SYNTHESIS OF SMALL MOLECULE INHIBITORS FOR THE TREATMENT OF DISEASE

by

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Abstract

Three aspects of the protecting-group-free (PGF) synthesis of small molecules have been described in this thesis. In the first part, the PGF azasugar synthesis methodology was applied to 2-deoxy-D-glucose with the intention of selectively forming the six-membered azasugar 5-*epi*-fagomine. Surprisingly, four products were formed in the key I₂-mediated carbamate annulation step, with a pyrrolidine being the major product after optimisation. This was formed in 15% yield. A mechanism that explains the formation of the four carbamates was proposed, which was supported by an investigation into related halocyclisation reactions.

The next part of this thesis describes the development of a new PGF methodology for the synthesis of conduramines, another class of biologically interesting molecules. Conduramines are amino polyhydroxy cyclohexenes and some conduramines have glycosidase inhibitory activity. These molecules are also useful precursors to a variety of biologically useful molecules including aminocyclitols and azasugars. The key steps in the PGF synthesis of conduramines are a Vasella-Barbier amination, a reaction that forms new C-C and C-N bonds concomitantly, and a ring closing metathesis in the presence of free hydroxyl and amine groups. To this end, a 4-deoxy 3-conduramine was prepared in just four steps and in 27% yield.

Finally, the preparation of an amine library and its biological testing for the identification of a new anti-tuberculosis drug is described. Two short syntheses were used to prepare alkenylamines and amines from the corresponding sugar, with various lipophilic groups attached to the amine. A 20-member amine library was prepared, and the compounds were tested for anti-mycobacterial activity in a mycobacterial growth inhibition assay. The most active compounds were subjected to further biological testing to determine their general cytotoxic properties. Two amines, arabinohexadecylamine and arabinohexadecylmethylaniline, were identified as having the best potential for use as anti-tuberculosis drugs, and have been sent to Colorado State University for subsequent *in vivo* testing in a mouse model of tuberculosis.

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“It’s not easy being green.”

- Kermit the Frog

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List of Abbreviations

Ac	acetyl	EQ	environmental quotient
AcOH	acetic acid	equiv.	equivalents
ADMET	adsorption, distribution, metabolism, and excretion – toxicity	ER	endoplasmic reticulum
AD-mix	asymmetric dihydroxylation mix	ESI	electron spray ionisation
<i>aq.</i>	aqueous	Et	ethyl
Ar	aryl	FDA	Food and Drug Administration
AraT	arabinosyltransferase	h	hour(s)
atm	atmosphere	HL60	human leukaemia cell line
ATP	adenosine triphosphate	HMBC	heteronuclear multiple bond correlation
BCG	Bacillus Calmette-Guérin, weakened strain of <i>M. bovis</i>	HRMS	high resolution mass spectrometry
Bn	Benzyl	HSQC	heteronuclear single quantum coherence
Boc	<i>tert</i> -butoxycarbonyl	Hz	hertz
br	broad	IC ₅₀	half maximal inhibitory concentration
Calcd.	calculated	Imid.	imidazole
Cbz	carboxybenzyl	IND	investigational new drug
COSY	correlated NMR spectrum	<i>i</i> Pr	isopropyl
cpd	compound	IR	infrared spectroscopy
d	doublet	<i>J</i>	coupling constant
d	day(s)	LAM	lipoarabinomannan
DBU	1,8-Diazabicycloundec-7-ene	LHMDS	lithium hexamethyldisilazide
DCM	dichloromethane	Lit.	literature
dd	doublet of doublets	LogP	octanol/water partition coefficient
de	diastereomeric excess	LSD	lysosomal storage disorder
DGJ	1-deoxygalactonojirimycin	m	multiplet
DIBAL-H	diisobutylaluminium hydride	MDR-TB	multi-drug-resistant tuberculosis
DiPEA	<i>N,N</i> -diisopropylethylamine	Me	methyl
DMAP	4-dimethylaminopyridine	MHz	mega hertz
DME	dimethoxyethane	MIC	minimum inhibitory concentration
DMF	dimethylformamide	MIMR	Malaghan Institute of Medical Research
DMSO	dimethyl sulfoxide	min	minutes
DNA	deoxyribonucleic acid	mRNA	messenger ribonucleic acid
DOX	doxorubicin	MS	mass spectrometry
E	electrophile	<i>Mtb</i>	<i>Mycobacterium tuberculosis</i>
<i>E. coli</i>	<i>Escherichia coli</i>		
E-factor	environmental factor		
EMB	ethambutol		

MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide	PyBroP	Bromotripyrrolidino-phosphonium hexafluorophosphate
<i>M. bovis</i>	<i>Mycobacterium bovis</i>	PZA	pyrazinamide
<i>M. smegmatis</i>	<i>Mycobacterium smegmatis</i>	quant.	quantitative
<i>m/z</i>	mass to charge ratio	RCM	ring closing metathesis
NAD ⁺ /NADH		Red-Al	reducing aluminium, sodium bis(2-methoxyethoxy)aluminum hydride
	Nicotinamide adenine dinucleotide redox pair	ref.	reference
NADP ⁺ /NADPH		<i>R_f</i>	retention factor
	Nicotinamide adenine dinucleotide phosphate redox pair	rt	room temperature
		s	singlet
		SAR	structure activity relationship
NBS	<i>N</i> -bromosuccinimide		
NDA	new drug approval	sat.	saturated
NIS	<i>N</i> -iodosuccinimide	SDS	sodium dodecyl sulfate
NMO	<i>N</i> -methylmorpholine <i>N</i> -oxide	SI	Selectivity Index
		S _N 2	bimolecular nucleophilic substitution
NMR	nuclear magnetic resonance	t	triplet
OADC	oleic albumin dextrose catalase	TAB	Tween albumin broth
		TB	tuberculosis
Obsd.	observed	Tf	trifluoromethanesulfonate
¹ O ₂	singlet oxygen	TFA	trifluoroacetic acid
<i>p</i>	para	THF	tetrahydrofuran
PBS	phosphate-buffered saline	TLC	thin layer chromatography
PG	protecting group	TMS	trimethylsilyl
PGF	protecting-group-free	tRNA	transfer ribonucleic acid
Ph	phenyl	TS	transition state
ppm	parts per million	Ts ₂ O	<i>para</i> -toluenesulfonyl anhydride
PSA	polar surface area		
<i>p</i> TsOH	<i>para</i> -toluene sulfonic acid	v.	version
		XDR-TB	extensively drug-resistant tuberculosis

Chapter 1: Introduction

1.1 Preface

Many small molecules are effective drugs. Some are common in every household, such as aspirin, (**1**) (Figure 1.1), which is an analgesic, antipyretic, and anti-inflammatory medication that works by inhibiting the production of thromboxane, thereby thinning the blood.¹ Another example, Prozac[®] (**2**), is a selective serotonin re-uptake inhibitor for the treatment of major depression and other psychiatric disorders,² while cisplatin (**3**) is a chemotherapeutic used to treat various cancers and works by binding to and cross-linking DNA, which ultimately leads to cell death by apoptosis.^{3,4} While all three drugs contain vastly different chemical skeletons, each can be prepared in remarkably few steps.

Figure 1.1: Small molecule drugs: aspirin (**1**), Prozac[®] (**2**), and cisplatin (**3**).

An attraction of pursuing small molecules as potential drugs is their ease of synthesis, which allows for more rapid screening and therefore faster establishment of lead compounds for the treatment of disease. In contrast, the total syntheses of larger and more complex molecules can be difficult and time

consuming. These complex total syntheses often require protecting group manipulations that increase the number of steps and ultimately lead to lower yields and increased costs.

This thesis outlines research on the protecting-group-free (PGF) synthesis of small molecules as potential drug candidates. To this end, a number of different molecular scaffolds were prepared. First, azasugars were synthesised using an adaptation of the Vasella-reductive amination and I_2 -mediated carbamate annulation methodology developed within the Stocker-Timmer group.⁵ Azasugars are notable glycosidase inhibitors,⁶ and can therefore be used in the treatment of a variety of diseases. The next class of molecules targeted was the conduramines, a scaffold that provides access to many biologically useful molecules. The preparation of conduramines required the development of a new C-C bond forming methodology, which was based on a Vasella-Barbier amination reaction. Finally, the development of a small molecule amine library for the treatment of tuberculosis was undertaken. Here, novel amines and alkenylamines with different sugar and lipophilic amine components were prepared without the use of protecting groups and were subsequently screened for tuberculostatic and cytotoxic activities. From this work, a lead lipophilic amine was identified. Thus, with the development of a new PGF synthesis, the extension of an existing PGF methodology, and the application of PGF synthesis to the treatment of disease, a variety of factors relating to the efficient synthesis of small molecule drugs were explored.

1.2 Green Chemistry and Protecting-Group-Free Synthesis

1.2.1 Green Chemistry

Green chemistry is defined as “the design, development and implementation of chemical processes and products to reduce or eliminate substances hazardous to human health and the environment.”⁷ This theme has been further developed into the twelve principles of green chemistry, which include objectives such as maximising reaction efficiency (with respect to atom economy as well as yield), the use of safe, renewable, and environmentally benign chemicals (including solvents), energy efficiency (e.g. reactions performed at ambient temperature and pressure where possible), and waste prevention at the source rather than ‘end-of-pipe’ waste remediation.^{7,8} There are several ways to determine the ‘greenness’ of a chemical process. These include atom economy, environmental factor (E-factor), and environmental quotient (EQ).^{8,9} Atom economy, initially conceived by Trost in the 1980s, is a measure of how many of the reactant’s atoms end up in the product, but does not take yield, spent catalysts or solvents into account.^{9b} To take the additional waste into account, the concept of E-factor, defined as mass of waste per mass of product, was developed by Sheldon.^{8,9a} The E-factor, however, fails to take into account the nature of the waste produced and for that reason, the concept of the EQ has been defined, which is the product of the E-factor and an arbitrary quotient describing the nature of the waste, Q. Here Q is dependent on the recycling or ease of disposal of the waste.⁸ As society becomes more concerned with the overall efficiency of any given process and its effects on the environment, the challenge for today’s organic chemist is to prepare complex molecules as efficiently as possible and generate minimal waste in the process.¹⁰

One way to improve the overall efficiency of a synthesis is to avoid the use of protecting groups (PGs). The use of PGs has undoubtedly led to an explosive growth in the complexity and successful synthesis of targeted compounds, however the use of PGs also introduces higher costs in terms of extra reagents and

solvents, and increased number of steps, which often results in a reduction of the overall yield. PGF chemistry addresses these challenges via the design of new synthetic schemes to simplify chemical production (the invention of novel reactions) and allowing for the use of solvents that are environmentally and ecologically benign.^{11,12} Key in this approach is the avoidance of separate chemical transformations to temporarily block other potentially reactive functional groups. This can be achieved by the careful design of synthetic routes to generate a product chemoselectively.¹³

Several methods have been employed by chemists to avoid protecting groups. These include *in situ* protection and deprotection, the mimicking of biological pathways used in nature, skeleton-building reactions that are functional-group tolerant, changes to the sequence of functional group introduction, and the development of new methodology in order to avoid protecting groups.^{12,14} These approaches are briefly discussed below.

1.2.2 *In situ* Protection

In situ protection is the avoidance of a side reaction through the *in situ* transformation into a less reactive group, and is preferred when only one transformation is required. The approach includes protonation/deprotonation strategies, as in the case of the synthesis of (±)-fawcettimine (**10**) (Scheme 1.1).¹⁵ The alkaloid fawcettimine was originally extracted from *Lycopodium fawcettii*, a plant material collected from the Blue Mountain Range in Jamaica in 1959.¹⁶ The synthesis of fawcettimine was not PGF by design, but by necessity due to the incorporation of protecting groups negatively affecting the reactivity of the protected intermediates. First enone **4** and bis(trimethylsilyl)alkene **5** were combined in the presence of a Lewis acid in a Hosomi-Sakurai reaction to give allylic alcohol **6**.¹⁷ Although trimethylsilyl (TMS) groups are traditionally used as protecting groups, here the carbon-bonded TMS group is required for the allylation reaction and the TMS ether is simultaneously removed during the course of the reaction, and therefore is not used as a PG. Allylic alcohol **6** was

transformed into amine **7** in seven steps, which included an intramolecular Michael addition and an Arndt-Eistert homologation.¹⁸ Treatment of amine **7** with *p*-toluenesulfonic anhydride (Ts₂O) gave tosylated adduct **8** due to failure to selectively tosylate the primary alcohol group of intermediate **7**. Thus formation of the nine-membered ring under basic conditions, followed by detosylation gave tricyclic intermediate **9** in 65% yield (two-steps). It was necessary to remove the tosyl group at this stage, due to difficulty removing the *N*-tosyl group at a later stage in the synthesis; therefore, the *N*-tosyl group was not technically employed as a protecting group in this synthesis. Oxidation of the alcohol in tricycle **9** was followed by *in situ* protection by protonation of the amine with perchloric acid and ozonolysis of the double bond. Finally, deprotonation with sodium bicarbonate gave (±)-fawcettimine (**10**) in an overall yield of 17% over 13 steps, an improvement on an earlier synthesis, which yielded only 0.1% product after 26 steps.¹⁹ More recently, in 2007, Toste and co-workers reported the first asymmetric total synthesis of (+)-fawcettimine,²⁰ with the use of protecting groups, in a 13-step reaction sequence with an overall yield of 14%. There have subsequently been other reports of this synthesis,^{21,22} however the synthetic strategy used in the paper by Heathcock *et al.* has remained a key strategy, and one of the most efficient to date.²³

Scheme 1.1: PGF synthesis of (±)-fawcettimine.¹⁵

Other *in situ* protection PGF syntheses include the eight-step synthesis of welwitindolinone A (**14**, Scheme 1.2),²⁴ a natural product that was originally extracted from blue-green algae in 1994.²⁵ The first step of the synthesis involved the opening of an epoxide **11** by a Grignard reagent in the presence of a ketone group, which was made possible by the *in situ* formation of enolate **12**, before addition of the Grignard reagent to give **13**. Seven subsequent PGF steps allowed for the first total synthesis of welwitindolinone A (**14**).

Scheme 1.2: *In situ* protection step towards PGF synthesis of (+)-welwitindolinone A.²⁴

1.2.3 Synthesis of Compounds with Few Competing Reactivities

Some molecules contain few reactive groups that are likely to undergo side reactions, which readily allows for the avoidance of PGs. The presence of few competing reactivities in (±)-subincanadine F (**22**) allowed for the synthesis of this compound in a 33% yield over seven steps (Scheme 1.3).²⁶ Subincanadine F was originally isolated from a Brazilian medicinal plant *Aspidosperma subincanum* Mart, and early biological screening revealed that it had *in vitro* cytotoxic activity towards murine lymphoma L1210 cells ($IC_{50} = 2.4 \mu\text{g/mL}$) and human epidermoid carcinoma KB cells ($IC_{50} = 4.8 \mu\text{g/mL}$).²⁷ The synthesis of (±)-subincanadine F (**22**) began with the Pictet-Spengler²⁸ condensation of tryptamine

15 with methyl bromopyruvate to form **16**, which led to olefinic indoloazepine **17** after skeletal rearrangement in the presence of base. Reduction of the alkene in **17** with NaCNBH₃ afforded amine **18**, which was subjected to a Michael addition using methyl acrylate to give diester **19** in good yield. A Dieckmann condensation followed,²⁹ which was achieved in the presence of 2 equiv. of *t*-BuOK in THF at room temperature to give the subincanadine core **20** in 82% yield. Hydrolysis and decarboxylation gave ketone **21**, which underwent an aldol reaction with acetaldehyde, followed by dehydration, to afford (±)-subincanadine F (**22**).

Scheme 1.3: PGF synthesis of (±)-subincanadine F.²⁶

Another example of a synthesis involving few competing reactivities is the total synthesis of (±)-patchouli alcohol (**28**) by Danishefsky and Dumas (Scheme 4).³⁰ The synthesis began with a Diels-Alder reaction of diene **23** and methyl vinyl ketone (**24**) to establish the carbon framework in bicyclic ketone **25**. Hydrogenolysis of the alkene and base-mediated equilibration of the acetyl group to the equatorial position were undertaken before treatment with an organolithium reagent and conversion to the allylic chloride **26**. Substitution of the chloride,

followed by hydrogenation gave alcohol **27**, which was converted to (±)-patchouli alcohol (**28**) through treatment with PBr₃ and cyclisation with Na in THF.

Scheme 1.4: PGF synthesis of (±)-patchouli alcohol (**28**).³⁰

1.2.4 Biomimetic synthesis

Biomimetic synthesis is defined as the laboratory design of intermediates or reaction sequences inspired by the natural enzymatic pathway without using the enzyme.¹² A biomimetic synthesis of (±)-elysiapyrone A (**37**), which was originally isolated by Cueto *et al.* from the sea slug *Elysia diomedea*,³¹ took advantage of a cascade of 6 π and 8 π electrocyclic reactions that mimics the biosynthetic pathway used in nature (Scheme 1.5).³² The synthesis began with the conversion of ester **29** into aldehyde **30**, before enolate addition and Dess-Martin oxidation³³ formed diketone **31** as a mixture of stereoisomers. Base-mediated cyclocondensation, followed by regioselective methylation gave pyrone **32**, which underwent Stille coupling with a vinyl-tin reagent to form intermediate tetraene **33**. *In situ* 8 π electrocyclisation of **33** to **34**, followed by a 6 π electrocyclisation completed the cascade with the formation of the bicyclo[4.2.0]octadiene **35**, and its epimer, as the major and minor products, respectively. The formation of epimers can be explained by the formation of *cis* or *trans* products in the 6 π electrocyclisation. Singlet oxygen was used in a [4+2] cycloaddition to form peroxide **36**, which was isomerised to (±)-elysiapyrone A (**37**) in the presence of a Ru(II) catalyst.^{14,32}

Scheme 1.5: Biomimetic PGF synthesis of (±)-elysiapyrone A (37).³²

Another example of a biomimetic synthesis was the preparation of (±)-allamcin (40) by Trost and co-workers (Scheme 1.6).³⁴ Here, diol formation across the least hindered double bond of intermediate (38) was followed by periodate

cleavage to form hemi-acetal intermediate **39**. The intermediate was directly treated with NaOAc causing a cascade that selectively formed ($\pm$)-allamcin (**40**).

Scheme 1.6: Biomimetic synthesis of ($\pm$)-allamcin (**40**).³⁴

1.2.5 Invention oriented synthesis

Invention oriented synthesis is the term given to PGF methodology whereby the development of new methodology is required to reach a synthetic target.¹² An elegant example of an invention oriented synthesis of a natural product is that developed by Newhouse and Baran for the synthesis of ($\pm$)-psychotrimine (**45**, Scheme 1.7).³⁵ Psychotrimine (**45**) is a trimeric tryptamine alkaloid that was originally isolated from the leaves of the shrub *Psychotria rostrata*.³⁶ The invention of new methodology for the formation of the uncommon N1-C3 bond was required in the synthesis. Here, *o*-iodoaniline and *N*-iodosuccinimide (NIS) were used in combination with tryptophan derivative **41** to form pyrroloindoline **42** in the novel C-N bond-forming step. In the past, C-C tryptophan dimers and N1-C2 reactions have been observed under similar reaction conditions, and the N1-C3 bond formation was unprecedented. Detailed mechanistic analysis suggests the reaction proceeds via initial halogenation of *o*-iodoaniline, followed by nucleophilic attack of the corresponding tryptophan enamine and subsequent ring closure.³⁷ Pyrroloindoline **42** was then subjected to an iodide-selective Larock annulation and desilylation (**42** $\rightarrow$ **43**),³⁸ followed by a Buchwald-Goldberg-Ullmann reaction to give trimeric species **44**.³⁹ Finally, reduction with Red-Al gave ($\pm$)-psychotrimine **45** in a five-step sequence that was preferable to the previously reported 16-step synthesis.⁴⁰

Scheme 1.7: PGF synthesis of (±)-psychotrimine (**45**).³⁵

Other invention oriented PGF syntheses whereby new chemical methodologies were developed includes the single-step synthesis of 6-arylurine ribonucleosides (**46**, Scheme 1.8A) from inosine **47**, which involved a modified Suzuki-Miyaura coupling.⁴¹ An improved synthesis of (+)-welwitindolinone A (**14**) by the Baran group employs a novel synthetic step that involves an oxidative ring contraction of (-)-fischerindole I (**48**, Scheme 1.8B), itself prepared in a seven-step synthesis from a readily available terpenoid, using xenon difluoride to modify the C2-C3 indole double bond without affecting the remaining alkenes.⁴²

Scheme 1.8: Arylpurine nucleoside **46** and (+)-welwitindolinone A (**14**), made with newly developed PGF methodologies.^{41,42}

The PGF azasugar methodology is an example of invention oriented synthesis with particular relevance to this thesis. The development of two new reactions allowed five-membered azasugars to be prepared from readily available furanoses in a five-step synthesis (Scheme 1.9).^{5,43,44} The synthesis of 1,4-dideoxy-1,4-imino-L-lyxitol (**55**)^{5a} began with a Fischer glycosylation of D-ribose **49** to give methyl furanoside **50**, which was iodinated at the primary hydroxyl to form methyl iodoriboside **51** in moderate yield. The first novel reaction, a modified Vasella-reductive amination, gave primary amine **52** in excellent yield and chemoselectivity. In this reaction a methyl iodoglycoside is submitted to a zinc-mediated Vasella reaction followed by a reductive amination with ammonia *in situ*. Key in this reaction was the use of excess ammonia and ammonium acetate to yield the primary amine **52** rather than the secondary amine (**53**), with the latter being formed under normal reductive amination conditions.^{5b,45,46} Alkenylamine **52** was subjected to a novel carbamate annulation procedure where the

3,4-*cis* carbamate **54** was formed stereoselectively and in excellent yield. The reaction is suggested to proceed via initial halocyclisation, followed by attack of carbon dioxide (formed *in situ* from NaHCO₃) to give the desired carbamate.^{47,48} Hydrolysis using NaOH completed the synthesis to provide pyrrolidine **55** in five steps from D-ribose and in 55% overall yield, which is the best yield reported for the pyrrolidine.^{5a} This novel methodology has been applied to the synthesis of five- and six-membered azasugars, where stereoselective 3,4- or 4,5-*cis* azasugars were formed preferentially over the *trans* diastereomers.^{5a,c,43,44}

Scheme 1.9: Total synthesis of 1,4-dideoxy-1,4-imino-L-lyxitol (**55**).⁵

1.3 Azasugars as Glycosidase Inhibitors and Molecular Chaperones

1.3.1 Azasugars

Azasugars are carbohydrates with nitrogen replacing the ring oxygen. Due to their similarities to furanoses and pyranoses, azasugars can bind to glycosidases and other sugar binding enzymes, and thus have potential in the treatment of a range of diseases including Type 2 diabetes, viral and bacterial infections, lysosomal storage disorders (LSDs), cancer, and cystic fibrosis.^{49,50} There are several classes of azasugars (Figure 1.2) including the pyrrolidines (five membered azasugars), piperidines (six membered azasugars), pyrrolizidines (1-azabicyclo [3.3.0] octanes, fused five-five bicyclic rings), indolizidines (1-azabicyclo [4.3.0] nonanes, fused five-six bicyclic rings), and notropanes (8-azabicyclo [3.2.1] octanes, fused five-six bicyclic rings).⁵¹ Members of each class have been found in a variety of plants and microorganisms.⁵⁰

Figure 1.2: Different classes of azasugars.

1.3.2 Glycosidases as Potential Drug Targets

Glycosidases are enzymes that catalyse the hydrolysis of glycosidic linkages and are involved in numerous fundamental biological processes including the break down of polysaccharides driving intestinal digestion, cellular function such as post-translational processing of glycoproteins, and lysosomal catabolism of glycoconjugates.⁵⁰ Glycosidases play an important and varied role in a number of diseases. For example, cancerous tumours present unusual carbohydrate structures due to changes in the *N*- and *O*-glycan biosynthesis and the inhibition of key enzymes involved in glycosidase reactions,⁵² while LSDs (see Section 1.3.3), such

as Gaucher's disease, Fabry's disease and Tay-Sachs disease, result from various mutated glycosidase enzymes involved in the catabolic pathway for glycosphingolipids.⁵³ A number of bacteria and viruses target carbohydrates on mammalian cell surfaces and utilise glycosidases in their internalisation and/or pathogenesis mechanisms.⁵⁴ The neuraminidase (carbohydrate binding receptor) in influenza viruses cleaves the sialic acids from the glycoproteins on the surface of an infected cell, releasing the pathogen.⁵⁵ Inhibition of the neuraminidase effectively traps the virus on the cell surface, and therefore stops the virus from reproducing.

The enzymatic hydrolysis of glycosidic bonds occurs through one of two mechanisms, with either the retention or inversion of stereochemistry at the newly formed anomeric centre.⁵⁶ In an inverting mechanism the oligosaccharide binds to the enzyme active site along with a water molecule, and the glycosidic linkage is hydrolysed via an S_N2 -like transition state that has some oxocarbenium character (Figure 1.3A). The retaining glycosidase mechanism occurs through a two-step process. The oligosaccharide undergoes nucleophilic substitution by a residue in the enzyme active site, and the glycosidic linkage is hydrolysed via an S_N2 -like transition state, leaving the glycoside covalently bound to the enzyme (Figure 1.3B).⁵⁷ Nucleophilic attack on the anomeric centre by a water molecule occurs from the unhindered side of the sugar. The new bond is formed through a second inverting transition state, effecting retention of stereochemistry at the anomeric centre. An azasugar, which will be protonated at physiological pH, can potentially bind to the glycosidase active site by mimicking the charge density of the native glycoside transition state, inhibiting the enzyme (Figure 1.3C).⁵⁷

Figure 1.3: Proposed mechanisms of glycosidase enzyme catalysis, the enzyme active site, including residues involved in the catalysis, is shown. A: Inverting glycosidase mechanism of action. B: Retaining glycosidase mechanism of action. C: Protonated azasugar transition state mimic.⁵⁶

1.3.3 P F P C

For an enzyme or protein to function properly, the primary sequence(s) of amino acids must correctly fold to form the tertiary or quaternary structure of the functional protein. Under normal cellular conditions this may be aided by the presence of a molecular chaperone – a separate protein that guides the peptide folding to give a functional protein, without being involved in the final protein function or activity.⁵⁸ Certain genetically inherited diseases result from the misfolding and/or aggregation of proteins, causing reduction in enzymatic function and hence the build up of intermediate substrates in an enzymatic pathway. Such diseases include cystic fibrosis, LSDs, Alzheimer's disease, and cataracts.^{59,60}

LSDs are a collection of more than 50 inherited diseases characterised by defective lysosomal function, which results from the failure to transport or metabolise lipids, proteins and carbohydrates causing a build up of these substrates within the cell.⁶¹ These rare metabolic diseases cause a variety of physical and neurological symptoms including skeletal deformities, heart problems, enlarged liver and spleen, eyesight problems, and brain degeneration.⁶²

Often the genetic mutation resulting in protein misfolding is a simple point mutation resulting from a single amino acid substitution in the primary sequence. This may only slightly reduce the folded protein's activity, but often hinders the folding and thus quality control mechanisms stop the protein from exiting the endoplasmic reticulum (ER) for its cellular target. Pharmacological chaperones are molecules that have been developed to encourage protein folding in the treatment of protein misfolding disorders.⁶⁰ The pharmacological chaperone binds to the active site at an early stage in protein folding, thereby stabilising the protein and encouraging a conformation close to that of the native protein. This allows the protein to pass internal quality control mechanisms and exit the ER for its biological target within the cell.⁶³ For example, Fabry disease is a lysosomal storage disorder caused by lack of lysosomal α -galactosidase A; the misfolding of

this enzyme causes some forms of the disease. The azasugar 1-deoxy galactonojirimycin, DGJ **56**, is currently in clinical trials for use as a pharmacological chaperone in the treatment of Fabry disease (Figure 1.4).^{64,65} For an effective LSD drug, a molecule must support protein folding by binding to the enzyme active site, while not binding preferentially to the native substrate. DGJ is a nanomolar inhibitor of α -galactosidase A *in vitro*, and at sub-inhibitory concentrations *in vivo*, is an effective pharmacological chaperone.⁶⁴

Figure 1.4: Azasugars of clinical significance for the treatment of lysosomal storage disorders.

Azasugars may also be used as glycosidase inhibitors in the treatment of misfolded protein disorders by way of substrate reduction therapy. An example of this is in the use of Miglustat (**57**) for the treatment of Gaucher's disease.⁶⁶ Gaucher's disease is a lysosomal storage disorder caused by a build up of glucosylceramide in cells due to poor activity of the glucocerebrosidase enzyme.⁶⁷ Rather than repairing the function of the faulty enzyme, Miglustat inhibits the synthesis of glucosylceramide, reducing the amount of substrate produced so that there is no glycolipid build up within the cells.

1.4 C-C Bond Forming Reactions for the Synthesis of Conduramines

1.4.1 Conduramines

For PGF chemistry to be more widely incorporated into the design of synthetic strategies, new chemoselective methodologies need to be developed. Invention oriented synthesis has enabled the preparation of a number of interesting compounds by a PGF and therefore ‘greener’ approach,^{35,41,42} as illustrated by the development of the modified Vasella-reductive amination and carbamate methodologies for synthesis of azasugars.^{5,43} A natural extension of this work is the development of chemoselective C-C bond forming reactions, which will allow access to a variety of molecules, including conduramines.

Conduramines such as conduramine A-1 (**58**, Figure 1.5) are derivatives of the natural products conduritols,⁶⁸ of which conduritol A (**59**) was isolated from the bark of the vine *Marsdenia condurango*.^{69,70} Some conduramines have activity as glycosidase inhibitors,⁷¹ and these molecules provide access to many other biologically useful compounds including azasugars⁷² and aminocyclitols.^{73,74} Two examples of conduramine derivatives with medical relevance are Tamiflu® (**60**) and *N*-octyl valienamine (**61**). Tamiflu (**60**) is a well-known aminocyclitol that is used for the treatment of influenza. It is a glycosidase inhibitor that acts on the neuraminidase enzyme of the influenza virus.⁷⁵ *N*-Octyl valienamine (**61**), on the other hand, is being investigated for use as a chemical chaperone for the treatment of Gaucher’s disease.^{76,77} Although protected conduramine analogues have been prepared earlier,⁷⁸ the fully deprotected amines have not been reported. The development of PGF methodology to synthesise conduramines therefore provides access to an array of biologically significant molecules.

Figure 1.5: Conduramine A-1 (**58**) and conduritol A (**59**) and conduramine derivatives Tamiflu[®] (**60**) and *N*-octyl valienamine (**61**).

1.4.2 The Barbier Reaction for C-C bond Formation

The PGF preparation of conduramines requires the development of new methodology for the C-C bond forming reaction, a PGF Barbier-type reaction was proposed for this key step. A Barbier reaction is the reaction of an aldehyde or ketone with an alkyl halide and a metal (*e.g.*, Zn, Mg, In) *in situ* to form a substituted alcohol.⁷⁹ The Barbier reaction has been combined with other reactions to perform multiple steps in one pot. Madsen and coworkers prepared a tandem Vasella-Barbier reaction on methyl iodoriboside **62**, using allyl bromide **63** as the nucleophile, to give triol **64** in quantitative yield and moderate selectivity (Scheme 1.10).⁸⁰ Key in the reaction was the use of Zn in the Vasella reductive elimination and as the activating agent for the Barbier reaction. The reaction required sonication, presumably to ensure the Zn was well dispersed and the reaction mixture was homogeneous, but dry conditions were not necessary. Indium was also used in the Vasella-Barbier reaction, which led to slightly reduced yields and the opposite diastereoselectivity.⁸¹

Scheme 1.10: Vasella-Barbier reaction of methyl iodoriboside.⁸⁰

The addition of benzylamine (**65**) to the Vasella-Barbier reaction was an extension of the methodology, which was undertaken in the presence of an isopropylidene protected methyl iodoriboside **66** (Scheme 1.11).⁸¹ Here the Vasella reaction leads to the generation of an aldehyde, which is subject to nucleophilic attack by benzylamine (**68**) to give an imine. The carbon nucleophile formed from the Zn insertion in the C-Br bond of allyl bromide (**66**), attacks the imine to form diene **67** in high yield and selectivity. The use of sonication was required. Dry conditions were employed, however an earlier paper by the same group reported a similar yield when the reaction was carried out in the presence of water.⁸⁰

Scheme 1.11: Vasella-Barbier-amination reaction of a protected methyl iodoriboside.⁸¹

Finally, Behr *et al.* subjected D-xylose (**68**) to benzylamine (**68**) to quantitatively form glycosylamine **69** (Scheme 1.12).⁸² The hemiaminal ether **72** was treated with indium and allyl bromide (**66**) at rt in MeOH to achieve a Barbier reaction producing alkene **70** in good yield (72%) and excellent diastereoselectivity

(>95%). The reaction sequence could also be performed in a single pot with no reduction in yield or selectivity. Key to the reaction was the use of indium powder; the use of either Sn or Zn in place of In was unsuccessful.

Scheme 1.12: Barbier amination reaction on D-xylose.⁸²

The Vasella-Barbier amination reaction is amenable to the development of a PGF protocol. This is supported by the facts that a Vasella-Barbier reaction has been reported in the absence of PGs,⁸⁰ a Vasella-Barbier amination reaction has been described with protected amine and alcohol groups,⁸¹ and a Barbier amination has been achieved with unprotected hydroxyl groups.⁸² When considered as a whole, these results suggest that a PGF Vasella-Barbier amination with a primary amine such as benzylamine is plausible. An extension of this methodology would be the use of ammonia as the amine source, utilizing an ‘unprotected amine’ in the reaction.

1.4.3 Ring Closing Metathesis

Ring closing metathesis (RCM) is the cyclisation of a dialkene aided by an organometallic catalyst (Scheme 1.13A). The reaction is entropically driven through the generation of two products, the desired cyclic alkene and a small alkene by-product that is often volatile, hence the reaction usually goes to completion.⁸³ The four main catalysts: Grubbs I and II, and Hoveyda-Grubbs I and II, are shown in Scheme 1.13B. The catalysts vary in substrate scope and specificity, functional group tolerance, and activity.

Scheme 1.13: A: Example of ring closing metathesis. B: The four main RCM catalysts.

RCM with Grubbs' catalyst I was shown to tolerate a range of functional groups including acids, alcohols and aldehydes.⁸⁴ For example, RCM of triol **71** with Grubbs' 1st generation catalyst afforded the anticipated cyclohexene triol **72** in excellent yield (Scheme 1.14A).^{80,81} Poor conversion was noted in the presence of secondary amine **73**, even with a higher catalyst loading (10 mol%), and acetylation of the amine was required to achieve cyclisation to cyclohexene **74** in good yield (Scheme 1.14B). The incompatibility of amines with RCM catalysts has been attributed to amine-mediated degradation of a catalyst that contains a phosphine ligand (*e.g.*, Grubbs I, Grubbs II, Hoveyda-Grubbs I)⁸⁵ in the presence of a primary or secondary amine. Degradation occurs via ligand exchange between the amine and the phosphine on the catalyst. Here, the benzylidene precatalyst undergoes one cycle of RCM to form the methylidene catalyst, the amine group binds to the metal, substituting the phosphine ligand, which is free to attack the methylidene moiety, thereby degrading the catalyst (Scheme 1.15). Strategies to avoid this problem include protection of the amine functionality with

bulky or electron withdrawing groups,^{86,87} *in situ* protection by protonation to form the corresponding ammonium salt,^{84,88} or utilisation of a catalyst without a phosphine ligand, such as Hoveyda-Grubbs II.^{83,89,90}

Scheme 1.14: Functional group tolerance of RCM.

Scheme 1.15: Amine-mediated catalyst degradation pathway, L= ligand.⁸⁵

Metathesis catalysts were originally used under oxygen free and dry conditions,⁸⁴ however, catalyst design has improved to the extent that examples of olefin metathesis reactions carried out in aqueous or protic solvents have recently been reported.^{83,91} RCM reactions have been carried out in dimethoxyethane/water and acetone/water mixtures using the Hoveyda-Grubbs II catalyst as a heterogeneous catalyst.⁸⁹ Additionally, homogeneous catalysts based on the Grubbs and Hoveyda-Grubbs catalysts depicted in Scheme 1.13B have been developed with polyethylene glycol (PEG), or quaternary amine salts attached to a ligand (Figure 1.6), to increase water solubility and therefore improve activity.^{92,93}

Figure 1.6: Water soluble RCM catalysts.

In 2011, Robinson and coworkers reported the successful olefin metathesis of primary amines (Scheme 1.16).⁹⁴ Diene **75** was cyclised to cyclopentene **76** in >95% yield, as determined by ¹H NMR. This was the first example of a primary amine being successfully cyclised by RCM in such a high yield, and took advantage of both the quaternary amine protection strategy, and the use of the Hoveyda-Grubbs II catalyst that has no labile phosphine ligands. The metathesis reactions were also tested on the free amine, as well as the chloride, triflate, and tetrafluoroborate ammonium salts. The free amine metathesis reaction was unsuccessful, while the reaction with the ammonium chloride salt resulted in a significantly reduced yield (38% yield). The triflate and tetrafluoroborate salts proceeded to give conversions comparable to the tosylate (83 – 91% yield). This is the strategy that will be applied to the synthesis of conduramines, due to the simple ‘protection’ strategy for the amine, and the use of a readily available catalyst.

Scheme 1.16: RCM of tosylate salt of a primary amine.

1.5 Small Molecules as Tuberculosis Drugs

1.5.1 Tuberculosis

Tuberculosis (TB), the disease caused by infection with *Mycobacterium tuberculosis* (*Mtb*), is a worldwide problem. Each year TB kills around 1.3 million people, with millions more infected with the bacteria.⁹⁵ In New Zealand, on average one person a day is diagnosed with TB. The rate of infection for Māori and Pacific Island people is also significantly higher than for European ethnic groups.⁹⁶ With the increased mobility of individuals worldwide, and increasing disparity between the classes, TB is a problem in both developed, and developing nations.

1.5.2 Pathogenesis of Tuberculosis

The most common form of tuberculosis is pulmonary TB (infection of the lungs), which is caused by the inhalation of the *Mtb* bacilli that are dispersed when an infected individual coughs or sneezes.⁹⁷ The infection may spread from the lungs to cause extrapulmonary TB, which can infect almost any organ in the body. Symptoms of TB include a cough, fever, weight loss, fatigue, chest pain and blood in the sputum.^{97,98} TB is diagnosed by culturing the sputum for identification of the bacteria, and by a chest x-ray for the identification of lung infections.⁹⁸

Once the bacteria reach the lungs they are phagocytosed by the alveolar macrophages. The bacteria are either immediately killed through the activation of the immune system, or the bacilli are able to avoid the innate immune response by preventing the phagosome from fusing with a lysosome, stalling the killing process.⁹⁹ The bacteria multiply until they eventually burst the macrophage, expelling bacilli into the lungs, before being phagocytosed once again by the surrounding macrophages, and the process is repeated. Additionally, the

engulfment of bacilli by macrophages marks the beginning of granuloma formation. A granuloma is a collection of immune cells surrounding the living and lysed macrophages that contain the mycobacterial infection.^{100,101} Often the formation of a granuloma does not kill the bacteria but instead keeps the infection controlled and contained. The granuloma contains a variety of immune cells that work to stabilise bacterial growth, causing the bacilli to enter a non-replicating state known as latent tuberculosis.¹⁰¹ Latent TB does not give the patient symptoms of active TB, however it may become active at some later stage, potentially years after the initial infection, as a result of a weakened immune system.¹⁰² If the granuloma fails to contain the disease, or latent TB is reactivated, then the bacteria will replicate unchecked in the lungs causing active TB that may be spread throughout the body (causing extrapulmonary TB) or transmitted by coughing.¹⁰¹

Mycobacteria have a complicated cell wall structure that protects the bacterium from its environment (Figure 1.7). At the base of the cell wall is the peptidoglycan, a cross-linked polymer of peptides and amino sugars. The peptidoglycan forms a backbone, to which the arabinogalactan is linked via a rhamnose-*N*-acetyl-glucosamine-phosphate linker unit.¹⁰³ Arabinogalactans are polymers of arabinose and galactose and are essential for the structural integrity of the mycobacterial cell wall.¹⁰⁴ At the reducing end of the arabinogalactan, mycolic acids are covalently bound. The lipophilic mycolic acids represent the outmost layer of the bacterial cell wall, and are attached to either the arabinogalactan, or to a trehalose unit in the form of trehalose dimycolates (TDMs) and trehalose monomycolates (TMMs), which are present on the cell surface. The mycolic acids consist of alkyl chains of up to C₉₀ in length,¹⁰⁴ and therefore reduce the permeability of the cell wall, providing a physical barrier that protects the bacteria from antibiotics and the host's immune response.¹⁰² Also present in the cell wall are the lipoarabinomannans (LAMs) that are anchored to the plasma membrane by a phosphoryl-inositol-mannose (PIM) linker.¹⁰⁴ The LAMs are involved in the virulence of *Mtb* by modulating the host's immune system.¹⁰⁵

Figure 1.7: Cartoon of the structure of the Mycobacterial cell wall.¹⁰⁶

1.5.3 Treatment of Tuberculosis

1.5.3.1 Vaccination

The ideal treatment of *Mtb* would be prevention by vaccination. The current TB vaccine, Bacillus Calmette-Gerin (BCG), which contains a weakened strain of bovine tuberculosis *M. bovis*,¹⁰⁷ protects against severe childhood TB but has varied efficacy against adult pulmonary TB.¹⁰⁸ Research into a new, more effective TB vaccination is underway,^{109,110} however, a recent phase two clinical trial of a booster vaccine candidate demonstrated insignificant protection against TB.¹¹¹ There is only a limited understanding of the memory immune response required for protection against *Mtb* infection¹¹² and no good animal model to accurately represent the human disease.¹¹⁰ These factors explain why no new TB vaccinations have been developed since BCG. Furthermore, as a significant proportion of the world's population is already infected with the disease, treatment of TB in the future will still require both vaccination and medication strategies.

1.5.3.2 Anti-TB Drugs

Tuberculosis is a difficult disease to treat because the causative agent, *Mtb*, has several defense mechanisms. The ability of the bacteria to prevent phagosome-lysosome fusion, to form granulomas to evade the immune system, the presence of persistent, latent TB, and the protective lipophilic cell wall have all been discussed in Section 1.5.2. As a result of the difficulty in treating tuberculosis, the treatment involves the use of a selection of first and second line drugs. First line drugs are generally the more effective and specific antimycobacterial drugs, and usually have fewer side effects than the second line drugs. The second line drugs are broad spectrum antibacterials, most of which are injectable rather than orally available, and accordingly, are more expensive than the first line drugs.^{106,113} Normal treatment of drug-sensitive TB begins with an intensive two month course of all first line drugs: isoniazid, rifampin, pyrazinamide, and ethambutol (EMB), followed by a further four months of treatment with just rifampin and isoniazid (Figure 1.8).¹¹⁴ Following the six months of treatment the patient is considered cured, however recurrence of the disease occurs in 2-3% of all new TB cases.¹¹⁴

Figure 1.8: First line TB drugs and commonly used second line drugs.¹⁰⁶

Drug resistant tuberculosis arises from infected individuals not complying to the lengthy drug regimens required for treatment. Patients become non-infectious after a few weeks but must finish the drug course to remove the non-replicating bacteria and achieve a clinical cure. Side effects of the drugs, including hepatotoxicity (liver damage), can force termination of treatment and facilitate drug resistance.¹¹⁵ Multi-drug resistant TB (MDR-TB) is defined as resistance to both isoniazid and rifampin, while extensively drug resistant TB (XDR-TB) occurs when a *Mtb* strain is resistant to isoniazid, rifampin, a fluoroquinolone, and at least one of kanamycin, capreomycin, or amikacin.^{115,116} There have also been reported cases of drug resistance beyond XDR-TB, where the bacteria may be

resistant to all known anti-TB medications. This is termed totally drug resistant TB (TDR-TB).¹¹⁷ Treatment of the drug resistant forms of TB varies depending on what drugs the patient is resistant to, but usually involves administration of more of the fluoroquinolone and injectable second line drugs (streptomycin, capreomycin, amikacin and kanamycin).^{114,118} The increasing presence of drug resistant TB underlies the need for new anti-TB medications.

The current TB drugs have a variety of different modes of action. Isoniazid is a prodrug that is oxidised *in vivo* to the active species, which inhibits mycolic acid synthesis, thereby compromising the integrity of the bacterial cell wall.^{106,115,119} EMB also inhibits cell wall biosynthesis by targeting the arabinosyl transferase enzymes involved in the synthesis of the arabinogalactan.^{106,115,119} Pyrazinamide is a prodrug that is converted to its active form, pyrazinoic acid, *in vivo*. Pyrazinoic acid inhibits trans-translation, a process that manages non-functioning proteins and rescues stalled ribosomes in RNA translation.¹²⁰ Rifampin, another drug that inhibits RNA translation, works by binding to RNA polymerase and blocking RNA synthesis.^{106,115,119} Of the second line drugs, the fluoroquinolones inhibit enzymes involved in DNA replication, while streptomycin, kanamycin, amikacin, capreomycin are all inhibitors of protein synthesis.^{106,115,119} Kanamycin and capreomycin both contain mixtures of components (as depicted in Figure 1.5), all of which are active against *Mtb*.^{121,122} There are many validated targets that anti-TB drugs act on and any new antituberculosis drug that acts with a novel mechanism may provide the best chance of success against drug resistant TB.

1.5.3.3 New Drugs in the Pipeline

In recent years many potential new antituberculosis drugs have entered clinical trials.¹²³ Fluoroquinolones moxifloxacin (Figure 1.8) and gatifloxacin (Figure 1.9) are broad spectrum antibiotics and are already approved as second line anti-TB drugs for MDR-TB. Moxifloxacin and gatifloxacin are in phase III clinical trials for their use as first line TB drugs in the treatment of drug sensitive tuberculosis, with the objective of reducing the combination drug therapy treatment time from

six months to four months.¹¹⁸ Fluoroquinolones inhibit DNA gyrase and/or topoisomerase IV - enzymes that are involved in the supercoiling of DNA necessary for replication.¹¹⁹

Gatifloxacin
(Fluoroquinolone)

Bedaquiline

PA-824

SQ109

Delamanid

Figure 1.9: Emerging tuberculosis drugs.

Diarylquinoline bedaquiline (Figure 1.9) is the first new approved drug for the treatment of tuberculosis in over 40 years, and was granted advanced approval for the treatment of MDR- and XDR-TB.¹²⁴ It is currently in Phase II trials for the treatment of MDR-TB and also for the treatment of drug sensitive tuberculosis.^{125,126} Bedaquiline inhibits a proton pump involved in ATP synthesis, and is also active against non-replicating (dormant) TB.^{127,128} That said, the drug

may cause heart arrhythmias,¹²⁹ and the drug's early approval is controversial as the successful clinical trial outcome was based on the clearance of the bacteria as measured by sputum cultures rather than the number of patient deaths.¹³⁰

Nitroimidazoles are pro-drugs that are reduced by bacterial nitroreductases to form the active drugs *in vivo*. The nitroimidazole drugs PA-824 and delamanid (Figure 1.9) are in phase II and phase III clinical trials, respectively. Both drugs inhibit mycolic acid biosynthesis under aerobic conditions and thereby inhibit actively replicating bacteria, while they kill non-replicating bacteria in anaerobic conditions through the release of nitric oxide within the cell.^{131,132} Delamanid is the second new anti-TB drug recently approved for the treatment of MDR-TB, although it is also still in clinical trials.¹³³

Ethylenediamine SQ109, an analogue of EMB, was identified from a 63000 compound library, and is now in phase II trials.¹³⁴ SQ109 inhibits mycolic acid biogenesis by targeting a membrane transporter of trehalose monomycolate (TMM).¹³⁵ This is a different target to that of EMB, thus SQ109 is also active against EMB resistant TB.¹¹⁹

There are several drugs currently in phase II and III clinical trials for the treatment of tuberculosis, some of which have been discussed. While there are drug candidates in pre-clinical trials, there are currently no drugs in phase I trials. Accordingly, there is a need to fill this gap in the TB drug development pipeline, as some of the later stage drug candidates will inevitably fail phase II or III clinical trials.^{123,119}

1.6 The Process of Drug Discovery

Drug discovery and development is a long and challenging process. In recent years the process has become even more demanding as stricter health and safety regulations and increasing economic pressures develop.^{136,137} An overview of the drug discovery process is given in Figure 1.10.

Figure 1.10: The drug discovery process, IND = investigational new drug, NDA = new drug approval. Reproduced from Pharmaceutical Research and Manufacturers of America with permission.¹³⁸

1.6.1 Screening Methods

Either whole cell or enzyme based screening methods are used to screen for active compounds. A biological target (*e.g.*, an enzyme) that can bind a drug molecule causing growth inhibition or toxicity needs to be identified and validated to ensure that the target is effective and accessible *in vitro* and *in vivo*.¹³⁹ Alternatively, for whole cell screening the cell model needs to be validated as a representative model of the disease (*e.g.*, the use of BCG as a model for *Mtb* infection).¹⁴⁰ There is much debate about the utility of whole cell versus target based drug screening strategies and there is no consensus about which model is best.^{141,142}

1.6.2 Screening and Lead Identification

Drug screening is used to determine which compounds are the best inhibitors. There are many ways in which the inhibitory concentration of a drug candidate is determined from an assay.^{143,144} A common assay readout is fluorescence, whereby a fluorescent protein or dye displays a measurable change in fluorescence intensity at the inhibitory concentration for a given compound. In this way, the high throughput screening of large compound libraries (>10,000 compounds) can be automated.

Compounds may be obtained through synthesis, extraction of natural product sources, or purchasing of a compound library, and there are two general strategies for the identification of leads. The first is a randomised screening approach, which is compatible with high throughput screening methods for large compound libraries, while a structure-based molecular design approach focuses on the design of specific molecules to inhibit an enzymatic target.¹⁴⁵

The screening process is designed to provide lead compounds that inhibit the target or cell at low concentrations. Optimisation of the lead compounds through the systematic structural variation of the molecular scaffold gives a second generation compound library and provides a better understanding of the structure activity relationship (SAR) of the class of molecules. The optimisation process may be repeated several times until a drug candidate with the desired activity (often nanomolar inhibition) has been discovered.

1.6.3 Pharmacokinetic and other Biological Testing

A newly identified lead compound needs to be tested in a variety of models to validate its efficacy and safety. If the optimised drug lead was originally screened in an isolated target enzyme then *in vitro* whole cell screening is the next logical step. By comparison, if the compound was initially tested in a whole cell model,

then the drug mechanism of action, and enzyme target still needs to be identified at some stage. However, often *in vivo* studies precede studies for identification of the mechanism of action. Absorption, distribution, metabolism, excretion, and toxicity (ADMET) studies should also be carried out at the earliest possible stage and, if possible, in parallel with target screening.¹⁴⁶ Without the appropriate pharmacokinetic properties, a potential drug will not reach its biological target in the human body and will therefore fail to treat the disease. Lipinski's rule of five has been developed as a useful predictor of the absorption properties of a compound. By adhering to these guidelines for bioavailability (*i.e.* molecular weight ≤ 500 g/mol, no more than five hydrogen-bond donors, the octanol/water partition coefficient $\text{cLogP} \leq 5$, and no more than 10 hydrogen bond acceptors) orally available compounds with good absorption properties are favoured.¹⁴⁷ That said, Lipinski's rule of five only applies to compounds that are absorbed by passive diffusion through the cell membrane, and compounds that act on, or are transported by biological transporter proteins are therefore exempt.

There are numerous other pharmacokinetic properties and physiological targets to be screened.^{139,148} In particular, cytotoxicity testing *in vitro* (*e.g.*, anti-proliferation assays on human T cells) to confirm that the lead compound is not toxic is important. Next, the lead compound is subjected to an animal model to confirm efficacy of the drug against the disease *in vivo* and to further explore the compound's safety and pharmacological profile.¹⁴⁹ If at any stage a compound fails, then it may be sent back for further optimisation through repetition of the previous stages. A successful lead compound will progress to an investigational new drug (IND) and be submitted for clinical trials.¹³⁹

1.6.4 Clinical Trials of the Investigational New Drug

With proven efficacy in an animal model and promising pharmacokinetic data, the IND will enter clinical trials, as highlighted herein. In phase I clinical trials, the drug is tested on a small group (~20-80 people) of healthy adults to determine its safety, maximum dose, dose treatment range, and potential side effects.^{150,151} The

main goal of phase II clinical trials is to explore whether a drug works in adult volunteers (~100-500 people) who are affected by the disease or illness.^{138,150,151} Phase III clinical trials are carried out on thousands of volunteer patients and the objective of the trial is to confirm the efficacy of the drug on a statistically and clinically relevant sample size.^{150,151}

The drug discovery and development process often takes more than 10 years, from library screening to new drug on the market. The monitoring of efficacy, safety and side effects once the drug is on the market (phase IV) continues for many years to identify long-term effects, unexpected side effects, and optimal methods of use. This is essential as the larger sample size when in clinical use provides evidence for rare side effects or uncommon drug interactions that may have been missed in the clinical trials.^{150,151}

1.7 Thesis Overview

This thesis describes the use of PGF methodologies for the synthesis of biologically significant molecules.

Chapter 2 describes the application of the PGF methodology to the synthesis of six-membered azasugars, first by providing an overview of work performed in this research field, and then by exploring how different chiral scaffolds affect the PGF carbamate annulation reaction. It then elaborates on the use of halocyclisations in the synthesis of piperidines, and provides mechanistic insight and an explanation of why different products are formed from different chiral starting materials.

The second part of the thesis, described in **Chapter 3**, explores development of a new C-C bond forming methodology to enable the PGF synthesis of conduramines. A PGF Vasella-Barbier amination was developed, and then a PGF RCM undertaken to afford a 4-deoxy-3-conduramine in few steps.

Finally, the development of a potential new anti-TB drug using a PGF approach is discussed (**Chapter 4**). This involved the preparation of a 20-member lipophilic amine library and the *in vitro* antimycobacterial screening of these compounds. Additional cytotoxicity testing was also undertaken within the group of Prof. Michael Berridge at the Malaghan Institute of Medical Research.¹⁵² From this work, arabino hexadecylmethamphetamine was identified as the lead compound, and its single step synthesis was optimised allowing for its preparation in 90% yield.

Chapter 5 provides a brief summary of the work, along with future directions for these projects.

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Chapter 2: Applications and Limitations of the I₂-mediated Carbamate Annulation for the Synthesis of Piperidines

2.1 Introduction

2.1.1 Six-membered Ring Iminosugars and the Treatment of Disease

Iminosugars have enormous therapeutic potential in the treatment of a number of diseases such as cancer, diabetes, and lysosomal storage disorders.¹ Notable members of the piperidine iminosugar family include *N*-hydroxyethyl-DNJ (Miglitol **77**), which is approved for the treatment of non-insulin-dependent diabetes,² and 1-deoxygalactonojirimycin (DGJ, **56**), recently used in a Phase 2 clinical trial for the treatment of Fabry's disease (Figure 2.1).³ The corresponding 2-deoxy piperidines, fagomine (**78**), and the unnatural 4-*epi*-fagomine **79** also exhibit promising biological activities. Fagomine isomer **79** selectively inhibits coffee bean α -galactosidase activity with a 50% inhibition concentration (IC₅₀) of 8 μ M.⁴ Fagomine (**78**) was found to lower peak blood glucose levels in a rat model when fed with glucose or starch, without causing insulin release.⁵ A

clinical trial on the effects of fagomine (**78**) on glycaemic responses to sucrose has also recently been completed.⁶

Figure 2.1: Piperidines.

2.1.2 Retrosynthesis

Given the biological activities of piperidines, the application of the PGF pyrrolidine synthesis strategy⁷ to prepare piperidines was of interest.⁸ According to this strategy, the target piperidines **80** could be attained following the hydrolysis of the intermediate cyclic carbamates **81**, themselves prepared via the I₂-mediated cyclisation of alkenylamine precursors **82** (Scheme 2.1). Alkenylamines **82** would in turn be prepared from methyl iodoglycosides **83** via the recently reported protecting-group-free Vasella-reductive amination methodology.⁹ This approach was based on the assumption that when R¹ = OH, alkenylamine **82** should cyclise to form the desired piperidine (*N*-cyclisation) rather than undergoing *O*-cyclisation, which would lead to the undesired aminomethyl furan.¹⁰

Scheme 2.1: Retrosynthetic analysis for the synthesis of piperidines.

2.1.3 Previous Cyclisations from Hexose Scaffolds

1-Deoxygalactonojirimycin (DGJ, **56**) was the first piperidine prepared via the PGF azasugar synthesis methodology (Scheme 2.2).^{11,12} Beginning with the methyl galactoside **84**, iodination of the primary hydroxyl gave **85** in only 49% yield due to the formation of 3,6-anhydro- α -D-galactopyranoside **86** by competing intramolecular cyclisation. Following this, subjection of iodide **85** to the Vasella-reductive amination conditions provided alkenylamine **87** in good (85%) yield. Next, the carbamate annulation gave a 3:1:1 ratio of *cis*-piperidine **88a**, *trans*-piperidine **88b**, and furanose **88c**, respectively. Cyclisation to the piperidine framework was much slower and resulted in lower stereoselectivity compared to the previously reported pyrrolidine annulation methodology.⁷ Hydrolysis of the carbamate and purification by silica gel flash column chromatography allowed for DGJ (**56**) to be prepared in a respectable 17% yield (from **84**).

Scheme 2.2: Synthesis of DGJ (**56**).^{11,12}

Following on from the successful synthesis of DGJ (**56**) from galactose, the preparation of a glucose-derived azasugar, 1,5-dideoxy-1,5-imino-L-idoitol (**89**), was attempted (Scheme 2.3).^{10,12} To this end, methyl glucopyranoside **90** was subjected to triphenylphosphine, imidazole and iodine to give the corresponding iodosugar **91** in 98% yield.⁹ Next, a Vasella-reductive amination led to the smooth formation of alkenylamine **92**, again in excellent (93%) yield and with only minor (<5%) traces of the secondary amine.¹³ Alkenylamine **92** was then treated with I_2 and NaHCO_3 in attempted formation of carbamate **93**, however characterisation suggested formation of either the aminomethyl furan **94** or piperidine **95**. Acetylation of the crude reaction mixture revealed furan **96**, and hence the

product from the I_2 -mediated annulation reaction was assigned as aminomethyl furan **94**. While piperidine **95** could have been hydrolysed to give the desired piperidine **89**, furan **94** indicated a dead-end in the PGF synthesis from glucose. Attempted optimisation to encourage attack of the nitrogen atom during the annulation reaction was unsuccessful with no observable formation of the desired carbamate **93**. The inherent problem of controlling chemoselectivity during protecting-group-free syntheses is well known and explains why the use of protecting groups, though undesirable in terms of atom economy, is still required for many syntheses.

Scheme 2.3: Formation of undesired iodo-furanoside by I_2 -mediated annulation.¹²

2-*O*-cyclisation was observed when the glucose and galactose scaffolds were subjected to the carbamate annulation conditions. As 2-deoxy-D-glucose and 2-deoxy-D-galactose scaffolds are devoid of a hydroxyl functionality at C-2, it was purported that 6-*N* cyclisation would prevail when the corresponding alkenylamines were subjected to the PGF annulation methodology. This would provide access to the fagomine family, six-membered azasugars that retain interesting biological properties without a C-2 hydroxyl group ($R^1 = H$, Scheme 2.1). The synthesis of 4-*epi*-fagomine **79** began with Fischer glycosidation of 2-deoxy-D-galactose (**97**) to give the corresponding methyl glycoside **98** in modest (40%) yield (Scheme 2.4),^{10,14} with the kinetically favoured furan formed as a by-product. Iodination of methyl glycoside **98** gave only bicyclic pyranoside **99**,¹⁵ due to intramolecular cyclisation similar to that observed *en route* to DGJ.¹¹ To avoid this, the installation of an isopropylidene protecting group was required, and concomitant installation of the methyl group gave galactoside **100**. Iodination yielded primary iodide **101**, and removal of the isopropylidene group produced the desired methyl iodoglycoside **102** in 94% yield. Subjection of **102** to the Vasella-reductive amination conditions yielded alkenylamine **103** in excellent (99%) yield. Alkenylamine **103** was then subjected to a carbamate annulation reaction. Again, cyclisation to the six-membered ring proved sluggish with no appreciable product formation after seven days at room temperature. Optimisation of the reaction conditions revealed carbamates **104a** and **104b** formed in a 3:1 ratio when the reaction was carried out at 50 °C over three days. This was improved to give a diastereoselectivity of >95:5 (*i.e.* solely *cis* isomer **104a** by NMR) when the reaction was performed at 35 °C over five days, with the improved diastereoselectivity suggesting kinetic control of the reaction. Under these conditions carbamate **104a** was isolated in 86% yield. To complete the synthesis, carbamate **104a** was then hydrolysed via treatment with NaOH in EtOH at reflux. This resulted in the formation of 4-*epi*-fagomine (**79**) in 92% yield, completing this remarkably efficient six-step synthesis with an overall yield of 52%. The need for a protecting group could not be avoided in this synthesis, because of the inherent reactivity of the *galacto* configuration. The protecting group manipulations added one additional step to the synthesis; however, an expedient

synthesis of 4-*epi*-fagomine (**79**) was still achieved with the best overall yield to date.¹⁶

Scheme 2.4: Synthesis of 4-*epi*-fagomine (**79**).¹⁴

2.2 Application of the PGF Azasugar Methodology to 2-Deoxy Glucose

The PGF strategy for the synthesis of fagomine analogues was applied to 2-deoxy-D-glucose (**105**) for the synthesis of 5-*epi*-fagomine (**108**) (Scheme 2.5). To begin, 2-deoxy-D-glucose (**105**) was converted to methyl glycoside **106** under Fischer glycosidation conditions and then treated with iodine, triphenylphosphine and imidazole at 75 °C for 1.5 h to give primary iodide **107** in good overall yield (64%, 2 steps).^{17,18} Reaction times and temperatures were important to avoid over-iodination or incomplete consumption of starting material. Doubly iodinated glucoside **109** was a key by-product that was observed by TLC. This was further evidenced by high resolution mass spectrometry ($[M+Na]^+ = 420.8773$) in combination with NMR characterisation, where a downfield shift of the H-3 (from δ 3.97 ppm in **107** to δ 4.62 in **109**), and a carbon shift of C-3 from δ 69.1 to δ 33.1 ppm, indicated a different substituent on the 3-position. Over-iodination of 2-deoxy glucose has been observed previously,¹⁷ and as the 3-OH is less sterically hindered than the 4-OH, this is anticipated to be the more reactive centre. While the *galacto*-sugars were seen to undergo intramolecular ring closure under iodination conditions, this was not observed for the *gluco* analogues. This demonstrates how differences in stereochemistry can affect reactivity.

Scheme 2.5: Synthesis of alkenylamine **110** derived from 2-deoxy-D-glucose (**105**).

Iodide **107** was then treated with Zn , $NaCNBH_3$, NH_3 and NH_4OAc according to the Vasella-reductive amination protocol to effect smooth transformation into alkenylamine **110** in good (86%) yield. The mechanism of the Vasella-reductive amination is shown in Scheme 2.6, whereby zinc insertion in the C-I bond then an electron cascade ejecting the methoxide group forms the corresponding aldehyde. The aldehyde then undergoes a reductive amination with ammonia to form the desired primary alkenylamine **110**. The normal method of alkenylamine purification (elution from Dowex- H^+ cation exchange resin using 35% aqueous ammonia) was unsuccessful here due to the strong affinity of alkenylamine **110** for the resin leading to low product recovery. Purification by reverse phase chromatography and dialysis techniques were attempted before a ‘salting out’ work up procedure was developed. This involved transformation of the initial saturated NH_4OAc solution into $NaCl$ through the addition of acid and then base, allowing for removal of large quantities of salt before column purification. Briefly, the crude alkenylamine reaction mixture was concentrated to remove excess ammonia and solvent, then 1.2 equiv. $HCl_{(aq)}$ was added to the remaining salt mixture converting NH_4OAc into NH_4Cl and $AcOH$. Concentration of the

AcOH and excess HCl, followed by addition of 1.0 equiv. of aqueous NaOH allowed conversion of NH_4Cl into $\text{NaCl}_{(\text{s})}$ and $\text{NH}_3_{(\text{aq})}$, which could also be removed *in vacuo*. Due to differences in solubility, alkenylamine **110** could be dissolved in EtOH, while the NaCl was only minimally soluble, hence filtration could be used to remove much of the salt. The remainder of the NaCl could be removed with a short SiO_2 column (eluting in DCM/EtOH/MeOH/35% aqueous NH_3 , 25/2/2/1, v/v/v/v), to provide clean alkenylamine **110**, as a white solid.

Scheme 2.6: Mechanism of the Vasella-reductive amination of iodide **107**.

After the successful purification of alkenylamine **110**, this was then subjected to a variety of annulation conditions (Table 2.1). As previously highlighted, slightly warmer temperatures were required for the annulation of alkenylamine **103**, and because of this, alkenylamine **110** was first treated with I_2 and NaHCO_3 (sat. aq.) at 40 °C (entry 1, Table 2.1). These conditions led to a complex mixture of four carbamate products later identified as pyrrolidine **111**, the two piperidine diastereomers **112a** and **112b**, and iodide **113**, with iodide **113** being the major product in this instance. In an attempt to limit the formation of so many carbamate products, the reaction temperature was lowered and the number of equivalents of NaHCO_3 reduced (entry 2). After stirring the reaction mixture at room

temperature for nine days, however, no product formation was observed. Next, iodine was added in portions over the course of the reaction and this was stirred at room temperature until all starting material was consumed, as gauged by TLC analysis (entry 3). While these conditions invoked annulation reactions, and a slight improvement in the ratio of the piperidine carbamate **112a** was observed, iodide **113** was once again the major product and all four carbamates were still formed. The reaction was then attempted at room temperature again using a saturated solution of NaHCO₃ and I₂ (5 equiv.) added in one portion at the start of the reaction (entry 4). Under these conditions, formation of iodide **113** was reduced and pyrrolidine **111** was formed with modest selectivity in a ratio of 4:2:2:3 (**111:112a:112b:113**). The role of the halide in influencing the ratio of products was then investigated through the use of Br₂ (entry 5), however this proved futile with degradation being observed. Further changes were then made to the number of equivalents of reagents and the temperature at which the reaction was performed, however, in all instances, complex mixtures of products were observed and the best yield of any single product, apart from iodide **113**, was still found to be that of pyrrolidine **111** (as highlighted in entry 4). Though disappointing, pyrrolidine **111** could nevertheless be isolated as a pure compound in 10-30% yield following flash silica gel column chromatography (eluting in 2% MeOH in EtOAc) and reverse phase column chromatography (C8, H₂O). The range of yield reflects the difficulty of this chromatographic separation. The piperidines **112a** and **112b** could not be separated from one another and were isolated in a poor (*ca.* 20%) combined yield.

Table 2.1: Carbamate annulation reaction for alkenylamine **110**

	110	111 112a 112b 113
Entry	Conditions	Products (111 : 112a : 112b : 113) ^a
1	I ₂ (5 equiv.), NaHCO ₃ (sat.), 40 °C, 18 h	3 : 2 : 2 : 8
2	I ₂ (5 equiv.), NaHCO ₃ (1.5 equiv.), r.t., 9 d	Starting material 110
3	I ₂ (3 x 1.5 equiv. added over 22 d), NaHCO ₃ (sat.), r.t.	3 : 2 : 1 : 7
4	I ₂ (5 equiv.), NaHCO ₃ (sat.), r.t., 5 d	4 ^b : 2 : 2 : 3
5	Br ₂ (5 equiv.), NaHCO ₃ (sat.), r.t., 3 d	Decomposition

^aAs determined by ¹H NMR analysis of the crude reaction mixture.

^b**111** was isolated as a pure compound in 10-30% yield following column chromatography.

To confirm the structure of pyrrolidine **111** and alkyl iodide **113**, extensive 1D and 2D NMR spectral analysis was undertaken. For pyrrolidine **111**, a correlation was observed in the HMBC spectrum between H-4 and C-1 that allowed the central 5-membered ring system of **111** to be established. High resolution mass spectrometry gave a mass the same as that of carbamates **112a** and **112b** ([M + Na]⁺ = 196.0579), and a characteristic carbon shift of δ (¹³C) 154.4 ppm confirmed the formation of a carbamate. An HMBC cross-peak was observed

between the carbamate carbonyl and H-1 of pyrrolidine **111**, but overlapping ^1H NMR signals for H-3 and H-6a (δ 4.24 ppm) meant that the observed correlation between H-6a and C=O could not be confirmed. NMR analysis of the acetylated derivative **114** was more beneficial. Analysis of the ^1H NMR spectrum for azasugar **114** (see appendix) reveals baseline impurities, which can be assigned as small amounts of acetylated piperidines **112a** and **112b** or iodide **113**. As the product was of sufficient purity to enable the required characterisation, further purification was unnecessary. In pyrrolidine **114**, downfield shifts of the H-3 and H-5 protons (to δ 5.19 ppm and δ 5.12 ppm respectively) separated these signals from the H-6 proton signals, which did not move significantly. The observed proton shifts indicated that the 3- and 5-positions were acetylated and this was confirmed by the corresponding correlations in the HMBC spectrum. An HMBC cross-peak between the carbamate carbonyl and H-6a in the acetylated adduct **114** supported establishment of the 5/6 ring system of carbamates **111** and **114**. The overlapping proton signals in the NMR for pyrrolidine **111** meant that the configuration of the stereocentres could not be determined unequivocally, however the acetylated derivative **114** allowed determination of the relative configurations of the H-5, H-4 and H-6b stereocentres. Indeed, in pyrrolidine **114**, two large ($J_{4,5} = J_{5,6b} = 9$ Hz) *trans*-diaxial couplings, and the smaller coupling constant between H-5 and H-6a ($J_{5,6a} = 4.7$ Hz), indicated an axial/equatorial relationship between these two protons. As the stereochemistry at C-3 is known from the starting material **110**, NOE correlations were then used to solve the stereochemistry of the remaining centres. NOE correlations were observed between H-5 and H-3, H-3 and H-2a, H-2a and H-1a, and H-6a and H-5 (Figure 2.2), indicating these protons to be on the bottom face of the molecule, while NOE correlations between H-2b and H-4, and H-4 and H-6b identified these protons to be on the opposite face thereby allowing for the assignment of the L-*allo*-stereochemistry. Pyrrolidine **111** was then treated with NaOH in EtOH, which resulted in the smooth hydrolysis of the carbamate providing previously undisclosed trihydroxylated pyrrolidine **115** in 5-15% yield over five steps (Scheme 2.7). The group of Stephen Pyne previously prepared the enantiomer of **115**.¹⁹ Comparison of NMR data showed only minor differences in chemical

shifts, which can be explained by the use of a different NMR solvent, and differences in pH (in our hands, **115** was treated with conc. DCl to ensure the amine was fully protonated). Optical rotation of **115** was measured as $[\alpha]_D^{26} = -1.8$ ($c = 0.41$, MeOH), while the optical rotation of the enantiomer was of the opposite sign, and a slightly larger magnitude, reported as $[\alpha]_D^{22} = +4.7$ ($c = 4.0$, MeOH), which can again be explained by difference in concentration, and protonation state of the two compounds.

Figure 2.2: NOE correlations for acetylated carbamate **114**.

Scheme 2.7: NOE correlations for acetylated carbamate **114** and synthesis of trihydroxylated pyrrolidine **115**.

At this stage, the piperidine carbamates **112a** and **112b** were also hydrolysed to give 5-*epi*-fagomine (**108**) and fagomine (**78**), which were separated by flash silica gel column chromatography (Scheme 2.8). Spectral data was similar to that previously reported,^{16d,20} again with some variation due to slight differences in pH.²¹

Scheme 2.8: Hydrolysis of piperidine carbamates to give fagomine (**78**) and 5-*epi*-fagomine (**108**).

Confirmation of the structure of iodide **113** was more challenging. Mass spectrometric analysis (m/z $[M+H]^+ = 301.9888$) indicated the presence of an iodide in the molecule, and a characteristic ^{13}C NMR chemical shift at δ 6.2 ppm suggested that this belonged to a primary iodide group, and therefore was most likely at the C-6 position. The characteristic ^{13}C NMR peak at δ 154.7 ppm suggested the presence of a carbamate group. No HMBC cross-peak was observed between the carbonyl and H-3, and the correlation between the carbamate and H-1 was difficult to confirm due to overlap in the proton signals of H-1 and H-6b. The acetylated adduct **116** (Figure 2.3, Scheme 2.9) was thus prepared to aid structural elucidation. Upon acetylation, downfield shifts of protons H-4 and H-5 were observed (from overlapping at δ 3.76 ppm, to δ 5.34 and 5.14 ppm, respectively), indicating acetylation of the 4- and 5-OH groups, and confirming the product to be linear rather than cyclic. Proton H-3, which was already at a relatively low field (δ 4.50 ppm), did not move significantly upon acetylation indicating that

there was no free hydroxyl on C-3 that could be acetylated. There was an NH peak in the proton NMR of **116**, predicting that the amine was not acetylated. An HMBC cross-peak observed between the carbamate carbonyl and H-1 explained why the amine group was not acetylated, and confirmed the iodo methylene group to be at the C-6 position. Again no correlation in the HMBC spectrum was observed between the carbamate and H-3, but a weak HMBC cross-peak was observed between the carbamate and H-4, presumably a long-range correlation. When looked at as a whole, this leads to the characterisation of acetylated carbamate **116** and hence iodide **113**.

Figure 2.3: ^1H NMR spectrum of acetylated iodide **116** (CDCl_3 , 500 MHz).

Scheme 2.9: Determination of structure of iodo-carbamate **113** via transformation into alkenylamine **(+)-110**.

As the stereochemistry of compound **113** also needed to be confirmed, it was proposed that the synthesis of the corresponding alkenylamine (Scheme 2.9) would allow for confirmation of the structure of **113**, including the chirality of all stereogenic centres. To this end, iodide **113** was converted to the corresponding acetonide **117**. On the basis of a slightly smaller $\Delta\delta$ for the acetonide methyl group proton signals ($\Delta\delta$ ^1H = 0.039 ppm),²² and diagnostic δ ^{13}C $\approx$ 27 ppm for both methyl groups (δ ^{13}C = 27.0 and δ ^{13}C = 27.6 ppm),²³ the relative stereochemistries of the 4- and 5-substituents was determined as *trans* in the acetonide 5-membered ring. There is less difference in the chemical environment of the acetonide methyl groups when in the *trans* configuration compared with those in the *cis* configuration (Figure 2.4). Therefore in iodide **113**, as drawn in the zig-zag conformation in Scheme 2.9, a *syn* relationship would be expected between the 4- and 5-OHs. Opening of the acetonide, via the oxidative addition of zinc and subsequent reductive ring opening then provided the truncated carbamate **118** in good yield. Finally, hydrolysis of the carbamate in **118** via treatment with NaOH under reflux for 4 h gave the desired alkenylamine **110**. To confirm the methodology, alkenylamine **(+)-110** was also synthesised from the acetylated

adduct **116**, by way of reductive elimination of 5-OAc ($\rightarrow$ **119**) and subsequent carbamate hydrolysis. Comparison of the ^1H NMR and ^{13}C NMR spectra of the synthesised alkenylamine with (+)-**110** revealed identical spectra, and the optical rotation was of the same sign and comparable magnitude ($[\alpha]_{\text{D}}^{27} = +37.5$ ($c = 1.0$, MeOH) and $[\alpha]_{\text{D}}^{27} = +31.8$ ($c = 0.2$, MeOH)), thus establishing the structure of the synthesised alkenylamine to be that of the starting material, (+)-**110**. Using this information and the previously deduced *trans* relationship in acetone **117** the starting iodo-carbamate **113** was thus determined to be 3(*R*), 4(*R*), 5(*R*), as depicted in Scheme 2.9.

Figure 2.4: Characteristic *cis* and *trans* acetone **117** NMR shifts^{22,23} for the determination of relative stereochemistry of iodide **113**.

2.3 Proposed Models to Explain the Formation of Pyrrolidines and Piperidines via the I₂-mediated Carbamate Annulation

Four carbamates were formed in the I₂-mediated cyclisation of the 2-deoxy-glucose derived amine **110**,¹⁰ while only one carbamate was formed when 2-deoxy-galacto alkenylamine **103** was subjected to the optimised carbamate annulation conditions.¹⁴ It is important to propose an explanation for the formation of the different products in the I₂-mediated carbamate annulation of hexenylamines. For clarity of understanding, it is necessary to first explain the mechanism of the I₂-mediated cyclisation in the formation of pyrrolidines, as a similar transition state model has been proposed for the 6-*N*-cyclisations to form piperidines.

The formation of pyrrolidines via the I₂-mediated carbamate annulation has been shown to be highly stereoselective in favour of the 3,4-*cis* pyrrolidine.⁷ Here, the stereochemistry of the new chiral centre is established during the formation of the intermediate iodide **121** from alkenylamine **120** (Figure 2.5A),²⁴ and the preference for the 3,4-*cis* configuration can be explained by considering transition state models proposed by Chamberlin²⁵ and Gouverneur,²⁶ where the selective formation of the 3,4-*cis* pyrrolidine **122** relies on stereodirecting effects of the allylic hydroxyl (Figure 2.5B).^{7b} Assuming the transition state adopts an envelope structure, with the amine nucleophile attacking the alkene-iodine complex through a Bürgi-Dunitz trajectory, then there are two transition states (TSs) that can be adopted: the OH-in-plane transition state (**I**), and the H-in-plane transition state (**II**), whereby the alignment of the allylic OH or H with the plane of the double bond is considered. In the H-in-plane TS (**II**), where the allylic H is in the plane of the double bond, the C-O antibonding (σ^*) and the C=C π orbitals are aligned and electron density is therefore withdrawn from the alkene-iodine complex. This destabilises the H-in-plane TS, and disfavours the formation of the 3,4-*trans* pyrrolidine. Conversely, in the OH-in-plane TS (**I**), the unfavourable C-O σ^* to C=C π orbital overlap is minimised, hence this TS, which leads to the *cis* pyrrolidine, is favoured.^{7b,24}

A.

B.

Figure 2.5: Formation of pyrrolidines via the I_2 -mediated carbamate annulation.^{7c,24} **A.** The stereoselectivity of the reaction is determined by the formation of the intermediate iodo-amine **121**. **B.** Two possible transition states *en route* to the formation of the diastereomeric pyrrolidines: the OH-in-plane TS **I**, which leads to the 3,4-*cis* product, and the H-in-plane TS **II**, which would give the 3,4-*trans* product.

For the formation of piperidines via the I_2 -mediated carbamate annulation, similar OH-in-plane and H-in-plane TS models can be used (Figure 2.6).¹² When applying this model to the formation of six-membered rings, the relative orientation of the I_2 -alkene complex (*i.e.* either pseudo equatorial or pseudo axial) also needs to be considered. Here, it is assumed that the pseudo-axial alkene conformation is less stable due to steric interactions. Thus, for the I_2 -mediated carbamate annulation reaction in the synthesis of piperidines there are four chair-like transition states that need to be considered (**III-VI**, Figure 2.6). Of the two transition states (**III** and **IV**) that lead to the preferred *cis* piperidine (formed during the synthesis of DGJ¹¹ and 4-*epi*-fagomine,¹⁰ see Section 2.1.3), the OH-

in-plane, pseudo-equatorial alkene conformer **III** is likely to be more favourable than the H-in-plane, pseudo-axial alkene conformer **IV**. Similarly, the two *trans* TSs (**V** and **VI**) are likely to be less stable than **III**, with the OH-in-plane TS **V** having a pseudo-axial alkene group, while the H-in-plane conformer **VI** would have unfavourable C-O σ^* to C=C π orbital overlap. It should also be mentioned that when comparing the 5N and 6N cyclisation transition states there is greater overlap between the C-O σ^* to C=C π orbitals in the H-in-plane conformers of the five-membered rings. Thus the *cis* directing effect of the allylic OH has less influence during 6N cyclisations compared to 5N cyclisations. In addition to the above effects, the ring substituents (*e.g.* hydroxyl groups) of the six-membered rings are also expected to influence the selectivity of the cyclisation if the orientation of these substituents results in unfavourable 1,3-diaxial interactions.

Figure 2.6: Six-membered transition states for the I_2 -mediated halocyclisation of amino hexenols in the formation of piperidines.

2.4 Comparison of the Outcome of the 2-Deoxy-Glucose and 2-Deoxy-Galactose derived Alkenylamine Cyclisations using Proposed Transition State Models

As described in Sections 2.1 and 2.2, the use of the I₂-mediated carbamate annulation for the PGF synthesis of piperidines led to a range of outcomes. The PGF synthesis of pyrrolidines proceeds smoothly and with excellent *cis*-selectivity for the carbamate annulation,²⁴ however, this methodology does not always translate readily to the synthesis of piperidines. DGJ (**56**) and 4-*epi*-fagomine (**79**) were formed with good chemo- and stereoselectivity, while attempts at forming other piperidines (*e.g.* 1,5-dideoxy-1,5-imino-L-iditol and 5-*epi*-fagomine) were less successful.

The first factor to consider when applying the carbamate annulation methodology to the synthesis of piperidines is *O*- versus *N*-cyclisation. As illustrated *en route* to the synthesis of DGJ,¹¹ when using D-galactose as a starting material, it was possible to favour formation of the desired piperidine, which is remarkable given the general preference for 5- versus 6-membered ring formation.^{27,28} Conversely, when using D-glucose as a starting material,¹² only the furan was observed.

To prevent formation of the furan as a competing reaction product, the synthesis of piperidines devoid of the hydroxyl functionality at C-2 was undertaken. As illustrated, the chirality of the alkenylamine plays a crucial role in determining the number of products formed during the annulation reaction. The alkenylamine derived from 2-deoxy-D-galactose (**103**) was transformed into *cis* carbamate **104a** with excellent chemo- and diastereoselectivity (>95:5, *cis:trans*). However, subjecting the corresponding 2-deoxy-D-glucose alkenylamine (**110**) to the I₂-mediated carbamate annulation conditions resulted in the formation of four products: pyrrolidine **111**, *cis* and *trans* piperidines **112a** and **112b**, and iodide **113** despite efforts to optimise the reaction.

To explain why the two seemingly similar alkenylamines **103** and **110** led to such different reaction outcomes, the corresponding six-membered iodocyclisation transition states need to be considered (Figures 3.3 and 3.4). For the 2-deoxy-D-galactose series, the four transition states **VII** to **X** are possible (Figure 2.7). Transition state **VII** for the 2-deoxy-D-galactose series depicts the lowest energy conformer that leads to the *cis* product, due to a favourable pseudo-equatorial I₂-alkene configuration and more stable allylic OH-in-plane conformation. In contrast, TS **VIII** could also lead to the major *cis* product, however, this conformation has an unfavourable pseudo-axial I₂-alkene orientation and H-in-plane conformation that increases the energy of TS **VIII** relative to TS **VII**. Of the transition states that lead to the minor *trans* carbamate, it is unclear whether TS **IX**, with an unfavourable pseudo-axial alkene, or TS **X**, with an unfavourable H-in-plane conformation, would have the lower energy TS and hence be responsible for formation of the minor product.

Figure 2.7: Six-membered iodocyclisation transition states for 2-deoxy-D-galactose.

For the 2-deoxy-D-glucose series, the relative axial or equatorial orientation of the hydroxyl substituents also need to be considered. The transition states for the 2-deoxy-D-glucose series **XI** to **XIV** are given in Figure 2.8. In this case, all four transition states involve a combination of favourable and unfavourable

interactions. For the two transition states that lead to the major *cis* carbamate, OH-in-plane TS **XI** has a favourable pseudo-equatorial alkene configuration, but ring hydroxyl substituents in an unfavourable axial orientation, while TS **XII** has a pseudo-axial alkene and H-in-plane conformation, both of which are unfavourable, but equatorial ring hydroxyls. The minor *trans* carbamate could be formed from OH-in-plane TS **XIII**, with three unfavourable axial ring substituents, or the H-in-plane TS **XIV**, which has all equatorial substituents. The presence of fewer unfavourable interactions in TSs **XI** and **XIV** suggest that these are the lower energy transition states for the major and minor products, respectively. The unfavourable axial ring OHs of TS **XI** would increase the overall energy of this transition state making it closer in energy to TS **XIV**.

Figure 2.8: Six-membered iodocyclisation transition states for 2-deoxy-D-glucose.

In summary, for the 2-deoxy-D-galactose series there is one favoured transition state (TS **VII**), which leads to the preferential formation of the *cis*-piperidine carbamate **104a**. For the 2-deoxy-D-glucose series, however, there does not appear to be one iodo-cyclisation transition state of substantially lower energy. Thus a significant yield of numerous products were observed in the carbamate annulation, where products pyrrolidine **110**, *cis* and *trans* piperidines **112a** and **112b**, and iodide **113** were formed in a 4:2:2:3 ratio, respectively.

2.5 Proposed Explanation of the Multiple Products formed in the 2-Deoxy-Glucose PGF Azasugar Synthesis

To explain the formation of multiple products from the treatment of alkenylamine **110** with I_2 and $NaHCO_3$, the following reaction mechanisms were proposed (Scheme 2.10). First, attack of iodine from the top face of the alkenylamine **110** (via **XV**) leads to an intermediate that can be transformed into piperidine **112a**, iodide **113** and pyrrolidine **111**. Here, pathway 'A' involves amine attack on the iodine complex at C-5, followed by the addition of CO_2 (formed via the dissociation of carbonic acid) to give the *cis* carbamate **112a** in accordance with the previously published annulation methodology.²⁴ Iodocarbamate **113**, contains a chiral backbone including a new C-5 stereocenter that was not present in parent alkenylamine **110**. The synthesis of iodocarbamate **113**, is thought to occur via S_N2 attack of water on the I_2 -alkene complex (pathway 'B'), followed by the addition of CO_2 to the linear iodoamine. The carbamate formation in iodide **113** is thought to occur via carbamic acid formation with the addition of a proton from the aqueous solvent. Following this, the nucleophilic attack of the 3-OH is expected to lead to carbamate **113**. There are examples in the literature for the formation of similar carbamic acids,¹² however the formation of a carbamate between an alcohol and amine under comparable conditions is unreported. There is literature precedent for carbamate formation from CO_2 under high pressure and temperature,²⁹ or in the presence of a catalyst,³⁰ however none of these reactions were carried out in water. $NaHCO_3$ is more soluble in water than in other solvents, hence CO_2 would be more readily available for carbamate formation under these conditions. Additionally, iodine may act as a catalyst in this carbamate annulation. The regioselectivity observed in the attack on the I_2 -alkene complex follows Markovnikov's rule, while the stereoselectivity is more difficult to rationalise. The observation of only one iodocarbamate diastereomer, however, suggests that **XV** is lower in energy than **XVI** and hence the intermolecular nucleophilic attack of H_2O on **XV** (pathway B) is more energetically favourable than the equivalent pathway on **XVI**.

Scheme 2.10: Proposed mechanism for the formation of pyrrolidine **111**, piperidines **112a** and **112b**, and iodide **113** following the reaction of alkenylamine **110** to iodine and aqueous NaHCO_3 .

While there is no literature precedent for the selective formation of a linear terminal iodide similar to iodocarbamate **113** through the reaction of iodine with an unsubstituted allylic alcohol, a number of halohydrin reactions have been reported for similar substrates. In these reactions, both the substitution pattern and accessibility of the halide-alkene complex influenced regio-^{31,32} and stereochemical^{33,34} outcomes. In the most closely related system involving halohydrin formation, an allylic alcohol **123** was subjected to iodic acid in acetone and a mixture of the terminal alcohol **124** and terminal iodide **125** were observed as the major and minor products, respectively (Scheme 2.11).³¹ Unfortunately, no stereochemical data was reported for this reaction. In the I_2 -mediated carbamate annulation of alkenylamine **110**, however, there was no indication of products

formed due to the opening of the iodine complex by water at the terminal carbon or from water attack on the diastereomeric iodine complex. It should also be noted that the possibility of **113** being formed via a Payne rearrangement³⁵ of the intermediate epoxide (pathway C) is unlikely as this reaction typically requires strongly basic conditions,³⁶ as illustrated by the conversion of allylic alcohol **126** to allylic epoxide **127** under the mediation of NaOH (Scheme 2.12).³⁷ It is also conceivable that formation of a cyclic carbamate at the 3-position, or the formation of a carbamic acid that ‘protects’ the amine,³⁸ takes place prior to halohydrin formation and this provides an alternative route to **113**. Such a cyclic carbamate intermediate, however, does not add to a conceivable mechanism for the formation of either pyrrolidine **111** or piperidine **112a**.

Scheme 2.11: Halohydrin reaction of a terminal alkene (stereochemistry not given in the literature).³¹

Scheme 2.12: Payne rearrangement under strongly basic conditions.³⁷

To explain the formation of pyrrolidine **111**, a mechanism that supports the inversion of stereochemistry at C-4, and formation of a chiral centre at C5 is required. Accordingly, an iodo-epoxide (pathway ‘C’, Scheme 2.10) is proposed

as a key intermediate.^{39,40} Here, nucleophilic 5-*exo-tet* ring opening⁴¹ of the epoxide, followed by the addition of CO₂ forms carbamate **111**. Finally, the formation of *trans*-piperidine **112b** can be envisioned to occur via the S_N2 attack of the diastereomeric iodine complex (pathway D), whereby **XVI** leads to the conformation of the iodine-alkene complex. Subsequent addition of CO₂ to the intermediate cyclic iodoamine would then give piperidine **112b**. Finally, it should be noted that attack at the 5-position of the intermediate epoxide discussed in pathway C could also lead to *trans* piperidine **112b** through a 6-*endo-trig* cyclisation. According to Baldwin's rules, this is less favored than the corresponding 5-*exo-trig* cyclisation ($\rightarrow$ **112b**),⁴¹ especially in the absence of a bulky group α - to the nitrogen.⁴²

When comparing the proposed mechanism for the formation of carbamates **111** to **113** in Scheme 2.10, with the results obtained from the reaction optimisation (Table 2.1), it is important to note that the mechanism was proposed to explain the products formed under optimal conditions (*i.e.* entry 4, Table 2.1). As a result of this, there is some disconnect between the variation in reaction conditions, and the proposed mechanism. When a reduced amount (1.5 equiv.) of NaHCO₃ was used in the reaction, no product formation was observed, which could suggest that NaHCO₃ (as a source of CO₂) was the limiting reagent. A similar result was observed in the galactose derived carbamate formation, where minimal NaHCO₃ resulted in an incomplete reaction after 7 d.¹² It is surprising that no iodide intermediates (or degradation products as a result of unstable intermediates) were observed under these conditions, given that proposed first step in the mechanism in Scheme 2.10 does not involve CO₂. There are several possible explanations for this observation. As well as being a source of CO₂ in the reaction, NaHCO₃ could also influence the pH of the solution, which could be important in deprotonating alkenylamine **110**, which was added as the HCl salt. It could also be that the ionic strength is important in the reaction, or that the excess NaHCO₃ aids the solubility of the iodine, which is involved in the rate determining step of the carbamate annulation. Another possibility is that the carbamate annulation occurs though an alternative mechanism where CO₂ is involved in the first step. A possible

mechanism for the formation of iodide **113** is displayed in Scheme 2.13. Iodine association with the top face of amine **110**, is followed by the attack of the 3-OH on CO₂ to form a carbonate ester. The subsequent attack of this species at C5 could lead to the formation of a cyclic six-membered carbonate, which is then proposed to undergo amine attack on the carbonyl group leading to the formation of iodo carbamate **113**. However this mechanism is unlikely, as carbonates have never been made by the reaction of alcohols with aqueous NaHCO₃. Carbonates can be prepared from allylic alcohols using CO₂ and a strong base (*e.g.* BuLi)^{43,44} however these reactions would not be possible under aqueous conditions. Additionally Venturello *et al.* described the formation of cyclic carbonates from halohydrins and a Me₄NHCO₃/CO₂ solution in MeCN.⁴⁵ If the PGF carbamate annulation of amine **110** were occurring by this mechanism, the isolation of halohydrin **113** would not be observed. Alternatively, the formation of a piperidine carbonate was recently reported with evidence of aziridine formation, rather than carbonate substitution, as the key cyclisation step,⁴⁶ however the presence of an aziridine intermediate has been ruled out under the conditions used in this study.²⁴

Scheme 2.13: Alternative mechanism for the formation of iodo carbamate **113**.

By proposing reaction mechanisms to account for the formation of products **111**, **112a**, **112b**, and **113**, it may be possible to tailor the starting materials to favour one particular product over the others, and further support the proposed mechanism. Indeed the preparation of a protected version of the hypothesised iodo epoxide intermediate, and successful carbamate annulation from this would support the mechanism. This will be further discussed in Chapter 5 (Future Prospects).

2.6 Wider Relevance: Related 6-*N*-Cyclisations

To control the regioselectivity of cyclisation reactions in the preparation of piperidines, most synthetic strategies involve the use of protected scaffolds, such as amine **128**, to avoid the formation of smaller (*e.g.* five-membered) rings, and rely on the preference for six- over seven-membered rings (*i.e.* **129** vs. **130**) to favour the piperidine scaffold (Scheme 2.14). The control of stereochemistry, however, can be more challenging. While the OH-in-plane vs. H-in-plane transition state model has some success in predicting the stereochemical outcome of the cyclisations, other factors such as across-the-ring *trans* directing effects, choice of the cyclisation mediator, and thermodynamic vs. kinetic reaction control can also influence product formation. These will be highlighted in the following examples.

Scheme 2.14: Protecting group controlled regiochemistry during halocyclisations.

In addition to the successful PGF-synthesis of DGJ and 4-*epi*-fagomine via an I₂-mediated carbamate annulation, there are also a number of other halocyclisations involving the use of PGs whereby the 4,5- (or 5,6-) *cis* product was the major isomer formed (Table 2.2). As summarised in the PGF-synthesis of 4-*epi*-fagomine (Section 2.1.3), 2-deoxy-galactose **103** underwent iodocyclisation and carbamate annulation to selectively give *cis* carbamate **104a** under kinetic conditions (entry 1). The PGF synthesis of *cis* carbamate **88a** *en route* to DGJ was also discussed earlier. Although this iodocyclisation of *galacto* alkenylamine **87**

was less selective, with *trans* piperidine **88b** and amino furan **88c** also observed, the reaction conditions could be optimised so that the *cis* carbamate **88a** was the major product formed (entry 2). NIS has been used to induce cyclisations of protected amino heptenols with high stereocontrol, as illustrated by the 5,6-*cis* selective cyclisation of **131**, **133** and **135** to give the corresponding iodoamines **132**, **134**, and **136**, respectively (entries 3-5). Of particular note is that cyclisation of **133** forms **134** stereoselectively (entry 4) despite the unfavourable 2,6-*cis* relationship across the ring, which, in other instances, has been shown to adversely effect the stereochemical outcome (see Scheme 2.15 and the nearby discussion).

Table 2.2: *Cis* selective 6-*N*-cyclisations

Entry	Alkenylamine	Reaction conditions	Cyclic Product	Ratio: <i>cis:trans</i>	Yield	Ref.
1	<p>103</p>	I ₂ , NaHCO ₃ , 35 °C, H ₂ O, 5 d	<p>104a</p>	>95:5	86%	¹⁰
2	<p>87</p>	I ₂ , NaHCO ₃ , 50 °C, H ₂ O, 2 d	<p>88a</p> <p>88b</p> <p>88c</p>	3:1:1 ^a	60% ^a	¹¹
3	<p>131</p>	NIS, DCM, rt, 3 h	<p>132</p>	>95:5	80%	⁴⁷
4	<p>133</p>	NIS, DCM, rt, 3 h	<p>134</p>	>95:5	90%	⁴⁷
5	<p>135</p>	NIS, DCM, rt, 3 h	<p>136</p>	>95:5	69%	⁴⁷

Entry	Alkenylamine	Reaction conditions	Cyclic Product	Ratio: <i>cis/trans</i>	Yield	Ref.
6	137	i) $\text{Hg}(\text{TFA})_2$, THF ii) NaBH_4 , DMF, O_2	138 + 139	7:1	quant.	⁴⁸
7	140	$\text{Hg}(\text{TFA})_2$	141 + 142	7.5:1	85%	⁴⁹
8	143	$\text{Hg}(\text{TFA})_2$	144	>95:5	76%	⁵⁰
9	145	$\text{Hg}(\text{OAc})_2$	146	>95:5	71%	⁵¹

^a Carbamate products **88a:88b:88c** observed in a 3:1:1 ratio by ^1H NMR spectroscopy. Yield of **88a** was estimated based on ratios observed by ^1H NMR spectroscopy.

$\text{Hg}(\text{II})$ salts have also been shown to give good *cis*-selectivity in the 6-*N*-cyclisation reactions (entries 6-9, Table 2.2). $\text{Hg}(\text{TFA})_2$ was reported to provide the corresponding *cis*-piperidines in high yields and good to excellent selectivity. *Manno* derivative **137** displayed good selectivity, and quantitative yield, when subjected to $\text{Hg}(\text{TFA})_2$ followed by oxidative demercuration, giving a 7:1 ratio of *cis* **138** : *trans* **139** isomers (entry 6). A notable improvement in selectivity was

observed for the cyclisation of *galacto* alkenylamine **140** in entry 7 when kinetic conditions were used instead of thermodynamic conditions (see Scheme 2.19), providing a good selectivity for *cis* piperidine **141** over the *trans* isomer **142**. A CH₂OBn group α to the amine in alkenylamines **143** and **145**, encouraged the selective formation of *cis* piperidines **144** and **146** (entries 8 and 9), respectively. Here the 2,6 *trans* directing effect (due to across-the-ring steric interactions) and the 5,6 *cis* preferences are in agreement, giving excellent selectivity. Comparison of the PGF iodo-cyclisation of *galacto* alkenylamine **87** (entry 2) and the Hg-mediated cyclisation of the corresponding benzyl-protected alkenylamine **140** (entry 7) suggests that the protected derivative is more *cis* selective. The *cis* selectivity of the protected alkenylamine may be due to the steric interactions of the bulky protecting groups increasing the energy of the less favoured (*trans*) transition state. However, it is also possible that the differing cyclisation conditions are responsible for the observed differences in selectivity.

While the above examples illustrate a preference for the 4,5- (or 5,6-) *cis* products during aminocyclisation reactions, there are also examples where mixtures of *cis* and *trans* products are formed. Both the ring substituents and the annulation conditions used have been shown to influence the regio- and stereochemical outcome of annulation reactions. The use of PGs allows for better control of the regiochemistry of the annulation, yet even with PGs, the stereochemical outcome of the reaction is not easy to predict.

An example of a PG synthesis of piperidines leading to the 5,6-*trans* product is the iodocyclisation for the preparation of aza-C-glycosides displayed in Scheme 2.15.⁴⁷ Here, benzyl protected aminoheptenols **131** and **147** were submitted to NIS (1 equiv.) in DCM at rt effecting cyclisation to the corresponding aza-C-glycosides **132** and **148**, respectively, in excellent yield and stereoselectivity. When amine **131** was submitted to the cyclisation conditions, the 5,6-*cis*, 2,6-*trans* piperidine **132** was formed (see entry 2, Table 2.2), however, cyclisation of amine **147** gave the 2,6-*trans*, 5,6-*trans* piperidine **148**. The difference in the stereochemical preference of the two reactions can be explained by the reduction

in 1,3-diaxial interactions when the groups at the 2- and 6-positions adopt a *trans* relationship. The configuration at the 2-position appears to be the major contributing factor when explaining which of the stereoisomers at the 6-position will be preferred. The across-the-ring *trans* directing effect has also been observed during the formation of pyrrolidines with substituents at the 2- and 5-positions,⁵² although in these examples, often mixtures of diastereomers were obtained.

Scheme 2.15: Benzyl protected iodocyclisation giving the 5,6-*trans* product as the major diastereomer.⁴⁷

Examples of *gluco* alkenylamines that were seen to favour the formation of the 4,5-*trans* piperidines have been given in Scheme 2.16. Benzylated alkenylamine **149** was subjected to I₂-mediated annulation conditions to give *cis* and *trans* piperidine iodides **150** and **151**, in a ratio of 35:59, respectively.⁵³ Similarly, Hg-mediated cyclisation of alkenylamine **149** gave *cis* and *trans* bromomercurials **152** and **153**, respectively,⁴⁸ also in *ca.* 1:1.6 ratio. The same ratio of *cis:trans* piperidines **155** and **156** was observed in the Hg mediated annulation of benzyl protected hydroxyethyl amine **154**.⁵⁴ Transition state models similar to those discussed earlier for the cyclisation of the 2-deoxy-D-glucose alkenylamine **110**

(Figure 2.8) can explain the poor selectivity. The position of the electron withdrawing allylic OBn group, and steric interactions of the alkene and OBn ring substituents have similar effects on the transition state energies, thus a mixture of diastereomers results.

Scheme 2.16: Halocyclisations of *gluco* amines that favour *trans* piperidine formation.^{48,53,54}

Another example where the stereochemical outcome of the amino-cyclisation reaction changes depending on the substrate used is the Pd(II) mediated chlorocyclisation of alkenylamines.⁵⁵ Here, the Pd(II) catalysed chlorocyclisation from D-*gluco* amine **157** was remarkably selective, with the 4,5-*cis* azasugar **158** formed as the major product, and only minor amounts of the corresponding diastereomer **159** observed (Scheme 2.17A).⁵⁵ Given the poor selectivity observed for the cyclisation of the *gluco* alkenylamines in Scheme 2.16, the excellent *cis*

selectivity observed here was unforeseen. In contrast, three products were observed in the Pd(II) catalysed cyclisation of galactose-derived alkenylamine **160** (Scheme 2.17B).⁵⁵ The inversion of stereochemistry at C4 enabled the formation of bicycle **161** in addition to the anticipated *cis*- and *trans*-piperidines **162** and **163**, respectively. A variety of reaction conditions were studied in the Pd(II) catalysed cyclisation. Here, AcOH as solvent favoured formation of bicycle **161** (conditions A), while DMF gave the best selectivity for the *trans* piperidine **163** (conditions B). A ¹C₄ chair of the transition state and coordination of Pd to the 3-O-Bn play predominant roles in the formation of the products. In particular, the formation of bicycle **161** has been proposed to occur via the bis-coordination of the alkene and the C-3 benzyl ether to the metal, followed by nucleophilic attack of the amine on the alkene/ π -complex (TS **XVII**, Scheme 2.17C). Reductive elimination in the presence of CuCl₂ then gives bicycle **161**.

Scheme 2.17: Pd(II) catalysed cyclisations. **A:** Cyclisation of the glucose derivative. **B:** Cyclisation of the galactose derivative. **C:** Transition state involved in the formation of bicycle **161**.⁵⁵

When performing a Pd(II) catalysed aminocarbonylation reaction using protected *gluco* alkenylamine **157**, the selectivity under each set of conditions (A or B, Scheme 2.18) differed.^{56,57} *Cis* bicycle **165** was favoured when CuCl₂ was used as the co-oxidant (conditions A), while *trans* piperidine **164** was favoured when LiCl and *p*-benzoquinone were used in place of CuCl₂ (conditions B). A variety of other reaction conditions were tested, however none improved on the yields and selectivities noted for conditions A and B. In all instances a mixture of the *cis* and *trans* bicyclic lactones **164** and **165** were observed, and when CuCl₂ was used as the co-oxidant, *cis*-piperidine **158** was a major by-product. These results are in contrast to the previously mentioned Pd(II) catalysed chlorocyclisation from *gluco* amine **157** (Scheme 2.17A), where only the 5,6-*cis* product was formed. It is unclear why conditions A and B favour different diastereomers, or why the conditions are less selective than the chlorocyclisation in the previous scheme, but this may be related to a change in the mechanism of the reaction when carried out under each set of conditions.

Scheme 2.18: Aminocarbonylation of *gluco* alkenylamine **157**.^{56,57}

Finally, it should also be noted that thermodynamic or kinetic reaction control can sometimes be used to influence the stereoselectivity of an amino cyclisation. For example, Ganem and co-workers observed thermodynamic and kinetic control gave different stereoselectivity of amino mercuration cyclisations (Scheme 2.19).⁴⁹ *Galacto*-configured amine **140** was subjected to mercury-mediated cyclisation under thermodynamic (conditions A) and kinetic control (conditions B). A mixture of products containing 4,5-*cis*-azasugar **141**, the 4,5-*trans*-azasugar

142, and an unidentified by-product were obtained under both sets of conditions, however the kinetic conditions provided *cis*-piperidine **141** preferentially in 75% yield. Amine **141** is likely to be formed through an OBn-in-plane transition state, which is anticipated to be the lower energy transition state, and hence gives rise to the kinetic product. Under thermodynamic conditions, a 1:1 ratio of the *cis:trans* isomers **141:142** is observed, indicating that the energies of the products are similar.

Scheme 2.19: Thermodynamic (A) vs. kinetic (B) control in Hg-mediated cyclisations.⁴⁹

These literature examples of 6-*N*-halocyclisations show that the selectivity of these reactions varies depending on the reaction conditions used and the stereochemistry of the starting material. This indicates that it is difficult to predict the selectivity of such a reaction, and helps to explain the formation of multiple products in the PGF halocyclisations. The observation of consistently poor selectivity in *gluco* derived alkenylamines further supports the observation of reduced selectivity (*i.e.* the formation of four carbamate products) in the carbamate annulation of the 2-deoxy-glucose analogue.

2.7 Conclusion

The PGF azasugar methodology has been applied to the 2-deoxyglucose scaffold. The key cyclisation step, the I₂-mediated carbamate annulation, resulted in a series of competing intramolecular reactions that produced four carbamates. A variety of synthetic and spectroscopic techniques were used to identify the carbamates as: the anticipated *cis* piperidine **112a**, the corresponding *trans* isomer **112b**, pyrrolidine **111**, and iodide **113**. To this end, previously undisclosed enantiomer of trihydroxypyrrolidine **115** was prepared in five steps and modest (up to 15%) overall yield, using a completely PGF strategy. These studies highlight the limitations of using the carbamate annulation for the preparation of piperidines in protecting-group-free syntheses, although the work also points to the potential of this methodology for the synthesis of interesting chiral scaffolds when using either protected or suitably functionalised alkenylamines as starting materials.

2.8 Experimental

General Experimental

Unless otherwise stated, all reactions were performed under atmospheric air. THF (Lab-Scan) was distilled from LiAlH_4 prior to use. Pyridine was distilled over KOH prior to use. H_2O , MeOH (Pure Science), and EtOH (absolute, Pure Science), were distilled prior to use. EtOAc (Pure Science), and petroleum ether (Pure Science), HCl (PanReac), AcOH (Ajax Finechem), DCM (LabServ), 35% aqueous NH_3 (J. T. Baker Chemical Co.), NaHCO_3 (PureScience), 2-deoxy-D-glucose (Sigma-Aldrich), AcCl (B&M), PPh_3 (Merck), imidazole (Aldrich), I_2 (BDH), NaCNBH_3 (Aldrich), NaOH (Pure Science), and NH_4OAc (AnalaR), Ac_2O (Acros) were used as received. All chemicals obtained from commercial suppliers were used without further purification. Zn dust was activated by the careful addition of concentrated H_2SO_4 , followed by decantation and washing with EtOH (3 $\times$) and hexanes (3 $\times$) and storage under dry hexanes. All solvents were removed by evaporation under reduced pressure. Reactions were monitored by TLC analysis on Macherey-Nagel silica gel coated plastic sheets 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 Pure Science silica gel (40-63 micron). Dowex® W50-X8 acidic resin (Sigma) and Dowex® 1X4-50 basic resin (Sigma) were used for ion exchange chromatography and HP-20 (Supelco) for reverse phase chromatography. High-resolution mass spectra were recorded on a Waters Q-TOF Premier™ Tandem Mass Spectrometer using positive electrospray ionisation. Optical rotations were recorded using an Autopol II (Rudolf Research Analytical) at 589 nm (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 at 20 °C in D_2O , CD_3OD , or CDCl_3 using either a Varian Unity-INOVA operating at 300

MHz or a Varian Unity operating at 500 MHz. Chemical shifts are given in ppm (δ) and were internally referenced to the residual solvent peak. NMR peak assignments are based on 2D NMR experiments (COSY, HSQC, and HMBC).

Methyl 2-deoxy- α/β -D-glucopyranoside (106): To a solution of AcCl (240 μ L) in MeOH (240 mL), was added 2-deoxy-D-glucose (**105**) (1200 mg, 7.3 mmol) and the reaction was stirred at room temperature for 18 h. The reaction was neutralised by the addition of Dowex-OH⁻, filtered and concentrated. The resulting oil was used without further purification. The methyl 2-deoxy- α/β -D-glucopyranoside (**106**) was obtained as a colourless oil in an 8:1 α/β ratio. **α isomer:** R_f = 0.27 (10% MeOH in EtOAc); ¹H NMR (600 MHz, D₂O) δ 4.86 (d, $J_{1,2b}$ = 3.5 Hz, 1H, H-1), 3.83 (dd, $J_{5,6a}$ = 2.1, $J_{6a,6b}$ = 12.0 Hz, 1H, H-6a), 3.79-3.87 (m, 1H, H-3), 3.73 (dd, $J_{5,6b}$ = 5.5, $J_{6a,6b}$ = 12.0 Hz, 1H, H-6b), 3.56 (ddd, $J_{5,6a}$ = 2.1, $J_{5,6b}$ = 5.5, $J_{4,5}$ = 9.6 Hz, 1H, H-5), 3.32 (s, 3H, OMe), 3.31 (m, 1H, H-4), 2.10 (dd, $J_{2a,3}$ = 5.3, $J_{2a,2b}$ = 13.5 Hz, 1H, H-2a), 1.67 (ddd, $J_{1,2b}$ = 3.5, $J_{2b,3}$ = 12.0, $J_{2a,2b}$ = 13.5 Hz, 1H, H-2b); ¹³C NMR (150 MHz, D₂O) δ 98.1 (C1), 71.9 (C5), 70.8 (C4), 68.0 (C3), 60.5 (C6), 54.2 (OMe), 36.4 (C2); HRMS(ESI) m/z calcd. for [C₇H₁₄O₅+Na]⁺: 201.0733, obsd.: 201.0735. **β isomer:** R_f = 0.27 (10% MeOH in EtOAc); ¹H NMR (600 MHz, D₂O) δ 4.59 (dd, $J_{1,2a}$ = 1.8, $J_{1,2b}$ = 9.9 Hz, 1H, H-1), 3.89 (dd, $J_{5,6a}$ = 2.0, $J_{6a,6b}$ = 12.0 Hz, 1H, H-6a), 3.65-3.72 (m, 2H, H3 and H-6b), 3.48 (s, 3H, OMe), 3.32 (m, 1H, H-5), 3.21 (t, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, 1H, H-4), 2.21 (ddd, $J_{1,2a}$ = 1.8, $J_{2a,3}$ = 5.1, $J_{2a,2b}$ = 12.9 Hz, 1H, H-2a), 1.43 (ddd, $J_{1,2b}$ = 9.9, $J_{2b,3}$ = 12.0, $J_{2a,2b}$ = 12.9 Hz, 1H, H-2b); ¹³C NMR (150 MHz, D₂O) δ 100.6 (C1), 75.9 (C5), 70.9 (C4), 70.2 (C3), 60.8 (C6), 56.4 (OMe), 38.0 (C2); HRMS(ESI) m/z calcd. for [C₇H₁₄O₅+Na]⁺: 201.0733, obsd.: 201.0735.

Methyl 2,6-dideoxy-6-iodo- α -D-glucoside (107): To a solution of methyl 2-deoxy-D-glucoside (70.1 mg, 0.4 mmol) in dry THF (4 mL) under an atmosphere of argon, was added imidazole (81.6 mg, 1.2 mmol), PPh_3 (157 mg, 0.6 mmol), and I_2 (152 mg, 0.6 mmol). The reaction was heated to 75°C for 1.5 h, then cooled and concentrated. The product was purified by silica gradient flash column chromatography (Petroleum Ether/EtOAc, 2/1, v/v), then reverse phase HP20 chromatography (MeOH/water, 1/5, v/v) to give the methyl 2,6-dideoxy-6-iodo- α -D-glucopyranoside (**107**) as a colourless oil (74.1 mg, 0.26 mmol, 64% over 2 steps from 2-deoxy glucose **105**). $R_f = 0.42$ (1% MeOH in EtOAc); $[\alpha]_D^{23} = +84.0$ ($c = 1.0$, CHCl_3) (lit $[\alpha]_D^{27} = +97$, $c = 0.9$ in chloroform⁵⁸); IR (film) 3387, 2934, 1442, 1377, 1211, 1128, 1044, 966, 938 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 4.83 (d, $J_{1,2b} = 3.4$ Hz, 1H, H-1), 3.97 (ddd, $J_{2a,3} = 5.1$, $J_{3,4} = 8.7$, $J_{2b,3} = 11.7$ Hz, 1H, H-3), 3.57-3.62 (m, 1H, H-6a), 3.40 (s, 3H, OMe), 3.35-3.41 (m, 2H, H-5 and H-6b), 3.24-3.30 (m, 1H, H-4), 2.16 (ddd, $J_{1,2a} = 1.0$, $J_{2a,3} = 5.1$, $J_{2a,2b} = 12.9$ Hz, 1H, H-2a), 1.71 (ddd, $J_{1,2b} = 3.2$, $J_{2b,3} = 11.7$, $J_{2a,2b} = 12.9$ Hz, 1H, H-2b); ^{13}C NMR (125 MHz, CDCl_3) δ 98.7 (C1), 77.6 (C4), 70.4 (C5), 69.1 (C3), 55.3 (OMe), 37.8 (C2), 7.9 (C6); HRMS(ESI) m/z calcd. for $[\text{C}_7\text{H}_{13}\text{O}_4\text{I}+\text{Na}]^+$: 310.9751, obsd.: 310.9751.

Methyl 2,3,6-trideoxy-3,6-di-iodo- α -D-glucoside (109): $R_f = 0.76$ (10% MeOH in EtOAc); ^1H NMR (500 MHz, CDCl_3) δ 4.87 (d, $J = 4.1$ Hz, 1H, H-1), 4.62 (dd, $J = 4.0$, $J = 8.0$ Hz, 1H, H-3), 3.66-3.51 (m, 2H, H-5 and H-6a), 3.44-3.34 (m, 4H, H-6b and OMe), 2.66-2.41 (m, 3H, H-2a, H-2b, and H-4); ^{13}C NMR (125 MHz, CDCl_3) δ 97.6 (C1), 70.8 (C4), 70.3 (C5), 55.4 (OMe), 37.4 (C2), 33.1 (C3) 7.2 (C6); HRMS(ESI) m/z calcd. for $[\text{C}_7\text{H}_{12}\text{O}_3\text{I}_2+\text{Na}]^+$: 420.8768, obsd.: 420.8773.

(3*R*,4*R*)-1-Amino-hex-5-ene-3,4-diol hydrochloride (110**):**

To a solution of methyl 2,6-dideoxy-6-iodo- α -D-glucoside (**107**) (111 mg, 0.39 mmol) in a saturated solution of NH_4OAc in EtOH (7.3 mL) was added activated Zn (450 mg, 6.9 mmol), NaCNBH_3 (85 mg, 1.3 mmol) and 30% aqueous NH_3 (2.9 mL). The mixture was stirred at reflux for 4.5 h, cooled to room temperature, filtered through celite and concentrated under reduced pressure. 30 mL HCl (2 M) was added and AcOH was removed by evaporation. Coevaporation with water/EtOH was performed to remove traces of AcOH, before an aqueous solution of NaOH (40 mL, 1.25 M) was added and the NH_3 was evaporated. The resulting solid was filtered and washed with EtOH, to remove the bulk of the insoluble NaCl salt, and the filtrate and combined washings were concentrated before dry loading on silica for column chromatography (DCM/EtOH/MeOH/35% aqueous NH_3 , 55/2/2/1 to 5/2/2/1, v/v/v/v). Alkenylamine **110**, was obtained as the HCl salt after the addition of HCl (1.2M, aq) and concentration to obtain a white solid (55.5 mg, 0.33 mmol, 86%). R_f = 0.16 (DCM/EtOH/MeOH/35% aqueous NH_3 , 5/2/2/1, v/v/v/v); $[\alpha]_D^{27} = +37.5$ (c = 1.0, MeOH); IR (film) 3336, 2924, 1621, 1505, 1468, 1396, 1312, 1255, 1122, 1020, 997, 931, 854 cm^{-1} ; ^1H NMR (500 MHz, D_2O) δ 5.87 (ddd, $J_{4,5} = 6.7$, $J_{5,6\text{-cis}} = 10.5$, $J_{5,6\text{-trans}} = 17.3$ Hz, 1H, H-5), 5.34 (d, $J_{5,6\text{-trans}} = 17.3$ Hz, 1H, H-6-*trans*), 5.28 (d, $J_{5,6\text{-cis}} = 10.5$ Hz, 1H, H-6-*cis*), 4.03 (dd, $J_{4,5} = 6.7$, $J_{3,4} = 6.8$ Hz, 1H, H-4), 3.70 (ddd, $J_{2a,3} = 2.9$, $J_{2b,3} = 5.4$, $J_{3,4} = 6.8$ Hz, 1H, H-3), 3.13 (m, 2H, H-1), 1.89 (m, 1H, H-2a), 1.77 (m, 1H, H-2b); ^{13}C NMR (125 MHz, D_2O) δ 136.1 (C5), 118.1 (C6), 75.4 (C4), 71.7 (C3), 37.2 (C1), 29.4 (C2); HRMS(ESI) m/z calcd. for $[\text{C}_6\text{H}_{13}\text{O}_2\text{N}+\text{H}]^+$: 132.1019, obsd.: 132.1031.

Iodo-cyclisation/carbamate annulation: To a solution of alkenylamine hydrochloride **110** (240 mg, 1.4 mmol) in saturated aqueous NaHCO_3 (7.2 mL) (made fresh immediately prior to use) was added I_2 (1900 mg, 7.5 mmol) and NaHCO_3 (1400 mg, 17 mmol). The solution was stirred at room temperature for 5 days, filtered, and concentrated under reduced pressure. The products were

separated by repeated silica plug (0-10% MeOH in EtOAc, v/v), and reverse phase columns (octyl bonded endcapped silica beads) eluting in H₂O (for carbamates **111**, **112a** and **112b**) and MeOH (for iodide **113**), all as white solids. Yield of **111** = 10 – 30%.

1-N,6-O-Carbonyl-1,2,4-trideoxy-1,4-imino-L-allitol (111):

$[\alpha]_D^{25} = -0.5$ ($c = 0.4$, MeOH); IR (film) 3390, 3331, 2965, 2914, 1719, 1634, 1517, 1477, 1455, 1133, 1110, 1070 cm^{-1} ; ^1H NMR (500 MHz, D₂O) δ 4.19-4.27 (m, 2H, H-3, H-6a), 3.94-4.03 (m, 2H, H-5, H-6b), 3.47 (dd, $J_{1,2a} = 6.1$, $J_{1,2b} = 9.0$ Hz, 2H, H-1), 3.28 (t, $J_{4,5} = J_{3,4} = 7.7$ Hz, 1H, H-4), 2.24 (dq, $J_{1,2a} = J_{2a,3} = 6.1$, $J_{2a,2b} = 12.6$ Hz, 1H, H-2a), 1.80 (dq, $J_{1,2b} = J_{2b,3} = 9.0$, $J_{2a,2b} = 12.6$ Hz, 1H, H-2b); ^{13}C NMR (150 MHz, D₂O) δ 154.4 (C=O), 74.0 (C3), 69.2 (C6), 64.8 (C4), 64.2 (C5), 44.2 (C1), 30.2 (C2); HRMS(ESI) m/z calcd. for $[\text{C}_7\text{H}_{11}\text{O}_4\text{N}+\text{Na}]^+$: 196.0580, obsd.: 196.0579.

1-N,6-O-Carbonyl-5-*epi*-fagomine (112a) and 1-N,6-O-Carbonyl-fagomine (112b): IR (film) 3409, 2948, 2840, 1632, 1462, 1403, 1092, 1010 cm^{-1} . **Major isomer (112a):** ^1H NMR (500 MHz, D₂O) δ 4.53 (t, $J_{5,6a} = J_{6a,6b} = 9.0$ Hz, 1H, H-6a), 4.34 (dd, $J_{5,6b} = 6.0$, $J_{6a,6b} = 9.0$ Hz, 1H, H-6b), 4.23 (ddd, $J_{4,5} = 2.5$, $J_{5,6b} = 6.0$, $J_{5,6a} = 9.0$ Hz, 1H, H-5), 4.06 (dd, $J_{3,4} = 3.2$, $J_{2a,3} = 6.3$ Hz, 1H, H-3), 3.73 (m, 1H, H-4), 3.56 (dd, $J_{1a,2a} = 6.3$, $J_{1a,1b} = 13.5$ Hz, 1H, H-1a), 3.23 (td, $J_{1b,2a} = 3.7$, $J_{1a,1b} = J_{1b,2b} = 13.5$ Hz, 1H, H-1b), 1.95-2.02 (m, 1H, H-2a), 1.64 (d, $J_{2a,2b} = 15.0$ Hz, 1H, H-2b); ^{13}C NMR (125 MHz, D₂O) δ 159.2 (C=O), 66.5 (C3), 66.5 (C4), 64.1 (C6), 53.2 (C5), 35.2 (C1), 24.4 (C2); HRMS(ESI) m/z calcd. for $[\text{C}_7\text{H}_{11}\text{O}_4\text{N}+\text{Na}]^+$: 196.0586, obsd.: 196.0587. **Minor isomer (112b):** ^1H NMR (500 MHz, D₂O) δ 4.53 (t, $J_{5,6a} = J_{6a,6b} = 9.0$ Hz, 1H, H-6a), 4.31 (dd,

$J_{5,6b} = 5.0$, $J_{6a,6b} = 9.0$ Hz, 1H, H-6b), 3.68-3.71 (m, 1H, H-1a), 3.67 (ddd, $J_{5,6b} = 5.0$, $J_{5,6a} = 9.0$, $J_{4,5} = 9.5$ Hz, 1H, H-5), 3.62 (ddd, $J_{2b,3} = 5.0$, $J_{3,4} = 9.5$, $J_{2a,3} = 11.5$ Hz, 1H, H-3), 3.34 (t, $J_{3,4} = J_{4,5} = 9.4$ Hz, 1H, H-4), 3.03 (td, $J_{1b,2a} = 3.2$, $J_{1a,1b} = J_{1b,2b} = 13.0$ Hz, 1H, H-1b), 1.92-1.98 (m, 1H, H-2a), 1.51 (ddt, $J_{2b,3} = 5.0$, $J_{1a,2b} = 11.7$, $J_{1b,2b} = J_{2a,2b} = 13.0$ Hz, 1H, H-2b); ^{13}C NMR (125 MHz, D_2O) δ 158.7 (C=O), 74.5 (C4), 71.4 (C3), 66.5 (C6), 57.6 (C5), 38.7 (C1), 30.8 (C2); HRMS(ESI) m/z calcd. for $[\text{C}_7\text{H}_{11}\text{O}_4\text{N}+\text{Na}]^+$: 196.0580, obsd.: 196.0587.

1-Amino-1-N,3-O-carbonyl-1,2,6-trideoxy-6-iodo-L-gulitol

(113): $R_f = 0.26$ (10% MeOH in EtOAc); $[\alpha]_D^{26} = -8.9$ ($c = 0.67$, MeOH); IR (film) 3367, 2928, 1680, 1488, 1457, 1300, 1223, 1108, 1021, 523 cm^{-1} ; ^1H NMR (500 MHz, D_2O) δ 4.50 (dt, $J = 3.3$, $J = 9.9$ Hz, 1H, H-3), 3.72-3.81 (m, 2H, H-4 and H-5), 3.44 (dd, $J = 3.9$, $J = 10.5$ Hz, 1H, H-6a), 3.29-3.37 (m, 3H, H-1ab and H-6b), 1.90-2.01 (m, 2H, H-2ab); ^{13}C NMR (125 MHz, D_2O) δ 154.7 (C=O), 76.1 (C3), 71.8, 68.1 (C4, C5), 35.8 (C1), 19.6 (C2), 6.2 (C6); HRMS(ESI) m/z calcd. for $[\text{C}_7\text{H}_{12}\text{O}_4\text{NI}+\text{H}]^+$: 301.9884, obsd.: 301.9888.

3,5-Di-O-acetyl-1-N,6-O-carbonyl-1,2,4-trideoxy-1,4-imino-L-

allitol (114): Carbamate **111** (124.7 mg, 0.7 mmol) was subjected to dry pyridine (1.0 mL) and acetic anhydride (1.0 mL) at rt, under an argon atmosphere, overnight (14 h). After concentration, the crude compound was dry-loaded on silica before gradient column chromatography (Petroleum Ether to Petroleum Ether/EtOAc, 1/1, v/v). Acetylated carbamate **114** was recovered as a white solid. $R_f = 0.44$ (5% MeOH in EtOAc); $[\alpha]_D^{27} = +19.9$ ($c = 0.1$, MeOH); IR (film) 2957, 2925, 1741, 1711, 1430, 1367, 1238, 1070, 754 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 5.16 (dt, $J_{3,4} = 6.5$, $J_{2a,3} = J_{2b,3} = 7.5$ Hz, 1H, H-3), 5.06 (td, $J_{5,6a} = 4.9$, $J_{4,5} = J_{5,6b}$

= 9.0 Hz, 1H, H-5), 4.32 (dd, $J_{5,6a} = 4.9$, $J_{6a,6b} = 11.0$ Hz, 1H, H-6a), 4.05 (dd, $J_{5,6b} = 9.0$, $J_{6a,6b} = 11.0$ Hz, 1H, H-6b), 3.79 (dt, $J_{1a,2a} = J_{1a,2b} = 7.5$, $J_{1a,1b} = 11.0$ Hz, 1H, H-1a), 3.50-3.57 (m, 2H, H-1b and H-4), 2.32 (dt, $J_{1a,2a} = J_{1b,2a} = J_{2a,3} = 7.5$, $J_{2a,2b} = 14.5$ Hz, 1H, H-2a), 2.11 (s, 3H, Me), 2.08 (s, 3H, Me), 1.88 (dt, $J_{1a,2b} = J_{1b,2b} = J_{2b,3} = 7.5$, $J_{2a,2b} = 14.5$ Hz, 1H, H-2b); ^1H NMR (300 MHz, CD_3OD) δ 5.19 (dd, $J_{3,4} = 7.2$, $J_{2b,3} = 7.9$ Hz, 1H, H-3), 5.12 (dd, $J_{5,6a} = 4.7$, $J_{4,5} = J_{5,6b} = 8.7$ Hz, 1H, H-5), 4.32 (dd, $J_{5,6a} = 4.7$, $J_{6a,6b} = 10.8$ Hz, 1H, H-6a), 4.11 (dd, $J_{5,6b} = 8.7$, $J_{6a,6b} = 10.8$ Hz, 1H, H-6b), 3.66 (dt, $J_{1a,2a} = J_{1a,2b} = 8.0$, $J_{1a,1b} = 11.1$ Hz, 1H, H-1a), 3.62 (dd, $J_{3,4} = 7.2$, $J_{4,5} = 8.7$ Hz, 1H, H-4), 3.49 (ddd, $J_{1b,2b} = 4.7$, $J_{1b,2a} = 9.1$, $J_{1a,1b} = 11.1$ Hz, 1H, H-1b), 2.34 (dddd, $J_{1b,2b} = 4.7$, $J_{2b,3} = 7.9$, $J_{1a,2b} = 8.0$, $J_{2a,2b} = 12.9$ Hz, 1H, H-2a), 2.07 (s, 3H, Me), 2.05 (s, 3H, Me), 1.88 (ddt, $J_{1a,2b} = J_{2b,3} = 7.9$, $J_{1b,2b} = 9.1$, $J_{2a,2b} = 12.9$ Hz, 1H, H-2b); ^{13}C NMR (125 MHz, CDCl_3) δ 170.1 (C=O, Ac), 169.9 (C=O, Ac), 151.6 (C=O), 75.2 (C3), 66.7 (C6), 65.7 (C5), 63.2 (C4), 44.9 (C1), 28.8 (C2), 20.9 (CH_3 , Ac), 20.8 (CH_3 , Ac); HRMS(ESI) m/z calcd. for $[\text{C}_{11}\text{H}_{15}\text{O}_6\text{N}+\text{Na}]^+$: 280.0792, obsd.: 280.0791.

1,2,4-Trideoxy-1,4-imino-L-allitol (115): Carbamate **111** (10.6 mg, 0.061 mmol) was dissolved in EtOH (1 mL) and NaOH (26 mg, 0.6 mmol) was added. The solution was refluxed for 2 h, then neutralised with aqueous HCl (1 M) before loading on silica for chromatography (DCM/EtOH/MeOH/35% aqueous NH_3 , 55/2/2/1 to 15/2/2/1, v/v/v/v). The product **115** was obtained as a white solid (8.0 mg, 0.054 mmol, 89%). $R_f = 0.19$ (DCM/EtOH/MeOH/35% aqueous NH_3 , 5/2/2/1, v/v/v/v); $[\alpha]_{\text{D}}^{26} = -1.8$ ($c = 0.41$, MeOH) (lit (**115-enantiomer**) = $[\alpha]_{\text{D}}^{22} = +4.7$, $c = 4.0$, MeOH¹⁹); IR (film) 3309, 2956, 2927, 1629, 1420, 1089, 1041 cm^{-1} ; ^1H NMR (500 MHz, D_2O) δ 4.33 (dt, $J_{2b,3} = J_{3,4} = 4.3$, $J_{2a,3} = 6.2$ Hz, 1H, H-3), 3.78 (dd, $J_{4,5} = 4.3$, $J_{5,6} = 10.3$ Hz, 1H, H-5), 3.44-3.52 (m, 2H, H-6), 3.35 (t, $J_{3,4} = J_{4,5} = 4.3$ Hz, 1H, H-4), 3.21 (dd, $J_{1,2b} = 6.3$, $J_{1,2a} = 8.1$ Hz, 2H, H-1), 2.02 (ddd, $J_{2a,3} = 6.2$, $J_{1,2a} = 8.1$, $J_{2a,2b} = 14.2$ Hz, 1H, H-2a), 1.78 (ddd, $J_{2b,3} = 4.3$, $J_{1,2b} = 6.3$, $J_{2a,2b} = 14.2$ Hz, 1H, H-2b); ^{13}C NMR (125 MHz,

D₂O) δ 69.4 (C3), 68.4 (C5), 67.1 (C4), 62.5 (C6), 44.1 (C1), 32.6 (C2); HRMS(ESI) m/z calcd. for [C₆H₁₃O₃N+H]⁺: 148.0968, obsd.: 148.0978.

5-*epi*-Fagomine (108) and Fagomine (78): Carbamates **112a** and **112b** (11.7 mg, 0.07 mmol) were dissolved in EtOH (1 mL) and NaOH (80 mg, 2 mmol) was added. The solution was refluxed for 2 h, then neutralised with aqueous HCl (1 M), followed by concentration *in vacuo*. Purification by gradient silica column chromatography (DCM/EtOH/MeOH/35% aqueous NH₃, 45/2/2/1 to 15/2/2/1, v/v/v/v) gave the products **78** and **108** as a white solid and a mixture of diastereomers (12.4 mg, 0.07 mmol, quant.). **5-*epi*-Fagomine (108):** R_f = 0.24 (DCM/EtOH/MeOH/35% aqueous NH₃, 5/2/2/1, v/v/v/v); $[\alpha]_D^{29}$ = -8.9 (c = 0.63, MeOH) (lit = $[\alpha]_D$ = -11.6, c = 0.94, H₂O^{16d}); IR (film) 3364, 2970, 1425, 1366, 1217, 1079 cm⁻¹; ¹H NMR (500 MHz, D₂O) δ 4.02 (m, 1H, H-3), 3.93 (m, 1H, H-4), 3.85 (dd, $J_{5,6a}$ = 4.9, $J_{6a,6b}$ = 12.0 Hz, 1H, H-6a), 3.80 (dd, $J_{5,6b}$ = 8.5, $J_{6a,6b}$ = 12.0 Hz, 1H, H-6b), 3.54 (dd, $J_{5,6a}$ = 4.9, $J_{5,6b}$ = 8.5 Hz, 1H, H-5), 3.20-3.31 (m, 2H, H-1ab), 2.16-2.24 (m, 1H, H-2a), 1.84 (d, $J_{2a,2b}$ = 14.5 Hz, 1H, H-2b); ¹³C NMR (125 MHz, D₂O) δ 65.8 (C4), 64.8 (C3), 59.5 (C6), 55.8 (C5), 38.9 (C1), 23.6 (C2); HRMS(ESI) m/z calcd. for [C₆H₁₃O₃N+H]⁺: 148.0974, obsd.: 148.0968. **Fagomine (78):** R_f = 0.33 (DCM/EtOH/MeOH/35% aqueous NH₃, 5/2/2/1, v/v/v/v); $[\alpha]_D^{29}$ = +8.4 (c = 0.27, MeOH) (lit = $[\alpha]_D$ = +12.2, c = 0.33, MeOH^{16d}); IR (film) 3367, 2930, 1461, 1271, 1073 cm⁻¹; ¹H NMR (500 MHz, D₂O) δ 3.93 (dd, $J_{5,6a}$ = 3.2, $J_{6a,6b}$ = 12.5 Hz, 1H, H-6a), 3.88 (dd, $J_{5,6b}$ = 5.5, $J_{6a,6b}$ = 12.5 Hz, 1H, H-6b), 3.71 (ddd, $J_{2a,3}$ = 4.9, $J_{3,4}$ = 9.0, $J_{2b,3}$ = 11.7 Hz, 1H, H-3), 3.52 (t, $J_{3,4}$ = $J_{4,5}$ = 9.0 Hz, 1H, H-4), 3.44 (ddd, $J_{1a,2a}$ = 2.5, $J_{1a,2b}$ = 4.1, $J_{1a,1b}$ = 13.5 Hz, 1H, H-1a), 3.12-3.16 (m, 1H, H-5), 3.10 (dd, $J_{1b,2a}$ = 2.5, $J_{1b,2b}$ = $J_{1a,1b}$ = 13.5 Hz, 1H, H-1b), 2.21 (ddd, J = 2.5, $J_{2a,3}$ = 4.9, $J_{2a,2b}$ = 13.5 Hz, 1H, H-2a), 1.72 (ddt, $J_{1a,2b}$ = 4.1, $J_{2b,3}$ = 11.7, $J_{1a,2b}$ = $J_{2a,2b}$ = 13.5 Hz, 1H, H-2b); ¹³C NMR (125 MHz, D₂O) δ 70.4 (C3), 69.5 (C4), 60.0 (C5), 57.6 (C6), 41.8 (C1), 28.5 (C2); HRMS(ESI) m/z calcd. for [C₆H₁₃O₃N+H]⁺: 148.0968, obsd.: 148.0968.

4,5-Di-O-acetyl-1-amino-1-N,3-O-carbonyl-1,2,6-trideoxy-6-

iodo-L-gulitol (116): Iodide **113** (20.4 mg, 0.07 mmol) was subjected to dry pyridine (0.5 mL) and acetic anhydride (0.5 mL) at room temperature under an argon atmosphere overnight (9 h). After concentration and repeated coevaporation with toluene to remove pyridine, the crude compound was dry-loaded on silica before gradient column chromatography (Petroleum Ether to Petroleum Ether/EtOAc, 1/1, v/v). Acetylated iodide **116** was recovered as a white solid in 48% yield (12.5 mg, 0.03 mmol). $R_f = 0.44$ (10% MeOH in EtOAc); $[\alpha]_D^{25} = +6.6$ ($c = 0.23$, CHCl_3); IR (film) 3266, 3056, 2927, 1742, 1716, 1373, 1266, 1219, 1117, 1059, 1024, 598.7 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 5.51 (br s, 1H, NH), 5.34 (dd, $J_{3,4} = 3.7$, $J_{4,5} = 5.5$ Hz, 1H, H-4), 5.14 (dd, $J_{5,6a} = 4.5$, $J_{4,5} = J_{5,6b} = 5.5$ Hz, 1H, H-5), 4.47 (d, $J_{2b,3} = 10.8$ Hz, 1H, H-3), 3.46 (dd, $J_{5,6a} = 4.5$, $J_{6a,6b} = 11.4$ Hz, 1H, H-6a), 3.37-4.43 (m, 2H, H-1ab), 3.36 (dd, $J_{5,6b} = 5.5$, $J_{6a,6b} = 11.4$ Hz, 1H, H-6b), 2.16 (s, 3H, 4-OAc), 2.13 (s, 3H, 5-OAc), 2.00 (d, $J_{2a,2b} = 14.2$ Hz, 1H, H-2a), 1.79-1.89 (m, 1H, H-2b); ^{13}C NMR (125 MHz, CDCl_3) δ 170.2 (C=O, 4-Ac), 169.9 (C=O, 5-Ac), 152.9 (C=O), 75.0 (C3), 73.4 (C4), 70.4 (C5), 38.9 (C1), 22.8 (C2), 20.9 (CH_3 , Ac), 20.6 (CH_3 , Ac), 3.3 (C6); HRMS(ESI) m/z calcd. for $[\text{C}_{11}\text{H}_{16}\text{O}_6\text{NI}+\text{H}]^+$: 386.0095, obsd.: 386.0094.

1-Amino-1-N,3-O-carbonyl-1,2,6-trideoxy-6-iodo-4,5-O-

isopropylidene-L-gulitol (117): Iodide **113** (8.8 mg, 0.3 mmol) was dissolved in acetone (0.45 mL), dimethoxypropane (0.45 mL), and MeOH (0.25 mL). 100 μL $p\text{TsOH} \cdot \text{H}_2\text{O}$ in acetone (2.85 mg/mL) was added and the reaction was stirred at room temperature overnight (17 h). The reaction was neutralised with Dowex- OH^-

, filtered and concentrated to give a white solid **117** (7.2 mg, 0.02 mmol, 72%) that was used without further purification. $R_f = 0.49$ (10% MeOH in EtOAc); $[\alpha]_D^{23} = -23.0$ ($c = 0.48$, CHCl_3); IR (film) 3282, 2985, 2930, 1700, 1487, 1456, 1421, 1372, 1294, 1239, 1214, 1107, 1069, 554 cm^{-1} ; ^1H NMR (500 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$ 95/5) δ 4.40 (app dt, $J_{3,4} = 2.2$, $J_{2a,3} = 10.0$ Hz, 1H, H-3), 4.20 (dt, $J_{5,6a} = J_{5,6b} = 4.7$, $J_{4,5} = 7.5$ Hz, 1H, H-5), 3.83 (dd, $J_{3,4} = 2.2$, $J_{4,5} = 7.5$ Hz, 1H, H-4), 3.41 (ddd, $J_{1a,2b} = 3.7$, $J_{1a,2a} = 5.8$, $J_{1a,1b} = 11.0$ Hz, 1H, H-1a), 3.36 (m, 1H, NH), 3.31-3.35 (m, 1H, H-1b), 3.31 (dd, $J_{5,6a} = 4.7$, $J_{6a,6b} = 10.8$ Hz, 1H, H-6a), 3.28 (dd, $J_{5,6b} = 4.7$, $J_{6a,6b} = 10.8$ Hz, 1H, H-6b), 2.06 (dt, $J_{1a,2a} = 5.8$, $J_{1b,2a} = J_{2a,3} = 10.0$, $J_{2a,2b} = 14.0$ Hz, 1H, H-2a), 1.98 (m, 1H, H-2b), 1.43 (s, 3H, Me), 1.40 (s, 3H, Me); ^{13}C NMR (125 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$ 95/5) δ 154.4 (C=O), 110.7 (iPr-C(CH₃)₂), 82.4 (C4), 75.1 (C3), 74.6 (C5), 38.7 (C1), 27.6, 27.0 (iPr-C(CH₃)₂), 23.3 (C2), 6.1 (C6); HRMS(ESI) m/z calcd. for $[\text{C}_{10}\text{H}_{16}\text{O}_4\text{NI}+\text{H}]^+$: 342.0197, obsd.: 342.0201.

D-threo-1-Amino-1-O,3-N-carbonyl-1,2,5,6-tetradecoxy-hex-5-enose (118): Iodo-acetonide (**117**) (6.8 mg, 0.02 mmol) was subjected to DCM (0.3 mL), AcOH (0.1 mL) and zinc (27.6 mg, 0.2 mmol), and stirred at 40 – 55 °C for 12 d. The reaction mixture was filtered through silica, and concentrated, to remove zinc. Crude product was purified by silica column chromatography (Petroleum Ether to Petroleum Ether/EtOAc, 1/3, v/v) yielding **118** as a white solid (2.4 mg, 0.015 mmol, 77 %). $R_f = 0.22$ (10% MeOH in EtOAc); $[\alpha]_D^{29} = -12.8$ ($c = 0.16$, MeOH); IR (film) 3373, 2926, 2856, 1682, 1489, 1459, 1294, 1108, 1071, 1028, 946 cm^{-1} ; ^1H NMR (500 MHz, D_2O) δ 5.92 (ddd, $J_{4,5} = 6.6$, $J_{5,6-cis} = 10.5$, $J_{5,6-trans} = 17.3$ Hz, 1H, H-5), 5.40 (app dt, $J_{4,6-trans} = 1.2$, $J_{5,6-trans} = 17.3$ Hz, 1H, H-6-trans), 5.32 (app dt, $J_{4,6-cis} = 1.2$, $J_{5,6-cis} = 10.5$ Hz, 1H, H-6-cis), 4.35 (ddd, $J_{2a,3} = 3.0$, $J_{3,4} = 5.7$, $J_{2b,3} = 10.5$ Hz, 1H, H-3), 4.24 (ddt, $J_{4,6-trans} = J_{4,6-cis} = 1.2$, $J_{3,4} = 5.7$, $J_{4,5} = 6.6$ Hz, 1H, H-4), 3.33 (ddd, $J_{1a,2a} = 3.0$, $J_{1a,2b} = 6.9$, $J_{1a,1b}$

= 12.0 Hz, 1H, H-1a), 3.32 (ddd, $J_{1b,2a} = 4.5$, $J_{1b,2b} = 10.5$, $J_{1a,1b} = 12.0$ Hz, 1H, H-1b), 1.99 (ddt, $J_{2a,3} = J_{1a,2a} = 3.0$, $J_{1b,2a} = 4.5$, $J_{2a,2b} = 14.1$ Hz, 1H, H-2a), 1.84 (dtd, $J_{1a,2b} = 6.9$, $J_{1b,2b} = J_{2b,3} = 10.5$, $J_{2a,2b} = 14.1$ Hz, 1H, H-2b); ^{13}C NMR (150 MHz, D_2O) δ 156.8 (C=O), 134.9 (C5), 118.4 (C6), 80.2 (C3), 73.5 (C4), 37.8 (C1), 21.7 (C2); HRMS(ESI) m/z calcd. for $[\text{C}_7\text{H}_{11}\text{O}_3\text{N}+\text{H}]^+$: 158.0812, obsd.: 158.0805.

d-threo-4-O-Acetyl-1-amino-1-O,3-N-carbonyl-1,2,5,6-

tetradexoxy-hex-5-enose (119): Iodide **116** (10.7 mg, 0.03 mmol) was dissolved in THF (0.3 mL) and H_2O (0.06 mL), AcOH (0.06 mL) and zinc (62 mg, 0.6 mmol) were added. The resulting mixture was then stirred at room temperature for 1 h. The reaction mixture was filtered through celite, washed with DCM and EtOH, and concentrated before coevaporation with H_2O and toluene to remove traces of AcOH. The crude product was purified by silica column chromatography (Petroleum Ether to Petroleum Ether/EtOAc, 1/3, v/v) to give **119** as a white solid (5.5 mg, 0.03 mmol, quant.). $R_f = 0.38$ (10% MeOH in EtOAc); $[\alpha]_{\text{D}}^{25} = -11.0$ ($c = 0.13$, CHCl_3); IR (film) 3278, 2925, 2854, 1703, 1489, 1459, 1424, 1372, 1294, 1227, 1104, 1023, 950, 763 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 5.88 (ddd, $J_{4,5} = 6.5$, $J_{5,6\text{-cis}} = 10.5$, $J_{5,6\text{-trans}} = 17.0$ Hz, 1H, H-5), 5.51 (br s, 1H, NH), 5.44 (d, $J_{4,5} = 6.5$ Hz, 1H, H-4), 5.43 (d, $J_{5,6\text{-trans}} = 17.0$ Hz, 1H, H-6-trans), 5.37 (d, $J_{5,6\text{-cis}} = 10.5$ Hz, 1H, H-6-cis), 4.39 (ddd, $J = 2.5$, $J = 5.5$, $J_{2b,3} = 10.5$ Hz, 1H, H-3), 3.35-3.45 (m, 2H, H-1ab), 2.13 (s, 3H, Me), 1.94-2.01 (m, 1H, H-2a), 1.86 (dtd, $J = 6.5$, $J = J_{2b,3} = 10.5$, $J_{2a,2b} = 14.0$ Hz, 1H, H-2b); ^{13}C NMR (125 MHz, CDCl_3) δ 170.0 (C=O, Ac), 153.8 (C=O), 131.5 (C5), 120.4 (C6), 77.5 (C3), 74.5 (C4), 39.0 (C1), 22.5 (C2), 21.2 (CH_3 , Ac); HRMS(ESI) m/z calcd. for $[\text{C}_9\text{H}_{13}\text{O}_4\text{N}+\text{H}]^+$: 200.0917, obsd.: 200.0918.

(3*R*,4*R*)-6-Amino-hex-1-ene-3,4-diol hydrochloride (110):

Acetylated alkene carbamate (**119**) (5.7 mg, 0.03 mmol) was dissolved in EtOH (1 mL) and NaOH (80 mg, 2 mmol) was added. The solution was refluxed for 1 h, neutralised with aqueous HCl (1.2 M), then concentrated *in vacuo*. Purification by gradient silica column chromatography (DCM/EtOH/MeOH/35% aqueous NH₃, 65/2/2/1 to 5/2/2/1, v/v/v/v) gave the alkenylamine, which was converted into the HCl salt (**110**) by the addition of HCl (1.2M, aq) and concentration to obtain a white solid (3.3 mg, 0.02 mmol, 69%). $R_f = 0.16$ (DCM/EtOH/MeOH/35% aqueous NH₃, 5/2/2/1, v/v/v/v); $[\alpha]_D^{27} = +31.8$ ($c = 0.2$, MeOH).

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Chapter 3: Development of a C-C Bond Forming Reaction for the Synthesis of Conduramines

3.1 Introduction

A variety of biologically significant molecules contain conduramine motifs, some of which have been depicted in Figure 3.1. Aminoshikimic acid **166** was recently identified as a competitive inhibitor of the *Mtb* shikimate kinase enzyme,¹ an enzyme that is essential to many pathogenic bacteria including *Mtb*, which is also absent from human cells.² The shikimate kinase enzyme is responsible for the phosphorylation of shikimic acid (**167**), hence the conduramine containing analogue, aminoshikimic acid **166** has potential as an anti-tuberculosis drug. Another conduramine containing motif is that of narciclasine (**168**), a natural product that was extracted from daffodil bulbs.³ Narciclasine (**168**) exhibits promising anti-cancer activity, including cytotoxicity against the HeLa (cervical cancer) cell line down to 0.1 $\mu\text{g/mL}$.³ The validamycin (**169**) and acarbose (**170**) scaffolds both contain the same conduramine motif, however each molecule has a different biological target.⁴ Validamycin (**169**) is a potent trehalase inhibitor and is used as a fungicide in the treatment of sheath blight disease in rice plants.⁴ Acarbose, on the other hand, is a drug used to treat of type 2 diabetes. The conduramine moiety in acarbose is the active pharmacophore responsible for the observed α -glucosidase inhibition in the intestine.⁴ Additionally, the second line

anti-tuberculosis drugs streptomycin, kanamycin and amikacin (Chapter 1, Figure 1.8) contain related aminoglycoside scaffolds that could also be prepared from conduramines.

Figure 3.1: Biologically relevant structures containing a conduramine motif.

To gain access to the conduramine family, an efficient PGF method for their preparation was sought and hence the 4-deoxy-3-conduramines were pursued. Retrosynthetic analysis suggested that 4-deoxy-3-conduramine **171** could be made from diene **172** via a PGF ring closing metathesis (RCM) reaction (Scheme 3.1). The preparation of diene **172** required a new PGF C-C and C-N bond forming reaction to install the allyl and amine functional groups simultaneously, which was envisioned through the development of a PGF Vasella-Barbier amination on methyl iodoxylofuranoside **173**. The 4,5-*anti* stereochemistry in **172** was anticipated to result from chelation control during the Barbier reaction. The iodide **173** could be prepared in two steps from D-xylose (**174**) using literature

procedures.⁵ A similar method for the synthesis of 4-deoxy-3-conduramines was employed by Verhelst *et al.*, to produce the protected conduramines in three steps and 59% overall yield from the protected furanoses,^{6,7} however, the final deprotection steps were not reported.

Scheme 3.1: Retrosynthesis of 4-deoxy-3-conduramine **171**.

3.2 Development of a PGF C-C bond Forming Reaction

The development of a PGF Vasella-Barbier amination began with the preparation of the corresponding methyl iodoxylofuranoside **173** from D-xylose (**174**) (Scheme 3.2). This was achieved in 78% yield over two steps using literature procedures.⁵ Next, a PGF Vasella-Barbier amination was attempted through the reaction of iodide **173** with indium powder, AcOH, 35% NH_{3(aq.)}, and allyl bromide in refluxing THF. The proposed mechanism for the Vasella-Barbier amination is depicted in Scheme 3.3. Indium inserts in the C-I bond of iodide **173** and an electron cascade to form the aldehyde follows. The aldehyde is then attacked by the amine to give the corresponding imine. Indium insertion in the C-Br bond of allyl bromide leads to nucleophilic attack on the imine to give the desired diene **172**. Unfortunately, under the conditions shown in Scheme 3.2, although diene **172** could be identified by mass spectrometry, no product was isolated. Purification of the reaction mixture by silica column chromatography revealed a product that appeared to be triol **175** on the basis of a downfield shift in ¹³C NMR signal for the C5 (δ 75.3 ppm), where the corresponding amine C5 would be expected close to δ 50 ppm. To confirm the formation of this product, triol diastereomers **175S** and **175R** were prepared according to a literature procedure (Scheme 3.4),⁸ and indeed, the ¹H NMR spectrum of triols **175S** and **175R** was found to be identical to the spectrum obtained from the attempted Vasella-Barbier amination reaction of methyl iodoxylofuranoside **173**. Although full conversion of the iodide starting material **173** was observed, triol **175** was the only product isolated, hence the low yield suggests that degradation was also occurring.

Scheme 3.2: Attempted synthesis of diene **172** and resultant product triol **175**.

Scheme 3.3: Proposed mechanism of the PGF Vasella-Barbier amination.

Scheme 3.4: A Vasella-Barbier reaction to form triols **175**.

As triol **175** was the major product formed from the initial Vasella-Barbier amination conditions, a number of other reaction conditions for the PGF C-C bond forming reaction were trialled. During the course of these studies, it was determined that the presence of water in the reaction mixture encouraged the formation of triol **175**. The use of both excess NH_3 , and allyl bromide was necessary to promote product formation, as degradation was also observed, however when iodide **173** was subjected to excess reagents a number of by-products were observed by mass spectrometry. These were identified as products from the nucleophilic attack of ammonia on allyl bromide (**176** to **179**, Figure 3.2), as well as the secondary and tertiary amine dienes, **180** and **181**, which were most likely formed from mono- or di-allyl amines **176** and **177** (which are more nucleophilic than ammonia) reacting with iodide **173** in the Vasella-Barbier amination. While these findings provide an idea of how to reduce the formation of triol **175**, and what was responsible for the formation of the over-allylated products, it was still difficult to determine how to best optimise for the formation of the desired product **172**. Accordingly, the preparation of a benzylamine diene **182** was proposed as an intermediate target (Scheme 3.5). The preparation of diene **182** would allow for the development of the C-C bond forming methodology with the use of a primary amine source (instead of NH_3) and it was predicted that this would limit the by-products formed from the reaction of the amine with allyl bromide. Ultimately, the choice of amine could be revisited, with the intention of attempting the synthesis of diene **172** again when the successful PGF preparation of benzylamine diene **182** was established.

Figure 3.2: Allylamine by-products observed by mass spectrometry.

Scheme 3.5: Proposed reaction to target benzylamine diene **182**.

Before the PGF synthesis of the new target, benzylamine *xylo* diene **182** (Scheme 3.5), was attempted, the preparation of an analogous *ribo* diene **183** via a literature protection/deprotection methodology was undertaken (Scheme 3.6), with two goals in mind: Firstly, returning to a literature method would confirm that the reported synthetic procedure was reliable, and that a protected Vasella-Barbier amination was possible. More importantly, the preparation of *ribo* diene

183 would provide a TLC reference for use in the development of the Vasella-Barbier amination for the synthesis of the *xyl*o diene **182**. Similarly, the ^1H NMR and ^{13}C NMR spectra of diene isomer **183**, although not identical, could also be used for comparison purposes. To this end, D-ribose (**62**) was subjected to Fischer methylation with concomitant isopropylidene protection of the *syn* diol and the primary hydroxyl was subsequently iodinated to provide protected iodide **66** in excellent yield (Scheme 3.6). Next, the Vasella-Barbier amination was undertaken according to the procedure developed by Madsen and coworkers⁸ which involved the sonication of iodide **66**, benzylamine **184**, and Zn in THF under dry conditions, followed by the portion-wise addition of allyl bromide. The reaction was complete after 4 h, at which stage workup and purification by silica gel column chromatography provided protected benzylamine diene **67S** as a single isomer in a modest 32% yield. Comparison the ^1H NMR couplings with those reported previously⁸ confirmed that diene **67S** was the *syn* diastereomer. Finally, to complete the synthesis, diene **67S** was deprotected under aqueous acidic conditions, providing diol **183** in 94% yield. Thus the synthesis was successfully completed, providing *ribo* diene **183** in a modest (unoptimised) yield of 24%. As the main goal of this synthesis was to provide *ribo* diene **183** for use as a reference standard, optimisation of the protected Vasella-Barbier amination reaction was not undertaken, and the modest yield could be attributed to the isolation of a single diastereomer, as well as the formation of some alcohol **185** as a by-product.

Scheme 3.6: Acetonide protected Vasella-Barbier amination reaction.

With the successful preparation of reference *ribo* diene **183** completed, the Vasella-Barbier amination on iodo methylxyloside **173** without the use of protecting groups, to give benzylamine *xylo* diene **182**, was then pursued (Scheme 3.5). Here, iodo methylxyloside **173** was subjected to the same Vasella-Barbier amination conditions as iodo riboside **66** (Zn, THF, sonication, allyl bromide added portion-wise), however no product formation was observed. The Vasella-Barbier reaction was then subjected to variations in the reaction time, solvent (EtOH or THF), heat vs. sonication, and the addition of AcOH (to favour imine formation), however in all attempted reactions, no product was observed. Monitoring the formation of *xylo* diene **182** by TLC revealed multiple product spots, including allylbenzylamine **186** (Figure 3.3). This by-product **186** had the same retention factor as the reference *ribo* diene **183** (and *xylo* diene **182**) hence, the use of benzylamine diene **183** as a TLC reference was not very useful in monitoring reaction progress. However, it was noted from the disappearance of

the starting material by TLC, that the Vasella component of the reaction required either sonication or reflux for the metal insertion and reductive elimination to proceed. Additionally, the stepwise addition of reagents to pre-form the aldehyde or imine led to degradation, as has been noted previously for hydroxyaldehydes.⁹ As the use of Zn in the Vasella-Barbier amination did not yield much success, the reagent was then substituted with indium powder.¹⁰ First, a Vasella-reductive amination with benzylamine **184** and NaCNBH₃ was undertaken to confirm that indium could also be used for the Vasella component (Scheme 3.7), and indeed alkenylamine **187** was successfully formed in 71% yield. A variety of Vasella-Barbier amination conditions were then explored using indium as the metal and eventually the formation of diene **182** was achieved when using 10 equiv. of both indium and benzylamine **184** and 2 equiv. of both allyl bromide and AcOH in THF (5 mL/mmol) at reflux overnight. *Xylo* diene **182** was initially identified by ¹H NMR spectroscopy of the crude reaction mixture whereby ¹H resonances at 2.4 ppm, corresponding to H-6ab could be identified. The formation of product was further supported by high resolution mass spectrometry. Purification of the reaction mixture by silica gel column chromatography then led to the isolation of benzylamine diene **182**, albeit in a modest (*ca.* 5%) yield.

Figure 3.3: Allylbenzylamine by-product **186**.

Scheme 3.7: Vasella reductive amination of iodide **173** to give benzylalkenylamine **187**.¹¹

In order to optimise the synthesis of diene **182**, a rapid method to estimate the yield of this product was required. Ideally, such methodology would allow for the quantification of the product yield directly from the crude reaction mixture, as ^1H NMR analysis of the crude reaction mixture was also ineffective due to the number of products formed, and similarly, isolation and purification of the product was difficult and time consuming. Accordingly, a high resolution mass spectrometry quantification method was attempted. To this end, an internal standard, tertiary amine **188** (Figure 3.4), was added to the mass spectrometry samples to compare the relative amount of product formed under a given set of reaction conditions. To estimate the quantity of diene **182** that was produced in a given reaction, one drop ($\sim 20\ \mu\text{L}$) of the reaction mixture was triturated and decanted from a methanolic solution of the internal standard (10 mL) and submitted for direct injection high resolution mass spectrometry. The peak height ratio for the protonated internal standard amine **188** (m/z : $[\text{C}_{15}\text{H}_{27}\text{NO}_3+\text{H}]^+ = 270.2064$) versus the diene **182** (m/z : $[\text{C}_{15}\text{H}_{21}\text{NO}_2+\text{H}]^+ = 248.1645$) then gave an indication of the amount of product formed in the reaction. In a similar vein, the mass spectrometry method could also be used to identify relative ratios of amine by-product, allylbenzylamine **186** (m/z : $[\text{C}_{10}\text{H}_{13}\text{N}+\text{H}]^+ = 148.1121$).

Figure 3.4: Mass spectrometry internal standard, tertiary amine **188**.

Using the MS method, optimal reaction conditions (10 equiv. AcOH, 6 equiv. In, 10 equiv. BnNH₂, and 4 x 2 equiv. allyl bromide, refluxed in 5 mL/mmol THF for 6 h) were identified (Scheme 3.8), however, unfortunately the isolated yield of diene **182** was only 16%, which was significantly lower than the anticipated yield based on MS quantification. The disparity between the expected and isolated yields indicates a limitation of the MS quantification methodology with variations in drop size, reaction concentration due to solvent evaporation over the course of the reaction, and the formation of insoluble products in the reaction mixture, limiting the accuracy of the MS method. Moreover, the difficulty in separating product **182** from the by-product allylbenzylamine **186** by silica column chromatography may have led to the degradation of diene **182** on the silica column and contributed to the low isolated yield. Accordingly, while the MS technique did provide an indication of whether an alteration to the reaction conditions was beneficial to product formation, the technique was not an accurate method to quantify the amount of diene **182** formed, and thus, for the subsequent PGF Vasella-Barbier optimisation studies, the MS technique was not employed.

Scheme 3.8: PGF Vasella-Barbier benzylamination.

Having achieved the successful Vasella-Barbier amination with benzylamine **184** to form diene **182**, albeit in modest yield, the development of a PGF Vasella-Barbier amination to form primary amine **172** was resumed with the confidence that unprotected diol **173** was amenable to the reaction conditions. To avoid over-allylation of the amine, an amine source that was less nucleophilic towards allyl bromide was sought. To this end, a literature search revealed that NH₄OAc had been previously used in a Barbier-Mannich reaction to form secondary amine ‘dimers’,¹² and hence the PGF Vasella-Barbier amination was attempted using

NH₄OAc rather than NH₃ as the amine source (Scheme 3.9). Analysis of the crude reaction mixture by ¹H NMR spectroscopy provided evidence that amino diene **172** was formed, whereby a spectrum similar, but not identical to that of triol **175** was observed. Indeed, purification by Dowex-H⁺ ion exchange chromatography, and subsequent silica gel column chromatography enabled the successful isolation of diene **172** in *ca.* 5% yield. The ¹H NMR spectrum of this compound is given in Figure 3.5, where full ¹H assignments are made.

Scheme 3.9: Vasella-Barbier reaction with unprotected amine.

Figure 3.5: ^1H NMR spectrum of amino diene **172** (D_2O , 500 MHz).

Having determined a methodology that allowed for the synthesis of diene **172**, optimisation of the reaction conditions was undertaken (Table 3.1). All reactions were performed in dry conditions, under Ar, with the dropwise addition of allyl bromide to the refluxing reaction mixture over 10 min. From the initial conditions of 6 equiv. of indium powder, 2 equiv. of each NH₄OAc and allyl bromide, refluxed in MeOH (5 mL/mmol of xyloside **173**) overnight (entry 1), a larger excess (6 equiv.) of both ammonium acetate and allyl bromide gave a small improvement in yield (8%, entry 2). A dilution of the reaction mixture to 10 mL/mmol, with the use of additional NH₄OAc (24 equiv., entry 3) also resulted in an improved (20%) yield. Next, the concentration of NH₄OAc was further increased to a nearly saturated solution (48 equiv., entry 4), however, no improvement in yield was observed in this case (18% yield). To test the necessity of the dropwise addition of the allyl bromide, a reaction under the same conditions as entry 3 was performed, with the only difference being the addition of allyl bromide in one portion at the beginning of the reaction (entry 5). Very little product was obtained under these conditions, with an insoluble solid (presumably a polymer) being observed. Changes to the solvent were then made, with EtOH and THF replacing MeOH (entries 6 and 7, respectively), though both reactions provided insubstantial amounts of product. In the case of entry 6, the EtOH was not dry and hence triol **175** was predominantly formed, however, freshly distilled dry THF was used in entry 7 and this reaction was also very poor yielding. That said, the reaction in THF was notably slower, with incomplete reaction after 2 d refluxing, which suggests poor solubility of one of the starting materials.

Table 3.1: Reaction conditions for the Vasella-Barbier amination

Entry	Conditions	Yield of 172
1	In (6 equiv.), NH ₄ OAc (2 equiv.), allyl bromide dropwise (2 equiv.), MeOH (5 mL/mmol), reflux, 24 h	~5%
2	In (6 equiv.), NH ₄ OAc (6 equiv.), allyl bromide dropwise (6 equiv.), MeOH (5 mL/mmol), reflux, 16 h	8%
3	In (6 equiv.), NH ₄ OAc (24 equiv.), allyl bromide dropwise (6 equiv.), MeOH (10 mL/mmol), reflux, 20 h	20%
4	In (6 equiv.), NH ₄ OAc (48 equiv.), allyl bromide dropwise (6 equiv.), MeOH (10 mL/mmol), reflux, 20 h	18%
5	In (6 equiv.), NH ₄ OAc (24 equiv.), allyl bromide 1 portion (6 equiv.), MeOH (10 mL/mmol), reflux, 21 h	<5%
6	In (6 equiv.), NH ₄ OAc (24 equiv.), allyl bromide dropwise (6 equiv.), EtOH (10 mL/mmol), reflux, 17 h	<5%
7	In (6 equiv.), NH ₄ OAc (24 equiv.), allyl bromide dropwise (6 equiv.), THF (10 mL/mmol), reflux, 44 h	Incomplete <<5%
8	In (6 equiv.), NH ₄ OAc (24 equiv.), allyl bromide dropwise (6 equiv.), MeOH (10 mL/mmol), sonicate 11 h	Alcohol only
9	In (6 equiv.), NH ₄ OAc (24 equiv.), allyl bromide dropwise (6 equiv.), MeOH (20 mL/mmol), reflux, 15 h	7%

Entry	Conditions	Yield of 172
10	In (6 equiv.), NH ₄ OAc (24 equiv.), allyl bromide dropwise (6 equiv.), MeOH (10 mL/mmol), 28% NH ₃ /MeOH (6 equiv.) reflux, 15 h	9%
11	In (6 equiv.), NH ₄ OAc (24 equiv.), allyl bromide dropwise (6 equiv.), MeOH (10 mL/mmol), AcOH (6 equiv.), reflux, 21 h	9%
12 ^a	In (6 equiv.), NH ₄ OAc (24 equiv.), allyl bromide dropwise (6 equiv.), MeOH (10 mL/mmol), reflux, 20 h	35%

^a Performed on a 2 mmol scale, when all other reactions were performed on a 0.5 mmol scale.

As many literature procedures for Barbier reactions use sonication,^{10a,13} this technique was used in entry 8, however, disappointingly, only alcohol **175**, was observed. Next, a more dilute solution of 20 mL/mmol of furanoside **173** was used with the aim of reducing the formation of the dimer **189** as well as reducing polymerisation (entry 9). Although never isolated, dimer **189** was observed by high resolution mass spectrometry (m/z : [C₁₆H₂₇NO₄+H]⁺ calcd. = 298.2013, obsd. = 298.2025). This could be formed from the reaction of aldehyde **190** and product amine **172**, and the subsequent Barbier reaction of the resulting imine (Scheme 3.10). Unfortunately, the lowered concentration of entry 9 resulted in a low yield of 7%, which may occur as a result of degradation of the corresponding aldehyde or imine.¹¹ Upon further assessment of the literature, it was noted that the pH of the reaction mixture was important to favour primary amine formation in the development of the PGF Vasella-reductive amination.¹¹ Thus, the reaction conditions were adjusted through the addition of small amounts (6 equiv.) of either AcOH (pH 7, entry 10), or NH₃ (28% methanolic, pH 8, entry 11). Surprisingly, although the pH of the reaction mixtures varied only slightly (*cf.* pH 7.5 observed in entry 3), both reactions resulted in a lowered yield of amine **172**. Because of the presence of electrophilic allyl bromide in the C-C bond forming

reaction, the use of even a small excess of ammonia to increase the pH appeared undesirable, presumably due to the formation of over-allylated products. Therefore, with a variety of conditions attempted and only a low yield achieved, the reaction was scaled up in order to produce enough diene **172** to continue to the RCM step. Surprisingly, when the reaction was scaled four times to a 600 mg (2.2 mmol) scale reaction (entry 12), the yield was also improved to 35%. This result may be attributed to the dryness of the reaction, as large scale reactions are less sensitive to the small amounts of water present.

Scheme 3.10: Mechanistic explanation of dimer **189** formation.

Purification of amino diene **172** by Dowex- H^+ and then silica column chromatography provided the product as a single isomer (the 4,5-*anti* isomer). The 4,5-*anti* relationship was established from the conformationally locked cyclohexene **171** following the RCM of the linear diene **172** (see Section 3.3). Accordingly, a Cram-chelate model can be used to explain the 4,5-*anti* selectivity of the C-C bond forming reaction (Figure 3.6), whereby after imine formation

indium chelates between the 5-NH and the 4-OH groups,¹⁴ locking the conformation of the molecule. With a bulky allyl alcohol group (R) blocking one side of the molecule, the nucleophilic Barbier reagent can only attack from the *Re* face, leading to the *anti* relationship between C-4 and C-5 substituents. Here, it should be noted that baseline impurities in the ¹H NMR spectra were occasionally noted, which may indicate the presence of tiny amounts of the *syn* **172** isomer or the dimer **189**.

Figure 3.6: Cram-chelate model for the stereoselectivity of the Vasella-Barbier amination to form diene **172**.

3.3 RCM for the Synthesis of a Conduramine Analogue

Having successfully prepared amino diene **172** via a PGF route, the RCM reaction to form conduramine derivative **171** was then explored (Scheme 3.11). RCM on a molecule containing a primary amine has only been reported once in the literature,¹⁵ and this utilised a protection by protonation strategy and the Hoveyda-Grubbs 2nd generation catalyst. Using this approach, amine **172** was thus transformed into the ammonium triflate salt and subjected to 5 mol% of the Hoveyda-Grubbs II catalyst in dry THF. TLC analysis revealed that the reaction was complete after 1 h, and following purification by flash silica column chromatography (DCM:MeOH:EtOH:35% NH_{3(aq.)} 55:2:2:1), the target conduramine **171** was obtained in a quantitative yield. To test the scope of this reaction, and confirm the necessity of the ammonium triflate salt, the reaction was also attempted on the free amine **172**. No reaction was observed by TLC after several hours stirring at rt, and gentle heating and then refluxing of the reaction mixture with the addition of more catalyst also failed to induce the desired reaction. In contrast, when the ammonium chloride salt of diene **172** was used in the RCM, conduramine **171** was successfully isolated in 72% yield. The RCM reaction of the HCl salt required an 8 mol% catalyst loading and heating, thus the quantitative reaction of the triflate salt was still preferred.

Scheme 3.11: PGF conduramine synthesis.

Confirmation of the structure of conduramine **171** by NMR spectroscopy involved the identification of ¹H couplings between the CH₂ group at C-6 and the alkene CH at C-1. The disappearance of the terminal alkene peaks in the ¹H NMR spectrum, as well as reduced integration of the alkene signals supported this assignment (Figure 3.8), as did high resolution mass spectrometry, where an ion at the mass of the protonated amine was clearly visible (m/z : [C₆H₁₁NO₂+H]⁺ calcd. = 130.0863, obsd. = 130.0865). Having confirmed the formation of a substituted cyclohexene, the stereochemistry at C-5 could thus be determined as *anti* to C-4 hydroxyl based on large couplings between H-4 and H-5 and also between H-5 and H-6b ($J_{4,5} = J_{5,6b} = 10.5$ Hz) suggesting a diaxial relationship between these protons (Figure 3.7).

Figure 3.7: Coupling constants for the determination of the 4,5-*anti* relationship in conduramine **171**.

Figure 3.8: ¹H NMR spectrum of conduramine **171** (D₂O, 500 MHz).

3.4 Conclusion

In summary, a short, stereoselective PGF synthesis of 4-deoxy-3-conduramines has been presented, as illustrated by the preparation of conduramine **171** which was synthesised in four steps and in an overall yield of 27%. The synthesis of conduramine **171** required the installation of both a C-C and a C-N bond and hence the PGF Vasella-Barbier amination was developed. A Cram chelate model could be used to explain the 4,5-*anti* selectivity of the novel C-C bond forming methodology. In the final step, an *in situ* protection strategy was utilised to allow the quantitative RCM of diene **172**, and the formation of the desired 4-deoxy-3-conduramine **171**. Taken as a whole, this methodology provides the first step towards a useful synthetic route that has potential to be extended for the development of a variety of products, as will be elaborated on in Chapter 5.

3.5 Experimental

General Experimental

Unless otherwise stated, all reactions were performed under atmospheric air. allyl bromide (Merck), EtOH (absolute, Pure Science), H₂O, MeOH (Pure Science), and TfOH (Sigma) were distilled prior to use. EtOAc (Pure Science), petroleum ether (Pure Science), AcOH (Ajax Finechem), AcCl (B&M), Acetone (Fischer), benzylamine (Sigma-Aldrich), DCM (LabServ), HCl (PanReac), Hoveyda Grubbs II Catalyst (Aldrich), Indium powder (AlfaAesar), MgSO₄ (PureScience), 35% aqueous NH₃ (LabServ), NaCNBH₃ (Aldrich), NaHCO₃ (PureScience), NH₄OAc (AnalaR), D-ribose (Sigma-Aldrich), and D-xylose (Sigma) were used as received. All chemicals obtained from commercial suppliers were used without further purification. Zn dust was activated by the careful addition of concentrated H₂SO₄, followed by decantation and washing with EtOH (3×) and hexanes (3×) and storage under dry hexanes. All solvents were removed by evaporation under reduced pressure. Reactions were monitored by TLC analysis on Macherey-Nagel silica gel coated plastic sheets with detection by coating with 20% H₂SO₄ 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% KMnO₄ and 1% NaIO₄ in H₂O followed by heating. Column chromatography was performed on Pure Science silica gel (40-63 micron). Dowex® W50-X8 acidic resin (Sigma) and Dowex® 1X4-50 basic resin (Sigma) were used for ion exchange chromatography and HP-20 (Supelco) for reverse phase chromatography. High-resolution mass spectra were recorded on a Waters Q-TOF Premier™ Tandem Mass Spectrometer using positive electro-spray ionisation. Optical rotations were recorded using an Autopol II (Rudolf Research Analytical) at 589 nm (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 at 20 °C in D₂O or CDCl₃ using either a Varian Unity-INOVA operating at 300 MHz or a Varian Unity

operating at 500 MHz. Chemical shifts are given in ppm (δ) and were internally referenced to the residual solvent peak. NMR peak assignments are based on 2D NMR experiments (COSY, HSQC, and HMBC).

Methyl 5-deoxy-5-iodo- α/β -D-xyloside (173):⁵

To a solution of D-xylose (10.04 g, 67 mmol) in MeOH (330 mL), AcCl (1 mL) was added and the reaction stirred at room temperature for 22 h. The reaction was neutralised by the addition of Dowex (OH⁻), filtered and concentrated to give the methyl xyloside. The resulting oil was used in the next step without further purification. To a solution of methyl xyloside (11.0 g, 67 mmol) in dry THF (300 mL), imidazole (9.0 g, 132 mmol), PPh₃ (26.0 g, 99 mmol), and I₂ (25 g, 99 mmol) were added under an atmosphere of argon. The reaction was refluxed for 1.5 h, then cooled and concentrated under reduced pressure. The product was taken up in petroleum ether/EtOAc, 2/1, v/v, and filtered through a silica plug to remove excess iodine. The product was further purified by reverse phase HP20 beads (10% to 40% MeOH/water, v/v) to give xyloside **173** as colourless oil in a 1:1 ratio of α - and β -anomers (78%, 14.27 g, 52.1 mmol). Characterisation data are in full agreement with that reported.¹⁶

(175S/R):⁸ To a solution of methyl 5-deoxy-5-iodo- α/β -D-xyloside (**173**) (100 mg, 0.36 mmol) in THF/H₂O (1.1 mL, 4/1, v/v) was added Zn (234 mg, 3.6 mmol) and allyl bromide (95 μ L, 1.1 mmol). The solution was sonicated for 4 h, before filtration through celite provided the crude product **175** as a 3:7 mixture of *S*:*R* diastereomers. Characterisation data are in agreement with that reported in the literature.⁸

Methyl 5-deoxy-5-iodo-2,3-*O*-isopropylidene- α/β -D-ribose

(66): To a solution of D-ribose (**62**) (5.04 g, 33.6 mmol) in acetone/MeOH (68 mL, 1/1, v/v) was added conc. HCl (350 μ L) dropwise, and the reaction refluxed for 1.5 h. The reaction was then cooled and neutralised with NaHCO_{3(aq.)}, and the acetone/MeOH solvent removed under reduced pressure. The aqueous layer was then extracted with EtOAc (3x), washed with NaCl_(aq.), dried over MgSO₄, filtered and concentrated to provide methyl 2,3-*O*-isopropylidene- α/β -D-ribose as a colourless oil which was used in the next step without further purification (6.25 g, 31, mmol, 91%). To a solution of methyl riboside (2.02 g, 9.9 mmol) in dry toluene/MeCN (36 mL, 5/1, v/v) was added I₂ (3.03 g, 11.9 mmol), PPh₃ (3.02 g, 11.5 mmol), and imidazole (1.09 g, 16.0 mmol) and the reaction refluxed under Ar for 30 min. Upon cooling, 100 mL Et₂O was added and the organic layer was washed with Na₂S₂O_{3(aq.)}, H₂O, then NaCl_(aq.), dried over MgSO₄, filtered and concentrated under reduced pressure. Purification by silica gel flash column chromatography (petroleum ether/EtOAc, 19/1, v/v) provided methyl 5-deoxy-5-iodo-2,3-*O*-isopropylidene- α/β -D-ribose (**66**) as a pale yellow oil (2.72 g, 8.6 mmol, 88%). R_f = 0.71 (petroleum ether/EtOAc, 1/1, v/v); $[\alpha]_D^{26}$ = -64.5 (c = 1.0, CHCl₃), lit.¹⁷ $[\alpha]_D^{25}$ = -65.7 (c = 1.0, CHCl₃); IR (film) 2989, 2936, 1374, 1103, 1066, 868, 515 cm⁻¹. ¹H NMR (500 MHz, CDCl₃) δ 5.03 (s, 1H, H-1), 4.75 (d, $J_{2,3}$ = 5.9 Hz, 1H, H-3), 4.61 (d, $J_{2,3}$ = 5.9 Hz, 1H, H-2), 4.42 (dd, $J_{4,5a}$ = 6.0, $J_{4,5b}$ = 10.0 Hz, 1H, H-4), 3.35 (s, 3H, OMe), 3.27 (dd, $J_{4,5a}$ = 6.0, $J_{5a,5b}$ = 10.0 Hz, 1H, H-5a), 3.14 (t, $J_{4,5b}$ = $J_{5a,5b}$ = 10.0 Hz, 1H, H-5a), 1.46 (s, 3H, iPr), 1.31 (s, 3H, iPr); ¹³C NMR (75 MHz, CDCl₃) 112.6 (iPr-C(CH₃)₂), 109.6 (C1), 87.4 (C4), 85.3 (C2), 83.0 (C3), 55.2 (OMe), 26.4 (iPr-C(CH₃)₂), 25.0 (iPr-C(CH₃)₂), 6.7 (C5); HRMS(ESI) m/z calcd. for [C₉H₁₅O₄I+H]⁺: 315.0088, obsd.: 315.0120.

5*S*-Benzylamino-3*R*,4*S*-O-isopropylidene-1,7-octadiene

(67*S*):⁸ To a solution of methyl 5-deoxy-5-iodo-2,3-*O*-isopropylidene- α/β -D-ribose (**66**) (2.09 g, 6.7 mmol) in dry THF (4.3 mL) was added Zn (540 mg, 8.3 mmol) and benzylamine (157 μ L, 1.4 mmol) and the reaction was sonicated under Ar for 30 min. allyl bromide (137 μ L, 1.6 mmol) was added dropwise to the sonicating reaction mixture in portions over 2.5 h, and then sonication was continued for another 1 h. The reaction mixture was then filtered through celite and washed with EtOAc, petroleum ether, and DCM before concentration under reduced pressure. Purification by silica gel flash column chromatography (petroleum ether/EtOAc, 19/1, v/v) provided 5*S*-benzylamino-3*R*,4*S*-O-isopropylidene-1,7-octadiene (**67*S***) (60.7 mg, 0.21 mmol, 32%). R_f = 0.56 (petroleum ether/EtOAc, 1/1, v/v). Characterisation data are in full agreement with that reported in the literature.⁸

5*S*-Benzylamino-3*R*,4*S*-dihydroxy-1,7-octadiene (183):

A solution of 5*S*-benzylamino-3*R*,4*S*-O-isopropylidene-1,7-octadiene (**67*S***) (53.4 mg, 0.19 mmol), AcOH (1 mL) and H₂O (0.2 mL) was stirred at 60 °C for 3 d. Concentration followed by purification by silica gel flash column chromatography (petroleum ether/EtOAc, 1/1, v/v) afforded 5*S*-Benzylamino-3*R*,4*S*-dihydroxy-1,7-octadiene (**183**) as a pale oil (43.3 mg, 0.18 mmol, 94%). R_f = 0.53 (DCM/EtOH/MeOH/30% NH_{3(aq.)}, 105/2/2/1, v/v/v/v); $[\alpha]_D^{26}$ = 27.4 (c = 0.6, MeOH); IR (film) 3363, 3031, 2957, 2925, 2857, 1639, 1494, 1454, 1079, 920, 748, 698 cm⁻¹. ¹H NMR (500 MHz, CDCl₃) δ 7.37-7.24 (m, 5H, H_{arom}), 5.91 (J =

1.7, $J_{2,3} = 7.2$, $J_{1cis,2} = 10.3$, $J_{1trans,2} = 17.3$ Hz, 1H, H-2), 5.82 ($J_{7,8cis} = 9.0$, $J_{7,8trans} = 16.9$ Hz, 1H, H-7), 5.37 (d, $J_{1trans,2} = 17.3$ Hz, 1H, H-1 $_{trans}$), 5.29 (d, $J_{1cis,2} = 10.3$ Hz, 1H, H-1 $_{cis}$), 5.20 (d, $J_{7,8cis} = 9.0$ Hz, 1H, H-8 $_{cis}$), 5.17 (d, $J_{7,8trans} = 16.9$ Hz, 1H, H-8 $_{trans}$), 4.10 (app. t, $J_{2,3} = J_{3,4} = 7.5$ Hz, 1H, H-3), 3.87 (d, $J_{Ha,Hb} = 12.4$ Hz, 1H, CH $_{\underline{a}}$ H $_{\underline{b}}$ Bn), 3.75 (d, $J_{Ha,Hb} = 12.4$ Hz, 1H, CH $_{\underline{a}}$ H $_{\underline{b}}$ Bn), 3.27 (app. t, $J_{3,4} = J_{4,5} = 8.0$ Hz, 1H, H-4), 2.84 (ddd, $J = 4.2$, $J = 6.1$, $J_{4,5} = 7.6$ Hz, 1H, H-5), 2.58-2.45 (m, 2H, H-6); ^{13}C NMR (125 MHz, CDCl_3) δ 139.0 (C- i , Bn), 138.5 (C2), 134.0 (C7), 128.8, 128.5, 127.6 (CH-Bn), 119.4 (C8), 118.3 (C1), 77.8 (C3), 71.6 (C4), 60.6 (C5), 51.6 (CH $_{\underline{2}}$ Bn), 33.6 (C6); HRMS(ESI) m/z calcd. for $[\text{C}_{15}\text{H}_{21}\text{O}_2\text{N}+\text{H}]^+$: 248.1645, obsd.: 248.1654.

1-Benzylamino-2S,3S-dihydroxy-pent-4-ene (187): To

a solution of methyl 5-deoxy-5-iodo- α/β -D-xyloside (**173**) (45 mg, 0.16 mmol) in EtOH (3.3 mL), was added benzylamine (36 μL , 0.33 mmol), indium (94 mg, 0.83 mmol), NaCNBH_3 (31 mg, 0.5 mmol), water (100 μL), and AcOH (50 μL). The reaction was stirred under reflux for 17 h, cooled, filtered through celite and the solvent was removed under reduced pressure. The reaction mixture was dry loaded onto silica gel and purified by gradient flash column chromatography (DCM/EtOH/MeOH/35% $\text{NH}_3(\text{aq.})$, 205/2/2/1 to 155/2/2/1, v/v/v/v) to give benzylalkenylamine **187** (24 mg, 0.12 mmol, 71%). $R_f = 0.63$ (DCM/EtOH/MeOH/35% $\text{NH}_3(\text{aq.})$, 35/2/2/1, v/v/v/v); $[\alpha]_D^{26} = -36.9$ ($c = 0.9$, MeOH); IR (film) 3381, 3086, 2902, 2844, 1646, 1454, 1106, 1045, 994 cm^{-1} . ^1H NMR (500 MHz, D_2O) δ 7.41-7.30 (m, 5H, H_{arom}), 5.81 (ddd, $J_{3,4} = 6.6$, $J_{4,5cis} = 10.5$, $J_{4,5trans} = 17.2$ Hz, 1H, H-4), 5.29 (d, $J_{4,5trans} = 17.2$ Hz, 1H, H-5 $_{trans}$), 5.22 (d, $J_{4,5cis} = 10.5$ Hz, 1H, H-5 $_{cis}$), 3.99 (app t, $J_{2,3} = J_{3,4} = 6.1$ Hz, 1H, H-3), 3.76 (d, $J_{Ha,Hb} = 13.3$ Hz, 1H, CH $_{\underline{a}}$ H $_{\underline{b}}$ Bn), 3.70 (d, $J_{Ha,Hb} = 13.3$ Hz, 1H, CH $_{\underline{a}}$ H $_{\underline{b}}$ Bn), 3.67 (ddd, $J_{1a,2} = 3.4$, $J_{2,3} = 5.6$, $J_{1b,2} = 9.0$ Hz, 1H, H-2), 2.67 (dd, $J_{1a,2} = 3.4$, $J_{1a,1b} = 12.7$ Hz, 1H, H-1a), 2.57 (dd, $J_{1b,2} = 9.0$, $J_{1a,1b} = 12.7$ Hz, 1H, H-1b); ^{13}C NMR (125 MHz, D_2O) δ 138.7 (C- i , Bn), 136.1 (C5), 128.6, 128.5, 127.4 (CH-Bn),

117.7 (C5), 74.6 (C3), 72.2 (C2), 52.2 ($\underline{\text{CH}_2\text{Bn}}$), 49.6 (C1); HRMS(ESI) m/z calcd. for $[\text{C}_{12}\text{H}_{17}\text{O}_2\text{N}+\text{H}]^+$: 208.1332, obsd.: 208.1338.

5-Benzylamino-3S,4S-dihydroxy-1,7-octadiene (182): To a solution of methyl iodoxyloside **173** (138 mg, 0.5 mmol) in THF (2.4 mL), was added indium powder (380 mg, 3.3 mmol), AcOH (300 μL , 5.2 mmol), benzylamine (600 μL , 5.5 mmol), and allyl bromide (100 μL , 1.2 mmol). The reaction mixture was refluxed, with additional allyl bromide (100 μL) added after 1 h, 2 h, and 3 h. After 6 h of refluxing, the reaction was cooled and 5 mL of aqueous 1.2 M HCl added. The reaction mixture was then stirred at room temperature for 5 min before being filtered through celite and concentrated *in vacuo*. The crude reaction mixture was dry loaded on silica gel for gradient flash chromatography (DCM/EtOH/MeOH/35% $\text{NH}_3(\text{aq.})$, 705/2/2/1 to 305/2/2/1, v/v/v/v). Repeated silica column chromatography was required to separate allyl benzylamine **186** from the desired diene (**182**), which was isolated as a white solid (20 mg, 0.08 mmol, 16%). R_f = 0.53 (DCM/EtOH/MeOH/30% $\text{NH}_3(\text{aq.})$, 105/2/2/1, v/v/v/v); $[\alpha]_D^{23}$ = -49.5 (c = 1.0, MeOH); IR (film) 3321, 3075, 3028, 2978, 2916, 2852, 1659, 1496, 1453, 1071, 1028, 993, 919, 741, 699 cm^{-1} . ^1H NMR (500 MHz, CDCl_3) δ 7.40-7.26 (m, 5H, aromatics), 5.96-5.76 (m, 2H, H-2 and H-7), 5.42 (dd, $J_{1\text{cis},1\text{trans}}$ = 1.6, $J_{1\text{trans},2}$ = 17.1 Hz, 1H, H-1 trans), 5.26 (dd, $J_{1\text{cis},1\text{trans}}$ = 1.6, $J_{1\text{cis},2}$ = 10.5 Hz, 1H, H-1 cis), 5.19 (d, $J_{7,8\text{cis}}$ = 11.1 Hz, 1H, H-8 cis), 5.16-5.13 (m, 1H, H-8 trans), 4.29-4.23 (m, 1H, H-3), 3.98 (d, $J_{\text{Ha},\text{Hb}}$ = 12.3 Hz, 1H, $\underline{\text{CH}}_{\text{a}}\text{H}_{\text{b}}$ Bn), 3.76 (d, $J_{\text{Ha},\text{Hb}}$ = 12.3 Hz, 1H, $\text{CH}_a\underline{\text{H}}_{\text{b}}$ Bn), 3.49 (br t, $J_{3,4}$ = $J_{4,5}$ = 2.4 Hz, 1H, H-4), 2.84 (ddd, J = 3.3, J = 5.1, J = 7.5 Hz, 1H, H-5), 2.57-2.37 (m, 2H, H-6); ^{13}C NMR (125 MHz, CDCl_3) δ 139.2 (C- i , Bn), 137.9, 134.8 (C2 and C7), 128.8, 128.5, 127.6 (CH-Bn), 118.4 (C8), 116.3 (C1), 75.1 (C3), 71.8 (C4), 59.9 (C5), 51.5 ($\underline{\text{CH}_2\text{Bn}}$), 35.8 (C6); HRMS(ESI) m/z calcd. for $[\text{C}_{15}\text{H}_{21}\text{O}_2\text{N}+\text{H}]^+$: 248.1645, obsd.: 248.1654.

5R-Amino-3S,4S-dihydroxy-1,7-octadiene (172): To a solution of methyl iodoxyloside **173** (602 mg, 2.2 mmol) in dry distilled MeOH (21.6 mL), was added indium powder (1.49 g, 13 mmol), and NH₄OAc (4.0 g, 51 mmol) and the reaction mixture was heated to reflux. allyl bromide (1.2 mL, 13.9 mmol) was then added drop-wise over 10 min and the reaction mixture was refluxed under Ar for 20 h before being cooled to rt. 20 mL of aqueous 1.2 M HCl was added and the solution stirred at room temperature for 5 min before being filtered through celite, and concentrated *in vacuo*. The residue was loaded onto Dowex-H⁺ cation exchange resin and eluted with 5-35% NH_{3(aq.)}. Further purification by gradient silica gel flash column chromatography (DCM/EtOH/MeOH/35% NH_{3(aq.)}, 205/2/2/1 to 125/2/2/1, v/v/v/v) provided amino diene (**172**) as a yellow oil (122 mg, 0.78 mmol, 35%). $R_f = 0.53$ (DCM/EtOH/MeOH/30% NH_{3(aq.)}, 15/2/2/1, v/v/v/v); $[\alpha]_D^{23} = -17.6$ (c = 1.2, MeOH); IR (film) 3355, 3076, 2978, 2906, 1640, 1581, 1437, 1047, 993, 918 cm⁻¹. ¹H NMR (500 MHz, D₂O) δ 5.93 (ddd, $J_{2,3} = 6.4$, $J_{1cis,2} = 10.5$, $J_{1trans,2} = 17.2$ Hz, 1H, H-2), 5.85 (dddd, $J_{6a,7} = 6.8$, $J_{6b,7} = 7.6$, $J_{7,8cis} = 10.2$, $J_{7,8trans} = 17.1$ Hz, 1H, H-7), 5.37 (dt, $J_{1,3} = J_{1cis,1trans} = 1.2$, $J_{1trans,2} = 17.2$ Hz, 1H, H-1trans), 5.28 (dt, $J_{1,3} = J_{1cis,1trans} = 1.2$, $J_{1cis,2} = 10.5$ Hz, 1H, H-1cis), 5.17 (dd, $J_{6,8trans} = 1.9$, $J_{7,8trans} = 17.1$ Hz, 1H, H-8trans), 5.14 (dd, $J_{6,8cis} = 1.1$, $J_{7,8cis} = 10.2$ Hz, 1H, H-8cis), 4.26 (ddd, $J_{1,3} = 1.2$, $J_{3,4} = 5.3$, $J_{2,3} = 6.4$ Hz, 1H, H-3), 3.45 (t, $J_{3,4} = J_{4,5} = 5.3$ Hz, 1H, H-4), 2.96 (dt, $J_{4,5} = J_{5,6a} = 5.3$, $J_{5,6b} = 7.6$ Hz, 1H, H-5), 2.34 (dddd, $J_{6a,8} = 1.2$, $J_{5,6a} = 5.3$, $J_{6a,7} = 6.8$, $J_{6a,6b} = 14.0$ Hz, 1H, H-6a), 2.15 (dt, $J_{5,6b} = J_{6b,7} = 7.6$, $J_{6a,6b} = 14.0$ Hz, 1H, H-6b); ¹³C NMR (125 MHz, D₂O) δ 136.9 (C2), 134.8 (C7), 117.9 (C8), 117.1 (C1), 74.8 (C4), 73.4 (C3), 51.2 (C5), 37.4 (C6); HRMS(ESI) m/z calcd. for [C₈H₁₅O₂N+H]⁺: 158.1176, obsd.: 158.1178.

5R-Amino-3S,4S-dihydroxy-cyclohex-1-ene (171): To a

solution of amino diene **172** (29.8 mg, 0.2 mmol) in H₂O (3 mL) was added 20 μ L of distilled TfOH. The solution was then concentrated under reduced pressure to give the ammonium triflate salt of diene **172**, and the triflate salt co-evaporated with dry toluene before being subjected to dry THF (1.9 mL) and the Hoveyda-Grubbs 2nd generation catalyst (6.0 mg, 0.01 mmol) under Ar at rt for 1 h. The reaction mixture was then concentrated *in vacuo* before purification by gradient silica gel flash column chromatography (DCM/EtOH/MeOH/35% NH_{3(aq.)}, 105/2/2/1 to 55/2/2/1, v/v/v/v) provided cyclohexene (**171**) as a pale oil (24.5 mg, 0.2 mmol, quant.). R_f = 0.16 (DCM/EtOH/MeOH/30% NH_{3(aq.)}, 15/2/2/1, v/v/v/v); $[\alpha]_D^{23}$ = +24.5 (c = 0.61, MeOH); IR (film) 3357, 3040, 2916, 1621, 1519, 1441, 1242, 1225, 1168, 1071, 1026, 949, 692, 638 cm⁻¹. ¹H NMR (500 MHz, D₂O) δ 5.71 (ddd, $J_{1,6b}$ = 2.4, $J_{1,6a}$ = 5.4, $J_{1,2}$ = 10.2 Hz, 1H, H-1), 5.57 (d, $J_{1,2}$ = 10.2 Hz, 1H, H-2), 4.17 (ddd, $J_{2,3}$ = 1.8, J = 3.6, $J_{3,4}$ = 7.8 Hz, 1H, H-3), 4.46 (dd, $J_{3,4}$ = 7.8, $J_{4,5}$ = 10.5 Hz, 1H, H-4), 3.17 (dt, $J_{5,6a}$ = 5.4, $J_{4,5}$ = $J_{5,6b}$ = 10.5 Hz, 1H, H-5), 2.50 (dt, $J_{1,6a}$ = $J_{5,6a}$ = 5.4, $J_{6a,6b}$ = 17.5 Hz, 1H, H-6a), 2.12 (dddd, $J_{1,6b}$ = 2.4, $J_{5,6b}$ = 10.5, J = 6.9, $J_{6a,6b}$ = 17.5 Hz, 1H, H-6b); ¹³C NMR (125 MHz, D₂O) δ 128.2 (C2), 125.6 (C1), 75.3 (C4), 72.0 (C3), 50.2 (C5), 30.5 (C6); HRMS(ESI) m/z calcd. for [C₆H₁₁O₂N+H]⁺: 130.0863, obsd.: 130.0865.

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Chapter 4: Lipophilic Amines as Potential TB Drugs

4.1 Introduction

4.1.1 Tuberculosis and the need for New Anti-TB Drugs

Tuberculosis (TB) is a global health problem. According to the WHO Global TB report¹ there were an estimated 8.6 million new cases of TB worldwide in 2012. Of the 1.3 million TB deaths in 2012, approximately a quarter of these were from HIV positive TB sufferers. It was also estimated that approximately one third of the multi drug resistant (MDR) cases of TB were fatal. If left untreated, approximately 70% of infected (HIV negative) individuals will die within ten years of contracting the disease.²

The increasing drug resistance of *Mtb* and the inherent difficulty in killing the bacteria makes the continued development of TB drugs a pressing goal. Most drugs for TB currently in use were developed more than forty years ago and it is only in the last two years that any new anti-TB drugs have come onto the market.^{1,3} The development of new anti-TB drugs with unique modes of action are required to prevent further drug resistance arising. To this end, a protecting-group-free (PGF) approach amenable to the efficient synthesis of potential TB drugs will be described.

4.1.2 Alkenylamines as Potential TB drugs

The anti-TB drug ethambutol (EMB) and drug candidate SQ109 (Figure 4.1) are both inhibitors of cell wall biosynthesis, although they act on different enzymatic pathways.^{4,5} Key features of EMB and SQ109 are the secondary amine groups and the amphiphilic nature of the molecules, both of which are presumed to be important for penetration through the lipophilic cell wall, and anti-TB activity. Following on from this, it was proposed that alkenylamines **191** (Scheme 4.1), as structural analogues of EMB and SQ109, would also possess anti-TB activity.

Figure 4.1: Emerging tuberculosis drug SQ109, and current first line TB drug EMB.

Previously within the Stocker-Timmer group, a first generation amine library was prepared and screened in a tuberculostatic Alamar Blue[®] BCG assay (Scheme 4.1).⁶ A colour change in the Alamar Blue[®] dye indicated the ability of a compound to inhibit the growth of the mycobacteria BCG (see Section 4.3).^{7,8} From this initial screening, alkenylamine **191a** was identified as having modest growth inhibition (MIC = 500 $\mu\text{g/mL}$) against BCG.⁶ This compound was readily prepared in three steps without the use of protecting groups and provided a molecular scaffold around which to base the synthesis of other potential TB drugs. To this end, a variety of alkenylamines **191b-i** were prepared via a PGF synthesis that involved few competing reactivities.⁶ Here the alkenylamines **191** were made via a modified Vasella-reductive amination of iodoglycosides **192** with a primary amine as the amine source. Iodoglycosides **192** were in turn prepared from the corresponding sugars **193** by Fischer glycosidation followed by iodination of the primary hydroxyl.⁹

Initial trends for compounds **191a-i** indicated the arabinose derived alkenylamines to be slightly more active than the xylose or ribose derived compounds, and that long straight chain amines gave slightly better activity than the aromatic alkenylamines. The latter finding may be because mycobacteria have very thick cell walls consisting of mycolic acids,¹⁰ suggesting that the alkenylamines containing more lipophilic amine groups are better able to penetrate the lipophilic cell wall.

Scheme 4.1: First generation amine library, with the minimum inhibitory concentration (MIC) for growth inhibition against BCG,⁶ NI = no inhibition at or below 500 µg/mL.

4.2 Second Generation Amine Library Synthesis

To further explore the structure activity relationship (SAR) of the lipophilic amines, a second generation amine library was prepared. The BCG Alamar Blue[®] Growth Inhibition Assay^{7,8} was used as a model for the growth inhibition of tuberculosis and, for the most promising compounds, cytotoxicity studies were also undertaken. The molecular scaffold **194** around which the second generation amine library was based is shown in Figure 4.2. Variations in antimycobacterial activity induced by changes in the structure at C4 and C5 were investigated. Here, C4/5 alkene or dihydroxylated analogues were prepared using Vasella-reductive amination and reductive amination conditions, respectively. The use of arabinose, fucose and rhamnose derivatives in the reductive aminations provided variation in the stereochemistry of the hydroxyls, while the presence of an additional methyl group at R¹ occurred in the fucose and rhamnose analogues. The use of different amines in the reductive aminations provided access to a range of alkyl groups. Linear and cyclic amines of sizes ranging from C6 to C16 were prepared. The variety of amine and sugar scaffolds used in the reactions aimed to provide a better understanding of how molecular size, three-dimensional shape, hydrophobicity, and sugar configuration affect antimycobacterial activity.

Figure 4.2: Molecular scaffold for the second generation amine library.

The purpose of these syntheses was to prepare a large library of compounds, hence the optimisation of the reaction conditions would only be undertaken for the most active compounds. Initial reaction yields varied considerably due to variations in solubility and reactivity of the sugar and amine components and difficulty in separating the product from the unreacted amine during purification. A variety of conditions were used to encourage product formation for specific reductive amination reactions. Early literature precedent on the formation of related (unreduced) glycosylamines with long (C₁₂-C₁₈) lipid amines reported stirring mixtures of amine and sugar in *i*PrOH/H₂O or EtOH/H₂O at rt for 2–15 days,¹¹ while up to C₁₂ glucosylamines (**195**) were reported by stirring a suspension of sugar **196** and amine **197** in MeOH at rt overnight (Scheme 4.2).¹² To form the corresponding reduced amines, changes in solvent (DMSO/AcOH^{13,14} to MeOH/H₂O); reducing agent (NaCNBH₃, NaBH(OAc)₃,¹⁵ NaBH₄,¹⁶ ZnCl₂¹⁷) or hydrogenation,^{12,18,19} time of addition (one-pot or two-steps-one-pot); temperature (rt to reflux); and means of heating (conventional or microwave irradiation¹⁴) have all been reported. A variety of these literature procedures were trialled accordingly.

Scheme 4.2: Example amination reaction to form *N*-dodecylglucosylamine (**195**).¹²

To begin preparation of the second generation amine library, a series of reductive aminations of D-arabinose (**198**) using several different amines was undertaken (Table 4.1). In this way the amine products could be compared with the alkenylamines (made via Vasella-reductive aminations) to test the importance of

the double bond on biological activity. A wide variety of amines were used to study the influence of the size and shape of the amine on anti-mycobacterial activity. To explore whether cyclic amines also inhibit mycobacterial growth, a variety of cyclic amines were used in reductive aminations with arabinose. To this end cyclohexylamine **199**, cyclooctylamine **200**, adamantylamine **201**, and cyclododecylamine **202** were subjected to the reductive amination conditions yielding amines **203**, **204**, **205**, and **206**, respectively, in low (14-45%) yields (entries 1-4). Key to the reaction was the use of EtOH as the solvent for optimal solubility of all reagents, whileß AcOH was also important to aid the solubility of the larger amines by protonation. The use of the amine as the limiting reagent avoided difficulties in separating unreacted amine and product amine during purification.

Table 4.1: Reductive amination reactions

Entry	Sugar	Amine	Product	Reaction Time	Yield
1				23 h	43%
2				4 h	45%
3				20 h	14%
4				5 h	26%
5 ^a			 208 + 209	21 h	208: 42% 209: 10%
6 ^b			 208 + 210	18 h	208: 34% 210: 4%

Entry	Sugar	Amine	Product	Reaction Time	Yield
7	17	199	212	3 d	30%
8	17	200	213	22 h	12%
9	17	202	214	7 d	18%
10	21	200	216	19 h	10%

^a Reaction carried out in MeOH at 45 °C, some formaldehyde was present in the reagent grade solvent. ^b Reaction carried out in EtOH at 50 °C.

To study the effect of lipophilicity on biological activity, the reductive amination of D-arabinose (**198**) with hexadecylamine (**207**) was carried out (to give **208**), providing a larger linear amine for anti-mycobacterial testing (entries 5 and 6). Because of difficulties in solubilising the amine and sugar simultaneously, a variety of solvents (EtOH, DCM, PE, EtOH/DCM, DMSO/AcOH, DMSO/EtOH/H₂O, MeOH) and reaction temperatures (ranging from rt to reflux) were trialled. Step-wise synthesis through imine formation followed by reduction was then attempted in refluxing methanol and AcOH, however this led to a rapid degradation of the sugar. Gratifyingly, it was discovered that both the amine and the sugar were soluble in MeOH (7 mL/mmol amine) with AcOH (0.4 mL/mmol amine) at *ca.* 45 °C (entry 5); the additional AcOH and the more dilute conditions helped to protonate and solubilise the amine. The reaction mixture was stirred overnight with NaCNBH₃, achieving the synthesis of amine **208** in 42% yield. In addition to the successful synthesis of the desired arabinohexadecylamine **208**, methylated by-product **209** was also formed as a result of further reductive amination of formaldehyde (impurity from the reagent grade MeOH) with amine

208. The solvent was subsequently changed to EtOH (entry 6), and in the absence of formaldehyde, the formation of methylamine **209** was not observed. Potentially as a result of the improved solubility of the D-arabinose (**198**) relative to amine **207** in EtOH, diarabinoamine **210** was made via subsequent reductive amination of arabinohexadecylamine **208** with another molecule of D-arabinose (**198**). Although unexpected, methylamine **209** and diarabinoamine **210** were successfully isolated in sufficient quantities, which expanded the scope of the reaction and thus provided additional amines for anti-mycobacterial testing.

In the workup of amine **208**, removal of the borane reducing agent was required to stop the additional reductions that led to by-product formation. A variety of workup techniques that were tested (lyophilisation, Dowex-H⁺ chromatography, reversed phase chromatography) led to either insolubility of the amine, or excessive foaming on the rotary evaporator, due to the amphiphilic amine **208** having soap-like properties in water. Fortunately removal of the boranes using methanolic HCl (5%, v/v) was successful and the resulting crude material could be purified by silica column chromatography. Separation of the amines was achievable with careful purification via normal phase chromatography and the amphiphilic arabinohexadecylamine **208** was successfully purified by taking advantage of the amine's marginal solubility in the DCM:EtOH:MeOH:35% NH_{3(aq)} solvent mixture (as amine **208** was insoluble in many solvents). Hexadecylamine **208** eluted slowly from the column, over a gradient from 25:2:2:1 to 5:2:2:1 DCM:EtOH:MeOH:35% NH_{3(aq)}, which led to a slightly reduced yield when the synthesis of **208** was later optimised (see Section 5.4).

Characterisation of the amines was straight-forward, with a correlation in the HMBC spectrum observed between the C1 of the sugar and C1' of the amine lipid for almost all amines prepared. Arabinocyclododecylamine **206** and diarabinoheptylamine **210** had no observable correlation, however the connectivity of these amines was supported by the downfield shift of the C1' of the amine upon binding to the sugar. The amine with two sugars coupled (**210**) was evidenced by a much lower running spot on TLC compared to

arabinohexadecylamine **208** ($R_f = 0.17$ for **210** *cf.* $R_f = 0.40$ for **208** in DCM/EtOH/MeOH/35% $\text{NH}_3(\text{aq})$, 5/2/2/1, v/v/v/v), as well as a change in the peak integrations in the ^1H NMR spectrum, and a much larger molecular mass (m/z : $[\text{C}_{26}\text{H}_{55}\text{O}_8\text{N}+\text{H}]^+$ calcd. = 510.4004, obsd. = 510.4004). Methylamine **209** could be identified by a higher running spot on TLC ($R_f = 0.57$ in DCM/EtOH/MeOH/35% aqueous NH_3 , 5/2/2/1, v/v/v/v), as well as the appearance of a singlet in the ^1H NMR spectrum (δ 2.93) that had HMBCs to both H5ab and H1' confirming the presence of the methyl substituent. The mass spectrum (m/z : $[\text{C}_{22}\text{H}_{47}\text{O}_4\text{N}+\text{H}]^+$ calcd. = 390.3578, obsd. = 390.3594) further supports the identification of amine **209**.

To investigate how the polyhydroxylated backbone of the compounds affect anti-mycobacterial activity, the reductive amination products of L-fucose (**211**) and cyclohexylamine (**199**), cyclooctylamine (**200**), and cyclododecylamine (**202**) were also prepared to give amines **212**, **213**, and **214**, respectively (entries 7-9, Table 4.1). L-Fucose has the same stereochemistry as D-arabinose at C2-4, and has *S*-stereochemistry defined at C5 due to the additional (C6) methyl group present. Comparison of the L-fucosylamines and D-arabinosylamines gives an indication of how the additional methyl group and 5*S* stereochemistry affects activity. The L-rhamnose (**215**) scaffold, with very different stereochemistry to both L-fucose and D-arabinose was also used for the preparation of L-rhamnocyclooctylamine **216** to further expand the amine library (entry 10).

To complete the second generation amine library, alkenylamines were prepared using Vasella-reductive amination conditions (Table 4.2). The arabino configuration was retained, as this was observed to be more active in the first generation alkenylamines, and amines of varying size and configuration were prepared. To this end, methyl iodoarabinoside (**217**) was treated with Zn, AcOH, adamantylamine (**201**), and NaCNBH_3 in refluxing EtOH, to give adamantyl alkenylamine **218** in 49% yield (entry 1). Cyclododecylamine **202** was next used to prepare alkenylamine **219** in modest 37% yield (entry 2), while the use of

dibutylamine **220**, allowed for the preparation of tri-substituted alkenylamine **221** (entry 3), which could be used to assess the effect of a tertiary amine with two shorter (C₄) alkyl groups in comparison to secondary amines with longer linear or cyclic amine groups attached. Two longer linear amines, tetradecylamine **222** and hexadecylamine **207**, were then used in the Vasella-reductive amination to prepare alkenylamines **223** and **224**, respectively (entries 4 and 5), to aid the understanding of how lipid length influences anti-mycobacterial activity (also see Figure 4.3). Fortunately, the alkenylamines were readily soluble in a variety of solvents. Isolation of the alkenylamines was challenging due to the similar polarities of the uncoupled amines and the product alkenylamines. The successful isolation of the alkenylamines was achieved with careful purification via silica column chromatography using a long column and eluting slowly (*ca.* DCM/MeOH/EtOH/35% NH_{3(aq.)}; 205:2:2:1 v/v/v/v), providing clean compounds ready for biological testing. The ¹H NMR spectrum of alkenylamine **223** is presented in Figure 4.3 and the others are available in the appendices.

Table 4.2: Products from the Vasella-reductive amination reaction of methyl iodoarabinoside **217** with various amines

Entry	Amine	Product	Yield
1	 201	 218	49%
2	 202	 219	37%
3	 220	 221	22%
4	 222	 223	44%
5	 207	 224	69%

Figure 4.3: ¹H NMR spectrum of arabino tetradecylalkenylamine **223** (CD₃OD, 500 MHz).

Finally, to explore the effect of *N*-methylation of an alkenylamine, a reductive amination of formaldehyde and hexadecylalkenylamine **224** was performed to give methylalkenylamine **225** in 73% yield (Scheme 4.3). Preparation of alkenylamine **225** allows for activity comparisons between the alkene and the ‘dihydroxylated’ analogue methylamine **209**, and also between the series of methylated (tertiary) and unmethylated (secondary) amines. Having prepared a variety of different amines and alkenylamines with varied stereochemistry, size, shape, and hydrophobicity, this completed the second generation library. The amines were thus submitted for biological testing and analysis.

Scheme 4.3: Reductive methylation to give alkenylamine **225**.

4.3 Biological Testing: Results and Discussion

4.3.1 Tuberculostatic Activity: BCG Alamar Blue® Growth Inhibition Screening

With the second generation amine library in hand, biological testing commenced with a whole cell BCG model for the screening of anti-tuberculosis activity. Named after its developers, Bacillus Calmette-Guérin (BCG) is a strain of *Mycobacterium bovis* that has been weakened by culturing on artificial medium for many years, and is therefore no longer virulent to humans.²⁰ As a species of mycobacteria, BCG is related to *Mtb* and hence displays very similar drug susceptibilities making it a good initial screen for anti-TB compounds.²¹ The BCG Alamar Blue® growth inhibition assay measures the smallest concentration of a compound that can effect growth inhibition of the bacteria (the minimum inhibitory concentration, MIC), providing an indication of the compound's tuberculostatic properties.^{22,23} In this work, the MIC is defined as the concentration of compound required to induce 100% growth inhibition, a fact worthy of mention as MIC values are sometimes reported as 50% or 90% inhibition.²⁴ The Alamar Blue® dye contains resazurin, a blue coloured, non-fluorescent, redox active dye (Figure 4.4). The dye is reduced to the pink, fluorescent resorufin, in the presence of NADH/NADPH (produced by cell respiration). Accordingly, while the bacteria are growing the dye will turn pink, but when treated with a compound that kills the bacteria or inhibits their growth, the dye will remain blue. The assay is safe, sensitive, and inexpensive.

Figure 4.4: Alamar Blue[®] dye redox activity.

Two methods were used to screen the second generation amine library. Initially, to identify any active compounds, four amines were tested on a 96-well plate over three serial dilutions, with each compound tested in triplicate for reproducibility. From this, any compound displaying activity was retested over a range of six serial dilutions (with two compounds to a plate) to get a more accurate measure of a compound's MIC (see Section 5.6.2 for details of the assay protocol). The significantly active compounds were screened twice more to confirm the MIC value; where these values differed, an MIC range was given. Ethambutol (EMB, entry 1, Table 4.3) was used as a positive control on each plate. EMB has an MIC of 39 μM in this assay, which is comparable to (if not slightly larger than) the measured MIC *in vitro* for *Mtb* (H37Rv, MIC = 2.4 μM)²⁵ and BCG (MIC = 9.8 μM).²⁶ Arabinooctylalkenylamine (**191i**, entry 2) the lead compound from the first generation library, with an MIC of 105 $\mu\text{g/mL}$ or 1400 μM , is also reported in Table 4.3 for activity comparisons. The starting material amines cyclododecylamine **202**, hexadecylamine **207**, and tetradecylamine **222** were also tested to identify how the sugar backbone altered the anti-TB activity of the amines. These also display activity in the BCG model (entries 10, 14, and 19, respectively); however the product amines and alkenylamines derived from these generally displayed improved activity.

Table 4.3: BCG Alamar Blue[®] growth inhibition assay

Entry	Compound	MIC (μM)	Entry	Compound	MIC (μM)
1	EMB (Control)	39 μM	12	214	680 μM
2	191i	1400 μM	13	219	200 μM
3	203	NI	14	207	130 μM
4	204	NI	15	208	83-170 μM
5	212	NI	16	209	20-40 μM
6	213	NI	17	210	61 μM
7	216	NI	18	221	NI
8	205	NI	19	222	140-290 μM
9	218	NI	20	223	45 μM
10	202	680 μM	21	224	21-41 μM 300 μM ^a 30 μM ^b
11	206	160 μM	22	225	22 μM

NI = no inhibition at or below 250 μg/mL. ^a Tested on *M. smegmatis*. ^b Tested on *M. tuberculosis* H37Ra.²⁷

For the reductive amination products it was seen that cyclohexylamines and cyclooctylamines, when coupled to arabinose (to give amines **203** and **204**, entries 3 and 4, respectively), fucose (to give **212** and **213**, entries 5 and 6, respectively) or rhamnose (**216**, entry 7) were inactive in the assay. This suggests that with the smaller cyclohexyl- or cyclooctylamines attached, the amines may not be lipophilic enough to pass through the hydrophobic bacterial cell wall. It is notable that the linear arabinooctylalkenylamine **191i** (entry 2) was more active than the cyclooctylamines tested. This may be due to reduced hydrophobicity, or reduced flexibility of the cyclic amines, which affects their ability to penetrate the cell wall. The idea that more conformationally restrained amines are less active is supported by the observation that both adamantyl amine **205** and adamantyl alkenylamine **218** (entries 8 and 9) were also inactive, even though adamantylamine has two additional carbons compared with octylamine lead **191i**.

The larger cyclic amines, arabinocyclododecylamine **206**, fucocyclododecylamine **214**, and arabinocyclododecylalkenylamine **219** (entries 11-13) showed more promising anti-mycobacterial activity. Both *arabino* configured amines **206** (160 μ M) and **219** (200 μ M) were more active than the fucose derived cyclododecylamine **214** (680 μ M), which had activity comparable to that of uncoupled cyclododecylamine **202** (entry 10). It was also observed that the presence or absence of the double bond in the molecules did not significantly affect activity (compare entry 11 with entry 13). Alternatively, this could be viewed as an indication that the addition of the two terminal hydroxyls in the reductive amination series also does not affect anti-mycobacterial activity.

The linear amines **208**, **209**, and **210** (entries 15-17) had improved activity compared with the cyclododecylamines. Methylamine **209** (20 – 40 μ M) was the most active, with activity comparable to that of first line TB drug EMB (39 μ M).

The amine with two sugars attached (**210**) had slightly reduced activity (61 μ M), and arabinohexadecylamine **208** (83-170 μ M) was *ca.* half as active as methylamine **209**. The reduced activity of hexadecylamine **208** may be due to insolubility, a result of the amphiphilic nature of the compound. As the amphiphilic amine was only soluble in DMSO at *ca.* 80 °C, or partially soluble in MeOH at 50 °C, the delivery the amine to the bacteria in the assay was possibly affected.

Dibutylalkenylamine **221** was inactive at or below 250 μ g/mL (entry 18), while its linear isomer, octylalkenylamine **191i**, had some activity (1400 μ M, 105 μ g/mL). This supports that suggestion that spatial arrangement and lipophilicity are important for activity. To determine how lipid length affects activity tetradecylalkenylamine **223** (45 μ M) and hexadecylalkenylamine **224** (21-41 μ M) were found to have comparable activity (entries 20-21). The addition of two more carbons to the amine group does not significantly improve the activity of hexadecylalkenylamine **224** compared to that of tetradecylalkenylamine **223**. These alkenylamines also have similar activity to methylamine **209**.

Finally, methylated alkenylamine **225** was tested for comparisons with alkenylamine **224** and methylamine **209**. Methylated alkene **225** had an MIC of 22 μ M (entry 22) which is comparable to the dihydroxylated analogue **209** (20 – 40 μ M) again indicating that the alkene functionality does not confer improved anti-mycobacterial activity. By comparison of alkenes **224** and **225** it appears that N-methylation does not significantly improve activity, except in cases where it aids solubility (as observed in hexadecylamine **208** and the methylated counterpart **209**).

To further test the scope and selectivity of the amines, one of the alkenylamine leads, hexadecylalkenylamine **224**, was also tested for growth inhibition against

other mycobacteria - *Mycobacterium smegmatis* (*M. smegmatis*) and an avirulent *Mtb* strain, H37Ra, by collaborators from the O'Toole group in the School of Biological Sciences (VUW).²⁷ Although *M. smegmatis* is known to be more resistant than TB,²¹ it is often used as a compound screening model as it is a faster growing bacterium than BCG, and hence gives more rapid feedback in drug screening. Alkenylamine **224** was found to have an MIC of 300 μ M against *M. smegmatis*, which is less active than against BCG (21-41 μ M). When tested against H37Ra bacteria, hexadecylalkenylamine **224** was shown to have improved activity (MIC = 30 μ M), which is promising for the class of compounds, as H37Ra is a strain of *Mtb*, and thus a more accurate predictor of activity than either BCG or *M. smegmatis*.

In terms of the ability of the amines to inhibit *Mtb* growth, there are a few general observations that can be made. First, the lipid size and shape appear to be important for anti-mycobacterial activity. This is evidenced by the reduced activity of the smaller and more conformationally restrained cyclic and adamantyl amines compared with the linear tetradecyl- and hexadecylamines. This may be due to the linear amines being more hydrophobic and therefore more able to pass through the waxy cell wall of *Mtb* to inhibit cell growth. The preferred linear structure may also make the amines more flexible and improve their ability to get inside the cell. Here, it should be noted that related compounds with various sugar and alkylamine components have been previously noted for both their surfactant properties and anti-fungal and anti-microbial activity.^{12,16,28} As for the chirality and number of hydroxyl groups on the hydrophilic portion of the compounds and how these influence activity, the initial studies from the first generation library suggest that the arabinose configuration is more active than the xylose or ribose configured alkenylamines. In the second generation amine library, it is unclear whether the arabinose configuration is preferred over rhamnose, as both cyclooctylamine derivatives (**204** and **216**) were inactive in the assay. The cyclododecylamine fucose derivative **214** was significantly less active than arabinocyclododecylamine **206**, suggesting that the arabinose scaffold was

preferred over the fucose scaffold. To gain a better understanding of the SAR with respect to sugar configurations, further chiral scaffolds of the most active amines (*e.g.* tetradecyl- or hexadecylamines) and a larger variety of sugars could be prepared and screened for anti-mycobacterial activity. It was also noted that an alkene group is not necessary for activity, as illustrated by alkenes **219** and **225**, which have similar activities to those of the corresponding dihydroxylated amines **206** and **209**. This finding is important because the dihydroxy amines can be prepared from sugars in a single-step synthesis, an improvement on the three steps required to make alkenylamines using the Vasella-reductive amination conditions.

4.3.2 Anti-proliferative Activity and Selectivity

Compounds that showed promising anti-mycobacterial activity in the BCG assay were screened for cytotoxicity using anti-proliferative HL60 and human T cell MTT assays. These give an indication of the selectivity of the compounds by measuring toxicity against a cancer cell line (HL60) and a healthy human blood cell line (T cells). The choice of cell model for cytotoxicity screening varies depending on the aims of the research and the drug target. Generally immortalised cell lines or primary cell lines are used as human cytotoxicity models.²⁹ The human leukaemia cell line HL60 is an immortalised cell line that is non adherant, inexpensive, easy to grow, and reproducible. A downside of immortalised cell lines is that they are not always representative of native cell types.³⁰ Primary cells lines (such as the T cells), on the other hand, are very similar to their native cells, but have a short culture lifespan, and are more difficult to grow.²⁹ For a potential anti-TB drug a low MIC against BCG is required as well as a high 50% inhibition concentration (IC_{50}) for the human T cells. The IC_{50} for the HL60 cell line gives a further indication of the selectivity of the drug, and a low IC_{50} against the HL60 cell line, coupled with a high IC_{50} for T cells may indicate a promising anti-cancer drug. To quantify the selectivities for comparison of the properties of different amines, the selectivity index (SI) was calculated, whereby a larger SI indicates a better selectivity for the diseased cells over the normal human cells. For anti-mycobacterial selectivity:

$$SI = \frac{IC_{50}(Tcells)}{MIC(BCG)}$$

And for anti-cancer selectivity:

$$SI = \frac{IC_{50}(Tcells)}{IC_{50}(HL60)}$$

As a comparison for the anti-mycobacterial activity, cytotoxicities and selectivities, first line TB drug EMB and Doxorubicin (DOX), a clinically used anti-cancer drug, were included in Table 4.4 (entries 1 and 2).

Table 4.4: Second generation amine library - biological testing overview

Entry	Compound	Alamar Blue [®] BCG inhib. - MIC (μM)	Cytotoxicity – T cells IC ₅₀ (μM)	Cytotoxicity – HL60 cells IC ₅₀ (μM)	BCG SI (T cells) (IC ₅₀ / MIC)	Cancer SI (Tcell/HL60)	cLogP
1	EMB (Control)	39	380	160	9.7	2.4	0.12
2	DOX (Control)	31-62	3.3- 4.5	1.3- 1.7	0.053- 0.15	1.9- 3.5	-0.44
3	191i	1400	280	44-85	0.20	3.3- 6.4	3.2
4	202	680	21	16	0.031	1.3	4.7
5	206	160	48	26-95	0.30	0.51 -1.8	4.0
6	214	680	86	44	0.13	2.0	4.3
7	219	200	45	32-80	0.13	0.56 -1.4	4.9

Entry	Compound	Alamar Blue [®] BCG inhib. - MIC (μM)	Cytotoxicity - T cells IC ₅₀ (μM)	Cytotoxicity - HL60 cells IC ₅₀ (μM)	BCG SI (T cells) (IC ₅₀ / MIC)	Cancer SI (Tcell/HL60)	cLogP
8	207	130	47-82	19-20	0.36- 0.63	2.4- 4.3	7.3
9	208	83- 170	11-20	7.1- 9.2	0.065- 0.24	1.2- 2.8	6.9
10	209	20-40	7.7- 9.5	3.3- 4.1	0.19- 0.48	1.9- 2.9	7.2
11	210	61	24-31	2.5	0.39- 0.51	9.6-12	5.1
12	222	140- 290	27	11-78	0.093- 0.19	0.35- 2.5	6.2
13	223	45	12	6.4-13	0.27	0.92- 1.9	6.4
14	224	21-41	6.0- 9.7	3.4- 4.3	0.15- 0.46	1.4- 2.9	7.4
15	225	22	9.4-11	4.2- 4.9	0.43- 0.50	1.9- 2.6	8.1

SI = selectivity index, calculated as described in Section 5.3.2. cLogP = computational octanol/water partition coefficient, as calculated by ChemDrawStd v.11.0[®].

A correlation between anti-mycobacterial activity and cytotoxicity (HL60 and T cells) was observed for the amines and alkenylamines in Table 4.4. The large linear amines and alkenylamines showed greater inhibition towards the human and bacterial cells compared to the smaller and/or cyclic amines. Comparison of the biological properties of the first generation lead octylalkenylamine **191i** showed minimal cytotoxicity against HL60 cancer cells or human T cells, which corresponds to the limited growth inhibition observed for this compound against mycobacteria. The cyclododecylamine series (entries 5-7), regardless of the sugar attached, were less cytotoxic, displaying moderate anti-proliferative activity against HL60 (IC_{50} = 26-95 μ M) and T cells (IC_{50} = 45-86 μ M), in line with the moderate anti-mycobacterial activity noted earlier for these compounds. It is noteworthy that uncoupled cyclododecylamine **202** (entry 4) is more cytotoxic towards both cell lines than all corresponding reductive amination products. Poorer selectivity for bacterial cells (SI = 0.031) was also observed for cyclododecylamine **202** compared to amines **206**, **214**, and **219** (SI = 0.13-0.30).

The trend of increasing cytotoxicity or growth inhibition towards all cell lines was seen across the second generation amine library. The hexadecylamine reductive amination products **208** to **210** (entries 9-11), while having improved anti-mycobacterial activity, also had increased anti-proliferative activity against both HL60 cells (IC_{50} = 2.5-9.2 μ M) and T cells (IC_{50} = 7.7-31 μ M). The uncoupled hexadecylamine **207** (entry 8) also followed the trend with moderate toxicities observed across the anti-mycobacterial and anti-proliferative assays. The selectivity of amines **207** to **210** for the bacteria over the human T cells was significantly reduced when compared to EMB (SI < 0.65 *cf.* EMB SI = 9.7). Linear alkenylamines tetradecylalkenylamine **223**, hexadecylalkenylamine **224**, and hexadecylmethylalkenylamine **225** (entries 13-15) also continued the toxicity trend with the amines showing promising anti-mycobacterial activity (MIC = 21-45 μ M), but also cytotoxicity that may limit therapeutic application (HL60 IC_{50} = 3.4-13 μ M, T cell IC_{50} = 6.0-12 μ M) resulting in poor selectivities for BCG (SI < 0.50).

The biological profile for cancer drug Doxorubicin (DOX) is also useful to frame the parameters important in cancer drug selectivity (entry 2). Doxorubicin is active against BCG (MIC = 31-62 μ M), however as it is also toxic to human T cells, the selectivity for the bacteria is poor (SI = 0.053-0.15). The cancer drug is highly toxic to HL60 cells and the selectivity for cancer cells (Cancer SI = 1.9-3.5) suggests some preference for the fast growing HL60 cells over the T cells. Although some selectivity is observed, doxorubicin is known to have poor selectivity for tumour tissues, and has noted side effects as a result of this narrow therapeutic window.³¹

Most compounds in the second generation library display anti-cancer selectivity similar to that of doxorubicin. In particular, hexadecylalkenylamines **224** and **225** had reasonable anti-proliferative activity against HL60 cells (IC₅₀ = 3.4-4.9 μ M). However, as this cytotoxicity is still approximately three times that of doxorubicin (IC₅₀ = 1.3-1.7 μ M) it is unlikely that these amines are active enough to be successful anti-cancer drugs.

The best anti-cancer selectivity observed was for diarabinohexadecylamine **210** (SI (T cell/HL60) = 9.6-12, entry 11), with a selectivity index that was significantly improved compared to that of anti-cancer drug doxorubicin (SI = 1.9-3.5), which is quite remarkable. There could be potential for this compound as an anti-cancer rather than anti-TB drug, and further biological testing of amine **210** is needed to establish its anti-cancer properties.

4.3.3 Lipinski's Rule of Five and cLogP

Lipinski's rule of five for oral bioavailability outlines four requirements for drug-likeness. It states that compounds with molecular weight < 500 g/mol, no more than five hydrogen bond donors, ten or less hydrogen bond acceptors, and $\text{cLogP} \leq 5$ are likely to be better absorbed into target cells within the body.³² These rules were derived from observations of already known drugs and therefore are not absolute. Lipinski's rule of five is only applicable to compounds that are delivered orally and pass through the cell membrane by passive diffusion.

The LogP value gives an indication of the lipophilicity of a compound and therefore its ability to reach the desired target within the cell.^{32,33} The octanol/water partition coefficient, P, is the ratio of compound that would be partitioned in the octanol layer vs. the water layer, so a LogP value between 1 and 5 indicates that a compound is 10 to 100000 times more soluble in octanol than water, thus $\text{LogP} > 5$ indicates an extremely lipophilic compound, that will generally have poor solubility in the blood, and not be freely available for passive transport across membranes in the body. $\text{LogP} < 1$ implies a more hydrophilic compound that may be too soluble in water, and therefore fail to displace water from binding pockets. LogP values are rarely determined experimentally due to the large amount of compound needed, and the computational octanol/water partition coefficient (cLogP) is therefore used instead. cLogP is a computational prediction for LogP, which combines atomic contributions from smaller fragments of the compound with measured LogP values to predict the LogP for a given molecule. A variety of different algorithms have been used to calculate computational LogP values, using different parameters and molecular contribution libraries. The accuracy of these cLogP values vary,^{34,35} however cLogP can still be qualitatively applied to a series of molecules to predict trends, and give an estimation of a compound's bioavailability. For compounds of the second generation amine library, cLogP was calculated using ChemDrawStd v.11.0[®] (Table 4.4).

Interestingly, across the second generation amine library increasing anti-mycobacterial activity corresponds with increasing cLogP values. The most active compounds were observed to have $\text{cLogP} > 5$. This indicates that the amines are highly lipophilic, and therefore likely to have reduced oral bioavailability. The trend in improved anti-mycobacterial activity corresponding with increased lipophilicity of the amines was noted earlier and is presumably due to the improved ability of the amines to penetrate the waxy mycobacterial cell wall. In this case, the lipophilicity that conveys activity of the amines is also responsible for the high cLogP values. Although the cLogP values for the active second generation amines are disappointing, these values are only an estimation, and as with the rest of Lipinski's rules, there are exceptions. Therefore the *in vivo* testing of the most active compounds should be pursued and will provide a better indication of oral bioavailability. It is also worth considering an alternative method of drug delivery,³⁶ other than an oral route, that could be applied to help the amine drug candidate reach its target in the body. For example, when applying Lipinski's rule of five to the anti-cancer drug Doxorubicin, the cLogP is -0.44 and DOX has too many H-bond donors and acceptors, thus it would not be expected to be bioavailable as an oral drug. Indeed, DOX is not orally bioavailable and is therefore administered intravenously.³⁷

From the second generation amine library, the only other exception to Lipinski's rule of five is the potential anti-cancer drug, diarabinohexadecylamine **210**, which has a molecular weight of 509 g/mol, and eight hydrogen bond donor atoms. Given that the mechanism of action of these compounds is currently unknown, it could be that these lipophilic amines are membrane disruptors (indeed other amphiphilic molecules have been found to act as membrane disruptors)³⁸ and therefore Lipinski's rule of five would not apply. Thus determination of the mechanism of action of potential anti-cancer candidate amine **210** should be a priority.

4.3.4 Alternative Measures of Drug-likeness

As discussed in the previous section, Lipinski's rule of five suggested that the lead compounds from the second generation amine library are too lipophilic for oral bioavailability; however due to exceptions to Lipinski's rules, and it has been suggested that molecules that pass on three of the four the rules should not be discounted from drug discovery efforts.³⁹ Additionally, since the development of Lipinski's rules, there has been many other selection criteria suggested for identifying drug-like molecules. Some of the alternative drug-likeness predictors have been described in this section.

Polar surface area (PSA) is the sum of the surface area of the polar atoms within a molecule. Kelder *et al.* have linked PSA to the bioavailability of a drug-like molecule.⁴⁰ For drugs that are not active in the central nervous system (*i.e.* drugs that do not cross the blood-brain barrier) a PSA below 120 Å² is preferred, while central nervous system active drugs require a PSA less than 60-70 Å².

Another set of rules for determining drug-likeness was established by Ghose *et al.*⁴¹ After quantitative analysis of a different molecular library, a cLogP between -0.4 and 5.6, and molecular weight in the range 160 – 480 g/mol were identified. In addition the total number of atoms (20 to 70) and the property of molar refractivity (the polarizability of a bulk substance, between 40 and 130) were also included. The total number of atoms in a molecule is important, as it relates to molecular weight, while the molar refractivity relates to the size and polarity of the bulk compound, hence relating to its solubility and absorption properties.

Oprea identified other properties important to predicting drug-likeness.⁴² In addition to the properties identified in Lipinski's rule of five, the number of rotatable bonds, and the number of rings in a molecule were considered important as they reflect the complexity of a molecule with respect to its flexibility and rigidity. It was established that 8 or fewer rotatable bonds, and 4 or fewer rings were necessary to maintain drug-like properties in a molecule.

A simplified model for drug-likeness was established by measurements of over 1100 drug candidates at GlaxoSmithKline,⁴³ wherein Veber *et al.* suggested that molecular flexibility and polarity are key properties in predicting oral bioavailability. From this it was proposed that compounds that contain 10 or fewer rotatable bonds, and a PSA less than 140 Å² (or fewer than 13 H-bond donors and acceptors) were likely to have good oral bioavailability.

Consideration of these alternative measures of bioavailability with respect to the second generation amine library could be used to provide further insight on the drug-likeness of the lead compounds. The PSA for amine leads **208**, **209**, **223**, **224**, and **225** ranged from 44 to 93 Å² according to the programme Daylight.⁴⁴ However, diarabinoheptadecylamine **210** had a PSA of 165 Å², which exceeds the guideline of PSA < 140 Å². The molar refractivity of the amines is also anticipated to be large, due to the amphiphilic nature of the molecules. Furthermore, the lead amines would be very flexible due to the presence of more than eight rotatable bonds. Overall, there is a mixture of positive and negative factors influencing the bioavailability of the amines. Ultimately, the best predictor of bioavailability is success in an animal model for the disease. Future biological testing of the second generation amine leads will be discussed in the following section.

4.3.5 Future Biological Testing

Amine **209**, and alkenylamines **223**, **224**, and **225** were identified as having the best anti-mycobacterial activity from the second generation amine library (MIC = 20-45 µM). This activity is comparable to that of first line TB drug EMB (MIC = 39 µM) in the BCG Alamar Blue[®] assay, and up to a 70-fold improvement in the activity of the first generation lead octylalkenylamine **191i** (MIC = 1400 µM) was achieved. Methylamine **209** was identified as the most promising target for further biological testing due to its simple, one-step synthesis with comparable activity to

the alkenylamines (which require a three-step preparation), making it a more attractive target.

The unmethylated analogue of methylamine **209**, arabinohexadecylamine **208** was slightly less active in the BCG growth inhibition assay (MIC = 83-170 μ M). This may be due to solubility, as the amphiphilic nature of amine **208** makes delivery to the bacterial cells more challenging. Despite its reduced anti-mycobacterial activity, the pursuit of hexadecylamine **208** as an anti-TB drug candidate was also of interest, to determine any marked difference between the *in vitro* and *in vivo* activity, and for SAR comparisons with methylamine **209**. The pharmacokinetics of a compound can greatly influence its potential as a drug, and although amines **208** and **209** have promising anti-TB activity, cytotoxicity may limit therapeutic potential of these amines. That said, although *in vitro* screening gives a good starting point for lead optimisation in drug discovery, as it is a model, *in vivo* work is a better predictor of anti-TB activity and cytotoxicity. An example of this was the discovery of first line TB drug pyrazinamide, which is inactive *in vitro*, however *in vivo* activity studies were undertaken regardless, and the compound was found to be highly active.⁴⁵ Both arabinohexadecylamine **208** and methylamine **209** can be made remarkably quickly and efficiently as the syntheses have been optimised to provide amines **208** and **209** on a 250 mg scale (see Section 5.4). These amines have recently been sent to the group of Assoc. Prof. Anne Lenaerts at Colorado State University for *in vivo* testing in a mouse model of tuberculosis.

Diarabinohexadecylamine **210**, is of interest for its impressive anti-cancer selectivity (SI = 9.6-12). As it does not obey Lipinski's rule of five, it may not be orally bioavailable, or it may indeed be a membrane disruptor hence determination of the mechanism of action of amine **210**, and indeed this entire class of amines is of interest. An investigation into the *in vivo* anti-cancer activity of diarabinohexadecylamine **210** would also be beneficial.

4.3.6 Related Amphiphilic Amines

A number of amphiphilic amines with relevant biological activity related to lead compounds **208**, **209**, and **210** have been reported (Figure 4.5). The surfactant properties of these amphiphilic molecules (*e.g.* their ability to form micelles) were also frequently reported. Glucose derived dodecylamine **226** was shown to suppress protein adsorption, and therefore reduce platelet adhesion when this molecule was used as a surfactant on polypropylene nonwoven fabrics. Amphiphilicity at the fabric surface is unfavourable for protein accumulation, and this property can have biomedical applications.⁴⁶ Analogues of methylamine **208** and diarabinohexadecylamine **210**, were also shown to have biological activity, with methylated 2-hydroxy-dodecylamine **227** and glucose dimer **228** being patented for use in formulation as surfactants for plant growth regulators, herbicides or pesticides.⁴⁷ The molecules were shown to improve activity of the agrichemicals in formulation. Another related molecule with biological activity is lactose derived amine **229**, which was identified as having antibacterial and antifungal activity.^{16a,28b} Although this molecule has a slightly different structure, the amphiphilic nature and biological activity of the molecule is maintained. Gemini surfactant **230** maintains the same components as amphiphilic amines **208** to **210** but in a different structural arrangement. Due to their charged, amphiphilic nature, gemini surfactants have lower critical micelle concentrations compared to the corresponding monomeric amines such as **226**. The surfactant **230** was also shown to have promising antimicrobial and antifungal activity,⁴⁸ and such molecules are thought to convey biocidal activity through adsorption of the alkylammonium cation to the cell surface followed by diffusion into the cell and disruption of the cytoplasmic membrane ultimately leading to cell death.⁴⁹ 3-Deoxy-3-dodecylamino-allose **231** was identified as an inhibitor or promoter of plant growth, depending on the concentration of amine used.⁵⁰ At a concentration of 2 mM, plant growth was inhibited, while at a much lower concentration of 0.1 mM, the plant growth rate was increased by approximately 40% compared to an untreated control. It is interesting to note that despite the use of a ring closed sugar of a different stereochemistry attached to the amine at a different position,

biological activity in the form of plant growth inhibition was still observed. This may suggest that the bioactivity of these amines, and second generation leads **208** to **210**, is not structure specific or due to favourable binding interactions, but rather general activity across the class of molecules due to the lipophilic/surfactant properties of these molecules.

Figure 4.5: Biologically active lipophilic amines.

Finally, another amphiphilic amine, 1-deoxysphingoid **232**, was recently extracted from a marine alga.⁵¹ This amine differs from the amine leads **208** to **210** however it maintains a hydrophilic part, an amine group, and a lipid portion. Amine **232** was identified as having activity against *Mtb* H37Ra (IC₅₀ = 19.4 μ M) and HL60 cells (IC₅₀ = 8.8 μ M) suggesting a general cytotoxicity of the compound, similar to that observed in the second generation amine library. Indeed, 1-deoxysphingosines have been previously identified as having a broad range of antimicrobial and cytotoxic activity.⁵² To inform on the mode of action of sphingosine **232**, the compound was screened in a yeast knock out assay, against a library of 4800 nonessential gene deletion mutants of the yeast *Saccharomyces cerevisiae*.⁵¹ Genes involved in lipid metabolism and actin cytoskeleton organisation were identified as possible targets for amine **232**. This could potentially inform on the mode of action of the amphiphilic amines in general, however both lipid metabolism and actin cytoskeleton organization pathways had been previously implicated in sphingolipid activity and therefore may be targets specific to the class of molecules.

A variety of amphiphilic amines have been discussed in this section. Some amines, such as **226** to **228**, are close analogues to the amine leads **208** to **210**, while others are more varied, while maintaining the key sugar and lipophilic amine portions that define the class of molecules. Growth inhibition or cytotoxicity of the amines was observed in a variety of different organisms including bacteria, fungi, yeast and plants suggesting that this class of amphiphilic amines displays non-specific inhibitory activity. This is in agreement with the activity observed in the testing of the second generation amine library (Section 5.3.2). These observations support the suggestion that the molecules may be acting non-specifically as membrane disruptors. While some drugs work through membrane disruption pathways, specificity in a drug is essential, and the general bioactivity observed for this class of molecules against a range of different organisms suggests a lack of specificity. Confirmation of the mechanism of action of these molecules would therefore be beneficial.

4.4 Optimised Syntheses of Lead Compounds

The synthesis of arabinohexadecylamine **208** was optimised by the addition of excess hexadecylamine **207** (10 equiv.) with EtOH (that did not contain the formaldehyde impurity) as the solvent to give amine **208** in 78% yield (Scheme 4.4). As the imine formation step is in equilibrium, it follows that the large excess of amine **207** pushes the equilibrium in favour of imine formation. By favouring the imine formation, the D-arabinose (**198**) is rapidly used up in the reaction, reducing the likelihood of the product amine **208** forming and then attacking remaining sugar **198**, to form the diarabinohexadecylamine **210** by-product. The difference in polarities of the reacting amine **207** and the amphiphilic arabinohexadecylamine **208** was significant hence chromatographic separation of these was straightforward.

Scheme 4.4: Optimised synthesis of arabinohexadecylamine **208**.

The optimised synthesis of methylamine **209** also required a large excess of amine **207**. To this effect, 10 equiv. of hexadecylamine **207** in methanol was reacted with D-arabinose (**198**) and a small amount of NaCNBH₃ first to form amine **208** *in situ* before the addition of 20 equiv. formaldehyde (as a 37% aqueous solution), and then additional reducing agent, portion-wise to form methylamine **209** (Scheme 4.5). This could be isolated in an excellent 90% yield, and also was easily separable from the excess amine.

Scheme 4.5: Optimised synthesis of arabinohexadecyl(methyl)amine **209**.

4.5 Conclusion

A variety of different amines have been successfully synthesised and isolated using Vasella-reductive amination and reductive amination conditions. The amine yields vary considerably, due to challenges in solubility of the amine and sugar components and difficulty in purification of the product amines. In the interest of rapidly finding a new lead compound for drug discovery, only the syntheses of lead amines hexadecylamine **208** and methylamine **209** were optimised. The optimisation provided clean compounds **208** and **209** in excellent yields of 78% and 90%, respectively.

Biological activity and bioavailability data was reported for the second generation library. The compounds were tested for anti-mycobacterial activity in the BCG Alamar Blue[®] growth inhibition assay. Longer, linear alkylamines were observed to be more active than shorter or more conformationally restricted alkylamines (*e.g.* cyclic or adamantylamines), and the alkene functionality present in the alkenylamines did not significantly affect activity. Methylation of the secondary amine only appeared to aid activity when this also improved solubility. Lead amine **209**, and alkenylamines **223** to **225** were identified as the most active compounds, and when compared with the first generation lead compound **191i**, up to a 70-fold improvement in activity was achieved, giving the amines activity comparable to that of first line TB drug ethambutol. Additionally, alkenylamine **224** exhibited good (30 μ M) inhibition against an avirulent *Mtb* strain, H37Ra, and this is a promising result for the class of compounds as a whole.

Further biological testing and bioavailability studies revealed a trend in toxicities where increased cytotoxicity was observed with increased anti-mycobacterial activity. This implies the amines are generally toxic, rather than selective for mycobacteria, and this was supported by a review of related bioactive amphiphilic amines in the literature. Bioavailability studies revealed that the more active amines and alkenylamines fail Lipinski's rule of five based on $cLogP > 5$. Amine

210 also failed based on molecular weight > 500 g/mol, and the presence of greater than five hydrogen bond donors.

The second generation compound library has shown varied toxicity towards both cancer cells and human T cells, thus there is scope for tuning these compounds to improve their activity towards HL60 cancer cells. Diarabinohexadecylamine **210** is a promising example of this; with remarkable selectivity for cancer cells over T cells (SI = 9.6-12) and investigations into possible collaborations for the *in vivo* testing of the amine in a cancer model are underway.

Finally, arabinohexadecylamine **208** and arabinohexadecylmethylamine **209** were identified as promising anti-mycobacterial leads, and the single-step syntheses of these compounds were optimised. The *in vivo* testing of these amines in a mouse model of *Mtb* is underway at Colorado State University and results will be reported in due course.

4.6 Experimental

General Methods

Unless otherwise stated, all reactions were performed under atmospheric air. H₂O, MeOH (Pure Science), and EtOH (absolute, Pure Science), were distilled prior to use. EtOAc (Pure Science), and petroleum ether (Pure Science), HCl (PanReac), AcOH (Ajax Finechem), DCM (LabServ), 35% aqueous NH₃ (LabServ), AcCl (B&M), NaCNBH₃ (Aldrich), NH₄OAc (AnalaR), D-arabinose (Sigma-Aldrich), L-fucose (Sigma-Aldrich), L-rhamnose (Sigma-Aldrich), cyclohexylamine (Riedel), cyclooctylamine (Aldrich), 1-adamantylamine (Sigma), cyclododecylamine (Aldrich), hexadecylamine (Aldrich), formaldehyde (Sigma-Aldrich), di-*n*-butylamine (Sigma), and tetradecylamine (Aldrich) were used as received. All chemicals obtained from commercial suppliers were used without further purification. Zn dust was activated by the careful addition of concentrated H₂SO₄, followed by decantation and washing with EtOH (3×) and hexanes (3×) and storage under dry hexanes. All solvents were removed by evaporation under reduced pressure. Reactions were monitored by TLC analysis on Macherey-Nagel silica gel coated plastic sheets with detection by coating with 20% H₂SO₄ 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% KMnO₄ and 1% NaIO₄ in H₂O followed by heating. Column chromatography was performed on Pure Science silica gel (40-63 micron). Dowex® W50-X8 acidic resin (Sigma) and Dowex® 1X4-50 basic resin (Sigma) were used for ion exchange chromatography and HP-20 (Supelco) for reverse phase chromatography. High-resolution mass spectra were recorded on a Waters Q-TOF PremierTM Tandem Mass Spectrometer using positive electro-spray ionisation. Optical rotations were recorded using an Autopol II (Rudolf Research Analytical) at 589 nm (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 at 20 °C in D₂O, CD₃OD,

pyridine-d₅, CDCl₃, or DMSO-d₆ at (80 °C) using either a Varian Unity-INOVA operating at 300 MHz or a Varian Unity operating at 500 MHz. Chemical shifts are given in ppm (δ) and were internally referenced to the residual solvent peak. NMR peak assignments are based on 2D NMR experiments (COSY, HSQC, and HMBC).

4.6.1 Synthetic Experimental

(203): To a solution of D-arabinose (156 mg, 1.0 mmol) in EtOH (20 mL), was added cyclohexylamine (89 mg, 0.9 mmol), NaCNBH₃ (194 mg, 3 mmol), water (0.5 mL), and AcOH (0.07 mL). The reaction was stirred at room temperature for 23 h. The solvent was then removed under reduced pressure, the residue redissolved in H₂O and loaded onto Dowex-H⁺ cation exchange resin. The product was eluted with 25% aqueous NH₃, to give a white solid (90 mg, 0.39 mmol, 43%) which was then acidified with 1 M HCl and concentrated to afford 5-cyclohexylamino-5-deoxy-D-lyxitol hydrochloride **(203)**. $R_f = 0.16$ (DCM/EtOH/MeOH/30% NH_{3(aq.)}, 15/2/2/1, v/v/v/v); $[\alpha]_D^{25} = +12.8$ (c = 1.0, EtOH); IR (film) 3324, 2935, 2858, 1636, 1455, 1032, 896 cm⁻¹. ¹H NMR (500 MHz, D₂O) δ 4.17 (dd, $J_{4,5} = 3.2$, $J_{3,4} = 8.8$ Hz, 1H, H-4), 3.80 (dd, $J_{1a,2} = 2.6$ Hz, $J_{1a,1b} = 11.7$ Hz, 1H, H-1a), 3.70 (ddd, $J_{1a,2} = 2.6$, $J_{1b,2} = 5.8$, $J_{2,3} = 8.7$ Hz, 1H, H-2), 3.63 (dd, $J_{1b,2} = 5.8$, $J_{1a,1b} = 11.7$ Hz, 1H, H-1b), 3.48 (d, $J_{3,4} = 8.8$ Hz, 1H, H-3), 3.25-3.19 (m, 2H, H-5), 3.18-3.11 (m, 1H, H-1'), 2.10-2.04 (m, 2H, H-2'a), 1.82 (d, $J = 12.5$ Hz, 2H, H-3'a), 1.64 (d, $J = 13.2$ Hz, 2H, H-4'a), 1.42-1.25 (m, 4H, H-2'b, H-3'b) 1.18-1.11 (m, 2H, H-4'b); ¹³C NMR (125 MHz, D₂O) δ 71.0 (C3), 70.4 (C2), 65.9 (C4), 62.7 (C1), 57.5 (C1'), 47.3 (C5), 28.9, 28.5 (C2'), 24.4 (C4'), 23.9, 23.9 (C3'); HRMS(ESI) m/z calcd. for [C₁₁H₂₃O₄N+H]⁺: 234.1700, obsd.: 234.1705.

5-cyclooctylamino-5-deoxy-D-lyxitol (204): To a solution of D-arabinose (100 mg, 0.67 mmol) in EtOH (13.3 mL), was added cyclooctylamine (77 mg, 0.60 mmol), NaCNBH₃ (126 mg, 2 mmol), and water (0.4 mL). The reaction was stirred at room temperature for 4 h. The solvent was then removed under reduced pressure, and the residue loaded onto Dowex-H⁺ cation exchange resin and eluted with 5-35% NH₃ in EtOH/H₂O to give 5-deoxy-5-cyclooctylamino-5-deoxy-D-lyxitol (**204**) as a white solid (70 mg, 0.27 mmol, 45%). *R_f* = 0.18 (DCM/EtOH/MeOH/30% NH_{3(aq.)}, 15/2/2/1, v/v/v/v); [α]_D²⁵ = +4.9 (c = 1.0, EtOH); IR (film) 3348, 2925, 2857, 1642, 1469, 1446, 1054, 879 cm⁻¹. ¹H NMR (500 MHz, CD₃OD) δ 3.97 (td, *J*_{3,4} = 2.1, *J*_{4,5} = 6.2 Hz, 1H, H-4), 3.79 (dd, *J*_{1a,2} = 3.2, *J*_{1a,1b} = 10.9 Hz, 1H, H-1a), 3.66 (ddd, *J*_{1a,2} = 3.2, *J*_{1b,2} = 5.8, *J*_{2,3} = 8.0 Hz, 1H, H-2), 3.62 (dd, *J*_{1b,2} = 5.8, *J*_{1a,1b} = 10.9 Hz, 1H, H-1b), 3.42 (dd, *J*_{3,4} = 2.1, *J*_{2,3} = 8.0 Hz, 1H, H-3), 2.83 (d, *J*_{4,5} = 6.2 Hz, 2H, H-5), 2.83-2.79 (m, 1H, H-1'), 1.87-1.73 (m, 4H, H-2'-5'), 1.61-1.59 (m, 4H, H-2'-5'), 1.59-1.46 (m, 6H, H-2'-5'); ¹³C NMR (125 MHz, CD₃OD) δ 74.3 (C3), 73.0 (C2), 69.7 (C4), 64.9 (C1), 59.2 (C1'), 51.0 (C5), 33.0, 28.1, 26.9, 25.3 (C2'-5'); HRMS(ESI) *m/z* calcd. for [C₁₃H₂₇O₄N+H]⁺: 262.2013, obsd.: 262.2016.

5-adamantylamino-5-deoxy-D-lyxitol hydrochloride (205): To a solution of D-arabinose (100 mg, 0.67 mmol) in EtOH (3.4 mL), was added 1-adamantanamine (121 mg, 0.80 mmol), NaCNBH₃ (126 mg, 2 mmol), water (0.34 mL), and AcOH (0.05 mL). The reaction was stirred at room temperature for 20 h. The solvent was then removed under reduced pressure, and the residue loaded onto Dowex-H⁺ cation exchange resin and eluted with 5-35% NH₃ in EtOH/H₂O. Further purification by C₈ reverse phase chromatography (H₂O to H₂O/MeOH, 1/1, v/v) and then gradient flash chromatography (DCM/EtOH/MeOH/35% NH_{3(aq.)}, 75/2/2/1 to 35/2/2/1, v/v/v/v), followed by acidification with 1M HCl and then concentration afforded 5-

adamantylamino-5-deoxy-D-lyxitol hydrochloride (**205**) as a white solid (30 mg, 0.09 mmol, 14%). R_f = 0.09 (DCM/EtOH/MeOH/30% $\text{NH}_3(\text{aq.})$, 15/2/2/1, v/v/v/v); $[\alpha]_{\text{D}}^{25}$ = +6.6 (c = 0.27, EtOH); IR (film) 3365, 2916, 2854, 1645, 1455, 1365, 1310, 1071, 1020 cm^{-1} . ^1H NMR (500 MHz, D_2O) δ 4.13 (td, $J_{3,4}$ = 1.7, $J_{4,5}$ = 6.4 Hz, 1H, H-4), 3.82 (dd, $J_{1a,2}$ = 2.8, $J_{1a,1b}$ = 11.7 Hz, 1H, H-1a), 3.71 (ddd, $J_{1a,2}$ = 2.8, $J_{1b,2}$ = 5.9, $J_{2,3}$ = 8.9 Hz, 1H, H-2), 3.65 (dd, $J_{1b,2}$ = 5.9, $J_{1a,1b}$ = 11.7 Hz, 1H, H-1b), 3.50 (dd, $J_{3,4}$ = 1.7, $J_{2,3}$ = 8.9 Hz, 1H, H-3), 3.18 (d, $J_{4,5}$ = 6.4 Hz, 1H, H-5), 2.19 (br s, 3H, H-3'), 1.95-1.87 (m, 6H, H-2'), 1.74 (d, $J_{4'a,4'b}$ = 12.2 Hz, 3H, H-4'a), 1.65 (d, $J_{4'a,4'b}$ = 12.2 Hz, 3H, H-4'b); ^{13}C NMR (125 MHz, D_2O) δ 71.1 (C3), 70.4 (C2), 66.2 (C4), 62.7 (C1), 57.8 (C1'), 42.7 (C5), 37.8 (C2'), 34.8 (C4'), 28.7 (C3'); HRMS(ESI) m/z calcd. for $[\text{C}_{15}\text{H}_{27}\text{O}_4\text{N}+\text{H}]^+$: 286.2013, obsd.: 286.2022.

5-cyclododecylamino-5-deoxy-D-lyxitol

hydrochloride (206): To a solution of D-arabinose (100 mg, 0.67 mmol) in EtOH (13.3 mL), was added cyclododecylamine (243 mg, 1.33 mmol), NaCNBH_3 (126 mg, 2 mmol), and water (0.4 mL). The reaction was stirred at room temperature for 5 h then solvent removed under reduced pressure. The product was purified first by gradient flash chromatography (DCM/EtOH/MeOH/35% $\text{NH}_3(\text{aq.})$, 305/2/2/1 to 5/2/2/1, v/v/v/v), followed Dowex- H^+ cation exchange (5-35% NH_3 in EtOH/ H_2O) to give a white solid, **206**, as the HCl salt upon acidification and subsequent concentration (61 mg, 0.17 mmol, 26%). R_f = 0.24 (DCM/EtOH/MeOH/30% $\text{NH}_3(\text{aq.})$, 15/2/2/1, v/v/v/v); $[\alpha]_{\text{D}}^{25}$ = +8.1 (c = 0.93, EtOH); IR (film) 3330, 2929, 2862, 1576, 1471, 1446, 1074 cm^{-1} . ^1H NMR (500 MHz, CD_3OD) δ 4.20-4.17 (m, 1H, H-4), 3.80 (dd, $J_{1a,2}$ = 2.8, $J_{1a,1b}$ = 10.5 Hz, 1H, H-1a), 3.68 (ddd, $J_{1a,2}$ = 2.8, $J_{1b,2}$ = 5.4, $J_{2,3}$ = 8.1 Hz, 1H, H-2), 3.65 (dd, $J_{1b,2}$ = 5.4, $J_{1a,1b}$ = 10.5 Hz, 1H, H-1b), 3.47 (dd, $J_{3,4}$ = 1.7, $J_{2,3}$ = 8.1 Hz, 1H, H-3), 3.31 (dt, $J_{1',2'a}$ = 1.6, $J_{1',2'b}$ = 3.2 Hz, 1H, H-1'), 3.21 (dd, $J_{4,5a}$ = 8.4, $J_{5a,5b}$ = 12.8 Hz, 1H, H-5a), 3.18 (dd, $J_{4,5b}$ = 4.4, $J_{5a,5b}$ = 12.8 Hz, 1H, H-5b), 1.91-1.78 (m, 2H, H-2'a), 1.74-1.66 (m, 2H, H-2'b), 1.57-1.35 (m, 18H, H-3'-H-7'); ^{13}C NMR (125

MHz, CD₃OD) δ 73.3 (C3), 72.7 (C2), 67.4 (C4), 64.6 (C1), 57.9 (C1'), 49.7 (C5), 27.3, 26.7 (C2'), 25.7, 25.6, 25.4, 24.1, 24.0, 23.9, 21.6, 21.3 (C3'-C7'); HRMS(ESI) m/z calcd. for [C₁₇H₃₅O₄N+H]⁺: 318.2639, obsd.: 318.2648.

hexadecylamino-D-lyxitol hydrochloride (208) and 5-deoxy-5-hexadecylmethylamino-D-lyxitol hydrochloride (209): To a solution of D-arabinose (200 mg, 1.3 mmol) in MeOH (16.8 mL), was added hexadecylamine (574 mg, 2.4 mmol), NaCNBH₃ (126 mg, 2 mmol), and AcOH (0.96 mL). The reaction was stirred at 45 °C for 21 h then the solvent was removed under reduced pressure to give **208**, and **209** as a by-product. These could be separated by silica column chromatography (DCM/EtOH/MeOH/35% NH_{3(aq.)}, 105/2/2/1 to 35/2/2/1, v/v/v/v) to give **208** as a white solid (211.2 mg, 0.56 mmol, 42%,) and **209**, also as a white solid that was converted into the HCl salt by addition of HCl (1.2 M, aq.) and concentration (50 mg, 0.13 mmol, 10%).

5-deoxy-5-hexadecylamino-D-lyxitol hydrochloride (208): R_f = 0.40 (DCM/EtOH/MeOH/35% NH_{3(aq.)}, 5/2/2/1, v/v/v/v); IR (film) 3268, 2917, 2849, 1467, 1093, 1040, 878 cm⁻¹. ¹H NMR (300 MHz, DMSO-d₆, 80 °C) δ 3.78-3.71 (m, 1H, H-4), 3.60 (dd, $J_{1a,2}$ = 3.7, $J_{1a,1b}$ = 10.4 Hz, 1H, H-1a), 3.50 (ddd, $J_{1a,2}$ = 3.7, $J_{1b,2}$ = 5.7, $J_{2,3}$ = 7.5 Hz, 1H, H-2), 3.42 (dd, $J_{1b,2}$ = 5.7, $J_{1a,1b}$ = 10.4 Hz, 1H, H-1b), 3.31 (dd, $J_{3,4}$ = 2.5, $J_{2,3}$ = 7.5 Hz, 1H, H-3), 2.71-2.62 (m, 2H, H-5), 2.59-2.52 (m, 2H, H-1'), 1.47-1.36 (m, 2H, H-2'), 1.36-1.21 (m, 26H, H-3'-H-15'), 0.87 (t, $J_{15',16'}$ = 6.8 Hz, 3H, H-16'); ¹³C NMR (75 MHz, DMSO-d₆, 80 °C) δ 72.9 (C3), 71.7 (C2), 68.5 (C4), 63.5 (C1), 52.4 (C5), 48.9 (C5), 30.9, 28.6 (br. s), 26.4, 21.6 (C2'-C15'), 13.5 (C16'); HRMS(ESI) m/z calcd. for [C₂₁H₄₅O₄N+H]⁺: 376.3421, obsd.: 376.3421.

5-deoxy-5-hexadecylmethylamino-D-lyxitol hydrochloride (209): R_f = 0.57 (DCM/EtOH/MeOH/35% NH_{3(aq.)}, 5/2/2/1, v/v/v/v); $[\alpha]_D^{28}$ = +8.0 (c = 1.0, MeOH); IR (film) 3333, 2953, 2918, 2850, 1467, 1081, 1033, 736 cm⁻¹. ¹H NMR (300 MHz, CD₃OD) δ 4.29 (d, J = 9.3 Hz, 1H, H-4), 3.80 (app. d, J = 8.0 Hz, 1H,

H-1a), 3.72-3.60 (m, 2H, H-1b and H-2), 3.48-3.34 (m, 2H, H-3 and H-5a), 3.26-3.10 (m, 3H, H-1' and H-5b), 2.93 (s, 3H, N-Me), 1.84-1.69 (m, 2H, H-2'), 1.46-1.24 (m, 26H, H-3'-H-15'), 0.90 (t, $J_{15',16'} = 6.6$ Hz, 3H, H-16'); ^{13}C NMR (125 MHz, CD_3OD) δ 71.4 (C3), 71.2 (C2), 64.7 (C4), 63.3 (C1), 55.5 (C5), 56.6 (C1'), 40.2 (N-Me), 31.7, 29.4, 29.4, 29.3, 29.2, 29.1, 28.9, 26.3, 22.3 (C3'-C15'), 23.8 (C2'), 13.1 (C16'); HRMS(ESI) m/z calcd. for $[\text{C}_{22}\text{H}_{47}\text{O}_4\text{N}+\text{H}]^+$: 390.3578, obsd.: 390.3594.

hexadecylamino-D-lyxitol hydrochloride (208) and 5-deoxy-5-hexadecylamino-bis-D-lyxitol (210): To a solution of D-arabinose (197 mg, 1.3 mmol) in EtOH (16.8 mL), was added hexadecylamine (576 mg, 2.4 mmol), NaCNBH_3 (124 mg, 2.0 mmol), and AcOH (0.96 mL). The reaction was stirred at 50 °C for 18 h then the solvent was removed under reduced pressure. The crude reaction mixture was co-evaporated with 2 x 25 mL of 5% HCl in distilled methanol (w/v), then purified by gradient flash silica chromatography (DCM/EtOH/MeOH/35% $\text{NH}_3(\text{aq.})$, 105/2/2/1 to 15/2/2/1, v/v/v/v) to give **208** as a white solid (168.6 mg, 0.45 mmol, 34%) and **210**, also a white solid, as a by-product (14 mg, 0.03 mmol, 4%). Amine **208** was subsequently acidified and concentrated to give the HCl salt for characterisation.

5-deoxy-5-hexadecylamino-bis-D-lyxitol (210): $R_f = 0.17$ (DCM/EtOH/MeOH/35% $\text{NH}_3(\text{aq.})$, 5/2/2/1, v/v/v/v); $[\alpha]_{\text{D}}^{26} = +9.0$ (c = 1.0, pyridine); IR (film) 3357, 2954, 2922, 2853, 1466, 1203, 1135, 1080 cm^{-1} . ^1H NMR (500 MHz, Pyridine- d_5) δ 4.98-4.89 (m, 1H, H-4), 4.59-4.54 (m, 1H, H-2), 4.52 (dd, $J_{1a,2} = 3.8$, $J_{1a,1b} = 10.7$ Hz, 1H, H-1a), 4.39 (dd, $J_{1b,2} = 5.6$, $J_{1a,1b} = 10.7$ Hz, 1H, H-1b), 4.32 (d, $J_{2,3} = 8.0$ Hz, 1H, H-3), 3.27 (dd, $J_{4,5a} = 7.9$, $J_{5a,5b} = 12.9$ Hz, 1H, H-5a), 3.12 (dd, $J_{4,5b} = 5.4$, $J_{5a,5b} = 12.9$ Hz, 1H, H-5b), 2.78-2.65 (m, 2H, H-1'), 1.63-1.54 (m, 2H, H-2'), 1.32-1.15 (m, 26H, H-3'-H-15'), 0.88 (t, $J_{15',16'} =$

6.9 Hz, 3H, H-16'); ^{13}C NMR (125 MHz, Pyridine- d_5) δ 73.9 (C3), 73.5 (C2), 68.2 (C4), 65.5 (C1), 59.4 (C5), 56.5 (C1'), 32.5, 30.4, 30.3, 30.1, 30.0, 27.9, 23.3 (C3'-C15'), 26.6 (C2'), 14.6 (C16'); HRMS(ESI) m/z calcd. for $[\text{C}_{26}\text{H}_{55}\text{O}_8\text{N}+\text{H}]^+$: 510.4000, obsd.: 510.4004.

Optimised synthesis of 5-deoxy-5-hexadecylamino-D-lyxitol hydrochloride (208): To a solution of D-arabinose (100 mg, 0.67 mmol) in EtOH (46.6 mL), was added hexadecylamine (1.66 g, 6.9 mmol), NaCNBH_3 (62.1 mg, 1.0 mmol), and AcOH (2.6 mL). The reaction was stirred at 50 °C for 4 h then the solvent was removed under reduced pressure. The crude reaction mixture was co-evaporated with 2x 25 mL of 5% HCl in distilled methanol (w/v), then purified by gradient flash silica chromatography (DCM/EtOH/MeOH/35% $\text{NH}_3(\text{aq.})$, 105/2/2/1 to 15/2/2/1, v/v/v/v) to give **208** as a white solid (195 mg, 0.52 mmol, 78%).

Optimised synthesis of 5-deoxy-5-hexadecylmethylamino-D-lyxitol hydrochloride (209): To a solution of D-arabinose (101.5 mg, 0.67 mmol) in MeOH (46.6 mL), was added hexadecylamine (1.65 g, 6.8 mmol), NaCNBH_3 (64.8 mg, 1 mmol), and AcOH (2.6 mL). The reaction was stirred at 45 °C for 4 h before 1 mL of 37% aqueous formaldehyde (13.3 mmol) was added. After a further 3 h, additional NaCNBH_3 (840 mg, 13.3 mmol) was added and the reaction was left to stir overnight (16 h), then the solvent was removed under reduced pressure. The crude reaction mixture was co-evaporated with 2x 25 mL of 5% HCl in distilled methanol (w/v), then the residue was purified by gradient flash silica chromatography (DCM/EtOH/MeOH/35% $\text{NH}_3(\text{aq.})$, 105/2/2/1 to 85/2/2/1, v/v/v/v) affording **209** as a white solid that was converted into the HCl salt by addition of HCl (1.2 M, aq.) and concentration (236 mg, 0.61 mmol, 90%).

1-cyclohexylamino-1,6-dideoxy-L-galactitol (212):

To a solution of L-fucose (100 mg, 0.63 mmol) in EtOH (3 mL), was added cyclohexylamine (54 mg, 0.55 mmol), NaCNBH₃ (113 mg, 1.8 mmol), water (0.3 mL), and AcOH (0.04 mL). The reaction was stirred at room temperature for 3 d. The solvent was then removed under reduced pressure, and the residue loaded onto Dowex-H⁺ cation exchange resin and eluted with 5-35% NH₃ in H₂O. Gradient flash chromatography (DCM/EtOH/MeOH/35% NH_{3(aq.)}, 105/2/2/1 to 15/2/2/1, v/v/v/v) afforded 1-cyclohexylamino-1,6-dideoxy-L-galactitol (**212**) (41 mg, 0.17 mmol, 30%). *R_f* = 0.40 (DCM/EtOH/MeOH/30% NH_{3(aq.)}, 15/2/2/1, v/v/v/v); [α]_D²⁵ = +10.6 (c = 0.86, EtOH); IR (film) 3351, 2948, 2836, 1651, 1450, 1410, 1114, 1017 cm⁻¹. ¹H NMR (500 MHz, D₂O) δ 4.14 (ddd, *J*_{2,3} = 1.6, *J*_{1b,2} = 4.5, *J*_{1a,2} = 8.5 Hz, 1H, H-2), 4.10 (qd, *J*_{4,5} = 1.7, *J*_{5,6} = 6.7 Hz, 1H, H-5), 3.58 (dd, *J*_{2,3} = 1.6, *J*_{3,4} = 9.3 Hz, 1H, H-3), 3.47 (dd, *J*_{4,5} = 1.7, *J*_{3,4} = 9.3 Hz, 1H, H-4), 3.11 (dd, *J*_{1a,2} = 8.5, *J*_{1a,1b} = 13.0 Hz, 1H, H-1a), 3.09 (dd, *J*_{1b,2} = 4.5, *J*_{1a,1b} = 13.0 Hz, 1H, H-1b), 3.00-2.94 (m, 1H, H-1'), 2.07-2.01 (m, 2H, H-2'a), 1.84-1.77 (m, 2H, H-3'a), 1.69-1.64 (m, 1H, H-4'a), 1.34-1.28 (m, 4H, H-2'b, H-3'b), 1.25 (d, *J*_{5,6} = 6.7 Hz, 3H, H-6), 1.23-1.12 (m, 1H, H-4'b); ¹³C NMR (125 MHz, D₂O) δ 72.7 (C4), 70.9 (C3), 67.1 (C2), 65.8 (C5), 57.0 (C1'), 47.7 (C1), 29.9, 29.6 (C2'), 24.8 (C4'), 24.1, 24.1 (C3'), 18.6 (C6); HRMS(ESI) *m/z* calcd. for [C₁₂H₂₅O₄N+H]⁺: 248.1856, obsd.: 248.1866.

1-cyclooctylamino-1,6-dideoxy-L-galactitol

hydrochloride (213): To a solution of L-fucose (100 mg, 0.63 mmol) in EtOH (3 mL), was added cyclooctylamine (84 mg, 0.66 mmol), NaCNBH₃ (113 mg, 1.8 mmol), water (0.3 mL), and AcOH (0.04 mL). The reaction was stirred at room temperature for 22 h. The solvent was then removed under reduced pressure, and the residue loaded onto Dowex-H⁺ cation exchange resin and eluted with 5-35% NH₃ in H₂O. Gradient flash chromatography (DCM/EtOH/MeOH/35% NH_{3(aq.)},

105/2/2/1 to 55/2/2/1, v/v/v/v), followed by acidification with 1M HCl and concentration afforded 1-cyclooctylamino-1,6-dideoxy-L-galactitol hydrochloride (**213**) as a white solid (19 mg, 0.07 mmol, 12%). $R_f = 0.16$ (DCM/EtOH/MeOH/30% $\text{NH}_3(\text{aq.})$, 15/2/2/1, v/v/v/v); $[\alpha]_D^{25} = +10.5$ ($c = 1.0$, EtOH); IR (film) 3355, 2928, 2858, 1642, 1450, 1285, 1052, 1023 cm^{-1} . ^1H NMR (500 MHz, D_2O) δ 4.13 (ddd, $J_{2,3} = 1.6$, $J_{1b,2} = 4.4$, $J_{1a,2} = 9.1$ Hz, 1H, H-2), 4.01 (qd, $J_{4,5} = 1.7$, $J_{5,6} = 6.6$ Hz, 1H, H-5), 3.51 (dd, $J_{2,3} = 1.6$, $J_{3,4} = 9.4$ Hz, 1H, H-3), 3.39 (dd, $J_{4,5} = 1.7$, $J_{3,4} = 9.4$ Hz, 1H, H-4), 3.35 (tt, $J_{1',2'a} = 3.5$, $J_{1',2'b} = 9.3$ Hz, 1H, H-1'), 3.18 (dd, $J_{1a,2} = 9.1$, $J_{1a,1b} = 13.2$ Hz, 1H, H-1a), 3.14 (dd, $J_{1b,2} = 4.4$, $J_{1a,1b} = 13.2$ Hz, 1H, H-1b), 1.92-1.85 (m, 2H, H-2'a), 1.73-1.65 (m, 4H, H-2'b, H-3'a), 1.60-1.40 (m, 7H, H-3'b-5'a), 1.37-1.30 (m, 1H, H-5'b), 1.17 (d, $J_{5,6} = 6.6$ Hz, 3H, H-6); ^{13}C NMR (125 MHz, D_2O) δ 72.6 (C4), 70.7 (C3), 66.2 (C2), 65.6 (C5), 59.0 (C1'), 47.8 (C1), 29.2, 28.7 (C2'), 25.6, 25.6 (C4'), 25.2 (C5'), 23.3 (C3'), 18.6 (C6); HRMS(ESI) m/z calcd. for $[\text{C}_{14}\text{H}_{29}\text{O}_4\text{N}+\text{H}]^+$: 276.2169, obsd.: 276.2170.

1-cyclododecylamino-1,6-dideoxy-L-

galactitol hydrochloride (214): To a solution of L-fucose (165 mg, 1.0 mmol) in EtOH (20 mL), was added cyclododecylamine (294 mg, 1.5 mmol), NaCNBH_3 (189 mg, 3.0 mmol), water (0.5 mL), and AcOH (0.07 mL). The reaction was stirred at room temperature for 7 d. The solvent was then removed under reduced pressure, and the residue loaded onto Dowex- H^+ cation exchange resin and eluted with 5-35% NH_3 in EtOH/ H_2O . Reverse phase chromatography with HP20 beads (eluting in 10% to 100% MeOH), afforded a white solid (59.8 mg, 0.18 mmol, 18%) that was subsequently acidified with 1 M HCl and concentrated to provide 1-cyclododecylamino-1,6-dideoxy-L-galactitol hydrochloride (**214**) for characterisation. $R_f = 0.29$ (DCM/EtOH/MeOH/30% $\text{NH}_3(\text{aq.})$, 5/2/2/1, v/v/v/v); $[\alpha]_D^{25} = +6.0$ ($c = 0.29$, EtOH); IR (film) 3366, 2928, 2859, 1637, 1471, 1448, 1414, 1052, 996 cm^{-1} . ^1H NMR (500 MHz, D_2O) δ 4.21 (t, $J_{1,2} = 6.3$ Hz, 1H, H-2), 4.07 (q, $J_{5,6} = 6.6$ Hz, 1H, H-5), 3.56 (d, $J_{3,4} = 9.5$ Hz, 1H, H-3), 3.44 (d, $J_{3,4} =$

9.5 Hz, 1H, H-4), 3.32 -3.38 (m, 1H, H-1'), 3.24 (d, $J_{1,2} = 6.3$ Hz, 2H, H-1), 1.74-1.84 (m, 2H, H-2'a), 1.63-1.73 (m, 2H, H-2'b), 1.32-1.51 (m, 18H, H-3' - H-7'), 1.22 (d, $J_{5,6} = 6.6$ Hz, 3H, H-6); ^{13}C NMR (125 MHz, D_2O) δ 72.5 (C4), 70.7 (C3), 66.0 (C2), 65.6 (C5), 56.9 (C1'), 48.0 (C1), 25.7, 25.3, 24.0, 24.0, 23.9, 22.5, 22.3, 22.3, 22.2, 19.9, 19.7 (C2'-C7'), 18.5 (C6); HRMS(ESI) m/z calcd. for $[\text{C}_{18}\text{H}_{37}\text{O}_4\text{N}+\text{H}]^+$: 332.2795, obsd.: 332.2802.

1-cyclooctylamino-1,6-dideoxy-L-mannitol

hydrochloride (216): To a solution of L-rhamnose (100 mg, 0.63 mmol) in EtOH (5.5 mL), was added cyclooctylamine (62 mg, 0.49 mmol), NaCNBH_3 (104 mg, 1.7 mmol), water (0.3 mL), and AcOH (0.15 mL). The reaction was stirred at room temperature for 19 h. The solvent was then removed under reduced pressure, and the residue loaded onto Dowex- H^+ cation exchange resin and eluted with 5-35% NH_3 in EtOH/ H_2O . Gradient flash chromatography (DCM/EtOH/MeOH/35% $\text{NH}_{3(\text{aq})}$, 105/2/2/1 to 5/2/2/1, v/v/v/v), followed by acidification with 1M HCl and concentration afforded 1-cyclooctylamino-1,6-dideoxy-L-mannitol hydrochloride (**216**) as a white solid (16 mg, 0.05 mmol, 10%). $R_f = 0.36$ (DCM/EtOH/MeOH/30% $\text{NH}_{3(\text{aq})}$, 5/2/2/1, v/v/v/v); $[\alpha]_D^{25} = +1.3$ (c = 0.38, EtOH); IR (film) 2930, 2851, 1641, 1450, 1306, 1071, 1019 cm^{-1} . ^1H NMR (500 MHz, D_2O) δ 3.94 (ddd, $J_{1a,2} = 3.0$, $J_{2,3} = 8.2$, $J_{1b,2} = 9.5$ Hz, 1H, H-2), 3.82 (dq, $J_{5,6} = 6.3$, $J_{4,5} = 8.1$ Hz, 1H, H-5), 3.78 (dd, $J_{3,4} = 1.5$, $J_{2,3} = 8.2$ Hz, 1H, H-3), 3.51 (dd, $J_{3,4} = 1.5$, $J_{4,5} = 8.1$ Hz, 1H, H-4), 3.46-3.40 (m, 1H, H-1'), 3.41 (dd, $J_{1a,2} = 3.0$, $J_{1a,1b} = 13.0$ Hz, 1H, H-1a), 3.11 (dd, $J_{1b,2} = 9.5$, $J_{1a,1b} = 13.0$ Hz, 1H, H-1b), 1.98-1.90 (m, 2H, H-2'a), 1.81-1.70 (m, 4H, H-2'b, H-3'a), 1.67-1.45 (m, 7H, H-3'b-5'a), 1.42-1.35 (m, 1H, H-5'b), 1.25 (d, $J_{5,6} = 6.3$ Hz, 3H, H-6); ^{13}C NMR (125 MHz, D_2O) δ 72.9 (C4), 70.9 (C3), 66.9 (C2), 66.8 (C5), 59.0 (C1'), 47.5 (C1), 29.3, 28.6 (C2'), 25.6, 25.6 (C4'), 25.1 (C5'), 23.3, 23.3 (C3'), 18.9 (C6); HRMS(ESI) m/z calcd. for $[\text{C}_{14}\text{H}_{29}\text{O}_4\text{N}+\text{H}]^+$: 276.2169, obsd.: 276.2175.

General Procedure for the synthesis of alkenylamines

To a solution of methyl 5-deoxy-5-iodo- α/β -D-arabinoside (1 mmol) in EtOH (20 mL), was added amine (1.1 - 1.2 mmol), activated Zn (5 mmol), NaCNBH₃ (3 mmol), water (0.6 mL), and AcOH (0.3 mL). The reaction was stirred under reflux overnight (18 h), then cooled, filtered and the solvent removed under reduced pressure. The product was dry loaded on silica and purified by repeated gradient flash chromatography (DCM/EtOH/MeOH/35% NH_{3(aq.)}, 305/2/2/1 to 5/2/2/1, v/v/v/v).

(2*R*, 3*R*)-1-adamantylamino-pent-4-ene-2,3-diol

hydrochloride (218): Methyl 5-deoxy-5-iodo- α/β -D-arabinoside (134 mg, 0.49 mmol) and 1-adamantanamine (96 mg, 0.64 mmol) was subjected to the general procedure for the synthesis of alkenylamines. Further purification by C₈ reverse phase chromatography (H₂O to H₂O/MeOH, 1/4, v/v) followed by acidification with 1M HCl and concentration gave (2*R*, 3*R*)-1-adamantylamino-pent-4-ene-2,3-diol hydrochloride (**218**) as a white solid (70 mg, 0.24 mmol, 49%). R_f = 0.42 (DCM/EtOH/MeOH/30% NH_{3(aq.)}, 15/2/2/1, v/v/v/v); $[\alpha]_D^{25}$ = +24.9 (c = 1.0, EtOH); IR (film) 3364, 2915, 2855, 1644, 1454, 1365, 1310, 1071, 1022, 938 cm⁻¹. ¹H NMR (500 MHz, D₂O) δ 5.90 (ddd, $J_{3,4}$ = 5.9, $J_{4,5-cis}$ = 10.6, $J_{4,5-trans}$ = 17.2 Hz, 1H, H-4), 5.39 (d, $J_{4,5-trans}$ = 17.2 Hz, 1H, H-5-*trans*), 5.33 (d, $J_{4,5-cis}$ = 10.6 Hz, 1H, H-5-*cis*), 4.14 (dd, $J_{2,3}$ = 5.1, $J_{3,4}$ = 5.9 Hz, 1H, H-3), 3.84 (ddd, $J_{1a,2}$ = 2.6, $J_{2,3}$ = 5.1, $J_{1b,2}$ = 10.2 Hz, 1H, H-2), 3.18 (dd, $J_{1a,2}$ = 2.6, $J_{1a,1b}$ = 12.7 Hz, 1H, H-1a), 3.04 (dd, $J_{1b,2}$ = 10.2, $J_{1a,1b}$ = 12.7 Hz, 1H, H-1b), 2.22-2.17 (m, 3H, H-3'), 1.95-1.85 (m, 6H, H-2'), 1.75 (d, $J_{4'a,4'b}$ = 12.5 Hz, 3H, H-4'a), 1.66 (d, $J_{4'a,4'b}$ = 12.5 Hz, 3H, H-4'b); ¹³C NMR (125 MHz, D₂O) δ 135.4 (C4), 118.4 (C5), 73.9 (C3), 69.6 (C2), 57.9 (C1'), 41.7 (C1), 37.8 (C2'), 34.8 (C4'), 28.8 (C3'); HRMS(ESI) m/z calcd. for [C₁₅H₂₅O₂N+H]⁺: 252.1958, obsd.: 252.1961.

(2R, 3R)-1-cyclododecylamino-pent-4-ene-2,3-

diol hydrochloride (219): By subjecting methyl 5-deoxy-5-iodo- α/β -D-arabinoside (89.1 mg, 0.33 mmol) and cyclododecylamine (65.5 mg, 0.36 mmol) to the general procedure for the synthesis of alkenylamines, the (2R, 3R)-1-cyclododecylamino-pent-4-ene-2,3-diol (**219**) was obtained as the HCl salt, a white solid (38 mg, 0.12 mmol, 37%). $R_f = 0.69$ (DCM/EtOH/MeOH/30% $\text{NH}_3(\text{aq.})$, 35/2/2/1, v/v/v/v); $[\alpha]_D^{21} = -0.7$ (c = 1.0, EtOH); IR (film) 3335, 2932, 2863, 1642, 1471, 1446, 1096, 1025, 926, 719 cm^{-1} . ^1H NMR (500 MHz, CD_3OD) δ 5.98 (ddd, $J_{3,4} = 5.6$, $J_{4,5\text{-cis}} = 10.7$, $J_{4,5\text{-trans}} = 17.1$ Hz, 1H, H-4), 5.40 (d, $J_{4,5\text{-trans}} = 17.1$ Hz, 1H, H-5-*trans*), 5.26 (d, $J_{4,5\text{-cis}} = 10.5$ Hz, 1H, H-5-*cis*), 4.16-4.11 (m, 1H, H-3), 3.93-3.86 (m, 1H, H-2), 3.34-3.28 (m, 1H, H-1'), 3.18 (d, $J_{1a,1b} = 12.7$ Hz, 1H, H-1a), 3.05 (dd, $J_{1a,2} = 9.7$, $J_{1a,1b} = 12.7$ Hz, 1H, H-1b), 1.80 (ddd, $J = 7.3$ Hz, $J = 14.3$ Hz, $J = 22.0$ Hz, 2H, H-2'a), 1.75-1.64 (m, 2H, H-2'b), 1.58-1.34 (m, 18H, H-3' – H-7'); ^{13}C NMR (125 MHz, CD_3OD) δ 138.1 (C4), 117.3 (C5), 75.1 (C3), 70.5 (C2), 57.9 (C1'), 49.3 (C1), 27.3, 26.7, 25.7, 25.6, 25.4, 24.0, 24.0, 23.9, 23.9, 21.6, 21.3 (C2'–C6') HRMS(ESI) m/z calcd. for $[\text{C}_{17}\text{H}_{33}\text{O}_2\text{N}+\text{H}]^+$: 284.2584, obsd.: 284.2584.

(2R, 3R)-1-di-*n*-butylamino-pent-4-ene-2,3-diol (221):

By subjecting methyl 5-deoxy-5-iodo- α/β -D-arabinoside (100.2 mg, 0.37 mmol) and di-*n*-butylamine (74 μL , 0.44 mmol) to the general procedure for the synthesis of alkenylamines, the (2R, 3R)-1-di-*n*-butylamino-pent-4-ene-2,3-diol (**221**) was obtained as a white solid (19 mg, 0.08 mmol, 22%). $R_f = 0.90$ (DCM/EtOH/MeOH/30% $\text{NH}_3(\text{aq.})$, 35/2/2/1, v/v/v/v); $[\alpha]_D^{20} = +48.3$ (c = 0.82, CHCl_3); IR (film) 3370, 3086, 2957, 2931, 2872, 2863, 2821, 1644, 1467, 1378, 1305, 1267, 1163, 1069, 994, 921, 735 cm^{-1} . ^1H NMR (500 MHz, CDCl_3) δ 5.92 (ddd, $J_{3,4} = 5.8$, $J_{4,5\text{-cis}} = 10.5$, $J_{4,5\text{-trans}} = 17.3$ Hz, 1H, H-4), 5.38 (ddd, $J_{3,5\text{-trans}} =$

1.5, $J_{5-cis,5-trans} = 1.5$, $J_{4,5-trans} = 17.3$ Hz, 1H, H-5-*trans*), 5.24 (ddd, $J_{3,5-cis} = 1.4$, $J_{5-cis,5-trans} = 1.5$, $J_{4,5-cis} = 10.5$ Hz, 1H, H-5-*cis*), 3.99 (ddd, $J_{3,5} = 1.4$, $J_{2,3} = 4.2$, $J_{3,4} = 5.8$ Hz, 1H, H-3), 3.65 (ddd, $J_{2,3} = 4.2$, $J_{1b,2} = 4.3$, $J_{1a,2} = 8.6$ Hz, 1H, H-2), 2.62 (ddd, $J = 8.7$, $J = 11.7$, $J_{1'a,1'b} = 15.9$ Hz, 2H, H-1'a), 2.59 (dd, $J_{1a,2} = 8.6$, $J_{1a,1b} = 12.9$ Hz, 1H, H-1a), 2.54 (dd, $J_{1b,2} = 4.3$, $J_{1a,1b} = 12.9$ Hz, 1H, H-1b), 2.47 (ddd, $J = 5.2$, $J = 8.6$, $J_{1'a,1'b} = 15.9$ Hz, 2H, H-1'b), 1.53-1.40 (m, 4H, H-2'), 1.38-1.25 (m, 4H, H-3'), 0.92 (t, $J_{3',4'} = 7.3$ Hz, 6H, H-4'); ^{13}C NMR (125 MHz, CDCl_3) δ 137.5 (C4), 116.5 (C5), 74.4 (C3), 69.2 (C2), 57.0 (C1), 54.2 (C1'), 28.8 (C2'), 20.5 (C3'), 14.0 (C4'); HRMS(ESI) m/z calcd. for $[\text{C}_{13}\text{H}_{27}\text{O}_2\text{N}+\text{H}]^+$: 230.2115, obsd.: 230.2122.

(2R, 3R)-1-tetradecylamino-pent-4-ene-2,3-diol (223):

By subjecting methyl 5-deoxy-5-iodo- α/β -D-arabinoside (146 mg, 0.53 mmol) and tetradecylamine (136 mg, 0.64 mmol) to the general procedure for the synthesis of alkenylamines, the white solid (2R, 3R)-1-tetradecylamino-pent-4-ene-2,3-diol (**223**) was obtained (74 mg, 0.24mmol, 44%). $R_f = 0.65$ (DCM/EtOH/MeOH/30% $\text{NH}_3(\text{aq.})$, 35/2/2/1, v/v/v/v); $[\alpha]_D^{21} = +0.3$ (c = 0.66, EtOH); IR (film) 3380, 3275, 2954, 2916, 2847, 1646, 1468, 1420, 1326, 1252, 1162, 1119, 1095, 1053, 1030, 987, 926, 859 cm^{-1} . ^1H NMR (500 MHz, CD_3OD) δ 5.93 (ddd, $J_{3,4} = 6.3$, $J_{4,5-cis} = 10.6$, $J_{4,5-trans} = 17.2$ Hz, 1H, H-4), 5.32 (ddd, $J_{3,5-trans} = 1.5$, $J_{5-cis,5-trans} = 1.6$, $J_{4,5-trans} = 17.2$ Hz, 1H, H-5-*trans*), 5.19 (ddd, $J_{3,5-cis} = 1.5$, $J_{5-cis,5-trans} = 1.6$, $J_{4,5-cis} = 10.6$ Hz, 1H, H-5-*cis*), 3.99 (ddd, $J_{3,5} = 1.5$, $J_{2,3} = 5.2$, $J_{3,4} = 6.3$ Hz, 1H, H-3), 3.65 (ddd, $J_{1a,2} = 3.7$ Hz, $J_{2,3} = 5.2$, $J_{1b,2} = 8.8$ Hz, 1H, H-2), 2.72 (dd, $J_{1a,2} = 3.7$, $J_{1a,1b} = 12.2$ Hz, 1H, H-1a), 2.63 (dt, $J_{1'a,2'} = 7.6$, $J_{1'a,1'b} = 11.7$ Hz, 1H, H-1'a), 2.61 (dd, $J_{1b,2} = 8.8$, $J_{1a,1b} = 12.2$ Hz, 1H, H-1b), 2.58 (dt, $J_{1'b,2'} = 7.6$, $J_{1'a,1'b} = 11.7$ Hz, 1H, H-1'b), 1.56-1.49 (m, 2H, H-2'), 1.36-1.27 (m, 22H, H-3' – H-13'), 0.91 (t, $J_{13',14'} = 7.0$ Hz, 3H, H-14'); ^{13}C NMR (125 MHz, CD_3OD) δ 139.0 (C4), 116.7 (C5), 76.1 (C3), 73.5 (C2), 52.6 (C1), 50.6 (C1'), 33.0, 30.7, 30.7, 30.6, 30.6, 30.4, 29.5, 28.3, 28.2, 27.9, 23.7 (C2'–C13'),

14.3 (C14'); HRMS(ESI) m/z calcd. for $[C_{19}H_{39}O_2N+H]^+$: 314.3054, obsd.: 314.3058.

(2R, 3R)-1-hexadecylamino-pent-4-ene-2,3-diol

hydrochloride (224): By subjecting methyl 5-deoxy-5-iodo- α/β -D-arabinoside (102 mg, 0.37 mmol) and hexadecylamine (110 mg, 0.44 mmol) to the general procedure for the synthesis of alkenylamines, (2R, 3R)-1-hexadecylamino-pent-4-ene-2,3-diol (**224**) was obtained as a white solid as the HCl salt (96 mg, 0.25 mmol, 69%). R_f = 0.19 (DCM/EtOH/MeOH/30% $NH_3(aq.)$, 35/2/2/1, v/v/v/v); $[\alpha]_D^{22}$ = +28.4 (c = 1.0, EtOH); IR (film) 3352, 2955, 2919, 2850, 1739, 1650, 1470, 1373, 1146, 1016 cm^{-1} . 1H NMR (500 MHz, CD_3OD) δ 5.96 (ddd, $J_{3,4}$ = 5.2, $J_{4,5-cis}$ = 10.7, $J_{4,5-trans}$ = 16.9 Hz, 1H, H-4), 5.38 (d, $J_{4,5-trans}$ = 16.9 Hz, 1H, H-5-trans), 5.25 (dd, $J_{5-cis,5-trans}$ = 0.9, $J_{4,5-cis}$ = 10.7 Hz, 1H, H-5-cis), 4.09 (dd, $J_{2,3}$ = 4.4, $J_{3,4}$ = 5.2 Hz, 1H, H-3), 3.87 (dd, $J_{1,2}$ = 1.7, $J_{2,3}$ = 4.4 Hz, 1H, H-2), 3.12 (dd, $J_{1a,2}$ = 2.5 Hz, $J_{1a,1b}$ = 12.6 Hz, 1H, H-1a), 3.06-2.95 (m, 3H, H-1b, H-1'), 1.75-1.66 (m, 2H, H-2'), 1.42-1.24 (m, 26H, H-3' – H-15'), 0.90 (t, $J_{15',16'}$ = 6.7 Hz, 3H, H-16'); ^{13}C NMR (125 MHz, CD_3OD) δ 138.1 (C4), 117.3 (C5), 75.0 (C3), 70.5 (C2), 50.8 (C1), 48.5 (C1'), 33.1, 30.8, 30.8, 30.8, 30.7, 30.6, 30.5, 30.2, 27.6, 27.0, 23.7 (C2'–C15'), 14.5 (C16'); HRMS(ESI) m/z calcd. for $[C_{21}H_{43}O_2N+H]^+$: 342.3367, obsd.: 342.3371.

(2R, 3R)-1-hexadecylmethylamino-pent-4-ene-2,3-diol

(225): To a solution of hexadecylalkenylamine **224** (15.8 mg, 0.04 mmol) in MeOH (1 mL) and AcOH (0.05 mL), was added 37% aqueous formaldehyde (60 μ L, 0.8 mmol), and $NaCNBH_3$ (75.6 mg, 1.2 mmol). This was stirred under reflux for 24 h with additional formaldehyde (100 μ L) and $NaCNBH_3$ (100 mg) added after 15 h and 21 h, respectively. For workup, an additional 1 mL of formaldehyde was added to remove the excess reducing agent, and the reaction mixture was concentrated *in vacuo*. Purification by reverse phase column chromatography

(octadecyl bonded endcapped silica preppacked column), eluting in MeOH gave methylated alkenylamine **225** as a white solid (11 mg, 0.03 mmol, 73%). $R_f = 0.69$ (DCM/EtOH/MeOH/30% $\text{NH}_3(\text{aq.})$, 35/2/2/1, v/v/v/v); $[\alpha]_D^{22} = +28.0$ ($c = 0.5$, MeOH); IR (film) 3373, 3156, 2923, 2853, 1658, 1643, 1466, 1379, 1266, 1072, 994, 921 cm^{-1} . ^1H NMR (500 MHz, CD_3OD) δ 5.97 (ddd, $J_{3,4} = 5.0$, $J_{4,5\text{-cis}} = 10.5$, $J_{4,5\text{-trans}} = 17.5$ Hz, 1H, H-4), 5.33 (d, $J_{4,5\text{-trans}} = 17.5$ Hz, 1H, H-5-*trans*), 5.20 (d, $J_{4,5\text{-cis}} = 10.5$ Hz, 1H, H-5-*cis*), 4.04 (dd, $J_{2,3} = 4.4$, $J_{3,4} = 5.0$ Hz, 1H, H-3), 3.72 (ddd, $J_{2,3} = 4.4$, $J = 7.5$, $J = 9.5$ Hz, 1H, H-2), 2.53 (dd, $J = 4.6$, $J = 13.0$ Hz, 1H, H-1a), 2.45-2.40 (m, 3H, H-1b, H-1'), 2.28 (s, 3H, N-Me), 1.52-1.48 (m, 2H, H-2'), 1.37-1.22 (m, 26H, H-3' – H-15'), 0.90 (t, $J_{15',16'} = 7.0$ Hz, 3H, H-16'); ^{13}C NMR (125 MHz, CD_3OD) δ 139.1 (C4), 116.4 (C5), 76.1 (C3), 71.7 (C2), 61.1 (C1), 59.5 (C1'), 43.0 (N-Me), 33.1, 30.8, 30.8, 30.8, 30.7, 30.7, 30.5, 28.5, 28.0, 23.7 (C2'–C15'), 14.4 (C16'); HRMS(ESI) m/z calcd. for $[\text{C}_{22}\text{H}_{45}\text{O}_2\text{N}+\text{H}]^+$: 356.3523, obsd.: 356.3523.

4.6.2 BCG Alamar Blue[®] Growth Inhibition Assay Protocol

Bacteria: Stock solutions of BCG (1.5×10^7 bugs per mL) were stored at -80°C . These were defrosted with repeated cycles of 10 seconds sonication, followed by 10 seconds on ice. A stock sample of BCG was diluted to a concentration of 8×10^4 bugs per mL using sterile Tween albumin broth [Dubos broth base/OADC] to suspend.

Compounds: Compounds were dissolved in one of two ways, depending on solubility:

1) For compounds soluble in phosphate buffered saline (PBS) with up to 5% DMSO, a solution was prepared with sterile PBS and sterile DMSO at double the desired concentration and these were filter sterilised using Acrodisc 13 mm syringe filters, with 0.22 μm pore size into sterile eppendorf tubes.

2) Compounds soluble only in DMSO were prepared at forty times the desired starting concentration, in sterile eppendorf tubes. Sterility filtering was not necessary because DMSO is toxic to bacteria, but if required, the compounds were sterility tested separately, by incubating compounds at a concentration of 2.5% DMSO in PBS on blood agar, and observing bacterial growth after 3 days.

Plate Set-up: Optical bottom black 96 well plates were used to screen 4 compounds at one time, each in triplicate, and tested over three serial dilutions (Figure 4.6). For the compounds dissolved in PBS, 200 μ L of the compound solutions were added to each of three wells before serial dilutions. For the compounds dissolved in DMSO, 190 μ L of PBS was added to the three initial wells, followed by 10 μ L of compound solution. Subsequent two-fold serial dilutions of the compounds were carried out, by removing 100 μ L of compound from the first wells and diluting with 100 μ L PBS, then repeating this procedure. As a control, 200 μ L EMB at a concentration of 64 μ g/mL was added to the plate in duplicate. EMB titrations, by serial two-fold dilutions, gave concentrations of 32 to 1 μ g/mL EMB after addition of the bacteria. To determine if the compounds had any inherent redox potential, 200 μ L solutions of compounds and PBS were added to individual control wells (*i.e.* no bacteria were added to these wells). Control wells of bacteria only, and PBS medium only were also prepared. 100 μ L of BCG solution was added to all wells except the PBS only, and compound only wells, and the plate was incubated at 37 °C and 5% CO₂ for 7 days. The outer wells were left free of compound and filled with water throughout the course of the incubation to avoid uneven evaporation of compounds. 20 μ L of Alamar Blue[®] dye was added to all test wells on day 7, and the plate was incubated for another 24 hours before fluorescence was measured (excitation at 544 nm, emission at 590 nm) using a FLUOstar OPTIMA plate reader. The results were graphed to determine the MICs. Any compounds with significant anti-mycobacterial activity were retested at the necessary lower concentrations over 6 titrations of two-fold dilutions to more accurately determine the MIC. The lowest drug concentration effecting 100% growth inhibition is considered the MIC.

A:

		Compound 3			Compound 4			EMB			3	4	
											1	2	
		Compound 1			Compound 2						PBS only		

B:

					EMB		Cpd 2 Only	PBS only		
							Cpd 1 only	BCG only		
	Compound 1		Compound 2							

C:

Figure 4.6: 96 well plate layout for BCG Alamar Blue[®] growth inhibition assay: A. 96 well plate layout for 4 compounds per plate screening. B. Plate layout for 2 compounds per plate MIC calculations. C. Photograph of an example plate with the MIC noted for compounds 1 and 2.

4.6.3 Anti-proliferative HL60 and T cell MTT Assays

James Baty (MIMR) carried out cytotoxicity testing on HL60 (cancer) cells, and human T cells in an anti-proliferative MTT assay.⁵³ The experimental procedure is briefly summarised here. HL60 or T cells were plated into a 96 well plate in the presence of different concentrations of compounds (final cell concentration = 0.2×10^6 cells/mL in complete RPMI), and incubated for 48 h (37 °C, 5% CO₂). 20 µL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 2.5 mg/mL in HBSS) was added before incubating for a further 2 h, then adding 100 µL SDS lysing buffer and incubating overnight. The absorbance at 570 nm was read with a FLUOstar OPTIMA plate reader.²²

4.7 References:

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Chapter 5: Global Conclusion and Future Prospects

In this thesis the use of PGF synthetic methodology for drug discovery has been explored and this chapter highlights the main conclusions of the research and discusses possible future directions for these projects. With that in mind, some of the future prospects discussed in this chapter involve the use of PGs to either aid in mechanistic understanding, or to allow for the synthesis of a desired synthetic target. Although the use of PGs is not generally in the interest of the green chemist, the understanding of chemoselective mechanisms and the development of the most efficient synthetic route is in everybody's best interest.

5.1 The PGF Synthesis of Azasugars

In **Chapter 2** the PGF azasugar methodology was applied to 2-deoxy-D-glucose (**105**) with the anticipation of selectively forming 5-*epi*-fagomine **108**. Instead, the I₂-mediated carbamate annulation of the *gluco* alkenylamine **110** resulted in the formation of four products (Scheme 5.1). *Cis* and *trans* piperidines **112a** and **112b**, pyrrolidine **111**, and linear iodohydrin **113** were identified using a variety of spectroscopic and synthetic techniques. Hydrolysis of the three cyclic carbamates provided 5-*epi*-fagomine **108** and fagomine **78** as minor products, and the trihydroxylated pyrrolidine **115** as the major product in 15% yield.

Scheme 5.1: Carbamate annulation of 2-deoxy-D-glucose derived alkenylamine **110** and the subsequent hydrolysis of the cyclic carbamates.

Insight into the carbamate annulation mechanism for the 2-deoxy-D-glucose derived alkenylamine **110** was also provided in this chapter. Analysis of the six-membered iodocyclisation transition state models indicated no obvious low energy TS in the *gluco* cyclisation, which could explain why a variety of products were formed. The preferred mechanisms to explain the formation of the four carbamates were then proposed in Scheme 2.10. An extension of the iodocyclisation model that was originally proposed by Chamberlin¹ was used to explain the formation of the *cis* and *trans* piperidines **112a** and **112b**, while the formation of pyrrolidine **111** could be explained via an iodo-epoxide intermediate. The formation of the iodohydrin **113** was suggested to form through the direct attack of water on the I₂-alkene complex, with carbamate formation at the 3-position. Future experiments and calculations to confirm these proposed mechanisms would also be useful.

The I₂-mediated carbamate annulation for piperidines was then discussed in relation to other cyclisations. It was observed that, in addition to the stereodirecting effect of the allylic hydroxyl, factors such as the position of ring-substituents, across-ring *trans* directing effects, and the reaction conditions could

also influence the selectivity of annulations. Accordingly, to aid in understanding of the 2-deoxy-D-glucose derived alkenylamine annulation mechanism, and to favour the formation of a specific product, a variety of selective protection strategies are proposed (Scheme 5.2). Herein it should be noted that the selective protection of one hydroxyl group may require multiple protecting group manipulation steps and thereby reduce the overall efficiency of the process.

Scheme 5.2: Selective protection strategies favouring particular products.

Of the strategies proposed to probe the reaction mechanism and explore the conditions required to favour certain *gluco* annulation products, the first involves the use of an electron donating amine PG, such as a benzyl group in amine **226**.

This would make the amine more nucleophilic, hence more reactive, and therefore more likely to rapidly react *via* a six-membered transition state to form the corresponding piperidine iodides, **227** and **228**. Another strategy involves the selective protection of the 4-OH (as in **229**), which would prevent the formation of the iodo-epoxide intermediate and therefore pyrrolidine **111**. The selective protection of the 3-OH (as in **240**) may prevent the formation of an iodohydrin and, by doing so, increase the yields of piperidines **241** and **242**, and pyrrolidine **243**. This 3-OH protection strategy could provide insight into the mechanism of formation of iodide **113**. As discussed in **Chapter 2**, the formation of iodide **113** may occur through initial water attack on the I₂-alkene complex, or through initial carbamate formation at C3. Therefore, prevention of the carbamate formation at the 3-OH may encourage the amine attack on the I₂-alkene complex, suggesting that the initial carbamate formation in iodide **113** precedes the iodohydrin formation. Similarly, the protection of both hydroxyls in alkenylamine **244** could lead to the selective formation of piperidines **245** and **246**.

As described in Scheme 2.15 the presence of a group α to the amine has a *trans* directing effect across the ring. To exploit this effect, a 2-deoxy-D-glucose alkenylamine analogue with a bulky group at C1 could be prepared. To this end, the development of a new PGF C-C bond forming addition to the Vasella reductive amination methodology (similar to that developed in the synthesis of conduramines in **Chapter 3**) could potentially be utilised to prepare an α -substituted alkenylamine that could then be submitted to the carbamate annulation conditions. This will be discussed in Section 5.2.

As the selective formation of pyrrolidine **111** is difficult to favour through PG manipulations, an alternative synthetic route for its formation, which would support the predicted iodoepoxide intermediate, is suggested (Scheme 5.3). Retrosynthetically, pyrrolidine **111** can be envisaged to be prepared via the deprotection and carbamate annulation of protected iodoepoxide **247**. The iodide **247** can be prepared via a Sharpless asymmetric epoxidation² of the protected amine **248**, which should itself be available from the cross metathesis of protected

alkenylamine **249** and allyliodide (**250**).³ The protected alkenylamine **249** can be made by the Vasella-reductive amination of 2-deoxy-D-xylose (**251**) and an appropriate primary amine. In addition to supporting the proposed mechanism for the carbamate annulation step, this synthetic route could be used to prepare pyrrolidine **111** in better yield, and also be a means to provide access to diastereomers of **111** and its hydrolysis product, pyrrolidine **115**.

Scheme 5.3: Retrosynthetic plan for alternative route to pyrrolidine carbamate **111**.

5.2 The PGF Synthesis of Conduramines

The development of a PGF methodology for the synthesis of conduramines was described in **Chapter 3**. 4-Deoxy-3-conduramine **171** was prepared from D-xylose in a 27% yield over four steps, with the critical step being the C-C bond forming adaptation of the Vasella-reductive amination reaction (Scheme 5.4). To this end, a stereoselective PGF Vasella-Barbier amination provided 4,5-*anti* amino diene **172** in 35% yield. Key to the success of this reaction was the use of NH_4OAc as the amine source, and indium as the metal. To complete the synthesis, a RCM provided conduramine **171** quantitatively in the presence of free hydroxyl groups and an *in situ* protected amine.

Scheme 5.4: The PGF synthesis of 4-deoxy-3-conduramine **171**.

In addition to the PGF Vasella-Barbier reaction developed in **Chapter 3**, there is also potential for the development of other C-C bond forming variations of the PGF Vasella-reductive amination. The use of different carbon nucleophiles in a similar reaction would provide access to differently functionalised alkenylamines, and hence, different products. One example, as alluded to earlier, is the

preparation of a hexenylamine **252** with an across-the-ring *trans*-directing cyano group α to the amine, whereby hexenylamine **252** is prepared through the use of Zn, NH₄OAc, and TMSCN in a modified PGF Vasella-Strecker reaction (Scheme 5.5).⁴ After the key C-C bond forming step, an adaptation of the synthesis of five-membered amino-iminosugars⁴ via an I₂-mediated carbamate annulation to give **253**, followed by hydrolysis ($\rightarrow$ **254**) and then hydrogenation is envisioned to provide the 2,6-*trans*-5,6-*cis*-amino-imino-heptitol **255** in a PGF manner.

Scheme 5.5: Proposed development of a PGF Vasella-Strecker reaction for the PGF synthesis of amino-imino-heptitol **255**.

Due to the interesting biological properties of aminocyclitols,⁵ their preparation would be a useful extension to the PGF methodology for the synthesis of conduramines. To this end, a dihydroxylation of 4-deoxy-3-conduramine **171** is expected to provide deoxyaminocyclitol **256**. An initial dihydroxylation of conduramine **171** was attempted using Upjohn conditions⁶ but this was unsuccessful (conditions A, Scheme 5.6). Instead, the use of a Sharpless asymmetric dihydroxylation⁷ (conditions B) may prove more effective. It is unclear which asymmetric dihydroxylation mix (AD mix- α or - β) would produce the desired *anti* selectivity, therefore reactions using either catalyst may need to

be explored. Furthermore the application of the aminocyclitol synthesis methodology to several different furanose scaffolds, and the use of both AD mixes (α and β) in the dihydroxylation, would provide access to a variety of 2-deoxyaminocyclitols, which could then be screened for glycosidase activity.

Scheme 5.6: A. Upjohn conditions for dihydroxylation of conduramine **171**.
B. Sharpless dihydroxylation of conduramine **171**.

A variety of other molecules can also be prepared from the conduramine scaffold. For example, subjecting conduramine **171** to (-)-diethyl tartrate and $\text{Ti}(\text{O}^i\text{Pr})_4$ in a Sharpless asymmetric epoxidation² would provide *syn* epoxide **257** (Scheme 5.7), however, the amine group in conduramine **171** may need to be protected to achieve the desired transformation. From epoxide **257**, nucleophilic attack with different nucleophiles would then provide a range of products including aziridines, ethers, and diamines.⁸ This could also provide an alternative route to deoxyaminocyclitols with an *anti* relationship between the newly formed hydroxyls, compared with the *syn* dihydroxylation described above.

Scheme 5.7: Sharpless asymmetric epoxidation of conduramine **171**.

5.3 The Synthesis and Testing of Lipophilic Amines as Anti-TB Drugs

The development of a potential amine anti-tuberculosis drug was described in **Chapter 4**. To this end, a 20-member amine library was prepared using reductive amination and Vasella-reductive amination reactions. Variation in the stereochemistry of the sugar, and the use of several different lipophilic amines provided a wide range of amines and alkenylamines for anti-mycobacterial testing. The BCG Alamar Blue growth inhibition assay was used to test for potential anti-TB activity. The trends in BCG growth inhibition for the amine library revealed that the arabinose configuration was the favoured stereochemistry for the sugar group, and that larger, linear amines conferred more potent anti-mycobacterial activity compared to the shorter chain alkylamines or the cyclic amines. It was proposed that the lipophilicity and flexibility of the amine affected the ability of a molecule to pass through the cell wall and inhibit cell growth. Additional biological testing of the more active amines revealed that with increasing anti-mycobacterial activity, an increase in anti-proliferative activity was also observed for both human T cells and the human leukaemia cell line HL60. The *in vitro* trend in general cytotoxicity for the amines was disappointing, however, as *in vivo* data provides a better prediction of anti-TB activity and cytotoxicity, two promising alkylamines, arabinohexadecylamine **208** and arabinohexadecylmethylanine **209** (Figure 5.1), both prepared in a single step, were sent to the group of Assoc. Prof. Anne Lenaerts at Colorado State University for further biological testing in a mouse model of TB. The results of these studies will be reported in due course.

Figure 5.1: Lead amines **208** and **209** with promising anti-TB activity that have been submitted for *in vivo* testing, and potential anti-cancer lead **210**.

Future work on the anti-TB drug discovery project includes the preparation of a third generation amine library, with the aim of further improving anti-mycobacterial activity while also reducing the cytotoxicity of the compounds. Although the stereochemistry of the sugar group was studied to some extent in the first and second generation amine libraries, a more structured approach to the design of future inhibitors would be useful. To this end, a series of inhibitors with the active hexadecylamine lipid but with different pentose, hexose, and ketose sugars could be prepared to better understand how the stereochemistry of the sugar affects activity. As arabinohexadecylamine **210** was poorly soluble in most solvents, it is anticipated that the proposed sugar series in the third generation amine library may also be poorly soluble, hence *N*-methylation of the corresponding amines could also be carried out. It has been shown in this thesis that *N*-methylated amines retained anti-mycobacterial activity, and in some cases improved solubility, hence a series of *N*-alkylated amines would also be of interest from a SAR perspective.

In addition to the potential anti-TB drugs **208** and **209**, di-arabino hexadecylamine **210** was notably more active towards the cancer cell line, HL60, compared with the human T cell line (Figure 5.1). This selectivity suggests potential for di-arabino hexadecylamine **210** to act as an anti-cancer drug and further investigations into the biological testing of this compound would be valuable. The optimisation of the synthesis of amine **210** is anticipated through the use of excess arabinose, and

one equivalent of hexadecylamine as the limiting reagent. Moreover, an anticancer amine library based on amine **210** with varied sugar groups and lipophilic tails could be used to assess the SAR of these compounds, with the idea that this would lead to the identification of a more selective and more potent anticancer drug.

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Appendix

Chapter 2: NMR Spectra

106

$\alpha:\beta = 8:1$
 $^1\text{H NMR}$
 D_2O
 300 MHz

106

$\alpha:\beta = 8:1$
 ^1H NMR
 D_2O
 150 MHz

^{13}C NMR
 CDCl_3
 125 MHz

¹H NMR
D₂O
500 MHz

¹³C NMR
D₂O
125 MHz

¹³C NMR
 D₂O
 125 MHz

113

¹H NMR
D₂O
300 MHz

113

^{13}C NMR
 D_2O
 125 MHz

¹H NMR
CDCl₃
500 MHz

^{13}C NMR
 CDCl_3
 125 MHz

¹H NMR
CD₃OD
300 MHz

2D NOESY
CD₃OD
500 MHz

¹H NMR
D₂O
500 MHz

115

^{13}C NMR
 D_2O
 125 MHz

¹H NMR
D₂O
500 MHz

^{13}C NMR
 D_2O
125 MHz

¹H NMR
 D₂O
 500 MHz

78

^{13}C NMR
 D_2O
125 MHz

116

¹H NMR
CDCl₃
500 MHz

116

^{13}C NMR
 CDCl_3
 125 MHz

117

¹H NMR
CDCl₃/CD₃OD 95/5
500 MHz

117

^{13}C NMR
 $\text{CDCl}_3/\text{CD}_3\text{OD}$ 95/5
125 MHz

¹H NMR
D₂O
300 MHz

118

^{13}C NMR
 D_2O
 600 MHz

119

^{13}C NMR
 CDCl_3
 125 MHz

Chapter 3: NMR Spectra

66

¹H NMR
CDCl₃
500 MHz

66

^{13}C NMR
 CDCl_3
 125 MHz

183

¹H NMR
CDCl₃
500 MHz

183

¹³C NMR
CDCl₃
125 MHz

187

¹H NMR
D₂O
500 MHz

182

¹H NMR
CDCl₃
500 MHz

182

¹³C NMR
CDCl₃
125 MHz

172

¹H NMR
D₂O
500 MHz

172

^{13}C NMR
 D_2O
 125 MHz

171

^1H NMR
 D_2O
 500 MHz

171

^{13}C NMR
 D_2O
 125 MHz

Chapter 4: NMR Spectra

203

¹H NMR

D₂O

500 MHz

203

^{13}C NMR

D_2O

125 MHz

204

¹H NMR
CD₃OD
500 MHz

204

^{13}C NMR

CD_3OD

125 MHz

205

¹H NMR
D₂O
500 MHz

205

^{13}C NMR

D_2O

125 MHz

206

¹H NMR

CD₃OD

500 MHz

206

^{13}C NMR
 CD_3OD
 125 MHz

208

¹H NMR

DMSO-d₆, 80 °C

300 MHz

209

¹H NMR
CD₃OD
300 MHz

¹³C NMR
$$\text{CD}_3\text{OD}$$

125 MHz

¹H NMR
D₂O
500 MHz

212

^{13}C NMR

D_2O

125 MHz

213

^{13}C NMR

D_2O

125 MHz

214

¹H NMR
D₂O
500 MHz

214

^{13}C NMR

D_2O

125 MHz

216

¹H NMR
D₂O
500 MHz

216

^{13}C NMR
 D_2O
 125 MHz

218

¹H NMR
D₂O
500 MHz

218

^{13}C NMR

D_2O

125 MHz

219

¹H NMR
CD₃OD
500 MHz

^{13}C NMR
 CD_3OD
 125 MHz

221

¹H NMR
CDCl₃
500 MHz

221

¹³C NMR
CDCl₃
125 MHz

223

¹H NMR
CD₃OD
500 MHz

223

¹³C NMR
CD₃OD
125 MHz

224

¹³C NMR
CD₃OD
125 MHz

225

¹H NMR
CD₃OD
500 MHz

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Applications and Limitations of the I₂-Mediated Carbamate Annulation for the Synthesis of Piperidines: Five- versus Six-Membered Ring Formation

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S Supporting Information

ABSTRACT: A protecting-group-free synthetic strategy for the synthesis of piperidines has been explored. Key in the synthesis is an I₂-mediated carbamate annulation, which allows for the cyclization of hydroxy-substituted alkenylamines into piperidines, pyrrolidines, and furans. In this work, four chiral scaffolds were compared and contrasted, and it was observed that with both D-galactose and 2-deoxy-D-galactose as starting materials, the transformations into the piperidines 1-deoxygalactonojirimycin (DGJ) and 4-*epi*-fagomine, respectively, could be achieved in few steps and good overall yields. When D-glucose was used as a starting material, only the furan product was formed, whereas the use of 2-deoxy-D-glucose resulted in reduced chemo- and stereoselectivity and the formation of four products. A mechanistic explanation for the formation of each annulation product could be provided, which has improved our understanding of the scope and limitations of the carbamate annulation for piperidine synthesis.

INTRODUCTION

Iminosugars have enormous therapeutic potential in the treatment of a number of diseases, such as cancer, diabetes, and lysosomal storage disorders.¹ Notable members of the piperidine iminosugar family include *N*-hydroxyethyl-DNJ (Miglitol, **1**), which has been approved for the treatment of non-insulin-dependent diabetes,² and 1-deoxygalactonojirimycin (DGJ, **2**), recently used in a phase-2 clinical trial for the treatment of Fabry's disease (Figure 1).³ The corresponding 2-deoxypiperidines, fagomine (**3**)^{4,5} and the unnatural 4-*epi*-isomer **4**, also exhibit promising biological activities. Fagomine was found to have activity against mammalian gut α -glucosidases and β -galactosidase⁶ and a potent antihyperglyce-

mic effect in streptozotocin-induced diabetic mice,⁷ while fagomine isomer **4** inhibits lysosomal α -galactosidase A activity in Fabry lymphoblasts.⁸

Given the interesting biological activity of piperidines, we became interested in extending our previously reported protecting-group-free strategy for the synthesis of pyrrolidines^{9–11} to the synthesis of piperidines.¹² To this end, we proposed that the target piperidines **1** could be attained following the hydrolysis of the intermediate cyclic carbamate **II**, itself prepared via the I₂-mediated cyclization of alkenylamine precursor **III** (Scheme 1). Alkenylamine **III** would in turn be prepared from methyl iodoglycoside **IV** via our recently reported protecting-group-free Vasella reductive amination methodology.¹³ Key in this approach was the provision that when R¹ = OH, alkenylamine **III** should cyclize to form the desired piperidine (N-cyclization) rather than undergoing O-cyclization, which would lead to the undesired amino-methylfuran.

To explore the potential of our annulation methodology for the synthesis of piperidines, we first set out to prepare 1-deoxygalactonojirimycin (DGJ, **2**). As previously described,¹⁴ alkenylamine **5**, itself available in three steps from D-galactose, was subjected to an I₂-mediated carbamate annulation to give, under optimized conditions, piperidine isomers **6a** and **6b** and furan **6c** in a 3:1:1 ratio, respectively (Scheme 2). Whereas our previously reported pyrrolidine annulation methodology

Figure 1. Piperidines.

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Scheme 1. Retrosynthetic Analysis for the Synthesis of Piperidines

Scheme 2. Synthesis of DGJ (2)

proceeded in 18 h at room temperature and with >95:5 diastereoselectivity in favor of the *cis* product,^{9–11} cyclization to the piperidine framework was much slower and resulted in lower stereoselectivity. Hydrolysis of the carbamate and purification by silica gel flash column chromatography, however, allowed **2** to be prepared in a respectable 40% yield (from **5**) and in five steps in total. Given this, we were keen to explore whether a similar methodology could be used for the preparation of other piperidines. The results of our studies in this area are reported herein.

RESULTS AND DISCUSSION

Following our successful synthesis of DGJ, we then explored the potential of our I₂-mediated annulation strategy for the synthesis of 1,5-dideoxy-1,5-imino-L-iditol (**7**) (Scheme 3). To this end, methyl glucopyranoside **8** was subjected to triphenylphosphine, imidazole, and iodine to give the corresponding iodosugar **9** in 98% yield.¹³ Subsequent Vasella reductive amination led to the smooth formation of alkenyl-

amine **10**, again in excellent yield (93%) with only minor traces of the secondary amine (<5%).¹⁵ Alkenylamine **10** was then treated with I₂ and NaHCO₃ in anticipation that carbamate **11** would be formed. Though TLC analysis revealed the smooth transformation into a higher-running product (*R_f* = 0.23, DCM/EtOH/MeOH/30% aq. NH₃, 5/2/2/1 v/v/v/v), ¹³C NMR analysis of the crude reaction mixture revealed the presence of an iodomethylene carbon (−0.3 ppm, −CH₂I), thus indicating that the desired carbamate had not been formed. Furthermore, an aminomethylene signal at 39.4 ppm and four oxymethine signals (82–76 ppm) were observed, which indicated the presence of aminomethylfuran **13** rather than piperidine **12**, both of which satisfy the HRMS result ([*M* + *H*]⁺ = 273.9938). Attempts to purify the material to allow for further characterization proved futile, as Dowex-H⁺ or silica gel chromatography led to degradation of the product. Full characterization was therefore achieved after acetylation of the crude product with acetic anhydride and pyridine. The ¹H NMR spectral data clearly revealed an NH proton at 5.84 ppm with a COSY correlation to both H1a and H1b and no correlation to H5. In addition, the HMBC correlations between both H1 protons and the NHAc carbonyl were also present. As acetylation of pyran **12** would protect the amine, leaving no observable NH proton, these observations allowed the product of the acetylation reaction to be determined as furan **14**. Following on from this, the product of the I₂-mediated annulation reaction could then be assigned as furan **13**.

In an attempt to encourage attack of the nitrogen atom and formation of the desired carbamate **11** during the annulation reaction, alkenylamine **10** was treated with a stronger base, Na₂CO₃, to favor formation of the free amine. After the reaction mixture was stirred at room temperature overnight, no starting material remained; concentration and NMR analysis of the crude reaction mixture, however, revealed that the carbamic acid derivative of furan **13**, formed via CO₂ absorption onto the amine,^{16,17} had been prepared. Numerous other changes were

Scheme 3. Formation of an Undesired Iodofuranoside by I₂-Mediated Annulation

Scheme 4. Formation of Linear Alkenylamine 24

then made to the reaction conditions, including changes to the temperature (0 °C to reflux) and the reagent concentrations. However, under all of the conditions attempted, there was no observable formation of the desired carbamate **11** as determined by ^1H NMR analysis of the crude reaction mixture, and furan products always appeared to dominate. Though this result was disappointing, the inherent problem of controlling the chemoselectivity during protecting-group-free syntheses is well-known and thus explains why the development of protecting groups, though undesirable in terms of atom economy, still remains an important objective for the synthetic chemist.¹⁸

Having observed competing O-cyclization in an attempt to prepare 1,5-dideoxy-1,5-imino-L-iditol (**7**) and to a lesser extent DGJ (**2**), we thus proposed the synthesis of members of the fagomine family. Fagomine analogues retain interesting biological properties but are devoid of the hydroxyl functionality at C2 ($R^1 = \text{H}$; Scheme 1), and hence, the synthesis would be more readily accomplished. Accordingly, we first set out to synthesize 4-*epi*-fagomine (**4**) (Scheme 4). To this end, 2-deoxy-D-galactose (**17**) was first subjected to a Fischer glycosylation using AcCl and MeOH to give the corresponding methyl glycoside **18** in modest (40%) yield, with a second major product being the kinetically favored furan. Methyl glycoside **18** was then treated with I_2 , triphenylphosphine, and imidazole in THF. Under these conditions, however, only bicyclic pyranoside **19** was observed,¹⁹ a consequence of intramolecular substitution of the activated phosphonium group at C6 by the hydroxyl at the 3-position. Indeed, a similar observation was seen en route to the synthesis of DGJ,¹⁴ and given the tendency for this intramolecular cyclization in iodination of methyl glycosides of the *galacto* configuration, we resorted to the installation of an isopropylidene protecting group at the 3- and 4-positions via the treatment of 2-deoxy-D-

galactose (**17**) with MeOH, acetone, dimethoxypropane, and AcCl. Conveniently, these conditions led to the concomitant installation of the methyl group at the anomeric position to give galactoside **20** in one step. Galactoside **20** was then subjected to the standard iodination conditions to give the required primary iodide **21** in 71% yield (from **17**), and the isopropylidene group was then removed to give the desired methyl iodoglycoside **22** in 94% yield. Here it is also interesting to note that **20** can be prepared from D-galactal (**23**) by protonation of the alkene with TsOH followed by nucleophilic attack of methanol.²⁰ Under these conditions, pyranose **20** was formed in ~78% yield with small amounts (ca. 10%) of methyl 5,6-O-isopropylidene-D-galactofuranoside, which was more readily separable following conversion of **20** into iodide **21**.

With primary iodide **22** in hand, we then applied the Vasella reductive amination conditions and obtained alkenylamine **24** in excellent yield (99%). Alkenylamine **24** was then subjected to a solution of I_2 and NaHCO_3 in H_2O to affect the desired carbamate annulation reaction (Table 1). Again, cyclization to the desired six-membered ring proved to be sluggish, and no appreciable reaction was observed after alkenylamine **24** was stirred in NaHCO_3 (sat. aq.) in the presence of excess I_2 (10 equiv) at room temperature (ca. 20 °C) for 7 days (entry 1). When the solution was heated to 50 °C, however, complete conversion of the starting material was observed after 3 days (entry 2), and following filtration, carbamates **25a** and **25b** were observed in a 3:1 ratio, as determined by ^1H NMR analysis of the crude reaction mixture. Lowering the reaction temperature to 40 °C resulted in an increased reaction time (5 days) but led to improved diastereoselectivity in favor of the *cis* isomer **25a** (**25a**:**25b** = 9:1; entry 3). Remarkably, a diastereoselectivity of >95:5 was achieved when the reaction was performed at 35 °C with a reaction time of 5 days (entry 4). Indeed, using the latter conditions, carbamate **25a** was

Table 1. Diastereoselectivity of Carbamate Annulation

entry	T (°C)	t (days)	25a:25b ^a
1	20	>7	no reaction
2	50	3	3:1
3	40	5	9:1
4	35	5	>95:5 ^b

^aAs determined by ¹H NMR analysis of the crude reaction mixture.^b25a was isolated in 86% yield.

isolated in 86% yield following silica gel column chromatography.

To complete the synthesis, carbamate 25a was then hydrolyzed via treatment with NaOH in EtOH at reflux (Scheme 5). This resulted in the smooth formation of 4-*epi*-

Scheme 5. Hydrolysis of *cis*-Carbamate 25a

fagomine (4) in 92% yield, thus completing this remarkably efficient six-step synthesis with an overall yield of 52%. 4-*epi*-Fagomine has been prepared on several other occasions.^{21–24} The first synthesis required 10 steps from 3²¹ using the method of Heiker and Schueller²² (no yield reported), and the synthesis with the best reported yield (28%) proceeded via an asymmetric route from a benzyl-protected galactal.²⁵ Although the need for a protecting group here was unavoidable because of the inherent reactivity of the *galacto* configuration, protecting group manipulation added just one step to the synthesis, and an expedient synthesis of 4 was achieved with the best yield to date.

To further demonstrate the potential of our strategy for the synthesis of fagomine diastereomers, we then set about to synthesize 5-*epi*-fagomine (26) (Scheme 6). 2-Deoxy-D-glucose

Scheme 6. Synthesis of Alkenylamine 29 Derived from 2-Deoxy-D-glucose

(27a) was converted to the methyl glycoside (27b) under Fischer glycosylation conditions, and subsequent treatment with iodine, triphenylphosphine, and imidazole at 75 °C for 1.5 h gave primary iodide 28 in good overall yield (64% for two steps).^{26,27} The reaction times and temperatures were critical to avoid overiodination or incomplete consumption of starting material. Iodide 28 was then treated with Zn, NaCNBH₃, NH₃, and NH₄OAc according to the standard Vasella reductive amination protocol to effect its smooth transformation into alkenylamine 29 in good yield (86%).

Alkenylamine 29 was then subjected to a variety of annulation conditions (Table 2). As previously highlighted, slightly warmer temperatures were required for the annulation of alkenylamine 24, and because of this, alkenylamine 29 was first treated with I₂ and NaHCO₃ (sat. aq.) at 40 °C (entry 1). These conditions led to a complex mixture of four carbamate products later identified as pyrrolidine 30, the two piperidine diastereomers 31a and 31b, and iodide 32 (vide infra), with iodide 32 being the major product in this instance. In an attempt to limit the formation of so many carbamate products, the reaction temperature was then lowered and the number of equivalents of NaHCO₃ was reduced (entry 2). After the reaction mixture was stirred at room temperature for 9 days, however, no product formation was observed. Next, iodine was added in portions over the course of the reaction while the reaction mixture was stirred at room temperature until all of the starting material was consumed, as gauged by TLC analysis (entry 3). While these conditions invoked annulation reactions and a slight improvement in the ratio of the piperidine carbamate 31a was observed, iodide 32 was once again the major product and all four carbamates were still formed. The reaction was then attempted at room temperature, again using a saturated solution of NaHCO₃ and I₂ (5 equiv) added in one portion at the start of the reaction (entry 4). Under these conditions, the formation of iodide 32 was reduced and pyrrolidine 30 was formed with modest selectivity in a 30:31a:31b:32 ratio of 4:2:2:3. The role of the halide in influencing the ratio of products was then investigated through the use of Br₂ (entry 5); however, this proved futile, with degradation being observed. Further changes were then made to the number of equivalents of reagents and the temperature at which the reaction was performed, but in all instances complex mixtures of products were observed, and the best yield of any single product apart from iodide 32 was still found to be that of pyrrolidine 30 (as highlighted in entry 4). Although the yield was disappointing (10–30%), pyrrolidine 30 could nevertheless be isolated as a pure compound following repeated flash silica gel column chromatography (eluting in 2% MeOH in EtOAc) and reversed-phase column chromatography (C8, H₂O). Here the range of yields reflects the difficulty of this chromatographic separation. Piperidines 31a and 31b, however, could not be separated from one another and were isolated in a poor combined yield (ca. 20%). Attempts to separate the products at a later stage in the synthesis (i.e., following hydrolysis) also did not lead to any overall improvement in the isolated yields.

To confirm the structures of pyrrolidine 30 and alkyl iodide 32, extensive 1D and 2D NMR spectral analysis was undertaken. For pyrrolidine 30, an HMBC correlation was observed between H4 and C1, thus enabling the 5,6-bicyclic ring system of 30 to be established. Unfortunately, overlapping signals in the proton spectra meant that the configurations of the stereocenters could not be determined unequivocally. However, NMR analysis of the acetylated derivative 33 proved

Table 2. Carbamate Annulation Reaction for Alkenylamine 29

entry	conditions	products (30:31a:31b:32) ^a
1	I ₂ (5 equiv), NaHCO ₃ (sat.), 40 °C, 18 h	3:2:2:8
2	I ₂ (5 equiv), NaHCO ₃ (1.5 equiv), rt, 9 days	starting material
3	I ₂ (3 × 1.5 equiv added over 22 days), NaHCO ₃ (sat.), rt	3:2:1:7
4	I ₂ (5 equiv), NaHCO ₃ (sat.), rt, 5 days	4 ^b :2:2:3
5	Br ₂ (5 equiv), NaHCO ₃ (sat.), rt, 3 days	decomposition

^aAs determined by ¹H NMR analysis of the crude reaction mixture ^b30 was isolated as a pure compound in 10–30% yield following column chromatography

to be more beneficial. The relative configurations of H5, H4, and H6b were apparent through two large *trans*-daxial couplings ($J_{4,5} = J_{5,6b} = 9$ Hz), and the coupling constant between H5 and H6a ($J_{5,6a} = 4.9$ Hz) indicated an axial/equatorial relationship between these two protons. As the stereochemistry at C3 is known, NOEs were then used to determine the stereochemistries of the remaining centers. NOEs were observed between H5 and H3, H3 and H2a, H2a and H1a, and H6a and H5 (Scheme 7), indicating these

Scheme 7. Synthesis of Previously Undisclosed Trihydroxylated Pyrrolidine 34 and NOE Correlations for Acetylated Carbamate 33

protons to be on the bottom face of the molecule, while NOEs between H2b and H4 and between H4 and H6b identified these protons to be on the opposite face, thereby allowing for the assignment of the *L-allo* stereochemistry. Pyrrolidine 30 was then treated with NaOH in EtOH, which resulted in the smooth hydrolysis of the carbamate and allowed for the synthesis of the previously undisclosed trihydroxylated pyrrolidine 34 in 5–15% yield over five steps. The enantiomer of 34 has been prepared once previously.²⁸ Comparison of NMR data showed minor differences in the chemical shifts, which can be explained by the use of a different solvent and differences in pH (34 was treated with conc. DCl to ensure that the amine was fully protonated). The optical rotation of 34 was measured as $[\alpha]_D^{26} = -1.8$ ($c = 0.41$, MeOH), while that of the enantiomer was of the opposite sign and slightly larger in magnitude, reported as $[\alpha]_D^{22} = +4.7$ ($c = 4.0$, MeOH), which can be explained by the differences in concentration and protonation state of the two compounds. At this stage, the piperidine carbamates 31a and 31b were also hydrolyzed to give 5-*epi*-fagomine (26) and fagomine (3), which were separated following flash silica gel column chromatography (Scheme 8). The spectral data were similar to those previously

reported,^{24,29} once again with some variation due to slight differences in pH.³⁰

Scheme 8. Hydrolysis of Piperidine Carbamates To Give Fagomine (3) and 5-*epi*-Fagomine (26)

Confirmation of the structure of iodide 32 proved to be more difficult. No HMBC correlation was observed between the carbonyl and H3, and accordingly, the acetylated adduct 35 (Scheme 9) was prepared to aid with structural elucidation. Indeed, upon acetylation, downfield shifts of the resonances for protons H4 (5.34 ppm) and H5 (5.14 ppm), which were previously at ca. 3.76 ppm in the starting material, were observed. Proton H3, which was already at a relatively high chemical shift, did not move significantly upon acetylation. An HMBC correlation between H1 and the carbonyl was observed, though unfortunately, again no HMBC correlation was observed between the carbamate carbonyl and H3. As the stereochemistry of compound 32 also needed to be confirmed, we proposed that the synthesis of the corresponding alkenylamine (Scheme 9) would allow for confirmation of the structure of 32, including the chirality of all stereogenic centers. To this end, iodide 32 was first converted to the corresponding acetone 36, and on the basis of a slightly smaller $\Delta\delta$ of the acetone methyl group proton signals ($\Delta\delta_{\text{H}} = 0.039$ ppm)³¹ and diagnostic $\delta^{13}\text{C} \approx 27$ ppm for both methyl groups ($\delta^{13}\text{C} = 27.0$ ppm and $\delta^{13}\text{C} = 27.6$ ppm),^{32,33} this allowed for the relative stereochemistries of the 4- and 5-substituents to be determined as *trans* in the acetone five-membered ring, indicating a *syn* relationship in iodide 32. Opening of the acetone via oxidative addition of zinc and subsequent reductive ring opening then provided the truncated carbamate 37 in good yield. Finally, hydrolysis of the carbamate in 37 via treatment with NaOH under reflux for 4 h gave the desired alkenylamine 29. Comparison of the ¹H NMR and ¹³C NMR spectra of the synthesized alkenylamine with those of (+)-29 revealed them to be identical, and the optical rotations were of the same sign and comparable magnitude $\{[\alpha]_D^{27} = +37.5$ ($c = 1.0$, MeOH) and $[\alpha]_D^{27} = +31.8$ ($c = 0.2$, MeOH) $\}$, thus

Scheme 9. Determination of the Structure of Iodocarbamate **32** via Transformation into Alkenylamine (+)-**29**

Figure 2. Six-membered iodocyclization transition states for 2-deoxy sugar series.

establishing the structure of the synthesized alkenylamine to be that of the starting material, (+)-**29**. From this information and the previously deduced *trans* relationship in acetone **36**, the starting iodocarbamate **32** was thus determined to be (3*R*,4*R*,5*R*), as depicted. To confirm our methodology, alkenylamine (+)-**29** was also synthesized from the acetylated adduct **35** by way of reductive elimination of 5-OAc ($\rightarrow$ **38**) and subsequent carbamate hydrolysis.

Taken as a whole, the range of products formed en route to the synthesis of the target piperidines highlights the inherent problems of chemoselectivity in protecting-group-free chemistry. While we have previously illustrated that the protecting-group-free synthesis of pyrrolidines proceeds relatively smoothly with good to excellent *cis* selectivity for the carbamate annulation,³⁴ the present results illustrate that this methodology does not always translate readily to the synthesis of piperidines. The first factor to be considered when applying the carbamate annulation methodology to the synthesis of piperidines is O- versus N-cyclization. As illustrated when D-galactose was used as the starting material en route to the synthesis of DGJ,¹⁴ it is possible to favor formation of the desired pyran. This observation is remarkable in its own right given the general preference for five- versus six-membered ring formation. Indeed, when D-glucose was used as the starting material, only the furan was observed.

To eliminate formation of the furan as a competing reaction, the synthesis of piperidines devoid of the hydroxyl functionality at C2 can then be attempted using the corresponding 2-deoxy sugars as starting materials. Here, the chirality of the alkenylamine is crucial in determining the number of products formed during the annulation reaction. While the alkenylamine derived from 2-deoxy-D-galactose was transformed into the 4-*epi*-fagomine carbamate scaffold with excellent chemo- and diastereoselectivity (*cis:trans* > 95:5), subsection of the corresponding alkenylamine derived from 2-deoxy-D-glucose

to the annulation conditions resulted in poor selectivity and the formation of four products despite much effort to optimize this reaction to favor piperidine formation. The difference in selectivity for the two annulation reactions can be explained by considering the corresponding six-membered iodocyclization transition states, wherein, according to the seminal work of Chamberlin and co-workers,³⁵ the allylic hydroxyl has a stereodirecting effect in the formation of both five- and six-membered rings. Here, Chamberlin proposed that the unfavorable C–O σ^* to C=C π orbital overlap can be minimized by an OH-in-plane (with the double bond) transition state. In the case of our I₂-mediated carbamate annulations, this is illustrated in Figure 2, wherein transition state **I** for the 2-deoxy-D-galactose series depicts the major OH-in-plane conformer while **II** depicts the unfavorable H-in-plane conformer. Similarly, the OH-in-plane transition state **III** and the H-in-plane transition state **IV** are also given for the 2-deoxy-D-glucose series.

In addition to the conformation of the allylic hydroxyl, for six-membered rings the relative axial or equatorial orientation of the hydroxyl substituents needs to be considered. For the 2-deoxy-D-galactose series, both the OH-in-plane transition state **I** and the H-in-plane transition state **II** have one axial and one equatorial hydroxyl group, and as a consequence, the OH-in-plane transition state is lower in energy because there is no unfavorable C–O σ^* to C=C π orbital overlap. For the 2-deoxy-D-glucose series however, the OH-in-plane transition state **III** has the two hydroxyl substituents placed in an unfavorable axial orientation, thereby increasing the overall energy of this transition state and making it closer in energy to transition state **IV**, which, while having the unfavorable H-in-plane conformation, has the hydroxyl substituents in a more favorable equatorial orientation. In summary, the 2-deoxy-D-galactose series thus leads to the preferential formation of the *cis*-piperidine carbamate, while the 2-deoxy-D-glucose series,

Scheme 10. Proposed Mechanism for the Formation of Pyrrolidine 30, Piperidines 31a and 31b, and Iodide 32

with iodocyclization transition states of similar energy, leads to a variety of products.

To gain further insight into how to better design starting materials to favor the formation of the desired piperidine or pyrrolidine, reaction mechanisms have been proposed to explain the formation of the four products following the subjection of alkenylamine **29** to the carbamate annulation conditions (Scheme 10). First, attack of iodine from the top face of alkenylamine **29** (via transition state **III** in Figure 2) leads to an intermediate that can be transformed into piperidine **31a**, iodide **32**, and pyrrolidine **30**. Here, pathway A involves amine attack on the iodine complex at C5 followed by the addition of CO_2 to give *cis*-carbamate **31a** in accordance with our previously published annulation methodology.³⁴ The synthesis of iodocarbamate **32**, which contains a chiral backbone with a C5 stereocenter that differs from the parent alkenylamine **29**, is proposed to occur via S_N2 attack of water on the iodine complex (pathway B) followed by the addition of CO_2 to the linear iodoamine, which itself can be formed via the dissociation of carbonic acid. While there is no literature precedent for the reaction of terminal unsubstituted allylic alcohols with iodine, a number of halohydrin reactions have been performed on similar substrates with both the substitution pattern and the accessibility of the halonium ion effecting regio-^{36–38} and stereochemical^{39,40} outcomes. In the most closely related system involving hydrobromination, a variety of products were observed.^{41,42} In our case, however, 1H NMR analysis revealed no product that could have arisen from opening of the iodine complex by water at the terminal carbon or from attack on the diastereomeric iodine complex. The observed regioselectivity can be explained by Markovnikov's rule, while the stereoselectivity is more difficult to rationalize but is correlated to the relative reaction rates for formation of the intermediate iodine complex and the subsequent intra-

molecular or intermolecular nucleophilic ring opening. It is also conceivable that formation of a cyclic carbamate at the 3-position takes place prior to halohydrin formation⁴³ and that this provides an alternative route to **32**. The possibility that **32** is formed via a Payne rearrangement⁴⁴ of the intermediate epoxide is unlikely, as this reaction would require strongly basic conditions.⁴⁵ Such a cyclic carbamate intermediate, however, does not add to a conceivable mechanism for the formation of either **31a** or **30**.

To explain the formation of pyrrolidine **30**, a mechanism that supports the inversion of the C4 and C5 chiral centers is required, and thus an iodoepoxide (pathway C) is proposed as a key intermediate.^{42,46} Nucleophilic 5-*exo-tet* ring opening of the epoxide followed by the addition of CO_2 then allows for the formation of **30**.⁴⁷ Finally, the formation of *trans*-piperidine **31b** can be envisioned to occur via S_N2 attack of the diastereomeric iodine complex (pathway D), with the iodine complex itself arising from transition state **IV** (Figure 2). Subsequent addition of CO_2 to the intermediate cyclic iodoamine then gives the piperidine. Here it should also be noted that attack at the 5-position of the intermediate epoxide discussed in pathway C (6-*endo-trig*) could also lead to **31b**, but this is less favored than the corresponding 5-*exo-trig* cyclization ($\rightarrow$ **31b**),⁴⁷ especially in the absence of a bulky group α to the nitrogen.⁴⁸

Taken together, a number of factors should thus be considered before using the carbamate annulation reaction as a key step in the synthesis of piperidines containing multiple reactive sites. First, the orientations of the ring substituents affect the relative energies of the OH-in-plane or H-in-plane transition states, and where there is no obvious low-energy conformer, this can lead to a variety of products. One could thus envisage the use of judiciously placed bulky substituents to favor one conformer over the other, while the positioning of a

protecting group at the 4-position of the alkenylamine could be used to inhibit formation of the intermediate epoxide and hence the associated pyrrolidine products (e.g., 30). We have previously shown that the carbamate annulation can occur in the presence of protecting groups,^{49,50} which supports the validity of installing one or more protecting groups to control the chemoselectivity of the annulation when required.

CONCLUSION

We have presented two highly efficient syntheses of the biologically important piperidines DGJ (2) and 4-*epi*-fagomine (4) in good overall yields. Key in these reactions is the use of an I₂-mediated carbamate annulation. The use of the carbamate annulation for the synthesis of other piperidines, however, was less successful, as either O-cyclization or a series of competing intramolecular reactions occurred. To this end, trihydroxypyrrolidine 34 was prepared in five steps and modest overall yield (up to 15%). These studies highlight the limitations of using the carbamate annulation for the preparation of piperidines in protecting-group-free syntheses, although the work also points to the potential of this methodology for the synthesis of interesting chiral scaffolds when either protected or suitably functionalized alkenylamines are used as starting materials.

EXPERIMENTAL SECTION

General Procedures. Unless otherwise stated, all reactions were performed under atmospheric air. THF was distilled from LiAlH₄ prior to use. Pyridine was distilled over KOH prior to use. All chemicals obtained from commercial suppliers were used without further purification. Zn dust was activated by the careful addition of concentrated H₂SO₄ followed by decantation, washing with EtOH (3×) and hexanes (3×), 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 with detection by coating with 20% H₂SO₄ 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% KMnO₄ and 1% NaIO₄ in H₂O followed by heating. ¹H and ¹³C chemical shifts (δ) were internally referenced to the residual solvent peak. NMR peak assignments are based on 2D NMR experiments (COSY, HSQC, and HMBC). High-resolution electrospray ionization mass spectrometry [HRMS(ESI)] was performed on a Q-TOF instrument.

1-Amino-2,5-anhydro-1,6-dideoxy-6-iodo-L-idoitol (13). To a solution of alkenylamine 10¹³ (310 mg, 1.69 mmol) in sat. aq. NaHCO₃ (7 mL) was added I₂ (645 mg, 2.54 mmol), and the solution was stirred for 7 days. The reaction mixture, a crude oil, was concentrated and used without further purification. *R*_f = 0.23 (DCM/EtOH/MeOH/30% aq. NH₃, 5/2/2/1 v/v/v/v); ¹H NMR (500 MHz, D₂O) δ 4.45 (dt, *J*_{4,5} = 3.1, *J*_{5,6a} = *J*_{5,6b} = 7.3 Hz, 1H, HS), 4.40 (ddd, *J*_{2,3} = 3.8, *J*_{1a,2} = 4.4, *J*_{1b,2} = 5.6 Hz, 1H, H2), 4.38 (dd, *J*_{3,4} = 1.3, *J*_{2,3} = 3.8 Hz, 1H, H3), 4.28 (dd, *J*_{3,4} = 1.3, *J*_{4,5} = 3.1 Hz, 1H, H4), 3.27 (dd, *J*_{5,6a} = 7.3, *J*_{6a,6b} = 9.8 Hz, 1H, H6a), 3.26 (dd, *J*_{5,6b} = 7.3, *J*_{6a,6b} = 9.8 Hz, 1H, H6b), 3.258 (dd, *J*_{1a,2} = 4.4, *J*_{1a,1b} = 13.6 Hz, 1H, H1a), 3.23 (dd, *J*_{1b,2} = 5.6, *J*_{1a,1b} = 13.6 Hz, 1H, H1b); ¹³C NMR (125 MHz, D₂O) δ 81.7 (C5), 77.5 (C3), 76.52, 76.50 (C2 and C4), 39.4 (C1), 0.6 (C6); HRMS(ESI) *m/z* calcd for [C₆H₁₂O₃NI + H]⁺ 273.9935, found 273.9938.

1-Acetamido-3,4-di-O-acetyl-2,5-anhydro-1,6-dideoxy-6-iodo-L-idoitol (14). A solution of iditol 13 was stirred overnight at rt in a solution of acetic anhydride (4 mL) and pyridine (4 mL). The reaction mixture was concentrated, dissolved in EtOAc, washed with sat. aq. NaHCO₃ and brine, dried over MgSO₄, filtered, and concentrated in vacuo to give acetylated iditol 14 as a colorless oil. *R*_f = 0.12 (EtOAc); ¹H NMR (500 MHz, CDCl₃) δ 5.84 (t, *J*_{1a,NH} = *J*_{1b,NH} = 6.2 Hz, 1H, NH), 5.38 (dd, *J*_{3,4} = 1.4, *J*_{4,5} = 4.0 Hz, 1H, H4), 5.27 (dd, *J*_{3,4} = 1.4, *J*_{2,3} = 3.4 Hz, 1H, H3), 4.52 (ddd, *J*_{4,5} = 4.0, *J*_{5,6a} = 6.3, *J*_{5,6b} = 8.8 Hz, 1H, HS), 4.30 (ddd, *J*_{2,3} = 3.4, *J*_{1b,2} = 6.2, *J*_{1a,2} = 7.3

Hz, 1H, H2), 3.47 (ddd, *J*_{1a,NH} = 6.2, *J*_{1a,2} = 7.3, *J*_{1a,1b} = 14.0 Hz, 1H, H1a), 3.27 (td, *J*_{1b,NH} = *J*_{1b,2} = 6.2, *J*_{1a,1b} = 14.0 Hz, 1H, H1b), 3.21 (dd, *J*_{5,6a} = 6.3, *J*_{6a,6b} = 9.7 Hz, 1H, H6a), 3.17 (dd, *J*_{5,6b} = 8.8, *J*_{6a,6b} = 9.7 Hz, 1H, H6b), 2.16 (s, 3H, Ac), 2.15 (s, 3H, Ac), 2.00 (s, 3H, NHAc); ¹³C NMR (125 MHz, CDCl₃) δ 170.5 (C=O, NHAc), 170.1 (C=O, Ac), 169.4 (C=O, Ac), 79.6 (C5), 78.9 (C2), 76.4 (C3), 76.1 (C4), 38.1 (C1), 23.5 (CH₃, NHAc), 20.9 (CH₃, Ac), 20.8 (CH₃, Ac), -0.6 (C6); HRMS(ESI) *m/z* calcd for [C₁₂H₁₈O₆NI + Na]⁺ 422.0076, found 422.0076.

Methyl 3,6-Anhydro-2-deoxy-α-D-galactopyranoside (19).

To a solution of methyl glycoside 18 (28 mg, 0.16 mmol) in dry THF (3 mL) were added PPh₃ (83 mg, 0.32 mmol), imidazole (43 mg, 0.63 mmol), and I₂ (80 mg, 0.32 mmol). The mixture was refluxed overnight (18 h) and then cooled and concentrated. The resulting oil was purified by flash column chromatography (petroleum ether/EtOAc, 5/1 to 1/1 v/v) to give bicyclic galactoside 19. *R*_f = 0.3 (EtOAc/MeOH, 98/2 v/v); ¹H NMR (500 MHz, D₂O) δ 4.69 (d, *J*_{1,2b} = 6.2 Hz, 1H, H1), 4.31–4.32 (m, 2H, H3 and H6a), 4.27–4.28 (m, 1H, H5), 4.02 (d, *J*_{3,4} = 1.8 Hz, 1H, H4), 3.98 (dd, *J*_{5,6b} = 3.4, *J*_{6a,6b} = 9.9 Hz, 1H, H6b), 3.40 (s, 3H, OMe), 2.19 (dd, *J*_{2a,3} = 4.6, *J*_{2a,2b} = 14.5 Hz, 1H, H2a), 2.02 (ddd, *J*_{2b,3} = 1.6, *J*_{1,2b} = 6.2, *J*_{2a,2b} = 14.5 Hz, 1H, H2b); ¹³C NMR (125 MHz, D₂O) δ 97.2 (C1), 77.8 (C3), 77.3 (C5), 74.8 (C4), 70.2 (C6), 55.7 (OMe), 37.6 (C2); HRMS(ESI) *m/z* calcd for [C₇H₁₂O₄ + Na]⁺ 183.0633, found 183.0631.

Methyl 2-Deoxy-3,4-O-isopropylidene-α/β-D-galactopyranoside (20). From 17. AcCl (180 μL) was added to a solution of 2-deoxy-D-galactose (17) (200 mg, 0.61 mmol) in MeOH/acetone/dimethoxypropane (6 mL, 2/2/2 v/v/v), and the solution was stirred for 18 h at room temperature. The reaction was quenched with Dowex-OH⁻, and the mixture was filtered and concentrated. The resulting oil, 20, was used without purification.

From 23. To a solution of D-galactal 23 (200 mg, 1.36 mmol) in MeOH (8 mL) and acetone (8 mL) was added pTsOH-H₂O (24 mg, 0.14 mmol), and the reaction was stirred at room temperature for 18 h. The reaction mixture was then neutralized by the addition of Dowex-OH⁻, filtered, and concentrated in vacuo. The crude reaction product, 20, was used in the iodination reaction without further purification.

*R*_f = 0.13 (petroleum ether/EtOAc, 1/1 v/v); ¹H NMR (500 MHz, CDCl₃) δ 4.86 (dd, *J*_{1,2a} = 5.1, *J*_{1,2b} = 6.0 Hz, 1H, H1), 4.47 (ddd, *J*_{2b,3} = 4.1, *J*_{2a,3} = 5.1, *J*_{3,4} = 6.9 Hz, 1H, H3), 4.16 (dd, *J*_{4,5} = 1.7, *J*_{3,4} = 6.9 Hz, 1H, H4), 3.89 (dd, *J*_{5,6a} = 5.6, *J*_{6a,6b} = 10.6 Hz, 1H, H6a), 3.80 (ddd, *J*_{4,5} = 1.7, *J*_{5,6b} = 4.3, *J*_{5,6a} = 5.6 Hz, 1H, H5), 3.77 (dd, *J*_{5,6b} = 4.3, *J*_{6a,6b} = 10.6 Hz, 1H, H6b), 3.37 (s, 3H, OMe), 2.17 (dt, *J*_{2a,2b} = 14.8, *J*_{1,2a} = *J*_{2a,3} = 5.1 Hz, 1H, H2a), 1.73 (ddd, *J*_{2b,3} = 4.1, *J*_{1,2b} = 6.0, *J*_{2a,2b} = 14.8 Hz, 1H, H2b), 1.47 (s, iPr), 1.31 (s, iPr); ¹³C NMR (75 MHz, CDCl₃) 109.2 (iPr-C(CH₃)₂), 97.4 (C1), 73.4 (C4), 70.5 (C3), 68.5 (C5), 62.8 (C6), 55.0 (OMe), 30.8 (C2), 26.8, 25.3 (iPr-C(CH₃)₂); HRMS(ESI) *m/z* calcd for [C₁₀H₁₈O₅ + Na]⁺ 241.1052, found 241.1046.

Methyl 2,6-Dideoxy-6-iodo-3,4-O-isopropylidene-α/β-D-galactopyranoside (21). From 17. To a solution of 20 (0.61 mmol) in dry THF (7 mL) were added PPh₃ (456 mg, 1.74 mmol), imidazole (180 mg, 2.62 mmol), and I₂ (442 mg, 1.74 mmol). The mixture was refluxed for 18 h and then cooled and concentrated. The syrup was redissolved in ethyl acetate (20 mL), washed with sat. aq. Na₂S₂O₃ (2 × 15 mL) and then brine (20 mL), dried over MgSO₄, filtered, and concentrated. The resulting oil was purified by flash column chromatography (petroleum ether/EtOAc, 15/1 v/v) followed by crystallization from hexanes to give iodogalactoside 21 as white crystals (142 mg, 0.434 mmol, 71% from 17).

From 23. Iodination of 20 performed as above yielded iodogalactoside 21 as white crystals (241 mg, 0.73 mmol, 54% over two steps from 23).

*R*_f = 0.77 (petroleum ether/EtOAc, 1/1 v/v); Mp 91–92 °C; [α]_D²¹ = +72.4 (c = 1.0, CHCl₃); IR (film) 2982, 2936, 2912, 2833, 1439, 1373, 1246, 1214, 1186, 1088, 1047, 969, 880, 738 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 4.86 (dd, *J*_{1,2a} = 5.4, *J*_{1,2b} = 6.6 Hz, 1H, H1), 4.49 (ddd, *J*_{2b,3} = 3.7, *J*_{2a,3} = 4.6, *J*_{3,4} = 7.3 Hz, 1H, H3), 4.26 (dd, *J*_{4,5} = 2.0, *J*_{3,4} = 7.3 Hz, 1H, H4), 3.86 (ddd, *J*_{5,4} = 2.0, *J*_{5,6a} = 5.8, *J*_{5,6b} = 8.1 Hz, 1H, H5), 3.47 (s, 3H, OMe), 3.34 (dd, *J*_{5,6a} = 5.8, *J*_{6a,6b} = 10.2 Hz, 1H,

H6a), 3.28 (dd, $J_{5,6b} = 8.1$, $J_{6a,6b} = 10.2$ Hz, 1H, H6b), 2.24 (ddd, $J_{2a,3} = 4.6$, $J_{1,2a} = 5.4$, $J_{2a,2b} = 15.1$ Hz, 1H, H2a), 1.64 (ddd, $J_{2b,3} = 3.7$, $J_{1,2b} = 6.6$, $J_{2a,2b} = 15.1$ Hz, 1H, H2b), 1.48 (s, 3H, iPr), 1.34 (s, 3H, iPr); ^{13}C NMR (75 MHz, CDCl_3) 109.3 (iPr-C(CH_3)₂), 97.6 (C1), 73.4 (C4), 70.9 (C3), 70.0 (C5), 55.2 (OMe), 30.2 (C2), 26.6, 25.2 (iPr-C(CH_3)₂), 3.2 (C6); HRMS(ESI) m/z calcd for $[\text{C}_{10}\text{H}_{17}\text{O}_4\text{I} + \text{Na}]^+$ 351.0069, found 351.0072.

Methyl 2,6-Dideoxy-6-iodo- α/β -D-galactopyranoside (22). To a solution of iodogalactoside **21** (237 mg, 0.79 mmol) in MeOH (10 mL), at 0 °C was added AcCl (200 μL). The reaction mixture was stirred at room temperature for 5 days, after which the reaction was quenched with Dowex- OH^- and the mixture was concentrated in vacuo. The product was purified via gradient flash column chromatography (petroleum ether/EtOAc, 3/1 to 1/1 v/v) to provide galactoside **22** as a colorless oil (200 mg, 0.69 mmol, 94%). $R_f = 0.11$ (EtOAc); $[\alpha]_D^{21} = +0.11$ ($c = 1.0$, MeOH); IR (film) 3292, 2925, 2854, 1735, 1457, 1209, 1130, 1034, 668 cm^{-1} ; ^1H NMR (500 MHz, CD_3OD) δ 4.71 (d, $J_{1,2a} = 3.6$ Hz, 1H, H1), 3.81 (ddd, $J_{3,4} = 3.2$, $J_{2b,3} = 4.1$, $J_{2b,3} = 12.6$ Hz, 1H, H3), 3.78–3.72 (m, 2H, H4 and H5), 3.31 (s, 3H, OMe), 3.30–3.22 (m, 2H, H6a and H6b), 1.82 (td, $J_{2a,2b} = J_{2a,3} = 12.6$, $J_{1,2a} = 3.6$ Hz, 1H, H2a), 1.62 (dd, $J_{2b,3} = 5.1$, $J_{2a,2b} = 12.6$ Hz, 1H, H2b); ^{13}C NMR (125 MHz, CD_3OD) δ 100.3 (C1), 72.9 (C4), 70.5 (C5), 66.7 (C3), 55.5 (OCH₃), 33.1 (C2), 4.5 (C6); HRMS(ESI) m/z calcd for $[\text{C}_7\text{H}_{13}\text{O}_4\text{I} + \text{Na}]^+$ 310.9756, found 310.9756.

(3R,4S)-1-Aminohex-5-ene-3,4-diol (24). To a solution of iodogalactoside **22** (58 mg, 0.21 mmol) in a saturated solution of NH_4OAc in EtOH (3 mL) were added activated Zn (36 mg, 0.55 mmol), NaCNBH_3 (21 mg, 0.33 mmol), and 30% aq. NH_3 (1 mL). The mixture was stirred at reflux for 18 h, filtered, and concentrated in vacuo. The mixture was loaded on a Dowex- H^+ column, eluted in 15% aq. NH_3 , and concentrated in vacuo. Acidification with 1 M HCl yielded alkenylamine **24**, a white solid, as the HCl salt (35 mg, 0.21 mmol, 99%). $R_f = 0.11$ (DCM/EtOH/MeOH/30% aq. NH_3 , 5/2/2/1 v/v/v/v); $[\alpha]_D^{25} = -114$ ($c = 0.1$, EtOH); IR (film) 3370, 3321, 2962, 2836, 1642, 1504, 1435, 1378, 1025, 652 cm^{-1} ; ^1H NMR (500 MHz, D_2O) δ 5.87 (ddd, $J_{4,5} = 6.2$, $J_{5,6\text{-cis}} = 10.3$, $J_{5,6\text{-trans}} = 17.1$ Hz, 1H, H5), 5.30 (d, $J_{5,6\text{-trans}} = 17.1$ Hz, 1H, H6-*cis*), 5.27 (d, $J_{5,6\text{-cis}} = 10.3$ Hz, 1H, H6-*trans*), 4.03 (dd, $J_{3,4} = 4.6$, $J_{4,5} = 6.2$ Hz, 1H, H4), 3.69 (ddd, $J_{2a,3} = 3.2$, $J_{3,4} = 4.6$, $J_{2b,3} = 9.6$ Hz, 1H, H3), 2.85 (ddd, $J_{1a,2b} = 5.9$, $J_{1a,2a} = 8.5$, $J_{1a,1b} = 12.8$ Hz, 1H, H1a), 2.77 (ddd, $J_{1b,2a} = 7.0$, $J_{1b,2b} = 8.0$, $J_{1a,1b} = 12.8$ Hz, 1H, H1b), 1.72 (dddd, $J_{2a,3} = 3.2$, $J_{1b,2a} = 7.0$, $J_{1a,2a} = 8.5$, $J_{2a,2b} = 17.5$ Hz, 1H, H2a), 1.55 (dddd, $J_{1a,2b} = 5.9$, $J_{1b,2b} = 8.0$, $J_{2b,3} = 9.6$, $J_{2a,2b} = 17.5$ Hz, 1H, H2b); ^{13}C NMR (75 MHz, D_2O) δ 135.5 (C5), 118.0 (C6), 75.4 (C4), 71.6 (C3), 37.1 (C1), 28.9 (C2); HRMS(ESI) m/z calcd for $[\text{C}_6\text{H}_{13}\text{O}_2\text{N} + \text{H}]^+$ 132.1025, found 132.1022.

1-N,6-O-Carbonyl-4-*epi*-fagomine (25a). To a solution of alkenylamine hydrochloride **24** (17 mg, 0.13 mmol) in saturated NaHCO_3 (aq) (0.6 mL) was added I_2 (32 mg, 0.25 mmol). The solution was stirred for 5 days at 35 °C and then filtered and concentrated in vacuo. Purification by flash chromatography (EtOAc/MeOH, 99/1 v/v) yielded carbamate **25a** as a white solid (15.1 mg, 0.0873 mmol, 86%). $[\alpha]_D^{19} = +4.0$ ($c = 1$, MeOH); IR (film) 3338, 2945, 2833, 2360, 2342, 1653, 1449, 1417, 1114, 1021 cm^{-1} ; ^1H NMR (500 MHz, D_2O) δ 4.48 (t, $J_{5,6} = J_{6a,6b} = 9.0$ Hz, 1H, H6a), 4.37 (dd, $J_{5,6} = 5.5$, $J_{6a,6b} = 9.0$ Hz, 1H, H6b), 4.03 (ddd, $J_{4,5} = 1.8$, $J_{5,6b} = 5.5$, $J_{5,6a} = 9.0$ Hz, 1H, H5), 3.89 (m, 1H, H4), 3.86 (dd, $J_{3,4} = 2.5$, $J_{2,3} = 5.5$ Hz, 1H, H3), 3.76 (ddd, $J_{1a,2a} = 1.8$, $J_{1a,2b} = 5.5$, $J_{1a,1b} = 13.5$ Hz, 1H, H1a), 3.06 (app dt, $J_{1b,2a} = J_{1b,2b} = 4.5$, $J_{1a,1b} = 13$ Hz, 1H, H1b), 1.70–1.80 (m, 2H, H2a and H2b); ^{13}C NMR (125 MHz, D_2O) δ 159.2 (C=O), 68.6 (C3), 67.9 (C4), 64.1 (C6), 57.0 (C5), 38.5 (C1), 25.1 (C2); HRMS(ESI) m/z calcd for $[\text{C}_7\text{H}_{11}\text{NO}_4 + \text{Na}]^+$ 196.0586, found 196.0590.

4-*epi*-Fagomine (4). To a solution of carbamate **25a** (11 mg, 0.06 mmol) in EtOH (2 mL) was added NaOH (32 mg, 0.80 mmol). The solution was stirred at reflux for 2 h and then cooled and purified directly using a Dowex- H^+ column. The product was eluted in 5 to 15% aq. NH_3 and concentrated in vacuo. Purification by flash chromatography (DCM/EtOH/MeOH/30% aq. NH_3 , 25/2/2/1 to 5/2/2/1 v/v/v/v) yielded piperidine **4** as a white solid (8.1 mg, 0.055 mmol, 92%). $[\alpha]_D^{19} = +11.8$ ($c = 0.1$, MeOH); IR (film) 3286, 2923,

1653, 1398, 1145, 1100, 1046, 1022 cm^{-1} ; ^1H NMR (600 MHz, D_2O) δ 3.92 (bs, 1H, H4), 3.76 (ddd, $J = 2.8$, $J = 8.4$ Hz, $J = 10.5$ Hz, 1H, H3), 3.69 (dd, $J = 5.8$, $J = 11.7$ Hz, 1H, H6a), 3.65 (dd, $J = 7.6$, $J = 11.6$ Hz, 1H, H6b), 3.17 (dt, $J = 3.6$, $J = 12.7$ Hz, 1H, H1a), 2.92 (app t, $J = 7.1$ Hz, 1H, H5), 2.73 (m, 1H, H1b), 1.74–1.79 (m, 2H, H2a and H2b); ^{13}C NMR (150 MHz, D_2O) δ 67.4 (C3), 65.8 (C4), 60.0 (C6), 59.4 (C5), 42.1 (C1), 24.0 (C2); HRMS(ESI) m/z calcd for $[\text{C}_6\text{H}_{13}\text{NO}_3 + \text{H}]^+$ 148.0974, found 148.0974.

Methyl 2-Deoxy- α/β -D-glucopyranoside (27b). To a solution of AcCl (240 μL) in MeOH (240 mL) was added 2-deoxy-D-glucose (**27a**) (1200 mg, 7.3 mmol), and the mixture was stirred at room temperature for 18 h. The reaction mixture was then neutralized by the addition of Dowex- OH^- , filtered, and concentrated. The resulting oil was used without further purification. Methyl 2-deoxy- α/β -D-glucopyranoside (**27b**) was obtained as a colorless oil in an 8:1 α/β ratio.

Data for the α isomer: $R_f = 0.27$ (10% MeOH in EtOAc); ^1H NMR (600 MHz, D_2O) δ 4.86 (d, $J_{1,2b} = 3.5$ Hz, 1H, H1), 3.83 (dd, $J_{5,6a} = 2.1$, $J_{6a,6b} = 12.0$ Hz, 1H, H6a), 3.79–3.87 (m, 1H, H3), 3.73 (dd, $J_{5,6b} = 5.5$, $J_{6a,6b} = 12.0$ Hz, 1H, H6b), 3.56 (ddd, $J_{5,6a} = 2.1$, $J_{5,6b} = 5.5$, $J_{4,5} = 9.6$ Hz, 1H, H5), 3.32 (s, 3H, OMe), 3.31 (m, 1H, H4), 2.10 (dd, $J_{2a,3} = 5.3$, $J_{2a,2b} = 13.5$ Hz, 1H, H2a), 1.67 (ddd, $J_{1,2b} = 3.5$, $J_{2b,3} = 12.0$, $J_{2a,2b} = 13.5$ Hz, 1H, H2b); ^{13}C NMR (150 MHz, D_2O) δ 98.1 (C1), 71.9 (C5), 70.8 (C4), 68.0 (C3), 60.5 (C6), 54.2 (OMe), 36.4 (C2); HRMS(ESI) m/z calcd for $[\text{C}_7\text{H}_{14}\text{O}_5 + \text{Na}]^+$ 201.0733, found 201.0735.

Data for the β isomer: $R_f = 0.27$ (10% MeOH in EtOAc); ^1H NMR (600 MHz, D_2O) δ 4.59 (dd, $J_{1,2a} = 1.8$, $J_{1,2b} = 9.9$ Hz, 1H, H1), 3.89 (dd, $J_{5,6a} = 2.0$, $J_{6a,6b} = 12.0$ Hz, 1H, H6a), 3.65–3.72 (m, 2H, H3 and H6b), 3.48 (s, 3H, OMe), 3.32 (m, 1H, H5), 3.21 (t, $J_{3,4} = J_{4,5} = 9.6$ Hz, 1H, H4), 2.21 (ddd, $J_{1,2a} = 1.8$, $J_{2a,3} = 5.1$, $J_{2a,2b} = 12.9$ Hz, 1H, H2a), 1.43 (ddd, $J_{1,2b} = 9.9$, $J_{2b,3} = 12.0$, $J_{2a,2b} = 12.9$ Hz, 1H, H2b); ^{13}C NMR (150 MHz, D_2O) δ 100.6 (C1), 75.9 (C5), 70.9 (C4), 70.2 (C3), 60.8 (C6), 56.4 (OMe), 38.0 (C2); HRMS(ESI) m/z calcd for $[\text{C}_7\text{H}_{14}\text{O}_5 + \text{Na}]^+$ 201.0739, found 201.0735.

Methyl 2,6-Dideoxy-6-iodo- α -D-glucoside (28). To a solution of methyl 2-deoxy-D-glucoside (70.1 mg, 0.4 mmol) in dry THF (4 mL) under an atmosphere of argon were added imidazole (81.6 mg, 1.2 mmol), PPh_3 (157 mg, 0.6 mmol), and I_2 (152 mg, 0.6 mmol). The reaction mixture was heated to 75 °C for 1.5 h and then cooled and concentrated. The product was purified by silica gradient flash column chromatography (petroleum ether/EtOAc, 2/1 v/v) and then reversed-phase HP20 chromatography (MeOH/water, 1/5 v/v) to give methyl 2,6-dideoxy-6-iodo- α -D-glucopyranoside (**28**) as a colorless oil (74.1 mg, 0.26 mmol, 64% over two steps from 2-deoxyglucose **27**). $R_f = 0.42$ (1% MeOH in EtOAc); $[\alpha]_D^{23} = +84.0$ ($c = 1.0$, CHCl_3) (lit $[\alpha]_D^{27} = +97$, $c = 0.9$ in chloroform⁵¹); IR (film) 3387, 2934, 1442, 1377, 1211, 1128, 1044, 966, 938 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 4.83 (d, $J_{1,2b} = 3.4$ Hz, 1H, H1), 3.97 (ddd, $J_{2a,3} = 5.1$, $J_{3,4} = 8.7$, $J_{2b,3} = 11.7$ Hz, 1H, H3), 3.57–3.62 (m, 1H, H6a), 3.40 (s, 3H, OMe), 3.35–3.41 (m, 2H, H5 and H6b), 3.24–3.30 (m, 1H, H4), 2.16 (ddd, $J_{1,2a} = 1.0$, $J_{2a,3} = 5.1$, $J_{2a,2b} = 12.9$ Hz, 1H, H2a), 1.71 (ddd, $J_{1,2b} = 3.2$, $J_{2b,3} = 11.7$, $J_{2a,2b} = 12.9$ Hz, 1H, H2b); ^{13}C NMR (125 MHz, CDCl_3) δ 98.7 (C1), 77.6 (C4), 70.4 (C5), 69.1 (C3), 55.3 (OMe), 37.8 (C2), 7.9 (C6); HRMS(ESI) m/z calcd for $[\text{C}_7\text{H}_{13}\text{O}_4\text{I} + \text{Na}]^+$ 310.9756, found 310.9751.

(3R,4R)-1-Amino-hex-5-ene-3,4-diol Hydrochloride (29). To a solution of methyl 2,6-dideoxy-6-iodo- α -D-glucoside (**28**) (111 mg, 0.39 mmol) in a saturated solution of NH_4OAc in EtOH (7.3 mL) were added activated Zn (450 mg, 6.9 mmol), NaCNBH_3 (85 mg, 1.3 mmol), and 30% aq. NH_3 (2.9 mL). The mixture was stirred at reflux for 4.5 h, cooled to room temperature, filtered through Celite, and concentrated under reduced pressure. HCl (30 mL, 2 M) was added, and AcOH was removed by evaporation. Coevaporation with water/EtOH was performed to remove traces of AcOH, after which an aqueous solution of NaOH (40 mL, 1.25 M) was added and the NH_3 was evaporated. The resulting solid was filtered and washed with EtOH to remove the bulk of the insoluble NaCl salt, and the filtrate and combined washings were concentrated and then dry-loaded on silica for column chromatography (DCM/EtOH/MeOH/35% aq.

NH₃, 55/2/2/1 to 5/2/2/1 v/v/v/v). Alkenylamine **29** was obtained as the HCl salt after the addition of aq. HCl (1.2 M) and concentration to obtain a white solid (55.5 mg, 0.33 mmol, 86%). R_f = 0.16 (DCM/EtOH/MeOH/35% aq. NH₃, 5/2/2/1 v/v/v/v); $[\alpha]_D^{27}$ = +37.5 (c = 1.0, MeOH); IR (film) 3336, 2924, 1621, 1505, 1468, 1396, 1312, 1255, 1122, 1020, 997, 931, 854 cm⁻¹; ¹H NMR (500 MHz, D₂O) δ 5.87 (ddd, $J_{4,5}$ = 6.7, $J_{5,6-cis}$ = 10.5, $J_{5,6-trans}$ = 17.3 Hz, 1H, H5), 5.34 (d, $J_{5,6-trans}$ = 17.3 Hz, 1H, H6-*trans*), 5.28 (d, $J_{5,6-cis}$ = 10.5 Hz, 1H, H6-*cis*), 4.03 (dd, $J_{4,5}$ = 6.7, $J_{3,4}$ = 6.8 Hz, 1H, H4), 3.70 (ddd, $J_{2,3}$ = 2.9, $J_{2b,3}$ = 5.4, $J_{3,4}$ = 6.8 Hz, 1H, H3), 3.13 (m, 2H, H1), 1.89 (m, 1H, H2a), 1.77 (m, 1H, H2b); ¹³C NMR (125 MHz, D₂O) δ 136.1 (C5), 118.1 (C6), 75.4 (C4), 71.7 (C3), 37.2 (C1), 29.4 (C2); HRMS(ESI) m/z calcd for [C₆H₁₃O₂N + H]⁺ 132.1025, found 132.1031.

Iodocyclization/Carbamate Annulation. To a solution of alkenylamine hydrochloride **29** (240 mg, 1.4 mmol) in saturated aq. NaHCO₃ (7.2 mL) (made fresh immediately prior to use) were added I₂ (1900 mg, 7.5 mmol) and NaHCO₃ (1400 mg, 17 mmol). The solution was stirred at room temperature for 5 days, filtered, and concentrated under reduced pressure. The products, all as white solids, were separated by repeated use of silica plug (MeOH in EtOAc, 0–10% v/v) and reversed-phase columns (octyl-bonded end-capped silica beads) eluting in H₂O (for carbamates **30**, **31a**, and **31b**) and MeOH (for iodide **32**). Yield of **30** = 10–30%.

1-N,6-O-Carbonyl-1,2,4-trideoxy-1,4-imino-L-allitol (30). $[\alpha]_D^{25}$ = –0.5 (c = 0.4, MeOH); IR (film) 3390, 3331, 2965, 2914, 1719, 1634, 1517, 1477, 1455, 1133, 1110, 1070 cm⁻¹; ¹H NMR (500 MHz, D₂O) δ 4.19–4.27 (m, 2H, H3 and H6a), 3.94–4.03 (m, 2H, H5 and H6b), 3.47 (dd, $J_{1,2a}$ = 6.1, $J_{1,2b}$ = 9.0 Hz, 2H, H1), 3.28 (t, $J_{4,5}$ = $J_{3,4}$ = 7.7 Hz, 1H, H4), 2.24 (dq, $J_{1,2a}$ = $J_{2a,3}$ = 6.1, $J_{2a,2b}$ = 12.6 Hz, 1H, H2a), 1.80 (dq, $J_{1,2b}$ = $J_{2b,3}$ = 9.0, $J_{2a,2b}$ = 12.6 Hz, 1H, H2b); ¹³C NMR (150 MHz, D₂O) δ 154.4 (C=O), 74.0 (C3), 69.2 (C6), 64.8 (C4), 64.2 (C5), 44.2 (C1), 30.2 (C2); HRMS(ESI) m/z calcd for [C₇H₁₁O₄N + Na]⁺ 196.0586, found 196.0579.

1-N,6-O-Carbonyl-5-*epi*-fagomine (31a) and 1-N,6-O-Carbonyl-fagomine (31b). IR (film) 3409, 2948, 2840, 1632, 1462, 1403, 1092, 1010 cm⁻¹.

Data for the major isomer (**31a**): ¹H NMR (500 MHz, D₂O) δ 4.53 (t, $J_{5,6a}$ = $J_{6a,6b}$ = 9.0 Hz, 1H, H6a), 4.34 (dd, $J_{5,6b}$ = 6.0, $J_{6a,6b}$ = 9.0 Hz, 1H, H6b), 4.23 (ddd, $J_{4,5}$ = 2.5, $J_{5,6b}$ = 6.0, $J_{5,6a}$ = 9.0 Hz, 1H, H5), 4.06 (dd, $J_{3,4}$ = 3.2, $J_{2a,3}$ = 6.3 Hz, 1H, H3), 3.73 (m, 1H, H4), 3.56 (dd, $J_{1a,2a}$ = 6.3, $J_{1a,1b}$ = 13.5 Hz, 1H, H1a), 3.23 (td, $J_{1b,2a}$ = 3.7, $J_{1a,1b}$ = $J_{1b,2b}$ = 13.5 Hz, 1H, H1b), 1.95–2.02 (m, 1H, H2a), 1.64 (d, $J_{2a,2b}$ = 15.0 Hz, 1H, H2b); ¹³C NMR (125 MHz, D₂O) δ 159.2 (C=O), 66.5 (C3), 66.5 (C4), 64.1 (C6), 53.2 (C5), 35.2 (C1), 24.4 (C2); HRMS(ESI) m/z calcd for [C₇H₁₁O₄N + Na]⁺ 196.0586, found 196.0587.

Data for the minor isomer (**31b**): ¹H NMR (500 MHz, D₂O) δ 4.53 (t, $J_{5,6a}$ = $J_{6a,6b}$ = 9.0 Hz, 1H, H6a), 4.31 (dd, $J_{5,6b}$ = 5.0, $J_{6a,6b}$ = 9.0 Hz, 1H, H6b), 3.68–3.71 (m, 1H, H1a), 3.67 (ddd, $J_{5,6b}$ = 5.0, $J_{5,6a}$ = 9.0, $J_{4,5}$ = 9.5 Hz, 1H, H5), 3.62 (ddd, $J_{2b,3}$ = 5.0, $J_{3,4}$ = 9.5, $J_{2a,3}$ = 11.5 Hz, 1H, H3), 3.34 (t, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, 1H, H4), 3.03 (td, $J_{1b,2a}$ = 3.2, $J_{1a,1b}$ = $J_{1b,2b}$ = 13.0 Hz, 1H, H1b), 1.92–1.98 (m, 1H, H2a), 1.51 (ddt, $J_{2b,3}$ = 5.0, $J_{1a,2b}$ = 11.7, $J_{1b,2b}$ = $J_{2a,2b}$ = 13.0 Hz, 1H, H2b); ¹³C NMR (125 MHz, D₂O) δ 158.7 (C=O), 74.5 (C4), 71.4 (C3), 66.5 (C6), 57.6 (C5), 38.7 (C1), 30.8 (C2); HRMS(ESI) m/z calcd for [C₇H₁₁O₄N + Na]⁺ 196.0586, found 196.0587.

1-Amino-1-N,3-O-carbonyl-1,2,6-trideoxy-6-iodo-L-gulitol (32). R_f = 0.26 (10% MeOH in EtOAc); $[\alpha]_D^{26}$ = –8.9 (c = 0.67, MeOH); IR (film) 3367, 2928, 1680, 1488, 1457, 1300, 1223, 1108, 1021, 523 cm⁻¹; ¹H NMR (500 MHz, D₂O) δ 4.50 (dt, J = 3.3, J = 9.9 Hz, 1H, H3), 3.72–3.81 (m, 2H, H4 and H5), 3.44 (dd, J = 3.9, J = 10.5 Hz, 1H, H6a), 3.29–3.37 (m, 3H, H1a,b and H6b), 1.90–2.01 (m, 2H, H2a,b); ¹³C NMR (125 MHz, D₂O) δ 154.7 (C=O), 76.1 (C3), 71.8, 68.1 (C4 and C5), 35.8 (C1), 19.6 (C2), 6.2 (C6); HRMS(ESI) m/z calcd for [C₇H₁₂O₄NI + H]⁺ 301.9889, found 301.9888.

3,5-Di-O-acetyl-1-N,6-O-carbonyl-1,2,4-trideoxy-1,4-imino-L-allitol (33). Carbamate (**30**) (124.7 mg, 0.7 mmol) was subjected to dry pyridine (1.0 mL) and acetic anhydride (1.0 mL) at rt under an argon atmosphere overnight (14 h). After concentration, the crude

compound was dry-loaded on silica before gradient column chromatography (petroleum ether to petroleum ether/EtOAc, 1/1 v/v). Acetylated carbamate **33** was recovered as a white solid. R_f = 0.44 (5% MeOH in EtOAc); $[\alpha]_D^{27}$ = +19.9 (c = 0.1, MeOH); IR (film) 2957, 2925, 1741, 1711, 1430, 1367, 1238, 1070, 754 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 5.16 (dt, $J_{3,4}$ = 6.5, $J_{2a,3}$ = $J_{2b,3}$ = 7.5 Hz, 1H, H3), 5.06 (td, $J_{5,6a}$ = 4.9, $J_{4,5}$ = $J_{5,6b}$ = 9.0 Hz, 1H, H5), 4.32 (dd, $J_{5,6a}$ = 4.9, $J_{6a,6b}$ = 11.0 Hz, 1H, H6a), 4.05 (dd, $J_{5,6b}$ = 9.0, $J_{6a,6b}$ = 11.0 Hz, 1H, H6b), 3.79 (dt, $J_{1a,2a}$ = $J_{1a,2b}$ = 7.5, $J_{1a,1b}$ = 11.0 Hz, 1H, H1a), 3.50–3.57 (m, 2H, H1b and H4), 2.32 (dt, $J_{1a,2a}$ = $J_{1b,2a}$ = $J_{2a,3}$ = 7.5, $J_{2a,2b}$ = 14.5 Hz, 1H, H2a), 2.11 (s, 3H, Me), 2.08 (s, 3H, Me), 1.88 (dt, $J_{1a,2b}$ = $J_{1b,2b}$ = $J_{2b,3}$ = 7.5, $J_{2a,2b}$ = 14.5 Hz, 1H, H2b); ¹H NMR (300 MHz, CD₃OD) δ 5.19 (dd, $J_{3,4}$ = 7.2, $J_{2b,3}$ = 7.9 Hz, 1H, H3), 5.12 (dd, $J_{5,6a}$ = 4.7, $J_{4,5}$ = $J_{5,6b}$ = 8.7 Hz, 1H, H5), 4.32 (dd, $J_{5,6a}$ = 4.7, $J_{6a,6b}$ = 10.8 Hz, 1H, H6a), 4.11 (dd, $J_{5,6b}$ = 8.7, $J_{6a,6b}$ = 10.8 Hz, 1H, H6b), 3.66 (dt, $J_{1a,2a}$ = $J_{1a,2b}$ = 8.0, $J_{1a,1b}$ = 11.1 Hz, 1H, H1a), 3.62 (dd, $J_{3,4}$ = 7.2, $J_{4,5}$ = 8.7 Hz, 1H, H4), 3.49 (ddd, $J_{1b,2b}$ = 4.7, $J_{1b,2a}$ = 9.1, $J_{1a,1b}$ = 11.1 Hz, 1H, H1b), 2.34 (dddd, $J_{1b,2b}$ = 4.7, $J_{2b,3}$ = 7.9, $J_{1a,2b}$ = 8.0, $J_{2a,2b}$ = 12.9 Hz, 1H, H2a), 2.07 (s, 3H, Me), 2.05 (s, 3H, Me), 1.88 (ddt, $J_{1a,2b}$ = $J_{2b,3}$ = 7.9, $J_{1b,2b}$ = 9.1, $J_{2a,2b}$ = 12.9 Hz, 1H, H2b); ¹³C NMR (125 MHz, CDCl₃) δ 170.1 (C=O, Ac), 169.9 (C=O, Ac), 151.6 (C=O), 75.2 (C3), 66.7 (C6), 65.7 (C5), 63.2 (C4), 44.9 (C1), 28.8 (C2), 20.9 (CH₃, Ac), 20.8 (CH₃, Ac); HRMS(ESI) m/z calcd for [C₁₁H₁₅O₆N + Na]⁺ 280.0797, found 280.0791.

1,2,4-Trideoxy-1,4-imino-L-allitol (34). Carbamate (**30**) (10.6 mg, 0.061 mmol) was dissolved in EtOH (1 mL), and NaOH (26 mg, 0.6 mmol) was added. The solution was refluxed for 2 h, neutralized with aq. HCl (1 M), and loaded on silica for chromatography (DCM/EtOH/MeOH/35% aq. NH₃, 55/2/2/1 to 15/2/2/1 v/v/v/v). Product **34** was obtained as a white solid (8.0 mg, 0.054 mmol, 89%). R_f = 0.19 (DCM/EtOH/MeOH/35% aq. NH₃, 5/2/2/1 v/v/v/v); $[\alpha]_D^{26}$ = –1.8 (c = 0.41, MeOH); IR (film) 3309, 2956, 2927, 1629, 1420, 1089, 1041 cm⁻¹; ¹H NMR (500 MHz, D₂O) δ 4.33 (dt, $J_{2b,3}$ = $J_{3,4}$ = 4.3, $J_{2a,3}$ = 6.2 Hz, 1H, H3), 3.78 (dd, $J_{4,5}$ = 4.3, $J_{5,6}$ = 10.3 Hz, 1H, H5), 3.44–3.52 (m, 2H, H6), 3.35 (t, $J_{3,4}$ = $J_{4,5}$ = 4.3 Hz, 1H, H4), 3.21 (dd, $J_{1,2b}$ = 6.3, $J_{1,2a}$ = 8.1 Hz, 2H, H1), 2.02 (ddd, $J_{2a,3}$ = 6.2, $J_{1,2a}$ = 8.1, $J_{2a,2b}$ = 14.2 Hz, 1H, H2a), 1.78 (ddd, $J_{2b,3}$ = 4.3, $J_{1,2b}$ = 6.3, $J_{2a,2b}$ = 14.2 Hz, 1H, H2b); ¹³C NMR (125 MHz, D₂O) δ 69.4 (C3), 68.4 (C5), 67.1 (C4), 62.5 (C6), 44.1 (C1), 32.6 (C2); HRMS(ESI) m/z calcd for [C₆H₁₃O₃N + H]⁺ 148.0974, found 148.0978.

5-*epi*-Fagomine (26) and Fagomine (3). Carbamates **31a** and **31b** (11.7 mg, 0.07 mmol) were dissolved in EtOH (1 mL), and NaOH (80 mg, 2 mmol) was added. The solution was refluxed for 2 h, neutralized with aq. HCl (1 M), and concentrated in vacuo. Purification by gradient silica column chromatography (DCM/EtOH/MeOH/35% aq. NH₃, 45/2/2/1 to 15/2/2/1 v/v/v/v) gave the products **3** and **26** as a white solid and a mixture of diastereomers (12.4 mg, 0.07 mmol, quant.).

Data for **5-*epi*-fagomine (26)**: R_f = 0.24 (DCM/EtOH/MeOH/35% aq. NH₃, 5/2/2/1 v/v/v/v); $[\alpha]_D^{29}$ = –8.9 (c = 0.63, MeOH); IR (film) 3364, 2970, 1425, 1366, 1217, 1079 cm⁻¹; ¹H NMR (500 MHz, D₂O) δ 4.02 (m, 1H, H3), 3.93 (m, 1H, H4), 3.85 (dd, $J_{5,6a}$ = 4.9, $J_{6a,6b}$ = 12.0 Hz, 1H, H6a), 3.80 (dd, $J_{5,6b}$ = 8.5, $J_{6a,6b}$ = 12.0 Hz, 1H, H6b), 3.54 (dd, $J_{5,6a}$ = 4.9, $J_{5,6b}$ = 8.5 Hz, 1H, H5), 3.20–3.31 (m, 2H, H1a,b), 2.16–2.24 (m, 1H, H2a), 1.84 (d, $J_{2a,2b}$ = 14.5 Hz, 1H, H2b); ¹³C NMR (125 MHz, D₂O) δ 65.8 (C4), 64.8 (C3), 59.5 (C6), 55.8 (C5), 38.9 (C1), 23.6 (C2); HRMS(ESI) m/z calcd for [C₆H₁₃O₃N + H]⁺ 148.0974, found 148.0968.

Data for **fagomine (3)**: R_f = 0.33 (DCM/EtOH/MeOH/35% aq. NH₃, 5/2/2/1 v/v/v/v); $[\alpha]_D^{29}$ = +8.4 (c = 0.27, MeOH); IR (film) 3367, 2930, 1461, 1271, 1073 cm⁻¹; ¹H NMR (500 MHz, D₂O) δ 3.93 (dd, $J_{5,6a}$ = 3.2, $J_{6a,6b}$ = 12.5 Hz, 1H, H6a), 3.88 (dd, $J_{5,6b}$ = 5.5, $J_{6a,6b}$ = 12.5 Hz, 1H, H6b), 3.71 (ddd, $J_{2a,3}$ = 4.9, $J_{3,4}$ = 9.0, $J_{2b,3}$ = 11.7 Hz, 1H, H3), 3.52 (t, $J_{3,4}$ = $J_{4,5}$ = 9.0 Hz, 1H, H4), 3.44 (ddd, $J_{1a,2a}$ = 2.5, $J_{1a,2b}$ = 4.1, $J_{1a,1b}$ = 13.5 Hz, 1H, H1a), 3.12–3.16 (m, 1H, H5), 3.10 (dd, $J_{1b,2a}$ = 2.5, $J_{1b,2b}$ = $J_{1a,1b}$ = 13.5 Hz, 1H, H1b), 2.21 (ddd, J = 2.5, $J_{2a,3}$ = 4.9, $J_{2a,2b}$ = 13.5 Hz, 1H, H2a), 1.72 (ddt, $J_{1a,2b}$ = 4.1, $J_{2b,3}$ = 11.7, $J_{1a,2b}$ = $J_{2a,2b}$ = 13.5 Hz, 1H, H2b); ¹³C NMR (125 MHz, D₂O) δ 70.4 (C3), 69.5 (C4), 60.0 (C5), 57.6 (C6), 41.8 (C1), 28.5 (C2); HRMS(ESI) m/z calcd for [C₆H₁₃O₃N + H]⁺ 148.0974, found 148.0968.

4,5-Di-O-acetyl-1-amino-1-N,3-O-carbonyl-1,2,6-trideoxy-6-iodo-L-gulitol (35). Iodide 32 (20.4 mg, 0.07 mmol) was subjected to dry pyridine (0.5 mL) and acetic anhydride (0.5 mL) at room temperature under an argon atmosphere overnight (9 h). After concentration and repeated coevaporation with toluene to remove pyridine, the crude compound was dry-loaded on silica before gradient column chromatography (petroleum ether to petroleum ether/EtOAc, 1/1 v/v). Acetylated iodide 35 was recovered as a white solid in 48% yield (12.5 mg, 0.03 mmol). R_f = 0.44 (10% MeOH in EtOAc); $[\alpha]_D^{25}$ = +6.6 (c = 0.23, CHCl₃); IR (film) 3266, 3056, 2927, 1742, 1716, 1373, 1266, 1219, 1117, 1059, 1024, 598.7 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 5.51 (br s, 1H, NH), 5.34 (dd, $J_{3,4}$ = 3.7, $J_{4,5}$ = 5.5 Hz, 1H, H4), 5.14 (dd, $J_{5,6a}$ = 4.5, $J_{4,5}$ = $J_{5,6b}$ = 5.5 Hz, 1H, H5), 4.47 (d, $J_{2b,3}$ = 10.8 Hz, 1H, H3), 3.46 (dd, $J_{5,6a}$ = 4.5, $J_{6a,6b}$ = 11.4 Hz, 1H, H6a), 3.37–4.43 (m, 2H, H1a,b), 3.36 (dd, $J_{5,6b}$ = 5.5, $J_{6a,6b}$ = 11.4 Hz, 1H, H6b), 2.16 (s, 3H, 4-OAc), 2.13 (s, 3H, 5-OAc), 2.00 (d, $J_{2a,2b}$ = 14.2 Hz, 1H, H2a), 1.79–1.89 (m, 1H, H2b); ¹³C NMR (125 MHz, CDCl₃) δ 170.2 (C=O, 4-Ac), 169.9 (C=O, 5-Ac), 152.9 (C=O), 75.0 (C3), 73.4 (C4), 70.4 (C5), 38.9 (C1), 22.8 (C2), 20.9 (CH₃, Ac), 20.6 (CH₃, Ac), 3.3 (C6); HRMS(ESI) m/z calcd for [C₁₁H₁₆O₆NI + H]⁺ 386.0101, found 386.0094.

1-Amino-1-N,3-O-carbonyl-1,2,6-trideoxy-6-iodo-4,5-O-isopropylidene-L-gulitol (36). Iodide 32 (8.8 mg, 0.3 mmol) was dissolved in acetone (0.45 mL), dimethoxypropane (0.45 mL), and MeOH (0.25 mL). A 100 μ L aliquot of pTsOH·H₂O in acetone (2.85 mg/mL) was added, and the reaction mixture was stirred at room temperature overnight (17 h). The mixture was then neutralized with Dowex-OH⁻, filtered, and concentrated to give a white solid 36 (7.2 mg, 0.02 mmol, 72%) that was used without further purification. R_f = 0.49 (10% MeOH in EtOAc); $[\alpha]_D^{23}$ = -23.0 (c = 0.48, CHCl₃); IR (film) 3282, 2985, 2930, 1700, 1487, 1456, 1421, 1372, 1294, 1239, 1214, 1107, 1069, 554 cm⁻¹; ¹H NMR (500 MHz, CDCl₃/CD₃OD 95/5) δ 4.40 (app dt, $J_{3,4}$ = 2.2, $J_{2a,3}$ = 10.0 Hz, 1H, H3), 4.20 (dt, $J_{5,6a}$ = $J_{5,6b}$ = 4.7, $J_{4,5}$ = 7.5 Hz, 1H, H5), 3.83 (dd, $J_{3,4}$ = 2.2, $J_{4,5}$ = 7.5 Hz, 1H, H4), 3.41 (ddd, $J_{1a,2b}$ = 3.7, $J_{1a,2a}$ = 5.8, $J_{1a,1b}$ = 11.0 Hz, 1H, H1a), 3.36 (m, 1H, NH), 3.31–3.35 (m, 1H, H1b), 3.31 (dd, $J_{5,6a}$ = 4.7, $J_{6a,6b}$ = 10.8 Hz, 1H, H6a), 3.28 (dd, $J_{5,6b}$ = 4.7, $J_{6a,6b}$ = 10.8 Hz, 1H, H6b), 2.06 (dtd, $J_{1a,2a}$ = 5.8, $J_{1b,2a}$ = $J_{2a,3}$ = 10.0, $J_{2a,2b}$ = 14.0 Hz, 1H, H2a), 1.98 (m, 1H, H2b), 1.43 (s, 3H, Me), 1.40 (s, 3H, Me); ¹³C NMR (125 MHz, CDCl₃/CD₃OD 95/5) δ 154.4 (C=O), 110.7 (iPr-C(CH₃)₂), 82.4 (C4), 75.1 (C3), 74.6 (C5), 38.7 (C1), 27.6, 27.0 (iPr-C(CH₃)₂), 23.3 (C2), 6.1 (C6); HRMS(ESI) m/z calcd for [C₁₀H₁₆O₄NI + H]⁺ 342.0202, found 342.0201.

D-threo-1-Amino-1-O,3-N-carbonyl-1,2,5,6-tetra-deoxyhex-5-enose (37). Iodoacetone 36 (6.8 mg, 0.02 mmol) was subjected to DCM (0.3 mL), AcOH (0.1 mL), and zinc (27.6 mg, 0.2 mmol) and stirred at 40–55 °C for 12 days. The reaction mixture was filtered through silica and concentrated to remove zinc. The crude product was purified by silica column chromatography (petroleum ether to petroleum ether/EtOAc, 1/3 v/v), yielding 37 as a white solid (2.4 mg, 0.015 mmol, 77%). R_f = 0.22 (10% MeOH in EtOAc); $[\alpha]_D^{29}$ = -12.8 (c = 0.16, MeOH); IR (film) 3373, 2926, 2856, 1682, 1489, 1459, 1294, 1108, 1071, 1028, 946 cm⁻¹; ¹H NMR (500 MHz, D₂O) δ 5.92 (ddd, $J_{4,5}$ = 6.6, $J_{5,6-cis}$ = 10.5, $J_{5,6-trans}$ = 17.3 Hz, 1H, H5), 5.40 (app dt, $J_{4,6-trans}$ = 1.2, $J_{5,6-trans}$ = 17.3 Hz, 1H, H6-trans), 5.32 (app dt, $J_{4,6-cis}$ = 1.2, $J_{5,6-cis}$ = 10.5 Hz, 1H, H6-cis), 4.35 (ddd, $J_{2a,3}$ = 3.0, $J_{3,4}$ = 5.7, $J_{2b,3}$ = 10.5 Hz, 1H, H3), 4.24 (ddt, $J_{4,6-trans}$ = $J_{4,6-cis}$ = 1.2, $J_{3,4}$ = 5.7, $J_{4,5}$ = 6.6 Hz, 1H, H4), 3.33 (ddd, $J_{1a,2a}$ = 3.0, $J_{1a,2b}$ = 6.9, $J_{1a,1b}$ = 12.0 Hz, 1H, H1a), 3.32 (ddd, $J_{1b,2a}$ = 4.5, $J_{1b,2b}$ = 10.5, $J_{1a,1b}$ = 12.0 Hz, 1H, H1b), 1.99 (ddt, $J_{2a,3}$ = 3.0, $J_{1b,2a}$ = 4.5, $J_{2a,2b}$ = 14.1 Hz, 1H, H2a), 1.84 (dtd, $J_{1a,2b}$ = 6.9, $J_{1b,2b}$ = $J_{2b,3}$ = 10.5, $J_{2a,2b}$ = 14.1 Hz, 1H, H2b); ¹³C NMR (150 MHz, D₂O) δ 156.8 (C=O), 134.9 (C5), 118.4 (C6), 80.2 (C3), 73.5 (C4), 37.8 (C1), 21.7 (C2); HRMS(ESI) m/z calcd for [C₇H₁₁O₃N + H]⁺ 158.0817, found 158.0805.

D-threo-4-O-Acetyl-1-amino-1-O,3-N-carbonyl-1,2,5,6-tetra-deoxyhex-5-enose (38). Iodide 35 (10.7 mg, 0.03 mmol) was dissolved in THF (0.3 mL), and H₂O (0.06 mL), AcOH (0.06 mL), and zinc (62 mg, 0.6 mmol) were added. The resulting mixture was then stirred at room temperature for 1 h. The reaction mixture was filtered through Celite, washed with DCM and EtOH, and

concentrated before coevaporation with H₂O and toluene to remove traces of AcOH. The crude product was purified by silica column chromatography (petroleum ether to petroleum ether/EtOAc, 1/3 v/v) to give 38 as a white solid (5.5 mg, 0.03 mmol, quant.). R_f = 0.38 (10% MeOH in EtOAc); $[\alpha]_D^{25}$ = -11.0 (c = 0.13, CHCl₃); IR (film) 3278, 2925, 2854, 1703, 1489, 1459, 1424, 1372, 1294, 1227, 1104, 1023, 950, 763 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 5.88 (ddd, $J_{4,5}$ = 6.5, $J_{5,6-cis}$ = 10.5, $J_{5,6-trans}$ = 17.0 Hz, 1H, H5), 5.51 (br s, 1H, NH), 5.44 (d, $J_{4,5}$ = 6.5 Hz, 1H, H4), 5.43 (d, $J_{5,6-trans}$ = 17.0 Hz, 1H, H6-trans), 5.37 (d, $J_{5,6-cis}$ = 10.5 Hz, 1H, H6-cis), 4.39 (ddd, J = 2.5, J = 5.5, $J_{2b,3}$ = 10.5 Hz, 1H, H3), 3.35–3.45 (m, 2H, H1a,b), 2.13 (s, 3H, Me), 1.94–2.01 (m, 1H, H2a), 1.86 (dtd, J = 6.5, J = $J_{2b,3}$ = 10.5, $J_{2a,2b}$ = 14.0 Hz, 1H, H2b); ¹³C NMR (125 MHz, CDCl₃) δ 170.0 (C=O, Ac), 153.8 (C=O), 131.5 (C5), 120.4 (C6), 77.5 (C3), 74.5 (C4), 39.0 (C1), 22.5 (C2), 21.2 (CH₃, Ac); HRMS(ESI) m/z calcd for [C₉H₁₃O₄N + H]⁺ 200.0923, found 200.0918.

(3R,4R)-6-Aminohex-1-ene-3,4-diol Hydrochloride (29). Acetylated alkene carbamate 38 (5.7 mg, 0.03 mmol) was dissolved in EtOH (1 mL), and NaOH (80 mg, 2 mmol) was added. The solution was refluxed for 1 h, neutralized with aq. HCl (1.2 M), and then concentrated in vacuo. Purification by gradient silica column chromatography (DCM/EtOH/MeOH/35% aq. NH₃, 65/2/2/1 to 5/2/2/1 v/v/v/v) gave the alkenylamine, which was converted into the HCl salt 29 as a white solid (3.3 mg, 0.02 mmol, 69%) by the addition of HCl (1.2 M, aq.) and concentration. R_f = 0.16 (DCM/EtOH/MeOH/35% aq. NH₃, 5/2/2/1 v/v/v/v); $[\alpha]_D^{27}$ = +31.8 (c = 0.2, MeOH).

■ ASSOCIATED CONTENT

● Supporting Information

NMR spectra (¹H and ¹³C) for all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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