The Synthesis of Azasugars

Using an I_2-mediated Carbamate Annulation

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Abstract

The biological activity of azasugars has largely been attributed to their ability to mimic the oxocarbenium ion-like transition state formed during reactions with carbohydrate-processing enzymes and, for this reason, functional and stereochemical modifications of the azasugar scaffold have led to the development of specific and potent glycosidase inhibitors. Given the potential of azasugars as glycosidase inhibitors, we were interested in developing efficient methodology for their synthesis.

This thesis highlights synthetic methodology developed to produce amino-imino-hexitols as azasugar scaffolds. Key in the synthesis of the amino-imino-hexitols was the application of a stereoselective Strecker reaction, without the need for chiral Lewis acids or catalysts, and an extension of an I_2-mediated carbamate annulation to cyclise functionalised and protected alkenylamines. Sixteen amino-imino-hexitols were synthesized, including ten previously undisclosed substrates with the D-*galacto*, D-*talo*, and L-*altro* configurations. The novel amino-imino-hexitols were then tested for their ability to act as glycosidase inhibitors and substrates of the D-*talo* configuration showed promising inhibitory effects.

Mechanistic considerations of the I_2-mediated carbamate annulation are discussed and although the exact annulation mechanism has yet to be determined, experimental studies have revealed that an aziridine is not an intermediate in the reaction. Factors influencing the diastereoselectivity of the carbamate annulation are also explored. Furthermore, an in depth analysis of the high *cis*-selectivity of the carbamate annulation is investigated using density functional theory to calculate the transition states of iodocyclisations *en route* to the formation of carbamates. Taken as a whole, the applicability of the

carbamate annulation to a variety of alkenylamines and an understanding of the factors controlling the diastereoselectivity of the reaction should make this methodology a valuable addition to the synthetic chemist’s toolbox.

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Contents

List of Abbreviations

ABG	β-D-glucosidase (*Agrobacterium* sp.)
Ac	acetyl
AcOH	acetic acid
aq.	aqueous
ASSC	active-site-specific chaperone
Ar	aryl
B3LYP	Becke, three-parameter, Lee-Yang-Parr
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
br	broad
Bz	benzoyl
ca.	approximately
calcd.	calculated
conc.	concentrated
COSY	correlation NMR spectroscopy
d	days
d	doublet
δ	chemical shift
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DBAD	dibenzyl azodicarboxylate
DCM	dichloromethane
dd	doublet of doublets
ddd	doublet of doublet of doublets
DFT	density functional theory
DGJ	deoxygalactonojirimycin
DMDP	2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
DNJ	deoxynojirimycin
DOWEX-H^+	acidic ion-exchange resin
DOWEX-OH^-	basic ion-exchange resin
dr	diastereomeric ratio
dt	doublet of triplets
ee	enantiomeric excess
equiv.	equivalents
ESI	electro-spray ionisation
ERT	enzyme replacement therapy
Et	ethyl

FDP	fructose 1,6-diphosphate
h	hours
HBTU	*O*-Benzotriazole-*N*,*N*,*N'*,*N'*-tetramethyl-uronium-hexafluoro-phosphate
HMBC	heteronuclear multiple bond correlation
HMPA	hexamethylphosphoric triamide
HRMS	high resolution mass spectroscopy
HSQC	heteronuclear single quantum coherence
Hz	hertz
IC_{50}	half maximal inhibitory concentration
Imid.	imidazole
*i*Pr	*iso*-propyl
IR	infrared spectroscopy
IRC	intrinsic reaction coordinate
J	coupling constant
GCase	β-D-glucocerebrosidase
K_i	inhibition dissociation constant
KIE	kinetic isotope effect
LANL2DZ	Los Alamos National Laboratory 2-double-zeta
M	molar
m	multiplet
Me	methyl
MHz	mega Hertz
min.	minutes
MOM	methoxymethyl
MP	melting point
Ms	methanesulfonyl
m/z	mass to charge ratio
n-Bu	*normal*-butyl
NBO	natural bond order
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
NMR	nuclear magnetic resonance
obsd.	observed
PMB	*para*-methoxybenzyl
Ph	phenyl
ppm	parts per million
quant.	quantitative
ref.	reference
rds	rate determining step

R_f	retention factor
rt	room temperature
s	singlet
sat.	saturated
SM	starting material
*Sp*HEX	*N*-acetyl-β-D-hexosaminidase (*Streptomyces plicatus*)
SRT	substrate reduction therapy
t	triplet
TBAF	tetrabutylammoniumflouride
TBDPS	*tert*-butyldiphenylsilyl
*t*Bu	*tert*-butyl
TBS	*tert*-butyldimethylsilyl
TES	triethylsilane
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromotography
TMS	trimethylsilyl
TS	transition state
Tr	triphenylmethyl

Chapter 1

Introduction

1.1 General Introduction

Azasugars, or iminosugars, are structural analogues of native carbohydrates in which the ring oxygen has been replaced by a nitrogen. These sugar mimics are a valuable class of compounds that play an important role in drug development, primarily due to their ability to mimic the charge density in the transition state of glycosidic hydrolase enzymes.[1–3] Other biological activities of azasugars have also been noted, such as their ability to act as molecular chaperones,[4–7] as immunomodulators,[8] or as inhibitors of other enzymes and proteins.[9,10]

Azasugars sparked scientific interest in the early 1960's with the almost simultaneous reports of the syntheses of "piperidinoses", sugar derivatives containing a nitrogen atom in the ring, by the groups of Jones,[11,12] Paulsen,[13] and Hanessian.[14] Shortly thereafter, the first azasugar, nojirimycin (**1**), was isolated from *Streptomyces reseochromogenes*

and found to possess anti-microbial activity.[15] Its 1-deoxy analogue deoxynojirimycin (DNJ, **2**), initially prepared by Paulsen *et al.*,[16] was later isolated from Mulberry trees[17] and shown to be a potent α-glycosidase inhibitor.[18] The D-*gluco* chiral scaffold of the nojirimycin family has subsequently played an important role in the development of clinical drugs, such as Glyset® for non-insulin dependent diabetes (**3**),[19] and Zavesca® (**4**, Fig. 1.3), for the control of Gaucher disease. 1-Deoxygalactonojirimycin (DGJ, **5**), currently in clinical trials, has shown great promise in the treatment of Fabry's disease.[20] These nojirimycin carbohydrate mimics represent just one of the many types of azasugar that have been isolated, synthesised, and their biological activity explored in an effort to find therapeutic activities.[21–28] In addition to the aforementioned nojirimycin derivatives (**1**-**5**), which belong to the piperidine (6-membered ring) class of azasugars, other structural classes of azasugars include the pyrrolidines (5-membered) [e.g. 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine **6**, (DMDP, R = H)],[8,29,30] the pyrrolizidines (1,2-fused 5-5-membered bicyclic rings), [e.g. casuarine (**7**)],[31] the indolizidines (1,2-fused 5-6-membered bicyclic rings) [e.g. swainsonine (**8**)],[32] and the nortropanes (2,6-bridged 5-6-membered bicyclic rings) [e.g. calystegine A_3 (**9**)].[33]

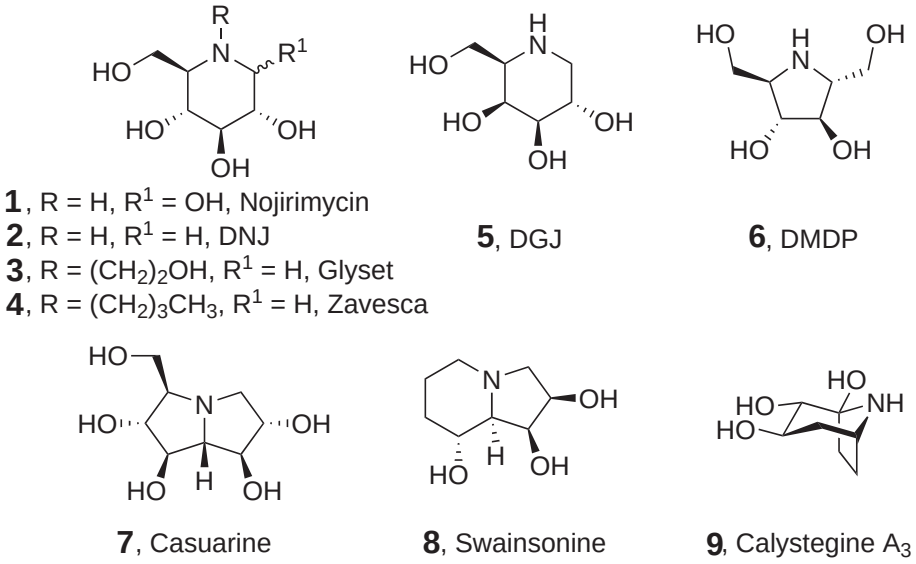
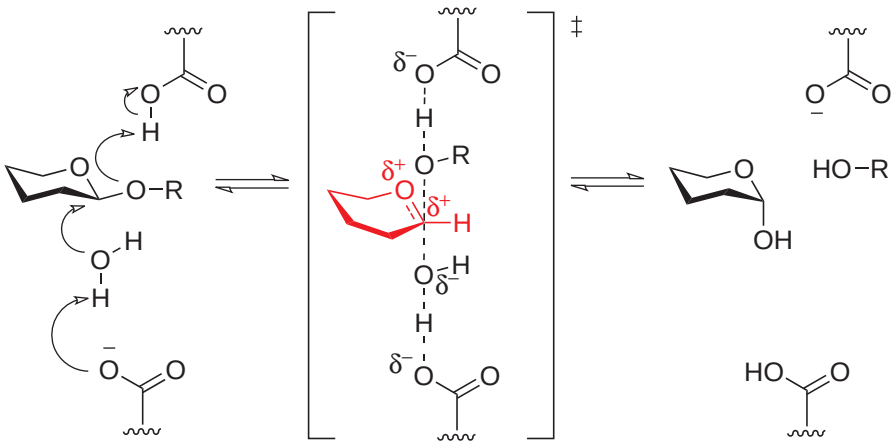
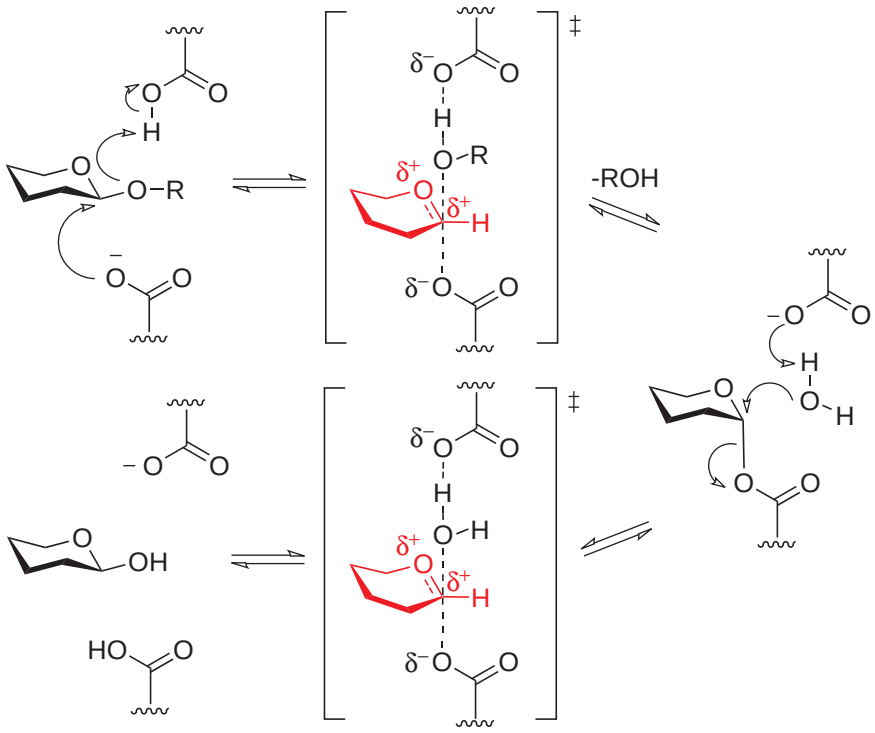


Fig. 1.1 Classes of azasugars

Glycosidases are carbohydrate processing enzymes that catalyse the degradation of glycosidic linkages to break down carbohydrate chains into smaller sugar units. There are two general mechanisms by which glycosidases hydrolyse a glycosidic bond, with either inversion or net retention of anomeric configuration.[34,35] An inverting glycosidase follows a one step mechanism ((a), Fig. 1.2) in which both a water molecule and the substrate are bound by the enzyme. In the active site of the glycosidase, acid and base residues catalyse a nucleophilic substitution by water via the generation of an oxocarbenium ion-like transition state to produce the inverted hemiacetal. A retaining glycosidase follows a double displacement mechanism (b) catalysed by both nucleophilic and acid/base residues in the active site. Here, the substrate is attacked by a nucleophilic residue to displace the leaving group via the generation of an oxocarbenium ion-like transition state, which leads to formation of a covalent glycosyl-enzyme intermediate.



(a) **Inversion**



(b) **Retention**

(c)

Fig. 1.2 Prototypical glycosidase mechanisms for hydrolysis, (a) inversion of configuration, (b) retention of configuration, (c) protonated piperidines and pyrrolidines can act as transition state inhibitors

Nucleophilic attack by water at the anomeric carbon of the bound substrate then displaces the glycoside via a second oxocarbenium ion-like transition state, leading to retention of configuration. The ability of azasugars to inhibit glycosidases is illustrated with both a piperidine and pyrrolidine (c). Here, the nitrogen atom of the azasugar is protonated at physiological pH and it is this charged species that mimics the developing charge density of the transition state during glycosidic hydrolysis.[36] The adoption of a chair conformation by the piperidine and envelope conformation by the pyrrolidine allow both classes of azasugar to resemble the structure and electronics of the glycosyl cation formed during the reaction.[23]

1.1.1 Evaluation of Glycosidase Inhibition

There are many classes of glycosidase enzymes[37] and the inhibition of each specific class can have potential in the treatment of different diseases.[35] For example, the inhibition of α-mannosidases has potential for the treatment of cancer,[38] the inhibition of α-amylases can be a treatment for type II diabetes,[19] and the inhibition of α-galactosidases has the potential to treat lysosomal storage disorders.[39] Two types of measurements are used predominantly to determine the glycosidase inhibitory activity of azasugars.[40] For both measurements, assays comprise a source of purified enzyme and its native substrate, along with the inhibitor being evaluated. The inhibition constant, K_i, denotes the equilibrium constant of the dissociation of the inhibitor-bound glycosidase complex. A second measurement, the IC_{50} value, quantifies the concentration of inhibitor necessary to halve the reaction rate of a glycosidase-catalysed reaction observed under specific assay conditions. For both the IC_{50} and K_i, a smaller value denotes stronger inhibition. Typically IC_{50} values are reported as they are less time consuming to measure and

require only one concentration of substrate over a range of inhibitor concentrations. The IC_{50} value, however, is relative to the concentration of native substrate used and can therefore vary across different assays. In comparison, determining the K_i involves measuring the rates of the glycosidase-catalysed reactions while independently varying the concentration of substrate and the concentration of inhibitor. These assays require more experiments and are more time consuming to perform, however, the K_i measured is a constant value for any given inhibitor with a particular glycosidase.[40]

1.2 Amino-imino-hexitols as Glycosidase Inhibitors

The focus of this thesis concerns the synthesis of amino-imino-hexitols and their ability to act as glycosidase inhibitors. Of particular note is the pioneering work of Wong *et al.* who observed that derivatisation of the naturally occurring 2,5-dihydroxymethyl-3,4-dihydroxy-pyrrolidine (DMDP, **6**, Fig. 1.1) to the 1-amino-1-deoxy-DMDP analogue **10** (Fig. 1.3) resulted in the generation of a compound with pronounced inhibitory activity against *N*-acetyl-β-D-glucosaminidase.[41] The subsequent synthesis of a library of *N*-functionalised DMDP analogues[42–44] then led to the identification of potential drug candidates for osteoarthritis[45,46] and bacterial infection.[47] Moreover, the *N*-methyl amino-hexitol **11** was found to be the most promising drug candidate with the strongest inhibitory activity against hexosaminidase (K_i = 24 nm).[46]

The groups of Stütz and Wrodnigg have also developed a series of functionalised amino-imino-hexitols. Here, the Amadori rearrangement formed a key part of their synthetic strategy.[48–53] Stütz and Wrodnigg found that the one-step *N*-derivatisation of amine-derivative (**12**) led to the generation of powerful β-glycosidase inhibitors,

while amino imino-hexitol **12** itself was a moderate β-glucosidase inhibitor.[48,49,52] More recently, Ramesh and co-workers prepared several selective glycosidase inhibitors based on modifications to the L-*ido* azasugar **13**.[54] While the parent compound showed no glycosidase inhibitory activity, *N*-tosyl, *N*-methyl, and *N*-ethyl derivatives all exhibited good, and often selective, activity against the glycosidases tested. An *N*-acyl derivative of imino-D-glucitol **14** was also found to exhibit potent and selective activity against β-glucosidase while the parent compound was inactive.[55]

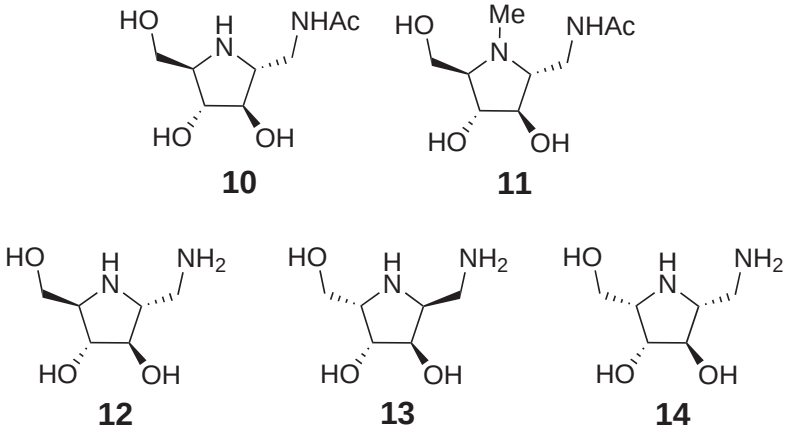


Fig. 1.3 Representative amino-imino-hexitols

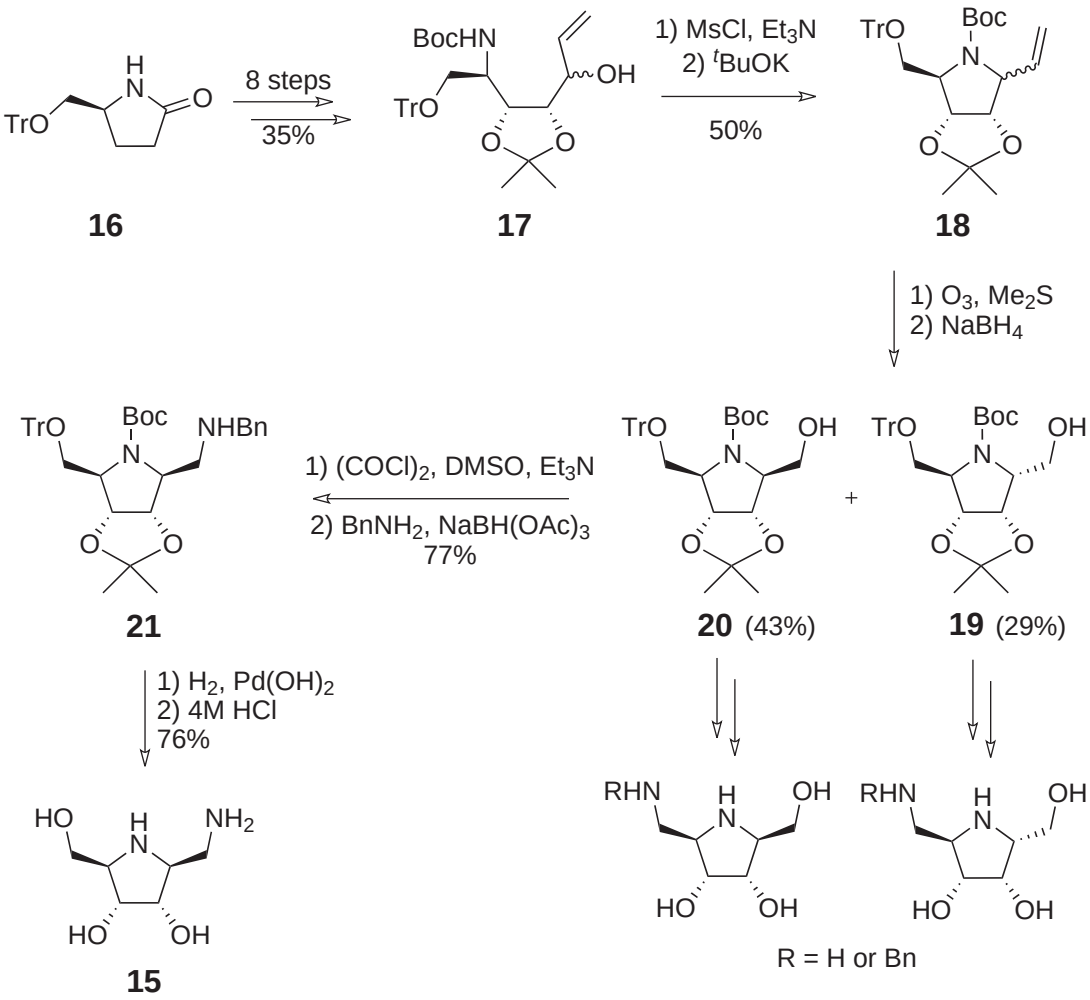
1.2.1 Synthetic Routes to Amino-imino-hexitols

A number of routes have been developed for the synthesis of amino-imino-hexitols. These utilise a variety of key synthetic transformations including dihydroxylation,[56] reductive aminations,[41,48,56] substitution reactions,[54,56–58] enzymatic synthesis,[41] nucleophilic additions,[42,57,58] and rearrangements.[48,49] A few synthetic routes are summarised below.

Substitution Reaction

In 2004, Vogel and co-workers[56] developed a synthesis of amino-imino-hexitols based on the use of a lactam scaffold derived from (*S*)-pyroglutamic acid. The synthesis of one such derivative, the L-*allo* configured hexitol (**15**), is illustrated (Scheme 1.1). Key steps in the synthesis included a nucleophilic substitution to form the imino-hexitol ring, and reductive amination to install the second amine. Following the procedure reported by Ikota and co-workers,[59] lactam derivative **16** was converted into the allylic alcohol **17**, obtained as a mixture of inseparable diastereomers. Mesylation of the secondary alcohol **17** followed by treatment with *t*BuOK induced cyclisation to give pyrrolidine **18**. Subsequent ozonolysis and reduction of the corresponding aldehyde then provided key imino-hexitol intermediates, **19** and **20**, in moderate yields. The major pyrrolidine isomer **20** was subjected to a Swern oxidation, and subsequent reductive amination with benzylamine to generate protected amine **21**. Global deprotection then afforded amino-imino-hexitol **15** produced in 16 steps and 5% overall yield. While the route to L-*allo* imino-hexitol **15** was not high yielding, Vogel was able to synthesise six amino-imino-hexitol derivatives with the D- and L-*allo* and D-*altro* configurations, by extending

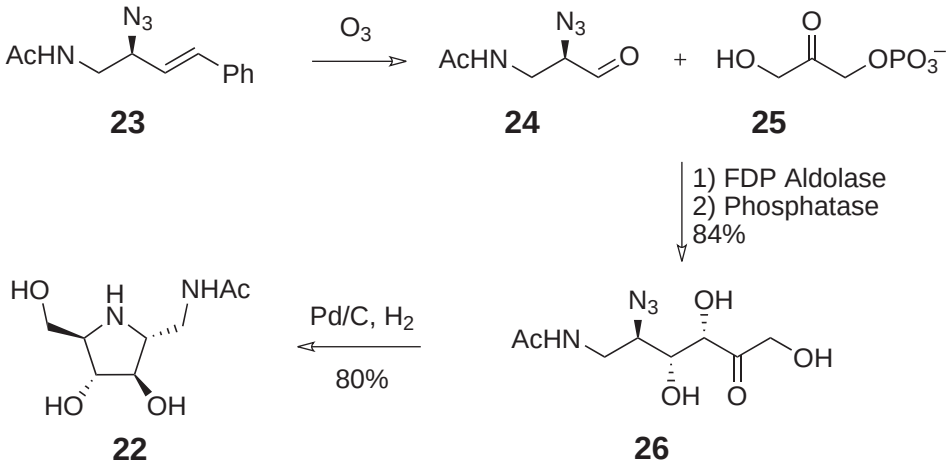
the route with either pyrrolidine isomer (**19** or **20**) and altering the protection strategy. The synthesised amino-hexitols were tested against a panel of glycosidases for their inhibition activity. A D-*altro* derivative was found to be a good broad ranging inhibitor against five classes of glycosidases assayed, whereas amino-hexitol **15** was found to be a selective moderate β-glucosidase inhibitor.



Scheme 1.1 Synthesis of amino-imino-hexitols from an (*S*)-pyroglutamic acid derivative

Enzymatic Synthesis

In 1993, Wong and co-workers[41] utilised an enzyme catalysed aldol condensation as a key step for the synthesis of *N*-acetyl amino-imino-hexitol **22** (Scheme 1.2). Here, azido alkene **23** underwent ozonolysis to produce aldehyde **24**. Condensation of acetamido propanal **24** with dihydroxyacetone phosphate **25** in the presence of fructose 1,6-diphosphate aldolase (FDP-aldolase), followed by removal of the phosphate ester by phosphatase produced aldol product **26** in excellent (84%) yield. Subsequent hydrogenation of azido hydroxyketone **26** allowed cyclisation upon reductive amination to give D-*manno* acetamide **22**, which was found to be a potent inhibitor of *N*-acetyl-β-D-glucosaminidase (Jack beans, K_i = 1.9 μM).

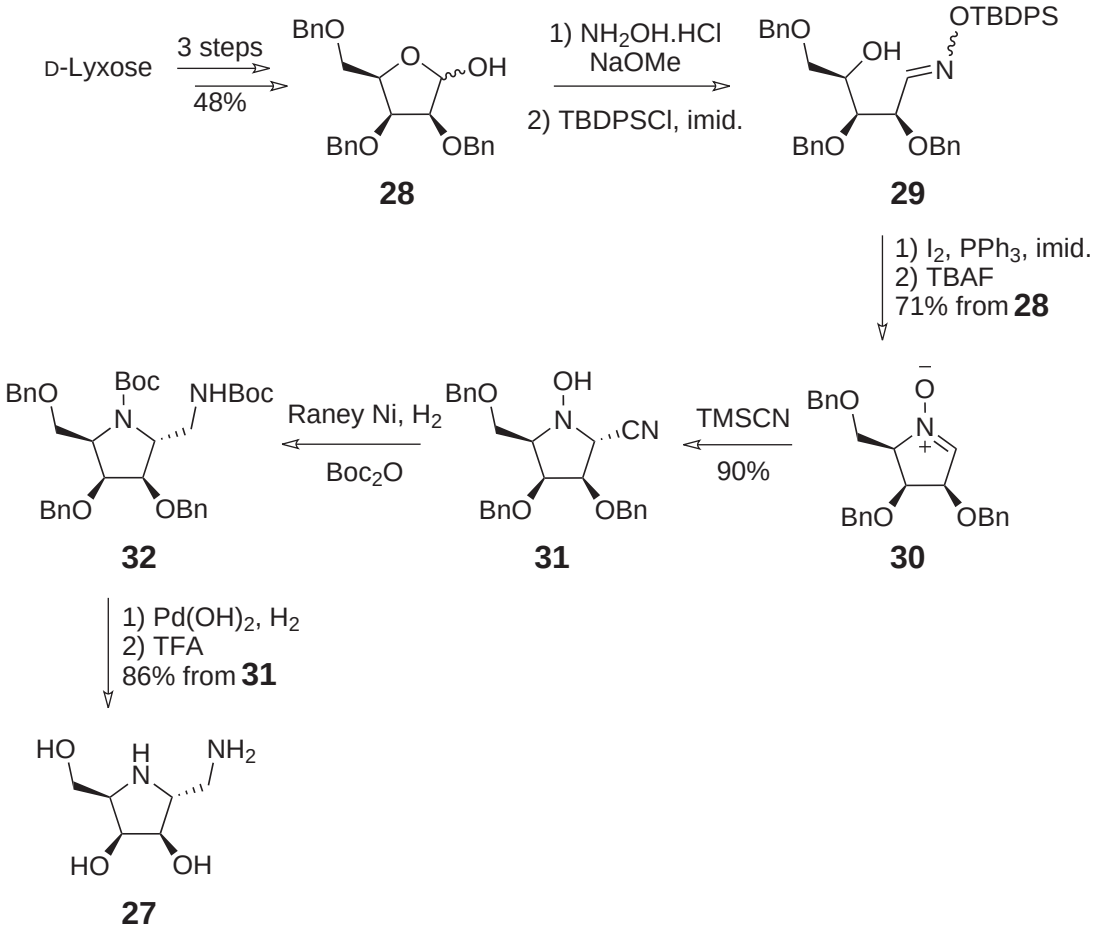


Scheme 1.2 Enzyme catalysed aldol condensation key in the synthesis of an amino-imino-hexitol

Nucleophilic additions

Another approach to the synthesis of amino-imino-hexitols was reported in 2009 by Cheng and co-workers.[58] Here, the nucleophilic addition of cyanide to a cyclic nitrone

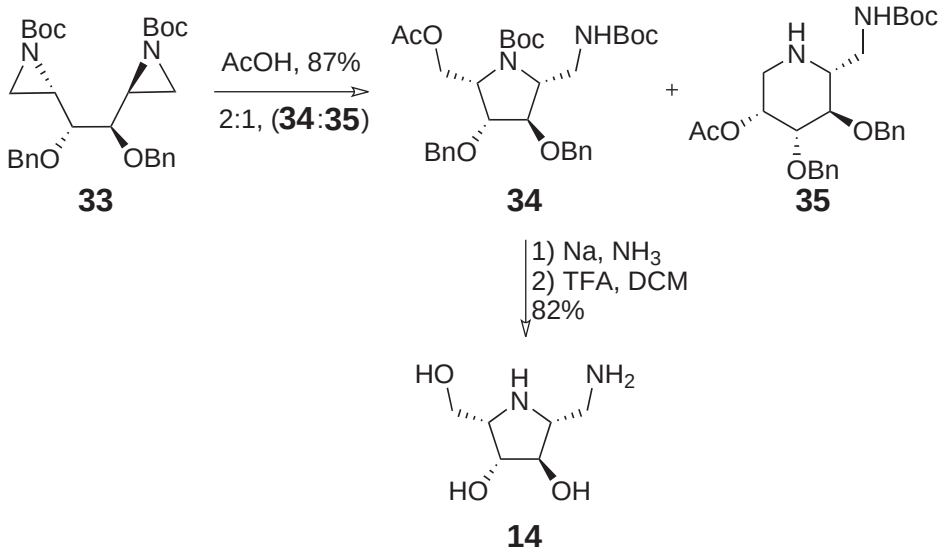
intermediate formed a key step in the synthesis. A representative synthesis of the D-*altro* amino-imino-hexitol **27** is illustrated (Scheme 1.3). In this strategy, D-lyxose was converted to tri-*O*-benzyl derivative **28** via the methyl furanoside followed by per-benzylation and hydrolysis. Tri-*O*-benzyl derivative **28** was then treated with hydroxylamine hydrochloride and the resultant oxime subsequently protected with TBDPSCl to provide a mixture of *E*/*Z*-oximes **29**. Iodination of **29** followed by intramolecular nucleophilic displacement in the presence of anhydrous TBAF provided the key intermediate nitrone **30** in 71% yield from lactol **28**. Nitrone **30** then underwent a stereoselective cyanation to produce nitrile **31** in a diastereomeric ratio of 19:1. The nitrile in **31** was reduced using Raney Ni/H_2 in the presence of Boc_2O to give the bis-*N*-Boc protected diamine **32**. Subsequent hydrogenolysis and Boc removal of **32** afforded the desired D-*altro* amino-imino-hexitol **27**, in 11 steps from D-lyxose and 27% overall yield. D-*Altro* hexitol **27** proved to be a moderately selective α-mannosidase inhibitor (Jack beans, IC_{50} = 32 μM). This synthetic route was also applied to the synthesis of amino-imino-hexitols with the L-*allo*, D-*manno* and L-*gulo*, L-*manno*, and D-*allo*, configurations, which was achieved by starting from D-ribose, D-arabinose, D-xylose and D-lyxose, respectively.



Scheme 1.3 Cyclic nitrones key in the synthesis of amino-imino-hexitols

McCort and Duréalt reported[55] the nucleophilic ring opening of a bis-aziridine as a key step in the synthesis of D-*gluco* amino-imino-hexitol **14** (Scheme 1.4). Here D-mannitol derived bis-aziridine **33** underwent nucleophilic ring opening with acetic acid, followed by subsequent aminocyclisation via ring opening of the remaining aziridine to produce a mixture of pyrrolidine **34** and piperidine **35** (2:1, 87%). Deprotection of pyrrolidine **34** under Birch conditions and subsequent acid hydrolysis produced D-*gluco* configured amino-hexitol **14**, which displayed no inhibition towards five glycosidases it was evaluated against. However, 1-acetamido-1,2,5-trideoxy-2,5-imino-D-glucitol

derived from pyrrolidine **34** was found to be a good inhibitor of β-glucosidase (K_i = 10 μM).

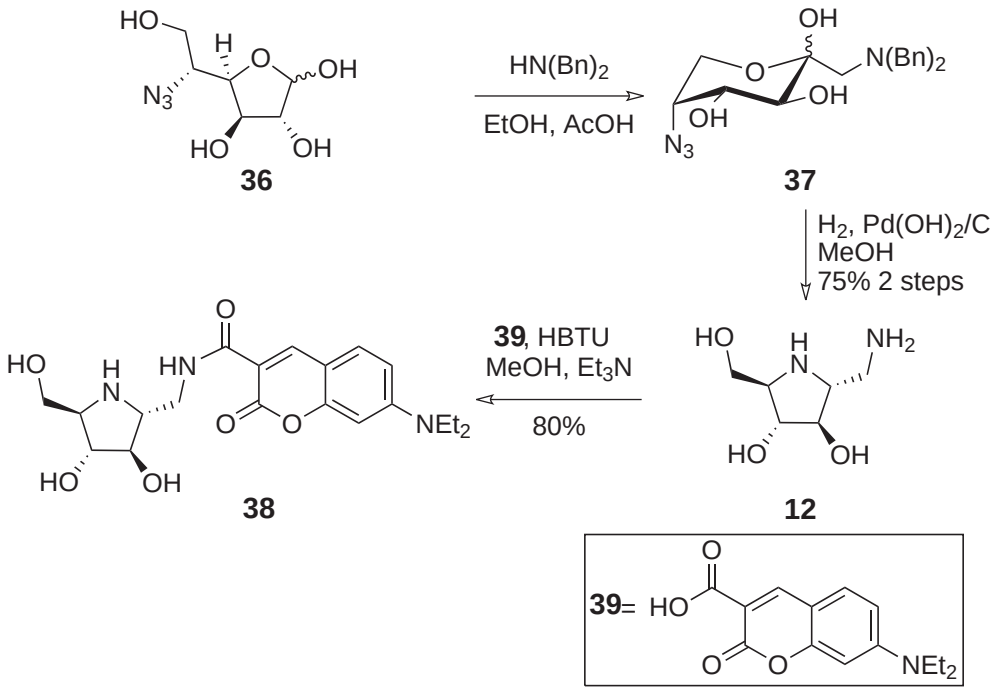


Scheme 1.4 Synthetic route to an amino-imino-hexitol via the ring opening of an aziridine

Rearrangement

In 1997, Wrodnigg and co-workers[48] were the first to report the syntheses of the D-*manno* and D-*gluco* amino-imino-hexitol scaffolds. They devised an elegant route to the novel scaffolds utilising the Amadori rearrangement. As illustrated (Scheme 1.5), readily available 5-azido-5-deoxy-glucofuranose **36**[60] was reacted with dibenzylamine in the presence of acetic acid to produce D-fructopyranose **37** with introduction of the C-1 amino group. Subsequent cyclisation with reductive amination and removal of the *N*-benzyl groups upon treatment with $Pd(OH)_2/C$ and H_2, provided D-*manno* hexitol **12** in good (75%) yield from azido-furanose **36**. In subsequent work, elaboration of the D-*manno* scaffold **12**, via the one-step selective coupling with a variety of lipophilic side

chains, provided a library of 1-*N*-acyl or -*N*-sulfonylated derivatives, some of which proved to be potent glycosidase inhibitors.[49,50,52,53] For example, coumarin derivative **38** was produced in good (80%) yield via the HBTU mediated coupling of hexitol **12** with acid **39**, and proved to be a powerful β-glucosidase inhibitor (*Agrobacterium* sp.) with a K_i of 1.2 nM.

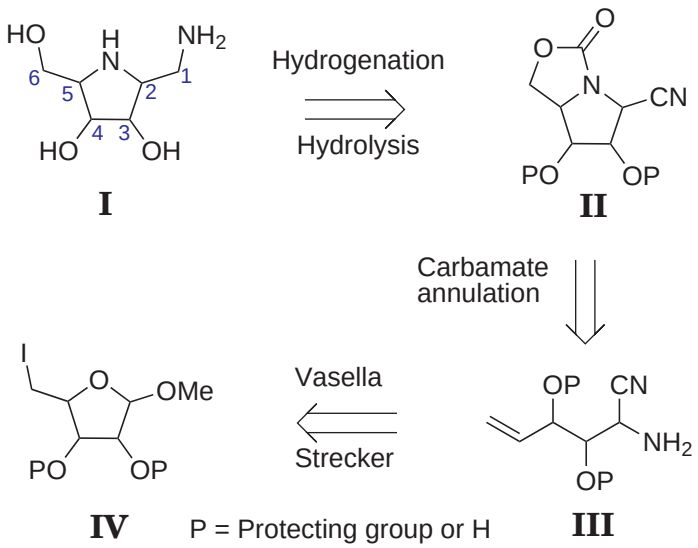


Scheme 1.5 Synthesis of the D-*manno* scaffold using an Amadori rearrangement with further 1-*N*-derivatisation

1.3 The Synthesis of Amino-imino-hexitols

Given the potential of amino-imino-hexitols as glycosidase inhibitors, we were interested in developing an efficient methodology for their synthesis. To this end, we

envisioned using cyclic carbamates **II** as key intermediates, which could be readily hydrolysed to give the target amino-imino-hexitols **I** (Scheme 1.6). Azasugar scaffolds **I** could be derivatised further as 1-*N*-functionalisation has been shown to produce potent glycosidase inhibitors.[42–44,49,50,52–55] The key intermediate carbamates should be accessible from α-aminonitriles **III** via the I_2-mediated carbamate annulation reaction recently developed in our group.[61–64] Application of this annulation methodology to protected and functionalised alkenylamines, such as **III**, would greatly enhance the scope of the reaction. The nitrile functionalised alkenylamines **III** could in turn be prepared via a Strecker reaction[65] of the intermediate aldehydes generated from the Vasella reaction[66,67] of readily available methyl iodoglycosides **IV**. Here, the inherent chirality of the carbohydrate-derived aldehyde would be used to control the diastereoselectivity of the Strecker reaction.[68–70]

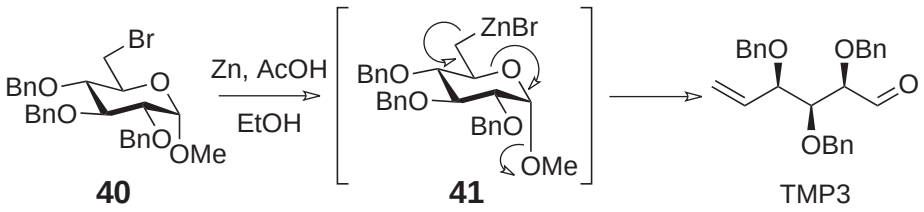


I **II** **IV** **III**

Scheme 1.6 Proposed retrosynthesis for the formation of amino-imino-hexitols utilising the I_2-mediated carbamate annulation

1.3.1 Vasella Reaction

In 1979, Bernet and Vasella reported the preparation of aldehydes via a Zn-mediated reductive elimination of haloglycosides (Scheme 1.7).[66,67] Here, activated zinc and acetic acid were added to a solution of methyl bromoglucoside **40** in ethanol and the solution was stirred at reflux to give aldehyde **41**. The Vasella reaction proceeds via oxidative insertion of zinc into the carbon-bromide bond, this is followed by reductive elimination with the loss of methoxide to give the aldehyde product. Thus, in our proposed methodology for the preparation of amino-imino-hexitols we anticipate the application of Vasella conditions to methyl iodoglycosides (**IV**, Scheme 2.1) will produce the corresponding aldehydes.



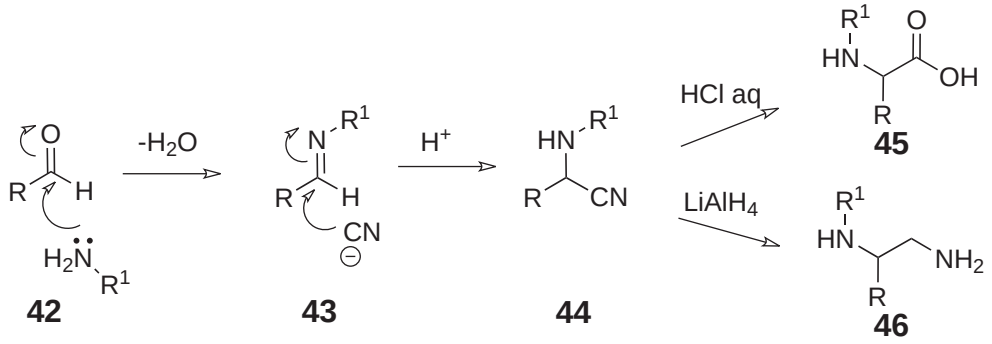
Scheme 1.7 Vasella reaction

1.3.2 Strecker Reaction

One of the key aspects of our proposed methodology for the synthesis of amino-imino-hexitols is the Strecker reaction, which provides a convenient means to prepare α-aminonitriles from aldehydes. The Strecker reaction was one of the earliest one-pot, multi-component reactions discovered[71] and has found wide application since the pioneering synthesis of α-amino acids by Strecker in 1850.[65] Since this time,

the development of modified and asymmetric Strecker reactions have received much attention.[71–75]

The Strecker reaction to produce α-aminonitriles follows a general mechanism, whereby an aldehyde **42** reacts with an amine to form an imine **43**, which undergoes subsequent nucleophilic attack by a cyanide to produce the desired α-aminonitrile **44** (Scheme 1.8). α-Aminonitriles **44** are useful synthetic precursors and, for example, can be transformed into amino acids via acid hydrolysis[76] (**45**), or reduced with $LiAlH_4$ to give primary amines (**46**).[77,78]

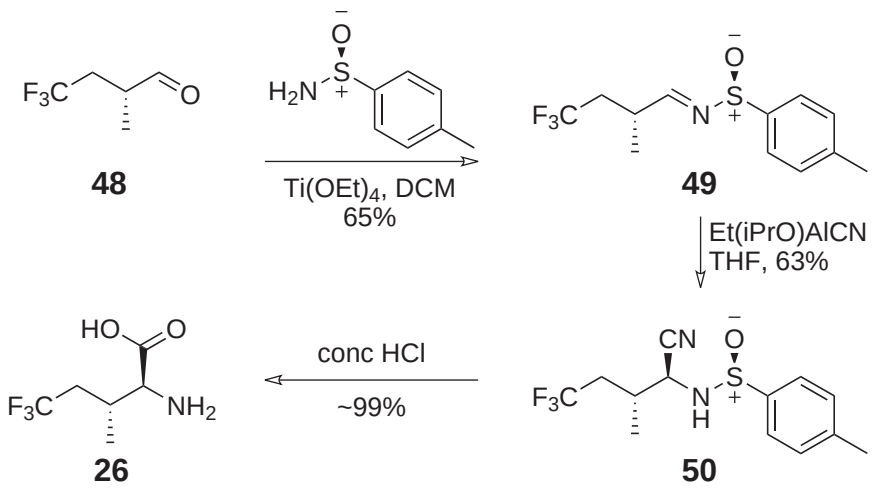


Scheme 1.8 General mechanism of the Strecker reaction with examples of the synthetic usage of the α-aminonitrile formed

In Strecker's original conditions hydrogen cyanide was used as the source of nitrile nucleophile, however, due to the highly toxic nature of HCN, modifications to the Strecker reaction have since involved the addition of alternative sources of nitrile. Alkaline cyanide salts such as KCN or NaCN have been utilised,[79] however, these salts are not very soluble in organic solvents and can lead to low yields of α-aminonitriles and tedious reaction work-ups. Improved cyanide solubility in organic solvents has

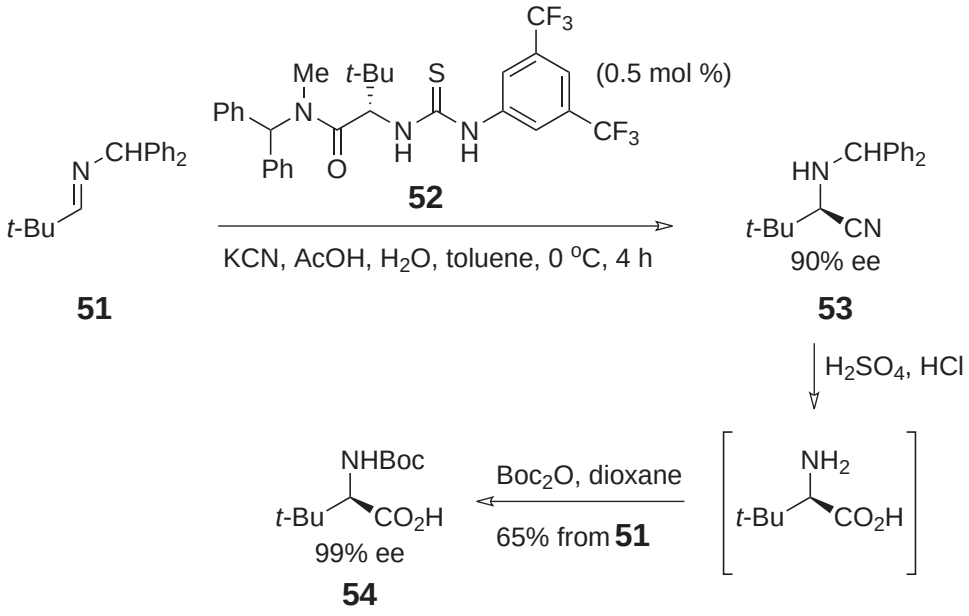
been obtained by the use of nitrile sources such as diethyl phosphorocyanidate[80] and TMSCN,[81] resulting in the efficient preparation of α-aminonitriles under mild reaction conditions.

There are a number of synthetic methodologies that can be used to achieve a stereoselective Strecker reaction. These include the use of a chiral Lewis acid,[82,83] or the generation of a chiral imine (auxiliary).[84–87] A recent example of auxiliary use was reported by Wang and Resnick,[88] who utilised a chiral Strecker reaction via Davis' sulfinimine protocol[85,89] as a key step in the synthesis of a novel γ-sectretase inhibitor (Scheme 1.9). Here, condensation of (*S*)-(+)-toluenesulfinamide with aldehyde **48** provided sulfinimine **49**, which was subjected to a Strecker reaction with Et(*i*-OPr)AlCN to give aminonitrile **50** with good (7:1) diastereoselectivity. Subsequent crystallisation enhanced the diastereomeric ratio (100:1). Aminonitrile **50** was then hydrolysed to give the unnatural α-amino acid **26** in excellent (99%) yield.



Scheme 1.9 Sulfinimine auxillary mediated asymmetric Strecker reaction

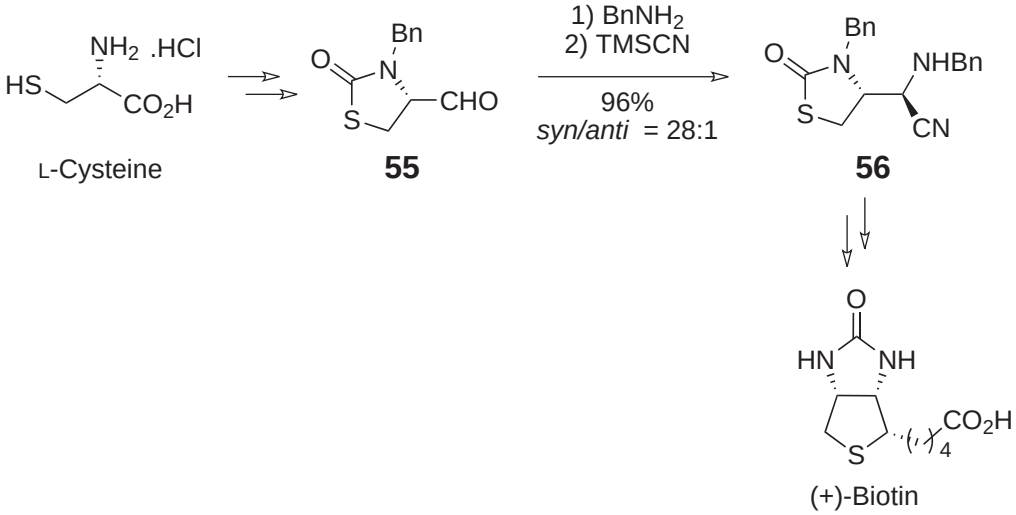
Another strategy to achieve an asymmetric Strecker reaction is via the catalytic enantioselective cyanation of achiral imines, of which there have been many examples over the past decade.[75] For example, Jacobsen and co-workers[90] recently reported a robust asymmetric catalytic method to provide non-natural amino acids with high enantioselectivities via the use of aqueous cyanide salts (Scheme 1.10). Here, cyanation of imine **51** with KCN in the presence of a catalytic amount of amido-thiourea derivative **52** produced aminonitrile **53** with good enantioselectivity (90% ee). Subsequent acid-mediated hydrolysis followed by treatment of the resulting aqueous amino acid solution with di-*tert*-butyl dicarbonate then provided amino acid **54** on the multi-gram scale and with improved enantiomeric excess upon recrystallisation.



Scheme 1.10 Catalytic asymmetric Strecker synthesis of an unnatural α-amino acid

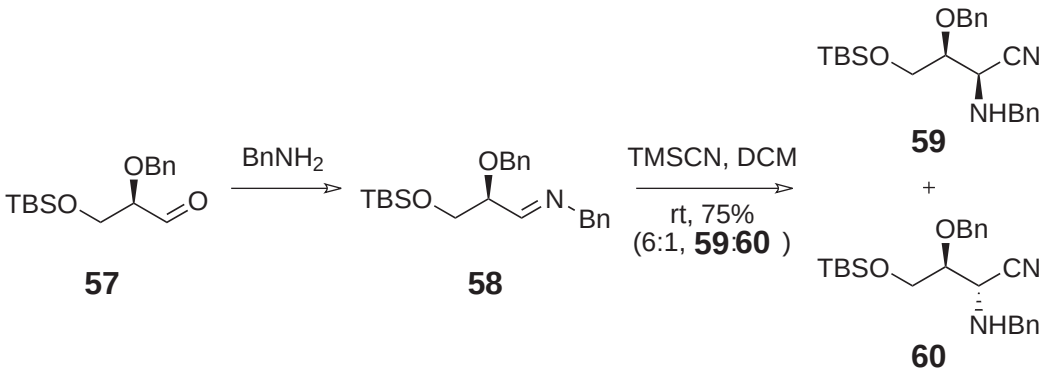
Strecker reactions in which the chiral matrix is the carbonyl or imine reagent, however, have many advantages as they avoid additional steps and the added complexities

associated with the use of an auxiliary or a catalyst. Some recent examples in this field include a highly diastereoselective Strecker reaction reported by Seki *et al.*[68,69] used as a key step in the synthesis of (+)-biotin (Scheme 1.11). Here, α-amino-aldehyde **55**, derived from L-cysteine, was first treated with benzylamine and then TMSCN to provide the protected α-aminonitrile **56** in excellent (96%) yield and high *syn*-diastereoselectivity (28:1, *syn*/*anti*).



Scheme 1.11 Stereoselective Strecker reaction key in the synthesis of (+)-biotin

Furthermore, Gálvez, Díaz-de-Villegas and co-workers reported the synthesis of synthetic precursors for the preparation of β-hydroxy-α-amino acids that utilised a stereoselective Strecker reaction as a key step (Scheme 1.12).[70] Here, protected D-glyceraldehyde **57**, was reacted with benzylamine to form imine **58**, which upon reaction with TMSCN afforded the corresponding α-aminonitriles in 75% yield and good diastereoselectivity (6:1, **59**:**60**).



Scheme 1.12 Stereoselective Strecker reaction with a glyceraldehyde derivative

Gálvez and Díaz-de-Villegas then explained the observed diastereoselectivity of their Strecker reaction by the glyceraldehyde derived imine **58** adopting an anti-Felkin-Anh[91] conformation in which the α-OBn group is situated perpendicular to the imine nitrogen such that the cyanide can preferentially attack from the *Re*-face to produce the major *syn*-product (Fig. 1.4). Based on the stereochemical outcomes of the Strecker reactions with both glyceraldyde **57** and α-amino-aldehyde **55** we could expect to observe similar *syn*-diastereoselectivity in the formation of our α-aminonitriles **III** (Scheme 1.6, pg. 15).

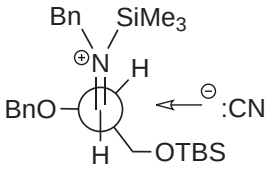
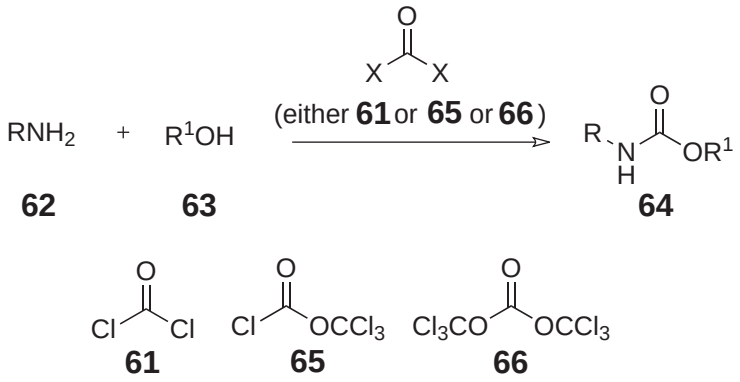


Fig. 1.4 Anti-Felkin-Anh model to explain observed *syn*-diastereoselectivity

1.3.3 Organic Carbamate Synthesis

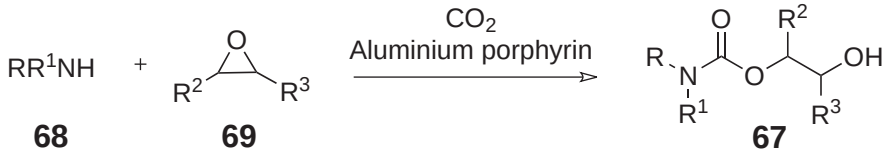
Organic carbamates have found wide application in chemistry with their use as pharmaceuticals and pharmaceutical intermediates, as agrochemicals, as linkers in combinatorial chemistry, and as protecting-groups during peptide couplings.[92] Indeed, in our own retrosynthesis for the preparation of amino-imino-hexitols, an organic carbamate forms a key intermediate (Scheme 1.6). A number of strategies have been developed for the synthesis of carbamates and these include the reaction of amines with phosgene (or derivatives), carbon dioxide (gaseous, electrochemical, and supercritical), carbonate esters and salts, or the use of amides in, for example, Hoffmann, Curtius and Lossen rearrangements.[92]

Phosgene (**61**) is a potentially useful building block for the addition of a carbonyl equivalent to bind amines (**62**) and alcohols (**63**) in the synthesis of carbamates (**64**) (Scheme 1.13).[92] However, as phosgene (**61**) is extremely toxic, safer derivatives have also been utilised for the synthesis of carbamates **64**, such as di-phosgene **65** or tri-phosgene **66**.[92]



Scheme 1.13 The use of phosgene and derivatives for the synthesis of carbamates

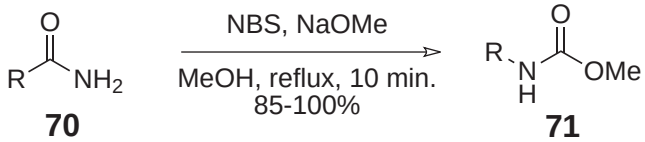
The synthesis of carbamates with CO_2 has also been well utilised.[92] Kojima and co-workers have reported the formation of carbamates **67** from addition of CO_2 to amines **68** (Scheme 1.14).[93] Here, CO_2 was trapped by an aluminium porphyrin and thus activated to react with secondary amines **68** in the presence of epoxides **69** to produce hydroxy carbamates **67**.



68 **69** **67**

Scheme 1.14 The use of carbon dioxide for the synthesis of carbamates

Huang and Keillor have reported[94] a modified Hoffman rearrangement for the synthesis of carbamates (Scheme 1.15). Here, a series of aliphatic and aromatic amides **70** were treated with NBS and NaOMe and, via rearrangement to the isocyanate with addition of methanol, produced the corresponding carbamates **71** in excellent (85%-100%) yields.

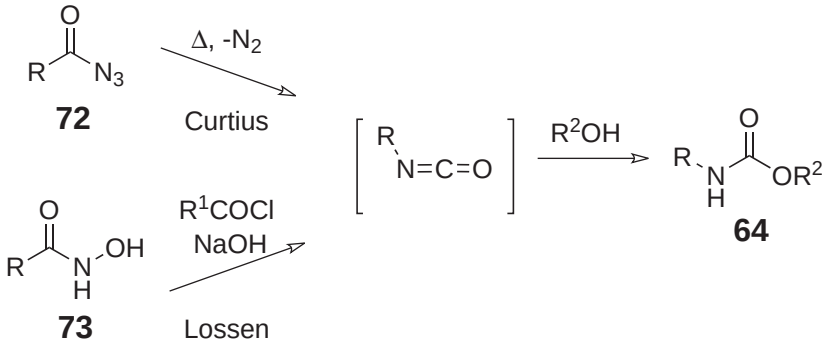


70 **71**

Scheme 1.15 A modfied Hoffman rearrangement for the preparation of carbamates

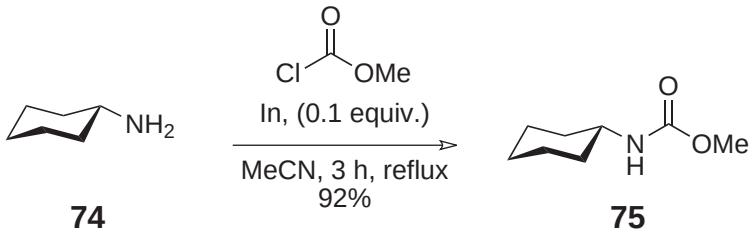
Curtius and Lossen rearrangements have also produced carbamates from azides and hydroxamic acids via isocyanate intermediates (Scheme 1.16).[92] The Curtius rearrangement involves heating acyl azides **72** to give isocyanates with the loss of N_2. The Lossen rearrangement generally involves the reaction of hydroxamic acids with an acyl

or sulfonyl chloride, which under basic conditions rearranges to form an isocyanate. For both rearrangements, subsequent reaction of the intermediate isocyanates with alcohols provides carbamates **64**.



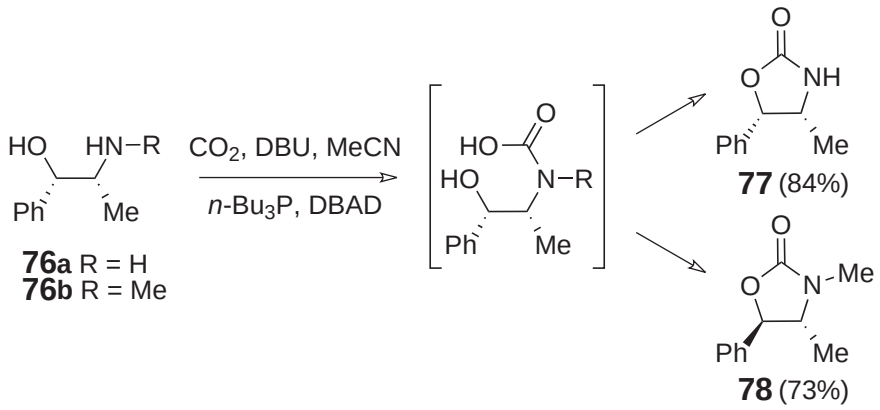
Scheme 1.16 Carbamates prepared via a Curtius or Lossen rearrangement

Other recent examples of organic carbamate synthesis include the use of alkyl chloroformates, as illustrated by Kim and Jung[95] who reported on the simple and efficient conversion of a series of amines to their carbamate derivatives via indium metal catalysis (Scheme 1.17). Here, amine **74** reacted with an equimolar amount of methyl chloroformate in the presence of indium to produce carbamate **75** in excellent (92%) yield. This general route to carbamates was then further exemplified by starting with a wide variety of aliphatic and aromatic amine precursors.



Scheme 1.17 Indium catalysed formation of carbamates

A stereoselective synthesis of cyclic carbamates from linear amino alcohol precursors was reported by Dinsmore and Mercer in 2004[96] (Scheme 1.18). Here, carboxylation of amino alcohols **76** followed by a Mitsunobu reaction provided either carbamate **77** with retention of configuration or **78** with inversion (Scheme 1.18). Using this method a series of cyclic carbamates were produced in good yields from a variety of amino alcohols. Unexpectedly, the stereochemical outcome of the Mitsunobu reaction was dependent on whether the carbamic acid intermediate was *N*-substituted with hydrogen (retention) or carbon (inversion).

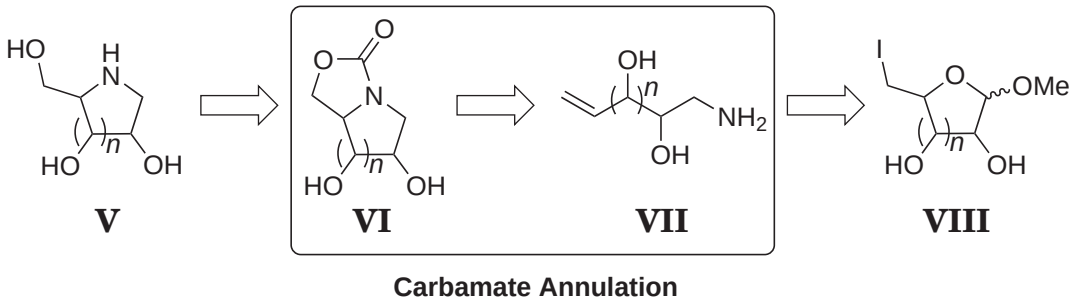


Scheme 1.18 Formation of cyclic carbamates from amino alcohols

1.3.4 I_2-mediated Carbamate Annulation

More recently, Stocker, Timmer and co-workers developed a highly stereoselective I_2-mediated carbamate annulation as a key step in the protecting-group-free synthesis of azasugars.[61–64] This methodology was inspired by seminal work of Hassner and Burke[97] and Inesi *et al.*[98] who prepared cyclic carbamates, in modest yield, from the reaction of acyclic amines equipped with a halogen leaving group and either sodium carbonate or

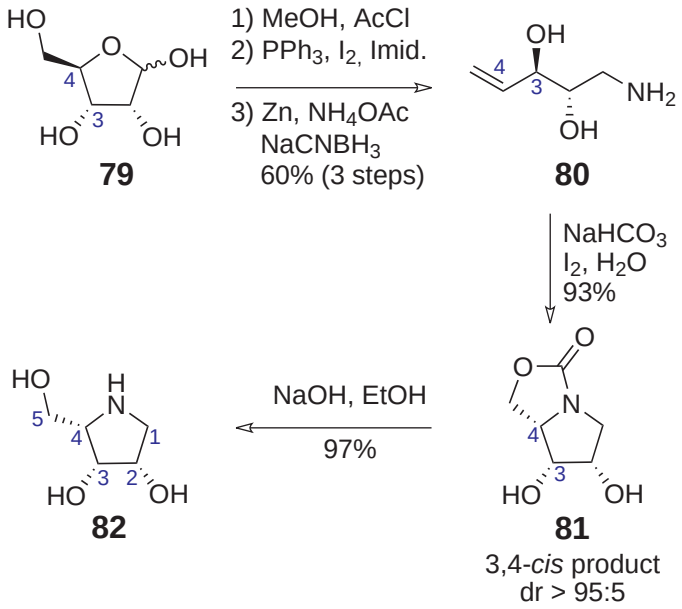
tetraethylammonium bicarbonate. Stocker and Timmer thus envisioned a retrosynthetic strategy for the preparation of azasugars **V** whereby the target compounds could be readily prepared from the precursor carbamates **VI** via base-mediated hydrolysis (Scheme 1.19). Carbamates **VI**, in turn, could be formed via the I_2-mediated annulation, with the required alkenylamines **VII** being prepared from the parent sugars **VIII** via a Vasella reductive amination.[66,67,99] In this manner, both five-membered (n = 1) or six-membered (n = 2) azasugars, without an 'anomeric' substituent, could be readily prepared. As this thesis concerns the synthesis of pyrrolidines, our attention will thus focus on the scope and limitations of the I_2-mediated annulation for this ring size.



Scheme 1.19 Retrosynthesis for the protecting-group-free preparation of azasugars

Stocker and Timmer's general synthetic approach was applied to the protecting-group-free synthesis of pyrrolidines (Scheme 1.20)[61–63] In their seminal work, the parent pentose **79** was readily converted into the corresponding alkenylamine **80** via a Vasella reaction followed by a protecting-group-free reductive amination.[99] Subjection of alkenylamine **80** to a solution of I_2 and excess $NaHCO_3$ in H_2O then formed carbamate **81** in one step and in excellent yield and diastereoselectivity.[61] The carbamate annulation favours the formation of 3,4-*cis* pyrrolidines (dr >20:1), with the stereochemistry at the 3-position exerting stereocontrol on the cyclisation.[100–103] Carbamate **81** was

subsequently treated with NaOH in EtOH to provide the 1,4-dideoxy-1,4-imino-L-lyxitol **82** in excellent (99%) yield. Using this strategy, 1,4-dideoxy-1,4-imino-D-lyxitol **83** ,[63] 1,4-dideoxy-1,4-imino-D-xylitol **84**,[61] 1,4-dideoxy-1,4-imino-L-xylitol **85**,[63] and 1,2,4-trideoxy-1,4-imino-L-xylitol **86** (Fig. 1.5),[62] were prepared in excellent (48%-57%) overall yields.



Scheme 1.20 Carbamate annulation used in the synthesis of pyrrolidines

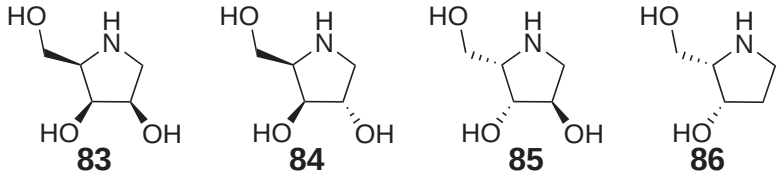
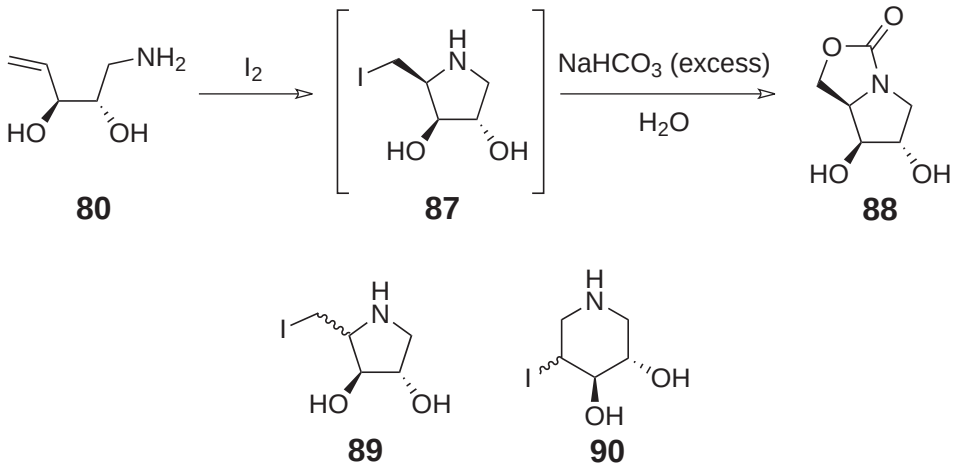


Fig. 1.5 Application of the carbamate annulation to a series of pyrrolidines

To explain the mechanism of the annulation, Stocker and Timmer proposed a reaction involving the initial formation of a haloamine followed by *in situ* carbonylation, similar

to that described for the addition of CO_2 to 1,2-hydroxyhalides[104] (cf. **87** → **88**, Scheme 1.21) To support this idea the role of the base in the reaction was investigated[61] and it was observed that subjection of alkenylamine **80** to *N*-iodosuccinimide (NIS), or iodine in the presence of DBU or NaOH gave an inseparable mixture of five- and six-membered iodoazasugars **89** and **90** (Scheme 1.21) presumably formed from **80** via aziridine formation and subsequent opening of the three-membered ring by nucleophilic iodide.[105] Moreover, in all experiments, there was no evidence for the formation of the corresponding carbamate, suggesting that the CO_2 required for the reaction is generated via the dissolution of $NaHCO_3$ in water. Attempts were also made to determine if the unprotected iodoamine **87** was indeed an intermediate in the reaction though unfortunately iodoamine **87** was unable to be isolated despite numerous attempts.[61]



Scheme 1.21 Proposed iodoamine intermediate in the carbamate annulation

1.3.5 Diastereoselectivity in the I_2-mediated Carbamate Annulation

The high diastereoselectivity observed in the I_2-mediated carbamate annulation of alkenylamines without an α-amine substituent[62] was explained by adapting a transition state model originally proposed by Chamberlin *et al.*,[100–102] and in line with more recent theoretical studies by Gouverneur and co-workers.[103] Here it was proposed that during electrophilic cyclisations, the stereo directing hydroxyl could be positioned either in the plane of the double bond (**A**, O-in-plane), or almost perpendicular to that plane (**B**, H-in-plane) (Fig. 1.6). Of these two transition states, **A** had minimal overlap between the electron-withdrawing $\sigma^*_{C\text{-}O}$ and reacting $\pi_{C=C}$ orbitals, thereby forming the lowest energy transition state. The H-in-plane conformation (**B**) had overlapping hydroxyl $\sigma^*_{C\text{-}O}$ and double bond $\pi_{C=C}$ orbitals, which destabilised the I_2–π complex and was hence disfavoured.

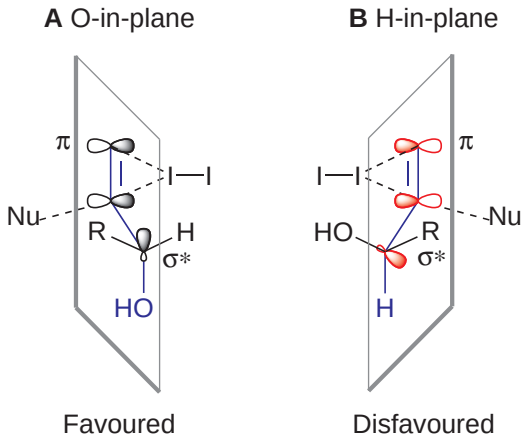
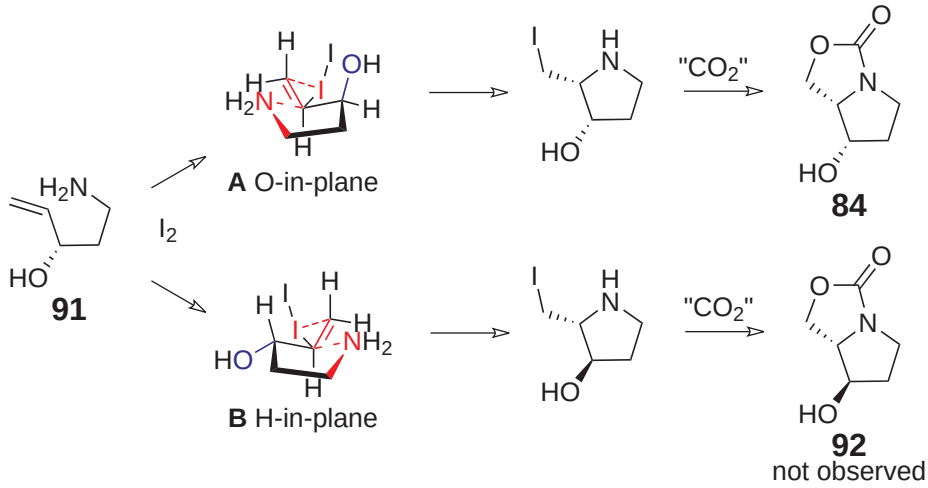


Fig. 1.6 O-in-plane vs H-in-plane

Accordingly, during the protecting-group-free carbamate annulation, attack of the amine on the I_2–olefin complex was envisioned to take place via a 5-membered ring transition structure, in which the ring nitrogen approached the double bond in an ^{N}E

(envelope) conformation and followed a Bürgi-Dunitz-like trajectory[106] (Scheme 1.22). Applying the transition state model, it is apparent that the favoured O-in-plane (**A**) conformation leads to the observed 3,4-*cis*-product **91** and the less favoured H-in-plane (**B**) conformation leads to the unobserved 3,4-*trans*-product **92**.

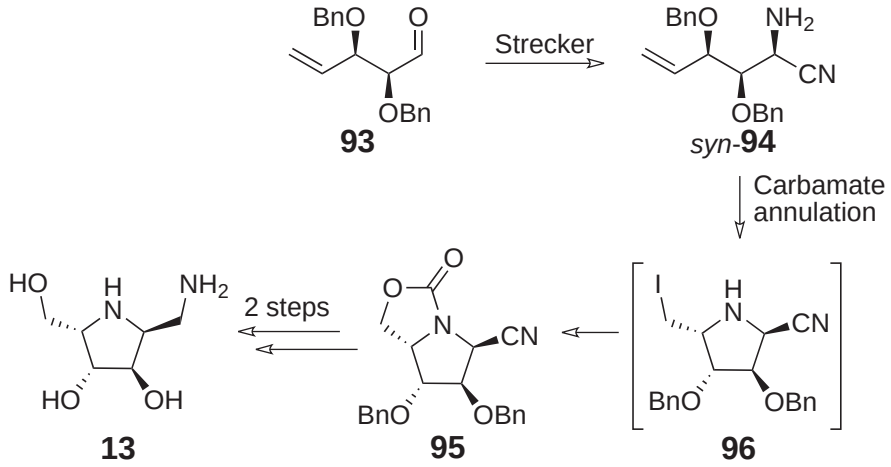


Scheme 1.22 Stocker and Timmer's proposed transtion state for the protecting-group-free I_2-mediated carbamate annulation

Based on the stereochemical outcomes of carbamate annulations with alkenylamines without an α-amine substituent, we could expect to observe similar high *cis*-diastereoselectivity in the production of carbamates **II** from α-aminonitriles **III** (Scheme 1.6, pg. 15). However, the nitrile substituent at the α-amine position may play a role in the transition states of our carbamate annulation.

1.4 Thesis Outline

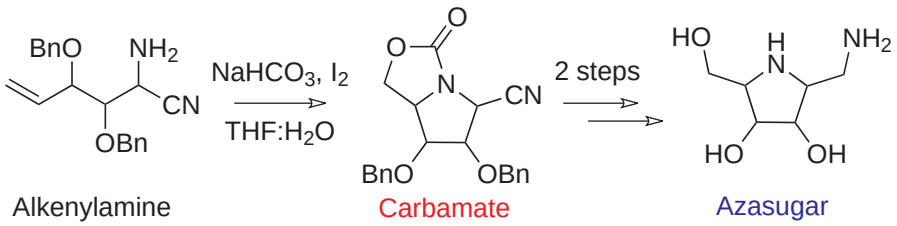
The work in this thesis focuses on the synthesis of amino-imino-hexitols as glycosidase inhibitors via an I_2-mediated carbamate annulation and includes investigations into the mechanism and diastereoselectivity of the carbamate annulation. **Chapter 2** describes novel methodology for the synthesis of 1-amino-1,2,5-trideoxy-2,5-imino-L-iditol **13** starting from D-arabinose (Scheme 1.23). Key in the synthesis is a modified Strecker reaction with aldehyde **93** to produce α-aminonitrile *syn*-**94** in excellent diastereoselectivity and the annulation of alkenylamine *syn*-**94** to give carbamate **95** as the only observed diastereomer. Iodoamine **96** is identified as an intermediate formed during the carbamate annulation.



Scheme 1.23 Novel methodology to synthesise an amino-imino-hexitol

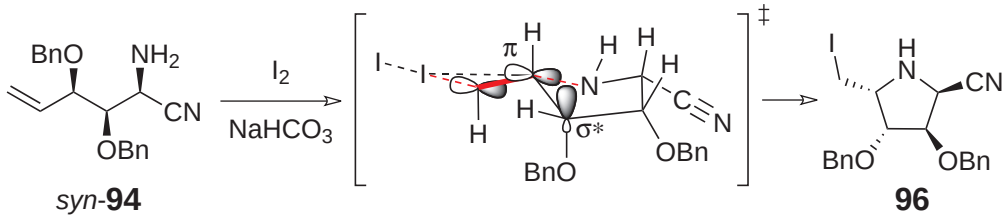
Chapter 3 reports on the application of the methodology presented in Chapter 2 to prepare five more amino-imino-hexitol scaffolds with the D-*gluco*, D-*manno*, and previously undisclosed D-*galacto*, D-*talo* and L-*altro* configurations (Scheme 1.24). Here, the variety of azasugar scaffolds are accessed via carbamate annulations of

alkenylamines derived from D-arabinose and D-ribose.



Scheme 1.24 Preparation of amino-imino-hexitols

Chapter 4 focuses on evaluation of the glycosidase inhibitory activity of a number of amino-imino-hexitols, along with *N*-acylation of the compounds with novel configurations. In **Chapter 5**, mechanistic considerations of the I_2-mediated carbamate annulation are presented along with a discussion of the diastereoselectivity of the reaction and proposed transition state models for the initial iodocyclisation (e.g. *syn*-**94** → **96**, Scheme 1.25).



Scheme 1.25 Proposed transition state in the iodocyclisation of α-aminonitrile *syn*-**94**

The work in **Chapter 6** comprises a computational investigation of the diastereoselectivity of the carbamate annulation using density functional theory to find transition states of the initial iodocyclisation step. Calculations starting with several alkenylamine precursors with and without an α-amine substituent are presented. **Chapter 7** focuses on future prospects.

Each Chapter in this thesis has been written in a self contained format and thus has its own compound numbering, experimental details and reference list.

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Chapter 2

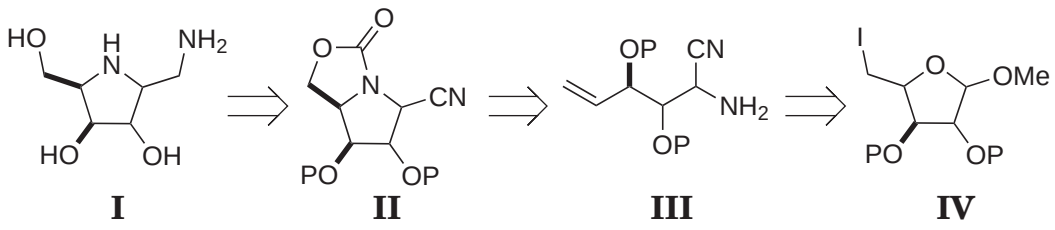
Development of Stereoselective Strecker and I_2-mediated Carbamate Annulation for the Synthesis of an Amino-imino-hexitol

2.1 Introduction

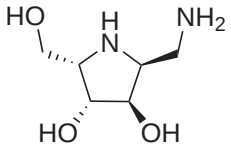
Azasugars, or iminosugars, are a valuable class of compounds that play an important role in drug discovery, primarily due to their ability to mimic oxocarbenium ion-like transition states of glycosidase reactions (Chapter 1).[1,2] In particular, the therapeutic potential of amino-imino-hexitols as glycosidase inhibitors has led to a number of past syntheses of these azasugars and their *N*-acyl derivatives.[3–6] We sought to develop

amino-imino-hexitols as scaffolds for drug development and therefore devise a synthetic strategy that would enable access to the various stereoisomers. We aimed to do this by taking advantage of the I_2-mediated carbamate annulation recently developed within the Stocker and Timmer research group.[7–10]

The focus of the work in this chapter is the development of methodology that allowed for the efficient and stereoselective synthesis of amino-imino-hexitols (**I**, Scheme 2.1).[11] Key to achieving this goal was the extension of the I_2-mediated carbamate annulation to functionalised alkenylamines (**III** to **II**), and the application of a diastereoselective Strecker[12] reaction (**IV** to **III**). At the start of this project we chose to synthesise the previously undisclosed L-*ido* configured amino-imino-hexitol (**1**, Fig. 2.1) as a 'proof-of-concept' for the synthesis of a variety of amino-imino-hexitols. However, during the course of the research the synthesis of L-*ido*-hexitol (**1**) was published by Ramesh and co-workers.[13]



Scheme 2.1 Retrosynthetic analysis of amino-imino-hexitols

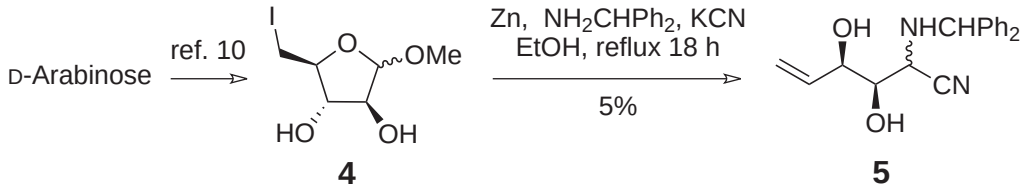


1

Fig. 2.1 L-*Ido* configured amino-imino-hexitol (**1**)

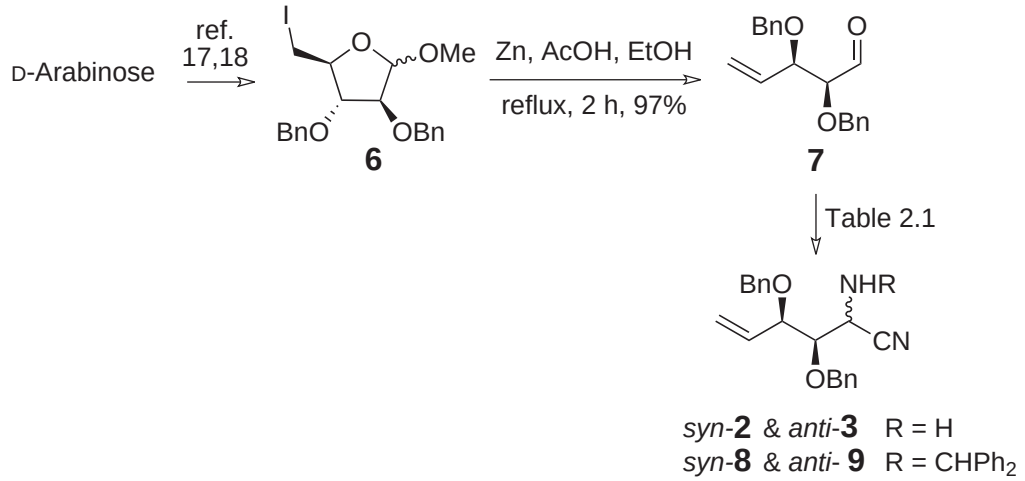
2.2 Development of a Stereoselective Strecker Reaction

With our synthetic plan in place, we set out to prepare the D-arabinose derived α-aminonitriles *syn*-**2** and *anti*-**3** (Scheme 2.3). Previously, work was carried out by Dangerfield,[11] within the Stocker and Timmer research group, to attempt the Strecker reaction without the use of protecting-groups, as this would provide a shorter synthetic route if successful (Scheme 2.2). To this end, methyl iodoglycoside **4**, readily prepared from D-arabinose in 64% yield (2 steps),[9,14] was subjected to Vasella[15,16] conditions. Though a lower running spot was observed via TLC, isolation of the corresponding aldehyde proved futile with product degradation being observed. A two-step one pot Vasella/Strecker reaction was then attempted by first treating glycoside **4** with activated zinc in EtOH at reflux, followed by cooling and treatment with amine (either Ph_2CHNH_2 or NH_4Cl) and nitrile (either KCN or TMSCN). Unfortunately, under these conditions, significant product degradation was observed. At best only a minor amount (ca. 5%) of the desired α-aminonitrile **5** could be obtained when Ph_2CHNH_2 was used as the nucleophile.



Scheme 2.2 Attempts to produce nitrile-functionalised alkenylamines via a protecting-group-free protocol

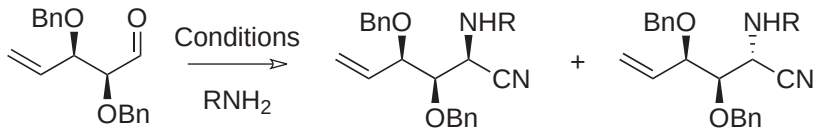
In light of the issues in preparing and isolating the α-aminonitriles without protecting groups, we decided to develop the Strecker reaction with the use of protecting groups (Scheme 2.3). Here, D-arabinose was converted into the benzyl-protected methyl iodoglycoside **6** in 5 steps and 62% overall yield following literature procedures.[9,17,18] Subsequent treatment of iodide **6** with a solution of activated zinc in EtOH, according to the conditions of Vasella, gave the corresponding aldehyde **7** in an excellent 97% yield. Given the chirality of **7**, we then envisioned that a diastereoselective Strecker reaction could be developed to favour the selective formation of one α-aminonitrile, either *syn*-**2** or *anti*-**3**, representing the D-*lyxo* and D-*xylo* configurations, respectively.



Scheme 2.3 Preparation of nitrile-functionalised alkenylamines

Aldehyde **7** was first treated with KCN in the presence of NH_4Cl in acetonitrile (entry 1, Table 2.1). Unfortunately, the poor solubility of NH_4Cl in acetonitrile resulted in formation of the corresponding cyanohydrin, as observed by ^1H NMR (illustrated by the downfield shift of the α-protons to 4.53 and 4.45 ppm from 3.89 and 3.70 ppm, respectively), and the desired α-aminonitrile was not observed. Studies of the Strecker reaction by Taillades and Commeyra demonstrated that cyanohydrin formation is kinetically favoured;[19] it is interesting to note that in this case none of the desired α-aminonitriles *syn*-**2** or *anti*-**3** were observed, even after prolonged reaction times (24 h). To prevent formation of the cyanohydrin and other impurities, the much more soluble and nucleophilic Ph_2CHNH_2 has been utilised,[20,21] which, with increased steric bulk, could also lead to enhanced diastereoselectivity. Aldehyde **7** was thus treated with Ph_2CHNH_2, KCN, as the nitrile source, and Al_2O_3 (entry 2, Table 1). The reaction was sluggish, and a reaction time of two weeks was required for the complete disappearance of aldehyde **7** starting material. Although the yield of aminonitriles *syn*-**8** and *anti*-**9** was poor (ca. 30%), the diastereoselectivity was excellent, being 11:1 in favour of the *S* diastereomer *syn*-**8**, and both isomers could be separated via column chromatography. Next, in an attempt to improve the yield and decrease the reaction time, sonication[22] was employed (entry 3). This improved the reaction yield (78%), though the diastereoselectivity was reduced (7:1, *syn*:*anti*).

Table 2.1 Optimisation of the Strecker reaction



7

syn-**2** R = H
syn-**8** R = $CHPh_2$

anti-**3** R = H
anti-**9** R = $CHPh_2$

Entry	R-NH_2 (equiv.)	Nitrile (equiv.)	Conditions	Ratio[a] [b] *syn*:*anti*	Yield (%)
1	NH_4Cl (2.1)	KCN (2)	MeCN, sonication, 50 °C, 18 h	-	0[c]
2	Ph_2CHNH_2 (2)	KCN (2)	Al_2O_3, MeCN, r.t., 14 d	11:1	33
3	Ph_2CHNH_2 (2)	KCN (2)	Al_2O_3, MeCN, sonication, 50 °C, 2 d	7:1	78
4	Ph_2CHNH_2 (2)	$(EtO)_2P(O)CN$ (1.2)	MeCN, r.t., 2 h	7:1	52
5	Ph_2CHNH_2 (2)	$(EtO)_2P(O)CN$ (1.2)	THF, r.t., 6 h	2:1	72
6	Ph_2CHNH_2 (2)	KCN (2)	TMSCl, MeCN, r.t., 2 d	8.5:1	49
7	Ph_2CHNH_2 (2)	TMSCN (1.2)	MeCN, r.t., 16 h	12.5 :1	85
8	NH_4OAc (10)	TMSCN (1.2)	MeCN, r.t., 24 h	5.5:1	80
9	NH_4OAc (10)	TMSCN (1.2)	THF/H_2O, r.t., 18 h	-	0[c]
10	NH_4OAc (10)	TMSCN (1.2)	EtOH, r.t., 18 h	8:1	82
11	NH_4OAc (10)	TMSCN (2)	EtOH, r.t., 18 h	9:1	88

[a] As determined from analysis of the crude reaction mixture by ^{1}H NMR spectroscopy.

[b] The configurations of *syn*-**2** and *anti*-**3** were determined after cyclisation by ^{1}H NMR NOE correlations and comparison of the optical rotation values with those of known products. The configuration of *anti*-**3** was further confirmed through X-ray crystallography. For *syn*-**8** and *anti*-**9**, the diphenylmethyl group was removed by treatment with TFA and the resulting ^{1}H NMR spectrum was compared to that of *syn*-**2** and *anti*-**3**.

[c] Only the cyanohydrin was observed.

We then explored nitrile sources with improved solubility in organic solvents. When diethyl phosphorocyanidate[23] was added to a solution of aldehyde **7** and Ph_2CHNH_2 in acetonitrile (entry 4), the diastereoselectivity (7:1, *syn*:*anti*) was similar to that observed using KCN and sonication (entry 3), however the yield was only 52%. Changing the solvent to THF (entry 5) improved the overall yield, but led to a dramatic decrease in diastereoselectivity (2:1). The use of TMSCN as a nitrile source was then investigated. First, TMSCN was generated in situ via the addition of KCN to a solution of TMSCl, this was then added to a solution of the aldehyde in acetonitrile (entry 6). This reaction proved to be slow, taking 2 days to go to completion, and although the diastereoselectivity was good (8.5:1, *syn*:*anti*), the overall yield was modest (49%). Unreacted imine was observed by ^1H NMR analysis of the crude reaction mixture. A change to TMSCN,[24] however, led to a rapid improvement in yield (85%) and diastereoselectivity (12.5:1, *syn*:*anti*, entry 7), providing the conditions of choice for the Strecker reaction with Ph_2CHNH_2 as the amine source.

Although the initial experiments with NH_4Cl and KCN (entry 1) failed to give the desired aminonitriles, we were keen to explore whether the optimised Strecker conditions, using Ph_2CHNH_2 as the nucleophile, were also applicable when using an ammonium salt. This would allow us to pursue our initial goal of using an unprotected amine source and remove one step from the reaction sequence. To this end, the more soluble NH_4OAc was used in combination with TMSCN as the nitrile source, and to our delight, this resulted in a respectable 80% yield for the two diastereomers and good diastereoselectivity (5.5:1, *syn*/*anti*), (entry 8). The stereochemistry of *syn*-**2** was determined via subsequent NOE correlations and comparison with known optical rotation values following cyclisation (*vide infra*), while a crystal structure of *anti*-

3 was obtained (Fig. 2.2), proving the stereochemistry unambiguously. To further explore the effects of solvent on reaction yield and diastereoselectivity, the reaction was repeated, this time using a solution of THF/H_2O (1:1) to dissolve both aldehyde **7** and the ammonium salt (entry 9). Unfortunately, these conditions yielded only the cyanohydrin. A change to EtOH, however, led to both an improvement in yield (82%) and diastereoselectivity (8:1, *syn*/*anti*) (entry 10), with only a minor amount of the cyanohydrin formed. A further improvement was observed with the addition of 2 equivalents of TMSCN (entry 11), which gave the desired α-aminonitrile in excellent (88%) yield and diastereoselectivity (9:1, *syn*/*anti*). This result was remarkable in that both the yield and diastereoselectivity were excellent, and moreover, that the diastereomers were readily separable via flash chromatography [*syn*-**2**, R_f = 0.47; *anti*-**3**, R_f = 0.38 (hexanes/EtOAc; 1:1)].

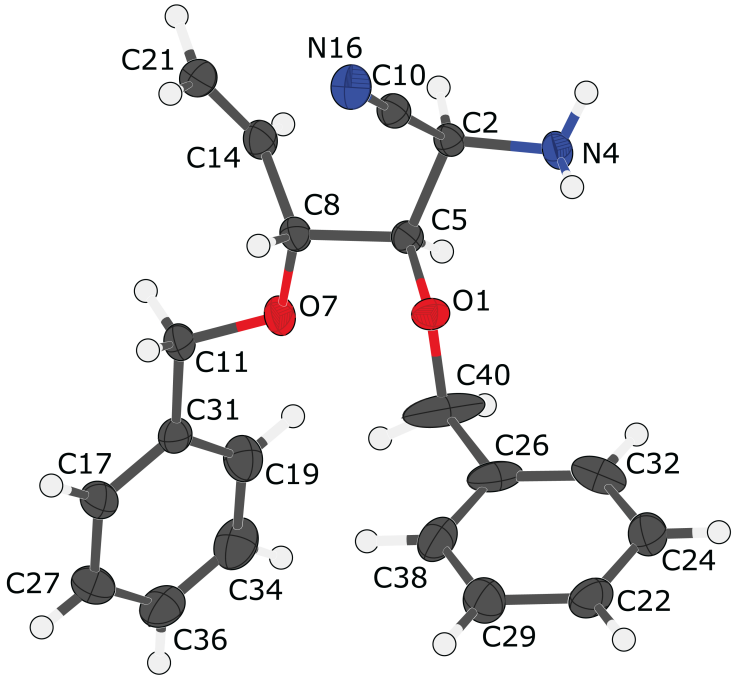


Fig. 2.2 Crystal structure of *anti*-**3**[25]

The stereochemical outcome of the reaction can be explained by considering the approach of the cyanide nucleophile through a Cram-model of a chelation controlled transition state (Fig. 2.3). Here chelation occurs via the iminium proton and the α-benzyloxy group to give a five-membered ring. The cyanide then attacks from the least hindered *Si* face of the ring.

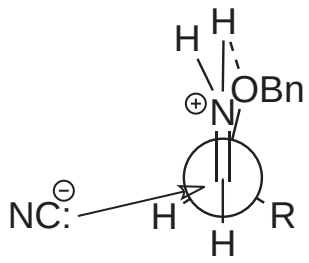


Fig. 2.3 Cram chelate model for the Strecker reaction to give α-aminonitrile *syn*-**10**

2.3 Extension of the I_2-mediated Carbamate Annulation

With the desired α-aminonitriles in hand, an iodine mediated carbamate annulation was investigated. Previous work from our research group illustrated that iodine-mediated cyclisations of hydroxy-functionalised alkenylamines led to the rapid formation of pyrrolidine-derived cyclic carbamates with excellent diastereoselectivity in favour of the 4,5-*cis* product.[7] These cyclisations were performed in saturated aqueous $NaHCO_3$, and were in contrast to previous scientific dogma whereby iodine-mediated cyclisations of alkenes containing an internal nucleophile (oxygen or nitrogen) were reported to give a halide-functionalised product.[26] Given the limited solubility of benzyl-protected α-aminonitrile *syn*-**2** in H_2O, the carbamate annulation would need to occur in a mixed solvent system, and moreover, generation of a primary iodide was suggested by the literature. To this end, a number of I_2-mediated electrophilic cyclisations were

performed.

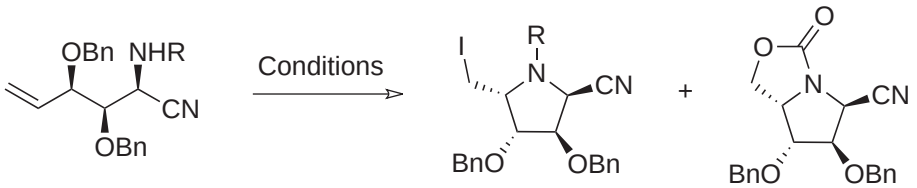
First, we treated protected amine *syn*-**8** with *N*-iodosuccinimide (NIS) as the source of iodine, and unsurprisingly, in the absence of a 'CO_2' source, generated the primary iodide **10** in good (79%) yield (entry 1, Table 2.2). A similar result was observed when NIS was used with unprotected amine *syn*-**2** with the corresponding iodo azasugar **11** being prepared in 75% yield (entry 2). A change of base to $NaHCO_3$ (1.5 equiv.) and iodine source to I_2 with the use of a mixed solvent system, THF/H_2O (1:1) led to the generation of only a minor amount of carbamate **12** (<10%) when the reaction was stirred at room temperature for 4 h (entry 3). Here, the major product was the primary iodide **11**. Increasing the reaction time to 20 h, however, led to a dramatic increase in the yield of **12** (ca. 50%) and suggests that iodo iminosugar **11** is an intermediate en route to the formation of **12** (entry 4). Only the 4,5-*cis* diastereomer was formed for both **12** and **11**, as was predicted by our previous carbamate annulations to form dideoxy pyrrolidines,[7–9] and literature precedence for the iodocyclisation of substrates carrying an alkoxy substituent in the allylic position.[27–30]

Given that the primary iodide appears to be a key intermediate during the carbamate annulation (**2** → [**11**] → **12**), it is the formation of the iodide that determines the diastereoselectivity of the reaction. Seminal work by Chamberlin et al.,[31,32] and the more recent theoretical studies by Gouverneur and coworkers[33] highlight the effects of the substitution pattern on I_2-mediated electrophilic halocyclisations and the role that the allylic group has on influencing the diastereoselectivity of the reaction. The attack of the amine on the I_2-alkene complex is thought to take place via a five-membered ring transition state in which the internal nucleophile approaches the double bond in an envelope conformation and follows a Bürgi-Dunitz-like trajectory. In our studies, the

benzyloxy substituent on the ring would be preferentially positioned in the plane of the double bond, resulting in the 4,5-*cis*-product. The addition of 'CO_2' to **11** then provides carbamate **12**. A more detailed discussion on the mechanism and diastereoselectivity of the I_2-mediated carbamate annulation, will be provided in Chapters 5 and 6.

The number of equivalents of iodine and $NaHCO_3$ were then increased to further improve carbamate formation. The use of 2.5 equiv. of iodine and 15 equiv. of $NaHCO_3$ led to the preferential formation of **12** (1:20, **11**:**12**) after 20 h (entry 5). Finally, optimal conditions for the formation of the carbamate **12** were found when 20 equiv. of $NaHCO_3$ were added to a solution of iodine in THF/H_2O (1/1) so that the final concentration of $NaHCO_3$ in H_2O was approximately 6M (entry 6). Under these conditions there was no trace of iodide **11**, as evidenced by ^{1}H NMR of the crude reaction mixture. Again, conversion of iodide **11** to carbamate **12** was observed via TLC analysis (hexanes:ethyl acetate, 2:1; R_f = 0.52 for iodide **11**, R_f = 0.35 for α-aminonitrile *syn*-**2**; R_f = 0.24 for carbamate **12**). Here it is important to note that the ability to form carbamate **12**, when starting with a functionalised and protected alkenylamine, greatly expands the scope of this annulation methodology.

Table 2.2 Formation of cyclic carbamate **12**



syn-**2** R = H
syn-**8** R = $CHPh_2$

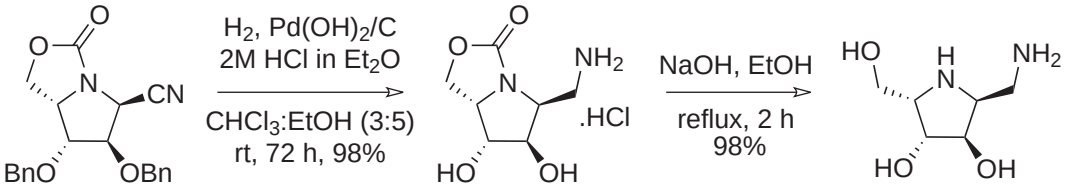
11 R = H
10 R = $CHPh_2$

12

Entry	R	Conditions	Ratio[ab] **11**/**12**	Yield (%)
1	CH_2Ph_2	NIS, (1.2 equiv.), DCM, r.t., 5 h	**10** only	79
2	H	NIS (1.2 equiv.), DCM, r.t., 5 h	>20:1	75
3	H	I_2 (1.1 equiv.), $NaHCO_3$ (1.5 equiv.), THF/H_2O (1/1), r.t., 4 h	9:1	80
4	H	I_2 (1.1 equiv.), $NaHCO_3$ (1.5 equiv.), THF/H_2O (1/1), r.t., 20 h	1:1.5	76
5	H	I_2 (2.5 equiv.), $NaHCO_3$ (15 equiv.), THF/H_2O (1/1), r.t., 20 h	1:20	84
6	H	I_2 (2.5 equiv.), $NaHCO_3$ (20 equiv.), THF/H_2O (1/1), r.t., 20 h	<1:20	85

[a]Determined by integration of the relevant signals in the ^{1}H NMR spectrum of the crude reaction mixture.
[b]Configuration determined by NOE correlations and comparison of spectra with that of known aminoiminohexitols (*vide infra*)

To complete the synthesis of target amino-imino-hexitol **1** (Scheme 2.4), carbamate **12** was treated with $Pd(OH)_2/C$ in the presence of 2M HCl and H_2 (7 bar) to give amine **13** in 98% yield. This one pot reduction and deprotection strategy gave clean product as evidenced by the ^1H NMR (Fig. 2.4) and ^{13}C NMR (Fig. 2.5) spectra of amino carbamate **13**. Here, characteristic NMR resonances were observed at (^1H δ) 4.64 (t) and 4.52 ppm (dd) for the H-1 methylene protons, 1_A and 1_B, respectively, which had a long range correlation according to HMBC, to the C-3 carbonyl peak (^{13}C δ 164.9 ppm). Also the CH_2NH_2 methylene protons at (^1H δ) 3.30 (dd) and 3.21 ppm (dd) for CH_ANH_2 and CH_BNH_2, respectively, correlated by HSQC to the upfield CH_2NH_2 carbon resonance at (^{13}C δ) 39.3 ppm (full characterisation details can be found in the experimental section).



12 **13** **1**

Scheme 2.4 Nitrile reduction and deprotection

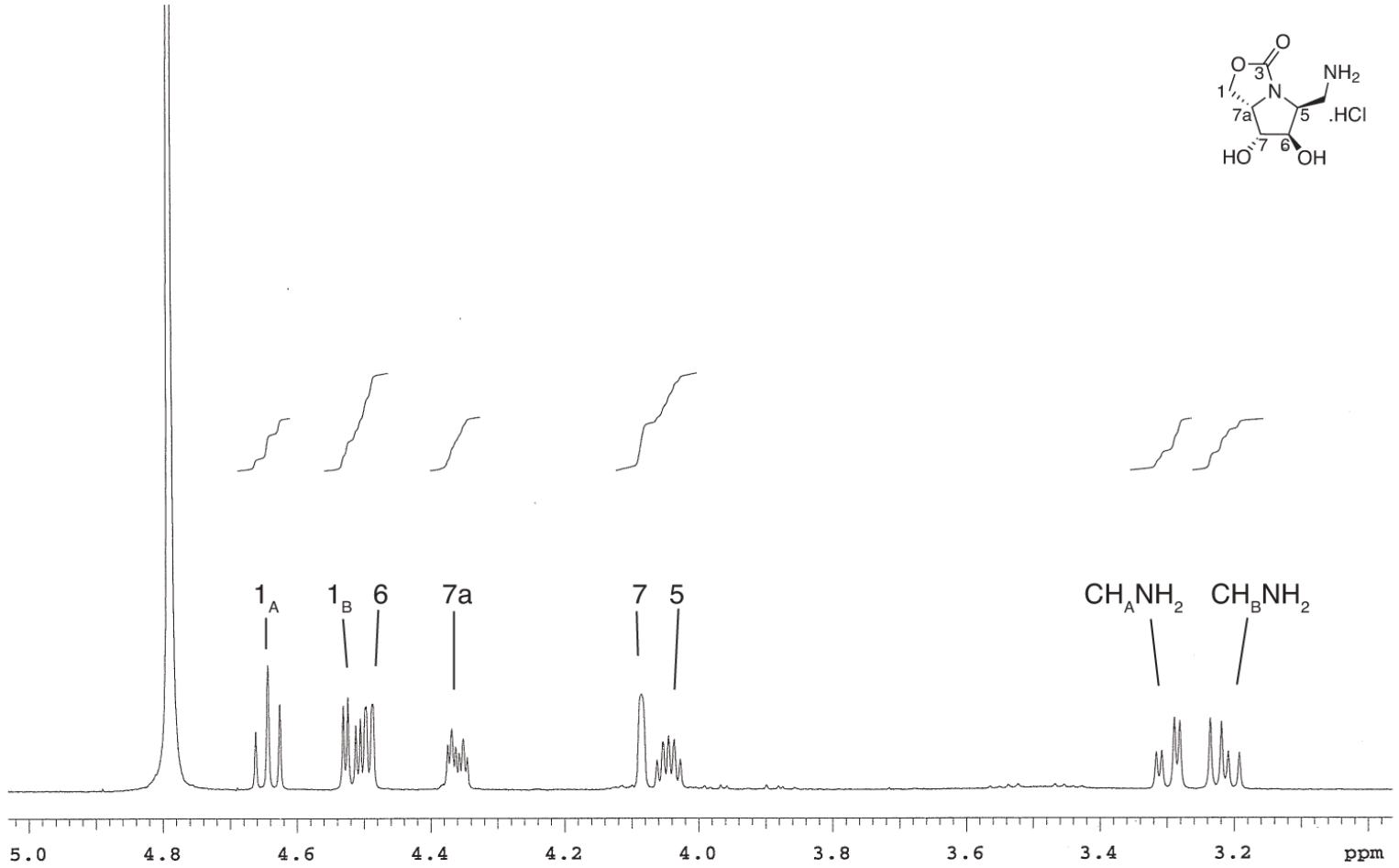


Fig. 2.4 ^{1}H NMR spectrum of amino carbamate **13**, (500 MHz, HCl salt in D_2O)

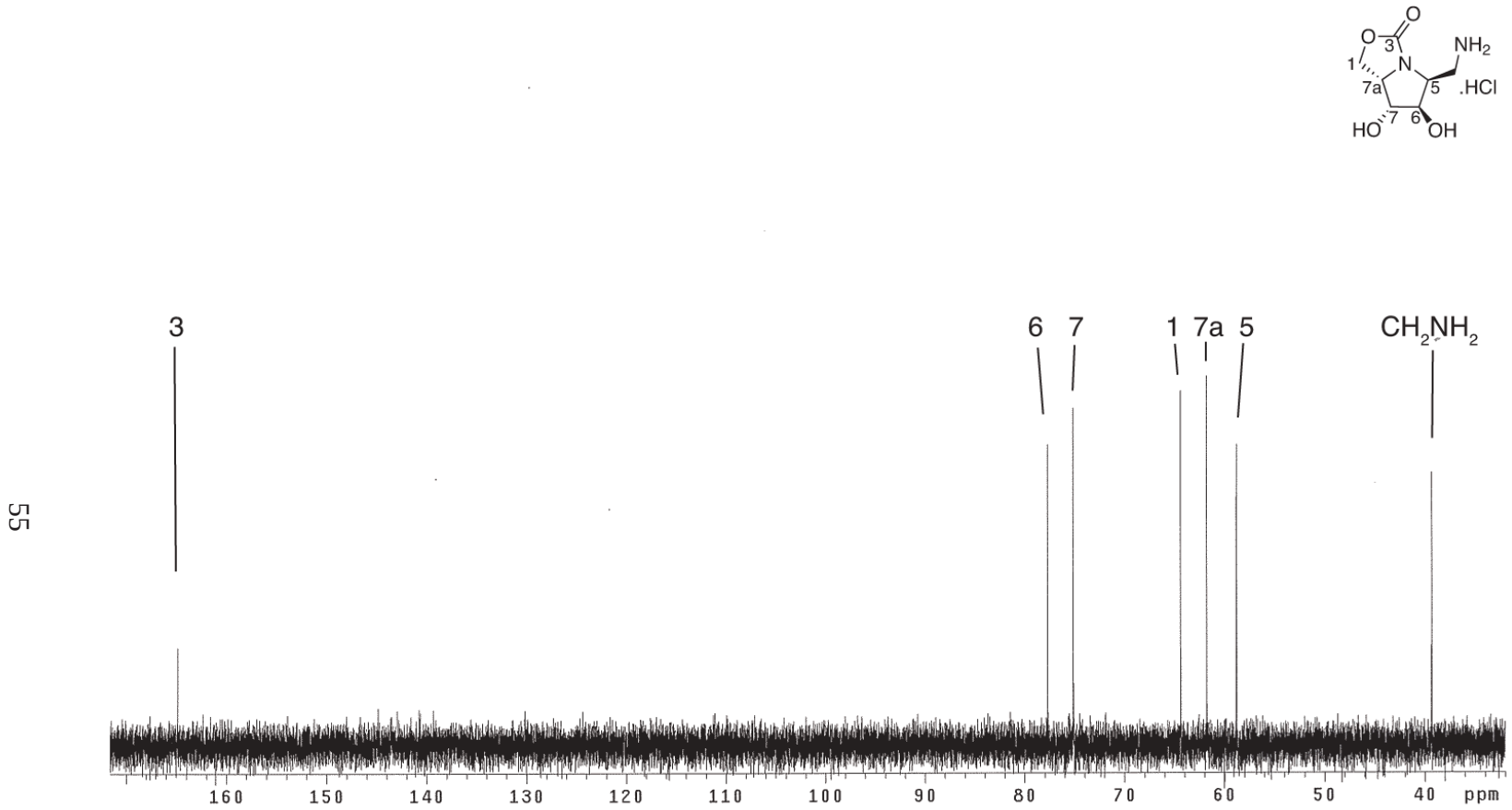
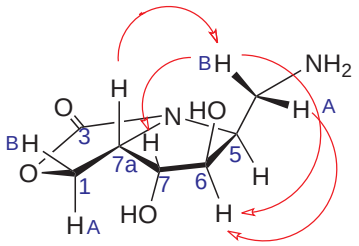


Fig. 2.5 ^{13}C NMR spectrum of amino carbamate **13**, (133 MHz, HCl salt in D_2O)

NOE correlations between the methylene proton H_B of the CH_2NH_2 group of **13**, the methine proton at C-7, the bridge proton at C-7a and the methine proton at C-6, and between the methylene proton H_A of the CH_2NH_2 and the methine proton at C-6 confirmed the stereochemistry of this product (Fig. 2.6). The carbamate in **13** was then hydrolysed using a solution of NaOH in EtOH, followed by neutralisation with DOWEX-H^+ to complete the synthesis and generate the known L-*ido* amino-imino-hexitol **1**,[13] in good (39%) overall yield from D-arabinose.



13

Fig. 2.6 NOE of amine **13**

2.4 Conclusion

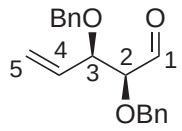
In summary, an efficient route for the preparation of the L-*ido* amino-imino-hexitol (**1**) has been developed. This has been demonstrated via the preparation of amino-imino-hexitol **1** in 10 steps from D-arabinose and a good 39% overall yield. During the course of this work, the power of a highly diastereoselective Strecker reaction, without the need for chiral Lewis acids or catalysts, has been demonstrated. The stereoselectivity of the Strecker reaction can be explained by the Cram-chelation control model to favour the *syn*-aminonitrile **2**. Furthermore, an I_2-mediated carbamate annulation has been optimised for alkenylamine *syn*-**2** to produce carbamate **12**. A primary iodoamine has also been identified as a key intermediate in the carbamate annulation reaction. Moreover, the potential of our novel carbamate annulation for the cyclisation of protected and functionalised alkenylamine precursors has greatly expanded the scope of this reaction.

2.5 Experimental

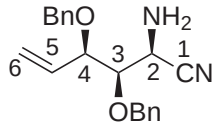
General Experimental

Unless otherwise stated all reactions were performed under air. THF was distilled from $LiAlH_4$ and DCM was distilled from P_2O_5 prior to use. All chemicals obtained from commercial suppliers were used without further purification. Zn dust was activated by the careful addition of conc. H_2SO_4, followed by decantation and washing with EtOH (3 x) and hexanes (3 x), and storage under dry hexanes. All solvents were removed by evaporation under reduced pressure. Reactions were monitored by TLC analysis on silica gel coated plastic sheets (0.20 mm, Polygram SIL G/UV254) with detection by coating with 20% H_2SO_4 in EtOH followed by charring at *ca.* 150 °C, by coating with a solution of ninhydrin in EtOH followed by charring at *ca.* 150 °C, by coating with Hanessian's stain followed by charring at *ca.* 150 °C, or by coating with a solution of 5% $KMnO_4$ and 1% $NaIO_4$ in H_2O followed by heating. Column chromatography was performed on silica gel (40-63 μm). Dowex Monosphere M-31 acidic resin was used for ion exchange chromatography. Melting points [°C] were obtained with Gallenkamp apparatus and are uncorrected. High-resolution mass spectra were recorded on a Waters Q-TOF Premier^{TM} Tandem Mass Spectrometer using positive electro-spray ionisation. Optical rotations were recorded using a Perkin-Elmer 241 or a Rudolf Research Analytical Autopol II polarimeter at the sodium D-line. Infrared spectra were recorded as thin films using a Bruker Tensor 27 FTIR spectrometer, equipped with an Attenuated Total Reflectance (ATR) sampling accessory, and are reported in wave numbers (cm^{-1}). Nuclear magnetic resonance spectra were recorded using a Varian Inova operating at 500 MHz for ^{1}H and 125 MHz ^{13}C or a Varian Direct Drive operating at 600 MHz and 133 MHz. ^{1}H and ^{13}C chemical shifts (δ) were internally referenced to

the residual solvent peak, ($CDCl_3$ = 7.26 ppm and 77.16 ppm, D_2O = 4.79 ppm). NMR peak assignments are based on 2D NMR experiments (COSY, HSQC, and HMBC).



(2S,3*R*)-2,3-Dibenzyloxypent-4-enal (7). To a solution of methyl iodo arabinoside **6**[9,17,18] (397 mg, 0.87 mmol) in EtOH/H_2O/AcOH (40/2/1, v/v/v, 13 mL) was added Zn (284 mg, 4.35 mmol). The mixture was stirred at reflux for 2 h, cooled to room temperature and filtered through a celite plug with EtOAc. The resulting mixture was further diluted with EtOAc and washed twice with sat. aq.˙ $NaHCO_3$, then H_2O and brine, dried ($MgSO_4$), filtered and concentrated *in vacuo* to provide aldehyde **7** as a colourless oil (251 mg, 0.85 mmol, 97%), which was used without further purification. R_f = 0.41 (hexanes/EtOAc, 3/1, v/v); $[\alpha]_D^{22}$ = -48.5 (c = 0.73, $CHCl_3$); IR (film) 3064, 3031, 1732, 1454, 1066, 735, 696 cm^{-1}. ^1H NMR: (500 MHz, $CDCl_3$, 20 °C) δ 9.68 (d, $J_{1,2}$ = 1.6 Hz, 1H, H-1), 7.35–7.26 (m, 10H, H-Ar), 5.94 (ddd, $J_{4,5}$ = 18.2, $J_{4,5a}$ = 10.5, $J_{3,4}$ = 7.8 Hz, 1H, H-4), 5.37 (ddd, $J_{4,5a}$ = 10.5, $^2J_{5a,5b}$ = 1.5, $^4J_{3,5a}$ = 1 Hz, 1H, H-5a), 5.35 (ddd, $J_{4,5b}$ = 18.2, $^2J_{5a,5b}$ = 1.5, $^4J_{3,5b}$ = 1 Hz, 1H, H-5b), 4.76 (d, $^2J_{a,b}$ = 11.9 Hz, 1H, CHa 2-*O*-Bn), 4.64 (d, $^2J_{a,b}$ = 12.2 Hz, 1H, CHa 3-*O*-Bn), 4.63 (d, $^2J_{a,b}$ = 11.9 Hz, 1H, CHb 2-*O*-Bn), 4.36 (d, $^2J_{a,b}$ = 12.2 Hz, 1H, CHb 3-*O*-Bn), 4.17 (ddt, $J_{3,4}$ = 7.8, $^2J_{2,3}$ = 4.2, $^4J_{3,5a}$ = $^4J_{3,5b}$ = 1 Hz, 1H, H-3), 3.83 (dd, $J_{2,3}$ = 4.2, $J_{1,2}$ = 1.6 Hz, 1H, H-2); ^{13}C NMR: (125 MHz $CDCl_3$, 20 °C) δ 202.7 (C-1), 137.6 (C-*i* 3-*O*-Bn), 137.2 (C-*i* 2-*O*-Bn), 133.9 (C-4), 128.6 (C-Ar), 128.5 (C-Ar), 128.3 (C-Ar), 128.3 (C-Ar), 128.0 (C-Ar), 127.9 (C-Ar), 120.0 (C-5), 85.3 (C-2), 80.0 (C-3), 73.6 (CH_2 2-*O*-Bn), 70.8 (CH2 3-*O*-Bn); HRMS (ESI) *m/z* calcd. for $[C_{19}H_{20}O_3+Na]^+$: 319.1305, obsd.: 319.1311.



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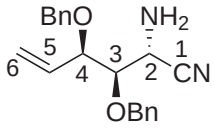
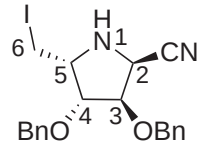


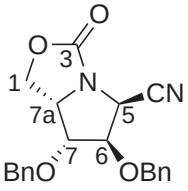
(2*S*,3*R*,4*R*)-2-Amino-3,4-dibenzyloxy-hex-5-enenitrile (*syn*-2) and (2*R*,3*R*,4*R*)-2-Amino-3,4-dibenzyloxy-hex-5-enenitrile (*anti*-3). To a solution of aldehyde **7** (32.0 mg, 0.11 mmol) in EtOH (2.2 mL) was added NH_4OAc (83.2 mg, 1.07 mmol). The mixture was stirred at room temperature for 15 min, at which point TMSCN (28.9 μL, 0.22 mmol) was added dropwise. The clear colourless solution was stirred at room temperature for 18 h, then diluted with EtOAc, washed with sat. aq. $NaHCO_3$, H_2O and brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification of the α-amino nitriles using silica gel gradient flash chromatography (hexanes/EtOAc, 10/1 $\rightarrow$ 1/1, v/v) first gave α-amino-nitrile *syn*-**2** as a pale yellow oil (27.5 mg, 0.09 mmol, 79%) and then α-amino-nitrile *anti*-**3** as a pale yellow oil (3.06 mg, 0.01 mmol, 9%). Crystallisation of *anti*-**3** (minimum EtOAc/hexanes) yielded fine colourless needles, for which a single crystal X-ray diffraction was obtained then solved and refined using Olex2.[34] *Syn*-**2**: R_f = 0.47 (hexanes/EtOAc, 1/1, v/v); $[\alpha\text{-}]_D^{22}$ = -1.08 (c = 0.90, $CHCl_3$); IR (film) 3393, 3327, 3064, 3031, 2868, 2361, 1497, 1067, 738, 698 cm^{-1}. ^{1}H NMR: (500 MHz, $CDCl_3$, 20 °C) δ 7.39–7.29 (m, 10H, H-Ar), 5.86 (ddd, $J_{5,6a}$ = 17.4, $J_{5,6b}$ = 10.4, $J_{4,5}$ = 7.7 Hz, 1H, H-5), 5.44 (ddd, $J_{5,6a}$ = 17.4, $^2J_{6a,6b}$ = 1.5, $^4J_{4,6a}$ = 1 Hz, 1H, H-6a), 5.41 (ddd, $J_{5,6b}$ = 10.4, $^2J_{6a,6b}$ = 1.5, $^4J_{4,6b}$ = 0.7 Hz, 1H, H-6b), 4.91 (d, $^2J_{a,b}$ = 11.0 Hz, 1H, CHa 3-*O*-Bn), 4.71 (d, $^2J_{a,b}$ = 11.0 Hz, 1H, CHb 3-*O*-Bn), 4.64 (d, $^2J_{a,b}$ = 11.7 Hz, 1H, CHa 4-*O*-Bn), 4.40 (d, $^2J_{a,b}$ = 11.7 Hz, 1H, CHb 4-*O*-Bn), 4.16 (dd, $J_{4,5}$ = 7.7, $J_{3,4}$ = 6.8 Hz, 1H, H-4), 3.89 (d, $J_{2,3}$ = 3.3 Hz, 1H, H-2), 3.70 (dd, $J_{3,4}$ = 6.8, $J_{2,3}$ = 3.3 Hz, 1H, H-3), 1.63 (br s, 2H, NH2); ^{13}C NMR: (125 MHz $CDCl_3$, 20 °C) δ 138.0 (C-*i* 4-*O*-Bn), 137.7 (C-*i* 3-*O*-Bn), 134.6 (C-5), 128.6 (C-Ar), 128.6 (C-Ar), 128.5 (C-Ar), 128.2 (C-Ar), 128.1

(C-Ar), 127.9 (C-Ar), 121.3 (C-1), 120.5 (C-6), 81.8 (C-3), 81.0 (C-4), 75.7 (CH_2 3-*O*-Bn), 71.1 (CH_2 4-*O*-Bn), 44.7 (C-2); HRMS (ESI) *m/z* calcd. for $[C_{20}H_{22}N_2O_2+Na]^+$: 345.1573, obsd.: 345.1572. *Anti*-**3**: R_f = 0.38 (hexanes/EtOAc, 1/1, v/v); MP = 64.5 – 64.7 °C; $[\alpha\text{-}]_D^{23}$ = -15.5 (c = 1.0, $CHCl_3$); IR (film) 3389, 3325, 3064, 3031, 2919, 2870, 2358, 1497, 1068, 737, 698 cm^{-1}. ^{1}H NMR: (500 MHz, $CDCl_3$, 20 °C) δ 7.36–7.30 (m, 10H, H-Ar), 5.83 (ddd, $J_{5,6b}$ = 17.5, $J_{5,6a}$ = 10.5, $^2J_{4,5}$ = 7.9 Hz, 1H, H-5), 5.49 (dd, $J_{5,6a}$ = 17.5, $^2J_{6a,6b}$ = 1 Hz, 1H, H-6a), 5.44 (dd, $J_{5,6b}$ = 10.5, $J_{6a,6b}$ = 1 Hz, 1H, H-6b), 4.96 (d, $^2J_{a,b}$ = 11.4 Hz, 1H, CHa 3-*O*-Bn), 4.72 (d, $^2J_{a,b}$ = 11.4 Hz, 1H, CHb 3-*O*-Bn), 4.64 (d, $^2J_{a,b}$ = 11.6 Hz, 1H, CHa 4-*O*-Bn), 4.41 (d, $^2J_{a,b}$ = 11.6 Hz, 1H, CHb 4-*O*-Bn), 4.13 (t, $J_{4,5}$ = 7.9, $J_{3,4}$ = 6.6 Hz, 1H, H-4), 3.80 (d, $J_{2,3}$ = 4.4 Hz, 1H, H-2), 3.64 (dd, $J_{3,4}$ = 6.6, $J_{2,3}$ = 4.4 Hz, 1H, H-3), 1.68 (br s, 2H, NH_2); ^{13}C NMR: (125 MHz $CDCl_3$, 20 °C) δ 138.0 (C-*i* 4-*O*-Bn), 137.9 (C-*i* 3-*O*-Bn), 133.9 (C-5), 128.6 (C-Ar), 128.6 (C-Ar), 128.3 (C-Ar), 128.1 (C-Ar), 127.9 (C-Ar), 121.3 (C-1), 120.4 (C-6), 82.1 (C-3 and C-4), 75.5 (CH_2 3-*O*-Bn), 71.0 (CH_2 4-*O*-Bn), 45.3 (C-2); HRMS (ESI) *m/z* calcd. for $[C_{20}H_{22}N_2O_2+Na]^+$: 345.1573, obsd.: 345.1573.



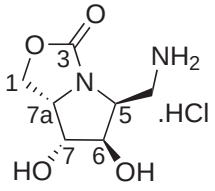
(2*S*,3*R*,4*R*,5*R*)-3,4-Bisbenzyloxy-5-iodomethylpyrrolidine-2-carbonitrile (11). α-Aminonitrile *syn*-**2** (30.0 mg, 0.09 mmol) was co- evaporated with toluene and dissolved in DCM (450 μL) under a nitrogen atmosphere. N-Iodosuccinimide (25.1 mg, 0.11 mmol) was added, and the pink solution was stirred at room temperature for 5 h, over which time the solution became dark red in color. The solution was quenched with sat. aq. $Na_2S_2O_3$ and extracted with EtOAc, and the organic layer was washed with H_2O and brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The

residue was purified using silica gel gradient flash chromatography (hexanes/ EtOAc, 20/1 → 3/1) to give iodoazasugar **11** as a pale orange oil (31.3 mg, 0.07 mmol, 75%). R_f = 0.52 (hexanes/EtOAc, 2/1). $[\alpha]_D^{20.6}$ = -21.0 (c = 1.0, $CHCl_3$). IR (film) 3336, 3062, 3030, 2922, 2867, 2245, 1496, 1454, 1354, 1207, 1091, 1027, 913, 736, 697 cm^{-1}. ^1H NMR: (500 MHz, $CDCl_3$, 20 °C) δ 7.39–7.28 (m, 10 H, H-Ar), 4.66 (d, $^2J_{a,b}$ = 11.8 Hz, 1H, CHa 3-*O*-Bn), 4.59 (d, $^2J_{a,b}$ = 11.8 Hz, 1H, CHb 3-*O*-Bn), 4.54 (d, $^2J_{a,b}$ = 11.6 Hz, 1H, CHa 4-*O*-Bn), 4.51 (d, $^2J_{a,b}$ = 11.6 Hz, 1H, CHb 4-*O*-Bn), 4.27 (d, $J_{4,5}$ = 5.4 Hz, 1H, H-2), 4.09 (dd, $J_{2,3}$ = 5.4 Hz, $J_{3,4}$ = 2.7 Hz, 1H, H-3), 4.03 (dd, $J_{4,5}$ = 5.4 Hz, $J_{3,4}$ = 2.7 Hz, 1H, H-4) 3.84 (td, $J_{5,6a}$ = $J_{5,6b}$ = 7.3 Hz, $J_{4,5}$ = 5.4 Hz, 1H, H-5), 3.27 (dd, $^2J_{6a,6b}$ = 9.6 Hz, $J_{5,6a}$ = 7.3 Hz, 1H, H-6a), 3.15 (dd, $^2J_{6a,6b}$ = 9.6 Hz, $J_{5,6b}$ = 7.3 Hz, 1H, H-6b), 2.32 (br. s, 1H, NH) ppm. ^{13}C NMR: (125 MHz $CDCl_3$, 20 °C) δ 137.3 (C-*i* 4-*O*-Bn), 136.8 (C-*i* 3-*O*-Bn), 128.8 (C-Ar), 128.7 (C-Ar), 128.5 (C-Ar), 128.3 (C-Ar), 128.3 (C-Ar), 128.1 (C-Ar), 117.9 (CN), 82.4 (C-3), 81.8 (C-4), 73.1 (CH_2 3,4-*O*-Bn), 61.5 (C-5), 51.4 (C-2), 4.1 (C-6); HRMS (ESI) *m/z* calcd. for $[C_{20}H_{21}IN_2O_2+Na]^+$: 449.0720: found 449.0726.



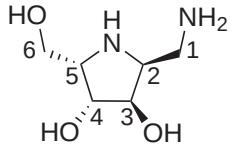
(5*S*,6*R*,7*R*,7a*S*)-6,7-Dibenzyloxy-3-oxo-tetrahydro-pyrrolo[1,2-c]-oxaza-5-carbonitrile (12). To a solution of α-amino-nitrile *syn*-**2** (34.0 mg, 0.11 mmol) in THF (0.4 mL), was added I_2 (66.9 mg, 0.26 mmol), H_2O (0.4 mL) and $NaHCO_3$ (177 mg, 2.11 mmol). The reaction mixture was stirred at room temperature for 20 h, quenched via the addition of sat. aq. $Na_2S_2O_3$ and extracted with EtOAc. The organic layer was washed with H_2O and brine, dried ($MgSO_4$), filtered and concentrated

in vacuo. Purification of the residue using silica gel gradient flash chromatography (hexanes/EtOAc, 10/1 → 1/1, v/v) provided carbamate **12** as a colourless viscous oil (32.6 mg, 0.09 mmol, 85%). R_f = 0.24 (hexanes/EtOAc, 2/1, v/v); $[\alpha]_D^{20}$ = +23.7 (c = 1.0, $CHCl_3$); IR (film) 3064, 3032, 2925, 2872, 2254, 1755, 1469, 1316, 1216, 1093, 1051, 1001, 814, 736, 698 cm^{-1}. ^1H NMR: (500 MHz, $CDCl_3$, 20 °C) δ 7.43–7.32 (m, 10H, H-Ar), 7.16 (m, 2H, H-Ar), 4.75 (d, $^2J_{a,b}$ = 12 Hz, 1H, CHa 6-*O*-Bn), 4.76 (d, $J_{2,3}$ = 5.2 Hz, 1H, H-5), 4.56 (d, $^2J_{a,b}$ = 12 Hz, 1H, CHb 6-*O*-Bn), 4.50 (d, $^2J_{a,b}$ = 12.6, Hz, 1H, CHa 7-*O*-Bn), 4.45 (t, $^2J_{1a,1b}$ = 8.7, $J_{1a,7a}$ = 8.2 Hz, 1H, H-1a), 4.38 (t, $^2J_{1a,1b}$ = 6.2, $J_{1b,7a}$ = 3.0 Hz, 1H, H-1b), 4.35 (d, $^2J_{a,b}$ = 12.6 Hz, 1H, CHb 7-*O*-Bn), 4.27 (dt, $J_{1a,7a}$ = 8.2, $J_{1b,7a}$ = $J_{7,7a,}$ = 3.0 Hz, 1H, H-7a), 4.23 (dd, $J_{5,6}$ = 5.2, $J_{6,7}$ = 1.0 Hz, 1H, H-6), 3.77 (dd, $J_{7,7a}$ = 3.0, $J_{6,7}$ = 1.0 Hz, 1H, H-7); ^{13}C NMR: (125 MHz $CDCl_3$, 20 °C) δ 161.0 (C-3), 136.5 (C-*i* 7-*O*-Bn), 136.3 (C-*i* 6-*O*-Bn), 128.9 (C-Ar), 128.9 (C-Ar), 128.7 (C-Ar), 128.6 (C-Ar), 128.3 (C-Ar), 127.9 (C-Ar), 115.4 (CN), 81.6 (C-6), 79.9 (C-7), 73.8 (CH_2 6-*O*-Bn), 72.0 (CH_2 7-*O*-Bn), 63.2 (C-1), 61.3 (C-7a), 53.2 (C-5); HRMS (ESI) *m/z* calcd. for $[C_{21}H_{20}N_2O_2+Na]^+$: 387.1315, obsd.: 387.1317.



(5*S*,6*R*,7*R*,7a*S*)-5-Aminomethyl-6,7-dihydroxy-tetrahydro-pyrrolo [1,2-c]oxazol-3-one hydrochloride (13). Carbamate **12** (100 mg, 0.27 mmol) was dissolved in $CHCl_3$ (4.5 mL) and transferred into a Fischer-Porter bottle, EtOH (7.5 mL), HCl in ether (1.4 mL 2M) and $Pd(OH)_2/C$ (230 mg) were then added and the vessel was charged with H_2 (7 bar). The reaction mixture was stirred vigorously at room temperature for 3 d. After releasing the H_2 pressure, the reaction mixture was

filtered through a plug of celite, washing with EtOH and H_2O, and concentrated *in vacuo* to provide amine **13** as the HCl salt (50.1 mg, 0.26 mmol, 98%). R_f = 0.25 (DCM/MeOH/EtOH/30% aq. NH_3, 5/2/2/1, v/v); $[\alpha]_D^{19}$ = -50.0 (c = 0.1, H_2O); IR (film) 3928, 2925, 2854, 1727, 1642, 1404, 1243, 1083, 1014, 783 cm^{-1}. ^1H NMR: (500 MHz, 2% DCl in D_2O, 20 °C) δ 4.64 (t, $^2J_{1a,1b}$ = 9.2, $J_{1a,7a}$ = 8.9 Hz, 1H, H-1a), 4.52 (dd, $^2J_{1a,1b}$ = 9.2, $J_{1b,7a}$ = 3.3 Hz, 1H, H-1b), 4.49 (dd, $J_{5,6}$ = 4.4, $J_{6,7}$ = 0.5 Hz, 1H, H-6), 4.36 (dt, $J_{1a,7a}$ = 8.9, $J_{1b,7a}$ = 3.3, $J_{7,7a}$ ¡1 Hz, 1H, H-7a), 4.09 (br s, 1H, H-7), 4.04 (dt, $J_{CHbNH2,5}$ = 8.5, $J_{5,6}$ = $J_{CHaNH2,5}$ = 4.4 Hz, 1H, H-5), 3.30 (dd, $^2J_{a,b}$ = 13.4, $J_{CHaNH2,5}$ = 4.4 Hz, 1H, $CHaNH_2$), 3.21 (dd, $^2J_{a,b}$ = 13.4, $J_{CHbNH2,5}$ = 8.5 Hz, 1H, H-$CHbNH_2$); ^{13}C NMR: (125 MHz, 2% DCl in D_2O, 20 °C) δ 164.9 (C-3), 77.7 (C-6), 75.1 (C-7), 64.4 (C-1), 61.8 (C-7a), 58.8 (C-5), 39.3 (CH_2NH_2); HRMS (ESI) *m/z* calcd. for $[C_7H_{12}N_2O_4+Na]^+$: 211.0689, obsd.: 211.0684.



1-Amino-1,2,5-trideoxy-2,5-imino-L-iditol (1). A solution of NaOH in EtOH (0.5 ml, 2M) was added to amino carbamate **13** (16.0 mg, 0.09 mmol) and stirred at reflux for 2 h. The resulting reaction mixture was loaded directly onto a Dowex-H^+ ion-exchange resin column and washed with H_2O to remove excess salt. The hydrolysed amine was then eluted with 30% aq.˙ NH_3 and concentrated *in vacuo* to provide amino hexitol **1** (13.5 mg, 0.08 mmol, 98%). R_f = 0.01 (DCM/MeOH/EtOH/30% aq. NH_3, 5/2/2/1, v/v); (HCl salt) $[\alpha]_D^{26}$ = +6.0 (c = 1.0, H_2O); IR (film, free base) 3376, 2881, 2854, 1651, 1549, 1393, 1218, 1115, 1059, 946, 836 cm^{-1}. ^1H NMR: (500 MHz, 2% NaOD in D_2O, 20 °C) δ 4.14 (d, $J_{2,3}$ = 4.0, $J_{3,4}$ <1 Hz, 1H, H-3), 4.09 (d, $J_{4,5}$ = 4.0, $J_{3,4}$ <1 Hz, 1H, H-4), 3.73 (dd, $^2J_{6a,6b}$ = 11.2, $J_{5,6a}$ = 6.5

Hz, 1H, H-6a), 3.64 (dd, $^{2}J_{6a,6b}$ = 11.2, $J_{5,6b}$ = 6.5 Hz, 1H, H-6b), 3.35 (td, $J_{5,6a}$ = $J_{5,6b}$ = 6.5, $J_{4,5}$ = 4.0 Hz, 1H, H-5), 3.28 (td, $J_{1a,2}$ = $J_{1b,2}$ = 6.9, $J_{2,3}$ = 4.0 Hz, 1H, H-2), 2.77 (dd, $^{2}J_{1a,1b}$ = 12.7, $J_{1a,2}$ = 6.9 Hz, 1H, H-1a), 2.66 (dd, $^{2}J_{1a,1b}$ = 12.7, $J_{1b,2}$ = 6.9 Hz, 1H, H-1b); ^{13}C NMR: (125 MHz, 2% NaOD in D_2O, 20 °C) δ 77.3 (C-3), 77.0 (C-4), 61.1 (C-5), 60.4 (C-6), 59.9 (C-2), 40.1 (C-1); HRMS (ESI) *m/z* calcd. for $[C_6H_{14}N_2O_3+H]^+$: 163.1077, obsd.: 163.1077. ^{1}H NMR (500 MHz, D_2O, 2HCl salt, 20 °C) δ 4.41 (dd, $J_{2,3}$ = 3.4, $J_{3,4}$ = 1.7 Hz, 1H, H-3), 4.40 (dd, $J_{4,5}$ = 3.6, $J_{3,4}$ = 1.7 Hz, 1H, H-4), 4.17 (td, $J_{1a,2}$ = $J_{1b,2}$ = 6.8, $J_{2,3}$ = 3.4 Hz, 1H, H-2), 4.07 (ddd, $J_{5,6a}$ = 8.8, $J_{5,6b}$ = 4.6, $J_{4,5}$ = 3.6 Hz, 1H, H-5), 4.00 (dd, $^{2}J_{6a,6b}$ = 12.2, $J_{5,6a}$ = 4.6 Hz, 1H, H-6a), 3.91 (dd, $^{2}J_{6a,6b}$ = 12.2, $J_{5,6b}$ = 8.8 Hz, 1H, H-6b), 3.58 (dd, $^{2}J_{1a,1b}$ = 13.7, $J_{1a,2}$ = 6.8 Hz, 1H, H-1a), 3.49 (dd, $^{2}J_{1a,1b}$ = 13.7, $J_{1b,2}$ = 6.8 Hz, 1H, H-1b); ^{13}C NMR (125 MHz, D_2O, 2HCl salt, 20 °C) δ 74.5 (C-3), 74.2 (C-4), 63.8 (C-5), 58.2 (C-2), 57.2 (C-6), 35.8 (C-1).

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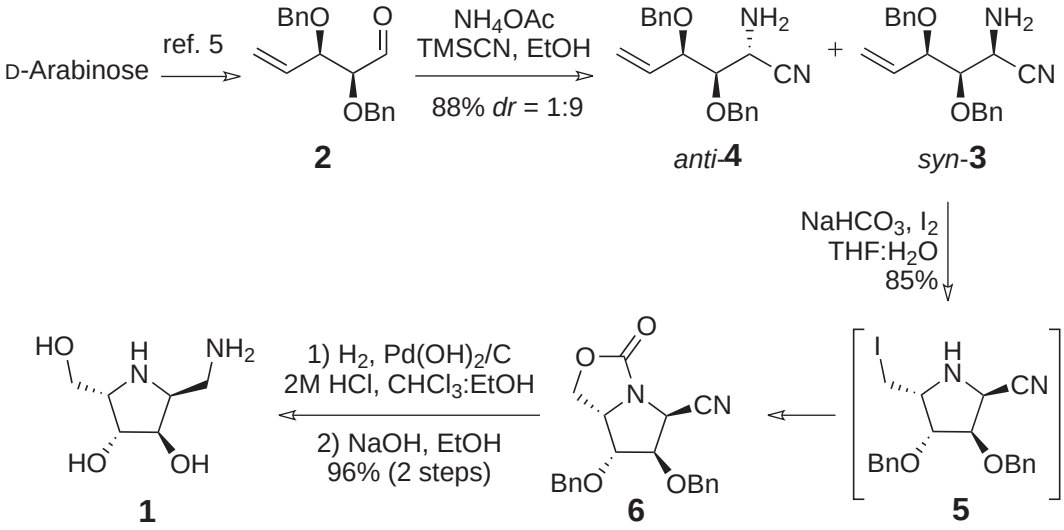
Chapter 3

Application of I_2-mediated Carbamate Annulation to the Synthesis of Amino-imino-hexitols

3.1 Introduction

Given the potential of pyrrolidines as glycosidase inhibitors, (see Sections 1.1 and 1.2 and references cited therein), we were interested in developing efficient methodology for the preparation of amino-imino-hexitols. To this end, we demonstrated that the combination of a diastereoselective Strecker reaction and the recently developed I_2-promoted carbamate annulation methodology[1–3] allowed for the synthesis of the L-*ido* derived amino-imino-hexitol **1** in 39% overall yield from D-arabinose (Scheme 3.1).[4] Here, we illustrated that benzyl-protected aldehyde **2**, readily prepared from D-arabinose, underwent a highly diastereoselective Strecker[5] reaction to give the *syn*-**3**

and *anti*-**4** alkenylamines (*dr* = 9:1), which were readily separated by flash column chromatography. The major *syn*-isomer was subjected to I_2 and excess $NaHCO_3$ in THF:H_2O to give, by way of intermediate iodide **5**, the 4,5-*cis* carbamate **6** in excellent yield and diastereoselectivity (*dr* >95:5). Treatment of carbamate **6** with $Pd(OH)_2$/C in the presence of 2M HCl followed by hydrolysis then gave known L-*ido* amino-imino-hexitol **1**.[4]



Scheme 3.1 Preparation of **1** from D-arabinose

To further explore this methodology, we herein report[6] on the application of our carbamate annulation to the synthesis of other amino-imino-hexitols including 1-amino-1,2,5-trideoxy-2,5-imino-D-mannitol[7] and 1-amino-1,2,5-trideoxy-2,5-imino-D-glucitol,[7] and the previously undisclosed stereoisomers with the D-*galacto* **7**, D-*talo* **8**, and L-*altro* **9** configurations (Fig. 3.1). Factors influencing the diastereoselectivity of the I_2-mediated carbamate annulation will also be noted.

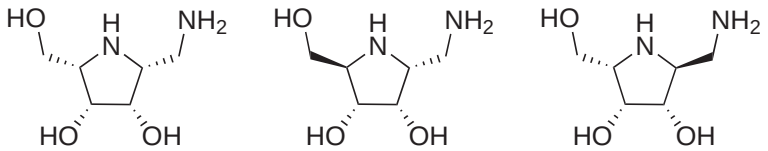
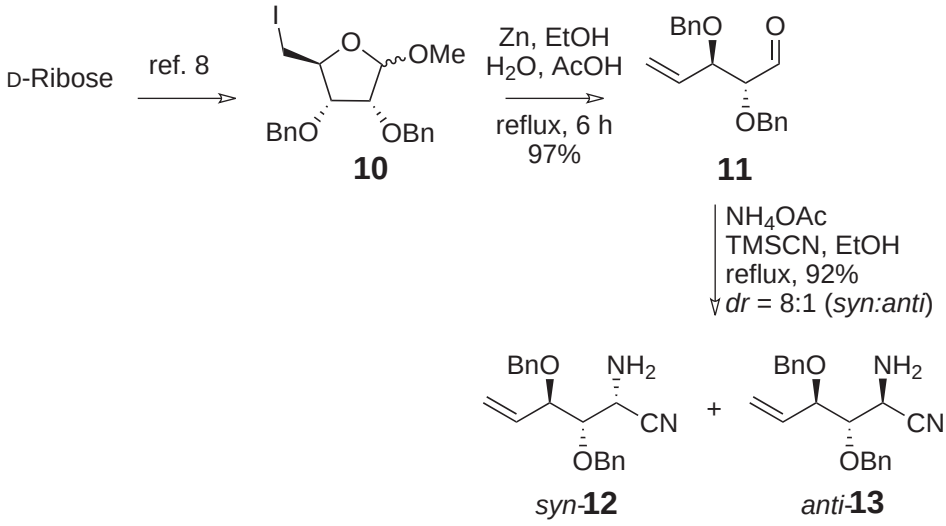


Fig. 3.1 Novel amino-imino-hexitols

3.2 Synthesis of Amino-imino-hexitols

To gain access to the 3,4-*cis*-series of hydroxylated iminosugars, we required the 3,4-*anti*-substituted α-aminonitrile precursors, and consequently, D-ribose as the starting material (Scheme 3.2). Methyl iodoriboside **10** was conveniently prepared according to literature precedent[8] and subjected to a Vasella reaction[9,10] to give aldehyde **11** in excellent (97%) yield. Aldehyde **11** was then treated with NH_4OAc and TMSCN to give α-aminonitrile *syn*-**12**, the chelation-controlled Cram product, as the major diastereomer (*syn*-**12**:*anti*-**13**, 8:1) and in excellent (92%) overall yield. It is interesting to note that the *syn*-stereoselectivity of the Strecker reaction does not appear to be affected by the relative configuration between C-2 and C-3 of the linear aldehyde, with similar diastereoselectivities being obtained for both the arabinose (*cf.* Scheme 3.1)[4] and ribose derived aldehydes. The diastereomers, *syn*-**12** and *anti*-**13**, were readily separated via flash column chromatography (hexanes:EtOAc, 2:1; R_f = 0.27 for α-aminonitrile *syn*-**12**, R_f = 0.16 for α-aminonitrile *anti*-**13**) and their relative configurations confirmed following subsequent cyclisation (*vide infra*).

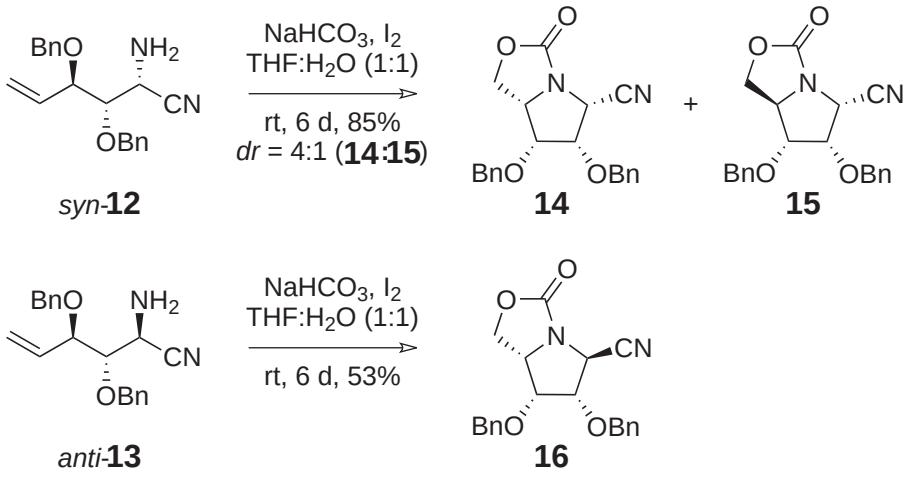
The major Strecker product *syn*-aminonitrile **12** was then subjected to a carbamate annulation via treatment with $NaHCO_3$ and I_2 (Scheme 3.3). Surprisingly, in contrast to the usual high selectivity of this reaction[1–4] two diastereomeric carbamates **14** and



Scheme 3.2 Preparation of α-aminonitriles commencing with D-ribose

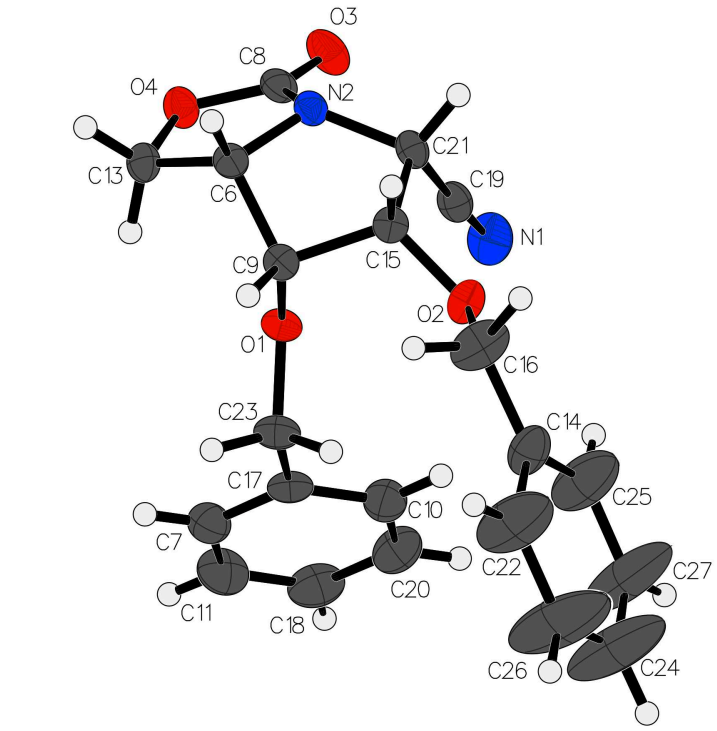
15 (*dr* = 4:1) were formed. Here, the major carbamate was the 4,5-*cis*-isomer **14**, as confirmed by X-ray analysis (Fig. 3.2(a)). Cyclisation of the minor Strecker product, *anti*-aminonitrile **13**, however, gave only one diastereomer **16**, in 53% yield. This was also crystallised and a crystal structure obtained (Fig. 3.2(b)). Though the yield of *anti*-aminonitrile **13** was modest, there was no evidence by ^{1}H NMR of the other carbamate diastereomer or the intermediate primary iodide. ^{1}H NMR of the crude reaction mixture, however, did indicate that retro-Strecker products (e.g. aldehydes) were formed in addition to the desired product. It is also interesting that both ribose-derived alkenylamines took longer to cyclise (6 days), when compared to the cyclisation of arabinose-derived *syn*-**3** (20 h). This suggests that the activation energies for the formation of **14**, **15** and **16** are higher than for the formation of **10**, which could be due to steric constraints encountered by the benzyloxy groups at C-3 and C-4 when transforming from the linear (3,4-*anti*) to cyclised (3,4-*cis*) structures. In earlier studies by Tamaru *et al.*,[11] it was also observed that cyclisation of 1,2-alkoxy substituted

pentenylic alcohols proceeded much more slowly when forming the 2,5-*cis*, as compared to the 2,5-*trans*, products.

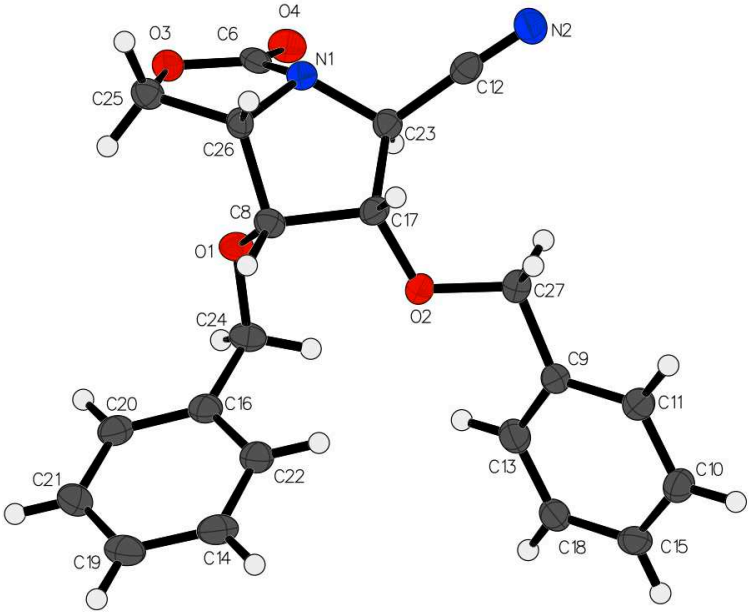


Scheme 3.3 Carbamate annulations with D-ribose-derived α-aminonitriles

To further tease out the effects that the substituents have on the diastereoselectivity of the annulation, the minor arabinose derived aminonitrile *anti*-**17** was also subjected to the carbamate annulation conditions (Scheme 3.4). Here, cyclisation resulted in the generation of two carbamate products (**18**:**19**, *dr* = 6:1) following stirring at room temperature for 48 h, with the configuration of each diastereomer being determined at subsequent stages in the synthesis (*vide infra*). A full discussion of the observed diastereoselectivities of the I_2-mediated carbamate annulation will be provided in chapters five and six.

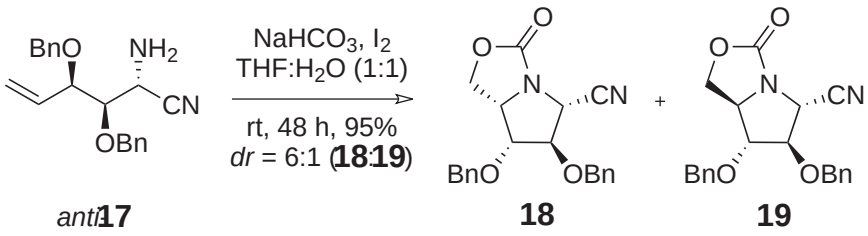


(a)



(b)

Fig. 3.2 Crystal structures of carbamates **14** (a) and **16** (b)



anti-**17** **18** **19**

Scheme 3.4 I_2-mediated carbamate annulation using D-arabinose derived *anti*-**17**

Having noted the interesting diastereoselectivity of the carbamate annulation, we were then interested in using the said carbamates as intermediates for the synthesis of amino-imino-hexitols. To this end, carbamate **14** was first hydrogenated using $Pd(OH)_2/C$ in the presence of 2M HCl with the objective of simultaneously removing the benzyl groups and reducing the nitrile to the amine (Scheme 3.5). According to TLC analysis, this reaction appeared to proceed smoothly and following silica gel chromatography (DCM/EtOH/MeOH/30% aq. NH_3, 5/2/2/1, v/v/v/v) only one product was observed. HRMS also gave a molecular ion of 189.0869 $[M+H]^+$ corresponding to the expected $[C_7H_{12}N_2O_4+H]^+$. Close analysis of the NMR spectral data, however, revealed the presence of a carbonyl peak at δ 164.0 ppm in the ^{13}C NMR spectra, which correlated by HMBC to two methylene protons at δ 3.59 and 3.49 ppm (Fig. 3.3) instead of the downfield methylene protons at δ 4.13 and 3.72 ppm. This HMBC correlation indicated that the carbonyl was next to a CH_2NH group rather than a CH_2O group due to the comparative upfield proton chemical shifts. Further analysis by 2D NMR (COSY, HMBC, HSQC) revealed that, rather than the desired amino sugar, urea-derivative **20** was formed in 30% yield (unoptimised). We presume the urea was formed via the ring closure of the intermediate amine on the carbamate in **21** due to the *cis*-relationship across the ring nitrogen, which allowed the basic amino group close proximity to the carbonyl for migration. After silica gel chromatography the product fractions

required heating *in vacuo* to remove the aqueous solvents, this could have aided in the transformation from carbamate **21** to urea **20**.

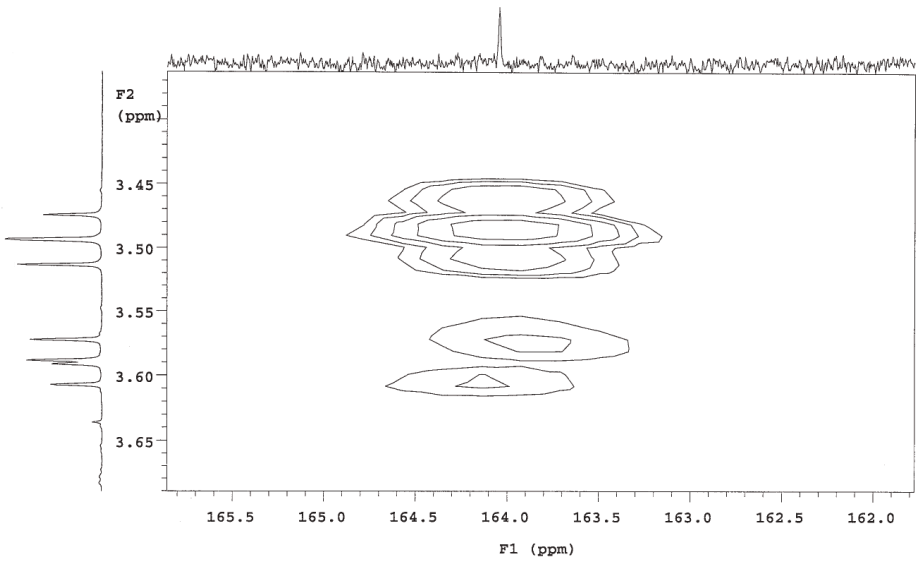
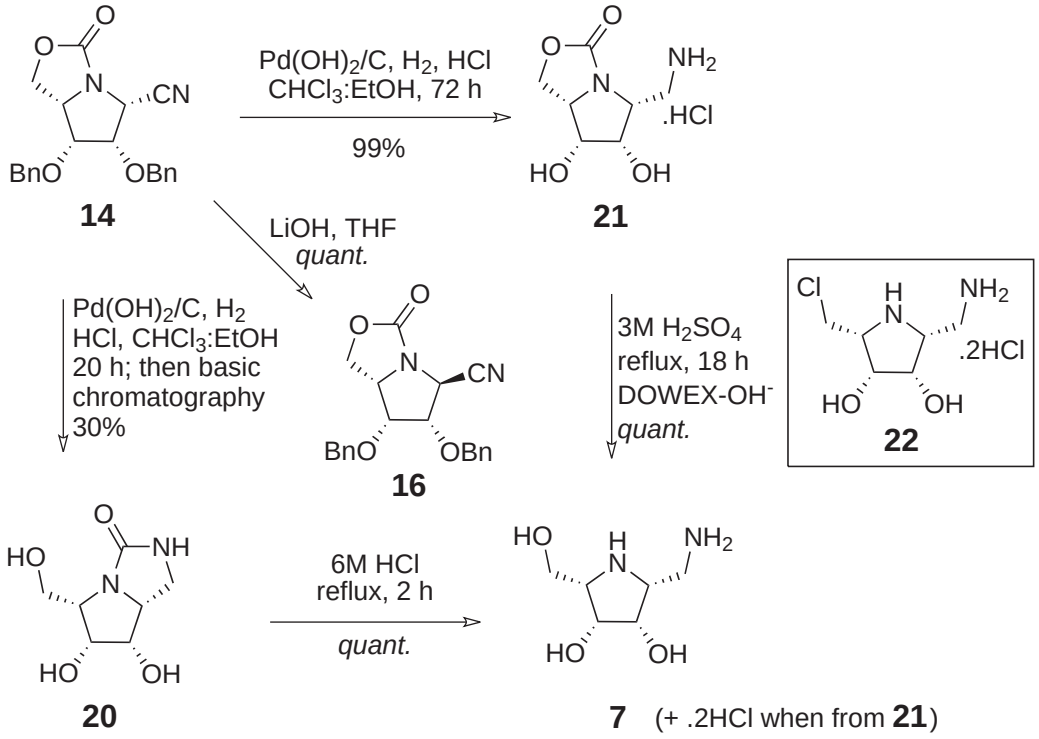


Fig. 3.3 HMBC spectrum of urea **20**, (500 MHz in D_2O)



Scheme 3.5 Deprotection of carbamate **14**

An attempt was made to prevent urea formation by changing the deprotection order through the hydrolysis of carbamate **14** with LiOH, however, this led to epimerisation of the proton α to the nitrile and carbamate **16** was isolated in quantitative yield. Carbamate **16** was identified by analysis of the ^{1}H and ^{13}C NMR spectra. Features of note were a change of chemical shift for the H-5 epimerised proton from δ (^{1}H) 4.52 in **14** to 4.57 ppm and a shift of the carbonyl peak from δ (^{13}C) 157.4 in **14** to 160.8 ppm. While not effecting the desired transformation, this epimerisation to favour the thermodynamically more stable *trans* relationship between the carbamate and the nitrile, provides a high yielding route for the preparation of carbamate **16**. Previously, carbamate **16** could only be prepared from the minor ribose-derived Strecker product. The hydrogenation of carbamate **14** using $Pd(OH)_2/C$ was then repeated, and during workup it was observed

that if the amine remained protonated, by keeping the conditions acidic throughout, the desired amine functionalised carbamate **21** could be obtained in excellent (99%) yield and without the need for further purification. Attempts to hydrolyse carbamate **21** under acidic conditions then followed but surprisingly the use of 6M HCl gave chloro-amino-imino-hexitol **22** as the major product and only minor amounts of the desired D-*galacto* amino-imino-hexitol **7**. Acidic conditions were needed to hydrolyse the carbamate in **21** as using the previously used basic conditions would lead to formation of the urea derivative **20**. Urea **20** was very stable under the basic hydrolysis conditions that were tried earlier. Though a potentially useful synthetic intermediate, chloride **22** decomposed upon purification by silica gel chromatography and thus could not be isolated in sufficient purity for subsequent biological assessment. Subjecting carbamate **21** to 3M H_2SO_4 at reflux, however, followed by neutralisation with basic DOWEX allowed for amino-imino-hexitol **7** to be obtained in quantitative yield as the free base. Urea-derivative **20** could also be quantitatively converted to the desired amino-imino-hexitol **7** as the hydrochloride salt following treatment with refluxing 6M HCl. Using the optimised route (**14** $\rightarrow$ **21** $\rightarrow$ **7**), the target amino-imino-hexitol **7**, can now be prepared in 36% overall yield from D-ribose.

Armed with this knowledge, the remaining carbamates were then transformed into their corresponding amino-imino-hexitols (Scheme 3.6). Treatment of carbamate **15** with $Pd(OH)_2/C$ in the presence of 2M HCl led to the smooth conversion into amine **23** (98% yield). The carbamate in **23** was then hydrolysed using a solution of NaOH in EtOH, followed by neutralisation with DOWEX-H^+ to complete the synthesis and generate novel D-*talo* amino-imino-hexitol **8**. Here, it is important to note that when there is a 2,5-*trans* relationship between the aminomethyl and carbamate moieties, the

corresponding urea derivative is not formed and base-mediated hydrolysis can be used. Amino-imino-hexitol **8** was characterised by a combination of NMR spectroscopic (^{1}H, ^{13}C, COSY, HSQC, HMBC) analysis and mass spectrometry (full characterisation can be found in the experimental section). Analyses of the data revealed characteristic NMR resonances at (^{1}H δ) 2.77 (dd) and 2.62 ppm (dd) for the H-1 methylene protons, 1_A and 1_B, respectively (Fig. 3.4), which correlated in the HSQC to the upfield C-1 chemical shift (^{13}C δ 40.4 ppm, Fig. 3.5). Here, also the H-6 methylene proton resonances were observed at (^{1}H δ) 3.68 (dd) and 3.56 ppm (dd), for 6_A and 6_B, respectively, and correlated in the HSQC to the C-6 methylene carbon at (^{13}C δ) 62.4 ppm. HRMS also gave an exact m/z of 163.1084 $[M+Na]^+$ corresponding to the expected $[C_6H_{14}N_2O_3+H]^+$.

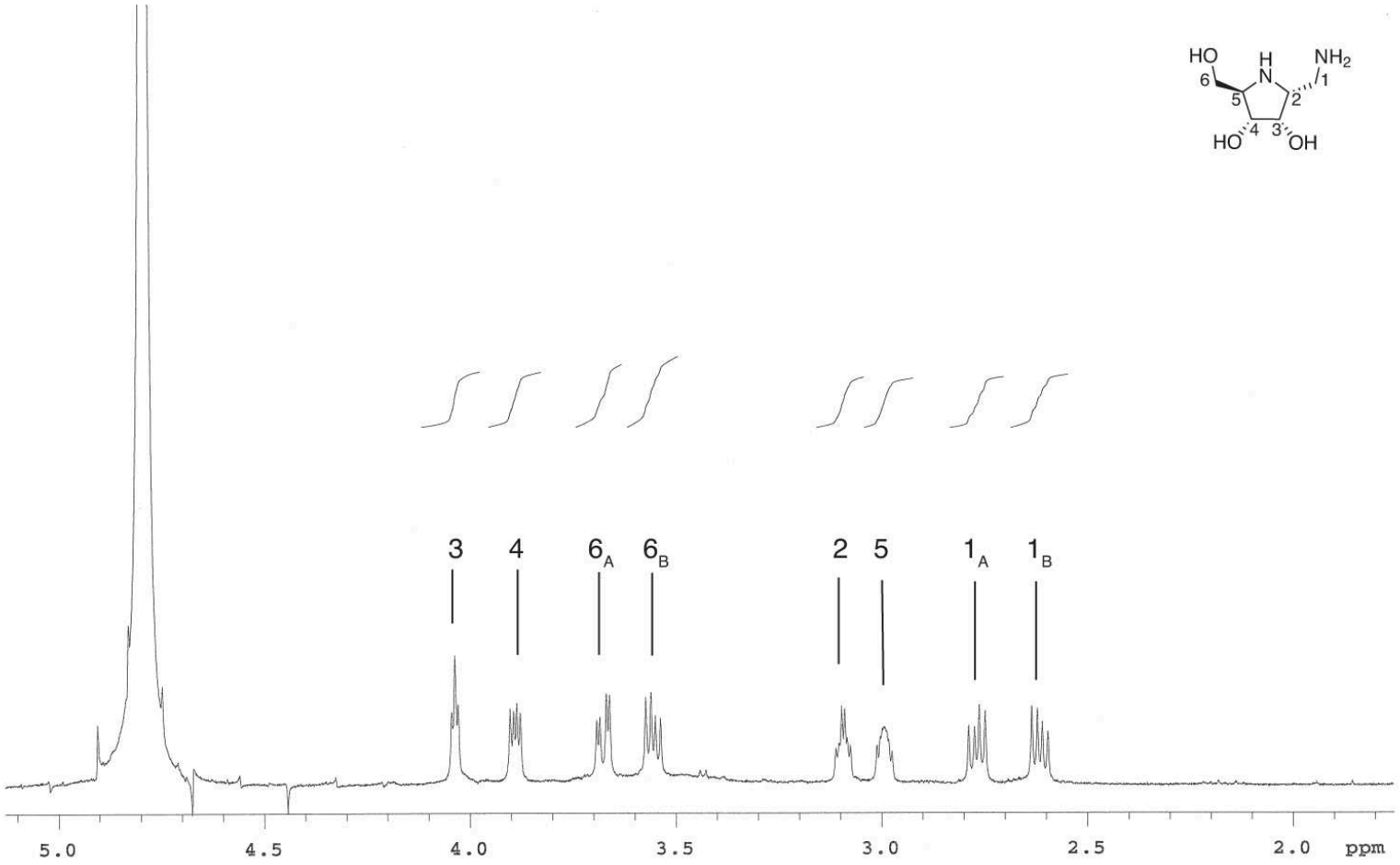


Fig. 3.4 ^{1}H NMR spectrum for amino-imino-hexitol **8**, (500 MHz, 2% NaOD in D_2O)

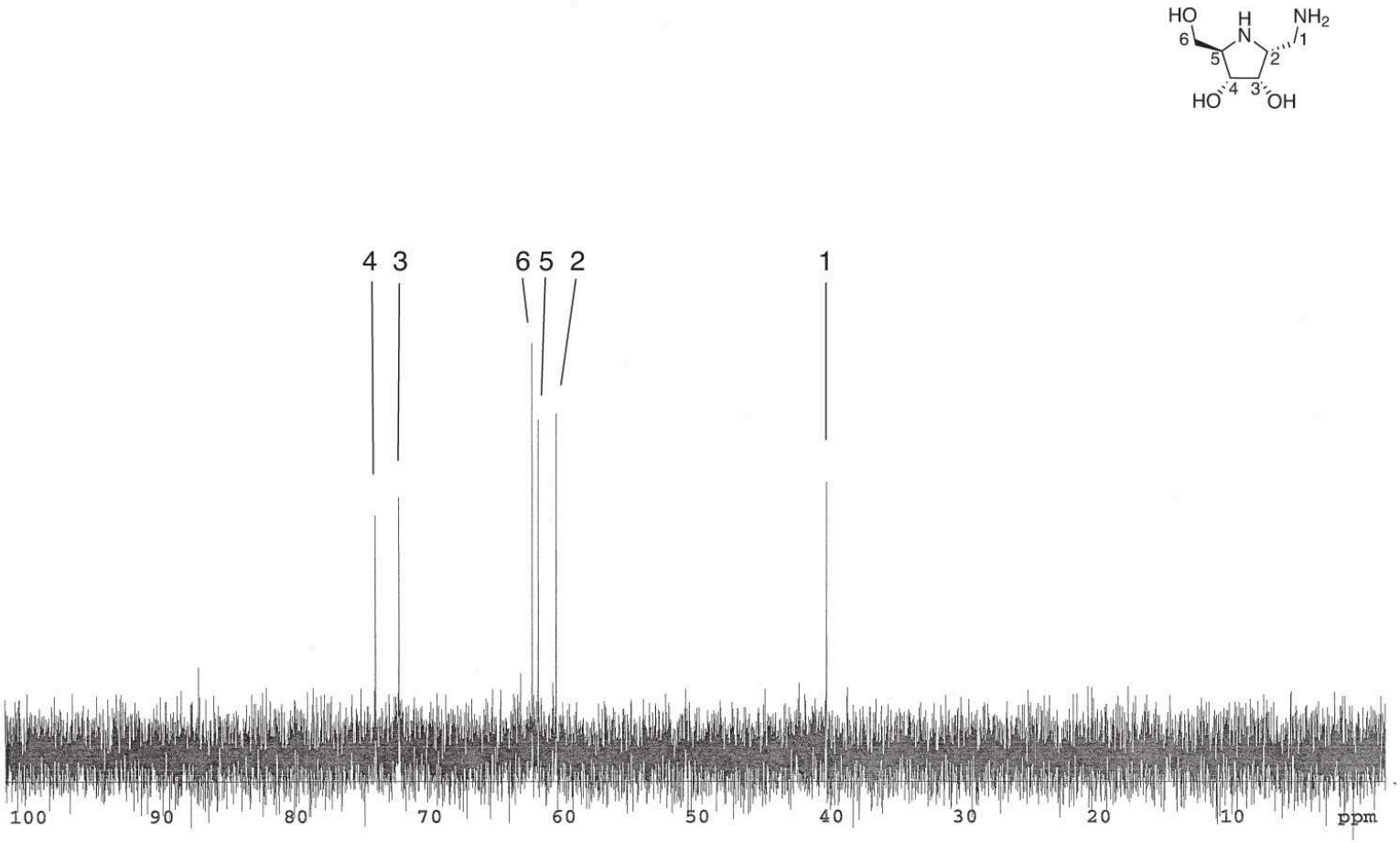
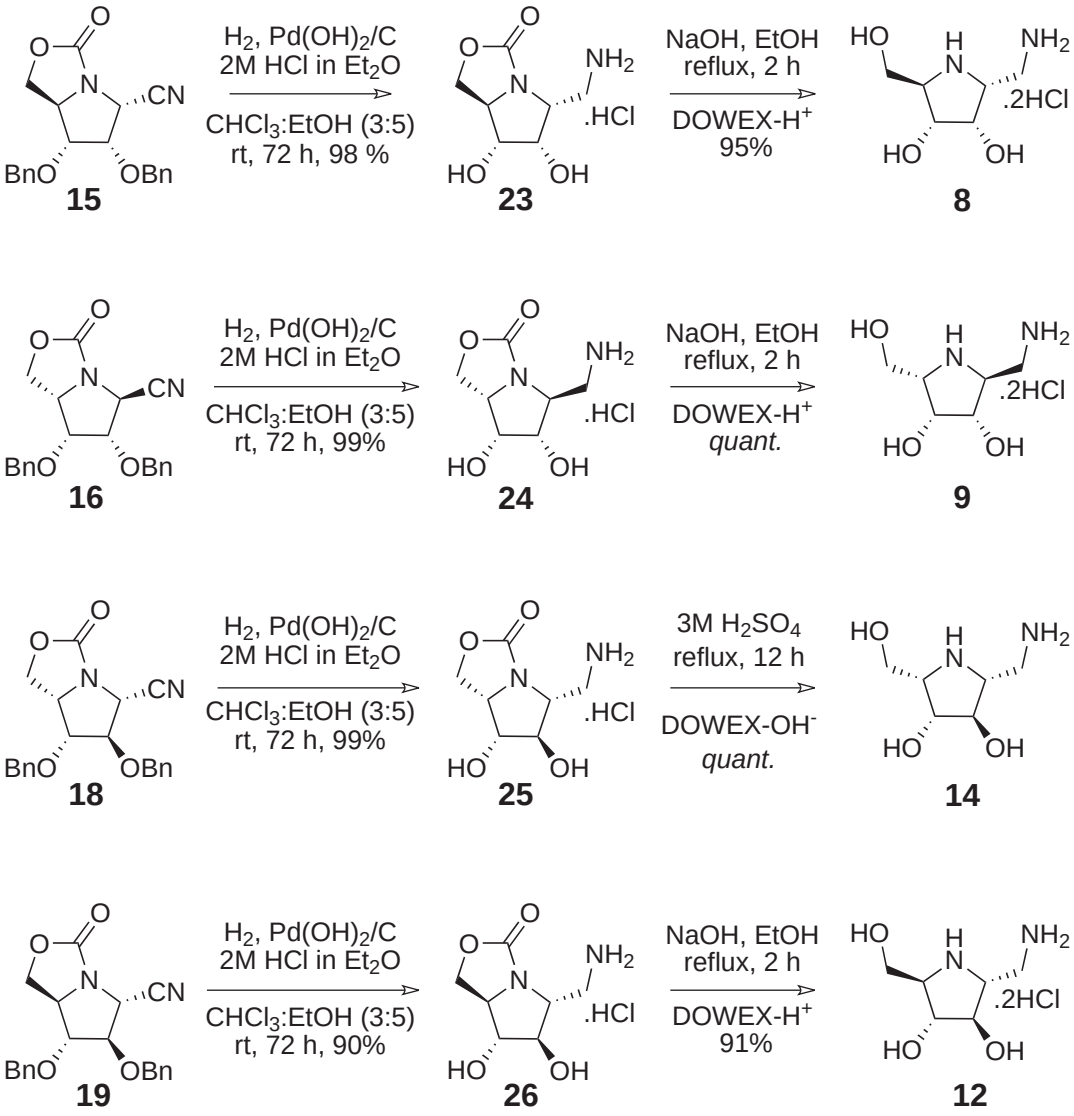


Fig. 3.5 ^{13}C NMR spectrum for amino-imino-hexitol **8**, (125 MHz, 2% NaOD in D_2O)

Carbamate **16** was then hydrogenated to give the corresponding amine **24** (99% yield), and subsequent hydrolysis proceeded uneventfully to yield the novel L-*altro* derived amino-imino-hexitol **9** in 37% overall yield from D-ribose. Though we had already assigned the stereochemistry of **9** from the crystal structure of the precursor carbamate **24**, the ^{1}H NMR and ^{13}C NMR data was consistent with that of the known D-*altro* enantiomer and the optical rotation of similar magnitude but opposite sign.[12,13] Carbamate **18**, with the *cis*-relationship between the aminomethyl and carbamate functionalities, was then hydrogenated and the ammonium salt **25** obtained in excellent (99%) yield. The acid catalysed hydrolysis of **25** using 3M H_2SO_4 then gave the D-glucitol derived amino-imino-hexitol **14**, again in excellent yield. Spectral data for **14** matched that previously reported.[7] Finally, carbamate **19**, derived from D-arabinose, was hydrogenated in 90% yield to generate the deprotected amine **26**, which was subsequently hydrolysed under basic conditions to give the known D-mannitol derived amino-imino-hexitol **12**.[7,14]



Scheme 3.6 Amine reduction and carbamate hydrolysis

3.3 Conclusion

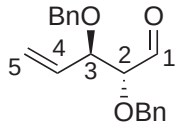
In summary, an efficient route for the preparation of a number of amino-imino-hexitols has been presented. In particular, the two novel amino-imino-hexitols **7** and **9** were efficiently prepared in 36% and 37% overall yield from D-ribose, respectively. During the course of this work, the power of a highly diastereoselective Strecker reaction without the need for chiral Lewis acids or catalysts has been demonstrated. The diastereoselectivity of the reaction can be explained by the Cram-chelation control model for both D-arabinose and D-ribose derived aldehydes. Moreover, the potential of our novel carbamate annulation for the cyclisation of protected and functionalised alkenylamine precursors has been highlighted. The applicability of the carbamate annulation to a variety of alkenylamines and an understanding of the factors controlling the diastereoselectivity of the reaction should make this methodology a valuable addition to the synthetic chemist's toolbox.

3.4 Experimental

General Experimental

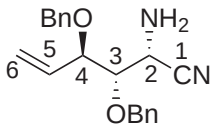
Unless otherwise stated all reactions were performed under air. THF was distilled from $LiAlH_4$ prior to use. All chemicals obtained from commercial suppliers were used without further purification. Zn dust was activated by the careful addition of conc. H_2SO_4, followed by decantation and washing with EtOH (3 x) and hexanes (3 x), and storage under dry hexanes. All solvents were removed by evaporation under reduced pressure. Reactions were monitored by TLC analysis on silica gel coated plastic sheets (0.20 mm, Polygram SIL G/UV254) with detection by coating with 20% H_2SO_4 in EtOH followed by charring at *ca.* 150 °C, by coating with a solution of ninhydrin in EtOH followed by charring at *ca.* 150 °C, by coating with Hanessian's stain followed by charring at *ca.* 150 °C, or by coating with a solution of 5% $KMnO_4$ and 1% $NaIO_4$ in H_2O followed by heating. Column chromatography was performed on silica gel (40-63 μm). Dowex Monosphere M-31 acidic resin and Dowex 1x4-50 basic resin were used for ion exchange chromatography. Melting points [°C] were obtained with a Gallenkamp apparatus and are uncorrected. High-resolution mass spectra were recorded on a Waters Q-TOF Premier^{TM} Tandem Mass Spectrometer using positive electro-spray ionisation. Optical rotations were recorded using a Perkin-Elmer 241 or Rudolf Research Analytical Autopol II polarimeter at the sodium D-line. Infrared spectra were recorded as thin films using a Bruker Tensor 27 FTIR spectrometer, equipped with an Attenuated Total Reflectance (ATR) sampling accessory, and are reported in wave numbers (cm^{-1}). Nuclear magnetic resonance spectra were measured using a Varian Inova operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C or a Varian Direct Drive

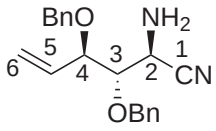
operating at 600 MHz and 133 MHz. ^{1}H and ^{13}C chemical shifts (δ) were internally referenced to the residual solvent peak, ($CDCl_3$ = 7.26 ppm and 77.16 ppm, D_2O = 4.79 ppm). NMR peak assignments are based on 2D NMR experiments (COSY, HSQC, and HMBC).



(2*R*,3*R*)-2,3-Dibenzyloxypent-4-enal (11). To a solution of methyl iodoriboside **10**[8] (1.63 g, 3.59 mmol) in EtOH/H_2O/AcOH (40/2/1, v/v/v, 54 mL) was added Zn (1.17 g, 18.0 mmol). The mixture was stirred at reflux for 3 h, cooled to room temperature and filtered through a celite plug with EtOAc. The resulting mixture was further diluted with EtOAc and washed twice with sat. aq.˙ $NaHCO_3$, then H_2O and brine, dried (MgSO4), filtered and concentrated *in vacuo* to provide the aldehyde **11** as a colourless oil (1.03 g, 3.48 mmol, 97%). R_f = 0.57 (hexanes/EtOAc, 2/1, v/v); $[\alpha]_D^{17.6}$ = -29 (c = 1.0, $CHCl_3$); IR (film) 3064, 3031, 2925, 2868, 1729, 1455, 1207, 1069, 1027, 933, 735, 696 cm^{-1}. ^{1}H NMR: (500 MHz, $CDCl_3$, 20 °C) δ 9.64 (d, $J_{1,2}$ = 2.0 Hz, 1H, H-1), 7.38–7.27 (m, 10H, H-Ar), 5.88 (ddd, $J_{4,5b}$ = 17.7, $J_{4,5a}$ = 10.4, $J_{3,4}$ = 7.6 Hz, 1H, H-4), 5.38 (dt, $J_{4,5a}$ = 10.4, $^{2}J_{5a,5b}$ = $^{4}J_{3,5a}$ = 1.2 Hz, 1H, H-5a), 5.35 (dt, $J_{4,5b}$ = 17.7, $^{2}J_{5a,5b}$ = $^{4}J_{3,5b}$ = 1.2 Hz, 1H, H-5b), 4.71 (d, $^{2}J_{a,b}$ = 12.0 Hz, 1H, CHa 2-*O*-Bn), 4.66 (d, $^{2}J_{a,b}$ = 12.0 Hz, 1H, CHb 2-*O*-Bn), 4.65 (d, $^{2}J_{a,b}$ = 12.0 Hz, 1H, CHa 3-*O*-Bn), 4.42 (d, $^{2}J_{a,b}$ = 12.0 Hz, 1H, CHb 3-*O*-Bn), 4.17 (ddt, $J_{3,4}$ = 7.6, $J_{2,3}$ = 4.7, $^{4}J_{3,5}$ Hz, 1H, H-3), 3.90 (dd, $J_{2,3}$ = 4.7, $J_{1,2}$ = 2Hz, 1H, H-2); ^{13}C NMR: (125 MHz $CDCl_3$, 20 °C) δ 201.8 (C-1), 137.9 (C-*i* 3-*O*-Bn), 137.3 (C-*i* 2-*O*-Bn), 134.2 (C-4), 128.6 (C-Ar), 128.5 (C-Ar), 128.2 (C-Ar), 128.2 (C-Ar), 127.9 (C-Ar), 128.8 (C-Ar), 120.2 (C-5), 85.0 (C-2), 80.3 (C-3), 73.1 (CH_2 2-*O*-Bn), 70.6 (CH_2 3-*O*-Bn); HRMS (ESI) *m/z* calcd. for

$[C_{19}H_{20}O_3+Na]^+$: 319.1305, obsd.: 319.1311.



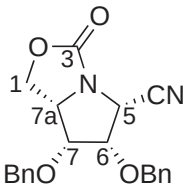
and


(2*S*,3*S*,4*R*)-2-Amino-3,4-dibenzyloxyhex-5-enenitrile (*syn*-12) and (2*S*,3*S*,4*R*)-2-Amino-3,4-dibenzyloxyhex-5-enenitrile (*anti*-13). To a solution of aldehyde **11** (166 mg, 0.56 mmol) in EtOH (11.2 mL) was added NH_4OAc (471 mg, 5.60 mmol). The mixture was stirred at room temperature for 15 mins, at which point TMSCN (147 μL, 1.12 mmol) was added dropwise. The clear colourless solution was stirred at room temperature for 48 h. The resulting solution was diluted with EtOAc, washed with sat. aq.˙ $NaHCO_3$, H_2O and brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification of the α-aminonitriles using gradient flash chromatography (hexanes/EtOAc, 10/1 $\rightarrow$ 1/1, v/v), gave first α-aminonitrile *syn*-**12** as a pale yellow oil (147 mg, 0.46 mmol, 82%) and then α-aminonitrile *anti*-**13** as a pale yellow oil (18.9 mg, 0.06 mmol, 10%), (dr = 8:1, *syn*-**12**:*anti*-**13**). *syn*-**12**: R_f = 0.27 (hexanes/EtOAc, 2/1, v/v); $[\alpha]_D^{19}$ = -22.4 (c = 1.0, $CHCl_3$); IR (film) 3397, 3327, 3088, 3064, 3032, 2871, 2372, 1710, 1497, 1455, 1392, 1214, 1088, 1072, 935, 741, 698 cm^{-1}. ^1H NMR: (500 MHz, $CDCl_3$, 20 °C) δ 7.37–7.30 (m, 10H, H-Ar), 5.84 (ddd, $J_{5,6a}$ = 17.2, $J_{5,6b}$ = 10.5, $J_{4,5}$ = 7.9 Hz, 1H, H-5), 5.46 (ddd, $J_{5,6a}$ = 17.2, $^2J_{6a,6b}$ = 1.7, $^4J_{4,6a}$ = 0.7 Hz, 1H, H-6a), 5.45 (ddd, $J_{5,6b}$ = 10.5, $^2J_{6a,6b}$ = 1.7, $^4J_{4,6b}$ = 0.7 Hz, 1H, H-6b) 4.76 (d, $^2J_{a,b}$ = 10.8 Hz, 1H, CHa 3-*O*-Bn), 4.68 (d, $^2J_{a,b}$ = 10.8 Hz, 1H, CHb 3-*O*-Bn), 4.62 (d, $^2J_{a,b}$ = 11.4 Hz, 1H, CHa 4-*O*-Bn), 4.36 (d, $^2J_{a,b}$ = 11.4 Hz, 1H, CHb 4-*O*-Bn), 4.08 (d, $J_{2,3}$ = 2.0 Hz, 1H, H-2), 4.06 (tt, $J_{4,5}$ = $J_{3,4}$ = 7.9, $^4J_{4,6}$ = 0.7 Hz, 1H, H-4), 3.66 (dd, $J_{3,4}$ = 7.9, $J_{2,3}$ = 2.0 Hz, 1H, H-3), 1.82 (br s, 2H, NH_2); ^{13}C NMR: (125 MHz $CDCl_3$, 20 °C) δ 137.7 (C-*i* 4-*O*-Bn), 137.2 (C-*i* 3-*O*-Bn), 135.3 (C-5), 128.7 (C-Ar), 128.6 (C-Ar), 128.6

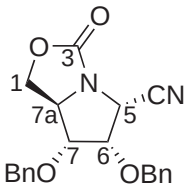
(C-Ar), 128.5 (C-Ar), 128.5 (C-Ar), 128.4 (C-Ar), 128.2 (C-Ar), 128.2 (C-Ar), 128.0 (C-Ar), 121.3 (C-1), 120.8 (C-6), 81.5 (C-3), 79.0 (C-4), 74.8 (CH_2 3-*O*-Bn), 70.8 (CH_2 4-*O*-Bn), 44.4 (C-2); HRMS (ESI) *m/z* calcd. for $[C_{20}H_{22}N_2O_2+H]^+$: 323.1754, obsd.: 323.1762. *anti*-**13**: R_f = 0.16 (hexanes/EtOAc, 2/1, v/v); $[\alpha]_D^{19}$ = -2.8 (c = 1.0, $CHCl_3$); IR (film) 3388, 3325, 3088, 3065, 3031, 2869, 2231, 1643, 1497, 1455, 1392, 1210, 1086, 1070, 1028, 936, 738, 698 cm^{-1}. ^1H NMR: (500 MHz, $CDCl_3$, 20 °C) δ 7.38–7.30 (m, 10H, H-Ar), 5.90 (ddd, $J_{5,6a}$ = 17.3, $J_{5,6b}$ = 10.3, $J_{4,5}$ = 7.8 Hz, 1H, H-5), 5.51 (ddd, $J_{5,6a}$ = 17.3, $^2J_{6a,6b}$ = 1.7, $^4J_{4,6a}$ = 1.0 Hz, 1H, H-6a), 5.47 (ddd, $J_{5,6b}$ = 10.3, $^2J_{6a,6b}$ = 1.7, $^4J_{4,6a}$ = 1.0 Hz, 1H, H-6b) 4.81 (d, $^2J_{a,b}$ = 11.2 Hz, 1H, CHa 3-*O*-Bn), 4.64 (d, $^2J_{a,b}$ = 11.3 Hz, 1H, CHa 4-*O*-Bn), 4.62 (d, $^2J_{a,b}$ = 11.2 Hz, 1H, CHb 3-*O*-Bn), 4.39 (d, $^2J_{a,b}$ = 11.3 Hz, 1H, CHb 4-*O*-Bn), 4.08 (d, $J_{2,3}$ = 4.2 Hz, 1H, H-2), 4.05 (tt, $J_{4,5}$ = $J_{3,4}$ = 7.8, $^4J_{4,6}$ = 0.7 Hz, 1H, H-4), 3.64 (dd, $J_{3,4}$ = 7.8, $J_{2,3}$ = 4.2 Hz, 1H, H-3), 1.76 (br s, 2H, NH_2); ^{13}C NMR: (125 MHz $CDCl_3$, 20 °C) δ 137.8 (C-*i* 4-*O*-Bn), 137.6 (C-*i* 3-*O*-Bn), 135.2 (C-5), 128.6 (C-Ar), 128.1 (C-Ar), 128.1 (C-Ar), 128.0 (C-Ar), 128.0 (C-Ar), 121.2 (C-1), 120.5 (C-6), 82.6 (C-3), 81.0 (C-4), 75.2 (CH_2 3-*O*-Bn), 70.9 (CH_2 4-*O*-Bn), 45.9 (C-2); HRMS (ESI) *m/z* calcd. for $[C_{20}H_{22}N_2O_2+H]^+$: 323.1754, obsd.: 323.1763.

General Procedure for the Carbamate Annulation. To a solution of α-aminonitrile (1 mmol) in THF (3.5 mL), were added I_2 (634 mg, 2.5 mmol), H_2O (3.5 mL) and $NaHCO_3$ (1.68 g, 20 mmol). The reaction mixture was stirred at room temperature for varying lengths of time (20 h – 6 d), quenched with sat. aq. $Na_2S_2O_3$ and extracted with EtOAc. The organic layer was washed with H_2O and brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification was achieved using gradient flash chromatography

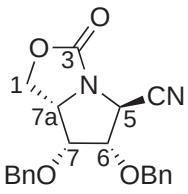
(hexanes/EtOAc, v/v) or (DCM/EtOAc, v/v).



(5*R*,6*S*,7*R*,7a*S*)-6,7-Dibenzyloxy-3-oxotetrahydropyrrolo[1,2-c]oxazol-5-carbonitrile (14). By subjecting α-aminonitrile *syn*-**12** (492 mg, 1.53 mmol) to the general procedure for the carbamate annulation for 6 d, carbamate **14** was obtained as a white solid (378 mg, 1.04 mmol, 68%, dr = 4:1, 18:19). Crystallisation (DCM) yielded fine white needles for which a single crystal X-ray diffraction was obtained then solved and refined using Olex2.[15] R_f = 0.19 (DCM/EtOAc, 50/1, v/v); MP = 198.9 – 199.9 °C; $[\alpha]_D^{20}$ = +34.5 (c = 1.0, $CHCl_3$); IR (film) 3066, 3028, 2953, 2921, 2887, 2251, 1737, 1395, 1269, 1256, 1150, 1101, 1046, 1002, 702, 694 cm^{-1}. ^{1}H NMR: (500 MHz, $CDCl_3$, 20 °C) δ 7.43–7.30 (m, 10H, H-Ar), 5.18 (d, $^2J_{a,b}$ = 11.8 Hz, 1H, CHa 7-*O*-Bn), 4.78 (d, $^2J_{a,b}$ = 11.9 Hz, 1H, CHa 6-*O*-Bn), 4.71 (d, $^2J_{a,b}$ = 11.9 Hz, 1H, CHb 6-*O*-Bn) 4.68 (d, $^2J_{a,b}$ = 11.8 Hz, 1H, CHb 7-*O*-Bn), 4.51 (dd, $^2J_{1a,1b}$ = 8.8, $J_{1a,7a}$ = 6.8, Hz, 1H, H-1a), 4.42 (d, $J_{5,6}$ = 7.1 Hz, 1H, H-5), 4.35 (t, $J_{1a,1b}$ = $J_{1b,7a}$ = 8.8 Hz, 1H, H-1b), 4.37 (dd, $J_{5,6}$ = 7.1, $J_{6,7}$ = 2.7 Hz, 1H, H-6), 4.09 (ddd, $J_{1b,7a}$ = 8.8, $J_{1a,7a}$ = 6.8, $J_{7,7a}$ = 2.7 Hz, 1H, H-7a), 3.87 (t, $J_{7,7a}$ = $J_{6,7}$ = 2.7 Hz, 1H, H-7); ^{13}C NMR: (125 MHz $CDCl_3$, 20 °C) δ 157.4 (C-3), 137.4 (C-*i* 7-*O*-Bn), 136.1 (C-*i* 6-*O*-Bn), 129.0 (C-Ar), 128.9 (C-Ar), 128.7 (C-Ar), 128.3 (C-Ar), 128.3 (C-Ar), 128.2 (C-Ar), 112.9 (CN), 83.7 (C-6), 74.5 (C-7), 73.8 (CH_2 6-*O*-Bn), 73.6 (CH_2 7-*O*-Bn), 64.1 (C-1), 58.8 (C-7a), 47.2 (C-5); HRMS (ESI) *m/z* calcd. for $[C_{21}H_{20}N_2O_4+Na]^+$: 387.1315, obsd.: 387.1321.



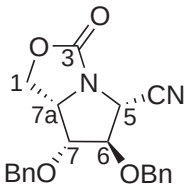
(5*R*,6*S*,7*R*,7a*R*)-6,7-Dibenzyloxy-3-oxotetrahydropyrrolo[1,2-c]oxazol-5-carbonitrile (15). By subjecting α-aminonitrile *syn*-**12** (492 mg, 1.53 mmol) to the general procedure for the carbamate annulation for 6 d, carbamate **15** was obtained as a colourless oil (94.5 mg, 0.26 mmol, 17%, dr = 4:1, 18:19). R_f = 0.31 (DCM/EtOAc, 20/1, v/v); $[\alpha]_D^{20}$ = +45.2 (c = 1.0, $CHCl_3$); IR (film) 3064, 3032, 2919, 2872, 1761, 1455, 1393, 1306, 1214, 1136, 1027, 1010, 737, 699 cm^{-1}. ^{1}H NMR: (500 MHz, $CDCl_3$, 20 °C) δ 7.45–7.27 (m, 10H, H-Ar), 4.96 (d, $^2J_{a,b}$ = 11.5 Hz, 1H, CHa 6-*O*-Bn), 4.77 (d, $J_{2,3}$ = 5.0 Hz, 1H, H-5), 4.73 (d, $^2J_{a,b}$ = 11.5 Hz, 1H, CHb 6-*O*-Bn), 4.64 (d, $^2J_{a,b}$ = 11.9 Hz, 1H, CHa 7-*O*-Bn), 4.46 (dd, $^2J_{1a,1b}$ = 9.6, $J_{1a,7a}$ = 8.3 Hz, 1H, H-1a), 4.36 (d, $^2J_{a,b}$ = 11.9 Hz, 1H, CHb 7-*O*-Bn), 4.29 (t, $J_{6,7}$ = $J_{5,6}$ = 5.0 Hz, 1H, H-3), 4.23 (td, $J_{1a,7a}$ = $J_{7,7a}$ = 8.3, $J_{1b,7a}$ = 2.7 Hz, H-7a), 4.08 (dd, $^2J_{1a,1b}$ = 9.6, $J_{1b,7a}$ = 2.7 Hz, 1H, H-1b), 3.56 (dd, $J_{7,7a}$ = 8.3, $J_{6,7}$ = 5.0 Hz, 1H, H-7); ^{13}C NMR: (125 MHz $CDCl_3$, 20 °C) δ 160.2 (C-3), 136.6 (C-*i* 7-*O*-Bn), 136.5 (C-*i* 6-*O*-Bn), 129.0 (C-Ar), 128.8 (C-Ar), 128.8 (C-Ar), 128.6 (C-Ar), 128.6 (C-Ar), 128.0 (C-Ar), 115.0 (CN), 80.7 (C-7), 77.1 (C-6), 74.5 (CH_2 6-*O*-Bn), 73.2 (CH_2 7-*O*-Bn), 66.3 (C-1), 60.0 (C-7a), 53.1 (C-5); HRMS (ESI) *m/z* calcd. for $[C_{21}H_{20}N_2O_4+Na]^+$: 387.1315, obsd.: 387.1328.



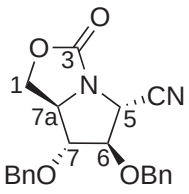
(5*S*,6*S*,7*R*,7a*S*)-6,7-Dibenzyloxy-3-oxotetrahydropyrrolo[1,2-c]oxazol-5-carbonitrile (16). By subjecting α-aminonitrile *anti*-**13** (25.0 mg, 0.08 mmol) to the general procedure for the carbamate annulation for 6 d, carbamate **16** was obtained as

a white solid (14.9 mg, 0.04 mmol, 53%). Crystallisation (DCM) yielded colourless plates for which a single crystal X-ray diffraction was obtained then solved and refined using Olex2.[15] R_f = 0.45 (DCM/EtOAc, 20/1, v/v); $[\alpha]_D^{20}$ = +13.6 (c = 0.5, $CHCl_3$); MP = 97.4 – 97.6 °C; IR (film) 3034, 2953, 2923, 2852, 1757, 1456, 1395, 1207, 1141, 1075, 1005, 772, 735, 696 cm^{-1}. ^1H NMR: (500 MHz, $CDCl_3$, 20 °C) δ 7.43–7.25 (m, 10H, H-Ar), 4.93 (d, $^2J_{a,b}$ = 11.9 Hz, 1H, CHa 7-*O*-Bn), 4.79 (d, $^2J_{a,b}$ = 11.9 Hz, 1H, CHa 6-*O*-Bn), 4.69 (d, $^2J_{a,b}$ = 11.9 Hz, 1H, CHb 6-*O*-Bn), 4.59 (d, $^2J_{a,b}$ = 11.9 Hz, 1H, CHb 7-*O*-Bn), 4.57 (d, $J_{5,6}$ = 6.8 Hz, 1H, H-5), 4.46 (dd, $J_{6,7}$ = 3.4, $J_{5,6}$ = 6.8 Hz, 1H, H-6), 4.40 (dd, $^2J_{1a,1b}$ = 8.6, $J_{1a,7a}$ = 3.7 Hz, 1H, H-1a), 4.34 (t, $^2J_{1a,1b}$ = $J_{1a,7a}$ = 8.6 Hz, 1H, H-1b), 4.03 (td, $J_{1a,7a}$ = $J_{1b,7a}$ = 8.6, $J_{7,7a}$ = 3.4 Hz, 1H, H-7a), 3.95 (t, $J_{7,7a}$ = $J_{6,7}$ = 3.4 Hz, 1H, H-7); ^{13}C NMR: (125 MHz $CDCl_3$, 20 °C) δ 160.8 (C-3), 137.2 (C-*i* 7-*O*-Bn), 136.1 (C-*i* 6-*O*-Bn), 129.0 (C-Ar), 128.8 (C-Ar), 128.8 (C-Ar), 128.4 (C-Ar), 128.1 (C-Ar), 128.0 (C-Ar), 117.9 (CN), 86.0 (C-6), 76.2 (C-7), 73.8 (CH_2 7-*O*-Bn), 73.8 (CH_2 6-*O*-Bn), 63.7 (C-1), 59.5 (C-7a), 50.9 (C-5); HRMS (ESI) *m/z* calcd. for $[C_{21}H_{20}N_2O_4+Na]^+$: 387.1315, obsd.: 387.1320.

Synthesis of 16 via Epimerisation. To a solution of carbamate **14** (11.0 mg, 0.03 mmol) in THF (0.6 mL) was added LiOH aq. (2M, 0.3 mL). The reaction mixture was stirred at room temperature for 4 h, then quenched with sat. aq. NH_4Cl and extracted with EtOAc. The organic layer was washed with H_2O and brine, dried ($MgSO_4$) and concentrated *in vacuo* to provide carbamate **16** as a colourless oil (11.0 mg, 0.03 mmol, *quantitative*). Spectral data as above.

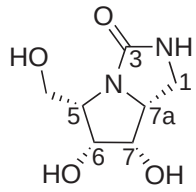


(5*R*,6*R*,7*R*,7a*S*)-6,7-Dibenzyloxy-3-oxotetrahydropyrrolo[1,2-c]oxazol-5-carbonitrile (18). By subjecting α-aminonitrile *anti*-**17**[4] (140 mg, 0.43 mmol) to the general procedure for the carbamate annulation for 48 h, carbamate **18** was obtained as a colourless oil (129 mg, 0.35 mmol, 81%, dr = 6:1, 21:22). R_f = 0.29 (hexanes/EtOAc, 1/1, v/v); $[\alpha]_D^{24}$ = +25.7 (c = 1.0, $CHCl_3$); IR (film) 3064, 3032, 2919, 2872, 2247, 1752, 1471, 1395, 1246, 1096, 1077, 1007, 740, 698 cm^{-1}. ^{1}H NMR: (500 MHz, $CDCl_3$, 20 °C) δ 7.42–7.27 (m, 10H, H-Ar), 4.73 (d, $^2J_{a,b}$ = 12 Hz, 1H, CHa 7-*O*-Bn), 4.60 (d, $^2J_{a,b}$ = 11.9 Hz, 1H, CHa 6-*O*-Bn), 4.55 (s, 1H, H-6), 4.53 (d, $^2J_{a,b}$ = 11.9 Hz, 1H, CHb 6-*O*-Bn), 4.46 (dd, $^2J_{1a,1b}$ = 8.6, $J_{1a,7a}$ = 5.6 Hz, 1H, H-1a), 4.44 (d, $^2J_{a,b}$ = 12 Hz, 1H, CHb 7-*O*-Bn), 4.42 (t, $^2J_{1a,1b}$ = $J_{1b,7a}$ = 8.6 Hz, 1H, H-1b), 4.36 (ddd, $J_{1b,7a}$ = 8.6, $J_{1a,7a}$ = 5.6, $J_{7,7a}$ = 3.2 Hz, 1H, H-7a), 4.22 (d, $J_{5,6}$ = 0.5 Hz, 1H, H-5), 3.79 (dd, $J_{7,7a}$ = 3.2, $J_{6,7}$ = 1.0 Hz, 1H, H-7); ^{13}C NMR: (125 MHz $CDCl_3$, 20 °C) δ 157.5 (C-3), 136.3 (C-*i* 7-*O*-Bn), 135.9 (C-*i* 6-*O*-Bn), 129.0 (C-Ar), 128.9 (C-Ar), 128.9 (C-Ar), 128.6 (C-Ar), 128.4 (C-Ar), 128.2 (C-Ar), 113.5 (CN), 87.0 (C-6), 78.2 (C-7), 73.2 (CH_2 6-*O*-Bn), 71.9 (CH_2 7-*O*-Bn), 62.6 (C-1), 62.0 (C-7a), 50.6 (C-5); HRMS (ESI) *m/z* calcd. for $[C_{21}H_{20}N_2O_4+Na]^+$: 387.1315, obsd.: 387.1324.



(5*R*,6*R*,7*R*,7a*R*)-6,7-Dibenzyloxy-3-oxotetrahydropyrrolo[1,2-c]oxazol-5-carbonitrile (19). By subjecting α-aminonitrile *anti*-**17**[4] (140 mg, 0.43 mmol) to the general procedure for the carbamate annulation for 48 h, carbamate **19** was obtained as a

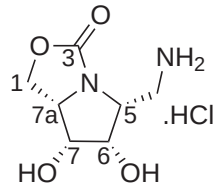
colourless oil (21.4 mg, 0.06 mmol, 13.6%, dr = 6:1, 21:22). R_f = 0.50 (hexanes/EtOAc, 1/1, v/v); $[\alpha]_D^{24}$ = +5.7 (c = 1.0, $CHCl_3$); IR (film) 3064, 3032, 2919, 2872, 2250, 1762, 1455, 1391, 1211, 1099, 1072, 740, 698 cm^{-1}. ^1H NMR: (500 MHz, $CDCl_3$, 20 °C) δ 7.41–7.28 (m, 10H, H-Ar), 4.80 (d, $J_{5,6}$ = 2.1 Hz, 1H, H-5), 4.65 (d, $^2J_{a,b}$ = 12.0 Hz, 1H, CHa 7-*O*-Bn), 4.63 (d, $^2J_{a,b}$ = 11.5 Hz, 1H, CHa 6-*O*-Bn), 4.51 (ddd, $J_{1a,7a}$ = 8.7, $^2J_{1a,1b}$ = 7.5, $^4J_{1a,7}$ = 4.0 Hz, 1H, H-1a), 4.50 (d, $^2J_{a,b}$ = 12.0 Hz, 1H, CHb 7-*O*-Bn), 4.46 (d, $^2J_{a,b}$ = 11.5 Hz, 1H, CHb 6-*O*-Bn), 4.37 (dd, $J_{5,6}$ = 2.1, $J_{6,7}$ = 2.7 Hz, 1H, H-6), 4.12 (dd, $J_{1a,1b}$ = 7.5, $J_{1b,7a}$ = 4.4 Hz, 1H, H-1b), 4.09 (ddd, $J_{1a,7a}$ = 8.7, $J_{1b,7a}$ = 4.4, $J_{7,7a}$ = 0.5 Hz, 1H, H-7a), 3.87 (ddd, $^4J_{1a,7}$ = 4.0, $J_{6,7}$ = 2.7, $J_{7,7a}$ = 0.5 Hz, 1H, H-7); ^{13}C NMR: (125 MHz $CDCl_3$, 20 °C) δ 160.0 (C-3), 136.6 (C-*i* 7-*O*-Bn), 135.7 (C-*i* 6-*O*-Bn), 129.1 (C-Ar), 129.0 (C-Ar), 128.9 (C-Ar), 128.8 (C-Ar), 128.7 (C-Ar), 128.6 (C-Ar), 128.3 (C-Ar), 128.1 (C-Ar), 115.9 (CN), 86.8 (C-7), 86.4 (C-6), 73.0 (CH_2 7-*O*-Bn), 72.8 (CH_2 6-*O*-Bn), 67.4 (C-1), 62.7 (C-7a), 52.3 (C-5); HRMS(ESI) m/z calcd. for $[C_{21}H_{20}N_2O_4+Na]^+$: 387.1315, obsd.: 387.1321.



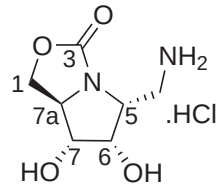
(5*S*,6*R*,7*S*,7a*R*)-6,7-Dihydroxy-5-(hydroxymethyl)tetrahydro-1H-pyrrolo[1,2-c]imidazol-3(2H)-one (20). Carbamate **14** (147 mg, 0.40 mmol) was dissolved in $CHCl_3$ (3.0 mL) and transferred into a Fischer-Porter bottle, EtOH (5.0 mL), HCl in ether (1.0 mL 2M) and $Pd(OH_2)/C$ (110 mg) were then added and the vessel was charged with 7 bar pressure of H_2. The reaction mixture was stirred vigorously at room temperature for 3 d. After releasing H_2 pressure the reaction mixture was filtered through a plug of celite, washing with EtOH and DCM/MeOH/EtOH/30% aq. NH_3,

(5/2/2/1, v/v/v/v) and concentrated *in vacuo*. Purification was achieved by dry-loading using gradient flash chromatography (DCM/MeOH/EtOH/30% aq. NH_3, 50/2/2/1 → 5/2/2/1, v/v/v/v) to provide the urea **20** as a colourless oil (23.0 mg, 0.12 mmol, 30%). R_f = 0.48 (DCM/MeOH/EtOH/30% aq. NH_3, 5/2/2/1, v/v/v/v); $[\alpha]_D^{21}$ = -22.0 (c = 1.0, H_2O); IR (film) 3288, 2927, 1658, 1492, 1451, 1372, 1130, 1025, 978, 757, 700 cm^{-1}. ^1H NMR: (500 MHz, D_2O, 20 °C) δ 4.61 (dd, $J_{5,6}$ = 8.1, $J_{6,7}$ = 4.0 Hz, 1H, H-6), 4.13 (dd, $^2J_{a,b}$ = 12.0, $J_{5,CHaOH}$ = 3.2, Hz, 1H, H-CHaOH) 4.12 (ddd, $J_{1b,7a}$ = 9.5, $J_{1a,7a}$ = 8.1, $J_{7,7a}$ = 4.0 Hz, 1H, H-7a), 3.99 (t, $J_{6,7}$ = $J_{7,7a}$ = 4.0 Hz, 1H, H-7), 3.76 (dt, $J_{5,6}$ = 8.1, $J_{5,CHaOH}$ = $J_{5,CHbOH}$ = 3.2 Hz, 1H, H-5), 3.72 (dd, $^2J_{a,b}$ = 12.0, $J_{5,CHbOH}$ = 3.2 Hz, H-CHbOH) 3.59 (dd, $^2J_{1a,1b}$ = 9.5, $J_{1a,7a}$ = 8.1 Hz, 1H, H-1a), 3.49 (t, $^2J_{1a,1b}$ = $J_{1b,7a}$ = 9.5 Hz, 1H, H-1b); ^{13}C NMR: (125 MHz D_2O, 20 °C) δ 164.0 (C-3), 74.2 (C-6), 69.1 (C-7), 60.1 (C-7a), 58.5 (C-5), 55.3 (CH_2OH), 38.7 (C-1); HRMS (ESI) *m/z* calcd. for $[C_7H_{12}N_2O_4+H]^+$: 189.0870, obsd.: 189.0869.

General Procedure for Hydrogenation of Carbamates. Protected carbamate (0.27 mmol) was dissolved in $CHCl_3$ (4.5 mL) and transferred into a Fischer-Porter bottle, EtOH (7.5 mL), HCl in ether (1.4 mL 2M) and $Pd(OH)_2/C$ (230 mg) were then added and the vessel was charged with 7 bar pressure of H_2. The reaction mixture was stirred vigorously at room temperature for 3 d. After releasing H_2 pressure the reaction mixture was filtered through a plug of celite, washing with EtOH and H_2O, and concentrated in vacuo to provide the amino carbamate as the hydrochloride salt.

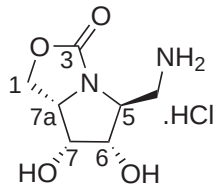


(5*R*,6*S*,7*R*,7a*S*)-5-(Aminomethyl)-6,7-dihydroxytetrahydropyrrolo [1,2-c]oxazol-3(1H)-one hydrochloride (21). By subjecting carbamate **14** (60 mg, 0.16 mmol) to the general procedure for the hydrogenation of carbamates, ammonium salt **21** was obtained as a white solid (30.8 mg, 0.16 mmol, 99%) and further crystallised from MeOH. R^f = 0.29 (DCM/MeOH/EtOH/30% aq. NH_3, 5/2/2/1, v/v/v/v); $[\alpha]_D^{26.1}$ = +30.0 (c = 1.0, H_2O); MP = 230.0 – 230.5 °C; IR (film) 3362, 2960, 2930, 1724, 1633, 1433, 1261, 1127, 1075, 949, 897 cm^{-1}. ^1H NMR: (500 MHz, D_2O, 20 °C) δ 4.66 (dd, $J_{5,6}$ = 7.6, $J_{6,7}$ = 3.4 Hz, 1H, H-6), 4.56 (t, $^2J_{1a,1b}$ = $J_{1a,7a}$ = 9.3 Hz, 1H, H-1a), 4.49 (dd, $^2J_{1a,1b}$ = 9.3, $J_{1a,7a}$ = 7.1 Hz, 1H, H-1b), 4.25 (ddd, $J_{1a,7a}$ = 9.3, $J_{1b,7a}$ = 7.1, $J_{7,7a}$ = 3.4 Hz, 1H, H-7a), 4.13 (t, $J_{7,7a}$ = $J_{6,7}$ = 3.4 Hz, 1H, H-7), 4.04 (td, $J_{5,6}$ = $J_{5,CHbNH2}$ = 7.6, $J_{5,CHaNH2}$ = 3.4 Hz, 1H, H-5), 3.59 (dd, $^2J_{a,b}$ = 14.2, $J_{5,CHaNH2}$ = 3.4 Hz, 1H, $CHaNH_2$), 3.38 (dd, $^2J_{a,b}$ = 14.2, $J_{5,CHbNH2}$ = 7.6 Hz, 1H, $CHbNH_2$); ^{13}C NMR: (125 MHz D_2O, 20 °C) δ 159.7 (C-3), 74.3 (C-6), 69.1 (C-7), 64.5 (C-1), 60.3 (C-7a), 55.0 (C-5), 36.7 (CH_2NH_2); HRMS (ESI) *m/z* calcd. for $[C_7H_{12}N_2O_4+H]^+$: 189.0870, obsd.: 189.0869.

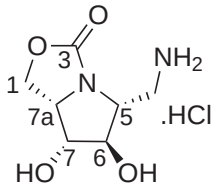


(5*R*,6*S*,7*R*,7a*R*)-5-(Aminomethyl)-6,7-dihydroxytetrahydropyrrolo [1,2-c]oxazol-3(1H)-one hydrochloride (23). By subjecting carbamate **15** (4 mg, 0.01 mmol) to the general procedure for the hydrogenation of carbamates, ammonium salt 26 was obtained as a colourless oil (2.0 mg, 0.01 mmol, 98%). R_f = 0.25 (DCM/MeOH/EtOH/30% aq. NH_3, 5/2/2/1, v/v/v/v); $[\alpha]_D^{25.7}$ = -10.0 (c = 0.7, H_2O);

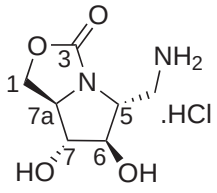
IR (film) 3382, 3284, 2927, 2855, 1735, 1630, 1402, 1243, 1221, 1114, 999, 768 cm^{-1}. ^{1}H NMR: (500 MHz, D_2O, 20 °C) δ 4.59–4.56 (m, 1H, H-1a), 4.36–4.34 (m, 2H, H-6 and H-1b), 4.00–3.94 (m, 3H, H-5, H-7 and H-7a), 3.17 (dd, $^{2}J_{a,b}$ = 13.2, $J_{5,CHaNH2}$ = 3.6 Hz, 1H, $CHaNH_2$), 3.12 (dd, $^{2}J_{a,b}$ = 13.2, $J_{5,CHbNH2}$ = 7.8 Hz, 1H, $CHbNH_2$); ^{13}C NMR: (125 MHz D_2O, 20 °C) δ 163.7 (C-3), 74.3 (C-6), 73.3 (C-7), 67.9 (C-1), 60.2 (C-7a), 58.7 (C-5), 39.6 (CH_2NH_2); HRMS (ESI) *m/z* calcd. for $[C_7H_{12}N_2O_4+H]^+$: 189.0870, obsd.: 189.0873.



(5*S*,6*S*,7*R*,7a*S*)-5-(Aminomethyl)-6,7-dihydroxytetrahydropyrrolo [1,2-c]oxazol-3(1H)-one hydrochloride (24). By subjecting carbamate **16** (104 mg, 0.29 mmol) to the general procedure for the hydrogenation of carbamates, ammonium salt **24** was obtained as a colourless oil (53.0 mg, 0.28 mmol, 99%). R_f = 0.22 (DCM/MeOH/EtOH/30% aq. NH_3, 5/2/2/1, v/v/v/v); $[\alpha]_D^{25}$ = +14.8 (c = 1.0, H_2O); IR (film) 3233, 2978, 2903, 2837, 1736, 1622, 1506, 1403, 1236, 1089, 1013, 916, 823, 775 cm^{-1}. ^{1}H NMR: (500 MHz, D_2O, 20 °C) δ 4.57 (t, $^{2}J_{1a,1b}$ = $J_{1a,7a}$ = 9.3 Hz, 1H, H-1a), 4.54 (dd, $^{2}J_{1a,1b}$ = 9.3, $J_{1b,7a}$ = 3.7 Hz, 1H, H-1b), 4.24 (dd, $J_{5,6}$ = 7.8, $J_{6,7}$ = Hz, 1H, H-6), 4.21–4.20 (m, 1H, H-7a) 4.06 (br s, 1H, H-7), 3.62 (td, $J_{5,6}$ = $J_{5,CHbNH2}$ = 7.8, $J_{5,CHaNH2}$ = 3.4 Hz, 1H, H-5), 3.28 (dd, $^{2}J_{a,b}$ = 13.2, $J_{5,CHaNH2}$ = 3.4 Hz, 1H, $CHaNH_2$), 3.01 (dd, $^{2}J_{a,b}$ = 13.2, $J_{5,CHbNH2}$ = 7.8 Hz, 1H, $CHbNH_2$); ^{13}C NMR: (125 MHz D_2O, 20 °C) δ 164.4 (C-3), 76.6, (C-6), 71.4 (C-7), 64.6 (C-1), 60.7 (C-5), 60.4 (C-7a), 42.0 (CH_2NH_2); HRMS (ESI) *m/z* calcd. for $[C_7H_{12}N_2O_4+H]^+$: 189.0870, obsd.: 189.0872.

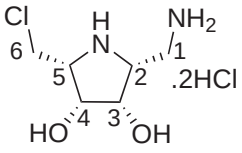


(5*R*,6*R*,7*R*,7a*S*)-5-(Aminomethyl)-6,7-dihydroxytetrahydropyrrolo [1,2-c]oxazol-3(1H)-one hydrochloride (25). By subjecting carbamate **18** (100 mg, 0.27 mmol) to the general procedure for the hydrogenation of carbamates, ammonium salt **25** was obtained as a white solid (51.4 mg, 0.27 mmol, 99%) and further crystallised from EtOH. R_f = 0.62 (DCM/MeOH/EtOH/30% aq. NH_3, 5/2/2/1, v/v/v/v); $[\alpha]_D^{25.8}$ = +60.2 (c = 0.83, H_2O); MP = 183.3 – 184.0 °C; IR (film) 3356, 2961, 2929, 1716, 1633, 1428, 1262, 1143, 1094, 1063, 1007, 949, 893 cm^{-1}. ^1H NMR: (500 MHz, D_2O, 20 °C) δ 4.63–4.59 (m, 1H, H-1a), 4.52–4.47 (m, 2H, H-7a, and H-1b), 4.15 (t, $J_{6,7}$ = $J_{5,6}$ = 1.9 Hz, 1H, H-6), 4.01 (dd, $J_{7,7a}$ = 2.8, $J_{6,7}$ = 1.9 Hz, 1H, H-7), 3.72 (ddd, $J_{5,CHbNH2}$ = 10.0, $J_{5,CHaNH2}$ = 3.2, $J_{5,6}$ = 1.9 Hz, 1H, H-5), 3.59 (dd, $^2J_{a,b}$ = 13.9, $J_{5,CHaNH2}$ = 3.2 Hz, 1H, $CHaNH_2$), 3.53 (dd, $^2J_{a,b}$ = 13.9, $J_{5,CHbNH2}$ = 10.0 Hz, 1H, $CHbNH_2$); ^{13}C NMR: (125 MHz D_2O, 20 °C) δ 160.2 (C-3), 82.2 (C-6), 73.3 (C-7), 63.9 (C-1), 63.2 (C-7a), 62.5 (C-5), 39.3 (CH_2NH_2); HRMS (ESI) *m/z* calcd. for $[C_7H_{12}N_2O_4+H]^+$: 189.0870, obsd.: 189.0871.



(5*R*,6*R*,7*R*,7a*R*)-5-(Aminomethyl)-6,7-dihydroxytetrahydropyrrolo [1,2-c]oxazol-3(1H)-one hydrochloride (26). By subjecting carbamate **19** (16 mg, 0.04 mmol) to the general procedure for the hydrogenation of carbamates, ammonium salt **26** was obtained as a colourless oil (7.0 mg, 0.04 mmol, 90%). R_f = 0.41 (DCM/MeOH/EtOH/30% aq. NH_3, 5/2/2/1, v/v/v/v); $[\alpha]_D^{24.8}$ = +10.0 (c = 0.4, H_2O); IR

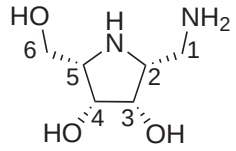
(film) 3330, 3247, 2924, 1731, 1641, 1404, 1235, 1084, 1012, 918, 773 cm^{-1}. ^{1}H NMR: (500 MHz, D_2O, 20 °C) δ 4.70 (dd, $^{2}J_{1a,1b}$ = 9.6, $J_{1a,7a}$ = 8.1 Hz, 1H, H-1a), 4.48 (dd, $^{2}J_{1a,1b}$ = 9.6, $J_{1b,7a}$ = 4 Hz, 1H, H-1b), 4.07–4.02 (m, 3H, H-6, H-7 and H-7a), 3.84 (ddd, $J_{5,CHbNH2}$ = 11, $J_{5,6}$ = 4.9, $J_{5,CHaNH2}$ = 3.3 Hz, 1H, H-5), 3.37 (dd, $^{2}J_{a,b}$ = 13.1, $J_{5,CHaNH2}$ = 3.3 Hz, 1H, $CHaNH_2$), 3.19 (dd, $^{2}J_{a,b}$ = 13.1, $J_{5,CHbNH2}$ = 11, 1H, $CHbNH_2$); ^{13}C NMR: (125 MHz D_2O, 20 °C) δ 163.6 (C-3), 79.2 (C-6), 78.4 (C-7), 68.4 (C-1), 61.6 (C-5), 60.9 (C-7a), 40.8 (CH_2NH_2); HRMS (ESI) *m/z* calcd. for $[C_7H_{12}N_2O_4+H]^+$: 189.0870, obsd.: 189.0874.



1-Amino-6-chloro-1,2,5,6-tetradeoxy-2,5-imino-D-galactitol dihydrochloride (22). Amino carbamate **21** (4 mg, 0.07 mmol) was dissolved in HCl (1.2 mL, conc.). The solution was stirred at reflux for 3 h, then cooled and concentrated in vacuo. Purification was attempted using gradient flash chromatography (DCM/MeOH/EtOH/30% aq. NH_3, 15/2/2/1 → 5/2/2/1, v/v/v/v) to provide chloro-imino-galactitol **22** as a white solid with an inseparable decomposition product also observed. R_f = 0.37 (DCM/MeOH/EtOH/30% aq. NH_3, 5/2/2/1, v/v/v/v); ^{1}H NMR: (500 MHz, 2% DCl in D_2O, 20 °C) δ 4.65 (dd, $J_{2,3}$ = 7.1, $J_{3,4}$ = 4.4 Hz, 1H, H-3), 4.47 (t, $J_{4,5}$ = $J_{3,4}$ = 4.4 Hz, 1H, H-4), 4.08 (td, $J_{2,3}$ = $J_{1a,2}$ = 7.1, $J_{1b,2}$ = 5.4 Hz, 1H, H-2), 4.02 (dd, $^{2}J_{6a,6b}$ = 13.2, $J_{5,6a}$ = 4.4 Hz, 1H, H-6a), 4.01 (td, $J_{5,6b}$ = 11.5, $J_{5,6a}$ = $J_{4,5}$ = 4.4, Hz, 1H, H-5), 3.90 (dd, $^{2}J_{6a,6b}$ = 13.2, $J_{5,6b}$ = 11.5 Hz, 1H, H-6b), 3.61 (dd, $^{2}J_{1a,1b}$ = 13.7, $J_{1a,2}$ = 7.1 Hz, 1H, H-1a), 3.44 (dd, $^{2}J_{1a,1b}$ = 13.7, $J_{1b,2}$ = 5.4 Hz, 1H, H-1b); ^{13}C NMR: (125 MHz, 2% DCl in D_2O, 20 °C) δ 70.2 (C-3), 70.2 (C-4), 62.4 (C-5), 55.8 (C-2), 39.5 (C-6), 36.9 (C-1); HRMS (ESI) *m/z* calcd. for $[C_6H_{13}{}^{35}ClN_2O_2+H]^+$: 181.0738, obsd.:

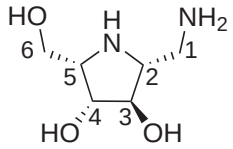
181.0741; *m/z* calcd. for $[C_6H_{13}{}^{37}ClN_2O_2+H]^+$: 183.0709, obsd.: 183.0713.

General Procedure for Acidic Hydrolysis of Carbamates. Amino carbamate (0.05 mmol) was dissolved in H_2SO_4 aq.˙ (1 mL, 3M). The solution was refluxed for 12 – 18 h, then cooled and neutralised by the addition of Dowex-OH^- ion-exchange resin. The resin mixture was filtered with H_2O and the filtrate concentrated *in vacuo* to provide the pure amino-imino-hexitols as the free base. Imino-hexitols were also characterised as the dihydrochloride salt with the addition of deuterium chloride.



1-Amino-1,2,5-trideoxy-2,5-imino-D-galactitol (7). By subjecting amino carbamate **21** (9.7 mg, 0.05 mmol) to the general procedure for the acidic hydrolysis of carbamates for 18 h, imino-D-galactitol **7** was obtained as a white solid (8.4 mg, 0.05 mmol, *quantitative*). R_f = 0.01 (DCM/MeOH/EtOH/30% aq. NH_3, 5/2/2/1, v/v/v/v); (HCl salt) $[\alpha]_D{}^{19.6}$ = -2.0 (c = 0.47, H_2O); IR (film) 3316, 2965, 1644, 1364, 1315, 1081, 1019, 835 cm^{-1}. ^1H NMR (500 MHz, D_2O, 2HCl salt) δ 4.59 (dd, $J_{2,3}$ = 7.2, $J_{3,4}$ = 4.7 Hz, 1H, H-3), 4.42 (t, $J_{4,5} = J_{3,4}$ = 4.7 Hz, 1H, H-4), 4.03 (td, $J_{2,3} = J_{1a,2}$ = 7.2, $J_{1b,2}$ = 5.7 Hz, 1H, H-2), 3.97 (dd, $^2J_{6a,6b}$ = 12.5, $J_{5,6a}$ = 4.7 Hz, 1H, H-6a), 3.88 (dd, $^2J_{6a,6b}$ = 12.5, $J_{5,6b}$ = 9.0 Hz, 1H, H-6b), 3.75 (dt, $J_{5,6b}$ = 9.0, $J_{5,6a} = J_{4,5}$ = 4.7 Hz, 1H, H-5), 3.57 (dd, $^2J_{1a,1b}$ = 13.9, $J_{1a,2}$ = 7.2 Hz, 1H, H-1a), 3.42 (dd, $^2J_{1a,1b}$ = 13.9, $J_{1b,2}$ = 5.7 Hz, 1H, H-1b); ^{13}C NMR (125 MHz, D_2O, 2HCl salt) δ 70.1 (C-3), 69.7 (C-4), 62.1 (C-5), 57.3 (C-6), 55.7 (C-2), 36.8 (C-1); HRMS (ESI) *m/z* calcd. for $[C_6H_{14}N_2O_3+H]^+$: 163.1077, obsd.: 163.1084. ^1H NMR: (500 MHz, 2% NaOD in D_2O, 20 °C) δ 4.21 (dd, $J_{4,5}$ = 6.2, $J_{3,4}$ = 5.1 Hz, 1H, H-4), 4.03 (t, $J_{3,4} = J_{2,3}$ = 5.1 Hz, 1H, H-3), 3.63 (dd, $^2J_{6a,6b}$

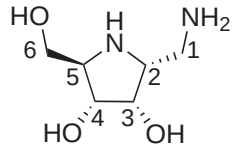
= 11.4, $J_{5,6a}$ = 5.1 Hz, 1H, H-6a), 3.58 (dd, $^{2}J_{6a,6b}$ = 11.4, $J_{5,6b}$ = 5.1 Hz, 1H, H-6b), 3.07 (dt, $J_{4,5}$ = 6.2, $J_{5,6a}$ = $J_{5,6b}$ = 5.1 Hz, 1H, H-5), 2.92 (td, $J_{1a,2}$ = $J_{1b,2}$ = 6.5, $J_{2,3}$ = 5.1 Hz, 1H, H-2), 2.76 (dd, $^{2}J_{1a,1b}$ = 13, $J_{1a,2}$ = 6.5 Hz, 1H, H-1a), 2.61 (dd, $^{2}J_{1a,1b}$ = 13, $J_{1b,2}$ = 6.5 Hz, 1H, H-1b); ^{13}C NMR: (125 MHz, 2% NaOD in D_2O, 20 °C) δ 73.0 (C-4), 71.6 (C-3), 61.3 (C-2), 60.4 (C-6), 60.3 (C-5), 40.2 (C-1).



1-Amino-1,2,5-trideoxy-2,5-imino-D-glucitol (14).[7] By subjecting amino carbamate **25** (4.2 mg, 0.02 mmol) to the general procedure for the acidic hydrolysis of carbamates for 12 h, imino-D-glucitol **14** was obtained as a white solid (3.6 mg, 0.02 mmol, *quantitative*). R_f = 0.01 (DCM/MeOH/EtOH/30% aq. NH_3, 5/2/2/1, v/v/v/v); (HCl salt) $[\alpha]_D^{20.1}$ = +8.0 (c = 0.38, H_2O); IR (film) 3121, 3050, 2930, 2799, 1633, 1401, 1082, 964, 879 cm^{-1}. ^{1}H NMR (500 MHz, D_2O, 2HCl salt) δ 4.38 (t, $J_{4,5}$ = $J_{3,4}$ = 3.0 Hz, 1H, H-4), 4.29 (t, $J_{3,4}$ = $J_{2,3}$ = 3.0 Hz, 1H, H-3), 4.03 (dd, $^{2}J_{6a,6b}$ = 9.8, $J_{5,6a}$ = 3.0 Hz, 1H, H-6a), 3.99 (dt, $J_{5,6b}$ = 7.3, $J_{5,6a}$ = J4,5 = 3.0 Hz, 1H, H-5), 3.96 (dd, $^{2}J_{6a,6b}$ = 9.8, $J_{5,6b}$ = 7.3 Hz, 1H, H-6b), 3.84 (td, $J_{1,2}$ = 6.9, $J_{2,3}$ = 3.0 Hz, 1H, H-2), 3.57 (d, $J_{1,2}$ = 6.9 Hz, 2H, H-1); ^{13}C NMR (125 MHz, D2O, 2HCl salt) δ 77.5 (C-3), 74.0 (C-4), 64.1 (C-5), 62.5 (C-2), 56.9 (C-6), 39.2 (C-1); HRMS (ESI) *m/z* calcd. for $[C_6H_{14}N_2O_3+H]^+$: 163.1077, obsd.: 163.1081. ^{1}H NMR: (500 MHz, 2% NaOD in D_2O, 20 °C) δ 3.97 (dd, $J_{4,5}$ = 5.5, $J_{3,4}$ = 2.7 Hz, 1H, H-4), 3.67 (dd, $^{2}J_{6a,6b}$ = 11.5, $J_{5,6a}$ = 6.2 Hz, 1H, H-6a), 3.65 (t, $J_{3,4}$ = $J_{2,3}$ = 2.7 Hz, 1H, H-3), 3.55 (dd, $^{2}J_{6a,6b}$ = 11.5, $J_{5,6b}$ = 6.2 Hz, 1H, H-6b), 3.16 (td, $J_{5,6a}$ = $J_{5,6b}$ = 6.2, $J_{4,5}$ = 5.5 Hz, 1H, H-5), 2.78 (ddd, $J_{1b,2}$ = 7.0, $J_{1a,2}$ = 5.1, $J_{2,3}$ = 2.7 Hz, 1H, H-2), 2.74 (dd, $^{2}J_{1a,1b}$ = 12.8, $J_{1a,2}$ = 5.1 Hz, 1H, H-1a), 2.60 (dd, $^{2}J_{1a,1b}$ = 12.8, $J_{1b,2}$ = 7.0 Hz, 1H, H-1b); ^{13}C NMR: (125 MHz, 2% NaOD in

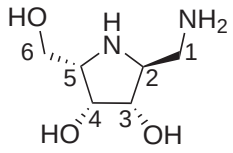
D_2O, 20 °C) δ 80.7 (C-3), 77.6 (C-4), 65.5 (C-2), 60.7 (C-5), 60.3 (C-6), 43.5 (C-1). The structural characterisation data were consistant with those reported in the literature,[7] key data includes: (free base) $[\alpha]_D^{20}$ = +24.0 (c = 0.7, H_2O); ^{13}C NMR (D_2O, pH 1) δ 57.8 (C-6), 40.2 (C-1).

General Procedure for Basic Hydrolysis of Carbamates. A solution of NaOH in EtOH (0.5 ml, 2M) was added to the amino carbamate (0.08 mmol) and stirred under reflux for 2 h. The resulting reaction mixture was loaded directly on to a Dowex-H^+ ion-exchange resin column and washed with H_2O to remove excess salt. The amine product was then eluted with 30% aq.˙ NH_3 and concentrated *in vacuo* to provide the pure amino-imino-hexitols as a mixture of protonation states, which were characterised as both the free base by adding sodium deuteroxide and the dihydrochloride salt with the addition of deuterium chloride.



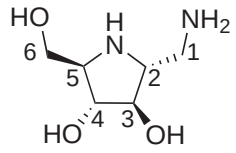
1-Amino-1,2,5-trideoxy-2,5-imino-D-talitol (8). By subjecting amino carbamate **23** (5 mg, 0.03 mmol) to the general procedure for the basic hydrolysis of carbamates, imino-talitol **8** was obtained as a colourless oil (4.1 mg, 0.03 mmol, 95%). R_f = 0.01 (DCM/MeOH/EtOH/30% aq. NH_3, 5/2/2/1, v/v/v/v); (HCl salt) $[\alpha]_D^{27.4}$ = +11.1 (c = 0.27 H_2O); IR (film) 3322, 2927, 1622, 1497, 1403, 1278, 1127, 1074, 1035, 981 cm^{-1}. ^1H NMR: (500 MHz, 2% DCl in D_2O, 20 °C) δ 4.42 (t, $J_{3,4}$ = $J_{2,3}$ = 3.6 Hz, 1H, H-3), 4.31 (dd, $J_{4,5}$ = 8.6, $J_{3,4}$ = 3.6 Hz, 1H, H-4), 3.99–3.97 (m, 1H, H-2), 3.97 (dd, $^2J_{6a,6b}$ = 12.7, $J_{5,6a}$ = 3.4 Hz, 1H, H-6a), 3.83 (dd, $^2J_{6a,6b}$ = 12.7, $J_{5,6b}$ = 5.8 Hz, 1H, H-6b), 3.70 (ddd, $J_{4,5}$ = 8.6, $J_{5,6b}$ = 5.8, $J_{5,6a}$ = 3.4 Hz, 1H, H-5), 3.60 (dd, $^2J_{1a,1b}$ = 13.9,

$J_{1a,2}$ = 7.1 Hz, 1H, H-1a), 3.43 (dd, $^{2}J_{1a,1b}$ = 13.9, $J_{1b,2}$ = 6.1 Hz, 1H, H-1b); ^{13}C NMR: (125 MHz, 2% DCl in D_2O, 20 °C) δ 71.2 (C-4), 70.0 (C-3), 62.4 (C-5), 57.9 (C-6), 57.6 (C-2), 35.9 (C-1); HRMS (ESI) *m/z* calcd. for $[C_6H_{14}N_2O_3+H]^+$: 163.1077, obsd.: 163.1084; ^{1}H NMR: (500 MHz, 2% NaOD in D_2O, 20 °C) δ 4.03 (t, $J_{3,4}$ = $J_{2,3}$ = 4.1 Hz, 1H, H-3), 3.89 (dd, $J_{4,5}$ = 8.3, $J_{3,4}$ = 4.1 Hz, 1H, H-4), 3.68 (dd, $^{2}J_{6a,6b}$ = 11.7, $J_{5,6a}$ = 3.9 hz, 1H, H-6a), 3.56 (dd, $^{2}J_{6a,6b}$ = 11.7, $J_{5,6b}$ = 6.3 Hz, 1H, H-6b), 3.09 (td, $J_{1a,2}$ = $J_{1b,2}$ = 7.1, $J_{2,3}$ = 4.1 Hz, 1H, H-2), 2.99 (ddd, $J_{4,5}$ = 8.3, $J_{5,6b}$ = 6.3, $J_{5,6a}$ = 3.9 Hz, 1H, H-5), 2.77 (dd, $^{2}J_{1a,1b}$ = 13.0, $J_{1a,2}$ = 7.1 Hz, 1H, H-1a), 2.62 (dd, $^{2}J_{1a,1b}$ = 13.0, $J_{1b,2}$ = 7.1 Hz, 1H, H-1b); ^{13}C NMR: (125 MHz, 2% NaOD in D_2O, 20 °C) δ 74.1 (C-4), 72.4 (C-3), 62.4 (C-6), 62.0 (C-5), 60.6 (C-2), 40.4 (C-1).



1-Amino-1,2,5-trideoxy-2,5-imino-L-altritol (9). By subjecting amino carbamate **24** (12.3 mg, 0.06 mmol) to the general procedure for the hydrolysis of carbamates, imino-hexitol **9** was obtained as a colourless oil (10.6 mg, 0.06 mmol, *quantitative*). R_f = 0.01 (DCM/MeOH/EtOH/30% aq. NH_3, 5/2/2/1, v/v/v/v); (HCl salt) $[\alpha]_D^{27.4}$ = -20.0 (c = 0.45 H_2O); IR (film) 3302, 2928, 1622, 1402, 1342, 1221, 1124, 1039, 1027 cm^{-1}. ^{1}H NMR: (500 MHz, 2% DCl in D_2O, 20 °C) δ 4.18 (dd, $J_{3,4}$ = 3.7, $J_{4,5}$ = 2.9 Hz, 1H, H-4), 4.14 (dd, $J_{2,3}$ = 9.7, $J_{2,3}$ = 3.7 Hz, 1H, H-3), 3.84 (dd, $^{2}J_{6a,6b}$ = 16.1, $J_{5,6a}$ = 8.7 Hz, 1H, H-6a), 3.75 (dd, $^{2}J_{6a,6b}$ = 16.1, $J_{5,6b}$ = 8.7 Hz, 1H, H-6b), 3.74 (td, $J_{5,6a}$ = $J_{5,6b}$ = 8.7, $J_{4,5}$ = 2.9 Hz, 1H, H-5), 3.66 (ddd, $J_{2,3}$ = 9.7, $J_{1a,2}$ = 7.9, $J_{1b,2}$ = 5.9 Hz, 1H, H-2), 3.41 (dd, $^{2}J_{1a,1b}$ = 14.0, $J_{1a,2}$ = 7.9 Hz, 1H, H-1a), 3.36 (dd, $^{2}J_{1a,1b}$ = 14.0, $J_{1b,2}$ = 7.9 Hz, 1H, H-1b); ^{13}C NMR: (125 MHz, 2% DCl in D_2O, 20 °C) δ 74.0 (C-4), 69.3 (C-3), 62.8 (C-5), 57.3 (C-6), 56.9 (C-2), 39.0 (C-1); HRMS (ESI) *m/z* calcd. for

$[C_6H_{14}N_2O_3+H]^+$: 163.1077, obsd.: 163.1079. ^{1}H NMR: (500 MHz, 2% NaOD in D_2O, 20 °C) δ 4.05 (t, $J_{4,5} = J_{3,4}$ = 4.1 Hz, 1H, H-4), 3.79 (dd, $J_{2,3}$ = 8.1, $J_{3,4}$ = 4.1 Hz, 1H, H-3), 3.70 (dd, $^2J_{6a,6b}$ = 11.2, $J_{5,6a}$ = 6.3 Hz, 1H, H-6a), 3.58 (dd, $^2J_{6a,6b}$ = 11.2, $J_{5,6b}$ = 6.3 Hz, 1H, H-6b), 3.17 (td, $J_{5,6a} = J_{5,6b}$ = 6.3, $J_{4,5}$ = 4.1 Hz, 1H, H-5), 2.93 (td, $J_{2,3} = J_{1b,2}$ = 8.1, $J_{1a,2}$ = 4.4 Hz, 1H, H-2), 2.76 (dd, $^2J_{1a,1b}$ = 13.2, $J_{1a,2}$ = 4.4 Hz, 1H, H-1a), 2.57 (dd, $^2J_{1a,1b}$ = 13.2, $J_{1b,2}$ = 8.1 Hz, 1H, H-1b); ^{13}C NMR: (125 MHz, 2% NaOD in D_2O, 20 °C) δ 75.6 (C-3), 72.6 (C-4), 62.7 (C-2), 60.6 (C-6), 59.6 (C-5), 43.8 (C-1). The structural characterisation data were consistant with those reported in the literature[13] for the enantiomer, except for the optical rotation being of similar magnitude but opposite sign: $[\alpha]_D^{20}$ = +18 (c = 0.1, H_2O).



1-Amino-1,2,5-trideoxy-2,5-imino-D-mannitol (12).[7,14] By subjecting amino carbamate **26** (4.0 mg, 0.02 mmol) to the general procedure for the basic hydrolysis of carbamates, imino-mannitol **12** was obtained as a colourless oil (3.2 mg, 0.02 mmol, 91%). R_f = 0.01 (DCM/MeOH/EtOH/30% aq. NH_3, 5/2/2/1, v/v/v/v); (HCl salt) $[\alpha]_D^{27.4}$ = +40.0 (c = 0.10, H_2O); IR (film) 3209, 2925, 1603, 1503, 1406, 1123, 1065, 1034, 813 cm^{-1}. ^{1}H NMR: (500 MHz, 2% DCl in D_2O, 20 °C) δ 4.08 (t, $J_{3,4} = J_{2,3}$ = 7.0 Hz, 1H, H-3), 4.04 (t, $J_{4,5} = J_{3,4}$ = 7.0 Hz, 1H, H-4), 3.89 (dd, $^2J_{6a,6b}$ = 12.7, $J_{5,6a}$ = 3.9 Hz, 1H, H-6a), 3.79 (dd, $^2J_{6a,6b}$ = 12.7, $J_{5,6b}$ = 7.0 Hz, 1H, H-6b), 3.73 (td, $J_{1,2}$ = 7.2, $J_{2,3}$ = 7.0 Hz, 1H, H-2), 3.61 (td, $J_{4,5} = J_{5,6b}$ = 7.0, $J_{5,6a}$ = 3.9 Hz, 1H, H-5), 3.36 (d, $J_{1,2}$ = 7.2 Hz, 2H, H-1); ^{13}C NMR: (125 MHz, 2% DCl in D_2O, 20 °C) δ 76.4 (C-3), 73.9 (C-4), 63.4 (C-5), 58.4 (C-6), 58.0 (C-2), 38.6 (C-1); HRMS (ESI) *m/z* calcd. for $[C_6H_{14}N_2O_3+H]^+$: 163.1077, obsd.: 163.1084. ^{1}H NMR: (500 MHz, 2% NaOD in D_2O,

20 °C) δ 3.83 (t, $J_{4,5}$ = $J_{3,4}$ = 7.6 Hz, 1H, H-4), 3.75 (t, $J_{3,4}$ = $J_{2,3}$ = 7.6 Hz, 1H, H-3), 3.72 (dd, $^{2}J_{6a,6b}$ = 11.7, $J_{5,6a}$ = 4.4 Hz, 1H, H-6a), 3.64 (dd, $^{2}J_{6a,6b}$ = 11.7, $J_{5,6b}$ = 6.1 Hz, 1H, H-6b), 2.99 (ddd, $J_{4,5}$ = 7.6, $J_{5,6b}$ = 6.1, $J_{5,6a}$ = 4.4 Hz, 1H, H-5) 2.95 (td, $J_{2,3}$ = $J_{1b,2}$ = 7.6, $J_{1a,2}$ = 4.4 Hz, 1H, H-2), 2.83 (dd, $^{2}J_{1a,1b}$ = 13.2, $J_{1a,2}$ = 4.4 Hz, 1H, H-1a), 2.67 (dd, $^{2}J_{1a,1b}$ = 13.2, $J_{1b,2}$ = 7.6 Hz, 1H, H-1b); ^{13}C NMR: (125 MHz, 2% NaOD in D_2O, 20 °C) δ 79.4 (C-3), 77.7 (C-4), 62.0 (C-2), 61.9 (C-6), 61.5 (C-5), 43.5 (C-1). The structural characterisation data were consistant with those reported in the literature,[7,14] key data includes: (free base) $[\alpha]_D^{20}$ = +49.4 (c = 0.9, H_2O); ^{13}C NMR (D2O, pH 1) δ 76.4 (C-3), 73.6 (C-4), 38.6 (C-1).

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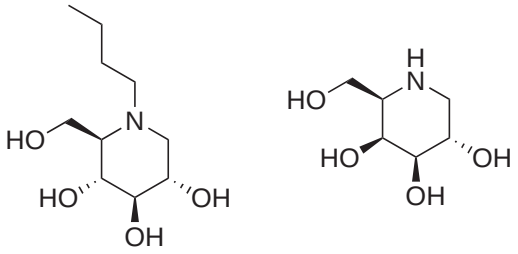
Chapter 4

Biological Evaluation of Amino-imino-hexitols

4.1 Introduction

Having developed methodology for the synthesis of amino-imino-hexitols (Chapters 2 and 3) we sought to evaluate their potential glycosidase inhibitory activity.[1] Azasugars have shown great promise for the treatment of lysosomal storage disorders as eluded to in Chapter 1. In fact, Zavesca,® (**1**, Fig. 4.1) as a potent inhibitor of glucosylceramide synthase, is used clinically to treat Gaucher disease in the form of substrate reduction therapy (SRT).[2] Lysosomal storage disorders are a group of over 50 rare metabolic disorders that result from genetic deficiencies in lysosomal enzymes.[3] As a result of mutations to metabolic enzymes, macromolecules such as glycolipids and glycoproteins build up in the lysosome, which leads to pathogenesis.[3] Gaucher disease, the most prevalent of the lysosomal storage disorders, is caused by deficiency of β-

glucocerebrosidase (GCase), the enzyme responsible for glucosylceramide degradation.[2,3] The accumulation of glucosylceramide in the lysosome causes severe symptoms such as liver or spleen damage, bone pains, and skeletal lesions.[2] Treatments for Gaucher disease include enzyme replacement therapy (ERT), which is costly and does not treat the neuropathogenic types of the disease due to the inability of proteins to cross the blood-brain barrier.[4] SRT, on the other hand, reduces the amount of glucosylceremide in the lysosome by inhibiting the enzyme responsible for its biosynthesis. A downside of this therapy is the requirement for large doses of inhibitor, which can lead to unwanted side-effects.[2] A third strategy is active-site-specific chaperone (ASSC) therapy.[2] An ASSC is a small molecule that can bind specifically to the active site of a misfolded enzyme to stabilise the structure and prevent it from being degraded in the cell as a mutant protein.[3] Thus, finding specific inhibitors of β-glucocerebrosidase can lead to treatments of Gaucher disease, as administration of ASSCs at a sub-inhibitory dose has been shown to be therapeutic.[5] An example of an ASSC is the piperidene deoxygalactonojirimycin (DGJ, **2**), an inhibitor of α-galactosidase,[6] which is currently in phase three clinical trials for the treatment of the lysosomal storage disorder Fabry's disease.[7]



1 **2**

Fig. 4.1 Azasugars as clinical drugs

Deficiencies in β-hexosaminidases are also responsible for a number of lysosomal storage diseases.[8] Absence of enzymes to cleave a terminal *N*-acetyl-β-D-glucosamine gives rise to Sanfilippo,[9,10] Tay-Sachs and Sandhoff[11] diseases. Finding potent inhibitors of β-hexosaminidases could help elucidate the catalytic mechanism of the enzymes, and hence lead to the design of ASSCs to treat these debilitating genetic diseases.[9] The inhibition of *N*-acetyl-β-D-hexosaminidase by the D-*manno* acetamide (**3**, Fig. 4.2) first prepared by Wong and co-workers,[12] has also led to potential treatments for osteoarthritis[13,14] and bacterial infection[15] (discussed in Chapter 1.2). Other amino-imino-hexitols have proven potent glycosidase inhibitors such as the library of compounds based on the D-*manno* scaffold (**4**) prepared by Wrodnigg and co-workers,[16–20] in which coumarin derivative **5** proved to be a powerful β-glucosidase inhibitor.[17]

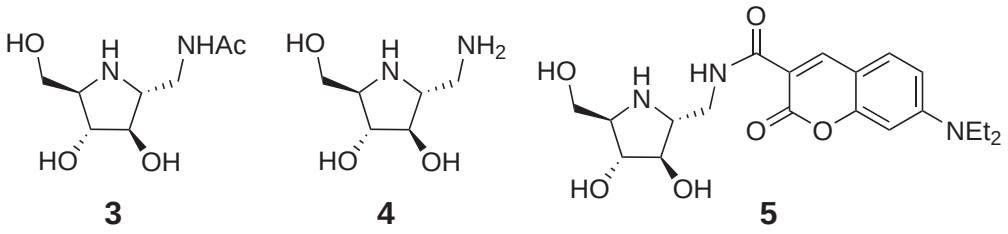


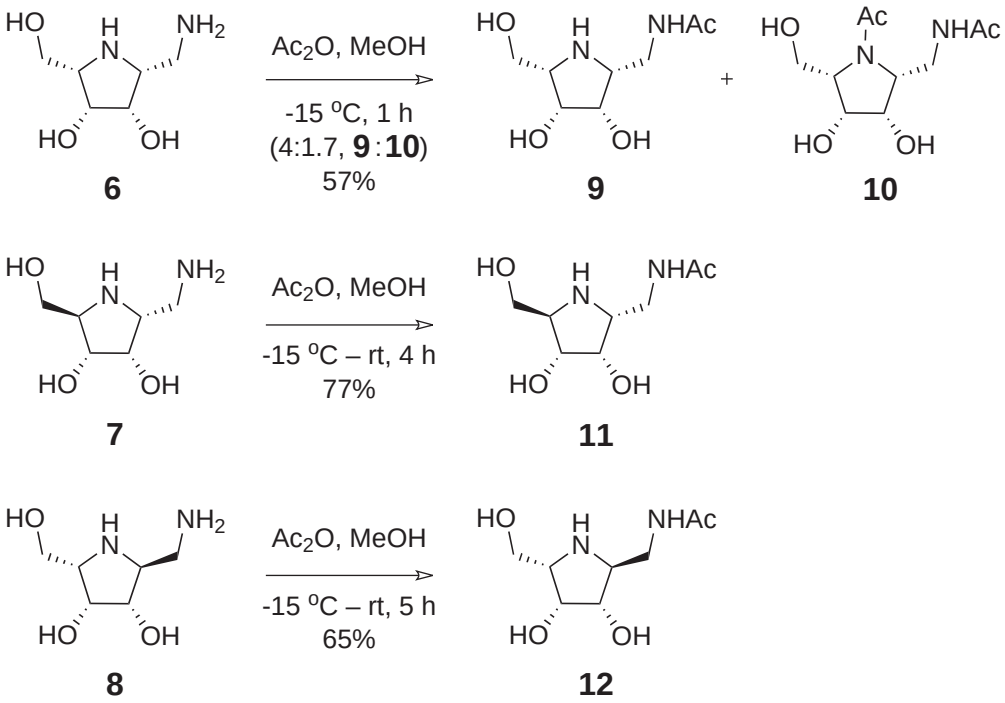
Fig. 4.2 Amino-imino-hexitols as glycosidase inhibitors

The work in this chapter outlines the synthesis of *N*-acylated amino-imino-hexitols from the previously synthesised hexitols (Chapter 3), along with subsequent evaluation of the glycosidase kinetics of these amino-hexitols, their precursors, and that of the L-*ido* scaffold. To gauge the potential glycosidase inhibitory activity of our synthesised amino-imino-hexitols we sent samples to the laboratory of Professor Stephen Withers (University of British Columbia, Canada) for kinetic evaluation. We chose a panel of glycosidases based on their importance and availability, to determine the inhibition

dissociation constant (K_i, discussed in Chapter 1.1) for each of the azasugars.

4.2 *N*-Acylation of Amino-imino-hexitols

Acetylated derivatives of the novel stereoisomers, D-*galacto* **6**, D-*talo* **7** and L-*altro* **8**, were prepared as literature precedence suggests that *N*-acylation improves glycosidase activity (Scheme 4.1).[16,18–26] Subjecting D-*galacto* hexitol **6** to acetic anhydride in MeOH[20] gave acetamide **9** along with the bis-acetamide **10** in 57% combined yield in a ratio of 4:1.7 (**9**:**10**) and they could be separated by chromatography. Selective acetylation of D-*talo* **7** and L-*altro* **8**, however, proceeded smoothly to give the corresponding acetamides **11** and **12**, in 77% and 65% yield, respectively.

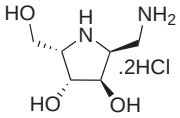
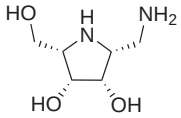
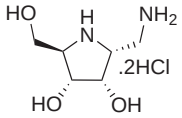
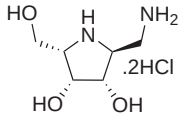
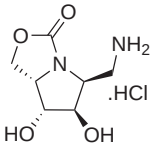
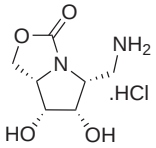
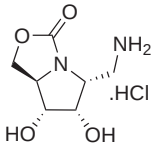
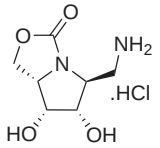
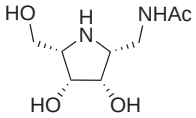
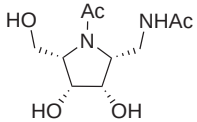
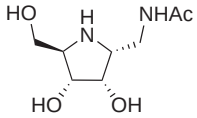
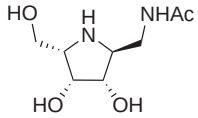


Scheme 4.1 Preparation of acetamides from amino-imino-hexitols **6**–**8**

4.3 Glycosidase Inhibition

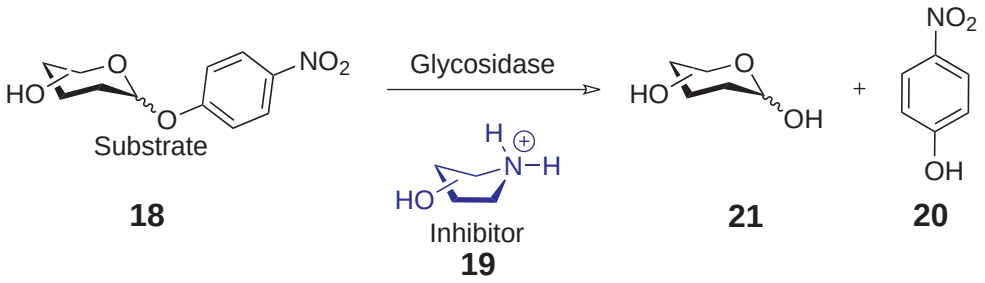
The novel amino-imino-hexitols (**6**–**8**), their carbamate precursors (**13**, **14** and **15**) and acetamides (**9**–**12**), and the previously reported L-*ido* derivative (**16**)[25] and carbamate precursor (**17**) were then sent to the laboratory of Professor Stephen Withers for kinetic studies against three chosen glycosidases. The compounds were tested for their ability to inhibit human lysosomal β-D-glucocerebrosidase (GCase), N-acetyl-β-D-hexosaminidase from *Streptomyces plicatus* (*Sp*HEX) and β-D-glucosidase from *Agrobacterium* sp. (ABG) (Table 4.1). ABG is a benchmark enzyme that libraries of compounds have been tested against, to compare the kinetics of all azasugars evaluated in the Withers laboratory.[17–20]

Table 4.1 Glycosidase inhibition studies[a]

Enzyme	Amino-imino-hexitols			
	 **16**	 **6**	 **7**	 **8**
GCase	9	n.i.*	n.i.	n.i.*
*Sp*HEX	n.i.	n.i.	n.i.	n.i.‡
ABG	n.i.‡	n.i.‡	0.006	1.1
	 **17**	 **13**	 **14**	 **15**
GCase	n.i.*	n.i.	n.i.	n.i.
*Sp*HEX	n.i.	n.i.‡	n.i.‡	n.i.‡
ABG	n.i.‡	n.i.‡	0.06	2.3
	 **9**	 **10**	 **11**	 **12**
GCase	n.i.*	n.i.*	n.i.*	n.i.*
*Sp*HEX	0.5	n.i.‡	n.i.‡	0.5
ABG	0.8	n.i.‡	0.07	1.3

[a]Key: K_i values (mM) of compounds with GCase = β-D-glucocerebrosidase from human lysosome, *Sp*Hex = *N*-acetyl-β-D-hexosaminidase from *Streptomyces plicatus*, ABG = β-D-glucosidase from *Agrobacterium* sp., n.i. = no inhibition was observed up to 10 mM inhibitor concentration, n.i.* = no inhibition was observed up to 2 mM inhibitor concentration, n.i.‡ = no inhibition was observed up to 1 mM inhibitor concentration.

Assays were performed in a similar manner for all three glycosidases tested to determine the inhibition dissociation constant (K_i) for each amino-imino-hexitol.[1] Kinetic experiments were run in 96-well plates at 37 °C comprising the purified enzyme, the natural substrate as the *p*-nitrophenylglycoside[27] (**18**) and the potential azasugar inhibitor (**19**) being evaluated (Scheme 4.2). The concentrations of both the substrate and inhibitor were varied independently for the glycosidase catalysed reactions. Glycosidase activity rates, or enzyme velocity, were determined by measuring the amount of *p*-nitrophenol (**20**) released by the hydrolysis of substrate **18** to the hemiacetal product (**21**), as measured by its absorbance at 405 nm.[28] The data gained from the rate experiments provided information on the K_i value from initial analysis of a linear plot of the inverse velocity against inhibitor concentration. Subsequent analysis fit the data directly to the Michaelis-Menton equation, which describes the enzymatic reaction in the presence of a competitive inhibitor.



Scheme 4.2 Schematic of a general enzymatic kinetic assay

As can be seen in Table 4.1, L-*ido* amine **16** exhibited weak selective inhibition of GCase (K_i = 9 mM), the enzyme involved in Gaucher disease, however, this level of inhibition is likely to be ineffective for any therapeutic effect. Changing the stereochemistry around the pyrrolidine ring at C-2 and C-3 for D-*galacto* configured amine **6**, resulted in a loss of any glycosidase inhibitory activity (no inhibition observed up to 1, 2 and 10 mM

concentrations). However, inverting the stereochemistry at C-5 for D-*talo* configured amine **7** provided an azasugar with very good selective glycosidase inhibitory activity for ABG (K_i = 0.006 mM), this may be due to the D-*talo* configuration being more favourable for residing in the glycosidase active-site. With the opposite 2,5-*trans*-configuration across the imino-ring for L-*altro* amine **8**, selective inhibition of ABG was also observed (K_i = 1.1 mM), however, with only moderate inhibition of the enzyme.

The glycosidase inhibitory activity of the carbamate precursors follows a similar trend to that observed with the respective amines. Thus, L-*ido* carbamate **17** and D-*galacto* carbamate **13** showed no glycosidase inhibitory activity for any of the enzymes tested. Furthermore, the D-*talo* carbamate **14** exhibited good selective inhibition of ABG (K_i = 0.06 mM), although it was an order of magnitude less potent than amine **7**. Moderate selective inhibitory activity was also observed for L-*altro* carbamate **15** (K_i = 2.3 mM).

Examining the glycosidase inhibitory activity of the *N*-acyl derivatives, it can be seen that D-*galacto* acetamide **9** inhibits both *Sp*HEX and ABG with a K_i of 0.5 mM and 0.8 mM, respectively. However, the addition of a second *N*-acyl group in bis-acetamide **10** resulted in loss of all glycosidase inhibitory activity. The D-*talo* acetamide **11** exhibited good selective inhibitory activity for ABG (K_i = 0.07 mM), with a similar value to D-*talo* carbamate **14**, however, an order of magnitude less potent than D-*talo* amine **7**. In the case of L-*altro* acetamide **12** inhibitory activities were observed for both *Sp*HEX and ABG (K_i = 0.5 and 1.3 mM), similar to the inhibitory activities observed for acylated amino-imino-hexitol **9**. Given that the D-*manno* isomer of these compounds (**3**, refer Fig. 4.2) has been shown to inhibit Jack bean *N*-acetyl-β-D-hexosaminidase (K_i = 1.9

μm),[12] it appears as though the functional group pattern around the pyrrolidine scaffold is more important than stereochemistry for the inhibition of this enzyme.

Of the five compounds found to be selective ABG inhibitors, it is altogether surprising that D-*talo* amine **7** had the most potent inhibitory activity as generally *N*-acylated derivatives of amino-imino-hexitols are better inhibitors than their parent compounds.[16,18–26] Here, it should also be noted that more lipophilic *N*-substituted derivatives generally exhibit improved inhibitory activity. For example, D-*manno* acetamide **3** and amine **4** both exhibited a K_i of 0.025 mM against ABG,[20] whereas *N*-functionalised lipophilic D-*manno* derivatives, such as **5**, showed potent inhibition in the nanomolar range.[18] In view of this, the preparation of lipophilic derivatives of the D-*talo* scaffold has the potential to produce noteworthy glycosidase inhibitors. Moreover, our amino-imino-hexitols have only been evaluated against a small range of glycosidases and may well show inhibitory activity for other glycosyl hydrolytic enzymes, therefore testing a broader range of glycosidase enzymes may be of interest.

4.4 Conclusion

In summary, we have synthesised four novel *N*-acylated amino-imino-hexitols in one step from the amine precursors. A library of amino-imino-hexitols, including the *N*-acyl derivatives has been evaluated for glycosidase inhibitory activity against three enzymes: human lysosomal β-D-glucocerebrosidase (GCase), *N*-acetyl-β-D-hexosaminidase from *Streptomyces plicatus* (*Sp*Hex), and β-D-glucosidase from *Agrobacterium* sp. (ABG). Unfortunately, no good inhibitors were found for GCase, the glycosidase involved in Gaucher disease. L-*Ido* amine **16** is unlikely to have any potential therapeutic effect

as an active-site-specific chaperone (ASSC) for GCase, with a K_i of only 9 mM. In the case of *Sp*HEX, two inhibitors were identified with activity in the sub-millimolar range. Both the D-*galacto* (**9**) and L-*altro* (**12**) isomers of the acylated amino-imino-hexitols had inhibitory activities of 0.5 mM and in spite of the varied stereochemistry around the pyrrolidine ring the acetamido moiety of the azasugar influences inhibitory activity for *Sp*HEX. Furthermore, several amino-imino-hexitol derivatives have shown promising selective glycosidase activity against ABG (e.g. **7**, **8**, **14**, and **11**), with the D-*talo* configuration exhibiting good selective inhibition of β-D-glucosidase. Moreover D-*talo* amine **7** was found to be the most potent inhibitor in the micromolar range and may show inhibitory activity for other types of glycosidases. In light of these findings, further derivatisation of the D-*talo* scaffold has the potential to provide powerful glycosidase inhibitors.

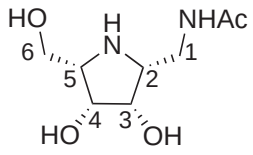
4.5 Experimental

General Experimental

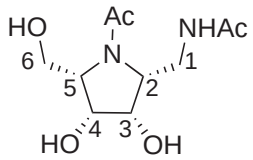
All chemicals obtained from commercial suppliers were used without further purification. All solvents were removed by evaporation under reduced pressure. Reactions were monitored by TLC analysis on silica gel coated plastic sheets (0.20 mm, Polygram SIL G/UV254) with detection by coating with 20% H_2SO_4 in EtOH followed by charring at *ca.* 150 °C, by coating with a solution of ninhydrin in EtOH followed by charring at *ca.* 150 °C, or by coating with a solution of 5% $KMnO_4$ and 1% $NaIO_4$ in H_2O followed by heating. Column chromatography was performed on silica gel (40-63 μm). High-resolution mass spectra were recorded on a Waters Q-TOF Premier™ Tandem Mass Spectrometer using positive electro-spray ionisation. Nuclear magnetic resonance spectra were recorded using a Varian Inova operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C or a Varian Direct Drive operating at 600 MHz and 133 MHz. ^{1}H and ^{13}C chemical shifts (δ) were internally referenced to the residual solvent peak (D_2O = 4.79 ppm for ^{1}H). NMR peak assignments are based on 2D NMR experiments (COSY, HSQC, and HMBC).

General Procedure for the Acetylation of Amino-imino-hexitols[20] Amino-imino-hexitol (0.05 mmol) was co-evaporated with dry toluene (0.5 mL) three times, placed under argon and dissolved in dry MeOH (500 μL). Acetic anhydride (0.05 mmol) was added to the solution at -15 °C. The solution was concentrated in vacuo when TLC analysis (DCM/MeOH/EtOH/30% aq. NH_3, 5/2/2/1, v/v/v/v) revealed the reaction was complete. Purification was achieved using flash chromatography to provide the pure

acetamides (DCM/MeOH, 5/1, v/v containing 1% of 30% aq. NH_3).

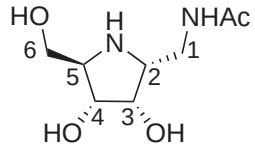


1-Acetamido-1,2,5-trideoxy-2,5-imino-D-galactitol (9). By subjecting imino-D-galactitol **6** (8.0 mg, 0.05 mmol) to the general procedure for the acetylation of amino-imino-hexitols for 1 hour at -15 °C, acetamide **9** was obtained as a colourless oil (4.0 mg, 0.02 mmol, 40%). R_f = 0.29 (DCM/MeOH/EtOH/30% aq. NH_3, 5/2/2/1, v/v/v/v); ^{1}H NMR: (500 MHz, D_2O, 20 °C) δ 4.48 (dd, $J_{3,4}$ = 4.7, $J_{2,3}$ = 6.3 Hz, 1H, H-3), 4.41 (t, $J_{3,4}$ = $J_{4,5}$ = 4.7 Hz, 1H, H-4), 3.94 (dd, $J_{6a,6b}$ = 12.2, $J_{5,6a}$ = 4.5 Hz, 1H, H-6a), 3.87 (dd, $J_{6a,6b}$ = 12.2, $J_{5,6b}$ = 8.6 Hz, 1H, H-6b), 3.77–3.74 (m, 2H, H-2, H-5), 3.64 (dd, $J_{1a,1b}$ = 14.5, $J_{1a,2}$ = 5.4 Hz, 1H, H-1a), 3.59 (dd, $J_{1a,1b}$ = 14.5, $J_{1b,2}$ = 7.8 Hz, 1H, H-1b), 2.00 (s, 3H, $COCH_3$); ^{13}C NMR: (125 MHz D_2O, 20 °C) δ 175.2 (C=O), 69.9, 69.7 (C-3, C-4), 61.2, 59.4, 57.8 (C-2, C-5, C-6), 36.8 (C-1), 21.6 ($COCH_3$). HRMS (ESI) *m/z* calcd. for $[C_8H_{17}N_2O_4+H]^+$: 205.1183, obsd.: 205.1185.



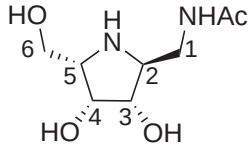
1-Acetamido-1,2,5-trideoxy-2,5-acetimido-D-galactitol (10). By subjecting imino-D-galactitol **6** (8.0 mg, 0.05 mmol) to the general procedure for the acetylation of amino-imino-hexitols for 1 hour at -15 °C, bis-acetamide **10** was obtained as a colourless oil and appeared as a 1:1 mixture of rotamers in the NMR spectra (2.0 mg, 0.008 mmol, 17%). R_f = 0.50 (DCM/MeOH/EtOH/30% aq. NH_3, 5/2/2/1, v/v/v/v); ^{1}H NMR: (500 MHz, D_2O, 20 °C) δ 4.38 (dd, $J_{4',5'}$ = 6.3, $J_{3',4'}$ = 5.6 Hz, 1H, H-4'), 4.34 (dd, $J_{4,5}$ = 7.6, $J_{3,4}$ = 5.4 Hz, 1H, H-4), 4.27 (dd, $J_{2,3}$ = 7.0, $J_{3,4}$ = 5.4 Hz, 1H,

H-3), 4.24 (dd, $J_{2',3'}$ = 3.0, $J_{3',4'}$ = 5.6 Hz, 1H, H-3'), 4.18–4.12 (m, 4H, H-2, H-2', H-5, H-5'), 3.89 (dd, $J_{6a,6b}$ = 12.2, $J_{5,6a}$ = 5.0 Hz, 1H, H-6a), 3.87 (dd, $J_{6a',6b'}$ = 11.5, $J_{5',6a'}$ = 6.0 Hz, 1H, H-6a'), 3.81 (dd, $J_{6a',6b'}$ = 11.5, $J_{5',6b'}$ = 4.5 Hz, 1H, H-6b'), 3.79 (dd, $J_{6a,6b}$ = 12.2, $J_{5,6b}$ = 5.5, 1H, H-6b), 3.60 (dd, $J_{1a,1b}$ = 14.1, $J_{1a,2}$ = 6.3 Hz, 1H, H-1a), 3.57 (dd, $J_{1a',1b'}$ = 13.4, $J_{1a',2'}$ = 5.8 Hz, 1H, H-1a'), 3.50 (dd, $J_{1a,1b}$ = 14.1, $J_{1b,2}$ = 6.3 Hz, 1H, H-1b), 3.48 (dd, $J_{1a',1b'}$ = 13.4, $J_{1b',2'}$ = 7,7 Hz, 1H, H-1b'), 2.12 (s, 3H, $NCOCH_3$), 2.11 (s, 3H, $NCOCH_3$'), 1.97 (s, 3H, $NHCOCH_3$), 1.94 (s, 3H, $NHCOCH_3$'); ^{13}C NMR: (125 MHz D_2O, 20 °C) δ 175.1 (NC=O), 175.0 (NC=O'), 174.3 (NHC=O), 174.1 (NHC=O'), 70.3 (C-3), 70.2 (C-4), 70.1 (C-4'), 69.6 (C-3'), 62.2 (C-5), 61.1 (C-5'), 60.0 (C-2'), 59.3 (C-6), 59.0 (C-6'), 58.7 (C-2), 38.9 (C-1), 38.1 (C-1'), 21.8 ($NHCOCH_3$), 21.8 ($NHCOCH_3$'), 21.7 ($NCOCH_3$), 21.2 ($NCOCH_3$'). HRMS (ESI) *m/z* calcd. for $[C_{10}H_{18}N_2O_5+Na]^+$: 269.1108, obsd.: 269.1115.



1-Acetamido-1,2,5-trideoxy-2,5-imino-D-talitol (11). By subjecting imino-D-talitol dihydrochloride **7** (4.0 mg, 0.017 mmol) to the general procedure for the acetylation of amino-imino-hexitols for 3 hours at -15 °C, then 1 hour at room temperature, acetamide **11** was obtained as a colourless oil (2.0 mg, 0.009 mmol, 77%). R_f = 0.30 (DCM/MeOH/EtOH/30% aq. NH_3, 5/2/2/1, v/v/v/v); ^{1}H NMR: (500 MHz, D_2O, 20 °C) δ 4.17 (t, $J_{2,3}$ = $J_{3,4}$ = 3.8 Hz, 1H, H-3), 4.11 (dd, $J_{4,5}$ = 8.8, $J_{3,4}$ = 3.8 Hz, 1H, H-4), 3.83 (dd, $J_{6a,6b}$ = 12.2, $J_{5,6a}$ = 3.5 Hz, 1H, H-6a), 3.71 (dd, $J_{6a,6b}$ = 12.2, $J_{5,6b}$ = 5.8 Hz, 1H, H-6b), 3.55–3.49 (m, 1H, H-1a), 3.45–3.36 (m, 3H, H-1b, H-2, H-5), 1.97 (s, 3H, $COCH_3$); ^{13}C NMR: (125 MHz D_2O, 20 °C) δ 174.7 (C=O), 72.2, 71.0 (C-3, C-4), 61.7, 59.9, 59.1 (C-2, C-5, C-6), 37.8 (C-1), 21.7 ($COCH_3$). HRMS (ESI) *m/z*

calcd. for $[C_8H_{17}N_2O_4+H]^+$: 205.1183, obsd.: 205.1184.



1-Acetamido-1,2,5-trideoxy-2,5-imino-L-altritol (12). By subjecting imino-L-altritol dihydrochloride **8** (5.0 mg, 0.021 mmol) to the general procedure for the acetylation of amino-imino-hexitols for 2 hours at -15 °C, then 3 hours at room temperature, acetamide **12** was obtained as a colourless oil (2.6 mg, 0.013 mmol, 65%). R_f = 0.31 (DCM/MeOH/EtOH/30% aq. NH_3, 5/2/2/1, v/v/v/v); ^{1}H NMR: (500 MHz, D_2O, 20 °C) δ 4.18 (t, $J_{4,5} = J_{3,4}$ = 3.9 Hz, 1H, H-4), 3.98 (dd, $J_{2,3}$ = 8.5, $J_{3,4}$ = 3.9 Hz, 1H, H-3), 3.80 (dd, $J_{6a,6b}$ = 11.4, $J_{5,6a}$ = 6.8 Hz, 1H, H-6a), 3.66 (dd, $J_{6a,6b}$ = 11.4, $J_{5,6b}$ = 6.8 Hz, 1H, H-6b), 3.47 (dd, $J_{1a,1b}$ = 14.1, $J_{1a,2}$ = 4.4 Hz, 1H, H-1a), 3.41 (td, $J_{5,6a} = J_{5,6b}$ = 6.8, $J_{4,5}$ = 3.9 Hz, 1H, H-5), 3.32 (dd, $J_{1a,1b}$ = 14.1, $J_{1b,2}$ = 7.4 Hz, 1H, H-1b), 3.22–3.25 (m, 1H, H-2), 2.00 (s, 3H, $COCH_3$); 174.9 (C=O), 74.5 (C-3), 71.3 (C-4), 60.0 (C-5), 59.7 (C-2), 59.6 (C-6), 41.1 (C-1), 21.7 ($COCH_3$). HRMS (ESI) *m/z* calcd. for $[C_8H_{17}N_2O_4+H]^+$: 205.1183, obsd.: 205.1185.

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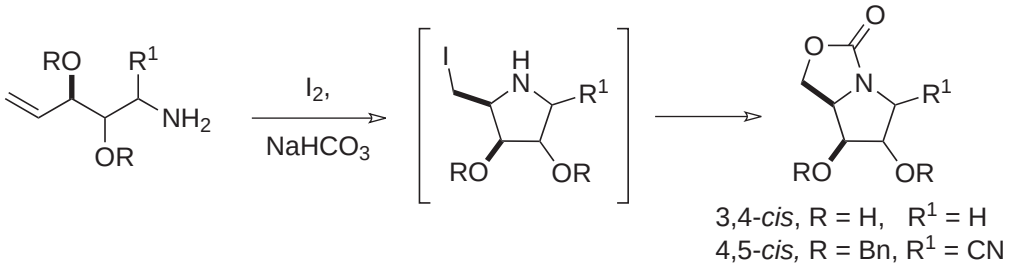
Chapter 5

Mechanistic Considerations and Diastereoselectivity of the I_2-mediated Carbamate Annulation

5.1 Introduction

The I_2-mediated carbamate annulation is highly diastereoselective in favour of 3,4- and 4,5-*cis*-carbamates, (Scheme 5.1).[1–5] Indeed, where R^1 = H, only the 3,4-*cis* isomer is observed.[1–3] However, as illustrated in chapter three of this thesis, a decrease in diastereoselectivity can be observed when R^1 = CN.[5] Given that the I_2-mediated carbamate annulation is paramount to our synthesis of amino-imino-hexitols, we wanted to investigate the mechanism and reasons for the diastereoselectivity of the reaction.

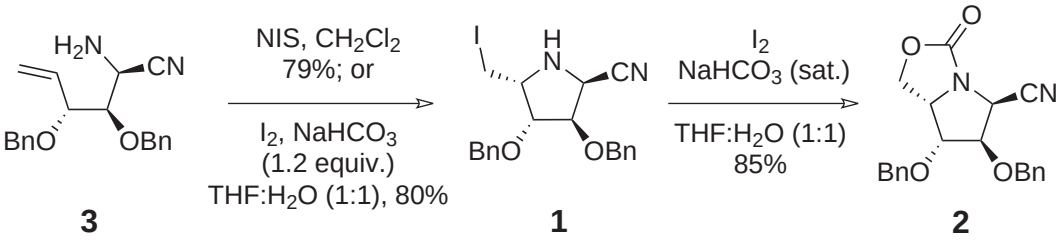


Scheme 5.1 General scheme for I_2-mediated carbamate annulation

5.2 Mechanistic Considerations

As a first step in understanding more about the mechanism of this annulation, it needed to be determined whether the proposed primary iodide (e.g. **1**, Scheme 5.2) was indeed an intermediate in the reaction. Previous attempts to isolate the suspected iodoazasugar intermediate in the protecting-group-free carbamate annulation had been unsuccessful[1] (Chapter 1.3.3), however, when extending the I_2-mediated carbamate annulation to the synthesis of amino-imino-hexitols, we isolated the iodoamine, which was formed during reactions to produce carbamate **2** (As highlighted in Chapter 2 and also illustrated in Scheme 5.2 below).[4] Here, alkenylamine *syn*-**3** was treated with either NIS in DCM or 1.2 equiv. of $NaHCO_3$ and I_2 in THF/H_2O (1:1) to give iodoamine **1** in good (80% yield). Subjection of iodide **1** to excess $NaHCO_3$ then led to the smooth transformation of **1** into carbamate **2**, again in good (85%) yield. Having established that iodoamine **1** could be converted to carbamate **2**, we then repeated the carbamate annulation and through careful analysis by TLC, NMR spectroscopy and mass spectrometry, observed that iodoamine **1** was formed *en route* to **2**. This confirmed the role of iodoamine **1** as an intermediate formed in the I_2-mediated carbamate annulation. Furthermore, as

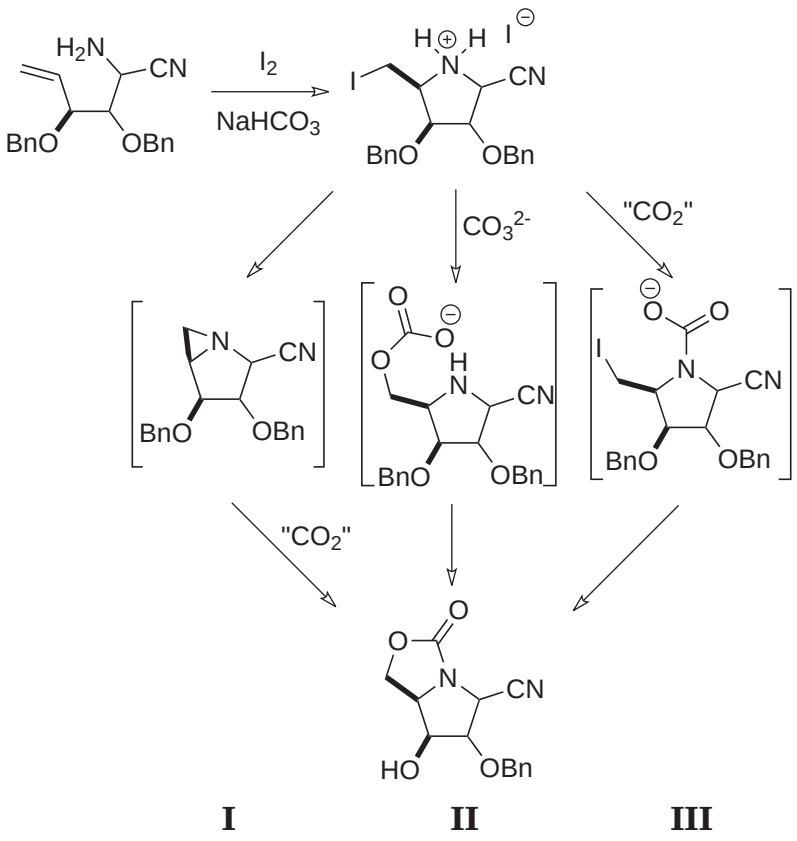
the formation of iodoamine **1** is irreversible, it is highly likely that this step determines the diastereoselectivity of the reaction. These findings are also supported by literature precedent whereby the reactions of alkenylamines with $NaHCO_3$ (*ca.* 1–3 equiv.) and I_2 are found to yield iodoamines.[6–8] The diastereoselectivities of these literature iodamine forming reactions are discussed further in Section 5.3.



3 **1** **2**

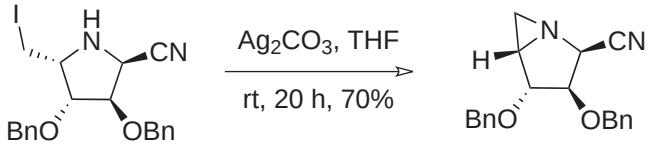
Scheme 5.2 Formation of iodoazasugar **1** *en route* to the synthesis of carbamate **2**

Having established that an iodoamine is initially formed in the carbamate annulation, the next question to address was the mechanism of CO_2 addition. As discussed in the introduction (Section 1.3.2), the source of CO_2 has been shown to come from the dissolution of $NaHCO_3$ in H_2O *en route* to the carbamate. To this end, we have proposed three reaction pathways from the intermediate iodoamine (Scheme 5.3).[9] The first involves formation of an aziridine, which has been previously reported by Verhelst *et al.*[6] and also by Diaba and Bonjoch.[10] Subsequent ring opening of the aziridine by CO_2 then provides the carbamate. The second pathway involves a carbonate as a key intermediate, while the third proposed mechanism occurs via the formation of carbamic acid, which subsequently cyclises with displacement of iodide.



Scheme 5.3 Possible mechanisms for the carbamate annulation

To gain insight into the potential reaction pathways for the I_2-mediated annulation, we decided to synthesise aziridine **4** to determine if it was an intermediate *en route* to the carbamate. To this end, iodoamine **1** was treated with Ag_2CO_3 (2 equiv.) to provide aziridine **4** [R_f = 0.19 (hexanes/EtOAc, 1:1, v/v) in good (70%) yield, (Scheme 5.4).[9] The structure of **4** was established by 2D (COSY, HSQC and HMBC) and 1D (^{1}H and ^{13}C) NMR in combination with high resolution mass spectrometry ($[C_{20}H_{20}N_2O_2+Na]^+$ = 343.1416, full characterisation data can be found in the experimental section).



1 **4**

Scheme 5.4 Formation of aziridine **4** from iodoamine **1**

Analysis of these data revealed characteristic ^{1}H NMR resonances, (Fig. 5.1) observed at δ 1.87 (dd) and 1.76 ppm (dd), for the aziridine methylene protons 6_A and 6_B, respectively, and δ 2.66 ppm (ddd) for the aziridine methine proton (H-5). The aziridine methylene protons correlated in the HSQC to the upfield ^{13}C NMR resonance at δ 30.2 ppm (C-6, Fig. 5.2), with the carbon signal for C-5 at δ 39.8. The nitrile carbon chemical shift, observed at (^{13}C NMR δ) 116.6 ppm, was confirmed by HMBC correlations to H-2 and H-3.

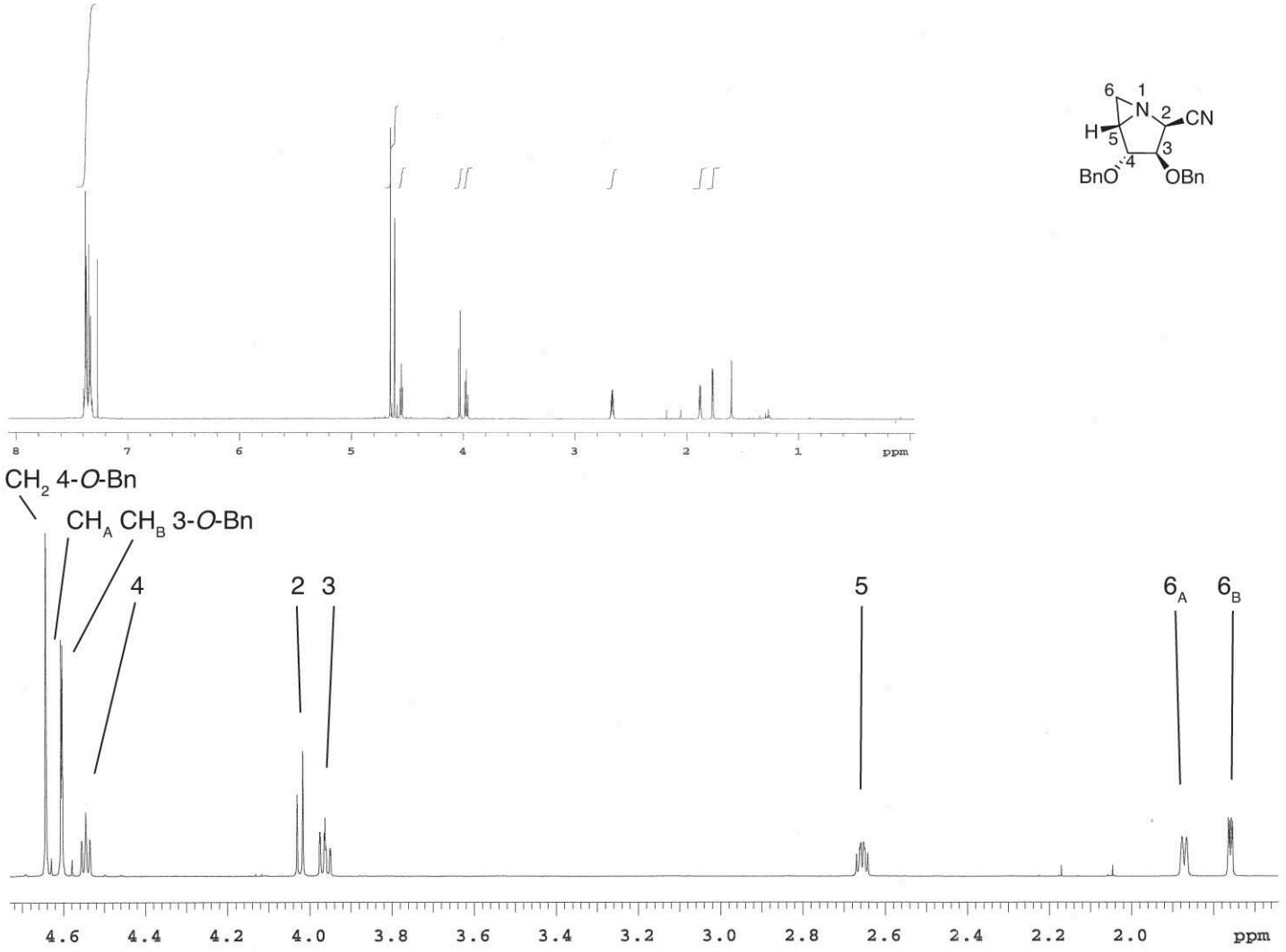


Fig. 5.1 ^{1}H NMR spectrum for aziridine **4**, (500 MHz, in $CHCl_3$)

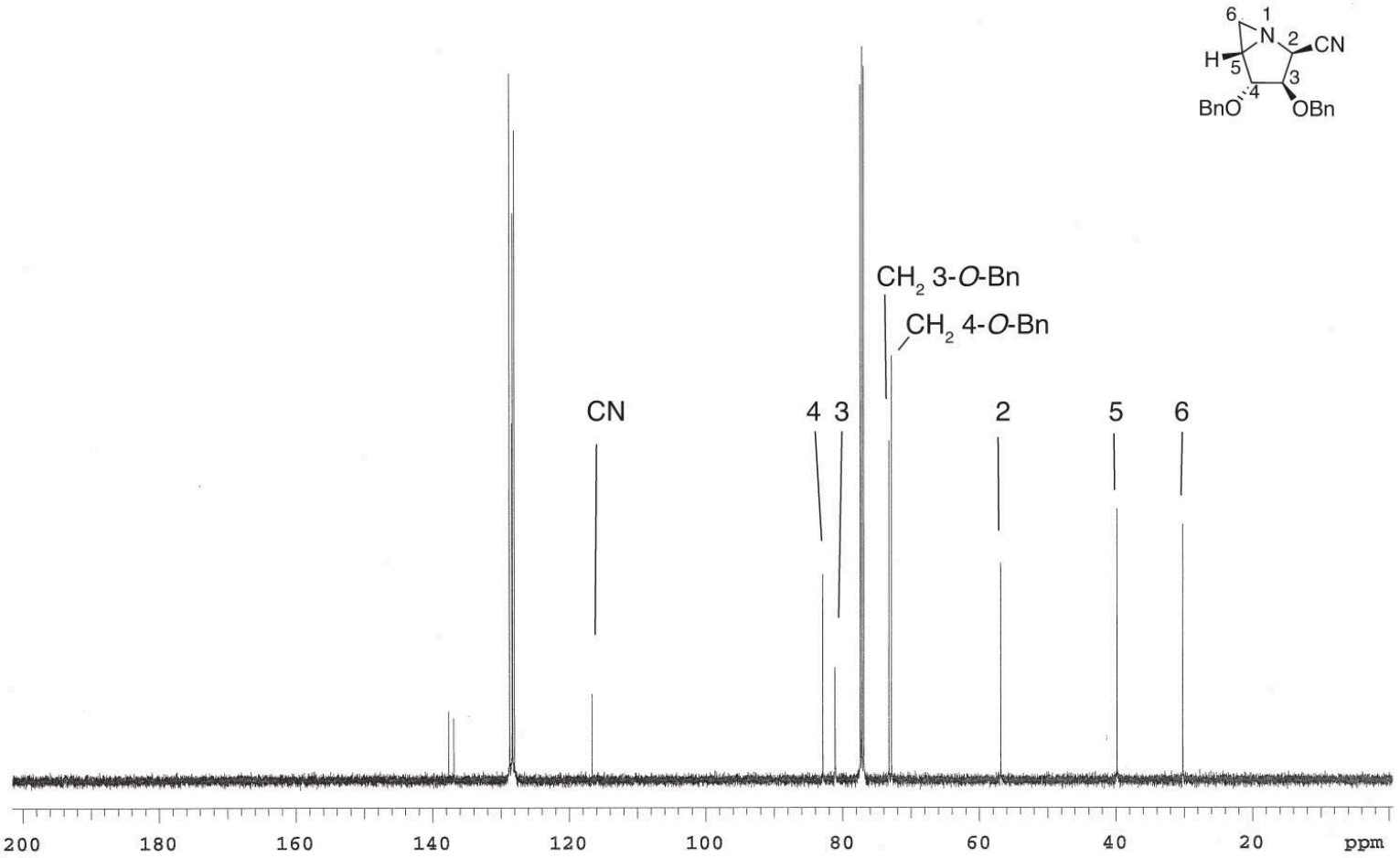
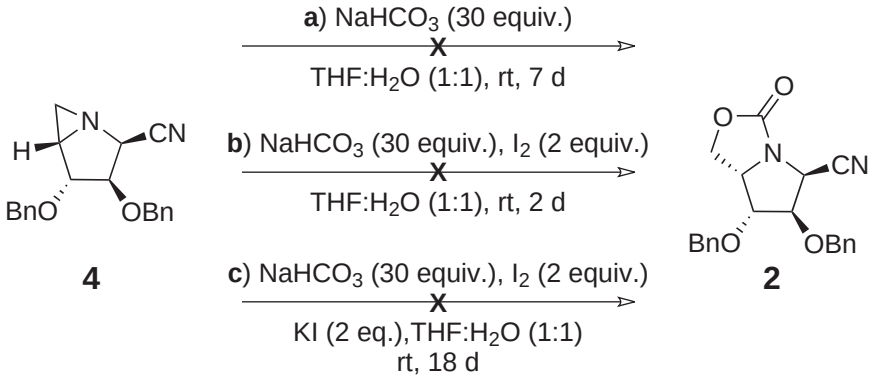


Fig. 5.2 ^{13}C NMR spectrum for aziridine **4**, (125 MHz, in $CHCl_3$)

Having formed aziridine **4**, attempts were then made to convert **4** into carbamate **2** using the previously disclosed carbamate annulation conditions (Scheme 5.5). Thus, aziridine **4** was treated with, **a**) excess $NaHCO_3$ for seven days, **b**) for two days with the addition of I_2, and **c**) for eighteen days with the addition of KI; however in all cases, there was no trace of the corresponding carbamate **2** by TLC or ^1H NMR of the crude reaction mixture and only starting material was observed. Failure to form the carbamate suggests that the annulation does not occur via an aziridine intermediate (Pathway **I**, Scheme 5.3) and that either the carbonate (Pathway **II**) or the carbamic acid (Pathway **III**) is formed during the annulation. Literature precedent suggests that carbamic acids readily form upon exposure of amines to CO_2[11–13] and therefore an annulation mechanism involving a carbamic acid seems most likely. That said, further experiments (such as determining kinetic isotope effects (KIEs)) would need to be undertaken before any conclusion can be made.

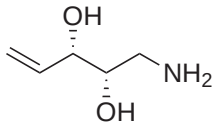
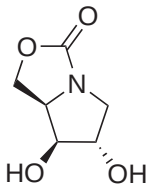
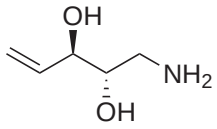
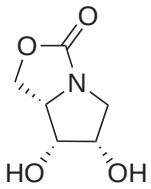
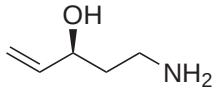
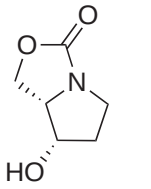
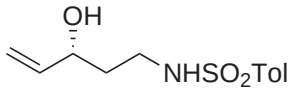
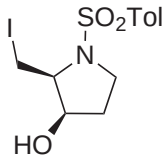
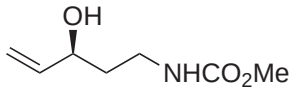
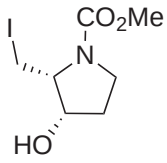


Scheme 5.5 Attempted transformation of aziridine **5** into carbamate **6**

5.3 Diastereoselectivity

The high degree of diastereoselectivity observed in the I_2-mediated carbamate annulation reaction can be discussed by first considering the previous reactions to produce 3,4-*cis*-carbamates performed by Stocker, Timmer and co-workers,[1–3] along with other iodocyclisations from the literature, since we now know that an iodoamine is formed as the key intermediate in the carbamate annulation. As illustrated (Table 5.1), the stereochemistry at the allylic position exerts stereocontrol on the carbamate annulation and this appears to be independent of stereochemistry at the homoallylic position (entries 1 and 2)[1] or lack thereof (entry 3).[3] As already discussed in the introduction (Chapter 1) the observed diastereoselectivities for the protecting-group-free annulations can be explained by Stocker and Timmer[2] via a transition state model originally proposed by Chamberlin *et al.*[14–16] and in line with theoretical studies by Gouverneur and co-workers,[17] where the *cis*-isomer is produced via the electronically favoured 'O-in-plane' envelope conformation that positions the allylic hydroxyl in plane with the double bond.[2] Similarly, 3,4-*cis*-diastereoselectivity has been observed for the halocyclisation or amidomercuration of a number of *N*-substituted alkenylamines, including *N*-sulfonyl (e.g., entry 4),[7] *N*-*t*-butyloxycarbonyl,[18] *N*-methoxycarbonyl (e.g., entry 5),[19] and *N*-benzyloxycarbonyl[20] derivatives and although slight differences in diastereoselectivity can be observed when changes to the solvent, reaction temperature, or choice of iodinating reagent are made, the *cis*-isomer is the major product. Moreover, the reactions presented in Table 5.1 show that iodocyclisations on alkenylamines without an α-amine substituent give predominantly 3,4-*cis*-products.

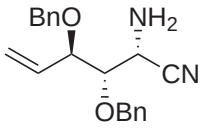
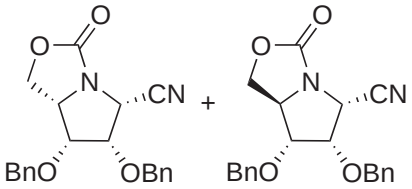
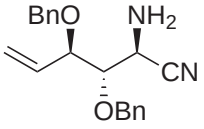
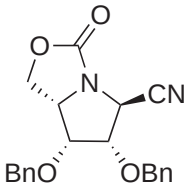
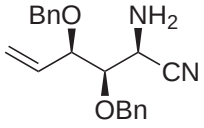
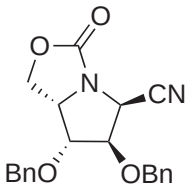
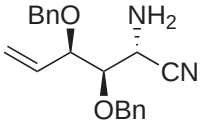
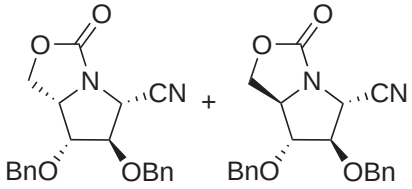
Table 5.1 *cis*-Diastereoselectivity of previous carbamate annulations from Stocker and Timmer and other iodocyclisations from the literature

Entry	Alkenylamine	Cyclic product[a]	dr (*cis*/*trans*)	Yield (%)
1			>95:5	99[1]
2			>95:5	99[1]
3			>95:5	90[3]
4			96:4	86[7]
5			93:7	83[19]

[a]Reactions performed via I_2-mediated cyclisation in the presence of $NaHCO_3$

When the carbamate annulation methodology was applied during the synthesis of amino-imino-hexitols (Chapter 3), however, a decrease in diastereoselectivity was observed in a couple of instances. As illustrated (Table 5.2), the D-ribose derived major Strecker product, aminonitrile *syn*-**5**, gave a mixture of diastereomers (4:1, *cis*/*trans*, entry 1), while the minor Strecker product α-aminonitrile *anti*-**6** led to exclusive formation of one diastereomer, (entry 2).[5] However, for the carbamate annulation of the D-arabinose derived α-aminonitriles, the major Strecker product *syn*-**3** provided the 4,5-*cis*-product exclusively (entry 3),[4] whereas a mixture of diastereomers was produced in the annulation of *anti*-**7** (6:1, *cis*/*trans*, entry 4).[5] To explain the diastereoselectivities observed in the carbamate annulation of these alkenylamines, possessing a nitrile substituent α to the amine, the transition states for the formation of the observed carbamates need to be considered. Therefore, we propose a transition state model that builds on Stocker and Timmer's original model for the protecting-group-free carbamate annulation[2] where attack of the internal-nucleophile on the I_2-alkene complex takes place via a 5-membered ring transition state in which the nitrogen approaches the double bond in an envelope conformation and follows a Bürgi-Dunitz-like trajectory.[21] However, unlike the earlier model, the effect of the α-amine substituent on the transition states can also be considered. (A computational investigation of iodocyclisation transition states is presented in Chapter 6).

Table 5.2 Diastereoselectivity of carbamate annulations presented in this thesis

Entry	Alkenylamine	Cyclic product[a]	dr (*cis*/*trans*)	Yield (%)
1	 *syn*- **5**	 **8** + **9**	4:1	85[5]
2	 *anti*- **6**	 **10**	>95:5	53[5]
3	 *syn*-**3**	 **2**	>95:5	85[4]
4	 *anti*- **7**	 **11** + **12**	6:1	95[5]

[a]Reactions performed via I_2-mediated cyclisation in the presence of $NaHCO_3$

In the case of the cyclisation of the D-ribose-derived Strecker product *syn*-**5** (Fig. 5.3), the benzyloxy substituent on the ring (depicted in blue) can be positioned either in the plane of the double bond (**A**, OBn-in-plane), or almost perpendicular to that plane (**B**, H-in-plane). Of these two transition states, **A**, which ultimately leads to the major carbamate **8**, has minimal overlap between the electron-withdrawing $\sigma^*_{C–O}$ and reacting $\pi_{C=C}$ orbitals, thereby resulting in a lower energy transition state. In contrast, transition state **B** has overlapping benzyloxy $\sigma^*_{C–O}$ and double bond $\pi_{C=C}$ orbitals, which results in the removal of electron density from the π-bond and a higher energy transition state. Similarly, one can view the more favourable transition state (**A**) as having the C–O bond in the plane of the olefin to minimise unfavourable electrostatic interactions.[22] At this point, however, the influence of the nitrile substituent on the transition state also needs to be considered. In **A**, the dihedral angle between the plane of the nitrile dipole and the lone pair of electrons on the amine is approximately 180° (depicted in green). This results in electron density being withdrawn from the nitrogen nucleophile thus raising the transition state energy of conformer **A**. In contrast, the dihedral angle between the nitrogen dipole and the lone pair of electrons of the amine in transition state **B** is approximately 90° and thus has less of a negative influence on the nucleophilicity of the amine. Accordingly, the transition states for **A** and **B** become closer in energy and some of the *trans*-carbamate product **9** is also formed in the cyclisation.

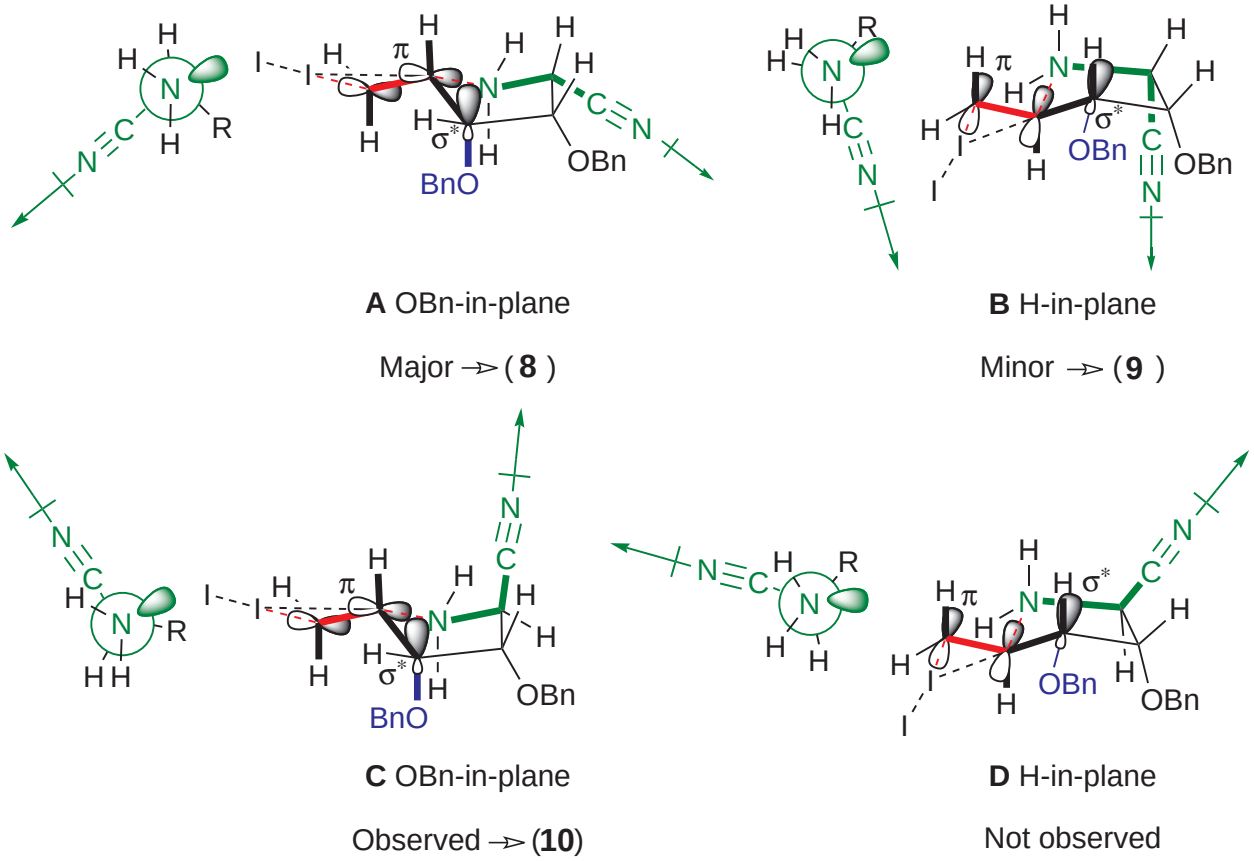


Fig. 5.3 Proposed transitions states leading to the formation of carbamate **8** (**A**, major product) and **9** (**B**, minor product) from alkenylamine *syn*-**5** and the formation of carbamate (**10**) (**C**) from alkenylamine *anti*-**6**

When considering the formation of carbamate **10** from the *anti*-α-aminonitrile **6** (Fig. 5.3), similar transition states can be drawn with the benzyloxy substituent in the plane of the double bond (**C**, OBn-in-plane, lower energy TS), or almost perpendicular to that plane (**D**, H-in-plane, higher energy TS). In addition, the nitrile group in transition state **C** does not reduce the nucleophilicity of the amine, while in transition state **D** the nitrile dipole leads to electron density being withdrawn from the amine through n–σ^*_{CCN} overlap. For this reason, there is a large energy difference between the two transition states and carbamate **10** (from **C**) is the only product observed.

Similar analyses can also be used when explaining the observed diastereoselectivity of the carbamate annulation for the D-arabinose derived alkenylamines, *syn*-**3** and *anti*-**7** (Fig. 5.4). Here, as for the ribose derivatives (refer Fig. 5.3), there are two influences on the transition state energy of the cyclisation. Thus, for the major Strecker product *syn*-**3**, the electronically preferred transition state (**E**, OBn-in-plane, lower energy TS) leads to the only carbamate observed (**2**), as the dihedral angle of the nitrile substituent is also favourable (90°), compared with the least preferred conformer (**F**, 180°, H-in-plane, higher energy TS) where the nitrile dipole reduces the nucleophilicity of the amine. However, in the cyclisation of alkenylamine *anti*-**7** the two transition states are closer in energy, as the preferred conformation (**G**, OBn-in-plane $\rightarrow$ **11**) has reduced amine nucleophilicity due to the dihedral angle of the nitrile dipole (180°, compared to the least preferred conformer (**H**, 90°, H-in-plane $\rightarrow$ **12**) where the nitrile dipole has limited electron withdrawing influence on the nucleophile. Furthermore, we can not rule out that the *trans*-products being preferred in the carbamate annulation are not due to the steric influence of the nitrile group positioned α to the amine, during the iodocyclisation of both arabinose derived *anti*-**7** and ribose derived *syn*-**5**.

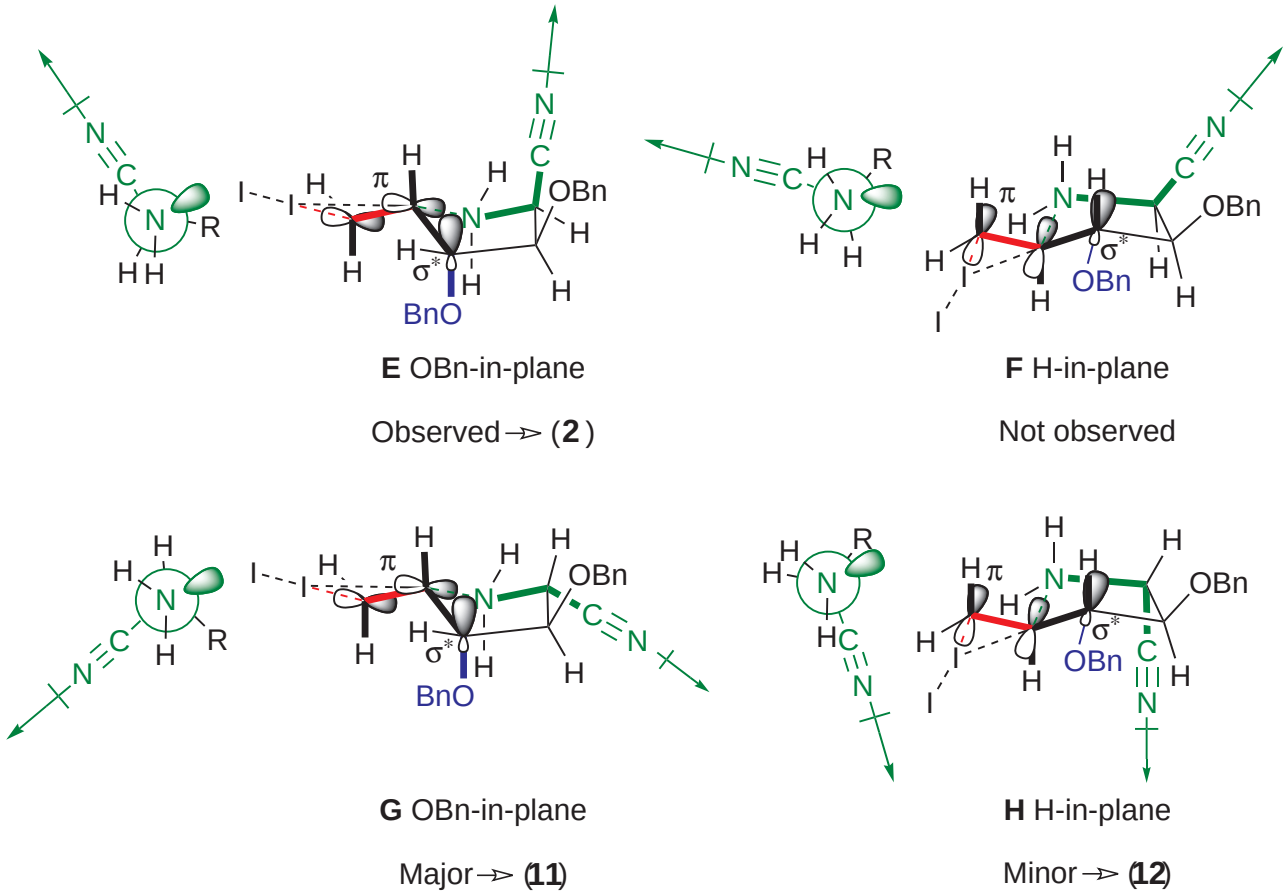
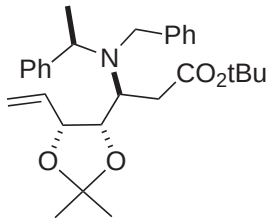
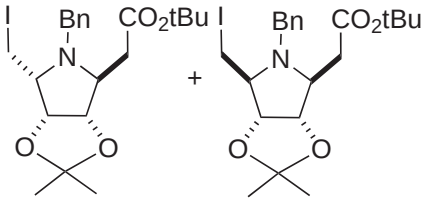
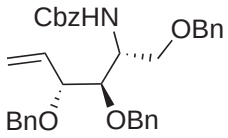
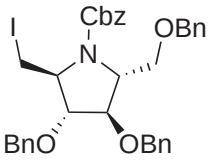


Fig. 5.4 Proposed transitions states leading to the formation of carbamate **2** (**E**) from alkenylamine *syn*-**3** and the formation of carbamates (**11**) (**G**, major product) and **12** (**H**, minor product) from alkenylamine *anti*-**7**

The reduced *cis*-diastereoselectivity observed in the carbamate annulations of α-aminonitriles can also be rationalised by examining the literature for iodocyclisations performed on alkenylamines with a substituent at the α-amine position. There are not many examples, however, Davies et *al*.[8] illustrated (Table 5.3) that the presence of a CH_2CO_2tBu group α to the amine influences the stereochemical outcome of iodoaminocyclisations. Though the 4,5-*cis* product is the major diastereomer following concomitant *N*-debenzylation (entry 1), the relative stereochemistry of the chiral-auxiliary and α-amine position can reduce, or even reverse, this selectivity. This *cis*-selectivity is thought to occur via the rapid equilibriation of the two diastereomeric iodonium ions and subsequent cyclisation as the rate-determining step. Surprisingly, halocyclisation or amidomercuration of a per-*O*-benzylated *N*-benzyloxycarbonyl protected alkenylamine derived from D-arabinose, however, resulted in preferential formation of the 4,5-*trans*-diastereomer (entry 2).[23] This *trans*-selectivity was attributed to the differential steric requirements in the transition states, and may reflect the added steric-considerations that need to be taken into account when an alkenylamine possesses both an α-amine substituent and an *N*-protecting group.[23,24] For example, *O*-cyclisation of a similar system (where NHCbz is replaced by OH) gives the *cis*-diastereomer preferentially (9:1, *cis*/*trans*)[25] Moreover, a substituent at the α-amine position of alkenylamines has been shown to influence the stereochemical outcome of the carbamate annulation, and may reduce the high *cis*-diastereoselectivity of the iodocyclisation.

Table 5.3 Diastereoselectivity of literature halocyclisations with alkenylamines possessing an α-amino substituent

Entry	Alkenylamine	Cyclic product[a]	dr (*cis*/*trans*)	Yield (%)
1			3:1	80[8]
2			<5:95	50[23]

[a]Entry 1 performed via I_2-mediated cyclisation in the presence of $NaHCO_3$; entry 2 performed via NIS-mediated cyclisation in the presence of HMPA

5.4 Conclusion

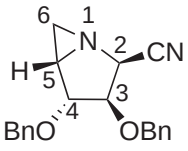
The mechanism of the I_2-mediated carbamate annulation has been investigated. An aziridine (**4**) was formed from the intermediate iodoamine (**1**) isolated *en route* to the formation of the carbamate (**2**). Subsequent attempts to form the carbamate (**2**) from the aziridine (**4**) were unsuccessful, which suggests that an aziridine is not an intermediate during the annulation. The diastereoselectivity of the I_2-mediated carbamate annulations of α-aminonitriles derived from D-ribose (*syn*-**5** and *anti*-**6**) and D-arabinose (*syn*-**3** and *anti*-**7**) have been considered. Comparisons to the previous carbamate annulations from Stocker and Timmer, along with halocyclisations or amidomercurations from the literature have been drawn, where it was revealed that alkenylamines without

a substituent at the α-amine position have a high degree of *cis*-diastereoselectivity. Furthermore, alkenylamines possessing an α-amino substituent can reduce or even reverse the 4,5-*cis*-diastereoselectivity, particularly in the presence of an *N*-protecting group. Moreover, transition states for the iodocyclisation of the α-aminonitriles have been postulated based on a preferred 'O-in-plane' conformation, and to reflect the influence of the nitrile substituent on the diastereoselectivity of the reaction. Here, it was proposed that D-ribose derived *syn*-aminonitrile **5** and D-arabinose derived *anti*-aminonitrile **7** both had reduced *cis*-selectivity due to the negative effect of the nitrile dipole on the nucleophile in the O-in-plane' conformation. As a result, the 'H-in-plane' becomes relatively less disfavoured, thereby lowering the energy of the TS and leading to some of the *trans*-product being observed.

5.5 Experimental

General Experimental

All chemicals obtained from commercial suppliers were used without further purification. All solvents were removed by evaporation under reduced pressure. Reactions were monitored by TLC analysis on silica gel coated plastic sheets (0.20 mm, Polygram SIL G/UV254) with detection by coating with 20% H_2SO_4 in EtOH followed by charring at *ca.* 150 °C, or by coating with a solution of 5% $KMnO_4$ and 1% $NaIO_4$ in H_2O followed by heating. Column chromatography was performed on silica gel (40-63 μm). High-resolution mass spectra were recorded on a Waters Q-TOF Premier^{TM} Tandem Mass Spectrometer using positive electro-spray ionisation. Nuclear magnetic resonance spectra were measured using a Varian Inova operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C or a Varian Direct Drive operating at 600 MHz and 133 MHz. ^{1}H and ^{13}C chemical shifts (δ) were internally referenced to the residual solvent peak, ($CDCl_3$ = 7.26 ppm and 77.16 ppm). NMR peak assignments are based on 2D NMR experiments (COSY, HSQC, and HMBC).



(2*S*,3*R*,4*R*,5*S*)-1-Aza-2-cyano-3,4-dibenzyloxybicyclo[3.1.0]hexane (**4**).

To a solution of iodoamine **1** (113 mg, 0.25 mmol) in THF (2.5 mL), under an argon atmosphere, was added Ag_2CO_3 (137 mg, 0.50 mmol). The resulting reaction mixture was stirred at room temperature for 20 h then filtered through a celite plug and washed with EtOAc. The filtrate was washed with H_2O and brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification was achieved using gradient flash chromatography

(hexanes/EtOAc, 20/1 → 1/1, v/v) to provide aziridine **4** as a colourless oil (57 mg, 0.17 mmol, 70%). R_f = 0.19 (hexanes/EtOAc, 1/1, v/v); ^{1}H NMR: (500 MHz, $CDCl_3$, 20 °C) δ 7.39–7.31 (m, 10H, H-Ar), 4.64 (s, 2H, CH_2 4-*O*-Bn), 4.62 (d, $^2J_{a,b}$ = 11.8 Hz, 1H, CHa 3-*O*-Bn), 4.59 (d, $^2J_{a,b}$ = 11.8 Hz, 1H, CHb 3-*O*-Bn), 4.54 (t, $J_{3,4}$ = $J_{4,5}$ = 5.1 Hz, 1H, H-4), 4.02 (d, $J_{2,3}$ = 6.8 Hz, 1H, H-2), 3.96 (dd, $J_{2,3}$ = 6.8, $J_{3,4}$ = 5.1 Hz, 1H, H-3), 2.66 (ddd, $J_{5,6a}$ = 5.4, $J_{4,5}$ = 5.1, $J_{5,6b}$ = 3.6 Hz, 1H, H-5), 1.87 (dd, $J_{5,6a}$ = 5.5, $J_{6a,6b}$ = 1.5 Hz, 1H, H-6a), 1.76 (dd, $J_{5,6b}$ = 3.6, $J_{6a,6b}$ = 1.5 Hz, 1H, H-6b); ^{13}C NMR: (125 MHz $CDCl_3$, 20 °C) δ 137.7 (C-*i* 4-*O*-Bn), 136.9 (C-*i* 3-*O*-Bn), 128.73 (C-Ar), 128.72 (C-Ar), 128.4 (C-Ar), 128.27 (C-Ar), 128.25 (C-Ar), 128.0 (C-Ar), 116.6 (CN), 82.9 (C-4), 81.1 (C-3), 73.2 (CH_2 3-*O*-Bn), 72.8 (CH_2 4-*O*-Bn), 56.9 (C-2), 39.8 (C-5), 30.2 (C-6); HRMS (ESI) m/z calcd. for $[C_{20}H_{20}N_2O_2+Na]^+$: 343.1417, obsd.: 343.1416.

Protocol for Attempted Conversion of Aziridine (4) into Carbamate (2). To a solution of aziridine **4** (6.0 mg, 0.018 mmol) in THF (0.25 mL), was added H_2O (0.25 mL) and $NaHCO_3$ (50.0 mg, 0.60 mmol). The reaction mixture was left to stir at room temperature for seven days, at this point TLC analysis indicated only starting material present (aziridine **4** R_f = 0.27 [hexanes/EtOAc, 1/2, v/v], carbamate **2** R_f = 0.50 [hexanes/EtOAc, 1/2, v/v]). Upon addition of I_2 (10.2 mg, 0.04 mmol) the reaction mixture was stirred for a further two days, again with only starting material indicated by TLC analysis. To further attempt reproduction of the I_2-mediated carbamate annulation conditions, KI (6.6 mg, 0.04 mmol) was added. The reaction mixture was stirred at room temperature for a further 18 days, then quenched with sat. aq. $Na_2S_2O_3$ and extracted with EtOAc. The organic layer was washed with H_2O and brine, dried ($MgSO_4$), filtered and concentrated *in vacuo* to obtain aziridine **4** as the only isolated compound according to ^{1}H NMR analysis of the crude material.

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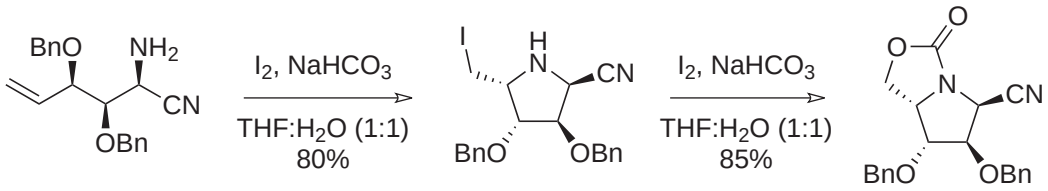
Chapter 6

Theoretical Studies of the I_2-mediated Carbamate Annulation

6.1 Introduction

The remarkable diastereoselectivity of the I_2-mediated carbamate annulation of alkenylamines makes this an important reaction for the synthesis of azasugars. Gaining further understanding of the diastereoselectivity could allow the prediction of outcomes for future carbamate annulations with structurally diverse alkenylamines. This information could then lead to improved design of synthetic routes for the preparation of azasugars. As such, we decided to perform a theoretical investigation of the carbamate annulation using computational modelling to explore the factors governing the diastereoselectivity of the reaction. Previously we have established that the initial iodocyclisation of alkenylamines *en route* to the formation of carbamates is the key stereoselective step in the I_2-mediated carbamate annulation (discussed in Chapter 5). For example, iodoamine

1 (Scheme 6.1) was isolated from the cyclisation of alkenylamine **2** with I_2 and $NaHCO_3$ and then subsequently transformed into carbamate **3** under those same carbamate annulation conditions.[1,2] Therefore, we wanted to use density functional theory (DFT) to find transition states (TSs) for the possible stereoisomers formed in the iodocyclisation of a variety of alkenylamines.



2 **1** **3**

Scheme 6.1 Isolation of iodoamine **4** *en route* to the formation of carbamate **3**

The analysis of previous experimental halocyclisations to form iodolactones by Chamberlin and co-workers,[3] showed that they were under kinetic rather than thermodynamic control to provide the *cis*-cyclic products. Further work by Chamberlin, Hehre, and co-workers,[4–6] centred on developing reactivity models by assessing the stereoselectivity of electrophilic cyclisations. They considered the relative affinities of diastereotopic olefin faces toward a test electrophile (H^+) using DFT calculations. The proposed reactivity models of the electrophilic halocyclisations of acyclic allylic alcohols and ethers suggest that there are two lowest energy conformations that can lead to the two *cis* and *trans* cyclic products: one conformation where the electrophile attacks *syn* to H in an 'O-in-plane' conformation (**A**, Fig. 6.1), and one where the electrophile attacks *syn* to OH in an 'H-in-plane conformation' (**B**).[6] These models provide an indication of which face of an allylic π bond will be attacked by an electrophile in a kinetically controlled addition reaction.

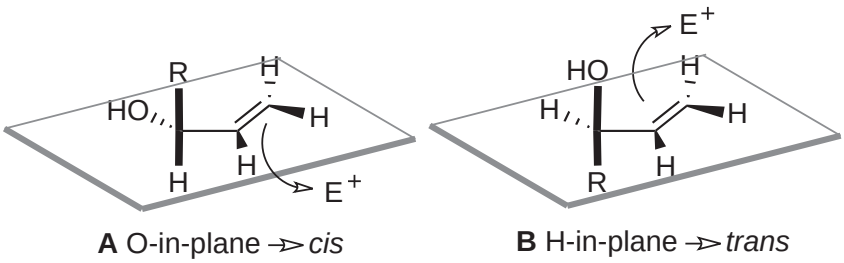
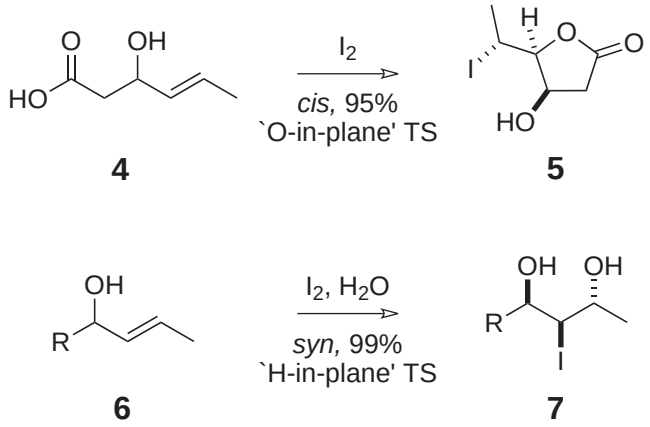


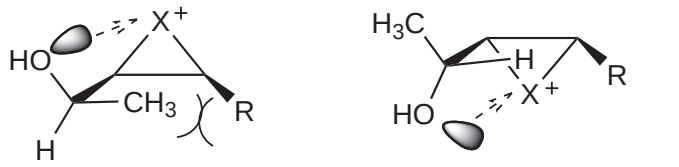
Fig. 6.1 Preferred conformers for electrophilic attack of an olefin

Furthermore, empirical data suggested that electrophilic attack on an acyclic allylic alcohol or ether with an internal nucleophile preferred the 'O-in-plane' conformer, (**A**) to give the major *cis*-cyclic product.[5,6] In this conformation the internal nucleophile was constrained to be in a position for backside attack on the (activated) π bond. However, attack on the less abundant 'H-in-plane' conformer gave rise to the minor *trans*-diastereomer. An example is shown in Scheme 6.2, where iodolactonisation of hydroxyacid **4** produced the *cis*-iodolactone **5** in ca. 20:1 selectivity via the 'O-in-plane' conformer. These findings were the reverse of the diastereoselectivity observed when there was an external nucleophile, such as in the reaction of alcohol **6** with I_2 and water to form iodohydrin **7**.



Scheme 6.2 Iodolactonisation compared with iodohydrin formation

It was believed that for halocyclisations the initially formed, favoured ('O-in-plane') I_2-π complex was trapped by an internal nucleophile before collapse or as it collapsed to an energetically unfavourable iodonium ion. However, when no internal nucleophile was present, as for haloadditions, the reaction would proceed through to an iodonium ion TS and the initial 'O-in-plane' conformation would change to that favoured by repositioning the allylic OH *syn* to the iodonium ion (**D**, Fig. 6.2 or 'H-in-plane').



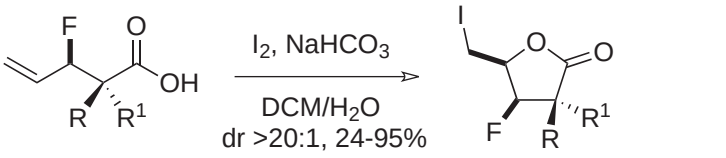
C Least preferred → *cis* **D** Most preferred → *trans*

Fig. 6.2 Lowest energy conformers for simple onium ion TSs

Chamberlin argued that the reason for the difference in the observed diastereoselectivities for the halocyclisation vs. haloaddition of allylic alcohols was due to a change in the rate determining step (rds) of the reaction, i.e., π-complex trapping for cyclisations vs. onium ion formation for additions.[6] Thus, the difference in rds would alter the position of the transition state along the reaction coordinate, presuming an 'early' reactant-type TS for the electrophile-π complex and 'late' product-type TS for onium ion formation. The change in TS timing would then affect which face of the π bond preferentially reacts with the electrophile. These postulations were based on calculations of simple onium ion TSs, where the lowest energy conformers for the two diastereomers, **C** and **D**, positioned the hydroxyl *syn* to the halogen. This was presumed to be due to the stabilising effect of the oxygen electron pair on the developing positive charge in the TSs. Here, the lowest energy onium ion conformation (**D**) resembled the 'H-in-plane' TS, seen in the

formation of iodohydrin **7**. However, conformation **C**, which resembled the 'O-in-plane' TS after C_2-C_3 bond rotation, was higher in energy due to the unfavourable steric interactions of the methyl group and alkyl chain.

More recently, work by Gouverneur and co-workers investigated the directing abilities of allylic fluorides on the diastereoselectivity of iodolactone formation (Scheme 6.3).[7] Here, they found that a series of allylic fluorides (**8a-c**) provided the *cis* iodolactones (**9a-c**) as the predominant diastereomers upon iodocyclisation with I_2 and $NaHCO_3$. Furthermore, they investigated this effect using DFT to determine the role of fluorine in the excellent diastereoselectivities observed.



8a R = H, R^1 = Bn
8b R = H, R^1 = H
8c R = Me, R^1 = Me

9a R = H, R^1 = Bn
9b R = H, R^1 = H
9c R = Me, R^1 = Me

Scheme 6.3 Iodocyclisation of allylic fluorides

Calculations were made of an unsubstituted iodonium ion (**I**, Fig. 6.3) to discover that the iodonium complex was somewhere between an iodonium ion and iodonium-π complex, with bond lengths for C–C of 1.44 Å, and C–I of 2.29 Å.[8] They then made calculations of the rotamers of an iodonium ion substituted with a fluoromethyl group and discovered that the fluorine substituent prefers to sit *inside* (**II**) by 12.55 kJ mol^{-1} with respect to *outside*, and 20.92 kJ mol^{-1} with respect to the *anti* conformer. They also looked at the TS of I_2-π complexes of 2-fluorobut-3-ene with an external formate nucleophile. Here, they found that the rotamers where the fluoro group was *inside* with

the methyl group either *outside* (**III**) or *anti* were equal in energy. These isomers, with the fluorine substituent *inside*, were preferred versus *outside* by 4.2 kJ mol^{-1} and *anti* by 12.6 kJ mol^{-1}. Finally they found TS structures for the iodoetherification of an allylic fluoride containing an internal alcohol nucleophile. The minimum energy structures were those with the conformation needed for backside nucleophilic attack of the I_2-π complex. Here, the structure with fluorine *inside* (**IV**) was 8.78 kJ mol^{-1} more favoured than the conformer with the fluorine *outside* (**V**). In both cases the rest of the molecule sat *anti* to the I_2-π complex.

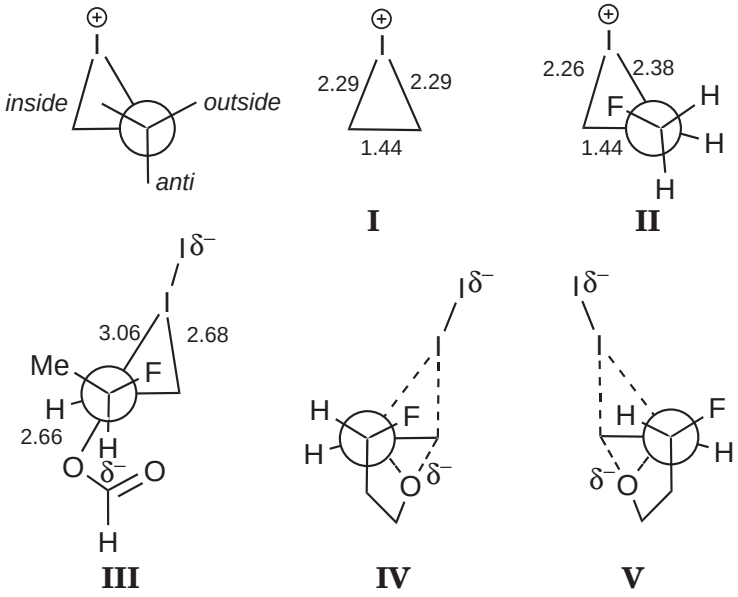
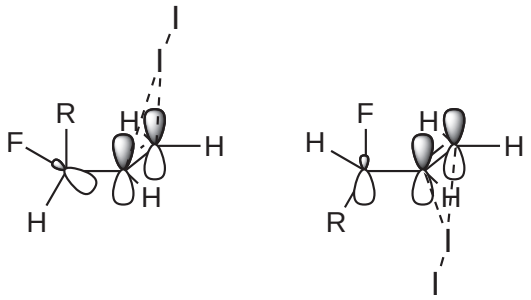


Fig. 6.3 Preferred TS conformations of fluorine substituted iodonium complexes

Gouverneur proposed that an *anti* fluorine substituent (**F**, Fig. 6.4) was energetically disfavoured as this position aligns the $\sigma^*_{C–F}$ orbital with one of the C–I bonds, having a destabilising effect on the I_2-π-complex. However, the preferred *inside* fluorine substituent position (**E**) would minimise the $\sigma^*_{C–F}$ orbital and C–I bond interactions. The fluorine *inside* would also enable its lone pairs of electrons to have a stabilising

effect on the partial positive charge of the I_2-π-complex.



E *Inside* fluorine **F** *Anti* fluorine

Fig. 6.4 Preferred *inside* fluorine conformation (**E**) and disfavoured *anti* fluorine conformation (**F**)

The work presented in this chapter builds on past computational models of the halocyclisations to form furans from work by Chamberlin et al.[3,4,6] and Gouverneur and co-workers.[7] Our aim was to determine the differences in transition state energy or structure that could account for the high *cis*-selectivity observed in the I_2-mediated carbamate annulation. We also wanted to refine our previous TS model,[9] which postulated about the effects of the dipole interactions of a nitrile substituent at the α-amine position on the amine nucleophile of alkenylamines in I_2-mediated carbamate annulations (see Chapter 5).

6.2 Computational Methods

All calculations were performed with the programme package Gaussian09, Rev B.01,[10] using density functional theory (DFT) with the B3LYP[11] density functional and the LANL2DZ[12] basis set. B3LYP with the LANL2DZ basis set has been shown to effectively model the geometries and energies of compounds that contain iodine.[13,14]

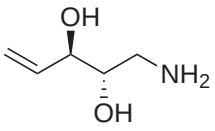
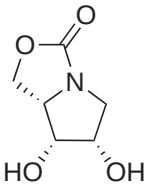
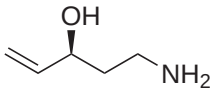
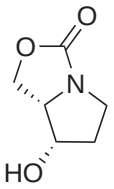
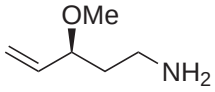
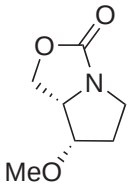
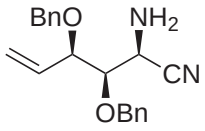
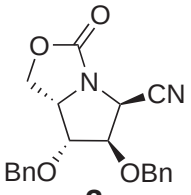
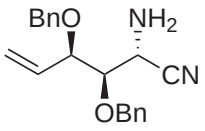
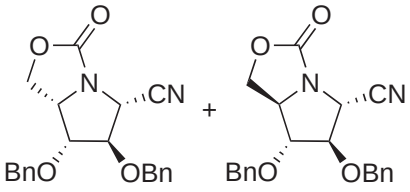
Geometrically optimised structures were confirmed using frequency calculations, where minima must have no imaginary frequencies and transition states exactly one imaginary frequency. The found transition states were verified by performing an intrinsic reaction coordinate calculation (IRC)[15] with subsequent optimisation to the preceding and following minima. The electronic structures of stationary points were also analysed by the natural bond order (NBO) method.[16]

6.3 DFT Calculations of Iodocyclisations

To determine what factors govern the transition state energies that lead to the stereoselectivity in the iodocyclisations, a number of linear alkenylamine structures possessing allylic-directing groups were used as starting points in the calculations (Table 6.1). We wanted to explore the scope of the carbamate annulations with and without protecting groups and α-amine substituents, and to compare with the previous computational halocyclisation models.[3,4,6,7] Here, we chose to analyse the carbamate annulation of three alkenylamines with a methylene group α to the amine; the diol derived from D-ribose (**10** $\rightarrow$ **11**),[17] the alcohol from 2-deoxy-D-ribose (**12** $\rightarrow$ **13**)[18] and the methyl ether from 2-deoxy-D-ribose (**14** $\rightarrow$ **15**). To ascertain the influence of the α-amine position on carbamate annulation diastereoselectivity, we chose to investigate the iodocyclisations of both the *syn*- (**2** $\rightarrow$ **3**) and *anti*- (**16** $\rightarrow$ **17** and **18**) α-aminonitriles possessing benzyl ether protecting groups. For all of the alkenylamines in Table 6.1 we used density functional theory to calculate the optimised geometries corresponding to starting material, iodine complexes, transitions states and products along the reaction coordinate of the initial iodocyclisation *en route* to the formation of carbamates. For ease of

comparison of all the different structural alkenylamines the atom numbering has been changed for this Chapter. The numbering starts at 1 for the nitrogen atom and moves down the carbon chain ending at 6.

Table 6.1 Diastereoselectivity of iodocyclisations observed experimentally

Alkenylamine	Cyclic product[a]	dr[b] (*cis*/*trans*)	Yield (%)
 **10**	 **11**	>95:5	99[17]
 **12**	 **13**	>95:5	90[18]
 **14**	 **15**	>95:5	75
 **2**	 **3**	>95:5	85[1]
 **16**	 **17** **18**	6:1	95[9]

[a]Reactions performed via I_2-mediated cyclisation in the presence of $NaHCO_3$
[b]As determined from analysis of the crude reaction mixture by ^1H NMR spectroscopy.

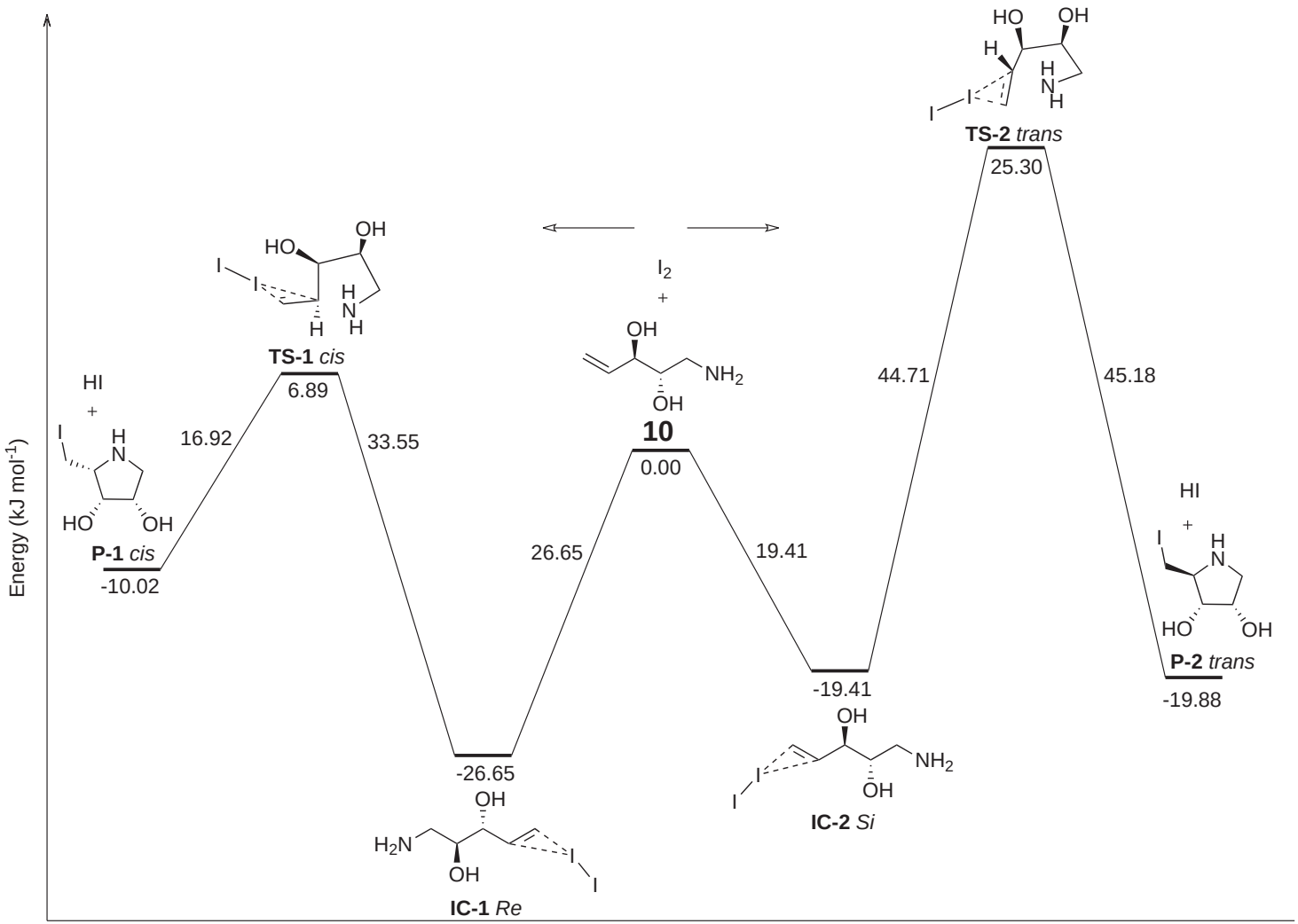
6.3.1 Alkenylamines Possessing an α-Amine Methylene

The iodocyclisation reaction profile of D-ribose derived alkenylamine (**10**, Scheme 6.4) plots the electronic energies (ΔE_{elec}) of the optimised geometries relative to the starting materials, alkenylamine **10** and I_2. Here, the reaction proceeds with an exothermic process upon complexation of I_2 to the alkene π-bond (**IC-1** -26.65 kJ mol^{-1} and **IC-2** -19.41 kJ mol^{-1}). The two iodine complexes, **IC-1** and **IC-2** exist in a pre-equilibrium, favouring I_2 approach to the *Re*-face of the alkene over *Si*-face approach, before passing through the transition states, *cis* **TS-1** and *trans* **TS-2**, which lead to the diastereomeric iodoamines *cis* **P-1** (experimentally observed) and *trans* **P-2** (experimentally not observed). The activation energy of **TS-1** (33.55 kJ mol^{-1}) was 11.16 kJ mol^{-1} less than that of **TS-2** (44.71 kJ mol^{-1}). Interestingly, the *cis* iodoamine **P-1**, (-10.02 kJ mol^{-1}) leading to the observed carbamate, (**11**) was 9.86 kJ mol^{-1} less stable than the *trans* iodoamine **P-2** (-19.88 kJ mol^{-1}). It was also found that the difference in transition state electronic energy between the *cis* **T-1** (6.89 kJ mol^{-1}) and *trans* **T-2** (25.30 kJ mol^{-1}) was significant at 18.40 kJ mol^{-1}. We found the relative Gibb's free energies from the frequency calculations of **TS-1** and **TS-2**, where $\Delta G = \Delta E_{\text{elec}} + \Delta E_{\text{vib}} + \Delta E_{\text{rot}} + \Delta E_{\text{trans}}$. The value of ΔG was then related to the equilibrium constant ($\Delta G = -RTlnK$) to find the theoretical diastereomeric product distribution of the iodoamines **P-1** and **P-2**, (*cis*/*trans*, Table 6.2). It was found that the theoretical diastereoselectivity was higher than the detectable experimental value of $>$95:5 in favour of the 4,5-*cis*-carbamate **11**.

Table 6.2 Relative transition-state Gibbs energies (kJ mol^{-1}) and diastereoselectivities in the iodocyclisation of alkenylamine **10**

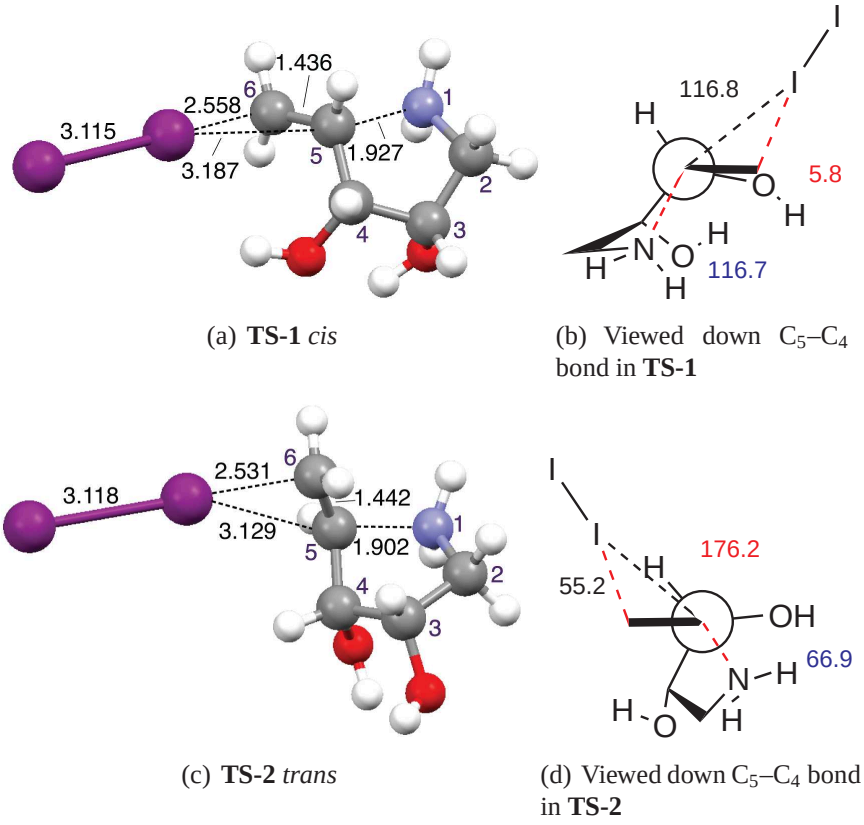
Transition State	ΔG^{ab} (kJ mol^{-1})	dr^a (%)	dr^c (%)
TS-1 *cis*	0.00	99.7	>95.0
TS-2 *trans*	14.05	0.3	<5.0

[a]B3LYP/LANL2DZ
[b]Relative to the *cis* TS
[c]Experimental results



Scheme 6.4 Reaction coordinate of the iodocylisation of alkenylamine **10** to form iodoamines *cis* **P-1** and *trans* **P-2**. Energy (kJ mol^{-1}) is shown relative to alkenylamine **10**

The optimised 3D TS structures, *cis* **TS-1** (a) and *trans* **TS-2** (c) depict the calculated bond lengths of nucleophile approach and I_2-π-complex in Fig. 6.5. Here, Newman projections of *cis* (b) and *trans* (d) TSs show a view down the C_5–C_4 bond to depict the calculated dihedral angles of both the allylic hydroxyl ($C_6C_5C_4O(H)$, in red) and hydrogen ($C_6C_5C_4H$, in black) to the C_5–C_6 bond, and the line of nucleophilic approach to the allylic hydroxyl ($N_1C_5C_4O(H)$, in blue). The transition state structures (**TS-1** and **TS-2**) both possess an I_2-π complex rather than an iodonium ion, as postulated by Chamberlin[6] and found by Gouverneur.[7] However, there is a degree of pyramidalisation in the C_5–C_6 olefin. The I_2–C_5–C_6 bond lengths have progressed from the initial I_2-π complexes (**IC-1** and **IC-2**) to a more asymmetric system in which the I–C_6 and N_1–C_5 bonds are forming, and I–I bond lengthening, suggestive of product-like (late) transition states. Furthermore, the amine nucleophile (N_1) approaches C_5 of the I_2-π-complex from either the *Si*-face to give a *cis*-TS, or the *Re*-face to give the *trans*-TS. The difference in the calculated distances between **TS-1** and **TS-2** is small, however, *cis*-**TS-1** is a slightly earlier TS due to the longer N_1–C_5 nucleophile approach (1.927 Å vs 1.902 Å), longer I–C_6 distance (2.558 Å vs 2.531 Å) and shorter lengthening C5–C6 bond (1.436 Å vs 1.442 Å), where the uncoordinated alkene is 1.348 Å. Both of the TSs position the allylic hydroxyl (C_4–OH) in plane with the C_5–C_6 bond, however, in **TS-1** C_4-OH is *cis* with a small dihedral angle (5.8°, (b)), whereas in **TS-2** the C_4-OH is *trans* with respect to C_5–C_6 (176.2°, (d)). The allylic hydrogen (C_4–H) is also positioned out of the plane by 116.8°, (**TS-1**) and 55.4°, (**TS-2**).



(a) **TS-1** *cis*

(b) Viewed down C_5–C_4 bond in **TS-1**

(c) **TS-2** *trans*

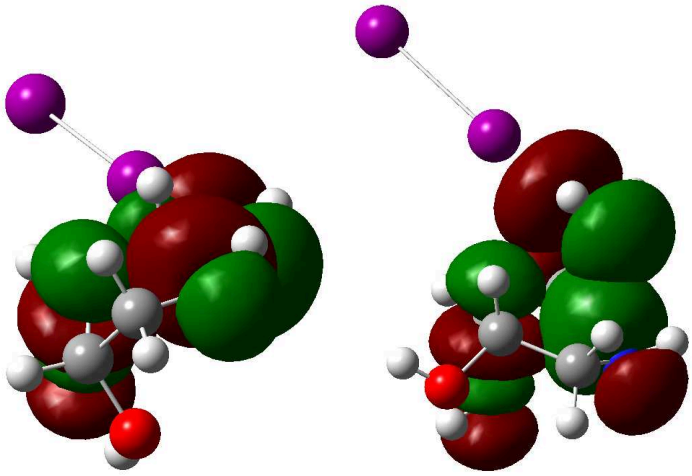
(d) Viewed down C_5–C_4 bond in **TS-2**

Fig. 6.5 Calculated transition-state structures for the iodocyclisation of alkenylamine **10** *en route* to the formation of carbamate **11**. Newman projections show dihedral angles of $C_6C_5C_4O(H)$ ('O-in-plane', in red), $C_6C_5C_4H$ ('H-in-plane', in black) and the $N_1C_5C_4O(H)$ (in blue). [Distances in Å, angles in deg.]

What appears to account for the significant energy difference between the *cis* and *trans* TSs is the position of C_4-OH *inside* (**TS-1**) vs *outside* (**TS-2**) or the '*inside*-alkoxy effect'.[7] Interestingly, both the *cis* and *trans* optimised TS structures sit in an envelope conformation that positions the nucleophile in such a way that the line of attack (N_1–C_5) has an approximate 90° dihedral angle with the allylic directing C_4–OH ($N_1C_5C_4O(H)$ = 116.7° (b) and 66.9° (d)). The constraint of this envelope conformation also allows for backside attack of the amine on C_5 of the expanded I_2-π-complex with an attack angle of ca. 113°.[19] Alternatively the 5-membered TS envelopes could be conformed in

such a way that the $N_1C_5C_4O(H)$ dihedral angle was approximately 180° and still allow for backside nucleophilic attack. The reason for the approximately 90° $N_1C_5C_4O(H)$ dihedral angle could be due to the need to limit the interaction of the dipoles by positioning the partial negative lone pair of electrons on the amine perpendicular to the lone pair of electrons on the allylic oxygen.

A single point calculation with Natural Bond Orbital (NBO) analysis was performed on both TS structures, **TS-1** ((a), Fig. 6.6) and **TS-2** (b), to determine the effect the relative position the allylic-OH has on the electronic nature of the transition states. In the *cis* **TS-1** the calculated electron withdrawing allylic $\sigma^*_{C_4–OH}$ orbital has minimal overlap with the newly forming $N_1–C_5$ bond and lone pair of electrons on C_6; the late stages of the I_2-π-complex. This is also the case for the allylic $\sigma^*_{C4–OH}$ orbital in the *trans* **TS-2**. These electronic interactions illustrate the favourable conformation of the 'O-in-plane' model.[4–7]



(a) **TS-1** *cis* (b) **TS-2** *trans*

Fig. 6.6 Calculated natural bond orbital (NBO) images that show the minimal orbital interactions of, $\sigma^*_{C4–OH} \rightarrow \sigma_{N1–C5}$ and n_{C6}, for *cis* **TS-1** (a) and *trans* **TS-2** (b)

Next we calculated the iodocyclisation of the more simple 2-deoxy-D-ribose derived alkenylamine without a homoallylic substituent (**12**, Scheme 6.5), which proceeds through similar structural transformations as did alkenylamine **10**. Here, it was found that the ΔE_{elec} to form the I_2-π-bond-complex with approach from the *Re*-face (**IC-3**) leading to the observed product, was favoured by -25.38 kJ mol^{-1}, compared to -16.66 kJ mol^{-1} for the *Si*-face complex (**IC-4**) which leads to the experimentally unobserved product. However, a small difference in energy of 0.48 kJ mol^{-1} was found between the two transition states in favour of *cis* **TS-3** (5.49 kJ mol^{-1}) vs *trans* **TS-4** (5.97 kJ mol^{-1}). When calculating the theoretical diastereoselectivity from the **TS-3** and **TS-4** ΔG values, it was found that the experimental carbamate annulation gave higher selectivity for the 4,5-*cis*-carbamate **13** by around 10%, (Table 6.3). The margin of error in these type of DFT calculations is $\pm$20 kJ mol^{-1}. Here, it is important to note that the calculated thermodynamic energies of the iodocyclisations presented should all have a margin of error of a similar magnitude because they are all the same type of electronic system.[20]

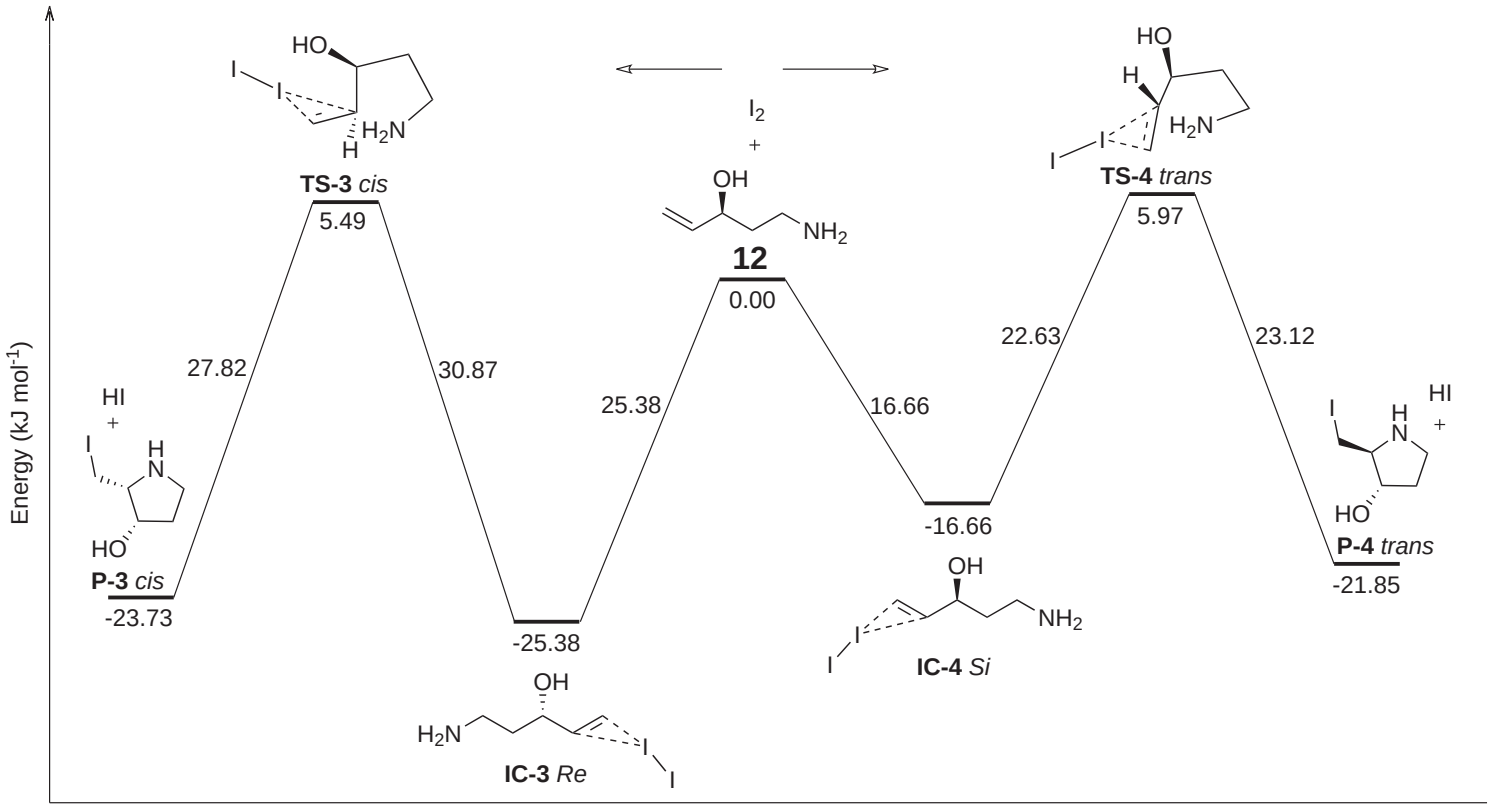
Table 6.3 Relative transition-state Gibbs energies (kJ mol^{-1}) and diastereoselectivities in the iodocyclisation of alkenylamine **12**

Transition State	ΔG^{ab} (kJ mol^{-1})	dr^a (%)	dr^c (%)
TS-3 *cis*	0.00	83.5	$>$95.0
TS-4 *trans*	3.88	17.5	$<$5.0

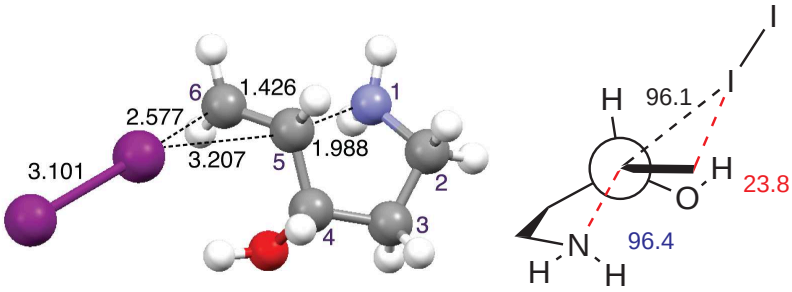
[a] B3LYP/LANL2DZ
[b] Relative to the *cis* TS
[c] Experimental results

Looking in more detail at the calculated 3D transition state structures for the iodocyclisation of **12** (**TS-3** (a) and **TS-4** (c), Fig. 6.7) we can see that both I_2–C_5–C_6 complexes

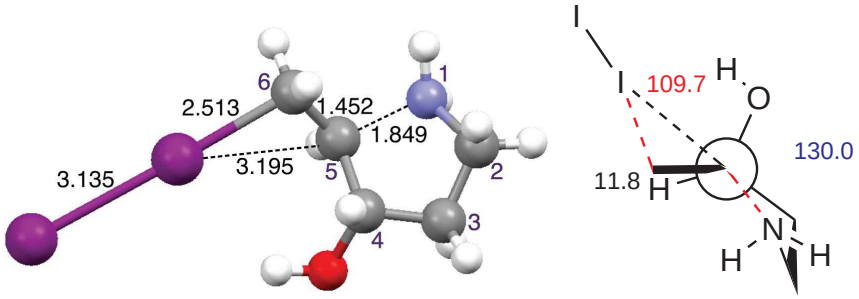
have progressed along the reaction coordinate towards a more product-like TS, although the *cis* **TS-3** is a relatively earlier transition state than the *trans* **TS-4**. The difference in reaction coordinate position is illustrated by a much longer nucleophile approach of N_1–C_5 for the *cis*-TS by 0.14 Å (1.988 Å, (a) vs 1.849 Å, (c)) and 0.03 Å shorter C_5–C_6 bond length (1.426 Å, (a) vs 1.452 Å, (c)). The Newman projections of *cis* **TS-3** (b) and *trans* **TS-4** (d), depict a different picture to those of **TS-1** and **TS-2** (see Fig. 6.5). Here, the C_4–OH is approximately in-plane *cis* to C_5–C_6 in **TS-3** (23.8°), whereas for **TS-4** it is the C_4–H that is in-plane (11.8°), such that the I_2 electrophile approaches *syn* to the C_4-OH.[6] Both *cis* and *trans* TSs also have a 5-membered envelope conformation where the nucleophile approach (N_1–C_5) is approximately 90° to the plane of the C_4–OH bond, ($N_1C_5C_4O(H)$ = 96.4° (b) and = 130.0° (d)) as was the case for the TSs in the iodocyclisation of **10**. NBO analysis shows that there is some energetically unfavourable $\sigma^*_{C4–OH} \rightarrow \sigma_{N1–C5}$ and $\sigma_{C5–I}$ orbital overlap in *trans* **TS-4** ((b) Fig. 6.8) compared to minimal $\sigma^*_{C4–OH} \rightarrow n_{N1}$ and $\pi_{C5–C6}$ orbital overlap for *cis* **TS-3** (a).



Scheme 6.5 Reaction coordinate of the iodocylisation of alkenylamine **12** to form iodoamines *cis* **P-3** and *trans* **P-4**. Energy (kJ mol^{-1}) is shown relative to alkenylamine **12**

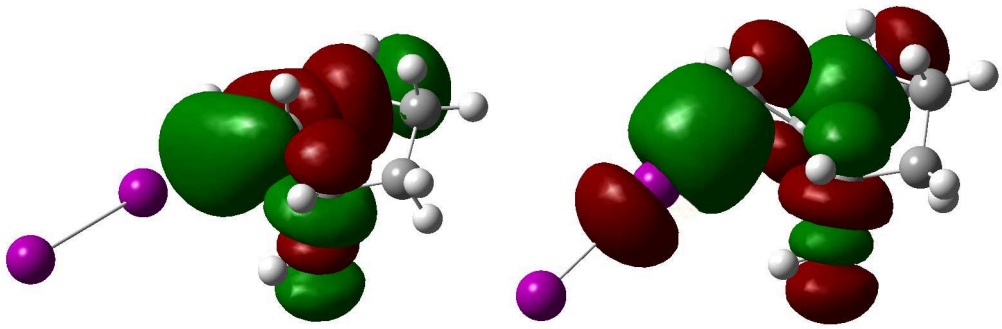


(a) **TS-3** *cis* (b) Viewed down C_5–C_4 bond in **TS-3**



(c) **TS-4** *trans* (d) Viewed down C_5–C_4 bond in **TS-4**

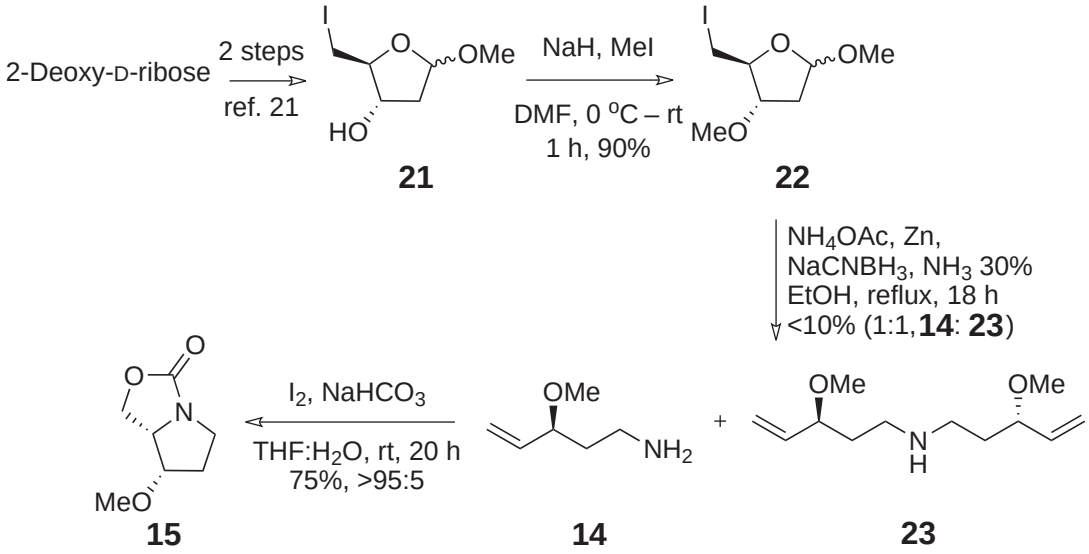
Fig. 6.7 Calculated transition-state structures for the iodocyclisation of alkenylamine **12** *en route* to the formation of carbamate **13**. Newman projections show dihedral angles of $C_6C_5C_4O(H)$ ('O-in-plane', in red), $C_6C_5C_4H$ ('H-in-plane', in black) and the $N_1C_5C_4O(H)$ (in blue). [Distances in Å, angles in deg.]



(a) **TS-3** *cis* (b) **TS-4** *trans*

Fig. 6.8 Calculated natural bond orbital (NBO) images that show the minimal orbital interactions of $\sigma^*_{C4–OH} \rightarrow$ nN1 and $\pi_{C5–C6}$ for *cis* **TS-3** (a) and small $\rightarrow \sigma_{N1–C5}$ and $\sigma_{C5–I}$ orbital overlap for *trans* **TS-4** (b)

We then wanted to add a methyl group to the deoxy-ribose derived alkenylamine (**14**, Scheme 6.6) to ascertain the effects of an ether on the allylic directing properties in the iodocyclisation. Here, methyl iodoriboside **21**, prepared in two steps from 2-deoxy-D-ribose,[21] was subjected to a mixture of Ag_2O and MeI to produce the iodoriboside methyl ether **22** in excellent yield (90%). A Vasella reaction with reductive amination, as pioneered previously in our research group,[17,22] was then carried out on iodomethoxyriboside **22** in the hope of producing the primary methyl ether alkenylamine **14** as the major product. This was the case when the unprotected iodoriboside **21** was subjected to a Vasella reductive amination and the primary alkenylamine **12** was formed as the only product and in excellent yield (>95:5, 81%).[17] However, using the optimised conditions for the Vasella reductive amination, with a large excess of NH_4OAc and NH_3 aq. 30%, a mixture of primary/secondary amines (1:1, **14**:**23**) were obtained and in poor isolated yield. The primary/secondary amine ratio was established by the ^1H NMR and ^{13}C spectra of the crude material.



Scheme 6.6 Formation of methyl ether alkenylamine **14**

The selectivity in the Vasella reductive amination of iodoriboside **22** is not particularly surprising as the reductive amination of substrates with benzyl ethers have also produced approximate 1:1 mixtures of primary/secondary amines.[22] The poor yield can be attributed to the volatility of the methyl ether alkenylamine **14** in that isolation was difficult with the reaction conditions used, including a DOWEX-H^+ column and subsequent silica chromatography. However, enough material was isolated to perform the I_2-mediated carbamate annulation on the methyl ether alkenylamine, **14**, to produce 4,5-*cis*-carbamate **15** in a diastereoselectivity of $>$95/5, (refer Table 6.1). Carbamate **15** was proven to have the 4,5-*cis* configuration by methylating a sample of known 4,5-*cis*-hydroxycarbamate **11** with MeI and NaH in DMF, and finding that the NMR spectral data matched for both methyl ether compounds.

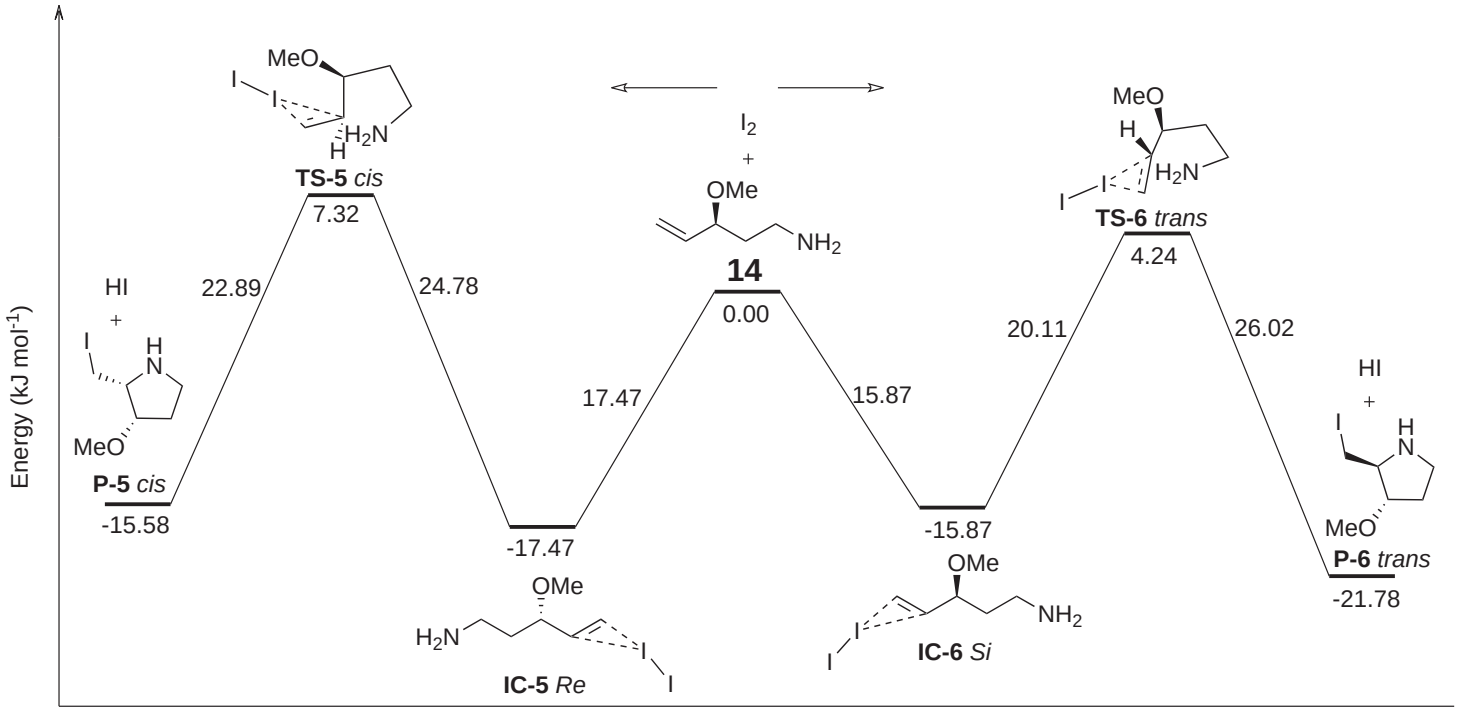
The calculated reaction coordinate of the iodocyclisation of methyl ether alkenylamine **14** follows the reaction mechanism described for the previous alkenylamines discussed (Scheme 6.7). Here, the relative transition state electronic energies are reversed so that *trans* **TS-6** is lower in energy than *cis* **TS-5**. There is also little energetic preference for *Re*-face approach over *Si*-face approach (**IC-5** = -17.47 kJ mol^{-1} vs **IC-6** = -15.87 kJ mol^{-1}), and the *trans* iodoamine **P-6** is 6.2 kJ mol^{-1} energetically more stable than the the *cis* iodoamine **P-5**. However, the product distribution calculated from TS ΔG's (**TS-5** vs **TS-6**) predicts an almost 50:50 ratio of *cis*/*trans* products (Table 6.4). This can not account for the observed experimental diastereoselectivity of $>$95:5 (*cis*/*trans*) within a reasonable margin of error, compared to the previous two iodocyclisations. The anomaly exhibited with the iodocyclisation of methyl ether protected alkenylamine **14** can also not be explained by examining the 3D transition state structures and Newman projections (**TS-5** (a), (b) and **TS-6** (c), (d), Fig. 6.9).

Table 6.4 Relative transition-state Gibbs energies (kJ mol^{-1}) and diastereoselectivities in the iodocyclisation of alkenylamine **14**

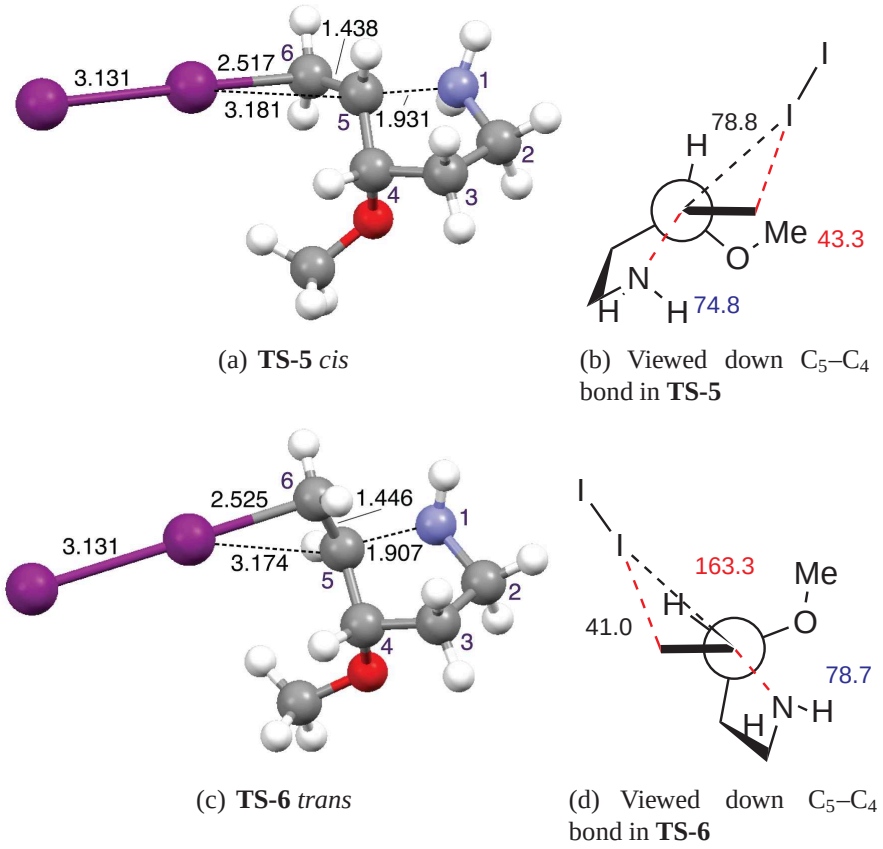
Transition State	ΔG^{ab} (kJ mol^{-1})	dr^a	dr^c (%)
TS-5 *cis*	0.00	51.8	>95.0
TS-6 *trans*	0.19	48.2	<5.0

[a]B3LYP/LANL2DZ
[b]Relative to the *cis* TS
[c]Experimental results

Here, the trend of later product-like TSs continue with *trans* (**TS-6**) later than *cis* (**TS-5**), albeit by a small degree; the nucleophile approach (N_1–C_5) is shorter by 0.02 Å (1.907 Å, (c) vs 1.931 Å, (a)) and C_5–C_6 bond longer by 0.008 Å (1.446 Å, (c) vs 1.438 Å, (a)). The $C_6C_5C_4O$(Me) dihedral angle is more acute for *cis* **TS-5** ((b), 43.3°) so that OMe is not in the plane of the pyramidalised olefin (C_5–C_6) as much as for *trans* **TS-6** ((d), 163.3°). However, the envelope conformation of both *cis* and *trans* TSs position the nucleophile so that the $N_1C_5C_4O$(Me) dihedral angle is very close to 90°, as has been discussed for the previous iodocyclisation TS structures. We can only speculate as to why the TS energy difference for the cyclisation of methyl ether **14** does not correlate with experimental results, it may be that there is a complexity in the reaction pathway that we have not proficiently accounted for in our calculations or model of the reaction.



Scheme 6.7 Reaction coordinate of the iodocylisation of alkenylamine **14** to form iodoamines *cis* **P-5** and *trans* **P-6**. Energy (kJ mol^{-1}) is shown relative to alkenylamine **14**



(a) **TS-5** *cis*

(b) Viewed down C_5–C_4 bond in **TS-5**

(c) **TS-6** *trans*

(d) Viewed down C_5–C_4 bond in **TS-6**

Fig. 6.9 Calculated transition-state structures for the iodocyclisation of alkenylamine **14** *en route* to the formation of carbamate **15**. Newman projections show dihedral angles of $C_6C_5C_4O(Me)$ ('O-in-plane', in red), $C_6C_5C_4H$ ('H-in-plane', in black) and the $N_1C_5C_4O(Me)$ (in blue). [Distances in Å, angles in deg.]

6.3.2 Alkenylamines Possessing an α-Amine Nitrile

Next we wanted to determine what adding an α-amine substituent to alkenylamines while also retaining an ether allylic directing group would do to the structure of the transition states in the iodocyclisation. Hence, the calculated reaction profile of arabinose derived benzyl protected *syn*-α-aminonitrile **2** is shown in Scheme 6.8. Here, there is virtually no energetic preference in the iodine complex pre-equilibrium for either

Re- or *Si*-face approach of I_2 to the alkene double bond, (**IC-7** *Re* = -18.31 kJ mol^{-1} vs **IC-8** *Si* = -18.05 kJ mol^{-1}). However, the two TS geometries found for *cis* (**TS-7** = 18.46 kJ mol^{-1}) and *trans* (**TS-8** = 28.49 kJ mol^{-1}) have a difference in activation energy of 10.02 kJ mol^{-1}. Interestingly, the *trans* iodoamine **P-8** is 9.74 kJ mol^{-1} more energetically favoured than the *cis* **P-7**. Taking into account the full thermodynamic picture of the TSs by relating ΔG (*cis*/*trans*) to the equilibrium constant, we found that the calculated values almost relate to the experimental diastereoselectivity though predict a lower *cis*-selectivity by 6% (Table 6.5).

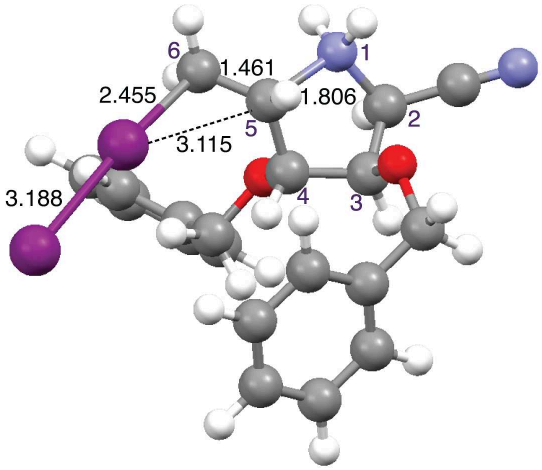
Table 6.5 Relative transition-state Gibbs energies (kJ mol^{-1}) and diastereoselectivities in the iodocyclisation of alkenylamine **2**

Transition State	ΔG^{ab} (kJ mol^{-1})	dr^a	dr^c (%)
TS-7 *cis*	0.00	89.0	>95.0
TS-8 *trans*	5.25	11.0	<5.0

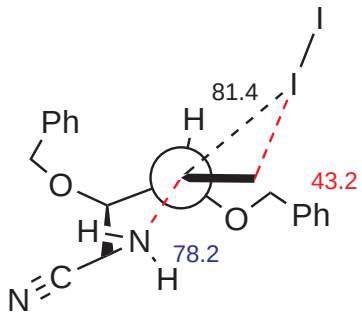
[a]B3LYP/LANL2DZ
[b]Relative to the *cis* TS
[c]Experimental results

The 3D transition state structures *cis* **TS-7** ((a), Fig. 6.10) and *trans* **TS-8** (c) show the same late TS position on the reaction coordinate, with an asymmetric I_2-C_6–C_5 complex and pyramidalised C_6–C_5 olefin bond, as already discussed for the iodocyclisation of the previous alkenylamines possessing an α-amino methylene. Here, there are two potential structural influences on the TS energy, the relative position of the C_4-OBn group, and the α-nitrile substituent, which will be discussed shortly in comparison to the *anti*-aminonitrile (**16**) TSs. The position of C_4-OBn in the favoured **TS-7** (b) is in-plane, ($C_6C_5C_4O(Bn)$ = 43.2°) in a similar magnitude to the C_4-OBn for **TS-8** ((d), $C_6C_5C_4O(Bn)$ = 149.9°), however, situated *inside* (b) vs *outside* (d) the I_2-C_6–C_5

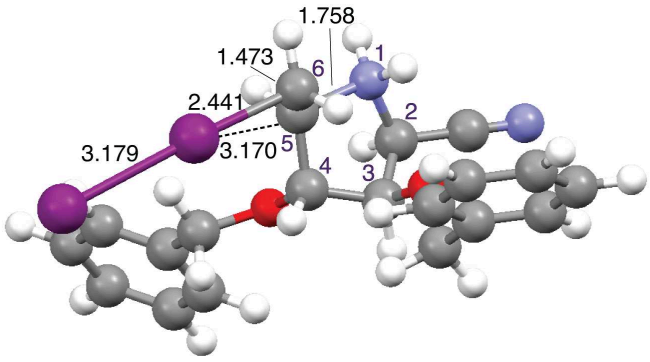
complex. Furthermore, the $N_1C_5C_4O$(Bn) dihedral angle is very close to 90° for both *cis* (78.2°, (b)) and *trans* (92.2°, (d)) TSs, following the trend seen in the previous TSs discussed. It appears that the benzyl ether groups do not impede the preferred 'O-in-plane' conformation or perpendicular positioning of the amine attack to the C_4-O(Bn).



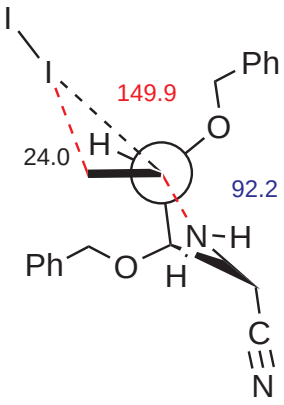
(a) **TS-7** *cis*



(b) Viewed down C_5–C_4 bond in **TS-7**

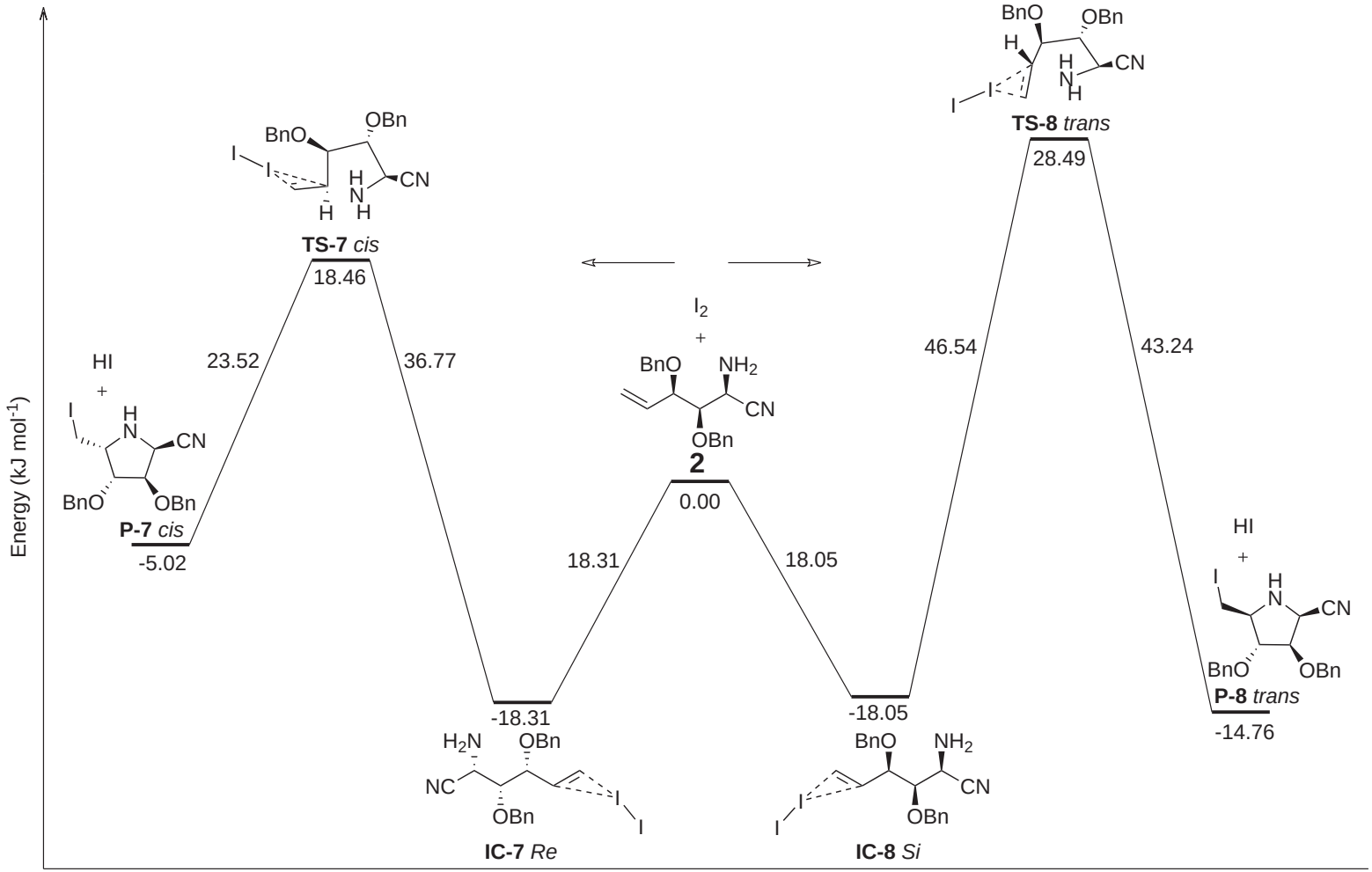


(c) **TS-8** *trans*



(d) Viewed down C_5–C_4 bond in **TS-8**

Fig. 6.10 Calculated transition-state structures for the iodocyclisation of alkenylamine **2** *en route* to the formation of carbamate **3**. Newman projections show dihedral angles of $C_6C_5C_4O$(Bn) ('O-in-plane', in red), $C_6C_5C_4H$ ('H-in-plane', in black) and the $N_1C_5C_4O$(Bn) (in blue). [Distances in Å, angles in deg.]



Scheme 6.8 Reaction coordinate of the iodocylisation of alkenylamine **2** to form iodoamines *cis* **P-7** and *trans* **P-8**. Energy (kJ mol^{-1}) is shown relative to alkenylamine **2**

We then wanted to examine the iodocyclisation of *anti*-α-aminonitrile **16** to compare with those iodocyclisations already discussed, as this alkenylamine shows reduced *cis*-diastereoselectivity in the experimental I_2-mediated carbamate annulation (6:1, *cis*/*trans*, refer Table 6.1). The calculated reaction coordinate of the iodocyclisation of aminonitrile **16** depicts a similar reaction path as for the rest of the alkenylamines (Scheme 6.9). Here, there is a small energetic preference of I_2-complexation for the *Re* diastereotopic olefin face of 3.35 kJ mol^{-1}, (**IC-9** = -10.59 kJ mol^{-1} vs **IC-10** = -7.24 kJ mol^{-1}). Furthermore, the difference in activation energy to form *cis* **TS-9** is a small degree (1.98 kJ mol^{-1}) more than *trans* **TS-10**. However, when calculating the diastereoselectivity from TS ΔG's it was found that the theoretical selectivity was greater for the *cis* diastereomer by approximately 12% more than the experimentally observed ratio of dr = 83.3:16.7, (*cis*/*trans*, Table 6.6).

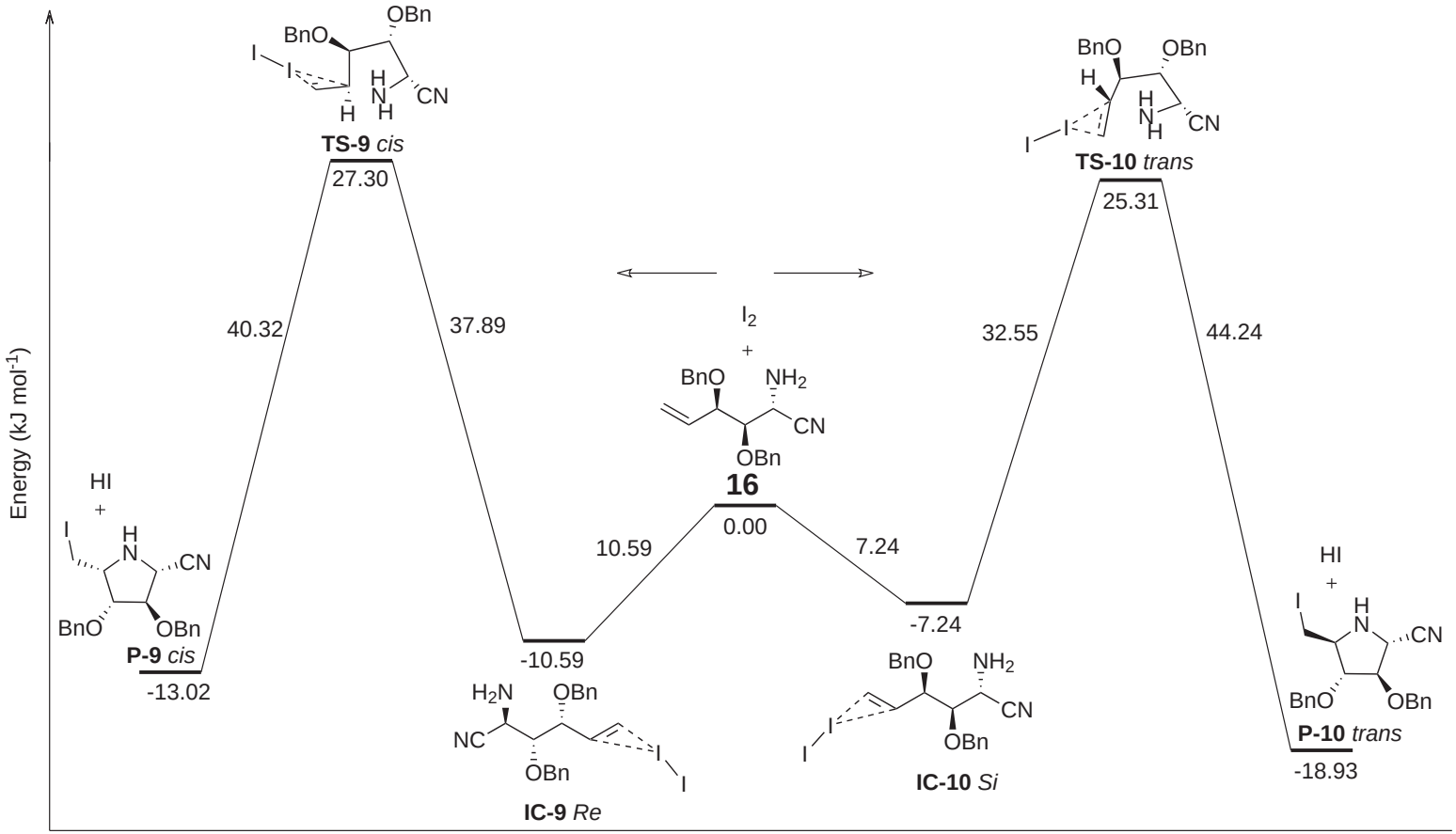
Table 6.6 Relative transition-state Gibbs energies (kJ mol^{-1}) and diastereoselectivities in the iodocyclisation of alkenylamine **16**

Transition State	ΔG^{ab} (kJ mol^{-1})	dr^a	dr^c (%)
TS-9 *cis*	0.00	95.0	83.3
TS-10 *trans*	7.51	5.0	16.7

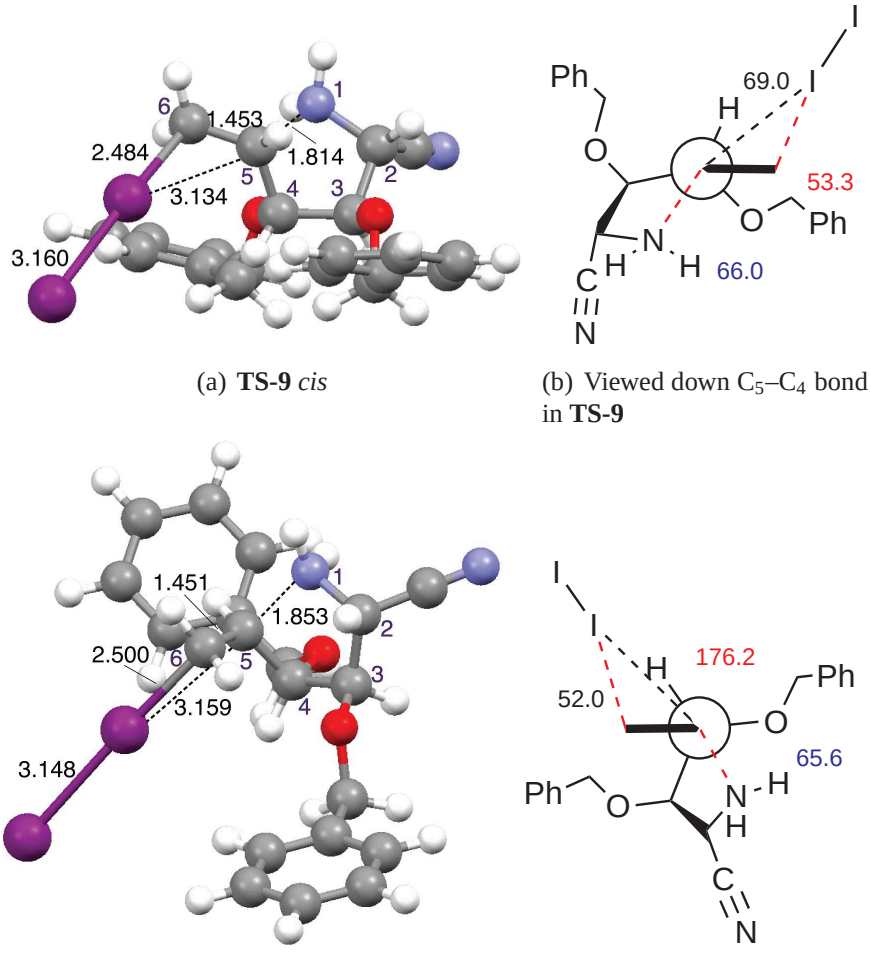
[a]B3LYP/LANL2DZ
[b]Relative to the *cis* TS
[c]Experimental results

Considering the optimised 3D structures of *cis* **TS-9** ((a), Fig. 6.11) and *trans* **TS-10** (c), we can see that both are late-stage TS structures on the reaction coordinate as previously discussed in terms of the progression of I_2-π-complex. However, the *cis* TS is slightly later than the *trans* TS indicated by shorter a N_1–C_5 approach by 0.04 Å (1.814 Å, (a) vs 1.853 Å, (c)) and I–C_6 bond (2.484 Å, (a) vs 2.500 Å, (c)). This is the reverse of the

trend seen for all the other iodocyclisation TSs. The position of C_4-OBn in plane for **TS-9** (b) is calculated to be almost *anti* the C_6–C_5 bond ($C_6C_5C_4O(Bn)$ dihedral angle = 53.3°), compared to **TS-10** (d) where the $C_6C_5C_4O(Bn)$ dihedral angle of 176.2° puts the C_4-OBn in the plane, but *outside* the C_6–C_5 bond. Interestingly, here, as for all of the other calculated iodocyclisation TSs, the 5-membered ring envelope conformation positions the line of nucleophilic attack almost perpendicular to the C_4–O(Bn) bond for both the *cis* and *trans* TS ($N_1C_5C_4O(Bn)$ = 66.0 Å (b) and 65.6 Å (d)).



Scheme 6.9 Reaction coordinate of the iodocylisation of alkenylamine **16** to form iodoamines *cis* **P-9** and *trans* **P-10**. Energy (kJ mol^{-1}) is shown relative to alkenylamine **16**



(a) **TS-9** *cis*

(b) Viewed down C_5–C_4 bond in **TS-9**

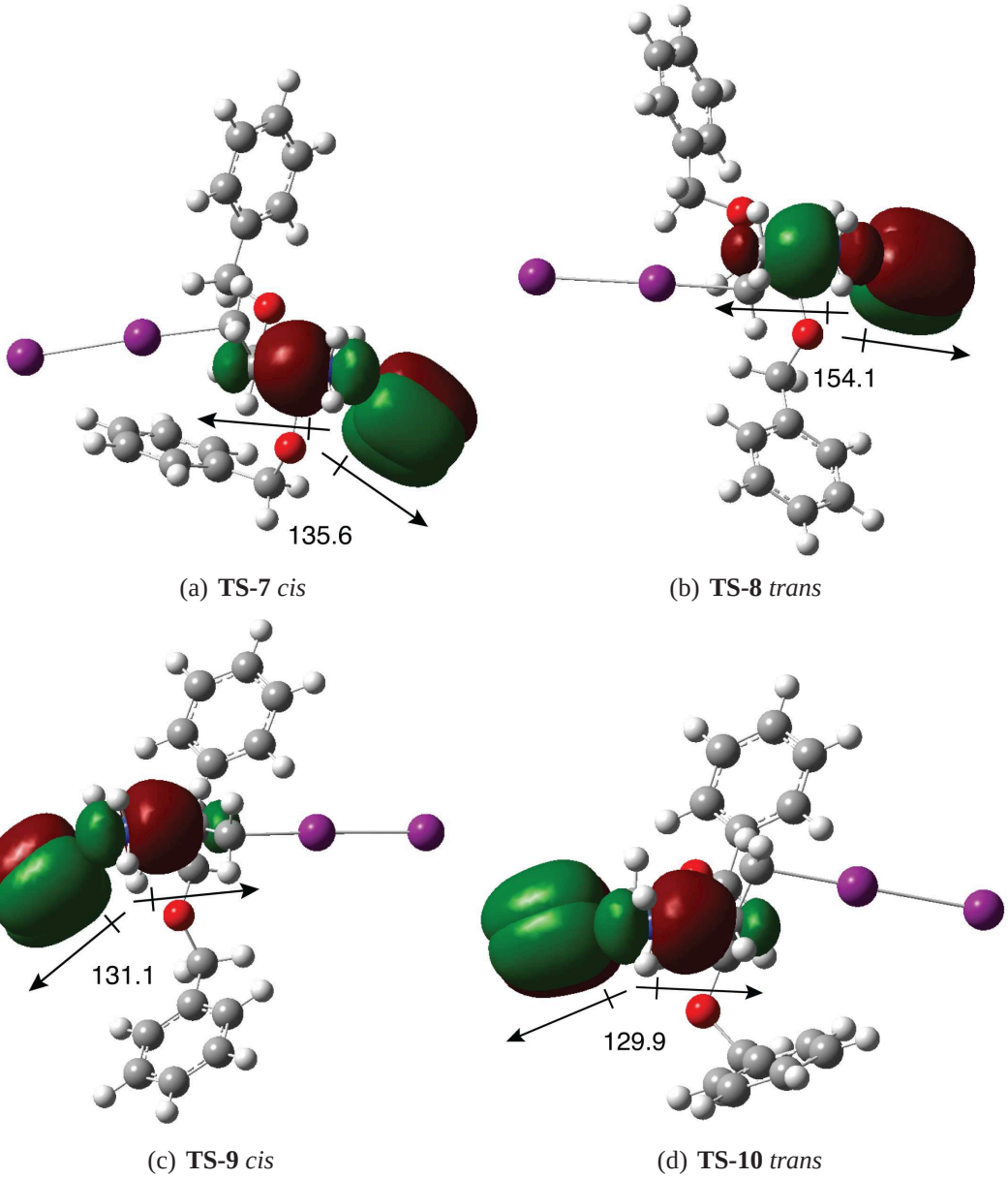
(c) **TS-10** *trans*

(d) Viewed down C_5–C_4 bond in **TS-10**

Fig. 6.11 Calculated transition-state structures for the iodocyclisation of alkenylamine **16** *en route* to the formation of carbamates **17** and **18**. Newman projections show dihedral angles of $C_6C_5C_4O$(Bn) ('O-in-plane', in red), $C_6C_5C_4H$ ('H-in-plane', in black) and the $N_1C_5C_4O$(Bn) (in blue). [Distances in Å, angles in deg.]

The extent to which the α-amino nitrile affects TS energy for the iodocyclisations of both *syn*- (**2**, **TS-7** and **TS-8**) and *anti*-aminonitrile (**16**, **TS-9** and **TS-10**) can be determined by looking at the influence of the nitrile substituent on the nucleophilicity of the amine (N_1). This can be inferred by measuring the NCN_1C_5 dihedral angle (Fig. 6.12). Here,

the measured dihedral angle in *cis* **TS-7** (135.6°, (a)) shows the nitrile dipole has an effect on N_1 by withdrawing some electron density. In contrast, the NCN_1C_5 dihedral angle in *trans* **TS-8** (154.1°, (b)) related to the nitrile withdrawing electron density from N_1, and to a greater extent than *cis* **TS-7**. Whereas, the nitrile dipoles in **TS-9** and **TS-10** have a similar electronic influence on the nucleophile, as the NCN_1C_5 dihedral angles in **TS-9** (131.1°, (c)) and **TS-10** (129.9°, (d)) show that both have some electron withdrawing on the amine. Therefore, the sizeable difference in relative energy between **TS-7** and **TS-8** could be due to the difference in electronic influence of the α-amino nitrile substituent. This is compared to **TS-9** and **TS-10** where the nitrile dipole exerts a similar effect in both optimised TS structures and the energy gap between the TSs is significantly smaller. This explanation of nitrile dipole influence on TS energy provides an update on our previous postulations, (Chapter 5).[2,9]



(a) **TS-7** *cis* (b) **TS-8** *trans*

(c) **TS-9** *cis* (d) **TS-10** *trans*

Fig. 6.12 Calculated natural bond orbital (NBO) images that show the difference in NCN_1C_5 dihedral angle for *cis* **TS-7** (a) and *trans* **TS-8** (b) and the similar NCN_1C_5 dihedral angle for *cis* **TS-9** (c) and *trans* **TS-10** (d)

6.4 Conclusions

We have gained further insight into the factors dictating the diastereoselectivity in the I_2-mediated carbamate annulation of alkenylamines, with and without protecting groups and α-amine substituents. Iodocyclisations, to produce iodoamine intermediates *en route* to the formation of carbamates were investigated using density functional theory calculations. Stationary points were found on the energy surface of five different iodocyclisations corresponding to starting alkenylamines, iodine complexes, transition states and final products. It was found that the theoretical TS Gibb's free energies roughly matched the experimental diastereoselectivities within a theoretical error of $\pm$12% (less than the error for these calculations of $\pm$20 kJ mol^{-1}) for all of the iodocyclisations except one. The calculation did not reproduce the experimentally determined *cis*-selectivity as there was no theoretical diastereoselectivity for the iodocyclisation of methyl ether alkenylamine **14**. This anomaly could be due to further complexity in the mechanism not accounted for in the calculated iodocyclisation reactions. Taken as a whole, both *cis* and *trans* TSs were found to be late-stage on the reaction coordinate with the *cis*-TSs earlier than the *trans*-TSs by varying degrees for most of the computed iodocyclisations. Evidence was found for the *cis* 'O-in-plane' model[3–7] by noting the $C_5C_6C_4O(X)$ dihedral angles and examining the TS molecular orbitals. However, in the current calculations the *trans*-TSs were also found to prefer the 'O-in-plane' conformation rather than the 'H-in-plane' conformation, which was not predicted by earlier models.[3–7] There was also a trend in the *cis*-TSs for the allylic-O(X) group to prefer to reside *inside* the C_6C_5 complex and prefer to reside *outside* in *trans*-TSs. The role of the nitrile on TS energy for the iodocyclisation of α-aminonitriles, (**2**, and **16**) was found to be important for both sets of TSs. Here, measuring the NCN_1C_5 dihedral

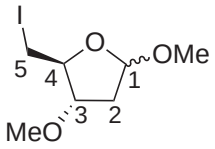
angle indicated that the nitrile dipole was more electron withdrawing for the *trans* **TS-8** than *cis* **TS-7** (**2**), in contrast, the nitrile dipole had the same electronic effect for *cis* **TS-9** and *trans* **TS-10** (**16**) so that the two TSs were much closer in energy. It is also interesting to note that the perpendicular nucleophilic attack relative to the allylic-O(X) holds true whether the alkenylamine TSs have constraints or not, e.g. bulky benzyl groups (**TS-7**–**TS-10**) through to no other substituents (**TS-3** and **TS-4**). The fact that all of the TS structures discussed in this chapter exhibit perpendicular nucleophile attack to the allylic directing group indicates there may be a strong electronic preference for the 5-membered ring envelope that positions the amine in this conformation, which limits the positioning of the rest of the molecule in the TS. This feature could be useful in determining the preferred 5-membered ring TS conformation for future iodocyclisations of alkenylamines.

6.5 Experimental

General Experimental

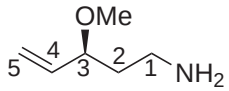
Unless otherwise stated all reactions were performed under air. THF was distilled from $LiAlH_4$ prior to use. All chemicals obtained from commercial suppliers were used without further purification. Zn dust was activated by the careful addition of conc. H_2SO_4, followed by decantation and washing with EtOH (3 x) and hexanes (3 x), and storage under dry hexanes. All solvents were removed by evaporation under reduced pressure. Reactions were monitored by TLC analysis on silica gel coated plastic sheets (0.20 mm, Polygram SIL G/UV254) with detection by coating with 20% H_2SO_4 in EtOH followed by charring at *ca.* 150 °C, by coating with a solution of ninhydrin in EtOH followed by charring at *ca.* 150 °C, or by coating with a solution of 5% $KMnO_4$ and 1% $NaIO_4$ in H_2O followed by heating. Column chromatography was performed on silica gel (40-63 μm). Dowex Monosphere M-31 acidic resin was used for ion exchange chromatography. High-resolution mass spectra were recorded on a Waters Q-TOF Premier^{TM} Tandem Mass Spectrometer using positive electro-spray ionisation. Optical rotations were recorded using a Rudolf Research Analytical Autopol II polarimeter at the sodium D-line. Infrared spectra were recorded as thin films using a Bruker Tensor 27 FTIR spectrometer, equipped with an Attenuated Total Reflectance (ATR) sampling accessory, and are reported in wave numbers (cm^{-1}). Nuclear magnetic resonance spectra were measured using a Varian Inova operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C or a Varian Direct Drive operating at 600 MHz and 133 MHz. ^{1}H and ^{13}C chemical shifts (δ) were internally referenced to the residual solvent peak, ($CDCl_3$ = 7.26 ppm and 77.16 ppm). NMR peak assignments are based on 2D NMR experiments (COSY,

HSQC, and HMBC).



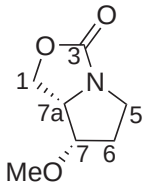
Methyl 2,5-dideoxy-5-iodo-3-methoxy-α/β-D-ribofuranoside 22. Methyl riboside **21**[21] (156 mg, 0.60 mmol) was co-evaporated with dry toluene (0.5 mL) three times, placed under argon and dissolved in dry DMF (1.8 mL). MeI (0.05 mmol) was added to the solution, which was cooled to 0 °C for 15 mins. NaH (0.66 mmol) was added and the resulting mixture allowed to warm to room temperature with stirring. After 1 hour, the reaction was quenched by the careful addition of H_2O, diluted with further H_2O, and extracted twice with EtOAc. The organic layer was washed with brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified using silica gel gradient flash chromatography (hexanes/ EtOAc, 50/1 → 10/1) to give the methyl methoxyribosides **22** as a colourless oil in a 3:2 ratio of α and β anomers (147 mg, 0.54 mmol, 90%). R_f α = 0.47, β = 0.38 (hexanes/EtOAc, 2/1). $[\alpha]_D^{20.9}$ = -54 (c = 1.0, $CHCl_3$); IR (film) 2985, 2927, 2829, 1441, 1371, 1203, 1100, 1034, 948, 934 cm^{-1}. α-**22**: ^{1}H NMR: (500 MHz, $CDCl_3$, 20 °C) δ 5.15 (dd, $J_{1,2b}$ = 5.4, $J_{1,2a}$ = 2.2 Hz, 1H, H-1), 4.20 (ddd, $J_{4,5b}$ = 8.4, $J_{4,5a}$ = 6.3, $J_{3,4}$ = 3.0 Hz, 1H, H-4), 3.99 (ddd, $J_{2a,3}$ = 6.8, $J_{2b,3}$ = 5.4 Hz, 1H, H-3), 3.36 (s, 3H, 1-OMe), 3.34 (s, 3H, 3-OMe), 3.29 (dd, $^2J_{5a,5b}$ = 10.1, $J_{4,5a}$ = 6.3 Hz, 1H, H-5a), 3.22 (dd, $^2J_{5a,5b}$ = 10.1, $J_{4,5b}$ = 8.4 Hz, 1H, H-5b), 2.28 (ddd, $^2J_{2a,2b}$ = 13.8, $J_{2a,3}$ = 6.8, $J_{1,2a}$ = 2.2 Hz, 1H, H-2a), 2.12 (dt, $^2J_{2a,2b}$ = 13.8, $J_{3,2b}$ = $J_{1,2b}$ = 5.4 Hz, 1H, H-2b); ^{13}C NMR: (125 MHz $CDCl_3$, 20 °C) δ 106.0 (C-1), 84.0 (C-4), 83.8 (C-3), 57.3 (3-OMe), 55.5 (1-OMe), 39.6 (C-2), 8.1 (C-5); β-**22**: ^{1}H NMR: (500 MHz, $CDCl_3$, 20 °C) δ 5.10 ($J_{1,2a}$ = 5.4, $J_{1,2b}$ = 1 Hz, 1H, H-1), 3.97 (dd, $J_{4,5a}$ = 8.5, $J_{4,5b}$ = $J_{3,4}$ = 4.5 Hz, 1H, H-4), 3.68 (ddd, $J_{2a,3}$ = 8.0, $J_{3,4}$ = 4.5, $J_{2b,3}$ = 2.2 Hz, 1H,

H-3), 3.39 (s, 3H, 1-OMe), 3.36 (s, 3H, 3-OMe), 3.35 (dd, $^2J_{5a,5b}$ = 10.8, $J_{4,5a}$ = 8.5 Hz, 1H, H-5a), 3.33 (dd, $^2J_{5a,5b}$ = 10.8, $J_{4,5a}$ = 4.5 Hz, 1H, H-5b), 2.25 (ddd, $^2J_{2a,2b}$ = 13.9, $J_{2a,3}$ = 8.0, $J_{1,2a}$ = 5.4 Hz, 1H, H-2a), 2.02 (ddd, $^2J_{2a,2b}$ = 13.9, $J_{2b,3}$ = 2.2, $J_{1,2b}$ = 1 Hz, 1H, H-2b); ^{13}C NMR: (125 MHz $CDCl_3$, 20 °C) δ 105.6 (C-1), 84.9 (C-3), 81.6 (C-4), 57.8 (3-OMe), 55.4 (1-OMe), 38.6 (C-2), 8.6 (C-5); α/β-**22**: HRMS (ESI) *m/z* calcd. for $[C_7H_{13}IO_3+Na]^+$: 294.9802, obsd.: 294.9814.



(3*S*)-1-Amino-3-methoxy-pent-5-ene 14. To a solution of iodoriboside **22** (1.55 g, 5.7 mmol) in a saturated solution of NH_4OAc in EtOH (114 mL) were added activated Zn (1.86 g, 28.5 mmol), $NaCNBH_3$ (1.07 g, 17.1 mmol), and 30% aqueous NH_3 (46 mL). The mixture was stirred at reflux for 18 h, cooled to room temperature, and concentrated under reduced pressure. The residue was redissolved in H_2O, loaded onto a Dowex-H^+ ion-exchange resin, and washed several times with H_2O to remove excess salt. The amine product was then eluted with 30% aqueous NH_3, and further purification was achieved using gradient flash chromatography (DCM/EtOH/MeOH/30% aq NH_3, 100/2/2/1 → 25/2/2/1, v/v/v/v), to provide methoxyalkenylamine **14** as a colourless liquid (10 mg, 0.09 mmol, <5% yield). R_f = 0.11 (DCM/EtOH/MeOH/30% aq NH_3, 50/2/2/1, v/v/v/v). IR (film) 3294, 3077, 2928, 2853, 2823, 1661, 1451, 1423, 1377, 1105, 993, 927 cm^{-1}. ^{1}H NMR: (500 MHz, $CDCl_3$, 20 °C) δ 5.67 (ddd, $J_{3,4}$ = 13.5, $J_{4,5b}$ = 10.1, $J_{4,5b}$ = 8.4 Hz, 1H, H-4), 5.24–5.19 (m, 2H, H-5), 3.65 (dt, $J_{3,4}$ = 13.5, $J_{2a,3}$ = $J_{2b,3}$ = 7.1 Hz, 1H, H-3), 3.27 (s, 3H, OMe), 2.78 (t, $J_{1,2a}$ = $J_{1,2b}$ = 7.1 Hz, 1H, H-1), 1.74 (dt, $^2J_{2a,2b}$ = 13.8, $J_{1,2a}$ = $J_{2a,3}$ = 7.1 Hz, 1H, H-2a), 1.63 (dt, $^2J_{2a,2b}$ = 13.8, $J_{1,2b}$ = $J_{2b,3}$ = 7.1 Hz, 1H, H-2b), 1.39 (br-s, 2H, NH_2); ^{13}C NMR: (125 MHz $CDCl_3$, 20 °C) δ 138.7 (C-4), 117.4 (C-5), 81.4 (C-3), 56.3 (OMe), 39.4 (C-

2), 38.8 (C-1); HRMS (ESI) *m/z* calcd. for $[C_6H_{13}NO+H]^+$: 116.1070, obsd.: 116.1074.



(7*S*,7a*S*)-7-Methoxy-tetrahydro-pyrrolo[1,2-*c*]oxazol-3-one 15. To a solution of methoxyalkenylamine **14** (10 mg, 0.09 mmol) in THF (0.9 mL), were added I_2 (68.5 mg, 0.27 mmol), H_2O (0.9 mL) and $NaHCO_3$ (226.8 g, 2.7 mmol). The reaction mixture was stirred at room temperature for 20 h, quenched with sat. aq. $Na_2S_2O_3$ and extracted with EtOAc. The organic layer was washed with H_2O and brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification was achieved using gradient flash chromatography (hexanes/EtOAc, 20/1 → 1/2, v/v) to provide methoxycarbamate **15** as a colourless viscous liquid (10 mg, 0.07 mmol, 75% yield). IR (film) 2954, 2923, 2853, 1741, 1467, 1397, 1244, 1206, 1089, 1073, 1000, 777 cm^{-1}. 4.52 (dd, $^2J_{5a,5b}$ = 8.5, $J_{4,5a}$ = 3.6 Hz, 1H, H-5a), 4.39 (t, $^2J_{5a,5b} = J_{4,5b}$ = 8.5 Hz, 1H, H-5b), 3.90 (dt, $J_{4,5b}$ = 8.5, $J_{4,5a} = J_{3,4}$ = 3.6 Hz, 1H, H-4), 3.70 (t, $J_{3,4} = J_{2b,3}$ = 3.6 Hz, 1H, H-3), 3.60 (dd, $^2J_{1a,1b}$ = 10.4, $J_{1a,2a}$ = 8.6 Hz, 1H, H-1a), 3.31 (s, 3H, OMe), 3.25, (t, $^2J_{1a,1b} = J_{1b,2b}$ = 10.4 Hz, 1H, H-1b), 2.31 (dd, $^2J_{2a,2b}$ = 13.4, $J_{1a,2a}$ = 8.6 Hz, 1H, H-2a), 3.85 (ddd, $^2J_{2a,2b}$ = 13.4, $J_{1b,2b}$ = 10.4, $J_{2b,3}$ = 3.6 Hz, 1H, H-2b); ^{13}C NMR: (125 MHz $CDCl_3$, 20 °C) δ 79.0 (C-3), 63.6 (C-4), 63.0 (C-5), 56.3 (OMe), 44.0 (C-1), 30.1 (C-2); HRMS (ESI) *m/z* calcd. for $[C_7H_{11}NO_3+H]^+$: 158.0812, obsd.: 158.0822.

Cartesian coordinates for stationary points

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HI
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10
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I -0.268631 -2.908748 0.900425

P-4
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14
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IC-5
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H -2.015559 0.434445 1.414493
H -1.943061 1.492334 -0.006346
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H 0.429632 -0.124462 1.224261
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H 1.354893 -1.149679 -1.556309
C 2.386122 -1.654920 0.234580
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TS-5
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H 5.763234 0.366187 0.363347
C 3.027352 0.755163 -0.554811
H 2.262457 1.268927 -1.152081
C 2.592685 -0.697547 -0.327045
H 2.546145 -1.276559 -1.254287
C 1.613777 -1.050957 0.665721
H 1.355043 -2.103752 0.761015
H 1.625199 -0.493871 1.599952
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H 4.322742 -1.162270 1.200420
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H 4.805769 1.725296 -1.363785

TS-6
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H -2.482354 -0.328949 -1.592011
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P-5
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P-6
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IC-7
48
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IC-8
48
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TS-7
48
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TS-8
48
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P-6
46
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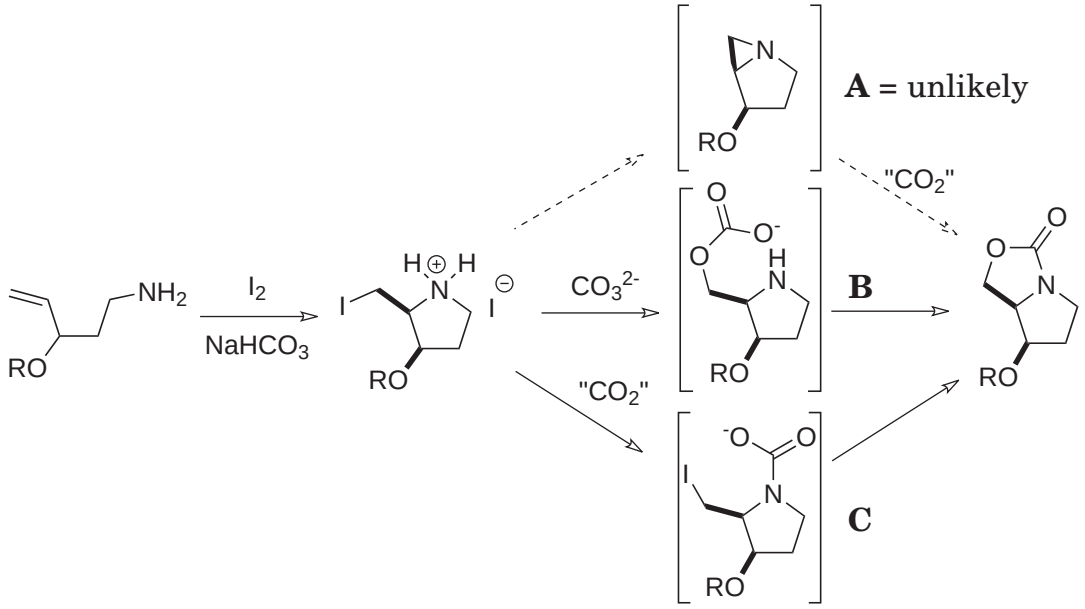
Chapter 7

Future Prospects

The biological activity of azasugars has been largely attributed to their ability to mimic the oxocarbenium ion-like transition state formed during a glycosidase reaction. This ability has led to functional and stereochemical modifications of the azasugar scaffold in the development of specific and potent glycosidase inhibitors.[1–8] The work presented in this thesis has described the development of synthetic methodology to prepare a series of amino-imino-hexitols as azasugar scaffolds. The amino-imino-hexitols were evaluated for their biological activity against a panel of glycosidases with the D-*talo* configuration exhibiting good selective inhibition of β-D-glucosidase. The key transformation in the synthetic route to produce amino-imino-hexitols was an I_2-mediated carbamate annulation of alkenylamines possessing an α-aminonitrile moiety. As eluded to in Chapter 5, we have proposed three potential pathways for the mechanism of addition of CO_2 to the intermediate iodoamine in the carbamate annulation, which could proceed via an aziridine (Pathway **A**, Scheme 7.1), carbonate (Pathway **B**) or carbamic acid (Pathway **C**) intermediate. We suggested that an aziridine is not an intermediate in

the carbonylation as it was not transformed to the carbamate when subjected to the annulation conditions. To determine whether carbonylation proceeds via pathway **B** (carbonate) or **C** (carbamic acid), we can utilise the measurement of kinetic isotope effects (KIEs). The measurement of KIEs is a powerful tool for probing reaction mechanisms that has been used to elucidate events taking place in the rate determining step (rds) of a wide range of reactions.[9–15]



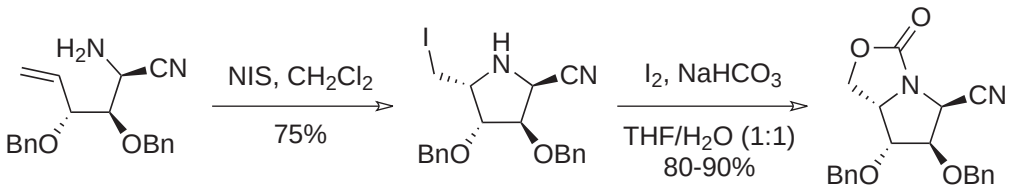
Scheme 7.1 Proposed pathways for carbonlyation of iodoamine

Isotope effects have generally been measured by kinetic competition reactions using isotopically labeled and unlabelled reactants.[16] However, the synthesis of isotopically labeled reactants can be tedious, and a new synthesis and competition reaction is required for each KIE determination. Seminal work by Singleton revealed that carbon KIEs could be measured at natural abundance using ^{13}C NMR analysis,[16] to provide information on the fundamental bonding changes of carbon atoms occurring in the rds of

a reaction. With Singleton's technique, KIEs can be derived from the ratio of $^{12}C/^{13}C$ in the carbons of the reacting starting material (SM). The carbons involved in the rds of the reaction will have an isotopic enhancement or diminishment of ^{13}C over ^{12}C, which can be determined by recovering a sample (R) of SM from a reaction taken to a high degree of conversion and comparing the measured ^{13}C NMR spectrum with that of a standard (R_0) of SM. Any difference in the isotopic ratio of R/R_0 will be apparent by examining the comparative integrations of the carbon signals in the ^{13}C NMR spectrum of the SM. The integrations are referenced to the chemical shift of an internal carbon known not to be involved in the transformation. However, the observed difference in R/R_0 is small due to the low natural abundance of ^{13}C (1.1%). Thus, high precision NMR data is necessary to accurately determine the difference in the integrations of carbon signals within experimental error and this requires a large amount of material to be measured in the ^{13}C NMR (ca. 2 mmol at 125 MHz). For successful KIE analysis of a reaction; the unreacted SM must be recovered in sufficient quantity for NMR measurements; the reaction must be irreversible and the mechanism not change as the reaction proceeds and the reaction must be clean with no side product formation. Therefore, we believe the I_2-mediated carbamate annulation is an ideal reaction to study using the $^{12}C/^{13}C$ natural abundance KIE technique.

Iodoamine **1** was considered as a starting material to investigate KIEs for the mechanism of CO_2 addition to produce carbamate **2**, since a route to gram quantities of **1** had previously been established via the halocyclisation of alkenylamine **3** (Scheme 7.2). However, with a molecular weight of 448 $gmol^{-1}$ iodoamine **1** was not ideal to use as a starting material for natural abundance $^{12}C/^{13}C$ KIE analysis, due to the difficultly of dissolving enough material (2 mmol, 896 mg) in deuterated solvent in a 5 mm i.d. NMR

tube.

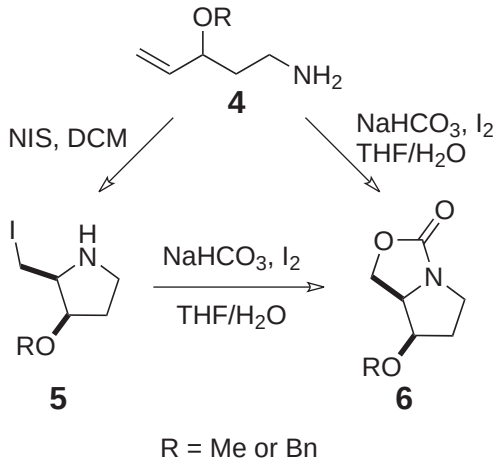


3 **1** **2**

Scheme 7.2 Formation of iodoazasugar **1** *en route* to the synthesis of carbamate **2**

An ideal molecule to study KIEs for the I_2-mediated carbamate annulation would have a low molecular weight for the dissolution of ca. 2 mmol in an NMR tube, while also possessing the necessary functionality to undergo an annulation, and a carbon to act as an internal NMR standard. Thus alkoxy alkenylamine (**4**, Scheme 7.3) derived from 2-deoxy-D-ribose fits these criteria, with a molecular weight of 115 $gmol^{-1}$ when R = Me, and 191 $gmol^{-1}$ when R = Bn. KIEs could be determined for the cyclisation of alkenylamine **4** to iodoamine **5**, for the annulation of alkenylamine **4** to carbamate **6**, and for the CO_2 addition of iodoamine **5** to carbamate **6**. The combined results from these KIE experiments could then be compared to theoretical KIEs derived using density functional theory (DFT). This comparison would indicate whether the initial iodocyclisation or subsequent CO_2 addition was the rds of the carbamate annulation and whether the addition of CO_2 proceeded via a carbonate or carbamic acid intermediate.

We produced the simplified alkenylamine **4** (R = Me, as discussed in Chapter 6), utilising the Vasella reductive amination[17–19] on methyl 2,5-dideoxy-5-iodo-3-methoxy-α/β-D-ribofuranoside. However, this route was not high yielding due to the formation of a major byproduct and difficulties in isolating the alkenylamine. As we needed

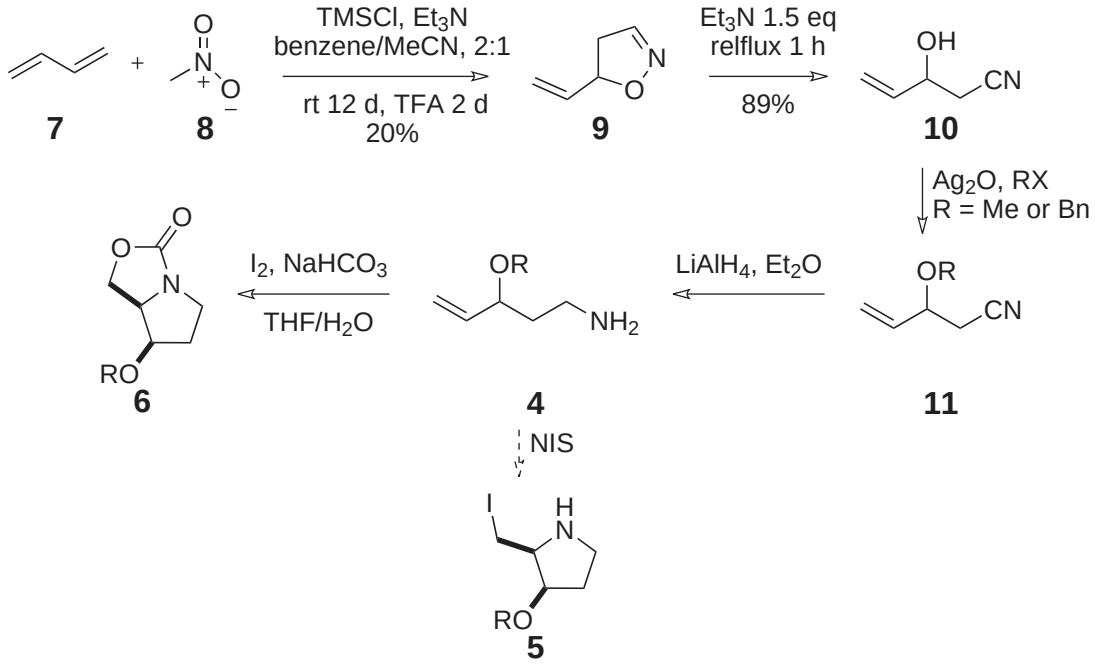


Scheme 7.3 Proposed routes to determine KIEs for the I_2-mediated carbamate annulation

large quantities of material to run the KIE experiments protected amines were used in the reductive amination. Thus, a Vasella reductive amination in the presence of diphenylmethylamine was performed, however, all attempts to remove the diphenylmethyl group were unsuccessful whilst also keeping the methyl ether intact. When *p*-methoxybenzylamine was used as an amine source in the Vasella reductive amination a secondary amine byproduct was formed along with the desired primary amine and problems were also encountered removing the *p*-methoxybenzyl moiety.

In light of the difficulties with the Vasella reductive amination protocol other ways to make an alkoxy alkenylamine framework have been explored, thus a concise racemic route was anticipated involving a [2+3] dipolar cycloaddition as the key step based on the procedure by Torssell and Das (Scheme 7.4).[20] It is important to note that a racemic alkenylamine will not be detrimental to obtaining KIEs, as the carbamate annulation of (3*S*)-1-amino-3-methoxy-pent-4-ene is known to produce the *cis*-diastereomer as the only observable product (Chapter 6), therefore, both enantiomers should cyclise via the

same mechanistic pathway. Furthermore, as enantiomers have identical spectral data, measurement of the racemic alkenylamine (**4**) will not affect the determination of R/R_0 in the ^{13}C NMR spectra. Here, the [2+3] dipolar cycloaddition of butadiene (**7**) and nitromethane (**8**) produced the cyclic oxime **9** in 20% yield and, although this reaction was low yielding, further optimisation could be achieved. Ring opening of oxime **9** under basic conditions gave hydroxy nitrile **10** in good (89%) yield. At this point alkylation of nitrile **10** in the presence of Ag_2O could be carried out with either MeI or BnBr to produce the alkoxy nitrile **11**, which could be subsequently reduced to the alkenylamine **4** with treatment by $LiAlH_4$.



Scheme 7.4 Preparation of a racemic alkoxy alkenylamine for KIE analysis

Methoxy alkenylamine (**4**, R = Me) was prepared using the racemic route shown in Scheme 7.4, however, this compound is highly volatile which makes it difficult to

reliably recover in a quantitative amount from KIE experiments. Although carbamate annulation of alkenylamine **4** (R = Me) successfully produced the *cis*-carbamate **6**, attempts to isolate the iodoamine **5** were yet unsuccessful. However, it is anticipated that the benzyloxy alkenylamine (**4**, R = Bn) will not be volatile, and can be prepared through the use of BnBr and Ag_2O (**10** → **11**) followed by reduction of the nitrile via treatment with $LiAlH_4$. A benzyl ether increases the weight of the alkenylamine compared to a methyl ether, however, we anticipate the trade-off in molecular weight should facilitate the smooth re-isolation of the SM in the KIE experiments. We also believe the iodoamine (**5**, R = Bn) can be synthesised from alkenylamine **4** (R = Bn) using NIS in DCM. With this racemic route, gram quantities of benzyloxy alkenylamine (**4**, R = Bn) can be prepared and utilised as the SM for the KIE experiments. Moreover, analysis of the experimental KIE data will enable further elucidation of the I_2-mediated carbamate annulation mechanism.

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