DESIGN AND SYNTHESIS OF CHEMICAL PROBES FOR THE PROTEIN KINASE B PH DOMAIN

Joseph Nemeth

A Thesis Submitted for the Degree of PhD at the University of St Andrews

2008

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Design and synthesis of chemical probes for the protein kinase B PH domain

University of St Andrews

School of Chemistry and Centre for Biomolecular Sciences

Joseph Nemeth

April 2008

Thesis submitted to the University of St Andrews in the application for the degree of Doctor of Philosophy

Supervisor: Dr Stuart J. Conway
Abstract

Phosphatidyl d-myo-inositol (3,4,5)-trisphosphate [PtdIns(3,4,5)P₃] contributes to the activation of protein kinase B (PKB) by interacting with the PKB PH domain. PKB is known to be up-regulated in several cancer cell types. Compounds that can display selective inhibition of this kinase have promising chemotherapeutic potential, and inhibition of the PH domain of PKB represents a realistic means by which to achieve this. Analysis of the X-ray crystal structures of apo PKBαPH and PKBαPH bound to d-myo-inositol 1,3,4,5-tetrakisphosphate [InsP₄, the inositol head group of PtdIns(3,4,5)P₃] led to the design of PtdIns(3,4,5)P₃ and InsP₄ analogues as potential PKB PH domain inhibitors. The synthesis of PtdIns(3,4,5)P₃ analogues modified at the C-4 position was investigated, but it was discovered that such compounds were prone to migration of the 1-position phosphate. Subsequently, a range of racemic InsP₄ analogues, modified at the C-1 or C-4 position, were successfully synthesised. Advanced progress has also been made towards the synthesis of enantiomerically pure analogues of InsP₄.
Declarations

I, Joseph Nemeth, hereby certify that this thesis, which is approximately 52,000 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 ___________ Signed _______________

I was admitted as a research student in September 2004 and as a candidate for the degree of Doctor of Philosophy in February 2008; the higher study for which this is a record was carried out in the University of St Andrews between 2004 and 2008.

Date ___________ Signed _______________

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

Date ___________ Signed _______________
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<th>Description</th>
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</thead>
<tbody>
<tr>
<td>AGC</td>
<td>protein kinases A, G and C</td>
</tr>
<tr>
<td>All</td>
<td>allyl</td>
</tr>
<tr>
<td>AllBr</td>
<td>allyl bromide</td>
</tr>
<tr>
<td>ADP</td>
<td>adenosine diphosphate</td>
</tr>
<tr>
<td>Arg</td>
<td>arginine</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine trisphosphate</td>
</tr>
<tr>
<td>BAD</td>
<td>Bcl-2-associated death promoter</td>
</tr>
<tr>
<td>BnBr</td>
<td>benzyl bromide</td>
</tr>
<tr>
<td>c</td>
<td>concentration</td>
</tr>
<tr>
<td>CAN</td>
<td>ceric(III) ammonium nitrate</td>
</tr>
<tr>
<td>CSA</td>
<td>camphor sulfonic acid</td>
</tr>
<tr>
<td>DAPP</td>
<td>Dual adaptor of phosphotyrosine and 3-phosphoinositides</td>
</tr>
<tr>
<td>DAG</td>
<td>diacylglycerol</td>
</tr>
<tr>
<td>DCC</td>
<td>dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>DDQ</td>
<td>dichlorodicyanoquinone</td>
</tr>
<tr>
<td>DIBAL-H</td>
<td>diisobutylaluminium hydride</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
</tr>
<tr>
<td>ENTH</td>
<td>Epsin N-terminal homology</td>
</tr>
<tr>
<td>FKHR</td>
<td>Forkhead (Drosophila) homolog 1 (rhabdomyosarcoma)</td>
</tr>
<tr>
<td>FMLP</td>
<td>N-formylmethionylleucylphenylanaline</td>
</tr>
<tr>
<td>FRET</td>
<td>fluorescence resonance energy transfer</td>
</tr>
<tr>
<td>FYVE</td>
<td>Fab1, YOTB/ZK632.12, Vac1, EEA1</td>
</tr>
<tr>
<td>Glu</td>
<td>glutamate</td>
</tr>
<tr>
<td>GSK3β</td>
<td>glycogen synthase kinase 3-beta</td>
</tr>
<tr>
<td>Grp1</td>
<td>general receptor for phosphoinositides-1</td>
</tr>
<tr>
<td>HM</td>
<td>hydrophobic motif</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>inhibitory concentration 50%</td>
</tr>
<tr>
<td>InsP&lt;sub&gt;3&lt;/sub&gt;</td>
<td>1D-&lt;i&gt;myo&lt;/i&gt;-inositol (1,4,5)-trisphosphate</td>
</tr>
</tbody>
</table>
**InsP₄** 1D-**myo**-inositol (1,3,4,5)-tetrakisphosphate

**InsP₆** 1D-**myo**-inositol (1,2,3,4,5,6)-hexakisphosphate

**InsP₇** 1D-**myo**-inositol (1,2,3,4,5,6)-heptakisphosphate

**InsP₈** 1D-**myo**-inositol (1,2,3,4,5,6)-octakisphosphate

**IR** infra red spectroscopy

**KD** kinase domain

**Kᵢ** inhibition constant

**lys** lysine

**mCPBA** 3-chloroperoxybenzoic acid

**mTOR** mammalian target of rapamycin

**NF-kB** nuclear factor – kappa B

**NSCLC** non-small cell lung carcinoma

**PDK 1/2** phosphoinositide dependent kinase 1 or 2

**Pg** protecting group

**PH** pleckstrin homology

**Phe** phenylalanine

**PI** phosphoinositide

**PI3-K** phosphoinositide 3-kinase

**PIA** phosphatidylinositol ether lipid analogue

**PIF** PKC-related kinase 2 interacting factor

**PIK3CA** phosphatidylinositol 3-kinase, catalytic, alpha polypeptide

**PLC** phospholipase C

**PKA** protein kinase A

**PKB** protein kinase B

**PKBα** protein kinase B α isoform

**PKB PH** protein kinase B pleckstrin homology

**PKC** protein kinase C

**PMB** 4-methoxybenzyl

**PMBCl** 4-methoxybenzyl chloride

**PMB-TCA** (4'′-methoxy)benzyl-2,2,2-trichloroacetimidate

**PRK2** protein kinase C-related kinase 2
PtdIns          phosphatidylinositol
PtdIns(3)P     phosphatidylinositol (3)-phosphate
PtdIns(3,4)P₂  phosphatidylinositol (3,4)-bisphosphate
PtdIns(3,4,5)P₃ phosphatidylinositol (3,4,5)-trisphosphate
PtdIns(4)P     phosphatidylinositol (4)-phosphate
PtdIns(4,5)P₂  phosphatidylinositol (4,5)-bisphosphate
PTEN           phosphatase and tensin homologue
PX             PHOX
RT             room temperature
Ser            serine
SGK            serum/glucocorticoid regulated kinase
SHIP 1/2       Src homology 2-containing inositol 5'-phosphatase 1 or 2
TBAF           tetrabutylammonium fluoride
TBAI           tetrabutylammonium iodide
THF            tetrahydrofuran
Thr            threonine
TIPS           triisopropylsilyl
TLC            thin layer chromatography
TMS            tetramethyl silane
4-TsOH·H₂O    4-toluenesulfonic acid monohydrate
VL             variable loop
1. INTRODUCTION

1.1 Inositols: Structure, nomenclature and natural occurrence

In 1850 Scherer isolated from heart muscle an optically inactive cyclitol possessing an empirical formula of a carbohydrate \([\text{C}_n(\text{H}_2\text{O})_n]\). This compound was termed “inosit” after the Greek root \(\text{inos}\), meaning “muscle”, and became known in English as “inositol”. It is now understood that this was one of nine possible stereoisomers of hexahydroxy cyclohexane (Figure 1.1), and is currently known as \textit{myo}-inositol 1.

![Figure 1.1. The nine inositol stereoisomers.](image)

As Figure 1.1 depicts, the stereoisomers \textit{myo}-, \textit{allo}-, \textit{cis}-, \textit{epi}-, \textit{scyllo}-, \textit{neo}- and \textit{muco}-inositol 1-7 all contain internal elements of symmetry and are therefore optically inactive. \textit{L-}\((\cdot)\)-\textit{chiro}-inositol 8 and \textit{D-(+)}-\textit{chiro}-inositol 9 are unsymmetrical and form an enantiomeric pair. \textit{myo}-Inositol, a \textit{meso} compound, is by far the most naturally abundant of the series, and is synthesised in both plants and animals from \textit{D}-glucose-6-phosphate. Therefore, often the term “inositol”, without the prefix, refers to \textit{myo}-inositol whereas “inositols” refers to all of the nine diastereomers.

The large number of inositols and their derivatives has caused much confusion in the scientific literature in relation to nomenclature. \textit{myo}-Inositol 1 is represented in its more thermodynamically stable chair form with five equatorial hydroxyl groups and one axial
hydroxyl. The carbon bearing the axial hydroxyl is designated C-2 (Figure 1.2), and the other carbons on the ring can be numbered from C-1 to C-6 starting from a C-1 atom either side of C-2 and proceeding either clockwise or anti-clockwise around the ring. According to convention, an anti-clockwise numbering in an asymmetrically substituted inositol leads to the D-configuration, while a clockwise numbering gives a substituted inositol with an L-prefix. The choice of prefix would be derived by giving preference to the lowest numbering of substituents. However, complications arise in multiply substituted derivatives and, in particular, when a biological pathway is traversed, confusing changes in nomenclature can occur. Consequently, an IUPAC recommendation allowing all biologically relevant compounds to be denoted as D-derivatives was proposed in 1989.\(^2\) This convention has largely been adhered to in the literature since, and is adopted in this thesis. It is the function of these biologically significant phosphorylated derivatives of myo-inositol, in particular the phospholipids, that this research project is focussed on.

![Figure 1.2. The numbering of myo-inositol.](image)

**1.2 History of the inositol phosphates**

**1.2.1. Phospholipids and the Phosphoinositide effect**

The function and regulation of the inositol phosphates has been of long standing interest to the biologist and biochemist alike.\(^3\) Although Scherer's discovery of myo-inositol was as early as 1850, it was not until 1942 that Folch reported the discovery of inositol-containing phospholipids in the brain.\(^4\) In 1953 Lowell and Mabel Hokin established that acetylcholine stimulates the turnover of inositol-containing phospholipids in the pancreas and brain cortex slices.\(^5\) This type of stimulus-response coupling was subsequently observed in a number of other systems exposed to agonist stimulation, and became
known as the phosphoinositide (or “PI”) effect. By the late 1950s, new analytical techniques allowed the separation of inositol-containing phospholipids, permitting the isolation of phosphatidylinositol (PtdIns) \[10\], phosphatidylinositol (4)-phosphate [PtdIns(4)P] \[11\], and phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P\[2\]] \[12\] (Figure 1.3). The chemical structures of these compounds were soon established by Ballou and co-workers.\(^7\) The structures of these intracellular, membrane-bound molecules are essentially defined by a myo-inositol core, bearing a “phosphatidyl” moiety (i.e. a phosphate ester bearing glycerol-linked, fatty chains) at the 1-position of the ring.

![Figure 1.3. The major phosphatidylinositols and InsP\(_3\).](image)

**1.2.2. Inositol (1,4,5)-trisphosphate and Ca\(^{2+}\) release**

Despite the observation of the so-called “PI effect”, little progress had been made in understanding the physiological significance of the stimulated turnover of phosphoinositides until a key breakthrough in 1975. Michell realised a connection between agonist-stimulated phospholipid turnover and increased intracellular Ca\(^{2+}\) levels.\(^8\) However, the chemical link between these events was not known until Berridge and co-workers published their findings on a seminal breakthrough in 1983.\(^9\) 1d-myoinositol (1,4,5)-trisphosphate (InsP\(_3\)) \[16\] was correctly proposed to mobilise Ca\(^{2+}\) from intracellular stores. Phospholipase C (PLC) hydrolyses PtdIns(4,5)P\(_2\) leading to the accumulation of InsP\(_3\) and diacylglycerol (DAG) following activation of G-protein coupled receptors (Figure 1.4). The lipophilic DAG remains bound to the cell membrane and effects signal transduction by the activation of protein kinase C (PKC). InsP\(_3\), which is hydrophilic, acts as a second messenger by diffusing into the cytoplasm and binding to
InsP$_3$ receptors which, upon activation, promote Ca$^{2+}$ release from intracellular Ca$^{2+}$ stores. This discovery helped stimulate even greater interest in the role of inositol phosphates in signal transduction. Gradually, this resulted in the mapping out of a complex metabolic pathway involving a large number of different phosphorylated inositol phosphates, including higher inositol phosphates such as InsP$_6$ and even the pyrophosphate-containing InsP$_7$ and InsP$_8$. However, some years following Berridge’s discovery of InsP$_3$ as a second messenger, it became evident that several lipid-bound phosphoinositides themselves may be considered as second messengers in their own right.

**Figure 1.4.** Schematic representation of the InsP$_3$ signalling cascade.$^{13}$

### 1.2.3. PI3-Kinase and $\delta$3-phosphorylated inositol lipids

In 1988 Whitman et al. observed the phosphorylation of PtdIns 10 at the $\delta$3-position by a phosphoinositide 3-kinase to give the novel phospholipid phosphatidylinositol (3)-phosphate (PtdIns(3)P) $^{13,14}$ In the same year, Traynor-Kaplin et al. reported the appearance of a novel phosphoinositide that contained four phosphates (a phosphatidylinositol trisphosphate), which was found only in activated, and not in unstimulated, human neutrophils.$^{15}$ It was shown that in such neutrophils N-formylmethionylleucylphenylanaline (FMLP) causes the rapid accumulation of the trisphosphate, as well as a novel phospholipid bisphosphate. These compounds were
thought to be phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5)\(P_3\)] 15 and phosphatidylinositol (3,4)-bisphosphate [PtdIns(3,4)\(P_2\)] 14 respectively; products of the action of the phosphoinositide 3-kinase (PI3-K). Indeed, the enzyme responsible for producing these lipids was later purified and shown to phosphorylate PtdIns(4)\(P\) and PtdIns(4,5)\(P_2\) to produce PtdIns(3,4)\(P_2\) and PtdIns(3,4,5)\(P_3\) respectively, with the structures of these products having finally been characterised.\(^{16}\) Given the discovery that these compounds were virtually absent in unstimulated cells, and accumulated rapidly after agonist induced stimulation, there was a clear indication that these phosphoinositides act as intracellular second messengers. Eventually, it was established that three classes of PI3-K exist in mammalian cells, types I, II and III, the former two consisting of multiple isoforms.\(^{17}\) These different classes display varying substrate preferences, contributing towards a multifaceted regulation cycle for phosphoinositides. Class I PI3-Ks are recognised as the most important in the phosphorylation of PtdIns(4,5)\(P_2\) and hence the production of PtdIns(3,4,5)\(P_3\).\(^{18}\) It also emerged that the phospholipid products of Class I PI3-Ks do indeed act as important second messenger signalling molecules following PI3-K activation, recruiting a range of proteins to the cell membrane.\(^{19}\) It is this pathway that has generated most interest while extensive research attempts to decipher the diverse roles that phosphoinositides play in cellular processes.

1.3. Metabolism and activity of D3-phosphorylated phosphoinositides

1.3.1. Phosphoinositide Regulation

Metabolic regulation of the known phosphoinositides has proved to be highly complex. As well as the existence of kinase driven phosphorylation of such species at the \(\alpha_3\)-, \(\alpha_4\)- and \(\alpha_5\)-positions, phosphatases that can specifically remove the phosphates at each of these positions have also been discovered (Figure 1.5).\(^{20}\) Furthermore, these enzymes are often expressed as more than one class and/or isoform (such as PI3-Ks) and have been shown to be prevalent in distinct intracellular locations. Consequently, the phosphoinositide products of their action also exhibit characteristic subcellular
distribution patterns. For instance, PtdIns(4,5)P₂ and PtdIns(3,4,5)P₃ are found mainly in the plasma membrane, whereas PtdIns(3)P, PtdIns(4)P and PtdIns(5)P are mainly present in endosomes, Golgi and nuclei respectively. Figure 1.5 demonstrates how different phosphoinositide species can be reversibly interconverted through tightly regulated kinase/phosphatase processes. This can result in short-term alterations of the protein-binding properties and function of a given membrane domain. Such plastic properties render phosphoinosities ideally suited to integrating cell signalling events and membrane trafficking.

1.3.2. Regulation of PtdIns(3,4,5)P₃ and PtdIns(3,4)P₂

The regulation of PtdIns(3,4)P₂ and PtdIns(3,4,5)P₃ within the cell appears to demonstrate the significance of these type of complex phosphorylation/dephosphorylation cycles. Their metabolism clearly demonstrates that, during cell activation, both positive and negative regulatory signalling processes are vital for homeostasis. For instance, while PtdIns(3,4)P₂ can be formed by the action of a PI3-K on PtdIns(4)P as outlined above, the action of Src homology domain 2-containing inositol 5'-phosphatases (SHIP) on PtdIns(3,4,5)P₃ also results in PtdIns(3,4)P₂ production (Figure 1.5). Cellular concentration of PtdIns(3,4)P₂ appears to be lower than the concentration of PtdIns(3,4,5)P₃, and it is currently thought that SHIP
phosphatase action on PtdIns(3,4,5)P$_3$ is the dominant mechanism by which PtdIns(3,4)P$_2$ is formed.$^{28}$ SHIP mediates an important negative feedback mechanism in lymphocytes. Loss of SHIP results in an unbalanced immune response, potentially leading to autoimmunity.$^{29,30}$ Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) catalyses the dephosphorylation of both PtdIns(3,4,5)P$_3$ and PtdIns(3,4)P$_2$ at the 3-position.$^{31}$ Cells lacking PTEN have elevated levels of these two phosphoinositides. The significance of such negative feedback regulation is demonstrated by the fact that PTEN is known to act as a tumour suppressor that is mutated/deleted in a variety of different human tumours.$^{32}$ The importance of elevated PtdIns(3,4,5)P$_3$ levels in relation to human malignancy is discussed later. Suffice to say, at this early stage of discussion it is clear how vital these D-3 phosphoinositides and the proteins with which they interact are to fundamental cellular processes. The implication is that proteins exist within the cell that selectively recognise and interact with these phosphoinositides in a highly regulated manner.

### 1.3.3. The targets for D3 phosphoinositides

For a given protein to bind to a membrane-bound phosphoinositide, a particular recognition domain is required through which the enzyme can interact with the phospholipid. The number of domains or modules known to bind to phosphoinositides has increased dramatically in recent years.$^{33}$ Figure 1.6 indicates the major binding domains that the most studied phosphoinositides are known to interact with, including some of the relevant proteins. The selectivity of such domains and how they bind is variable. Those that target only the rare D3-phosphorylated phosphoinositides must bind with very high affinity and selectivity. In the case of certain pleckstrin homology (PH) domains which bind PtdIns(3,4,5)P$_3$ and/or PtdIns(3,4)P$_2$, this is achieved almost solely via the inositol headgroup interactions.$^{34}$ Although, as indicated, monophosphorylated phosphatidylinositolos (e.g. PtdIns(3)P) have also been reported to bind PH domains, at least in vitro, this is achieved with reduced affinity, and the physiological relevance of such interactions is unclear. In the case of FYVE$^{35}$ and phox$^{36,37}$ homology domains,
which bind PtdIns(3)P preferentially, high affinity interactions are achieved via a membrane insertion and/or oligomerisation component in addition to head group interaction.\textsuperscript{33} Domains that target PtdIns(4,5)P\textsubscript{2}, which is more abundant by some 25-fold, do not seem to require the same stringent affinity and specificity characteristics, and tend to be more structurally diverse. Some of these domains, such as the PH domain of PLC\textsubscript{δ1}, do bind PtdIns(4,5)P\textsubscript{2} strongly and selectively, although this is possibly more of an exception rather than the rule. Such high affinity PH domain binding is generally reserved for PtdIns(3,4)P\textsubscript{2} and PtdIns(3,4,5)P\textsubscript{3}. Thus, variability between binding domain-types affords the targeting of different phosphoinositides, while variability in preference and specificity within the domain types allow for further differentiation of inositol lipid targeting. Therefore, the apparent range that exists in phosphoinositide-substrate binding modes allows a large number of proteins to be selectively recruited to specific membrane locations. Of all known phosphoinositide recognition modules, the PH domain is the most thoroughly characterised. By far the most common target for PH domains is PtdIns(3,4,5)P\textsubscript{3}.

\textbf{Figure 1.6.} Phosphinositide binding domains and associated proteins.\textsuperscript{26} BD = binding domain. Domains types: PX (phox); FYVE (Fab1, YOTB/ZK632.12, Vac1, EEA1); PH (pleckstrin homology); TUBBY; ENTH (Epsin N-terminal homology).
As noted, PtdIns(3,4,5)P₃ is the major product of agonist-stimulated PI3-kinases, and its concentration increases by more than 40-fold within seconds of cell stimulation. Thus, proteins containing a domain capable of specific and preferential PtdIns(3,4,5)P₃ binding, such as certain PH domains, can be recruited rapidly to the plasma membrane of agonist-treated cells, in a PI3-K dependent manner. PH domains are composed of approximately 120 amino acid residues and, although sequence homology between different PH domain family members is relatively low, all contain a common β-sandwich fold. Since the discovery of this domain in 1993, it has been found in some 250+ human proteins. In fact, those that are highly selective for PtdIns(3,4,5)P₃ are rather few, and several also bind PtdIns(3,4)P₂ with appreciable affinity. Prominent examples are protein kinase B (PKB, also known as Akt), phosphoinositide-dependent protein kinase 1 (PDK1), Bruton’s tyrosine kinase (Btk), the general receptor for phosphoinositides-1 (Grp1) and the dual adaptor for phosphotyrosine and 3-phosphoinositides-1 (DAPP1). Of these, only Btk and Grp1 are considered highly selective for PtdIns(3,4,5)P₃.

Of the PH domain-containing proteins mentioned, PKB in particular, a serine/threonine kinase belonging to the ‘AGC’ superfamily of protein kinases, has aroused significant interest in recent years due to the crucial role it plays in a range of fundamental cellular processes (‘AGC’ stands for protein kinases A, G and C). PKB’s PH domain binds selectively to PtdIns(3,4)P₂ and PtdIns(3,4,5)P₃ with similar affinity, and its activation is therefore PI3-kinase driven. This PI3-K/PKB pathway, as it has become known, is now recognised as one of the most crucial signal transduction pathways in cellular metabolism, with the second messenger activity of the two aforementioned phosphoinositides at its core. An overview of this signalling cascade serves to highlight this fact.
1.4. PI3-K/PKB signalling pathway overview

PI3-kinases are activated by a host of stimulants including growth factors, cytokines, and insulin. They consist of a p110 catalytic subunit and a regulatory adaptor subunit p85, which binds to specific tyrosine-phosphorylated sequences within the cytoplasmic tail of the receptor (see Figure 1.7). This binding process of the p85 subunit recruits the p110 catalytic subunit to the plasma membrane where it can phosphorylate PtdIns(4,5)P$_2$ thereby generating PtdIns(3,4,5)P$_3$. As discussed, this phosphoinositide then binds to the PH domain of several proteins, in this case PKB, PDK1 and PDK2, thus recruiting them to the cell membrane. The tandem translocation of these proteins may be crucial as it brings them in close proximity to one another, facilitating their interaction.

PKB is fully activated by phosphorylation at two specific sites by PDK1 and PDK2, following which it disperses into the cytoplasm and the nucleus, phosphorylating a number of proteins downstream of the signalling cascade. Identifying the target substrates for PKB has been a major challenge in the study of the PI3-K pathway, and several have been determined. One such example is the mammalian target of

![Fig.1.7. An overview of the phosphoinositide 3-kinase (PI3-K) signalling pathway. The diagram shows how the activation of PI3-K from extracellular stimuli leads to the accumulation of PtdIns(3,4,5)P$_3$ at the cell membrane. The binding of PtdIns(3,4,5)P$_3$ to PKB, PDK1 and PDK2 brings these proteins together to allow the phosphorylation of PKB. PKB then phosphorylates a number of proteins downstream of the signalling cascade.](image-url)
rapamycin (mTOR), a major effector in cell growth and translation, which PKB activates through at least two mechanisms. PKB is also recognised to phosphorylate several proteins that mediate apoptosis. For instance, it inhibits the proteins BAD and FKHR which are activators of apoptosis. Also, it activates NF-κB, a protein known to inhibit apoptosis. Thus, PKB activity promotes cell growth and survival. Also, PKBβ is highly expressed in insulin responsive tissue such as adipose tissue, and is believed to be heavily involved in glucose metabolism via the inactivation of glycogen synthase kinase 3β (GSK3β). Clearly, deregulation of the PI3-K/PKB signalling pathway would have profound effects on cellular function.

1.5 PKB structure and activation

1.5.1. A structural overview

Given the crucial role that PKB plays in such fundamental cellular processes, it is unsurprising that the precise structure and activation mechanism of this protein has commanded great interest in recent years. Consequently, it has been well studied, although its mechanism of activation is complex and remains somewhat controversial. Three isoforms of PKB (α, β and γ) are known to exist in mammals, each consisting of three conserved domains: the N-terminal PH domain, a central kinase catalytic domain and a C-terminal extension containing a regulatory hydrophobic motif (HM) (Figure 1.8.A). Among the PKB isoforms, the PH domains are ~80 % identical and the kinase domains ~90 % identical. The kinase domain is structurally similar to other members of the AGC kinase family and is closely related to the PKC, PKA and SGK subfamilies. The linker (LINK) region is poorly conserved and the structure of the 25 residue gap between the PH and kinase domains is currently unknown.

Figure 1.8 shows a composite of the crystal structures of the PH (yellow) and CAT (blue) domains, with the PH domain positioned arbitrarily with respect to the kinase domain and connected by a modelled linking peptide. The crystal structure of the kinase domain for PKBβ in activated and unactivated forms have been solved. Crystal structures of PKB PH bound to naturally occurring PtdIns(3,4,5)P₃ or shortened glyceryl-chain analogues of PtdIns(3,4,5)P₃ have not been obtained. However, a crystal structure
of the inositol head group of PtdIns(3,4,5)P₃, inositol(1,3,4,5)tetrakisphosphate (InsP₄ 17, which has a similar affinity for PKB), bound to the PH domain of PKBα has been solved and was reported in 2003 by van Aalten and co-workers. Analysis of the binding of InsP₄ revealed several interesting features. For instance, as Figure 1.8.B shows, the 1-, 3- and 4- position phosphates of InsP₄ interact strongly with the binding site, while the 5-position phosphate is largely solvent exposed. This explains the similar affinity that PtdIns(3,4)P₂ and PtdIns(3,4,5)P₃ have for PKB. Furthermore, on comparison with the InsP₄ bound structure and the uncomplexed apo structure, it was observed that such binding induced a large conformational change in PKBα PH, not previously observed in PH domain–phosphoinositide interactions.

![Figure 1.8. A. A composite of the crystal structure of the PKBαPH (yellow) and PKBβ kinase (CAT, blue) domains.](image)

![Figure 1.8. B. PyMOL representation of the crystal structure of InsP₄ bound to PKBαPH.](image)

The 3- and 4-position phosphates interact strongly with the binding site residues, while the 5-position phosphate is significantly solvent exposed.
1.5.2. The binding of the PtdIns(3,4,5)P$_3$ head group to PKB PH produces a conformational change

Prior to the report of van Aalten, it had been postulated that the binding of PtdIns(3,4,5)P$_3$ to PKB PH did not only serve to co-localise the protein with PDK1 at the cell membrane. It had been speculated that such binding also induced an activating conformational change in PKB, supported by the observation that PDK1 is unable to phosphorylate wild-type PKB under conditions where it does efficiently phosphorylate a mutant form of PKB that lacks its PH domain.$^{55,56}$ Van Aalten's study provided the first direct structural evidence that a conformational change does indeed occur.

![Fig.1.9](image_url) PyMOL representations of the crystal structure of PKBαPH in the apo form (left) and as the InsP$_4$ complex (right). Variable loops VL1 and VL3 move towards the charged phosphates of the inositol substrate. VL2 also undergoes a conformational change, forming an acidic $\alpha$-helix in the process.$^{54}$

The ribbon representations of the structures determined in the study are presented in Figure 1.9. As expected, the apo PKBα PH possessed the standard PH domain fold, together with three loops (VL1, VL2 and VL3), that are variable in both length and sequence in all known PH domains.$^{38,40}$ In the case of PKBα PH, these loops help form a highly basic pocket into which the head groups of PtdIns(3,4)P$_2$ and PtdIns(3,4,5)P$_3$ bind. Significantly, conformational changes in all three variable loops were observed upon InsP$_4$ binding. For instance, the VL3 loop was positioned up to 7.4 Å away from the
phosphoinositide binding pocket in the apo structure compared to its position in the InsP$_4$ complex. The most dramatic difference, however, was observed by the absence of the short acidic α-helix of VL2 in the apo structure, present in the complex. The VL2 loop was not well defined in the apo structure, suggesting that in this form the loop is flexible. However, it was evident that VL2 did not assume ordered secondary structure, such as the α-helix observed in the InsP$_4$ complex. According to the analysis, in order to achieve the formation of the α-helix, VL2 must undergo a 7.6 Å shift upon InsP$_4$ binding. The formation of this helix is defined by the clustering of 3-4 negative charges (Asp-44, Asp-46, Glu-49 and to a lesser extent Glu-40), forming an acidic patch facing the solvent.

It has been proposed that a consequence of these conformational changes observed in VL2 and/or VL3 could be to effect interactions of these loops with the PKB kinase domain, and in doing so expose one of the protein’s sites of phosphorylation by either PDK1 or PDK2. So far, this kind of ligand-induced conformational change for PH domains appears to be unique to PKB. Such changes in other PH domains bound to InsP$_4$ are limited to movement of VL1, typically to accommodate the δ5-phosphate group.$^{57-59}$

1.5.3. The Activation cycle of PKB

The binding of PKB PH to PtdIns(3,4,5)P$_3$ is merely the initial step in a complex activation process for PKB. The precise mechanism of activation is, in fact, far more complicated than that implied in the simplified PI3-K/PKB pathway overview presented in Figure 1.7. The protein is phosphorylated at a threonine residue in the activation T loop (Thr308 in PKBα) on the kinase domain, which stimulates enzymatic activity by at least 100-fold, and is essential for activity.$^{60}$ Mutation of Thr308 to non-phosphorylatable alanine greatly reduces activity.$^{61}$ Evidence suggests that PDK1 is responsible for this phosphorylation. PDK1 is a ‘master kinase’ responsible for the phosphorylation of numerous AGC kinases. Over-expression of PDK1 leads to elevated Thr308 phosphorylation in the absence of natural agonists.$^{62}$ Also, phosphorylation of Thr308 is lost in cells where PDK1 has been genetically disrupted, and PKB is unresponsive to mitogenic stimulation as a result.$^{63}$ PDK1 appears to phosphorylate several of its
substrates in a non-regulated manner. However, when translocated to the cell membrane by phosphoinositide binding, the targets it phosphorylates at this location are PI3-K signal-dependent. Thus, PDK1 only encounters some of its kinase substrates at the cell membrane, and PKB is one of these.\textsuperscript{64}

The second point of PKB activation occurs via the phosphorylation of a serine residue (Ser473 in PKB\(\alpha\)) located on the C-terminal hydrophobic motif (HM). This process increases PKB activity by another 10-fold. However, the means by which this occurs remains unknown. The existence of a “PDK2” which performs this phosphorylation has been inferred, but so far it has not yet been discovered. In fact, a number of enzymes responsible for this modification have been touted. These include PDK1 (when in a complex with the kinase PRK2),\textsuperscript{65} PKC\(\alpha\),\textsuperscript{66} PKC\(\beta\)I,\textsuperscript{67} DNA-dependent kinase,\textsuperscript{68} and the rictor-mTOR complex.\textsuperscript{69} It has even been suggested that PDK2 may not exist at all, and that activation occurs via autophosphorylation.\textsuperscript{70}

The role that the phosphorylated HM plays in PKB activation is also not certain. It has been suggested that phosphorylation of the HM stabilises the interaction between PKB and PDK for the crucial T-loop phosphorylation.\textsuperscript{71} The HM present in PKB is found in many other AGC kinases, and is believed to act as a docking site for PDK1.\textsuperscript{72} PDK1, like many AGC kinases, contains what has been termed the ‘PKC related kinase 2 interacting factor pocket’ or ‘PIF pocket’, to which HM binds. PDK1 has a PIF pocket, but no HM. On the other hand, the HM of PKB binds with lower affinity to PDK1 PIF than the HM of several other AGC kinases do. Evidence suggests that this type of PIF pocket binding is more important for the PDK1-induced activation of kinases not bearing a PH domain (and therefore not PI3-K dependent).\textsuperscript{73} Thus, the assumption is that co-localisation at the cell membrane itself, as well as the conformational change that lipid binding induces, does contribute significantly to an effective interaction of PDK1 with PKB. Again, the significance of phosphoinositide binding is highlighted. Perhaps the tenfold increase in PKB activity seen by HM phosphorylation may well be related to the association of HM with its own PIF pocket following Thr308 activation (Figure 1.10). This, it has been postulated, may further stabilise PKB, resulting in its release from the cell membrane to then diffuse into the cytoplasm.
The precise mechanism of PKB activation remains uncertain. The order in which Ser473 and Thr308 are phosphorylated has not been confirmed, nor has the means by which Ser473 phosphorylation occurs. However, both phosphorylation processes appear to be dependent on PI3-K activation. What is clear is that the process of PKB activation lies at a crucial juncture in a complex and critically important signalling cascade.
1.6. The PI3-K/PKB pathway in human cancer

The discussion so far has presented how an intricate and tightly regulated activation process of PKB is essential for cell growth, proliferation, glucose metabolism and protein synthesis. The fact that upregulation of this pathway is now strongly associated with malignancy is, therefore, not surprising. In fact, the PI3-K/PKB pathway is one of the most frequently hyperactivated signalling pathways in human cancer.\textsuperscript{74,75} A crucial feature of this pathway is the role of the second messenger phosphoinositides produced by PI3-K. As alluded to earlier, one frequent route of overactivity is derived directly from phosphoinositide deregulation. PTEN dephosphorylates PtdIns(3,4,5)\textsubscript{P}\textsuperscript{3} and PtdIns(3,4)\textsubscript{P}\textsuperscript{2} at the D3-position, preventing them from binding to PH domain-containing proteins such as PKB.\textsuperscript{76} Thus, loss of PTEN leads to constitutive signalling of the PI3-K pathway, resulting in uncontrolled cell growth, survival and proliferation.\textsuperscript{77} It is now known that a major path through which PTEN acts as a tumour suppressor is by negatively regulating PKB activity specifically. PTEN was originally isolated as a tumour suppressor gene in breast cancer and glioblastomas\textsuperscript{78} and has subsequently been implicated in various human cancers (Table 1). Reintroduction of wild-type PTEN into tumour cells that are mutant for PTEN have resulted in inhibition of PKB activation leading to either cell cycle arrest or apoptosis.\textsuperscript{79-81}

Several oncogenic mutations are associated with PI3-K itself, and PI3-K is constitutively active in a number of human malignancies.\textsuperscript{74} Mutations have been observed in both the p110 and p85 subunits of PI3-K. Again, the immediate result of such hyperactivity is the increased levels of PtdIns(3,4,5)\textsubscript{P}\textsuperscript{3}, leading to constitutive phosphoinositide signalling through PKB activity.

PKB was originally identified as a retroviral oncogene product,\textsuperscript{82} and amplification and overexpression of PKB was subsequently observed in several cancer types including ovarian, breast and pancreatic cancer.\textsuperscript{83,84} Site mutations in PKB are rare, although a recent report did describe a Glu→Lys mutation at amino acid 17 of the PH domain of PKB\textsubscript{α}.\textsuperscript{85} The crystal structures of this mutant PH domain in apo form and bound to the PtdIns(3,4,5)\textsubscript{P}\textsuperscript{3} head group Ins\textsubscript{P}\textsubscript{4} were obtained, revealing enhanced interaction of the binding site with the 6-OH and 5-phosphate groups of Ins\textsubscript{P}\textsubscript{4}. Thus, the mutation is
believed to result in increased binding efficacy of the PH domain to PtdIns(3,4,5)P$_3$. This led to pathological localisation of PKBα to the plasma membrane, thus stimulating downstream signalling and induced leukaemia in mice.$^{85}$

<table>
<thead>
<tr>
<th>Target</th>
<th>Alteration in PI3-K/PKB pathway</th>
<th>Type of cancer</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTEN</td>
<td>Mutation or deletion</td>
<td>Brain, bladder, breast, prostate and endometrial cancers, glioblastoma</td>
<td>76, 86-90</td>
</tr>
<tr>
<td></td>
<td>Epigenetic silencing</td>
<td>NSCLC, endometrial and gastric cancer, prostate, ovarian and melanocytic tumours</td>
<td>91-94</td>
</tr>
<tr>
<td>PI3-K</td>
<td>Amplification of PIK3CA (encodes p110α)</td>
<td>Ovarian and cervical cancers</td>
<td>95-97</td>
</tr>
<tr>
<td></td>
<td>Mutation in PIK3CA</td>
<td>Colorectal, gastric, breast, ovarian cancers, glioblastoma and hepatocellular carcinoma</td>
<td>97-100</td>
</tr>
<tr>
<td></td>
<td>Mutation in p85α</td>
<td>Colon and ovarian cancers</td>
<td>101</td>
</tr>
<tr>
<td>PKB</td>
<td>PKBα amplification</td>
<td>Gastric adenocarcinoma</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>PKBβ amplification</td>
<td>Ovarian, breast and pancreatic cancers</td>
<td>83, 103, 104</td>
</tr>
<tr>
<td></td>
<td>PKBγ amplification</td>
<td>Hormone-independent breast and prostate</td>
<td>84</td>
</tr>
</tbody>
</table>

Table 1. Genetic alterations resulting in activation of the PI3-K/PKB pathway and their associated human malignancies.$^{105}$

Importantly, the alterations in the PI3-K/PKB pathway, such as those described in Table 1, are frequently associated with advanced disease, and are often features of a poor prognostic factor in many cancers.$^{106,107}$ Hyperactivation of the pathway has also been linked with resistance to chemotherapeutic drugs in cancer treatment. Inhibition of the pathway by biochemical or genetic means increases the efficacy of chemotherapy and/or radiation both in vivo and in vitro.$^{108,109}$ All such factors provide strong justification and rationale for the development of compounds that potently and directly inhibit the PI3-K/PKB signalling pathway for use in the treatment of cancer.
1.7. Targeting PKB inhibition for cancer therapy

Inhibition of the PI3-K/PKB pathway can theoretically be achieved at several junctures along the signalling cascade. Indeed, in the search for effective chemotherapeutic agents, this is exactly what has taken place in recent years, with varying success.\textsuperscript{110} As well as PKB itself, targets have included the tyrosine receptor kinases (TRKs), PI3-K, PDK1 and downstream targets such as mTOR. The synthesis of targets upstream of PKB, such as PI3-K, which are selective and exhibit acceptable toxicity profiles, has met with very limited success. Some older compounds, such as wortmannin, are well characterised and target the p110 subunit of PI3-K.\textsuperscript{111} Wortmannin has displayed effective inhibition of tumour cell growth in vivo and in vitro,\textsuperscript{112} but poor solubility and high toxicity have limited its clinical application.\textsuperscript{113} The inhibition of PDK1 appears more encouraging, given the decent potency and reduced toxicity of most of the available inhibitors compared with the available PI3-K inhibitors.\textsuperscript{105} However, as noted earlier, PDK1 also activates a number of other kinases that regulate cell survival and proliferation. Thus, although potentially advantageous from a therapeutic standpoint, establishing such compounds as inhibitors specifically of the PKB pathway has become complicated, given that their anti-tumour effects may be related to an alternative pathway.

mTOR is a downstream target of PKB which plays a role in protein synthesis, angiogenesis and cell cycle progression. Studies have shown that mTOR inhibitors, rapamycin and its derivatives CCI-779 and RAD001, induce cell cycle arrest and/or apoptosis in tumours exhibiting activation of PKB.\textsuperscript{114,115} However, inhibition of PKB itself may be considered preferable as this would be expected to inhibit most, if not all, PKB substrates. Not all PKB substrates have been identified and the “critical substrates” can vary with cell type, therefore inhibition of individual downstream components may miss key substrates involved in cancer cell survival and proliferation. Thus PKB inhibition may offer greater efficacy, albeit at the expense of potential greater toxicity.

The last three years in particular have seen a number of structurally variable PKB inhibitors reported. The mode of action of such compounds can generally be divided into four categories: ATP-competitive, PH domain, allosteric and substrate-mimetic inhibitors.
However, the precise nature of inhibition for some compounds has not been conclusively determined.

**ATP-competitive inhibitors**

Several small molecule leads are being used as the basis for developing potent and selective PKB inhibitors which target the ATP binding site. As there is a high degree of homology between the ATP-binding pocket of PKB, PKA and PKC, achieving a high degree of selectivity has proved challenging. However, several leads have been derived from the modulation of PKA inhibitors which have been subjected to optimisation for PKB selectivity. An example of such efforts led to the discovery of compound 18, displaying potent inhibition of PKBα (IC$_{50}$ 20 nM) with enhanced selectivity against PKA (IC$_{50}$ 1900 nM).

![Compound 18](image1.png)

![Compound 19](image2.png)

![Compound 20](image3.png)

![Compound 21](image4.png)

![Compound 22](image5.png)

*Figure 1.11. Representative ATP-competitive PKB inhibitors.*

A hit from high throughput screening enabled the group from Abbott laboratories to develop highly potent indole/indazole-pyridine based inhibitors 21 and 22. These compounds slowed the growth of tumours when used alone or in combination with paclitaxel or rapamycin. However, selectivity against PKA was very moderate and the compounds displayed a narrow therapeutic window with significant toxicities observed. Optimisation to provide more selective compounds from this series is still underway.
collaboration between Astex Therapeutics and the Institute of Cancer Research led to the development of low molecular weight phenylpurine and phenylpyrazole ATP-binding inhibitors \(19\) and \(20\), via fragment-based screening techniques.\(^{119,120}\) Again, however, selectivity against PKA was somewhat low.

**Allosteric inhibitors**

Improved selectivity for PKB has been achieved in the synthesis of allosteric inhibitors (Figure 1.12).\(^{121,122}\) These are in fact PKB\(\alpha\) and PKB\(\beta\) isoform selective and highly selective against closely related kinases such as PKA. Such compounds do not compete with ATP or peptide substrate. They are active only against the full-length enzyme requiring the PH domain for full inhibition, but do not bind to this domain. Interestingly, it was observed that inhibition of both the PKB\(\alpha\) and PKB\(\beta\) isoforms, using dual inhibitor \(25\) or compounds \(23\) and \(24\) together, was required for maximal apoptotic response in tumour cells when used in combination with chemotherapeutic agents. Also, it was shown that inhibition of PKB\(\alpha\) and PKB\(\beta\) sensitised only tumour cells but not normal cells to apoptotic stimuli. Unfortunately, these compounds have poor solubility and pharmacokinetic properties that have precluded their evaluation in animal tumour models.

![Figure 1.12. Allosteric inhibitors of PKB.](image-url)
Pseudosubstrate inhibitors

Substrate mimetic inhibitors of PKB have also been reported. These have included the identification of a 14-mer peptide, AKTide-2T, which binds to the substrate binding region of PKBα catalytic domain and inhibits the enzyme with a $K_i$ of 12 μM.\textsuperscript{123} Subsequent manipulation resulted in a further 10-fold increase in potency ($K_i$ 0.11 μM). However, the size of this and other peptide-based inhibitors makes them poor leads for development of small molecule inhibitors. Truncation of the peptide chains generally led to reduced potency. A recent report has described the development of a small molecule, substrate mimetic inhibitor derived from modification of the GSK3β substrate sequence.\textsuperscript{124} Potency was rather moderate, though, with an IC$_{50}$ of 14 μM. The cytotoxic effects of such compounds have yet to be fully investigated.

PH domain inhibitors

Several examples now exist where the binding of the PKB PH domain to PtdIns(3,4,5)P$_3$ is inhibited. It is understood that this process prevents membrane localisation and thus activation of PKB. One example supporting this is the discovery of inositol(1,3,4,5,6)-pentakisphosphate [Ins(1,3,4,5,6)P$_5$]\textsuperscript{26} as an inhibitor of PKB, which induced apoptosis in ovarian, lung and breast cancer cells.\textsuperscript{125} Conversely, other inositol polyphosphates such as Ins(1,4,5,6)P$_4$, Ins(3,4,5,6)P$_4$ and InsP$_6$ had little or no effect on cell apoptosis. Concentrations of synthetic Ins(1,3,4,5,6)P$_5$ (20-50 μM) required for activity were higher than concentrations normally found in mammalian cells (5-15 μM). However, the biological role of indigenous Ins(1,3,4,5,6)P$_5$ has not been well studied, and poor cellular penetration may be a problem for extracellular Ins(1,3,4,5,6)P$_5$.

Several lipid-based inhibitors of PKB PH have also been synthesised. Perifosine\textsuperscript{27} is the best characterised of these. \textit{In vitro}, perifosine is believed to inhibit membrane
translocation of PKB.\textsuperscript{126} This is evidenced by the fact that perifosine-mediated inhibition of PKB phosphorylation was substantially relieved by introduction of MYR-PKB, which bypasses the requirement for PH domain-mediated membrane recruitment. Perifosine inhibited the growth of melanoma, prostate, lung, colon and breast cancer cell lines \textit{in vitro}, and is currently in Phase II trials alone and in combination to treat multiple forms of cancer. The precise mechanism of PKB inhibition for this drug could not be determined, however. Modulation of PKB has not been assessed in clinical studies so far, and perifosine is not specific for PKB, interacting with additional pathways downstream of growth factors. Thus, it will be important to correlate its clinical effects with modulation of PKB signalling, as with all such drugs.

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{figure14.png}
\caption{Phosphatidylinositol ether lipid analogues.}
\end{figure}

Another group of lipid-based PKB inhibitors are those developed by Kozikowski and co-workers, known as phosphatidylinositol ether lipid analogues (PIAs).\textsuperscript{127-129} These were designed to interact selectively with the PH domain of PKB, and five PIAs (28-32) were observed to inhibit PKB with $IC_{50}$s of between 2.5 and 5 μM.\textsuperscript{129} These compounds inhibited PKB activation and phosphorylation of several downstream substrates of PKB in tumour cells, without affecting the activities of upstream kinases PI3-K and PDK1. The result was increased apoptosis 20-30-fold in cancer cell lines with high levels of PKB activity, and only 4-5-fold in those with low levels of PKB activity. It is assumed that it is the phosphorylated derivatives of such compounds that are active, if they are to competitively interact with the highly charged PH domain binding site. However, their phosphorylated versions were not obtained in order to ascertain this. Furthermore,
selectivity against other PH domain-containing proteins was not well established, and in vivo efficacy has yet to be presented.

To conclude, the PKB pathway appears to represent an attractive target for anticancer drug discovery. The fact that there are several examples where apoptosis or cell cycle arrest is observed selectively for PKB-upregulated cells suggests that there is an acceptable therapeutic index to be exploited. Many challenges remain though, not least the need for compounds which display genuine selectivity for the PI3-K pathway and, in particular, for PKB inhibition. Even compounds which may not necessarily be suitable for drug development, if highly selective for PKB, will also be of great use as biological tools in the continuing effort to probe this complex pathway.

1.8. Summary

Over the many years during which inositol phosphates have been studied, it has become evident that the controlled metabolism of phosphoinositides is fundamental for signal transduction in eukaryotic cells. It has emerged that many of these inositol-containing phospholipids act as second messengers in complex intracellular systems. PtdIns(3,4,5)P$_3$, a product of phosphoinositide-3 kinase, is one such lipid. Notably, PtdIns(3,4,5)P$_3$ recruits protein kinase B, among other proteins, to the cell membrane. This process partially activates PKB, facilitating its phosphorylation. Once fully activated, PKB proceeds to phosphorylate a number of other kinases involved in several crucial processes such as cell growth and proliferation. Up-regulation of the PI3-K/PKB pathway is a feature of numerous cancer cell lines. Thus, recent years have seen a drive towards the synthesis of selective PKB inhibitors. Whilst potency has often been achieved, selectivity has proved more challenging. Many of the reported inhibitors bind to the ATP binding site of the PKB kinase domain. However, due to the high homology between the kinase domains of AGC kinases, selectivity is often compromised. PH domain inhibitors offer a potential advantage in terms of selectivity, given that there are far fewer PH domain-containing proteins than proteins bearing an ATP binding site. Furthermore, different PH domains possess different binding affinities towards different inositol
phosphates. This increases the possibility of selectively inhibiting a particular membrane-targeted protein.

The discussion has detailed how the binding of PtdIns(3,4,5)P₃ to PKB PH recruits PKB to the cell membrane and induces a conformational change in the protein, presumably resulting in a conformation more active to subsequent phosphorylation. Inhibition of this interaction can result in reduction of PKB activity, as suggested by the activity of PIAs 28-32. Further probing of the PKB PH domain with novel PtdIns(3,4,5)P₃ analogues is required to ascertain whether such compounds can demonstrate higher selectivity than other types of the reported PKB inhibitors. It would be useful if such compounds could demonstrate whether inhibition of membrane recruitment alone is sufficient for effective inhibition, or if conformational changes in PKB PH are crucial. Thus, analysis of any crystal structure data for active compounds bound to PKB PH would contribute greatly to our understanding of PKB activity and activation.
Chapter 2. Results and Discussion Part 1

2.1. Project Aims

The aim of this project is to synthesise analogues of PtdIns(3,4,5)P$_3$ 15, and/or analogues of its inositol head group InsP$_4$ 17, with the view to obtaining novel selective inhibitors of the PKB PH domain. Furthermore, it will be desirable to obtain crystal structures of such compounds bound to PKB PH. This would help to ascertain precisely how modification of the inositol ring substituents affects substrate binding and how it may affect PH domain conformation in PKB. Such a study would offer further insight into the extent by which unnatural substrates are tolerated by PKB PH, and thus aid the development of potent and selective inhibitors of the protein.

The crystal structure of InsP$_4$ bound to PKBαPH, obtained by van Aalten and co-workers, was compared with the apo PKBαPH structure.$^{54}$ A complex hydrogen bonding network exists in the binding site of the apo structure, involving several amino acid residues and a number of water molecules. The binding of InsP$_4$ disrupts this arrangement, as the 1-, 3- and 4- phosphate groups interact with several of the proximal basic amino acids, and in doing so form a new hydrogen bonded network (Figure 2.2).

Thus, upon InsP$_4$ binding, Arg-86 moves 2.3 Å towards the 4-phosphate and Lys-14 moves 1.2 Å to interact with both the 3- and 4-position phosphates. Acidic Glu-17 is repelled from the ligand binding site by the negatively charged phosphates. This repulsion of Glu-17 away from the ligand binding pocket could enable the movement of Arg-86 towards the 4-phosphate. Arg-86 is found at the base of the VL3 loop, and its movement upon ligand binding could mediate the observed movement in this loop noted
earlier, contributing to a significant conformational change. Furthermore, Arg-23 is observed to move 6.2 Å inwards to make contact with the 1- and 3-position phosphates. Meanwhile, Arg-25 is also seen to interact significantly with the 3-position phosphate. Additionally, van Aalten and co-workers also obtained a third PKBoPH crystal structure in the study, in which the ligand binding pocket contained an ordered sulfate molecule rather than the expected inositol ligand. In this complex, the water-mediated hydrogen bonding network present in the unbound apo structure was significantly disrupted by the presence of the sulfate. The study also showed that the sulfate molecule was located approximately 2.2 Å from the 4-phosphate of InsP4 in the complex structure, forming similar interactions as InsP4 with Arg-86 and Asn-53. However, the disruption of the hydrogen bonding network was apparently not sufficient to induce the major conformational changes seen between the apo and InsP4 complex structures. For instance, the VL1 and VL3 loops are found in the same positions in the PKBoPH-sulfate structure as in the apo structure. The VL2 loop in the PKBoPH-sulfate complex is disordered and possesses no detectable secondary structure.

Together, such observations suggest that if the movement of particular binding site residues is reduced or altered upon the binding of an analogue of the PtdIns(3,4,5)P3 head group, formation of the activating conformation of PKBoPH may be restricted. A
compound which can achieve this and prevent the membrane translocation of PKB should act as an effective PKB inhibitor. Potentially, suitable modification at either the 1-, 3- or 4-positions of the inositol ring of PtdIns(3,4,5)P_3 could achieve this. Given that the movement of Arg-86 towards the 4-position phosphate may be a major factor in conformational change in VL3, the first objective of this project was to synthesise analogues of the type displayed in Figure 2.3, which are modified at the 4-position of the inositol ring. No 4-position analogues of PtdIns(3,4,5)P_3 have been reported in the literature to date.

![Figure 2.3. Initial target compounds 33-36 as 4-position modified PtdIns(3,4,5)P_3 analogues.](image)

With respect to 4-position modification, the primary aim was to synthesise the dimethylphosphinate 33. The phosphinate moiety would approximate the tetrahedral geometry of the phosphate group, but lack the H-bonding capacity to trigger the full movements of Arg-86 and Lys-14 seen upon InsP_4 binding. An acidic group alternative to a phosphate could also be introduced at the 4-position, such as the methylenecarboxylate 34. This type of acidic group may be necessary to maintain affinity for the basic binding site whilst potentially offering an altered binding mode to the parent phosphate. The introduction of other functionality via the synthesis of carbonates or sulfonates 35 and 36 were also to be synthesised in order to further probe the binding site, including the introduction of aromatic groups at the 4-position. Phe-55 lies in close proximity to the 4-position phosphate of the InsP_4-PKBαPH complex (Figure 2.2), and is associated with an aromatic, hydrophobic pocket in the apo structure. Aromatic 4-position analogues may be able to interact with this hydrophobic region leading to altered conformation while maintaining binding affinity.

In the proposed compounds, the long-chain diester groups of the phosphoglyceryl unit of PtdIns(3,4,5)P_3 are truncated to simple diacyl groups for the rationally designed analogues. The reason for this truncation is to increase the chances of obtaining crystal structures of the active analogues bound to PKB PH. Lengthy diester-chains historically
make co-crystallisation of the inositol with PH domain-containing proteins unlikely, and are not considered crucial for PH domain binding. Once an effective inhibitor is discovered, the phosphate groups could potentially be masked with suitable bioactivatable protecting groups to provide cell-permeable derivatives, enhancing their value as potential modulatory tools for cell biology.

2.2. Retrosynthesis

A number of syntheses of PtdIns(3,4,5)P$_3$ have been reported in the literature. However, none of the approaches described are directly compatible with the synthesis of 4-position analogues. A retrosynthesis of the primary target, the 4-dimethylphosphinate analogue 33, is presented in Scheme 2.1. As outlined, the 1-position phosphoglyceryl group is introduced as the penultimate step prior to global deprotection, and is derived from compound 37. The mild conditions for PMB-deprotection, required for compound 37, are known to be highly compatible with the presence of phosphate functionality. It was envisaged that the 3,5-bisphosphate 37 could be obtained from intermediate (-)-38, which in turn could be derived from the versatile alcohol (-)-39 via a phosphinylation reaction. Clearly, the synthesis of other 4-position-modified PtdIns(3,4,5)P$_3$ analogues could be achieved by the introduction of other relevant groups at the 4-position at this stage. Compound (-)-39 itself was to be synthesised via a selective allylation of the vicinal diol (-)-40. Many of the reported syntheses of phosphorylated inositols involve a resolution step within the synthetic route to obtain the optically active final product. It is this type of approach that was to be adopted in the attempted synthesis of analogue 33. Thus, intermediate (-)-40 was to be obtained from the resolution of racemic triol 42 using the camphor acetal methodology. This serves to protect the 3,4-diol as well as producing enantiopure material. Finally, the racemic triol can be synthesised in 6 steps from myo-inositol 1 using literature procedures.
2.3. Synthesis of the enantiopure 3,4 vicinal diol (-)-40

Following the chemistry developed by Holmes and co-workers, the synthesis of key diastereomeric intermediate (-)-41 began with the formation of the inositol orthoformate 43 from myo-inositol (Scheme 2.2). Indeed, compound 43 has proved an effective starting point for the synthesis of several phosphoinositides and associated analogues. All starting materials are inexpensive and highly conducive to large scale synthesis. Therefore, reacting myo-inositol with 2 equivalents of triethylorthoformate in the presence of 4-toluenesulfonic acid monohydrate gave the desired orthoformate 43 in 71% yield. This process was regularly performed on a 40 g scale.
Scheme 2.2. Synthesis of the camphor acetal (-)-41. Reagents and conditions: i. (EtO)₃CH (3 equiv), TsOH·H₂O (0.3 equiv.), DMF, 100 °C, 71% yield; ii. NaH (1.3 equiv), PMBCl (1.1 equiv), DMF, 0 °C to RT, 85% yield; iii. NaH (2.5 equiv), BnBr (2.5 equiv), DMF, -5 °C to RT, 98% yield; iv. DIBAL-H (2.5 equiv), CH₂Cl₂, 0 °C to RT, 96% yield; v. NaH (1.5 equiv), allyl bromide (1.5 equiv), imidazole (cat.), DMF, -5 °C to RT, 84% yield; vi. HCl, MeOH, reflux, 83% yield; vii. a. (-)-(S)-Camphor dimethyl acetal (3.5 equiv), TsOH·H₂O (0.3 equiv.) CH₂Cl₂, reflux, b. Silica gel column chromatography diastereomeric resolution, 24% yield.

Regioselective protection of triol 43 at an axial hydroxyl upon treatment with 1.3 equivalents of sodium hydride and 1.1 equivalents of 4-(methoxy)benzyl chloride (PMBCl) gave the diol 44 as a racemate (85% yield). Ensuring controlled reaction conditions by adding the optimal amount of sodium hydride portionwise to a cooled solution of compound 43 in N,N-dimethylformamide (DMF) allows the formation of a di-axial sodium chelate complex (Scheme 2.3). Here, the sodium ion ionically bonded to an axial alkoxide ion is able to chelate to the other axial hydroxyl group. This stabilises the complex increasing the favourability of its formation over that of the equatorial sodium alkoxide species. Further studies by Billington and co-workers confirmed this experimental outcome, as a loss of regioselectivity is observed when either the counter-ion or solvent are changed. The 1.3 equivalents of sodium hydride ensure complete consumption of the triol 43 with minimal formation of di-protected product. Subsequent reaction of the sodium chelate with PMBCl affords the PMB ether. Naturally, the sodium hydride does not distinguish between the two axial alcohols, i.e. this desymmetrisation procedure is not stereocontrolled and thus the enantiomer of compound 44 is also produced. The formation of di-protected material is also observed, but can be removed by silica gel column chromatography.
Dibenzylation of diol 44 proceeded in excellent yield (98%) to afford the fully protected orthoformate 45. This compound was then treated with 2.5 equivalents of 1 M diisobutylaluminium hydride (DIBAL-H) in hexane to give the nonane 46. The mechanism of this regioselective reduction has been studied with deuterium labelling, and is understood to occur as outlined in Scheme 2.4. The DIBAL-H acts as a Lewis acid by coordinating to the least hindered orthoformate oxygen atom of the 5-position, as the equatorial O-benzyl group hinders approach of the bulky DIBAL-H group towards the 1- and 3-position oxygen atoms. Thus, the formation of intermediate 48 is the more favourable, and spontaneous rearrangement to give an oxacarbenium species occurs. This species is considered to be more stable in a boat conformer to avoid unfavourable 1,3-diaxial interactions, and thus following a ring flip the intermediate 50 accepts a hydrogen from a second equivalent of DIBAL-H to give the desired compound in excellent yield, without the need for chromatographic purification.

Allyl protection of the resulting alcohol by treatment with sodium hydride and allyl bromide gave compound 47. Acid hydrolysis of this compound allowed removal of the PMB group as well as hydrolysis of the acetal to give the racemic triol 42 in good yield.
At this stage, resolution of the racemic mixture was performed by 1,2-diol protection of the 3- and 4-position hydroxyl groups using the chiral auxiliary (1S)-(−)-camphor dimethyl acetal (−)-52. This compound was prepared by stirring (1S)-(−)-camphor (−)-51 with trimethylorthoformate in the presence of Montmorillonite® clay K-10 (Scheme 2.5). The reaction afforded a crude mixture of 75% converted acetal (−)-52 and unreacted starting material, as adjudged by 1H NMR analysis.

Scheme 2.5. Synthesis of (S)-(−)-camphor dimethyl acetal (−)-52. Reagents and conditions: i. (MeO)3CH, K-10 clay, hexane, RT, 75% yield.

This crude mixture was reacted directly with triol 42 in the presence of 4-toluenesulfonic acid monohydrate in dichloromethane under reflux, to give a mixture of the four possible diastereomeric products (Scheme 2.5). Lengthy silica gel column chromatography was required to obtain compound (−)-41, the only isolable diastereomer from the mixture. The other three diastereomers 53, 54 and 55 were not separable from each other using this technique, including the other desired product 53. This being the case, the desired diastereomer (−)-41 was only acquired in 24% yield. The observed specific rotation for camphor derivative (−)-41 ([α]D20 -10.8) compared well with the literature value ([α]D20
It should be noted that the L-enantiomer can be synthesised at this point, simply by using the (+)-R-camphor dimethyl acetal. This may potentially be desirable in order to synthesise the opposite enantiomer of any active analogues, to use as a control in biological testing, or simply to assess the activity of the alternative enantiomer.

2.4 Synthesis of the 3,5-diallyl protected intermediate (-)-39

Following chromatographic resolution, the alcohol (-)-41 was converted to the enantiomeric 3,4-diol (-)-40 in two steps, in a manner similar to that reported by Lim et al. PMB protection of alcohol (-)-41 under modified conditions provided intermediate (-)-56 in good yield. Acidic methanolysis of the camphor acetal, using acetyl chloride in methanol/dichloromethane, afforded the intermediate (-)-40 (82% yield).

\[
\begin{align*}
\text{(-)-41} & \quad \text{NaH, PMBCl, THF/DMF, TBAI, 0 °C to RT, 83% yield;} \\
\text{(-)-56} & \quad \text{AcCl, MeOH/CH}_2\text{Cl}_2, \text{RT, 82% yield;} \\
\text{(-)-40} & \quad \text{Scheme 2.7. Synthesis of enantiomeric diol (-)-40. Reagents and conditions: i. NaH, PMBCl, THF/DMF, TBAI, 0 °C to RT, 83% yield; ii. AcCl, MeOH/CH}_2\text{Cl}_2, \text{RT, 82% yield;}}
\end{align*}
\]

The following crucial step in the synthesis involved selective allyl protection of the 3-position alcohol over the 4-position alcohol (see Scheme 2.9). Selective protection of this nature has been reported on compound (-)-40 within the group, and in the literature, in good yield (72%) involving benzyl as opposed to allyl protection (Scheme 2.8). According to the literature, the ratio of isomers formed in this benzylation was approximately 5:1 in favour of the desired product. The structure of the major isomer was determined by crystal structure analysis. The reaction conditions involved heating under reflux a mixture of the diol, 1.1 equivalents of dibutyltin oxide, 4.8 equivalents of benzyl bromide and 1 equivalent of tetrabutylammonium iodide in acetonitrile. Soxhlet apparatus filled with 3 Å molecular sieves served to remove water generated during the reaction. Allyl protection of diol (-)-40 has not been previously reported.
The analogous reaction using allyl bromide instead of benzyl bromide proved less successful. Attempts to optimise this reaction are summarised in Table 1. Exposing diol (-)-40 to the same conditions as above using allyl bromide (Table 1, Experiment 1) gave only very low conversion of starting material into products after 48 h as indicated by TLC analysis, even following the addition of further equivalents of allyl bromide. This was possibly due to the loss of volatile allyl bromide at the temperature required for efficient reflux of the reaction mixture (~100 °C).

In order to minimise the loss of allyl bromide it was decided that the desired allyl-protection be carried out over two steps. The mechanism for dibutyltin oxide-based protection, previously discussed in the literature, allows this to be performed. The reaction proceeds via the initial formation of a stannylene acetal upon treatment of the diol with dibutyltin oxide in a suitable solvent (Scheme 2.9). The intermediate 58 is believed to form in polar solvents, where co-ordination of a solvent molecule to the tin atom gives a trigonal bipyramidal stannylene complex. In this structure, the oxygen atoms of the stannylene are in apical (the 3-position oxygen as depicted in compound 58), and equatorial positions. The bond between the apical oxygen atom and the tin is longer than the equatorial tin-oxygen bond, and hence more reactive. This results in increased reactivity of this oxygen towards the allyl bromide electrophile, resulting in monosubstitution. Once formed via reflux of the diol with dibutyltin oxide, the stannylene acetal is sufficiently stable to allow the reaction conditions to be altered to favour the following allylation.

The first attempt at the two-step approach towards allyl protection (Table 1, Experiment 2) followed a procedure reported by Gigg, where the dibutyltin oxide and diol mixture was heated under reflux in toluene using Dean Stark apparatus for the azeotropic removal of water. The toluene was then removed in vacuo and replaced with DMF as
solvent. The allyl bromide was then added and the mixture heated to 50 °C. However, consumption of the starting material was still extremely slow. It was clear that an additive was required to promote efficient allylation. A number of analogous and highly selective reactions reported in the literature have utilised caesium fluoride as an additive for tin-mediated allyl protection. Thus, following the initial pre-formation of the stannylene acetal as before, caesium fluoride was added in the allylation step and after 24 h almost full consumption of the starting material was seen to have occurred by TLC analysis (Experiment 3). However, analysis by 1H NMR of the crude product following work-up...
revealed poor selectivity for the allyl protection; the ratio of isomers being 1.6:1 in favour of the desired compound (-)-39 (Figure 2.4). The compound structure was assigned on the basis of characteristic proton NMR shifts of the hydroxyl groups for each of the isomers. An attempt to see if selectivity could be increased by maintaining a low temperature (< 0 °C) throughout the reaction (Table 1, Experiment 4) offered little improvement. Further difficulty in separating the two isomers efficiently by silica gel column chromatography resulted in a maximum isolated yield for the intermediate (-)-39 of 36% following successive separation attempts. The unwanted isomer 59 could not be isolated by column chromatography without contaminants of (-)-39 present.

![Integrals ratio 1.6:1](image)

**Figure 2.4.** Partial $^1$H spectrum (300 MHz, CDCl$_3$) of a crude mixture of compounds (-)-39 and 59 after the allyl protection under the conditions in Table 2.1, Expt. 3.

The large difference in selectivity between allyl and benzyl protection for this step suggests that the outcome of the reaction is at least in part dependent on steric interactions. Benzyl bromide must be of sufficient size to afford significant steric clash with the 5-O-allyl group upon reaction with the stannane acetal. The axial O-benzyl group at the 2-position is believed to lie sufficiently far away from the 3-position oxygen to provide only minimal steric hindrance. π-Stacking interactions of the 2-O-benzyl with benzyl bromide may also contribute towards enhanced selectivity. Allyl bromide is apparently too small to provide significant steric interaction with the 5-O-allyl group.
2.5. Attempted synthesis of bisphosphate 37

Having obtained the di-allyl protected intermediate (-)-39, the introduction of the dialkyl phosphinate group at the 4-position, as indicated in the retrosynthesis, was now required. One method by which this could be achieved was to react the alcohol with the desired dialkyl phosphinic chloride. The dimethyl phosphinic chloride 62, although commercially available in small quantities, can be prepared from inexpensive materials in two steps using literature procedures (Scheme 2.10). The first step involved the formation of tetramethyl biphosphine disulfide 61, a dimeric compound, from the reaction of thiophosphoryl chloride with a suitable Grignard reagent. Although the yield for this step was poor (23%), a sufficient amount of pure material was obtained to produce significant quantities of the desired phosphinic chloride. Thus, dimethyl phosphinic chloride 62 was subsequently formed by reacting the tetramethyl biphosphine disulfide with thionyl chloride. The dimethyl phosphinic chloride was very moisture sensitive, presumably undergoing hydrolysis to give the corresponding free acid. It was therefore prepared fresh for use in any subsequent reaction. A range of dialkyl phosphinic chlorides can be prepared in a similar fashion by varying the Grignard reagent used for the formation of the biphosphine disulfide.

![Scheme 2.10. Preparation of the dimethyl phosphinic chloride reagent 62. Reagents and conditions: i. CH₃MgBr, Et₂O, 0 °C - 8 °C, reflux, 1 h, 23% yield; ii. SOCl₂, toluene, -5 °C to RT, 30 min, reflux, 1 h, 57% yield.]

Phosphinylation of alcohol (-)-39 was performed under similar conditions employed for this type of reaction in the literature. Therefore, intermediate (-)-38 was obtained by the addition of dimethyl phosphinic chloride in DMF with 2,6-lutidine as a base, in high yield (89%, Scheme 2.11). Allyl deprotection at the 3- and 5-positions was then required. Conditions reported by Chen et al. resulted in allyl deprotection in high yields for several inositol-based compounds bearing a PMB-ether as one of the substituents. According to this procedure, the palladium catalyst would effect the isomerisation of the allyl groups, followed by acidic cleavage of the resultant vinyl ether. However, employing
such conditions for the allyl deprotection of (-)-38 by heating a mixture of (-)-39, palladium on carbon and 4-toluenesulfonic acid in methanol/water under reflux afforded diol 63 in a modest yield of 31%. PMB deprotection was also observed under such conditions, owing to the low yield. A standard de-allylation procedure using Wilkinson’s catalyst for the allyl isomerisation under reflux, followed by acidic cleavage of the vinyl ether at room temperature also resulted in a poor yield (30%) and less pure product. Despite this, sufficient material was obtained in order to attempt the next step, directly following the de-allylation.

**Scheme 2.11.** Attempted synthesis of intermediate 37. **Reagents and conditions:** i. a. $^1$Bu$_2$SnO, toluene, reflux; b. AllBr, CsF, N,N-dimethylformamide; 36% yield; ii. Dimethylphosphinic chloride, 2,6-lutidine, N,N-dimethylformamide, 89% yield; iii. Pd/C, 4-TsOH·H$_2$O, MeOH/H$_2$O, reflux, 31% yield; iv. a. bis(benzyloxy)-N,N-diisopropylamino phosphine, 1H-tetrazole; b. mCPBA, inseparable mixture of products.

Phosphitylation at the 3- and 5-positions of the inositol ring was attempted. Standard conditions for phosphitylation were employed. For the synthesis of benzyl protected phosphates, such conditions require the synthesis of the phosphoramidite reagent bis(benzyloxy)-N,N-diisopropylamino phosphine 65, which was derived from phosphorus trichloride in two steps (Scheme 2.12).$^{150}$ Addition of the diol 63 to a mixture of excess phosphoramidite 65 and 1H-tetrazole in dichloromethane followed by oxidation by mCPBA resulted in full consumption of the starting material. However, the desired di-phosphorylated product 37 was not obtained. A mixture of two mono-phosphorylated products, inseparable by silica gel column chromatography, were identified by $^1$H and $^{31}$P NMR. Mass spectrometric data of the product mixture revealed only one $M^+$ ion, suggesting that the products were regioisomers. There are two possible scenarios that
could have led to this isomeric mixture. Firstly, the two compounds could have arisen from two separate phosphitylation events at the 3- and 5-positions respectively, with a second phosphitylation of both resultant intermediates proving unfavourable in the chosen reaction conditions. A second possibility is that the phosphinate group migrated following phosphitylation of one of the alcohols, again resulting in an isomeric mixture. Incompatibility of the phosphinate moiety on the inositol ring with standard phosphitylation conditions has proved not to be an isolated example, as this has since been observed within the research group on a different substrate.

Scheme 2.12. Synthesis of bis(benzyloxy)-N,N-diisopropylamino phosphine 65. Reagents and conditions: i. a. Pyridine, diethyl ether, benzyl alcohol, -78 °C to RT; b. Diisopropylamine, diethyl ether, -5 °C to RT, 62% yield; ii. 1-H tetrazole (0.43 M in CH₃CN), benzyl alcohol, CH₂Cl₂, 71% yield.

2.6. Summary

Analysis of the crystal structure of InsP₄ bound to PKBαPH₅₄ and comparison with the apo PKBαPH structure, has enabled the rational design of novel PtdIns(3,4,5)P₃ analogues. Such compounds are altered at the 4-position to prevent the formation of an activating conformational change in the PH domain of PKB upon ligand binding, thus potentially acting as selective inhibitors of this oncogenic protein. A synthetic strategy towards the primary target, the dimethylphosphinate 33, was devised. Although advanced intermediate 63, bearing the required phosphinate at the 4-position, was successfully synthesised enantiomerically pure in 12 steps, phosphitylation of this compound failed. It would appear that the dimethylphosphinate group is susceptible to migration under the conditions for phosphitylation. In order to circumvent this problem, an alternative synthetic approach was to be devised.
Chapter 3. Results and Discussion Part 2

3.1. Second synthetic approach

The initial synthetic route towards the 4-dimethylphosphinate PtdIns(3,4,5)\(P_3\) analogue 33 presented a series of problems which undermined the viability of this route (see chapter 2). It seemed necessary to introduce the phosphate groups prior to the dimethylphosphinate to avoid migration of the phosphinate moiety. Thus, in order to introduce the dimethylphosphinate group at a late stage in the synthesis, a route was conceived whereby the 4-position hydroxyl is functionalised as the penultimate step, prior to global debenzylation (Scheme 3.1). Although lengthier than the initial strategy, this synthetic approach would facilitate the synthesis of a greater number of analogues. Potentially, key intermediate 66 could be reacted with a range of electrophiles, enabling the introduction of the type of functionality desired for probing the PKB PH domain, followed by the final deprotection step. The phosphoglyceryl unit was to be introduced at the 1-position of alcohol 67, synthesised from the 3,5-diol 68. Given the poor outcome of the tin-mediated allyl protection of the 3-position of compound (-)-40 discussed in chapter 2, an alternative protecting group would be employed for the selective protection of intermediate 70. This compound could again be derived from the racemic triol 42.

Scheme 3.1. Retrosynthesis for the racemic PtdIns(3,4,5)\(P_3\) analogue 33.
Given the low yield of the resolution step, the synthetic complexity of the target molecule and the potential requirement for extensive optimisation of reaction conditions, it was decided that the first series of the 4-position analogues using this route were to be synthesised as a mixture of diastereomers, derived from a racemic synthesis. With respect to PKB PH domain inhibition, the protein would “select” the active enantiomer from a racemic mixture, presumably the D-enantiomer, given that the L-isomer of PtdIns(3,4,5)P₃ does not bind to the PKB PH domain. Crystal structure analysis of active compounds bound to PKB PH would help confirm which enantiomer of each analogue is active. The synthetic approach outlined was devised to allow compatibility with the camphor acetal resolution, permitting the subsequent synthesis of enantiopure analogues as required. The intention was, therefore, to maximise the output of analogues for biological testing at as early a stage as possible. Information gained from the testing of the racemic compounds would direct the synthesis of the enantiomeric analogues.

3.2. Synthesis of 2,6-bis-O-benzyl-4-O-(4’-methoxybenzyl)-3,5-bis(dibenzylphosphate)-myo-inositol 67

The primary objective for this route was to synthesise key intermediate 67 (Scheme 3.2). This compound represents a versatile intermediate, at which point the phosphodiester is introduced at the 1-position. The length of the diester group can be varied at this stage, with the diacetyl glyceryl moiety being employed in the first instance, as indicated. For this racemic synthesis, the 3,4-vicinal diol was protected as the acetonide, instead of the camphor acetal, in good yield to afford compound 71 (Scheme 3.2). The 1-position hydroxyl group was then protected as the triisopropylsilyl (TIPS) ether, followed by acidic methanolysis of the acetonide, both proceeding in excellent yield, to provide the 3,4-diol 70.

Tin-mediated allyl protection of the 3-position hydroxyl of diol 70, using the optimal conditions described in the previous chapter, was attempted once. However, the outcome was again poor, providing the desired product in only 21% yield. Thus attention was turned to formation of the benzoate ester 73. This was attempted by adding benzoyl
chloride to a cooled mixture of the pre-formed stannylene acetal derived from diol 70 in toluene. After reaching room temperature, the reaction was complete within 3 h. This reaction proved to be highly selective for the 3-position, as only the desired 3-O-benzoyl protected compound 73 was isolated upon purification of the crude mixture, in an optimum yield of 73%. Protection at the 4-position was not observed. Regioselectivity was confirmed by single X-ray crystal structure analysis of the product (Figure 3.1).

Scheme 3.2. Racemic synthesis of key intermediate 67. Reagents and conditions: i. 2,2-Dimethoxypropane, 4-TsOH·H$_2$O, DMF, 89% yield; ii. TIPS triflate, 2,6-lutidine, CH$_2$Cl$_2$, 94% yield; iii. 4-TsOH·H$_2$O, MeOH, 95% yield; iv. a. Bu$_2$SnO, toluene, reflux, b. Benzoyl chloride, 0 °C → RT, 73% yield; v. PMB-trichloroacetimidate 78, Y(OTf)$_3$ (1.2 mol%), PhCH$_3$, -78 → -10 °C, 80% yield (impure); vi. NaOH, MeOH/Et$_2$O, 85% yield, vii. a. Wilkinson’s Catalyst, Hünig’s base, EtOH, reflux; b. 4-TsOH·H$_2$O, MeOH, 72% yield; viii. a. bis(benzyloxy)-N,N-diisopropylamino phosphine, 1H-tetrazole, CH$_2$Cl$_2$, b. mCPBA -78 °C → RT; ix. TBAF, THF, 76% yield over 2 steps.

High selectivity for this type of benzylation process is well documented.$^{142}$ The observation that the 4-benzoate ester is not formed in this instance may be due to an equilibrium in which intramolecular transesterification occurs (Scheme 3.3). In non-polar solvents, the stannylene exists as a dimer which reacts with the benzoyl chloride. The resulting esterified compound can undergo transesterification via formation of a cyclic tetrahedral intermediate.$^{152}$ The equilibrium promotes accumulation of the less hindered 4-substituted intermediate in the reaction mixture, prior to work-up.

Scheme 3.3. Proposed transesterification process in the tin-mediated benzylation of diol 70.
With only the 4-position hydroxyl group exposed, 4-methoxybenzyl (PMB) protection of alcohol 73 was then attempted. The use of sodium hydride and PMB chloride for this process failed, as benzoyl migration and partial deprotection could not be prevented under these conditions. Therefore, the employment of the PMB-trichloroacetimidate (PMB-TCA) chemistry was required.\textsuperscript{153} The PMB-TCA 78 was initially synthesised from PMB alcohol and trichloroacetonitrile using phase transfer catalysis in aqueous KOH and dichloromethane (Scheme 3.4).\textsuperscript{154} However, following purification by alumina gel column chromatography, the trichloroacetimidate 78 was isolated in only 22\% yield. Alternative reaction conditions involved stirring the PMB alcohol with a catalytic amount (0.15 equiv.) of sodium hydride in diethyl ether for 45 min.\textsuperscript{155} Subsequent addition of trichloroacetonitrile resulted in full consumption of the PMB alcohol. Work up involved solvent exchange for petroleum ether. Addition of a few drops of methanol resulted in the precipitate of unwanted by-products which could be removed by filtration through Celite. Facile purification of the crude filtrate by alumina gel column chromatography provided acetimidate 78 in 90\% yield.

Scheme 3.4. Synthesis of 4-methoxybenzyl trichloroacetimidate (PMB-TCA) 78. Reagents and conditions: i. \textsuperscript{5}Bu\textsubscript{4}NHSO\textsubscript{4}, 50\% aqueous KOH, Cl\textsubscript{3}CCN, CH\textsubscript{2}Cl\textsubscript{2}, 22\% yield; ii. NaH, 0 °C → RT, Cl\textsubscript{3}CCN, 0 °C → RT, Et\textsubscript{2}O, 90\% yield.
The protection of alcohol 73 using PMB-TCA required significant optimisation, the results of which are summarised in Table 3.1. Many acid catalysts have been employed for such transformations in the literature, although the commonly used camphor sulfonic acid,156 triflic acid157 and boron trifluoride diethyl etherate155 (Experiments 1-3) all failed to promote the reaction. However, some transition metal triflates have been identified in the literature as highly activating catalysts for this type of process, with complete PMB protection observed in only 5 minutes in certain instances.158 Indeed, complete consumption of starting material 73 was observed after 2 h of stirring the alcohol with PMB-TCA and 5 mol% Sc(OTf)$_3$ at -40 °C (Experiment 4). However, inositol 74 was obtained in a modest 43% yield. The use of 5 mol% Y(OTf)$_3$ with the reaction proceeding at -10 °C gave a similar yield (Experiment 5). For such reactions, formation of an isomeric by-product was identified from a complex product mixture. It is believed that this material was derived from the migration of the benzoyl group to the 4-position, prior to reaction with the PMB-TCA. Performing the reaction on a large scale facilitated
the lowering of the catalyst loading to 2 or even 1.2 mol%, which afforded the desired compound in an optimal yield of 80%. One remaining problem with the reaction was that it did not prove possible to completely remove a PMB-related impurity from the material by silica gel column chromatography, although the impurity was a very minor component of the product as adjudged by $^1$H NMR analysis. Thus, the yields quoted in Table 3.1 are approximate. In any case, the impurity could be easily separated from the product of the subsequent reaction.

Following PMB-protection of the 4-position alcohol, the benzoyl group was removed, using 2 M NaOH in methanol/ether (3:1), in 85% yield providing alcohol 75. Subsequent allyl deprotection was performed in reasonable yield. This deprotection was achieved by refluxing the inositol 75, Wilkinson’s catalyst [(Ph$_3$P)$_3$RhCl] and Hünig’s base in ethanol for 2.5 h to perform isomerisation of the allyl group, followed by cleavage of the resulting vinyl ether with 4-toluenesulfonic acid in methanol. The 3,5-diol 68 thus formed was subjected to the standard conditions for phosphitylation. Treatment with excess bis(benzyloxy)-N,N-diisopropylamino phosphine and 1-$H$ tetrazole, followed by oxidation of the resulting phosphite groups by mCPBA, afforded the benzyl-protected 3,5-bisphosphate 76 in high yield. This material was again difficult to purify. For this type of reaction on an inositol substrate, the desired compound, bearing the polar phosphate groups, is typically easy to separate from the less polar phosphoramidite by-products by silica gel chromatography. However, the triisopropylsilyl ether group at the 1-positon of compound 76 renders this compound relatively non-polar. This made chromatographic separation of 76 from such by-products difficult by column chromatography, although a number of solvent elution systems were attempted. Once the TIPS group was removed in the following step, using TBAF in THF, the alcohol 67 could then be separated from any remaining phosphoramidite impurities by silica gel chromatography. Thus, overall, pure intermediate 67 was obtained from compound 68 in 76% yield over two steps. Several grams of this versatile intermediate could be obtained using the route described.
3.3. Synthesis of the key 4-hydroxy intermediate 66

The next key step in the synthesis involved the introduction of the phosphodiester group at the 1-position of alcohol 67. This reaction required the synthesis of a phosphoramidite bearing the diacyl glyceryl moiety, achieved in five steps from commercially available (S)-(+-)2,2-dimethyl-1,3-dioxolane-4-methanol (+-)79 (Scheme 3.5). Thus, benzyl protection of compound (+-)79159 followed by acidic methanolysis afforded diol (-)-81 in high yield.159 Acylation of the 1- and 2-positions gave (+)-3-O-benzyl-1,2-diacetylglycerol (+)-82.160 The observed specific rotation for intermediate (+)-82 ([α]20 = +16.4) compared favourably with the literature value ([α]20 = +16.2).160 However, hydrogenolysis of the benzyl group of this glycerol did not prove straightforward. Following the conditions previously reported in the literature,161 which involved stirring compound (+)-82 with palladium on carbon in methanol under hydrogen for 12 hours, afforded a mixture of two products as adjudged by 1H NMR analysis. These compounds had identical TLC Rf values and were inseparable by silica gel column chromatography. Analysis of the 1H NMR indicated that partial migration of an acetyl group had occurred following debenzylation, to give a mixture of meso 1,3-diacetyl glycerol as well as the desired product (-)-83. Attempts to separate these compounds by silica gel chromatography appeared to result in an increase in the ratio of migrated compound. Repeating the reaction, ensuring minimum reaction time for full consumption of starting material, gave the same result.

It was evident that the reaction conditions had to be altered to ensure that clean product was obtained. Using THF as the solvent instead of methanol, the migration process was avoided, giving the alcohol (-)-83 in 96% yield without the need for chromatographic purification. However, reaction times under these conditions were significantly extended, with 5-7 days typically required for full consumption of starting material. Replacing palladium on carbon with palladium hydroxide (Pearlman’s catalyst), resulted in a more efficient hydrogenolysis process. Under such conditions, the reaction was complete in 22 h (91% yield). It should be noted that the observed specific rotation for product (-)-83 for the two debenzylation conditions were in good agreement ([α]30 = -3.40, c 0.6 and
$\left[\alpha\right]_D^{20} = -4.10, \, c \, 1.0 \text{ in CHCl}_3 \text{ where Pd/C and Pd(OH)$_2$ are used respectively). These values differ from the only value reported in the literature ($\left[\alpha\right]_D^{20} = +8.3, \, c \, 0.4 \text{ in CHCl}_3$),\textsuperscript{161} derived from the report describing the method where the debenzylation was performed in methanol. As noted, however, the use of this procedure did not appear conducive to providing pure material, due to acetyl migration.

Finally, intermediate (-)-83 was successfully reacted with benzyloxy bis(N,N-diisopropylamino)phosphine with 1-$H$ tetrazole in dichloromethane to give the desired phosphoramidite 84. It should be noted that compound 84 was unstable and, therefore, this compound had to be synthesised shortly before the time of use.

\[ \text{Scheme 3.5. Synthesis of phosphoramidite 84. Reagents and conditions: i. NaH, BnBr, DMF, 0 °C → RT 87% yield;} \]
\[ \text{ii. Conc. HCl, MeOH, reflux, 91% yield;} \]
\[ \text{iii. Acetic anhydride, DMAP, triethylamine, THF, 91% yield;} \]
\[ \text{iv. Pd/C, H}_2, \text{ THF, 5-7 days, 96% yield;} \]
\[ \text{v. Pd(OH)$_2$, H}_2, \text{ THF, 22 h, 91% yield;} \]
\[ \text{vi. Benzyloxy bis(N,N-diisopropylamino)phosphine, 1-$H$-tetrazole, CH}_2\text{Cl}_2, 76% yield.} \]

The reaction of phosphoramidite 84 and 1-$H$ tetrazole with the 1-hydroxyl 67 in dichloromethane proceeded smoothly, giving compound 85 in 84% yield following mCPBA oxidation and subsequent purification (Scheme 3.6). One problem with the product of this reaction is that as the inositol starting material was racemic, a mixture of four diastereomers resulted, due to the chiral nature of the 1-position phosphate being introduced. The consequence of this, coupled with the complexity of the molecule bearing seven benzyl groups, is that the $^1H$, $^{13}C$ and $^{31}P$ NMR spectra are convoluted (see Figure 3.2). This remains the case for any subsequent intermediates, until the benzyl group of the 1-position phosphodiester is removed in the final deprotection step to give a racemic final product.
Scheme 3.6. Synthesis of key 4-OH intermediate 66. Reagents and conditions: i. a. Phosphoramidite 84, 1H-tetrazole, CH₂Cl₂, b. mCPBA -78 °C → RT, 84% yield; ii. CAN, MeCN/H₂O (4:1), 84% yield.

The synthesis of key intermediate 66 was completed by the oxidative cleavage of the PMB protecting group using ceric ammonium nitrate (CAN) in acetonitrile/water (84% yield). From alcohol 66, the 4-dimethylphosphinate 33 and several other 4-position analogues could be synthesised in two steps.

Figure 3.2. The partial ^1H NMR spectrum (300 MHz, CDCl₃) of compound 85. The complexity of the spectrum is partly due to the introduction of the chiral phosphate at the 1-position, producing 4 diastereomers, with the stereocentre of the glyceryl moiety fixed. The appearance of the two acetyl CH₃ groups as a complex multiplet demonstrates this.

3.4. The synthesis of 4-position-modified PtdIns(3,4,5)P₃ analogue precursors

3.4.1 Synthesis of the 4-dimethylphosphinate precursor

In order to complete the synthesis of the 4-phosphinate target compound, phosphinylation of alcohol 66 was now required, followed by global deprotection. Several conditions for this reaction were tested before an efficient approach was found (Table 3.2). Compound 66 proved unreactive under the conditions previously employed.
for the phosphinylation of inositol intermediate (-)-39 described in chapter 2. Thus, the reaction of alcohol 66 with dimethylphosphinic chloride and 2,6-lutidine in DMF provided the desired product in only 5% yield after 3 days at room temperature (Table 3.2, Experiment 1), with recovered starting material as the major by-product. Changing the solvent to acetonitrile resulted in gradual decomposition of the starting material (Experiment 2), with several products forming as adjudged by TLC analysis. Returning to DMF as solvent (Experiment 3), but trying a different base while heating the reaction mixture, did not promote the reaction to any noticeable extent.

It was evident that an alternative phosphinylation reagent was required. The 4-position hydroxyl of alcohol 66 was clearly somewhat hindered by the 3- and 5-position bisbenzyl-protected phosphates. It was believed that the use of a smaller, more reactive electrophile might improve the outcome of the reaction. Dimethylchlorophosphine was therefore employed, using triethylamine as base in dichloromethane (Experiment 4). However, the reaction again proved sluggish. A thick precipitate quickly formed during the reaction and additional equivalents of reagents were required throughout in order to

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Reagents</th>
<th>Solvent</th>
<th>Time, Temperature</th>
<th>Yield of 86</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Excess Me₂P(O)Cl and 2,6-lutidine</td>
<td>DMF</td>
<td>3 days, RT</td>
<td>5%</td>
</tr>
<tr>
<td>2</td>
<td>Excess Me₂P(O)Cl and 2,6-lutidine</td>
<td>CH₃CN</td>
<td>18 h, RT</td>
<td>Decomposition of starting material</td>
</tr>
<tr>
<td>3</td>
<td>Excess Me₂P(O)Cl and Hünig’s base</td>
<td>DMF</td>
<td>48 h, 50 °C</td>
<td>No significant reaction</td>
</tr>
<tr>
<td>4</td>
<td>i. Excess Me₂PCl and triethylamine ii. mCPBA</td>
<td>CH₂Cl₂</td>
<td>48 h, RT</td>
<td>9%</td>
</tr>
<tr>
<td>5</td>
<td>Excess Me₂PCl</td>
<td>Pyridine/CH₂Cl₂ (5:1)</td>
<td>2.5 h, RT</td>
<td>60%</td>
</tr>
</tbody>
</table>

Table 3.2. Experimental conditions for the phosphinylation of compound 66. RT = room temperature.
force full consumption of the starting material after 48 h. mCPBA was then added to ensure formation of the phosphinate. Following chromatographic purification, compound 86 was isolated in a disappointing 9% yield. Although the starting material was consumed, prolonged reaction times under the conditions employed appeared to result in decomposition, probably of both starting material and product. A far more favourable outcome was achieved by reacting the alcohol with dimethylchlorophosphine in pyridine/CH$_2$Cl$_2$ (5:1) (Experiment 5). The reaction completed in 2.5 h, and oxidation of the resulting phosphine to the phosphinate occurred spontaneously during work-up of the reaction, providing compound 86 in 60% yield.

3.4.2 Synthesis of 4-sulfonate precursors

While optimisation of the phosphinylation was being performed, the synthesis of other analogue precursors was attempted. The introduction of sulfonates to the 4-position via an appropriate sulfonyl chloride offered an attractive means by which to probe the PKB PH binding site with the derived analogues.

![Scheme 3.7. Synthesis of sulfonates 87 and 88. Reagents and conditions: i. CH$_3$SO$_2$Cl, triethylamine, DMAP, CH$_2$Cl$_2$, 55% yield of 87; ii. PhSO$_2$Cl, triethylamine, DMAP, THF, 40% yield of 88.](image)

Reaction of alcohol 66 with methanesulfonyl chloride, triethylamine and dimethylaminopyridine (DMAP) in dichloromethane gave the 4-mesylate 87 in 55% yield (Scheme 3.7). The introduction of aromatic functionality at the 4-position was sought through the synthesis of the 4-phenylsulfonate 88. This was achieved by using the electrophile benzenesulfonyl chloride effectively as a co-solvent, as otherwise the reaction would not proceed at an appreciable rate. Again, this is most likely due to steric hindrance from the the 3- and 5-position phosphates. Reaction under these conditions with the addition of triethylamine and DMAP afforded the 4-sulfonate 88 in 40% yield.
However, this compound was again difficult to fully purify, and was found to be relatively unstable, decomposing over time. Therefore, an alternative approach for the introduction of aromatic groups at the 4-position was required.

### 3.4.3 Synthesis of the 4-phenylcarbonate and 4-O-phenoxyacetyl precursors

In order to probe the aromatic hydrophobic pocket proximal to the PKB PH ligand binding site, the synthesis of 4-position-modified PtdIns(3,4,5)P$_3$ analogues with an aromatic group of variable distance from the inositol ring was attempted (Scheme 3.8). Thus, the 4-O-phenoxyacetyl intermediate 89 was synthesised by reaction of compound 66 with excess phenoxyacetyl chloride, triethylamine and DMAP in THF in 67% yield. Rapid silica gel chromatographic purification was required following completion of the reaction to prevent degradation of the product and to afford the optimum yield. The analogous reaction using phenylchloroformate as the electrophile, to form the carbonate 90, was inefficient, resulting in isolation of impure product 90 in only 11% yield. Alternative conditions, where alcohol 66 is stirred with phenylchloroformate and DMAP in pyridine/CH$_2$Cl$_2$ (5:1), provided the desired 4-phenylcarbonate in 60% yield.

The introduction of a phenolic group at the 4-position of a PtdIns(3,4,5)P$_3$ analogue was attractive as it offered the potential to interact with Phe-55 of the PH domain, as well as possessing hydrogen bonding capacity for binding to the basic amino acid residues in the binding site around where the 4-position of the ligand is located. Therefore, as a precursor to such an analogue, the synthesis of the benzyl-protected phenolic ester 91 was attempted. Alcohol 66 was thus stirred with (4-benzyloxy)benzoyl chloride, triethylamine and DMAP in THF. However, conversion of the starting material to product was slow, and after two days degradation of the inositol material was seen to occur by
TLC analysis. Consequently, compound 91 was only formed in approximately 20% yield, and could not be isolated sufficiently pure by chromatography. Performing the reaction in pyridine resulted in negligible conversion of starting material.

3.4.4 Attempted synthesis of acidic 4-position derivatives

![Chemical structures](image)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Target compound</th>
<th>Electrophile</th>
<th>Conditions</th>
<th>Reaction outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>92</td>
<td>BrCH₂CO₂Bn</td>
<td>NaH, THF, -5 °C to RT</td>
<td>Inseparable mixture of products</td>
</tr>
<tr>
<td>2</td>
<td>92</td>
<td>TfOCH₂CO₂Bn</td>
<td>NaH, THF, -5 °C to RT</td>
<td>Inseparable mixture of products</td>
</tr>
<tr>
<td>3</td>
<td>93</td>
<td>ClC(O)CH₂CO₂Bn</td>
<td>DMF</td>
<td>29% yield of 93 (impure)</td>
</tr>
<tr>
<td>4</td>
<td>94</td>
<td>1H-tetrazole-5-acetic acid</td>
<td>DCC, DMAP, DMF, RT</td>
<td>Formation of 94 not observed</td>
</tr>
<tr>
<td>5</td>
<td>94</td>
<td>1H-tetrazole-5-acetic acid</td>
<td>Hünig's base, HOBt, EDCI, DMF, RT to 50 °C</td>
<td>Formation of 94 not observed</td>
</tr>
</tbody>
</table>

Table 3.3. Conditions used for the attempted synthesis of 4-O-acetylbenzoate 92, 4-malonylbenzoate 93 and 4-O-acetyl(1'H-tetrazole) 94.

Given the highly basic nature of the PKB PH ligand binding site, it was considered that replacing the 4-phosphate with an alternative acidic group may be necessary to maintain reasonable affinity. Thus, synthesis of the 4-O-acetylbenzoate 92 was attempted under various conditions (Table 3.3). Addition of sodium hydride to a mixture of alcohol 66 and excess benzyl 2-bromoacetate in THF resulted in slow consumption of starting material, yielding a complex mixture of products, inseparable by silica gel column chromatography. It is believed that the reaction conditions may have resulted in the migration of the 3- and/or 5-position phosphates to the 4-position, prior to the reaction of alkoxide with benzyl 2-bromoacetate. Attempts to limit phosphate migration by maintaining the reaction temperature below 0 °C merely prolonged reaction times.
further, resulting in gradual decomposition of starting material and product. It was reasoned that if the electrophile was more reactive, reaction with the 4-position alkoxide may occur prior to phosphate migration. However, using the triflate of benzyl glycolate as the reagent, instead of the bromide, again resulted in formation of a complex mixture of products (Experiment 2)

The introduction of a malonate ester at the 4-position appeared more promising. Alcohol 66 could be reacted with mono-benzyl malonyl chloride to give analogue precursor 93 (Experiment 3). However, again the reaction was sluggish, and addition of excess reagents was once more required to drive consumption of the starting material. Purification of the desired compound 93 was difficult, and the desired product was only obtained in 29% yield as an impure gum.

A bid was also made to synthesise analogue precursor 94 bearing a tetrazole group, an isostere of a carboxylic acid, at the 4-position (Experiments 4 and 5). Coupling of alcohol 66 with 1H-tetrazole-5-acetic acid was attempted using 1,3-dicyclocarbodiimide (DCC) and DMAP in DMF. A second attempt involved heating to 50 °C a mixture of the inositol 66, Hunig’s base, 1-hydroxy-benzotriazole, EDCI and 1H-tetrazole-5-acetic acid in DMF. Both reactions were monitored by TLC and LC-MS, but formation of the desired product was not observed in either case, with recovered starting material the major product.

3.5. Global deprotection of PtdIns(3,4,5)P₃ analogue precursors

The final step in the synthesis of the PtdIns(3,4,5)P₃ analogues required global debenzylation under hydrogenolysis conditions. This reaction was performed on those precursor compounds that had been successfully synthesised, purified and that were sufficiently stable. The global debenzylation of inositol polyphosphates, including PtdIns(3,4,5)P₃, has strong literature precedent.\textsuperscript{134,162} Several varying conditions have been employed successfully. Previously within the group, a range of bisphosphate InsP₃ analogues were synthesised by hydrogenolysis using palladium black (20 equivalents) in t-butanol/water (6:1) in the presence of sodium bicarbonate to generate bisphosphate sodium salts 95-98 (Scheme 3.9).\textsuperscript{136,163} Migration of one of the phosphate groups is
always a possibility during such reactions, where a free alcohol can potentially attack a phosphate in an intra- or intermolecular migration process. However, migration was only observed in attempts to synthesise compound 99. All other reactions gave clean products after 5-8 h.

Scheme 3.9. The reported synthesis of InSP$_3$ analogues following global debenzylation.$^{15,16}$ Reagents and conditions: i. Pd black (20 equiv.), NaHCO$_3$ (4 equiv.), t-BuOH/H$_2$O (6:1), H$_2$. 5-8 h. Yields shown in parenthesis.

These conditions were therefore the starting point for the synthesis of the PtdIns(3,4,5)P$_3$ analogues. In an initial attempt, the 4-dimethlyphosphinate 86 was subjected to the same conditions (Scheme 3.10). After 4 h reaction time, debenzylation was incomplete as adjudged by $^1$H NMR analysis, with no migration observed, and the material was returned to reaction conditions for a further 3 h. Although debenzylation was complete by this time, some migration is believed to have occurred, as indicated by analysis of the $^{31}$P NMR. The reaction was repeated, but with the addition of further Pd black after 3 h in an effort to reduce reaction time and aid completion of the debenzylation. The reaction was allowed to proceed for an additional 3 h, after which time full deprotection was complete. However, $^1$H and $^{31}$P NMR analysis revealed small amounts of an impurity, ~ 3% (Figure 3.4); probably a compound which is produced as a result of migration of one of the phosphate groups. This result raised serious concerns over the tendency of analogue 33 to undergo migration in aqueous conditions. Such a process could disrupt any biological testing that would be carried out in an aqueous environment.
Scheme 3.10. Attempted global debenzylation of compounds 86, 87, 89, and 90. Reagents and conditions: i. Pd black (20 equiv.), NaHCO$_3$ (7 equiv.), tBuOH/H$_2$O (6:1), H$_2$, 3-7 h; ii. Pd(OH)$_2$ (20% w/w), NaHCO$_3$ (7 equiv.), tBuOH/H$_2$O (6:1), H$_2$, 2-3 h. Yields were not determined for compounds 33, 101 and 102 due to the presence of impurities.

Figure 3.4. Partial $^{31}$P NMR spectrum (300 MHz, D$_2$O) for compound 33, following debenzylation of inositol 86. The NMR indicates migration of the 1-position phosphate.

In an effort to reduce reaction times, Pearlman’s catalyst was used as an alternative to Pd black for several substrates. However, although debenzylation was quicker, the conditions appeared to promote phosphate migration further, providing complicated $^{31}$P
NMR spectra. Returning to the use of Pd black, global deprotection of the 4-mesylate intermediate to give compound 100 completed in a much reduced reaction time compared with the deprotection of the phosphinate 66 (3 h, 80% yield). This compound was, therefore, initially isolated without any evidence of migration. However, following several weeks of cold storage, analysis by $^1$H and $^{31}$P NMR did reveal that migration occurred over time to a degree, even with isolated material. Judging from the $^{31}$P NMR of analogue 33, it appeared that it was the 1-position phosphate which underwent migration in the debenzylation of compound 86. This contrast with the lack of migration observed with similar compounds in Scheme 3.9 can be rationalised. It is believed that the formation of the disodium salt following phosphate debenzylation may stabilise a phosphate group against migration. Furthermore, it is understood that phosphate esters are more susceptible to migration than both the acid and the sodium salt. This would explain why migration is observed only in the formation of compound 99 in the Ins$P_3$ analogue syntheses (Scheme 3.9), where the dimethylphosphate group at the 4-position is thought to have migrated. Since the 1-position phosphate of the PtdIns(3,4,5)$P_3$ analogues bears an ester unit, it seems logical that this phosphate group is most susceptible to migration. Although phosphatidylinositols have been synthesised under the same conditions previously in the literature, $^{141,164}$ such compounds possess considerably longer diester chains on the 1-position phosphate. Such long chains presumably prevent the phosphate from migrating, due to steric interactions. Although analogues bearing relatively short-chain esters on the glyceryl unit, such as dibutyryl or dioctanoyl groups, have been reported on several occasions, $^{164,165}$ there are few reports of acetyl groups being employed for the phosphodiester chain. Possibly, PtdIns(3,4,5)$P_3$ analogues will require relatively lengthy diester chains to stabilise the compounds against migration.

Problems were also encountered with the final deprotection step in the synthesis of the 4-phenylcarbonate 90. Following initial reaction of 3 hours, $^1$H and $^{31}$P NMR analysis indicated the presence of two compounds; one major and one minor. To ensure completion of the debenzylation process, the material was returned to reaction conditions for a further 1 hour. However, two compounds remained, and it emerged following $^1$H and $^{31}$P NMR analysis that what was the minor component was now the
major one. Comparison of the data revealed that the amount of material bearing the carbonate at the 4-position was diminishing over time under the reaction conditions (see Figure 3.5). It was therefore apparent that the carbonate group was being cleaved under such conditions.

![Diagram](image)

**Figure 3.5.** $^1$H NMR spectra in D$_2$O demonstrating the cleavage of the carbonate group during the debenzylation of 86. Comparison of the integrals for the CH$_2$OAc glyceryl proton (multiplet) and the 4-H proton of the inositol ring (triplet) indicates a reduction in material bearing the carbonate group over time. **A.** $^1$H NMR spectrum after 3 hours reaction; **B.** $^1$H NMR spectrum after 4 hours reaction.

A similar outcome was observed for the deprotection of 1-O-phenoxyacetyl 89. Partial cleavage of the ester group was observed, even when reaction time was minimised. Presumably the sodium bicarbonate used to generate the sodium salts of the phosphates must play a role in this unwanted side-reaction. Potentially, this problem
may be overcome simply by omitting the sodium bicarbonate for the duration of the debenzylation, then adding it to rapidly form the desired salt before work-up. This way, the length of time that the compound is exposed to the bicarbonate is minimised. However, given the apparent tendency of the 1-phosphate of this class of compounds to migrate over time, further efforts to optimise conditions for this step could not be justified.

3.6 Summary

Following the problems encountered in the first synthetic approach towards novel PtdIns(3,4,5)P₃ analogues, a second approach was devised. This involved the introduction of appropriate functionality to the 4-position as the penultimate step in the synthesis. A racemic synthesis of the key 4-hydroxy intermediate 66, bearing protected phosphates at the 3- and 5-positions and a phosphodiester at the 1-position, was achieved in high overall yield. Despite steric hindrance from the neighbouring phosphate groups, the 4-position of alcohol 66 was successfully functionalised to synthesise analogue precursors with either phosphinate, sulfonate, carbonate or ester groups at this position. Several attempts had been made to synthesise further analogue precursors, but the lack of reactivity of alcohol 66 and purification difficulties curtailed the success of such efforts. Unfortunately, final debenzylation of the penultimate compounds was problematic. Prolonged exposure to aqueous conditions facilitated the migration of the 1-position phosphodiester to a neighbouring alcohol of the inositol ring. This was observed to occur during reaction in basic conditions, as with the synthesis of the 4-dimethylphosphinate 33, or with the isolated material over time as with the 4-mesylate 100. Furthermore, the carbonate and ester groups of compounds 89 and 90 were partially cleaved under the deprotection conditions employed.

In order to reduce the chances of migration of the 1-position phosphate, both during the final step, in storage and in conditions for biological testing, it seemed that modification of the target compound was required. Also, alteration of debenzylation conditions
appears necessary in order to isolate carbonate and ester-bearing analogues such as 89 and 90. The introduction of less base-labile groups at the 4-position may well be required if analogues bearing an aromatic group are to be synthesised.
Chapter 4. Results and Discussion Part 3

4.1 The rationale for Ins\textsubscript{P\textsubscript{4}} analogue synthesis

The second route towards the rationally designed PtdIns(3,4,5)\textsubscript{P\textsubscript{3}} analogues (chapter 3) enabled the synthesis of several precursor compounds. However, the fact that the 1-phosphodiester was prone to migration either during the final deprotection, or over time with the stored product, suggested that the design of the target compounds should be modified. One option was to increase the size or length of the diester group to provide the more commonly reported dibutanoyl or dihexanoyl phosphodiester, to give analogues of the type 103 (Figure 4.1). The added steric bulk of the diester should prevent migration. However, one potential problem with this approach is that additional lipophilicity, and the resulting disordered nature, of the phosphodiester is likely to reduce the chances of achieving co-crystallisation of the analogues with PKB PH. This is evidenced by the fact that biologists have failed to obtain crystal structures of short chain PtdIns(3,4,5)\textsubscript{P\textsubscript{3}} compounds bound to PKB PH, and have only achieved co-crystallisation with the inositol head group Ins\textsubscript{P\textsubscript{4}}.\textsuperscript{54} Another option would be to replace the phosphodiester altogether with a simple phosphate, to give trisphosphates of the type 104 (Figure 4.1). If anything, this modification should increase the chances of obtaining ligand-PKB PH crystal structure complexes, as compared to the original design. Given the potential utility of such crystal structures for active compounds, it was decided that this latter approach would be adopted. Such a modification should also significantly reduce the likelihood of phosphate migration, as there would be no phosphate ester in the molecule.

Figure 4.1. Modified target compounds as potential PKB PH inhibitors.
Compounds such as trisphosphate 104 can be seen as 4-position-modified \( \text{InsP}_4 \) analogues. \( \text{InsP}_4 \) itself is synthesised from \( \text{Ins}(1,4,5)\text{P}_3 \) by \( \text{Ins}(1,4,5)\text{P}_3 \)-3-kinase.\textsuperscript{166} Its characteristic short life-time has led to the proposition that this inositol may also act as a second messenger.\textsuperscript{167} As noted earlier, \( \text{InsP}_4 \) binds PKBoPH with similar affinity to PtdIns(3,4,5)\( \text{P}_3 \) \textit{in vitro}.\textsuperscript{34} This is not the case for all the PH domain-containing proteins that bind to PtdIns(3,4,5)\( \text{P}_3 \), where \( \text{InsP}_4 \) affinity is sometimes significantly less than for the phospholipid. Proteins that are selective for \( \text{InsP}_4 \) are rare. Also, the ability of proteins such as PKB to distinguish between \( \text{InsP}_4 \) and PtdIns(3,4,5)\( \text{P}_3 \) \textit{in vivo} remains unclear. Clearly, the crucial difference between these inositols involves the presence of the fatty glyceryl unit at the PtdIns(3,4,5)\( \text{P}_3 \) 1-position phosphate. As it is the phosphate interactions which are seen as most significant for binding, it is possible that the glyceryl unit acts more as a recognition module, the extent to which may vary between the protein substrate. The lipid chains themselves serve only to anchor the molecule in the membrane. Such debate further underlines the complexity of inositol signalling.

It is, therefore, conceivable that omitting the glyceryl unit in the proposed analogues may improve selectivity against some PtdIns(3,4,5)\( \text{P}_3 \)-binding proteins that display reduced affinity for \( \text{InsP}_4 \). A long-term aim for the project would involve the initial discovery of an active \( \text{InsP}_4 \) analogue, followed by the synthesis of a related PtdIns(3,4,5)\( \text{P}_3 \) analogue which bears a long-chain phosphodiester at the 1-position. Comparison of activity could reveal useful information concerning the nature of PtdIns(3,4,5)\( \text{P}_3 \)/\( \text{InsP}_4 \) selectivity for PH domain binding.

4.2. The racemic synthesis of 4-position \( \text{InsP}_4 \) analogues

4.2.1 Synthesis of the 4-dimethylphosphinate \( \text{InsP}_4 \) analogue 108

The synthesis of the racemic bisphosphate 67 was outlined in chapter 3. This material was used to synthesise the key 1-hydroxy intermediate 106 in two steps. Thus, phosphitylation of the 1-position of 67 was achieved by reaction with bis(benzyloxy)-\( N,N \)-diisopropylamino phosphine and 1\( H \)-tetrazole followed by oxidation by \( m \)CPBA, giving
intermediate 105 in 87% yield (Scheme 4.1). Efficient PMB-deprotection of compound 105 using ceric ammonium nitrate afforded the 4-hydroxy-trisphosphate 106.

![Scheme 4.1. Synthesis of key intermediate 106. Reagent and conditions: i. a. bis(Benzyloxy)-N,N-disopropylamino phosphine, 1H-tetrazole, CH2Cl2; b. mCPBA -78 °C → RT, 87% yield; ii. CAN, CH3CN/H2O (4:1), 85% yield.](image)

The synthesis of the first 4-position modified InsP4 analogue was achieved as outlined in Scheme 4.2. The previously optimised conditions for phosphinylation were employed, in which alcohol 106 was reacted with dimethylchlorophosphine in pyridine/dichloromethane to give compound 107 in 62% yield. Global debenzylation was then performed under the same conditions employed previously, using 20 equivalents of Pd black in the presence of sodium bicarbonate (6 equivalents), with t-butanol/water (6:1) as the solvent under an atmosphere of hydrogen. Clean product 108 was obtained in quantitative yield after 5 hours. As anticipated, no phosphate migration was observed by 1H and 31P NMR analysis. However, obtaining useful mass spectrometric data proved difficult. It was found that the addition of diethylamine to a dilute solution of the sample in methanol/water was required to obtain interpretable spectra. This promoted the association of the phosphate groups with H+ as opposed to Na+ to form the phosphate acid. Thus, in ES⁻ mode, the (M-6Na+5H)⁻ ion for the resulting acid of compound 108 was identified, as well as the ions corresponding to the mono- and di-sodiated material (see Figure 4.2). The fully sodiated material, however, was not observed by this method. Also, due to the hygroscopic nature of polyphosphorylated compounds such as analogue 108, as well as the likely preference of mixed sodium salts of the compound, accurate elemental analysis could not be obtained.

![Scheme 4.2. Synthesis of the 4-dimethylphosphinate InsP4 analogue 108. Reagents and conditions: i. Me2PCl, pyridine/CH2Cl2 (5:1), 62% yield; ii. Pd black (20 equiv.), NaHCO3 (6 equiv.), t-BuOH/H2O (6:1), H2, 100% yield.](image)
4.2.2. Synthesis of a 4-sulfate InsP₄ analogue

The introduction of an alternative acidic group to the 4-position, in order to enhance analogue binding affinity, remained an important aim. A sulfate group is frequently incorporated into bioactive molecules, and was deemed an appropriate group to employ for a 4-position-modified InsP₄ analogue. A common method for the formation of a sulfate from an alcohol involves treating the alcohol with an excess of powdered sulfur trioxide–pyridine complex in DMF.¹⁶⁸ For the sulfation of intermediate 106 to complete using this reagent, heating to 50 °C was required (Scheme 4.3). It was generally found that full consumption of the starting material would not occur, for an unknown reason, limiting the eventual yield. Purification of the intermediate sulfate by silica gel chromatography was performed, although obtaining pure product proved challenging, with repeated attempts at purification required. The sodium salt was then formed, by stirring a solution of the inositol in methanol with strongly acidic Dowex Na⁺ form resin. After freeze-drying a solution of the product, compound 109 was obtained in 44% yield as a colourless solid. The compound proved to be very hygroscopic, turning into a gum
after brief exposure to the air. In fact, this intermediate was not found to be very stable as, after several days, degradation of the material was observed by TLC analysis. Therefore, the material had to be prepared immediately prior to use. It should also be noted that it was not possible to prove that the product was indeed the sodium salt of the sulfate, as the hygroscopic nature and instability of the compound precluded the possibility of reliable elemental analysis. The mass spectrometric analysis obtained in ES⁻ mode provided the expected (M-Na)⁻ ion, as opposed to the ion for the sodium salt. Such difficulties in obtaining proof of sodium salt formation are not unexpected for this type of compound.

![Scheme 4.3](image1.png)

Scheme 4.3. Synthesis of 4-sulfate InsP₄ analogue 110. Reagents and conditions: i. a. SO₃·Pyridine complex, DMF, 50 °C; b. Dowex Marathon C resin Na⁺ form, MeOH, 44% yield; ii. Pd black (20 equiv.), NaHCO₃ (6 equiv.), t-BuOH/H₂O (6:1), H₂, 99% yield.

The debenzylation of intermediate 109 appeared to proceed smoothly under the conditions previously employed, using 6 equivalents of sodium bicarbonate, to give the analogue 110 in high yield (Scheme 4.3). This compound was thus submitted for biological testing along with the 4-dimethylphosphinate 108, although it was subsequently discovered that the compound 110 may have contained traces of sodium bicarbonate impurity. Thus, the tetrasodium salt of the 4-sulfate analogue was also synthesised to ensure pure product was obtained. In this instance, 3 equivalents of sodium bicarbonate were used in the debenzylation of compound 109 (Scheme 4.4). Therefore, compound 111 was obtained as a pure analogue in high yield.

![Scheme 4.4](image2.png)

Scheme 4.4. Synthesis of the 4-sulfate InsP₄ analogue 111. i. Pd black (20 equiv.), NaHCO₃ (3 equiv.), t-BuOH/H₂O (6:1), H₂, 97% yield.
The 4-dimethylphosphinate 108 and the 4-sulfate 110 have yet to be tested for their affinity for the PKB PH domain. However, their affinity for GRP1 PH and PLCδ PH has been assessed (Figure 4.3). FRET ratio analysis revealed that the InsP₄ analogues 108 and 109 do not appear to bind to the GRP1 PH domain at concentrations of up to 100 μM. The 4-dimethylphosphinate also failed to bind PLCδPH at this concentration, although the data do indicate that the 4-sulfate does bind very weakly at 100 μM. As noted, this analogue may contain traces of bicarbonate impurity, although it is not believed that this would interfere significantly with the results. However, it is perhaps not...
surprising that this compound displays some affinity for PLCδPH at high concentration, given that this PH domain can bind several of the natural inositol polyphosphates \textit{in vitro} with reasonable affinity, including \text{Ins}(1,4,5)P_3 as Figure 4.3 shows. The activity of an enantiomeric form of the 4-sulfate may enhance the activity of the analogue. Further testing of such compounds for activity towards other PH domain containing proteins, particularly PKB PH, is ongoing.

\textbf{4.2.3 Attempted synthesis of a 4-O-phenoxyacetyl InsP_4 analogue}

In a continuing effort to synthesise a suitable analogue with which to probe the aromatic pocket of PKB PH, the 4-O-phenoxyacetyl InsP_4 analogue precursor 112 was prepared from alcohol 106 (Scheme 4.5). As noted in chapter 3, the phenoxyacetyl ester is not stable to the usual debenzylation conditions employed for the final step. In order to avoid cleavage of the ester group, the sodium bicarbonate was omitted from the hydrogenolysis conditions. The acid was thus isolated following complete debenzylation as indicated by $^1$H NMR, with the ester group intact. However, it was anticipated that the sodium salt of the phosphates would help stabilise the compound against potential transesterification during storage. The acid was therefore passed through an ion exchange resin (Dowex Marathon C $\text{Na}^+$ form, strongly acidic) in an attempt to form the sodium salt. However, the $^{31}$P NMR spectra was unaffected by this process, indicating that the resin used was not able to sodiate the phosphates. In another attempt to form the sodium salt, sodium bicarbonate (6 equivalents) was added to an aqueous solution of the acid, rapidly followed by freeze-drying of the solution. However, a small degree of
ester cleavage was again observed. Attention was thus turned to an alternative method by which an aromatic 4-position InsP₄ analogue could be synthesised.

### 4.2.4 Synthesis of 4-position carbamate InsP₄ analogues 115 and 118

It was envisioned that the synthesis of a carbamate, *via* the reaction of alcohol 106 with an appropriate isocyanate, could provide an alternative method by which to introduce aromatic functionality at the 4-position of the ring. Carbamates are more stable to basic cleavage than the carbonate or ester groups previously employed for this purpose, and should therefore remain stable to the final deprotection conditions. Therefore, a cooled solution of alcohol 106 and DMAP in pyridine/dichloromethane (5:1) was treated with excess phenylisocyanate (Scheme 4.6). After warming to room temperature, the reaction was complete within 2 hours. The reaction mixture was immediately subjected to silica gel chromatography. This procedure was necessary, otherwise yields were found to be impaired. Compound 114 was thus obtained in 80% yield. Final deprotection, using the same conditions as for the 4-dimethylphosphinate, gave the InsP₄ analogue 115 in high yield, with no evidence of migration or carbamate cleavage by ¹H or ³¹P NMR.

![Scheme 4.6. Synthesis of the 4-phenylcarbamate InsP₄ analogue 115. Reagent and conditions: i. phenylisocyanate (20 equiv.), DMAP (cat.), pyridine/CH₂Cl₂ (5:1), 80% yield; ii. Pd black (20 equiv.), NaHCO₃ (6 equiv.), t-BuOH/H₂O (6:1), H₂, 84% yield.](image)

Encouraged by the success of the phenylcarbamate synthesis, this method was then considered as a route towards an analogue bearing a phenol group. As described in chapter 3, a previous attempt to achieve this using an aromatic acid chloride had failed. However, the reaction of alcohol 106 with excess 4-(benzyloxy)phenyl isocyanate in...
pyridine/dichloromethane was considerably more efficient (Scheme 4.7). Again, rapid purification was required to provide the carbamate 116 as a gum in good yield.

**Scheme 4.7.** Synthesis of the 4-phenoxy carbamate InsP₄ analogue 118. **Reagent and conditions:** i. 4-(benzyloxy)phenyl isocyanate (17 equiv.), DMAP (cat.), pyridine/CH₂Cl₂ (5:1), 76 % yield; ii. Pd black (20 equiv.), NaHCO₃ (7 equiv.), t-BuOH/H₂O (6:1), H₂, 92 % yield.

Debenzylation of analogue precursor 116 did not prove as straightforward as with the phenylcarbamate 114. Exposing the precursor to the same hydrogenolysis conditions, using 6 equivalents of sodium bicarbonate, did not provide clean product. The ¹H and ³¹P NMR analysis suggested that some unexpected phosphate migration may have occurred. Given that this migration was not observed for the phenylcarbamate deprotection, it was presumed that the presence of the acidic phenol group must have affected the outcome of the reaction in some way, promoting migration. The reaction was repeated using an extra equivalent of sodium bicarbonate (7 equivalents in total), in order to see if this would result in the formation of a fully sodiated product, where the sodium phenolate 118 is formed. Under these conditions, the reaction did indeed proceed more smoothly, providing pure carbamate 118 in 92% yield. Comparison of the ³¹P NMR spectra of the two experiments demonstrates the occurrence of migration in the first attempt, and how the second experiment using 7 equivalents of sodium bicarbonate provided clean inositol material (Figure 4.4). No carbonate peak was observed in the ¹³C NMR of product 118, indicating that all the sodium bicarbonate was consumed, resulting in the formation of a heptakis-sodium salt.
4.2.5 Towards an enantiopure 4-position analogue synthesis

As outlined in the previous chapter, the synthesis of enantiopure analogues was highly desirable. This could be achieved using the synthetic route already optimised for the synthesis of the racemic analogues as described. The synthesis of the enantiopure camphor derivative (-)-41 was outlined in chapter 2, and could be prepared in multi-gram quantities. The diol (-)-17 was then formed by TIPS protection of the 1-position alcohol followed by efficient acidic methanolysis of the camphor acetal (Scheme 4.8). The following steps proceeded in the same manner, and in similar yields, as described in chapter 3 for the analogous racemic synthesis, up until the synthesis of alcohol (+)-75 had been achieved.

The allyl deprotection of intermediate (+)-75 gave an unexpected result, as the additional, unwanted cleavage of the PMB group at the 4-position occurred during the reaction/work-up conditions. Isomerisation of the allyl group proceeded as normal. For the racemic synthesis, 4-toluenesulfonic acid was used to affect the cleavage of the vinyl ether. For de-allylation of compound (+)-75, acetyl chloride was used for this purpose, and the acid was quenched by the addition of triethylamine as normal. TLC

Figure 4.4. $^{31}$P NMR spectra of the products of the hydrogenolysis of carbamate 116 (162 MHz, D$_2$O). A. Product of the hydrogenolysis conditions using 6 equivalents of sodium bicarbonate. Note the apparent by-product formation, presumably due to phosphate migration. B. Product of the hydrogenolysis conditions using 7 equivalents of sodium bicarbonate. No migration is observed in this instance.
analysis did not indicate significant cleavage of the PMB group prior to work-up. It is proposed that this cleavage occurred during work-up, possibly due to incomplete quenching of the acid by triethylamine. Thus, two products were formed; the desired diol (+)-68 as the minor product and the triol (+)-120 as the major. This outcome was unfortunate, as only 74 mg (5% yield) of the desired enantiopure 3,5-diol was isolated from 1.63 g of the precursor inositol (+)-75. With significant PMB-deprotection observed, 658 mg of the 3,4,5-triol (+)-120 was obtained. However, potentially this enantiomerically pure material could be used for the synthesis of 1-position-modified InsP₄ analogues (as described below), or for the production of biologically relevant phosphatidylinositol derivatives. In order to ensure that the unwanted PMB deprotection does not occur in the future, alternative reaction conditions will be employed. Such conditions involve stirring a solution of the allyl-protected compound and palladium tetrakis(triphenylphosphine) in acetic acid either at room temperature or with gentle heating.

Scheme 4.8. Enantiomeric synthesis of key intermediate (-)-106. Reagents and conditions: i. TIPS triflate, 2,6-lutidine, CH₂Cl₂, 85% yield; ii. 4-TsOH·H₂O, MeOH, 80% yield; iii. a. Bu₂SnO, toluene, reflux, b. Benzoyl chloride, 0 °C→RT, 74 % yield; iv. PMB-trichloroacetimidate, Y(OTf)₃ (2 mol%), PhCH₃, -78 → -10 °C, 64% yield (impure); v. NaOH, MeOH/Et₂O, 80% yield, vi. a. Wilkinson’s catalyst, Hüning’s base, EtOH, reflux; b. AcCl, CH₂Cl₂/MeOH, 5% yield of compound X; vii. TBAF, THF, viii. a. bis(Benzyloxy)-N,N-diisopropylamino phosphine, 1H-tetrazole, CH₂Cl₂, b. mCPBA -78 °C → RT, 40% yield over 2 steps; ix. CAN, MeCN/H₂O (4:1), 72% yield.
The 3,5-diol (+)-68 that was obtained from the de-allylation step was subjected to TIPS deprotection followed by formation of the trisphosphate, under previously described conditions, to provide intermediate (-)-105. Deprotection of the PMB group afforded the desired 1-hydroxy trisphosphate (-)-106. However, due to the low-yielding allyl deprotection step, only 55 mg of this enantiopure material was acquired. As this is only enough material to synthesise one 4-position analogue, it will only be used once testing of the racemic compounds is complete, to assess which analogue is the most active, and thus most necessary to synthesise in enantiopure form.

4.3 The synthesis of racemic and enantiopure 1-position InsP₄ analogues

As discussed in chapter 2, the rationale behind the synthesis of PtdIns(3,4,5)P₃ analogues modified at the 4-position was to inhibit the activating conformational change in PKB PH that occurs upon binding of the natural ligand. Modification of the 4-position was seen as a good starting point, due to the possibility of altering the conformational change observed for variable loop VL3 in particular. However, alteration of the 1- or 3-position phosphates could potentially affect conformational change in VL1 or VL2, and thus may also result in the desired PH domain inhibition. For instance, as noted in chapter 2, Arg-23 is seen to move a full 6.2 Å towards these two phosphates upon binding of InsP₄ to PKBαPH.⁵⁴ The synthesis of novel InsP₄ analogues modified at the 1-position (Figure 4.5) was therefore proposed as another route towards potential PKB PH inhibitors.

![Figure 4.5: 1-Position InsP₄ or PtdIns(3,4,5)P₃ analogues.](image-url)
The activity of some myo-inositol compounds possessing phosphates at the 3-, 4- and 5-positions, with an alternative group at the 1-position, has been reported in the literature. In 1995, Sawada et al. reported the synthesis of 3,4,5 trisphosphates bearing simple 1-O-alkyl and 1-O-acyl groups of varying length as analogues of PtdIns(3,4,5)P$_3$. Such compounds were tested only against PtdIns(3,4,5)P$_3$ 5-phosphatase. A few of the analogues synthesised did display high activity against the phosphatase, suggesting that in some instances, a substitute for the 1-phosphate group may be tolerated.

In another study, Shirai et al. reported the use of analogue 122 attached to a solid phase in affinity chromatography experiments, to detect PtdIns(3,4,5)P$_3$ binding proteins. It was found that this compound showed less affinity and specificity for PH-domain-containing proteins than the analogue 123, although PKB itself was not discussed in the study. The implication is, though, that the potential for electrostatic interactions that the 1-position phosphate provides may be crucial for high affinity binding to PKB PH. It would be intriguing to see if replacing the phosphate with an alternative acidic group could be tolerated with respect to PKB PH binding, and if so, whether an activating conformational change results. Thus, the objective was to synthesise 1-position sulfate and phosphonate analogues, test their binding affinity for the PH domain, and obtain crystal structures of such ligands bound to PKB PH where possible.

4.3.1 Attempted synthesis of the racemic 1-sulfate InsP$_4$ analogue 127

The racemic synthesis of the required 1-hydroxy trisphosphate intermediate 125 was straightforward, as it could be derived from the previously synthesised diol 70 in three steps (Scheme 4.9). De-allylation of 70 was achieved in moderate yield, using the Wilkinson’s catalyst-mediated isomerisation and 4-toluenesulfonic acid cleavage conditions described earlier. The 3-, 4- and 5-positions were then subjected to the standard phosphitylation and oxidation conditions to provide the benzyl-protected trisphosphate 124. The facile cleavage of the TIPS ether was then performed using TBAF to afford alcohol 125 in good yield.
Scheme 4.9. Synthesis of the 1-hydroxy trisphosphate intermediate 125. Reagents and conditions: i. a. Wilkinson’s Catalyst, Hünig’s base, EtOH, reflux; b. 4-TsOH·H₂O, MeOH, 53% yield; ii. a. bis(benzylxy)-N,N-diisopropylamino phosphine, 1H-tetrazole, CH₂Cl₂, b. mCPBA -78 °C → RT, 74% yield; iii. TBAF, THF, 80%, yield.

Sulfation of the 1-position was performed under the conditions optimised for the synthesis of the 4-sulfate 109. Compound 126 was thus obtained in 44% yield and, like the 4-sulfate, was again very hygroscopic and seemingly unstable (Scheme 4.10). Hydrogenolysis was performed using six equivalents of sodium bicarbonate to give the 1-sulfate analogue 127. However, on this occasion a very clear, unexpected peak was present in the $^{13}$C spectrum at 160.8 ppm. This peak presumably corresponded to the presence of a carbonate group. The intensity of the peak relative to those corresponding to the carbons of the inositol ring suggested that a significant amount of carbonate impurity was present, and was unlikely to be due to accidental addition of excess sodium bicarbonate. In order to confirm this, the reaction was repeated under the same conditions. However, the outcome was the same. Furthermore, mass spectrometric analysis provided the expected (M-nNa+nH)$^+$ ions, suggesting that an ionic complex may have formed between the inositol salt and a carbonate species.

Scheme 4.10. Attempted synthesis of 1-sulfate InsP₄ analogue 127. Reagents and conditions: i. a. SO₃·Pyridine complex, DMF, 50 °C; b. Dowex Marathon C resin Na⁺ form, MeOH, 44% yield; ii. Pd black (20 equiv.), NaHCO₃ (6 equiv.), t-BuOH/H₂O (6:1), H₂, impure product.

Purification of the sulfate 127 was attempted by reverse phase silica gel column chromatography. However, the carbonate impurity remained. Another attempt at purification involved the application of ion exchange chromatography, using Sepharose fast flow resin, eluting with TEAB using a procedure reported by Potter and co-
workers. However, no inositol-based material was isolated from the ion exchange column.

4.3.2 Synthesis of the racemic 1-methylenephosphonate InsP$_4$ analogue 134

The preparation of a suitable reagent was required for the synthesis of a 1-methylenephosphonate analogue. Initially, the synthesis of bromide 130 was attempted. Thus, using conditions described in the literature, dibenzyl(hydroxymethyl)phosphonate 129 was obtained from the reaction of dibenzyl phosphite with paraformaldehyde using catalytic triethylamine (Scheme 4.11). The reaction required heating the mixture at 130 °C until the paraformaldehyde was fully consumed/dissolved after which, according to the literature, the reaction was complete. However, the reaction appeared incomplete after this time by TLC analysis, and further heating resulting in the formation of by-products. Compound 129 was therefore obtained in only 31% yield following silica gel column chromatography, although this was sufficient for the purpose of the InsP$_4$ analogue synthesis.

Synthesis of the bromide 130 was then attempted (Scheme 4.11). Literature bromination conditions using carbon tetrabromide and triphenylphosphine in dry acetonitrile were employed, but failed to yield the desired product. It was noted that maintaining the reaction temperature below 0° C did not allow the reaction to proceed, but allowing the mixture to slowly warm to room temperature resulted in benzyl alcohol formation, without isolation of the desired bromide. Treatment of the hydroxyphosphonate with phosphorous tribromide in diethyl ether appeared to give similar results. Attention was therefore turned to the synthesis of the triflate 131. Although the synthesis of this triflate has been reported in the literature, the formation of this compound was also problematic. A solution of the alcohol 129 in dichloromethane at -78 °C was treated with triflic anhydride. In order for the reaction to proceed, the reaction temperature had to be slowly raised to -40 °C, then to -15 °C at which it was maintained until full consumption of starting material. If the temperature was raised to 0 °C or higher, a complex mixture formed. Even so, despite efforts to minimise the formation of unwanted by-products, side reactions were observed. The major products isolated by silica gel column
chromatography were the desired triflate 131 and the dimer 132. Consequently, the reagent 131 was isolated in only 26% yield. The formation of compound 132 as an unwanted by-product of this reaction has been reported in the literature.\textsuperscript{12} However, this method provided sufficient material for subsequent reaction with the requisite inositol.

![Scheme 4.11. The synthesis of methylenephosphonate reagents. Reagents and conditions: i. Paraformaldehyde, triethylamine, 130 °C, 15 min, 31% yield; ii. CBr₄, Ph₃P, MeCN, product not isolated; iii. Trifluoromethanesulfonic anhydride, 2,6-lutidine, CH₂Cl₂, 26% yield.](image)

The introduction of the phosphonate to alcohol 125 is outlined in Scheme 4.12. Sodium hydride was added to a cooled mixture of the alcohol and the triflate 131 in THF (Figure 4.12). Given the possibility that intermolecular attack of the alkoxide on a phosphate group could lead to phosphate migration, the reaction temperature was initially kept below 0 °C. However, the mixture had to warm to room temperature for reaction to occur, after which the starting material was fully consumed within 2 h. Initial purification by silica gel chromatography provided moderately pure material in a yield of about 46%. However, further chromatographic purification was required. After exhaustive column chromatography, the desired phosphonate of satisfactory purity was isolated in 24% yield.

Debenzylation of intermediate 133 proceeded smoothly. For this reaction, 8 equivalents of sodium bicarbonate were used to synthesise the fully sodiated 1-position methylenephosphonate InsP₄ analogue 134 (Scheme 4.12). No evidence for migration or the presence of carbonate was found on the \(^1\)H, \(^{13}\)C or \(^{31}\)P NMR spectra.

![Scheme 4.12. Synthesis of the 1-methylenephosphonate InsP₄ analogue 134. Reagents and conditions: i. Dibenzyl phosphonomethyl triflate 131, NaH, THF, -78 °C to RT, 24% yield; ii. Pd black (20 equiv.), NaHCO₃ (8 equiv.), t-BuOH/H₂O (6:1), H₂, 93% yield.](image)
4.3.3 Synthesis of enantiopure 1-position InsP₄ analogues

The enantiopure syntheses of the 1-sulfate and 1-methylenephosphonate analogues were attempted. The enantiomeric 1-hydroxy (-)-125 was synthesised in the same manner as the racemic compound, and was derived from the previously prepared diol (-)-70 (Scheme 4.13). The 1-phosphonate (-)-133 was synthesised using the same conditions as outlined above. As with the synthesis of the racemic phosphonate, purification of the intermediate again proved difficult. Pure inositol was therefore isolated in only 28% yield. Global deprotection provided the desired analogue 134. However, a significant peak in the ¹³C NMR spectrum at 160.7 ppm relating to carbonate impurity was present. This was unexpected, as it was not observed with the analogous racemic phosphonate synthesis, although the chemical shift of the anomalous peak had the same chemical shift as the impurity present in the ¹³C spectrum of 1-sulfate 127 described above. The mass spectrometric data provided the expected (M-8Na+7H)⁻ ion for the acid derivative, thus it is presumed that the carbonate species is ionically associated with the inositol phosphate. Attempts to purify compound 134 or re-synthesise the compound without the use of sodium bicarbonate have yet to be attempted.

Scheme 4.13. Attempted synthesis of the enantiopure 1-methylenephosphonate 134. Reagents and conditions: i. a. Wilkinson’s Catalyst, Hüning’s base, EtOH, reflux; b. 4-TsOH·H₂O, MeOH, 54% yield; ii. a. bis(Benzyloxy)-N,N-diisopropylamino phosphine, ¹H-tetrazole, CH₂Cl₂, b. mCPBA -78 °C → RT, 86% yield; iii. TBAF, THF, 81%, yield; iv. Dibenzyl phosphonomethyl triflate 131, NaH, THF, -78 °C→RT, 28% yield; v. Pd black (20 equiv.), NaHCO₃ (8 equiv.), t-BuOH/H₂O (6:1), H₂, impure product.
In order to synthesise an enantiopure 1-position sulfate analogue, the alcohol \((-)-125\) was sulfated in the manner described above to give compound \((+)-126\) in 68\% yield (Scheme 4.14). In an attempt to avoid the problem of bicarbonate impurity appearing in the $^{13}$C NMR of the final product, hydrogenolysis was performed with a reduced 3
equivalents of sodium bicarbonate, instead of 6 equivalents. This reaction proceeded in 80% yield to provide pure 1-sulfate InsP₄ analogue (−)-135. Thus, the tetrasodium salt of (−)-135 was formed, instead of the fully sodiated heptasodium salt. There was no evidence of phosphate or sulfate migration from ¹H NMR analysis, and there was no indication of the presence of bicarbonate in the ¹³C NMR spectrum (see Figure 4.6).

It is apparent from the ¹³C and ³¹P NMR data for the racemic sulfate 127 (Figure 4.6, A.1 and A.2) and the enantiopure sulfate (−)-135 (Figure 4.6, B.1 and B.2) that only one inositol trisphosphate was formed in the synthesis of each compound. The differences in the chemical shifts between the NMR spectra for the two compounds can be attributed to differences in counterion formation and the degree of ionisation of each compound (compound (−)-135 can only form the tetrasodium salt). Given that the mass spectrometric data for both compounds provided the expected M⁺ ions, the implication is, therefore, that the expected product was obtained in both instances, and that the carbonate impurity possibly arose due to carbonate interaction with compound 127.
4.4. Future Work

With respect to the synthesis of C-4 position-modified InsP₄ analogues, future work will depend partly on the outcome of the biological testing on those compounds already obtained. A 4-position methylphosphonate analogue such as compound 136 (Figure 4.7) may also be synthesised as another potential inhibitor which bears an alternative acidic group at the C-4 position. All compounds need to be assessed for activity against PKB PH, as well as other PH domain-containing proteins in order to assess their selectivity. Analogues displaying significant activity will be synthesised as a single enantiomer using the methods outlined above.

![Figure 4.7. Structure of a 4-methylphosphonate InsP₄ analogue 136 and C-3 position-modified analogues 137 and 138 to be synthesised.](image)

Additionally, the PKB PH domain ligand binding pocket will be probed further by the production of compounds which are modified at the 3-position of the inositol ring. Examples of target compounds include the 3-methylphosphonate 137 and the 3-sulfate 138 (Figure 4.7). It is predicted that such compounds may also act as inhibitors of the PKB PH domain. Any active compounds that are altered at the C-3 or C-4 positions can also be modified to include a long-chain phosphodiester group at the C-1 position, in order to assess the influence of this group on binding and selectivity.
4.5. Summary and Conclusions

The discovery of selective inhibitors of protein kinase B remains a primary target for chemotherapeutic research. Furthermore, such inhibitors would act as useful biological tools in the ongoing processes of delineating the PI3-K/PKB signalling cascade. This project has investigated the synthesis of PtdIns(3,4,5)P$_3$ and InsP$_4$ analogues modified at the C-4 position. Such compounds have been designed to bind to the PH domain of PKB, but not induce the activating conformational change that the protein undergoes upon binding of the natural ligand. It has been discovered that in order to produce PtdIns(3,4,5)P$_3$ analogues that possess the phosphoglyceryl diester at the 1-position, relatively lengthy ester chains on the glyceryl moiety are required in order to produce stable compounds. Thus, in the search for novel PKB PH inhibitors C-4 and C-1 position-modified InsP$_4$ analogues have been synthesised (Figure 4.8).

![Figure 4.8. Structure of the C-4 and C-1 position InsP$_3$ analogues that have been synthesised.](image)

These compounds were produced via an efficient divergent synthesis, using orthogonal protecting group strategies. The routes that have been devised have been shown to be compatible with the synthesis of single enantiomers of the InsP$_4$ analogues. The information gained from the biological testing of such compounds will be used to synthesise a second generation of such inositol-based analogues, with the view to increasing potency and selectivity for PKB inhibition.
Experimental Section

General

$^1$H NMR spectra were recorded on a Bruker Advance 300 (300 MHz) or Bruker Advance 400 (400 MHz) instrument using deuterated solvent as a reference. The chemical shift data for each signal are given as $\delta$ in units of parts per million (ppm) relative to tetramethylsilane (TMS) where $\delta$ (TMS) = 0.00 ppm. The multiplicity of each signal is indicated by: s (singlet); br s (broad singlet); d (doublet); t (triplet); dd (doublet of doublets); ddd (doublet of doublet of doublets); dt (doublet of triplets); dddd (doublet of doublet of triplets) or m (multiplet). The number of protons (n) for a given resonance is indicated by “n H”. Coupling constants ($J$) are quoted in Hz and are recorded to the nearest 0.1 Hz.

$^{13}$C NMR spectra were recorded on a Bruker Advance 300 (75.5 MHz) or Bruker Advance 400 (101 MHz) instrument using the PENDANT sequence and internal deuterium lock. The chemical shift data for each signal are given as $\delta$ in units of ppm relative to tetramethylsilane (TMS) where $\delta$ (TMS) = 0.00 ppm. Signals are assigned as C, CH, CH$_2$ or CH$_3$ according to the number of protons bonded to the carbon atom. Where appropriate, coupling constants ($J$) are quoted in Hz and are recorded to the nearest 0.1 Hz.

$^{31}$P NMR data were recorded on a Bruker Advance 300 (121.5 MHz) instrument using proton decoupling and internal deuterium lock. The chemical shift data for each signal are given as $\delta$ in units of ppm relative to an external standard of 85% H$_3$PO$_4$.

Melting points were determined using a Gallenkamp MF-370 or an Electrothermal 9100 melting point instrument and are uncorrected.

Optical specific rotations were measured using either an Optical Activity AA-1000 automatic polarimeter, a Bellingham and Stanley Ltd ADP220 instrument or a Perkin Elmer Model 341 Polarimeter, in cells with a path length of 2 or 1 dm. The concentration (c) is expressed in g/100 mL. Specific rotations are given in implied units of 10$^{-1}$ deg cm$^2$ g$^{-1}$ (T = ambient temperature in °C).

Infra red (IR) spectra were recorded on a Perkin Elmer Spectrum GX FT-IR system.
Analytical thin layer chromatography (TLC) was carried out on pre-coated 0.25 mm ICN Biomedicals GmbH 60 F254 silica gel plates. Visualisation was achieved by absorption of UV light, or by an ethanolic phosphomolybdcic acid or potassium permanganate dip staining system followed by thermal development.

Silica gel column chromatography was carried out on silica gel (Apollo Scientific Ltd 40-63 micron) under a positive pressure of compressed air. Kugelrohl bulb-to-bulb distillations were carried out using a Büchi GKR-51 machine. Boiling points are the actual oven temperatures.

Dry dichloromethane was distilled from calcium hydride in a recycling still. Diethyl ether was distilled from sodium in a recycling still using benzophenone ketyl as an indicator. Dry dichloromethane, THF, diethyl ether and toluene were also obtained by being passed through two columns of alumina using an MBRAUN (SPS-800) solvent purification system. Anhydrous DMF was purchased from Aldrich UK and dried by distillation from 4 Å molecular sieves onto 4 Å molecular sieves under nitrogen atmosphere. Chemicals were purchased from Acros UK, Aldrich UK, Avocado UK, Fisher UK or Fluka UK. All solvents and reagents were purified and dried, where necessary, by standard techniques. Where appropriate and if not stated otherwise, all non-aqueous reactions were performed under an inert atmosphere of nitrogen, using a vacuum manifold with nitrogen passed through 4 Å molecular sieves and self-indicating silica gel. In vacuo refers to the use of a rotary evaporator attached to a diaphragm pump. Brine refers to a saturated aqueous solution of sodium chloride. Hexane refers to n-hexane and petroleum ether to the fraction boiling between 40-60 °C. Room temperature (RT) refers to an ambient temperature of approximately 25 °C.
2,4,10-Trioxatricyclo[3.3.1.1^{3,7}]decane-6,8,9-triol 43\textsuperscript{138}

myo-Inositol (40.0 g, 222 mmol, 1 equiv.) and 4-toluenesulfonic acid monohydrate (12.7 g, 67 mmol, 0.3 equiv.) were dissolved in dry N,N-dimethylformamide (250 mL). Triethyl orthoformate (98.9 g, 111 mL, 666 mmol, 3 equiv.) was added and the reaction mixture was stirred at 100 °C for 30 h. The reaction was adjudged to be complete by TLC analysis. The mixture was allowed to cool to room temperature and the 4-toluenesulfonic acid was quenched with a saturated aqueous solution of sodium bicarbonate (50 mL). The resulting sodium tosylate was removed by filtration, washing with methanol (20 mL), and the filtrate was concentrated under reduced pressure. Crystallisation from methanol gave the desired orthoformate (13.20 g) as a colourless solid. The mother liquor was concentrated and adsorbed onto silica gel. Purification by silica gel column chromatography, eluting with methanol/dichloromethane (5:95) gave the desired product (16.90 g) as a colourless crystalline solid. This solid was combined with the product obtained from crystallisation to give 2,4,10-trioxatricyclo[3.3.1.1^{3,7}]decane-6,8,9-triol 43 (30.10 g, 71% yield); R\textsubscript{f} 0.53 (methanol/chloroform, 20/80); mp 220 °C dec. (Lit.,\textsuperscript{138} 300-302 °C sealed tube); \(\delta\textsuperscript{H} (300 \text{ MHz, DMSO-D}_6) 5.47 (1 \text{ H, d, } J 6.1, \text{ equatorial OH}), 5.44 (2 \text{ H, d, } J 1.3, 2 \times \text{ axial OH}), 5.31 (1 \text{ H, d, } J 6.4, 3\text{-H}), 4.30-4.22 (2 \text{ H, m, } 2 \times \text{ inositol ring}), 4.08-4.04 (1 \text{ H, m, inositol ring}), 4.03-4.00 (1 \text{ H, m, inositol ring}), 3.96-3.92 (2 \text{ H, m, } 2 \times \text{ inositol ring}). The data are in good agreement with literature values.\textsuperscript{138}

(\pm)-6-[(4'-Methoxy)benzyloxy]-2,4,10-trioxatricyclo[3.3.1.1^{3,7}]decane-8,9-diol 44\textsuperscript{134}

Sodium hydride (60 % dispersion in mineral oil, 3.28 g, 82.0 mmol, 1.3 equiv.) was added portionwise to a stirred solution of 2,4,10-trioxatricyclo[3.3.1.1^{3,7}]decane-6,8,9-triol 43 (12.00 g, 63.1 mmol, 1 equiv.) in dry N,N-dimethylformamide (220 mL) under nitrogen at 0 °C. The mixture was stirred at 0 °C for 30 min then left to stir at RT for a
further 2 h. The suspension was re-cooled to 0 °C and 4-(methoxy)benzyl chloride (10.9 g, 9.41 mL, 69.4 mmol, 1.1 equiv.) was added dropwise. The mixture was left to stir for 30 min at 0 °C and then overnight at RT. The reaction mixture was quenched with ice-water (20 mL) and concentrated under reduced pressure. The resulting residue was dissolved in ethyl acetate (100 mL) and water (100 mL). The organic layer was collected and the aqueous phase was further extracted with ethyl acetate (3 × 80 mL). The combined organic extracts were dried (MgSO$_4$), filtered and the solvent was removed in vacuo. The resulting solid was purified by silica gel column chromatography, eluting with ethyl acetate/petroleum ether (20:80, 30:70, 40:60 then 50:50) to give (±)-6-[(4'-methoxy)benzyl]oxy]-2,4,10-trioxa-tricyclo[3.3.1.1$^{3,7}$]decane-8,9-diol 44 (16.70 g, 85% yield) as a colourless solid; R$_f$ 0.34 (1:1 ethyl acetate/petroleum ether); mp 98-100 °C (diethyl ether/petroleum ether) (Lit.,$^{177}$ 99-101 °C); δ$_H$ (300 MHz, CDCl$_3$) 7.28-7.24 (2 H, m, OCH$_2$C$_6$H$_4$OCH$_3$), 6.94-6.88 (2 H, m, OCH$_2$C$_6$H$_4$OCH$_3$), 5.44 (1 H, d, J 1.2, 3-H), 4.64 (1 H, d, J$_{AB}$ 11.5, OCH$_A$H$_B$), 4.58 (1 H, d, J$_{AB}$ 11.5, OCH$_A$H$_B$), 4.41-4.40 (2 H, m, 2 × inositol ring), 4.25-4.20 (3 H, m, 3 × inositol ring), 3.84 (3 H, s, OCH$_3$), 3.79 (1 H, d, J 10.5, OH), 3.14 (1 H, d, J 11.7, OH). The data are in good agreement with literature values.$^{134,177}$

(±)-8,9-Bis-O-benzyl-6-[(4'-methoxy)benzyl]oxy]-2,4,10-trioxa-tricyclo[3.3.1.1$^{3,7}$]decane 45$^{34}$

Sodium hydride (60 % dispersion in mineral oil, 4.49 g, 112.3 mmol, 2.5 equiv.) was suspended in N,N-dimethylformamide (175 mL) and the suspension was cooled to -5 °C under argon. A solution of (±)-6-[(4'-methoxy)benzyl]oxy]-2,4,10-trioxa-tricyclo[3.3.1.1$^{3,7}$]decane-8,9-diol 44 (13.20 g, 42.6 mmol, 1 equiv.) in N,N-dimethylformamide (90 mL) was transferred via cannula to the cooled sodium hydride suspension. The mixture was stirred for 30 min at -5 °C and for 1 h at RT, then re-cooled to -5 °C and benzyl bromide (19.3 g, 13.4 mL, 112.3 mmol, 2.5 equiv.) was added dropwise, keeping the temperature between 0 °C and -5 °C. The reaction mixture was
stirred for 30 min at 0 °C and for 20 h at RT, after which time the reaction was judged to be complete by TLC analysis. The reaction was quenched by the addition of water (20 mL), and all volatile components were removed in vacuo. The resulting residue was reconstituted in ethyl acetate (150 mL) and water (100 mL). The organic layer was collected and the aqueous portion was extracted further with ethyl acetate (3 × 100 mL). The combined organic extracts were washed with water (100 mL) and brine (100 mL), dried (MgSO₄), filtered and the solvent was removed in vacuo. The resulting oil was purified by silica gel column chromatography, eluting with ethyl acetate/petroleum ether (20:80 then 30:70) to give (±)-8,9-bis-O-benzyl-6-[(4'-methoxy)benzyl]oxy]-2,4,10-trioxa-tricyclo[3.3.1.1³,⁷]decane 45 (20.50 g, 98% yield) as a colourless oil; Rᵣ 0.32 (ethyl acetate/petroleum ether 25:75); δ₇ (300 MHz, CDCl₃) 7.40-7.19 (10 H, m, 2 × Ph), 7.10 (2 H, d, J 8.7, OCH₂C₆H₄OCH₃), 6.81 (2 H, d, J 8.7, OCH₂C₆H₄OCH₃), 5.55 (1 H, d, J 1.3, 3-H), 4.66 (2 H, s, OCH₂Ph), 4.64 (1 H, d, Jₐₐ 11.5, OCH₆H₄Ph), 4.56 (1 H, d, Jₐₐ 11.2, OCH₆H₄-C₆H₄OCH₃), 4.50 (1 H, d, Jₐₐ 11.5, OCH₆H₄Ph), 4.46 (1 H, d, J 11.2 OCH₆H₄-C₆H₄OCH₃), 4.40-3.37 (1 H, m, inositol ring), 4.30-4.20 (4 H, m, inositol ring), 4.07-4.04 (1 H, m, inositol ring), 3.82 (3 H, s, OCH₃). The data are in good agreement with literature values.¹³⁴

(±)-6,8-Bis-O-benzyl-9-[(4'-methoxy)benzyl]oxy]-2,4-dioxabicyclo[3.3.1]nonan-7-ol 46

![Chemical Structure](image)

The protected orthoformate (±)-8,9-bis-O-benzyl-6-[(4'-methoxy)benzyl]oxy]-2,4,10-trioxa-tricyclo[3.3.1.1³,⁷]decane 45 (15.02 g, 30.6 mmol, 1 equiv.) was dissolved in dry dichloromethane (150 mL) under nitrogen and cooled to 0 °C. Diisobutylaluminium hydride (1.0 M solution hexanes, 79 mL, 79.2 mmol, 2.5 equiv.) was added dropwise, maintaining a temperature of 0 °C. The mixture was stirred at RT for 5 h, after which time the reaction was adjudged to be complete by TLC analysis. To quench the reaction, the mixture was cannulated onto a vigorously stirred solution of 1.0 M aqueous sodium potassium tartrate (100 mL) and saturated aqueous ammonium chloride solution (100
mL), and the resulting mixture was stirred overnight. The aqueous and organic layers were separated and the organic phase was collected. The aqueous layer was extracted with dichloromethane (3 × 100 mL). The combined organic extracts were washed with brine (100 mL), dried (MgSO₄), filtered and the solvent removed under reduced pressure to give (±)-6,8-O-benzyl-9-[(4′-methoxy)benzyloxy]-2,4-dioxabicyclo[3.3.1]nonan-7-ol 46 (14.52 g, 96% yield) as a colourless oil; \( R_f \) 0.28 (ethyl acetate/petroleum ether 40:60); \( \delta^H \) (300 MHz, CDCl₃) 7.36-7.28 (10 H, m, 2 × Ph), 7.20 (2 H, d, J 8.7, OCH₂C₆H₄OCH₃), 6.84 (2 H, d, J 8.7, OCH₂C₆H₄OCH₃), 5.57 (1 H, d, J 4.9, 3-H), 4.68 (1 H, d, Jₐₐ 12.0, OCHₐHₐ-Ph), 4.66 (1 H, d, J 4.9, 3-H), 4.62 (2 H, s, OCH₂-Ph), 4.60 (1 H, d, Jₐₐ 11.5, OCHₐHₐ-C₆H₄OCH₃), 4.59 (1 H, d, Jₐₐ 12.0, OCHₐHₐ-Ph), 4.53 (1 H, d, Jₐₐ 11.5, OCHₐHₐ-C₆H₄OCH₃), 4.45-4.41 (2 H, m, inositol ring), 4.32-4.29 (1 H, m, inositol ring), 4.03-4.00 (2 H, m, inositol ring), 3.96 (1 H, br s, inositol ring), 3.81 (3 H, s, OCH₃), 2.98 (1 H, br s, OH). The data are in good agreement with literature values.¹³⁴

(±)-7-Allyoxy-8,9-bis-O-benzyl-8-(4-methoxybenzyloxy)-2,4-dioxabicyclo[3.3.1]nonane 47¹³⁴

Sodium hydride (60 % dispersion in mineral oil, 1.83 g, 45.8 mmol, 1.5 equiv.) was suspended in \( N,N \)-dimethylformamide (100 mL) under nitrogen and cooled to -5 °C. The alcohol (±)-8,9-bis-O-benzyl-6-[(4′-methoxy)benzyloxy]-2,4-dioxabicyclo[3.3.1]nonan-7-ol 46 (14.53 g, 30.5 mmol, 1 equiv) was dissolved in \( N,N \)-dimethylformamide (75 mL) and transferred via cannula to the cooled sodium hydride suspension. The resulting mixture was stirred at -5 °C for 20 min and for 1 h at RT. The mixture was again cooled to -5 °C and imidazole (catalytic amount) was added followed by dropwise addition of allyl bromide (5.6 g, 4.0 mL, 45.8 mmol, 1.5 equiv.). The reaction mixture was stirred for 30 min at -5 °C and then left to stir overnight at RT. Analysis by TLC showed that the reaction was complete and the mixture quenched with water (10 mL). The solvent was removed in vacuo and the resulting oil was partitioned between ethyl acetate (100 mL) and water (100 mL). The organic layer was collected and the aqueous portion was
extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were washed with water (50 mL) and brine (50 mL), dried (MgSO₄), filtered and the solvent removed under reduced pressure. The resulting crude oil was purified by flash silica gel column chromatography eluting with ethyl acetate/petroleum ether (20:80 then 30:70) to give (±)-7-allyloxy-8,9-bis-O-benzyl-8-(4-methoxybenzoyloxy)-2,4-dioxabicyclo[3.3.1]nonane 47 (13.16 g, 84% yield) as a colourless oil; Rᵣ 0.47 (ethyl acetate/petroleum ether 40/60); δᵣ (300 MHz, CDCl₃) 7.40-7.30 (10 H, m, 2 × Ph), 7.26 (2 H, d, J 8.7, OCH₂C₆H₄OCH₃), 6.88 (2 H, d, J 8.7, OCH₂C₆H₄OCH₃), 5.90 (1 H, dddd, J 17.2, 10.3, 5.7, 5.7, CH=CH₂), 5.24 (1 H, dddd, J 17.2, 1.8, 1.6, 1.6, CH=CHH), 5.20 (1 H, d, J 5.5, 3-H), 5.20-5.17 (1 H, m, CH=CHH), 4.84 (1 H, d, J 5.4, 3-H), 4.66 (1 H, d, Jₐₜ 11.8, OCH₆H₆-Ph), 4.65 (2 H, s, OCH₂-Ph), 4.61 (1 H, d, Jₐₜ 11.8, OCH₆H₆-Ph), 4.60 (1 H, d, Jₐₜ 11.5, OCH₆H₆-Ph-C₆H₄OCH₃), 4.54 (1 H, d, Jₐₜ 11.5, OCH₆H₆-Ph-C₆H₄OCH₃), 4.28-4.25 (2 H, m, inositol ring), 4.15-4.10 (2 H, m, OCH₂CH=CH), 3.91-3.88 (1 H, m, inositol ring), 3.80 (3 H, s, OCH₃), 3.53 (1 H, t, J 5.6, inositol ring). The data are in good agreement with literature values.¹³⁴

(±)-5-O-Allyl-2,6-bis-O-benzyl-myo-inositol 42¹³⁴

Concentrated hydrochloric acid (80 mL) was slowly poured onto a stirred solution of (±)-7-allyloxy-8,9-bis-O-benzyl-8-(4-methoxybenzoyloxy)-2,4-dioxabicyclo[3.3.1]nonane 47 (60.50 g, 113.7 mmol, 1 equiv.) in methanol (500 mL). The mixture was heated under reflux for 4 h, then allowed to cool to room temperature. The reaction was quenched by the addition of sodium hydrogen carbonate (100 g). The solid components were removed by filtration and rinsed with methanol (30 mL) and the crude filtrate was adsorbed onto silica gel. Silica gel column chromatography, eluting with ethyl acetate/petroleum ether (30:70, 40:60 then 80:20) gave (±)-5-O-allyl-2,6-O-dibenzyl-myoinositol 42 (37.8 g, 83% yield) as a colourless solid; Rᵣ 0.43 (ethyl acetate); mp 118-120 °C (ethyl acetate/petroleum ether), (Lit.,¹³⁴ 111-112 °C); δᵣ (300 MHz, CDCl₃) 7.39-7.22 (10 H, m, 2 × Ph) 5.98 (1 H, dddd, J 17.2, 10.3, 5.6, 5.6, CH=CH₂), 5.30 (1 H, dddd, J 17.2, 1.6, 1.6, 1.6, CH=CHH), 5.20 (1 H, dddd, J 10.3, 1.6, 1.2, 1.2, CH=CHH), 4.91 (1
H, d, $J_{AB}$ 11.5, OCH$_3$H$_3$-Ph), 4.88 (1 H, d, $J_{AB'}$ 11.3, OCH$_3$H$_3$-Ph), 4.78 (1 H, d, $J_{AB'}$ 11.3, OCH$_3$H$_3$-Ph), 4.74 (1 H, d, $J_{AB}$ 11.5, OCH$_3$H$_3$-Ph), 4.38-4.35 (2 H, m, OCH$_2$CH=CH$_2$), 4.03 (1 H, dd, J 2.7, 2.7, 2-H), 3.82 (1 H, dd, J 9.5, 9.5, inositol ring), 3.74 (1 H, dd, J 9.4, 9.4, inositol ring), 3.58 (1 H, dd, J 9.5, 2.6, inositol ring), 3.48 (1 H, dd, J 9.7, 2.6, inositol ring), 3.23 (1 H, dd, J 9.3, 9.3 inositol ring), 2.54 (1 H, br s, OH), 2.33 (2 H, br s, 2 × OH). The data are in agreement with the literature values.$^{134}$

(1S)-(−)-Camphor dimethyl acetal (−)-52$^{178}$

![diagram](diagram.png)

Trimethylorthoformate (33.5 g, 34.5 mL, 315.6 mmol, 4 equiv.) was added to a mixture of (1S)-(−)-camphor (12.0 g, 78.9 mmol) and Montmorillonite K-10 clay (22 g) in hexane (100 mL) under an atmosphere of nitrogen and the mixture stirred for 32 h. The clay was removed by filtration and washed with hexane (100 mL) and the solvent was then removed under reduced pressure to give (1S)-(−)-camphor dimethyl acetal (−)-52 (11.85 g, 75% conversion to the dimethyl acetal as estimated from $^1$H NMR) as a colourless oil; R$_f$ 0.62 (diethyl ether/petroleum ether 30:70); $\delta_H$ (300 MHz, CDCl$_3$) 3.21 (3 H, s, OCH$_3$), 3.15 (3 H, s, OCH$_3$), 2.22-2.13 (1 H, m, camphor ring), 1.80-1.60 (3 H, m, camphor ring), 0.95 (3 H, s, 1-CH$_3$), 0.89 (3 H, s, 7-CH$_3$), 0.81 (3 H, s, 7-CH$_3$). The data are in agreement with literature values.$^{178}$

(−)-1D-5-O-Allyl-2,6-bis-O-benzyl-3-O-endo-4-O-exo-(L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidine)-myo-inositol (−)-41$^{134}$

![diagram](diagram.png)

The crude (1S)-(−)-camphor dimethyl acetal (−)-52 (8.66 g, 43.8 mmol, 2.5 equiv.), the triol (±)-5-O-allyl-2,6-bis-O-benzyl-myoinositol 42 (7.00 g, 17.5 mmol, 1 equiv.) and 4-
toluenesulfonic acid monohydrate (0.99 g, 5.25 mmol, 0.3 equiv.) were dissolved in dry dichloromethane (80 mL) under nitrogen and heated under reflux for 48 h. The acidic reaction mixture was quenched with triethylamine (3 mL), and all volatile components were removed under reduced pressure. The crude product was purified by silica gel column chromatography eluting with the following solvent systems to allow maximum isolation of the desired pure diastereomer: ethyl acetate/petroleum ether (5:95, 6:94, 7:93, 8:92, 9:91, 10:90 – the undesired diastereomers were collected in this solvent system) then 20:80 elution gave the required diastereomer (-)-1D-5-O-allyl-2,6-bis-O-benzyl-3-O-endo-4-O-exo-(L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidine)-myo-inositol (-)-41 as a colourless gum. Additional purification by flash silica gel column chromatography of impure fractions from the first column, eluting with the solvent system as above, was required to obtain fully the title compound (combined weight and yield: 2.32 g, 24% yield) as a colourless gum. Rf 0.32 (ethyl acetate/petroleum ether 20:80); [α]D -10.8, (c 0.5 in CHCl₃) [(Lit.,134 [α]D -11.7 (c 1.3 in CHCl₃)]; δH (300 MHz, CDCl₃) 7.40-7.30 (10 H, m, 2 × Ph), 5.95 (1 H, dddd, J 17.3, 10.6, 5.5, 5.5, CH=CH₂), 5.32 (1 H, dddd, J 17.2, 1.7, 1.7, 1.7 CHC=CH), 5.16 (1 H, dddd, J 10.3, 1.7, 1.3, 1.3, CHC=CHH), 5.02 (1 H, d, JAB 11.5, OCHAHB-Ph), 4.95 (1 H, d, JAB' 11.1, OCHAHB-Ph), 4.81 (1 H, d, JAB' 11.1, OCHAHB-Ph), 4.70 (1 H, d, JAB 11.5, OCHAHB-Ph), 4.41 (1 H, dddd, J 12.7, 5.5, 1.7, 1.3, CHH=CH₂), 4.21-4.17 (2 H, m, 1 × CHH=CH₂, 1 × inositol ring), 3.96 (1 H, dd, J 9.7, 9.7, inositol ring), 3.70-3.50 (3 H, m, inositol ring), 3.31 (1 H, dd, J 9.7, 1.8, inositol ring), 2.46 (1 H, d, J 7.6, OH), 2.17 (1 H, dt, J 13.6, 3.9, camphor ring), 1.94-1.90 (1 H, m, camphor ring), 1.78-1.75 (2 H, m, 2 × camphor ring), 1.47 (1 H, d, J 13.5, camphor ring), 1.30-1.23 (2 H, m, 2 × camphor ring), 1.03 (3 H, s, CH₃), 1.28-1.10 (3 H, m, 3 × camphor ring), 0.86 (3 H, s, CH₃ camphor bridge), 0.85 (3 H, s, CH₃ camphor bridge). The data are in good agreement with literature values.134
A solution of the alcohol (-)-1D-5-O-allyl-2,6-bis-O-benzyl-3-O-endo-4-O-exo-(L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidine)-myo-inositol (-)-41 (583 mg, 1.09 mmol, 1 equiv.) in THF (10 mL) was transferred via cannula into a suspension of sodium hydride (60 % dispersion in mineral oil, 66 mg, 1.65 mmol, 1.5 equiv.) in THF (20 mL) at 0 °C. The mixture was stirred at RT for 1 h, then cooled to 0 °C after which N,N-dimethylformamide (10 mL), 4-(methoxy)benzyl chloride (254 mg, 220 μL, 1.64 mmol, 1.5 equiv.) and tetrabutylammonium iodide (catalytic amount) were added. The resulting mixture was allowed to warm to RT and stirred for 24 h. The reaction was then stirred for 24 h at RT. The reaction was quenched with a 10 % aqueous solution of sodium hydrogen carbonate (5 mL). The solvent was removed in vacuo and the resulting residue was reconstituted with ethyl acetate (50 mL) and water (50 mL). The layers were separated and the aqueous layer was extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were washed with brine (50 mL), dried (MgSO₄), filtered and concentrated under reduced pressure to give a yellow oil. Purification by silica gel column chromatography, eluting with ethyl acetate/petroleum ether (10:90) gave (-)-1D-5-O-allyl-2,6-bis-O-benzyl-3-O-endo-4-O-exo-(L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidine)-myo-inositol (-)-56 (577 mg, 83% yield) as a colourless oil. Rf 0.46 (ethyl acetate/petroleum ether 20/80); [α]D²⁰ -18.3, (c 0.5 in CHCl₃) [Lit.₁₃⁴ [α]D²⁰ -20.1 (c 2.6 in CHCl₃)]; δH (300 MHz; CDCl₃) 7.45-7.29 (10 H, m, 2 × Ph), 7.20 (2 H, d, J 8.7, OCH₂C₆H₄OCH₃), 6.82 (2 H, d, J 8.7, OCH₂C₆H₄OCH₃), 5.95 (1 H, dddd, J 17.2, 10.3, 5.6, 5.6, CH=CH₂), 5.32 (1 H, dddd, J 17.2, 1.7, 1.7, 1.7, CH=CHH), 5.18 (1 H, dddd, J 10.3, 1.7, 1.3, 1.3, CH=CHH), 4.91 (1 H, d, JAB, 12.2, OCH₃H₆Ph), 101
4.90 (1 H, d, $J_{AB}$ 10.7, OCH$_A$H$_B$Ph), 4.84 (1 H, d, $J_{AB}$ 10.7, OCH$_A$H$_B$Ph), 4.81 (1 H, d, $J_{AB}$ 12.2, OCH$_A$H$_B$Ph), 4.51 (2 H, s, OCH$_C$H$_4$OCH$_3$), 4.38 (1 H, dddd, $J$ 13.2, 5.6, 1.7, 1.3, CHHCH=CH$_2$), 4.20 (1 H, dddd, $J$ 13.2, 5.6, 1.7, 1.3, CHHCH=CH$_2$), 4.11 (1 H, dd, $J$ 2.8, 1.9, inositol ring), 4.03 (1 H, dd, $J$ 9.6, 9.6, inositol ring), 3.85 (1 H, dd, $J$ 9.6, 9.6, inositol ring), 3.80 (3 H, s, OCH$_3$), 3.52-3.42 (2 H, m, inositol ring), 3.17 (1 H, dd, $J$ 9.1, 9.1, inositol ring), 3.00 (1 H, m, inositol ring), 2.12 (1 H, dt, $J$ 13.6, 3.8, camphor ring), 1.96-1.87 (1 H, m, camphor ring), 1.74-1.70 (2 H, m, camphor ring), 1.43 (1 H, d, $J$ 13.6, camphor ring), 1.44-1.34 (1 H, m, camphor ring), 1.25-1.13 (1 H, m, camphor ring), 1.02 (3 H, m, camphor bridge), 0.86 (3 H, s, camphor bridge), 0.85 (3 H, s, camphor bridge). These data are in good agreement with the literature values.\textsuperscript{134}

(-)-1D-5-O-allyl-2,6-bis-O-benzyl-1-O-(4'-methoxybenzyl)-myo-inositol (-)-40\textsuperscript{141}

Acetyl chloride (46 mg, 42 μL, 0.52 mmol, 0.6 equiv.) was added to a solution of (-)-1D-5-O-allyl-2,6-di-O-benzyl-1-O-(4'-methoxybenzyl)-3-O-endo-4-O-exo-(L-$1',7',7'$-trimethylbicyclo[2.2.1]hept-2'-ylidine)-myo-inositol (-)-56 (550 mg, 0.86 mmol, 1 equiv.) in dichloromethane (12 mL) and methanol (8 mL) and the solution was stirred for 5 h. The reaction mixture was quenched by the addition of triethylamine (1 mL), and all volatile components were removed \textit{in vacuo} to give a colourless solid. Purification by silica gel column chromatography, eluting with ethyl acetate/petroleum ether (20:80, 30:50, 50:50) then 100 % ethyl acetate gave (-)-1D-5-O-allyl-2,6-bis-O-benzyl-1-O-(4'-methoxybenzyl)-myo-inositol (-)-40 (366 mg, 82% yield) as a colourless solid. $R_f$ 0.28 (ethyl acetate/petroleum ether 1:1); mp 124-126 °C, (ethyl acetate/hexane, Lit.\textsuperscript{141} mp 125-126 °C); $[\alpha]_D^{20}$ -24.3, (c 0.14 in CHCl$_3$) [Lit.,\textsuperscript{141} $[\alpha]_D^{20}$ -26.4, (c 1.2 in CHCl$_3$)]; $\delta_H$ (300 MHz; CDCl$_3$) 7.30-7.18 (12 H, m, 10 × Ph and 2 × OCH$_2$C$_6$H$_4$OCH$_3$), 6.80 (2 H, d, $J$ 8.7, OCH$_2$C$_6$H$_4$OCH$_3$), 5.90 (1 H, dddd, $J$ 17.2, 10.2, 5.5, 5.5, CH=CH$_2$), 5.24 (1 H, dddd, $J$ 17.2, 1.7, 1.6, 1.6, CH=CHH), 5.12 (1 H, dddd, $J$ 10.2, 1.7, 1.2, 1.2, CH=CHH), 5.01 (1 H, d, $J_{AB}$ 11.5, OCH$_A$H$_B$Ph), 4.83 (1 H, d, $J_{AB}$ 10.6, OCH$_A$H$_B$Ph), 4.75 (1 H, d, $J_{AB}$ 10.6, OCH$_A$H$_B$Ph), 4.63-4.53 (3 H, m, 2 × OCH$_2$C$_6$H$_4$OCH$_3$ and 1 × OCH$_A$H$_B$Ph), 4.33 (1 H, dddd, $J$ 12.5, 5.5, 1.6, 1.2, CHHCH=CH$_2$), 4.20 (1 H, dddd, $J$ 12.5, 5.5, 1.6, 1.2,
CHHCH=CH₂), 3.92 (1 H, dd, J 2.5, 2.5, inositol ring), 3.88 (1 H, dd, J 9.5, 9.5, inositol ring), 3.75 (3 H, s, OCH₃), 3.75-3.71 (1 H, m, inositol ring), 3.37 (1 H, dd, J 9.7, 2.5, inositol ring), 3.31-3.28 (1 H, m, inositol ring), 3.13 (1 H, dd, J 9.2, 9.2, inositol ring), 2.43 (1 H, br s, OH), 2.18 (1 H, d, J 1.8, OH). These data are in good agreement with the literature values.¹⁴¹

(-)-1D-3,5-bis-O-allyl-2,6-bis-O-benzyl-1-O-(4'-methoxybenzyl)-myo-inositol (-)-39

A mixture of (-)-1D-5-O-allyl-2,6-bis-O-benzyl-1-O-(4'-methoxybenzyl)-myo-inositol (-)-40 (288 mg, 0.56 mmol, 1 equiv.) and dibutyltin oxide (279 mg, 1.12 mmol, 2 equiv.) in toluene (12 mL) was heated under reflux, using Dean-Stark apparatus for azeotropic removal of water, for 24 h. The solvent was removed in vacuo and the residue dissolved in N,N-dimethylformamide (10 mL). Allyl bromide (531 mg, 380 μL, 4.45 mmol, 8.0 equiv.) and caesium fluoride (170 mg, 1.12 mmol, 2.0 equiv.) were both added and the reaction mixture was stirred for 24 h. Solvents and any remaining volatile compounds were removed under reduced pressure and the resultant solid material was partitioned between ethyl acetate (30 mL) and water (30 mL). The aqueous layer was extracted with ethyl acetate (3 × 30 mL). The combined organic extracts were washed with saturated aqueous sodium bicarbonate solution (30 mL) then brine (30 mL), dried (MgSO₄), filtered and concentrated under reduced pressure. Exhaustive purification by silica gel column chromatography, eluting with ethyl acetate/petroleum ether (10:90, 15:85 then 20:80) gave (-)-1D-3,5-bis-O-allyl-2,6-bis-O-benzyl-1-O-(4'-methoxybenzyl)-myo-inositol (-)-39 (116 mg, 37% yield) as a colourless solid. Rf 0.54 (ethyl acetate/petroleum ether 60/40); mp 55-57 °C (diethyl ether/hexane); [α]D²⁰ -3.02 (c 0.5 in CHCl₃); νmax (thin film)/cm⁻¹ 2924 (m), 2874 (m), 1515 (w), 1254 (w), 1077 (m); δH (300 MHz; CDCl₃) 7.34-7.18 (10 H, m, 2 × Ph), 7.19 (2 H, d, J 8.6, OCH₂C₆H₄OCH₃), 6.79 (2 H, d, J 8.6, OCH₂C₆H₄OCH₃), 5.89 (1 H, dddd, J 17.2, 10.4, 5.6, 5.6, CH=CH₂), 5.66 (1 H, dddd, J 17.2, 10.4, 5.6, 5.6, CH=CH₂), 5.23 (1 H, dddd, J 17.2, 1.7, 1.7, 1.7, CH=CH₂Hₖ), 5.18 (1
H, dddd, J 17.2, 1.7, 1.7, 1.7, CH=CH₂H₂), 5.11 (1 H, dddd, J 10.4, 1.7, 1.3, 1.3, CH=CH₂H₂), 5.08 (1 H, dddd, J 10.4, 1.7, 1.3, 1.3, CH=CH₂H₂), 4.83 (1 H, d, J_AB, 10.5, OCH₃H₂Ph), 4.80 (1 H, d, J_AB, 12.1, OCH₃H₆Ph), 4.74 (1 H, d, J_AB, 10.5, OCH₃H₆Ph), 4.75 (1 H, d, J_AB, 12.1, OCH₃H₆Ph), 4.56 (1 H, d, J 11.4, OCH₂C₆H₄OCH₃), 4.50 (1 H, d, J 11.4, OCH₂C₆H₄OCH₃), 4.30-4.26 (2 H, m, CH₂(CH=CH₂), 4.01-3.96 (5 H, m, 2 × CH₂CH₂CH=CH₂ and 3 × inositol ring), 3.74 (3 H, s, OCH₃), 3.27 (1 H, dd, J 9.7, 2.3, inositol ring), 3.16 (1 H, dd, J 9.2, 9.2, inositol ring), 3.01 (1 H, dd, J 9.9, 2.3, inositol ring), 2.43 (1 H, d, J 1.5 OH); δ_C (75 MHz, CDCl₃); 159.6 (C), 139.3 (C), 135.7 (CH), 134.9 (CH), 130.9 (C), 129.6 (2C, C₆H₄), 128.7 (CH), 128.6 (CH), 128.5 (CH), 128.2 (CH), 128.0 (CH), 127.8 (CH), 117.8 (CH₂), 117.2 (CH₂), 114.2 (2C, C₆H₄), 83.3 (CH), 81.8 (CH), 81.2 (CH), 80.1 (CH), 76.2 (CH₂), 74.6 (CH₂), 74.4 (CH₂), 73.7 (CH), 73.0 (CH₂), 72.9 (CH), 71.5 (CH₂), 55.7 (CH₃); HRMS m/z (ES+) [Found: (M+Na)⁺ 583.2655. C₃₄H₄₀O₇Na requires M⁺ 583.2672], m/z (ES⁺) 583 ([M+Na]⁺, 100%).

**Tetramethyl biphosphine disulfide 61**

Thiophosphoryl chloride (8.3 g, 5.0 mL, 49.2 mmol, 1 equiv.) was added dropwise over a period of 40 min to a cooled solution of methylmagnesium bromide (3 M solution in diethyl ether, 66 mL, 4.0 equiv.). The reaction was very exothermic and cautious addition of the thiophosphoryl chloride ensured the reaction mixture temperature remained between 0 °C and 8 °C. Following addition, the mixture was stirred at room temperature for 20 min, then heated under reflux for 1 h. The mixture was poured into a flask containing ice and conc. HCl (10 mL), and diluted with diethyl ether (100 mL). The colourless precipitate thus formed was filtered from the organic/aqueous mixture and recrystallised from ethanol to give the first batch of tetramethyl biphosphine disulfide product. The organic and aqueous mixture was then separated and the aqueous layer extracted with diethyl ether (3 × 100 mL). The combined organic extracts were washed with water (50 mL), concentrated under reduced pressure and the crude product recrystallised from ethanol and combined with the first crystallised batch to give tetramethyl biphosphine disulfide 61 (2.13 g, 23% yield) as a colourless crystalline solid.
mp 220-222 °C (ethanol), (Lit.\textsuperscript{146} mp 221-223 °C); $\delta_H$ (300 MHz; CDCl\textsubscript{3}) 2.05-1.90 (12 H, m, 4 $\times$ CH\textsubscript{3}); $\delta_P$ (122 MHz, CDCl\textsubscript{3}) 36.5 (2 P, s); $m/z$ (ES+) 209 ([M+Na]\textsuperscript{+}, 100%). The data are in good agreement with the literature values.\textsuperscript{146}

**Dimethylphosphinic chloride 62\textsuperscript{147}**

Thionyl chloride (2.04 g, 1.25 mL, 17.1 mmol, 4.8 equiv.) was added dropwise to a cooled suspension of tetramethyl biphosphine disulfide 61 (665 mg, 3.57 mmol, 1 equiv.) in dry toluene (4 mL) at -5 °C. The mixture turned a peach colour during initial reaction. Following complete mixing of reactants, the mixture was stirred for 30 min at room temperature, then heated under reflux for 1 h with the mixture turning a yellow colour on heating. Analysis by $^{31}$P NMR showed complete consumption of the tetramethyl biphosphine disulfide. Most of the toluene and excess thionyl chloride were removed \textit{in vacuo}. Kugelrohl distillation of the crude yellow residue first yielded a yellow liquid impurity (70 °C, 32 Torr) then dimethylphosphinic chloride 62 (140 °C, 28 Torr) as a colourless solid (458 mg, 57% yield) which was stored in a desiccator under nitrogen. mp – material unstable to air; $\delta_H$ (300 MHz; CDCl\textsubscript{3}) 2.05 (6 H, d, $J_{P,H}$ 13.6, 2 $\times$ CH\textsubscript{3}); $\delta_P$ (122 MHz, CDCl\textsubscript{3}) 61.0. The data are in agreement with the literature values.\textsuperscript{147}

\textbf{(-)-1D-3,5-bis-O-Allyl-2,6-bis-O-benzyl-1-O-(4'-methoxybenzyl)-4-O-(dimethylphosphinyl)-myo-inositol (-)-38}

A solution of dimethylphosphinic chloride (46 mg, 0.41 mmol, 4 equiv.) in N,N-dimethylformamide (1 mL) was added \textit{via} cannula to a stirred solution of alcohol (-)-1D-3,5-bis-O-allyl-2,6-bis-O-benzyl-1-O-(4'-methoxybenzyl)-myo-inositol (-)-39 (57 mg, 0.10 mmol, 1 equiv.) and 2,6-lutidine (31 mg, 23 μL, 0.20 mmol, 2 equiv.) in N,N-dimethylformamide (2 mL) at -5 °C. The mixture was allowed to slowly warm to room
temperature and stirred for 20 h, after which time TLC analysis showed the consumption of starting materials. The solvent was removed in vacuo to give a light brown solid. Purification by silica gel column chromatography, eluting with methanol/dichloromethane (2:98) gave 1D-3,5-bis-O-allyl-2,6-bis-O-benzyl-1-O-(4’-methoxybenzyl)-4-O-(dimethylphosphinyl)-myo-inositol (-)-38 (58 mg, 89% yield) as a colourless crystalline solid. Rf 0.49 (methanol/dichloromethane 5:95); mp 79-82 °C (diethyl ether/hexane); $[\alpha]_{D}^{20}$ -0.24 (c 0.42 in CHCl$_3$); $\nu_{\max }$ (nujol)/cm$^{-1}$: 2726 (w), 1211 (m), 817 (w); $\delta_{H}$ (300 MHz; CDCl$_3$) 7.36-7.20 (10 H, m, 2 × Ph), 7.19 (2 H, d, J 8.7, OCH$_2$C$_6$H$_4$OCH$_3$), 6.81 (2 H, d, J 8.6, OCH$_2$C$_6$H$_4$OCH$_3$) 5.97-5.74 (2 H, 2 × CH=CH$_2$), 5.25-5.23 (1 H, m, CH=CH$_2$), 5.20-5.18 (1 H, m, CH=CH$_2$), 5.12-5.09 (2 H, m, CH=CH$_2$), 4.84 (1 H, d, $J_{AB}$ 10.6, OCH$_3$H$_4$BPh), 4.77 (2 H, s, OCH$_2$Ar), 4.74 (1 H, d, $J_{AB}$ 10.6, OCH$_3$H$_4$BPh), 4.56-4.46 (1 H, m, CH$_2$CH=CH$_2$) 4.51 (1 H, s, CH$_2$Ar), 4.49 (1 H, s, CH$_2$Ar), 4.36-4.29 (1 H, m, CH$_2$CH=CH$_2$), 4.25-4.17 (1 H, m, CH$_2$CH=CH$_2$), 3.98-3.90 (4 H, m, 1 × CH$_2$CH=CH$_2$, 3 × inositol ring), 3.76 (3 H, s, OCH$_3$), 3.24 (1 H, dd, J 9.3, 9.3, inositol ring), 3.28-3.21 (1 H, m, inositol ring), 3.10 (1 H, dd, J 9.9, 2.2, inositol ring), 1.50 (3 H, d, $^{1}J_{P-H}$ 14.0, P(O)CH$_3$), 1.46 (3 H, d, $^{1}J_{P-H}$ 14.0, P(O)CH$_3$); $\delta_{C}$ (75 MHz, CDCl$_3$) 159.6 (C), 139.1 (C), 139.06 (C), 135.4 (CH), 134.7 (CH), 130.7 (C), 129.7 (CH), 128.8 (CH), 128.6 (CH), 128.5 (CH), 128.3 (CH), 128.0 (CH), 127.9 (CH), 118.0 (CH$_2$), 117.1 (CH$_2$), 114.2 (CH), 82.0 (1 C, d, $^{3}J_{P-C}$ 2.3, CH), 81.8 (CH), 80.6 (CH), 79.1 (CH), 76.6 (1 C, d, $^{2}J_{P-C}$ 8.3, CH), 76.2 (CH$_2$), 74.7 (CH$_2$), 74.5 (CH$_2$), 73.7 (CH), 72.9 (CH$_2$), 71.7 (CH$_2$), 55.7 (CH$_3$), 17.7 (1 C, d, $^{1}J_{P-C}$ 93.0, PCH$_3$), 17.6 (1 C, d, $^{1}J_{P-C}$ 93.0, PCH$_3$); $\delta_{P}$ (122 MHz, CDCl$_3$) 54.8; HRMS m/z (ES+) [Found: (M+Na)$^{+}$ 659.2736. C$_{36}$H$_{45}$O$_{8}$PNa requires $M^{+}$ 659.2750]; m/z (ES+) 659 ([M+Na]$^{+}$, 100%).
Attempted synthesis of 1D-2,6-bis-O-benzyl-1-O-(4'-methoxybenzyl)-4-O-(dimethylphosphinyl)-3,5-bis(dibenzylphosphate)-myo-inositol 37

A mixture of 1D-3,5-bis-O-allyl-2,6-bis-O-benzyl-1-O-(4'-methoxybenzyl)-4-O-(dimethylphosphinyl)-myo-inositol (41 mg, 0.065 mmol, 1.0 equiv.), palladium on carbon (10 % w/w, 10 mg) and 4-toluenesulfonic acid (7 mg, 0.039 mmol, 0.6 equiv.) in methanol (2 mL) and water (0.5 mL) was heated under reflux for 18 h. The mixture was then filtered through a pad of Celite and the filtrate concentrated under reduced pressure. Purification by silica gel column chromatography, eluting with dichloromethane then methanol/dichloromethane (1:99 then 2:98) gave 1D-2,6-bis-O-benzyl-1-O-(4'-methoxybenzyl)-4-O-(dimethylphosphinyl)-myo-inositol 63 (11.5 mg, 32 % yield) as a yellow oil. Analysis by 1H and 31P NMR confirmed de-allylation was complete and only the desired product was formed as a colourless gum. Rf 0.22 (methanol/dichloromethane 5:95); 6H (300 MHz; CDCl3) 7.40-7.22 (12 H, m, 2 × Ph, 2 × OCH2C6H4OCH3), 6.86 (2 H, d, J 8.7, CH2C6H4OCH3), 4.98 (2 H, d, J 11.2, CH2Ph), 4.81 (1 H, d, J 11.7, CH2Ph), 4.79 (1 H, d, J 11.2, CH2Ph), 4.58 (2 H, s, CH2Ar), 4.36 (1 H, ddd, J 9.1, 9.1, 9.1, inositol ring 4-H), 4.04 (1 H, dd, J 2.4, 2.4, inositol ring 2-H), 3.94-3.84 (2 H, m, 2 × inositol ring), 3.81 (3 H, s, OCH3), 3.56-3.49 (2 H, m, 1 × OH), 3.45 (1 H, dd, J 9.8, 2.4, inositol ring), 1.60 (3 H, d, J 14.1, P(O)CH3), 1.55 (3 H, d, J 14.1, P(O)CH3); 6P (122 MHz, CDCl3) 60.8. The product was then used immediately for the next step. 1H-Tetrazole in acetonitrile (3 % w/v, 209 μL, 0.09 mmol, 5.0 equiv.) was added to a solution of bis(benzyloxy)-N,N-diisopropylamino phosphine 65 (31 mg, 0.09 mmol, 5.0 equiv.) in dichloromethane (0.8 mL) and the mixture stirred for 20 min. A solution of 1D-2,6-bis-O-benzyl-1-O-(4'-methoxybenzyl)-4-O-(dimethylphosphinyl)-myo-inositol 63 (10 mg, 0.018 mmol, 1.0 equiv.) in dichloromethane (0.6 mL) was added via cannula and the resulting mixture stirred for 20 h. The mixture was cooled to -78 °C and 3-chloroperbenzoic acid (assume 60 %, 26 mg, 0.09 mmol, 5 equiv.) was added. The
reaction mixture was allowed to reach RT and stirred for 3 h. The 3-chloroperbenzoic acid was quenched with a 10 % aqueous solution of sodium bisulfite (30 mL). The mixture was stirred for 10 min and the layers separated. The aqueous layer was extracted with dichloromethane (3 × 50 mL). The combined organic extracts were washed with saturated aqueous sodium bicarbonate solution (100 mL), brine (100 mL), dried (MgSO₄), filtered and concentrated under reduced pressure. Purification by silica gel chromatography, eluting with dichloromethane then methanol/dichloromethane (1:99) gave an inseparable mixture of products (8 mg) as a yellow gum.

**Benzyloxy bis(N,N-diisopropylamino)phosphine 64**

\[ \text{BnO} - \text{P} \]
\[ \text{N} \cdot \text{Pr}_2 \]

Dry pyridine (16.7 mL, 206 mmol, 1.0 equiv.) was added to a stirred solution of phosphorus trichloride (18.0 mL, 206 mmol, 1.0 equiv.) in dry diethyl ether (200 mL). The mixture was cooled to -78 °C and a solution of benzyl alcohol (21 mL, 206 mmol, 1.0 equiv.) in diethyl ether (150 mL) was added slowly over 1 h. The mixture was allowed to reach RT and stirred for 1.5 h. The resulting colourless precipitate was removed by filtration under a blanket of nitrogen and washed with dry diethyl ether (2 × 50 mL). The filtrate was placed under nitrogen and cooled to -10 °C. Dry \(N,N\)-diisopropylamine (111 mL, 855 mmol, 4.1 equiv.) was added dropwise over 15 min. The mixture was allowed to reach RT and stirred for 20 h. The resulting precipitate was removed by filtration under an a blanket of nitrogen and the filtrate was concentrated under reduced pressure to give benzyloxy bis(\(N,N\)-diisopropylamino)phosphine 64 (43.0 g, 62% yield) as a yellow oil. The crude product was stored in the fridge under an atmosphere of nitrogen. \(\delta_H\) (300 MHz; CDCl₃) 7.40-7.20 (5 H, m, Ph), 4.67 (2 H, d, J 7.3, OCH₂Ph), 3.65-3.50 (4 H, m, NCH(CH₃)₂), 1.20 (24 H, dd, J 6.7, 3.5, NCH(CH₃)₂); \(\delta_P\) (122 MHz, CDCl₃) 124.8. The data are in good agreement with literature values.¹⁷⁹
**Bis(benzyloxy)-N,N-diisopropylamino phosphine 65**

$\text{BnO} \quad \text{Me}_2\text{N} \quad \text{BnO}$

$\text{H}_2\text{N} \quad \text{BnO} \quad \text{Me}_2\text{N}$

$1H$-Tetrazole in acetonitrile (3 % w/v, 73 mL, 31.3 mmol, 0.4 equiv.) was added to a stirred solution of benzyloxy bis($N,N$-diisopropylamino)phosphine (26.50 g, 78.3 mmol, 1.0 equiv.) in dichloromethane (200 mL). Dry benzyl alcohol (7.62 g, 7.30 mL, 70.5 mmol, 0.9 equiv.) was added dropwise over a period of 40 min. The resulting mixture was stirred for 2 h. The solvent was removed *in vacuo* to give a colourless paste which was purified by silica gel column chromatography, eluting with triethylamine, ethyl acetate and petroleum ether (5:15:80) to give bis(benzyloxy)-$N,N$-diisopropylamino phosphine **65** (19.30 g, 71% yield) as a colourless oil. $\delta_H$ (300 MHz; CDCl$_3$) 7.40-7.24 (10 H, m, Ph), 4.81 (2 H, dd, $J_{12.7, 8.2}$, OCH$_A$H$_B$Ph), 4.72 (2 H, dd, $J_{12.7, 8.6}$, OCH$_A$H$_B$Ph), 3.78-3.65 (2 H, m, NCH(CH$_3$)$_2$), 1.23 (12 H, d, $J_{6.8}$, NCH(CH$_3$)$_2$); $\delta_P$ (122 MHz, CDCl$_3$) 148.8. The data are in good agreement with literature values.$^{150}$

**(+)-5-O-Allyl-2,6-bis-O-benzyl-3,4-isopropylidine-myoinositol 71**

The triol (+)-5-O-allyl-2,6-O-dibenzyl-myoinositol **42** (11.07 g, 27.68 mmol) and 4-toluenesulfonic acid monohydrate (1.58, 8.3 mmol, 0.3 equiv.) were dissolved in $N,N$-dimethylformamide (150 mL). 2,2-Dimethoxypropane (86.3 g, 102 mL, 830 mmol, 30 equiv.) was added to the solution and the mixture was allowed to stir at RT for 5 h. The reaction was quenched with triethylamine (5 mL) and all volatile components were removed *in vacuo*. The resulting oil was partitioned between ethyl acetate (50 mL) and water (50 mL). The aqueous phase was extracted with ethyl acetate (3 × 50 mL) and the combined organic extracts were washed with aqueous saturated sodium bicarbonate (100 mL), brine (100 mL), dried (MgSO$_4$), filtered and concentrated. The resulting crude product was adsorbed onto silica gel and purified by silica gel column chromatography, eluting with ethyl acetate and petroleum ether (20:80 then 30:70) to give (+)-5-O-allyl-2,6-bis-O-benzyl-3,4-isopropylidine-myoinositol **71** (10.8 g, 89% yield) as a colourless
solid. \( R_f \) 0.28 (ethyl acetate/petroleum ether 25:75); mp 88-90 °C (ethyl acetate/petroleum ether); \( \nu_{\text{max}} \) (thin film)/cm\(^{-1} \) 3438 (m), 2923 (m), 2874 (m), 1719 (w), 1369 (m), 1047 (m); \( \delta_H \) (300 MHz, CDCl\(_3\)) 7.40-7.29 (10 H, m, 2 × Ph), 5.96 (1 H, dddd, \( J \) 17.3, 10.5, 5.5, 5.5, \( CH=CH_2 \)), 5.30 (1 H, dddd, \( J \) 17.3, 1.7, 1.6, 1.6, \( CH=CH_H \)), 5.15 (1 H, dddd, \( J \) 10.5, 1.7, 1.3, 1.3, \( CH=CH_H \)), 5.03 (1 H, d, \( J_{AB} 11.3 \), O\( CH_AH_B-\)Ph), 4.92 (1 H, d, \( J_{AB} 10.9 \), O\( CH_AH_B-\)Ph), 4.83 (1 H, d, \( J_{AB} 10.9 \), O\( CH_AH_B-\)Ph), 4.68 (1 H, d, \( J_{AB} 11.3 \), O\( CH_AH_B-\)Ph), 4.40 (1 H, dddd, \( J \) 12.7, 5.5, 1.6, 1.3, \( CHCH=CH_2 \)), 4.24-4.20 (1 H, m, CH\( HHCH=CH_2 \)), 4.19-4.12 (2 H, m, inositol ring 2-H, 4-H), 3.71-3.67 (1 H, m, inositol ring 1-H), 3.66-3.53 (2 H, m, inositol ring 5-H, 6-H), 3.49 (1 H, dd, \( J \) 9.9, 1.8, inositol ring 3-H), 2.43 (1 H, d, \( J \) 7.8, OH), 1.48 (3 H, s, CH\(_3\)), 1.45 (3 H, s, CH\(_3\)); \( \delta_C \) (75 MHz, CDCl\(_3\)) 139.1 (C), 138.5 (C), 135.4 (CH), 128.8 (CH), 128.5 (CH), 128.2 (CH), 128.2 (CH), 128.1 (CH), 117.1 (CH\(_2\)), 112.2 (C), 84.6 (CH), 80.6 (CH), 78.3 (CH), 77.0 (CH), 76.4 (CH\(_2\)), 74.8 (CH), 74.7 (CH\(_2\)), 74.0 (CH), 72.6 (CH\(_2\)), 27.5 (CH\(_3\)), 27.1 (CH\(_3\)); HRMS \( m/z \) (ES+) [Found: (M+Na)\(^+\) 463.2079. C\(_{26}\)H\(_{32}\)O\(_6\)Na requires M\(^+\) 463.2097], \( m/z \) (ES+) 463 ([M+Na]\(^+\), 100%); Anal. Calcd for C\(_{26}\)H\(_{32}\)O\(_6\): C, 70.89; H 7.32. Found: C, 70.86; H, 7.40.

(±)-5-O- Allyl-2,6-bis-O-benzyl-3,4-O-isopropylidene-1-O-triisopropylsilyl-myoinositol 72

Triisopropylsilyl triflate (15.60 g, 50.8 mmol, 2.2 equiv.) was added to a stirred solution of the (±)-5-O-allyl-2,6-bis-O-benzyl-3,4-O-isopropylidene-myoinositol 71 (10.17 g, 23.1 mmol, 1.0 equiv.) and 2,6-lutidine (12.40 g, 13.4 mL, 115.5 mmol, 5.0 equiv.) in dichloromethane (200 mL) at 0 °C. The mixture was allowed to warm to RT and stirred for 36 h. Water (100 mL) was added to the mixture and the organic and aqueous phases separated. The organic phase was collected and the aqueous portion extracted with dichloromethane (3 × 100 mL). The combined organic extracts were washed with brine (200 mL), then dried (MgSO\(_4\)), filtered and concentrated under reduced pressure. Purification by silica gel column chromatography, eluting with ethyl acetate/petroleum
ether (5:95), gave (±)-5-O-allyl-2,6-bis-O-benzyl-3,4-isopropylidene-1-O-triisopropylsilyl-
myo-inositol 72 (12.9 g, 94% yield) as a colourless gum. \( R_f \) 0.64 (ethyl acetate/petroleum ether 30:70); \( \nu_{\text{max}} \) (thin film)/cm\(^{-1}\) 3031 (w), 2867 (s), 1454 (m), 1370 (m), 1086 (s); \( \delta_H \) (300 MHz, CDCl\(_3\)) 7.41-7.24 (10 H, m, 2 \( \times \) Ph), 5.89 (1 H, dddd, \( J \) 17.2, 10.5, 5.4, \( CH=CH_2 \)), 5.26 (1 H, dddd, \( J \) 17.2, 1.7, 1.7, 1.7, \( CH=CHH \)), 5.11 (1 H, dddd, \( J \) 10.5, 1.7, 1.3, 1.3, \( CH=CHH \)), 4.98 (1 H, d, \( J_{AB} \) 11.3, OCH\(_A\)H\(_B\)-Ph), 4.94 (1 H, d, \( J'_{A'} \) 11.3, OCH\(_A\)H\(_B\)-Ph), 4.81 (1 H, d, \( J_{A'B'} \) 11.2, OCH\(_A\)H\(_B\)-Ph), 3.92 (1 H, ddd, \( J \) 12.6, 5.4, 1.7, 1.3, CHHCH=CH\(_2\)), 3.87 (1 H, d, \( J \) 9.7, 9.7, inositol ring), 3.92 (1 H, ddd, \( J \) 12.6, 5.4, 1.7, 1.3, CHHCH=CH\(_2\)), 4.14-4.12 (1 H, m, inositol ring), 3.77 (1 H, dd, \( J \) 8.7, inositol ring), 3.55 (1 H, dd, \( J \) 9.6, 8.3, inositol ring), 3.46 (1 H, dd, \( J \) 9.8, 1.5, inositol ring), 1.45 (6 H, s, 2 \( \times \) CCH\(_3\)), 1.08-1.06 (21 H, m, 3 \( \times \) SiCH, 18 \( \times \) SiCHCH\(_3\)); \( \delta_C \) (75 MHz, CDCl\(_3\)) 139.7 (C), 139.3 (C), 135.5 (CH), 128.5 (CH), 128.4 (CH), 127.9 (CH), 127.8 (CH), 127.7 (CH), 127.5 (CH), 116.9 (CH\(_2\)), 112.1 (C), 83.4 (CH), 81.4 (CH), 78.3 (CH), 77.3 (CH), 76.5 (CH), 76.3 (CH\(_2\)), 75.2 (CH), 74.2 (CH\(_2\)), 72.6 (CH\(_2\)), 27.6 (CH\(_3\)), 27.1 (CH\(_3\)), 18.6 (CH\(_3\)), 13.3 (CH); HRMS \( m/z \) (ES+) [Found: (M+Na)]\(^+\) 619.3455. C\(_{35}\)H\(_{52}\)O\(_6\)NaSi requires \( M^+ \) 619.3431, \( m/z \) (ES+) 619 [(M+Na)]\(^+\), 100%).

(±)-5-O-Allyl-2,6-bis-O-benzyl-1-O-triisopropylsilyl-myo-inositol 70

A mixture of (±)-5-O-allyl-2,6-bis-O-benzyl-3,4-isopropylidene-1-O-triisopropylsilyl-myoinositol 72 (26.2 g, 43.96 mmol, 1.0 equiv.) and 4-toluenesulfonic acid monohydrate (4.18 g, 21.98 mmol, 0.5 equiv.) in methanol (300 mL) was stirred at RT for 2.5 h. The reaction was quenched with triethylamine (15 mL) and all volatile components were removed in vacuo. Purification of the crude product by silica gel column chromatography, eluting with ethyl acetate/petroleum ether (25:75 then 30:70) gave (±)-5-O-allyl-2,6-bis-O-benzyl-1-O-triisopropylsilyl-myoinositol 70 (23.3 g, 95% yield) as a colourless oil. \( R_f \) 0.18 (ethyl acetate/petroleum ether 30:70); \( \nu_{\text{max}} \) (thin film)/cm\(^{-1}\) 3421 (m), 2943 (s), 1457 (m), 1060 (s); \( \delta_H \) (300 MHz, CDCl\(_3\)) 7.39-7.23 (10 H, m, 2 \( \times \) Ph), 5.88 (1 H, dddd, \( J \) 17.1, 10.4, 5.7, 5.7, \( CH=CH_2 \)) 5.20 (1 H, dddd, \( J \) 17.1, 1.7, 1.6, 1.6,
CH=CH₂), 5.10 (1 H, d, J_{AB} 11.4, OCH₆H₆-Ph), 5.12-5.07 (1 H, m, CH=CH₂), 4.91 (1 H, d, J_{AB} 11.7, OCH₆H₆-Ph), 4.86 (1 H, d, J_{AB} 11.7, OCH₆H₆-Ph), 4.75 (1 H, d, J_{AB} 11.3, OCH₆H₆-Ph), 4.30 (1 H, dddd, J 12.3, 5.7, 1.6, 1.3, CHCH=CH₁), 4.24 (1 H, dddd, J 12.3, 5.7, 1.6, 1.3, CHHCH=CH₂), 4.01-3.99 (1 H, m, inositol ring 2-H), 3.87-3.84 (2 H, m, 2 × inositol ring 1-H, 6-H), 3.81 (1 H, dd, J 9.3, 1.2, inositol ring 4-H), 3.46 (1 H, dddd, J 10.0, 7.8, 2.7, inositol ring 3-H), 3.24-3.14 (1 H, m, inositol ring 5-H), 2.63 (1 H, d, J 1.9, OH), 2.42 (1 H, d, J 7.8, OH), 1.10-1.07 (21 H, m, 3 × SiCH, 18 × SiCHCH₃); δ_C (75 MHz, CDCl₃) 139.1 (C), 139.0 (C), 135.1 (CH), 128.4 (CH), 128.1 (CH), 127.6 (CH), 127.4 (CH), 127.1 (CH), 116.9 (CH₂), 83.1 (CH), 81.7 (CH), 81.5 (CH), 75.3 (CH₂), 75.2 (CH₂), 74.5 (CH), 74.3 (CH), 74.3 (CH₂), 72.4 (CH), 18.3 (CH₃), 12.9 (CH); HRMS m/z (ES⁺) [Found: (M+Na)⁺ 579.3100. C₃₂H₄₈O₆NaSi requires M⁺ 579.3118], m/z (ES⁺) 579 ([M+Na]^+, 100%).

(±)-3-O-Benzoyl-5-O-allyl-2,6-bis-O-benzyl-1-O-triisopropylsilyl-myoinositol 73

A mixture of (±)-5-O-allyl-2,6-bis-O-benzyl-1-O-triisopropylsilyl-myoinositol 70 (2.45 g, 4.41 mmol, 1.0 equiv.) and dibutyltin oxide (3.29 g, 13.22 mmol, 3.0 equiv.) in dry toluene (40 mL) was heated under reflux for 16 h with Dean-Stark apparatus for the azeotropic removal of water. The resulting solution was allowed to cool to RT and 4 Å molecular sieves were added. The mixture was cooled to -5 °C and benzoyl chloride (1.2 g, 1.0 mL, 8.8 mmol, 2.0 equiv.) was added slowly. After 10 min, the mixture was warmed to RT and stirred for 2.5 h. The molecular sieves were removed by filtration and the toluene removed in vacuo. The residue was partitioned between ethyl acetate (30 mL) and water (20 mL). The organic layer was collected and the aqueous phase extracted with ethyl acetate (3 × 30 mL). The combined organic extracts were washed with aqueous saturated sodium bicarbonate solution (80 mL), saturated aqueous ammonium chloride solution (80 mL), brine (80 mL), dried (MgSO₄), filtered and concentrated under reduced pressure. Purification by silica gel column chromatography, eluting with ethyl acetate/petroleum ether (10:90) gave (±)-3-O-benzoyl-5-O-allyl-2,6-bis-O-benzyl-1-O-triisopropylsilyl-myoinositol 73 (2.1 g, 73% yield) as a colourless solid. R_f
0.56 (ethyl acetate/petroleum ether 30:70); mp 94-96 °C (dichloromethane/petroleum ether); \( \nu_{\text{max}} \) (thin film)/cm\(^{-1} \) 3518 (m), 2948 (m), 1696 (s), 1101 (m); \( \delta \text{H} \) (300 MHz, CDCl\(_3\)) 8.10-8.05 (2 H, m, C\(_6\)H\(_5\)C(O)CH), 7.61-7.56 (1 H, m, C\(_6\)H\(_5\)C(O)CH), 7.47-7.42 (2 H, m, C\(_6\)H\(_5\)C(O)CH), 5.22 (1 H, dddd, J 17.1, 1.7, 1.6, 1.6, CH=CH\(_2\)), 5.00 (1 H, dd, J 10.3, 2.4, inositol ring 3-H), 4.90 (2 H, s, OCH\(_2\)-Ph), 4.86 (1 H, d, J\(_{AB}\) 11.2, OCH\(_A\)H\(_B\)-Ph), 4.76 (1 H, d, J\(_{AB}\) 11.4, OCH\(_A\)H\(_B\)-Ph), 4.36 (1 H, dddd, J 12.5, 5.6, 1.6, 1.1, CHHCH=CH\(_2\)), 4.27-4.28 (1 H, m, inositol ring 4-H), 4.25 (1 H, dddd, J 12.5, 5.6, 1.6, 1.1, CHHCH=CH\(_2\)), 4.15 (1 H, dd, J 2.4, 2.4, inositol ring 2-H), 4.00-3.92 (2 H, m, inositol ring 1-H, 6-H), 3.31 (1 H, dd, J 8.7, 8.7, inositol ring 5-H), 2.36 (1 H, br s, OH), 1.10-1.07 (21 H, m, 3 × SiC\(_H\)), 18 × SiCHC\(_3\)); \( \delta \text{C} \) (75 MHz, CDCl\(_3\)) 166.7 (C=O), 139.5 (C), 139.3 (C), 135.4 (CH), 133.7 (CH), 130.3 (CH), 130.2 (CH), 128.9 (CH), 128.6 (CH), 128.5 (CH), 127.8 (CH), 127.6 (CH), 127.5 (CH), 117.4 (CH\(_2\)), 84.3 (CH), 81.9 (CH), 80.8 (CH), 75.74 (CH\(_2\)), 75.7 (CH\(_2\)), 74.9 (CH\(_2\)), 74.8 (CH), 74.3 \( m/z \) (ES+) [Found: (M+Na)+ 683.3392, m/z (ES+) 683 ([M+Na]+, 100%); Anal. Calcd for C\(_{39}\)H\(_{52}\)O\(_7\)NaSi: 70.87; H 7.93. Found: C, 70.45; H, 7.82.

(±)-3-0-Benzoyl-5-0-allyl-2,6-bis-O-benzyl-4-0-(4'-methoxybenzyl)-1-O-triisopropylsilyl-my o-inositol 74

To a solution of 4-methoxybenzyltrichloroacetimidate (10.94 g, 38.73 mmol, 1.5 equiv.) and (±)-3-O-benzoyl-5-O-allyl-2,6-bis-O-benzyl-4-O-(4'-methoxybenzyl)-1-O-triisopropylsilyl-myo-inositol 73 (17.04 g, 25.82 mmol, 1.0 equiv.) and 4 Å molecular sieves in toluene (500 mL) was added yttrium (III) triflate (110 mg, 0.206 mmol, 0.008 equiv.) at -78 °C. The mixture was stirred at -78 °C for 30 min, then at -40 °C. After 5 h at this temperature the mixture was re-cooled to -78 °C. 4-Methoxybenzyltrichloroacetimidate (7.29 g, 5.36 mL, 25.82 mmol, 1.0 equiv.) and yttrium(III) triflate (55 mg, 0.103 mmol, 0.004 equiv.) were added, and the mixture was allowed to warm slowly to RT overnight. After 16 h the mixture was again cooled to -78 °C and 4-methoxybenzyl-trichloroacetimidate (3.65 g, 2.70 mL,
12.91 mmol, 0.5 equiv.) was added. The reaction mixture was quenched by the addition of saturated aqueous sodium bicarbonate solution (1 mL) and filtered through a pad of Celite. The filtrate was concentrated under reduced pressure, and the residue partitioned between ethyl acetate (100 mL) and water (100 mL). The aqueous phase was extracted with ethyl acetate (3 × 100 mL) and the combined organic fractions were washed with saturated aqueous sodium bicarbonate solution (150 mL) and brine (150 mL), dried (MgSO$_4$), filtered and concentrated under reduced pressure. Exhaustive purification by silica gel column chromatography eluting with ethyl acetate/petroleum ether (2.5:97.5 then 5:95) gave (±)-3-O-benzoyl-5-O-allyl-2,6-bis-O-benzyl-4-O-(4'-methoxybenzyl)-1-O-triisopropylsilyl-myo-inositol 74 (16.2 g, 80% yield) as an impure, colourless gum which was used without further purification. $R_f$ 0.47 (ethyl acetate/petroleum ether 25:75); $\delta$$_H$ (300 MHz, CDCl$_3$) 8.01-7.97 (2 H, m, C$_6$H$_5$C(O)CH), 7.61-7.55 (1 H, m, C$_6$H$_5$C(O)CH) 7.46-7.41 (2 H, m, C$_6$H$_5$C(O)CH), 7.37-7.23 (10 H, m, 2 × Ph), 7.07 (2 H, d, J 8.7, C$_6$H$_4$OCH$_3$), 6.67 (2 H, d, J 8.7, C$_6$H$_4$OCH$_3$) 5.87 (1 H, dddd, J 17.2, 10.5, 5.7, 5.7, CH=CH$_2$), 5.21 (1 H, dddd, J 17.2, 1.8, 1.6, 1.6, CH=CH$_2$), 5.10 (1 H, dddd, J 10.5, 1.8, 1.1, 1.1, CH=CH$_2$), 5.03 (1 H, dd, J 10.0, 2.3, inositol ring), 4.91 (1 H, d, J$_{AB}$ 11.3, OCH$_A$H$_B$-Ph), 4.88 (1 H, d, J$_{AB}$ 11.4, OCH$_A$H$_B$-Ph), 4.87 (1 H, d, J$_{AB}$ 11.3, OCH$_A$H$_B$-Ph), 4.72 (1 H, d, J$_{AB}$ 10.5, OCH$_A$H$_B$C$_6$H$_4$OCH$_3$), 4.71 (1 H, d, J$_{AB}$ 11.4, OCH$_A$H$_B$-Ph), 4.65 (1 H, d, J$_{AB}$ 10.5, OCH$_A$H$_B$C$_6$H$_4$OCH$_3$), 4.38-4.24 (2 H, m, CH$_2$CH=CH$_2$), 4.16 (1 H, dd, J 10.0, 9.3, inositol ring) 4.10-4.08 (1 H, m, inositol ring), 3.98-3.89 (2 H, m, inositol ring), 3.72 (3 H, s, OCH$_3$), 3.43-3.39 (1 H, m, inositol ring), 1.08-1.05 (21 H, m, 3 × SiCH, 18 × SiCHCH$_3$). HRMS m/z (ES+) [Found: (M+Na)$^+$ 803.3955]. C$_{47}$H$_{60}$O$_6$NaSi requires M$^+$ 803.3954, m/z (ES+) 803 ([M+Na]$^+$, 100%).

(±)-5-O- Allyl-2,6-bis-O-benzyl-4-O-(4'-methoxybenzyl)-1-O-triisopropylsilyl-myoinositol 75

To a solution of (±)-3-O-benzoyl-5-O-allyl-2,6-bis-O-benzyl-4-O-(4'-methoxybenzyl)-1-O-triisopropylsilyl-myoinositol 74 (16.08 g, 20.6 mmol, 1.0 equiv.) in diethyl ether (120 mL) and methanol (60 mL) was added 2 M aqueous sodium hydroxide solution (15.5 mL,
30.9 mmol, 1.5 equiv.). The solution was stirred at RT for 18 h. The mixture was then neutralised with saturated aqueous ammonium chloride solution (60 mL). All volatile components were removed in vacuo and the residue partitioned between diethyl ether (100 mL) and water (100 mL). The aqueous phase was extracted with diethyl ether (3 × 100 mL) and the combined organic extracts were washed with saturated aqueous ammonium chloride solution (20 mL), brine (50 mL), dried (MgSO₄), filtered and concentrated under reduced pressure. Purification by silica gel column chromatography eluting with ethyl acetate/petroleum ether (5:95 then 10:90) gave (±)-5-O-allyl-2,6-bis-O-benzyl-4-O-(4’-methoxybenzyl)-1-O-triisopropylsilyl-myoinositol 75 (11.80 g, 85% yield) as a colourless oil. R̃f 0.53 (ethyl acetate/petroleum ether 30:70); νmax (thin film)/cm⁻¹ 3550 (m), 2942 (s), 1612 (m), 1514 (s), 1249 (s), 1068 (s); δH (300 MHz, CDCl₃) 7.38-7.24 (12 H, m, 10 × C₆H₅, 2 × C₆H₄OCH₃), 6.89 (2 H, d, J 8.7, C₆H₄OCH₃) 5.89 (1 H, dddd, J 17.1, 10.5, 5.6, 5.6, CH=CH₂), 5.22 (1 H, dddd, J 17.1, 1.7, 1.6, 1.6, CH=CHH), 5.11 (1 H, dddd, J 10.5, 1.7, 1.3, 1.3, CH=CHH), 5.00 (1 H, d, JAB 11.3, OCH₆H₅-Ph), 4.88 (1 H, d, JAN 11.5, OCH₆H₅-Ph), 4.86 (1 H, d, JAN 11.5, OCH₆H₅-Ph), 4.83 (1 H, d, JAN 10.7, OCH₆H₅C₆H₄OCH₃), 4.77 (1 H, d, JAB 11.3, OCH₆H₅-Ph), 4.67 (1 H, d, JAB 10.7, OCH₆H₅C₆H₄OCH₃), 4.32-4.27 (2 H, m, CH₂CH=CH₂), 3.96 (1 H, dd, J 2.2, 2.2, inositol ring 2-H), 3.88 (1 H, dd, J 9.2, 9.2, inositol ring 6-H), 3.81-3.77 (1 H, m, inositol ring 1-H), 3.80 (3 H, s, OCH₃), 3.75 (1 H, dd, J 9.2, 9.2, inositol ring 4-H), 3.51-3.48 (1 H, m, inositol ring 3-H), 3.30 (1 H, dd, J 9.2, 9.2, inositol ring 5-H), 2.20 (1 H, d, J 5.4, OH), 1.08-1.05 (21 H, m, 3 × SiCH, 18 × SiCH₂CH₃); δC (75 MHz, CDCl₃) 159.7 (C), 139.6 (C), 139.5 (CH), 135.5 (C), 131.2 (CH), 130.2 (CH), 128.7 (CH), 128.4 (CH), 127.9 (CH), 127.8 (CH), 127.5 (CH), 127.4 (CH), 117.1 (CH₂), 114.3 (CH), 84.4 (CH), 82.4 (CH), 82.3 (CH), 82.0 (CH), 75.8 (CH₂), 75.4 (2 × CH₂), 74.8 (CH₂), 74.6 (CH), 72.9 (CH), 55.7 (CH₃), 18.7 (CH₃), 18.6 (CH₃), 13.2 (CH); HRMS m/z (ES+) [Found: (M+Na)⁺ 699.3696. C₄₀H₅₆O₇NaSi requires M⁺ 699.3693], m/z (ES+) 699 ([M+Na]⁺, 100%).
A stirred solution of (±)-5-O-allyl-2,6-bis-O-benzyl-4-O-(4′-methoxybenzyl)-1-O-triisopropylsilyl-myoinositol 75 (3.08 g, 4.56 mmol, 1 equiv.), Wilkinson’s catalyst (422 mg, 0.46 mmol, 0.2 equiv.) and diisopropylethyl amine (588 mg, 793 μL, 4.56 mmol, 1 equiv.) in absolute ethanol (50 mL) was heated under reflux for 2.5 h. Analysis by 1H NMR showed that full isomerisation of the allyl double bonds had occurred. The reaction mixture was then filtered through a pad of Celite and concentrated under reduced pressure. The residue was dissolved in methanol (40 mL) and 4-toluenesulfonic acid monohydrate (260 mg, 1.37 mmol, 0.3 equiv.) was added. The mixture was stirred for 4 h after which the reaction was quenched with triethylamine (3 mL). All volatile components were removed in vacuo and the residue partitioned between ethyl acetate (30 mL) and water (30 mL). The aqueous phase was extracted with ethyl acetate (3 × 30 mL) and the combined organic extracts were washed with aqueous saturated sodium bicarbonate solution (80 mL) and brine (80 mL), dried (MgSO₄), filtered and concentrated in vacuo. Purification of the crude product by silica gel column chromatography, eluting with ethyl acetate and petroleum ether (5:95, 10:90, then 15:85) gave (±)-2,6-bis-O-benzyl-4-O-(4′-methoxybenzyl)-1-O-triisopropylsilyl-myoinositol 68 (2.10 g, 72% yield) as a colourless oil. Rf 0.42 (ethyl acetate/petroleum ether 30:70); νmax (thin film)/cm⁻¹ 3561 (m), 3470 (m), 2944 (s), 1612 (m), 1514 (s), 1250 (s), 1065 (s); δH (300 MHz, CDCl₃) 7.38-7.26 (10 H, m, 2 × Ph), 7.29 (2 H, d, J 8.6, C₆H₄OCH₃), 6.89 (2 H, d, J 8.6, C₆H₄OCH₃), 5.01 (1 H, d, JAB 11.3, OCH₃H₆-Ph), 4.98 (1 H, d, JAB’ 11.7, OCH₃H₆-Ph), 4.82 (1 H, d, JAB 10.8, OCH₃H₆C₆H₄OCH₃), 4.80 (1 H, d, JAB 11.3, OCH₃H₆-Ph), 4.74 (1 H, d, JAB’ 11.7, OCH₃H₆-Ph) 4.67 (1 H, d, JAB 10.8, OCH₃H₆C₆H₄OCH₃), 3.98 (1 H, dd, J 2.2, 2.2, inositol ring 2-H), 3.87-3.77 (2 H, m, inositol ring 3-H, 4-H), 3.80 (3 H, s, OCH₃), 3.67 (1 H, dd, J 9.1, 9.1, inositol ring 6-H), 3.49 (1 H, dd, J 2.2, 9.1, inositol ring 1-H), 3.43 (1 H, m, inositol ring 5-H), 2.38 (1 H, br s, OH), 2.33 (1 H, d, J 5.9, OH), 1.11-1.09 (21 H, m, 3 × SiCH, 18 × SiCH₂CH₃); δC (75 MHz, CDCl₃) 159.3 (C), 139.0 (C), 138.9 (C), 130.8 (CH), 129.7 (CH), 128.4 (CH), 128.3 (CH), 127.7 (CH), 127.6 (CH), 127.5 (CH), 127.4 (CH), 113.9 (CH), 81.9 (CH), 81.6
(CH), 81.5 (CH), 75.3 (CH), 75.25 (CH), 74.9 (CH), 74.4 (CH), 74.3 (CH), 72.4 (CH), 55.3 (CH), 18.2 (CH), 12.8 (CH); HRMS m/z (ES+) [Found: (M+Na)$^+$ 659.3354. $C_{37}H_{52}O_7NaSi$ requires $M^+$ 659.3380], m/z (ES+) 659 ([M+Na]$^+$, 100%).

(±)-2,6-bis-O-Benzyl-4-O-(4'-methoxybenzyl)-3,5-bis(dibenzylphosphate)-myo-inositol 67

1H-Tetrazole in acetonitrile (3 % w/v, 37 mL, 15.7 mmol, 5.0 equiv.) was added to a solution of bis(benzyloxy)-N,N-diisopropylamino phosphine (5.42 g, 15.7 mmol, 5.0 equiv.) in dichloromethane (10 mL) and the mixture stirred for 10 min. A solution of (±)-2,6-bis-O-benzyl-4-O-(4'-methoxybenzyl)-1-O-triisopropylsilyl-myoinositol 68 (2.00 g, 3.14 mmol, 1.0 equiv.) in dichloromethane (30 mL) was added via cannula and the resulting mixture stirred for 6 h. After this time, bis(benzyloxy)-N,N-diisopropylamino phosphine (1.62 g, 4.71 mmol, 1.5 equiv.) in dichloromethane (5 mL) was added via cannula, followed by 1H-tetrazole in acetonitrile (3 % w/v, 11 mL, 1.5 mmol, 1.5 equiv.), and the mixture was stirred for 16 h. The mixture was cooled to -78 °C and 3-chloroperbenzoic acid (assume 60 %, 5.87 g, 20.4 mmol, 6.5 equiv.) was added. The reaction mixture was allowed to reach RT and stirred for 1.5 h. The 3-chloroperbenzoic acid was quenched with a 10 % aqueous solution of sodium bisulfite (30 mL). The mixture was stirred for 10 min and the organic and aqueous phases were separated. The aqueous layer was extracted with dichloromethane (3 × 50 mL). The combined organic extracts were washed with saturated aqueous sodium bicarbonate solution (100 mL), brine (100 mL), dried (MgSO$_4$), filtered and concentrated under reduced pressure. Purification by silica gel chromatography, eluting with ethyl acetate/petroleum ether (15:85, 20:80 then 30:70) gave (±)-2,6-bis-O-benzyl-4-O-(4'-methoxybenzyl)-1-O-triisopropylsilyl-3,5-bis(dibenzylphosphate)-myo-inositol 76 (3.40 g) as an impure, colourless gum which was used without further purification. The gum was dissolved in THF (40 mL) and tetrabutylammonium fluoride (1 M in THF, 3.8 mL, 1.3 equiv.) was
added. The mixture was stirred for 3 h then diluted with diethyl ether (50 mL). Water (50 mL) was added and the layers separated and the aqueous phase was extracted with diethyl ether (3 × 50 mL). The combined organic extracts were washed with aqueous saturated bicarbonate solution (80 mL) and brine (80 mL), dried (MgSO₄), filtered and concentrated. Purification by silica gel column chromatography eluting with ethyl acetate/petroleum ether (30:70, 50:50 then 70:30) gave 2,6-bis-O-benzyl-4-O-(4'-methoxybenzyl)-3,5-bis(dibenzyolphosphate)-myo-inositol 67 (2.38 g, 76% yield over 2 steps) as a colourless oil. Rf 0.47 (ethyl acetate/petroleum ether 80:20); νmax 3387 (m), 3053 (m), 2954 (m), 1514 (m), 1266 (s), 1014 (s); δH (300 MHz, CDCl₃) 7.37-7.16 (28 H, m, 26 × Ph, 2 × C₆H₄OCH₃), 7.14-7.09 (4 H, m, Ph), 6.73 (2 H, d, J 8.8, C₆H₄OCH₃), 4.97-4.66 (14 H, m, 12 × OCH2Ph, 2 × OCH2C₆H₄OCH₃), 4.41 (1 H, ddd, J 9.5, 9.5, 9.5, inositol ring 5-H), 4.29-4.19 (2 H, m, inositol ring 3-H, 2-H), 4.03 (1 H, ddd, J 9.5, 9.5, inositol ring 4-H), 3.82 (1 H, ddd, J 9.5, 9.5, inositol ring 6-H), 3.72 (3 H, s, OCH₃), 3.52-3.46 (1 H, m, inositol ring 1-H), 2.07 (1 H, d, J 5.3, OH); δC (75 MHz, CDCl₃) 159.3 (C), 139.0 (C), 138.7 (C), 136.5 (C), 136.4 (C), 136.1 (C), 136.0 (C), 130.7 (C), 129.7 (CH), 129.0 (CH), 128.97 (CH), 128.8 (CH), 128.7 (CH), 128.5 (CH), 128.32 (CH), 128.3 (CH), 128.2 (CH), 128.1 (CH), 128.0 (CH), 113.9 (CH), 80.7 (1 C, d, 2JF-C 5.7, CH), 80.4 (CH), 79.0 (CH), 78.6 (1 C, d, 2JF-C 5.4, CH), 75.9 (CH2), 75.0 (CH2), 74.9 (CH2), 71.8 (CH), 70.0 (1 C, d, 2JF-C 5.6, CH2), 69.9 (1 C, d, 2JF-C 5.5, CH2), 69.7 (1 C, d, 2JF-C 5.0, CH2), 69.6 (1 C, d, 2JF-C 5.0, CH2), 55.6 (CH3); δP (121 MHz, CDCl₃) -0.18, -0.41; HRMS m/z (ES+) [Found: (M+NH₄)⁺ 1018.3689. C₅₆H₆₂O₁₃NP₂ requires M⁺ 1018.3691]; m/z (ES+) 1023 ([M+Na]⁺, 100%).

4-Methoxybenzyl-2,2,2-trichloroacetimidate 78

A solution of (4-methoxy)benzyl alcohol (2.50 g, 18.09 mmol, 1 equiv.) in diethyl ether (50 mL) was cannulated onto a suspension of sodium hydride (60 % dispersion in mineral oil, 109 mg, 2.71 mmol, 0.15 equiv.) in diethyl ether (15 mL) at 0 °C. The mixture was stirred at RT for 45 min then cooled to 0 °C. Trichloroacetonitrile (2.87 g,
2.0 mL, 19.9 mmol, 1.1 equiv.) was added slowly, and the mixture was allowed to warm to RT and stirred for 2.5 h. Wet diethyl ether (20 mL) was added and the solvent removed in vacuo. To the resulting orange oil was added hexane (30 mL) and several drops of methanol. The mixture was filtered through Celite and concentrated. Purification by basic alumina column chromatography eluting with ethyl acetate/petroleum ether (2:98) gave 4-methoxybenzyl-2,2,2-trichloroacetimidate 78 (4.59 g, 90% yield), as a colourless oil. Rf 0.38 (alumina, ethylacetate/hexane 5:95); δH (300 MHz, CDCl3) 8.38 (1 H, s, C=N), 7.36 (2 H, d, J 10.2, ArH), 6.90 (2 H, d, J 10.2, ArH), 5.28 (2 H, s, CH2Ar), 3.78 (3 H, s, OCH3). The data are in agreement with literature values.180

(+)-Benzyl-2,2-dimethyl-1,3-dioxolane (+)-80

A solution of (S)-(+)2,2-dimethyl-1,3-dioxolane-4-methanol (2.00 g, 15.13 mmol, 1 equiv.) in N,N-dimethylformamide (20 mL) was cannulated onto a cooled suspension of sodium hydride (60 % dispersion in mineral oil, 1.51 g, 37.83 mmol, 2.5 equiv.) in N,N-dimethylformamide (30 mL) at -5 °C. The mixture was stirred for 30 min at -5 °C then 1 h at RT. The alkoxide that formed was insoluble in the N,N-dimethylformamide. The mixture was cooled to -5 °C and benzyl bromide (4.50 mL, 37.83 mmol, 2.5 equiv.) was added slowly. The mixture was allowed to slowly warm to room temperature and stirred for 20 h. The reaction was quenched by the addition of water (10 mL). All volatile components were removed in vacuo and the residue partitioned between ethyl acetate (20 mL) and water (20 mL). The organic layer was collected and the aqueous phase extracted with ethyl acetate (3 × 15 mL). The combined organic extracts were washed with brine (40 mL), dried (MgSO4), filtered and concentrated in vacuo. Purification by silica gel column chromatography, eluting with ethyl acetate/petroleum ether (10:90) gave (+)-benzyl-2,2-dimethyl-1,3-dioxolane (+)-80 (2.92 g, 87% yield) as a colourless oil. Rf 0.51 (ethyl acetate/petroleum ether 20:80); [α]D20 + 21.9, (c 0.51 in CHCl3) [Lit.,181 [α]D20 + 20.3, (c 2.56 in CHCl3)]; δH (300 MHz; CDCl3) 7.36-7.26 (5 H, m, Ph), 4.60 (1 H, d, J 12.1, CH2HbPh), 4.58 (1 H, d, J 12.1, CHaHbPh), 4.32 (1 H, ddd, J 12.0, 6.3, 5.8,
CH$_2$CHCH$_2$), 4.07 (1 H, dd, J 8.3, 6.4, CHHCHCH$_2$), 3.75 (1 H, dd, J 8.3, 6.4, CHHCHCH$_2$), 3.57 (1 H, dd, J 9.8, 5.7, CH$_2$CHCHH), 3.47 (1 H, dd, J 9.8, 5.5, CH$_2$CHCHH), 1.43 (3 H, s, CH$_3$), 1.38 (3 H, s, CH$_3$). The data are in good agreement with literature values.$^{181}$

(-)-3-O-Benzyl-sn-glycerol (-)-81$^{182}$

(S)-(S)-Benzy1-2,2-dimethyl-1,3-dioxolane (+)-80 was dissolved in methanol (10 mL) and concentrated hydrochloric acid (870 μL) was added slowly with stirring. The mixture was heated under reflux for 2 h. The acid was neutralised with NaHCO$_3$(s) (5 g), and the resulting precipitate removed by filtration. The solvent was removed under reduced pressure and the residue reconstituted with ethyl acetate (10 mL) and water (10 mL). The organic phase was collected and the aqueous phase extracted with ethyl acetate (3 × 10 mL). The combined organic extracts were washed with brine (10 mL), dried (MgSO$_4$), filtered and concentrated under reduced pressure. Purification of the crude product by silica gel column chromatography, eluting with ethyl acetate/petroleum ether (20:80) gave (-)-3-O-benzyl-sn-glycerol (-)-81 (470 mg, 91% yield) as a colourless oil. $R_f$ 0.21 (ethyl acetate/petroleum ether 50:50); $[\alpha]^{20}_D$ -0.23, (c 0.61 in CHCl$_3$) [Lit.,$^{182}$ $[\alpha]^{20}_D$ -3.65, (c 5.0 in CHCl$_3$)]; $\delta$$_H$ (300 MHz; CDCl$_3$) 7.40-7.28 (5 H, m, Ph), 4.57 (2 H, s, CH$_2$Ph), 3.93-3.89 (1 H, m, CH$_2$CHCH$_2$), 3.75-3.66 (2 H, m, CH$_2$CHCH$_2$), 3.59 (1 H, dd, J 9.6, 4.0, CH$_2$CHCHH), 3.56 (1 H, dd, J 9.6, 6.1, CH$_2$CHCHH), 2.66 (1 H, d, J 3.7, OH), 2.16 (1 H, s, OH). The data are in good agreement with literature values.$^{182}$

(+)-3-O-Benzyl-1,2-diacyetyl-sn-glycerol (+)-82$^{160}$

To a stirred solution of (-)-3-O-benzyl-sn-glycerol (-)-81 (448 mg, 2.46 mmol, 1.0 equiv.) and 4-dimethylamino pyridine (256 mg, 0.49 mmol, 0.2 equiv.) in THF (10 mL) were
added triethylamine (1.03 mL, 7.38 mmol, 3.0 equiv.) and acetic anhydride (0.93 mL, 9.85 mmol, 4.0 equiv.). The mixture was stirred for 14 h, then diluted with diethyl ether (20 mL). Water (20 mL) was added and the layers separated. The organic phase was collected and the aqueous layer was extracted with diethyl ether (3 × 20 mL). The combined organic extracts were washed with saturated aqueous sodium bicarbonate solution (20 mL), saturated aqueous ammonium chloride solution (20 mL) and brine, dried (MgSO₄), filtered and concentrated under reduced pressure. Purification by silica gel column chromatography, eluting with ethyl acetate/petroleum ether (10:90, 15:85 then 25:75) gave (+)-3-O-benzyl-1,2-diacetyl-sn-glycerol (+)-82 (654 mg, 91% yield) as a colourless oil. R₇ 0.59 (ethyl acetate/petroleum ether 50:50); [α]₂⁰⁺16.4, (c 0.5 in CHCl₃) [Lit.,[160] [α]₂⁰⁺16.2, (c 1.01 in CHCl₃)]; δH (300 MHz; CDCl₃) 7.39-7.28 (5 H, m, Ph), 5.24-5.20 (1 H, m, CH₂CH₂C₆H₄Ph), 4.59 (1 H, d, J 12.1, CH₂CH₂C₆H₄Ph), 4.54 (1 H, d, J 12.1, CH₂CH₂C₆H₄Ph), 4.36 (1 H, dd, J 11.9, 3.8, CH₂CH₂C₆H₄Ph), 4.20 (1 H, dd, J 11.9, 6.3, CH₂CH₂C₆H₄Ph), 3.61 (2 H, d, J 5.2, CH₂CH₂C₆H₄Ph). The data are in good agreement with literature values.[160]

(-)-1,2-O-Diacetyl-sn-glycerol (-)-83[161]

Method A.

A mixture of (+)-3-O-benzyl-1,2-diacetyl-sn-glycerol (+)-82 (250 mg, 0.939 mmol, 1.0 equiv.) and palladium on carbon (10% on carbon, 115 mg, 10% w/w of glycerol) in THF (10 mL) was stirred under an atmosphere of hydrogen for 7 days. The mixture was filtered through a pad of Celite and the solvent removed in vacuo to give (-)-1,2-O-diacetyl-sn-glycerol (-)-83 (159 mg, 96% yield) as a colourless oil. R₇ 0.18 (ethyl acetate/petroleum ether 50:50); [α]₂⁰[161] -3.40 (c 0.6 in CHCl₃) [Lit.,[161] [α]₂⁰⁺8.3, (c 0.4 in CHCl₃)]; δH (300 MHz; CDCl₃) 5.11-5.08 (1 H, m, CHOAc), 4.34 (1 H, dd, J 12.9, 4.8, CH₂), 4.24 (1 H, dd, J 12.0, 5.6, CH₂), 3.75 (2 H, d, J 4.8, CH₂), 2.11 (3 H, s, CH₃), 2.09 (3 H, s, CH₃); m/z (ES+) [Found: (M+Na)⁺ 199.0578. C₇H₁₂O₅Na requires M⁺ 199.0582],
m/z (ES+) 199 ([M+Na]+, 100%). The ¹H NMR data are in good agreement with the literature values.¹⁶¹

Method B
A mixture of (+)-3-0-benzyl-1,2-diacetyl-sn-glycerol (±)-82 (1.47 g, 5.52 mmol, 1.0 equiv.) and Pd(OH)₂ (20 % on carbon, 147 mg, 10 % w/w) in THF (5 mL) was stirred under an atmosphere of hydrogen for 22 h. The mixture was filtered through a pad of Celite and the solvent removed in vacuo to give (-)-1,2-O-diacetyl-sn-glycerol (−)-83 (890 mg, 91%) as a colourless oil. R_f 0.18 (ethyl acetate/petroleum ether 50:50); [α]_D^2⁰ +4.10 (c 1.0 in CHCl₃) [Lit.,¹⁶¹ [α]_D^2⁰ +8.3, (c 0.4 in CHCl₃); δ_H (300 MHz; CDCl₃) 5.12-5.09 (1 H, m, C_HOAc), 4.32 (1 H, dd, J 12.9, 4.8, CH₂), 4.23 (1 H, dd, J 12.0, 5.6, CH₂), 3.73 (2 H, d, J 4.8, CH₂), 2.10 (3 H, s, CH₃), 2.08 (3 H, s, CH₃). The ¹H NMR data are in good agreement with the literature values.¹⁶¹

Benzyloxy-1,2-O-diacyl-sn-glycerol N,N-diisopropylamino phosphine 84

1H-Tetrazole (141 mg, 2.0 mmol, 0.4 equiv.) was added to a stirred solution of benzyloxy bis(N,N-diisopropylamino)phosphine (1.86 g, 5.5 mmol, 1.1 equiv.) in dichloromethane (10 mL). (-)-1,2-O-Diacetyl-sn-glycerol (−)-83 (0.88 g, 5 mmol, 1.0 equiv.) was added dropwise via cannula over 30 min. The resulting mixture was stirred for 2 h. The solvent was removed in vacuo to give a colourless paste which was purified by silica gel column chromatography, eluting with triethylamine/ethyl acetate/petroleum ether (5:10:85) to give benzyloxy-1,2-O-diacetyl-sn-glycerol N,N-diisopropylamino phosphine 84 (1.57 g, 76% yield) as a colourless oil. The compound was unstable and used directly for phosphitylation. R_f 0.65 (ethyl acetate/petroleum ether 50:50); δ_H (300 MHz; CDCl₃) 7.39-7.26 (5 H, m, Ph), 5.21-5.15 (1 H, m, CHOAc), 4.78-4.60 (2 H, m, OCH₂Ph), 4.34 (1 H, ddd, J 11.9, 6.9, 3.7, CHHOAc), 4.18 (1 H, ddd, J 11.9, 6.1, 2.4, CHHOAc), 3.84-3.58 (4 H, m, 2 × NCH(CH₃)₂, 2 × CH₂OP), 2.07-2.06 (6 H, m, 2 ×
COCH$_3$), 1.21 (3 H, s, NCHCH$_3$), 1.19 (3 H, s, NCHCH$_3$), 1.18 (3 H, s, NCHCH$_3$), 1.17 (3 H, s, NCHCH$_3$); $\delta_p$ (121 MHz, CDCl$_3$) 150.1, 149.9.

2,6-bis-O-Benzyl-4-O-(4'-methoxybenzyl)-1-[(1',2'-di-O-acetyl-sn-glycerol)-(3')-benzylphosphate]-3,5-bis(dibenzylphosphate)-myo-inositol 85

1H-Tetrazole in acetonitrile (3 % w/v, 885 µl, 0.38 mmol, 2.5 equiv.) was added to a solution of benzylxyo-1,2-O-diacyl-sn-glycerol N,N-diisopropylamino phosphine (157 mg, 0.38 mmol, 2.5 equiv.) in dichloromethane (2 mL) and the mixture stirred for 10 min. A solution of 2,6-bis-O-benzyl-4-O-(4'-methoxybenzyl)-3,5-bis(dibenzylphosphate)-myo-inositol 67 (152 mg, 0.152 mmol, 1.0 equiv.) in dichloromethane (4 mL) was added via cannula and the resulting mixture stirred for 2.5 h. The mixture was cooled to -78 °C and 3-chloroperbenzoic acid (assume 60 %, 109 mg, 0.38 mmol, 2.5 equiv.) was added. The reaction mixture was allowed to reach RT and stirred for 2 h. The 3-chloroperbenzoic acid was quenched with a 10 % aqueous solution of sodium bisulfite (5 mL). The mixture was stirred for 10 min, then the organic and aqueous phases were separated. The aqueous layer was extracted with dichloromethane (3 × 5 mL). The combined organic extracts were washed with saturated aqueous sodium bicarbonate solution (10 mL), brine (10 mL), dried (MgSO$_4$), filtered and concentrated under reduced pressure. Purification by silica gel chromatography, eluting with ethyl acetate/petroleum ether (30:70 then 60:40) gave 2,6-bis-O-benzyl-4-O-(4'-methoxybenzyl)-1-[(1',2'-di-O-acetyl-sn-glycerol)-(3')-benzylphosphate]-3,5-bis(dibenzylphosphate)-myo-inositol 85 (169 mg, 84% yield), a colourless gum, as a mixture of 4 diastereomers. $R_f$ 0.58 (ethyl acetate/petroleum ether 80:20); $\delta$H (300 MHz, CDCl$_3$) 7.39-7.16 (33 H, m, 33 × Ar), 7.05-6.99 (4 H, m, Ar), 6.70 (2 H, d, J 8.7, C$_6$H$_4$OCH$_3$), 5.03-4.66 (17 H, m, 1 × COAc, 14 × OCH$_2$Ph, 2 × OCH$_2$C$_6$H$_4$OCH$_3$), 4.53 (1 H, m, inositol ring), 4.48-3.77 (9 H, m, 5 × inositol ring, 2 × POCH$_2$CHOAc, 2 × CH$_2$OAc), 3.71 (3 H, s, OCH$_3$), 1.98-1.91 (6 H, m, 2 × COCH$_3$); $\delta$C (75 MHz, CDCl$_3$) 170.3 (C=O), 170.0 (C=O), 159.0 (C), 138.4 (C), 138.1
A mixture of 2,6-bis-O-benzyl-4-O-(4'-methoxybenzyl)-1-[(1',2'-di-O-acetyl-sn-glycerol)-(3')-benzylphosphate]-3,5-bis(dibenzylphosphate)-myo-inositol 85 (165 mg, 0.124 mmol, 1.0 equiv.) and ceric ammonium nitrate (170 mg, 0.310 mmol, 2.5 equiv.) in acetonitrile (4 mL) and water (1 mL) was stirred for 1.5 h open to the air. The solvent was removed in vacuo and the resultant residue partitioned between ethyl acetate (5 ml) and water (5 mL). The aqueous layer was extracted with ethyl acetate (3 × 5 mL), and the combined organic extracts were washed with saturated aqueous sodium bicarbonate solution (10 mL), brine (10 mL), dried (MgSO₄), filtered and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography, eluting with ethyl acetate/petroleum ether (80:20) then ethyl acetate to give 2,6-bis-O-benzyl-1-[(1',2'-di-O-acetyl-sn-glycerol)-(3')-benzylphosphate]-3,5-bis(dibenzylphosphate)-myo-inositol 66 (127 mg, 84% yield), a colourless oil, as a mixture of 4 diastereomers. Rf 0.41 (ethyl acetate/petroleum ether 80:20); νmax (thin film)/cm⁻¹ 3370 (m), 3034 (m), 2956 (m), 1746 (s), 1456 (s), 1263 (s), 1012 (s); δH (300 MHz, CDCl₃) 7.34-7.14 (35 H, m, Ph), 5.13 (1 H, s, OCH₂Ph), 5.11 (1 H, s, OCH₂Ph), 5.07-4.86 (9 H, m, 1 × CHOAc, 8 × OCH₂Ph), 4.78-4.65 (4 H, m, 4 × OCH₂Ph), 4.62-4.59 (1 H, m, inositol ring), 4.37-4.34 (1 H, m,
inositol ring), 4.32-3.79 (8 H, m, 4 × inositol ring, 2 × POCH₂CHOAc, 2 × CH₂OAc), 1.97-1.91 (6 H, m, 2 × COCH₃); δ_C (75 MHz, CDCl₃) 170.0 (C=O), 169.9 (C=O), 138.2 (C), 137.8 (C), 137.9 (C), 135.9 (1 C, d, 3_J_P-C 7.2, C), 135.7 (1 C, d, 3_J_P-C 7.1, C), 135.6 (1 C, d, 3_J_P-C 6.6, C), 135.5 (1 C, d, 3_J_P-C 7.1, C), 128.7 (CH), 128.62 (CH), 128.6 (CH), 128.5 (CH), 128.7 (CH), 128.3 (CH), 128.0 (CH), 127.9 (CH), 127.7 (CH), 127.6 (CH), 127.43 (CH), 127.4 (CH), 81.9, (CH) 78.1 (CH), 78.0-77.6 (1 C, m), 77.3 (CH), 75.8 (CH₂), 75.3 (CH₂), 75.2 (CH₂), 71.1 (CH), 69.9-69.5 (4 C, m, 4 × CH₂), 69.4 (CH), 69.3 (CH), 65.7-65.4 (1 C, m, CH₂), 61.6 (CH₂), 20.8 (CH₃), 20.6 (CH₃); δ_P (121 MHz, CDCl₃) 1.4 - -0.7 (3 P); HRMS m/z (FAB) [Found: (M+Na)⁺ 1231.3394. C₆₂H₆₇O₁₉NaP₃ requires M⁺ 1231.3382]; m/z (ES+) 1231 ([M+Na]⁺, 100%).

2,6-bis-O-Benzyl-1-[(1',2'-di-O-acetyl-sn-glycerol)-(3')-benzylphosphate]-3,5-bis(dibenzylphosphate)-4-dimethylphosphinyl-myo-inositol 86

To a solution of 2,6-bis-O-benzyl-1-[(1',2'-di-O-acetyl-sn-glycerol)-(3')-benzylphosphate]-3,5-bis(dibenzyl phosphate) myo-inositol 66 (198 mg, 0.164 mmol, 1 equiv.) in pyridine (1.5 mL) and dichloromethane (0.3 mL) was added dimethylchlorophosphine (126.5 mg, 104 µL, 1.311 mmol, 8.0 equiv.). The mixture was stirred for 2 h at RT after which time the reaction was adjudged to be incomplete by TLC analysis. Dimethylchlorophosphine (47 mg, 39 µL, 0.492 mmol, 3.0 equiv.) was added and the reaction mixture was stirred for a further 0.5 h. The mixture was diluted with chloroform (10 mL). Water (10 mL) was added and the layers separated. The aqueous phase was extracted with chloroform (3 × 10 mL) and the combined organic extracts were washed with water (2 × 10 mL), brine (15 mL), dried (MgSO₄), filtered and concentrated. The crude product was adsorbed onto silica gel and purification by silica gel column chromatography eluting with ethyl acetate/hexane (60:40, 80:20 then 100% ethyl acetate) followed by methanol/dichloromethane (5:95) to give 2,6-bis-O-benzyl-1-[(1',2'-di-O-acetyl-sn-glycerol)-(3')-benzylphosphate]-3,5-bis(dibenzylphosphate)-4-dimethylphosphinyl-myo-
inositol 86 (125 mg, 60% yield), light yellow gum, as a mixture of 4 diastereomers. Rf 0.44 (ethyl acetate); ν_{max} (thin film)/cm^{-1} 3468 (w), 3033 (m), 2955 (m), 1746 (s), 1456 (s), 1277 (s), 1019 (s); \delta_H (300 MHz, CDCl_3) 7.37-7.17 (33 H, m, Ph), 7.07-7.05 (2 H, m, Ph), 5.11-4.68 (16 H, m, 1 × CHOAc, 1 × inositol, 14 × OCH_2Ph), 4.63-4.60 (1 H, m, inositol ring), 4.40-3.77 (8 H, m, 4 × inositol ring, 2 × POCH_2CHOAc, 2 × CH_2OAc), 1.99-1.93 (6 H, m, 2 × COCH_3), 1.47 (3 H, d, J 13.6, POCH_3), 1.43 (3 H, d, J 12.5, POCH_3); \delta_C (75 MHz, CDCl_3) 170.3 (C=O), 170.0 (C=O), 138.03 (C), 138.0 (C), 135.9-135.3 (5 C, m, 5 × ArC), 128.8 (CH), 128.7 (CH), 128.67 (CH), 128.4 (CH), 128.33 (CH), 128.3 (CH), 128.27 (CH), 128.2 (CH), 128.1 (CH), 128.06 (CH), 128.0 (CH), 127.9 (CH), 127.88 (CH), 127.8 (CH), 127.7 (CH), 127.6 (CH), 127.44 (CH), 127.4 (CH), 127.38 (CH), 127.3 (CH), 78.5 (CH), 77.7 (CH), 77.4 (CH), 76.0 (CH_2), 75.5 (CH), 75.0 (CH_2), 74.9 (CH_2), 72.0 (CH), 70.1-69.5 (4 C, m, 4 × CH_2), 69.4 (CH), 69.3 (CH), 65.7-65.4 (1 C, m, CH_2), 61.6 (CH_2), 20.8 (CH_3), 20.6 (CH_3), 17.8 (1 C, d, ^1J_P-C 91.5, CH_3), 17.1 (1 C, d, ^1J_P-C 96.2, CH_3); \delta_P (121 MHz, CDCl_3) 58.7, 0.0 − -0.3 (3 P); HRMS m/z (ES+) [Found: (M+Na)^+ 1307.3457. C_{64}H_{72}O_{20}NaP_4 requires M^+ 1307.3465], m/z (ES+) 1307 ([M+Na]^+, 100%).

2,6-bis-O-Benzyl-1-[(1',2'-di-O-acetyl-sn-glycerol)-(3')-benzylphosphate]-3,5-bis(dibenzylphosphate)-4-methanesulfonyl-myoinositol 87

![Chemical Structure](image)

To a stirred mixture of 2,6-bis-O-benzyl-1-[(1',2'-di-O-acetyl-sn-glycerol)-(3')-benzylphosphate]-3,5-bis(dibenzylphosphate)-myo-inositol 66 (15 mg, 12.4 μmol, 1.0 equiv.) and triethylamine (5 mg, 7 μL, 49.6 μmol, 4.0 equiv.) in dichloromethane (1.5 mL) at 0 °C was added methanesulfonyl chloride (6 mg, 4 μL, 49.6 μmol, 4.0 equiv.). The mixture was allowed to warm to RT and stirred for 2 h. Dichloromethane (3 mL) and water (3 mL) was added and the layers separated. The aqueous phase was extracted with dichloromethane (3 × 5 mL) and the combined organic extracts were washed with
saturated aqueous sodium bicarbonate solution (10 mL), saturated aqueous ammonium chloride (10 mL) and brine (10 mL), dried (MgSO₄), filtered and concentrated. Purification by silica gel column chromatography gave 2,6-bis-O-benzyl-1-[(1',2'-di-O-acetyl-sn-glycerol)-(3')-benzylphosphate]-3,5-bis(dibenzylphosphate)-4-methanesulfonyl-myo-inositol 87 (9 mg, 56% yield), a colourless gum, as a mixture of 4 diastereomers. Rᵣ 0.55 (ethyl acetate/petroleum ether 80:20); νₓmax (thin film)/cm⁻¹ 3479 (w), 3034 (m), 2956 (m), 1746 (s), 1456 (s), 1359 (s), 1266 (s), 1019 (s); δₓ (300 MHz, CDCl₃) 7.39-7.21 (33 H, m, Ph), 7.07-7.04 (2 H, m, Ph), 5.18-4.64 (17 H, m, 1 × CHOAc, 2 × inositol, 14 × OCH₂Ph), 4.45-4.18 (3 H, m), 4.10-3.76 (5 H, m), 2.99 (3 H, SO₂CH₃), 1.99-1.92 (6 H, m, 2 × COCH₃); δₓ (75 MHz, CDCl₃) 170.3 (C=O), 170.0 (C=O), 137.9 (C), 137.8 (C), 135.7 (1 C, d, 3JₓC=7.3, C), 135.6 (1 C, d, 3JₓC=6.6, C), 135.5 (1 C, d, 3JₓC=6.3, C), 135.5 (1 C, d, 3JₓC=7.3, C), 135.3 (1 C, d, 3JₓC=7.3, C), 128.7 (CH), 128.67 (CH), 128.65 (CH), 128.5 (CH), 128.46 (CH), 128.35 (CH), 128.3 (CH), 128.2 (CH), 128.1 (CH), 128.0 (CH), 127.96 (CH), 127.92 (CH), 127.88 (CH), 127.75 (CH), 127.7 (CH), 127.5 (CH), 127.3 (CH), 78.2-78.0 (1 C, m, CH), 77.8 (CH), 77.3 (CH), 77.2 (CH), 77.1 (CH₂), 75.1 (CH₂), 75.0 (CH₂), 74.3 (CH), 70.2 (1 C, d, 2JₓC=5.5, CH₂), 70.0 (2 C, d, 2JₓC=5.5, CH₂), 69.7 (1 C, d, 2JₓC=5.5, CH₂), 69.4 (1 C, d, JₓC=4.8, CH), 69.3 (1 C, d, JₓC=4.5, CH), 65.5 (1 C, d, 2JₓC=5.3, CH₂), 61.6 (CH₂), 39.2 (CH₃), 20.8 (CH₃), 20.6 (CH₃); δₓP (121 MHz, CDCl₃) -1.6 -2.2 (3 P); HRMS m/z (ES+) [Found: (M+Na)⁺ 1309.3179. C₆₃H₉₉O₂₁NaP₃S requires M⁺ 1309.3163], m/z (ES+) 1309 ([M+Na⁺], 100%).

2,6-bis-O-Benzyl-1-[(1',2'-di-O-acetyl-sn-glycerol)-(3')-benzylphosphate]-3,5-bis(dibenzylphosphate)-4-methanesulfonyl-myo-inositol 88

A mixture of 2,6-bis-O-benzyl-1-[(1',2'-di-O-acetyl-sn-glycerol)-(3')-benzylphosphate]-3,5-bis(dibenzylphosphate) 66 myo-inositol (51 mg, 0.042 mmol, 1 equiv.), triethylamine (17 mg, 23 µl, 0.168 mmol, 4.0 equiv.) and DMAP (catalytic amount) in benzenesulfonyl
chloride (0.5 mL) was stirred at RT for 40 h. Dichloromethane (200 µl) and benzenesulfonyl chloride (200 µl) were added and the mixture stirred for a further 30 h. The mixture was diluted with dichloromethane (5 mL). Water (5 mL) was added and the layers separated. The aqueous phase was extracted with dichloromethane (3 × 10 mL) and the combined organic extracts were washed with saturated aqueous sodium bicarbonate solution (20 mL), brine (20 mL), dried (MgSO₄), filtered and concentrated. The crude product was adsorbed onto silica gel and purification by silica gel column chromatography eluting with ethyl acetate:petroleum ether (50:50, 60:40 then 70:30) to give 2,6-bis-O-benzyl-1-[(1’,2’-di-O-acetyl-sn-glycerol)-(3’)-benzylphosphate]-3,5-bis(dibenzylyphosphate)-4-benzenesulfonate-myoinositol (26 mg, 45% yield) as an unstable, colourless gum, and as a mixture of four diastereomers. Rf 0.60 (ethyl acetate/petroleum ether 80:20); δH (500 MHz, CDCl₃) 7.91 (2 H, d, J 8.1, SO₂Ph), 7.40-7.16 (36 H, m, ArH), 7.01 (2 H, d, J 7.1, ArH), 5.31-5.27 (1 H, m, inositol ring 4-H), 5.07-4.62 (15 H, m, 1 × CHOAc, 14 × OC₆H₄Ph), 4.49-3.77 (9 H, m, 5 × inositol ring, 2 × POCH₂CHOAc, 2 × CH₂OAc), 1.98-1.92 (6 H, m, 2 × C(O)CH₃); δP (202 MHz, CDCl₃) -0.2 - -0.9 (3 P); m/z (ES+) 1371 ([M+Na]⁺, 100%).

2,6-bis-O-Benzyl-1-[(1’,2’-di-O-acetyl-sn-glycerol)-(3’)-benzylphosphate]-3,5-bis(dibenzylyphosphate)-4-phenoxyacetyl-myoinositol 89

To a solution of 2,6-bis-O-benzyl-1-[(1’,2’-di-O-acetyl-sn-glycerol)-(3’)-benzylphosphate]-3,5-bis(dibenzylyphosphate)-myoinositol 66 (99 mg, 0.082 mmol, 1 equiv.), triethylamine (33 mg, 46 µL, 0.328 mmol, 4.0 equiv.) and DMAP (catalytic amount) in THF (2 mL) was added phenoxyacetyl chloride (56 mg, 45 µL, 0.328 mmol, 4.0 equiv.) at 0 °C. The mixture was stirred for 18 h during which time a precipitate formed. The mixture was diluted with ether (5 mL) and quenched by the addition of saturated aqueous sodium
bicarbonate (5 mL). The aqueous phase was extracted with ether (3 × 5 mL) and the combined organic extracts were washed with brine (15 mL), dried (MgSO₄), filtered and concentrated. The crude product was immediately adsorbed onto silica gel and purification by silica gel column chromatography eluting with ethyl acetate/petroleum ether (60:40 then 70:30) gave a yellow gum. Repeated silica gel column chromatography using the same elution system gave 2,6-bis-O-benzyl-1-[(1',2'-di-O-acetyl-sn-glycerol)-(3')-benzylphosphate]-3,5-bis(dibenzylphosphate)-4-phenoxyacetyl-myoinositol 89 (74 mg, 67% yield), a mixture of 4 diastereomers, as a light yellow gum. Rf 0.41 (ethyl acetate/petroleum ether 70:30); ν_max (thin film)/cm⁻¹ 3476 (w), 3034 (m), 2897 (m), 1772 (s), 1745 (s), 1497 (s), 1271 (s), 1009 (s); δ_H (300 MHz, CDCl₃) 7.33-7.11 (35 H, m, Ph), 7.06-7.03 (2 H, m, Ph), 6.88 (1H, m, Ph), 6.78-6.75 (2 H, m, Ph), 5.77 (1 H, dd, J 9.8, 9.8, inositol ring 4-H), 5.08-4.64 (15 H, m, 1 × CHOAc, 14 × OCH₂Ph), 4.56-3.80 (11 H, m, 5 × inositol ring, 2 × POCH₂CHOAc, 2 × CH₂OAc, 2 × CH₂OPh), 1.97-1.91 (6 H, m, 2 × COCH₃); δ_C (101 MHz, CDCl₃) 170.4 (C=O), 170.0 (C=O), 168.5 (C=O), 157.7 (C), 137.9 (C), 137.7 (C), 135.5-135.2 (5 C, m, 5 × C), 129.4 (CH), 128.8 (CH), 128.7 (CH), 128.5 (CH), 128.46 (CH), 128.42 (CH), 128.4 (CH), 128.3 (CH), 128.25 (CH), 128.2 (CH), 128.0 (CH), 127.94 (CH), 127.93 (CH), 127.9 (CH), 127.8 (CH), 127.77 (CH), 127.7 (CH), 127.6 (CH), 127.5 (CH), 127.48 (CH), 127.4 (CH), 127.4 (CH), 114.8 (CH), 77.8 (CH), 77.7 (CH), 76.9 (CH), 76.0 (CH₂), 75.3 (CH₂), 75.2 (CH₂), 75.0 (1 C, dd, 3J_P-C 4.3, 4.3, CH), 71.4, (CH), 70.0 (1 C, d, 2J_P-C 5.8, CH₂), 69.8 (1 C, d, 2J_P-C 5.0, CH₂), 69.7 (1 C, d, 2J_P-C 5.6, CH₂), 69.6 (1 C, d, 2J_P-C 5.4, CH), 69.4 (1 C, d, 2J_P-C 3.0, CH₂), 69.3 (1 C, d, 2J_P-C 3.3, CH), 65.8-65.5 (1 C, m, CH₂), 64.9 (CH₂), 61.6 (CH₂), 20.8 (CH₃), 20.7 (CH₃); δ_P (121 MHz, CDCl₃) 0.0 – -0.2 (3 P); HRMS m/z (FAB) [Found: (M+Na)⁺ 1365.3771. C₆₂H₇₀O₁₉NaP₃ requires M⁺ 1365.3749]; m/z (ES+) 1365 ([M+Na]⁺, 100%).
To a solution of 2,6-bis-O-benzyl-1-[(1',2'-di-O-acetyl-sn-glycerol)-(3')-benzylphosphate]-3,5-bis(dibenzylphosphate)-myo-inositol 66 (72 mg, 60 µmol, 1 equiv.) in pyridine (0.75 mL) was added phenyl chloroformate (56 mg, 45 µL, 358 µmol, 6.0 equiv.) and a thick precipitate formed. The mixture was stirred for 2 h at RT after which time the reaction was adjudged to be incomplete by TLC analysis. Phenyl chloroformate (56 mg, 45 µl, 358 µmol, 6.0 equiv.) was added. The reaction mixture was stirred for a further 2 h after which phenyl chloroformate (29 mg, 23 µL, 183 µmol, 3.0 equiv.) was again added. After 1 h the mixture was transferred directly onto a silica gel column. Purification by successive attempts at silica gel column chromatography, eluting with ethyl acetate/petroleum ether (50:50 then 70:30), gave 2,6-bis-O-benzyl-1-[(1',2'-di-O-acetyl-sn-glycerol)-(3')-benzylphosphate]-3,5-bis(dibenzylphosphate)-4-phenylcarbonate-myo-inositol 90 (36 mg, 46% yield), a mixture of 4 diastereomers, as a colourless gum. Rf 0.39 (ethyl acetate/petroleum ether 70:30); νmax (thin film)/cm⁻¹ 3460 (w), 3034 (m), 2955 (m), 1772 (s), 1745 (s), 1456 (s), 1248 (s), 1011 (s); δH (300 MHz, CDCl₃) 7.36-7.16 (31 H, m, Ph), 7.10-7.09 (2 H, m, Ph), 7.00-6.98 (2 H, m, Ph), 5.50 (1 H, dd, J 9.8, 9.8, inositol ring), 5.02-4.13 (19 H, m, 14 × CH₂Ph, 1 × CHOAc, 4 × inositol ring), 4.07-3.73 (5 H, m, 1 × inositol ring, 2 × POCH₂CHOAc, 2 × CH₂OAc), 1.99-1.93 (6 H, m, 2 × COCH₃); δP (121 MHz, CDCl₃) 0.5–0.0 (3 P, m); HRMS m/z (FAB) [Found: (M+Na)⁺ 1351.3580. C₆₉H₇₁O₂₁NaP₃ requires M⁺ 1351.3593]; m/z (ES+) 1351 ([M+Na]⁺, 100%).
(±)-1-[(1′,2′-di-O-Acetyl-sn-glycerol)-(3′)-benzylphosphate]-4-O-methanesulfonyl-
myo-inositol 3,5-bisphosphate pentakis-sodium salt 100

Palladium black (58 mg, 0.544 mmol, 20 equiv.) and sodium bicarbonate (11 mg, 0.136
mmol, 5 equiv.) were added to a solution of 2,6-bis-O-benzyl-1-[(1′,2′-di-O-acetyl-sn-
glycerol)-(3′)-benzylphosphate]-3,5-bis(dibenzylphosphate)-4-methanesulfonyl
myo-inositol 87 (35 mg, 0.027 mmol, 1 equiv.) in t-butanol (3.0 ml) and water (0.5 ml) under
nitrogen. The mixture was then placed under an atmosphere of hydrogen and stirred for
3 h. The organic phase was decanted by pipette and water added to the black residue.
The palladium was removed by filtration and the aqueous filtrate was freeze-dried to
give 1-[(1′,2′-di-O-acetyl-sn-glycerol)-(3′)-benzylphosphate]-4-O-methanesulfonyl-
myo-inositol 3,5-bisphosphate pentakis-sodium salt 100 (19 mg, 90% yield) as a colourless
solid. This compound proved unstable over time. δH (300 MHz, D2O) 5.08-5.04 (1 H, m,
CHOAc), 4.54 (1 H, dd, J 9.7, 9.7, 4-H inositol ring), 4.38 (1 H, dd, J 2.3, 2.3, 2-H inositol
ring), 4.17 (1 H, dd, J 3.5, 12.2, CH2), 4.06 (1 H, ddd, J 3.5, 6.2, 12.2, CH2), 3.96-3.71 (6
H, m, 2 × CH2, 4 × inositol ring), 3.15 (3 H, s, SO2Me), 1.93 (3 H, s, COCH3), 1.89 (3 H,
s, COCH3); δP (162 MHz, D2O) 3.7, 2.5, -0.9.

(±)-2,6-bis-O-Benzyl-4-O-(4′-methoxybenzyl)-1,3,5-tris(dibenzylphosphate)-myo-
inositol 105

1H-Tetrazole in acetonitrile (3 % w/v, 4.2 mL, 1.98 mmol, 2.0 equiv.) was added to a
solution of bis(benzyloxy)-N,N-diisopropylamino phosphine (684 mg, 1.98 mmol, 2.0
equiv.) in dichloromethane (5 mL) and the mixture stirred for 10 min. A solution of (±)-
2,6-bis-O-benzyl-4-O-(4′-methoxybenzyl)-3,5-bis(dibenzylphosphate)-myo-inositol 67
(992 mg, 0.99 mmol, 1.0 equiv.) in dichloromethane (5 mL) was added via cannula and the resulting mixture stirred for 26 h. The mixture was cooled to -78 °C and 3-chloroperbenzoic acid (assume 60 %, 570 mg, 1.98 mmol, 2.0 equiv.) was added. The reaction mixture was allowed to reach RT and stirred for 1.5 h. The 3-chloroperbenzoic acid was quenched with a 10 % aqueous solution of sodium bisulfite (10 mL). The mixture was stirred for 10 min and the organic and aqueous phases were separated. The aqueous layer was extracted with dichloromethane (3 x 20 mL). The combined organic extracts were washed with saturated aqueous sodium bicarbonate solution (20 mL), brine (20 mL), dried (MgSO₄), filtered and concentrated under reduced pressure. Purification by silica gel chromatography, eluting with ethyl acetate/petroleum ether (50:50, 60:40 then 70:30) gave (±)-2,6-bis-O-benzyl-4-O-(4’-methoxybenzyl)-1,3,5-tris(dibenzylphosphate)-myo-inositol 105 (1.08 g, 87% yield) as a colourless oil. Rf 0.56 (ethyl acetate/petroleum ether 80:20); νmax (thin film)/cm⁻¹ 3483 (m), 3033 (s), 2895 (s), 1514 (s), 1455 (s), 1272 (s), 1016 (s); δH (400 MHz, CDCl₃) 7.42-7.40 (2 H, m, Ph), 7.34-7.14 (36 H, m, 34 × Ph, 2 × C₆H₄OCH₃), 7.08-7.04 (4 H, m, Ph), 6.73 (2 H, d, J 8.7, C₆H₄OCH₃), 5.00-4.70 (18 H, m, 16 × OC₂H₂Ph, 2 × OCH₂C₆H₄OCH₃), 4.59 (1 H, dd, J 2.4, 2.4, inositol ring, 2-H), 4.43 (1 H, ddd, J 9.2, 9.2, 9.2, inositol ring, 5-H), 4.33-4.27 (2 H, m, inositol ring, 1-H and 3-H), 4.09 (2 H, dd, J 9.4, 9.4, inositol ring, 4-H and 6-H), 3.70 (3 H, s, OCH₃); δC (75 MHz, CDCl₃) 159.4 (C), 138.9 (C), 138.5 (C), 136.3 (1 C, d, 2JPC 7.1, C), 136.6 (1 C, d, 2JPC 7.1, C), 136.1-136.0 (4 C, m, 4 × C), 130.6 (C), 129.8 (CH), 129.0 (CH), 128.9 (CH), 128.8 (CH), 128.7 (CH), 128.65 (CH), 128.6 (CH), 128.45 (CH), 128.4 (CH), 128.3 (CH), 128.24 (CH), 128.2 (CH), 128.1 (CH), 127.0 (CH), 127.8 (CH), 113.9 (CH), 80.3, (1 C, d, 2JPC 6.9, CH), 78.6-78.4 (1 C, m, CH), 78.3 (CH), 78.2-78.1 (1 C, m, CH), 77.8 (CH), 77.7 (CH), 76.2 (CH₂), 75.1 (CH₂), 74.8 (CH₂), 70.1 (CH₂), 69.8-69.7 (4 C, m, 4 × CH₂), 55.6 (CH₃); δP (121 MHz, CDCl₃) -1.5, -1.7, -1.7; HRMS m/z (ES+) [Found: (M+NH₄)⁺ 1278.4312. C₇₀H₇₅O₁₆NP₃ requires M⁺ 1278.4293]; m/z (ES+) 1284 ([M+Na]⁺, 100%).
A mixture of (±)-2,6-bis-O-benzyl-4-(4'-methoxybenzyl)-1,3,5-tris(dibenzylphosphate)-myo-inositol 105 (1.02 g, 0.81 mmol, 1.0 equiv.) and ceric ammonium nitrate (1.10 g, 2.02 mmol, 2.5 equiv.) in acetonitrile (20 mL) and water (5 mL) was stirred for 1.5 h open to the air. Aqueous saturated sodium bicarbonate solution (5 mL) was added and the solvent was removed in vacuo. The residue was partitioned between dichloromethane (20 mL) and water (20 mL). The aqueous layer was extracted with dichloromethane (3 × 20 mL), and the combined organic extracts were washed with saturated aqueous sodium bicarbonate solution (40 mL), brine (40 mL), dried (MgSO₄), filtered and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography, eluting with ethyl acetate/petroleum ether (50:50, 70:30 then 80:20) to give (±)-2,6-bis-O-benzyl-1,3,5-tris(dibenzylphosphate)-myo-inositol 106 (779 mg, 85% yield) as a slow-crystallising colourless solid. Rᶠ 0.41 (ethyl acetate/petroleum ether 80:20); mp 82-84 °C; ν max (thin film)/cm⁻¹ 3367 (m), 3033 (m), 1456 (s), 1266 (s), 1014 (s); δH (300 MHz, CDCl₃) 7.38-7.16 (40 H, m, Ph), 5.14 (1 H, s, OCH₂Ph), 5.12 (1 H, s, OCH₂Ph), 5.06-4.89 (10 H, m, 5 × OCH₂Ph), 4.73 (2 H, s, OCH₂Ph), 4.69 (2 H, s, OCH₂Ph), 4.55 (1 H, d, J 1.9, OH), 4.36-4.34 (1 H, m, inositol ring), 4.27-4.13 (4 H, m, 4 × inositol ring), 4.00 (1 H, dd, J 9.3, 9.3, inositol ring); δC (101 MHz, CDCl₃) 138.3 (C), 137.9 (C), 135.9 (1 C, d, 2JPC 7.4, C), 135.7 (1 C, d, 2JPC 7.4, C), 135.63 (1 C, d, 2JPC 7.4, C), 135.62 (1 C, d, 2JPC 7.4, C), 135.61 (1 C, d, 2JPC 7.4, C), 135.5 (1 C, d, 2JPC 7.4, C), 128.65 (CH), 128.6 (CH), 128.55 (CH), 128.5 (CH), 128.25 (CH), 128.2 (CH), 128.1 (CH), 128.0 (CH), 127.9 (CH), 127.8 (CH), 127.7 (CH), 127.65 (CH), 127.6 (CH), 127.5 (CH), 127.4 (CH), 82.0 (1 C, d, 2JPC 6.3, CH), 78.1 (1 C, d, 2JPC 2.7, CH), 77.9 (1 C, dd, 3JPC 7.0, 7.0, CH), 77.7 (1 C, d, 2JPC 5.9, CH), 77.1-77.3 (1 C, m, CH), 75.8 (CH₂), 75.3 (CH₂), 71.1 (1 C, d, 2JPC 4.5, CH), 69.9-69.8 (3 C, m, CH₂), 69.6 (1 C, d, 2JPC 5.5, CH₂), 69.5 (1 C, d, 2JPC 5.5, CH₂), 69.4 (1 C, d, 2JPC 5.5, CH₂); δP (121 MHz, CDCl₃) 0.2, -1.1, -1.8; HRMS m/z (ES⁺) [Found: (M+Na)⁺ 1163.3275. C₆₂H₆₃O₁₅NaP₃ requires M⁺
1163.3278, m/z (ES+) 1163 ([M+Na]+, 100%). Anal. Calcd for C_{62}H_{63}O_{15}P_{3}: C, 65.26%; H 5.56%. Found: C, 65.30%; H, 5.56%.

(±)-2,6-bis-O-Benzyl-4-dimethylphosphinyl-1,3,5-tris(dibenzylphosphate)-myo-inositol 107

To a solution of (±)-2,6-bis-O-benzyl-1,3,5-bis(dibenzylphosphate)-myo-inositol 106 (83 mg, 0.07 mmol, 1 equiv.) in pyridine (1 mL) and dichloromethane (0.2 mL) was added dimethylchlorophosphine (56 mg, 46 µl, 0.58 mmol, 8.0 equiv.). The mixture was stirred for 3 h at RT then diluted with chloroform (10 mL). Water (10 mL) was added and the layers separated. The aqueous phase was extracted with chloroform (3 × 10 mL) and the combined organic extracts were washed with water (2 × 15 mL), brine (15 mL), dried (MgSO₄), filtered and concentrated. The crude product was adsorbed onto silica gel and purification by silica gel column chromatography eluting with ethyl acetate/hexane (60:40), ethyl acetate (100%) then methanol/dichloromethane (2:98, then 5:95) gave (±)-2,6-bis-O-benzyl-4-dimethylphosphinyl-1,3,5-tris(dibenzylphosphate)-myo-inositol 107 (55 mg, 62%) as a colourless gum. Rf 0.39 (ethyl acetate); ν max (thin film)/cm⁻¹ 3464 (s), 3064 (m), 3034 (m), 2955 (m), 1497 (m), 1455 (s), 1273 (s), 1216 (s), 1013 (s), 874 (s); δ_H (300 MHz, CDCl₃) 7.39-7.04 (40 H, m, 8 × Ph), 5.09 (1 H, dd, J 11.7, 6.3, CH₂Ph), 5.01-4.66 (16 H, m, 1 × inositol, 15 × OC₂H₂Ph), 4.62 (1 H, dd, J 2.4, 2.4, inositol ring), 4.35-4.24 (2 H, m, 2 × inositol ring), 4.14-4.01 (2 H, m, inositol ring), 1.46 (3 H, d, J 14.2, POCH₃), 1.41 (3 H, d, J 14.2, POCH₃); δ_C (101 MHz, CDCl₃) 138.1 (C), 138.0 (C), 135.9-135.4 (6 C, m, C), 128.8 (CH), 128.7 (CH), 128.6 (CH), 128.5 (CH), 128.4 (CH), 128.2 (CH), 128.15 (CH), 128.1 (CH), 128.0 (CH), 127.9 (CH), 127.6 (CH), 127.5 (CH), 127.4 (CH), 127.3 (CH), 78.4 (CH), 77.6 (1 C, d, J_P-C 7.4, CH), 77.4 (CH), 77.3 (CH), 75.9 (CH₂), 75.6 (1 C, d, J_P-C 5.0, CH), 75.0 (CH₂), 72.1-71.9 (1 C, m, CH), 70.0 (1 C, d, J_P-C 5.6, CH₂), 69.8 (1 C, d, J_P-C 5.5, CH₂), 69.7 (1 C, d, J_P-C 5.3, CH₂), 69.6 (1 C, d, J_P-C 5.6, CH₂), 69.4 (1 C, d, J_P-C 5.3, CH₂), 69.3 (1 C, d, J_P-C 5.5, CH₂), 17.7 (1 C, d,
$^1J_{P\text{-C}}$ 67.8, CH$_3$), 16.7 (1 C, d, $^1J_{P\text{-C}}$ 71.9, CH$_3$); $\delta_P$ (121 MHz, CDCl$_3$) 58.8, 0.2, 0.0, −0.1;
HRMS $m/z$ (ES+) [Found: (M+Na)$^+$ 1239.3330. C$_{64}$H$_{68}$O$_{16}$NaP$_4$ requires $M^+$ 1239.3356];
m/z (ES+) 1239 ([M+Na]$^+$, 100%).

(±)-4-Dimethylphosphinyl-myoinositol 1,3,5-trisphosphate hexakis-sodium salt 108

Palladium black (39 mg, 0.36 mmol, 20 equiv.) and sodium bicarbonate (9 mg, 0.11 mmol, 6 equiv.) were added to a solution of (±)-2,6-bis-O-benzyl-4-dimethylphosphinyl-1,3,5-tris(dibenzylphosphate)-myoinositol 107 (22 mg, 0.018 mmol, 1 equiv.) in t-butanol (3.0 mL) and water (0.5 mL) under nitrogen. The mixture was then placed under hydrogen and stirred for 5 h. The organic phase removed and water (1 mL) was added to the black residue. The palladium was removed by filtration and washed with water. The aqueous filtrate was freeze-dried to give (±)-4-dimethylphosphinyl-myoinositol 1,3,5-trisphosphate hexakis-sodium salt 108 (11 mg, 100% yield) as a colourless solid.

$\delta_H$ (300 MHz, D$_2$O) 4.52 (1 H, dd, J 2.2, 2.2, inositol ring 2-H), 4.26 (1 H, ddd, J 10.0, 10.0, 10.0, inositol ring), 3.94-3.75 (4 H, m, 4 × inositol ring), 1.62 (3 H, s, POCH$_3$), 1.57 (3 H, s, POCH$_3$); $\delta_C$ (75 MHz, D$_2$O) 76.2 (CH), 75.3 (1C, d, $^2J_{P\text{-C}}$ 6.9, CH), 73.9 (1C, d, $^2J_{P\text{-C}}$ 5.0, CH), 72.3 (1C, d, $^2J_{P\text{-C}}$ 7.2, CH), 72.0 (CH), 70.0 (CH), 16.5 (CH$_3$), 15.2 (CH$_3$);

$\delta_P$ (121 MHz, D$_2$O) 66.6, 3.7, 3.0, 2.9; HRMS $m/z$ (FAB) [Found: (M−6Na+5H)$^-$ 494.9625. C$_8$H$_{19}$O$_{16}$P$_4$ requires $M^-$ 494.9629]; m/z (ES-) 247 (100%), 495 ([M−6Na+5H]$^-$, 18%); 517 ([M−5Na+4H]$^-$, 18%); 539 ([M−4Na+3H]$^-$, 7%).

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A solution of (±)-2,6-bis-O-benzyl-1,3,5-tris(dibenzylphosphate)-myo-inositol 106 (30 mg, 0.026 mmol, 1.0 equiv.), sulfur trioxide pyridine complex (41 mg, 0.263 mmol, 10 equiv.) and triethylamine (10.5 mg, 15 μL, 0.104 mmol, 4.0 equiv.) in DMF (1 mL) was heated to 50 °C for 24 h. After this time, the reaction was adjudged to have completed by TLC analysis. Triethylamine (1 mL) and methanol (2 mL) was added and the solvent was removed in vacuo. The residue was adsorbed onto silica gel and subjected to silica gel column chromatography eluting with ethyl acetate/hexane (60:40, 70:30, 80:20 then 100 % ethyl acetate) then methanol/chloroform (5:95). The product was dissolved in methanol and stirred with Dowex Marathon C sodium form resin for 20 min. The resin was removed by filtration and washed with methanol. Water (5 mL) was added to the filtrate which was freeze-dried overnight to give (±)-2,6-bis-O-benzyl-1,3,5-tris(dibenzylphosphate)-4-sulfate-myoinositol sodium salt 109 (14 mg, 44% yield) as a colourless, hygroscopic solid. Rf 0.28 (methanol/chloroform 10:90); δH (400 MHz, CD3OD) 7.42-7.15 (40 H, m, 8 × Ph), 5.25-4.82 (14 H, m, CH2Ph), 4.96 (1 H, d, J 11.1, CH2Ph), 4.75 (1 H, d, J 11.1, CH2Ph), 4.62-4.53 (5 H, m, 5 × inositol ring), 4.06 (1 H, dd, J 8.8, 8.8, inositol ring); δC (101 MHz, CD3OD) 139.6 (C), 139.5 (C), 137.8-136.9 (6 C, m, 6 × ArC), 129.64 (CH), 129.60 (CH), 129.57 (CH), 129.5 (CH), 129.48 (CH), 129.41 (CH), 129.35 (CH), 129.33 (CH), 129.3 (CH), 129.2 (CH), 129.17 (CH), 129.0 (CH), 128.8 (CH), 128.7 (CH), 128.6 (CH), 128.4 (CH), 79.5 (1 C, d, 2JPC 6.1, CH), 78.6 (CH), 78.3 (1 C, d, 2JPC 5.9, CH), 78.0 (CH), 77.0 (1 C, dd, 3JPC 6.1, CH), 76.6 (1 C, d, 2JPC 5.6, CH), 76.4 (CH2), 75.5 (CH2), 76.4 (1 C, d, 2JPC 5.2, CH2), 71.33 (1 C, d, 2JPC 5.2, CH2), 71.3 (1 C, d, 2JPC 5.2, CH2), 71.2 (1 C, d, 2JPC 5.8, CH2), 71.1 (2 C, d, 2JPC 6.6, CH2), 70.8 (1 C, d, 2JPC 6.2, CH2); δP (162 MHz, CD3OD) −2.4, −3.0, −3.3; HRMS m/z (FAB) [Found: (M−Na)− 1219.2866. C62H62O18P3S requires M − 1219.2875]; m/z (ES−) 1219 ([M−Na]−, (100%).
(±)-myo-Inositol 4-sulfate-1,3,5-trisphosphate heptakis-sodium salt 110

Palladium black (24 mg, 0.23 mmol, 20 equiv.) and sodium bicarbonate (6.2 mg, 0.07 mmol, 6.5 equiv.) were added to a solution of (±)-2,6-bis-O-benzyl-1,3,5-tris(dibenzylphosphate)-4-sulfonato-myosinositol sodium salt 109 (14 mg, 0.011 mmol, 1 equiv.) in t-butanol (3.0 mL) and water (0.5 mL) under nitrogen. The mixture was then placed under hydrogen and stirred for 2.5 h. The organic phase was decanted by pipette and water added to the black residue. The palladium was removed by filtration, washed with water and the aqueous filtrate was freeze-dried to give (±)-myo-inositol 4-sulfate-1,3,5-trisphosphate heptakis-sodium salt 110 (7 mg, 99% yield) as a colourless solid. δ_H (300 MHz, D_2O) 4.47 (1 H, br s, inositol ring 2-H), 4.37 (1 H, ddd, J 9.3, 9.3, 9.3, inositol ring), 3.89-3.72 (4 H, m, 4 × inositol ring); δ_C (75 MHz, D_2O) 79.1 (CH), 75.9 (1 C, d, ^2J_{P-C} 4.0, CH), 73.8 (1 C, d, ^2J_{P-C} 5.0, CH), 72.5 (1 C, d, ^2J_{P-C} 6.8, CH), 71.9 (CH), 69.9 (CH); δ_P (121 MHz, D_2O) 4.0, 3.1, 3.0; HRMS m/z (FAB) [Found: (M−7Na+6H)^− 498.9113. C_{6}H_{14}O_{18}P_{3}S requires M^− 498.9119]; m/z (ES−) 123 (100%), 499 ([M−7Na+6H]^−, 11%).

(±)-myo-Inositol 4-sulfate-1,3,5-trisphosphate tetrakis-sodium salt 111

Palladium black (42 mg, 0.39 mmol, 20 equiv.) and sodium bicarbonate (5.0 mg, 0.06 mmol, 3.0 equiv.) were added to a solution of (±)-2,6-bis-O-benzyl-1,3,5-tris(dibenzyl phosphate)-4-sulfonato-myosinositol sodium salt 109 (24.5 mg, 19.7 μmol, 1 equiv.) in t-butanol (3.0 mL) and water (0.5 mL) under nitrogen. The mixture was then placed under hydrogen and stirred for 3 h. The organic phase was decanted by pipette and water (2
mL) added to the black residue. The palladium was removed by filtration, washed with water (5 mL) and the aqueous filtrate was freeze-dried to give (±)-myo-inositol 4-sulfate-1,3,5-trisphosphate tetrakis-sodium salt 110 (11 mg, 97% yield) as a colourless solid. δH (300 MHz, D2O) 4.59 (1 H, dd, J 9.7, 9.7, inositol ring), 4.41 (1 H, dd, J 2.5, 2.5, inositol ring 2-H), 4.12-3.94 (3 H, m, 3 × inositol ring), 3.87 (1 H, dd, J 9.4, 9.4, inositol ring); δC (75 MHz, D2O) 79.2 (1 C, dd, 3JPC 5.8, 4.5 CH), 76.9 (1 C, d, JPC 5.8, CH), 74.5 (1 C, d, JPC 5.5, CH), 73.1 (1 C, d, JPC 5.3, CH), 71.0 (1 C, d, JPC 5.8, CH), 69.9 (CH); δP (121 MHz, D2O) 0.4, -0.3, -0.5; HRMS m/z (FAB) [Found: (M−3Na+2H)− 520.8937. C6H13O18NaP3S requires M− 520.8939]; m/z (ES-) 499 ([M−4Na+3H]−, 100%); 521 ([M−3Na+2H]−, 30%); 543 ([M−2Na+H]−, 10%).

(±)-2,6-bis-O-Benzyl-4-phenoxyacetyl-1,3,5-tris(dibenzylphosphate)-myo-inositol 112

To a solution of (±)-2,6-bis-O-benzyl-1,3,5-tris(dibenzylphosphate)-myo-inositol 106 (86 mg, 0.075 mmol, 1 equiv.), triethylamine (38 mg, 53 µL, 0.377 mmol, 5.0 equiv.) and DMAP (catalytic amount) in THF (2 mL) was added phenoxyacetyl chloride (51 mg, 42 µL, 0.302 mmol, 4.0 equiv.) at 0 °C. The mixture was allowed to warm to RT and stirred for 3 h, after which time the reaction was adjudged to be incomplete by TLC analysis. Triethylamine (38 mg, 53 µL, 0.377 mmol, 5.0 equiv.) and phenoxyacetyl chloride (51 mg, 42 µL, 0.302 mmol, 4.0 equiv.) were added at 0 °C. The mixture was stirred for a further 1.5 h, then diluted with diethyl ether (6 mL). Water (5 mL) was added and the layers separated. The aqueous phase was extracted with diethyl ether (3 × 8 mL) and the combined organic extracts were washed with saturated aqueous sodium bicarbonate solution (15 mL) and brine (15 mL), dried (MgSO4), filtered and concentrated. The crude product was immediately adsorbed onto silica gel and purification by silica gel column
chromatography eluting with ethyl acetate/petroleum ether (50:50 then 60:40) gave (±)-2,6-bis-O-benzyl-4-phenoxycarbonyl-1,3,5-tris(dibenzylphosphate)-myo-inositol 112 (51 mg, 53% yield), as a colourless gum. Rf 0.41 (ethyl acetate/petroleum ether 70:30); ν max (thin film)/cm⁻¹ 3034 (m), 2953 (m), 1773 (s), 1497 (s), 1456 (s), 1272 (s), 1011 (s); δH (300 MHz, CDCl₃) 7.35-7.14 (40 H, m, Ph), 7.09-7.05 (2 H, m, Ph), 6.94-6.88 (1 H, m, Ph), 6.82-6.79 (2 H, m, Ph), 5.79 (1 H, dd, J 9.8, 9.8, inositol ring 4-H), 4.98-4.75 (14 H, m, CH₂Ph), 4.70 (2 H, s, CH₂Ph), 4.60 (1 H, d, J 16.3, C(O)CHHO), 4.48 (1 H, dd, J 2.4, 2.4, inositol ring 2-H), 4.40 (1 H, ddd, J 9.2, 9.2, 9.2, inositol ring), 4.33-4.24 (2 H, m, 2 × inositol ring), 4.10 (1 H, dd, J 9.5, 9.5, inositol ring); δC (101 MHz, CDCl₃) 168.5 (C=O), 157.7 (C), 138.0 (C), 137.7 (C), 135.6-135.2 (6 C, m, 6 × ArC), 129.4 (CH), 128.73 (CH), 128.7 (CH), 128.6 (CH), 128.55 (CH), 128.5 (CH), 128.45 (CH), 128.4 (CH), 128.3 (CH), 128.2 (CH), 128.04 (CH), 128.0 (CH), 127.9 (CH), 127.84 (CH), 127.82 (CH), 127.7 (CH), 127.6 (CH), 127.5 (CH), 121.4 (CH), 114.8 (CH), 77.8 (CH), 77.7 (CH), 77.4 (CH), 76.8 (1 C, d, 2J_P-C 5.3, CH), 76.0 (CH₂), 75.2 (CH₂), 75.0 (1 C, d, 2J_P-C 5.3, CH), 71.3 (1 C, d, 2J_P-C 4.4, CH), 70.0 (1 C, d, 2J_P-C 5.7, CH₂), 69.8 (1 C, d, 2J_P-C 5.6, CH₂), 69.7 (1 C, d, 2J_P-C 5.7, CH₂), 69.6 (1 C, d, 2J_P-C 5.6, CH₂), 69.5 (1 C, d, 2J_P-C 5.6, CH₂), 69.4 (1 C, d, 2J_P-C 5.7, CH₂), 64.9 (CH₂); δp (162 MHz, CDCl₃) −1.6, −1.7, −1.8; HRMS m/z (ES+) [Found: (M+Na)⁺ 1297.3665. C₇₀H₇₀O₁₇NaP₃ requires M⁺ 1297.3645], m/z (ES⁺) 1297 (100%).

(±)-2,6-bis-O-Benzyl-4-phenoxycarbonyl-1,3,5-tris(dibenzylphosphate)-4-phenylcarbamate-myoinositol 114

To a solution of (±)-2,6-bis-O-benzyl-1,3,5-tris(dibenzylphosphate)-myo-inositol 106 (83 mg, 0.073 mmol, 1.0 equiv.) and 4-dimethylaminopyridine (catalytic amount) in pyridine (1 mL) and dichloromethane (0.2 mL) at 0 °C was added phenylisocyanate (173
mg, 158 µL, 1.46 mmol, 20 equiv.). Precipitate was immediately formed, and the mixture was allowed to warm to RT. After 2 h stirring, the reaction was adjudged to be complete by TLC analysis. The reaction mixture was transferred directly onto a silica gel chromatography column. Elution with ethyl acetate and hexane (50:50, 60:40, then 80:20) followed by ethyl acetate (100 %) gave (±)-2,6-bis-O-benzyl-1,3,5-tris(dibenzylphosphate)-4-phenylcarbamate-myoinositol 114 (74 mg, 80% yield), as a colourless gum. R f 0.26 (ethyl acetate); ν max (thin film)/cm -1 3449 (m), 3065 (m), 3034 (m), 1738 (s), 1602 (m), 1542 (m), 1268 (s), 1221 (s), 1016 (s); δ H (400 MHz, CDCl 3 ) 7.33-7.07 (40 H, m, 8 × Ph), 6.99-6.96 (5 H, m, Ph), 5.64 (1 H, dd, J 9.9, 9.9, inositol ring 4-H), 4.95-4.62 (16 H, m, CH 2 Ph), 4.52 (1H, ddd, J 9.4, 9.4, 9.4, inositol ring), 4.46 (1 H, dd, J 2.5, 2.5, inositol ring 2-H), 4.40-4.31 (2 H, m, 2 × inositol ring), 4.13 (1 H, dd, J 9.6, 9.6, inositol ring); δ C (101 MHz, CDCl 3 ) 152.6 (C=O), 138.2 (C), 137.9 (C), 137.8 (C), 135.7-135.3 (6 C, m, 6 × C), 128.9 (CH), 128.6 (CH), 128.59 (CH), 128.56 (CH), 128.52 (CH), 128.5 (CH), 128.4 (CH), 128.33 (CH), 128.29 (CH), 128.28 (CH), 128.2 (CH), 127.89 (CH), 127.87 (CH), 127.8 (CH), 127.7 (CH), 127.6 (CH), 127.57 (CH), 127.5 (CH), 127.4 (CH), 127.36 (CH), 123.2 (CH), 118.6 (CH), 78.02 (CH), 77.95 (CH), 77.9 (CH), 77.0 (1 C, d, 2 J P,C 5.4, CH), 75.9 (CH 2 ), 75.6 (1 C, d, 2 J P,C 5.2, CH), 74.9 (CH 2 ), 71.7 (CH), 69.7-69.2 (6 C, m, 6 × CH 2 ); δ P (162 MHz, CDCl 3 ) -1.3, -1.7, -1.8; HRMS m/z (FAB) [Found: (M+Na) + 1282.3644. C 69 H 68 O 16 NNaP 3 requires M + 1282.3643]; m/z (ES+) 1282 ([M+Na] + , 100%).

(±)-4-Phenylcarbamate-myoinositol-1,3,5-trisphosphate hexakis-sodium salt 115

Palladium black (121 mg, 1.14 mmol, 20 equiv.) and sodium bicarbonate (29 mg, 0.343 mmol, 6 equiv.) were added to a solution of (±)-2,6-bis-O-benzyl-1,3,5-tris(dibenzyl phosphate)-4-phenylcarbamate-myoinositol 114 (72 mg, 57 µmol, 1 equiv.) in t-butanol
(3.0 mL) and water (0.5 mL) under nitrogen. The mixture was then placed under hydrogen and stirred for 3 h. The organic phase was decanted by pipette and water added to the black residue. The palladium was removed by filtration and the aqueous filtrate was freeze-dried to give (±)-4-phenylcarbamate-myoinositol-1,3,5-trisphosphate hexakis-sodium salt 115 (32 mg, 84% yield) as a colourless solid.\[\delta H\ (400\ MHz,\ D_2O)\ 7.29-7.27\ (2\ H,\ m,\ Ph),\ 7.23-7.19\ (2\ H,\ m,\ Ph),\ 6.99-6.94\ (1\ H,\ m,\ Ph),\ 4.89\ (1\ H,\ dd,\ J\ 9.7,\ 9.7,\ inositol\ ring\ 4-H),\ 4.41\ (1\ H,\ dd,\ J\ 2.4,\ 2.4,\ inositol\ ring\ 2-H),\ 3.95\ (1\ H,\ ddd,\ J\ 11.1,\ 8.6,\ 2.4,\ inositol\ ring),\ 3.90-3.72\ (3\ H,\ m,\ 3\ \times\ inositol\ ring);\ \delta C\ (75\ MHz,\ D_2O)\ 155.8\ (C=O),\ 137.7\ (C),\ 129.2\ (CH),\ 124.0\ (CH),\ 120.0\ (CH),\ 76.1\ (1\ C,\ d,\ ^2J_{P-C}\ 5.2,\ CH\ inositol\ ring),\ 74.3\ (1\ C,\ d,\ ^2J_{P-C}\ 5.2,\ CH\ inositol\ ring),\ 72.9\ (CH,\ inositol\ ring),\ 72.6\ (1\ C,\ d,\ ^2J_{P-C}\ 4.5,\ inositol\ ring),\ 71.8\ (CH,\ inositol\ ring);\ \delta P\ (121\ MHz,\ D_2O)\ 2.4,\ 2.0,\ 1.1;\ HRMS\ m/z\ (FAB)\ [Found:\ (M−6Na+5H)^−\ 537.9916.\ C_{13}H_{19}O_{16}NP_3\ requires\ M^−\ 537.9922];\ m/z\ (ES−)\ 97\ (100%),\ 538\ ([M−6Na+5H]^−,\ 62%);\ 560\ ([M−5Na+4H]^−,\ 50%);\ 582\ ([M−4Na+3H]^−,\ 21%).

(±)-2,6-bis-O-Benzyl-1,3,5-tris(dibenzylphosphate)-4-(4'-benzyloxy)phenyl carbamate-myoinositol 116

To a solution of (±)-2,6-bis-O-benzyl-1,3,5-tris(dibenzylphosphate)-myoinositol 106 (72 mg, 0.063 mmol, 1.0 equiv.) and 4-dimethylaminopyridine (catalytic amount) in pyridine (1 mL) and dichloromethane (0.2 mL) at 0 °C was added 4-(benzyloxy)phenyl isocyanate (245 mg, 1.09 mmol, 17 equiv.). A precipitate was immediately formed, and the mixture was allowed to warm to RT. After 7 h, the reaction was adjudged to be complete by TLC analysis. The reaction mixture was transferred directly onto a silica gel chromatography column. Elution with ethyl acetate and hexane (50:50, 60:40 then 80:20) followed by ethyl acetate (100 %) gave a mixture of two products.
was added to the product mixture and the solids removed by filtration. The filtrate was concentrated to give (±)-2,6-bis-O-benzyl-1,3,5-tris(dibenzylphosphate)-4-(4’-benzyloxy)phenyl carbamate-\textit{myo}-inositol 116 (65 mg, 76% yield), as a colourless gum. R\textsubscript{f} 0.48 (ethyl acetate); \( \nu \)\textsubscript{max} (thin film)/cm\textsuperscript{-1} 3279 (m), 3064 (m), 1734 (s), 1539 (s), 1268 (s), 1218 (s); \( \delta \)\textsubscript{H} (400 MHz, CDCl\textsubscript{3}) 7.51-7.16 (43 H, m, Ar), 7.10-7.03 (4 H, m, Ar), 6.84-6.82 (2 H, d, J 9.1, NHC\textsubscript{2}H\textsubscript{4}OBn), 5.77 (1 H, dd, J 9.8, 9.8, inositol ring 4-H), 5.07 (2 H, s, C\textsubscript{H}\textsubscript{2}Ar), 5.02-4.72 (16 H, m, 16 \times C\textsubscript{H}\textsubscript{2}Ar), 4.66 (1 H, ddd, J 9.5, 9.5, 9.5, inositol ring 5-H), 4.57 (1 H, dd, J 2.5, 2.5, inositol ring 2-H), 4.42-4.49 (2 H, m, 2 \times inositol ring 1-H and 3-H), 4.22 (1 H, ddd, J 9.5, 9.5, 9.5, inositol ring 6-H); \( \delta \)\textsubscript{C} (101 MHz, CDCl\textsubscript{3}) 154.9 (C), 152.9 (C=O), 138.2 (C), 137.9 (C), 135.7-135.4 (6 C, m, 6 \times C), 131.3 (C), 128.60 (CH), 128.58 (CH), 128.55 (CH), 128.51 (CH), 128.49 (CH), 128.4 (CH), 128.33 (CH), 128.30 (CH), 128.0 (CH), 127.89 (CH), 127.87 (CH), 127.8 (CH), 127.7 (CH), 127.6 (CH), 127.56 (CH), 127.5 (CH), 127.4, 127.3 (CH), 120.3 (CH), 115.2 (CH), 78.04 (CH), 78.00 (CH), 77.9 (CH), 77.0 (1 C, d, \( \text{J}_{P-C} \) 5.1, CH), 75.9 (CH\textsubscript{2}), 75.6 (1 C, d, \( \text{J}_{P-C} \) 5.1, CH), 74.9 (CH\textsubscript{2}), 71.6 (CH), 70.3 (CH\textsubscript{2}), 69.7 (1 C, d, \( \text{J}_{P-C} \) 6.1, CH\textsubscript{2}), 69.6 (1 C, d, \( \text{J}_{P-C} \) 5.5, CH\textsubscript{2}), 69.5 (1 C, d, \( \text{J}_{P-C} \) 5.3, CH\textsubscript{2}), 69.46 (1 C, d, \( \text{J}_{P-C} \) 6.1, CH\textsubscript{2}), 69.4 (1 C, d, \( \text{J}_{P-C} \) 5.7, CH\textsubscript{2}), 69.2 (1 C, d, \( \text{J}_{P-C} \) 5.3, CH\textsubscript{2}); \( \delta \)\textsubscript{P} (162 MHz, CDCl\textsubscript{3}) -1.3, -1.6, -1.7; HRMS m/z (FAB+) [Found: (M+Na)+ 1388.4074. C\textsubscript{76}H\textsubscript{74}O\textsubscript{17}NNaP\textsubscript{3} requires \( M^+ \) 1388.4062]; m/z (ES+) 1384 ([M+NH\textsubscript{4}]\textsuperscript{+}, 100%).

\((\pm)-(4’-\text{Carbamoyl-}N\text{-phenolate})-\text{myo}-\text{inositol 1,3,5-trisphosphate heptakis-sodium salt 118}\)

\[
\text{\begin{center}
\includegraphics[width=0.5\textwidth]{palladium-black-and-sodium-bicarbonate-added-to-base-paper}
\end{center}}
\]

Palladium black (78 mg, 0.74 mmol, 20 equiv.) and sodium bicarbonate (21.9 mg, 0.26 mmol, 7 equiv.) were added to a solution of (±)-2,6-bis-O-benzyl-1,3,5-tris(dibenzyl phosphate)-4-dimethylphosphinyl \textit{myo}-inositol 116 (51 mg, 37 \textmu m mol, 1 equiv.) in \( t-\)
butanol (3.0 mL) and water (0.5 mL) under nitrogen. The mixture was then placed under hydrogen and stirred for 3 h. The organic phase removed and water (1 mL) was added to the black residue. The palladium was removed by filtration and washed with water. The aqueous filtrate was freeze-dried to (±)-4-(4'-carbamoyl-N-phenolate)-myo-inositol 1,3,5-trisphosphate heptakis-sodium salt 118 (24 mg, 92% yield) as a colourless solid. δ_H (400 MHz, D_2O) 7.21 (2 H, d, J = 9.0, NHC_6H_4OBn), 6.79 (2 H, d, J = 9.0, NHC_6H_4OBn), 5.01 (1 H, dd, J = 9.9, 9.9, inositol ring 4-H), 4.41 (1 H, dd, J = 2.5, 2.5, inositol ring 2-H), 4.10 (1 H, ddd, J = 9.6, 2.5, inositol ring), 4.05-3.86 (3 H, m, 3 × inositol ring); δ_C (75 MHz, D_2O) 156.4 (C=O), 152.2 (C), 130.3 (C), 122.9 (CH), 115.7 (CH), 76.2 (1 C, d, J_P-C = 5.4, CH), 74.3 (1 C, d, J_P-C = 5.4, CH), 72.9 (1 C, d, J_P-C = 5.4, CH), 72.8 (1 C, d, J_P-C = 5.3, CH), 71.7 (1 C, d, J_P-C = 5.3, CH), 70.17 (CH); δ_P (162 MHz, D_2O) 1.9, 1.6, 0.6; HRMS m/z (FAB) [Found: (M − 7Na+6H)− 553.9865. C_{62}H_{62}O_{18}P_{3}S requires M− 553.9871]; m/z (ES−) 576 ([M−6Na+5H]−, 100%); 598 ([M−5Na+4H]−, 65%).

(-)-1D-5-O-Allyl-2,6-di-O-benzyl-1-O-triisopropylsilyl-3-O-endo-4-O-exo-(L-1',7',7'-'trimethylbicyclo[2.2.1]hept-2'-ylidine)-myo-inositol (-)-119

Triisopropylsilyl triflate (7.15 g, 6.20 mL, 23.3 mmol, 2.2 equiv.) was added to a stirred solution of (-)-1D-5-O-allyl-2,6-di-O-benzyl-3-O-endo-4-O-exo-(L-1',7',7'-'trimethylbicyclo[2.2.1]hept-2'-ylidine)-myo-inositol (-)-41 (5.66 g, 10.60 mmol, 1.0 equiv.) and 2,6-lutidine (3.40 g, 3.70 mL, 31.8 mmol, 3.0 equiv.) in dry dichloromethane (50 mL) at 0 °C. The mixture was allowed to warm to RT and stirred for 18 h. Water (50 mL) was added to the mixture and the organic and aqueous phases separated. The organic phase was collected and the aqueous portion extracted with dichloromethane (3 × 50 mL). The combined organic extracts were washed with brine (100 mL), then dried (MgSO_4), filtered and concentrated under reduced pressure. Purification by silica gel column chromatography, eluting with ethyl acetate and petroleum ether (5:95), gave (-)-1D-5-O-allyl-2,6-di-O-benzyl-1-O-triisopropylsilyl-3-O-endo-4-O-exo-(L-1',7',7'-'...
trimethylbicyclo[2.2.1]hept-2'-ylidine)-myo-inositol \((-\text{-119})\) (6.20 g, 85% yield) as a colourless gum. Rf 0.62 (ethyl acetate/petroleum ether 10:90); [$\alpha$]$^0$D -2.91, (c 1.1 in CHCl$_3$); $v_{\text{max}}$ (thin film)/cm$^{-1}$ 3386 (w), 2944 (s), 2867 (s), 1455 (m), 1098 (s); $\delta$H (400 MHz, CDCl$_3$) 7.34-7.15 (10 H, m, 2 × Ph), 5.83 (1 H, dddd, J 17.2, 10.4, 5.6, 5.6, CH=CH$_2$), 5.20 (1 H, dddd, J 17.2, 1.8, 1.7, 1.7, CH=CHH), 4.90 (1 H, d, J$_{\text{AB}}$ 11.5, OCH$_A$H$_B$-Ph), 4.88 (1 H, d, J$_{\text{AB'}}$ 11.1, OCH$_A$H$_B$-Ph), 4.70 (1 H, d, J$_{\text{AB'}}$ 11.1, OCH$_A$H$_B$-Ph), 4.64 (1 H, d, J$_{\text{AB}}$ 11.5, OCH$_A$H$_B$-Ph), 4.29 (1 H, dddd, J 12.7, 5.6, 1.7, 1.2, CHHCH=CH$_2$), 4.11 (1 H, dd, J 2.8, 1.6, inositol ring 2-H), 4.05 (1 H, dddd, J 12.7, 5.6, 1.7, 1.2, CHHCH=CH$_2$), 3.95 (1 H, dd, J 9.7, 9.7, inositol ring), 3.84 (1 H, dd, J 9.3, 2.8, inositol ring), 3.67 (1 H, dd, J 8.5, 8.5, inositol ring), 3.45 (1 H, dd, J 9.5, 8.5, inositol ring), 3.22 (1 H, dd, J 9.7, 1.6, inositol ring), 2.08 (1 H, dt, J 13.5, 3.8, camphor ring), 1.84 (1 H, ddd, J 12.9, 9.6, 3.8, camphor ring), 1.68-1.62 (2 H, m, camphor ring), 1.37-1.29 (1 H, m, camphor ring), 0.98-0.96 (21 H Si[CH(CH$_3$)$_2$]$_3$), 0.94 (3 H, s, CH$_3$), 0.78 (3 H, s, CH$_3$ camphor bridge), 0.74 (3 H, s, CH$_3$ camphor bridge); $\delta$C (75 MHz, CDCl$_3$) 139.5 (C), 138.9 (C), 135.4 (CH), 128.1 (CH), 128.0 (CH), 127.6 (CH), 127.5 (CH), 127.3 (CH), 127.1 (CH), 120.4 (C), 116.5 (CH$_2$), 83.3 (CH), 81.6 (CH), 77.5 (CH), 76.8 (CH), 76.0 (CH), 75.9 (CH$_2$), 74.9 (CH), 73.8 (CH$_2$), 71.7 (CH$_2$), 52.9 (C), 48.3 (C), 46.3 (CH$_2$), 45.0 (CH), 28.9 (CH$_2$), 26.8 (CH$_2$), 20.3 (CH$_3$), 20.2 (CH$_3$), 18.2 (CH$_3$), 12.9 (CH), 9.7 (CH$_3$); HRMS m/z (ES+) [Found: (M+H)$^+$ 691.4394. C$_{42}$H$_{63}$O$_6$Si requires M$^+$ 691.4388]; m/z (ES+) 691 ([M+H]$^+$, 100%).

\((-\text{-1d-5-O-Allyl-2,6-bis-O-benzyl-1-O-triisopropylsilyl-myo-inositol (-)-70})\)

Acetyl chloride (211 mg, 191 $\mu$L, 2.69 mmol, 0.3 equiv.) was added to a solution of \((-\text{-1d-5-O-allyl-2,6-di-O-benzyl-1-O-triisopropylsilyl-3-O-endo-4-O-exo-(-L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidine)-myo-inositol (-)-119})\) (6.19 g, 8.97 mmol, 1 equiv.) in dichloromethane (80 mL) and methanol (40 mL) and the solution was stirred for 3.5 h. The reaction mixture was quenched by the addition of triethylamine (2 mL), and all
volatile components were removed in vacuo. The residue was partitioned between dichloromethane (30 mL) and water (30 mL), and the aqueous phase was extracted with dichloromethane (3 × 30 mL). The combined organic extracts were washed with brine, dried (MgSO₄), filtered and concentrated under reduced pressure. Purification by silica gel column chromatography, eluting with ethyl acetate/petroleum ether (20/80 then 30/70) gave (-)-1D-5-O-allyl-2,6-bis-O-benzyl-1-O-triisopropylsilyl-myoinositol (−)-70 (4.01 g, 80% yield) as a colourless gum. \([\alpha]_{\text{D}}^{20}\) -16.9, (c 1.7 in CHCl₃); \(\delta_H\) (300 MHz, CDCl₃) 7.38-7.24 (10 H, m, 2 × Ph), 5.87 (1 H, dddd, J 17.3, 10.4, 5.7, 5.7, CH=CH₂) 5.22 (1 H, dddd, J 17.3, 1.7, 1.6, 1.6, CH=CHH), 5.13-5.07 (1 H, m, CH=CHH), 5.11 (1 H, d, Jₐₐ 11.4, OCHₐHₐ-Ph), 4.92 (1 H, d, Jₐₐ 11.7, OCHₐHₐ-Ph), 4.86 (1 H, d, Jₐₐ 11.7, OCHₐHₐ-Ph), 4.76 (1 H, d, Jₐₐ 11.4, OCHₐHₐ-Ph), 4.31 (1 H, dddd, J 12.4, 5.7, 1.6, 1.3, CHHCH=CH₂), 4.24 (1 H, dddd, J 12.4, 5.7, 1.6, 1.3, CHH=CHH=CH₂), 4.02-3.99 (1 H, m, inositol ring 2-H), 3.87-3.79 (3 H, m, 3 × inositol ring 1-H, 4-H, 6-H), 3.48 (1 H, dd, J 9.6, 2.6, inositol ring 3-H), 3.26-3.15 (1 H, m, inositol ring 5-H), 2.64 (1 H, br s, OH), 2.44 (1 H, br s, OH), 1.10-1.07 (21 H, m, 3 × SiCH, 18 × SiCH₂CH₃). The data are in agreement with the values reported for (+)-70.

(+)-1D-3-O-Benzoyl-5-O-allyl-2,6-bis-O-benzyl-1-O-triisopropylsilyl-myoinositol (+-73

A mixture of 5-O-allyl-2,6-bis-O-benzyl-1-O-triisopropylsilyl-myoinositol (+)-70 (3.90 g, 7.01 mmol, 1.0 equiv.) and dibutyltin oxide (5.24 g, 21.03 mmol, 3.0 equiv.) in dry toluene (40 mL) was heated under reflux for 16 h with Dean-Stark apparatus. The mixture was then reacted with benzoyl chloride (1.08 g, 900 μL, 7.71 mmol, 3.0 equiv.) using the procedure described for compound (±)-73 to give (+)-1D-3-O-benzoyl-5-O-allyl-2,6-bis-O-benzyl-1-O-triisopropylsilyl-myoinositol (+)-73 (3.43 g, 74% yield) as a colourless solid. \([\alpha]_{\text{D}}^{20}\) +26.0, (c 1.6 in CHCl₃); mp 94-96 °C (dichloromethane/petroleum ether); \(\delta_H\) (300 MHz, CDCl₃) 8.10-8.06 (2 H, m, C₆H₅C(O)CH), 7.63-7.59 (1 H, m, C₆H₅C(O)CH), 7.47-7.42 (2 H, m, C₆H₅C(O)CH), 7.39-7.25 (10 H, m, 2 × Ph), 5.89 (1 H,
To a solution of 4-methoxybenzyltrichloroacetimidate (2.81 g, 9.94 mmol, 2.0 equiv.) and (+)-1D-3-O-benzoyl-5-O-allyl-2,6-bis-O-benzyl-1-O-triisopropylsilyl-myoinositol (-)-73 (3.28 g, 4.97 mmol, 1.0 equiv.) and 4 Å molecular sieves in toluene (100 mL) was added yttrium (III) triflate (53 mg, 0.099 mmol, 0.02 equiv.) at -78 °C. The mixture was stirred at -78 °C for 30 min, then the temperature was raised to -40 °C and the progress of the reaction was monitored by TLC analysis. After 3 h at this temperature 4-methoxybenzyltrichloroacetimidate (702 mg, 520 μL, 2.49 mmol, 0.5 equiv.) was added to the mixture. The temperature was raised to -20 °C and allowed to slowly warm to RT overnight. The reaction mixture was quenched by the addition of saturated aqueous sodium bicarbonate solution (1 mL) and filtered through a pad of Celite. The filtrate was concentrated under reduced pressure, and the residue partitioned between ethyl acetate (50 mL) and water (50 mL). The aqueous phase was extracted with ethyl acetate (3 × 50 mL) and the combined organic fractions were washed with saturated aqueous sodium bicarbonate solution (70 mL) and brine (70 mL), dried (MgSO₄), filtered and concentrated under reduced pressure. Exhaustive purification by silica gel column chromatography eluting with ethyl acetate/petroleum ether (2.5:97.5 then 5:95) gave 1D-3-O-benzoyl-5-O-allyl-2,6-bis-O-benzyl-4-O-(4'-methoxybenzyl)-1-O-triisopropylsilyl-myoinositol 74 (2.49 g, 64% yield) as an impure, colourless gum which was used.
without further purification. Rf 0.47 (ethyl acetate/petroleum ether 25:75); δH (300 MHz, CDCl3) 8.02-7.92 (2 H, m, C6H5C(O)CH), 7.64-7.58 (1 H, m, C6H5C(O)CH) 7.49-7.43 (2 H, m, C6H5C(O)CH), 7.41-7.25 (10 H, m, 2 × Ph), 7.10 (2 H, d, J 8.7, C6H4OCH3), 6.71 (2 H, d, J 8.7, C6H4OCH3) 5.92 (1 H, dddd, J 17.0, 10.5, 5.6, 5.6, CH=CH2), 5.23 (1 H, dddd, J 17.0, 1.8, 1.6, 1.6, CH=CHH), 5.13 (1 H, dddd, J 10.5, 1.8, 1.1, 1.1, CH=CHH), 5.03 (1 H, dd, J 10.0, 2.3, inositol ring), 4.93 (1 H, d, JAB 11.3, OCHAHB-Ph), 4.90 (1 H, d, JAB 11.4, OCHAHB-Ph), 4.89 (1 H, d, JAB 11.3, OCHAHB-Ph), 4.72 (1 H, d, JAB 10.5, OCHAHB-C6H4OCH3), 4.71 (1 H, d, JAB 11.4, OCHAHB-Ph), 4.65 (1 H, d, JAB 10.5, OCHAHB-C6H4OCH3), 4.42-4.27 (2 H, m, CH2CH=CH2), 4.18 (1 H, dd, J 10.0, 9.3, inositol ring) 4.12-4.09 (1 H, m, inositol ring), 3.99-3.89 (2 H, m, inositol ring), 3.72 (3 H, s, OCH3), 3.44-3.40 (1 H, m, inositol ring), 1.09-1.04 (21 H, m, 3 × SiCH, 18 × SiCHCH3).

The data are in agreement with the values reported for (+)-74.

(±)-1D-5-O-Allyl-2,6-bis-O-benzyl-4-O-(4′-methoxybenzyl)-1-O-triisopropylsilyl-myoinositol (+)-75

1D-3-O-Benzoyl-5-O-allyl-2,6-bis-O-benzyl-4-O-(4′-methoxybenzyl)-1-O-triisopropylsilyl-myoinositol 74 (2.47 g, 3.17 mmol, 1 equiv.) was reacted with 2 M aqueous sodium hydroxide solution (2 mL, 4.96 mmol, 1.25 equiv.) in diethyl ether (14 mL) and methanol (7 mL) using the procedure described for compound (±)-75 to give (+)-1D-5-O-allyl-2,6-bis-O-benzyl-4-O-(4′-methoxybenzyl)-1-O-triisopropylsilyl myo-inositol (+)-75 (1.72 g, 80% yield) as a colourless oil. [α]D +24.3, (c 1.0 in CHCl3); δH (300 MHz, CDCl3) 7.37-7.20 (12 H, m, 10 × C6H5, 2 × C6H4OCH3), 6.87 (2 H, d, J 8.7, C6H4OCH3) 5.88 (1 H, dddd, J 17.1, 10.4, 5.7, 5.7, CH=CH2), 5.23 (1 H, dddd, J 17.1, 1.7, 1.6, 1.6, CH=CHH), 5.13 (1 H, dddd, J 10.4, 1.7, 1.3, 1.3, CH=CHH), 5.00 (1 H, d, JAB 11.3, OCHAHB-Ph), 4.88 (1 H, d, JAB 11.5, OCHAHB-Ph), 4.86 (1 H, d, JAB 11.5, OCHAHB-Ph), 4.83 (1 H, d, JAB 10.7, OCHAHB-C6H4OCH3), 4.77 (1 H, d, JAB 11.3, OCHAHB-Ph), 4.67 (1 H, d, JAB 10.7, OCHAHB-C6H4OCH3), 4.30 (2 H, m, CH2CH=CH2), 3.98 (1 H, dd, J 2.4, 2.4, inositol ring 2-H), 3.88 (1 H, dd, J 9.2, 9.2, inositol ring 6-H), 3.81-3.77 (1 H, m, inositol ring 1-H).
3.80 (3 H, s, OCH₃), 3.75 (1 H, dd, J 9.2, 9.2, inositol ring 4-H), 3.51-3.48 (1 H, m, inositol ring 3-H), 3.32 (1 H, dd, J 9.2, 9.2 inositol ring 5-H), 2.20 (1 H, br s, OH), 1.09-1.05 (21 H, m, 3 × SiCH₃, 18 × SiCH₂CH₃). The data are in agreement with the values reported for (+)-75.

(+)-1D-2,6-bis-O-Benzyl-4-O-(4’-methoxybenzyl)-1-O-triisopropylsilyl-myo-inositol (+)-68

A stirred solution of (+)-1D-5-O-allyl-2,6-bis-O-benzyl-4-O-(4’-methoxybenzyl)-1-O-triisopropylsilyl-myo-inositol (+)-75 (1.63 g, 2.41 mmol, 1 equiv.), Wilkinson’s catalyst (557 mg, 0.60 mmol, 0.25 equiv.) and diisopropylethyl amine (342 mg, 461 μL, 2.65 mmol, 1.1 equiv.) in absolute ethanol (25 mL) was heated under reflux for 3 h. Analysis by 1H NMR showed that full isomerisation of the allyl double bonds had occurred. The reaction mixture was then filtered through a pad of Celite and concentrated under reduced pressure. The residue was dissolved in methanol (8 mL) and dichloromethane (5 mL) and acetyl chloride (57 mg, 52 μL, 0.72 mmol, 0.3 equiv.) was added. The mixture was stirred for 4 h after which the reaction was quenched with triethylamine (2 mL). All volatile components were removed in vacuo and the residue partitioned between ethyl acetate (20 mL) and water (20 mL). The aqueous phase was extracted with ethyl acetate (3 × 20 mL) and the combined organic extracts were washed with aqueous saturated sodium bicarbonate solution (40 mL) and brine (40 mL), dried (MgSO₄), filtered and concentrated in vacuo. Purification of the crude product by silica gel column chromatography, eluting with ethyl acetate and petroleum ether (15:85, 25:75 then 40:60) gave the desired product (+)-1D-2,6-bis-O-benzyl-4-O-(4’-methoxybenzyl)-1-O-triisopropylsilyl-myo-inositol (+)-68 (74 mg, 5% yield) as a colourless gum, and 2,6-bis-O-benzyl-1-O-triisopropylsilyl myo-inositol (+)-120 (658 mg) as an undesired by-product. Data for compound (+)-68: [α]D₂⁰ +28.6, (c 0.9 in CHCl₃); δH (300 MHz, CDCl₃) 7.39-7.25 (10 H, m, 2 × Ph), 7.26 (2 H, d, J 8.6, C₆H₄OCH₃), 6.87 (2 H, d, J 8.6, C₆H₄OCH₃), 5.02 (1 H, d, JₐB 11.3, OCH₃H₃-Ph), 4.99 (1 H, d, JₐB 11.6,
OCH₆H₃Ph), 4.83 (1 H, d, Jₐb 10.8, OCH₆H₃C₆H₄OCH₃), 4.80 (1 H, d, Jₐₐb 11.3, OCH₆H₃Ph), 4.73 (1 H, d, Jₐb 11.6, OCH₆H₅Ph) 4.67 (1 H, d, Jₐb 10.8, OCH₆H₅C₆H₄OCH₃), 3.98 (1 H, dd, J 2.0, 2.0, inositol ring 2-H), 3.89-3.78 (2 H, m, inositol ring 3-H, 4-H), 3.81 (3 H, s, OCH₃), 3.67-3.65 (1 H, m, inositol ring 6-H), 3.58-3.47 (2 H, m, inositol ring 1-H, 5-H), 2.34 (1 H, br s, OH), 2.28 (1 H, d, J 4.7, OH), 1.11-1.09 (21 H, m, 3 × SiCH, 18 × SiCH₂). The data are in agreement with the values reported for (±)-68. Data for compound (+)-120: [α]D° +14.1 (c 1.1 in CHCl₃) δH (300 MHz, CDCl₃) 7.37-7.27 (10 H, m, 2 × Ph), 5.09 (1 H, d, Jₐb 11.1, OCH₆H₃-Ph), 4.96 (1 H, d, Jₐb 11.7, OCH₆H₅-Ph), 4.78 (1 H, d, Jₐb 11.7, OCH₆H₅-Ph) 4.78 (1 H, d, Jₐb 11.1, OCH₆H₅-Ph), 4.00 (1 H, dd, J 2.1, 2.1, inositol ring 2-H), 3.89 (1 H, dd, J 9.4, 2.1, inositol ring 1-H), 3.78 (1 H, dd, J 9.0, 9.0, inositol ring 6-H), 3.73 (1 H, dd, J 9.0, 9.0, inositol ring 4-H), 3.50-3.47 (1 H, m, inositol ring 3-H), 3.37-3.34 (1 H, m, inositol ring 5-H), 2.99 (1 H, br s, OH), 2.58 (2 H, br s, 2 × OH), 1.14-1.10 (21 H, m, 3 × SiCH, 18 × SiCH₂). The data are in agreement with the values reported for the synthesis of 2,6-bis-O-benzyl-1-O-triisopropylsilyl myo-inositol (+)-120 below.

(-)-1d-2,6-bis-O-Benzyl-4-O-(4'-methoxybenzyl)-1,3,5-tris(dibenzylphosphate)-myo-inositol (-)-105

To a solution of (+)-1d-2,6-bis-O-benzyl-4-O-(4'-methoxybenzyl)-1-O-triisopropylsilyl-myoinositol (+)-68 (71 mg, 0.11 mmol, 1.0 equiv.) in THF (2 mL) was added tetrabutylammonium fluoride (1 M in THF, 134 μL, 1.2 equiv.). The mixture was stirred for 2.5 h after which the reaction was adjudged to be complete by TLC analysis. The mixture was diluted with diethyl ether (5 mL). Water (5 mL) was added and the layers separated and the aqueous phase was extracted with diethyl ether (3 × 5 mL). The combined organic extracts were washed with aqueous saturated bicarbonate solution (10 mL) and brine (10 mL), dried (MgSO₄), filtered and concentrated. The crude product was passed through a silica plug, eluting with ethyl acetate/hexane (35:75, then 70:30).
The inositol material obtained was then dissolved in dichloromethane (1 mL). This solution was added to a stirred mixture of 1H-tetrazole (3 % w/v in acetonitrile, 1.0 mL, 0.45 mmol, 6.0 equiv.) and bis(benzyloxy)-N,N-diisopropylamino phosphine (155 mg, 0.45 mmol, 6.0 equiv.) in dichloromethane (2 mL). After 18 h, bis(benzyloxy)-N,N-diisopropylamino phosphine (52 mg, 0.15 mmol, 2 equiv.) in dichloromethane (1 mL) was added via cannula, followed by 1H-tetrazole in acetonitrile (3% w/v, 350 μL, 0.15 mmol, 2 equiv.), and the mixture was stirred for 3 h. The mixture was cooled to -78 °C and 3-chloroperbenzoic acid (assume 60 %, 173 mg, 0.60 mmol, 8.0 equiv.) was added. The temperature was raised to RT and the mixture stirred for 2 h. The 3-chloroperbenzoic acid was quenched with a 10 % aqueous solution of sodium bisulfite (3 mL). The mixture was stirred for 10 min and the organic and aqueous phases were separated. The aqueous layer was extracted with dichloromethane (3 × 5 mL). The combined organic extracts were washed with saturated aqueous sodium bicarbonate solution (10 mL), brine (10 mL), dried (MgSO₄), filtered and concentrated under reduced pressure. Purification by silica gel chromatography, eluting with ethyl acetate and hexane (40:60, 50:50 then 60:40) gave (-)-1D-2,6-bis-O-benzyl-4-O-(4′-methoxybenzyl)-1,3,5-tris(dibenzylphosphate)-myo-inositol (-)-105 (56 g, 40% yield over two steps) as a colourless gum. \( [\alpha]_{D}^{20} \) -1.3 (c 1.1 in CHCl₃); \( \delta_H \) (400 MHz, CDCl₃) 7.42-7.40 (2 H, m, Ph), 7.37-7.15 (36 H, m, 34 × Ph, 2 × C₆H₄OCH₃), 7.10-7.05 (4 H, m, Ph), 6.76 (2 H, d, \( J \) 8.7, C₆H₄OCH₃), 5.00-4.71 (18 H, m, 16 × OCH₂Ph, 2 × OCH₂C₆H₄OCH₃), 4.59 (1 H, dd, \( J \) 2.4, 2.4, inositol ring 2-H), 4.42 (1 H, ddd, \( J \) 9.2, 9.2, 9.2, inositol ring 5-H), 4.34-4.26 (2 H, m, inositol ring 1-H and 3-H), 4.10 (2 H, dd, \( J \) 9.5, 9.5, inositol ring 4-H and 6-H), 3.70 (3 H, s, OCH₃); \( \delta_P \) (121 MHz, CDCl₃) -1.4, -1.6 (2 P). The data are in agreement with the values reported for (±)-105.
(-)-1\(\alpha\)-2,6-bis-O-Benzyl-1,3,5-tris(dibenzylphosphate)-myo-inositol (-)-106

(-)-1\(\alpha\)-2,6-bis-O-Benzyl-4-O-(4'-methoxybenzyl)-1,3,5-tris(dibenzylphosphate)-myo-inositol (-)-105 (55 mg, 0.044 mmol, 1 equiv.) was reacted with ceric ammonium nitrate (1.10 g, 2.02 mmol, 2.5 equiv.) in acetonitrile (20 mL) and water (5 mL) using the procedure described for compound (±)-106 to give (-)-1\(\alpha\)-2,6-bis-O-Benzyl-1,3,5-tris(dibenzylphosphate)-myo-inositol (-)-106 (36 mg, 72% yield) as a colourless gum. 

\([\alpha]^{20}_D -5.1, (c 1.8 \text{ in CHCl}_3); \delta_H (300 \text{ MHz, CDCl}_3) 7.35-7.14 (40 \text{ H, m, Ph}), 5.13 (1 \text{ H, s, OCH}_2\text{Ph}), 5.11 (1 \text{ H, s, OCH}_2\text{Ph}), 5.05-4.99 (4 \text{ H, m, OCH}_2\text{Ph}), 4.98-4.85 (6 \text{ H, m, OCH}_2\text{Ph}), 4.72 (2 \text{ H, s, OCH}_2\text{Ph}), 4.68 (2 \text{ H, s, OCH}_2\text{Ph}), 4.57 (1 \text{ H, br s, OH}), 4.34-4.33 (1 \text{ H, m, inositol ring}), 4.27-4.16 (4 \text{ H, m, 4 \times inositol ring}), 3.99 (1 \text{ H, dd, J 9.3, 9.3, inositol ring}); \delta_P (121 \text{ MHz, CDCl}_3) 0.2, -1.1, -1.8. The data are in agreement with the values reported for (±)-106.

(±)-2,6-bis-O-Benzyl-1-O-triisopropylsilyl-myosinositol 120

A stirred solution of (±)-5-O-allyl-2,6-bis-O-Benzyl-1-O-triisopropylsilyl-myosinositol 70 (1.82 g, 3.27 mmol, 1 equiv.), Wilkinsons catalyst (303 g, 0.33 mmol, 0.1 equiv.) and diisopropylethyl amine (422 mg, 569 \(\mu\)L, 3.33 mmol, 1.0 equiv.) in absolute ethanol (25 mL) was heated under reflux for 3 h. Analysis by \(^1\text{H} \text{NMR}\) showed that full isomerisation of the allyl double bond had occurred. The reaction mixture was then filtered through a pad of Celite and concentrated under reduced pressure. The residue was dissolved in methanol (20 mL) and 4-toluenesulfonic acid (186 mg, 0.98 mmol, 0.3 equiv.) was added. The mixture was stirred for 2 h after which 4-toluenesulfonic acid (124 mg, 0.65 mmol, 0.2 equiv.) was again added. The mixture was stirred for a further 2 h and the reaction was then quenched with triethylamine (3 mL). All volatile components were removed \textit{in vacuo} and the residue partitioned between ethyl acetate (30 mL) and water.
(20 mL). The aqueous phase was extracted with ethyl acetate (3 × 30 mL) and the combined organic extracts were washed with aqueous saturated sodium bicarbonate solution (50 mL) and brine (50 mL), dried (MgSO₄), filtered and concentrated in vacuo. Purification of the crude product by silica gel column chromatography, eluting with ethyl acetate and hexane (25:75, 30:70 then 40:60) gave (±)-2,6-bis-O-benzyl-1-O-triisopropylsilyl-myoinositol 120 (902 mg, 53%) as a colourless oil. R_f 0.14 (ethyl acetate/petroleum ether 50:50); δ_H (300 MHz, CDCl₃) 7.38-7.28 (10 H, m, 2 × Ph), 5.09 (1 H, d, J_AB 11.1, OCH_AH_B-Ph), 4.96 (1 H, d, J_AB 11.7, OCH_AH_B-Ph), 4.78 (1 H, d, J_AB 11.7, OCH_AH_B-Ph) 4.77 (1 H, d, J_AB 11.1, OCH_AH_B-Ph), 4.00 (1 H, dd, J 2.2, 2.2, inositol ring 2-H), 3.88 (1 H, dd, J 9.4, 2.2, inositol ring 1-H), 3.78 (1 H, dd, J 9.0, 9.0, inositol ring 6-H), 3.73 (1 H, dd, J 9.0, 9.0, inositol ring 4-H), 3.49-3.47 (1 H, m, inositol ring 3-H), 3.36 (1 H, ddd, J 9.0, 9.0, 1.9, inositol ring 5-H), 2.99 (1 H, d, J 1.8, OH), 2.59 (1 H, d, J 1.9, OH), 2.55 (1 H, d, J 7.6, OH), 1.16-1.08 (21 H, m, 3 × SiCH, 18 × SiCH₃). The data are in agreement with the values reported for (+)-120.

(±)-2,6-bis-O-Benzyl-1-O-triisopropylsilyl-3,4,5-tris(dibenzylphosphate)-myo-inositol 124

(±)-2,6-bis-O-Benzyl-1-O-triisopropylsilyl-3,4,5-tris(dibenzylphosphate)-myo-inositol 124 (1.46 g, 74% yield) was obtained from (±)-2,6-bis-O-benzyl-1-O-triisopropylsilyl-myoinositol 120 (783 mg, 1.51 mmol) using the procedure described for (+)-124. R_f 0.68 (ethyl acetate/petroleum ether 60:40); δ_H (400 MHz, CDCl₃) 7.41-7.11 (38 H, m, Ph), 6.90-6.88 (2 H, m, Ph), 5.15-4.74 (15 H, m, 14 × CH₂Ph, 1 × inositol ring 4-H), 4.81 (1 H, dd, J 11.8, 7.4, CH_AH_B-Ph), 4.61 (1 H, ddd, J 9.4, 9.4, 9.4, inositol ring 5-H), 4.56 (1 H, dd, J 11.8, 8.6, CH_AH_B-Ph), 4.44 (1 H, dd, J 1.9, 1.9, inositol ring 2-H), 4.30 (1 H, ddd, J 9.4, 7.1, 1.9, inositol ring 3-H), 4.03 (1 H, dd, J 9.4, 9.4, inositol ring 6-H), 3.91 (1 H, dd, J 9.4, 1.9, inositol ring 1-H), 1.07-1.04 (21 H, m, 3 × SiCH, 18 × SiCH₃); δ_P (121 MHz, CDCl₃) -1.3 (2 P), -1.7; HRMS m/z (ES+) [Found: (M+NH₄)⁺ 1314.5063. C₇₁H₈₇O₁₅NP₃Si
requires $M^+ 1314.5052$, $m/z$ (ES+) 1297 ([M+H]$^+$, 100%). The data are in agreement with the values reported for (+)-124.

(±)-2,6-bis-O-Benzyl-3,4,5-tris(dibenzylphosphate)-myo-inositol 125

(±)-2,6-bis-O-Benzyl-3,4,5-tris(dibenzylphosphate)-myo-inositol 125 (953 g, 80% yield) was obtained from (±)-2,6-bis-O-benzyl-1-O-trisopropylsilyl-3,4,5-tris(dibenzylphosphate)-myo-inositol 124 (1.36 g, 1.05 mmol) using the procedure described for compound (-)-125. $R_f$ 0.24 (ethyl acetate/petroleum ether 60:40); $\delta_H$ (400 MHz, CDCl$_3$) 7.35-7.14 (40 H, m, Ph), 5.15-4.95 (12 H, m, $11 \times$ CH$_2$Ph, $1 \times$ inositol ring 4-H), 4.89-4.79 (3 H, m, CH$_2$Ph), 4.70-4.64 (2 H, m, CH$_2$Ph), 4.47 (1 H, ddd, $J$ 9.5, 9.5, 9.5, inositol ring 5-H), 4.30 (1 H, dd, $J$ 2.5, 2.5, inositol ring 2-H), 4.26 (1 H, ddd, $J$ 9.5, 7.7, 2.5, inositol ring 3-H), 3.84 (1 H, dd, $J$ 9.5, 9.5, inositol ring 6-H), 3.52 (1 H, ddd, $J$ 9.5, 6.2, 2.5, inositol ring 1-H), 1.94 (1 H, d, $J$ 6.2, OH); $\delta_P$ (162 MHz, CDCl$_3$) -1.2, -1.3, -1.9; $m/z$ (ES+) [Found: (M+NH$_4$)$^+$ 1158.3702. $C_{62}H_{67}O_{15}NP_3$ requires $M^+$ 1158.3718], $m/z$ (ES+) 1141 ($M^+$, 100%). The data are in agreement with the values reported in the literature.$^{183}$

(±)-2,6-bis-O-Benzyl-3,4,5-tris(dibenzylphosphate)-1-sulfate-myo-inositol sodium salt 126

A solution of (±)-2,6-bis-O-benzyl-3,4,5-tris(dibenzylphosphate)-myo-inositol 125 (96 mg, 0.084 mmol, 1.0 equiv.) and sulfur trioxide pyridine complex (131 mg, 0.84 mmol, 10 equiv.) in DMF (1 mL) was heated to 50 °C for 8 h. After this time, the reaction was adjudged to be complete by TLC analysis. Triethylamine (1 mL) and methanol (2 mL)
was added and the solvent was removed in vacuo. The residue was subjected to silica gel column chromatography eluting with ethyl acetate/hexane (80:20 then 100 % ethyl acetate) then methanol/chloroform (5:95). The purified product was dissolved in methanol (5 mL) and stirred with Dowex Marathon C sodium form resin for 20 min. The resin was removed by filtration and washed with methanol (3 mL) and methanol/water (3 mL). Water (2 mL) was added to the filtrate which was freeze-dried to give (±)-2,6-bis-O-benzyl-3,4,5-tris(dibenzylphosphate)-1-sulfate-myo-inositol sodium salt 126 (46 mg, 44% yield) as a hygroscopic, colourless solid. δH (300 MHz, CD3OD) 7.56 (2 H, d, J 7.6, Ph), 7.42 (2 H, d, J 7.6, Ph), 7.31-7.08 (36 H, m, Ph), 5.16-4.55 (21 H, m, 16 × CH2Ph, 5 × inositol ring), 4.07 (1 H, dd, J 9.8, 9.8, inositol ring); δC (75 MHz, CD3OD) 140.2 (C), 139.7 (C), 137.3-136.9 (6 C, m, 6 × ArC), 130.1 (CH), 129.7 (CH), 129.6 (CH), 129.51 (CH), 129.49 (CH), 129.47 (CH), 129.43 (CH), 129.36 (CH), 129.3 (CH), 129.2 (CH), 129.15 (CH), 129.1 (CH), 129.0 (CH), 128.8 (CH), 128.5 (CH), 128.4 (CH), 80.0 (1 C, d, 2JPC 4.9, CH), 79.2 (CH), 78.5-78.7 (1 C, m, CH), 78.4 (CH), 78.2 (CH), 77.0 (CH2), 76.8 (CH), 75.2 (CH2), 71.4 (1 C, d, 2JPC 6.0, CH2), 71.2-70.9 (4 C, m, 4 × CH2), 70.7 (1 C, d, 2JPC 5.6, CH2); δP (121 MHz, CD3OD) −2.4, −2.8, −3.0. The data are in agreement with the values reported for (+)-126.

(±)-2,6-bis-O-Benzyl-1-(dibenzyl)methylene phosphonate-3,4,5-tris(dibenzylphosphate)-myo-inositol 133

Dibenzyl phosphonomethyl triflate 131 (160 mg, 0.38 mmol, 3.0 equiv.) in THF (1 mL) was added to a solution of (±)-2,6-bis-O-benzyl-3,4,5-tris(dibenzylphosphate)-myo-inositol 125 (145 mg, 0.127 mmol, 1.0 equiv.) in THF (1 mL). The mixture was cooled to -78 °C and sodium hydride (60 % dispersion in mineral oil, 8 mg, 1.6 equiv.) was added, and the reaction monitored by TLC analysis. After stirring at -78 °C for 1 h, the mixture was allowed to warm to -15 °C and the mixture was stirred at this temperature for 1 h.
The reaction temperature was then raised to RT and stirred for a further 2 h. After this time, the reaction was observed to be incomplete by TLC analysis. The mixture was cooled to -78 °C and sodium hydride (60 % dispersion in mineral oil, 8 mg, 1.6 equiv.) was added. The mixture was allowed to warm to RT and stirred for a further 2 h. After this time, the reaction was adjudged to be complete by TLC analysis. The mixture was diluted with diethyl ether (5 mL) and quenched by the addition of aqueous saturated sodium bicarbonate solution (3 mL). The layers were separated and the aqueous phase was extracted with diethyl ether (3 × 5 mL). The combined organic extracts were washed with brine, dried (MgSO₄), filtered and concentrated. Repeated silica gel column chromatography, eluting with ethyl acetate and hexane (50:50, 60:40, 70:30 then 80:20) gave (±)-2,6-bis-O-benzyl-1-(dibenzyl)methylene phosphonate-3,4,5-tris(dibenzyolphosphate)-myo-inositol 133 (43 mg, 24% yield) as a colourless gum. Rf 0.24 (ethyl acetate/petroleum ether 60:40); νmax (thin film)/cm⁻¹ 3475 (m), 3065 (m), 3034 (m), 2956 (m), 1456 (s), 1274 (s), 1019 (s); δH (300 MHz, CDCl₃) 7.44-7.20 (48 H, m, Ph), 7.07-7.04 (2 H, m, Ph), 5.19-4.69 (21 H, m, 20 × C₂H₂Ph, 1 × inositol ring 4-H), 4.51 (1 H, ddd, J 9.5, 9.5, 9.5, inositol ring 5-H), 4.50 (1 H, dd, J 2.3, 2.3, inositol ring 2-H), 4.24 (1 H, ddd, J 9.6, 9.6, 7.3, 2.3, inositol ring 3-H), 4.07 (1 H, dd, J 9.5, 9.5, inositol ring 6-H), 3.85 (1 H, dd, J 13.6, 9.2, CH₂P), 3.77 (1 H, dd, J 13.6, 8.6, CH₂P), 3.34 (1 H, dd, J 9.5, 2.5, inositol ring 1-H); δC (75 MHz, CDCl₃) 138.4 (C), 138.3 (C), 136.3-135.5 (8 C, m, P(O)OCH₂Ph), 128.6 (CH), 128.5 (CH), 128.4 (CH), 128.3 (CH), 128.2 (CH), 128.1 (CH), 128.11 (CH), 128.07 (CH), 128.0 (CH), 127.95 (CH), 127.9 (CH), 127.6 (CH), 127.5 (CH), 127.4 (CH), 127.2 (CH), 82.8 (CH, inositol ring), 82.6 (CH, inositol ring), 78.5 (CH, inositol ring), 76.4 (CH, inositol ring), 75.8 (CH, inositol ring), 75.4 (CH, inositol ring), 75.3 (CH₂), 74.7 (CH₂), 69.8 (1 C, d, ²Jₚ₋ₖ 5.7, CH₂Ph), 69.7 (1 C, d, ²Jₚ₋ₖ 5.7, CH₂Ph), 69.6 (1 C, d, ²Jₚ₋ₖ 5.5, CH₂Ph), 69.5 (1 C, d, ²Jₚ₋ₖ 5.0, CH₂Ph), 69.4 (1 C, d, ²Jₚ₋ₖ 5.6, CH₂Ph), 69.2 (1 C, d, ²Jₚ₋ₖ 5.2, CH₂Ph), 68.0 (1 C, d, ²Jₚ₋ₖ 6.6, CH₂Ph), 67.8 (1 C, d, ²Jₚ₋ₖ 6.6, CH₂Ph), 66.4 (1 C, d, ¹Jₚ₋ₖ 167.1, CH₂P(O)(OBn)₂); δP (121 MHz, CDCl₃) 22.6, -0.0, -0.2, -0.8; HRMS m/z (FAB) [Found: (M+NH₄)⁺ 1432.4498. C₇₇H₈₂O₁₈NP₄ requires M⁺ 1432.4477]; m/z (ES⁺) 1432 ([M+NH₄]⁺, 100%).
(±)-1-Methylenephosphonate-myoinositol 3,4,5-trisphosphate octakis-sodium salt

Palladium black (65 mg, 0.68 mmol, 20 equiv.) and sodium bicarbonate (20.4 mg, 0.24 mmol, 8 equiv.) were added to a solution of (±)-2,6-bis-O-benzyl-1-(dibenzyl)methylenephosphonate-3,4,5-tris(dibenzylphosphate)-myoinositol (43 mg, 30.4 μmol, 1 equiv.) in t-butanol (3.0 mL) and water (0.5 mL) under nitrogen. The mixture was then placed under hydrogen and stirred for 3 h. The organic phase was removed and water (1 mL) was added to the black residue. The palladium was removed by filtration and washed with water. The aqueous filtrate was freeze-dried to give (±)-1-methylenephosphonate-myoinositol 3,4,5-trisphosphate octakis-sodium salt (19.6 mg, 93% yield) as a colourless solid. δH (300 MHz, D2O) 4.40 (1 H, d, J 2.6, inositol ring 2-H), 4.36 (1 H, ddd, J 9.4, 9.4, 9.4, inositol ring 4-H), 4.04 (1 H, ddd, J 9.4, 9.4, 2.6, inositol ring 3-H), 3.95 (1 H, ddd, J 9.2, 9.2, 9.2 inositol ring 5-H), 3.82 (1 H, dd, J 9.5, 9.5, inositol ring 6-H), 3.77 (1 H, dd, J 12.7, 9.8, CH2P), 3.57 (1 H, dd, J 12.7, 9.8, CH2P), 3.37 (1 H, dd, J 9.5, 2.6, inositol ring 1-H); δC (75 MHz, D2O) 80.3 (1 C, d, J P-C 13.5, CH), 78.3 (CH), 76.1 (CH), 74.5 (CH), 70.9 (CH), 67.2 (CH), 66.7 (1 C, d, J P-C 155.3, CH2); δP (162 MHz, D2O) 15.7, 1.0, 0.8, 0.3; HRMS m/z (FAB) [Found: (M−8Na+7H)− 512.9369. C7H17O18P4 requires M+ 512.9371]; m/z (ES−) 170 (100%), 513 ([M−8Na+7H]+, 1%), 535 ([M−7Na+6H]+, 2%), 557 ([M−6Na+5H]+, 3%), 579 ([M−5Na+6H]+, 2%).

Dibenzyl(hydroxymethyl)phosphonate 129

A mixture of dibenzyl phosphite (85 %, 8.26 g, 26.8 mmol, 1.0 equiv.), paraformaldehyde (0.83 g, 26.8 mmol, 1.0 equiv.) and triethylamine (270 mg, 373 μL,
2.7 mmol, 0.1 equiv.) was heated to 130 °C for 10 min until the mixture became clear. The mixture was allowed to cool to RT and directly purified by silica gel column chromatography eluting with diethyl ether then diethyl ether/ethanol (99:1) to give dibenzyl (hydroxymethyl)phosphonate 129 (2.40 g, 31%) as a colourless oil. Rf 0.27 (diethyl ether); δH (400 MHz, CDCl3) 7.40-7.35 (10 H, m, 2 × Ph), 5.15 (2 H, dd, J 11.8, 8.8, PhCH2), 5.08 (2 H, dd, J 11.8, 8.2, CH2Ph), 3.92 (2 H, app t, J 6.3, PCH2OH), 3.17 (1 H, br s, OH); δC (75 MHz, D2O) 136.1 (C), 136.0 (C), 128.7 (CH), 128.6 (CH), 128.1 (CH), 68.1 (CH2), 68.0 (CH2), 58.6 (1 C, d, 1Jp-CH 160, CH2); δP (162 MHz, CDCl3) 24.8. The data are in good agreement with literature values.175

Dibenzyl phosphonomethyl triflates 131175

2,6-Lutidine (301 mg, 308 μL, 3.8 mmol, 1.2 equiv.) was added to a solution of dibenzyl (hydroxymethyl)phosphonate 129 (927 mg, 3.2 mmol, 1.0 equiv.) in dichloromethane (8 mL) at -78 °C. Triflic anhydride (993 mg, 592 μL, 3.5 mmol, 1.1 equiv.) was added dropwise and the mixture stirred at -78 °C for 1 h. The temperature was raised to -40 °C and stirred for 1 h, then stirred at -10 °C for 1 h. The mixture was diluted with diethyl ether (50 mL) and the precipitate was removed by filtration. The filtrate was washed with water, 1 M HCl then brine, dried (MgSO4), filtered and concentrated. The crude oil was purified by silica gel column chromatography, eluting with ethyl acetate/hexane (3:1). The product obtained was again subjected to silica gel column chromatography, eluting with diethyl ether/hexane (1:1) to give dibenzyl phosphonomethyl triflate 131 (358 mg, 26% yield) as a colourless oil. Rf 0.56 (diethyl ether); δH (400 MHz, CDCl3) 7.42-7.36 (10 H, m, 2 × Ph), 5.18 (2 H, dd, J 11.9, 9.5, CH2Ph), 5.13 (2 H, dd, J 11.6, 9.7, CH2Ph), 4.53 (2 H, d, J 9.0, PCH2OTf); δP (162 MHz, CDCl3) 13.3; δF (375 MHz, CDCl3) −73.9. The data are in good agreement with literature values.175
A stirred solution of (-)-1D-5-O-allyl-2,6-bis-O-benzyl-1-O-triisopropylsilyl myo-inositol (-)-70 (1.58 g, 2.84 mmol, 1 equiv.), Wilkinsons catalyst (263 g, 0.28 mmol, 0.1 equiv.) and diisopropylethyl amine (403 mg, 544 μL, 3.13 mmol, 1.1 equiv.) in absolute ethanol (25 mL) was heated under reflux for 3 h. Analysis by 1H NMR showed that full isomerisation of the allyl double bond had occurred. The reaction mixture was then filtered through a pad of Celite and concentrated under reduced pressure. The residue was dissolved in methanol (5 mL) and dichloromethane (8 mL) and acetyl chloride (89 mg, 81 μL, 1.14 mmol, 0.4 equiv.) was added. The mixture was stirred for 4 h after which the reaction was quenched with triethylamine (3 mL). All volatile components were removed in vacuo and the residue partitioned between ethyl acetate (20 mL) and water (20 mL). The aqueous phase was extracted with ethyl acetate (3 × 20 mL) and the combined organic extracts were washed with aqueous saturated sodium bicarbonate solution (50 mL) and brine (50 mL), dried (MgSO4), filtered and concentrated in vacuo. Purification of the crude product by silica gel column chromatography, eluting with ethyl acetate and hexane (25:75, 30:70 then 40:60) gave (+)-1D-2,6-bis-O-benzyl-1-O-triisopropylsilyl myo-inositol (+)-120 (789 mg, 54%) as a colourless oil. Rf 0.14 (ethyl acetate/petroleum ether 50:50); [α]D 15.4, (c 1.8 in CHCl3); νmax (thin film)/cm⁻¹ 3405 (s), 2943 (s), 1455 (s), 1150 (s), 1061 (s); δH (300 MHz, CDCl3) 7.38-7.28 (10 H, m, 2 × Ph), 5.09 (1 H, d, JAB 11.1, OCHAHB-Ph), 4.96 (1 H, d, JAB 11.7, OCHAHb-Ph), 4.78 (1 H, d, JAB 11.7, OCHAHB-Ph) 4.77 (1 H, d, JAB 11.1, OCHAHB-Ph), 4.00 (1 H, dd, J 2.2, 2.2, inositol ring 2-H), 3.89 (1 H, dd, J 9.4, 2.2, inositol ring 1-H), 3.78 (1 H, dd, J 9.0, 9.0, inositol ring 6-H), 3.73 (1 H, dd, J 9.0, 9.0, inositol ring 4-H), 3.50-3.47 (1 H, m, inositol ring 3-H), 3.36 (1 H, ddd, J 9.0, 9.0, 1.9, inositol ring 5-H), 2.99 (1 H, d, J 1.8, OH), 2.59 (1 H, d, J 1.9, OH), 2.55 (1 H, d, J 7.6, OH), 1.15-1.09 (21 H, m, 3 × SiCH, 18 × SiCH3); δC (75 MHz, CDCl3) 139.3 (C), 139.2 (C), 128.82 (CH), 128.8 (CH), 128.1 (CH), 128.0 (CH), 127.8 (CH), 82.2 (CH), 81.7 (CH), 75.8 (CH2), 75.5 (CH2), 75.3 (CH), 74.9 (CH), 74.4
(CH), 72.7 (CH), 18.7 (CH₃), 13.2 (CH); HRMS m/z (ES+) [Found: (M+H)+ 517.2978. C₂₉H₄₅O₆Si requires M⁺ 517.2980], m/z (ES+) 517 ([M+H]+, 100%).

(+)-1D-2,6-bis-O-Benzyl-1-O-triisopropylsilyl-3,4,5-tris(dibenzylphosphate)-myo-inositol (+)-124

1H-Tetrazole in acetonitrile (3 % w/v, 21 mL, 9.17 mmol, 6.0 equiv.) was added to a solution of bis(benzyloxy)-N,N-diisopropylamino phosphine (3.17 g, 9.17 mmol, 6.0 equiv.) in dichloromethane (10 mL) and the mixture stirred for 10 min. A solution of (+)-1D-2,6-bis-O-benzyl-1-O-triisopropylsilyl myo-inositol (+)-120 (789 mg, 1.53 mmol, 1.0 equiv.) in dichloromethane (10 mL) was added via cannula and the resulting mixture stirred for 5 h. After this time, bis(benzyloxy)-N,N-diisopropylamino phosphine (1.06 g, 3.06 mmol, 2 equiv.) in dichloromethane (10 mL) was added via cannula, followed by 1H-tetrazole in acetonitrile (3 % w/v, 7.00 mL, 3.06 mmol, 2 equiv.), and the mixture was stirred for 16 h. The mixture was cooled to -78 °C and 3-chloroperbenzoic acid (assume 60%, 3.52 g, 12.25 mmol, 8.0 equiv.) was added. The temperature was raised to RT and the mixture stirred for 2 h. The 3-chloroperbenzoic acid was quenched with a 10% aqueous solution of sodium bisulfite (30 mL). The mixture was stirred for 10 min and the organic and aqueous phases were separated. The aqueous layer was extracted with dichloromethane (3 x 30 mL). The combined organic extracts were washed with saturated aqueous sodium bicarbonate solution (50 mL), brine (50 mL), dried (MgSO₄), filtered and concentrated under reduced pressure. Purification by silica gel chromatography, eluting with ethyl acetate and petroleum ether (15:85, 20:80, 25:75 then 35:65) gave (+)-1D-2,6-bis-O-benzyl-1-O-triisopropylsilyl-3,4,5-tris(dibenzylphosphate)-myo-inositol (+)-124 (1.71 g, 86% yield) as a colourless gum. Rf 0.67 (ethyl acetate/petroleum ether 60:40); [α]D⁺ 5.5, (c 1.3 in CHCl₃); νmax (thin film)/cm⁻¹ 3033 (m), 2945 (s), 1954 (w), 1456 (s), 1278 (s), 1016 (s); δH (400 MHz, CDCl₃) 7.41-7.15 (38 H, m, Ph), 6.90-6.88 (2 H, m, Ph), 5.15-4.73 (15 H, m, 14 ×
CH$_2$Ph, 1 × inositol ring 4-H), 4.82 (1 H, dd, J 11.8, 7.4, CH$_3$H$_8$Ph), 4.62 (1 H, ddd, J 9.4, 9.4, 9.4, inositol ring 5-H), 4.56 (1 H, dd, J 11.8, 8.6, CH$_3$H$_8$Ph), 4.44 (1 H, dd, J 1.9, 1.9, inositol ring 2-H), 4.30 (1 H, ddd, J 9.5, 7.1, 1.9, inositol ring 3-H), 4.03 (1 H, dd, J 9.4, 9.4, inositol ring 6-H), 3.91 (1 H, dd, J 9.4, 1.9, inositol ring 1-H), 1.07-1.04 (21 H, m, 3 × SiCH, 18 × SiCHCH$_3$); $\delta$ C (75 MHz, CDCl$_3$) 139.2 (C), 139.1 (C), 136.7 (1 C, d, $^2$J$_{P-C}$ 7.7, C), 136.6 (1 C, d, $^2$J$_{P-C}$ 7.1, C), 136.5 (1 C, d, $^2$J$_{P-C}$ 8.2, C), 136.2 (1 C, d, $^2$J$_{P-C}$ 7.2, C), 136.1 (1 C, d, $^2$J$_{P-C}$ 7.3, C), 136.0 (1 C, d, $^2$J$_{P-C}$ 8.2, C), 129.0 (CH), 128.8 (CH), 128.7 (CH), 128.66 (CH), 128.6 (CH), 128.56 (CH), 128.5 (CH), 128.4 (CH), 128.37 (CH), 128.3 (CH), 128.2 (CH), 127.9 (CH), 127.7 (CH), 127.0 (CH), 126.7 (CH), 80.5 (CH), 79.8 (CH), 77.4-77.2 (CH), 76.8 (CH), 75.9 (CH$_2$), 75.2 (CH$_2$), 73.9 (CH), 70.2 (1 C, d, $^2$J$_{P-C}$ 5.7, CH$_2$), 70.0-69.8 (3 C, m, CH$_2$), 69.8 (1 C, d, $^2$J$_{P-C}$ 5.9, CH$_2$), 69.4 (1 C, d, $^2$J$_{P-C}$ 5.0, CH$_2$), 18.6 (CH$_3$), 13.1 (CH); $\delta$ P (121 MHz, CDCl$_3$) -1.4, -1.5, -1.9; HRMS m/z (FAB) [Found: (M+H)$^+$ 1297.4777. C$_{71}$H$_{84}$O$_{15}$P$_3$Si requires M$^+$ 1297.4787], m/z (ES$^+$) 149 (100%), 1298 ([M+H]$^+$, 40%); Anal. calcd for C$_{71}$H$_{83}$O$_{15}$P$_3$Si: C, 65.73%; H, 6.45%. Found: C, 65.49%; H, 6.67%.

(-)-1d-2,6-bis-O-Benzyl-3,4,5-tris(dibenzylphosphate)-myo-inositol (-)-125$^{183}$

To a solution (+)-1d-2,6-bis-O-benzyl-1-O-triisopropylsilyl-3,4,5-tris(dibenzylphosphate)-myo-inositol (+)-124 (1.71 g, 1.32 mmol, 1.0 equiv.) in THF (20 mL) was added tetrabutylammonium fluoride (1 M solution in THF, 1.6 mL, 1.58 mmol, 1.2 equiv.) and the solution was stirred for 1.5 h. The solution was diluted with ether (50 mL) and water was added (40 mL). The layers were separated and the aqueous phase was extracted with ether (3 × 50 mL). The combined extracts were washed with brine (80 mL), dried (MgSO$_4$) and filtered. The crude product was adsorbed onto silica and purified by silica gel column chromatography, eluting with ethyl acetate and petroleum ether (40:60, 60:40 then 80:20) to give (-)-1d-2,6-bis-O-benzyl-3,4,5-tris(dibenzylphosphate)-myo-inositol (-)-125 (1.22 g, 81% yield) as a colourless gum. $R_f$ 0.24 (ethyl acetate/petroleum
ether 60:40); $[\alpha]_{D}^{20}$ $-8.9$ (c 1.1 in CHCl$_3$), [Lit.,$^{183}$ $[\alpha]_{D}^{20}$ $-8.2,$ (c 1.0 in CHCl$_3$)]; $\nu_{\max}$ (thin film)/cm$^{-1}$ 3379 (s), 3065 (m), 2954 (m), 1456 (s), 1273 (s), 1017 (s); $\delta_H$ (400 MHz, CDCl$_3$) 7.40-7.19 (40 H, m, Ph), 5.18-4.99 (12 H, m, $11 \times$ CH$_2$Ph, $1 \times$ inositol ring 4-H), 4.93-4.84 (3 H, m, CH$_2$Ph), 4.75-4.70 (2 H, m, CH$_2$Ph), 4.51 (1 H, ddd, J 9.5, 9.5, 9.5, inositol ring 5-H), 4.35 (1 H, dd, J 2.5, 2.5, inositol ring 2-H), 4.31 (1 H, ddd, J 10.0, 7.6, 2.5, inositol ring 3-H), 3.89 (1 H, dd, J 9.5, 9.5, inositol ring 6-H), 3.58 (1 H, dd, J 9.5, 2.5, inositol ring 1-H); $\delta_C$ (101 MHz, CDCl$_3$) 138.24 (C), 138.23 (C), 136.2 (1 C, d, $2J_{P-C}$ 7.3, C), 136.1 (1 C, d, $2J_{P-C}$ 6.8, C), 136.0 (1 C, d, $2J_{P-C}$ 7.3, C), 135.9 (1 C, d, $2J_{P-C}$ 7.3, C), 135.7 (1 C, d, $2J_{P-C}$ 6.8, C), 135.5 (1 C, d, $2J_{P-C}$ 7.3, C), 128.6 (CH), 128.5 (CH), 128.4 (CH), 128.3 (CH), 128.25 (CH), 128.17 (CH), 128.15 (CH), 128.1 (CH), 128.0 (CH), 127.80 (CH), 127.78 (CH), 127.76 (CH), 79.6 (CH), 78.5 (CH), 77.8 (CH), 76.3 (CH), 76.2 (CH), 75.6 (CH$_2$), 74.8 (CH$_2$), 71.4 (CH), 69.8 (1 C, d, $2J_{P-C}$ 5.8, CH$_2$), 69.7 (1 C, d, $2J_{P-C}$ 5.8, CH$_2$), 69.63 (1 C, d, $2J_{P-C}$ 5.8, CH$_2$), 69.57 (1 C, d, $2J_{P-C}$ 5.8, CH$_2$), 69.5 (1 C, d, $2J_{P-C}$ 5.3, CH$_2$), 69.4 (1 C, d, $2J_{P-C}$ 5.3, CH$_2$); $\delta_P$ (162 MHz, CDCl$_3$) -1.2, -1.3, -1.9; HRMS m/z (ES+) [Found: (M+Na)$^+$ 1163.3290. C$_{62}$H$_{63}$O$_{15}$NaP$_3$ requires M$^+$ 1163.3272], m/z (ES+) 1163 ([M+Na]$^+$, 100%); Anal. calcd for C$_{62}$H$_{63}$O$_{15}$P$_3$: C, 65.26%; H, 5.56%. Found: C, 65.09%; H, 5.42%. The data are in agreement with the literature values.$^{183}$

(-)-1D-2,6-bis-O-Benzyl-1-(dibenzyl)methylene phosphonate-3,4,5-tris(dibenzylphosphate)-myo-inositol (-)-133

Dibenzy phosphonomethyl triflate 131 (195 mg, 0.46 mmol, 3.0 equiv.) in THF (1 mL) was added to a solution of (-)-1D-2,6-bis-O-benzyl-3,4,5-tris(dibenzylphosphate)-myo-inositol (-)-125 (175 mg, 0.153 mmol, 1.0 equiv.) in THF (1 mL). The mixture was cooled to -15 °C and sodium hydride (60 % dispersion in mineral oil, 9 mg, 1.5 equiv.) was added. After stirring at -15 °C for 1.5 h, the mixture was allowed to warm to RT and stirred for a further 1 h. After this time, the reaction was adjudged to be complete by TLC
analysis. The mixture was diluted with diethyl ether (5 mL) and quenched by the addition of aqueous saturated sodium bicarbonate solution (3 mL). The layers were separated and the aqueous phase was extracted with diethyl ether (3 × 5 mL). The combined organic extracts were washed with brine, dried (MgSO₄), filtered and concentrated. Repeated silica gel column chromatography, eluting with ethyl acetate and hexane (50:50, 60:40, 70:30 then 80:20) gave (-)-1D-2,6-bis-O-benzyl-1-(dibenzyl)methylene phosphonate-3,4,5-tris(dibenzylphosphate)-myo-inositol (-)-133 (61 mg, 28% yield) as a colourless gum. \( \rho \) 0.24 (ethyl acetate/petroleum ether 60:40); \([\alpha]_{D}^{20} \) −6.6 (c 1.2 in CHCl₃); \( \nu_{\text{max}} \) (thin film)/cm\(^{-1}\) 3476 (m), 3065 (m), 3034 (m), 2956 (m), 1456 (s), 1274 (s), 1019 (s), 737 (s), 697 (s); \( \delta_{H} \) (400 MHz, CDCl₃) 7.40-7.23 (48 H, m, Ph), 7.05-7.03 (2 H, m, Ph), 5.13-4.80 (21 H, m, 20 × C\(_{6}\)H\(_{5}\)Ph, 1 × inositol ring 4-H), 4.52-4.45 (2 H, m, inositol ring 2-H, 5-H), 4.25-4.23 (1 H, m, inositol ring 3-H), 4.04 (1 H, dd, J 9.5, 9.4, inositol ring 6-H), 3.85-3.75 (2 H, m, CH\(_{2}\)P), 3.35 (1 H, m, inositol ring 1-H); \( \delta_{C} \) (75 MHz, CDCl₃) 138.8 (C), 138.7 (C), 136.6-136.0 (8 C, m, P(O)OCH\(_{2}\)Ph), 129.0 (CH), 128.9 (CH), 128.8 (CH), 128.7 (CH), 128.6 (CH), 128.5 (CH), 128.4 (CH), 128.34 (CH), 128.3 (CH), 128.0 (CH), 127.8 (CH), 127.6 (CH), 83.2 (CH, inositol ring), 83.0 (CH, inositol ring), 78.9 (CH, inositol ring), 76.7 (CH, inositol ring), 76.2 (CH, inositol ring), 75.8 (CH, inositol ring), 75.7 (CH\(_{2}\)), 75.1 (CH\(_{2}\)), 70.2-69.6 (6 C, m, CH\(_{2}\)Ph), 68.4 (1 C, d, \( ^{2}J_{P-C} \) 6.3, CH\(_{2}\)Ph), 68.2 (1 C, d, \( ^{2}J_{P-C} \) 6.3, CH\(_{2}\)Ph), 66.8 (1 C, d, \( ^{1}J_{P-C} \) 166.9, CH\(_{2}\)P(O)(OBn)\(_{2}\)); \( \delta_{P} \) (121 MHz, CDCl₃) 21.4, -1.20, -1.40, -2.00; HRMS m/z (FAB) [Found: (M+Na)\(^{+}\) 1437.4019. C\(_{77}H\(_{78}\)O\(_{18}\)NaP\(_{4}\) requires M\(^{+}\) 1437.4031]; m/z (ES+) 1437 ([M+Na]\(^{+}\), 100%).

\(+\)-1D-2,6-bis-O-Benzyl-3,4,5-tris(dibenzylphosphate)-1-sulfate-myoinositol sodium salt (+)-126

A solution of (-)-1D-2,6-bis-O-benzyl-3,4,5-tris(dibenzylphosphate)-myo-inositol (-)-125 (89 mg, 0.078 mmol, 1.0 equiv.) and sulfur trioxide pyridine complex (122 mg, 0.781
mmol, 10 equiv.) in DMF (2 mL) was heated to 50 °C for 20 h. After this time, the reaction was adjudged to be complete by TLC analysis. Triethylamine (1 mL) and methanol (2 mL) was added and the solvent was removed in vacuo. Silica gel column chromatography, eluting with ethyl acetate/hexane (70:30, 80:20 then 100% ethyl acetate) then methanol/chloroform (5:95), gave a colourless gum. The gum was dissolved in methanol (6 mL) and stirred with Dowex Marathon C sodium form resin for 1 h. The resin was removed by filtration and washed with methanol (5 mL) and methanol/water (2 mL). Water (2 mL) was added to the filtrate which was freeze-dried to give (+)-126 (66 mg, 68% yield) as a hygroscopic, colourless solid. R_f 0.25 (methanol/chloroform 10:90); [α]_D^20 +8.8 (c 1.2 in MeOH); ν_{max} (thin film)/cm\(^{-1}\) 3463 (m), 3034 (m), 1456 (m), 1264 (s), 1016 (s); δ_H (300 MHz, CD_3OD) 7.58 (2 H, d, J 7.6, Ph), 7.45 (2 H, d, J 7.6, Ph), 7.32-7.11 (36 H, m, Ph), 5.18-4.56 (21 H, m, 16 × C\_H\_2Ph, 5 × inositol ring), 4.09 (1 H, dd, J 9.8, 9.8, inositol ring); δ_C (75 MHz, CD_3OD) 140.2 (C), 139.7 (C), 137.3-136.9 (6 C, m, 6 × ArC), 130.1 (CH), 129.7 (CH), 129.6 (CH), 129.51 (CH), 129.49 (CH), 129.47 (CH), 129.43 (CH), 129.36 (CH), 129.3 (CH), 129.2 (CH), 129.15 (CH), 129.1 (CH), 129.0 (CH), 128.8 (CH), 128.5 (CH), 128.4 (CH), 80.0 (1 C, d, 2 J_{P-C} 4.9, CH), 79.2 (CH), 78.5-78.7 (1 C, m, CH), 78.4 (CH), 78.2 (CH), 77.0 (CH\_2), 76.8 (CH), 75.2 (CH\_2), 71.4 (1 C, d, 2 J_{P-C} 6.0, CH\_2), 71.2-70.9 (4 C, m, 4 × CH\_2), 70.7 (1 C, d, 2 J_{P-C} 5.6, CH\_2); δ_P (121 MHz, CD_3OD) -2.1, -2.68, -2.72; HRMS m/z (FAB) [Found: (M–Na+2H)^+] 1221.3041. C_{62}H_{62}O_{18}P_3S requires M^+ 1221.3021; m/z (ES+) 1238 ([M–Na+H+NH_4]^+, 100%).

(-)-1D-myoinositol-1-sulfate-3,4,5-trisphosphate tetrakis-sodium salt (-)-135

Palladium black (67 mg, 0.628 mmol, 20 equiv.) and sodium bicarbonate (7.9 mg, 94.2 μmol, 3 equiv.) were added to a solution of (+)-126 (39 mg, 31.4 μmol, 1 equiv.) in
t-butanol (3.0 mL) and water (0.5 mL) under nitrogen. The mixture was then placed under hydrogen and stirred for 3 h. The organic phase was decanted by pipette and water (2 mL) added to the black residue. The palladium was removed by filtration and the aqueous filtrate was freeze-dried to give (-)-1-O-myo-inositol-1-sulfate-3,4,5-trisphosphate tetrakis-sodium salt (135) (14.8 mg, 80% yield) as a colourless solid. 

\[ \alpha_{\text{D}}^{20} -8.0 \text{ (c 1.3 in H}_2\text{O)}; \delta_H (300 \text{ MHz, D}_2\text{O}) 4.44 \text{ (1 H, dd, J 2.7, 2.7, inositol ring 2-H)}, \]

4.35 (1 H, ddd, J 9.4, 9.4, 9.4, inositol ring 4-H), 4.22 (1 H, dd, J 9.9, 2.7, inositol ring 1-H), 4.06 (1 H, ddd, J, 9.9, 2.7, inositol ring 3-H), 4.00 (1 H, ddd, J 9.2, 9.2, 9.2, inositol ring 5-H), 3.84 (1 H, dd, J 9.5, 9.5, inositol ring 6-H); \delta_C (75 \text{ MHz, D}_2\text{O}) 78.1 (CH), 77.8 (CH), 75.9 (CH), 74.3 (1 C, \text{ d}^2J_{\text{P-C}} 5.0, \text{ CH}), 69.8 (1 C, \text{ d}, \text{ d}^2J_{\text{P-C}} 2.3, \text{ CH}), 69.4 (CH); \delta_P (121 \text{ MHz, D}_2\text{O}) 1.1, 0.8, 0.2; \text{ HRMS m/z (ES-)} \text{ [Found: (M-3Na+2H)\textsuperscript{−} 520.8933; C}_{6}\text{H}_{13}\text{O}_{18}\text{NaP}_3\text{S requires M}\textsuperscript{−} 520.8939]; m/z (ES-) 499 ([M-4Na+3H]\textsuperscript{−}, 100 %); 521 ([M-3Na+2H]\textsuperscript{−}, 52%).

\textbf{Benzzyloxy carbonylmethyl trifluoromethanesulfonate}\textsuperscript{184}

Dry pyridine (262 mg, 268 \mu L, 3.31 mmol, 1.1 equiv.) was added to a solution of benzyl glycolate (500 mg, 3.31 mmol, 1.0 equiv.) in dichloromethane (12 mL) at -15 °C. Triflic anhydride (934 mg, 557 \mu L, 3.31 mmol, 1.1 equiv.) was added dropwise and the mixture stirred at -10 °C for 20 min. The temperature was raised to RT and stirred for 30 min, after which time the reaction was adjudged to be complete by TLC analysis. The mixture was concentrated \textit{in vacuo} and the crude product was purified by silica gel column chromatography, eluting with dichloromethane/hexane (1:1) to give benzzyloxy carbonylmethyl trifluoromethanesulfonate (590 mg, 66% yield) as an unstable, colourless solid. \textit{Rf} 0.51 (dichloromethane/hexane 1:1); \delta_H (300 MHz, CDCl\textsubscript{3}) 7.40-7.42 (5 H, m, Ph), 5.13 (2 H, s, CH\textsubscript{2}OPh), 4.96 (2 H, s, CH\textsubscript{2}OTf); \delta_F (375 MHz, CDCl\textsubscript{3}) -74.5. The data are in good agreement with literature values.\textsuperscript{184}
6 References


4-Dimethylphosphinyl-"myo"-inositol 1,3,5-trisphosphate hexakis-sodium salt 108

Appendix 1 – Selected NMR Spectra
4-Dimethylphosphinyl-myo-inositol 1,3,5-trisphosphate hexakis-sodium salt

NAME  03292507-8-joseph1
EXPNO  10
EXPERIMENTER

TIME  20070329
RUN   20070329
INSTRUMENT  HP
NUMagnet  500 MHz
NUMQP  34 kHz
POLESPEC  100.73 Hz
REDUCE  65536
SOLVENT  500
DS  6000
FS  10000
DSW  10000000 Hz
Q0  1.00084262 sec
QX  5.00084262 sec
SM  250000 ussec
DS  6000 ussec
DSW  100000000 ussec
Q  1.6000000000 sec
QX  1.0000000000 sec
S  0.0014828 sec
DSW  0.00000014828 sec

 disabled CHANNEL 1

 disabled CHANNEL 2

CPDPRG2  waltie16
CF2  120.0000000000 sec
P1  18.0000000000 sec
P2  2.0000000000 sec
SP2  75.46774920 Hz
SP  75.46774920 Hz
SMW  50 Hz
SNB  5.00 Hz
SW  2.0000000000 Hz
PC  1.00 Hz

ppm

78 77 76 75 74 73 72 71 70 69

220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
4-Dimethylphosphinyl-myoinositol 1,3,5-trisphosphate hexakis-sodium salt 108
myo-Inositol 4-sulfate-1,3,5-trisphosphate hexakis-sodium salt 111
myo-Inositol 4-sulfate-1,3,5-trisphosphate hexakis-sodium salt 111
myo-Inositol 4-sulfate-1,3,5-trisphosphate hexakis-sodium salt

\[
\begin{align*}
\text{Na}^+ & \quad \text{PO}_4^3- \\
\text{O} & \quad \text{Na}^+ \\
\text{Na}^+ & \quad \text{O} \\
\end{align*}
\]
4-Phenylcarbamate-myoinositol-1,3,5-trisphosphate hexakis-sodium salt 115
4-Phenylcarbamate-mylo-inositol-1,3,5-trisphosphate hexakis-sodium salt

NAME 01262008-40-joseph
EXPRID 11
PROCNO 1
Date 20080127
Time 4:21
INSTRUM av300
PRBID 5 mm QNP 18/13
POLPROG deptq35
TD 600K
SOLVENT D2O
NS 4096
DS 4
SNR 1815.94 Hz
FIBRES 0.276427 Hz
AQ 1.00986436 sec
MS 20642.5
DW 27.600 usec
DE 6.00 usec
TE 297.5 K
CNST2 145.00000000 sec
D1 1.50000000 sec
D2 0.00344928 sec
D11 0.03000000 sec

CHANNEL f1
MCI1 13C
P1 9.00 usec
P2 18.00 usec
PL1 2.00 usec
PL1W 0.00340884 W
SFO1 75.4760000 MHz

CHANNEL f2
CP20PRG2 Wait316
MCI2 1H
P1 7.00 usec
P4 14.00 usec
P2P2 50.00 usec
PL2 0.00 dB
PL12 21.16 dB
SFO2 300.1300000 MHz
S1 65336
SF 75.4677490 MHz
M2W 8M
SSB 2.00 Hz
LB 0
GC 1.40
PC 0

115
4-Phenylcarbamate-myoinositol-1,3,5-trisphosphate hexakis-sodium salt 115
4-(4'-Carbamoyl-N-phenolate)-myo-inositol 1,3,5-trisphosphate heptakis-sodium salt 118
4-(4'-Carbamoyl-N-phenolate)-myo-inositol 1,3,5-trisphosphate heptakis-sodium salt 118
4-(4'-Carbamoyl-N-phenolate)-myo-inositol 1,3,5-trisphosphate heptakis-sodium salt 118
1-Methylene phosphonate-\textit{myo}-inositol 3,4,5-trisphosphate octakis-sodium salt 134
1-Methyleneephosphonate-myoinositol 3,4,5-trisphosphate octakis-sodium salt 134
1-Methylene phosphonate-\textit{myo}-inositol 3,4,5-trisphosphate octakis-

sodium salt 134
myo-Inositol 4-sulfate-1,3,5-trisphosphate tetrakis-sodium salt (-)-135
myo-Inositol 4-sulfate-1,3,5-trisphosphate tetrakis-sodium salt (-)-135
myo-Inositol 4-sulfate-1,3,5-trisphosphate tetrakis-sodium salt (\(-\)-135)

\[
\begin{align*}
\text{H}_2\text{O} & \quad \text{O} \\
\text{O} & \quad \text{H} \\
\text{O} & \quad \text{H} \\
\text{O} & \quad \text{O} \\
\text{P} & \quad \text{O} \\
\text{O} & \quad \text{H} \\
\text{H} & \quad \text{P} \\
\text{O} & \quad \text{O} \\
\text{Na}^+ & \quad \text{O} \\
\text{Na}^+ & \quad \text{O} \\
\end{align*}
\]

---

**NMR spectra**

- **Sample**: myo-Inositol 4-sulfate-1,3,5-trisphosphate tetrakis-sodium salt (\(-\)-135)
- **Solvent**: D2O
- **Temperature**: 298 K
- **Field strength**: 400 MHz

---

** Parameters**

- **T1**: 300.0 μsec
- **T2**: 10.75 μsec
- **T1**: 6.00 μsec
- **T2**: 200.0 μsec
- **δ**: 0.000 μsec
- **Δ**: 1.100 μsec

---

**Channel 1**

- **µH2O**: 1.00 μsec
- **F1L**: 15.00 MHz
- **F01**: 121.4500 MHz

---

**Channel 2**

- **Channel 3**

**Chemical shifts**

- **δ**: 3.00 ppm
- **δ**: 1.00 ppm
- **δ**: 0.40 ppm

---

**FID parameters**

- **T1**: 300.0 μsec
- **T2**: 10.75 μsec
- **T1**: 6.00 μsec
- **T2**: 200.0 μsec
- **δ**: 0.000 μsec
- **Δ**: 1.100 μsec
Appendix 2 – Crystallographic Data

Crystal structure of compound 73

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Atom coordinates (x \times 10^4) and equivalent isotropic displacement parameters (Å²\times 10^3) for 73. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

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Bond lengths [Å] and angles [°] for 73

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\[-2\pi^2 h^2 a^2 U_{11} + \ldots + 2 h k a^* b^* U_{12} \]

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<td>C(10)-Si(1)-C(7)-C(9)</td>
<td>169.0(2)</td>
</tr>
<tr>
<td>C(13)-Si(1)-C(7)-C(9)</td>
<td>45.1(3)</td>
</tr>
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<td>156.8(2)</td>
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<td>O(1)-Si(1)-C(10)-C(12)</td>
<td>64.2(3)</td>
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### Hydrogen bonds for 73 [Å and °]

<table>
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<tr>
<th>D-H...A</th>
<th>d(D-H)</th>
<th>d(H...A)</th>
<th>d(D...A)</th>
<th>(&lt;\text{DHA})</th>
</tr>
</thead>
<tbody>
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<td>O(4)-H(4O)...O(23)#1</td>
<td>0.9799(10)</td>
<td>1.823(9)</td>
<td>2.788(3)</td>
<td>168(3)</td>
</tr>
</tbody>
</table>

Symmetry transformations used to generate equivalent atoms: #1 -x,y-1/2,-z+3/2