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**Synthesis of substrate analogues and
inhibitors for phosphoribosyl
anthranilate isomerase and indole-3-
glycerolphosphate synthase.**

Benjamin Joseph Mulchin

2008

**Synthesis of substrate analogues and
inhibitors for phosphoribosyl
anthranilate isomerase and indole-3-
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Abstract

The general biosynthetic pathway for tryptophan is known. However, little information has been gathered on how substrates and enzymes interact when phosphoribosylanthranilate isomerase (PRAI) and indole-3-glycerolphosphate synthase (IPGS) convert a substituted phenyl ring, PRA, into an indole moiety, IGP, *via* 1-(*O*-carboxyphenylamino)-1-deoxyribulose-5-phosphate (CdRP). There has been no serious synthetic approach to develop methodology to produce a plethora of substrate and product analogues of CdRP. The studies described in this thesis cover methodology focusing on secondary aryl amine formation, using reductive amination, nucleophilic substitution and epoxide ring opening, leading to CdRP analogues. Reductive aminations with D-ribose failed to produce any aryl glycosylamine precursor, possibly due to the low nucleophilicity of aryl amines such as aniline. Removing the aromaticity and using cyclohexylamine produced secondary amines in moderate yield in the presence of benzylpentanal, and NaBH₃CN, at a pH of 5.5. This led to a successful reductive amination using anthranilate methyl ester. Secondary aryl amine synthesis *via* epoxide ring opening proved consistently reproducible. Using LiNTf₂ and high equivalents of cyclohexylamine or aniline in neat conditions opened protected epoxides. This has led to the formation of advanced secondary aryl amine synthons and the development of methodology leading to target compounds with functionality at the 1,2 and 5 positions. Nucleophilic substitution using caesium base, high equivalents aniline at room temperature, gave a moderate yield of secondary aryl amines from sulfonyl and bromide good leaving groups. Raising the reaction temperature improved yields using low equivalents of aniline, with the optimal temperature being 50 °C. Ultimately using both the high equivalents of aniline or anthranilate methyl ester and warming the reaction in DMF gave the highest yields of secondary aryl amines. No overalkylated tertiary amine was isolated when a caesium base was used. Boc *N*-protection of 1-phenylamino-4-pentene and asymmetric dihydroxylation gave the corresponding diol, which was phosphorylated giving the protected target 1,4,5 compound. The methodology leading to the protected target 1,4,5 compound synthesis provides a means to the synthesis additional of CdRP analogues.

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Abbreviations

Ac	Acetyl
ACE-Cl	α -Chloroethyl chloroformate
AD-mix	Asymmetric dihydroxylation reagent
Aq	Aqueous
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
BINAP	2,2'-Bis(diphenylphosphino)-1,1'-binaphthyl
bmim	1-Butyl-3-methylimidazolium
Bn	Benzyl
Boc	<i>tert</i> -Butyloxycarbonyl
BTP	1,3-Bis(tris(hydroxymethyl)methylamino)propane
Bz	Benzoyl

CdRP	1-(<i>O</i> -Carboxyphenylamino)-1-deoxyribulose-5-phosphate
¹ H/ ¹ H COSY	Proton correlation spectroscopy
<i>m</i> -CPBA	<i>meta</i> -Chloroperoxybenzoic acid
CSA	Camphorsulfonic acid
Cys	Cysteine
DAHP	3-Deoxy-D- <i>arabino</i> -heptulosonate 7-phosphate
DAH7P	3-Deoxy-D- <i>arabino</i> -heptulosonate 7-phosphate
DCP	1,2-Dichloropropane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DEAE	Diethylamino ethanol
DHP	3,4-Dihydro-2 <i>H</i> -pyran
DHQ	Dehydroquinone
(DHQ) ₂ -PHAL	Dihydroquinone phthalazine
DHS	Dehydroshikimate
Diab-H	Disiamylborane
Dibal-H	Di- <i>iso</i> -butylaluminum hydride
DIEA	Di- <i>iso</i> -propylethylamine
DMAP	4-Dimethylaminopyridine
DMF	<i>N,N</i> -Dimethylformamide
DMP	2,2-Dimethoxypropane
DMP	Dess-Martin periodinane
DMSO	Dimethyl sulfoxide
<i>e</i> IGPS	<i>Escherichia coli</i> IGPS
<i>e</i> PRAI	<i>Escherichia coli</i> PRAI
E4P	D-Erythrose 4-phosphate
EDG	Electron-donating groups
EDTA	Ethylenediamine tetra-acetic acid (di-sodium salt)
EPSP	5- <i>Enol</i> pyruvyl-shikimate 3-phosphate
Eq	Equivalent
EWG	Electron-withdrawing groups
Fmoc	9-Fluorenylmethoxycarbonyl

FT	Fourier transform
GL	Good leaving group
Glu	Glutamic acid
His	Histidine
HMPA	Hexamethylphosphoric triamide
HREIMS	High Resolution Electron Impact Mass Spectrometry
$^1\text{H}/^{13}\text{C}$ HMQC	Heteronuclear multiple quantum coherence
IEX	Ion exchange chromatography
IGPS	Indole-3-glycerolphosphate synthase
Ile	Isoleucine
im	Imidazole
k_{cat}	Catalytic rate/turnover number
$k_{\text{cat}}/K_{\text{M}}$	Catalytic efficiency
KDO8P	3-Deoxy-D- <i>manno</i> -octulosonate 8-phosphate
K_{i}	Enzyme inhibitor affinity
K_{M}	Michaelis constant
K_{p}	Enzyme product affinity
L. Pet.	Light petroleum
Leu	Leucine
Lys	Lysine
MEM	β -Methoxyethoxymethyl
4 Å M.S.	4 Å Molecular sieves
MW	Molecular weight
Ms	Mesyl, methanesulfonate
N. P.	No product
NMP	<i>N</i> -Methyl pyrrolidone
NMR	Nuclear magnetic resonance
Nu	Nucleophile
PB	Pyridine-borane
PEP	Phospho <i>enol</i> pyruvate

Ph	Phenyl
Phe	Phenylalanine
p <i>K</i> _a	Acid dissociation constant
PMB	<i>para</i> -Methoxybenzyl
PMHS	Polymethylhydrosiloxane
PPG	Primary protecting groups
ppm	Parts per million
Pro	Proline
PRAI	Phosphoribosyl anthranilate isomerase
Psi	Pounds per square inch
py	Pyridine
rCdRP	1-(<i>O</i> -Carboxyphenylamino)-1-deoxyribose-5-phosphate
<i>R</i> _f	Retardation factor/retention factor
R5P	D-Ribose 5-phosphate
RT	Room temperature
<i>s</i> IGPS	<i>Sulfolobus solfataricus</i> IGPS
Ser	Serine
<i>t</i> IGPS	<i>Thermotoga maritima</i> IGPS
<i>t</i> PRAI	<i>Thermotoga maritima</i> PRAI
TBAB	Tetrabutylammonium bromide
TBAF	Tetrabutylammonium fluoride
TBAI	Tetrabutylammonium iodide
TBDMS	<i>tert</i> -Butyldimethylsilyl
TBDPS	<i>tert</i> -Butyldiphenylsilyl
TBHP	<i>tert</i> -Butyl hydroperoxide
Tf	Trifluoromethane sulfonate
TFA	Trifluoroacetyl
THCP	Tributylammonium hydrobenzoin cyclic phosphate
THF	Tetrahydrofuran
THP	Tetrahydropyran
TLC	Thin layer chromatography

TMS	Tetramethylsilane
TMSBr	Trimethylsilyl bromide
Tr	Trityl, triphenyl methyl
Trp	Tryptophan
Ts	Tosyl, toluene-sulfonate
<i>p</i> TSA	<i>para</i> -Toluenesulfonic acid
Tyr	Tyrosine
UV	Ultraviolet
V_{\max}	Maximum reaction velocity
<i>y</i> PRAI	<i>Saccharomyces cerevisiae</i> PRAI

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CHAPTER 1: Tryptophan biosynthesis.

1.1. Thesis introduction:

Phosphoribosyl anthranilate isomerase (PRAI) and indole-3-glycerolphosphate synthase (IGPS) are distantly related (β/α)₈-barrel enzymes that catalyse two sequential steps of tryptophan biosynthesis.^{1,2} My project is based on developing methodology for synthesising substrate analogues and inhibitors for PRAI and IPGS. Testing of these analogues and inhibitors will provide information on how the substrates and enzymes interact, and illuminate the mechanisms by which these enzymes catalyse these isomerisation and ring-forming reactions. The information gathered will be used in the design, synthesis and analysis of refined inhibitors of these two enzymes. Inhibitors of these enzymes may be useful for the development of new antibacterial drugs or herbicides, as pathways for the synthesis of the aromatic amino acids, tryptophan, phenylalanine and tyrosine, are found only in microorganisms and plants and not in animals.³

1.1.2. Roles of tryptophan and other aromatic amino acids:

The aromatic amino acids, tyrosine, phenylalanine and tryptophan (figure 1.01), have two key biosynthetic roles in plants: they are required for protein synthesis, and they are precursors to a range of aromatic primary and secondary metabolites.⁴ These metabolites include the UV-absorbing flavonoids, the plant growth regulator auxin (indole 3-acetic acid), antimicrobial alkaloids and polyphenolic compounds such as lignin.⁴ Secondary metabolites are necessary for normal development of a multicellular plant but unlike primary metabolites they are not essential for cell survival. It is also known that some secondary metabolites can be produced in response to stimulation by different environmental conditions.⁴ Interest in the chemical synthesis of these secondary metabolites is great, due to their wide and varying properties.

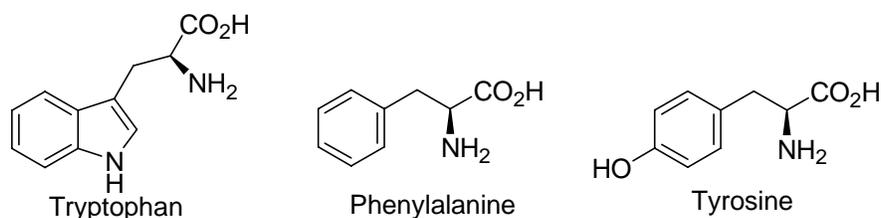


Figure 1.01. Aromatic amino acids.

The essential amino acid, tryptophan, is required by animals for protein synthesis as well as production of other compounds, including the neurohormone serotonin and the vitamin nicotinic acid.⁵ Animals, and some eubacteria, lack the ability to synthesise tryptophan and the other aromatic amino acids from the aromatic precursor chorismate, which is synthesised in the shikimate pathway (scheme 1.01).³ Hence these amino acids must be obtained from plant and microbial sources such as fungi and bacteria.³ Inhibitors of the enzymes may therefore be safely used as herbicides, fungicides and antimicrobial agents. In part, this is the reason for research into finding potent inhibitors of PRAI and IGPS. An illustration of a good enzyme inhibitor of a shikimate pathway enzyme is the herbicide glyphosate, more commonly known by its commercial name Roundup[®]. Glyphosate terminates plant growth by inhibiting the sixth enzyme of the pathway.

1.2. Pathways:

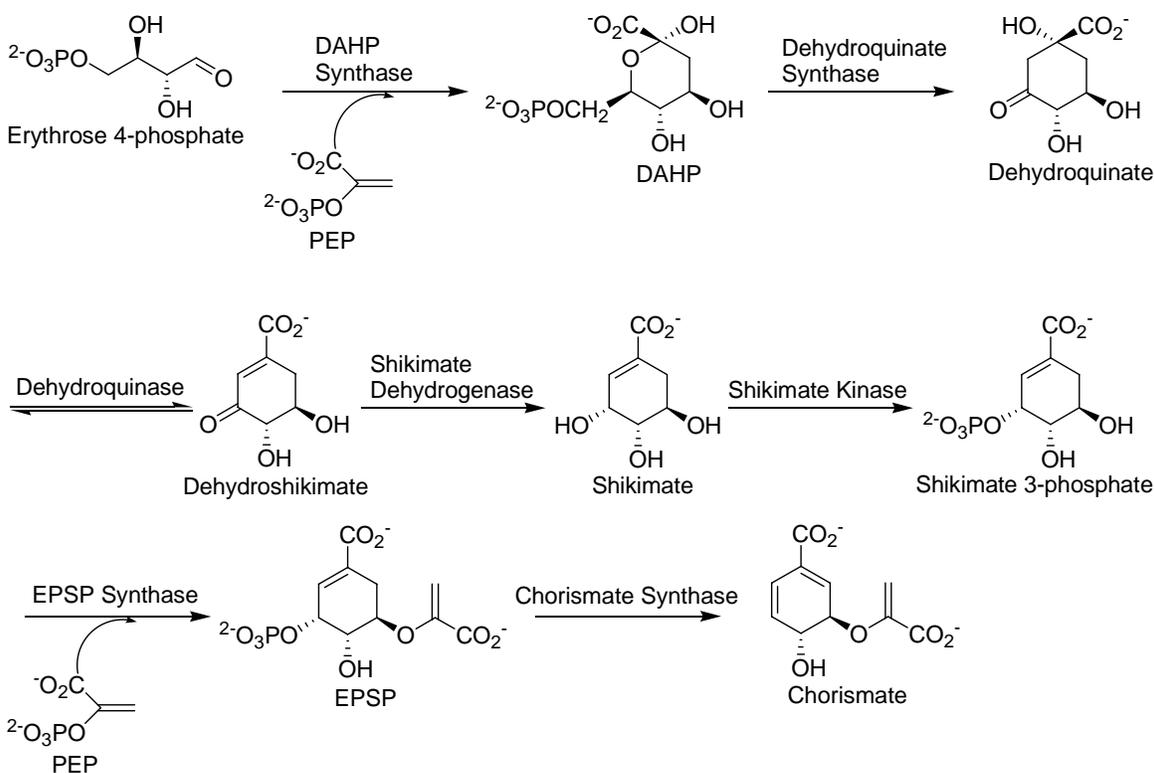
1.2.1. Shikimate pathway:

The starting material for tryptophan biosynthesis is chorismate, which is generated by the seven enzyme-catalysed reactions of the shikimate pathway. The shikimate pathway (scheme 1.01), which precedes tryptophan biosynthesis, is outlined below.

3-Deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP) synthase catalyses the condensation reaction between erythrose 4-phosphate (E4P) and phospho*enol*pyruvate (PEP). The conversion of DAHP to dehydroquinate (DHQ) requires a range of chemical steps, specifically oxidation, β -elimination, reduction and finally an intramolecular aldol reaction.⁶ Elimination of water from DHQ leads to dehydroshikimate (DHS). This is the

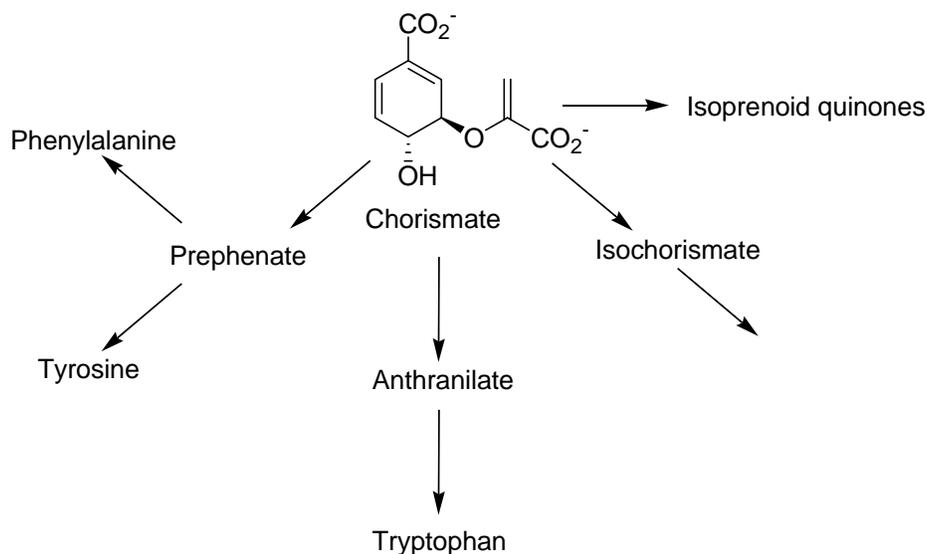
initial step in the aromatisation process. Reduction of DHS by shikimate dehydrogenase produces shikimate, which is then phosphorylated by shikimate kinase to form shikimate 3-phosphate. Shikimate 3-phosphate then reacts *via* an addition-elimination mechanism with PEP to form 5-*enol*pyruvyl-shikimate 3-phosphate (EPSP). EPSP synthase is the target for glyphosate (Roundup[®]). The last step shown in scheme 1.01 shows EPSP being converted to chorismate by a 1,4 elimination of phosphate, producing the second double bond necessary for aromatisation.⁶

Scheme 1.01. The shikimate pathway.



Chorismate, the last compound in the shikimate pathway, is the branch point to many different biosynthetic routes (scheme 1.02). Some of these routes produce aromatic compounds such as vitamin K, which are nutritionally essential for humans. Another biosynthetic route from chorismate produces tryptophan as the end product.

Scheme 1.02. Chorismate, the precursor to the aromatic amino acids and aromatic compounds.

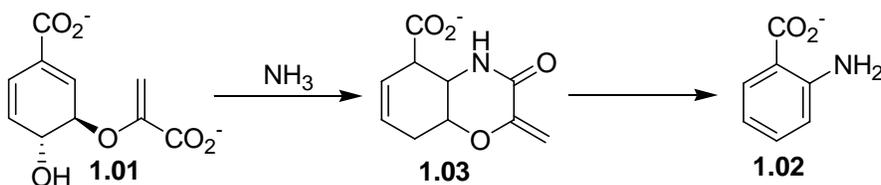


1.2.2. Biosynthesis of tryptophan:

Scheme 1.05 shows how tryptophan is generated from chorismate over six enzyme-catalysed steps. The first reaction in this pathway is conversion of chorismate **1.01** to anthranilic acid **1.02** by anthranilate synthase. This reaction requires a nitrogen source as well as chorismate. Different microorganisms have the ability to use either glutamine or free ammonia as the nitrogen source.⁷ A number of possible reaction mechanisms have been proposed for this transformation.^{7,8}

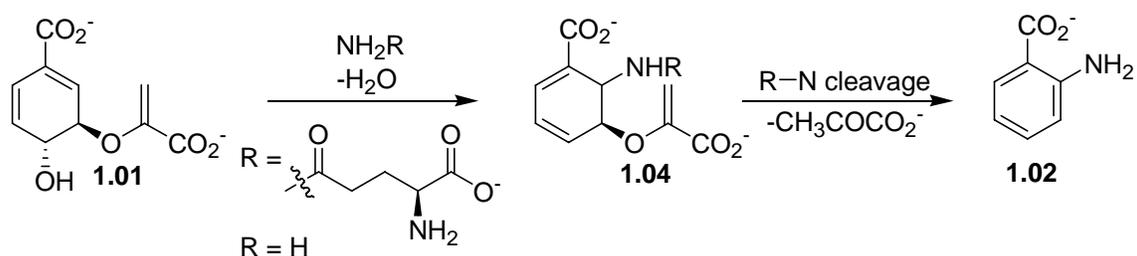
The first mechanism proposed by Ratledge,⁹ involves forming a *N*-acyl anthranilate bicyclic derivative **1.03**, which upon hydrolysis gives anthranilate **1.02** (scheme 1.03).⁷

Scheme 1.03. Formation of anthranilate **1.02** via bicyclic derivative **1.03**.



The second mechanism was put forward by Sprinson *et al.*¹⁰ in the mid 1960s, where it was proposed the nitrogen source attacks the more electrophilic double bond of chorismate in a Michael-type addition (scheme 1.04). This in turn aromatises by the elimination of pyruvate. In support of the intermediacy of compound **1.04**, McCormick *et al.*¹¹ and Bauerle *et al.*^{12,13} found that closely related compounds undergo a similar transformation. Bauerle *et al.*¹³ synthesised substrate intermediates for anthranilate synthase, which all but confirmed the mechanism was Michael-type addition.

Scheme 1.04. Formation of anthranilate **1.02** *via* Michael-type addition and loss of pyruvate.

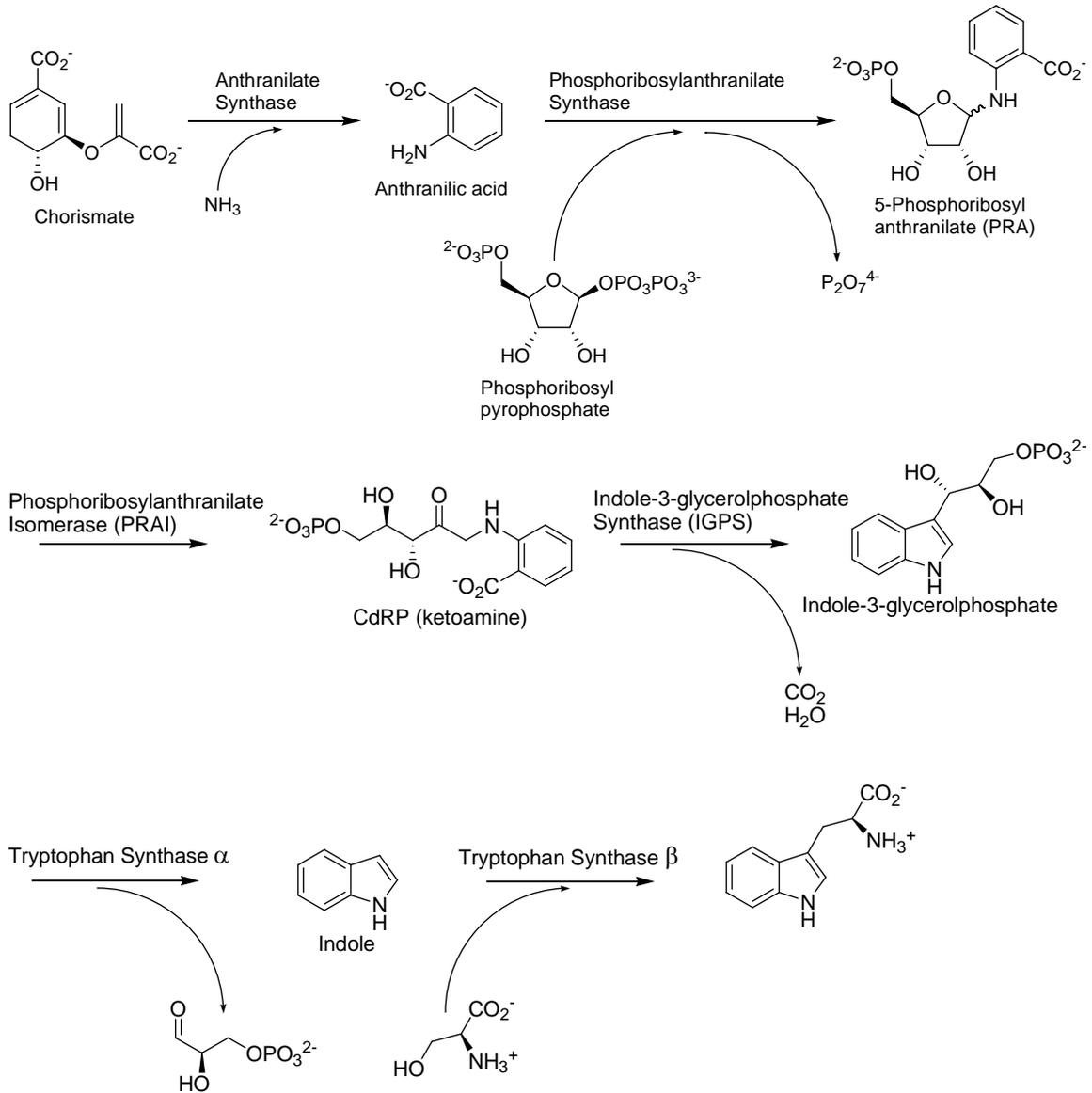


Formation of 5-phosphoribosyl anthranilate then occurs *via* nucleophilic attack of the amine group of anthranilate at the C-1 carbon on phosphoribosyl pyrophosphate (scheme 1.05). Pyrophosphate is a good leaving group. The next two enzymatic steps are the focus of our research and will be discussed in the proceeding paragraphs in more detail. 5-Phosphoribosyl anthranilate is converted to 1-(*O*-carboxyphenylamino)-1-deoxyribulose-5-phosphate (CdRP) by a reaction catalysed by phosphoribosylanthranilate isomerase (PRAI). Then, indole-3-glycerolphosphate synthase (IGPS) catalyses the transformation of CdRP to indole-3-glycerol phosphate (IGP). These two enzymatic reactions have had little previous research attention with respect to substrate analogues or inhibitors, and how these potentially bind with the enzymes and give chemical evidence for the proposed mechanisms.

IGP goes on to form tryptophan in a two-step process catalysed by the α and β subunits of tryptophan synthase. A retroaldol reaction eliminates glyceraldehyde 3-phosphate from

IGP to form indole. Tryptophan synthase β catalyses electrophilic aromatic substitution of serine onto the benzylic position of indole, forming tryptophan.

Scheme 1.05. Tryptophan biosynthesis.



1.3. Enzyme structure basics of PRAI and IGPS:

1.3.1. Introduction:

In 2002,¹⁴ roughly 10% of all known enzyme structures had an $(\beta/\alpha)_8$ -barrel fold.¹⁵⁻¹⁷ Arguments in favour of convergent evolution to yield a stable $(\beta/\alpha)_8$ -barrel fold have been published;¹⁸ however, there is substantial evidence that several subfamilies of this fold have each arisen by divergent evolution from a single common ancestor.^{2,19,20} One such subfamily comprises three consecutive enzymes in the tryptophan biosynthetic pathway. These are phosphoribosyl anthranilate isomerase, indoleglycerol phosphate synthase and the α subunit of tryptophan synthase. The active site of most $(\beta/\alpha)_8$ -barrel enzymes is located at the bottom of a funnel-shaped pocket created by the loops connecting the carboxy-terminal end of the β -strands with the amino-terminal end of the α helices that form the barrel. Substrate binding takes place within the barrel while the catalytic residues mainly occur within the connecting loop regions.^{21,22}

1.3.2. Sources of PRAI and IGPS:

Presently PRAI has been characterised from *Escherichia coli* (ePRAI), *Saccharomyces cerevisiae* (γ PRAI), and *Thermotoga maritima* (tPRAI). IGPS has been characterised from *Escherichia coli* (eIGPS), *Sulfolobus solfataricus* (sIGPS), and *Thermotoga maritima* (tIGPS).

1.3.3. PRAI and IGPS enzymes:

Enzymes from *E. coli* are examples of bifunctional enzymes containing independent domains, the N-terminal domain having PRAI activity, and the C-terminal domain having IGPS activity. The active sites are on one polypeptide chain, which folds to give two separate non-overlapping domains, each catalysing a different reaction. Both of these active sites are located at the C-terminal end of the central β barrels.²⁰ The positions of the substrate binding sites within the $(\beta/\alpha)_8$ -barrels are identical in terms of phosphate-

ester group and the hydrogen-bonding network between the phosphate ester group and the protein. In addition there are similar hydrophobic pockets on both domains that bind the anthranilate regions of the substrates. Monofunctional forms of *e*PRAI and *e*IGPS have been engineered recently^{23,24}, and it was shown that their catalytic properties are similar to the native bifunctional enzyme.

The two enzymes from *T. maritima* *t*PRAI and *t*IGPS are known to be monofunctional homodimers of $(\beta/\alpha)_8$ -barrel subunits, which are extremely stable to acidic pH, proteolytic attack and heat.²⁵ Another enzyme from a hyperthermophilic source is *s*IGPS, which is a monomeric enzyme containing the common $(\beta/\alpha)_8$ -barrel subunit.²⁶ It is thought like *t*PRAI and *t*IGPS, *s*IGPS contains intramolecular ion pairing which contributes significantly to its thermostability.²⁶

1.4. Biochemical details of PRAI:

1.4.1. Enzyme kinetics for PRAI:

All the K_M and k_{cat} values (table 1.01) from *E. coli* for PRAI are from the bifunctional enzyme PRAI:IGPS,²⁷⁻²⁹ apart from the monofunctional engineered enzyme, *e*PRAI^m.^{23,24} Variation in kinetic values between *e*PRAI:IGPS and *e*PRAI^m is minimal. The naturally occurring monofunctional *y*PRAI displayed higher k_{cat} and k_{cat}/K_M values than *e*PRAI at the same temperatures.²⁴

One notable feature of table 1.01 is the high catalytic efficiency parameter k_{cat}/K_M values for *t*PRAI over a range of temperatures. The catalytic efficiency parameter k_{cat}/K_M of $13.3 \mu M^{-1} s^{-1}$ is fivefold higher at 25 °C for *t*PRAI than that of *e*PRAI at the same temperature.²⁷ Even at the optimal growth temperatures of both *e*PRAI (37 °C) and *t*PRAI (80 °C), *t*PRAI is more active by a factor of 35.²⁷ This high catalytic efficiency for the transformation of PRA to CdRP is attributed to the fact that at the optimal growth temperature of 80 °C for *T. maritima*, PRA is highly labile and undergoes rapid

hydrolysis.²⁷ This in turn forces the enzyme to evolve a higher efficiency than otherwise would be needed. Typically, low activity of thermostable enzymes at room temperature is explained by their conformational rigidity, which is relieved at the higher physiological temperatures.²⁷ However, the chemical transformation that PRAI catalyses, the Amadori rearrangement,^{14,19,24,30,31} requires general acid-base catalysis and probably involves minimal conformational rearrangements at the active site.²⁷

The reduced carbonyl form of 1-(*O*-carboxyphenylamino)-1-deoxyribulose-5-phosphate (CdRP) is abbreviated as rCdRP and it is interesting that the inhibitor affinity values, K_i^{rCdRP} obtained with *e*PRAI, *e*PRAI^m, and *t*PRAI are similar to the product values of K_p^{CdRP} respectively, despite the structural differences of the two compounds.

Enzyme	Temperature (° C)	K_M (μM)	k_{cat} (sec^{-1})	k_{cat}/K_M ($\mu M^{-1} sec^{-1}$)	K_p^{CdRP} (μM)	K_i^{rCdRP} (μM) ^a
<i>e</i> PRAI ²⁷	25	12.2	34.5	2.8		
	37	22.9	74.7	3.3		
<i>e</i> PRAI ²⁹	25	7.1	38.9	5.5		
<i>e</i> PRAI ²⁸	25	5.0 ± 0.8	40 ± 4	8.0		
<i>e</i> PRAI ²³	25	4.9	40	8.2		6.5
<i>e</i> PRAI ^{m23}	25	4.7	32	6.8	6.2	6.8
<i>y</i> PRAI ²⁴	25	3.2	69	22	3.8	1.7
<i>y</i> PRAI ²⁸	25	4.0 ± 0.6	50 ± 5	12.5		
<i>t</i> PRAI ²⁷	25	0.280	3.7	13.3	0.21	0.5
	45	0.390	13.5	34.4	0.46	
	60	0.730	38.5	52.1		
	80	1.030 ^b	116.8 ^b	113.4		

^a Product inhibition constant.

^b Extrapolated from an Arrhenius plot.³²

References: 23, 24, 27-29.

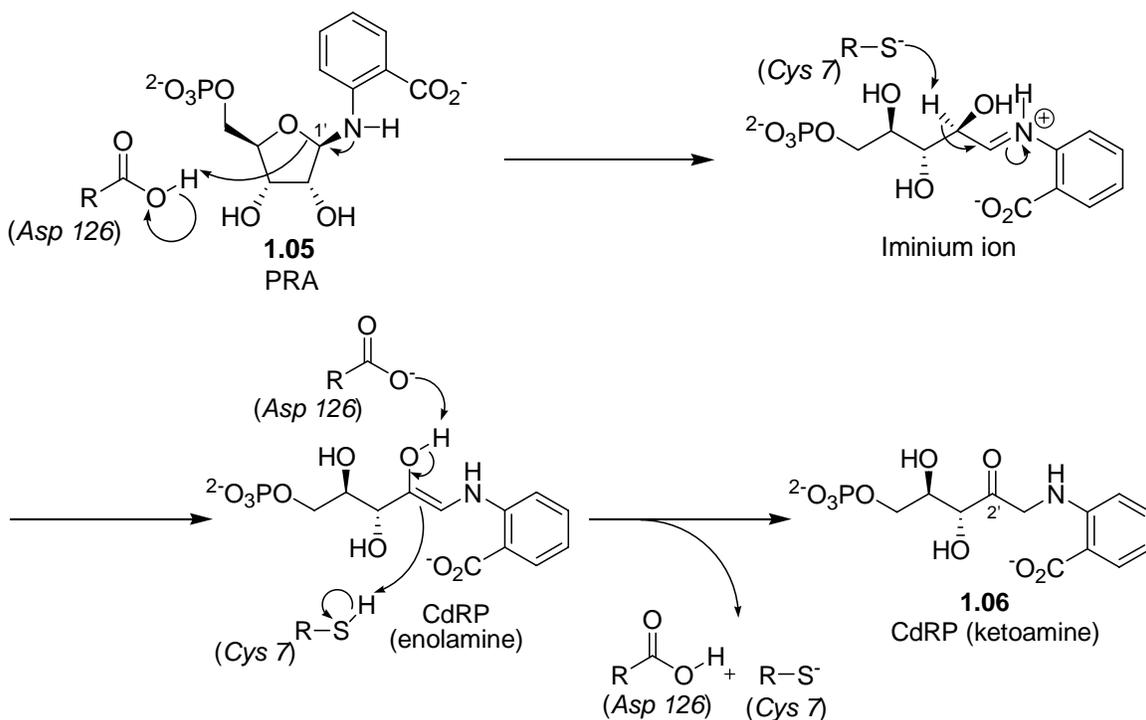
Table 1.01. Enzyme kinetics of PRAI enzymes.

1.4.2. Proposed mechanism catalysed by PRAI – The Amadori rearrangement:

As mentioned, increasing our understanding of the conversion of PRA to CdRP catalysed by PRAI is one of the aims of these studies. Crystal structures of product analogues in the active site of *t*PRAI have allowed Sterner *et al.*³³ to propose a mechanism for this transformation, as shown in scheme 1.06.^{14,19,27} The PRAI reaction is an intramolecular redox reaction, otherwise known as the Amadori rearrangement.^{14,19,24,30,31} The furanose ring oxygen on PRA **1.05** is protonated by Asp initiating cleavage of the O-C1' bond. This leads to the formation of the iminium ion, which has a proton abstracted from C2' by Cys, yielding the enolamine CdRP.^{14,19,20} The prediction of the specific amino acids involved in this transformation has been made by comparing crystal structures containing

bound product analogues in *t*PRAI and superimposing this on the apoenzyme. A spontaneous nonenzymatic reaction forms the ketoamine of CdRP **1.06**, since thermodynamically the ketoamine is more stable than the enolamine.^{14,19,24} It has been shown that the rate of the tautomerism step is not enhanced by enzyme concentration.²⁴

Scheme 1.06. Plausible mechanism of the Amadori rearrangement forming CdRP **1.06**. The most likely residues catalysing this reaction in *t*PRAI are Asp 126 and Cys 7.^{14,19}



1.4.3. X-ray structural analysis of product analogue rCdRP crystallized in *t*PRAI :

Recently Sterner *et al.*¹⁴ have obtained crystals for X-ray structural analysis of a complex of rCdRP, which has the C2' keto functionality reduced to an hydroxyl group, with *t*PRAI. rCdRP is generated by a non-selective chemical reduction of CdRP and the product is of unknown configuration at C2'. Structural resolution of the complex was to 2.8 Å. Four main enzyme residue-rCdRP interactions were identified. A salt bridge is formed between the carboxylate group of the anthranilate moiety of rCdRP with the

conserved Arg 36 (N ϵ -O1 distance of 2.8 Å, N ϵ -O2 distance of 3.1 Å). Hydrogen bonding occurs between the oxygen atom of the carboxylate group of the invariant residue Asp 126 and the C4' hydroxyl oxygen of rCdRP (O-O of 3.0 Å distance). The C4' hydroxyl oxygen corresponds to the substrate PRA furanose ring oxygen. The C2' carbon of rCdRP is in close proximity to the thiol group of the conserved residue Cys 7 (S-C distance of 3.9 Å). Importantly, the orientation of the C2' hydroxyl oxygen of rCdRP hydrogen bonds with His 83 (N-O distance of 2.8 Å).³³ Electron density suggests the (*S*) diastereomer is favoured to bind to the enzyme giving non-diastereomeric reduction of rCdRP.

Interestingly *s*IGPS prefers to bind the (*R*) configuration of rCdRP,³⁴ suggesting both diastereomers are formed in the chemical reaction. It is believed that the difference in configuration is due to the different residue binding to the C2' hydroxyl oxygen. In the case of *s*IGPS it is Lys 110 (N-O distance of 2.5 Å).³⁴

1.5. Biochemical details of IGPS:

1.5.1. Enzyme kinetics for IGPS:

All the K_M and k_{cat} values (table 1.02) from *E. coli* for IGPS are from the bifunctional enzyme PRAI:IGPS,^{23,29,35-37} apart from *e*IGPS^m, which is an engineered monofunctional enzyme.^{23,38} The monofunctional IGPS domain, *e*IGPS^m gives similar catalytic properties and similar competitive substrate analogue inhibition values to K_i^{rCdRP} , and to those obtained with the bifunctional enzyme.^{23,38}

The IGPS enzyme from the hyperthermophile *S. solfataricus* at 25 °C gave tenfold lower k_{cat}/K_M values than *e*IGPS at the same temperature. This is generally explained by conformational rigidity that is common for enzymes from thermophilic sources.²⁷ A search of literature has not yielded enzyme kinetic values at higher temperatures than 25 °C for *e*IGPS.

As with *t*PRAI, *t*IGPS gave an unexpectedly high $k_{\text{cat}}/K_{\text{M}}$ value, which is twofold higher at 25 °C than for *e*IGPS.^{39,40} This is attributed to the highly labile nature of CdRP at 80 °C, hence, the enzyme must ensure CdRP substrate survival by having a high catalytic efficiency.^{25,39}

It is interesting to note that the inhibitor affinity values $K_{\text{i}}^{\text{rCdRP}}$ for *e*IGPS, *e*IGPS^m, and *s*IGPS are similar to the K_{M} values respectively, despite the structural differences of the two compounds.^{23,29,37,38,41}

Enzyme	Temperature (° C)	K_{M} (μM)	k_{cat} (sec^{-1})	$k_{\text{cat}}/K_{\text{M}}$ ($\mu\text{M}^{-1} \text{sec}^{-1}$)	$K_{\text{i}}^{\text{rCdRP}}$ (μM) ^a
<i>e</i> IGPS ²³	25	0.42	3.6	8.6	0.19
<i>e</i> IGPS ³⁶	25	0.34	2.2	6.5	
<i>e</i> IGPS ²⁹	25	1.2	7.2	6.0	
<i>e</i> IGPS ^{29,37}	25				0.25
<i>e</i> IGPS ^{m23}	25	0.47	3.2	6.8	0.26
<i>e</i> IGPS ^{m35}	25	0.3	2.7	9.0	0.34
<i>s</i> IGPS ^{36,39}	25	0.045	0.033	0.73	
	60	0.069	0.98	14.2	
<i>s</i> IGPS ³⁴	25	NA	NA	NA	0.014
<i>t</i> IGPS ^{39,40}	25	0.006	0.11	18	
	45	0.014	0.75	54	
	60	0.053	3.24	61	
	80	0.123	15.4	125	

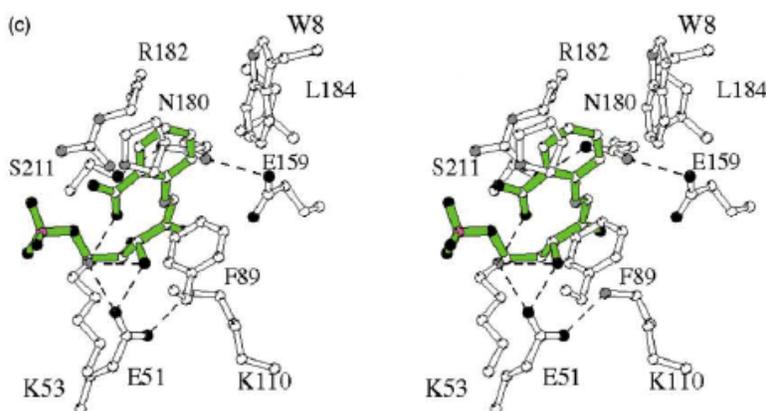
^a Substrate inhibition constant.

References: 23, 29, 32-37.

Table 1.02. Enzyme kinetic parameters of IGPS enzymes.

1.5.2. Proposed mechanism catalysed by IGPS – indole ring formation:

Kirschner *et al.*⁴¹ has crystallised *s*IGPS in complex with CdRP (resolution of 2.4 Å, figure 1.02) and *s*IGPS with IGP (resolution of 2.0Å) leading them to propose the following mechanism and residue interactions (scheme 1.07).⁴¹ This led to modelling studies of the intermediates 1 and 2 into the active site of *s*IGPS (figure 1.03).⁴¹

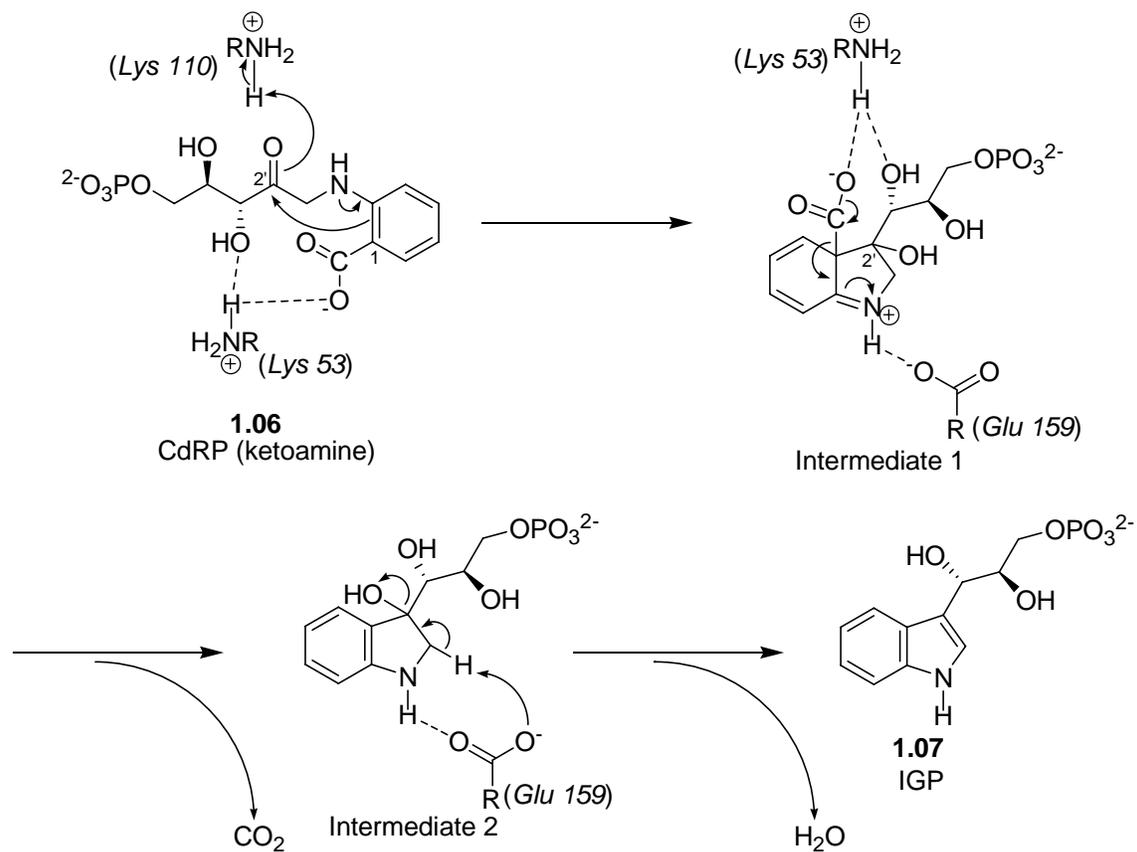


Legend: W8-trp, L184-leu, E159-glu, K110-lys, E51-glu, K53-lys, S211-ser, R182-arg, N180-asn, F89-phe.

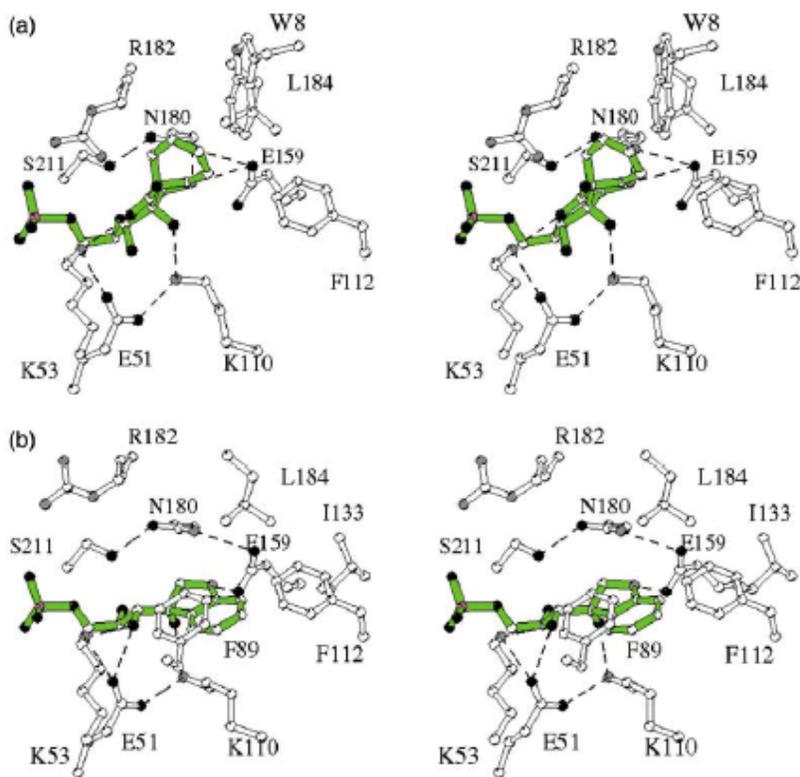
Figure 1.02. Stereoview of substrate CdRP bound to the active site of *s*IGPS.⁴¹

As seen in scheme 1.07, π electrons from the anthranilate moiety of CdRP nucleophilically attack the electrophilic C2' carbonyl on ribulose under acidic conditions. Decarboxylation of intermediate 1 follows giving intermediate 2, which dehydrates under basic conditions to give the IGP indole moiety of **1.07**.

Scheme 1.07. Indole ring formation showing substrate interactions with Lys 53, Lys 110, and Glu 159 from sIGPS.⁴¹



The residues Trp 8, Pro 57, Phe 89, Arg 182, and Leu 184 line the anthranilate-binding pocket. Interactions of the catalytically important residues Lys 53, Lys 110 and Glu 159 with substrates are shown in figures 1.02 and 1.03.⁴¹



Legend: W8-trp, L184-leu, E159-glu, F112-phe, K110-lys, E51-glu, K53-lys, S211-ser, R182-arg, N180-asn, I133-ile, F89-phe.

Figure 1.03. Stereoview of the bound catalytic intermediate 1 (a) and intermediate 2 (b) from molecular models of the active site in *sIGPS*.⁴¹

1.5.3. Residue interactions and model studies of intermediates of indole ring formation:

According to recent work^{19,33} and previous crystal structures (figure 1.02),⁴¹ the keto form is the predominant tautomer of CdRP **1.06** at equilibrium. Hence the carbonyl C2' carbon atom of the ribulose moiety is nucleophilically attacked by the carbon atom C1 of the anthranilate moiety of CdRP.⁴¹ Residue Lys 110 acts as a Brønsted acid on the C2' carbonyl (N-O distance of 2.6 Å) aiding the nucleophilic attack, resulting in a hydroxyl group being formed. Also the residue Lys 53 simultaneously acts as a salt bridge to the carboxylate group (N-O distance of 3.0 Å) on the anthranilate moiety and forms a hydrogen bond with the C3' hydroxyl group (N-OH distance of 2.7 Å).⁴¹ A synthetic equivalent to this synthesis of the indole moiety would be a Bischler reaction, which uses

a Lewis acid to promote carbon-carbon bond formation in the same way as Lys 110 catalyses the reaction. Of note is: (i) the large separation of 4.8 Å between C1 and C2' before cyclisation occurs, which in modeling studies is reduced to an estimated 2.0 Å by mobility of the ribulose moiety being able to flex into a more favourable conformation, and (ii) the role that the salt bridge plays with the Lys 53, drawing the two moieties together. The conclusion has been drawn that without the Lys 53 salt bridge or the carboxylate group formation of intermediate 1 would be difficult.⁴¹ This residue is conserved in all known IGPS enzymes.

Residue orientations, N-O distances and modelling define that the diastereomer intermediate 1 has the absolute configuration of (*S*) at C1 and (*R*) at C2'. This is consistent with the apparent selectivity for the (*R*) configuration of rCdRP.⁴¹

A salt bridge is importantly formed in intermediate 1 (figure 1.03a), between the positively charged NH group of the substrate and the carboxylate group of Glu 159 (O-NH distance of 3.6Å), resulting in aromaticity being restored to the anthranilate moiety by decarboxylation, giving intermediate 2 (figure 1.03b).⁴¹ Dehydration then occurs using Glu 159 to deprotonate the C1' position with Lys 110 acting as a Brønsted acid. It is believed that Lys 53, Glu 51 and Lys 110 mediate the reprotonation of the amino group of Lys 110 after the first reaction step, reverting the group to a lower p*K*_a, which helps cleave the hydroxyl group at C2' producing the indole ring system of IGP **1.07**.⁴¹

1.5.4. Reaction catalysed by IGPS – Indole ring formation:

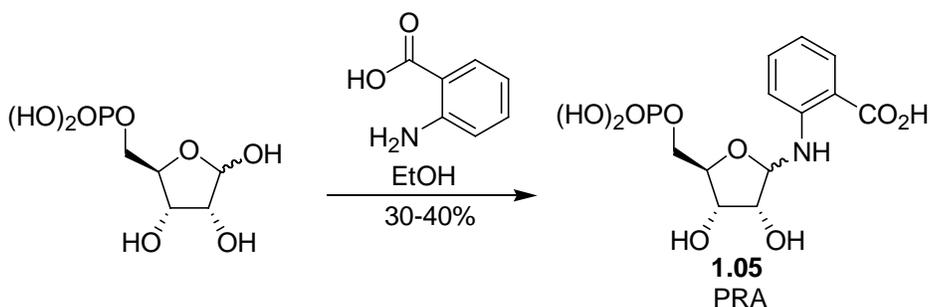
Crystal structures for CdRP complexed to *s*IGPS, rCdRP complexed to *s*IGPS, and IGP complexed to *s*IGPS have all been determined to resolutions of 2.40 Å, 2.05 Å and 2.00 Å, respectively.³⁴ Overall the three-dimensional structures are very similar. The structure of the monofunctional enzyme *e*IGPS^m has also been determined and compared with the structures of both *e*IGPS and *s*IGPS. The comparison showed that the conformations of the catalytically important residues are very similar.³⁴

1.6. Previous synthetic research on PRA and CdRP:

1.6.1. PRA synthesis:

Creighton⁴² first synthesised PRA in a condensation reaction in 1968 by simply mixing equal volumes of 1.0 M anthranilic acid in 95% ethanol and 1.0 M D-ribose-5-phosphate at room temperature (scheme 1.08). The maximum quantity of PRA **1.05** was formed within the first four minutes after mixing.⁴² A 30-40% yield was estimated, calculated by the quantity of PRA transformed by PRAI into the keto form of CdRP, which was catalysed by IGPS to form the measurable IGP product.⁴²

Scheme 1.08. First synthesis of PRA **1.05** by Creighton.⁴²



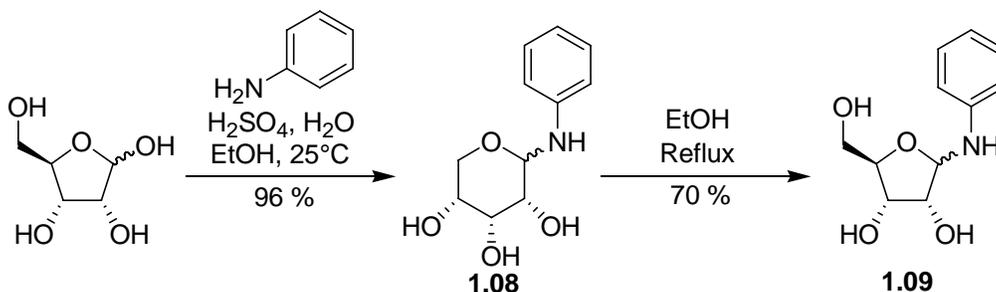
Upon standing at room temperature for an hour, a third of the PRA rearranged *via* the Amadori rearrangement to form CdRP. Creighton⁴² also showed, as did Ellis *et al.*⁴³ and Doy,⁴⁴ that PRA was liable to decompose back into the original starting materials of amines and carbohydrates. Earlier attempts at synthesising PRA were not successful;⁴⁴⁻⁴⁶ this problem has been ascribed to its instability.⁴² CdRP also was found to decompose to starting materials of amines and carbohydrates.⁴⁷ This problem could be overcome to some degree by storing these compounds at 0 °C at pH 8.6.

Creighton⁴² gave no purification methods for PRA **1.05**. After synthesis it was immediately used in enzymatic studies involving PRAI.

1.6.2. Synthesis of PRA derivatives:

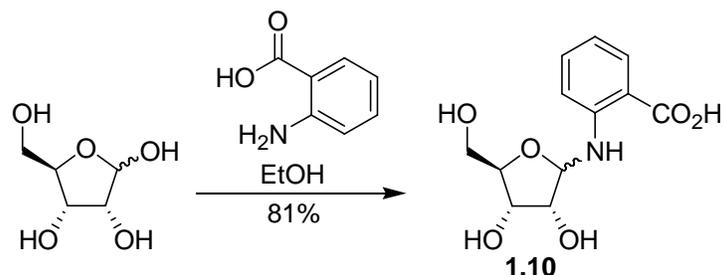
Berger and Lee⁴⁸ were the first to synthesise a dephosphorylated, decarboxylated form of PRA (scheme 1.09). In this experiment a solution of D-ribose in distilled water was adjusted to a pH of 4.0, and a solution of aniline in absolute ethanol was added. The solution was stirred at 25 °C for 10 minutes before being refrigerated overnight at 5 °C. The crystalline pyranoside ribosyl aniline moiety **1.08** was filtered off and washed with cold absolute ethanol and finally with diethyl ether.⁴⁸ This condensation reaction was catalysed by acid (pH 4.0) without any improvement in yield. At a pH of 8.0 the reaction took several hours at room temperature before **1.08** was formed to the same quantity as **1.08** in acidic conditions.⁴⁹ Upon refluxing with ethanol, **1.08** rearranged to form ribosyl aniline furanoside **1.09**. To our knowledge **1.09** has not been used in any activity studies with PRAI.

Scheme 1.09. Berger *et al.*⁴⁸ synthesis of ribosyl aniline **1.09**.



In 1958 Doy and Gibson⁴⁶ synthesised a dephosphorylated form of PRA **1.10** via the condensation method of Berger *et al.*⁴⁸ (scheme 1.10). This preliminary investigation did not undertake any kinetic activity studies involving PRAI.

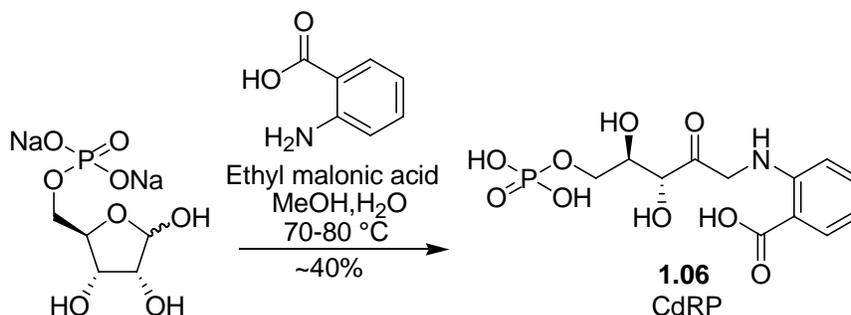
Scheme 1.10. Synthesis of dephosphorylated PRA **1.10**.⁴⁶



1.6.3. CdRP synthesis:

Yanofsky *et al.*⁵⁰ was credited with the first synthesis of CdRP **1.06** in 1960. He modified^{51,52} reaction conditions such as heat and the use of a proton donor⁵³ known to favour Amadori-type rearrangements. As a result the reaction yield was increased to 40%. The optimised condensation reaction conditions⁵¹ involved a 3:1 mixture of anthranilic acid dissolved in methanol and sodium ribose 5-phosphate dissolved in water, being heated at 70-80 °C for 30 minutes. This solution included the proton donor ethyl malonic acid. The reaction was then chilled to 4 °C in order to stop the reaction and halt the formation of any degradation products, and the reaction was then acidified to a pH of 5 using HCl. The product was then extracted with ethyl acetate giving CdRP **1.06** in 40% yield (scheme 1.11).⁵¹

Scheme 1.11. Heat-induced synthesis of CdRP **1.06**.⁵¹



Room temperature condensation chemical synthesis of CdRP **1.06** is described by Bisswanger *et al.*⁵⁴ whereby a 1:1 mixture of anthranilic acid and sodium ribose 5-

phosphate in 50% ethanol is adjusted to a pH of 5.0 and then stirred for 4 hours at 25 °C. The reaction was stopped by addition of water.⁵⁴ No isolated yield was given.

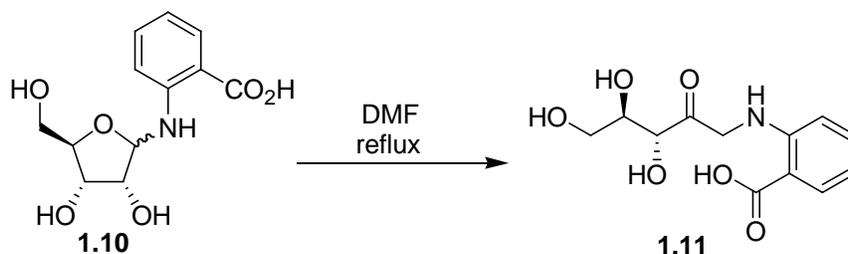
The most recent paper published describing CdRP synthesis is by Kirschner *et al.*⁵⁵ The method follows a similar procedure at room temperature to Bisswanger *et al.*⁵⁴ except the mixture is covered and stored in the dark, removing the product's liability to decompose in light. The reaction length was prolonged to 17 hours, giving the highest yield (60%) in the literature of CdRP **1.06**.

Up until the Kirschner *et al.*⁵⁵ report, no satisfactory method had been found to purify CdRP from unreacted ribose 5-phosphate and other side products. Anion exchangers such as Dowex have been employed but the instability of the compound has reportedly made it impossible to elute and purify.⁵² Some purification has been achieved by chromatography on DEAE-cellulose and elution with a NaCl gradient, but the results were poor.⁵² Kirschner *et al.*,⁵⁵ however, have successfully purified CdRP on a reverse-phase C₁₈ column.

1.6.4. Synthesis of CdRP derivatives:

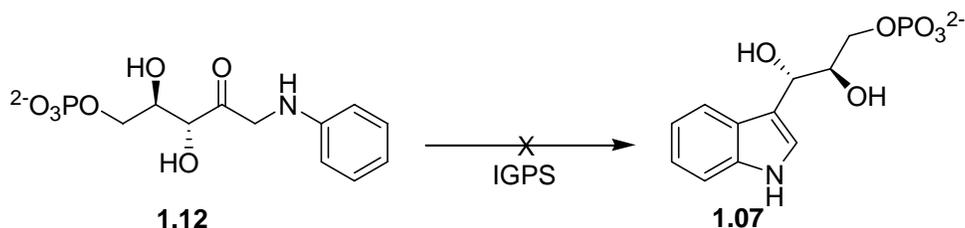
A dephosphorylated form of CdRP **1.06** was prepared by the Amadori rearrangement (scheme 1.12), induced by refluxing the furanoside **1.09** in DMF for 2 minutes.⁴⁶ No yield was reported for this reaction nor has there been enzyme kinetic data collected for 1-(*O*-carboxyphenylamino)-1-deoxyribulose **1.11**.

Scheme 1.12. Synthesis of 1-(*O*-carboxyphenylamino)-1-deoxyribulose **1.11**.⁴⁶



Yanofsky *et al.*⁵⁰ synthesised 1-(phenylamino)-1-deoxyribulose 5'-phosphate **1.12** using a similar procedure to that which had been used for the preparation of CdRP **1.06**. It was found that the **1.12** was not a substrate for eIGPS.^{38,50} This suggests that the carboxylate functionality is a key group for substrate recognition and mechanistic transformation, as noted in section 1.5.

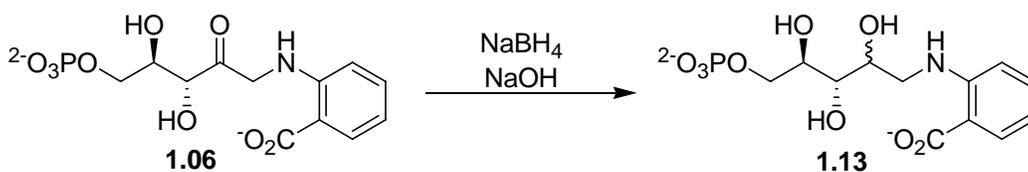
Scheme 1.13. Inhibition of IGPS by 1-(phenylamino)-1-deoxyribulose 5'-phosphate **1.12**.⁵⁰



1.6.5. rCdRP synthesis:

The keto group at C2' of CdRP **1.06** has been reduced to the alcohol **1.13**, which is known to bind to the active site of IGPS. Bisswanger *et al.*⁵⁴ followed a similar reduction procedure to that developed by Lingens *et al.*⁵⁶ rCdRP **1.13** was simply formed by adding 0.05M sodium borohydride in 0.05 M NaOH to a freshly prepared sample of CdRP **1.06** (scheme 1.14).⁵⁴ After an hour of intense stirring the pH was adjusted to 4.8 and excess anthranilic acid was extracted with ethyl acetate. A purification technique using a DEAE-cellulose column was described and purity was confirmed by Thin Layer Chromatography (TLC) and absorbance spectra. However, no yield was given, nor was there a discussion on diastereomeric selectivity.⁵⁴ It is assumed that both (*R*) and (*S*) are formed at the C2' stereocenter. It was noted that the product was more stable than CdRP **1.06** but it was still light sensitive.⁵⁴ rCdRP **1.13** is not a substrate for IGPS; it is a competitive inhibitor with respect to CdRP **1.06**, as discussed in section 1.5.⁵⁴

Scheme 1.14. Successful reduction of CdRP **1.06** to rCrRP **1.13**.⁵⁴



Although **1.13** has been formed by reducing CdRP **1.06** we aim to synthesize this molecule using a general synthetic pathway allowing us the option of modifying the substituents, and changing the stereochemistry on the basic structure of CdRP **1.06**. Our target compounds and the reasons that we are interested in synthesising them are detailed in section 1.7.

1.7. Target compounds:

1.7.1. PRA analogue targets for the investigation of PRAI reaction:

Analogues of PRA **1.05** were considered for studies into PRAI substrate specificity (figure 1.04). Functional group analogues of PRA **1.05** involved in the reaction mechanism could be synthesised, such as replacing the ring oxygen with a nitrogen or carbon (to create a carbocycle) and modifying the secondary amine functionality on the anthranilate moiety. The carbocycle would not be expected to be able to undergo the Amadori reaction catalysed by PRAI. Changing the C2' substituents on PRA **1.05**, for example, from a hydroxyl group to a fluorine was also considered. Fluorine is a highly electronegative element, having a slightly higher electronegativity than a hydroxyl group. However, unlike a hydroxyl group, fluorine lacks hydrogen-bonding capabilities. In terms of atomic size, fluorine is comparable with a hydrogen atom. Comparing hydroxyl, fluorine and hydrogen at C2' and then at C3' would give us a range of different atomic sizes, hydrogen-bonding capabilities and electronegativity. Altering the primary phosphate group could provide useful information on the enzyme recognition and binding of this functionality, as would modifying the aromatic carboxylate group.

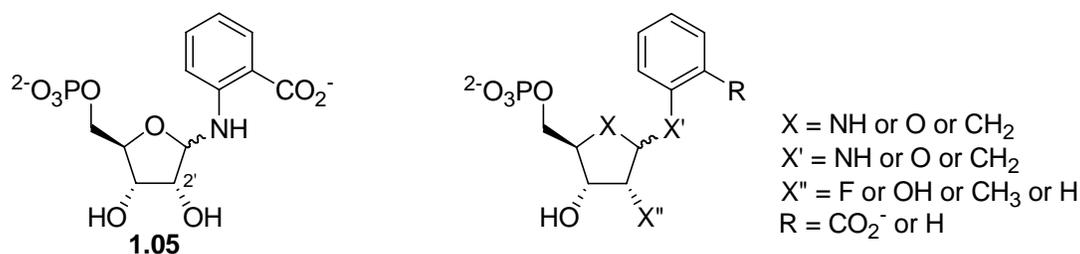


Figure 1.04. Ring analogue target compounds for potential inhibition of PRAI.

Another more straightforward option is to change the orientation of the C2' and C3' hydroxyl groups on PRA **1.05** by using different five-carbon carbohydrates, such as arabinose **1.14**, lyxose **1.15** and xylose **1.16** (figure 1.05). It is important to note that the hydroxyl groups are not directly involved in the reaction mechanism, but it is likely that they are involved in the binding of the substrate at the catalytic site.

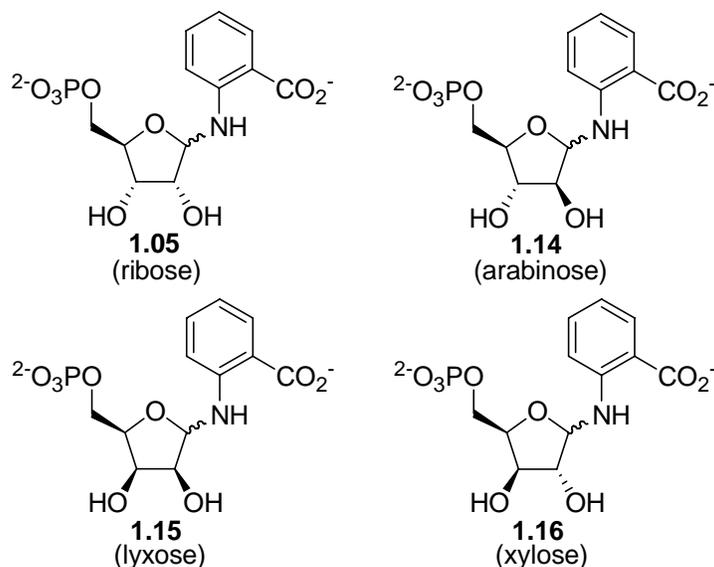


Figure 1.05. PRA **1.05** carbohydrate analogues target compounds for potential inhibition of PRAI.

1.7.2. CdRP analogue targets for the investigation of PRAI and IGPS:

Since CdRP is the product of PRAI as well as the substrate of IGPS, it is therefore capable of binding to each of the different active sites, and hence has the potential to

illuminate both reaction mechanisms. There has been little biochemical kinetic research on CdRP analogues in studies with PRAI and IGPS, hence a range of possible targets are available.

By dipping into the chiral pool and using different five-carbon carbohydrates in synthesis of CdRP analogues, we can potentially produce the hydroxyl group (rCdRP) at the C2' carbon in different orientations, and alter the stereochemistry of the C3' and C4' hydroxyl groups (figure 1.06).

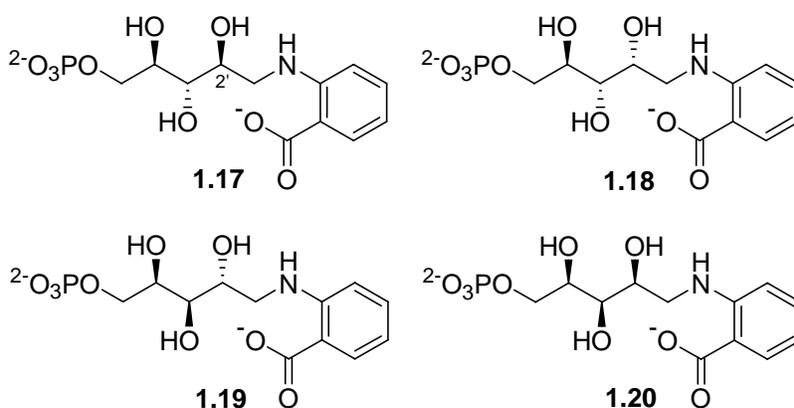


Figure 1.06. rCdRP carbohydrate-analogue target compounds for potential inhibition of PRAI and IGPS.

By using D-ribose in the synthesis of 1-*o*-carboxyphenylamino-1'-deoxyribose-5'-phosphate **1.17** we have access to the (*S*) stereocentre of C2'. Use of D-arabinose in 1-*o*-carboxyphenylamino-1'-deoxyarabinose-5'-phosphate **1.18** gives us access to the (*R*) stereocenter of C2'. 1-*o*-carboxyphenylamino-1'-deoxylyxose-5'-phosphate **1.19** and 1-*o*-carboxyphenylamino-1'-deoxyxylose-5'-phosphate **1.20** can be synthesised from D-lyxose and D-xylose, respectively.

The change of a carbonyl ketone (CdRP) to a hydroxy group (rCdRP) would be expected to prevent indole formation as catalysed by IGPS (section 1.5.2). This is due to the first step in indole ring cyclisation taking place by nucleophilic attack on the carbonyl. Replacing this carbonyl functionality with a hydroxyl group would hinder this *ipso* attack

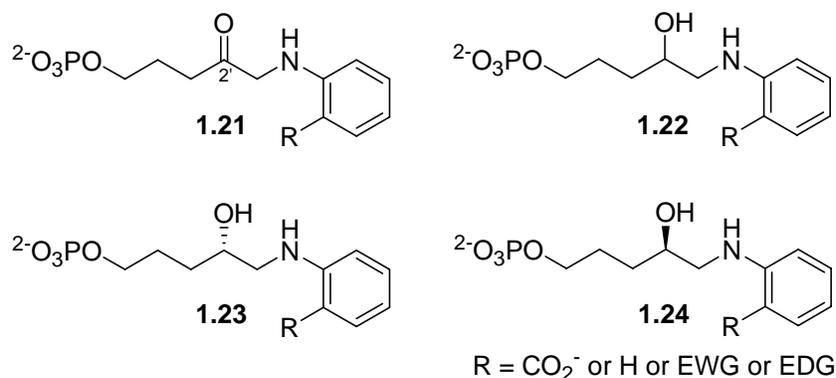


Figure 1.08. Ring analogue target compounds for PRAI.

Once the inhibition properties of ketone **1.21** is known, alcohol **1.22** will be synthesised and tested with IGPS to compare inhibition properties. It should be noted that **1.22** can exist as a mixture of stereoisomers as C2' is a stereogenic centre. The intention is to synthesise initially a racemic mixture and use both enantiomers in enzyme testing. If strong inhibition or substrate ability is observed, it would be interesting to pinpoint the influence that the C2' hydroxyl stereochemistry plays on this. Due to the potential twisting of the carbon chain, backbone the carbonyl of **1.21** may sit in an orientation further up or down in relation to other functionalities. It should be noted that the phosphate group possibly anchors the chain limiting this potential twisting. Formation of enantiomerically pure forms of alcohol, with the hydroxyl group lying in either the (*S*) **1.23**, or the (*R*) **1.24** orientation can ultimately address stereochemical interactions.

1.7.4. Target 1,4,5 compounds for the investigation of the PRAI and IGPS:

Another variant on the above CdRP analogues is to completely remove the C2' and C3' functional groups (figure 1.09). Changing the C4' hydroxyl group of the 2,3-deoxy compound from one that has the (*S*) stereochemistry of CdRP, **1.25**, to a racemic mixture, **1.26**, and the opposite (*R*) stereochemistry, **1.27**, could provide some interesting information about the role of the C4' hydroxyl group. The 1,4,5 naming of these target compounds is due to the functionalities at the respective positions along the pentyl moiety.

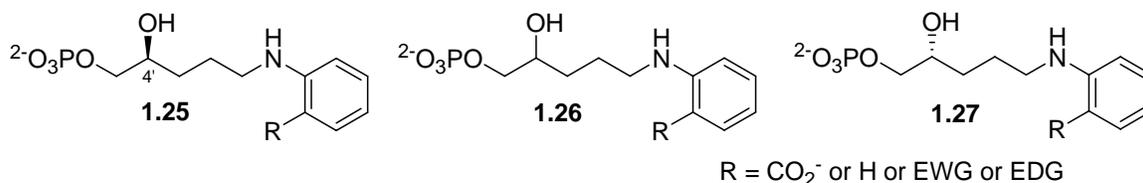


Figure 1.09. Target 1,4,5 compounds for inhibition of PRAI and IGPS.

1.8. Synthetic ideas:

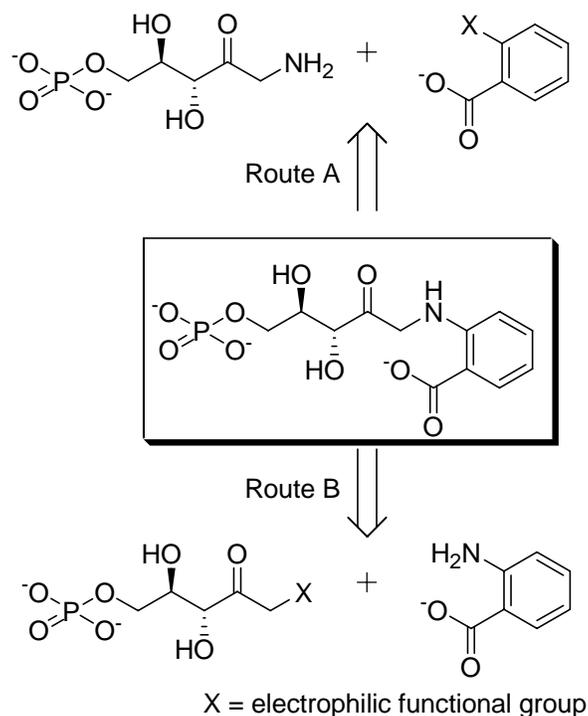
1.8.1 Synthetic goals:

The predominant theme in the synthesis of the target compounds is the formation of secondary amines. As shown in section 1.6, historically PRA- and CdRP-like compounds have been synthesised by condensation of anthranilate moieties and simple five-carbon carbohydrates. Expanding the methods of synthesis for PRA- and CdRP-like compounds and allowing more complex five-carbon carbohydrates and functionalities to be present during the secondary amine formation is paramount, as is the development of general synthetic procedures for formation of CdRP-like compounds.

1.8.2. Retrosynthesis of CdRP-like compounds:

Two routes can be taken in the synthesis of the secondary amines of CdRP-like compounds. Route A (scheme 1.15), where a five-carbon carbohydrate is the nucleophilic amine source and an aromatic moiety is the electrophile or route B, a more traditional approach, where a five-carbon carbohydrate is the electrophile and an anthranilate moiety is the amine nucleophile. Both use amine as the nucleophile. With this in mind a brief review of secondary amine synthesis will follow.

Scheme 1.15. Retrosynthesis of CdRP-like compounds.



1.9. Formation of secondary amines:

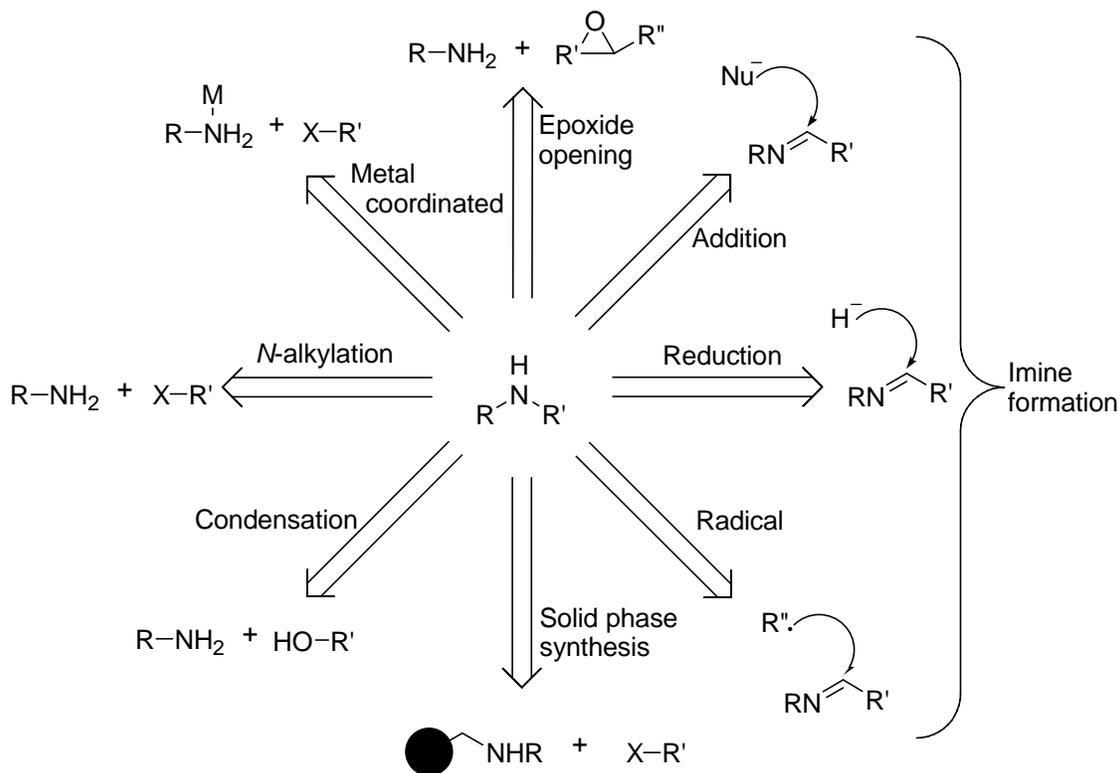
1.9.1. Introduction:

Efficient synthesis of secondary amines has perhaps received more attention than the preparation of many other functional groups in organic chemistry.^{57,58} This can be attributed to numerous known biologically active compounds containing secondary amines and the potential for many more to be discovered.^{57,59-62}

Despite this widespread interest, traditional methods are susceptible to low chemical selectivity, and/or harsh reaction conditions, and/or generally poor yields.^{57,63} Tertiary amine formation predominates if reaction conditions are not carefully selected. Scheme 1.16 outlines some of these traditional methods, which will be developed to synthesise CdRP analogues. The following sections will cover the advantages and disadvantages of these methods and why or why not they will be developed in this project. Developing similar chemistry for the synthesis of CdRP analogues, target 1,2,5 and target 1,4,5

compounds allows for potential crossover knowledge and a simplification of the synthesis. Literature examples similar to our target compounds will be discussed.

Scheme 1.16. Retrosynthesis of secondary amines.



1.9.2. Condensation:

As described in section 1.6, condensation-type chemistry was used in the synthesis of PRA- and CdRP-like compounds. Most of the major advances in this field of chemistry were well over a hundred year ago.

The first synthesis of glycosylamines using aryl amines was by Schiff^{64,65} who heated aniline with dry D-glucose. Later Sorokin^{66,67} found that by using a lower reaction temperature and by adding hot ethanol after the reaction crystalline *N*-phenyl-D-glucosylamine, -D-galactosylamine, and -D-fructosylamine could be formed. The most generally used method of preparation, also described by Sorokin,^{66,67} consists of heating

the amine and the reducing sugar in boiling methanol or ethanol, containing up to 10% of water.⁴⁷

Formation of glycosylamines with electron-withdrawing groups on the aryl amine ring was devised in the early twentieth century by Irvine *et al.*⁶⁸⁻⁷⁰ The method developed used cold aqueous ethanol to produce *N*-*o*-carboxyphenylglycosylamines from D-galactose, D-mannose, L-rhamnose and maltose. Weygand⁷¹ produced aryl glycosylamines simply by dissolving the sugar in the minimum amount of hot water and heating with the arylamine.⁶⁸⁻⁷⁰

Condensation reactions with carbohydrates and aryl amines generally involve polar solvents, heat and potentially small amounts of catalytic acids.⁴⁷ On paper they seem a quick and easy method to simple secondary amines but by potentially using some acid catalysis and heat, they may not be ideal for some functional or hydroxyl-protecting groups.

1.9.3. *N*-alkylation *via* nucleophilic substitution:

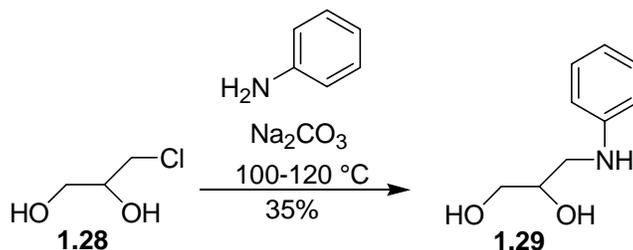
In principle, the most common and straightforward route to secondary amine formation is direct amination. This is also known as Hofmann alkylation⁷² and involves treatment of good leaving groups, alkyl halides or dialkyl sulfates (sulfonates), with a primary amine.⁵⁷ The presence of a base is also common.

Mixtures of mono- and dialkylated products obtained from primary aromatic amines and alkylating agents can be separated by fractional distillation. The mono-alkylated derivative can be easily converted into a non-volatile derivative such as the tosylate from which the dialkylated product can be easily recovered.⁷³

Examples of the synthesis of similar compounds to our targets includes *N*-alkylation of 3-chloro-1,2-propanediol **1.28** (scheme 1.17) by Okahara *et al.*⁷⁴ A mixture of neat aniline

and powdered Na_2CO_3 had **1.28** added to it and was heated between 100-120 °C for 17 hours with stirring. Distillation purified the aryl secondary amine **1.29**.

Scheme 1.17. Okahara *et al.*⁷⁴ *N*-alkylation to form aryl secondary amine **1.29**.



The low $\text{p}K_a$ values of aryl amines, argues against the use of them as nucleophiles. Section 1.8.2. discusses different retrosynthesis ideas for the formation of CdRP-like compounds. Route B showed a five-carbon carbohydrate as the electrophile and an anthranilate moiety as the amine nucleophile (scheme 1.15). This was a more traditional approach to secondary amines and brought about our initial general synthetic strategy in chapter 2 (scheme 2.01) involving reductive amination reactions with aryl amines and lactol moieties. A literature investigation, whether route A, where a five-carbon carbohydrate is the nucleophilic amine source and an aromatic moiety is the electrophile, was plausible (scheme 1.15). Electrophilic aromatic moieties could be synthesised *via* aniline and anthranilic acid **1.02**, due to an onsite supply. Potential synthesis of these electrophilic aromatic moieties can come about by two steps:

- i) formation of diazonium salts;
- ii) subsequent reactions involving substitution to form an aryl halide.⁷³

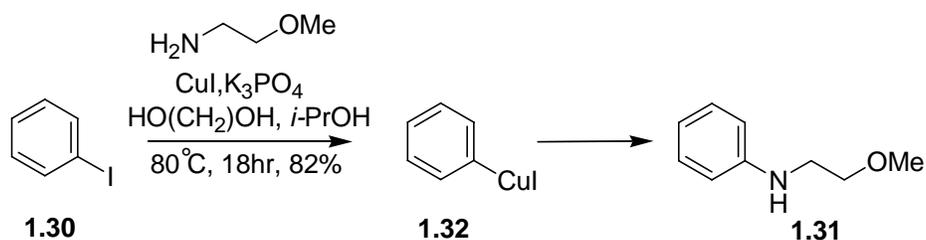
Highly reactive diazonium salts can be formed in cool conditions by mixing nitrous acid and primary aryl amines. The nitrous acid required is formed *in situ* from sodium nitrite and hydrochloric acid. This process is known as diazotisation. Griess⁷⁵ discovered it in 1858. Replacement of the diazonium group with a halogen by using cuprous halides is referred to as the Sandmeyer reaction⁷⁶ and is one of the main methods to form aryl halides from aryl amines. Chlorine and bromine substituents can replace the diazo group

by mixing the corresponding diazonium halide salt with cuprous chloride or cuprous bromide at ambient or slightly elevated temperatures over several hours.⁷⁶ Substituting in iodine is much simpler and is accomplished by merely adding potassium iodide to the diazonium salt, forming iodobenzene **1.30** (scheme 1.18).⁷³

There are several methods featuring different mechanisms to form secondary aryl amines from aryl halides. One example is with aryl halides containing *ortho* or *para* electron-withdrawing substituents. These readily react with amines to produce secondary aryl amines *via* straightforward nucleophilic aromatic substitution.⁷³ In the case of unactivated aromatic halides without electron-withdrawing groups, amines can react *via* benzyne intermediates, in the presence of strong base or generally at temperatures in excess of 200° C with a copper catalyst.^{73,77,78}

Copper-mediated Ullmann⁷⁹ and Goldberg⁸⁰ couplings are attractive for industrial scale applications.⁸¹⁻⁸³ These involve oxidative addition of a copper (I) salt to the aryl halide (scheme 1.18). The reaction usually generates secondary and tertiary amines, however, and requires separation of the resulting mixture. Buchwald *et al.*⁸² have limited the tertiary amine production, forming the secondary aryl amine **1.31** from the aryl copper intermediate **1.32** in good yield (scheme 1.18).

Scheme 1.18. Example of Ullmann⁷⁹ and Goldberg⁸⁰ couplings by Buchwald *et al.*⁸²



The Buchwald *et al.*⁸² method has been further developed by Fukuyama *et al.*⁸⁴ to take place under milder reaction conditions, forming similar secondary aryl amines to **1.31**. Perhaps the best yielding, milder methodology for secondary aryl amine formation *via* an

aryl halide is by the Buchwald-Hartwig palladium-catalysed cross-coupling reaction, as discussed below.

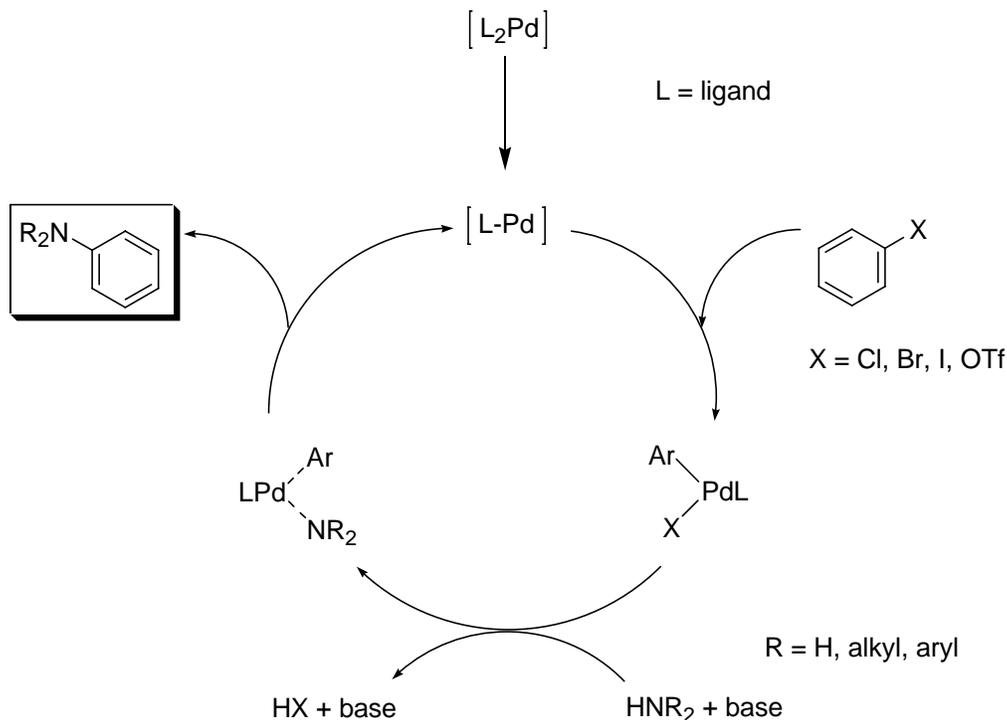
1.9.4. Nucleophilic substitution with metal coordination:

Secondary amine formation *via* nucleophilic substitution with metal coordination typically involves palladium, nickel^{57,85} or copper^{57,82,83} catalysis and an aromatic halide or triflate (scheme 1.15, route A). The amine can be aryl or alkyl in nature.

Palladium-catalysed reactions have been extensively studied in the past few years by Hartwig *et al.*,⁸⁵⁻⁹⁰ Buchwald *et al.*⁹¹⁻⁹⁴, and others.⁹⁴⁻⁹⁹ Scheme 1.19 shows this catalytic cycle. In the mid 1990s Buchwald *et al.*⁹¹ and Hartwig⁸⁶ started modifying this reaction and managed to find milder conditions instead of needing the presence of a strong base such as NaOEt. These amination reactions can be carried out using K₂CO₃ or Cs₂CO₃, and a palladium-based catalyst in the form of L₂Pd or L₂PdCl₂. The ligands or L are typically chelating phosphines such as BINAP (2,2'-bis(diphenylphosphino)-1,1'-binaphthyl), and P(*o*-tolyl)₃ but can also be (OAc)₂.

The combination of Pd(OAc)₂ and BINAP is an excellent catalyst system for the coupling of primary amines with aryl bromides.^{85,92,100} Although this processes enjoys excellent functional group tolerance, high yields and wide substrate scope, due to the lack of glycosylamines in the literature we chose to follow other avenues for secondary amine formation.

Scheme 1.19. General scheme of palladium-catalysed secondary amine formation.⁸⁵



1.9.5. Epoxide ring opening:

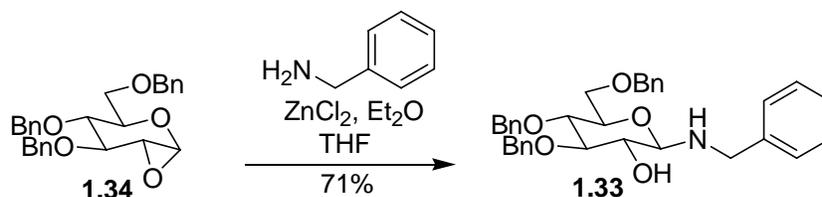
The strained nature of the epoxide ring, together with the polarisation of the C-O bonds, makes epoxides prone to react with a large variety of nucleophiles,^{101,102} which includes aryl and alkyl amines. Formation of secondary amines or β -amino alcohols, as is the case with aminolysis of an epoxide ring, has been the subject of extensive studies in recent years.

The classical synthetic approach towards β -amino alcohols involves aminolysis of epoxides at elevated temperatures, in protic solvents and with an excess of the amine.¹⁰³⁻¹⁰⁵ Elevated temperatures can be detrimental to certain functional groups and to the control of regioselectivities.^{101,106} Other limitations arise from low nucleophilicity and steric factors resulting from bulky crowded amines or epoxides.¹⁰⁶ Various

activators/promoters, usually Lewis acids or metal salts, have been used in order to overcome these problems and this will be discussed in more detail in Chapter Four.

Gordon and Danishefsky¹⁰⁷ have developed a potential method for the formation of our target compounds by using ZnCl_2 as a potent activator for glycosylamine formation *via* epoxide ring opening (scheme 1.20). Note that the diastereomeric product **1.33** is due to the chiral epoxide **1.34**. Protection of the surrounding hydroxyl groups is important since they can potentially undergo intra- and inter-molecular epoxide ring opening.

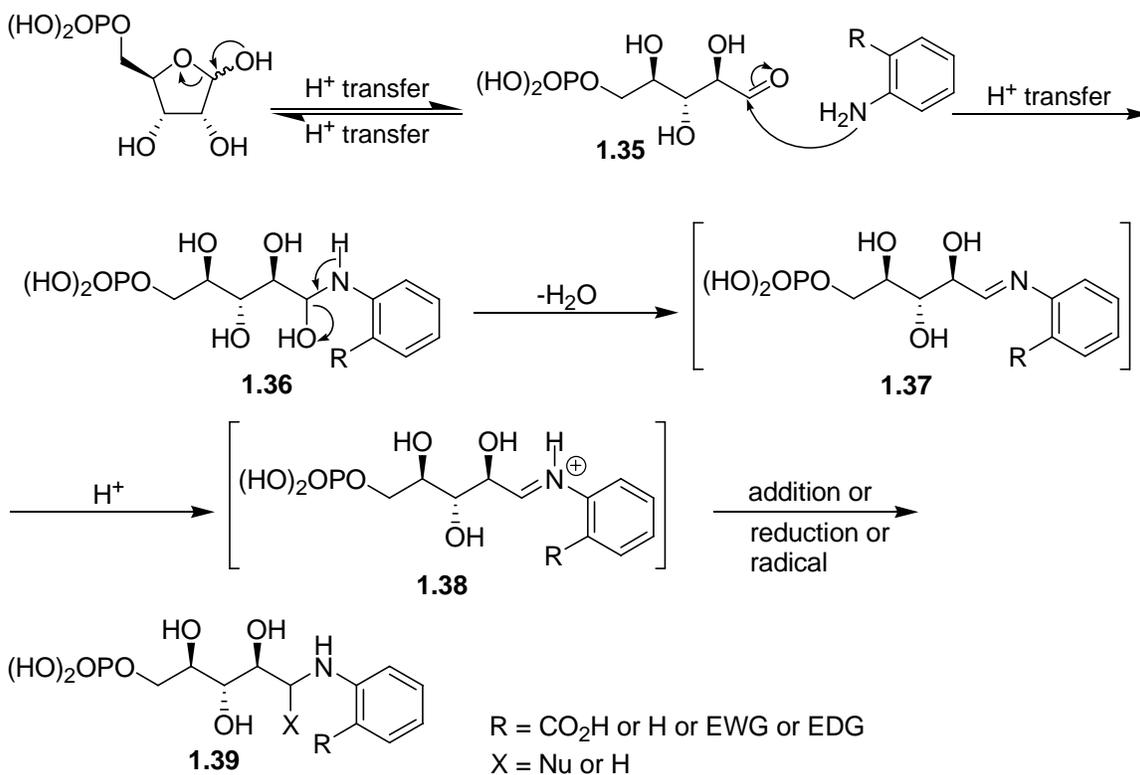
Scheme 1.20. Glycosylamine formation *via* an epoxide.¹⁰⁷



1.9.6. Imine formation:

Imine formation is a prerequisite to addition, reduction (reductive amination) or radical approaches to secondary amines.¹⁰⁸ Imine formation involves the aryl amine attacking the aldehyde of an open-chain carbohydrate **1.35** (scheme 1.21), forming the intermediate carbinol amine **1.36**, which dehydrates to form an imine **1.37**.¹⁰⁸ Under the reaction conditions, which are usually weakly acidic to neutral, the imine is protonated to form the highly electrophilic iminium ion **1.38**.¹⁰⁸⁻¹¹⁰ Subsequent addition/reduction/radical chemistry on the iminium ion produces the secondary amine product **1.39**. Stanaszek *et al.*¹¹¹ have, however, provided evidence suggesting the direct reduction of the carbinol amine **1.36** as a possible pathway leading to **1.39**.¹¹¹

Scheme 1.21. Imine formation and subsequent formation of secondary amine.



Addition of nucleophiles to the iminium ion would be a great source for C1' CdRP analogues. This method and radical addition of nucleophiles will not be covered in this review or thesis. The reductive amination method looks to be the prime candidate for production of CdRP analogues, target 1,2,5 and 1,4,5 compounds, as long as there is a comprehensive protecting strategy to ensure the carboxylate functionality on the anthranilate moiety does not interfere or react. A mini review into this chemistry and results we obtained using it follow in Chapter Two.

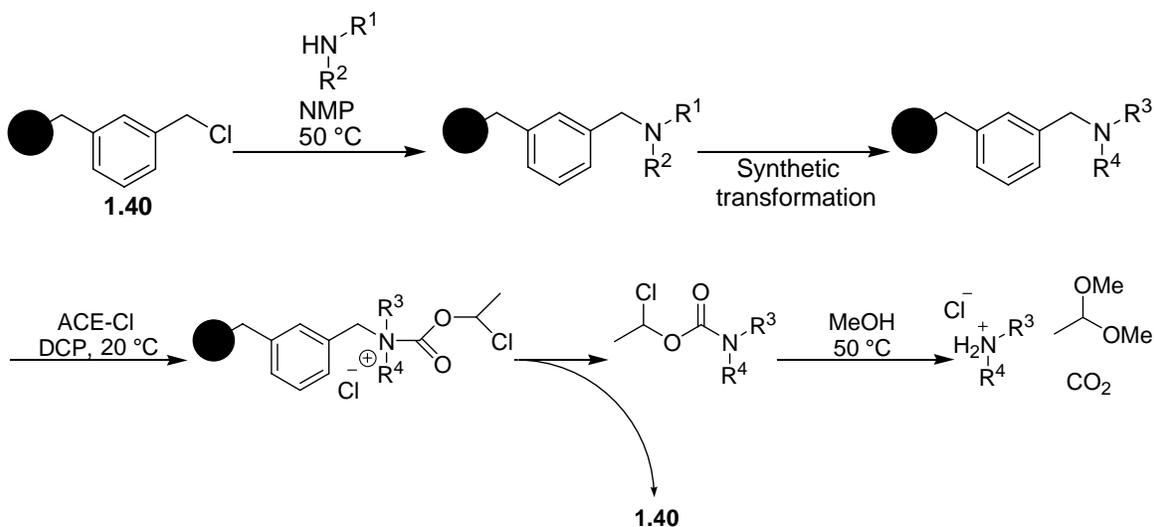
1.9.7. Solid phase synthesis:

As previously discussed, many drugs contain the secondary amine functionality, creating a demand for swift preparation and screening for biological activity. It has been shown that combinatorial chemistry^{112,113} has an advantage over classical one-pot synthetic methods since it has the ability to produce a large range of target compounds simultaneously with a minimal of effort. Combinatorial synthetic chemistry can be

generally divided into two domains, solution-phase synthesis and solid-phase synthesis.¹¹²

Solid-phase chemistry¹¹³ involves a reactant bound to a resin, having its functional group synthetically transformed *via* classical chemistry and then being cleaved to give the isolated product and unbound resin. Solid-phase chemistry has been at the forefront of combinatorial chemistry. The development of vast chemical libraries is due to the ease of purification and the ability of the resin to be recycled. Disadvantages of synthesis on solid supports include the additional linking and cleavage steps, and the development of an appropriate reactant to be the resin linker. One such linker is benzyl carbamate, which is known to be stable under diverse conditions.¹¹⁴ Work by Rees *et al.*¹¹⁵ in 1996 has shown that solid-phase synthesis of tertiary amines can proceed in the absence of a linker to the resin. This development has resulted in advances in secondary amine formation requiring no resin linker with the reactant (scheme 1.22).¹¹⁶

Scheme 1.21. General synthesis of secondary amine *via* resin **1.40**.¹¹⁶



Methods developed in this thesis for the formation of secondary aryl amines could be used in combinatorial solid-phase chemistry, which would increase the breadth of target compounds synthesised.

1.10. Summary of thesis aims:

The general biosynthetic pathway for tryptophan is known (scheme 1.05). However, little information has been gathered on how substrates and enzymes interact when phosphoribosylanthranilate isomerase (PRAI) and indole-3-glycerolphosphate synthase (IGPS) convert a substituted phenyl ring, PRA, into an indole moiety, IGP. Previous work on the overall pathway has been focused on the earlier enzymatic reactions with minimal attention being directed towards these two key enzymatic steps immediately preceding tryptophan formation. Nor has there been a serious synthetic approach to develop methodology to produce a plethora of substrate and product analogues. My project is based on finding methodology to synthesise substrate analogues and inhibitors for PRAI and IPGS in order to provide clear information on how the substrates and enzymes interact, and how these enzymes catalyse the isomerisation and ring-forming reactions.

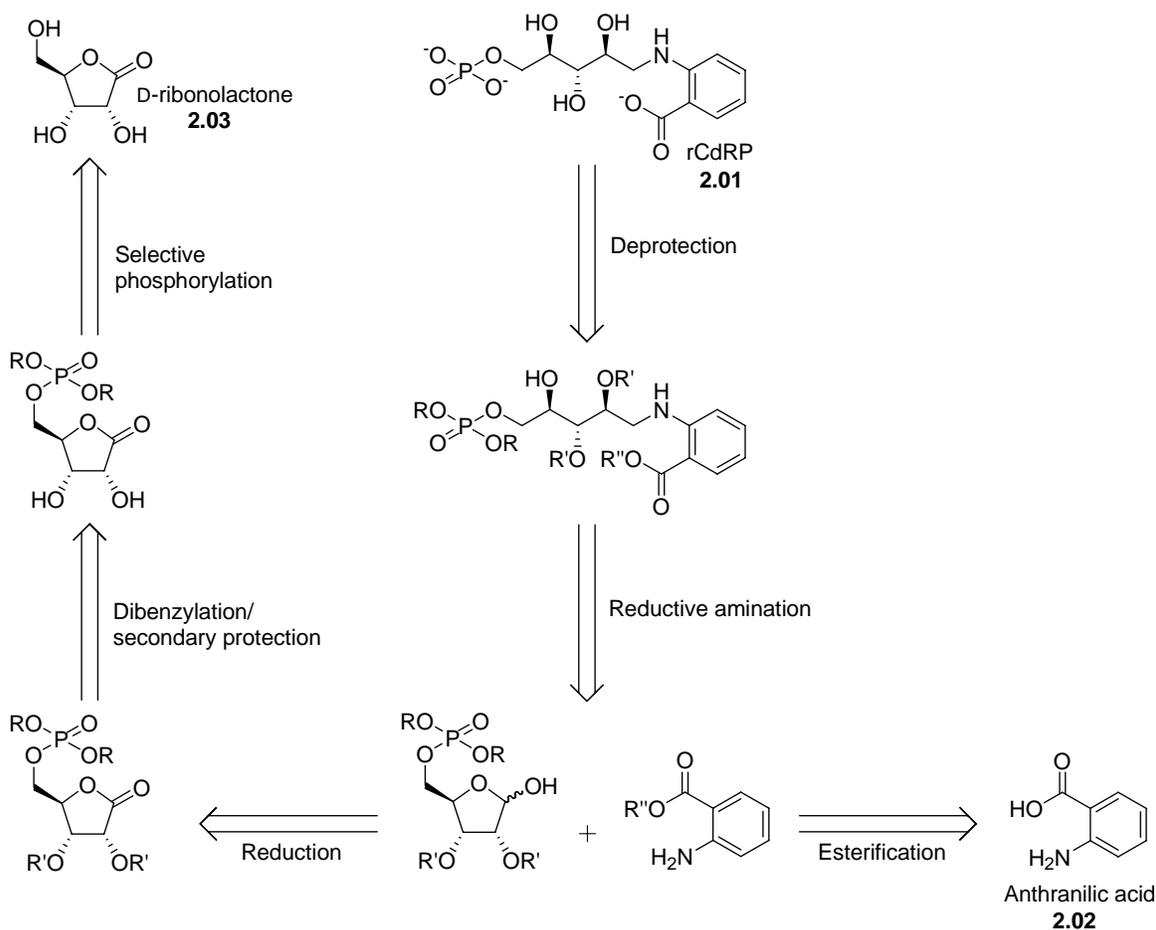
Our initial target compounds are based around the structure of CdRP. Target compounds for inhibition of IGPS may also inhibit PRAI. This is due to the target compounds being based on CdRP, the end product of the PRAI-catalysed reaction. Our initial emphasis has been on producing a reduced form of CdRP. Other potential targets are shown in figure 1.05, and are modified variants of ribose 5-phosphate and deoxy compounds. The methodology leading to these target compounds will focus on secondary amine formation, using reductive amination, nucleophilic substitution and epoxide ring opening, rather than the previously studied condensation reaction. These three types of chemistry will comprise the following three chapters. Each chapter will loosely contain retrosynthesis of target compounds, including protection strategy details, and synthesis of active target precursors, which lead to the investigation into secondary amine formation. Chapters five and six will summarise and contain experimental procedures respectively.

CHAPTER 2: Reductive aminations.

2.1. The initial retrosynthetic plan:

Our initial target was the reduced form of CdRP **2.01**, which we aimed to synthesise *via* a general strategy shown in scheme 2.01. Developing a general synthetic strategy would allow the use of different five-carbon sugars to produce target compounds with minimal need to diverge from chemistry previously used to produce the alcohol **2.01**. Our initial retrosynthetic plan for the preparation of target alcohol **2.01** hinged on coupling phosphorylated protected D-ribonolactol with esterified anthranilic acid *via* a reductive amination reaction. Phosphorylated protected D-ribonolactol would come from the reduction of phosphorylated protected D-ribonolactone. Esterification of anthranilic acid **2.02** and selective primary phosphorylation of D-ribonolactone **2.03** and would be the first steps in this convergent synthesis. One advantage of starting with D-ribonolactone **2.03** is that the lactone functionality essentially traps the carbohydrate as a five-membered ring with an exposed primary hydroxyl group, unlike starting with D-ribose sugar, which could be present in either pyranose or furanose forms.

Scheme 2.01. General synthetic strategy.

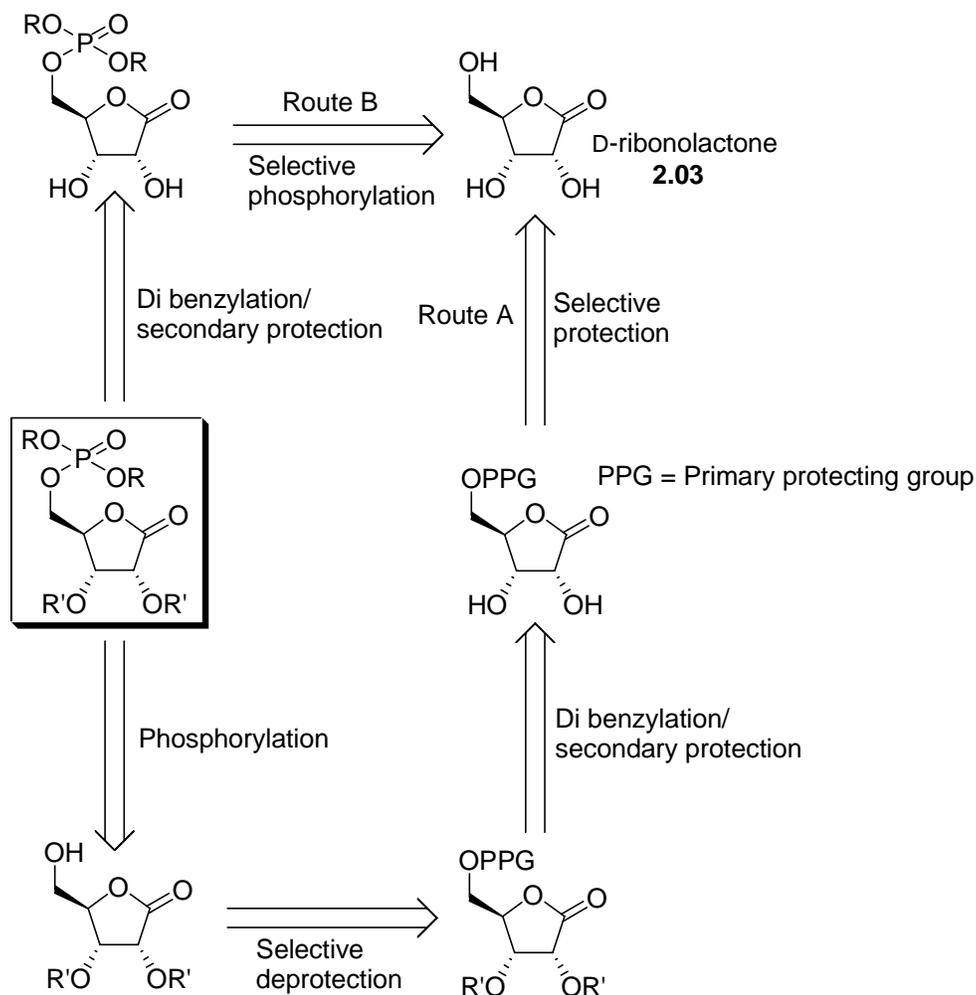


2.1.2. Phosphorylation introduction:

It is commonly found that the introduction of functionality at the C5-hydroxyl group, in this case introduction of a phosphate group, on five-carbon lactones or carbohydrates is done *via* a protection/deprotection strategy. This involves selective protection of the less hindered primary hydroxyl group (scheme 2.02, route A).¹¹⁷ The bulky sterically demanding primary protecting groups (PPG) typically used are the *tert*-butyldiphenylsilyl (TBDPS) and triphenyl methyl (trityl, Tr) groups. Protection of the remaining secondary hydroxyl groups is followed by selective removal of the C5-hydroxyl PPG allowing manipulation of this free hydroxyl to the desired functionality. Removal of the secondary hydroxyl protecting groups after the desired C5-hydroxyl functionality forms is common, but since these hydroxyl groups need protection during

the reduction and reductive amination stages the protecting groups would be carried through subsequent reactions and removed at a later stage in the synthetic route. We initially chose to follow route B (scheme 2.02) since it is two steps shorter, would potentially provide a higher overall yield and was only partially developed in the literature as explained in section 2.1.3.

Scheme 2.02. Phosphorylation route options.



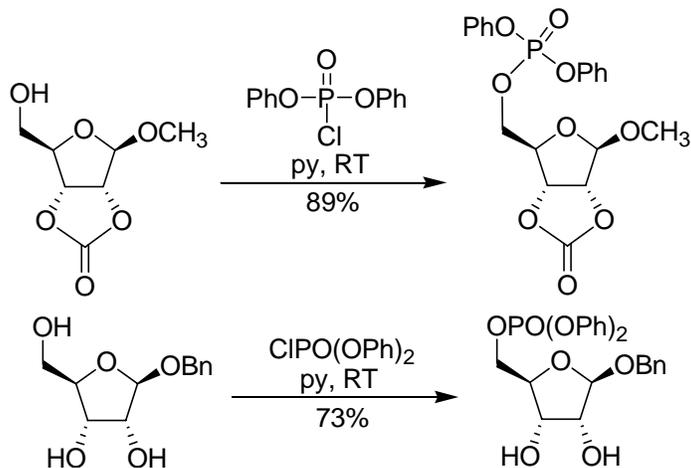
2.1.3. Literature background on phosphorylations:

Due to the importance of the phosphate group on PRA and CdRP as a potential anchor to the enzyme active site, the following will briefly describe some of the current synthetic literature procedures involved in phosphorylation.

Phosphorylating protected or unprotected lactones is rarely reported in literature. Phosphorylating five-carbon carbohydrates, however, is widely reported due to the fundamental importance of this moiety in biochemistry.¹¹⁸⁻¹²⁰ A plethora of conditions and reagents have been developed because of this. Typically the carbohydrate is in the form of a glycoside with secondary and primary hydroxyl groups unprotected. Selective phosphorylation on the C5-hydroxyl group has been developed and its selectivity is mainly attributed to the steric hindrance of the phosphorylating reagent. The report of Whiteside *et al.*¹²¹ is one of the few descriptions of this chemistry involving unprotected D-ribose. Formation of ribose-5-phosphate (R5P) in very low yields involved stirring D-ribose with phosphorus oxychloride in the presence of 2,6-lutidine in triethyl phosphate.¹²¹ Generally phosphorylating reagents fall into two categories depending on the phosphorus oxidation state, P(III) or P(V).

One of the first methods of converting a carbohydrate to a phosphate moiety using phosphorus(V) was developed by Tener and Khorana¹²² as shown in scheme 2.03. The simple substitution reaction obtains the desired phosphate in high yields. The use of diphenyl phosphorochloridate (phosphoryl chloride) as the phosphorylating reagent has the advantage of stability and commercial availability compared with other disubstituted phosphorochloridate reagents such as dibenzyl, which are unstable and require preparation before use.¹²³ A range of different Lewis bases can be used such as imidazole (im), pyridine (py) and 4-dimethylaminopyridine (DMAP).

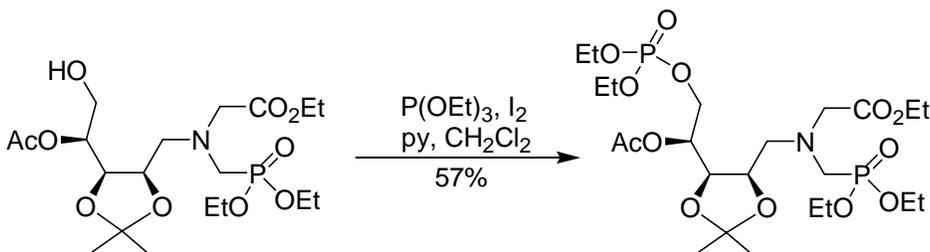
Scheme 2.03. Carbohydrate phosphate formation by use of diphenyl phosphorochloridate at room temperature (RT).¹²²



Another unique factor of the diphenyl phosphorochloridate is that the deprotection of the phenyl groups is selective and only possible with platinum, while palladium on carbon is ineffective.^{124,125}

A two-step reaction developed by Stowell and Widlanski¹²⁶ has led to a more reactive phosphorylating species. This involves the activation and oxidation of a trialkyl phosphate with molecular iodine giving rise to phosphoryl iodide, which is then added to a solution containing the carbohydrate and pyridine. The phosphoryl iodide acts in the same manner as phosphoryl chloride but is more reactive. It is mild enough to be used with a variety of functional groups as shown in scheme 2.04 by Walker and Parker.¹²⁷

Scheme 2.04. Phosphorylation by triethyl phosphate and iodine.¹²⁷

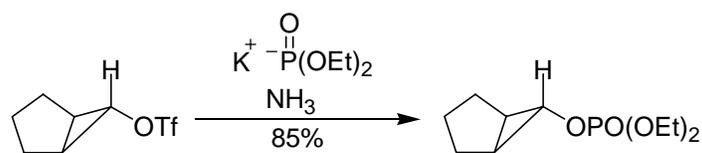


Using diphenyl phosphorochloridate and a Lewis base is a well-established method and has worked well previously in our laboratory,¹²⁸ however, difficulties in removing the phenyl protecting groups have led to the use of other phosphorylating reagents.¹²⁹ By using tetrabenzylpyrophosphate and *n*-butyllithium, Frost *et al.*¹²⁹ avoid using the unstable dibenzyl phosphorochloridate reagent and could deprotect the dibenzyl phosphate carbohydrate *via* hydrogenolysis using palladium on carbon.

Other methods involving the pyrophosphate moiety include adding the disubstituted phosphorochloridate to a solution of tributylammonium hydrobenzoin cyclic phosphate (THCP), and tributylamine as shown by Aimi *et al.*¹³⁰ The desired carbohydrate is then added to the reactive pyrophosphate moiety.¹³¹ This method, however, suffers from moderate yields and poor atom economy.

Phosphate production is not only limited to the reaction of electrophilic reagents with a nucleophilic hydroxyl group. Hilton *et al.*¹³² developed a method where potassium diethyl phosphite in liquid ammonia has been shown to substitute alkyl triflates and form phosphate esters (scheme 2.05).¹³² The phosphate ester product represents a formal oxidation of phosphorus.

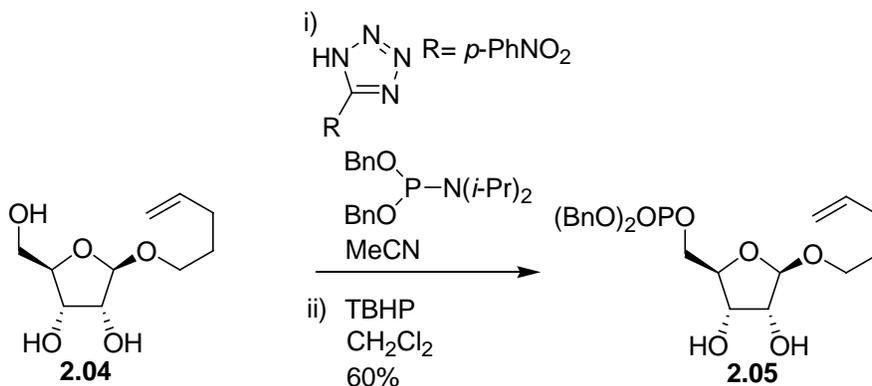
Scheme 2.05. Example of electrophilic phosphorylation.¹³²



Reports of phosphorylating reagents using phosphorus(III) have become frequent, mainly due to Beaucage and Caruthers¹³³ developing methods involving phosphoramidite in the early 1980s. In a recent example by Graham and Pope (scheme 2.06),¹¹⁷ the C5-hydroxyl of D-ribose **2.04** attacks the reactive tetrazole and phosphoramidite producing a phosphite triester, which is subsequently oxidised by *tert*-butyl hydroperoxide (TBHP) to the phosphate ester **2.05**. If recovery of starting D-ribose **2.04** is allowed for, then the yields improve further, exceeding 85%.¹¹⁷ A main point of difference between the

phosphoramidite and most phosphorus(III) phosphorylating methods is the removal of the need to rely on alkaline conditions to catalyse the reaction.

Scheme 2.06. Phosphorylation of C5-hydroxyl using the phosphoramidite method.¹¹⁷



Variations of the phosphoramidite method include changing the oxidant to *m*-chloroperoxybenzoic acid (*m*-CPBA) and the phosphate protecting groups to ethyl or *tert*-butyl.^{134,135}

2.1.4. Literature deprotection of phosphate esters:

As mentioned, the diphenyl and dibenzyl phosphate ester protecting groups can be removed by hydrogenolysis with H₂ with platinum and palladium on carbon respectively.^{124,125} Dimethyl and diethyl phosphate ester protecting groups can be cleaved using trimethylsilyl bromide (TMSBr) and NEt₃ in CH₃CN.¹³⁶ Similar deprotections of alkyl phosphate esters have been reported by employing NaI in acetone¹³⁷ or the generation of Me₃SiI *in situ* from Me₃SiCl and NaI in CH₃CN.¹³⁸⁻¹⁴¹

One of the problems we could foresee with the alkyl deprotection step is that the phosphate ester moiety could also be cleaved at the carbohydrate moiety. In terms of reactivity we believe this should follow cleavage of the phosphate ester alkyl groups. Taking care in ending the reaction before the phosphate ester has time to cleave is of up-most importance. Also there is a possibility, when using NaI in acetone, that after the first

alkyl group cleaves the resultant sodium salt may precipitate, thereby inhibiting the cleavage of the second ethyl group and yielding only the mono-protected rCdRP product **2.06** (figure 2.01). This has been discussed in literature previously.¹³⁷

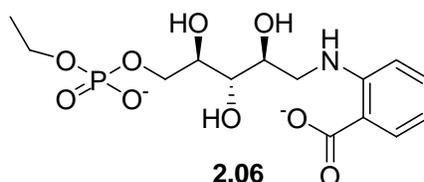


Figure 2.01. Mono-protected product.

2.2. Phosphorylation of D-ribonolactone:

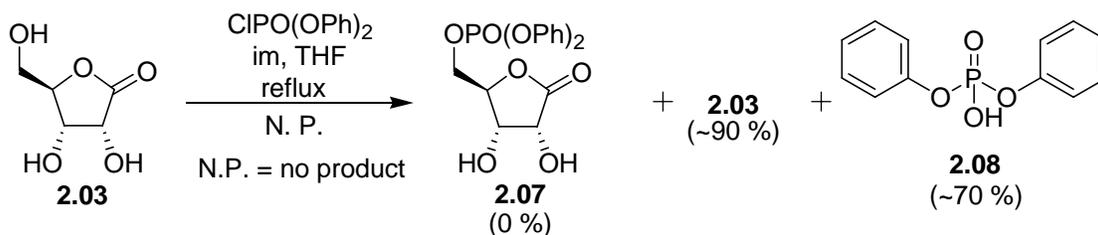
Following the retrosynthesis plan shown in scheme 2.02, route B, we went about attempting to synthesise D-ribonolactone-5-diphenyl phosphate **2.07**. With two unprotected secondary hydroxyl groups exposed, selectively phosphorylating the primary hydroxyl group of D-ribonolactone **2.03** was paramount. Diphenyl phosphorochloridate was selected for this task due to its sterically bulky phenyl groups. In a procedure based on work done by Tener and Khorana,¹²² D-ribonolactone **2.03**, imidazole and diphenyl phosphorochloridate were stirred in dry CH₂Cl₂ for several hours at room temperature. Thin layer chromatography (TLC) monitoring by spotting crude aliquots of reaction mixtures on silica plates proved difficult due to the poor solubility of **2.03** in CH₂Cl₂. The polar nature of **2.03** demanded a solvent or conditions that would improve solubility and allow reactivity with diphenyl phosphorochloridate. Keeping the other reaction condition parameters constant, the solvent was altered from CH₂Cl₂ to the more polar *N,N*-dimethylformamide (DMF). However, these conditions failed to produce the phosphorylated product **2.07**. A low *R_f* spot on TLC, however, was apparent unlike the reaction carried out in CH₂Cl₂. Upon work-up, this spot disappeared when the crude reaction mixture was washed with aqueous HCl. Concentrating the aqueous layer produced a cream coloured syrup, which was later determined to be the unreacted starting material **2.03** by ¹H NMR (D₂O). The organic phase of the work-up contained diphenyl phosphate **2.08**, the hydrolysis product of diphenyl phosphorochloridate. A ³¹P NMR

shift change from -4.1 ppm to -9.9 ppm was noted with the hydrolysis of diphenyl phosphorochloridate.

Changing the solvent to tetrahydrofuran (THF) did not produce phosphorylated product **2.07**. Neither did warming the reaction mixture to 40 °C over a period of several hours with or without the use of an ultrasonic bath. Increasing the ratio of phosphorylating reagent and imidazole or use of additional base in the form of pyridine also did not produce any phosphorylated product **2.07**. Finally after refluxing with a high ratio of phosphorylating reagent and imidazole to D-ribonolactone **2.03**, in ultrasonic conditions (scheme 2.07), a decision was made to explore other avenues to a phosphorylated precursor (scheme 2.02), since no product (N. P.) was formed.

It is likely the solvents used did not fully solubilise D-ribonolactone **2.03**, or produce conditions for reactivity with the phosphorylating reagent. This is evident by the crude yield of recovered starting material, D-ribonolactone **2.03** (~ 90 %, scheme 2.07). Approximate yield of diphenyl phosphate **2.08** (70%) was based on the number of moles of starting reagent, diphenyl phosphorochloridate. Although other phosphorylating procedures could have been attempted, it was obvious that phosphorylating D-ribonolactone **2.03** was not trivial. As there was also an ominous lack of reports in the literature of reductive aminations with phosphate groups present, we discarded the idea of firstly phosphorylating the C5 position of D-ribonolactone. This led to the protecting strategy shown in Scheme 2.02 route A being followed.

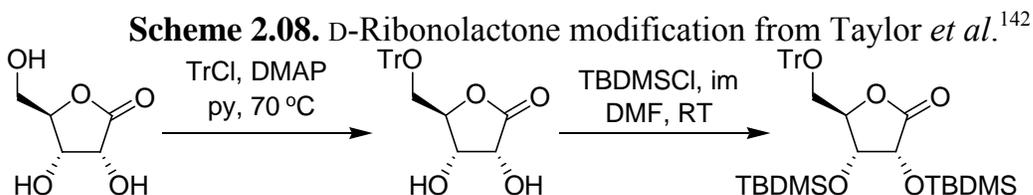
Scheme 2.07. Failed synthesis of D-ribonolactone-5-diphenyl phosphate **2.07**.



2.3. D-Ribonolactone modifications:

2.3.1. D-Ribonolactone protection literature background:

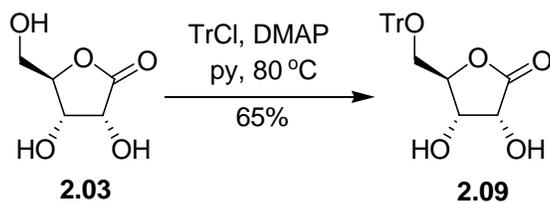
By incorporating a protection strategy (scheme 2.02, route A), it was vital that the inclusion and removal of the chosen protecting groups would proceed selectively and in a manner that did not interfere with functionality already present in the molecule. Numerous strategies were looked at until it was decided to follow the synthetic work of Taylor *et al.*¹⁴² In addition to the protection/deprotection selectivity the following reasons contributed to our decision: the protections were high-yielding, access to reagents was plentiful, Associate Professor Taylor was on site and the paper¹⁴² published on this work described a general synthesis strategy of D-ribonolactone, L-arabinonolactone, and L-lyxonolactone. This general strategy allowed us to protect different lactone configurations, which potentially could have led to a range of target compounds. Scheme 2.08 shows part of the synthesis involving D-ribonolactone.¹⁴²



2.3.2. D-Ribonolactone protections:

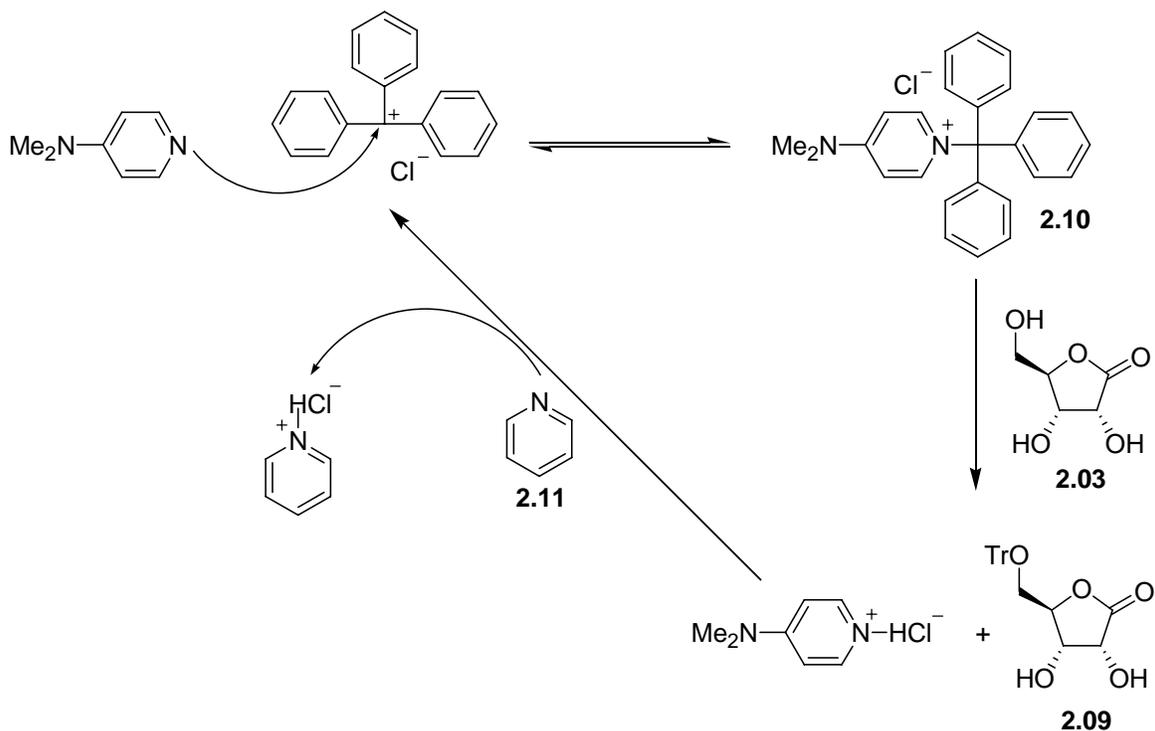
Following literature procedure,^{142,143} the primary hydroxyl was protected as a trityl ether, 5-*O*-tritylmethyl-D-ribonolactone **2.09** or *cis* diol (scheme 2.09). This was achieved by stirring a mixture of D-ribonolactone, DMAP and chlorotriphenylmethane (trityl chloride, TrCl) in dry pyridine for 17 hours at 80 °C. The maximum yield obtained was 65% under these conditions.

Scheme 2.09. Primary tritylation of D-ribonolactone.



Excellent selectivity for the primary hydroxyl over the secondary hydroxyl groups was displayed with no secondary protected material observed. This can be attributed to TrCl being sterically bulky, hence hindering reaction of the secondary hydroxyl groups. DMAP is known to increase TrCl steric bulk by forming the proposed intermediate **2.10** postulated by Chaudhary and Hernandez^{144,145} (scheme 2.10). Pyridine **2.11** is used to regenerate the DMAP catalyst.

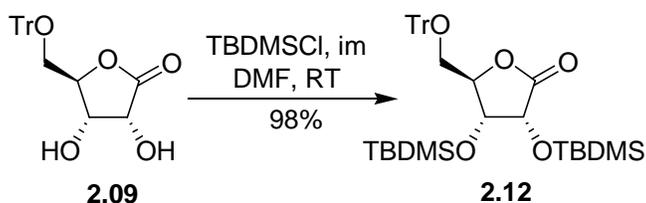
Scheme 2.10. Formation of intermediate **2.10** from catalytic DMAP.



Removing DMAP lowered the yield of the tritylated **2.09**, as did heating at 70 °C for 16 hours as described in literature.¹⁴²

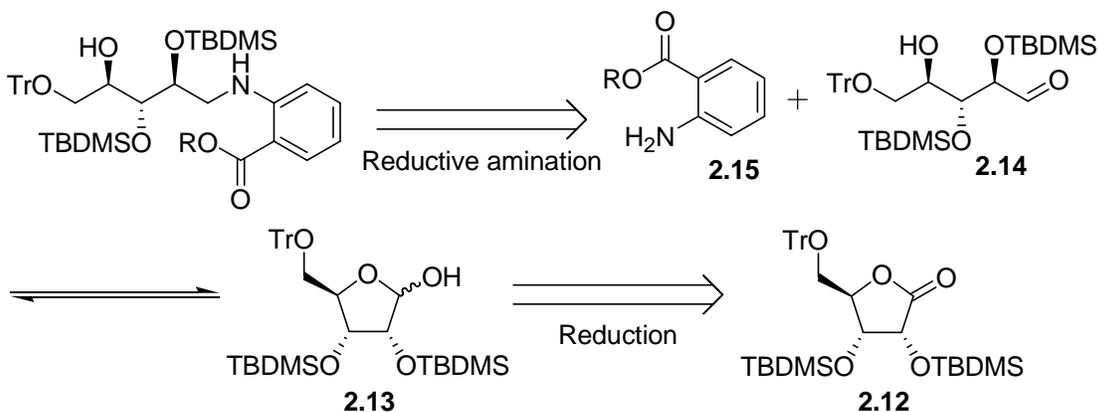
Disilyl protection¹⁴² (scheme 2.11) of the secondary alcohols was achieved using 3.4 equivalents of *tert*-butyldimethylsilylchloride¹⁴⁶ (TBDMSCl) mixed with *cis* diol **2.09**, and imidazole (5.4 equivalents) dissolved in DMF. This mixture was stirred for 66 hours at room temperature and monitored by thin layer chromatography (TLC), which showed *cis* diol **2.09** still present. A further 0.21 equivalents of TBDMSCl were added. The reaction mixture was stirred for a further 6 hours before work up. Isolated yields of the diprotected product were high and reproducible at over 95%.

Scheme 2.11. Disilylation of trityl **2.09**.



The disilyl TBDMS-protected lactone **2.12** needs to be reduced to form the lactol **2.13**, in order to access the open chain aldehyde **2.14**, allowing for reductive amination chemistry with esterified anthranilic acid **2.15** as shown in scheme 2.12.

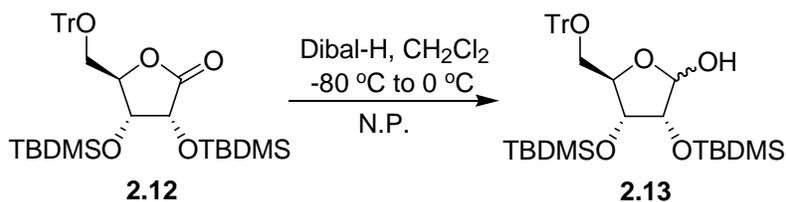
Scheme 2.12. Retrosynthesis for the reductive amination of lactol.



2.3.3. Di-*iso*-butylaluminum hydride reductions:

Standard di-*iso*-butylaluminum hydride (Dibal-H) reducing procedures¹⁴⁷⁻¹⁴⁹ were employed, such as adding the reducing agent slowly dropwise, at low reaction temperatures ($-78\text{ }^{\circ}\text{C}$) and in large equivalents. Unfortunately, reduction of the disilyl TBDMS-protected lactone **2.12** to the lactol **2.13** with Dibal-H proved to be more difficult than expected (scheme 2.13). At temperatures between $-80\text{ }^{\circ}\text{C}$ to $-45\text{ }^{\circ}\text{C}$ over 6-7 hour period the carbonyl moiety showed no sign of reduction even with eight additional equivalents of Dibal-H.

Scheme 2.13. Dibal-H reduction of disilyl protected lactone **2.12**.

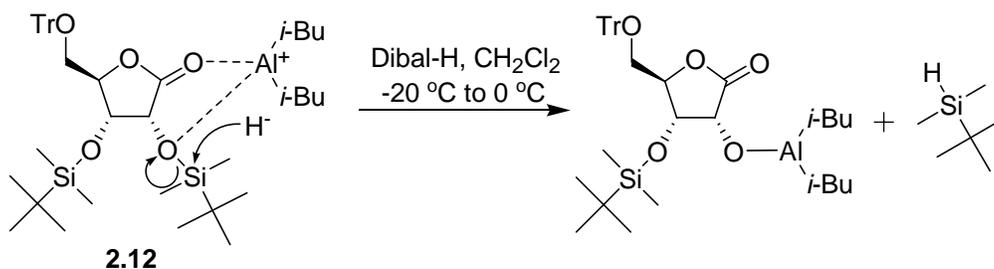


A fresh source of Dibal-H was acquired from a commercial supplier and the reduction was repeated under similar conditions. Increasing the reaction temperature was necessary since reductions at $-78\text{ }^{\circ}\text{C}$ were fruitless. Monitoring this reaction with TLC and NMR proved rewarding since it was apparent that the TBDMS protecting groups started to cleave off when the reaction was allowed to warm to around $-20\text{ }^{\circ}\text{C}$ and higher. No reduction of disilyl TBDMS-protected lactone **2.12** was evident. This could possibly be partially attributed to the lower solubility of disilyl TBDMS-protected lactone **2.12** at $-78\text{ }^{\circ}\text{C}$ to $-20\text{ }^{\circ}\text{C}$.

It is possible that this desilylation would lead to monosilyl ether or diol compounds (scheme 2.14), which in turn might be reduced from lactones to corresponding lactols as hinted by numerous polar TLC spots. A literature review of Dibal-H desilylation will follow in the next section. Migration of the TBDMS protecting group to neighbouring hydroxyl groups could also explain the number of spots in a slightly less polar position than the starting material **2.12** on the TLC plate. This has been observed by both Son and

Fleet,¹⁵⁰ and Weir and Taylor.¹⁵¹ Purification of these potential lactols and desilylation compounds, from other by-products would have proven difficult as shown by complex NMR spectra and numerous spots on the TLC plate.

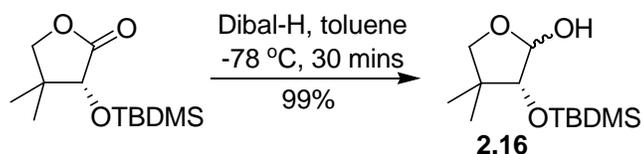
Scheme 2.14. Desilylation by Dibal-H.



2.3.4. Literature background of Dibal-H reductions and silyl ethers:

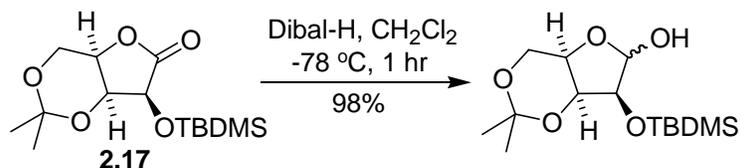
There are several high yielding Dibal-H reductions in the literature of silyl-protected lactones to silyl-protected lactols. Conditions include the use of toluene as the solvent, such work done by Ley *et al.*¹⁵² producing near-quantitative yields of lactol **2.16** in a relatively short reaction time (scheme 2.15).

Scheme 2.15. Dibal-H reduction by Ley *et al.*¹⁵²



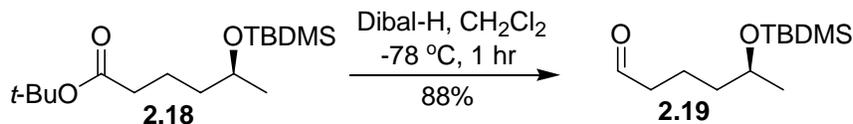
Ireland and Wilcox¹⁵³ had success using CH₂Cl₂ on the acetal moiety **2.17** (scheme 2.16).

Scheme 2.16. Dibal-H reduction by Ireland and Wilcox.¹⁵³



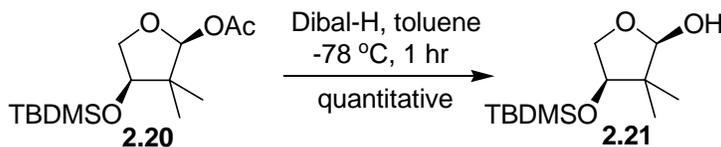
Under similar conditions to those in scheme 2.16, Pamies and Backvall¹⁵⁴ reduced the *tert*-butyl ester **2.18** to the aldehyde **2.19** (scheme 2.17).

Scheme 2.17. Dibal-H ester reduction by Pamies and Backvall.¹⁵⁴



Yamada *et al.*¹⁵⁵ provided another example of ester reduction, this time of the acetate **2.20** to the aldehyde **2.21** in quantitative yield (scheme 2.18). Toluene was the solvent.

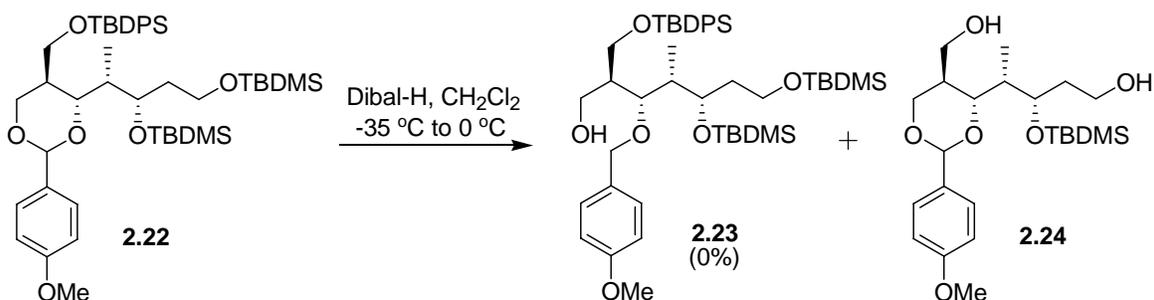
Scheme 2.18. Dibal-H acetate reduction by Yamada *et al.*¹⁵⁵



Overall the ratio of Dibal-H equivalents compared with the lactone/ester starting material was no more than 1.5 in the above examples, with the notable exception of the sterically bulky lactone **2.17** of Ireland and Wilcox,¹⁵³ which required three equivalents (scheme 2.16). All four examples kept stringent control of their reaction temperature at -78 °C and all proceeded at a relatively fast rate in high yield.

Selective deprotection by Iqbal *et al.*¹⁵⁶ of acetal **2.22** with Dibal-H at temperatures ranging from -35 °C to 0 °C did not form the free primary hydroxyl **2.23** (scheme 2.19). Instead an unexpected desilylation of both TBDMS protecting groups led to the diol **2.24**, possibly due to the higher temperatures used. Yields of the diol **2.24** were not given.

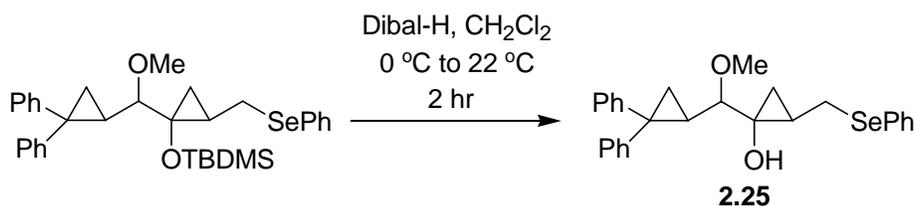
Scheme 2.19. Dibal-H acetal reduction by Iqbal *et al.*¹⁵⁶



Further literature searching provided strong evidence that TBDMS protecting groups are not necessarily stable in reducing media. Typically silyl ethers are removed with fluoride ions or with aqueous acid.^{124,125,146,157} There are now incidental examples of removal of the TBDMS protecting group by exposure to NaH,¹⁵⁷ LiAlH₄,¹⁵⁷ Dibal-H,¹⁵⁸ or DDQ.¹⁵⁷

Corey and Jones¹⁵⁸ developed a method to specifically cleave TBDMS protecting groups using Dibal-H. This involved using three equivalents of Dibal-H, CH₂Cl₂ and, critically, reaction temperatures around 23 °C. Sporadic reports of using this procedure specifically include Xu and Newcomb¹⁵⁹ (scheme 2.20), and Bening and Willis.¹⁶⁰ Xu and Newcomb¹⁵⁹ found that removal of TBDMS protecting groups with fluoride under a variety of conditions resulted in fragmentation of the cyclopropyl ring. Using the method of Corey and Jones¹⁵⁸ method gave acceptable yields of the secondary hydroxyl **2.25**. Bening and Willis¹⁶⁰ extended the Corey and Jones¹⁵⁸ method to successfully cleave terminal trimethylsilyl protecting groups.

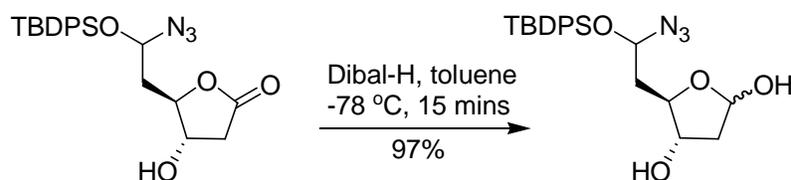
Scheme 2.20. Dibal-H desilylation by Xu and Newcomb¹⁵⁹



Note that there has been no report of cleavage of TBDMS protecting groups on lactones using Dibal-H.

It is possible that by increasing the stability of the silyl protecting group from TBDMS to TBDPS (*tert*-butyldiphenylsilyl) cleavage would not occur. Gorrichon *et al.*¹⁶¹ have reduced a lactone with Dibal-H in the presence of TBDPS protecting groups (scheme 2.21).

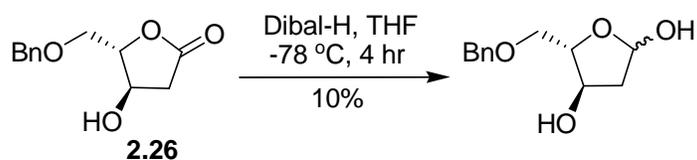
Scheme 2.21. Dibal-H reduction in the presence of TBDPS.¹⁶¹



A literature search has not provided any information on TBDPS protecting group stability at temperatures similar to Corey and Jones¹⁵⁸ TBDMS cleavage method. Changing the protecting strategy to remove the need to use silyl-protected lactones is another consideration, as is using a different reducing agent.

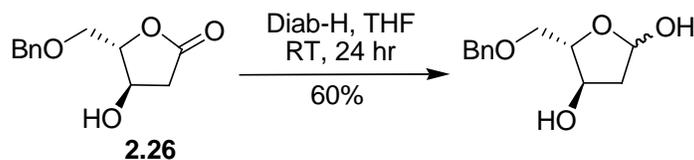
Fazio and Schneider^{162,163} found Dibal-H reduction for benzyl (Bn)-protected lactone **2.26** to be low yielding, even at -50 °C for several hours (scheme 2.22).

Scheme 2.22. Dibal-H reduction for benzyl-protected lactone **2.26**.^{162,163}



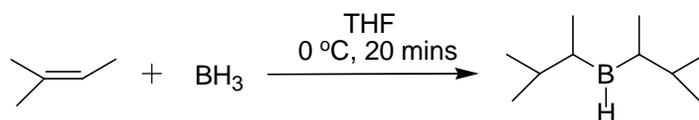
This led to disiamylborane¹⁶⁴⁻¹⁶⁷ (Diab-H) being used as the reducing agent. The reaction progressed with three equivalents of Diab-H, in THF, for duration of 24 hours (scheme 2.23). Yields were greatly improved compared with the Dibal-H reduction. However, a literature search has not provided any information on TBDMS protecting group stability in relation to Diab-H.

Scheme 2.23. Diab-H reduction for benzyl-protected lactone **2.26**.^{162,163}



Diab-H is not commercially available, but can be easily prepared *in situ* from BH_3 and 2-methyl-2-butene (scheme 2.24),¹⁶² making it a viable option in our reduction of lactones.

Scheme 2.24. Diab-H preparation.¹⁶²



Clearly the conditions used in the reduction of disilyl TBDMS-protected lactone **2.12** induce cleavage of TBDMS protecting groups. Synthesis of a protected lactol would have to come about by:

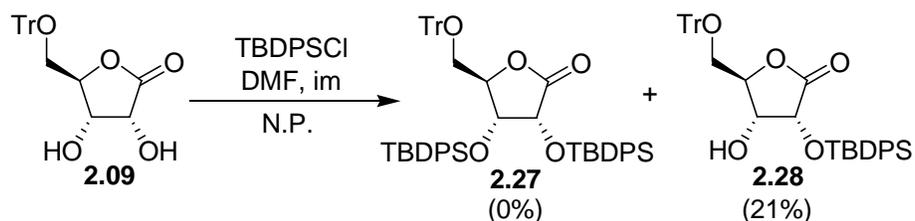
- (i) finding suitable secondary protecting groups that are stable to Dibal-H reduction at temperature above -78 °C;
- (ii) using a different reducing agent;
- (iii) finding a solvent, which increases the solubility of disilyl TBDMS-protected lactone **2.12** at -78 °C.

2.3.5. Alternative D-ribonolactone protections:

Increasing the steric bulk around the silyl groups by using TBDPSCl on *cis* diol **2.09** would give more robust protecting groups (scheme 2.25). Unfortunately the bulky phenyl groups on TBDPSCl proved too sterically demanding for the *cis* diol **2.09** to be successfully diprotected to form the protected lactone **2.27**. Mono TBDPS protected lactone **2.28** was formed due to the C2 hydroxyl's hydrogen having increased acidity from the nearby lactone carbonyl. Alternative protecting groups for the *cis* diol **2.09** were

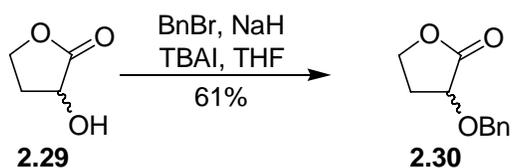
considered, such as benzylidene or *iso*-propylidene groups, but protecting with alkyl groups, specifically benzyl seemed the next logical method, due to the availability of reagents.

Scheme 2.25. TBDPSCI protection of diol **2.09**.



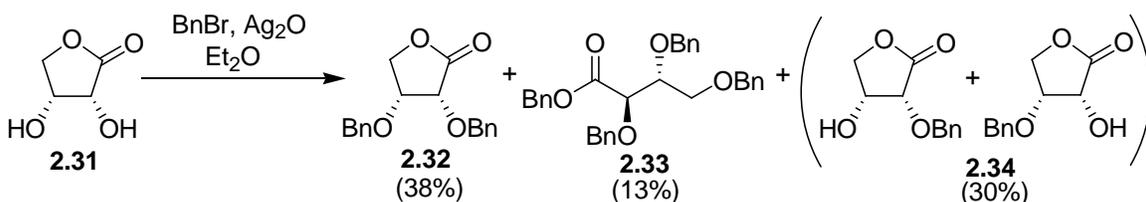
It has been shown by Motherwell *et al.*¹⁶⁸ that racemic α -hydroxy- γ -butyrolactone **2.29**, can be protected at room temperature using benzyl bromide (BnBr) and sodium hydride (NaH) in the presence of a catalytic quantity of tetrabutylammonium iodide (TBAI) (scheme 2.26). The resulting α -benzyloxy- γ -butyrolactone **2.30** was then reduced to the corresponding lactol with Dibal-H.¹⁶⁸

Scheme 2.26. Lactone benzylation using BnBr and NaH.¹⁶⁸



Silver oxide (Ag_2O) and MeI are another combination of reagents used in alkylations, this time to methylate lactone **2.29** in high yield, as shown by Gill *et al.*¹⁶⁹ A C5 hydroxyl of a similar lactone moiety was methylated in high yield by Ireland *et al.*¹⁷⁰ The only literature example of a lactone containing a *cis* diol similar to our *cis* diol **2.09** that was benzylated, was the work done by Luke *et al.*¹⁷¹ D-Erythronolactone **2.31** was only partially dibenzylated **2.32**, with a mixture of by-products being isolated, including the oxidised ester **2.33** and a mixture of monobenzylated lactones **2.34** (scheme 2.27).

Scheme 2.27. *Cis* diol benzylation using BnBr and Ag₂O.



With the Motherwell *et al.*¹⁶⁸ and the Luke *et al.*¹⁷¹ work in mind, various reagents, equivalents (Eq) of activators, solvents and methods were used as shown in table 2.01, in order to find the best conditions for the formation of the dibenzylated lactone **2.35** (scheme 2.28). Reaction 1 (rxn 1) in table 2.01 failed to produce any product of any kind or allow for the recovery of the starting material. After the addition of sodium hydride (NaH) to the *cis* diol **2.09**, TLC plates began almost immediately to show streaking of the starting material. NMR spectra of the crude aliquots of the reaction solution showed benzyl bromide (BnBr) and a multitude of ¹H peaks around 1.4 ppm to 4.0 ppm. It was thought that perhaps NaH had interfered with the lactone moiety, which Pedersen *et al.*¹⁷² briefly allude to. Hence, freshly precipitated silver oxide (Ag₂O) was used (rxn 2-6).

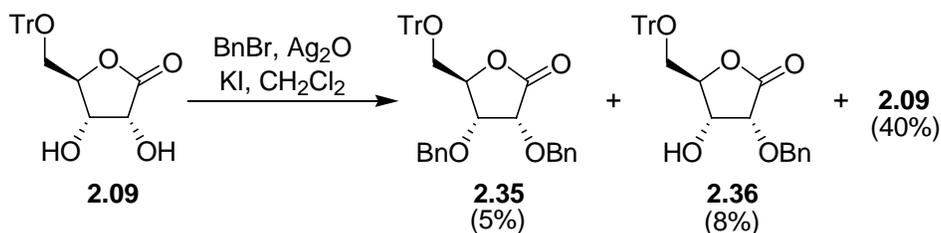
Rxn #	Activator	Eq	Cat	Reagent	Eq	Solvent	Duration	Yield 2.35	Yield 2.36	Yield 2.09
1 ¹⁶⁸	NaH	4.6	KI	BnBr	2.8	THF	4 days	0%	0%	0%
2 ¹⁷¹	Ag ₂ O	3.2	KI	BnBr	3.2	CH ₂ Cl ₂	28 hrs	Trace	5%	24%
3 ¹⁷¹	Ag ₂ O	3.2	KI	BnBr	3.2	DMF	11 hrs	Trace	11%	25%
4 ¹⁷¹	Ag ₂ O	4.6	KI	BnBr	3.8	DMF	4 hrs	Trace	11%	53%
5 ¹⁷¹	Ag ₂ O	4.6	KI	BnBr	3.8	CH ₂ Cl ₂	8.5 hrs	5%	8%	40%
6 ¹⁷¹	Ag ₂ O	4.6	KI	BnBr	3.8	DMF	8 hrs	Trace	12%	33%
7 ¹⁷¹	Ag ₂ O	4.6	KI	BnBr	3.8	DMF	24.5 hrs	3%	15%	13%
8 ^{172,173}	TfOH	0.1		BnOC (=NH)CCl ₃	3.2	Et ₂ O	16 hrs	3%	12%	73%

References: ^{168,171-173}

Table 2.01. Conditions used in benzylation reactions.

Finding conditions to produce an acceptable yield was not easy. This was the case using either DMF or CH₂Cl₂ as the reaction solvent. The starting material, *cis* diol **2.09**, was isolated in all of the reactions 2 to 7, with silver oxide. Note all attempts at benzylation (rxn 1-8) were done at room temperature.

Scheme 2.28. Reaction 5 - the best conditions for the formation of dibenzylated lactone **2.35**.



A mere 5% yield of the dibenzylated lactone **2.35** was isolated from a mixture of Ag₂O, potassium iodide (KI) and BnBr in CH₂Cl₂ (rxn 5). Even lower yields were recorded when DMF was employed as the solvent. The majority of the product isolated in all of the reactions 2 to 8 was the C2 monobenzylated **2.36**. The maximum yield of this was 15% achieved over 24.5-hour duration with Ag₂O, KI and BnBr in DMF (rxn 7). DMF almost exclusively formed monobenzylated **2.36**. Again the C2 position was the only hydroxyl group protected bar the small amount of dibenzyl product as indicated by the ¹H NMR shift of 4.07 ppm for C3 of monobenzylated **2.36** to 3.80 ppm for the dibenzylated lactone **2.35**. Sole monobenylation can be contributed to the lactone carbonyl increasing the acidity of the C2 hydroxyl groups hydrogen. The remaining material isolated was comprised of a complex mixture of starting materials that had decomposed and a multitude of decomposed by-products, as shown by TLC and NMR. Reducing the reaction time while using DMF (rxn 6) to eight hours lessened the decomposition of the product mixture, whereas lowering the reaction time further to four hours (rxn 5) led to virtually no dibenzylated lactone **2.35** and more monobenzylated **2.36** and unreacted *cis* diol **2.09**.

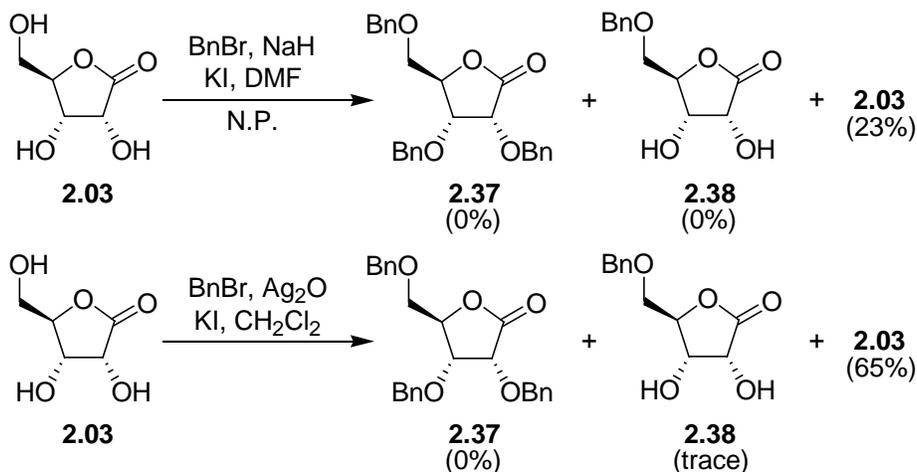
Benzyl trichloroacetimidate (BnOC(=NH)CCl₃)¹⁷²⁻¹⁷⁴ catalysed by trifluoromethanesulfonic acid (TfOH) has been shown by Iversen and Bundle¹⁷³ to benzylate numerous

carbohydrates. Following their methodology gave little improvement over the Ag₂O procedures; however, nearly all of the starting material, *cis* diol **2.09**, was isolated or accounted for.

Due to the low yield of dibenzylated lactone **2.35**, the subsequent Dibal-H reduction was not attempted. After benzylidene and *iso*-propylidene protections (section 2.3.6) the focus of my thesis shifted to other areas, where it was later discovered that under strong alkaline conditions, lactones isomerise and perform elimination reactions.¹⁷² Pedersen *et al.*¹⁷² has shown that lactone moieties can be benzylated with benzyl trichloroacetimidate in the presence of an acid catalyst, but, in order to achieve this, it is necessary to use 2-2.5 equivalents of benzyl trichloroacetimidate per hydroxyl group. A decision was made not to revisit lactone protections using this improved method.

Two attempts to tribenzylate D-ribonolactone **2.03** were run concurrently with the dibenzylations (scheme 2.29). These reactions were based on work by Barker and Fletcher¹⁷⁵ on tribenzylating furanosides. Due to solubility issues of D-ribonolactone **2.03** in both CH₂Cl₂ and DMF, the reactions were placed in an ultrasonic bath and warmed to 40 °C. After 4 hours both CH₂Cl₂- and DMF-based reactions were quenched with H₂O, and worked up by standard methods including washing the diluted reaction solution with a further addition of H₂O. By NMR characterisation, the aqueous layers for both reactions contained the starting material D-ribonolactone **2.03**. No evidence for the tribenzylated D-ribonolactone **2.37** was found by TLC or NMR analysis before or after workup for both reactions. A small spot, however, with a slightly higher *R_f* than D-ribonolactone **2.03** was seen on TLC when using Ag₂O. The ¹H NMR spectrum was consistent with this compound being 5-*O*-benzyl-D-ribonolactone **2.38** due to the change of the C5' hydrogen's chemical shift from 3.87 ppm in D-ribonolactone **2.03** to 3.49 ppm with the benzyl group attached. The quantity obtained was so small, however, that full characterisation of 5-*O*-benzyl-D-ribonolactone **2.38** was not possible.

Scheme 2.29. Attempts at tribenzylating D-ribonolactone **2.03**.



Protecting the secondary hydroxyl groups of the *cis* diol **2.09** or D-ribonolactone **2.03** with independent protecting groups proved frustratingly difficult. The lactone moiety was intolerant to strong alkaline conditions and the C3 hydroxyl group was particularly less reactive than the C2 hydroxyl group. A way to overcome these problems was to use hydroxyl-protecting groups that;

- (i) could be added under non-alkaline conditions, or
- (ii) were specifically designed for diol protection.

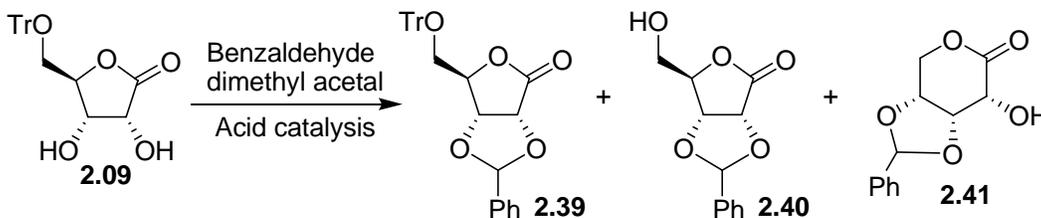
Benzylidene acetals fulfill both of these conditions.

2.3.6. Benzylidene and *iso*-propylidene acetal D-ribonolactone protections:

Two common reagents are used in benzylidene acetal acid-catalysed protections, benzaldehyde^{151,176-179} and benzaldehyde dimethyl or diethyl acetal.^{176,180} Initially we chose to employ a similar method to Ortuno *et al.*,¹⁷⁷ refluxing benzaldehyde dimethyl acetal, the *cis* diol **2.09** and a catalytic amount of *p*-toluenesulfonic acid (*p*TSA) in CH₂Cl₂. Unexpectedly the trityl group was cleaved in the presence of the *p*TSA, as shown by the trityl-protected C5-hydroxyl hydrogen signal disappearing in the ¹H NMR spectrum (at 3.43 ppm) and TLC spots forming at lower *R_f* than for the *cis* diol **2.09**.

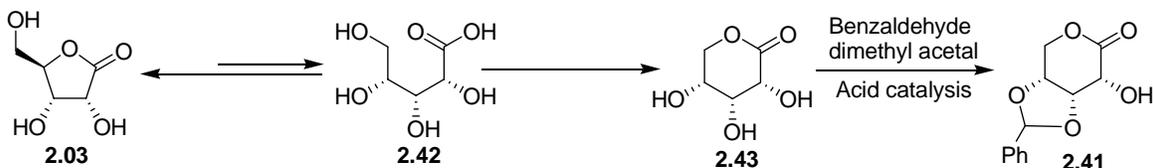
Complicated NMR spectra and numerous spots on the TLC plate, discouraged the isolation of the potential trityl benzylidene acetal **2.39** product and by-products (scheme 2.30). These could have included the furanose lactone acetal **2.40** and pyranose lactone acetal **2.41**.

Scheme 2.30. Potential benzylidene acetal products.



We chose the trityl-protected *cis* diol **2.09** to form the acetal rather than the C5'-hydroxyl unprotected *D*-ribonolactone **2.03** due to the latter's propensity to ring-open under acid conditions forming the carboxylic acid **2.42**, which can ring-close forming either the furanose or pyranose lactone **2.43** (scheme 2.31).^{177,181,182} The pyranose lactone **2.43** could potentially form the pyranose lactone acetal **2.41**.

Scheme 2.31. Unprotected C5 hydroxyl group leads to acid-catalysed rearrangement.

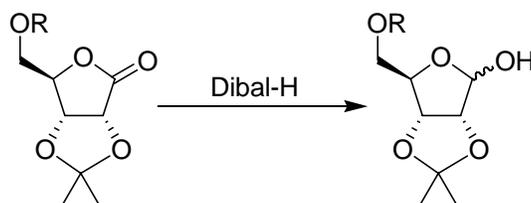


Following the same conditions, except exchanging *p*TSA with camphorsulfonic acid (CSA),¹⁷⁶ which has a higher pK_a ,¹⁸³ again resulted in a mixture of products (scheme 2.30). A ^1H NMR spectrum of the crude material indicated that the trityl group on *cis* diol **2.09** was less labile in these conditions, with some starting material still present as well as some probable product, trityl benzylidene acetal **2.39**. Although no product was isolated and the ^1H NMR spectrum obtained is neither quantitative nor definitive, trityl

benzylidene acetal **2.39** formation can be deduced by an upfield shift of the C2 and C3 protons.

The formation of *iso*-propylidene acetals occurs in non-alkaline conditions and is specifically designed for diol protection. It is well documented that *iso*-propylidene acetal D-ribonolactone moieties can be successfully reduced.^{178,184} This includes Dibal-H reductions shown in scheme 2.32.^{185,186}

Scheme 2.32. Dibal-H reduction of *iso*-propylidene acetal D-ribonolactone moieties.



Key: R = CH₂OCH₃, CH₂OCH₂CH₂OCH₃, TBDMS, Si-*i*-Pr₃¹⁸⁵ or Tr.¹⁸⁶

With this in mind, it was decided to form 2,3-*O*-*iso*-propylidene-D-ribo-1,4-lactone **2.44**, by treating D-ribonolactone **2.03** with 2,2-dimethoxypropane (DMP) in the presence of various acid catalysts (scheme 2.33).

Scheme 2.33. Potential *iso*-propylidene acetal products.

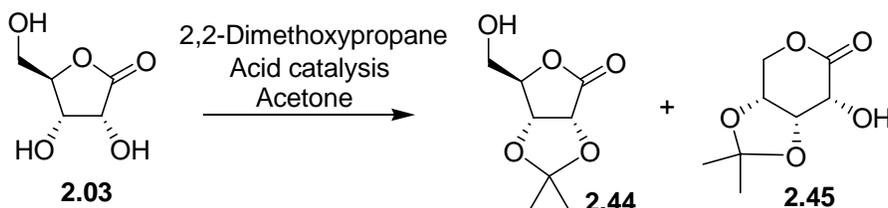


Table 2.02 shows the various acid catalyst and reaction conditions that were employed for the formation of the acetal. Ultimately, it was found that reaction 4, a boron trifluoride etherate-catalysed reaction produced the highest yields of 2,3-*O*-*iso*-propylidene-D-ribo-1,4-lactone **2.44**. Limiting the quantity of acid present was crucial in reducing the formation of 3,4-*O*-*iso*-propylidene-D-ribo-1,5-lactone **2.45**, shown by higher yields in

reaction 5 and 8 where conditions were more acidic than in other reactions. It was also found that unreacted starting material, D-ribonolactone **2.03**, was recovered as shown by its isolation after reaction 2 in good quantity (55%). This essentially makes the formation of 2,3-*O*-*iso*-propylidene-D-ribo-1,4-lactone **2.44** high yielding.

Rxn #	Activator	Eq	Reagent	Eq	Solvent	Duration	Temp	Yield 2.44	Yield 2.45
1 ¹⁸⁷	<i>p</i> TSA	0.3	DMP	1.5	Acetone	14+7 hrs	rt+reflux	17%	Trace
2 ¹⁸⁷	<i>p</i> TSA	0.3	DMP	1.5	Acetone	36 hrs	rt	34%	Trace
3 ¹⁸⁷	<i>p</i> TSA	0.1	DMP	3.6	Acetone	22 hrs	rt	51%	0%
4 ¹⁸⁸	BF ₃ .Et ₂ O	0.1	DMP	3.0	Acetone	3.25 hrs	rt	54%	Trace
5 ¹⁸⁸	BF ₃ .Et ₂ O	1.0	DMP	3.3	Acetone	2 hrs	0 °C	35%	23%
6 ^{177,189}	HCl	1.2	Acetone		Acetone	20 hrs	rt	16%	0%
7 ^{177,189}	HCl	1.8	Acetone		Acetone	21 hrs	rt	31%	Trace
8 ^{177,189}	HCl	2.2	Acetone		Acetone	22 hrs	rt	15%	10%

References: ^{177,187-189}

Table 2.02. Conditions used in *iso*-propylidene acetal reactions.

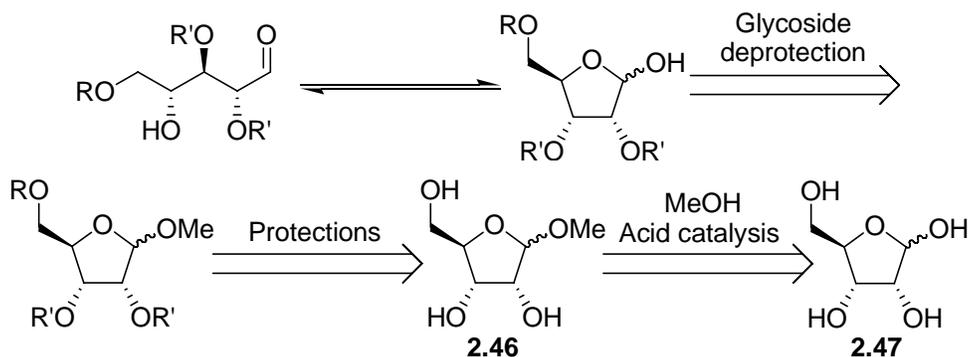
The next logical step would be to follow literature protections^{185,186} for the C5 hydroxyl group and the subsequent Dibal-H reduction. However, it was decided to investigate:

- (i) the preparation of a suitable ribose derivative, which avoids the trouble of reducing a lactone to a lactol since C1 of ribose is already in the correct oxidation state;
- (ii) model reductive amination reactions.

2.4. D-Ribose modifications:

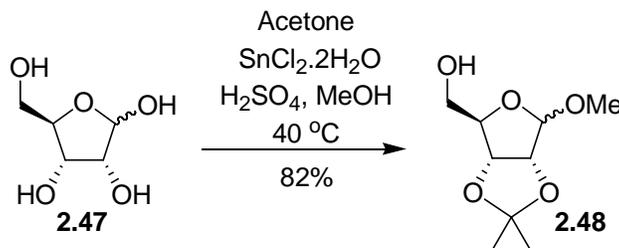
D-Ribose can be transformed into a moiety ready for reductive amination by firstly methylating its C1 anomeric hydroxyl (scheme 2.34) then protecting its primary hydroxyl with a primary selective protecting group such as TBDPS or trityl. The secondary protections are put in place to minimise the possibility of potentially interfering side reactions during the reductive aminations. TBDMS groups or *iso*-propylidene acetals could be used to protect the secondary alcohols. The lack of a lactone moiety may allow the use of NaH as a base for benzylation protections.

Scheme 2.34. Retrosynthesis of protected lactol *via* D-ribose methyl glycoside.



Synthesis of ribose methyl glycoside **2.46** *via* a known procedure¹⁹⁰ by stirring D-ribose **2.47** in dry methanol with acetyl chloride ultimately failed. Recovery of most of the starting material D-ribose **2.47** and little of the product and its isomers possibly points towards the reactivity of acetyl chloride and its freshness. Acetyl chloride is used to generate catalytic amounts of HCl in the solution. It would also have been plausible to use small amounts of concentrated H₂SO₄ or HCl but synthesis of 2,3-*O*-*iso*-propylidene-β-D-ribofuranoside **2.48**^{191,192} (scheme 2.35) seemed more advantageous.

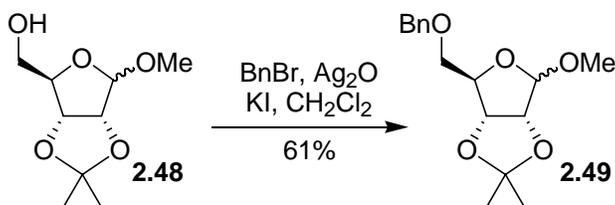
Scheme 2.35. One-step formation of 2,3-*O*-*iso*-propylidene- β -D-ribofuranoside **2.48** from D-ribose **2.47**.



A mixture of D-ribose **2.47** and tin(II) chloride (SnCl₂·2H₂O) was suspended in a solution of methanol:acetone with a catalytic amount of concentrated H₂SO₄ and heated at 45 °C for 20 hours. The reaction was high yielding (82%) and had no major by-products. Levene and Stiller¹⁹³ originally formed the protected ribofuranoside **2.48** by using the above procedure with anhydrous copper sulfate instead of tin(II) chloride. Tin(II) chloride was at hand so this was used as an alternative.

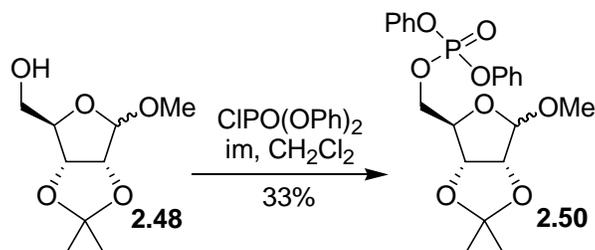
Benylation (scheme 2.36) of the primary hydroxyl group then followed using benzyl bromide, Ag₂O and KI in a solution of CH₂Cl₂, producing primary benzylated ribofuranoside **2.49** in reasonable yields (61%). The formation was evident by the slight up-field shift of the C5 hydrogen from 3.61 to the 3.50 ppm. Compared with the Ag₂O and benzyl bromide reactions involving lactone moieties (section 2.3.5), benzylating the protected ribofuranoside **2.48** was highly successful. Using the NaH benzylation procedure of Mootoo *et al.*¹⁹⁴ and Johansson and Samuelsson¹⁹⁵ yielded little of the benzylated ribofuranoside **2.49** (20%), however.

Scheme 2.36. Primary hydroxyl group benzylation.



Phosphorylation of the primary hydroxyl group (scheme 2.37) was also attempted using a procedure based on work by Tener and Khorana.¹²² This was primarily to get a first-hand understanding of phosphorylations. The reaction was clean with little by-product but the yield of the primary phosphorylated product **2.50** was low (33%). Starting material was recovered, and the yield of the phosphorylated compound based on reacted starting material was >85 %. A ³¹P NMR shift from -4.1 for the diphenyl phosphorochloridate reagent to -10.9 ppm for the phosphorylated product **2.50** was observed. In an attempt to increase yields, the reaction temperature was raised on two separate occasions to 45 °C and 80 °C. This produced a messy mixture of by-products as shown by the ¹H NMR spectra. No phosphorylated ribofuranoside **2.50** was isolated.

Scheme 2.37. Primary hydroxyl group phosphorylation.



Selectively demethylating the primary benzylated ribofuranoside **2.49** to produce the protected lactol would have proven time consuming and problematic regarding the selective cleavage of the methyl glycoside moiety over the *iso*-propylidene acetal. Therefore it was not attempted.

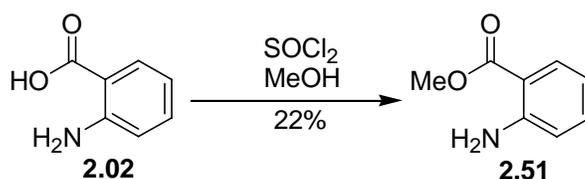
Methods developed in this section using D-ribose gave options other than D-ribonolactone to form an aldehyde precursor for reductive aminations with aryl amines.

During these glycoside-protecting reactions we carried out other experiments involving reductive aminations that suggested that reacting a lactol with an aryl amine, such as esterified anthranilic acid, would prove problematic. This work is discussed in section 2.7.

2.5. Esterification of anthranilic acid:

As previously discussed in section 2.1, the initial scheme for the formation of the target inhibitors involves a crucial reductive amination reaction in order to join the protected aliphatic aldehyde with the esterified anthranilic acid. There are a plethora of experimental conditions for esterification of aromatic acids.¹⁹⁶⁻¹⁹⁸ There are two general mechanisms for the conversion of carboxylic acids into esters: nucleophilic attack by an alcohol on the carboxyl carbon involving a tetrahedral intermediate or alkylation of the carboxyl oxygen. In the former, an acid catalyst must be employed to form a better leaving group out of the hydroxyl present. This activation allows the conversion of the acid to an acid halide, anhydride or thiol ester.¹⁹⁹ We chose to use and investigate the formation of an acid chloride *in situ* to produce a good leaving group in the form of a chloride ion for the carboxylic acid functional group on anthranilic acid. Formation of the acid chloride is typically achieved by using thionyl chloride (SOCl₂)¹⁹⁷ or phosphorus trichloride¹⁹⁶ as the chlorinating agent. It was found that using phosphorus trichloride produced very low yields (1-2%) of anthranilate methyl ester **2.51**, whereas initially using thionyl chloride (rxn 1, table 2.03) and following the methodology developed by Hosangadi and Dave¹⁹⁷ yields were increased somewhat to around 20% (scheme 2.38).

Scheme 2.38. Methyl esterification of anthranilic acid.



Various conditions were used in an attempt to increase esterification yields. The duration of the experiment did not seem to increase or decrease the yield dramatically (rxn 2 and 3), with longer reaction times giving a slightly lower yield of the methyl ester **2.51**, and an increase in production of other by-products. When shorter reaction times were used (rxn 2) no starting material was recovered.

Rxn #	Reagent added	Induction duration	Eq of SOCl ₂	Temp	Rxn duration	Yield
1 ¹⁹⁷	MeOH (1 st)		1.5	rt	3.5 hrs	22%
2 ¹⁹⁷	MeOH (1 st)		2.5	rt	1.5 hrs	17%
3 ¹⁹⁷	MeOH (1 st)		1.5	rt	4.5 hrs	20%
4 ¹⁹⁷	EtOH (1 st)		1.5	rt	3.5 hrs	22%
5 ¹⁹⁷	<i>i</i> -PrOH (1 st)		2.0	rt	3.5 hrs	3%
6 ^{a,197}	MeOH (1 st)		1.5	rt	3.5 hrs	29%
7 ¹⁹⁷	MeOH (2 nd)	3 hr	2.5	rt	4.5 hrs	35%
8 ¹⁹⁷	MeOH (2 nd)	3 hr	2.5	4 °C	4.5 hrs	40%
9 ^{b,197}	MeOH (2 nd)	3 hr	4.2	4 °C	12 hrs	46%

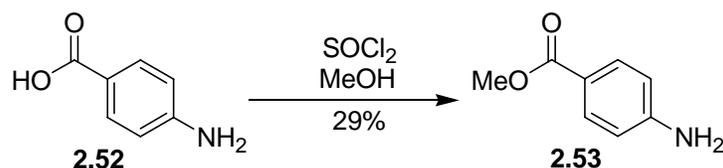
^a Carboxylic acid is 4-aminobenzoic acid.

^b Cooling of acid chloride present.

Table 2.03. Conditions used in esterification reactions.¹⁹⁷

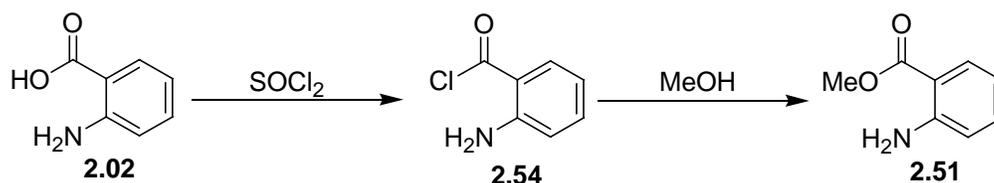
Following the same procedure as reaction 1, ethanol (EtOH) was used as the reagent producing anthranilate ethyl ester in similar yield (rxn 4). Increasing the steric hindrance of the esterifying reagent by the use of *iso*-propanol (*i*-PrOH) limited the formation of the corresponding ester (rxn 5). Removing the steric hindrance of the neighbouring amino group by using 4-aminobenzoic acid **2.52** gave the highest yields (29%) of all the esters using the prescribed method (rxn 6, scheme 2.39).¹⁹⁷ The methyl,^{200,201} ethyl²⁰² and *iso*-propyl esters,²⁰³ and 4-aminobenzoic methyl ester²⁰⁴ **2.53** synthesised have ¹H NMR spectra corresponding to literature. Overall this method reported by Hosangadi and Dave¹⁹⁷ was low yielding and difficult to reproduce in our hands.

Scheme 2.39. Methyl esterification of 4-aminobenzoic acid.



The lower yields than expected may be due to intramolecular hydrogen bonding owing to the *ortho*-substituted amine and possible amide formation. Another important factor contributing to low yields is that the alcohol solvent could be interfering with the thionyl chloride. Removing the alcohol reagent, and reacting thionyl chloride solely with anthranilic acid could eliminate this. Isolating the acid chloride **2.54** then adding in the desired alcohol reagent potentially could give rise to the anthranilate ester (scheme 2.40).

Scheme 2.40. Esterification *via* isolated acid chloride.



Allowing thionyl chloride to react with anthranilic acid **2.02** for 3 hours before adding methanol increased the yield (rxn 7). Keeping the temperature at 4 °C instead of room temperature also increased yields (rxn 8). Reaction 9 included cooling the acid chloride **2.54** to -30 °C before adding methanol. Ultimately, this reaction produced the highest yield (46%).

Other methods such as using chlorotrimethylsilane in methanol²⁰⁵ or ethanol in the presence of HCl²⁰⁶ could have been attempted in order to find a higher yielding way to form anthranilate methyl ester **2.51**. Formation of the methyl ester by the generation of diazomethane *in situ* was another possibility.^{199,207}

2.6. Introduction to reductive aminations:

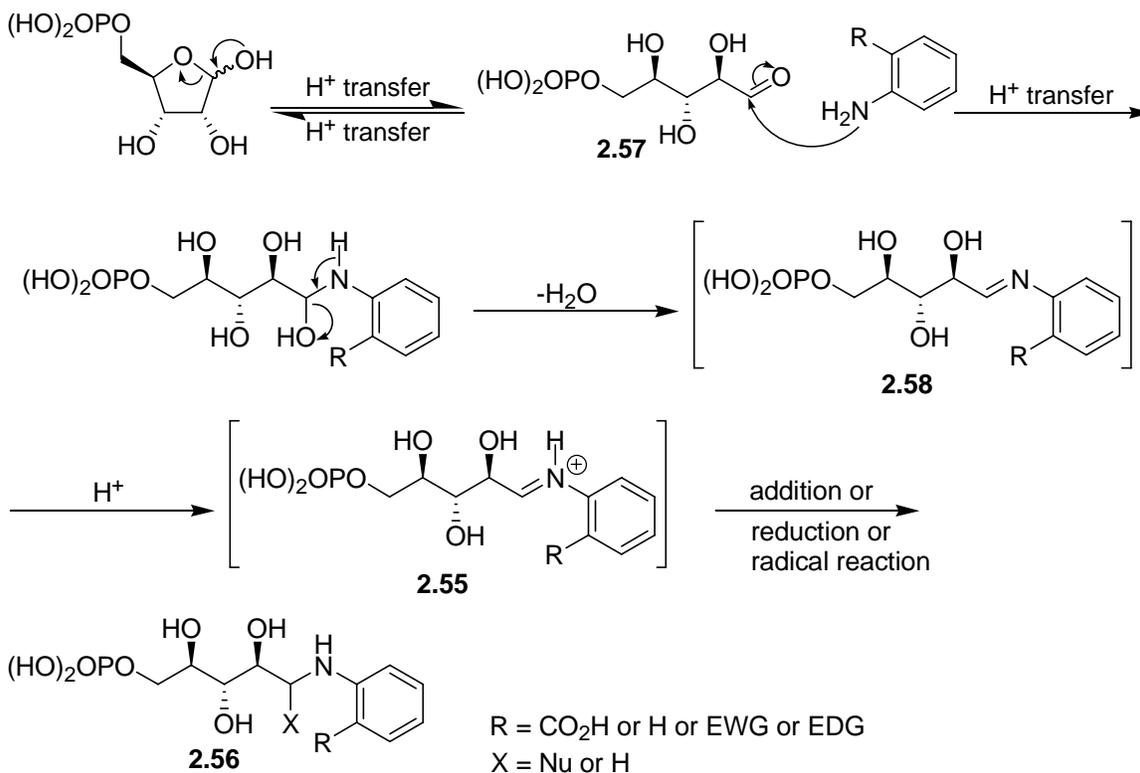
2.6.1. Reductive amination using metal catalysis:

The reactions of aldehydes or ketones with ammonia, primary amines or secondary amines in the presence of reducing agents to give primary, secondary or tertiary amines, respectively, known as reductive aminations (of the carbonyl compounds) or reductive

alkylations (of the amines) are among the most useful and important tools in the synthesis of different kinds of amines.^{57,108,110,208-216}

As mentioned in section 1.9.6, reduction of the iminium ion **2.55** produces the secondary amine **2.56** (scheme 2.41). For the overall process to succeed there needs to be chemoselectivity between the initial open-chain carbohydrate **2.57** and the iminium ion **2.55** during the reduction phase.^{108,110} Hence, the choice of the reducing agent is critical to the success of the reaction.^{108,110} The reducing agent also needs to be stable at conditions primed for iminium ion formation **2.55**.^{108,110}

Scheme 2.41. Imine formation and subsequent formation of secondary amine.



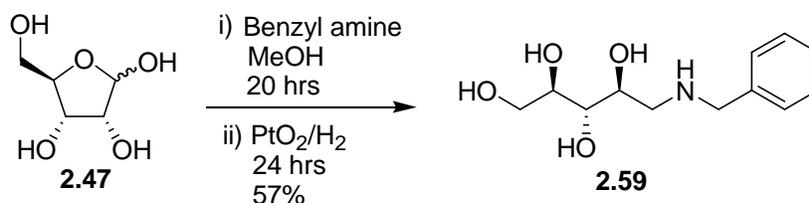
A reductive amination reaction is simply described as the direct reaction to form an amine product when the amine and carbonyl compound are mixed with a reducing agent in a single operation without prior formation of the intermediate imine or iminium ion.^{108,208} Separation of the imine **2.58** from the initial open-chain carbohydrate **2.57** and then reducing it in a separate step is a stepwise or indirect reaction. The stepwise reaction

eliminates the need for the reducing agent to discriminate between an initial open-chain carbonyl functionality of **2.57** and the imine **2.58**. Stork and Dowd²¹⁷ have developed a method to obtain more stable highly conjugated aryl imines but due to imine lability reports of direct reactions are more predominate in literature.

There are two commonly used direct reductive amination methods.¹⁰⁸ These differ in the nature of the reducing agent.¹⁰⁸ The first method is catalytic hydrogenation with platinum, palladium, or nickel noble metal catalysts.^{108,208} Generally this is an effective and economical method, particularly in large-scale reactions.¹⁰⁸ A mixture of products and low yields can result, however, depending on the molar ratio and the structure of the reactants.¹⁰⁸ Another restraint is the lack of selectivity when used with compounds containing carbon-unsaturated bonds and reducible functional groups such as nitro and cyano.^{108,208} Divalent sulfur is also known to inhibit the catalyst.¹⁰⁸

There are a number of examples in the literature of using noble-metal catalysts in the reaction of alkyl amines and carbohydrates to form secondary amines.^{108,208,218-221} Most are stepwise reactions. For example, an aryl amine D-ribose moiety **2.59** was formed in 57% yield when benzyl amine was added to a suspension of D-ribose **2.47** in methanol (scheme 2.42). After 20 hours, the clear solution was hydrogenated over platinum oxide for 24 hours.²¹⁹

Scheme 2.42. Platinum oxide use in indirect reductive amination.



2.6.2 Reductive amination using hydride reducing agents:

The second method utilises hydride reducing agents particularly sodium cyanoborohydride (NaBH₃CN) for reduction. In recent years there has been a flurry of

hydride reducing agents developed and clear move away from using the noble metal agents. NaBH_3CN ^{212,222-224} and sodium borohydride analogue reducing agents include: LiBH_3CN ,²²⁵ $\text{NaBH}_3\text{CN-ZnCl}_2$,²²⁶ $\text{NaBH}_3\text{CN-Ti(OiPr)}_4$,²²⁷ $\text{NaBH}_3\text{CN-Mg(ClO}_4)_2$,²²⁸ $(n\text{-Bu})_4\text{NBH}_3\text{CN}$,²²⁹ NaBH(OAc)_3 ,¹⁰⁸ $\text{NaBH}_4\text{-NiCl}_2$,^{230,231} $\text{NaBH}_4\text{-ZnCl}_2$,²³² $\text{NaBH}_4\text{-ZrCl}_4$,²³³ $\text{Ti(OiPr)}_4\text{-NaBH}_4$,²³³ and $\text{NaBH}_4\text{-H}_2\text{SO}_4$.²³⁴ Resins (borohydride exchange resin)²³⁵ and clays (NaBH_4 wet clay microwave)²³⁶ are now being employed.

The success and widespread use of NaBH_3CN is due to its stability in relatively strong acid solutions (pH 3), and its solubility in hydroxylic solvents such as methanol.^{108,222} It also has different selectivities at different pH values.^{108,222} At pH 3-4 it reduces aldehydes and ketones effectively, but this reduction becomes very slow at higher pH values.^{108,225} At pH 6-8, the more basic imines are protonated preferentially and reduced faster than aldehydes or ketones.^{108,222} This selectivity allows for a convenient and controlled direct reductive amination procedure.¹⁰⁸

Sodium cyanoborohydride and most reductive amination reducing agents have one drawback or another explaining why so many different reagents have been employed.²⁰⁸ Sodium cyanoborohydride is highly toxic and generates toxic by-products such as HCN and NaCN upon workup and may result in the contamination of the product with the toxic compounds.^{108,208,210,211,237} Other limitations of this reducing agent include requiring up to a five-fold excess relative to the amine,^{108,222} sluggish reactivities for the reductive amination of aldehydes with aniline moieties bearing electron-withdrawing groups and with weakly nucleophilic amines.^{227,238}

Sodium triacetoxyborohydride (NaBH(OAc)_3) in the presence of acetic acid eliminates some of the problems associated with reductive aminations of aniline moieties bearing electron-withdrawing groups.¹⁰⁸ It still has the drawback of being corrosive and of suffering from long reaction times with moderate yield.^{108,210}

Some of the problems with acidity and corrosiveness, which could hinder synthesis of acid-sensitive CdRP-protected analogues, have been circumvented by using Lewis-acid

catalysis with reagents such as $\text{NaBH}_3\text{CN-ZnCl}_2$,²²⁶ $\text{NaBH}_3\text{CN-Ti(OiPr)}_4$,²²⁷ $\text{NaBH}_4\text{-ZnCl}_2$,²³² $\text{NaBH}_4\text{-ZrCl}_4$,²³³ and $\text{Ti(OiPr)}_4\text{-NaBH}_4$.²³³ Some of these milder Lewis acids, ZnCl_2 , have also been shown to stabilise the hydride reducing agent in aqueous media.^{226,232}

One of the latest reductive amination reagents,²⁰⁸ sodium borohydride activated by boric acid, reacts in a solvent-free chemoselective manner and has been reported to reduce imines in the presence of other reducible functional groups, such as ketone, carboxylic acid, ester, nitrile, amide, nitro, furyl and alkenyl groups, to the corresponding functionalised amine compounds.^{208,211,236,239,240} This method is not only of interest from an ecological point of view, but also proves to be a clean, rapid and very simple procedure for the reduction of imine derivatives.²⁰⁸ It should be mentioned that some sodium borohydride reducing agents have been found to have poor discrimination between the aldehyde and the iminium ion.

A long list of alternative hydride sources include: $\text{Zn(BH}_4)_2$,²⁴¹ $\text{Zn(BH}_4)_2\text{-ZnCl}_2$,²⁴² $\text{Zn(BH}_4)_2\text{-SiO}_2$,²⁴³ Zn-AcOH ,²⁴⁴ polymethylhydrosiloxane PMHS-Ti(OiPr)_4 ,^{245,246} PMHS-ZnCl_2 ,²⁴⁷ PMHS-BuSn(OCOR)_3 ,²⁴⁸ $\text{Et}_3\text{SiH-CF}_3\text{CO}_2\text{H}$,²⁴⁹ $(\text{PhMe}_2)\text{SiH-(C}_6\text{F}_6)_3$,²⁵⁰ $\text{Cl}_3\text{SiH-DMF}$,²⁵¹ $\text{PhSiH}_3\text{-Bu}_2\text{SnCl}_2$,²⁵² $n\text{-Bu}_3\text{SnH-DMF}$ or HMPA ,^{216,253} $n\text{-Bu}_3\text{SnH-SiO}_2$ ²⁴⁰ and $n\text{-Bu}_2\text{SnIH}$ or $n\text{-Bu}_2\text{SnClH}$.^{254,255}

However, again most of these reagents have drawbacks. Tin hydride reagents are highly toxic and generate toxic by-products upon workup such as organotin compounds.²⁰⁸ Other hydrides such as zinc borohydride,^{208,256} nickel boride,²³¹ and PMHS-Ti(OiPr)_4 ²⁴⁶ may be unsuitable for use in the chemoselective reduction of imines having ketone, ester, amide and nitro groups, since these reagents can reduce those functional groups.²⁰⁸

Use of borane reducing agents is widespread: pyridine-borane,^{110,257} picoline-borane,²¹¹ diborane-MeOH,²⁵⁸ decaborane.²⁵⁹ Pyridine-borane (PB) works well for reactions using aryl amines as does picoline-borane but the reaction must be performed under acidic

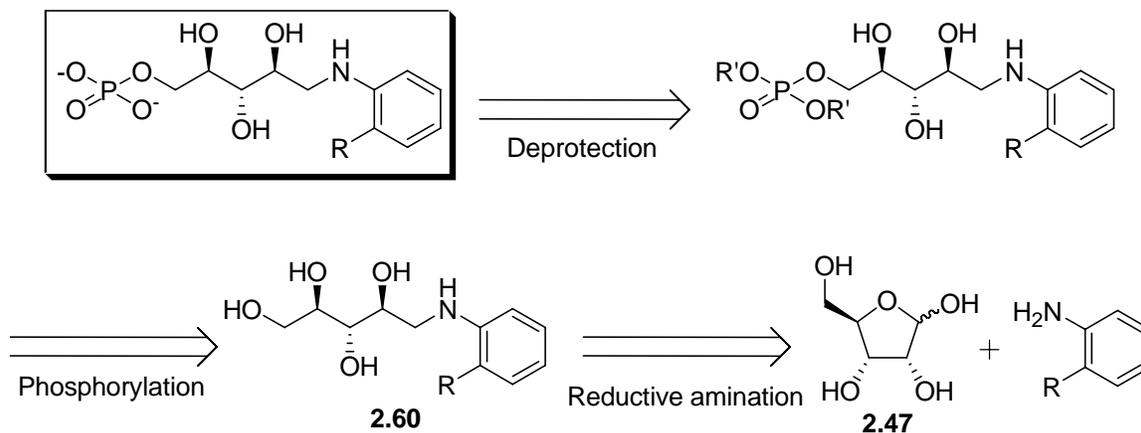
conditions.^{110,211,216} Pyridine-borane also has the added drawback of being unstable to heat and having a relatively short shelf life of six months.²¹¹

2.7. Reductive aminations of lactols:

2.7.1. Introduction:

Lactols are in equilibrium with their aldehydes, which can then undergo a reductive amination reaction with aryl amines, hence forming precursors to potential inhibitors. However, as reducing a lactone to a lactol seems to be a problematic step an alternative strategy was developed as shown in scheme 2.43, which avoids this problem by using D-ribose **2.47**. Protecting strategies proved to be difficult, so it was decided to investigate whether unprotected D-ribose **2.47** could be employed in the formation of aryl glycosylamine precursor **2.60**.

Scheme 2.43. Retrosynthesis of potential inhibitors *via* D-ribose **2.47**.

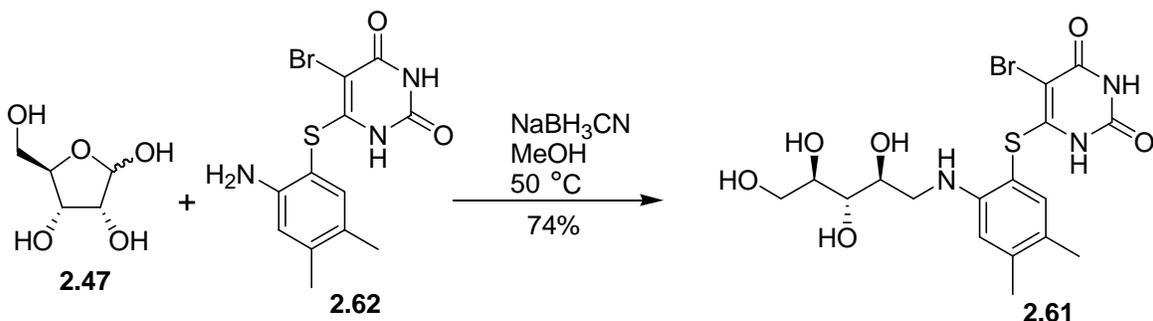


2.7.2. Literature background to the reductive aminations of lactols:

Two examples in the literature have a similarity to our aryl glycosylamine precursor **2.60** and offer a potential methodology for formation of similar compounds. Hirota *et al.*²⁶⁰ regioselectively synthesised the glycosylamine **2.61** by treating the aryl amine **2.62** with D-ribose **2.47** and NaBH₃CN (scheme 2.44). The mixture was stirred overnight in

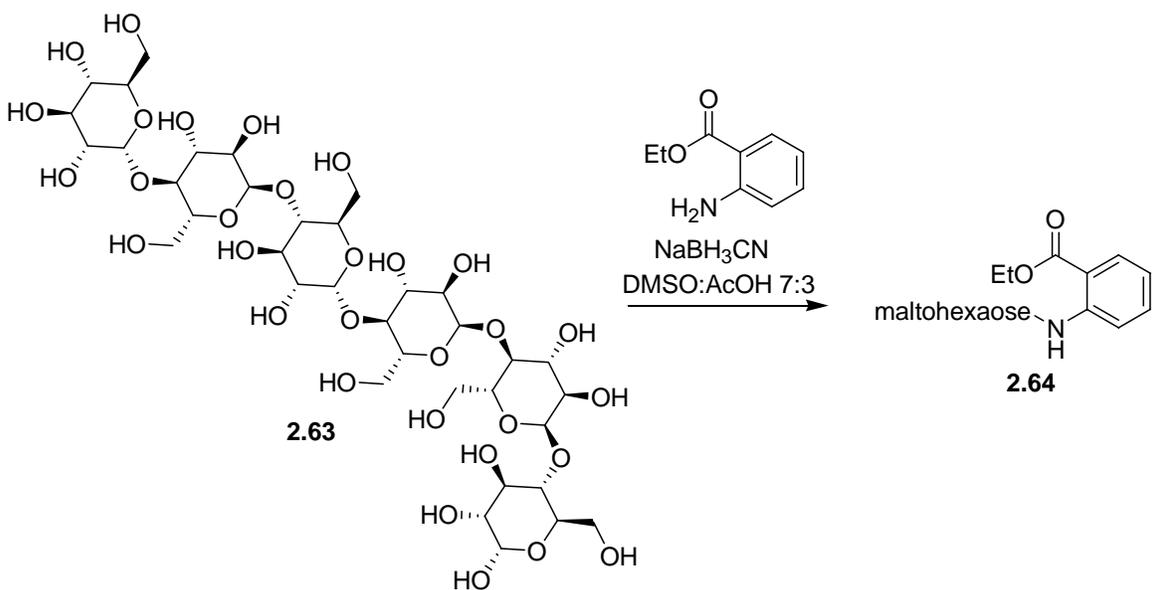
methanol at 50 °C. Small amounts of the debrominated derivative of glycosylamine **2.61** were also formed. pH adjustment was not mentioned.

Scheme 2.44. Glycosylamine formation using D-ribose **2.47**.²⁶⁰



Use of the electron-deficient anthranilate ethyl ester and a carbohydrate, maltohexaose **2.63**, has been reported by Kitada *et al.* (scheme 2.45).^{261,262} A dry sample of **2.63** was dissolved in a 7:3 solution of dimethyl sulfoxide (DMSO) and acetic acid containing NaBH₃CN. No yield, reaction time or other parameters were given, but an optimised reaction using a different electron-deficient aryl amine, 3-aminobenzamide, was reported to give a 100% yield. This was achieved by heating the reagents in DMSO:AcOH (7:3) at 50 °C for one hour.

Scheme 2.45. Glycosylamine **2.64** formation using anthranilate ethyl ester.²⁶¹



2.7.3. Reductive aminations of D-ribose with aryl amines:

Initially we followed the procedure reported by Hirota *et al.*²⁶⁰ Aniline **2.65** was dissolved in absolute methanol and added to a methanolic solution of D-ribose **2.47** (scheme 2.46). NaBH₃CN was added to the stirred solution, which was left to react over a 20-hour period at room temperature. The reaction was monitored by TLC (run in *n*-butanol:EtOH mixtures) and by taking crude aliquots of the reaction mixture and running ¹H NMR in DMSO-*d*₆. No imine or aryl glycosylamine precursor **2.66** was apparent by either ¹H NMR or TLC over this 20-hour period. This is reaction 1 (rxn 1) of table 2.04. It is also the general procedure followed in all reductive aminations involving D-ribose **2.47**.

Scheme 2.46. Parameters used in reductive aminations involving D-ribose **2.47**.

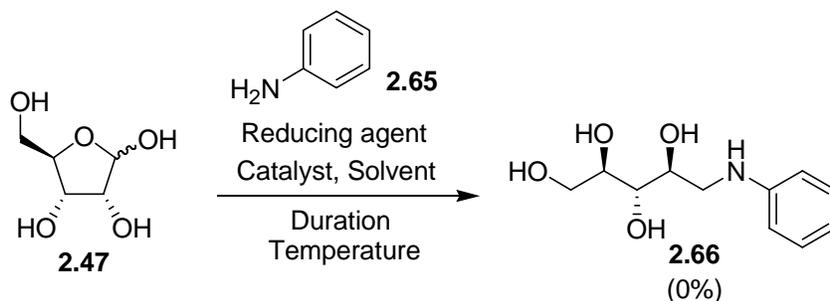


Table 2.04 shows the specific conditions used in each subsequent reaction and scheme 2.46 shows the parameters of these reactions. It is important to note that, unlike reaction 1, the pH of each reaction was adjusted upon the addition of aniline to the solution containing D-ribose **2.47** by addition of glacial acetic acid (rxn 3-15). Different parameters include:

- amine equivalents, note the use of anthranilic acid **2.02** in reaction 2;
- reducing agents such as pyridine-borane (PB) (rxn 7-8), and their equivalents;
- Lewis acid catalysis (rxn 9-15);
- Solvents, where all precautions were taken to ensure solvents were as H₂O-free as possible;

- reaction duration;
- reaction temperatures.

Rxn #	Amine Eq	Reducing agent	Eq	pH	Catalyst	Solvent	Duration	Temp
1 ²⁶⁰	2.25	NaBH ₃ CN	1	9.0		MeOH	20 hrs	rt
2 ^{a,260}	2.25	NaBH ₃ CN	1	9.0		MeOH	20 hrs	rt
3 ²⁶¹	10	NaBH ₃ CN	10	5.0		MeOH	7 days	rt
4 ²⁶¹	10	NaBH ₃ CN	35	5.0		MeOH	7 days	rt
5 ²⁶¹	10	NaBH ₃ CN	35	5.5		MeOH	7 days	rt
7 ^{110,257}	10	PB	10	4.4		L. Pet.	6 days	rt
8 ^{110,257}	10	PB	10	5.2		L. Pet.	6 days	rt
9 ^{227,263}	1.5	NaBH ₃ CN	2	5.5	Ti- <i>i</i> Pr	EtOH	24 hrs	rt
10 ^{b,227,263}	1.5	NaBH ₃ CN	2	5.5	Ti- <i>i</i> Pr	EtOH	24 hrs	45 °C
11 ^{b,c,227,263}	1.5	NaBH ₃ CN	2	5.5	Ti- <i>i</i> Pr	EtOH	24 hrs	45 °C
12 ^{b,d,226}	4	NaBH ₃ CN	6.5	5.5	ZnCl ₂	EtOH	48 hrs	45 °C
13 ^{b,226}	4	NaBH ₃ CN	10	5.5	ZnCl ₂	EtOH	48 hrs	rt
14 ^{b,226}	4	NaBH ₃ CN	10	5.5	ZnCl ₂	EtOH	48 hrs	45 °C
15 ^{b,226}	4	NaBH ₃ CN	16	4.5	ZnCl ₂	EtOH	48 hrs	45 °C

^a Anthranilic acid **2.02** was used instead of aniline.

^b 4 Å M.S. added.

^c Reducing agent added after 90 minutes of stirring aniline **2.65** with D-ribose **2.47** solution.

^d 1 M HCl was used to lower the pH.

References: ^{110,226,227,257,260,261,263}

Table 2.04. Conditions used in lactol reductive amination reactions.

4 Å molecular sieves (4 Å M.S.) have been shown to absorb H₂O generated, causing a definite improvement in yield.²²² They were used in reactions 10-15 in a quantity that would minimise any H₂O present in the solvents and potentially generated in the reaction.

It must also be stated that the first third of the reductive aminations reactions above was done in dry methanol before realising the problems associated with the amines reacting with the formaldehyde present in methanol. Changing to dry ethanol or a mixture of light petroleum (L. Pet.) and glacial acetic acid removed any problems associated with this.

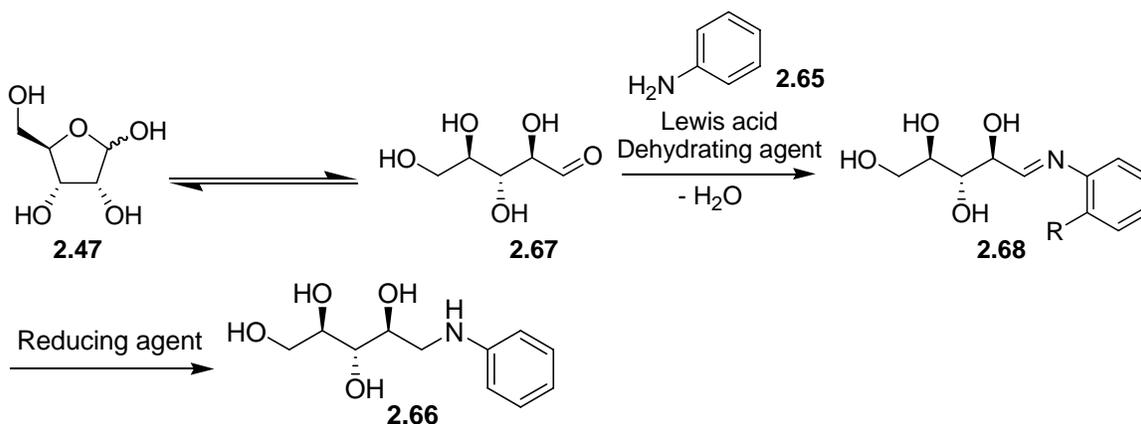
Coupling unprotected D-ribose **2.47** with aniline **2.65** with a raft of different Lewis acids such as $ZnCl_2$ and Ti(IV) *iso*-propoxide ($Ti(iPr)_4$), dehydrating agents and reducing agents, including freshly bought reducing agents, all proved unsuccessful. Crude aliquots of the reaction mixtures taken at different periods of the reaction showed distinctive sets of aniline peaks between 6.5-7.2 ppm using 1H NMR in $DMSO-d_6$. There was no hint of the aryl glycosylamine precursor **2.66** forming or of a shift in aniline peak positions. The lack of reaction could also be seen with the D-ribose **2.47** 1H NMR peaks between 3.3-4.1 ppm not shifting. Crucially the anomeric hydrogen remained and integrated with the rest of D-ribose **2.47** suggesting no aryl glycosylamine precursor **2.66** was formed, nor was there any sign of the anomeric hydrogen shifting up-field. 1H NMR was also performed using D_2O and $CDCl_3:D_2O$ mixtures in case the solubility of aryl glycosylamine precursor **2.66** prevented its appearance in $DMSO-d_6$. 1H NMR and ^{13}C NMR provided confirmation of the starting materials, D-ribose **2.47** and aniline **2.65**.

Isolating the reaction mixture, once a decision was made to terminate the reaction, involved adding a few drops of H_2O and diluting the reaction mixture in EtOAc. The solution would then be washed with H_2O and the phases separated. The aqueous phase would be concentrated by reduced pressure evaporation and NMR spectra would be recorded of the residue D-ribose **2.47**, checking for any sign of aryl glycosylamine precursor **2.66**. The organic layer was washed with 1 M HCl, a saturated solution of $NaHCO_3$, and a brine solution before being dried with $MgSO_4$ and concentrated. NMR spectra confirmed the presence of aniline in almost quantitative amounts from what was initially present in the reaction.

It is possible reaction rates are slow between the open-chain aldehyde **2.67** and aniline **2.65** (scheme 2.47). The aromatic amine, aniline or anthranilic acid may be insufficiently

nucleophilic to successfully attack the aldehyde. The Lewis acids used such as ZnCl_2 and Ti(IV) *iso*-propoxide will activate any small quantities of aldehyde present and make the aldehyde more susceptible to amine attack. Any imine or iminium ion formed should hopefully be converted to the amine by the reducing agents, hence driving the equilibrium to produce more aldehyde. The dehydrating agent, in this case molecular sieves, will remove water, assisting imine formation and pulling the equilibrium again to produce more aldehyde to replace the aldehyde consumed. Critically, imines are more basic than carbonyl compounds and hence should protonate preferentially. Thus the reduction should be directed mainly at the iminium ion species, rather than the initial carbonyl (section 2.61, scheme 2.41). All evidence points towards the aromatic amine not reacting with the aldehyde.

Scheme 2.47. Aldehyde equilibrium and formation of the imine **2.68** before reduction to the aryl glycosylamine precursor **2.66**.



From our results, reductive aminations do not appear to be a reliable method to link aryl amines with D-ribose **2.47**. This led us to investigate simplified model reductive aminations (section 2.9) and other potential synthetic methods of forming secondary aryl amines. In the following section we elaborate on some of the properties of aryl amines.

2.8. Properties of aryl amines:

2.8.1. pK_a :

Aniline **2.65** is a weaker nucleophile than methylamine by a factor of about a million (table 2.05).⁷³ It is also a much weaker nucleophile than its saturated analogue cyclohexylamine. Cyclohexylamine has a pK_a of 10.66, which is in line with other alkyl amines such as methylamine (pK_a 10.62), but aniline has only a pK_a of 4.63.⁷³ This cannot be explained by the inductive effect of the phenyl group relative to the saturated cyclohexyl ring or methyl group and is best accounted for in terms of delocalisation of the unshared electron pair of the nitrogen by orbital overlap with the π electrons of the phenyl ring.^{73,264-266} Electrons donate from the nitrogen to the aromatic ring, hence lowering the nucleophilicity of the amine group but increasing the reactivity of the benzene towards electrophiles (figure 2.02).^{73,264-266}

Compound ^a	pK_a
Ammonia ⁷³	9.27
Methylamine ⁷³	10.62
Cyclohexylamine ⁷³	10.66
Benzylamine ²⁶⁵	9.34
Aniline ²⁶⁶ 2.65	4.59
<i>N</i> -methylaniline ²⁶⁶	4.85
Anthranilic acid ²⁶⁷ 2.02	2.11, 4.95
Benzoic acid ²⁶⁵	4.18

^a Listed values for ammonia and amines are the pK_a values for the corresponding ammonium ions.

Table 2.05. pK_a of different potential nucleophiles at 25 °C.

The pK_a values for anthranilic acid **2.02** are 2.11 and 4.95 for the carboxylate and amino functionalities respectively.

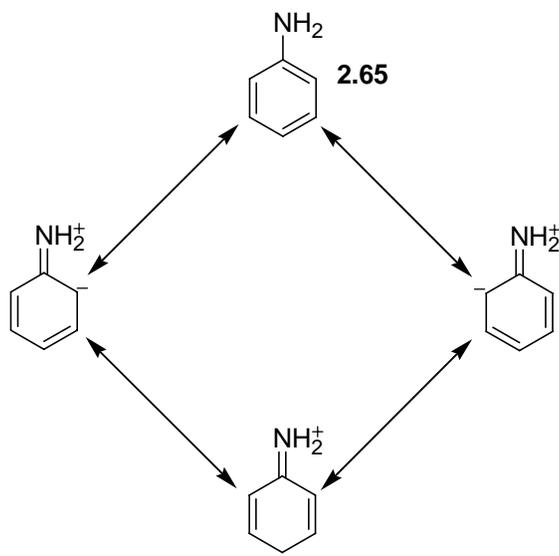


Figure 2.02. Resonance structures for aniline **2.65**, lowering pK_a .⁷³

2.8.2. The influence on pK_a by substituents:

Planarity is the key condition for resonance; hence resonance can be inhibited by steric effects such as the presence of bulky phenyl ring substituents or of *N*-substituents on the amine nitrogen. *N*-Substituents increase nucleophilic strength by more than could be expected from their inductive effect, and are, apparently, by their bulk, pushing the amino-group out of the plane of the benzene ring, a process which interferes with the nucleophilic-weakening resonance.^{265,266} This can be seen when comparing *N*-methylaniline (pK_a 4.85) a secondary amine, and aniline (pK_a 4.59). In other words for resonance to be maximally effective, approximate coplanarity of the groups on the nitrogen with the ring plane is required, a fact that makes resonance subject to steric influences.^{265,266,268,269}

The pK_a values of aniline are very susceptible to the influence of *ortho* and *para* substituents, owing to the pronounced ability of the benzene ring to transmit electronic

Synthesis of aryl glycosylamine precursor **2.66** will produce a more nucleophilic secondary amine, which will compete and, in terms of pK_a , be more reactive towards the aldehyde than aniline **2.65** (figure 2.04).

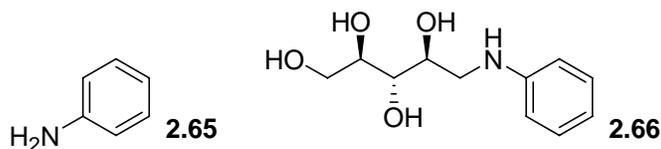


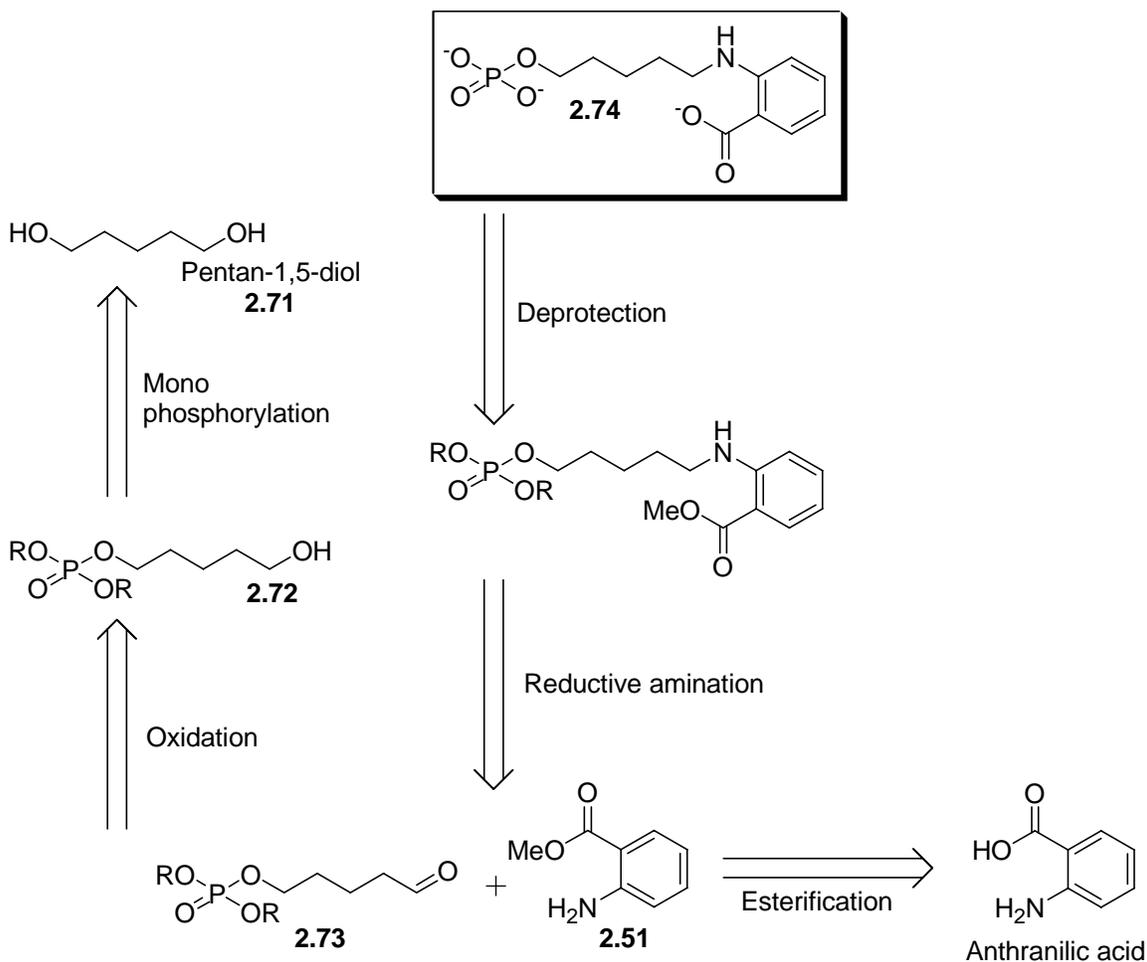
Figure 2.04. Primary and secondary amines.

2.9. Model reductive amination chemistry:

2.9.1. Introduction:

It was decided to test our initial general synthetic strategy (scheme 2.01) by using similar chemistry on test molecules. Hence, it was decided to target model reductive amination reactions involving simpler compounds before attempting to synthesise our target inhibitors. This was done in order to get an early grasp on the important reductive amination reaction, its conditions, limitations and sensitivity. We choose to use pentan-1,5-diol **2.71** as our test subject. This should remove the difficulty involved in protecting and deprotecting the free hydroxyl groups, allowing us to focus on the reductive amination reaction. Attaching a phosphate moiety early on in the synthesis was also a priority since one of the ideas behind the general synthetic strategy (scheme 2.01) was to attach the protected phosphate moiety at the first step and carry it through the reductive amination to the end product. Monophosphorylation is followed by oxidation of the free primary hydroxyl group on **2.72** to give the aldehyde **2.73**, which will undergo a reductive amination with anthranilate methyl ester **2.51** ultimately leading to 5-phosphonopentyl anthranilic acid **2.74** (scheme 2.48).

Scheme 2.48. Retrosynthetic pathway of 5-phosphonopentyl anthranilic acid **2.74**.

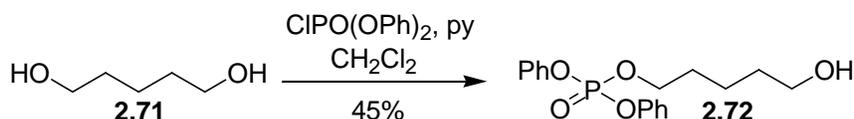


2.9.2. Preparation of aldehyde:

The diol **2.71**, diphenyl phosphorochloridate (1.2 equivalents), and pyridine (one equivalent) were dissolved in dichloromethane in a procedure based on work performed by Tener and Khorana.¹²² The reaction was cooled to 0 °C for 45 minutes, allowed to warm to room temperature and then stirred for 21.5 hours (scheme 2.49). After working the reaction up, ¹H NMR spectra of the diol **2.71** and the crude reaction mixture were compared with each other. These showed that the original triplet at 3.63 ppm, which integrated as four hydrogens now integrated to only two hydrogens. In addition a new set of peaks formed a multiplet at 4.26 ppm. This was deduced to be the result of the loss of symmetry of the diol **2.71** as monophosphorylated product **2.72** was formed. The yield of

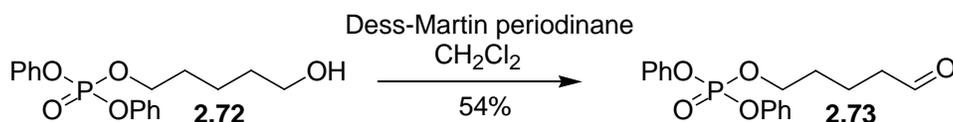
the reaction was 45%. Increasing the equivalents of diphenyl phosphorochloridate and/or of base led to an increase in the amount diphosphorylated product formed, and a similar yield of monophosphorylated product **2.72**. When using NaH as a base with THF as the solvent, the yields were lowered to 25%. It was decided not to lower the equivalents of diphenyl phosphorochloridate, which would increase the yield of the product based on the phosphorylating reagent being the limiting reagent. Also we did not recover the unreacted diol **2.71** and, therefore did not attribute that into the product yield.

Scheme 2.49. Synthesis of monophosphorylated product **2.72**.



Oxidation of the primary alcohol to an aldehyde **2.73** using Dess-Martin periodinane²⁷³ (2.3 equivalents) in dry CH₂Cl₂ was successfully achieved over 20.5 h in 54% yield (scheme 2.50). This was an optimised yield brought about by a further addition of Dess-Martin periodinane after 18 hours. The isolated product clearly showed an aldehyde peak at 9.72 ppm in the ¹H NMR spectrum. The by-products were uncharacterisable.

Scheme 2.50. Synthesis of aldehyde **2.73**.



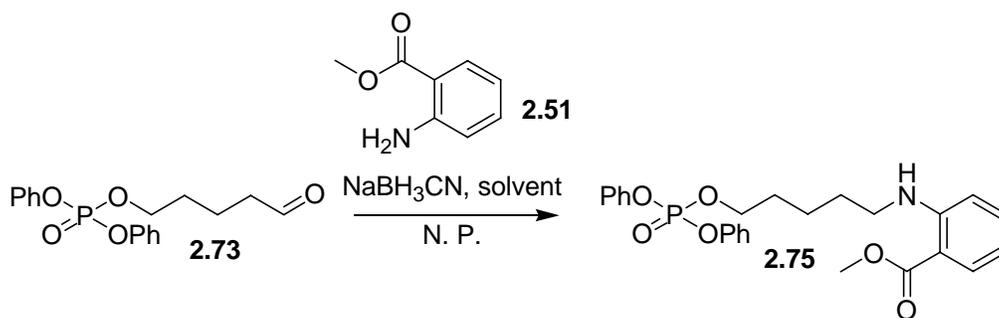
2.9.3. Model reductive aminations with phosphate present:

Unfortunately, under various conditions the reductive amination involving the aldehyde **2.73** and anthranilate methyl ester **2.51** failed to give the desired secondary amine, 5-phosphonopentyl anthranilate methyl ester **2.75** (scheme 2.51).

Using methods based on literature NaBH₃CN^{212,222-224} reductive aminations, a general procedure follows: anthranilate methyl ester **2.51** was stirred in either MeOH, EtOH or

THF before being added to a solution of aldehyde **2.73** in the appropriate solvent. The solution was adjusted to a pH of between 5-6 using acetic acid or left at its natural pH of 8.6, after which the solution was allowed to stir for between 5 minutes and 5 hours in an attempt to form the imine. NaBH₃CN was added in various quantities (scheme 2.51). A procedure based on pyridine-borane^{110,257} was also used.

Scheme 2.51. Attempted synthesis of 5-phosphonopentyl anthranilate methyl ester **2.75**.



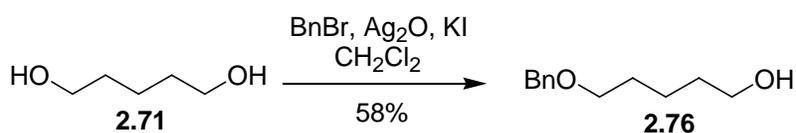
NMR analysis of aliquots of the above reaction mixtures indicated that the phosphate moiety was unstable when in the presence of anthranilate methyl ester **2.51**. Aniline **2.65** was substituted for anthranilate methyl ester **2.51** and reaction conditions were kept constant with previous attempts at forming the secondary amine **2.75**. The spectra obtained using both nucleophilic amines suggest that the amine may have interfered with the phosphate functionality. After an hour with either amine the aldehyde peak at 9.72 ppm (¹H NMR) diminished as did the integration of the phosphate phenyl groups. The ¹³C NMR spectrum showed a number of peaks between 110 to 155 ppm and 17 to 50 ppm, which did not match starting materials. TLC silica plates run with 3:1 hexanes-EtOAc, showed two broad streaks from *R_f* 0.42 to an *R_f* of 0.55, using anthranilate methyl ester **2.51**. Altering the solvent from MeOH to THF or the amount of NaBH₃CN²²² or using pyridine-borane^{110,257} did not change the reaction outcome. Neither the desired product 5-phosphonopentyl anthranilate methyl ester **2.75** nor the aniline equivalent were isolated out of this reaction mixture, nor was any of the by-products isolated. The possibility exists that the weakly nucleophilic aryl amines attacked the phosphate diester. There are reports in literature of similar occurrences.²⁷⁴⁻²⁷⁸

Due to the fact that the amine appeared to be interacting with the phosphate group we went about replacing the monophosphate group with a protecting group, in this case benzyl.

2.9.4. Preparation of protected aldehyde:

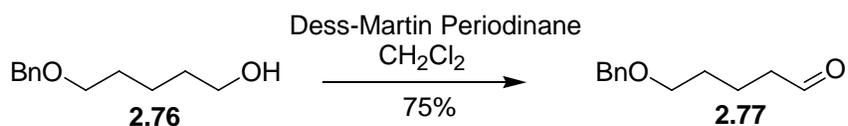
Monobenzylation yields were good with the best results (58%) coming from the diol **2.71** being dissolved in CH_2Cl_2 containing Ag_2O (one equivalent) and KI (catalytic) with stirring in ice-bath conditions (scheme 2.52). Benzyl bromide was added dropwise, and after 10 minutes the solution was allowed to warm to room temperature and then was placed in an ultrasonic bath for a 5 minute period before being removed and stirred for 23 hours. Using NaH and benzyl bromide gave similar yields.¹⁹⁵ Again a decision was made not to recover starting material or limit the amount of benzyl bromide used.

Scheme 2.52. Synthesis of benzyl-protected product **2.76**.



An ice-bath cooled solution of Dess-Martin periodinane²⁷³ (five equivalents) in CH_2Cl_2 was used to form protected aldehyde 5-benzylpentanal **2.77** in 75% yield as shown by the characteristic ^1H NMR peak at 9.76 ppm.

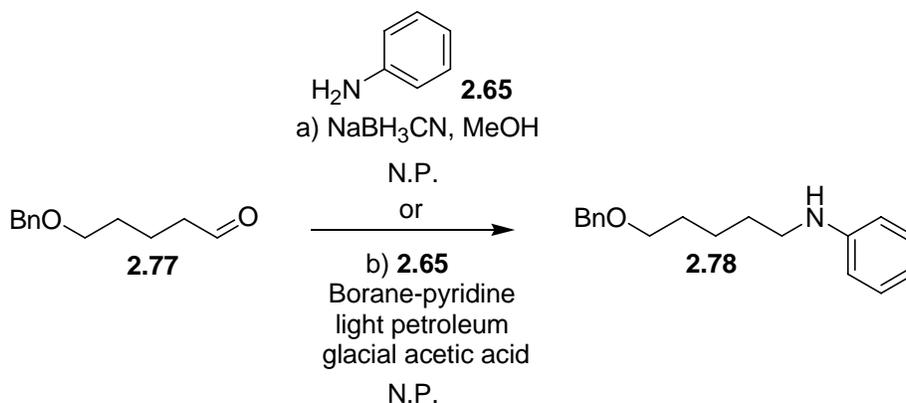
Scheme 2.53. Synthesis of protected aldehyde **2.77**.



2.9.5. Model reductive aminations with protected aldehyde:

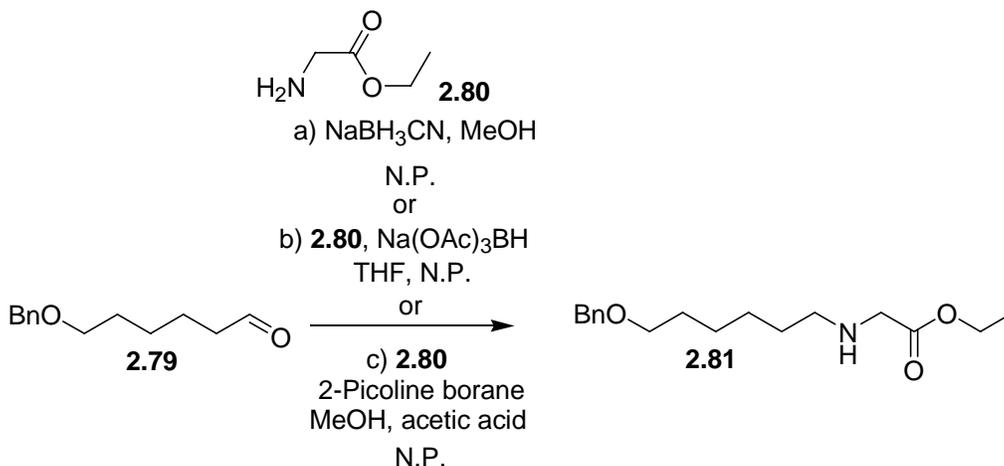
It was decided to exchange anthranilate methyl ester **2.51** with aniline **2.65** and see whether the reaction proceeded smoothly without the ester group. Section 2.9.3 outlines a general method used in NaBH_3CN ^{212,222-224} reductive aminations. Disappointingly no secondary amine **2.78** was isolated, either by using several equivalents of NaBH_3CN or by using pyridine-borane^{110,257} (scheme 2.54). The protected aldehyde **2.77** was isolated from the reaction mixture even after several hours in the presence of aniline **2.65** and NaBH_3CN . The amount of protected aldehyde **2.77** recovered would vary between 15 to 35 % with the remaining material being uncharacterisable decomposed by-products at a lower R_f on TLC silica plates. Aniline **2.65** was not recovered. Freshly acquired aniline **2.65** did not change the outcome of the reaction.

Scheme 2.54. Unsuccessful synthesis of benzyl-protected pentylaminophenyl **2.78**.



A work colleague²⁷⁹ has had similar difficulties with reductive aminations. Comparing Harrison's²⁷⁹ aldehyde moiety **2.79** with the protected aldehyde **2.77**, the carbon backbone was hexyl rather than pentyl. The amine, ethyl glycine **2.80** was aliphatic, unlike aniline **2.65**, although electron density was withdrawing due to the ethyl ester (scheme 2.55).

Scheme 2.55. Harrison's²⁷⁹ attempts at synthesis of benzyl-protected hexylamino-acetic ethyl ester **2.81**.



Ultimately, benzyl-protected hexylamino-acetic ethyl ester **2.81**, was not synthesised by Harrison²⁷⁹ using a) NaBH_3CN ,^{212,222-224} b) $\text{NaBH}(\text{OAc})_3$,^{108,210} or c) 2-picoline-borane²¹¹ reducing agents following procedures based on their respective literature. The low nucleophilic nature of ethyl glycinate **2.80** was attributed to its ethyl ester moiety.

Our results (scheme 2.54), Harrison's²⁷⁹ and literature^{110,211,257} all imply that reductive amination reactions are difficult to advance with poor nucleophiles. With this in mind, removing the aromatic properties of the cyclic amine should allow easier imine formation; hence cyclohexylamine **2.82** was used instead of aniline **2.65**. Following a similar procedure discussed for the failed formation of the secondary amine **2.78**, pentylcyclohexylamine **2.83** was formed in 25% yield, using NaBH_3CN in unoptimised conditions (scheme 2.56). TLC silica plates showed a spot forming at an R_f of 0.45 (3:1 hexanes-EtOAc) one hour after the addition of NaBH_3CN .

Scheme 2.56. Successful synthesis of benzyl-protected pentylcyclohexyl amine **2.83**.



As the reaction proceeded a further spot developed at a higher R_f of 0.63 (3:1 hexanes-EtOAc). This was later isolated and tentatively assigned as the tertiary amine **2.84** by NMR, although it was not fully characterised (figure 2.05). The isolated yield was 5%. As with all amination reactions, formation of the tertiary amine product will be possible since the secondary amine, in this case **2.83**, is a better nucleophile than cyclohexylamine **2.82**.

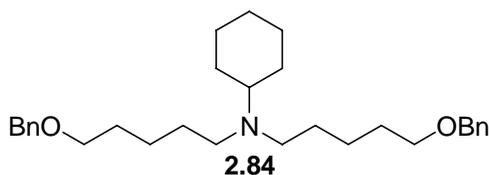


Figure 2.05. Possible by-product, tertiary amine **2.84**.

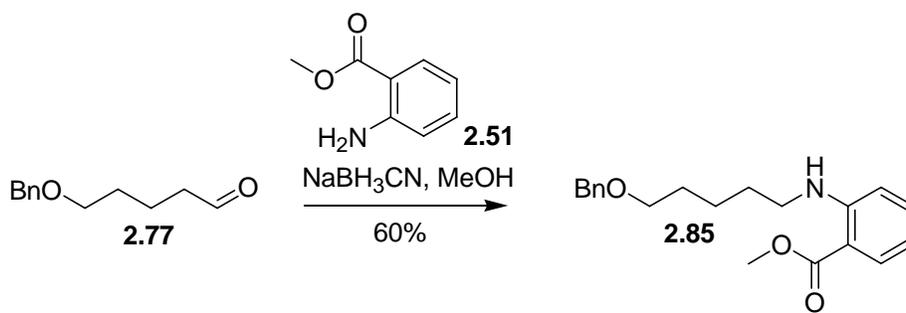
2.9.6. Model reductive aminations with protected aldehyde and anthranilate methyl ester:

After the success of pentylcyclohexyl amine **2.83**, synthesis of pentyl anthranilate methyl ester **2.85** was attempted. It was anticipated that the poor nucleophilic nature of anthranilate methyl ester **2.51** would have made the formation of the imine difficult, as was the case with aniline **2.65** (scheme 2.54). Steric hindrance of the *ortho* methyl ester was also thought to be detrimental to imine formation.

A solution of anthranilate methyl ester **2.51** (2.25 equivalents) in methanol was added to protected aldehyde **2.77** (one equivalent), the pH was adjusted to 5.5 with acetic acid and

the solution was stirred for 15 minutes before NaBH₃CN (one equivalent) was added. The reaction was stirred for a further 5.5 hours at room temperature, during which time the protected aldehyde **2.77** disappeared (TLC silica plate, *R_f* 0.52, 5:1 hexanes-EtOAc) and a new spot formed at *R_f* 0.41 (3:1 hexanes-EtOAc). The reaction was worked up upon the complete disappearance of the protected aldehyde **2.77** spot. The new spot formed was found to be pentyl anthranilate methyl ester **2.85** (60%) *via* NMR spectroscopy (scheme 2.57). No tertiary amine was isolated.

Scheme 2.57. Successful synthesis of benzyl-protected pentyl anthranilate methyl ester **2.85**.



Possible explanations for the formation of pentyl anthranilate methyl ester **2.85**, but not of the pentylaminophenyl secondary amine **2.78**, could be attributed to product stability and better solubility of anthranilate methyl ester **2.51** than aniline **2.65** in MeOH, however this is speculation.

2.10. Conclusions:

This chapter evolves from initial attempts to synthesise rCdRP **2.01** *via* reductive aminations with phosphorylated lactols and anthranilate alkyl esters to the successful synthesis of 5-benzylpentyl anthranilate methyl ester **2.85**.

D-Ribonolactone **2.03** was difficult to solubilise in solvents typically used for phosphorylation reactions. This led to the formation of a trityl, disilyl TBDMS-protected lactone **2.12**, which would not reduce with Dibal-H to form the lactol at temperatures

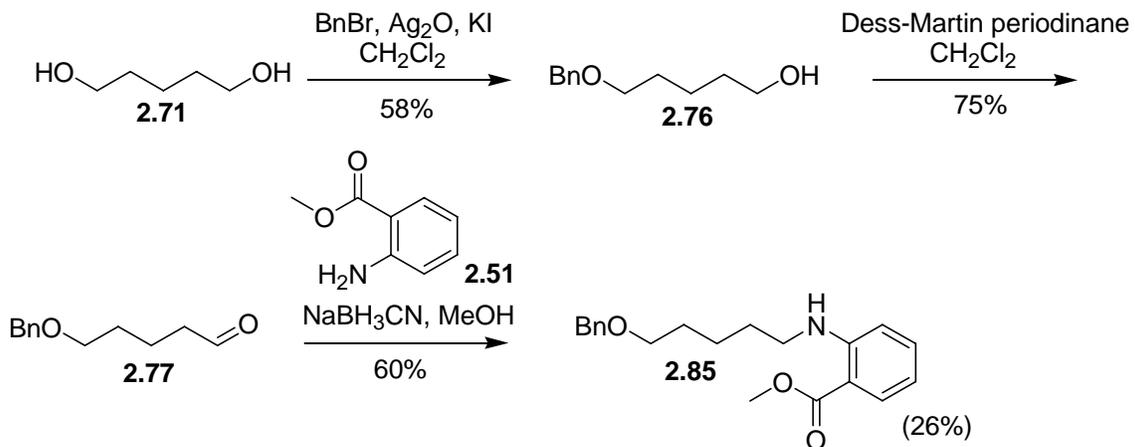
under $-45\text{ }^{\circ}\text{C}$. Warming the reaction to $-20\text{ }^{\circ}\text{C}$ and higher led to cleavage of the protecting TBDMS groups. There is a precedent for this in the literature, originally noted by Corey and Jones.¹⁵⁸ Changing the disilyl groups to dibenzyl was not as easy as first thought, with alkaline conditions using NaH and Ag_2O degrading the lactone moiety. Non-alkaline protections were developed, attempting to form a benzylidene acetal first, and then an *iso*-propylidene acetal. The acidic conditions, however, cleaved off the trityl protecting group when trying to form the benzylidene acetal, but using $\text{BF}_3\cdot\text{Et}_2\text{O}$ and 2,2-dimethoxypropane protection of tritylated *cis* diol **2.09** was finally achieved. Lactol formation was held off until more first-hand knowledge of reduction aminations was gained.

Protected D-ribose moieties were synthesised. These moieties gave us alternatives to using D-ribonolactone **2.03** to form the lactols for reductive aminations. Methyl esterification of anthranilic acid proved less than straightforward, which could be attributed to steric hindrance and hydrogen bonding.

Reductive aminations with D-ribose **2.47** failed to produce any aryl glycosylamine precursor **2.66**, possibly due to the low nucleophilicity of aryl amines such as aniline **2.65**. Reductive aminations with monophosphorylated aldehyde **2.73** also failed, but gave important information that the aryl amine interferes with the phosphate moiety. Pentylcyclohexyl amine **2.83** was synthesised from benzyl-protected aldehyde **2.77**, cyclohexylamine **2.82** and NaBH_3CN , at a pH of 5.5.

This led to the synthesis of pentyl anthranilate methyl ester **2.85** at an overall yield of 26% over three steps, summarised in scheme 2.58.

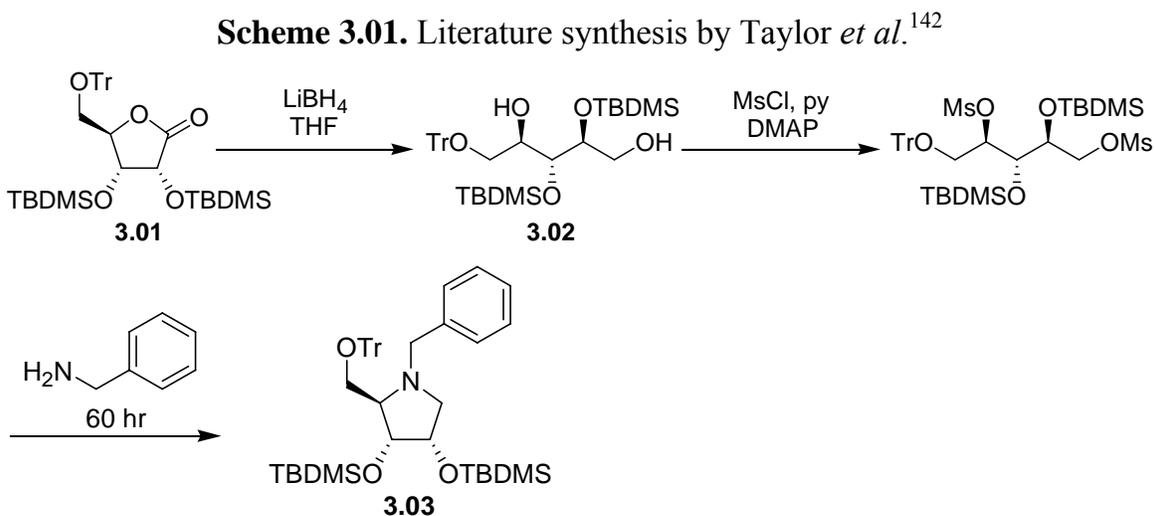
Scheme 2.58. Formation of benzyl-protected pentyl anthranilate methyl ester **2.85** over three steps.



CHAPTER 3: Nucleophilic substitution of good leaving groups.

3.1. Synthesis of rCDRP-like target compounds:

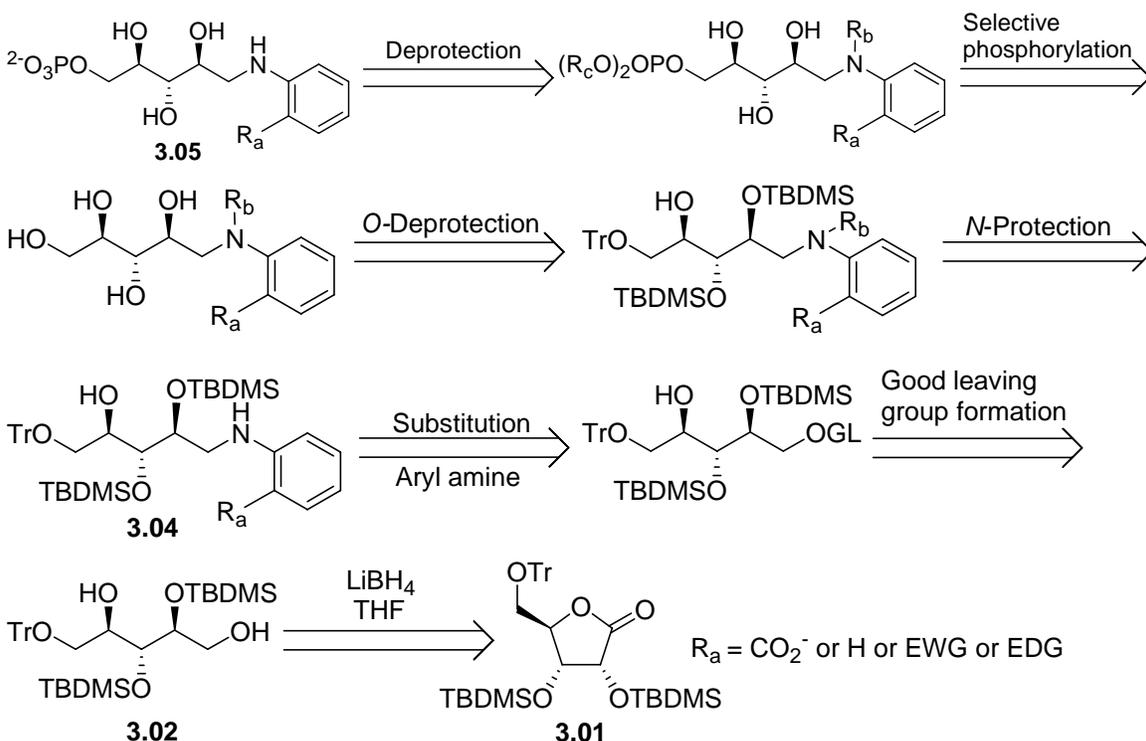
The experiences with reductive amination chemistry detailed in chapter two led to an investigation of alternative methods to form secondary aryl amines. Nucleophilic substitution of a good leaving group appears to be historically reliable chemistry^{57,196,280} so it was decided to examine a procedure based on work by Taylor *et al.*¹⁴² (scheme 3.01). This utilises the previously synthesised disilyl TBDMS-protected lactone **3.01** (section 2.3.2). This compound is reportedly reduced to the diol **3.02** by LiBH₄ in THF and subsequently converted to a mesylate with mesyl chloride (MsCl) in order to form a cyclised tertiary amino sugar **3.03** on reaction with the nucleophile benzylamine.¹⁴²



Our aim was to form a good leaving group (GL) at the primary hydroxyl on diol **3.02**, then substitute it with aniline or anthranilate methyl ester or aryl amines containing electron-withdrawing or electron-donating groups producing a secondary aryl amine **3.04**

(EWG, EDG, scheme 3.02). Subsequent protections and deprotections would lead to the formation of the rCdRP-like target compound **3.05**.

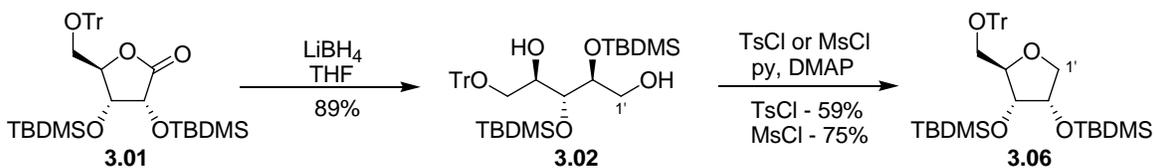
Scheme 3.02. General retrosynthetic strategy for CdRP-like compounds.



3.1.2. Synthesis of secondary rCdRP-like aryl amines:

Reduction of the protected lactone **3.01** (scheme 3.03) to the diol **3.02** was accomplished in 89% yield by adding LiBH_4 in THF dropwise over 50 minutes to a $-15\text{ }^\circ\text{C}$ solution of protected lactone **3.01** in THF. After 18 hours stirring at room temperature the lactone ^{13}C NMR signal disappeared at 175.2 ppm and a new ^{13}C NMR signal appeared at 61.9 ppm and a ^1H multiplet at 4.06 ppm. This represented the carbon at C1', verifying the formation of the open-chained diol **3.02**. The yields were consistently high (78-89%) over several reduction reactions with the upper figure arising from a reaction where the protected lactone **3.01** solution was cooled to $-15\text{ }^\circ\text{C}$ instead of a reaction at room temperature as described by Taylor *et al.*¹⁴² Silyl migrations to unprotected hydroxyl groups were not apparent during or after the reduction process.

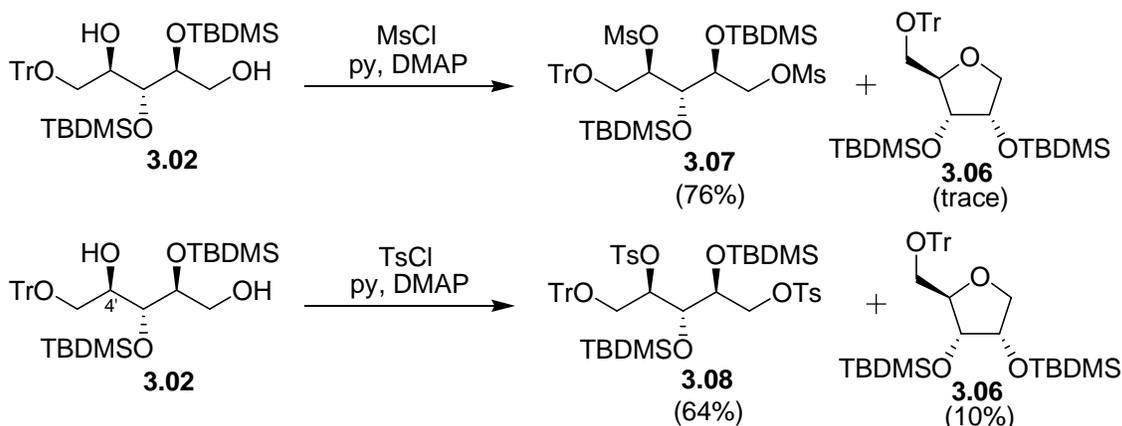
Scheme 3.03. Initial attempts at forming a primary-mono good-leaving group.



The next step was to attempt to mono-tosylate the diol **3.02** on the primary hydroxyl group. A solution of the diol **3.02** in dry pyridine was added dropwise to an ice cold premixed suspension of tosyl chloride (TsCl, one equivalent) and DMAP in dry pyridine. The resulting orange solution gave a less polar spot on TLC analysis as the reaction was stirred over several hours at 4 °C. Perhaps unsurprisingly the less polar spot, the major product isolated, was the furan derivative **3.06** (scheme 3.03). This is due to the use of alkaline conditions, which caused the secondary unprotected hydroxyl group intramolecularly substitute the tosylate to form the furan derivative **3.06**. The distinguishing ^1H NMR multiplet at 4.06 ppm on the diol **3.02** disappeared and a new multiplet was observed at 3.95 ppm, corresponding to the C1' hydrogens on **3.06**. Simultaneously, a similar result was observed on trying to mono-mesylylate the diol **3.02** using 1.3 equivalents of mesyl chloride (MsCl).

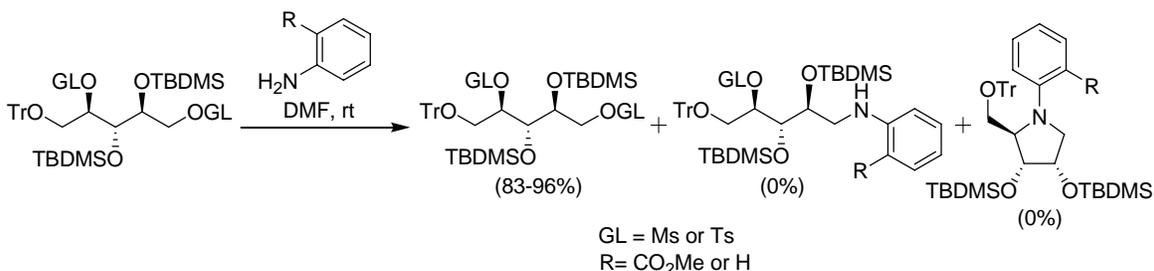
One option to avoid forming the furan derivative **3.06** was to place a good leaving group on the secondary hydroxyl group while forming the primary good leaving group, hence reducing the possibility of intramolecular cyclisation. Following the Taylor *et al.*¹⁴² procedure, bis-mesyl **3.07** was formed in 76% yield with trace amounts of the furan derivative **3.06** isolated (scheme 3.04). Four equivalents of MsCl were used to ensure the secondary hydroxyl was transformed into a good leaving group. Similar conditions were used in the formation of bis-tosyl **3.08** (64%), with the higher yield of furan derivative **3.06** (10%) possibly being due to the larger steric bulk of the tosyl compared to mesyl group hindering protection of the secondary hydroxyl group at C4'. This hindrance increases the likelihood of the secondary hydroxyl reacting intramolecularly with the primary C1' carbon (scheme 3.04).

Scheme 3.04. Synthesis of bis-mesyl **3.07** and bis-tosyl **3.08** compounds.



In an attempt to form a secondary aryl amine, bis-tosyl **3.08** was initially dissolved in a solution of anthranilate methyl ester (one equivalent) in DMF and allowed to stir at room temperature over several days. An almost quantitative return of starting material, bis-tosyl **3.08**, was achieved (scheme 3.05). Using the bis-mesyl **3.07** as an alternative did little to change the outcome, since the weakly nucleophilic anthranilate methyl ester and the bis-mesyl **3.07** were recovered in high yield. Using similar conditions, aniline and either bis-mesyl **3.07** or bis-tosyl **3.08** did not return any substituted product. Anthranilate methyl ester and aniline were also recovered. Using *N*-alkylation promoters for catalysis was not investigated.

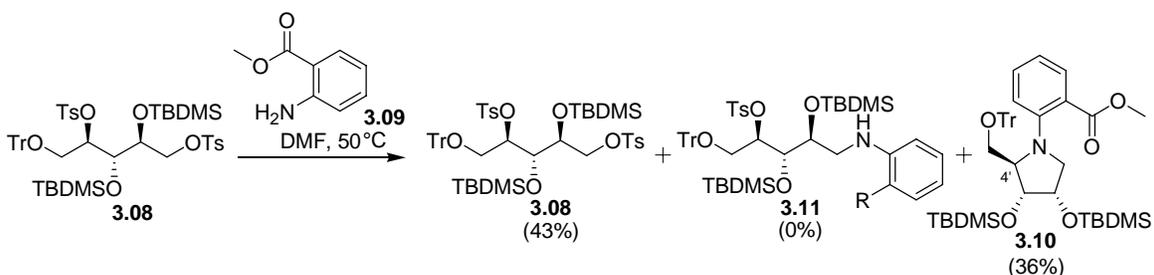
Scheme 3.05. Lack of aryl amine reactivity at room temperature.



When heat was applied, somewhat predictably, we could not selectively displace the primary good leaving group on the bis-mesyl **3.07** or bis-tosyl **3.08** without forming pyrrolidine structures. After warming the solution of bis-tosyl **3.08** and anthranilate methyl ester **3.09** (one equivalent) in DMF to 50 °C, it was noticeable on TLC that a

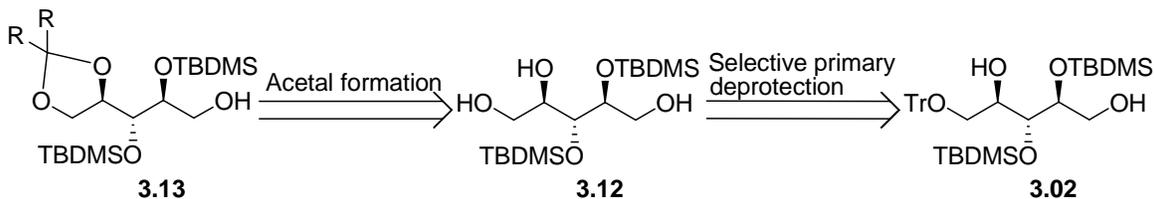
reduction in starting material and formation of a less-polar compound was occurring. An aliquot of crude reaction mixture was extracted and analysed by NMR spectroscopy to determine the identity of the non polar compound, which was characterised to be the tertiary pyrrolidine derivative **3.10** due to single resonance of one hydrogen at 4.40 ppm at C4' and appropriate ^{13}C and ^1H NMR spectra. There was no suggestion the target secondary aryl amine **3.11** was being formed (scheme 3.06). Replacing anthranilate methyl ester **3.09** with aniline, increasing the equivalents of the aryl amine and/or changing the electrophile to bis-mesyl **3.07** produced the same results, no target secondary aryl amine instead formation of a tertiary pyrrolidine derivative after a few hours of heating at 50 °C.

Scheme 3.06. Nucleophilic substitution forming pyrrolidine derivative **3.10**.



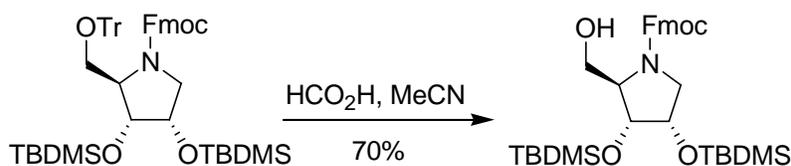
Finding conditions for synthesis of secondary aryl amine **3.11** while the secondary good leaving group was present on bis-mesyl **3.07** or bis-tosyl **3.08** was deemed problematic, so an alternative strategy was devised. This hinged on protecting the secondary alcohol of the diol **3.02** before formation of the good leaving group at the primary hydroxyl. The most logical way to do this was to selectively deprotect the trityl group on the diol **3.02** to form the 1,2 diol **3.12**, which could be protected using an acetal,^{124,125,281} exposing the primary hydroxyl **3.13** (scheme 3.07).

Scheme 3.07. Synthesis of precursor towards formation of a primary good leaving group.



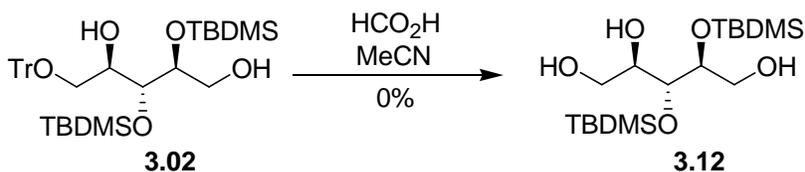
There is evidence^{125,282} that trityl groups are more labile than TBDMS-protecting groups in acidic conditions. Taylor *et al.*¹⁴² showed that a trityl group could be removed by formic acid in the presence of TBDMS-protecting groups (scheme 3.08). However, it was noted that this was not straightforward.¹⁴²

Scheme 3.08. Formic acid removal of trityl group by Taylor *et al.*¹⁴²



Selective removal of the trityl group on diol **3.02** failed using methodology described by Taylor *et al.*¹⁴² since formic acid exerted little selectivity in cleaving the trityl group preferentially to the TBDMS groups (scheme 3.09). Limiting the equivalents of formic acid did not lessen the amount of by-products produced.

Scheme 3.09. Attempted removal of trityl group.



3.1.3. Proposed synthesis of secondary rCdRP-like aryl amines:

The general idea of selectively protecting the 1,2 diol **3.12** using an acetal, freeing the primary hydroxyl group to be converted to a good leaving group remains a sensible one. Due to the difficulties of selective cleavage of the trityl over the TBDMS groups a new strategy must be devised. It was necessary to either replace the TBDMS groups with other secondary protecting groups or to develop a totally redesigned strategy. Both avenues must take into consideration the conditions used in the formation of:

- (i) secondary aryl amine;
- (ii) good leaving groups;
- (iii) acetal;
- (iv) open-chain diol (reduction);
- (v) other protections (*N*- and *O*-).

It has been shown by Antonakis *et al.*²⁸² that *iso*-propylidene and benzylidene acetals were retained in the selective removal of the respective trityl groups (figure 3.01). An acid-stable resilient acetal could therefore replace the problematic TBDMS groups. Another candidate to replace the TBDMS groups is methoxyethoxymethyl (MEM) ethers, which exhibit a large differential in acidic lability over the trityl group.^{283,284}

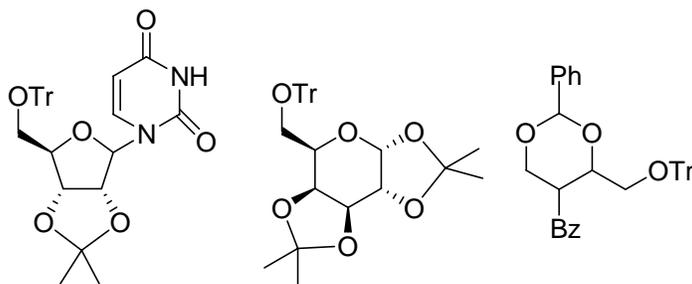
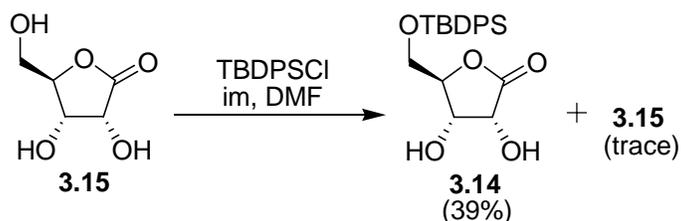


Figure 3.01. Selective removal of trityl groups in the presence of acetals.²⁸²

Preliminary steps at redesigning the synthetic strategy by replacing the trityl group with the primary selective TBDPS-protecting group were taken. Changing to this silyl group removes the need for problematic acidic deprotection of trityl since TBDPS is cleaved by

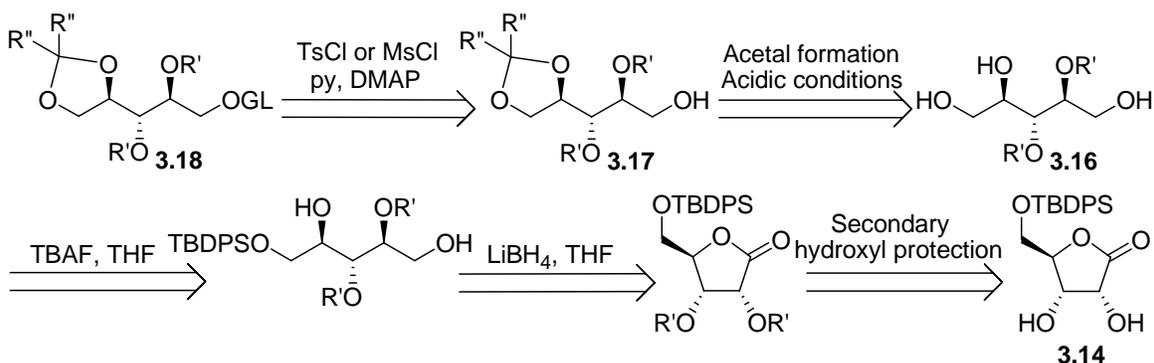
fluoride ions.^{124,125,146} Protection was achieved using a procedure similar to that of Dodd *et al.*²⁸⁵ The *cis* diol **3.14** was produced in 39% yield over 2 hours, with trace amounts of starting material D-ribonolactone **3.15** recovered (scheme 3.10).

Scheme 3.10. Synthesis of 5-*O*-*tert*-butyldiphenylsilyl-D-ribonolactone **3.14**.²⁸⁵



The secondary hydroxyl protection would involve using either β -methoxyethoxymethyl (MEM) ethers,^{283,284} methylene acetals²⁸¹ or allyl ethers.^{174,286,287} Reduction of the corresponding protected lactone and deprotection of the TBDPS-protecting group with tetrabutylammonium fluoride (TBAF), giving 1,2 diol **3.16** would occur. The 1,2 diol **3.16** would then be protected with an acetal giving the primary hydroxyl **3.17**, which is transformed into a good leaving group **3.18** (scheme 3.11).

Scheme 3.11. Proposed alternative strategy to primary good leaving group **3.18**.



Before attempting further protections and developing the pathway to a suitable primary good leaving group, it was decided to test a more general synthetic strategy by using nucleophilic substitution on more simple molecules developed from pentan-1,5-diol and 4-penten-1-ol. This was propagated by the shift in focus of this thesis to formation of secondary aryl amines and away from complicated protection and deprotection strategies. Once these simpler compounds were synthesised and the conditions, limitations and

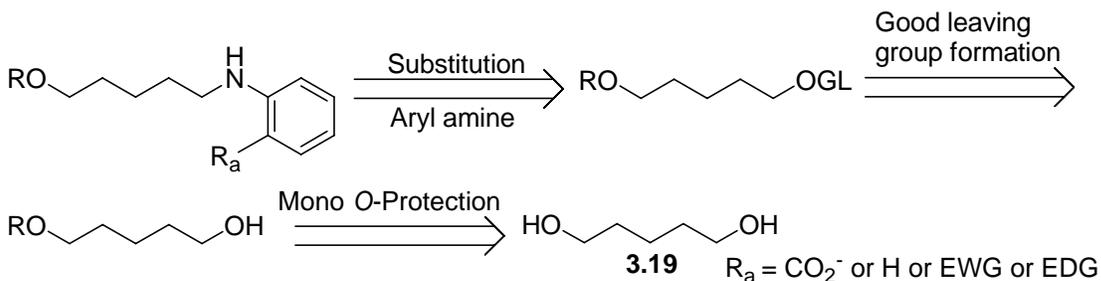
sensitivity of nucleophilic substitution were known first hand, development of rCdRP-like target compounds would continue.

3.2. Synthesis of sulfonyl leaving groups:

3.2.1. Introduction:

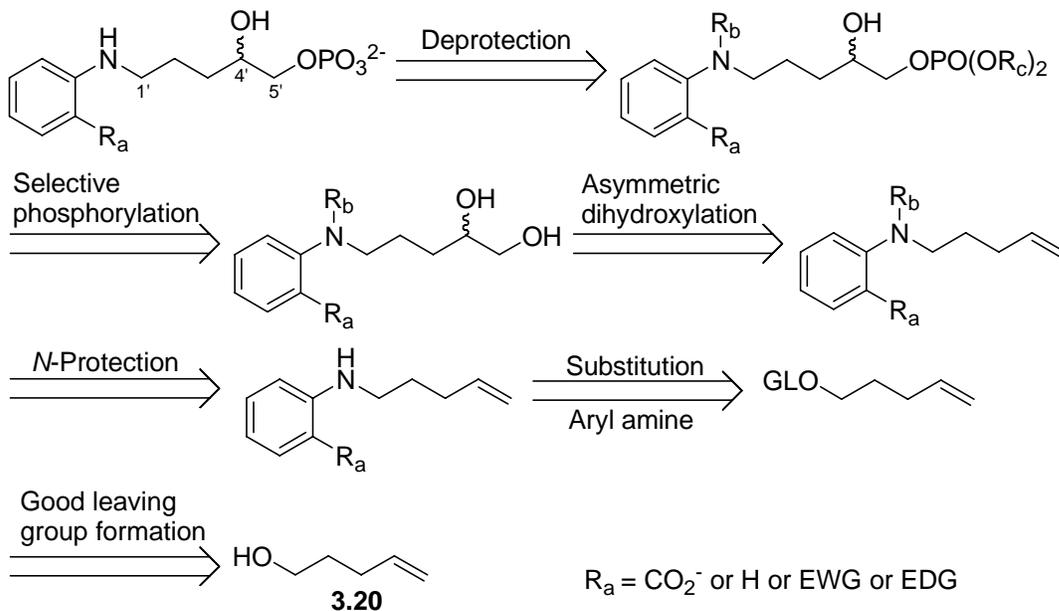
Two retrosynthetic schemes were designed to synthesise simple secondary aryl amines by nucleophilic substitutions (scheme 3.12). Mono-protected pentan-1,5-diol **3.19** would allow the unprotected hydroxyl group to be transformed into a good leaving group. Nucleophilic substitution by an aryl amine would follow to give a secondary aryl amine.

Scheme 3.12. Retrosynthetic pathway for preparation of secondary aryl amines from pentan-1,5-diol **3.19**.



4-Penten-1-ol **3.20** was the other starting material candidate due to its straightforward two-step transformation into a secondary aryl amine and its potential to form target 1,4,5 compounds (section 1.7.4) *via* asymmetric dihydroxylation (scheme 3.13).

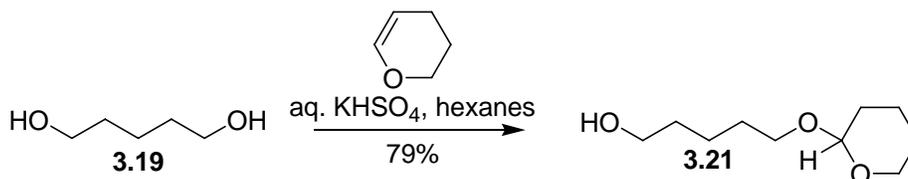
Scheme 3.13. Retrosynthetic pathway for target 1,4,5 compounds from 4-penten-1-ol **3.20**.



3.2.2. Preparation of sulfonyl leaving groups on pentan-1,5-diol:

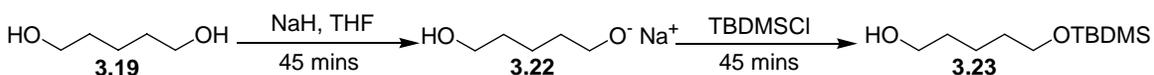
The symmetrical diol **3.19** was treated with 3,4-dihydro-2*H*-pyran (DHP) with aqueous KHSO_4 and toluene in a manner similar to Saitoh *et al.*²⁸⁸ The mono-protected tetrahydropyranyloxy pentanol **3.21** was formed in moderate yields of 54% as noted by the ^1H NMR spectrum upfield shift in neighbouring hydrogens due to the tetrahydropyranyl protecting group and an increase in the number of hydrogen signals due to the loss of symmetry. The selectivity can be attributed to: the diol **3.19** existing in the aqueous layer in a higher percentage than the mono-protected pentandiol **3.21**; the reaction occurring in the aqueous layer containing DHP and the hydronium ion; the mono-protected pentandiol **3.21** upon formation migrating from the aqueous into the toluene layer.²⁸⁸ Exchanging the aprotic solvent from toluene to hexane increased the yield to 79% (scheme 3.14).

Scheme 3.14. Pentan-1,5-diol **3.19** protection with DHP in hexanes.



The nature of the pentandiol moiety and the tetrahydropyranyl-protecting group produced a large number of overlapping signals complicating ¹H NMR spectrum, which could have prevented the ease of characterisation of subsequent reactions, hence, another mono-protection was sought. McDougal *et al.*²⁸⁹ offered a simple modification of the silylation reaction, which utilises the limited solubility of the monosodium alkoxide salt **3.22** in tetrahydrofuran (THF, scheme 3.15). The small amount of dissolved monosodium alkoxide salt **3.22** reacts with TBDMSCl, upon its addition.²⁸⁹ Critically, the rate of monosilylation of the monosodium alkoxide salt **3.22** proceeds faster than the rate of silylation of the formed monosilyl **3.23**.²⁸⁹ This can be attributed to the possibility of monosilyl **3.23** behaving like a surfactant or reverse micelles, which are expected to shield the polar hydroxyl group from further interactions with the reagents.

Scheme 3.15. Silylation of pentan-1,5-diol **3.19** using NaH.²⁸⁹



Following the McDougal *et al.*²⁸⁹ procedure for monosilyl **3.23** synthesis, the diol **3.19** was vigorously mixed with one equivalent of NaH in THF for 45 minutes before the addition of TBDMSCl (one equivalent). After 45 minutes, the time allotted by McDougal *et al.*,²⁸⁹ the reaction was shown to be only partially complete by TLC analysis since diol **3.19** was still present. After 70 minutes the reaction was stopped and subsequent purification provided a large percentage of unreacted diol **3.19** and only small amounts of monosilyl **3.23** (20%, reaction 1, table 3.01). Marquet *et al.*²⁹⁰ found slightly longer reaction durations for monosodium alkoxide salt **3.22** formation and monosilyl **3.23** synthesis improved yields. This was mimicked in reaction 2 (rxn2); however, the product yield was low and starting material was still recovered. A longer reaction duration with

TBDMSCl (rxn 3) and a higher equivalent of NaH (rxn 4) slightly improved yields, as did withholding TBDMSCl addition and potentially increasing monosodium alkoxide salt **3.22** formation (rxn 5). Attempts at improving the solubility of the monosodium alkoxide salt **3.22** by using an ultrasonic bath and gently warming did little to improve product yields, which were considerably lower than those described by McDougal *et al.*²⁸⁹

Rxn ^a #	NaH (Eq)	Duration of alkoxide salt formation	Duration ^b	Yield
1	1	45 mins	70 mins	20%
2	1	1 hr	6 hrs	40%
3	1	1 hr	13 hrs	48%
4	2	1 hr	6 hrs	44%
5	1	4 hrs	13 hrs	63%

^a 2 mL of THF per 1 mmol of diol **3.19**.

^b Duration of reaction after addition of one equivalent of TBDMSCl.

Table 3.01. Conditions used in silylation of pentan-1,5-diol **3.19** using NaH.

A more traditional approach employing imidazole and a limited quantity of TBDMSCl was more successful in our hands.²⁹¹ A general statistical mixture of diol **3.19** (30%), monosilyl **3.23** (44%) and disilyl product (22%) was recovered using stoichiometric amounts of imidazole and TBDMSCl over 14 hours (rxn 1, table 3.02). Lowering the equivalents of TBDMSCl gave moderate yields of monosilyl **3.23** with little disilyl product isolated (rxn 2-3). Ultimately 0.66 equivalents of TBDMSCl was found to form almost exclusively monosilyl **3.23** (rxn 4), as shown by the correct number of ¹H NMR signal integrals.

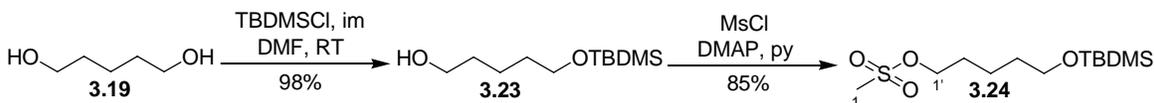
Rxn ^a #	Imidazole (Eq)	TBDMSCl (Eq) (Limiting reagent)	Duration	Monosilyl 3.23 yield
1	1	1	14 hrs	44%
2	0.8	0.8	6 hrs	70%
3	0.8	0.33	21 hrs	71%
4	0.8	0.66	20 hrs	98%

^a 1 mL of CH₂Cl₂ per 2 mmol of diol **3.19**.

Table 3.02. Conditions used in silylation of pentan-1,5-diol **3.19** using imidazole.

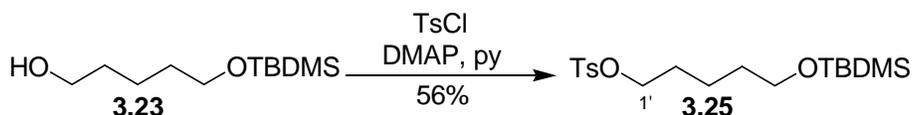
Forming a sulfonyl leaving group on the free hydroxyl initially was achieved by stirring monosilyl **3.23** in pyridine with mesyl chloride.²⁹²⁻²⁹⁴ Including DMAP in the reaction mixture increased yields of mesyl **3.24** by 20% to 85% (scheme 3.16). ¹H NMR spectroscopy showed the C1' hydrogens shifted downfield to 4.21 ppm and a new methyl peak at 2.98 ppm corresponding to the hydrogens at C1 was present.

Scheme 3.16. Mesylation of monosilyl **3.23**.



Tosylation of monosilyl **3.23** followed a procedure similar to that of mesyl **3.24** and Mash *et al.*²⁹⁵ A downfield shift of the C1' hydrogen's signal and new signals arising from hydrogens of the tosyl moiety were shown on ¹H NMR spectroscopy. DMAP improved yields in the unoptimised synthesis of tosyl **3.25** to 56% (scheme 3.17).

Scheme 3.17. Tosylation of monosilyl **3.23**.



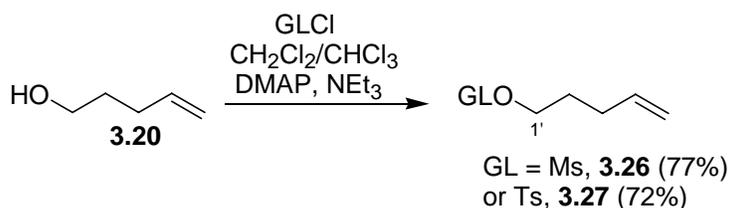
Nucleophilic substitution of mesyl **3.24** and tosyl **3.25** is described in section 3.3.2.

3.2.3. Preparation of sulfonyl leaving groups on 4-penten-1-ol:

Formation of 4-pentenyl methanesulfonyl **3.26** initially followed the procedure of Li and Marks,²⁹⁶ who described adding one equivalent of mesyl chloride slowly to a CH₂Cl₂ solution of 4-penten-1-ol **3.20** and Et₃N (3 equivalents) cooled in a ice-salt bath. After 40 minutes stirring in the ice-salt bath the reaction was quenched with ice water to give 4-pentenyl methanesulfonyl **3.26** in a reported isolated yield of 98%.²⁹⁶ In this candidate's hands following the procedure of Li and Marks²⁹⁶ gave isolated yields of only 68%. Increasing the equivalents of mesyl chloride or NEt₃ respectively did little to improve yields, nor did prolonging the reaction duration and/or allowing the reaction to warm to room temperature. The inclusion of a catalytic amount of DMAP increased yields of 4-pentenyl methanesulfonyl **3.26** to an acceptable percentage of 77% (scheme 3.18).

Tosylation of 4-penten-1-ol **3.20** followed a procedure based on White and Hrnčiar's²⁹⁷ synthesis of 4-pentenyl toluenesulfonyl **3.27**. Yields were improved in this candidate's hands by increasing the reaction duration from 3 hours to 8 hours (scheme 3.18). Nucleophilic substitution of 4-pentenyl methanesulfonyl **3.26** and 4-pentenyl toluenesulfonyl **3.27** is described in section 3.3.3.

Scheme 3.18. Formation of leaving groups on 4-penten-1-ol **3.20**.

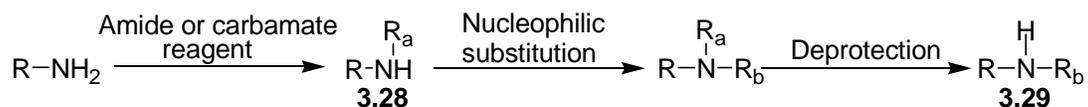


3.3. *N*-nucleophilic substitution of sulfonyl leaving groups:

3.3.1. Introduction to *N*-nucleophilic substitution:

N-Alkylation by nucleophilic substitution suffers from overalkylation forming mixtures of secondary and tertiary amines, as well as quaternary ammonium salts.^{57,196,298} Employment of the alkylating agent as the limiting reagent works to a degree to enable the selective formation of secondary amines but due to the increased nucleophilicity of secondary amines over their primary counterparts, tertiary amines will nearly always form.^{196,280} Other factors such as solubility in solvent media, amine nucleophilicity, and steric demands²⁷⁰ contribute to selectivity and yield.⁵⁷ Transforming the primary amine into a protected derivative **3.28** such as an amide (acetyl, tosyl) or carbamate (Boc, Fmoc) is widely practiced but adds additional steps to form the secondary amine **3.29** (scheme 3.19).²⁹⁹

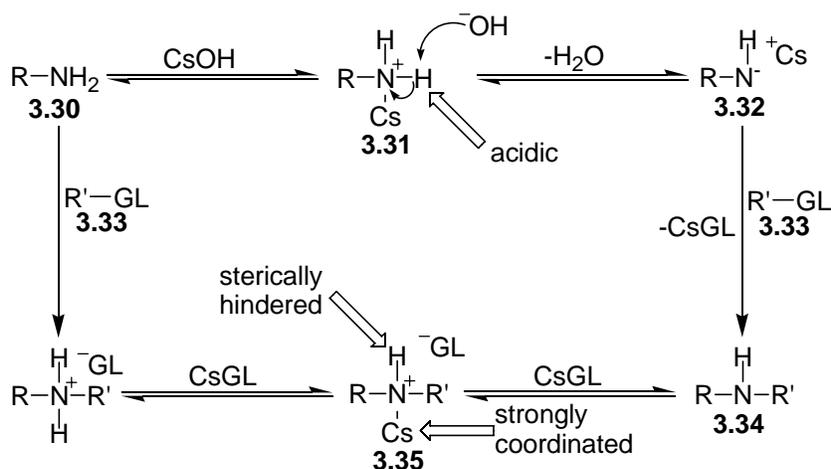
Scheme 3.19. Protection/deprotection strategy leading to the synthesis of secondary amine **3.29**.



Tuning reaction parameters so that secondary amines become predominant avoids the protection strategy and can be achieved by adjusting time, reaction temperature, and the use of additives.^{57,73} Traditionally inorganic alkali-metal bases such as K₂CO₃ and NaOH and to a lesser degree organic bases have been used to non-selectively form secondary amines. Dehmlow *et al.*³⁰⁰ showed the nucleophilic substitution between aryl amines and good leaving groups is catalysed by phase-transfer catalysts (Bu₄N⁺Cl⁻ and Bu₄N⁺Br⁻) forming subsequent intermediate complexes in the presence of inorganic bases.⁵⁷ Use of caesium bases promotes alkylation of primary amines but also suppresses overalkylation of the formed secondary amines.^{57,280,298,301,302} Jung *et al.*²⁸⁰ describe how a solution of amine **3.30**, CsOH.H₂O and activated powdered 4 Å molecular sieves (4 Å MS) in DMF

forms an amine-caesium complex **3.31** (scheme 3.20). The formation of the amine-caesium complex **3.31** is aided by the solvation of the caesium ion, weakly coordinating it to the hydroxide anion in polar aprotic solvents such as DMF.²⁸⁰ Removal of the acidic hydrogen by the hydroxide anion and water by molecular sieves forms the reactive caesium amide **3.32**, which undergoes nucleophilic substitution of a good leaving group **3.33**. The synthesised secondary amine **3.34** has stronger nucleophilicity than the corresponding primary amine **3.30** hence it forms a stronger more stable amine-caesium complex **3.35**. Due to the amine-caesium complex's **3.35** decreased nucleophilicity and steric bulk, which minimises hydrogen removal, further alkylation is suppressed.²⁸⁰

Scheme 3.20. Chemoselectivity of secondary amine **3.34** by caesium base.²⁸⁰

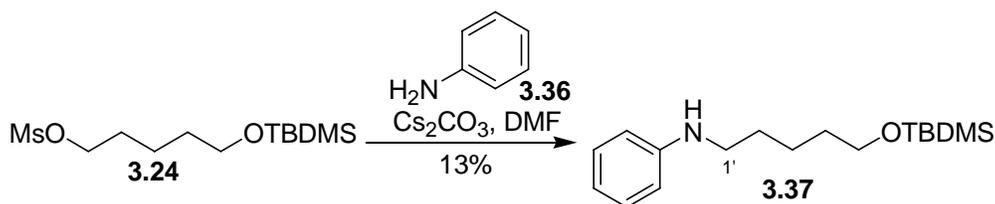


As of writing there are many examples of secondary amine synthesis using caesium bases in literature; however, there are no instances where an aryl amine has been used as the nucleophile. This gives the candidate the opportunity to build on and extend current methodology.

3.3.2. Nucleophilic substitution of sulfonyl leaving groups on pentan-1,5-diol by aniline:

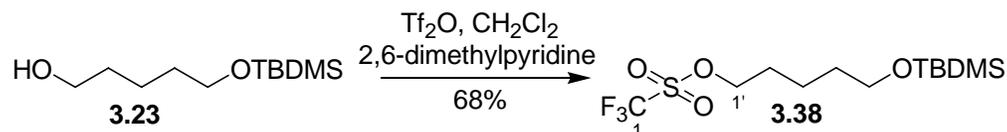
Initial attempts at substituting the mesyl group on 5-*O*-*tert*-butyldimethylsilyl-1-*O*-methanesulfonyl pentanol **3.24** with aniline **3.36** (1.5 equivalents) and the caesium base, Cs₂CO₃ (1.5 equivalents), over a prolonged reaction period at room temperature, produced low yields of the secondary aryl amine **3.37** (scheme 3.21). The secondary aryl amine **3.37** was clearly identified from mesyl **3.24** by the upfield shift in ¹H and ¹³C NMR spectra of H1' from 4.21 to 3.11 ppm and of C1' from 69.9 to 44.3 ppm. The majority of mesyl **3.24** and aniline **3.36** starting materials was recovered. An analogous substitution using tosyl **3.25** yielded a similar result.

Scheme 3.21. Use of caesium carbonate and low equivalents of aniline **3.36** in the synthesis of secondary aryl amine **3.37**.



A fresh source of Cs₂CO₃ was acquired since it was first thought that its condition was probably responsible for the low yields of secondary aryl amine **3.37**. The electrophile was changed to the more reactive sulfonyl leaving group, trifluoromethanesulfonyl as shown in scheme 3.22 by adding a cooled solution of trifluoromethanesulfonic anhydride (Tf₂O) to a cooled mixture of monosilyl **3.23** and 2,6-dimethylpyridine.^{303,304} After quenching the reaction in ice-cold H₂O the triflate **3.38** was washed with 1 M HCl and concentrated *in vacuo* to give a crude yield of 68%. An aliquot of the triflate **3.38** was withdrawn and ¹H NMR spectrum showed little starting material or impurities.

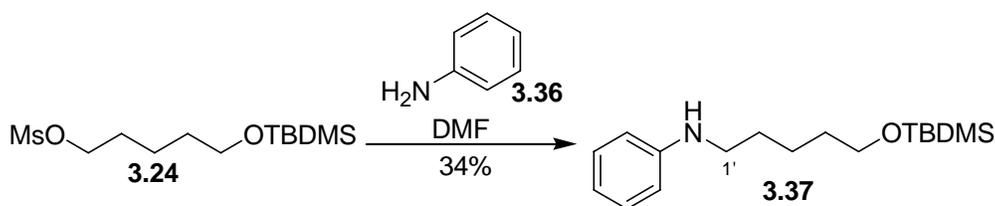
Scheme 3.22. Synthesis of 5-*O*-*tert*-butyldimethylsilyl-1-*O*-trifluoromethanesulfonyl pentanol **3.38**.^{303,304}



The unpurified triflate **3.38** was added to an ice-cooled DMF solution containing aniline **3.36** (1.5 equivalents) and Cs_2CO_3 . After 45 minutes trace amounts of secondary aryl amine **3.37** were detected on TLC at an R_f of 0.18 (20:1 hexanes- EtOAc). As the reaction proceeded over 4 hours under ice-bath conditions, more polar compounds were detected by TLC analysis. After the triflate **3.38** was completely consumed, as shown by TLC, the reaction was worked-up to give crude secondary aryl amine **3.37** and more polar by-products. Due to the low yield, purification was not attempted. It could be speculated that the triflate **3.38** decomposed at such a rate as to limit the substitution reaction occurring to any great degree. The low nucleophilic nature of aniline **3.36** could also have contributed to the lack of reactivity.

Increasing the ratio of the weakly nucleophilic aniline **3.36** to five equivalents and stirring mesyl **3.24** in DMF for a prolonged period of 48 hours gave the secondary aryl amine **3.37** in an isolated yield of 34% (scheme 3.23). Small amounts of the mesyl **3.24** were recovered accounting for the low yield, again suggesting an increase in reactivity of the nucleophile could improve yields.

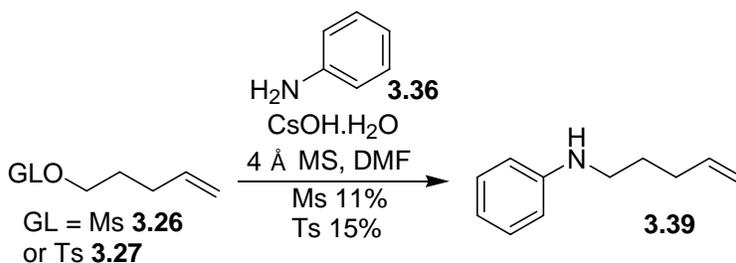
Scheme 3.23. Use of higher equivalents of aniline **3.36** in the synthesis of secondary aryl amine **3.37**.



3.3.3. Nucleophilic substitution of sulfonyl leaving groups on 4-penten-1-ol by aniline:

It has been shown by Jung *et al.*^{280,298} that using CsOH.H₂O is more efficient at forming secondary amines than Cs₂CO₃. Using the same conditions as described in section 3.3.2, scheme 3.21, except using CsOH.H₂O and changing the electrophile to 4-pentenyl methanesulfonyl **3.26**, gave disappointingly low yields of the secondary aryl amine **3.39**. Mixing crushed 4 Å molecular sieves with aniline **3.36**, and repeating the reaction did little to improve yields (scheme 3.24). Yields still remained low when the electrophile was changed to 4-pentenyl toluenesulfonyl **3.27** (scheme 3.24). The products isolated for all three reactions contained the starting material electrophile and aniline **3.36**.

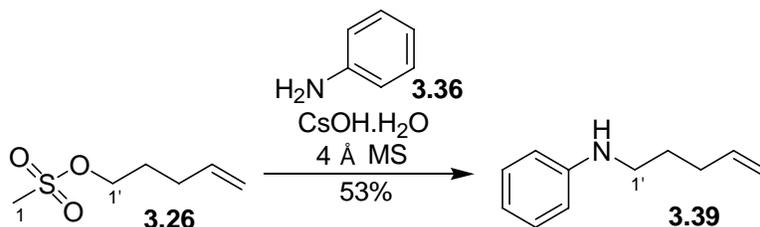
Scheme 3.24. Use of low equivalents of aniline **3.36** in the synthesis of secondary aryl amine **3.39**.



Increasing the ratio of aniline **3.36** to 30 equivalents and removing the solvent gave a dramatic increase in yield of secondary aryl amine **3.39** (scheme 3.25). TLC clearly showed a disappearance of 4-pentenyl methanesulfonyl **3.26** at an R_f of 0.35 (3:1 hexanes-EtOAc) and formation of a less polar spot, which was deduced by NMR spectroscopy to be the secondary aryl amine **3.39**. ¹H NMR spectra of the crude mixture showed H1' from 4-pentenyl methanesulfonyl **3.26** shifted from 4.21 ppm to 3.13 ppm with formation of the product. There was no signal for 4-pentenyl methanesulfonyl **3.26** methyl hydrogens (H1) at 2.99 ppm nor was there any overalkylated tertiary amine present. The purification *via* flash column chromatography proved challenging as aniline **3.36** and the secondary aryl amine **3.39** have similar R_f values. This could explain the encouraging yet only moderate isolated yields of product. The downfield region of the ¹H

NMR spectra between 6.50 and 7.50 ppm was defined by three distinct signals corresponding to the hydrogens of the secondary aryl amine and of the excess aniline.

Scheme 3.25. Neat aniline **3.36** in the synthesis of secondary aryl amine **3.39**.



Clearly using high equivalents of aniline **3.36** increased the rate of substitution of sulfonyl leaving groups. An investigation into whether changes in the leaving groups from sulfonyl to halogens, specifically bromides, improves the yields follows.

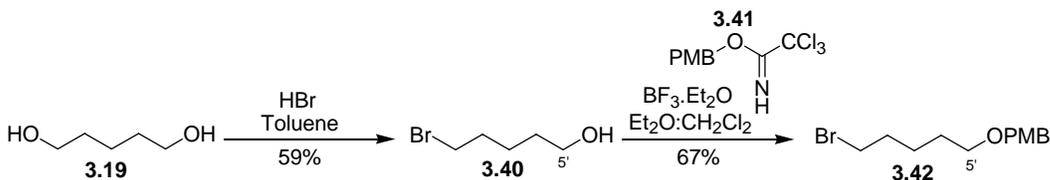
3.4. *N*-nucleophilic substitution of bromide moieties:

3.4.1. Preparation of bromide moieties from pentan-1,5-diol and 4-penten-1-ol:

Monobromination of pentan-1,5-diol **3.19** is amply described in literature. Many examples include heating diol **3.19** with aqueous HBr using a continuous extraction apparatus and employing non-polar solvents. Kang *et al.*³⁰⁵ found a more efficient, selective and convenient method to produce bromopentan-5-ol **3.40** by azeotropic removal of water, eliminating the need for continuous extraction of the product mixture. More recently Chong *et al.*³⁰⁶ have debated the need for removing water and have produced higher yields of bromopentan-5-ol **3.40** by simply refluxing HBr in toluene.

Procedures by both Chong *et al.*³⁰⁶ and Kang *et al.*³⁰⁵ were followed as shown in the first two reactions in table 3.03. Both employed a mole ratio of approximately 3:2 HBr to diol **3.19**. In this candidate's hands both procedures produced lower yields than reported in literature. Similar yields of bromopentan-5-ol **3.40** were produced with or without Dean-Stark apparatus, as shown in reactions 3 and 4 (scheme 3.26).

Scheme 3.26. Synthesis of 5-*O*-*para*-methoxybenzyl bromopentanol **3.42** from pentan-1,5-diol **3.19**.



All other parameters were kept constant. The crude mixture from either method contained bromopentan-5-ol **3.40** and very small amounts of dibromo product, with the remainder containing the starting material diol **3.19**. The non-statistical mixtures of products and starting materials can be potentially explained by the relatively lower reactivity of bromo alcohols under these reaction conditions and the possibilities that these behave like surfactants and reverse micelles.^{289,306} All three products were separated by Kugelrohr distillation. Interestingly changing pentan-1,5-diol **3.19** to hexan-1,6-diol increased yields of the monobromo product (rxn 5).

Rxn #	Solvent	Method	Duration	Yield of 3.40
1 ³⁰⁶	Toluene	Reflux	9 hrs	38%
2 ³⁰⁵	Benzene	Water removal	29 hrs	56%
3 ³⁰⁶	Toluene	Water removal	21 hrs	57%
4 ³⁰⁵	Toluene	Reflux	21 hrs	59%
5 ^{306,a}	Toluene	Reflux	13 hrs	74%

^a Hexan-1,6-diol was used instead of pentan-1,5-diol.

References: ^{305,306}

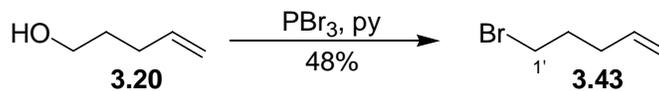
Table 3.03. Conditions used in the synthesis of bromopentan-5-ol **3.40**.

para-Methoxybenzyl trichloroacetimidate is known to protect hydroxyl groups under acid conditions, which was necessary due to the nature of bromopentan-5-ol **3.40**.³⁰⁷⁻³⁰⁹ Once the secondary aryl amine was synthesised, selective removal of the *para*-methoxybenzyl (PMB) group could be achieved by oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) thus eliminating any interference with the aryl

amine.^{310,311} *para*-Methoxybenzyl trichloroacetimidate **3.41** was synthesised according to the procedure of Patil.¹⁷⁴ A solution of the acetimidate **3.41** (1.6 equivalents) in Et₂O was treated with bromopentan-5-ol **3.40** with various different acid catalysts (~2-3% per mol), which included titanium chloride, tosyl sulfonic acid, trifluoromethane sulfonic acid and boron trifluoride etherate. Titanium chloride, tosyl sulfonic acid, and trifluoromethane sulfonic acid³⁰⁷ were found to result in isolated yields of 5-*O*-*para*-methoxybenzyl bromopentanol **3.42** of between 42 and 50%. TLC analysis and ¹H NMR spectroscopy monitored the reaction's progress and without optimisation boron trifluoride etherate³⁰⁸ gave the highest isolated yield of 67% (scheme 3.26). Isolation of the crude product *via* flash chromatography removed the numerous but small amounts of by-products and ¹H NMR spectra verified the product's identity to be 5-*O*-*para*-methoxybenzyl bromopentanol **3.42** by H5' shift from 3.65 to 3.45 ppm and the addition of signals from the methoxybenzyl moiety.

After the successful synthesis of a protected bromo alcohol attention turned to synthesising bromo-4-pentene **3.43** from 4-penten-1-ol **3.20**. Using the procedure of Iranpoor *et al.*,³¹² stirring 4-penten-1-ol **3.20** with triphenylphosphine (Ph₃P), DDQ and tetrabutylammonium bromide at room temperature did not produce the desired product in any great yield. Large amounts of starting materials were recovered. A method originally described by Johnson and Owyang³¹³ and later modified by Kitching *et al.*³¹⁴ using neat phosphorus tribromide and pyridine, proved however, more successful (scheme 3.27). Vacuum distillation of the crude oil furnished pure bromo-4-pentene **3.43** in 48% yield and was verified by literature boiling point value³¹⁴ and by NMR spectra showing upfield shifts of C1' and H1'.

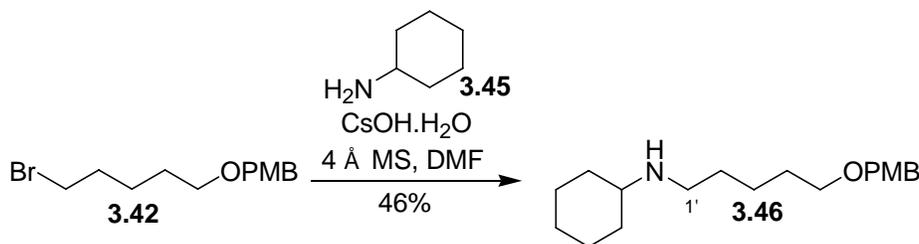
Scheme 3.27. Synthesis of bromo-4-pentene **3.43** by PBr₃ and pyridine.^{313,314}



3.4.2. *N*-nucleophilic substitution of 5-*O*-*para*-methoxybenzyl bromopentanol and bromo-4-pentene:

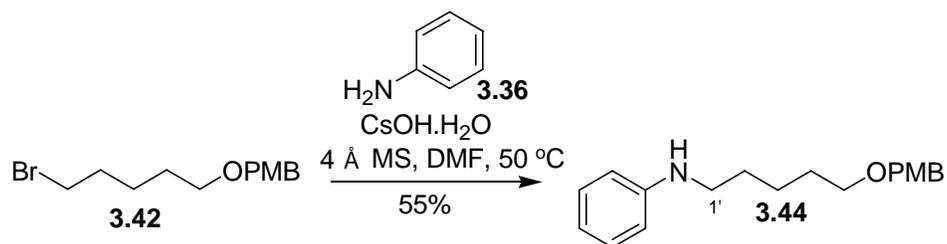
Two equivalents of aniline **3.36** were added to 5-*O*-*para*-methoxybenzyl bromopentanol **3.42** in an experiment parallel to those carried out in section 3.3.3 and to those described by Jung *et al.*^{280,298} The secondary aryl amine **3.44** was synthesised after stirring at room temperature for 18 hours and isolated *via* flash chromatography (rxn 1, table 3.04). As with other reactions using low equivalents of aniline **3.36**, yields of the desired product were low with the starting materials being recovered and no tertiary amine isolated. Exchanging aniline **3.36** for cyclohexylamine **3.45** and following the same procedure gave considerably higher yields of the secondary amine **3.46**; however, trace amounts of starting materials were still isolated (scheme 3.28). Isolated secondary amine **3.46** was clearly distinguished from 5-*O*-*para*-methoxybenzyl bromopentanol **3.42** by an upfield shift in the ¹H NMR signal of H1' from 3.40 to 2.50 ppm and by a proportional increase in integration of ¹H signals between 1.17-1.66 ppm corresponding to the cyclohexyl hydrogens.

Scheme 3.28. Secondary amine **3.46** synthesis using low equivalents of cyclohexylamine **3.45**.



Increasing the equivalents of aniline **3.36** to 30 and removing DMF as the solvent increased yields of secondary aryl amine **3.44** to 51% (rxn 2, table 3.04). The large volume of aniline **3.36** made purification difficult. Gently warming the reaction to 40 °C (rxn 3) increased the yield slightly over 18 hours compared to reaction 1. Starting materials were recovered so the temperature was raised to 50 °C (rxn 4), ultimately giving the highest yields of secondary aryl amine **3.44** (scheme 3.29).

Scheme 3.29. Synthesis of secondary aryl amine **3.44** *via* heating.



The product's identity was confirmed by an upfield shift in the signal of H1' to 3.16 ppm and by the aryl hydrogen's signals integrating to the expected value. No tertiary aryl amine or starting materials were recovered. Heating the reaction to 110 °C produced a large mixture of by-products and decomposed starting materials (rxn 5), while slightly increasing the temperature from reaction 4 did not increase yields and returned the same unidentified non-polar by-products (rxn 6).

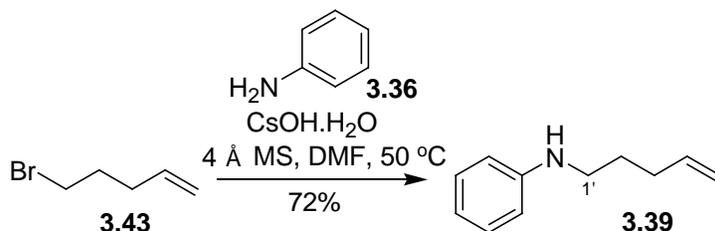
Rxn #	Aniline (Eq)	Temperature	Duration	Yield of 3.44
1	1.5	rt	18 hrs	18%
2 ^a	30	rt	19 hrs	51%
3	2	40 °C	18 hrs	24%
4	2	50 °C	19 hrs	55%
5	2	110 °C	12 hrs	5%
6	2	55 °C	18 hrs	51%

^a Neat.

Table 3.04. Conditions used in the synthesis of secondary aryl amine **3.44**.

Reaction conditions in table 3.04 were transferred to synthesise 1-phenylamino-4-pentene **3.39** (scheme 3.30). Results mirrored the outcomes of previous reactions with low yields of product isolated when low equivalents of aniline were used (rxn 1, table 3.05).

Scheme 3.30. Synthesis of 1-phenylamino-4-pentene **3.39** using heat and high equivalents of aniline **3.36**.



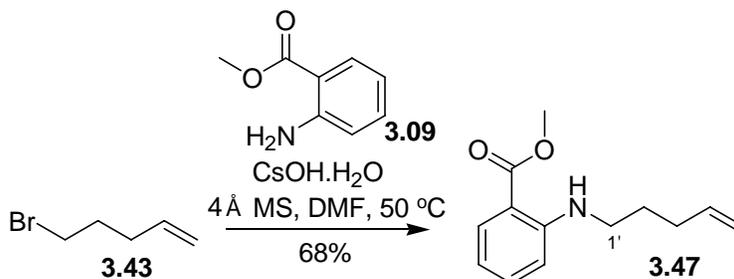
Warming the reaction to 50 °C increased yields of 1-phenylamino-4-pentene **3.39** (rxn 2), as did using large equivalents of aniline **3.36**, this time in the presence of DMF (rxn 3). Combining these parameters produced a 72% isolated yield of 1-phenylamino-4-pentene **3.39**, the highest yield of a secondary aryl amine involving nucleophilic substitution (rxn 4).

Rxn #	Aniline (Eq)	Temperature	Duration	Yield of 3.39
1	1.5	rt	20 hrs	11%
2	2	50 °C	18 hrs	55%
3	30	rt	18 hrs	56%
4	30	50 °C	15 hrs	72%

Table 3.05. Conditions used in the synthesis of 1-phenylamino-4-pentene **3.39**.

Stirring excess equivalents of anthranilate methyl ester **3.09** in a 50 °C solution of bromo-4-pentene **3.43**, CsOH.H₂O and 4 Å molecular sieves over 72 hours produced anthranilate methyl ester-4-pentene **3.47** in good yield (scheme 3.31). ¹H NMR spectra confirmed this, due to the appropriate H1' upfield signal shift, aryl and methyl ester hydrogen signals appearing and the expected integral ratios. Bromo-4-pentene **3.43** was completely consumed and no tertiary amine was recovered. Now that reliable methodology to produce secondary aryl amines had been identified, attention was directed towards formation of target 1,4,5 compounds as depicted in scheme 3.13 (section 3.2.1).

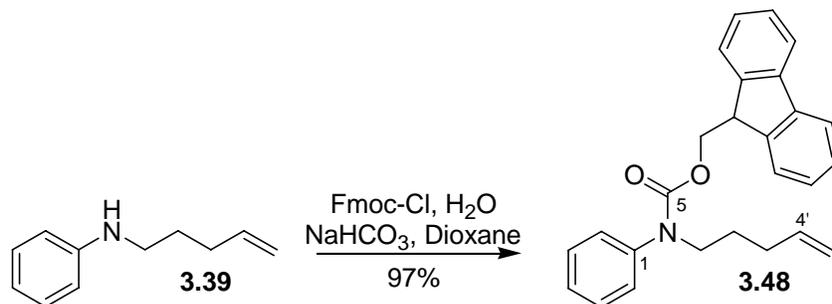
Scheme 3.31. Synthesis of anthranilate methyl ester-4-pentene **3.47** using heat and high equivalents of anthranilate methyl ester **3.09**.



3.4.3. Synthesis of a target 1,4,5 compound *N*-*tert*-butyloxycarbonyl-1-phenylamino-5-diethyl phosphate-4-pentanol:

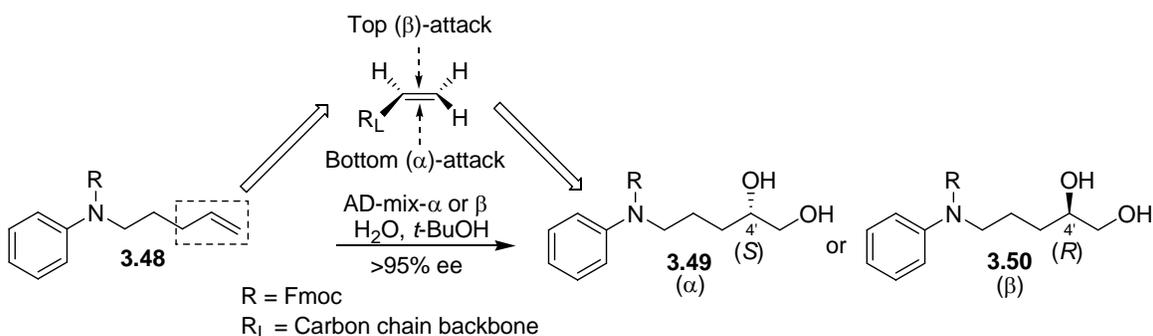
1-Phenylamino-4-pentene **3.39** was the amine initially chosen to develop methodology leading to target 1,4,5 compounds, compounds with functionalities at the respective 1,4,5 positions along the pentyl moiety. Protecting the amine group before oxidation of the alkene and subsequent phosphorylation was the next step. Aryl amines are sensitive to oxidation due to the electron availability in the system and they are also known to interact with phosphate moieties as discussed in section 2.9.3.²⁶⁶ A method described by Carpino and Han³¹⁵ employed an inorganic base and 9-fluorenylmethyl chloroformate (Fmoc-Cl), a high yielding amino-protecting reagent. After 3 hours stirring 1-phenylamino-4-pentene **3.39** with K_2CO_3 in H_2O /dioxane following Carpino and Han's³¹⁵ procedure, a faint spot on TLC at a lower R_f than the starting material appeared. A ~5% aliquot of the crude reaction mixture showed two signals in the ^{13}C NMR spectrum for C1, 148.8 ppm corresponding to the amine **3.39** and 141.7 ppm from the suspected *N*-protected amine **3.48** product (scheme 3.32). A large excess of Fmoc-Cl complicated the spectrum between 115 to 145 ppm. A signal further downfield at 155.8 ppm suggested this was the product's C5 signal. Driving the reaction to completion over 20 hours gave 63% isolated yield of *N*-protected amine **3.48**. The ^1H NMR spectrum showed correct integral ratios and appropriate ^1H signal shifts, and ^{13}C NMR confirmed the identity. Changing the inorganic base to NaHCO_3 drove isolated yields up to 97%.

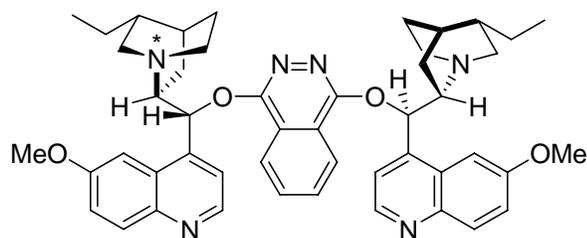
Scheme 3.32. Synthesis of *N*-9-fluorenylmethoxycarbonyl-1-phenylamino-4-pentene **3.48**.³¹⁵



Asymmetric dihydroxylation of the *N*-protected amine **3.48** could come about using commercially available “AD-mix”. The premix contains reagents, potassium osmate [$K_2OsO_2(OH)_4$], chiral ligand, K_2CO_3 and potassium ferricyanide [$K_3Fe(CN)_6$] a stoichiometric reoxidant. Sharpless *et al.*³¹⁶⁻³¹⁸ developed this osmium-catalyst for asymmetric dihydroxylation of alkenes in the late 1980’s to early 1990’s. Two varieties of the mix, “ α ” or “ β ”, denote the different ligands. The alkene is selectively oxidised, relating to the ligand acceleration effect, which ensures that the reaction is funneled through a less hindered pathway involving the chiral catalyst giving either respective diol **3.49** or **3.50** in high enantiomeric excess (>95% ee, scheme 3.33). Dihydroquinine phthalazine (DHQ)₂-PHAL is the chiral ligand coupled to osmium, which is employed in AD-mix- α (figure 3.02).³¹⁷

Scheme 3.33. Overview of asymmetric dihydroxylation using either AD-mix- α or β .³¹⁶⁻³¹⁸



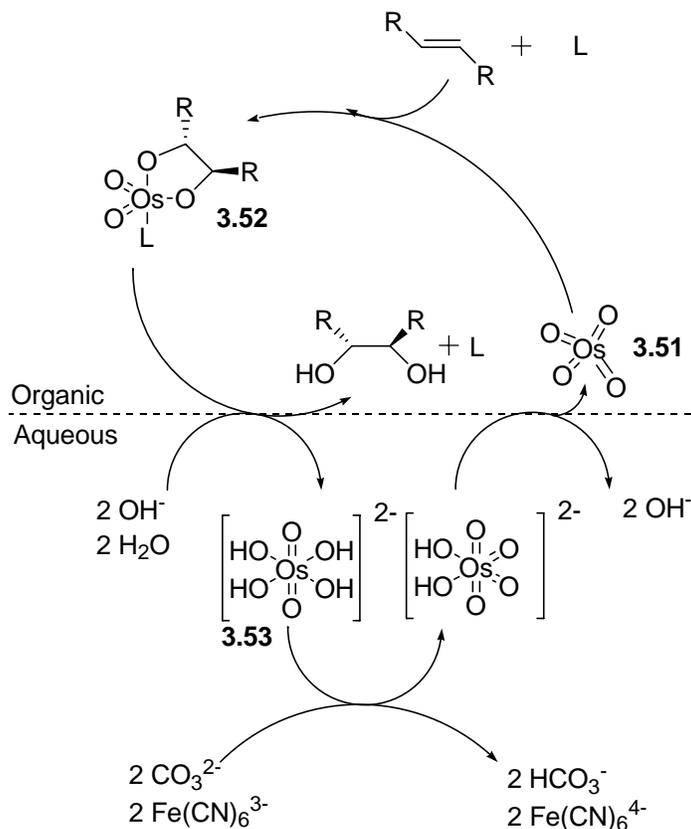


Legend: The osmium-nitrogen bonding site is noted by the symbol *.

Figure 3.02. Chiral ligand of AD-mix- α .³¹⁷

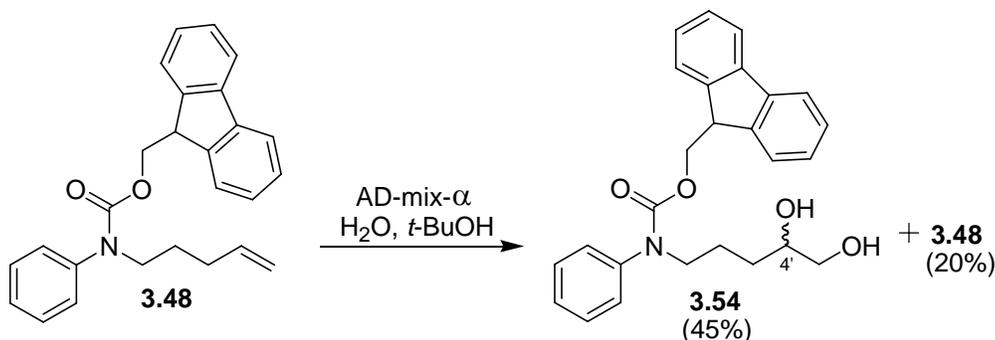
AD-mix is best used under two-phase conditions where the oxidant, OsO_4 **3.51**, reacts with the alkene in the presence of ligand (L). The osmium(VI) monoglycolate ester **3.52** undergoes hydrolysis, releasing diol and ligand to the organic layer and Os(VI) **3.53** to the aqueous layer before it is reoxidised by potassium ferricyanide (scheme 3.34).³¹⁶⁻³¹⁸

Scheme 3.34. Catalytic cycle of the AD reaction with $\text{K}_3\text{Fe(CN)}_6$ as the cooxidant.^{317,319}



Following the procedure prescribed by Sharpless *et al.*³¹⁸ one mmol of *N*-protected amine **3.48** was added to a heterogeneous mixture of H₂O, *t*-BuOH and 1.4 g of AD-mix- α . After stirring the reaction for 24 hours at 0 °C, diol **3.54** and starting material **3.48** were isolated in 35% and 45% yield respectively. TLC clearly showed quantities of the diol **3.54** forming at a lower *R_f* than the starting material **3.48** early in the reaction. The ¹H NMR spectrum clearly showed an upfield shift of H4' and H5' signals and a change from 138.3 and 115.4 ppm to 71.9 and 67.0 ppm for the ¹³C signals from the diols C4' and C5' atoms respectively. Enantiomeric excess was not measured but the predicted stereochemical outcome would be (*S*) for C4'. In an effort to boost yields of diol **3.54**, higher equivalents of AD-mix- α were added to subsequent reactions. The highest yield of diol **3.54** occurred when 1.9 g of AD-mix- α was added per one mmol of *N*-protected amine **3.48** and the reaction was allowed to stir at 4 °C for 18 hours (scheme 3.35).

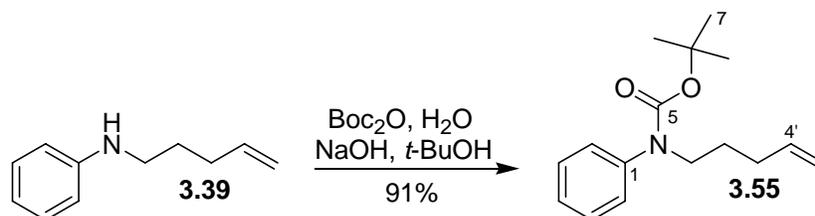
Scheme 3.35. Synthesis of *N*-9-fluorenylmethoxycarbonyl-1-phenylamino-4,5-pentan-diol **3.54** using AD-mix- α .³¹⁸



Since literature boasts of high yields for similar reactions, it was thought the Fmoc *N*-protecting group might be sterically hindering access to the alkene. Fmoc was replaced with *tert*-butyloxycarbonyl (Boc)²⁹⁹ based on a procedure Kemp and Carey³²⁰ developed. This involved adding 1-phenylamino-4-pentene **3.39** to a solution of di-*tert*-butyl dicarbonate anhydride (Boc₂O) and di-*iso*-propylethylamine (DIEA) in MeCN. Stirring at room temperature for 96 hours produced a good isolated yield of Boc *N*-protected amine **3.55**. Different reaction temperatures and higher equivalents of the reagents were used to increase the yield. The best results came from using 1.3 equivalents of Boc₂O and 1.1

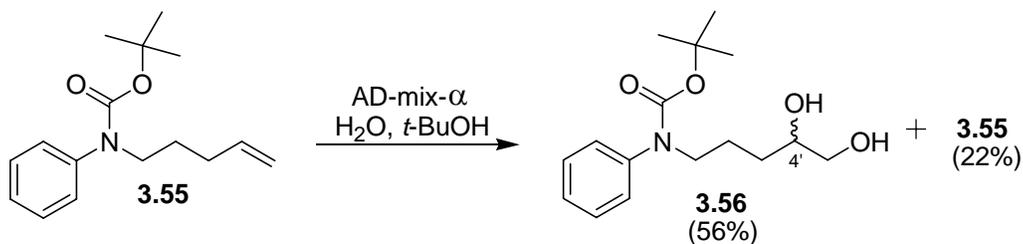
equivalents of DIEA stirring at 40 °C for 48 hours, giving Boc *N*-protected amine **3.55** in 82% yield. An alternative procedure based on work described by Kocienski¹²⁴ and Greene and Wutz¹²⁵ used a heterogeneous solution of 2.5% NaOH, Boc₂O, *t*-BuOH stirred with the amine **3.39** (scheme 3.36). After 96 hours at room temperature TLC of the crude reaction mixture showed no starting material, instead a large spot at *R_f* 0.17 (6:1 hexanes- EtOAc) was visible. This was confirmed to be Boc *N*-protected amine **3.55** *via* a ¹³C NMR spectrum, which showed a shift in the C1 signal from 148.8 ppm, corresponding to the amine **3.39**, to 143.0 ppm and a 155.1 ppm signal from C5 further downfield. A ¹H NMR spectrum showed correct integral ratios and appropriate ¹H signal shifts, including the *tert*-butyl's methyl hydrogens (H7) at 1.40 ppm.

Scheme 3.36. Synthesis of *N*-*tert*-butyloxycarbonyl-1-phenylamino-4-pentene **3.55** using a heterogeneous system and NaOH.



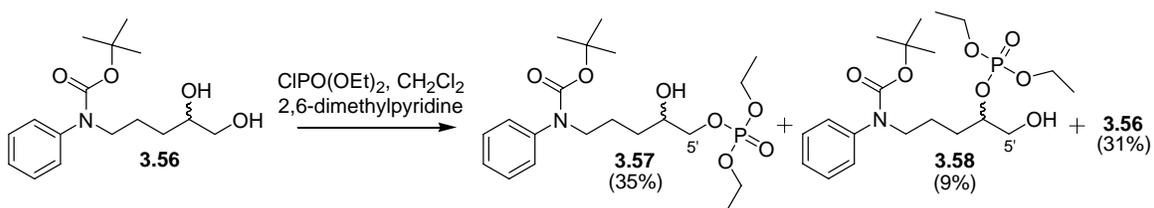
Disappointingly following the AD procedure of Sharpless *et al.*³¹⁸ with the Boc *N*-protected amine **3.55** produced similar yields to the synthesis of Fmoc *N*-protected diol **3.54** of around 50%. Increasing the amount of AD-mix- α to 1.9 g per one mmol of Boc *N*-protected amine **3.55** and allowing the reaction to stir at 4 °C for 25 hours gave the highest isolated yields (scheme 3.37). Lengthening the reaction duration and/or adding additional amounts of AD-mix- α did not improve yields or reduce the amount of starting material recovered. NMR spectra of diol **3.56** showed appropriate shifts, including the definitive ¹H signals from H4' and H5' between 3.30 and 3.64 ppm respectively. Enantiomeric excess again was not measured.

Scheme 3.37. Synthesis of *N-tert*-butyloxycarbonyl-1-phenylamino-4,5-pentanol **3.56** using AD-mix- α .³¹⁸



Phosphorylating the diol **3.56** formed the protected target 1,4,5 compound, *N-tert*-butyloxycarbonyl-1-phenylamino-5-diethyl phosphate-4-pentanol **3.57**, in moderate yield using one equivalent of 2,6-dimethylpyridine and 1.1 equivalents of diethyl chlorophosphate over eight hours (scheme 3.38).³²¹ The product's formation was apparent by a downfield shift of the H5' signal on a ¹H NMR spectrum compared with the diol **3.56**. The desired product formed in a 3:1 ratio with the secondary phosphate **3.58**. Isolation of the two products by flash chromatography proved challenging since their *R_f* values were close together. The tedious nature of the purification may account for the loss of material. The ¹H NMR spectrum for the target compound **3.57** showed a signal for H4' between 3.45 and 3.59 ppm, whereas the secondary phosphate **3.58** H4' signal was between 3.53 and 3.70 ppm. The ¹³C NMR spectrum also showed a C4' signal shift from 70.2 ppm with the target compound **3.57** to 72.6 ppm for the secondary phosphate **3.58**. Continuing the reaction for more than 8 hours proved problematic since more secondary phosphate **3.58** was produced, further complicating purification. Using pyridine lowered the selectivity of phosphorylation. It is hoped that deprotected target compound **3.57** will be used in future PRAI and IGPS enzyme studies.

Scheme 3.38. Synthesis of *N-tert*-butyloxycarbonyl-1-phenylamino-5-diethyl phosphate-4-pentanol **3.57**.



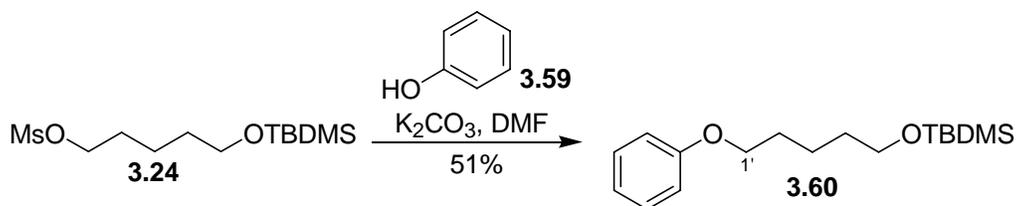
3.5. Nucleophilic substitution of *O*- and *C*- nucleophiles:

3.5.1. Substitution of good leaving groups on pentan-1,5-diol moieties by *O*- and *C*- nucleophiles:

After successfully forming secondary aryl amines using nucleophilic substitution, the next task was preliminary synthesis of model *O*- and *C*- target compounds. Alternative *O*- and *C*- target compounds were discussed in section 1.7.2.

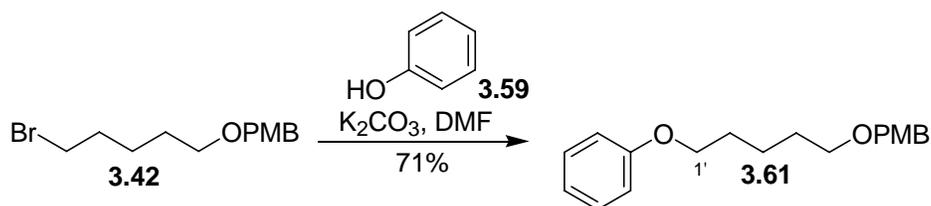
Phenol **3.59** was added to a DMF solution containing mesyl silyl pentanol **3.24** and K_2CO_3 . The reaction was stirred at room temperature until no mesyl silyl pentanol **3.24** remained visible on TLC (scheme 3.39). This was replaced with a more non-polar spot at an R_f of 0.35 (6:1 hexanes- EtOAc), which was later deduced by NMR spectroscopy to be 5-*O*-*tert*-butyldimethylsilyl-1-phenoxy-pentane **3.60**. The phenol ether moiety induced a notable 1H NMR signal shift from 4.21 to 3.70 ppm for H1'. Using aqueous NaOH instead of K_2CO_3 produced slightly lower yields.

Scheme 3.39. Substitution of mesyl **3.24** by phenol **3.59** at room temperature.



A second reaction involving phenol **3.59**, on this occasion using a procedure detailed by Miyata *et al.*,³²² displaced the bromo group on the *O*-protected bromopentanol **3.42** giving high yields of 5-*O*-*para*-methoxybenzyl-1-phenoxy-pentane **3.61**. Yields were slightly improved to 71% by lowering the temperature from 75 °C as described by Miyata *et al.*³²² to room temperature (scheme 3.40). The proximity of the phenol ether moiety shifted H1' 1H NMR signal from 3.40 to 3.55 ppm. As with the mesyl substitution, the small isolated amount of by-product was unidentifiable.

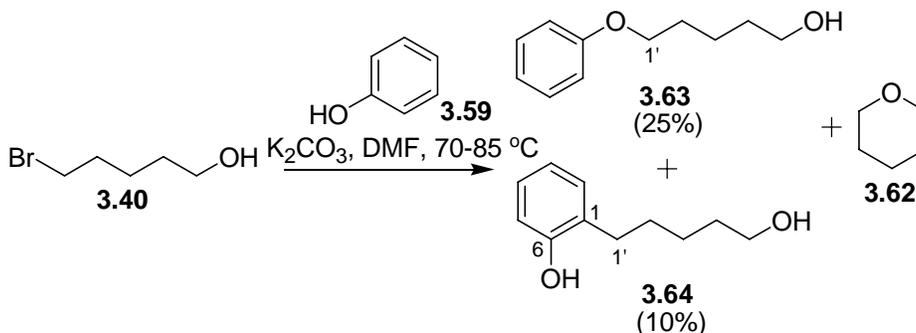
Scheme 3.40. Substitution of bromide **3.42** by phenol **3.59** at room temperature.



Miyata *et al.*³²² showed phenol **3.59** could react with bromohexan-6-ol forming phenoxyhexan-6-ol in 96% by heating the DMF solution with K_2CO_3 at 80 °C for an hour. This procedure avoids the need to protect bromopentan-5-ol **3.40**. After heating bromopentan-5-ol **3.40** and phenol **3.59** at 70 °C for 90 minutes in a parallel procedure to Miyata *et al.*,³²² TLC still showed trace amounts of bromopentan-5-ol **3.40**. Two strong non-polar spots relative to the starting material were also visible. The temperature was increased to 85 °C for a further 15 minutes before all trace of bromopentan-5-ol **3.40** disappeared as determined by TLC. After working up the reaction, 1H and ^{13}C NMR spectra of the crude material indicated tetrahydropyran **3.62** had been formed along with a potential mixture of compounds as shown by the numerous signals between 6.8 and 7.4 ppm on 1H and 114 and 160 ppm on ^{13}C NMR spectrum. Further investigation of the rotary evaporator's volatiles trap revealed a large amount of the tetrahydropyran **3.62** had been evaporated off the crude mixture prior to obtaining the NMR spectrum. A combined yield of the tetrahydropyran **3.62** formed was not acquired. Purification of the crude material by flash chromatography afforded the desired product 1-phenoxy-pentanol **3.63** (25%) and 2-(5-hydroxy-pentyl)-phenol **3.64** (10%, scheme 3.41). Confirmation of 1-phenoxy-pentanol **3.63** synthesis can be deduced from a downfield signal shift to 3.96 ppm for H1' from 3.43 ppm on bromopentan-5-ol **3.40**. NMR spectra of 1-phenoxy-pentanol **3.63** also showed definitive 1H signals and integral ratios for the phenol ether ring and pentanol chain. 2-(5-Hydroxy-pentyl)-phenol **3.64** formed through aromatic electrophilic substitution and was characterised by a number of NMR techniques. 1H NMR spectrum showed a considerable upfield shift for H1' to 2.65 ppm, lower than what was expected if it was bonded to the phenol moiety. Four signals for aryl hydrogens between 6.99 and 7.31 ppm were displayed, which signified the aryl moiety was non-symmetrical in nature. 1H coupling *via* $^1H/^1H$ COSY spectroscopy confirmed the

positions of the hydrogens. Integral ratios and signal shifts verified the structure to be 2-(5-hydroxy-pentyl)-phenol **3.64**. A ^{13}C NMR spectrum showed C6 bonding to the hydroxyl due to its highly deshielded signal at 159.5 ppm. The upfield signal of C1' at 34.0 ppm showed the carbon was not bonding directly to the phenol ether oxygen but rather C1. Interestingly there was no 4-(5-hydroxy-pentyl)-phenol (*para* substituted) product isolated suggesting bromopentan-5-ol **3.40** stabilised the formation of the *ortho* product **3.64**. Little information or precedent was found in the literature for aromatic electrophilic substitution reactions solely directing to the *ortho* position when the *para* position was available. Further investigation into this *ortho* substitution is needed in order to fully explain its occurrence.

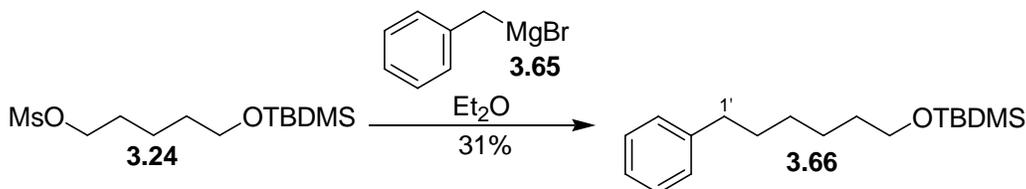
Scheme 3.41. Mixture of products formed when phenol **3.59** substituted bromopentan-5-ol **3.40**.



A C-nucleophile in the form of benzyl magnesium bromide **3.65** was synthesised using the procedure of Zimmerman *et al.*³²³ Mesyl **3.24** in Et_2O was added dropwise to an 0 °C solution of benzyl magnesium bromide³²³ **3.65** (1.6 equivalents) in Et_2O . Less polar spots compared to mesyl **3.24** started appearing instantly on TLC. After three hours stirring at 0 °C the mesyl **3.24** spot was diminishing so the reaction was allowed to warm to room temperature and stir overnight for a total of 17 hours. The crude reaction mixture showed an absence of starting materials. Isolation of the less polar spots provided 5-(*tert*-butyldimethylsilyl ether)-1-phenyl-hexane **3.66** in 31% yield (scheme 3.42). A ^1H NMR spectrum showed a signal of 2.65 ppm for H1', a significant shift from the starting materials H1' position. The by-products were uncharacterisable.

Use of benzyl magnesium bromide³²³ was extended to 4-pentenyl methanesulfonyl **3.26**. The corresponding alkene was epoxidised, leading to an attempt to ring open the epoxide with a phosphate moiety in an effort to synthesise a target 1,4,5 compound. This reaction pathway is described in the following section.

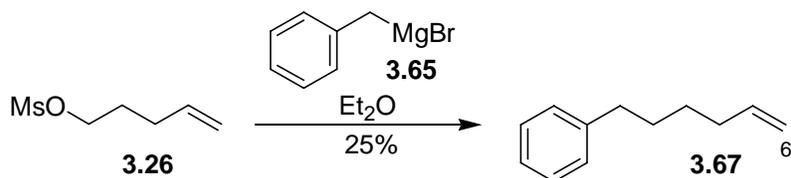
Scheme 3.42. Substitution of mesyl **3.24** with benzyl magnesium bromide **3.65**.



3.5.2. Attempted synthesis of target 1,4,5 compound using a C- nucleophile:

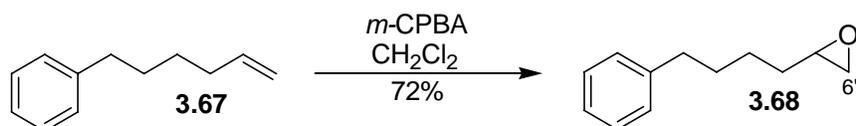
Using a similar method for the formation of protected phenyl-hexane **3.66**, 4-pentenyl methanesulfonyl **3.26** was added dropwise to a 0 °C solution of benzyl magnesium bromide³²³ **3.65** in Et₂O. A slow reduction of starting materials was perceptible by monitoring TLC silica plates over an 8-hour period during which, the reaction was stirred at 0 °C. As with the synthesis of protected phenyl-hexane **3.66**, several extremely non-polar spots became evident over this period. The reaction was allowed to warm to room temperature and stir for 32 more hours at which time the starting materials had been completely consumed. A ¹H NMR spectrum showed a complex mixture of products after the crude reaction mixture was worked up. Isolation of a spot on TLC at an *R_f* of 0.46 (100% hexanes) *via* flash chromatography on silica produced pure hex-5-enylbenzene **3.67** in low yield (scheme 3.43). This compounds ¹³C NMR spectrum was characterised by four alkane signals between 28.6 (C3') and 35.8 ppm (C1'), two alkene signals at 114.4 (C6') and 138.8 ppm (C5'), and four signals corresponding to the aryl carbon centers of the benzene moiety. Separation of the additional by-product was not achieved due to their high non-polar nature. It is possible benzyl magnesium bromide³²³ used was not at its best condition.

Scheme 3.43. Synthesis of hex-5-enylbenzene **3.67** via substitution of 4-pentenyl methanesulfonyl **3.26** with benzyl magnesium bromide **3.65**.



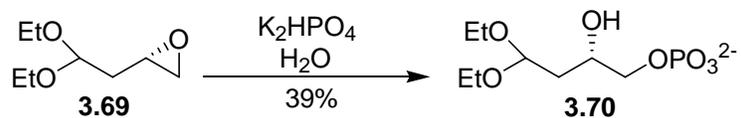
Epoxidation of hex-5-enylbenzene **3.67** by *m*-CPBA (*meta*-chloroperoxybenzoic acid) was achieved in 72% yield over 18 hours (scheme 3.44). 5,6-epoxyhexylbenzene **3.68** was identified via a ^{13}C NMR spectrum containing the signal shift of 47.1 ppm for $\text{C}6'$, and 53.2 ppm for the $\text{C}5'$ center. Further epoxidation methodology is described in section 4.2.2.

Scheme 3.44. Synthesis of 5,6-epoxyhexylbenzene **3.68** by *m*-CPBA.³²⁴



It has been shown by Bolte *et al.*³²⁵ that epoxide **3.69** can be ring opened in the presence of inorganic phosphate (scheme 3.45).

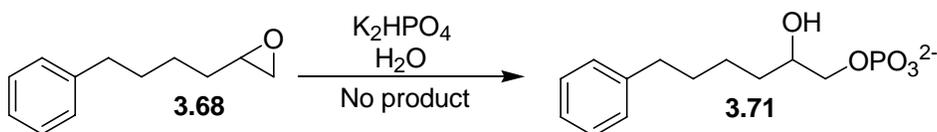
Scheme 3.45. Epoxide ring opening by inorganic phosphate described by Bolte *et al.*³²⁵



Following this procedure, 5,6-epoxyhexylbenzene **3.68** and K_2HPO_4 were refluxed in water over 24 hours (scheme 3.46). The solution was extracted with diethyl ether and the aqueous layer was added to a solution of barium acetate. The pH of the mixture was adjusted to 8 and the precipitate formed was removed by centrifugation.³²⁵ The procedure of Bolte *et al.*³²⁵ then calls for ethanol to be added and the solution kept at 4 °C

overnight. This is how the barium salt of (2*S*)-4,4-diethoxy-2-hydroxybutyl phosphate **3.70** was formed.³²⁵ In this candidate's hands no barium salt of 2-hydroxy-6-phenylhexyl phosphate **3.71** was precipitated after 48 hours at 4 °C in a solution of ethanol. The reaction was not monitored either by TLC or NMR analysis during the refluxing stage so the reason for the lack of product can only be speculated. Limited solubility in water of the starting material during reflux and the di-polar nature of the potential product could be factors.

Scheme 3.46. Attempted ring opening of 5,6-epoxyhexylbenzene **3.68** with inorganic phosphate.



3.6. Conclusions:

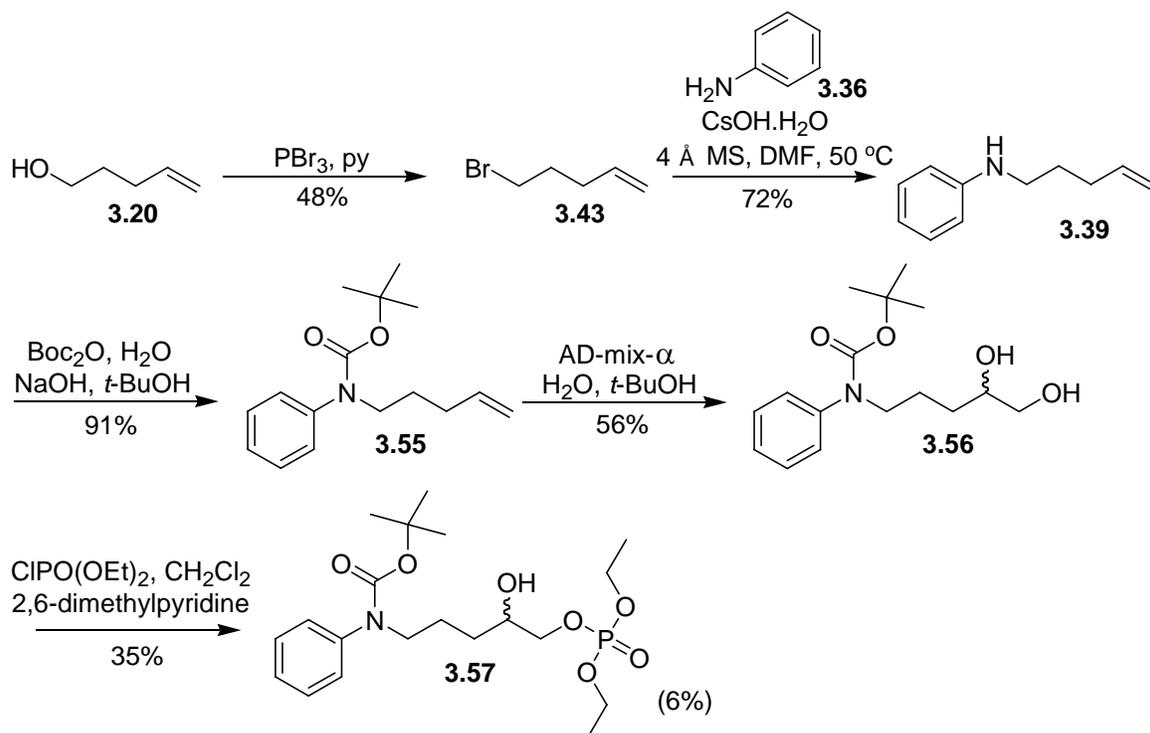
In an effort to synthesise CdRP like target compounds by nucleophilic substitution of good leaving groups on primary carbons with aryl amines, bis mesyl **3.07** and bis tosyl **3.08** were formed. Tertiary pyrrolidine compounds were produced with no desired secondary aryl amine products forming. A retrosynthetic scheme was proposed to synthesis mono good leaving groups on primary carbons leading to CdRP like target compounds. It was decided to first explore less complicated synthetic strategies using pentan-1,5-diol **3.19** and 4-penten-1-ol **3.20**.

Mesyl, tosyl and triflate sulfonyl analogues of 5-*O*-*tert*-butyldimethylsilyl pentanol **3.23** were synthesised. Using Cs_2CO_3 , low equivalents of aniline **3.36** and different sulfonyl leaving groups produced the same low yield of secondary aryl amine **3.37** at room temperature. Starting materials were recovered after prolonged reaction durations. Similar results occurred when using different sulfonyl leaving groups on 4-penten-1-ol **3.20**, $CsOH.H_2O$ and low equivalents of aniline **3.36**. Changing the sulfonyl leaving groups to bromides did little to improve yields, however removing the aromaticity by

using cyclohexylamine **3.45** did. Increasing the ratio of aniline **3.36** and removing the solvent gave a dramatic increase in yield of secondary aryl amines from sulfonyl and bromide good leaving groups. Both bromide and sulfonyl good leaving groups produced similar yields of the respective secondary aryl amine. Raising the reaction temperature improved yields using low equivalents of aniline **3.36**, with the optimal temperature being 50 °C. Ultimately using both the high equivalents of aniline **3.36** or anthranilate methyl ester **3.09** and warming the reaction in DMF gave the highest yields of secondary aryl amines. To this candidates knowledge this is the first successful *N*-alkylation using aryl amines and CsOH.H₂O. No overalkylated tertiary amine was isolated in any of the nucleophilic substitutions involving a caesium base.

A protected target 1,4,5 compound **3.57** was synthesised over five steps in a total yield of 6%. The secondary aryl amine 1-phenylamino-4-pentene **3.39** was *N*-protected in high yield using both Fmoc and Boc groups. Asymmetric dihydroxylation using AD-mix- α gave the corresponding diols of which *N*-*tert*-butyloxycarbonyl-1-phenylamino-4,5-pentan-diol **3.56** was phosphorylated giving protected target 1,4,5 compound **3.57** (scheme 3.47).

Scheme 3.47. Synthesis of a protected target 1,4,5 compound **3.57** over 5 steps.



Preliminary synthesis of model *O*- and *C*- target compounds occurred using phenol and benzyl magnesium bromide, respectively as the nucleophiles. This led to the epoxidation of hex-5-enylbenzene **3.67** by *m*-CPBA producing 5,6-epoxyhexylbenzene **3.68**. An attempt at opening the epoxide ring with inorganic phosphate failed.

CHAPTER 4: Epoxide ring opening.

4.1. Introduction:

Epoxides are important and versatile synthetic intermediates in organic synthesis.^{102,326} Their ability to react with a wide range of reagents, including nucleophiles such as aryl amines to produce β -amino alcohols, led us to investigate their viability to produce target 1,2,5 compounds (section 1.7.3). Initially the target compounds synthesised would be in racemic form as **4.01** (figure 4.01). Synthesis of the different enantiomers, (*R*) **4.02** and (*S*) **4.03** at C2', would follow at a later stage.

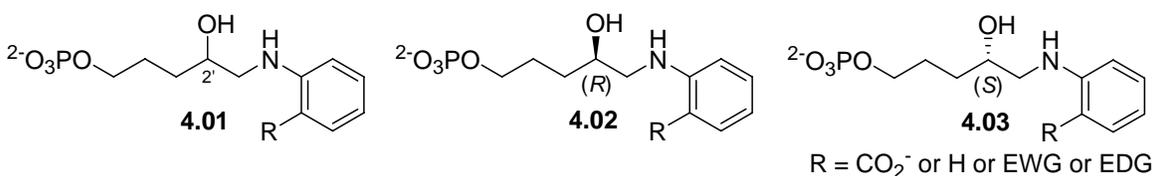
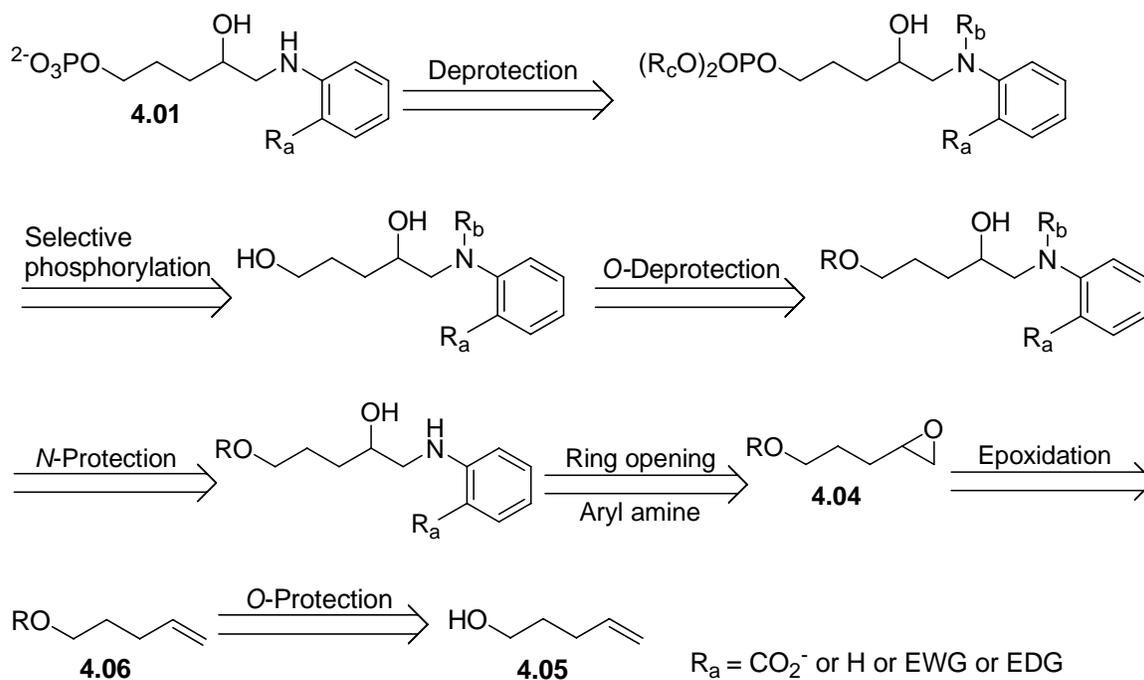


Figure 4.01. Target 1,2,5 compounds.

4.1.2. Retrosynthetic plan:

The general retrosynthetic plan for racemic β -amino alcohol **4.01**, a secondary aryl amine, hinged on ring opening the epoxide **4.04** with an aryl amine (scheme 4.01). The epoxide **4.04** would come from protecting 4-penten-1-ol **4.05** with a suitable group and then treating the protected alkene **4.06** with an epoxidation reagent. Subsequent protections and deprotections would lead to the formation of racemic **4.01**.

Scheme 4.01. General retrosynthetic strategy.

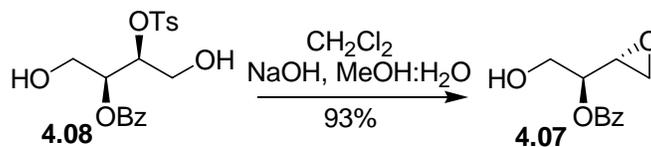


4.2. Literature on epoxide ring formation:

4.2.1. Good leaving groups:

A number of different reagents and methods can be used to form epoxides. Typically alkenes are treated with the appropriate reagents, but epoxides can be formed from 1,2-diols by substitution reactions. Adam and Seebach³²⁷ produced the enantiomerically pure (enantiomeric excess, *ee*, >99%) epoxide **4.07** by treating the tosyl **4.08** with NaOH for ten minutes (scheme 4.02).

Scheme 4.02. Epoxide formation from good leaving group.³²⁷

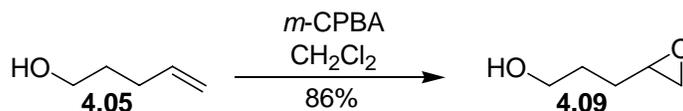


In light of the success in using asymmetric dihydroxylation (AD)-mix- α to form stereoselective diols as described in section 3.4.3, synthesis of enantiomerically pure epoxides using substitution chemistry could be attempted.

4.2.2. Epoxidation from alkenes:

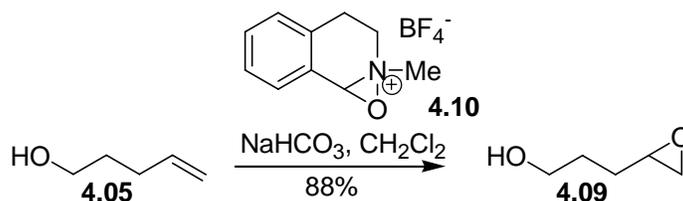
Epoxides can be prepared conveniently by reacting alkenes with organic peroxyacids such as *meta*-chloroperoxybenzoic acid (*m*-CPBA), peroxyacetic acid (CH₃CO₃H), peroxybenzoic acid (PhCO₃H) and peroxytrifluoroacetic acid (CF₃CO₃H).³²⁸ These are particularly convenient reagents due either to their simplicity of application or for their strong oxidizing properties. Lusinchi and Hanquet reported³²⁹ that 4-penten-1-ol **4.05** can be transformed to 4,5-epoxypentan-1-ol **4.09** by *m*-CPBA (scheme 4.03).

Scheme 4.03. 4,5-Epoxypentan-1-ol formation from *m*-CPBA.³²⁹



The reaction required stirring at room temperature for 8 hours using 1.2 equivalents of *m*-CPBA to complete the conversion of 4-penten-1-ol **4.05** to 4,5-epoxypentan-1-ol **4.09**. The reaction duration was reduced to 3 hours and only one equivalent was needed using oxaziridinium salt³³⁰ **4.10** and NaHCO₃ (scheme 4.04).³²⁹

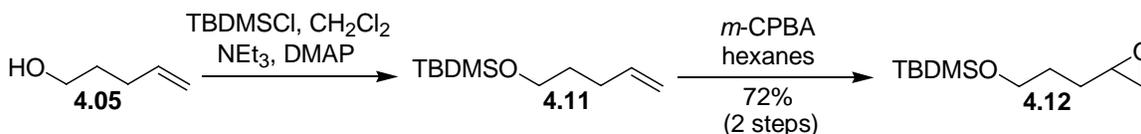
Scheme 4.04. 4,5-Epoxypentan-1-ol formation from oxaziridinium salt.³²⁹



Yang *et al.*³³¹ and Kishi *et al.*³³² showed that silyl-protected alkene **4.11** can be converted to 1-*O*-*tert*-butyldimethylsilyl-4,5-epoxypentanol **4.12** by *m*-CPBA; however, on both occasions the preparation of **4.11** and **4.12** was not described in detail (scheme 4.05).

Yang *et al.*³³¹ did note that the silylation proceeded under reflux for 3 hours and the epoxide **4.12** was formed over 4 hours at room temperature.

Scheme 4.05. Protected epoxide **4.12** formation by Yang *et al.*³³¹



Instead of using asymmetric epoxidation methods to form (*R*) or (*S*) enantiomers from the silyl-protected alkene **4.11**, Kishi *et al.*³³² treated the racemic epoxide **4.12** with Jacobsen's catalyst³³³⁻³³⁵ **4.13** (0.2 mol %), *t*-BuOMe and H₂O, to produce the (*R*) enantiomer of epoxide **4.14** and the (*S*) diol **4.15** in high *ee* (scheme 4.06). Distilling (*R*) epoxide **4.14** from the (*S*) diol **4.15** provided separation. An exact *ee* of both products was not given nor was the yield for the (*S*) diol **4.15**.

Scheme 4.06. Hydrolytic kinetic resolution of racemic epoxide **4.12**.³³²



Hydrolytic resolution was achieved by reaction-kinetic differences of the (*R*) and (*S*) epoxide binding to Jacobsen's catalyst³³³⁻³³⁵ **4.13** and the nucleophile, H₂O, attacking the respective epoxide (figure 4.01). This can be attributed to the location of the substituents of chiral salen ligands, since they are located proximal to the cobalt center and they can interact intensely with the incoming substrate, hence effecting enantiotopic selection.³³⁶

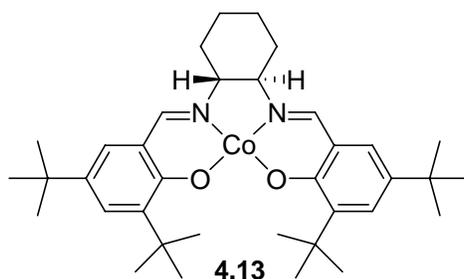


Figure 4.01. Jacobsen's catalyst³³³⁻³³⁵ **4.13**, a cobalt-based salen ligand complex.

Other non-enantioselective epoxidation reagents that could be used on the silyl-protected alkene **4.11** include H₂O₂ catalysed by methyltrioxorhenium (VII, MeReO₃)³³⁷ and 3,3-dimethyldioxirane.^{338,339} Other reagents for the production of enantiopure epoxides from the silyl-protected alkene **4.11** could include: chiral (salen) Mn(III) complexes (Jacobsen)³⁴⁰ with a range of oxidants including 3,3-dimethyldioxirane³⁴¹ and NaOCl,³⁴² or Sharpless' method³⁴³ using Ti(IV) *iso*-propoxide, *t*-BuOOH and di-*iso*-propyl-L-tartrate²⁹⁷ or diethyl-L-tartrate.^{344,345}

4.3. Formation of protected epoxide:

4.3.1. 1-*O*-*tert*-Butyldimethylsilyl-4,5-epoxypentanol:

The retrosynthetic plan (section 4.1.2) led to the synthesis of *tert*-butyldimethylsilyl ether silyl-protected alkene **4.11**. Due to the lack of experimental detail in the work of Yang *et al.*,³³¹ different parameters were used to optimise the yield of protected alkene **4.11** (table 4.01, scheme 4.07). The concentration of penten-1-ol **4.05** was kept constant, as was the generous equivalents of NEt₃ used (rxn 1-4). The yields of protected alkene **4.11** were insensitive to changes in equivalents of DMAP or TBDMSCl (rxn 1-3). Adding TBDMSCl to the solution of DMAP, NEt₃ and penten-1-ol **4.05** slightly raised yields to 94% (rxn 4). Formation of protected alkene **4.11** was monitored by ¹H NMR and identified by the downfield shift of the terminal hydrogens from 3.39 to 3.60 ppm.

Rxn #	Base ^a	DMAP (Eq)	TBDMSCl (Eq)	Solvent ^b	Temp	Duration	Yield
1	NEt ₃	0.3	2	CH ₂ Cl ₂	rt	15 hrs	89%
2	NEt ₃	0.1	2	CH ₂ Cl ₂	rt	22 hrs	88%
3	NEt ₃	0.1	1.6	CH ₂ Cl ₂	rt	22 hrs	90%
4 ^c	NEt ₃	0.1	2	CH ₂ Cl ₂	rt	20 hrs	94%

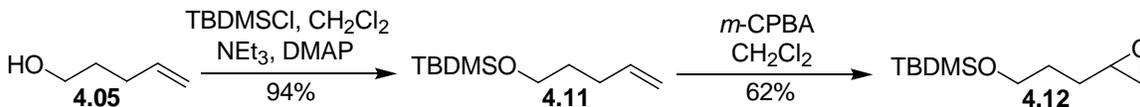
^a 3 equivalents of NEt₃.

^b 1 mL of CH₂Cl₂ per 1 mmol of penten-1-ol **4.05**.

^c TBDMSCl was added to the solution of DMAP, NEt₃ and penten-1-ol **4.05** under ice-bath conditions.

Table 4.01. Conditions used in silylation reactions.³³¹

Scheme 4.07. Synthesis of protected epoxide **4.12**.



Forming 1-*O*-*tert*-butyldimethylsilyl-4,5-epoxypentanol **4.12** in good yield proved more difficult than suggested in the literature. The protected alkene **4.11** was treated with *m*-CPBA in a procedure described by Yang *et al.*³³¹ (table 4.02). After 4 hours stirring at room temperature, TLC showed the starting material, protected alkene **4.11**, was still present in large amounts. An aliquot of the reaction mixture was worked up and ¹H NMR analysis confirmed the presence of starting material **4.11**, as shown by the alkene-hydrogens at 4.93, 4.99 and 5.80 ppm. Hydrogen peaks at 2.47, 2.75 and 2.94 ppm represented small amounts of epoxide **4.12**. The reaction was allowed to stir for 19 hours in total yielding only 5% of the isolated epoxide **4.12** and a large portion of starting material **4.11** (rxn 1). Slightly increasing the equivalents of *m*-CPBA improved the yield to 20% (rxn 2). Again a large portion of unreacted starting material **4.11** was recovered. It was decided to attempt to form 4,5-epoxypentan-1-ol **4.09** from 4-penten-1-ol **4.05** as described by Lusinchì and Hanquet³²⁹ (scheme 4.03). *m*-CPBA (4.2 equivalents) stirred in a solution of 4-penten-1-ol **4.05** in CH₂Cl₂ produced the desired epoxide **4.09** in 55% yield over 20 hours at room temperature (rxn 3). A small amount (5%) of starting

material **4.05** was recovered. The moderate yield of the epoxide **4.09** compared with Lusinchi and Hanquet's³²⁹ yield of 86% and the recovered starting material **4.05**, suggests that the *m*-CPBA used may not be in the best condition. Using a greater equivalents of *m*-CPBA was considered.

Following the procedure of reaction 3 (table 4.02) for the synthesis of epoxide **4.12** increased the yield to 51% (rxn 4). Keeping the temperature at a constant 4 °C slightly increased the yield of epoxide **4.12**, as did allowing the reaction to continue for 56 hours (rxn 5 and 6). Again small amounts of unreacted starting material **4.11** were recovered in both reactions. It is possible that changing the solvent from CH₂Cl₂ to hexanes, may have helped increase the solubility of the starting material **4.11**. The protected epoxide **4.12** was formed in 58% yield over two steps (scheme 4.07).

Rxn #	<i>m</i> -CPBA (Eq)	Solvent ^a	Temp	Duration	Yield
1	1.2	2:1	rt	19 hrs	5%
2	1.4	2:1	rt	22 hrs	20%
3 ^b	4.2	1:1	rt	20 hrs	55%
4	4.2	1:1	rt	20 hrs	51%
5	4.2	1:1	4 °C	16 hrs	55%
6	4.2	1:1	4 °C	56 hrs	62%

^a mL of CH₂Cl₂ per mmol of *m*-CPBA.

^b penten-1-ol **4.05** was used.

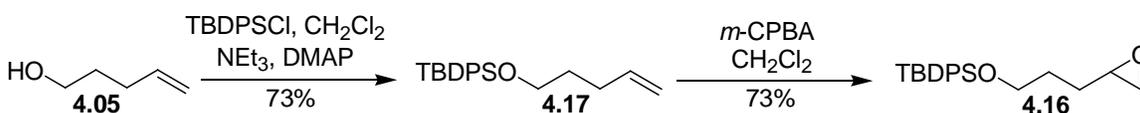
Table 4.02. Conditions used in epoxidation reactions.³²⁹

4.3.2. 1-*O*-*tert*-Butyldiphenylsilyl-4,5-epoxypentanol:

Due to the relatively low overall yield for the formation of the protected epoxide **4.12**, synthesis of an alternative protected epoxide **4.16** was attempted (scheme 4.08). Following a similar procedure to that used for the formation of *tert*-butyldimethylsilyl ether silyl-protected alkene **4.11**, TBDPSCl was added to a cooled solution of DMAP,

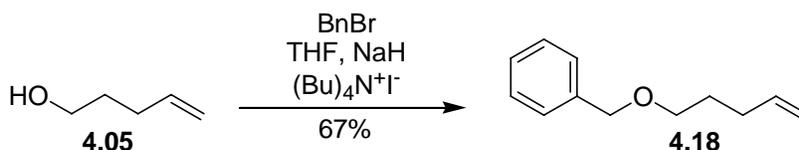
NEt₃ and penten-1-ol **4.05** in CH₂Cl₂. After warming to room temperature, the reaction was allowed to stir for 18 hours before 1-*O*-*tert*-butyldiphenylsilyl-4-penten-1-ol **4.17** was worked up and isolated in a yield of 73%. Synthesis of protected epoxide **4.16** followed a similar method used in the formation of epoxide **4.12**. Using ten equivalents of *m*-CPBA possibly helped increase the yield of epoxide **4.16** to 73% compared with 62% for epoxide **4.12**. The protected epoxide **4.16** was formed in 53% yield over two steps.

Scheme 4.08. Synthesis of 1-*O*-*tert*-butyldiphenylsilyl-4,5-epoxypentanol **4.16**.



In order to see the effect of different electron-withdrawing protecting groups on 4-penten-1-ol **4.05**, and whether they increase the reactivity of the alkene group to *m*-CPBA and of the subsequent epoxide to ring opening in the presence of a nucleophile, 5-*O*-benzylpentene **4.18** was synthesised by a procedure similar to that described by Mootoo *et al.*¹⁹⁴ Benzyl bromide was added to a solution of NaH, tetrabutylammonium iodide (Bu₄N⁺I⁻) and 4-penten-1-ol **4.05** in THF and stirred at room temperature for 3 hours. After work up the isolated material (67%) was shown to be 5-*O*-benzylpentene **4.18** by ¹H NMR analysis that showed downfield shifts of the terminal hydrogens from 3.39 to 3.60 ppm and the phenyl hydrogen peaks between 7.36 and 7.50 ppm (scheme 4.09).

Scheme 4.09. Synthesis of 5-*O*-benzylpentene **4.18**.



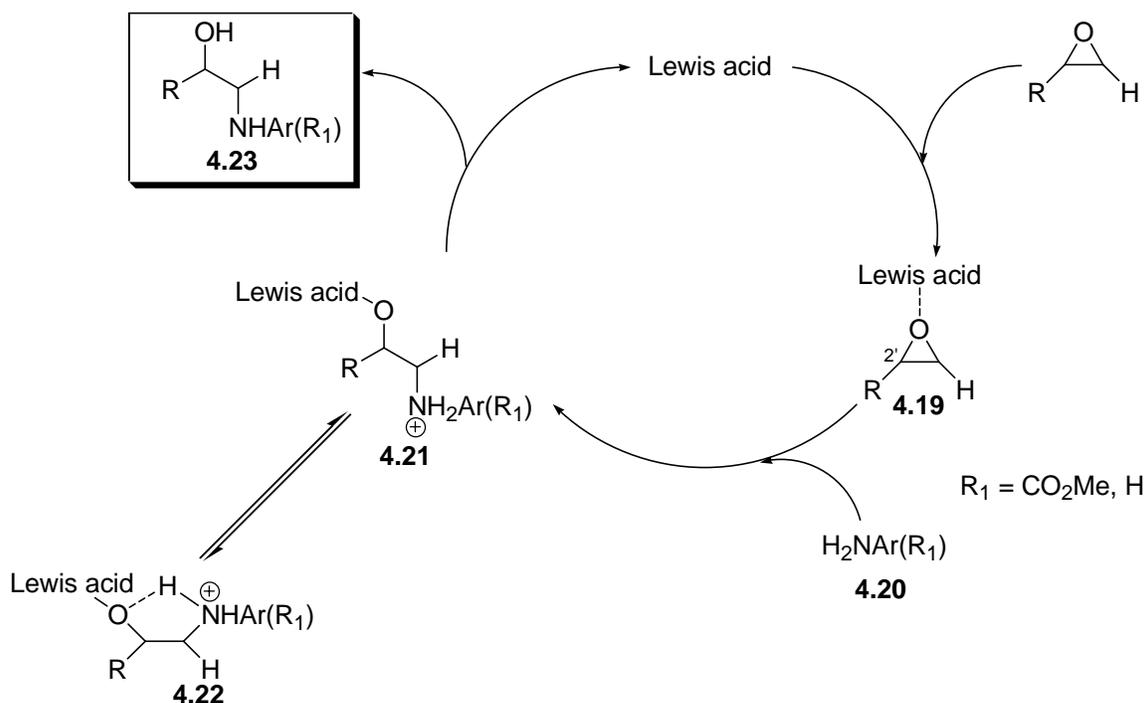
Due to time constraints and the need to focus on the formation of secondary amines, the testing of different epoxidation reagents to improve the yields of protected epoxides **4.12** and **4.16** was not attempted nor was 5-*O*-benzylpentene **4.18** epoxidised.

4.4. Literature on epoxide ring opening:

As briefly discussed in section 1.9.5, the classic synthetic approach towards epoxide ring opening with aryl amines involves elevated temperatures, protic solvents and an excess of amine.¹⁰³⁻¹⁰⁵ Lewis acids or metal salts have been increasingly used to activate or promote ring opening with weakly nucleophilic aryl amines.

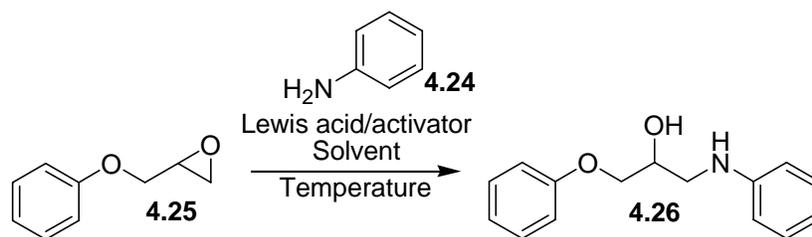
A speculative Lewis acid catalytic cycle for epoxide ring opening is depicted in scheme 4.10. The Lewis acid coordinates to the epoxide oxygen **4.19** rendering the epoxide susceptible to S_N2-type nucleophilic attack by the aryl amine **4.20**.^{346,347} This leads to the ring-opened intermediate structures **4.21** and **4.22**, which undergo rapid proton transfer *via* intermolecular or intramolecular routes respectively, forming the desired secondary aryl amine **4.23** with liberation of the Lewis acid catalyst.^{346,347} The epoxide ring **4.19** opens regioselectively due to the steric hindrance of the R group on C2'.

Scheme 4.10. Lewis acid catalytic cycle for epoxide ring opening.^{346,347}



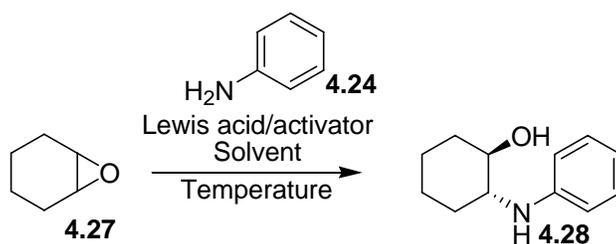
A list of reported literature Lewis acids for this transformation and some of the conditions used in the ring opening reactions with weakly nucleophilic aniline **4.24** follow. The phenyl *O*-protected epoxide **4.25** is used extensively as a model epoxide to test the effectiveness of different Lewis acids (scheme 4.11). These include: ZrCl_4 (neat, rt, 100%),³⁴⁶ $\text{Cu}(\text{BF}_4)_2 \cdot x\text{H}_2\text{O}$ (neat, rt, 97%),¹⁰⁵ silica gel (10% w/w, neat, rt, 100%),³⁴⁸ $\text{Bi}(\text{TFA})_3$ (bismuth trifluoroacetate, tetrabutylammonium bromide [TBAB], 70 °C, 82%),³⁴⁷ $\text{Bi}(\text{OTf})_3$ (TBAB, 70 °C, 84%),³⁴⁷ and 1-butyl-3-methylimidazolium tetrafluoroborate ($[\text{bmim}]\text{BF}_4$) (rt, 89%).³⁴⁹ The durations of the reactions range from 12 minutes with $\text{Cu}(\text{BF}_4)_2 \cdot x\text{H}_2\text{O}$ ¹⁰⁵ to 6 hours with $[\text{bmim}]\text{BF}_4$ ³⁴⁹ an ionic liquid with no Lewis acids present.

Scheme 4.11. Parameters to form secondary aryl amine **4.26**.



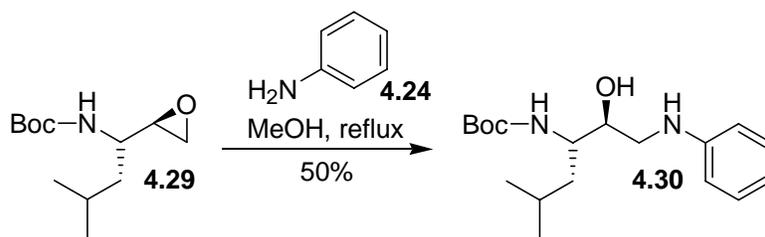
Synthesis of target 1,2,5 compounds **4.01** using the parameters above is a possible starting point to ring-open epoxide **4.04**. Another model epoxide used extensively in literature is epoxycyclohexyl **4.27** (scheme 4.12). Lewis acids and conditions to open epoxycyclohexyl **4.27** with aniline **4.24** include: Yb(OTf)₃ (THF, reflux, 94%),³⁵⁰ ZnCl₂ (MeCN, reflux, 76%),³⁵¹ LiNTf₂ (lithium bistrifluoromethanesulfonimide, CH₂Cl₂, rt, 89%),¹⁰² α -Zr(O₃PCH₃)_{1.2}(O₃PC₆H₄SO₃H)_{0.8} (neat, 40 °C, 92%),³⁵² Sn(OTf)₂ (Et₂O, rt, 94%),³⁵³ Cu(OTf)₂ (Et₂O, rt, 95%),³⁵³ ZrCl₄ (neat, rt, 100%),³⁴⁶ Bi(TFA)₃ (TBAB, 70 °C, 68%),³⁴⁷ Bi(OTf)₃ (TBAB, 70 °C, 78%),³⁴⁷ and Bi(OTf)₃ (H₂O, rt, 83%),³⁵⁴ Cu(BF₄)₂.xH₂O (neat, rt, 97%),¹⁰⁵ and ([bmim]BF₄) (rt, 83%).³⁴⁹ The durations of the reactions range from 5 minutes with Cu(BF₄)₂.xH₂O¹⁰⁵ to 20 hours with Sn(OTf)₂,³⁵³ Cu(OTf)₂,³⁵³ α -Zr(O₃PCH₃)_{1.2}(O₃PC₆H₄SO₃H)_{0.8},³⁵² and LiNTf₂ (CH₂Cl₂, rt, 89%).¹⁰²

Scheme 4.12. Parameters to form secondary aryl amine **4.28**.



Lewis acids are not always necessary for epoxide ring opening with weakly nucleophilic aryl amines as shown by Luly *et al.*³⁵⁵ (scheme 4.13). To a stirred solution of epoxide **4.29** in methanol aniline **4.24** (one equivalent) was added. The solution was refluxed for 20 hours to give secondary aryl amine **4.30** (50%).³⁵⁵

Scheme 4.13. Epoxide ring opening by refluxing aniline.³⁵⁵

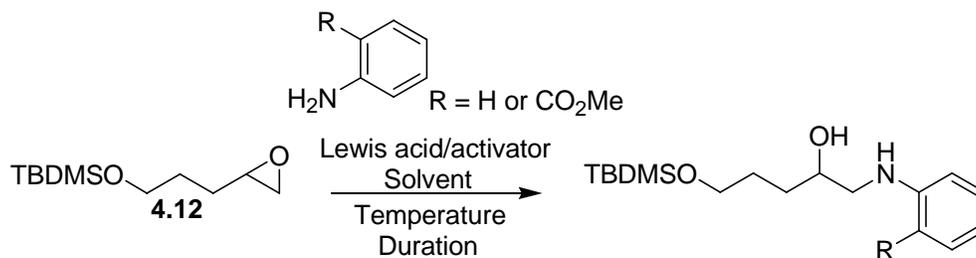


4.5. Epoxide ring opening:

4.5.1. Ring opening of 1-*O*-*tert*-butyldimethylsilyl-4,5-epoxypentanol:

Ring opening of the epoxide 1-*O*-*tert*-butyldimethylsilyl-4,5-epoxypentanol **4.12** was first attempted using anthranilate methyl ester (R = CO₂Me, scheme 4.14). The Lewis acid/activator chosen for this transformation was LiNTf₂, which was commercially available, inexpensive and non-hazardous.¹⁰² LiNTf₂ had also been shown by Hamoir *et al.*¹⁰² to ring-open epoxide **4.27** with two equivalents of aniline **4.24** at room temperature in dichloromethane over 20 hours, producing the secondary aryl amine **4.28** (scheme 4.12). In neat condition the yield was 100%, including dichloromethane the yield was 89%.¹⁰²

Scheme 4.14. Parameters for epoxide ring opening by aryl amines.



Following the reported procedure,¹⁰² LiNTf₂ was added to a CH₂Cl₂ solution of anthranilate methyl ester and the protected epoxide **4.12** (table 4.03, rxn 1). The reaction mixture was stirred under N₂ at 4 °C for 20 hours. Although TLC showed no reaction after 20 hours at 4°C, the reaction was worked up as described by Hamoir *et al.*¹⁰² ¹H

NMR analysis showed starting materials, which were almost completely recovered to the amount initially used. Repeating the experiment with 0.5 equivalents of LiNTf₂, and allowing the reaction mixture to stir at room temperature did not yield any product (rxn 2). Using a stronger aryl amine nucleophile such as aniline (R = H, rxn 3-5) and increasing the concentration of the CH₂Cl₂ solution did not produce any product even after stirring over a 9-day period. Hamoir *et al.*¹⁰² noted that some epoxide ring openings benefit from solvent being excluded from the reaction. This was investigated by stirring the reaction mixture at room temperature as a neat solution of two equivalents of aniline, LiNTf₂ and the protected epoxide **4.12** over 22 hours (rxn 5). Again starting materials were almost completely recovered and ¹H NMR or TLC analysis detected no product.

Rxn #	Aryl amine (Eq)	Lewis acid (Eq)	Solvent	Temp	Duration	Yield
1	R = CO ₂ Me, 2.75	LiNTf ₂ , 0.25	CH ₂ Cl ₂ ^a	4 °C	20 hrs	0%
2	R = CO ₂ Me, 2.75	LiNTf ₂ , 0.5	CH ₂ Cl ₂ ^a	rt	15 hrs	0%
3	R = H, 2.0	LiNTf ₂ , 0.5	CH ₂ Cl ₂ ^b	rt	20 hrs	0%
4	R = H, 2.2	LiNTf ₂ , 0.5	CH ₂ Cl ₂ ^a	rt	9 days	0%
5	R = H, 2.0	LiNTf ₂ , 0.5	Neat	rt	22 hours	0%

^a 1 mL of CH₂Cl₂ per 0.5 mmol of epoxide **4.12**.

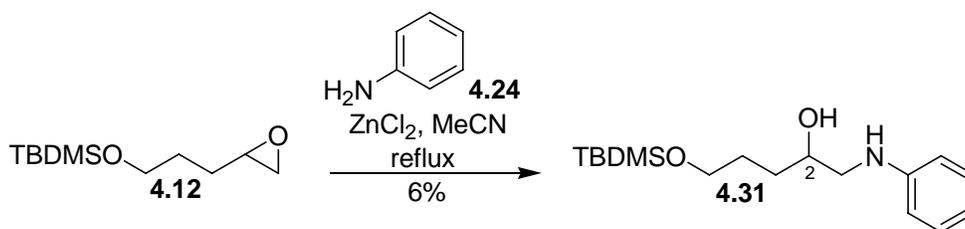
^b 1 mL of CH₂Cl₂ per 2.5 mmol of epoxide **4.12**.

Table 4.03. Reactions using low equivalents of aryl amine at room temperature to open the TBDMS-protected epoxide **4.12**.¹⁰²

It was clear conditions needed to be more forceful to ring-open the protected epoxide **4.12**. Refluxing the protected epoxide **4.12** with aniline **4.24** and LiNTf₂ in CH₂Cl₂ over 14 hours returned only starting materials (table 4.04, rxn 1). Changing the Lewis acid from LiNTf₂ to ZnCl₂³⁵¹ and refluxing the protected epoxide **4.12** with aniline **4.24** in MeCN produced small amounts of the desired secondary aryl amine (rxn 2). An array of spots appeared on TLC four hours after the reagents were refluxed. These spots increased in intensity until the protected epoxide **4.12** spot completely disappeared after 46 hours. Isolation of the numerous spots by flash chromatography afforded unidentifiable by-

products and the desired product (6%, scheme 4.15). Distinguishing NMR features of the secondary amine **4.31** include a downfield shift of hydrogen-2 ^1H to 3.85 ppm and a shift in carbon-2 ^{13}C 52.2 to 70.0, and carbon-1 ^{13}C from 47.2 to 50.1 ppm. Changing the solvent to THF and using $\text{BF}_3 \cdot \text{Et}_2\text{O}$ ³⁵⁶ instead of ZnCl_2 produced similar results as in reaction 2. A multitude of spots were apparent *via* TLC after all of the protected epoxide **4.12** was consumed.

Scheme 4.15. Low-yielding opening of TBDMS-protected epoxide **4.12** using aniline **4.24**, and ZnCl_2 in refluxing MeCN.



Increasing the nucleophilic nature of the amine by using cyclohexylamine **4.32** did not produce the desired product under refluxing conditions with LiNTf_2 (rxn 4). Surprisingly cyclohexylamine **4.32** and the protected epoxide **4.12** were recovered without reacting.

Rxn #	Aryl amine (Eq)	Lewis acid (Eq)	Solvent	Temp	Duration	Yield
1	R = H, 3.5	LiNTf ₂ , 0.5	CH ₂ Cl ₂ ^a	reflux	14 hrs	0%
2	R = H, 1.0	ZnCl ₂ , 0.05	MeCN ^b	reflux	46 hrs	6%
3	R = H, 10	BF ₃ .Et ₂ O, 0.5	THF ^c	reflux	19 hrs	trace
4	cyclohexylamine, 3.5	LiNTf ₂ , 0.5	CH ₂ Cl ₂ ^a	reflux	14 hours	0%

^a 1 mL of CH₂Cl₂ per 1 mmol of epoxide **4.12**.

^b 5 mL of MeCN per 1 mmol of epoxide **4.12**.

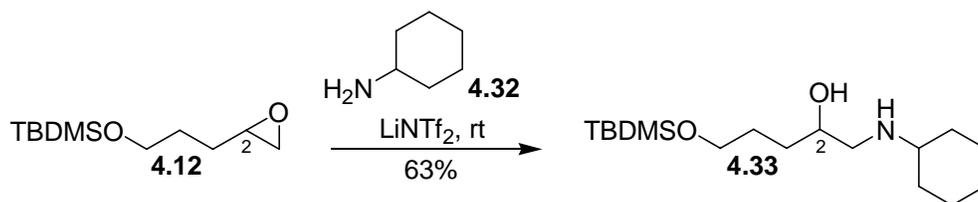
^c 5 mL of THF per 1 mmol of epoxide **4.12**.

References: ^{102,351,356}

Table 4.04. Reactions using low equivalents of aryl amine at reflux to open the protected epoxide **4.12**.

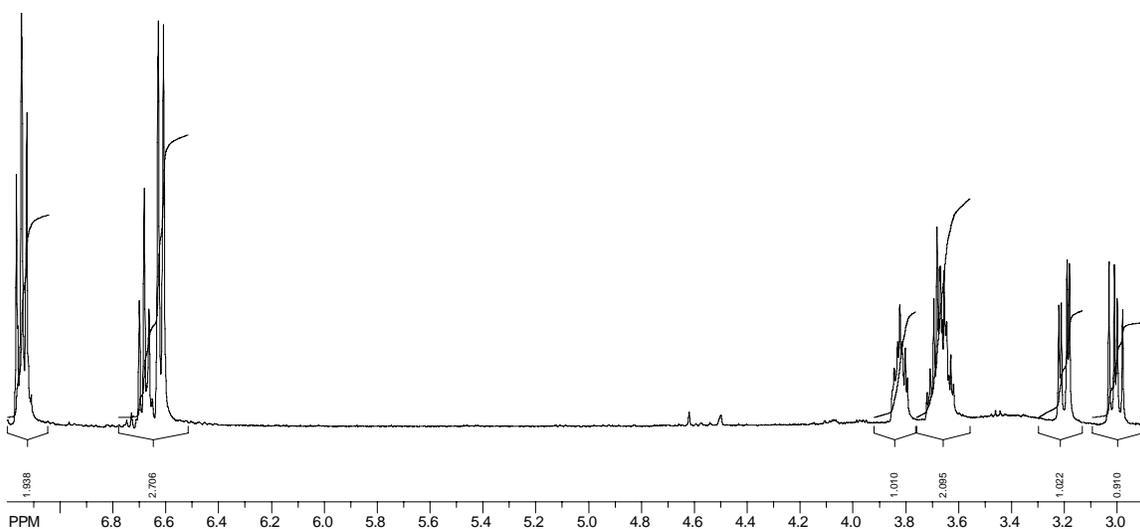
It was decided to dramatically increase the equivalents of cyclohexylamine **4.32** and remove the solvent. After a few hours stirring at room temperature TLC analysis showed an emergence of a more polar spot and a gradual decline of the protected epoxide **4.12**. After 14 hours the protected epoxide **4.12** reacted completely to form a single product, which after isolation was characterised by ¹H NMR spectroscopy to be the secondary amine **4.33** (scheme 4.16). Distinguishing NMR features of the secondary amine **4.33** include a downfield shift of hydrogen-2 ¹H to 3.58 ppm, and a shift in carbon-2 ¹³C from 52.2 to 69.7, and carbon-1 from 47.2 to 56.7 ppm. Excellent regioselectivity was observed in forming the terminal secondary amine **4.33**, with none of the other regioisomer isolated. The secondary amine **4.33** was assumed to be a racemic mixture. The parameters of this reaction can be seen in table 4.05, reaction 1.

Scheme 4.16. Cyclohexylamine **4.32** opening of the TBDMS-protected epoxide **4.12**.



Using aniline **4.24** and the parameters of reaction 1 table 4.05, the secondary aryl amine **4.31** was synthesised in a yield of 55% (rxn 2). This was the only product seen by ^1H NMR analysis of the crude reaction mixture apart from trace amounts of the protected epoxide **4.12** (scheme 4.17). A portion of the ^1H NMR of secondary aryl amine **4.31** can be seen in graph 4.01. A pair of doublet of doublets is at δ 3.03 and δ 3.23 corresponding to hydrogen-1, the multiplet at δ 3.69 to hydrogen-5 and the multiplet at δ 3.85 to hydrogen-2. The downfield hydrogens between δ 6.6 and δ 7.15 correspond to the aryl hydrogens. Excellent regioselectivity was again observed in forming the terminal secondary aryl amine **4.31** and it was assumed to be a racemic mixture.

Scheme 4.17. Aniline **4.24** opening of TBDMS-protected epoxide **4.12**.



Graph 4.01. ^1H NMR spectrum of secondary aryl amine **4.31** between 3.0 and 7.15 ppm.

Asymmetric epoxidation or hydrolytic kinetic resolution to produce chiral epoxides would have been considered if epoxide ring opening was not as challenging or as time consuming to develop.

A comparison of reaction 5 of table 4.03 and reaction 2 of table 4.05, using 15 equivalents of aniline appeared to be key in opening the epoxide ring. After numerous failures to open the protected epoxide **4.12** using LiNTf₂ and lower equivalents of amines the question remained: was LiNTf₂ necessary in forming **4.31** or was the higher equivalence of aniline **4.24** in neat conditions the only critical factor? Reaction 3 on table 4.05 shows upon the removal of LiNTf₂ the protected epoxide **4.12** reacts extremely slowly with aniline **4.24**. Starting materials were recovered and only 5% of secondary aryl amine **4.31** was isolated after stirring for 9 days at room temperature.

Rxn #	Aryl amine (Eq)	Lewis acid (Eq)	Solvent	Temp	Duration	Yield
1	cyclohexylamine, 15	LiNTf ₂ , 0.5	Neat	rt	14 hrs	63%
2	R = H, 15	LiNTf ₂ , 0.5	Neat	rt	14 hrs	55%
3	R = H, 15		Neat	rt	9 days	5%

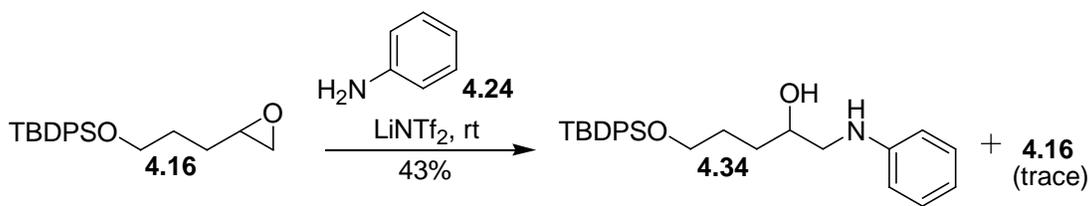
Table 4.05. Reactions using high equivalents of neat aryl amine at room temperature to open the TBDMS-protected epoxide **4.12**.¹⁰²

When solvents were present (CH₂Cl₂), the highest equivalents of amines used in these ring-opening reactions were 3.5 equivalents (rxn 1 and 4, table 4.04). Both these reactions failed to produce product. It would have been interesting to use 15 equivalents of the amine in CH₂Cl₂. In the interest of time this was not investigated nor were the secondary aryl amines **4.31** and **4.33** *N*-protected and developed further along the pathway to target 1,2,5 compounds (scheme 4.01).

4.5.2. Ring opening of 1-*O*-*tert*-butyldiphenylsilyl-4,5-epoxypentanol:

Following the general parameters shown in table 4.05, 1-*O*-*tert*-butyldiphenylsilyl-4,5-epoxypentanol **4.16** was ring-opened using 30 equivalents of aniline **4.24** after stirring for 14.5 hours at room temperature (scheme 4.18). The racemic secondary aryl amine **4.34** was isolated in 43% yield, slightly lower than the parallel reaction involving 1-*O*-*tert*-butyldimethylsilyl-4,5-epoxypentanol **4.12**. The lower reactivity of the protected epoxide **4.16** could be attributed to the bulky phenyl groups sterically hindering the approach of aniline **4.24** to the epoxide ring. Apart from trace amounts of the protected epoxide **4.16** no other products were isolated.

Scheme 4.18. Aniline **4.24** opening of TBDPS-protected epoxide **4.16**.

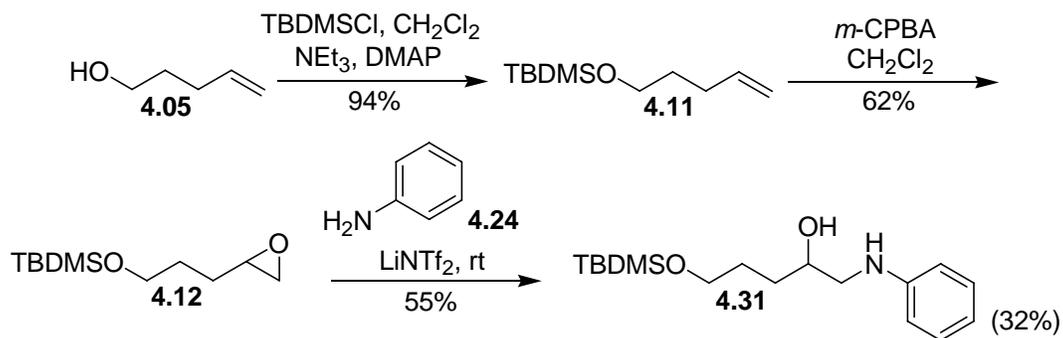


4.6. Conclusions:

This chapter details formation of silyl-protected epoxides and various attempts at ring opening them with amines. The protected epoxides were initially formed in low yields until an increase in equivalents of the epoxidation reagent *m*-CPBA was used. The Lewis acid LiNTf₂ was chosen to catalyse the epoxides's ring opening initially with anthranilate methyl ester, then with stronger nucleophilies such as aniline **4.24** and cyclohexylamine **4.32**. Reactions using low equivalents (2-2.75) of aryl amines and LiNTf₂ in CH₂Cl₂ failed to ring-open the TBDMS-protected epoxide **4.12** at room temperature. Reactions using low equivalents (1-3.5) of aniline **4.24** or cyclohexylamine **4.32**, using either LiNTf₂, ZnCl₂ or BF₃.Et₂O in various solvents also failed or poorly opened the protected epoxide **4.12** ring under refluxing conditions. Reactions using high equivalents (15) of aniline **4.24** or cyclohexylamine **4.32** in neat conditions with LiNTf₂ as a catalyst opened the protected epoxide **4.12** at room temperature. This has led to the formation of

advanced secondary aryl amine synthons over three steps in 32% yield and developed the pathway leading to target 1,2,5 compounds. The three steps are summarised in scheme 4.19.

Scheme 4.19. Formation of secondary aryl amine synthon **4.31** over three steps.



CHAPTER 5: Summary of thesis.

5.1. Overall conclusions:

The studies described in this thesis cover methodology focusing on secondary aryl amine formation, using reductive amination, nucleophilic substitution and epoxide ring opening, leading to 1-(*O*-carboxyphenylamino)-1-deoxyribose-5-phosphate (CdRP) analogues. These analogues may inhibit both phosphoribosylanthranilate isomerase (PRAI) and indole-3-glycerolphosphate synthase (IGPS) due to the target compounds being based on CdRP, the end product of the PRAI-catalysed reaction and the starting material for IGP formation.

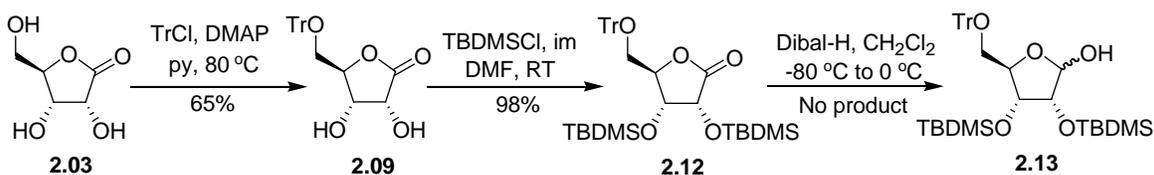
5.1.2. Conclusions of reductive amination chemistry:

Our initial target was the reduced form of CdRP **2.01**, which we aimed to synthesise *via* a general strategy allowing the use of different five-carbon sugars. The retrosynthetic plan for the preparation of rCdRP **2.01** hinged on coupling phosphorylated protected D-ribonolactol with esterified anthranilic acid *via* a reductive amination reaction. Phosphorylated protected D-ribonolactol would come from the reduction of phosphorylated protected D-ribonolactone. Phosphorylation of D-ribonolactone **2.03** using pyridine, imidazole, high equivalents of diphenyl phosphorochloridate, different reaction temperatures and a number of different solvents proved difficult. It is likely the solvents used did not fully solubilise D-ribonolactone **2.03** or produce conditions for reactivity with the phosphorylating reagent. This is evident by the approximate crude yield of recovered starting materials, D-ribonolactone **2.03** (90%) and diphenyl phosphate **2.08** (70%), which was based on the number of moles of starting reagent, diphenyl phosphorochloridate. Although other phosphorylating procedures could have been attempted, model reductive aminations using anthranilate methyl ester **2.51** and 5-diphenyl phosphate pentanal **2.73** indicated that the amine may have interfered with the phosphate functionality. TLC silica plates of the reaction showed broad streaks from R_f

0.42 to an R_f of 0.55 (3:1 hexanes-EtOAc), and no 5-diphenyl phosphate pentanal **2.73** spot. NMR analysis of aliquots of the above reaction mixtures indicated that the phosphate moiety was unstable when in the presence of anthranilate methyl ester **2.51**, possibly being attacked by the weakly nucleophilic aryl amine. There are reports in literature of similar occurrences.²⁷⁴⁻²⁷⁸ Due to the fact that the amine appeared to be interacting with the phosphate group we discarded the idea of firstly phosphorylating the primary hydroxyl position of D-ribonolactone **2.03**, and instead focused on protecting the primary and secondary hydroxyl groups. This led to the formation of 2,3-bis-*O*-*tert*-butyldimethylsilyl-5-*O*-triphenylmethyl-D-ribonolactone¹⁴² **2.12** (64% over two steps), which needed to be reduced to form the lactol **2.13**, in order to access the open chain aldehyde **2.14**, allowing for reductive amination chemistry.

Standard di-*iso*-butylaluminum hydride (Dibal-H) reducing procedures¹⁴⁷⁻¹⁴⁹ were employed, such as adding the reducing agent slowly dropwise, at low reaction temperatures (-78 °C) and in high equivalent. Unfortunately, reduction of the disilyl TBDMS-protected lactone **2.12** did not occur, even at temperatures between -80 °C to -45 °C over 6-7 hour period with eight additional equivalents of Dibal-H (scheme 5.01). A fresh source of Dibal-H did not change the outcome. Increasing the reaction temperature and monitoring with TLC and NMR showed the TBDMS protecting groups start to cleave off when the reaction warmed to around -20 °C and higher. No reduction of disilyl TBDMS-protected lactone **2.12** was evident. This could possibly be partially attributed to the lower solubility of disilyl TBDMS-protected lactone **2.12** at -78 °C to -20 °C in CH_2Cl_2 . Further search through literature showed Corey and Jones¹⁵⁸ developed a method to specifically cleave TBDMS protecting groups using Dibal-H.

Scheme 5.01. Failed Dibal-H reduction of disilyl protected lactone **2.12**.

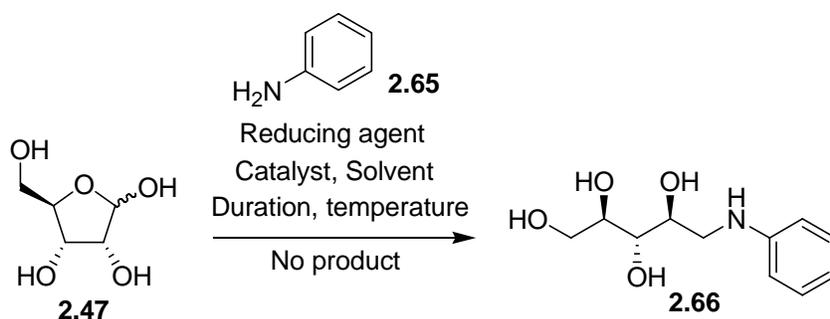


Increasing the stability of the secondary protecting groups to Dibal-H, from TBDMS protecting groups to *tert*-butyldiphenylsilyl (TBDPS) was proposed in order to access a protected lactol for reductive aminations; however, it was found that the TBDPS groups are too sterically demanding for the *cis* diol, 5-*O*-triphenylmethyl-D-ribonolactone **2.09**. Alternative protecting groups for the *cis* diol **2.09** were considered, such as benzyl, benzylidene or *iso*-propylidene groups. Finding conditions to produce an acceptable yield of 2,3-*O*-dibenzyl-5-*O*-triphenylmethyl-D-ribonolactone **2.35** was not easy. The *cis* diol **2.09** was found to be intolerant to strong alkaline conditions, as shown by a complex mixture of materials that had decomposed and a multitude of decomposed by-products, revealed by TLC and NMR spectroscopy. The C3 hydroxyl group was particularly less reactive than the C2 hydroxyl group, attributed to the lactone carbonyl increasing the acidity of the C2 hydroxyl's hydrogen group. An attempt at forming 2,3-*O*-benzylidene-5-*O*-triphenylmethyl-D-ribonolactone **2.39** by refluxing benzaldehyde dimethyl acetal, the *cis* diol **2.09** and a catalytic amount of *p*-toluenesulfonic acid or camphorsulfonic acid in CH₂Cl₂, cleaved the trityl group. In order to protect the secondary hydroxyl groups and avoid using the acid-sensitive trityl group, the unprotected D-ribonolactone **2.03** was treated with 2,2-dimethoxypropane in the presence of various acid catalysts. Ultimately it was found that a boron trifluoride etherate-catalysed reaction over 3.25 hours produced the highest yields of 2,3-*O*-*iso*-propylidene-D-ribo-1,4-lactone **2.44** (54%). The unreacted starting material, D-ribonolactone **2.03**, can be recovered in good quantity, essentially making the formation of 2,3-*O*-*iso*-propylidene-D-ribo-1,4-lactone **2.44** high yielding. Limiting the quantity of acid present was crucial in reducing the formation of 3,4-*O*-*iso*-propylidene-D-ribo-1,5-lactone **2.45** due to D-ribonolactone's **2.03** susceptibility to ring-open under acidic conditions. The next logical step would be to follow literature protections^{185,186} for the primary hydroxyl group and the subsequent Dibal-H reduction to give the lactol; however, it was decided to investigate reductive amination reactions involving D-ribose **2.47** and model aldehydes.

Using D-ribose **2.47** avoided needing to use a protection strategy and a Dibal-H reduction to give the lactol. Reductive aminations with D-ribose **2.47** and aniline **2.65** were based on the procedure reported by Hirota *et al.*²⁶⁰ Generally aniline **2.65** was dissolved in

absolute methanol and added to a methanolic solution of D-ribose **2.47** at a pH between 4.4 and 5.5. NaBH₃CN was added to the stirred solution, after which a number of parameters were tested in attempts to form the aryl glycosylamine precursor. These include using different Lewis acids such as ZnCl₂ and Ti(IV) *iso*-propoxide, molecular sieves, increasing the equivalents of aniline **2.65**, changing the solvent to ethanol, increasing the temperature and allowing the reactions to run over several hours (scheme 5.02).

Scheme 5.02. Parameters used in reductive aminations involving D-ribose **2.47**.



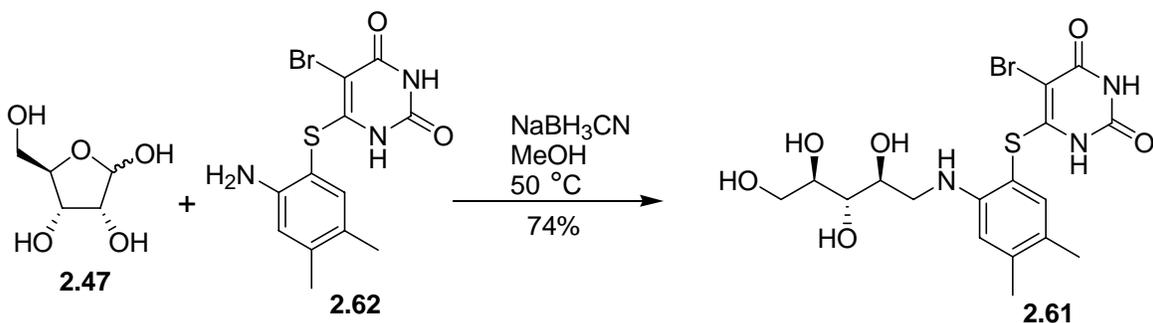
Crude aliquots of the reaction mixtures taken at different periods of the reaction showed distinctive sets of aniline peaks between 6.5-7.2 ppm using ¹H NMR spectroscopy in DMSO-*d*₆. There was no hint of the aryl glycosylamine precursor forming or of a shift in aniline signal positions. The lack of reaction could also be seen with the D-ribose **2.47** ¹H NMR peaks between 3.3-4.1 ppm not shifting. Crucially the anomeric hydrogen remained and integrated with the rest of D-ribose **2.47** suggesting no aryl glycosylamine precursor **2.66** was formed, nor was there any sign of the anomeric hydrogen shifting up-field. ¹H NMR spectroscopy was also performed using D₂O and CDCl₃:D₂O mixtures in case the solubility of aryl glycosylamine precursor prevented its appearance in DMSO-*d*₆. ¹H NMR and ¹³C NMR spectroscopy provided confirmation of the starting materials, D-ribose **2.47** and aniline **2.65**. It is possible that:

- reaction rates are slow between the open-chain aldehyde **2.67** of D-ribose **2.47** and aniline **2.65**;

- the aromatic amine, aniline **2.65** or anthranilic acid **2.02** may be insufficiently nucleophilic to successfully attack the open-chain aldehyde **2.67**;
- the limited solubility of aniline **2.65** in methanol or ethanol reduces the chance of aniline **2.65** and the open-chain aldehyde **2.67** reacting.

All evidence points towards the aromatic amine not reacting with the open-chain aldehyde **2.67**. This is contradictory to Hirota *et al.*²⁶⁰ reductive amination work, which described formation of an aryl glycosylamine in 74% yield when 6-(2-amino-4,5-dimethylphenyl)thio-5-bromouracil **2.62**, D-ribose **2.47** and NaBH₃CN were stirred overnight in methanol at 50 °C (scheme 5.03). 6-(2-Amino-4,5-dimethylphenyl)thio-5-bromouracil is more soluble in methanol than aniline **2.65** due to a larger number of polar functional groups, possibly explaining the difference in outcome between Hirota *et al.*²⁶⁰ work and when aniline **2.65** was used.

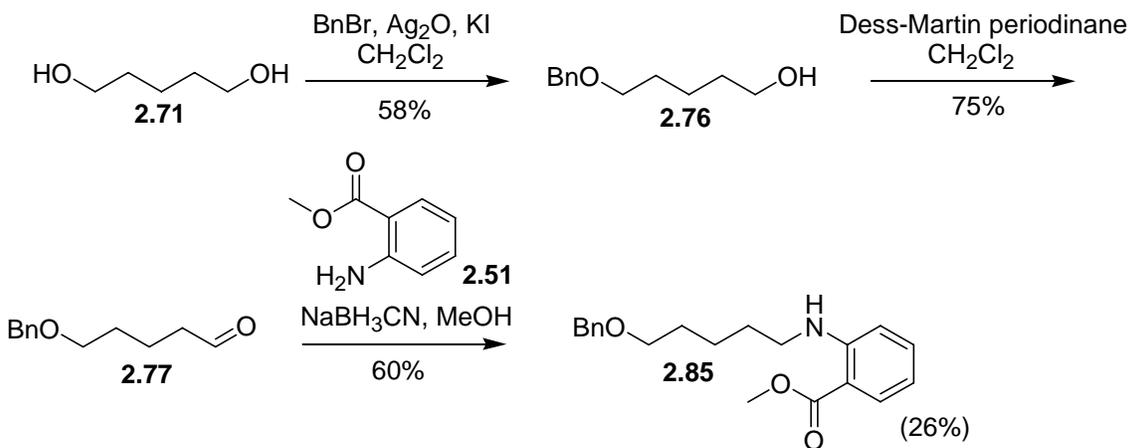
Scheme 5.03. Hirota *et al.*²⁶⁰ glycosylamine formation using D-ribose **2.47**.



Changing the aldehyde from D-ribose **2.47** to 5-*O*-benzylpentanal **2.77** (44% over two steps) removed some of the solubility issues with D-ribose **2.47** and difficulties analyzing the reaction on TLC. In changing the aryl amine from aniline **2.65** to anthranilate methyl ester **2.51**, it was anticipated that the poor nucleophilic nature of anthranilate methyl ester **2.51** in addition to the steric hindrance of the *ortho* methyl ester would be even more detrimental to imine formation. A solution of anthranilate methyl ester **2.51** in methanol was added to protected 5-*O*-benzylpentanal **2.77**, the pH was adjusted to 5.5 with acetic acid and the solution was stirred for 15 minutes before NaBH₃CN was added. The reaction was stirred for a further 5.5 hours at room temperature, during which time 5-*O*-

benzylpentanal **2.77** started disappearing via TLC silica plate analysis, and a new spot formed. The new spot formed was found to be the secondary aryl amine, 5-*O*-benzylpentyl anthranilate methyl ester **2.85** (60%) *via* NMR spectroscopy (scheme 5.04).

Scheme 5.04. Synthesis of 5-*O*-benzylpentyl anthranilate methyl ester **2.85**.



It was decided to exchange anthranilate methyl ester **2.51** with aniline **2.65** and see whether the reaction proceeded smoothly without the ester group. Disappointingly no 5-*O*-benzylpentylphenylamino **2.78** was isolated, either by the procedure used in the formation of 5-*O*-benzylpentyl anthranilate methyl ester **2.85** or by using several equivalents of NaBH₃CN or by using a procedure involving pyridine-borane.^{110,257} 5-*O*-Benzylpentanal **2.77** was isolated from the reaction mixture even after several hours in the presence of aniline **2.65** and NaBH₃CN. Freshly acquired aniline **2.65** did not change the outcome of the reaction. Possible explanations for the formation of 5-*O*-benzylpentyl anthranilate methyl ester **2.85**, but not 5-*O*-benzylpentylphenylamino **2.78**, could be attributed to product stability and better solubility of anthranilate methyl ester **2.51** than aniline **2.65** in methanol; however, this is speculation. 5-*O*-Benzylpentylcyclohexylamine **2.83** was formed in low yield (25%) using in a procedure similar to that in the synthesis of 5-*O*-benzylpentyl anthranilate methyl ester **2.85**. The successful reductive amination involving cyclohexylamine **2.82** showed that removing the aromatic properties of the cyclic amine contributed to the reaction; however, the increased solubility of cyclohexylamine **2.82** in methanol compared to aniline **2.65** could have also played a role.

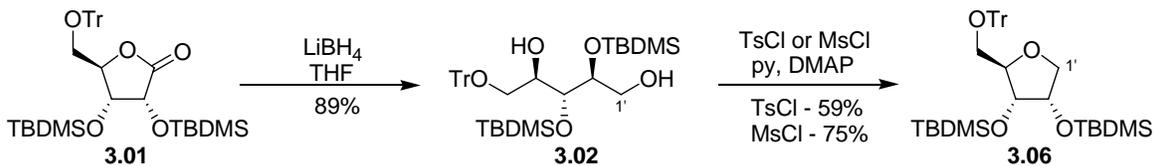
Overall, the synthesis of secondary aryl amines *via* reductive amination procedures proved difficult. The majority of reactions resulted in the recovery of the starting materials, aldehyde and aryl amine. This can be partly attributed to the lack of reasonable solubility of the starting materials, specifically aryl amines, in the reaction solvent. It can be argued the modest reactivity of the weakly nucleophilic aryl amines did not help the reaction to progress. Protections and reductions to form a lactol from D-ribonolactone **2.03** added additional complications, which ultimately proved time-consuming and difficult to overcome. CdRP analogues will have to be synthesised through other methods than reductive aminations.

5.1.3. Conclusions of nucleophilic substitution of good leaving groups:

The experiences with reductive amination chemistry led to an investigation of methodology used in nucleophilic substitution to form secondary aryl amines. Reducing the previously synthesised 2,3-bis-*O*-*tert*-butyldimethylsilyl-5-*O*-triphenylmethyl-D-ribonolactone **3.01** by a procedure based on work by Taylor *et al.*¹⁴² gave 2,3-bis-*O*-*tert*-butyldimethylsilyl-5-*O*-triphenylmethyl-D-ribitol **3.02** in good yield (89%). Our aim was to form a good leaving group at the primary hydroxyl on diol **3.02**, then substitute it with aniline **3.36** or anthranilate methyl ester **3.09**. Subsequent protections and deprotections would lead to the formation of the rCdRP-like target compounds.

Due to the alkaline conditions involved in placing a sulfonyl leaving group on the primary hydroxyl, the secondary unprotected hydroxyl group intramolecularly substituted the mesylate or tosylate group to form (2*S*,3*R*,4*R*)-3,4-bis-*O*-*tert*-butyldimethylsilyl-1-*O*-triphenylmethyl tetrahydrofuran **3.06** (scheme 5.05).

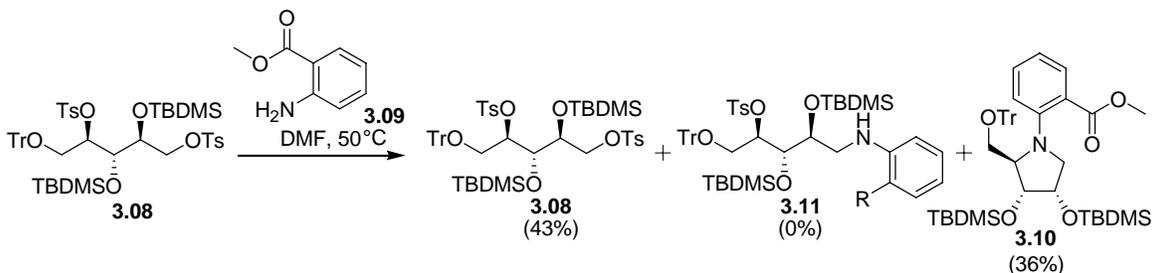
Scheme 5.05. Formation of the furan derivative **3.06**, after attempting to synthesise a sulfonyl leaving group on diol **3.02** primary hydroxyl group.



One option to avoid forming the furan derivative **3.06** was to place a good leaving group on the secondary hydroxyl group, while forming the primary good leaving group, hence reducing the possibility of intra-molecular cyclisation. Following a modified procedure by Taylor *et al.*,¹⁴² 3,4-bis-*O*-*tert*-butyldimethylsilyl-2,5-bis-*O*-methanesulfonyl-1-*O*-triphenylmethyl-D-ribitol **3.07** and 3,4-bis-*O*-*tert*-butyldimethylsilyl-2,5-bis-*O*-toluenesulfonyl-1-*O*-triphenylmethyl-D-ribitol **3.08** were formed in 76% and 64% yield respectively, with only small amounts of the furan derivative **3.06** isolated. In an attempt to form a secondary aryl amine, bis-tosyl **3.08** was initially dissolved in a solution of anthranilate methyl ester **3.09** (one equivalent) in DMF and allowed to stir at room temperature over several days. An almost quantitative return of starting material, bis-tosyl **3.08**, was achieved. Using the bis-mesyl **3.07** as an alternative did little to change the outcome, since the weakly nucleophilic anthranilate methyl ester **3.09** and the bis-mesyl **3.07** were recovered in high yield. Using similar conditions, aniline **3.36** and either bis-mesyl **3.07** or bis-tosyl **3.08** did not return any substituted product. Warming a solution of either bis-mesyl **3.07** or bis-tosyl **3.08** and anthranilate methyl ester **3.09** (one equivalent) in DMF to 50 °C, gave a noticeable reduction in starting material and formation of a less-polar compound *via* TLC analysis. Somewhat predictably, no selectivity was shown in displacing the primary good leaving group on the bis-mesyl **3.07** or bis-tosyl **3.08** without forming (2*S*,3*R*,4*S*)-*N*-5-anthranilate methyl ester-3,4-bis-*O*-*tert*-butyldimethylsilyl-1-*O*-triphenylmethyl **3.10** (scheme 5.06). There was no suggestion the target *N*-5-anthranilate methyl ester-3,4-bis-*O*-*tert*-butyldimethylsilyl-2-*O*-methanesulfonyl-1-*O*-triphenylmethyl-D-ribitol **3.11** was being formed by TLC or NMR spectroscopy. Replacing anthranilate methyl ester **3.09** with aniline **3.36**, and/or increasing the equivalents of the aryl amine produced the same results, that is, no target secondary aryl amine but instead formation of a tertiary pyrrolidine derivative,

(2*S*,3*R*,4*S*)-*N*-5-phenylamino-3,4-bis-*O*-*tert*-butyldimethylsilyl-1-*O*-triphenylmethyl, after a few hours of heating at 50 °C.

Scheme 5.06. Nucleophilic substitution forming pyrrolidine derivative **3.10**.



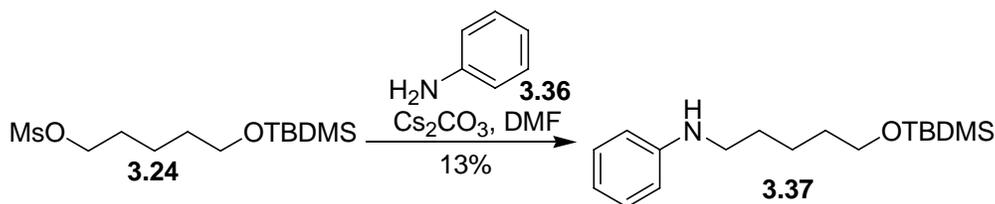
Finding conditions for synthesis of secondary aryl amine **3.11** while a good leaving group was present on the secondary hydroxyl group on bis-mesyl **3.07** or bis-tosyl **3.08** was deemed problematic, so an alternative strategy was devised. This hinged on protecting the secondary alcohol of the diol **3.02** before formation of the good leaving group at the primary hydroxyl. The most logical way to do this was to selectively deprotect the trityl group on the diol **3.02** to form 2,3-bis-*O*-*tert*-butyldimethylsilyl-D-ribitol **3.12**, which could be protected using an acetal,^{124,125,281} exposing the primary hydroxyl group. Selective removal of the trityl group on diol **3.02** failed using methodology described by Taylor *et al.*¹⁴² since formic acid exerted little selectivity in cleaving the trityl group preferentially to the TBDMS groups. Limiting the equivalents of formic acid did not lessen the amount of by-products produced; however, the general idea of selectively protecting the 1,2 diol **3.12** using an acetal, freeing the primary hydroxyl group to be converted to a good leaving group, remains a sensible one. An alternative protection strategy was proposed.

Before attempting this alternative protection strategy, it was decided to test a more general synthetic strategy using nucleophilic substitution on more simple molecules developed from pentan-1,5-diol **3.19** and 4-penten-1-ol **3.20**. This was propagated by the shift in focus of this thesis to formation of secondary aryl amines and away from complicated protection and deprotection strategies. The alkene functionality on 4-penten-1-ol **3.20** could be used in the synthesis of CdRP-like target compounds, specifically

target 1,4,5 compounds with functionalities at the respective 1,4,5 positions along the pentyl moiety. Mono-protection of the diol **3.19** was sought using a monosilylation procedure described by McDougal *et al.*²⁸⁹ This utilises the limited solubility of the monosodium alkoxide salt, sodium 5-hydroxypentanoate **3.22**, in tetrahydrofuran (THF). A more traditional approach employing imidazole and a limited quantity of TBDMSCl was more successful in forming 5-*O*-*tert*-butyldimethylsilyl pentanol **3.23** (98%) in our hands than the McDougal *et al.*²⁸⁹ procedure, which was problematic due to the difficulties in producing the monosodium alkoxide salt **3.22**.²⁹¹ Forming a sulfonyl leaving group on the free hydroxyl initially was achieved by stirring monosilyl **3.23** in pyridine and 4-dimethylaminopyridine (DMAP) with either mesyl chloride (85%) or tosyl chloride (56%).²⁹²⁻²⁹⁴ The lower yield of 5-*O*-*tert*-butyldimethylsilyl-1-*O*-toluenesulfonyl pentanol **3.25** was possibly due to the greater susceptibility to hydrous conditions. Formation of 4-pentenyl methanesulfonyl **3.26** (77%) and 4-pentenyl toluenesulfonyl **3.27** (72%) followed a similar procedure.²⁹⁶

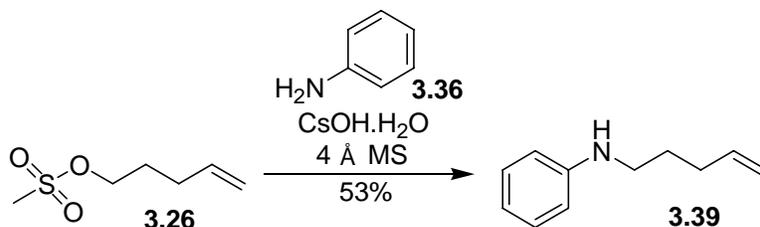
It has been shown that caesium carbonate and caesium hydroxide promote alkylation of primary amines but also suppress overalkylation of the formed secondary amines.^{57,280,298,301,302} There are no instances in literature as of writing where caesium carbonate or hydroxide and an aryl amine have been used in formation of a secondary aryl amine. Initial attempts at substituting the sulfonyl leaving group on 5-*O*-*tert*-butyldimethylsilyl-1-*O*-methanesulfonyl pentanol **3.24** or 5-*O*-*tert*-butyldimethylsilyl-1-*O*-toluenesulfonyl pentanol **3.25** with one equivalent each of aniline **3.36** and of Cs₂CO₃, over a prolonged reaction period at room temperature, produced low yields of 5-*O*-*tert*-butyldimethylsilyl-1-phenylamino-pentane **3.37** (13%, scheme 5.07).

Scheme 5.07. Use of caesium carbonate and low equivalents of aniline **3.36** in the synthesis of secondary aryl amine **3.37**.



The majority of mesyl **3.24** or tosyl **3.25** starting materials were recovered, indicating a lack of reactivity. A more reactive triflate leaving group was synthesised on the monosilyl **3.23**, yielding 5-*O*-*tert*-butyldimethylsilyl-1-*O*-trifluoro-methanesulfonyl pentanol **3.38**, which was stirred with aniline **3.36** and Cs₂CO₃. After 4 hours the triflate **3.38** was completely consumed, by TLC analysis, and the reaction was worked-up to give crude secondary aryl amine **3.37** and more polar by-products. Due to the low crude yield of secondary aryl amine **3.37**, purification was not attempted. It could be speculated that the triflate **3.38** decomposed at such a rate as to limit the substitution reaction occurring to any great degree. The poor nucleophilic nature of aniline **3.36** could also have contributed to the lack of reactivity. Changing the caesium species from Cs₂CO₃ to CsOH.H₂O, reportedly a more efficient base did little to improve yields of secondary aryl amines when low equivalents of aniline **3.36** were reacted with either 4-pentenyl methanesulfonyl **3.26** or 4-pentenyl toluenesulfonyl **3.27**. This was in an attempt to form 1-phenylamino-4-pentene **3.39**, a key secondary aryl amine along the pathway leading to target 1,4,5 compounds. Increasing the ratio of aniline **3.36** to 30 equivalents and removing the solvent gave a dramatic increase in yield of 1-phenylamino-4-pentene **3.39** (53%) and produced no overalkylated tertiary amine (scheme 5.08).

Scheme 5.08. Neat aniline **3.36** in the synthesis of secondary aryl amine **3.39**.

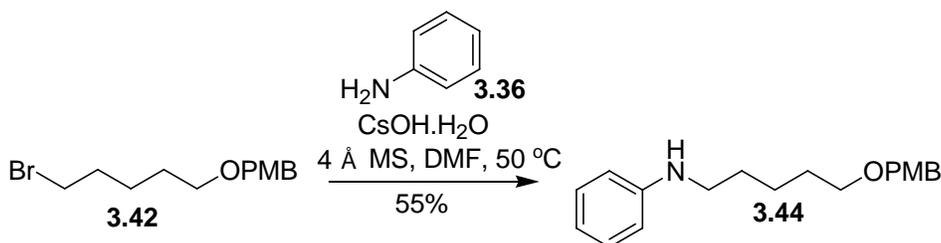


It was decided to develop bromide moieties as leaving groups in an attempt to increase yields of secondary aryl amines from nucleophilic substitution and advance the methodology toward synthesis of target 1,4,5 compounds. Monobromination of pentan-1,5-diol **3.19** was carried out by following literature methodology either by heating aqueous HBr in non polar solvents while removing water using Dean-Stark apparatus, or by simply refluxing HBr in non polar solvents.^{305,306} Similar yields of bromopentan-5-ol **3.40**, 57 and 59% respectively, were produced with or without Dean-Stark apparatus,

indicating the presence of water was not detrimental to the reaction. The non-statistical mixtures of products and starting materials can be potentially explained by the relatively lower reactivity of bromo alcohols under these reaction conditions and the possibilities that these behave like surfactants and reverse micelles.^{289,306} Bromopentan-5-ol **3.40** was protected with *para*-methoxybenzyl trichloroacetimidate **3.41** in the presence of various acid catalysts, of which boron trifluoride etherate gave the highest yields of 5-*O*-*para*-methoxybenzyl bromopentanol **3.42** (67%). 4-Penten-1-ol **3.20** was transformed into bromo-4-pentene **3.43** (48%) using a method described by Kitching *et al.*,³¹⁴ involving neat phosphorus tribromide and pyridine.

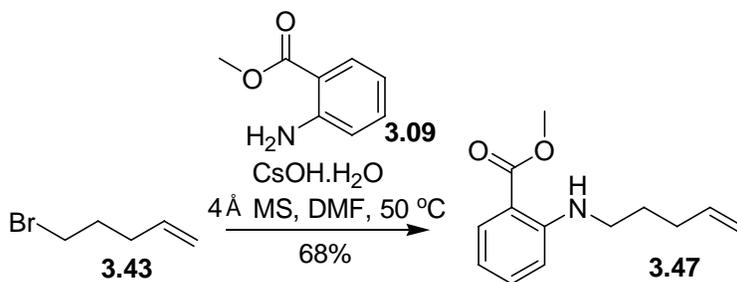
There was little difference in the yields of secondary aryl amine formation between sulfonyl leaving groups and bromides when low equivalents of aniline **3.36** were used at room temperature. At low equivalents of cyclohexylamine **3.45** considerably higher yields of the secondary amine, 5-*O*-*para*-methoxybenzyl pentylcyclohexylamine **3.46**, resulted (46%), compared with the analogous reaction with aniline **3.36**; however, trace amounts of starting materials were still isolated even after 18 hours. Removing the amines aromaticity improved the substitution rate but reaction parameters needed further enhancement. Following the same conditions used in the substitution of mesyl species, 4-pentenyl methanesulfonyl **3.26**, with 30 equivalents of aniline **3.36** gave a similar increase in yield, resulting in the formation of 5-*O*-*para*-methoxybenzyl-1-phenylamino-pentane **3.44** (51%). Clearly an increase in equivalents of aniline **3.36** improved the rate of substitution of the leaving groups. Raising the reaction temperature improved yields of 5-*O*-*para*-methoxybenzyl-1-phenylamino-pentane **3.44** (55%) using low equivalents of aniline, with the optimal temperature being 50 °C (scheme 5.09).

Scheme 5.09. Synthesis of secondary aryl amine **3.44** *via* heating.



Lower temperatures slowed the reaction rate, while increasing it past 50 °C produced a mixture of by-products and decomposed starting materials. When bromo-4-pentene **3.43** was the electrophile, using both the high equivalents of aniline **3.36** and warming the reaction in DMF gave the highest yields of 1-phenylamino-4-pentene **3.39** (72%). Anthranilate methyl ester **3.09** gave anthranilate methyl ester-4-pentene **3.47** (68%) in an analogous reaction (scheme 5.10). Overall mesyl and bromide leaving groups reacted at a similar rate. No overalkylated tertiary amine was isolated in any of the nucleophilic substitutions involving a caesium hydroxide base. To this candidate's knowledge this is the first successful nucleophilic substitution using aryl amines and CsOH.H₂O.

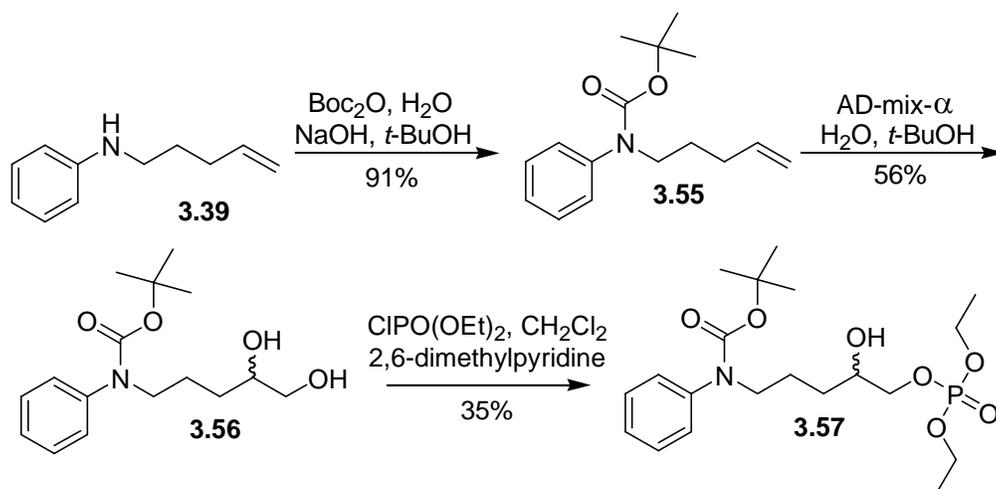
Scheme 5.10. Synthesis of anthranilate methyl ester-4-pentene **3.47** using heat and high equivalents of anthranilate methyl ester **3.09**.



Now that reliable methodology to produce secondary aryl amines using high equivalents of amine and heat had been identified, attention was directed towards formation of target 1,4,5 compounds. Protecting the amine group on 1-phenylamino-4-pentene **3.39** before oxidation of the alkene and subsequent phosphorylation was the next step. *N*-9-fluorenylmethoxycarbonyl-1-phenylamino-4-pentene **3.48** was synthesised in 97% yield by adding 9-fluorenylmethyl chloroformate to 1-phenylamino-4-pentene **3.39** in a 10% NaHCO₃/dioxane solution and stirring over 12 hours. Asymmetric dihydroxylation of the *N*-protected amine **3.48** came about using AD-mix- α . The highest yield of diol, *N*-9-fluorenylmethoxycarbonyl-1-phenylamino-4,5-pentan-diol **3.54** (45%) occurred when 1.9 g of AD-mix- α was added per one mmol of *N*-protected amine **3.48** and the reaction was allowed to stir at 4 °C for 18 hours. *N*-protected amine **3.48** was the only other material recovered (20%). Enantiomeric excess was not measured. Since literature boasts of high

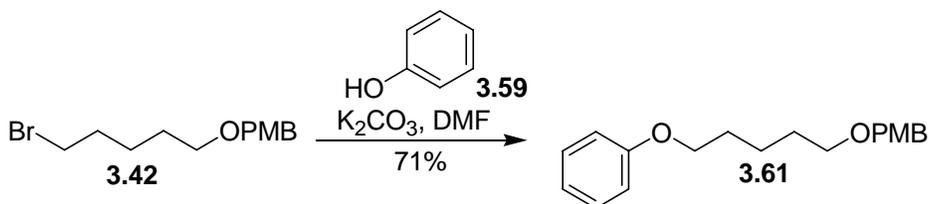
yields for similar reactions, it was thought the Fmoc *N*-protecting group might be sterically hindering access to the alkene **3.48**. Fmoc was replaced with *tert*-butyloxycarbonyl (Boc) by stirring 1-phenylamino-4-pentene **3.39** in a heterogeneous solution of 2.5% NaOH, Boc₂O, *t*-BuOH for 96 hours at room temperature. Disappointingly *N-tert*-butyloxycarbonyl-1-phenylamino-4-pentene **3.55** reacted with AD-mix- α in a similar manner to the synthesis of Fmoc *N*-protected diol **3.54**. Increasing the amount of AD-mix- α to 1.9 g per one mmol of Boc *N*-protected amine **3.55** and allowing the reaction to stir at 4 °C for 25 hours gave the highest isolated yields (56%). Lengthening the reaction duration and/or adding additional amounts of AD-mix- α did not improve yields or reduce the amount of starting material recovered. It is possible that the AD-mix- α degrades before reacting completely with either *N*-protected alkenes due to their sterically hindrance. Phosphorylating *N-tert*-butyloxycarbonyl-1-phenylamino-4,5-pentan-diol **3.56** formed the protected target 1,4,5 compound, *N-tert*-butyloxycarbonyl-1-phenylamino-5-diethyl phosphate-4-pentanol **3.57** (35%), in moderate yield using one equivalent of 2,6-dimethylpyridine and 1.1 equivalents of diethyl chlorophosphate over eight hours (scheme 5.11). The desired product formed in a 3:1 ratio with the secondary phosphate, *N-tert*-butyloxycarbonyl-1-phenylamino-4-diethyl phosphate-5-pentanol **3.58**. Isolation of the two products by flash chromatography proved challenging since their *R_f* values were close together. The tedious nature of the purification may account for the loss of material. It is hoped that deprotected target compound **3.57** will be used in future PRAI and IGPS enzyme studies.

Scheme 5.11. Synthesis of a protected target 1,4,5 compound **3.57**.



Preliminary synthesis of model *O*- and *C*- target compounds arose from using phenol **3.59** and benzyl magnesium bromide **3.65**, respectively, as the nucleophiles. Bromide leaving groups produce higher yields of ethers than mesyl leaving groups, as shown by bromide **3.42** forming 5-*O*-*para*-methoxybenzyl-1-phenoxy-pentane **3.61** in 71% yield compared with an analogous reaction yielding 51% of 5-*O*-*tert*-butyldimethylsilyl-1-phenoxy-pentane **3.60**, using mesyl **3.24**, phenol **3.59**, K_2CO_3 and DMF (scheme 5.12). Changing the base to NaOH increased the number of by-products as did warming the reaction, possibly due to the increased likelihood of eliminating the leaving group.

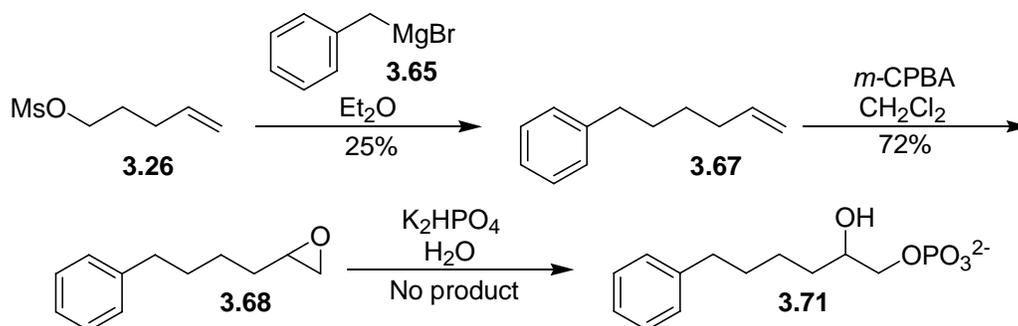
Scheme 5.12. Substitution of bromide **3.42** by phenol **3.59** at room temperature.



Benzyl magnesium bromide **3.65** was shown to displace mesyl leaving groups on both mesyl **3.24** and mesyl **3.26**. Yields were low in both reactions possibly due to the condition of benzyl magnesium bromide **3.65**, evident by the slow reaction rates. Epoxidation of hex-5-enylbenzene **3.67** by *meta*-chloroperoxybenzoic acid (*m*-CPBA) produced 5,6-epoxyhexylbenzene **3.68** (72%). An attempt at opening the epoxide ring of

5,6-epoxyhexylbenzene **3.68** with inorganic phosphate in refluxing water failed (scheme 5.13). The epoxide's limited solubility in water during reflux and the di-polar nature of the potential product, 2-hydroxy-6-phenyl-hexyl phosphate **3.71**, could be factors.

Scheme 5.13. Attempted ring opening of 5,6-epoxyhexylbenzene **3.68** with inorganic phosphate.



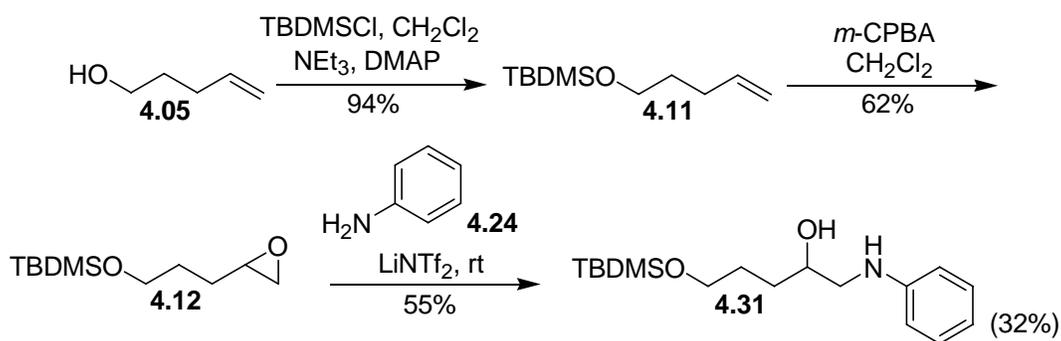
Protection/deprotection complications stopped the production of a suitable leaving group on a D-ribonolactone **3.15** moiety, which could have led to rCDRP target analogues through nucleophilic substitution. Substitution of leaving groups on pentan-1,5-diol and 4-penten-1-ol protected moieties was shown to be successful with a variety of nucleophilicities, producing several *N*-, *O*- and *C*- pentyl compounds. Methodology for the synthesis of protected target 1,4,5 compound **3.57** could be used to produce a range of target 1,4,5 compound analogues. *O*- and *C*- target 1,4,5 compound analogues could also be synthesised through the methodology developed in this thesis. These 1,4,5 compounds will be used in inhibition studies of PRAI and IGPS.

5.1.4. Conclusions of epoxide ring opening methodology:

Compounds with functionalities at the respective 1,2,5 positions along the pentyl moiety were developed by ring opening epoxides with aryl amines. The protected epoxide, 1-*O*-*tert*-butyldimethylsilyl-4,5-epoxypentanol **4.12**, was initially formed in low yields until an increase in equivalents of the epoxidation reagent *m*-CPBA was used. Epoxidations of 4-penten-1-ol **4.05** and 1-*O*-*tert*-butyldiphenylsilyl-4-penten-1-ol **4.17** gave moderate yields similar to epoxide **4.12** (62%), indicating *m*-CPBA may have been in poor

condition. The Lewis acid LiNTf_2 was chosen to catalyse the ring opening of epoxide **4.12** with anthranilate methyl ester, aniline **4.24** and cyclohexylamine **4.32**. Reactions using low equivalents of aryl amines and LiNTf_2 in CH_2Cl_2 failed to ring-open the epoxide **4.12** at room temperature. Recovery of starting materials showed the reaction rate was slow. Reactions using low equivalents of aniline **4.24** or cyclohexylamine **4.32**, using either LiNTf_2 , ZnCl_2 or $\text{BF}_3 \cdot \text{Et}_2\text{O}$ in various solvents, also failed or poorly opened the protected epoxide **4.12** ring under refluxing conditions. Increasing the equivalents of cyclohexylamine **4.32** or aniline **4.24** opened the epoxide **4.12** at room temperature, giving racemic 5-*O*-*tert*-butyldimethylsilyl-1-cyclohexylamino-pentan-2-ol **4.33** (63%) and racemic 5-*O*-*tert*-butyldimethylsilyl-1-phenylamino-pentan-2-ol **4.31** (55%), respectively. Hence, methodology to synthesise target 1,2,5 compounds (scheme 5.14) was established. These advanced secondary aryl amine synthons should lead to target 1,2,5 compounds after deprotection and phosphorylation.

Scheme 5.14. Formation of secondary aryl amine synthon **4.31** over three steps.



Synthesis of protected target 1,4,5 compound **3.57** and advanced 1,2,5 synthon, 5-*O*-*tert*-butyldimethylsilyl-1-phenylamino-pentan-2-ol **4.31**, can lead to gaining information how substrates and enzymes interact with PRAI and IGPS. Secondary aryl amine synthesis *via* nucleophilic substitution and epoxide ring opening proved consistently reproducible and allows for future synthesis of a range of CdRP-like target compounds.

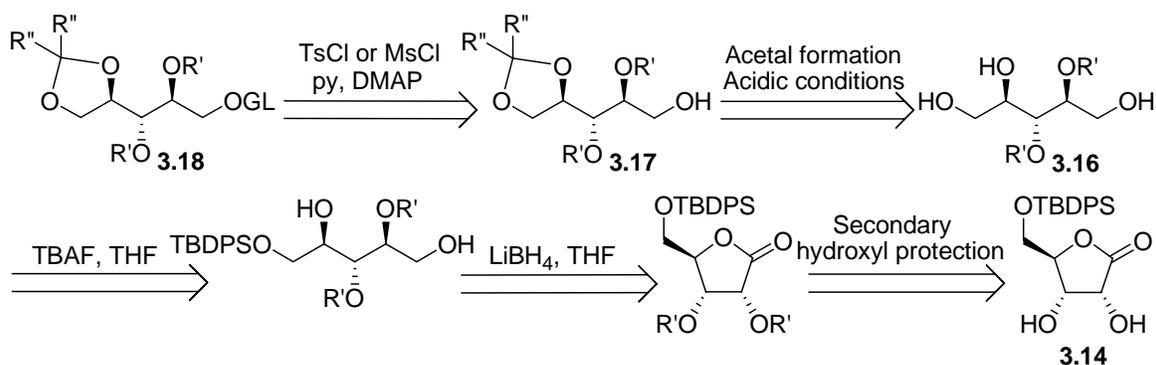
5.2. Possible future experiments:

Based on this work, a number of synthetic strategies and experiments can be pursued. First, the protected target 1,4,5 compound, *N-tert*-butyloxycarbonyl-1-phenylamino-5-diethyl phosphate-4-pentanol **3.57** should be deprotected and then tested for PRAI and IGPS activity. Second, the asymmetric dihydroxylation of *N-tert*-butyloxycarbonyl-1-phenylamino-4-pentene **3.55** with both AD-mix- α and AD-mix- β needs to be optimised. Optimisation of the phosphorylation reaction involving the 1,2 diol, *N-tert*-butyloxycarbonyl-1-phenylamino-4,5-pentan-diol **3.56**, should also be investigated. A chiral support could aid in the selectivity of the phosphorylation reaction.

To increase the range of synthesised target 1,4,5 compounds *via* nucleophilic substitution, different aryl amines can be used following the methodology created in the synthesis of protected target 1,4,5 compound **3.57**. Using the methods developed for nucleophilic substitution with phenol **3.59** and benzyl magnesium bromide **3.65** should lead to the synthesis of *O*- and *C*- target 1,4,5 compounds. Testing for PRAI and IGPS activity with these different target 1,4,5 compounds can lead to gaining information how substrates interact with PRAI and IGPS.

To gain access to 1,2,5 compounds, the protection strategies used for the synthesis of a mono primary good leaving group on a protected D-ribose moiety **3.17** (scheme 5.15) can be implemented. Secondary hydroxyl protection of the synthesised 5-*O-tert*-butyldiphenylsilyl-D-ribonolactone **3.14** leading to such a moiety could involve either β -methoxyethoxymethyl (MEM) ethers,^{283,284} methylene acetals²⁸¹ or allyl ethers.^{174,286,287} Reduction of the corresponding protected lactone and deprotection of the TBDPS-protecting group with tetrabutylammonium fluoride (TBAF), could give a 1,2,5 triol moiety **3.16**. The 1,2 diol functionality would then be protected with an acetal, freeing the primary hydroxyl to be subsequently transformed into a good leaving group. Following nucleophilic substitution methodology developed in this thesis, compound **3.18** could lead to CdRP-like target compounds.

Scheme 5.15. Proposed alternative strategy to primary good leaving group **3.18**.



To increase the range of synthesised target 1,2,5 compounds using methodology developed in the synthesis of the advanced synthon 5-*O*-*tert*-butyldimethylsilyl-1-phenylamino-pentan-2-ol **4.31**, different aryl amines, such as anthranilate methyl ester **3.09**, can be used to ring-open the protected epoxide 1-*O*-*tert*-butyldimethylsilyl-4,5-epoxypentanol **4.12**. This can lead to a reduced deoxy form of CdRP. Producing (*R*) and (*S*) enantiomers of racemic epoxide **4.12** using a procedure described by Kishi *et al.*,³³² could allow for the synthesis of enantiomerically pure target 1,2,5 compounds to test on PRAI and IGPS.

CHAPTER 6: Experimental.

6.1. General Methods:

6.1.2. Reagents and solvents:

All starting materials were obtained from commercial sources and used without further purification unless otherwise noted. All other reagents and solvents were obtained from commercial sources and used as supplied.

4 Å molecular sieves (4 Å M.S.) were acquired from Aldrich Chemical Co. and were activated by heating at 120 °C for 24 h prior to use.

Solvents were purified as follows unless stated differently. Tetrahydrofuran (THF) and diethyl ether (Et₂O) were freshly distilled from sodium benzophenone ketyl. Acetonitrile (MeCN), toluene and dichloromethane (CH₂Cl₂) were freshly distilled from calcium hydride (CaH₂).

Pyridine, benzylamine, and triethylamine (Et₃N) were dried and distilled from CaH₂ and stored over potassium hydroxide (KOH) pellets. *tert*-Butyl alcohol (*t*-BuOH) was dried over magnesium sulfate (MgSO₄), distilled from CaH₂, and stored over 4 Å M.S. Methanol (MeOH) was dried and distilled from CaH₂ and stored over 4 Å M.S. *N,N*-dimethylformamide (DMF) and dimethylsulfoxide (DMSO) were dried and distilled under reduced pressure from barium oxide (BaO) and stored over 4 Å M.S. Prior to use, the DMF solvent was evacuated (~0.1 mmHg) for 20 minutes to remove residual dimethylamine. Hexanes and ethyl acetate (EtOAc) were distilled to remove non-volatile contaminants prior to use in chromatography. The petroleum ether used consisted of the fraction with a boiling range of 50-70 °C.

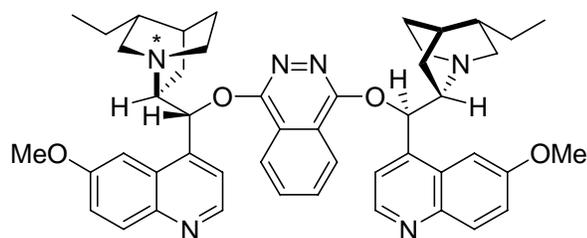
Methanesulfonyl chloride (MsCl) was dried and distilled from phosphorus pentoxide under vacuum and stored over 4 Å molecular sieves. *Para*-toluenesulfonyl chloride (TsCl) was recrystallised from petroleum ether and stored in a desiccator.

Aqueous dilutions of hydrochloric acid (HCl) and sodium hydroxide (NaOH) were produced in the laboratory. All sodium bicarbonate (NaHCO₃) washings are with saturated aqueous NaHCO₃ solutions. All brine (NaCl) washings are with saturated aqueous brine solutions.

NaH, 60% suspension in oil, was washed multiple times with hexane and pressed between two sheets of filter paper to dry to remove oil. *Meta*-chloroperbenzoic (*m*-CPBA) was purchased from Lancaster and was 55% pure. Dess-Martin periodinane²⁷³ (DMP) was prepared according to the modified procedures of Ireland and Liu.³⁵⁷ Benzyl trichloroacetimidate (BnOC(=NH)CCl₃) was synthesised according to the procedure of Patil.¹⁷⁴ *para*-Methoxybenzyl trichloroacetimidate (PMBOC(=NH)CCl₃) was synthesised according to the procedure of Patil.¹⁷⁴ Silver oxide (Ag₂O) was prepared according to the procedure of Janssen and Wilson.³⁵⁸ Benzyl magnesium bromide reagent was prepared according to the procedure of Zimmerman *et al.*³²³

AD-mix- α ³¹⁷ (asymmetric dihydroxylation) was purchased from Aldrich. The mix contains:

- Potassium osmate [K₂OsO₂(OH)₄] as the source of osmium tetroxide;
- Potassium ferricyanide [K₃Fe(CN)₆] as the re-oxidant in the catalytic cycle;
- Potassium carbonate;
- Chiral ligand (figure 6.01).



Legend: * Denotes osmium-nitrogen bonding site.

Figure 6.01. Chiral ligand of AD-mix- α ³¹⁷.

6.1.3. Synthetic methods:

Unless otherwise stated, all reactions were carried out in flame- or oven-dried glassware under an atmosphere of nitrogen or argon, with magnetic stirring. The reaction temperature refers to the external bath temperature. The progression of the reaction was monitored by thin layer chromatography (TLC). All organic extracts were dried over anhydrous magnesium sulfate. After filtration of the solution to remove the solids, the solvents were removed under reduced pressure on a Büchi rotary evaporator (~12 mmHg). All reported yields are isolated yields, unless otherwise stated. When necessary, a high vacuum pump (~0.1 mmHg) was used to remove the last traces of solvent from purified compounds.

6.1.4. Chromatography:

TLC was carried out on Merck Silica Gel 60 F₂₅₄ aluminum sheets, and observed under short- or long-wave ultraviolet (UV) light. Staining with dips could also identify the compounds.

Dips included;

- ninhydrin (staining nitrogen-containing compounds orange to red);
- alkali potassium permanganate (oxidises susceptible functional groups).

Flash column chromatography was performed on Scharlau Silica Gel 60 (230-400 mesh) using a ratio of hexanes to EtOAc unless stated otherwise. The compound is loaded onto the column, and then the initial solvent, which is stated in each procedure, is used to start

eluting the column. As products are run through the column, solvent polarity was generally increased in order to flush any remaining compounds from the column.

6.1.5. Characterisation:

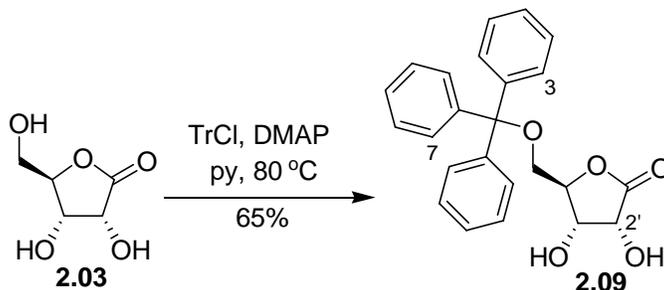
The nuclear magnetic resonance spectra were recorded on a Bruker Avance[®] 400 MHz NMR, at room temperature (23 °C) in deuterated chloroform (CDCl₃), unless otherwise stated. All chemical shifts are reported in parts per million (ppm) on the δ scale, relative to CHCl₃ (δ H 7.26 ppm) or CDCl₃ (δ C 77.0 ppm) as internal standards, respectively. Where this is not possible external tetramethyl silane (TMS) is used. If D₂O was used, chemical shifts are reported relative to HOD (δ H 4.70 ppm). All signal assignments were confirmed by 2D experiments (¹H/¹H COSY and ¹H/¹³C HMQC).

Infrared spectra were recorded using an FT-IR spectrometer, ThermoElectron Nicolet 5700, and absorptions are reported in reciprocal centimeters (cm⁻¹). Spectra of oils were run neat on KBr plates.

Mass spectra were recorded either by the Department of Chemistry, University of Auckland, or the Department of Chemistry, University of Canterbury on a VG-7070SE mass spectrometer or a Micromass LCT respectively. High Resolution Electron Impact Mass Spectrometry (HREIMS) or High Resolution Mass Spectrometry positive Electro-Spray Ionization, (HRMS ESI) were carried out by the University of Auckland or the University of Canterbury respectively.

6.2. Experiments described in Chapter Two:

Procedure for preparation of 5-*O*-triphenylmethyl-D-ribonolactone **2.09**.



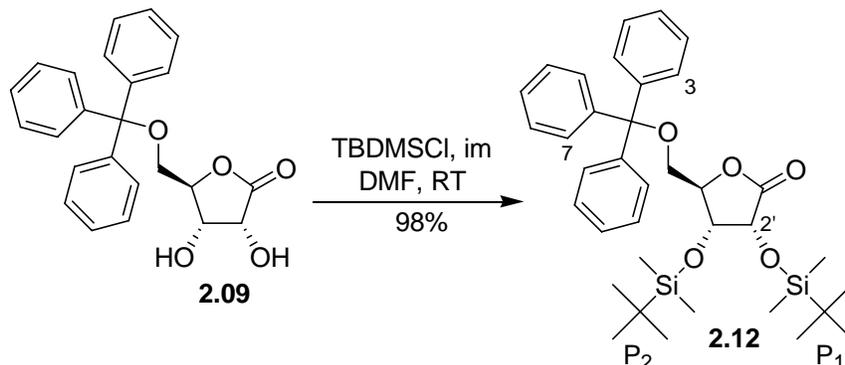
A procedure similar to those of Ireland *et al.*¹⁴³ and Taylor *et al.*¹⁴² was employed. Chlorotriphenylmethane (TrCl, 2.28 g, 8.18 mmol) was added to a solution of D-ribonolactone **2.03** (1.01 g, 6.88 mmol) and dimethylaminopyridine (DMAP, 165 mg, 1.35 mmol) in pyridine (49 mL). The resulting solution was heated at 80 °C for 17 hours. The cooled reaction mixture was diluted with 75 mL of CH₂Cl₂, washed with 1 M aqueous HCl (40 mL × 3), NaHCO₃ (20 mL × 2), H₂O (25 mL) and brine solution (25 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 1:1 hexanes-EtOAc afforded title compound **2.09** as a white solid (1.72 g, 65%).

R_f 0.22 (1:1 hexanes- EtOAc).

¹H NMR (400 MHz, CDCl₃) δ 3.43 (m, 2 H, H5'), 4.25 (dd, 1 H, *J* = 5.5, 1.0 Hz, H3'), 4.52 (app. t, 1 H, *J* = 2.7 Hz, H4'), 4.89 (d, 1 H, *J* = 5.5 Hz, H2'), 7.20-7.39 (m, 15 H, H3-H5, H7-H9).

¹³C NMR (400 MHz, CDCl₃) δ 63.1 (C5'), 67.8 (C3'), 83.8 (C4'), 87.9 (C2'), 88.3 (C1), 125.3 (C5) 125.9 (C9), 127.9 (C3), 128.5 (C7), 128.9 (C4), 129.5 (C8), 144.1 (C2), 144.9 (C6), 178.7 (C1').

Procedure for preparation of 2,3-bis-*O*-*tert*-butyldimethylsilyl-5-*O*-triphenylmethyl-D-ribonolactone **2.12.**



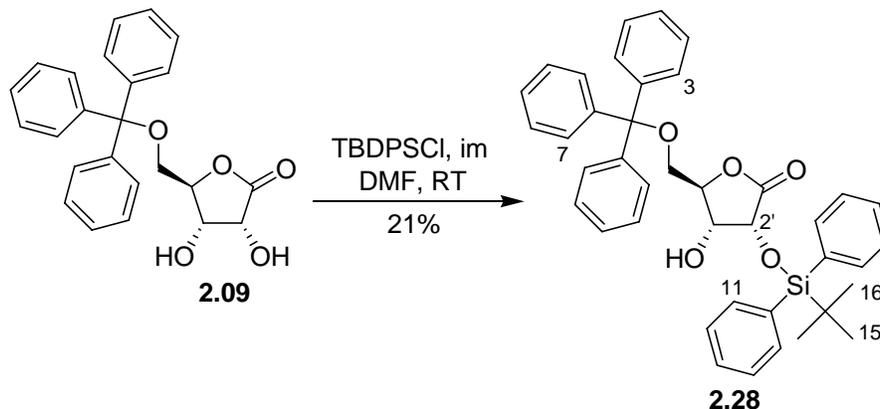
A procedure similar to that of Taylor *et al.*¹⁴² was employed. A solution of the 5-*O*-triphenylmethyl-D-ribonolactone **2.09** (2.34 g, 5.99 mmol) in DMF (6 mL) was added dropwise to a stirring solution of *tert*-butyldimethylsilyl chloride (TBDMSCl, 3.07 g, 20.37 mmol) and imidazole (im, 2.20 g, 32.35 mmol) in DMF (6 mL). The resulting solution was diluted with additional DMF (6 mL) and stirred at room temperature for 66 hours. Starting material was still evident by TLC so additional TBDMSCl (188 mg, 1.25 mmol) was added. After 6 hours, the reaction was diluted with CHCl₃ (200 mL) and washed with H₂O (250 mL × 2). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 5:1 hexanes-EtOAc afforded title compound **2.12** as a colourless oil (3.63 g, 98%).

R_f 0.46 (5:1 hexanes-EtOAc).

¹H NMR (400 MHz, CDCl₃) δ -0.06 (s, 3 H, P₂ -SiCH₃), 0.01 (s, 3 H, P₂ -SiCH₃), 0.09 (s, 3 H, P₁ -SiCH₃), 0.17 (s, 3 H, P₁ -SiCH₃), 0.79 (s, 9 H, P₂ -SiC(CH₃)₃), 0.92 (s, 9 H, P₁ -SiC(CH₃)₃), 3.40 (m, 2 H, H5'), 3.95 (dd, 1 H, *J* = 5.2, 1.0 Hz, H3'), 4.29 (app. t, 1 H, *J* = 2.7 Hz, H4'), 4.67 (d, 1 H, *J* = 5.2 Hz, H2'), 7.20-7.39 (m, 15 H, H3-H5, H7-H9).

¹³C NMR (400 MHz, CDCl₃) δ -5.2 (P₂ -SiCH₃), -4.9 (P₂ -SiCH₃), -4.7 (P₁ -SiCH₃), -4.6 (P₁ -SiCH₃), 18.1 (P₂ -SiC(CH₃)₃), 18.4 (P₁ -SiC(CH₃)₃), 25.6 (P₂ -SiC(CH₃)₃), 25.7 (P₁ -SiC(CH₃)₃), 62.3 (C5'), 70.3 (C3'), 72.0 (C2'), 84.6 (C4'), 87.5 (C1), 124.2 (C5), 124.8 (C9), 126.8 (C3), 127.4 (C7), 127.9 (C4), 128.5 (C8), 142.3 (C2), 143.1 (C6), 175.2 (C1').

Procedure for preparation of 2-*O*-*tert*-butyldiphenylsilyl-5-*O*-triphenylmethyl-D-ribose-5-phosphate 2.28.



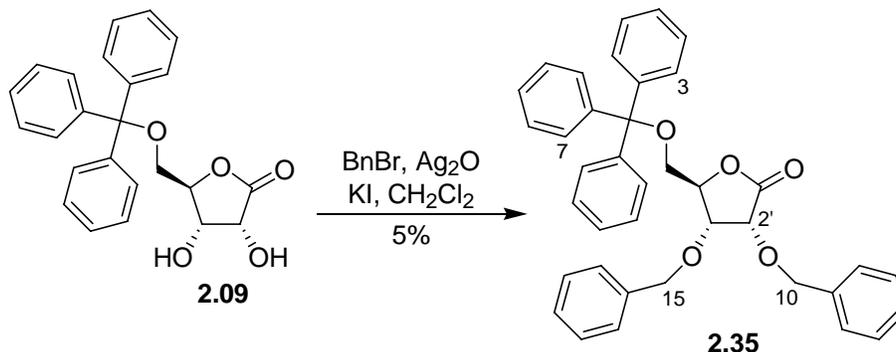
The procedure was based on work done by Taylor *et al.*¹⁴² and Bartlett *et al.*³⁵⁹ *tert*-Butyldiphenylsilyl chloride (TBDPSCl, 174 mg, 0.63 mmol) in DMF (0.8 mL) was added dropwise to a solution of 5-*O*-triphenylmethyl-D-ribose-5-phosphate **2.09** (73 mg, 0.19 mmol) and imidazole (50 mg, 1.00 mmol) in DMF (0.4 mL). The resulting solution had additional DMF (0.6 mL) added and was stirred at room temperature for 4 days. Starting material was still evident on TLC so a DMF solution (1 mL) of TBDPSCl (115 mg, 0.42 mmol) and imidazole (50 mg, 1.00 mmol) was added and stirred for another 24 hours. The reaction mixture was diluted with CH₂Cl₂ (15 mL) and washed with H₂O (15 mL × 2). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 10:1 hexanes-EtOAc afforded trace amounts of disilyl-protected product and title compound **2.28** (24 mg, 21%).

R_f 0.14 (10:1 hexanes-EtOAc).

¹H NMR (400 MHz, CDCl₃) δ 1.12 (s, 3 H, H15), 1.52 (s, 6 H, H16), 3.14 (dd, 1 H, *J* = 5.1, 1.0 Hz, H3'), 3.43 (m, 2 H, H5'), 4.33 (app. t, 1 H, *J* = 2.8 Hz, H4'), 4.74 (d, 1 H, *J* = 5.1 Hz, H2'), 7.20-7.47 (m, 21 H, H3-H5, H7-H9, H12, H13), 7.90 (d, 4 H, *J* = 7.1 Hz, H11).

¹³C NMR (400 MHz, CDCl₃) δ 18.9 (C14), 26.1 (C15), 26.3 (C16), 62.5 (C5'), 67.8 (C3'), 72.9 (C2'), 84.8 (C4'), 87.6 (C1), 124.2 (C5), 124.8 (C9), 126.8 (C3), 127.4 (C7), 127.9 (C4), 128.1 (C12), 128.5 (C8), 129.6 (C13), 133.9 (C10), 135.6 (C11), 142.3 (C2), 143.1 (C6), 175.2 (C1').

Procedure for preparation of 2,3-*O*-dibenzyl-5-*O*-triphenylmethyl-D-ribonolactone 2.35.



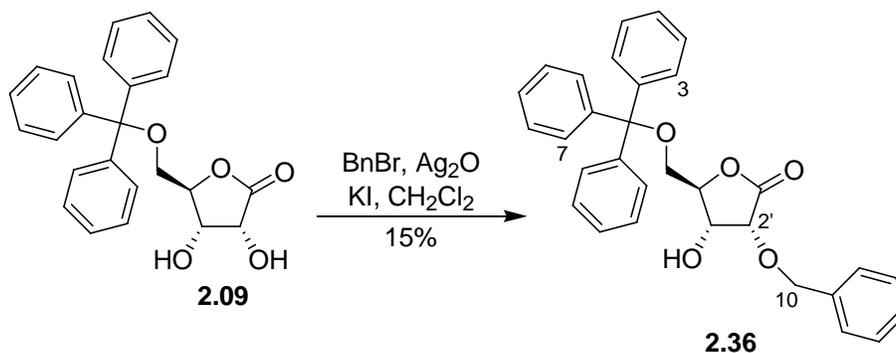
The procedure was based on work done by Luke *et al.*¹⁷¹ To an ice-bath solution of 5-*O*-triphenylmethyl-D-ribonolactone **2.09** (97 mg, 0.25 mmol) in CH₂Cl₂ (4 mL), potassium iodide (15 mg, 0.02 mmol), and Ag₂O (259 mg, 1.12 mmol) were added. Benzyl bromide (113 μ L, 0.94 mmol) was added by syringe and the solution was warmed to room temperature where it was stirred for 8.5 hours. Ag₂O was filtered off *via* celite pad, CH₂Cl₂ (10 mL \times 3) washings were combined, and washed with NaHCO₃ (25 mL), H₂O (15 mL) and brine solution (25 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 10:1 hexanes-EtOAc afforded monobenzylated lactone **2.36** (10 mg, 8%) and title compound **2.35** (7 mg, 5%).

R_f 0.26 (10:1 hexanes-EtOAc).

¹H NMR (400 MHz, CDCl₃) δ 3.31 (m, 2 H, H5'), 3.80 (dd, 1 H, *J* = 5.7, 1.0 Hz, H3'), 4.38 (app. t, 1 H, *J* = 1.0 Hz, H4'), 4.44 (m, 4 H, H10, H15), 4.47 (d, 1 H, *J* = 5.7 Hz, H2'), 7.17-7.39 (m, 25 H, H3-H5, H7-H9, H12- H14, H17-H19).

¹³C NMR (400 MHz, CDCl₃) δ 63.2 (C5'), 73.3 (C3'), 74.0 (C2'), 74.2 (C15), 74.4 (C10), 82.3 (C4'), 87.7 (C1), 124.2-129.3 (C3-C5, C7-C9, C12-C15, C17-C19), 138.2 (C16), 138.3 (C11), 142.4 (C2), 143.1 (C6), 177.0 (C1').

Procedure for preparation of 2-*O*-benzyl-5-*O*-triphenylmethyl-D-ribonolactone 2.36.



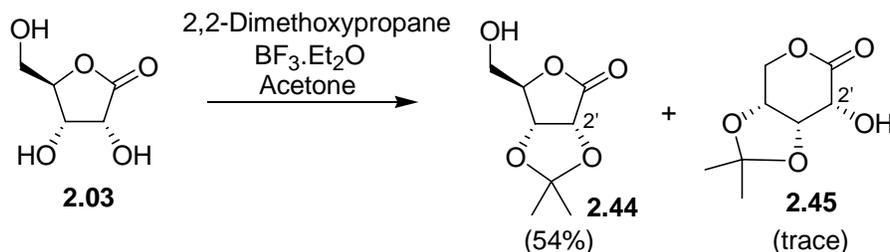
The procedure was based on work done by Luke *et al.*¹⁷¹ 5-*O*-Triphenylmethyl-D-ribonolactone **2.09** (41 mg, 0.11 mmol), potassium iodide (5 mg, 0.006 mmol), and Ag₂O (111 mg, 0.48 mmol) were dissolved in DMF (4 mL). Benzyl bromide (48 μ L, 0.39 mmol) was syringed in and stirred at room temperature for 24.5 hours. Ag₂O was filtered off *via* celite pad, CH₂Cl₂ (5 mL \times 5) washings were combined, and washed with NaHCO₃ (15 mL), H₂O (15 mL), and brine solution (15 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 10:1 hexanes-EtOAc afforded small amounts of dibenzylated lactone **2.35** (3%) and title compound **2.36** (10 mg, 15 %).

R_f 0.19 (5:1 hexanes-EtOAc).

¹H NMR (400 MHz, CDCl₃) δ 3.31 (m, 2 H, H5'), 4.07 (dd, 1 H, *J* = 5.7, 1.0 Hz, H3'), 4.40 (app. t, 1 H, *J* = 1.0 Hz, H4'), 4.44 (s, 2 H, H10), 4.48 (d, 1 H, *J* = 5.7 Hz, H2'), 7.18-7.39 (m, 20 H, H3-H5, H7-H9, H12- H14).

¹³C NMR (400 MHz, CDCl₃) δ 62.6 (C5'), 68.3 (C3'), 74.1 (C2'), 74.2 (C10), 82.6 (C4'), 87.5 (C1), 124.2 (C5), 124.5 (C14), 124.8 (C9), 126.8 (C3), 127.4 (C7), 127.6 (C12), 127.9 (C4), 128.5 (C8), 129.1 (C13), 138.2(C11), 142.3 (C2), 143.1 (C6), 176.9 (C1').

Procedure for preparation of 2,3-*O*-iso-propylidene-D-ribo-1,4-lactone 2.44.



A procedure similar to that of Salvini *et al.*¹⁸⁸ was employed. A solution of 2,2-dimethoxypropane (DMP, 0.90 mL, 7.33 mmol) and acetone (3.6 mL) was added to D-ribonolactone **2.03** (0.36 g, 2.44 mmol). Boron trifluoride etherate ($\text{BF}_3 \cdot \text{Et}_2\text{O}$, 0.031 mL, 0.24 mmol) was added dropwise and the reaction was stirred for 3.25 hours. The reaction was quenched with H_2O (3 mL), and allowed to stir overnight, before the layers were separated, and the aqueous phase extracted with EtOAc (10 mL \times 4). The organic extracts were combined, washed with water (10 mL \times 2), dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 2:1 EtOAc-hexanes to afford trace amounts of 3,4-*O*-iso-propylidene-D-ribo-1,5-lactone **2.45** and title compound **2.44** (0.25 g, 54%).

R_f 0.57 (4:1 EtOAc-hexanes).

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 1.37 (s, 3 H, - CCH_3), 1.46 (s, 3 H, - CCH_3), 3.79 (ddd, 1 H, $J = 12.3, 5.6, 1.6$ Hz, $\text{H}5'$), 3.96 (ddd, 1 H, $J = 12.3, 5.3, 2.3$ Hz, $\text{H}5'$), 4.60 (app. t, 1 H, $\text{H}4'$), 4.73 (d, 1 H, $J = 5.5$ Hz, $\text{H}3'$), 4.81 (d, 1 H, $J = 5.5$ Hz, $\text{H}2'$).

$^{13}\text{C NMR}$ (400 MHz, CDCl_3) δ 25.4 (- CCH_3), 26.6 (- CCH_3), 61.7 ($\text{C}5'$), 75.3 ($\text{C}3'$), 78.6 ($\text{C}4'$), 83.1 ($\text{C}2'$), 113.5 (- $(\text{O})_2\text{C}(\text{CH}_3)_2$), 175.0 ($\text{C}1'$).

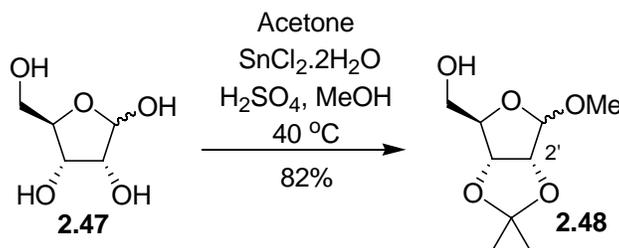
Trace amounts of 3,4-*O*-iso-propylidene-D-ribo-1,5-lactone **2.45** were also isolated.

R_f 0.25 (4:1 EtOAc-hexanes).

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 1.37 (s, 3 H, - CCH_3), 1.46 (s, 3 H, - CCH_3), 4.22 (dd, 1 H, $J = 13.2, 7.7$ Hz, $\text{H}5'$), 4.37 (d, 1 H, $J = 3.5$ Hz, $\text{H}2'$), 4.40 (br. d, 1 H, $J = 13.2$ Hz, $\text{H}5'$), 4.59 (br. d, 1 H, $J = 7.7$ Hz, $\text{H}4'$), 4.82 (dd, 1 H, $J = 7.8, 3.6$ Hz, $\text{H}3'$).

$^{13}\text{C NMR}$ (400 MHz, CDCl_3) δ 24.4 (- CCH_3), 25.0 (- CCH_3), 67.8 ($\text{C}5'$), 68.6 ($\text{C}4'$), 72.9 ($\text{C}2'$), 74.9 ($\text{C}3'$), 111.0 (- $(\text{O})_2\text{C}(\text{CH}_3)_2$), 170.7 ($\text{C}1'$).

Procedure for preparation of methyl-2,3-*O*-iso-propylidene- β -D-ribofuranoside **2.48.**



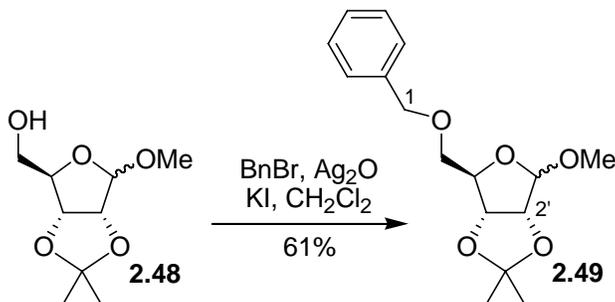
A procedure similar to that of Chandra *et al.*¹⁹¹ was employed. D-ribose **2.47** (3.63 g, 24.19 mmol) and tin (II) chloride (5.46 g, 24.19 mmol) were suspended in a mixture of acetone (73 mL) and methanol (19 mL) containing a catalytic amount of concentrated sulfuric acid (0.27 mL, 5.10 mmol). The resulting mixture was stirred at 40 °C for 21.5 h. After this period, the mixture was allowed to cool, filtered through celite and the filter cake was washed with a mixture of acetone and methanol (1:1 mixture, 50 mL \times 3). The resulting solution was neutralised with NaHCO₃ solution, and the methanol and acetone were removed under reduced pressure. The residue was extracted with EtOAc (50 mL \times 3), combined and washed with brine. The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 5:1 hexanes-EtOAc afforded the title compound **2.48** as a white syrup (4.05 g, 82%).

R_f 0.37 (2:1 hexanes-EtOAc).

¹H NMR (400 MHz, CDCl₃) δ 1.32 (s, 3 H, -CCH₃), 1.49 (s, 3 H, -CCH₃), 3.44 (s, 3 H, -COCH₃), 3.61 (m, 2 H, H5'), 4.33 (app. t, 1 H, J = 3.1 Hz, H4'), 4.59 (d, 1 H, J = 5.9 Hz, H3'), 4.83 (d, 1 H, J = 5.9 Hz, H2'), 4.97 (app. s, 1 H, H1').

¹³C NMR (400 MHz, CDCl₃) δ 25.1 (-CCH₃), 26.9 (-CCH₃), 56.0 (-COCH₃), 64.5 (C5'), 82.1 (C3'), 86.1 (C4'), 88.9 (C2'), 110.5 (C1'), 112.5 (-(O)₂C(CH₃)₂).

Procedure for preparation of methyl-2,3-*O*-iso-propylidene-5-*O*-benzyl- β -D-ribofuranoside **2.49.**



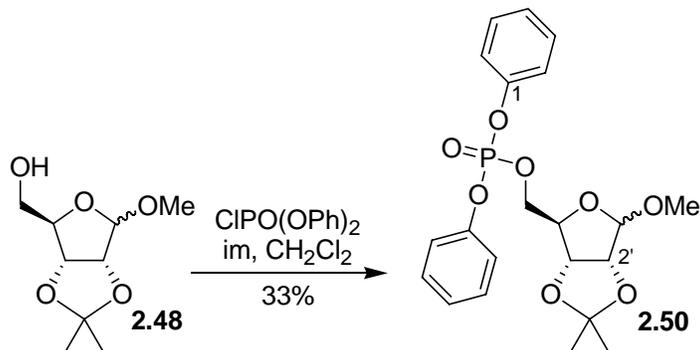
The procedure was based on work done by Luke *et al.*¹⁷¹ Methyl-2,3-*O*-isopropylidene- β -D-ribofuranoside **2.48** (0.28 g, 1.35 mmol), potassium iodide (23 mg, 0.14 mmol) and Ag₂O (0.41 g, 1.76 mmol) were dissolved or suspended in CH₂Cl₂ (5 mL). Benzyl bromide (0.19 mL, 1.56 mmol) was syringed in and the solution was allowed to stir at room temperature for 18 hours. Ag₂O was filtered off *via* celite pad, CH₂Cl₂ (15 mL \times 3) washings were combined, and washed with NaHCO₃ (50 mL), H₂O (50 mL), and brine solution (55 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 10:1 hexanes-EtOAc afforded the title compound **2.49** (0.24 g, 61 %).

R_f 0.79 (2:1 hexanes-EtOAc).

¹H NMR (400 MHz, CDCl₃) δ 1.32 (s, 3 H, -CCH₃), 1.49 (s, 3 H, -CCH₃), 3.44 (s, 3 H, -COCH₃), 3.50 (m, 2 H, H5'), 4.29 (app. t, 1 H, *J* = 3.0 Hz, H4'), 4.51 (m, 2 H, H1), 4.59 (d, 1 H, *J* = 5.9 Hz, H3'), 4.83 (d, 1 H, *J* = 5.9 Hz, H2'), 4.97 (app. s, 1 H, H1'), 7.26-7.37 (m, 5 H, H3-H5).

¹³C NMR (400 MHz, CDCl₃) δ 25.1 (-CCH₃), 26.9 (-CCH₃), 56.0 (-COCH₃), 70.1 (C5'), 78.1 (C1), 82.2 (C3'), 84.0 (C4'), 88.9 (C2'), 110.4 (C1'), 112.5 (-(O)₂C(CH₃)₂), 127.3 (C5), 128.2 (C3), 128.9 (C4), 138.3 (C2).

Procedure for preparation of methyl-2,3-*O*-*iso*-propylidene-5-*O*-diphenylphosphate- β -D-ribofuranoside **2.50.**



The procedure was based on work done by Tener and Khorana.¹²² To an ice-bath mixture of methyl-2,3-*O*-*iso*-propylidene- β -D-ribofuranoside **2.48** (0.66 g, 3.25 mmol) and imidazole (0.31 g, 4.54 mmol), CH₂Cl₂ (5 mL) was added. The solution was stirred and diphenyl phosphorochloridate (0.89 mL, 4.29 mmol) was added dropwise slowly over a minute. The ice-bath was removed after 10 minutes and the solution was stirred at room temperature for 7 hours. The reaction was quenched with H₂O (2 mL), then diluted with EtOAc (30 mL), and the organic layer washed with 1 M HCl (40 mL), H₂O (50 mL \times 2) and brine solution (25 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 5:1 hexanes-EtOAc afforded the title compound **2.50** (0.46 g, 33%).

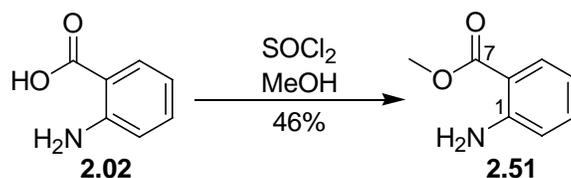
R_f 0.51 (2:1 hexanes-EtOAc).

¹H NMR (400 MHz, CDCl₃) δ 1.28 (s, 3 H, -CCH₃), 1.45 (s, 3 H, -CCH₃), 3.31 (s, 3 H, -COCH₃), 4.20 (m, 2 H, H5'), 4.39 (app. t, 1 H, *J* = 7.2 Hz, H4'), 4.55 (d, 1 H, *J* = 5.9 Hz, H3'), 4.63 (d, 1 H, *J* = 5.9 Hz, H2'), 4.96 (app. s, 1 H, H1'), 7.14-7.39 (m, 10 H, H2-H4).

¹³C NMR (400 MHz, CDCl₃) δ 25.1 (-CCH₃), 26.8 (-CCH₃), 55.5 (-COCH₃), 68.8 (C5'), 81.8 (C3'), 85.3 (C4'), 88.4 (C2'), 109.8 (C1'), 113.0 (-(O)₂C(CH₃)₂), 120.5 (C2), 125.9 (C4), 130.1 (C3), 151.2 (C1).

³¹P NMR (400 MHz, CDCl₃) δ -10.9 (P1).

Procedure for preparation of anthranilate methyl ester 2.51.



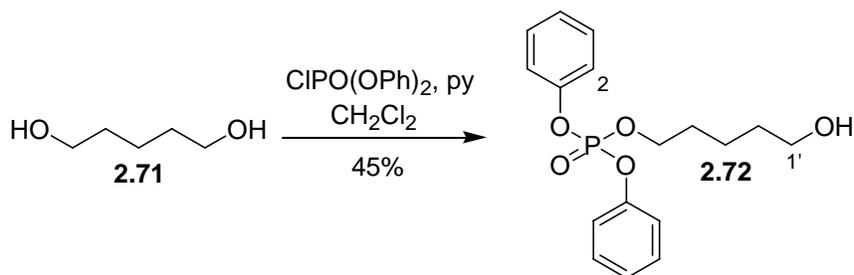
A procedure similar to that of Hosangadi and Dave was employed.¹⁹⁷ Thionyl chloride (SOCl₂, 19.67 mL, 269.7 mmol) was added to anthranilic acid **2.02** (12.3 g, 89.9 mmol) in an ice-bath. The solution was stirred and kept at ~0 °C for 0.5 hours and then room temperature for 3 hours. The thick solution was cooled to -30 °C before methanol (12.0 mL) was added dropwise over a period of 10 minutes. The solution was kept at -30 °C for 0.5 hours and then allowed to warm to 4 °C where it was stirred for 15 hours. The reaction was quenched by the slow addition of MeOH (20 mL), and after a few minutes H₂O was added dropwise (60 mL). The reaction mixture was diluted in EtOAc (200 mL) and aqueous layer was extracted with Et₂O (20 mL × 4). The organic layers were combined, dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 10:1 hexanes-EtOAc afforded the title compound **2.51** as a white solid (6.26 g, 46%).

R_f 0.36 (5:1 hexanes- EtOAc).

¹H NMR (400 MHz, CDCl₃) δ 3.85 (s, 3 H, H8), 6.59 (dd, 1 H, *J* = 8.4, 8.1 Hz, H4), 6.68 (d, 1 H, *J* = 8.4 Hz, H6), 7.41 (t, 1 H, *J* = 8.4 Hz, H5), 7.90 (d, 1 H, *J* = 8.1 Hz, H3).

¹³C NMR (400 MHz, CDCl₃) δ 52.8 (C8), 112.1 (C2), 116.9 (C6), 117.4 (C4), 131.9 (C5), 134.7 (C3), 150.2 (C1), 169.3 (C7).

Procedure for preparation of 5-diphenyl phosphate pentanol 2.72.



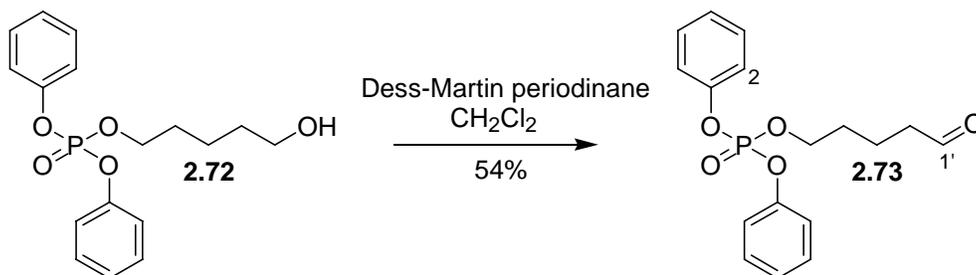
The procedure was based on work done by Tener and Khorana.¹²² Pentan-1,5-diol **2.71** (1.01 mL, 9.54 mmol) was dissolved in CH_2Cl_2 (30 mL) and stirred in an ice-bath. Pyridine (0.77 mL, 9.54 mmol) and then diphenyl phosphorochloridate (3.05 mL, 11.35 mmol) were syringed in and stirred at $\sim 0^\circ\text{C}$ for 0.75 hours before allowing the reaction to warm to room temperature with stirring over a period of 21.5 hours. The reaction was quenched by slowly adding 1 M HCl (25 mL). This mixture was then diluted with CH_2Cl_2 (15 mL \times 2). The organic layer was washed with H_2O (45 mL) and brine solution (45 mL), dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 1:1 hexanes-EtOAc afforded the title compound **2.72** (1.45 g, 45%).

R_f 0.15 (1:1 hexanes- EtOAc).

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 1.43 (tt, 2 H, $J = 7.8, 7.1$ Hz, $\text{H}_{3'}$), 1.55 (tt, 2 H, $J = 7.8, 7.1$ Hz, $\text{H}_{2'}$), 1.73 (tt, 2 H, $J = 7.1$, $\text{H}_{4'}$), 3.60 (t, 2 H, $J = 7.1$ Hz, $\text{H}_{1'}$), 4.26 (m, 2 H, $\text{H}_{5'}$), 7.14-7.39 (m, 10 H, H_2 - H_4).

$^{13}\text{C NMR}$ (400 MHz, CDCl_3) δ 23.0 ($\text{C}_{3'}$), 31.2 ($\text{C}_{4'}$), 33.2 ($\text{C}_{2'}$), 63.6 ($\text{C}_{1'}$), 70.7 (d, 1 C, $J = 6.2$ Hz, $\text{C}_{5'}$), 121.5 (C2), 126.8 (C4), 131.2 (C3), 151.9 (d, 1 C, $J = 7.8$ Hz, C1).

Procedure for preparation of 5-diphenyl phosphate pentanal 2.73.



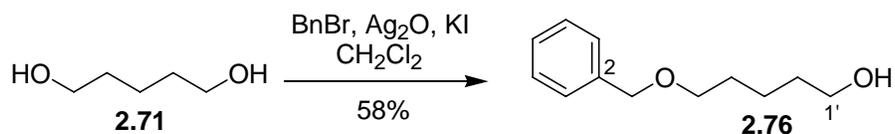
The procedure was based on work done by Dess and Martin.²⁷³ 5-Diphenyl phosphate pentanol **2.72** (1.12 g, 3.33 mmol) was dissolved in CH_2Cl_2 (20 mL) and stirred in an ice-bath. Dess-Martin periodinane (DMP) (3.22 g, 7.59 mmol) was added and the resulting white solution was stirred at $\sim 0^\circ\text{C}$ for 10 minutes before allowing the reaction to warm to room temperature and to stir for a further 18 hours. After this time, starting material was still evident by TLC so another addition of DMP (1.28 g, 3.01 mmol) was added and the solution stirred for a further 2.5 hours. The reaction mixture was diluted with CH_2Cl_2 (60 mL) and excess DMP was filtered off *via* a silica glass plug. The washings, CH_2Cl_2 (20 mL \times 4), of the silica plug were combined and washed with H_2O (55 mL), NaHCO_3 (80 mL), $\text{Na}_2\text{S}_2\text{O}_3$ (50 mL), and brine solution (45 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 3:2 hexanes-EtOAc afforded the title compound **2.73** of yellowish oil (0.60 g, 54%).

R_f 0.48 (1:1 hexanes- EtOAc).

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 1.64-1.78 (m, 4 H, H3'-H4'), 2.44 (dt, 2 H, $J = 6.6, 1.7$ Hz, H2'), 4.27 (m, 2 H, H5'), 7.16-7.38 (m, 10 H, H2-H4), 9.72 (br. s, 1 H, H1').

$^{13}\text{C NMR}$ (400 MHz, CDCl_3) δ 18.4 (C3'), 29.8 (C4'), 43.4 (C2'), 69.2 (d, 1 C, $J = 6.2$ Hz, C5'), 120.5 (C2), 125.8 (C4), 130.2 (C3), 150.9 (s, 1 C, $J = 7.8$ Hz, C1), 202.2 (C1').

Procedure for preparation of 5-*O*-benzylpentanol **2.76**.



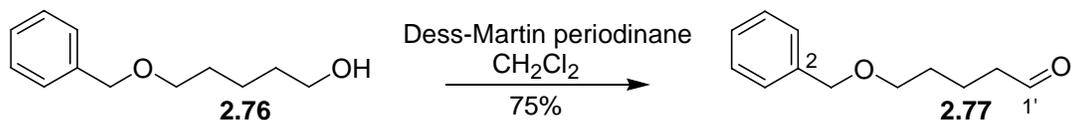
The procedure was based on examples by Greene,¹²⁵ Kocienski¹²⁴ and Luke *et al.*¹⁷¹ Pentan-1,5-diol **2.71** (1.37 g, 13.2 mmol) was dissolved in CH₂Cl₂ (40 mL) and stirred in an ice-bath. Potassium iodide (0.11 g, 0.66 mmol) and Ag₂O (3.05 g, 13.2 mmol) were added, and the solution was stirred for 10 minutes before benzyl bromide (1.57 mL, 13.2 mmol) was added in a continuous dropwise fashion. Following this addition, the ice-bath was removed and the solution was placed in ultrasonic bath for 5 minutes. The solution was removed from the ultrasonic bath and stirred at room temperature for 23 hours. The Ag₂O was removed from the solution *via* filtration using a celite pad. The combined washings, CH₂Cl₂ (20 mL × 5), and filtrate were washed with NaHCO₃ (60 mL), H₂O (60 mL), and brine solution (60 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 3:2 hexanes-EtOAc afforded the title compound **2.76** (1.48 g, 58%).

*R*_f 0.50 (2:1 hexanes- EtOAc).

¹H NMR (400 MHz, CDCl₃) δ 1.45 (tt, 2 H, *J* = 6.8, 6.5 Hz, H3'), 1.59 (tt, 2 H, *J* = 6.8, 6.6 Hz, H2'), 1.66 (tt, 2 H, *J* = 6.6, 6.5 Hz, H4'), 3.49 (t, 2 H, *J* = 6.5 Hz, H5'), 3.65 (t, 2 H, *J* = 6.6 Hz, H1'), 4.51 (s, 2 H, H1), 7.26-7.37 (m, 5 H, H3-H5).

¹³C NMR (400 MHz, CDCl₃) δ 22.8 (C3'), 29.9 (C4'), 32.9 (C2'), 63.3 (C1'), 70.7 (C5'), 73.3 (C1), 127.5 (C5), 128.1 (C3), 128.8 (C4), 138.9 (C2).

Procedure for preparation of 5-*O*-benzylpentanal **2.77**.



The procedure was based on work done by Dess and Martin.²⁷³ 5-Benzylpentanol **2.76** (2.6 g, 13.38 mmol) was dissolved in CH₂Cl₂ (65 mL) and stirred in an ice-bath. Dess-Martin periodinane (12.2 g, 28.76 mmol) was added and the resulting white solution was

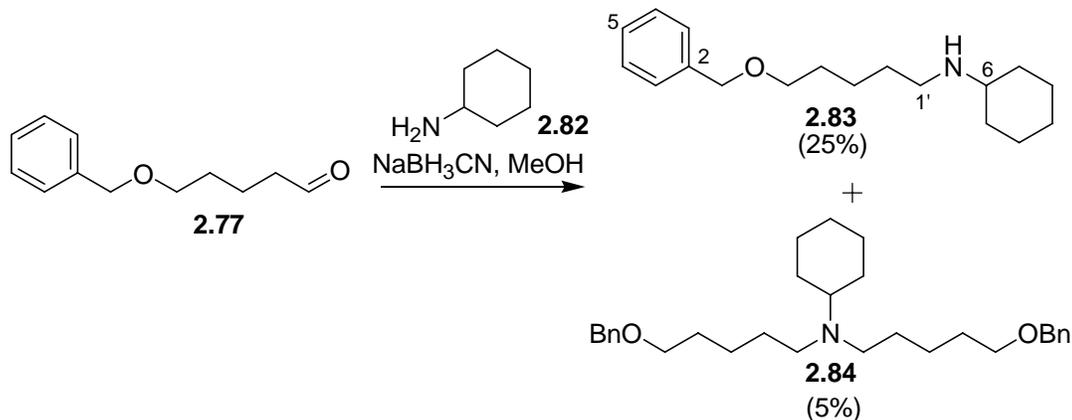
stirred at ~ 0 °C for 20 minutes before removing the ice-bath and allowing the reaction to warm to room temperature and stir for a further 16 hours. The reaction mixture was diluted with CH_2Cl_2 (60 mL) and filtered using a silica glass plug. The washings, CH_2Cl_2 (20 mL \times 4), of the silica plug were combined and washed with NaHCO_3 (50 mL), $\text{Na}_2\text{S}_2\text{O}_3$ (50 mL), H_2O (50 mL), and brine solution (50 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 5:1 hexanes-EtOAc afforded the title compound **2.77** of yellowish oil (1.93 g, 75%).

R_f 0.52 (5:1 hexanes- EtOAc).

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 1.62-1.78 (m, 4 H, H3'-H4'), 2.46 (dt, 2 H, $J = 7.0, 1.7$ Hz, H2'), 3.50 (t, 2 H, $J = 6.6$ Hz, H5'), 4.51 (s, 2 H, H1), 7.28-7.41 (m, 5 H H3-H5), 9.76 (br. s, 1 H, H1').

$^{13}\text{C NMR}$ (400 MHz, CDCl_3) δ 19.3 (C3'), 29.5 (C4'), 44.0 (C2'), 70.1 (C5'), 73.4 (C1), 128.0 (C5), 128.1 (C3), 128.8 (C4), 138.8 (C2), 203.0 (C1').

Procedure for preparation of 5-O-benzylpentylcyclohexylamine **2.83**.



Cyclohexylamine **2.82** (29 μL , 0.255 mmol) was added to a solution of 5-benzylpentanal **2.77** (35 mg, 0.182 mmol) in methanol (3 mL). The solution was adjusted to a pH of 5.5 with acetic acid and stirred at room temperature for 5 minutes before sodium cyanoborohydride (NaBH_3CN , 67 mg, 1.07 mmol) was added. The solution was stirred for 17 hours before being diluted in EtOAc (8 mL \times 3), and washed with H_2O (15 mL) and brine solution (15 mL). The organic layer was dried and concentrated *in vacuo*.

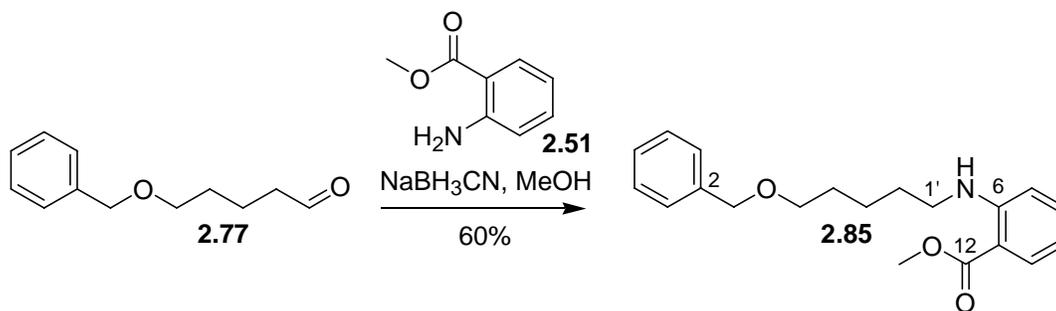
Purification of the crude material by flash chromatography on silica, eluting with 15:1 hexanes-EtOAc potentially afforded the tertiary amine **2.84** (4 mg, 5%) and title compound **2.83** (12 mg, 25%).

R_f 0.45 (3:1 hexanes- EtOAc).

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 0.88-1.91 (m, 16 H, H2'-H4', H7-H9), 2.63 (quin, 1 H, $J = 6.8$ Hz, H6), 2.70 (t, 2 H, $J = 7.0$ Hz, H1'), 3.49 (t, 2 H, $J = 6.6$ Hz, H5'), 4.51 (s, 2 H, H1), 7.22-7.38 (m, 5 H, H3-H5).

$^{13}\text{C NMR}$ (400 MHz, CDCl_3) δ 23.1 (C8), 24.3 (C3'), 26.4 (C9), 29.8 (C4'), 32.0 (C7), 32.3 (C2'), 46.2 (C1'), 55.8 (C6), 70.2 (C5'), 73.4 (C1), 128.0 (C5), 128.1 (C3), 128.7 (C4), 138.7 (C2).

Procedure for preparation of 5-*O*-benzylpentyl anthranilate methyl ester **2.85**.



Anthranilate methyl ester **2.51** (29 mg, 0.19 mmol), dissolved in methanol (0.5 mL \times 2), was added to a solution of 5-benzylpentanal **2.77** (16 mg, 0.085 mmol) in methanol (3 mL). The solution was adjusted to a pH of 5.5 with acetic acid and was stirred at room temperature for 15 minutes before sodium cyanoborohydride (10 mg, 0.17 mmol) was added. The solution was stirred for 5.5 hours before being quenched by the addition of H_2O (1 mL), then diluted with Et_2O (10 mL), and washed with H_2O (10 mL) and brine solution (15 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 15:1 hexanes-EtOAc afforded the title compound **2.85** (17 mg, 60%).

R_f 0.41 (3:1 hexanes- EtOAc).

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 1.49-1.56 (m, 2 H, H3'), 1.66-1.79 (m, 4 H, H2', H4'), 3.22 (t, 2 H, $J = 7.0$ Hz, H1'), 3.49 (t, 2 H, $J = 6.6$ Hz, H5'), 3.88 (s, 3 H, H13), 4.51 (s, 2

H, H1), 6.64 (dd, 1 H, $J = 8.2, 8.1$ Hz, H9), 6.74 (d, 1 H, $J = 8.2$ Hz, H7), 7.29 (t, 2 H, $J = 7.9$ Hz, H4), 7.33-7.37 (m, 3 H, H3, H5), 7.40 (t, 1 H, $J = 8.2$ Hz, H8), 7.97 (d, 1 H, $J = 8.1$ Hz, H10).

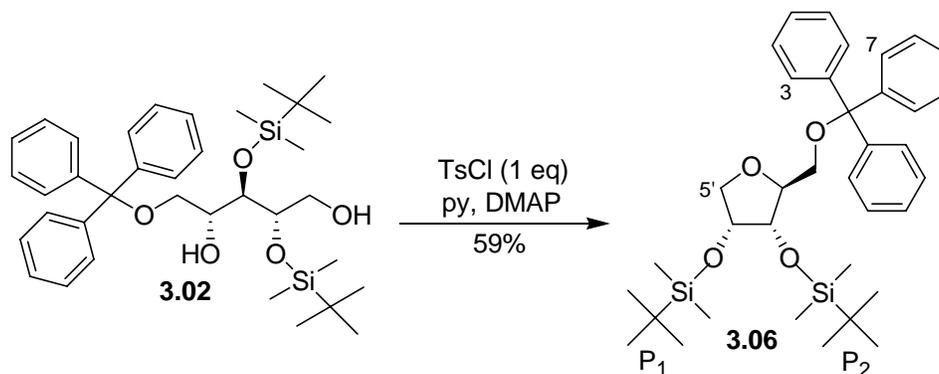
^{13}C NMR (400 MHz, CDCl_3) δ 23.8 (C3'), 28.8 (C2'), 29.5 (C4'), 43.4 (C1'), 61.9 (C13), 70.1 (C5'), 73.4 (C1), 109.5 (C7), 112.1 (C11), 115.2 (C9), 128.0 (C5), 128.1 (C3), 128.7 (C4), 132.6 (C10), 135.6 (C8), 138.5 (C2), 151.1 (C6), 172.9 (C12).

IR (KBr) 1655 cm^{-1} .

HREIMS: calculated $\text{C}_{20}\text{H}_{25}\text{NO}_3$ 327.1834; observed 327.1701.

6.3. Experiments described in Chapter Three:

Procedure for preparation of (2*S*,3*R*,4*R*)-3,4-bis-*O*-*tert*-butyldimethylsilyl-1-*O*-triphenylmethyl tetrahydrofuran **3.06**.



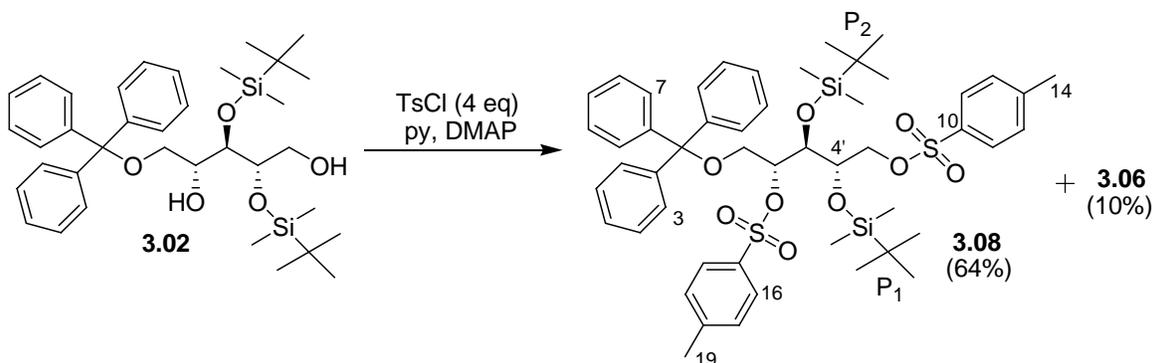
The procedure was based on work done by Taylor *et al.*¹⁴² and Mash *et al.*²⁹⁵ A solution of the diol **3.02** (130 mg, 0.21 mmol) in dry pyridine (0.4 mL) was added dropwise to a cooled ($0\text{ }^\circ\text{C}$), premixed suspension of TsCl (40 mg, 0.21 mmol) and DMAP (5 mg, 0.04 mmol) in dry pyridine (0.2 mL). The resulting orange solution was removed from the ice-bath and stirred for 7 hours at $4\text{ }^\circ\text{C}$. The reaction mixture was diluted with chloroform (10 mL) and washed with 1 M HCl (10 mL \times 2), NaHCO_3 (10 mL), and water (10 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 20:1 hexanes-EtOAc afforded the title compound **3.06** (73 g, 59%).

R_f 0.36 (10:1 hexanes-EtOAc).

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ -0.06 (s, 3 H, P_2 - SiCH_3), 0.01 (s, 3 H, P_2 - SiCH_3), 0.09 (s, 3 H, P_1 - SiCH_3), 0.17 (s, 3 H, P_1 - SiCH_3), 0.79 (s, 9 H, P_2 - $\text{SiC}(\text{CH}_3)_3$), 0.92 (s, 9 H, P_1 - $\text{SiC}(\text{CH}_3)_3$), 3.00 (dd, 1 H, $J = 10.0, 4.2$ Hz, $\text{H1}'$), 3.26 (dd, 1 H, $J = 10.0, 3.3$ Hz, $\text{H1}'$), 3.73 (dd, 1 H, $J = 8.2, 5.7$, $\text{H4}'$), 3.91-3.98 (m, 3 H, $\text{H2}'$, $\text{H5}'$), 4.15 (m, 1 H, $\text{H3}'$), 7.19-7.39 (m, 15 H, H3-H5 , H7-H9).

$^{13}\text{C NMR}$ (400 MHz, CDCl_3) δ -4.8 (P_2 - SiCH_3), -4.5 (P_2 - SiCH_3), -4.3 (P_1 - SiCH_3), -4.2 (P_1 - SiCH_3), 18.5 (P_2 - $\text{SiC}(\text{CH}_3)_3$), 18.6 (P_1 - $\text{SiC}(\text{CH}_3)_3$), 26.2 (P_2 - $\text{SiC}(\text{CH}_3)_3$), 26.4 (P_1 - $\text{SiC}(\text{CH}_3)_3$), 64.6 ($\text{C1}'$), 72.4 ($\text{C3}'$), 73.1 ($\text{C4}'$), 74.3 ($\text{C5}'$), 83.6 ($\text{C2}'$), 87.0 (C1), 127.4 (C5) 127.6 (C9), 128.2 (C3), 128.6 (C7), 129.1 (C4), 129.6 (C8), 144.1 (C2), 143.4 (C6).

Procedure for preparation of 3,4-bis-*O*-*tert*-butyldimethylsilyl-2,5-bis-*O*-toluenesulfonyl-1-*O*-triphenylmethyl-D-ribose 3.08.



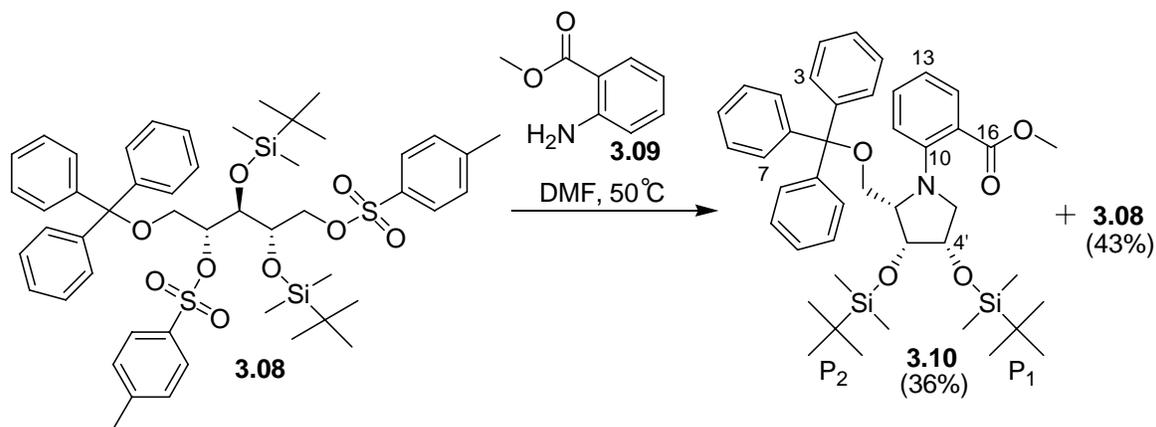
The procedure was based on work done by Taylor *et al.*¹⁴² and Mash *et al.*²⁹⁵ A solution of the diol **3.02** (0.78 g, 1.25 mmol) in dry pyridine (2.6 mL) was added dropwise to a cooled (0 °C), premixed suspension of TsCl (0.97 g, 5.07 mmol) and DMAP (0.04 g, 0.33 mmol) in dry pyridine (2.4 mL). The resulting orange solution was removed from the ice-bath and stirred for 17 hours at room temperature. The reaction mixture was diluted with chloroform (40 mL) and washed with 1 M HCl (30 mL \times 2), NaHCO_3 (25 mL), and water (25 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 5:1 hexanes-EtOAc, afforded the tetrahydrofuran **3.06** (0.17 g, 10%) and title compound **3.08** (0.75 g, 64%).

R_f 0.50 (3:1 hexanes-EtOAc).

¹H NMR (400 MHz, CDCl₃) δ -0.04 (s, 3 H, P₂ -SiCH₃), 0.03 (s, 3 H, P₂ -SiCH₃), 0.10 (s, 3 H, P₁ -SiCH₃), 0.19 (s, 3 H, P₁ -SiCH₃), 0.81 (s, 9 H, P₂ -SiC(CH₃)₃), 0.94 (s, 9 H, P₁ -SiC(CH₃)₃), 2.42 (s, 6 H, H14, H19), 3.43 (dd, 1 H, *J* = 11.1, 8.1 Hz, H1'), 3.57 (dd, 1 H, *J* = 11.1, 2.8 Hz, H1'), 3.87-3.94 (m, 1 H, H3'), 4.04 (dd, 1 H, *J* = 5.8, 3.6 Hz, H4'), 4.19 (dd, 1 H, *J* = 10.3, 5.8 Hz, H5'), 4.41 (dd, 1 H, *J* = 10.3, 3.6 Hz, H5'), 5.27 (dt, 1 H, *J* = 8.1, 2.8 Hz, H2'), 7.16-7.44 (m, 19 H, H3-H5, H7-H9, H12, H17), 7.90 (d, 4 H, *J* = 8.3 Hz, H11, H16).

¹³C NMR (400 MHz, CDCl₃) δ -5.2 (P₂ -SiCH₃), -4.9 (P₂ -SiCH₃), -4.7 (P₁ -SiCH₃), -4.6 (P₁ -SiCH₃), 18.1 (P₂ -SiC(CH₃)₃), 18.4 (P₁ -SiC(CH₃)₃), 25.6 (P₂ -SiC(CH₃)₃), 25.7 (P₁ -SiC(CH₃)₃), 22.0 (C14, C19), 62.8 (C1'), 70.2 (C3'), 72.3 (C4'), 75.4 (C5'), 82.9 (C2'), 87.4 (C1), 124.2 (C5), 124.8 (C9), 126.8 (C3), 127.4 (C7), 127.9 (C4), 128.3 (C11), 128.4 (C16), 128.5 (C8), 130.3 (C12), 130.5 (C17), 133.5 (C10), 133.6 (C15), 142.2 (C2), 142.9 (C6), 143.2 (C13), 143.3 (C18).

Procedure for preparation of (2*S*,3*R*,4*S*)-*N*-5-anthranilate methyl ester-3,4-bis-*O*-*tert*-butyldimethylsilyl-1-*O*-triphenylmethyl **3.10.**



The procedure was based on work done by Taylor *et al.*¹⁴² The bis-tosyl **3.08** (136 mg, 0.17 mmol) was dissolved in a solution of anthranilate methyl ester **3.09** (26 mg, 0.19 mmol) in DMF (2.5 mL) and heated at 50 °C for 72 hours. The solution was allowed to cool to room temperature before diluting in EtOAc (10 mL) and washing with water (100 mL/mmol). The organic layer was dried and concentrated *in vacuo*. Purification of the

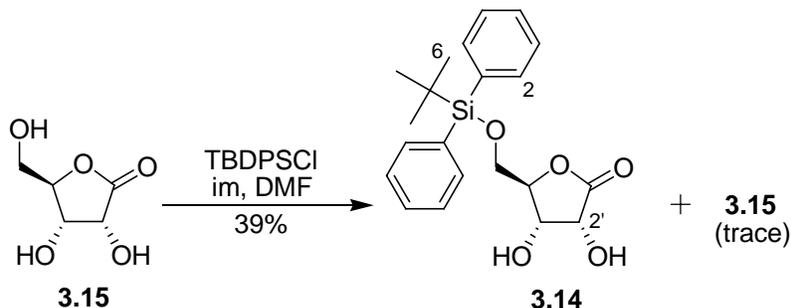
crude material by flash chromatography on silica, eluting with 10:1 hexanes-EtOAc, afforded the title compound **3.10** (15 mg, 36%).

R_f 0.28 (5:1 hexanes-EtOAc).

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ -0.05 (s, 3 H, P_2 - SiCH_3), 0.01 (s, 3 H, P_2 - SiCH_3), 0.10 (s, 3 H, P_1 - SiCH_3), 0.17 (s, 3 H, P_1 - SiCH_3), 0.79 (s, 9 H, P_2 - $\text{SiC}(\text{CH}_3)_3$), 0.91 (s, 9 H, P_1 - $\text{SiC}(\text{CH}_3)_3$), 2.74 (dd, 1 H, $J = 10.1, 5.3$ Hz, $\text{H1}'$), 3.01 (dd, 1 H, $J = 10.1, 3.4$ Hz, $\text{H1}'$), 3.33-3.50 (m, 2 H, $\text{H4}'$, $\text{H5}'$), 3.61-3.72 (m, 1 H, $\text{H5}'$), 3.86 (s, 3 H, H17), 4.01 (m, 1 H, $\text{H3}'$), 4.40 (d, 1 H, $J = 5.3$ Hz, $\text{H2}'$), 6.61 (dd, 1 H, $J = 8.3, 8.1$ Hz, H13), 6.71 (d, 1 H, $J = 8.2$ Hz, H11), 7.20-7.42 (m, 16 H, H3-H5 , H7-H9 , H12), 7.95 (d, 1 H, $J = 8.3$ Hz, H14).

$^{13}\text{C NMR}$ (400 MHz, CDCl_3) δ -4.7 (P_2 - SiCH_3), -4.4 (P_2 - SiCH_3), -4.2 (P_1 - SiCH_3), -4.1 (P_1 - SiCH_3), 18.5 (P_2 - $\text{SiC}(\text{CH}_3)_3$), 18.6 (P_1 - $\text{SiC}(\text{CH}_3)_3$), 26.2 (P_2 - $\text{SiC}(\text{CH}_3)_3$), 26.4 (P_1 - $\text{SiC}(\text{CH}_3)_3$), 59.9 ($\text{C5}'$), 61.9 (C17), 61.2 ($\text{C1}'$), 66.3 ($\text{C2}'$), 73.0 ($\text{C4}'$), 74.6 ($\text{C3}'$), 87.5 (C1), 109.9 (C11), 113.7 (C15), 116.6 (C13), 124.2 (C5), 124.8 (C9), 126.8 (C3), 127.4 (C7), 127.9 (C4), 128.5 (C8), 133.2 (C14), 136.2 (C12), 142.1 (C2), 143.0 (C6), 151.8 (C10), 173.9 (C16).

Procedure for preparation of 5-*O*-*tert*-butyldiphenylsilyl-D-ribonolactone **3.14**.



A procedure similar to those of Dodd *et al.*²⁸⁵ To a solution of D-ribonolactone **3.15** (0.54 g, 3.6 mmol) in DMF (3.6 mL) in an ice-bath, imidazole (0.55 g, 8.0 mmol) and TBDPSCI (1.05 mL, 4.0 mmol) were added. After 45 min of stirring at 0 °C, the mixture was warmed to room temperature and stirred for an additional 75 min. The reaction solution was diluted with EtOAc (5 mL) and water (10 mL). The layers were separated, and the aqueous phase was extracted with ethyl acetate (10 mL \times 2). The organic extracts

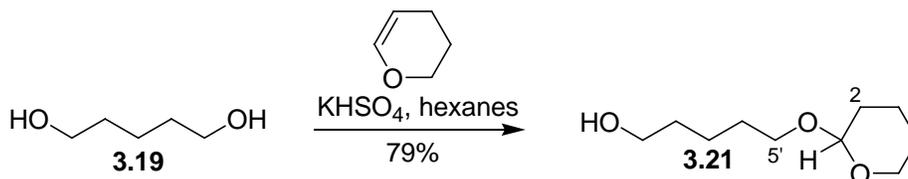
were combined, washed with water (10 mL × 2), dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 3:1 hexane-EtOAc to afford trace amounts of the starting material **3.15** and title compound **3.14** as a white solid (0.53 g, 39%).

R_f 0.19 (10:1 hexanes- EtOAc).

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 0.97 (br. s, 9H, H6-H7), 3.76 (m, 2 H, H5'), 4.20 (m, 1 H, H3'), 4.58 (m, 1 H, H4'), 4.88 (d, 1 H, $J = 5.4$ Hz, H2'), 7.20-7.64 (m, 10 H, H2-H4).

$^{13}\text{C NMR}$ (400 MHz, CDCl_3) δ 19.0 (C5), 26.3 (C7), 26.5 (C6), 63.0 (C5'), 68.3 (C3'), 75.9 (C4'), 86.6 (C2'), 128.1 (C3), 130.6 (C4), 134.9 (C1), 135.6 (C2), 177.5 (C1').

Procedure for preparation of 5-tetrahydropyranyloxy pentanol **3.21**.



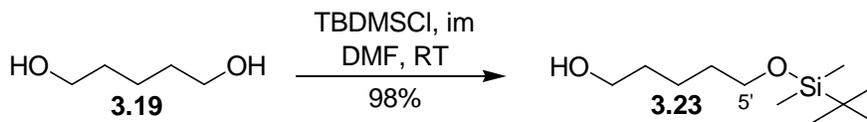
The procedure was based on work done by Saitoh *et al.*²⁸⁸ Pentan-1,5-diol **3.19** (0.42 g, 4.1 mmol), a saturated solution of KHSO₄ (0.81 ml) and 5:95 (vol/vol) 3,4-dihydro-2H-pyran -hexane (24.3 mL) were stirred at 34 °C for 3 hours, with a reflux condenser attached. The solution was diluted in CH_2Cl_2 (30 mL). K_2CO_3 (50 mg) was added to the solution, stirred, and then filtered off. The organic and aqueous layers were separated and the organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 3:1 hexanes-EtOAc afforded the title compound **3.21** as a colourless oil (0.60 g, 79%).

R_f 0.45 (1:1 hexanes- EtOAc).

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 1.41-1.85 (m, 12 H, H2'-H4', H2-H4), 3.36-3.89 (m, 6 H, H1', H5', H5), 4.57 (m, 1 H, H1).

$^{13}\text{C NMR}$ (400 MHz, CDCl_3) δ 19.9 (C3), 22.8 (C3'), 25.7 (C4), 29.7 (C4'), 31.0 (C2), 32.7 (C2'), 62.6 (C1'), 67.9 (C5'), 99.2 (C1).

Procedure for preparation of 5-*O*-*tert*-butyldimethylsilyl pentanol **3.23**.



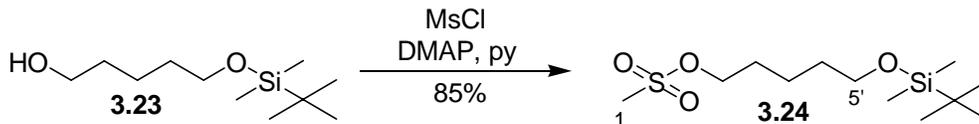
The procedure was based on work done by Kozikowski *et al.*²⁹¹ To a solution of pentan-1,5-diol **3.19** (4.11 g, 39.5 mmol), and imidazole (2.68 g, 39.5 mmol) in CH₂Cl₂ (79 mL) was added TBDMSCl (3.96 g, 26.3 mmol) slowly over a period of 5 minutes. The mixture was stirred at room temperature for 20 hours, then diluted with H₂O (150 mL), and extracted with EtOAc (100 mL × 3). The combined extracts were washed with H₂O (150 mL) and brine (150 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 5:1 hexanes-EtOAc afforded the title compound **3.23** (5.63 g, 98%).

R_f 0.71 (1:1 hexanes- EtOAc).

¹H NMR (400 MHz, CDCl₃) δ -0.01 (s, 6 H, Si(CH₃)₂), 0.86 (s, 9 H, SiC(CH₃)₃), 1.28-1.61 (m, 6 H, H₂'-H₄'), 3.53-3.69 (m, 4 H, H₁' , H₅').

¹³C NMR (400 MHz, CDCl₃) δ -4.9 (Si(CH₃)₂), 18.8 (SiC(CH₃)₃), 22.3 (C₃'), 26.3 (SiC(CH₃)₃), 32.7 (C₂'), 32.8 (C₄'), 62.9 (C₁'), 63.6 (C₅').

Procedure for preparation of 5-*O*-*tert*-butyldimethylsilyl-1-*O*-methanesulfonyl pentanol **3.24**.



The procedure was based on work done by Burke *et al.*²⁹³, Kellogg *et al.*²⁹⁴ and Tsukube *et al.*²⁹² To a mixture of monosilyl pentanol **3.23** (0.71 g, 3.26 mmol), and DMAP (0.28 g, 2.28 mmol) in pyridine (9.6 mL) was added MsCl (0.63 mL, 8.14 mmol) dropwise at 0 °C. After the mixture was stirred for 25 minutes at the same temperature, Et₂O (100 mL) was added and the solution was allowed to warm to room temperature while stirring over a period of 80 minutes. 1 M HCl (10 mL) was added slowly to the solution. After separating the layer, the organic layer was washed again with 1 M HCl (100 mL), then

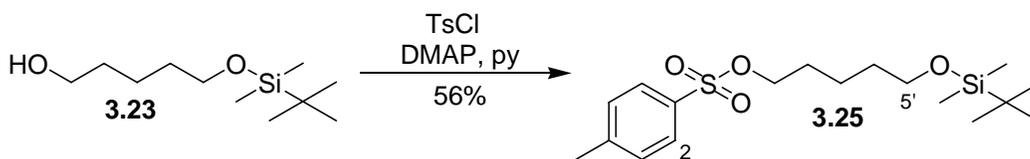
NaHCO₃ (100 mL), and brine solution (60 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 7:1 hexanes-EtOAc afforded the title compound **3.24** (247 mg, 85%).

R_f 0.45 (3:1 hexanes- EtOAc).

¹H NMR (400 MHz, CDCl₃) δ 0.03 (s, 6 H, Si(CH₃)₂), 0.88 (s, 9 H, SiC(CH₃)₃), 1.47 (m, 2 H, H3'), 1.57 (m, 2 H, H4'), 1.76 (tt, 2 H, *J* = 7.1, 6.3 Hz, H2'), 2.98 (s, 3 H, H1), 3.62 (t, 2 H, *J* = 6.0 Hz, H5'), 4.21 (t, 2 H, *J* = 6.3 Hz, H1').

¹³C NMR (400 MHz, CDCl₃) δ -4.9 (Si(CH₃)₂), 18.8 (SiC(CH₃)₃), 22.2 (C3'), 26.2 (SiC(CH₃)₃), 28.9 (C2'), 31.9 (C4'), 37.8 (C1), 62.5 (C5'), 69.9 (C1').

Procedure for preparation of 5-*O*-*tert*-butyldimethylsilyl-1-*O*-toluenesulfonyl pentanol **3.25**.



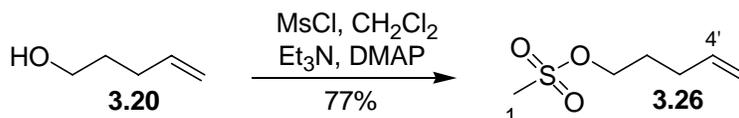
The procedure was based on work done by Mash *et al.*²⁹⁵ To a mixture of monosilyl pentanol **3.23** (95 mg, 0.43 mmol), and DMAP (5 mg, 0.04 mmol) in pyridine (0.32 mL) at 0 °C was added in one portion TsCl (128 mg, 0.67 mmol). The resulting solution was maintained at 0-4 °C for 8 hours. The mixture was then diluted Et₂O (5 mL) and 1 M HCl (1 mL) was added dropwise to the solution. After separating the layer, the organic layer was washed again with 1 M HCl (10 mL), then NaHCO₃ (10 mL), and brine solution (10 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 10:1 hexanes-EtOAc afforded the title compound **3.25** (90 mg, 56%).

R_f 0.25 (6:1 hexanes- EtOAc).

¹H NMR (400 MHz, CDCl₃) δ 0.03 (s, 6 H, Si(CH₃)₂), 0.91 (s, 9 H, SiC(CH₃)₃), 1.39 (m, 2 H, H3'), 1.50 (tt, 2 H, *J* = 7.3, 6.3 Hz, H4'), 1.66 (tt, 2 H, *J* = 7.3, 6.5 Hz, H2'), 2.43 (s, 3 H, H5), 3.58 (t, 2 H, *J* = 6.3 Hz, H5'), 4.01 (t, 2 H, *J* = 6.5 Hz, H1'), 7.34 (d, 2 H, *J* = 8.3 Hz, H3), 7.77 (d, 2 H, *J* = 8.3 Hz, H2).

^{13}C NMR (400 MHz, CDCl_3) δ -4.9 ($\text{Si}(\underline{\text{C}}\text{H}_3)_2$), 18.8 ($\text{Si}\underline{\text{C}}(\text{CH}_3)_3$), 21.8 (C5), 22.2 (C3'), 25.9 (C2'), 26.1 ($\text{Si}\underline{\text{C}}(\underline{\text{C}}\text{H}_3)_3$), 32.3 (C4'), 63.5 (C5'), 67.7 (C1'), 128.3 (C2), 130.3 (C3), 133.5 (C1), 143.2 (C4).

Procedure for preparation of 4-pentenyl methanesulfonyl 3.26.



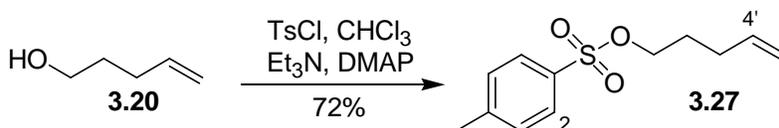
A procedure similar to that of Li and Marks.²⁹⁶ A solution of 4-penten-1-ol **3.20** (1.24 g, 14.4 mmol), NEt_3 (12 mL, 86.3 mmol), DMAP (0.18 g, 1.44 mmol) and CH_2Cl_2 (15 mL) was cooled in an ice-bath, and MsCl (1.78 mL, 23.0 mmol) was slowly added dropwise over a period of 10 minutes. The solution was stirred in ice-bath conditions for 30 minutes, then room temperature for 90 minutes, after which ice-water (3 mL) was added to quench the reaction. The organic phase was diluted with Et_2O (50 mL), separated and washed successively with 1 M HCl (55 mL), saturated Na_2CO_3 (55 mL) solution, and brine (55 mL). The organic layer was dried and concentrated *in vacuo* to yield the title compound **3.26** as an oil (1.81 g, 77%).

R_f 0.35 (3:1 hexanes- EtOAc).

^1H NMR (400 MHz, CDCl_3) δ 1.84 (m, 2 H, H2'), 2.16 (m, 2 H, H3'), 2.99 (s, 3 H, H1), 4.21 (t, 2 H, $J = 6.5$ Hz, H1'), 4.97-5.00 (m, 1 H, H5'), 5.01-5.07 (m, 1 H, H5'), 5.07 (m, 1 H, H5'), 5.76 (m, 1 H, H4').

^{13}C NMR (400 MHz, CDCl_3) δ 28.4 (C2'), 29.7 (C3'), 37.4 (C1), 70.0 (C1'), 116.2 (C5'), 137.1 (C4').

Procedure for preparation of 4-pentenyl toluenesulfonyl 3.27.



A procedure similar to that of White and Hrnčiar.²⁹⁷ A solution of 4-penten-1-ol **3.20** (0.92 g, 10.7 mmol), TsCl (2.90 g, 15.2 mmol), triethylamine (3.43 mL, 24.6 mmol),

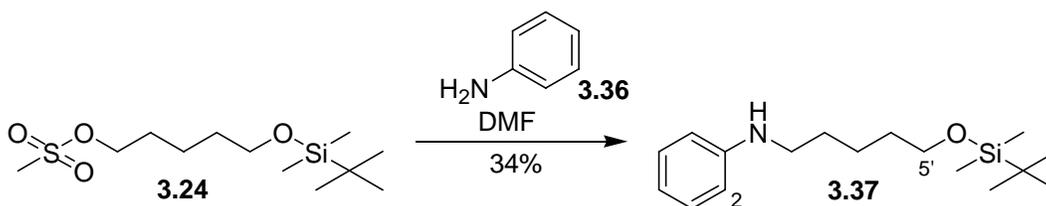
DMAP (67 mg, 0.55 mmol), and CHCl_3 (0.2 mL) was stirred for 8 hours at room temperature. A solution of 1 M HCl (1 mL) was added, and the organic phase was diluted with Et_2O (20 mL), separated, and washed with brine (5 mL). The organic layer was dried and concentrated *in vacuo* to yield title compound **3.27** as an oil (1.85 g, 72%).

R_f 0.42 (8:1 hexanes- EtOAc).

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 1.67-1.74 (m, 2 H, $\text{H}_{2'}$), 2.06 (dt, 2 H, $J = 7.6, 1.2$ Hz, $\text{H}_{3'}$), 2.42 (s, 3 H, H_5), 4.01 (t, 2 H, $J = 6.4$ Hz, $\text{H}_{1'}$), 4.89-4.91 (m, 1 H, $\text{H}_{5'}$), 4.92-4.95 (m, 1 H, $\text{H}_{5'}$), 5.66 (ddt, 1 H, $J = 16.5, 9.8, 7.6$ Hz, $\text{H}_{4'}$), 7.33 (d, 2 H, $J = 8.3$ Hz, H_3), 7.78 (d, 2 H, $J = 8.3$ Hz, H_2).

$^{13}\text{C NMR}$ (400 MHz, CDCl_3) δ 21.8 (C_5), 28.2 ($\text{C}_{2'}$), 29.5 ($\text{C}_{3'}$), 70.1 ($\text{C}_{1'}$), 116.0 ($\text{C}_{5'}$), 128.1 (C_2), 130.0 (C_3), 133.4 (C_1), 136.0 ($\text{C}_{4'}$), 144.9 (C_4).

Procedure for preparation of 5-*O*-*tert*-butyldimethylsilyl-1-phenylamino-pentane **3.37**.



The procedure was based on work done by Jung *et al.*²⁸⁰ Aniline **3.36** (0.51 mL, 5.55 mmol) was added to a solution of mesyl silyl pentanol **3.24** (0.33 g, 1.11 mmol) in DMF (5.5 mL). The solution was stirred for 48 hours at room temperature before being diluted in Et_2O (30 mL) and washed with H_2O (25 mL). The organic layer was separated and washed again with brine solution (30 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 20:1 hexanes-EtOAc afforded the title compound **3.37** (0.11 g, 34%).

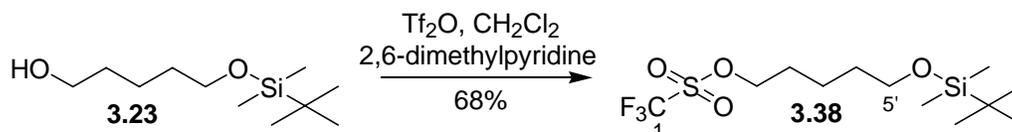
R_f 0.18 (20:1 hexanes- EtOAc).

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 0.04 (s, 6 H, $\text{Si}(\text{CH}_3)_2$), 0.89 (s, 9 H, $\text{Si}(\text{CH}_3)_3$), 1.44 (m, 2 H, $\text{H}_{3'}$), 1.53-1.65 (m, 4 H, $\text{H}_{2'}$, $\text{H}_{4'}$), 3.11 (t, 2 H, $J = 7.1$ Hz, $\text{H}_{1'}$), 3.62 (t, 2 H, $J = 6.2$ Hz, $\text{H}_{5'}$), 6.60 (d, 2 H, $J = 7.3$ Hz, H_2), 6.67 (t, 1 H, $J = 7.3$ Hz, H_4), 7.18 (t, 2 H, $J = 7.3$ Hz, H_3).

^{13}C NMR (400 MHz, CDCl_3) δ -4.9 ($\text{Si}(\underline{\text{C}}\text{H}_3)_2$), 18.8 ($\text{Si}\underline{\text{C}}(\text{CH}_3)_3$), 23.8 ($\text{C}3'$), 26.4 ($\text{Si}\underline{\text{C}}(\underline{\text{C}}\text{H}_3)_3$), 29.7 ($\text{C}2'$), 32.9 ($\text{C}4'$), 44.3 ($\text{C}1'$), 63.2 ($\text{C}5'$), 113.1 ($\text{C}2$), 117.5 ($\text{C}4$), 129.6 ($\text{C}3$), 148.8 ($\text{C}1$).

HREIMS: calculated $\text{C}_{17}\text{H}_{31}\text{NOSi}$ 293.2175; observed 293.2173.

Procedure for preparation of 5-O-tert-butyl dimethylsilyl-1-O-trifluoromethanesulfonyl pentanol 3.38.



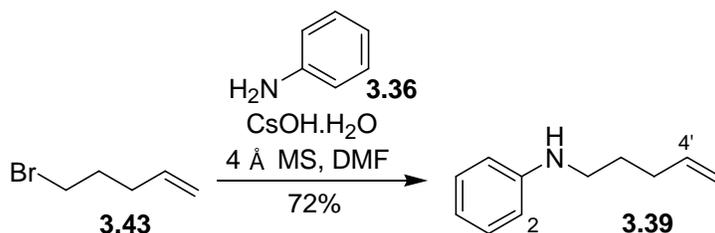
The procedure was based on work done by Paquette *et al.*³⁰³ and Grakauskas *et al.*³⁰⁴ To -50 °C cooled mixture of monosilyl pentanol **3.23** (0.20 g, 0.93 mmol), and 2,6-dimethylpyridine (0.11 g, 0.97 mmol) in CH_2Cl_2 (2.5 mL) was added dropwise a -50 °C cooled solution of trifluoromethanesulfonic anhydride (Tf_2O , 0.18 mL, 1.07 mmol) in CH_2Cl_2 (3.0 mL). After 7 minutes the Tf_2O solution was completely added and the mixture was stirred for 70 minutes at -50 °C. The mixture was then allowed to warm to room temperature over an hour before quenching the reaction with ice cold H_2O (0.5 mL) and diluting with CH_2Cl_2 (25 mL). The layers were separated and the organic layer was washed with ice cold H_2O (25 mL), and 1 M HCl (10 mL). The organic layer was dried and concentrated *in vacuo*, affording the title compound **3.38** (0.22 g, 68%).

R_f 0.18 (3:1 hexanes- EtOAc).

^1H NMR (400 MHz, CDCl_3) δ 0.04 (s, 6 H, $\text{Si}(\underline{\text{C}}\text{H}_3)_2$), 0.90 (s, 9 H, $\text{Si}\underline{\text{C}}(\text{CH}_3)_3$), 1.40 (m, 2 H, $\text{H}3'$), 1.51-1.61 (m, 4 H, $\text{H}2'$, $\text{H}4'$), 3.62 (m, 4 H, $\text{H}1'$, $\text{H}5'$).

^{13}C NMR (400 MHz, CDCl_3) δ -4.9 ($\text{Si}(\underline{\text{C}}\text{H}_3)_2$), 18.8 ($\text{Si}\underline{\text{C}}(\text{CH}_3)_3$), 22.4 ($\text{C}3'$), 24.5 ($\text{C}2'$), 26.3 ($\text{Si}\underline{\text{C}}(\underline{\text{C}}\text{H}_3)_3$), 32.9 ($\text{C}4'$), 63.3 ($\text{C}1'$), 63.5 ($\text{C}5'$), 153.5 ($\text{C}1$).

Procedure for preparation of 1-phenylamino-4-pentene 3.39.



The procedure was based on work done by Jung *et al.*²⁸⁰ Aniline **3.36** (6.8 mL, 74.6 mmol) was dissolved in DMF (4.5 mL), and activated 4 Å molecular sieves (0.75 g) and CsOH.H₂O (0.42 g, 2.50 mmol) were added to the mixture and stirred for 30 minutes at room temperature. Bromo-4-pentene **3.43** (0.34 g, 2.27 mmol) in DMF (2.5 mL) was added in one portion to the white suspension, and the reaction was gently warmed to 50 °C and stirred for 15 hours. The suspension was then filtered and rinsed with EtOAc. The filtrate was concentrated, and the residue was taken up in 1 M NaOH (15 mL) and extracted with ethyl acetate (10 mL × 5). The organic layer was washed with water (20 mL × 2) and brine (30 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 15:1 hexanes-EtOAc afforded the title compound **3.39** as clear oil (0.26 g, 72%).

R_f 0.47 (10:1 hexanes- EtOAc).

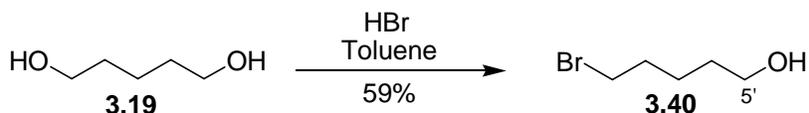
¹H NMR (400 MHz, CDCl₃) δ 1.72 (tt, 2 H, *J* = 7.7, 6.6 Hz, H2'), 2.18 (dt, 2 H, *J* = 7.7, 6.8 Hz, H3'), 3.13 (t, 2 H, *J* = 6.6 Hz, H1'), 5.00 (dd, 1 H, *J* = 10.1, 1.5 Hz, H5'), 5.05 (dd, 1 H, *J* = 16.9, 1.5 Hz, H5'), 5.85 (ddt, 1 H, *J* = 16.9, 10.1, 6.8 Hz, H4'), 6.60 (d, 2 H, *J* = 7.7 Hz, H2), 6.90 (t, 1 H, *J* = 8.3 Hz, H4), 7.18 (dd, 2 H, *J* = 8.3, 7.7 Hz, H3).

¹³C NMR (400 MHz, CDCl₃) δ 29.1 (C2'), 31.7 (C3'), 43.8 (C1'), 113.1 (C2), 115.5 (C5'), 117.6 (C4), 129.7 (C3), 138.5 (C4'), 148.8 (C1).

IR (KBr) 3411, 3052, 2930, 1601 cm⁻¹.

HRMS ESI: calculated [C₁₁H₁₅N+H] 162.1283; observed 162.1280.

Procedure for preparation of bromopentan-5-ol **3.40**.



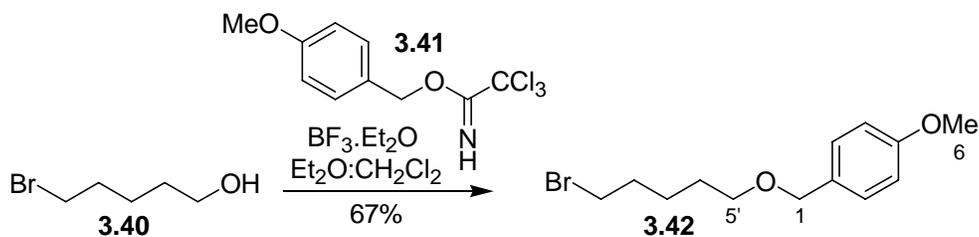
A procedure similar to those of Chong *et al.*³⁰⁶ was employed. To a solution of pentan-1,5-diol **3.19** (15.6 g, 0.15 mol) and toluene (300 mL) was added concentrated HBr (48%, 9 M aqueous solution, 19 mL, 0.24 mol). The heterogeneous mixture was heated at reflux for 21 h. The reaction mixture was allowed to cool to room temperature, and the phases were separated. The organic layer was diluted with Et₂O (300 mL) and washed with 1 M NaOH (350 mL), brine (350 mL), and phosphate buffer (3 M, pH 7, 100 mL). The organic layer was dried and concentrated *in vacuo*. This was distilled (Kugelrohr, bath temperature 110-120 °C, 0.2 mmHg), providing the title compound **3.40** (16.2 g, 59%).

R_f 0.80 (1:1 hexanes- EtOAc).

¹H NMR (400 MHz, CDCl₃) δ 1.50 (m, 2 H, H3'), 1.60 (m, 2 H, H4'), 1.88 (tt, 2 H, *J* = 7.5, 6.9 Hz, H2'), 3.43 (t, 2 H, *J* = 6.9 Hz, H1'), 3.65 (m, 2 H, H5').

¹³C NMR (400 MHz, CDCl₃) δ 24.1 (C3'), 31.6 (C4'), 32.8 (C2'), 34.3 (C1'), 62.6 (C5').

Procedure for preparation of 5-*O*-*para*-methoxybenzyl bromopentanol **3.42**.



The procedure was based on work done by Danishefsky *et al.*³⁰⁸ *para*-Methoxybenzyl trichloroacetimidate **3.41** (8.39 g, 29.7 mmol) was dissolved in Et₂O (40 mL), and a solution of bromopentan-5-ol **3.40** (3.10 g, 18.6 mmol) in CH₂Cl₂ (20 mL) was added. The resulting solution was cooled to 0 °C and treated with BF₃·Et₂O (19 μL, 0.14 mmol). The reaction mixture was warmed to room temperature after 10 minutes and stirred for 18 hours. The solution was filtered through celite, and the solids were washed with Et₂O:CH₂Cl₂ (2:1, 2 × 55 mL). The filtrate was washed with saturated aqueous NaHCO₃

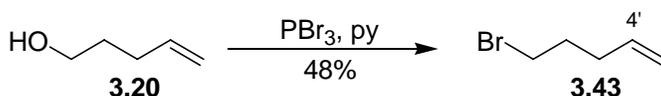
(150 mL), dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 10:1 hexanes-EtOAc, afforded the title compound **3.42** (3.57 g, 67%).

R_f 0.18 (10:1 hexanes- EtOAc).

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 1.52 (tt, 2 H, $J = 7.2, 6.9$ Hz, H3'), 1.62 (tt, 2 H, $J = 6.9, 6.7$ Hz, H4'), 1.88 (tt, 2 H, $J = 7.2, 6.8$, H2'), 3.40 (t, 2 H, $J = 6.8$ Hz, H1'), 3.45 (t, 2 H, $J = 6.7$ Hz, H5'), 3.80 (s, 3 H, H6), 4.44 (s, 2 H, H1), 6.89 (d, 2 H, $J = 8.4$ Hz, H4), 7.26 (d, 2 H, $J = 8.4$ Hz, H3).

$^{13}\text{C NMR}$ (400 MHz, CDCl_3) δ 23.0 (C3'), 28.9 (C4'), 32.6 (C2'), 33.8 (C1'), 55.3 (C6), 69.7 (C5'), 72.6 (C1), 113.8 (C4), 129.3 (C3), 130.7 (C2), 159.2 (C5).

Procedure for preparation of bromo-4-pentene **3.43**.



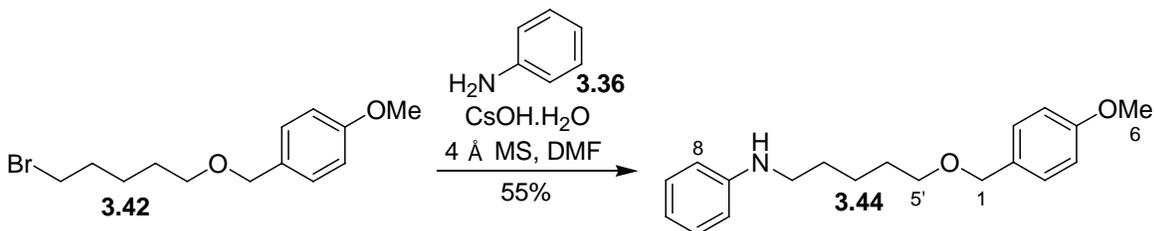
A procedure similar to that of Kitching *et al.*³¹⁴ and Johnson and Owyang.³¹³ PBr_3 (2.47 g, 9.14 mmol) was placed into a dry 25 mL two necked round bottom-flask fitted with a pressure-equalizing dropping funnel through which a gentle stream of N_2 gas was passed. The flask was cooled in an ice-salt bath (-5°C). A mixture of 4-penten-1-ol **3.20** (2.36 g, 27.4 mmol) and pyridine (0.74 mL, 9.14 mmol) was then added dropwise to the well-stirred neat PBr_3 reagent during a period of 45 minutes. After complete addition, stirring was maintained at -5°C for 15 minutes and then at room temperature for 80 minutes. The reaction mixture was then vacuum distilled, and all volatile material was collected. The distillate was taken up in Et_2O (25 mL) and washed with H_2O (25 mL). The organic layer was dried and concentrated *in vacuo*. Vacuum distillation (60°C , 75 mmHg) of the crude oil furnished the title compound **3.43** (0.65 g, 48%).

R_f 0.27 (20:1 hexanes- EtOAc).

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 1.62 (tt, 2 H, $J = 6.7, 6.5$ Hz, H2'), 2.10 (dt, 2 H, $J = 6.7, 6.5$ Hz, H3'), 3.62 (t, 2 H, $J = 6.5$ Hz, H1'), 4.93 (dd, 1 H, $J = 10.3, 1.5$ Hz, H5'), 5.01 (dd, 1 H, $J = 17.0, 1.5$ Hz, H5'), 5.80 (ddt, 1 H, $J = 17.0, 10.3, 6.7$ Hz, H4').

^{13}C NMR (400 MHz, CDCl_3) δ 29.5 (C3'), 32.9 (C2'), 33.8 (C1'), 115.7 (C5'), 136.9 (C4').

Procedure for preparation of 5-*O*-*para*-methoxybenzyl-1-phenylamino-pentane 3.44.



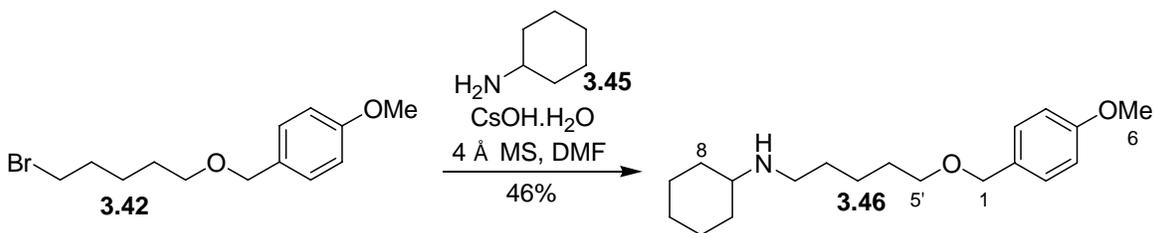
The procedure was based on work done by Jung *et al.*²⁸⁰ Aniline **3.36** (0.43 mL, 4.73 mmol) was dissolved in DMF (4 mL), and activated 4 Å molecular sieves (0.71 g) and CsOH.H₂O (0.44 g, 2.60 mmol) were added to the mixture and stirred for 30 min at room temperature. Protected bromo pentanol **3.42** (0.68 g, 2.37 mmol) in DMF (2.5 mL) was added in one portion to the white suspension, and the reaction was gently warmed to 50 °C and stirred for 19 hours. The suspension was then filtered and rinsed with EtOAc. The filtrate was concentrated, and the residue was taken up in 1 M NaOH (35 mL) and extracted with ethyl acetate (30 mL \times 3). The organic layer was washed with water (30 mL \times 2) and brine (80 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 12:1 hexanes-EtOAc afforded the title compound **3.44** as a clear oil (0.39 g, 55%).

R_f 0.32 (7:1 hexanes- EtOAc).

^1H NMR (400 MHz, CDCl_3) δ 1.52 (m, 2 H, H3'), 1.65-1.72 (m, 4 H, H2', H4'), 3.16 (t, 2 H, $J = 7.0$ Hz, H1'), 3.50 (t, 2 H, $J = 6.5$ Hz, H5'), 3.84 (s, 3 H, H6), 4.48 (s, 2 H, H1), 6.66 (d, 2 H, $J = 7.7$ Hz, H8), 6.75 (t, 2 H, $J = 7.5$ Hz, H10), 6.93 (d, 2 H, $J = 8.3$ Hz, H4), 7.21 (dd, 2 H, $J = 7.7, 7.5$ Hz, H9), 7.29 (d, 2 H, $J = 8.3$ Hz, H3).

^{13}C NMR (400 MHz, CDCl_3) δ 23.9 (C3'), 29.3 (C4'), 29.6 (C2'), 44.0 (C1'), 58.1 (C6), 69.9 (C5'), 72.6 (C1), 112.9 (C8), 113.8 (C4), 117.3 (C10), 129.3 (C3), 129.3 (C9), 130.7 (C2), 148.3 (C7), 159.2 (s, 1 C, C5).

Procedure for preparation of 5-*O*-*para*-methoxybenzyl pentylcyclohexylamine 3.46.



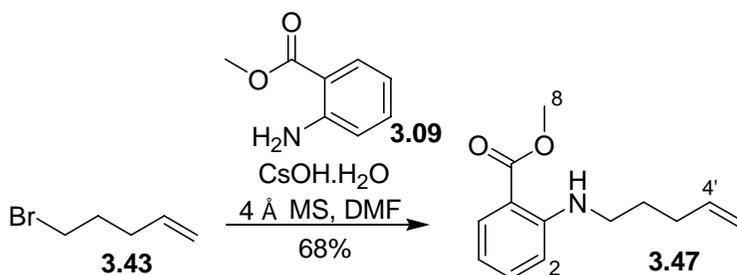
The procedure was based on work done by Jung *et al.*²⁸⁰ Cyclohexylamine **3.45** (92 μ L, 0.81 mmol) was dissolved in DMF (1.2 mL), and activated 4 Å molecular sieves (130 mg) and CsOH.H₂O (74 mg, 0.44 mmol) were added to the mixture and stirred for 30 minutes at room temperature. Protected bromo pentanol **3.42** (115 mg, 0.41 mmol) in DMF (0.5 mL) was added in one portion to the white suspension, and the reaction was allowed to proceed for 18 hours. The suspension was then filtered and rinsed with EtOAc. The filtrate was concentrated, and the residue was taken up in 1 M NaOH (15 mL) and extracted with ethyl acetate (20 mL \times 3). The organic layer was washed with water (20 mL \times 2), brine (20 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 10:1 hexanes-EtOAc afford the title compound **3.46** as a clear oil (56 mg, 46%).

R_f 0.40 (3:1 hexanes- EtOAc).

¹H NMR (400 MHz, CDCl₃) δ 1.17-1.66 (m, 14 H, H3', H4', H8-H10), 1.80 (tt, 2 H, *J* = 7.1, 6.8, H2'), 2.50 (t, 2 H, *J* = 7.1 Hz, H1'), 2.56 (m, 1 H, H7), 3.45 (t, 2 H, *J* = 6.7 Hz, H5'), 3.80 (s, 3 H, H6), 4.42 (s, 2 H, H1), 6.89 (d, 2 H, *J* = 8.3 Hz, H4), 7.26 (d, 2 H, *J* = 8.3 Hz, H3).

¹³C NMR (400 MHz, CDCl₃) δ 23.1 (C3'), 23.2 (C9), 26.4 (C10), 28.8 (C4'), 32.1 (C8), 32.3 (C2'), 46.8 (C1'), 55.7 (C7), 58.1 (C6), 70.2 (C5'), 72.5 (C1), 113.8 (C4), 129.3 (C3), 130.7 (C2), 159.2 (C5).

Procedure for preparation of anthranilate methyl ester-4-pentene 3.47.



The procedure was based on work done by Jung *et al.*²⁸⁰ Anthranilate methyl ester **3.09** (0.27 g, 1.75 mmol) was dissolved in DMF (0.35 mL), and activated 4 Å molecular sieves (60 mg) and CsOH.H₂O (33 mg, 0.19 mmol) were added to the mixture and stirred for 2 hours at room temperature. Bromo-4-pentene **3.43** (26 mg, 0.18 mmol) in DMF (0.25 mL) was added in one portion to the white suspension, and the reaction was gently warmed to 50 °C and stirred for 72 hours. The suspension was then filtered and rinsed with EtOAc. The filtrate was concentrated, and the residue was taken up in 1 M NaOH (5 mL) and extracted with ethyl acetate (7 mL × 5). The organic layer was washed with water (20 mL × 2) and brine (20 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 10:1 hexanes-EtOAc afforded the title compound **3.47** as clear oil (27 mg, 68%).

R_f 0.33 (10:1 hexanes- EtOAc).

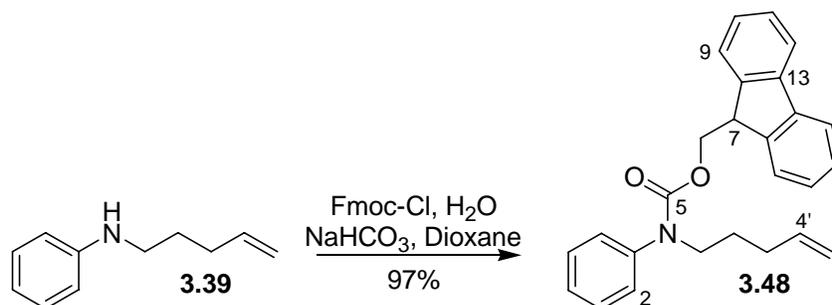
¹H NMR (400 MHz, CDCl₃) δ 1.76 (tt, 2 H, *J* = 7.4, 7.2 Hz, H2'), 2.18 (dt, 2 H, *J* = 7.4, 6.5 Hz, H3'), 3.18 (t, 2 H, *J* = 7.2 Hz, H1'), 3.82 (s, 3 H, H8), 4.97 (dd, 1 H, *J* = 10.4, 1.5 Hz, H5'), 5.04 (dd, 1 H, *J* = 17.0, 1.5 Hz, H5'), 5.82 (ddt, 1 H, *J* = 17.0, 10.4, 6.5 Hz, H4'), 6.54 (dd, 1 H, *J* = 8.3, 8.0 Hz, H4), 6.64 (d, 1 H, *J* = 8.3 Hz, H2), 7.32 (t, 1 H, *J* = 8.3 Hz, H3), 7.88 (d, 1 H, *J* = 8.0 Hz, H5).

¹³C NMR (400 MHz, CDCl₃) δ 28.3 (C2'), 31.2 (C3'), 42.5 (C1'), 63.6 (C8), 111.8 (C2), 114.2 (C6), 115.2 (C4), 115.3 (C5'), 131.6 (C5), 134.5 (C3), 137.8 (C4'), 151.3 (C1), 168.9 (C7).

IR (KBr) 3363, 2928, 2854, 1682, 1582, 1244 cm⁻¹.

HRMS ESI: calculated [C₁₃H₁₇NO₂+H] 220.1338; observed 220.1327.

Procedure for preparation of *N*-9-fluorenylmethoxycarbonyl-1-phenylamino-4-pentene 3.48.



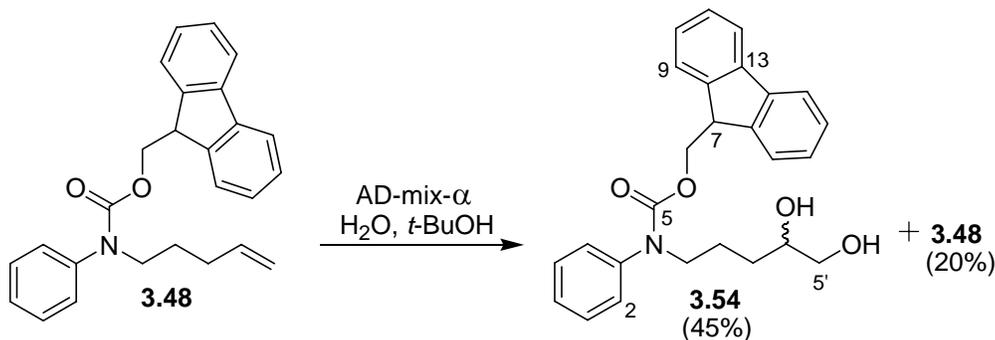
The procedure was based on work done by Carpino and Han.³¹⁵ 9-Fluorenylmethyl chloroformate (Fmoc-Cl, 60 mg, 0.23 mmol) in dioxane (0.5 mL) was added slowly to an ice-bath cooled solution of secondary amine **3.39** (15 mg, 0.09 mmol) in dioxane (1.0 mL) and 10% NaHCO₃ (1.0 mL). The mixture was stirred in the ice-bath for 30 minutes and then warmed to room temperature where it was allowed to stir for 12 hours. The heterogeneous solution was poured into H₂O (3 mL), and extracted with Et₂O (1.5 mL × 3). The organic layer was separated and washed again with H₂O (3 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 15:1 hexanes-EtOAc afforded the title compound **3.48** as clear oil (35 mg, 97%).

R_f 0.36 (10:1 hexanes- EtOAc).

¹H NMR (400 MHz, CDCl₃) δ 1.59 (m, 2 H, H2'), 1.99 (m, 2 H, H3'), 3.61 (m, 2 H, H1'), 4.06 (m, 1 H, H7), 4.36 (m, 2 H, H6), 4.90-4.99 (m, 2 H, H5'), 5.72 (m, 1 H, H4'), 7.10-7.20 (m, 5 H, H2, H4, H10), 7.26-7.40 (m, 6 H, H3, H9, H11), 7.69 (d, 2 H, *J* = 7.5 Hz, H12).

¹³C NMR (400 MHz, CDCl₃) δ 27.8 (C2'), 31.2 (C3'), 47.6 (C7), 50.4 (C1'), 67.7 (C6), 115.4 (C5'), 120.2 (C12), 125.5 (C2), 127.3 (C11), 127.9 (C9), 128.2 (C4), 129.5 (C10), 130.1 (C3), 138.1 (C13), 138.3 (C4'), 141.7 (C1), 144.3 (C8), 155.8 (C5).

Procedure for preparation of *N*-9-fluorenylmethoxycarbonyl-1-phenylamino-4,5-pentan-diol **3.54.**



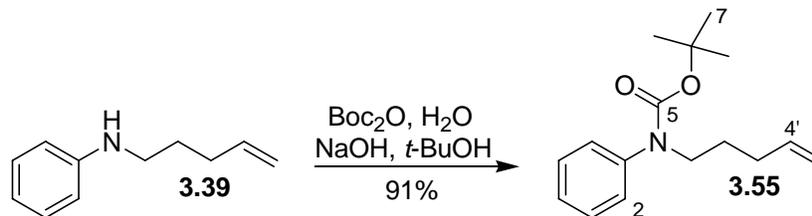
The procedure was based on work done by Sharpless *et al.*³¹⁸ *t*-BuOH (0.5 mL), H₂O (0.5 mL), and AD-mix- α (0.17 g) were stirred at room temperature producing two clear phases. The heterogeneous solution was cooled to 0 °C and Fmoc protected secondary aryl amine **3.48** (34 mg, 0.09 mmol) was added. The solution was stirred vigorously at 0 °C for 30 minutes then over an 18 hour period at 4 °C. Solid sodium sulfite (0.17 g) was added and the mixture was allowed to warm to room temperature and stirred for 30 minutes. EtOAc (2 mL) was added to the reaction mixture, and after separation of the layers, the aqueous phase was further extracted with the organic solvent (2 mL \times 3). The combined organic extracts was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 10:1 hexanes-EtOAc afforded the starting material **3.48** (6 mg, 20%) and title compound **3.54** (16 mg, 45%).

R_f 0.31 (4:1 hexanes- EtOAc).

¹H NMR (400 MHz, CDCl₃) δ 1.23 (m, 2 H, H3'), 1.58 (m, 2 H, H2'), 3.37 (dd, 1 H, *J* = 10.8, 7.5 Hz, H5'), 3.56 (dd, 1 H, *J* = 10.8, 2.7 Hz, H5'), 3.70-3.50 (m, 3 H, H1', H4'), 4.05 (m, 1 H, H7), 4.36 (m, 2 H, H6), 7.10-7.20 (m, 5 H, H2, H4, H10), 7.26-7.40 (m, 6 H, H3, H9, H11), 7.69 (d, 2 H, *J* = 7.5 Hz, H12).

¹³C NMR (400 MHz, CDCl₃) δ 24.6 (C2'), 28.0 (C3'), 47.6 (C7), 49.9 (C1'), 67.0 (C5'), 67.7 (C6), 71.9 (C4'), 120.2 (C12), 125.4 (C2), 127.3 (C11), 127.8 (C9), 128.2 (C4), 129.5 (C10), 130.0 (C3), 138.1 (C13), 141.7 (C1), 144.3 (C8), 155.8 (C5).

Procedure for preparation of *N*-tert-butyloxycarbonyl-1-phenylamino-4-pentene 3.55.



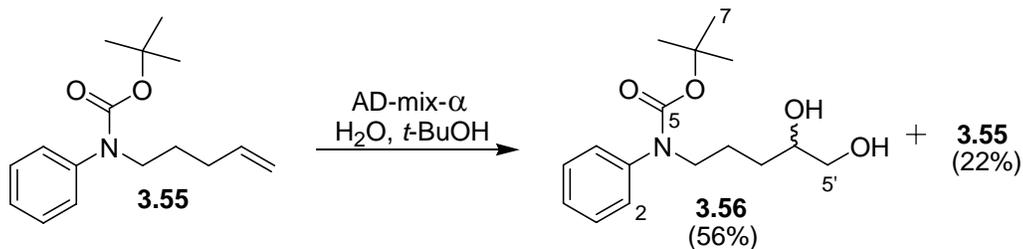
The procedure was based on work done by Carpino and Han.³¹⁵ and described by Kocienski¹²⁴ and Greene and Wutz.¹²⁵ Di-*tert*-butyl dicarbonate anhydride (Boc_2O , 0.14 g, 0.63 mmol) in *t*-BuOH (0.5 mL) was added slowly to an ice-bath cooled solution of secondary amine **3.39** (85 mg, 0.52 mmol) in *t*-BuOH (0.75 ml) and 2.5% NaOH (1.0 ml). The mixture was stirred in the ice-bath for 30 minutes and then warmed to room temperature where it was allowed to stir for 96 hours. The heterogeneous solution was poured into H_2O (10 mL), and extracted with Et_2O (5 mL \times 5). The organic layer was separated and washed again with H_2O (10 ml \times 2). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 8:1 hexanes-EtOAc afforded the title compound **3.55** as clear oil (0.125 g, 91%).

R_f 0.17 (6:1 hexanes- EtOAc).

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 1.40 (s, 9 H, H7), 1.61 (tt, 2 H, $J = 7.6, 7.3$ Hz, H2'), 2.02 (dt, 2 H, $J = 7.3, 7.1$ Hz, H3'), 3.60 (t, 2 H, $J = 7.6$ Hz, H1'), 4.89-4.98 (m, 2 H, H5'), 5.75 (ddt, 1 H, $J = 16.9, 10.3, 7.1$ Hz, H4'), 7.12-7.19 (m, 3 H, H2, H4), 7.30 (dd, 2 H, $J = 7.9, 7.6$ Hz, H3).

$^{13}\text{C NMR}$ (400 MHz, CDCl_3) δ 28.1 (C2'), 28.7 (C7), 31.3 (C3'), 50.0 (C1'), 80.4 (C6), 115.5 (C5'), 126.4 (C2), 127.5 (C4), 129.1 (C3), 138.3 (C4'), 143.0 (C1), 155.1 (C5).

Procedure for preparation of *N*-*tert*-butyloxycarbonyl-1-phenylamino-4,5-pentandiol **3.56.**



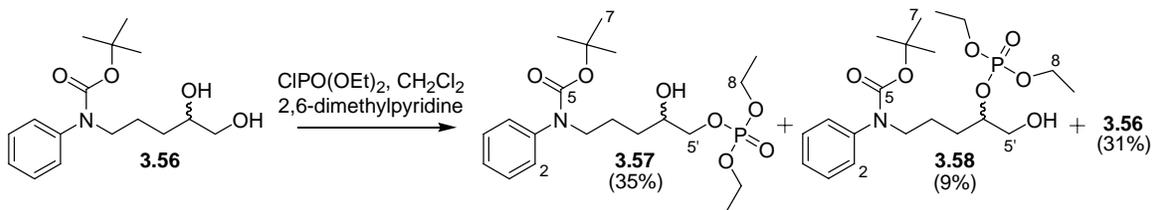
The procedure was based on work done by Sharpless *et al.*³¹⁸ *t*-BuOH (1.6 mL), H₂O (1.6 mL), and AD-mix- α (0.63 g) were stirred at room temperature producing two clear phases. The heterogeneous solution was cooled to 0 °C and Boc protected secondary aryl amine **3.55** (82 mg, 0.31 mmol) was added. The solution was stirred vigorously at 0 °C for 30 minutes then over an 25 hour period at 4 °C. Solid sodium sulfite (0.65 g) was added and the mixture was allowed to warm to room temperature and stirred for 30 minutes. EtOAc (5 mL) was added to the reaction mixture, and after separation of the layers, the aqueous phase was further extracted with the organic solvent (3 mL \times 3). The combined organic extracts was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 3:1 hexanes-EtOAc to afford the starting material **3.55** (17 mg, 22%) and title compound **3.56** (52 mg, 56%).

R_f 0.12 (2:1 hexanes- EtOAc).

¹H NMR (400 MHz, CDCl₃) δ 1.20 (app. t, 2 H, *J* = 7.1 Hz, H3'), 1.35 (s, 9 H, H7), 1.59 (tt, 2 H, *J* = 7.5, 7.1 Hz, H2'), 3.30 (dd, 1 H, *J* = 11.1, 7.4 Hz, H5'), 3.48 (dd, 1 H, *J* = 11.1, 2.9 Hz, H5'), 3.49-3.64 (m, 3 H, H1', H4'), 7.08-7.15 (m, 3 H, H2, H4), 7.26 (dd, 2 H, *J* = 7.8, 7.7 Hz, H3).

¹³C NMR (400 MHz, CDCl₃) δ 24.9 (C2'), 28.7 (C7), 30.2 (C3'), 50.2 (C1'), 67.1 (C5'), 72.2 (C4'), 80.7 (C6), 126.6 (C2), 127.8 (C4), 129.2 (C3), 142.7 (C1), 155.5 (C5).

Procedure for preparation of *N*-*tert*-butyloxycarbonyl-1-phenylamino-5-diethyl phosphate-4-pentanol **3.57.**



The procedure was based on work done by Milewski *et al.*³²¹ Diethyl chlorophosphate (57 mL, 0.39 mmol) was added to a 0 °C solution of 4,5-diol **3.56** (105 mg, 0.36 mmol) in 2,6-dimethylpyridine (41 μL , 0.36 mmol) and CH_2Cl_2 (1.5 mL). The solution was warmed to 4 °C and stirred for 8 hours before H_2O (5 mL) was added dropwise. EtOAc (3 mL \times 4) was used to wash the solution. The combined organic layers was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 3:1 hexanes-EtOAc afforded starting material **3.56** (48 mg, 31%), *N*-*tert*-butyloxycarbonyl-1-phenylamino-4-diethyl phosphate-5-pentanol **3.58** (14 mg, 9%) and title compound **3.57** (54 mg, 35%).

R_f 0.21 (1:1 hexanes- EtOAc).

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 1.20 (m, 2 H, H3'), 1.23-1.31 (m, 15 H, H7, H9), 1.56 (m, 2 H, H2'), 3.45-3.59 (m, 3 H, H1', H4'), 3.98-4.04 (m, 5 H, H5', H8), 4.13 (m, 1 H, H5'), 7.06-7.13 (m, 3 H, H2, H4), 7.24 (dd, 2 H, $J = 7.8, 7.7$ Hz, H3).

$^{13}\text{C NMR}$ (400 MHz, CDCl_3) δ 16.3 (C9), 16.5 (C9), 24.0 (C2'), 28.6 (C7), 30.4 (C3'), 50.1 (C1'), 53.8 (d, 1 C, $J = 14.3$ Hz, C8), 54.1 (d, 1 C, $J = 14.3$ Hz, C8), 70.2 (C4'), 72.1 (d, 1 C, $J = 8.5$ Hz, C5'), 80.4 (C6), 126.4 (C2), 127.5 (C4), 129.1 (C3), 142.7 (C1), 155.2 (C5).

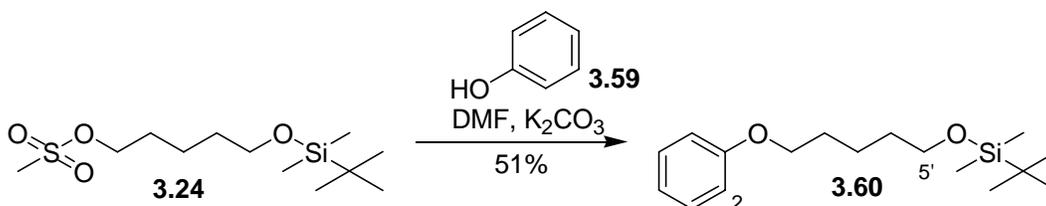
N-*tert*-butyloxycarbonyl-1-phenylamino-4-diethyl phosphate-5-pentanol **3.58**.

R_f 0.17 (1:1 hexanes- EtOAc).

$^1\text{H NMR}$ (400 MHz, CDCl_3) 1.23-1.41 (m, 17 H, H7, H9, H3'), 1.58 (m, 2 H, H2'), 3.53-3.70 (m, 3 H, H1', H4'), 3.77 (m, 1 H, H5'), 3.88 (m, 1 H, H5'), 4.00 (m, 4 H, H8), 7.06-7.13 (m, 3 H, H2, H4), 7.24 (dd, 2 H, $J = 7.8, 7.7$ Hz, H3).

^{13}C NMR (400 MHz, CDCl_3) δ 16.6 (C9) 16.7 (C9) 24.1 (C2'), 28.6 (C7), 29.9 (C3'), 50.1 (C1'), 53.9 (d, 1 C, $J = 14.1$ Hz, C8), 54.2 (d, 1 C, $J = 14.1$ Hz, C8), 66.9 (C5'), 72.6 (d, 1 C, $J = 6.9$ Hz, C4'), 80.4 (C6), 126.4 (C2), 127.5 (C4), 129.1 (C3), 142.7 (C1), 155.2 (C5).

Procedure for preparation of 5-*O*-*tert*-butyldimethylsilyl-1-phenoxy-pentane 3.60.



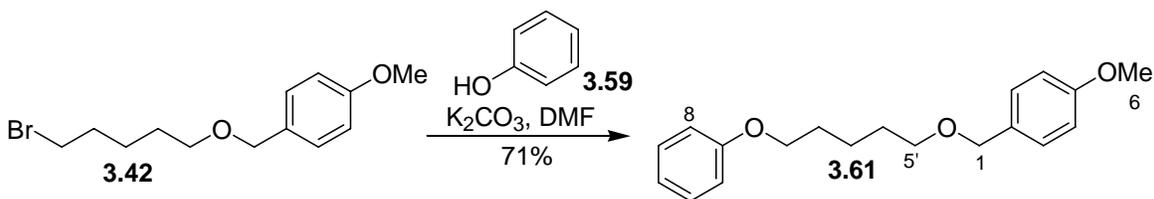
Phenol **3.59** (0.12 g, 1.27 mmol) was added to a solution of mesyl silyl pentanol **3.24** (0.19 g, 0.64 mmol) and K₂CO₃ (0.18 g, 1.27 mmol) in DMF (1.3 mL). The solution was stirred at room temperature for 44 hours before 1 M NaOH (5 mL) was added. The solution stirred for a further 3 hours before being diluted with Et₂O (30 mL), the layers were then separated, with the organic layer being washed with 1 M HCl (25 mL), NaHCO₃ (20 mL), and brine solution (20 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 10:1 hexanes-EtOAc afforded the title compound **3.60** (95 mg, 51%).

R_f 0.35 (6:1 hexanes- EtOAc).

^1H NMR (400 MHz, CDCl_3) δ 0.03 (s, 6 H, Si(CH₃)₂), 0.88 (s, 9 H, Si(CH₃)₃), 1.48 (m, 2 H, H3'), 1.64 (m, 2 H, H4'), 1.84 (m, 2 H, H2'), 3.57 (t, 2 H, $J = 6.4$ Hz, H5'), 3.70 (t, 2 H, $J = 6.1$ Hz, H1'), 6.86 (d, 2 H, $J = 7.1$ Hz, H2), 6.96 (t, 1 H, $J = 6.8$ Hz, H4), 7.27 (dd, 2 H, $J = 7.1, 6.8$ Hz, H3).

^{13}C NMR (400 MHz, CDCl_3) δ -4.9 (Si(CH₃)₂), 18.7 (Si(CH₃)₃), 22.4 (C3'), 26.4 (Si(CH₃)₃), 28.0 (C2'), 32.3 (C4'), 63.1 (C5'), 67.6 (C1'), 115.5 (C2), 120.8 (C4), 129.8 (C3), 146.9 (C1).

Procedure for preparation of 5-*O*-*para*-methoxybenzyl-1-phenoxy-pentane 3.61.



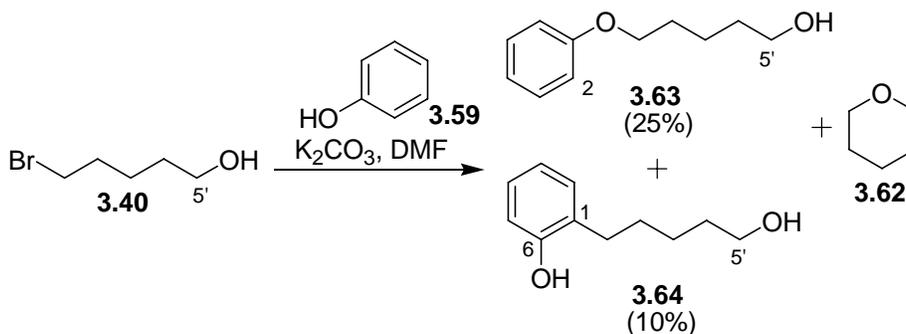
The procedure was based on work done by Miyata *et al.*³²² K₂CO₃ (0.28 g, 2.0 mmol) was added to a solution of phenol **3.59** (0.20 g, 2.1 mmol) and protected bromo pentanol **3.42** (0.29 g, 1.0 mmol) in DMF (4.2 mL) and the mixture was stirred at room temperature for 48 hours. The reaction mixture was then diluted with Et₂O (30 mL). The solution was then washed with 1 M HCl (25 mL), the organic layer was separated, and washed with NaHCO₃ (20 mL), and brine solution (20 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 8:1 hexanes-EtOAc afforded the title compound **3.61** (0.21 g, 71%).

R_f 0.35 (5:1 hexanes- EtOAc).

¹H NMR (400 MHz, CDCl₃) δ 1.44 (tt, 2 H, *J* = 7.0, 6.8 Hz, H3'), 1.54-1.70 (m, 4 H, H2', H4'), 3.43 (t, 2 H, *J* = 6.6 Hz, H5'), 3.55 (t, 2 H, *J* = 6.5 Hz, H1'), 3.81 (s, 3 H, H6), 4.45 (s, 2 H, H1), 6.82-6.90 (m, 4 H, H4, H8), 6.96 (t, 1 H, *J* = 6.8 Hz, H10), 7.22-7.28 (m, 4 H, H3, H9).

¹³C NMR (400 MHz, CDCl₃) δ 23.1 (C3'), 29.1 (C4'), 32.0 (C2'), 55.3 (C6), 69.7 (C5'), 71.0 (C1'), 73.1 (C1), 113.8 (C4), 115.5 (C8), 120.8 (C10), 129.3 (C3), 129.8 (C3), 130.8 (C2), 154.9 (C7), 159.2 (C5).

Procedure for preparation of 1-phenoxy-pentanol **3.63**.



The procedure was based on work done by Miyata *et al.*³²² To a solution of phenol **3.33** (2.12 g, 22.6 mmol) and bromopentanol-5-ol **3.40** (1.96 g, 11.7 mmol) in DMF (30 mL) was added K_2CO_3 (3.15 g, 22.8 mmol) and the mixture was stirred at 70 °C for 90 minutes. The temperature was increased to 85 °C for a further 15 minutes. The reaction mixture was allowed to cool and then diluted with Et_2O (150 mL). The solution was then washed with 1 M HCl (150 mL), the organic layer was separated, and washed with $NaHCO_3$ (120 mL), and brine solution (120 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 5:1 hexanes-EtOAc afforded 2-(5-Hydroxy-pentyl)-phenol **3.64** (0.21 g, 10%) and the title compound **3.63** (0.54 g, 25%).

R_f 0.30 (4:1 hexanes- EtOAc).

1H NMR (400 MHz, $CDCl_3$) δ 1.53 (m, 2 H, H3'), 1.63 (tt, 2 H, $J = 7.3, 6.3$ Hz, H4'), 1.81 (tt, 2H, $J = 7.2, 6.5$ Hz, H2'), 3.66 (t, 2 H, $J = 6.3$ Hz, H5'), 3.96 (t, 2 H, $J = 6.5$ Hz, H1'), 6.85-6.94 (m, 3 H, H2, H4), 7.26 (t, 2 H, $J = 7.4$ Hz, H3).

^{13}C NMR (400 MHz, $CDCl_3$) δ 22.8 (C3'), 29.5 (C4'), 32.8 (C2'), 63.2 (C5'), 68.1 (C1'), 114.9 (C2), 120.9 (C4), 129.8 (C3), 159.4 (C1).

IR (KBr) 3355, 2938, 2867, 1600, 1242, 1172 cm^{-1} .

HRMS ESI: calculated $[C_{11}H_{16}O_2+H]$ 181.1229; observed 181.1237.

2-(5-Hydroxy-pentyl)-phenol **3.64**.

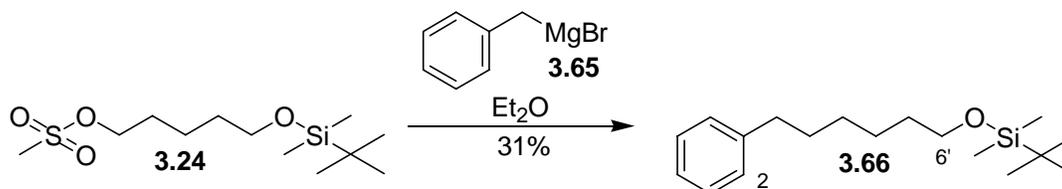
R_f 0.27 (4:1 hexanes- EtOAc).

1H NMR (400 MHz, $CDCl_3$) δ 1.00 (m, 2 H, H3'), 1.39 (m, 2 H, H4'), 1.73 (m, 2 H, H2'), 2.65 (t, 2 H, $J = 6.5$ Hz, H1'), 3.69 (t, 2 H, $J = 6.3$ Hz, H5'), 6.99 (d, 1 H, $J = 7.4$

Hz, H2), 7.11 (t, 1 H, $J = 7.4$ Hz, H3), 7.20 (d, 1 H, $J = 7.7$ Hz, H5), 7.31 (dd, 1 H, $J = 7.7, 7.4$ Hz, H4).

^{13}C NMR (400 MHz, CDCl_3) δ 27.7 (C3'), 29.5 (C4'), 32.8 (C2'), 34.0 (C1'), 63.5 (C5'), 115.8 (C5), 121.9 (C3), 123.9 (C1), 129.3 (C4), 130.7 (C2), 159.5 (C6).

Procedure for preparation of 5-*O*-*tert*-butyldimethylsilyl-1-phenyl-hexane 3.66.



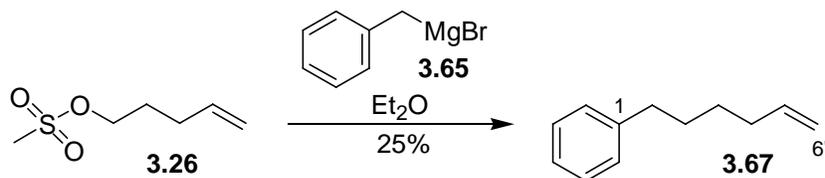
Mesityl silyl pentanol **3.24** (0.14 g, 0.49 mmol) in Et_2O (1.5 mL) was added dropwise to an $0\text{ }^\circ\text{C}$ solution of benzyl magnesium bromide³²³ **3.65** (0.16 g, 0.81 mmol) in Et_2O (2 mL). The solution was kept at $0\text{ }^\circ\text{C}$ for 3 hours before allowing the reaction to warm to room temperature and stir overnight for a total of 17 hours. The reaction was quenched slowly with ice cold H_2O (1 mL), before being diluted with Et_2O (20 mL). The solution was washed with 1 M HCl (20 mL), NaHCO_3 (20 mL), H_2O (15 mL), and brine solution (25 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 20:1 hexane-EtOAc afforded the title compound **3.66** (44 mg, 31%).

R_f 0.48 (20:1 hexanes- EtOAc).

^1H NMR (400 MHz, CDCl_3) δ 0.04 (s, 6 H, $\text{Si}(\underline{\text{C}}\text{H}_3)_2$), 0.88 (s, 9 H, $\text{SiC}(\underline{\text{C}}\text{H}_3)_3$), 1.29-1.40 (m, 4 H, H3', H4'), 1.50-1.65 (m, 4 H, H2', H5'), 2.65 (t, 2 H, $J = 6.5$ Hz, H1'), 3.62 (t, 2 H, $J = 6.2$ Hz, H6'), 7.15-7.28 (m, 5 H, H2-H4).

^{13}C NMR (400 MHz, CDCl_3) δ -4.9 ($\text{Si}(\underline{\text{C}}\text{H}_3)_2$), 18.8 ($\text{SiC}(\underline{\text{C}}\text{H}_3)_3$), 26.4 ($\text{SiC}(\underline{\text{C}}\text{H}_3)_3$), 27.8 (C3'), 29.1 (C4'), 31.5 (C2'), 33.0 (C4'), 35.9 (C1'), 63.3 (C6'), 125.5 (C4), 128.1 (C2), 128.6 (C3), 142.8 (C1).

Procedure for preparation of hex-5-enylbenzene **3.67**.



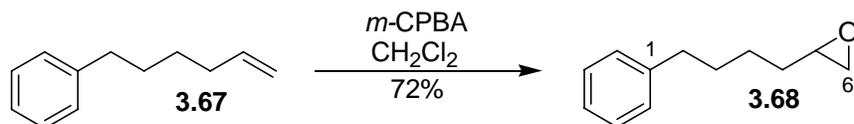
To an 0 °C solution of benzyl magnesium bromide³²³ **3.65** (3.17 g, 16.2 mmol) in Et₂O (8 mL), 4-pentenyl methanesulfonyl **3.26** (0.76 g, 4.6 mmol) in Et₂O (3 mL) was added dropwise. The solution was kept at 0 °C for 8 hours before allowing the reaction to warm to room temperature and stir for a total of 40 hours. The reaction was quenched slowly with ice cold H₂O (2 mL), before being diluted with Et₂O (75 mL). The solution was washed with 1 M HCl (60 mL), NaHCO₃ (60 mL), H₂O (65 mL), and brine solution (60 mL). The organic layer was dried and the solvent was removed gently by evaporation at atmospheric pressure. The resulting syrup residue was purified by flash chromatography on silica, eluting with 15:1 hexane-Et₂O to afford the title compound **3.67** (0.19 g, 25%).

R_f 0.46 (100% hexanes).

¹H NMR (400 MHz, CDCl₃) δ 1.45 (tt, 2 H, *J* = 6.8, 6.5 Hz, H3'), 1.65 (tt, 2 H, *J* = 6.8, 6.6 Hz, H2'), 2.11 (dt, 2 H, *J* = 7.1, 6.5 Hz, H4'), 2.63 (t, 2 H, *J* = 6.6 Hz, H1'), 4.96 (dd, 1H, *J* = 9.7, 1.0 Hz, H6'), 5.04 (dd, 1H, *J* = 16.1, 1.0 Hz, H6'), 5.83 (ddt, 1H, *J* = 16.1, 9.7, 7.1 Hz, H5'), 7.34-7.16 (m, 5 H, H2-H4).

¹³C NMR (400 MHz, CDCl₃) δ 28.6 (C3'), 31.6 (C2'), 33.6 (C4'), 35.8 (C1'), 114.4 (C6'), 125.6 (C4), 128.2 (C2), 128.4 (C3), 138.8 (C5'), 142.7 (C1).

Procedure for preparation of 5,6-epoxyhexylbenzene **3.68**.



The procedure was based on work done by Gerkin and Rickborn.³²⁴ *meta*-Chloroperoxybenzoic acid (*m*-CPBA, 0.36 g, 1.1 mmol) was added to a 4 °C solution of hex-5-enylbenzene **3.67** (160 mg, 1.0 mmol) in CH₂Cl₂ (5 mL). The solution was allowed to warm to room temperature where it was stirred for 18 hours. The solution was diluted

in CH₂Cl₂ (50 mL) before the organic phase was washed successively with 1 M NaOH (40 mL × 2) and H₂O (50 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 8:1 hexanes-EtOAc afforded the title compound **3.68** as a clear colourless oil (1.26 g, 72%).

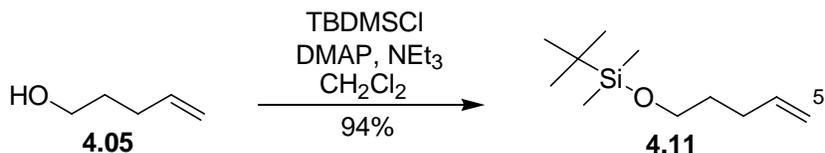
R_f 0.52 (8:1 hexanes-EtOAc).

¹H NMR (400 MHz, CDCl₃) δ 1.51-1.65 (m, 4 H, H3', H4'), 1.74 (tt, 2 H, *J* = 6.8, 6.6 Hz, H2'), 2.50 (m, 1H, H6'), 2.68 (t, 2 H, *J* = 6.6 Hz, H1'), 2.78 (overlapping dd, 1 H, H6'), 2.94 (overlapping ddt, 1 H, H5'), 7.20-7.35 (m, 5 H, H2-H4).

¹³C NMR (400 MHz, CDCl₃) δ 25.7 (C3'), 31.3 (C2'), 32.4 (C4'), 35.9 (C1'), 47.1 (C6'), 53.2 (C5'), 125.8 (C4), 128.3 (C2), 128.4 (C3), 142.4 (C1).

6.4. Experiments described in Chapter Four:

Procedure for preparation of 1-*O*-*tert*-butyldimethylsilyl-4-penten-1-ol **4.11**.



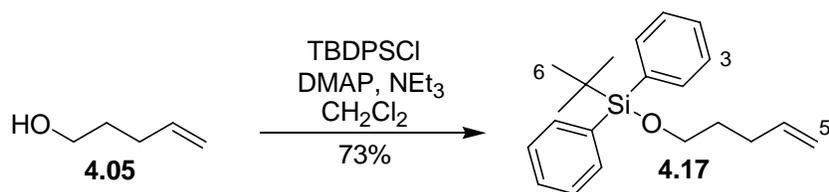
The procedure was based on work done by Coates *et al.*³⁶⁰ and Yang *et al.*³³¹ A solution of DMAP (0.53 g, 43.4 mmol) and NEt₃ (18 mL, 130.3 mmol) was added to a solution of 4-penten-1-ol **4.05** (3.74 g, 43.4 mmol) in CH₂Cl₂ (40 mL). The resulting solution was stirred in an ice-bath. TBDMSCl (10.8 g, 71.7 mmol) was added slowly over 5 minutes, after which the ice-bath was allowed to warm over a period of an hour and then removed. After 20 hours stirring at room temperature the solution was diluted in CH₂Cl₂ (100 mL). The organic phase was washed successively with 1 M HCl (120 mL × 3), NaHCO₃ (120 mL), H₂O (100 mL), and brine (125 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 10:1 hexanes-Et₂O afforded the title compound **4.11** as a clear colourless oil (8.37 g, 94%).

R_f 0.69 (10:1 hexanes-EtOAc).

¹H NMR (400 MHz, CDCl₃) δ 0.04 (s, 6 H, Si(CH₃)₂), 0.89 (s, 6 H, SiC(CH₃)₂), 0.91 (s, 3 H, SiC(CH₃)), 1.60 (tt, 2 H, $J = 7.6, 6.6$ Hz, H2'), 2.09 (dt, 2 H, $J = 7.6, 7.1$ Hz, H3'), 3.60 (t, 2 H, $J = 6.6$ Hz, H1'), 4.93 (dd, 1H, $J = 10.2, 1.4$ Hz, H5'), 4.99 (dd, 1H, $J = 15.6, 1.4$ Hz, H5'), 5.80 (ddt, 1H, $J = 15.6, 10.2, 7.1$ Hz, H4').

¹³C NMR (400 MHz, CDCl₃) δ -5.3 (Si(CH₃)₂), 18.3 (SiC(CH₃)₃), 25.6 (SiC(CH₃)), 25.9 (SiC(CH₃)₂), 30.0 (C3'), 32.0 (C2'), 62.5 (C1'), 114.5 (C5'), 138.5 (C4').

Procedure for preparation of 1-*O*-*tert*-butyldiphenylsilyl-4-penten-1-ol 4.17.



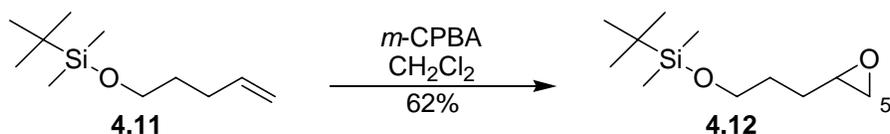
The procedure was based on work done by Coates *et al.*³⁶⁰ and Panek *et al.*³⁶¹ A solution of DMAP (47 mg, 0.38 mmol) and NEt₃ (1.2 mL, 11.4 mmol) was added to a solution of 4-penten-1-ol **4.05** (0.33 g, 3.81 mmol) in CH₂Cl₂ (5 mL). The resulting solution was stirred in an ice-bath. TBDPSCI (2.09 g, 7.6 mmol) was added slowly over 5 minutes, after which the ice-bath was allowed to warm over a period of an hour and then removed. The solution was stirred for 18 hours at room temperature before being diluted in CH₂Cl₂ (25 mL). The organic phase was washed successively with 1 M HCl (25 mL \times 3), NaHCO₃ (25 mL), H₂O (25 mL), and brine solution (25 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 12:1 hexanes-Et₂O afforded the title compound **4.17** as a clear colourless oil (0.90 g, 73%).

R_f 0.83 (22:1 hexanes-EtOAc).

¹H NMR (400 MHz, CDCl₃) δ 1.18 (s, 6 H, H7), 1.26 (s, 3 H, H6), 1.78 (tt, 2 H, $J = 7.3, 6.4$ Hz, H2'), 2.28 (dt, 2 H, $J = 7.3, 7.2$ Hz, H3'), 3.80 (t, 2 H, $J = 6.4$ Hz, H1'), 5.06 (dd, 1H, $J = 10.0, 1.2$ Hz, H5'), 5.13 (dd, 1H, $J = 15.3, 1.2$ Hz, H5'), 5.91 (ddt, 1H, $J = 15.3, 10.0, 7.2$ Hz, H4'), 7.45-7.53 (m, 6 H, H3, H4), 7.89 (d, 4 H, $J = 7.7$ Hz, H2).

¹³C NMR (400 MHz, CDCl₃) δ 18.5 (C5), 25.6 (C6), 26.0 (C7), 30.0 (C3'), 32.1 (C2'), 63.5 (C1'), 114.5 (C5'), 127.9 (C3), 129.5 (C4), 134.1 (C1), 136.1 (C2), 138.7 (C4').

Procedure for preparation of 1-*O*-*tert*-butyldimethylsilyl-4,5-epoxypentanol 4.12.



The procedure was based on work done by Yang *et al.*³³¹ and Lusinchi and Hanquet.³²⁹ *m*-CPBA (14.6 g, 42.3 mmol) was added to a 4 °C solution of 1-*O*-*tert*-butyldimethylsilyl-4-penten-1-ol **4.11** (2.01 g, 10.0 mmol) in CH₂Cl₂ (10 mL). Additional CH₂Cl₂ (8 mL) was added and the solution was stirred at 4 °C for 56 hours. The solution was diluted in CH₂Cl₂ (150 mL) before the organic phase was washed successively with 1 M NaOH (150 mL × 2) and H₂O (150 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 14:1 hexanes-EtOAc afforded the title compound **4.12** as a clear colourless oil (1.35 g, 62%).

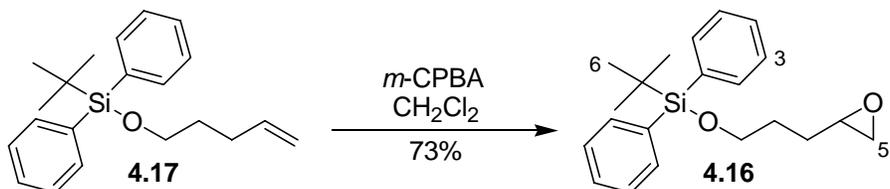
*R*_f 0.36 (14:1 hexanes-EtOAc).

¹H NMR (400 MHz, CDCl₃) δ 0.04 (s, 6 H, Si(CH₃)₂), 0.89 (s, 9 H, Si(CH₃)₃), 1.56-1.73 (m, 4 H, H2', H3'), 2.47 (m, 1H, H5'), 2.75 (overlapping dd, 1 H, H5'), 2.94 (overlapping ddt, 1 H, H4'), 3.65 (t, 2 H, *J* = 6.4 Hz, H1').

¹³C NMR (400 MHz, CDCl₃) δ -5.3 (Si(CH₃)₂), 18.3 (Si(CH₃)₃), 25.9 (Si(CH₃)), 29.0 (C2'), 29.1 (C3'), 47.2 (C5'), 52.2 (C4'), 62.6 (C1').

HREIMS: calculated C₁₁H₂₄O₂Si 216.1546; observed 216.1711.

Procedure for preparation of 1-*O*-*tert*-butyldiphenylsilyl-4,5-epoxypentanol 4.16.



The procedure was based on work done by Yang *et al.*³³¹ and Lusinchi and Hanquet.³²⁹ *m*-CPBA (7.22 g, 20.4 mmol) was added to a 4 °C solution of 1-*O*-*tert*-butyldiphenylsilyl-4-penten-1-ol **4.17** (0.66 g, 2.0 mmol) in CH₂Cl₂ (6 mL). Additional

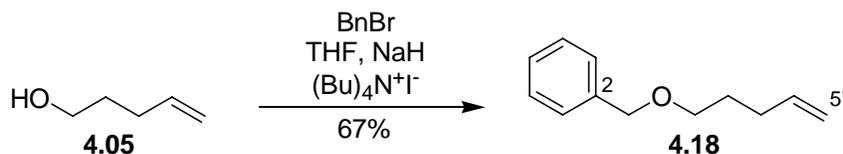
CH₂Cl₂ (3 mL) was added and the solution was stirred at 4 °C for 13 hours. The solution was diluted in CH₂Cl₂ (30 mL) before the organic phase was washed successively with 1 M NaOH (35 mL × 2), H₂O (50 mL) and brine (25 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 14:1 hexanes-EtOAc afforded the title compound **4.16** as a clear colourless oil (0.51 g, 73%).

R_f 0.69 (14:1 hexanes-EtOAc).

¹H NMR (400 MHz, CDCl₃) δ 1.06 (s, 9 H, H6, H7), 1.58-1.79 (m, 4 H, H2', H3'), 2.46 (m, 1H, H5'), 2.74 (m, 1 H, H5'), 2.91 (m, 1H, H4'), 3.71 (t, 2 H, *J* = 5.9 Hz, H1'), 7.38-7.49 (m, 6 H, H3, H4), 7.71 (d, 4 H, *J* = 7.4 Hz, H2).

¹³C NMR (400 MHz, CDCl₃) δ 19.2 (C5), 26.8 (C6, C7), 28.9 (C2'), 29.0 (C3'), 47.1 (C5'), 52.2 (C4'), 63.4 (C1'), 127.7 (C3), 129.6 (C4), 134.4 (C1), 135.6 (C2).

Procedure for preparation of 5-*O*-benzylpentene **4.18**.



The procedure was based on work done by Mootoo *et al.*¹⁹⁴ NaH (0.13 g, 5.35 mmol) was added to a solution of the 4-penten-1-ol **4.05** (0.38 g, 4.46 mmol) and tetrabutylammonium iodide ((Bu)₄N⁺I⁻, 17 mg, 0.05 mmol) in THF (4.5 mL) at 0 °C and stirred for 10 minutes. Benzyl bromide (0.61 mL, 5.13 mmol) was then added in a continuous dropwise fashion. After 30 minutes the reaction was warmed to room temperature and stirred for 3 hours. The reaction was quenched slowly with cold water (1 mL), and diluted in Et₂O (15 mL) before the organic phase was washed successively with 1 M HCl (10 mL × 2) and H₂O (15 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 8:1 hexanes-Et₂O afforded the title compound **4.18** as a clear colourless oil (0.53 g, 67%).

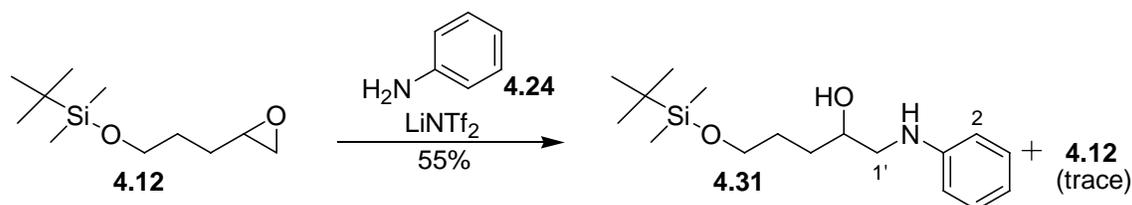
R_f 0.63 (8:1 hexanes-Et₂O).

¹H NMR (400 MHz, CDCl₃) δ 1.85 (tt, 2 H, *J* = 7.3, 6.5 Hz, H2'), 2.28 (dt, 2 H, *J* = 7.3, 7.1 Hz, H3'), 3.60 (t, 2 H, *J* = 6.5 Hz, H1'), 4.56 (s, 2 H, H1), 5.06 (dd, 1H, *J* = 9.9, 1.1

Hz, H5'), 5.14 (dd, 1H, $J = 15.2, 1.1$ Hz, H5'), 5.95 (ddt, 1H, $J = 15.2, 9.9, 7.1$ Hz, H4'), 7.36-7.50 (m, 5 H, H3-H5).

^{13}C NMR (400 MHz, CDCl_3) δ 29.1 (C2'), 30.5 (C3'), 69.8 (C1'), 73.0 (C1), 114.9 (C5'), 127.7 (C5), 127.8 (C3), 128.5 (C4), 137.9 (C2), 138.4 (s, 1 C, C4').

Procedure for preparation of 5-*O*-*tert*-butyldimethylsilyl-1-phenylamino-pentan-2-ol 4.31.



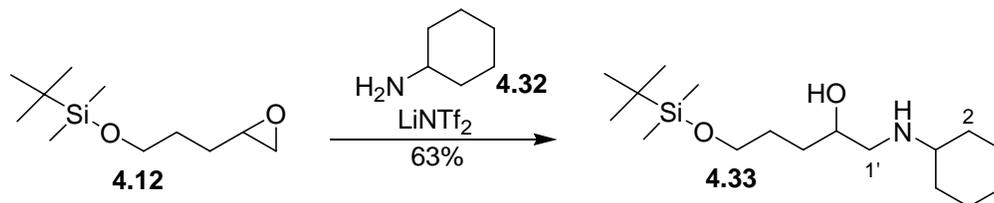
The procedure was based on work done by Hamoir *et al.*¹⁰² LiNTf_2 (86 mg, 0.30 mmol) was added to a solution of aniline **4.24** (0.82 mL, 9.04 mmol) and 1-*O*-*tert*-butyldimethylsilyl-4,5-epoxypentanol **4.12** (130 mg, 0.60 mmol). The solution was stirred at room temperature for 14.5 hours. The solution was diluted in CH_2Cl_2 (10 mL) and quenched with NaHCO_3 (2 mL) and extracted with CH_2Cl_2 (10 mL \times 3) before the organic phase was washed successively with 1 M HCl (20 mL), 1 M NaOH (20 mL \times 3), H_2O (20 mL) and brine (25 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 15:1 hexanes-EtOAc afforded starting material **4.12** (trace), and title compound **4.31** as a clear colourless oil (101 mg, 55%).

R_f 0.39 (7:1 hexanes-EtOAc).

^1H NMR (400 MHz, CDCl_3) δ 0.06 (s, 6 H, $\text{Si}(\text{CH}_3)_2$), 0.90 (s, 9 H, $\text{SiC}(\text{CH}_3)_3$), 1.54-1.77 (m, 4 H, H3', H4'), 3.03 (dd, 1 H, $J = 12.4, 8.0$ Hz, H1'), 3.23 (dd, 1 H, $J = 12.4, 3.5$ Hz, H1'), 3.69 (m, 2 H, H5'), 3.85 (m, 1 H, H2'), 6.64 (d, 2 H, $J = 7.5$ Hz, H2), 6.71 (t, 1 H, $J = 7.3$ Hz, H4), 7.17 (dd, 2 H, $J = 7.5, 7.3$ Hz, H3).

^{13}C NMR (400 MHz, CDCl_3) δ -5.4 ($\text{Si}(\text{CH}_3)_2$), 18.3 ($\text{SiC}(\text{CH}_3)_3$), 25.9 ($\text{SiC}(\text{CH}_3)$), 29.2 (C4'), 32.9 (C3'), 50.1 (C1'), 63.5 (C5'), 70.0 (C2'), 113.1 (C2), 117.6 (C4), 129.2 (C3), 145.5 (C1).

Procedure for preparation of 5-*O*-*tert*-butyldimethylsilyl-1-cyclohexylamino-pentan-2-ol **4.33.**



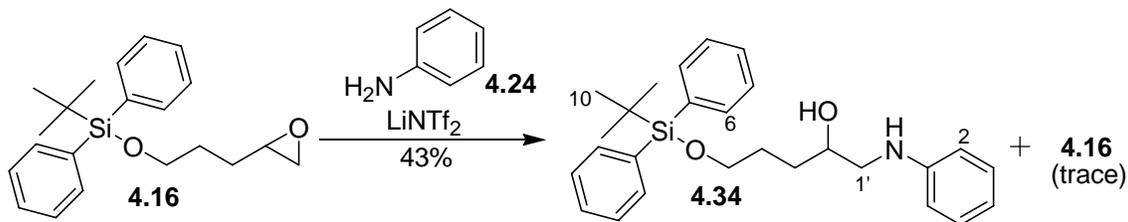
The procedure was based on work done by Hamoir *et al.*¹⁰² Lithium bistrifluoromethanesulfonimide (LiNTf₂, 88 mg, 0.31 mmol) was added to a solution of cyclohexylamine **4.32** (1.06 mL, 9.23 mmol) and 1-*O*-*tert*-butyldimethylsilyl-4,5-epoxypentanol **4.12** (133 mg, 0.62 mmol). The solution was stirred at room temperature for 14.5 hours. The solution was diluted in CH₂Cl₂ (4 mL) and quenched with NaHCO₃ (2 mL) and extracted with CH₂Cl₂ (10 mL × 3) before the organic phase was washed successively with 1 M HCl (20 mL), 1 M NaOH (20 mL × 3), H₂O (20 mL) and brine (25 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 15:1 hexanes-EtOAc afforded the title compound **4.33** as a clear colourless oil (122 mg, 63%).

R_f 0.44 (7:1 hexanes-EtOAc).

¹H NMR (400 MHz, CDCl₃) δ 0.05 (s, 6 H, Si(CH₃)₂), 0.88 (s, 9 H, SiC(CH₃)₃), 1.01-1.29 (m, 6 H, H3, H4), 1.41-1.56 (m, 4 H, H2), 1.57-1.75 (m, 4 H, H3', H4'), 2.38-2.46 (m, 2H, H1', H1), 2.77 (dd, 1H, *J* = 11.9, 3.6 Hz, H1'), 3.58 (m, 1 H, H2'), 3.63 (t, 2 H, *J* = 6.2 Hz, H5').

¹³C NMR (400 MHz, CDCl₃) δ -5.3 (Si(CH₃)₂), 18.3 (SiC(CH₃)₃), 25.1 (SiC(CH₃)), 25.9 (C3), 29.1 (C4), 32.1 (C2), 33.5 (C4'), 33.9 (C3'), 52.5 (C1), 56.7 (C1'), 63.4 (C5'), 69.7 (C2').

Procedure for preparation of 5-*O*-*tert*-butyldiphenylsilyl-1-phenylamino-pentan-2-ol 4.34.



The procedure was based on work done by Hamoir *et al.*¹⁰² LiNTf₂ (0.43 g, 1.48 mmol) was added to a solution of aniline **4.24** (4.06 mL, 44.5 mmol) and 1-*O*-*tert*-butyldiphenylsilyl-4,5-epoxypentanol **4.16** (0.51 g, 1.48 mmol). The solution was stirred at room temperature for 14.5 hours. The solution was diluted in CH₂Cl₂ (20 mL) and quenched with NaHCO₃ (5 mL) and extracted with CH₂Cl₂ (20 mL × 3) before the organic phase was washed successively with 1 M HCl (40 mL), 1 M NaOH (30 mL × 3), H₂O (30 mL) and brine (50 mL). The organic layer was dried and concentrated *in vacuo*. Purification of the crude material by flash chromatography on silica, eluting with 20:1 hexanes-EtOAc afforded starting material **4.16** (trace), and title compound **4.34** as a clear colourless oil (0.28 g, 43%).

R_f 0.42 (10:1 hexanes-EtOAc).

¹H NMR (400 MHz, CDCl₃) δ 1.05 (s, 9 H, H10, H11), 1.54-1.78 (m, 4 H, H3', H4'), 3.06 (dd, 1 H, *J* = 12.3, 8.0 Hz, H1'), 3.27 (dd, 1 H, *J* = 12.3, 3.5 Hz, H1'), 3.71 (t, 2 H, *J* = 6.3 Hz, H5'), 3.82 (m, 1 H, H2'), 6.65 (d, 2 H, *J* = 7.5 Hz, H2), 6.72 (t, 1 H, *J* = 7.3 Hz, H4), 7.19 (dd, 2 H, *J* = 7.5, 7.3 Hz, H3), 7.38-7.49 (m, 6 H, H7, H8), 7.71 (d, 4 H, *J* = 7.4 Hz, H6).

¹³C NMR (400 MHz, CDCl₃) δ 18.5 (C9), 25.6 (C10), 26.0 (C11), 29.1 (C4'), 32.1 (C3'), 50.2 (C1'), 63.9 (C5'), 70.2 (C2'), 113.1 (C2), 117.6 (C4), 127.9 (C7), 129.2 (C3), 129.5 (C8), 134.2 (C5), 136.1 (C6), 145.6 (C1).

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