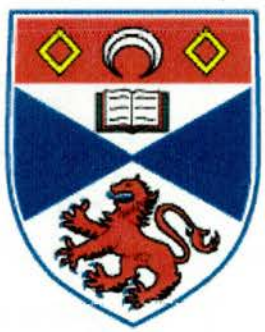
Synthesis of new substrates and inhibitors for Inositol Monophosphatase

Martin W Beaton

April 2001



University Of St. Andrews

Declaration

I, Martin Beaton, hereby certify that this thesis, which is approximately 55, 000 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

Date 11/6/2001 Signature of candidate.

I was admitted as a research student in September 1995 and as a candidate for the degree of doctor in philosophy; the higher study for which this is a record was carried out in the University of St Andrews between 1995 and 2000.

Date 11/6/2001 Signature of candidate.

I hereby certify that the candidate had fulfilled the condition of the Resolution and Regulations appropriate for the degree of Doctor in Philosophy in the University of St. Andrews and that the candidate is qualified to submit this thesis in application for that degree.

Date _____ Signature of supervisor _____

Copyright Statement

Acknowledgements

The success of this research and the completion of the manuscript could not have been possible without the assistance and support of many colleagues, friends, and family. So many people have been involved at some point over the long five years that it would be impossible to name them all however I am eternally grateful to all concerned.

I thank all technical staff in the University of St Andrews for their help. Special thanks to Melanja for helping me to figure out the temperamental NMR machines, gossip and benchball. I would also like to thank Sylvia for her invaluable support in microanalysis. For their support following my group's departure I am truly grateful to Prof. Woollins for acting as an invaluable point of contact and Dr Nigel Botting for proof reading the manuscript and pointing out all my mistakes.

Throughout my research in St Andrews I received huge support and assistance from Dr Mahmoud Ahktar - a legend in his own lifetime - for all your help I am truly grateful. I am indebted to all that I knew in Lab 434, for all the useful discussion, jokes and abuse – thank-you for breaking up the monotony. From the inositol monophosphatase team – Jurgen, Dave M and Louis, I couldn't ask for a better team to work with. Also thanks to Dave M for the first proofing of the R&D. Other friends and colleagues include Arwel, Tony, Thierry 'beep beep' Jenn, Krushida and Ian (still struggling to acknowledge the PC's supremacy over the Mac).

Extra special thanks are reserved for Nigel, Saqib and Roger. Moral support, friendship and exercise are a few of the many services provided by these exceptional individuals. I cannot thank them enough and I wish them all the best for their futures.

Without the constant nagging of my wife I would never have got this far and I thank her for believing in me. Also near the top of the nagging list star my parents – thanks for all the support. To those that helped by not mentioning the 'T' word I also thank-you.

Finally thanks go to whoever first uttered the following phrase which was perpetuated by Gillian and a constant source of energy:

"There are NO failures in this house....!"

Abstract

Inositol monophosphatase (IMPase) plays a pivotal role in the biosynthesis of the secondary messengers and is believed to be a target for lithium therapy. It is established how lithium works but details of the mechanism for the direct magnesium ion activated hydrolysis of the substrate remain unclear. It is known that substrates of IMPase require a minimal 1,2-diol phosphate structural motif which in D-*myo*-inositol 1-phosphate relates to the fragment comprising the 1-phosphate ester and 6-hydroxy groups. This research focuses on the role of the 6-hydroxy group adjacent to the phosphate ester.

It has been demonstrated that inhibitors of IMPase which are D-*myo*-inositol 1-phosphate substrate analogues possessing 6-substituents larger than the 6-hydroxy group of the substrate, for example, the O^6-methyl analogue, are able to bind to IMPase in a congruous manner to the substrate. It was demonstrated, however, that such compounds show no substrate activity whatsoever. This research shows that a 6-amino group is able to fulfil the role of the 6-hydroxy group of the substrate in conferring substrate activity and that a 6-methylamino group is similarly able to support catalysis. The results indicate that a 6-substituent capable of serving as a hydrogen bond donor is required in the catalytic mechanism for hydrolysis.

Two mechanisms for the above hydrolysis have been proposed, one in which a water molecule associated with a buried magnesium ion ($Mg^{2+}1$) attacks the phosphate ester *via* an inline displacement of the inositol moiety, and one in which adjacent displacement occurs from a water molecule associated with a second magnesium ion ($Mg^{2+}2$). The results obtained here fit very well with the latter mechanism and further indictate that the role of the 6-hydroxyl group of the substrate is to position and stabilise the formation of hydroxide ion on $Mg^{2+}2$ such that it is orientated correctly for the adjacent displacement of inositolate from the phosphorus atom.

Table of Figures

Table of Schemes

Table of Abbreviations

2' AMPs	Adenosine 2'-monophosphorothioate
2'AMP	Adenosine 2'-monophosphate
Ala	Alanine
Asp	Aspartate
BnOH	Benzyl alcohol
DABCO	1,4-diazobycyclo[2.2.2]octane
DAG	Diacylglycerol
DCC	Dicyclohexyl carboiimide
DCE	Dichloroethane
DCM	Dichloromethane
DMAP	Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethylsulphoxide
FTIR	Fourier Transform Infra-Red
GDP	Guanosine diphosphate
Glu	Glutamate
Gly	Glycine
G-Protein	GTP-binding protein
GTP	Guanosine triphosphate
H	Hours
HCl	Hydrochloric Acid
HRMS	High resolution mass spectrometry
Ile	Isoleucine
IMPase	Inositol Monophosphatase
Ins-1P	Inositol 1-Phosphate
KJ	Kilo Joule
LDA	Lithium diisopropylamide
MCPBA	Meta-chloroperoxybenzoic acid
Min	Minute
NMR	Nuclear Magnetic Resonance
PtdIns	Phosphatidylinositol
Ser	Serine
SPOS	Solid phase organic synthesis
TBDPSCl	Tertiary butyldiphenylsilyl chloride
TBPP	Tetrabenzylpyrophosphate
TEA	Triethylamine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
Thr	Threonine
TLC	Thin layer chromatography
TMEDA	N,N,N',N'-tetramethylethylenediamine
UV	Ultra violet
WHO	World Health Organisation
[dias]	Diastereoisomer
[PtdIns(4,5)P_2]	Phosphatidylinositol 4,5-bisphosphate

Introduction

1.1 Manic depression

Manic depression or bipolar disorder is a debilitating affliction that affects around 1% of the world's population according to a World Health Organisation (WHO) report.[1] The disease is characterised by extreme chronic mood swings from elation to depression.

The cycle may last many months and have a profound effect on the sufferers some of whom succumb to suicidal thoughts. The disease is thought to be the product of a brain chemical imbalance which at present is not understood. The only respite that those affected have is through the treatment of the symptoms by means of oral or intravenous drugs.

1.2 Treatment with lithium carbonate

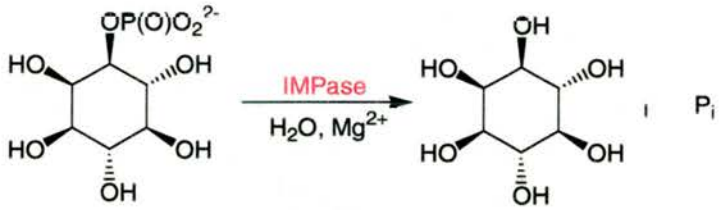
The origins of lithium treatment for mania are unclear and the dosages formulated as a result of trial and error. It is known that William Hammond was using the drug in the United States as early as 1871 to treat severe mania and depression.[2] However, lithium was 'officially' discovered in 1949 by the Australian psychiatrist John F.J. Cade as an effective treatment for bipolar disorder.[2]

This drug is still being used in almost the same form today. Some of the earliest work on the mechanism of action of lithium began in the early 1970s and found that administration of the drug caused a decrease in inositol concentrations in rat brains (Jope and Williams, 1994).[3] It has also been shown that the lithium ions inhibit a brain enzyme inositol monophosphatase (IMPase) (which is responsible for the hydrolysis of inositol monophosphates - Scheme 1.1) in an uncompetitive manner *in vitro* (Scheme 1.2, page 3).[4] Consequently, since it was known that the

other source of inositol monophosphate from the *de novo* synthesis (Section 1.13, page 13) from glucose was insufficient to compensate it was proposed that IMPase may be the target of lithium during drug therapy.[5-8]

If IMPase was indeed the target of lithium therapy it was expected that treatment would result in a large decrease in the rate of cell signalling especially in those cells that were abnormally overactive as found in patients suffering manic depression.[4]

The drug has been shown to have surprising specificity to the central nervous system. It is thought that this arises from the impermeability of the blood-brain barrier to inositol. This means that areas of the body out-with the brain can absorb dietary inositol even though the cell has been prevented from recycling existing stocks. It has also been proposed that areas of hyperactivity may have an increased permeability to lithium resulting in a concentration of lithium ions in overactive areas.[9]



Scheme 1.1– *Hydrolysis of Inositol-1-phosphate by IMPase*

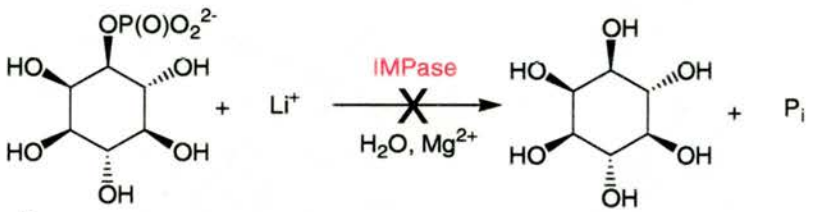
The effect of lithium carbonate on the brains of rats has been well known for a long time namely a decrease in the amount of free inositol and an increase in concentration of inositol monophosphates.[7] However it was only more recently that the same parallels could be conclusively proven with humans.[10-13]

1.3 Cause of affliction

Research has shown that manic depression appears to be associated with a pathway within the brain known as the phosphatidylinositol pathway (Scheme 1.4, page 10).[14] A key enzyme in this pathway is inositol monophosphatase (IMPase) which hydrolyses inositol monophosphate to give free inositol and inorganic phosphate. The enzyme has been shown to have an absolute requirement for two divalent metal ions and a molecule of water to function efficiently. [15,16]

1.4 Side effects

The use of lithium carbonate for therapy has serious side effects – it has been shown that lithium affects other areas of phosphatidylinositol signal transduction which means that it is not specific.[17] Lithium carbonate is known to be a toxic drug. It has many side effects and a narrow therapeutic window which means that patients must have their blood plasma concentrations of Li^+ monitored continuously.[18]



Scheme 1.2 – *Lithium ions inhibit hydrolysis*

The optimum lithium concentration level in blood serum is between 0.5 and 1.0 mmol dm^{-3} the side effects at this concentration include weight gain, increased urine production and slight hand tremors. At higher concentrations around 2 mmol dm^{-3} toxicity begins to occur resulting in nausea, fine tremors, ataxia, confusion

and slurred speech. At double this concentration around 4 mmol dm^{-3} coma and/or death is likely.[19]

1.5 New drugs

Drugs other than lithium carbonate can be used to target IMPase resulting in a similar therapeutic effect on the overactive phosphatidylinositol cell signalling pathway.[20-22] As a direct result of the drawbacks with lithium treatment new techniques are being sought.

1.6 Introduction to biological messengers

The cells in multi-cellular organisms need to communicate with the rest of the cells in the body in order to sustain life. This communication is done mainly by means of hormones and neurotransmitters. Some hormones, for instance steroids, of which testosterone is an example can pass through the lipid bi-layer of a cell membrane. However, many of the chemical messengers travelling the body *via* the vascular system are too hydrophilic to pass through membranes. The brain with it's highly specialised role is even more selective than normal cells about the messengers that can diffuse through.

To enable brain cells access to specific messengers the cell membranes contain specialised receptors that the messengers can bind to. Once bound the receptors activate trans-membrane mechanisms that transmit the signal through the membrane resulting in an intracellular response. This is known as "trans-membrane signalling" or "signal transduction". An intracellular response could be

the production of *secondary messengers* which then go on to induce a cellular response.

1.7 Signal transduction and calcium ions

The result of a stimulation of membrane receptors by agonists leads to, in many cases, calcium release within the cell. For instance protein phosphorylation which controls a great number of intracellular processes responds directly to the intracellular concentrations of calcium ions. Thus the intracellular concentration of calcium ions needs to be carefully controlled; this is regulated by agonists for instance hormones in one of two ways.

- The first is that the agonist operates on a receptor bound protein on the exterior of the cell membrane. This induces a change in the potential difference across the membrane. The result is the opening of voltage sensitive transmembrane Ca^{2+} channels. Most intracellular calcium in the non-excited cell is found either bound to proteins or the cell membrane therefore intracellular calcium ion concentrations are low. The resulting ion gradient causes the influx of calcium ions resulting in an increase in the cytosolic calcium concentration.
- The second method of controlling the concentration of intracellular calcium is by triggering the release of calcium ions from intracellular stores. This again causes the cytosolic concentration of calcium to rise resulting in intracellular responses for instance the activation of calcium dependant enzymes.

1.8 Signal transduction in brain cells

Brain cells have a far more selective membrane than ordinary cells of the body. This is because the brain plays such an important role in the body that it must be protected. Penetrating the membrane of a brain cell therefore is a very difficult task and specialised structures are present to specifically transfer information or nutrients.

Many water soluble proteins make use of a signal transduction system whereby a receptor attached to the outside of the cell membrane is coupled to the production of an internal signalling molecule *via* an intracellular effector. The exterior receptors are made to be stimulated only by a specific molecule or molecules, once this has occurred a membrane bound GTP-binding protein (G-protein) specific to the receptor is activated. It is these G-proteins that transmit the signal.

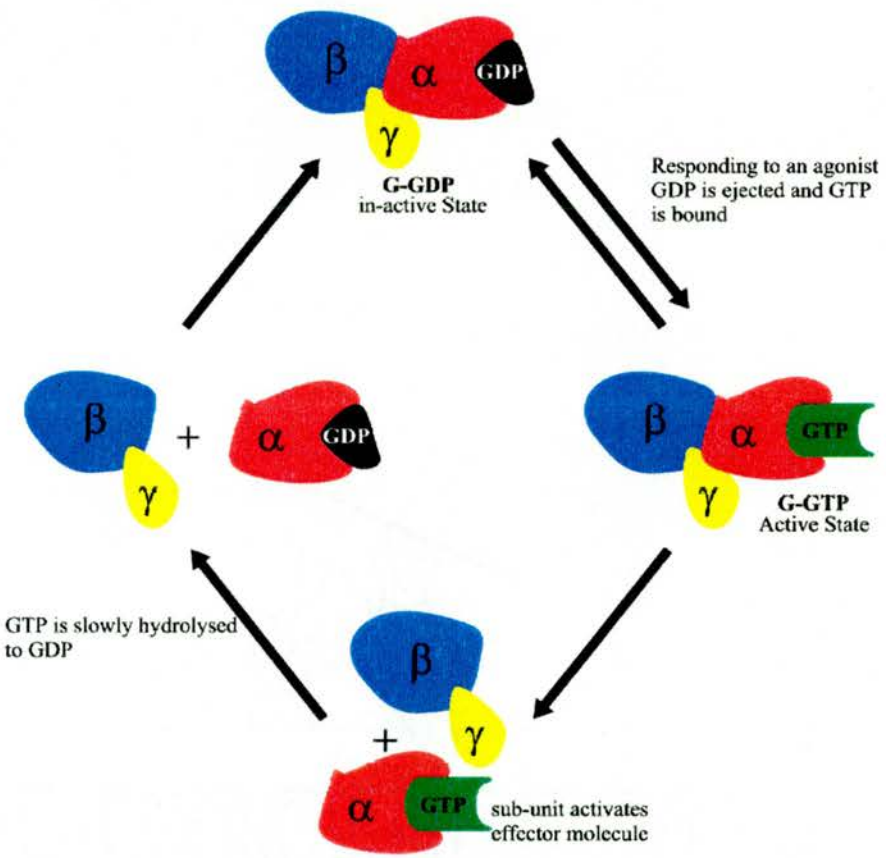
1.9 G-Proteins

G-proteins are membrane proteins consisting of three subunits α (45 KD), β (35 KD) and γ (7 KD). The G-protein can inter-convert between a GDP (guanosine diphosphate) and a GTP (guanosine triphosphate) form.

In the absence of the specific hormone almost all the G-protein is in the inactive GDP form (Scheme 1.3, page 7). It is the binding of the hormone to the receptor that triggers a conformational change which ejects GDP from the α-subunit and allows the entry of GTP. This causes the dissociation of the α-sub-unit bearing the GTP. The activated sub-units can then stimulate or inhibit other membrane bound enzymes acting as amplifiers, for instance K^+ channels, which in turn generate secondary messengers on the membrane side of the cell. Many subunits are

formed for each bound agonist and it is this way that a weak signal acting on the exterior of the cell can be amplified.

GTP bound to the sub-unit of the G-protein is slowly hydrolysed to GDP by the inherent GTPase activity of the α-subunit thus returning the system to rest. The α-subunit containing the bound GDP then reassociates with the βγ-dimer (Scheme 1.3) to return the system to its original state. Therefore if the hormone levels drop the amplified response in the cell quickly declines.



Scheme 1.3 - *The G-Protein cycle*

1.10 Inositol phosphates as secondary messengers

It is known that derivatives of *myo*-inositol **7** (page 12) have an important role in numerous secondary messenger systems throughout the body by virtue of the phosphoinositide pathway (Scheme 1.4, page 10).[23,24] This system acts on calcium concentrations within cells to evoke an intracellular response.

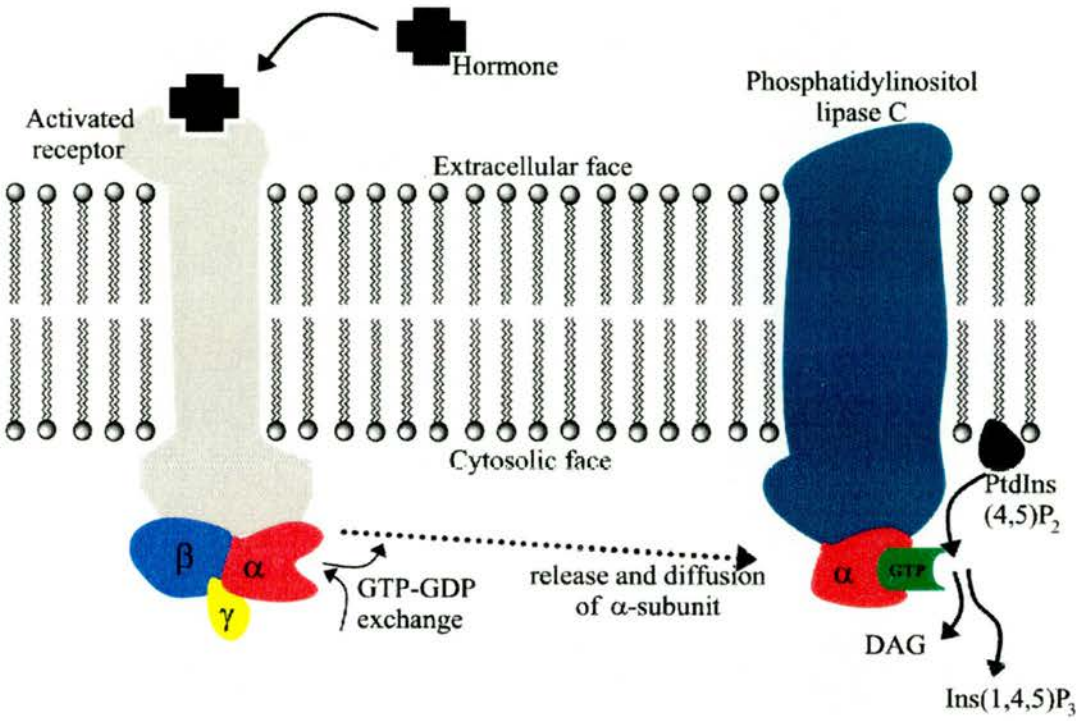


Figure 1.1– *Production of the two secondary messengers diacylglycerol (DAG) and inositol (1,4,5) trisphosphate from phosphatidylinositol 4,5-bisphosphate through the action of the G-protein controlled phospatidylinositol lipase C.*

The intracellular messengers are formed from phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2]. After the action of an agonist this inactive molecule is directed towards a G-protein controlled enzyme (phosphatidylinositol lipase C) where it is hydrolysed to form two secondary messengers Ins(1,4,5) trisphosphate and diacylglycerol (DAG) (Figure 1.1).

DAG is hydrophobic and remains within the membrane, it acts as a secondary messenger by activating protein kinase C which promotes the phosphorylation of

target proteins in the cell. DAG can be further metabolised either through phosphorylation to phosphatidic acid or hydrolysed to produce hormone precursors.

Ins(1,4,5) trisphosphate is released into the cell causing the release of calcium from the intracellular stores - the endoplasmic reticulum and, in smooth muscle cells, the sacroplasmic reticulum. Calcium release has profound effects on a cell leading to effects like glycogen breakdown, smooth muscle contraction or exocytosis.

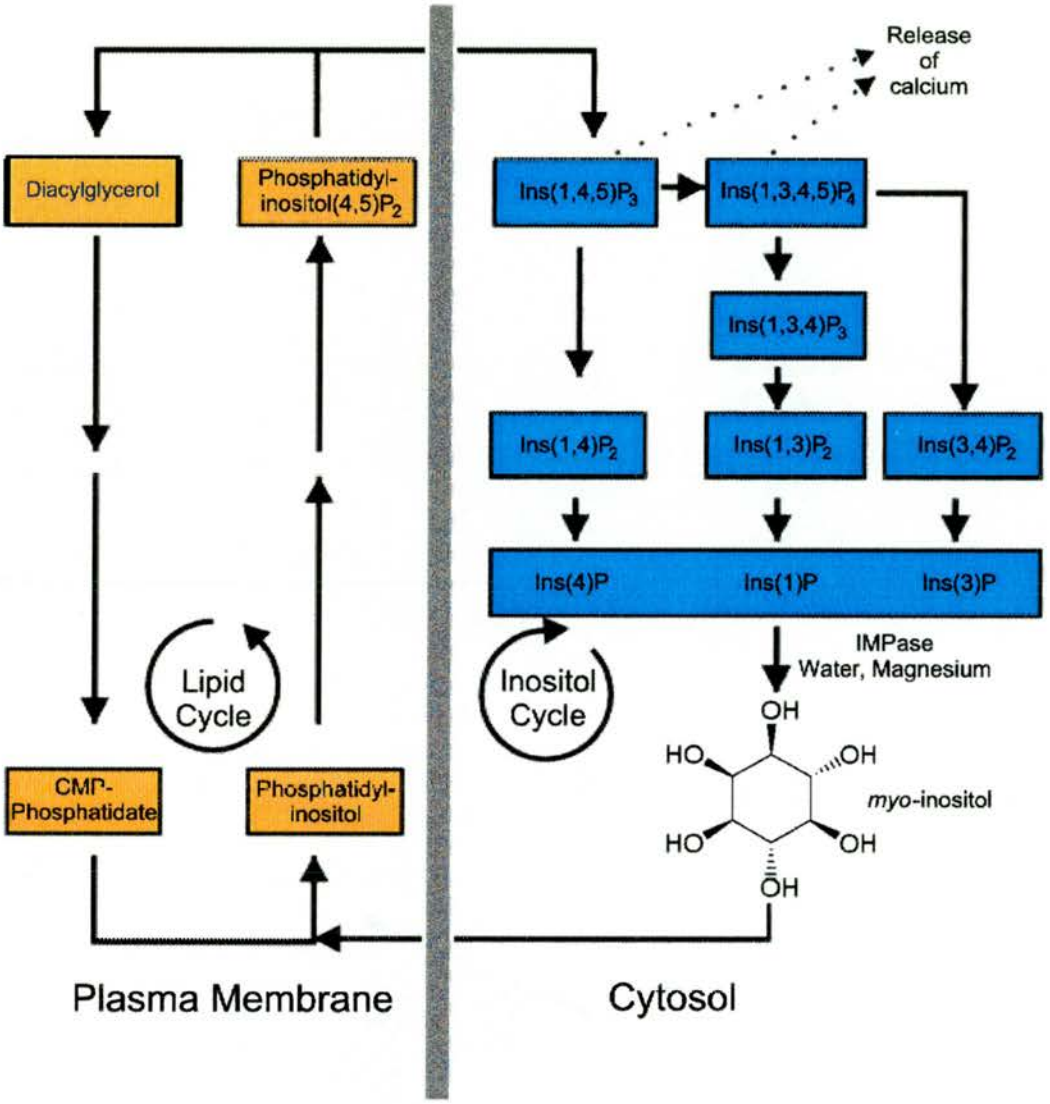
These secondary messengers are 'deactivated' within the phosphatidylinositol pathway by a series of dephosphorylations by specific enzymes to give inositol monophosphate. This is finally dephosphorylated to make inositol which can then re-enter the cycle.

1.11 Phosphatidylinositol pathway

In order to biologically 'switch off' the secondary messenger signalling system it is necessary to breakdown the messengers. DAG is removed through the processes of hydrolysis or phosphorylation while Ins (1,4,5)-P_3 is recycled by a complex cycle to regenerate free inositol. This cycle is called the phosphatidylinositol pathway (Scheme 1.4, page 10).

Ins(1,4,5)P_3 is a short lived messenger, lasting only a few seconds. It is converted to inositol through the sequential action of three phosphatases. Removal of the 5-phosphate group terminates it's messenger role. Inositol 1,4-bisphosphate is hydrolysed to inositol 4-phosphate and then to inositol through the action of the enzyme inositol monophosphatase.

Alternatively, $Ins(1,4,5)P_3$ can be phosphorylated to inositol 1,3,4,5-tetrakisphosphate – also thought to have secondary messenger roles, which is then hydrolysed to the inactive derivative inositol 1,3,4-trisphosphate. This 1,3,4-isomer is converted to inositol by successive dephosphorylations.



Scheme 1.4 – *The phosphotidylinositol pathway showing the lipid and cytosolic cycles*

Most of the phospholipids within a cell spend their time in a 'futile cycle' (see below). Phosphatidylinositol 4,5-bisphosphate is the immediate precursor for receptor mechanisms and resides within the plasma membrane. It is formed by a

two stage phosphorylation of phosphatidylinositol. This is done by first phosphorylating the 4 position of the inositol by a specific kinase which is in turn phosphorylated at the 5 position to give the PtdIns(4,5)P_2.

The lipid cycle is known as the 'futile cycle' because if not used the secondary messenger precursor is continually dephosphorylated by two phosphomonoesterases to return phosphatidylinositol (Scheme 1.4, page 10). This futile cycle serves to provide a pool of PtdIns(4,5)P_2 which is continually replenished ensuring the rapid availability of the trisphosphate secondary messenger.

1.12 Role played by inositol monophosphatase

Within the phosphatidylinositol pathway there is but one enzyme that functions as a unique enzyme capable of metabolising the variety of substrates that present themselves to it (as opposed to a team of enzymes) and this is inositol monophosphatase (IMPase) which produces inositol. It is this unique attribute that provides a means of interfering with the cycle. At all other stages within the pathway there are either a variety of enzymes that may metabolise the substrate or a way of bypassing an enzyme should it be blocked. Obviously it would not make sense to target several enzymes at once as drug specificity is of great importance therefore the most sensible choice of enzyme in the pathway is IMPase.

It is important to note that although inhibition of IMPase would break the overactive cycle it is possible for the brain to synthesise inositol from a *de novo* synthesis using glucose as a starting material (Section 1.13, Page 13), however

this is a slow process and would not seriously affect the situation generated by inhibiting inositol monophosphatase.

1.13 Types of inositol

Inositol is a six substituted hexane ring known as a "cyclohexane hexol". The hexanol exists in many stable stereochemical configurations giving rise to a family of related cyclitols. All nine configurations have all been identified and given names that prefix inositol (i.e *myo*-inositol) they are:- 1) *allo*-, 2) (+)-*chiro*-, 3) (-)-*chiro*-, 4) *cis*-, 5) *epi*-, 6) *myo*-, 8) *neo*-, and 9) *scyllo-myo*-inositol.

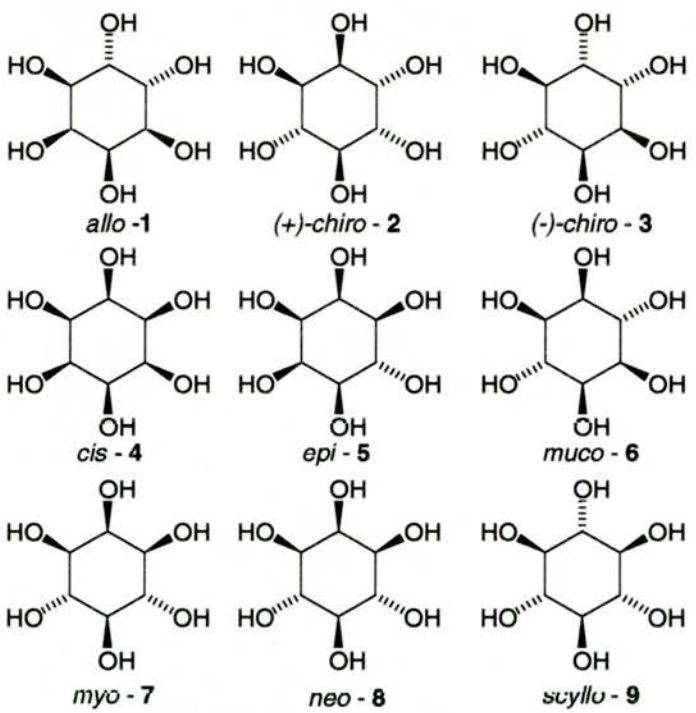


Figure 1.2 – *Various possible forms of the inositol*

myo-Inositol **7** is ubiquitous in all of nature through both the plant and animal kingdoms. In plants inositol derivatives (hexakisphosphates) play a vital role as a

source of phosphorus in seed formation and in animals an important use of inositol derivatives is the formation of phospholipids present in cell membranes.

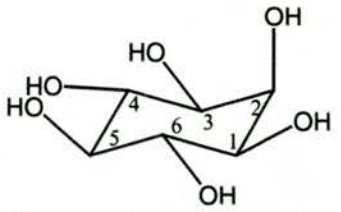


Figure 1.3 – *myo-Inositol showing symmetry plane*

myo-Inositol **7** has a plane of symmetry running through C-2 and C-5 (Figure 1.3). The molecule has one hydroxyl in an axial position with the rest residing equatorial [The axial position is numbered as C-2 in the conventional number system].

Incorporation of a substituent at C-2 or C-5 of *myo*-inositol **7** leads to an optically inactive *meso*- compound, whereas incorporation of a substituent at C-1 or C-3 leads to a pair of enantiomers (+)-**10** and (-)-**10**.

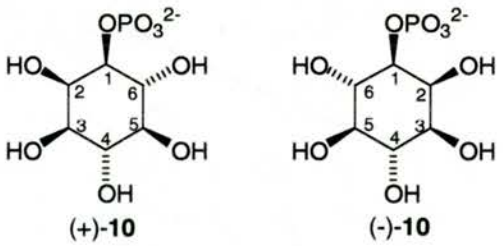


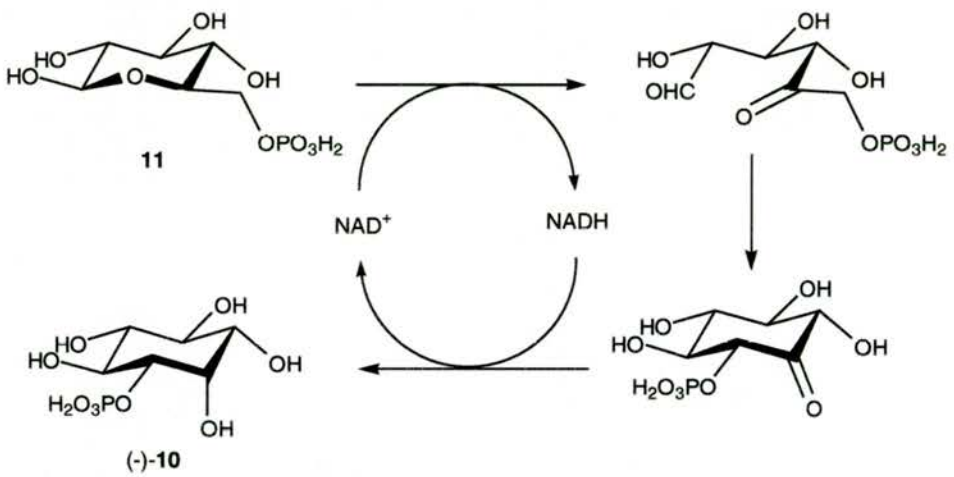
Figure 1.4 – *The two enantiomers of inositol –1-phosphate*

1.14 Biosynthesis of myo-inositol

In humans the majority of *myo*-inositol **7** present is received through consumption of plants. It is however possible to synthesise this essential molecule in animals by the isomerisation of *D*-glucose 6-phosphate **11** catalysed by an enzyme called L-

myo-inositol 1-phosphate synthase (Scheme 1.5). *Myo*-inositol **7** is then synthesised through the action of inositol monophosphatase on the inositol 1-phosphate **10** as part of the phosphatidylinositol pathway.

There has been much interest in the mechanism of the enzyme as it performs a coupled stereospecific ring closure and an inosine reduction.[25-27] The inositol 1-phosphate **10** is then hydrolysed to free inositol **7** by IMPase (Scheme 1.5). This ability of IMPase to catalyse the de-phosphorylation of both isomers of inositol 1-monophosphate **10**, one from the phosphatidylinositol pathway and the other from glucose biosynthesis among other molecules, makes it a key enzyme in the phosphatidylinositol cycle (Scheme 1.4, page 10).



Scheme 1.5 - *Aldolase and coupled oxidoreductase activities of L-myo-inositol 1-phosphate synthase*

1.15 Inositol monophosphatase

Inositol monophosphatase has been purified from a number of different sources including rat, bovine and human brain tissue.[28,29] The enzyme has also been produced through the use of recombinant strains of *Eschericia coli*.[30,31] It is accepted that the enzyme is in the form of a dimer of roughly 58,000 Da.

Early research used bovine sources of the enzyme - this had undergone extensive comparisons with the human example and showed only minor differences. This meant it could be used as a viable surrogate for the human IMPase in research [Human enzyme was hard to source].

Inositol monophosphatase has an essential requirement for a divalent metal ion to function. The natural cofactor is Mg^{2+} but the enzyme has been shown to function with Mn^{2+}, Co^{2+} and Zn^{2+}. Other divalent ions have been shown to be competitive inhibitors for Mg^{2+} including Ca^{2+}, Mn^{2+} and Cu^{2+}.[28,29,32,33]

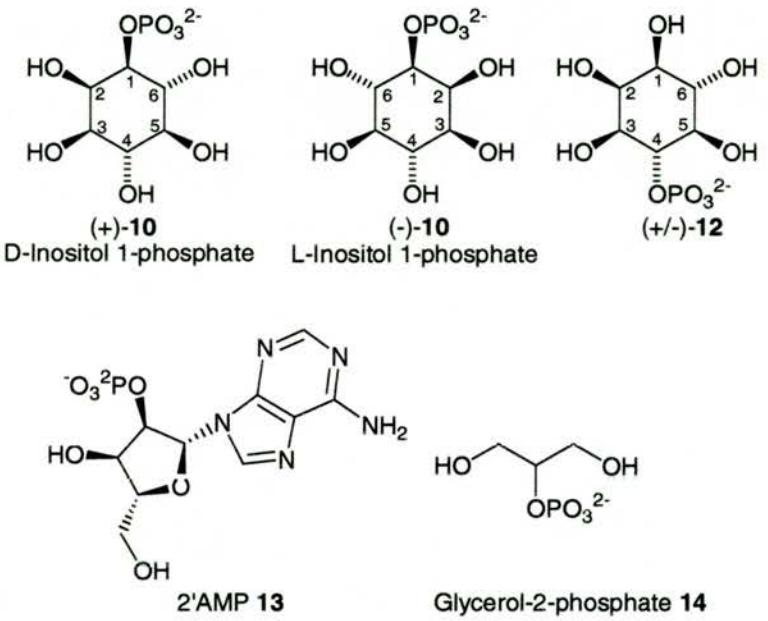


Figure 1.5 – *Various Substrates of IMPase.*

Li^{+} has been shown however to be a powerful non-competitive inhibitor with respect to Mg^{2+} and has also been shown to be an uncompetitive inhibitor of inositol monophosphatase (Section 1.18, page 19). This is thought to be the source of the therapeutic effect of lithium carbonate on manic depressives.

The enzyme has the ability to hydrolyse a broad range of monophosphate esters including both enantiomers of *myo*-inositol 1- phosphate **10** and 4- phosphate **12** as already stated, glycerol-2-phosphate **14**, 2'AMP (adenosine 2'-monophosphate)

13 and other nucleoside 2'-phosphates (Figure 1.5, page 15).[33] The K_m of *myo*-inositol-1-phosphate **10** is ~0.1 mmol dm^{-3}.

1.16 Isolation of IMPase

Bovine brain inositol monophosphatase (Section 1.25, Page 34) is used as an alternative to the human source. Originally the enzyme was recovered from the purification of pulverised brain matter. A more reliable method was developed using recombinant techniques with *E. coli* and it is this source that is generally used in the studies.[33]

1.17 Early studies

Early investigations into the mechanism of inositol monophosphatase showed that the uncompetitive mode of inhibition exhibited by Li^+ on IMPase only held true at low concentrations. At higher concentrations the mode of inhibition became far more complex.[34] Also demonstrated was the fact that at saturating levels of Li^+, dephosphorylation by IMPase to form inositol was observed to occur at rates far higher than predicted by steady state calculations quickly followed by a complete cessation of enzyme activity.[33]

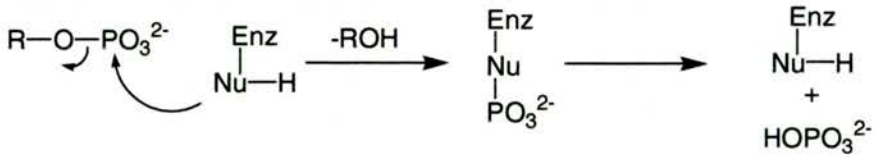
It was known that Mg^{2+} enters the enzyme to bind after the substrate has bound and then leaves before inorganic phosphate is ejected. The kinetic data showed an absolute necessity of two Mg^{2+} for catalysis suggesting that each half of the IMPase dimer required a Mg^{2+}.[33] It was presumed that the binding of the first Mg^{2+} facilitated the binding of the second in a co-operative manner either by adjusting the binding site or creating a new one. The binding of the second Mg^{2+}

was sensitive to the type of substrate reinforcing the view that the substrate binds first and in some way aids the binding of the second Mg^{2+}. High concentrations of Mg^{2+} (>1 mmol dm^{-3}) resulted in uncompetitive inhibition showing kinetic similarities with Li^{+}. This data seemed to suggest that lithium ions acted through binding to sites vacated by magnesium ions.

To ascertain the order of product release the inhibitory characteristics of the various hydrolysis components were studied. Inorganic phosphate was found to be a competitive inhibitor of inositol-1-phosphate implying that P_i is released last. Increasing the pH increased the K_i of inorganic phosphate as the phosphate dianion bound more strongly to the enzyme. At pH 6.5 inorganic phosphate had a K_i of approximately 80 mmol dm^{-3}, at pH 8.0 it's Ki decreased markedly to 0.3 mmol dm^{-3}. As inositol was expected to be the first product to be released it was expected that it should be an uncompetitive inhibitor for the hydrolysis of inositol-1-phosphate as it does not compete directly for the active site. As expected this was the result, $K_{i\ apparent}$ = 250 mmol dm^{-3} at pH 6.5 and 400 mmol dm^{-3} at pH 8.0. The fact that inositol is such a poor inhibitor for inositol 1-phosphate suggest that inositol release following hydrolysis is not a slow step. It was also proposed that the conformation of the binding site may change slightly allowing Li^{+} to bind with greater affinity than Mg^{2+}.[35]

1.18 Mechanism prediction

The results of kinetic studies (Section 1.16, page 16) seemed initially to suggest a well documented phosphate ester hydrolysis mechanism commonly exhibited by alkaline phosphatases,[36] and acid phosphatases[37] known as a '*in-line enzyme mechanism*' (ping-pong).

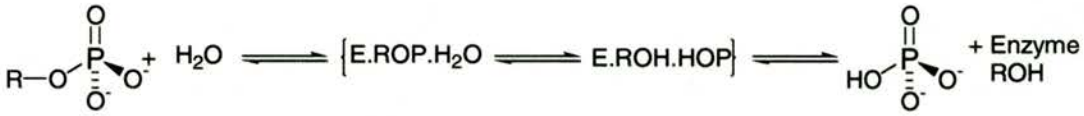


Scheme 1.6 – *Phosphate ester ping-pong mechanism*

The mechanism is known to operate through two half reactions (Scheme 1.6). Usually in the first part of the reaction the phosphate group is transferred to a group on the enzyme to produce what is known as the phosphorylated enzyme intermediate (E-P). Following this the phosphate group is transferred to water to give the desired product, inorganic phosphate and the regenerated free enzyme. Enzymes which follow this mechanism can incorporate ^{18}O labelled water from a stock solution into a pool of unlabeled inorganic phosphate as the 'second half' of the mechanism can work to equilibrate the labelled water in the absence of the product from the first half reaction. When this method was attempted with IMPase, however no labelling was evident except following the addition of inositol-1-phosphate demonstrating that IMPase had an absolute requirement for a suitable substrate for isotopic mixing.[38] The K_m for inositol was 190 mmol dm^{-3} at pH 8.0.[39] All attempts to identify a phosphorylated enzyme intermediate species failed.[33,34]

In addition, isotope mixing with IMPase did not occur in the absence of Mg^{2+}, it was also inhibited by Li^+ and the rate of exchange did not increase in-line with pH, it instead showed an optimum similar to that for the hydrolysis reaction. Also it was shown that the enzyme processed phosphorothioates only at a slightly lower rate than the natural substrates – this was unusual because phosphatases that operate *via* an *in-line displacement mechanism* can usually only process phosphorothioates very slowly.

From these results and others,[40] it became clear that the enzyme did not follow the substituted enzyme mechanism originally suggested and an alternative mechanism called a *ternary complex mechanism* (Scheme 1.7) was proposed where the alcohol is directly displaced from the substrate by a water molecule, so there exists no enzyme-phosphate intermediate.

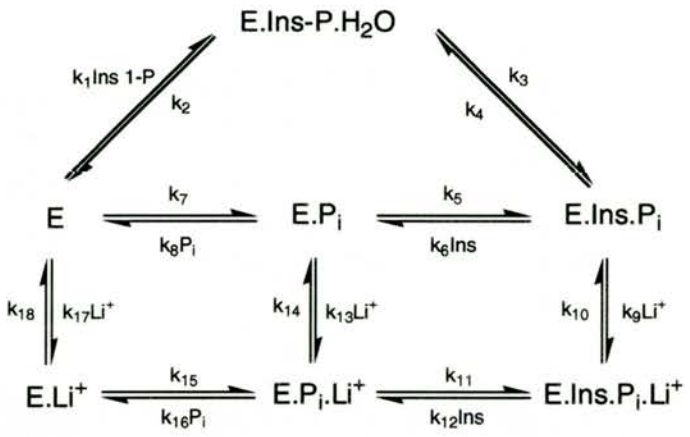


Scheme 1.7- *Ternary complex mechanism.*

1.19 Inhibition by Lithium

Much research has been performed by various groups into the mode of inhibition by lithium acting on IMPase using various substrates.[28,29,33,41-44] The investigations led to important advances in the understanding of IMPase and it's inhibition. It was discovered that lithium ions act as a linear uncompetitive inhibitor at low concentrations (K_i with Ins 1-P as the substrate was approx. 1 mmol dm^{-3}). The result indicated that Li^+ could not bind to the free enzyme but only the enzyme/product and or enzyme/substrate species (Scheme 1.8, page 20). The same investigations concluded that at similar concentrations Li^+ acted as a non-competitive inhibitor for Mg^{2+} with Ins1-P as the substrate. This meant that Li^+ did not prevent Mg^{2+} from binding and Li^+ does not trap any E-Mg^{2+} complex.[33]

It was found that at high Li^+ concentrations (>5 mmol dm^{-3}) the mode of inhibition changed to non-competitive. This indicated that Li^+ can also bind to the enzyme when present in high concentrations.[33]



Scheme 1.8 – *Inhibition by lithium at high and low concentrations*

It was found that the presence of free inositol made no difference either way to the action of Li^+, also the inhibitory effects of both entities were independent. This indicated that Li^+ could bind to an inositol – enzyme species but in doing so could not prevent inositol from debinding resulting in an E, P_i, Li^+ species.

The inhibitory effect of the hydrolysis product P_i was found to be enhanced in the presence of Li^+ and that Mg^{2+} had a similar effect.[33] This result means because of the presence of P_i in cells (1-3 mmol dm^{-3}) that *in vivo* the inhibitory effect of Li^+ could be far more potent than first predicted (Scheme 1.8).

The conclusion drawn from the observations was that IMPase operated *via* a ternary complex mechanism (Scheme 1.7, page 19) in which *P*i was partially rate limiting and Li^+ bound selectively, at low concentrations, to the non-covalent species E.P_i in the site vacated by Mg^{2+} (Scheme 1.8). The other rate limiting step in the mechanism was the catalytic step.[43]

1.20 Deletion studies for structural analysis

The early investigations concerning IMPase substrates were devised to ascertain the roles of each of the functional groups on Ins-1 P. This work was carried out by the Merck, Sharp and Dohme group at Harlow, UK.[38,45,46] The group carried out a series of elegant deletion studies on the natural substrate to define the important binding interactions of the molecule with the enzyme. They demonstrated that the hydroxyl functionality at the 3- and 5- carbons on the inositol ring were not necessary for binding or catalysis. Surprisingly, deletion of these two groups gave a compound with an even greater affinity for the enzyme (K_m= 0.025 mmol dm^{-3}) than the natural substrate. This indicates that the 3- and 5- hydroxy groups actually have a detrimental effect of binding (compound 15; Figure 1.6).[48]

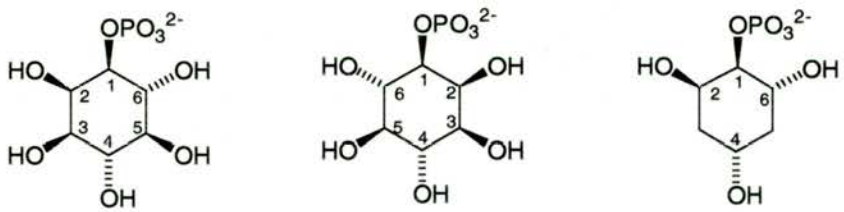


Figure 1.6 – *Substrates and inhibitors of inositol monophosphatase*

An unusual characteristic of the enzyme is that it can hydrolyse both enantiomers of Ins-1P **10** (Figure 1.6). Three dimensional analysis through molecular modelling techniques of the two enantiomers demonstrated that the phosphate group, both α-hydroxy groups and the 4-hydroxy of each enantiomer could be superimposed.[45] The 3- and 5- hydroxy groups however were not superimposible providing further evidence towards the non-essential nature of these groups.[46]

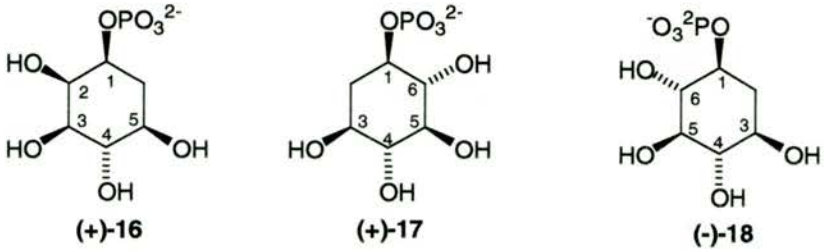


Figure 1.7 – *Various substrates and inhibitors*

Removal of the hydroxyl functionality at the 6 position (±)-6-deoxyinositol 1-phosphate **16**, (Figure 1.7) which was found to be a competitive inhibitor (K_i= 70μmol dm^{-3}).[45] Deleting the functionality at the 2' position, replacing the –OH with an –H, gave (+)-2-deoxyinositol 1-phosphate **17** (Figure 1.7) which was a weak substrate (V_{max}= 78% of V_{max} for Ins-1-P, K_m = 1.3mmol dm^{-3}). This is around 10 times poorer a substrate than the natural substrate indicating that the hydroxyl plays an important role.

Its enantiomer **18** (Figure 1.7) was found to be a competitive inhibitor of the substrate (K_i = 50 μmol dm^{-3}).[45]

It is evident from the deletion studies that both the 6' and the 2' –OH groups have discrete and separate roles to play in hydrolysis. These studies led to the hypothesis that the 2' –OH acted in a binding interaction while the 6' –OH was used somehow for catalysis of the reaction within the enzyme.

It was found that 2,4,6-tris-deoxyinositol 1-phosphate **19** (Figure 1.8) failed to act as either a substrate or an inhibitor. The *trans*-4-hydroxy **20** and the (±)-*cis*-2-hydroxy-cyclohexanol phosphates **21** both appeared to bind to the enzyme indicating that the 4'- and 2'-hydroxy groups were indeed important for binding (Figure 1.8).

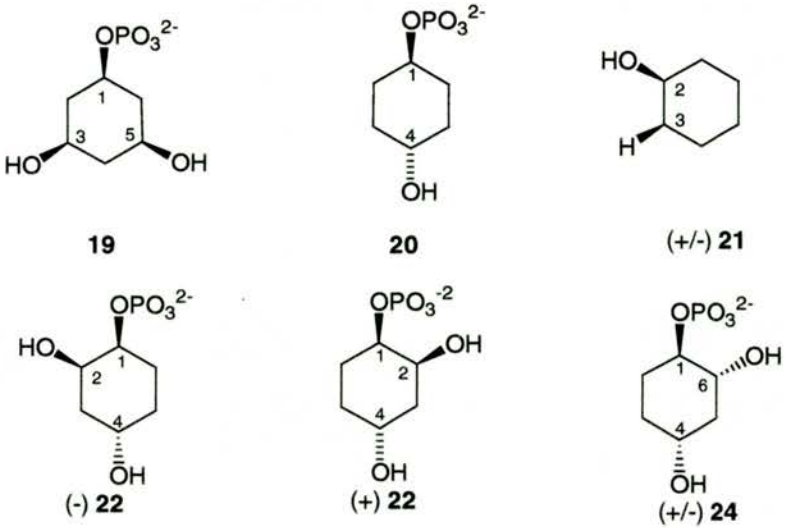
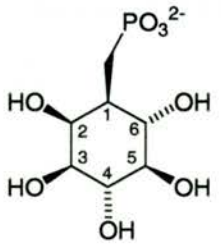


Figure 1.8 – *various substrates and inhibitors*

To confirm the ascribed binding and catalytic roles of the functional groups a series of compounds were prepared. Racemic **22** and **24** were synthesised where the compounds had the 3' and 5' hydroxy groups removed (unnecessary for binding) and either the 6'-hydroxyl or the 2' hydroxy replaced by –H (Figure 1.8). The racemates lacking important functional groups on testing were as expected failures as substrates, in fact the racemates performed as potent competitive inhibitors (K_i= 7 and 90 μmol dm^{-3} respectively).[44] The more potent inhibitor (±) **22** (Figure 1.8) was resolved into it's individual enantiomers. As expected **(-)-22** which lacks the 6' –OH proposed to act in a catalytic role was an extremely potent competitive inhibitor (K_i= 3 μmol dm^{-3}) wherease it's enantiomer (+) **22** was a weak substrate.[46]



25

Figure 1.9-*Inositol 1-phosphonate*

The O-P functionality was also examined to find it's role in hydrolysis. Inositol 1-phosphonate **25** was synthesised and was found to have neither substrate or inhibitor characteristics implying that the bridging oxygen atom was essential for binding.[46]

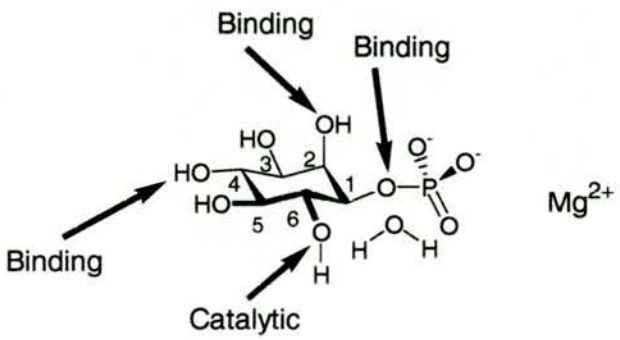


Figure 1.10 – *Pictorial representation of enzyme – substrate interactions*

A summary of all the known binding and catalytic interactions of inositol monophosphate with the enzyme in question – inositol monophosphatase - is shown above

1.21 Early inhibitory studies

Inositol monophosphatase was found to be capable of catalysing the hydrolysis of a range of 2'nucleoside phosphates containing purine and pyrimidines. An example of this was adenosine 2'-monophosphate (2'AMP) **13** which was found to act as a good substrate (V_{max} = 90% of Ins-1*P*, K_m=0.86 mmol dm^{-3}) (see

Section 1.24). As the bulky adenine group does not greatly affect binding of the substrate there must be a certain level of tolerance in the active site for bulky side-groups.[38]

1.21.1 Discovery of 6' position's catalytic and lipophillic properties

In order to design more potent inhibitors for the enzyme the most potent known inhibitor 3,5,6-tri-deoxy-inositol 1-phosphate **22** was examined by molecular modelling (Figure 1.11).

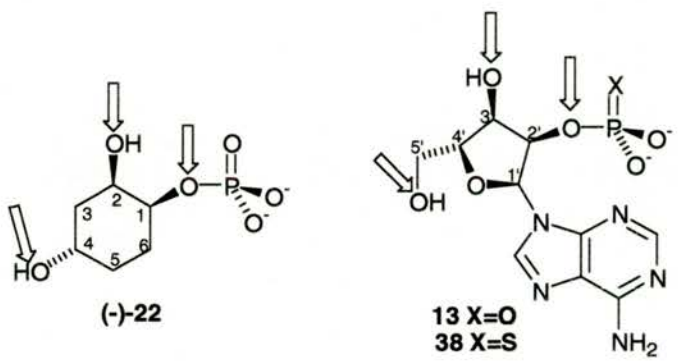


Figure 1.11 – *Predicted interactions of 2'AMP with the 3,5,6-tri-deoxyinositol inhibitor X = O*

The 3-dimensional structure was compared with that of the good substrate 2'AMP **13** and differences examined. It was found that three of the hydroxyl functionality's (2', 3' and 5' –OH positions) of **13** mimic the spacial co-ordinates of three of the hydroxyl groups of **22** (1-O, 2-OH and 4-OH groups) (section 1.24, page 30). This suggests that the three O-atoms of **13** could be involved in the same binding functions as **22**. A direct comparison also leads to the further conclusion that the adenine group must play a part in catalysis (Section 1.24, page 30).

1.21.2 Exploration of lipophillic area

Based on the hypothesis that a lipophilic arm as well as a removal of the catalytic OH group would produce strong binding inhibitors two candidates **26** and **27** based on **22** were synthesised and tested for catalytic properties (Figure 1.11). Both the compounds have long lipophilic arms filling the space utilised by the adenine group which should increase binding affinity and have had the essential catalytic OH group removed.[38] The results were very promising with compound **26** showing good inhibitory characteristics (K_i= 3 μmol dm^{-3}). The results from **27** were even more dramatic giving a very strong inhibitor (K_i= 70 nmol dm^{-3}). This indicated that the aromatic group on the lipophillic arm provided a strong binding contribution.

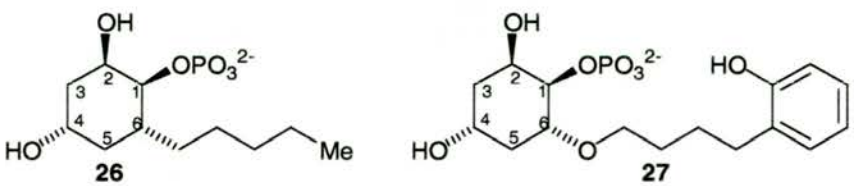


Figure 1.12 – *Deoxyinositol 1-phosphate inhibitors with lipophilic side chains*

1.22 Bisphosphonate inhibitors and the C-1 O atom

The inhibitory characteristics of some *bis*-phosphinate compounds were discovered through the screening of some *bis*-phosphinic acids. The hydroxymethylene-bisphosphinic acid **28** (Figure 1.13, page 27) was found to be a moderate competitive inhibitor of inositol monophosphatase (K_i = 0.18 mmol dm^{-3}). It was thought that the inhibitory properties of this compound may occur through the compound mimicking the action of phosphate in the enzyme active site.[47]

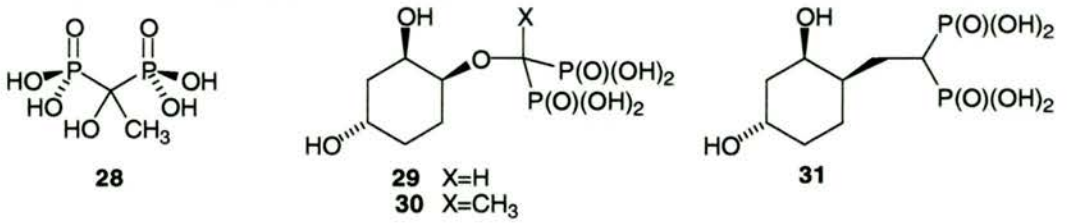


Figure 1.13 – *Bisphosphinic acid inhibitors*

To test this hypothesis the *bis*-phosphonic acid moiety of compound **28** was combined with the known inhibitor **22** to produce a new compound (±)-3,5,6-trisdeoxyinositol-1,1-bisphosphinic acid **30**. Surprisingly although this was a potent inhibitor for IMPase (K_i= 2.5 μmol dm^{-3} for the racemate) there was no enhancement of the inhibitory characteristics over those of **22**. Removal of the methyl group gave an inhibitor **29** with a slightly reduced affinity (K_i = 7.4 μmol dm^{-3} for the racemate, 4.3 μmol dm^{-3} for the (-) enantiomer). Removal of the C-1 oxygen to give the methylene analogue **31** (Figure 1.13) led to a considerable reduction in the compounds affinity (K_i = 0.68 mmol dm^{-3}) compared with **22**. This data confirms the importance of the C-1 oxygen atom in binding.

Although some of these compounds display good inhibitory characteristics they cannot be used *in vivo*. The reason for this is because the deoxy analogues are highly charged species which makes it difficult for membrane penetration and the fact that they are highly susceptible to hydrolysis by non-specific phosphatase enzymes. This results in low concentrations of inhibitor within the cell cytoplasm.

1.23 Novel inhibitors

To avoid the difficulty concerned with non-specific phosphatase enzyme hydrolysis *in vivo* a group of targets that substituted the deoxyinositol substituent on **30** (Figure 1.13, page 27) was investigated.[48,49]

Studies led to the discovery of a potent non-hydrolysable analogue **32** (Figure 1.14) (K_i=0.08 mmol dm^{-3}).[49] This compound was too complex for commercial development but a simpler analogue **33** was made (Figure 1.14) (K_i=0.33 μmol dm^{-3}) this exhibited some inhibitory behaviour towards inositol monophosphatase and was selected for development into a *pro*-drug.

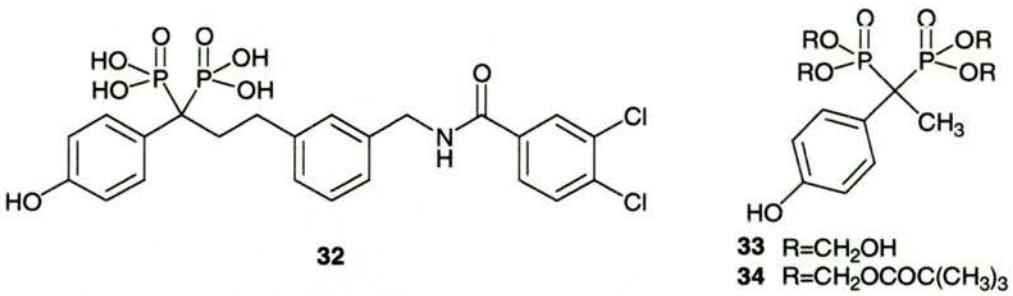


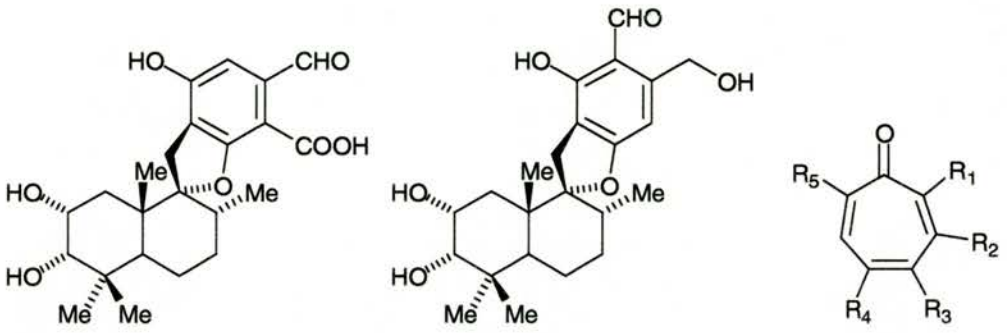
Figure 1.14 – *Novel non-hydrolysable targets*

In an effort to reduce the polarity of **33** the groups attached to the phosphate O-atoms were changed in the hope that the compound **34** would cross the cell membrane more readily. Once inside the cell the protecting groups would be hydrolysed by esterases to return the alcohol **33** (Figure 1.14).

Compound **34** proved to be a far better inhibitor than **33**, indicating that the pivolate group strategy indeed aided cell permeability. It was also found that the drug mimicked the effect of lithium on the phosphoinonositide cycle. Testing however, showed that the drug had zero penetration of the brain from plasma suggesting that the drugs were too insoluble to leave the injection site.[20]

There exist other novel inhibitors for inositol monophosphatase unrelated to the natural product that were isolated from various fungal sources.[50,51,52] The sesquiterpenic compounds **35** and **36** are non-competitive inhibitors for *D*-Ins 1-P and show higher potency than lithium (K_i = 0.5 mmol dm^{-3}).

Recently another class of potent inhibitors of inositol monophosphatase α-hydroxytropolones, based on structure **37** have also been reported.[15] It is proposed that when R5 and or R1 are oxygen functions the resulting configuration can bind strongly in some enzymes with bi-metallic co-catalysts. It was found that IMPase inhibition became more efficient with an increasing number of oxygens



35 **36** **37**

Figure 1.15 – *novel inhibitor classes sesquiterpenic and α-hydroxytropolones*

1.24 Proposal of second magnesium ion

Although good inhibitors were being designed on the available information there remained four important features of the proposed system which were difficult to rationalise:

- The first was the apparent ability of the enzyme to process ribonucleoside 2' phosphates for instance compound **13** which lacks the catalytically 'essential' hydroxy group.[47,45]
- The second concerned a X-ray crystal structure of Gd^{3+} sulfate complex of the enzyme (Section 1.25, page 34).[32] It appeared that the position of the Gd^{3+} ion (which is a competitive inhibitor of Mg^{2+}) was buried so deeply within the active site that it could not possibly bind after the substrate or debind before

the product. This is in direct disagreement with the expectations predicted by kinetic studies.[33]

- Thirdly was the fact that the optimum concentration of Mg^{2+} varied with the structure of substrate,[33,43] and that Mg^{2+} binding was co-operative for some substrates but not for others.[33]
- Finally, the mode of inhibition of the enzyme by Li^{+} changed from uncompetitive to noncompetitive with increasing concentration.[33] Also, the K_i values for uncompetitive inhibition by Li^{+} depended acutely on the structure of the substrate.[9]

Further studies into the ability of the enzyme to recognise a range of substrates including ribonucleotide 2'-phosphates like 2' AMP led to the proposal that there was a *second* magnesium ion participating in the enzyme mechanism (See below).[16,53]

1.25 2'AMP and IMPase

2' AMP **13** acts as a good substrate for inositol monophosphatase (V_{max} is 90% of V_{max} for Ins 1-*P*; K_m = 0.86 mmol dm^{-3}) and is hydrolysed to give adenosine and inorganic phosphate (P_i). The binding affinity of 2'AMP is ten-fold lower than that for Ins 1-P inferring that structural organisation of the protein to accommodate the extra bulk is not quite compensated by extra binding interactions between adenosine and the active site.

Deletion studies indicated that the 2'-, 3'- and 5'-oxygen atoms of 2' AMP serve the same roles as the 1-, 2-, and 4-oxygen atoms in *D*-Ins 1-P respectively in binding to the enzyme (Section 1.19, page 21). 2' AMP however does not possess a similar 6-OH group found to be so important in *D*-Ins 1-P. The purine ring was

found to have no binding role and although the 4'-hydroxymethyl group was used in binding it's orientation was not optimal for this purpose.[16] It was also found that the ribofuranosyl ring O-atom was found to be *essential* for efficient phosphate hydrolysis.

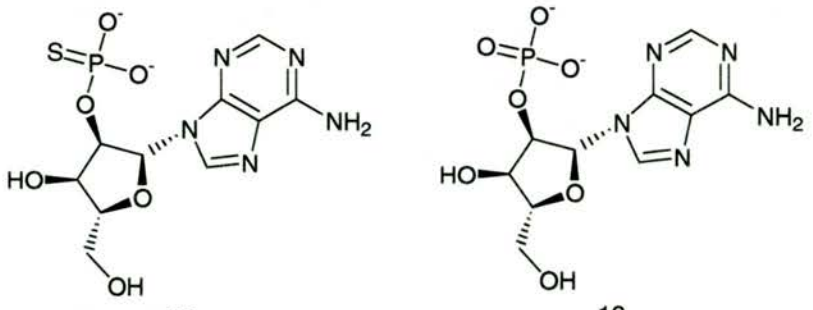
Adenosine – as a hydrolysis product of 2' AMP – was expected to mediate ^{18}O oxygen exchange into inorganic phosphate (P_i) on the basis of microscopic reversibility. This was plainly exhibited by inositol – the hydrolysis product of inositol monophosphate. It was found that no incorporation of ^{18}O occurred which indicates, for the adenosine system, that there exists at least one very slow step which is essentially irreversible.[16] There were only two possible explanations:

- Either 2' AMP is hydrolysed by an alternative mechanism so that adenosine is not the true primary product of hydrolysis or,
- The product-binding complex for adenosine exists in a high energy state.

The result of either of these two possibilities was that adenosine would not be recognised by the active site and therefore would be unable to bind.

The first possibility for exchange failure (alternative mechanism) was discounted as studies demonstrated that no transphosphorylation products were discovered.[33]

The other possibility, which seemed more convincing, was that the product-binding complex for adenosine existed in a high-energy state so it was impossible for adenosine to bind. [53]



38
Adenosine 2'-phosphorothioate

13
Adenosine 2'-phosphate

Figure 1.16 – *2' AMP and 2' AMPS*

Studies with 2'-adenosine monophosphorothioate (2' AMP_S) **38** indicated that it did not act as a substrate for the enzyme in the presence of Mg^{2+}. However on substituting Mg^{2+} with the thiophilic Mn^{2+} ions the expected hydrolysis reaction occurred at approximately 20% of the rate of 2' AMP **13**. The findings indicated the need for a strong binding interaction between the enzyme-bound metal ion and the phosphate or phosphorothioate group in order to confer substrate activity. It was thus reasonable to propose that the primary binding interaction of the phosphate group with the metal ion must be strong in order to off-set the energy required to reorganise the adenosine moiety into a high-energy active configuration.

To deduce the conformation of the high-energy arrangement of the adenosine system it was reasonable to use inositol – which being a product from the inositol monophosphatase reaction, must exist in its low energy configuration- as a template.

Forcing 2' AMP into a similar configuration as the low energy configuration of inositol meant that, relative to all other binding and catalytically important groups, the furanosyl ring O-atom must move toward the 2'-O atom. This forces their respective lone pairs to point towards each other and so bring the adenine moiety

into an unfavourable position. The proposed confirmation also results in an adverse 1,3-interaction between the adenine moiety and the 4'-hydroxymethyl group and from a 1,2-interaction between the 2'-O and 3'-O atoms. This forced structure was calculated to be as high as 100 – 105 kJ mol^{-1} less stable than the unconstrained form which explained the reason adenosine was not recognised by the enzyme as a substrate.[40]

It was proposed that the high energy of this strained system could be substantially stabilised by chelation to a second Mg^{2+} ion producing a five membered metallocycle. This second Mg^{2+} could also provide a possible site for a hydroxide ion suitably orientated for attacking the P-atom. The role of the first Mg^{2+} in this system would be to bind the phosphate of the substrate in the enzyme active site while simultaneously acting as a Lewis acid increasing the electrophilicity of the P-atom.

Further studies refined the calculated energy of the system. The catalytic ribofuranosyl 1'-O-atom which was predicted to make direct contact with the second Mg^{2+} could instead hydrogen bond to it through two water molecules. This conformation was calculated to exist in a very stable state which was only 20.1 KJ mol^{-1} less stable than the ground state conformation of 2' AMP (section 1.24 page 30).[40]

Transposition of the interaction of Mg^{2+} with 2' AMP onto the structure of D-Ins 1-P gave a possible 3-D structure for the key interactions of both metal ions with the substrate.

1.26 Structure of inositol monophosphatase

The first X-ray crystallographic structure of inositol monphosphatase was released in 1992 at a resolution of 2.1Å. The structure confirmed that the enzyme existed as a homodimer of approximately 58,000 Da.[32]

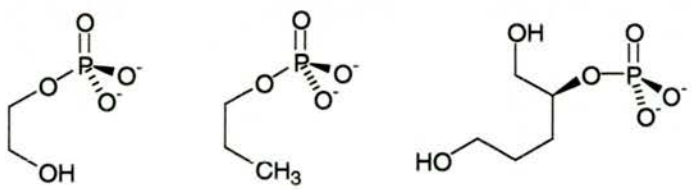
Each of the subunits are folded into a five-layer sandwich of three α-helices and 2 β-sheets. The structure was solved with a lanthanide (Gd^{3+}) and a sulfate bound at identical sites on each subunit. This served to identify the positions of the active sites in the large hydrophobic cavity at the base of two central helices where several segments of the secondary structure meet. Since Gd^{3+} and sulfate are competitive inhibitors for Mg^{2+} and phosphate respectively it is a reasonable assumption to expect that the inhibitors would occupy the same sites as their rivals in the enzyme-product complex.[32]

It was found that Gd^{3+} was located in a position deeper in the active site than the sulfate ion. This implies that the magnesium should bind to inositol monophosphatase before the substrate. This finding was in conflict with the conclusions of kinetic studies (Section 1.16, page 16) where it was found that Ins 1-P bound to the active site first, then followed by a magnesium ion. The solution to this apparent contradiction was the proposal that two magnesium ions were involved in the enzyme reaction.

More recent X-ray structures have provided improved resolution. Notably one containing Gd^{3+} and either *D*- or *L*-Ins 1-P has been released which provides information on the conformation and position of the substrate within the active site.[32] Another X-ray structure contains inorganic phosphate and two Mn^{2+} ions.[57] This provides additional evidence for the involvement of two metal ions in catalysis.

1.27 Binding of Alternative Substrates

If the proposal that only the two adjacent 6-O and 1-O atoms of Ins-1P interact with $Mg^{2+}2$ and the phosphate moiety interacts with $Mg^{2+}1$ then the simplest compound that should act as a substrate for the enzyme will be ethane 1,2-diol 1-phosphate **39**. Molecular modelling revealed that the compound could bind in either both or one of the two conformations proposed to be adopted by 2' AMP and *D*-Ins1-P. This proposal was confirmed when **39** was shown indeed to act as a substrate with moderate activity (V_{max} = 12% of the V_{max} value for Ins 1-P, K_m = 0.7 mmol dm^{-3}), and behaved as a competitive inhibitor as expected on the basis of it's properties as a substrate (K_i = 1.0 mmol dm^{-3}). [53,58]



39 **40** **41**

Figure 1.17 – *Minimal Substrates*

The importance of the 2-OH group was further verified by the synthesis and testing of the propyl phosphate **40** which proved to be completely inactive as a substrate or inhibitor.[58]

The minimal substrate **39** was treated as a basis to further test the environment of Mg^{2+}. The structure was elaborated by adding extra oxygen functionality into specific positions. (2*S*)-Pentane-1,2,5-triol 2-phosphate **41** serves as a competitive inhibitor of inositol monophosphatase (K_i = 0.12 mmol dm^{-3}). The observed 9-fold reduction in K_i over diol **39** implied that both the 1-OH and 5-OH groups interact with the enzyme presumably at the sites for the 2-OH and 4-OH groups of the Ins 1-P and the effective inhibitor **22** Figure 1.8, page 23). The more elaborate

derivative (2R)-pentane-1,2,5-triol 2-phosphate **41** was expected to serve as a substrate by analogy to the weak deoxyinositol substrate (+)-**22**, but actually turned out to act as a weak inhibitor (K_i = 3.8 mmol dm^{-3}). The proposed reason for this was due to the flexibility of the 5-OH group which meant that it could bind to a site on $Mg^{2+}2$ and therefore disrupt the geometry of the complex such that hydrolysis was prevented.[58]

Another series of compounds **42** and **43** were prepared which concentrated on analysing the effect produced by elaboration of the free hydroxyl group of diol **39** (Figure 1.17, page 35). It was expected that this would produce derivatives that might displace the nucleophilic water molecule from it's proposed site on $Mg^{2+}2$. It was not expected that any of these compounds would display substrate activity.

The effect of replacing the 2-OH group of **39** with a methoxy group gave 2-methoxyethyl phosphate **44** which showed no activity as a substrate and also exhibited a 25 fold reduction in binding affinity as an inhibitor ($K_i \geq 25$ mmol dm^{-3}). This result compared well with the result from the previously synthesised 6-O-methylinositol 1-phosphate **45** which also showed no substrate activity and is an inhibitor.[54]

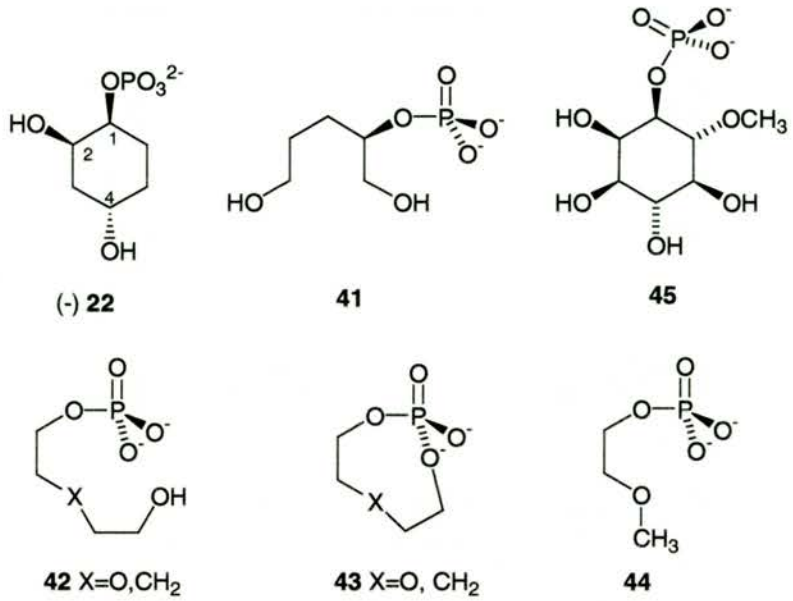


Figure 1.18 – *Minimum substrate designed to probe the internal environment of IMPase*

The results of these studies indicated that the ability of the O-atoms in the substrate (6 – O in *D*-Ins-1P and 2-O in **39**) to interact with $Mg^{2+}2$ was severely compromised by alkylation. It was proposed therefore that steric crowding or adverse hydrophobic effects within the active site was to blame for preventing the lone pairs of these O- atoms from assuming an orientation favourable to catalysis. Only in the highly ordered and constrained 2'-AMP could an ether O-atom support catalysis.

Diethyleneglycol monophosphate **42** (X=O) showed an 8-fold increase in binding affinity to inositol monophosphatase (K_i = 3.5 mmol dm^{-3}) relative to **44**. The reason for this was ascribed to the direct interaction of the flexible 5-OH arm with $Mg^{2+}2$ in a similar manner to the proposed behaviour of triol monophosphate **41** with the possible effect of displacing the nucleophillic water molecule. The affinity of **42** (X=O) did not alter when the ether O atom was replaced to give propane diol monophosphate **42** (X = CH_2), thus confirming the fact that the 5-OH was responsible for the major interaction with $Mg^{2+}2$.

Cyclic diester **43** (X=O) has a reduced negative charge on the phosphate moiety and was thus expected to bind less well to $Mg^{2+}1$ in the enzyme active site than acyclic **42** (X=O), and consequently decrease the affinity of the enzyme bound species for $Mg^{2+}2$. In the event **43** (X=O) was a weak inhibitor with a K_i-value of 8 mmol dm^{-3}. This result was considered surprising for such a monoanionic compound. An altered binding arrangement of **43** (X=O) with the enzyme as the surrogate for the nucleophile was now bonded directly to the P atom was cited as a reason for the low K_i value.[58] The fact that this compound is not a substrate for inositol monophosphatase is consistent with the proposed mechanism in which $Mg^{2+}2$ rather that $Mg^{2+}1$ delivers the nucleophile. Again, removal of the ether O-atom of **43** to give the cyclic propane diester **43** ($X=CH_2$) exerted little effect on the affinity of the compound.

As a result of the observed low reactivity of the ether O-atoms of **42** (X = O, 43 X = O) and **44** with $Mg^{2+}2$ and the substrate activity of ethane 1,2-diol phosphate **39**, it was proposed that the catalytically essential O-atom in substrates did not interact directly with $Mg^{2+}2$. Instead they provide a lone pair to position the $Mg^{2+}2$-chelated nucleophile, water, properly and hold it in place on the metal atom.[58] This arrangement would give a seven membered trioxametallocycle containing an internal hydrogen bond (Figure 1.20, page 42).

1.28 Non-inline associative mechanism (adjacent displacement mechanism)

Many of the phosphate monoester probes which contained suitable oxygen functionality's for binding the proposed second Mg^{2+} ion also serve as competitive inhibitors. If $Mg^{2+}1$ participates in activating the water nucleophile then these compounds should also act as substrates with $Mg^{2+}2$ acting to stabilise the leaving alkoxide group. This mechanism predicts an in-line displacement with inversion of configuration.

However, if the second Mg^{2+} ion provides the binding site for the water nucleophile then none of the compounds **41**, **42** (X = O & CH_2), **44** or **45** can provide a ligand to displace the nucleophile or cause the reorganisation of the co-ordination chemistry about Mg^{2+} would be hydrolysed. This information and the results of other studies[54] strongly indicate that there are two magnesium ions involved in the mechanism where the second Mg^{2+} chelates and activates the nucleophile. This mechanism predicts adjacent displacement at phosphorus with pseudorotation and places the inositol leaving group in an apical leaving position and resulting in retention of configuration.[55]

IMPase is the first enzyme identified to possibly operate *via* this unusual mechanism (Section 1.28, Page 40), although there is considerable evidence that a pseudo-rotation mechanism operates in non-enzymic 1,2-phosphoryl transfer reactions.[56]

The conclusion that two magnesium ions are involved in the mechanism are supported by the reported X-ray crystal structure for human inositol monophosphatase containing two divalent Mn^{2+} ions and phosphate.[57]

1.29 Analysis of pseudorotation

The pseudorotation thought to occur in the hydrolysis reaction is an intramolecular process beginning with a trigonal bipyramidal molecule. The initially apical nucleophile moves to an equatorial position while the leaving group simultaneously moves to an apical position before departing.

The pseudorotation is a mechanism where the apical components (RO) and the nucleophile are in a trigonal bipyramidal conformation (Figure 1.19). The apical component's bond length then shrinks to give a tetragonal pyramidal intermediate before the O^- and the leaving group bond lengths extend resulting in a new trigonal bipyramid with the leaving group now in an apical position. It appears that the trigonal bipyramid has been rotated by 90° around an interatomic bond – however this is only an illusion which is why it is called a *pseudo*rotation.

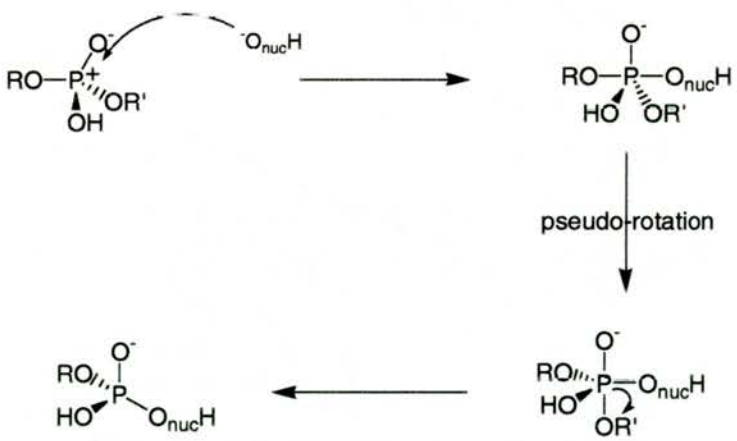


Figure 1.19 *Mechanism of pseudorotation*

1.30 Accuracy of modelling approximations

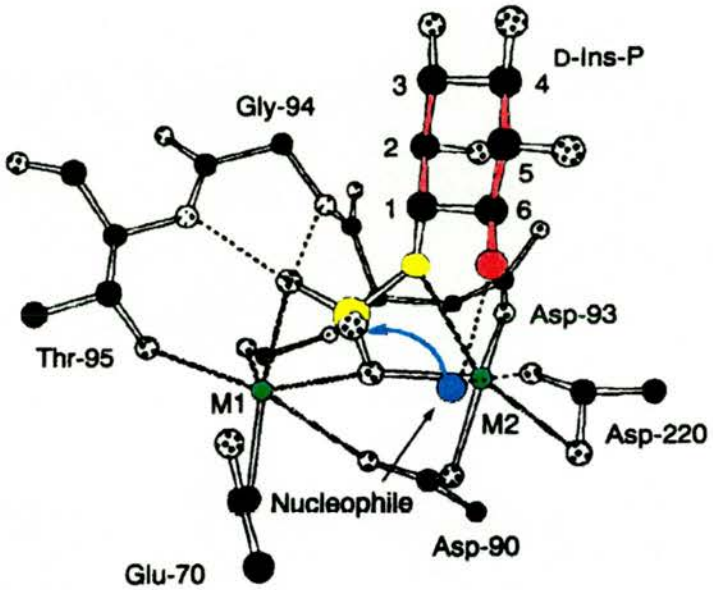
Most of the kinetic data associated with IMPase was derived from bovine brain extracts. It was argued that the mechanistic arguments pertinent to the enzyme may not be accurate when applied to the human version of the brain enzyme. It was discovered that when the two enzymes were compared the human IMPase differed from bovine by around 11.5% (32 residues per subunit).[40] However the majority of the differences were in the form of highly conserved mutations and only four were reported to lie within 10 Å of the active site. Studies by Gani *et al* have demonstrated that none of the differences seem likely to affect the conformation of the substrate – enzyme complex or influence the proposed enzyme mechanism.[40]

1.31 Calculation of Enzyme - Substrate Binding Interactions

The best available structure of the enzyme is the 2.3 Å resolution X-ray crystal structure for a Gd^{3+} - sulphate form of the human enzyme.[32] As previously mentioned it was known that both Gd^{3+} and sulphate were competitive inhibitors for Mg^{2+} and phosphate respectively so it was a reasonable assumption to presume that they bound in the same place as Mg^{2+} and phosphate within the active site.[32,39] To produce an accurate representation of human IMPase binding interactions and determine the structure of the active complex the published structure was modified computationally.[39] The Gd^{3+} ion in the crystal structure was replaced by a Mg^{2+} and the phosphate ion was modelled to replace the sulphate.[32]

The dihedral angles of the substrate were then adjusted to take into account the available kinetic information. A second Mg^{2+} ion was then placed in a site created by the bridging O – atom of the substrate and three asparate residues from the

enzyme. This position compared well with analysis of structure – activity relationships with the identified binding and catalytic centres if Ins-1P all participating.[58] It was found that, as mentioned above (See section 1.25. page 34) the structures generated for the human and bovine versions of the enzyme were identical in the vicinity of the active site. Figure 1.20 shows a representation of the model showing both metals, key amino acid residues and the proposed position of the nucleophilic water molecule.



Blue = Nucleophile, Green = Mg, Yellow = Oxygen and Phosphate

Figure 1.20 – *Optimised active-site structure for the substrate-enzyme complex*

1.32 Analysis of calculated model

It is proposed that there are two metal binding sites within the active site. The position of the first binding site ($Mg^{2+}1$, site 1) closely corresponds with the published X-ray structure data for the Gd^{3+} -*D*-Ins 1-P enzyme substrate complex.[35,59] This Mg^{2+} ion co-ordinates to the enzyme side chain carboxylate groups of Glu-70 and Asp-90, the hydroxy group of Thr-95 and to the carbonyl O-atom of Ile-92 as well as two of the non-bridging phosphate O-atoms. On the opposite side from which the phosphate is attached there is a vacant co-ordination site which in the Gd^{3+} X-ray crystal structure there is a protein-enclosed water molecule.[32] This water molecule completes the co-ordination sphere of $Gd^{3+}$and is hydrogen bonded to Asp-47. This water molecule appeared to be unable to interact with the phosphate group of the substrate and is incapable of accessing bulk solvent because of the protein barrier and the bound substrate. This implies that it is very unlikely that this water molecule is directly involved in substrate hydrolysis. There is one other water molecule attached to Gd^{3+} which H-bonds to Thr-95 in the Gd^{3+} -*D*-Ins 1-P X-ray structure.[59] This water molecule however, is probably unimportant because it is likely to be displaced by one of the inorganic phosphate atoms in the product complex. Studies have shown that in the di-Mn^{2+} ion complex with phosphate that a phosphate linked O indeed occupies this position.[57]

$Mg^{2+}1$ occupies a position deep within the enzyme at the bottom of the active site cleft. An α-helix (residues 44-61) runs along the bottom of this cleft preventing gross movement of the enzyme ligands. The Mg^{2+} ion therefore, must enter the active site cleft by the top of the active site and once in place is fully encapsulated

by the protein. Access by any other route other than the top of the active site cleft is very unlikely as it would require large movements in protein. The $Mg^{2+}1$ must then enter the active site through the top of the active site cleft before any of the other species and will be unable to escape even with saturating concentrations of substrates between individual catalytic events. This finding is completely consistent with kinetic findings,[33] where it was shown with two Mg^{2+} ions that the binding of the substrate to the enzyme occurs after $Mg^{2+}1$ but before $Mg^{2+}2$.[58]

The metal ion coordination site for $Mg^{2+}2$ is formed in the enzyme-*D*-Ins 1-P substrate complex comprising of both substrate and enzyme ligands. All but one of the six co-ordination sites are occupied as for $Mg^{2+}1$. There are three co-ordination ligands from the carboxylate groups of the enzyme (Asp-90, Asp-93, and Asp-220) and two to the substrate through one of three equivalent phosphate O-atoms and to the bridging ester O-atom. The final free face of the metal is accessible to bulk solvent.

The inositol ring binds in the active site cleft by forming several hydrogen bonds with it's hydroxy groups. One hydrogen bond is formed from the 4-OH group to the carboxylate group of Glu-213, one from the 4-OH group to the side chain of Asp-93 and one from the backbone NH of Ala-196 to the 2-OH atom. There is a hydrogen bond from the 6-OH group with Asp-220 which additionally provides a ligand to $Mg^{2+}2$. The phosphate ester forms several interactions with both metals and to the backbone NH groups of Thr-95 and Gly-94 of the enzyme.

Kinetic studies have shown the importance of the 6-OH of Ins-1P in hydrolysis as a catalytic OH group,[9] however it does not form any direct interaction with Mg^{2+} 2 and lies around 4.02 Å away. This observation is in keeping with the kinetic behaviour of several substrates and substrate analogues.[58] The catalytic OH group

is positioned to coordinate a water molecule from the bulk solvent which can then form the final coordination ligand to $Mg^{2+}2$ giving approximate octahedral geometry. This water molecule is well placed to act as a nucleophile in the hydrolytic reaction, and is also within hydrogen bonding distance of the carboxylate group Asp-220 which could conceivably act as a base.[40]

There exist other areas of the enzyme that may assist in substrate binding or stabilisation of the hydrolysis intermediate through the phosphate. The N-terminal (positive pole)[60,61] of an α-helix spanning residues 195-205 is directed at the phosphate ester group and could possibly play a part in binding the substrate. There is another α-helix spanning residues 95-100 which is targeted towards the nucleophilic water molecule and the phosphate ester bridging O-atom. As the bridging O-atom develops a negative charge during the hydrolysis by becoming an alcoholate leaving group and the nucleophile becomes more hydroxide in nature an α-helix in this position could stabilise the developing negative charges.[60,61]

1.33 Kinetic results and the mechanism

Analysis of the kinetic data (Section 1.16 page 16) indicates the possibility of a non-inline associative mechanism (adjacent displacement mechanism). Applying this to the developing computer model allowed a hypothetical mechanism to be advanced. When all components are present in the active site the nucleophile is H-bonded to the 6-OH of the substrate and is also constrained by $Mg^{2+}2$ and Asp-220. Hydrolytic attack of the phosphorus occurs from opposite the O-atom that is bound to the enzyme NH residues of Gly-94 and Thr-95.

As the nucleophile approaches the P-O bonds begins to lengthen and the unbound phosphate O-atom moves towards $Mg^{2+}1$ such that it's position is stabilised by interactions between the side chain hydroxy group of Thr-95 and by $Mg^{2+}1$.

As the nucleophile approaches further the O-1-P bond lengthens even further as the other phosphate O-atoms begin to shorten. The O-atom of the nucleophile leaves the co-ordination sphere of $Mg^{2+}2$ as O-1 of the substrate stabilised as an alkoxide, becomes protonated by (the now acidic) Asp-220, to give the product complex.[40]

In the product complex it was observed that the side chain of Thr-95 and $Mg^{2+}1$ interacted with the pseudorotated phosphate O-atom. This closely resembles the arrangement of phosphate found in the X-ray crystal structure of the di-Mn^{2+}-phosphate product complex.[57]

1.34 The alternative hydrolysis mechanism

Based on the above evidence alone it has not been possible to disprove the possibility of another mechanism of hydrolysis named the in-line displacement mechanism (in-line substitution mechanism) (Section 1.34.1, page 48). This alternative mechanism has been proposed by a group at Merck, Sharp and Dohme using a combination of X-ray crystallographic studies of protein-substrate complexes,[32,57,59] and mutagenesis.[62-67] The binding interactions they found between the enzyme and the natural substrate *D*-Ins 1-P were almost identical to the interactions discussed. Although both proposed mechanisms involved two Mg^{2+} ions the role that the Merck group assigns to the Mg^{2+} ions differ from those given by Gani and co-workers.[35]

The Merck group propose that it is actually $Mg^{2+}1$ that plays the most important role during hydrolysis. The nucleophilic water molecule is thought to be H-bonded to the 3-OH group of Thr-95. $Mg^{2+}1$ and Glu-70 then proceed to activate and orientate the nucleophile.[35] Attack on the phosphorus occurs from opposite the 1-O atom of the substrate to give a trigonal bipyramidal intermediate. As the nucleophile approaches the 1-O atom of the substrate begins to lengthen and weaken until it finally departs, stabilised as the alkoxide. The phosphate now will have been inverted. Before the substrate can be released from the active site the 1-O atom must be protonated this occurs possibly through bulk solvent to give the hydrolysis product along with inorganic phosphate.

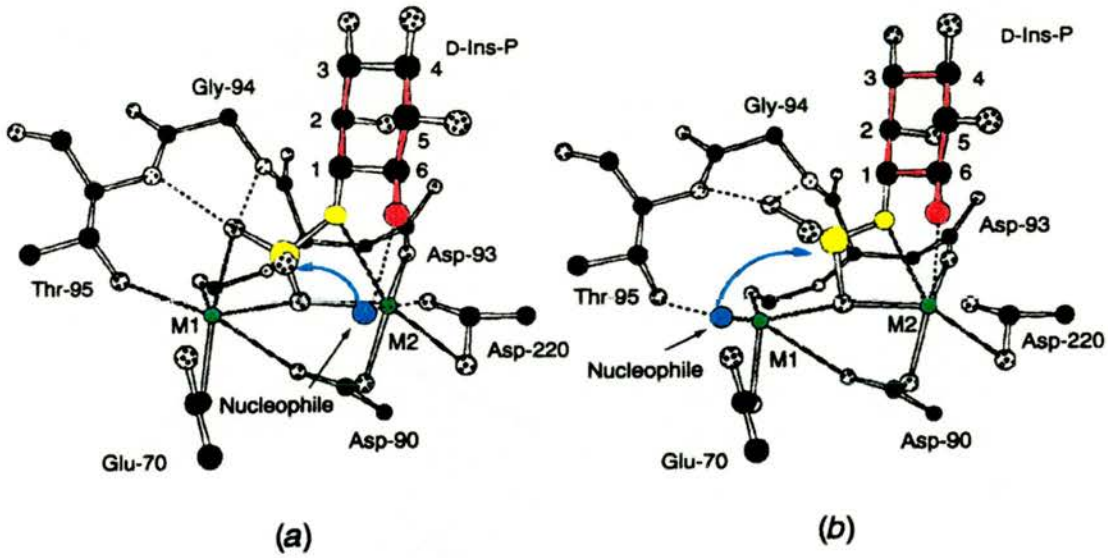


Figure 1.21 – *The two proposals for the location of the nucleophilic water molecule*

1.35 Comparison of in-line and adjacent mechanisms

1.35.1 Support for in-line mechanism

The in-line mechanism [Figure 1.21 (b)] proposes that the hydrolytic nucleophile is situated on $Mg^{2+}1$ and attacks to give inversion at phosphorus. There is much evidence quoted to support this proposal.

Analysis of the Gd^{3+} X-ray crystal structure of the enzyme-substrate complex showed that there existed a long Gd^{3+} to Thr-95 distance (3.3 Å),[43] (the Mg^{2+} model calculates a distance of 2.32 Å). The in-line displacement model proposed that the nucleophile which was attached to $Mg^{2+}1$ was activated by Thr-95. The long distance separating the two species indicates that there was no interaction which could imply that there was space for a water molecule at an ideal distance (3.6 Å) from the phosphate making it an equivalent nucleophile to the one proposed to be co-ordinated to $Mg^{2+}2$.

Site-specific mutagenesis gave results initially thought to support the in-line displacement theory.[35,63] Operating on the principle that Glu-70 activated the nucleophile on Mg^{2+} 1 through H-bonding it was proposed that mutation of this residue would result in a drastic decrease in K_{cat}. The residue was altered to glutamine or aspartate which reduced K_{cat} by 7000 and 400 fold respectively. Although this seemed to support the in-line mechanism there were compelling arguments against this conclusion (Section 1.34.3, page 50).

Mutatation of Thr-95 (which is thought to form an H-bond to the water molecule) to serine and alanine gave results which were consistent with the proposed in-line displacement model namely a reduced K_{cat} and inhibitory Li^{+}/Mg^{2+} binding properties consistent with considerably reduced concentration of E-Mg^{2+} -P_i indicating a severely disabled enzyme. This result however could also be applied

to the other mechanism satisfactorily. Other mutations to important residues gave results that were also equally applicable to both proposed mechanisms.

Unfortunately the results of the mutation studies were not conclusive in proving or disproving either proposed mechanism. In section 1.34.3 the evidence presented in support of an in-line displacement mechanism is demonstrated to be ambiguous or flawed.

1.35.2 Support for adjacent mechanism

There is much evidence that directly supports the proposed adjacent mechanism. For instance there were observations of a very fast rate of ^{18}O exchange from water to inorganic phosphate (70% V_{max} for the hydrolysis reaction for *D*-Ins 1-P at saturating inositol and P_i).[33,39] It was expected that this higher rate could only be possible through the shallow buried Mg^{2+} ion. This metal ion is very easily accessible to bulk solvent as it must debind from the enzyme and it's associated hydration ligands every time the enzyme turns over to allow the release of phosphate. A high rate of exchange is unlikely to occur if the Mg^{2+} ion activating the nucleophilic water molecule was the deep buried $Mg^{2+}2$ ion. At high concentrations of substrates or P_i this Mg^{2+} ion is likely to be trapped within the enzyme thus restricting it's access to bulk solvent.

Important evidence for the likelihood of the nucleophile being positioned on $Mg^{2+}2$ comes from the study of phosphorothioate analogues of Ins 1-P and 2'-AMP with IMPase.[68] This research demonstrated that these sulphur analogues were processed by the enzyme albeit at a lower rate than for the natural substrate. It is known that $Mg^{2+}1$ makes a strong binding interaction with the phosphate group of the substrate. Based on this knowledge it would be expected that a

dramatic decrease in the reaction rate due to the larger size of the sulphur atom which binds to the Mg^{2+} ion would cause the P-atom to be forced away from $Mg^{2+}1$ (Mn^{2+} for 2'-AMP$_S$) to the order of 0.2-0.3 Å. The larger size of the sulphur atom would also increase the steric hindrance for an in-line attack. Although these factors would be encountered with an adjacent mechanism,[56] where the nucleophile is supplied by $Mg^{2+}2$ on the opposite face from the sulphur atom,[68] the combined effect would be expected to be severe if $Mg^{2+}1$ supplied the nucleophile. The actual effect was found to be quite mild (typically 10-20% rate of phosphomonoester hydrolysis).

The ability of the enzyme to be inhibited by compounds that possess the correct functional groups required for in-line displacement *e.g.* *D*-6-O-methylinositol 1-phosphate **44** (Figure 1.25, page 56)[45,69] and 6-deoxyinositol 1-phosphate **16** (Figure 1.7, page 22) also support the adjacent displacement mechanism.[45,69]

1.35.3 Arguments against in-line mechanism

Although there has been much evidence in favour of the in-line displacement mechanism all of the available information proposed can be shown to be ambiguous.

The fact that there is space for a water molecule in the Gd^{3+} model is not uniquely consistent with the in-line displacement at the P-atom as this water molecule could be displaced from the metal ion by one of the phosphate O-atoms during the pseudorotation mechanism. The conclusions drawn from this work should be treated with caution as the X-ray crystal structure that this evidence was based upon was only solved to 2.2-2.3 Å resolution and within the active site there was only one metal ion of the wrong size which possessed a higher than optimum

charge of +3 instead of +2. Finally there is no experimental evidence that can conclusively prove the presence of a $Mg^{2+}1$ bound water molecule.

The mutation studies seem to provide strong evidence for a nucleophilic role of a $Mg^{2+}1$ bound water molecule as site mutation of proposed nucleophile activators e.g. Glu-70 have a drastic effect on the reaction rate. However the observed drop in rate may be caused by a decrease in the binding affinity of the mutant enzyme for $Mg^{2+}1$. Also the kinetic arguments that are proposed to accompany this result may be open to interpretation.[40]

When the 6-OH group of Ins 1-P is removed the resulting compound is a potent competitive inhibitor (K_i=0.003 mmol dm^{-3})[46] and replacement of the 6-OH group by OMe gave an equally potent inhibitor (K_i=0.002 mmol dm^{-3}).[70] These compounds contain all the functionality's required for in-line displacement so should act as substrates, only the adjacent displacement mechanism which proposes that the 6-OH goup of Ins 1-P H-bonds to a water molecule coordinated to $Mg^{2+}2$ can accommodate this observed property.

1.35.4 Prediction of correct mechanism

Computational determination of the reaction pathways for the alternative pathways has shown that differentiation of the two pathways on energetic grounds is inconclusive.[71] Although there is convincing evidence to support the adjacent mechanism, it is not at present possible to completely rule out the in-line displacement mechanism. Work is ongoing to prove the adjacent displacement mechanism through the use of phosphorothioates.[72] The rational being that if the phosphorus could be made chiral then the inorganic phosphate hydrolysis product could be analysed and shown not to be inverted at phosphorus. The in-line

displacement mechanism predicts inversion of stereochemistry at phosphorus on account of its S_N2 type mechanism.

1.36 The 2' AMP – enzyme 3-dimensional interactions

The proposed conformation of 2' AMP **13** within the active site was of very high energy due to strain.[33, 16] As information about the mechanism was gained it became clear that it was possible to propose a more reasonable, lower energy conformation for 2' AMP. A important difference between 2' AMP and *D*-Ins 1-P was that 2' AMP did not appear to possess an equivalent of the essential catalytic 6-OH moiety in *D*-Ins 1-P. This was the reason that the ribofuranosyl 1'O-atom was forced into a high energy position. It has now been proposed that the ribofuranosyl atom may instead bind to $Mg^{2+}2$ through two water molecules (OW1 and OW2). This conformation predicted a energy penalty of only 20.1 kJ mol^{-1} compared to the ground-state conformation of 2'-AMP.[40]

Analysis of the structure reveals that the key H-bonding interactions between the peripheral hydroxy groups of the ribofuranosyl ring and the enzyme are similar to those observed for *D*-Ins 1-P, and that the position of the second water molecule (OW2) is co-incident with the 6-OH of *D*-Ins 1-P. The nucleophilic water molecule (OW1) is properly aligned for an attack on the phosphate group in an adjacent manner (Figure 1.22, page 53).

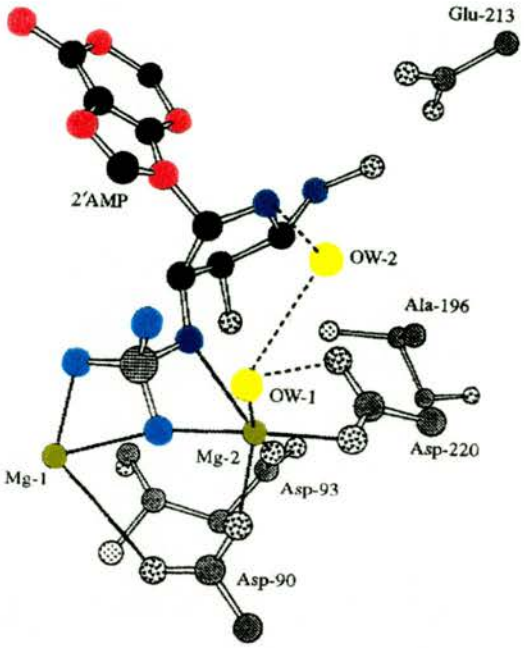


Figure 1.22 - *Optimised active structure for 2' AMP in active site*

1.37 Modelling of the Lithium Inhibited Complex

Kinetic studies have shown that at the therapeutic levels of Li^+ the ion acts as an uncompetitive inhibitor of IMPase by preventing the release of inorganic phosphate from the active site. [9,55]

Modelling has shown that lithium occupies the site usually used by $Mg^{2+}2$ and that the Li^+ ion can interact with the four closest ligands. These ligands are carboxylate O- atoms of Asp-93, Asp-90 and Asp-220 along with one of the phosphate O-atoms of the hydrolysis product which form an approximate tetrahedral geometry in which the phosphate dianion is found slightly closer to the Mg^{2+} 1 ion.

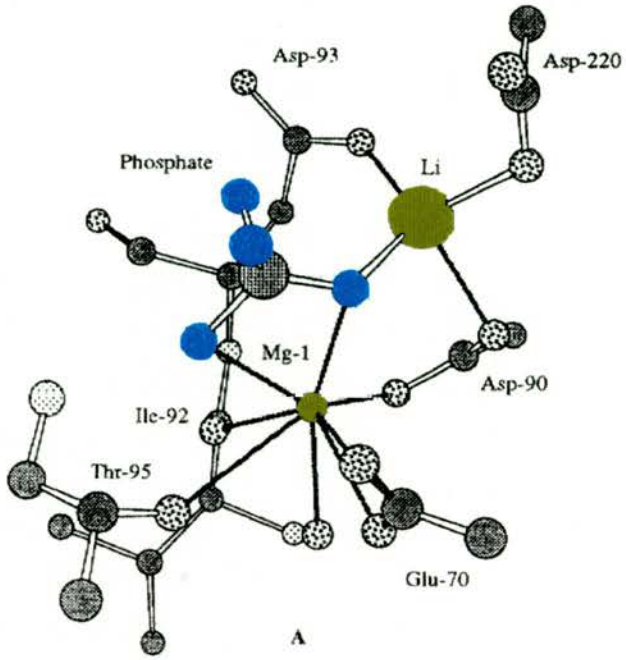


Figure 1.23 – *Optimised active site structure for the lithium-inhibited complex*

1.38 Rationally developed inhibitors

The catalytic mechanism proposed by Cole and Gani [40,53,58,73] involved the adjacent nucleophilic attack on phosphorus by water co-ordinated to $Mg^{2+}2$. Consequently, a series of inositol derivatives based on deoxyinositol substrate **15** were designed to displace the nucleophile to give efficient, tight binding inhibitors of inositol monophosphatase.[70,74,75] The compounds have been prepared via a chiral epoxide route,[70] or from (-)quinic acid **49** which possesses much of the ring stereochemistry already.[75]

Hydroxyethyl analogue **46** exhibited no substrate activity and proved to be a potent competitive inhibitor (K_i = 1.8 μmol dm^{-3} for the racemate, K_i = 0.5 mmol dm^{-3} for the enantiomer shown) implying that the pendant arm chelated $Mg^{2+}2$ rather than hydrogen bonding to a residue across the active site (Ser-165) as partly predicted by molecular modelling studies.

Compounds **47** and **48** were designed to represent the intermediate and product respectively of a hypothetical intramolecular transesterification reaction if the pendant arm 2'-OH group of **46** were to act as the active site nucleophile on $Mg^{2+}2$. The inhibitory properties of both compounds were excellent,[70] and neither compound exhibited substrate activity – again strongly supporting the validity of the proposed enzyme mechanism.

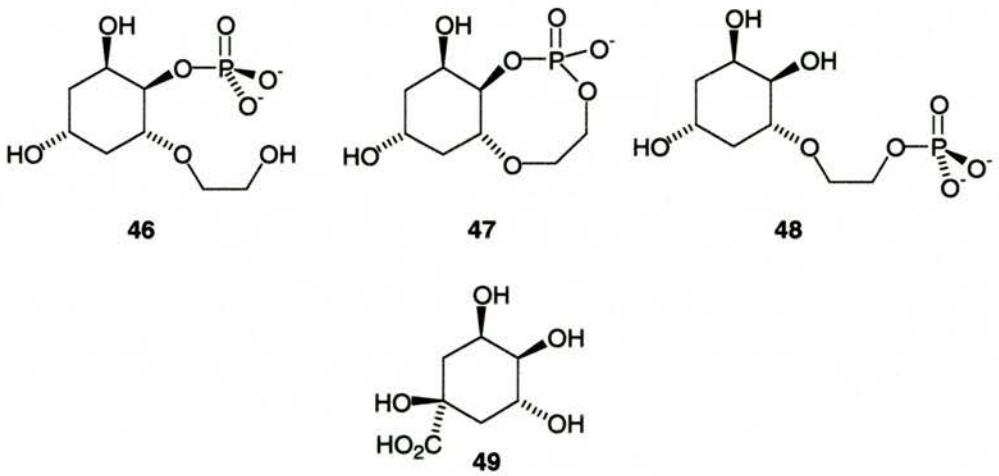


Figure 1.24 – *Inhibitors based on proposed adjacent displacement mechanism, and quinic acid **49**.*

Although there was strong evidence that these hydrophilic side-chains could interact with the co-ordination sphere of $Mg^{2+}2$ excluding water all the evidence had come from compounds containing flexible side chains. In order to fix the positions of the side chains whilst maintaining the key interactions of the peripheral oxygen atoms with the enzyme the constrained bicyclic phosphate diester was prepared **47** (Figure 1.24). This compound differed markedly from the predecessors as it was a monoanion which would affect it's binding performance. When tested it was found that it acted as a moderate competitive inhibitor displaying a K_i value of 160 μmol dm^{-3}. This was the most potent monoanionic

inhibitor known for IMPase [This is important because monoanionic targets may cross the blood-brain barrier easier than dianionic compounds].

Other derivatives were prepared to investigate the internal structure of the active site. The racemic methoxy derivative **44** was prepared to ascertain the importance of the H-atom on the 6-OH. It was found that the compound acted as a competitive inhibitor and showed a K_i value of 2.5 μmol dm^{-3}. The racemic 6-propyl ether analogue **50** proved to be a slightly more potent competitive inhibitor and displayed a K_i value of 1.2 μmol dm^{-3}. This finding indicated that the presence of a lipophilic side-chain appended to 6-C enhanced inhibitory efficiency.

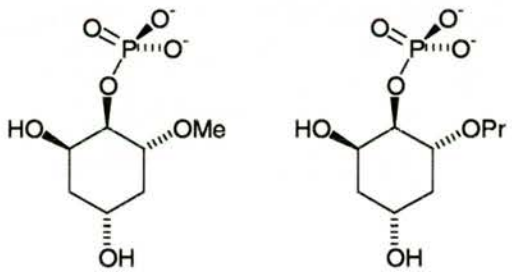
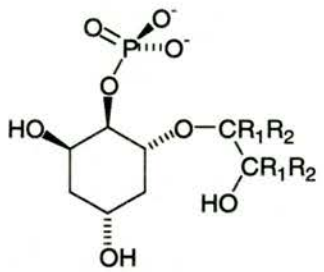


Figure 1.25 – *Two inhibitors based on interfering with the co-ordination sphere of magnesium and seeking out the lipophilic area within the active site.*

The findings of this research indicated that there was a water molecule bound to $Mg^{2+}2$ but do not prove that this water molecule is the nucleophile. The research also did not disprove the theory that the nucleophilic water molecule originates from a position near $Mg^{2+}1$ in an in-line displacement manner. However, it was unclear how the replacement of the 6-OH group by methoxy or alkoxy gave such tight binding, non-hydrolysable compounds.

Kinetic and modelling studies have demonstrated the presence of two different environments within the active site.[40] There is one hydrophobic (lipophilic) area

created by the residues Val-40 and Leu-42, and another hydrophilic area (created by the co-ordination sphere of $Mg^{2+}2$). These two environment can be accessed simultaneously by side chains on target compounds to create tighter binding inhibitors (Figure 1.26).

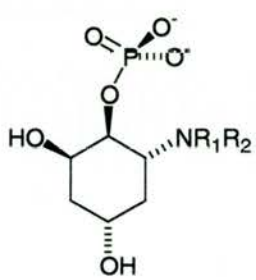


51

Figure 1.26 – *General structure of substituted derivatives able to access discrete environments within the enzyme*

1.39 Nitrogen derivatives as precursors to dual action inhibitors

It has already been proposed that compounds capable of accessing both the lipophillic and the metal co-ordination sphere simultaneously would make potent inhibitors through their tight binding action. The preparation of such compounds however are complex due to the number of chiral centres. To produce compounds capable of accessing both areas of the enzyme it was proposed to use attach specified ligands to 6-C . Naturally it is impossible to tri-substitute an alcohol so it was proposed to substitute the oxygen atom for a nitrogen **52**.



52

Figure 1.27 – *General design of nitrogen derivatives*

A second important reason for the creation of nitrogen targets was to provide additional evidence towards the role of the 6-OH, it's relationship with the nucleophilic water molecule and $Mg^{2+}2$.

1.40 General approach to the creation of nitrogen targets

The creation of this novel class of target would involve several steps.

1. Synthesis of nitrogen nucleophiles
2. Assurance that nitrogen is an acceptable surrogate for oxygen
3. Comparison with methoxy inhibitor
4. Preparation of more complex targets.

1.41 Synthetic Routes to epoxide

The synthesis of all potential targets begins at a common intermediate - the key epoxide. This may then be opened using a suitable nucleophile to give the desired 6' functionality. There are two known routes to the key epoxide from which all inhibitors are derived. It is a difficult and elaborate synthesis because of the stereo- and regio- constraints of the molecule.

The early route to the epoxide started from the commercially available 1,4-cyclohexanediol, however, it included a wasteful resolution step towards the end of the synthesis. The new route to the key epoxide developed in our laboratories start from a natural product (quinic acid) and therefore do not require a resolution step.

1.42 Opening of epoxide

Opening of the epoxide is the key reaction in the synthetic route. There are various well documented procedures that are available and all required scrutiny for research purposes

1.42.1 Azidolysis

A common method for the creation of β-amino alcohols involves reaction with azide salts. The azide then needs to be reduced to provide the necessary amino functionality. This method was thoroughly investigated in our laboratories and not pursued further as the disadvantages outweighed possible benefit.

1.42.2 Phosphoramidites

An alternative method to produce the desired target compounds using phosphoramidites to simultaneously introduce the amine and phosphorolate was attempted. The advantage of using this approach was that it would reduce the number of steps required and therefore lead to increased overall yields.

1.42.3 Alcoholysis and aminolysis

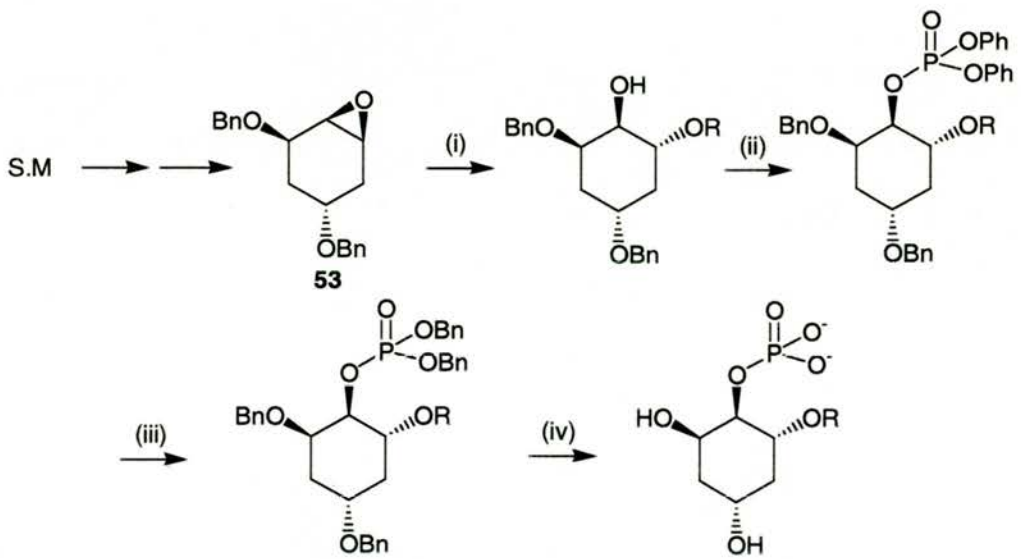
The simplest way to introduce an amine/alcohol to an epoxide is simply to react the epoxide with an excess of nucleophile. This has been shown to be a highly successful method with unhindered nucleophiles and epoxides however, the reaction begins to fail as the reactants become more complex.

This route would allow easy addition of various amines/alcohols if the difficulties associated with the reaction could be overcome

Results and Discussion

2.1 Work Targets

In order to find new and potent cyclic and acyclic inhibitors for IMPase it was necessary to formulate an efficient synthetic pathway to the desired compounds. To do this a reliable and quick synthetic route to nitrogen substituted derivatives from a key advanced precursor was required before determining if nitrogen analogues of IMPase substrates were acceptable surrogates within the active site. Once satisfied that the nitrogen analogues were acceptable a variety of amine derivatives could be designed to probe the active site.



Reagents and Conditions: (i) Lewis acid, alcohol; (ii) ^{t}BuSiPh$_2$Cl, TEA, DCM, DMAP, r.t, 12 h; (iii) NaH, BnOH, THF, -70°C, 3 h; (iv) Na/NH$_3$(liq), -70 °C, 1 h.

Scheme 2.1 *General reaction sequence for the preparation of alcohol derivatives.*

Ongoing work within our laboratories on alcohol derivatives provided clues towards the development of a good synthetic route. The established route at the time required the lengthy synthesis of a key epoxide intermediate **53** prepared using modifications of literature procedures.[38,28,76,139] The epoxide **53** was then reacted with an alcoholic nucleophile to give substrate/inhibitor precursors. After

opening of the epoxide most routes proceed to phosphorylate the 1-OH using one of two methods (section 3.1.1, page 110) followed by a global deprotection to give the target molecule (Scheme 2.1, Page 60).

In modifying this work to produce nitrogen derivatives two problems with the existing design were acknowledged.

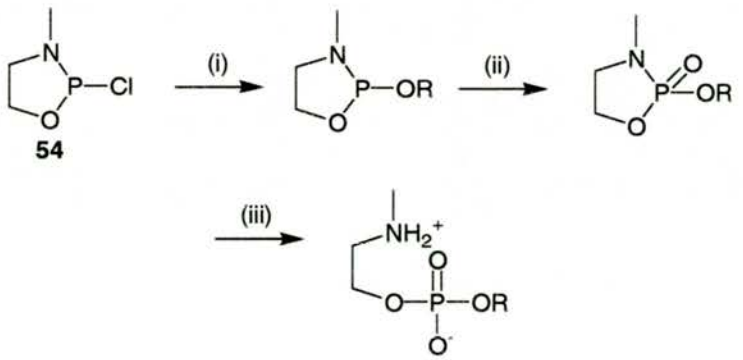
- Firstly, the established route was lengthy and the epoxide **53** was produced using racemic starting material which meant a wasteful resolution step late in the synthesis. As a consequence this route was low yielding. (It should be noted that in parallel with this work, investigations into a more efficient synthetic route to the key epoxide were taking place.)

- Secondly, the key epoxide cleavage reaction with the alcohol was found to be most efficient using a boron trifluoride.etherate catalyst – this method would be unlikely to be successful with nitrogen nucleophiles as the boron reagent and the nitrogen atoms could associate removing the nucleophilicity of the nitrogen lone pair. Also aminolysis of epoxides with hindered reactants is generally accepted to be a difficult and low yielding reaction.

In light of the complexities inherent with the established route to the epoxide new methods of creating target cyclic and acyclic nitrogen derivatives were investigated. A promising lead came from phosphoramidite chemistry.

2.2 Possible synthesis using phosphoramidite methodology

The production of novel phospholipids using phosphoramidite methodology had been investigated by McGuigan *et al.*[77,79,80] They reported a rapid three step synthesis of phospholipids from 2-chloro-3-methyl-1,3,2-oxaphosphacyclo pentane **54**. This was reacted with an alcohol to provide a cyclic phosphoramidite intermediate. The product was then oxidised with dinitrogen tetraoxide and the resulting product was reacted with water to produce the target phospholipid (Scheme 2.2).



Reagents and Conditions: (i) ROH, TEA, DCM; (ii) DCM, N_2O_4; (iii) H_2O.

Scheme 2.2 *General scheme using phosphoramidites to produce phospholipids*

Phosphoramidites have a bond from phosphorus to both oxygen and nitrogen. However the N-P bond is very weak and is easily cleaved. The bond from phosphorus to oxygen is very strong due to backbonding through *p*-orbitals. This makes it very unlikely that a P-O bond will be broken through water treatment with the end result that the P-N bond is replaced by a P-OH bond. This method could be readily adapted to prepare the desired target compound (Figure 2.1).

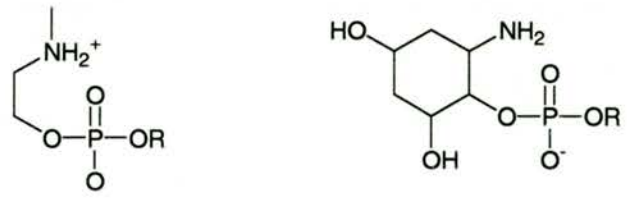
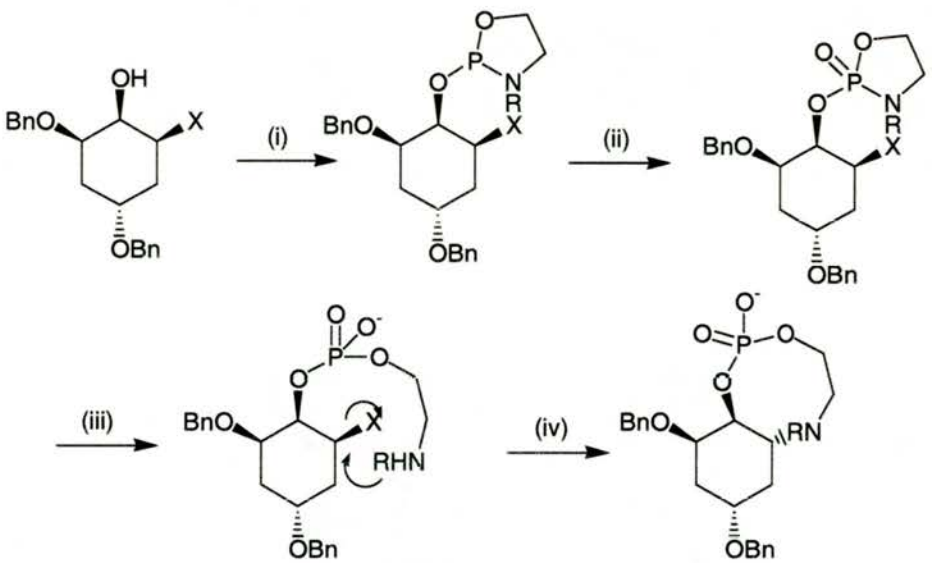


Figure 2.1 *Comparison of similarities between phospholipid and target compound*

Scheme 2.3 shows the proposed route to the amino phosphate target material. The first step involved the attack of an alcohol onto the phosphitylating agent. It was assumed that the nitrogen substituent on the phosphitylating agent could be easily varied during preparation.



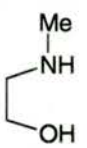
Reagents and Conditions: (i) TEA, DCM, 2-chloro-3-methyl-1,3,2-oxaphosphacyclopentane; (ii) DCM, N_2O_4; (iii) & (iv) H_2O.

Scheme 2.3 *Hypothetical synthetic scheme to bicyclic amine targets showing stereochemistry.*

It was proposed that if the alcohol was the 1-OH of a suitable inositol derivative it should be possible to produce a bicyclic precursor of the nitrogen targets using the phospholipid method detailed above. This precursor could then form the target molecule through ring closure.

It was not known whether or not the nitrogen ligand would/could perform a ring closure. Seven membered ring closure reactions are not unknown ,[78] however it seemed likely that the reaction could be encouraged through the use of a reactive leaving group. This method if successful would enable the synthesis of a multitude of varied targets in three steps from a simple epoxide derivative **53**.

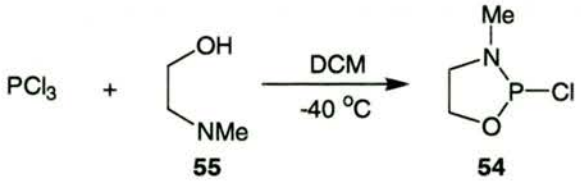
Before reacting the epoxide **53** with the phosphitylating agent it would be necessary to first transform the epoxide for the substitution reaction. This would involve opening the key epoxide to give a trans diol followed by a specific substitution by a halogen to give the undesired stereochemistry at the 6-position. When ring closure elimination (S_N2) occurred it was expected that the desired stereochemistry would be returned through inversion.



Structure 55 *N-methylethanolamine*

The route necessary to prepare the key epoxide **53** had been previously published (Section 2.10, page 91),[74] so the research was first concentrated on the novel phosphoramidite scheme.

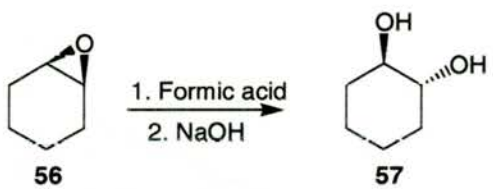
The synthetic scheme (Scheme 2.4) used a cyclic phosphoramidite **54** which was synthesised following literature methods in high yield from phosphorus trichloride and *N*-methyl ethanolamine **55**.[79,80,81]



Scheme 2.4 *Synthesis of cyclic phosphoramidite.*

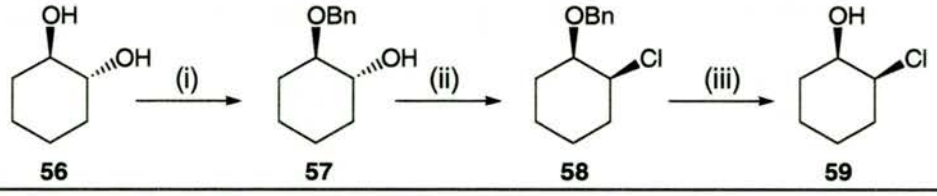
The structure of **54** was fully supported by ^{31}P NMR spectroscopy and other spectroscopic evidence. The ^{13}C spectrum showed three signals at 30.85, 48.67 and 70.69 ppm, two bond coupling to phosphorus were clearly evident. The OCH_2 resonance was shifted 10 ppm downfield relative to the spectrum of the starting *N*-methylethanolamine **55**, whereas the other two resonances (Methyl and CH_2N) were shifted around 5ppm upfield.

It was important to first evaluate the likelihood of ring closure occurring on a simple model before potentially wasting time preparing the target molecule. The model chosen was cyclohexene oxide (±)-**56** because it possessed a cyclohexane ring with an epoxide ring.



Scheme 2.5 *Opening epoxide with formic acid*

The epoxide (±)-**56** was cleaved by a literature method,[82] involving treatment with formic acid followed by an aqueous solution of sodium hydroxide to produce the desired trans diol (±)-**57** in a moderate yield of 73%. Another method involved the treatment of cyclohexene with peroxide and formic acid followed by sodium hydroxide although this gave a poor yield of only 23% and was rejected.



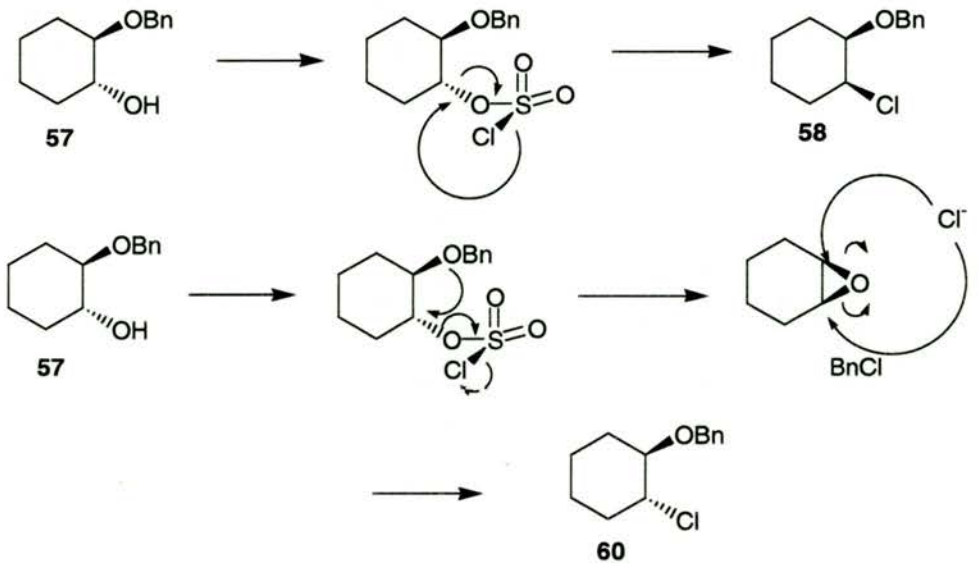
Reagents and Conditions: (i) BnBr, NaH, DMF, 0 °C, 12 h; (ii) DCM, Py, Sulfuryl chloride, -70 °C, 2 h; (iii) DCM, BF_3OEt_2, DMS, -40 °C, 12 h.

Scheme 2.6 - *Preparation of chlorohydrin model*

The diol (±)-**56** was then subjected to a selective protection of one of the alcohol groups on the molecule. The trans diol has C_2 symmetry so the protection was achieved with one molar equivalent of benzyl bromide and NaH. The resultant mixture of di and mono substituted diol was easily separated by silica column chromatography to give a 42% yield of the monobenzylated product (±)-**57**.

It was now possible to substitute the free alcohol functionality with a suitable leaving group. It was decided that chloride would be used for this purpose although it was acknowledged that chloride is not a particularly good leaving group it was worthwhile trying it in a test case. The chloride substitution on the alcohol (±)-**57** was carried out using a general method utilising sulfuryl chloride.[83] This gave mainly inversion of stereochemistry at OH, producing a mixture of cis-trans chlorohydrin in 82% yield. It was discovered that the reaction produced a 2.5:1 mixture of cis (±)-**58** and trans (±)-**60** products respectively. The stereo – mixing was thought to be caused by an inter-molecular reaction (Scheme 2.7). To combat this problem high dilution techniques were employed which improved the cis/trans ratios to 6:1 respectively.

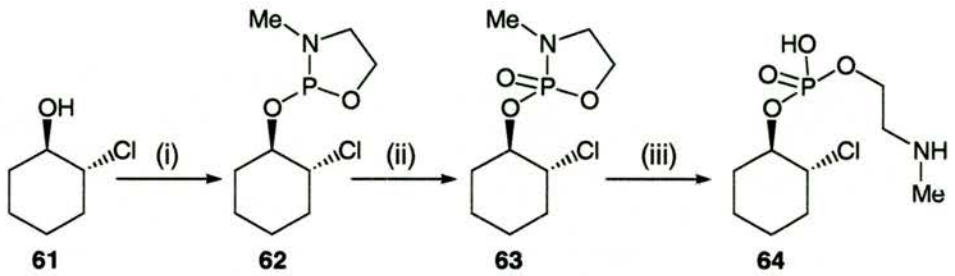
An attempt to avoid the benzylation step by carrying out the chloride substitution directly on the diol with the premise that the products could be separated failed as it resulted in an inseparable mixture of products.



Scheme 2.7 *Proposed mechanisms for chlorination.*

It was then necessary to remove the temporary benzyl protection from the remaining alcohol on **57** in preparation for reaction with the phosphitylating reagent. This proved to be problematic as palladium on carbon hydrogenation was found to cause loss of the chloride. The best method was found to be the use of $BF_3.Et_2O$ and dimethyl sulphide as a mild and selective deprotecting agent to give a mixture which was purified by distillation at 62°C at 20mm Hg to give a clear liquid.[84] ^{13}C NMR spectroscopy showed a 50:50 mixture of signals only slightly offset from one another. Experiments which raised the temperature of the sample during ^{13}C NMR spectroscopic analysis showed no coalescence of the signals proving that this was not a temperature effect. The conclusion was that there was a mixture of the cis (±)-**59** and trans (±)-**61** products present. Comparisons with the commercial sources indicated that the two isomers boil at the same temperature and so were not separable by distillation. Separation was found to be impossible by ordinary purification methods so it was proposed that the *trans* isomer (±)-**61** could be converted to the epoxide through treatment with sodium

hydroxide. It was not expected that the cis isomer (±)-**59** would ring close.[85] The reaction was a success returning a clear liquid at 76-82°C (13 torr) found by ^{13}C NMR spectroscopic analysis to be pure *cis*-2-chloro-cyclohexanol (±)-**59**.



Reagents and Conditions: (i) Phosphoramidite, DCM, TEA; (ii) N_2O_4, DCM, -70 °C; (iii) H_2O

Scheme 2.8: *Ring opening of the phosphoramidite*

To speed up the research it was decided to utilise commercial sources of the chlorohydrin (±)-**59** while the chemistry of the ensuing phosphoramidite steps were explored. Unfortunately the commercial supply of chlorohydrin was of undesired *trans* isomer (±)-**61**, however it was decided for the purposes of this research that this would not be an issue.

The phosphoramidite (±)-**54** was reacted with the commercially available chlorohydrin (±)-**61** in dichloromethane to yield the phosphate triester (±)-**62** in 97% yield. The product was characterised by ^{31}P and ^{13}C NMR spectroscopy. The ^{31}P NMR spectrum showed a shift from + 167 ppm in **54** to +138 ppm in **62** which was entirely consistent with previous reports [e.g. δ_P 143 ppm for $PNMe_2(OEt)_2$].[86] The ^{1}H NMR spectrum of the product (±)-**62** showed that the *N*-methyl signal was almost unchanged relative to (±)-**61,** however the magnitude of the phosphorus coupling was reduced (from 15 to 13 Hz). The ^{13}C NMR spectrum was of particular interest, all carbons within 3 bonds of phosphorus were coupled to it and appeared as doublets. The magnitude of the coupling constant varied

between carbons – couplings appeared larger through oxygen (10-13 Hz) than nitrogen (4-6 Hz).

Oxidation of the phosphate triester (±)-**62** was achieved using dinitrogen tetraoxide which has been used previously for phosphite oxidation.[81,87] It had been proposed that an excess of reagent would be deleterious to the integrity of the (labile) phosphate heterocycle, and thus a standard solution of the oxidant was used.[79] By assuming 1:4 phosphite:oxidant stoichiometry,[89] it was possible to use only the required amount of oxidant. The reaction proceeded cleanly at low temperature in dichloromethane to gave the phosphoramidate (±)-**63** in quantitative yield. The oxidation was, as anticipated, accompanied by a marked shift to lower frequency of the resonance in the ^{31}P NMR spectrum. Compound (±)-**63** displayed a resonance at 21 ppm close to that recorded with analogous phosphates e.g. δ_P 11 ppm for $[OPNMe_2(OEt)_2]$.[88] The most marked change in the ^{13}C NMR spectrum on proceeding from phosphite to phosphate was the change in the P-N$\underline{C}H_3$ coupling constant from 13 Hz to 2 Hz. This coupling constant appears to be highly susceptible to the oxidation state of the phosphorus atom.

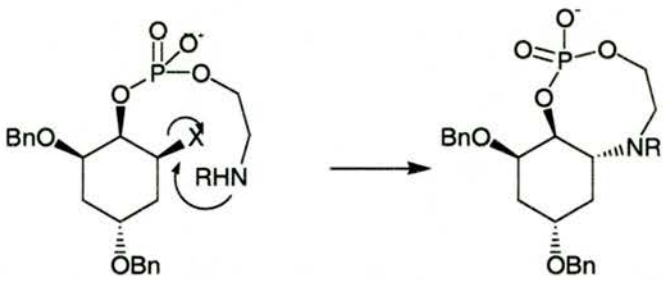
The advantage to this route lay in the hydrolysis of the P-N group. The phosphoramidite bond was known to be acid labile and had previously been reported to be cleaved using rather forcing conditions of THF/2M HCl,[77] or acetic acid.[89] McGuigan and co-workers investigated the kinetics of the hydrolysis reaction using ^{31}P NMR spectroscopy to follow the course of the reaction since it was expected that P-N cleavage would be accompanied by a major shift to lower frequency.[90] During the course of their studies they noticed that a relatively weak (5% molar %) solution of HCl in THF-D_2O succeeded in cleaving the P-N bond in various compounds in minutes.[80] Having noted this they decided to investigate the

least acidic conditions capable of bringing about hydrolysis. This was important because the neutralisation step necessary after the acidic cleavage had been shown to hydrolyse the phosphate. They discovered that the hydrolysis reaction could proceed in the absence of acid and although the precise mechanism is unclear they proposed that it may have been catalysed by traces of acid left from the previous step.

Following this literature method compound (±)-**63** was stirred in H_2O overnight followed by lyophilisation to give the ring opened amine (±)-**64** in 99% yield.[80] This was then characterised. The ^{13}C NMR spectrum was particularly informative as the *N*-methyl resonance was shifted to higher frequency on P-N cleavage from 32.05 ppm to 33.69 ppm (as expected), and it also appeared as a singlet having lost its coupling ($J_{C\text{-}P}$ 21 Hz) to the phosphorus atom. The resonances of the two carbon atoms nearest to the phosphorus displayed a shift to lower frequency and while the 2-bond P-C couplings were greatly reduced the 3-bond couplings increased. The ^{1}H NMR spectrum displayed additional evidence that the reaction had been successful with the *N*-methyl signal appearing as a singlet having lost the phosphorus coupling. This was further confirmed by the resonance in the ^{31}P NMR spectrum which displayed a single peak close to 0 ppm. This is the expected shift for a phosphate diester [c.f. δ_P 4 ppm for sodium diethyl phosphate].[90]

It had been hoped, although not expected, that the amine would spontaneously eliminate the chloride to effect ring closure. This would be accompanied by a marked shift in the C-6 resonance. Monitoring using ^{13}C & ^{1}H NMR spectroscopy showed no evidence of ring closure over 12 h. Longer time periods also demonstrated that ring closure would not occur without encouragement.

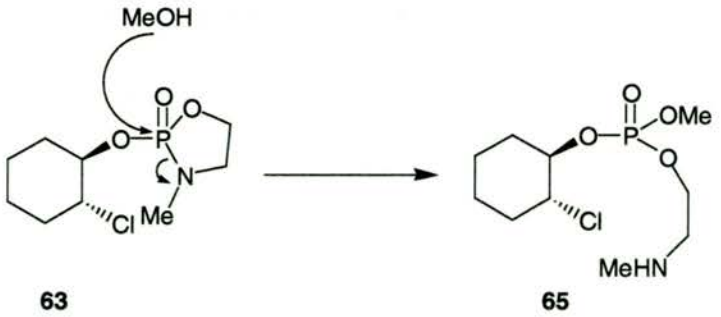
To encourage ring closure various attempts were made using lithium naphthalene,[91] or, on the premise that the addition of a base would catalyse the reaction, triethylamine, LDA, and a potassium carbonate, water and methanol mixture. Disappointingly however, all attempts returned only starting material as identified by ^{1}H NMR spectroscopy.



Scheme 2.9 *Proposed ring closure mechanism*

It was proposed that the presence of the phosphate group was having a deleterious effect on the positioning of the amine arm preventing it, through electrostatic effects, from achieving optimal alignment for elimination. To prevent the phosphate from interfering with the reaction attempts were made to protect it as an ester. Reacting the ring opened phosphoramidite with diazomethane was unsuccessful returning only starting material identified by ^{1}H NMR spectroscopy.

It was decided to attempt the phosphate protection step at an earlier stage of the synthesis. The oxidised (but not ring opened) phosphoramidite was refluxed in methanol for 2 days returned the ring opened, esterified phosphoramidite analogue (±)-**65** in quantitative yield (Figure 2.2) identified through ^{31}P NMR and ^{13}C NMR spectroscopy.



63 65

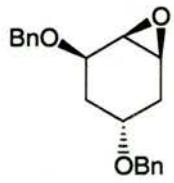
Figure 2.2 *Esterification of phosphate using methanol*

The methyl signals of the methoxy groups on the phosphorus of the product were particularly noticeable in the ^{13}C NMR spectrum, forming a pair of doublets with identical coupling constants thus confirming the success of the reaction. However, repeating the ring closure experiments with base catalysis once again showed no sign of ring closure having occurred.

The lack of ring closure was disappointing although not entirely unexpected due to the entropy of the relatively long pendant arm combined with the use of chloride as a poor leaving group. This model work demonstrated a reliable and high yielding route to pre ring-closed bicyclic target molecules and a novel way of introducing the phosphate group. The route however remains largely useless as long as ring closure cannot be effected reliably. Although it was acknowledged that the use of leaving groups with higher reactivity e.g. bromide or tosyl would probably have been successful in ring closure it was decided to abandon this route while more traditional routes based around epoxide cleavage were investigated.

Previous investigations,[98] into the preparation of novel amino targets had demonstrated a technique of opening an epoxide centred around the 1-C and 6-C positions with azide which could subsequently be reduced to the corresponding amine. Before exploring this area of research it was necessary to first synthesise a stock of the key epoxide intermediate **53**.

2.3 Racemic route from cyclohexane-1,4-diol



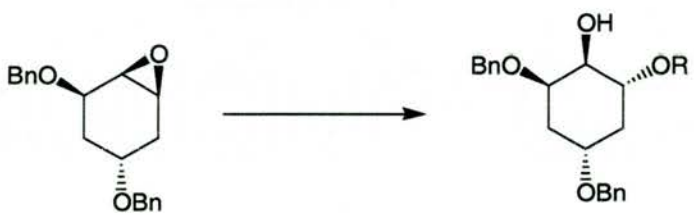
53

Scheme 2.10 *Preparation of the key epoxide from 1,4-cyclohexanediol*

The existing route to the key epoxide (±)-**53** was accomplished in ten steps starting from a commercially available cis/trans mixture of 1,4-cyclohexanediol **85**. Although a new stereospecific route from (-) quinic acid was at the time under development it had not been completed. The required stereogenic centres at 1-C, 2-C, 4-C and 6-C were then constructed in a sequence of seven steps following a route first communicated by Baker *et al.*[38] The product was a racemic mixture of 2,4-bis(benzyloxy)-1,6-epoxycyclohexane **53**.

2.4 Alcohol based cleavage of the epoxide

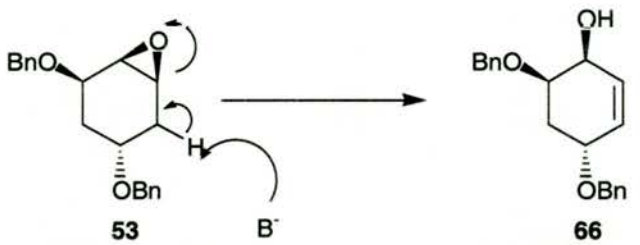
Before planning methods of nitrogen based epoxide cleavage it was important to review the accepted methods of alcohol cleavages. It had been envisaged that the reaction of epoxide **53** with a range of suitably derivatised oxygen nucleophiles would facilitate the introduction of required functionality at the 6-C position.



53

Figure 2.3 *Cleavage of epoxides with alcohol nucleophiles*

Attempts to open the oxirane ring with 2-benzyloxyethanol in the presence of alumina failed.[92] This reaction had been shown previously to have been successful for the addition of simpler alcohols to epoxide **53**, it was assumed that the 1,2 arrangement of the Lewis bases in the nucleophile had prevented the reaction from occuring.

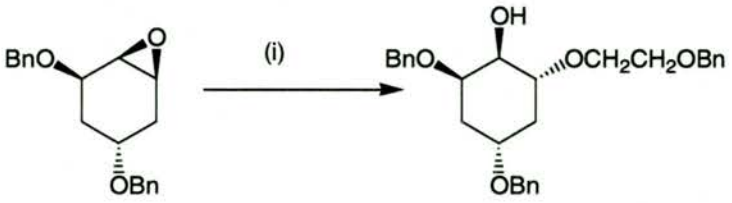


53 **66**

Figure 2.4 *Elimination of epoxide by TMEDA*

Generation of 2-benzyloxy-ethoxide using NaH and subsequent reaction with the epoxide in the presence of N,N,N',N'-tetramethylethylenediamine (TMEDA) at 100 °C,[93] led to cleavage of the oxirane ring on **53** to give the allyl alcohol **66** but none of the required ether **67**. It was proposed that weakly acidic conditions may help to polarise the epoxide ring e.g. with camphorsulfonic acid, but this gave no reaction at all.[94]

The first successful cleavage occurred in the presence of a catalytic amount of a strong Lewis acid, $BF_3.Et_2O$, and the nucleophile 2-benzyloxyethanol. The oxirane ring was shown to open smoothly to give the required racemic 2-benzyloxyethyl ether **67**.[95]



53 **67**

Reagents and Conditions: (i) $BF_3.OEt_2$, 2-benzyloxyethanol, toluene.

Scheme 2.11 *Successful alcohol cleavage of epoxide with borontrifluoroetheratecatalyst*

It was noted that the reaction could be carried out successfully either in the absence of solvent or in the presence of a small amount of dry toluene and that the ^{13}C & ^{1}H NMR spectra for the 2-benzyloxyethyl ether **67** were in keeping with those expected for the required regio- and stereo- specificity for the ring opening reaction. To confirm the correct assignment of the structure the X-ray crystal structure of the camphanate ester derivative was solved by Gani and co-workers.[70] This work confirmed that nucleophilic attack on the oxirane ring by an alcohol in the presence of the appropriate catalyst resulted in the desired ring opening reaction with the correct regio- and stereo- chemistry. There appeared to be no reason why a similar reaction could not occur for nitrogen nucleophile analogues if a suitable catalyst could be found.

2.5 Considerations

To proceed from the epoxide to a target molecule requires opening the key epoxide intermediate **53** both stereospecifically and regioselectively with a prepared nucleophile. Epoxides cannot easily be cleaved by naked nucleophiles but the epoxide can be activated using a lone pair acceptor (Lewis acid) which enhances the electrophilicity of the oxirane ring. In the synthesis of the alkoxy derivatives from the epoxide, it had been shown that alkali metal alkoxides were too basic and caused C-5 deprotonation and oxirane ring-opening to give the allylic alcohol.[74]

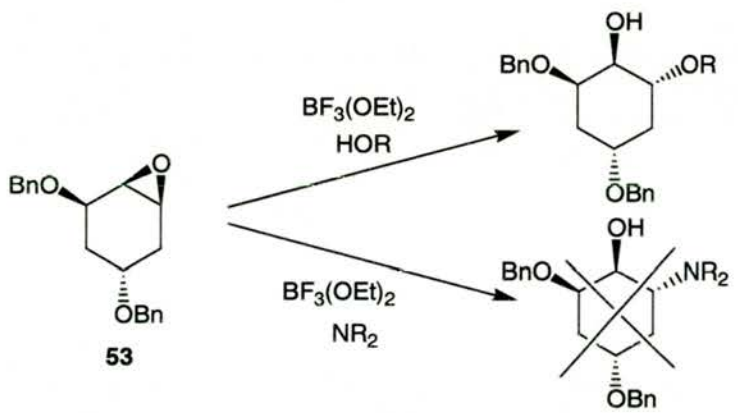
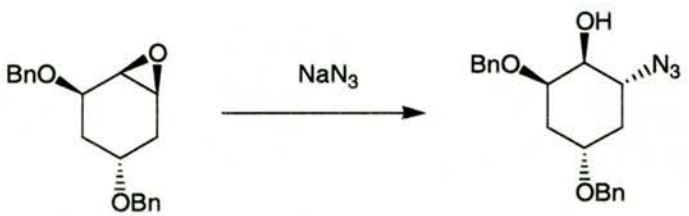


Figure 2.5 *Epoxide cleavage using boron trofluoroetherate with alcohols and amines*

However, under acidic conditions, in the presence of catalytic quantities of boron trifluoride, good yields of the required 6-ethers were obtained.[74] Using the same protocol for aminolysis failed to give the desired product as expected, therefore it was necessary to search for a milder method of ring cleavage before continuing with the synthetic pathway. Previous work indicated that azides could be used to produce the target compounds.[98]

2.6 Review of early work on opening epoxide with azide

The standard method of opening the epoxide with azide is to react the epoxide with NaN_3 and NH_4Cl in the presence of methanol.[96,97] This step was carried out on **53** with 10 eq of sodium azide and required a prolonged reaction time (3-4 days). The resulting yield of the opened epoxide **68** was a disappointing 22% although the starting material was recoverable.



53 **68**

Figure 2.6 *Opening of the key epoxide **53** with azide*

Work done previously indicated that literature methods of opening the epoxide **53** with azide had been investigated.[98] The use of alumina[92] or camphorsulfonic acid,[94] in the presence of NaN_3 did not yield the desired product. Treatment with a catalytic amount of $BF_3.OEt_2$ the method used to open epoxides with alcohols, also was shown to be ineffective.

A procedure described by Choukroun and Gervais involved the use of bis azido complexes of titanium to deliver the azide to the molecule.[99]

This titanium complex was prepared from a tetraisopropyloxy derivative, using $SiMe_3(N_3)$ as the azide source. Treating $Ti(OPr^i)_4$ with two equivalents of $SiMe_3(N_3)$ led to the corresponding azido complex $(Ti(OPri)_2(N_3)_2)$ **69** within 5 days.

Figure 2.7 *Synthesis of bis-azido titanium complex*

Using this bis-azido complex of Ti in dry toluene was found to be faster and more efficient than with NaN_3. The reaction was carried out at 75-80 °C for 7 hours using the key epoxide **53** giving the desired product in 89% yield. The reaction was shown to occur with high regio- and stereoselectivity and the formation of the desired 6-azido alcohol **68** was unequivocally assigned through the use of NMR spectroscopy COSY and CH correlation techniques.

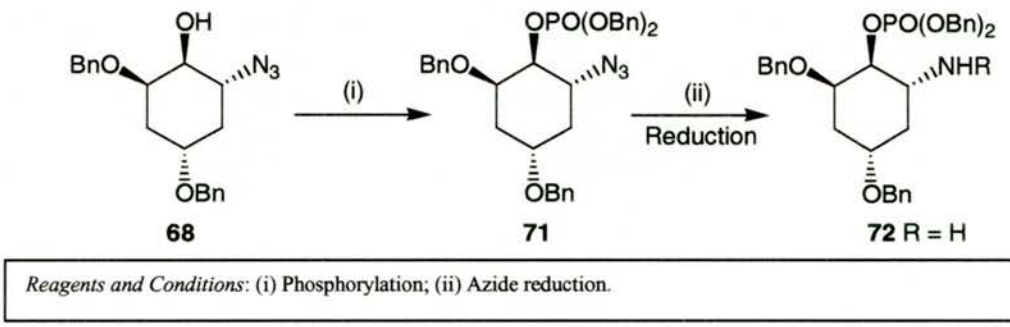


Figure 2.8 *General scheme showing phosphorylation and reduction*

Before reducing to the amine the 1-C alcohol was phosphorylated. There were two commonly used methods for this explained in more detail later (see section 3.1.1, page 110). Phosphorylation to give compound **71** was successful in 62% yield. The molecule was then prepared to undergo azide reduction.

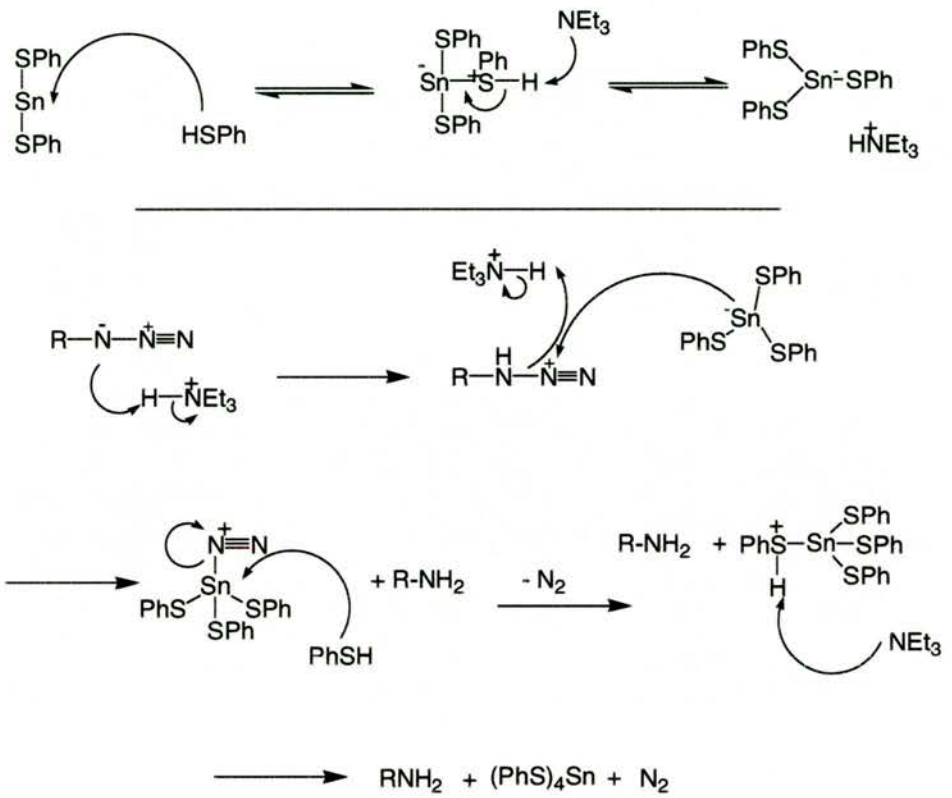
Previous research,[98] had planned - in order to prepare derivatives for testing as inhibitors - to selectively reduce the azide to the primary amine which would then be reacted with various electrophiles to produce functionalised amines. The available literature indicated that the common reagents employed for reducing azides into primary amines include $LiAlH_4$,[100] $NaBH_4$[101,102] or catalytic hydrogenation over Pd/C, nickel or platinum oxide.[103] The most common method

of hydrogenation could not be used because of the sensitivity of the benzyl ether protecting groups to this reagent and so it was necessary to find a mild method of reduction. The use of stannous chloride was found to be well established in organic synthesis for the reduction of nitro groups and Micetich *et al.* have reported research into the use of this reagent in azide reduction.[104] The initial results appeared promising with azido-cylohexane being reduced in 15 min to give the primary amine in 90% yield. The rate of reduction was found to vary considerably with the electronic environment of the azido group and the method was ruled out altogether for derivatives of epoxide **53**. An attempt was also made using the Staudinger reaction carried out by adding triphenyl phosphine and water to the azide, this unfortunately gave only a low yield (11%) of the desired product **72**.[105]

Good yields were reported by using tin (II) complexes.[106,107] The tin complexes were formed by the treatment of Sn^{2+} ions from $SnCl_2$ with 3 eq. of PhSH and Et_3N giving a yellow solution in toluene which reacted almost immediately with the azide **71** to give the primary amine **72** identified through FTIR data where no evidence of the triple bond could be identified (Scheme 2.12, page 80). The success of the reaction using Sn(II)Et_3N/PhSH was attributed to the formation of the $Sn(SPh)_3^-$ complex.

In order to promote the formation of this species it was found that reaction rates were enhanced by reacting $SnCl_2$, dry Et_3N and freshly distilled PhSH for approximately one hour before the addition of the azide in a THF solution. Moisture was found to have a major deleterious effect on the reaction so as a consequence the reactions were carried out in a Dean-Stark apparatus.

This work appeared to provide a reliable method of opening the epoxide with nitrogen and a good scheme for the production of the completed primary amine derivative.



Scheme 2.12 *Possible mechanism of azide reduction using tin*

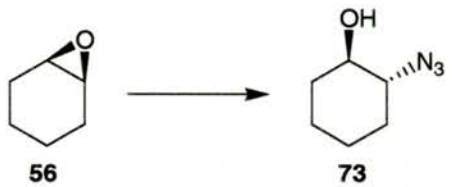
There were some problems with this work that required modifications to make the scheme more relevant to this project.

- No evidence for the production of a variety of amine derivatives were proposed although there are several literature methods for converting primary amines to secondary amines which needed investigated.[108] Other methods would include the addition of functionalised azides or conversion of the azide directly to a secondary amine.
- No successful method of taking the reduced azide derivative through to an inositol monophosphate derivative had been proposed

2.7 Completion of azidolysis investigations

2.7.1 Methods of azide reductions

Before embarking on any attempted synthesis with the valuable *key epoxide* **53** it was decided to carry out reactions with the model cyclohexene oxide (±)-**56**. This was designed to preserve the valuable epoxide while viable routes were being evaluated.



56 **73**

Scheme 2.13 *Preparation of azide from cyclohexene oxide*

Using the established method cyclohexene oxide (±)-**56** was stirred for 12 hours at 60 °C with the azide complex to give cyclohexan-2-azido-ol (±)-**73** (49%) as a clear oil after silica column chromatography. This was identified through the use of NMR spectroscopy and FTIR which clearly showed the azide triple bond at 2100 cm^{-1}.

A modification of the reduction step was required in order to build in flexibility of amino derivatives. Literature research into azide reduction uncovered a promising method by Brown *et al.* using alkyldichloroboranes which would directly reduce the azide into a secondary amines.[109] It was reported that alkyldichloroboranes[110,111] $RBCl_2$ as well as aryl-dichloroboranes reacted readily with organic azides in benzene/toluene solution, producing an intermediate $RR'NBCl_2$, readily converted by alkaline hydrolysis to the corresponding secondary amines RR'NH in yields of 84-100%. The reaction was shown to possess wide generality and proceeded with retention of stereochemistry of the alkyl group in the alkyldichloroborane.

Following a modification of the literature method,[112] the model azide (±)-**73** was added dropwise to commercially available phenyldichloroborane in toluene at room temperature. The reaction mixture was then heated to 80 °C before cooling and workup.

On analysis of the product there was found only starting material present. The failure of the reaction was attributed to the solvent which was not benzene as stipulated in the literature. Changing the solvent to 1,2-dichloroethane[109] was found to provide the corresponding secondary amine (±)-**74** in 94% yield. By collecting the nitrogen released in a water filled measuring cylinder the reaction progress could be followed closely by the volume of water displaced. The product displayed the expected analytical characteristics with the characteristic triple bond stretch in FTIR disappearing and the C-6 carbon moving downfield (7 ppm) in the ^{13}C NMR spectrum.

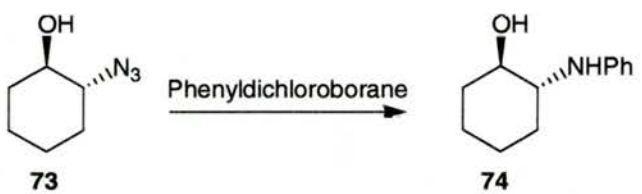


Figure 2.9 *Reducing azide with phenyldichloroborane*

Phenyldichloroborane was chosen to test the reaction because it was commercially available, the route although successful in this case would not have been very practical without an easily flexible synthesis of alkyldichloroboranes available. In the preparation of inhibitors, a flexible synthesis of a wide range of alkyl dichloroboranes would be vitally important.

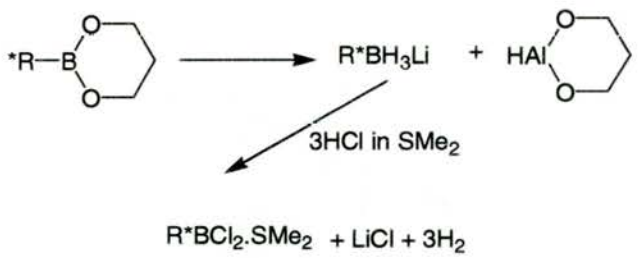
2.7.2 Methods of alkyldichloroborane synthesis

Further literature research indicated that a variety of methods for producing organodichloroboranes were available. A promising route was found to proceed via boronic esters as a starting material.

Figure 2.10 *Preparation of organyldichloroboranes using boron trichloride*

The first method of this type reported in 1956 by Lappert *et. al.* involved the interaction of the neat boronic esters, $RB(OR')_2$, with gaseous boron trichloride at –78 °C in the presence of a catalytic amount of ferric chloride to give the corresponding organodichloroboranes, $RBCl_2$, in good yields.

A second method involved treatment of boronic esters with $LiAlH_4$ to give alkylboronhydrides. These, upon treatment with 3 equiv of HCl in dimethyl sulfide yield the organodichloroborane –dimethyl sulfide complexes in excellent yield.



Scheme 2.14 *Preparation of organodichloroboranes using LiAlH*

This two step procedure involved the separation of the dialkoxyalane, which apparently in some cases did not separate cleanly, especially in the case of acyclic boronic esters.

The final method involved an improved procedure for the conversion of chiral alkyldichloroboranes in very high enantiomeric purities.[91] The applicability of the

reaction was demonstrated by the preparation of (E) and (Z)–1, alkenyldichloroborane and phenyldichloroborane as a representative aryl derivative, from the respective boronic esters.

2.7.3 Preparation of chiral organodichloroboranes using boronic esters

The accepted procedure for the preparation of organodichloroboranes involves the interaction of neat boronic esters with 2 eq. of gaseous boron trichloride in the presence of a catalytic amount of ferric chloride at low temperature (–78 °C). Using this method Brown and co-workers studied various cyclic and acyclic ester derivatives of boronic acids, such as dimethyl, diethyl and ethylene glycol in order to find the most suitable ester derivative under previously optimised reaction conditions.[91] The dimethyl and diethyl derivatives of the boronic acid were found to give the best results. Hence, the diethyl ester derivatives of the boronic acids readily prepared from the corresponding boronic acids and absolute alcohol were adopted as the best method for preparing the organodichloroboranes.

The general production of alkyl dichloroboranes began with a boronic acid which was made from the asymmetric hydroboration of an alkene. The acid was then reacted with ethanol to give the corresponding alkyl borate ester. This borate ester was then reacted with $LiAlH_4$ to give the corresponding lithium monoalkylborohydride **75** stereospecifically. The reason for this was that the lithium monoalkylborohydrides were more highly reactive than the alkyl boronic esters.[113] This was then treated with two equivalents of a 1 N solution of boron trichloride in dichloromethane, in the presence of a catalytic amount of anhydrous ferric chloride (3 mol %) to give the corresponding monoalkyldichloroborane **76** within one hour.

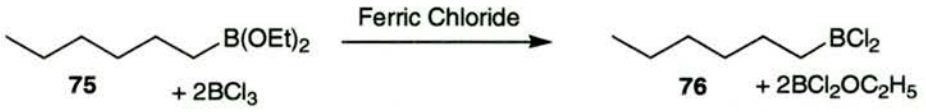


Figure 2.11 *Proposed partial synthesis of alkyl dichloroboranes*

The optically active organoborane intermediates $R^*B(OEt)_2$ were prepared by asymmetric hydroboration of an appropriate prochiral olefin e.g. **77** with either (+)-diisopinocampheylborane, $^dIpc_2BH$ **78** (>99% ee),[114] or (+)-iso-pinocampheylborane, $^dIpcBH_2$ X (≥99% ee),[115] both easily prepared from (+)-α-pinene. Asymmetric hydroboration of cis-2-butene **77** with $^dIPc_2BH$ **78** gave trialkylborane,[116] which upon treatment with 1.8 equiv of benzaldehyde resulted in the selective facile elimination of the chiral auxiliary, providing the corresponding boronic ester. This on extraction with 3 N NaOH followed by acidification with 3 N HCl provided (R)-2-butylboronic acid in very high enantiomeric purity **79**.[115] The chiral diethyl (R)-2-butylboronate **80** was then prepared by esterification of (*R*)-2-butylboronic acid with absolute alcohol.[117]

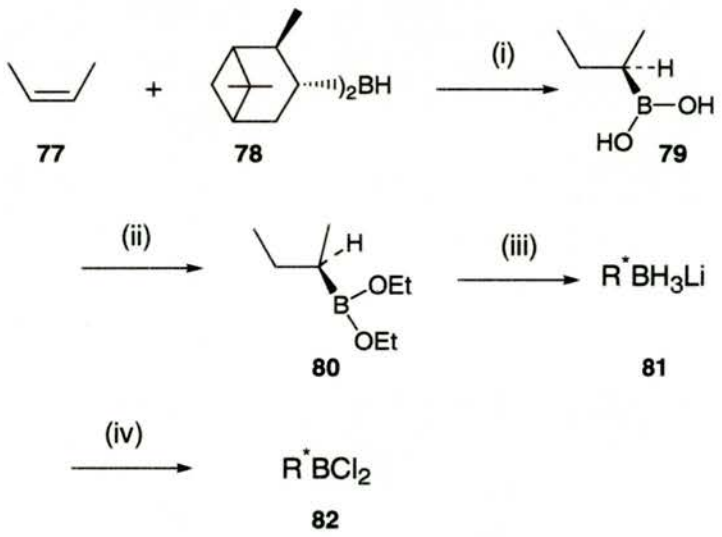
The conversion of the boronic ester **80** into the corresponding alkyl dichloroborane **82** was performed in the same manner as for the achiral example (above). The boronic ester **80** was converted into lithium monoalkylborohydride **81** with $LiAlH_4$ and then treated with 2 eq. of a 1 M solution of boron trichloride in dichloromethane in the presence of a catalytic amount of ferric chloride (3 mol %) at 0 °C for 1 h to give the alkyl dichloroborane **82**.

The formation of alkyl dichloroborane **82** was based on observations using ^{11}B NMR spectroscopic analysis.

This example demonstrates a simple, convenient and efficient procedure for the preparation of chiral and achiral alkydichloroboranes from the respective chiral or achiral alkyboronic esters, in very high enantiomeric purity.

This example showed that the route had the potential to produce enantiomerically pure dichloroboranes as desired which could be used in the production of a wide variety of enantiomerically pure derivatives via azides to test the internal environment of the IMPase active site

The only transformation left in the sequence to the target molecule was to phosphorylate the free alcohol in the presence of the secondary amine.



Reagents and Conditions: (i) –25 °C, 6h, PhCHO, 12 h, H_2O, NaOH, H^+; (ii) EtOH, H^+, reflux, 12 h; (iii) $LiAlH_4$; (iv) BCl_3, DCM, $FeCl_3$.

Scheme 2.15 *Preparation of chiral dichloroboranes from prochiral alkenes*

2.8 Phosphorylation of the azide derivative

In the original scheme the free alcohol was phosphorylated before attempting to reduce the azide. This was achieved using diphenylchlorophosphate and triethylamine followed by transesterification with BnOH in the presence of NaH in moderate yield or through the use of tetrabenzylpyrophosphate (TBPP) **83**.[98]

Low yields of the phosphorylation reaction were thought to be caused by the free amine (nitrogen reacts more readily with phosphorus than oxygen). This was partially alleviated by using excess amounts of phosphorylating reagent. An attempt was made using an alternative phosphorylating reagent tetrabenzyl pyrophosphate (TBPP) which was prepared in three steps by a literature method.[118,119,120]

The original method of synthesising this phosphorylating reagent developed by Todd *et. al.*[120] involved hazardous chemicals, including CCl_4 therefore the method was modified within our laboratories such that less hazardous reagents were employed (See section 3.1.2, page 111 for full scheme).

Treatment of PCl_3 with BnOH in dry toluene, in the presence of Et_3N led to the formation of dibenzylphosphite **118**. This was not purified but was directly oxidised to dibenzylphosphate **119** using bromine as the oxidising agent in diethyl ether (instead of CCl_4). Finally treatment of dibenzylphosphate with DCC afforded tetrabenzylpyrophosphate **83** which was purified using silica column chromatography and the product subsequently recrystallised from diethyl ether/petroleum ether.

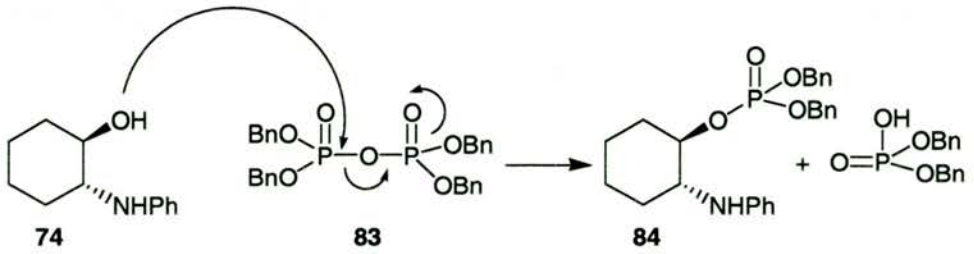


Figure 2.12 *Phosphorylation with tetrabenzylpyrophosphate*

TBPP and NaH were reacted with the amine (±)-**74** in the presence of DMF. Analysis of the reaction product was found to contain only starting material.

It was postulated that the amine was preferentially bonding to the phosphorus which then hydrolysed during aqueous work up. The addition of an excess of phosphorylating agent did not improve the reaction yield possibly due to the steric bulk of the phosphorylating agent attached to the nitrogen molecule.

To combat this effect an attempt was made to protect the amine with benzylchloroformate. This delocalises the amine lone pair thus destroying its ability to co-ordinate the phosphorylating compound. The secondary amine **74** was reacted with benzylchloroformate in the presence of triethylamine dissolved in dichloromethane. No reaction was found to have taken place. An alternative reaction in the presence of water, potassium bicarbonate and benzylchloroformate returned only starting material probably due to the lack of solvation of the reactant in the predominantly aqueous solution.

An attempt to reverse the phosphorylation/azide reduction step was to be investigated however new results indicated that a simpler method to the target compounds by direct attack of amines on the epoxide could be more efficient. Thus this scheme was temporarily abandoned while investigations continued into aminolysis. A new method for the production of the key derivative epoxide **53**

was devised at this time with the ultimate aim of providing a plentiful supply of high purity enantiomeric key epoxide. This scheme began with (-) quinic acid **49**.

2.9 Functional constraints of target molecule

In order to prepare the target molecule (-)-**53**, care must be taken to generate the required stereochemistry at position 1, 2, 4 and 6 on the cyclohexyl ring.

As a result it takes over 10 steps to prepare the key epoxide (-)-**53** from a reasonably simple starting molecule. There have been two routes used mainly for the preparation of the molecule both using different starting compounds. Both routes begin with the cyclohexane backbone already in place however this is where the similarity ends. The cyclohexane-1,4-diol route (Section 2.3, Page 73) begins with the racemic 1,4-diol **85** and uses hazardous reagents for some steps and ends with a late low yielding resolution step. The quinic acid route devised in our own laboratories begins with an enantiomerically pure form of quinic acid **49** which is a natural product.

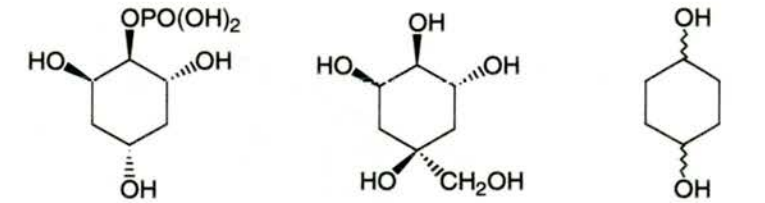
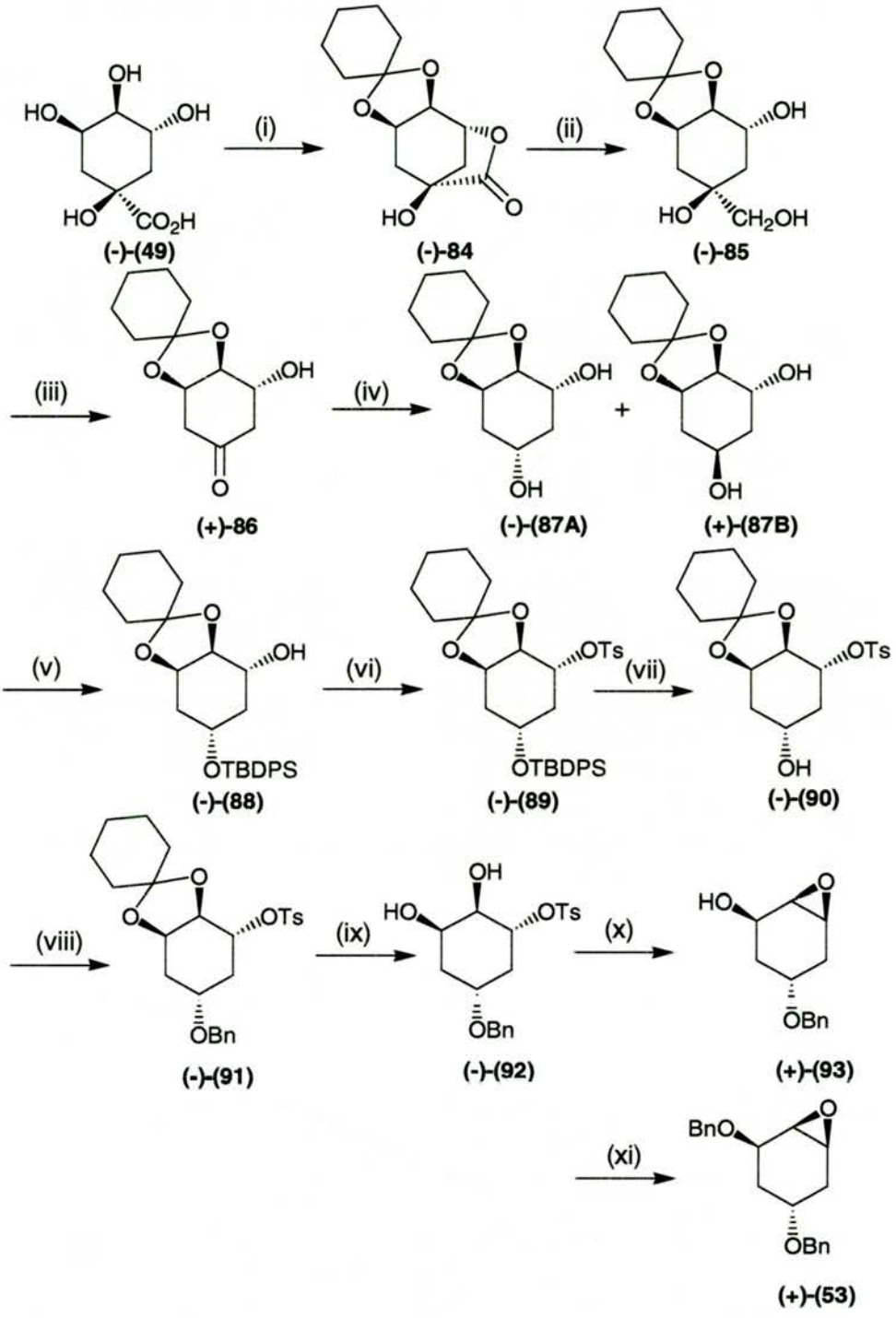


Figure 2.13 *Structures of key molecules*



Reagents and Conditions: (i) Cyclohexanone, H_3PO_4, 155 °C, 30 min; (ii) $NaBH_4$, EtOH, 0 °C→rt, 12 h; (iii) $NaIO_4$, H_2O, pH5-6, 0 °C→rt, 6 h; (iv) $LaCl_3.5H_2O$, $NaBH_4$, MeOH, -78 °C→rt, 12 h; (v) $tBuSiPh_2Cl$, Et_3N, DMAP, DCM, 0 °C→rt, 3 days; (vii)TBAF, THF, rt, 6 h; (viii) BnBr, NaH, DMF, -40 °C→rt, 12 h; (ix) $TFA_{(cat.)}$, MeOH, tr, 2 days; (x) K_2CO_3, MeOH, rt, 30 min; (xi) KH, BnBr, DCM, 12 h, -78 °C→rt, 12 h.

Scheme 2.16 *Quinic acid route to 3,5-doxy- inositol monophosphate*

2.10 Route from quinic acid[121]

(-)-Quinic acid **49** was converted to the cyclohexylidene lactone **84** in 85% yield following a literature procedure.[122] Reduction of lactone **84** with sodium borohydride in ethanol gave the primary alcohol **85** which was, without further purification, converted to the ketone **86** in 95% overall yield using sodium periodate.[123,124] This sequence was optimised in our labs to give a better yield than the reported literature preparations of the ketone which used lithium aluminium hydride to reduce the acetylated form of lactone **84**.[122,123,124,125]

The La^{3+} assisted reduction of ketone **84** (to give a 6:1 to 9:1 mixture of alcohols **87A** & **87B**) and could be performed successfully on a 20 g scale in one litre of solvent to maintain high dilution. Direct separation of the isomers,[83,124] was difficult on such a large scale but treatment with the bulky silylating group TBDPS-Cl (which demands an accessible nucleophile),[126] gave a mixture of mono-silylated products, containing predominantly the desired 4-silyl ether **88**, which could be isolated easily by column chromatography on silica in 38% overall yield from the ketone **86**. [Note that the remaining material could be recycled *via* the sequence: desilylation, reaction with TBDPS-Cl and chromatographic resolution of the isomers to give further quantities of 4-silyl ether]. Tosylation of the free 6-OH group gave **89**, followed by removal of the silyl group with TBAF gave the 4-hydroxy 6-tosylate **90** in excellent yield. Benzylation with NaH and benzylbromide afforded the 4-benzylether **91** in good yield. Hydrolysis of the cyclohexylidene protection in methanol containing a catalytic amount of TFA gave the diol **92** which was treated with potassium carbonate, in the same "pot", to give the required homochiral 1,6-epoxy-4-

benzyloxycyclohexan-2-ol **93** in 80% yield, 16% overall yield from (-)-quinic acid **49** (Scheme 2.16, page 90). Compound **93** {mp 64-65 °C, $[\alpha]_D$ +56.4° (c. 0.33 in MeOH), $[\alpha]_D$ +19.2° (c. 0.21 in $CHCl_3$) {lit.[76] for 87% ee material obtained as an oil, $[\alpha]_D$ +18.6° (c. 4.4 in $CHCl_3$)} and all of its intermediates were fully characterised and showed the expected properties.

At this stage epoxide **93** may be attached to solid support (Section 3.3, page 113) using the free hydroxyl group or for solution chemistry the hydroxyl can be protected with a benzyl group. This was achieved using KH and benzyl bromide. The resulting thick oil could be purified through silica chromatography to give the pure, fully protected key epoxide **53**. Initially NaH was used but it was found to react unfavourably with the epoxide so KH was used instead.

2.11 Key stereospecific cleavage of the ketone

The enantioselective reduction via the ketone **86** was essential to the success of this synthetic route. The reduction lay in the middle of the synthetic pathway while the amount of material was still large. If this step was low yielding then it was possible that the final product would contain unacceptable concentrations of the undesired isomer or that yields would be so low that the wastage became excessive.

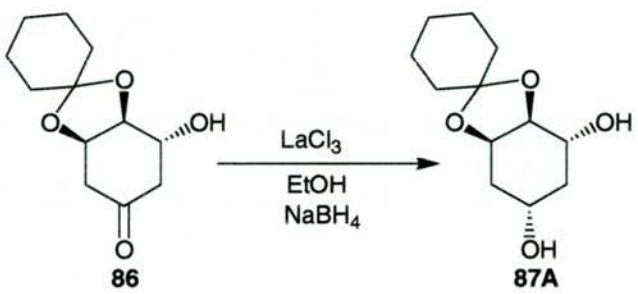


Figure 2.14 *The key reduction step in the epoxide synthesis from quinic acid*

Following the literature method of sodium borohydride in refluxing diethyl ether gave the unwanted 4-axial alcohol **87B** exclusively. Under similar conditions but at 20 °C, in either diethyl ether, or, in ethanol, or, at -60 °C in methanol, the axial alcohol **87B** was still the predominant product. This is expected because the approach of borohydride from the 4-*re*-face of the ketone is hindered by the cyclohexylidene moiety. As it seemed possible that the 4-*re*-face of the ketone might be better exposed to the reductant if the 4-carbonyl O-atom and the 6-OH group could be persuaded to occupy axial positions through chelation to a highly charged metal ion, reductions were repeated in the presence of various lanthanide ions.[127]

At -60 to -78 °C in methanol in the presence of La^{3+} or Ce^{3+} over a range of conditions and concentrations, borohydride reduction gave a mixture the 4-equatorial alcohol **87A** and 4-axial alcohol **87B** in 95% yield of which the desired alcohol constituted to 80-90% of the total product mixture (as determined by ^{1}H- and ^{13}C-NMR spectroscopy). Similar reductions performed in the presence of Pr^{3+}, Er^{3+} and Nd^{3+} were less successful, Ca^{2+} was totally ineffective and Nd^{3+} and Sm^{3+} gave viscous slurries at -60 °C. The use of ethanol in place of methanol also gave higher proportions of the axial alcohol **87B**. Note that Ca^{2+} and La^{3+} assisted borohydride reduction have been utilised for the partially selective reduction of other ketones and, as yet, the structures of the transition state complexes for such systems are not well understood.[127,128]

Preliminary studies on the cause for reversed selectivity for reduction of the ketone **86** observed here were performed in methanol, in the absence and presence of La^{3+}. ^{1}H- and ^{13}C-NMR spectroscopic data indicate that the 4-carbonyl O-atom of ketone **86** binds directly to the lanthanide ion and that there are no gross

changes in the conformation of the ketone. The reduction was found to be most efficient when carried out at –73 °C so the NMR comparisons were also analysed at +30 °C and –20 °C (the lowest practical temp for the deuteriated solvent) to exaggerate gross changes in the cyclohexane backbone. The ^{1}H NMR spectra and the spectra of the controls were found to be identical so no conclusions could be drawn from this experiment about conformation changes after complexing to the lanthanum ion. 2-D spectra also showed no evidence of intermolecular bonding.

It is difficult to account for the large increase in selectivity with increased dilution. It is possible that two or more molecules may associate together in high concentrations perhaps with a lanthanum linker and resulting in decreased selectivity.

Once a stock of epoxide had been built up it was then possible to pursue leads provided by aminolysis experiments on models.

2.12 Literature methods of aminolysis

A literature search indicated that a variety of different reagents may be used to open the oxirane ring with amines in fact the literature indicated that this was a valuable transformation to make synthetically useful 1,2-amino alcohols.[129] Reactions rarely gave yields above 60% for all but the most simple cleavages. As steric bulk increased the success of the reaction dropped dramatically.

The literature indicated that the simplest classic method to achieve aminolysis was to reflux the epoxide with the amine.[130] This was reported to be successful with a wide range of amines and epoxides. Closer analysis however indicated that this method was only successful with poorly nucleophilic amines.[130,131] Moreover,

drastic conditions were often involved, further limitations are encountered with sensitive epoxides.[132]

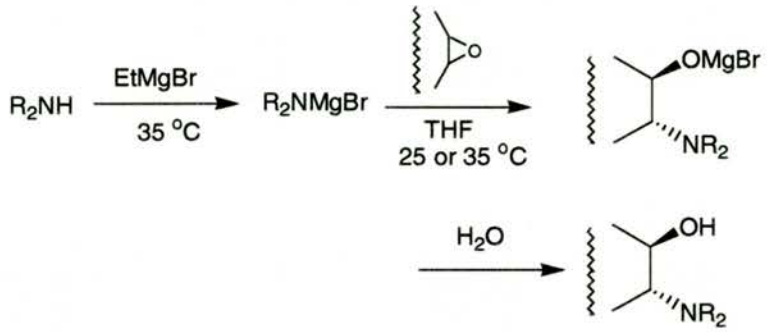
To preserve the valuable key epoxide **53**, research into aminolysis was frequently attempted with (±)-cyclohexene oxide (±)-**56** unless otherwise indicated in the text. It was believed that (±)-**56** was a suitable test molecule because it was far less hindered than the key epoxide **53**, and as a consequence, if the reaction was unsuccessful with the model (±)-**56** it was very unlikely that it would work with the key epoxide **53**. Successful reactions were then transferred to the key epoxide **53**.

Attempts were made to react cyclohexene oxide (±)-**56** with ammonia, benzylamine and dibenzylamine. Stirring at room temperature or at reflux returned only starting material in each case. This was an expected result due to the steric considerations of the cyclohexyl epoxide. The use of catalysts in amine epoxide cleavage are well known and a large variety of techniques exist for the cleavage of sterically hindered epoxides with hindered amines.

The first catalyst used was BF_3 etherate. This was catalyst of choice in the analogous epoxide cleavage with alcohols as mentioned previously (Section 2.4, page 73). Attempts were made at aminolysis using BF_3 etherate under similar conditions to those utilised for the alcoholysis. Reaction of cyclohexene oxide (±)-**53** with benzylamine in the presence of BF_3 etherate returned only starting material. It was proposed that the failure of the reaction was due to the complete sequestration of the Lewis acid catalyst by the excess amine employed.

It has been reported that magnesium amides were very good reagents for the conversion of epoxides to 1,2-amino alcohols.[133] This method was derived from work by Overman and Flippin who efficiently used diethylaluminium amides to

open epoxides.[134] Caubere and co-workers attempted to improve this method using amino magnesium derivatives, easily prepared from the corresponding amine and ethyl magnesium bromide.[133] In testing their hypothesis they found that the halomagnesium alkylamides reacted with epoxides under mild conditions to give, after hydrolysis β-amino alcohols in good yields.



Scheme 2.17 *Using halomagnesium alkylamides to cleave epoxides*

To re-produce the epoxide cleavage 1.2 eq. of the amine in 5 cm^3 of THF was added dropwise to a stirred 1.1-1.2 M solution of ethylaminomagnesium bromide (1.2 eq.) previously prepared in THF and the reaction sequence maintained at 35 °C for 1 h. The epoxide (±)-**56** (1 eq.) in 5 cm^3 of THF was then added to the stirred solution of magnesium amide at room temperature. The reaction mixture was stirred at the specified temperature, for the time indicated in the table, and the cooled mixture then poured into a saturated NH_4Cl solution. The aqueous solution was then acidified with cold 2N HCl solution and extracted twice with diethyl ether. The aqueous solution was then made alkaline by the addition of cold 10% NaOH solution to pH 8 and the amine extracted with diethyl ether. The organic phase was then dried, extracted and the solvent removed to provide the crude amino alcohol product which was purified by silica column chromatography.

The technique was attempted using cyclohexene oxide (±)-**56** as a model for the key epoxide **53** and a number of 1,2 amino alcohols were produced. Magnesium benzylamide freshly prepared from ethyl magnesium bromide and phenylamine, gave 2-*N*-phenylaminocyclo-hexanol **97** (R = Ph) in 70% yield in 90 minutes. A parallel reaction with the slightly more hindered benzylamine **98** gave 76% yield in 36 hours (Figure 2.16).

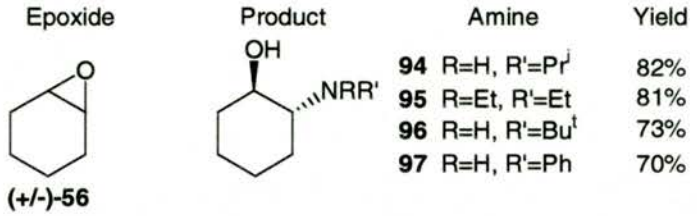


Figure 2.15 *Extract from published results of aminolysis using halomagnesium alkylamides*

The indication was that although this method was an improvement over simple amine reflux the overall yield dropped sharply as steric bulk increased. Using this method with the more sterically hindered key epoxide **53** and benzylamine gave a much poorer 19% yield of the required amino alcohol **99** together with a complex mixture of other products.

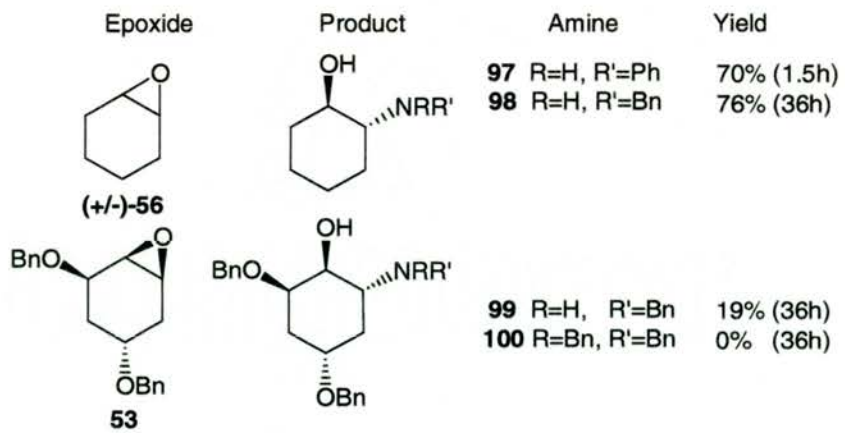


Figure 2.16 *Results from attempted epoxide cleavage experiments using halomagnesium alkylamides*

Using dibenzylamine with the key epoxide gave a variety of unidentifiable products. This method had been shown to be ineffective at the steric levels required for the planned scheme and so other protocols were sought and attempted.

A thorough literature research indicated that although there were many methods available there were still many drawbacks, for instance nucleophilic metal amides e.g. aluminium,[134] and silicon,[135] are potentially incompatible with certain functional groups e.g. alcohols or the reagents were expensive.

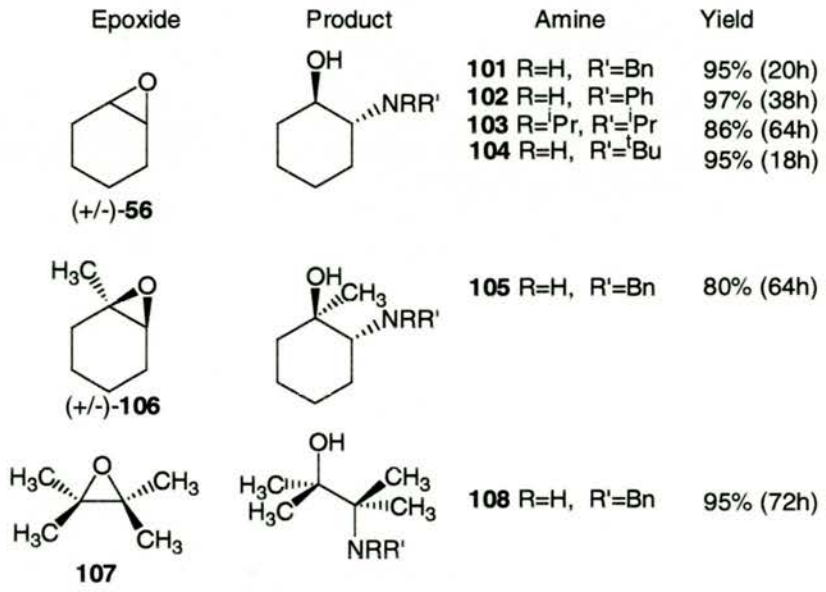


Figure 2.17 *Extract from published results of epoxide cleavage using perchlorate salts*

It was reported by Macchia and co-workers that several readily available metal salts in particular lithium tetrafluoroborate and perchlorate salts were claimed to be efficient in the amino cleavage of 1,2-epoxides with a variety of amines (Figure 2.17).[136] The figure below (Figure 2.18) shows a representative group of results from the research.

It was indicated that the observed catalytic effect of the Lewis-acid metal ions on epoxide aminolysis reactions was related to the ions ability to co-ordinate with the oxirane oxygen.

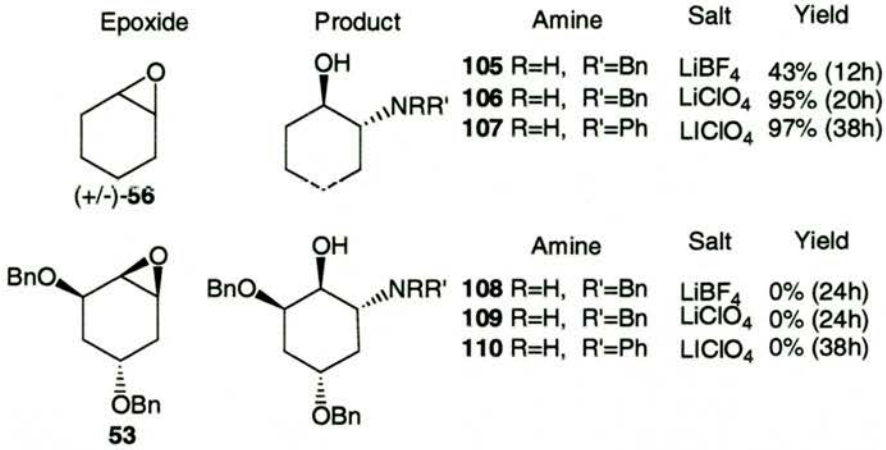
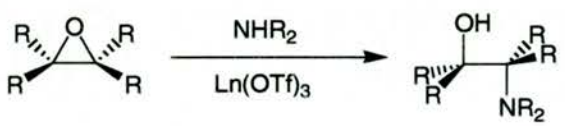


Figure 2.18 *Results from attempted aminolysis experiments using metal salts*

In model reactions in the presence of $LiBF_4$ and benzylamine, cyclohexene oxide (±)-**56** was converted overnight to the corresponding amino alcohol (R = Bn) in 43% yield using lithium tetrafluoroborate salts in acetonitrile. Attempting the same reaction using the same conditions using the more sterically hindered key epoxide **53** returned only starting material. The same reaction was attempted using lithium perchlorate salts as the catalyst for the cleavage of cyclohexene oxide (±)-**56** with benzylamine (20 h) and phenylamine (38 h) giving good yields of 95% and 97% respectively. Once again however the reaction failed using the key epoxide **53**.



Scheme 2.18 *General reaction sequence of aminolysis using lanthanide(III) trifluoromethanesulfonates as catalysts*

It is a recognised characteristic of oxirane cleavages that as the oxirane becomes more functionalised and the nucleophile becomes more bulky (and therefore less

nucleophilic) the reaction yield will decrease.[136] The group also noted that a shortcoming of the metal salt e.g. $LiClO_4$ approach was that equimolar amounts of the salt was required.

Crotti and co-workers more recently published work based on the search for more efficient aminolysis catalysts.[137] It was found that lanthanide (III) trifluoromethane sulfonates [lanthanide triflates, $Ln(OTf)_3$] such as $Yb(OTf)_3$, $Nd(OTf)_3$ and $Gd(OTf)_3$ promoted the aminolysis reaction of 1,2-epoxides in low-polar, non-protic solvents such as toluene or CH_2Cl_2 at room in good yields.[138]

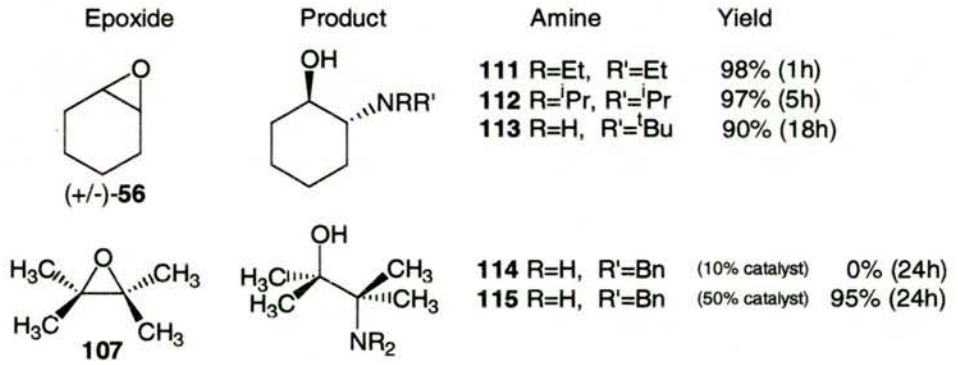


Figure 2.19 *Extract from published results of aminolysis using lanthanide(III) Trifluoromethanesulfonates.*[137]

The paper demonstrated the use of the lanthanide ytterbium (III) triflate as the Lewis acid catalyst in dichloromethane.[137] For simple to moderately hindered primary amines the yields were good (85 - 100%), however the more hindered amines and epoxides required long reaction times (>24 hrs) and high catalyst concentrations (50%) to work. The order of effectiveness for the various lanthanide (III) triflates was Yb(III) ≡ Gd(III) > Nd(III) (see table). Ytterbium was chosen as an initial catalyst based on this finding and its availability. The reported yields they claimed to have achieved were promising, so various test reactions were attempted based on their work.

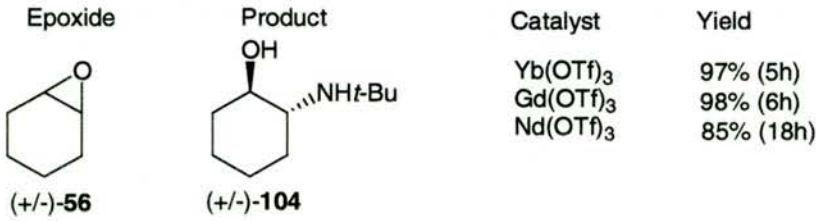


Figure 2.20 *Comparison of the catalytic ability of various lanthanide trifluoromethanesulfonates*

2.13 Lanthanide triflates

Using the information from this new method two slightly different protocols for cleaving the epoxide with volatile and liquid amines were developed.

2.13.1 Cleavage with liquid amines

Crotti and co-workers had shown that ytterbium (III) triflate,[139] a Lewis acid, was useful in the preparation of 2-amino alcohols from oxiranes in aprotic solvents. Following their protocols using the model cyclohexene oxide (±)-**56** with benzylamine and 0.2 equivalents of the salt in DCM solution at 20 °C for 12 h quantitatively afforded the amino alcohol **105**. Repeating the reaction with O-benzyl hydroxylamine in DCM with 0.2 equivalents of the salt gave the corresponding amino alcohol **125** in 90% yield after chromatographic purification.

However the more sterically hindered dibenzylamine did not react with epoxide **53** in DCM over 48 hours. Likewise when the catalyst loading was increased to 0.5 mole equivalents only starting material was returned after 12 hours of reaction.

It appeared that once again promising results on the model epoxide (±)-**56** could not be transferred to the key epoxide **53** because of steric considerations. On the assumption that heat may accelerate the reaction the same reaction with

dibenzylamine was carried out in refluxing dichloromethane at 40 °C. Once again this was met with little success, but in a last attempt to react the two compounds the solvent was changed from DCM for the higher refluxing temperature of dichloroethane (83 °C). This proved to be the key to the reaction – repeating the reaction with only 0.2 catalyst concentration at reflux temperature for 1 h returned the desired amino alcohol **116** in quantitative yield as judged by ^{1}H & ^{13}C NMR spectroscopy.

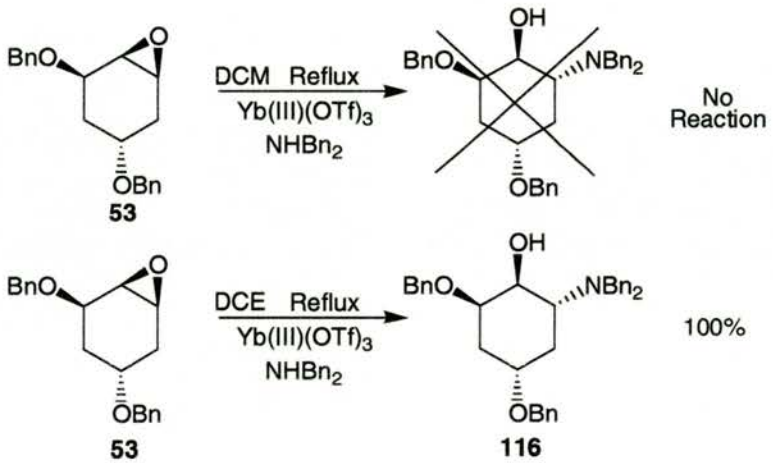


Figure 2.21 *Effect of changing to a higher boiling solvent on aminolysis*

Once the reaction conditions with the model epoxide (±)-**56** had been optimised the reaction was transfered to the more hindered key epoxide **53**.

Following the protocol offered by Crotti *et. al.* the key epoxide **53** was refluxed in dichloromethane in the presence of dibenzylamine and 0.2 equivelents of the ytterbium salt. As expected from the results of the model epoxide (±)-**56** no reaction occurred. Changing the solvent to 1,2-dichloroethane following the results of the model reaction and refluxing the mixture resulted in a 98% yield of the desired product **116** and the reaction was complete in less than two hours. This reaction was carried out on a variety of primary and secondary alkyl amines (Section 4.1, page 114) and in every case a quantitative yield was returned.

In the course of the research a few nucleophiles failed to react with the epoxide in the presence of ytterbium triflate, these included acylamine and sodium azide. This was probably due to delocalisation of the amine lone pair for the former acylamine and lack of solvation on the latter. Standard phase transfer reagents were utilised to aid solvation of the sodium azide e.g. 15-crown-5 but with no success.

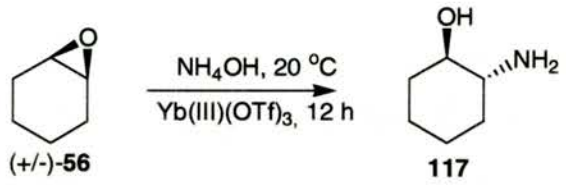
The result of this modification to the literature method has led to the discovery of a mild method of opening epoxides with primary or secondary amines in quantitative yield and very short reaction times. The reaction has also been shown to proceed with complete anti-stereoselectivity and regioselectivity. The reaction has also been extended to include nucleophiles other than the previously mentioned primary and secondary amines e.g aqueous amines (Section 2.13.2, page 103), alcohols (Section 2.13.3, page 106) and on the solid phase (Section 2.13.4, page 108).

2.13.2 Cleavage with volatile amines

Although a reliable method of producing secondary or tertiary amines via aminolysis of the epoxide had been discovered the method which involved reflux could not be used with volatile amines e.g. ammonium hydroxide or methylamine. It was our initial aim to create an ammonium derivative **137** to act as the 'first in the series' of amine derivatives. Naturally this could not be done with the existing refluxing method so many attempts to use a protected source of ammonia (e.g. benzylamine) which could easily cleave the epoxide and then later be deprotected to provide the primary amine derivative were sought. This however was more

difficult than first anticipated and it became necessary to find a way of cleaving with the unprotected volatile amine.

Using cyclohexene oxide (±)-**56** as a model, the epoxide was added to a 15N solution of ammonium hydroxide containing a 0.2 equivalents of ytterbium triflate at 20 °C and stirred for 12 hours. Extraction and purification by silica column gave a white solid which was the amino alcohol **117** in 60% conversion (51% isolated yield). Longer reaction times were observed to give better yields.



Scheme 2.19 *Cleavage of model epoxide with aqueous ammonia*

It was postulated that the yield for this reaction appeared so low was because the epoxide (±)-**56** was not dissolving properly in the aqueous solution. Small amounts of methanol were added to aid solvation of the epoxide in the aqueous solution, it was not expected that the methanol would react preferentially to the more nucleophilic amine. This indeed served to lower the overall reaction times.

Transferring this method to the key epoxide **53** disappointingly returned only starting material. It seemed that the reason was that the lipophilic key epoxide was not dissolving enough in the aqueous solvent to react. In an attempt to improve the reaction a solution of ammonia in dioxane was used instead of the aqueous solution however this also failed to react and no aminolysis was observed.

It had been shown (Section 2.13, page 101) when cleaving the epoxide (±)-**56** with non-volatile amines that heating the mixture proved to be the key to the reaction. Transferring this result was not possible because as soon as the aqueous

solution was warmed the volatile amine evaporated. However, when the key epoxide **53** or cyclohexene oxide (±)-**56** was heated to 65 °C with either aqueous ammonia, methylamine or ethylamine in a sealed tube (to prevent evaporation) in the presence of 0.2 equivalents of $Yb(OTf)_3$ and a few drops of methanol (to aid phase transfer) for 24 h, each of the amino alcohols **A** (R = H **117**, Me **197** and Et **198**) and **B** (R' = H **133**, Me **139** and Et **148**) were obtained in quantitative yield.

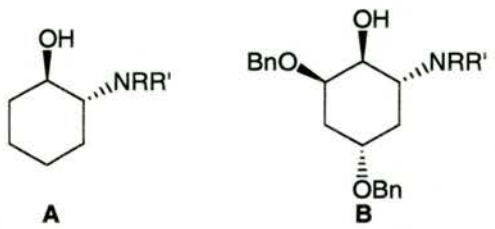


Figure 2.22 *Successful cleavage reactions*

The control without the presence of catalyst indicated no reaction showing that pressure alone had not accelerated the reaction. The compounds displayed the expected analytical and spectroscopic properties and analysis of the ^1H NMR spectra of the **133**, **139** & **148** showed that the reaction had proceeded with inversion of configuration at C-6 and that no or very little reaction had occurred *via* attack at C-1. Thus, it was possible to convert the key epoxide **53** to amino alcohols of the required configuration through direct aminolysis. This was a very exciting result as it increased the scope of the modified ytterbium method to include all primary and secondary amines whether in water or organic solvents (Table 1, page 109).

The likely purpose of the sealed tube and its contribution to the success of the reaction probably lies not in the increase in pressure but in the heating of the reaction medium which forces more epoxide into solution as well as speeding the

reaction up. At room temperature it is proposed that the epoxide cannot dissolve sufficiently to allow a speedy reaction.

Crotti had noted that treatment of 4-benzyloxy-1,2-epoxycyclohexane with diethylamine in the presence of 0.5 equivalents of $Yb(OTf)_3$ in ethanol for 5 days at 55 °C had afforded the amino alcohol in only 50% yield. It was also noted that non-protic, non-coordinating solvents worked best and that increasing amounts of water severely inhibited catalysis. In contrast here we found that water had little or no effect on the reaction moreover it acted as an ideal solvent.

2.13.3 Cleavage with alcohols

Following the success of the aminolysis of epoxide **56** using the new catalyst the protocol was attempted with alcohols. Although a method was available using boron trifluoroetherate the yields were not always high.

To test the efficiency of the reaction it was tested against the then current method with boron trifluoro etherate. Thus the key epoxide **56** was reacted with 1-propanol in 1,2-dichloroethane at reflux for 2h. The product **161** was analytically compared with a standard **160** produced from the boron trifluoro etherate method and was found to be identical in all respects. The yield for the ytterbium method was quantitative whereas the boron trifluoetherate method gave a 51% yield in 3 hours. Comparison of the ^{13}C NMR spectra (Figure 2.23, page 107) showed side products in the boron trifluoroetherate catalysed product while the ytterbium triflate catalysed reaction had proceeded cleanly.

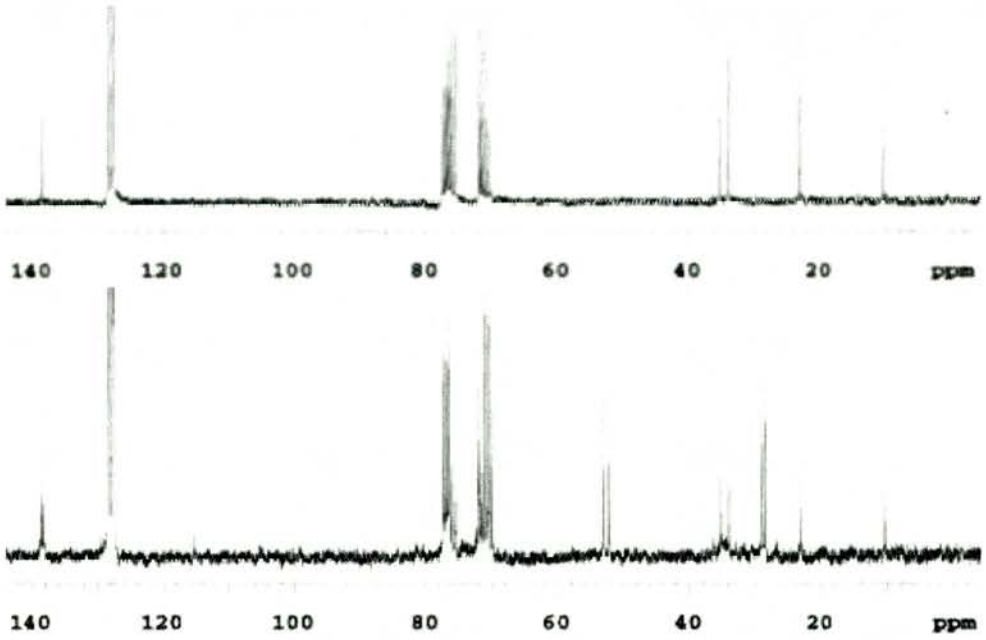


Figure 2.23 *^{13}C NMR spectra comparison of crude products of epoxide cleavage using ytterbium triflate (top) and boron trifluoroetherate (bottom)*

To explore the practical value of this catalyst the epoxide **56** was also cleaved with other more hindered alcohols including 2-phenylethanol **165**, isopropanol and 1-benzyl, 2-TBDPS-ethanediol which all gave quantitative amounts of the desired isolated products.

Following this experiment the reagent of choice for oxirane cleavage in either refluxing dichloromethane or dichloroethane was ytterbium. The tables (Table 1, page 109) indicate the reactions carried out with the accompanying yields.

2.13.4 Cleavage on solid phase

It was likely that the synthesis of the target molecules could be carried out in the solid phase. There was no reliable method of opening the epoxide **56** on the solid phase without causing elimination or solid support cleavage. Ytterbium triflate catalysis was proposed as a mild method of oxirane cleavage and was employed successfully within our laboratories.[141] Using an identical protocol to the one successfully used for solution work gave excellent results. Thus, refluxing the key epoxide attached to solid support (Merryfield resin) with propanol in refluxing 1,2-dichloroethane for 3 hours gave the desired and expected product in quantitative yield (Section 3.3, page 113).

2.13.5 Summary of advance

The excellent catalytic effect of lanthanide (III) triflates in the aminolysis of 1,2-epoxides was tentatively attributed to the strong oxophilicity of the lanthanide (III) triflates which allows the metal to tightly co-ordinate to the oxirane oxygen, thus directly favouring the nucleophilic ring opening reaction. The results obtained represented a marked improvement over the original work carried out by Crotti and co-workers.[137]

This new procedure provides a generally applicable, cheap and mild method of amine and alcohol cleavage of 1,2-epoxides giving quantitative yields with most types of amine and epoxide. The reaction times are very quick, the catalyst cheap and success can be achieved with a variety of different nucleophiles in different physical forms and using substrate friendly solvents in solution or on solid support.

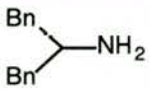
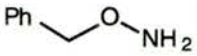
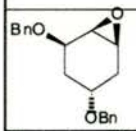
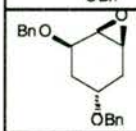
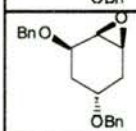
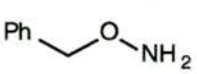
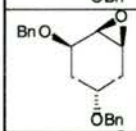
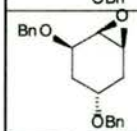
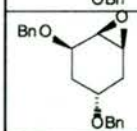
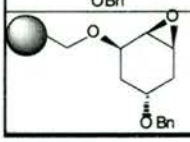
Epoxide	Amine	Solvent	Time	Conditions	Yield
		Dichloromethane	1h	reflux	99%
	$BnNH_2$	Dichloromethane	1h	reflux	99%
		Dichloromethane	1h	reflux	87%
		Dichloromethane	1h	reflux	68%
	$NH_3.OH$	Water	6h	rt	99%
	$BnNH_2$	1,2-Dichloroethane	4h	reflux	99%
		1,2-Dichloroethane	6h	reflux	82%
		1,2-Dichloroethane	5h	reflux	83%
	$NH_3.OH$	Water	6h	65 C	87%
	$MeNH_2$	Water	6h	65 C	94%
		Dichloromethane	3h	reflux	99%
		1,2-Dichloroethane *This experiment was carried out by others	3h	reflux	99%

Table 1 *Various cleavage reactions carried out with ytterbium triflate*

3 Synthesis of general target molecules

3.1 Preparation of fully protected precursors

Once the key epoxide **53** has been cleaved to provide the desired intermediate the molecule requires phosphorylation, transesterification and then global deprotection. Different methods were employed to provide the fully protected target molecule depending on the type of molecule.

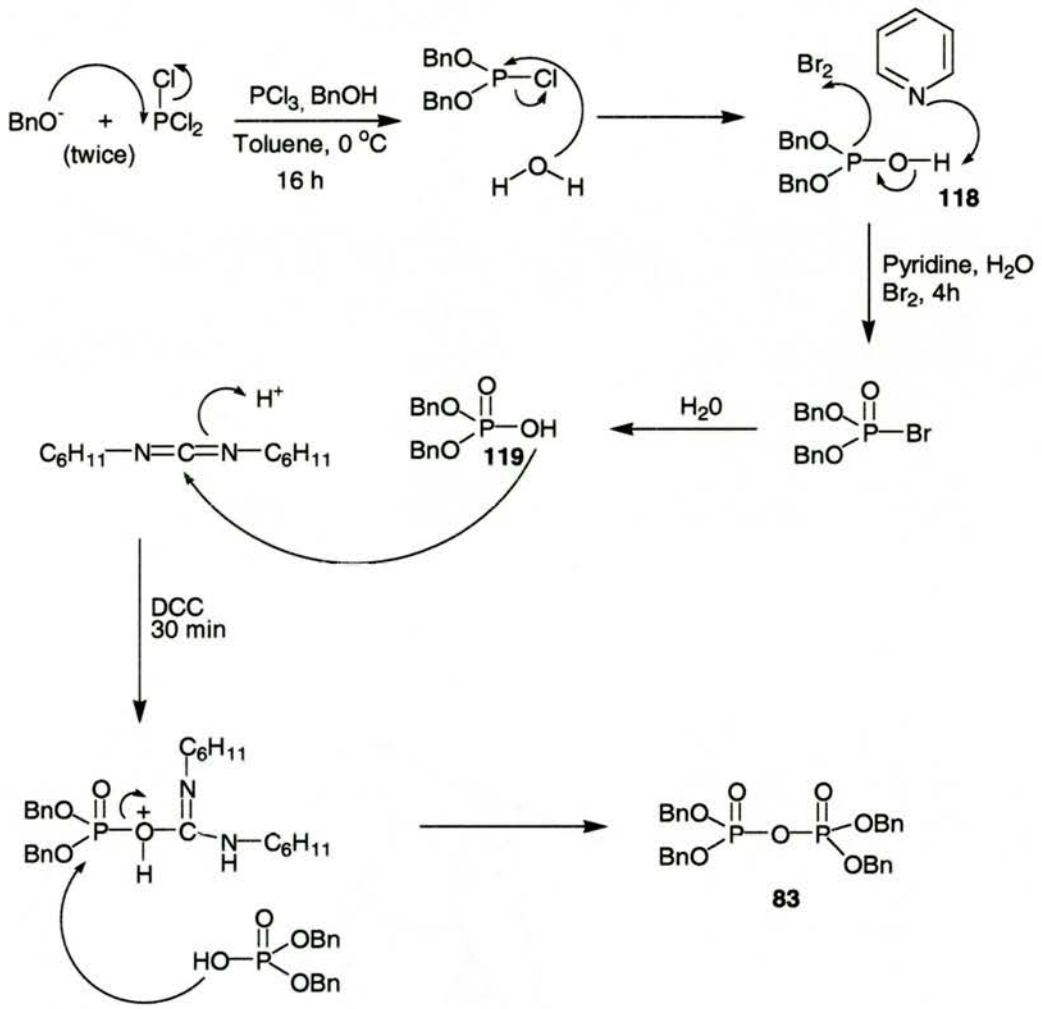
3.1.1 Phosphorylation and transesterification

Once the key epoxide **53** has been cleaved the free alcohol can be phosphorylated. On some occasions it became necessary to protect the functionality at the 6 – position as it interfered with the phosphorylation. This will be discussed with the specific cases (Section 4.1, page 114). The general method for phosphorylation employed diphenylchlorophosphate in dichloromethane, a suitable base and DMAP for covalent catalysis. After overnight stirring reactions gave a yield ranging from 50 – 95%. The mixture required purification by silica column chromatography to give the pure phosphorylated intermediate as an oil.

The next step was to transesterify the diphenyl groups on the phosphate to prepare the molecule for global deprotection. This was achieved with benzyl alcohol and sodium hydride at –70 °C for a few hours to furnish the completed fully protected target ready for global deprotection.

3.1.2 Other methods of phosphorylation

Another method of transesterification has been shown to be achieved through the use of 'TBPP' **83**.[119,120] By using this reagent two steps in the phosphorylation process could be avoided. This reagent is not commercially available and could only be prepared in small amounts.



Scheme 3.1 *Preparation of dibenzyl phosphate*

The first step in the preparation of TBPP **83** was the formation of dibenzyl phosphite **118**.[118] This was made by the reaction of phosphorus trichloride and benzyl alcohol. The product was hydrolysed and then oxidised with bromine to give dibenzyl phosphate **119** which was coupled using DDC (dicyclohexyl carboiimide) to give the desired product.[119,120]

3.2 Methods of global deprotection

3.2.1 Sodium/liquid ammonia

The standard method of benzyl de-protection was a sodium in liquid ammonia reduction. This is a highly reactive reagent and can only be used if the target compound can tolerate harsh reagents and conditions.[144] After deprotection it is necessary to purify the product as there exists a large amount of inorganic salt side-product. This was achieved through the use of Amberlite acidic resin (IR118 H^+) to give cation exchange.

Although very effective with alcohol based derivatives this method of purification was attempted, and found to be unsuccessful with, deprotected amine targets probably because the amine atom bound to the resin.

3.2.2 Hydrogenation

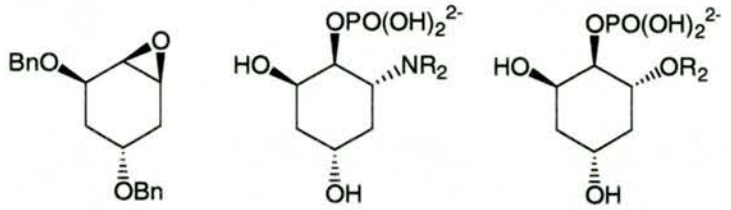
This was another method of benzyl deprotection attempted. Using a Pd/C catalyst in an atmosphere of hydrogen it has been demonstrated that benzyl protecting groups can be removed in a facile and selective manner. This method has the added advantage that the product can be purified by simple filtration.

3.3 Solid phase synthesis

Solid phase organic synthesis (SPOS) is an indespensible tool for rapidly producing large numbers of structurally diverse molecules in analytical amounts.[140] Originally developed with peptide synthesis in mind it is beginning to be used for more mainstream organic synthesis. The advantages of SPOS include high yields, simple purification steps and eventually automation. This technology when applied to mainstream organic chemistry however is still at an early stage. Drawing on techniques optimised in the conventional synthesis others were successful using solid phase techniques in preparing analytical amounts of the propyl showing that the technology especially the ring opening could be almost directly applied to solid phase synthesis. [142-144]

4 Targets Synthesised

Once the various sections required to construct the desired target molecules were completed namely key epoxide synthesis, epoxide cleavage and the subsequent phosphorylation and deprotection it became possible to design and construct rationally designed targets based on the 3,5 deoxy analogue containing either an oxygen or nitrogen atom at the catalytic 6' position.



53

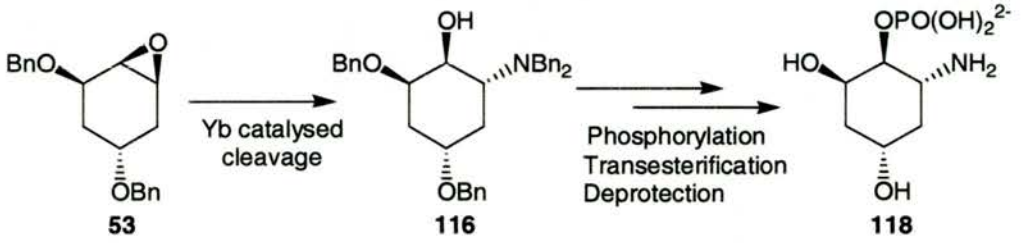
Figure 4.1 *Starting epoxide and the target general target molecules*

4.1 Nitrogen derivatives

Using the new reaction sequences detailed and optimised above (Section 2.13, page 101) the first compound that was proposed was the ammonia derivative **137** for reasons detailed below. At that time there were no reported methods for opening epoxides with volatile amines e.g. ammonia so it was necessary to find an easily deprotected 'masked' derivative of ammonia that could be used to cleave the epoxide and protect the amine while further transformations were performed on the molecule.

The first target molecule attempted was via the dibenzylamine derivative **116**. This was attempted before the aqueous method was found and it was hoped that the dibenzyl groups could be deprotected in the final global deprotection sequence to return the ammonia derivative. The reaction was initially attempted using

cyclohexenoxide (±)-**56** as the model. As detailed above the aminolysis reaction was a success using ytterbium (III) triflate as the catalyst returning the desired compound in quantitative yield. This reaction when applied to the key epoxide **53** also was found to be a complete success returning the target compound **116** in quantitative yield.



Scheme 4.1 *General method using benzylamine as a masked ammonia derivative*

Phosphorylation was then attempted using TBPP and NaH in DMF however even after overnight stirring the reaction was found to be a failure. An attempt to phosphorylate using diphenylchlorophosphate was also found to be unsuccessful. The alcohol was dissolved in DCM along with diphenylchlorophosphate, TEA and DMAP and stirred overnight. After an aqueous workup it was found that the reaction had failed returning a mixture of products including starting material. It was thought that the reason for the failure was likely to be the interference of the amine with the phosphorylating reagent. Nitrogen - phosphorus bonds are easily formed and equally easily broken on aqueous workup so it seemed likely that the phosphorylating agent was bonding with the nitrogen atom, preventing other molecules from approaching through its steric bulk but subsequently disappearing on exposure to water. This hypothesis appeared to fit the facts therefore it was necessary to somehow reduce the amine's nucleophilicity. Using high concentrations (>3) of the phosphorylating reagent was found to be reasonably

successful however complex mixtures were still formed and purification was always necessary.

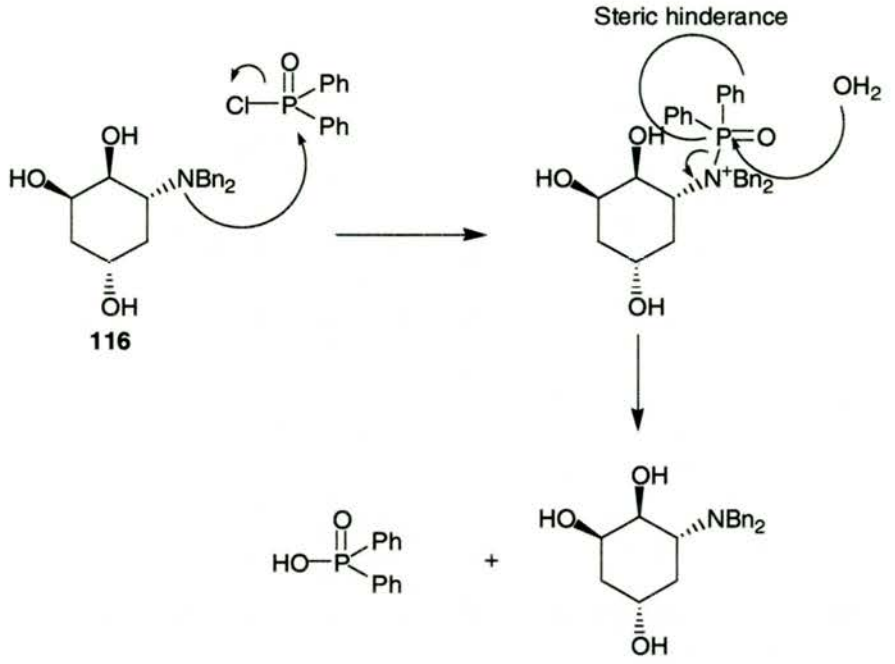


Figure 4.2 *Proposed mechanism of phosphate hydrolysis*

In an attempt to improve the efficiency of the phosphorylation reaction the effect of the amine group on the reaction was examined as a possible disrupting influence.

The amine was already trisubstituted so urethane protection was out of the question. A common technique used in amino acid chemistry to temporarily protect tertiary amines is to form the hydrochloride salt of the amine. Borrowing this idea the amine was dissolved in ethyl acetate and HCl gas produced from sulphuric acid and sodium chloride was bubbled through the mixture for 30 minutes. It was noticed that lengthy exposures to HCl gas had a deleterious effect on the molecule resulting in fragmentation .

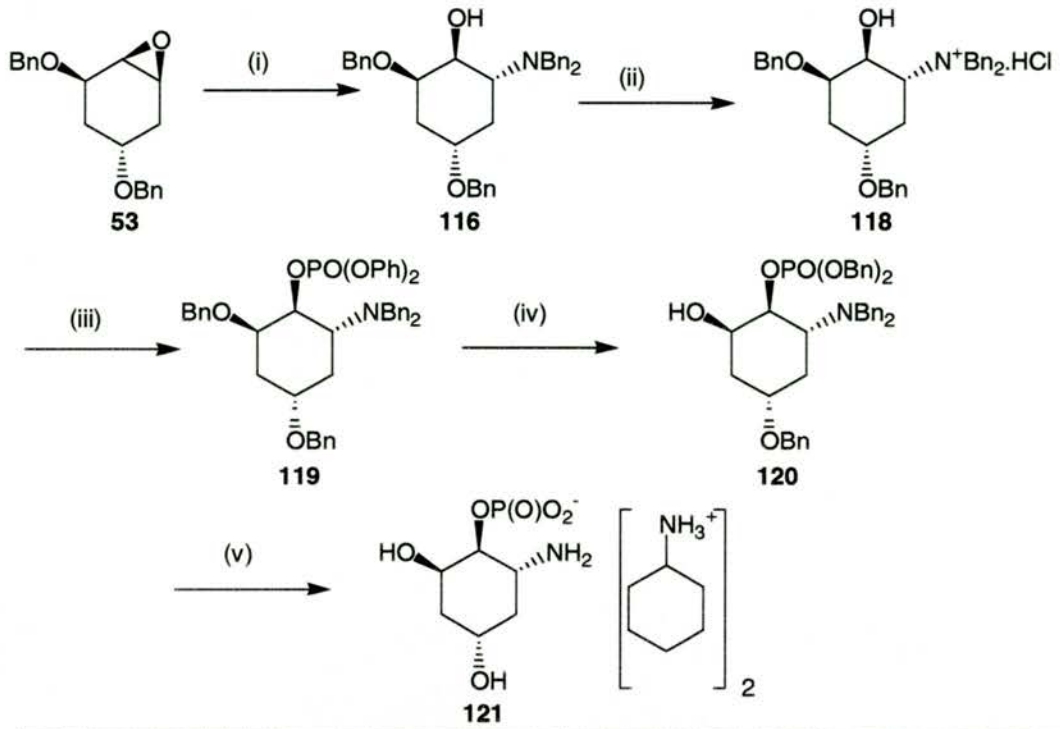
The salt formation was found to be a success, the product could be recrystallised from an ethyl acetate/pet. ether mixture to give the product salt in quantitative

yield. The product was identified through the use of microanalysis, mass spectrometry and NMR spectroscopic analysis. Phosphorylation of the amine salt was attempted using diphenylchlorophosphate, DMAP and TEA in DCM overnight. After isolation the product **119** was recovered as a golden oil in excellent yield (98%).

This was identified through ^{13}C, ^{1}H & ^{31}P NMR spectroscopic analysis and mass spectrometry. Transesterification of the compound was achieved using benzyl alcohol and NaH. The mixture was stirred overnight and the product **120** recovered after column chromatography as a yellow oil (87%).

This fully protected precursor to the ammonia target derivative was then ready for global deprotection. Deprotection was attempted using the standard Na/NH_3 method used exclusively in the analogous alcohol derivative chemistry. The fully protected precursor was placed into a blue solution of liquid ammonia and sodium metal. This was stirred for 1 h before methanol was added to quench the reaction and the solution warmed to room temperature. The solvent was then evaporated to leave a white solid **121**.

The standard protocol for purification was carried out using Amberlite IR-118 H+ strongly acidic resin. However this was unlikely to work with the amine as it would stick to the resin permanently. What was required was actually a strongly basic anionic resin. A suitable resin was found to be Dowex 1 (1 x 8 – 400) anionic exchanger. This was prepared as detailed above and the product extracted using a weak solution of formic acid. The acid fractions were collected and lyophilised to give pale yellow crystals (65% overall yield after salt formation with cyclohexylamine).



Reagents and Conditions: (i) $Yb(OTf)_3$, dibenzylamine, 1,2-dichloroethane, reflux, 2 h, 93%; (ii) DCM, $HCl_{(g)}$; (iii) DCM, TEA, diphenyl chlorophosphate, DMAP, 12 h, 20 °C, 88%; (iv) THF, NaH, BnOH, -40 °C, 12 h, 87%; (v) Na/NH_3, -70 °C; b) Dowex (1 x 8 – 400) (OH^-); c) Cyclohexylamine, H_2O, 34%.

Scheme 4.2 *Preparation of ammonia target from dibenzylamine*

Analysis of this compound by ^1H NMR spectroscopy indicated that while deprotection of most of the molecule was successful there were aromatic signals which were identified as belonging to the N-Bn group or groups.

Literature research indicated that while the first N-Bn group was simple to remove the second was very difficult even under the strongly reducing conditions presented by the Na liquid NH_3 combination.

It was apparent that the dibenzylamine method of amine protection was not ideal and several different groups were tested for their ability to endure the rigours of the reaction sequence yet deprotect at the appropriate time. Benzylamine **122** was disregarded as a candidate due to the aforementioned difficulty of deprotection of a single Bn group attached to a nitrogen. Another possibility was through the use of a urethane protected compound e.g. benzyl carbamate **123** which was a resilient

amine protecting group yet simple to deprotect under the same conditions as the O-Benzyl groups. However epoxide cleavage was found to be unsuccessful probably due to the delocalised nature of the amine.

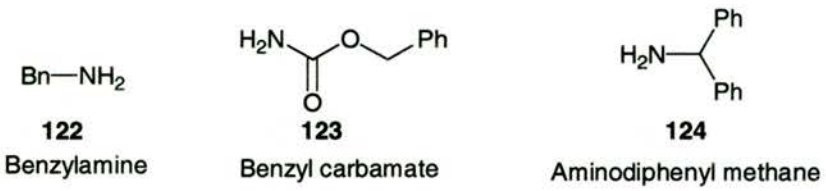


Figure 4.3 *Various masked ammonia examples attempted*

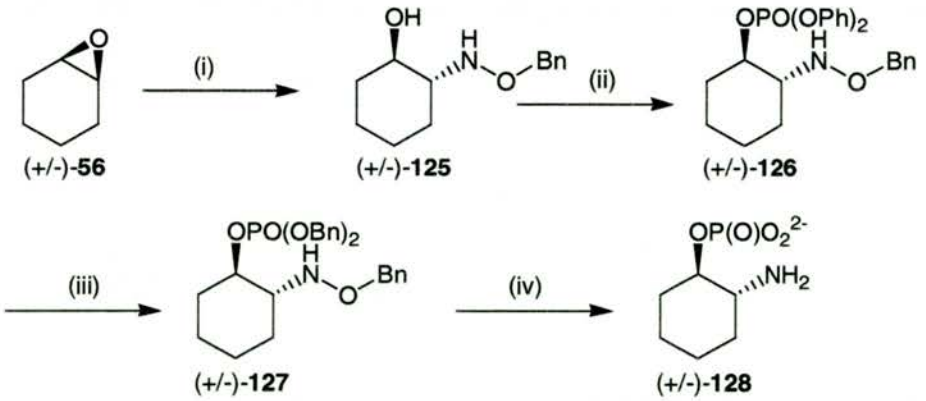
Aminodiphenyl methane **124** was another candidate which acts as a protecting group for ammonia yet was easy to cleave at the desired time. Thus aminodiphenyl methane was stirred with the model cyclohexene oxide (±)-**56** in refluxing 1,2-dichloroethane in the presence of the ytterbium catalyst for 2h. The reaction was followed by tlc and was found to be complete at this time. The organic layer was washed with water, separated and evaporated to yield the crude product. The target **199** was isolated following column chromatography as a yellow oil in 96% yield. Following phosphorylation problems the compound was first converted to the salt by bubbling HCl gas. Phosphorylation was carried out using diphenychlorophosphate in the presence of DMAP and TEA in DCM. Following purification the product **200** was found to be an oil (56%). Transesterification was achieved using the standard benzyl alcohol method to return target **201** in 43% yield. Following the success of this method it was decided to attempt the route using the key epoxide **53**.

The key epoxide **53** was reacted in the standard method with aminodiphenylmethane and ytterbium (III) triflate over several hours to give the target compound **202** in 92% yield identified through a number of analytical

techniques. The salt was formed using HCl gas before attempting the phosphorylation. To phosphorylate diphenylchlorophosphate was used along with DMAP and TEA in the standard manner. Unfortunately the compound failed to phosphorylate successfully resulting in an inseparable mixture of products. It was proposed that the salt was not forming properly. Cbz protection of the molecule was unsuccessful and the use of aminodiphenyl methane was abandoned.

The use of O-benzyl hydroxylamine was recommended as a potentially successful method. Using the ytterbium triflate method of aminolysis the model epoxide (±)-**56** was reacted with O-benzyl hydroxylamine. The mixture was refluxed in 1,2-dichloroethane for 3 h before isolating the product **125**. The reaction was found to have been successful giving the desired opened epoxide in 90% yield.

Although unsure whether or not the direct phosphorylation would be successful two equivelents of diphenylchlorophosphate was reacted with the free alcohol of the aminolysis product. Isolation of the product after overnight stirring resulted in an excellent yield of the desired phosphate **126** (88%) identified by ^{1}H, ^{13}C & ^{31}P NMR spectroscopy. The ensuing transesterification with benzyl alcohol in the presence of NaH was also successful returning the fully protected compound **127** in 42% yield after purification by column chromatography. The compound was deprotected using the mild hydrogenolysis method to return a white solid **128** after filtering and lyophilisation.



Reagents and Conditions: (i) $Yb(OTf)_3$, O-benzylhydroxylamine, 1,2-dichloroethane, 2,6-lutidine, reflux, 12 h, 90%; (ii) DCM, TEA, diphenyl chlorophosphate, DMAP, 12 h, 20 °C, 88%; (iii) THF, NaH, BnOH, -40 °C, 2 h, 42%; (v) a) Pd/C (10%), H_2, 12 h 30%.

Scheme 4.3 *Successful synthesis of model ammonium target using O-benzylhydroxylamine as the masked ammonia molecule*

^1H, ^{13}C & ^{31}P NMR spectroscopic analysis appeared to indicate that the reaction had been a success although there were some impurities visible on the ^1H & ^{13}C NMR spectra probably due to deprotection fragments. Mass spectral analysis showed that the target molecule had indeed been succesfully produced.

The reaction sequence was once again transferred to the key epoxide. Aminolysis was successful giving the product after purification in excellent yield (87%). This line of work was temporarily paused while a new method of opening the epoxide directly with the volatile amines was researched.

4.2 Opening the epoxide with volatile amines

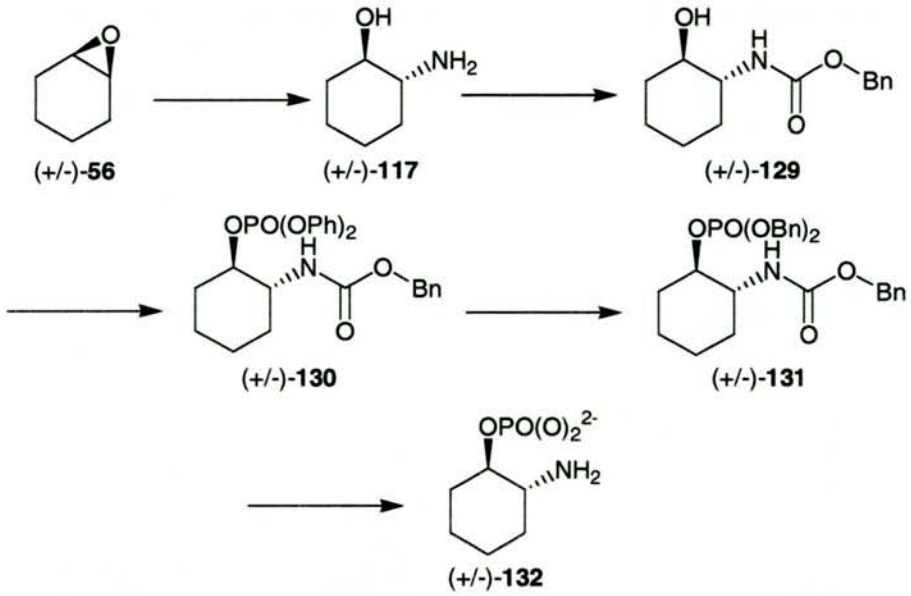
Using the methods previously discussed three nitrogen derivatives were synthesised from ammonia, methylamine and ethylamine.

Epoxides **53** or (±)-**56** were heated to 65 °C with either aqueous ammonia, methylamine, ethylamine or benzylamine in a sealed tube in the presence of 0.2 equivalents of $Yb(OTf)_3$ for 24 h, each of the amino alcohols were obtained in

quantitative conversion. Thus, it was possible to convert the key epoxide **53** to amino alcohols of the required configuration through direct amine cleavage of the epoxide.

4.2.1 Opening cyclohexenoxide with ammonia

Compound **117** was carried forward as a model. Treating the compound with diphenychlorophosphate resulted in a low yield of product following silica purification. It was found that to achieve good yields of phosphorylation it was necessary to use many (>3) equivalents of the diphenylchlorophosphate. Although those problems were likely to remain exclusive to the ammonia derivative it was decided to attempt an amine protection prior to the phosphorylation step. Following general procedures the amine **117** was treated with benzylchloroformate in water overnight.[144] The product **129** was isolated using column chromatography to give a white powder (97%) which was identified as the desired carbamate. Phosphorylation was then achieved simply using diphenylchlorophosphate in DCM overnight and the product **130** was purified using column chromatography (90%). This was identified through the ^{1}H, ^{13}C & ^{31}P NMR spectroscopic data. Transesterification was achieved overnight using the normal procedure with NaH, benzyl alcohol and DMAP (51%). The product **131** was identified from NMR ^{1}H, ^{13}C & ^{31}P NMR spectroscopic analysis following column chromatographic purification.



Reagents and Conditions: (i) $Yb(OTf)_3$, NH_4OH, sealed flask, 65 °C, 4 h, 99%; (ii) Sodium bicarbonate, benzylchloroformate, 0 °C, 12 h, 97%; (iii) DCM, TEA, diphenyl chlorophosphate, DMAP, 12 h, 20 °C, 90%; (iii) THF, NaH, BnOH, -70 °C, 2 h, 51%; (v) a) Pd/C (10%), H_2, 12 h, 67%.

Scheme 4.4 *Successful synthesis of model ammonia derivative using aqueous ammonia*

The fully protected model **131** was then deprotected using the Na/NH_3 protocol. The resulting crude mixture was analysed by ^{31}P NMR spectroscopy and no evidence of the expected signal around 0 ppm was found. It was thought that the method may have been too harsh for the fragile molecule so alternative methods were sought.

A common method of benzyl and carbamate deprotection is hydrogenolysis and this method was attempted. The fully protected precursor **131** was dissolved in methanol and was stirred for 48h under a hydrogen atmosphere in the presence of 10% Pd/C. The mixture was then filtered through celite with water before removing the solvent and analysing the product **132** as its cyclohexylamine salt. The ^{31}P NMR spectrum showed the desired signal at 0 ppm.

It was not expected that the compound would demonstrate any enzymatic activity - due to its lack of the proposed binding functionalities - however this premise

was tested by incubating the substrate with the enzyme and following any reaction by ^{31}P NMR spectroscopy. The substrate was monitored overnight by ^{31}P NMR spectroscopy for any depletion of starting material however none was identified (Section 7.1.2, page 149).

Although the molecule itself was not found to be a substrate it proved that the proposed route to amine derivatives was viable and research then began into the preparation of new derivatives based on the 3,5-deoxyinositol model.

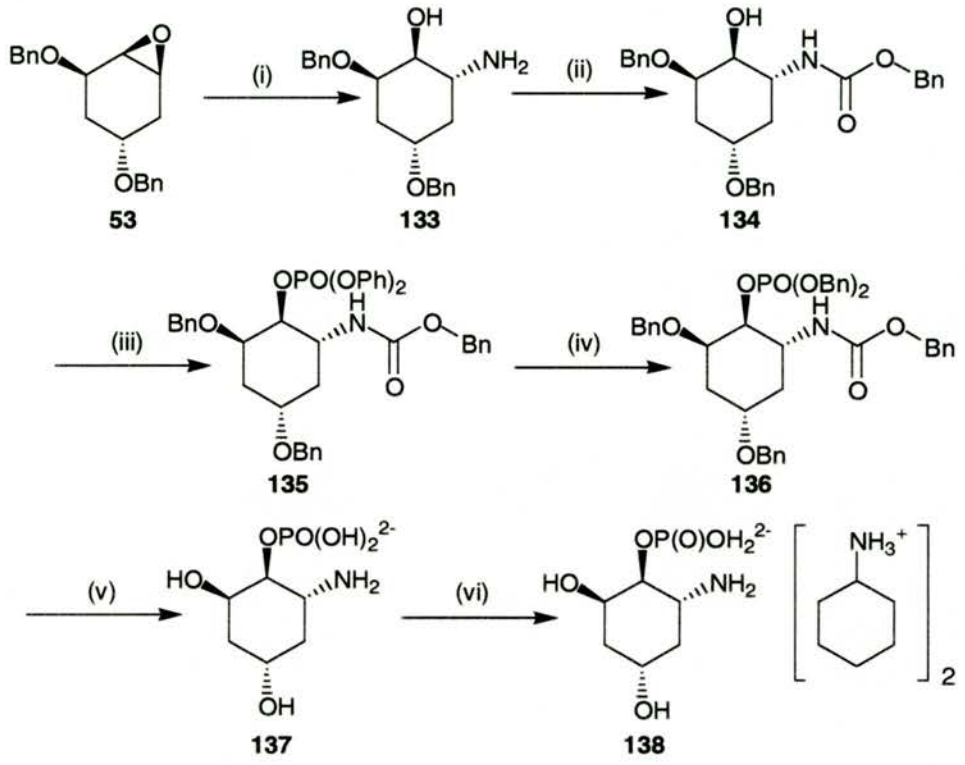
4.2.2 Opening key epoxide with ammonia

The first amine derivative target was proposed to be primary amine derivative **137**. This was a suitable initial target for the following reasons:

1) This is the simplest target allowing direct comparisons with the analogous hydroxyl substrate **15**;
2) The results of testing would not be affected by steric considerations making the derivative a good reference molecule.

The preparation of the ammonium derivative **137** required special considerations (detailed in the aminolysis research above) as ammonia is a volatile compound. Using the technique discovered in earlier research key epoxide **53** was dissolved in excess ammonia solution containing a few drops of ethanol as a phase transfer catalyst and 10% ytterbium (III) trifluoromethansulfonate catalyst. This was heated in a sealed flask to 70 °C for 12 h, the reaction progress was followed by tlc analysis. The resulting mixture was then extracted with ethyl acetate and

purified by column chromatography to yield the desired product **133** as white crystals (87%), m.p. (81-83 °C).



Reagents and Conditions: (i) $Yb(OTf)_3$, NH_4OH, sealed flask, 65 °C, 12 h, 87%; (ii) Sodium bicarbonate, benzylchloroformate, 0 °C, 12 h, 57%; (iii) DCM, TEA, diphenyl chlorophosphate, DMAP, 12 h, 20 °C, 68%; (iii) THF, NaH, BnOH, -70 °C, 2 h, 88%; (v) Pd/C (10%), acetic acid, H_2, 48 h, 100%; (vi) Cyclohexylamine.

Scheme 4.5 *Successful preparation of ammonia derivative*

Armed with the knowledge from previous experiments with the model that the phosphorylating reagent diphenylchlorophosphate would react with the free amine and the fact that chromatographic purification of the triesters was hampered by the high polarity of the amino groups and their salt derivatives it was decided to protect the amine as a urethane identical to the procedure used for the model (Section 2.13.2, page 103). The amine **133** was reacted with benzylchloroformate in water for 3 h to provide **134** in good yield (57%) identified by ^{13}C NMR spectroscopy. The molecule was then treated with diphenyl chlorophosphate in the presence of TEA and DMAP to give the required ureathane triesters **135** in

excellent yield. The compounds displayed the required spectral and analytical properties and were transesterified with sodium benzyloxide to give the dibenzyl phosphate ester **136** in a yield of 88%.

It was decided to de-protect this molecule by the mild method of hydrogenolysis rather than Na/NH_3 reduction as demonstrated on the model. The target precursor **136** was subjected to hydrogenolysis using a 10% solution of Pd/C in methanol with a few drops of acetic acid as a catalyst under an atmosphere of hydrogen. The reaction was followed by tlc analysis and was judged complete after 48 h. The product was gained by filtration through celite, evaporation and lyophilisation to give a white solid identified as the desired target molecule **137**. The compound was isolated as it's cyclohexylammonium salt **138**, formed by reacting with cyclohexylamine. The overall yield for the two steps of dehydrogenation and salt formation was 42%.

Analogous to the model **132** the cyclohexylamine salt **138** was analysed in the presence of enzyme by ^1H NMR spectroscopy. All the product was consumed within 6 h indicating that the amine derivative was a very good substrate for the enzyme. (Section 7.1.3, page 150).

This was a highly significant result. It meant inositol 1-phosphate derivatives with an amine in the place of the 6-OH group were acceptable surrogates in inositol monophosphatase hydrolysis and a novel line of amine derivatives could be produced to investigate the internal environment of inositol monophosphatase.

4.2.3 Opening key epoxide with methylamine

To further probe the internal structure of the enzyme it was decided to attempt the creation of the methylamine derivative **146**. This was done for several reasons:

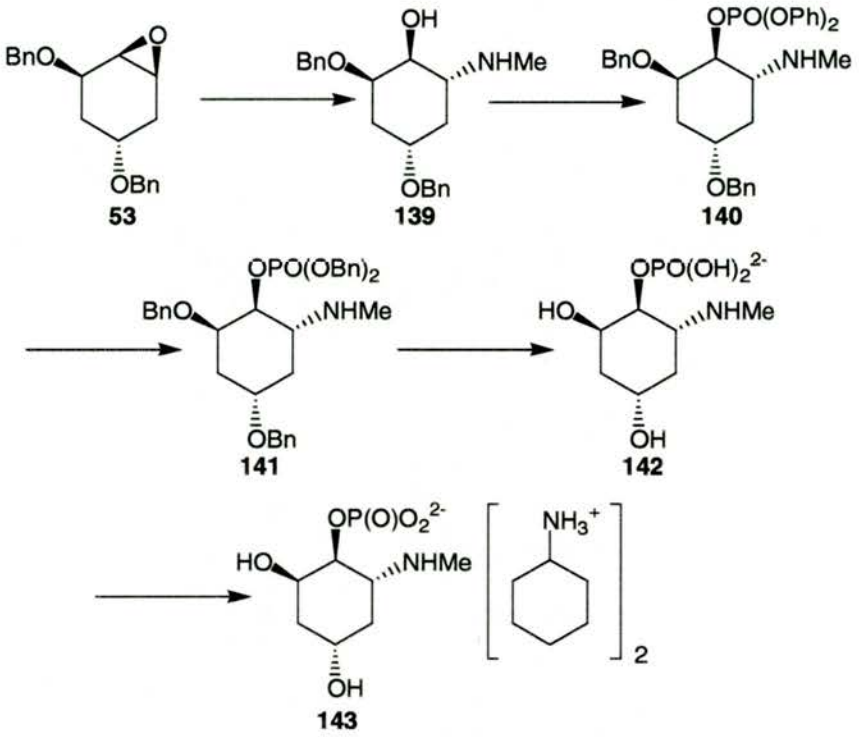
1) It is the next simplest target in the amine sequence
2) The analogous oxygen derivative **196** had been prepared and tested in the enzyme so would make a good reference molecule.
3) If our hypothesis (Section 1.31, page 43) was correct then the methylamine molecule **146** should act as a substrate in contrast to the inhibitory activity demonstrated by the methoxy derivative **196**.

In the preparation of this target the same problems faced in the preparation of the ammonium target were discovered. Fortunately the same solutions worked successfully.

Following the procedure given before, the epoxide **53** was reacted with aqueous methylamine in a sealed flask at 60 °C for 48 h. The reaction was followed by tlc and after separation and purification the desired product **139** was isolated in quantitative yield. The product was identified through ^{13}C & ^{1}H NMR analysis and comparisons drawn with the ammonium **133** and methoxy **196** analogues.

It was hoped, in this case that the urethane formation step could be avoided, so a phosphorylation of the compound **139** was attempted. The reaction with diphenylchlorophosphate to give **140** was found to be successful (83%) so transesterification was attempted. Compound **141** was highly polar and was difficult to purify. It is important to have a very pure compound at this point

because purity levels are directly related with the purity of the final deprotected product.



Reagents and Conditions: (i) $Yb(OTf)_3$, NH_4OH, sealed flask, 65 °C, 12 h, 87%; (ii) Sodium bicarbonate, benzylchloroformate, 0 °C, 12 h, 57%; (iii) DCM, TEA, diphenyl chlorophosphate, DMAP, 12 h, 20 °C, 68%; (iii) THF, NaH, BnOH, -70 °C, 2 h, 88%; (v) Pd/C (10%), acetic acid, H_2, 48 h, 100%; (vi) Cyclohexylamine.

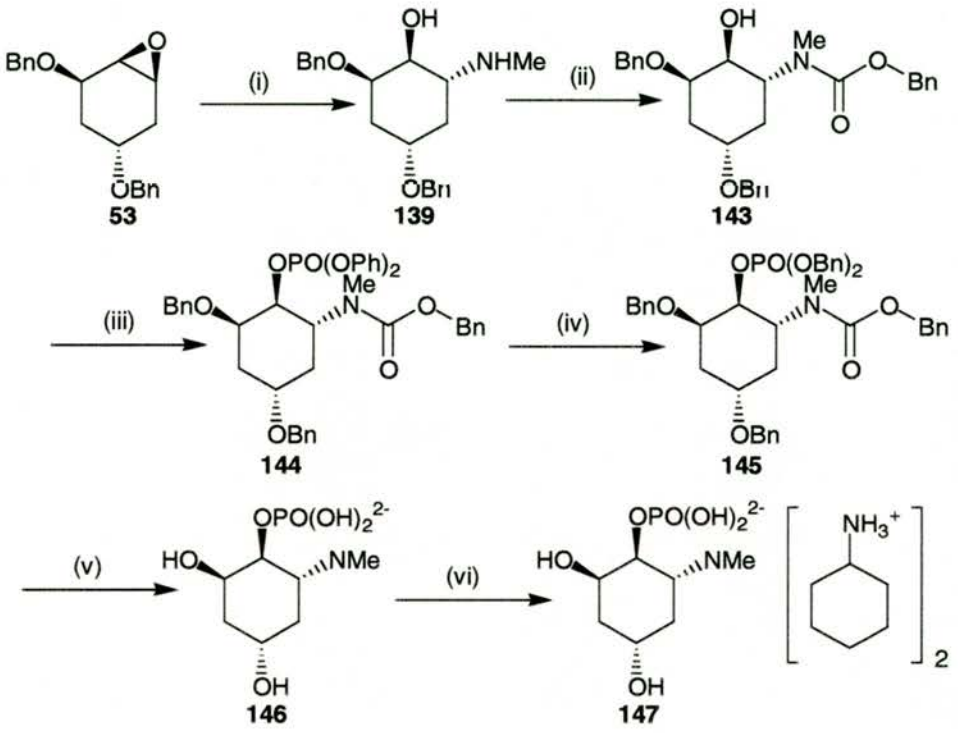
Scheme 4.6 *Unsuccessful first attempt at the preparation of the methylamine derivative*

This product was de-protected in the previously successful manner of hydrogenation with Pd/C in a hydrogen atmosphere however although the desired product **143** appeared to be present it was stuck in an inseparable mixture of products indicated by ^{31}P NMR spectroscopy.

The product was too impure to be accurately used in enzymatic analysis where accurate weights are essential so it was decided to use the urethane protection step after all.

Using previously optimised techniques the amino alcohol **139** was reacted with benzylchloroformate at 0 °C and the mixture stirred for several hours. Purification

of the product was done in the normal manner to give **143** as a solid (m.p. 98-100 °C) in good yield (65%). The target molecule was identified through ^{13}C and ^{1}H NMR analysis and comparisons with analogous compounds.



Reagents and Conditions: (i) $Yb(OTf)_3$, $NMeH_3OH$, sealed flask, 65 °C, 48 h, 100%; (ii) Sodium bicarbonate, benzylchloroformate, 0 °C, 3h, 65%; (iii) DCM, TEA, diphenyl chlorophosphate, DMAP, 12 h, 20 °C, 56%; (iii) THF, NaH, BnOH, -70 °C, 2 h, 89%; (v) Pd/C (10%), acetic acid, H_2, 48 h, 100%; (vi) Cyclohexylamine.

Scheme 4.7 *Successful preparation of methylamine derivative*

The free alcohol group was then phosphorylated with diphenylchlorophosphate as before, the reaction proceeding in a satisfactory manner overnight to **144** as a clear oil in reasonable yield (56%). Transesterification of phosphate **144** was achieved in the standard manner using benzyl alcohol and NaH to give, following purification, the fully protected precursor to the target **145** as a clear oil in good yield (89%). This compound was positively identified as the desired product through the use of ^{13}C, ^{1}H & ^{31}P NMR spectroscopy.

The product **145** was then deprotected in an analogous manner to the ammonia derivative using Pd/C in a hydrogen atmosphere followed by filtration through

celite to give the desired product as a white solid following lyophilisation. The product **146** was identified as it's cyclohexylamine salt **147** through ^{13}C, ^{1}H & ^{31}P NMR spectroscopy and was ready to undergo testing with the enzyme. NMR spectral analysis of the compound **146** with the enzyme demonstrated that the methylamine derivative was a good substrate for the enzyme (Section 7.1.4, page 151).

4.2.4 Opening key epoxide with other amines

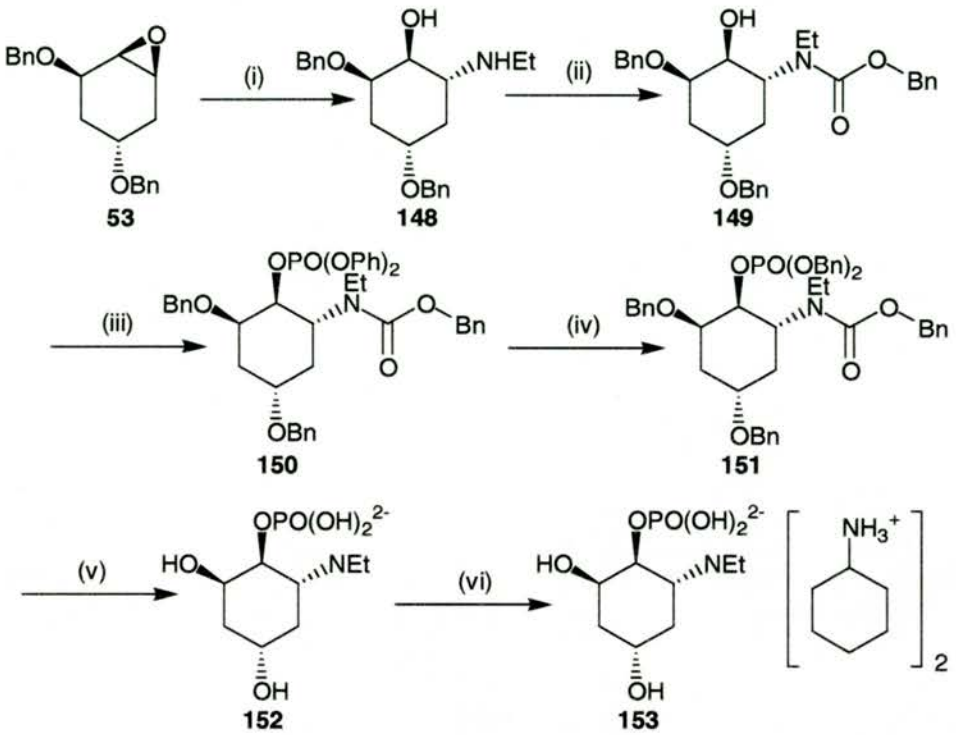
Further studies into derivatives were planned and although not completed were at an advanced stage when research was required to terminate.

The first planned derivative was the ethylamine analogue **152**. This was a natural next step as an upper limit of alkyl chain needed to be established. Ethylamine is once again a volatile amine so the same protocols used for the ammonia **137** and methylamine **146** compounds could be used.

Following the procedure the epoxide **53** was reacted in the presence of aqueous amine and the catalyst in a sealed flask at 65 °C for 48 hours. The product was isolated in the usual manner to give **148** as an oil in quantitative yield. Mindful of previous phosphorylation problems the amine was protected as a urethane using Cbz as a protecting group. This transformation was successful giving the fully protected compound **149** in 69% yield. The product was readily identified through ^{13}C & ^{1}H NMR spectroscopic analysis and comparisons with analogous molecules.

This compound was then phosphorylated in the standard manner using diphenychlorophosphate and DMAP. The product of this was a complex mix indicating that the phosphorylation step had not been completely successful. This may have been because of blocking characteristics displayed by the bulky groups

attached to the amine. An amount of the desired product **150** was isolated by column chromatography and identified through ^{1}H NMR spectroscopic analysis.



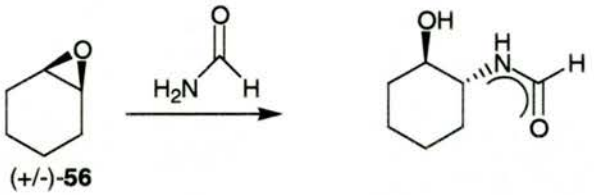
Reagents and Conditions: (i) $Yb(OTf)_3$, $NEtH_3OH$, sealed flask, 65 °C, 48 h, 100%; (ii) Sodium bicarbonate, benzylchloroformate, 0 °C, 3h, 69%; (iii) DCM, TEA, diphenyl chlorophosphate, DMAP, 12 h, 20 °C, 63%; (iii) THF, NaH, BnOH, -70 °C, 2 h, 49%; (v) Pd/C (10%), acetic acid, H_2, 48 h, 100%; (vi) Cyclohexylamine.

Scheme 4.8 *Attempted synthesis of ethylamine derivative*

Transesterification of the compound proceeded as planned and column chromatography was used to isolate the fully protected product **151** ready for deprotection. Deprotection was achieved using the standard palladium catalysed hydrogenation method to give an impure sample of the target ethylamine derivative **152**.

Another planned target was the formamide derivative **159**. This molecule was designed to mimic the configuration of the methylamine derivative **146** while at the same time delocalising the amine lone pair. If this compound was found to be inactive in the enzyme then that would provide evidence towards participation of the lone pair on the 6-C heteroatom in enzyme hydrolysis.

It was expected that the introduction of the formamide group would be the hardest part of the synthesis of this molecule. To avoid wasting valuable epoxide, tests were initially carried out using cyclohexene oxide (±)-**56** as the simple model. Reacting (±)-**56** with formamide in the presence of ytterbium (III) triflate at elevated temperatures in 1,2-dichloroethane the method used successfully with previous amine was a failure. The reaction returned only starting material even after sustained heating times.



Scheme 4.9 *Failed attempt to cleave epoxide with carbamate*

The most likely reason for the failure is the delocalising effect of the carbonyl group muting the nucleophilic nature of the amine lone pair. The introduction of the carbamate to the epoxide was therefore an unlikely proposition so it became clear that the carbamate would have to be formed from the amine already attached to the target molecule precursor.

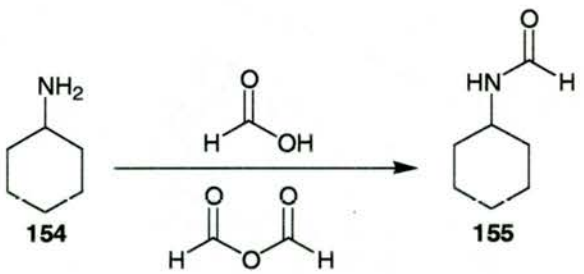


Figure 4.4 *Formylation of cylohexylamine using oxalic acid*

The standard method of transforming an amine to a carbamate is termed a formylation. There are a few different methods of carrying this formylation out.

Before attempting the formylation on the actual compound a test reaction was carried out. Cyclohexylamine **154** was used to represent the ammonia opened epoxide. A mixture of oxalyl chloride in DCM, imidiazole and formic acid was stirred for 30 min to produce the oxalic intermediate before the addition of the cyclohexlamine.

The reaction was stirred for several hours and followed by tlc. The reaction was found to be low yielding and thus abandoned.

A further attempt at formylation was performed using acetic anhydride and formic acid. The formic acid was added to the acetic anhydride and stirred for 2 hours at 55 °C. The amine **154** was then added slowly to the mixture and stirred for 3 h, the reaction progress followed by tlc.

The reaction was deemed complete after this time. The product was isolated by extraction with ethyl acetate, separation and evaporation to yield the crude product **155** which was purified by column chromatography. The product was identified as **155** through the use of ^{13}C, ^{1}H & ^{31}P NMR spectroscopic analysis.

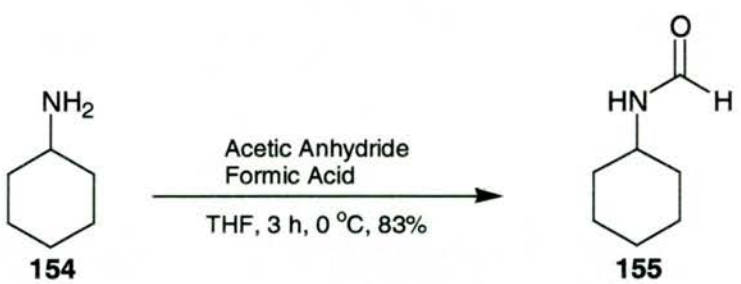
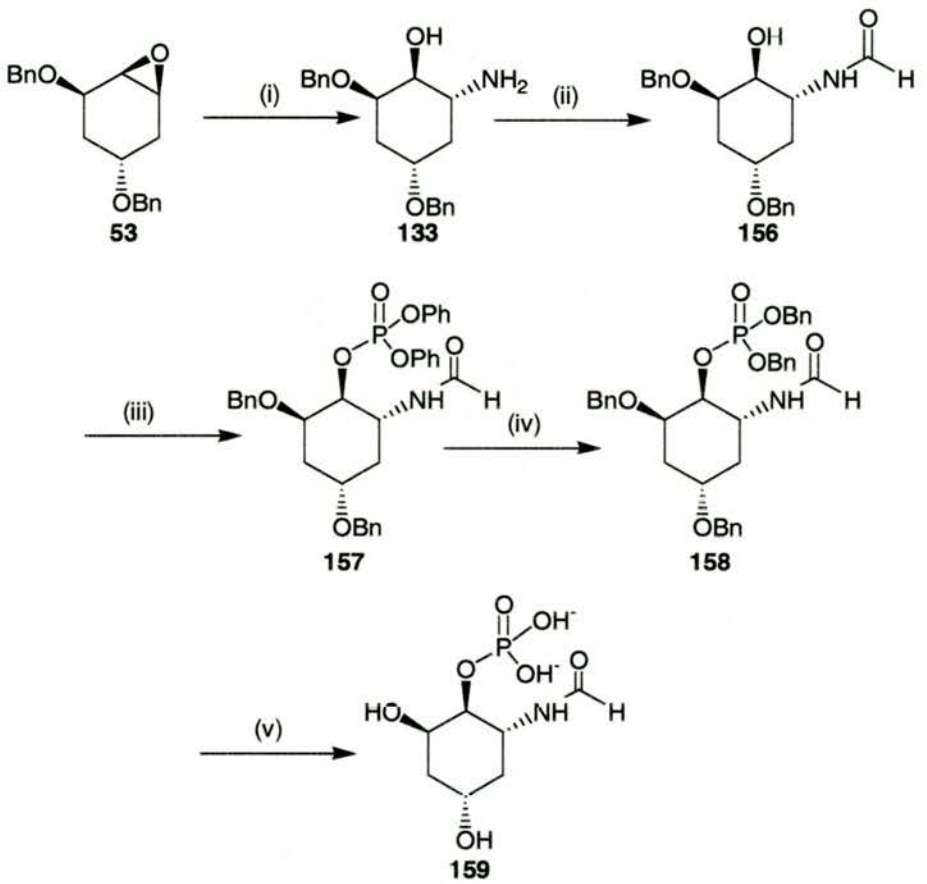


Figure 4.5 *Formylation of cyclohexylamine using oxalic acid*

Using a further stock of the ammonia derivative **133** the amine was added dropwise to a solution of acetic anhydride and formic acid which had been stirred at 55 °C for several hours. The mixture was followed by tlc and was complete after 2 hours to give the product in reasonable yield (36%). The product **156** was identified through NMR spectroscopic analysis.



Reagents and Conditions: (i) $Yb(OTf)_3$, NH_4OH, sealed flask, 65 °C, 48 h, 100%; (iiAcetic anhydride, formic acid, 55 °C, 3 h, 36%; (iii) DCM, TEA, diphenyl chlorophosphate, DMAP, 12 h, 20 °C, 45%; (iii) THF, NaH, BnOH, -70 °C, 2 h, 49%; (v) (proposed) Pd/C (10%), acetic acid, H_2, 48 h, 100%.

Scheme 4.10 *Preparation of formamide derivative*

Phosphorylation was once again achieved through the use of diphenylchlorophosphate and DMAP in DCM. After aqueous workup and purification using silica column chromatography gave the product **157** identified by NMR analysis in reasonable yield (45%). Transesterification of the phosphate was also achieved by the standard method using benzyl alcohol and NaH and

overnight stirring in THF. Purification by silica column chromatography of the small amount of product proved difficult and the product **158** was found to have a number of impurities included. The target was identified through ^{13}C, ^{1}H & ^{31}P NMR spectroscopic analysis however the number of impurities made it impractical to carry forward to the hydrogenation step.

5 Oxygen derivatives[145]

Although the specific aim of research concerned epoxide cleavage with amines, preliminary work into alcohol cleavage was also undertaken. The standard method for cleaving 1,2-epoxides with oxygen nucleophiles was by Lewis acid catalysis using boron trifluoride diethyl etherate. This procedure could be complex and low yielding so the reaction was attempted with the catalyst ytterbium triflate.[121]

1-Propanol was chosen as the test alcohol for the cleavage reaction because the analogous reaction with boron trifluoride diethyl etherate had been previously successful thus spectroscopic analysis of the product was available and the product was simply separated from the volatile alcohol. The two catalysts were directly compared by running the reactions concurrently and using equal quantities of key epoxide **53**.

The epoxide **53** was reacted with boron trifluoride diethyl etherate and propan-1-ol in toluene following the established procedure. The reaction progress was followed by tlc and it was found that after 3 h no further reaction occurred. The crude product **160** was isolated by evaporation and the resultant oil was purified by column chromatography. The yield from the procedure was calculated to be 51%.

The same experiment was carried out using the ytterbium triflate catalyst in the place of boron trifluoride diethyl etherate. Using the same conditions optimised for amine epoxide cleavage the epoxide **53** was dissolved in 1,2-dichloroethane along with 1-propanol and the ytterbium catalyst. The reaction mixture was refluxed for 2 h before evaporation and purification by silica column chromatography. It was found that the desired product **161** was in highly pure

state and was isolated in 100% yield. This pleasing result meant that the use of the ytterbium catalyst could be expanded to include the highly efficient cleavage of epoxides with alcohols.

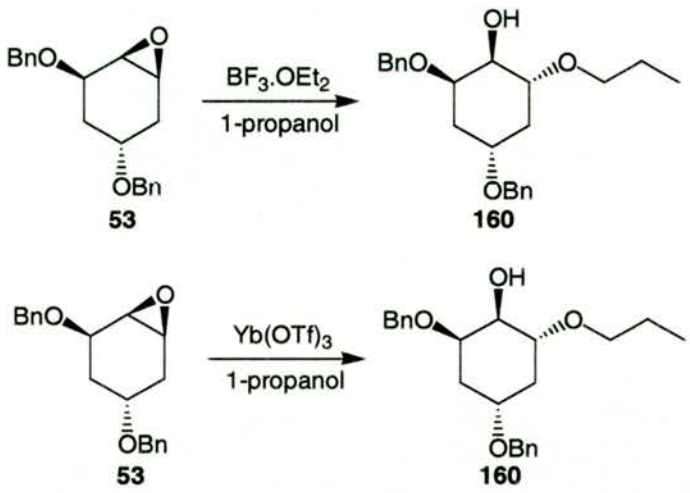


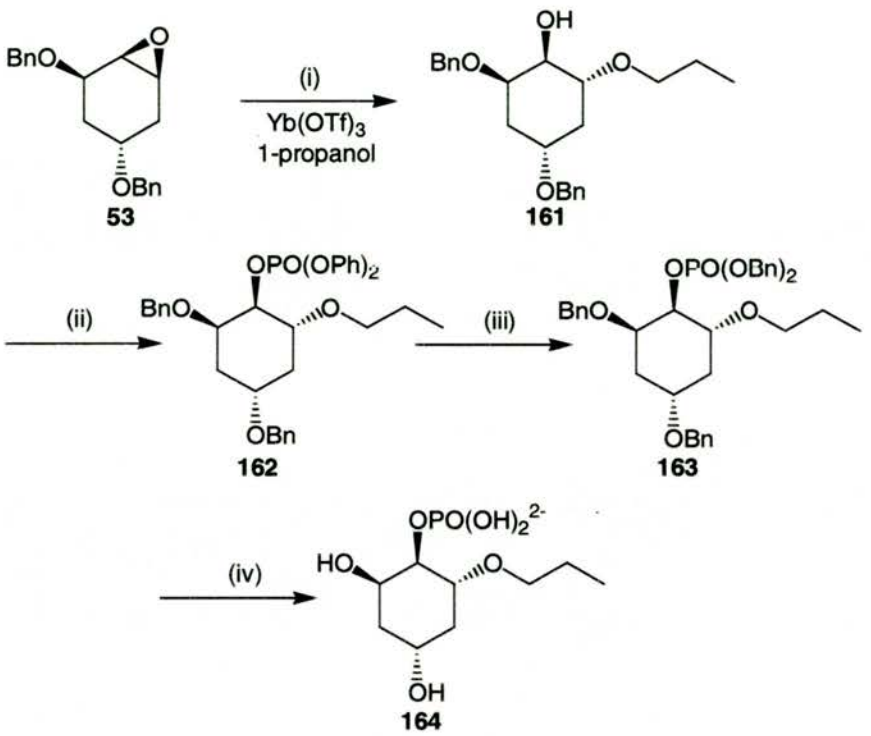
Figure 5.1 *Alcoholysis catalysts compared directly*

To demonstrate the superiority of the reaction sequence the ^{1}H NMR spectra of the crude products can be directly compared (Figure 2.23, page 107)

The propanol derivative **160** from the ytterbium cleavage reaction was phosphorylated using diphenylchlorophosphate and DMAP. The reaction was found to be successful and after purification by column chromatography the product was isolated as a colourless oil (79%) **162**. The product was identified using ^{13}C, ^{1}H & ^{31}P NMR spectroscopic analysis and comparisons with data from identical products synthesised using the boron trifluoroetherate method.

Transesterification was accomplished using the standard benzyl alcohol/NaH method to produce after purification the desired fully protected target molecule **163** in 67% yield. It was decided that further deprotection would be fruitless as the reaction and the resulting enzyme activity had already been documented. The important information gained from this reaction sequence was that the later steps

following epoxide cleavage were equally successful using ytterbium triflate as with boron trifluoride diethyletherate.

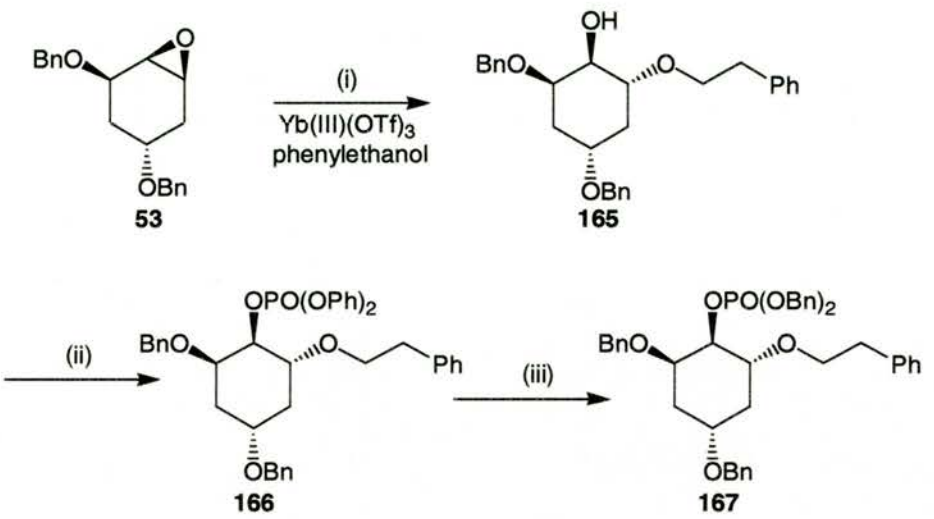


Reagents and Conditions: (i) $Yb(OTf)_3$, 1-propanol, 1, 2-dichloroethane, reflux, 3 h, 98%; (ii) DCM, TEA, diphenyl chlorophosphate, DMAP, 3 h, 20 °C, 92%; (iii) THF, NaH, BnOH, -70 °C, 2 h, 67%.; Na/NH_3 (liq.), 65%

Scheme 5.1 *Completion of fully protected 1-propanol derivative using ytterbium catalyst*

To test the flexibility of the new procedure a variety of alcohols were tested using ytterbium triflate. The first target was the phenylethanol derivative **165**. Reacting the epoxide **53** with the alcohol in the presence of ytterbium triflate overnight gave the desired product in 65% yield after silica column chromatography. The reason for the low yield was found to be as a result of the similar r.f. values of the opened epoxide product **165** and phenylethanol which caused mixing and proved difficult to separate. Phosphorylation was achieved using diphenylchlorophosphate in the presence of DMAP and TEA. The mixture was stirred overnight and the product **166** after standard isolation techniques was a clear oil (75%).

The phosphate was transesterified using benzyl alcohol and NaH in DCM. The resultant mixture was purified using silica column chromatography to give a low yield (18%) of the product **167**. There was insufficient starting material for a reasonable amount of target material to be isolated following deprotection.

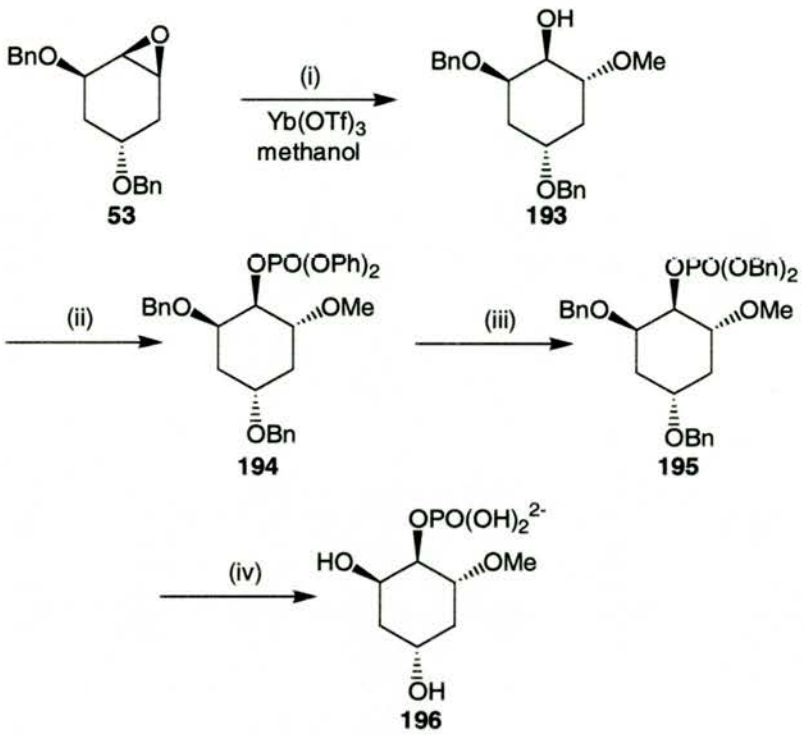


Reagents and Conditions: (i) $Yb(OTf)_3$, phenylethanol, 1,2-dichloroethane, reflux, 2 h, 65%; (ii) DCM, TEA, diphenyl chlorophosphate, DMAP, 3 h, 20 °C, 96%; (iii) THF, NaH, BnOH, -70 °C, 2 h, 18%.

Scheme 5.2 *Completion of fully protected phenyl ethyl derivative using ytterbium catalyst*

Previous research had demonstrated the ability of boron trifluoro etherate in opening the epoxide with methanol. However the cleavage had never been attempted with the ytterbium catalyst. This was a reasonable reaction to carry out because parallels could be drawn with the well documented BF_3 method to ensure the correct functioning of the catalyst. The alcohol was shown to cleave the epoxide within 3 hours in the presence of the ytterbium catalyst to give **193** in 97% yield. The enantiomer was fully characterised and shown to be the target compound. Phosphorylation with diphenylchlorophosphate **194** (37%) and transesterification **195** (55%) gave the fully protected precursor which was deprotected by dehydrogenation to give the fully deprotected target **196** which

was isolated as it's cyclohexyl-ammonium salt (51%). [Testing was not carried out due to time constraints]



Reagents and Conditions: (i) $Yb(OTf)_3$, methanol, 1, 2-dichloroethane, reflux, 3 h, 97%; (ii) DCM, TEA, diphenyl chlorophosphate, DMAP, 3 h, 20 °C, 37%; (iii) THF, NaH, BnOH, -70 °C, 2 h, 55%.; Na/NH_3 (liq.), 51%

Scheme 5.3 *Completion of fully protected methyl derivative using ytterbium catalyst*

While research had been continuing into the design of a procedure for amino derivatives of the IMPase substrate inositol monophosphate much work had been continuing into the design of advanced alcohol based targets.

These targets are discussed in detail above (Section 1.37, page 54) however they were based on designs that incorporated mono- and bi-cyclic structures to produce mono and di anionic derivatives that might be capable of binding near the active site bound by Ser 160, Val 40 and Leu 42.

6 Di-alkylated alcohol derivatives

The research into those interesting targets was continued using the ytterbium catalysed alcohol epoxide cleavage technique optimised above.[74]

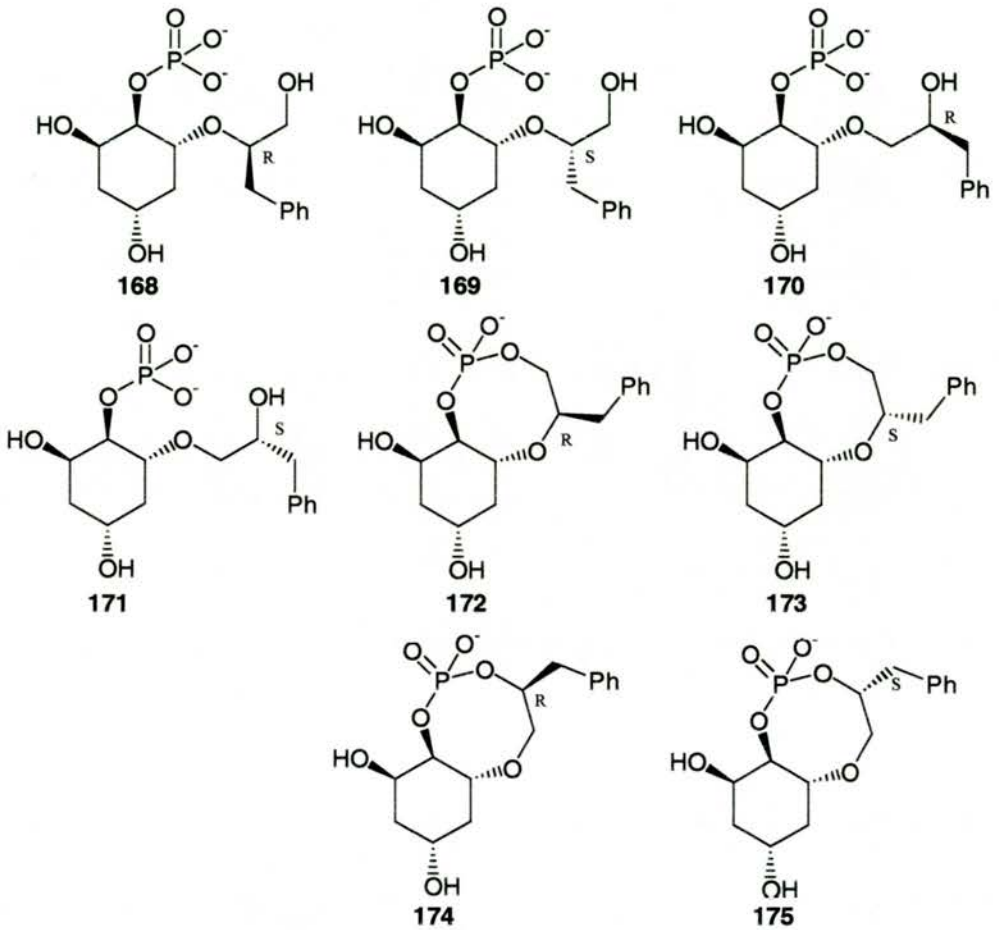


Figure 6.1 *Various alcohol based targets proposed*

Before epoxide cleavage could be achieved it was necessary to first produce the alcohols that would be used to perform the cleavage (Figure 6.2).

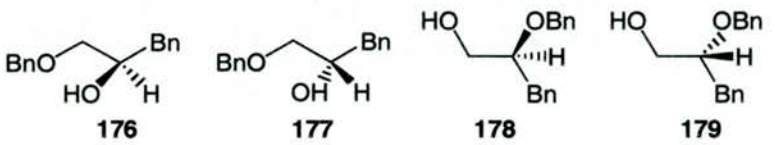
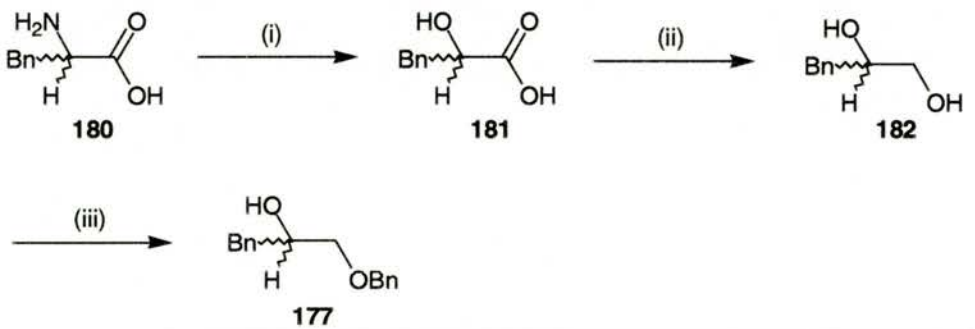


Figure 6.2 *Alcohols necessary for the cleavage of the epoxide*

The preparation of compound **168** and **169** began with the synthesis of (*D*) and (*L*) – phenyl lactic acid **181**. (*D*) or (*L*) Phenylalanine **180** was converted to the corresponding phenyl lactic acid **181** by dissolving in a solution of sulphuric acid at 0 °C followed by addition of sodium nitrite.

The mixture was stirred overnight before the addition of solid sodium bicarbonate. The mixture was then filtered and the elutant acidified with HCl to pH 2. The product was extracted with THF which was evaporated and the resulting solid recrystallised from toluene the acids (*D*) & (*L*) **181** were obtained in 41% and 46% yield respectively.



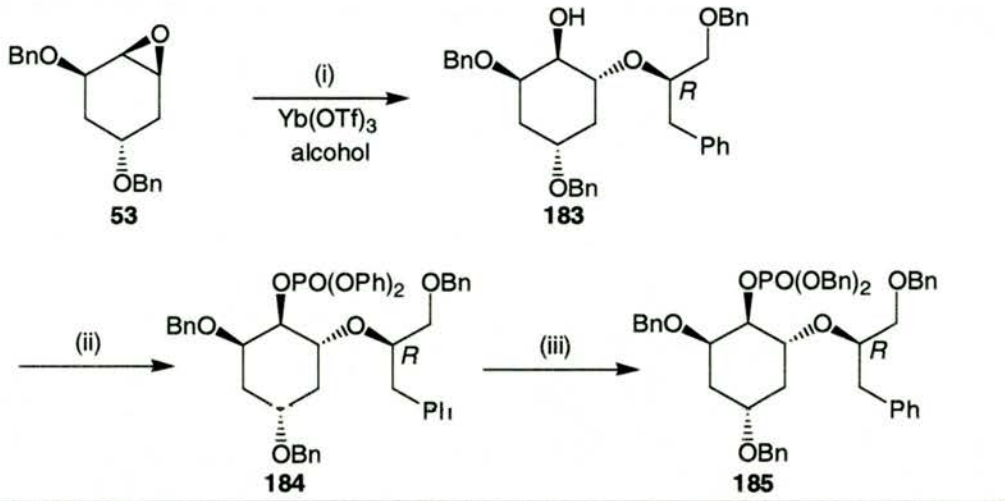
Reagents and Conditions: (i) Sulphuric acid, sodium nitrite 0 °C, 12 h, 41%; (ii) BH_3 1 h, 0 °C, 99%; (iii) THF, NaH, BuOH, -70 °C, 12 h, 18%.

Scheme 6.1 *Preparation of 1' alcohol target precursors.*

The acid was identified from comparisons with library ^1H NMR spectra.[146] It was then necessary to reduce the resulting acid groups **181** to the desired alcohol **182**. This was achieved using borane dissolved in THF. Thus the respective alcohols (*D*) and (*L*) **181** were added to a solution of BH_3 (1 M solution in THF). The reaction was stirred for 1 h before quenching in a sodium bicarbonate/ice bath. The mixture was extracted with DCM and after purification the respective alcohols **182** were isolated as oils (in quantitative yield).

Before the alcohol **182** could be used in the cleavage reaction it was necessary to selectively protect the primary alcohol in the presence of the secondary alcohol. This was easily achieved using an equimolar quantity of benzyl bromide and alcohol. The principle behind the experiment was that primary alcohols usually react faster than secondary alcohols in S_N2 reactions so would become protected preferentially. The reaction proceeded satisfactorily in THF with KH overnight. The reaction mixture was washed, separated and evaporated to produce a thick oil. Analysis of the oil showed that some benzylation of the secondary alcohol had occurred but the mixtures were easily separated to give the desired product **177** as a yellow oil (18%).

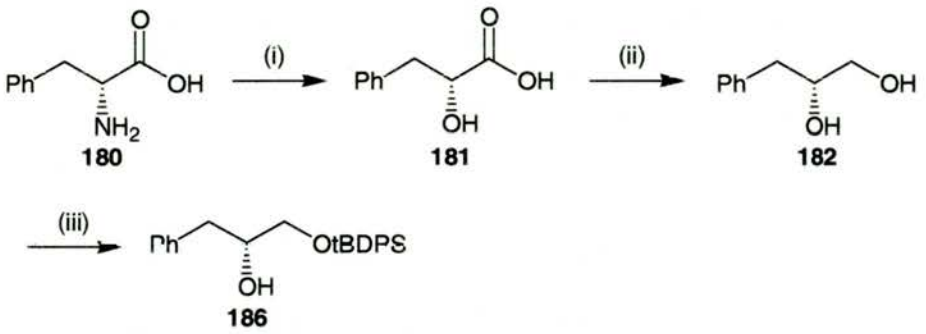
The alcohol (*R*) **177** was then used to open the epoxide under the standard conditions. The alcohol was reacted with the epoxide **53** in the presence of ytterbium triflate to give, after the appropriate work-up conditions, **183** as an oil in reasonable yield (67%). Phosphorylation was achieved using diphenylchlorophosphate, TEA and DMAP, following overnight stirring the product **184** was isolated as a clear oil in excellent yield (85%). Transesterification was carried out using benzyl alcohol and NaH. The reaction mixture was stirred overnight to return **185** in reasonable yield (53%) as an oil. The purity level of this molecule identified through ^{13}C, ^{1}H & ^{31}P NMR spectroscopic analysis was insufficient to complete the sequence. It was proposed that the number of inseparable impurities was based on the poor purity level of the alcohol used for the cleavage.



Reagents and Conditions: (i) $Yb(OTf)_3$, alcohol (X), 1,2-dichloroethane, reflux, 2 h, 67%; (ii) DCM, TEA, diphenyl chlorophosphate, DMAP, 48 h, 20 °C, 85%; (iii) THF, NaH, BnOH, -70 °C, 2 h, 53%.

Scheme 6.2 *Synthesis of alcohol target*

The disappointing yield of this reaction encouraged research into modifications of the technique which could return better yields of the desired alcohols.



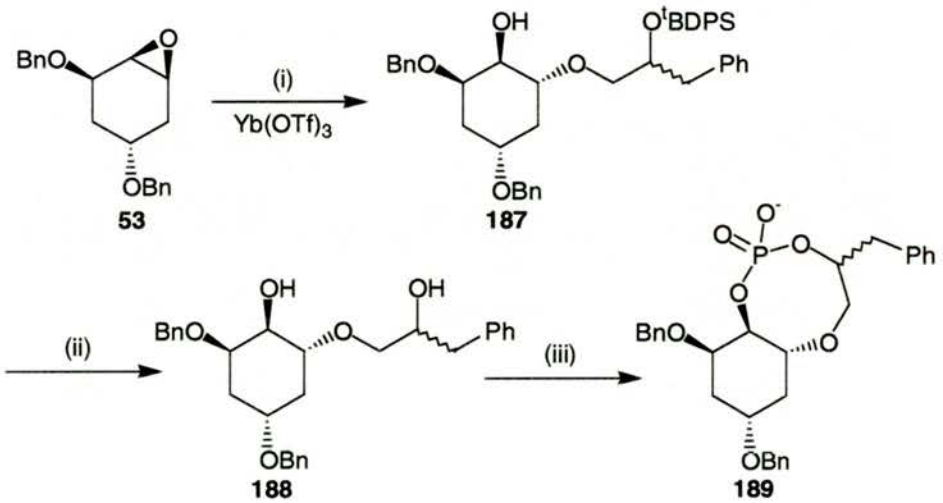
Reagents and Conditions: (i) Sulphuric acid, sodium nitrite 0 °C, 12 h, 41%; (ii) BH_3 1 h, 0 °C, 99%; (iii DCM, TEA, diphenyl chlorophosphate, DMAP, 48 h, 20 °C, 89%.

Scheme 6.3 *Using tert-butly diphenylsilylchloride as the alcohol protecting agent*

As an alternative to the benzylation technique of protecting the primary alcohol **182** tert-butyldiphenylsilylchlorophosphate was used as a protecting group. This bulky silyl group is rather hindered and as a consequence should avoid the more hindered secondary alcohol. The diol **182** was dissolved in DCM and stirred in the presence of TBDPSCl and TEA and DMAP overnight. Once purified it was found

that the reaction had been highly successful giving the correctly protected alcohol **186** in good yield (89%).

Cleavage of the epoxide **53** with the (*R*) variant of the silylated alcohol was attempted using the normal conditions. The epoxide **53** was stirred in refluxing 1,2-dichloroethane for 4 h in the presence of the ytterbium triflate catalyst. The product was isolated in the normal fashion producing **187** as an oil in quantitative yield. The product was identified using ^{13}C, ^{1}H & ^{31}P NMR spectroscopy.



Reagents and Conditions: (i) $Yb(OTf)_3$, alcohol (X), 1,2-dichloroethane, reflux, 4 h, 100%; (ii) TBAF/THF, 20 °C, 12 h, 100%; (iii) DCM, di-isopropyl-N-ethylamine, diisopropyl-o-methyl phosphonamidic chloride, MCPBA

Scheme 6.4 *Synthesis of cyclic derivative*

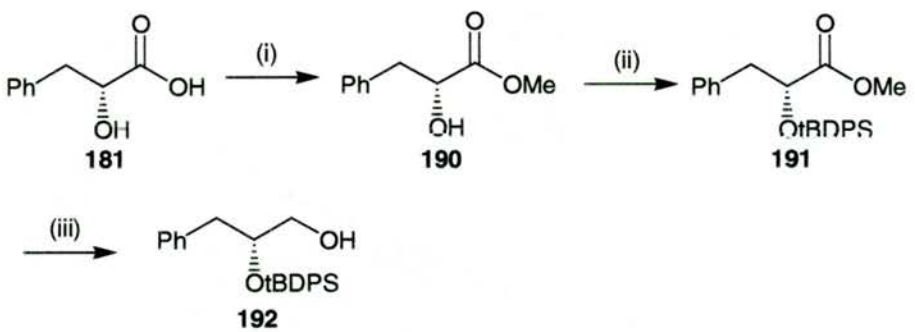
The test reaction continued by attempting to desilylate the protected alcohol **187** in preparation for the cyclisation reaction. The deprotection was achieved using TBAF in THF. The reaction was a complete success, returning the diol **188** in quantitive yield.

[Cyclisation of such molecules to produce **189** had been successfully achieved by Schulz *et. al.* by the following method: The diol was dissolved in DCM and treated with di-isopropyl-N-ethylamine followed by diisopropyl-o-methyl

phosphonamidic chloride. The mixture was stirred for 1 h and the solvent then removed. The resulting oil was dissolved in a solution of tetrazole in THF-acetonitrile and the reaction mixture stirred for 14 h. To complete the reaction the solvents were removed under reduced pressure and a solution of MCPBA in DCM was added. After stirring for a further hour DCM and water was added. The reaction mixture was separated and extracted before evaporation to yield **189**.]

It was also necessary to produce an alcohol with the secondary alcohol protected while leaving the primary free **178** & **179**.

To do this it was necessary to attempt the protection earlier in the sequence before the acid group **181** was reduced to the primary alcohol **182**. To prevent interference from the acid group **181** during silylation of the secondary alcohol it was necessary to mask it. The simplest way to do this was found to be via a simple methyl ester formation **190**.



Reagents and Conditions: (i) Thionyl chloride, methanol, 3 h, 0 °C, 95%; (ii) DCM, TEA, diphenyl chlorophosphate, DMAP, 12 h, 20 °C, 100%; (iii) $LiAlH_4$, EtOH, -70 °C, 5 h, 100%.

Scheme 6.5 *Protection of secondary alcohol while leaving primary free*

Thus the lactic acid **181** was dissolved in methanol and thionyl chloride was added. After 3 h reaction the reaction was quenched with sodium bicarbonate and **190** extracted using organic solvents. The esterification reaction was found to

have occurred in high yield (95%). With the acid group masked as the ester it was then a simple procedure to protect the remaining alcohol with the silyl group. This was achieved as before using TBDPSCl, TEA and DMAP in DCM. The product **191** after purification was a clear oil. Reduction of the ester to the alcohol was accomplished using $LiAlH_4$ in EtOH at –70 °C for 5 hours. Analytical analysis of the resulting product showed that the ester had indeed been reduced giving **192** quantitatively. This alcohol was then ready to be used in epoxide cleavage reactions. Due to time constraints this cleavage reaction was never attempted.

7 Enzymatic Analysis

Testing of the molecules as enzyme substrates was achieved in qualitative studies. The candidate molecules were incubated in the presence of the enzyme in NMR tubes whilst monitoring a chosen signal (usually the ^{31}P resonance) for changes.

7.1.1 General method of NMR spectroscopic studies

The substrates to be tested by NMR spectroscopy were dissolved in buffered deuterium oxide solutions (0.5 cm^3) of each of the amino phosphate esters (15 mM) containing magnesium chloride (2mM) were monitored by ^1H NMR spectroscopy and spectra were acquired at 15 minute intervals in a solution of $MgCl_2$ (2mM) at pH 8.0 in ^2H_2O to a concentration of 60 mmol dm^{-3} in a 5 mm NMR tube. The ^1H NMR spectrum was recorded and recombinant enzyme solution IMPase (30 units) was added. The ^1H NMR spectrum was recorded immediately to account for any signals arising from the enzyme buffer system. The samples were incubated at 37 °C for periods up to 72 h and the ^1H and ^{31}P NMR spectra were recorded at regular time intervals (15 min) to assess the extent of hydrolysis.

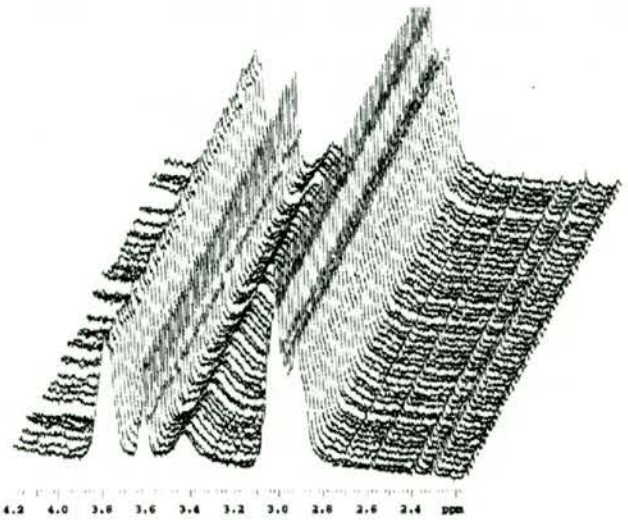


Figure 7.1 *^{1}H NMR array spectra of 1 amino-2 phosphate model **132** showing no time dependent hydrolysis*

7.1.2 Ammonia model

The 1-amino-2-phosphate model **132** was incubated for 48 hours in the presence of the enzyme and the reaction followed by ^{1}H NMR spectroscopy. However there was no evidence of hydrolysis as illustrated by the ^{1}H NMR spectroscopic array shown (Figure 7.1). The data shows the CHN group at 3.0 ppm and the CHOP proton at 3.8 ppm. If hydrolysis were to occur it would be expected that these signals would change dramatically. This result was not unexpected as the molecule is missing several of the identified important binding groups (section 1.19, page 21).

7.1.3 Ammonia target

The same reaction with the 6-amino phosphate **137** gave a more promising result. NMR analysis showed that the compound was hydrolysed completely within 6 h to give the expected product, indicating that it was a moderate substrate. Note that the physiological substrate **10** is hydrolysed completely within 15 minutes under these conditions. The result indicates that a 6-amino group is able to fully support catalysis and, indeed, is the first example of a compound possessing any substituent other that a hydroxyl group at C-6 to have been shown to display substrate activity. The peak that is obviously reducing in size is the CHOP peak at 3.41 ppm which would be expected to disappear if hydrolysis were to occur.

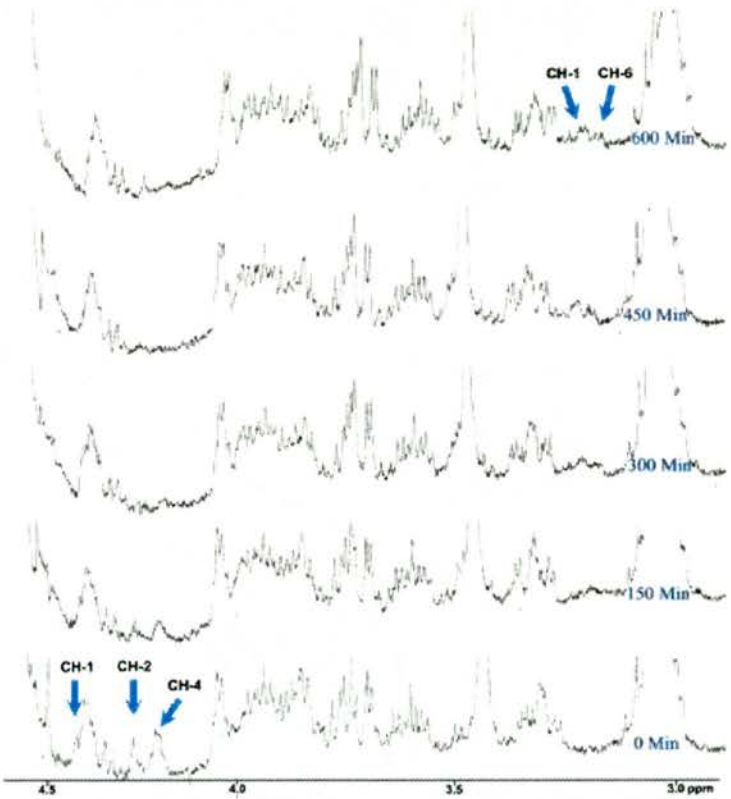


Figure 7.2 *Time dependent hydrolysis of ammonia derivative by inositol monophosphatase*

The phosphate **137** was tested for biological activity using standard enzyme assay conditions except that a solution of Mg2+ ions at a concentration of 6 mM was

used.[33] Fresh batches of the substrate was prepared and all traces of inorganic phosphate were removed by recrystallisation. The primary amine displayed a υ_{max} value of 8.00 μM min^{-1} using 15 mg of enzyme per assay and a *K*m value of 300 μM, which is nine times the υ_{max} value for 2'-AMP under standard conditions.[147]

7.1.4 Methylamino target

The 6-methylamino phosphate **146** took over 10 h before a change could be observed in the ^{1}H NMR spectrum. The result indicated that the 6-methylamino phosphate **146** was a poor substrate for IMPase. Albeit encouraging, the very low rate of reaction precludes a firm conclusion on the basis of the observation alone because the presence of trace levels of contaminating non-specific phosphatases which can act upon the 6-methylamino phosphate **146** cannot be excluded. Nevertheless, it was established that the 6-amino phosphate was a moderate substrate and that analogues of the compound with which to probe the mechanism further could be prepared in useful quantities from the key epoxide **53** through its direct cleavage with amines.

The phosphate **146** was also tested for biological activity using the same conditions as **137**[33] This preliminary study gave values of 4.00 μM min^{-1} for υ_{max} and 140 μM for *K*m.[147]

Conclusion

8 Conclusion

The quinic acid route to the key epoxide **53** provides a quick and easy way of producing large amounts of the intermediate which is important in the preparation of alcohol and amino inositol monophosphate derivatives.

It has been discovered that although it is possible to use azides to open the epoxide and methods exist for the subsequent reduction step this route seems unwieldy and inefficient compared to the new direct aminolysis reaction using ytterbium triflate.

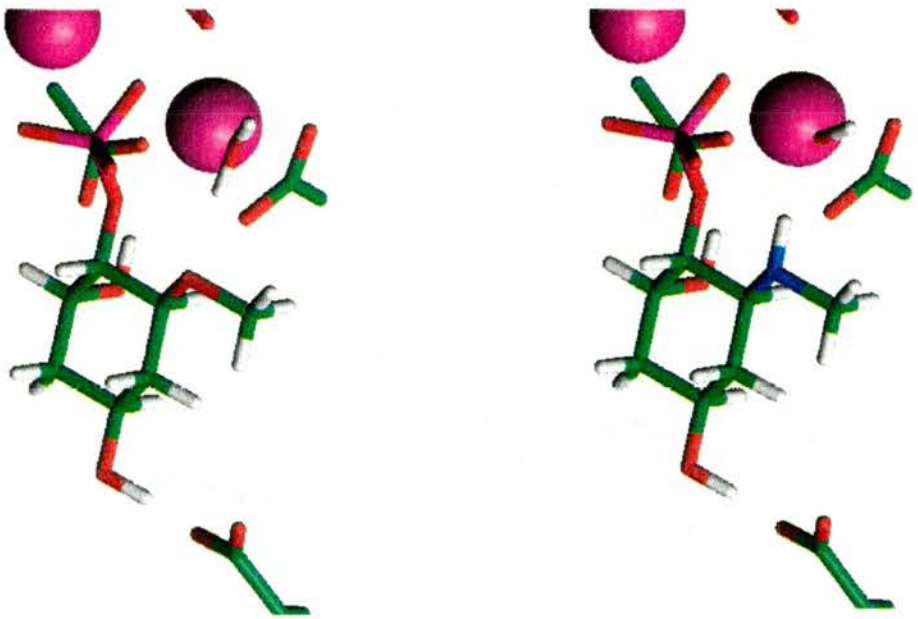
This catalyst, ytterbium triflate, used under the right conditions has been found to be highly successful in the cleavage of a range of epoxides with nucleophiles. The scope of the reaction has been extended to include a range of and has been shown to work well in a variety of solvents.

The fact that the ammonia **137** and methylamine **146** derivatives are substrates for IMPase is completely in accord with the predictions of earlier molecular modelling work and provides additional support for the structural detail of the active complex.[40] The results indicate that there is a nucleophilic hydroxide ion bound to $Mg^{2+}2$ and simultaneously hydrogen bonded to the 6-OH proton of Ins 1-P. The results do not rule-out the alternative in-line mechanism but, if such a mechanism is followed, it is extremely difficult to understand why the replacement of the 6-OH group in a substrate by an alkoxy group or by a hydrogen atom should give a tight-binding non-hydrolysable compound. Yet, (as in the methylamine **146** example) when using an amine to replace the 'lost' lone pair on the methoxy inhibitor **196** the compound becomes a substrate. Molecular modelling of the proposed mechanism clearly shows how the methylamine group

would be able to support hydrolysis wherease the methoxy cannot (Figure 8.1). Clearly such inhibitors would not prevent a water molecule from binding to (or remaining on) $Mg^{2+}2$ in ternary complexes.

The finding that the 6-amino phosphate esters serve as substrates was expected and implies that hydroxide ion chelated to $Mg^{2+}2$ and H-bonded through to the C-6 heteroatom serves as the nucleophile in an adjacent displacement reaction on the phosphate P-atom. Such a mechanism would proceed with retention of configuration. It was expected that the ethylamine derivative would also act as a substrate using the same mechanism as the ammonia and methylamine derivatives, however based on the above analysis the formamide derivative should be unable to support hydrolysis.

The results of this research introduce for the first time novel amino derivatives of inositol monophosphate while also providing strong independent proof for the mechanism employing the nucleophillic water molecule on $Mg^{2+}2$.[147]



A **B**

Figure 8.1 *Methoxy (A) and methylamine (B) modelled in active site*

Experimental

NMR spectra were recorded on a Varian 300 (300 MHz; fourier transform. ^{1}H NMR and 75 MHz; ^{13}C NMR) or a Varian Gemini f.t. spectrometer (200 MHz; f.t. ^{1}H NMR and 50 MHz; ^{13}C NMR). Chemical shifts for both ^{1}H and ^{13}C nmr spectra are described in parts per million shift from TMS and are reported consecutively as position (δ_H or δ_C), relative integral, multiplicity (s-singlet, d-doublet, t-triplet, q-quartet, dd-double of doublets, sep-septet, m-multiplet, and br-broad), coupling constant (Hz) and assignment (numbering according to the IUPAC nomenclature for the compound). ^{1}H-NMR were referenced internally on ^{2}HOH (4.68 ppm), $CHCl_3$ (7.00ppm) or DMSO (2.47ppm). ^{13}C-NMR were referenced on CH_3OH (49.9 ppm), $CHCl_3$ (77.5 ppm), or DMSO (39.70 ppm).

IR spectra were recorded on a Perkin-Elmer 1710 f.t. IR spectrometer. The samples were prepared as Nujol mulls, solutions in chloroform or thin films. The frequencies (ν) as absorption maxima are given in wavenumbers (cm^{-1}) relative to a polystyrene standard. Mass spectra and accurate mass measurements were recorded on a VG 70-250 SE, and a Kratos MS-50.

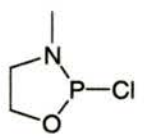
Melting points were taken on an Electrothermal melting point apparatus and are uncorrected. Optical rotations were measured at room temperature on an optical activity AA-100^{o} polarimeter using 10 or 20 cm path length cells; $[\alpha]_D$-values are given in 10^{-1} deg cm^2 g^{-1}.

Analytical thin layer chromatography (TLC) was carried out on 0.25 mm precoated silica gel plates and compounds were visualised using UV fluorescence, iodine vapour, or ethanolic phosphomolybdic acid.

The solvents used were either distilled or of Analar quality and petrol ether refers to that portion boiling between 40 and 60 ^{o}C. Solvents were dried according to literature procedures. Ethanol and methanol were dried over magnesium turnings.

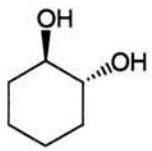
DMF, toluene, dichloromethane (methylene chloride), triethylamine and pyridine were dried over CaH_2. THF and diethyl ether were dried over sodium/benzophenone and distilled under nitrogen.

9.1 2-chloro-3-methyl-1,3,2-oxazaphosphacyclo pentane 54[77]



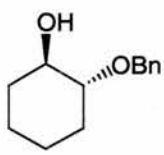
A solution of dry methylaminoethanol **55** (16 cm^3, 0.2 mol) and triethylamine (32 cm^3, 0.23 mol) in dichloromethane (32 cm^3) were added dropwise with vigorous stirring to dichloromethane (40 cm^3) at –40 °C under an atmosphere of nitrogen. Simultaneously, phosphorus trichloride (20 cm^3, 0.23 mol) in dichloromethane (50 cm^3) was added dropwise. After warming to –30 °C, a further portion of triethylamine (32 cm^3, 0.23 mol) in dichloromethane (20 cm^3) was added dropwise, with vigorous stirring. The solution was allowed to warm to room temperature and stirred for a further 2 hours. The solvent was then removed under reduced pressure and the residue extracted with dry diethylether (3 x 100 cm^3). The extract was then filtered and the filtrate concentrated under reduced pressure to give a yellow oil. This oil was purified by vacuum distillation to give **54** as a clear colourless oil (10.52 g, 43%); (b.p. 30-35 °C @ 0.1 mmHg); υ_{max} (Nujol)/cm^{-1} 1478 s, 1200 s and 708 s; δ_H (300 MHz; C^2HCl_3) 2.75 (3 H, d, $^2J_{P\text{-}H}$ 21, Me), 30.85 (2 H, m, CH_2N), 4.30-4.60 (2 H, b, CH_2O); δ_C (75.4 MHz; C^2HCl_3) 30.85 (CH_3N, $^2J_{C\text{-}P}$ 14), 48.67 (CH_2N, $^2J_{C\text{-}P}$ 7), 70.69 (CH_3O, $^2J_{C\text{-}P}$ 10); δ_P (121 MHz; C^2HCl_3) 170.09.

9.2 *Trans*-1,2-cyclohexanediol 203



(±)-Cyclohexene oxide **56** (5 g, 50.9 mmol) was stirred for 3 h in the presence of a 98-100% formic acid solution (60 cm^3). The formic acid was then removed under reduced pressure and the residue refluxed with 20% aqueous sodium hydroxide containing a a few drops of ethanol. The diol was extracted by washing with diethyl ether (2 x 50 cm^3) and the organic solution evaporated at reduced pressure to yield the product, *trans*-1,2-cyclohexanediol **203** as colourless crystals (4.31g, 73%); m.p 101-103 °C; δ_H (300 MHz; C^2HCl_3) 1.07 (4 H, m, 2 x cyclohexylidine CH_2), 1.60 & 1.92 (4 H, m, 2 x C*H*$_2$OH), 3.31 (2 H, m, 2 x C*H*O), 3.80-4.25 (2 H, b, 2 x OH); δ_C (75.4 MHz; C^2HCl_3) 24.67 (2 x *C*H_2), 33.24 (2 x *C*H_2OH), 78.30 (2 x *C*HOH).

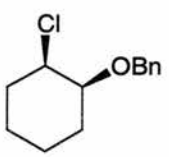
9.3 *Trans*-2-benzyloxy-cyclohexanol 57



Sodium hydride (1.5 g, 6.25 mmol) was added to a suspension of trans-1,2-cyclohexanediol **203** (4 g, 3.44 mmol) in dry DMF (20 cm^3) at 0 °C. After 20 min, benzyl bromide (4.1 cm^3, 1.5 mmol) was added. The reaction mixture was warmed to room temperature and stirred overnight. Water (5 cm^3) was then added to quench remaining sodium hydride before the solvents were removed by evaporation. The residual oil was then re-dissolved in diethyl ether (2 x 50 cm^3),

washed with brine (2 x 50 cm^3), separated the organic layer, dried (Mg_2SO_4) and evaporated to give the mono and di-benzylated mixture as a brown oil. The oil was purified by silica column chromatography (ethyl acetate-petroleum ether, 1:10) to give the product *trans*-2-benzyloxy-cyclohexanol **57** as a lightly coloured liquid (2.78 g, 42%); δ_H (200 MHz; C^2HCl_3) 1.13-1.37 (4H, m, 2 x CH_2), 1.61-1.75 (2H, m, CH_2), 1.95-2.19 (2H, m, CH_2), 3.11-3.26 (1H, m, C<u>H</u>OH), 3.42-3.56 (1H, m, CHOBn), 4.60 (2H, dd $^2J_{H-H}$ 24 Hz, $^3J_{H-H}$ 4 Hz, CH_2Ph), 7.25-7.42 (5H, ar, Ph); δ_C (50 MHz; C^2HCl_3) 24.52, 24.71, 29.75, 32.67 (cyclohexane CH_2), 71.31 (CHOH), 74.18 (CHOBn), 83.89 (CH_2Ph), 128.13, 128.23, 128.93 and 139.23 (Ar-C); *m/z* (CI) 207 (94%, $[M + H]^+$), 116 (62, $[MH - CH_2Ph]^+$) and 91 (14, $[C_6H_5]^+$).

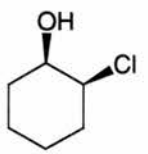
9.4 *Cis*-(2-Chloro-cyclohexyloxymethyl)-benzene 58



Trans-2-benzyloxy-cyclohexanol **57** (200 mg, 1.04 mmol) was dissolved in dichloromethane (15 cm^3). Pyridine (5 cm^3) was added and the reaction was stirred. The temperature was reduced to -70 °C and sulfuryl chloride (0.28 cm^3) was added drop wise. The temperature was then maintained at -40 °C and the solution stirred for 2 h. The solvent was then removed by evaporation and the residue re-dissolved in methanol (15 cm^3). Saturated sodium hydrogen carbonate solution (15 cm^3) was added followed by stirring for 3 h. The resulting precipitate was filtered and the filtrate separated and evaporated, the residue was dissolved in

diethyl ether (2 x 20 cm^3), washed with water (2 x 20 cm^3) and, following separation, evaporated to give the product as a light brown oil. This was purified by silica column chromatography (ethyl acetate-petroleum ether, 1:15) to give **58** as a clear liquid (0.15 g, 64%); (Shown by ^1H NMR spectroscopy to be 74% of the *cis* isomer). δ_H (300 MHz; C^2HCl_3) 1.10-2.18 (8H, m, CH_2 ring), 3.30 (0.5H, m, CHCl *trans* isomer), 3.50 (1H, m, CHCl *cis* isomer) 3.85 (0.5, m, CHOH *trans* isomer), 4.30 (1H, m, CHOH *cis* isomer), 4.58 (2H, m, CH_2Bn), 7.30 (5H, m, aromatic); δ_C (50 MHz; C^2HCl_3) 22.00, 22.94, 27.97 & 32.73 (CH_2, cis ring), 62.16 (CHOBn, cis), 70.76 (CHCl, cis), 128.87 (Ph), 138.97 (CH_2Ph), 23.69, 25.77, 28.86, 35.22 (CH_2, trans ring), 63.40 (CHOBn, trans), 72.36 (CHCl, trans).

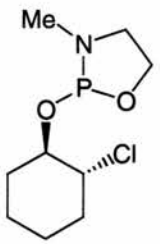
9.5 (±)-*Cis*-2-chloro-cyclohexanol 59



Cis-(2-Chloro-cyclohexyloxymethyl)-benzene **58** (800 mg, 3.57 mmol) was dissolved in dichloromethane (25 cm^3). Boron trifluoroetherate (5.0 g, 35.7 mmol, 4.3 cm^3) was added along with dimethyl sulphide (4.44g, 71.4 mmol, 5.25 cm^3). The mixture was stirred for two and a half hours before adding another 10 equivalents dimethylsulphide (2.22 g, 35.7 mmol, 2.63 cm^3) and stirring overnight. The dichloromethane was then removed by evaporation and the residue taken up with a 10% ammonia solution (20 cm^3) and extracted with ethyl acetate (2 x 20 cm^3). The solvent was evaporated off to give a yellow oil which solidified after prolonged vacuum drying. This was purified by silica column chromatography (ethyl acetate-petroleum ether, 1:10) to give a yellow liquid found to be a mixture of *cis* and *trans* isomers. The isomers were separated by

refluxing the *cis/trans* mixture in a 0.5N NaOH solution for 1 h resulting in elimination of the Cl moiety on the trans isomer. The *cis* product was extracted and isolated by evaporating and distilling the residue to give **59** as a clear liquid. (0.16g, 33%). b.p. 76-82 °C (20 mmHg); δ_H (300 MHz; C^2HCl_3) 1.20-1.25 (8H, m, CH_2 ring), 2.65 (1H, b, OH), 3.44-3.57 (1H, m, CHO) 3.67-4.78 (1H, m, CHCl); δ_C (50 MHz; C^2HCl_3) 21.95 & 22.64 (2 x CH_2), 31.01 & 32.17 (2 x CH_2), 66.53 (CHOH), 70.90 (CHCl).

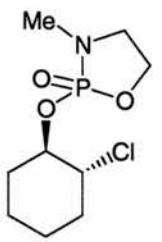
9.6 *(±)-Trans*-2-(2-Chloro-cyclohexyloxy)-3-methyl-[1,3,2]oxazaphospholidine 62



A solution of commercially available *trans*-2-chloro-cyclohexanol (±)-**60** (2.5g, 18.6 mmol) and triethylamine (1.88g, 18.6 mmol) in dichloromethane (dry) (50 cm^3) was added dropwise with vigorous stirring to the phosphoramidite **54** (2.51g, 18 mmol) in dichloromethane (50 cm^3) at 0 °C in an atmosphere of nitrogen. The solution was warmed to ambient temperature, with stirring for 30 min, and then extracted with a saturated solution of sodium hydrogen carbonate (100 cm^3) followed by saturated brine (100 cm^3). The solution was then dried (Mg_2SO_4) and the solvent removed under reduced pressure to give (±)-**62** as a lightly coloured oil. ^{13}C NMR spectroscopic analysis showed the presence of the two diastereoisomers which were not separated. 0.85g (97%); δ_P (121 MHz; C^2HCl_3) 141.88 & 138.81; δ_H (300 MHz; C^2HCl_3) 1.19-2.39 (8H, m, CH_2 ring), 2.65 (2 x 3

H, d, $^2J_{P-H}$ 12, 2 x Me), 2.88 & 3.07 (2 x 2H, m, CH_2N) 3.69 (1H, m, CHCl), 3.78 (1H, m, CHOH), 4.20 & 4.32 (2H, m, CH_2O); δ_C (50 MHz; C^2HCl_3) 23.22 & 23.51 (2 x CH_2), 24.20 & 24.67 (2 x CH_2 [dias]), 31.50 & 32.10 (2 x CH_3N, $^2J_{C-P}$ 21 Hz), 33.50 & 33.98 (2 x CH_2), 34.38 & 34.93 (2 x CH_2 [dias]), 49.26 (CH_2N, $^2J_{C-P}$ 5 Hz), 63.61 (CHCl), 63.86 (CHCl [dias]), 68.42 (CH_2O, $^2J_{C-P}$ 10 Hz), 68.78 (CH_2O [dias], $^2J_{C-P}$ 10 Hz), 75.70 (CHO, $^2J_{C-P}$ 13 Hz), 76.21 (CHO, $^2J_{C-P}$ 13 Hz).

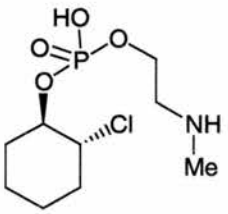
9.7 *(±)-Trans*-2-(2-Chloro-cyclohexyloxy)-3-methyl-[1,3,2]oxazaphospholidine 2-oxide 63



A saturated DCM solution of dinitrogen tetraoxide (4.67 cm^3, 4.9 mg, 0.53mmol) was added dropwise to *trans*-2-(2-Chloro-cyclohexyloxy)-3-methyl-[1,3,2] oxazaphospholidine (±)-**62** (0.5 g, 2.11 mmol) in DCM (15 cm^3) at –70 °C. The solution was warmed to ambient temperature with stirring for 30 min and the solvent removed under reduced pressure to yield (±)-**63** as an oil (0.52 g, 99%); δ_H (300 MHz; C^2HCl_3) 1.11-2.32 (8H, m, CH_2 ring), 2.63 (3 H, d, $^2J_{P-H}$ 11 Hz), 2.68 (3 H [dias], d, $^2J_{P-H}$ 11 Hz), 3.08 & 3.29 (2 x 2H, m, CH_2N) 3.69-3.88 (1H, m, CHCl), 4.08-4.44 (3H, m, CHOH & CH_2O); δ_C (50 MHz; C^2HCl_3) 23.31 & 23.61 (2 x CH_2), 24.50 & 24.70 (2 x CH_2 [dias]), 31.71 & 32.40 (2 x CH_3N, $^2J_{C-P}$ 21 Hz), 34.12 & 34.99 (2 x CH_2 [dias]), 49.37 (CH_2N, $^2J_{C-P}$ 5 Hz), 63.93 (CHCl), 64.02 (CHCl [dias]), 68.54 (CH_2O, $^2J_{C-P}$ 10 Hz), 68.67 (CH_2O [dias], $^2J_{C-P}$ 10 Hz), 80.39 (CHO), 80.64 (CHO, [dias]); δ_P (121 MHz; C^2HCl_3) 20.71 & 20.79.

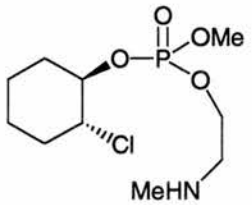
[A saturated solution of N_2O_2 was made by dissolving 0.2 cm^3 of liquid N_2O_4 in 50 cm^3 of ice cold DCM (dry). This equated to 0.524 g (in 50 cm^3) which translated as 5.69 mmol per 50 cm^3.]

9.8 *Trans*-Phosphoric acid 2-chloro-cyclohexyl ester 2-methylamino-ethyl ester 64



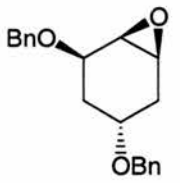
2-(2-Chloro-cyclohexyloxy)-3-methyl-[1,3,2]oxazaphospholidine 2-oxide (±)-**63** (0.25 g, 1.05 mmol) was suspended in of water (5 cm^3) and stirred at ambient temperature for 3 h. The mixture was lyophilised to yield (±)-**64** as a white solid. m.p. 43-45 °C (0.28 g, 99%); (HRMS: found: M^+, 271.0782 $C_9H_{19}ClNO_4P$ requires 271.0740); δ_H (300 MHz; C^2HCl_3) 1.16-2.27 (8H, m, CH_2 ring), 2.62 (3 H, s, NMe), 3.12 (2H, m, CH_2N) 3.79 (1H, m, CHCl), 3.99 (1H, m, CHOP), 4.16 (2H, m, CH_2OP); δ_C (50 MHz; C^2HCl_3) 22.98 & 24.17 (2 x CH_2), 32.16 (CH_2), 33.46 (CH_3N), 34.77 (CH_2), 49.82 (CH_2N), 60.96 (CH_2O), 62.68 (CHCl, $^2J_{C\text{-}P}$ 6), 78.23 (CHO); δ_P (121 MHz; C^2HCl_3) –1.11; *m/z* (EI) 272 (18%, $[M + H]^+$), 236 (37, $[M + H - Cl]^+$) and 80 (100, $[PO_3H]^+$).

9.9 *(±)-Trans*-Phosphoric acid 2-chloro-cyclohexyl ester methyl ester 2-methylamino-ethyl ester 65



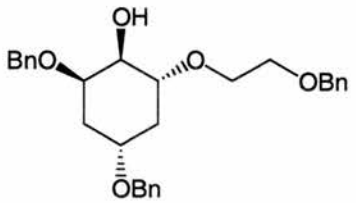
2-(2-Chloro-cyclohexyloxy)-3-methyl-[1,3,2]oxazaphospholidine 2-oxide (±)-**63** (0.5 g, 1.97 mmol) was dissolved in dry methanol (10 cm^3). Triethylamine (2.2 mmol, 0.22 g) was then added and the mixture was heated to reflux temperature for 3 hours. The solvent was removed under reduced pressure to give the product 2-(2-Chloro-cyclohexyloxy)-3-methyl-[1,3,2]oxazaphospholidine 2-oxide (±)-**65** as a light oil (0.52 g, 92%); δ_H (300 MHz; C^2HCl_3) 1.10-2.25 (8H, m, CH_2 ring), 2.61 (3 H, s, NMe), 3.12 (2H, m, CH_2N), 3.39 (OMe) 3.79 (1H, m, CHCl), 4.01 (1H, m, CHOP), 4.17 (2H, m, CH_2OP); δ_C (50 MHz; C^2HCl_3) 23.15 & 24.36 (2 x CH_2), 32.32 (CH_2), 33.69 (CH_3N), 34.58 (CH_2), 50.18 (CH_2N), 50.62 (OMe), 61.28 (CH_2O), 62.76 (CHCl), 78.59 (CHO); δ_P (121 MHz; C^2HCl_3) –0.86; *m/z* (EI) 286 (31%, $[M + H]^+$) and 251 (44, $[M + H - Cl]^+$).

9.10 (+)-(1*R*,2*R*,4*R*,6*S*)-4-Bis(benzyloxy)-1,6-epoxycyclohexane 53[74]



To a stirred solution of dry THF (50 cm^3) and 2,4-Bis(benzyloxy)-1,6-epoxycyclohexane (+)-**93** (1.33 g, 6.03 mmol), were added 1.2 eq of NaH (0.36 g, 9 mmol, 60% dispersion in oil). After 30 min, 1.2 eq of benzyl bromide were added (11 cm^3, 9 mmol) and the solution was stirred at room temperature till completion and then water (100 cm^3) was cautiously added. The mixture was extracted with diethyl ether (2 x 100 cm^3) and the combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. The residual oil was purified by silica column chromatography (ethyl acetate-petroleum ether, 1:5) to give, (+)-**53**, as a colourless oil (1.35 g, 72%); $[\alpha]_D$ +72.6 (*c* 0.208 in MeOH); δ_H(300 MHz, C^2HCl_3) 1.68-1.72 (1 H, m, 3-H), 2.00-2.10 (3 H, m, 3-H and 2 x 5-H), 3.29-3.32 (1 H, m, 6-H), 3.42-3.46 (1 H, m, 1-H), 3.70-3.77 (1 H, m, 4-H), 4.15-4.23 (1 H, m, 2-H, 4.45 (2 H, s, OCH_2Ph), 4.70 (1 H, d, $^2J_{H\text{-}H}$ 12.1, OCH_2Ph), 4.74 (1 H, d, $^2J_{H\text{-}H}$ 12.1, OCH_2Ph) and 7.25-7.50 (10 H, m, Ar-H); δ_C(75.4 MHz, C^2HCl_3) 28.60 and 29.09 (3-C and 5-C), 52.25 and 53.12 (1-C and 6-C), 70.04 and 70.50 (OCH_2Ph), 71.12 (4-C), 72.09 (2-C), 127.48, 127.62, 127.75 and 128.39 (Ar-CH) and 138.36 & 138.58 (Ar-C quaternary); *m/z* (EI): 310 ($[M]^+$, 1 %), 219 ($[M\text{-}OC_7H_7]^+$, 5%), 91 ($[C_7H_7]^+$, 100 %).

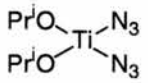
9.11 (-)-(1*R*,2*R*,4*R*,6*R*)-2,4-Bis-benzyloxy-6-(2-benzyloxy-ethoxy)-cyclohexanol 67



(+)-(1R,2R,4R,6S)-2,4-Bis(benzyloxy)-1,6-epoxycyclohexane (+)-**53** (1.5 g, 4.8 mmol) and 2-benzyloxyethanol (1.5 g, 10 mmol) were cooled in an ice bath. Three drops of $BF_3.OEt_2$ in diethyl ether were added with stirring, and stirring at room temperature was continued for 30 min. The reaction mixture was partitioned between water (50 cm^3) and diethyl ether (50 cm^3). The diethyl ether phase was dried (Mg_2SO_4), evaporated to dryness, and excess 2-benzyloxyethanol was distilled off in a Kugelrohr apparatus under reduced pressure. The residual oil was chromatographed on silica (light petroleum-ethyl acetate, 2 : 1) to give (-)-(1R,2R,4R,6R)-2,4-Bis-benzyloxy-6-(2-benzyloxy-ethoxy)-cyclohexanol **67** as a colourless oil (1.1 g, 50 %) (Found: C, 75.0; H, 7.6. $C_{29}H_{34}O_5$ requires C, 75.3; H, 7.4%); $[\alpha]_D$ -23.1 (*c* 0.192 in EtOAc); IR υ_{max} (neat/cm^{-1}) 3475s, 2948s, 2873s and 1094s; δ_H(200 MHz; C^2HCl_3) 1.25-1.48 (2 H, m, 2 x secondary-H), 2.22-2.40 (1 H, m, secondary-H), 2.39-2.56 (1 H, m, secondary-H), 3.5-4.0 (8 H, m, OC_2H_4O and 4 x tertiary), 4.50 (2 H, s, OCH_2Ph), 4.55-4.65 (4 H, m, 2 x OCH_2Ph) and 7.20-7.50 (15 H, m, Ar-H); δ_C(50.3 MHz; C^2HCl_3) 34.9 and 36.3 (3-C and 5-C), 69.7 and 70.2 (OC_2H_4O), 71.1 (OCH_2Ph), 72.2 (4-C), 72.7 (OCH_2Ph), 73.7 (OCH_2Ph), 76.4 and 76.7 (2-C and 6-C), 78.3 (1-C), 128.1, 128.2, 128.3, 128.8, 128.9 and 128.9 (Ar-CH) and 139.1 and 139.4 (Ar-C quaternary);

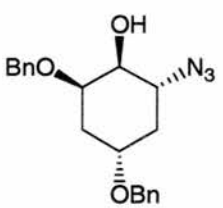
m/z (EI) 371 (8%, $[M - C_7H_7]^+$), 281 {10, $[M + H - (2 \times C_7H_7)]^+$}, 105 (42, $[PhCO]^+$) and 91 (100, $[C_7H_7]^+$).

9.12 Preparation of titanium complex 69[98]



To a stirred solution of $Ti(OPr^i)_4$ (0.89 cm^3, 3 mmol) and hexane (2 cm^3), 2 eq of $SiMe_3(N_3)$ (0.79 cm^3, 6 mmol) were added. After being stirred at room temperature for five days, the precipitate was filtered off and washed with hexane. Drying *in vacuo* afforded $Ti(OPr^i)_2(N_3)_2$ **69** as a yellow solid (0.7g, 90%); IR υ_{max} (neat)/cm^{-1}: 2118 (N_3).

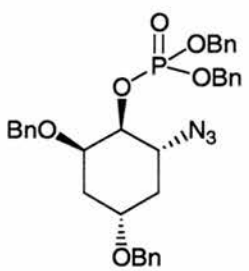
9.13 (-)-(1*S*,2*R*,4*S*,6*R*)-(2,4-Bis-benzyloxy-6-azido)-cyclohexanol 68



To a stirred solution of (+)-(1R,2R,4R,6S)-2,4-Bis(benzyloxy)-1,6-epoxycyclohexane **53** (0.5 g, 1.6 mmol) in dry toluene (15 cm^3), was added 1.2 eq of ($Ti(O\text{-}i\text{-}Pr^2)_2$ $(N_3)_2$) (0.78 g, 1.9 mmol). The reaction mixture was stirred at 75 °C for 7 hours. The mixture was cooled to room temperature and the toluene removed by evaporation to give an oil. Silica column chromatography (ethyl acetate-petroleum ether: 1:1) afforded (-)-(1S,2R,4S,6S)-(2,4-Bis-benzyloxy-6-azido)-cyclohexanol **68** as a colourless oil (0.5 g, 77%); (Found: C, 68.3; H, 6.5; N, 11.8. $C_{20}H_{23}O_3N_3$ requires C, 68.0; H, 6.6; N, 11.9 %); $[\alpha]_D$ -15.1 (*c* 0.163 in

EtOAc); υ_{max} (neat)/cm^{-1} 3441 (O-H), 2099 (N_3); δ_H(500 MHz, C^2HCl_3): 1.36-1.45 (2H, m, CH_2), 2.35-2.46 (2H, m, CH_2), 2.53 (1H, m, OH), 3.51 (1H, m, 1-H), 3.61-3.67 (1H, m), 3.74-3.81 (1H, m, 4-H), 3.92-3.96 (1H, m), 4.46-4.63 (4H, m, OCH_2Ph), 7.28-7.43 (10 H, m, Ar-H). δ_C(75.42 MHz, C^2HCl_3): 33.29, 35.18 (3-C, 5-C), 60.56 (6-C), 70.6 (4-C), 70.8, 71.55 (OCH_2Ph), 74.95 (2-C),76.46 (l-C) 127.58, 127.70, 127.79, 127.98, 128.43, 128.54, (Ar-CH), 137.72, 138.24 (Ar-C); *m/z* (El): 354 ([M+H]$^+$, 4 %), 326 ([M+H-N_2]$^+$, 30 %), 234 ([M-N_2-C_7H_7]$^+$, 55 %).

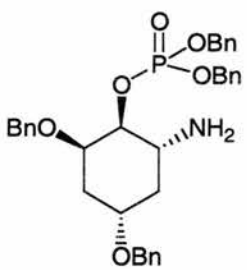
9.14 (-)-(1*S*,2*R*,4*S*,6*R*)-Phosphoric acid dibenzyl ester 2,4-bis-benzyloxy-6-azido-cyclohexyl ester 71



To a stirred solution of (-)-(1S,2R,4S,6S)-(2,4-Bis-benzyloxy-6-azido)-cyclohexanol **68** (0.42 g, 1.18 mmol) in dry DMF (50 cm^3) 1.2 eq of tetrabenzyl pyrophosphate (0.76 g, 1.42 mmol) were added. The reaction was cooled to 0 °C and 2 eq of NaH (0.09 g, 60% dispersion in oil, 2.3 mmol) were added. After being stirred for 2 h, the reaction mixture was partitioned between water (50 cm^3) and ether (3 x 50 cm^3). The pooled ether extracts were dried over $MgSO_4$ and evaporated *in vacuo*. Silica column chromatography of the residual oil (petroleum ether-ethyl acetate, 1:2) yielded (-)-(1S,2R,4S,6R)-phosphoric acid dibenzyl ester 2,4-bis-benzyloxy-6-azido-cyclohexyl ester **71** as an oil (0.45 g, 62%); (Found:

C, 66.7; H, 6.3; N, 6.5. $C_{34}H_{36}O_6N_3P$ requires C, 66.6; H, 5.9; N, 6.8 %); $[\alpha]_D$ -23.9 (*c* 0.2.16 in EtOAc); υ_{max} (neat)/cm^{-1}: 1018 (P-O-C), 1267 (P-0), 2109 (C-N_3); δ_H(500 MHz, C^2HCl_3): 1.31-1.46 (2H, m), 2.26 (1H, m), 2.42 (1H, m), 3.75-3.83 (1H, m, 4-H), 3.94-3.96 (1H, m), 4.06-4.11 (1H, m), 4.24-4.23 (1H, m, 1-H), 4.43-4.60 (4H, m, OCH_2Ph), 5.02-5.17 (4H, m, $POCH_2Ph$), 7.25-7.40 (20H, m, Ar-H); δ_C(75.42 MHz, C^2HCl_3): 33.96, 35.33 (3-C, 5-C), 58.23 (6-C, d, $^3J_{CP}$ 7.6Hz), 69.23 ($POCH_2Ph$, d, $^2J_{CP}$ 5.35 Hz), 69.46 ($POCH_2Ph$, d, $^2J_{CP}$ 5.35 Hz), 69.51 (OCH_2Ph), 70.5 (4-C), 70.8 (OCH_2Ph), 72.42 (OCH_2Ph), 75.03 (2-C), 81.10 (1-C, d, $^2J_{CP}$ 6.48Hz), 127.59, 127.66, 127.70, 127.79, 127.90, 127.98, 128.02, 128.38, 128.51, 128.53, 128.59, 128.62 (Ar-CH), 135.76, 135.86, 138.25 (Ar-C); δ_P(121.2 MHz, C^2HCl_3) –1.61; *m/z* (El): 614 ($[M+H]^+$, 4 %), 586 ($[MH-N_2]^+$,100 %), 400 ([MH-$O_2C_{14}H_{14}$, 42 %);

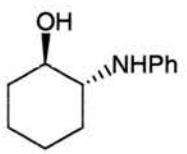
9.15 (-)-(1*S*,2*R*,4*S*,6*R*)-Phosphoric acid 6-amino-2,4-bis-benzyloxy-cyclohexyl ester dibenzyl ester 72



A mixture of SnO (1.9 mmol, 256 mg), PhSH (3.81 mmol, 0.39 cm^3) in dry toluene was heated at reflux in a Dean-Stark apparatus. To the resulting green precipitate, PhSH (0.11 cm^3, 1.10 mmol), Et_3N (0.15 cm^3, 1.10 mmol) were added to afford a yellow solution. To the mixture was added (-)-(1*S*,2*R*,4*S*,6*R*)-phosphoric acid dibenzyl ester 2,4-bis-benzyloxy-6-azido-cyclohexyl ester **71** (450 mg, 0.73 mmol) in 5 cm^3 of dry toluene and the reaction mixture was stirred

at room temperature for 2 hours. Then NaOH (2N, 50 cm^3) and DCM (50 cm^3) was added. Separation of the two phases and extraction of the aqueous phase twice more with DCM (2 x 50 cm^3), drying of the solvent (Mg_2SO_4) and evaporation afforded phosphoric acid (-)-(1*S*,2*R*,4*S*,6*R*)-6-amino-2,4-bis-benzyloxy-cyclohexyl ester dibenzyl ester **72** as a crude solid which was purified by silica column chromatograhy (DCM-MeOH, 1:5) (260 mg, 60 %); $[\alpha]_D$ –19.5 (*c* 0.194 in EtOAc); υ_{max} (neat)/cm^{-1}: 1013 (P-O-C), 1257 (P-O), 3382 (NH_2); δ_H(500 MHz, C^2HCl_3): 1.25 (1H, m), 1.62 (1H, m), 1.85 (1H, m), 2.02-2.13 (1H, m), 3.15-3.23 (1H, m, 4-H), 3.44-3.46 (IH, m, 6-H), 4.00-4.03 (1H, m, 2-H), 4.11-4.15 (1H, m, 1-H), 4.46-4.60 (4H, m, OCH_2Ph), 5.00-5.13 (4H, m, $POCH_2Ph$), 7.22-7.44 (20 H, m, Ar-H); δ_C(300 MHz, C^2HCl_3): 23.02-24.09 (3-C, 5-C), 54.30 (6-C, d, $^3J_{C\text{-}P}$ 6.48 Hz), 69.13 ($POCH_2Ph$, d, $^2J_{C\text{-}P}$ 5.42 Hz), 69.13 ($POCH_2Ph$, d, $^2J_{C\text{-}P}$ 5.42), 71.36, 71.57 (OCH_2Ph), 74.86 (2-C), 81.31 (4-C), 83.00 (l-C, d, $^2J_{C\text{-}P}$ 6.2 Hz); δ_P(121.2 MHz, C^2HCl_3) -1.033; *m/z* (CI): 587 ($[M]^+$), 470 ($[M\text{-}NH_3]^+$, 1 %), 480 ($[MH\text{-}OC_{17}H_7]^+$, 1 %), 279 ($[(C_7H_7O)_2PO_2H_2]^+$, 64 %), 107 ($[OC_7H_7]^+$, %), 91 ($[C_7H_7]^+$, %).

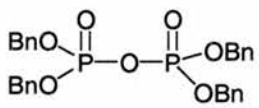
9.16 (±)-Trans-6-Phenylamino-cyclohexanol 74



Phenyldichloroborane (2.83 mmol, 0.45 g, 0.70 cm^3 was dissolved in of 1,2-dichloroethane (8 cm^3). The solution was carefully heated to 60 °C before (±)-2-azido-cyclohexanol **73** (0.4g 2.83 mmol) was added dropwise over 2 hours. The

amount of nitrogen released by the reaction (58 cm^3) was measured in order to follow the progress of the reaction. After addition was complete the reaction was stirred for an additional 30 minutes until gas evolution had ceased. The mixture was then cooled in ice and very carefully quenched with the drop wise addition of water (10 cm^3). The solution was then made strongly basic with the addition of 40% potassium hydroxide solution. The liberated amine was extracted with diethyl ether (2 x 10 cm^3) and dried (Mg_2SO_4). Removal of the solvent by evaporation gave (±)-*trans*-6-phenylamino-cyclohexanol **74** as an oil which was purified by column chromatography (petroleum ether-ethyl acetate,1:1) to give brown crystals (0.51g, 94%); m.p. 36-39 °C; υ_{max} (neat)/cm^{-1} 3384 (OH) 2945 (CH), 1603 (Ph); δ_H(200 MHz, C^2HCl_3) 0.91-2.22 (8H, m, ring protons), 3.01-3.20 (1H, m, CHN), 3.28-3.43 (1H, m, CHOH), 3.71-3.80 (1H, b, OH) 6.65-7.28 (5 H, m, Aryl-H), 7.29-7.32 (1 H, b, NH); δ_C(300 MHz, C^2HCl_3) 24.30 & 24.71 (3-C, 4-C), 32.04 (5-C), 33.59 (2-C), 60.54 (6-C), 74.79 (1-C), 114.90, 118.83 & 129.84, (Aryl-C ortho, meta, para) and 148.33 (Aryl-C quaternary);

9.17 Tetrabenzylpyrophosphate 83[119]



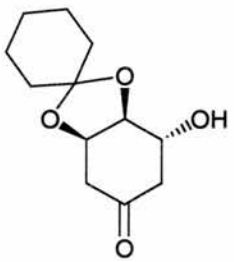
To a solution of PCl_3 (13.8 g, 8.8 cm^3) in dry toluene (100 cm^3) at 0 °C was added a mixture of dry Et_3N (15.8 cm^3) and dry benzyl alcohol (20.8 cm^3) slowly over 3 h and with vigorous stirring.The mixture was then stirred for a further 30 min. and benzyl alcohol (10 cm^3) was added over 20 min. The mixture was then stirred for 16 hours at room temperature. The toluene was washed with water (20 cm^3) and

ammonium hydroxide solution (20 cm^3) before separation and drying (Mg_2SO_4). The toluene was removed under reduced pressure to yield a light coloured oil **118**. This was not stored but carried on immediately to the next step:

A mixture of crude dibenzyl phosphite (20 g, 85.49 mmol), diethyl ether (25 cm^3) and aqueous pyridine (20 g, 19.6 cm^3) in 60 cm^3 water was cooled to 0 °C. A solution of bromine (11 g) was added with stirring over 3 h. Stirring was continued for a further 1 h, concentrated HCl (~ 5 drops) was added and the layers separated. The ether layer was washed with water and neutralised to around pH 9 (addition of sodium phosphate oxidised remaining bromine) with sodium hydroxide. Acidification of the neutral aqueous layer with HCl precipitated dibenzyl hydrogen phosphate which was extracted with DCM. The solvent was removed by evaporation to give the product **119** as a solid. This was recrystallised from diethyl ether. This intermediate was also used immediately in the next step:

DCC (1 M) was dissolved in 20 cm^3 of dry diethyl ether (20 cm^3) and added to dibenzyl hydrogen phosphate (1 M) dissolved in dry ether. After 30 min the solution was filtered and the colourless filtrate was evaporated. The residue was recrystallised from DCM-petroleum ether giving **83** as white crystals (0.64 g 66%) m.p. 61-62 °C; δ_H(75 MHz; C^2HCl_3), 5.10 (8H, m, CH_2Bn), 7.31 (20H, m, aromatic); δ_P(200 MHz C^2HCl_3) -12.57.

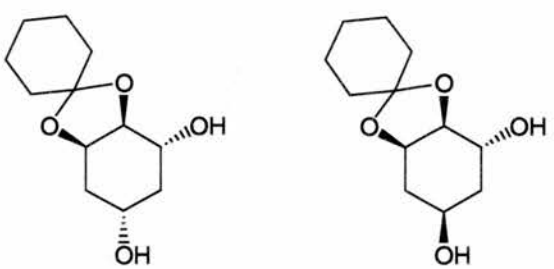
9.18 (+)-(1*S*,2*R*,6*R*)-1,2-Cyclohexylidenedioxy-4-oxocyclohexan-6-ol 86[74]



(-)-Quinic acid (-)-**49** (30 g, 156 mmol) and cyclohexanone (48.6 cm^3, 46 g, 470 mmol) were treated with conc. phosphoric acid (3 drops) and heated under reflux for 30 min. The water produced was removed by distillation for 1-2 h and the reaction mixture was allowed to cool to room temperature whereupon it solidified. The crude solid was recrystallised from DCM to remove most of the cyclohexanone and the crude lactone (-)-**84** was collected by filtration. The crude lactone (-)-**84** was dissolved in ethanol (300 cm^3) and the solution cooled in an ice bath. $NaBH_4$ (3.8 g, 100 mmol) was added in 4 batches with vigorous stirring and the mixture was allowed to warm to room temperature with stirring overnight. The solvent was removed under reduced pressure and the residue was dissolved in water (300 cm^3). The pH of this solution was adjusted to pH 6 by the dropwise addition of conc. phosphoric acid. The weakly acidic solution was cooled in an ice bath and $NaIO_4$ (33.4 g, 156 mmol) was added slowly in batches over a period of 30 min with stirring. After a further 5 h, the mixture was extracted with diethyl ether (2 x 150 cm^3) followed by ethyl acetate (150 cm^3). The combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. The residual oil solidified upon drying *in vacuo* and the crude solid was recrystallised (ethyl acetate-petroleum ether; 1:10) to give ketone (+)-**86** as a white solid (27.5

g, 78%); work-up of the mother liqueur by chromatography on silica (ethyl acetate-petroleum ether; 1:2) afforded further quantities of the ketone (+)-**86** (1.1 g, 3%), m.p. 97-98 °C. (Found: C, 63.9, H, 8.1. $C_{12}H_{18}O_4$ requires C, 63.7, H, 8.0%); $[\alpha]_D$ +100.3 (*c* 0.44 in MeOH); υ_{max} (Nujol)/cm^{-1} 3775 s, 1720 s, 1471 s, 1386 s and 1092 s; δ_H(300 MHz; C^2HCl_3) 1.30-1.45 (2 H, br s, cyclohexylidene), 1.50-1.70 (8 H, m, cyclohexylidene), 2.00-2.25 (1 H, broad, OH), 2.43 (1 H, ddd, $^2J_{H\text{-}H}$ 17.85, $^3J_{H\text{-}H}$ 3.85 and 1.9, 5-H), 2.63-2.73 (2 H, m, 3-H and 5-H), 2.80 (1 H, dd, $^2J_{H\text{-}H}$ 17.6, $^3J_{H\text{-}H}$ 3.85, 3-H), 4.21-4.26 (1 H, br s, 6-H), 4.27-4.33 (1 H, m, 1-H), 4.66-4.72 (1 H, m, 2-H); δ_C(75.4 MHz; C^2HCl_3) 23.39, 23.77, 25.01, 33.17, 36.15, 40.17 & 41.59 (5 x C secondary of cyclohexylidene, 3-C and 5-C), 68.21 (6-C), 71.71 (2-C), 74.61 (1-C), 109.52 (C quaternary of cyclohexylidene) and 208.66 (4-C); *m/z* (CI) 227 (35%, $[M + H]^+$), 129 (56, $[M + H - C_6H_{10}O]^+$) and 99 (100, $[C_6H_{11}O]^+$).

9.19 1,2-Cyclohexylidenedioxycyclohexane-4,6-diol (-)-(1*S*,2*R*,4*S*,6*R*)-87A and (+)-(1*S*,2*R*,4*R*,6*R*)-87B[74]



To a stirred solution of the ketone (+)-**86** (13.56 g, 60 mmol) in methanol (700 cm^3) was added $LaCl_3.7H_2O$ (22.3 g, 60 mmol), the mixture was then cooled to -78 °C. $NaBH_4$ (2.66 g, 70 mmol) was added in small batches whilst maintaining vigorous stirring. The mixture was allowed to warm slowly to room temperature

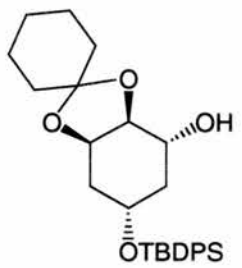
over 12 h. The solvent was removed under reduced pressure and the residual oil was partitioned between water (100 cm^3) and ethyl acetate (100 cm^3) and the aqueous phase was extracted with ethyl acetate (2 x 100 cm^3). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure to give 13 g of a crude mixture of the *cis*-diol (-)-**87A** and the *trans*-diol (+)-**87B** in a ratio of 5:1, as judged by ^1H NMR spectroscopy. For analytical purposes a small amount of the diol mixture was separated using silica column chromatography (ethyl acetate-petroleum ether; 2:1) where the less polar *trans*-4,6 diol (+)-**87B** was extracted next.

For the *trans*-diol (+)-**87B**: m.p. 129-130 °C; $[\alpha]_D$ +6 (*c* 0.16 in MeOH); δ_H(300 MHz; C^2HCl_3) 1.33-1.83 (10 H, m, cyclohexylidene), 1.90 (1 H, dt, $^2J_{H\text{-}H}$ 15.4, $^3J_{H\text{-}H}$ 4.1, secondary-H), 2.03-2.14 (1 H, m, secondary-H), 2.20-2.30 (1 H, m, secondary-H), 2.58 (1 H, d, $^3J_{H\text{-}H}$ 8.0, OH), 3.30-3.70 (1 H, broad, OH), 3.90 (1 H, t, $^3J_{H\text{-}H}$ 5.6, 1-H), 4.07-4.23 (2 H, m, 4-H and 6-H) and 4.35-4.44 (1 H, m, 2-H); δ_C(75.4 MHz; C^2HCl_3) 23.56, 23.93, 24.83, 33.07, 34.98, 37.17 & 38.36 (5 x C secondary of cyclohexylidene, 3-C and 5-C), 65.96 (4-C), 68.43 (6-C), 73.98 (2-C), 80.02 (1-C) and 109.75 (C quaternary of cyclohexylidene); *m/z* (CI) 229 (100%, $[M + H]^+$), 211 (38, $[M + H - H_2O]^+$) and 99 (4, $[C_6H_{11}O]^+$).

For *cis*-diol **(-)-87A**: m.p. 119-120 °C; (Found: C 62.65, H 9.1. $C_{12}H_{20}O_4$ requires C, 63.1; H, 8.8%); (HRMS: found: $[M + H]^+$, 229.1432. $C_{12}H_{21}O_4$ requires 229.1440); $[\alpha]_D$-70.6 (*c* 0.16 in MeOH); δ_H(300 MHz; C^2HCl_3) 1.30-1.70 (11 H, m, cyclohexylidene and 5-H), 1.75 (1 H, ddd, $^2J_{H\text{-}H}$ 14.3, $^2J_{H\text{-}H}$ 9.33 and 4.7, 3-H), 2.04-2.13 (1 H, m, 5-H), 2.24-2.33 (1 H, m, 3-H), 3.77-3.85 (1 H, m, 6-H), 3.86-3.92 (1 H, t, $^3J_{H\text{-}H}$ 6.3 and 5.2, 1-H), 4.02-4.14 (1 H, m, 4-H) and 4.34-4.41 (1 H, m, 2-H); δ_H (75.4 MHz; C^2HCl_3) 23.61, 23.94, 24.90 & 35.10 (4 x C secondary of

cyclohexylidene), 35.70 (3-C), 38.07 (C secondary of cyclohexylidene), 38.11 (5-C), 65.31 (4-C), 70.8 (6-C), 72.66 (2-C), 79.62 (1-C) and 109.59 (C quaternary of cyclohexylidene); *m/z* (CI) 229 (100%, $[M + H]^+$), 211 (44, $[MH - H_2O]^+$) and 99 (10, $[C_6H_{11}O]^+$).

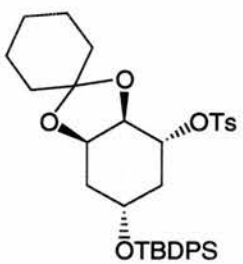
9.20 (-)-(1*S*,2*R*,4*S*,6*R*)-1,2-Cyclohexylidene-dioxy-4-(tert-butyldiphenylsilyl)cyclohexan-6-ol 88[74]



The above described crude mixture of *cis* and *trans*-4,6 diols (+)-**87B** and (-)-**87A** (10 g, 43.86 mmol) was dissolved in dry dichloromethane (100 cm^3), and DMAP (1.22 g, 10 mmol) and dry TEA (6.26 cm^3, 4.55 g, 45 mmol) were added. The mixture was cooled in an ice bath and *tert*-butyldiphenylsilyl chloride (11.7 cm^3, 12.37 g, 45 mmol) was added dropwise with vigorous stirring. The mixture was stirred for a further 16 h at room temperature and then extracted with water (100 cm^3). The aqueous phase was extracted with dichloromethane (3 x 100 cm^3) and the pooled organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. The residual oil was purified by silica column chromatography (ethyl acetate-petroleum ether; 10:1 and then 5:1) to give the silylated compound (-)-**88** as a white, sticky foam (9.9 g, 48%); (Found: C, 71.9, H, 8.15. $C_{28}H_{38}O_4Si$ requires C, 72.05, H, 8.2%); (HRMS: found: $[M + H]^+$, 467.2608. $C_{28}H_{39}O_4Si$ requires 467.2618); $[\alpha]_D$ -23.0 (*c* 0.398 in MeOH); υ_{max} (Nujol)/cm^{-1} 3404 s; δ_H (300 MHz; C^2HCl_3) 1.06 (9 H, s, ^{t}butyl), 1.30-1.40 (1 H, br. s, 3-H), 1.45-1.60 (10

H, m, cyclohexylidene), 1.65-1.75 (1 H, m, 5-H), 1.78-1.93 (2 H, m, 3-H and 5-H), 3.07-3.16 (1 H, bs, 6-OH), 3.73-3.84 (1 H, bs, 6-H), 4.0 (1 H, t, 1-H, $^3J_{H\text{-}H}$ 5.3), 4.06-4.16 (1 H, m, 4-H), 4.36-4.44 (1 H, m, 2-H), 7.30-7.50 (6 H, m, $SiPh_2$) and 7.6-7.7 (4 H, m, $SiPh_2$); δ_C (75.4 MHz; C^2HCl_3) 18.92 ($C(CH_3)_3$), 23.58, 23.88 & 24.30 (3 x C secondary of cyclohexylidene), 26.84 ($C(CH_3)_3$), 35.19 (C secondary of cyclohexylidene), 35.72 (3-C), 36.55 (5-C), 38.06 (C secondary of cyclohexylidene), 68.02 (4-C), 69.93 (6-C), 71.81 (2-C), 78.79 (1-C), 109.26 (C quaternary of cyclohexylidene) and 127.78, 127.81, 129.89, 129.95, 133.49, 135.78, 135.81 & 135.84 (Aryl-C ortho, meta, para and quaternary of $SiPh_2$); *m/z* (CI) 467 (100%, $[M + H]^+$), 369 (33, $[MH - C_6H_{10}O]^+$), 211 (29, $[M - OSiC_{16}H_{19}]^+$) and 99 (41, $[C_6H_{11}O]^+$).

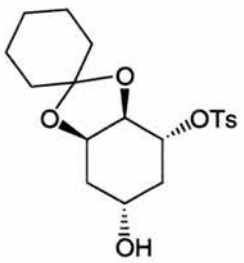
9.21 (-)-(1*R*,2*R*,4*S*,6*R*)-1,2-Cyclohexylidenedioxy-4-(tert-butyl-diphenylsilyl)-6-(4'-methylphenylsulfonyloxy)cyclohexane 89[74]



To a stirred solution of the silyated compound (-)-**88** (9 g, 19.3 mmol) in dry dichloromethane (100 cm^3), was added DMAP (488 mg, 4 mmol) and dry TEA (3.01 cm^3, 2.18 g, 21.6 mmol). The mixture was cooled in an ice bath and 4-methylbenzenesulfonyl chloride (4.78 g, 25.1 mmol) was added with vigorous stirring. Stirring was continued for three days at room temperature and then water

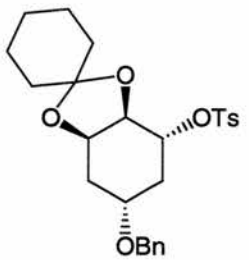
(100 cm^3) was added. The two phases were separated and the aqueous phase was extracted with dichloromethane (3 x 100 cm^3). The combined organic layers were dried ($MgSO_4$) and the solvent removed under reduced pressure. The residual oil was purified by silica column chromatography (ethyl acetate-petroleum ether, 1:10) to give the tosylate (-)-**89** as a white, sticky foam which solidified upon treatment with MeOH (8 g, 67%) and some unreacted starting material (-)-**88** (710 mg, 8%); m.p. 98-99 °C; (Found: C, 67.75; H, 6.8. $C_{35}H_{44}O_6SSi$ requires C, 67.7; H, 7.1%); (HRMS: found: M^+, 620.2638. $C_{35}H_{44}O_6SSi$ requires 620.2628); $[\alpha]_D$-51.5 (*c* 0.68 in MeOH); δ_H(300 MHz; C_2HCl_3) 1.0 (9 H, s, ^{t}butyl), 1.10-1.50 (10 H, m, secondary-H of cyclohexylidene), 1.52-1.66 (1 H, m, 5-H), 1.68-174 (1 H, m, 3-H), 2.12-2.26 (2 H, m, 3-H and 5-H), 2.4 (3 H, s, Tosyl-CH_3), 3.88-4.02 (2 H, m, 1-H and 4-H), 4.08-4.29 (2 H, m, 2-H and 6-H), 7.23-7.28 (2 H, m, Aryl-H), 7.33-7.47 (6 H, m, Aryl-H ortho, meta and para), 7.60-7.65 (4 H, m, Aryl-H ortho, meta and para) and 7.72 (2 H, d, tosyl-H, $^3J_{H\text{-}H}$ 8.25); δ_C (75.4 MHz; C^2HCl_3) 18.94 ($C(CH_3)_3$), 21.48 (Tosyl-CH_3), 23.39, 23.68 & 24.84 (3 x C secondary of cyclohexylidene), 26.76 ($C(CH_3)_3$), 34.62, 35.34, 37.22 & 38.19 (2 x C secondary of cyclohexylidene, 3-C and 5-C), 65.46 (4-C), 72.94 (2-C), 76.11 (6-C), 81.21 (1-C), 109.51 (C quaternary of cyclohexylidene) and 127.69, 127.76, 128.05, 129.61, 129.77, 129.84, 133.71, 133.94, 135.72, 135.76 & 144.4 (Aryl-C ortho, meta, para and quaternary); *m/z* (EI) 620 (3%, M^+), 353 (100) and 193 (17, $[M - C_7H_7SO_3 - C_{16}H_{19}OSi]^+$).

9.22 (-)-(1*R*,2*R*,4*R*,6*R*)-1,2-Cyclohexylidenedioxy-6-(4'-methyl-phenylsulfonyloxy)cyclohexan-4-ol 90[74]



To a stirred solution of the tosylate (-)-**89** (8 g, 12.9 mmol) in THF was added TBAF (15 cm^3 of a 1 mol dm^{-3} solution in THF, 15 mmol) and the resulting mixture stirred at room temperature for 16 h. The solvent was concentrated under reduced pressure and the residual oil was purified by silica column chromatography (ethyl acetate-petroleum ether, 1:1) to give alcohol (-)-**90** as a white solid (dihydrate: 5.39 g, 100%); m.p. 140-142 °C; (Found: C, 55.0, H, 6.6. $C_{19}H_{26}O_6S{\cdot}2H_2O$ requires C, 54.5; H, 6.3%); $[\alpha]_D$ -88.5 (*c* 0.3 in MeOH); υ_{max} (Nujol)/cm^{-1} 3394 s, 1464 s and 1381 s; δ_H(300 MHz; C^2HCl_3) 1.22-1.70 (12 H, m, cyclohexylidene, 3-H and 5-H), 2.32-2.45 (5 H, m, 3-H, 5-H and Tosyl-CH_3), 3.95 (1 H, dd, 1-H; $^3J_{H\text{-}H}$ 6.9 and 5.3), 3.98-4.10 (1 H, m, 4-H), 4.30-4.35 (1 H, m, 2-H), 4.45 (1 H, ddd, 6-H, $^3J_{H\text{-}H}$ 11.5, 7.1 and 4.4), 7.29 (2 H, d, $^3J_{H\text{-}H}$ 8.3, Aryl-H) and 7.80 (2 H, d, $^3J_{H\text{-}H}$ 8.3, Aryl-H); δ_C(75.4 MHz; C^2HCl_3) 21.53 (Tosyl-CH_3), 23.49, 23.76, 24.86, 34.72, 35.0, 37.44 & 38.25 (5 x C secondary of cyclohexylidene, 3-C and 5-C), 63.94 (4-C), 73.07 (2-C), 76.16 (6-C), 81.29 (1-C), 109.65 (C quaternary of cyclohexylidene), 128.10 & 129.75 (Aryl-C ortho and meta) and 134.04 & 144.71 (Aryl-C quaternary); *m/z* (EI) 383 (100%, $[M + H]^+$), 285 (11, $[MH - C_6H_{10}O]^+$), 211 (33, $[MH - C_7H_8O_3S]^+$) and 99 (34, $[C_6H_{11}O]^+$).

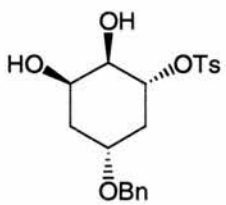
9.23 (-)-(1*R*,2*R*,4*R*,6*R*)-1,2-Cyclohexylidenedioxy-4-benzyl-6-(4'-methylphenylsulfonyloxy)cyclohexane 91[74]



Under an atmosphere of N_2, alcohol (-)-**90** (5 g, 12 mmol) was dissolved in dry DMF (100 cm^3) and treated with benzyl bromide (2.8 cm^3, 4.1 g, 24 mmol). The solution was cooled to –50 °C and NaH (600 mg, 15 mmol; 60% dispersion in oil) was added with continued stirring. The mixture was allowed to slowly warm up to room temperature and then water (100 cm^3) was cautiously added. The mixture was extracted with diethyl ether (2 x 100 cm^3) and the combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. The residual oil was purified by silica column chromatography (ethyl acetate-petroleum ether, 1:5) to give, after recrystallisation from methanol the benzyl ether (-)-**91**, as a white solid (4.53 g, 80%); m.p. 83-84 °C; (Found: C, 65.8, H, 6.9. $C_{26}H_{32}O_6S$ requires C, 66.1, H, 6.8%); (HRMS: found: M^+, 472.1927. $C_{26}H_{32}O_6S$ requires 472.1920); $[\alpha]_D$ -85.3 (*c* 0.214 in MeOH); υ_{max} (CH_2Cl_2)/cm^{-1} 3063 s, 2946 s, 1605 s, 1444 s, 1346 s, 1273 s, 1183 s, 942 s, 816 s and 742; δ_H (300 MHz; C^2HCl_3) 1.25-1.52 (10 H, m, cyclohexylidene), 1.52-1.61 (1 H, m, 5-H), 1.71 (1 H, ddd, $^2J_{H\text{-}H}$ 13.6, $^3J_{H\text{-}H}$ 10.4 and 4.7, 3-H), 2.43 (3 H, m, Tosyl-CH_3), 2.46-2.56 (1 H, m, 3-H and 5-H), 3.7-3.81 (1 H, m, 4-H), 3.97 (1 H, dd, $^3J_{H\text{-}H}$ 5.5 and 7.1, 1-H), 4.32-4.37 (1 H, m, 2-H), 4.46 (1 H, ddd, $^3J_{H\text{-}H}$ 11.8, 7.1 and 4.4, 6-H), 4.49 (1 H, d, $^2J_{H\text{-}H}$ 11.5, one of OCH^2Ph), 4.55 (1 H, d, $^2J_{H\text{-}H}$ 11.5, one of OCH_2Ph), 7.20-7.30 (7 H, m, Aryl-H

ortho, meta and para), 7.82 (2 H, d, $^3J_{H\text{-}H}$ 8.3, Aryl-H); δ_C(75.4 MHz; C^2HCl_3) 21.47 (Tosyl-CH_3), 23.44, 23.72, 24.83, 32.5, 34.71, 35.18 & 37.41 (5 x C secondary of cyclohexylidene, 3-C and 5-C), 70.71 (OCH_2Ph), 70.97 (4-C), 72.98 (2-C), 76.42 (6-C), 81.43 (1-C), 109.72 (C quaternary of cyclohexylidene), 127.52, 127.69, 128.05, 128.45 & 129.64 (Aryl-C ortho, meta, para) and 134.10, 138.2 & 144.56 (Aryl-C quaternary); *m/z* (EI) 472 (31%, M^+), 382 (7, [MH – C_7H_7]$^+$) and 91 (100, $[C_7H_7]^+$).

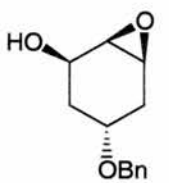
9.24 (-)-(1*R*,2*R*,4*R*,6*R*)-4-Benzyloxy-6-(4'-methylphenylsulfonyl-oxy)cyclohexane-1,2-diol 92. [74]



To a stirred solution of the ketal (-)-**91** (4 g, 8.5 mmol) in MeOH (50 cm^3) was added TFA (0.1 cm^3). After 2 days, the solvent was removed under reduced pressure and the residual oil was purified by silica column chromatography (ethyl acetate-petroleum ether, 2:1) to give diol (-)-**92** as a colourless oil (2.6 g, 78%) and some unreacted starting material (-)-**91** (0.6 g, 15%); (Found: C, 60.8, H, 6.6. $C_{20}H_{24}O_6S$ requires C, 61.2, H, 6.2%); (HRMS: found: $[M + H]^+$, 393.1382. $C_{20}H_{25}O_6S$ requires 393.1372); $[\alpha]_D$ -42.0 (*c* 0.67 in MeOH); υ_{max} (neat/cm^{-1}) 3472, 2926, 1600, 1444, 1361 and 1084; δ_H(300 MHz; C^2HCl_3) 1.37-1.48 (1 H, m, 3-H), 1.49-1.61 (1 H, m, 5-H), 2.24-2.39 (2 H, m, 3-H, 5-H), 2.46 (3 H, m, Tosyl-CH_3), 2.54-2.62 (1 H, broad, OH), 3.06-3.14 (1 H, broad, OH), 3.63 (1-H, dd, $^3J_{H\text{-}H}$ 8.8 and 2.8, 1-H), 3.75-3.86 (1 H, m, 4-H), 4.12-4.17 (1 H, m, 2-H), 4.46

(2 H, s, OCH_2Ph), 4.70 (1 H, ddd, $^3J_{H\text{-}H}$ 11.8, 9.05 and 4.95, 6-H) and 7.20-7.30 (7 H, m, Aryl-H ortho, meta and para) and 7.82 (2 H, d, $^3J_{H\text{-}H}$ 8.3, tosyl-H); δ_C(75.4 MHz; C^2HCl_3) 21.62 (tosyl-CH_3), 35.48 (3-C), 36.16 (5-C), 68.59 (2-C), 70.86 (OCH_2Ph), 70.95 (4-C), 73.61 (1-C), 80.42 (6-C), 127.59, 127.75, 127.94, 128.48 & 130.05 (Aryl-C ortho, meta and para) and 133.42, 138.25 & 145.34 (Aryl-C quaternary); *m/z* (CI) 393 (67%, $[M + H]^+$), 221 (63, $[MH - C_7H_8O_3S]^+$), 203 (51, $[MH - C_7H_8O_3S - H_2O]^+$), 113 (100) and 91 (22, $[C_7H_7]^+$).

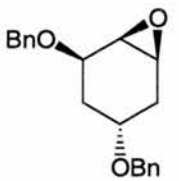
9.25 (+)-(1*S*,2*R*,4*R*,6*R*)-4-Benzyloxy-1,6-epoxycyclohexane-2-ol 93[74]



To a stirred solution of the diol (-)-**92** (2.5 g, 6.4 mmol) in MeOH (30 cm^3) was added K_2CO_3 (1.77 g, 12.8 mmol) in small portions. The viscous reaction mixture was stirred at room temperature for 30 min, filtered, and the pad washed thoroughly with diethyl ether (3 x 50 cm^3) followed by ethyl acetate (2 x 50 cm^3). The combined organic solutions were concentrated under reduced pressure and the residual oil was purified by silica column chromatography (ethyl acetate-petroleum ether, 1:1) to give epoxy alcohol (+)-**93** as a white solid (1.27 g, 90%), m.p. 64-65 °C; (Found: C, 70.7, H, 7.2. $C_{13}H_{16}O_3$ requires C, 70.9, H, 7.3%); $[\alpha]_D$ +56.4 (*c* 0.329 in MeOH); δ_H (300 MHz; C^2HCl_3) 1.56 (1 H, ddd, $^2J_{H\text{-}H}$ 13.7, $^3J_{H\text{-}H}$ 9.3 and 2.0, 3-H), 1.90-2.10 (3 H, m, 2 x 5-H and 3-H), 3.32-3.42 (2 H, m, 1-H and 6-H), 3.62-3.71 (1 H, m, 4-H), 4.26-4.38 (1 H, br. s, 2-H), 4.42 (1 H, d, $^2J_{H\text{-}H}$ 11.7, one of OCH_2Ph), 4.50 (1 H, d, $^2J_{H\text{-}H}$ 11.7, one of OCH_2Ph) and 7.25-

7.50 (5 H, m, Aryl-H ortho, meta and para); δ_C(75.4 MHz; C^2HCl_3) 29.14 & 32.50 (3-C and 5-C), 54.05 & 55.40 (1-C and 6-C), 64.83 (2-C), 70.13 (OCH_2Ph), 71.63 (4-C), 127.51, 127.70 & 128.49 (Aryl-C ortho, meta para) and 138.39 (Aryl-C quaternary); *m/z* (CI) 221 (65% $[M + H]^+$), 203 (100 $[MH - H_2O]$).

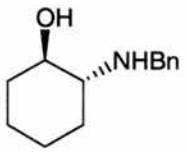
9.26 (+)-(1*S*,2*R*,4*R*,6*R*)-2,4-Bis(benzyloxy)-1,6-epoxycyclohexane 53[74]



Under an atmosphere of N_2, a solution of epoxy alcohol (+)-**93** (1.1 g, 5 mmol) in dry THF (50 cm^3) was cooled in an ice bath and benzyl bromide (0.66 cm^3, 940 mg, 5.5 mmol) and then KH (washed with petroleum ether prior to use, 220 mg, 5.5 mmol) were added with stirring. Stirring was continued at room temperature for 3 h and then water was added cautiously. The mixture was extracted with diethyl ether (2 x 50 cm^3), and the organic phases combined, dried ($MgSO_4$) and then concentrated under reduced pressure. The residual oil was purified using silica column chromatography (ethyl acetate-petroleum ether; 1:5) to give epoxide (+)-**53** as a colourless oil (1.44 g, 93%); $[\alpha]_D$ +72.6 (*c* 0.208 in MeOH); δ_H(300 MHz; C^2HCl_3) 1.68-1.72 (1H, m, 3-H), 2.00-2.10 (3H, m, 3-H and 2 x 5-H), 3.29-3.32 (1H, m, 6-H), 3.42-3.46 (1H, m, 1-H), 3.70-3.77 (1H, m, 4-H), 4.15-4.23 (1H, m, 2-H), 4.45 (2H, s, OCH_2Ph), 4.70 (1H, d, $^2J_{H\text{-}H}$ 12.1, OCH_2Ph), 4.74 (1H, d, $^2J_{H\text{-}H}$ 12.1, OCH_2Ph) and 7.25-7.50 (10H, m, Aryl-H ortho, meta and para); δ_C(75.4 MHz; C^2HCl_3) 28.60 & 29.09 (3-C and 5-C), 52.25 & 53.12 (1-C and 6-C), 70.04 & 70.50 (OCH_2Ph), 71.12 (4-C), 72.09 (2-C), 127.48, 127.62, 127.75 &

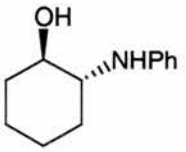
128.39 (Aryl-C ortho, meta and para) and 138.36 & 138.58 (Aryl-C quaternary); *m/z* (CI) 221 (8%, $[MH_2 - C_7H_7]^+$) and 91 (100, $[C_7H_7]^+$).

9.27 (±)-*Trans*-2-Benzylamino-cyclohexanol 98



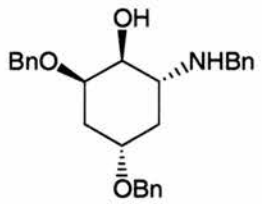
A solution of benzylamine (0.65 g, 0.67 cm^3, 6.11 mmol) in THF (2 cm^3) was added slowly to a stirred solution of ethylmagnesium bromide (6.11 cm^3, 6.11 mmol) and THF. After stirring the mixture for 1 h at 35 °C the solution was allowed to cool to r.t. and a solution of (±)-cyclohexene oxide **56** (0.5 g, 5.10 mmol, 0.51 cm^3) in THF (2 cm^3) was carefully added. The mixture was stirred continuously for 12 h before pouring into a saturated solution of NH_4Cl. The alkaline mixture was acidified with 2N HCl and extracted with diethyl ether (2 x 50 cm^3) which was discarded before treating the aqueous layer with a cold solution of 10% NaOH. The mixture was extracted with diethyl ether (2 x 50 cm^3), and the organic phases combined, dried ($MgSO_4$) and then concentrated under reduced pressure to give *trans*-2-benzylamino-cyclohexanol (±)-**98** as a white solid (0.78 g, 76%); m.p. 41-43 °C; δ_H(200 MHz, C^2HCl_3) 0.87-2.01 (8H, m, ring protons), 2.22-2.31 (1H, m, CHN), 2.41-2.82 (2H, b, OH & NH) 3.15-3.39 (1H, m, CHOH), 3.56-3.86 (2H, m, CH_2Ph), 7.13-7.39 (5 H, m, Aryl-H); δ_C(300 MHz, C^2HCl_3) 24.18 & 24.83 (3-C, 4-C), 30.17 (5-C), 33.32 (2-C), 50.63 (CH_2Ph), 62.90 (6-C), 73.46 (1-C), 126.95, 128.08 & 128.38, (Aryl-C ortho, meta, para) and 140.39 (Aryl-C quaternary);

9.28 (±)-*Trans*-2-Phenylamino-cyclohexanol 97



A solution of phenylamine (0.57 g, 6.11 mmol) in THF (2 cm^3) was added slowly to a stirred solution of ethylmagnesium bromide (6.11 cm^3, 6.11 mmol) and THF. After stirring the mixture for 1 h at 35 °C the solution was allowed to cool to r.t. and a solution of (±)-cyclohexene oxide **56** (0.5 g, 5.10 mmol, 0.51 cm^3) in THF (2 cm^3) was carefully added. The mixture was stirred continuously for 12 h before pouring into a saturated solution of NH_4Cl. The alkaline mixture was acidified with 2N HCl and extracted with diethyl ether (2 x 50 cm^3) which was discarded before treating the aqueous layer with a cold solution of 10% NaOH. The mixture was extracted with diethyl ether (2 x 50 cm^3), and the organic phases combined, dried ($MgSO_4$) and then concentrated under reduced pressure to give 97 as a white solid (0.83 g, 70%); m.p. 36-39 °C; υ_{max} (neat)/cm^{-1}; 3384 (OH) 2945 (CH), 1603 (Ph); δ_H(200 MHz, C^2HCl_3) 0.91-2.22 (8H, m, ring protons), 3.01-3.20 (1H, m, CHN), 3.28-3.43 (1H, m, CHOH), 3.71-3.80 (1H, b, OH) 6.65-7.28 (5 H, m, Aryl-H), 7.29-7.32 (1 H, b, NH); δC (300 MHz, C^2HCl_3) 24.30 & 24.71 (3-C, 4-C), 32.04 (5-C), 33.59 (2-C), 60.54 (6-C), 74.79 (1-C), 114.90, 118.83 & 129.84, (Aryl-C ortho, meta, para) and 148.33 (Aryl-C quaternary);

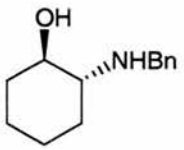
9.29 (-)-(1*S*,2*R*,4*S*,6*R*)-6-Benzylamino-2,4-bis-benzyloxy-cyclohexanol 99



A solution of benzylamine (0.08 g, 0.08 cm^3, 0.77 mmol) in THF (2 cm^3) was added slowly to a stirred solution of ethylmagnesium bromide (0.39 cm^3, 0.77 mmol) and THF. After stirring the mixture for 1 h at 35 °C the solution was allowed to cool to r.t. and a solution of (+)-(1*S*,2*R*,4*R*,6*R*)-2,4-bis(benzyloxy)-1,6-epoxycyclohexane **53** (0.09 g, 0.29 mmol) in THF (2 cm^3) was carefully added. The mixture was stirred continuously for 12 h before pouring into a saturated solution of NH_4Cl. The alkaline mixture was acidified with 2N HCl and extracted with diethyl ether (2 x 50 cm^3) [found to contain some unreacted starting material] before treating the aqueous layer with a cold solution of 10% NaOH. The mixture was extracted with diethyl ether (2 x 50 cm^3), and the organic phases combined, dried ($MgSO_4$) and then concentrated under reduced pressure to give oil. The oil was purified by silica chromatography (ethyl acetate-petroleum ether, 2:1) to give (-)-(1S,2R,4S,6R)-6-benzylamino-2,4-bis-benzyloxy-cyclohexanol **99** as an oil (0.02 g, 19%); $[\alpha]_D$ -28.0 (*c* 0.151 in EtOAc); δ_H(200 MHz, C^2HCl_3) 1.27-1.39 (1H, m, ring protons), 1.83-2.20 (2H, b, OH & NH), 2.21-2.39 (3H, m, ring protons), 2.77-2.86 (1H, m, CHN), 3.23-3.38 (1H, m, CHOH), 3.56-3.87 (2H, m, 2 x CHOBn), 3.82-3.87 (NCH_2Ph), 4.32-4.58 (4H, m, 2 x OCH_2Ph), 7.11-7.32 (15 H, m, Aryl-H); δ_C(300 MHz, C^2HCl_3): 33.67 & 35.06 (3-C, 5-C), 50.98 (NCH_2Ph), 56.00 (6-C), 70.60 (OCH_2Ph), 71.71 (4-C), 72.19 (OCH_2Ph), 74.49 (2-

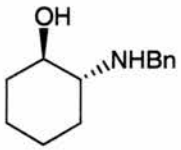
C), 76.15 (1-C), 127.16, 127.72, 128.28, 128.50, 128.54, 128.80 (Aryl-C ortho, meta, para), 138.65 & 139.99 (Aryl-C quaternary);

9.30 (±)-Trans-2-Benzylamino-cyclohexanol 105



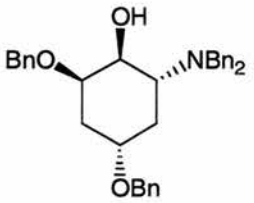
A solution of (±)-cyclohexene oxide **56** (5 mmol, 0.49 g) in acetonitrile (5 cm^3) was treated with $LiBF_4$ (5 mmol, 0.47 g). Benzylamine (0.53 g, 0.55 cm^3, 5 mmol) was added to the mixture and stirring was continued for 12 hours. Water was then added to the mixture before extracting with diethyl ether (2 x 50 cm^3), the organic phases were combined, dried ($MgSO_4$) and then concentrated under reduced pressure to give an oil. The oil was purified by silica column chromatography (ethyl acetate-petroleum ether, 2:1) to give the target compound (±)-**105** (0.44 g, 43 %); δ_H(200 MHz, C^2HCl_3): 1.27-1.39 (1H, m, ring protons), 1.83-2.20 (2H, b, OH & NH), 2.21-2.39 (3H, m, ring protons), 2.77-2.86 (1H, m, CHN), 3.23-3.38 (1H, m, CHOH), 3.56-3.87 (2H, m, 2 x CHOBn), 3.82-3.87 (NCH_2Ph), 4.32-4.58 (4H, m, 2 x OCH_2Ph), 7.11-7.32 (15 H, m, Aryl-H); δ_C(300 MHz, C^2HCl_3): 33.67 & 35.06 (3-C, 5-C), 50.98 (NCH_2Ph), 56.00 (6-C), 70.60 (OCH_2Ph), 71.71 (4-C), 72.19 (OCH_2Ph), 74.49 (2-C), 76.15 (1-C), 127.16, 127.72, 128.28, 128.50, 128.54, 128.80 (Aryl-C ortho, meta, para), 138.65 & 139.99 (Aryl-C quaternary); *m/z* (CI) 206 (36%, $[M + H]^+$) and 107 (100, $[(M+H)-NHBn]^+$).

9.31 *(±)-Trans*-2-Benzylamino-cyclohexanol 204



To a stirred solution of (±)-cyclohexene oxide **56** (0.1 g, 1.0 mmol) in dichloromethane (5 cm^3) was added $Yb(OTf)_3$ (85 mg, 0.2 mmol) followed by benzylamine (1.2 mmol, 130 mg, 0.13 cm^3). The mixture was stirred for 12 h followed by tlc, and then the solvent was removed under reduced pressure. The residue was then washed with water (10 cm^3) and then extracted with ethyl acetate (2 x 10 cm^3). The combined organic layers were dried (Na_2SO_4) and the solvent removed under reduced pressure to give a light coloured oil purified by silica chromatography (ethyl acetate-petroleum ether, 2:1) to give the target amine *trans*-2-benzylamino-cyclohexanol (±)-**204** as an oil (0.18 g, 88%); δ_H(200 MHz, C^2HCl_3) 1.27-1.39 (1H, m, ring protons), 1.83-2.20 (2H, b, OH & NH), 2.21-2.39 (3H, m, ring protons), 2.77-2.86 (1H, m, CHN), 3.23-3.38 (1H, m, CHOH), 3.56-3.87 (2H, m, 2 x CHOBn), 3.82-3.87 (NCH_2Ph), 4.32-4.58 (4H, m, 2 x OCH_2Ph), 7.11-7.32 (15 H, m, Aryl-H); δ_C(300 MHz, C^2HCl_3) 33.67 & 35.06 (3-C, 5-C), 50.98 (NCH_2Ph), 56.00 (6-C), 70.60 (OCH_2Ph), 71.71 (4-C), 72.19 (OCH_2Ph), 74.49 (2-C), 76.15 (1-C), 127.16, 127.72, 128.28, 128.50, 128.54, 128.80 (Aryl-C ortho, meta, para), 138.65 & 139.99 (Aryl-C quaternary); *m/z* (CI) 206 (36%, $[M + H]^+$) and 107 (100, $[(M+H)\text{-}NHBn]^+$).

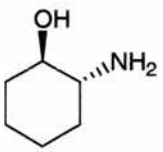
9.32 (-)-(1S,2R,4S,6R)-2,4-Bis-benzyloxy-6-dibenzylamino-cyclohexanol 116



To a solution of the key epoxide (+)-(1*S*,2*R*,4*R*,6*R*)-2,4-bis(benzyloxy)-1,6-epoxycyclohexane **53** (400 mg, 1.29 mmol) in 1,2-dichloroethane (15 cm^3) was added $Yb(OTf)_3$ (160 mg, 0.26 mmol) and dibenzylamine (250 mg, 1.29 mmol, 0.25 cm^3). The mixture was refluxed for 2 h before removal of the solvent by reduced pressure. The residue was washed with water (2 x 100 cm^3) and then extracted with ethyl acetate (2 x 100 cm^3). The combined organic layers were dried (Mg_2SO_4) and concentrated leaving a yellow oil purified by column chromatography (ethyl acetate-pet. ether, 1:15) to give the product (-)-(1*S*,2*R*,4*S*,6*R*)-2,4-bis-benzyloxy-6-dibenzylamino-cyclohexanol **116** as a pale yellow solid (390 mg, 93%); m.p. 52-53 °C; (Found: C, 80.4, H, 7.4, N, 2.6. $C_{34}H_{37}O_3N$ requires C, 80.5, H, 7.3, N, 2.8%); (HRMS: found: M^+, 508.2841 $C_{34}H_{38}NO_3$ requires 508.2852); $[\alpha]_D$ –60.9 (*c* 0.50 in AcOEt); δ_H(300 MHz; C^2HCl_3) 1.14-1.34 (2 H, m, 2 x secondary-H), 2.20-2.30 (2 H, m, 2 x secondary-H), 3.02-3.11 (1 H, dt J 3.02, 12.63, CHN), 3.34 & 3.38 (2 H, J 13.18, NCH_2Ph), 3.48-3.52 (2 H, m, secondary-H & OH), 3.57-3.68 (1 H, m, secondary-H), 3.75-3.79 (2 H, d J 13.18, NCH_2Ph), 3.91-3.92 (1 H, m, secondary-H), 4.02-4.57 (4 H, m, 2 x OCH_2Ph) and 7.11-7.27 (20 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 28.96 (3-C), 35.16 (5-C), 53.56 (2 x NCH_2Ph), 55.38 (6-C), 70.70 and 72.55 (2 x OCH_2Ph), 72.07 (4-C), 73.38 (2-C), 75.24 (1-C), 127.09, 127.19, 127.23, 127.59,

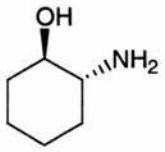
127.66, 128.12, 128.42, 128.51, 129.01 (Ar-CH), 138.74 and 139.23(2) (Aryl-C quaternary);

9.33 (±)-*Trans*-2-Amino-cyclohexanol 117



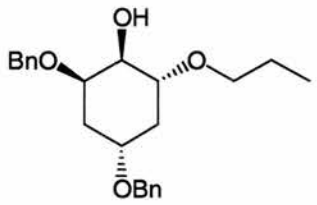
(±)-Cyclohexene oxide **56** (2 g, 20.38 mmol) was placed in a round bottomed flask to which 80 cm^3 of ammonium hydroxide was added along with a small amount of ethanol to dissolve the epoxide. The catalyst $Yb(OTf)_3$ was also added (20% equivalent). The flask was then sealed to prevent evaporation ammonia gas and the solution stirred at room temperature for 4 hours. The flask was then opened and the ammonia allowed to evaporate before eluting the product with ethyl acetate (2 x 20 cm^3). This was then dried (Mg_2SO_4) and the solvent removed under reduced pressure to give a solid. Recrystallisation from 1:10 ethyl acetate/petroleum ether gave **117** as a white solid 1.2g (51%); m.p. 40-42 °C; (Found: C, 62.6, H, 11.4, N, 12.2. $C_6H_{13}NO$ requires C, 62.6, H, 11.29, N, 12.2%); (HRMS: found: M^+, 115.1511 $C_6H_{13}NO$ requires 115.1736); δ_H(300 MHz; C^2HCl_3) 0.89-1.21 (4H, m, 3 & 4 CH_2), 1.60-2.01 (4H, m, 2 & 5 CH_2), 2.35-2.92 (1H, m, CH-6), 3.06-3.63 (2H, $^2J_{H\text{-}H}$ 13.18, NCH_2Ph), 3.48-3.52 (2H, m, secondary-H & OH), 3.57-3.68 (4H, m, CH-1, OH, NH_2); δ_C(75.4 MHz; C^2HCl_3) 24.54 (4-C), 24.71 (3-C), 33.71 (5-C), 34.08 (2-C), 56.69 (6-C), 75.57 (1-C); *m/z* (CI) 116 (56%, $[M + H^+]$) and 100 (100).

9.34 (±)-*Trans*-2-Amino-cyclohexanol 117



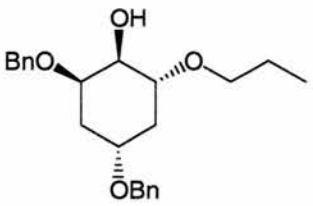
To a stirred solution of the (±)-cyclohexene oxide **56** (2 g, 20.38 mmol) in ammonium hydroxide (80 cm^3) was added $Yb(OTf)_3$ (1.6 g, 1 mmol) and a little ethanol (1 cm^3) to aid solvation. The flask was sealed and stirred at 65 °C for 1 h, the solvent was then removed under reduced pressure. The residue was washed with water (2 x 50 cm^3) and extracted twice with ethyl acetate (2 x 50 cm^3). The combined layers were dried (Mg_2SO_4) and the solvent removed under reduced pressure to give the product *Trans*-2-Amino-cyclohexanol (±)-**117** as white crystals (2.30 g, 99%); m.p. 40-42 °C; (Found: C, 62.6, H, 11.4, N, 12.2. $C_6H_{13}NO$ requires C, 62.6, H, 11.29, N, 12.2%); (HRMS: found: M^+, 115.1511 $C_6H_{13}NO$ requires 115.1736); δ_H(300 MHz; C^2HCl_3) 0.89-1.21 (4 H, m, 3 & 4 CH_2), 1.60-2.01 (4 H, m, 2 & 5 CH_2), 2.35-2.92 (1 H, m, CH-6), 3.06-3.63 (2 H, $^2J_{H\text{-}H}$ 13.18, NCH_2Ph), 3.48-3.52 (2 H, m, secondary-H & OH), 3.57-3.68 (4 H, m, CH-1, OH, NH_2); δ_C(75.4 MHz; C^2HCl_3) 24.54 (4-C), 24.71 (3-C), 33.71 (5-C), 34.08 (2-C), 56.69 (6-C), 75.57 (1-C); *m/z* (CI) 116 (56%, [M + H^+) and 100 (100).

9.35 (-)-(1*S*,2*R*,4*S*,6*R*)-2,4-Bis(benzyloxy)-6-propyloxycyclohexanol 160



To an ice cold, stirred solution of the key epoxide (+)-(1*S*,2*R*,4*R*,6*R*)-2,4-bis(benzyloxy)-1,6-epoxycyclohexane **53** (620 mg, 2 mmol) and propan-1-ol (0.37 cm^3, 300 mg, 5 mmol) in dry toluene (5 cm^3) was added $BF_3.OEt_2$ (3 drops of a 1:15 mixture in dry toluene). After stirring at room temperature for 3 hours the solvent was removed under reduced pressure and the residual oil purified by silica column chromatography (ethyl acetate-petroleum ether, 1:2) to give alcohol (-)-(1*S*,2*R*,4*S*,6*R*)-2,4-bis(benzyloxy)-6-propyloxycyclohexanol **160** as a colourless oil (380 mg, 65%) (HRMS: found: $[M + H]^+$ 371.2214. $C_{36}H_{41}O_5$ requires 371.2222); $[\alpha]_D$ -34.1 (*c* 0.18 in MeOH); δ_H(300 MHz; C^2HCl_3) 0.92 (3H, t, $^3J_{H\text{-}H}$ 7.4, $OCH_2CH_2CH_3$), 1.23-1.43 (2H, m, 3-H and 5-H), 1.54-1.67 (2H, m, $OCH_2CH_2CH_3$), 2.31-2.40 (1H, m, secondary-H), 2.45-2.53 (1H, m, secondary-H), 3.39-3.61 (4 H, m, $OCH_2CH_2CH_3$, 1-H and 6-H), 3.68-3.8 (1H, m, 4-H), 3.93-3.98 (1H, m, tertiary-H), 4.46 (1H, d, $^2J_{H\text{-}H}$ 12.8, OCH_2Ph), 4.53 (1H, d, $^2J_{H\text{-}H}$ 12.8, OCH_2Ph), 4.60 (2H, s, OCH_2Ph) and 7.20-7.50 (10H, Ar-H ortho, meta and para); δ_C(75.4 MHz; C^2HCl_3) 10.49 ($OCH_2CH_2CH_3$), 23.24 ($OCH_2CH_2CH_3$), 34.05 & 35.31 (3-C and 5-C), 70.60 (OCH_2Ph), 71.17 ($OCH_2CH_2CH_3$), 71.67 (4-C), 72.02 (OCH_2Ph), 75.59 & 76.18 (2-C and 6-C), 76.73 (1-C), 127.67 & 128.46 (Ar-C ortho, meta and para) and 138.62 (Ar-C quaternary); *m/z* (CI) 371 (26%, $[M + H^+]$) and 107 (100).

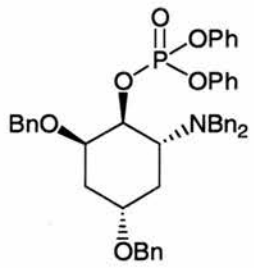
9.36 (-)-(1*S*,2*R*,4*S*,6*R*)-2,4-Bis(benzyloxy)-6-propyloxycyclohexanol 161



To a stirred solution of the epoxide (+)-(1*S*,2*R*,4*R*,6*R*)-2,4-bis(benzyloxy)-1,6-epoxycyclohexane **53** (180 mg, 0.58 mmol) in 1,2-dichloroethane (25 cm^3) was added $Yb(OTf)_3$ (128 mg, 0.08 mmol) followed by propan-1-ol (1.28 mmol, 80 mg, 0.1 cm^3). The mixture was refluxed for 3 h, and then the solvent was removed under reduced pressure. The residue was washed with water (10 cm^3) and then extracted with ethyl acetate (2 x 10 cm^3). The combined organic layers were dried (Na_2SO_4) and the solvent removed under reduced pressure to give the product (-)-(1*S*,2*R*,4*S*,6*R*)-2,4-Bis(benzyloxy)-6-propyloxycyclohexanol **161** as a light coloured oil (210 mg, 98%) (HRMS: found: $[M + H]^+$ 371.2229. $C_{36}H_{41}O_5$ requires 371.2222); $[\alpha]$ –25.0 (*c* 1.1 in EtOAc); υ_{max}(neat)/cm^{-1} 3490 s, 2975 s, 1728 s and 1090 s; δ_H(300 MHz; C^2HCl_3) 0.85 (3H, t, $^3J_{H\text{-}H}$ 7.42 Hz, $OCH_2CH_2CH_3$), 1.16-1.34 (2H, m, 3-H and 5-H), 1.47-1.58 (2H, qt, $OCH_2CH_2CH_3$), 2.25-2.49 (3H, m, 2 x secondary-H and OH), 3.32-3.63 (4H, m, $OCH_2CH_2CH_3$, 4-H and 6-H), 3.64-3.88 (2 H, m1-H and 2-H), 4.37-4.60 (4 H, m, 2 x OCH_2Ph) and 7.17-7.27 (10H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 10.40 ($OCH_2CH_2CH_3$), 23.15 (CH_3), 33.92 (3-C), 35.21 (5-C), 70.49 (OCH_2Ph), 71.06 ($OCH_2CH_2CH_3$), 71.56 (4-C), 71.92 (OCH_2Ph), 75.46 (6-C), 76.11 (2-C), 76.64 (1-C), 127.63 & 128.40 (Ar-C ortho, meta and para) and 138.60 (Ar-C

quaternary); *m/z* (CI) 371 (100%, $[M + H]^+$), 281 (49), 263 (31), 107 (78) and 91 (20).

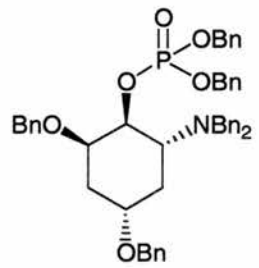
9.37 (-)-(1*S*,2*R*,4*S*,6*R*)-Phosphoric acid 2,4-bis-benzyloxy-6-dibenzylamino-cyclohexyl ester diphenyl ester 119



The hydrochloric salt of (-)-(*1*S,*2*R,*4*S,*6*R)-2,4-bis-benzyloxy-6-dibenzylamino-cyclohexanol **116** (150 mg, 0.27 mmol) was dissolved in around 5 cm^3 of DCM. Et_3N was added (0.34 mmol, 300 mg, 0.05 cm^3) followed by diphenyl chlorophosphate (0.34 mmol, 90mg, 0.07 cm^3) and DMAP (0.07 mmol, 10 mg). After overnight stirring the mixture was evaporated to give a residue which was washed with water (2 x 10 cm^3) and extracted with ethyl acetate (2 x 10 cm^3). The mixture was separated, dried ($NaSO_4$), and evaporated to give an oil. The crude product was purified by column chromatography (ethyl acetate-light petroleum,1:15) to give the product (-)-(1*S*,2*R*,4*S*,6*R*)-phosphoric acid 2,4-bis-benzyloxy-6-dibenzylamino-cyclohexyl ester diphenyl ester **119** as a yellow oil (180 mg, 88%);□ δ_H(300 MHz; C^2HCl_3) 0.83-1.34 (3 H, m, 2 x secondary-H), 2.20-2.27 (1 H, m, secondary-H), 3.38-3.41 (1 H, m, CHN), 3.41–3.59 (2 H, m, NCH_2Ph), 3.63-3.79 (2 H, m, NCH_2Ph), 3.97-4.03 (1 H, m, CH-4), 4.16-4.20 (1 H, m ,CH-2), 4.22-4.39 (4H, m, 2 x OBn), 4.73-4.80 (1H, m, CH-1) and 7.04-7.18 (30 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 26.74 (3-C), 34.07 (5-C), 53.25 (6-C, d $^2J_{C\text{-}P}$ 9.66 Hz), 53.55 (2 x NCH_2Ph), 70.73 (OCH_2Ph), 71.39 (4-C), 72.15

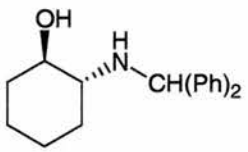
(OCH_2Ph), 75.95 (2-C), 81.26 (1-C, d, $^2J_{C\text{-}P}$ 6.49) 119.87, 119.95, 120.02, 120.08, 125.23, 126.67, 126.72, 127.12, 127.62, 127.86, 128.09, 128.38, 128.84, 129.49, 129.69, 129.80, 130.03, 130.13 (Ar-CH), 134.85, 135.62, 135.73, 138.54, 138.68, and 139.94 (Aryl-C quaternary); δ_P(121.4 MHz; C^2HCl_3) -12.36.

9.38 (-)-(1*S*,2*R*,4*S*,6*R*)-Phosphoric acid dibenzyl ester 2,4-bis-benzyloxy-6-dibenzylamino-cyclohexyl ester 120



The phosphate (-)-(1*S*,2*R*,4*S*,6*R*)-phosphoric acid 2,4-bis-benzyloxy-6-dibenzylamino-cyclohexyl ester diphenyl ester **119** (100 mg, 0.13 mmol) was dissolved in dry THF (5 cm^3). NaH was then added (0.23 mmol, 0.01 g - 60% dispersion). Benzyl alcohol (0.23 mmol, 0.02 g, 0.016 cm^3) was then added dropwise. After stirring overnight at room temperature 10 cm^3 was added to the solution before being extracted with ethyl acetate (2 x 10 cm^3). This was then separated, dried ($NaSO_4$) and evaporated to give an oil. The product was then columned on silica (ethyl acetate-petroleum ether, 1:4) to give the product (-)-(1*S*,2*R*,4*S*,6*R*)-phosphoric acid dibenzyl ester 2,4-bis-benzyloxy-6-dibenzylamino-cyclohexyl ester **120** as a yellow oil (90 mg, 87%); δ_H(300 MHz; C^2HCl_3) 1.12-1.40 (2 H, m, secondary-H), 2.10-2.20 (2 H, m, secondary-H), 3.33-3.37 (1 H, m, CHN), 3.39-3.55 (3 H, m, NCH_2Ph & CH-4), 3.76-3.80 (2 H, m, NCH_2Ph), 4.03-4.06 (1 H, m, CH-2), 4.30-4.60 (5H, m, 2 x OBn & CH-1), 4.86-

5.14 (4 H, m, 2 x POBn) and 6.70-7.30 (30 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 32.72 (3-C), 34.40 (5-C), 53.29 (6-C, d $^2J_{C\text{-}P}$ 8.66 Hz), 53.59 (2 x NCH_2Ph), 69.15 (OCH_2Ph, d, *J*p 6.49 Hz), 69.37 (OCH_2Ph, d, $^2J_{C\text{-}P}$ 6.49 Hz), 70.75 (4-C), 71.84 & 72.29 (2 x OCH_2Ph), 75.98 (2-C), 79.97 (1-C, d, $^2J_{C\text{-}P}$ 7.58) 126.69, 126.85, 126.98, 127.18, 127.61, 127.68, 127.81, 128.10, 128.15, 128.42, 128.58, 128.69, 128.70, 129.03 (Ar-CH), 135.85, 135.95, 138.62, 138.90 & 140.13 (Aryl-C quaternary); δ_P(121.4 MHz, C^2HCl_3) –1.30.

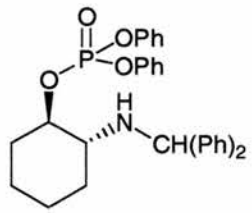


9.39 (±)-*Trans*-2-(Benzhydryl-amino)-cyclohexanol 199

(±)-Cyclohexene oxide **56** (0.25 g, 2.54 mmol) was dissolved in 1,2-dichloroethane (10 cm^3) along with aminodiphenylmethane (3.05 mmol, 0.55 g, 0.59 cm^3). The catalyst ytterbium triflate (0.3 g) was added to the solution and the mixture refluxed for two hours followed by tlc. The solvent was removed by evaporation and the residue washed with brine (2 x 20 cm^3) and extracted with ethyl acetate (2 x 20 cm^3). The solvent was removed under reduced pressure and the resulting oil purified using silica chromatography (ethyl acetate-petroleum ether, 1:5) giving the product *trans*-2-(benzhydryl-amino)-cyclohexanol (±)-**199** as a yellow oil (0.79 g, 96%); δ_H(300 MHz; C^2HCl_3) 0.89-1.37 (4H, m, 3 & 4 CH_2), 1.64-1.75 (2 H, m, 4 & 5 CH_2), 2.02-2.08 (1 H, m, CH-6), 2.23-2.36 (2 H, m, 4 & 5 CH_2), 3.24-3.32 (1 H, m, CH-1), 5.12 (1 H, s, CH), 7.22-7.49 (Ar-H); δ_C(75 MHz C^2HCl_3) 23.94 (4-C), 24.77 (3-C), 30.18 (5-C), 32.76 (2-C), 60.35

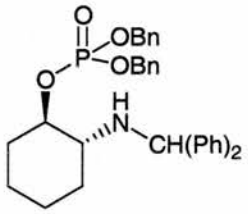
(CH), 63.21 (6-C), 74.14 (C-1), 127.00, 127.03, 127.35, 128.45, 128.48, 128.54 (Ar-CH), 143.13 & 144.88 (quaternary C).

9.40 (±)-*Trans*-Phosphoric acid 2-(benzhydryl-amino)-cyclohexyl ester diphenyl ester 200



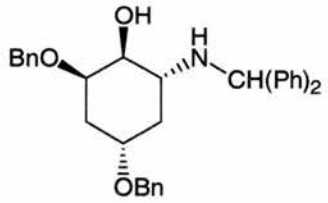
The hydrochloride salt of *trans*-2-(benzhydryl-amino)-cyclohexanol (±)-**199** (390 mg, 1.4 mmol) was partially dissolved in dichloromethane (20cm^3). Et_3N (1.6 mmol, 0.17 g, 0.23 cm^3), diphenylchlorophosphate (0.31 g, 0.31 cm^3) and DMAP (0.32 mmol, 0.04 g) was added and the mixture stirred for 12 hours. The solvent was then removed under reduced pressure and the residue washed with brine (30 cm^3). The aqueous solution was extracted with ethyl acetate (2 x 20 cm^3) and the combined organic layers dried (Mg_2SO_4). The solvent was removed under reduced pressure to give an oil which was purified by silica column chromatography (ethyl acetate – petroleum ether, 1:4) to give the product *trans*-phosphoric acid 2-(benzhydryl-amino)-cyclohexyl ester diphenyl ester (±)-**200** as an oil (0.4 g, 56%); δ_H(300 MHz C^2HCl_3) 0.68-1.71 (5 H, m, 3, 4 & 2 CH_2), 1.83-2.16 (1 H, m, CH-6), 2.50-2.69 (1 H, m, CH-2), 4.52-4.66 (1 H, m, CH-1), 5.04 (1 H, s, CH), 7.01-7.52 (20 H, Ar-H); δ_C(75 MHz C^2HCl_3) 23.23 (4-C), 23.30 (3-C), 29.46 (5-C), 31.81 (2-C), 58.14 (6-C), 63.63 (CH), 82.90 (C-1), 125.20, 125.30, 127.20, 127.21, 127.43, 127.51, 127.61, 128.19, 128.44 (Ar-CH), 137.23, 137.64, 142.05 & 144.75 (quaternary C); δ_P(121.4 MHz; C^2HCl_3) –12.22.

9.41 (±)-*Trans*-Phosphoric acid 2-(benzhydryl-amino)-cyclohexyl ester dibenzyl ester 201



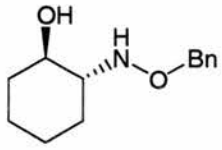
The phosphate *trans*-phosphoric acid 2-(benzhydryl-amino)-cyclohexyl ester diphenyl ester (±) **200** (0.4 g, 0.78 mmol) was dissolved in THF (20 cm^3) and added to a solution of NaH (1.00 mmol, 0.024 g) in THF (10 cm^3). Benzyl alcohol was added to the mixture (1.14 mmol, 0.12 g, 0.12 cm^3) and the solution stirred for 12 h. The solvent was removed by evaporation and the residue washed with brine (10 cm^3). The aqueous phase was extracted with ethyl acetate (2 x 15 cm^3) and the combined organic layers dried (Mg_2SO_4) and evaporated under reduced pressure to give an oil. The product *trans*-phosphoric acid 2-(benzhydryl-amino)-cyclohexyl ester dibenzyl ester (±)-**201** was gained following silica chromatography (ethyl acetate – petroleum ether, 1:5) as an oil (180 mg, 43%). δ_H(300 MHz C^2HCl_3) 0.89-2.11 (5 H, m, 3, 4 & 2 CH_2), 2.47-2.52 (1 H, m, CH-6), 3.28-3.59 (1 H, m, CH-2), 4.15-4.22 (1 H, m, CH-1), 4.90-4.98 (5 H, m, CH & 2 x Bn), 7.06-7.26 (20H, Ar-H); δ_C(75 MHz C^2HCl_3) 23.41 (4-C), 23.60 (3-C), 29.13 (5-C), 31.86 (2-C), 58.25 (6-C), 63.93 (CH), 69.00 & 69.08 (2 x $POCH_2$), 82.05 (d, 7.58 Hz, C-1), 126.77, 126.89, 127.38, 127.48, 127.51, 127.69, 127.81, 128.35, 128.40, 128.48, 128.51, 128.57 (Ar-CH), 136.00, 136.10, 144.02 & 144.85 (quaternary C); δ_P (121.4 MHz; C^2HCl_3) –0.98.

9.42 (-)-(1*S*,2*R*,4*S*,6*R*)-6-(Benzhydryl-amino)-2,4-bis-benzyloxy-cyclohexanol 202



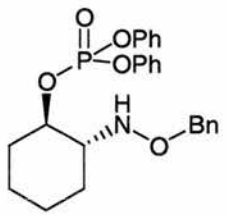
The key epoxide (+)-(1*S*,2*R*,4*R*,6*R*)-2,4-bis(benzyloxy)-1,6-epoxycyclohexane **53** (0.6 g, 1.9 mmol) was dissolved in 1,2-dichloroethane (10 cm^3). To the solution was added aminodiphenyl methane (2.1 mmol, 0.39 g) followed by the catalyst ytterbium triflate (0.42 mmol, 0.26 g). The mixture was refluxed for 3 h, after which time the reaction was deemed complete by tlc. The solvent was removed and the residue washed with brine (20 cm^3). The brine was extracted with ethyl acetate (2 x 20 cm^3) and the combined organic layers dried (Mg_2SO_4). The solvent was then removed by evaporation under reduced pressure and the resulting oil purified by column chromatography (ethyl acetate-petroleum ether, 1:4) to give the product (-)-(1*S*,2*R*,4*S*,6*R*)-6-(benzhydryl-amino)-2,4-bis-benzyloxy-cyclohexanol **202** as an oil (0.88 g, 92%); δ_H(300 MHz; C^2HCl_3) 2.07-2.27 (4 H, m, 2 x secondary-H), 2.78 (1 H, dt $^2J_{H\text{-}H}$ 4.12, $^3J_{H\text{-}H}$ 14.3 Hz, CHN), 3.43 (1H, dd, $^2J_{H\text{-}H}$ 2.75, $^3J_{H\text{-}H}$ 8.5 Hz, CH-1), 3.53-3.62 (1 H, m, CH-4), 3.81-3.85 (1 H, m, CH-2), 4.32 & 4.45 (4 H, 2 x OCH_2Ph), 4.93 (1 H, s, NCH) and 7.07-7.31 (10 H, m, Ar-H); δ_C (75.4 MHz; C^2HCl_3) 33.22 (3-C), 34.48 (5-C), 54.13 (6-C), 64.22 (2 x NCH_2Ph), 70.53 and 71.56 (2 x OCH_2Ph), 72.83 (4-C), 74.70 (2-C), 75.46 (1-C), 126.98, 127.02, 127.33, 127.45, 127.53, 127.58, 127.61, 128.37, 128.44, 128.47, 128.50, 128.58 (Ar-CH), 138.61, 138.65, 143.69 & 144.78 (Aryl-C quaternary).

9.43 (±)-*Trans*-2-Benzyloxyamino-cyclohexanol 125



(±)-Cyclohexene oxide **56** (0.4 g, 4.07 mmol) was dissolved in 1,2-dichloroethane (10 cm^3). To this solution was added O-benzyl hydroxylamine (4.07 mmol, 0.65 g) followed by the catalyst ytterbium triflate (0.8 mmol, 0.5 g) and 2,6-lutidine (4.07 mmol, 0.44 g, 0.47 cm^3). The mixture was stirred for 12 h before the solvent was removed by evaporation to give an oil. This was washed with brine (10 cm^3), extracted with ethyl acetate (2 x 10 cm^3) and dried (Mg_2SO_4). The solvent was removed under reduced pressure to leave an oil which was purified by silica column chromatography (ethyl acetate-petroleum ether, 1:2) to give the product *trans*-2-benzyloxyamino-cyclohexanol **125** as an oil (180 mg, 90%); δ_H(200 MHz, C^2HCl_3): 1.12-1.40 (4 H, m, ring protons), 1.63-1.79 (2 H, m, ring protons), 1.85-2.19 (2 H, m, ring protons) 2.59-2.77 (1H, m, CHN), 3.37-3.52 (1 H, m, CHOH), 4.70 (2 H, s, CH_2Ph), 7.29-7.43 (5 H, m, Aryl-H); δ_C(300 MHz, C^2HCl_3): 24.65 & 25.00 (3-C, 4-C), 29.01 (5-C), 34.15 (2-C), 66.00 (OCH_2Ph), 72.89 (6-C), 76.92 (1-C), 128.21, 129.03, 129.10, (Aryl-C ortho, meta, para), 138.97 (Aryl-C quaternary); *m/z* (CI) 222 (72%, $[M + H]^+$) and 99 (100, $[C_6H_{11}O]^+$).

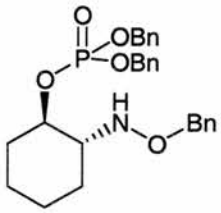
9.44 (±)-*Trans*-Phosphoric acid 2-benzyloxyamino-cyclohexyl ester diphenyl ester 126



The alcohol *trans*-2-benzyloxyamino-cyclohexanol (±)-**125** (50 mg, 0.23 mmol) was dissolved in dichloromethane (10 cm^3). Diphenylchlorophosphate (0.12 g, 0.09 cm^3, 0.46 mmol) was dropped in slowly followed by the addition of triethylamine (0.46 mmol, 0.05 g, 0.06 cm^3) and DMAP (0.01 g). The mixture was then stirred for 12 h before removing the solvent by evaporation under reduced pressure. The residue was washed with brine (10 cm^3) and extracted with ethyl acetate (2 x 10 cm^3). The combined organic fractions were dried (Mg_2SO_4) and the solvent removed under reduced pressure to leave a residue which was purified by silica column chromatography (ethyl acetate-petroleum ether, 1:4) to give the target *trans*-phosphoric acid 2-benzyloxyamino-cyclohexyl ester diphenyl ester (±)-**126** as an oil (90 mg, 88%); δ_H(200 MHz, C^2HCl_3) 1.12-1.40 (4 H, m, ring protons), 1.63-1.82 (2 H, m, ring protons), 2.01-2.31 (2 H, m, ring protons) 2.72-2.93 (1 H, m, CHN), 4.56-4.71 (1 H, m, CHOH & CH_2Ph), 7.03-7.57 (15 H, m, Aryl-H); δ_C(300 MHz, C^2HCl_3) 23.72 & 23.77 (3-C, 4-C), 28.52 (5-C), 32.00 (2-C), 63.60 (6-C, d, $^2J_{C\text{-}P}$ 7.58 Hz), 77.00 (OCH_2Ph), 79.32 (1-C, d, $^2J_{C\text{-}P}$ 7.58 Hz), 128.25, 128.28, 128.31, 128.35, 128.38, 129.03, 129.66, 129.70, 129.73, (Aryl-C ortho, meta, para), 135.67, 137.83, 150.69 (Aryl-C quaternary);

δ_P(121.4 MHz; C^2HCl_3) –12.49; *m/z* (CI) 454 (66%, $[M + H]^+$), 332 (24%, $[M - NHOBn]^+$) and 249 (63, $[P(O)O(OPh)_2]^+$).

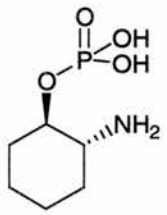
9.45 (±)-*Trans*-Phosphoric acid dibenzyl ester 2-benzyloxyamino-cyclohexyl ester 127



The amine *trans*-phosphoric acid 2-benzyloxyamino-cyclohexyl ester diphenyl ester (±)-**126** (900 mg, 2.0 mmol) was dissolved in THF (10 cm^3) and added dropwise to a THF (50 cm^3) solution of NaH (2.40 mmol, 0.1 g) at 0 °C. To the stirred solution was added benzyl alcohol (2.4 mmol, 200 mg, 0.2 cm^3) followed by stirring for 2 h. The solvent was then removed under reduced pressure to leave a residue which was washed with brine (2 x 20 cm^3). The aqueous mixture was extracted with ethyl acetate (2 x 20 cm^3) and the combined organic phases dried (Mg_2SO_4). The oil remaining after the solvent was removed by evaporation was subjected to silica column chromatography chromatography (ethyl acetate-petroleum ether, 1:4) to give the target phosphoric acid dibenzyl ester 2-benzyloxyamino-cyclohexyl ester (±)-**127** as an oil (400 mg, 42%); δ_H(200 MHz, C^2HCl_3) 1.22-1.38 (2 H, m, ring protons), 1.51-1.64 (2 H, m, ring protons), 1.90-2.14 (4H, m, ring protons), 2.63-2.75 (1 H, m, CHN), 4.23-4.35 (1 H, m, CHOP), 4.54 (2 H, s, $NOCH_2Ph$), 4.89 (2 H, d, $^2J_{H\text{-}H}$ 8.66 Hz, $POCH_2Ph$), 4.92 (2 H, d, $^2J_{H\text{-}H}$ 8.66 Hz, $POCH_2Ph$) 7.12-7.24 (15 H, m, Aryl-H); δ_C(300 MHz, C^2HCl_3): 23.71 & 23.74 (3-C, 4-C), 28.53 (5-C), 32.00 (2-C), 63.64 (6-C, d, 6.49 Hz),

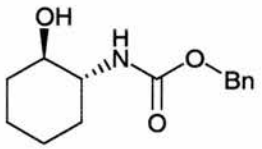
68.91 ($POCH_2Ph$, d, $^2J_{C\text{-}P}$ 3.25 Hz), 69.01 ($POCH_2Ph$, d, $^2J_{C\text{-}P}$ 3.25 Hz), 76.90 (OCH_2Ph), 77.76 (1-C, d, 6.49 Hz), 127.53, 127.58, 127.65, 127.71, 128.14, 128.18, 128.24, 128.37, 128.45, (Aryl-C ortho, meta, para), 135.81, 135.98, 137.82 (Aryl-C quaternary); δ_P(121.4 MHz; C^2HCl_3) –1.16.

9.46 *Trans*-Phosphoric acid mono-(2-amino-cyclohexyl) ester 128



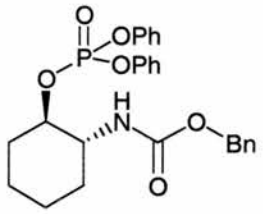
The fully protected amine *trans*-phosphoric acid dibenzyl ester 2-benzyloxyamino-cyclohexyl ester (±)-**127** (100 mg, 0.21 mmol) was dissolved in a 95% solution of ethanol. Pd/C (10%) (50 mg) was added and the mixture stirred under an atmosphere of hydrogen for 12 h. The mixture was extracted with diethyl ether and the aqueous layer filtered through celite by repeated washings with water and methanol. The solvent was removed by freeze drying to leave a white powder identified as the target compound *trans*-phosphoric acid mono-(2-amino-cyclohexyl) ester (±)-**128** (12.4 mg, 30 %); m.p. >200 °C; δ_H(200 MHz, D_2O): 1.11-2.12 (8 H, m, ring protons), 2.92-3.19 (1 H, m, CHN), 3.82-3.98 (1 H, m, CHOP); δ_C(300 MHz, D_2O) 25.41 & 26.92 (3-C, 4-C), 28.54 (5-C), 32.77 (2-C), 51.98 (6-C), 72.35 (1-C); δ_P (121.4 MHz; C^2HCl_3) +2.24.

9.47 (±)-*Trans*-(2-Hydroxy-cyclohexyl)-carbamic acid benzyl ester 129



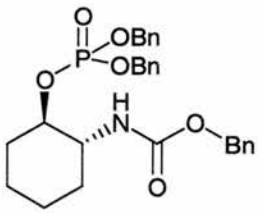
The amino alcohol *trans*-2-amino-cyclohexanol (±)-**117** (1 g, 8.7 mmol) was dissolved in 15 cm^3 of water. Sodium bicarbonate was then added (5.48 g, 19.0 mmol) followed by benzyl chloroformate (1.63 g, 9.6 mmol, 1.36 cm^3). This was stirred overnight. The product was isolated by eluting the aqueous solution with ethyl acetate which was separated, dried and evaporated to give a white solid which was recrystallised from 1:10 ethyl acetate/pet. ether to give *trans*-(2-hydroxy-cyclohexyl)-carbamic acid benzyl ester (±)-**129** as a white powder. (2 g, 97%) m.p (91-93 °C); %); (HRMS: found: M^+, 249.1356 $C_{14}H_{19}NO_3$ requires 249.1365); δ_H(300 MHz C^2HCl_3) 0.98-1.30 (4 H, m, 3 & 4-CH_2), 1.57-1.64 (2 H, m, 5-CH_2), 1.88-1.96 (2 H, m, 2-CH_2), 2.98-3.17 (1 H, b, OH), 3.20-3.35 (2 H, m, 1 & 6-CH), 4.90-4.97 (1 H, b, NH), 5.01-5.02 (2 H, s, CH_2Ph), 7.19-7.33 (5 H, ar, aromatic); δ_C(75 MHz C^2HCl_3) 23.93 (4-CH_2), 24.47 (3-CH_2), 31.63 (5-CH_2), 33.97 (2-CH_2), 56.97 (6-C), 66.89 (CH_2Ph), 74.62 (1-C), 128.18, 128.40, 128.51, 128.55, 128.61, 128.65, (Ar-CH), 136.39 (Ar-C quaternary), 157.37 (Urethane); *m/z* (EI^+) 249 (20%, $[M]^+$), 158 (17%, $[M - Bn]^+$) and 91 (100).

9.48 (±)-*Trans*-[2-(Diphenoxy-phosphoryloxy)-cyclohexyl]-carbamic acid benzyl ester 130



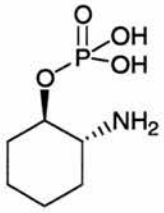
The alcohol *trans*-(2-hydroxy-cyclohexyl)-carbamic acid benzyl ester (±)-**129** (0.5 g, 2.0 mmol) was dissolved in dry DCM (20 cm^3). Et_3N was then added (3.21 mmol, 0.33 g, 0.45 cm^3) followed by diphenyl chlorophosphate (3.21 mmol, 0.86 g, 0.66 cm^3) and DMAP (0.53 mmol, 0.06 g). This was then stirred for 12 hours. The solvent was then evaporated and the residue washed with water. This was then extracted with ethyl acetate, which was separated, dried and evaporated to give *trans*-[2-(diphenoxy-phosphoryloxy)-cyclohexyl]-carbamic acid benzyl ester (±)-**130** as a white solid. The product was recrystallised from 1:15 ethyl acetate/petroleum ether to give white crystals (0.91 g, 90%); mp 96-98 °C; (Found: C, 64.7, H, 5.8, N, 2.9. $C_{26}H_{28}NO_6P$ requires C, 64.9, H, 5.9, N, 2.9%); (HRMS: found: M^+, 481.1610 $C_{26}H_{28}NO_6P$ requires 481.1654); δ_H(300 MHz C^2HCl_3) 1.18-2.14 (8 H, m, 4 x CH_2), 3.68 (1 H, m, 6-CH), 4.31-4.42 (1 H, m, 1-CH), 4.89-5.08 (2 H, m, CH_2Ph), 5.41 (1 H, b, NH), 7.11-7.34 (15 H, Ar-H); δ_C(75 MHz C^2HCl_3) 23.61 (4-CH_2), 23.70 (3-CH_2), 31.80 (5-CH_2), 32.22 (2-CH_2), 54.69 (6-C, d, $^2J_{C\text{-}P}$ 5.43Hz), 66.36 (CH_2Ph), 80.92 (1-C, d, $^2J_{C\text{-}P}$ 7.54Hz), 120.10, 120.16, 125.20, 125.25, 127.82, 127.85, 128.32, 129.61, 129.67, (Ar-CH), 136.50, 150.46, 150.56 (Ar-C quaternary), 155.94 (Urethane); *m/z* (EI^+) 481 (12%, $[M]^+$), 388 (21%, $[M - OPh]^+$) , 249 (90%, $[[P(O)O(OPh)_2]^+$) and 91 (87).

9.49 (±)-*Trans*-[2-(Bis-benzyloxy-phosphoryloxy)-cyclohexyl]-carbamic acid benzyl ester 131



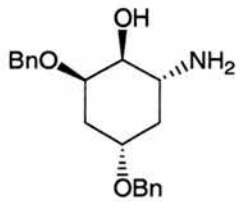
Trans-[2-(Diphenoxy-phosphoryloxy)-cyclohexyl]-carbamic acid benzyl ester (±)-**130** (0.24 g, 0.50 mmol) was dissolved in THF and added to a solution of NaH (0.02 g, 0.70 mmol). Benzyl alcohol (0.70 mmol, 0.07 g, 0.07 cm^3) was also added. The mixture was stirred overnight. The solvent was evaporated and the resulting residue washed with water (50 cm^3). The product was extracted with ethyl acetate (2 x 2 cm^3), separated, dried and evaporated to give *trans*-[2-(bis-benzyloxy-phosphoryloxy)-cyclohexyl]-carbamic acid benzyl ester (±)-**131** as an oil. This was subjected to silica column chromatography (ethyl acetate-petroleum ether, 1:10) to give the product as a brown oil (130 mg, 51%); δ_H(300 MHz C^2HCl_3) 1.00-2.08 (8 H, m, 4 x CH_2), 3.47-3.57 (1 H, m, 6-CH), 3.96-4.07 (1 H, m, 1-CH), 4.82-4.99 (6 H, m, 3 x CH_2Ph), 5.19-5.38 (1 H, b, NH), 7.16-7.30 (15H, ar, aromatic); δ_C(75 MHz C^2HCl_3) 23.80 (4 & 3-CH_2), 31.99 (5-CH_2), 32.40 (2-CH_2), 55.00 (6-CH, d, J 5.42 Hz), 66.38 (OCH_2Ph), 69.22 (2 x CH_2Ph), 79.31 (1-CH, d, J 6.49 Hz), 127.79, 127.84, 127.85, 127.86, 127.91, 127.94, 127.98, 128.05, 128.34, 128.38, 128.41, 128.44, 128.48, 128.50, 128.51, 128.54, 128.57, 128.61 (Ar-CH), 135.84, 135.90, 136.61 (Ar-C quaternary), 156.11 (Urethane); *m/z* (EI^+) 509 (4%, $[M]^+$), 312 (47%, $[M - OBn]^+$) and 91 (100).

9.50 (±)-*Trans*-phosphoric acid mono-(2-amino-cyclohexyl) ester 132



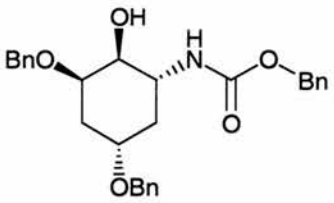
The protected amine *trans*-[2-(bis-benzyloxy-phosphoryloxy)-cyclohexyl]-carbamic acid benzyl ester (±)-**131** (130 mg, 0.25 mmol) was dissolved in methanol with 50 mg Pd/C under an atmosphere of hydrogen. This was then stirred for two days. The product was then filtered through celite with a methanol/water mixture and the solvents removed to give a yellow oil. The compound was then mixed with a dilute solution of cyclohexylamine until the pH was around 7-8 and the mixture was then lyophilised to give the *trans*-phosphoric acid mono-(2-amino-cyclohexyl) ester (±)-**132** as a white solid 50 mg (67%); m.p. > 200 °C; (HRMS: found: M^+, 196.0749 $C_6H_{15}NO_4P$ requires 196.0739); δ_H(300 MHz D_2O) 1.19-1.86 (18 H, m, 9 x CH_2), 2.96 (2 H, m, 6 and 1'-CHN), 3.78 (1 H, m, 1-CH); δ_C(75 MHz C^2HCl_3) 23.32, (4'-CH_2), 23.70 (2 x 3'-CH_2), 24.20 (2 x 2'-CH_2), 29.24, (4-CH_2), 30.27 (4-CH_2), 30.27 (3 & 5-CH_2), 32.41 (2-CH_2), 50.31-CH1'), 55.83 (6-CH), 73.99 (1-C); *m/z* (FAB$^+$) 196 (100%, $[M]^+$).

9.51 (-)-(1*S*,2*R*,4*S*,6*R*)-6-Amino-2,4-bis-benzyloxy-cyclohexanol 133



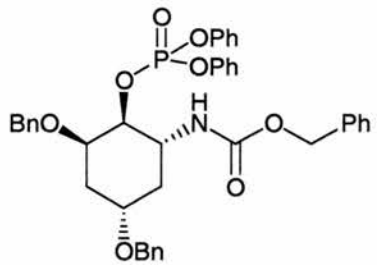
The key epoxide (+)-(1*S*,2*R*,4*R*,6*R*)-2,4-bis(benzyloxy)-1,6-epoxycyclohexane **53** (500 mg, 1.6 mmol) was dissolved in NH_3 solution (70 cm^3) accompanied by a few drops of ethanol to aid solvation. The catalyst ytterbium triflate was added (0.30 g, 0.48 mmol). The mixture was sealed in an airtight flask and heated to 70 °C for 12 h. The mixture was cooled and the aqueous solution was extracted with ethyl acetate. The combined organic fractions were pooled, dried (Mg_2SO_4), evaporated and purified using silica column chromatography (ethyl acetate-petroleum ether, 1:10) to give (-)-(1*S*,2*R*,4*S*,6*R*)-6-amino-2,4-bis-benzyloxy-cyclohexanol **133** as white crystals (459 mg, 87%); m.p. (81-83 °C); (HRMS: found: $[M + H]^+$, 328.1906 $C_{20}H_{26}NO_3$ requires 328.1913); $[\alpha]_D$ –10.6 (*c* 0.67, EtOAc); δ_H(300 MHz; C^2HCl_3) 1.08-1.34 (2 H, m, 2 x secondary-H), 2.15-2.26 (5 H, m, 2 x secondary-H, NH_2 & OH), 2.82 (1 H, s, CHN), 3.08-3.11 (1H, m, CH-1), 3.60-3.64 (1H, m, CH-4), 3.67-3.77 (1H, m, CH-2), 4.32-4.51 (4 H, m, 2 x OCH_2Ph) and 7.19-7.23 (10 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 33.61 (3-C), 38.26 (5-C), 49.82 (6-C), 70.36 and 71.32 (2 x OCH_2Ph), 71.85 (4-C), 76.57 (2-C), 76.94 (1-C), 127.32, 127.36, 127.52, 127.59, 128.32, 128.38 (Ar-CH), 138.28 & 138.58 (Aryl-C quaternary); *m/z* (CI) 328 (100), 278 (16), 238 (28), 123 (37) and 91 (29).

9.52 (-)-(1*S*,2*R*,4*S*,6*R*)-(2,4-Bis-benzyloxy-hydroxy-cyclohexyl)-6-carbamic acid benzyl ester 134



The amino alcohol (-)-(1*S*,2*R*,4*S*,6*R*)-6-amino-2,4-bis-benzyloxy-cyclohexanol **133** (150 mg, 0.46 mmol) was dissolved in dichloromethane (10 cm^3) containing a few drops of pyridine. The temperature was reduced to 0 °C and benzylchloroformate was added (0.14 cm^3, 1 mmol). The mixture was stirred overnight before adding NH_4Cl (10 cm^3) solution. The mixture was extracted with dichloromethane (3 x 10 cm^3) which was pooled then dried (Mg_2SO_4) and the solvent evaporated under reduced pressure. The residue was purified by silica column chromatography (ethyl acetate-petroleum ether, 1:1) to give the product (-)-(1*S*,2*R*,4*S*,6*R*)-(2,4-bis-benzyloxy-hydroxy-cyclohexyl)-6-carbamic acid benzyl ester **133** as a white powder (120 mg, 57%); m.p. 116-118 °C; $[\alpha]_D$ –8.7 (*c* 0.15, EtOAc); δ_H(300 MHz; C^2HCl_3) 1.51-2.28 (4 H, m, 2 x secondary-H), 2.82 (1 H, s, CHN), 3.08-3.11 (1H, m, CH-1), 3.60-3.64 (1H, m, CH-4), 2.50 (1H, b, OH), 3.73-3.90 (3H, m, CH-2, CH-1, CH-4), 3.98-4.03 (1H, m, CH-6), 4.45 (2 H, s, OCH_2Ph, 4.49 (2 H, s, OCH_2Ph), 5.05-5.17 (2 H, m, OCH_2Ph) 5.65 (1H, b, NH) and 7.26-7.36 (15 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 31.05 (3-C), 31.89 (5-C), 50.57 (6-C), 66.66 (OCH_2Ph), 70.78 and 71.15 (2 x OCH_2Ph), 71.17 (4-C), 73.51 (2-C), 74.07 (1-C), 127.71, 127.87, 127.97, 128.17, 128.63 (Ar-CH), 136.76, 138.24 (Aryl-C quaternary), 156.33 (Carbonyl C); *m/z* (CI) 462 (100), 322 (43), and 140 (72).

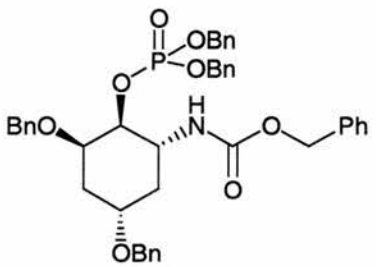
9.53 (-)-(1*S*,2*R*,4*S*,6*R*)-[2,4-Bis-benzyloxy-1-(diphenoxy-phosphoryloxy)-cyclohexyl]-6-carbamic acid benzyl ester 135



The amino alcohol (-)-(1*S*,2*R*,4*S*,6*R*)-(2,4-bis-benzyloxy-hydroxy-cyclohexyl)-6-carbamic acid benzyl ester **134** (120 mg, 0.26 mmol) was dissolved in dry DCM (10 cm^3) and Et_3N (0.52 mmol, 50 mg, 0.07 cm^3) was added followed by diphenyl chlorophosphate (0.52 mmol, 140 mg, 0.11 cm^3) and DMAP (10 mg). The mixture was stirred overnight at r.t. before the solvent was removed by evaporation under reduced pressure. The residue was washed with brine (10 cm^3) and extracted using ethyl acetate (2 x 10 cm^3) before drying (Mg_2SO_4). The solvent was removed by evaporation to give an oil which was purified using silica column chromatography (ethyl acetate-petroleum ether, 1:10) to give the product (-)-(1*S*,2*R*,4*S*,6*R*)-[2,4-bis-benzyloxy-1-(diphenoxy-phosphoryloxy)-cyclohexyl]-6-carbamic acid benzyl ester **135** as a clear oil (120 mg, 68%); $[\alpha]_D$ –15.9 (*c* 0.15, EtOAc); (HRMS: Found: $[M + H]^+$, 693.2483. $C_{40}H_{40}NO_8P$ requires 693.2492); δ_H(300 MHz; C^2HCl_3) 1.43-2.17 (4 H, m, 2 x secondary-H), 3.79-3.83 (3 H, m, CHN, CH-4, CH-2), 4.21-4.36 (6 H, m, 2 x OCH_2Ph), 4.44-4.51 (2H, m, OCH_2Ph), 4.70-4.85 (1H, m, CH-1), 4.88-5.05 (2H, m, OCH_2Ph [cbz]), 5.60-5.78 (1H, b, NH) and 7.06-7.24 (25 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 31.66 (3-C), 31.79 (5-C), 49.24 (6-C), 66.61 (OCH_2Ph), 70.80 (2 x OCH_2Ph), 71.32 (4-C), 73.10 (2-C), 77.21 (1-C), 120.23, 120.29, 125.25, 125.31, 127.62, 127.82, 128.05,

128.35, 128.51, 128.57, 129.71, 129.77 (Ar-CH), 136.63, 138.06, 138.24, 150.79 (x2) (Aryl-C quaternary), 155.62 (Carbonyl C); δ_P(121.4 MHz; C^2HCl_3) –11.70; *m/z* (EI) 693 (11), 602 (13), 352 (51), 251 (100) and 140 (56).

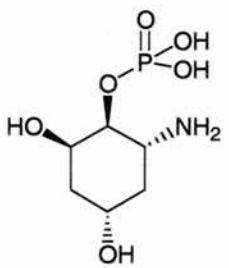
9.54 (-)-(1*S*,2*R*,4*S*,6*R*)-[2,4-Bis-benzyloxy-1-(bis-benzyloxy-phosphoryloxy)-cyclohexyl]-6-carbamic acid benzyl ester 136



To a THF (10 cm^3) solution of NaH (0.26 mmol, 0.01 g [60% dispersion]) at – 70 °C was added the amine (-)-(1*S*,2*R*,4*S*,6*R*)-[2,4-bis-benzyloxy-1-(diphenoxy-phosphoryloxy)-cyclohexyl]-6-carbamic acid benzyl ester **135** (120 mg, 0.18 mmol) in THF (10 cm^3). While stirring continued benzyl alcohol (0.26 mmol, 0.03 g, 0.03 cm^3) was added. The mixture was kept at – 70 °C for several hours before allowing to slowly warm to r.t.. After 12 h the solvent was removed and the residue taken up with brine (20 cm^3). The solution was extracted with ethyl acetate (2 x 20 cm^3) and the pooled organic fractions dried (Mg_2SO_4) and the solvent removed by evaporation. The resulting oil was purified by slica column chromatography (ethyl acetate-petroleum ether, 1:10) to give the product (-)-(1*S*,2*R*,4*S*,6*R*)-[2,4-bis-benzyloxy-1-(bis-benzyloxy-phosphoryloxy)-cyclohexyl]-6-carbamic acid benzyl ester **136** as an oil (110 mg, 88%); $[\alpha]_D$ –23.3 (*c* 0.20, EtOAc); (HRMS: found: $[M + H]^+$, 744.2689. $C_{42}H_{44}NO_8PNa$ requires 744.2702); δ_H(300 MHz; C^2HCl_3) 1.37-2.39 (4 H, m, 2 x secondary-H), 3.70-3.83 (2 H, m,

CHN, CH-4), 4.11-4.19 (1 H, m, CH-2), 4.31-4.59 (5 H, m, 2 x OCH_2Ph & CH-1), 4.83-5.02 (6 H, m, 3 x OCH_2Ph), 5.65-5.79 (1 H, b, NH) and 7.14-7.45 (25 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 32.04 (3-C), 32.07 (5-C), 49.30 (6-C), 66.49 (OCH_2Ph), 69.31 (2 x OCH_2Ph) 70.73 (2 x OCH_2Ph), 71.32 (4-C), 72.92 (2-C), 79.12 (1-C), 127.56, 127.74, 127.88, 128.02, 128.29, 128.40, 128.48, 129.66 (Ar-CH), 135.88, 135.97, 136.57, 138.03, 138.24 (Aryl-C quaternary), 155.66 (Carbonyl C); δ_P(121.4 MHz; C^2HCl_3) –0.50; *m/z* (FAB) 744 (100) and 242 (82).

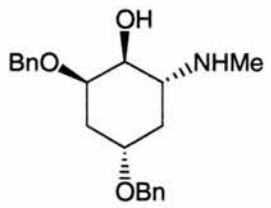
9.55 (-)-(1*S*,2*R*,4*S*,6*R*)-Phosphoric acid mono-(6-amino-2,4-dihydroxy-cyclohexyl) ester 137



The amine (-)-(1*S*,2*R*,4*S*,6*R*)-[2,4-bis-benzyloxy-1-(bis-benzyloxy-phosphoryloxy)-cyclohexyl]-6-carbamic acid benzyl ester **136** (60 mg, 0.08 mmol) was dissolved in methanol (10 cm^3). To the solution was added Pd/C (10%) (5 mg) and a few drops of acetic acid. The mixture was stirred under an atmosphere of H_2 (g) for 48 h. The solution was then filtered through a pad of celite and the pad washed with methanol (3 x 20 cm^3) and water (3 x 20 cm^3). The solvent was then evaporated under reduced pressure and the water removed by freeze drying to give a white solid **137** (17 mg, 100%). Stirring a solution of the presence of cyclohexylamine gave the target molecule (-)-(1*S*,2*R*,4*S*,6*R*)-phosphoric acid mono-(6-amino-2,4-dihydroxy-cyclohexyl) ester as the

cyclohexylamine salt; m.p. > 200 °C (decomp.) δ_H(300 MHz; D_2O) 1.31-1.55 (2 H, m, secondary-H), 2.03-2.28 (2 H, m, secondary-H), 3.41-3.56 (1 H, m, CHN), 4.11-4.19 (1H, m, CH-2), 4.31-4.59 (5 H, m, 2 x OCH_2Ph & CH-1), 3.90-4.18 (3H, m, CH-1, CH-2, CH-4); δ_C(75.4 MHz; D_2O) 35.06 (3-C), 35.14 (5-C), 51.12 (6-C), 63.22 (4-C), 72.36 (2-C), 75.27 (1-C); δ_P(121.4 MHz; C^2HCl_3) +0.45.

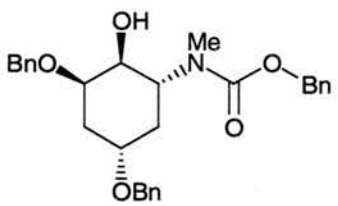
9.56 (-)-(1*S*,2*R*,4*S*,6*R*)-2,4-Bis-benzyloxy-6-methylamino-cyclohexanol 139



The key epoxide (+)-(1*S*,2*R*,4*R*,6*R*)-2,4-bis(benzyloxy)-1,6-epoxycyclohexane **53** (300 mg, 0.97 mmol) was added to a solution of methylamine in water (60 cm^3). To the mixture was added the catalyst ytterbium triflate (0.19 mmol, 114 mg) before sealing the flask and heating with stirring for 48 h at 60°C. The excess ammonia gas was allowed to escape by bubbling with nitrogen for several hours and the aqueous layer extracted with ethyl acetate (2 x 50 cm^3). After drying (Mg_2SO_4) the combined organic fractions the solvent was removed under reduced pressure. Remaining residues of water were removed by sustained drying in a desiccator in the presence of P_2O_5 and KOH under vacuum conditions to give after 48 h the product (-)-(1*S*,2*R*,4*S*,6*R*)-2,4-bis-benzyloxy-6-methylamino-cyclohexanol **139** as an oil (329 mg, 100%); $[\alpha]_D$ –36.1 (*c* 0.16, EtOAc); (HRMS: found: $[M + H]^+$, 342.2074 $C_{21}H_{28}NO_3$ requires 342.2069); δ_H(300 MHz; C^2HCl_3) 1.11-1.42 (4 H, m, 2 x secondary-H), 2.30-2.37 (1 H, m, CHN), 2.71 (3 H, d, 4.2

Hz, Methyl), 3.23 (1 H, b, OH), 3.34-3.42 (1 H, dd, 6.59 & 3 Hz, CH-1), 3.65-3.28 (1 H, m, CH-4), 3.84-3.89 (1 H, m, CH-2), 4.44-4.61 (4 H, m, 2 x OCH_2Ph) and 7.22-7.38 (10 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 32.65 (3-C), 33.51 (5-C), 34.10 (Methyl), 57.35 (6-C), 70.36 and 71.51 (2 x OCH_2Ph), 71.86 (4-C), 73.89 (2-C), 76.37 (1-C), 127.56, 127.61, 128.24, 128.28, 128.31, 128.34 (Ar-CH), 138.31 & 138.51 (Aryl-C quaternary); *m/z* (CI) 342 (100), 250 (21), 91 (7).

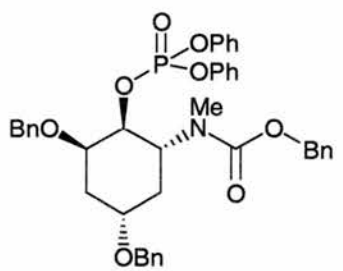
9.57 (-)-(1*S*,2*R*,4*S*,6*R*)-(2,4-Bis-benzyloxy-1-hydroxy-cyclohexyl)-6-methyl-carbamic acid benzyl ester 143



The amino alcohol (-)-(1*S*,2*R*,4*S*,6*R*)-2,4-bis-benzyloxy-6-methylamino-cyclohexanol **139** (340 mg, 1 mmol) was dissolved in a 2N solution of NaOH (50 cm^3) with a little ethanol to aid phase transfer. The temperature was reduced to 0 °C and benzylchloroformate was added (0.16 cm^3, 0.19 g, 1.1 mmol) and the mixture stirred for 3 h. The mixture was extracted with ethylacetate (3 x 30 cm^3) which was pooled then dried (Mg_2SO_4) and the solvent evaporated under reduced pressure. The residue was purified by silica column chromatography (ethyl acetate-petroleum ether, 1:1) to give the product (-)-(1*S*,2*R*,4*S*,6*R*)-(2,4-bis-benzyloxy-1-hydroxy-cyclohexyl)-6-methyl-carbamic acid benzyl ester **143** as a white powder (310 mg, 65%); m.p. 93-95 °C; δ_H (300 MHz; C^2HCl_3) 1.99-2.20 (2 H, m, 2 x secondary-H), 2.25-2.33 (2 H, m, 2 x secondary-H), 2.75 (3 H, s, Methyl), 3.37 (1 H, s, CHN), 3.66-3.73 (1 H, m, CH-4), 3.77-3.83 (1 H, m, CH-1),

4.30-4.52 (4 H, s, 2 x OCH_2Ph), 4.96-5.05 (2 H, s, OCH_2Ph [cbz]) and 7.14-7.26 (15 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 28.06 (Methyl), 33.75 (3-C), 33.88 (5-C), 53.82 (6-C), 66.94 (OCH_2Ph [cbz]), 70.57 and 71.10 (2 x OCH_2Ph), 71.51 (4-C), 71.69 (2-C), 76.58 (1-C), 127.13, 127.26, 127.35, 127.65, 127.68, 127.79, 128.15, 128.35, 128.40 (Ar-CH), 138.11, 138.41, 156.85 (Aryl-C quaternary), 157.85 (Carbonyl C).

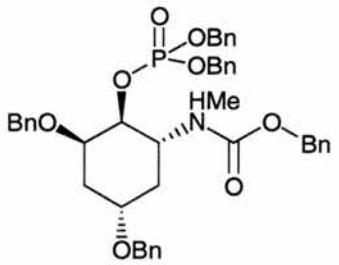
9.58 (-)-(1*S*,2*R*,4*S*,6*R*)-[2,4-Bis-benzyloxy-1-(diphenoxy-phosphoryloxy)-cyclohexyl]-6-methyl-carbamic acid benzyl ester 144



The amino alcohol (-)-(1*S*,2*R*,4*S*,6*R*)-(2,4-bis-benzyloxy-1-hydroxy-cyclohexyl)-6-methyl-carbamic acid benzyl ester **143** (300 mg, 0.63 mmol) was dissolved in dry DCM (20 cm^3) and Et_3N (0.75 mmol, 80 mg, 0.11 cm^3) was added followed by diphenyl chlorophosphate (0.75 mmol, 200 mg, 0.15 cm^3) and DMAP (20 mg, 0.13 mmol). The mixture was stirred overnight at r.t. before the solvent was removed by evaporation under reduced pressure. The residue was washed with brine (2 x 20 cm^3) and extracted using ethyl acetate (2 x 20 cm^3) before drying (Mg_2SO_4). The solvent was removed by evaporation to give an oil which was purified using silica column chromatography (ethyl acetate-petroleum ether, 1:10) to give the product (-)-(1*S*,2*R*,4*S*,6*R*)-[2,4-bis-benzyloxy-1-(diphenoxy-

phosphoryloxy)-cyclohexyl]-6-methyl-carbamic acid benzyl ester **144** as a clear oil (240 mg, 56%); $[\alpha]_D$ –19.1 (*c* 0.22, EtOAc); (HRMS: found: $[M + Na_2H]^{3+}$, 730.2534. $C_{39}H_{42}NO_8PNa_2$ requires 730.2522); δ_H(300 MHz; C^2HCl_3) 1.13-1.32 (2 H, m, 2 x secondary-H), 2.11-2.29 (2 H, m, 2 x secondary-H), 2.73 (3 H, s, Methyl), 3.78-3.86 (1 H, m, CH-4), 3.96-47.08 (2 H, m, CH-2 & CH-6), 4.25-4.43 (4 H, m, 2 x OCH_2Ph), 4.50-4.66 (1 H, m, CH-1), 4.83-5.10 (2 H, m, OCH_2Ph [cbz]) and 7.02-7.26 (25 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 29.46 (Methyl), 34.35 (3-C), 34.45 (5-C), 67.00 (OCH_2Ph [cbz]), 70.73 & 70.77 (2 x OCH_2Ph), 71.18 (6-C), 71.29 (4-C), 72.22 (2 x OCH_2Ph), 75.32 (2-C), 78.85 (1-C, d, $^2J_{C\text{-}P}$ 6.49 Hz), 127.18, 127.35, 127.45, 127.61, 127.65, 127.69, 127.74, 127.84, 128.25, 128.29, 128.38, 128.41, 129.67, 129.73, 129.80 (Ar-CH), 138.25, 138.34, 138.41, 150.49, 150.59 (Aryl-C quaternary), 156.14 (Carbonyl C); δ_P(121.4 MHz; C^2HCl_3) –12.14 & -11.98; *m/z* (FAB) 730 (100), 458 (87), 251 (38).

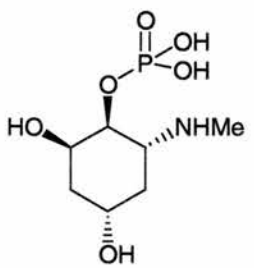
9.59 (-)-(1*S*,2*R*,4*S*,6*R*)-[2,4-Bis-benzyloxy-1-(bis-benzyloxy-phosphoryloxy)-cyclohexyl]-6-methyl-carbamic acid benzyl ester 145



To a THF (20 cm^3) solution of NaH (0.62 mmol, 0.02 g [60% dipersion]) at -70 °C was added the amine (-)-(1*S*,2*R*,4*S*,6*R*)-[2,4-bis-benzyloxy-1-(diphenoxy-phosphoryloxy)-cyclohexyl]-6-methyl-carbamic acid benzyl ester **144** (220 mg, 0.31 mmol) in THF (20 cm^3). While stirring continued benzyl alcohol (0.62 mmol, 0.07 g, 0.06 cm^3) was added. The mixture was kept at – 70 °C for several hours before allowing to slowly warm to r.t.. After 12 h the solvent was removed and the residue taken up with brine (20 cm^3). The solution was extracted with ethyl acetate (2 x 20 cm^3) and the pooled organic fractions dried (Mg_2SO_4) and the solvent removed by evaporation. The resulting oil was purified by slica column chromatography (ethyl acetate-petroleum ether, 1:10) to give the product [2,4-bis-benzyloxy-1-(bis-benzyloxy-phosphoryloxy)-cyclohexyl]-6-methyl-carbamic acid benzyl ester **145** as an oil (203 mg, 89%); $[\alpha]_D$ –20.7 (*c* 0.23, EtOAc); (HRMS: found: $[M + H]^+$, 758.2880. $C_{43}H_{46}NO_8PNa$ requires 758.2859); δ_H(300 MHz; C^2HCl_3) 0.80-0.98 (2 H, m, 2 x secondary-H), 1.50-1.66 (2 H, m, 2 x secondary-H), 2.84 (3 H, s, Methyl), 3.80-3.91 (1 H, m, CH-4), 4.04-4.14 (1 H, m, CH-2), 4.38-4.60 (5 H, m, 2 x OCH_2Ph & CH-6), 4.66-5.20 (7 H, m, 3 x OCH_2Ph & 1H), and 7.18-7.42 (25 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 29.56

(Methyl), 33.84 (3-C), 34.63 (5-C), 67.02 (OCH_2Ph [cbz]), 69.27 (OCH_2Ph), 70.28 (6-C), 71.30 (4-C), 71.46 (2 x OCH_2Ph) 72.41 (OCH_2Ph), 79.18 (1-C), 127.28, 127.49, 127.50, 127.68, 127.81, 127.94, 128.09, 128.32, 128.48, 128.55 (Ar-CH), 135.73, 135.97, 136.73, 138.59, 138.90 (Aryl-C quaternary), 156.54 (Carbonyl C); δ_P(121.4 MHz; C^2HCl_3) –1.45 & -1.63; *m/z* (FAB) 758 (100) 621 (34), 458 (45) and 242 (31).

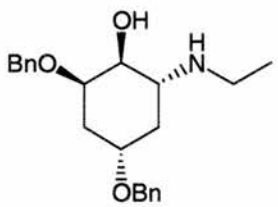
9.60 (-)-(1*S*,2*R*,4*S*,6*R*)-Phosphoric acid mono-(2,4-dihydroxy-6-methylamino-cyclohexyl) ester 146



The amine (-)-(1*S*,2*R*,4*S*,6*R*)-[2,4-bis-benzyloxy-1-(bis-benzyloxy-phosphoryl oxy)-cyclohexyl]-6-methyl-carbamic acid benzyl ester **145** (60 mg, 0.08 mmol) was dissolved in methanol (10 cm^3). To the solution was added Pd/C (10%) (5 mg) and a few drops of acetic acid. The mixture was stirred under an atmosphere of H_2 (g) for 48 h. The solution was then filtered through a pad of celite and the pad washed with methanol (3 x 20 cm^3) and water (3 x 20 cm^3). The solvent was then evaporated under reduced pressure and the water removed by freeze drying to give a white solid **146** (17 mg, 100%). Stirring a solution of the presence of cyclohexylamine gave the target molecule (-)-(1*S*,2*R*,4*S*,6*R*)-phosphoric acid mono-(2,4-dihydroxy-6-methylamino-cyclohexyl) ester as the cyclohexylamine salt; δ_H(300 MHz; D_2O) 1.40-1.53 (2 H, m, secondary-H), 2.19-2.28 (2 H, m, secondary-H), 2.64-2.89 (3 H, Methyl), 3.38-3.50 (1 H, m, CHN), 3.92-4.04 (2H,

m, CH-2, CH-4), 4.22-4.28 (1 H, m, CH-1); δ_C(75.4 MHz; D_2O) 37.46 (Methyl), 39.31 (3-C), 39.47 (5-C), 50.28 (6-C), 63.72 (4-C), 67.93 (2-C), 72.03 (1-C); δ_P(121.4 MHz; C^2HCl_3) +3.07.

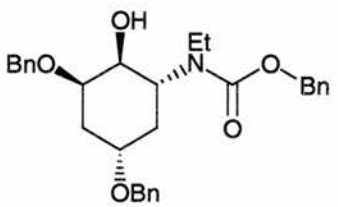
9.61 (-)-(1*S*,2*R*,4*S*,6*R*)-2,4-Bis-benzyloxy-6-ethylamino-cyclohexanol 148



The key epoxide (+)-(1*S*,2*R*,4*R*,6*R*)-2,4-bis(benzyloxy)-1,6-epoxycyclohexane **53** (520 mg, 1.68 mmol) was added to a solution of ethylamine in water (70 cm^3). To the mixture was added the catalyst ytterbium triflate (0.33 mmol, 200 mg) before sealing the flask and heating with stirring for 48 h at 65°C. The excess ethylamine gas was allowed to escape by bubbling with nitrogen for several hours and the aqueous layer extracted with ethyl acetate (2 x 50 cm^3). After drying (Mg_2SO_4) the combined organic fractions the solvent was removed under reduced pressure. Remaining residues of water was removed by sustained drying in a dessicator in the presence of P_2O_5 and KOH under vacuum conditions to give after 48 h the product (-)-(1*S*,2*R*,4*S*,6*R*)-2,4-bis-benzyloxy-6-ethylamino-cyclohexanol **148** as an oil (650 mg, 100%); $[\alpha]_D$ –23.6 (*c* 0.19, EtOAc); (HRMS: found: $[M + H]^+$, 355.2129 $C_{22}H_{29}NO_3$ requires 355.2147); δ_H(300 MHz; C^2HCl_3) 1.09-1.18 (3 H, t, Methyl), 1.23-1.71 (3 H, m, 2 x secondary-H), 2.03-2.20 (1 H, m, secondary-H), 2.31-2.46 (1 H, m, CHN), 2.55-2.68 (1H, m, NCH_2), 2.83-2.97 (1 H, m, NCH_2), 3.39 (1 H, b, OH), 3.42-3.50 (1 H, m, CH-1), 3.66-3.77 (1 H, m, CH-4), 3.88-3.94

(1 H, m, CH-2), 4.48-4.60 (4 H, m, 2 x OCH_2Ph) and 7.23-7.37 (10 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 31.63 (3-C), 33.54 (Methyl), 34.40 (5-C), 40.78 (NCH_2), 56.11 (6-C), 70.52 and 71.69 (2 x OCH_2Ph), 71.85 (4-C), 73.84 (2-C), 76.12 (1-C), 127.45, 127.56, 127.62, 127.76, 128.34, 128.40 (Ar-CH), 138.32, 138.55 (Aryl-C quaternary); *m/z* (CI) 356 (100), 264 (37), 91 (19).

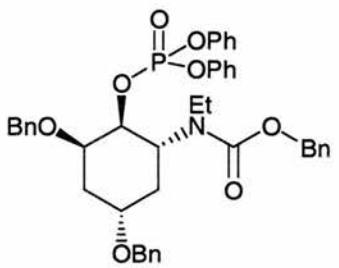
9.62 (-)-(1*S*,2*R*,4*S*,6*R*)-(2,4-Bis-benzyloxy-1-hydroxy-cyclohexyl)-6-ethyl-carbamic acid benzyl ester 149



The amino alcohol (-)-(1*S*,2*R*,4*S*,6*R*)-2,4-bis-benzyloxy-6-ethylamino-cyclohexanol **148** (600 mg, 1.69 mmol) was dissolved in a 2N solution of NaOH (50 cm^3) with a little ethanol to aid phase transfer. The temperature was reduced to 0 °C and benzylchloroformate was added (0.29 cm^3, 340 mg, 2.03 mmol). The mixture was extracted with ethyl acetate (3 x 30 cm^3) which was pooled then dried (Mg_2SO_4) and the solvent evaporated under reduced pressure. The residue was purified by silica column chromatography (ethyl acetate-petroleum ether, 1:1) to give the product (-)-(1*S*,2*R*,4*S*,6*R*)-(2,4-bis-benzyloxy-1-hydroxy-cyclohexyl)-6-ethyl-carbamic acid benzyl ester **149** as a white powder (570 mg, 69%); m.p. 92-95 °C; δ_H(300 MHz; C^2HCl_3) 1.12-1.12 (3 H, m, Methyl), 1.12-1.56 (3 H, m, secondary-H), 2.03-2.14 (1 H, m, secondary-H), 2.25-2.45 (2 H, m, 2 x secondary-H), 3.02-3.27 (2 H, m, NCH_2), 3.42-3.54 (1 H, s, CHN), 3.61-3.72 (1 H, m, CH-4), 3.76-3.81 (1 H, m, CH-1), 4.13-4.23 (1 H, m, CH-2), 4.30-4.53 (4 H, s, 2 x OCH_2Ph), 4.99-5.09 (2 H, s, OCH_2Ph [cbz]) and 7.13-7.67 (15 H, m, Ar-

H); δ_C(75.4 MHz; C^2HCl_3) 33.69 (Methyl), 34.90 (3-C), 34.95 (5-C), 37.95 (NCH_2), 54.84 (6-C), 66.80 (OCH_2Ph [cbz]), 70.55 and 71.53 (2 x OCH_2Ph), 71.65 (4-C), 71.79 (2-C), 76.76 (1-C), 127.16, 127.20, 127.38, 127.45, 127.51, 127.63, 127.72, 128.25, 128.28 (Ar-CH), 136.84, 138.14, 138.41 (Aryl-C quaternary), 157.91 (Carbonyl C).

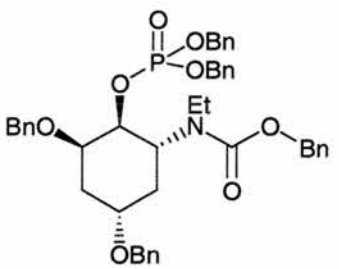
9.63 (-)-(1*S*,2*R*,4*S*,6*R*)-[2,4-Bis-benzyloxy-1-(diphenoxy-phosphoryloxy)-cyclohexyl]-6-ethyl-carbamic acid benzyl ester 150



The amino alcohol (-)-(1*S*,2*R*,4*S*,6*R*)-(2,4-bis-benzyloxy-1-hydroxy-cyclohexyl)-6-ethyl-carbamic acid benzyl ester **149** (530 mg, 1.08 mmol) was dissolved in dry DCM (25 cm^3) and Et_3N (1.30 mmol, 130 mg, 0.18 cm^3) was added followed by diphenyl chlorophosphate (130 mmol, 340 mg, 270 cm^3) and DMAP (40 mg, 0.3 mmol). The mixture was stirred overnight at r.t. before the solvent was removed by evaporation under reduced pressure. The residue was washed with brine (2 x 20 cm^3) and extracted using ethyl acetate (2 x 20 cm^3) before drying (Mg_2SO_4). The solvent was removed by evaporation to give an oil which was purified using silica column chromatography (ethyl acetate-petroleum ether, 1:10) to give the product (-)-(1*S*,2*R*,4*S*,6*R*)-[2,4-bis-benzyloxy-1-(diphenoxy-phosphoryloxy)-cyclohexyl]-6-ethyl-carbamic acid benzyl ester **150** as a clear oil (320 mg, 48%); $[\alpha]_D$ –28.6 (*c* 0.12, EtOAc); δ_H(300 MHz; C^2HCl_3) 1.08-1.21 (3 H, Methyl), 1.36-

1.44 (1 H, m, secondary-H), 1.91-2.38 (3 H, m, 2 x secondary-H), 3.21-3.43 (2 H, m, NCH_2), 3.76-3.94 (1 H, m, CH-4), 4.10-4.17 (2 H, m, CH-2 & CH-6), 4.37-4.54 (5 H, m, 2 x OCH_2Ph & CH-1), 4.82-5.05 (2 H, m, OCH_2Ph [cbz]) and 7.08-7.43 (25 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 34.28 (Methyl), 34.78 (3-C), 35.80 (5-C), 39.21 (NCH_2), 52.00 (6-C), 66.69 (OCH_2Ph [cbz]), (4-C), 70.59 & 71.30 (2 x OCH_2Ph), 72.29 (2-C), 79.35 (1-C), 127.25, 127.30, 127.39, 127.46, 127.53, 127.56, 127.59, 127.65, 127.72, 128.25, 128.32, 128.40, 129.67, 129.70, 129.76 (Ar-CH), 136.70, 136.85, 138.46, 138.93, 150.60 (Aryl-C quaternary), 156.51 (Carbonyl C); δ_P(121.4 MHz; C^2HCl_3) –6.12; *m/z* (FAB) 722 (100), 630 (43), 543 (19), 309 (23) and 91 (68).

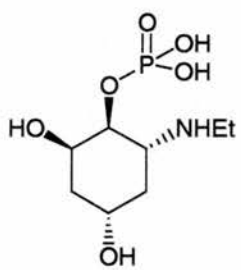
9.64 (-)-(1*S*,2*R*,4*S*,6*R*)-[2,4-Bis-benzyloxy-1-(bis-benzyloxy-phosphoryloxy)-cyclohexyl]-6-ethyl-carbamic acid benzyl ester 151



To a THF (20 cm^3) solution of NaH (0.53 mmol, 21.12 mg [60% dipersion]) at – 70 °C was added the amine (-)-(1*S*,2*R*,4*S*,6*R*)-[2,4-bis-benzyloxy-1-(diphenoxy-phosphoryloxy)-cyclohexyl]-6-ethyl-carbamic acid benzyl ester **150** (330 mg, 0.44 mmol) in THF (20 cm^3). While stirring continued benzyl alcohol (0.53 mmol, 57.3 mg, 0.05 cm^3) was added. The mixture was kept at – 70 °C for several hours before allowing to slowly warm to r.t.. After 12 h the solvent was removed

and the residue taken up with brine (20 cm^3). The solution was extracted with ethyl acetate (2 x 20 cm^3) and the pooled organic fractions dried (Mg_2SO_4) and the solvent removed by evaporation. The resulting oil was purified by slica column chromatography (ethyl acetate-petroleum ether, 1:10) to give the product **151** (-)-(1*S*,2*R*,4*S*,6*R*) -[2,4-bis-benzyloxy-1- (bis-benzyloxy - phosphoryloxy)-cyclohexyl]-6-ethyl-carbamic acid benzyl ester **151** as an oil (170 mg, 49%); δ_H(300 MHz; C^2HCl_3) 0.81-1.03 (2 H, m, 2 x secondary-H), 1.08-1.29 (3 H, Methyl), 1.44-1.63 (2 H, m, 2 x secondary-H), 3.21-3.47 (2 H, m, NCH_2), 3.88-3.97 (1 H, m, CH-4), 4.00-4.12 (1 H, m, CH-2), 4.35-4.59 (5 H, m, 2 x OCH_2Ph & CH-6), 4.66-5.20 (7 H, m, 3 x OCH_2Ph & 1H), and 7.11-7.45 (25 H, m, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 29.56 (Methyl), 33.79 (3-C), 34.29 (Methyl), 34.59 (5-C), 39.19 (NCH_2), 52.23 (6-C), 67.00 (OCH_2Ph [cbz]), 69.26 (OCH_2Ph), 71.33 (4-C), 71.46 (2 x OCH_2Ph) 72.41 (OCH_2Ph), 79.15 (1-C), 127.24, 127.41, 127.55, 127.69, 127.83, 127.94, 128.00, 128.33, 128.40, 128.56 (Ar-CH), 135.71, 135.99, 136.70, 138.44, 150.63 (Aryl-C quaternary), 156.09 (Carbonyl C); δ_P(121.4 MHz; C^2HCl_3) –1.53.

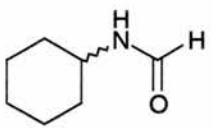
9.65 (-)-(1*S*,2*R*,4*S*,6*R*)-Phosphoric acid mono-(6-ethylamino-2,4-dihydroxy-cyclohexyl) ester 152



The amine (-)-(1*S*,2*R*,4*S*,6*R*)-[2,4-bis-benzyloxy-1-(bis-benzyloxy-phosphoryloxy)-cyclohexyl]-6-ethyl-carbamic acid benzyl ester **151** (170 mg,

0.23 mmol) was dissolved in methanol (10 cm^3). To the solution was added Pd/C (10%) (10 mg) and a few drops of acetic acid. The mixture was stirred under an atmosphere of H_2 (g) for 48 h. The solution was then filtered through a pad of celite and the pad washed with methanol (3 x 20 cm^3) and water (3 x 20 cm^3). The solvent was then evaporated under reduced pressure and the water removed by freeze drying to give a white solid (55 mg, 100%). Stirring a solution of the presence of cyclohexylamine gave the target molecule (-)-(1*S*,2*R*,4*S*,6*R*)-phosphoric acid mono-(6-ethylamino-2,4-dihydroxy-cyclohexyl) ester **152** as the cyclohexylamine salt; δ_H(300 MHz; D_2O) 1.02-1.20 (3 H, m, Methyl), 1.43-1.59 (2 H, m, secondary-H), 2.04-2.15 (1 H, m, secondary-H), 2.22-2.39 (1 H, m, secondary-H) 3.06-3.37 (2 H, m, CH_2), 3.42-3.58 (1 H, m, CHN), 3.90-4.14 (2 H, m, CH-2, CH-4), 4.22-4.28 (1 H, m, CH-1); δ_C(75.4 MHz; D_2O) 33.82 (Methyl), 34.65 (3-C), 34.81 (5-C), 37.54 (CH_2), 55.89 (6-C), 63.28 (4-C), 67.22 (2-C), 74.27 (1-C, d, 5.4 Hz); δ_P(121.4 MHz; C^2HCl_3) +3.48.

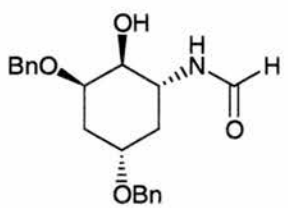
9.66 (±)-*N*-(2-Hydroxy-cyclohexyl)-formamide 155



Acetic anhydride (1.33 g, 1.16 cm^3, 13 mmol) was cooled to 0 °C before formic acid (0.7 g, 0.6 cm^3, 16 mmol) was added dropwise. The mixture was then heated to 50-60 °C for two hours before again cooling. (±)-Cyclohexylamine **154** (0.5 g, 5 mmol) dissolved in THF (50 cm^3) was added slowly and the mixture stirred under an atmosphere of nitrogen for 3 hours. The reagents were removed by evaporation and the residue washed with brine (100 cm^3). This was extracted with ether (2 x 50 cm^3) and the combined organic fractions dried (Mg_2SO_4) before

evaporating the solvent to give an oil. Purification by silica column chromatography (ethyl acetate-petroleum ether 1:5) gave the product *N*-(2-hydroxy-cyclohexyl)-formamide **155** as a white solid (83%); m.p. 33-35 °C; δ_H(300 MHz C^2HCl_3) 0.87-1.19 (4 H, m, 3 & 4 CH_2), 1.59-1.89 (4 H, m, 4 & 5 CH_2), 2.52-2.59 (1 H, m, CH-6), 3.70-3.84 (2 H, m, CH-2 & OH); δ_C(75 MHz C^2HCl_3) 24.57 (4-C), 24.84 (3-C), 33.92 (5-C), 34.43 (2-C), 51.62 (6-C), 74.39 (C-1).

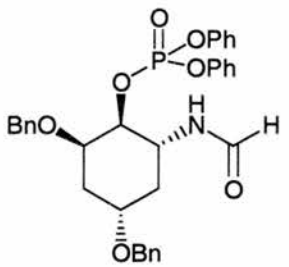
9.67 (-)-(1*S*,2*R*,4*S*,6*R*)-6-N-(2,4-Bis-benzyloxy-1-hydroxy-cyclohexyl)-formamide 156



Acetic anhydride (200 mg, 0.18 cm^3, 1.96 mmol) was cooled to 0 °C before formic acid (90 mg, 00.07 cm^3, 1.96 mmol) was added dropwise. The mixture was then heated to 50-60 °C for two hours before again cooling. The ammonia derivative product (-)-(1*S*,2*R*,4*S*,6*R*)-(2,4-bis-benzyloxy-hydroxy-cyclohexyl)-6-carbamic acid benzyl ester **133** (290 mg, 0.89 mmol), dissolved in THF (50 cm^3), was added slowly and the mixture stirred under an atmosphere of nitrogen for 3 hours. The reagents were removed by evaporation and the residue washed with brine (100 cm^3). This was extracted with ether (2 x 50 cm^3) and the combined organic fractions dried (Mg_2SO_4) before evaporating the solvent to give an oil. Purification by silica column chromatography (ethyl acetate-petroleum ether 1:5) gave the product (-)-(1*S*,2*R*,4*S*,6*R*)-6-N-(2,4-bis-benzyloxy-1-hydroxy-cyclohexyl)-formamide **156** as a white solid (112 mg, 36%); m.p. 69-71 °C;

$[\alpha]_D$ −15.3 (*c* 0.25, EtOAc); (HRMS: found: $[M + H]^+$, 355.1798 $C_{21}H_{25}NO_4$ requires 355.1784); δ_H(300 MHz C^2HCl_3) 1.21-2.19 (4 H, m, 3 & 4 CH_2), 2.53-2.59 (1 H, m, CHN), 3.70-3.84 (2 H, m, CH-2, CH-4), 4.21-4.32 (1H, m, CH-1), 4.39-4.51 (2 x CH_2Ph), 6.37-6.50 (1 H, m, C(O)H), 7.17-7.27 (10 H, Ar-H); δ_C(75 MHz C^2HCl_3) 30.62 (3-C), 31.59 (5-C), 47.84 (6-C), 70.92 and 71.02 (2 x OCH_2Ph), 71.47 (4-C), 73.68 (2-C), 76.88 (1-C), 127.32, 127.74, 127.90, 128.01, 128.66, 128.70 (Ar-CH), 138.13 & 138.98 (Aryl-C quaternary), 161.12 (carbonyl C); *m/z* (CI) 356 (100), 264 (25), 173 (15), 91 (33).

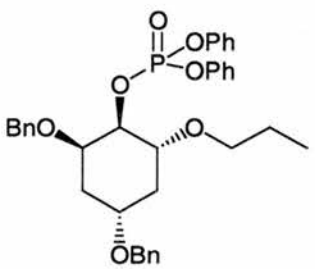
9.68 (-)-(1*S*,2*R*,4*S*,6*R*)-Phosphoric acid 2,4-bis-benzyloxy-6-formylamino-cyclohexyl ester diphenyl ester 157



The amino alcohol (-)-(1*S*,2*R*,4*S*,6*R*)-6-N-(2,4-bis-benzyloxy-1-hydroxy-cyclohexyl)-formamide **156** (40 mg, 0.11 mmol) was dissolved in dry DCM (20 cm^3) and Et_3N (0.24 mmol, 25 mg, 0.03 cm^3) was added followed by diphenyl chlorophosphate (0.24 mmol, 40 mg, 0.04 cm^3) and DMAP (5 mg, 0.01 mmol). The mixture was stirred overnight at r.t. before the solvent was removed by evaporation under reduced pressure. The residue was washed with brine (2 x 20 cm^3) and extracted using ethyl acetate (2 x 20 cm^3) before drying (Mg_2SO_4). The solvent was removed by evaporation to give an oil which was purified using silica column chromatography (ethyl acetate-petroleum ether, 1:10) to give the product (-)-(1*S*,2*R*,4*S*,6*R*)-phosphoric acid 2,4-bis-benzyloxy-6-formylamino-cyclohexyl

ester diphenyl ester **157** as a clear oil (30 mg, 45%); δ_H(300 MHz; C^2HCl_3) 1.50-2.30 (4 H, m, 3 & 4 CH_2), 2.44-2.57 (1 H, m, CHN), 3.71-3.77 (2 H, m, CH-2, CH-4), 4.13-4.19 (1 H, m, CH-1), 4.40-4.51 (2 x CH_2Ph), 7.07-7.38 (10 H, Ar-H); δ_C(75.4 MHz; C^2HCl_3) 33.56 (3-C), 36.08 (5-C), 50.87 (6-C), 70.95 and 71.00 (2 x OCH_2Ph), 72.40 (4-C), 74.31 (2-C), 80.26 (1-C), 127.25, 127.30, 127.39, 127.46, 127.53, 127.56, 127.59, 127.65, 127.72, 128.25, 128.32, 128.40, 129.67, 129.70, 129.76 (Ar-CH), 136.70, 136.85, 138.46, 138.93, (Aryl-C quaternary), 158.84 (Carbonyl C); δ_P(121.4 MHz; C^2HCl_3) –12.62.

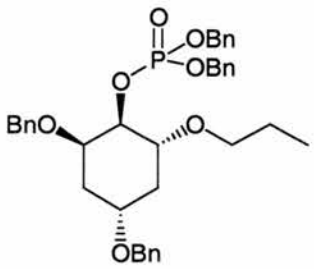
9.69 (-)-(1*S*,2*R*,4*S*,6*R*)-Phosphoric acid dibenzyl ester 2,4-bis-benzyloxy-6-formylamino-cyclohexyl ester 162



Under an atmosphere of N_2, a stirred solution of alcohol (-)-(1*S*,2*R*,4*S*,6*R*)-2,4-bis(benzyloxy)-6-propyloxycyclohexanol (-)-**161** (340 mg, 0.92 mmol) in dry dichloromethane (50 cm^3) was reacted with DMAP (37 mg, 0.3 mmol) and dry TEA (0.17 cm^3, 121 mg, 1.2 mmol) followed by chlorodiphenylphosphate (0.31 cm^3, 403 mg, 1.5 mmol) in dry dichloromethane (50 cm^3). After stirring at room temperature for 3 hours, water (50 cm^3) was added, the two phases separated, the aqueous phase extracted with dichloromethane (50 cm^3) and the combined organic phases dried ($MgSO_4$). The organic solvent was evaporated *in vacuo* and the residual oil chromatographed on silica (ethyl acetate-petroleum ether; 1:5) to give

phosphate triester phosphoric acid dibenzyl ester 2,4-bis-benzyloxy-6-formylamino-cyclohexyl ester **162** as a colourless oil (510 mg, 92%); (HRMS: found: $[M + H]^+$, 603.2504. $C_{35}H_{40}O_7P$ requires 603.2512); $[\alpha]_D$ -37.7 (*c* 0.17, MeOH); δ_H(300 MHz; C^2HCl_3) 0.84 (3 H, t, $^3J_{H\text{-}H}$ 7.8, $OCH_2CH_2CH_3$), 1.38-1.55 (4 H, m, $OCH_2CH_2CH_3$, 3-H and 5-H), 2.22-2.32 (1H, m, secondary-H), 2.40-2.49 (1H, m, secondary-H), 3.36-3.50 (2 H, m, $OCH_2CH_2CH_3$), 3.72-3.82 (2 H, m, 4-H and 6-H), 3.07-4.12 (1 H, m, 2-H), 4.41 (1 H, d, $^2J_{H\text{-}H}$ 11.4, OCH_2Ph), 4.45 (1 H, d, $^2J_{H\text{-}H}$ 12.3, OCH_2Ph), 4.46 (1H, d, $^2J_{H\text{-}H}$ 11.4, OCH_2Ph), 4.52 (1 H, d, $^2J_{H\text{-}H}$ 12.3, OCH_2Ph), 4.51-4.59 (1 H, m, 1-H) and 7.10-7.40 (20H, m, Ar-H ortho, meta and para); δ_C(75.4 MHz; C^2HCl_3) 10.35 ($OCH_2CH_2CH_3$), 23.09 ($OCH_2CH_2CH_3$), 33.89 & 35.53 (3-C and 5-C), 70.60 (OCH_2Ph), 71.07 (4-C), 71.78 ($OCH_2CH_2CH_3$), 72.19 (OCH_2Ph), 74.47 (C tertiary, $^3J_{C\text{-}P}$ 6.6), 75.35 (C tertiary, $^3J_{C\text{-}P}$ 2.8), 82.85 (1-C, $^2J_{C\text{-}P}$ 7.6), 120.12, 120.18, 120.20, 120.28, 125.15, 125.22, 127.55, 127.58, 127.61, 127.67, 128.35, 128.39, 128.44, 128.47 & 129.70 (Ar-C ortho, meta and para), 138.51 & 138.59 (Ar-C quaternary) and 150.87 (Ar-C quaternary); δ_P(121.5 MHz; C^2HCl_3) -12.32; *m/z* (EI) 603 (16), 341 (13), 251 (56), 140 (43) and 91 (100).

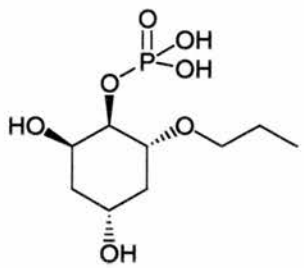
9.70 (-)-(1*S*,2*R*,4*S*,6*R*)-1-[Bis(benzyloxy)phosphoryloxy]-2,4-bis-(benzyloxy)-6-propyloxycyclohexane 163



Under an atmosphere of N_2, a stirred solution of the (-)-(1*S*,2*R*,4*S*,6*R*)-phosphate phosphoric acid dibenzyl ester 2,4-bis-benzyloxy-6-formylamino-cyclohexyl ester **162** (480 mg, 0.8 mmol) and benzyl alcohol (0.165 cm^3, 172 mg, 1.6 mmol) in dry THF (50 cm^3) was treated with NaH (60% dispersion in oil, 76 mg, 1.9 mmol). The solution was allowed to warm to room temperature over 3 h and water (50 cm^3) was added with caution. The mixture was extracted with diethyl ether (2 x 50 cm^3), the organic phases combined, dried ($MgSO_4$) and concentrated under reduced pressure. The residual oil was chromatographed on silica (ethyl acetate-petroleum ether, 1:10) to give phosphate (-)-(1*S*,2*R*,4*S*,6*R*)-1-[Bis(benzyloxy)phosphoryloxy]-2,4-bis-(benzyloxy)-6-propyloxycyclohexane **163** as a white solid (340 mg, 67%); m.p. 68-69 °C; (Found: C, 70.9, H, 7.1. $C_{37}H_{43}O_7P$ requires C, 70.5, H, 6.9%); (HRMS: found: $[M - C_7H_7]^+$, 539.2204. $C_{30}H_{36}O_7P$ requires 539.2199) $[\alpha]_D$ -33.4 (*c* 0.155, MeOH); δ_H(300 MHz; C^2HCl_3) 0.86 (3 H, t, $^3J_{H\text{-}H}$ 7.4, $OCH_2CH_2CH_3$), 1.34-1.46 (2 H, m, 3-H and 5-H), 1.48-1.58 (2 H, m, $OCH_2CH_2CH_3$), 2.09-2.29 (1 H, m, secondary-H), 2.38-2.47 (1 H, m, secondary-H), 3.4-3.54 (2 H, m, $OCH_2CH_2CH_3$), 3.70-3.82 (2 H, m, 4-H and 6-H), 4.07-4.12 (1 H, m, 2-H), 4.28-4.36 (1 H, m, 1-H), 4.45 (1 H, d, $^2J_{H\text{-}H}$ 11.5, OCH_2Ph), 4.49 (1 H, d, $^2J_{H\text{-}H}$ 11.8, OCH_2Ph), 4.52 (1 H, d, $^2J_{H\text{-}H}$ 11.8,

OC*H*$_2$Ph), 4.58 (1 H, d, $^2J_{H\text{-}H}$ 11.8, OC*H*$_2$Ph), 5.03-5.12 (4 H, m, 2 x POC*H*$_2$Ph) and 7.20-7.40 (20 H, m, Ar-H ortho, meta and para); δ_C(75.4 MHz; C^2HCl_3) 10.40 ($OCH_2CH_2CH_3$), 23.22 ($OCH_2CH_2CH_3$), 33.95 & 35.37 (3-C and 5-C), 69.00 (POCH$_2$Ph, $^2J_{C\text{-}P}$ 5.4), 69.11 (POCH$_2$Ph, $^2J_{C\text{-}P}$ 6.5), 70.59 (OCH$_2$Ph), 71.20 (4-C), 71.65 ($OCH_2CH_2CH_3$), 72.31 (OCH$_2$Ph), 74.65 (C tertiary, $^3J_{C\text{-}P}$ 6.5), 75.24 (C tertiary), 81.36 (1-C, $^2J_{C\text{-}P}$ 6.6), 127.56, 127.61, 127.65, 127.82, 127.89, 128.32, 128.38, 128.41, 128.45, 128.54 & 128.60 (Ar-C ortho, meta and para) and 138.64 (Ar-C quaternary); δ_P (121.5 MHz; C^2HCl_3) -1.55; *m/z* (EI) 539 (21), 279 (26), 261 (25) and 91 (100);

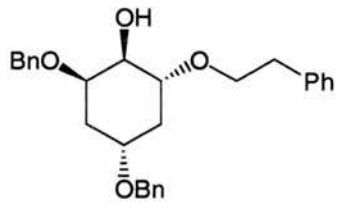
9.71 (-)-(1*R*,2*R*,4*R*,6*R*)-6-Propoxy-2,4-dihydroxycyclohexyl bis-(cyclohexyl ammonium) phosphate [bis(cyclohexylammonium) salt] 164



Under an atmosphere of N_2, gasous ammonia (15-20 cm^3) was condensed at -78 °C and sodium metal (110 mg, 4.8 mmol) was added. A solution of phosphate triester (-)-(1*S*,2*R*,4*S*,6*R*)-1-[bis(benzyloxy)phosphoryloxy]-2,4-bis-(benzyloxy)-6-propyloxycyclohexane **163** (300 mg, 0.48 mmol) in dry THF (0.5 cm^3) was added to the blue solution through a septum. After stirring at –78 °C for 30 minutes, methanol (2 cm^3) was added and the mixture allowed to warm up to room temperature. The solvents were removed under reduced pressure and the residual white solid was subjected to ion exchange chromatography (Amberlite

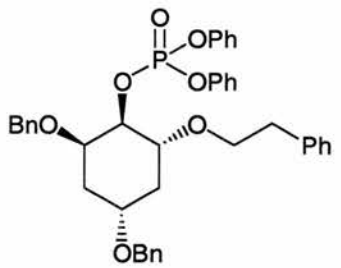
IR-118H) eluting with water. The acidic fractions containing the product were collected and an excess of freshly distilled cyclohexylamine was added and stirring a t room temperature was continued for 4 h. The aqueous layer was extracted with diethyl ether (3 x 50 cm^3) to remove the excess of cyclohexylamine, and lyphilised. The crude white solid was recrytstallised from water/acetone to give phosphate (-)-(1*R*,2*R*,4*R*,6*R*)-6-Propoxy-2,4-dihydroxycyclohexyl bis-(cyclohexyl ammonium) phosphate [bis(cyclohexylammonium) salt] **164** as a white solid (84 mg, 65%); m.p. >200 °C (decomp.); $[\alpha]_D$ -37.7 (*c* 0.526, MeOH); δ_H(500 MHz; ^2H_2O) 0.74 (3 H, t, $^3J_{H\text{-}H}$ 7.41, $OCH_2CH_2CH_3$), 0.94-1.12 (2 H, m, 2 x 4-H of Cha), 1.12-1.30 (9 H, m, 2 x {2 x 2-H and 3-H of Cha} and 5-H), 1.36-1.54 (5 H, m, $OCH_2CH_2CH_3$, 2 x 4-H of Cha and 3-H), 1.58-1.70 (4 H, m, 2 x {2 x 3-H of Cha}), 1.79-1.88 (4 H, m, 2 x {2 x 2-H of Cha}), 1.9-2.0 (1 H, m, 3-H), 2.12-2.20 (1H, m, 5-H), 2.94-3.06 (2 H, m, 2 x 1-H of Cha), 3.40-3.60 (3 H, m, $OCH_2CH_2CH_3$ and 6-H), 3.80-3.91 (2 H, m, 1-H and 4-H) and 4.16-4.22 (1 H, m, 2-H); δ_C(75.4 MHz; ^2H_2O) 9.40 ($OCH_2CH_2CH_3$), 22.08 ($OCH_2CH_2CH_3$), 23.42 (3-C of Cha), 23.91 (4-C of Cha), 29.97 (2-C of Cha), 36.68 (5-C), 36.95 (3-C), 50.0 (1-C of Cha), 64.08 (4-C), 67.13 (2-C), 71.72 ($OCH_2CH_2CH_3$), 75.12 (1-C, $^2J_{C\text{-}P}$ 6.6) and 77.29 (6-C, broad); δ_P(121.4 MHz; ^2H_2O) +3.11;

9.72 (-)-(1*S*,2*R*,4*S*,6*R*)-2,4-Bis-benzyloxy-6-phenethyloxy-cyclohexanol 165



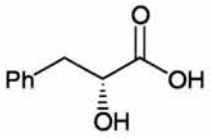
The key epoxide (+)-(1*S*,2*R*,4*R*,6*R*)-2,4-bis(benzyloxy)-1,6-epoxycyclohexane **53** (100 mg, 0.32 mmol) was placed in a round bottomed flask and dissolved in 10 cm^3 of dry dichloroethane. $Yb(OTf)_3$ was added (0.06 g, 0.06 mmol) followed by 2-phenylethanol (0.04 g, 0.32 mmol, 0.04 cm^3). This was refluxed for two hours before removing the solvent by evaporation. The residue was washed with water (10 cm^3) and extracted with ethyl acetate (2 x 10 cm^3). The organic was separated and dried with sodium sulphate before filtering and evaporation to yield the crude product as a brown oil. The oil was purified by silica column chromatography (ethyl acetate-petroleum ether, 1:5) to give the product (-)-(1*S*,2*R*,4*S*,6*R*)-2,4-Bis-benzyloxy-6-phenethyloxy-cyclohexanol **165** as a colourless oil (90 mg, 65% yield); (HRMS: found: $[M]^+$, 433.2370. $C_{28}H_{33}O_4$ requires 433.2379), $[\alpha]_D$ -70.6 (c 0.34 in EtOAc); δ_H(300 MHz; C^2HCl_3) 1.19-1.29 & 2.18-2.41 (4 H, m, 2 x secondary-H), 2.80 (2 H, t, 8-CH_2), 3.34-3.42 (2 H, m, 1-CH & 6-CH), 4.03-4.06 (1 H, m, CH-2), 3.58-3.80 (3 H, m, 4-CH & 7-CH_2), 3.83 (1 H, m, 2-CH), 4.33-4.57 (4 H, m, 2 x OCH_2Ph), 7.03-7.35 (15 H, Ar-H); δ_C(75 MHz; C^2HCl_3) 33.91 (3-C), 35.11 (5-C), 36.53 (8-CH_2), 70.20 (7-CH_2), 70.49 (OCH_2Ph), 71.51 (4-CH), 71.92 (OCH_2Ph), 75.42 (6-C), 76.01 (2-C), 77.01 (1-C), 126.26,127.55, 127.61, 127.63, 128.38, 128.41, 128.90 (Ar-CH), 138.54, 138.91 (Ar-C quaternary); *m/z* (CI) 433 (15), 123 (48), 107 (100) and 91 (19).

9.73 (-)-(1*S*,2*R*,4*S*,6*R*)-Phosphoric acid 2,4-bis-benzyloxy-6-phenethyloxy-cyclohexyl ester diphenyl ester 166



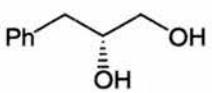
A solution of (-)-(1*S*,2*R*,4*S*,6*R*)-2,4-bis-benzyloxy-6-phenethyloxy-cyclohexanol **166** (50 mg, 0.11 mmol) in dichloromethane (20 cm^3) was stirred in the presence of triethylamine (0.16 mmol, 0.02 g, 0.02 cm^3), diphenyl chlorophosphate (0.16 mmol, 0.14 g, 0.03 cm^3) and DMAP (0.01 g) for 12 h. The solvents were then removed by evaporation and the residue washed with brine (20 cm^3) before extraction with diethyl ether (2 x 20 cm^3). The combined organic fractions were then dried (Mg_2SO_4) and the solvent removed by evaporation to give an oil which was purified by silica column chromatography (ethyl acetate-petroleum ether, 1:10) to give the product phosphoric acid 2,4-bis-benzyloxy-6-phenethyloxy-cyclohexyl ester diphenyl ester **166** as an oil (71 mg, 96%); δ_H(300 MHz; C^2HCl_3) 1.27-1.41 & 2.14-2.36 (4 H, m, 2 x secondary-H), 2.61 (2 H, t, 8-CH_2), 3.52-3.78 (4 H, m, 6-CH, 4-CH & 7-CH_2), 3.97-4.03 (1 H, m, CH-2), 4.31-4.55 (5 H, m, 2 x OCH_2Ph & CH-1), 7.03-7.30 (25 H, Ar-H); δ_C(75 MHz; C^2HCl_3) 33.81 (3-C), 35.31 (5-C), 36.43 (8-CH_2), 70.57 (2 x OCH_2Ph), 70.95 (7-CH_2), 71.03 (4-CH), 71.12 (2 x OCH_2Ph), 74.79 (2-C), 75.28 (6-C), 82.78 (1-C), 126.26, 126.14, 127.55, 127.61, 127.65, 127.68, 128.31, 128.38, 128.41, 128.44, 128.90, 128.98, 129.11, 129.55, 129.76 (Ar-CH), 138.56, 2 x 138.71, 2 x 138.87 (Ar-C quaternary); δ_P(121.5 MHz; C^2HCl_3) –12.20.

9.74 (*D*)-phenyl lactic acid 181



(*D*)-Phenylalanine **123** (5.5 g, 33.33 mmol) was dissolved in a 0.5 N solution of sulphuric acid (50 cm^3). The mixture was then cooled to 0 °C before the slow addition of sodium nitrite (5.06 g, 73.33 mmol in 19.2 cm^3 water [3.8 M solution]). The mixture was allowed to stir for 12 h in a sealed flask before the addition of $NaHCO_3$ until Ph 6. The solution was then acidified to pH 2 by the dropwise addition of phosphoric acid and the aqueous solution extracted with THF (4 x 50 cm^3). The combined organic fractions were washed with brine (2 x 50 cm^3) and the washed organic portion dried (Mg_2SO_4) before the solvent was removed by evaporation to give the product (D)-phenyl lactic acid **181** as a solid which was recrystallised by toluene (2.3 g, 41%); mp 122 - 123 °C; $[\alpha]_D$ +19 (*c* = 1.0, C_2H_5OH); δ_H(300 MHz; C^2HCl_3) 2.72-3.01 (2 H, m, CH_2Ph), 4.13-4.20 (1 H, m, CH), 7.17-7.30 (5 H, Ar-H); δ_C(75 MHz; C^2HCl_3) 39.91 (CH_2), 70.94 (CH), 125.92, 127.81, 129.24 (Ar-CH), 137.97 (Ar-C quaternary), 174.98 (quaternary C).

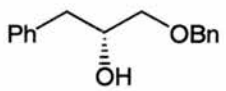
9.75 (2*R*)-3-Phenyl-propane-1,2-diol 182



(*D*)-phenyl lactic acid **181** (2 g, 12 mmol) was added slowly to an ice cold solution of BH_3 (1M in THF, 36 mmol, 36 cm^3). After 1 h the reaction was quenched by adding the mixture slowly to a $NaHCO_3$ – ice solution, the solution

extracted with diethyl ether. The ether was dried (Mg_2SO_4) and the solvent removed by evaporation to give the product (2*R*)-3-phenyl-propane-1,2-diol **182** as an oil (1.8 g, 99%); δ_H(300 MHz; C^2HCl_3) 2.69-2.75 (2 H, m, CH_2Ph), 3.10-3.35 (3 H, b, 3 x OH), 3.37-3.63 (2 H, m, CH_2), 3.80-3.92 (1 H, m, CH), 7.18-7.32 (5 H, Ar-H); δ_C(75 MHz; C^2HCl_3) 39.56 (CH_2), 65.77 (CH_2Ph), 73.04 (CH), 126.46, 128.50, 129.33 (Ar-CH), 137.95 (Ar-C quaternary).

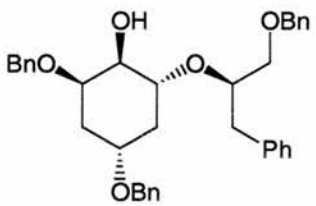
9.76 (2*R*)-benzyloxy-3-phenyl-propan-2-ol 177



The alcohol 3-phenyl-propane-1,2-diol **182** (1.8 g, 11.8 mmol) was dissolved in THF. The temperature was reduced to -40 °C and KH (14.2 mmol, 0.57 g) and benzyl bromide (11.8 mmol, 2.02 g, 1.40 cm^3) was added slowly. The mixture was stirred overnight and allowed to warm to room temperature. The mixture was then washed with water (50 cm^3) and extracted with diethyl ether (2 x 50 cm^3). The ether was dried (Mg_2SO_4) and the solvent removed by evaporation to give an oil which was a mixture of di-benzylated, primary alcohol substituted and secondary alcohol substituted. The compounds were separated by silica column chromatography (ethyl acetate-petroleum ether, 1:10) to give the product (2*S*)-(-)-benzyloxy-3-phenyl-propan-2-ol **177** – an oil - as the second spot (0.51 g, 18 %); δ_H(300 MHz; C^2HCl_3) 2.33-2.56 (1 H, b, OH), 2.80-2.87 (2 H, m, CH_2Ph), 3.38-3.56 (2 H, m, CH_2), 4.02-4.12 (1 H, m, CH), 4.56 (2 H, s, CH_2Ph), 7.22-7.37 (10 H, Ar-H); δ_C(75 MHz; C^2HCl_3) 39.76 (CH_2Ph), 71.35 (CH), 73.31 (OCH_2Ph),

73.44 (CH_2), 126.46, 127.82, 128.50, 128.71, 129.37, 129.43 (Ar-CH), 137.98 & 138.01 (Ar-C quaternary).

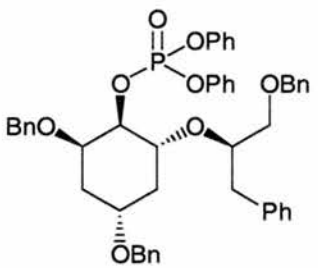
9.77 (-)-(1*R*,2*S*,4*R*,6*S*)-2,4-Bis-benzyloxy-6-(2'R-benzyloxymethyl-3-phenyl-propyl)-cyclohexanol 183



To a solution of the key epoxide (+)-(1*S*,2*R*,4*R*,6*R*)-2,4-bis(benzyloxy)-1,6-epoxycyclohexane **53** (250 mg, 0.81 mmol) in 1,2-dichloroethane (15 cm^3) was added (2*R*)-(-)-benzyloxy-3-phenyl-propan-2-ol **182** (190 mg, 0.80 mmol) and the ytterbium catalyst (100 mg, 0.16 mmol). The mixture was stirred for 2 h before the solvent was removed by evaporation and brine (20 cm^3) added to the residue. The solution was extracted with ethyl acetate (2 x 20 cm^3), the combined organic fractions dried (Mg_2SO_4) and the solvent removed by evaporation to give the product (-)-(1*R*,2*S*,4*R*,6*S*)-2,4-bis-benzyloxy-6-(2'*R*-benzyloxymethyl-3-phenyl-propyl)-cyclohexanol **183** as an oil which was purified by silica column chromatography (ethyl acetate-petroleum ether, 1:10), (300 mg, 67%); δ_H(300 MHz; C^2HCl_3) 1.90-2.02 & 2.25-2.34 (4 H, m, 5H & 3H), 2.80-2.85 (2 H, t, 3'H), 3.50-3.73 (5 H, m, 1-CH, 4-CH, 6-CH & 1'-CH_2), 3.97-4.01 (1 H, m, 2-CH), 4.03-4.13 (1 H, m, 2'-CH), 4.38-4.50 (2 H, m, OCH_2Ph), 4.60-4.67 (2 H, m, OCH_2Ph), 4.71-4.88 (2 H, m, OCH_2Ph), 7.23-7.50 (20 H, Ar-H); δ_C(75 MHz; C^2HCl_3) 34.61 & 36.71 (3-C & 5-C), 39.09 (3'-C), 70.13 (OCH_2Ph), 71.61 (4-C), 72.18 (OCH_2Ph), 72.85 (1'-C), 73.26 (OCH_2Ph), 76.01 (6-C), 76.57 (2-C), 78.58

(2'-C), 81.10 (1-C), 126.30, 127.18, 127.26, 127.39, 127.43, 127.48, 127.75, 127.78, 127.81, 128.11, 128.19, 128.24 (Ar-CH), 137.23, 137.99, 138.58 & 139.14 (Ar-C quaternary).

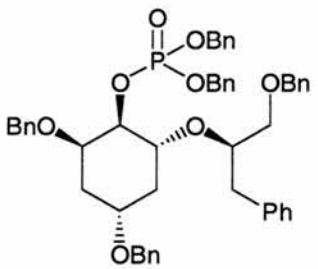
9.78 (-)-(1*R*,2*S*,4*R*,6*S*)-Phosphoric acid 2,4-bis-benzyloxy-6-(2'*R*-benzyloxymethyl-3-phenyl-propyl)-cyclohexyl ester diphenyl ester 184



To a solution of the alcohol (-)-(1*R*,2*S*,4*R*,6*S*)-2,4-bis-benzyloxy-6-(2'*R*-benzyloxymethyl-3-phenyl-propyl)-cyclohexanol **183** (200 mg, 0.36 mmol) in dry dichloromethane (15 cm^3) was added triethylamine (50 mg, 0.54 mmol, 0.08 cm^3), diphenyl chlorophosphate (140 mg, 0.54 mmol, 0.11 cm^3) and DMAP (0.01 g). The mixture was stirred for 48 h before the solvent was removed by evaporation and the residue washed with brine (2 x 20 cm^3). The solution was then extracted with ethyl acetate (2 x 30 cm^3), the combined organic fraction were dried (Mg_2SO_4) and the solvent removed to give (-)-(1*R*,2*S*,4*R*,6*S*)-phosphoric acid 2,4-bis-benzyloxy-6-(2'*R*-benzyloxymethyl-3-phenyl-propyl)-cyclohexyl ester diphenyl ester **184** as an oil which was purified by silica column chromatography (ethyl acetate-petroleum ether, 1:10) to give the product as a clear oil (240 mg, 85%); δ_H(300 MHz; C^2HCl_3) 1.82-1.93 & 2.05-2.14 (4 H, m, 5H & 3H), 2.48-2.56 & 2.77-2.84 (2 H, m, 3'H), 3.20-3.43 (2 H, m, 2'-H), 3.45-3.56 (1 H, m, 4H),

3.61-3.72 (1 H, m, 1'-CH), 3.75-3.88 (1 H, m, 6-H), 3.99-4.04 (1 H, m, 2-CH), 7.01-7.26 (30 H, Ar-H); δ_C(75 MHz; C^2HCl_3) 33.79 & 35.10 (3-C & 5-C), 39.10 (3'-C), 70.24 (OCH_2Ph), 71.69 (4-C), 71.88 (OCH_2Ph), 72.18 (1'-C), 73.21 (OCH_2Ph), 73.85 (6-C, d, 6.49 Hz), 75.06 (2-C), 78.85 (1'-C), 82.41 (1-C), 127.43, 127.46, 127.56, 127.59, 127.63, 127.69, 128.05, 128.08, 128.11, 128.14, 128.27, 128.29, 128.35, 128.38, 129.67, 129.70, 129.73, 129.76 (Ar-CH), 138.35, 138.46, 138.58, 138.77, 138.88 & 139.50 (Ar-C quaternary).

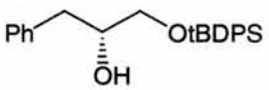
9.79 (-)-(1*R*,2*S*,4*R*,6*S*)-Phosphoric acid dibenzyl ester 2,4-bis-benzyloxy-6-(2'*R*-benzyloxymethyl-3-phenyl-propyl)-cyclohexyl ester 185



To a solution of the phosphate phosphoric acid (-)-(1*R*,2*S*,4*R*,6*S*)-2,4-bis-benzyloxy-6-(2'*R*-benzyloxymethyl-3-phenyl-propyl)-cyclohexyl ester diphenyl ester **184** (200 mg, 0.25 mmol) in THF (10 cm^3) at –40 °C was slowly added NaH (20 mg, 0.38 mmol) and benzyl alcohol (40 mg, 0.04 cm^3, 0.38 mmol). The solution was stirred for 12 h allowing to warm to room temperature before quenching the reaction with the slow addition of water (20 cm^3). The aqueous layer was extracted with ethyl acetate (2 x 20 cm^3) and the combined organic fractions dried (Mg_2SO_4) before removal of the solvent by evaporation. The residual oil was purified by silica column chromatography (ethyl acetate-

petroleum ether, 1:10) to give the product (-)-(1*R*,2*S*,4*R*,6*S*)-phosphoric acid dibenzyl ester 2,4-bis-benzyloxy-6-(2'*R*-benzyloxymethyl-3-phenyl-propyl)-cyclohexyl ester **185** as an oil (110 mg, 53%); δ_H(300 MHz; C^2HCl_3) 1.84-2.13 (4 H, m, 5H & 3H), 2.52-2.63 & 2.78-2.87 (2 H, m, 3'H), 3.30-3.57 (3 H, m, 2'-H & 4-CH), 3.59-3.78 (2 H, m, 6-CH & 2'-CH), 4.00-4.06 (1 H, m, 2-CH), 4.11-4.58 (6 H, m, 3 x OCH_2Ph), 4.71-5.03 (4 H, m, 2 x OCH_2Ph) 7.07-7.68 (30 H, Ar-H); δ_C(75 MHz; C^2HCl_3) 33.94 (3-C & 5-C), 39.12 (3'-C), 69.02 (2 x OCH_2Ph) 70.29 (OCH_2Ph), 70.85 (4-C), 71.88 (OCH_2Ph), 72.14 (1'-C), 73.21 (OCH_2Ph), 74.07 (6-C), 75.03 (2-C), 78.94 (1'-C), 81.12 (1-C), 126.19, 127.51, 127.53, 127.59, 127.66, 127.74, 127.79, 127.86, 127.98, 128.08, 128.15, 128.29, 128.34, 128.38, 128.42, 128.50, 128.54, 129.79 (Ar-CH), 135.95 (2), 138.38, 138.51, 138.64 & 138.84 (Ar-C quaternary).

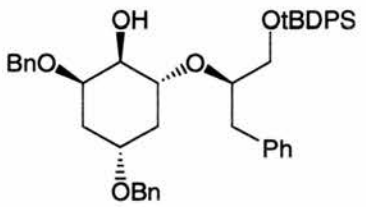
9.80 1-(2,2-Dimethyl-1,1-diphenyl-propylsilanyloxy)-3-phenyl-propan-2*R*-ol 186



To a solution of the diol 3-phenyl-propane-1,2*R*-diol **182** (0.5 g, 3.29 mmol) in dichloromethane (30 cm^3) was added *tert*-butyldiphenylsilyl chloride (900 mg, 0.85 cm^3, 3.29 mmol), triethylamine (330 mg, 0.46 cm^3, 3.29 mmol) and DMAP (80 mg, 0.66 mmol). The solution was stirred for 12 h before the solvent was removed by evaporation giving a residue which was re-dissolved in brine (30 cm^3) and extracted with ethyl acetate (2 x 30 cm^3). The combined organic fractions were dried (Mg_2SO_4) and the solvent removed by evaporation to give the product

1-(2,2-dimethyl-1,1-diphenyl-propylsilanyloxy)-3-phenyl-propan-2*R*-ol **186** as an oil which was purified by silica column chromatography (ethyl acetate-petroleum ether, 1:10) to give the product **186** as a clear oil (1115 mg, 89%); δ_H(300 MHz; C^2HCl_3) 1.08 (9 H, s, 3 x methyl), 2.79 (2 H, s, CH_2Ph), 3.55-3.72 (2 H, m, CH_2OSi), 3.91-4.01 (1 H, m, C*H*OH), 7.17-7.75 (15 H, Ar-H); δ_C(75 MHz; C^2HCl_3) 19.16 ($C(CH_3)_3$), 26.78 (3 x methyl), 39.43 (CH_2Ph), 67.03 (CHOH), 72.87 (CH_2OSi), 126.45, 127.87, 127.91, 128.53, 129.39 & 129.97 (Ar-CH), 135.72 (2) & 138.34.

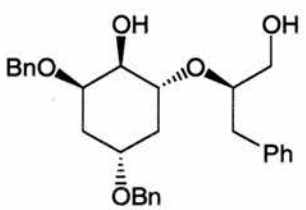
9.81 (-)-(1*R*,2*S*,4*R*,6*S*)-2,4-Bis-benzyloxy-6-[1-(2,2-dimethyl-1,1-diphenyl-propylsilanyloxymethyl)-2'R-phenyl-ethoxy]-cyclohexanol 187



To a solution of the key epoxide (+)-(1*S*,2*R*,4*R*,6*R*)-2,4-bis(benzyloxy)-1,6-epoxycyclohexane **53** (250 mg, 0.81 mmol) in 1,2-dichloroethane (20 cm^3) was added the alcohol 1-(2,2-dimethyl-1,1-diphenyl-propylsilanyloxy)-3-phenyl-propan-2*R*-ol (X) (380 mg, 0.97 mmol) and the ytterbium catalyst (100 mg, 0.16 mmol). The mixture was refluxed for 4 h before the solvent was removed by evaporation and brine (20 cm^3) added to the residue. The solution was extracted with ethyl acetate (2 x 20 cm^3), the combined organic fractions dried (Mg_2SO_4) and the solvent removed by evaporation to give the product (-)-(1*R*,2*S*,4*R*,6*S*)-2,4-bis-benzyloxy-6-[1-(2,2-dimethyl-1,1-diphenyl-propylsilanyloxymethyl)-2'*R*-

phenyl-ethoxy]-cyclohexanol **187** as an oil which was purified by silica column chromatography (ethyl acetate-petroleum ether, 1:10), (quantitative); δ_H(300 MHz; C^2HCl_3) 1.11 (9 H, s, 3 x methyl), 1.89-2.00 & 2.25-2.33 (4 H, m, 5H & 3H), 2.81 (2 H, s, CH_2Ph), 3.41-3.69 (5 H, m, 1-CH, 4-CH, CH_2OSi & 6-CH), 3.90 4.05 (2 H, m, 2-CH, & *CH*OH), 4.37-4.50 (2 H, m, OCH_2Ph), 4.60-4.67 (2 H, m, OCH_2Ph), 7.07-7.65 (25 H, Ar-H); δ_C(75 MHz; C^2HCl_3) 19.15 ($C(CH_3)_3$), 26.76 (3 x methyl), 34.58 & 36.68 (3-C & 5-C), 39.41 (CH_2Ph), 67.05 (CHOH), 70.09 (OCH_2Ph), 71.61 (4-C), 72.18 (OCH_2Ph), 72.86 (CH_2OSi), 75.86 (6-C), 76.48 (2-C), 81.09 (1-C), 126.33, 127.20, 127.23, 127.41, 127.45, 127.49, 127.75, 127.78, 127.81, 128.11, 128.15, 128.30 (Ar-CH), 135.72 (2), 137.23, 137.99, & 138.34 (Ar-C quaternary).

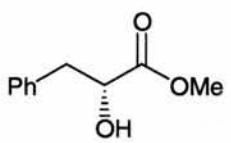
9.82 (-)-(1*R*,2*S*,4*R*,6*S*)-2,4-Bis-benzyloxy-6-(1'-hydroxymethyl-2'R-phenyl-ethoxy)-cyclohexanol 188



To (-)-(1*R*,2*S*,4*R*,6*S*)-2,4-bis-benzyloxy-6-[1-(2,2-dimethyl-1,1-diphenyl-propylsilanyloxymethyl)-2'*R*-phenyl-ethoxy]-cyclohexanol **187** (250 mg, 0.36 mmol) was added a solution of TBAF/THF (0.43 mmol, 0.43 cm^3). The mixture was stirred for 12 h before removal of the solvent by evaporation. The residue was taken up with brine (25 cm^3) and the aqueous extracted with diethyl ether (2 x 25 cm^3). The combined organic fractions were dried (Mg_2SO_4) and the solvent removed by evaporation to give an oil which was purified by silica column chromatography (ethyl acetate-petroleum ether, 1:10) to give the product (-)-

(1R,2S,4R,6S)-2,4-bis-benzyloxy-6-(1-hydroxymethyl-2'*R*-phenyl-ethoxy)-cyclohexanol **188** as an oil (quantitative); δ_H(300 MHz; C^2HCl_3) 1.70-1.80 & 2.23-2.39 (4 H, m, 5H & 3H), 2.67-2.75 (2 H, t, 3'H), 3.34-3.77 (5 H, m, 1-CH, 4-CH, 6-CH & 1'-CH_2), 3.82-3.87 (1 H, m, 2-CH), 3.95-4.02 (1 H, m, 2'-CH), 4.19-4.65 (4 H, m, OCH_2Ph), 7.22-7.42 (15 H, Ar-H); δ_C(75 MHz; C^2HCl_3) 33.56 & 36.85 (3-C & 5-C), 39.29 (3'-C), 65.41 (1'-C), 70.27 (OCH_2Ph), 70.93 (4-C), 71.46 (OCH_2Ph), 75.92 (1-C), 76.27 (2-C), 79.21 (6-C), 83.93 (2'-C), 81.10 (1-C), 126.65, 127.69, 127.81, 127.94, 128.37, 128.44, 128.55, 129.59 & 129.67 (Ar-CH), 134.88, 137.95 & 138.55 (Ar-C quaternary).

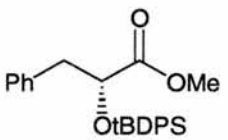
9.83 Methyl (2*R*)-hydroxy-3-phenyl propanoate 190



(*D*)-phenyl lactic acid **181** (500 mg, 3.12 mmol) was dissolved in dry methanol (90 cm^3) and the mixture cooled to 0 °C. Thionyl chloride (720 mg, 0.44 cm^3, 6.24 mmol) was added dropwise and the mixture then refluxed for 3 h. The solution was neutralised to pH 7 using saturated sodium bicarbonate solution. The resulting suspension was partitioned between water (60 cm^3) and extracted using ethyl acetate (3 x 25 cm^3). The organic extractes were combined, dried (Mg_2SO_4), filtered and concentrated under reduced pressure to yield a colourless oil which solidified on standing to give methyl (2*R*)-hydroxy-3-phenyl propanoate **190** as a white solid (515 mg, 95%), mp 43-45 °C (Found: C, 66.95; H, 6.9. $C_{10}H_{12}O_3$ requires C, 66.65; H, 6.7%); $[\alpha]_D$ –3.7 (*c*1.02 in methanol); $\upsilon_{max}(CHCl_3)/cm^{-1}$ 3600-3400 (Free, OH), 2900 (OCH_3), 1745 (CO, ester), 1450-1600 (aromatic) and

1200 (CO stretch); δ_H(300 MHz; C^2HCl_3) 2.74 (1 H, br s, OH), 2.96 (1 H, dd, *J* 14.0, 6.8, one of βCH_2), 3.13 (1 H, dd, $^2J_{H\text{-}H}$ 14.0, $^3J_{H\text{-}H}$ 4.4, one of βCH_2), 3.77 (3 H, s, OCH_3), 4.46 (1 H, dd, $^2J_{H\text{-}H}$ 6.8, $^3J_{H\text{-}H}$ 4.4, αCH) and 7.18-7.35 (5 H, m, Ar-H); δ_C(50.31 MHz; C^2HCl_3) 40.46 (CH_2Ph), 52.37 (OCH_3), 71.21 (αCH), 126.82 (Ar-CH *meta*), 128.35 (Ar-CH *ortho*), 129.38 (Ar-CH *para*), 136.25 (Ar-C quaternary) and 174.50 (CO, ester); *m/z* (CI) 181 (100%, $[M + H]^+$), 162 (64, $[M - H_2O]^+$), 121 (53, $[M + H - CH_3COOH]^+$), 103 (8, $[M - C_6H_5]^+$) and 91 (44, $PhCH_2^+$).

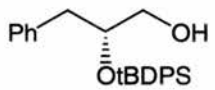
9.84 2*R*-(2,2-Dimethyl-1,1-diphenyl-propylsilanyloxy)-3-phenyl-propionic acid methyl ester 191



To a solution of the alcohol methyl (2*R*)-hydroxy-3-phenyl propanoate **190** (300 mg, 1.67 mmol) in dichloromethane (20 cm^3) was added triethylamine (20 mg, 2 mmol, 0.28 cm^3), tert-butyl diphenylsilyl chloride (550 mg, 2 mmol, 0.52 cm^3) and DMAP (40 mg, 0.33 mmol). The mixture was stirred for 12 h before removal of the solvent by evaporation. The residue was taken up with brine (25 cm^3) and the aqueous extracted with diethyl ether (2 x 25 cm^3). The combined organic fractions were dried (Mg_2SO_4) and the solvent removed by evaporation to give an oil which was purified by silica column chromatography (ethyl acetate-petroleum ether, 1:10) to give the 2*R*-(2,2-dimethyl-1,1-diphenyl-propylsilanyloxy)-3-phenyl-propionic acid methyl ester **191** product as an oil (quantitative); δ_H(300 MHz; C^2HCl_3) 1.06 (9 H, s, 3 x methyl), 3.00 (1 H, dd, $^2J_{H\text{-}H}$ 14.0, $^2J_{H\text{-}H}$ 6.8, one

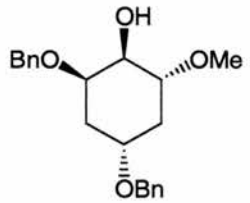
of βCH_2), 3.11 (1 H, dd, $^2J_{H\text{-}H}$ 14.0, $^3J_{H\text{-}H}$ 4.4, one of βCH_2), 3.74 (3 H, s, OCH_3), 4.73 (1 H, dd, $^2J_{H\text{-}H}$ 6.8, $^3J_{H\text{-}H}$ 4.4, CHOSi) and 7.16-7.54 (15 H, m, Ar-H); δ_C(50.31 MHz; C^2HCl_3) 40.49 (CH_2Ph), 52.29 (OCH_3), 72.96 (CHOSi), 126.44, 126.93, 127.79, 127.84, 128.31, 129.15 (Ar-CH), 135.64 (2) 138.48 (Ar-C quaternary) and 174.51 (CO, ester);

9.85 2*R*-(2,2-Dimethyl-1,1-diphenyl-propylsilanyloxy)-3-phenyl-propan-1-ol 192



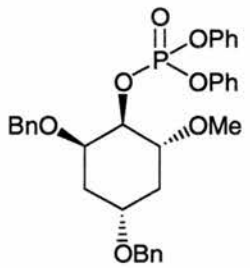
To a solution of the ester 2*R*-(2,2-dimethyl-1,1-diphenyl-propylsilanyloxy)-3-phenyl-propionic acid methyl ester **191** (0.75 g, 1.8 mmol) at –70 °C in dry EtOH (30 cm^3) was added lithium aluminum hydride (1.2 equiv.) with vigorous stirring. The mixture was allowed to warm slowly to room temperature over five hours before carefully quenching the reaction with water. The mixture was extracted with ethyl acetete (2 x 25 cm^3) and the combined organic fractions dried (Mg_2SO_4) before removal of the solvent by evaporation to give an oil. The oil was purified by silica column chromatography (ethyl acetate – petroleum ether, 1:5) to give the product 2*R*-(2,2-dimethyl-1,1-diphenyl-propylsilanyloxy)-3-phenyl-propan-1-ol **192** as an oil (quantitative); δ_H(300 MHz; C^2HCl_3) 1.11 (9 H, s, 3 x methyl), 2.29-2.52 (1 H, b, OH), 2.71-2.75 (2 H, m, CH_2Ph), 3.79-3.90 (2 H, s, CH_2OH), 4.89 (1 H, dd, $^2J_{H\text{-}H}$ 6.8, $^3J_{H\text{-}H}$ 4.4, CHOSi), 7.11-7.83 (15 H, Ar-H); δ_C(75 MHz; C^2HCl_3) 40.33 (CH_2Ph), 52.25 (OCH_3), 72.99 (CHOSi), 73.43 (CH_2), 126.39, 126.87, 127.77, 127.81, 128.33, 129.20 (Ar-CH), 135.59 (2) 138.68 (Ar-C quaternary).

9.86 (-)-(1*S*,2*R*,4*S*,6*R*)-2,4-Bis-benzyloxy-6-methoxy-cyclohexanol 193



The key epoxide (+)-(1*S*,2*R*,4*R*,6*R*)-2,4-bis(benzyloxy)-1,6-epoxycyclohexane **53** (100 mg, 0.32 mmol) was placed in a round bottomed flask and dissolved in dichloroethane (10 cm^3). $Yb(OTf)_3$ was then added (40 mg, 0.06 mmol) followed by methanol (1 cm^3). This mixture was refluxed for 3 h before removal of the solvent by evaporation. The residue was then washed with water (10 cm^3) and extracted with ethyl acetate (2 x 10 cm^3). The organic layer was then separated, dried (Na_2SO_4) and concentrated under reduced pressure to give the product (-)-(1*S*,2*R*,4*S*,6*R*)-2,4-bis-benzyloxy-6-methoxy-cyclohexanol **193** as a light coloured oil (107 mg, 97%); (HRMS: found: $[M]^+$ 342.1838. $C_{21}H_{26}O_4$ requires 342.1831); $[\alpha]_D$ -37.6 (*c* 0.2, EtOAc); (Found: C, 72.88; H, 7.48. $C_{21}H_{26}O_4$ requires C, 73.66; H, 7.65%); ν_{max} (neat)/cm^{-1} 3485s, 2927s, 1453s, 1364s and 1093s; δ_H(200 MHz, C^2HCl_3), 1.23-1.45 (2 H, m, 2 x secondary H), 1.73 (1 H, s, OH), 2.45 (1 H, m, secondary H), 2.45-2.64 (1 H, m, secondary H), 3.45 (3 H, s, OCH_3), 3.31-3.61 (2 H, m, 2 x tertiary-H), 3.65-3.85 (1 H, m, , 2 x tertiary-H), 3.90-4.01 (1 H, m, tertiary-H), 4.54 (2 H, s, O*C*H_2Ph), 4.66 (2 H, s, O*C*H_2Ph), 7.25-7.45 (10 H, Ar-H); δ_C(50.3 MHz, C^2HCl_3), 34.31 and 35.01 (3-C and 5-C), 57.54 (OCH_3), 71.16 (O*C*H_2Ph), 72.00 (4-C), 72.45 (O*C*H_2Ph), 75.86 and 76.86 (2-C and 6-C), 78.84 (1-C), 128.05, 128.14, 128.23, 128.74, 128.85, 130.05 & 130.27 (Ar-CH), and 138.87 & 138.95 (Ar-C quaternary); *m/z* (EI) 342 (8%, M^+), 250 (37, $[M - C_7H_7 - H]^+$, 235 (11, $[M - C_7H_7O]^+$ and 91 (100. $[C_7H]_7^+$).

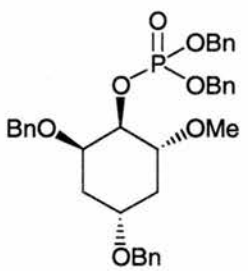
9.87 (-)-(1*S*,2*R*,4*S*,6*R*)-Phosphoric acid 2,4-bis-benzyloxy-6-methoxy-cyclohexyl ester diphenyl ester 194



To a solution of 2,4-Bis-benzyloxy-6-methoxy-cyclohexanol **193** (100 mg, 0.29 mmol) was added DMAP (10 mg, 0.09 mmol), triethylamine (40 mg, 0.44 mmol, 0.06 cm^3) and diphenylchlorophosphate (120 mg, 0.44 mmol, 0.10 cm^3) in DCM (10 cm^3). The mixture was stirred overnight before removal of the solvent by evaporation. The residue was then washed with water (10 cm^3) and extracted with ethyl acetate (2 x 10 cm^3). The organic layer was then separated, dried (Na_2SO_4) and concentrated under reduced pressure and purified by silica column (ethyl acetate-petroleum ether, 1:10) to give the product phosphoric acid 2,4-bis-benzyloxy-6-methoxy-cyclohexyl ester diphenyl ester **194** as a colourless oil (60 mg, 37%); $[\alpha]_D$ -23.9 (*c* 0.15, EtOAc); (HRMS: found: $[M + H]^+$ 575.2204. $C_{33}H_{36}O_7P$ requires 575.2199); δ_H(200 MHz, C^2HCl_3), 1.35-1.5 (2 H, m, 2 x secondary-H), 2.22-2.41 (1 H, m, secondary-H), 2.45-2.64 (1 H, m, secondary-H), 3.32 (3 H, s, OCH_3), 3.63-3.85 (2H, m, 2 x tertiary-H), 4.03-4.19 (1 H, m, tertiary-H), 4.46 (2 H, s, OCH_2Ph), 4.54 (2 H, s, OCH_2Ph), 4.59-4.64 (1 H, m, 1-H) and 7.25-7.46 (20 H, Ar-H); δ_C(50.3 MHz, C^2HCl_3), 34.56 and 35.34 (3-C and 5-C), 57.72 (OCH_3), 71.28 (OCH_2Ph), 71.53 (4-C), 72.74 (OCH_2Ph), 75.83 (C tertiary, $^3J_{C\text{-}P}$ 3 Hz), 76.54 (C tertiary, $^3J_{C\text{-}P}$ 5.1 Hz), 83.29 (1-C, $^2J_{C\text{-}P}$ 6.3 Hz), 120.50, 120.65, 120.74, 125.66, 125.76, 128.07, 128.13, 128.20, 128.85, 128.95,

130.10 and 130.26 (Ar-CH,), 138.87 and 138.98 (Ar-C quaternary), 150.40, 150.55 (POC_6H_5 quaternary); δ_P(121.5 MHz, C^2HCl_3), -12.05; *m/z* (EI): 575 (20%, $[M+H]^+$), 251 (58, $[(PhO)_2 PO_2H_2]^+$), 91 (100, $[C_7H_7]^+$).

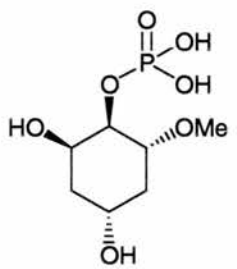
9.88 (-)-(1*S*,2*R*,4*S*,6*R*)-Phosphoric acid dibenzyl ester 2,4-bis-benzyloxy-6-methoxy-cyclohexyl ester 195



To a solution of NaH (60% dispersion in oil, 0.01 mg, 10 mmol) in THF (10 cm^3) was added benzylalcohol (10 mg, 10 mmol, 0.01 cm^3). The temperature was reduced to –70 °C and a solution of phosphoric acid 2,4-bis-benzyloxy-6-methoxy-cyclohexyl ester diphenyl ester **194** (50 mg, 0.09 mmol) dissolved in THF as added. This was stirred for four hours before removal of the solvent by evaporation. The residue was then washed with water (10 cm^3) and extracted with ethyl acetate (2 x 10 cm^3). The organic layer was then separated, dried (Na_2SO_4) and concentrated under reduced pressure and purified by silica column (1:10 ethyl acetate/pet.ether) to give the product phosphoric acid dibenzyl ester 2,4-bis-benzyloxy-6-methoxy-cyclohexyl ester **195** as a colourless oil (60 mg, 55%); $[\alpha]_D$ -26.6 (*c* 0.18, EtOAc); (HRMS: found: $[M+H]^+$, 603.2520. $C_{35}H_{40}O_7P$ requires 603.2512); υ_{max} (neat)/cm^{-1} 1273s, 1106s, 1023s, 931s, 740s, 696s; δ_H(500 MHz, C^2HCl_3), 1.36-1.42 (2 H, m, 2 x secondary-H), 2.18-2.23 (1 H, m, secondary-H), 2.43-2.46 (1 H, m, secondary-H), 3.35 (3 H, s, OCH_3), 3.6-3.65 (1

H, m, tertiary-H), 3.65-3.75 (1 H, m, 4-H), 3.98-4.03 (1 H, m, tertiary-H) 4.28-4.32 (1 H, m, 1-H), 4.44-4.52 (4 H, s, 2 x OCH_2Ph), 4.95-5.12 (4 H, m, 2 x $POCH_2Ph$), 7.25-7.54 (20 H, Ar-H); δ_C(50.3 MHz, C^2HCl_3), 34.57 and 35.23 (C secondary), 57.85 (OCH_3), 69.54 ($POCH_2Ph$, $^2J_{C\text{-}P}$ 5.1 Hz), 69.76 ($POCH_2Ph$, $^2J_{C\text{-}P}$ 5.5 Hz), 71.27 (OCH_2Ph), 71.68 (4-C), 72.82 (OCH_2Ph), 75.73 (C tertiary), 76.40 (C tertiary, $^3J_{C\text{-}P}$ 6.0 Hz), 81.81 (1-C, $^2J_{C\text{-}P}$ 6.3), 128.01, 128.10, 128.32, 128.80, 128.96 and 129.02 (Ar-CH) and 138.97 (Ar-C quaternary); δ_P(121.5 MHz, C^2HCl_3), -1.25; *m/z* (EI): 603 (15%, $[M+H]^+$) and 91 (100, $[C_7H_7]^+$).

9.89 (-)-(1*R*,2*R*,4*S*,6*R*)-Phosphoric acid mono-(2,4-dihydroxy-6-methoxy-cyclohexyl) ester 196



To a solution of phosphoric acid dibenzyl ester 2,4-bis-benzyloxy-6-methoxy-cyclohexyl ester **195** (50 mg, 0.07 mmol) in MeOH, was added Pd/C (10%) and a drop of acetic acid. This was stirred for 48 h under an atmosphere of hydrogen gas. The mixture was then filtered through celite with water and methanol and lyophilised to give the product phosphoric acid mono-(2,4-dihydroxy-6-methoxy-cyclohexyl) ester **196** as a white solid which was isolated as the bis-cyclohexyl-ammonium salt (16 mg, 51% overall); m.p.>200°C (decomp.); $[\alpha]_D$ –14.2 (*c* 0.05, H_2O); (HRMS: found: $[M - C_6H_{14}N - PO^4]^+$, 145.0869. $C_7H_{13}O_3P$ requires 145.0865); δ_H(500 MHz, ^2H_2O), 1.15-1.21 (2 H, m, 4-H of Cha), 1.25-1.35 (8 H,

m, 4 x 2-H and 4 x 3-H of Cha), 1.35-1.45 (1 H, m, 5-H), 1.55-1.6 (1 H, m, 3-H), 1.62-1.65 (2 H, m, 2 x 4-H of Cha), 1.72-1.82 (4 H, m, 4 x 3-H of Cha), 1.94-2.01 (4 H, m, 4 x 2-H of Cha), 2.05-2.17 (1 H, m, 3-H), 2.35-2.48 (1 H, m, 5-H), 3.10-3.15 (2 H, m, 2 x 1-H of Cha), 3.43 (3 H, s, OCH_3), 3.58-3.62 (1 H, m, 6-H), 3.94-3.98 (1 H, m, 4-H), 3.96-4.02 (1 H, m, 1-H) and 4.31-4.33 (1 H, m, 2-H); δ_C(125.8 MHz; ^2H_2O), 24.1 (3-C of Cha), 24.6 (4-C of Cha), 30.7 (2-C of Cha), 36.1 (5-C), 37.4 (3-C), 50.7 (1-C of Cha), 57.0 (OCH_3), 64.8 (4-C), 67.6 (2-C), 76.8 (1-C, $^2J_{C\text{-}P}$ 5.5 Hz) and 77.5 (6-C); δ_P(121.2 MHz, ^2H_2O) +3.0; *m/z* (FAB-MS): 319 (1%, $[M + 2K + H]^+$), 281 (8, $[M + K + 2H]^+$), 243 (7, $[M + 3H]^+$) and 147 (100).

Appendices

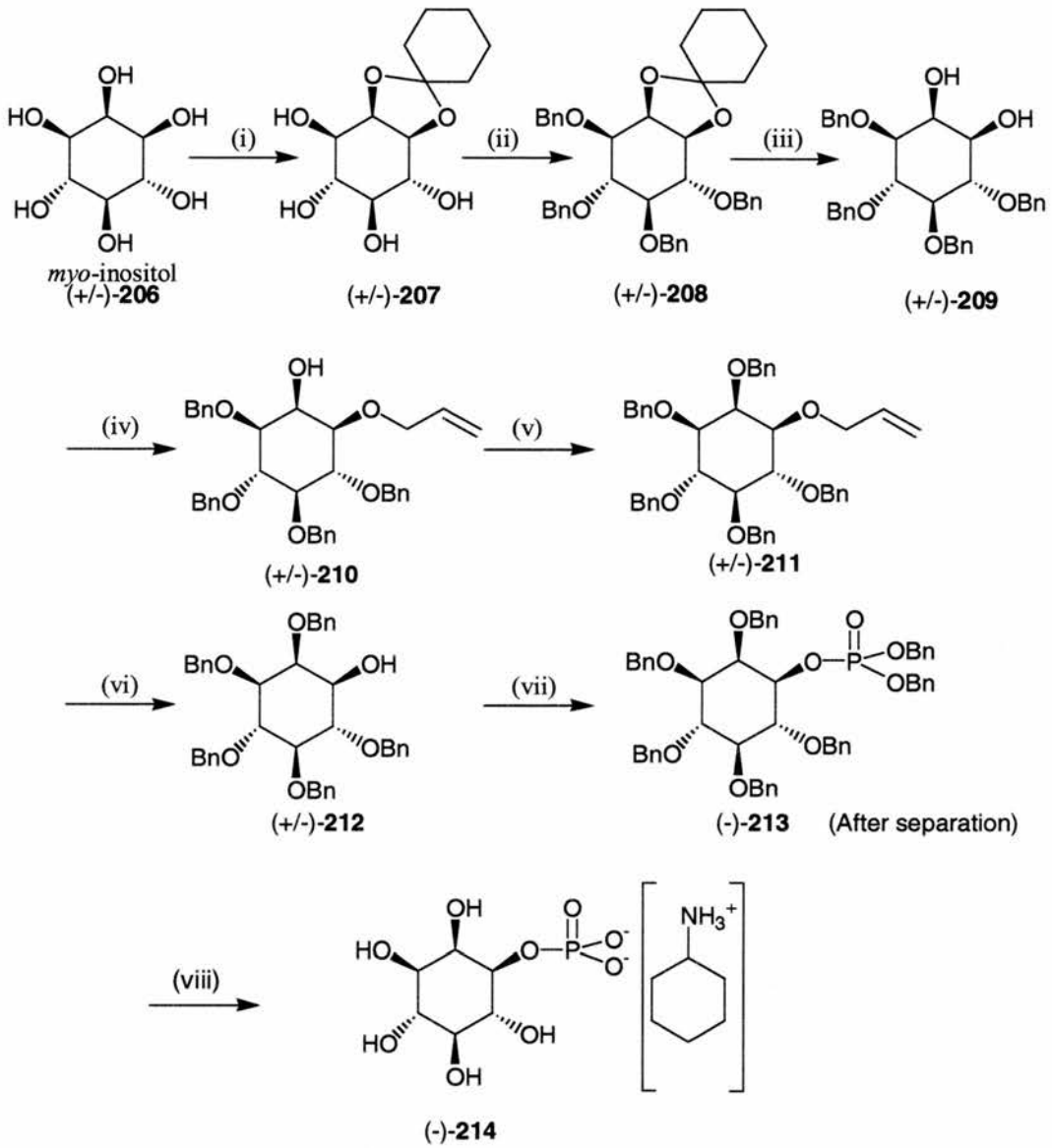
10 *Preparation of inositol monophosphate*

(-)*myo*-Ins-1-P **206** is a natural substrate for IMpase so this route was used to produce dervatives e.g. phosphorothiate compounds,[72] and the final product **214** was necessary for the calibration of enzyme assays. The compound was prepared by a protection-deprotection sequence following the method of Billington *et al.*

Reaction of the starting material myo-inositol **206** with cyclohexanone in the presence of an acid catalyst gave a mixture of three bisacetals. Mild hydrolysis of the mixture removed the less stable trans acetals and gave the desired product **207** in 86% yield. Protection of the remaining free hydroxyls was achieved by refluxing the acetal with benzyl chloride in the presence of potassium hydroxide giving the desired product **208** in 72% yield. The more stable cis acetal was hydrolysed by reacting with acetic acid to give **209** in 84% yield.

The next step requires allylation of the more reactive equatorial alcohol. The use of dibutyl tin oxide and allyl bromide was found to give allylation regio-specifically in the 1-position **210**. The product in the absence of the tin complex gave the desired regioisomer as the major product due to the higher reactivity of the equatorial hydroxyl group.

However, the use of the dibutyl tin complex ensured much greater selectivity in good yield (94%). The reason for this improvement was proposed to involve the formation of a reactive stannylidene intermediate under anhydrous conditions (Scheme 10.2, page 250).

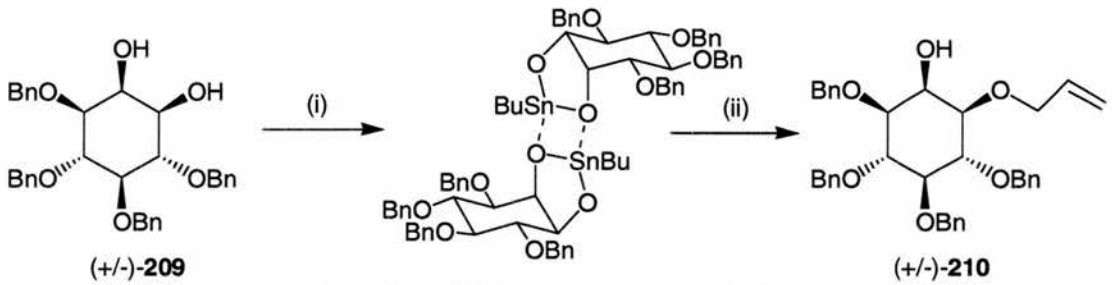


Reagents and Conditions: (i) C_6H_{10}, $PhCH_3$, 86%; (ii) BnCl, KOH, 72%; (iii) AcOH, H_2O, 84%; (iv) a) Bu_2SnO, PhH; b) Allyl bromide, 94%; (v) BnBr, NaH, DMF, 46%; (vi) a) $(PPh_3P)_3RhCl$, DABCO, EtOH, H_2O, 81%; (vii) Tetrabenzylpyrophosphate, DMF, NaH; (vii) a) Na/NH_3, -70 °C; b) Amberlite IRA 118H (H^+); c) Cyclohexylamine, H_2O, 34%.

Scheme 10.1: *Preparation of Ins-1-P from inositol*

The stannylidene intermediate exists as a dimer in solution in which the two oxygen atoms are not equivalent. 2-OH is co-ordinated to both tin atoms and is consequently relatively electron deficient, as well as being more hindered. In contrast 1-OH is only doubly co-ordinated, and more electron rich as only one tin atom is directly attached to it. It is thought that these factors along with the greater

reactivity of the equatorial hydroxy groups results in the observed preferential allylation of 1-OH.



Scheme 10.2: *C-1 OH allylation via a stannylidene intermediate*

The remaining hydroxy group of the allylated compound **210** was protected by benzylation with benzyl bromide and NaH to give the penta-benzylated mono-allylated fully protected cyclitol **211** in 46% yield.

Removal of the allyl group was then achieved by the method of Corey.[148] The use of a catalytic amount of both Wilkinson's catalyst (tristriphenylphosphine rhodium (I) chloride) and DABCO (1,4-diazobycyclo[2.2.2]octane) in aqueous ethanol resulted in the isomerisation of the allyl ether to the more thermodynamically favourable enol ether. The enol ether was then hydrolysed in aqueous acid (pH 2) to give the pentabenzyl-protected precursor to Ins 1-*P* **212**.

Racemic **212** was then resolved into its enatiomers by formation of the camphanate esters which were separated by chromatography. Commercially available (-)-(1R,4S)-camphanic acid was used for this purpose. Following separation the camphanate esters were hydrolysed by base hydrolysis.

To prepare Ins 1-P the remaining free alcohol of the pentabenzyl-protected cyclotol **212** was phosphorylated with tetrabenzyl-pyrophosphate (TBPP) and sodium hydride to give Ins 1-P **213**.

The final step in the synthesis of Ins 1-P involves a sodium/liquid ammonia reduction of **213**. Deprotection of **213** with sodium/ammonia gave the desired

product Ins 1-P **214** which was isolated as the biscyclohexylammonium salt. The final deprotection step gave racemic (±)-Ins 1-P isolated as it's biscyclohexylammonium salt **214**, in 34% yield [9 steps, 9.3% overall yield].

An alternative and simpler method of producing inositol monophosphate has recently been published by Terelli et al..[149] A mixture of myo-inositol and inorganic metaphosphate (prepared by freeze drying an aqueous solution at pH 4) produced, upon warming, in moderate yield every inositol monophosphate ester, thereby providing in a single step a complete library of these compounds. Inositol monophosphate could then be isolated by ion-exchange chromatography.

11 *Description of Assays*

The samples of bovine brain inositol monophosphatase were purified by others,[150] from a recombinant *E. coli* strain[151]in a routine yield of 20% and purity was assessed using polyacrylamide gel electrophoresis.[33] Enzyme activity assays were performed using a colourimetric assay developed by Itaya and Ui[152] employing molybdic acid and malachite green. Rate determinations were performed at 37 °C in triplicate in assay buffer A containing KCl (300 mmol dm^{-3}), $MgCl_2$ (2 mmol dm^{-3}) and Tris·HCl at pH 7.8 (50 mmol dm^{-3}). Incubation samples contained the following: assay buffer A (240 mm^3), substrate (aminocyclitol 1-phosphates **137** or **146**) at various concentrations in assay buffer (30 mm^3), and enzyme solution (activity pre-determined for the requirements of individual experiments) (30 mm^3). The reaction were started by the addition of enzyme and were incubated at 37 °C. The reaction was quenched by the addition of acidic colourimetric assay reagent (2.0 cm^3). The colour was allowed to develop over a period of 30 min, and the absorbance at 660 nm was measured in a 10 mm pathlength cuvette. Phosphate concentrations were determined by comparison of absorbance values to a preconstructed standard curve prepared using known phosphate concentrations. Background phosphatase activity was assessed in each experiment by performing parallel assays in the presence of Li^+ ion in buffer B (buffer B is buffer A plus 150 mmol dm^{-3} LiCl). One unit of IMPase hydrolyses 1 μmol of Ins 1-P per minute under these assay conditions. Rate data was analysed and processed graphically and by using non-linear regression analysis as described previously.[33]

12 References

1. World Health Organisation, Fact Sheet N 130, August 1996.
2. V. K. Yeragani and S. Gershon, *Biol. Psychiat.*, 1986, **21**, 1101-1102.
3. R. S. Jope and M. B. Williams, *Biochem. Pharmacol.*, 1994, **47**, 429-438.
4. S. R. Nahorski, C. I. Ragan and R. A. J. Challiss, *Trends, Pharmacol. Sci.*, 1991, **12**, 297-303.
5. J. F. J. Cade, *Med. J. Aust.*, 1949, **36**, 349-361.
6. G. Emilien, J. M. Maloteaux and A. Seghers, *Arch. Int. Pharmacod.*, 1995, **330**, 251-278.
7. J.R. Atack, *Biol. Psychiat.*, 1995, **37**, 761-763.
8. J. M. Baraban, P. F. Worley and S. H. Snyder, *Am. J. Psychiat.*, 1989, **146**, 1251-1260.
9. D. Gani, C. P. Downes and I. Batty, *Biochim. Biophys. Acta*, 1993, **1177**, 253-269.
10. K. E. Ackermann, B. G. Gish, M. P. Honchar and W. R. Sherman, *Biochem. J.*, 1987, **242**, 517-524.
11. E. D. Kennedy, R. A. J. Challiss, C. I. Ragan and S. R. Nahorski. *Biochem. J.*, 1990, **267**, 781-786.
12. S. E. Molchan, J. R. Atack and T. Sutherland. *Psychiat. Res.*, 1994, **53**, 103-105.
13. F. Kippert. *Biochem. Soc. Trans.*, 1997, **15**, S602-S602.
14 For a recent review see Proceedings of the 627th Meeting of the Biochemical Society, *Biochem. Soc. Trans.*, 1989, **17**, 1-7.

15. S. R. Piettre, A. J. Ganzhorn, J. Hoflack, K. Islam and J. Hornsperger, *J. Am. Chem. Soc.*, 1997, **119**, 3201-3204.

16. A. G. Cole and D. Gani, *J. Chem. Soc., Perkin Trans. I* 1995, **21**, 2685-2694.

17. C. I. Ragan, K. J. Watling, N. S. Gee, S. Aspley, R. G. Jackson, G. G. Reid, R. Baker, D. C. Billington, R. J. Barnaby and P. D. Leeson. *Biochem. J.*, 1988, **249**, 143-148.

18. J. Baraban, *Proc. Natl. Acad. Sci. U.S.A*, June, 1994, **91**, 5738-5739.

19. J. R. Atack, H. B. Broughton and S. J. Pollack. *Trends Neurosci.*, 1995, **18**, 343-349.

20. J. R. Atack, A. M. Prior, R. R. Fletcher, K. Quirk, R. McKernan and C. I. Ragan, *J. Pharmacol. Exp. Ther.*, 1994, **270**, 70-76.

21. J. R. Atack, S. M. Cook, A. P. Watt, S. R. Fletcher and C. I. Ragan, *J. Neurochem.*, 1993, **60**, 652-658.

22. J. R. Atack, *Med. Res. Rev.*, 1997, **17**, 215-224.

23. C. W. Taylor, *Biochem. J.*, 1990, **272**, 1-13.

24. S. K. Fisher, A. M. Heacock and B. W. Agranoff. *J. Neurochem.*, 1992, **58**, 18-38.

25. W. R. Sherman, A. Rasheed, I. A. Mauck and J. Wiecko, *J. Biol. Chem.*, 1977, **252**, 5672-5683.

26. F. A. Loewus and M. W. Loewus, *Annu. Rev. Plant Physiol.*, 1983, **34**, 137-161.

27. M. W. Loewus, F. A. Loewus, G. U. Brillinger, H. Otsuka and H. G. Floss, *J. Biol. Chem.*, 1980, **255**, 1710-1712.

28. K. Takimoto, M. Okada, Y. Matsuda and H. Nakagawa. *J. Biochem. (Tokyo)*, 1985, **98**, 363-370.

29. N. S. Gee, C. I. Ragan, K. J. Watling, S. Aspley, R. G. Jackson, G. G. Reid, D. Gani and J. K. Shute, *Biochem. J.* 1988, **249**, 883-889.

30. R. E. Diehl, P. Whiting, J. Potter, N. Gee, C. I. Ragan, D. Linemeyer, R. Schoepfer, C. Bennett and R. A. F. Dixon, *J. Biol. Chem.*, 1990, **265**, 5946-5949.

31. G. MacAllister, P. Whiting, E. A. Hammond, M. R. Knowles, J. R. Atack, F. J. Bailey, R. Maigetter and C. I. Ragan, *Biochem. J.* 1992, **284**, 749-754.

32. R. Bone, J. P. Springer and J. R. Atack, *Proc. Natl. Acad. Sci. U.S.A*, 1992, **89**, 10031-10035.

33. A. P. Leech, G. R. Baker, J. K. Shute, M. A. Cohen and D. Gani, *Eur. J. Biochem.*, 1993, **212**, 693-704.

34. R. Baker, D. C. Billington and D. Gani, *Tetrahedron*, 1991, **47**, 3895-3908.

35. S. J. Pollack, J. R. Atack, M. R. Knowles, G. McAllister, C. I. Ragan, R. Baker, S. R. Fletcher, L. L. Iversen and H. B. Broughton *Proc. Natl. Acad. Sci. U.S.A*, 1994, **91**, 5766-5770.

36. J. H. Schwartz, *Proc. Natl. Acad. Sci. U.S.A*, 1963, **49**, 871-879.

37. R. L. Van Etton, *Proc. Natl. Acad. Sci. U.S.A*, Oct. 1978. **75**, 4784-4796.

38. R. Baker, C. Carrick, P. D. Leeson, I. C. Lenon and N. Liverton, *J. Chem. Soc., Chem. Commun.*, 1991, 298-300.

39. G. R. Baker and D. Gani, *Bioorg. Med. Chem. Lett.*, 1991, **1**, 193-196.

40. J. Wilkie, A. G. Cole and D. Gani, *J. Chem. Soc., Perkin Trans 1.*, 1995, 2709-2727.

41. L. M. Hallcher and W. R. Sherman, *J. Biol. Chem.*, 25 Nov 1980, **255**, 10896-10903.

42. P. V. Attwood, J. B. Ducep and M. C. Chanal, *Biochem. J.*, 1988, **253**, 387-394.

43. A. J. Ganzhorn and M. C. Chanal, *Biochemistry*, 1990, **29**, 6065-6071.

44. R. Parthasarathy, L. Parthasarathy, T. G. Ramesh, C. S. S. Devi and R. E. Vadnal, *Life Sci.*, 1992, **50**, 1445-1450.

45. R. Baker, J. J. Kulagowski, D. C. Billington, P. D. Leeson, J. C. Lennon and N. J. Liverton. *J. Chem. Soc., Chem. Commun.*, 1989, 1383-1385.

46. R. Baker, P. D. Leeson, N. J. Liverton and J. J. Kulagowski., *J. Chem. Soc. Chem. Commun.*, 1990, 462-464.

47. J. J. Kulagowski, R. Baker and S. R. Fletcher, *J. Chem. Soc., Chem. Commun.*, 1991, 1649-1650.

48. S. R. Fletcher, R. Baker, P. D. Leeson, M. Teall, E. A. Harley and C. I. Ragan. *Bioorg. Med. Chem. Lett.*, 1992, **2**, 627-630.

49. S. R. Fletcher, R. Baker, T. Ladduwahetty, A. Sharp, M. Teal and J. R. Atack, *Bioorg. Med. Chem. Lett.*, 1993, **3**, 141-146.

50. Y. K. T. Lam, C. F. Wichmann, M. S. Meinz, L. Guariglia, R. A. Giacobbe, S. Mochales, L. Kong, S. S. Honeycutt, D. Zinc, G. F. Bills, L. Huang, R. W. Burg, R. L. Monaghan, R. Jackson, G. Reid, J. J. Maguire, A. T. McKnight and C. I. Ragan, *J. Antibiot.*, 1992, **45**, 1397-1403.

51. J. A. Pachter, *Mol. Pharmacol.*, 1991, **40**, 107-111.

52. S. Stefanelli, F. Sponga, P. Ferrari, C. Stottani, E. Corti, C. Brunnati and K. Islam, *J. Antibiot.*, 1996, **49**, 611-616.

53. A. G. Cole and D. Gani, *J. Chem. Soc., Chem. Commun.*, 1994, 1139-1141.

54. P. D. Leeson, K. James, I. C. Lennon, N. J. Liverton, S. Aspley and R. G. Jackson, *Biomed. Chem. Lett.*, 1993, **3**, 1925-1930.

55. P. A. Frey, *Adv. Enz.*, 1989, **62**, 119-201.

56. S. L. Buchwald, D. H. Pliura and J. R. Knowles, *J. Am. Chem. Soc.*, 1984, **106**, 4916-4922.

57. R. Bone, L. Frank, J. P. Springer and J. R. Atack., *Biochemistry*, 1994, **33**, 9468-9476.

58. A. G. Cole, J. Wilkie and D. Gani, *J. Chem. Soc., Perkin. Trans. I*, 1995, 2695-2707.

59. R. Bone, L. Frank, J. P. Springer, S. J. Pollack, S. Osborne, J. R. Atack, M. R. Knowles, G. McAllister, C. I. Ragan, H. B. Broughton, R. Baker and S. R. Fletcher, *Biochemistry*, 1994, **33**, 9460-9467.

60. P. J. Lodi and J. R. Knowles, *Biochemistry*, 1991, **30**, 6948-6956.

61. H. Nicholson, D. E. Anderson, S. Daopin and B. W. Matthews, *Biochemistry*, 1991, **30**, 9816-9828.

62. R. G. Jackson, N. S. Gee and C. I. Ragan, *Biochem. J.*, 1989, **264**, 419-422.

63. S. J. Pollack, M. R. Knowles, J. R. Atack, H. B. Broughton, C. I. Ragan, S. A. Osborne and G. MacAllister, *Eur. J. Biochem.*, 1993, **217**, 281-287.

64. M. R. Knowles, N. Gee, G. McAllister, C. I. Ragan, P. J. Greasley and M. G. Gore, *J. Biochem.*, 1992, **285**, 461-498.

65. M. G. Gore, P. J. Greasley, G. McAllister and C. I. Ragan, *J. Biochem*, 1993, **296**, 811-815.

66. P. J. Greasley, M. G. Gore, K. J. Rees-Milton and C. I. Ragan, *FEBS Lett.*, March 1993, **319**, 49-53.

67. K. J. Rees-Milton, P. J. Greasley, C. I. Ragan and M. G. Gore, *FEBS Lett.*, April 1993, **321**, 37-40.

68. P. M. Cullis and R. Misra, *J. Am. Chem. Soc.*, 1991, **113**, 9679-9680.

69. M. G. N. Russell, R. Baker and D. C. Billington, *Carbohydr. Res.*, 1992, **234**, 263-268.

70. J. Schulz, J. Wilkie, P. Lightfoot, T. Rutherford and D. Gani, *J. Chem. Soc., Chem. Commun.*, 1995, 2353-2356.

71. J. Wilkie and D. Gani, *J. Chem., Soc. Perkin Trans. 2*, 1996, 783-787.

72. R. Pybus, *University of St Andrews*, unpublished results.

73. A. G. Cole and D. Gani, *J. Chem. Soc., Perkin Trans 1*, 1995, 2695-2707.

74. J. Schulz and D. Gani, *J. Chem. Soc., Perkin Trans. 1*, 1997, 657-670.

75. J. Schulz and D. Gani, *Tetrahedron Lett.*, 1997, **38**, 111-114.

76. H. Suemune, K. Matsuno, M. Uchida and K. Sakai, *Tetrahedron: Asymmetry*, 1992, **3**, 297-306.

77. C. McGuigan, *J. Chem. Soc., Chem. Commun.*, 1986, 533-534.

78. Kerry Webster, *University of St Andrews*, unpublished results.

79. A. S. Jones, C. McGuigan and R. T. Walker, *J. Chem. Soc., Perkin. Trans. 1*, 1985, **80**, 199-202.

80. C. McGuigan and B. Swords, *J. Chem. Soc., Perkin. Trans. 1*, 1990, 783-787.

81. J. R. Cox and F. H. Westheimer, *J. Am. Chem. Soc.*, 1958, **80**, 5441-5446.

82. Fieser and Fieser, "*Reagents for Organic Synthesis*", ed. J Wiley and Sons., New York, 1967, pg 796.

83. L. Castellanos, J. Cleophax, C. Colas and S.D. Gero, *Carbohydr. Res.*, 1980, **82**, 283-301.

84. I. H. Sanchez, F. J. Lopez, J. J. Soria, M. I. Larraza and H. J. Flores, *J. Am. Chem. Soc.*, 1983, **105**, 7640-7643

85. H. Stevens and O. Grummitt, *J. Am. Chem. Soc.*, 1952, **74**, 4876-4889.

86. D. Houalla, M. Sanchez, and R. Wolf, *Bull. Soc. Chim. Fr.*, 1965, 2368-2381.

87. C. C. Addison and J. C. Sheldon, *J. Chem. Soc.*, 1956, 2705-2709.

88. V. Mark, C. H. Dungan, M. M. Crutchfield and J. R. Van Wazer., *'Tropics in Phosphorus Chemistry'*, vol. 5, ed. M Grayson and E. J. Griffith, Wiley, New York, **1969**, pg 353.

89. H. Eibl, *Proc. Natl. Acad. Sci. U.S.A*, 1978, **75**, 4074-4079.

90. M. L. Nielson, J. V. Pustinger, and J. Strobel, *J. Chem. Eng. Data*, 1964, **9**, 167-173.

91. H.C. Brown, A.M. Salunkhe and A.B. Argade, *Organometallics*, 1992, **11**, 3094-3097.

92. G. H. Posner and D. Z. Rogers, *J. Am. Chem. Soc.*, 1977, **99**, 8208-8213.

93. S. V. Ley, M. Parra, A. J. Redgrave and F. Sternfield, *Tetrahedron*, 1990, **46**, 4995-5026.

94. S. V. Ley and F. Sternfeld, *Tetrahedron*, 1989, **45**, 3463-3476.

95. A. Brandes, U. Eggert and H. M. R. Hoffmann. *Synlett.*, 1994, 745-747.

96 P. Kocovsky, *J. Chem. Soc., Perkin Trans. 1*, 1994, 1759-1763.

97 P. Chaimberlain, M. I. Roberts and G. H. Witham, *J. Chem. Soc.*, 1970, 1374-1381.

98. Emmanuelle Beushausen, *University of St. Andrews*, Unpublished Results.

99. R. Choukroun and D. Gervais, *J. Chem. Soc., Dalton Trans.*, 1980, 1800-1808.

100. J. Doussot, R. Garreau, L. Dallery and J. P. Guette, A. Guy, *Bull. Soc. Chim. Fr.*, 1995, **132**, 59-66.

101. F. Rolla, *J. Org. Chem.*, 1982, **47**, 4327-4329.

102. K. Soai, S. Yokoyama and A. Ookawa, *Synthesis*, 1987, 48-49.

103. P. V. Ramachandran, B. Gong and H. Brown, *J. Org. Chem.*, 1995, **60**, 41-46.

104. S. N. Maitai, M. P. Singh and R. G. Micetich, *Tetrahedron Lett.*, 1986, **27**, 1423-1424.

105. N. Knouzi and M. Vaultier, *Bull. Soc. Chim. Fr.*, 1985, **5**, 815-819.

106. M. Bartra, P. Romea, F. Urpi and J. Vilarrasa, *Tetrahedron Lett.*, 1987, **28**, 5941-5944.

107. M. Bartra, P. Romea, F. Urpi and J. Vilarrasa, *Tetrahedron.*, 1990, **46**, 587-593

108. J. March, "*Advanced Organic Chemistry*", ed. J. Wiley and Sons., Wiley Interscience, USA, 1992 and references therein.

109. H.C. Brown, A.M. Salunkhe and B. Singaram, *J. Org. Chem.*, 1991, **56**, 1170-1175.

110. H. C. Brown and A. B. Levy, *J. Organomet. Chem.*, 1972, **44**, 233-239.

111. H. C. Brown and N. Ravindran, *J. Am. Chem. Soc.*, 1973, **95**, 2396-2402.

112. H. C. Brown, M. M. Midland and Alan B. Levy, *J. Am. Chem. Soc.*, 1973, 2394-2396.

113. H. C. Brown, T. E. Cole, and B. Singaram, *Organometallics*, 1984, **3**, 774-777.

114. H. C. Brown and B. Singaram, *J. Org. Chem.* 1984, **49**, 945-947.

115. H. C. Brown, J. R. Schwier and B. Singaram, *J. Org. Chem.*, 1978, **43**, 4395-4402.

116. N. N. Joshi, C. Pyun, V. K Mahimdroo, B. Singaram and H. C. Brown, *J. Org. Chem.*, 1992, **57**, 504-511.

117. H. C. Brown, N. G. Bhat and V. Somayaji. *Organometallics*, 1983, **2**, 1311-1316.

118. O. M. Friedman, D. L. Klass and A. M. Seligman, *J. Am. Chem. Soc.*, 1954, **76**, 916-917.

119. F. R. Atherton, H. T. Howard and A. R. Todd, *J. Chem. Soc.*, 1948, 1106-1110.

120. H.G. Khorana and A.R. Todd, *J. Chem. Soc.*, 1953, 2257-2260.

121. J. Schulz, M. W. Beaton and D. Gani, *J. Chem. Soc., Perkin Trans. 1,* 2000, 943-954.

122. T. K. M. Shing and V. W. F. Tai, *J. Chem. Soc., Perkin Trans. 1,* 1994, 2017-2025.

123. C. D. Maycock, M. T. Barros, A. G. Santos and L. S. Godinho, *Tetrahedron Lett.*, 1992, **33**, 4633-4636.

124. J. Cléophax, S. D. Gero, J. Leboul, M. Akhtar, J. E. G. Barnett and C. J. Pearce, *J. Am. Chem. Soc.*, 1976, **98**, 7110.

125. R. Grewe and E. Nolte, *Liebigs Ann. Chem.*, 1952, **575**, 1-9.

126. M. Lalonde and T. H. Chan, *Synthesis*, 1985, 817-845.

127. A. L. Gemal and J. L. Luche, *J. Am. Chem. Soc.*, 1981, **103**, 5454-5459.

128. a) M. Taniguchi, H. Fujii, K. Oshima and K. Utimoto, *Tetrahedron*, 1995, **51**, 679-686; b) H. X. Zhai, P. S. Lei, J. C. Morris, K. Mensa-Wilmot and T. Y. Shen, *Tetrahedron Lett.*, 1995, **36**, 7403-7406.

129. See for example : Goodman and Gilman's, *"The Pharmacological Basis of Therapeutics"*, 6th Ed.; Goodman, L. S., Gilman, A., Eds, MacMillan; New York, 1980.

130. J. A. Deyrup and C. L. Moyer, *J. Org. Chem.*, 1969, **34**, 175-180; P. A. Crooks and R. Szyndler, R., *Chem. Ind. (London)*, 1973, 1111-1119.

131. M. Freifelder and G. R. Stone, *J. Org. Chem.*, 1961, **26**, 1477-1482.

149. R. E. Lutz, J. A. Freek and R. S. Murphey, *J. Am. Chem. Soc.*, 1948, **70**, 2015-2018.

133. M. C. Carre, J. P. Houmounou and P. Caubere, *Tetrahedron Lett.*, 1985, **26**, 3107-3110.

134. L. E. Overman and L. A. Flippin, *Tetrahedron. Lett.*, 1981, **22**, 195-198.

135. A. Papini, A. Ricci, M. Taddei, G. Seconi and P. Dembech, *J. Chem. Soc., Perkin Trans. 1*, 1984, 2261-2265.

136. M. Chini, P. Crotti and F. Macchia., *Tetrahedron Lett.*, 1990, **31**, 4661-4664,

137. M. Chini, P. Crotti, L. Favero, F. Macchia and M. Pineschi, *Tetrahedron Lett.*, 1994, 433-436.

138. A. E. Vougioukas and H. B. Kagan, *Tetrahedron. Lett.*, 1987, **28**, 6065-6068.

139 M. Chimi, P. Crotti, L. A. Flippin and F. Macchia, *J. Org. Chem.*, 1990, **55**, 4265-4272.

140. K.S. Lam, S. E. Salmon, E. M. Hersh, K. J. Hruby, W. M. Kazmiersky and R. J. Knapp. *Nature (London)*, 1991, **354**, 82-84.

141. D Stones, D. J. Miller, M. W. Beaton, T. J. Rutherford, and D. Gani, *Tetrahedron Lett.*, 1998, **39**, 4875-4878.

142. M. W. Beaton and D. Gani, *Tetrahedron Lett.*, 1998, 39, 8549-8552 .

143. P. Hormozdiari and D.Gani, *Tetrahedron Lett.*, 1996, 8227-8230.

144. T. W. Greene, P. G. M. Wuts, "*Protecting groups in Organic Synthesis*", John Wiley and Sons, 3d ed, 1999.

145. J. Schulz, J. Wilkie, M. W. Beaton, D. J. Miller and D. Gani, *Biochem. Soc. Trans.*, 1998, 315-322. Library of ^{13}C and ^{1}H ***NMR*** Spectra: 1(2),1024C.

146. Aldrich Library of ^{13}C and ^{1}H ***NMR*** Spectra: 1(2), 1024C.

147. M. W. Beaton and D. Gani, *Chemistry and Biology* (in press) 2000.

148. E. J Corey and J. W. Suggs, *J. Org. Chem.*, 1973, **58**, 3224-3229.

149. E. Tarelli, *J. Carbohydrate Chem.*, 2000, **19**, 233-241.

150 Inositol Monophosphatase purified and provided by Roger Pypus, University of St Andrews.

151 R. E. Diehl, and R.A.F. Dixon, *J.Biol. Chem.*, 1990, **265**, 5946-5949.

152 K. Itaya, and M. Ui, *Clin. Chim. Acta.*, 1966, **14**, 361-366.