involves the use of borane in THF.[49] This method was reported to give **88** in 63% yield.[28] However, the use of 9-bora-bicyclo[3.3.1]nonane (9-BBN) in THF, followed by oxidative work-up with alkaline hydrogen peroxide, was found to afford **88** in 86% yield, **Scheme 2.37**.[35]



135 **88**; D-*galacto*

Reagents and Conditions: (i) 9-BBN, THF then H_2O_2, NaOH, H_2O, 80°C

Scheme 2.37

The improvement in yield is presumed to be due to the greater selectivity of the bulky 9-BBN for the less hindered face of the double bond, opposite to the five-membered 1,2-*O*-isopropylidene ring, **Figure 2.5, p63**.

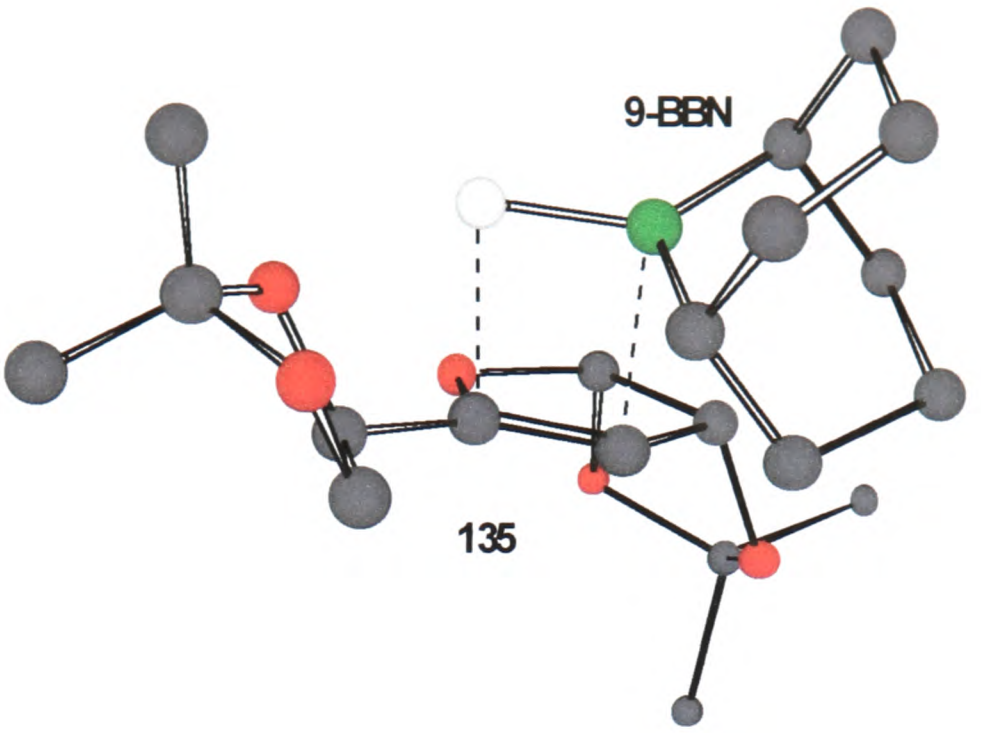
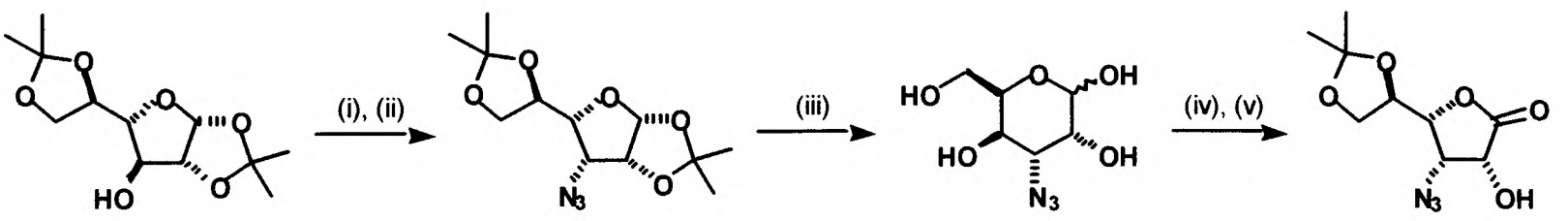


Figure 2.5. The transition state for the 9-BBN hydroboration of **135**.

The boron atom is depicted in green and borane hydrogen in white.

The non-borane hydrogen atoms have been omitted for clarity.

The introduction of azide at C-3 of diacetone-**D**-galactose **88** was found to proceed in good yields, with only a trace amount of by-product resulting from the elimination of triflic acid.[35] The C-3 hydroxyl was activated as the triflate, and displaced with sodium azide in DMF to give azido gulose **91** in 81% yield, **Scheme 2.38**. The acetonide groups were removed in aqueous trifluoroacetic acid to give pyranose **139**, which was not isolated but oxidised by bromine water and protected as the 5,6-acetonide **95**.



88; D-*galacto* **91**; D-*gulo* **139** **95**; D-*gulo*

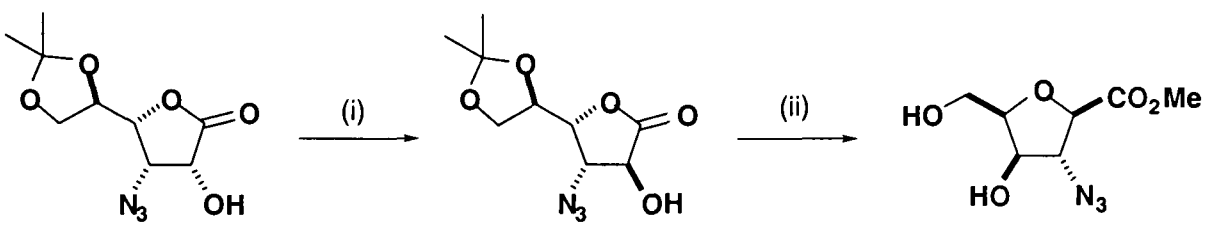
Reagents and Conditions: (i) Tf_2O, pyridine, DCM, -15ÞC; (ii) NaN_3, DMF; (iii) TFA, H_2O; (iv) Br_2, $BaCO_3$, H_2O, 0ÞC-r.t.; (v) CSA, acetone

Scheme 2.38

The yield for the three steps from diacetonide **91** to lactone **95** was 79%. The product was confirmed by comparison with the literature.[35]

2.2.10 *Gulo* series – synthesis of THF

Inversion of the C-2 hydroxyl group of lactone **95** was achieved in 57% yield by triflation and nucleophilic substitution by caesium trifluoroacetate, though this reaction was not optimised, **Scheme 2.39**. Triflation of inversion product **103** and treatment with acidic methanol gave β-azido ester target **107**.

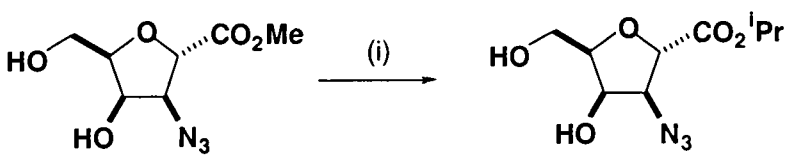


95; D-*gulo* **103**; D-*ido* **107**; D-*gulo*

Reagents and Conditions: (i) Tf_2O, pyridine, DCM, -30°C then CF_3CO_2Cs, butanone, -30°C-r.t.; (ii) Tf_2O, pyridine, DCM, -30°C then MeOH, HCl, -30°C-r.t.

Scheme 2.39

The conversion of methyl ester **107** to isopropyl ester **140**, which was required for use in the synthesis of oligomers (see **Chapter 3.2.7**) was effected by treatment with acidic isopropanol to form **140** in 67% yield, **Scheme 2.40**.



107; D-*gulo* **140**; D-*gulo*

Reagents and Conditions: (i) ^{i}PrOH, HCl

Scheme 2.40

2.3 Conclusion

This chapter has described the synthesis of the protected forms of five diastereomeric β-glycosamino acids with D-*manno*, D-*talo*, D-*galacto*, D-*allo* and D-*gulo* stereochemistry. They were all synthesised from diacetone-D-glucose, in total yields ranging from 4% to 40%, **Figure 2.6**.

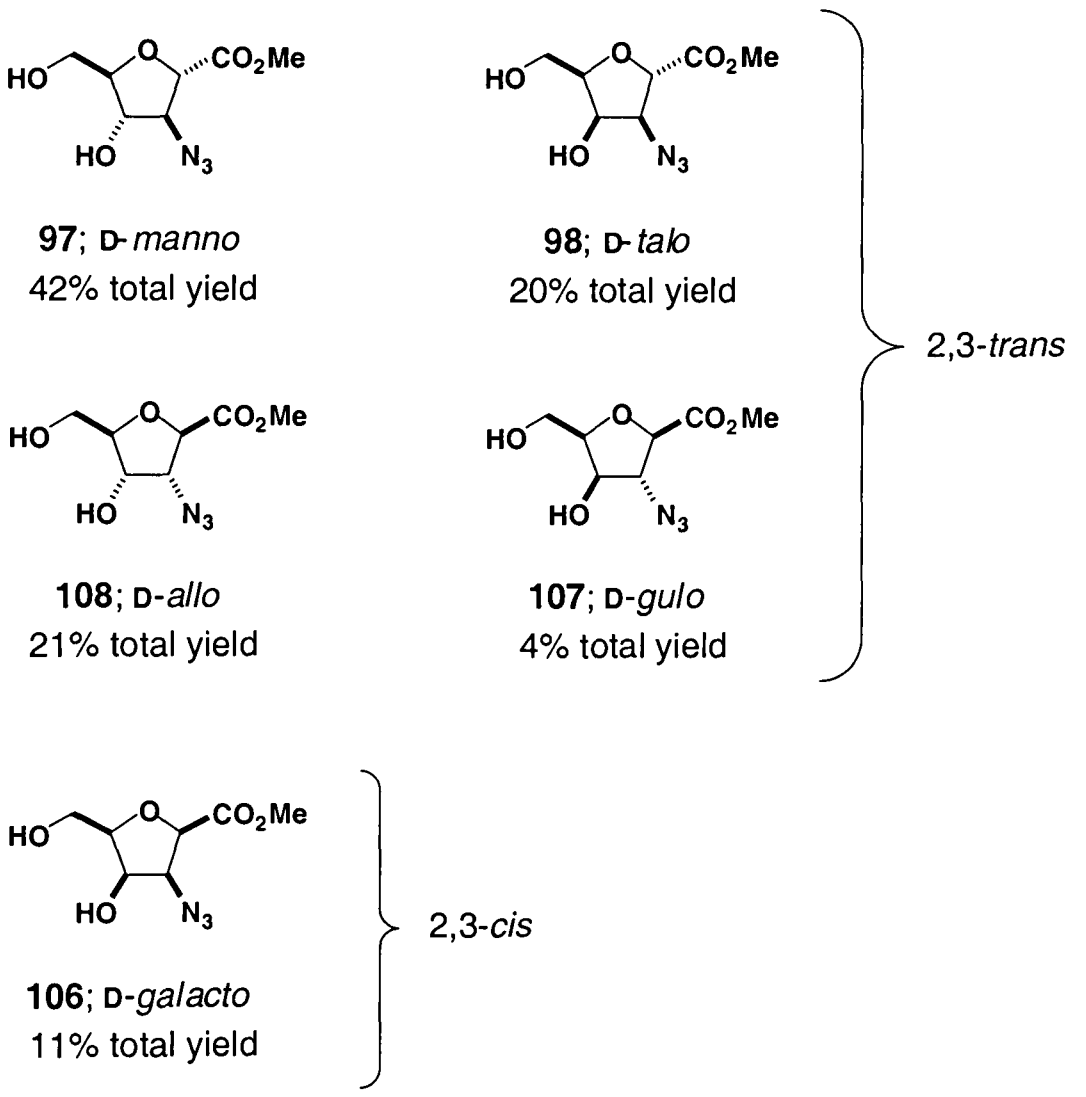


Figure 2.6

The following chapter will describe the synthesis of oligomers of the four β-amino acids **97**, **98**, **107** and **108** which have 2,3-*trans* configuration, in order to make a direct comparison with Gellman's helical homooligomers of *trans*-2-aminocyclopentanecarboxylic acid.

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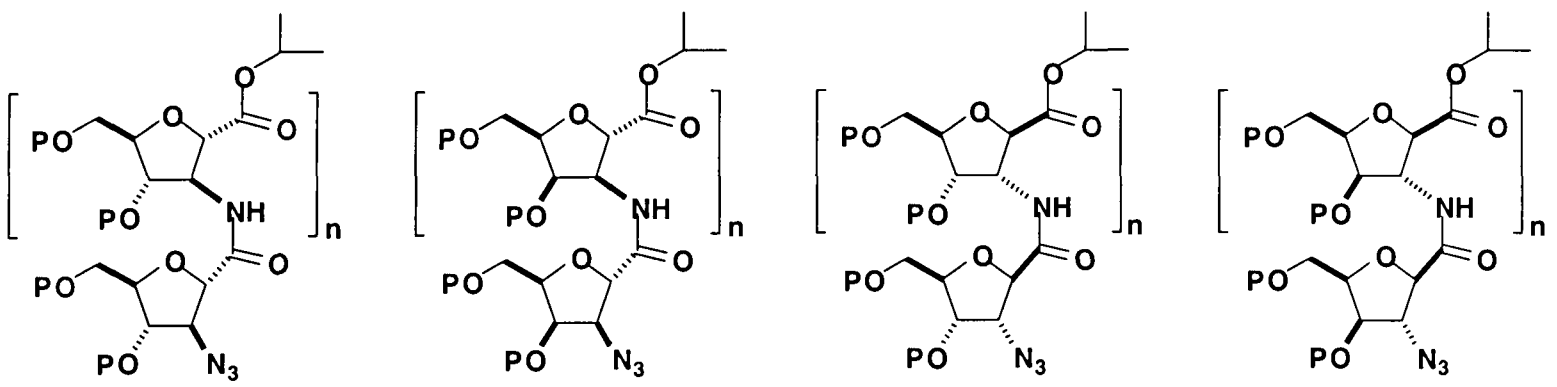
Chapter 3: Synthesis of oligomers

3.1 Introduction

3.1.1 Carbopeptoid targets

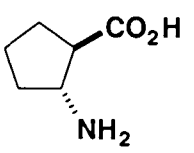
Previous published research by co-workers resulted in the synthesis of homooligomeric peptides containing up to eight sugar amino acid residues[1-8] (see **Chapter 1.5.1**). In addition, unpublished work includes the synthesis of a carbopeptoid that contains sixteen residues.[9] These were synthesised primarily in order to investigate the solution-phase conformations by NMR and CD spectroscopy.[3,5-7,10]

The synthesis of the protected forms of five diastereomeric β-glycosamino acids has been described in the previous chapter, and the four 2,3-*trans* azido esters **141-144** (n = 0) were selected for oligomerisation to enable a direct comparison with the helical oligomers of *trans*-2-aminocyclopentanecarboxylic acid **69**, **Figure 3.1**.



141; D-*manno* **142**; D-*talo* **143**; D-*allo* **144**; D-*gulo*

P = suitable protecting group



69

Figure 3.1

This chapter discusses the efforts to synthesise the oligomers **141-144** (n > 0).

3.1.2 Previous oligomerisation strategy

The group's solution phase approach that had previously been used to access the six diastereomeric δ-amino acid homooligomers (see **Chapter 1.5.1**) involved the selective protection of the acid and amine functionalities.[1-9]

The synthesis of the monomers involved the introduction of the amine group in protected form as the azide. The carboxylic acid was converted to the isopropyl carboxylic ester;[1,3,4,6,7] the use of a methyl ester as the acid protection in the amine component has been known to result in uncontrolled intermolecular condensation reactions[3] (comparable to the intramolecular cyclisation[1] mentioned in **Scheme 1.3**, **Chapter 1.5.1** leading to lactam **41**).

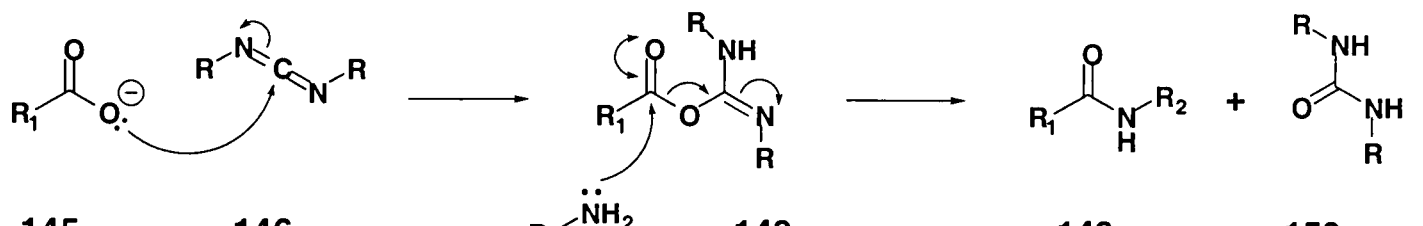
In order to perform a coupling reaction the amino terminus was deprotected by palladium catalysed reduction of the azide in an atmosphere of hydrogen gas. One equivalent of the ester was hydrolysed under basic aqueous conditions to give the acid component. Combination of the two components in the presence of an appropriate coupling reagent resulted in the formation of the required peptide bond.

In this way two monomeric units afforded the dimer, coupling of two dimers gave the tetramer, and coupling of two tetramers yielded the octamer.[5,7] In two cases the hexamer was formed by coupling of dimer to tetramer.[1,6] The reasons for this will be discussed later (see **Chapter 3.1.5**).

3.1.3 Coupling reagents

There are many carbodiimide based reagents commonly used in the conventional coupling of amino acid residues. They work by effecting the dehydration of the residues, driven by the highly favourable formation of a urea.

When a carboxylic acid **145** and an amine **147** are treated with carbodiimide **146** in the presence of basic catalysis acid **145** adds to the carbodiimide **146**, **Scheme 3.1**. **147** then reacts with the activated acid carbonyl group to give peptide **149** and urea **150**.

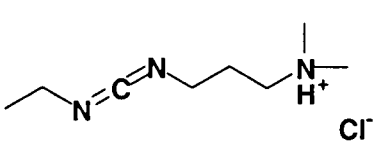


147

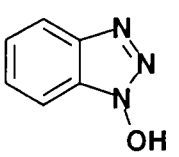
Scheme 3.1

Previous solution phase peptide synthesis by the group (see **Chapter 1.5.1**) has utilised EDCI **151**, **Figure 3.2**, **p72**, in DCM[4,5,7] or DMF[1,3] in the presence of HOBt **152** and diisopropylethylamine (DIPEA). Gellman's synthesis of the *trans*-aminocycloalkanecarboxylic acid oligomers[11] and Seebach's β- and γ-peptide syntheses[12-14] (see **Chapters 1.3.3-1.3.5**) used EDCI in the presence of 4-(*N*,*N*-dimethylamino)pyridine (DMAP) or *N*-methylmorpholine (NMM). Dicyclohexylcarbodiimide (DCC) **153** and DMAP[13] or *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HATU) **154** have also been employed.[14]

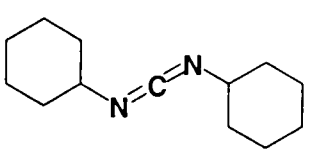
The coupling reactions were also found by co-workers to be highly efficient when *O*-(benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborate (TBTU) **155** was used as the coupling reagent,[6] significantly for the coupling of compounds similar to oligomer targets **141-144**.



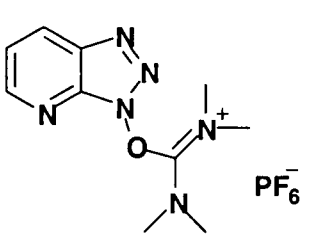
151; EDCI



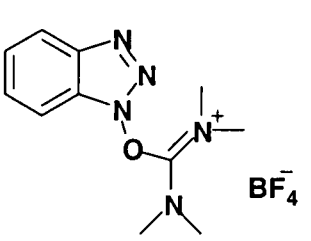
152; HOBt



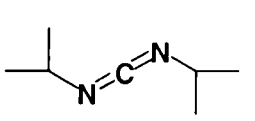
153; DCC



154; HATU



155; TBTU



156; DIC

Figure 3.2

Finally, the coupling of monomer residue to a solid-supported peptide (see **Scheme 1.4**, **Chapter 1.5.1**) was carried out using diisopropylcarbodiimide (DIC) **156** and HOBt[2] or DMAP[15] in DMF.

3.1.4 Hydroxyl protecting groups

Previous synthesis has utilised several different hydroxyl group protection schemes. While the free hydroxyl groups are not believed to affect the course of the coupling reaction,[1,3] the polarity they engender has been found to cause solubility problems which can lead to purification and handling difficulties.

The dihydroxyl moieties have therefore been protected as the isopropylidene[4-6] or cyclohexylidene[4,7] ketals or as the diacetic ester,[1,3] **Figure 3.3**. The principal advantage of the non-aromatic ketal protecting groups is their compatibility with NMR and CD spectroscopy (see **Chapter 4.2**). Acetates, however, contain an additional chromophore which complicates the CD spectrum and makes meaningful analysis difficult to achieve.

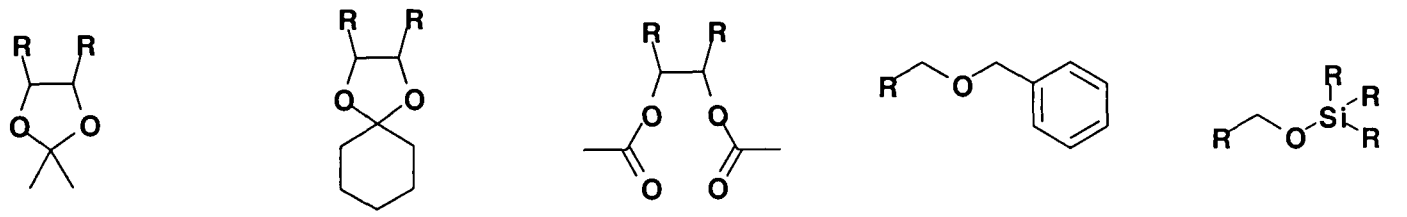


Figure 3.3

Other research in the group has utilised benzyl and silyl ether protecting groups where dihydroxyl protection was not possible.[8,16] However, the NMR signals relating to the aromatic protons of the benzyl ether appear in the amide region of the spectrum and cause assignment difficulties, while the benzyl chromophore again saturates the CD spectrum and renders the data too complex to interpret. The silyl ether is compatible with both NMR and CD spectroscopy.

3.1.5 Chain length and structural analysis

It was mentioned above that increasing chain length can present handling problems, which can be overcome by the use of suitable side-chain functionality protection. There is also the consideration of decreasing yields for each of the coupling steps as the chain length increases.

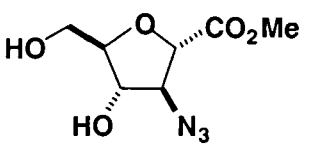
More difficulties arise in the assignment of NMR data as the number of residues increases. By the time the oligomer reaches eight residues in length the signals overlap to such an extent that they can no longer be resolved. This factor must be offset against the chain length that is required for any stable secondary structure to be observed.

The helical conformations discussed earlier (see **Chapters 1.3.3-1.3.5**) are stabilised by long-range interaction of residue *n* with residue $n \pm 1$, $n \pm 2$ or $n \pm 3$. Therefore the first signs of long-range order could be manifested in peptides of two or three residues in length. For the tetramer, however, there is the possibility for formation of any of the aforementioned hydrogen-bonded rings, and thus there should be large enough populations of any stabilised conformations for these to be observed by NMR and CD spectroscopy.

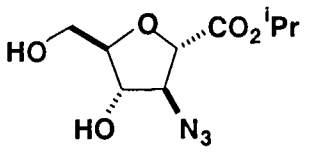
Taking into account each of these considerations, it was decided to target the oligomers consisting of four and, depending on availability of material, six residues in length.

3.2 Results and discussion I

As previously discussed, four diastereomeric azido esters had been selected for oligomerisation (see **Chapter 3.1.1**). The first of these to be chosen was the D-*manno* isomer **97**, **Figure 3.4**. This section deals with four different strategies of peptide formation for this isomer only. The D-*manno* isopropyl ester **116** was obtained as previously described (see **Chapters 2.2.1-2.2.2**).



97; D-*manno*



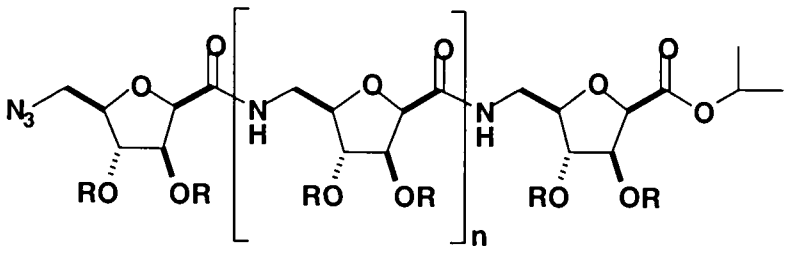
116; D-*manno*

Figure 3.4

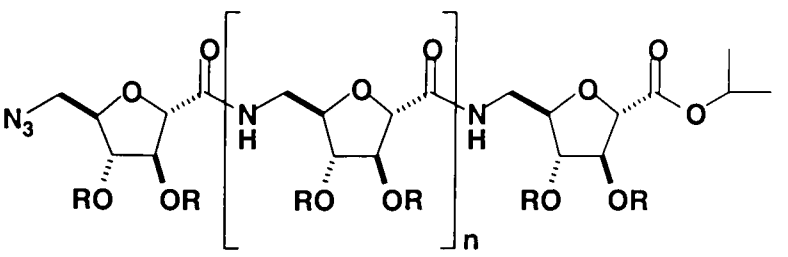
3.2.1 Strategies towards oligomers – *manno* series

While a conventional peptide coupling should be possible, it was proposed first to probe a novel single residue strategy for the oligomerisation. Feasibility studies would also be conducted on solid phase and microwave assisted approaches.

It was discussed earlier (see **Scheme 1.3**, **Chapter 1.5.1**) that the D-*gulo* **44**[1] and D-*manno* **61**[3] δ-peptide dimers, **Figure 3.5**, **p76**, were constructed using established methodology without the need for protection of the secondary hydroxyl groups of the monomers. When the oligomers reached four residues the high polarity engendered by the presence of eight free hydroxyl groups caused isolation difficulties. The solution to this problem was to treat the coupling mixture with acetic anhydride in pyridine, thus protecting the hydroxyl groups as the acetates.[1,3]



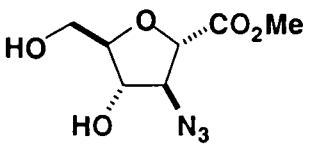
44; n = 0, R = H
47; n = 2, R = Ac
49; n = 4, R = Ac
} D-*gluco*



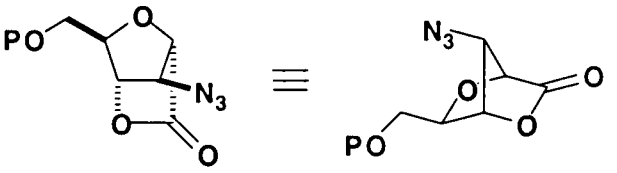
61; n = 0, R = H
62; n = 2, R = Ac
} D-*manno*

Figure 3.5

It was hoped that the peptide coupling reactions would be similarly unaffected by the primary hydroxyl functionality of azido ester **97**, **Figure 3.6**. The protecting schemes available for this compound include the peracetate, benzyl ether and a range of silyl ethers. The 4,6-trans relationship means that the use of ketal protecting groups is not possible.



97

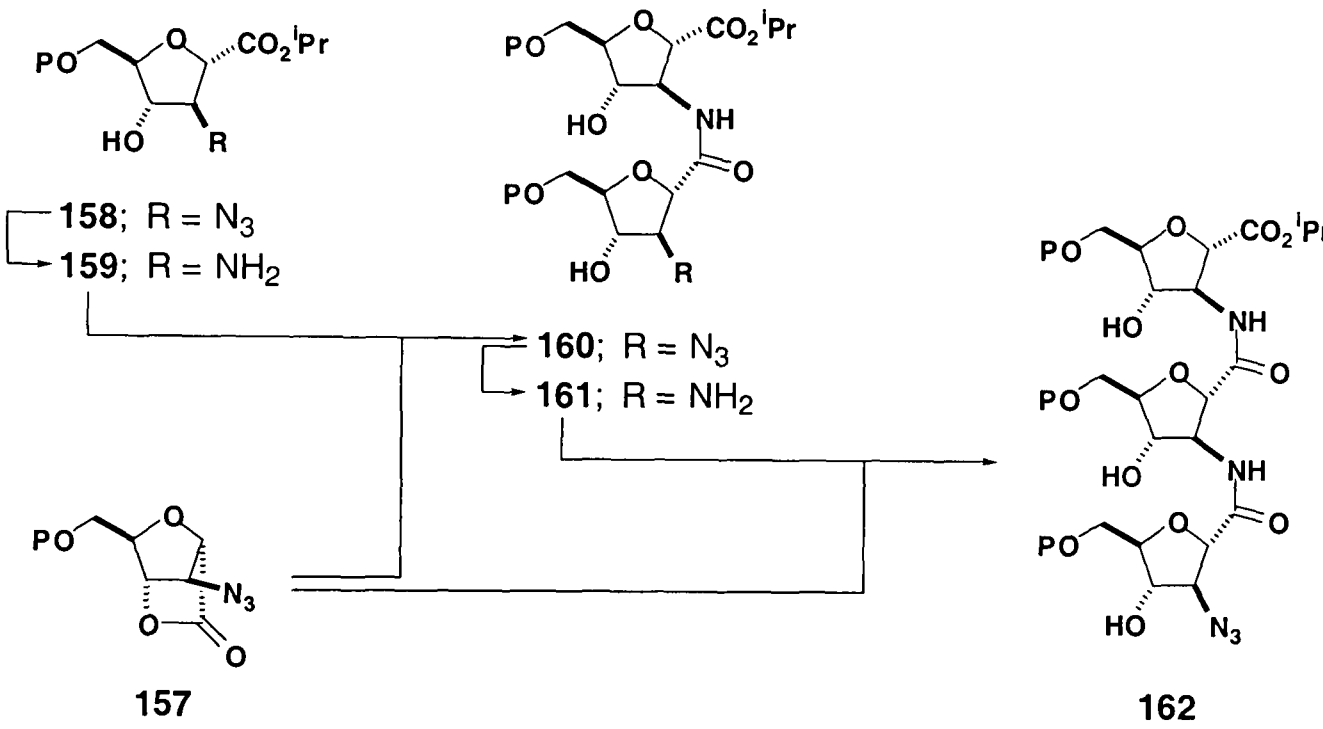


157; P = suitable protecting group

Figure 3.6

However, the fact that the C-4 hydroxyl substituent of azido ester **97** has a *cis* orientation with respect to the carboxylate lends the possibility of formation of a bicyclic system containing a lactone ring **157**.

Reaction of an amine with bicyclic lactone **157** should result in the direct formation of dimeric species without the need for coupling agents, **Scheme 3.2**. Reaction of amine **159** with **157** should give dimer **160**. Reduction of the azide functionality of dimer **160** to the amine **161** and treatment with excess bicyclic lactone **157** would give the trimer **162**. In this way the chain would be extended by a single residue with each coupling to afford the tetramer after three iterations. In addition to the simplicity of the coupling reaction, the purification of the oligomers formed should be facile.



P = suitable protecting group

Scheme 3.2

3.2.2 Bicyclic lactone coupling – *manno* series

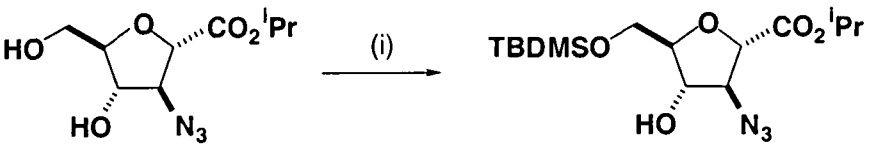
The first approach to be investigated was the use of a bicyclic lactone **157**, as described above. The primary hydroxyl protecting group was chosen as the *tert*-butyldimethylsilyl ether, on account of its orthogonality to the amine and acid protecting groups (azide and carboxylic ester respectively). Moreover, the use of a lipophilic protecting group should facilitate the handling of the oligomers, which otherwise would have increasing polarity as the number of free hydroxyl functionalities was augmented.

The acid component would therefore be the silyl protected bicyclic lactone **163**, and the amine component the isopropyl ester **164**, **Figure 3.7**.

163 **164**

Figure 3.7

Ester **164** would be acquired by deprotection of the amine terminus of silyl ether **165**, **Scheme 3.3**. Azido ester **165** was formed by silylation of isopropyl ester **116**, which had been synthesised from diacetone glucose in 42% overall yield (see **Chapters 2.2.1-2.2.2**). Treatment of **116** with 1.1 equivalents of *tert*-butyldimethylsilyl (TBDMS) chloride in the presence of imidazole gave silyl ether **165**. Only a small amount of disilylated product was observed, however the yield of the desired product was a disappointing 43%, due to some C-6 hydroxyl deprotection on the silica column during chromatographic purification.



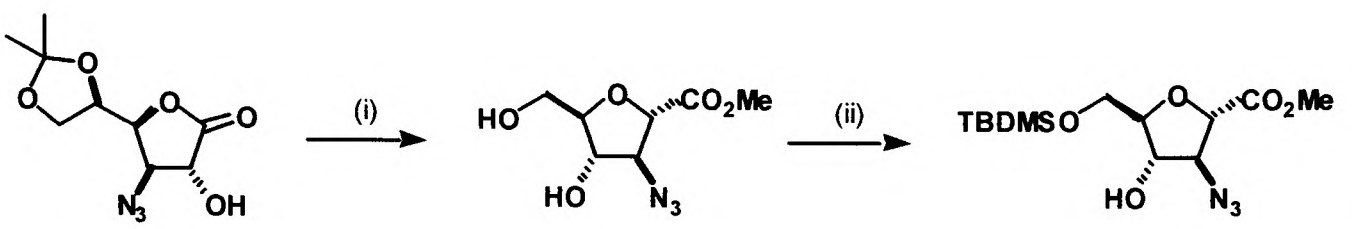
116 **165**

Reagents and Conditions: (i) TBDMSCl, imidazole, DMF, -10°C

Scheme 3.3

In order to access silyl protected bicyclic lactone **157**, it was decided to form the 6-*O*-silyl methyl ester **115**, which would then be cyclised by ester hydrolysis followed by intramolecular condensation. It was discussed previously (see **Chapter 2.2.2**) that methyl ester **97** is not readily

accessible in pure form, due to the presence of inorganic triflate salts. However, treatment of the crude **97** with TBDMS chloride in the presence of imidazole gave **115** in 45% from the lactone **93, Scheme 3.4**.



93 **97** **115**

Reagents and Conditions: (i) Tf_2O, pyridine, DCM, 0ÞC then MeOH, HCl, 0ÞC-r.t.;
(ii) TBDMSCl, imidazole, DMF, 0ÞC

Scheme 3.4

115 was readily crystallised from ethyl acetate and hexane, and x-ray diffraction confirmed the D-*manno* stereochemistry, **Figure 3.8**.

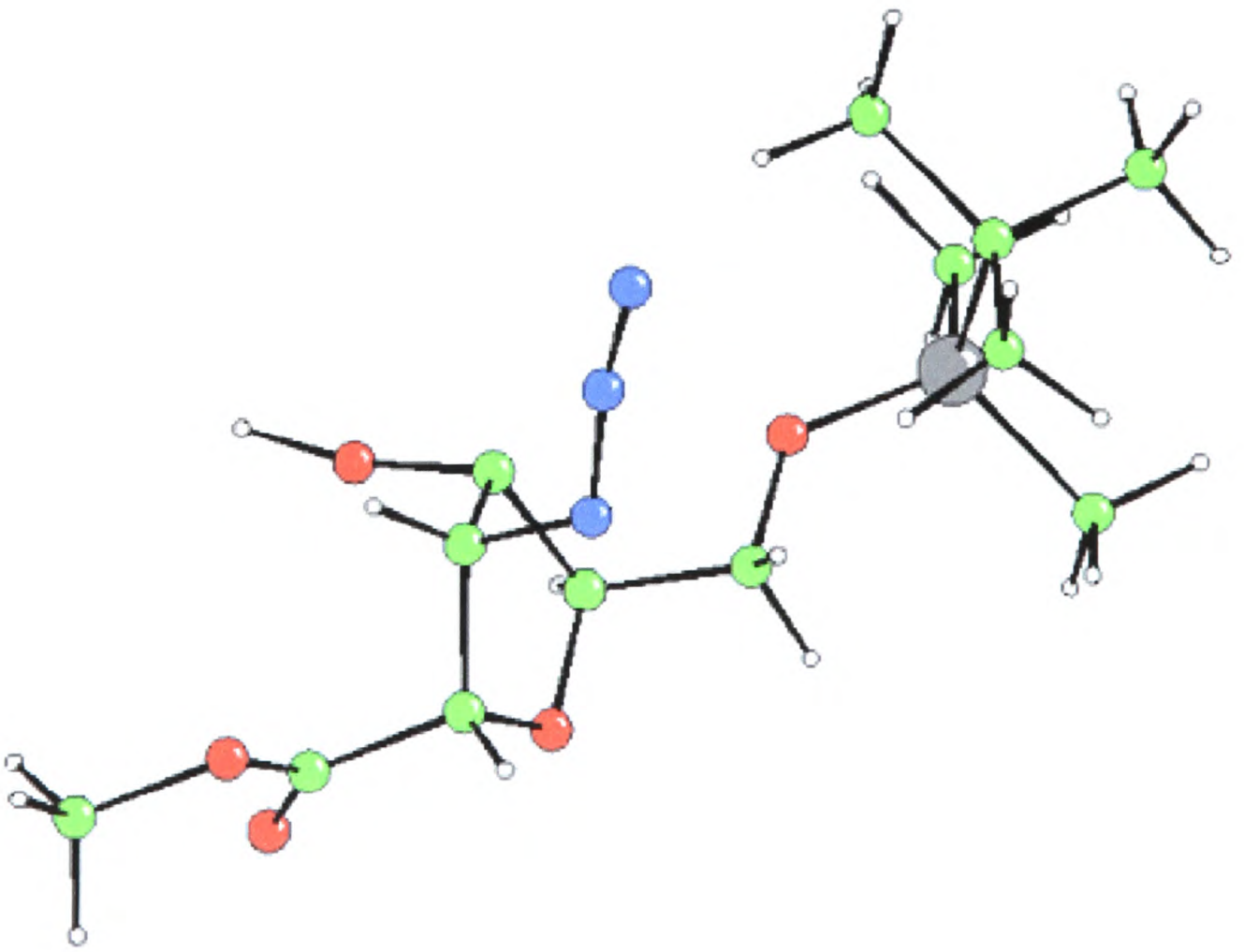
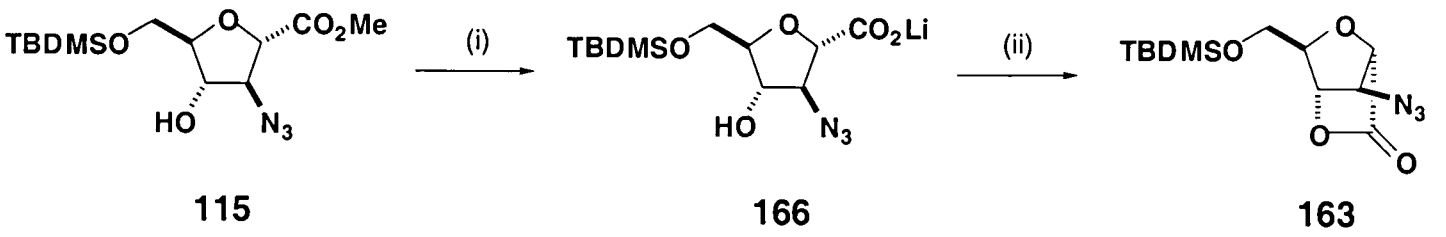


Figure 3.8. Crystal structure of 115.

Hydrolysis of the carboxylic ester was performed by treatment of silyl protected species **115** with lithium hydroxide in water and THF to give lithium salt **166** which was not isolated. Dehydration of **166** was effected by the use of acetic anhydride in the presence of pyridine to afford bicyclic lactone **163**, **Scheme 3.5**.

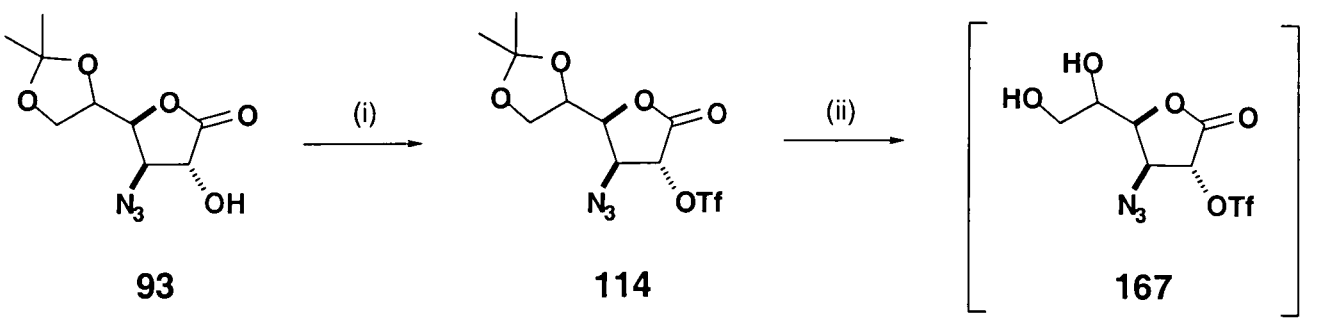


Reagents and Conditions: (i) $LiOH$, H_2O, THF; (ii) Ac_2O, pyridine, DCM

Scheme 3.5

However, the yields for this reaction were very poor, between 5% and 14%, presumed to be owing to the cleavage of the silyl ether during the hydrolysis step.

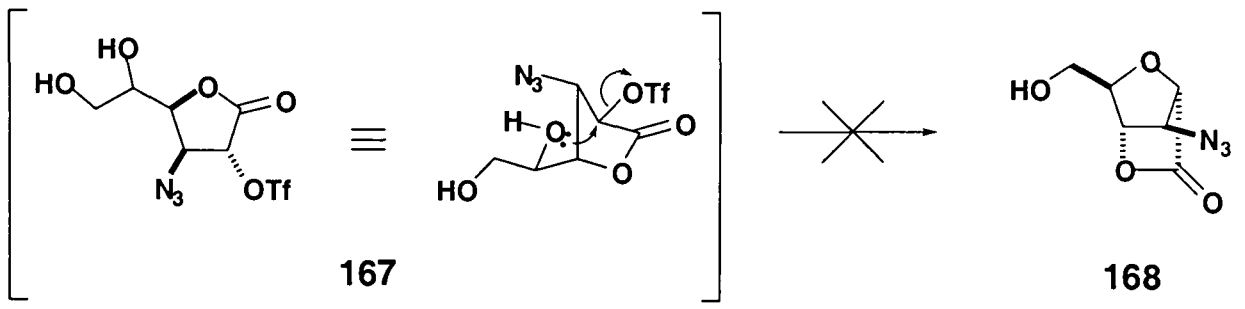
An alternative approach was conceived where the bicyclic compound is formed directly from lactone **93**, the THF ring being established by intramolecular S_N2 displacement of a sulphonate leaving group at C-2 without prior opening of the lactone ring. Accordingly, lactone **93** in DCM was treated with triflic anhydride in the presence of pyridine, **Scheme 3.6**. The triflate **114** was subjected to acidic conditions in the absence of methanol, with the aim of removal of the 5,6-acetonide without lactone opening.



Reagents and Conditions: (i) Tf_2O, pyridine, DCM, 0°C; (ii) AcOH, H_2O

Scheme 3.6

It was hoped that the C-5 hydroxyl of triflate **167** would then displace the triflate by nucleophilic attack to give bicyclic lactone **168, Scheme 3.7**. However, no bicyclic product was observed in the reaction mixture or after work-up. The aqueous acidic conditions were presumed to open the lactone to give the free acid.



Scheme 3.7

Finally, an attempt was made to form the bicycle by dehydration of acid azide **169** using a solid-supported carbodiimide. Hydrolysis of azido ester **116** with aqueous sodium hydroxide in dioxane followed by purification by ion exchange using Amberlite IR-120 (H^+) resin afforded acid **169** in 95% yield, **Scheme 3.8, p82**. It was hoped that treatment of **169** with commercially available *N*-cyclohexylcarbodiimide, *N'*-methylpolystyrene **170**[†] would activate the carboxyl group, enabling intramolecular attack by free C-4 hydroxyl to take place. While the starting material was consumed during the course of the reaction, no bicyclic product **168** was observed. It was postulated that solid-supported species **171** is too inert for the intramolecular displacement to take place, though no further studies were carried out.

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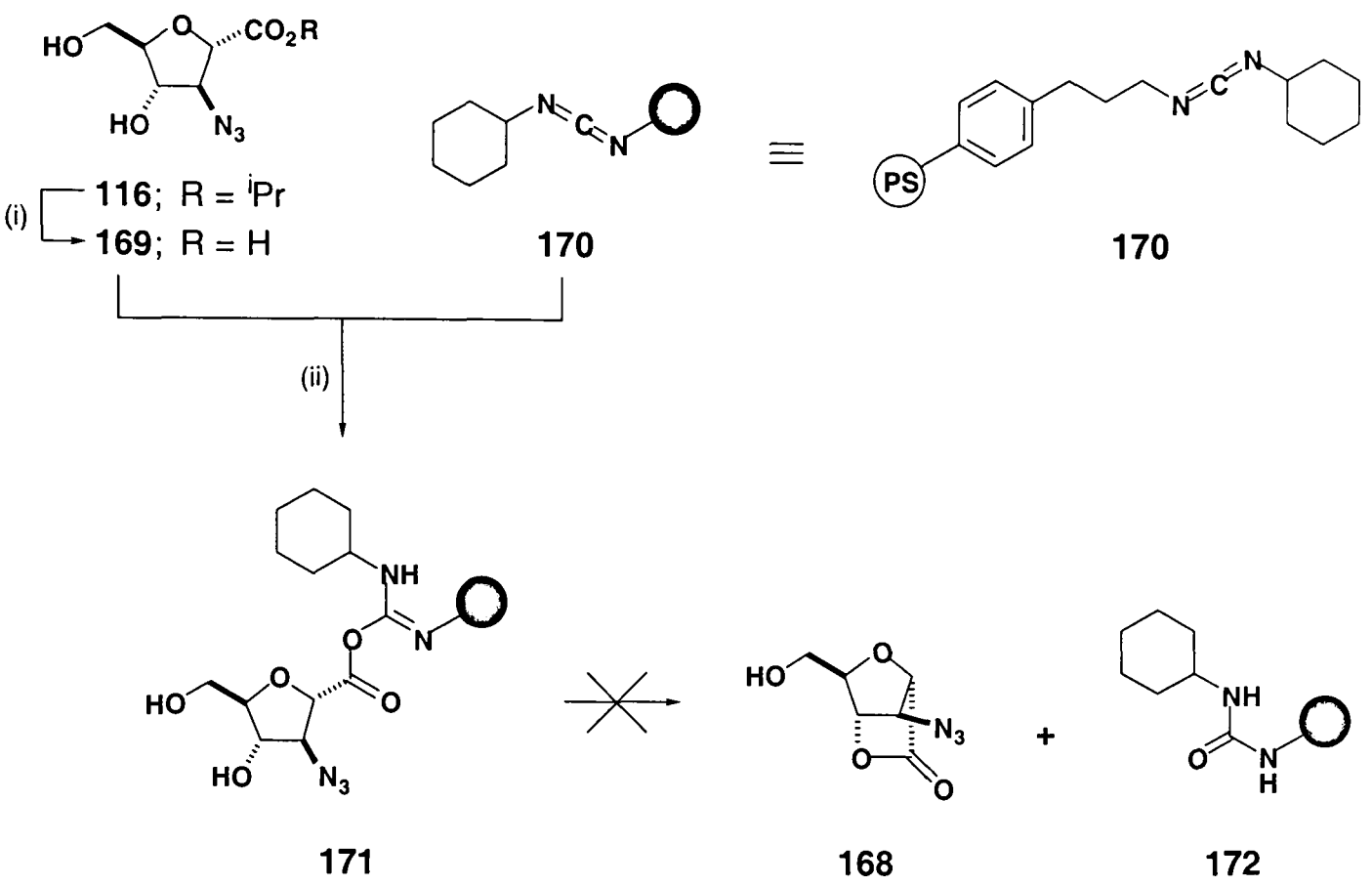


Reagents and Conditions: (i) NaOH, H_2O, dioxane then Amberlite IR120 (H^+), H_2O; (ii) DMF, DCM

Scheme 3.8

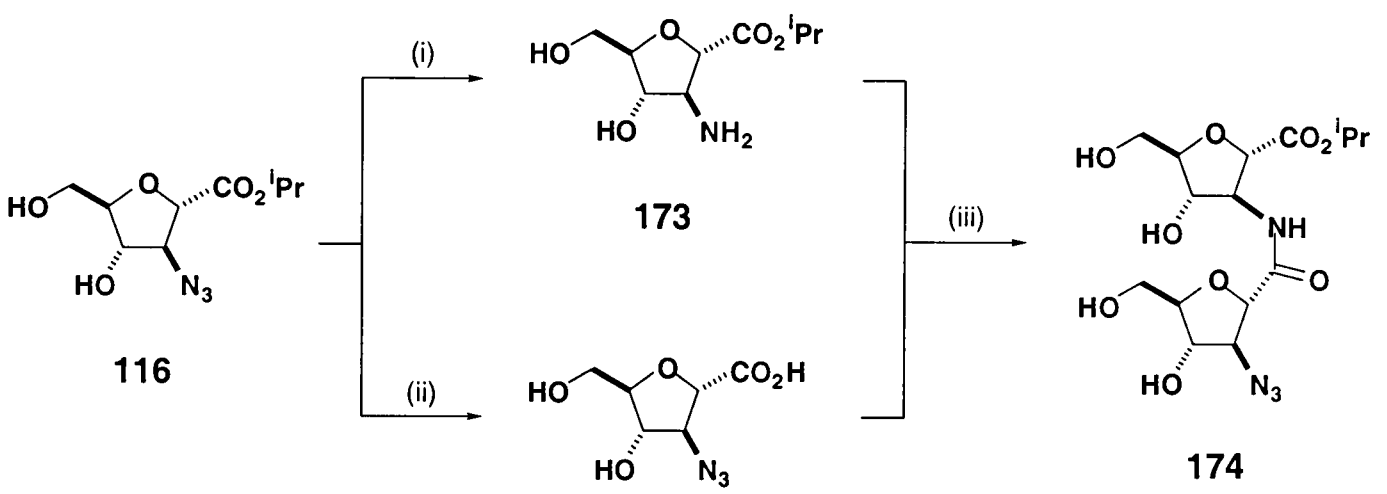
This iterative approach to oligomer synthesis relies on using an excess of bicyclic lactone **157** each time a residue is to be added to the molecule. The prohibitively low yields of formation of **157** dictated that a different strategy was required for the peptide bond creation.

3.2.3 Conventional coupling – *manno* series

In the light of the success of the conventional peptide coupling strategy for related compounds it was decided to utilise this approach to attempt the oligomerisation of azido ester **97**. The required monomer components were therefore azido acid **169** and amino ester **173**, **Figure 3.9**.

Figure 3.9

Accordingly, isopropyl azido ester **116** was subjected to hydrogenation in isopropanol in the presence of palladium black to give amino ester **173**, which was used without further purification, **Scheme 3.9**. Hydrolysis of the ester functionality of ester **116** with aqueous sodium hydroxide in dioxane was followed by ion exchange using Amberlite IR-120 (H^+) resin to give acid **169**. The coupling of monomer units **169** and **173** was performed using TBTU (see **Figure 3.2, Chapter 3.1.3**) in DMF in the presence of DIPEA to give unprotected dimer **174** in 80-90% yield from monomer **116**.

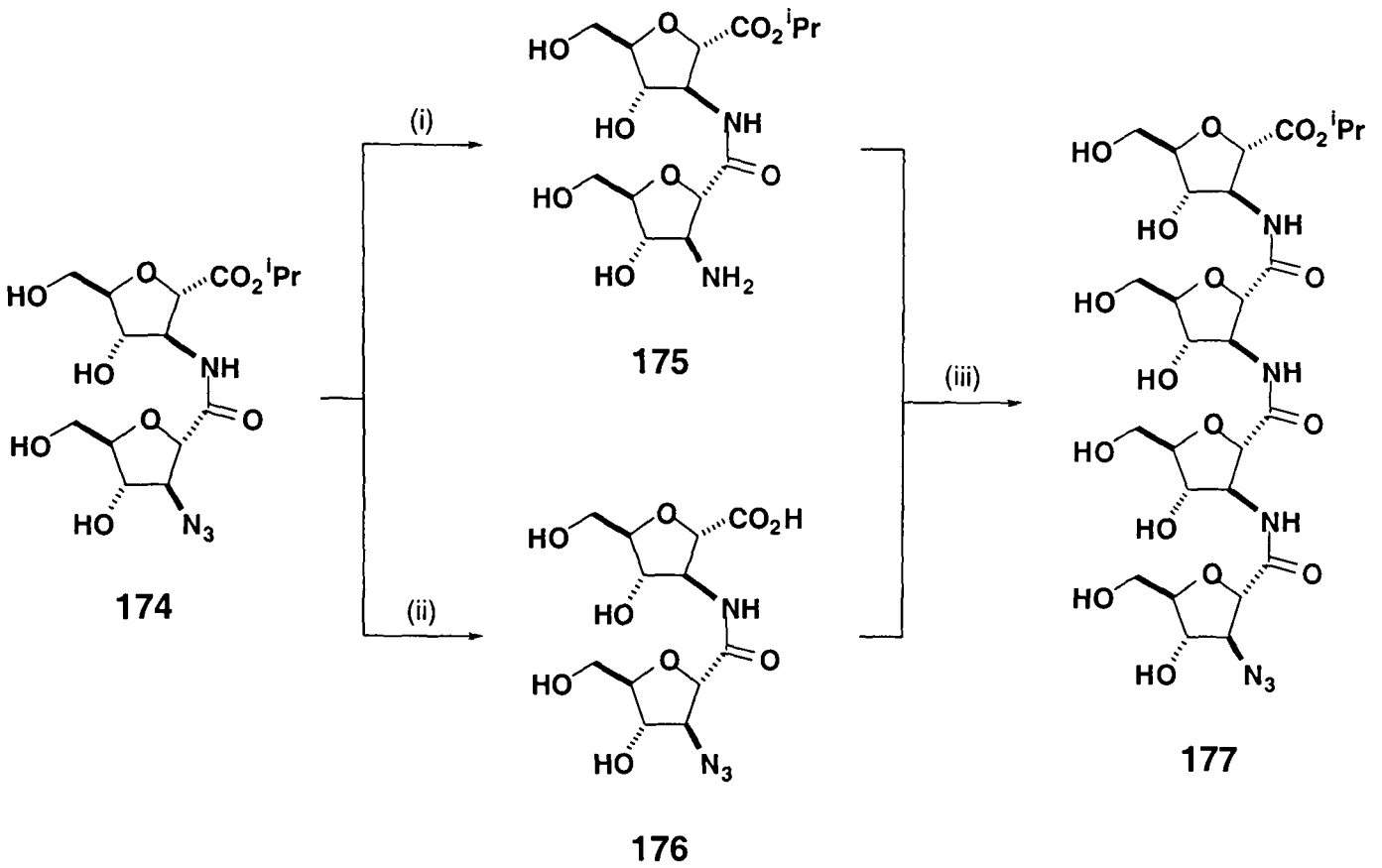


Reagents and Conditions: (i) H_2, Pd black, ^{i}PrOH; (ii) NaOH, H_2O, dioxane then Amberlite IR-120 (H^+), H_2O; (iii) TBTU, DIPEA, DMF

Scheme 3.9

Iteration of the deprotection and coupling procedures gave access to the tetramer **177**, **Scheme 3.10, p84**. The azide functionality of dimer **174** was reduced with hydrogen and palladium black to give amine component **175** which was used without further purification. The acid component **176** was formed by treatment of dimer **174** with aqueous sodium hydroxide in dioxane, followed by purification using Amberlite IR-120 (H+) ion exchange resin. The peptide coupling of **175** and **176** was achieved as previously using TBTU in DMF in the presence of

DIPEA to yield tetramer **177**, which was isolable by standard flash silica chromatography, in 70-80% yield.

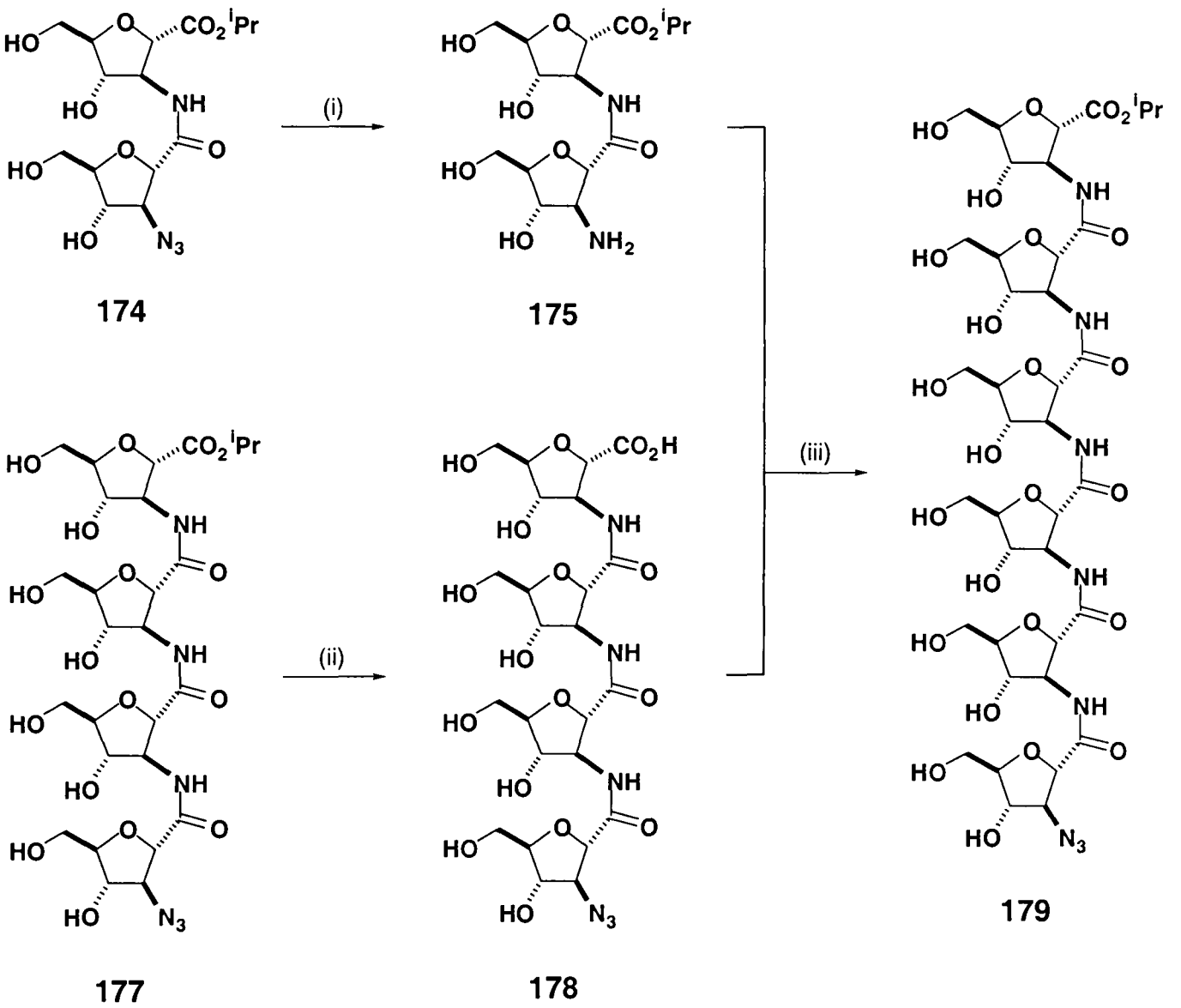


Reagents and Conditions: (i) H_2, Pd black, H_2O; (ii) NaOH, H_2O, dioxane then Amberlite IR-120 (H^+); (iii) TBTU, DIPEA, DMF

Scheme 3.10

Sufficient material was available for extension of the peptide backbone to be possible. For the reasons mentioned earlier (see **Chapter 3.1.5**) it was decided to target the hexamer rather than the octamer, and this was accomplished by the coupling of tetrameric acid with dimeric amine, **Scheme 3.11, p85**.

Accordingly, dimer **174** was hydrogenated in the presence of palladium black to give amine **175**, while tetramer **177** was hydrolysed with aqueous sodium hydroxide in dioxane then subjected to ion exchange to afford tetrameric acid component **178**. The coupling was carried out as before with TBTU in DMF in the presence of DIPEA.

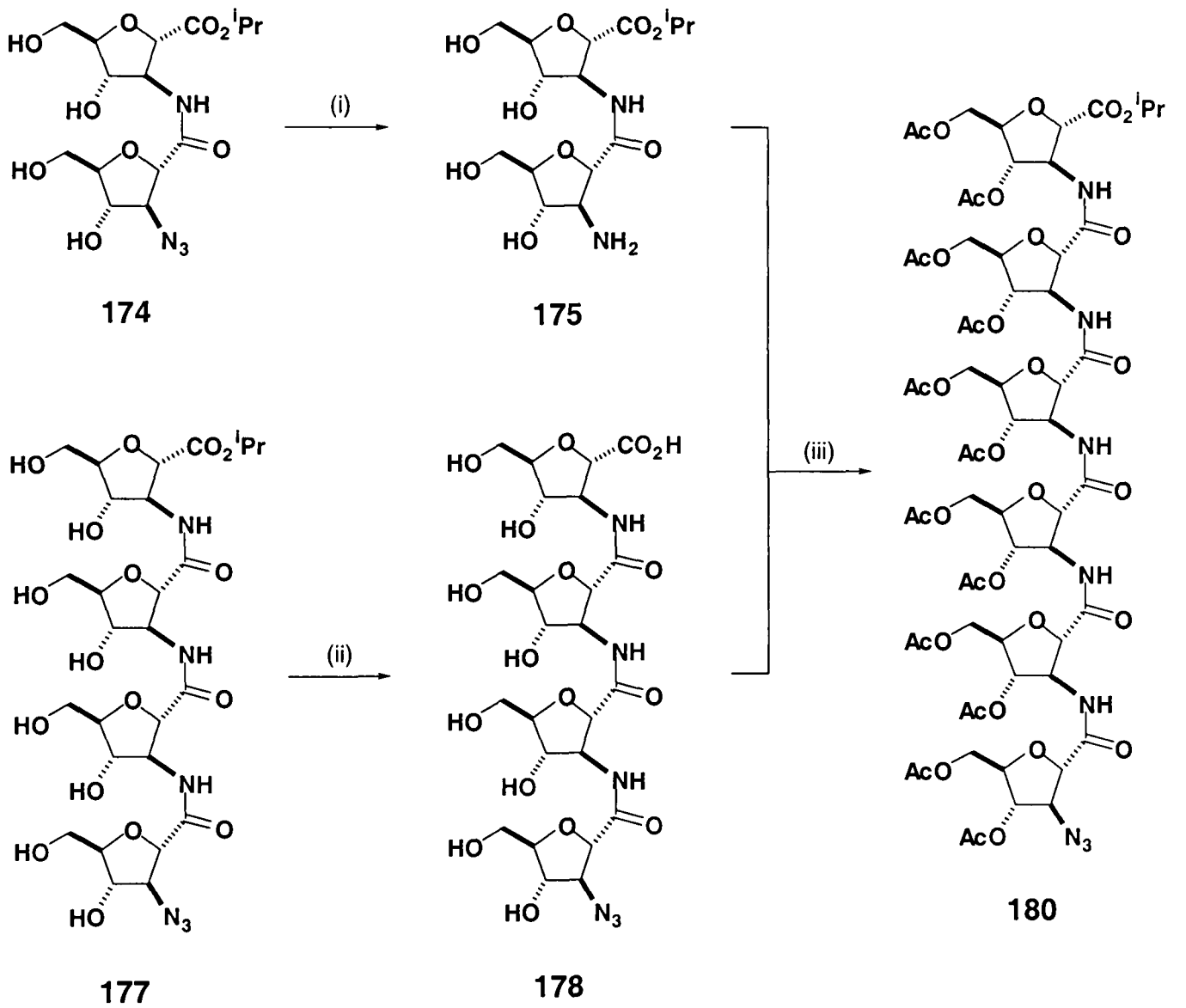


Reagents and Conditions: (i) H_2, Pd black, H_2O; (ii) NaOH, H_2O, dioxane then Amberlite IR-120 (H^+); (iii) TBTU, DIPEA, DMF

Scheme 3.11

However, the twelve free hydroxyl functionalities of the resulting hexamer **179** render it far too polar for conventional chromatographic techniques. Attempts were made to isolate **179** by size exclusion chromatography using Sephadex® LH-20 eluting with water, but these proved to be unsuccessful and it was deemed necessary to protect the hydroxyl groups as the acetic esters.

Following the established procedure of acetylation[1,3] (see **Scheme 1.3**, **Chapter 1.5.1**) the tetrameric acid and dimeric amine components were coupled as before, and on completion of the reaction the mixture was treated with acetic anhydride in pyridine, **Scheme 3.12**. The major product was purified by column chromatography on silica gel to afford peracetyl hexamer **180** in 19% yield from tetramer **177**.



Reagents and Conditions: (i) H_2, Pd black, H_2O; (ii) NaOH, H_2O, dioxane then Amberlite IR-120 (H^+); (iii) TBTU, DIPEA, DMF then Ac_2O, pyridine

Scheme 3.12

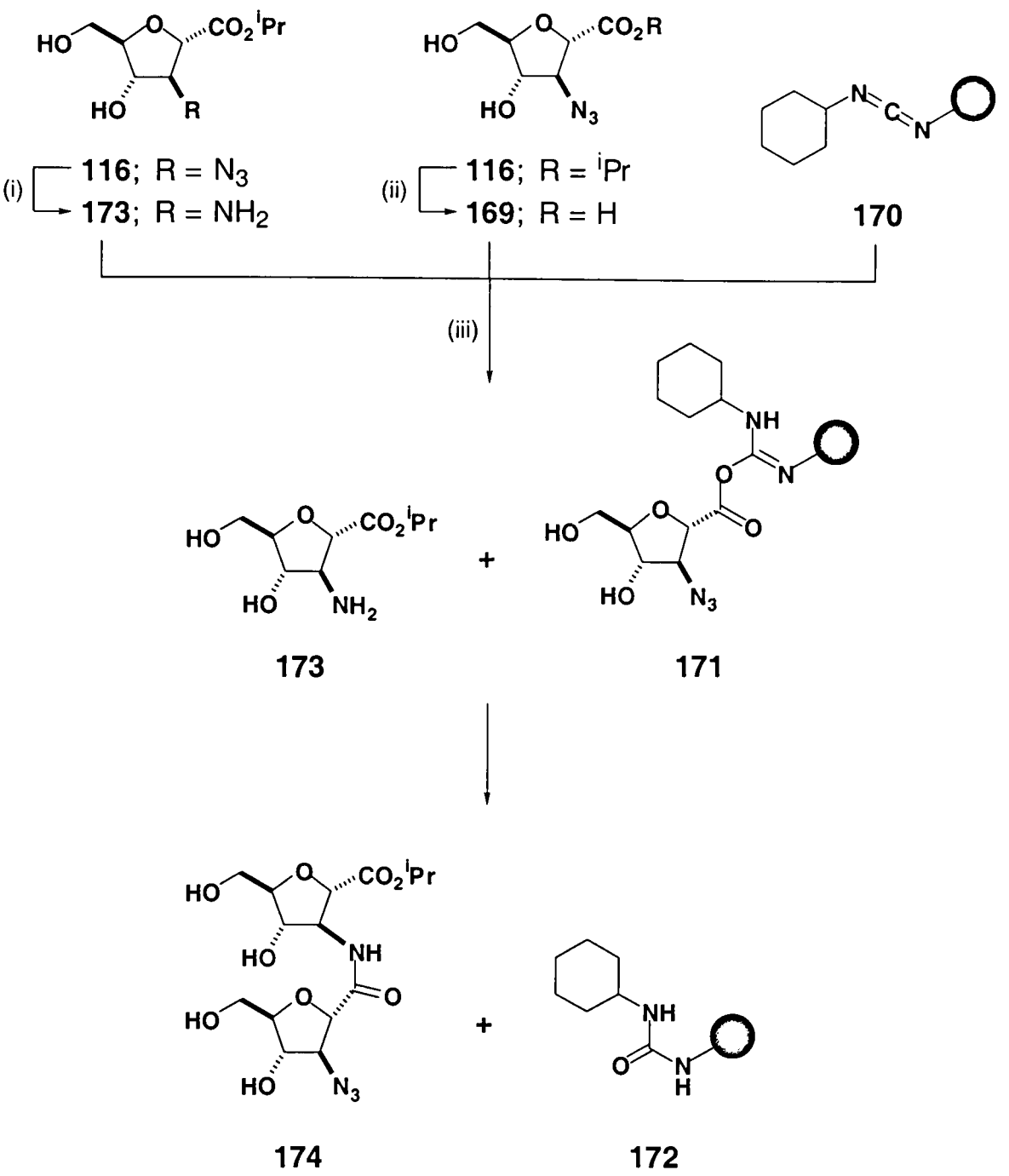
In conclusion, hexameric species **180** was synthesised in 14% over ten steps from azido ester starting material **116**, and in 6% overall yield from diacetone glucose.

3.2.4 Solid-phase coupling – *manno* series

A solid-phase synthesis of THF amino acid oligomers has previously been described[2] (see **Scheme 1.4**, **Chapter 1.5.1**). The immediate advantage of this approach is the ease of isolation of the coupled product due to the fact that the reagents are removed simply by washing the beads while the substrate remains secured to the support.

An alternative use of solid-phase support was investigated in the coupling of D-*manno* amino acid **97**. Instead of affixing the first residue to the bead and coupling each monomer iteratively, this method uses a solid-supported coupling reagent while the starting material and product remain in solution. This method has advantages over the solid-phase synthesis described above; purification is not required as the only compound in solution at completion of the reaction should be dimeric product, and the need for a separate step for cleavage of the product from the support is avoided.

The acid and amine components were prepared from azido ester **116** as for the conventional coupling (see **Scheme 3.9**, **Chapter 3.2.3**), **Scheme 3.13**, **p 88**. The reagent used was carbodiimide tethered to a polystyrene resin, **170** (see **Scheme 3.8**, **Chapter 3.2.2**). A small excess of acid component **169** was used, as the excess should remain secured to the support.



Reagents and Conditions: (i) H_2, Pd/C, ^{i}PrOH; (ii) NaOH, H_2O, dioxane then Amberlite IR120 (H^+), H_2O; (iii) THF, DCM

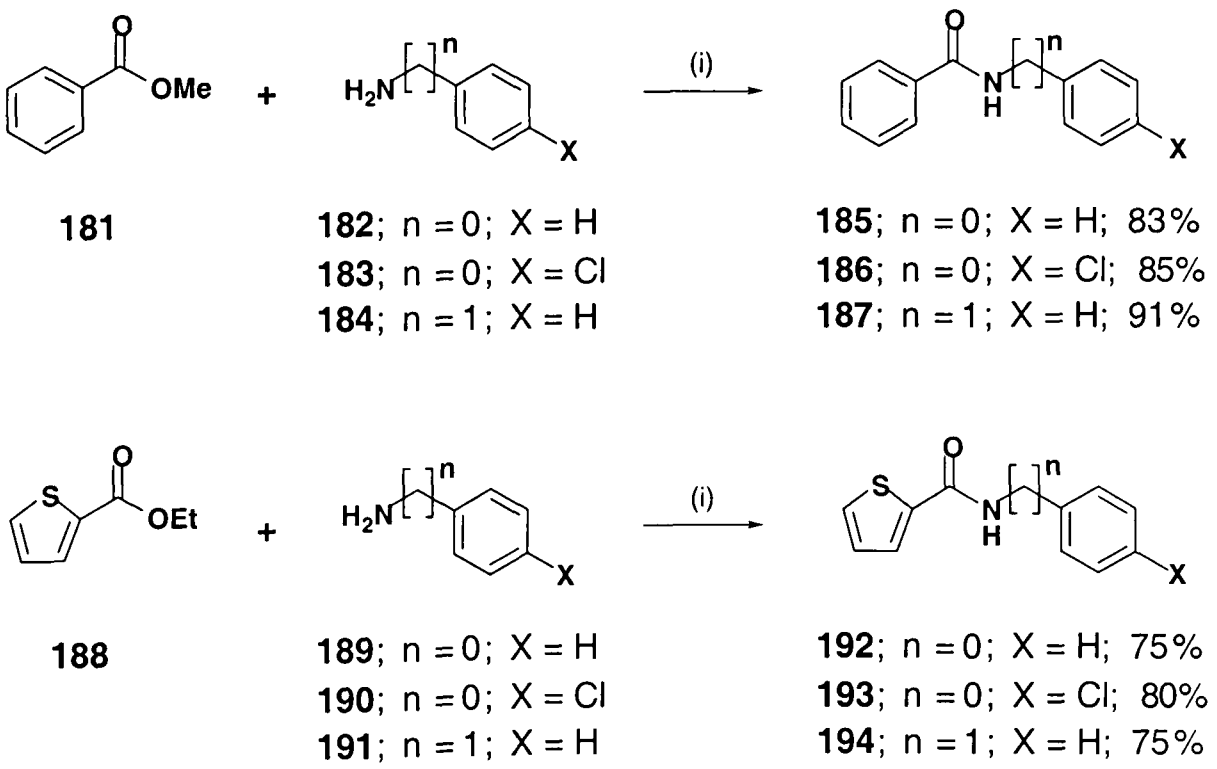
Scheme 3.13

While a small amount of dimer **174** was identified by liquid chromatography / mass spectrometry (LCMS) this reaction was not optimised. One possible reason for the low conversion is the relative chemical stability of the intermediate **171**, which was discussed earlier (see **Scheme 3.8**, **Chapter 3.2.2**).

3.2.5 Microwave activated coupling – *manno* series

Microwave technology has been increasingly used in organic synthesis in recent years.[17] Microwaves present a different method of heating reaction mixtures which can significantly enhance reaction rates and influence chemoselectivity relative to conventional heating.[18] These non-conventional effects are thought to be attributable to thermal effects – fast mass heating of the material, different boiling rates and overheating[18] – as well as non-thermal effects that are due to the inherent properties of the microwaves.[19] This use of microwave flash-heating has been found useful in high-speed combinatorial chemistry[20,21] where enhanced reaction times are highly desirable.

Microwave activation conditions have also been used in the synthesis of amide bonds by direct ester aminolysis, a step which generally requires high temperatures and extended reaction times.[17,22] The coupling under basic conditions of amines with non-enolisable esters was achieved in good yields and reaction times under seven minutes,[22] **Scheme 3.14**.

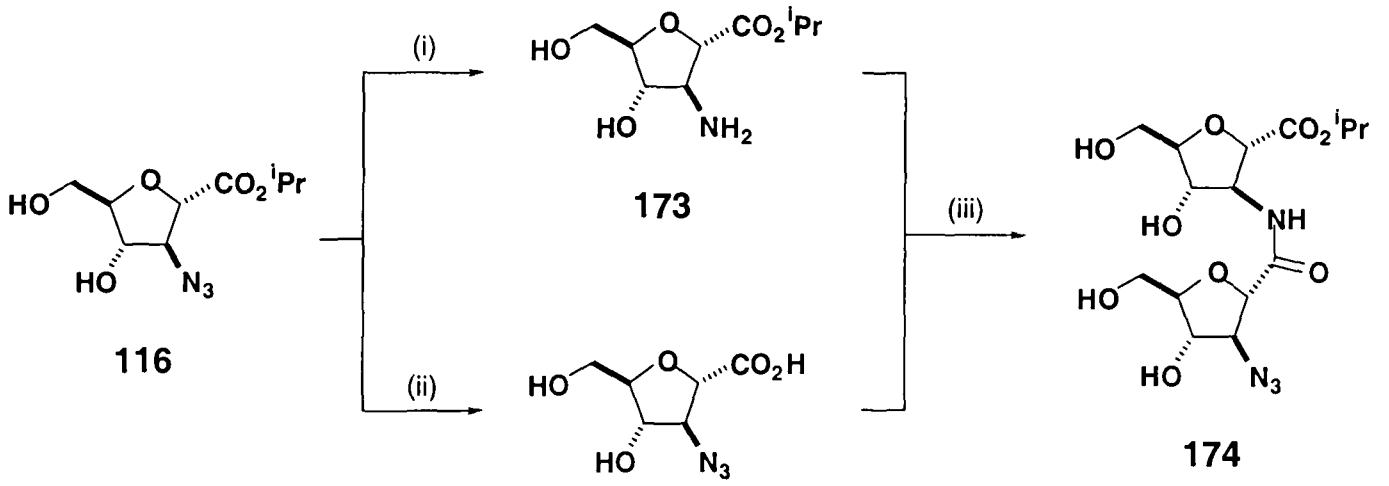


Reagents and Conditions: (i) ^{t}BuOK, microwave

Scheme 3.14

A microwave activated coupling of D-*manno* acid **169** and amine **173** was envisioned. It was hoped that microwave heating would cause the evaporation of any water from the reaction mixture, driving the reaction forward without the need for coupling reagents.

Accordingly, treatment of azido ester **116** with aqueous sodium hydroxide in dioxane followed by ion exchange chromatography with Amberlite IR-120 (H^+) gave azido acid **169**, **Scheme 3.15**. The azide functionality of **116** was hydrogenated in isopropanol in the presence of palladium black to give amino ester **173**. Several microwave activated coupling procedures were investigated, with variation of reaction time and power, **Table 3.1, p91**. 1-Methylpyrrolidin-2-one (NMP) was used as solvent on account of its high boiling point; only a drop was required to enable the acid and amine components to come into contact.



Reagents and Conditions: (i) H_2, Pd black, ^{i}PrOH; (ii) NaOH, H_2O, dioxane then Amberlite IR-120 (H^+), H_2O; (iii) NMP, microwave

Scheme 3.15

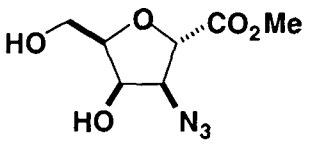
	Power	Time	Product
1	100%	6 min	Trace
2a	100%	1 min	Trace
2b	100%	2 min	Trace
2c	100%	3 min	Trace
2d	100%	4 min	Trace
2e	100%	5 min	Trace
3	60%	3 min	Trace
4	60%	6 min	Trace

Table 3.1

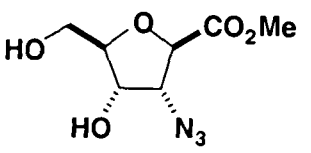
The composition of the reaction mixtures were elucidated by LCMS. Only trace amounts of dimeric product **174** were observed under any of the reaction conditions listed in **Table 3.1**.

3.3 Results and discussion II

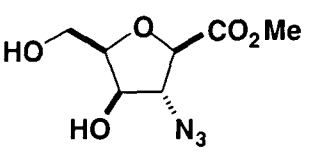
Following the success of conventional carbodiimide coupling in the synthesis of D-*manno* dimer, tetramer and acetyl-protected hexamer (see **Chapter 3.2.3**) it was decided to utilise this approach in the formation of the oligomers of the remaining three β-glycosamino acid precursors **98**, **107** and **108**, **Figure 3.10**.



98; D-*talo*



108; D-*allo*



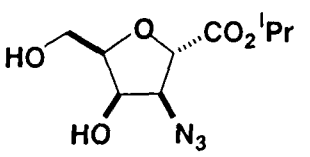
107; D-*gulo*

Figure 3.10

This section describes the syntheses of the homooligomers of **98**, **107** and **108**.

3.3.1 Conventional coupling – *talo* series

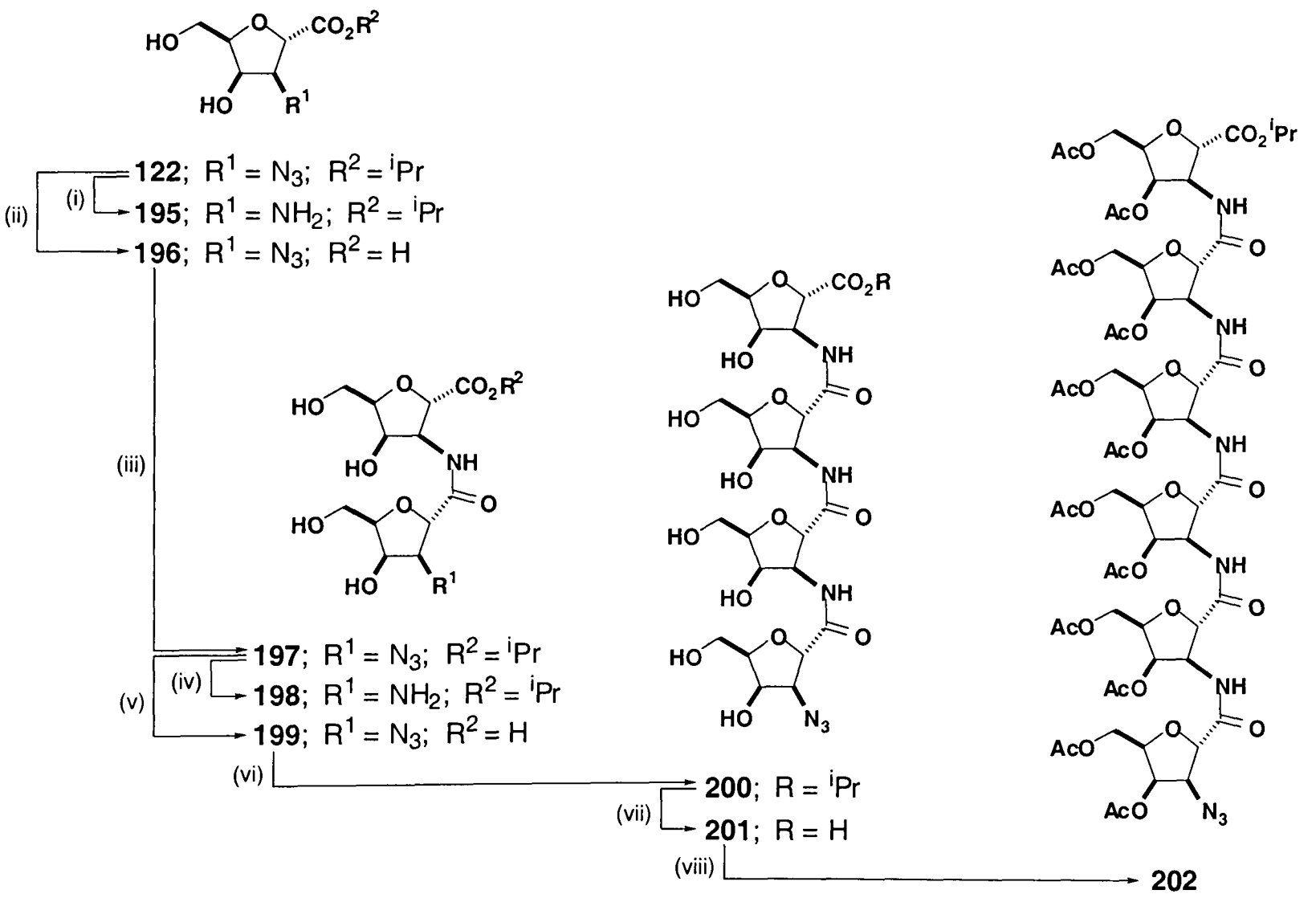
The synthesis of D-*talo* monomer starting material **122**, **Figure 3.11**, is described in the previous chapter (see **Chapters 2.2.3-2.2.4**).



122; D-*talo*

Figure 3.11

Reduction of the azide moiety of **122** was effected by hydrogenation in the presence of palladium black to give amine **195** which was used without purification, **Scheme 3.16**. Hydrolysis of the ester functionality of **122** followed by subjection to ion exchange afforded acid **196**. Coupling of amine **195** and acid **196** was achieved using TBTU and DIPEA in DMF to yield dimer **197**, 75-90%. Iteration of these procedures gave dimeric amine **198** and acid **199** which were coupled to provide tetramer **200** in 75-80% yield from the dimer. Finally, hydrolysis of tetramer **200** to give tetramer acid **201**, and coupling of **201** with dimer amine **198** followed by peracetylation afforded hexamer **202** in 17%.



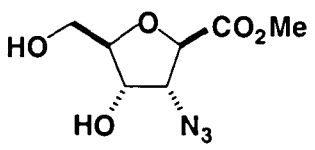
Reagents and Conditions: (i) H_2, Pd black, ^{i}PrOH; (ii) NaOH, H_2O, dioxane then Amberlite IR-120 (H^+); (iii) TBTU, DIPEA, DMF; (iv) H_2, Pd black, H_2O; (v) NaOH, H_2O, dioxane then Amberlite IR-120 (H^+); (vi) **198**, TBTU, DIPEA, DMF; (vii) NaOH, H_2O, dioxane then Amberlite IR-120 (H^+); (viii) **198**, TBTU, DIPEA, DMF then Ac_2O, pyridine

Scheme 3.16

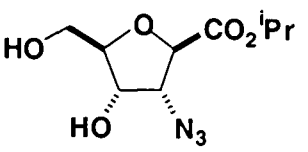
Hexamer **202** was thus synthesised from monomer **122** in ten steps and 12% yield, and from diacetone glucose in 2% overall yield.

3.3.2 Conventional coupling – *allo* and *gulo* series

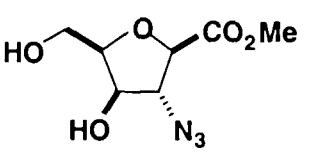
The same approach was employed to synthesise D-*allo* and D-*gulo* homooligomers. The syntheses of D-*allo* monomer **108** and **138** (see **Chapters 2.2.7-2.2.8**) and D-*gulo* monomer **107** and **140** (see **Chapters 2.2.9-2.2.10**), **Figure 3.12**, have been described in the previous chapter.



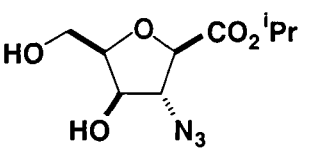
108; D-*allo*



138; D-*allo*



107; D-*gulo*

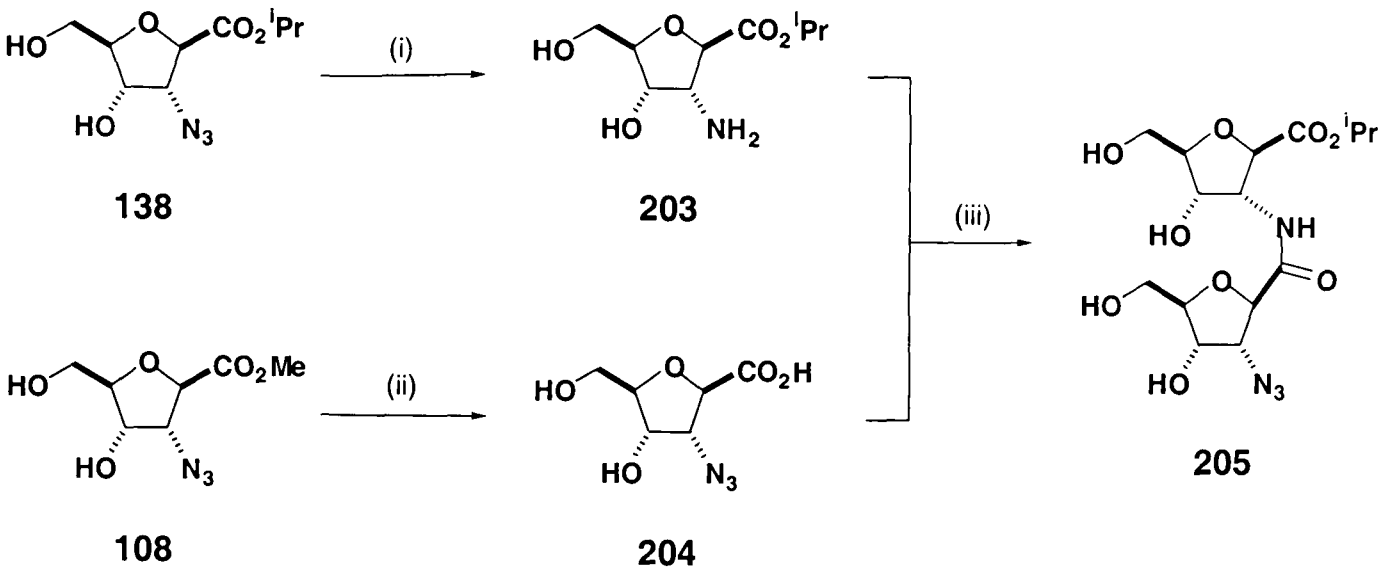


140; D-*gulo*

Figure 3.12

During the peptide coupling of D-*manno* and D-*talo* monomers, both the acid and amine components were formed from the isopropyl azido esters, in the D-*manno* case because of the isolation difficulties associated with the methyl ester (see **Chapter 2.2.2**). The D-*gulo* **107** and D-*allo* **108** methyl esters, however, were readily accessed in pure form and would therefore be used to form the acid components of the coupling mixtures.

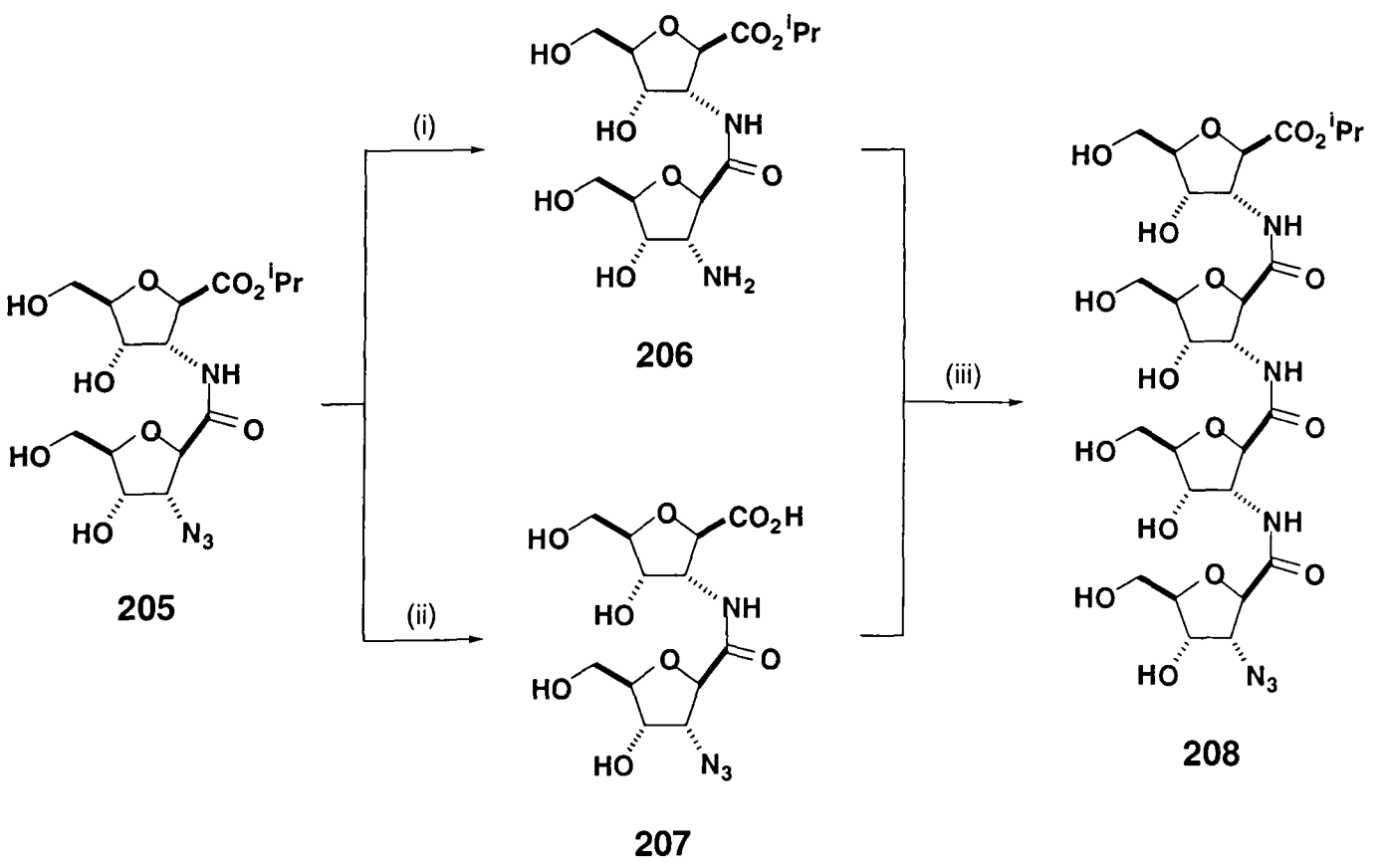
The D-*allo* amine component **203** was formed by reduction of isopropyl ester **138** with hydrogen in the presence of palladium black, and treatment of methyl ester **108** with aqueous sodium hydroxide in 1,4-dioxane followed by ion exchange chromatography gave acid component **204**, **Scheme 3.17**, **p95**. The coupling of **203** and **204** was carried out in DMF using TBTU in the presence of DIPEA to give dimer **205** in 86% yield.



Reagents and Conditions: (i) H_2, Pd black, ^{i}PrOH; (ii) NaOH, H_2O, dioxane then Amberlite IR-120 (H^+), H_2O; (iii) TBTU, DIPEA, DMF

Scheme 3.17

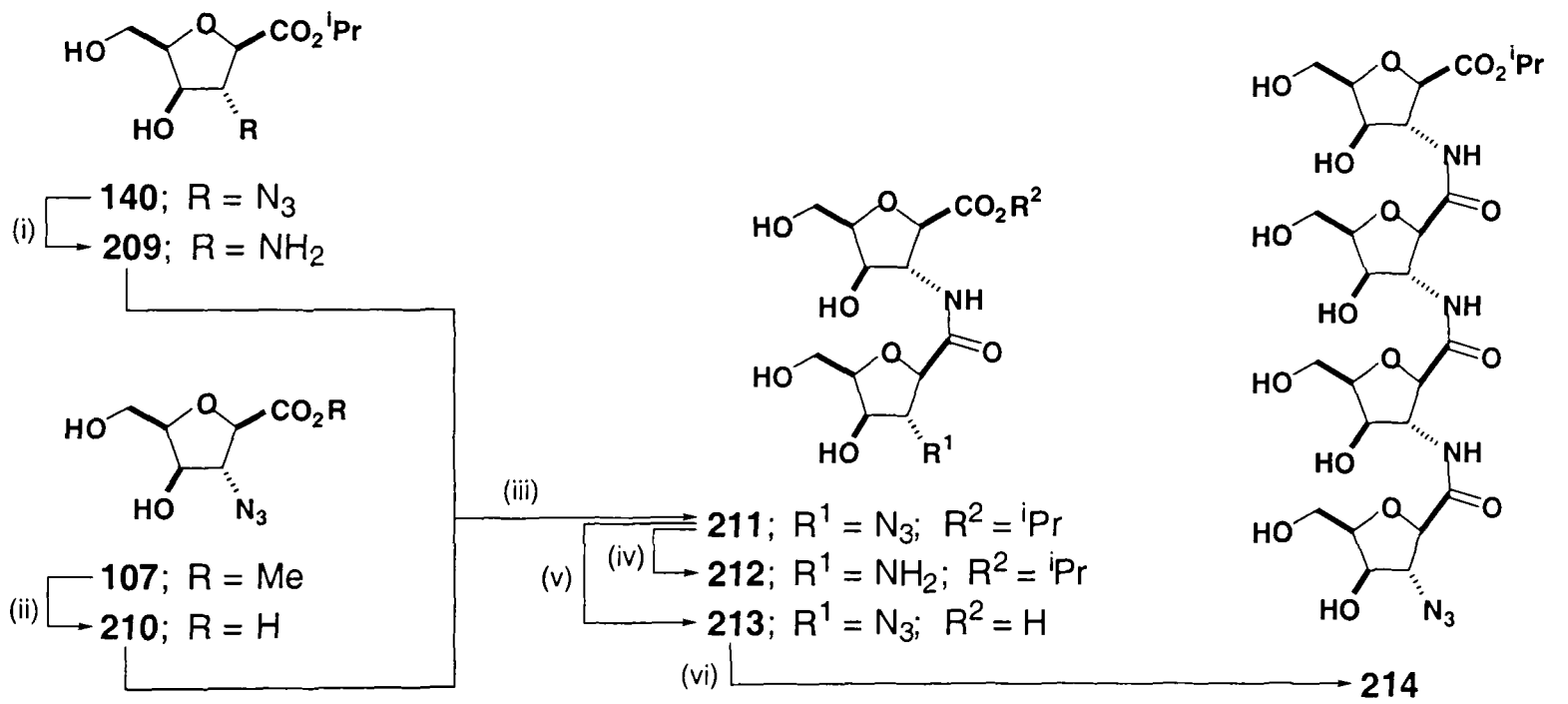
In order to access the D-*allo* tetramer the process was repeated. Dimer **205** was subjected to hydrogenation in water in the presence of palladium black to give amine **206**, **Scheme 3.18**. Hydrolysis by sodium hydroxide followed by ion exchange afforded acid **207**, which was coupled to amine **206** using TBTU and DIPEA in DMF. Tetramer **208** was formed in 68% yield from the dimer.



Reagents and Conditions: (i) H_2, Pd black, H_2O; (ii) NaOH, H_2O, dioxane then Amberlite IR-120 (H^+); (iii) TBTU, DIPEA, DMF

Scheme 3.18

The D-*gulo* dimer and tetramer were synthesised by the same route. Isopropyl ester **140** was hydrogenated in the presence of palladium black to give amine **209** which was used without further purification, **Scheme 3.19**. Methyl ester **107** was hydrolysed by sodium hydroxide and then subjected to ion exchange to give acid **210**. The coupling of **209** and **210** with TBTU and DIPEA in DMF afforded dimer **211** in 78% yield. Iteration of these reactions gave the tetramer **214**. Dimer **211** was hydrogenated to give amine **212**. Hydrolysis of dimer **211** also gave acid **213**. Acid **213** and amine **212** were coupled using TBTU and DIPEA in DMF to produce tetramer **214** in 57% yield.

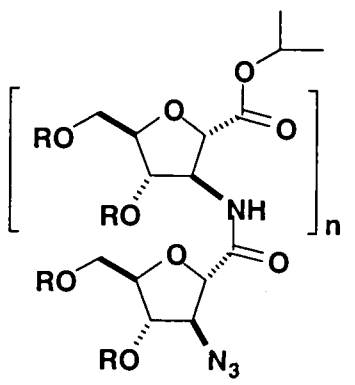


Reagents and Conditions: (i) H_2, Pd black, ^{i}PrOH; (ii) NaOH, H_2O, dioxane then Amberlite IR-120 (H^+); (iii) TBTU, DIPEA, DMF; (iv) H_2, Pd black, H_2O; (v) NaOH, H_2O, dioxane then Amberlite IR-120 (H^+); (vi) **212**, TBTU, DIPEA, DMF

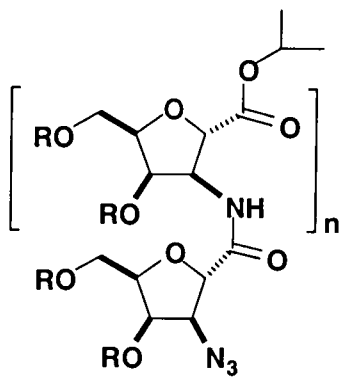
Scheme 3.19

3.4 Conclusion

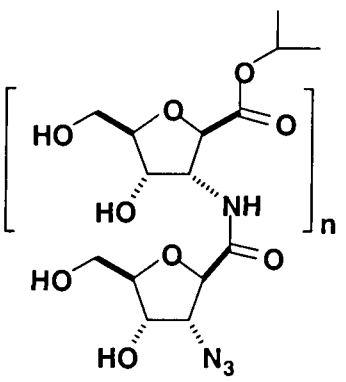
This chapter has described the synthesis of four diastereomeric carbopeptoids with D-*manno*, D-*talo*, D-*allo* and D-*gulo* stereochemistry, each as far as four residues and in two instances up to six residues in length, **Figure 3.13**. Each was synthesised in good yields using standard solution phase peptide coupling procedures.



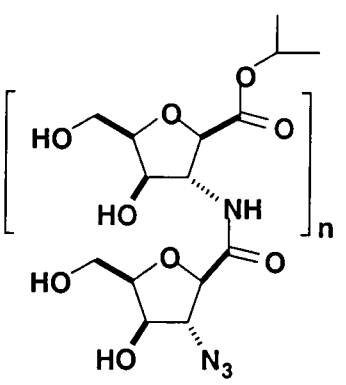
174; n = 1, R = H
177; n = 3, R = H
180; n = 5, R = Ac
} D-*manno*



197; n = 1, R = H
200; n = 3, R = H
202; n = 5, R = Ac
} D-*talo*



205; n = 1
208; n = 3
} D-*allo*



211; n = 1
214; n = 3
} D-*gulo*

Figure 3.13

Several other schemes were investigated for the formation of oligomers, including solid-phase and microwave activated coupling approaches, but these were not so successful.

The following chapter will describe the structural analysis of the oligomers **174-214**.

3.5 References

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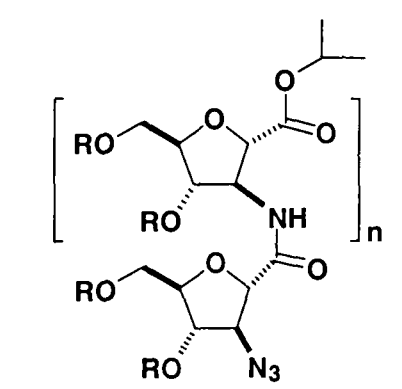
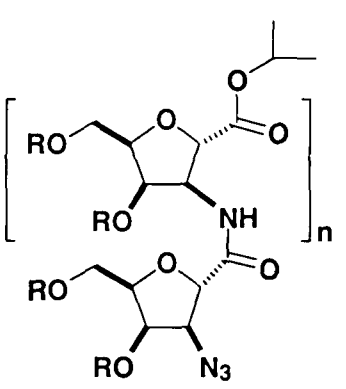
Chapter 4: Analysis of oligomers

4.1 Introduction

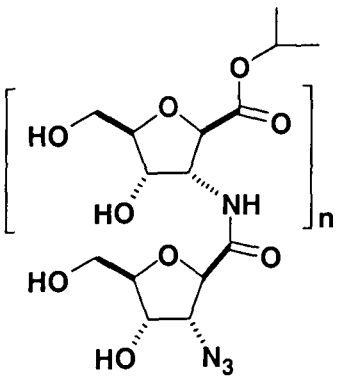
The previous two chapters have described the synthesis of four diastereomeric series of β-glycosamino acid homooligomers, **Figure 4.1**. It was hoped that these carbopeptoids would display stable secondary structures such as the 14-, 12- or 10/12-helices observed by other groups (see **Chapter 1.3**).



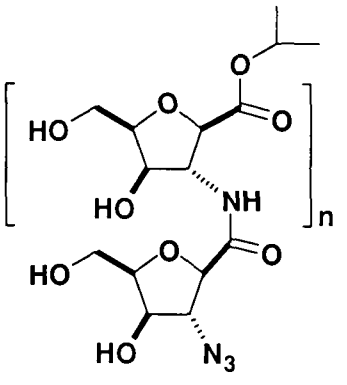
174; n = 1, R = H
177; n = 3, R = H
180; n = 5, R = Ac
} D-*manno*



197; n = 1, R = H
200; n = 3, R = H
202; n = 5, R = Ac
} D-*talo*



205; n = 1
208; n = 3
} D-*allo*



211; n = 1
214; n = 3
} D-*gulo*

Figure 4.1

Stable hydrogen bonding is expected to be manifested in oligomers consisting of four or more residues (see **Chapter 3.1.5**).

This chapter will discuss the characterisation and analysis of the oligomers shown in **Figure 4.1**, **p100**, the synthesis of which was described in the previous chapter.

4.1.1 Analytical tools

There are three techniques regularly used to probe the solid- and solution-phase conformations of potential foldamers. For a crystalline compound, x-ray crystallography can give the precise coordinates of the atoms in the molecule, thus an undisputable secondary structure. NMR spectroscopy can be used to investigate the secondary structure of molecules by means of identifying through-space interactions between atoms that are removed along the backbone. CD spectroscopy is a useful tool for the interpretation of peptide conformations.

The dimers **174** and **197** and tetramers **177** and **200** were isolated as crystalline solids. However, attempts to obtain crystals suitable for crystallographic analysis were unsuccessful. Co-workers have experienced similar difficulties in the crystallisation of δ-carbopeptoids.[1] Therefore, the remainder of this chapter will focus on CD and NMR spectroscopy for secondary structural investigation.

4.2 CD spectroscopy

Electronic CD spectroscopy measures the differential absorption of right and left circularly polarised light. This well-established technique is used for the analysis of secondary structure in proteins. Absorptions due to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ electronic transitions appear in the far UV between 180 nm and 260 nm. The exact positioning of the absorptions depends on the orientation of the peptide bonds, hence the adoption of a certain conformation leads to a characteristic CD spectrum.[2]

Although the CD spectrum is normalised for amide bond concentration, molecular terminal effects mean that the intensity tends to increase with chain length, as well as being dependent on the population of the relevant conformation. Another important point is that the sign of a CD spectrum depends on the handedness of the molecule. Two enantiomeric molecules display identical but inverse plots.

4.2.1 CD spectroscopy of proteins and α-peptides

Each major element of protein and peptide secondary structure (see **Chapter 1.2**) has a characteristic CD spectrum in the far UV region.[2]

An α-helical protein or peptide (see **Chapter 1.2.1**) gives a highly distinctive three-band pattern, as depicted in **Figure 4.2, p103**. Both the intense positive peak at 190 nm and the negative band at 208 nm arise from the exciton splitting of the amide $\pi_{nb} \rightarrow \pi^*$ (non-bonding π to antibonding π orbital) electronic transition.[2] Another negative band at about 220 nm is caused by the amide $n \rightarrow \pi^*$ (oxygen lone pair to antibonding π orbital).

An example of a peptide which exhibits the typical α-helix trace is Ac-Leu-Pro-NHMe in water.[3]

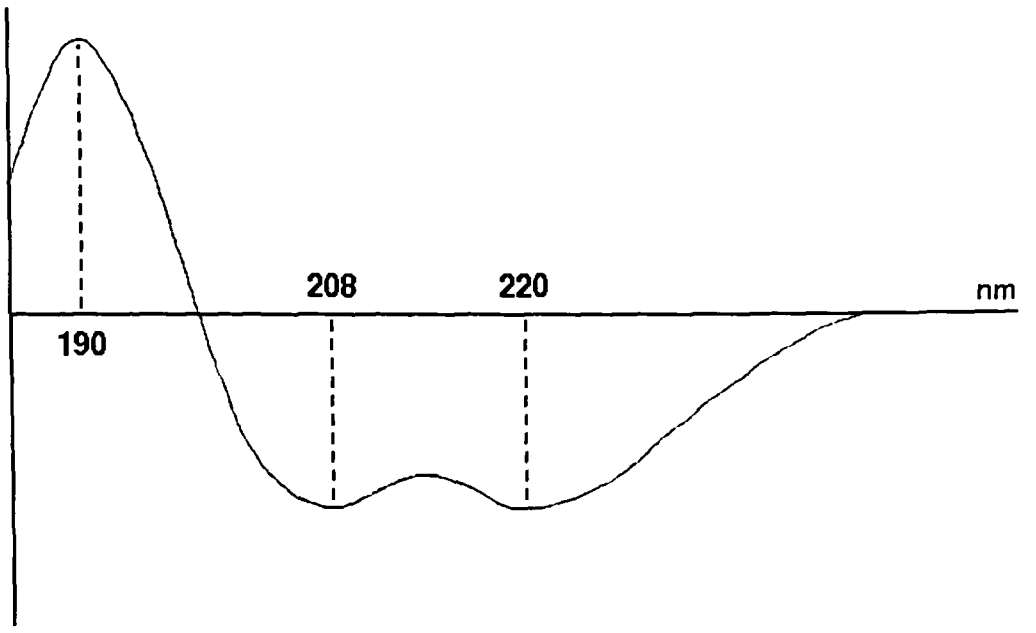


Figure 4.2. Schematic CD trace for an α-helix.

The THF-templated δ-carbopeptoids **67** and **68**, **Figure 4.3**, also gave rise to a trace that is similar to the α-helical pattern[4], **Figure 4.4, p104**. It was discussed previously (see **Chapter 1.5.1**) that **67** and **68** form 16-helices. While the 13-membered rings that stabilise the α-helix are unavailable, each residue of octamer **68** is a dipeptide isostere, and the molecule forms a helix that is not dissimilar to the α-helix in terms of amide bond orientation.

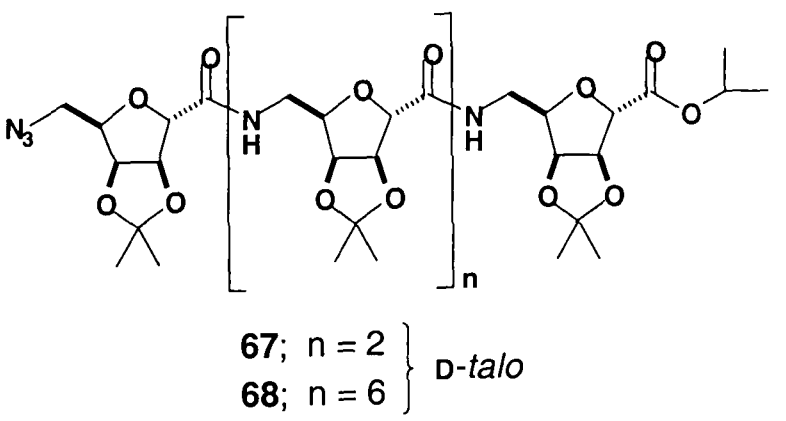


Figure 4.3

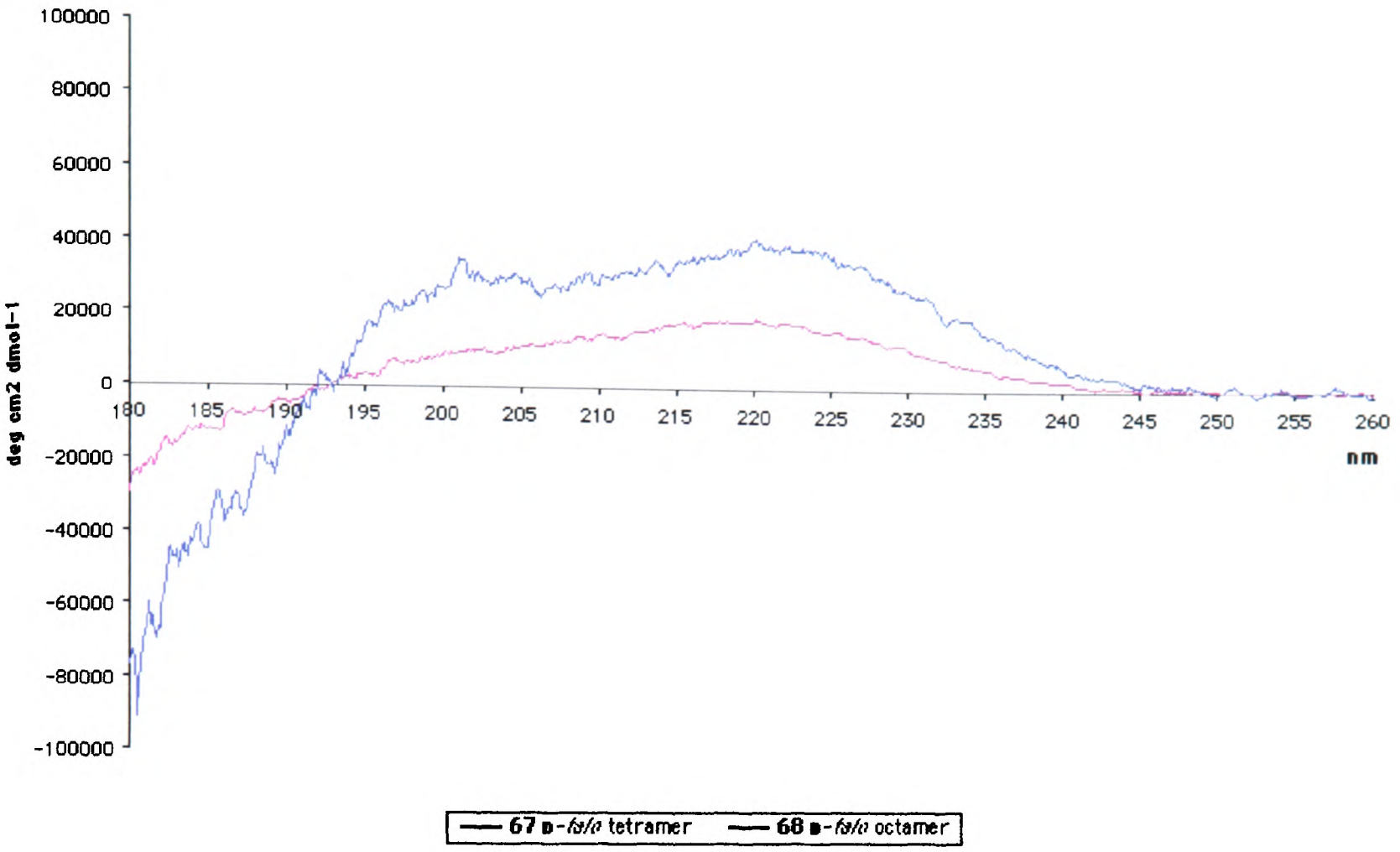


Figure 4.4. CD plots for **67** and **68** in TFE.

Peptides adopting the β-turn conformation (see **Chapter 1.2.2**) have previously been found to result in a CD spectrum containing a strong negative band at 188 nm, a strong positive band near 209 nm and a weaker negative band at around 227 nm,[3] **Figure 4.5**, **p105**. Ac-Ala-^{t}BuPro-NHMe and Ac-Leu-^{t}BuPro-NHMe give the CD spectrum of a peptide containing β-turns in both acetonitrile and water.[3]

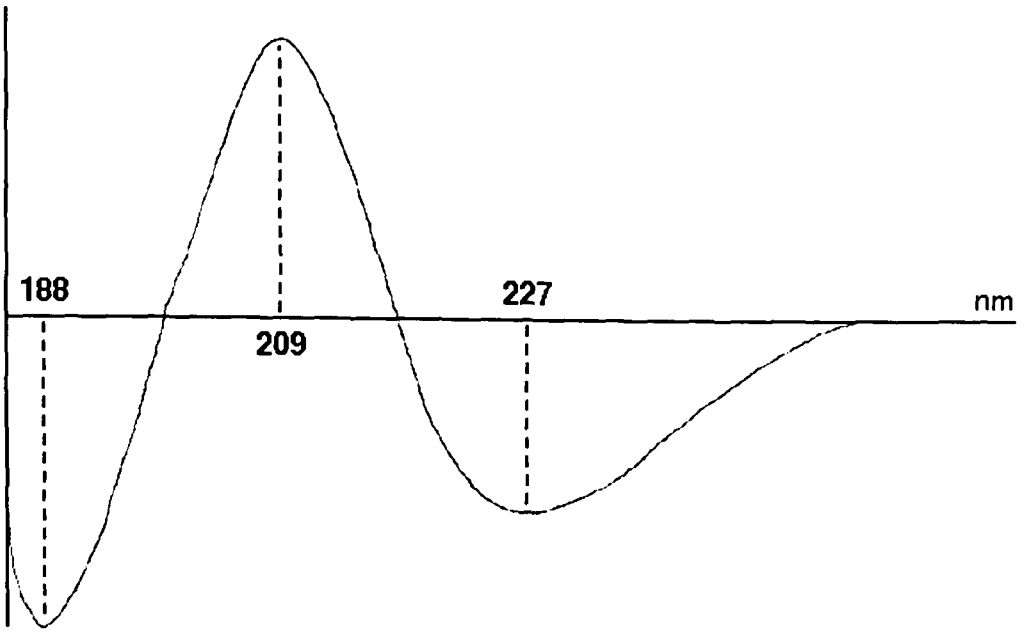
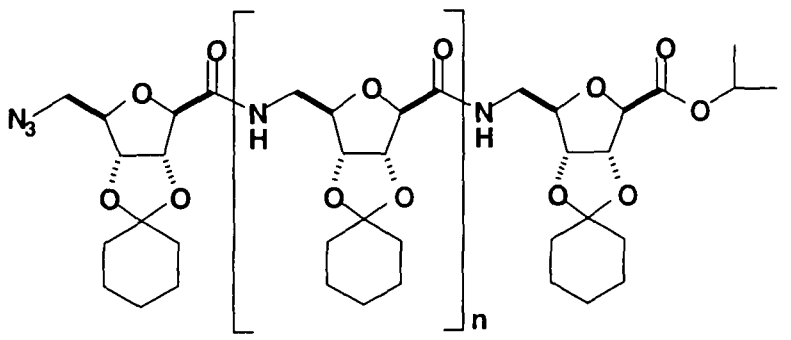
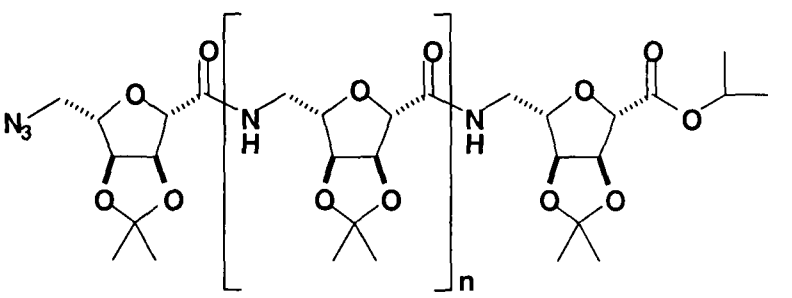


Figure 4.5. Schematic CD trace for a β-turn.

Several carbopeptoids, **Figure 4.6**, have been shown by NMR studies to adopt a repeating β-turn-type conformation (see **Chapter 1.5.1**) and exhibit similar CD spectra to the one above, **Figure 4.7, p106**.[4] The predicted inversion is seen between right- and left-handed **D**- and **L**-*allo* tetramers **57** and **60**.



56; n = 0
57; n = 2 } **D**-*allo*
58; n = 6



59; n = 0
60; n = 2 } **L**-*allo*

Figure 4.6

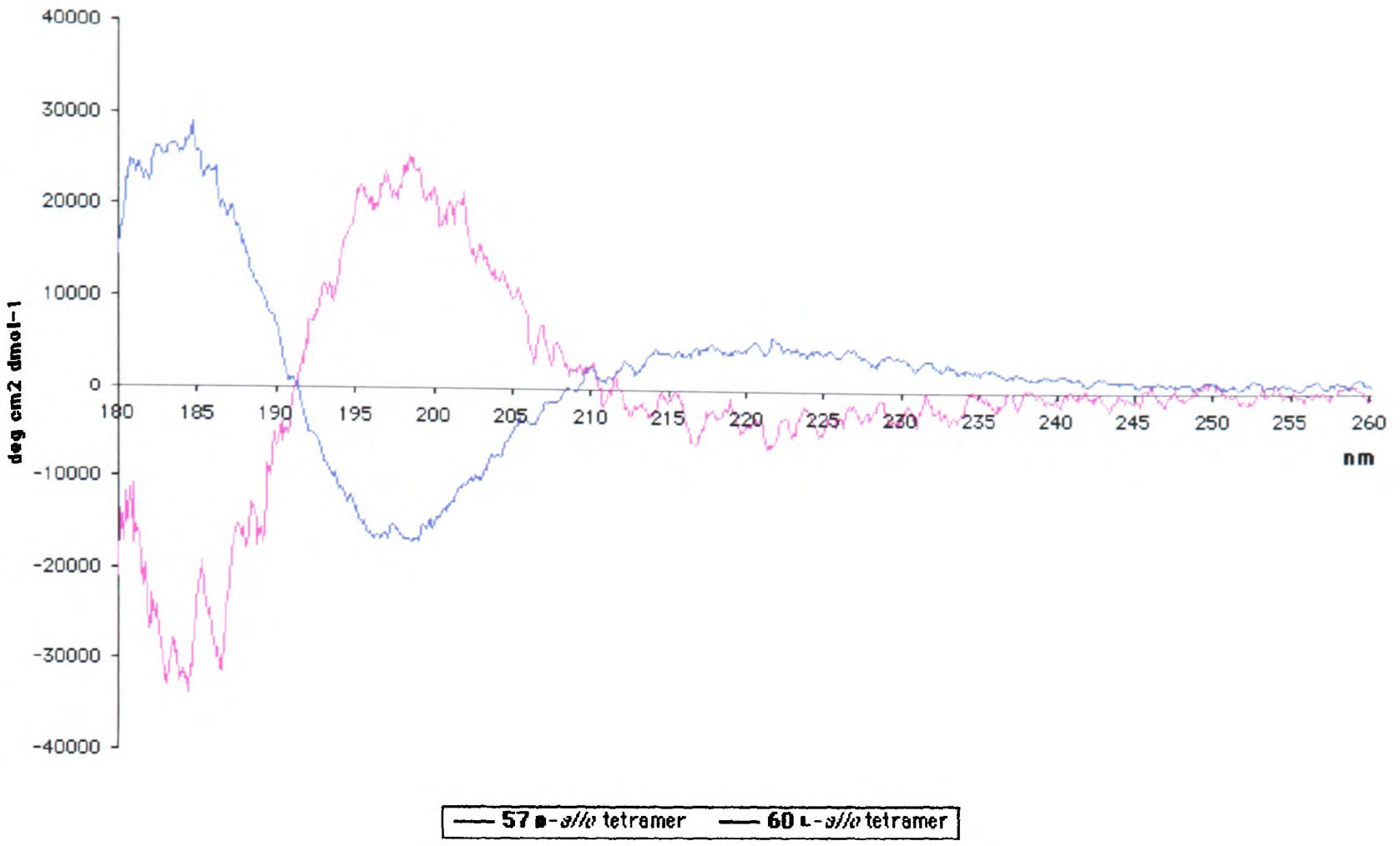


Figure 4.7. CD plots for **57** and **60** in TFE.

The CD spectrum of a peptide which adopts a random coil conformation shows an intense minimum at around 197 nm and a lack of significant intensity above 210 nm,[5] **Figure 4.8**.

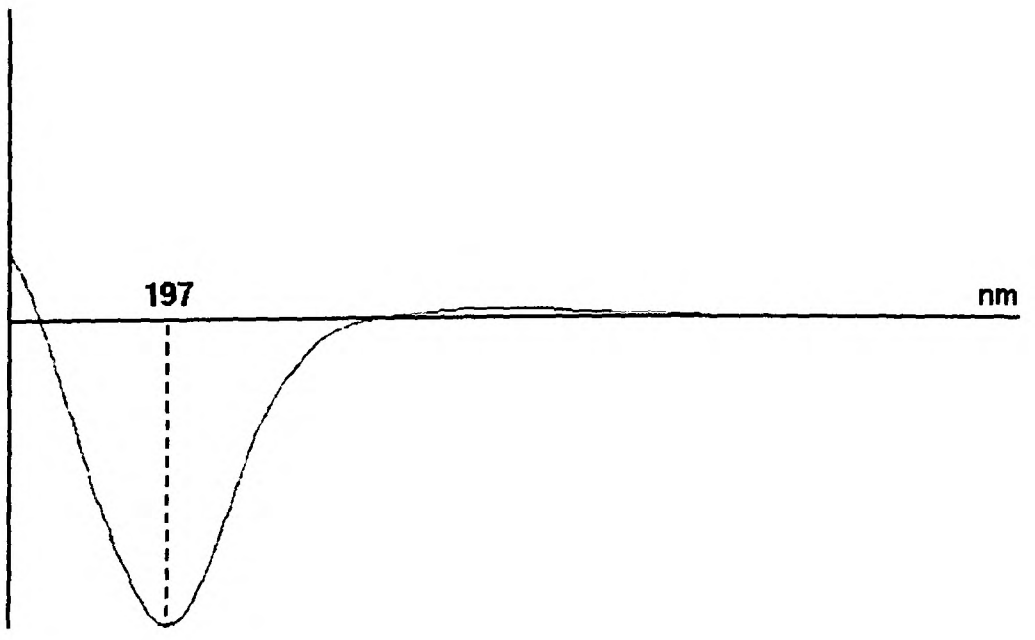


Figure 4.8. Schematic CD trace for a random coil.

Carbopeptoids **63-65**, **Figure 4.9**, show no hydrogen-bonding structure (see **Chapter 1.5.1**) and display a similar CD spectrum to the random coil, **Figure 4.10**.[4]

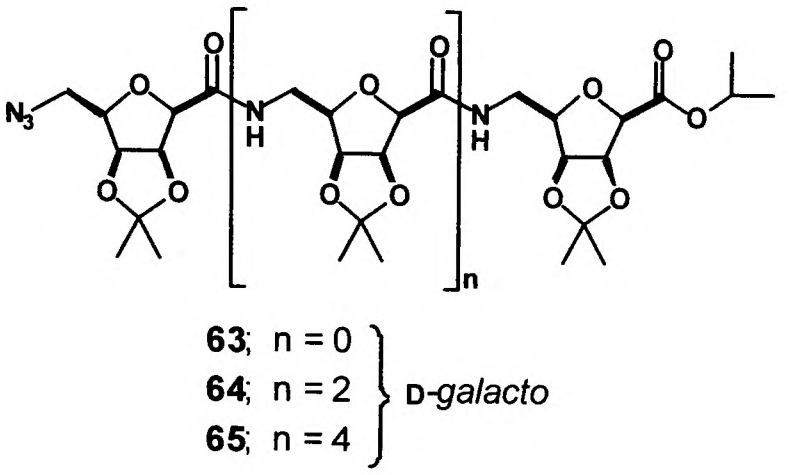


Figure 4.9

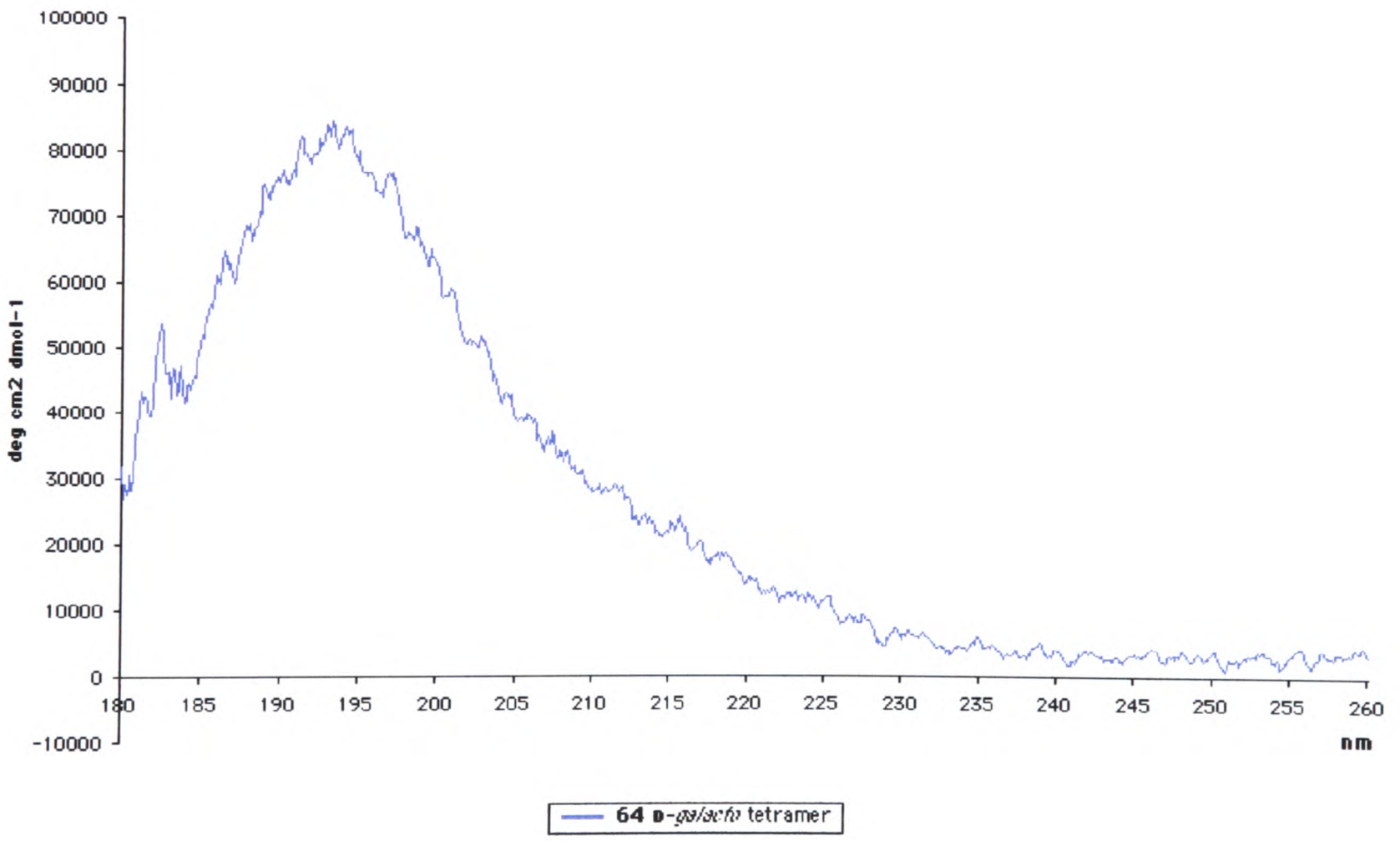
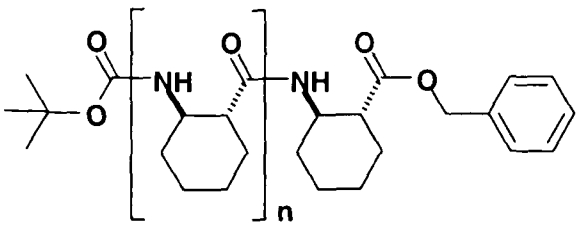


Figure 4.10. CD plot for **64** in TFE.

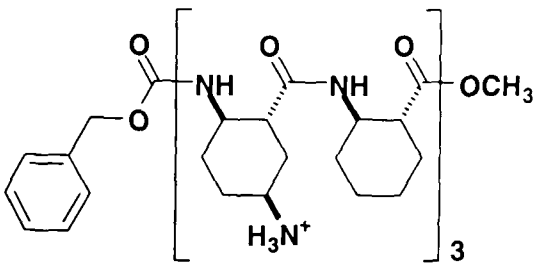
4.2.2 CD spectroscopy of β-peptides

CD spectroscopy was developed as a rapid qualitative method to analyse the conformational structure of α-peptides.[6] It has also been used recently as a diagnostic tool to study the structures of the oligomers of β-amino acids[6,7] as well as other peptidomimetics.

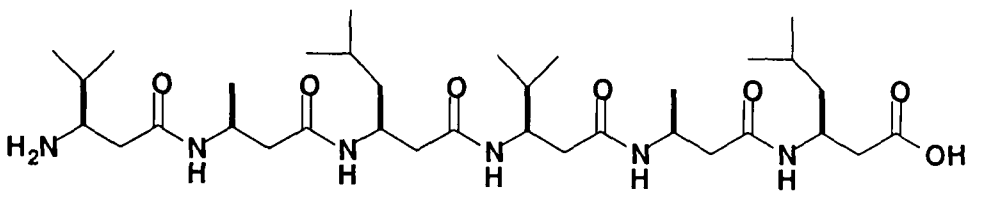
Gellman and Seebach have established the predominant conformations of β-peptides **1-9**, **Figure 4.11**, **p109**, by crystallography and/or NMR spectroscopy (see **Chapter 1.3**). As with the α-peptide counterparts, each different conformation of the β-amino acid oligomers was found to show a distinctive CD spectrum in the far UV region.



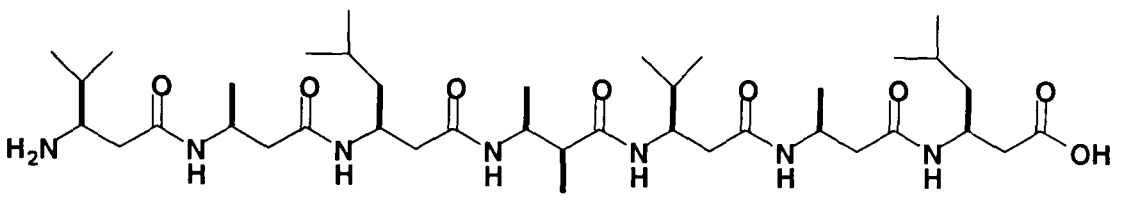
1; n = 0,1,2,3,5



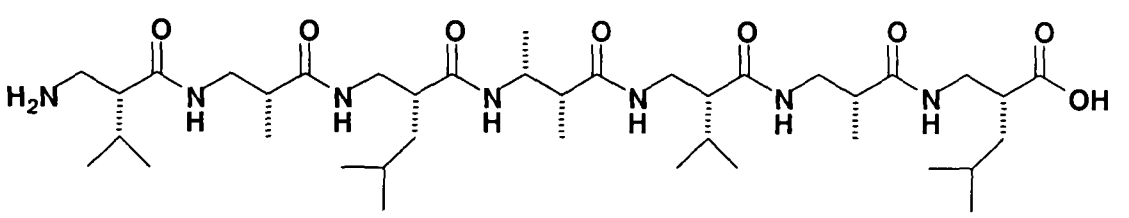
2



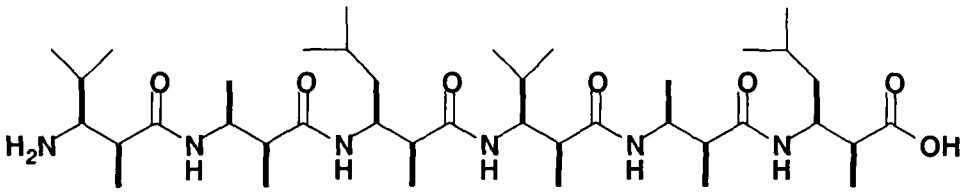
3; TFA salt



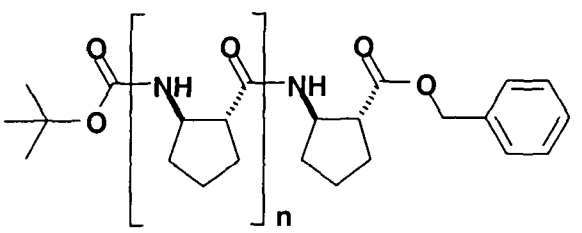
4; TFA salt



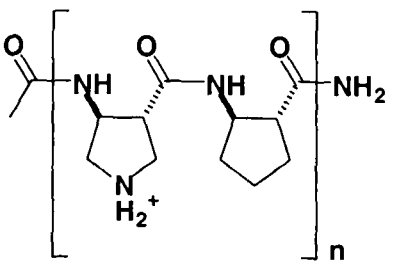
5; TFA salt



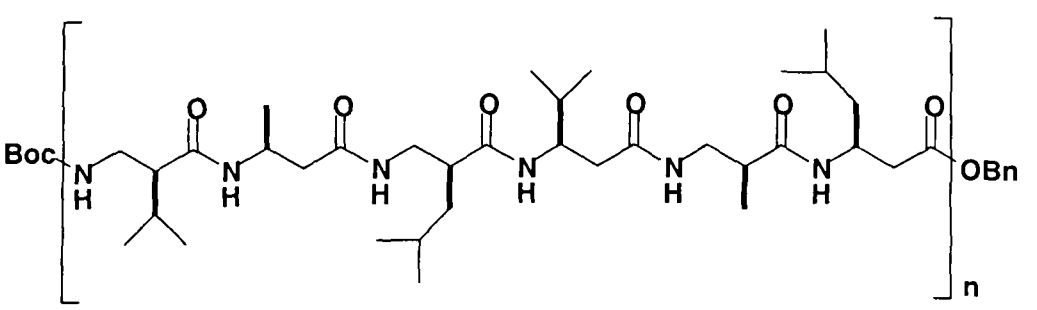
6; TFA salt



7; n = 0,1,2,3,5



8; n = 2, 3, 4; TFA salt



9; n = 1, 2

Figure 4.11

The CD spectra of oligomers **1-6**, which were shown by NMR spectroscopy to adopt left-handed 14-helices, display a maximum around 195-200 nm and a minimum near 215 nm,[6-10] **Figure 4.12**. These two energy bands arise due to the excitonic coupling of the $\pi \rightarrow \pi^*$ transition. For the right-handed 14-helix, the inverted spectrum is seen, with a minimum around 195 nm and a maximum near 215 nm.[6,9]

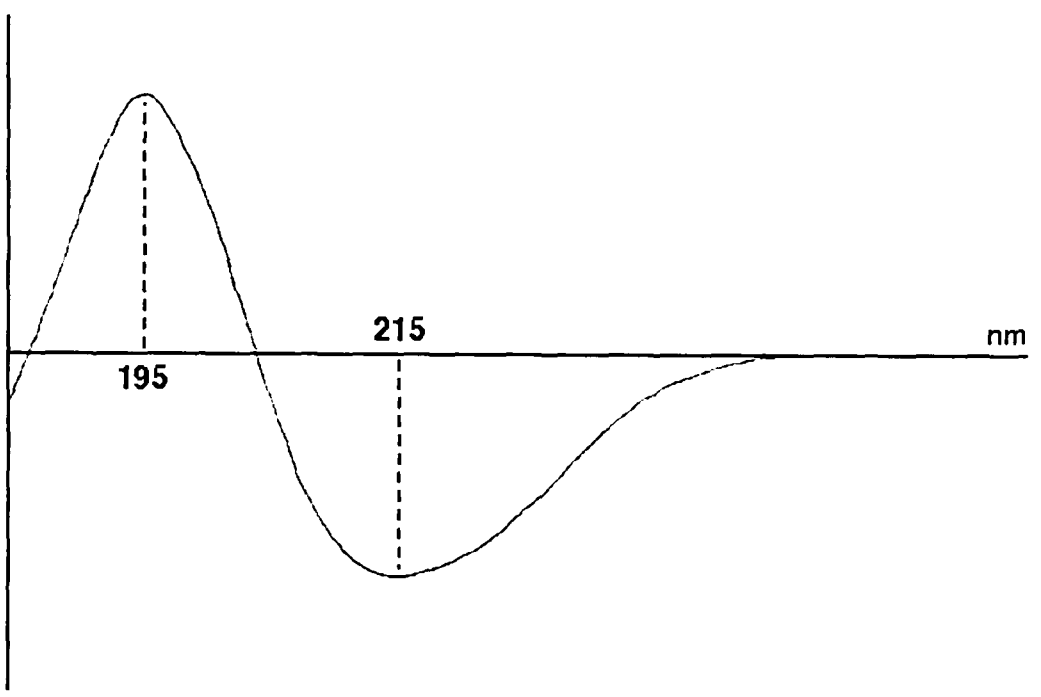


Figure 4.12. Schematic CD trace for a 14-helix.

The 12-helix, adopted by β-peptides **7-8**, shows a CD spectrum which contains similar $\pi \rightarrow \pi^*$ peaks to that of the 14-helix, but the signs are reversed,[6,7,9] **Figure 4.13, p111**. The bands near 190 nm and 205 nm arise due to the orthogonal splitting of the $\pi \rightarrow \pi^*$ transition, while a third band, associated with the $n \rightarrow \pi^*$ transition, appears at around 220 nm.

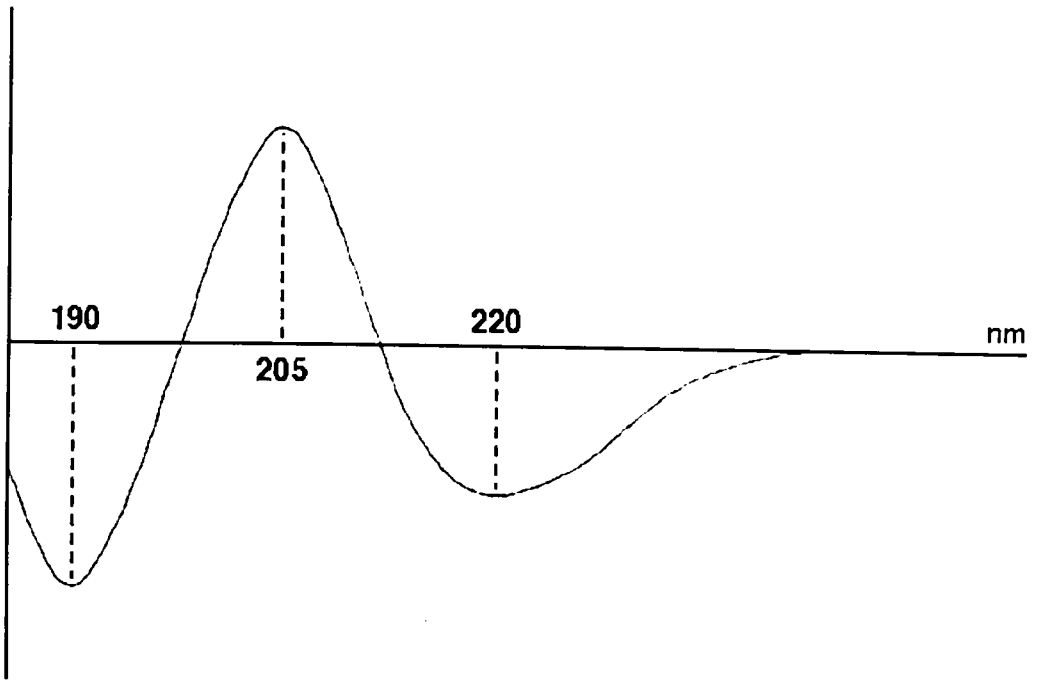


Figure 4.10. Schematic CD trace for a 12-helix.

The spectrum resulting from the 10/12-helical conformation of repeating (S)-α / (S)-β β-peptide **9** is different again, **Figure 4.11**. A single intense maximum is observed near 205 nm.[6,7,9]

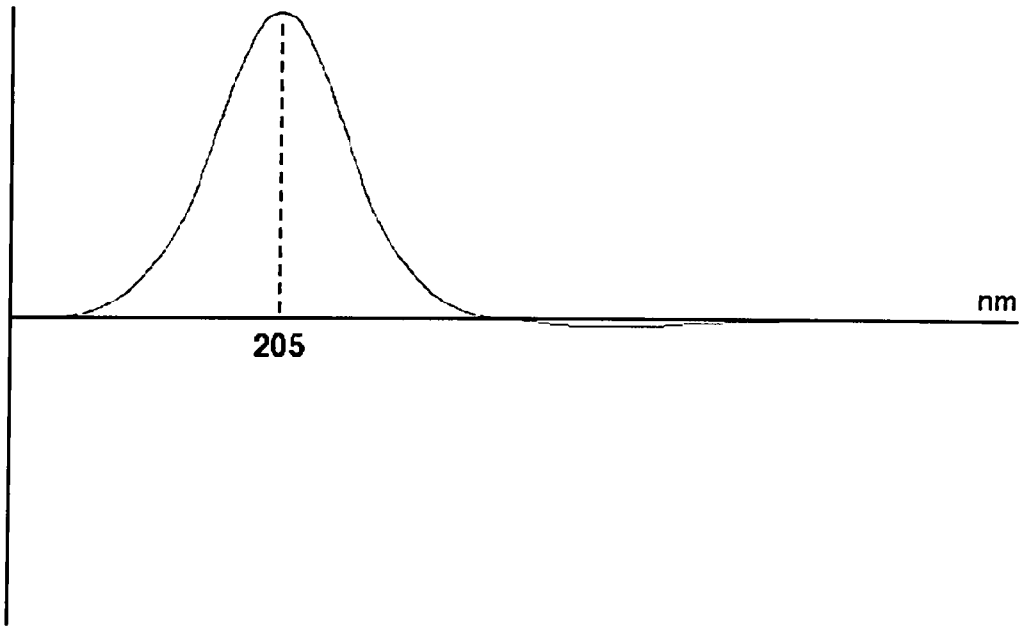
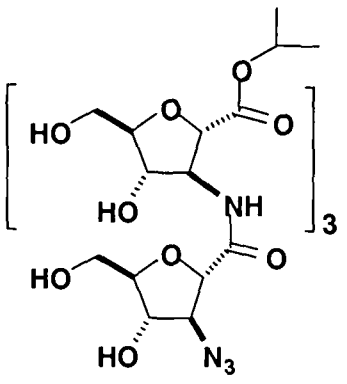


Figure 4.11. Schematic CD trace for a 10/12-helix.

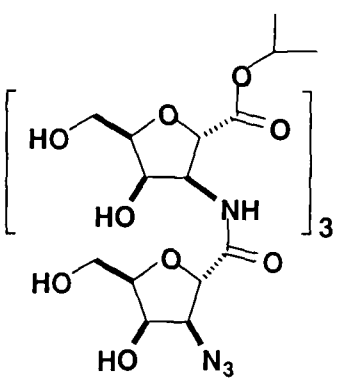
CD spectroscopy may be used as a tool for secondary structural analysis, not only for proteins but also for synthetic peptides consisting of α-, β- and other amino acids. The unique shapes and positions of maxima and minima in the spectra of compounds which adopt different known conformations mean that this method can be used to assign predominant structures rapidly and confidently.

4.2.3 CD spectroscopy for D-*manno* and D-*talo* oligomers

CD spectroscopic analysis was used in the characterisation of the D-*manno* **177** and D-*talo* **200** tetramers, **Figure 4.12**, the syntheses of which were described in the previous chapter.



177; D-*manno*



200; D-*talo*

Figure 4.12

The selection of solvent for CD experiments depends on several factors. Most importantly, there is a requirement for transparency to light in the appropriate far UV region of the spectrum, hence there can be no allowed electronic transitions at the relevant wavelength. Most of the common NMR solvents (chloroform, acetone, DMSO, benzene, pyridine etc.) contain a chromophore and are therefore not suitable for CD spectroscopy. The solvents used most frequently for CD studies are 1,1,1-trifluoroethanol (TFE) and water.

The effect of the solvent on the populations of specific conformations should also be considered. For example, a compound may contain both lipophilic and lipophobic moieties. A certain conformation may place the lipophilic components on the interior of the molecule and will be favoured in aqueous or polar organic media as opposed to a non-polar solvent.

TFE was selected as the solvent for the analysis of tetramers **177** and **200**. TFE is transparent to light at 180-260 nm, while its polarity ensures the solubility of the tetramer containing eight hydroxyl groups. CD experiments were conducted at low concentrations (126 μmol dm^{-3} of peptide linkages) in order to avoid any aggregation of molecules, which would affect the assignment of conformation.

The CD spectra of tetramers **177** and **200** display a distinctive two band pattern, **Figure 4.13**. A negative band appears near 192 nm for both **177** and **200**, and a broader positive band is positioned at 212 nm for **D-***talo* tetramer **200** and at 215 nm for **D-***manno* tetramer **177**.

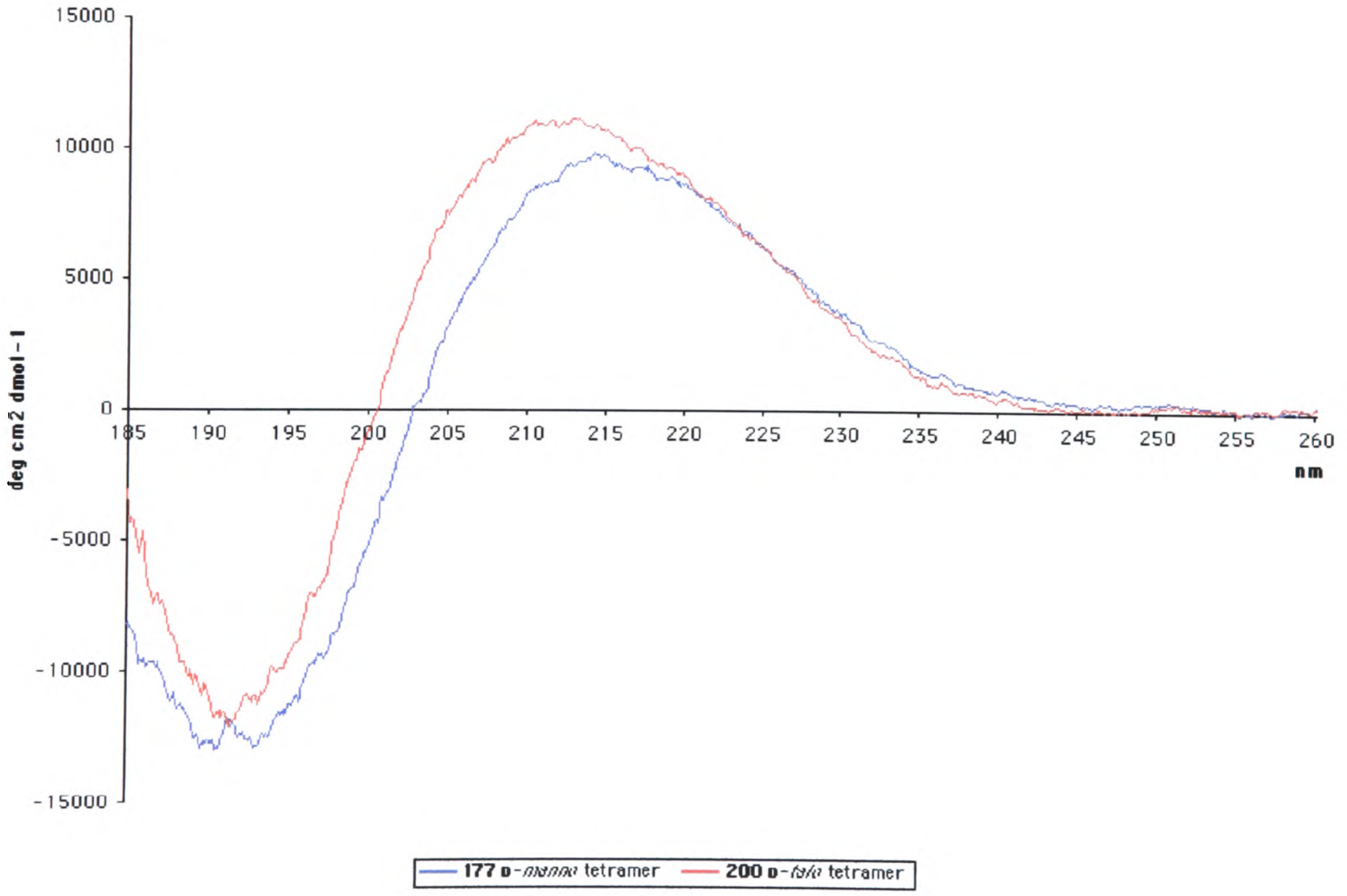


Figure 4.13. CD plots for **177** and **200** in TFE.

Comparing these minimum and maximum values with the results published by Gellman,[6] it is clear that there is close agreement with the CD spectra of the hexa- and heptapeptides that adopt

a regular left-handed 14-helix in solution, only the sign is reversed, **Figure 4.14**. The spectra of tetramers **177** and **200** are not consistent with any of the other regular conformations discussed previously (see **Chapters 4.2.1-4.2.2**), **Table 4.1**.

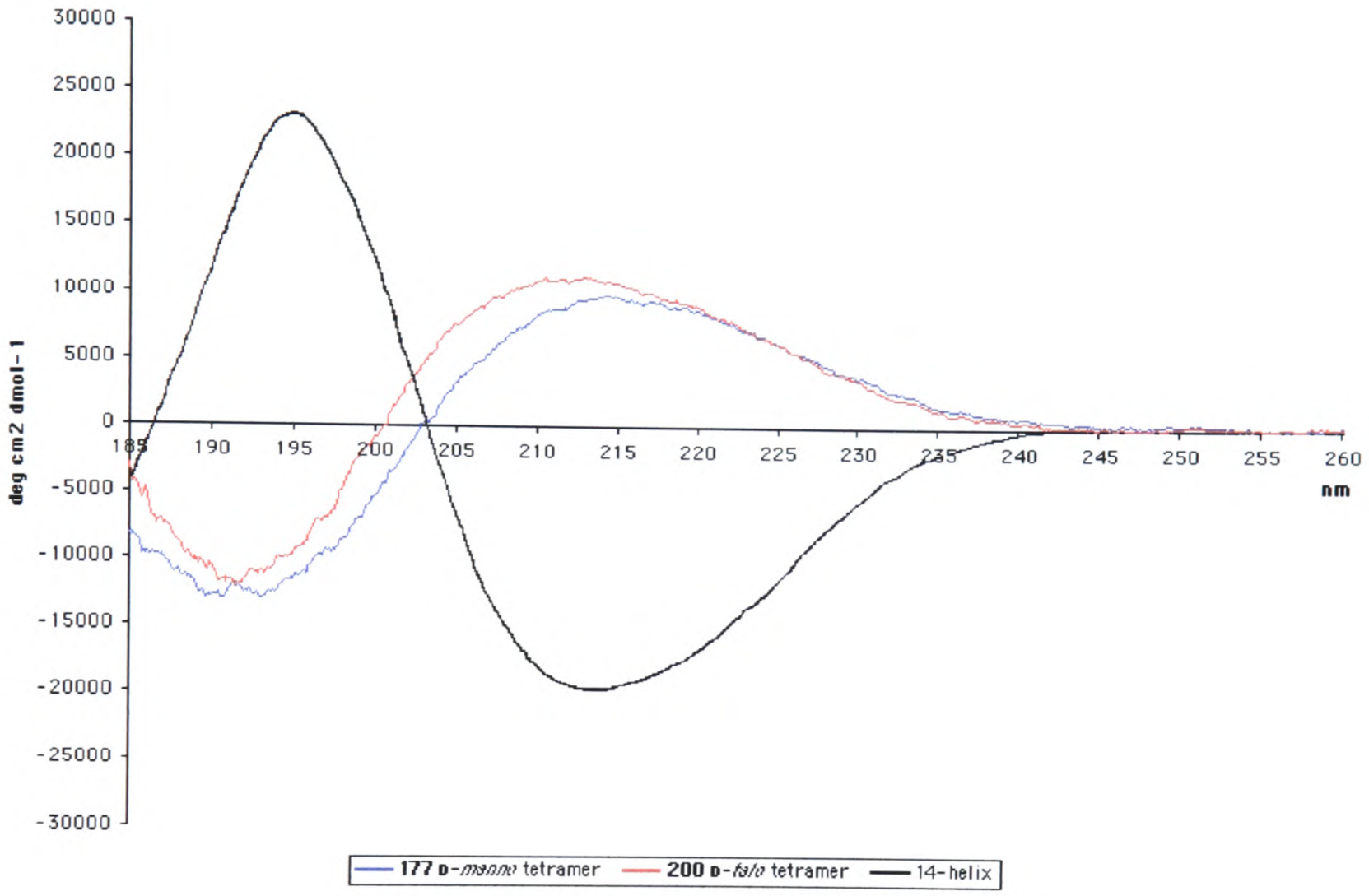


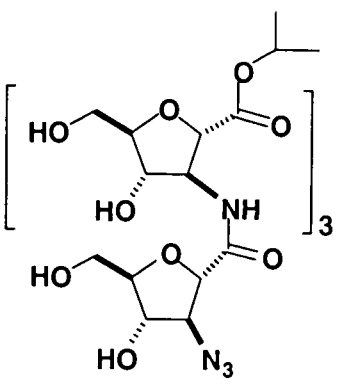
Figure 4.14. CD plots for **177**, **200** and schematic trace for a 14-helix.

	177	**200**	**1** (14-helix)[6]	**7** (12-helix)[6]	10/12-helix[6]
Band 1	192 (min)	192 (min)	195 (max)	190 (min)	205 (max)
Band 2	215 (max)	212 (max)	215 (min)	205 (max)	-
Band 3	-	-	-	220 (min)	-

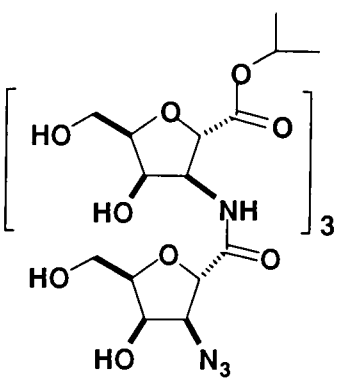
Table 4.1

It is therefore proposed on the basis of CD spectroscopic analysis that the predominant conformation of both **177** and **200** in TFE solution is a right-handed 14-helix.[11]

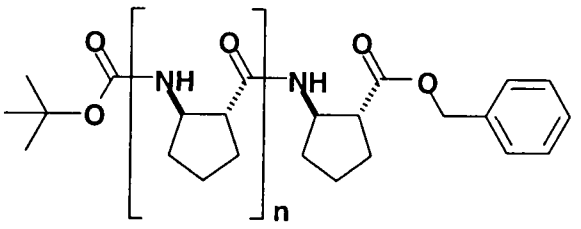
This postulation is not consistent with the expected results. Tetramers **177** and **200** have the same backbone structure as 12-helical 2-aminocyclopentanecarboxylic acid oligomers **7**, only with the opposite configurations at C-2 and C-3, **Figure 4.15**. The 12-helix was therefore anticipated to be the predominant structure of **177** and **200**. The evidence suggests, however, that THF templated oligomers **177** and **200** have similar conformational preferences to the cyclohexane-based β-peptides **1**.



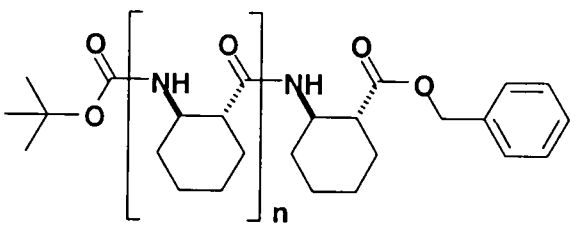
177; D-*manno*



200; D-*talo*



7; n = 0,1,2,3,5



1; n = 0,1,2,3,5

Figure 4.15

As mentioned earlier, the intensity of the spectrum increases with chain length as well as population of the relevant conformation. The amplitude of the spectra of tetramers **177** and **200** was therefore expected to be significantly lower than that of the longer oligomers. Average ellipticity for Gellman's hexa- and heptapeptides was found to approach 20,000 deg cm^2 $dmol^{-1}$,[6] while the amplitude of the peaks in the CD spectra of tetrapeptides **177** and **200** is in the region of 12,000 deg cm^2 $dmol^{-1}$. It is therefore proposed that the population of the 14-helical state by **177** and **200** is similar to the helical population adopted by **1**, and the lower spectrum intensity is due only to the difference in chain lengths.

4.3 NMR spectroscopy

The evidence gained from the CD analysis of the tetramers **177** and **200** suggested that it would be beneficial to study the ^{1}H NMR spectra of related compounds to assess their potential to adopt secondary structure. The acetyl protected hexamers **180** and **202** were selected for investigation as the greater number of residues relative to the tetramers may increase the potential to adopt a secondary structural motif. However, the inherent disadvantage of the longer oligomers is that the ^{1}H NMR spectra may prove to be more complicated to assign.

The initial ^{1}H NMR experiments were conducted in d-chloroform. However, it was found that this solvent gave an overlap of signals in the sugar region between 4.0 and 5.5 ppm which prevented full assignment, **Figure 4.16** and **Figure 4.17**, **p117**.

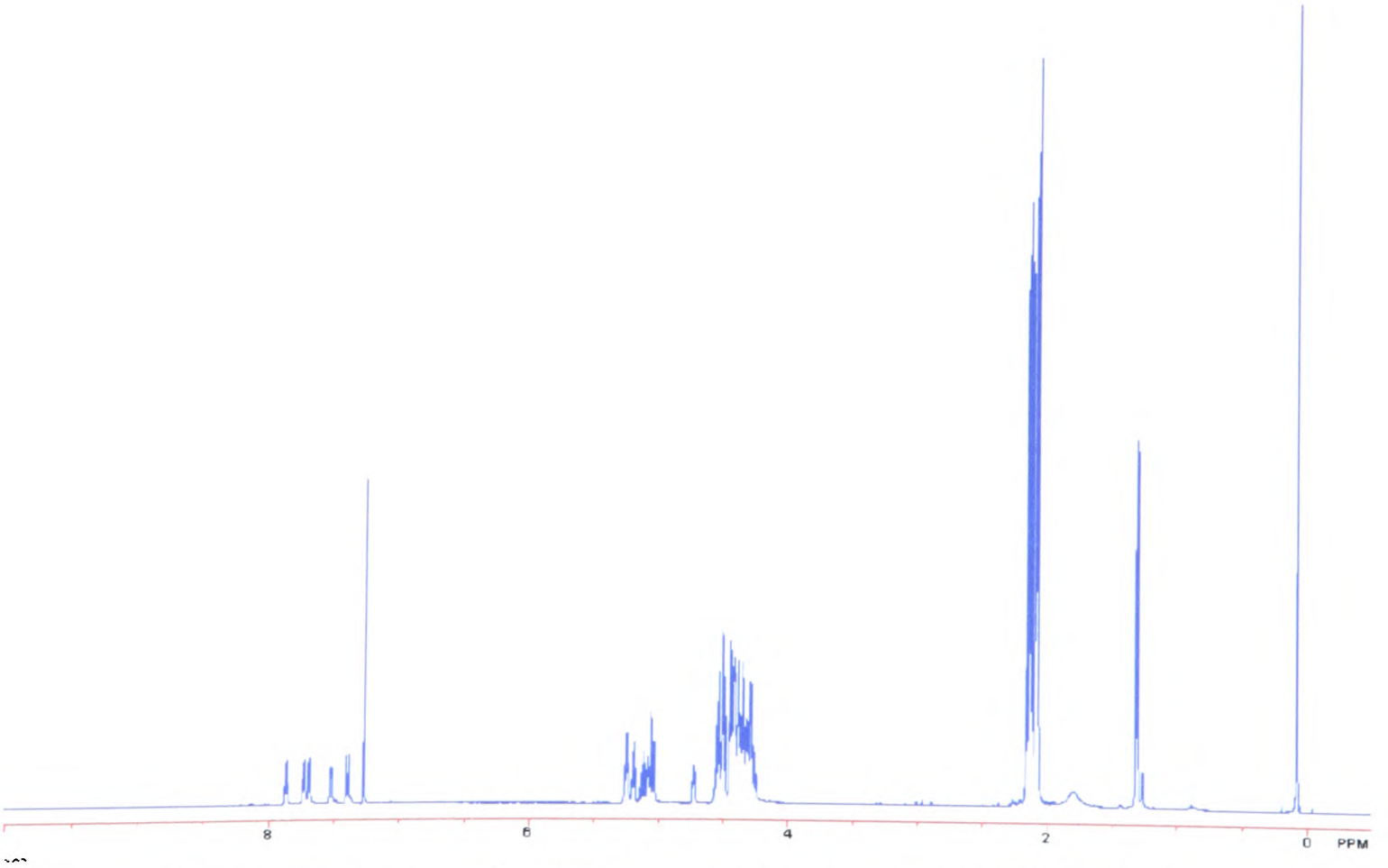


Figure 4.16. 500 MHz ^{1}H NMR spectrum of D-*manno* hexamer **180** in $CDCl_3$.

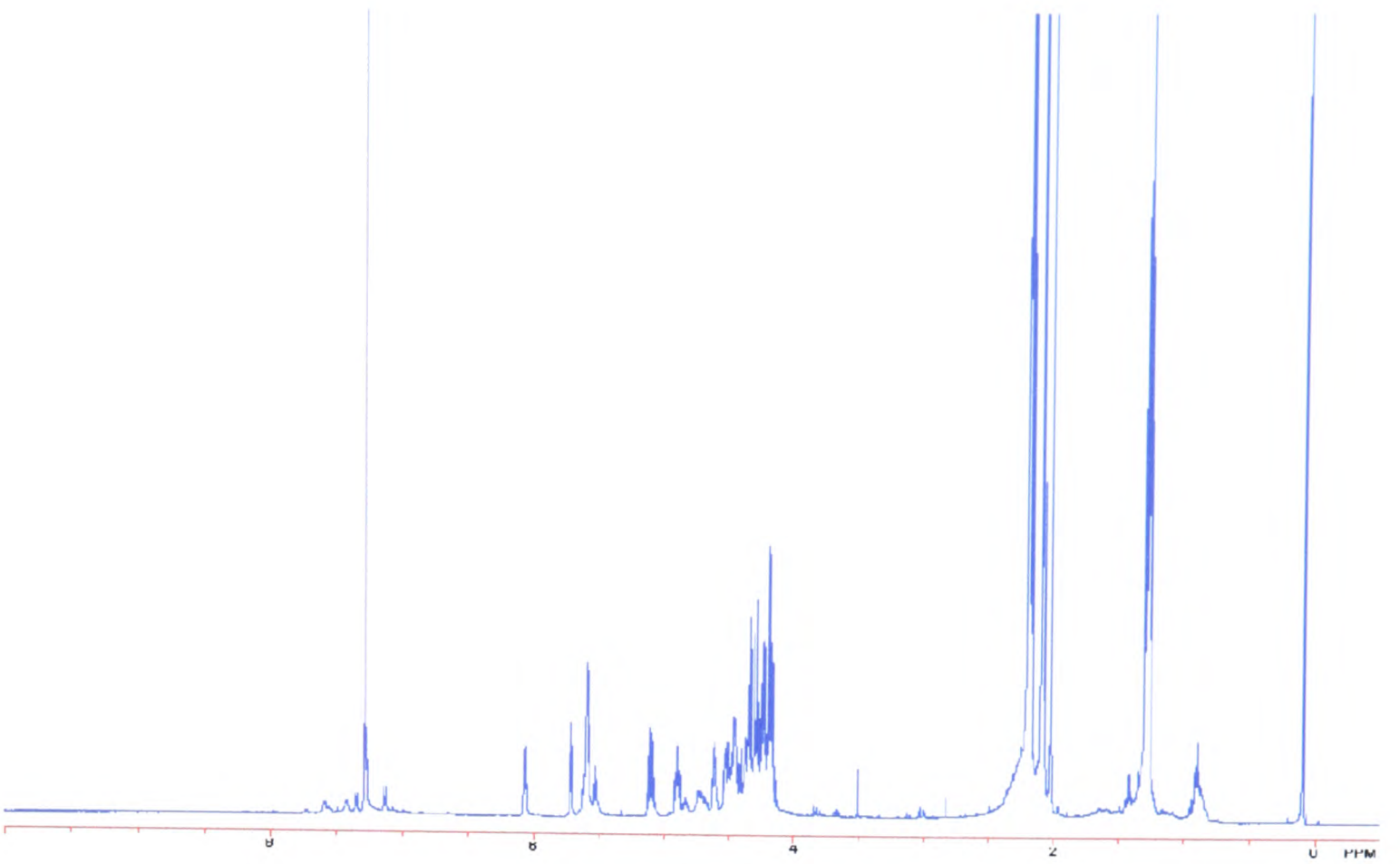


Figure 4.17. 500 MHz ^{1}H NMR spectrum of D-*talo* hexamer **202** in $CDCl_3$.

Further experiments were conducted on the D-*manno* hexamer **180** only, due to a lack of available D-*talo* hexamer **202**. It was assumed, however, that the similarities between **180** and **202** would mean that any conclusions drawn up for one compound should apply to the other.

4.3.1 Alternative solvents

The use of d^6-benzene in place of d-chloroform provided a much greater degree of dispersion in the sugar region of the ^{1}H NMR spectrum of hexamer **180**, **Figure 4.17**, **p118**, though the spectrum was still not sufficiently clear to allow full assignment of the sugar protons to their ring systems. A partial assignment was made and this is shown in **Table 4.2**, **p118**.

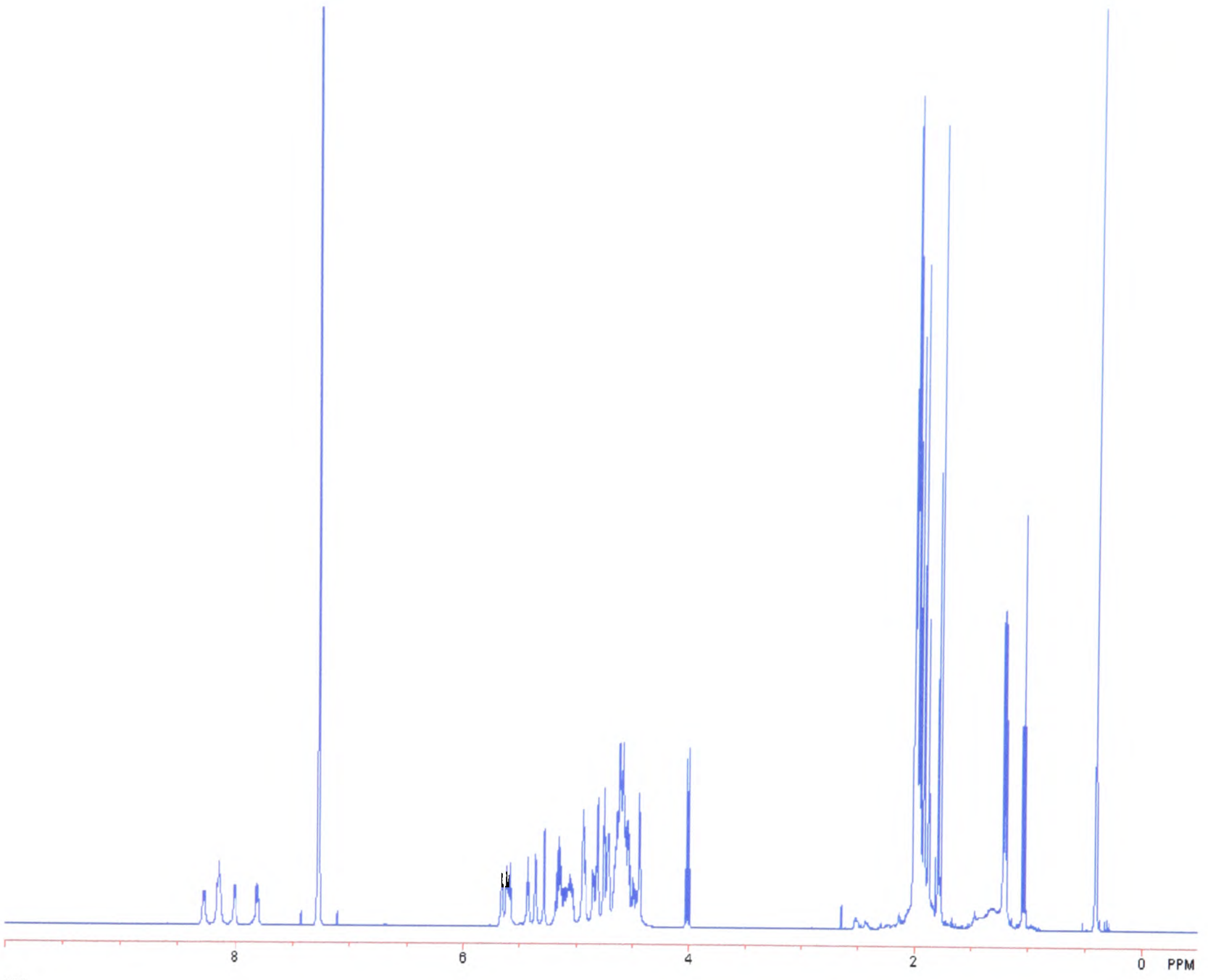


Figure 4.17. 500 MHz ^{1}H NMR spectrum of hexamer **180** in C_6D_6.

Residue	NH	H-3	H-2	H-4	H-5	H-6	Others
A	-	4.692	4.630	5.176	4.498	4.333	$OCOCH_3$ groups
B	7.917	4.960	4.748	5.322	4.614	4.518, 4.366	1.900, 1.887x2, 1.879,
C	8.178	4.994	4.717	5.481	4.609	4.453	1.871x2, 1.854, 1.837,
D	8.047	4.832	4.811	5.548	4.549	-[b]	1.813, 1.806, 1.771, 1.678
E	8.058	4.933	4.826	5.507	4.482	-[b]	$CH(CH_3)_2$ groups[a]
F	7.720	5.037	4.640	5.251	4.516	4.420	5.051 and 1.107, 1.086.

[a] Refers to the chemical shifts of isopropylic protons on the C-terminal protecting group.
[b] Unsuitable assignment (too crowded).

Table 4.2. ^{1}H chemical shifts of hexamer **180** in C_6D_6 at 298 K.

Addition of d^5-pyridine to the sample in d^6-benzene gave a much improved and well dispersed ^1H spectrum, **Figure 4.18**, which allowed assignment of the sugar protons to be completed, as shown in **Table 4.3**.

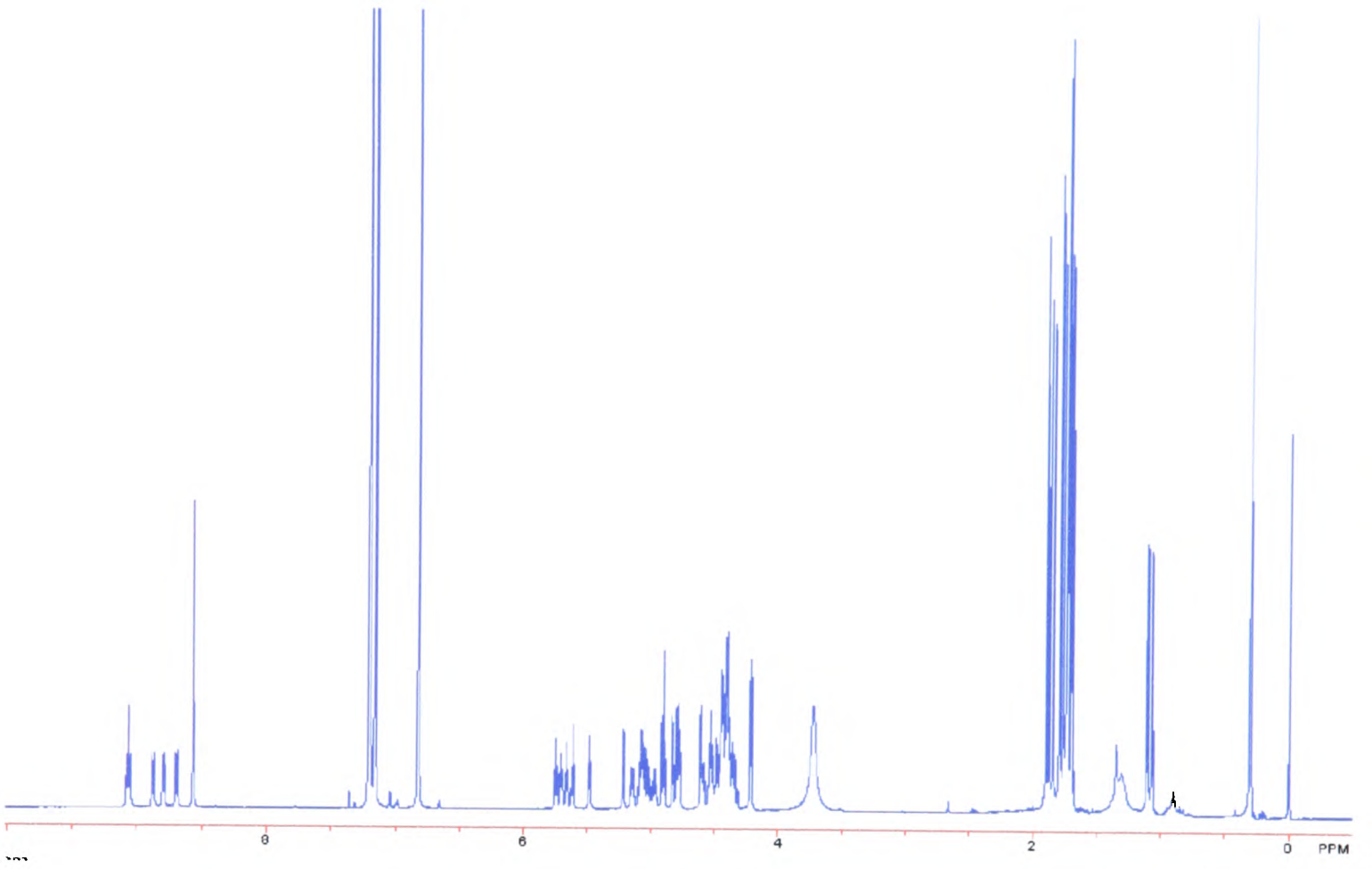


Figure 4.18. 500 MHz ^1H NMR spectrum of hexamer **180** in C_6D_6 + 50 µl pyridine-d^5.

Residue	NH	H3	H2	H4	H5	CH_2	Others
A		4.773	4.609	5.215	4.391	4.215	$OCOCH_3$ groups
B	9.081	5.082	4.795	5.615	4.526	4.347	1.897, 1.873, 1.843, 1.793,
C	9.061	5.022	4.798	5.745	4.535	4.400	1.788, 1.763, 1.744, 1.739,
D	8.803	4.973	4.895	5.702	4.441	4.408	1.735, 1.729, 1.706, 1.697.
E	8.885	5.072	4.911	5.662	4.406	4.460	$CH(CH_3)_2$ groups[a]
F	8.703	5.147	4.832	5.484	4.590	4.487, 4.440	5.056 and 1.104, 1.067.

[a] Refers to the chemical shifts of isopropylic protons on the C-terminal protecting group.

Table 4.3. ^1H Chemical shifts of hexamer **180** in C_6D_6 + 50 µl pyridine-d^5 at 298 K.

4.3.2 N.O.e. studies

The purpose of measurement of n.O.e. enhancements between protons on the sugar rings and the amide protons through use of ROESY experiments is twofold. Firstly, the order of the rings in the hexamer may be determined, as interactions are observed between protons in one ring system and the neighbouring ring systems on each side. Secondly, any observed long-range couplings may be indicative of ordered secondary structure. The results and assignments resulting from ROESY experiments on hexamer **180** in d^6-benzene and d^5-pyridine are shown in **Table 4.4**.

NOEs from ROESY 200 and 300ms in C_6D_6 + 50 μL pyr solution				
NH region		Weak	Medium	Strong
NH-B	H-2A			X
NH-B	H-3A	Overlap		
NH-B	H-5A		X	
NH-B	H-6A	X		
NH-B	H-2B	Overlap		
NH-B	H-3B			X
NH-B	H-4B			X
NH-B	H-6B	X		
NH-C	H-2B	Overlap		
NH-C	H-3B		X	
NH-C	H-5B		X	
NH-C	H-2C	Overlap		
NH-C	H-3C			X
NH-C	H-4C			X
NH-C	H-6C	X		
NH-D	H-3B	X		
NH-D	H-2C			X
NH-D	H-3C		X	
NH-D	H-5C		X	
NH-D	H-2D			X
NH-D	H-3D			X
NH-D	H-4D			X
NH-D	H-6D	X		
NH-E	H-3C	X		
NH-E	H-2D	Overlap		

NH-E	H-3D		X	
NH-E	H-5D		X	
NH-E	H-2E	Overlap		
NH-E	H-3E			X
NH-E	H-4E			X
NH-E	H-6E	X		
NH-F	H-3D	X		
NH-F	H-2E			X
NH-F	H-3E		X	
NH-F	H-5E		X	
NH-F	H-2F			X
NH-F	H-3F			X
NH-F	H-4F			X
NH-F	H-6F	X		
Sugar region		Weak	Medium	Strong
H-3B	H-2D		X	
H-3C	H-2E		X	
H4(all)	H3(all)			X
H4(all)	H2(all)		X	
H4(all)	H5(all)			X[a]
H3(all)	H5(all)		X[a]	
H2(all)	CH_2(all)		X[a]	

[a] Some of them overlap with other peaks

Table 4.4. ROESY experiment for hexamer **180**.

The following diagram displays a summary of the n.O.e. enhancements observed for hexamer **180** as determined by ^{1}H NMR and ROESY experiments, **Figure 4.19**, **p122**. While it was possible to confirm the assignment of ring systems, the observed n.O.e. enhancements did not provide sufficient evidence for a well defined secondary structural motif and are therefore inconclusive.

Figure 4.19

4.3.3 DMSO titrations

In order to further investigate the nature or presence of hydrogen bonding in hexamer **180**, DMSO titrations were performed in d^6-benzene. Protons which are involved in strong hydrogen bonds should show little change when exposed to DMSO, however weakly hydrogen bonded or non-hydrogen bonded protons may dramatically change in terms of their chemical shift as the increasingly polar solvent competes with the intramolecular hydrogen-bonding.

For hexamer **180**, the amide protons of rings B and F display the largest shift in ppm as the concentration of DMSO increases, **Figure 4.20**. This suggests that these NH protons are non-hydrogen bonding. The other NH protons each display a smaller shift and could therefore be involved in the formation of weak hydrogen bonds.

% dmso	NH(B)	NH(C)	NH(D)	NH(E)	NH(F)
0	8.0000	8.2099	8.0208	8.0504	7.6608
1	8.4834	8.5462	8.3610	8.3610	8.0943
2	8.6410	8.6330	8.4551	8.4735	8.2674
3	8.7153	8.6762	8.5096	8.5323	8.3681
4	8.7612	8.7036	8.5491	8.5711	8.4393
5	8.7906	8.7217	8.5778	8.5979	8.4878

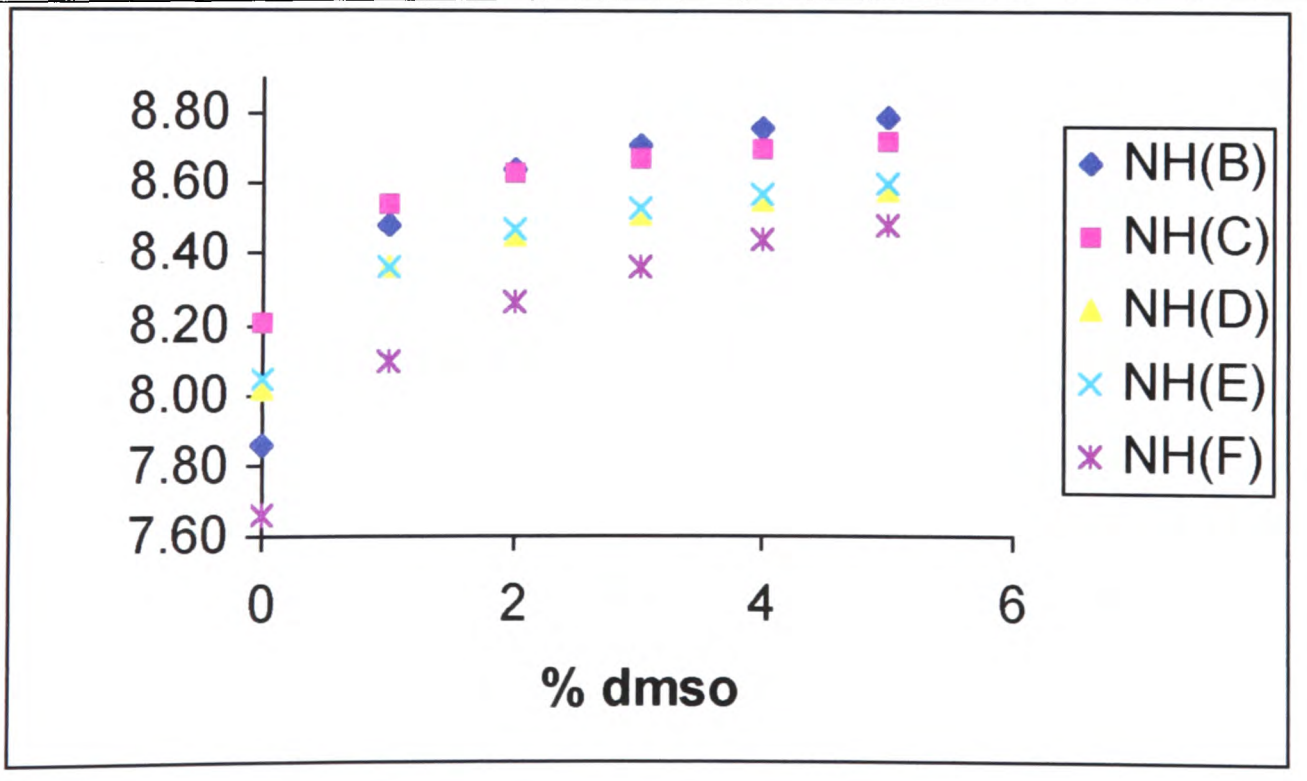
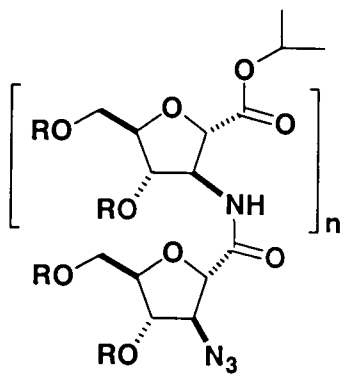


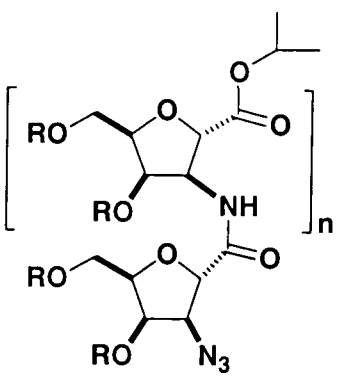
Figure 4.20. DMSO titration of hexamer **180** in C_6D_6.

4.4 Conclusion

This chapter has described the spectroscopic analysis of tetramers **177** and **200** and hexamers **180** and **202**, **Figure 4.21**.



174; n = 1, R = H
177; n = 3, R = H
180; n = 5, R = Ac
} D-*manno*



197; n = 1, R = H
200; n = 3, R = H
202; n = 5, R = Ac
} D-*talo*

Figure 4.21

Analysis of tetramers **177** and **200** by CD spectroscopy resulted in a distinctive curve containing bands at 192 nm and 215 nm. This plot is similar in both shape and peak positions to the spectra displayed by oligomers which have previously been shown to adopt a 14-helical conformation in solution. It can therefore be postulated that both **177** and **200** show a substantial population of the 14-helical hydrogen-bonded conformation in TFE solution.

NMR spectroscopy, however, did not prove to be as conclusive. Several experiments were performed, enabling the assignment of each signal in the proton spectrum for hexameric oligomer **180**. However the n.O.e. and DMSO titration data did not provide sufficient evidence for a well-defined secondary structural motif. It is not possible, therefore, to confirm the adoption of the stabilised secondary structure that was postulated on the basis of CD spectroscopy.

In summary, the results of the analytical experiments were inconclusive, and further investigation using molecular modelling would be required to develop a greater understanding of the conformational preferences of these potential carbopeptoid foldamers.

4.5 References

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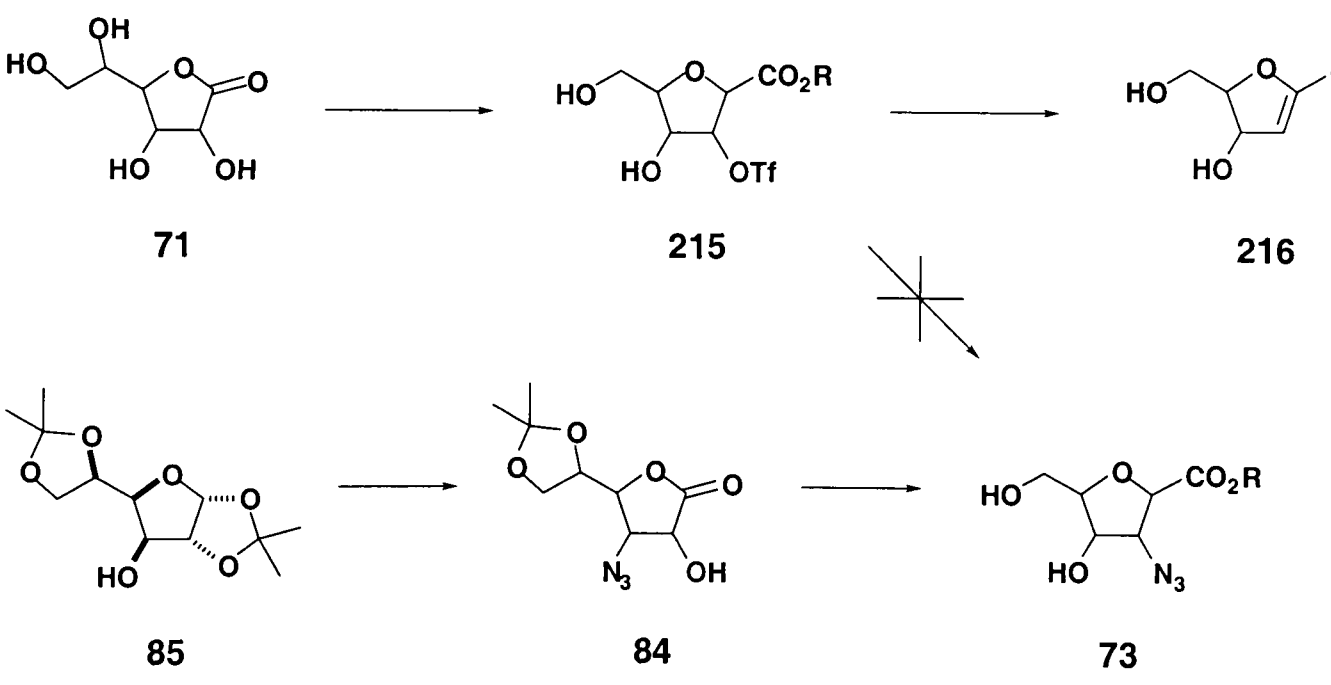
Chapter 5: Conclusions

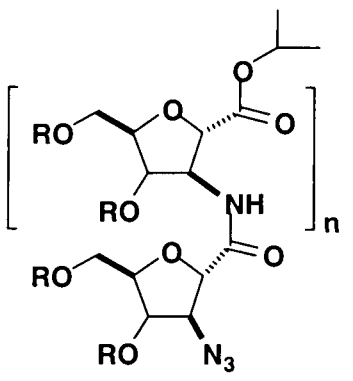
This thesis has described the synthesis of the oligomers of β-amino-THF carboxylic acids and analysis of their secondary structural conformations.

The protected forms of five diastereomeric 3-azido-THF carboxylates **73** were synthesised from diacetone-**D**-glucose **85** *via* related synthetic routes. The propensity of **215** for elimination reactions required the development of a novel strategy whereby the nitrogen functionality was introduced into the compound prior to formation of the THF ring, **Scheme 5.1**.

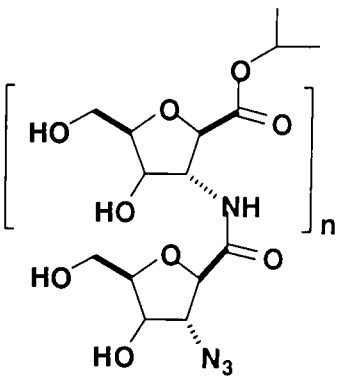


Scheme 5.1

The four β-glycosamino acids **73** with 2,3-*trans* stereochemistry were used to construct the homooligomers of β-amino-THF carboxylic acids, **217** and **218**, **Figure 5.1**, **p127**.



217; n = 1, 3, 5, R = H, Ac



218; n = 1, 3

Figure 5.1

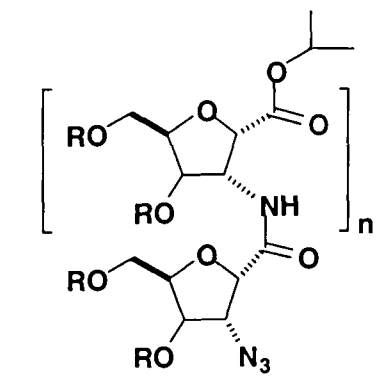
Standard solution phase peptide coupling techniques gave oligomers **217** and **218** in excellent yields. Several other coupling schemes were investigated, including solid-phase and microwave activated strategies and a novel approach using a bicyclic lactone, but these were not so successful.

Spectroscopic analysis of D-*manno* and D-*talo* carbopeptoids **217** did not provide sufficient evidence for any one secondary structural conformation. While analysis by CD spectroscopy suggested a substantial population of a right-handed 14-helix in TFE solution, it did not prove possible to confirm by NMR spectroscopy the adoption of this conformation. The application of molecular modelling may shed further light on the conformational preferences of oligomers **217**. However, the synthesis and analysis of these compounds has already shown that this class of carbopeptoid has potential for the formation of stable secondary structures.

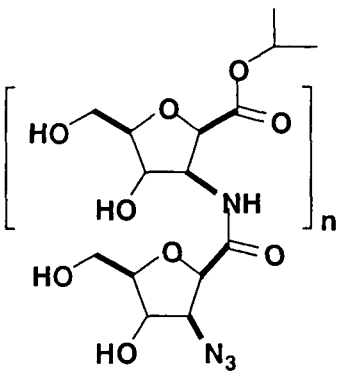
Further work involving the spectroscopic analysis of D-*allo* and D-*gulo* carbopeptoids **218** may provide further evidence for the 14-helical conformation, though a helix with the opposite handedness would be anticipated.

Should it be possible to confirm the adoption of this stable secondary structure, these β-amino-THF carboxylic acids may be used in peptidomimetics. Incorporation of the residues or oligomers into peptide sequences may induce helical conformations, while the introduction of hydrocarbon side chains on the two hydroxyl functionalities could result in the formation of a lipophilic layer on the outside of the molecule enabling cell membrane permeability.

On the other hand, research by co-workers on the synthesis and analysis of carbopeptoids **219** with 2,3-*cis* stereochemistry gave results which were not indicative of the formation of secondary structures stabilised by long-range hydrogen bonding interactions. The synthesis of the remaining 2,3-*cis* oligomers **220** was not therefore considered a priority for future research.



219; n = 1, 3, 5, R = H, Ac



220

Figure 5.2

Chapter 6: Experimental

6.1 General experimental

6.1.1 Solvents

Tetrahydrofuran was distilled under an atmosphere of dry nitrogen from sodium benzophenone ketyl or purchased dry from the Fluka chemical company in sure-seal™ bottles. Dichloromethane and pyridine were distilled from calcium hydride; distilled pyridine was stored over dried 4 Å molecular sieves. Water was distilled. *N*,*N*-Dimethylformamide was purchased dry from the Aldrich chemical company in sure-seal™ bottles. All other solvents were used as supplied (analytical or HPLC grade) without prior purification. Hexane refers to the fraction of petroleum ether that boils in the range 60-80°C and was purchased from Rathburn chemical company (HPLC grade).

6.1.2 Reagents

Reactions performed under an atmosphere of nitrogen, argon or hydrogen gas were maintained by an inflated balloon. pH7 buffer was prepared by dissolving KH_2PO_4 (85 g) and NaOH (14.5 g) in distilled water (950 ml). Imidazole was crystallised from dichloromethane. All other reagents were used as supplied, without prior purification.

6.1.3 Chromatography

Thin layer chromatography (t.l.c.) was performed on aluminium sheets coated with 60 F_{254} silica. Sheets were visualised using a spray of 0.2% w/v cerium (IV) sulphate and 5% ammonium molybdate in 2M sulphuric acid. Purification *via* flash chromatography and gel filtration was performed on Sorbsil C60 40/60 silica and Sephadex LH-20 respectively.

6.1.4 Melting points

Melting points were recorded on a Kofler hot block and are uncorrected.

6.1.5 Polarimetry

Optical rotations were recorded on a Perkin-Elmer 241 polarimeter with a path length of 1 dm. Concentrations are quoted in g/100 mL.

6.1.6 Infrared spectroscopy

Infrared (IR) spectra were recorded on a Perkin-Elmer 1750 IR Fourier Transform spectrophotometer using thin films on NaCl plates (film), KBr discs (KBr), Nujol® mulls (Nujol® mull), or in chloroform solution ($CHCl_3$) as stated. Only the characteristic peaks are quoted and in units of cm^{-1}. For solution measurements, spectroscopic grade $CHCl_3$ was dried over 4Å molecular sieves before use. Solution spectra were recorded at ambient temperature and a concentration of 2 mM, in cells equipped with CaF_2 windows and having a path length of either 0.1 mm or 10 mm. Spectra were obtained with 1 cm^{-1} resolution and solvent subtraction carried out by using background spectra obtained for the neat solvent.

6.1.7 Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) spectra were recorded on Bruker AM 500, AMX 500, and DRX 500 spectrometers (^{1}H: 500 MHz and ^{13}C: 125.7 MHz), a Bruker DPX 400 spectrometer (^{1}H: 400 MHz and ^{13}C: 100.6 MHz), and Bruker AC 200, Bruker DPX 200, and Varian Gemini 200 spectrometers (^{1}H: 200 MHz and ^{13}C: 50.3 MHz) in the deuterated solvent stated. All chemical shifts (δ) are quoted in ppm and scalar coupling constants (J) in Hz. Coupling constants are quoted twice and where assigned, given as the average of the two observed values. Residual signals from the solvents were used as an internal reference; ^{13}C NMR spectra in D_2O were referenced to added 1,4-dioxane (δ_C 67.3 ppm). ^{13}C resonances were assigned using either DEPT sequences, or from ^{1}H-^{13}C HSQC and HMBC spectra. Where two of a larger set of ^{13}C resonances overlap in the 1D spectrum the chemical shift is quoted twice.

2D Experiments (DQF-COSY, TOCSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC, ROESY, Tr-ROESY) were typically acquired with 2048 t_2 and 256 t_1 data points. 1D and 2D TOCSY experiments were recorded with an MLEV-17 spin lock of 80 ms. 1D TOCSY experiments used the double pulsed field gradient spin-echo technique (DPFGSE), with selective excitation in the ^{1}H channel achieved *via* a 40 ms 180° Gaussian pulse. ^{1}H-^{13}C gradient-selected HSQC spectra were optimised for $^1J_{CH}$ correlations of 145 Hz and generally acquired with multiplicity editing. ^{1}H-^{13}C HMBC spectra were recorded with a long-range evolution delay of 70 ms, using a one-step low-pass J-filter to suppress $^1J_{CH}$ correlations. Semi-selective ^{1}H-^{13}C HMBC spectra were acquired with selective excitation of the carbonyl region in the ^{13}C channel using a 2 ms 90° Gaussian pulse. ROESY and Tr-ROESY spectra were acquired with a 200-300 ms NOE mixing time and a continuous-wave spin lock (ROESY) or alternating phase (180°_x – 180°_{-x}) spin lock (Tr-ROESY).

6.1.8 Mass spectrometry

Low resolution mass spectra (*m/z*) were recorded using the following techniques: chemical ionisation (CI, NH_3), or atmospheric pressure chemical ionisation (APCI). CI mass spectra were recorded on a Micromass 500 OAT spectrometer. APCI mass spectra were recorded on a Micromass Platform 1 mass spectrometer *via* an 'Openlynx' system.

High resolution mass spectra (HRMS *m/z*) were recorded on a Micromass 500 OAT spectrometer by chemical ionisation (CI, NH_3) or a Waters 2790-Micromass LCT mass spectrometer by electrospray ionisation (ESI) as stated. For ESI mass spectra the spectrometer was operated at a resolution of 5000 full width half height. Positive ion spectra were calibrated relative to PEG with tetraoctylammonium bromide as the internal lock mass. Negative ion spectra were calibrated relative to poly-**DL**-alanine with Leu-enkephalin as the internal lock mass.

6.1.9 Elemental analysis

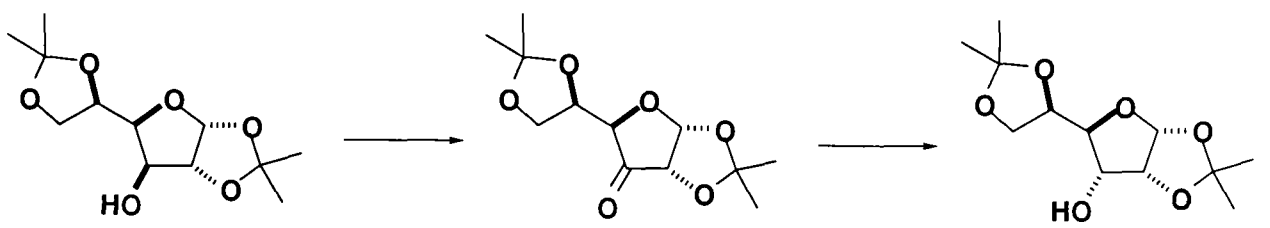
Elemental analyses were performed by the microanalysis service of the Dyson Perrins Laboratory, Oxford, or the Inorganic Chemistry Laboratory, Oxford.

6.1.10 Circular dichroism spectroscopy

Circular dichroism (CD) spectra were recorded in the Department of Biological Chemistry, Imperial College, London on a JASCO J600 circular dichroism spectrometer fitted with a bespoke thermostated cell holder. The sample cell was a quartz Suprasil cylindrical cell with a path length of 0.5 cm. Sample spectra were measured at 293 K in TFE. The following acquisition parameters were used: scan speed = 10 nm/min; time constant = 4 sec; band width = 1 nm; data interval = 0.1 nm; scan range = 260-185 nm. A baseline spectrum recorded in the same cell, at the same concentration, and at proximal time was subtracted from the sample spectra. The resultant spectra were normalised for path length and mean amide concentration.

6.2 Synthesis of monomers

1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranose 86

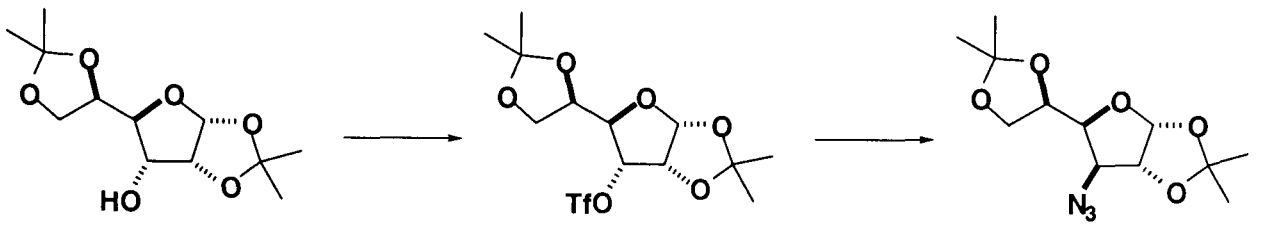


85 **109** **86**

1,2:5,6-Di-*O*-isopropylidene-α-**D**-glucofuranose **85** (30.0 g, 0.12 mol) was dissolved in dry dichloromethane (500 ml) and dry powdered 3Å molecular sieve (75 g) was added. Pyridinium chlorochromate (74.5 g, 0.35 mol) was added and the resultant mixture was stirred vigorously for 21 h. Ether (500 ml) was added and the solution was stirred for a further 1 h and then filtered through a silica plug topped with Celite and magnesium sulphate eluting with ether. The filtrate was concentrated *in vacuo* to give 1,2:5,6-di-*O*-isopropylidene-α-**D**-*ribo*-hexofuranos-3-ulose **109** with its hydrate, a colourless amorphous solid. The crude ketone was not further purified but dissolved in ethyl alcohol : water 9 : 1 (300 ml) and the solution was cooled to 0°C. Sodium borohydride (5.76 g, 0.15 mol) was added and the reaction mixture was stirred for 45 min, then allowed to warm to room temperature over a further 30 min. An excess of ammonium chloride (7.94 g, 0.16 mol) was added to quench the reaction and the solvent was removed *in vacuo*. The residue was partitioned between ethyl acetate (150 ml) and water (150 ml), and the aqueous phase was extracted with ethyl acetate (150 ml). The combined organic layers were washed with brine (150 ml), dried (magnesium sulphate), filtered and concentrated *in vacuo*. Purification by flash column chromatography (ethyl acetate : hexane 1 : 1) yielded 1,2:5,6-di-*O*-isopropylidene-α-**D**-allofuranose **86** (28.7 g, 96%), a white solid; m.p. 75°C (ethyl acetate) [lit.[1] 77-78°C (benzene/hexane)]; $[\alpha]_D^{24}$ +40.4° (c, 1.01 in chloroform) [lit.[2] $[\alpha]_D^{25}$ 35.4° (c, 1 in methanol)]; ν_{max} (Nujol® mull) 3471 (OH) cm^{-1}; δ_H (200 MHz,

$CDCl_3$) 1.22, 1.23, 1.31, 1.43 (12H, 4 x s, $C(C\underline{H}_3)_2$), 2.77 (1H, d, $J_{OH,3}$ 8.4, O$\underline{H}$), 3.69 (1H, dd, $J_{6,6'}$ 8.6, $J_{6,5}$ 4.4, H-6), 3.82-3.98 (3H, m, H-3, H-4, H-6'), 4.17 (1H, a-dt, J 6.5, $J_{5,4}$ 4.4, H-5), 4.46 (1H, a-t, J 4.4, H-2), 5.65 (1H, d, $J_{1,2}$ 3.7, H-1); δ_C (50.3 MHz, $CDCl_3$) 25.1, 26.1, 26.3, 26.4 (4 x q, $C(\underline{C}H_3)_2$), 65.5 (t, C-6), 72.2, 75.3, 78.9, 79.4 (4 x d, C-2, C-3, C-4, C-5), 103.7 (d, C-1), 109.6, 112.5 (2 x s, $\underline{C}(CH_3)_2$); *m/z* (APCI+) 261 (MH^+, 13%), 244 (100%), 203 ($MH^+ - (CH_3)_2CO$, 92%).

3-Azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose 89

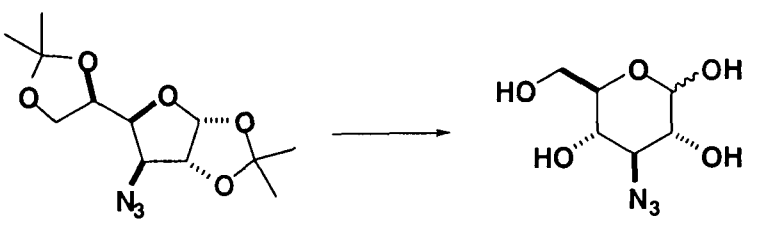


86 **110** **89**

1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranose **86** (13.3 g, 51 mmol) was dissolved in dry dichloromethane (175 ml) and dry pyridine (10.1 ml, 125 mmol) was added. The solution was cooled to -30°C in a nitrogen atmosphere and trifluoromethanesulphonic anhydride (10.5 ml, 63 mmol) was added slowly over 10 min. The resulting mixture was stirred at -30°C for 30 min after which t.l.c. (ethyl acetate : hexane 1 : 1) indicated the complete conversion of the starting material (R_f 0.3) to a single product (R_f 0.6). Methyl alcohol (21 ml) was added to quench the reaction and the solution was allowed to warm to room temperature. The solution was washed with water (120 ml) and brine (120 ml), dried (magnesium sulphate), filtered and concentrated *in vacuo* to give 1,2:5,6-di-*O*-isopropylidene-3-*O*-trifluoromethanesulphonyl-α-D-allofuranose **110**, a pale yellow oil; δ_H (200 MHz, $CDCl_3$) 1.35, 1.39, 1.45, 1.59 (12H, 4 x s, $C(C\underline{H}_3)_2$), 3.91 (1H, dd, $J_{6,6'}$ 8.4, $J_{6,5}$ 4.4, H-6), 4.11 (1H, a-t, J 6.1, H-4), 4.12-4.26 (2H, m, H-5, H-6'), 4.78 (1H, dd, $J_{2,3}$ 5.2, $J_{2,1}$ 3.8, H-2), 4.91 (1H, dd, $J_{3,4}$ 6.8, $J_{3,2}$ 5.2, H-3), 5.84 (1H, d, $J_{1,2}$ 3.8, H-1). The crude triflate was not further

purified but dissolved in dry *N,N*-dimethylformamide (120 ml), sodium azide (6.69 g, 103 mmol) added and the mixture stirred for 18 h, after which t.l.c. (ethyl acetate : hexane 1 : 1) indicated the complete conversion of the starting material (R_f 0.6) to a single product (R_f 0.7). The solvent was removed *in vacuo* and the residue was partitioned between chloroform (120 ml) and water (120 ml). The organic layer was washed with brine (120 ml), dried (magnesium sulphate), filtered and concentrated *in vacuo*. Purification by flash column chromatography (ethyl acetate : hexane 1 : 1) yielded 3-azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-**D**-glucofuranose **89** (13.9 g, 95%), a colourless oil; $[\alpha]_D^{24}$ -41.3° (c, 1.00 in chloroform) [lit.[3] $[\alpha]_D$ -41.5° (c, 2 in chloroform)]; ν_{max} (film) 2989 (C-H), 2109 (N_3) cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.33, 1.37, 1.44, 1.52 (12H, 4 x s, $C(C\underline{H}_3)_2$), 3.99 (1H, dd, $J_{6,6'}$ 8.5, $J_{6,5}$ 4.6, H-6), 4.06-4.15 (3H, m, H-3, H-4, H-6'), 4.24 (1H, m, H-5), 4.63 (1H, d, $J_{2,1}$ 3.4, H-2), 5.87 (1H, d, $J_{1,2}$ 3.4, H-1); δ_C (50.3 MHz, $CDCl_3$) 25.1, 26.1, 26.6, 26.8 (4 x q, $C(\underline{C}H_3)_2$), 66.3, 67.5, 72.9, 80.4, 83.3 (C-2, C-3, C-4, C-5, C-6), 105.0 (d, C-1), 109.5, 112.2 (2 x s, $\underline{C}(CH_3)_2$); *m/z* (APCI+) 258 ($MH^+ - N_2$, 93%), 200 ($MH^+ - N_2 - (CH_3)_2CO$, 100%).

3-Azido-3-deoxy-D-glucopyranose 111

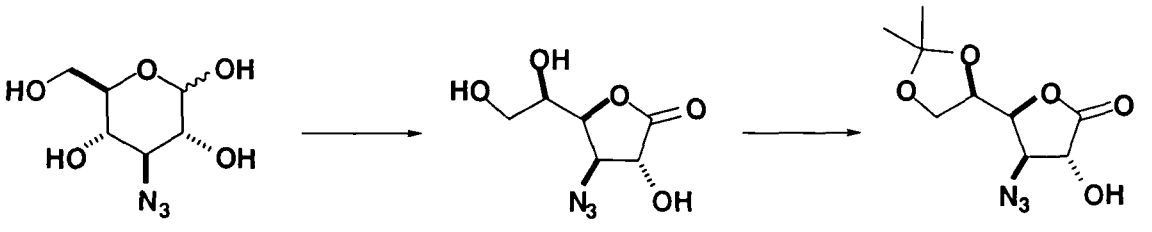


89 **111**

3-Azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-**D**-glucofuranose **89** (10.1 g, 35.3 mmol) was dissolved in 50% aqueous trifluoroacetic acid (100 ml) and stirred for 17 h. The solvent was removed *in vacuo*. Purification by flash column chromatography (ethyl acetate) yielded both anomers of 3-azido-3-deoxy-**D**-glucopyranose **111** (6.9 g, 95%), a white foam; δ_C (50.3 MHz, D_2O)

60.3, 60.4 (2 x t, C-6), 65.6, 68.3, 68.4, 70.2, 71.0, 72.6, 76.3 (7 x d, C-2, C-3, C-4, C-5), 91.3, 95.8 (2 x d, C-1).

***3-Azido-3-deoxy-5,6-O-isopropylidene-D-glucono-1,4-lactone* 93**

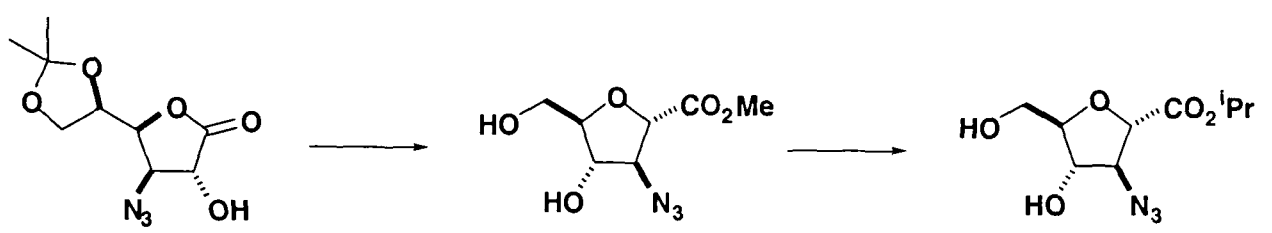


111 **113** **93**

3-Azido-3-deoxy-D-glucopyranose **111** (1.56 g, 7.6 mmol) was dissolved in water (25 ml), barium carbonate (2.25 g, 11.4 mmol) was added and the mixture was cooled to 0°C. Bromine (0.47 ml, 9.1 mmol) was added dropwise over 5 min. The mixture was stirred at 0°C for 30 min, allowed to warm to room temperature and stirred for 17 h in the dark. The solution was bubbled with nitrogen to remove excess bromine and the solvent was removed *in vacuo*. The white solid obtained was dissolved in water, subjected to ion exchange with Amberlite IR-120 (H^+) resin, and concentrated *in vacuo* to give 3-azido-3-deoxy-D-glucono-1,5-lactone **113**, an orange foam. The crude lactone was not further purified but dissolved in acetone (25 ml) and camphorsulphonic acid (2.25 g, 9.7 mmol) was added. The solution was heated under reflux with exclusion of moisture for 5 h, after which t.l.c. (ethyl acetate : hexane 1 : 1) indicated the complete conversion of baseline material to a single product (R_f 0.4). The solution was neutralised with sodium bicarbonate, filtered through Celite and concentrated *in vacuo*. Purification by flash column chromatography (ethyl acetate : hexane 1 : 1) yielded *3-azido-3-deoxy-5,6-O-isopropylidene-D-glucono-1,4-lactone* **93** (1.44 g, 78%), a colourless oil; $[\alpha]_D^{23}$ +71.1° (c, 1.11 in chloroform); ν_{max} (film) 3400 (OH), 2114 (N_3), 1798 (C=O) cm^{-1}; δ_H (500 MHz, DMSO-d^6) 1.31, 1.39 (6H, 2 x s, $C(C\underline{H}_3)_2$), 3.93 (1H, dd, $J_{6,6'}$ 8.7, $J_{6,5}$ 5.4, H-6), 4.12

(1H, dd, $J_{6',6}$ 8.7, $J_{6',5}$ 6.4, H-6'), 4.38 (1H, ddd, $J_{5,4}$ 7.5, $J_{5,6'}$ 6.4, $J_{5,6}$ 5.4, H-5), 4.39 (1H, a-t, J 5.5, H-2), 4.53 (1H, a-t, J 5.5, H-3), 4.68 (1H, dd, $J_{4,5}$ 7.5, $J_{4,3}$ 5.9, H-4), 5.77 (1H, d, $J_{OH,2}$ 5.4, O<u>H</u>); δ_C (50.3 MHz, $CDCl_3$) 24.9, 26.4 (2 x q, C(<u>C</u>H_3)$_2$), 66.4 (t, C-6), 63.8, 71.6, 73.2, 79.2 (4 x d, C-2, C-3, C-4, C-5), 111.2 (s, <u>C</u>$(CH_3)_2$), 174.4 (s, C-1); *m/z* (EPCI+) 261 (MNH_4^+, 23%), 244 (MH^+, 70%), 216 ($MNH_4^+ - N_2$, 43%), 158 (100%). HRMS *m/z* (CI+): Found 261.119585. $C_9H_{17}N_4O_5$ ($M + NH_4^+$) requires 261.119895.

***Methyl 2,5-anhydro-3-azido-3-deoxy-D-mannonate* 97 and *isopropyl 2,5-anhydro-3-azido-3-deoxy-D-mannonate* 116**



93 **97** **116**

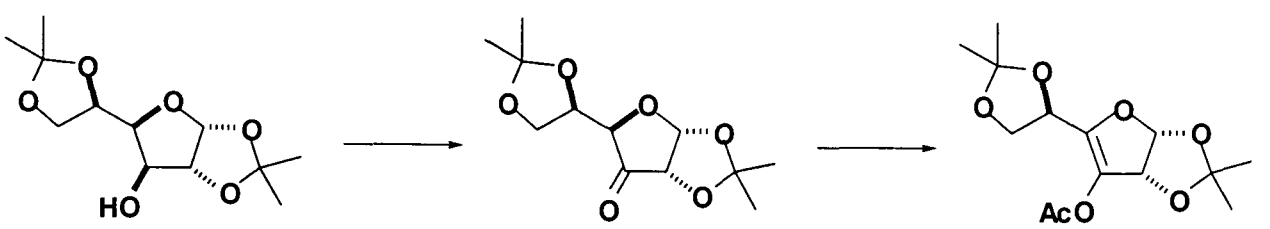
3-Azido-3-deoxy-5,6-*O*-isopropylidene-**D**-glucono-1,4-lactone **93** (3.20 g, 13.2 mmol) was dissolved in dry dichloromethane (40 ml) and the solution was cooled to 0°C in a nitrogen atmosphere. Pyridine (3.19 ml, 39.5 mmol) was added and the solution was stirred for 15 min before addition of trifluoromethanesulphonic anhydride (3.32 ml, 19.7 mmol) with stirring at 0°C. After 30 min t.l.c. (ethyl acetate : hexane 1 : 1) indicated the complete conversion of the starting material (R_f 0.5) to a single product (R_f 0.6). The reaction mixture was added directly to a solution of acetyl chloride (5.6 ml) in methyl alcohol (280 ml) at 0°C. The solution was stirred at 0°C for a further 30 min, then allowed to warm to room temperature and stirred for 42 h, after which t.l.c. (ethyl acetate : hexane 7 : 3) indicated the complete conversion to one product (R_f 0.2). The solvent was removed *in vacuo*. Purification by flash column chromatography (2% methanol in diethyl ether) gave methyl 2,5-

anhydro-3-azido-3-deoxy-**D**-mannonate **97** contaminated with an unknown compound, which was not purified further but dissolved in isopropyl alcohol (25 ml) and this solution was then added slowly to a solution of acetyl chloride (2.5 ml) in isopropyl alcohol (125 ml) at 0°C. The mixture was allowed to warm to room temperature and stirred for 22 h, after which t.l.c. (ethyl acetate : hexane 7 : 3) indicated incomplete conversion of the starting material (R_f 0.2) to a single product (R_f 0.3). The reaction mixture was heated to 80°C and stirred for 3 h, after which t.l.c. (ethyl acetate : hexane 7 : 3) indicated the complete conversion to a single product (R_f 0.3). The reaction mixture was neutralised with sodium hydrogencarbonate, filtered and the solvent was removed *in vacuo*. Purification by flash column chromatography (ethyl acetate : hexane 7 : 3) yielded *isopropyl 2,5-anhydro-3-azido-3-deoxy-**D**-mannonate* **116** (2.01 g, 62% from lactone), a white solid; m.p. 82-84°C (ethyl acetate); $[\alpha]_D^{22}$ +74.9° (c, 1.0 in chloroform); ν_{max} (Nujol® mull) 3279 (br, OH), 2119 (N_3), 1731 (C=O) cm^{-1}; δ_H (400 MHz, CD_3OD) 1.52 (6H, d, *J* 6.2, $CH(C\underline{H}_3)_2$), 3.84 (1H, dd, $J_{6,6'}$ 12.2, $J_{6,5}$ 4.5, H-6), 3.97 (1H, dd, $J_{6',6}$ 12.2, $J_{6',5}$ 3.4, H-6'), 4.14 (1H, ddd, $J_{5,4}$ 6.6, $J_{5,6}$ 4.5, $J_{5,6'}$ 3.4, H-5), 4.25 (1H, a-t, *J* 6.2, H-4), 4.30 (1H, a-t, *J* 5.8, H-3), 4.57 (1H, d, $J_{2,3}$ 6.0, H-2), 5.33 (1H, sept, *J* 6.2, $C\underline{H}(CH_3)_2$); δ_C (100.6 MHz, CD_3OD) 22.3, 22.3 (2 x q, $CH(\underline{C}H_3)_2$), 62.5 (t, C-6), 71.1 (d, $\underline{C}H(CH_3)_2$), 72.5 (d, C-3), 76.6 (d, C-4), 81.0 (d, C-2), 86.3 (d, C-5), 172.3 (s, C-1); *m/z* (APCI+) 218 ($MH^+ - N_2$, 30%), 176 ($MNH_4^+ - (CO_2{}^iPr)$, 100%), 158 ($M - (CO_2{}^iPr)^-$, 62%). Found C, 44.12; H, 6.08; N, 17.19%. $C_9H_{15}N_3O_5$ requires C, 44.08; H, 6.16; N, 17.13%. Further elution afforded *methyl 2,5-anhydro-3-azido-3-deoxy-**D**-mannonate* **97** (0.09 g, 3% from lactone), a colourless oil; $[\alpha]_D^{24}$ +32.6° (c, 1.01 in methanol); δ_H (500 MHz, CD_3OD) 3.63 (1H, dd, $J_{6,6'}$ 12.2, $J_{6,5}$ 4.6, H-6), 3.75 (1H, dd, $J_{6',6}$ 12.2, $J_{6',5}$ 3.6, H-6'), 3.81 (3H, s, $C\underline{H}_3$), 3.94 (1H, ddd, $J_{5,4}$ 6.6, $J_{5,6}$ 4.6, $J_{5,6'}$ 3.6, H-5), 4.07 (1H, a-t, *J* 6.1, H-4), 4.15 (1H, a-t, *J* 5.6, H-3), 4.41 (1H, d, $J_{2,3}$ 5.6, H-2); δ_C (50.3 MHz, $CDCl_3$) 52.9 (q, $\underline{C}H_3$), 61.3 (t, C-6), 69.9, 75.4, 79.2, 84.4 (4 x d, C-2, C-3, C-4, C-5), 171.3 (s, C-1); *m/z* (EPCI+)

255 (MK^+, 7%), 240 (MNa^+, 14%), 190 ($MH^+ - N_2$, 62%), 172 ($MH^+ - N_2 - H_2O$, 55%), 122 (100%).

HRMS *m/z* (CI+): Found 235.104780. $C_7H_{15}N_4O_5$ ($M + NH_4^+$) requires 235.104245.

3-*O*-Acetyl-1,2:5,6-di-*O*-isopropylidene-α-D-*erythro*-hex-3-enofuranose 117

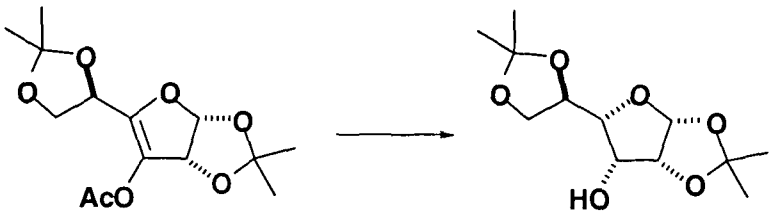


85 **109** **117**

1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose **85** (20.0 g, 76.8 mmol) was added to a stirred mixture of pyridinium dichromate (23.2 g, 61.5 mmol) and acetic anhydride (20 ml) in dichloromethane (200 ml) and the resultant solution was heated under reflux at 70°C for 2 h, after which t.l.c. (ethyl acetate : hexane 1 : 1) indicated the complete conversion of the starting material (R_f 0.3) to a single product (R_f 0.2). The reaction mixture was concentrated to one third of the volume. Ethyl acetate (400 ml) was added and the solution was stirred for a further 30 min and then filtered through Celite eluting with ethyl acetate. The filtrate was concentrated *in vacuo*. Flash column chromatography (ethyl acetate : hexane 1 : 1) yielded 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose **109**, a colourless oil. The crude ketone was not further purified but dissolved in a mixture of acetic anhydride (70 ml) and pyridine (110 ml) and the reaction mixture heated under reflux at 70°C for 5 h, after which t.l.c. (ethyl acetate : hexane 1 : 1) indicated the complete conversion of the starting material (R_f 0.2) to a single product (R_f 0.5). The solvent was removed *in vacuo* (co-evaporation with toluene). The residue was dissolved in ethyl acetate (400 ml), washed with 2M hydrochloric acid (130 ml) and pH7 buffer (160 ml), dried (magnesium sulphate), filtered and concentrated *in vacuo*. Purification by flash column chromatography (ethyl acetate : hexane

1 : 4) yielded 3-*O*-acetyl-1,2:5,6-di-*O*-isopropylidene-α-D-*erythro*-hex-3-enofuranose **117** (17.8 g, 77%), a white crystalline solid, m.p. 55-57°C (ethyl acetate) [lit.[4] 62°C (methanol)]; $[\alpha]_D^{25}$ -33.2° (c, 0.97 in chloroform) [lit.[4] $[\alpha]_D^{20}$ -33° (c, 1 in chloroform)]; ν_{max} (Nujol® mull) 1764 (C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.39, 1.46, 1.48, 1.55 (12H, 4 x s, $C(C\underline{H}_3)_2$), 2.23 (3H, s, $OCOC\underline{H}_3$), 4.07 (1H, dd, $J_{6,6'}$ 8.4, $J_{6,5}$ 6.2, H-6), 4.10 (1H, dd, $J_{6',6}$ 8.4, $J_{6',5}$ 6.6, H-6'), 4.72 (1H, a-t, *J* 6.4, H-5), 5.41 (1H, d, *J* 5.5), 5.79 (1H, d, *J* 5.5); δ_C (50.3 MHz, $CDCl_3$) 21.0 (q, $OCO\underline{C}H_3$), 26.1, 26.2, 28.3, 28.3 (4 x q, $C(\underline{C}H_3)_2$), 66.4 (t, C-6), 69.1, 81.3 (2 x d, C-2, C-5), 104.5 (d, C-1), 110.9, 113.9 (2 x s, $\underline{C}(CH_3)_2$), 129.5, 145.7 (2 x s, C-3, C-4), 169.4 (s, $O\underline{C}OCH_3$); *m/z* (APCI+) 323 (MNa^+, 12%), 125 (100%).

1,2:5,6-Di-*O*-isopropylidene-α-D-gulofuranose 87

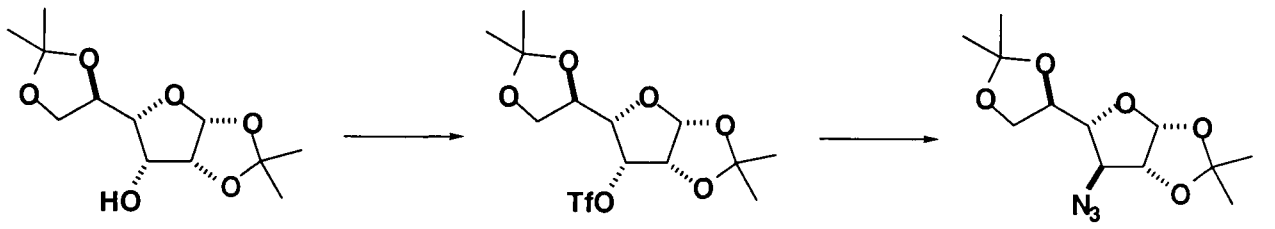


117 **87**

A solution of 3-*O*-acetyl-1,2:5,6-di-*O*-isopropylidene-α-D-*erythro*-hex-3-enofuranose **117** (14.0 g, 46.5 mmol) in methanol (140 ml) was stirred under hydrogen in the presence of palladium on carbon (1.40 g) for 20 h after which t.l.c. (ethyl acetate : hexane 1 : 1) indicated the complete conversion of the starting material (R_f 0.5) to a single product (R_f 0.3). The reaction mixture was filtered through Celite eluting with methanol and potassium carbonate (1.10 g) was added. The mixture was stirred for 4 d, after which t.l.c. (ethyl acetate : hexane 1 : 1) indicated the complete conversion to a single product (R_f 0.2). The reaction mixture was filtered through Celite and concentrated *in vacuo*. Purification by flash column chromatography (ethyl acetate : hexane 1 : 2) yielded 1,2:5,6-di-*O*-isopropylidene-α-D-gulofuranose **87** (7.60 g, 63%), a white crystalline solid; m.p. 103-104°C (ethyl

acetate) [lit.[4] 105-106°C (ethanol/ether/hexane)]; $[\alpha]_D^{24}$ +8.8° (c, 2.00 in chloroform) [lit.[4] $[\alpha]_D^{20}$ +7.5° (c, 1 in chloroform)]; ν_{max} (KBr) 3514, 3493 (OH), 2987, 2938, 2888 (C-H) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.38, 1.43, 1.45, 1.63 (12H, 4 x s, $C(C\underline{H}_3)_2$), 2.68 (1H, d, $J_{OH,3}$ 6.4, O$\underline{H}$), 3.72 (1H, dd, $J_{6,6'}$ 8.4, $J_{6,5}$ 7.5, H-6), 3.90 (1H, dd, $J_{4,5}$ 8.5, $J_{4,3}$ 5.8, H-4), 4.23 (1H, dd, $J_{6',6}$ 8.4, $J_{6',5}$ 6.6, H-6'), 4.24 (1H, a-q, *J* 6.0, H-3), 4.48 (1H, a-dt, $J_{5,4}$ 8.5, *J* 7.0, H-5), 4.67 (1H, dd, $J_{2,3}$ 6.1, $J_{2,1}$ 4.1, H-2), 5.79 (1H, d, $J_{1,2}$ 4.1, H-1); δ_C (100.6 MHz, $CDCl_3$) 25.6, 27.1, 27.5, 27.6 (4 x q, $C(\underline{C}H_3)_2$), 66.8 (t, C-6), 70.1 (d, C-3), 75.9 (d, C-5), 80.3 (d, C-2), 84.7 (d, C-4), 105.7 (d, C-1), 109.7, 115.4 (2 x s, $\underline{C}(CH_3)_2$); *m/z* (APCI+) 538 ($M_2NH_4^+$, 2%), 278 (MNH_4^+, 87%), 261 (MH^+, 8%), 203 ($MH^+ - (CH_3)_2CO$, 100%).

3-Azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose 90

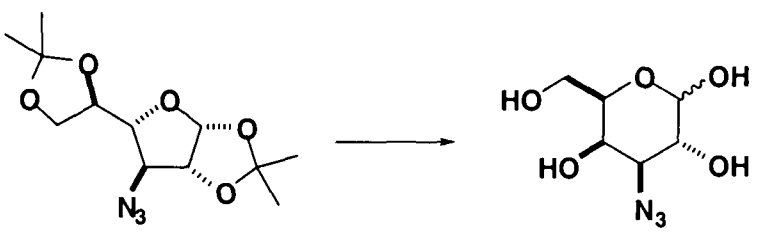


87 **118** **90**

1,2:5,6-Di-*O*-isopropylidene-α-D-gulofuranose **87** (3.41 g, 13.1 mmol) was dissolved in dry dichloromethane (60 ml) and dry pyridine (4.77 ml, 59.0 mmol) was added. The solution was cooled to 0°C in a nitrogen atmosphere and trifluoromethanesulphonic anhydride (3.31 ml, 19.7 mmol) was added slowly over 10 min. The resulting mixture was stirred at 0°C for 20 min after which t.l.c. (ethyl acetate : hexane 1 : 1) indicated the complete conversion of the starting material (R_f 0.2) to a single product (R_f 0.5). One drop of pH7 buffer was added to quench the reaction and the solvent was removed *in vacuo* (co-evaporation with toluene). The residue was dissolved in dichloromethane (300 ml), washed with 1M hydrochloric acid (60 ml) and pH7 buffer (90 ml), dried (magnesium

sulphate), filtered and concentrated *in vacuo* to give 1,2:5,6-di-*O*-isopropylidene-3-*O*-trifluoromethanesulphonyl-α-**D**-gulofuranose **118**, a pale yellow oil; ν_{max} (KBr) 2991, 2942, 2892 (C-H), 1421, 1416 (SO_3) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.39, 1.44, 1.47, 1.63 (12H, 4 x s, $C(C\underline{H}_3)_2$), 3.66 (1H, dd, $J_{6,6'}$ 8.5, $J_{6,5}$ 6.5, H-6), 4.06 (1H, dd, $J_{4,5}$ 8.8, $J_{4,3}$ 5.8, H-4), 4.16 (1H, dd, $J_{6',6}$ 8.5, $J_{6',5}$ 6.5, H-6'), 4.57 (1H, a-dt, $J_{5,4}$ 8.8, J 6.5, H-5), 4.81 (1H, dd, $J_{2,3}$ 5.8, $J_{2,1}$ 4.1, H-2), 5.09 (1H, a-t, J 5.8, H-3), 5.83 (1H, d, $J_{1,2}$ 4.1, H-1); δ_C (50.3 MHz, $CDCl_3$) 24.9, 26.6, 26.7, 27.2 (4 x q, $C(\underline{C}H_3)_2$), 65.8 (t, C-6), 74.0 (d, C-5), 78.9 (d, C-2), 80.6 (d, C-4), 82.4 (d, C-3), 104.9 (d, C-1), 109.9, 116.4 (2 x s, $\underline{C}(CH_3)_2$); *m/z* (APCI+) 410 (MNH_4^+, 99%), 393 (MH^+, 10%), 335 ($MH^+ - (CH_3)_2CO$, 100%). The crude triflate was not further purified but dissolved in dry *N,N*-dimethylformamide (35 ml), sodium azide (2.60 g, 40.0 mmol) added and the mixture was then stirred for 20 h, after which t.l.c. (ethyl acetate : hexane 1 : 1) indicated the complete conversion to a single product (R_f 0.7). The solvent was removed *in vacuo* (co-evaporation with toluene). The residue dissolved in dichloromethane (300 ml), washed with brine (60 ml) and water (60 ml), dried (magnesium sulphate), filtered and concentrated *in vacuo*. Purification by flash column chromatography (ethyl acetate : hexane 1 : 4) yielded 3-azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-**D**-galactofuranose **90** (3.63 g, 97%), a white crystalline solid; m.p. 35-36°C (ethyl acetate) [lit.[5] 30-32°C (ethyl acetate)]; $[\alpha]_D^{24}$ -17.6° (c, 0.50 in chloroform) [lit.[6] $[\alpha]_D$ -26.9° (c, 0.5 in chloroform)]; ν_{max} (KBr) 2106 (N_3), 2988, 2939, 2893 (C-H) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.37, 1.38, 1.46, 1.57 (12H, 4 x s, $C(C\underline{H}_3)_2$), 3.83 (1H, a-t, J 5.8, H-4), 3.88 (1H, dd, $J_{6,6'}$ 8.4, $J_{6,5}$ 6.6, H-6), 3.95 (1H, dd, $J_{3,4}$ 5.8, $J_{3,2}$ 1.8, H-3), 4.08 (1H, dd, $J_{6',6}$ 8.4, $J_{6',5}$ 6.8, H-6'), 4.35 (1H, a-q, J 6.0, H-5), 4.60 (1H, dd, $J_{2,1}$ 4.0, $J_{2,3}$ 1.8, H-2), 5.80 (1H, d, $J_{1,2}$ 4.0, H-1); δ_C (100.6 MHz, $CDCl_3$) 25.2, 26.4, 26.9, 27.5 (4 x q, $C(\underline{C}H_3)_2$), 65.5 (d, C-3), 65.6 (t, C-6), 74.6 (d, C-5), 83.1 (d, C-4), 85.8 (d, C-2), 104.9 (d, C-1), 110.1, 114.4 (2 x s, $\underline{C}(CH_3)_2$); *m/z* (APCI+) 303 (MNH_4^+, 100%), 228 ($MH^+ - (CH_3)_2CO$, 27%).

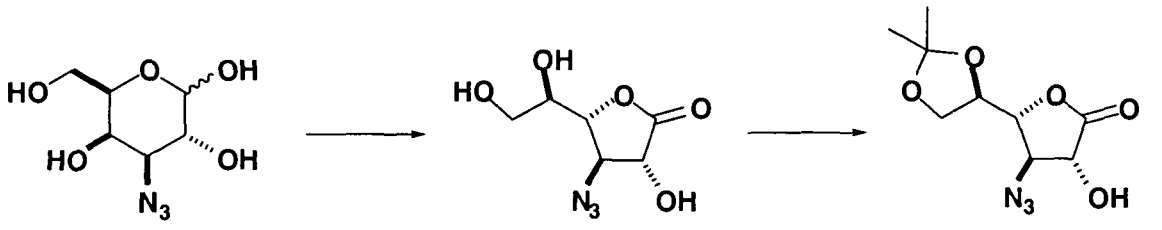
3-Azido-3-deoxy-D-galactopyranose 119



90 **119**

3-Azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose **90** (3.72 g, 13.0 mmol) was dissolved in 50% aqueous trifluoroacetic acid (60 ml) and stirred for 16 h. The solvent was removed *in vacuo* (co-evaporation with water and toluene) to give **119**, a colourless oil, which was used without further purification. Purification of a small sample by flash column chromatography (5% methanol in ethyl acetate) afforded 3-azido-3-deoxy-D-galactopyranose **119**, a colourless oil; $[\alpha]_D^{25}$ +90.6° (c, 0.25 in water) [lit.[7] $[\alpha]_D^{25}$ +90.8° (c, 0.25 in water)]; ν_{max} (film) 3351 (OH), 2112 (N_3) cm^{-1}.

3-Azido-3-deoxy-5,6-*O*-isopropylidene-D-galactono-1,4-lactone 94

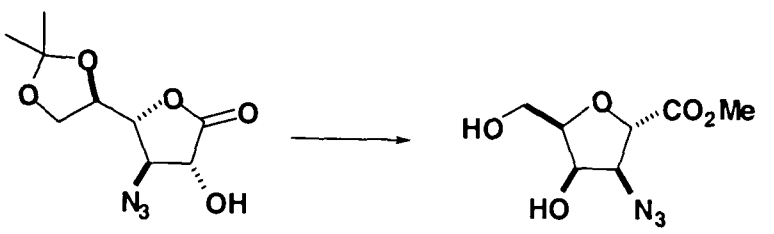


119 **120** **94**

3-Azido-3-deoxy-D-galactopyranose **119** (2.60 g, 12.7 mmol) was dissolved in water (50 ml) and the resulting solution was cooled to 0°C. Bromine (1.0 ml, 19.4 mmol) was added dropwise over 5 min. The mixture was stirred at 0°C for 30 min, allowed to warm to room temperature and stirred for 3 d in the dark, after which t.l.c. (5% methanol in ethyl acetate) indicated incomplete conversion of the

starting material (R_f 0.3) to a single product (R_f 0.5). Bromine (1.0 ml, 19.4 mmol) was added and the reaction mixture was stirred for 4 d in the dark, after which t.l.c. (5% methanol in ethyl acetate) indicated the complete conversion to a single product (R_f 0.5). The solution was bubbled with nitrogen to remove excess bromine and the solvent was removed *in vacuo* to give 3-azido-3-deoxy-**D**-glucono-1,4-lactone **120**, a yellow foam. The crude lactone was not further purified but dissolved in acetone (50 ml) and camphorsulphonic acid (0.59 g, 2.5 mmol) was added. The solution was heated under reflux with exclusion of moisture for 2 h, after which t.l.c. (ethyl acetate) indicated the formation of a single product (R_f 0.4). The solution was neutralised with sodium bicarbonate, filtered and concentrated *in vacuo*. Purification by flash column chromatography (ethyl acetate : hexane 1 : 3) yielded 3-azido-3-deoxy-5,6-*O*-isopropylidene-**D**-galactono-1,4-lactone **94** (2.22 g, 70% from furanose), a colourless oil; $[\alpha]_D^{24}$ -67.5° (c, 1.01 in chloroform) [lit.[7] $[\alpha]_D^{25}$ -68.2° (c, 1.00 in chloroform)]; ν_{max} (film) 3401 (OH), 2113 (N_3), 1799 (C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.41, 1.43 (6H, 2 x s, $C(C\underline{H}_3)_2$), 4.00 (1H, dd, $J_{6,6'}$ 8.5, $J_{6,5}$ 6.6, H-6), 4.08 (1H, dd, $J_{4,3}$ 7.9, $J_{4,5}$ 2.6, H-4), 4.16 (1H, dd, $J_{6',6}$ 8.5, $J_{6',5}$ 6.8, H-6'), 4.29 (1H, a-t, J 8.3, H-3), 4.34 (1H, a-dt, J 6.8, $J_{5,4}$ 2.6, H-5), 4.55 (1H, dd, $J_{2,3}$ 8.5, $J_{2,OH}$ 5.0, H-2), 4.72 (1H, d, $J_{OH,2}$ 5.0, OH); δ_C (50.3 MHz, $CDCl_3$) 25.3, 25.8 (2 x q, $C(\underline{C}H_3)_2$), 65.0 (t, C-6), 63.7, 73.2, 73.2, 77.7 (4 x d, C-2, C-3, C-4, C-5), 110.7 (s, $\underline{C}(CH_3)_2$), 173.5 (s, C-1); *m/z* (EPCI+) 216 ($MH^+ - N_2$, 20%), 114 (100%). HRMS *m/z* (CI+): Found 261.119757. $C_9H_{17}N_4O_5$ ($M + NH_4^+$) requires 261.119895. Found 244.092927. $C_9H_{14}N_3O_5$ (MH^+) requires 244.093346.

Methyl 2,5-anhydro-3-azido-3-deoxy-D-talonate 98



94 **98**

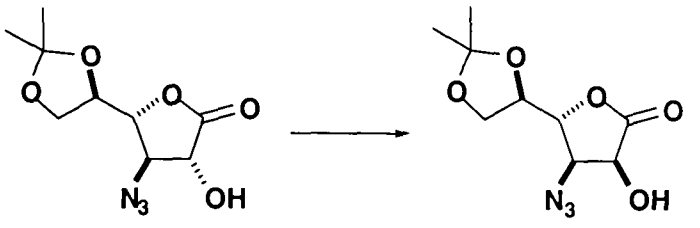
3-Azido-3-deoxy-5,6-*O*-isopropylidene-**D**-galactono-1,4-lactone **94** (1.50 g, 6.2 mmol) was dissolved in dry dichloromethane (30 ml) and the solution was cooled to -10°C in a nitrogen atmosphere. Pyridine (1.00 ml, 12.3 mmol) was added and the solution was stirred for 5 min before addition of trifluoromethanesulphonic anhydride (1.56 ml, 9.3 mmol) with stirring at -10°C. After 1 min t.l.c. (ethyl acetate : hexane 1 : 1) indicated the complete conversion of the starting material (R_f 0.5) to a single product (R_f 0.7). The reaction mixture was added directly to a solution of acetyl chloride (0.2 ml) in methyl alcohol (20 ml) at -10°C. The solution was stirred at -10°C for a further 10 min, then allowed to warm to room temperature and stirred for 17 h, after which t.l.c. (ethyl acetate : hexane 1 : 1) indicated the complete conversion to baseline products. The solution was neutralised with sodium bicarbonate, filtered and concentrated *in vacuo*. Flash column chromatography (5% methanol in chloroform) yielded methyl 2,5-anhydro-3-azido-3-deoxy-**D**-talonate **98** (0.82 g, 61%), a yellow oil; $[\alpha]_D^{25}$ +72.8° (c, 0.25 in chloroform) [lit.[8] $[\alpha]_D^{24}$ +75.3° (c, 1.01 in methanol)]; ν_{max} (film) 3369 (OH), 2114 (N_3), 1742 (C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 3.83 (3H, s, C$\underline{H}_3$), 3.92 (1H, dd, $J_{6,6'}$ 12.3, $J_{6,5}$ 4.1, H-6), 3.97 (1H, dd, $J_{6',6}$ 12.3, $J_{6',5}$ 4.6, H-6'), 4.09 (1H, dd, $J_{3,2}$ 7.3, $J_{3,4}$ 4.7, H-3), 4.22 (1H, a-q, *J* 4.3, H-5), 4.52 (1H, a-t, *J* 4.4, H-4), 4.57 (1H, d, $J_{2,3}$ 7.3, H-2); δ_C (50.3 MHz, $CDCl_3$) 52.8 (q, $\underline{C}H_3$), 61.0 (t, C-6), 66.2, 73.3, 78.0, 81.3 (4 x d, C-2, C-3, C-4, C-5), 171.0 (s, C-1); *m/z* (EPCI+) 240 (MNa^+, 100%), 190 ($MH^+ - N_2$, 25%). HRMS *m/z* (CI+): Found 235.104212. $C_7H_{15}N_4O_5$ ($M + NH_4^+$) requires 235.104245.

***Isopropyl 2,5-anhydro-3-azido-3-deoxy-D-talonate* 122**

98 **122**

A solution of methyl 2,5-anhydro-3-azido-3-deoxy-**D**-talonate **98** (0.82 g, 3.8 mmol) in isopropanol (5 ml) was added to a solution of acetyl chloride (2.5 ml) in isopropanol (60 ml) and stirred for 20 h, after which t.l.c. (5% methanol in chloroform, eluted twice) indicated incomplete conversion of the starting material (R_f 0.5) to a single product (R_f 0.6). The reaction mixture was heated to 70°C for 2 h, after which t.l.c. (5% methanol in chloroform, eluted twice) indicated the complete conversion to a single product (R_f 0.6). The mixture was neutralised with sodium bicarbonate, filtered and concentrated *in vacuo*. Purification by flash column chromatography (ethyl acetate : hexane 4 : 1) yielded *isopropyl 2,5-anhydro-3-azido-3-deoxy-**D**-talonate* **122** (0.79 g, 85%), a white crystalline solid; m.p. 63-64°C (ethyl acetate); $[\alpha]_D^{25}$ +65.2° (c, 0.25 in chloroform); ν_{max} (KBr) 3600-3100 (br, OH), 2983, 2939 (C-H), 2115 (N_3), 1740, 1735 (C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.31 (6H, d, *J* 6.2, $CH(C\underline{H}_3)_2$), 3.95 (1H, dd, $J_{6,6'}$ 12.2, $J_{6,5}$ 3.6, H-6), 4.01 (1H, dd, $J_{6',6}$ 12.2, $J_{6',5}$ 4.2, H-6'), 4.10 (1H, dd, $J_{3,2}$ 6.8, $J_{3,4}$ 4.6, H-3), 4.26 (1H, a-q, *J* 4.0, H-5), 4.53 (1H, d, $J_{2,3}$ 6.5, H-2), 4.54 (1H, m, H-4), 5.15 (1H, sept, *J* 6.2, $C\underline{H}(CH_3)_2$); δ_C (100.6 MHz, $CDCl_3$) 21.6, 21.7 (2 x q, $CH(\underline{C}H_3)_2$), 61.4 (t, C-6), 66.7 (d, C-3), 69.8 (d, $\underline{C}H(CH_3)_2$), 73.8 (d, C-4), 78.8 (d, C-2), 80.5 (d, C-5), 170.1 (s, C-1); *m/z* (EPCI+) 218 ($MH^+ - N_2$, 100%), 176 (95%). HRMS *m/z* (CI+): Found 263.136115. $C_9H_{19}N_4O_5$ ($M + NH_4^+$) requires 263.135545.

3-Azido-3-deoxy-5,6-O-isopropylidene-D-talono-1,4-lactone 102

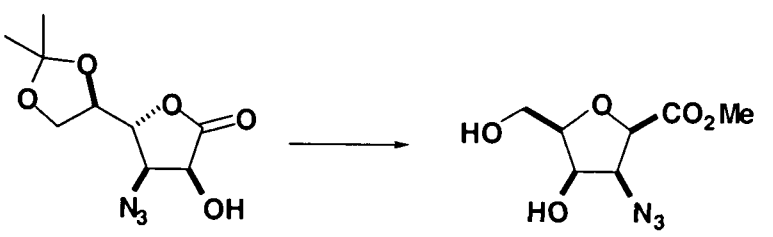


94 **102**

3-Azido-3-deoxy-5,6-*O*-isopropylidene-**D**-galactono-1,4-lactone **94** (93 mg, 0.38 mmol) was dissolved in dry dichloromethane (2 ml) and the solution was cooled to 0°C in a nitrogen atmosphere. Pyridine (62 µl, 0.76 mmol) was added and the solution was stirred for 5 min before addition of trifluoromethanesulphonic anhydride (77 µl, 0.46 mmol) with stirring at 0°C. After 1 min t.l.c. (ethyl acetate : hexane 1 : 1) indicated the complete conversion of the starting material (R_f 0.5) to a single product (R_f 0.7). The reaction mixture was diluted with butan-2-one (2 ml) at 0°C and caesium trifluoroacetate (282 mg, 1.15 mmol) added. The mixture was stirred at 0°C for a further 10 min, then allowed to warm to room temperature and stirred for 20 h, after which t.l.c. (ethyl acetate : hexane 1 : 1) indicated the complete conversion to a single product (R_f 0.8). Methanol (2ml) was added and the solution was stirred for a further 1 h and then concentrated *in vacuo*. Solid phase extraction (cyclohexane, increasing polarity to cyclohexane : dichloromethane 1:1) yielded *3-azido-3-deoxy-5,6-O-isopropylidene-D-galactono-1,4-lactone* **102** (62 mg, 66.7%), a yellow crystalline solid; m.p. 126-136°C (ethyl acetate); $[\alpha]_D^{25}$ -110.6° (c, 0.85 in chloroform); ν_{max} (KBr) 3600-3000 (br, OH), 2990, 2967, 2940, 2902 (C-H), 2128 (N_3), 1794, 1787, 1783 (C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.19, 1.23 (6H, 2 x s, $C(C\underline{H}_3)_2$), 3.87 (1H, dd, $J_{6,6'}$ 8.5, $J_{6,5}$ 7.0, H-6), 4.10 (1H, dd, $J_{6',6}$ 8.5, $J_{6',5}$ 7.1, H-6'), 4.30 (1H, br s, H-4), 4.36 (1H, a-dt, J 6.8, $J_{5,4}$ 1.5, H-5), 4.45 (1H, d, $J_{3,2}$ 6.2, H-3), 4.88 (1H, d, $J_{2,3}$ 6.2, H-2); δ_C (100.6 MHz, $CDCl_3$) 25.5, 25.5 (2 x q, $C(\underline{C}H_3)_2$), 62.1 (d, C-3), 65.2 (t, C-6), 69.2 (d, C-2), 75.2, 80.0 (2 x d, C-4, C-5), 110.8 (s, $\underline{C}(CH_3)_2$),

174.9 (s, C-1); *m/z* (APCI+) 261 (MNH_4^+, 100%), 244 (MH^+, 10%), 218 (85%). HRMS *m/z* (CI+): Found 261.119118. $C_9H_{17}N_4O_5$ ($M + NH_4^+$) requires 261.119895.

***Methyl 2,5-anhydro-3-azido-3-deoxy-D-galactonate* 106**

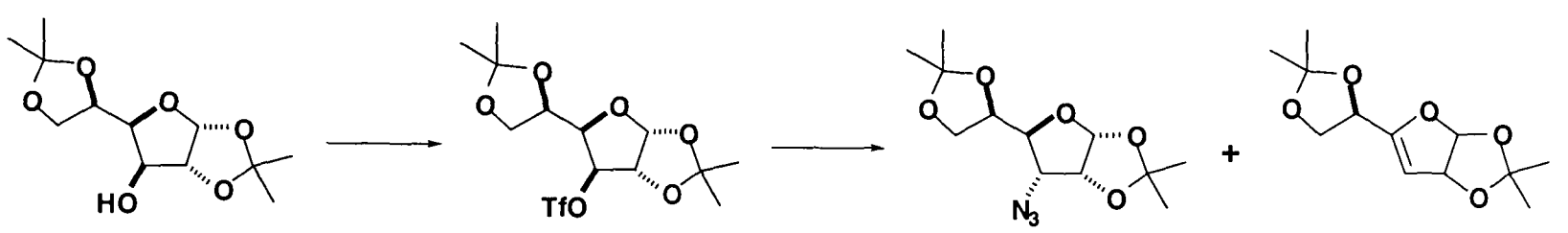


102 **106**

3-Azido-3-deoxy-5,6-*O*-isopropylidene-**D**-talono-1,4-lactone **102** (52 mg, 0.21 mmol) was dissolved in dry dichloromethane (1 ml) and the solution was cooled to 0°C in a nitrogen atmosphere. Pyridine (35 µl, 0.43 mmol) was added and the solution was stirred for 5 min before addition of trifluoromethanesulphonic anhydride (72 µl, 0.32 mmol) with stirring at 0°C. After 1 min t.l.c. (ethyl acetate : hexane 1 : 1) indicated the complete conversion of the starting material (R_f 0.7) to a single product (R_f 0.8). The reaction mixture was added directly to a solution of acetyl chloride (10 µl) in methyl alcohol (1 ml) at 0°C. The solution was stirred at 0°C for a further 10 min, then allowed to warm to room temperature and stirred for 19 h, after which t.l.c. (ethyl acetate : hexane 1 : 1) indicated the complete conversion to baseline products. The solution was neutralised with sodium bicarbonate, filtered and concentrated *in vacuo*. Flash column chromatography (5% methanol in chloroform) yielded *methyl 2,5-anhydro-3-azido-3-deoxy-D-galactonate* **106** (23 mg, 49.5%), a colourless oil; $[\alpha]_D^{25}$ –21.9° (c, 0.95 in methanol); ν_{max} (KBr) 3600-3000 (br, OH), 2960 (C-H), 2121 (N_3), 1748 (C=O) cm^{-1}; δ_H (400 MHz, CD_3OD) 3.65 (1H, dd, $J_{6,6'}$ 12.0, $J_{6,5}$ 4.5, H-6), 4.14 (1H, m, H-6'), 4.14 (3H, s, $CO_2C\underline{H}_3$), 4.42 (1H, a-dt, *J* 7.0, $J_{5,6}$ 4.5, H-5), 4.71 (1H, a-t, *J* 5.6, H-3), 4.95 (1H, d, $J_{2,3}$ 5.6, H-2), 5.01 (1H, dd, $J_{4,5}$ 6.4, $J_{4,3}$ 5.6, H-4); δ_C (100.6 MHz, $CDCl_3$) 53.7 (q,

$CO_2\underline{C}H_3$), 63.6 (t, C-6), 67.0 (d, C-3), 74.8 (d, C-4), 79.3 (d, C-2), 83.7 (d, C-5), 172.7 (s, C-1); *m/z* (APCI+) 235 (MNH_4^+, 100%), 218 (MH^+, 7%). Found C, 38.52; H, 5.36; N, 19.37%. $C_7H_{11}N_3O_5$ requires C, 38.71; H, 5.11; N, 19.35%.

3-Azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose 92 and 1,2:5,6-di-*O*-isopropylidene-α-D-*erythro*-hex-3-enofuranose 135



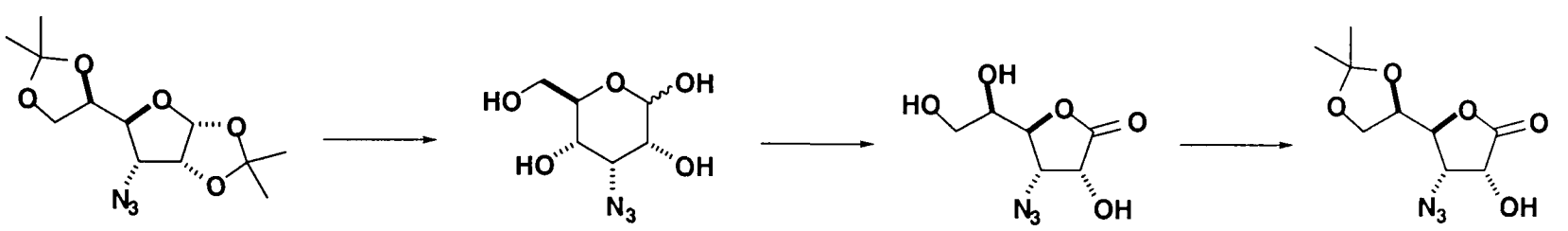
85 **131** **92** **135**

1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose **85** (10.0 g, 38.4 mmol) was dissolved in dry dichloromethane (100 ml) and dry pyridine (9.32 ml, 115.3 mmol) was added. The solution was cooled to -15°C in a nitrogen atmosphere and trifluoromethanesulphonic anhydride (7.76 ml, 46.1 mmol) was added slowly over 10 min. The resulting mixture was stirred at -15°C for 1 h after which t.l.c. (ethyl acetate : hexane 1 : 2) indicated the complete conversion of the starting material (R_f 0.3) to a single product (R_f 0.7). The reaction mixture was diluted with dichloromethane (700 ml), washed with 1M hydrochloric acid (200 ml) and pH7 buffer (2 x 200 ml), dried (magnesium sulphate), filtered and concentrated *in vacuo* to give 1,2:5,6-di-*O*-isopropylidene-3-*O*-trifluoromethanesulphonyl-α-D-glucofuranose **131**, a yellow crystalline solid; δ_H (500 MHz, $CDCl_3$) 1.34, 1.35, 1.43, 1.53 (12H, 4 x s, $C(C\underline{H}_3)_2$), 3.98 (1H, dd, $J_{6,6'}$ 8.4, $J_{6,5}$ 3.8, H-6), 4.16 (1H, dd, $J_{6',6}$ 8.4, $J_{6',5}$ 5.5, H-6'), 4.19-4.22 (2H, m, H-4, H-5), 4.77 (1H, d, $J_{2,1}$ 3.5, H-2), 5.27 (1H, s, H-3), 6.00 (1H, d, $J_{1,2}$ 3.5, H-1); δ_C (125.7 MHz, $CDCl_3$) 24.7, 26.1, 26.4, 26.6 (4 x q, $C(\underline{C}H_3)_2$), 67.4 (t, C-6), 71.5, 79.7 (2 x d, C-4, C-5), 83.1 (d, C-2), 88.0 (d, C-3), 104.9 (d, C-1), 109.7, 113.0 (2 x s,

$\underline{C}(CH_3)_2$), 118.3 (q, *J* 314.3, $\underline{C}F_3$);. The crude triflate was not further purified but dissolved in dry *N,N*-dimethylformamide (60 ml), sodium azide (3.00 g, 46.1 mmol) added and the mixture stirred for 2 d, after which t.l.c. (ethyl acetate : hexane 1 : 3) indicated the complete conversion to two products (R_f 0.5 and R_f 0.6). The solvent was removed *in vacuo* (co-evaporation with toluene). The residue was dissolved in dichloromethane (400 ml), washed with pH7 buffer (3 x 100 ml) and the combined aqueous layers were extracted with ethyl acetate (100 ml). The combined organic layers were dried (magnesium sulphate), filtered and concentrated *in vacuo*. Purification by flash column chromatography (ethyl acetate : hexane 1 : 9) yielded 3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-**D**-*erythro*-hex-3-enofuranose **135** (3.75 g, 40%), a white crystalline solid; m.p. 46-48°C (ethyl acetate) [lit.[9] 48-49°C (ethyl acetate)]; $[\alpha]_D^{23}$ +27.2° (c, 1.00 in chloroform) [lit.[9] $[\alpha]_D^{25}$ +25.4° (c, 1.5 in chloroform)]; ν_{max} (film) 1669 (C=C) cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.39, 1.45, 1.47, 1.47 (12H, 4 x s, $C(C\underline{H}_3)_2$), 3.97 (1H, dd, $J_{6,6'}$ 8.5, $J_{6,5}$ 5.7, H-6), 4.15 (1H, dd, $J_{6',6}$ 8.5, $J_{6',5}$ 6.8, H-6'), 4.59 (1H, a-ddt, $J_{5,6'}$ 6.8, $J_{5,6}$ 5.7, *J* 1.2, H-5), 5.25 (1H, dd, $J_{3,2}$ 2.3, $J_{3,5}$ 1.1, H-3), 5.30 (1H, ddd, $J_{2,1}$ 5.2, $J_{2,3}$ 2.3, $J_{2,5}$ 1.4, H-2), 6.08 (1H, d, $J_{1,2}$ 5.2, H-1); δ_C (50.3 MHz, $CDCl_3$) 25.5, 26.2, 27.9, 28.2 (4 x s, $C(\underline{C}H_3)_2$), 66.9 (t, C-6), 71.3, 83.4, 98.8, 106.6 (4 x d, C-1, C-2, C-3, C-5), 110.3, 112.3 (2 x s, $\underline{C}(CH_3)_2$), 160.0 (s, C-4); *m/z* (APCI+) 260 (MNH_4^+, 8%), 149 (58%), 101 (100%). Further elution afforded 3-azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-**D**-allofuranose **92** (5.41 g, 50%), a white crystalline solid; m.p. 37-38°C (hexane) [lit.[10] 38-39°C (hexane)]; $[\alpha]_D^{23}$ +76.5° (c, 1.00 in chloroform) [lit.[10] $[\alpha]_D^{25}$ +72° (c, 1 in chloroform)]; δ_H (200 MHz, $CDCl_3$) 1.36, 1.38, 1.48, 1.57 (12H, 4 x s, $C(C\underline{H}_3)_2$), 3.53 (1H, dd, $J_{6,6'}$ 9.1, $J_{6,5}$ 4.8, H-6), 3.95-4.24 (4H, m, H-3, H-4, H-5, H-6'), 4.73 (1H, dd, $J_{2,3}$ 4.7, $J_{2,1}$ 3.7, H-2), 5.78 (1H, d, $J_{1,2}$ 3.7, H-1); δ_C (50.3 MHz, $CDCl_3$) 25.0, 26.2, 26.4, 26.5 (4 x q, $C(\underline{C}H_3)_2$), 66.7 (t, C-6), 62.6, 75.7, 78.0, 80.5 (4 x d, C-2, C-3, C-4, C-5), 103.9 (d, C-1), 110.1,

113.2 (2 x s, $\underline{C}(CH_3)_2$); *m/z* (APCI+) 269 ($MH^+ - H_2O$, 12%), 258 ($MH^+ - N_2$, 21%), 200 (60%), 101 (100%).

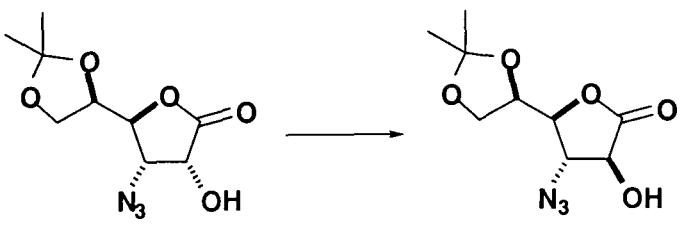
3-Azido-3-deoxy-5,6-*O*-isopropylidene-D-allono-1,4-lactone 96



3-Azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose **92** (2.85 g, 10 mmol) was dissolved in 50% aqueous trifluoroacetic acid (100 ml) and stirred for 4 h, after which t.l.c. (ethyl acetate : hexane 1 1) indicated the complete conversion of starting material (R_f 0.5) to baseline products. The solvent was removed *in vacuo* (co-evaporation with water and toluene) to give 3-azido-3-deoxy-D-allopyranose **136**. The crude pyranose was not further purified but dissolved in water (100 ml), barium carbonate (2.96 g, 15.0 mmol) added and the resulting mixture was cooled to 0°C. Bromine (0.62 ml, 12.0 mmol) was added dropwise over 5 min. The mixture was stirred at 0°C for 30 min, allowed to warm to room temperature and stirred for 5 h in the dark, after which t.l.c. (ethyl acetate : methanol 9 : 1) indicated the complete conversion of the starting material (R_f 0.4) to a single product (R_f 0.7). The solution was bubbled with nitrogen to remove excess bromine and the solvent was removed *in vacuo* to give 3-azido-3-deoxy-D-allono-1,5-lactone **137**, a white solid. The crude lactone was not further purified but suspended in acetone (150 ml) and camphorsulphonic acid (1.74 g, 7.5 mmol) was added. The solution was stirred at room temperature with exclusion of moisture for 3 h, after which t.l.c. (ethyl acetate) indicated the formation of a major product (R_f 0.7). The solution was neutralised with sodium bicarbonate, filtered and concentrated *in vacuo*.

Purification by flash column chromatography (ethyl acetate : hexane 2 : 3) yielded 3-azido-3-deoxy-5,6-*O*-isopropylidene-**D**-allono-1,4-lactone **96** (1.64 g, 68%), a white crystalline solid; m.p. 174-176°C (ethyl acetate) [lit.[11] 176-177°C (toluene/ethyl acetate)]; $[\alpha]_D^{23}$ +106.2° (c, 1.00 in acetone) [lit.[11] $[\alpha]_D^{25}$ +110.5° (c, 1.1 in acetone)]; ν_{max} (film) 3389 (OH), 2120 (N_3), 1752 (C=O) cm^{-1}; δ_H (200 MHz, d^6-acetone) 1.34, 1.47 (6H, 2 x s, C(C<u>H</u>$_3$)$_2$), 3.93 (1H, dd, $J_{6,6'}$ 9.0, $J_{6,5}$ 5.3, H-6), 4.21 (1H, dd, $J_{6',6}$ 9.0, $J_{6',5}$ 6.9, H-6'), 4.28 (1H, dd, $J_{4,5}$ 4.9, $J_{4,3}$ 0.6, H-4), 4.40 (1H, a-dt, $J_{5,6'}$ 6.9, *J* 5.1, H-5), 4.58 (1H, dd, $J_{3,2}$ 6.3, $J_{3,4}$ 0.6, H-3), 5.03 (1H, a-t, *J* 6.5, H-2), 5.71 (1H, d, $J_{OH,2}$ 6.8, O<u>H</u>); δ_C (50.3 MHz, $CDCl_3$) 24.3, 26.1 (2 x q, C(<u>C</u>H$_3$)$_2$), 66.0 (t, C-6), 60.2, 69.5, 74.8, 82.0 (4 x d, C-2, C-3, C-4, C-5), 110.5 (s, <u>C</u>(CH$_3$)$_2$), 174.0 (s, C-1); *m/z* (APCI+) 279 (10%), 216 ($MH^+ - N_2$, 12%), 160 (77%), 114 (100%).

***3-Azido-3-deoxy-5,6-O-isopropylidene-D-altrono-1,4-lactone* 104**

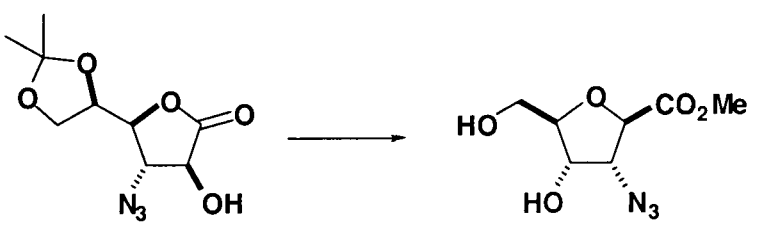


96 **104**

3-Azido-3-deoxy-5,6-*O*-isopropylidene-**D**-allono-1,4-lactone **96** (1.64 g, 6.7 mmol) was dissolved in dry dichloromethane (30 ml) and the solution was cooled to -30°C in a nitrogen atmosphere. Pyridine (3.27 ml, 40.5 mmol) was added and the solution was stirred for 5 min before addition of trifluoromethanesulphonic anhydride (1.70 ml, 10.1 mmol) with stirring at -30°C. After 5 min t.l.c. (ethyl acetate : hexane 1 : 1) indicated the complete conversion of the starting material (R_f 3) to a single product (R_f 6). The reaction mixture was diluted with butan-2-one (100 ml) at -30°C and caesium trifluoroacetate (4.97 g, 20.2 mmol) added. The mixture was stirred at -30°C for a further

10 min, then allowed to warm to room temperature and stirred for 2 h, after which t.l.c. (ethyl acetate : hexane 1 : 1) indicated the complete conversion to a single product (R_f 0.5). The solution was concentrated *in vacuo*. Purification by flash column chromatography (ethyl acetate : hexane 1 : 3) yielded *3-azido-3-deoxy-5,6-O-isopropylidene-D-altrono-1,4-lactone* **104** (1.48 g, 90%), a white crystalline solid; m.p. 101-103°C (ethyl acetate); $[\alpha]_D^{23}$ +15.4° (c, 0.85 in acetone); ν_{max} (Nujol® mull) 3423 (OH), 2112 (N_3), 1799 (C=O) cm^{-1}; δ_H (400 MHz, d^6-acetone) 1.38, 1.49 (6H, 2 x s, $C(C\underline{H}_3)_2$), 4.00 (1H, dd, $J_{6,6'}$ 8.9, $J_{6,5}$ 4.5, H-6), 4.19 (1H, dd, $J_{4,3}$ 8.3, $J_{4,5}$ 5.8, H-4), 4.21 (1H, dd, $J_{6',6}$ 8.9, $J_{6',5}$ 6.4, H-6'), 4.29 (1H, a-t, J 8.5, H-3), 4.40 (1H, a-dt, J 6.1, $J_{5,6}$ 4.5, H-5), 4.57 (1H, d, $J_{2,3}$ 8.9, H-2); δ_C (100.6 MHz, $CDCl_3$) 25.7, 27.0 (2 x q, $C(\underline{C}H_3)_2$), 66.9 (d, C-3), 67.2 (t, C-6), 73.7 (d, C-2), 76.5 (d, C-5), 78.9 (d, C-4), 111.1 (s, $\underline{C}(CH_3)_2$), 173.4 (s, C-1); *m/z* (APCI+) 244 (MH^+, 7%), 216 ($MH^+ - N_2$, 18%), 172 (44%), 153 (100%), 114 (82%), 113 (94%). HRMS *m/z* (CI+): Found 261.119813. $C_9H_{17}N_4O_5$ ($M + NH_4^+$) requires 261.119895. Found 244.092893. $C_9H_{14}N_3O_5$ (MH^+) requires 244.093346.

***Methyl 2,5-anhydro-3-azido-3-deoxy-D-allonate* 108**

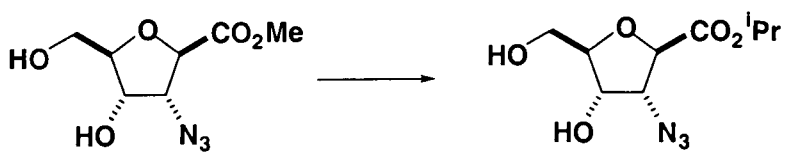


104 **108**

3-Azido-3-deoxy-5,6-*O*-isopropylidene-D-altrono-1,4-lactone **104** (0.33 g, 1.4 mmol) was dissolved in dry dichloromethane (15 ml) and the solution was cooled to -30°C in a nitrogen atmosphere. Pyridine (0.65 ml, 8.1 mmol) was added and the solution was stirred for 5 min before addition of trifluoromethanesulphonic anhydride (0.34 ml, 2.0 mmol) with stirring at -30°C. After 5 min t.l.c.

(ethyl acetate : hexane 1 : 1) indicated the complete conversion of the starting material (R_f 0.5) to a single product (R_f 0.7). The reaction mixture was added directly to a solution of acetyl chloride (0.71 ml) in methyl alcohol (15 ml) at -30°C. The solution was stirred at -30°C for a further 10 min, then allowed to warm to room temperature and stirred for 18 h, after which t.l.c. (ethyl acetate : hexane 1 : 1) indicated the complete conversion to a single product (R_f 0.2). The solution was neutralised with sodium bicarbonate, filtered and concentrated *in vacuo*. Flash column chromatography (ethyl acetate : hexane 1 : 1, increasing polarity to 7 : 3) yielded *methyl 2,5-anhydro-3-azido-3-deoxy-D-allonate* **108** (0.20 g, 69%), a colourless oil; $[\alpha]_D^{24}$ -35.7° (c, 1.07 in methanol); ν_{max} (film) 3435 (OH), 2117 (N_3), 1744 (C=O) cm^{-1}; δ_H (400 MHz, CD_3OD) 3.63 (1H, dd, $J_{6,6'}$ 12.2, $J_{6,5}$ 4.9, H-6), 3.76 (1H, dd, $J_{6',6}$ 12.2, $J_{6',5}$ 3.4, H-6'), 3.82 (3H, s, C$\underline{H}_3$), 3.95 (1H, a-dt, J 5.2, $J_{5,6'}$ 3.4, H-5), 4.12 (1H, a-t, J 5.2, H-3), 4.28 (1H, a-t, J 5.5, H-4), 4.42 (1H, d, $J_{2,3}$ 5.0, H-2); δ_C (100.6 MHz, CD_3OD) 53.7 (q, $\underline{C}H_3$), 63.1 (t, C-6), 67.2 (d, C-3), 73.8 (d, C-4), 80.7 (d, C-2), 86.6 (d, C-5), 173.6 (s, C-1); *m/z* (APCI+) 218 (MH^+, 10%), 192 (75%), 190 ($MH^+ - N_2$, 30%), 172 (64%), 132 (100%). Found C, 39.09; H, 4.75; N, 19.60%. $C_7H_{11}N_3O_5$ requires C, 38.71; H, 5.11; N, 19.35%.

***Isopropyl 2,5-anhydro-3-azido-3-deoxy-D-allonate* 138**

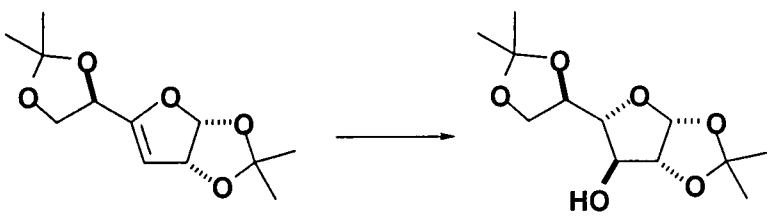


108 **138**

A solution of methyl 2,5-anhydro-3-azido-3-deoxy-**D**-allonate **108** (157 mg, 0.72 mmol) in isopropanol (8 ml) was added to a solution of acetyl chloride (0.2 ml) in isopropanol (2 ml) and stirred for 15 h, after which t.l.c. (ethyl acetate : hexane 1 : 1) indicated the complete conversion of

the starting material (R_f 0.2) to a single product (R_f 0.3). The mixture was neutralised with sodium bicarbonate, filtered and concentrated *in vacuo*. Purification by flash column chromatography (ethyl acetate : hexane 1 : 1) yielded *isopropyl 2,5-anhydro-3-azido-3-deoxy-D-allonate* **138** (62 mg, 35%), a colourless oil; $[\alpha]_D^{24}$ -56.6° (c, 0.99 in chloroform); ν_{max} (film) 3413 (OH), 2114 (N_3), 1734 (C=O) cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.31, 1.32 (6H, 2 x d, *J* 6.2, $CH(C\underline{H}_3)_2$), 2.90 (2H, br s, O<u>H</u>), 3.66 (1H, dd, $J_{6,6'}$ 12.6, $J_{6,5}$ 2.1, H-6), 3.94 (1H, dd, $J_{6',6}$ 12.6, $J_{6',5}$ 2.7, H-6'), 4.08 (1H, a-dt, $J_{5,4}$ 5.1, *J* 2.3, H-5), 4.19 (1H, a-t, *J* 4.9, H-3), 4.43 (1H, a-t, *J* 5.2, H-4), 4.46 (1H, d, $J_{2,3}$ 4.7, H-2), 5.13 (1H, sept, *J* 6.2, $C\underline{H}(CH_3)_2$); δ_C (125.7 MHz, $CDCl_3$) 21.4, 21.5 (2 x q, $CH(\underline{C}H_3)_2$), 61.3 (t, C-6), 66.7 (d, C-3), 70.3 (d, $\underline{C}H(CH_3)_2$), 71.8 (d, C-4), 79.3 (d, C-2), 84.8 (d, C-5), 171.3 (s, C-1); *m/z* (APCI+) 200 ($MH^+ - N_2 - H_2O$, 43%), 158 ($MH^+ - CO_2{}^iPr$, 86%), 140 (100%). Found C, 43.99; H, 6.34; N, 17.04%. $C_9H_{15}N_3O_5$ requires C, 44.08; H, 6.17; N, 17.13%.

1,2:5,6-Di-*O*-isopropylidene-α-D-galactofuranose 88

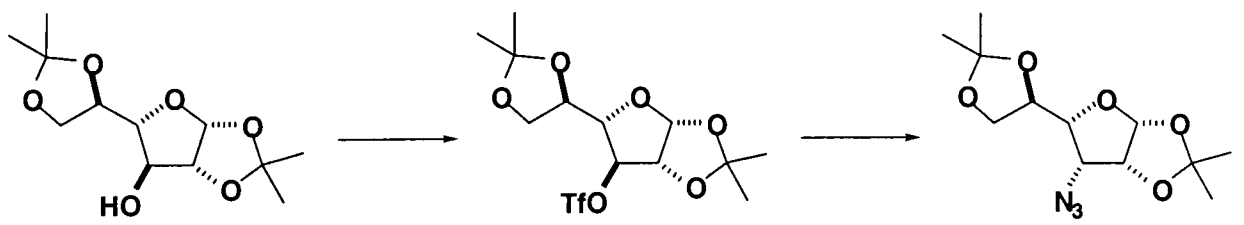


135 **88**

3-Deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*erythro*-hex-3-enofuranose **135** (4.62 g, 19.1 mmol) was dissolved in dry tetrahydrofuran (10 ml) and a 0.5M solution of 9-BBN in tetrahydrofuran (76.3 ml, 38.1 mmol 9-BBN) was added. The solution was stirred in a nitrogen atmosphere for 45 min after which t.l.c. (ethyl acetate : hexane 1 : 3) indicated the complete conversion of the starting material (R_f 0.4) to baseline products. The reaction mixture was cooled to 0°C, water (46 ml) added dropwise and the mixture was allowed to warm to room temperature. After 1 h the reaction mixture was

cooled to 0°C and a 1M solution of sodium hydroxide in water (95 ml, 95 mmol NaOH) and 35% aqueous hydrogen peroxide (10.2 ml, 105 mmol H_2O_2) added. The reaction mixture was heated under reflux for 1 h then allowed to cool and extracted with ethyl acetate (3 x 150 ml). The combined organic layers were washed with pH7 buffer (100 ml), dried (magnesium sulphate), filtered and concentrated *in vacuo*. Purification by flash column chromatography (ethyl acetate : hexane 1 : 2) yielded 1,2:5,6-di-*O*-isopropylidene-α-**D**-galactofuranose **88** (4.26 g, 86%), a white crystalline solid; m.p. 94-95°C (ethyl acetate) [lit.[12] 97.5-98°C (cyclohexane)]; $[\alpha]_D^{23}$ -20.5° (c, 1.02 in chloroform) [lit.[12] $[\alpha]_D^{20}$ -35.3° (c, 0.8 in methanol)]; ν_{max} (film) 3400 (OH) cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.35, 1.38, 1.45, 1.55 (12H, 4 x s, $C(C\underline{H}_3)_2$), 3.85 (1H, dd, $J_{6,6'}$ 8.4, $J_{6,5}$ 6.9, H-6), 3.87 (1H, dd, $J_{4,5}$ 6.8, $J_{4,3}$ 4.5, H-4), 4.08 (1H, dd, $J_{6',6}$ 8.4, $J_{6',5}$ 6.6, H-6'), 4.12 (1H, dd, $J_{3,4}$ 4.5, $J_{3,2}$ 1.3, H-3), 4.37 (1H, a-q, *J* 6.8, H-5), 4.55 (1H, dd, $J_{2,1}$ 4.0, $J_{2,3}$ 1.3, H-2), 5.88 (1H, d, $J_{1,2}$ 4.0, H-1); δ_C (50.3 MHz, $CDCl_3$) 25.3, 26.5, 26.7, 27.4 (4 x q, $C(\underline{C}H_3)_2$), 65.7 (t, C-6), 75.4, 76.1, 86.0, 87.6 (4 x d, C-2, C-3, C-4, C-5), 104.9 (d, C-1), 109.9, 113.6 (2 x s, $\underline{C}(CH_3)_2$); *m/z* (APCI+) 283 (MNa^+, 45%), 203 (96%), 145 (100%).

3-Azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-gulofuranose 91



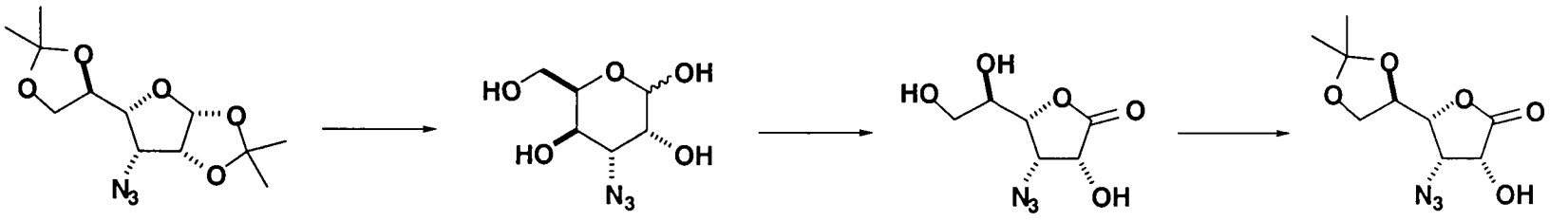
88 **215** **91**

1,2:5,6-Di-*O*-isopropylidene-α-**D**-galactofuranose **88** (2.13 g, 8.2 mmol) was dissolved in dry dichloromethane (15 ml) and dry pyridine (3.31 ml, 40.9 mmol) was added. The solution was cooled to -15°C in a nitrogen atmosphere and trifluoromethanesulphonic anhydride (2.07 ml, 12.3 mmol)

was added slowly over 10 min. The resulting mixture was stirred at -15°C for 1 h after which t.l.c. (ethyl acetate : hexane 1 : 1) indicated the complete conversion of the starting material (R_f 0.4) to a single product (R_f 0.7). The reaction mixture was diluted with dichloromethane (450 ml), washed with 1M hydrochloric acid (90 ml) and pH7 buffer (2 x 90 ml), dried (magnesium sulphate), filtered and concentrated *in vacuo* to give 1,2:5,6-di-*O*-isopropylidene-3-*O*-trifluoromethanesulphonyl-α-**D**-glucofuranose **215**, a yellow crystalline solid; ν_{max} (Nujol® mull) 1459, 1423, 1377 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.39, 1.39, 1.46, 1.59 (12H, 4 x s, $C(C\underline{H}_3)_2$), 3.87 (1H, dd, $J_{6,6'}$ 8.4, $J_{6,5}$ 6.8, H-6), 4.11 (1H, dd, $J_{6',6}$ 8.4, $J_{6',5}$ 6.6, H-6'), 4.13 (1H, dd, $J_{4,5}$ 5.9, $J_{4,3}$ 4.2, H-4), 4.36 (1H, a-q, J 6.6, H-5), 4.81 (1H, dd, $J_{2,1}$ 4.0, $J_{2,3}$ 1.1, H-5), 5.12 (1H, br d, $J_{3,4}$ 4.2, H-3), 5.95 (1H, d, $J_{1,2}$ 4.0, H-1); δ_C (50.3 MHz, $CDCl_3$) 25.3, 26.3, 26.6, 27.1 (4 x q, $C(\underline{C}H_3)_2$), 65.5 (t, C-6), 73.7, 82.8, 84.7, 87.7 (4 x d, C-2, C-3, C-4, C-5), 104.8 (d, C-1), 110.4, 114.8 (2 x s, $\underline{C}(CH_3)_2$); *m/z* (APCI+) 393 (MH+, 7%), 195 (100%). The crude triflate was not further purified but dissolved in dry *N,N*-dimethylformamide (35 ml), sodium azide (3.19 g, 49.1 mmol) added and the mixture was heated under reflux for 19 h, after which t.l.c. (ethyl acetate : hexane 1 : 4) indicated the complete conversion to a major product (R_f 0.2) and a minor product (R_f 0.4). The solvent was removed *in vacuo* (co-evaporation with toluene). The residue dissolved in dichloromethane (450 ml), washed with pH7 buffer (3 x 90 ml) and the combined aqueous layers were extracted with ethyl acetate (75 ml). The combined organic layers were dried (magnesium sulphate), filtered and concentrated *in vacuo*. Purification by flash column chromatography (ethyl acetate : hexane 1 : 4) yielded 3-azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-**D**-gulofuranose **91** (1.89 g, 81%), a white crystalline solid; m.p. 85-87°C (ethyl acetate) [lit.[11] 86-87°C (ether/hexane)]; $[\alpha]_D^{23}$ +144.3° (c, 0.98 in chloroform) [lit.[11] $[\alpha]_D^{25}$ +146.5° (c, 1.1 in chloroform)]; ν_{max} (Nujol® mull) 2115 (N_3) cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.37, 1.40, 1.45, 1.66 (12H, 4 x s, $C(C\underline{H}_3)_2$), 3.59 (1H, dd, $J_{6,6'}$ 8.6, $J_{6,5}$ 6.9, H-6), 4.01 (1H, dd, $J_{4,5}$

9.2, $J_{4,3}$ 6.9, H-4), 4.15 (1H, dd, $J_{3,4}$ 6.9, $J_{3,2}$ 5.5, H-3), 4.22 (1H, dd, $J_{6',6}$ 8.6, $J_{6',5}$ 6.6, H-6'), 4.54 (1H, a-dt, $J_{5,4}$ 9.2, J 6.8, H-5), 4.84 (1H, dd, $J_{2,3}$ 5.5, $J_{2,1}$ 4.0, H-2), 5.83 (1H, d, $J_{1,2}$ 4.0, H-1); δ_C (50.3 MHz, $CDCl_3$) 25.0, 26.5, 26.7, 26.7 (4 x q, $C(\underline{C}H_3)_2$), 66.5 (t, C-6), 61.3, 75.1, 80.3, 82.4 (4 x d, C-2, C-3, C-4, C-5), 105.1 (d, C-1), 109.2, 114.9 (2 x s, $\underline{C}(CH_3)_2$); *m/z* (APCI+) 308 (MNa^+, 5%), 258 ($MH^+ - N_2$, 8%), 142 (100%).

3-Azido-3-deoxy-5,6-*O*-isopropylidene-D-gulono-1,4-lactone 95

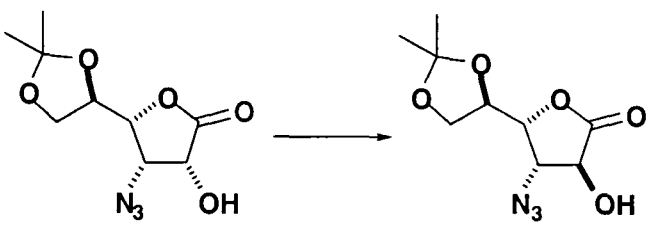


91 **139** **216** **95**

3-Azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-**D**-gulofuranose **91** (1.0 g, 3.5 mmol) was dissolved in 50% aqueous trifluoroacetic acid (50 ml) and stirred for 4 h. The solvent was removed *in vacuo* (co-evaporation with water and toluene) to give 3-azido-3-deoxy-**D**-gulopyranose **139**, a white foam. The pyranose was not further purified but was dissolved in water (100 ml), barium carbonate (1.04 g, 5.3 mmol) was added and the resulting mixture was cooled to 0°C. Bromine (0.22 ml, 4.2 mmol) was added dropwise over 5 min. The mixture was stirred at 0°C for 30 min, allowed to warm to room temperature and stirred for 3 d in the dark, after which t.l.c. (ethyl acetate : methanol 9 : 1) indicated the complete conversion of the starting material (R_f 0.4) to a single product (R_f 0.5). The solution was bubbled with nitrogen to remove excess bromine and the solvent was removed *in vacuo* to give 3-azido-3-deoxy-**D**-gulono-1,5-lactone **216**, a white solid. The crude lactone was not further purified but suspended in acetone (150 ml) and camphorsulphonic acid (0.61 g, 2.6 mmol) was added. The solution was stirred at room temperature with exclusion of moisture for 3 h, after which

t.l.c. (ethyl acetate) indicated the formation of a single product (R_f 0.7). The solution was neutralised with sodium bicarbonate, filtered and concentrated *in vacuo*. Purification by flash column chromatography (ethyl acetate : hexane 1 : 2) yielded 3-azido-3-deoxy-5,6-*O*-isopropylidene-**D**-gulono-1,4-lactone **95** (0.67 g, 79% from furanose), a white crystalline solid; m.p. 166-167°C (ethyl acetate) [lit.[11] 166-167°C (ethyl acetate)]; $[\alpha]_D^{23}$ +127.4° (c, 1.00 in acetone) [lit.[11] $[\alpha]_D^{24}$ +120.4° (c, 1.2 in acetone)]; ν_{max} (Nujol® mull) 3360 (OH), 2128 (N_3), 1774 (C=O) cm^{-1}; δ_H (200 MHz, d^6-acetone) 1.35, 1.39 (6H, 2 x s, C(C<u>H</u>$_3$)$_2$), 3.94 (1H, dd, $J_{6,6'}$ 8.5, $J_{6,5}$ 6.1, H-6), 4.24 (1H, dd, $J_{6',6}$ 8.5, $J_{6',5}$ 6.6, H-6'), 4.33 (1H, a-dt, $J_{5,4}$ 8.0, J 6.4, H-5), 4.51 (1H, dd, $J_{4,5}$ 8.0, $J_{4,3}$ 3.6, H-4), 4.79 (1H, dd, $J_{3,2}$ 5.4, $J_{3,4}$ 3.6, H-3), 5.04 (1H, dd, $J_{2,OH}$ 6.3, $J_{2,3}$ 5.4, H-2), 5.78 (1H, d, $J_{OH,2}$ 6.3, O<u>H</u>); δ_C (50.3 MHz, $CDCl_3$) 25.1, 26.5 (2 x q, C(<u>C</u>H$_3$)$_2$), 65.0 (t, C-6), 61.9, 71.5, 75.8, 79.1 (4 x d, C-2, C-3, C-4, C-5), 110.2 (s, <u>C</u>(CH$_3$)$_2$), 174.1 (s, C-1); *m/z* (APCI+) 244 (MH^+, 8%), 106 (43%), 104 (100%).

3-Azido-3-deoxy-5,6-O-isopropylidene-D-idono-1,4-lactone 103

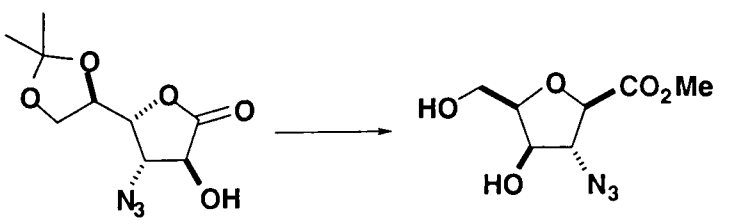


95 **103**

3-Azido-3-deoxy-5,6-*O*-isopropylidene-**D**-gulono-1,4-lactone **95** (0.99 g, 4.1 mmol) was dissolved in dry dichloromethane (20 ml) and the solution was cooled to -30°C in a nitrogen atmosphere. Pyridine (1.48 ml, 18.3 mmol) was added and the solution was stirred for 5 min before addition of trifluoromethanesulphonic anhydride (1.03 ml, 6.1 mmol) with stirring at -30°C. After 5 min t.l.c. (ethyl acetate : hexane 1 : 1) indicated the complete conversion of the starting material (R_f 0.4) to a

single product (R_f 0.6). The reaction mixture was diluted with butan-2-one (100 ml) at -30°C and caesium trifluoroacetate (3.00 g, 12.2 mmol) added. The mixture was stirred at -30°C for a further 10 min, then allowed to warm to room temperature and stirred for 16 h, after which t.l.c. (ethyl acetate : hexane 1 : 1) indicated the complete conversion to a single product (R_f 0.3). The solution was concentrated *in vacuo*. Purification by flash column chromatography (ethyl acetate : hexane 1 : 3) yielded *3-azido-3-deoxy-5,6-O-isopropylidene-D-idono-1,4-lactone* **103** (0.56 g, 57%), a white crystalline solid; m.p. 91-94°C (ethyl acetate); $[\alpha]_D^{23}$ -66.5° (c, 1.00 in chloroform); ν_{max} (film) 3420 (OH), 2115 (N_3), 1795 (C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.33, 1.37 (6H, 2 x s, $C(C\underline{H}_3)_2$), 3.96 (1H, dd, $J_{6,6'}$ 8.4, $J_{6,5}$ 7.2, H-6), 4.14 (1H, dd, $J_{6',6}$ 8.4, $J_{6',5}$ 7.1, H-6'), 4.41 (1H, a-dt, J 7.1, $J_{5,4}$ 1.0, H-5), 4.42 (1H, a-t, J 8.9, H-3), 4.51 (1H, dd, $J_{4,3}$ 8.3, $J_{4,5}$ 1.0, H-4), 4.82 (1H, d, $J_{2,3}$ 9.2, H-2); δ_C (100.6 MHz, $CDCl_3$) 25.4, 25.4 (2 x q, $C(\underline{C}H_3)_2$), 64.3 (d, C-3), 65.1 (t, C-6), 70.9 (d, C-2), 72.7 (d, C-5), 75.3 (d, C-4), 110.8 (s, $\underline{C}(CH_3)_2$), 174.5 (s, C-1); *m/z* (APCI+) 249 (5%), 248 (10%), 216 ($MH^+ - N_2$, 15%), 160 (50%), 158 (42%), 114 (68%), 102 (100%). HRMS *m/z* (CI+): Found 261.120041. $C_9H_{17}N_4O_5$ ($M + NH_4^+$) requires 261.119895. Found 244.093597. $C_9H_{14}N_3O_5$ (MH^+) requires 244.093346.

***Methyl 2,5-anhydro-3-azido-3-deoxy-D-gulonate* 107**

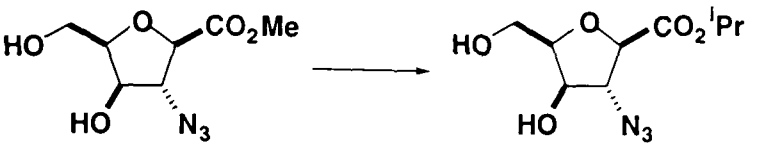


103 **107**

3-Azido-3-deoxy-5,6-*O*-isopropylidene-D-idono-1,4-lactone **103** (0.56 g, 2.3 mmol) was dissolved in dry dichloromethane (15 ml) and the solution was cooled to -30°C in a nitrogen atmosphere.

Pyridine (0.84 ml, 10.4 mmol) was added and the solution was stirred for 5 min before addition of trifluoromethanesulphonic anhydride (0.58 ml, 3.5 mmol) with stirring at -30°C. After 30 min t.l.c. (ethyl acetate : hexane 1 : 1) indicated the complete conversion of the starting material (R_f 0.3) to a single product (R_f 0.5). The reaction mixture was added directly to a solution of acetyl chloride (0.74 ml) in methyl alcohol (20 ml) at -30°C. The solution was stirred at -30°C for a further 10 min, then allowed to warm to room temperature and stirred for 7 h, after which t.l.c. (ethyl acetate : hexane 1 : 1) indicated the complete conversion to a major product (R_f 0.2). The solution was neutralised with sodium bicarbonate, filtered and concentrated *in vacuo*. Flash column chromatography (ethyl acetate : hexane 1 : 1, increasing polarity to 3 : 2) yielded *methyl 2,5-anhydro-3-azido-3-deoxy-**D**-gulonate* **107** (0.17 g, 35%), a colourless oil; $[\alpha]_D^{24}$ -94.5° (c, 1.01 in chloroform); ν_{max} (film) 3384 (OH), 2112 (N_3), 1743 (C=O) cm^{-1}; δ_H (400 MHz, CD_3OD) 3.79 (3H, s, C$\underline{H}_3$), 3.79-3.82 (2H, m, H-6, H-6'), 4.09 (1H, a-q, *J* 5.1, H-5), 4.22 (1H, dd, $J_{4,5}$ 4.5, $J_{4,3}$ 2.9, H-4), 4.28 (1H, a-t, *J* 3.2, H-3), 4.39 (1H, d, $J_{2,3}$ 3.6, H-2); δ_C (100.6 MHz, CD_3OD) 53.3 (q, $\underline{C}H_3$), 61.9 (t, C-6), 72.6 (d, C-3), 76.3 (d, C-4), 81.4 (d, C-2), 84.4 (d, C-5), 173.1 (s, C-1); *m/z* (APCI+) 218 (MH^+, 59%), 200 ($MH^+ - H_2O$, 37%), 102 (100%). HRMS *m/z* (CI+): Found 235.104281. $C_7H_{15}N_4O_5$ ($M + NH_4^+$) requires 235.104245.

***Isopropyl 2,5-anhydro-3-azido-3-deoxy-D-gulonate* 140**

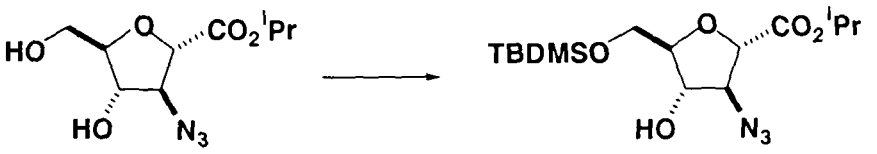


107 **140**

A solution of methyl 2,5-anhydro-3-azido-3-deoxy-D-gulonate **107** (127 mg, 0.58 mmol) in isopropanol (15 ml) was added to a solution of acetyl chloride (0.4 ml) in isopropanol (5 ml) and stirred for 6 h, after which t.l.c. (ethyl acetate : hexane 1 : 1) indicated the complete conversion of the starting material (R_f 0.2) to a single product (R_f 0.3). The mixture was neutralised with sodium bicarbonate, filtered and concentrated *in vacuo*. Purification by flash column chromatography (ethyl acetate : hexane 2 : 3, increasing polarity to 1 : 1) yielded *isopropyl 2,5-anhydro-3-azido-3-deoxy-D-gulonate* **140** (96 mg, 67%), a colourless oil; $[\alpha]_D^{25}$ -79.5° (c, 0.99 in chloroform); ν_{max} (film) 3400 (OH), 2111 (N_3), 1734 (C=O) cm^{-1}; δ_H (400 MHz, CD_3OD) 1.29, 1.30 (6H, 2 x d, *J* 6.3, $CH(C\underline{H}_3)_2$), 3.99 (1H, dd, $J_{6,6'}$ 13.7, $J_{6,5}$ 2.5, H-6), 4.18 (1H, dd, $J_{6',6}$ 13.7, $J_{6',5}$ 3.1, H-6'), 4.19 (1H, a-q, *J* 3.1, H-5), 4.26 (1H, a-t, *J* 1.8, H-3), 4.33 (1H, dd, $J_{4,5}$ 3.9, $J_{4,3}$ 1.9, H-4), 4.40 (1H, d, $J_{2,3}$ 1.7, H-2), 5.12 (1H, sept, *J* 6.3, $C\underline{H}(CH_3)_2$); δ_C (100.6 MHz, CD_3OD) 21.6, 21.7 (2 x q, $CH(\underline{C}H_3)_2$), 61.3 (t, C-6), 70.3 (d, $\underline{C}H(CH_3)_2$), 71.5 (d, C-3), 77.3 (d, C-4), 79.9 (d, C-2), 81.0 (d, C-5), 171.1 (s, C-1); *m/z* (APCI+) 246 (MH^+, 12%), 218 ($MH^+ - N_2$, 13%), 204 (65%), 176 ($MNH_4^+ - CO_2{}^iPr$, 15%), 158 ($M^+ - CO_2{}^iPr$, 100%). Found C, 44.27; H, 5.92; N, 17.11%. $C_9H_{15}N_3O_5$ requires C, 44.08; H, 6.17; N, 17.13%.

6.3 Synthesis of oligomers

***Isopropyl 2,5-anhydro-3-azido-6-O-tert-butyldimethylsilyl-3-deoxy-D-mannonate* 165**

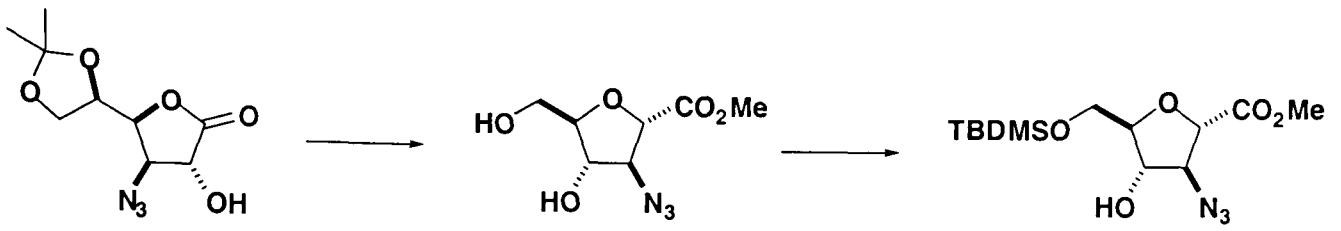


116 **165**

tert-Butyldimethylsilyl chloride (40 mg, 0.27 mmol) was added to a stirred solution of isopropyl 2,5-anhydro-3-azido-3-deoxy-**D**-mannonate **116** (64 mg, 0.26 mmol) and imidazole (40 mg, 0.59 mmol) in dry *N,N*-dimethylformamide (0.5 ml) at -20°C. The reaction mixture was stirred in a nitrogen atmosphere at -10°C for 1 h, after which t.l.c. (ethyl acetate : hexane 1 : 3) indicated the complete conversion of baseline material to a single product (R_f 0.4). The solvent was removed *in vacuo* (co-evaporation with toluene). The residue was dissolved in ethyl acetate (30 ml) and washed with pH7 buffer (3 x 10 ml). The aqueous phase was extracted with ethyl acetate (10 ml) and the combined organic extracts dried (magnesium sulphate), filtered and concentrated *in vacuo*. Purification by flash column chromatography (ethyl acetate : hexane 7 : 3) yielded *isopropyl 2,5-anhydro-3-azido-6-O-tert-butyldimethylsilyl-3-deoxy-D-mannonate* **165** (40 mg, 43%), a colourless oil; $[\alpha]_D^{24}$ +32.2° (c, 1.01 in chloroform); ν_{max} (film) 3454 (OH), 2104 (N_3), 1745 (C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 0.08 (6H, s, $Si(C\underline{H}_3)_2$), 0.90 (9H, s, $SiC(C\underline{H}_3)_3$), 1.30 (6H, d, *J* 6.3, $CH(C\underline{H}_3)_2$), 2.76 (1H, br s, O<u>H</u>), 3.72 (1H, dd, $J_{6,6'}$ 10.6, $J_{6,5}$ 6.2, H-6), 3.85 (1H, dd, $J_{6',6}$ 10.6, $J_{6',5}$ 4.3, H-6'), 4.02 (1H, a-dt, *J* 6.0, $J_{5,6'}$ 4.3, H-5), 4.09 (1H, a-t, *J* 5.5, H-3), 4.17 (1H, a-t, *J* 5.6, H-4), 4.34 (1H, d, $J_{2,3}$ 5.5, H-2), 5.13 (1H, sept, *J* 6.3, $C\underline{H}(CH_3)_2$); δ_C (100.6 MHz, $CDCl_3$) -5.5 (q, $Si(\underline{C}H_3)_2$), 18.3 (s, $Si\underline{C}(CH_3)_3$), 21.6, 21.7 (2 x q, $CH(\underline{C}H_3)_2$), 25.8 (q, $SiC(\underline{C}H_3)_3$), 63.0 (t, C-6), 69.7, 70.1, 77.2, 79.8, 83.9 (5 x d, C-2, C-3,

C-4, C-5, $\underline{C}H(CH_3)_2$), 170.3 (s, C-1); *m/z* (APCI+) 331 ($MH^+ - N_2$, 13%), 226 (57%), 132 (100%). Found C, 50.17; H, 8.02; N, 11.73%. $C_{15}H_{29}N_3O_5Si$ requires C, 50.12; H, 8.13; N, 11.69%.

***Methyl 2,5-anhydro-3-azido-6-O-tert-butyldimethylsilyl-3-deoxy-D-mannonate* 115**

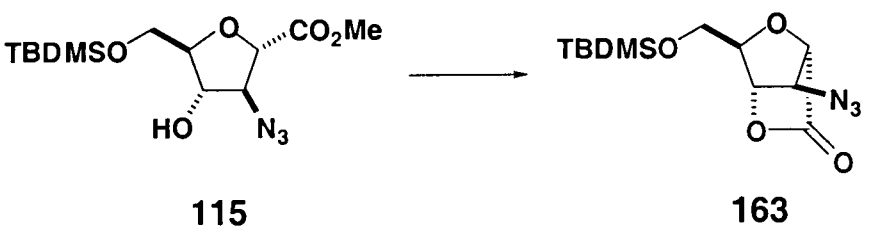


93 **97** **115**

3-Azido-3-deoxy-5,6-*O*-isopropylidene-**D**-glucono-1,4-lactone **93** (0.46 g, 1.9 mmol) was dissolved in dry dichloromethane (7 ml) and the solution was cooled to 0°C in a nitrogen atmosphere. Pyridine (0.46 ml, 5.7 mmol) was added and the solution was stirred for 15 min before addition of trifluoromethanesulphonic anhydride (0.48 ml, 2.9 mmol) with stirring at 0°C. After 30 min t.l.c. (ethyl acetate : hexane 1 : 1) indicated the complete conversion of the starting material (R_f 0.5) to a single product (R_f 0.6). The reaction mixture was added directly to a solution of acetyl chloride (0.85 ml) in methyl alcohol (42.5 ml) at 0°C. The solution was stirred at 0°C for a further 30 min, then allowed to warm to room temperature and stirred for 22 h, after which t.l.c. (ethyl acetate : hexane 7 : 3) indicated the complete conversion to a single product (R_f 0.2). The solvent was removed *in vacuo*. Flash column chromatography (2% methanol in diethyl ether) yielded methyl 2,5-anhydro-3-azido-3-deoxy-**D**-mannonate **97** contaminated with an unknown compound (1.07 g). The crude methyl ester was not purified further but dissolved in dry *N,N*-dimethylformamide (1.5 ml) and imidazole (0.26 g, 3.8 mmol) and *tert*-butyldimethylsilyl chloride (0.31 g, 2.1 mmol) added at 0°C. The reaction mixture was stirred at 0°C for 1 h, in a nitrogen atmosphere, after which t.l.c. (ethyl acetate : hexane 7 : 3) indicated the complete

conversion of the starting material (R_f 0.2) to a single product (R_f 0.6). The solvent was removed *in vacuo* (co-evaporation with toluene). The residue was dissolved in ethyl acetate (75 ml) and washed with pH7 buffer (3 x 25 ml). The aqueous phase was extracted with ethyl acetate (15 ml) and the combined organic extracts dried (magnesium sulphate), filtered and concentrated *in vacuo*. Purification by flash column chromatography (ethyl acetate : hexane 7 : 3) yielded *methyl 2,5-anhydro-3-azido-6-O-tert-butyldimethylsilyl-3-deoxy-D-mannonate* **115** (0.28 g, 45% from lactone), a white crystalline solid; m.p. 73-75°C (ethyl acetate); $[\alpha]_D^{24}$ +39.7° (c, 1.00 in chloroform); ν_{max} (film) 3463 (OH), 2105 (N_3), 1742 (C=O) cm^{-1}; δ_H (200 MHz, $CDCl_3$) 0.09 (6H, s, $Si(C\underline{H}_3)_2$), 0.90 (9H, s, $SiC(C\underline{H}_3)_3$), 2.47 (1H, d, $J_{OH,3}$ 4.4, O<u>H</u>), 3.72 (1H, dd, $J_{6,6'}$ 10.5, $J_{6,5}$ 6.3, H-6), 3.84 (3H, s, $C\underline{H}_3$), 3.87 (1H, dd, $J_{6',6}$ 10.5, $J_{6',5}$ 4.1, H-6'), 4.04 (1H, a-dt, J 6.1, $J_{5,6'}$ 4.2, H-5), 4.16 (1H, a-t, J 5.4, H-3), 4.23 (1H, a-q, J 5.4, H-4), 4.40 (1H, d, $J_{2,3}$ 5.5, H-2); δ_C (50.3 MHz, $CDCl_3$) -5.5 (q, $Si(\underline{C}H_3)_2$), 18.2 (s, $Si\underline{C}(CH_3)_3$), 25.8 (q, $SiC(\underline{C}H_3)_3$), 52.7 (q, $\underline{C}H_3$), 62.7 (t, C-6), 69.9, 76.6, 79.5, 84.3 (4 x d, C-2, C-3, C-4, C-5), 171.3 (s, C-1); *m/z* (APCI+) 304 ($MH^+ - N_2$, 43%), 286 (46%), 272 ($M - (CO_2Me)^-$, 18%), 154 (100%). Found C, 47.30; H, 7.35; N, 12.66%. $C_{13}H_{25}N_3O_5Si$ requires C, 47.11; H, 7.60; N, 12.68%.

(1S,4S,6R,7S)-7-azido-6-O-tert-butyldimethylsilyl-6-hydroxy-2,5-dioxa-bicyclo[2.2.1]heptan-3-one (2,5-anhydro-3-azido-6-O-tert-butyldimethylsilyl-3-deoxy-D-mannono-1,4-lactone) **163**

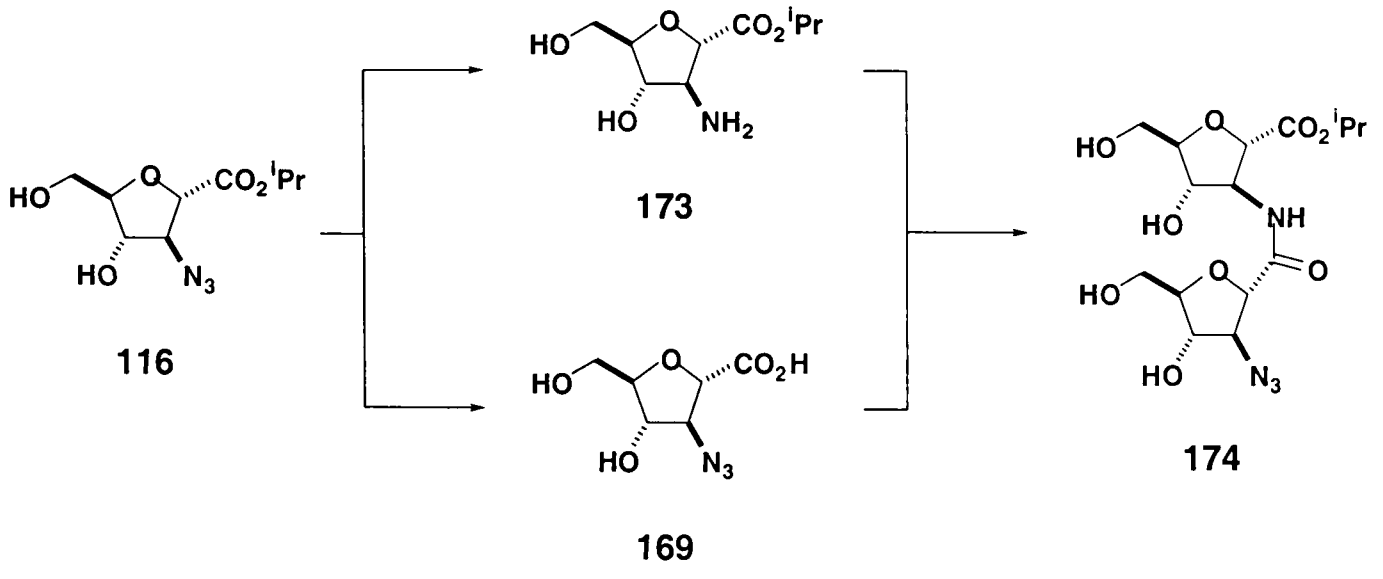


115 **163**

Methyl 2,5-anhydro-3-azido-6-*O-tert*-butyldimethylsilyl-3-deoxy-D-mannonate **115** (0.10 g, 0.30 mmol) was dissolved in tetrahydrofuran (2 ml) and a 0.5 M solution of lithium hydroxide in

water (0.60 ml, 0.30 mmol LiOH) added. The reaction mixture was stirred for 2 h, after which t.l.c. (ethyl acetate : hexane 1 : 3) indicated partial conversion of the starting material (R_f 0.3) to baseline products. 0.5 M solution of lithium hydroxide in water (0.27 ml, 0.26 mmol LiOH) was added and the mixture stirred for a further 2 h, after which t.l.c. (ethyl acetate : hexane 1 : 3) indicated the complete conversion to baseline products. The solvent was removed *in vacuo*. The resulting white solid was dissolved in dichloromethane (2 ml) and pyridine (59 μl, 0.73 mmol) and acetic anhydride (35 μl, 0.37 mmol) added. The reaction mixture was stirred for 16 h, after which t.l.c. (ethyl acetate : hexane 1 : 3) indicated incomplete conversion of the baseline material to a single product (R_f 0.6). Acetic anhydride (35 μl, 0.37 mmol) was added and the mixture stirred for a further 2 h, after which t.l.c. (ethyl acetate : hexane 1 : 3) indicated the complete conversion to a single product (R_f 0.6). The solution was diluted with dichloromethane, filtered through a silica plug eluting with dichloromethane and concentrated *in vacuo*. Purification by flash column chromatography (ethyl acetate : hexane 1 : 9) yielded *(1S,4S,6R,7S)-7-azido-6-O-tert-butyldimethylsilyl-6-hydroxy-2,5-dioxa-bicyclo[2.2.1]heptan-3-one* (or *2,5-anhydro-3-azido-6-O-tert-butyldimethylsilyl-3-deoxy-**D**-mannono-1,4-lactone*) **163** (11 mg, 14%), a colourless oil; $[\alpha]_D^{23}$ +67.3° (c, 0.97 in chloroform); ν_{max} (film) 2119 (N_3), 1818 (C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 0.09 (6H, s, $Si(C\underline{H}_3)_2$), 0.91 (9H, s, $SiC(C\underline{H}_3)_3$), 3.88 (1H, dd, $J_{6,6'}$ 9.7, $J_{6,5}$ 5.3, H-6), 3.98 (1H, dd, $J_{6',6}$ 9.7, $J_{6',5}$ 8.4, H-6'), 4.02 (1H, dd, $J_{5,6'}$ 8.4, $J_{5,6}$ 5.3, H-5), 4.13 (1H, d, $J_{3,2}$ 2.3, H-3), 4.58 (1H, s, H-4), 4.95 (1H, dd, $J_{2,3}$ 2.3, $J_{2,4}$ 0.4, H-2); δ_C (50.3 MHz, $CDCl_3$) -5.5, -5.4 (2 x q, $Si(\underline{C}H_3)_2$), 18.2 (s, $Si\underline{C}(CH_3)_3$), 25.8 (q, $SiC(\underline{C}H_3)_3$), 60.7 (t, C-6), 64.2, 77.3, 79.7, 81.9 (4 x d, C-2, C-3, C-4, C-5), 168.3 (s, C-1); *m/z* (APCI+) 272 ($MH^+ - N_2$, 21%), 228 (77%), 122 (100%). HRMS *m/z* (CI+): Found 317.164996. $C_{12}H_{25}N_4O_4Si$ ($M + NH_4^+$) requires 317.164505.

***Isopropyl 3-amino-2,5-anhydro-3-N-(2,5-anhydro-3-azido-3-deoxy-D-mannonyl)-3-deoxy-D-mannonate* 174**

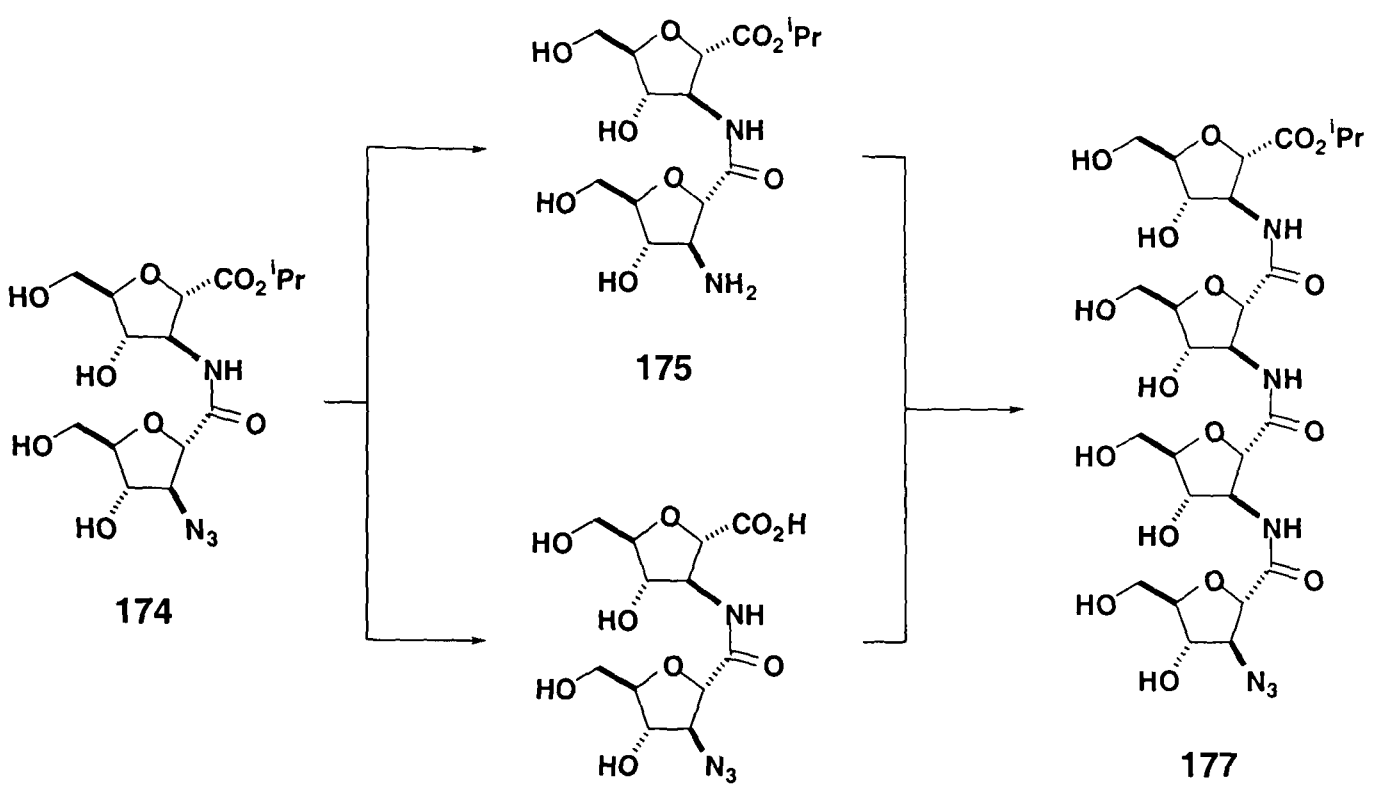


A 1 M solution of sodium hydroxide in water (1.37 ml, 1.37 mmol NaOH) was added to a stirred solution of isopropyl 2,5-anhydro-3-azido-3-deoxy-**D**-mannonate **116** (0.30 g, 1.22 mmol) in 1,4-dioxane (4.8 ml) and water (0.6 ml) at room temperature. After 4 h t.l.c. (ethyl acetate : hexane 7 : 3) indicated the complete conversion of the starting material (R_f 0.3) to baseline products. The solvent was removed *in vacuo* and the residue was subjected to ion exchange chromatography (Amberlite IR-120 (H^+)) to give 2,5-anhydro-3-azido-3-deoxy-**D**-mannonic acid **169** which was used without further purification.

A solution of isopropyl 2,5-anhydro-3-azido-3-deoxy-**D**-mannonate **116** (0.30 g, 1.22 mmol) in isopropanol (2.1 ml) was stirred in a hydrogen atmosphere in the presence of palladium black (0.03 g). After 4 h, t.l.c. (ethyl acetate : hexane 7 : 3) indicated the complete conversion of the starting material (R_f 0.3) to baseline products. The reaction was degassed, purged with nitrogen, filtered and concentrated *in vacuo* to give isopropyl 3-amino-2,5-anhydro-3-deoxy-**D**-mannonate **173** which was used without further purification.

N-Ethyldiisopropylamine (0.27 ml, 1.53 mmol) and *O*-benzotriazol-1-yl-*N*,*N*,*N'*,*N'*-tetramethyluronium tetrafluoroborate (0.49 g, 1.53 mmol) were added to a stirred solution of 2,5-anhydro-3-azido-3-deoxy-**D**-mannonic acid **169** (0.25 g, 1.22 mmol) and isopropyl 3-amino-2,5-anhydro-3-deoxy-**D**-mannonate **173** (0.27 g, 1.22 mmol) in *N*,*N*-dimethylformamide (0.90 ml) at room temperature in a nitrogen atmosphere. After 15 h t.l.c. (5% water in acetonitrile) indicated the complete conversion of baseline material to a single product (R_f 0.4). The solvent was removed *in vacuo*. Purification by flash column chromatography (5% methanol in ethyl acetate then 3% water in acetonitrile) yielded *isopropyl 3-amino-2,5-anhydro-3-deoxy-3-N-(2,5-anhydro-3-azido-3-deoxy-**D**-mannonyl)-**D**-mannonate* **174** (0.44 g, 88%), a white crystalline solid; m.p. 111-114°C (ethyl acetate); $[\alpha]_D^{21}$ +77.3° (c, 1.00 in methanol); ν_{max} (Nujol® mull) 3429 (br, OH, NH), 2113 (N_3), 1732 (C=O, ester), 1662 (C=O, amide I), 1550 (C=O, amide II) cm^{-1}; δ_H (400 MHz, pyridine-d^5) 1.20, 1.23 (6H, 2 x d, *J* 6.2, $CH(C\underline{H}_3)_2$), 4.04 (1H, dd, $J_{6A,6'A}$ 12.0, $J_{6A,5A}$ 4.2, H-6A), 4.05 (1H, dd, $J_{6B,6'B}$ 12.0, $J_{6B,5B}$ 2.5, H-6B), 4.12 (1H, dd, $J_{6'A,6A}$ 12.0, $J_{6'A,5A}$ 3.7, H-6'A), 4.26 (1H, dd, $J_{6'B,6B}$ 12.0, $J_{6'B,5B}$ 2.3, H-6'B), 4.54 (1H, a-dt, $J_{5A,4A}$ 6.7, *J* 3.9, H-5A), 4.70-4.75 (2H, m, H-2B, H-3A), 4.83 (1H, m, H-4A), 4.83 (1H, a-q, *J* 3.0, H-5B), 4.93 (1H, a-t, *J* 3.1, H-4B), 4.96 (1H, d, $J_{2B,3B}$ 2.9, H-2B), 5.05 (1H, br, O<u>H</u>-6), 5.23 (1H, sept, *J* 6.2, $C\underline{H}(CH_3)_2$), 5.54 (1H, a-dt, $J_{3B,NH\text{-}B}$ 9.2, *J* 2.8, H-3B), 6.71 (1H, br, O<u>H</u>-6), 7.56 (1H, br, O<u>H</u>-4B), 7.92 (1H, br, O<u>H</u>-4A), 9.46 (1H, d, *J* 9.2, N<u>H</u>-B); δ_C (100.6 MHz, pyridine-d^5) 23.7, 23.7 (2 x q, $CH(\underline{C}H_3)_2$), 63.4 (d, C-3B), 63.4, 64.0 (2 x t, C-6A, C-6B), 70.5 (d, $\underline{C}H(CH_3)_2$), 73.9 (d, C-3A), 77.6 (d, C-4A), 79.2 (d, C-4B), 83.3 (d, C-2B), 84.6 (d, C-2A), 88.0 (d, C-5A), 90.4 (d, C-5B), 172.9, 173.0 (2 x s, C-1A, C-1B); *m/z* (APCI+) 433 (3%), 405 (MH^+, 8%), 404 (33%), 363 ($MH^+ - N_3$, 12%), 362 ($MH^+ - {}^iPr$, 100%), 319 ($MH^+ - CO_2{}^iPr$, 11%), 316 (22%), 301 (13%), 271 (18%).

***Isopropyl 3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(2,5-anhydro-3-azido-3-deoxy-D-mannonyl)-3-deoxy-D-mannonyl)-3-deoxy-D-mannonyl)-3-deoxy-D-mannonate* 177**



176

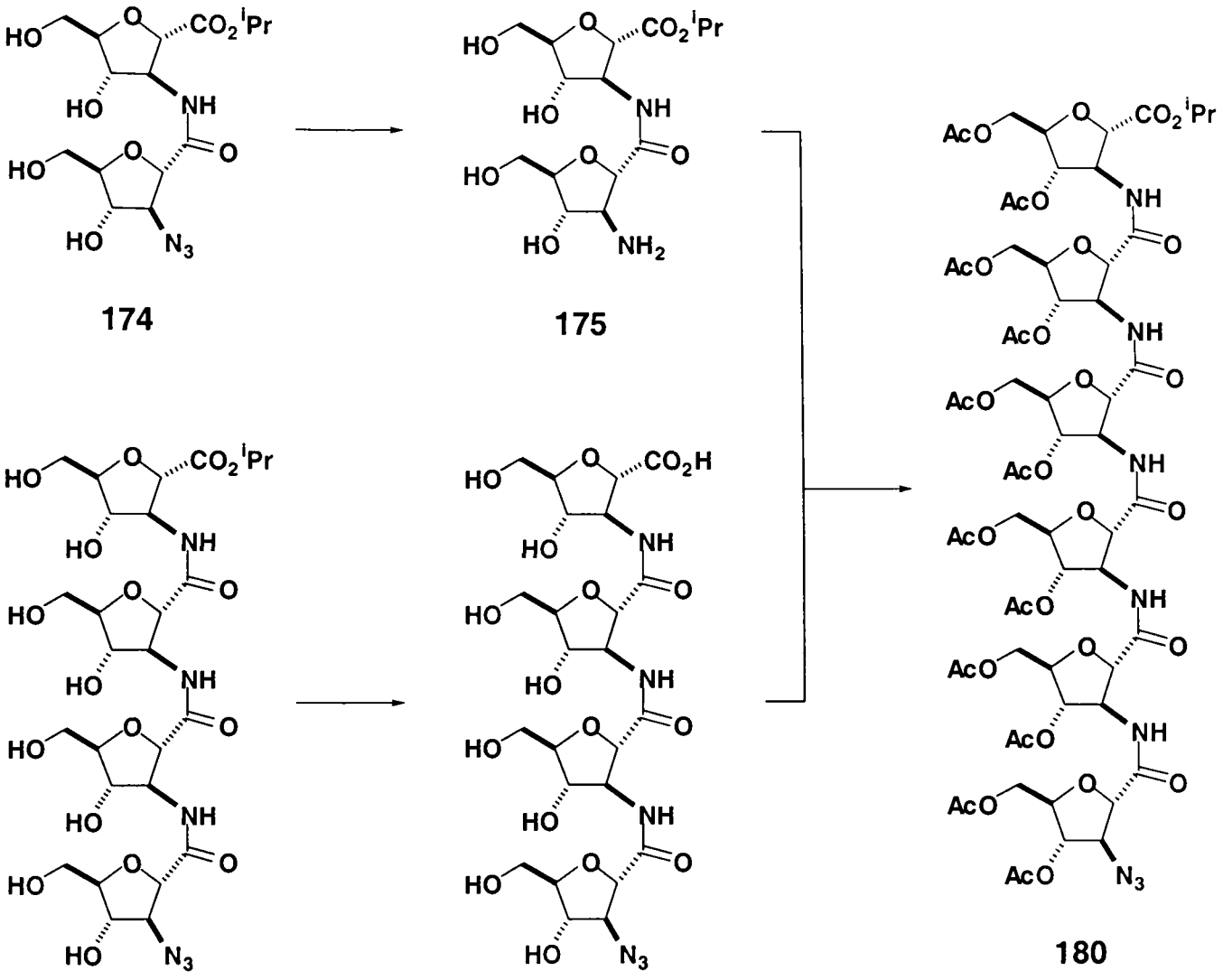
A 1 M solution of sodium hydroxide in water (297 μl, 297 μmol NaOH) was added to a stirred solution of isopropyl 3-amino-2,5-anhydro-3-*N*-(2,5-anhydro-3-azido-3-deoxy-**D**-mannonyl)-3-deoxy-**D**-mannonate **174** (100 mg, 247 μmol) in 1,4-dioxane (1 ml) and water (135 μl) at room temperature. After 5 h t.l.c. (5% water in acetonitrile) indicated the complete conversion of the starting material (R_f 0.3) to baseline products. The solvent was removed *in vacuo* and the residue was subjected to ion exchange chromatography (Amberlite IR-120 (H^+)) to give 3-amino-2,5-anhydro-3-*N*-(2,5-anhydro-3-azido-3-deoxy-**D**-mannonyl)-3-deoxy-**D**-mannonic acid **176** which was used without further purification.

A solution of isopropyl 3-amino-2,5-anhydro-3-*N*-(2,5-anhydro-3-azido-3-deoxy-**D**-mannonyl)-3-deoxy-**D**-mannonate **174** (100 mg, 247 μmol) in water (1.5 ml) was stirred in a hydrogen atmosphere in the presence of palladium black (10 mg). After 5 h, t.l.c. (5% water in acetonitrile) indicated the complete conversion of the starting material (R_f 0.3) to baseline products. The reaction was degassed, purged with nitrogen, filtered through Celite® and concentrated *in vacuo* to give isopropyl 3-amino-2,5-anhydro-3-*N*-(3-amino-2,5-anhydro-3-deoxy-**D**-mannonyl)-3-deoxy-**D**-mannonate **175** which was used without further purification.

N-Ethyldiisopropylamine (54 μl, 309 μmol) and *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (100 mg, 311 μmol) were added to a stirred solution of 3-amino-2,5-anhydro-3-*N*-(2,5-anhydro-3-azido-3-deoxy-**D**-mannonyl)-3-deoxy-**D**-mannonic acid **176** (90 mg, 247 μmol) and isopropyl 3-amino-2,5-anhydro-3-*N*-(3-amino-2,5-anhydro-3-deoxy-**D**-mannonyl)-3-deoxy-**D**-mannonate **175** (94 mg, 247 μmol) in *N*,*N*-dimethylformamide (300 μl) at room temperature in a nitrogen atmosphere. After 5 d t.l.c. (chloroform : methanol : acetic acid : water 60 : 30 : 3 : 5) indicated the formation of a major product (R_f 0.3). The solvent was removed *in vacuo*. Purification by flash column chromatography (chloroform : methanol : acetic acid : water 60 : 30 : 3 : 5) yielded *isopropyl 3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(2,5-anhydro-3-azido-3-deoxy-**D**-mannonyl)-3-deoxy-**D**-mannonyl)-3-deoxy-**D**-mannonyl)-3-deoxy-**D**-mannonate* **177** (130 mg, 73%), a white crystalline solid; m.p. 180-182°C (methanol); $[\alpha]_D^{24}$ +76.7° (c, 0.95 in methanol); ν_{max} (film) 3400 (br, OH, NH), 2116 (N_3), 1726 (C=O, ester), 1658 (C=O, amide I), 1536 (C=O, amide II) cm^{-1}; δ_H (400 MHz, pyridine-d^5) 1.15, 1.16 (6H, 2 x d, *J* 6.4, $CH(C\underline{H}_3)_2$), 3.90 (1H, dd, $J_{6B,6'B}$ 12.0, $J_{6B,5B}$ 2.1, H-6B), 3.95 (1H, dd, $J_{6C,6'C}$ 12.1, $J_{6C,5C}$ 2.3, H-6C), 3.99 (1H, dd, $J_{6A,6'A}$ 12.1, $J_{6A,5A}$ 4.1, H-6A), 4.03 (1H, m, H-6'B), 4.03-4.08 (2H, m, H-6D, H-6'A),

4.08 (1H, m, H-6'C), 4.25 (1H, dd, $J_{6'D,6D}$ 12.0, $J_{6'D,5D}$ 2.2, H-6'D), 4.49 (1H, a-dt, *J* 6.53, *J* 3.3, H-5A), 4.65-4.69 (2H, m, H-2A, H-3A), 4.75 (1H, m, H-5B), 4.77 (1H, m, H-4A), 4.81 (2H, m, H-5C, H-5D), 4.86 (2H, m, H-2B, H-4B), 4.93 (2H, m, H-2C, H-2D), 4.97 (2H, m, H-4C, H-4D), 5.18 (1H, sept, *J* 6.4, C<u>H</u>$(CH_3)_2$), 5.53 (1H, a-dt, $J_{3D,NH-D}$ 9.2, *J* 2.0, H-3D), 5.59 (1H, a-dt, $J_{3B,NH-B}$ 9.0, *J* 2.7, H-3B), 5.61 (1H, a-dt, $J_{3C,NH-C}$ 8.9, *J* 2.2, H-3C), 9.39 (1H, d, $J_{NH-D,3D}$ 9.2, N<u>H</u>-D), 9.51 (1H, d, $J_{NH-C,3C}$ 8.9, N<u>H</u>-C), 9.52 (1H, d, $J_{NH-B,3B}$ 9.0, N<u>H</u>-B); δ_C (100.6 MHz, pyridine-d^5) 21.6, 22.6 (2 x q, CH(<u>C</u>$H_3)_2$), 62.3, 62.5, 62.7, 63.0, 63.1, 63.2, 63.4 (C-3B, C-3C, C-3D, C-6A, C-6B, C-6C, C-6D), 69.3 (d, <u>C</u>H$(CH_3)_2$), 72.7 (d, C-3A), 76.5 (d, C-4A), 78.5, 78.5, 78.7 (3 x d, C-4B, C-4C, C-4D), 82.2 (d, C-2A), 83.7, 85.2, 85.5 (3 x d, C-2B, C-2C, C-2D), 86.9 (d, C-5A), 89.6, 89.7, 90.0 (3 x d, C-5B, C-5C, C-5D), 171.5, 171.6, 172.2, 172.4 (4 x s, C-1A, C-1B, C-1C, C-1D); δ_C (100.6 MHz, pyridine-d^5) 21.6, 22.6 (2 x q, CH(<u>C</u>$H_3)_2$), 62.3, 62.5, 62.7, 63.0, 63.1, 63.2, 63.4 (C-3B, C-3C, C-3D, C-6A, C-6B, C-6C, C-6D), 69.3 (d, <u>C</u>H$(CH_3)_2$), 72.7 (d, C-3A), 76.5 (d, C-4A), 78.5, 78.5, 78.7 (3 x d, C-4B, C-4C, C-4D), 82.2 (d, C-2A), 83.7, 85.2, 85.5 (3 x d, C-2B, C-2C, C-2D), 86.9 (d, C-5A), 89.6, 89.7, 90.0 (3 x d, C-5B, C-5C, C-5D), 171.5, 171.6, 172.2, 172.4 (4 x s, C-1A, C-1B, C-1C, C-1D); *m/z* (APCI+) 723 (MH^+, 12%), 619 (22%), 470 (40%), 279 (100%).

Isopropyl 3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(2,5-anhydro-3-azido-3-deoxy-4,6-di-O-acetoxy-D-mannonyl)-3-deoxy-4,6-di-O-acetoxy-D-mannonyl)-3-deoxy-4,6-di-O-acetoxy-D-mannonyl)-3-deoxy-4,6-di-O-acetoxy-D-mannonyl)-3-deoxy-4,6-di-O-acetoxy-D-mannonyl)-3-deoxy-4,6-di-O-acetoxy-D-mannonyl)-3-deoxy-4,6-di-O-acetoxy-D-mannonate **180**



A 1 M solution of sodium hydroxide in water (60 µl, 60 µmol NaOH) was added to a stirred solution of isopropyl 3-amino-2,5-anhydro-3-*N*-(3-amino-2,5-anhydro-3-*N*-(3-amino-2,5-anhydro-3-*N*-(2,5-anhydro-3-azido-3-deoxy-**D**-mannonyl)-3-deoxy-**D**-mannonyl)-3-deoxy-**D**-mannonyl)-3-deoxy-**D**-mannonate **177** (36 mg, 50 µmol) in 1,4-dioxane (420 µl) and water (54 µl) at room temperature. After 2 d t.l.c. (chloroform : methanol : acetic acid : water 60 : 30 : 3 : 5) indicated the complete

conversion of the starting material (R_f 0.3) to baseline products. The solvent was removed *in vacuo* and the residue was subjected to ion exchange chromatography (Amberlite IR-120 (H^+)) to give 3-amino-2,5-anhydro-3-*N*-(3-amino-2,5-anhydro-3-*N*-(3-amino-2,5-anhydro-3-*N*-(2,5-anhydro-3-azido-3-deoxy-**D**-mannonyl)-3-deoxy-**D**-mannonyl)-3-deoxy-**D**-mannonyl)-3-deoxy-**D**-mannonic acid **178** which was used without further purification.

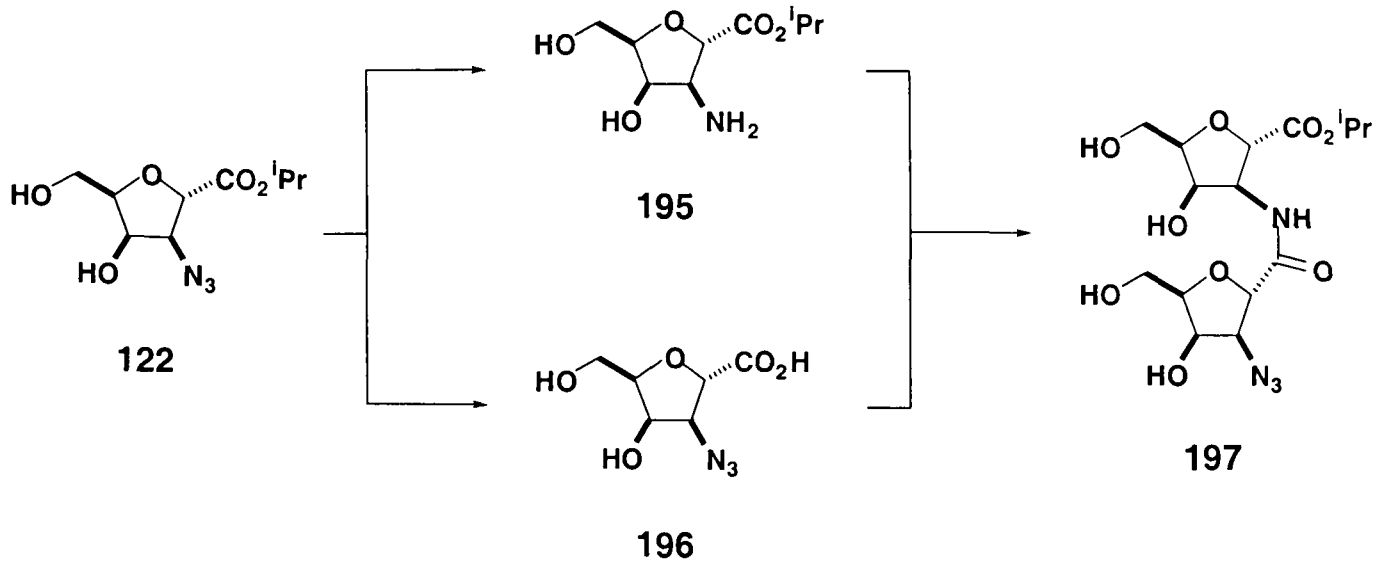
A solution of isopropyl 3-amino-2,5-anhydro-3-*N*-(2,5-anhydro-3-azido-3-deoxy-**D**-mannonyl)-3-deoxy-**D**-mannonate **174** (21 mg, 52 μmol) in water (300 μl) was stirred in a hydrogen atmosphere in the presence of palladium black (2 mg). After 5 h, t.l.c. (5% water in acetonitrile) indicated complete conversion of starting material (R_f 0.3) to baseline products. The reaction was degassed, purged with nitrogen, filtered and concentrated *in vacuo* to give isopropyl 3-amino-2,5-anhydro-3-*N*-(3-amino-2,5-anhydro-3-deoxy-**D**-mannonyl)-3-deoxy-**D**-mannonate **175** which was used without further purification.

N-Ethyldiisopropylamine (11 μl, 62 μmol) and *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (20 mg, 62 μmol) were added to a stirred solution of 3-amino-2,5-anhydro-3-*N*-(3-amino-2,5-anhydro-3-*N*-(3-amino-2,5-anhydro-3-*N*-(2,5-anhydro-3-azido-3-deoxy-**D**-mannonyl)-3-deoxy-**D**-mannonyl)-3-deoxy-**D**-mannonyl)-3-deoxy-**D**-mannonic acid **178** (34 mg, 50 μmol) and isopropyl 3-amino-2,5-anhydro-3-*N*-(3-amino-2,5-anhydro-3-deoxy-**D**-mannonyl)-3-deoxy-**D**-mannonate **175** (19 mg, 52 μmol) in *N*,*N*-dimethylformamide (42 μl) at room temperature in a nitrogen atmosphere. The mixture was stirred for 10 d at room temperature and the solvent removed *in vacuo*. The residue was dissolved in pyridine (0.5 ml) and acetic anhydride (0.5 ml) added. After 2 d t.l.c. (ethyl acetate) indicated the formation of a major product (R_f 0.4). The solvent was

removed *in vacuo*. Purification by flash column chromatography (ethyl acetate) yielded *isopropyl 3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(2,5-anhydro-3-azido-3-deoxy-4,6-di-O-acetoxy-**D**-mannonyl)-3-deoxy-4,6-di-O-acetoxy-**D**-mannonyl)-3-deoxy-4,6-di-O-acetoxy-**D**-mannonyl)-3-deoxy-4,6-di-O-acetoxy-**D**-mannonyl)-3-deoxy-4,6-di-O-acetoxy-**D**-mannonyl)-3-deoxy-4,6-di-O-acetoxy-**D**-mannonyl)-3-deoxy-4,6-di-O-acetoxy-**D**-mannonate* **180** (15 mg, 19%), a colourless oil; δ_H (500 MHz, benzene-d^6 + pyridine-d^5 (50µl)) 1.07, 1.10 (6H, 2 x d, *J* 6.4, CH(C$\underline{H}_3$)$_2$), 1.70, 1.71, 1.73, 1.74, 1.74, 1.74, 1.76, 1.79, 1.79, 1.84, 1.87, 1.90 (36H, 12 x s, OCOC$\underline{H}_3$), 4.22 (1H, d, *J* 5.2, H-6A), 4.35 (1H, dd, *J* 5.6, H-6B), 4.36-4.50 (13H, m, H-5A, H-5D, H-5E, H-6C, H-6D, H-6E, H-6F, H-6'A, H-6'B, H-6'C, H-6'D, H-6'E, H-6'F) 4.51-4.55 (2H, m, H-5B, H-5C), 4.59 (1H, a-q, *J* 4.2, H-5F), 4.61 (1H, d, $J_{2A,3A}$ 3.4, H-2A), 4.77 (1H, a-t, *J* 2.9, H-3A), 4.79 (1H, d, $J_{2B,3B}$ 6.6, H-2B), 4.80 (1H, d, $J_{2C,3C}$ 7.3, H-2C), 4.83 (1H, d, $J_{2F,3F}$ 4.5, H-2F), 4.90 (1H, d, $J_{2D,3D}$ 7.8, H-2D), 4.91 (1H, d, $J_{2E,3E}$ 7.3, H-2E), 4.97 (1H, a-q, *J* 6.7, H-3D), 5.02-5.09 (4H, m, H-3B, H-3C, H-3E, C$\underline{H}$(CH$_3$)$_2$), 5.15 (1H, a-dt, $J_{3F,NH-F}$ 7.6, *J* 4.4, H-3F), 5.21 (1H, a-t, *J* 3.0, H-4A), 5.48 (1H, a-t, *J* 4.5, H-4F), 5.61 (1H, a-t, *J* 5.4, H-4B), 5.66 (1H, a-t, *J* 5.2, H-4E), 5.70 (1H, a-t, *J* 5.1, H-4D), 5.74 (1H, a-t, *J* 5.4, H-4C), 8.70 (1H, d, $J_{NH-F,3F}$ 7.6, N$\underline{H}$-F), 8.80 (1H, d, $J_{NH-D,3D}$ 7.4, N$\underline{H}$-D), 8.88 (1H, d, $J_{NH-E,3E}$ 7.6, N$\underline{H}$-E), 9.06 (1H, d, $J_{NH-C,3C}$ 8.2, N$\underline{H}$-C), 9.07 (1H, d, $J_{NH-B,3B}$ 7.9, N$\underline{H}$-B).

Isopropyl 3-amino-2,5-anhydro-3-N-(2,5-anhydro-3-azido-3-deoxy-D-talonyl)-3-deoxy-D-talonate

197



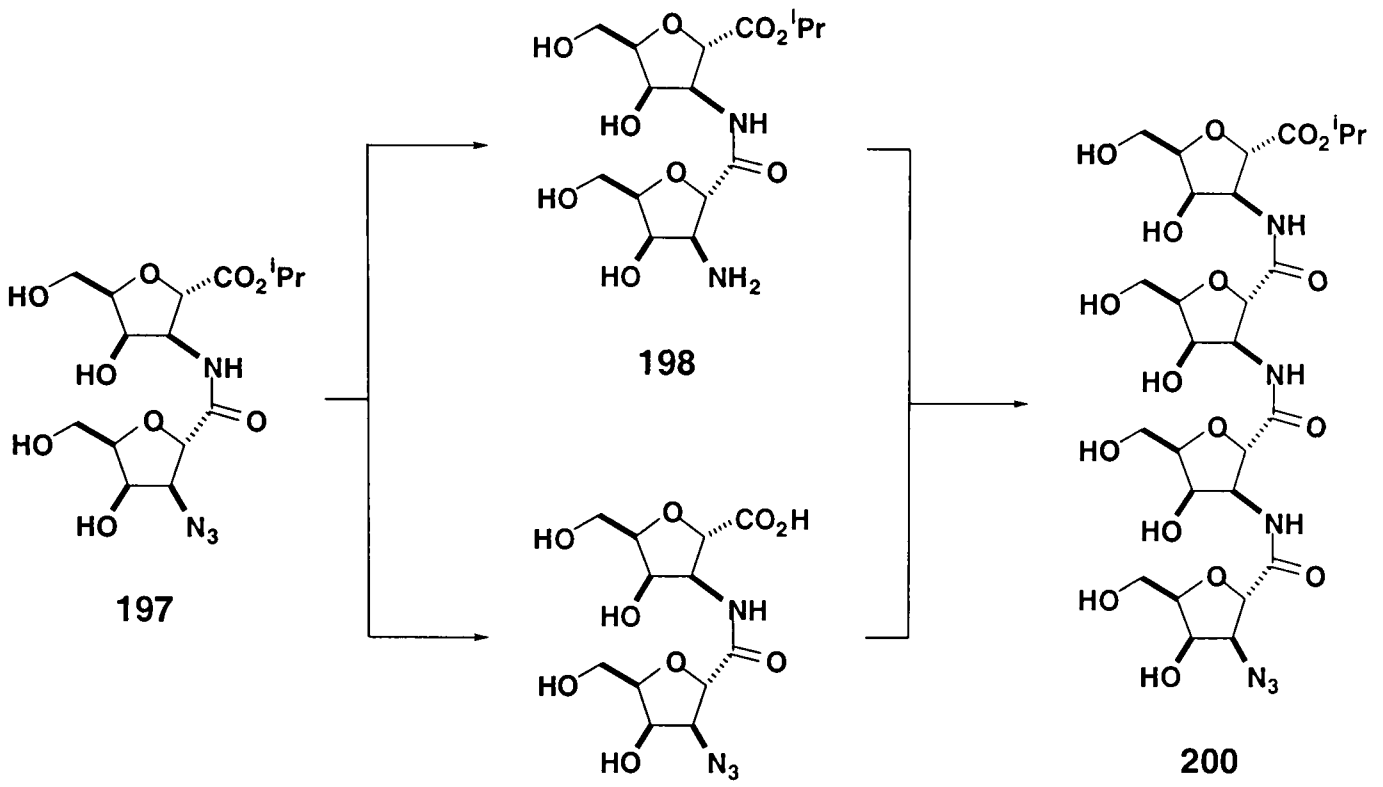
A 1 M solution of sodium hydroxide in water (1.37 ml, 1.37 mmol NaOH) was added to a stirred solution of isopropyl 2,5-anhydro-3-azido-3-deoxy-**D**-talonate **122** (0.30 g, 1.22 mmol) in 1,4-dioxane (4.8 ml) and water (0.6 ml) at room temperature. After 4 h t.l.c. (ethyl acetate : hexane 7 : 3) indicated the complete conversion of the starting material (R_f 0.3) to baseline products. The solvent was removed *in vacuo* and the residue was subjected to ion exchange chromatography (Amberlite IR-120 (H^+)) to give 2,5-anhydro-3-azido-3-deoxy-**D**-talonic acid **196** which was used without further purification.

A solution of isopropyl 2,5-anhydro-3-azido-3-deoxy-**D**-talonate **122** (0.30 g, 1.22 mmol) in isopropanol (2.1 ml) was stirred in a hydrogen atmosphere in the presence of palladium black (0.03 g). After 4 h, t.l.c. (ethyl acetate : hexane 7 : 3) indicated the complete conversion of the starting material (R_f 0.3) to baseline products. The reaction was degassed, purged with nitrogen, filtered and concentrated *in vacuo* to give isopropyl 3-amino-2,5-anhydro-3-deoxy-**D**-talonate **195** which was used without further purification.

N-Ethyldiisopropylamine (0.27 ml, 1.53 mmol) and *O*-benzotriazol-1-yl-*N*,*N*,*N'*,*N'*-tetramethyluronium tetrafluoroborate (0.49 g, 1.53 mmol) were added to a stirred solution of 2,5-anhydro-3-azido-3-deoxy-**D**-talonic acid **196** (0.25 g, 1.22 mmol) and isopropyl 3-amino-2,5-anhydro-3-deoxy-**D**-talonate **195** (0.27 g, 1.22 mmol) in *N*,*N*-dimethylformamide (0.90 ml) at room temperature in a nitrogen atmosphere. After 17 h t.l.c. (5% water in acetonitrile) indicated the formation of a single product (R_f 0.3). The solvent was removed *in vacuo*. Purification by flash column chromatography (5% water in acetonitrile then 3% water in acetonitrile) yielded *isopropyl 3-amino-2,5-anhydro-3-N-(2,5-anhydro-3-azido-3-deoxy-**D**-talonyl)-3-deoxy-**D**-talonate* **197** (0.44 g, 88%), a white crystalline solid; m.p. 180-186°C (ethyl acetate); $[\alpha]_D^{21}$ +85.4° (c, 0.50 in methanol); ν_{max} (Nujol® mull) 3266 (br, OH, NH), 2119 (N_3), 1732 (C=O, ester), 1661 (C=O, amide I), 1557 (C=O, amide II) cm^{-1}; δ_H (400 MHz, pyridine-d^5) 1.17, 1.24 (6H, 2 x d, *J* 6.3, $CH(C\underline{H}_3)_2$), 4.35 (1H, dd, $J_{6A,6'A}$ 11.2, $J_{6A,5A}$ 5.7, H-6A), 4.35 (1H, dd, $J_{6B,6'B}$ 11.2, $J_{6B,5B}$ 5.7, H-6B), 4.37 (1H, dd, $J_{3A,2A}$ 8.0, $J_{3A,4A}$ 4.4, H-3A), 4.42 (1H, dd, $J_{6'B,6B}$ 11.2, $J_{6'B,5B}$ 5.5, H-6'B), 4.46 (1H, dd, $J_{6'A,6A}$ 11.2, $J_{6'A,5A}$ 5.5, H-6'A), 4.66 (1H, a-dt, *J* 5.8, $J_{5A,4A}$ 3.4, H-5A), 4.81 (2H, m, H-4A, H-5B), 4.82 (1H, d, $J_{2B,3B}$ 7.5, H-2B), 4.87 (1H, br, H-4B), 5.06 (1H, d, $J_{2A,3A}$ 8.0, H-2A), 5.18 (1H, sept, *J* 6.3, $C\underline{H}(CH_3)_2$), 5.36 (1H, ddd, $J_{3B,NH-B}$ 9.0, $J_{3B,2B}$ 7.5, $J_{3B,4B}$ 4.9, H-3B), 6.70 (1H, br, O<u>H</u>-6), 6.91 (1H, br, O<u>H</u>-6), 7.86 (2H, br, O<u>H</u>-4A, O<u>H</u>-4B), 8.98 (1H, d, $J_{NH-B,3B}$ 9.0, N<u>H</u>-B); δ_C (100.6 MHz, pyridine-d^5) 21.9, 22.1 (2 x q, $CH(\underline{C}H_3)_2$), 57.3 (d, C-3B), 61.3, 61.5 (2 x t, C-6A, C-6B), 67.6 (d, C-3A), 69.0 (d, $C\underline{H}(CH_3)_2$), 72.1 (d, C-4B), 73.8 (d, C-4A), 79.6 (d, C-2A), 80.8 (d, C-2B), 84.6, 84.7 (2 x d, C-5A, C-5B), 172.0, 172.4 (2 x s, C-1A, C-1B); *m/z* (APCI+) 433 (14%), 405 (MH^+, 13%), 404 (72%), 386 (30%), 363 ($MH^+ - N_3$, 11%), 362 ($MH^+ - {}^iPr$, 100%), 360 (34%), 316 (20%), 301 (19%), 271 (44%), 177 (55%).

Isopropyl 3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(2,5-anhydro-3-azido-3-deoxy-D-talonyl)-3-deoxy-D-talonyl)-3-deoxy-D-talonyl)-3-deoxy-D-talonate

200



199

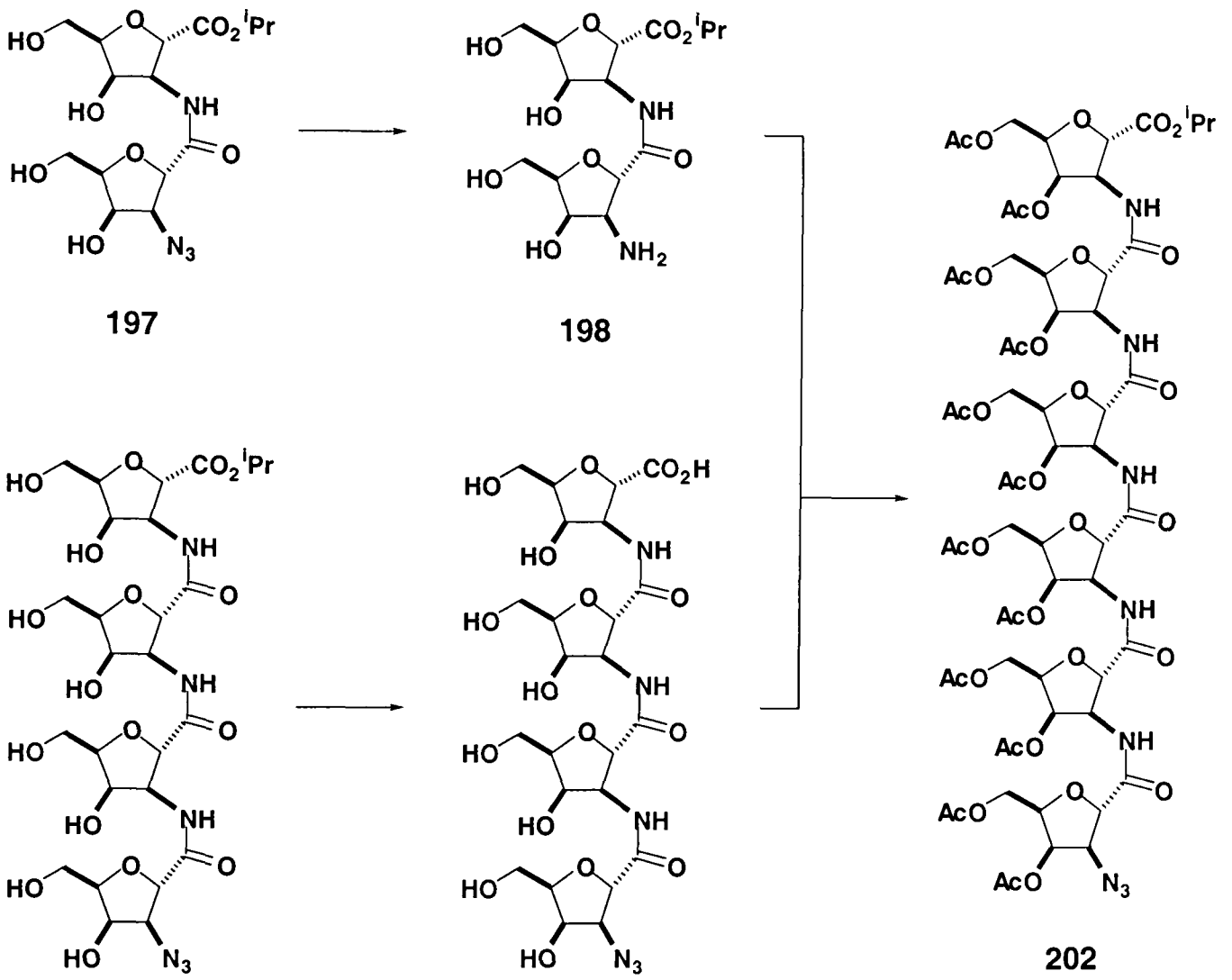
A 1 M solution of sodium hydroxide in water (297 μl, 297 μmol NaOH) was added to a stirred solution of isopropyl 3-amino-2,5-anhydro-3-*N*-(2,5-anhydro-3-azido-3-deoxy-**D**-talonyl)-3-deoxy-**D**-talonate **197** (100 mg, 247 μmol) in 1,4-dioxane (1 ml) and water (135 μl) at room temperature. After 5 h t.l.c. (5% water in acetonitrile) indicated the complete conversion of the starting material (R_f 0.3) to baseline products. The solvent was removed *in vacuo* and the residue was subjected to ion exchange chromatography (Amberlite IR-120 (H^+)) to give 3-amino-2,5-anhydro-3-*N*-(2,5-anhydro-3-azido-3-deoxy-**D**-talonyl)-3-deoxy-**D**-talonic acid **199** which was used without further purification.

A solution of isopropyl 3-amino-2,5-anhydro-3-*N*-(2,5-anhydro-3-azido-3-deoxy-**D**-talonyl)-3-deoxy-**D**-talonate **197** (100 mg, 247 μmol) in water (1.5 ml) was stirred in a hydrogen atmosphere in the presence of palladium black (10 mg). After 5 h, t.l.c. (5% water in acetonitrile) indicated the complete conversion of the starting material (R_f 0.3) to baseline products. The reaction was degassed, purged with nitrogen, filtered and concentrated *in vacuo* to give isopropyl 3-amino-2,5-anhydro-3-*N*-(3-amino-2,5-anhydro-3-deoxy-**D**-talonyl)-3-deoxy-**D**-talonate **198** which was used without further purification.

N-Ethyldiisopropylamine (54 μl, 309 μmol) and *O*-benzotriazol-1-yl-*N*,*N*,*N'*,*N'*-tetramethyluronium tetrafluoroborate (100 mg, 311 μmol) were added to a stirred solution of 3-amino-2,5-anhydro-3-*N*-(2,5-anhydro-3-azido-3-deoxy-**D**-talonyl)-3-deoxy-**D**-talonic acid **199** (90 mg, 247 μmol) and isopropyl 3-amino-2,5-anhydro-3-*N*-(3-amino-2,5-anhydro-3-deoxy-**D**-talonyl)-3-deoxy-**D**-talonate **198** (94 mg, 247 μmol) in *N*,*N*-dimethylformamide (300 μl) at room temperature in a nitrogen atmosphere. After 5 d t.l.c. (chloroform : methanol : acetic acid : water 60 : 30 : 3 : 5) indicated the formation of a major product (R_f 0.3). The solvent was removed *in vacuo*. Purification by flash column chromatography (chloroform : methanol : acetic acid : water 60 : 30 : 3 : 5) yielded *isopropyl 3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(2,5-anhydro-3-azido-3-deoxy-**D**-talonyl)-3-deoxy-**D**-talonyl)-3-deoxy-**D**-talonyl)-3-deoxy-**D**-talonate* **200** (144 mg, 81%), a white crystalline solid; m.p. 136-141°C (methanol); $[\alpha]_D^{24}$ +78.8° (c, 0.93 in methanol); ν_{max} (film) 3384 (br, OH, NH), 2111 (N_3), 1730 (C=O, ester), 1655 (C=O, amide I), 1531 (C=O, amide II) cm^{-1}; δ_H (500 MHz, pyridine-d^5) 1.14, 1.18 (6H, 2 x d, *J* 6.2, $CH(C\underline{H}_3)_2$), 4.25-4.36 (7H, m, H-3A, H-6A, H-6B, H-6C, H-6D, H-6'B, H-6'C), 4.41 (1H, dd, $J_{6'D,6D}$ 11.2, $J_{6'D,5D}$ 5.6, H-6'D), 4.42 (1H, dd, $J_{6'A,6A}$ 11.3, $J_{6'A,5A}$ 5.6, H-6'A), 4.60 (1H, a-q, *J* 4.5, H-5C), 4.64 (1H, a-q, *J* 4.6, H-5B), 4.67

(1H, a-dt, *J* 5.8, *J* 3.6, H-5A), 4.77 (1H, a-q, *J* 5.0, H-5D), 4.81 (1H, d, $J_{2D,3D}$ 7.6, H-2D), 4.81 (1H, m, H-4A), 4.84-4.88 (3H, m, H-4B, H-4C, H-4D), 4.89 (1H, d, $J_{2B,3B}$ 6.6, H-2B), 4.95 (1H, d, $J_{2C,3C}$ 7.4, H-2C), 5.00 (1H, d, $J_{2A,3A}$ 7.6, H-2A), 5.14 (1H, sept, *J* 6.2, C<u>H</u>$(CH_3)_2$), 5.27 (1H, a-dt, *J* 7.8, $J_{3C,4C}$ 5.0, H-3C), 5.30 (1H, a-dt, *J* 8.3, $J_{3D,4D}$ 5.1, H-3D), 5.36 (1H, ddd, $J_{3B,NH-B}$ 8.8, $J_{3B,2B}$ 6.6, $J_{3B,4B}$ 5.6, H-3B), 6.78 (8H, br, O<u>H</u>-4A, O<u>H</u>-4B, O<u>H</u>-4C, O<u>H</u>-4D, O<u>H</u>-6A, O<u>H</u>-6B, O<u>H</u>-6C, O<u>H</u>-6D), 8.86 (1H, d, $J_{NH-D,3D}$ 9.0, N<u>H</u>-D), 9.13 (1H, d, $J_{NH-B,3B}$ 8.8, N<u>H</u>-B), 9.20 (1H, d, $J_{NH-C,3C}$ 8.5, N<u>H</u>-C); δ_C (100.6 MHz, pyridine-d^5) 21.9, 22.1 (2 x q, CH(C<u>H</u>$_3)_2$), 57.4, 57.6, 58.0 (3 x d, C-3B, C-3C, C-3D), 61.2, 61.3, 61.4, 61.5 (4 x t, C-6A, C-6B, C-6C, C-6D), 67.7 (d, C-3A), 69.0 (d, <u>C</u>H$(CH_3)_2$), 71.6, 71.7, 72.1 (3 x d, C-4B, C-4C, C-4D), 73.8 (d, C-4A), 79.8 (d, C-2A), 80.6 (d, C-2D), 81.1 (d, C-2C), 81.8 (d, C-2B), 84.0, 84.3, 84.5, 84.7 (4 x d, C-5A, C-5B, C-5C, C-5D), 172.4, 172.5, 172.6, 172.7 (4 x s, C-1A, C-1B, C-1C, C-1D); *m/z* (APCI+) 723 (MH^+, 18%), 632 (30%), 361 (53%), 360 (67%), 178 (100%).

***Isopropyl** 3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(2,5-anhydro-3-azido-3-deoxy-4,6-di-O-acetoxy-D-talonyl)-3-deoxy-4,6-di-O-acetoxy-D-talonyl)-3-deoxy-4,6-di-O-acetoxy-D-talonyl)-3-deoxy-4,6-di-O-acetoxy-D-talonyl)-3-deoxy-4,6-di-O-acetoxy-D-talonyl)-3-deoxy-4,6-di-O-acetoxy-D-talonyl)-3-deoxy-4,6-di-O-acetoxy-D-talonate* **202**



A 1 M solution of sodium hydroxide in water (50 µl, 50 µmol NaOH) was added to a stirred solution of isopropyl 3-amino-2,5-anhydro-3-*N*-(3-amino-2,5-anhydro-3-*N*-(3-amino-2,5-anhydro-3-*N*-(2,5-anhydro-3-azido-3-deoxy-**D**-talonyl)-3-deoxy-**D**-talonyl)-3-deoxy-**D**-talonyl)-3-deoxy-**D**-talonate **200** (30 mg, 42 µmol) in 1,4-dioxane (350 µl) and water (45 µl) at room temperature. After 24 h t.l.c. (chloroform : methanol : acetic acid : water 60 : 30 : 3 : 5) indicated the complete conversion of the

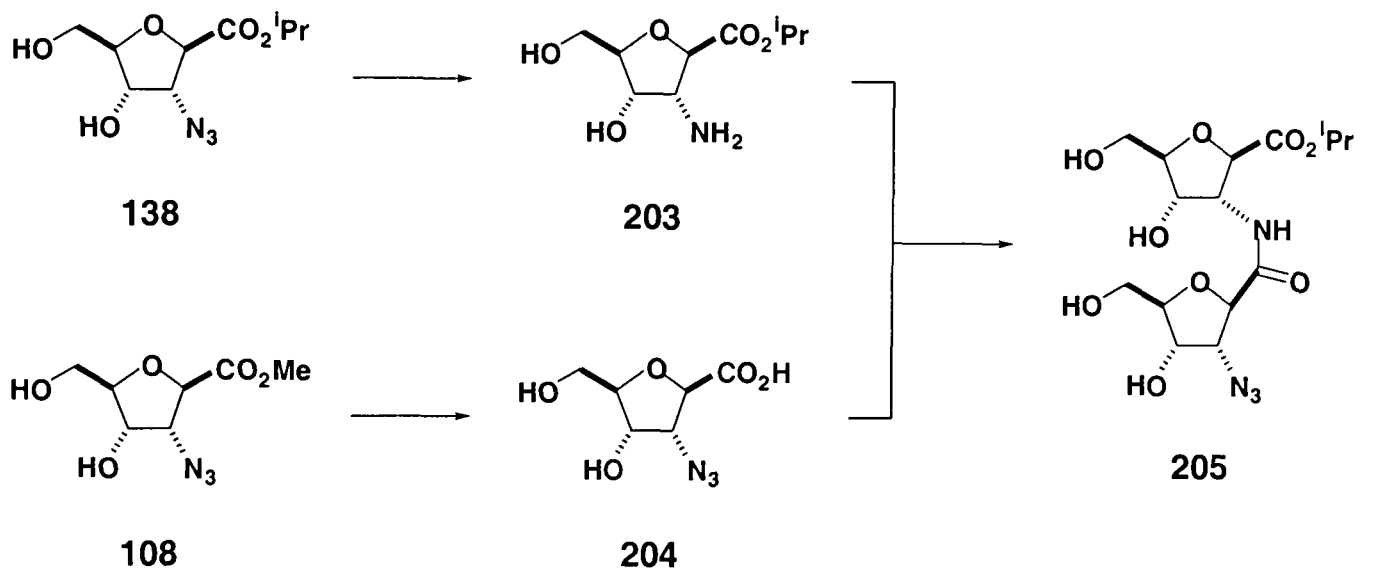
starting material (R_f 0.3) to baseline products. The solvent was removed *in vacuo* and the residue was subjected to ion exchange chromatography (Amberlite IR-120 (H^+)) to give 3-amino-2,5-anhydro-3-*N*-(3-amino-2,5-anhydro-3-*N*-(3-amino-2,5-anhydro-3-*N*-(2,5-anhydro-3-azido-3-deoxy-**D**-talonyl)-3-deoxy-**D**-talonyl)-3-deoxy-**D**-talonyl)-3-deoxy-**D**-talonic acid **201** which was used without further purification.

A solution of isopropyl 3-amino-2,5-anhydro-3-*N*-(2,5-anhydro-3-azido-3-deoxy-**D**-talonyl)-3-deoxy-**D**-talonate **197** (17 mg, 42 μmol) in water (250 μl) was stirred in a hydrogen atmosphere in the presence of palladium black (2 mg). After 4 h, t.l.c. (5% water in acetonitrile) indicated complete conversion to baseline products. The reaction was degassed, purged with nitrogen, filtered and concentrated *in vacuo* to give isopropyl 3-amino-2,5-anhydro-3-*N*-(3-amino-2,5-anhydro-3-deoxy-**D**-talonyl)-3-deoxy-**D**-talonate **198** which was used without further purification.

N-Ethyldiisopropylamine (9 μl, 51 μmol) and *O*-benzotriazol-1-yl-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborate (16 mg, 51 μmol) were added to a stirred solution of 3-amino-2,5-anhydro-3-*N*-(3-amino-2,5-anhydro-3-*N*-(3-amino-2,5-anhydro-3-*N*-(2,5-anhydro-3-azido-3-deoxy-**D**-talonyl)-3-deoxy-**D**-talonyl)-3-deoxy-**D**-talonyl)-3-deoxy-**D**-talonic acid **201** (28 mg, 42 μmol) and isopropyl 3-amino-2,5-anhydro-3-*N*-(3-amino-2,5-anhydro-3-deoxy-**D**-talonyl)-3-deoxy-**D**-talonate **198** (16 mg, 42 μmol) in *N*,*N*-dimethylformamide (35 μl) at room temperature in a nitrogen atmosphere. The mixture was stirred for 6 d at room temperature and the solvent removed *in vacuo*. The residue was dissolved in pyridine (0.5 ml) and acetic anhydride (0.5 ml) added. After 24 h t.l.c. (ethyl acetate) indicated the formation of a major product (R_f 0.4). The solvent was removed *in vacuo*. Purification by flash column chromatography (ethyl acetate) yielded *isopropyl 3-amino-2,5-anhydro-3-N-(3-*

*amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(2,5-anhydro-3-azido-3-deoxy-4,6-di-O-acetoxy-**D**-talonyl)-3-deoxy-4,6-di-O-acetoxy-**D**-talonyl)-3-deoxy-4,6-di-O-acetoxy-**D**-talonyl)-3-deoxy-4,6-di-O-acetoxy-**D**-talonyl)-3-deoxy-4,6-di-O-acetoxy-**D**-talonyl)-3-deoxy-4,6-di-O-acetoxy-**D**-talonate* **202** (11 mg, 17%), a colourless oil. δ_H (400 MHz) see **Chapter 4.3**.

***Isopropyl 3-amino-2,5-anhydro-3-N-(2,5-anhydro-3-azido-3-deoxy-D-allonyl)-3-deoxy-D-allonate* 205**



A 1 M solution of sodium hydroxide in water (461 µl, 461 µmol NaOH) was added to a stirred solution of methyl 2,5-anhydro-3-azido-3-deoxy-**D**-allonate **108** (89 mg, 410 µmol) in 1,4-dioxane (1.6 ml) and water (0.2 ml) at room temperature. After 5 h t.l.c. (ethyl acetate : hexane 7 : 3) indicated the complete conversion of the starting material (R_f 0.3) to baseline products. The solvent was removed *in vacuo* and the residue was subjected to ion exchange chromatography (Amberlite IR-120 (H^+)) to give 2,5-anhydro-3-azido-3-deoxy-**D**-allonic acid **204** which was used without further purification.

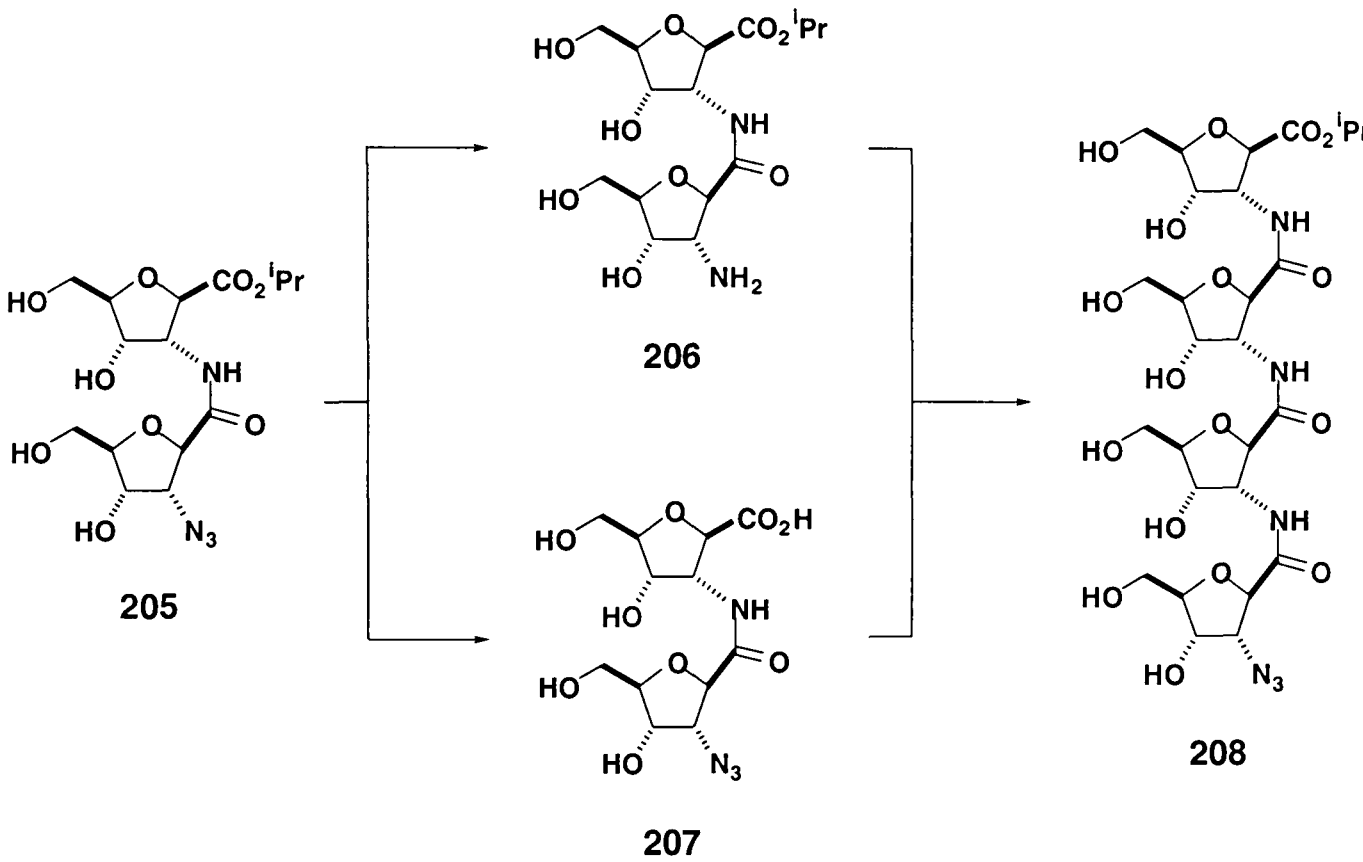
A solution of isopropyl 2,5-anhydro-3-azido-3-deoxy-**D**-allonate **138** (100 mg, 408 μmol) in isopropanol (0.7 ml) was stirred in a hydrogen atmosphere in the presence of palladium black (10 mg). After 5 h, t.l.c. (ethyl acetate : hexane 7 : 3) indicated the complete conversion of the starting material (R_f 0.4) to baseline products. The reaction was degassed, purged with nitrogen, filtered and concentrated *in vacuo* to give isopropyl 3-amino-2,5-anhydro-3-deoxy-**D**-allonate **203** which was used without further purification.

N-Ethyldiisopropylamine (89 μl, 510 μmol) and *O*-benzotriazol-1-yl-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborate (164 mg, 510 μmol) were added to a stirred solution of 2,5-anhydro-3-azido-3-deoxy-**D**-allonic acid **204** (83 mg, 410 μmol) and isopropyl 3-amino-2,5-anhydro-3-deoxy-**D**-allonate **203** (89 g, 408 μmol) in *N*,*N*-dimethylformamide (0.3 ml) at room temperature in a nitrogen atmosphere. After 18 h t.l.c. (5% water in acetonitrile) indicated the complete conversion of baseline material to a major product (R_f 0.5). The solvent was removed *in vacuo*. Purification by flash column chromatography (3.5% water in acetonitrile then 2.5% water in acetonitrile) yielded isopropyl 3-amino-2,5-anhydro-3-deoxy-3-*N*-(2,5-anhydro-3-azido-3-deoxy-**D**-allonyl)-**D**-allonate **205** (142 mg, 86%), a colourless oil; $[\alpha]_D^{24}$ -59.0° (c, 0.99 in methanol); ν_{max} (film) 3370 (br, OH, NH), 2114 (N_3), 1731 (C=O, ester), 1666 (C=O, amide I), 1536 (C=O, amide II) cm^{-1}; δ_H (400 MHz, pyridine-d^5) 1.13, 1.18 (6H, 2 x d, *J* 6.3, $CH(C\underline{H}_3)_2$), 4.05-4.15 (2H, m, H-6B, H-6'B), 4.20 (1H, dd, $J_{6A,6'A}$ 11.9, $J_{6A,5A}$ 2.1, H-6A), 4.30 (1H, dd, $J_{6'A,6A}$ 11.9, $J_{6'A,5A}$ 2.8, H-6'A), 4.58 (1H, a-dt, $J_{5A,4A}$ 7.6, *J* 2.4, H-5A), 4.65 (1H, a-q, *J* 4.3, H-5B), 4.74 (1H, dd, $J_{3A,4A}$ 4.9, $J_{3A,2A}$ 2.5, H-3A), 4.77 (1H, d, $J_{2B,3B}$ 7.1, H-2B), 4.87 (1H, dd, $J_{4B,3B}$ 5.6, $J_{4B,5B}$ 4.2, H-4B), 4.94 (1H, d, $J_{2A,3A}$ 2.5, H-2A), 5.11 (1H, sept, *J* 6.3, $C\underline{H}(CH_3)_2$), 5.28-5.34 (2H, m, H-4A, H-3B); δ_C (100.6 MHz, pyridine-d^5) 21.7, 21.9 (2 x q, $CH(\underline{C}H_3)_2$), 56.6 (d, C-3B), 60.5 (t, C-6A), 63.0 (t, C-6B), 68.2 (d, C-3A), 69.1 (d, $\underline{C}H(CH_2)_3$), 72.0

(d, C-4A), 72.2 (d, C-4B), 80.7 (d, C-2B), 82.3 (d, C-2A), 84.8 (d, C-5A), 88.2 (d, C-5B), 171.8, 171.9 (2 x s, C-1A, C-1B); *m/z* (APCI+) 407 (4%), 406 (17%), 405 (MH^+, 73%), 404 (85%), 363 ($MH^+ - N_3$, 89%), 362 ($MH^+ - {}^iPr$, 100%), 317 (14%), 220 (10%).

Isopropyl 3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(2,5-anhydro-3-azido-3-deoxy-D-allonyl)-3-deoxy-D-allonyl)-3-deoxy-D-allonyl)-3-deoxy-D-allonate

208



A 1 M solution of sodium hydroxide in water (99 µl, 99 µmol NaOH) was added to a stirred solution of isopropyl 3-amino-2,5-anhydro-3-*N*-(2,5-anhydro-3-azido-3-deoxy-**D**-allonyl)-3-deoxy-**D**-allonate **205** (33 mg, 82 µmol) in 1,4-dioxane (35 ml) and water (42 µl) at room temperature. After 5 h t.l.c. (5% water in acetonitrile) indicated the complete conversion of the starting material (R_f 0.5) to baseline products. The solvent was removed *in vacuo* and the residue was subjected to ion exchange

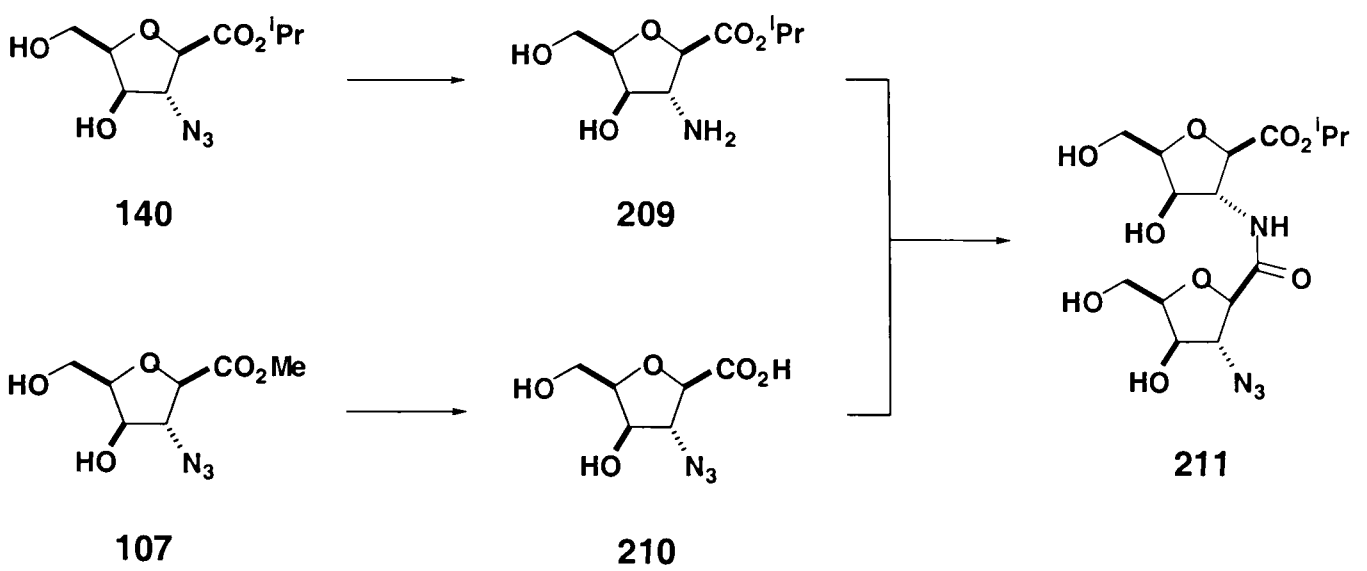
chromatography (Amberlite IR-120 (H^+)) to give 3-amino-2,5-anhydro-3-*N*-(2,5-anhydro-3-azido-3-deoxy-**D**-allonyl)-3-deoxy-**D**-allonic acid **207** which was used without further purification.

A solution of isopropyl 3-amino-2,5-anhydro-3-*N*-(2,5-anhydro-3-azido-3-deoxy-**D**-allonyl)-3-deoxy-**D**-allonate **205** (33 mg, 82 μmol) in water (0.5 ml) was stirred in a hydrogen atmosphere in the presence of palladium black (4 mg). After 5 h, t.l.c. (5% water in acetonitrile) indicated the complete conversion of the starting material (R_f 0.5) to baseline products. The reaction was degassed, purged with nitrogen, filtered and concentrated *in vacuo* to give isopropyl 3-amino-2,5-anhydro-3-*N*-(3-amino-2,5-anhydro-3-deoxy-**D**-allonyl)-3-deoxy-**D**-allonate **206** which was used without further purification.

N-Ethyldiisopropylamine (18 μl, 103 μmol) and *O*-benzotriazol-1-yl-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborate (33 mg, 104 μmol) were added to a stirred solution of 3-amino-2,5-anhydro-3-*N*-(2,5-anhydro-3-azido-3-deoxy-**D**-allonyl)-3-deoxy-**D**-allonic acid **207** (40 mg, 82 μmol) and isopropyl 3-amino-2,5-anhydro-3-*N*-(3-amino-2,5-anhydro-3-deoxy-**D**-allonyl)-3-deoxy-**D**-allonate **206** (31 mg, 82 μmol) in *N*,*N*-dimethylformamide (100 μl) at room temperature in a nitrogen atmosphere. After 5 d t.l.c. (chloroform : methanol : acetic acid : water 60 : 30 : 3 : 5) indicated the formation of a major product (R_f 0.5). The solvent was removed *in vacuo*. Purification by flash column chromatography (chloroform : methanol : acetic acid : water 60 : 30 : 3 : 5) yielded *isopropyl 3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(2,5-anhydro-3-azido-3-deoxy-**D**-allonyl)-3-deoxy-**D**-allonyl)-3-deoxy-**D**-allonyl)-3-deoxy-**D**-allonate* **208** (40 mg, 68%; $[\alpha]_D^{23}$ -62.5° (c, 0.97 in methanol); ν_{max} (film) 3292 (br, OH, NH), 2115 (N_3), 1729 (C=O, ester), 1655 (C=O, amide I), 1533 (C=O, amide II) cm^{-1}; δ_H (400 MHz, pyridine-d^5) 1.09, 1.12 (6H,

2 x d, J 6.3, CH(C<u>H</u>$_3$)$_2$), 3.99-4.14 (6H, m, H-6B, H-6C, H-6D, H-6'B, H-6'C, H-6'D), 4.17 (1H, dd, $J_{6A,6'A}$ 12.3, $J_{6A,5A}$ 1.6, H-6A), 4.27 (1H, dd, $J_{6'A,6A}$ 12.3, $J_{6'A,5A}$ 2.6, H-6'A), 4.52 (1H, a-dt, $J_{5A,4A}$ 8.1, J 2.1, H-5A), 4.55-4.59 (3H, m, H-5B, H-5C, H-5D), 4.67 (1H, dd, $J_{3A,4A}$ 5.0, $J_{3A,2A}$ 1.9, H-3A), 4.70 (1H, d, $J_{2D,3D}$ 8.0, H-2D), 4.77 (1H, dd, $J_{4D,3D}$ 5.6, $J_{4D,5D}$ 3.1, H-4D), 4.84 (1H, d, $J_{2A,3A}$ 1.9, H-2A), 4.86, 4.87 (2H, 2 x d, J 5.7, J 4.0, H-2B, H-2C), 4.99 (1H, a-t, J 5.6, H-4C), 5.06 (1H, sept, J 6.3, C<u>H</u>(CH$_3$)$_2$), 5.12 (1H, a-t, J 6.0, H-4B), 5.18 (1H, a-dt, J 8.2, $J_{3D,4D}$ 5.6, H-3D), 5.28 (1H, ddd, $J_{3C,NH-C}$ 8.5, $J_{3C,4C}$ 5.6, $J_{3C,2C}$ 4.0, H-3C), 5.33-5.39 (2H, m, H-3B, H-4A), 9.00 (1H, d, $J_{NH-D,3D}$ 8.7, N<u>H</u>-D), 9.26 (1H, d, $J_{NH-C,3C}$ 8.5, N<u>H</u>-C), 9.34 (1H, d, $J_{NH-B,3B}$ 8.8, N<u>H</u>-B); δ_C (100.6 MHz, pyridine-d^5) 22.2, 22.2 (2 x q, CH(C<u>H</u>$_3$)$_2$), 57.1 (d, C-3D), 58.3, 58.4 (2 x d, C-3C, C-3B), 60.7, 61.7, 62.3, 63.7 (4 x t, C-6A, C-6B, C-6C, C-6D), 69.1 (d, C-3A), 69.8 (d, <u>C</u>H(CH$_3$)$_2$), 71.2 (d, C-4B), 71.7 (d, C-4C), 72.3 (d, C-4A), 72.9 (d, C-4D), 81.1 (d, C-2D), 83.1, 83.2, 83.6 (3 x d, C-2A, C-2C, C-2B), 84.9 (d, C-5A), 86.6, 87.4, 89.3 (3 x d, C-5B, C-5C, C-5D), 172.6, 172.9, 173.8, 174.2 (4 x s, C-1A, C-1B, C-1C, C-1D); *m/z* (APCI+) 723 (MH$^+$, 16%), 632 (22%), 361 (60%), 178 (100%).

***Isopropyl 3-amino-2,5-anhydro-3-N-(2,5-anhydro-3-azido-3-deoxy-D-gulonyl)-3-deoxy-D-gulonate* 211**



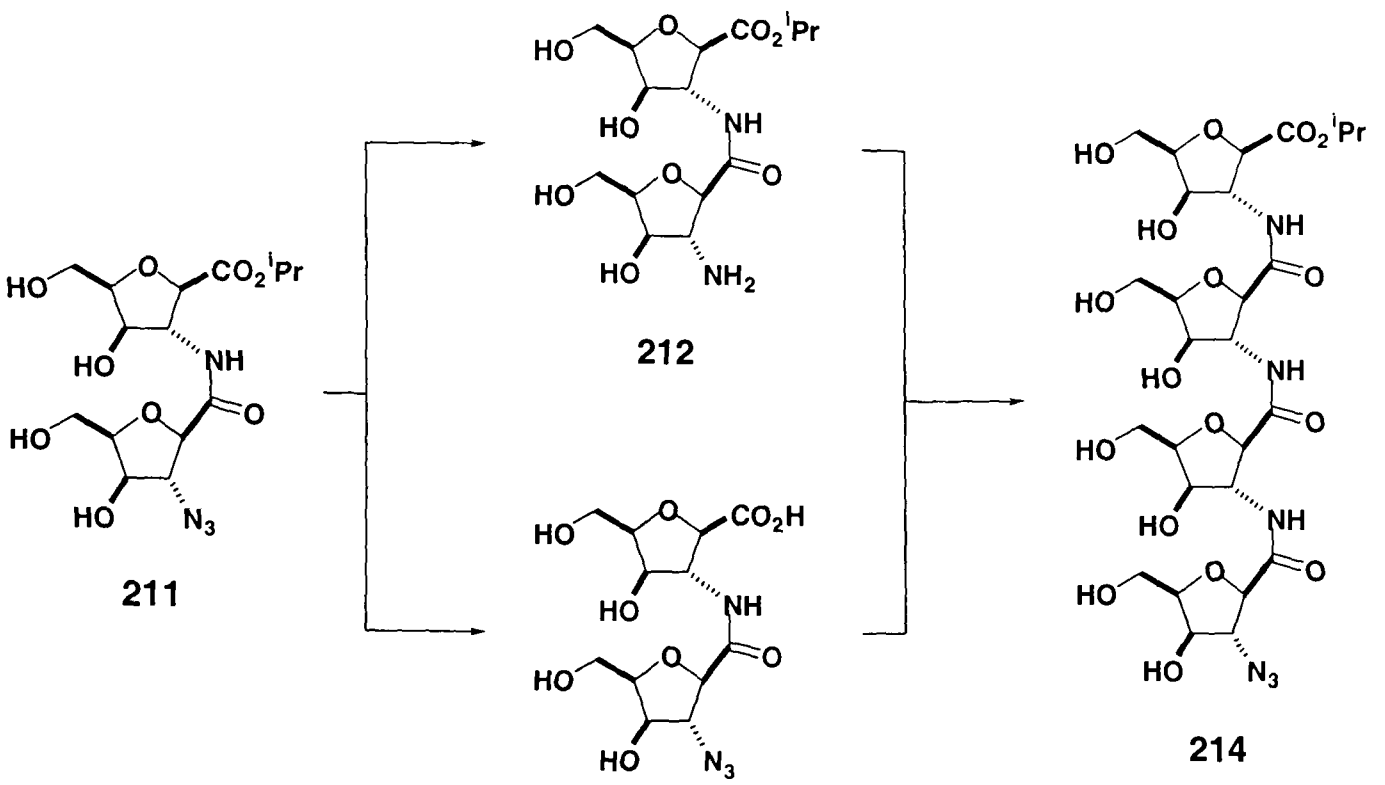
A 1 M solution of sodium hydroxide in water (461 µl, 461 µmol NaOH) was added to a stirred solution of methyl 2,5-anhydro-3-azido-3-deoxy-**D**-gulonate **107** (89 mg, 410 µmol) in 1,4-dioxane (1.6 ml) and water (0.2 ml) at room temperature. After 5 h t.l.c. (ethyl acetate : hexane 7 : 3) indicated the complete conversion of the starting material (R_f 0.3) to baseline products. The solvent was removed *in vacuo* and the residue was subjected to ion exchange chromatography (Amberlite IR-120 (H^+)) to give 2,5-anhydro-3-azido-3-deoxy-**D**-gulonic acid **210** which was used without further purification.

A solution of isopropyl 2,5-anhydro-3-azido-3-deoxy-**D**-gulonate **140** (100 mg, 408 µmol) in isopropanol (0.7 ml) was stirred in a hydrogen atmosphere in the presence of palladium black (10 mg). After 5 h, t.l.c. (ethyl acetate : hexane 7 : 3) indicated the complete conversion of the starting material (R_f 0.4) to baseline products. The reaction was degassed, purged with nitrogen, filtered and concentrated *in vacuo* to give isopropyl 3-amino-2,5-anhydro-3-deoxy-**D**-gulonate **209** which was used without further purification.

N-Ethyldiisopropylamine (89 µl, 510 µmol) and *O*-benzotriazol-1-yl-*N*,*N*,*N'*,*N'*-tetramethyluronium tetrafluoroborate (164 mg, 510 µmol) were added to a stirred solution of 2,5-anhydro-3-azido-3-deoxy-**D**-gulonic acid **210** (83 mg, 410 µmol) and isopropyl 3-amino-2,5-anhydro-3-deoxy-**D**-gulonate **209** (89 mg, 408 µmol) in *N*,*N*-dimethylformamide (0.3 ml) at room temperature in a nitrogen atmosphere. After 18 h t.l.c. (5% water in acetonitrile) indicated the complete conversion of baseline material to a major product (R_f 0.5). The solvent was removed *in vacuo*. Purification by flash column chromatography (3.5% water in acetonitrile then 2.5% water in acetonitrile) yielded isopropyl 3-amino-2,5-anhydro-3-deoxy-3-*N*-(2,5-anhydro-3-azido-3-deoxy-**D**-gulonyl)-**D**-gulonate **211** (128 mg, 78%), a colourless oil; $[\alpha]_D^{24}$ -71.8° (c, 0.97 in methanol); ν_{max} (film) 3332 (br, OH, NH), 2117 (N_3), 1732 (C=O, ester), 1663 (C=O, amide I), 1547 (C=O, amide II) cm^{-1}; δ_H (400 MHz, pyridine-d^5) 1.17, 1.21 (6H, 2 x d, *J* 6.3, $CH(C\underline{H}_3)_2$), 4.07 (1H, dd, $J_{6B,6'B}$ 11.9, $J_{6B,5B}$ 3.1, H-6B), 4.16 (1H, dd, $J_{6'B,6B}$ 11.9, $J_{6'B,5B}$ 2.8, H-6'B), 4.25 (1H, dd, $J_{6A,6'A}$ 11.7, $J_{6A,5A}$ 2.5, H-6A), 4.33 (1H, dd, $J_{6'A,6A}$ 11.7, $J_{6'A,5A}$ 2.8, H-6'A), 4.55 (1H, a-dt, $J_{5B,4B}$ 4.8, *J* 3.0, H-5B), 4.63 (1H, a-dt, $J_{5A,4A}$ 7.2, *J* 2.6, H-5A), 4.70-4.78 (2H, m, H-3A, H-4B), 4.84 (1H, d, $J_{2B,3B}$ 7.2, H-2B), 4.92 (1H, d, $J_{2A,3A}$ 2.7, H-2A), 5.09 (1H, dd, $J_{4A,5A}$ 7.2, $J_{4A,3A}$ 5.1, H-4A), 5.21 (1H, sept, *J* 6.3, $C\underline{H}(CH_3)_2$), 5.30 (1H, a-t, *J* 6.9, H-3B); δ_C (100.6 MHz, pyridine-d^5) 22.3, 22.4 (2 x q, $CH(\underline{C}H_3)_2$), 55.9 (d, C-3B), 60.4 (t, C-6A), 62.7 (t, C-6B), 68.0 (d, C-3A), 69.1 (d, $\underline{C}H(CH_2)_3$), 71.9 (d, C-4B), 72.3 (d, C-4A), 82.2 (d, C-2B), 81.3 (d, C-2A), 84.7 (d, C-5A), 89.0 (d, C-5B), 171.9, 172.4 (2 x s, C-1A, C-1B); *m/z* (APCI+) 406 (10%), 405 (MH^+, 56%), 404 (65%), 363 ($MH^+ - N_3$, 100%), 220 (30%).

Isopropyl 3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(2,5-anhydro-3-azido-3-deoxy-D-gulonyl)-3-deoxy-D-gulonyl)-3-deoxy-D-gulonyl)-3-deoxy-D-gulonate

214



213

A 1 M solution of sodium hydroxide in water (99 µl, 99 µmol NaOH) was added to a stirred solution of isopropyl 3-amino-2,5-anhydro-3-*N*-(2,5-anhydro-3-azido-3-deoxy-**D**-gulonyl)-3-deoxy-**D**-gulonate **211** (33 mg, 82 µmol) in 1,4-dioxane (35 ml) and water (42 µl) at room temperature. After 5 h t.l.c. (5% water in acetonitrile) indicated the complete conversion of the starting material (R_f 0.5) to baseline products. The solvent was removed *in vacuo* and the residue was subjected to ion exchange chromatography (Amberlite IR-120 (H^+)) to give 3-amino-2,5-anhydro-3-*N*-(2,5-anhydro-3-azido-3-deoxy-**D**-gulonyl)-3-deoxy-**D**-gulonic acid **213** which was used without further purification.

A solution of isopropyl 3-amino-2,5-anhydro-3-*N*-(2,5-anhydro-3-azido-3-deoxy-**D**-gulonyl)-3-deoxy-**D**-gulonate **211** (33 mg, 82 µmol) in water (0.5 ml) was stirred in a hydrogen atmosphere in

the presence of palladium black (4 mg). After 5 h, t.l.c. (5% water in acetonitrile) indicated the complete conversion of the starting material (R_f 0.5) to baseline products. The reaction was degassed, purged with nitrogen, filtered and concentrated *in vacuo* to give isopropyl 3-amino-2,5-anhydro-3-*N*-(3-amino-2,5-anhydro-3-deoxy-**D**-gulonyl)-3-deoxy-**D**-gulonate **212** which was used without further purification.

N-Ethyldiisopropylamine (18 µl, 103 µmol) and *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (33 mg, 104 µmol) were added to a stirred solution of 3-amino-2,5-anhydro-3-*N*-(2,5-anhydro-3-azido-3-deoxy-**D**-gulonyl)-3-deoxy-**D**-gulonic acid **213** (40 mg, 82 µmol) and isopropyl 3-amino-2,5-anhydro-3-*N*-(3-amino-2,5-anhydro-3-deoxy-**D**-gulonyl)-3-deoxy-**D**-gulonate **212** (31 mg, 82 µmol) in *N*,*N*-dimethylformamide (100 µl) at room temperature in a nitrogen atmosphere. After 5 d t.l.c. (chloroform : methanol : acetic acid : water 60 : 30 : 3 : 5) indicated the formation of a major product (R_f 0.4). The solvent was removed *in vacuo*. Purification by flash column chromatography (chloroform : methanol : acetic acid : water 60 : 30 : 3 : 5) yielded *isopropyl 3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(3-amino-2,5-anhydro-3-N-(2,5-anhydro-3-azido-3-deoxy-**D**-gulonyl)-3-deoxy-**D**-gulonyl)-3-deoxy-**D**-gulonyl)-3-deoxy-**D**-gulonate* **214** (34 mg, 57%), a colourless oil; $[\alpha]_D^{24}$ -68.1° (c, 0.99 in methanol); ν_{max} (film) 3373 (br, OH, NH), 2114 (N_3), 1726 (C=O, ester), 1656 (C=O, amide I), 1535 (C=O, amide II) cm^{-1}; δ_H (400 MHz, pyridine-d^5) 1.18, 1.23 (6H, 2 x d, *J* 6.3, $CH(C\underline{H}_3)_2$), 4.28-4.42 (8H, m, H-6A, H-6B, H-6C, H-6D, H-6'A, H-6'B, H-6'C, H-6'D), 4.54-4.61 (2H, m, H-3A, H-5B), 4.65 (1H, a-q, *J* 4.6, H-5C), 4.68 (1H, a-dt, *J* 5.9, *J* 3.8, H-5A), 4.78 (1H, a-q, *J* 4.4, H-5D), 4.82 (1H, d, $J_{2D,3D}$ 7.9, H-2D), 4.83 (1H, m, H-4A), 4.85-4.90 (3H, m, H-4B, H-4C, H-4D), 4.92 (1H, d, $J_{2B,3B}$ 7.3, H-2B), 4.96 (1H, d, $J_{2C,3C}$ 7.9, H-2C), 5.01 (1H, d, $J_{2A,3A}$ 7.6, H-2A), 5.16 (1H, sept, *J* 6.3, $C\underline{H}(CH_3)_2$), 5.28 (1H, a-dt, *J* 8.2, $J_{3C,4C}$ 5.1,

H-3C), 5.33 (1H, a-dt, *J* 8.4, $J_{3D,4D}$ 5.1, H-3D), 5.37 (1H, ddd, $J_{3B,NH-B}$ 8.7, $J_{3B,2B}$ 7.3, $J_{3B,4B}$ 5.6, H-3B), 8.76 (1H, d, $J_{NH-D,3D}$ 8.8, N<u>H</u>-D), 9.06 (1H, d, $J_{NH-B,3B}$ 8.7, N<u>H</u>-B), 9.14 (1H, d, $J_{NH-C,3C}$ 8.6, N<u>H</u>-C); δ_C (100.6 MHz, pyridine-d^5) 22.4, 22.6 (2 x q, CH(C<u>H</u>$_3$)$_2$), 58.4, 58.5, 58.9 (3 x d, C-3B, C-3C, C-3D), 61.4, 61.6, 61.7, 61.9 (4 x t, C-6A, C-6B, C-6C, C-6D), 68.3 (d, C-3A), 69.1 (d, <u>C</u>H(CH$_3$)$_2$), 71.6, 71.9, 72.0 (3 x d, C-4B, C-4C, C-4D), 73.5 (d, C-4A), 78.8 (d, C-2A), 80.4 (d, C-2D), 81.5 (d, C-2C), 81.9 (d, C-2B), 84.4, 84.7, 84.8, 85.2 (4 x d, C-5A, C-5B, C-5C, C-5D), 172.0, 172.4, 172.7, 172.9 (4 x s, C-1A, C-1B, C-1C, C-1D); *m/z* (APCI+) 723 (MH^+, 11%), 632 (36%), 361 (58%), 360 (76%), 178 (100%).

6.4 References

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“Trapped in a building
With the ghosts of all the people I betrayed,
Say what you want…

And did I choose to be
The one who is left?
Or is this something sinister?
Or is this proof against my ignorance?
Or am I meant to waste away?”

unbelievable truth