PHD

Synthesis of novel analogues of myo-inositol 1,4,5-trisphosphate

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SYNTHESIS OF NOVEL ANALOGUES OF MYO-INOSITOL 1,4,5-TRISPHOSPHATE

submitted by Dethard Lampe
for the degree of PhD
of the University of Bath
1993

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ABSTRACT

In this thesis, the synthesis of novel analogues of the second messenger D-myo-inositol 1,4,5-trisphosphate is described.

1-O-allyl-2,3,6-tri-O-benzyl-myo-inositol was prepared by a known method. Isomerisation of the allyl group gave the 1-O-propenyl compound, which was phosphorylated at the 4- and 5-positions. Removal of the propenyl protecting group followed by phosphitylation of the 1-hydroxyl group and sulfoxidation of the phosphite furnished the fully protected 1-phosphorothioate analogue of Ins(1,4,5)P₃. Deblocking gave DL-myo-inositol 4,5-bisphosphate 1-phosphorothioate [Ins(1,4,5)P₃-1S].

A fluorescent chromophore was selectively attached to the 1-position of this Ins(1,4,5)P₃ analogue. The precursor DL-2,3,6-tri-O-benzyl-4,5-bis-[di-(2-cyanoethoxy)phospho]-myo-inositol was used to synthesise [³⁵S]-labelled Ins(1,4,5)P₃-1S in co-operation with NEN-DuPont, USA.

Racemic 1-O-allyl-2,3,6-tri-O-benzyl-myo-inositol was resolved into the optically active D- and L-isomers via the (-)-(+)−biscamphanates. The D-isomer was transformed as outlined above for the racemic compound to give D-myo-inositol 4,5-bisphosphate 1-phosphorothioate. The L-isomer was used in the synthesis of L-myo-inositol 1,4,5-trisphosphorothioate, which was found to be a potent non Ca²⁺-mobilising inhibitor of myo-inositol 1,4,5-trisphosphate 5-phosphatase.

2,4,6-Tri-O-para-methoxybenzyl-myo-inositol was synthesised from myo-inositol via the orthoformate ester and was phosphitylated, sulfoxidised and deprotected to give DL-myo-inositol 1,3,5-trisphosphorothioate, which was also found to be an effective 5-phosphatase inhibitor.

Routes to scyllo-inositol analogues of Ins(1,4,5)P₃ were studied. 1-O-allyl-3,6-di-O-benzyl-4,5-O-isopropylidene-myo-inositol was trifluoromethylsulphonylated at the free 2-position. Nucleophilic substitution of the triflate with caesium acetate gave 2-O-acetyl-1-O-allyl-3,6-di-O-benzyl-4,5-O-isopropylidene-scyll-o-inositol. Successive removal of protecting groups gave 1-O-allyl-3,6-di-O-benzyl-scyll-o-inositol and 1,4-di-O-benzyl-scyll-o-inositol which were phosphorylated and deblocked yielding scyll-o-inositol 1,4,5-trisphosphate and scyll-o-inositol 1,2,4,5-tetrakisphosphate, respectively. These compounds were highly potent agonists in Ca²⁺-mobilisation. Thiophosphorylation and deprotection of 1,4-di-O-benzyl-scyll-o-inositol gave scyll-o-inositol 1,2,4,5-tetrakisphosphoro-thioate, which was found to be an effective partial agonist.

1-O-allyl-3,6-di-O-benzyl-4,5-O-isopropylidene-scyll-o-inositol, an intermediate in the previous synthesis, was isomerised to the 1-O-(cis-prop-1-enyl) derivative. The 2-position was trifluoromethylsulphonylated and then fluorinated using tetrabutylammonium fluoride to give 1-O-cis-prop-1-enyl-3,6-di-O-benzyl-4,5-O-isopropylidene-myo-inositol. Removal of acid labile protecting groups, phosphorylation of the free hydroxyl groups and deblocking yielded 2-deoxy-2-fluoro-myo-inositol 1,4,5-trisphosphate, a potent full agonist.

The diastereoisomers of the valuable intermediate DL-4,5-di-O-acetyl-3-O-allyl-6-O-benzyl-2-[(+)-(o)-camphanoyl]-1-O-p-methoxybenzyl-myo-inositol were synthesised and separated by crystallisation. Finally, methods to substitute the Ins(1,4,5)P₃ 1-phosphate group with a carboxymethylene moiety and to employ lactones as protecting groups were investigated. Thus, DL-1,4-di-O-benzyl-myo-inositol was either directly, or via a three step route, converted into the six-membered 1-O-methylene-2-O-lactone. The intermediate DL-3-O-carboxymethylene-1,4-di-O-benzyl-myo-inositol showed interesting ‘thermosalient’ properties.
Diese Arbeit ist meinen Eltern in Dankbarkeit gewidmet.
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Finally, I am grateful to The Wellcome Trust for providing me with a Wellcome Prize Studentship.
Publications

Part of the work described herein has appeared in the following publications:


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<tr>
<td>All</td>
<td>allyl</td>
</tr>
<tr>
<td>ADP</td>
<td>adenosine 5’-diphosphate</td>
</tr>
<tr>
<td>AMP</td>
<td>adenosine 5’-monophosphate</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine 5’-triphosphate</td>
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<tr>
<td>Bn</td>
<td>benzyl</td>
</tr>
<tr>
<td>Bn-OMe</td>
<td>p-methoxybenzyl</td>
</tr>
<tr>
<td>Bu^OK</td>
<td>potassium tert-butoxide</td>
</tr>
<tr>
<td>Bu^OOH</td>
<td>tert-butyl hydroperoxide</td>
</tr>
<tr>
<td>Bz</td>
<td>benzoyl</td>
</tr>
<tr>
<td>cAMP</td>
<td>adenosine 3’,5’-cyclic monophosphate</td>
</tr>
<tr>
<td>Camph</td>
<td>camphanate ester</td>
</tr>
<tr>
<td>COSY</td>
<td>correlated spectroscopy</td>
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<tr>
<td>mCPBA</td>
<td>meta-chloroperbenzoic acid</td>
</tr>
<tr>
<td>DABCO</td>
<td>1,4 diazabicyclo[2.2.2]octane</td>
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<td>DAG</td>
<td>1,2-diacylglycerol</td>
</tr>
<tr>
<td>DAST</td>
<td>diethylaminoisulphur trifluoride</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-(dimethylamino)pyridine</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulphoxide</td>
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<tr>
<td>DMF</td>
<td>dimethylformamide</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>E.C.</td>
<td>Enzyme Catalogue</td>
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<tr>
<td>GPI</td>
<td>glycosyl-phosphatidylinositol</td>
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<tr>
<td>GPI-PLC</td>
<td>GPI-specific PLC</td>
</tr>
<tr>
<td>G-protein</td>
<td>GTP-binding protein</td>
</tr>
<tr>
<td>GroPlns(4,5)P₂</td>
<td>glycerophospho-my-o-inositol 4,5-bisphosphate</td>
</tr>
<tr>
<td>GTP</td>
<td>guanosine 5’-triphosphate</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<td>--------------</td>
<td>------------</td>
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<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
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<tr>
<td>IANBD</td>
<td>(N-[2-(\text{iodoacetoxy})\text{ethyl}]-N-\text{methyl}]\text{amino-7-nitro-2,1,3-benzoxadiazole}</td>
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<td>Ins(1,4,5)P(_3)</td>
<td>1D-(\text{myo-inositol-1,4,5-trisphosphate})</td>
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<td>Ins(1,3,4,5)P(_4)</td>
<td>1D-(\text{myo-inositol-1,3,4,5-tetrasphosphate})</td>
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<td>1D-(\text{myo-inositol-1,4,5-trisphosphorothioate})</td>
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<tr>
<td>IR</td>
<td>infra-red</td>
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<tr>
<td>MEM</td>
<td>2-methoxyethoxymethyl</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>Ph</td>
<td>phenyl</td>
</tr>
<tr>
<td>PIM</td>
<td>putative insulin mediator</td>
</tr>
<tr>
<td>PLC</td>
<td>phospholipase C</td>
</tr>
<tr>
<td>PMB</td>
<td>(\text{para-methoxybenzyl})</td>
</tr>
<tr>
<td>PKC</td>
<td>protein kinase C</td>
</tr>
<tr>
<td>PtdIns</td>
<td>phosphatidylinositol</td>
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<tr>
<td>PtdIns(4,5)P(_2)</td>
<td>phosphatidylinositol-4,5-bisphosphate</td>
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<tr>
<td>PtdIns-PLC</td>
<td>phosphatidylinositol-specific PLC</td>
</tr>
<tr>
<td>PTSA</td>
<td>(\text{para-toluenesulphonic acid monohydrate})</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>TBPP</td>
<td>tetrabenzyl pyrophosphate</td>
</tr>
<tr>
<td>TEAB</td>
<td>triethylammonium bicarbonate</td>
</tr>
<tr>
<td>TIPS</td>
<td>1,1,3,3-tetraisopropylsiloxane</td>
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<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
</tr>
<tr>
<td>TPA</td>
<td>12-(\text{O-tetradecanoylphorbol-13-acetate})</td>
</tr>
<tr>
<td>UV</td>
<td>ultra-violet</td>
</tr>
<tr>
<td>VSG</td>
<td>variant surface glycoprotein</td>
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1. Introduction

1.1 Signal Transduction in Cells

The cells in multicellular organisms must communicate with each other in order to be able to work together. They convey signals by using substances such as hormones and neurotransmitters. Lipophilic hormones like steroids can pass through the lipid bilayer of cell membranes and bind to their receptors within the cell, thereby effecting a response. Many messenger substances, however, are too hydrophilic to cross membranes. In order to deliver their message they have to bind to specific receptors on the outside of the cell membrane and activate mechanisms which transmit the signal into the cell.

There are several different classes of receptors: If the receptor is part of an ion-channel, the opening of this channel can trigger the influx or efflux of ions into or out of the cell. A change of ion concentrations in the cytosol will then activate cellular enzymes, evoking a response. Since the extracellular concentrations of Na\(^+\) and Ca\(^{2+}\)-ions are 20 and 1000 fold higher, respectively, than the concentrations of these ions within the cell, they are particularly suitable for passive import through ligand-gated ion-channels. The K\(^+\)-concentration, on the other hand, is higher in the cytosol, so this ion is exported after channel opening.

Tyrosine kinase receptors are intrinsically enzymes. They are embedded in the membrane and have binding sites able to recognise agonists on the outer surface, and active sites on the inside of the cell membrane. Binding of agonists activates the enzyme and leads to the phosphorylation of tyrosine residues on target proteins within the cell. This signal transduction mechanism is used by many growth factors and hormones, including insulin [1].
Most water soluble hormones, however, make use of a somewhat more complex signal transduction system. After binding to their receptor-protein on the cell surface, a membrane-bound GTP-binding protein (G-protein)\(^2\)\(^4\), which is associated with the receptor, is activated. The family of G-proteins includes several members, regulating different intracellular pathways. G-proteins are composed of three subunits, designated \(\alpha\), \(\beta\) and \(\gamma\) in order of decreasing mass. On activation, guanosine diphosphate (GDP) bound to the \(\alpha\)-subunit is replaced by guanosine triphosphate (GTP) and the \(\beta\gamma\) subunit dissociates from the activated \(\alpha\)-GTP subunit. The free subunits can now stimulate or inhibit other membrane bound enzymes acting as amplifiers (e.g. \(K^+\) channels, \(Ca^{2+}\) channels, adenylate cyclase (AC), guanylate cyclase (GC), PtdIns(4,5)\(_2\)-specific phospholipase C (PtdIns-PLC)), which in turn generate so-called ‘second messengers’ on the cytosolic side of the cellular membrane. The intrinsic GTPase activity of the \(\alpha\) subunit then hydrolyses GTP to GDP, the \(\alpha\)-
GDP subunit recombines with the $\beta\gamma$ subunit and the G-protein returns to its basal state.

**Figure 1.2 GTP-binding proteins in signal transduction**

**Second Messengers**

The second messengers identified so far include cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), diacyl glycerol (DAG) and D-myo-inositol 1,4,5-trisphosphate [D-Ins(1,4,5)P₃] (in combination with Ca²⁺ ions). Recent studies suggest that cyclic adenosine diphosphate ribose (cADP-ribose), a metabolite of NAD⁺, may also have second messenger properties.

Compared to the large number of different hormones and neurotransmitters, there seem to be only few second messengers, suggesting that internal signalling pathways are surprisingly similar in spite of the plentitude of biochemical and physiological processes to be regulated.
The Signal Transduction Pathway

Thus, the signal transduction mechanism mediated by G-protein coupled receptors can be summarised as:

```
<table>
<thead>
<tr>
<th>external signal (hormone, neurotransmitter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>\rightarrow</td>
</tr>
<tr>
<td>receptor</td>
</tr>
<tr>
<td>\rightarrow</td>
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<tr>
<td>transducer (G-protein)</td>
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<td>\rightarrow</td>
</tr>
<tr>
<td>amplifier (e.g. adenylate cyclase, guanylate cyclase, PtdIns-PLC)</td>
</tr>
<tr>
<td>\rightarrow</td>
</tr>
<tr>
<td>second messenger (cAMP, cGMP, DAG and Ins(1,4,5)P₃)</td>
</tr>
<tr>
<td>\rightarrow</td>
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<tr>
<td>intracellular effector</td>
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<td>\rightarrow</td>
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<tr>
<td>cellular response (growth, secretion, etc.)</td>
</tr>
</tbody>
</table>
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1.2 The Role of Calcium in Signal Transduction

The first indication that calcium plays a role in the regulation of cellular events was an observation by Ringer in 1883 [5]. He examined muscle tissue and found that he could not induce the contraction of the tissue when he replaced the tap water in his medium with distilled water. The missing component was found to be calcium.

Today, it is known that a large number of different cellular processes are controlled by changes in the calcium concentration and the mobilisation of calcium has been found to be the primary function of many agonists. The cytosolic calcium concentration can be regulated by two different mechanisms:

1. Many agonists operate by inducing a change in the potential difference across the membrane. This causes voltage sensitive Ca²⁺ channels in the cell membrane to open. As most calcium within the cell is bound to membranes or
proteins the intracellular levels of free calcium are low and there is therefore a large gradient in favour of influx of ions into the cell. Thus, the cytosolic calcium concentration is increased.

2. Other agonists can mobilise sequestered calcium from intracellular stores. In each case, the calcium concentration in the cytosol is increased and Ca\(^{2+}\) dependent enzymes are activated. The cellular response depends on the type of cell targeted as well as the nature of the agonist.

1.3 Second Messengers

1.3.1 Adenosine 3'\(^{,}\)5'-cyclic monophosphate (cAMP)

In the late 1950's E.W. Sutherland and T.W. Rall discovered that the increased formation of phosphorylase in liver homogenates in the presence of epinephrine and glucagon was mediated by a heat-stable factor, which accumulated when the hormones were incubated with adenosine triphosphate and particulate fractions of liver homogenates. It was also observed that this factor stimulated the formation of phosphorylase in supernatant fractions in which the hormones had no effect. The factor was isolated and found to be the ribonucleotide adenosine 3'\(^{,}\)5'-cyclic monophosphate (cAMP) \(^{[5,7]}\). The mechanism by which this second messenger operates has been intensively studied and is now well understood \(^{[8]}\). Upon receptor stimulation cAMP is formed from adenosine 5'-triphosphate by the G-protein regulated enzyme adenylate cyclase. This enzyme is a large (185 kDa) integrated membrane protein. A wide variety of hormones have been found to activate adenylate cyclase, including corticotropin, adrenaline, noradrenaline, glucagon and vasopressin. There are two different types of receptors and G-proteins involved in the regulation of adenylate cyclase: stimulatory receptors (R\(_{s}\)) which are linked to a stimulatory G-protein (G\(_{s}\)) and inhibitory receptors (R\(_{i}\)) which in turn are linked to an inhibitory G-protein (G\(_{i}\)).

In order to exert its effect in the cell, cAMP must activate a mechanism resulting in a cellular response. All the known effects of cAMP in animal cells are due to the stimulation of a protein kinase. This protein is comprised of two regulatory (49 kDa) and two catalytic (38 kDa) subunits and is inactive in its tetrameric R\(_{2}\)C\(_{2}\) state. The catalytic activity of the protein kinase is however rapidly
increased by cAMP binding to the regulatory subunits, which results in the
dissociation of the R₂C₂ complex into a R₂ subunit and two C subunits, which
are catalytically active. cAMP is thus acting as an allosteric effector. Substrate
proteins are phosphorylated by the kinase and their properties changed. As
different cells contain different enzymes serving as substrates for the protein
kinase, the response to increased concentrations of cAMP varies with the type of
cell concerned. In liver cells for example, cAMP promotes glycogen breakdown
and gluconeogenesis. In adrenal cortex cells it leads to an increase in steroid
output and in stomach mucosa cells the secretion of hydrochloric acid is
stimulated.

The action of cAMP is terminated by its hydrolysis to adenosine 5'-
monophosphate (AMP) by a 3',5'-cyclic nucleotide phosphodiesterase (of which
subtypes with different specificities for cAMP versus cGMP exist).

Figure 1.3  Formation and deactivation of cAMP
1.3.2 Guanosine 3',5'-cyclic monophosphate (cGMP)

The role of cAMP in signal transduction was becoming widely accepted, when another compound with second messenger characteristics was discovered. Similar to cAMP, guanosine 3',5'-cyclic monophosphate (cGMP) is synthesised from GTP by a guanylate cyclase and is deactivated by hydrolysis to the 5'-monophosphate (GMP). In the photosensitive rod cells of the vertebrate retina, cGMP has been found to be the central regulatory molecule\textsuperscript{1,9}. In contrast to cAMP, whose levels are low before activation of the signal transduction process, the basal concentration of cGMP in rod cells is high. The cells respond to stimulation of the `receptor` rhodopsin by photons with the activation of the G-protein transducin, which in turn mobilises a specific cGMP phosphodiesterase. The resulting decrease in cGMP levels leads to the closure of an ion-channel in the plasma membrane \textsuperscript{10}, and the flow of both sodium and calcium ions is halted. Hyperpolarisation of the cells due to the reduced ion influx promotes the release of neurotransmitters into the synaptic gap, and the excited optical nerves transmit the signal to the brain. Deactivation of rhodopsin, regeneration of cGMP by guanylate cyclase and the re-opening of the ion channel leads to the return to the dark state.

![Figure 1.4 Formation of cGMP](image)

**Figure 1.4** Formation of cGMP
1.3.3 Cyclic adenosine diphosphate ribose (cADP-ribose)

Recently, the involvement of pyridine nucleotide metabolites in the mobilisation of intracellular calcium in sea urchin eggs has been reported \(^{[11]}\). The active metabolite was identified as cyclic ADP-ribose (cADP-ribose)\(^{[12]}\), derived from NAD\(^+\) by the action of the enzyme ADP-ribosyl cyclase \(^{[13]}\). It may be too early to categorise cADP-ribose as a novel second messenger, since the modulation of intracellular concentrations of this molecule by extracellular factors has not been demonstrated as yet. It has however, become clear that cADP-ribose is the most potent calcium mobilising agent described to date (EC\(_{50}\) for the release of Ca\(^{2+}\) from sea urchin microsomes 18 nM \(^{[11]}\)). A specific binding site for cADP-ribose on sea urchin microsomes has been demonstrated and it appears that the release of calcium is regulated by a ryanodine-sensitive, but Ins\((1,4,5)P_3\)-insensitive, Ca\(^{2+}\) channel of the endoplasmatic reticulum. The cADP-ribose sensitive and the Ins\((1,4,5)P_3\) sensitive calcium pools do however, seem to overlap substantially. cADP-ribose is very rapidly hydrolysed by cADP-ribose hydrolase, and this fast deactivation is another indication that cADP-ribose may indeed be a novel second messenger \(^{[14]}\).

![Figure 1.5 Formation and deactivation of cADP-ribose](image)

**Figure 1.5** Formation and deactivation of cADP-ribose
1.3.4 Inositol 1,4,5-trisphosphate and Diacylglycerol

There are three major inositol-containing phospholipids present in animal cells: phosphatidylinositol (PtdIns) 1, usually accounting for more than 90 % of the total inositol phospholipids, phosphatidylinositol 4-phosphate [PtdIns(4)P] 2 and phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P₂] 3. Recently, the presence of phosphatidylinositol 3-phosphate [PtdIns(3)P]ⁱⁱⁱⁱ ⁴ and phosphatidylinositol 3,4,5-trisphosphate [PtdIns(3,4,5)P₃]ⁱⁱⁱ ⁵ has also been demonstrated. Together, these inositol phospholipids constitute less than 10 % of the total phospholipid of animal cells.

![Figure 1.6 Inositol Phospholipids](image)

1: \( R^1 = R^2 = R^3 = H; \) PtdIns
2: \( R^1 = PO_3^{2-}; R^2 = R^3 = H; \) PtdIns(4)P
3: \( R^1 = R^2 = PO_3^{2-}; \) PtdIns(4,5)P₂
4: \( R^1 = R^2 = H; R^3 = PO_3^{2-} \) PtdIns(3)P
5: \( R^1 = R^2 = R^3 = PO_3^{2-} \) PtdIns(3,4,5)P₃

**Figure 1.6** Inositol Phospholipids
In 1953 Hokin and Hokin [17] reported that the stimulation of enzyme secretion in slices of pancreas and brain by the neurotransmitter acetylcholine was associated with the enhanced incorporation of $^{32}\text{P}_i$ into the total phospholipid fraction. Later, [18], phosphatidylinositol was identified as the phospholipid showing the most enhanced incorporation of radioactive phosphate. This observation, named the "phosphoinositide effect" (PI effect) was the first indication that inositol phospholipids may be playing a role in signal transduction.

By the mid-1970s it was known that a phosphatidylinositol-specific phospholipase C (PtdIns-PLC) catalysed the receptor-stimulated breakdown of inositol phospholipids [19].

In 1975, Michell [20] noted a correlation between the Ca$^{2+}$ mobilising properties of certain agonists and the PI effect in various tissues, and the observation that the phosphoinositide breakdown was not controlled by changes in extracellular or intracellular calcium concentrations led him to suggest that the PI effect must precede the intracellular liberation of calcium or the opening of calcium gates. The phospholipid hydrolysed by the PtdIns-PLC was then found to be phosphatidylinositol 4,5-bisphosphate [21], which led to the identification of D-myoinositol 1,4,5-trisphosphate [Ins(1,4,5)P$_3$] 6 as the Ca$^{2+}$ mobilising second messenger by Streb et al. in 1983 [22].

Diacylglycerol

1,2-$sn$-Diacylglycerol (DAG) 7, the other product derived from the PtdIns-PLC catalysed breakdown of PtdIns(4,5)P$_2$, has also been found to play a second
messenger role in cells. Diacylglycerol is lipophilic and remains in the cell membrane, where it exerts its function by activating a specialised protein kinase C (PKC). This signalling pathway initiated by DAG through the activation of PKC and the phosphorylation of various proteins by the kinase is separate from, but often synergistic to, the calcium signalling pathway.

Protein kinase C \textsuperscript{[23,24]}, of which there are several subspecies \textsuperscript{[25]}, was found to be highly stereospecific \textsuperscript{[26]}. For activation, the diacylglycerol has to be in the 1,2-\textit{sn}-configuration (\textit{sn} stands for stereospecific numbering \textsuperscript{[27]}), both 2,3-\textit{sn}- and 1,3-DAG are inactive. It also seems to be essential that one of the fatty acids is unsaturated. The chain length of the fatty acid moieties, however, does not seem to be important for recognition by the kinase. The activation of PKC depends on the presence of calcium and phosphatidylserine as well as DAG.

It has been reported that tumor-promoting phorbol esters such as 12-O-tetradecanoylphorbol-13-acetate (TPA) \textsuperscript{8}, with molecular structures similar to DAG, can activate PKC both \textit{in vitro} and \textit{in vivo} \textsuperscript{[28]}.

![Figure 1.7 Diacylglycerol and Phorbol ester](image-url)
Whilst DAG is degraded rapidly after formation, TPA is hardly metabolised. Thus TPA may extend a usually limited phase of cellular response, resulting in a distortion of the normal sequence of events leading eventually to tumor-growth. However, as PKC-stimulation by tumor promoters structurally unrelated to DAG has also been reported, this interpretation has to be treated with care. PKC has been found to be cleaved by calpain I in the presence of calcium, phosphatidylserine and DAG (or TPA). Thus the possibility of a sequential action of tumor promoters has been proposed: a short-term activation of PKC followed by the degradation of the enzyme over a longer period.

Deactivation of diacylglycerol is possible by two metabolic pathways: a) DAG is phosphorylated by a kinase to give phosphatidic acid, or b) the unsaturated fatty acid moiety from the 2-position of DAG is cleaved by phospholipase A₂, giving monoacylglycerol and the eicosanoid precursor arachidonic acid, which is used in prostaglandin synthesis [29].

1.4 The Ins(1,4,5)P₃ Receptor

The Ca²⁺ mobilising property of Ins(1,4,5)P₃ is mediated by an endoplasmic reticular receptor specific for D-Ins(1,4,5)P₃. Specific binding sites for Ins(1,4,5)P₃ have been identified in a number of peripheral tissues (see for example [30,31]) and in the brain, where sites are highly concentrated in the cerebellum [32,33].

The Ins(1,4,5)P₃ receptor has been isolated from rat cerebellar membranes by affinity chromatography on heparin-agarose [34]. The fact that the receptor also binds to concanavalin A-sepharose and can be eluted with α-methylmannopyranoside indicates that the receptor is a glycoprotein. The native receptor was found to be a homo-tetramer, but co-operativity between the four subunits has not been demonstrated. However, binding of at least 3 molecules of Ins(1,4,5)P₃ is required to effect Ca²⁺ release [35]. The relative molecular mass (M_r) of the monomer has been determined by electrophoretic analysis to be 260 KDa and the shape is globular with a Stokes' radius of ca. 10 nm. The receptor has now been cloned [36] and sequenced [37]. When reconstituted into
liposomes, the receptor mediates Ca$^{2+}$ release in response to Ins(1,4,5)P$_3$ suggesting that the calcium channel is an intrinsic component of the receptor protein \cite{38}. Two slightly different forms of the receptor were found (presumably generated by alternative splicing), comprising 2749 and 2734 amino acids, respectively. Hydrophobicity analyses have shown the presence of a cluster of hydrophobic sequences, there is however some disagreement about whether there are six \cite{39}, seven \cite{36} or eight \cite{37} transmembrane regions forming the calcium channel.

The Ins(1,4,5)P$_3$ receptor is regulated by phosphorylation by a cAMP-dependent protein kinase, by changes in pH and in the cytosolic calcium concentration. Calcium reversibly inhibits ligand binding to the particulate and detergent-solubilised receptor \cite{32}, suggesting that the Ca$^{2+}$ released by Ins(1,4,5)P$_3$ feeds back to inhibit further action of the second messenger, a process that may play a role in the calcium oscillations of many cells \cite{40-43}. Binding to the purified receptor protein was found to be unaffected by calcium \cite{34}, suggesting the involvement of a distinct calcium binding protein conferring calcium sensitivity to the receptor \cite{44}. Phosphorylation of the receptor on the other hand has no effect on ligand binding but prevents the ligand-induced opening of the calcium channel \cite{45}. Changes in pH also regulate Ins(1,4,5)P$_3$ binding, with a sharp increase in binding throughout the physiologic range, tripling between pH 7.5 and 8.5 \cite{32}. The influence of growth factors and hormones as well as phorbol esters on signal transduction may be due to their ability to alter the intracellular pH.

Both Alzheimer’s disease \cite{46} and chronic ethanol consumption \cite{47} have been linked to decreased brain $[^{3}$H$]$-Ins(1,4,5)P$_3$ binding.

1.5 Nomenclature of Inositol Derivatives

The nine different stereoisomers of 1,2,3,4,5,6-cyclohexanhexol or inositol (Fig. 1.6) are members of the family of cyclitols (by definition `cycloalkanes containing one hydroxyl group on each of three or more ring atoms`). Only two of these nine isomers are optically active: D- and L-chiro-inositol (16 and 17), all the other inositol have a plane of symmetry and are therefore optically inactive meso-compounds.
The IUPAC commission has issued rules for the nomenclature of cyclitols \(^{[48]}\), the most important of which are summarised here:

- the myo-inositol ring carbon bearing the only axial hydroxyl group in the chair conformation is C-2,
- substituents are listed in alphabetical order prior to the term `myo-inositol` unless they have to be named as suffixes (e.g. phosphates),
- when numbering substituents the lowest possible numbers have to be assigned according to which substituent has alphabetical priority,
- the prefix `-O-` is inserted between the numbering and the name of the substituent if the oxygen of the hydroxyl function has not been replaced in the substitution, (e.g. 1-O-benzyl-myoinositol),

Figure 1.8 The nine inositol stereoisomers

The IUPAC commission has issued rules for the nomenclature of cyclitols \(^{[48]}\), the most important of which are summarised here:
the prefix -C- is used in the same manner to denote substitution on the
ring carbon without loss of the hydroxyl function,
`deoxy` nomenclature is used if a hydroxyl group is replaced by another
univalent substituent,
phosphoesters of inositol are termed inositol phosphates, the quantity of
erster functions is denoted by using Greek numbers (e.g. myo-inositol
1,3,4,5-tetrakisphosphate,
to differentiate between enantiomers, the suffixes D- and L- are added,
the chiral centre used can also be stated (e.g. 1D-myoinositol 1,4,5-
trisphosphate if C-1 is the chiral centre used).

Myo-inositol 10 itself is a meso-compound, with a plane of symmetry going
through C-2 and C-5. However, on substitution at one of the stereogenic carbon
atoms (C-1, C-3, C-4 and C-6), a chiral derivative is obtained. In order to
discriminate between D- and L-configurations in such derivatives a numbering
system applies: if the numbering proceeds anticlockwise around the ring, the
compound has the D-configuration, if the numbering is clockwise, the compound
is assigned the L-configuration (see Figure 1.9). As the `lowest number` rule
applies, substitution of the hydroxyl group at C-1 or C-3 by a phosphate group
would in both cases give a mixture of D- and L-myoinositol 1-phosphate,
respectively.

Figure 1.9 The enantiomers of Ins(1,4,5)P3

D-myoinositol(1,4,5)P3  L-myoinositol(1,4,5)P3
Recent recommendations for the numbering of atoms in myo-inositol \[^{49}\] propose that, as all naturally occurring inositol phosphates are of the D-configuration, they may all be numbered for biological purposes as the D-inositol derivatives, thus overriding the `lowest number` rule. This new convention should make it easier to follow biochemical pathways. For example: according to the old IUPAC rules, D-myo-inositol 3,4-bisphosphate is converted into L-myo-inositol 1-phosphate by enzymatic hydrolysis. Use of the alternative name D-myo-inositol 3-phosphate for the product L-myo-inositol 1-phosphate clarifies the metabolic process involved, \textit{i.e.} hydrolysis of the phosphate group at C-4.

Although the number of isomers of the different myo-inositol phosphates is vast (there are 6 isomers of InsP and InsP\(_5\), respectively, 15 isomers of InsP\(_2\) and InsP\(_4\), respectively, and 20 isomers of InsP\(_3\)), only a few of these isomers occur naturally.

1.6 The Inositol Phosphate Cycle

The discovery of the second messenger role of Ins\((1,4,5)\)P\(_3\) has stimulated the interest in the formation and metabolism of this compound. The biochemistry of inositol phosphates has recently been reviewed \[^{50}\].

1.6.1 Biosynthesis of Inositol

The main source of myo-inositol for humans and animals is dietary intake, as inositol and inositol phosphates are present in many plants. \textit{De novo} synthesis is however possible and involves the enzyme L-myo-inositol 1-phosphate synthetase, which isomerises glucose-6-phosphate \(^{18}\) to L-Ins\((1)\)P \(^{19}\) [= D-Ins\((3)\)P, see above] by an interesting sequence of chemical transformations [Fig. 1.10]. The enzyme is very abundant in mammalian testis and brain and has been purified and characterised \[^{51-53}\].
Figure 1.10 Biosynthesis of L-\textit{myo}-inositol 1-phosphate

L-Ins(1)P is then hydrolysed to free \textit{myo}-inositol by the enzyme inositol monophosphatase.

Figure 1.11 \textit{De novo} synthesis of \textit{myo}-inositol

1.6.2 Inositol monophosphatase

Inositol monophosphatase (E.C. 3.1.3.25)\textsuperscript{[54-57]} not only catalyses the dephosphorylation of D- and L-\textit{myo}-inositol 1-phosphate but also D- and L-\textit{myo}-inositol 4-phosphate and \textit{myo}-inositol 5-phosphate. As the enzyme governs the production of inositol from inositol monophosphates arising either from agonist
activation of the cell or by de novo synthesis from glucose-6-phosphate it is one of the key enzymes in the inositol phosphate cycle.

Figure 1.12 Inositol monophosphatase, a key enzyme in the inositol phosphate cycle

Inositol monophosphatase is non-competitively inhibited by lithium [57-61] and the well known therapeutic effects of lithium in the treatment of manic-depressive patients can perhaps be attributed to its action on phosphoinositide signalling. However, as lithium does not only inhibit inositol monophosphatase but also has inhibitory effects on other metabolic enzymes of the inositol phosphate pathway, including Ins(1,4)P₂-phosphatase [62] and Ins(1,3,4)P₃-phosphatase [63,64], it is too early to say with certainty why lithium is effective in the treatment of manic depression. Lithium also affects the coupling of G-proteins to both muscarinic cholinergic and b-adrenergic receptors [65,66], thus interfering with signal transduction at another crucial point.

In order to discriminate between the different effects of lithium on signal transduction, more specific inhibitors of inositol monophosphatase would be helpful. Additionally, the narrow therapeutic index of lithium (constant monitoring
of blood concentrations is necessary to maintain effective lithium levels and prevent overdosage) would make the substitution of this drug by more effective medications desirable. This explains the great current interest in alternative inhibitors of inositol monophosphatase. Synthetic efforts in this direction by the Merck, Sharpe and Dohme group \cite{67-73} have shown encouraging results, and a number of inositol monophosphatase inhibitors have been prepared (Figure 1.14), although they are not non-competitive in nature like lithium.

It was found that the two \( \alpha \)-hydroxyl groups in the 2- and 6-position play very different roles in the hydrolysis of \( \text{Ins}(1)\text{P} \) by inositol monophosphatase. The 2-hydroxyl group is important for recognition of the substrate by the enzyme (2-deoxy-\( \text{Ins}(1)\text{P} \) is only a weak substrate), whereas the 6-hydroxyl group is involved in the mechanism of phosphate hydrolysis (thus 6-deoxy derivatives like 20 have inhibitory properties). The first approach to inhibitors was based on this observation, and by a ‘hydroxyl group deletion’ strategy D-3,5,6-tri-deoxy-\( \text{Ins}(1)\text{P} \) 21 was synthesised and found to be a potent inhibitor \cite{68}, indicating that the 3- and 5-hydroxyl groups are not necessary for enzyme recognition.

*Figure 1.13 Inositol monophosphatase: enzyme-substrate interaction*

It is known that inositol monophosphatase is very substrate unspecific and that the enzyme is capable of hydrolysing 2’-nucleotides including adenosine-2’-monophosphate (2’-AMP)\cite{55} as well as inositol monophosphates. After
superimposing the molecular structures of 2'-AMP and D-3,5,6-tri-deoxy-Ins(1)P 21 it was concluded that substituents in the 6-position (the purine heterocycle in 2'-AMP) would be tolerated by the enzyme. This led to the synthesis of 6-substituted 3,5-di-deoxy-Ins(1)P derivatives, amongst them 22, the most potent monophosphatase inhibitor reported to date [69].

However, phosphate esters are biologically labile and tend to have short half-lives due to hydrolysis by non-specific phosphatases. Therefore, attempts to improve the stability of the inhibitor by replacement of the phosphate ester with an isosteric, but stable, monophosphonate group were made. As myo-inositol 1-methylene phosphate did not show any inhibitory properties, the search for inhibitors was based on hydroxymethylene phosphonate, which had been identified as a weak inhibitor. In a series of analogues, the adamantyl ester 23 was the most potent inhibitor with a $K_i = 6.3$ mM. Further studies on hydroxymethylene-bisphosphonic acid derivatives [71] showed that compounds unrelated to the enzyme substrate like the tetralin derivative 24 can also be very potent.

![Chemical structures of inhibitors](image)

Figure 1.14 Inhibitors of Inositol Monophosphatase
potent monophosphatase inhibitors. The 3-(3,4-dichlorobenzamido)benzyl derivative 25 is the most potent, non-hydrolysable inhibitor of myo-inositol monophosphatase reported to date [73].

1.6.3 Synthesis of Phosphatidylinositol 4,5-bisphosphate

The product of inositol monophosphatase activity, myo-inositol, is used for the synthesis of phosphatidylinositol 4,5-bisphosphate 3 (Figure 1.6), the precursor of the second messenger Ins(1,4,5)P3: Phosphatidic acid, derived from DAG by phosphorylation of the free hydroxyl group by a kinase enzyme, can combine with cytidine monophosphate (CMP) to form CMP-phosphatidate. The enzyme Ptdlns synthetase (E.C. 2.7.8.11) can then fuse CMP-phosphatidate and myo-inositol to form Ptdlns 1, releasing CMP in the process. Ptdlns is phosphorylated stepwise by specific kinases first to PtdIns(4)P 2 and then to PtdIns(4,5)P2, which can be used once more for signalling.

Once Ins(1,4,5)P3 has mobilised Ca2+ and thus fulfilled its second messenger function, it has to be deactivated quickly so that the cell can return to a basal state in preparation for the next stimulus. Two enzymes are know to metabolise Ins(1,4,5)P3: a 3-kinase and a 5-phosphatase, producing Ins(1,3,4,5)P4 and Ins(1,4)P2, respectively.

1.6.4 Ins(1,4,5)P3 5-phosphatase

Different soluble and particulate subtypes of the 5-phosphatase have been characterised from brain [74,75]. The two soluble enzymes [74], referred to as type 1 and type 2 according to their order of elution from DEAE-Sepharose, show different substrate specificities with respect to Ins(1,4,5)P3 and Ins(1,3,4,5)P4. Type 1 5-phosphatase, a 60 kDa protein, has apparent K_m values of 3 and 0.8 μM for Ins(1,4,5)P3 and Ins(1,3,4,5)P4, respectively, and hydrolyses Ins(1,4,5)P3 ca. 12 times faster than Ins(1,3,4,5)P4. The substrate of the type 2 phosphatase, a 160 kDa protein, seems to be primarily Ins(1,4,5)P3 (K_m 18 μM), the K_m of this enzyme for Ins(1,3,4,5)P4 is greater than 150 μM.

A recent report [76] shows a high similarity between a protein encoded by the Lowe’s oculocerebrorenal syndrome (OCRL) gene and 5-phosphatase. OCRL is
a genetic disorder affecting eyes, brain and kidneys, and there is strong evidence that OCRL may be an inherent defect of inositol phosphate metabolism. There are also indications that HIV-infected cell show defects of inositol polyphosphate-mediated signalling, and evidence has been presented showing that \( \text{Ins}(1,4,5)P_3 / \text{Ins}(1,3,4,5)P_4 \) 5-phosphatase is the enzyme most affected.

\( \text{Ins}(1,4)P_2 \) does not seem to have any physiological role in this pathway, however it has been shown to act as an allosteric activator of 6-phosphofructo-1-kinase. \( \text{Ins}(1,4)P_2 \) is then further dephosphorylated to \( \text{Ins}(1)P \) and/or \( \text{Ins}(4)P \) and finally to inositol which is used for the re-synthesis of phosphoinositides.

### 1.6.5 \( \text{Ins}(1,4,5)P_3 \) 3-kinase

The existence of a second pathway of \( \text{Ins}(1,4,5)P_3 \) metabolism was first suggested by the discovery of \( \text{Ins}(1,3,4)P_3 \), which was found to be derived from \( \text{Ins}(1,3,4,5)P_4 \) via a 5-dephosphorylation. The source of \( \text{Ins}(1,3,4,5)P_4 \) was unclear at that time, and PtdIns(3,4,5)P_3, the possible parent phospholipid, could not be detected. An \( \text{Ins}(1,4,5)P_3 \) 3-kinase activity was then demonstrated in various tissues and the enzyme has been purified from rat brain. The 3-kinase (molecular mass 150-160 kDa) is a dimeric protein comprised of two catalytic subunits (53 kDa each) plus calmodulin. Its activity is mediated by \( \text{Ca}^{2+} \), which is intriguing since it suggests that the \( \text{Ins}(1,4,5)P_3 \)-induced rise of free cytosolic calcium acts as a regulator to promote the formation of \( \text{Ins}(1,3,4,5)P_4 \) at the expense of \( \text{Ins}(1,4,5)P_3 \).

The physiological significance of \( \text{Ins}(1,3,4,5)P_4 \) is not quite clear yet. Binding sites for this molecule as distinct from those for \( \text{Ins}(1,4,5)P_3 \) have been identified in a variety of tissues. In contrast to the \( \text{Ins}(1,4,5)P_3 \) binding sites they seem to be associated with plasma membranes rather than the ER membrane. Suggestions that the tetrakisphosphate may be involved in calcium homeostasis by regulating the influx of extracellular calcium ions through the plasma membrane and/or controlling calcium movement into various intracellular pools thus seem to be confirmed.
Ins(1,3,4,5)P$_3$ is hydrolysed to Ins(1,3,4)P$_3$ [79,82,90,91], possibly by the same 5-phosphatase that dephosphorylates Ins(1,4,5)P$_3$ [82,92]. Alternatively, dephosphorylation of Ins(1,3,4,5)P$_4$ to Ins(1,4,5)P$_3$ can occur [91]. Further hydrolysis of phosphates leads back to inositol.

Ins(1,3,4)P$_3$ is also known to be phosphorylated to Ins(1,3,4,6)P$_4$ [93]. This inositol tetrakisphosphate is capable of inducing Ca$^{2+}$ release by interacting with the Ins(1,4,5)P$_3$ receptor [94]. In contrast to Ins(1,4,5)P$_3$ however, Ins(1,3,4,6)P$_4$ appears to act as a partial agonist at the receptor, mobilising only 82% of the calcium in the Ins(1,4,5)P$_3$-dependent store. Further phosphorylation of Ins(1,3,4,6)P$_4$ leads to the higher polyphosphates now found in mammalian cells.

1.6.6 Higher Inositol Phosphates

For a long time higher inositol phosphates have been known to exist in plants; as early as 1872 a highly phosphorylated substance later shown to be myo-inositol hexakisphosphate (InsP$_6$, phytic acid) was isolated from seeds by Pfeffer [95]. InsP$_6$ is supposed to serve as a phosphorus and/or energy reservoir for the plant (it accounts for 60-90% of the organically bound phosphorus in seeds), and enzymes (phytases) able to degrade InsP$_6$ and release the bound phosphorus for use by the organism have been discovered.

The presence of Ins(1,3,4,5,6)P$_5$ in avian erythrocytes has also been known for some time [96], and it seems that it has a rather specialised function as a modulator of haemoglobin oxygen affinity in these cells [97], a role performed normally in mammalian cells by 2,3-bisphosphoglycerate.

Ins(1,3,4,5,6)P$_5$ and InsP$_6$ have now been found in a variety of cells, but their role is not clear. There is no evidence for rapid changes in the levels of these two compounds after agonist-stimulation of different receptors [98], rendering an involvement of Ins(1,3,4,5,6)P$_5$ and InsP$_6$ in the Ins(1,4,5)P$_3$ signal transduction process unlikely. However, the possibility that these highly phosphorylated inositols are used as extracellular messengers has to be considered after the observation of dose-dependent, reversible changes of blood pressure and heart rate following the injection of Ins(1,3,4,5,6)P$_5$ and InsP$_6$ into rat nucleus tractus
solitarius (a discrete brain stem nucleus implicated in cardio-vascular regulation) [99]. However, current opinion suggests that these observations should be treated with caution.

Reports (e.g. [100]) that the activation of PLC is accompanied by increases in levels of Ins(3,4,5,6)P$_4$ and that these increases are not due to an increased metabolic flux from Ins(1,4,5)P$_3$ have led to suggestions that agonists evoke this particular effect by modulating the activity of enzymes that interconvert Ins(1,3,4,5,6)P$_5$ and Ins(3,4,5,6)P$_4$ [101], and that Ins(1,3,4,5,6)P$_5$ may provide a precursor pool for Ins(3,4,5,6)P$_4$. The function of the Ins(3,4,5,6)P$_4$ signal, however, is still unclear.

1.6.7 Inositol pyrophosphates

A number of higher inositol phosphates have been found in Dictyostelium [98,102], and it was in this slime mould that inositol phosphates more polar than InsP$_6$ were first demonstrated [103]. Recent studies [104] have shown that these compounds are pyrophosphates of Ins(1,3,4,5,6)P$_5$ and InsP$_6$. As the turnover between pyrophosphates and parent inositol phosphates is rapid, a role for these pyrophosphates as a new form of high-energy phosphates has been proposed [105].

1.6.8 Summary

The inositol phosphate cycle as it is known today is shown in Figure 1.9. Whereas the role of Ins(1,4,5)P$_3$ and the immediate metabolism of this second messenger is now relatively well understood, it is clear that there are still many questions to be answered before the complex metabolic network of inositol phosphates and the physiological functions of the individual metabolites are fully unveiled.
Figure 1.15 The Inositol Phosphate Cycle
1.7 GPI membrane anchors and putative insulin mediators

Many proteins are bound to biological membranes. Some of these proteins (called integral membrane proteins) have one or more hydrophobic domains and are embedded to a great extent in the lipid bilayer (e.g. ion channels). Other proteins are covalently attached to lipids which serve as anchors to the cell membrane. Many of these latter proteins are linked to the membrane by phosphatidylinositol anchors attached to the C-terminal amino acid of the protein through an intervening glycan structure [glycosyl-phosphatidylinositol (GPI) anchor] \(^{106-109}\), (on the biosynthesis of GPI anchors see \(^{110}\)). More than 30 different cell surface proteins with a GPI anchor have been identified, including hydrolytic enzymes (e.g. alkaline phosphatase and acetylcholinesterase \(^{111}\)), mammalian antigens (e.g. Thy-1 \(^{112}\)) and cell adhesion proteins. The generic structure of GPI anchors is PtdIns - glucosamine - mannose\(_3\) - phosphoethanolamine - protein, however, modifications of the core mannosyl residues by additional side chains are common and other variations (e.g. in the fatty acid composition of PI) have also been observed.

Some metabolic fragments of the protein-GPI complex, formed by the combined actions of specific endogeneous proteases and a glycosyl-phosphatidylinositol specific phospholipase C (GPI-PLC) - a PLC distinct from the PtdIns-PLC which hydrolyses PtdIns, PtdIns(4)P and PtdIns(4,5)P\(_2\) and is located on the inner membrane rather than the cell surface - have displayed certain biochemical properties previously associated with crude preparations of putative insulin mediators (PIM). PIM are phosphorylated inositol-glycans which are generated following insulin cell surface receptor stimulation and can mimic some biochemical properties of insulin \textit{in vitro} \(^{113}\).

The discovery of GPI anchors and putative insulin mediators has attracted the interest of bioorganic chemists \(^{114-118}\).
Efforts to synthesise the GPI anchor of the variant surface glycoprotein (VSG) of the parasitic protozoan *Trypanosoma brucei* have recently been successful. The survival of African trypanosomes depends on the integrity of their cell-surface coat, which is formed by VSGs arranging into tightly packed monolayers and which protects the parasite from lytic factors of the host serum. By expressing different VSGs at different times the parasite is able to evade the host’s immune response. This antigenic variation makes it difficult for the immune system to supply the appropriate antibodies at the right time and it also renders drugs against specific VSGs ineffectual.
Inhibition of the biosynthesis of the VSG GPI-anchor however, or drugs targeting specific features of this GPI-anchor could prove successful. This explains the interest this particular GPI-anchor has attracted. Whereas the backbone structure of the VSG GPI-anchor is similar to other GPI-anchors, the α-galactose side chain of the VSG GPI-anchor seems to be parasite-specific, thus the design of drugs aiming at this side chain may prove successful.

The structures of other GPI anchors have been determined, including that of rat-brain Thy-1 glycoprotein \(^{112}\) and human erythrocyte acetylcholinesterase \(^{111}\).
2. The Synthesis of Inositol Phosphates

The inositol chemistry up to the mid-1960’s is summarised in a book by Posternak [120]. In 1980 a comprehensive review of the chemistry and biochemistry of inositol phosphates was published by Cosgrove [121], which was concluded with the words: "The lower phosphoric esters of myo-inositol do not appear to exist in other than small amounts as transient intermediates in biochemical reactions". Renewed interest in inositol phosphate chemistry was stimulated by the discovery of the second messenger role of Ins(1,4,5)P3 in 1983. Two review articles [122,123] and two books [124,125] summarise current developments in this area. In 1992, Carbohydrate Research published a special issue on the synthesis of inositol derivatives [126], which demonstrates the high degree of interest in this field today.

There are five major problems to be addressed when preparing inositol phosphates and analogues thereof. These problems are:

1 - synthesis of precursors with selectively protected hydroxyl functions,
2 - optical resolution of racemates,
3 - phosphorylation of free hydroxyl groups,
4 - deblocking of the fully protected compound,
5 - purification of the resulting highly charged inositol phosphate.

![Diagram of Synthesis of Inositol Phosphates and Analogues]

Figure 2.1 Synthesis of Inositol Phosphates and Analogues
2.1 Protecting Groups in the Synthesis of Inositol Phosphates

The starting material most commonly used in the synthesis of inositol phosphates is myo-inositol 1. In order to protect the six hydroxyl functions various blocking groups including ethers, esters, siloxanes, acetals have been employed. As all hydroxyl groups are secondary, they vary only slightly in reactivity, with the order of reactivity being 1- and 3-OH > 4- and 6-OH > 5-OH > 2-OH. The selective protection of hydroxyl functions is therefore difficult to achieve, and often product mixtures are obtained.

2.1.1 Ether protecting groups

Benzyl ethers

Benzyl ethers are stable to oxidising agents and reducing agents such as sodium borohydride and lithium aluminium hydride. They are also not cleaved by the methods used to remove temporary acid or base labile protecting groups and are therefore usually employed as permanent protecting groups which are only deblocked together with the phosphate protecting groups (which are often benzyl groups as well, see below) in the final step of the synthetic sequence. The preparation of benzyl ethers is usually performed under rather drastic conditions (ROH + PhCH₂Cl, powdered KOH, 130-140°C), but there are milder options, e.g. use of sodium hydride, PhCH₂Br and tetrabutyl ammonium iodide as phase transfer catalyst at r.t. \[^{[127]}\].

Removal of benzyl groups (as toluene) can be achieved by using palladium on carbon (platinum would hydrogenate the aromatic ring) as catalyst in catalytic hydrogenations or catalytic transfer hydrogenations with various hydrogen donors like cyclohexene \[^{[128]}\], 1,4-cyclohexadiene \[^{[129]}\], formic acid \[^{[130]}\], or ammonium formate \[^{[131]}\]. Other methods for deprotection are bromination-hydrolysis \[^{[132]}\], acetolysis \[^{[133]}\] or the use of sodium (or lithium) in liquid ammonia for chemical reduction \[^{[134]}\].

\textit{para-Methoxybenzyl ethers}

The preparation of p-methoxybenzyl ethers can be performed by using the methods described for benzyl ether formation. In contrast to unsubstituted
benzyl ethers \( p \)-methoxybenzyl ethers are acid sensitive. Selective deprotection in the presence of other acid-labile protecting groups however, is possible with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in dichloromethane-water [135].

**Allyl ethers**

Allyl and to a lesser extent crotyl protecting groups have been widely used in the synthesis of inositol phosphates. Allyl ethers are usually formed by reacting free hydroxyl groups with allyl bromide in DMF at r.t. in the presence of a strong base such as NaH [136]. Allyl protecting groups are similar to benzyl ethers in their stability under acidic and basic conditions. At a very high pH however, isomerisation to the prop-1-enyl group may occur.

Deprotection of allyl groups often involves their isomerisation to enol ethers by potassium tert.-butoxide in DMSO [137] (yielding the cis-isomer), tris(triphenylphosphine)rhodium(I) chloride \([(\text{Ph}_3\text{P})_3\text{RhCl}][138]\) or \(\text{PdCl}_2(\text{PhCN})_2\) (giving a mixture of cis- and trans-isomers) or iridium complexes[139] (giving selectively the trans-isomer) and the subsequent acidic hydrolysis [140] of these prop-1-enyl compounds. With compounds containing acid sensitive groups, cleavage of the prop-1-enyl group by treatment with mercuric (II) chloride in the presence of mercuric (II) oxide is possible [141]. Like benzyl groups, allyl ethers can also be cleaved by the use of sodium in liquid ammonia [142,143]. Recently the \(\text{SmCl}_3\)-catalysed electrochemical cleavage of allyl ethers [144] and the employment of low-valent titanium [145] for the removal of benzyl and allyl ethers have been described. Allyl deprotection methods have been reviewed [146].

**Silyl ethers**

The regioselectivity of different reagents in the protection of 1,2-\( \text{O}\)-cyclohexylylidene-\( \text{myo}\)-inositol was investigated [147]. It was found that selective protection of the 1-position can be achieved by employing sterically bulky reagents such as tert-butylidiphenylsilyl chloride (TBDPS-Cl), whereas the selectivity was considerably reduced when smaller silyl groups such as tert-butyldimethylsilyl or trimethylsilyl were used. This observation was used in the
synthesis of optically pure regioselectively silylated D-camphor dimethyl acetals of inositol. Reaction of 1D-2,3-O-(D-1',7',7'-trimethyl[2.2.1]bicyclohept-2'-ylidene)-myo-inositol with 1.1 equiv. of TBDPS-Cl gave the 1-silyl derivative in 88 % yield. When 2.1 equiv. of TBDPS-Cl were used, the 1,4-di-O-silyl ether was obtained in 84 % yield. Desilylation was effected by treatment with tetrabutylammonium fluoride or acid hydrolysis.

2.1.2 Ester protecting groups

Base labile esters complement the acid labile protection groups in inositol phosphate synthesis. Most widely used are benzoyl and acetyl esters, although propionyl and butyryl esters have also been employed. Butyryl esters of inositol phosphates are interesting lipophilic analogues which may allow the delivery of inositol phosphate prodrugs across the intact cell membrane. The action of esterases within the cell may then hydrolyse the prodrug to liberate the active compound. Acetate and butyrate esters have also been used in the synthesis of optically active intermediates by enzymatic catalysis (see below).

Benzoyl esters

Benzoylation is usually effected by reaction of the inositol derivative with benzoyl chloride in pyridine. Under suitable conditions, myo-inositol can be selectively benzoylated to give 1,3,4,5-tetra-O-benzoyl-myo-inositol or 1,3,5-tri-O-benzoyl-myo-inositol [148]. Removal of benzoates is possible under alkaline conditions.

2.1.3 Protection of diols

Isopropylidene and cyclohexylidene protecting groups

The use of these protecting groups allows the simultaneous protection of 1,2-diols. In several synthesis of inositol derivatives, the first step was the reaction of myo-inositol with 2,2-dimethoxypropane [149], cyclohexanone [150-152] or 1-ethoxycyclohexene [153,154] and an acid catalyst in DMF to give a mixture of three di-acetals (1,2:3,4, 1,2:4,5 and 1,2:5,6) or selectively the 1,2-mono-acetal,
depending on the reaction conditions. Separation of the di-O-cyclohexylidene acetals is possible by column-chromatography and crystallisation. 1,2:4,5-Di-O-isopropylidene-\textit{myo}-inositol was obtained by Gigg \textit{et al.} by converting the di-O-isopropylidene acetals into the dibenzoates. 3,6-Di-O-benzoyl-1,2:4,5-di-O-isopropylidene-\textit{myo}-inositol was found to be insoluble in water, acetone and ether and could thus be separated from the two other isomers by washing with these solvents \cite{149}. The same authors also succeeded in the isolation of 3,4-di-O-benzoyl-1,2:5,6-di-O-isopropylidene-\textit{myo}-inositol by fractional crystallisation of a mixture of the soluble dibenzoates (giving access to 1,2:5,6-di-O-isopropylidene-\textit{myo}-inositol) and in the preparation of 1,2:3,4-di-O-isopropylidene-\textit{myo}-inositol via the 5,6-di-O-allyl ether \cite{155}.

The 1,2-mono-acetals are obtainable by hydrolysis of the di-acetals under mild acidic conditions, since the \textit{trans}-acetals are less stable than the 1,2-\textit{cis}-acetal \cite{150}. Direct syntheses of 1,2-cyclohexylidene-\textit{myo}-inositol from \textit{myo}-inositol using 1,1-dimethoxycyclohexane and a strongly acidic resin-bound sulphonic acid catalyst (45-50 % yield) \cite{156} or cyclohexanone / PTSA in DMF / toluene followed by \textit{in situ} ethanolysis of the \textit{trans}-acetals (93 % yield) \cite{152} have also been reported.

The 1,1,3,3-tetraisopropyldisiloxane (TIPS) protecting group

More recently, the tetraisopropyldisiloxane-1,3-diyl group has been used in the synthesis of different inositol phosphates including Ins(1,3,4,5)P\textsubscript{4} \cite{157}. Reaction of \textit{myo}-inositol with 1,3-dichloro-1,1,3,3-tetraisopropyl disiloxane (TIPS-Cl) in pyridine at r.t. afforded in 66 % yield the symmetrical 1,6:3,4-bis(disiloxane) derivative with only minor amounts of other inositol derivatives. The 1,2-O-cyclohexylidene acetal of \textit{myo}-inositol gave selectively the 3,4-siloxane on treatment with TIPS-Cl \cite{157}. A number of enantiomerically pure regioselectively protected inositol derivatives have been synthesised by 1,2-acetalisation of \textit{myo}-inositol with D-camphor dimethyl acetal, protection of the 3,4-position with TIPS and various further protection/deprotection steps \cite{147}. Removal of the TIPS protecting groups was effected with aqueous HF in acetonitrile.
2.1.4 Orthoformate Esters

Simultaneous protection of the 1-, 3-, and 5-position of myo-inositol is possible by forming the orthoformate ester with triethylorthoformate and PTSA in DMSO [158] or preferably DMF [159]. The normal axial/equatorial relationship of the remaining free hydroxyl groups in this compound is reversed with the 2-position now equatorial and the 4- and 6-position axial. The spatial juxtaposition of the axial hydroxyl groups allows highly selective monoalkylations or monophosphorylations [160]. This intermediate has been employed in the synthesis of racemic [160,161] as well as optically resolved myo-inositol 1,3,4,5-tetrakisphosphate [159]. Myo-inositol orthoformate has also been used in the preparation of myo-inositol 2-phosphate, myo-inositol 4-phosphate and myo-inositol 1,3-bisphosphate [160,161]. Selective cleavage of the 2,4,6-tri-O-benzyl orthoester can be achieved by reduction with DIBAL or treatment with trimethyl aluminium, furnishing in high yields the 1,3-O-methylene and the 1,5-O-ethylidene derivative, respectively [162,163]. Rearrangement of the fully benzyl-protected 1,3-O-methylene derivative to the 1,2-acetal (with loss of the benzyl group in the 3-position) on treatment with stoichiometric amounts of Lewis acid (TiCl₄) has been observed [162,163].

Removal of the orthoformate ester is accomplished by acid hydrolysis.

2.1.5 The Use of Organotin Derivatives

As the differences between the reactivity of the equatorial hydroxyl groups in inositol derivatives are only small, attempts to selectively substitute one hydroxyl function often lead to a mixture of products. Organotin derivatives have proved to be useful to selectively activate hydroxyl groups [164], thus allowing the synthesis of compounds only difficult to obtain by other routes. They are especially useful in directing the regiochemistry of substitution in axial/equatorial pairs of vic-diols. An X-ray analysis of the 2,3-O-dibutylstannylene derivative of methyl-4,6-O-benzylidene-α-D-glucopyranoside showed a dimeric arrangement of the stannylenes (Figure 2.2). The two tin atoms are co-ordinated in a trigonal bipyramid structure with the butyl groups in equatorial positions and two sugar oxygen atoms each occupying the remaining equatorial and the apical positions.
Figure 2.2  The trigonal bipyramidal structure of sugar-stannylene complexes

Tin reagents show a greater affinity for 1,2-diols in an axial-equatorial cis-conformation (i.e. the 1:2 or 2:3 position myo-inositol derivatives) than for diequatorial trans-hydroxy I groups (i.e. the 3:4, 4:5, 5:6 and 1:6 positions), and it was for the selective allylation of the equatorial 1-hydroxyl group over the axial 2-hydroxyl group of 1,2-diols that dibutylstannylene derivatives were first employed in inositol chemistry \[165\].

The efficiency of tin-mediated alkylation has been found to be enhanced by quaternary ammonium halides \[166\] and caesium fluoride \[167\]. Both modifications have been used in the synthesis of inositol derivatives \[168,169\]. The observation that maximum yields in tin-mediated O-monoalkylations were obtained by the use of almost 2 equivalents of caesium fluoride \[167\] has led to the suggestion that the salt may act in two different ways to assist in the reaction: a) by activating the alkyl halide through interaction of the polarisable caesium cation with the halogen atom, and b) by activating the Sn-O bond by the formation of a pentacoordinate complex.

35
2.2 The Synthesis of Optically Active Inositol Derivatives

Chiral starting materials

If the meso-compound myo-inositol is used as starting material in the synthesis of inositol phosphates, optical resolution is required to separate D- and L-isomers of the inositol phosphate precursors. The problem of optical resolution can, however, be avoided by employing chiral starting materials, e.g. naturally occurring compounds such as L-quebrachitol (1L-2-O-methyl-choi-ro-inositol) 26 [in the synthesis of L-1-deoxy-1-fluoro-my/o-inositol [170], D-Ins(1)P [171], L-Ins(1)P [172], L-Ins(1,4,5)P₃ [173], L-Ins(2,4,5)P₃ and L-choi-ro-Ins(1,3,5)P₃ [174], a number of 3-modified D-choi-ro-inositol analogues [175], D-3-deoxy-Ins(1,4,5)P₃ and D-3-deoxy-Ins(1,5,6)P₃ [176], as well as in the synthesis of L-choi-ro-Ins(2,3,5)P₃ [177]]. Other chiral starting materials which have been used are D-pinitol (1D-3-O-methyl-choi-ro-inositol) 27 [in the synthesis of D-1-deoxy-1-fluoro-my/o-inositol and D-1,5-dideoxy-1,5-difluoro-neo-inositol [178], D-Ins(1,4,5)P₃ [173], D-Ins(2,4,5)P₃ and D-choi-ro-Ins(1,3,5)P₃ [174]], (-)-quinic acid 28 [in the synthesis of D-Ins(1,4,5)P₃ [179] and the 5-methylene phosphonate analogue thereof [180]], D-glucurono-6,3-lactone 29 [in the synthesis of precursors of D-Ins(1,4,5)P₃ [181]] and D-galactose 30 [in the synthesis of D-6-deoxy-Ins(1,4,5)P₃, D-6-deoxy-Ins(1,2-cyclic-4,5)P₃ and D-6-deoxy-Ins(1,5)P₂ via Ferrier rearrangement [182]]. Methyl α-D-glucopyranoside 31 has been employed in a biomimetic synthesis of enantiomerically pure D-my/o-inositol derivatives [183], which were then used to prepare an inositol 1,3,4,5-tetrakisphosphate analogue carrying an affinity label at the 1-position [184].

Enzymes in the synthesis of optically active inositol derivatives

In a number of studies, enzymes such as esterases, lipases and proteases have been examined for their ability to hydrolyse enantio- and regio-selectively racemic or meso-carboxylates of myo-inositol [159,185-187], 3-deoxy-epi-inositol and 1-deoxy-scyllo-inositol [188]. By employing the resolved precursors, D-Ins(1,4,5)P₃ [185,187], D-Ins(1,3,4)P₃ [186,187] and D-Ins(1,3,4,5)P₄ [159,186,187] as well as L-Ins(1,3,4,5)P₄ [159] have been synthesised. A new method involving enzymes is the enzyme-catalysed selective esterification of myo-inositol
derivatives, which has been applied to resolve racemic 1,2:5,6- and 1,2:3,4-di-O-cyclohexylidene-myoinositol \[189\].

![Chemical structures: L-quebrachitol 26, D-pinitol 27, (-)-quinic acid 28, D-glucurono-6,3-lactone 29, D-galactose 30, methyl-\(\alpha\)-D-glucopyranoside 31]

Figure 2.3 Chiral starting materials in the synthesis of inositol phosphates

Chiral HPLC-columns

Chiral HPLC-columns have been used to resolve 4,5-di-O-benzyl-1,6-di-O-PMB-myoinositol in the synthesis of \(\text{Ins}(1,3,4)\)P\(_3\) \[190\] and 1,3,4,5-tetra-O-benzoyl-myoinositol in the synthesis of \(\text{Ins}(1,3,4,5)\)P\(_4\) \[148\].

Resolving Agents

Most optical resolutions in the synthesis of inositol phosphates rely on the conversion of racemic inositol derivatives into a pair of diastereoisomers, which is then separated by crystallisation or chromatography. Resolving agents used include D-mannose derivatives 32 \[191\], \(\ell\)(-)-O-acetyl-mandelic acid 33 [in the synthesis of 1D- and 1L-4-O-benzyl-myoinositol \[192\]], \(S\)(-)-camphanic acid chloride 34 and its \(R\)(-)-isomer, D- and L-camphor dimethyl acetals 35 [in the synthesis of \(\text{Ins}(1,4,5)\)P\(_3\) and \(\text{Ins}(1,4)\)P\(_2\) \[193,194\], where the camphor acetal has also been used as protecting group for the 1,2-position], \(\ell\)-menthy1 chloroformate 36 \[185\], \(\ell\)-menthoxyl acetylchloride 37 [in the synthesis of \(\text{Ins}(1,4,5)\)P\(_3\) \[195-197\]...
and Ins(1,3,4,5)P₄ [197] and R-(+)-1-phenylethyl isocyanate 38 [in the synthesis of D-Ins(1,3,4,5)P₄ [159]]. Optical resolution via formation camphanate esters seems to be the most generally useful method available at the present time, both S-(−)- and R-(+)-camphamic acid chloride (stable crystalline reagents, which are commercially available in high optical purity) have been employed to obtain optically active inositol derivatives.

Figure 2.4 Resolving agents used in the synthesis of optically pure inositol derivatives
2.3 Phosphorylation Methodology

There are two different methods by which phosphorylation of free hydroxyl groups in suitably protected inositol derivatives can be achieved: employing a P(V) reagent in which the phosphorus is already on the phosphate oxidation level, or using a P(III) approach, i.e. phosphitylation of the hydroxyl group and subsequent oxidation to give the protected phosphate ester.

2.3.1 P(V) reagents

The advantages of P(V) reagents for phosphorylation are the relative stability of reagent and products. Problems have however been encountered in the application of some P(V) reagents because of the low reactivity of the secondary hydroxyl groups of inositol; the phosphorylation of vicinal diols has proved to be especially difficult. The intermediate phosphate triesters formed upon phosphorylation at the first free hydroxyl group are prone to attack by the second hydroxyl group, thus affording a 5-membered cyclic phosphate rather than the desired bisphosphate. Since P(V) reagents are usually more bulky than P(III) reagents, it may be sterically difficult for a second reagent molecule to gain access to the phosphorylation site after monosubstitution of the diol has taken place. Therefore cyclisation reactions are able to compete successfully with substitution reactions at the second hydroxyl group.

Phosphorochloridates

Phosphorochloridates are of the general structure: \((R\text{O})_{3-x}P(O)\text{Cl}_x\), with \(x = 1\) and \(x = 2\) for phosphoromonochloridates and phosphorodichloridates, respectively.

Phosphorus oxychloride (POCl\(_3\), \(x = 3\)) was first used as a phosphorylating reagent in 1857\(^{198}\) for the synthesis of methyl esters of phosphoric acid. More recently, it has been successfully employed in the regioselective synthesis of 5'-nucleotides from unprotected nucleosides\(^{199}\). In this synthesis, POCl\(_3\) was used partially hydrolysed in trimethyl or triethylphosphate as solvent, and the active phosphorylating species was probably \([\text{(RO)}_3\text{P-O-P(O)}\text{Cl}_2]^+\text{Cl}^-\).
A stepwise phosphorylation of the vicinal 4,5-diol in myo-inositol derivatives with two P(V) reagents has recently been reported \[^{[200]}\]. Phosphorylation with bis(2,2,2-trichloroethyl) phosphorochloridate (see below) only gave a mixture of the 4- and 5-monophosphorylated inositols but not the bisphosphate presumably due to steric hindrance. By using POCI\(_3\) in pyridine followed by addition of methanol, the mixture of 4- and 5-monophosphorylated inositols could however be converted into 4,5-bisphosphate triesters with different protecting groups in the 4- and 5-position (i.e. 2,2,2-trichloroethyl and methyl esters).

Diphenyl phosphorochloridate can be easily prepared from phosphorus oxychloride and phenol \[^{[201,202]}\] and has been employed in many phosphate ester preparations, including an early synthesis of D-myo-Ins(2)P \[^{[203]}\], and the synthesis of L-myo-Ins(1)P \[^{[172]}\].

The oxidation of dibenzyl phosphite with \(\text{N}\)-chlorosuccinimide furnishes dibenzyl phosphorochloridate, which was used in the first synthesis of a nucleotide coenzyme, adenosine 5\(^{-}\)-diphosphate (ADP) \[^{[204]}\], and later in the synthesis of adenosine 5\(^{-}\)-triphosphate (ATP) \[^{[205]}\].

Other diaryl phosphorochloridates have also been employed as phosphorylating agents. Bis(\(\text{para}\)-nitrobenzyl) phosphorochloridate and bis(\(\text{para}\)-nitrophenyl) phosphorochloridate have been used in the synthesis of 5\(^{-}\)-amino-5\(^{-}\)-deoxynucleosides \[^{[206]}\] and thymidine nucleotides \[^{[207]}\], respectively, and bis(2,2,2-trichloroethyl) phosphorochloridate has been applied in the synthesis of nucleotides \[^{[208]}\] as well as in the preparation of Ins(1,4,5)P\(_3\) and its 5-phosphorothioate analogue \[^{[209-211]}\].

Dianilino phosphorochloridate was employed in the first reported synthesis of D-Ins(1,4,5)P\(_3\) \[^{[195]}\], this phosphorylation method and the removal of the protecting groups with isoamyl nitrate in pyridine/acetic acid/acetic anhydride was however considered to be unsatisfactory.

Phosphorodichloridates can react with two different partners, thus forming a phosphate-bridge between these moieties. As phosphodiester-links are common features of many biologically interesting compounds (e.g. oligonucleotides and phospholipids), a number of phosphorodichloridates have been developed for the synthesis of these compounds, including \(\text{phenyl}\)-, \(\text{para}\)-nitrophenyl- and methyl-phosphoro-dichloridate. Dichlorophosphoric acid has also been used for
inter-nucleotide bond formation and in the synthesis of nucleoside 3',5'-cyclic phosphates [212].

Anhydrides

Polyphosphates and mixed anhydrides are widely used as phosphorylating reagents, especially on an industrial scale, in spite of their non-specific nature, which often leads to the formation of cyclic phosphates or other side products. Phosphoric acid, potassium hydrogen phosphate and pyrophosphoric acid have all been employed in the synthesis of carbohydrate phosphates, the latter has also been used to phosphorylate *epi*- and *muco*-inositols [213].

Tetrabenzyl pyrophosphate (TBPP) [214] is a commercially available crystalline solid formed by condensation of two molecules of dibenzyl phosphate with dicyclohexylcarbodiimide (DCC). It is the only P(V) reagent which has successfully been employed in inositol phosphate chemistry for the phosphorylation of vicinal diols [215-217]. In order to effect efficient phosphorylation with TBPP, it is necessary to convert the free hydroxyl groups into alkoxides. This has been achieved by different methods, including the use of butyl lithium or lithium diisopropylamide (LDA) [215,218], sodium hydride [216], NaH and catalytic amounts of imidazole or 18-crown-6 [160] or potassium hydride [217,219]. The strongly basic conditions needed for efficient phosphorylation can however cause problems with some compounds leading to decomposition rather than formation of alkoxide ions. Once the protected phosphate has been formed the benzyl protecting groups can easily be removed by catalytic hydrogenolysis, treatment with trimethylsilyl bromide or sodium / liquid ammonia.

Phosphoramidates

Phosphoramidates of the general structure \((RO)\_2P(O)(NR\,')_2\) have been extensively employed in the synthesis of nucleoside di- and triphosphates. Phosphate monoesters can be activated for example by reaction with N,N'-carbonyldiimidazole, and the phosphoramidate formed can then react with another nucleophile to afford the product. Depending on whether the nucleophile used is an alcohol or another phosphate monoester, a phosphate diester or a pyrophosphate is obtained [220,221].
2.3.2 P(III) reagents

P(III) reagents are more reactive than most P(V) reagents, and good results by phosphitylation and subsequent oxidation have been reported in cases where P(V) reagents failed to give the desired products, e.g. phosphorylation of vicinal diols such as the free hydroxyl groups in the 4- and 5-position of precursors of Ins(1,4,5)P₃. The possibility that undesired cyclic phosphodiesters are formed [which is often encountered when P(V) reagents are used] is reduced.

Phosphorochloridites

Phosphorochloridites (RR'PCI) have the disadvantage of being unstable towards moisture. In spite of this they have been widely used and have also been employed in the synthesis of several inositol phosphates. Dimethoxy chlorophosphine in the presence of the base N,N-diisopropylethylamine was used in the synthesis of Ins(1,4,5)P₃, Ins(1,3,4,5)P₄ and Ins(1,4,5,6)P₄ \[222\]. Bis(2-cyanoethoxy)chlorophosphine was for example applied in the preparation of Ins(1,3,4)P₃ \[223\] and Ins(1,4,5)P₃ \[224\].

Phosphoramidites

Phosphoramidites are more stable than phosphorochloridites and some can even be purified by flash chromatography. They are of the general type (RO)₂P-N(R')₂, with R being for example benzyl or cyanoethoxy and N(R')₂ being diisopropylamine, diethylamine or morpholine.

Both N,N-diisopropylamino bis(2-cyanoethoxy) phosphine and N,N-diisopropylamino dibenzyl phosphine \[225,226\] have been widely used in inositol phosphate chemistry \[159,169\], and N,N-diethyl bis(2-cyanoethoxy) phosphine \[227\] has also been used in the synthesis of Ins(1,4,5)P₃. The phosphitylating reagents have to be catalytically activated by a weak acid (1-H tetrazole). The benzyl reagent has the added advantage of giving phosphate triesters which readily crystallise.

The new o-xylylene phosphoramidite reagent 2-diethylamino-1,3,2-benzodioxaphosphane, prepared by the reaction of hexaethylphosphorous
triamide with 1,2-benzenedimethanol, has also been employed in the synthesis of inositol phosphates \[^{196,228-232}\], including Ins(1,4,5)P\(_3\) and Ins(1,3,4,5)P\(_4\).

\[
\begin{align*}
\text{HO-} & \text{OH} + \text{N-P-N} \rightarrow \text{HO-} \text{P-N} \\
\text{o-xylylene diethylphosphoramidite}
\end{align*}
\]

**Figure 2.5** O-xylylene diethylaminophosphine (OXDEP)

The efficiency of different methods for the oxidation of the phosphite triesters to the corresponding phosphate esters has been studied \[^{226}\]; in inositol phosphate chemistry oxidation is usually effected with tert.-butyl hydroperoxide (t-BuOOH) \[^{224,227}\] or m-chloroperoxybenzoic acid (mCPBA) \[^{169}\].

A further advantage of using a two-step procedure is that the oxidation of the phosphite triesters can be replaced by sulphoxidation with elemental sulphur in pyridine \[^{233}\] or phenacetyl disulfide \[^{234}\], thereby making available not only phosphates but also phosphorothioate esters, which have been shown to be useful non-hydrolysable analogues of nucleotides \[^{235}\] and inositol phosphates \[^{209,234,236}\].

### 2.3.3 Conversion of H-Phosphonates into Phosphorothioates

Recently, a new approach towards the synthesis of phosphorothioate derivatives has been reported \[^{237}\]. S-Benzyl and S-phenyl thiosuccinimides reacted with H-phosphonates and H-phosphonothioate diesters in the presence of \(N,N\)-diisopropylethylamine to give the corresponding S-benzyl(phenyl) phosphorothioates or phosphorodithioates, respectively. This method can be modified to give S,S-dibenzyl(phenyl) phosphorodithioates directly from hydroxylic compounds by reaction of the intermediate generated \textit{in situ} from ammonium phosphinate, the thiosuccinimide and the activating reagent 2,4,6-triisopropylbenzenesulphonyl chloride. Thus, the 1-S,S-dibenzyl(phenyl) phosphorodithioates of DL-2,3,4,5,6-penta-O-benzyl-my-o-inositol were prepared in a one-pot reaction from this derivative \[^{237}\].

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2.4 Deprotection and Purification of Final Compounds

Another problem to be addressed in the synthesis of inositol phosphates is phosphate migration, which can occur during deprotection when free hydroxyl groups are present adjacent to the phosphate ester moiety. Ideally, phosphate protecting groups should be removed prior to hydroxyl protecting groups. As the cleavage of benzylphosphate esters occurs much more rapidly than the deblocking of benzyl groups used for the protection of hydroxyl groups \[^{238}\], the use of benzyl groups has been proposed for the protection of phosphates as well as hydroxyl groups.

Purification of the deblocked inositol phosphates is usually achieved by ion-exchange h.p.l.c. After deprotection in sodium - liquid ammonia the compounds are accompanied by large amounts of salts, but the separation of the highly charged inositol phosphates is easily effected by the use of ion-exchange columns. If the pure fully protected inositol phosphate is subjected to catalytic hydrogenolysis, the inositol phosphate may be obtained without further purification.

The conventional method for detection and quantification of non-radiolabelled inositol phosphates relies on the complexation of free phosphate with molybdate ions \[^{239}\].

Recently two h.p.l.c. on-line systems for the detection of organic phosphate have been described \[^{240-242}\]. The first system \[^{240,241}\] generates inorganic phosphate by an enzyme-loaded post-column reactor (alkaline phosphatase immobilised on phenoxyacetyl cellulose) which is then detected by complexation with molybdate. The disadvantage of this method is that relatively large samples are required and strong interferences of incompletely dephosphorylated higher inositol phosphates with the molybdate complex are observed.

The second system \[^{242}\] does not require dephosphorylation. Here, the high affinity of tervalent transition-metal ions like \(\text{Y}^{3+}\) to both the cation-specific dye 4-(2-pyridylazo)resorcinol and polyanions has been exploited for picomolar-range detection of inositol polyphosphates.

Analysis of phosphorothioates is possible with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), a reagent developed by Ellman for the detection of sulphydryl groups.
The advantage of employing DTNB is that degradation of the phosphorothioate prior to analysis is not necessary, allowing a quick identification of the column fractions containing the deprotected compound.

2.5 Synthetic Inositol Phosphates

2.5.1 Inositol Monophosphates

All six myo-inositol monophosphates (the meso 2- and 5-phosphates and the enantiomeric 1- and 4-phosphates) have been synthesised.

myo-Inositol 1-phosphate

The D- [171,231] and L-enantiomers [172] of Ins(1)P have been prepared from 1L-quebrachitol via 1L-3,4:5,6-di-O-cyclohexylidene-2-O-methyl-chiro-inositol. In order to synthesise D-Ins(1)P this key compound was oxidised to the inosose (RuO2-NaIO4) and stereoselectively reduced with sodium borohydride to give L-1,2:5,6-di-O-cyclohexylidene-4-O-methyl-myio-inositol. Benzoylation of the 3-position, removal of methyl ether and trans-ketal with aluminium trichloride / sodium iodide, perbenzoylation, hydrolysis of the cis-ketal, selective silylation of the 1-position with triethylsilyl chloride and benzylation of the remaining free hydroxyl group afforded the 1-silyl pentabenzoate. Deprotection of the silyl group with PTSA-acetic acid, phosphorylation with o-xyylene diethylphosphoramide / mCPBA and successive deblocking of phosphates and hydroxyl groups gave D-Ins(P)1.

An elegant three-step conversion of myo-inositol into D-Ins(1)P has been reported [245]. Resolution of myo-inositol by formation of its 2,3-D-camphor acetal, selective phosphorylation of the 1-position of the acetal with dibenzyl or diethyl phosphorochloridate and subsequent hydrogenolysis and acid deprotection of the acetal afforded D-Ins(1)P.

L-Ins(1)P (= D-Ins(3)P), the product of the enzyme glucose-6-phosphate inositol-1-phosphate cyclase and the only primary source of inositol in nature, was obtained from 1L-3,4:5,6-di-O-cyclohexylidene-2-O-methyl-chiro-inositol by tosylation of the free hydroxyl group, removal of the other protecting groups with
boron trichloride, perbenzoylation and detosylation with NaF (which proceeded with inversion of configuration). The 1D-1,2,4,5,6-penta-O-benzoyl-myoinositol was then phosphorylated and deprotected to give L-Ins(1)P. Optically active Ins(1)P has also been synthesised from 1,2,4,5,6-penta-O-benzyl-myoinositol (resolved as the R-(-)-camphanate) \[238\] and the corresponding pentaacetate (resolved as acid oxalates) \[246\]. The pentaacetate was also used in the synthesis of racemic Ins(1)P and its phosphorothioate analogue \[247\].

**myo-Inositol 2-phosphate**

Ins(2)P was first synthesised by taking advantage of the highly selective oxidation of myo-inositol to scylo-inosose by Acetobacter suboxydans. The inosose was peracetylated and the ketone reduced to give myo-inositol 1,3,4,5,6-pentaacetate. Phosphorylation with diphenylchlorophosphate, removal of the phosphate protecting groups by hydrogenolysis and the acetates by catalytic saponification with sodium methyleate in MeOH gave Ins(2)P \[203\].

**myo-Inositol 4-phosphate**

Synthesis of Ins(4)P was possible by selectively protecting the 3-position of 1,2:4,5-di-O-cyclohexylidene-myoinositol, followed by phosphorylation of the remaining free hydroxyl group and deprotection \[248,249\]. Racemic as well as optically active Ins(4)P has been synthesised from 1,2:4,5-di-O-cyclohexylidene-3-O-benzyl-myoinositol, which was resolved using S-(-)-camphanic acid chloride \[238\]. Reaction of 1,2-O-cyclohexylidene-myoinositol with 1,3-dichloro-1,1,3,3-tetraisopropylsiloxane (TIPS) gave selectively the 3,4-disiloxane, which could be selectively benzyolated at the 6-position. Phosphorylation with TBPP and base resulted in the migration of the benzoate and phosphorylation at the 6-position. Removal of protecting groups afforded racemic Ins(4)P \[157\]. A short synthesis of racemic Ins(4)P by chelation-controlled selective phosphorylation of the mono anion of myo-inositol orthoformate with TBPP has also been reported \[160,161\].
myo-Inositol 5-phosphate

The meso-compound Ins(5)P was first prepared from 2-amino-2-deoxy-neo-inositol \(^{[249]}\), which can be obtained by hydrolysis of the antibiotic hygromycin A. Phosphitylation of 1,2-O-cyclohexyldene-3,4-disiloxane-myoinositol 6-benzoate with phosphorus trichloride and benzyl alcohol, oxidation and deblocking also gave Ins(5)P \(^{[157]}\).

2.5.2 Inositol Bisphosphates

**myo-Inositol 1,3-bisphosphate**

Ins(1,3)P\(_2\) is a meso-compound. A synthesis has been reported \(^{[160]}\) by regioselective diphenylchlorophosphate phosphorylation of 2,4,6-tri-O-benzyl-myoinositol, obtained via the myo-inositol orthoformate ester. The 1,3-bisphosphorylated product could be crystallised from the 82:18 mixture of 1,3- and 1,5-bisphosphorylated products. Deprotection with lithium in liquid ammonia gave Ins(1,3)P\(_2\).

**myo-Inositol 4,5-, 1,4-, and 3,4-bisphosphate**

Racemic Ins(4,5)P\(_2\), Ins(1,4)P\(_2\) and Ins(3,4)P\(_2\) have been synthesised by simply phosphorylating the three different di-cyclohexyldene or di-isopropylidene acetals of myo-inositol \(^{[249,250]}\). 1,2,3,4-Tetra-O-benzyl-myoinositol \(^{[251,252]}\) and conduritol B derivatives \(^{[253]}\) have also been used in the synthesis of Ins(4,5)P\(_2\). The different enantiomers of these bisphosphates were obtained by employing the optically active bisacetals which can be obtained by resolution with orthoesters of D-mannose \(^{[248]}\) or as bis-(−)-α-camphanates \(^{[217]}\).

**myo-Inositol 1,5-bisphosphate**

Both enantiomers of Ins(1,5)P\(_2\) \(^{[254]}\) have been prepared by phosphorylation of D- and L-1,2,4,6-tetra-O-benzyl-myoinositol with N,N-diethylamino bis(2-cyanoethoxy) phosphine, followed by oxidation and hydrogenolysis. Resolution of the tetrabenzy1 compounds (obtained by selective benzyla1ion of 2,4,6-tri-O-
benzyl-myoinositol derived from the orthoformate) could be effected as the mono-(-)-camphanic esters.

2.5.3 Inositol Trisphosphates

myo-Inositol 1,4,5-trisphosphate

The first synthesis of D-Ins(1,4,5)P_3 using myoinositol as starting material was reported by Ozaki et al. in 1986 \cite{1195} (Figure 2.6). DL-1,2:4,5-di-O-cyclohexylidene-myoinositol 39 was benzylated to give 40. Selective removal of the 4,5-acetal with ethylene glycol and PTSA afforded 41. Allylation and cleavage of the remaining cis-acetal gave the 1,2-diol 43, which could be resolved after regioselective conversion into the diastereomeric 1-L-menthoxyacetyl esters 44a and 44b. Hydrolysis of the menthoxyacetyl ester 44a and regioselective allylation gave D-1,4,5-tri-O-allyl-3,6-di-O-benzyl-myoinositol 45, which was benzylated and deallylated to 47. Phosphorylation with dianilidophosphoric chloride gave 48, and successive deblocking of phosphoryl and hydroxyl groups with isoamyl nitrite in pyridine / acetic acid / acetic anhydride and H_2 / 5 % Pd-C, respectively, gave D-Ins(1,4,5)P_3 6. The phosphorylation and deprotection procedures, however, were not deemed to be satisfactory.

The second reported synthesis of Ins(1,4,5)P_3 \cite{224} also employed 2,3,6-tri-O-benzyl-myoinositol 47, obtained by a similar route \cite{165}. The difficulties with phosphorylating this intermediate were overcome by the use of a P(III) approach: treatment of the triol with diisopropylamino (2-cyanoethoxy) chlorophosphine followed by displacement of the diisopropylamino group with 2-cyanoethanol gave the trisphosphite, which was subsequently oxidised with tBuOOH to give the protected trisphosphate. Deblocking with sodium / liquid ammonia afforded racemic Ins(1,4,5)P_3. This approach was later modified by using sulphur in pyridine to sulphotidise the trisphosphite, giving the trisphosphorothioate analogue of Ins(1,4,5)P_3 \cite{236}.
Figure 2.6  The first synthesis of D-\textit{myo}-inositol 1,4,5-trisphosphate

Optically active D-Ins(1,4,5)P$_3$ was synthesised by using chiral 1,2:5,6-di-O-cyclohexylidene-\textit{myo}-inositol \[185,187\], which could be prepared either by conversion into the diastereomeric di-\textit{\textit{\l}}-menthoxyacetic esters or by conversion
into the diacetate or dibutyrate followed by deacylation with cholesterol esterase or porcine pancreatic lipase. The enzymes regio- and enantiospecifically remove the acyl functions, yielding mixtures of (-)-1,2:5,6-di-O-cyclohexyldiene-myoinositol and the mono- or diacylated enantiomer (depending on enzyme and substrate), which on chemical deacylation afford 1D-(+)-2,3:4,5-di-O-cyclohexyldiene-myoinositol. Tin-mediated benzylation of the 6-position, acetylation of the 1-position, PTSA-catalysed hydrolysis of the trans-acetal and saponification afforded D-2,3-O-cyclohexyldiene-6-O-benzyl-myoinositol. Phosphorylation with N,N-diisopropylamino bis(benzyloxy)phosphine and mCPBA, hydrogenolysis of the benzyl protecting groups and acid hydrolysis of the acetal gave D-Ins(1,4,5)P₃.

A short synthesis of racemic Ins(1,4,5)P₃ starting from 1,4-di-O-benzoyl-myoinositol has been reported [222]. Phosphitylation of this tetrol at the 1-, 4-, and 5-positions with dimethyl chlorophosphite (3.3 equiv.) followed by acetylation at the 2-position and oxidation of the crude reaction mixture gave predominantly (94 %) the desired 1,4,5-tris(dimethyl phosphate). Demethylation and ester hydrolysis afforded Ins(1,4,5)P₃ contaminated with about 5 % bisphosphates.

The microbial oxidation of benzene by *Pseudomonas putida* affords cis-1,2-dihydroxycyclohexa-3,5-diene 49, which has been used in a synthesis of racemic Ins(1,4,5)P₃ [255] (Figure 2.7). The cyclic carbonate 50 derived from the diene by treatment with phosgene and triethylamine was stereoselectively oxidised to the α-epoxide 51 with mCPBA. Regioselective ring opening of the epoxide with benzyl alcohol and benzylation of the free hydroxyl group afforded the 4,5-di-O-benzyl-3-ene 53. The 1,2-carbonate was removed by hydrolysis, the double bond epoxidised and the cis-1,2-diol reprotected as the isopropylidene acetal to give 54. The β-epoxide was opened with sodium-2β-propoxy-5,5-dimethyl-1,3-dioxane 55 to give 4,5-di-O-benzyl-6-O-[2-(5,5-dimethyl-1,3-dioxan-2-yl)ethyl]-2,3-O-isopropylidene-myoinositol 56. Debenzylation and phosphorylation with TBPP afforded 57. Hydrogenolysis and treatment with moist trifluoroacetic acid afforded racemic Ins(1,4,5)P₃. This method has been modified to give D- and L-Ins(1,4,5)P₃ [256] by opening the α-epoxide 51 initially obtained with (R)-(−)-sec-phenethyl alcohol 58 rather than with benzyl alcohol, affording a mixture of diastereoisomers (59 a and 59 b) which could be separated by HPLC (Figure 2.7 b). The diastereoisomers were then processed analogous to the racemate to furnish the two enantiomers of Ins(1,4,5)P₃. This
approach has also been used to give analogues of inositol and Ins(1,4,5)P₃ modified at the 6-position.

Figure 2.7 Synthesis of Ins(1,4,5)P₃ via the microbial oxidation of benzene

Benzene was also the starting material in another synthesis of racemic Ins(1,4,5)P₃ [253] (Figure 2.8). Trans-1,2-dihydroxycyclohexa-3,5-diene 60 can be prepared from benzene in 40 % overall yield via Birch reduction, bromination, trans-hydroxylation, acetylation and dehydrobromination. Protection of the hydroxyl groups as MEM ethers and [4+2] addition to singlet oxygen followed by
thiourea reduction affords 1,4-dihydroxy-2,3-di-O-methoxyethoxymethyl-cyclohexa-5-ene 61 \(^{[257]}\). Inversion at C-1 is achieved by oxidation with pyridinium chlorochromate yielding the desired 4-hydroxy-5-en-1-one 62 in 50 % yield. Reduction with sodium borohydride / cerium trichloride gave the symmetrical conduritol B derivative 63, which could also be obtained in 70 % yield by oxidation of the 1,4-diol 61 to the ene-1,4-dione and subsequent reduction. Benzylation of 63 and osmium tetroxide-catalysed cis hydroxylation of the double bond gave 3,6-di-O-benzyl-4,5-di-O-methoxyethoxymethyl-myo-inositol 64, which was regioselective methoxyethoxymethylated at the 1-position to give 65. Benzylation and demethoxyethoxymethylation furnished 2,3,6-tri-O-benzyl-myo-inositol 47. Phosphorylation with TBPP followed by hydrogenolysis gave Ins(1,4,5)P₃.

![Chemical structures](image)

Figure 2.8 Synthesis of Ins(1,4,5)P₃ from benzene via trans-1,2-dihydroxy-cyclohexa-3,5-diene
Chiral starting materials have also been employed in the synthesis of Ins(1,4,5)P₃. Commercially available (-)-quinic acid 28 was converted to ester 66 in four steps according to literature procedure. Sequential protection of the C-1 alcohol as its β-(trimethylsilyl)ethoxymethyl (SEM) ether, diisobutylaluminium hydride (DIBAL-H) reduction of the ester, and selenylation of the resulting primary alcohol furnished 67. Rearrangement of the allylic selenoxide derived from 67 and benzylation generated a single stereoisomer 68. Transformation of 68 to silyl enol ether 69 by low temperature ozonolysis and treatment with excess tert-butyldimethylsilyl (TBDMS) triflate proceeded with nearly complete regiospecificity. Hydroboration of the enol ether followed by alkaline peroxide

![Synthesis of D-Ins(1,4,5)P₃ starting from (-)-quinic acid](image)

Figure 2.9 Synthesis of D-Ins(1,4,5)P₃ starting from (-)-quinic acid
oxidation furnished the alcohol 70, which on desilylation gave the known D-2,3-
O-cyclohexyldiene-6-O-benzyl-myoinositol 71. Phosphorylation with TBPP and
hydrogenolysis afforded D-Ins(1,4,5)P₃ \[^{179}\].

Another synthesis of D- and L-Ins(1,4,5)P₃ made use of D-pinitol 27 and L-
quebrachitol 26, respectively \[^{173}\]. The naturally occurring starting materials were
demethylated to give D- and L-chiro-inositol 16 and 17, respectively. Formation
of the more stable cis,cis-dicyclohexyldiene acetal 72 (from 16), benzylation of
the two remaining hydroxyl groups and hydrolysis of the acetals followed by
selective benzoylation with 3 equivalents of benzoyl chloride gave mainly D-
1,2,5-tri-O-benzoyl-3,4-di-O-benzyl-chiro-inositol 74. The free hydroxyl group

![Diagram of the synthesis process](image)

**Figure 2.10 Synthesis of D-Ins(1,4,5)P₃ from D-pinitol**
was converted into the triflate and the triflate displaced with inversion of configuration by sodium acetate / acetic acid to give predominantly L-1,2,4-tri-O-benzoyl-5,6-di-O-benzyl-myoinositol 75 and some 1,3,4-tribenzoate due to migration of benzoyl groups. Debenzylation, phosphitylation of 76 with N,N-diisopropylamino bis(benzyloxy)phosphine, followed by oxidation with mCPBA and deprotection by hydrogenolysis gave D- and L-Ins(1,4,5)P₃, respectively.

Several other preparations of racemic [¹⁶⁹] and optically active [¹⁹¹,¹⁹⁷,²¹⁷,²¹⁹,²²⁷,²³⁰,²⁵⁸] Ins(1,4,5)P₃ have been published, including the synthesis of [³H]-labelled D- and L-Ins(1,4,5)P₃ [²⁵⁹].

**myo-Inositol 1:2-cyclic,4,5-trisphosphate**

D-Ins(1:2,4,5)P₃ has been synthesised by treatment of D-3,6-di-O-benzyl-4,5-bis(dibenzylphospho)-myo-inositol with N-methyl-pyridinium phosphodi-chloridate and subsequent deprotection [²⁵⁸]. It was also possible to obtain D-Ins(1:2,4,5)P₃ from D-Ins(1,4,5)P₃ by the action of water soluble 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide [²⁶⁰].

**myo-Inositol 2,4,5-trisphosphate**

Racemic Ins(2,4,5)P₃ has been prepared from 3,6-di-O-benzyl-4,5-di-O-allyl-myoinositol [¹⁶⁵], which was selectively benzylated at the 1-position. Deallylation to give the 2,4,5-triol, phosphorylation with TBPP and hydrogenolysis gave Ins(2,4,5)P₃ [²¹⁶,²¹⁷].

Another synthesis of Ins(2,4,5)P₃ using the benzyl-protected 2,4,5-triol of myo-inositol (derived from benzylaion of 3,6-di-O-benzyl-2,4,5-di-O-MEM-myoinositol, a by-product obtained during the introduction of the MEM protecting group into the corresponding 1,2-diol) has been described [²⁵³].

An attempted synthesis of D-Ins(2,4,5)P₃ [²⁶¹] by resolution of a suitably protected precursor with L-menthoxycetyl chloride and stepwise phosphorylation to give the fully protected precursor of D-Ins(2,4,5)P₃ was not concluded. However, the same authors [²⁵⁸] succeeded in the preparation of D-Ins(2,4,5)P₃ by phosphorylating 1,2-O-cyclohexylidene-3,6-di-O-benzyl-myoinositol with
TBPP, removal of the acetal protecting group and resolution of this intermediate via the diastereomeric ℓ-menthoxycacetic esters. Silylation of the 1-position, phosphorylation of the 2-position and hydrogenolysis afforded D-Ins(2,4,5)P₃

D- and L-Ins(2,4,5)P₃ were also prepared from L- and D-1,3,4-tri-O-benzoyl-myoinositol, respectively [174]. Partial benzoylation of the 3,4-dibenzyl ethers of D- and L-chiro-inositol gave the 1,2,5-tri-O-benzoyl-3,4-di-O-benzyl-chiro-inositols, inversion of the free hydroxyl group and cleavage of the benzyl ethers by catalytic hydrogenolysis afforded D- and L-1,3,4-tri-O-benzoyl-myoinositol.

**myo-Inositol 1,3,4-trisphosphate**

Several syntheses of racemic [217,223] and optically active [186,187,190] Ins(1,3,4)P₃ have been reported:

1,6-Di-O-para-methoxybenzyl-4,5-di-O-benzyl-myoinositol (derived from both 1,2:3,4- and 1,2:5,6-di-O-cyclohexylidene-myoinositol) was resolved either by use of a chiral column or conversion into the diastereomeric ℓ-menthoxycacetic esters. The remaining equatorial hydroxyl group was methoxymethylated and the 2-OH benzylated. Removal of methoxybenzyl and methoxymethyl protecting groups gave D-2,5,6-tri-O-benzyl-myoinositol. Phosphorylation with TBPP and hydrogenolysis afforded D-Ins(1,3,4)P₃ [190].

Optically active 1,2:5,6-di-O-cyclohexylidene-myoinositol, obtained either by an enzymatic process or by conversion into the di-ℓ-methylcarbonates [185] was used in the synthesis of D-Ins(1,3,4)P₃ [186,187]. Allylation of the free hydroxyl groups, selective removal of the *trans*-acetal, benzylation, deprotection of the *cis*-acetal, tin-mediated allylation of the 1-position, benzylation of the 2-position and removal of the allyl groups gave D-2,5,6-tri-O-benzyl-myoinositol, which was phosphorylated and hydrogenolysed to yield D-Ins(1,3,4)P₃.
2.5.4 Inositol Tetrakisphosphates

**myo-Inositol 1,3,4,5-Tetrakisphosphate**

The first synthesis of \( \text{Ins}(1,3,4,5)\text{P}_4 \) employed the 1,3,5-orthoformate ester \([160,161]\), which could be selectively allylated at the 4-position. Benzylation of the remaining hydroxyl groups and removal of allyl group and orthoformate ester gave 2,4-di-O-benzyl-\( \text{myo} \)-inositol, which was phosphorylated using sodium hydride and TBPP and then deblocked by hydrogenolysis. Similar syntheses employing benzyloxymethyl ether rather than allyl ether for protection of the 4-position \([216]\) and different phosphorylation methods \([169]\) have also been published.

An even shorter route \([148]\) involves selective benzylation of \( \text{myo} \)-inositol with 3.5 molar equivalents of benzoyl chloride in pyridine. \( \text{myo} \)-Inositol 1,3,4,5-tetrabenzoate is isolated as the major product in 34% yield. Benzylation of the free hydroxyl groups with benzyl trichloracetimidate in the presence of trifluoromethanesulfonic acid and subsequent debenzoylation affords the 2,4-protected inositol, which was phosphitylated with \( \sigma \)-xylylene diethylphosphoramidite. Oxidation with \( m \text{CPBA} \) and hydrogenolysis afforded \( \text{Ins}(1,3,4,5)\text{P}_4 \). Resolution of the tetrabenzoate was accomplished by means of chiral column chromatography, thus giving access to optically active \( \text{Ins}(1,3,4,5)\text{P}_3 \).

The same authors have described the enantioselective acylation of inositol derivatives using tartaric acid monoester \([228]\). Thus symmetrical 1,3,5-tri-O-benzoyl-\( \text{myo} \)-inositol, obtained in modest yield by direct benzylation of \( \text{myo} \)-inositol, was enantioselectively transformed into the 4-tartrate and silylated at the 2- and 6-position. Resolution of the fully protected compound followed by deacylation with ethylmagnesium bromide, phosphorylation and hydrogenolysis afforded D-\( \text{Ins}(1,3,4,5)\text{P}_3 \) \([230]\).

D-\( \text{Ins}(1,3,4,5)\text{P}_3 \) was also prepared from D-1,2:5,6-di-O-cyclohexyldene-\( \text{myo} \)-inositol \([186,187]\), which can be obtained by chemical or enzymatic means \([185]\).
Further syntheses of racemic \textsuperscript{222} and optically active \textsuperscript{159,197,262} Ins(1,3,4,5)P\textsubscript{3} have been published.

\textit{myo-Inositol 1,2,4,5-tetrakisphosphate}

Racemic Ins(1,2,4,5)P\textsubscript{4} has been prepared from 1,4-dibenzoyl-\textit{myo}-inositol by phosphitylation with dimethoxychlorophosphate followed by oxidation to the tetrakisphosphate with hydrogen peroxide. Removal of the phosphate methyl groups was effected with bromotrimethylsilane or HBr in acetic acid, and basic hydrolysis of the benzoates with lithium hydroxide gave Ins(1,2,4,5)P\textsubscript{4} as the octalithium salt.

In another method \textsuperscript{253} 3,6-di-O-benzyl-4,5-di-O-(2-methoxyethoxy-methyl)-\textit{myo}-inositol, obtained from benzene via conduritol B derivatives was employed. Cleavage of the MEM ethers afforded 3,6-di-O-benzyl-\textit{myo}-inositol, which was phosphorylated with TBPP / sodium hydride and hydrogenolysed to give Ins(1,2,4,5)P\textsubscript{4}.

\textit{myo-Inositol 1,3,4,6-tetrakisphosphate}

The \textit{meso}-compound Ins(1,3,4,6)P\textsubscript{4} was obtained by treatment of \textit{myo}-inositol with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, which regioselectively gave the symmetrical 3,4:6,1-bis(disiloxane) in 66 \% yield. Benzoylation of the free hydroxyl groups followed by acidic removal of the siloxanes produced the 1,3,4,6-tetrol, which was phosphitylated with \textit{o}-xylene diethylphosphoramidite. Oxidation and hydrogenolysis afforded Ins(1,3,4,6)P\textsubscript{4} \textsuperscript{157}.

\textit{myo-Inositol 1,4,5,6-tetrakisphosphate}

Racemic Ins(1,4,5,6)P\textsubscript{4} \textsuperscript{222} was synthesised from the 1,2-cyclohexylidene acetal of \textit{myo}-inositol by phosphitylation with dimethoxychlorophosphite followed by oxidation, removal of the methyl esters with bromotrimethylsilane and self-catalysed hydrolysis of the acetal.
3 Analogues of Inositols and Inositol Phosphates

Analogues of natural products have an established place in the array of tools the biochemist uses to investigate enzyme functions and mechanisms. Substrate analogues have for example been constructed as reversible or irreversible inhibitors, transition state analogues, suicide substrates and probes carrying reporter groups.

3.1 Phosphorothioates

3.1.1 Phosphorothioates as Phosphate Analogues

Phosphorothioates are analogues of phosphates in which a non-bridging oxygen atom has been exchanged for sulphur.

![Figure 3.1 Phosphate vs. Phosphorothioate](image)

This replacement causes only a minimal change in the geometry of the molecule (van der Waals radii 1.4 Å for oxygen and 1.9 Å for sulphur). The electronegativities of oxygen and sulphur (Pauling index) are 3.5 and 2.5, respectively, and the second dissociation constant is reduced by ca. 0.5 units after substitution of oxygen for sulphur. At physiological pH, however, there is no difference in the number of formal negative charges associated with the phosphorus-containing group. The different dissociation energies (586 kJ mol\(^{-1}\) for the P=O bond vs. 377 kJ mol\(^{-1}\) for the P=S bond) are a reflection of the decreased extent of \(\pi\)-bonding in the P=S bond \(^{263}\), which is also the cause of the lower \(^{31}\)P-NMR field resonance of phosphorothioates.

Many biologically interesting compounds contain phosphate groups. As phosphorothioates are generally more stable towards enzymatic cleavage than the corresponding phosphate (for example, AMP is hydrolysed 2000 times faster than AMPS by alkaline phosphatase \(^{264}\)), phosphorothioate
analogues of naturally occurring phosphates are potential inhibitors of phosphatase enzymes. The phosphorothioate analogues of cAMP, (Sp)- and (Rp)-cAMPS (the Sp-analogue carrying an axial and the Rp-analogue carrying an equatorial exocyclic sulphur atom), have been used to probe the cyclic nucleotide binding sites of cAMP-dependent protein kinases I and II [265], and (Rp)-cGMPS was found to antagonise cGMP-dependent protein kinase [266].

![Figure 3.2 AMP and AMPS](image)

Phosphorothioate analogues of nucleosides and nucleotides have played a major role in helping the biochemist and the molecular biologist to elucidate the properties of these molecules [235,267]. Some of these phosphorothioates are substrates for enzymes and can thus participate in metabolic reactions i.e. the synthesis of phosphorothioate containing DNA. They are also useful in determining the stereochemical course of enzymatic phosphorylation and nucleotidyl transfer reactions, as the replacement of a non-bridging oxygen by sulphur leads to chirality in a phosphodiester and so the existence of a pair of diastereoisomers [235].

During the last five years phosphorothioate analogues of inositol phosphates have also found their place in the toolbox of the biochemist. The first synthetic Ins(1,4,5)P₃ analogue was myo-inositol 1,4,5-trisphosphorothioate [Ins(1,4,5)PS₃] [236], which was shown to be recognised with high affinity by intracellular sites mediating calcium release [268,269] and a specific D-
Ins(1,4,5)P₃ binding site in the cerebellum [270]. Ins(1,4,5)PS₃ is resistant to hydrolysis by the metabolic enzyme 5-phosphatase, inhibiting the enzyme potently with a $K_i$ of 6 µM (for the racemic mixture) [271]. Because the phosphorothioate is not degraded as rapidly as Ins(1,4,5)P₃ it causes sustained calcium release from intracellular stores [272].

![Phosphorothioate Analogues of Ins(1,4,5)P₃](image)

6: $X = Y = Z = O$; myo-Inositol 1,4,5-trisphosphate
77: $X = Y = Z = S$; myo-Inositol 1,4,5-trisphosphorothioate
78: $X = Y = O; Z = S$; myo-Inositol 1,4-bisphosphate 5-phosphorothioate
79: $X = O; Y = Z = S$; myo-Inositol 1-phosphate 4,5-bisphosphorothioate
80: $X = S; Y = Z = O$; myo-Inositol 4,5-bisphosphate 1-phosphorothioate

Figure 3.3 Phosphorothioate Analogues of Ins(1,4,5)P₃

Ins(1,4,5)PS₃ has now been supplemented by Ins(1,4,5)P₃-5S 78 [209-211] and Ins(1,4,5)P₃-4,5S₂ 79 [273]. Both compounds are also 5-phosphatase resistant and very similar to Ins(1,4,5)PS₃ in calcium releasing properties.

The synthesis of phosphorothioate analogues also provides an excellent method to introduce radiolabels into inositol phosphates. Because the sulphoxidation is usually the final synthetic step before deprotection and can be effected with elementary sulphur, the handling of radioactive material can be kept to a minimum. Thus, D-[³⁵S]Ins(1,4,5)PS₃ has been synthesised and used for enzyme [274] and receptor binding studies [275], in which it was found to label two different sites, one of them being the Ins(1,4,5)P₃ receptor and the other one (which is showing only a low affinity for Ins(1,4,5)P₃) possibly being a different conformation of the receptor.

The phosphorothioate group is a good nucleophile [276] and as such reacts preferentially with soft acids such as transition metals, halogens and sp³ hybridised carbons. In contrast, phosphate groups react preferentially with hard acids such as protons, carbonyl groups and tetrahedral phosphorus.
Selective attachment of reporter groups to phosphorothioates in the presence of phosphates is thus possible by choosing appropriate reagents.

### 3.1.2 Phosphorothioate Analogues of Inositol Monophosphates

**DL-myo-Inositol 1-phosphorothioate**

Myo-Inositol 2,3,4,5,6-pentaacetate can be thiophosphorylated with thiophosphoryl chloride in acetonitrile solution containing traces of pyridine followed by hydrolysis with potassium hydroxide to form the phosphorothioate derivative. Removal of protecting groups and precipitation from solution gave the 1-phosphorothioate compound in 21% yield [247].

An improved synthesis [277,278] has been reported by a different group. Racemic 2,3,4,5,6-penta-O-benzyl-myo-inositol produced the pentakis-O-benzyl myo-inositol 1-phosphoramidite ester on reaction with N,N-diisopropylamino(2-cyanoethoxy)chlorophosphine. Treatment with 3-hydroxypropionitrile and 1H-tetrazole afforded the phosphite triester which was dissolved in pyridine and treated with sulphur to give the 1-phosphorothioate triester. Deprotection produced myo-inositol 1-phosphorothioate in 70% overall yield from the pentabenzyl-protected inositol.

**D-myo-inositol 3-phosphorothioate**

DL-1,2,4-tri-O-benzyl-5,6-O-isopropylidene-myo-inositol was resolved using camphanic acid chloride and the D-enantiomer was converted to the protected 3-phosphorothioate by phosphitylation and oxidation using sulphur in pyridine. Deblocking produced the 3-phosphorothioate [279].

**DL-myo-inositol 1:2-cyclic-phosphorothioate**

Direct thiophosphorylation of DL-1,4,5,6-tetra-O-acetyl-myo-inositol (or the respective tetrabutyryl or tetrabenzyl derivatives) with thiophosphoryl chloride in acetonitrile solution containing traces of pyridine afforded a 1:1 mixture of the endo and exo diastereoisomers of the protected cyclic thiophosphate
which could be separated by reverse phase flash chromatography (yield 10%
of each isomer). Deprotection with triethylamine / methanol and precipitation with potassium chloride produced endo and exo DL-\textit{myo}-inositol 1:2-cyclic-phosphorothioate in quantitative yield \[280\].

3.1.3 Phosphorothioate Analogues of Inositol Bisphosphates

\textbf{\textit{myo}-Inositol 1,4-bisphosphorothioate}

DL-1,2:4,5-Di-O-isopropylidene-\textit{myo}-inositol reacted with diisopropylamino-(2-cyanoethoxy) chlorophosphine forming the bisphosphoramidite. Formation of the bisphosphite triester occurred on reaction with tetrazole and 3-hydroxypropionitrile. Oxidation with sulphur in pyridine produced the protected bisphosphorothioate derivative which was deprotected and purified to yield \textit{myo}-inositol 1,4-bisphosphorothioate \[250\].

\textbf{\textit{myo}-Inositol 4,5-bisphosphorothioate}

The starting material for the preparation was 1,2,3,4-tetra-O-benzyl-\textit{myo}-inositol which was phosphitylated with diisopropylamino(2-cyanoethoxy)chlorophosphine forming the bisphosphoramidite which was then converted to the tetracyanoethoxy bisphosphite triester. Sulphoxidation with sulphur in pyridine formed the bisphosphorothioate triester. Deblocking and purification produced the target compound \[251\].

3.1.4 Phosphorothioate Analogues of Inositol Trisphosphates

\textbf{\textit{myo}-Inositol 1,4,5-trisphosphorothioate}

\textit{Myo}-Inositol 1,4,5-trisphosphorothioate \[77\] \[224,236\] was the first \textit{Ins}(1,4,5)\textit{P}_3 analogue synthesised and has proved to be a valuable instrument in the elucidation of the biochemical role of \textit{Ins}(1,4,5)\textit{P}_3. \textit{Ins}(1,4,5)\textit{P}_3 was synthesised from the protected precursor DL-1,2,4-tri-O-benzyl-\textit{myo}-inositol, which was converted to the trisphosphoramidite by reaction with diisopropylamino(2-cyanoethoxy)chlorophosphine and then to the corresponding tris[di(2-cyanoethoxy)]phosphine on reaction with tetrazole and 2-cyanoethanol. Oxidation using sulphur in pyridine to the tris[di(2-}
cyanoethoxy)phosphorothioate was followed by deprotection and purification produced the trisphosphorothioate.

A second synthesis which also uses 2,3,6-tri-O-benzyl-myoinositol has been reported [234]. Phosphitylation with bisbenzylidiisopropylaminophosphine in the presence of 1-H tetrazole in a CH₂Cl₂ - CH₃CN mixture gave the intermediate trisphosphite triester. Sulphurisation with phenacetyl disulfide rapidly produced the protected phosphorothioate which was then deblocked and purified to afford myo-inositol 1,4,5-trisphosphorothioate in 51% yield.

**myo-Inositol 1,4-bisphosphate 5-phosphorothioate**

The synthesis of the 5-phosphorothioate Ins(1,4,5)P₃-5S 78 [209,211] followed the same route as the Ins(1,4,5)P₃ synthesis reported starting with 2,3,6-tri-O-benzyl-4,5-O-isopropylidene-myoinositol which was phosphorylated using bis(2,2,2-trichloroethyl) phosphorochloridate to form the bis(2,2,2-trichloroethyl) phosphate group at the 1-position. The 4,5-isopropylidene group was removed and phosphorylation of the diol with bis(2,2,2-trichloroethyl) phosphorochloridate afforded a mixture of the 1,4- and 1,5-bisphosphate triesters. The 1,4-bisphosphate triester could be obtained from this mixture by crystallisation and was then phosphitylated at the free 5-hydroxyl group using N,N-diisopropylamino-(2-cyanoethoxy) chlorophosphine, oxidised with sulphur and deblocked as before to give the desired 5-phosphorothioate analogue.

A second synthesis [234] of 78 has been reported. A fully benzyl protected inositol 1,4-bisphosphate with a free 5-hydroxyl group was phosphorylated with bisbenzylidiisopropylaminophosphine. Sulphoxidation with phenacetyl disulphide produced the protected 5-phosphorothioate derivative. Deprotection afforded myo-inositol 1,4-bisphosphate 5-phosphorothioate in 72 % yield.

**myo-Inositol 1-phosphate 4,5-bisphosphorothioate**

2,3,6-Tri-O-benzyl-1-[bis(2,2,2-trichloroethyl)phospho]-myo-inositol (obtained from phosphorylation of 2,3,6-tri-O-benzyl-4,5-O-isopropylidene-myoinositol followed by acid hydrolysis of the 4,5-acetal) was phosphitylated to form the 4,5-bisphosphite using either bis(2-cyanoethoxy)diisopropylaminophosphine or bisbenzylidiisopropylaminophosphine. Oxidation with sulphur in pyridine
gave the respective protected 4,5-bisphosphorothioates. Deprotection and purification produced myo-inositol 1-phosphate 4,5-bisphosphorothioate in 83% yield. The 4,5-bisphosphorothioate has been used in the synthesis of the pyrophosphate, which could be obtained by desulphurisation with N-bromosuccinimide (NBS) [273].

**myo-Inositol 4,5-bisphosphate 1-phosphorothioate**

A second synthesis of this Ins(1,4,5)P₃ analogue was reported by Dreef *et al.* [234] after work described in this thesis had been published. Thus, 2,3,6-tri-O-benzyl-4,5-di-O-(trans-prop-1-enyl)-myo-inositol 81 was reacted with (bisbenzyl)(diisopropylamino)phosphine followed by sulphurisation with phenacyl disulfide to form the 1-phosphorothioate triester 82. Mild acidic hydrolysis of the 4- and 5-(prop-1-enyl) groups gave 83. Phosphitylation at the 4- and 5-positions with the phosphoramidite and oxidation of the intermediate phosphate triester with Bu'OOH afforded the fully benzyl-protected inositol 1-phosphorothioate 4,5-bisphosphate 84. Deblocking with sodium in liquid ammonia gave 80 in 74% yield.

![Figure 3.4 Synthesis of Ins(1,4,5)P₃-1S (Dreef et al.)](image-url)
3.1.5 Phosphorothioate Analogues of Inositol Tetrakisphosphates

myo-Inositol 1,3,4-trisphosphate 5-phosphorothioate

Phosphitylation of 2,6-di-O-benzyl-1,3,4-tris[(dibenoxy)phospho]-myo-inositol with bisbenzylidiisopropylaminophosphine in the presence of 1H-tetrazole was followed by *in situ* sulphurisation to produce the fully protected Ins(1,3,4,5)P$_4$-5S analogue. Removal of the benzyl protecting groups yielded the 5-phosphorothioate derivative (which acts as a competitive inhibitor of 3-kinase) in 68% yield [234].

3.1.6 Phosphorothioate Analogues of Inositol Phospholipids

Syntheses of 1,2-dipalmitoyl-sn-glycero-3-thiophospho-myoinositol (DPPsI) have been described by two groups [281-283]. In the first synthesis [281,282] 1D-2,3:5,6-di-O-cyclohexyldene-4-O-methoxy-ethyl-myoinositol was phosphitylated with CIP(OCH$_3$)N(Pr$'$)$_2$ and then converted directly to the protected 1,2-dipalmitoyl-sn-glycero-3-thiophospho-myoinositol by reaction with 1,2-dipalmitoyl-sn-glycerol and sulphoxidation with elemental sulphur. Deprotection with 80% acetic acid afforded ($R_p+S_p$)-DPPsI as a mixture of diastereoisomers.

In the second approach 1,2-dipalmitoyl-sn-glycerol was reacted with chloro-$N,N$-diisopropylamino methoxycrophosphate to produce the phosphoramidite which was then condensed with 1D-2,3,4,5,6-penta-O-benzyl-myoinositol in the presence of tetrazole. Sulphoxidation afforded a mixture of the phosphorothioate diastereoisomers which could be separated by chromatography. Deprotection produced the individual diastereoisomers of 1,2-dipalmitoyl-sn-glycero-3-thiophospho-1-myoinositol [283].

![Figure 3.5](image-url)  
(R$_p$) and (S$_p$)-1,2-dipalmitoyl-sn-glycero-3-thiophospho-myoinositol
(RP)- and (SP)-1,2-dipalmitoyl-sn-glycero-3-thiophospho-myoinositol have been used to examine the stereochemical mechanism by which phosphatidylinositol-specific PLC’s cleave phosphatidylinositol (PtdIns) [281,282]. In contrast to PtdIns itself, these phosphorothioate diesters are chiral at the phosphorus atom, thus making the determination of enzyme stereospecificity possible. It was found that the RP isomer is the preferred substrate for PtdIns-PLC and that the formation of cyclic inositol(1:2)phosphate proceeds with inversion of configuration at phosphorus, suggesting that a direct attack of the 2-OH group (or in the case of non-cyclic products of water) on phosphorus affects the displacement of the diacylglycerol moiety, without involving a covalent enzyme-phosphoinositol intermediate.

3.2 Other Phosphate Analogues

3.2.1 Phosphonates

One of the first Ins(1,4,5)P₃ analogues was DL-myoinositol 1,4,5-tris-1-H-phosphonate 85 [284], prepared by the reaction of the ammonium salt of benzyl-1-H-phosphonic acid with 2,3,6-tri-O-benzyl-myoinositol and subsequent anionic debenzylation. However, no biological activity has been reported for this compound.

The 5-methylene phosphonate analogue of Ins(1,4,5)P₃ 86 has been prepared from (-)-quinic acid [180] and shown to be a long-lived agonist of calcium mobilisation.
Figure 3.6 Phosphonate analogues of Ins(1,4,5)P$_3$

Also synthesised were the 5-methylphosphonate analogues 87 and 89 and the 5-(difluoromethyl)phosphonate analogues 88 and 90 of Ins(1,4,5)P$_3$ and Ins(1,3,4,5)P$_4$, respectively, by employing the new bifunctional phosphenylating agents $\text{bis}[6\text{-}(\text{trifluoromethyl})\text{benzotriazol-1-yl}]\text{methylphosphonate}$ (for the preparation of the methylphosphonates) and (difluoromethyl)phosphonic-di(1,2,4-triazolide) (for the preparation of the difluoromethylphosphonate analogues) [285,286]. 87 has been reported to act as a calcium antagonist in permeabilised human platelets.

The synthesis of the racemic 3-methylphosphonates analogues of Ins(3,4)P$_2$ and Ins(1,3,4)P$_3$ has been described by the same group [287].
The synthesis of racemic myo-inositol 5-methylphosphonate, myo-inositol 4,5-bis(methylphosphonate) and myo-inositol 1,4,5-tris(methylphosphonate) was accomplished using a phosphinate P(III) approach followed by oxidation [288,289], a method which avoids the formation of undesired cyclic products.

**Phosphonate analogues of inositol phospholipids** have also been synthesised [290-293]. The bifunctional phosphorylating agent bis[6-(trifluoromethyl)-benzotriazol-1-yl]methylphosphonate was employed in the synthesis of optically active phosphonate analogues of PtdIns and PtdIns(4)P [291] as well as in the preparation of the diastereomeric uncharged methylphosphonate analogues of PtdIns [292]. The phosphonate analogue of PtdIns has proved to be a potent anti-inflammatory and analgesic agent [290]. Phosphonate derivatives of PtdIns which have a single alkyl chain in place of the diacylglycerol moiety have also been synthesised [293].

![Figure 3.7 Methylene phosphonate analogues of PtdIns and PtdIns(4)P](image)

**3.2.2 Sulphates, Sulphonamides and Carboxylates**

The preparation of racemic sulphonamide, sulphate and carboxymethyl analogues of Ins(1,4,5)P₃ has been described [289,294]. However, none of these compounds showed any affinity for the Ins(1,4,5)P₃ receptor.

![Figure 3.8 The trissulphate, trissulphonamide and triscarboxylate analogues of Ins(1,4,5)P₃](image)
3.3 Fluorinated Analogues

Of the 10 million compounds that had been registered in Chemical Abstracts by February 1990, 6.2% possess a carbon-fluorine bond. Every year more than 50000 novel organofluorine compounds are synthesised, the number of papers published in this area of research has reached 5000 per annum \[^{295}\]. These numbers show the immense interest that organofluorine compounds attract. A number of reviews on the subject have appeared \[^{295-298}\], including one on fluorinated carbohydrates \[^{296}\].

What is so special about fluorine? Fluorine has steric requirements very similar to hydrogen (van der Waals radius of fluorine 1.35 Å, hydrogen 1.20 Å). Additionally, there are similarities in bond length and polarisation between C-F and C-OH (bond length for the carbon-fluorine bond 1.39 Å, for the carbon-oxygen bond 1.43 Å, electronegativity of fluorine 4 vs. 3.5 for oxygen). By replacing hydrogen or hydroxyl with fluorine, however, the chemical properties of a compound can be dramatically changed. Once introduced, the high carbon-fluorine bond energy (489 kJ / mol vs. 413 kJ / mol for a C-H bond and 358 kJ / mol for a C-O bond) renders the substituent relatively resistant to metabolic transformations. These features can be exploited by the biochemist looking for analogues of naturally occurring compounds which may act as inhibitors of metabolic enzymes ('antimetabolites'). As the overall steric ‘appearance’ of the molecule has only been moderately changed, the fluoro-analogue will still fit into the active site of the enzyme, but it will not be processed in the same way as the original metabolite.

3.3.1 Fluorinated Analogues of Natural Products

A good illustration for this mode of action is given by fluoroacetate. When cattle feed on the leaves of Dichapetalum cymosum, a poisonous African plant, the citrate cycle is blocked. Within an hour the citrate levels in many organs are raised more than 10 fold. The cattle develop spasms and die shortly afterwards. The substance in the leaves causing the poisoning is fluoroacetate. Fluoroacetate, once ingested, is activated as fluoroacetyl-coenzyme A and then condensed with oxaloacetate to give fluorocitrate, a potent inhibitor of the enzyme aconitase. The active centre of aconitase contains Fe\(^{2+}\) as coenzyme which forms a chelate complex with the hydroxyl oxygen and the two carboxylate oxygens of citrate prior to the dehydration
step producing cis-aconitate. If the substrate is fluorocitrate, however, the fluorine atom replaces one of the carboxylate oxygens in the chelate complex with the Fe²⁺ ion. The high electronegativity of fluorine results in a strong interaction with the Fe²⁺, causing irreversible inhibition of the enzyme and blocking further steps in the metabolic pathway. In Australia thousands of cattle are lost every year due to the toxic effects of fluoroacetate even though it rarely exceeds 1% dry weight of the leaves or seeds of certain plants which the cattle feed on.

![Chemical structures](image)

**Figure 3.9** Fluoroacetate is metabolised to fluorocitrate

The same principle was applied in cancer chemotherapy with the development of 5-fluorouracil [299,300]. As cancer cells proliferate faster than ordinary cells, they also require more deoxythymidine monophosphate (dTMP) for DNA synthesis. The metabolic precursor of dTMP is deoxyuridine monophosphate (dUMP), the transformation (methylation of the 5-position of uracil) is catalysed by the enzyme thymidylate synthetase. *In vivo*, 5-fluorouracil is metabolised to 5-fluoro-2’-deoxyuridine-5’-monophosphate (F-dUMP), which irreversibly inhibits thymidylate synthetase. F-dUMP behaves like a normal substrate in the first step (formation of a covalent bond between C-6 and a sulphhydryl group of the enzyme) and the second step (addition of methylene-tetrahydrofolate - the donor of the C₁ unit required for methylation - to the 5-position) of the catalytic cycle. The next step (hydride-transfer to the methylene group and cleavage of the C-5 proton), however, is not possible with F-dUMP, where the C-5 proton has been replaced by fluorine.
Thus, the catalytic process is blocked and the enzyme inactivated as it remains covalently bound to its substrate.

Figure 3.10 Inhibition of Thymidylate Synthetase by F-dUMP

The effects of this inhibition are drastic. With thymidine no longer available, the DNA replication is stopped and with it the growth of any tumor. Unfortunately, the replacement of normal short-lived cells (e.g. leukocytes) is also affected by 5-fluorouracil, so that side effects common in cancer patients
undergoing chemotherapy (weakening of the immune system, loss of hair) cannot be avoided.

3.3.2 The Synthesis of Fluorinated Analogues of Carbohydrates

The synthesis of fluoro-analogues of carbohydrates and inositols is not unproblematic. In order to set up the desired hydroxyl group for the introduction of fluoride, protection of other hydroxyl functions in the molecule is necessary. The fluoride ion has only a low nucleophilicity, making substitution on a secondary carbon difficult unless good leaving groups like trifluoromethanesulphonates (triflates) are attached to the respective carbon atom. Another problem may arise from competing elimination reactions (which are catalysed by fluoride ion). It is therefore recommended to employ anhydrous fluoride salts with large counterions such as tetrabutylammonium (TBA+), tris(diethylamino)sulphur (TAS+) or Cs+ and use solvents which do not strongly solvate the fluoride ion (rendering the fluoride ion "naked") so as to maximise fluoride ion nucleophilicity, minimise elimination reactions and effect substitution under the mildest possible conditions.

In the synthesis of fluoro-analogues of inositol and inositol phosphates, diethylaminosulphur trifluoride \([\text{(C}_2\text{H}_5\text{)}_2\text{NSF}_3, \text{DAST})\] has been widely employed. DAST has the advantage of allowing direct replacement of a hydroxyl group by fluorine without prior activation of the hydroxyl function as a sulphonate. DAST is relatively mild, can be used on acid-sensitive substrates, and in the absence of neighbouring group participation, affords products resulting from Walden inversion. With some substrates DAST reacts regioselectively, so that protection of all the hydroxyl groups may not be required. Additionally, DAST can be employed to generate gem-difluoro compounds by reaction with inososes \([302-307]\).

3.3.3 Fluorinated Analogues of Inositol

Pharmacologically active analogues of inositol phosphates have to be injected into intact cells in order to be effective, since these highly charged molecules are not able to cross the lipid bi-layer of the plasma membrane.
The cellular inositol uptake system however, provides an alternative way of manipulating the In(1,4,5)P₃ signalling system. Analogues of \textit{myo}-inositol, which serve as substrates for PtdIns synthetase, may be incorporated into the cellular phosholipid and inhibit the formation of In(1,4,5)P₃ or subsequent metabolic processes. Fluorinated inositol analogues are particularly suitable for this purpose because of the reasons mentioned above.

1D- and 1L-1-Deoxy-1-fluoro-myoinositol

Different starting materials have been employed in the syntheses of 1D-1-deoxy-1-fluoro-myoinositol. Kozikowski \textit{et al.} \cite{178} were able to prepare the compound from optically active D-pinitol (3-O-methyl-D-chiro-inositol) in five steps by first converting pinitol into the 1,2:5,6-di-0-isopropylidene derivative (which is readily separable from the 1,2:4,5-diacetal also formed in the reaction). Methylation of the free 4-hydroxyl group, cleavage of the acetals and treatment of the 3,4-di-O-methyl-D-chiro-inositol with DAST gave selectively 1D-1-deoxy-1-fluoro-4,5-di-0-methyl-myoinositol with inversion of configuration on the fluorination site. Demethylation of this compound with BBr₃ afforded 1D-1-deoxy-1-fluoro-myoinositol.

The same group had earlier reported a 10-step synthesis of this fluorinated isostere from \textit{myo}-inositol \cite{170,308}, which involved fluorination at the 2-position of a suitably protected \textit{myo}-inositol derivative and the optical resolution of the resulting protected fluoro-scyllo-inositol. Recovery of the \textit{myo}-inositol configuration in the two enantiomers was achieved by Swern oxidation of the deprotected 1-hydroxyl group to give the 1-inosose followed by reduction of the keto-funtion with L-Selectride, which gave an axial alcohol (thus becoming the 2-position in the \textit{myo}-inositol configuration) and afforded 1D- and 1L-1-deoxy-1-fluoro-myoinositol after deprotection of the respective enantiomeric intermediates.

A similar synthesis also starting from \textit{myo}-inositol has been reported by Offer \textit{et al.} \cite{309,310}. Here, the problems of recovery of the \textit{myo}-inositol configuration and optical resolution were addressed efficiently in a single conversion, \textit{i.e.} a tosylate group in a fluorinated scyllo-inositol intermediate was displaced with S-(−)-caesium camphanate in 90% yield. The two resulting diastereoisomers were separated, deprotected and purified to produce the D- and L-1-fluoro analogues of \textit{myo}-inositol.

An efficient synthesis of 1L-1-deoxy-1-fluoro-myoinositol (= 1D-3-deoxy-3-fluoro-myoinositol) in two steps was possible by direct fluorination of L-
quebrachitol (L-3-O-methyl-\textit{chiro}-inositol). Treatment with DAST resulted in selective fluorination of the 1-position (which proceeded with inversion to give the \textit{myo}-inositol configuration) and subsequent demethylation with boron tribromide afforded the fluoro-inositol analogue \cite{170}. This compound is capable of entering into the PtdIns cycle and block the eventual production of both ln(1,3,4,5)P\textsubscript{4} and ln(1,3,4)P\textsubscript{3}, since the 3-hydroxyl group is missing in this compound and phosphorylation at this position is therefore no longer possible. 1L-1-deoxy-1-fluoro-\textit{myo}-inositol was found to inhibit growth of NIH 3T3 cells even in the presence of \textit{myo}-inositol \cite{178}.

3-Deoxy-3-fluoro-\textit{myo}-inositol inhibits cell growth in the presence of \textit{myo}-inositol on NIH 3T3 cells \cite{178}, although the medium used contained relatively high levels of \textit{myo}-inositol compared to other growth media. 3-Deoxy-3-fluoro-\textit{myo}-inositol competitively blocks the uptake of \textit{myo}-inositol which suggests that the 3-fluoro derivative acts as a substrate for the PtdIns synthase enzyme incorporating \textit{myo}-inositol into the cell phospholipid and so the 3-hydroxyl group is not required for activity but it is required for the action of the 3-kinase on ln(1,4,5)P\textsubscript{3} \cite{178}. The 1-deoxy-1-fluoro and the 1-deoxy-1,1-difluoro compounds also inhibited cell growth but not as potently.

1-Deoxy-1-fluoro-sc\textit{y}//lo-inositol

1-Deoxy-1-fluoro-sc\textit{y}//lo-inositol was the first fluorinated analogue of inositol prepared \cite{311}. DL-1-O-Benzoyl-3,4,5,6-tetra-O-benzyl-\textit{myo}-inositol was reacted with DAST under rather drastic conditions (toluene, 70-80°C; attempted fluorination under conventional conditions - dichloromethane, 0-25°C - did not succeed) to give the protected fluorinated sc\textit{y}//lo-inositol derivative, which was then deprotected by mild hydrolysis followed by hydrogenolysis to give the title compound.

1-Deoxy-1-fluoro-sc\textit{y}//lo-inositol has also been synthesised by a method which allowed the incorporation of radioactive tritium through the stereoselective reduction of the intermediate 2,3,4,5,6-penta-O-benzyl-sc\textit{y}//lo-inosose with sodium borotritide which produced mainly 1,3,4,5,6-penta-O-benzyl-\textit{myo}-[2-\textsuperscript{3}H]-inositol. Reaction of the tritium labelled inositol with DAST and deprotection gave 1-deoxy-1-fluoro-sc\textit{y}//lo-[1-\textsuperscript{3}H]-inositol \cite{312}. The synthesis of 1-deoxy-1-fluoro-sc\textit{y}//lo-inositol has also been reported by another group \cite{307}.  

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1D-1,5-Dideoxy-1,5-difluoro-neo-inositol

D-Pinitol reacted regioselectively with DAST to produce the difluoro compound 13 in 47% yield. Demethylation with boron tribromide then furnished 1D-1,5-dideoxy-1,5-difluoro-neo-inositol [178].

2-Deoxy-2-fluoro-myoinositol

A synthesis of [3H]-labelled and unlabelled 2-deoxy-2-fluoro-myoinositol has been reported by Lowe and McPhee [312]. Reaction with DAST usually occurs with inversion of configuration so in order to produce the 2-fluoro-myoinositol derivative from 1,3,4,5,6-penta-O-benzyl-myoinositol it was necessary to change the configuration at C-2 and convert the protected myo-inositol into 1,2,3,4,5-penta-O-benzyl-scyllo-inositol before fluorination. The inversion was effected by esterification of the free hydroxyl group with trifluoromethylsulphonic anhydride (triflic anhydride) and displacement of the triflate moiety with sodium trifluoroacetate. Base catalysed hydrolysis of the trifluoroacetate and reaction with DAST produced 1,3,4,5,6-penta-O-benzyl-2-deoxy-2-fluoro-myoinositol, and deprotection with dry HBr gave 2-deoxy-2-fluoro-myoinositol in 51% yield.

Oxidation of 1,3,4,5,6-penta-O-benzyl-myoinositol with Jones’ reagent (sodium dichromate / sulphuric acid) and reduction of the inosose with NaBH₃T followed by treatment with DAST gave some 1,3,4,5,6-penta-O-benzyl-2-deoxy-2-fluoro-myoinositol-[2-3H]-inositol (with predominant formation of the scyllo-inositol analogue, see above). This compound was then deprotected as before to yield 2-deoxy-2-fluoro-myoinositol-[2-3H]-inositol.

A further synthesis has been reported for 2-deoxy-2-fluoro-neo-inositol although the yield reported was low [313].

2-Deoxy-2,2-difluoro-myoinositol

1-O-Allyl-3,6-di-O-benzyl-4,5-O-isopropylidene-myoinositol was oxidised to the 2-inosose. The ketone was fluorinated using DAST in dry dichloromethane at room temperature to form the gem-difluoro derivative. Deprotection was carried out by first removing the allyl and isopropylidene groups and then hydrogenolysis of the benzyl groups to give 2-deoxy-2,2-difluoro-myoinositol compound [307].
2-C-Fluoromethyl-\textit{myo}-inositol

DL-1-O-Benzoyl-2-oxiranyl-3,4,5,6-tetra-O-benzyl-\textit{myo}-inositol was converted into DL-2-C-fluoromethyl-3,4,5,6-tetra-O-benzyl-\textit{myo}-inositol either by direct fluorination at the side chain with potassium hydrogen difluoride / 18-crown-6 via epoxide ring opening (66 % yield) and subsequent removal of the benzoate ester with lithium aluminium hydride (51 % yield), or by saponification and fluorination of the 1-hydroxyl derivative (64 % overall yield). Hydrogenolysis afforded the title compound in 60 % yield [314].

4-Deoxy-4-fluoro-\textit{myo}-inositol

3-O-Benzyl-1,2:5,6-di-O-cyclohexylidene-\textit{myo}-inositol reacted with DAST to give a mixture of the corresponding 4-fluoro-inositol derivatives in yields of 31 % (for the retention product) and 8.8 % (for the product obtained by inversion at C-4), respectively. The fact that this fluorination reaction proceeds predominantly with retention of configuration is probably due to steric crowding by the substituents in the 1,2, 3, and 5,6-positions which renders the usually occurring backside attack of F\textsuperscript{-} more difficult and therefore disfavours the product of inversion. Deprotection of the major product furnished racemic 4-deoxy-4-fluoro-\textit{myo}-inositol in 87% yield. Attempted deprotection of the minor \textit{epi}-inositol product led to decomposition products [313].

5-Deoxy-5-fluoro-\textit{myo}-inositol

Several methods of synthesis of 5-deoxy-5-fluoro-\textit{myo}-inositol have been published. The starting material for the first method [303] was 1,4,6-tri-O-benzyl-2,3-O-cyclohexylidene-\textit{neo}-inositol. Reaction with DAST and DMAP in toluene produced the 5-fluoro-\textit{myo}-inositol derivative due to inversion at C-5 in 61% yield followed by deprotection which produced the 5-deoxy-5-fluoro-\textit{myo}-inositol.

6-O-Benzyl-1,2:3,4-di-O-cyclohexylidene-\textit{myo}-inositol was employed as starting material for the preparation of 5-deoxy-5-fluoro-\textit{myo}-inositol by two different groups. Fluorination of this compound with DAST gave a mixture of the products of inversion (35 %) and retention (14.5 %), the latter was hydrogenated to afford the deprotected 5-fluoro-\textit{myo}-inositol [313].
The low yield obtained by this direct method was improved upon by inverting the 5-position prior to the DAST fluorination, which would then re-establish the myo-inositol configuration. This was accomplished by tosylation of the 5-hydroxyl group followed by nucleophilic displacement of the toluene sulphonate with caesium propionate in DMF to afford the neo-inositol derivative. The 5-hydroxyl function was recovered by base hydrolysis of the ester. Treatment with DAST gave the fluorinated derivative with the required conformation. Deprotection afforded the title compound in 73% yield from 6-O-benzyl-1,2:3,4-di-O-cyclohexylidene-myoinositol

5-Deoxy-5,5-difluoro-myoinositol

Oxidation of 1,4,6-tri-O-benzyl-2,3-O-cyclohexylidene-myoinositol with chromium trioxide-dipyridine complex gave the corresponding 5-inosose in good yield. Fluorination was accomplished with DAST (51% yield) to give the gem-5,5-difluoro derivative which was then deprotected to give the title compound in 83% yield.

A number of 2-, 3-, 4-, and 5-modified inositol analogues have been evaluated as substrates and inhibitors of PtdIns synthetase. It was found that the enzyme has very stringent requirements for the cyclitol substrate. All compounds tested (including deoxy-, fluoro-, chloro-, bromo-, iodo- and amino-analogues) were less effective than Ins(1,4,5)P_3 as substrates for PtdIns synthetase, with 5-deoxy-5-fluoro-myoinositol, the best analogue found in this study, being incorporated into the cellular phospholipid at a rate equal to 26 % of that of myo-inositol. This analogue was then further phosphorylated to PtdIns(4)P but not to PtdIns(4,5)P_2 as the compound lacks the 5-hydroxyl group necessary for phosphorylation. Replacement of the 5-hydroxyl group with other halogens produced very poor or inactive substrates, however the 5-deoxy analogue was also found to be well recognised. In the inhibition assay, the rank order of analogues was similar to their effectiveness as substrates, with the exception of 5-deoxy-5,5-difluoro-myoinositol which was inactive as a substrate for PtdIns synthase but an effective inhibitor of the enzyme.
3.3.4 Fluorine Analogues of Inositol Phosphates

2-Deoxy-2-fluoro-scyllo-inositol 1,4,5-trisphosphate

Reaction of 1-O-allyl-3,6-di-O-benzyl-4,5-O-isopropyldene-myo-inositol 93 (Figure 3.11) with DAST in dry dichloromethane at 0°C resulted in fluorination at the 2 position with inversion of configuration. Subsequent deprotections of 95 to 3,6-di-O-benzyl-2-deoxy-2-fluoro-scyllo-inositol 99 and phosphorylation with TBPP produced the fully protected fluorinated analogue 101, which was then deprotected by catalytic hydrogenolysis to give 2-deoxy-2-fluoro-scyllo-inositol 1,4,5-trisphosphate [2-F-scyllo-Ins(1,4,5)P₃] 103 [306].

Figure 3.11 Synthesis of 2-F-scyllo-Ins(1,4,5)P₃ and 2,2-F₂-Ins(1,4,5)P₃
Sawyer and Potter reported the synthesis of 103 by a similar method [307].

Reduction of the 2-inosose 94 with sodium borohydride resulted in the formation of a 2:1 mixture of myo- and scy/lo-inositol derivatives respectively, which could be separated by chromatography [168]. When the scy/lo-epimer was treated with DAST, fluorination surprisingly occurred with retention of configuration yielding the same derivative which was obtained from the myo-epimer.

2-Deoxy-2,2-difluoro-my/o-inositol 1,4,5-trisphosphate

DL-2-deoxy-2,2-difluoro-Ins(1,4,5)P$_3$ [2,2-F$_2$-Ins(1,4,5)P$_3$] 104 was synthesised from the same precursor used in the synthesis of 2-deoxy-2-fluoro-scy/lo-Ins(1,4,5)P$_3$ [306]. 93 was oxidised to the 2-inosose 94 in 66% yield. The ketone was fluorinated using an excess of DAST in dichloromethane at 25°C to give the difluoro compound 96 in 60% yield. The same sequence of deprotection and phosphorylation reactions as in the synthesis of 2F-scy/lo-Ins(1,4,5)P$_3$ produced the desired difluoro compound 104.

Racemic 2F$_2$-Ins(1,4,5)P$_3$ was also obtained by Sawyer and Potter using slightly different deprotection and phosphorylation procedures [305,307]. They succeeded in the optical resolution of 2-deoxy-2,2-difluoro-3,6-di-O-benzyl-4,5-isopropylidene-my/o-inositol by chromatographic separation of the 1-O-[(-)-o-camphanyl] esters of this compound and were thus able to prepare D- and L-2,2F$_2$-Ins(1,4,5)P$_3$ [304,316].

2-Deoxy-2-fluoro-scy/lo-Ins(1,4,5)P$_3$ and 2-deoxy-2,2-difluoro-my/o-Ins(1,4,5)P$_3$ act as full agonists for the release of Ca$^{2+}$ with only slightly lower affinity for the receptor than Ins(1,4,5)P$_3$ [305]. Thus it has been proposed that the unique 2 axial hydroxyl group is relatively unimportant for activity at the receptor. Both fluoro analogues are weaker substrates for the Ins(1,4,5)P$_3$ 3-kinase, with the difluoro compound better than the mono-fluoro compound, indicating that the 2-hydroxyl group is important for recognition by this enzyme although not essential. The mono-fluoro compound was a weak substrate for Ins(1,4,5)P$_3$ 5-phosphatase whilst the difluoro analogue was not hydrolysed by the enzyme but inhibited its activity potently [305]. The difluoro compound was then resolved into the enantiomers [307] and it was found that
D-2,2-F2-lns(1,4,5)P$_3$ was a substrate for 5-phosphatase, whereas the L-enantiomer was an effective inhibitor of both 5-phosphatase and 3-kinase [304,316].

**1D-3-Deoxy-3-fluoro-myсо-inositol 1,4,5-trisphosphate**

L-Quebrachitol was converted in two steps into D-3-deoxy-3-fluoro-myсо-inositol as described above. Subsequent protection and phosphorylation at the hydroxyl groups gave the 3-fluorodeoxy analogue of Ins(1,4,5)P$_3$ [317]. 3-Deoxy-3-fluoro-Ins(1,4,5)P$_3$ acts as a full agonist in releasing Ca$^{2+}$ from NIH 3T3 cells and is equipotent to Ins(1,4,5)P$_3$. This shows the equatorial 3-hydroxyl group is not required for receptor recognition or activity. This compound promises to be valuable for studying the release of Ca$^{2+}$ from the Ins(1,4,5)P$_3$ sensitive pools without interference from the Ins(1,3,4,5)P$_4$ regulated Ca$^{2+}$, as it will prevent the 3-kinase phosphorylating Ins(1,4,5)P$_3$ to Ins(1,3,4,5)P$_4$ [317].

**2-Deoxy-2-fluoro-scyllо-inositol 1,3,4-trisphosphate**

Reaction of 1,3,4-tri-O-allyl-5,6-di-O-benzyl-myсо-inositol with DAST resulted in monofluorination with inversion of configuration to give 2-deoxy-2-fluoro-1,3,4-tri-O-allyl-5,6-di-O-benzyl-scyllо-inositol. Removal of the allyl protecting groups with Pd on carbon followed by phosphorylation with TBPP and removal of the benzyl protecting groups by catalytic hydrogenolysis afforded 2-deoxy-2-fluoro-scyllо-inositol 1,3,4-trisphosphate [318].

**2-Deoxy-2,2-difluoro-myсо-inositol 1,3,4-phosphate**

1,3,4-Tri-O-allyl-5,6-di-O-benzyl-myсо-inositol was also used to prepare 2-deoxy-2,2-difluoro-myсо-inositol 1,3,4-trisphosphate [318]. Oxidation of this compound with DMSO / acetic anhydride furnished the 2-inosose, which reacted with two equivalents of DAST to give the 2,2-difluoro compound. Deprotection and phosphorylation as before produced 2-deoxy-2,2-difluoro-myсо-inositol 1,3,4-phosphate.
3.4.4 Fluorinated analogues of inositol lipids

The first synthesis of a fluoro-analogue of an inositol lipid was described as early as 1982. 2-Deoxy-2-fluoro-1-phosphatidyl-scylo-inositol was obtained by condensation of 2-fluoro-2-deoxy-3,4,5,6-tetra-O-benzyl-scylo-inositol with the sodium salt of dipalmitoyl-L-α-phosphatidic acid, followed by hydrogenolysis.

Recently, the preparation of D-3-deoxy-3-fluoro-1-phosphatidyl-mylo-inositol has been reported. This PtdIns analogue displayed cytostatic properties, inhibiting cell growth of NIH 3T3 cells at concentrations of ca. 100 μM.

![D-3-Deoxy-3-fluoro-phosphatidylinositol](image)

D-3-Deoxy-3-fluoro-phosphatidylinositol
4. Structure-Activity Studies on Ins(1,4,5)P₃ Analogues

4.1 Structural Requirements for Calcium Release

The Ins(1,4,5)P₃ receptor shows great stereo- and regiospecificity and structural requirements for Ins(1,4,5)P₃ analogues, in order to effect Ca²⁺-release, are quite strict.

Whereas Ins(1:2,4,5)P₃ > Ins(4,5)P₂ and GroPIns(4,5)P₂ have been shown to be 13-15 fold [320,321], 650 fold [322] and 3-10 fold [321,323] weaker than Ins(1,4,5)P₃, respectively, most other natural inositol phosphates including Ins(1)P, Ins(1:2)P, Ins(1,4)P₂ [221, Ins(1,3,4)P₃ [269,324] and InsP₆ (phytic acid) [269] are ineffective as agonists at the Ins(1,4,5)P₃ receptor.

The stereospecificity of the receptor is demonstrated by the fact that L-Ins(1,4,5)P₃, [ = D-Ins(3,5,6)P₃] is unable to mobilise calcium [269,326]. Receptor-binding of this synthetic enantiomer is ca. 2000 times weaker than that of natural D-Ins(1,4,5)P₃ [324,327].

Modifications at the 1-position

A number of semisynthetic 1-substituted Ins(1,4,5)P₃ analogues [323], able to release Ca²⁺, showed that large groups can be introduced at this position without major loss of activity.

Similarly, the 1-O-(3-aminopropyl) ester of Ins(1,4,5)P₃ and a photoaffinity analogue derived therefrom [328] were able to effect Ca²⁺ release, both being ca. 8-fold weaker than Ins(1,4,5)P₃. Receptor binding of these derivatives was equally good, and the photoaffinity derivatives were successfully used to label Ins(1,4,5)P₃ binding sites [329].

Modifications at the 2-position

Similar to the 1-position, the introduction of large groups is tolerated at the axial 2-position (Figure 4.1) without affecting the ability of the analogue to act as a full agonist at the Ins(1,4,5)P₃ receptor [320]. Removal of the hydroxyl group [in 2-
deoxy-Ins(1,4,5)P$_3$] resulted in only a slight loss of activity [EC$_{50}$ of racemic 2-deoxy-Ins(1,4,5)P$_3$ 0.5 µM, D-Ins(1,4,5)P$_3$ 0.2 µM] [320].

Figure 4.1  Ins(1,4,5)P$_3$ analogues modified at the 2-position

Recently, DL-Ins(1,2,4,5)P$_4$ has been pharmacologically evaluated [330]. This synthetic Ins(1,4,5)P$_3$ analogue with an additional phosphate group in the 2-position is the most potent inositol tetrakisphosphate described to date [EC$_{50}$ = 165 nM, compared with EC$_{50}$ = 52 nM for Ins(1,4,5)P$_3$]. The stereospecificity of the Ins(1,4,5)P$_3$ receptor was confirmed by the different agonist properties of the enantiomers D- and L-2-deoxy-2,2-difluoro-Ins(1,4,5)P$_3$ [304,316]. D-2-deoxy-2,2-difluoro-Ins(1,4,5)P$_3$ was a full agonist, slightly less potent than Ins(1,4,5)P$_3$ in releasing Ca$^{2+}$ from SH-SY5Y neuroblastoma cells [EC$_{50}$ 0.21 µM in comparison to an EC$_{50}$ of 0.13 µM for D-Ins(1,4,5)P$_3$], whereas the L-enantiomer was only a very poor agonist (EC$_{50}$ 53 µM).

DL-2-deoxy-2-fluoro-scy/eo-Ins(1,4,5)P$_3$ was also a full agonist, somewhat less potent than D-Ins(1,4,5)P$_3$ with EC$_{50}$ 0.77 [305,331]. The observation that 2,2-F$_2$-Ins(1,4,5)P$_3$ was a more potent analogue than 2-F-scy/eo-Ins(1,4,5)P$_3$ has led the authors to conclude that the axial fluorine atom in the first compound is able to make a more favorable interaction with the receptor protein than the axial hydrogen in the 2-position of the scyllo-analogue. This would imply that the 2-hydroxyl group of Ins(1,4,5)P$_3$ accepts rather than donates a hydrogen bond from the receptor: the less active 2-F-scy/eo-Ins(1,4,5)P$_3$ is still able to donate but not to accept a hydrogen bond, whereas the axial fluorine of the more potent 2,2-F$_2$-Ins(1,4,5)P$_3$ can still accept hydrogen bonds, but no longer donate them.
These data appear to indicate that the 2-position is relatively unimportant for receptor recognition.

** Modifications at the 3-position **

Both D-3-deoxy-Ins(1,4,5)P$_3$ [176] and D-3-deoxy-3-fluoro-Ins(1,4,5)P$_3$ [317] were shown to mobilise Ca$^{2+}$ from permeabilised 3T3 cells as full agonists. These two compounds were almost equipotent to Ins(1,4,5)P$_3$.

L-chiro-Ins(2,3,5)P$_3$ [177,332], an Ins(1,4,5)P$_3$ analogue with an axial rather than equatorial 3-hydroxyl group, was found to be a potent agonist for the mobilisation of sequestered calcium from permeabilised human neuroblastoma cells with an EC$_{50}$ only some 5 to 10-fold higher than Ins(1,4,5)P$_3$ [331,333]. The synthesis of racemic 6-deoxy-6-fluoro-chiro-Ins(2,3,5)P$_3$ has been reported [334], the pharmacological properties of this compound, however, are still due to be published.

Ins(1,3,4,5)P$_4$, the product of 3-kinase action on Ins(1,4,5)P$_3$, has been shown to inhibit [$^3$H]-Ins(1,4,5)P$_3$ binding to cerebellar membranes. The tetrakisphosphate has, however, a ca. 60 fold lower affinity to the receptor than Ins(1,4,5)P$_3$ [335]. A similar decrease in potency has been observed in the ability to release calcium [324].
These results show that the 3-hydroxyl group plays a relatively insignificant role in receptor binding and calcium release. An additional phosphate group in this position, however, reduces receptor binding properties considerably.

**Modifications at the 4-position**

No Ins(1,4,5)P$_3$ analogues selectively modified at the 4-position have yet been studied. The phosphate group in the 4-position appears, however, to be essential for recognition by the Ins(1,4,5)P$_3$ receptor, since all inositol phosphates lacking this moiety [e.g. Ins(1,3,5)P$_3$] are inactive.

**Modifications at the 5-position**

D-**myo**-inositol 1,4-bisphosphate 5-phosphorothioate [Ins(1,4,5)P$_3$-5S] [210,211] was found to be a full agonist at the Ins(1,4,5)P$_3$ receptor and a potent mobiliser of sequestered Ca$^{2+}$ from permeabilised SH-SY5Y neuroblastoma cell, being only some 7-fold less potent than Ins(1,4,5)P$_3$ [EC$_{50}$ values Ins(1,4,5)P$_3$ 0.11 µM, Ins(1,4,5)P$_3$-5S 0.8 µM]. Similar to the trisphosphorothioate analogue Ins(1,4,5)PS$_3$, Ins(1,4,5)P$_3$-5S is not dephosphorylated by the 5-phosphatase and is therefore able to evoke a sustained release of Ca$^{2+}$. However, Ins(1,4,5)P$_3$-5S is a substrate for 3-kinase unlike Ins(1,4,5)PS$_3$.

Only preliminary evaluations have been carried out on the 5-methylenephosphonate analogue of Ins(1,4,5)P$_3$, but these initial studies showed that this compound releases calcium from bovine adrenal gland microsomes in a sustained fashion similar to other non-hydrolysable analogues of Ins(1,4,5)P$_3$ [180].

The racemic 5-methylphosphonate analogue of Ins(1,4,5)P$_3$ has been reported to antagonise Ins(1,4,5)P$_3$-stimulated calcium release in permeabilised human platelets in a pH-dependent manner, and to act as a competitive inhibitor of [3H]-Ins(1,4,5)P$_3$ binding to bovine adrenocortical microsomes [285,286]. Full biological data for this compound and the 5-difluoromethylphosphonate analogue, which is also supposed to act as a weak antagonist [285,286], are still to be published and are awaited with interest, as this would represent the first small molecule antagonist of Ins(1,4,5)P$_3$ action.
Modifications at the 6-position

D-6-Deoxy-Ins(1,4,5)P₃ was found to be a full agonist for calcium release in permeabilised SH-SY5Y human neuroblastoma cells some 70-fold less potent than Ins(1,4,5)P₃ (EC₅₀ 6.4 μM) [182].

DL-Ins(1,4,5,6)P₄ exhibited no Ca²⁺ releasing activity and did not appear to influence the Ins(1,4,5)P₃ mediated calcium release at concentrations up to 10 μM [94].

Other 6-modified Ins(1,4,5)P₃ analogues have been synthesised by Ley et al., including the 6-deoxy-6-fluoro-, the 6-deoxy-6-methyl-, and the 6-O-methyl-derivative [255,256,336,337]. No biological data of these compounds have been reported by this group to date. DL-6-O-methyl-Ins(1,4,5)P₃ was, however, evaluated by another group [324] and found to mobilise calcium with an EC₅₀ of 65 μM [Ins(1,4,5)P₃ 0.3 μM].

Multiple Modifications

The unnatural inositol phosphates Ins(2)P [338], Ins(2,4)P₂, Ins(1,2,6)P₃ [339], Ins(1,3,5)P₃ [324] and 3-deoxy-Ins(1,5,6)P₃ [176] are all unable to release calcium, as is myo-inositol.

D-myo-Inositol 1,2-cyclic 4,5-trisphosphate [Ins(1:2,4,5)P₃] was initially reported as being equipotent to Ins(1,4,5)P₃ in mobilising calcium [340,341], and there were speculations that this cyclic inositol phosphate may be a second messenger in its own right [340]. Further studies [321], however, showed that Ins(1:2,4,5)P₃ is only a weak agonist more than an order of magnitude less potent than Ins(1,4,5)P₃ [EC₅₀ 3.6 μM, cf. Ins(1,4,5)P₃ 0.21 μM]. It was also demonstrated that treatment of Ins(1:2,4,5)P₃ with acid affords a mixture of Ins(1,4,5)P₃ and Ins(2,4,5)P₃ with receptor binding and calcium release properties similar to Ins(1,4,5)P₃, and it may therefore well be possible that the Ins(1:2,4,5)P₃ used in the initial experiments was contaminated with Ins(1,4,5)P₃ due to decomposition.

Synthetic Ins(2,4,5)P₃ was found to effect calcium release, being some 12-68 fold less potent than Ins(1,4,5)P₃ [320-322,324,342]. Tegge et al. [174,342] obtained
the same EC_{50} value [4 \mu M; \text{Ins}(1,4,5)P_3 0.13 \mu M] for D-\text{Ins}(2,4,5)P_3 and for D-\text{chiro}-\text{inositol} 1,3,4-trisphosphate, an analogue of \text{Ins}(2,4,5)P_3 with an axial 1-hydroxyl group. It thus appears that the difference between the two compounds, an equatorial \textit{versus} an axial hydroxyl group at the 1-position, is insignificant with respect to Ca^{2+} mobilising activity. The L-enantiomers of both compounds were considerably less active than the D-isomers, and some 800 - 960 fold less active than Ins(1,4,5)P_3 \cite{174,342}, this is another example for the stereospecificity of the Ins(1,4,5)P_3 receptor.

D-3-Azido-3-deoxy-\text{Ins}(2,4,5)P_3 was considerably less potent than \text{Ins}(1,4,5)P_3 in calcium release studies \cite{343}. The compound was found to mobilise 21 % of sequestered Ca^{2+} at a concentration of 100 \mu M, whereas \text{Ins}(1,4,5)P_3 released 53 % at 10 \mu M. In binding experiments, the azide exhibited a K_i of 18.9 \mu M \text{[Ins}(1,4,5)P_3: K_i = 10 \text{nM]} \cite{343}.

DL-Cyclohexane 1,2,4-trisphosphate [= DL-2,3,6-trideoxy-\text{Ins}(1,4,5)P_3] was shown to effect Ca^{2+} release from permeabilised smooth muscle cells with an EC_{50} of 40 \mu M \text{[Ins}(1,4,5)P_3 0.3 \mu M] \cite{324}. Benzene 1,2,4-trisphosphate was found to block \text{Ins}(1,4,5)P_3 binding to adrenal cortex microsomes competitively with an IC_{50} of 34 \mu M. The affinity of this compound to the receptor is about 10000 fold lower than that of \text{Ins}(1,4,5)P_3 \cite{344}.

The racemic trissulphate, trissulphonamide, triscarboxymethyl and trismethylphosphonate analogues of \text{Ins}(1,4,5)P_3 did not show any biological activity \cite{288,289}, neither did the 4,5-dimethylene phosphonate analogue of DL-\text{Ins}(4,5)P_2 \cite{345}.

There has been a report by Schultz \textit{et al.} \cite{346} that \textit{cis,cis}-cyclohexane 1,3,5-trisphosphate and related analogues with a 1,5-bisphosphate arrangement are able to effect calcium release from isolated vacuoles of \textit{Neurospora crassa}. This observation is however, to be treated with caution, as myo-inositol 1,3,5-trisphosphate has been shown not to act as an agonist in bovine aortic smooth muscle cells \cite{324}. If these results were to be confirmed, it would imply that the vacuolar receptor of \textit{Neurospora crassa} is different from mammalian receptors, which seems remarkable since \text{Ins}(1,4,5)P_3 receptors in different species have been found to be highly similar.
Summary

The data given above suggest that the vicinal 4,5 bisphosphate moiety is essential for receptor recognition and that the presence of an additional phosphate group at the 1-position greatly enhances Ca^{2+} mobilising properties. The fact that Ins(2,4,5)P_{3} is also a full and relatively potent agonist may possibly be explained by an interaction of the axial 2-phosphate with the receptor site normally occupied by the equatorial 1-phosphate of Ins(1,4,5)P_{3}.

Figure 4.3 Structure-activity relationship for the Ins(1,4,5)P_{3}-receptor

4.2 Partial Agonists at the Ins(1,4,5)P_{3} Receptor

A number of partial agonists at the Ins(1,4,5)P_{3} receptor have been identified: Ins(1,3,4,6)P_{4} has been found to have a high affinity for the receptor and to give a maximal calcium release of 49.5±2.5 % [Ins(1,4,5)P_{3}: 60.5±3.3 %]. If the maximally effective Ca^{2+}-releasing concentration of Ins(1,3,4,6)P_{4} is administered together with Ins(1,4,5)P_{3}, the EC_{50} value for the dose-response curve is dramatically increased [94]. This has led to the conclusion that the Ca^{2+} released is not only from the same intracellular store, but it also strongly
indicates that \( \text{Ins}(1,3,4,6)P_4 \) and \( \text{Ins}(1,4,5)P_3 \) compete for the same receptor site \(^{[94,347]}\). As \( \text{Ins}(1,3,4,6)P_4 \) does not nominally possess the vicinal 4,5-bisphosphate moiety normally required for agonist activity (see above), it may not be obvious why this tetrakisphosphate should release calcium. However, two alternative binding conformations of \( \text{Ins}(1,3,4,6)P_4 \) to the \( \text{Ins}(1,4,5)P_3 \) receptor can be visualised (Figure 4.3), in which a number of important recognition features are mimicked, including the 4,5-bisphosphate pairing and the 1-phosphate group.

![Figure 4.3 Alternative binding conformations of \( \text{Ins}(1,3,4,6)P_4 \) to \( \text{Ins}(1,4,5)P_3 \) receptor](image)

Figure 4.4 Alternative binding conformations of \( \text{Ins}(1,3,4,6)P_4 \)

Both of these alternative binding conformations of \( \text{Ins}(1,3,4,6)P_4 \) show an additional 2-phosphate moiety in the now equatorial 2-position. As discussed for analogues of \( \text{Ins}(1,4,5)P_3 \) with modifications at the 2-position, this should not cause a significant change in the binding and calcium release properties of the compound. The second feature that has changed is the 3-hydroxyl group (in the first alternative binding conformation) or 6-hydroxyl group (in the second binding conformation). In both cases the respective hydroxyl group is now axial rather than equatorial as in \( \text{Ins}(1,4,5)P_3 \).
Partial agonist behaviour was also found for L-chiro-Ins(2,3,5)PS$_3$ and 6-deoxy-Ins(1,4,5)PS$_3$ \cite{348}. They were able to mobilise only 34\% and 42\% of the calcium maximally mobilised by Ins(1,4,5)P$_3$, respectively. As very weak partial agonists \[\text{in comparison: Ins}(1,3,4,6)P$_3$ releases 82\% of the Ins(1,4,5)P$_3$ sensitive calcium, see above\] they can be used to antagonise Ins(1,4,5)P$_3$ induced Ca$^{2+}$ release at relatively high concentrations (IC$_{50}$ values of 26 \mu M and 171 \mu M, respectively). It should be noted that both compounds are 3 or 6-modified analogues of Ins(1,4,5)P$_3$, respectively, in addition to carrying phosphorothioate groups rather than phosphates in the 1-, 4-, and 5-positions.

### 4.3 Ins(1,4,5)P$_3$ Receptor Antagonists

Up to now, none of the Ins(1,4,5)P$_3$ analogues synthesised has shown any antagonistic properties apart from the 5-methylphosphonate analogue of Ins(1,4,5)P$_3$ \cite{285,286} and L-chiro-Ins(2,3,5)PS$_3$ \cite{348}, the latter, however, has significant intrinsic activity. The only molecules which have been clearly demonstrated to potently inhibit Ins(1,4,5)P$_3$-receptor binding are heparin \cite{32,349-354} and decavanadate \cite{22,355-357}; neither of them, however, show specificity for the Ins(1,4,5)P$_3$-receptor \cite{335,358}, and they are therefore of only limited use as pharmacological tools.

### Heparin

Heparin is a polysulphated polysaccharide with a molecular weight between 6000 and 20000, depending on origin and preparation. Its antagonistic action was first described in 1987 \cite{32} and it was also found that the heparin fragment TV84558-51 (average M$_r$ = 5100) is as potent as heparin itself in inhibiting $[^{3}H]$-Ins(1,4,5)P$_3$ binding, whereas the smaller heparin fragment OAM51126 (average M$_r$ = 2200) and less- or unsulphated glycosaminoglycans (chondroitin sulphate and hyaluronic acid) did not affect binding \cite{32}. This is also the case for O- and N-desulphated N-reacetylated heparin, N-desulphated heparin showing a decreased inhibitory activity at the Ins(1,4,5)P$_3$-receptor [but not at the Ins(1,3,4,5)P$_4$ binding site], whilst pentosan polyphosphate is another potent but non-selective inhibitor at both Ins(1,4,5)P$_3$- and Ins(1,3,4,5)P$_4$-binding sites \cite{335}. In contrast to the inhibitory effects of heparin and related substances observed...
in many animal and plant tissues, Ins(1,4,5)P$_3$-induced Ca$^{2+}$-mobilisation in fungi is heparin-insensitive [359].

Heparin also inhibits Ins(1,4,5)P$_3$ 3-kinase activity [but not Ins(1,4,5)P$_3$ 5-phosphatase] [350], the specific binding of Ins(1,3,4,5)P$_4$ to cerebellar membranes [335] and the ability of Ins(1,3,4,5)P$_4$ to release Ca$^{2+}$ from cerebellar microsomes [325].

It seems likely that the negatively charged sulphate groups of heparin interact with the hydrophilic pockets in the receptor binding site that usually accommodate the phosphate groups of Ins(1,4,5)P$_3$. However, it is interesting to note here that myo-inositol-1,4,5-trisulphate, in spite of carrying sulphate groups in the positions that are occupied by the phosphate groups in Ins(1,4,5)P$_3$ does not seem to have any antagonistic properties [289].

Vanadate

Different species of vanadate have been examined for their ability to inhibit Ins(1,4,5)P$_3$-receptor binding and Ca$^{2+}$ release, and it has been found that decavanadate (V$_{10}$O$_{28}^{6-}$ at pH 7 [360]) inhibits Ins(1,4,5)P$_3$-induced Ca$^{2+}$ mobilisation in permeabilised rat insulinoma and PC12 cells (IC$_{50}$ 5 µM) [355] and SH-SY5Y cells (K$_i$=1.2 µM) [356] and inhibits the binding of [$^3$H]-Ins(1,4,5)P$_3$ to its receptor in cerebellar and adrenal cortex membranes [356].

Orthovanadate and oligovanadate on the other hand do not inhibit Ins(1,4,5)P$_3$ receptor binding [355], possibly because they are unable to bridge multiple Ins(1,4,5)P$_3$ binding sites suggested by Meyer et al. [35].

It is interesting to note here that tetravanadate has been shown to be an inhibitor of PI-specific PLC [361].

Decavanadate also suppresses Ins(1,3,4,5)P$_4$-induced Ca$^{2+}$ release from permeabilised SH-SY5Y cells and inhibits Ins(1,4,5)P$_3$ 5-phosphatase, 3-kinase and Ins(1,3,4,5)P$_4$ 5-phosphatase [356].

Although decavanadate and heparin are potent and competitive antagonists at the Ins(1,4,5)P$_3$ receptor, the fact that their specificity is low may prevent them from becoming useful tools to investigate the second messenger role of Ins(1,4,5)P$_3$. 
4.4 Structure-Recognition Studies on Ins(1,4,5)P₃ 3-kinase

The specificity of 3-kinase is in some respects greater than that of the Ins(1,4,5)P₃ receptor itself [272], Ins(1,4,5)P₃ and Ins(1,3,4,5)P₃ being the only natural inositol polyphosphates known to be recognised with high affinity [324].

**Modifications at the 1-position**

Removal of the 1-phosphate group greatly decreases the affinity of the 3-kinase, Ins(4,5)P₂ and Ins(2,4,5)P₃ are both very poor substrates for this enzyme [362,363], and Ins(1:2,4,5)P₃ is not phosphorylated by this enzyme [92]. The 1-phosphate group therefore appears to be essential for substrate recognition, which is in agreement with the observation that whilst both Ins(1,4,5)P₃-5S [210,364] and Ins(1,4,5)P₃-4,5-S₂ [348] are substrates for 3-kinase, an additional phosphoro-thioate substitution in the 1-position is not tolerated: Ins(1,4,5)P₃ is not a substrate for 3-kinase [268,365].

**Modifications at the 2-position**

DL-2-deoxy-Ins(1,4,5)P₃ was shown to be recognised well by the kinase [320], the apparent Kᵢ in calcium-free medium was 1.6 μM [Ins(1,4,5)P₃ 1.0 μM]. Even the analogue 110 (Figure 4.1), carrying a rather bulky substituent in the 2-position, was recognised equally well as Ins(1,4,5)P₃. This analogue surprisingly showed an even greater affinity to the 3-kinase (Kᵢ 0.36 μM) than Ins(1,4,5)P₃ in calcium free medium. However, no data indicating whether these analogues act as substrates or inhibitors were given.

Both DL-2-deoxy-2-fluoro-scyllo-Ins(1,4,5)P₃ and DL-2-deoxy-2,2-difluoro-Ins(1,4,5)P₃ appeared to be substrates for 3-kinase [305]. After resolution of the racemic gem-difluoride, however, the substrate properties of D- and L-2-deoxy-2,2-difluoro-Ins(1,4,5)P₃ were found to be quite different. Whereas the D-isomer was a substrate for 3-kinase (apparent Kᵢ = 10.2 μM), the L-isomer was a potent inhibitor of this enzyme (Kᵢ 11.9 μM) [316]. The reason for this inhibitory activity is not yet known, however, L-2,2-F₂-Ins(1,4,5)P₃ represents a novel lead in the design of further effective inhibitors for the pharmacological intervention in the polyphosphoinositide pathway.
Modifications at the 3-position

As can be expected with 3-modified lnS(1,4,5)P₃ analogues, both D-3-deoxy-3-fluoro-lnS(1,4,5)P₃ (176,317) and L-chiro-lnS(2,3,5)P₃ (177,332), a D-lnS(1,4,5)P₃ analogue with inverted hydroxyl stereochemistry at the 3-position, are resistant to phosphorylation by the 3-kinase, and they are potent inhibitors of this enzyme ($K_i$ values of 7.1 and 8.6 μM respectively) [333].

![Diagram of L-chiro-lnS(2,3,5)P₃ and D-3-F-(1,4,5)P₃]

Figure 4.5 3-position modified lnS(1,4,5)P₃ analogues

lnS(1,3,4,5)P₄, the product of lnS(1,4,5)P₃ phosphorylation by 3-kinase, is a weak inhibitor of the enzyme (IC₅₀ 90 μM). However, based upon the IC₅₀ data it seems unlikely that sufficient lnS(1,3,4,5)P₄ can accumulate to affect the rate of lnS(1,4,5)P₃ phosphorylation significantly, suggesting that it plays no role in any "feedback" mechanism [324].

Modifications at the 4-position

No compounds with selective modifications at the 4-position have been pharmacologically evaluated to date. However, recognition of the 4,5-bisphosphorothioate analogue of lnS(1,4,5)P₃ [lnS(1,4,5)P₃-4,5S] was attenuated in comparison to lnS(1,4,5)P₃-5S ($K_m = 5$ μM and 46 μM, respectively) [348], indicating that the 4-phosphate group is important for substrate recognition.
Modifications at the 5-position

DL-Ins(1,4,5)P₃-5S was a substrate for the 3-kinase with $K_m = 5 \mu M$ [348], but phosphorylation of this compound occurred considerably more slowly than that of the natural substrate [ca. 15% the rate of Ins(1,4,5)P₃]. The 5-methylenephosphonate analogue of Ins(1,4,5)P₃, reported to evoke a sustained release of calcium [285, 286], is presumably not a substrate for 3-kinase, although biological details on this compound have not been published. It appears therefore that only conservative modifications are tolerated in this position.

Modifications at the 6-position

6-Deoxy-Ins(1,4,5)P₃ is one of the few compounds that is recognised by the highly selective 3-kinase [182]. The kinetics of its metabolism indicate that it is a substrate for this enzyme, and the phosphorylation of Ins(1,4,5)P₃ is inhibited competitively by 6-deoxy-Ins(1,4,5)P₃ with an apparent $K_i$ of 5.7 $\mu M$ [$K_m$ for Ins(1,4,5)P₃, 3.2 mM]. It appears that hydroxyl group deletion remote from the site of action of the 3-kinase has no major effect on the binding properties of the substrate.

In contrast to the 6-deoxy analogue, DL-6-methoxy-Ins(1,4,5)P₃ shows a marked drop in affinity (ca. 120-fold) for the 3-kinase [324]. Since the hydrogen bonding potential at the 6-position is removed in both analogues, it seems likely that the increased steric bulk at the 6-position of DL-6-methoxy-Ins(1,4,5)P₃ is the cause for the low affinity of this analogue for the enzyme.

Multiple modifications

DL-Cyclohexane 1,2,4-trisphosphate was only a weak inhibitor of [³H]-Ins(1,4,5)P₃ phosphorylation (IC₅₀ 327 $\mu M$) [324].
Summary

site of enzyme action: 3-deoxy-Ins(1,4,5)P₃, 3-F-Ins(1,4,5)P₃ and L-chiro-Ins(2,3,5)P₃ are all potent inhibitors

conservative modifications are tolerated:
Ins(1,4,5)P₃-5S is phosphorylated

not important for recognition by 3-kinase, 2-deoxy-Ins(1,4,5)P₃ and 2-fluoro analogues are good substrates some analogues carrying bulky groups show an even greater affinity than Ins(1,4,5)P₃

the 1-phosphate group appears to be an important but not essential feature:
Ins(4,5)P₂ is a weak substrate

deletion as in 6-deoxy-Ins(1,4,5)P₃ is tolerated, bulky substituents however appear to reduce the affinity

Figure 4.6 Structure-recognition relationship for 3-kinase

4.5 Structure-Recognition Studies on Ins(1,4,5)P₃ 5-phosphatase

In contrast to the Ins(1,4,5)P₃ receptor and the Ins(1,4,5)P₃ 3-kinase, the Ins(1,4,5)P₃ 5-phosphatase seems to be relatively non-specific. However, whilst many analogues bind to the phosphatase, only a few are substrates.

Modifications at the 1-position

The 1-phosphate group appears to be an important feature in substrate recognition by 5-phosphatase. Ins(4,5)P₂ is a very poor substrate (Kₘ = 215 μM) [348].

Modifications at the 2-position

A number of racemic Ins(1,4,5)P₃ analogues modified at the 2-hydroxyl group were examined for their ability to interact with erythrocyte ghost and brain cytosol 5-phosphatase [320] (Figure 4.1). It was found that all compounds were competitive inhibitors of the enzyme and that they could serve as substrates for
5-phosphatase, although the extent of hydrolysis varied. Surprisingly, 2-deoxy-\textit{Ins}(1,4,5)P\textsubscript{3} and most of the other 2-modified analogues showed an even greater affinity for 5-phosphatase than \textit{Ins}(1,4,5)P\textsubscript{3}.

2-Deoxy-2-fluoro-scyllo-\textit{Ins}(1,4,5)P\textsubscript{3} was found to be well recognised by the phosphatase but to be a weaker substrate than \textit{Ins}(1,4,5)P\textsubscript{3} [305].

The 5-phosphatase substrate properties of D- and L-2-deoxy-2,2-difluoro-\textit{Ins}(1,4,5)P\textsubscript{3} are quite different. Whereas D-2-deoxy-2,2-difluoro-\textit{Ins}(1,4,5)P\textsubscript{3} is a good substrate for the 5-phosphatase, the L-enantiomer was found to be a potent inhibitor of the enzyme (\(K_I = 19.0 \ \mu\text{M}\)) [316]. Since 5-phosphatase is known to be specific for the D-isomer of \textit{Ins}(1,4,5)P\textsubscript{3}, and analogues in the L-conformation are usually not recognised very well by this enzyme [cf. L-\textit{Ins}(1,4,5)P\textsubscript{3}, \(K_I = 124 \ \mu\text{M}\) [271]], this result is surprising. There have however been reports on the interaction of 2-substituted analogues in the L-configuration who also display inhibitory properties with respect to 5-phosphatase [366] and the L-isomer of 109 (Figure 4.1) was found to be a surprisingly potent inhibitor with \(K_I = 3.8 \ \mu\text{M}\) [D-\textit{Ins}(1,4,5)P\textsubscript{3}: \(K_I = 13.1 \ \mu\text{M}\)].

Thus, it may be concluded that the 2-hydroxyl group may be involved in substrate recognition by the \textit{Ins}(1,4,5)P\textsubscript{3} 5-phosphatase, although it does not appear to be an essential feature.

**Modifications at the 3-position**

3-Deoxy-\textit{Ins}(1,4,5)P\textsubscript{3} was found to be a good substrate, binding with a slightly higher affinity than \textit{Ins}(1,4,5)P\textsubscript{3} itself.

3-Deoxy-3-fluoro-\textit{Ins}(1,4,5)P\textsubscript{3} was a substrate for erythrocyte 5-phosphatase and inhibited the dephosphorylation of \(^{3}\text{H}\)-\textit{Ins}(1,4,5)P\textsubscript{3} with an apparent \(K_I\) of 3.9 \ \mu\text{M} [333].

L-\textit{chiro-Ins}(2,3,5)P\textsubscript{3}, which can be visualised as \textit{Ins}(1,4,5)P\textsubscript{3} with an inverted 3-hydroxyl group, was found to be a very potent inhibitor of 5-phosphatase (\(K_I = 7.7 \ \mu\text{M}\)) [177,332,333]. This is somewhat surprising, since the modification of the stereochemistry of the 3-hydroxyl group in this molecule is remote from the site of attack of the enzyme. Two possible explanations for this phenomenon have been given (Figure 4.7):
a) the conformation of L-chiro-Ins(2,3,5)P₃ in solution and / or bound to 5-phosphatase is sufficiently different from Ins(1,4,5)P₃ to interfere with the catalytic mechanism of the enzyme, but nevertheless the analogue binds to the enzyme in a similar mode to Ins(1,4,5)P₃ or;

b) the inhibition may be the result of non-productive binding of L-chiro-Ins(2,3,5)P₃ in an inverted and rotated mode. In this arrangement the analogue would mimic four elements of Ins(1,4,5)P₃ correctly, namely the ring pucker, the crucial vicinal 4,5-bisphosphate moiety (as the 2;3-bisphosphate pair) and the 3-hydroxyl group (as the 4-hydroxyl group). The 5-phosphate group of L-chiro-Ins(2,3,5)P₃ in this mode mimics an equatorial 2-phosphate group of an Ins(1,4,5)P₃ analogue, and such a phosphate could presumably still bind reasonably well to the hydrophilic pocket of the enzyme usually interacting with the equatorial 1-phosphate group of Ins(1,4,5)P₃ [Ins(2,4,5)P₃ is also a substrate for the 5-phosphatase [320]]. In this inverted binding mode the axial 1-hydroxyl group of L-chiro-Ins(2,3,5)P₃ now mimics the axial 6-hydroxyl group of an Ins(1,4,5)P₃ analogue. As discussed below (Modifications at the 6-position), the equatorial 6-hydroxyl group of Ins(1,4,5)P₃ may play an important role in the mechanism of 5-phosphatase catalysed hydrolysis and the inhibitory properties of L-chiro-Ins(2,3,5)P₃ may possibly be ascribed to the pseudo axial 6-hydroxyl group in the inverted binding conformation b).

Figure 4.7 Alternative binding conformations of L-chiro-Ins(2,3,5)P₃ to 5-phosphatase
Modifications at the 5-position

It has been demonstrated by the 5-phosphorothioate analogue Ins(1,4,5)P₃-5S, that modification at the 5-position creates a potent inhibitor of the enzyme Ins(1,4,5)P₃-5-phosphatase \( (K_i 6.8 \mu M) \) \cite{210,211}. The 5-methylenephosphonate analogue of D-Ins(1,4,5)P₃ is another long-lived agonist of calcium mobilisation \cite{180}. The sustained release of calcium by this compound indicates that it is not dephosphorylated and thus deactivated by the 5-phosphatase nor phosphorylated at the 3-position by the kinase. No data on interactions with the enzyme have however been published as yet. The synthesis and preliminary biological data of the 5-methylphosphonate and 5-difluoromethylphosphonate analogues of Ins(1,4,5)P₃ have been published, however no details on interactions of these 5-modified Ins(1,4,5)P₃ analogues with the 5-phosphatase have been reported to date.

Modifications at the 6-position

D-6-deoxy-Ins(1,4,5)P₃ \cite{182} inhibited the dephosphorylation of \[^{32}P\]-Ins(1,4,5)P₃ by erythrocyte 5-phosphatase with a \( K_i \) of 76 \( \mu M \) \( [K_m \text{ for } \text{Ins}(1,4,5)\text{P}_3 40 \mu M] \). D-6-deoxy-Ins(1,4,5)P₃ is therefore a relatively potent inhibitor of this enzyme. Both the only ca. 2-fold lower affinity of D-6-deoxy-Ins(1,4,5)P₃ and the approximately 4-fold lower affinity of DL-6-methoxy-Ins(1,4,5)P₃ for the aortic smooth muscle 5-phosphatase \cite{324} underline the non-selectivity of this enzyme. The resistance of these two analogues to dephosphorylation confirmed that the minimal structural requirements for substrate hydrolysis by the 5-phosphatase include the vicinal 4,5-bisphosphate moiety and a free 6-hydroxyl group \cite{324}. It is possible that the role of the 6-hydroxyl group of Ins(1,4,5)P₃ is similar to the role that the 6-hydroxyl group of Ins(1)P plays in the dephosphorylation of this compound by inositol monophosphatase, \( \text{i.e.} \) the absence of this group has no significant effect on binding, but its presence is essential for the enzyme to act on the substrate (mechanistic hydroxyl group, see Chapter 1.6.2).

Multiple modifications

DL-Ins(1,4,5)PS₃ was resistant to degradation by 5-phosphatase \cite{270}. DL-Cyclohexane 1,2,4-trisphosphate was a weak competitive inhibitor of 5-
phosphatase (Kᵢ 124 μM), and no inorganic phosphate release due to hydrolysis by the enzyme was detected [324].

Summary

3-deoxy-Ins(1,4,5)P₃ and 3-F-Ins(1,4,5)P₃ are good substrates, however, inversion at this position creates a potent inhibitor, L-chiro-Ins(2,3,5)P₃ analogues modified at the 5-position like Ins(1,4,5)P₃-5S and 5-methylenephosphonate-Ins(1,4,5)P₃ are potent inhibitors of 5-phosphatase.

not important for recognition by 5-phosphatase, 2-deoxy-Ins(1,4,5)P₃ and other 2-modified analogues are good substrates.

the 1-phosphate group appears to enhance recognition but not to be an essential feature: Ins(4,5)P₂ is a weak substrate.

mechanistic hydroxyl group: deletion as in 6-deoxy- and 6-O-methyl-Ins(1,4,5)P₃ gives inhibitors of 5-phosphatase.

Figure 4.8 Structure-recognition relationship for 5-phosphatase
RESULTS AND DISCUSSION

5 Aims of this work

As outlined in the General Section, there is currently great interest in the second messenger D-myoinositol 1,4,5-trisphosphate, as well as in other inositol phosphates involved in signal transduction, and in the interactions of these compounds with receptors and metabolic enzymes. To investigate the roles of the different components in this complex network, analogues of the naturally occurring inositol phosphates are needed which can, for example, serve to label relevant proteins or act as enzyme inhibitors.

In this thesis, the synthesis of novel analogues of the second messenger D-myoinositol 1,4,5-trisphosphate is reported. These analogues were prepared with different objectives in mind:

The first target compound was the 1-phosphorothioate analogue of Ins(1,4,5)P₃, myo-inositol 4,5-bisphosphate 1-phosphorothioate Ins(1,4,5)P₃-1S, which was to be prepared initially in racemic, and then in optically active form. This analogue was then to be used to link a fluorescent chromophore selectively to the 1-position of Ins(1,4,5)P₃. It was also thought that this method might be used to link Ins(1,4,5)P₃ to other reporter groups, e.g. spin labels, affinity and photoaffinity probes and affinity column matrices. An intermediate in the synthesis of Ins(1,4,5)P₃-1S was to be employed in the preparation of [³⁵S]-Ins(1,4,5)P₃-1S, a novel radioligand for the Ins(1,4,5)P₃ receptor which would allow the synthesis of radioactive S-alkyl reporter group adducts.

The trisphosphorothioate analogues of the moderately potent 5-phosphatase inhibitors Ins(1,3,5)P₃ and L-Ins(1,4,5)P₃ were then to be synthesised and evaluated as potential inhibitors of this enzyme. The aim here was to explore whether the improved binding of phosphorothioate analogues could be exploited to produce high affinity inhibitors which would not mobilise Ca²⁺.

The next objective was the preparation of analogues of Ins(1,4,5)P₃ modified at the 2-position in order to investigate the contribution of this position towards
receptor binding and interaction with metabolic enzymes. For this purpose, the scyllo-inositol analogue DL-scyllo-inositol 1,2,4-trisphosphate as well the fluorinated analogue DL-2-deoxy-2-fluoro-myo-inositol 1,4,5-trisphosphate were to be synthesised.

Two other scyllo-inositol derivatives, scyllo-inositol 1,2,4,5-tetrakisphosphate and scyllo-inositol 1,2,4,5-tetrakisphosphorothioate, were then to be prepared with the aim to mimic the phosphate arrangement of the known partial agonist Ins(1,3,4,6)P₄ and to find out more about structural requirements for compounds with these characteristics, with the overall aim of antagonist development.

A route to the novel proposed Ins(1,3,4,5)P₄ 3-phosphatase inhibitor, myo-inositol 1,4,5-trisphosphate-3-phosphorothioate, Ins(1,3,4,5)P₄-3S was devised. A key step included the optical resolution of a valuable intermediate in the synthesis of 3-position modified Ins(1,4,5)P₃ and Ins(1,3,4,5)P₄ analogues, DL-3-O-allyl-6-O-benzyl-1-O-ϕ-methoxybenzyl-4,5-O-isopropylidene-myo-inositol, which was to be attempted via the camphanate ester method. The resolved intermediate was then to be applied in the preparation of D-Ins(1,3,4,5)P₃-3S.

Methods to replace the Ins(1,4,5)P₃ 1-phosphate group with a carboxymethylene moiety (which should mimic a phosphate to some extent) and to employ lactones as protecting groups in the synthesis of inositol derivatives were to be investigated. We were also interested in ring-fused lacto-derivatives of inositol phosphates since compounds with a second ring fused to the inositol ring should be more rigid than ordinary inositol phosphates. Analogues with less flexibility than the natural substrates may display antagonistic or inhibitory properties and would help to define structure-activity parameters.

Finally, routes to C-allyl inositol derivatives via Claisen rearrangements of elimination products with a C=C bond in the inositol ring were to be studied. This new class of compounds was of interest to us because by oxidative cleavage of the allyl double bond the synthesis of derivatives with a -CH₂-COOH group linked directly to an inositol ring carbon should be possible. Sterically, the -CH₂-COOH moiety might appear to be a better surrogate for a phosphate group than a carboxymethylene group, and such compounds, when phosphorylated, may show novel activity.
Synthesis of DL-my-o-inositol 4,5-bisphosphate 1-phosphorothioate, and its fluorescent and radio-labelling

The few structure-activity studies which have been performed on Ins(1,4,5)P$_3$ analogues show that the vicinal 4,5-bisphosphate moiety of Ins(1,4,5)P$_3$ is essential for Ca$^{2+}$-releasing activity [272], the 1-phosphate group being thought to provide enhanced affinity for the receptor. Semisynthetic Ins(1,4,5)P$_3$ analogues with modifications at the 1-phosphate position have been prepared from the deacylated polyphosphoinositide phospholipid and are biologically potent in effecting calcium release [323]. It was thought that introduction of a nucleophilic phosphorothioate group into the Ins(1,4,5)P$_3$ molecule should permit the facile attachment of reporter groups to Ins(1,4,5)P$_3$, such as photoaffinity labels, spin labels and fluorescent probes. Previously synthesised inositol phosphorothioates like the trisphosphorothioate Ins(1,4,5)P$_3$S$_3$ and the 5-phosphorothioate Ins(1,4,5)P$_3$S$_3$S$_5$ have been shown to be highly potent analogues of Ins(1,4,5)P$_3$. Consequently, the synthesis of the novel Ins(1,4,5)P$_3$ analogue myo-inositol 4,5-bisphosphate 1-phosphorothioate [Ins(1,4,5)P$_3$-1S] in racemic and optically active form has been devised and its use in the fluorescent labelling of Ins(1,4,5)P$_3$ has been demonstrated. Whilst these studies were in progress other groups have also addressed the problem of attaching reporter groups to Ins(1,4,5)P$_3$ [328,367] or related compounds [368], and Ins(1,4,5)P$_3$ to affinity matrices [320,368,369].

Myo-inositol was chosen as a cheap (£ 18.50 for 250 g [370]) and readily available starting material for the synthesis of the 1-phosphorothioate analogue of Ins(1,4,5)P$_3$. In order to set up the inositol molecule for the introduction of phosphate groups at position 4 and 5 and a phosphorothioate group at position 1, it was necessary to synthesise a compound with protected 2-, 3- and 6-hydroxyl functions. It was also required that either the 4- and 5-hydroxyl groups or the 1-position of this intermediate were blocked (depending on whether the phosphorothioate group or the phosphate groups were introduced first). The protecting group(s) chosen to block the 1-position (or the 4- and 5-positions) had to be easily removable without affecting the blocked 2-, 3- and 6-positions, or the phosphate (phosphorothioate) triester functions or the protecting groups used for the 2-, 3- and 6-position.
The selected key intermediate that fulfilled the requirements outlined above, was 2,3,6-tri-O-benzyl-1-O-(prop-1-enyl)-myo-inositol. The 4- and 5-positions in this compound are free and can be selectively phosphorylated because all the other hydroxyl functions are protected. The prop-1-enyl ether at the 1-position can then be cleaved without affecting the benzyl ethers or the protected phosphate groups. Additionally, the compound is easily prepared by isomerisation of the known 1-O-allyl ether\textsuperscript{[168]}.

6.1 Synthesis of DL-2,3,6-Tri-O-benzyl-1-O-(prop-1-enyl)-myo-inositol

1-O- Allyl-2,3,6-tri-O-benzyl-myoinositol was synthesised essentially according to the procedure of Gigg \textit{et al.} \textsuperscript{[149,168]}.

\textit{Myo}-inositol was treated with 2,2-dimethoxypropane in DMF and PTSA to give a mixture of the three bis-acetals. The desired 1,2:4,5-di-O-isopropylidene-\textit{myo}-inositol was isolated by converting the three isomers into the dibenzoates with benzoyl chloride / pyridine. In contrast to the other two dibenzoates, 1,2:4,5-di-O-isopropylidene-3,6-di-O-benzoyl-\textit{myo}-inositol is insoluble in pyridine, water, acetone and ether and was obtained from the reaction mixture by washing with these solvents. Removal of the benzoyl groups by treatment with methanolic sodium hydroxide solution and benzylation with benzyl chloride, potassium hydroxide and the phase-transfer catalyst 18-crown-6 (the reaction is a modification of Gigg’s procedure, who used benzyl bromide and sodium hydride in \textit{N,N}-dimethylformamide) gave 3,6-di-O-benzyl-1,2:4,5-di-O-isopropylidene-\textit{myo}-inositol \textsuperscript{123}, with ‘permanent’ benzyl protecting groups in the 3- and 6-position as required.

After acid hydrolysis of the two acetals, the hydroxyl group in the 1-position of \textsuperscript{124} was allylated. Selective allylation of the tetrol was accomplished by first forming a tin-complex \textsuperscript{[164]} with dibutyltin oxide by heating under reflux in toluene with azeotropic removal of water of condensation by means of a Dean and Stark apparatus. The tin-complex reacted with allyl bromide in DMF to give selectively the 1-O-allyl ether. A cleaner reaction with higher yields was achieved by adding caesium fluoride (rather than tetrabutylammonium iodide \textsuperscript{[168]}) to the reaction mixture prior to the addition of allyl bromide \textsuperscript{[167]}. The role of caesium fluoride in this reaction is discussed in Chapter 2.1.5.
Figure 6.1 Synthesis of 1-O- Allyl-2,3,6-tri-O-benzyl-myoinositol
The 1-, 3- and 6-positions were now protected: the next objective was to introduce a benzyl group at the 2-position. This was effected by simultaneous protection of the 4- and 5-positions as the isopropylidene acetal using 2,2-dimethoxypropane and PTSA, followed by benzylation of the remaining free 2-hydroxyl group with benzyl chloride / KOH / 18-crown-6. The acetal was cleaved by acid hydrolysis to give DL-1-O-allyl-2,3,6-tri-O-benzyl-myoinositol 127, which could be obtained in crystalline form (m.p. 73-75°C) from ethanol / water. Gigg et al. obtained this compound only as a syrup after column chromatography on silica gel [168].

Isomerisation of the allyl ether to the corresponding prop-1-enyl derivative 128 was accomplished either by employing tris(triphenylphosphine)rhodium (l) chloride (Wilkinson’s catalyst) in the presence of 1,4-diazabicyclo[2.2.2]octane (DABCO) (to prevent premature hydrolysis of the propenyl ether) in 7:3:1 ethanol:benzene:water [371] or by using potassium tert.-butoxide (Bu'OK) in DMSO [137].

Figure 6.2 Isomerisation of the 1-O-allyl group

Yields were found to be higher when Bu'OK was used; additional advantages are the easier work-up and the fact that a homogenous product is obtained as only the cis-isomer is formed, whereas the use of Wilkinson’s catalyst gave a mixture of ca. 5:1 cis:trans prop-1-enyl isomers. The cis:trans ratio was
determined by the integration of the $^1$H-NMR signals at 1.56 ppm (0.5 H, dd, J 6.8, 1.55, trans-CH=CH-CH$_3$) and 1.68 ppm (2.5 H, dd, J 6.8, 1.6, cis-CH=CH-CH$_3$). The CH=CH-CH$_3$ and the CH=CH-CH$_3$ signals were part of multiplets and could therefore not be used to determine the exact amounts of the isomers present in the mixture. It was however clear from the CH=CH-CH$_3$ signal at 6.07-6.15 ppm that the cis-isomer (J 6.2 and 1.65 Hz) was the major component. The assignment of the 1.68 ppm signal to the cis-isomer was confirmed when pure 2,3,6-tri-O-benzyl-1-O-(cis-prop-1-enyl)-myo-inositol was prepared.

6.2 The Synthesis of bis(2-cyanoethoxy)(diisopropylamino) phosphine

The next step in the synthesis of Ins(1,4,5)P$_3$-1S was the phosphorylation of the 4- and 5-positions of 128. The P(III) reagent bis(2-cyanoethoxy)(diisopropylamino) phosphine was chosen to phosphitylate the free hydroxyl groups of the protected inositol derivative. P(III) reagents are more reactive than P(V) reagents and allow phosphorylation of the vicinal 4,5-diol without formation of cyclic phosphates (see Chapter 2.3). They have the additional advantage of providing access to both phosphates and phosphorothioates as the phosphite triesters obtained may be oxidised to give the corresponding phosphate triesters or sulphoxidised to give the protected phosphorothioates. The removal of the 2-cyanoethoxy protecting groups by β-elimination in sodium / liquid ammonia occurs faster than the deblocking of the benzyl protecting groups on the inositol ring hydroxyl functions. Undesirable phosphate migrations which may occur when free hydroxyl groups are present next to a protected phosphate, are therefore avoided.

The phosphitylating reagent 131 was synthesised in three steps from phosphorus trichloride according to the procedure of Bannwarth and Trzeciak [226]:

1) Phosphorus trichloride was treated under nitrogen with 3-hydroxypropionitrile and pyridine (1 equivalent each) in dry ether to give dichloro(2-cyanoethoxy)phosphine 129 after removal of the pyridine hydrochloride precipitate. $^{31}$P-NMR showed a single signal at $\delta = 179.77$ ppm. The compound was very moisture sensitive and partly decomposed during attempted distillation.
at reduced pressure. It was therefore used without further purification to prepare the alkoxybis(dialkylamino)phosphine 130.

2) (2-Cyanoethoxy)bis(diisopropylamino)phosphine 130 was prepared by adding 9 equivalents of $N,N$-diisopropylamine slowly to an ethereal solution of the alkoxydichlorophosphine 129 (addition of only 2 equivalents of secondary amine will give the $N,N$-diisopropylamino(2-cyanoethoxy)chlorophosphine $[^{372}]$). The crude product was obtained after filtering the precipitated amine hydrochloride. This compound is quite stable and was purified by distillation under reduced pressure from calcium hydride. $^{31}$P-NMR: $\delta = 122.56$ ppm.

The strong $p_\pi-d_\pi$ interaction between phosphorus and the nitrogen atoms $[^{127}]$ makes alkoxybis(dialkylamino)phosphines easily susceptible to protonation by very weak acids (such as diisopropylammonium tetrazolide), leading to an activated species which can react with another alcohol to give the dialkoxo(dialkylamino)phosphine. In this class of compounds the $p_\pi-d_\pi$ interaction is reduced due to the newly introduced alkoxy function, they are therefore less susceptible to protonation and behave like monofunctional reagents. Activation can still be achieved under slightly more acidic conditions (e.g. by employing 1-$H$-tetrazole), allowing the reaction with a hydroxyl group bearing substrate to give the desired phosphite triester.

![Chemical Structures]

Figure 6.3 Synthesis of bis(2-cyanoethoxy)(diisopropylamino)phosphine
3) The phosphitylating reagent bis(2-cyanoethoxy)(diisopropylamino)phosphine 131 was prepared by adding 1.1 equivalents of 3-hydroxypropionitrile to a solution of 130 and diisopropylammonium tetrazolide (prepared in quantitative yield by treatment of a solution of tetrazole in acetonitrile with an excess of diisopropylamine, followed by filtration and drying under vacuum [373]) in dichloromethane. The product was stable enough to allow purification by flash chromatography.

6.3 DL-mylo-Inositol 4,5-bisphosphate 1-phosphorothioate

Phosphorylation of the vicinal 4,5-diol of 128 was effected using a P(III) approach, thus avoiding difficulties which can arise by direct phosphorylation of the vicinal diols with P(V) reagents (e.g. the formation of undesirable cyclic phosphates). 2,3,6-Tri-O-benzyl-1-O-(prop-1-enyl)-myo-inositol was treated with bis(2-cyanoethoxy)-N,N-diisopropaminophosphine [226] and 1H-tetrazole in dry dichloromethane to give the bisphosphite triester. 31P-NMR showed signals at 148 ppm (excess phosphitylating agent), 139.21 and 138.64 ppm (inositol 4- and 5-phosphite) and 127 ppm (phosphitylating reagent-tetrazolide).

The protected P(III) bisphosphite was oxidised to the P(V) bisphosphate by the addition of excess tert.-butyl hydroperoxide (Bu'OOH) [236] to the cooled reaction mixture. The 31P-NMR spectrum showed a minor peak at 8 ppm (phosphonate) and two signals at -5.52 and -5.65 ppm. Although the oxidation of the phosphite was complete within a few minutes, stirring was usually continued overnight because it was found to make the chromatographic purification of the product easier, presumably because H-phosphonate by-products of a polarity similar to the product were oxidised to more polar derivatives.
Figure 6.4  Synthesis of DL-my-o-inositol 4,5-bisphosphate 1-phosphorothioate

The prop-1-enyl ether was cleaved by acidic hydrolysis with methanolic hydrochloric acid \(^{140}\) or HgO-HgCl\(_2\) \(^{141}\) to give DL-2,3,6-tri-O-benzyl-4,5-bis[di(2-cyanoethoxy)phospho]-myo-inositol. Initially, cleavage of the propenyl ether with HgO-HgCl\(_2\) was chosen in order to avoid any deprotecting of the sensitive phosphate triesters. It proved however, very difficult to separate the mercury compounds from the reaction product even by chromatographic means, so that in later experiments mild acidic hydrolysis was used which was found not to affect the protected phosphates and also to give cleaner products. The \(^1\)H-NMR signals of the inositol ring protons of this compound were assigned to the individual protons by coupling constants and correlation spectroscopy (COSY, Figure 6.5).
The free hydroxyl group was then phosphitylated and the phosphite sulphoxidised with an excess of sulphur in pyridine \textsuperscript{[233]} to give DL-2,3,6-tri-O-benzyl-4,5-bis[di(2-cyanoethoxy)phospho]-1-[bis(2-cyanoethoxy)thiophospho]-myo-inositol. \textsuperscript{31}P-NMR spectroscopy showed signals at 66.83 ppm for the phosphorothioate triester and peaks at -2.83 and -3.10 ppm for the phosphate triester.
Deblocking of benzyl and cyanoethoxy protecting groups was accomplished using sodium in liquid ammonia [236] to give the \( \text{Ins}(1,4,5)P_3 \) analogue DL-myo-inositol 4,5-bisphosphate 1-phosphorothioate [DL-Ins(1,4,5)P_3-1S] 80, which was purified by ion-exchange chromatography on DEAE Sephadex A-25. DL-Ins(1,4,5)P_3-1S was eluted at ca. 800 mmol dm\(^{-3}\) triethylammonium hydrogen carbonate (TEAB) buffer (yield 46 % as determined by quantitative phosphate analysis). \(^{31}\text{P}-\text{NMR} \) spectroscopy (Figure 6.6) showed that the product possessed a single phosphorothioate group (\( \delta_P \) 42.1 ppm) and two phosphate groups (\( \delta_P \) 4.6, 3.5 ppm). The signals in the proton NMR were assigned by their chemical shift and their coupling constants as well as their COSY spectrum (Figure 6.7).

![Figure 6.6 31P-NMR spectrum of 80](image)
6.4 $[^{35}\text{S}]-\text{DL-myoinositol 4,5-bisphosphate 1-phosphorothioate}$

The synthesis of the metabolically stable radioligand D-$[^{35}\text{S(U)]]-\text{myo-inositol 1,4,5-trisphosphorothioate} \, [^{35}\text{S}]-\text{Ins(1,4,5)PS}_3$ and its application in binding studies has been reported $^{275}$. This radioligand was shown to label two different sites in rat cerebellar membranes: the $\text{Ins(1,4,5)P}_3$ receptor and another site that displayed only low affinity for $\text{Ins(1,4,5)P}_3$.

In cooperation with NEN-DuPont, racemic 1,2,4-tri-$\text{O}$-benzyl-$5,6$-bis$[\text{di(2-cyanoethoxy)phospho}]$-$\text{myo-inositol was used to synthesise the radiolabelled DL-$[^{35}\text{S}]$-Ins(1,4,5)P}_3$-$1\text{S}$, which is a novel radioligand for the $\text{Ins(1,4,5)P}_3$ receptor.
This radiolabelled analogue was used in binding experiments with rat cerebellar membranes, the assay conditions are described in [275]. DL-[\(^{35}\)S]-Ins(1,4,5)P\(_3\)-1S was supplied by NEN-DuPont with a specific activity of ~ 81 Ci / mmol. Thus 1.03 nM of [\(^{35}\)S]-Ins(1,4,5)P\(_3\)-1S in an assay volume of 120 µl is equivalent to 22112 dpm. Figure 6.8 shows the displacement of [\(^{35}\)S]-Ins(1,4,5)P\(_3\)-1S by Ins(1,4,5)P\(_3\)-1S, and Figure 6.9 shows the Scatchard transformation of the same data.

![Figure 6.8](image)

**Figure 6.8** Displacement of [\(^{35}\)S]-Ins(1,4,5)P\(_3\)-1S by Ins(1,4,5)P\(_3\)-1S
Isotope-dilution analysis of [35S]InsP3-1S binding to rat cerebellar membranes

[35S]InsP3-1S = 22112 dpm/assay = 1.03 nM
95.1 ug membrane protein/assay

Scatchard analysis:

\( K_D = 44.0 \text{ nM} \)

\( B_{max.} = 20.73 \text{ pmol/mg} \)

Figure 6.9  Scatchard transformation of [35S]-Ins(1,4,5)P_3-1S binding data

These results indicate that the displacement of Ins(1,4,5)P_3-1S from cerebellar membranes is monophasic in contrast to Ins(1,4,5)PS_3, which has been found to be displaced in a biphasic mode \([275]\). This observation led to the suggestion that [35S]-Ins(1,4,5)PS_3 labels two different sites with equal affinity: the Ins(1,4,5)P_3 receptor and a site that displays low affinity for Ins(1,4,5)P_3. [35S]-Ins(1,4,5)P_3-1S on the other hand, being structurally closer to the natural ligand Ins(1,4,5)P_3 than [35S]-Ins(1,4,5)PS_3, appears to be specific for the Ins(1,4,5)P_3 receptor and does not interact with the low affinity binding site. This novel radioligand may thus be a better tool for examining the Ins(1,4,5)P_3 receptor than the radiolabelled trisphosphorothioate analogue.
6.5 Fluorescent Labelling

Fluorescent labelling methodology has already shown its versatility in the nucleic acid field \[^{374}\], where it is established as a valuable alternative to the use of radioactivity. The semisynthetic preparation of a fluorescent PtdIns analogue has been reported \[^{375}\], however, no fluorescent Ins(1,4,5)P\(_3\) analogue has yet been synthesised. We reasoned that such a compound may provide environmental information concerning receptor binding.

![Figure 6.10 Synthesis of fluorescently labelled DL-Ins(1,4,5)P\(_3\)-1S](image)

Nitrobenzoxadiazole (NBD) derivatives, such as the iodoacetate \(N\)-(2-(iodoacetoxy)ethyl)-\(N\)-methylamino-7-nitro-2,1,3-benzoxadiazole (IANBD), are unique in having long-wavelength fluorescein-like fluorescence spectral
properties, but with high environmental sensitivity of the quantum yield, coupled
with a relatively small molecular size \cite{376}. Such a probe appeared ideal to study
the interactions of a fluorescently tagged \textit{Ins}(1,4,5)P\textsubscript{3} with the intracellular
receptor and the metabolic enzymes 5-phosphatase and 3-kinase.

Reaction of the phosphorothioate analogue DL-\textit{Ins}(1,4,5)P\textsubscript{3}-1S with IANBD
proceeded smoothly to give the adduct 135, which was purified by ion exchange
chromatography on DEAE Sephadex A-25, and was eluted at \textit{ca.} 800 mmol dm\textsuperscript{-3}
TEAB (yield 57 % of a dark orange glass). \textsuperscript{31}P-NMR spectroscopy (Figure 6.11)
clearly showed that the signal for the 1-phosphorothioate group (\(\delta_P 42.1 \text{ ppm}\)) of
80 had disappeared. Instead, a new signal at \(\delta_P = 19.9 \text{ ppm}\) was found for 135.

![Figure 6.11 \textsuperscript{1}H-coupled \textsuperscript{31}P-NMR spectrum of 135](image)

\textit{Figure 6.11} \textsuperscript{1}H-coupled \textsuperscript{31}P-NMR spectrum of 135
Chemical shifts of about 20 ppm in the \(^{31}\text{P}-\text{NMR}\) are typical for S-alkyl phosphorothiolates. The signal appeared as a quartet with \(J = 9.5\) Hz, indicating coupling of the phosphorus nucleus to both C-1-H and the S-methylene protons. The two signals for the phosphate groups in positions 4 and 5 at 1.7 and 3.0 ppm remained unchanged (d, \(J = 8.5\)), proof that the alkylation was indeed selective at the more nucleophilic phosphorothioate.

The adduct 135 exhibited a UV spectrum consistent with the presence of an NBD chromophore and when excited at 460 nm showed the expected fluorescence at 540 nm.

![Excitation and fluorescence spectra](image)

**Figure 6.12 UV fluorescence spectrum of 135**
Compound 135 was potent at mobilising ATP-sequestered intracellular Ca²⁺ from permeabilised cells and was recognised by the Ins(1,4,5)P₃ receptor with surprisingly high affinity [only ca. 6 fold less than Ins(1,4,5)P₃] in spite of the bulky additional group. The synthesis of 135 thus provides the first example of a biologically active Ins(1,4,5)P₃ analogue labelled with a fluorescent reporter group.

Preliminary experiments on receptor site localisation with this probe were disappointing, the main problem was the lack of sensitivity of the NBD chromophore, which did not show any fluorescence enhancement on binding to the receptor protein. An explanation for this lack of sensitivity could be that after receptor binding the NBD probe is found in a hydrophilic environment (possibly the cytosol), which seems plausible since other 1-modified Ins(1,4,5)P₃ analogues with relatively bulky groups in this position (see Chapter 4.1) were also almost equipotent to Ins(1,4,5)P₃ in binding and calcium release studies, indicating that substituents at the 1-phosphate group show only little interaction with the receptor protein. To improve the fluorescence of 1-modified probes it would therefore be necessary to replace the environmentally sensitive NBD group with another chromophore like fluorescein whose fluorescence properties are only to a small degree dependent on the polarity of the solvent.

6.6 Pharmacology

All compounds whose synthesis is described in this chapter have now been pharmacologically evaluated:

Figure 6.13 summarises the Ca²⁺-release studies on DL-Ins(1,4,5)P₃-1S 80 and the fluorescent probe 135 (IP₃-1S-NBD). The assays were performed on electrically permeabilised SH-SY5Y neuroblastoma cells as described in [864], except that the cells were permeabilised using 3 pulses of a 3 μF capacitor rather than 12 pulses. The EC₅₀ values obtained under these conditions were 0.21 ± 0.02 μM for 80 and 0.64 ± 0.15 μM for 135. These results have to be seen in relation to the potency of the natural agonist D-Ins(1,4,5)P₃, which has EC₅₀ = 0.08 ± 0.02 μM. Considering that 80 was applied in racemic form and assuming
that the L-isomer is not biologically active, D-80 is almost equipotent to \( \text{lns}(1,4,5)\text{P}_3 \).

The kinetics of the \( \text{Ca}^{2+} \)-release were consistent with \( \text{lns}(1,4,5)\text{P}_3 \)-1S being a substrate for \( \text{lns}(1,4,5)\text{P}_3 \) 5-phosphatase and 3-kinase.

---

**Figure 6.13**

Ca\( ^{2+} \)-Release Profiles from Permeabilised SH-SY5Y Cells.
The Ca\textsuperscript{2+} mobilising properties of Ins(1,4,5)P\textsubscript{3}-1S have also been studied in platelets. For this assay, rabbit platelets were isolated and washed according to Murphy et al.\textsuperscript{[377]}. The platelets (10\textsuperscript{9} / ml) were suspended in an "intracellular like" buffer containing 5 mM ATP and permeabilised by treatment with saponin (40 µg / ml) for 1 min. The washed platelets were loaded with \textsuperscript{45}Ca\textsuperscript{2+} for 60 min and stimulated with the inositol phosphate analogue for 3 min at r.t. The remaining cell-associated \textsuperscript{45}Ca\textsuperscript{2+} was then determined by rapid filtration. The results for Ins(1,4,5)P\textsubscript{3}-1S are shown in Figure 6.14.

![Dose response curve to myo-inositol -4,5-bis phosphate-1-thiophosphate](image)

**Figure 6.14 Ca\textsuperscript{2+} mobilisation of Ins(1,4,5)P\textsubscript{3} and Ins(1,4,5)P\textsubscript{3}-1S in platelets**

The results of binding studies are shown in Figure 6.15. [\textsuperscript{3}H]-Ins(1,4,5)P\textsubscript{3} was displaced from cerebellar membranes by adding either Ins(1,4,5)P\textsubscript{3} or
Ins(1,4,5)P₃-1S. The IC₅₀ values obtained in this isotope dilution experiment were 22.7 nM and 37.6 nM for Ins(1,4,5)P₃ and Ins(1,4,5)P₃-1S, respectively.

Figure 6.15
7 Synthesis of D-Ins(1,4,5)P3-1S

7.1 Optical Resolution of 1-O-Allyl-2,3,6-tri-O-benzyl-myoinositol

Myo-inositol, a meso-compound, was used as starting material in the synthesis of Ins(1,4,5)P3-1S, and this analogue was initially obtained as the mixture of D- and L-enantiomers. In order to prepare the optically pure analogue of naturally occurring D-Ins(1,4,5)P3, it was necessary to resolve a precursor to synthesise 1D-Ins(1,4,5)P3-1S.

Thus racemic 1-O-allyl-2,3,6-tri-O-benzyl-myoinositol 127 was converted into its 4,5-bis-(-)ω-camphanate ester using (-)-camphamic acid chloride / pyridine. The camphanate method was chosen because (-)-camphamic acid chloride is commercially available in high optical purity (98 %) and camphanates are often highly crystalline, thus making separation of the diastereoisomers by crystallisation rather than by tedious chromatographic means possible. Camphanate esters have already been successfully employed for the resolution of various inositol derivatives [122,123,248,378-380]. With (+)-camphamic acid chloride now commercially available, the diastereoisomers which can not be crystallised from (-)-camphamic acid mixtures are now also accessible, making this route even more generally applicable. However, (+)-camphamic acid chloride is markedly more expensive than (-)-camphamic acid chloride (£ 25.70 for 250 mg vs. £ 8.60 for 1 g [370]).

The biscamphanate 136 of the protected D-isomer 137 readily crystallised initially from the diastereomeric mixture, however the crystalline biscamphanate 143 of the protected inositol, 144, could also be obtained by keeping the mother liquor left from the crystallisation of 136 at -20°C for several days. The supernatant was then filtered off and the solid that had formed was dissolved in hot ether. After leaving the solution in the refrigerator for two days crystals had appeared which were collected to give 143.

The 1H-NMR resonances (Figure 7.1) of the camphanate methyl groups were used as an initial guide to the efficiency of this resolution. After base-deblocking of the camphanate moieties both enantiomers of 1-O-allyl-2,3,6-tri-O-benzyl-
Figure 7.1  $^1$H-NMR of 136
myo-inositol were obtained. The absolute configuration of these enantiomers was assigned by conversion of one of them (144) to the known triol D-(-)-1,2,4-tri-O-benzyl-myoinositol 146 \cite{168} by isomerisation of the allyl group and subsequent removal of the cis-prop-1-enyl group with acid.

The melting point (97-98°C) and optical rotation ([\(\alpha\])\(_D\)\(^{18}\) = +21.5°) of 1D-1-O-allyl-2,3,6-tri-O-benzyl-myoinositol 137 are in good agreement with data for this compound (m.p. 98°C, [\(\alpha\])\(_D\)\(^{18}\) = +20°) measured by Gigg et al., who reported the synthesis of the resolved diol by a different route whilst our studies were in progress \cite{380}.

![Chemical structures](image)

Figure 7.2 Optical resolution of 127 and synthesis of D-Ins(1,4,5)P\(_3\)-1S
7.2 Synthesis of D-myo-inositol 4,5-bisphosphate 1-phosphorothioate

The 1D-isomer was used to prepare optically active 1D-Ins(1,4,5)P\textsubscript{3}-1S, by following the route outlined for the racemic compound. Thus, D-1-O-allyl-2,3,6-tri-O-benzyl-myo-inositol 137 was isomerised to the cis-1-O-prop-1-enyl derivative 138 (m.p. 116-118°C, [\alpha]_D^{11} = +40.6°) using Bu\textsuperscript{t}OK / DMSO and the product was phosphorylated to give the corresponding optically active protected 4,5-bisphosphate 139 (m.p. 131-132°C, [\alpha]_D^{19} = +11.5°). Removal of the propenyl group with 1 M HCl / methanol (1:5) and thiophosphorylation of the resulting alcohol 140 generated D-2,3,6-tri-O-benzyl-1-O-[bis(2-cyanoethoxy)thiophospho]-4,5-bis[di(2-cyanoethoxy)phospho]-myo-inositol 141 (neither 140 nor 141 could be crystallised). 141 was then deblocked with sodium in liquid ammonia to furnish D-myo-inositol 4,5-bisphosphate 1-phosphorothioate 142 in 44 % yield after ion-exchange chromatography. The optical rotation for this compound was found to be [\alpha]_D^{21} = -42.7° at pH 9.4.

7.3 Pharmacology

The pharmacological properties of D-Ins(1,4,5)P\textsubscript{3} are currently being evaluated. The optically active precursor 140 is now being applied in the preparation of radiolabelled [\textsuperscript{35}S]-D-Ins(1,4,5)P\textsubscript{3}-1S in cooperation with NEN-DuPont.
8 Synthesis of Novel Ins(1,4,5)P₃ 5-Phosphatase Inhibitors

Inhibitors of the metabolic enzymes 5-phosphatase and 3-kinase are of great current interest to the biochemist. Especially valuable for metabolic studies are inhibitors that selectively block the enzyme activity and do not interact with the Ins(1,4,5)P₃ receptor. Such compounds have not been prepared before, although in parallel with these studies Dr. Changsheng Liu in our group synthesised L-chiro-Ins(1,4,6)PS₃ [381], an analogue that showed similar characteristics. Thus, our aim was to develop potent 5-phosphatase inhibitors unable to release calcium by improving the binding properties of compounds known to be moderately potent, non calcium-mobilising inhibitors.

The cytosolic 5-phosphatase is relatively non-specific and recognises not only D-Ins(1,4,5)P₃ and D-Ins(1,3,4,5)P₄, which are the natural substrates of this enzyme, but also synthetic inositol phosphates such as L-Ins(1,4,5)P₃, Ins(1,3,5)P₃ and DL-Ins(2,4,5)P₃ [324]. Unlike D-Ins(1,4,5)P₃, DL-Ins(2,4,5)P₃ and DL-Ins(1,3,4,5)P₄ however, L-Ins(1,4,5)P₃ [= D-Ins(3,5,6)P₃] and Ins(1,3,5)P₃ are competitive inhibitors of this enzyme with Kᵢ values of 39 and 45 μM, respectively. In another study using human erythrocyte 5-phosphatase L-Ins(1,4,5)P₃ has also been found to inhibit the enzyme with a Kᵢ of 124 μM [271].

Cooke et al. [271] observed that the trisphosphorothioate analogue of Ins(1,4,5)P₃, DL-Ins(1,4,5)PS₃, acted as a potent competitive inhibitor of 5-phosphatase (Kᵢ 6 μM, cf. Ins(1,4,5)P₃: Kᵢ = 40 μM). Thus the substitution of phosphate groups by phosphorothioate moieties resulted in increased binding properties (i.e. decrease of the Kᵢ value) of the analogue. Racemic Ins(1,4,5)PS₃ does however mobilise intracellular calcium [269,326] and was therefore considered unsatisfactory with regard to the objective outlined above.

Considering the effect that replacement of phosphate groups by phosphorothioate groups had on Ins(1,4,5)P₃, we reasoned that phosphorothioate analogues of the competitive non calcium-mobilising, but weak 5-phosphatase inhibitors L-Ins(1,4,5)P₃ and Ins(1,3,5)P₃ would prove to be more potent inhibitors than the parent compounds and this idea was pursued by the synthesis of these analogues.
8.1 Synthesis of L-

\[
\text{myo-inositol 1,4,5-trisphosphorothioate}
\]

The L-enantiomer of 1-O-allyl-2,3,6-tri-O-benzyl-\textit{myo}-inositol 144 was isomerised to the corresponding 1-O-cis-(prop-1-enyl) derivative 145 and deprotected to give 1D-1,2,4-tri-O-benzyl-\textit{myo}-inositol 146. Melting point (116-118°C) and optical rotation ([\alpha]_D^{18} = -10.1°) measured for this previously described triol were in good agreement with the reported values (m.p. 118-120°C, [\alpha]_D^{18} = -9.0°) [168]. 146 was then thiophosphorylated, generating 1L-2,3,6-tri-O-benzyl-1,4,5-tris-[di(2-cyanoethoxy)thiophosphate]-\textit{myo}-inositol 147. Debloking with sodium / liquid ammonia furnished 1L-\textit{Ins}(1,4,5)PS_3 148, which
was purified by ion-exchange chromatography. The $^{31}$P-NMR spectrum of 148 showed as expected signals at 44.82, 44.69 and 42.06 ppm for the three phosphorothioate groups.

It was observed that treatment of the crude reaction product with Dowex 50 cation-exchange resin (H$^+$-form, added to reduce the ionic strength of the solution and thus facilitate purification by ion-exchange chromatography) resulted in decomposition of the deprotected phosphorothioate. Since decomposition was not reported for Dowex treatment of deblocked phosphates \cite{382} and a strong smell of sulphur could be noticed after Dowex treatment of the phosphorothioate, we assume that the cause of the decomposition is desulphurisation.

![Figure 8.2 $^{31}$P-NMR spectrum of 148](image)

Figure 8.2 $^{31}$P-NMR spectrum of 148
8.2 Synthesis of 2,4,6-tri-O-butyryl-myoinositol

Myo-inositol was used as starting material to prepare 2,4,6-tri-O-butyryl-myoinositol, a compound which was of interest to us for studies on the enantiospecific deacylation of inositol derivatives by enzymes. Selective protection of the 1-, 3- and 5-hydroxyl groups of myo-inositol can be effected by converting the starting material into its orthoformate ester 150 by Billington's modification \(^{[161]}\) of the method devised by Lee and Kishi \(^{[158]}\), \(i.e.\) DMF rather than DMSO was used as solvent for the reaction.

\[
\begin{align*}
\text{HO} & \quad \text{HO} \quad \text{HO} \\
\text{HO} & \quad \text{HO} \quad \text{HO}
\end{align*}
\]

Thus, the orthoformate was converted into the orthoformate tributyrate 151 by treatment with butyric anhydride in pyridine with DMAP as acylation catalyst. This compound was then treated with 80% trifluoroacetic acid to remove the orthoformate ester. Tlc (ether / hexane 2:1) showed the conversion of the starting material \((R_f 0.74)\) into a major and a minor product \((R_f 0.12 \text{ and } 0.28, \text{ respectively})\). Separation of the product mixture gave the desired tributyrate 152 as the major product (63 %) and the minor product 153 (14 %).

The \(^1\)H-spectrum of 153 showed the presence of 3 butyrate esters (with corresponding signals in the \(^{13}\)C-spectrum at 173.11, 173.30, 174.66 ppm for the butyrate carbonyl group). It was also obvious from the \(^1\)H-NMR spectrum that unlike 152, 153 was not a symmetrical compound. Only two \(D_2O\)-exchangeable protons were found, which excluded the possibility that a migration of the butyrate esters had occurred and implied the presence of a substituent in the 1(3)-position (substitution at the 5-position would have given a symmetrical compound). The only two NMR signals unaccounted for were assigned to this additional substituent: a singlet at 7.94 ppm in the \(^1\)H-spectrum (which was
Figure 8.4 \(^1\)H-NMR spectrum of 153
Figure 8.5  $^{13}$C-NMR spectrum of 153
assigned as an aldehyde proton signal) and a $^{13}$C-NMR doublet at 159.61 ppm (which was assigned as an aldehyde carbonyl signal). From this, it was concluded that the additional substituent in the 1-position was a formyl group derived from incomplete deprotection of the orthoformate ester.

After the presence of a formyl group in the 1-position had been established, the ring protons had to be assigned. The $^1$H-signal at 5.08 ppm, a dd with coupling constants of 10.4 Hz and 2.7 Hz, is found considerably further downfield than the other proton signal (3.85 ppm) showing axial / axial and axial / equatorial coupling ($J = 10.1, 2.9$ Hz). These signals were therefore assigned to C-1-H and C-3-H, respectively, the formate moiety causing the downfield shift of C-1-H. The signal at 3.65 ppm (dd, $J 9.8, 9.8$ Hz), which sharpened after D$_2$O exchange (indicating the presence of a hydroxyl group on the same ring carbon), was assigned to C-5-H. The C-2-H peak at 5.62 ppm was easily identified by the small J values, and the remaining two signals at 5.2 and 5.4 ppm (shifted downfield because of butyrate ester substitution) can be ascribed to C-4-H and C-6-H.
The Cl-mass spectrum, showing an (M+H)+ peak at m/z 419, is in agreement with the assignment of 153 as DL-2,4,6-tri-O-butyryl-1-O-formyl-myo-inositol.

Obviously, if this deprotection can be controlled it could be very useful as a way of partially deprotecting the orthoformate, thus affording a protected inositol which could be used for further manipulations.

Enzymes have been used by a number of groups to prepare optically active inositol derivatives (see Chapter 2.2). Baudin et al. [159] succeeded in enantioselectively converting 4,6-di-O-butyryl-myo-inositol orthoformate into the (-)-monobutyrate (ee > 95 %, yield 83 %) by employing porcine liver esterase (PLE). We wanted to investigate whether the major product obtained by deprotection of the tributyrate orthoformate, 2,4,6-tri-O-butyryl-myo-inositol 152, is also a substrate for this enzyme, and whether the enzyme would stereoselectively deacylate this compound to give either the D-2,4-di-O-butyrate or the D-2,6-di-O-butyrate, the latter being a useful intermediate in the synthesis of optically active D-Ins(1,3,4,5)P_4. This project was conducted in co-operation with Stephen J. Mills in our group.

152 was thus digested with porcine liver esterase (Sigma) in 0.1 M phosphate buffer, pH 7. However, even after several days of incubation, no deacylation product could be detected by tlc or NMR and the project was abandoned.

8.3 Synthesis of Bis(benzyloxy)(diisopropylamino) phosphine

For the phosphitylation of compounds prepared during the later stages of this study bis(benzyloxy)(diisopropylamino)phosphine [225] was preferred to the corresponding 2-cyanoethoxy phosphine because the resulting benzyl phosphate and phosphorothioate triesters tend to be more crystalline. Bis(benzyloxy)-(diisopropylamino)phosphine was easily accessible in two steps from phosphorus trichloride and was applied to the synthesis of inositol phosphates by Yu and Fraser-Reid [169] after initially having been developed for use in oligonucleotide chemistry and automated DNA synthesis [225].
Diisopropylamino dichlorophosphine 156 was prepared by the method of Tanaka et al. [383] by adding two equivalents of diisopropylamine to a solution of PCl₃ in dry ether at -78°C. The crude product was purified by distillation under reduced pressure (δ_P 166.4).

Reaction of 156 with two equivalents of benzyl alcohol in the presence of two equivalents of triethylamine then gave bis(benzyloxy)(diisopropylamino)phosphine 157 (δ_P 145.24) which could be purified by flash chromatography.

\[
\begin{align*}
\text{PCl}_3 & \quad \rightarrow \quad \text{Cl} \quad \text{P} \quad \text{N} \quad (\text{Pr}^i)_2 \\
156 & \quad \rightarrow \quad \text{Bn} \quad \text{O} \quad \text{P} \quad \text{N} \quad (\text{Pr}^i)_2 \\
& \quad 157
\end{align*}
\]

Figure 8.7 Synthesis of bis(benzyloxy)(diisopropylamino)phosphine

8.4 The synthesis of myo-inositol 1,3,5-trisphosphorothioate

Myo-inositol orthoformate 150 was then also used as the starting material for the synthesis of Ins(1,3,5)PS₃:

The remaining free hydroxyl groups of 150 were protected as p-methoxybenzyl ethers using p-methoxybenzyl chloride / sodium hydride in DMF. The orthoester was removed by treatment of the fully protected compound 154 with 80% trifluoroacetic acid yielding the desired 2,4,6-tri-O-p-methoxybenzyl-myoinositol. This compound was thiophosphorylated with bis(benzyloxy)(diisopropylamino)phosphine 157 to give the fully protected trisphosphorothioate 158, which was then deprotected with sodium in liquid ammonia to give Ins(1,3,5)PS₃ 159 as the triethylammonium salt after ion-exchange chromatography. ³¹P-NMR of this symmetrical trisphosphorothioate showed two signals at 47.74 and 46.55 ppm integrating for one and two P-nuclei, respectively (Figure 8.9). Both signals were doublets with J = 10.1 Hz. The corresponding ³J_H-P coupling constants were also observed in the ¹H-NMR spectrum for the signals at 4.08 ppm (1 H, ddd, J 10.3, 9.2, 9.2, C-5-H), and 4.19 ppm (2 H, ddd, J 10.3, 10.3, 2.5, C-1-H, C-3-H).
$^{13}$C-NMR spectrum of 159 showed $^2J_{\text{C-O-P}}$ coupling constants of 6.6 Hz for the signals at 74.29 ppm (2 C, dd, J 6.6 Hz, C-1, C-3) and 78.41 ppm (1 C, dd, J 6.6 Hz, C-5).

Figure 8.8  Synthesis of myo-inositol 1,3,5-trisphosphorothioate
The potential 5-phosphatase inhibitors L-myoinositol 1,4,5-trisphosphorothioate 148 and myo-inositol 1,3,5-trisphosphorothioate 159 have now been pharmacologically evaluated. The results showed that both 148 and 159 are highly potent inhibitors of the metabolic enzyme Ins(1,4,5)P3 5-phosphatase from human erythrocyte membranes, with submicromolar $K_i$ values of 0.43 $\mu$M and 0.50 $\mu$M, respectively [348].

As our objective was to prepare non-calcium mobilising 5-phosphatase inhibitors, the two trisphosphorothioates were also examined for their Ca$^{2+}$ releasing abilities. Figure 8.10 shows the effect of 148 and 159 on the concentration of free calcium in platelets in comparison to Ins(1,4,5)P$_3$. It is surprising to see 159 causing any mobilisation of calcium in this system, since
studies on calcium mobilisation in T-cells showed that Ins(1,3,5)PS₃ did not release any calcium in this system.

The Calcium releasing abilities of the two 5-phosphatase inhibitors

![Graph showing calcium release](image)

**Figure 8.10 Calcium mobilisation of 148 and 159 in platelets**

Both compounds were also assayed for their interactions with Ins(1,4,5)P₃ 3-kinase. However, whereas L-Ins(1,4,5)PS₃ is recognised surprisingly well by the enzyme (apparent $K_i = 1.46 \, \mu M$), Ins(1,3,5)PS₃ shows hardly any interaction (apparent $K_i 247 \, \mu M$). Thus, whilst both L-Ins(1,4,5)PS₃ and Ins(1,3,5)PS₃ are potent 5-phosphatase inhibitors, Ins(1,3,5)PS₃ has the advantage of being selective for this enzyme.

The pharmacological data of 5-phosphatase inhibitors are summarised below:
<table>
<thead>
<tr>
<th>inhibitor / substrate</th>
<th>5-phosphatase</th>
<th>3-kinase</th>
<th>Ins(1,4,5)P₃-receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-2,3-diphosphoglycerate</td>
<td>350 or 978 μM</td>
<td>inhibitor</td>
<td>no interaction</td>
</tr>
<tr>
<td>L-cho-Ins(1,4,6)P₃</td>
<td>44 μM</td>
<td>no interaction</td>
<td>no interaction</td>
</tr>
<tr>
<td>L-cho-Ins(1,4,6)PS₃₃</td>
<td>0.3 μM</td>
<td>no interaction</td>
<td>no interaction</td>
</tr>
<tr>
<td>Ins(1,3,5)P₃</td>
<td>45 μM</td>
<td>no interaction</td>
<td>no interaction</td>
</tr>
<tr>
<td>Ins(1,3,5)PS₃₃ 159</td>
<td>0.5 μM</td>
<td>no interaction</td>
<td>no interaction</td>
</tr>
<tr>
<td>DL-Ins(1,4,5)PS₃₃</td>
<td>1.7 μM</td>
<td>inhibitor</td>
<td>agonist</td>
</tr>
<tr>
<td>DL-Ins(1,4,5)P₃₃-4,5S</td>
<td>1.4 μM</td>
<td>substrate</td>
<td>agonist</td>
</tr>
<tr>
<td>DL-Ins(1,4,5)P₃₃-5S</td>
<td>6.8 μM</td>
<td>substrate</td>
<td>agonist</td>
</tr>
<tr>
<td>L-Ins(1,4,5)P₃</td>
<td>39 or 124 μM</td>
<td>40.3 μM</td>
<td>no interaction</td>
</tr>
<tr>
<td>L-Ins(1,4,5)PS₃₃ 148</td>
<td>0.43 μM</td>
<td>1.46 μM</td>
<td>no interaction</td>
</tr>
<tr>
<td>D-Ins(1,4,5)P₃, Kₘ</td>
<td>19 μM</td>
<td>substrate</td>
<td>agonist</td>
</tr>
</tbody>
</table>
The Synthesis of Scy/lo-Inositol Analogues

Modified analogues of Ins(1,4,5)P3 are of great current interest as potential Ins(1,4,5)P3 receptor antagonists and enzyme inhibitors. We chose to pursue scy/lo-inositol analogues in this regard, where the hydroxyl group at C-2 has a configuration inverse to that of the corresponding myo-inositol, i.e. the 2-hydroxyl group is in an equatorial rather than an axial position. 2-Modified Ins(1,4,5)P3 analogues including 2-fluoro and 2-deoxy derivatives have already been shown to have interesting properties (see Chapter 4).

The most obvious target compound was scy/lo-Ins(1,2,4)P3, the scy/lo-analogue of Ins(1,4,5)P3. In addition to the preparation of scy/lo-Ins(1,2,4)P3, the synthesis of scy/lo-Ins(1,2,4,5)P4 and its tetrakisphosphorothioate analogue was devised, but for a different reason: both compounds are analogues mimicking the phosphate arrangement in Ins(1,3,4,6)P4, which has been shown to act as a partial agonist at the Ins(1,4,5)P3 receptor. We were therefore interested in these scy/lo-inositol analogues to find out more about structural requirements for compounds acting as partial agonists.

9.1 Synthesis of 1-0-Allyl-3,6-di-0-benzyl-scy/lo-inositol

Thus, 1-0-allyl-3,6-di-0-benzyl-4,5-0-isopropylidene-myo-inositol 93, an intermediate with a free 2-hydroxyl group used to synthesise 1-0-allyl-2,3,6-tri-0-benzyl-myo-inositol, was employed to generate scy/lo-inositol compounds.

The axial 2-hydroxyl group was substituted in two steps: first, by action of trifluorosulphonic anhydride in pyridine to afford the triflate 160. This compound was only partially purified and characterised because of its instability (triflates have been reported to decompose on silica gel columns). Secondly, the triflate moiety was then displaced by substitution with caesium acetate in DMF in a reaction which proceeded with inversion of configuration at the 2-position of the inositol ring to give the scy/lo-inositol derivative 161. 1H-NMR (Figure 9.1) showed that the signal for the C-2 proton at 5.15 ppm (easily assigned to C-2-H by its downfield shift from all the other ring proton signals due to acetate-substitution on C-2) is a dd with J1 = J2 = 8.8. The coupling constants arise from axial-axial coupling to C-1-H and C-3-H, which made the assignment of the
compound as being of the scyllo-configuration safe. The C-2-H_{eq} - C-1(3)-H_{ax} arrangement found in myo-inositol derivatives usually results in J values in the order of 2 - 5 Hz according to the Karplus equation.

Figure 9.1  \(^1\)H-NMR spectrum of 161 (expansion 3.4 ppm to 5.3 ppm)
Removal of acetate and isopropylidene protecting groups by saponification followed by acid hydrolysis gave 1-0-allyl-3,6-tri-0-benzyl-scyllo-inositol 164 either via 162 or 163.

The synthesis of 1-0-allyl-3,6-di-0-benzyl-4,5-0-isopropylidene-scyllo-inositol 162 had previously been reported by Gigg et al. [168], who obtained this compound as a by-product (30 % yield) in the reduction of the 2-inosose derivative with sodium borohydride (the major product in this reaction was 1-0-allyl-3,6-di-0-benzyl-4,5-0-isopropylidene-myoinositol 93). The compound was presumed to be the scyllo-inositol derivative and melting point (105-106°C) and microanalysis data were reported. This derivative was, however, not further investigated by the authors.

9.2 Scyllo-Inositol 1,2,4-trisphosphate

Figure 9.2 Synthesis of DL-scyllo-inositol 1,2,4-trisphosphate 166
Phosphitylation of 164 with bis(benzylxyloxy)(diisopropylamino)phosphine / 1H-tetrazole followed by oxidation with Bu'OOH furnished 165. The 1H-coupled 31P-NMR spectrum for this fully protected trisphosphate showed a quartet integrating for 2 P at -1.72 (J = 7.9 Hz) and a second quartet with the same coupling constant which integrated for 1 P at -1.47. The corresponding signals in the 1H-NMR could not be observed due to overlapping signals from the CH2Ph and CH2CH=CH2 protons.

Figure 9.3 1H-coupled 31P-NMR spectrum of 165
Treatment with sodium-liquid ammonia deblocked both the benzyl and allyl protecting groups in one step. Ion-exchange chromatography on Q Sepharose Fast Flow gave pure 166.

9.3 *Scyllo*-Ins(1,2,4,5)P$_4$ and *Scyllo*-Ins(1,2,4,5)PS$_4$

We were interested in *scyllo*-inositol 1,2,4,5-tetrakisphosphate and its phosphorothioate analogue for two different reasons:

As mentioned in Chapter 4.2, Ins(1,3,4,6)P$_4$ has been found to be a good partial agonist at the Ins(1,4,5)P$_3$ receptor $^{[94,347]}$. We wanted to establish the individual contributions made by the 1,3,4,6-phosphate arrangement of this compound [which is also present in *scyllo*-Ins(1,2,4,5)P$_4$] and the axial hydroxyl group (which is missing in the *scyllo*-inositol analogues) towards this partial agonist behaviour.

The second motive to prepare these *scyllo*-inositol derivatives was the fact that myo-inositol 1,2,4,5-tetrakisphosphate has been found to be a very potent inhibitor of 5-phosphatase with a $K_i$ of 2.9 $\mu$M $^{[330]}$. We reasoned that the symmetrical *scyllo*-inositol analogue of this compound may show inhibitory properties similar to Ins(1,2,4,5)P$_4$ and that enzyme binding may be increased by replacing the phosphate groups with phosphorothioate moieties (see Chapter 8).

The synthesis of *scyllo*-Ins(1,2,4,5)P$_4$ 170 and *scyllo*-Ins(1,2,4,5)PS$_4$ 172 was relatively straightforward (Figure 9.4). 1-0-Allyl-3,6-di-O-benzyl-*scyllo*-inositol 164 was isomerised to the cis-prop-1-enyl derivative 167 using Bu$^+$OK / DMSO. The propenyl group of this intermediate was then removed by acidic hydrolysis to give the meso-compound 1,4-di-O-benzyl-*scyllo*-inositol 168. Phosphitylation with bis(benzyloxy)(diisopropylamino)phosphine followed by oxidation or sulphoxidation gave the fully protected tetrakisphosphate and phosphorothioate, respectively. Both compounds showed a single peak in the $^{31}$P-NMR spectrum (at -3.74 and 67.77 ppm, respectively), which was to be expected for these highly symmetrical derivatives. Deprotection with sodium in liquid ammonia afforded *scyllo*-Ins(1,2,4,5)P$_4$ 170 and *scyllo*-Ins(1,2,4,5)PS$_4$ 172, respectively.
9.5 Pharmacology

**DL-Scylo-Inositol 1,2,4-trisphosphate**

The ability of DL-scyllo-inositol 1,2,4-trisphosphate to mobilise sequestered calcium has now been studied in various tissues. Results for SH-SY5Y cells showed that this analogue is a full agonist almost equipotent to Ins(1,4,5)P₃ with EC₅₀ = 130 nM.

The dose response curves obtained using T-cells (Figure 9.5) and rabbit platelets (Figure 9.6) are shown below.
Figure 9.5  Calcium mobilisation of DL- scyllo-inositol 1,2,4-trisphosphate (□) and DL-2-deoxy-2-fluoro-myoinositol 1,4,5-trisphosphate (○) in T-cells

The fact that scyllo-inositol 1,2,4-trisphosphate is of a potency similar to Ins(1,4,5)P₃ is not surprising since other 2-modified Ins(1,4,5)P₃ analogues have also been found to be good agonists (see Chapter 4.1). The loss of activity observed when substituting the axial 2-hydroxyl group with an equatorial hydroxyl group is comparable to the effect caused by the deletion of this group [in 2-deoxy-Ins(1,4,5)P₃]. In each case, the modified compound in racemic form is ca. 2.5 times less active than D-Ins(1,4,5)P₃. Assuming that the L-isomers are not biologically active, the differences in activity between 2-modified analogues and Ins(1,4,5)P₃ are indeed minute, indicating that the 2-hydroxyl group does not contribute significantly towards receptor binding.
Dose response curve to scyllo—inositol trisphosphate

![Dose response curve to scyllo—inositol trisphosphate](image)

Figure 9.6 Ca\(^{2+}\) mobilisation of scyllo-Ins(1,2,4)P\(_3\) in platelets

The interactions of DL-scyllo-inositol 1,2,4-trisphosphate \(166\) with Ins(1,4,5)P\(_3\) 3-kinase and 5-phosphatase have also been examined. \(166\) was found to be a good substrate for 3-kinase (\(K_i\) 4.0 \(\mu\)M) and a moderately potent inhibitor of 5-phosphatase (\(K_i\) 24.2 \(\mu\)M).

**Scyllo-Inositol 1,2,4,5-tetrakisphosphate**

Scyllo-Ins(1,2,4,5)P\(_4\) was a very potent full agonist (\(EC_{50} = 82.56\) nM) at the Ins(1,4,5)P\(_3\) receptor [cf. Ins(1,4,5)P\(_3\): \(EC_{50} = 52\) nM], and binding data for this compound (obtained by displacing \[^3\text{H}\]-Ins(1,4,5)P\(_3\) from specific sites on bovine adrenal cortex membranes) are equally good (\(IC_{50} = 14.37\) nM, \(K_i = 10.82\) nM).
compound (obtained by displacing [3H]-Ins(1,4,5)P$_3$ from specific sites on bovine adrenal cortex membranes) are equally good (IC$_{50}$ = 14.37 nM, K$_i$ = 10.82 nM). The fact that unlike Ins(1,3,4,6)P$_4$, scyllo-Ins(1,2,4,5)P$_4$ does not show any partial agonist properties, is clearly due to the only difference between these two molecules, i.e. the stereochemistry of the 2-hydroxyl group of Ins(1,3,4,6)P$_4$, which is equatorial rather than axial in scyllo-Ins(1,2,4,5)P$_4$. Keeping in mind the alternative binding conformations that have been suggested for Ins(1,3,4,6)P$_4$ (Chapter 4.2), it is evident that the axial pseudo-3(6)-hydroxyl group arrangement does indeed contribute significantly to the partial agonist properties of this compound [but see below for Ins(1,3,4,6)PS$_4$].

**Scyllo-Inositol 1,2,4,5-tetrakisphosphorothioate**

Scyllo-Ins(1,2,4,5)PS$_4$ was found to be a partial agonist at the Ins(1,4,5)P$_3$ receptor in saponin-permeabilised SH-SY5Y cells with EC$_{50}$ = 1.6 μM. This compound is able to mobilise some 80% of Ins(1,4,5)P$_3$ sensitive calcium.

It is very surprising that scyllo-Ins(1,2,4,5)PS$_4$ is a partial agonist, since the parent compound scyllo-Ins(1,2,4,5)P$_4$ does not share these pharmacological characteristics. From what is known to date, one can only speculate as to why scyllo-Ins(1,2,4,5)PS$_4$ displays partial agonist properties. Comparison with the, now established, partial agonists L-chiro-Ins(2,3,5)PS$_3$ and 6-deoxy-Ins(1,4,5)PS$_3$ (which mobilise 34% and 42% of Ins(1,4,5)P$_3$ sensitive calcium, respectively) [384] shows that all compounds are phosphorothioate analogues of phosphate derivatives who are relatively potent full agonists. It therefore appears that substitution of phosphate groups with phosphorothioate moieties is a major element in creating compounds with partial agonist characteristics. However, since Ins(1,4,5)PS$_3$ is a full agonist, it can also be concluded that by replacing the 1-, 4-, and 5-phosphate groups with phosphorothioates alone, one does not obtain a partial agonist. Of considerable importance is also the observation that Ins(1,3,4,5)PS$_4$ is not a partial agonist.

Binding and Ca$^{2+}$ release data for scyllo-Ins(1,2,4,5)P$_4$ and scyllo-Ins(1,2,4,5)PS$_4$ are summarised below:
The results of calcium release studies performed on saponin-permeabilised rabbit platelets and T-cells are shown in Figure 9.7 and Figure 9.8.

![Dose response curves to scyllo-inositol-1,2,4,5-tetrakis phosphate & tetrakisthiophosphate](image)

**Figure 9.7** Calcium mobilisation of *scyllo*-Ins(1,2,4,5)P₄ and *scyllo*-Ins(1,2,4,5)PS₄ in platelets
Figure 9.8  Calcium mobilisation of Ins(1,4,5)P$_3$ (o), scylo-Ins(1,2,4,5)P$_4$ (•) and scylo-Ins(1,2,4,5)PS$_4$ (+) in T-cells

The interactions of these Ins(1,4,5)P$_3$ analogues with 5-phosphatase and 3-kinase are currently being investigated.
A number of fluorinated analogues of \( \text{Ins}(1,4,5)P_3 \) have been prepared, including the 2-fluoro analogues 2-deoxy-2-fluoro-scyllo-\( \text{Ins}(1,4,5)P_3 \) \(^{103}\) and 2-deoxy-2,2-difluoro-\( \text{Ins}(1,4,5)P_3 \) \(^{104}\) (see Chapter 3.3).

The synthesis of 2-deoxy-2-fluoro-\( \text{Ins}(1,4,5)P_3 \) \([2-F-\text{Ins}(1,4,5)P_3]\), the 2-fluoro analogue most similar to \( \text{Ins}(1,4,5)P_3 \), has however not been reported as yet. With regard to the interesting properties that both \(^{103}\) and \(^{104}\) displayed, we reasoned that an analogue resembling \( \text{Ins}(1,4,5)P_3 \) even closer like 2-F-\( \text{Ins}(1,4,5)P_3 \) may show improved characteristics as compared to the former analogues.

\[
\begin{align*}
\text{2-F-scyllo-Ins(1,4,5)P_3} & \\
\text{2,2-F_2-Ins(1,4,5)P_3} & \\
\text{2-F-myio-Ins(1,4,5)P_3} &
\end{align*}
\]

Figure 10.1 Analogues of \( \text{Ins}(1,4,5)P_3 \) fluorinated at the 2-position

1-O-allyl-3,6-di-O-benzyl-4,5-O-isopropylidene-scyllo-inositol, an intermediate in the synthesis of scyllo-inositol analogues of \( \text{Ins}(1,4,5)P_3 \) (see above), was chosen as the starting material to synthesise 2-F-\( \text{Ins}(1,4,5)P_3 \). An earlier attempt to synthesise 2-F-\( \text{Ins}(1,4,5)P_3 \) from the same precursor by fluorination with DAST failed \(^{306}\) because the reaction unexpectedly proceeded with retention of configuration, yielding the same 2-deoxy-2-fluoro-scyllo-inositol derivative which was obtained by DAST fluorination of the corresponding myo-inositol derivative. This may be an indication for steric effects or neighbouring group participation in the reaction mechanism, as DAST fluorinations usually give an inverted product and, in the special case of the reaction of 1,2,3,4,5-penta-O-benzyl-scyllo-inositol with DAST \(^{312}\), the fluorinated product did indeed have the myo-inositol configuration.
DL-1-O-Allyl-3,6-di-O-benzyl-4,5-isopropylidene-scyllo-inositol 162 was isomerised to the 1-O-cis-prop-1-enyl derivative 173 with Bu4OK / DMSO and converted into the triflate 174 by treatment with trifluoromethanesulfonic anhydride in pyridine. Substitution of the sulphonate at the 2-position with dry tetrabutylammonium fluoride gave 3,6-di-O-benzyl-2-deoxy-2-fluoro-4,5-isopropylidene-1-O-(cis-prop-1-enyl)-myo-inositol 175.

Observation of $^{2}J_{HF} = 50.0$ Hz and $^{3}J_{HF} = 29.5$ Hz in the $^{1}$H-NMR (Figure 10.3) and the $^{19}$F-NMR (Figure 10.4) spectra of this compound confirmed the presence of an axial 2-fluorine atom. The $^{2}J_{HF}$ and $^{3}J_{HF}$ coupling constants measured are in good agreement with those reported for 2-deoxy-2-fluoro-myoinositol $^{[312]}$, where $^{2}J_{HF}$ and $^{3}J_{HF}$ were found to be 52.1 Hz and 28.8 Hz, respectively. Inositol derivatives bearing equatorial fluorine atoms (e.g. 1-deoxy-1-fluoro-scyllo-inositol $^{[307,311]}$) show similar coupling constants for $^{2}J_{HF}$ (50.4 Hz), but $^{3}J_{HF}$ is much smaller (12.6 Hz) than the J value found in compounds with an axial fluorine.

Additional proof for the myo-inositol conformation of the product obtained are the $^{3}$J constants (2.4 Hz) found in the coupling between C-2-H and C-1(3)-H, which indicate an equatorial-axial arrangement of the respective protons.
Figure 10.3  $^1$H-NMR spectrum of 175 (expansion 1.4 - 5.1 ppm)
Figure 10.4 $^{19}$F-NMR spectrum of 175 (signal at -210.21 ppm)

Removal of the acid labile propenyl and isopropylidene protecting groups gave 176, which was phosphitylated with bis(benzoyloxy)(diisopropylamino) phosphine and oxidised to furnish 177. Deprotection with sodium / liquid ammonia furnished 2-deoxy-2-fluoro-myo-inositol 1,4,5-trisphosphate 178. Marecek and Prestwich [306] had reported that 2-fluoro-scy/lo-Ins(1,4,5)P$_3$ underwent slow defluorination ($t_{1/2} = 2$ weeks) at pH 13 and 50°C, whereas the difluoro analogue was stable under these conditions. No defluorination was observed during the deblocking of 177.
Figure 10.5 Synthesis of DL-2-deoxy-2-fluoro-Ins(1,4,5)P$_3$ 178

The $^{31}$P-NMR spectrum of 178 (Figure 10.6) showed three doublets integrating for 1 phosphorus each at 1.96, 1.56, and 0.37 ppm with $J = 10.1$, 6.7, and 10.1 Hz, respectively, and the $^{19}$F-spectrum of this compound showed a dt at -211.8 ppm with $J_{2-H,F} = 51.3$ Hz and $J_{1(3)-H,F} = 29.7$ Hz. The signals in the $^1$H-NMR spectrum could be assigned easily by their chemical shifts and $^1$H-$^1$H-, $^1$H-$^{31}$P-, and $^1$H-$^{19}$F-coupling constants.
Figure 10.6 1H-coupled 31P-NMR spectrum of 178

10.2 Pharmacology

The new fluorinated Ins(1,4,5)P₃ analogue 178 is now being evaluated, and preliminary results have shown it to be equipotent to Ins(1,4,5)P₃ in mobilising sequestered calcium assuming that only the D-isomer of 178 displays biological activity [EC₅₀ of 178 = 0.10 μM, EC₅₀ of D-Ins(1,4,5)P₃ = 0.05 μM]. Comparison of the calcium mobilising properties of 178 with those of 2F-scyllo-Ins(1,4,5)P₃ 103 (EC₅₀ = 0.77 μM) and 2,2-F₂-Ins(1,4,5)P₃ 104 (EC₅₀ = 0.41 μM) show that
the closer structural similarity of 178 to Ins(1,4,5)P₃ is reflected in its biological activity. The assumption [305,331] (see Chapter 4.1, Figure 4.2) that the 2-hydroxyl group of Ins(1,4,5)P₃ interacts with the receptor by accepting rather than donating a hydrogen bond thus appears to be confirmed: the 2-fluorine of 178 is still able to accept hydrogen bonds, but can no longer donate them, and the compound is nevertheless as potent as Ins(1,4,5)P₃.

178 is also a good substrate for 3-kinase, acting as a potent competitive inhibitor of the phosphorylation of [³H]-Ins(1,4,5)P₃ by this enzyme (apparent Kᵢ = 3.0 μM; for comparison: 2-F-scyllo-Ins(1,4,5)P₃ 103 and 2,2-F₂-Ins(1,4,5)P₃ 104 have Kᵢ values of 8.8 μM and 11.0 μM, respectively).

Interactions of racemic 2-F-hydroxy-Ins(1,4,5)P₃ with 5-phosphatase have also been studied, and the fluorinated derivative was found to be a moderately potent competitive inhibitor of this enzyme (Kᵢ 14.4 μM). It may tentatively be assumed that by analogy with the difluoro-analogue 104 the inhibitory effect of DL-178 is due to the presence of L-2-F-Ins(1,4,5)P₃ in the racemic mixture, whereas the D-isomer is probably a substrate for this enzyme like D-104. It will however, be necessary to resolve the enantiomers of this novel fluorinated analogue to determine the contributions made by the individual enantiomers towards the overall data obtained for the racemic compound.

The pharmacological results obtained for the different fluorinated Ins(1,4,5)P₃ analogues are summarised and compared to D-Ins(1,4,5)P₃ (= D-6) below:

<table>
<thead>
<tr>
<th></th>
<th>DL-103</th>
<th>DL-104</th>
<th>L-104</th>
<th>D-104</th>
<th>DL-178</th>
<th>D-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC₅₀</td>
<td>0.77</td>
<td>0.41</td>
<td>53</td>
<td>0.21</td>
<td>0.10</td>
<td>0.05</td>
</tr>
<tr>
<td>5-phosphatase, Kᵢ</td>
<td>0.7 (S)</td>
<td>26.0 (I)</td>
<td>19 (I)</td>
<td>60 (S)</td>
<td>14.4 (I)</td>
<td>16-40</td>
</tr>
<tr>
<td>3-kinase, Kᵢ</td>
<td>8.8 (S)</td>
<td>11.0 (S)</td>
<td>11.9 (I)</td>
<td>10.2 (S)</td>
<td>3.0 (S)</td>
<td>0.85-3.2</td>
</tr>
</tbody>
</table>

S = substrate, I = inhibitor, all values in μM.

Figure 10.7 Pharmacological activity of fluorinated Ins(1,4,5)P₃ analogues
A synthetic route to optically active myo-inositol 1,4,5-trisphosphate 3-phosphorothioate: resolution of DL-4,5-di-O-acetyl-3-O-allyl-6-O-benzyl-1-O-p-methoxybenzyl-myoinositol

The fully protected inositol derivative DL-3-O-allyl-2,6-di-O-benzyl-4,5-di-O-isopropylidene-1-O-p-methoxybenzyl-myoinositol would allow the selective deprotection of the 1-, the 3-, and the 4,5-positions.

Figure 11.1 Route to Ins(1,4,5)P₃-3S from 3-O-allyl-2,6-di-O-benzyl-4,5-di-O-isopropylidene-1-O-p-methoxybenzyl-myoinositol
Therefore this versatile compound represents a key intermediate in the synthesis of 1- and 3-modified analogues of the second messenger Ins(1,4,5)P₃ and of Ins(1,3,4,5)P₄, which is supposed to play an important role in calcium homeostasis [89].

Since the biochemical properties of enantiomers of the same derivative can be very different, it was thought essential to attempt the synthesis of this intermediate in optically pure form to gain access to the individual D- and L-Ins(1,4,5)P₃ and Ins(1,3,4,5)P₄ analogues, including the derivative we were most interested in, i.e. the 3-phosphorothioate analogue of D-Ins(1,3,4,5)P₄ (Figure 11.1), which would be resistant to hydrolysis by Ins(1,3,4,5)P₄ 3-phosphatase. For this purpose, the camphanate method (see Chapter 2.2), which had already proved to be effective in the optical resolution of 1-O-allyl-2,3,6-tri-O-benzyl-myoinositol (Chapter 7.1), was chosen. Thus the precursor to the key intermediate DL-3-O-allyl-2,6-di-O-benzyl-4,5-di-O-isopropylidene-1-O-p-methoxybenzyl-myoinositol, DL-3-O-allyl-2-O-benzyl-4,5-di-O-isopropylidene-1-O-p-methoxybenzyl-myoinositol 184 was synthesised by a 5-step route from DL-1:2,4:5-di-O-isopropylidene-myoinositol 122 [149] (Figure 11.2). Optical resolution was then attempted by forming the 2-O-(+)-(ω)-camphanate ester of 184.

Treatment of 1,2:4,5-di-O-isopropylidene-myoinositol 122 with 1 equivalent of allyl bromide and excess sodium hydride in DMF according to the procedure of Gigg et al. [165] gives the 3-allyl ether 179 in only 30 % yield. When a strong base like sodium hydride is used, the small differences in reactivity between the free 3- and 6-hydroxyl groups cannot be exploited to achieve selective alkylation at the more reactive 3-position. Therefore this procedure gives a product mixture of the two monoallylated inositols in a ratio of 1:1 and a minor amount of the bisallylated inositol.

If however 1,2:4,5-di-O-isopropylidene-myoinositol is treated with allyl bromide under mild conditions using barium oxide / barium hydroxide [385] rather than sodium hydride as base, selective alkylation of the 3-position is possible. The desired DL-3-O-allyl-1,2:4,5-di-O-isopropylidene-myoinositol 179 was obtained in 63 % yield and only minor amounts of DL-3,6-di-O-allyl-1,2:4,5-di-O-
isopropylidene-myoinositol 180 and DL-6-O-allyl-1,2:4,5-di-O-isopropylidene-myoinositol (not isolated) were formed.

Figure 11.2 Optical resolution of DL-4,5-di-O-acetyl-3-O-allyl-6-O-benzyl-1-O-p-methoxybenzyl-myoinositol
Benzylation of the free 6-position of 179 was effected with benzyl chloride, KOH and 18-crown-6 as phase transfer catalyst. DL-3-O-allyl-6-O-benzyl-1,2:4,5-di-O-isopropylidene-myoinositol 181 thus obtained was then converted into DL-1-O-allyl-4-O-benzyl-myoinositol 182 by acidic removal of the isopropylidene protecting groups.

Selective p-methoxybenzylation of 182 was achieved by first forming a stannylene complex with dibutyltin oxide, which was then O-alkylated with p-methoxybenzyl chloride with the assistance of caesium fluoride. Although several less polar by-products were also formed in this reaction as shown by tlc analysis, the major product DL-3-O-allyl-6-O-benzyl-1-O-p-methoxybenzyl-myoinositol 183 was isolated in 81% yield. The 4- and 5-positions of triol 183 were then simultaneously protected with an isopropylidene group by reaction with 2-methoxypropene and PTSA in DMF to give DL-3-O-allyl-6-O-benzyl-1-O-p-methoxybenzyl-4,5-di-O-isopropylidene-myoinositol 184.

Resolution of 184 was attempted by conversion into the diastereomeric S-(−)-camphanate esters using S-(−)-camphanic chloride / pyridine.

Tlc and high performance tlc (hptlc) analysis of the mixture of the D- and L-isomers of 3-O-allyl-6-O-benzyl-2-O-[(−)-(ω)-campanoyl]-1-O-p-methoxybenzyl-4,5-di-O-isopropylidene-myoinositol 185 showed only one single product spot for the two diastereoisomers, suggesting that chromatographic separation of the mixture would not be feasible. Efforts to crystallise selectively one diastereoisomer from polar (methanol) and non-polar (ether / petrol ether) solvents were unsuccessful. In each case a 1:1 mixture of the two diastereoisomers (as determined by the 1H-NMR signals of the camphanate methyl groups) was obtained.

Since the separation of D- and L-3-O-allyl-6-O-benzyl-2-O-[(−)-(ω)-camphanoyl]-1-O-p-methoxybenzyl-4,5-O-isopropylidene-myoinositol 185 was found to be impossible by conventional methods, it was decided to remove the isopropylidene group by mild acidic hydrolysis to give the 4,5-diol 186. Resolution of this more polar derivative was then to be attempted.
Separation of the diastereomers of 186 by flash chromatography on silica gel and by crystallisation proved to be impossible. However, as hptlc revealed two product spots for this compound, we reasoned that acetylation of 186 to give the 4,5-di-O-acetate 187 might afford a compound easier to crystallise than the diol. 187 was indeed highly crystalline and the two isomers showed up as two discrete spots on hptlc (chloroform : methanol 60:1, Rf 0.58 and 0.63). It was possible to obtain the less polar diastereoisomer in optically pure form by recrystallisation from ether. This compound had a melting point of 142-143°C and [α]_D^27 was found to be +19.0° (c = 4 in CHCl_3). The ^1H-NMR spectrum showed no detectable contamination of (+)-187 with the other isomer (Figure 11.3).

In order to establish the absolute configuration of this compound, attempts were made to obtain single crystals for X-ray analysis. Recrystallisation from several different solvents (ether, methanol, ethanol) however, gave long thin needles of (+)-187 unsuitable for X-ray analysis in each case. Since the removal of the base labile protecting groups was necessary in order to prepare the optically active, fully protected key intermediate 3-O-allyl-2,6-di-O-benzyl-4,5-di-O-isopropylidene-1-O-p-methoxybenzyl-mylo-inositol, both the camphanate and the acetate esters were cleaved by alkaline hydrolysis to give 188. This compound, with a sharp melting point of 157.5-158°C and [α]_D^{20} = +19.0°C, was obtained from ethyl acetate in beautiful crystals. Lack of time, however, precluded the determination of the absolute configuration of this compound.
Figure 11.3 $^1$H-NMR spectrum of (+)-187
A synthesis of the racemic triscarboxymethyl analogue of Ins(1,4,5)P₃ has been reported by Westerduin et al. [289]. This isosteric compound did, however, not shown any affinity for the Ins(1,4,5)P₃ receptor, nor did it induce platelet aggregation. Carboxymethyl substitution for phosphates is, however, in principle a useful substitution and we reasoned that a better chance for novel activity might come from mixed phosphate / carboxymethyl analogues.

We anticipated that an Ins(1,4,5)P₃ analogue with only a single carboxymethyl group in the 1-position and the 4,5-bisphosphate pair unchanged would be sufficiently close to Ins(1,4,5)P₃ to show biological activity. Comparison of the pharmacological properties of the 1-O-methylenecarboxylate analogue with those of Ins(1,4,5)P₃ and Ins(4,5)P₂ would then allow us to determine the effect of the modification, and give an indication of whether the same substitution performed on the 4- and / or 5-phosphate group(s) would be feasible.

Figure 12.1 Introduction of fluorescent reporter groups into the 1-position of Ins(1,4,5)P₃ analogues via amide links
Such an analogue would also allow the introduction of reporter groups into the molecule by linking them selectively to the 1-carboxylate (e.g. fluorescent labels containing amino groups could be attached to the inositol ring via a stable amide link, Figure 12.1). This might be an alternative to our alkylphosphorothioate route to biologically active probes (see Chapter 6.4).

We also wanted to explore the possibility of using the formation of a lactone between the 1-O-methylene carboxylate and 2-hydroxyl group as a means to temporarily protect the 2-position and to introduce a degree of rigidity into the structure of phosphorylated analogues. Lactone polyphosphates would be a new class of Ins(1,4,5)P₃ analogues.

The 1,2-O-methylene lactone 191 of DL-3,6-di-O-benzyl-‐myo-inositol 124 was prepared by two different methods (Figure 12.2). Direct synthesis albeit in low yield (16 %) was possible by treatment of 1,2-O-dibutylstannylene-3,6-di-O-benzyl-‐myo-inositol with ethyl bromoacetate in DMF.
The second route involved three steps, however, the overall yield was higher than that obtained by the direct method. Thus, DL-1-O-cyanomethylene-3,6-di-O-benzyl-myo-inositol 189 was prepared by tin-mediated alkylation of 1,4-di-O-benzyl-myo-inositol with bromoacetonitrile in 58 % yield. $^{13}$C-NMR showed signals at 56.24 ppm (t) for CH$_2$CN and at 116.21 ppm (s) for CN, and in the $^1$H-NMR spectrum the AB-system at 3.83 and 3.89 ppm was assigned to the CH$_2$CN group. The $\nu$(CH$_2$-CN) IR band (which may be found between 2260 and 2240 cm$^{-1}$ and is usually weak or even absent in unconjugated nitriles) was not observed in the IR spectrum of this compound.

Treatment of 189 with sodium hydroxide in ethanol followed by acidification resulted in conversion into DL-3,6-di-O-benzyl-1-O-carboxymethylene-myo-inositol 190 (81 %), which showed a strong carbonyl signal in the IR-spectrum at 1720 cm$^{-1}$. $^1$H-NMR (broad COOH signal at 12.6 ppm) and $^{13}$C-NMR (COOH singlet at 172.2 ppm) confirmed the presence of a carboxylate moiety.

**DL-1-O-carboxymethylene-3,6-di-O-benzyl-myo-inositol: a jumping crystal**

The 1-O-carboxymethylene derivative was found to display an interesting "thermosalient" behaviour, i.e. on heating, the crystals jumped over a range of 90-120°C before melting at 185-187°C. After the crystals had been warmed to 140°C the same phenomenon could be observed on cooling: the crystals jumped between 60-40°C. Similar properties have been reported by Gigg et al. for a different compound, DL-3,4-di-O-acetyl-1,2,5,6-tetra-O-benzyl-myo-inositol $^{155}$. It was suggested after preliminary studies in polarised light that the crystals "may be in a form of a bundle or parallel fibres or leaves ... with crystallographically non-identical faces in contact ... and that a composite crystal of this sort may act in the same way as a bimetallic strip at different temperatures".

The properties of DL-3,4-di-O-acetyl-1,2,5,6-tetra-O-benzyl-myo-inositol have been further investigated by calorimetric methods $^{386}$, where it was found that this compound underwent even two reversible solid / solid phase transitions, and by solid state magic angle spinning NMR studies $^{387}$, which led to the conclusion that there are no large differences in the structure or dynamic properties of the three solid phases of the diacetate, and that the thermosalient
behaviour may be the consequence of the cooperative effect of a number of subtle intermolecular interactions.

Lactonisation of the carboxymethylene derivative with a catalytic amount of acid and removal of water of condensation to shift the reaction equilibrium to the product side gave 191 identical to 191 prepared by the direct route in 92 % yield (43 % overall yield). The $^1$H-NMR spectrum of 191 showed unusually small $H_{ax}^1$-$H_{ax}^1$ J values (5.9 and 6.0 Hz) for the coupling between C-1-H and C-6-H and a relatively large $H_{ax}^1$-$H_{eq}^1$ coupling between C-1-H and C-2-H (3.3 Hz) (coupling constants of other inositol ring protons could not be measured because the signals were part of multiplets). This observation may be explained by the presence of the 1,2-lactone ring causing the inositol ring to adopt a slightly twisted position.

The lactone 191 was converted into the 4,5-diacetate 192 for characterisation. The $^1$H-NMR signals for C-5-H and C-4-H were found at 5.09 and 5.39 ppm, as expected shifted considerably downfield in comparison to the signals for these protons in the spectrum of the 4,5-diol.

In order to investigate whether the `jumping crystal’ behaviour of DL-1-O-carboxymethylene-3,6-di-O-benzyl-myoinositol 190 was a property shared by the different enantiomers of this compound, the optical resolution of this compound was attempted. 191 was converted into the 4,5-bis-(−)-ω-camphanate 193, and one diastereoisomer crystallised readily from methanol. Removal of the camphanate moieties by base hydrolysis followed by acidification gave the optically resolved 1-O-carboxymethylene derivative 194. This compound had a surprisingly high melting point (308-310°C) and showed a phase transition between 240 and 255°C, but in contrast to the racemic 190 no `thermosalient’ properties could be observed for 194.
13 Novel C-allyl inositols by Claisen rearrangement of inositol O-allyl ethers

Inositols linked via C-C bonds to other groups are not common (with the exception of methylenephosphonates) and are worth of investigation as analogues in the context of inositol phosphate synthesis. We devised a new approach to these derivatives as follows:

We anticipated that elimination rather than substitution reactions on 1-O-allyl-3,6-di-O-benzyl-4,5-O-isopropylidene-2-O-trifluoromethylsulphonyl-\textit{myo}-inositol 160 followed by Claisen-rearrangement of the resulting enol ether would give access to novel inositol analogues with a C-allyl group. By oxidation with ruthenium trichloride - sodium periodate such compounds could then be converted into C-methylenecarboxylates. Sterically, the \(-\text{CH}_2\text{-COOH}\) moiety might be a better phosphate surrogate than the \(-\text{O-CH}_2\text{-COOH}\) group obtained by oxidative cleavage of allyl ethers. Like compounds with an \(-\text{O-CH}_2\text{-COOH}\) group (see Chapter 12), C-methylenecarboxylates could be employed in the preparation of analogues carrying reporter groups linked to the 1-position of the inositol ring via a stable amide bond. Replacement of the 4- and / or 5-phosphate with a C-methylenecarboxylate may lead to derivatives with potential antagonistic properties.

Elimination products have been obtained in $S_N2$ displacement reactions on \textit{chiro}-inositol triflates, with potassium benzoate and 18-crown-6 catalyst in DMF at 80°C giving 85 % elimination product \textit{versus} 18 % (sic!) substitution product [231]. We therefore attempted to eliminate the triflate group of 160 by using the same reaction conditions. The sole product isolated was however, 1-O-allyl-2-O-benzoyl-3,6-di-O-benzyl-4,5-O-isopropylidene-\textit{scyllo}-inositol 195 (yield 67 %, Figure 13.2), the substitution product. That this compound is indeed in the \textit{scyllo}-configuration and that the displacement of the triflate moiety therefore proceeded with inversion at C-2 is obvious from the $^1$H-NMR spectrum (Figure 13.1): the signal for C-2-H at 5.43 ppm (the downfield shift as compared to the signals for the other ring protons at 3.57-3.81 ppm is caused by the benzoate ester group at C-2) is a dd with $J_1 = J_2 = 8.9$ Hz. Coupling constants this large are only found for ring protons in an axial-axial arrangement.
Figure 13.1 $^1$H-NMR spectrum of 195 (expansion 3.5 to 5.8 ppm)
195 was then converted into the diol 196 by acid hydrolysis of the 4,5-\(\text{O}\)-isopropylidene group. Removal of the allyl protecting group by isomerisation with Wilkinson's catalyst and in situ hydrolysis of the prop-1-enyl ether gave 197, a precursor for the synthesis of scyllo-inositol analogues of Ins(1,4,5)P\(_3\).

It was then attempted to effect elimination by treating the triflate with sodium carbonate in DMSO at 120°C. This however lead to the decomposition of 160. No reaction products could be isolated from the black tar obtained.

Elimination of the triflate group from 160 was finally successfully performed by employing the non-nucleophilic hindered base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) \(^{388}\). After stirring the reaction mixture for 24 h at r.t., tlc (petrol ether b.p. 60-80°C / ether 2:1) showed that the starting material (\(R_f\) 0.35) had disappeared and two major products (198, \(R_f\) 0.54 and 199, \(R_f\) 0.46) had been formed in a ratio of ca. 1:1. These compounds were isolated and characterised:

According to their mass spectra, both 198 and 199 had a molecular mass of \(M = 422\). Both compounds showed the presence of two benzyl groups, an allyl group and the isopropylidene group in their \(^{1}\text{H}-\text{NMR}\) spectra (see Figure 13.4 and 13.5). It was also evident from the \(^{1}\text{H}-\text{NMR}\) that there were only 5 protons
attached to the inositol ring in each compound, which was confirmed by the $^{13}$C-NMR spectra which showed only 5 doublets in the region between 70 and 100 ppm corresponding to the 5 C-H ring carbons. The signals for the remaining ring carbon were found to be singlets at 154.55 and 153.84 ppm, respectively.

It was thus safe to conclude that 198 and 199 corresponded to the two possible elimination products with a double bond between C-1 and C-2 or C-2 and C-3. The assignment of structures to the two products obtained proved to be impossible by reference to their spectroscopic data alone. The structures of 198 and 199 could, however, be assigned by chemical transformation: only 198 rearranged on heating for 15 min at 200°C to give a mixture of 200 and 201. In contrast, 199 decomposed under the same conditions. It may thus be tentatively assumed that the correct structure for 198 is the one drawn out in Figure 13.6, since 199 cannot undergo Claisen rearrangements.

Another way of structural assignment would be acid treatment of each product. In each case the enol ether should decompose to give an inosose with loss of either a benzyl or an allyl group, thus allowing the direction of ongoing elimination to be deduced:

![Figure 13.3](image-url)
Figure 13.4 $^1$H-NMR spectrum of 198
Figure 13.5  $^1$H-NMR spectrum of 199
Figure 13.6 Elimination and Claisen rearrangement

It should be mentioned here that 199 was also obtained as a by-product (7 % yield) in the reaction of 160 with tetrabutylammonium fluoride, which gave 1-O-allyl-2-deoxy-2-fluoro-3,6-di-O-benzyl-4,5-O-isopropylidene-scyllo-inositol 202 as the main product (Figure 13.7). The melting point measured for 202 (111-112°C, from hexane) differs considerably from the value reported by Sawyer (98-
100°C \cite{389}, who obtained this compound by DAST fluorination of 1-\textit{O}-allyl-3,6-di-\textit{O}-benzyl-4,5-\textit{O}-isopropylidene-\textit{myo}-inositol \textit{93}.

![Chemical structure](image)

**Figure 13.7 Reaction of 160 with tetrabutylammonium fluoride**

The assignment of the scyllo-configuration to the main product \textit{202} is based on the \textit{1H}-NMR signal for C-2-H at 4.50 ppm, which appears as a dt with $J_{H-C_F} = 49.3$ Hz and $J_{H-C_1(3)} = 7.9$ Hz. The large \textit{J} value measured for the coupling between the vicinal hydrogens at C-2 and C-1 / C-3 is typical for an axial-axial arrangement of the protons, thus the fluorine has to be found in an equatorial position. Further proof for the structure is provided by the proton signal at 3.71 ppm (1 H, ddd, $J = 12.8, 9.7, 7.9$), which can be assigned to C-1-H (or C-3-H). The $^3J_{H-F}$ value of 12.8 Hz (and the corresponding coupling constant of 14.7 Hz found in the $^{19}$F spectrum) are typical for proton and fluorine in an axial-equatorial relationship. $^3J_{H-F}$ coupling constants in a 2-fluoro derivative with \textit{myo}-inositol configuration can be expected to be much larger ($\approx 29$ Hz, see Chapter 10.1).

**Claisen rearrangement:**

When the product obtained by heating \textit{198} for 15 min at 200°C was examined, it was originally thought to be a homogenous compound, since tlc (petrol ether b.p. 60-80°C / ether 2:1) showed complete conversion of the starting material ($R_f 0.54$) into what appeared to be a single product ($R_f 0.45$). The $^1$H- \textit{(Figure 13.8)} and $^{13}$C-NMR spectra revealed however that it was a mixture of two compounds. Attempts to isolate these two products chromatographically were not successful. It was however clear from the $^{13}$C-NMR spectra of the product mixture that both compounds contained carbonyl groups ($\delta_C 203.69$ and 205.31), and it thus appears safe to conclude that the reaction mixture contained DL-2-deoxy-2-\textit{C-
allyl-3,6-di-O-benzyl-4,5-O-isopropylidene-myoinosose 200 and DL-2-deoxy-2-C-allyl-3,6-di-O-benzyl-4,5-O-isopropylidene-scylloinosose 201.

Figure 13.8 $^1$H-NMR of the Claisen rearrangement product (200 and 201)
Two explanations for this result can be imagined: as shown in Figure 13.9, the Claisen rearrangement can, in principle, proceed via two different transition states to yield either 200 or 201 or a mixture of both products.

Alternatively, either 200 or 201 could be formed selectively in the rearrangement. Epimerisation via the enol 203 (Figure 13.10) would then give rise to a mixture of the tautomers 200 and 201, since the addition of a proton to the enol could occur from either face of 203.
If separation of 200 and 201 was possible, selective reduction of the respective inososes using either sodium borohydride or the sterically hindered lithium trisiamylborohydride (‘LS-Selectride’ [394]), which affords predominantly the axial alcohol, would make available a number of new C-allyl inositol derivatives (204 - 207, Figure 13.11). Stereospecific reduction of the 1-position of 200 would transform this position either into the equatorial 1-OH of the 2-deoxy-2-C-allyl-myoinositol system 204 or into the axial 1-OH of the 6-deoxy-6-C-allyl-chiroinositol system 205. The same conversions performed on 201 would afford the C-allyl-scyllo-inositol derivative 206 and the 1-deoxy-1-C-allyl-myoinositol 207, respectively.

As outlined above, there is considerable potential for the preparation new inositol phosphate analogues by employing the synthetic sequence of triflate elimination, Claisen rearrangement and stereoselective reduction of the resulting inososes. Whilst the first steps into this direction have now been made, there are, however, still a number of problems (such as optimising the yield of the 1,2-eliminated product and the separation of the Claisen rearrangement products 200 and 201) to be addressed before this new route can be considered to be satisfactory.
Figure 13.11 Derivatives accessible by selective reduction of 200 and 201
Within the last decade, polyphosphoinositide research has been one of the most rapidly expanding fields in biochemistry. The number of research publications per annum in this area (Figure 14.1) increased almost 10-fold between 1983 (when the second messenger role of D-myo-inositol 1,4,5-trisphosphate was discovered) and 1988, and the average number of articles appearing is now at 500 per year.

Since 1983, the important role that inositol phosphates play in signal transduction has become increasingly clear. However, whilst the function of Ins(1,4,5)P₃ is now fairly well known, there are still many questions to be answered before the complex biochemical processes involved in the generation and metabolism of this second messenger are fully understood.
Apart from the most prominent inositol phosphate \( \text{Ins}(1,4,5)\text{P}_3 \), interest is now increasingly focussed on other biologically active inositol phosphates and phospholipids. The recent discovery that activation of a PtdIns(4,5)P\(_2\) 3-kinase by certain growth factors leads to the formation of PtdIns(3,4,5)P\(_3\) \(^{[390]}\), and that this inositol phospholipid can in turn stimulate the \( \zeta \) isozyme of protein kinase C \(^{[391]}\) is only one example of the important role that other inositol derivatives play in signal transduction and other physiological processes.

During this last decade, tools provided by pharmaceutical chemists have made a considerable contribution to unravelling various aspects in the \( \text{Ins}(1,4,5)\text{P}_3 \) signalling pathway:

Analogues of \( \text{Ins}(1,4,5)\text{P}_3 \) and \( \text{Ins}(1,3,4,5)\text{P}_4 \) have helped to elucidate structural requirements for receptor binding and enzyme interaction. A number of inhibitors for metabolic enzymes in the phosphoinositide pathway have been developed. Synthetic derivatives of \( \text{Ins}(1,4,5)\text{P}_3 \) have been used to prepare affinity matrices for the purification of receptor proteins. Radioactive analogues and photoaffinity derivatives have been employed to label \( \text{Ins}(1,4,5)\text{P}_3 \) and \( \text{Ins}(1,3,4,5)\text{P}_4 \) binding sites. The use of ‘caged’ \( \text{Ins}(1,4,5)\text{P}_3 \) \(^{[392]}\) has allowed the study of calcium mobilisation in intact cell preparations. The first lipophilic \( \text{Ins}(1,4,5)\text{P}_3 \) analogue has now been reported \(^{[393]}\), making possible the delivery of active substances across the cell membrane.

Impressive as this list of achievements may seem, there are many tasks ahead for the pharmaceutical chemist in inositol phosphate research:

The most interesting challenge for the future is arguably the preparation of \( \text{Ins}(1,4,5)\text{P}_3 \) antagonists. Heparin and decavanadate have already been identified as antagonists, but they are of only limited use in inositol phosphate research because of their lack of specificity, and the search for a specific small molecule \( \text{Ins}(1,4,5)\text{P}_3 \) antagonist continues. Analogues with partial agonist properties have given first indications on how this goal may be achieved, and the day may not be far when the synthesis of the first \( \text{Ins}(1,4,5)\text{P}_3 \) antagonist will be reported.
It may take considerably longer to find applications of inositol phosphate research in medicine, but one can imagine that cell-penetrating antagonists or inhibitors of metabolic enzymes may be devised who are able to serve as drugs.

The discovery of novel biologically active inositol phosphates and phospholipids like PtdIns(3,4,5)P₃ is likely to lead to equally strong efforts in the synthesis of analogues of these compounds as has been seen for Ins(1,4,5)P₃. Work in this area has only just started.

The concluding words of Potter's review from 1990 [123] still apply: "If the current pace of progress is maintained we can expect an exciting future".
EXPERIMENTAL SECTION

15 Materials and Methods

Chemicals were purchased from Aldrich, Fluka and Lancaster. IANBD was purchased from Molecular Probes. Diethyl ether was dried over sodium wire and distilled. Dichloromethane, triethylamine and dimethylformamide were dried over calcium hydride, distilled and stored over 4 Å molecular sieves. Pyridine was dried by refluxing with sodium hydroxide pellets, followed by distillation, and stored over 5 Å sieves.

Triethylammonium bicarbonate (TEAB) buffers (1 or 2 M) were prepared by bubbling carbon dioxide through a 1 or 2 M solution of triethylamine in deionised water until a pH of 8 was obtained.

Thin layer chromatography (tlc) and high performance tlc (hptlc) was performed on pre-coated plates (Merck tlc aluminium sheets silica 60 F254, Art. no. 5554 and Merck hptlc plates silica 60 F254, Art. no. 5635). Products were visualised by spraying phosphomolybdic acid in methanol followed by heating. Flash chromatography refers to the method of Still et al. [95] and was carried out using Sorbsil C60 silica gel.

\[ ^1H \text{- and } ^{13}C\text{-NMR spectra were recorded on either Bruker AM-300 or Jeol JNM GX-270 NMR spectrometers. Chemical shifts were measured in ppm relative to tetramethylsilane (TMS). } ^{31}P\text{-NMR and } ^{19}F\text{-NMR spectra were recorded on a Jeol FX-90Q spectrometer. } ^{31}P\text{- and } ^{19}F\text{-NMR chemical shifts were measured in ppm relative to external 85 % } \text{H}_3\text{PO}_4 \text{ and internal } \text{CFCI}_3, \text{ respectively. } J \text{ values are given in Hz. Melting points (uncorrected) were determined using a Reichert-Jung Thermo Galen Kofler block. Microanalysis was carried out at Butterworth Laboratories Ltd. and the University of Bath microanalysis service. Mass spectra were recorded at the SERC Mass Spectrometry Service Centre, Swansea, and at the University of Bath. Optical rotations were measured using a Optical Activity Ltd. AA-10 polarimeter. Ion exchange chromatography was performed on an LKB-Pharmacia Medium Pressure Ion Exchange Chromatograph using DEAE Sephadex or DEAE Sepharose with gradients of triethylammonium bicarbonate (TEAB) as eluent. } \]
Column fractions were assayed for phosphate by a modification of the Briggs test [239] as follows:
A molybdate solution (12.5 g of ammonium molybdate dissolved in 250 ml water and 35 ml conc. H₂SO₄), a hydroquinone solution (0.5 g hydroquinone dissolved in 100 ml water and a drop of conc. H₂SO₄) and a sulphite solution (20 % w/v sodium sulphite in water) were prepared. Aliquots (usually 250 μl) of the fractions to be assayed were transferred into test tubes and conc. H₂SO₄ (3 drops) was added to these samples. The samples were heated at 150°C for 1 h. After cooling, water (500 μl), molybdate solution (500 μl), hydroquinone solution (250 μl) and sulphite solution (250 μl) were added to each of the test tubes. The samples were then boiled for 7 seconds and allowed to cool. Phosphate containing fractions could be identified by their blue colour.

For quantitative analysis, samples containing known amounts of potassium dihydrogen phosphate were co-assayed with samples of unknown phosphate contents. After being processed as above, the test tube fractions were transferred to 10 ml volumetric flasks and water was added to give 10 ml of solution. The UV absorbance at 340 nm was recorded using 3 ml quartz cells. The concentration of the unknown samples was calculated from a standard curve derived from the absorbances of the reference samples.

Compounds containing phosphorothioates were assayed by a modification of the Ellman test [243] for sulphhydryl groups as follows. To 250 μl aliquots of the ion-exchange column fractions was added 1 ml of a buffered solution of Ellman's reagent [100 ml 10 mM Tris buffer, pH 8, containing 40 mg of 5',5-dithio-bis(2-nitrobenzoicacid)]. The fractions containing phosphorothioates were identified their deep yellow colour.

16 Synthesis of myo-inositol 4,5-bisphosphate 1-phosphorothioate

16.1 DL-3,6-Di-O-benzoyl-1,2:4,5-di-O-isopropylidene-myoinositol 121

This compound was prepared according to the method of Gigg et al. [149].
A mixture of myo-inositol 10 (250 g, 1.39 mol), N,N-dimethylformamide (1 litre), 2,2-dimethoxypropane (750 ml) and toluene-ρ-sulphonic acid (5 g) was heated under reflux for 2 h, after which only little solid remained. Triethylamine (50 ml)
was added to the cooled solution and the solid removed. Toluene (125 ml) was added to the filtrate and the low-boiling solvents were evaporated at 40°C. Pyridine (750 ml) was added to the $N,N$-dimethylformamide solution followed by benzyol chloride (1 l) dropwise with stirring and cooling during 30 min. After a further 2 h, the solid was collected and washed successively with pyridine, water, acetone and ether to give DL-3,6-di-$O$-benzoyl-$1,2:4,5$-di-$O$-isopropylidene-$myo$-inositol 121 (167 g, 357 mmol, 26% of reacted inositol).

m.p. 285-310°C (Lit. $^{[149]}$; 328-330°C)
$\delta_H$ (CDCl$_3$, 300 MHz) 1.30, 1.42, 1.51, 1.63 (12 H, 4 s, 4 CH$_3$), 3.73 (1 H, dd, J10.5, 9.75, C-5-H), 4.40 (2 H, m, C-1,4-H), 4.80 (1 H, dd, J 4.5, 4.5, C-2-H), 5.44 (1 H, dd, J 10.5, 4.5, C-3-H), 5.61 (1 H, dd, J 10.8, 7.2, C-6-H), 7.23-7.63 (6 H, m, C(O)Ph), 7.82-8.19 (4 H, m, C(O)Ph)

16.2 DL-1,2:4,5-Di-$O$-isopropylidene-$myo$-inositol 122

This compound was prepared according to the method of Gigg et al. $^{[149]}$.
A mixture of 121 (167 g, 357 mmol), sodium hydroxide (64 g) and methanol (4 litres) was heated under reflux for 30 min. The resulting clear solution was cooled, neutralised with solid carbon dioxide, diluted with water (3.2 litres) and evaporated to dryness. The title compound was extracted with dichloromethane, and after evaporation of the solvent 79 g (304 mmol, 85 %) of DL-1,2:4,5-di-$O$-isopropylidene-$myo$-inositol 122 were obtained.

m.p. 164-165°C (from ethyl acetate, Lit. $^{[149]}$; 171-173°C)
$\delta_H$ (CDCl$_3$, 270 MHz) 1.39, 1.46, 1.49, 1.54 (12 H, 4 s, 4 CH$_3$), 2.60-2.70 (2 H, brd, OH), 3.05-2.85 (2 H, brd, OH), 3.33 (1 H, dd, J 10.6, 9.5, C-5-H), 3.84 (1 H, dd, J 9.7, 9.7, C-H), 3.90 (1 H, dd, J 10.6, 6.4, C-H), 4.05 (1 H, dd, J 9.8, 4.5, C-H), 4.08 (1 H, d, J 6.0, C-H), 4.48 (1 H, dd, J 4.8, 4.8, C-2-H)

16.3 DL-3,6-Di-$O$-benzyl-$1,2:4,5$-di-$O$-isopropylidene-$myo$-inositol 123

DL-1,2:4,5-Di-$O$-isopropylidene-$myo$-inositol 122 (15.6 g, 60 mmol), 18-crown-6 (795 mg, 3 mmol), potassium hydroxide (40 g) and benzyl chloride (85 ml) were heated with vigorous stirring up to 120°C, at which time the reaction became exothermic. Heating was removed, and after 2 h the reaction mixture had cooled
to room temperature. Toluene (70 ml) was added and the mixture was washed with 1 M sodium chloride solution, until the aqueous phase remained neutral. The toluene layer was dried over magnesium sulphate and evaporated at 95°C (high vacuum) to remove remaining benzyl chloride. Recrystallisation of the product from ethanol gave DL-3,6-di-O-benzyl-1,2:4,5-di-O-isopropylidene-myoinositol 123 (25.7 g, 58 mmol, 98%).

m.p. 151-153°C (Lit. [149]: 153-155°C)
$\delta_H$ (CDCl$_3$, 300 MHz): 1.32, 1.39, 1.45, 1.48 (12 H, s, 4 CH$_3$), 3.34 (1 H, dd, J 10.5, 9.4, C-5-H), 3.67 (1 H, dd, J 10.6, 6.5, C-3-H), 3.74 (1 H, dd, J 10.1, 4.2, C-1-H), 3.98-4.07 (2 H, m, C-4-H, C-6-H), 4.30 (1 H, dd, J 4.6, 4.6, C-2-H), 4.81 (2 H, AB, CH$_2$Ph), 4.79, 4.88 (2 H, AB, J$_{AB}$ 12.6, CH$_2$Ph), 7.22-7.42 (10 H, m, CH$_2$Ph)

16.4 DL-1,4-Di-O-benzyl-myoinositol 124

This compound was prepared according to the method of Gigg et al. [149]. A solution of 123 (68.6 g, 156 mmol) in 80% acetic acid (1340 ml) was heated under reflux for 15 min, cooled and diluted with the same volume of water. The product was collected to give DL-1,4-di-O-benzyl-myoinositol 124 (53 g, 147 mmol, 95%).

m.p. 203-204°C (Lit. [149,396]: 205-207°C, 203-204°C)
$\delta_H$ (d$_6$-DMSO, 270 MHz) 3.09-3.19 (2 H, m, C-1,5-H), 3.30 (1 H, ddd, J 9.7, 4.2, 2.4, C-3-H), 3.45 (1 H, dd, J 9.3, 9.3, C-4-H), 3.62 (1 H, ddd, J 9.3, 9.3, 4.8, C-6-H), 3.98 (1 H, ddd, J 3.3, 2.8, 2.8, C-2-H), 4.58, 4.65 (2 H, AB, J$_{AB}$ 12.1, CH$_2$Ph), 4.67 (1 H, d, J 6.8, D$_2$O ex, OH), 4.76, 4.79 (2 H, AB, J$_{AB}$ 11.4, CH$_2$Ph), 4.76 (1 H, d, J 5.0, D$_2$O ex, OH), 4.79 (1 H, d, J 4.6, D$_2$O ex, OH), 4.83 (1 H, d, J 5.0, D$_2$O ex, OH), 7.23-7.44 (10 H, m, CH$_2$Ph)

16.5 DL-1-O-Allyl-3,6-di-O-benzyl-myoinositol 125

This compound was prepared by a modification of the method of Gigg et al. [168,155], i.e. caesium fluoride rather than tetrabutylammonium iodide was used. DL-1,4-Di-O-benzyl-myoinositol 124 (12 g, 33.3 mmol) and dibutyltin oxide (9 g, 36 mmol) were heated under reflux in toluene (300 ml) in a Dean and Stark
apparatus overnight. The solution was evaporated to complete dryness in vacuo. Cesium fluoride (7 g, 46 mmol) was added to the resulting white solid and the mixture was dried in vacuo for 2 h, and then suspended in a solution of allyl bromide (8 ml, 42 mmol) in dry dimethylformamide (200 ml). The mixture was stirred at r.t. for 20 h and evaporated to dryness. The residue was taken up in ethylacetate, washed with 1 M HCl, brine and saturated NaHCO₃ and the precipitated tin derivatives removed by filtration through Celite. The filtrate was concentrated and chromatographed on silica gel using ether as eluent to give 10.4 g (26 mmol, 78 %) of 125.

m.p. 103-104°C (Lit.₁₁₆₈: 107-108°C)
tlc (ether) Rₗ 0.3

δₛ (CDCl₃, 300 MHz) 2.53 (1 H, s, OH), 2.74-2.75 (1 H, d, J 2.2, OH) 2.79 (1 H, d, J 2.3, OH), 3.25 (1 H, dd, J 9.5, 2.8, C-3(1)-H), 3.30 (1 H, dd, J 9.45, 2.8, C-1(3)-H), 3.39 (1 H, ddd, J 9.4, 9.4, 2.0, C-5-H), 3.77 (1 H, dd, J 9.4, 9.4, C-6-H), 3.95 (1 H, ddd, J 9.4, 9.4, 2.0, C-4-H), 4.14, 4.18 (2 H, AB, ddd, Jₐₜ 12.6, J 5.7, 1.4, 1.4, CH₂-CH=CH₂), 4.24 (1 H, dd, J 2.8, 2.8, C-2-H), 4.69, 4.74 (2 H, AB, Jₐₜ 11.7, CH₂Ph), 4.74, 4.93 (2 H, AB, J 11.2, CH₂Ph), 5.19 (1 H, ddt, J 10.4, 1.4, 1.4, cis-CH₂-CH=CH₂), 5.29 (1 H, ddt, J 17.2, 1.6, 1.6, trans-CH₂-CH=CH₂), 5.92 (1 H, ddt, J 17.2, 10.4, 5.7, CH₂-C=H=CH₂), 7.25-7.40 (10 H, m, CH₂Ph)

16.6 DL-1-O-allyl-3,6-di-O-benzyl-4,5-O-isopropylidene-myoinositol 93

This compound was prepared according to the method of Gigg et al.₁₁₆₈. DL-1-O-allyl-3,6-di-O-benzyl-myoinositol 125 (4.1 g, 10 mmol), 2,2-dimethoxypropane (41 ml) and toluene-p-sulphonic acid (410 mg) were stirred for 2 h in 100 ml dry acetone, when tlc (ether-petrol ether b.p.40-60°C) showed a product (Rₗ 0.6). After cooling, 5 ml of triethylamine were added and stirring continued for 30 min. The solvents were evaporated and the crude product chromatographed on silica gel in ether / petrol ether (b.p. 40-60°C) to give DL-1-O-allyl-3,6-di-O-benzyl-4,5-O-isopropylidene-myoinositol 93 (3.27 g, 7.4 mmol, 74 %).

m.p. 102-103°C (Lit.₁₁₆₈: 103-105°C)
δₛ (CDCl₃, 300 MHz) 1.46 (3 H, s, CH₃), 1.47 (3 H, s, CH₃), 2.63 (1 H, s, OH), 3.25 (1 H, dd, J 8.4, 3.4, C-3(1)-H), 3.37 (1 H, dd, J 10.1, 10.1, C-5-H), 3.57 (1 H, dd, J 10.1, 3.1, C-1(3)-H), 3.93 (1 H, dd, J 9.7, 9.7, C-4(6)-H), 4.08 (1 H, dd, J
9.8, 9.8, C-6(4)-H), 4.16-4.21 (2 H, m, CH2-CH=CH2), 4.22 (1 H, dd, J 2.9, 2.9, C-2-H), 4.72, 4.86 (2 H, AB, JAB 11.6, CH2Ph), 4.76, 4.88 (2 H, AB, JAB 12.0, CH2Ph), 5.17 (1 H, ddt, J 10.25, 1.4, 1.4, cis-CH2-CH=CH2), 5.26 (1 H, ddt, J 17.2, 1.65, 1.65, trans-CH2-CH=CH2), 5.90 (1 H, ddt, J 17.2, 10.25, 5.5, CH2-CH=CH2), 7.28-7.41 (10 H, m, CH2Ph)

16.7 DL-1-0-Allyl-2,3,6-tri-O-benzyl-4,5-O-isopropylidene-myco-inositol 126

DL-1-0-Allyl-3,6-di-O-benzyl-4,5-O-isopropylidene-myco-inositol 93 (8.9 g, 20.2 mmol), 18-crown-6 (4.54 g, 1.7 mmol), potassium hydroxide (25 g, 446 mmol) and benzyl chloride (50 ml, 55 g, 434 mmol) were heated with vigorous stirring up to 120°C. After 30 min heating was removed, and the solution allowed to cool to room temperature. Toluene (70 ml) was added and the mixture was washed with 1 M sodium chloride solution, until the aqueous phase remained neutral. The toluene layer was dried (MgSO4) and evaporated at 95°C (high vacuum) to remove volatile benzylation by-products. Recrystallisation from ethanol gave 126 (10.5 g, 19.8 mmol, 98%).

m.p. 81-83°C (Lit.155: 82-84°C)
tlc (ether-petrol ether b.p.40-60°C 1:1): Rf 0.9
δH (CDCl3, 300 MHz) 1.46 (6 H, s, 2 CH3), 3.27 (1 H, dd, J 9.5, 3.2, C-3(1)-H), 3.41 (1 H, dd, J 9.8, 9.8, C-5-H), 3.57 (1 H, dd, J 9.7, 3.2, C-1(3)-H), 3.93 (1 H, dd, J 9.7, 9.7, C-4(6)-H), 4.08 (1 H, dd, J 9.8, 9.8, C-6(4)-H), 4.14-4.19 (2 H, m, CH2-CH=CH2), 4.18 (1 H, dd, J 2.7, 2.7, C-2-H), 4.56, 4.67 (2 H, AB, JAB 11.8, CH2Ph), 4.71, 4.85 (2 H, AB, JAB 11.8, CH2Ph), 4.76, 4.88 (2 H, AB, JAB 11.9, CH2Ph), 5.17 (1 H, ddt, J 10.25, 1.4, 1.4, cis-CH2-CH=CH2), 5.26 (1 H, ddt, J 17.2, 1.65, 1.65, trans-CH2-CH=CH2), 5.90 (1 H, ddt, J 17.2, 10.25, 5.5, CH2-CH=CH2), 7.28-7.41 (15 H, m, CH2Ph)

16.8 DL-1-0-Allyl-2,3,6-tri-O-benzyl-myco-inositol 127

This compound was prepared according to the method of Gigg et al.168. 126 (10 g, 18.8 mmol) was heated under reflux in a solution of methanol (360 ml) and 1 M hydrochloric acid (40 ml) for 30 min. After cooling, an excess of NaHCO3 was added and the solvents were evaporated. The product was extracted from the residue with ether, the extract was dried over magnesium
sulphate, and the solvent was evaporated to give 127 (8.6 g, 17.5 mmol, 93 %).

m.p. 73-75°C (from ethanol / water, Lit. [168] : 'syrup')

δH (CDCl3, 270 MHz) 2.56, 2.60 (2 H, 2 br s, D2O ex, OH), 3.20 (1 H, dd, J 9.7, 2.2, C-3-H), 3.28 (1 H, dd, J 9.7, 2.2, C-1-H), 3.42 (1 H, dd, J 9.3, 9.3, C-5-H), 3.87 (1 H, dd, J 9.4, 9.4, C-6-H), 4.04 (1 H, dd, J 9.5, 9.5, C-4-H), 4.06 (1 H, dd, J 2.5, 2.5, C-2-H), 4.10 (2 H, ddd, J 5.3, 1.4, 1.4, CH2CH=CH2), 4.55, 4.61 (2 H, AB, JAB 11.7, CH2Ph), 4.75, 4.96 (2 H, AB, JAB 11.9, CH2Ph), 4.79, 4.89 (2 H, AB, JAB 11.2, CH2Ph), 5.19 (1 H, ddt, J 10.4, 1.4, 1.4, cis-CH2CH=CH2), 5.31 (1 H, ddt, J 17.2, 1.6, 1.6, trans-CH2CH=CH2), 5.91 (1 H, ddt, J 17.2, 10.4, 5.3, CH2CH=CH2), 7.25-7.42 (15 H, m, CH2Ph);

δC (CDCl3, 68 MHz) 71.45, 72.26, 74.02, 75.35 (4 t, CH2CH=CH2 and CH2Ph), 72.20, 73.53, 74.60, 79.95, 80.76, 80.83 (6 d, inositol ring C), 116.73 (s, CH2CH=CH2), 127.37, 127.63, 127.70, 127.79, 128.12, 128.38, 128.44 (8 d, CH2Ph), 134.67 (d, CH2CH=CH2), 137.85, 138.76 (2 s, CH2Ph);

m/z (70 eV El) 399 [(M - C7H7)+, 2 %], 307, 181 (5), 131 (10), 109 (5), 91 [(C7H7)+*, 100];

m/z (Cl, Isobutane) 491 (M+H)+, 399 (10), 309, 181 (20), 131 (20), 107 (100), 91 (80), 69 (20)

16.9  DL-2,3,6-Tri-O-benzyl-1-O-(prop-1-enyl)-myo-inositol 128 (mixture of cis- and trans-isomers)

A solution of 127 (1.55 g, 3.16 mmol) and diazabicyclo[2.2.2]octane (71 mg, 0.65 mmol) in a mixture of ethanol / toluene / water (7:3:1, v/v/v) was heated. When the solution had reached reflux temperature, tris(triphenylphosphine)rhodium chloride (202 mg, 0.22 mmol) was added and the mixture was heated under reflux for 30 min. After cooling, the mixture was diluted with water and extracted twice with ether. The combined organic layers were dried over magnesium sulphate and the solvent was evaporated. Chromatography on silica gel (ether / hexane 2:1) gave 128 as a mixture of ca. 5:1 cis:trans prop-1-enyl isomers. Yield 1.27 g (82 %).

δH (CDCl3, 300 MHz) 1.56 (0.5 H, dd, J 6.8, 1.55, trans-CH=CH-CH3), 1.68 (2.5 H, dd, J 6.8, 1.6, cis-CH=CH-CH3), 2.67 (1 H, d, J 2.0, D2O ex, OH), 2.70 (1 H, d, J 1.8, D2O ex, OH), 3.22 (1 H, dd, J 9.7, 2.4, C-3-H), 3.46 (1 H, dd, J 9.3, 9.3,
C-5-H), 3.57 (1 H, dd, J 9.7, 2.3, C-1-H), 3.93 (1 H, dd, J 9.4, 9.4, C-6-H), 4.04 (1 H, dd, J 9.5, 9.5, C-4-H), 4.07 (1 H, dd, J 2.4, 2.4, C-2-H), 4.46-4.98 (7 H, m, CH=CH-CH₃ and 3 CH₂Ph), 6.07-6.15 (1 H, m, CH=CH-CH₃), 7.25-7.42 (15 H, m, CH₂Ph);

16.10 DL-2,3,6-Tri-O-benzyl-1-O-(cis-prop-1-enyl)-myo-inositol 128

A solution of 127 (2 g, 4.08 mmol) and freshly sublimed potassium tert-butoxide (2.28 g, 20 mmol) in dry DMSO (50 ml) was stirred for 3 h at 50°C when hptlc (ether) showed complete conversion of the starting material (Rf 0.78) to a single product (Rf 0.80). Water (50 ml) was added to the brown solution, which was then extracted with ether (2 x 100 ml). The combined organic layers were dried over anhydrous magnesium sulphate and evaporated to dryness to give 128 (1.97 g, 4.02 mmol, 98%)

m.p. 101-103°C (from ethanol / water)

Found: C, 73.28; H, 6.93. Calc for C₃₀H₃₄O₆ (490.60): C, 73.45; H, 6.99 %.

δₜ (CDCl₃, 270 MHz) 1.67 (3 H, dd, J 6.9, 1.65, CH=CH-CH₃), 2.76 (1 H, d, J 2.4, D₂O ex, OH), 2.79 (1 H, d, J 2.0, D₂O ex, OH), 3.21 (1 H, dd, J 9.7, 2.4, C-3-H), 3.42 (1 H, ddd, J 9.2, 2.2, D₂O shake gives dd, J 9.2, 2.2, C-5-H), 3.55 (1 H, dd, J 9.7, 2.4, C-1-H), 3.92 (1 H, dd, J 9.5, 9.5, C-6-H), 4.03 (1 H, ddd, J 9.5, 9.5, 2.0Hz, D₂O shake gives dd, J 9.5, 9.5, C-4-H), 4.07 (1 H, ddd, J 9.5, 9.5, C-2-H), 4.49 (1 H, dq, J 6.7, 6.7, CH=CH-CH₃), 4.49, 4.56 (2 H, AB, Jₐₜ 11.6, CH₂Ph), 4.70, 4.88 (2 H, AB, Jₐₜ 11.6, CH₂Ph), 4.77, 4.88 (2 H, AB, Jₐₜ 11.6, CH₂Ph), 6.08 (1 H, dd, J 6.2, 1.65, CH=CH-CH₃), 7.23-7.42 (15 H, m, CH₂Ph);

δ₀ (CDCl₃, 68 MHz) 9.44 (q, CH=CH-CH₃), 74.44, 75.35 (2 t, CH₂Ph), 72.07, 74.24, 74.96, 79.43, 80.28, 83.23 (6 d, inositol ring C), 100.97 (d, CH=CH-CH₃), 127.50, 127.66, 127.79, 128.18, 128.28, 128.41, 128.48 (7 d, CH₂Ph), 137.60, 138.43 (2 s, CH₂Ph), 145.34 (d, CH=CH-CH₃);

m/z (+ve ion FAB) 489 [(M-H)+, 0.5 %], 399 (3), 181 (15), 91 (100);

m/z (-ve ion FAB) 979 [(2M-H)-, 30 %], 643 [(M+NBA)-, 60], 489 [(M-H)-, 100], 399 (20), 381 (20)

16.11 Dichloro(2-cyanoethoxy)phosphine 129

This compound was prepared according to the procedure of Bannwarth and
Trzeciak \cite{226}, except that a larger volume of solvent (800 ml of ether rather than 200 ml) was used.

To a solution of PCl$_3$ (137.33 g, 85.5 ml, 1 mol) and dry pyridine (81 ml, 1 mol) in 800 ml of ether under N$_2$ at 78°C was added 3-hydroxypropionitrile (71.05 g, 68 ml, 1 mol) dropwise over a period of 90 min. After cooling had been removed, stirring was continued overnight at r.t. The precipitate was removed by filtration under N$_2$ and washed twice with 100 ml of ether. After evaporation of ether, the oily residue was dried for 3 h, giving crude 129 (104 g, 0.6 mol, 60%). Since 129 decomposed partly during distillation, the crude product was used for the preparation of 130.

$\delta$$_p$ (CDCl$_3$; 36 MHz) 179.77

16.12 (2-Cyanoethoxy)bis(diisopropylamino)phosphine 130

This compound was prepared according to the procedure of Bannwarth and Trzeciak \cite{226}.

To a solution of crude 129 (104 g, 0.6 mol) in 1 litre of dry ether under N$_2$, diisopropylamine (753 ml, 5.3 mol) was added over a period of 1 h at -10°C with stirring, which was continued overnight at r.t. The precipitated hydrochloride was removed by filtration under N$_2$ and washed twice with 300 ml of ether. The combined ether solutions were evaporated, and the oily residue was distilled under vacuum after the addition of 1.5 g of calcium hydride to give 130 (117 g, 388 mmol, 56%, Lit. \cite{226} 64%).

b.p. 120°C / 0.8 hPa (Lit. \cite{226} 117°C / 0.5 Torr)

$\delta$$_p$ (CDCl$_3$; 36 MHz) 122.56

16.13 Bis(2-cyanoethoxy)(diisopropylamino)phosphine 131

This compound was prepared according to the procedure of Bannwarth and Trzeciak \cite{226}.

To a solution of 130 (117 g, 388 mmol) and diisopropylammonium tetrazolide (33.4 g, 194 mmol) in 1400 ml dry dichloromethane, 3-hydroxypropionitrile (29 ml, 30.3 g, 427 mmol) was added dropwise under N$_2$ with stirring. After complete addition, stirring was continued for another 2 h. The mixture was poured into
1200 ml of saturated aqueous sodium bicarbonate, which was then extracted with dichloromethane (3 x 600 ml). The combined organic layers were dried over sodium sulphate and evaporated. Flash chromatography of the crude material (eluting with ether / pentane 3:1) gave 131 (72 g, 265 mmol, 68 %, Lit. [226] 81 %).

δ_H (CDCl₃; 300 MHz) 1.14 (12 H, d, J 6.8, CH(CH₃)₂), 2.62-2.57 (4 H, t, J 6.2, OCH₂CH₂CN), 3.47-3.65 (2H, m, CH(CH₃)₂), 3.68-3.93 (4 H, m, OCH₂CH₂CN)

δ_P (CDCl₃; 36 MHz) 149.15

16.14 DL-2,3,6-Tri-O-benzyl-4,5-bis-[di-(2-cyanoethoxy)phospho]-1-O-(cis-prop-1-enyl)-myo-inositol 132

A solution of bis(2-cyanoethoxy)(diisopropylamino)phosphine 131 (7 g, 26 mmol) in dichloromethane (50 ml) was added to a solution of 128 (1.24 g, 2.6 mmol) and tetrazole (2.19 g, 31.2 mmol) in dichloromethane (50 ml). The mixture was stirred at r.t. for 1 h (δ_P 140.70, 140.47 ppm). 10% water in THF (20 ml) was added and stirring continued for 30 min. 2,6-Lutidine (2 ml) followed by tert-butyl hydroperoxide (20 ml, 70 % in water) was then added and stirring continued overnight. The solution was washed with saturated aqueous sodium hydrogen carbonate (2 x 100 ml) and dried over magnesium sulphate. The solvents were evaporated and the residue chromatographed on silica gel with 0 % to 100 % ethyl acetate in hexane and then 0 % to 10 % ethanol in ethyl acetate. The product was recrystallised from ethanol to give 132 (1.43 g, 64 %).

m.p. 118-120°C (from ethanol)
tlc (ethyl acetate:ethanol 9:1) R_f 0.76

Found: C, 58.49; H, 5.57; N, 6.52. Calc for C₄₂H₄₈N₄O₁₂P₂ (862.81): C, 58.47; H, 5.61; N, 6.49 %.

δ_H (CDCl₃, 270 MHz) 1.63 (3 H, dd, J 6.8, 1.6, CH₂=CH-CH₃), 2.16-2.39 (4 H, m, CH₂CH₂CN), 2.65-2.69 (4 H, m, CH₂CH₂CN), 3.50 (1 H, dd, J 9.5, 2.0, C-3-H), 3.66 (1 H, dd, J 9.7, 2.0, C-1-H), 3.89-4.31 (10 H, m, CH₂CH₂CN, C-2-H, C-6-H), 4.38-4.99 (9 H, m, CH₂Ph, C-4-H, C-5-H, CH=CH-CH₃), 6.06 (1 H, dd, J 6.8, 1.6, CH=CH-CH₃), 7.26-7.40 (15 H, m, CH₂Ph);

δ_C (CDCl₃, 68 MHz) 9.34 (q, CH=CH-CH₃), 18.97, 19.10, 19.23, 19.33 (4 t,
132 (mixture of cis- and trans-isomers):
\( \delta_1 (\text{CDCl}_3, 270 \text{ MHz}) 1.51 \) (0.5 H, dd, J 6.8, 1.6, trans-\( \text{CH}_2=\text{CH}-\text{CH}_3 \)), 1.63 (2.5 H, dd, J 6.8, 1.6, cis-\( \text{CH}_2=\text{CH}-\text{CH}_3 \)), 2.10-2.45 (4 H, m, \( \text{CH}_2\text{CH}_2\text{CN} \)), 2.56-2.76 (4 H, m, \( \text{CH}_2\text{CH}_2\text{CN} \)), 3.50 (1 H, dd, J 9.5, 2.0, C-3-H), 3.66 (1 H, dd, J 9.5, 2.0, C-1-H), 3.89-4.31 (10 H, m, \( \text{CH}_2\text{CH}_2\text{CN}, \text{C}-2\text{-H}, \text{C}-6\text{-H} \)), 4.38-4.99 (9 H, m, \( \text{CH}_2\text{Ph}, \text{C}-4\text{-H}, \text{C}-5\text{-H}, \text{CH}=\text{CH}-\text{CH}_3 \)), 6.03-6.13 (1 H, m, \( \text{CH}=\text{CH}-\text{CH}_3 \)), 7.26-7.40 (15 H, m, \( \text{CH}_2\text{Ph} \));

16.15 DL-1,2,4-Tri-O-benzyl-5,6-bis[dil2-cyanoethoxy]phospho]-myo-inositol 133

a) A solution of mercuric chloride (300 mg, 1.1 mmol) in acetone / water (10:1 (v/v), 4 ml) was added dropwise with stirring to a mixture of 132 (949 mg, 1.1 mmol) and yellow mercuric oxide (300 mg) in acetone / water (10:1 (v/v), 10 ml). After the addition was complete, stirring was continued for a further 5 min. The mercuric oxide was removed by filtration through Celite, the solvents evaporated and the residue taken up in ethyl acetate (50 ml). The solution was washed with semisaturated aqueous potassium iodide solution (50 ml), dried and evaporated. Chromatography on silica gel using hexane → ethyl acetate → ethyl acetate / ethanol (9:1, v/v) gave the pure title compound (786 mg, 87 %) as a syrup.

b) A solution of 132 (470 mg, 0.54 mmol) in 30 ml 1 M HCl:methanol (1:5) was heated under reflux for 30 min when tlc (ethyl acetate:ethanol 9:1) showed complete conversion of the starting material (Rf 0.76) into a single product (Rf 0.76)
0.71). After cooling, the mixture was treated with an excess of sodium hydrogen carbonate and the solvents were evaporated. The title compound was extracted with ether (2 x 50 ml) and the solvent was evaporated to give 133 (426 mg, 95%) as a syrup which could not be crystallised.

δ_1H (CDCl₃, 300 MHz) 2.14-2.46 (4 H, m, CH₂CH₂CN), 2.60-2.72 (5 H, m, CH₂CH₂CN, OH, OH D₂O ex), 3.53 (1 H, dd, J 9.9, 2.1, C-3-H), 3.62 (1 H, ddd, J 9.9, 7.3, 2.4, D₂O ex gives dd, J 9.9, 2.4, C-1-H), 3.86 (1 H, dd, J 9.4, 9.4, C-4-H), 4.06 (1 H, dd, J 2.3, 2.3, C-2-H), 4.30-4.37 (8 H, m, CH₂CH₂CN), 4.43 (1 H, q, J 9.1, C-5-H), 4.56, 4.67 (2 H, AB, J_AB 11.3, CH₂Ph), 4.75, 4.93 (2 H, AB, J_AB 11.6, CH₂Ph), 4.79, 4.92 (2 H, AB, J_AB 11.4, CH₂Ph), 4.83 (1 H, q, J 9.3, C-6-H), 7.22-7.41 (15 H, m, CH₂Ph);

δ_13C (CDCl₃, 68 MHz) 18.65, 18.78, 18.84 (3 t, CH₂CH₂CN), 62.02, 62.08, 62.66 (3 t, CH₂CH₂CN), 71.97, 74.05, 74.86 (3 t, CH₂Ph), 71.55, 75.80, 77.65, 78.40, 79.23, (5 d, inositol ring C), 116.64, 116.86, 116.99 (3 s, CN), 127.08, 127.50, 127.57, 127.63, 127.86, 128.15, 128.22, 128.31 (8 d, CH₂Ph), 136.91, 137.85, 138.04 (3 s, CH₂Ph);

δ_P (CDCl₃, 36 MHz) -5.52, -5.65;

m/z (+ve ion FAB) 823 [(M+H)+, 1.3 %], 222 (8), 181 (6), 144 (8), 91 [(C₇H₇)+, 100];
m/z (-ve ion FAB) 768 [(M - CH₂CH₂CN)-, 45 %], 203 (100), 150 (95), 97 (80);

Accurate mass calc for C₃₉H₄₅O₁₂N₄P₂ (M+H)+ 823.2509, found 823.2509

16.16 DL-2,3,6-Tri-O-benzyl-4,5-bis[(2-cyanoethoxy)phospho]-1-[di(2-cyanoethoxy)thiophospho]-myo-inositol 134

Bis(2-cyanoethoxy)diisopropylamino phosphine (670 mg, 2.5 mmol) was added to a solution of 133 (412 mg, 0.5 mmol) and tetrazole (210 mg, 3 mmol) in dichloromethane (25 ml). The mixture was stirred at r.t. for 1 h. Dry pyridine (5 ml) and sulphur (320 mg, 10 mmol) was added and the solution stirred for another 24 h. The solvent was evaporated and the residue chromatographed on silica gel (eluent hexane → ethyl acetate → ethanol / ethyl acetate 10 % v/v) to give pure 134 as a syrup, yield 405 mg (79%).
δH (CDCl3, 270 MHz) 2.03-2.47 (6 H, m, CH2CH2CN), 2.49-2.79 (6 H, m, CH2CH2CN), 3.61 (1 H, dd, J 9.9, 1.8, C-3-H), 3.82-4.46 (14 H, m, CH2CH2CN, C-1-H, C-6-H), 4.40 (1 H, br s, C-2-H), 4.52 (1 H, q, J 9.2, C-5-H), 4.67, 4.75 (2 H, AB, JAB 11.3, CH2Ph), 4.87 (2 H, AB, CH2Ph), 4.85 (1 H, q, J 8.6, C-4-H), 4.84, 4.92 (2 H, AB, JAB 11.7, CH2Ph), 7.27-7.44 (15 H, m, CH2Ph)

δC (CDCl3, 68 MHz) 18.78, 18.91, 19.14, 19.20, 19.27 (5 t, CH2CH2CN), 62.08, 62.18, 62.24, 62.37, 62.44, 62.53, 62.63, 62.79, 62.83 (9 t, CH2CH2CN), 72.33, 74.34, 77.26 (3 t, CH2Ph), 74.63, 75.25, 78.04, 78.36, 78.46, 79.17 (6 d, inositol ring C), 116.41, 116.51, 116.57, 116.63, 116.96 (5 s, CN), 126.40, 127.60, 127.76, 128.05, 128.18, 128.31, 128.48 (7 d, CH2Ph), 136.86, 137.72, 137.85 (3 s, CH2Ph);

δP (CDCl3, 36 MHz) 66.83, -2.83, -3.10;

m/z (+ve ion FAB) 1025 [(M+H)+, 1.3 %], 181 (6), 144 (10), 91 [(C7H7)+, 100].

m/z (-ve ion FAB) 970 [(M - CH2CH2CN)-, 45 %], 219 (30), 203 (100), 150 (90), 97 (80);

Accurate mass calc for C₄₅H₅₂O₁₄N₆P₃S (M+H)+ 1025.2475 found 1025.2475

16.17 DL-myo-Inositol 4,5-bisphosphate 1-phosphorothioate 80

Ammonia was condensed into a three neck flask at -78°C. An excess of sodium was added to dry the liquid ammonia which was then distilled into a second three neck flask and kept at -78°C. Sodium was added until the solution remained blue. Compound 134 (120 mg, 117 μmol) was dissolved in dry dioxane (2 ml) and added to the sodium-liquid ammonia mixture. After stirring for 15 min the reaction was quenched by adding ethanol to the mixture, which became colourless. The ammonia was evaporated and the crude product taken up in water. The aqueous solution was treated with Dowex resin (H+) until a pH of 6 was reached. The resin was filtered off and washed well with water. A few drops of triethylamine were added to the filtrate which was then evaporated to dryness. The crude product was purified by ion-exchange chromatography on DEAE Sephadex A-25 eluting with a gradient of triethylammonium bicarbonate buffer (0.1 M to 1 M), pH 8.0. The triethylammonium salt of 80 eluted at approx. 800 mM and after evaporation of TEAB the product was obtained as a glass. Yield 54 μmol (46%).
δ\text{H} (D\text{2}O, pH 8, 300 MHz) 3.62 (1 H, dd, J 9.8, 2.5, C-3-H), 3.78 (1 H, dd, J 9.5, 9.5, C-6-H), 4.01 (1 H, q, J 9.2, C-5-H), 4.20-4.11 (2 H, m, C-2-H, C-1-H), 4.25 (1 H, q, J 9.4, C-4-H);

δ\text{P} (D\text{2}O, pH 8, 36 MHz) 42.13, 4.58, 3.50;

m/z (+ve ion FAB) 538 [(M+Et\text{3}NH\text{+}), 10\%], 436 (M\text{+}, 3), 102 (Et\text{3}NH\text{+}, 100)

Accurate mass calc for C\text{12}H\text{31}O\text{14}N\text{P}_{\text{3}}S (M+Et\text{3}NH\text{+}) 538.0678 found 538.0678

16.18 Fluorescent Labelling of Ins(1,4,5)P_{\text{3}}-1\text{S}

\textbf{}S-[2-[(N-Methyl-N-(7-nitro-2,1,3-benzoxadiazole-4-yl)amino]-ethoxycarbonylmethyl]-DL-myo-inositol 4,5-bisphosphate 1-phosphorothioate 135

A mixture of 80 (30 µmol) and 4-{N-2-(iodoacetoxy)ethyl-N-methylamino}-7-nitro-2,1,3-benzoxadiazole (15 mg, 33µmol) in ethanol was protected from light and stirred for 2 h at 0°C. The product was purified by ion-exchange chromatography on DEAE Sephadex A-25 eluting with a gradient of triethylammonium bicarbonate buffer (0.1 M to 1 M), pH 8.0. 135 eluted at ca. 800 mM and was obtained as a dark orange glass after evaporation of TEAB. Yield 17 µmol (57%).

δ\text{P} (D\text{2}O, 36 MHz, 1H-coupled) 1.72 (d, J 8.5, OPO\text{3}\text{2}\text{−}), 3.01 (d, J 8.5, OPO\text{3}\text{2}\text{−}), 19.86 (q, J 9.1, OPSO\text{2}\text{−});

m/z (+ve ion FAB) 815 [(M+Et\text{3}NH\text{+}, 3 %], (799 [(M+Et\text{3}NH\text{-O})\text{+}, 5], 714 (M\text{+}, 12), 698 [(M\text{-O})\text{+}, 14], 596 [(Ins(1,4,5)P\text{3}S-CH\text{2}CO\text{2} + Et\text{3}NH\text{+}, 27], 102 (Et\text{3}NH\text{+}, 100)

17 Synthesis of 1D-myo-Inositol 4,5-bisphosphate 1-phosphorothioate

17.1 1D-(+)-1-O-Allyl-2,3,6-tri-O-benzyl-4,5-di-O-[(-)ω-camphanoyl]-myo-inositol 136

A mixture of DL-1-O-allyl-2,3,6-tri-O-benzyl-myo-inositol (3.432 g, 7 mmol) and (-)-ω-camphamic acid chloride (6.067 g, 28 mmol) in dry pyridine (50 ml) was stirred for 12 h at r.t. The solution was cooled in ice-water then water (0.5 ml)
was added, and the solution was stirred for another 1 h at r.t., after which hptlc (ether:petroleum ether 1:1) showed two products (Rf 0.52 and 0.42). Ether (100 ml) and dichloromethane (50 ml) were added and the organic phase was washed successively with saturated aqueous potassium chloride, ice-cold M-hydrochloric acid, saturated aqueous potassium chloride and saturated aqueous sodium hydrogen carbonate (200 ml each) and then dried over magnesium sulphate. Evaporation of the solvents gave a syrup, which was taken up in ether (40 ml) and kept at -20°C overnight. The crystals formed (1.2 g) were filtered, the mother liquor evaporated, and the residue was dissolved in a mixture of ether (20 ml) and methanol (5 ml) to give more crystals (1 g). Overall yield: 2.2 g (2.6 mmol, 74%) of 136.

m.p. 142-143°C (from ethyl acetate / hexane);
\[\alpha_D^{21} = +19.4^\circ\] (c = 5 in CHCl₃);
Found C, 70.4; H 6.73. Calc for C₅₀H₅₈O₁₂ (851.00): C, 70.57; H, 6.87 %.

δ_H (CDCl₃, 300 MHz) 0.75 (6 H, 2 s, CH₃), 0.91 (3 H, s, CH₃), 0.95 (3 H, s, CH₃),
1.03 (6 H, s, 2 CH₃), 1.59-1.67 (2 H, m, CH₂), 1.77-1.88 (4 H, m, 2 CH₂), 2.27-
2.39 (2 H, m, CH₂), 3.44 (1 H, dd, J 9.7, 2.1, C-3-H), 3.55 (1 H, dd, J 10.7, 2.1,
C-1-H), 3.96-4.09 (2 H, m, CH₂CH=CH₂), 4.14 (1 H, dd, J 2.0, 2.0, C-2-H), 4.17
(1H, dd, J 9.6, 9.6, C-6-H), 4.45, 4.59 (2 H, AB, J_AB 11.5, CH₂Ph), 4.85, 4.87 (2
H, AB, J_AB 12.1, CH₂Ph), 4.62, 5.02 (2 H, AB, J_AB 11.3, CH₂Ph), 5.18 (1 H, ddt, J
10.4, 1.4, 1.4, cis-CH₂CH=CH₂), 5.27 (1 H, ddt, J 17.2, 1.6, 1.6, trans-
CH₂CH=CH₂), 5.34 (1 H, dd, J 9.6, 9.6, C-5-H), 5.76 (1 H, dd, J 9.9, 9.9, C-4-H),
5.83 (1 H, ddt, J 17.2, 10.4, 5.7, CH₂CH=CH₂), 7.20-7.42 (15 H, m, CH₂Ph);
δ_C (CDCl₃, 68 MHz) 16.28, 16.54, 16.64 (3 q), 28.80, 30.76 (2 t), 54.00, 54.78 (2
s), 71.39, 71.84, 74.15 (3 t, CH₂CH=CH₂ and CH₂Ph), 72.82, 73.37, 74.08,
78.07, 78.23, 80.18 (6 d, inositol ring C), 91.05 (s), 117.45 (t, CH₂CH=CH₂),
126.85, 127.24, 127.34, 127.708, 127.83, 127.96, 128.18, 128.28, 128.39 (9 d,
CH₂Ph), 134.12 (d, CH₂CH=CH₂), 137.04, 138.17 (3 s, CH₂Ph), 166.36, 166.62,
177.91 (3 s);
m/z (+ve ion FAB) 851 [(M+H)+, 7%], 181 (13), 109 (8), 91 (100)

17.2 1D-(+)-1-O- Allyl-2,3,6-tri-O-benzyl-my o-inositol 137

The (+)-biscamphanate 136 (1.26 g, 1.48 mmol) was dissolved in 100 ml methanol containing 1.3 g NaOH. The solution was heated under reflux for 1 h
when tlc (ether) showed complete conversion of the starting material (Rf 0.79) to a single product (Rf 0.59). After cooling, the solution was neutralised with solid CO₂. Water (100 ml) was added and the solution extracted twice with 100 ml chloroform each. The organic layers were dried over magnesium sulphate and the solvent evaporated to give 137 (703 mg, 1.44 mmol, 97 %).

m.p. 97-98°C (Lit. [168]: m.p. for the enantiomer 96-98 °C)

Found: C, 73.4; H, 6.92. Calc for C₃₀H₃₄O₆ (490.60): C, 73.45; H, 6.99 %.

[α]D¹⁸ = +21.5° (c = 4 in CHCl₃), (Lit. [168]: [α]D for the enantiomer - 20.5 °)

δH (CDCl₃, 270 MHz) 2.56, 2.60 (2 H, 2 br s, D₂O ex, OH), 3.20 (1 H, dd, J 9.7, 2.2, C-3-H), 3.28 (1 H, dd, J 9.7, 2.2, C-1-H), 3.42 (1 H, dd, J 9.3, 9.3, C-5-H), 3.87 (1 H, dd, J 9.4, 9.4, C-6-H), 4.04 (1 H, dd, J 9.5, 9.5, C-4-H), 4.06 (1 H, dd, J 2.5, 2.5, C-2-H), 4.10 (2 H, ddd, J 5.3, 1.4, 1.4, CH₂CH=CH₂), 4.55, 4.61 (2 H, AB, JAB 17.2, CH₂Ph), 4.75, 4.96 (2 H, AB, JAB 11.9, CH₂Ph), 4.79, 4.89 (2 H, AB, JAB 11.2, CH₂Ph), 5.19 (1 H, ddt, J 10.4, 1.4, 1.4, cis-CH₂CH=CH₂), 5.31 (1 H, ddt, J 17.2, 1.6, 1.6, trans-CH₂CH=CH₂), 5.91 (1 H, ddt, J 17.2, 10.4, 5.3, CH₂CH=CH₂), 7.25-7.42 (15 H, m, CH₂Ph);
δC (CDCl₃, 68 MHz) 71.45, 72.26, 74.02, 75.35 (4 t, CH₂CH=CH₂ and CH₂Ph), 72.20, 73.53, 74.60, 79.95, 80.76, 80.83 (6 d, inositol ring C), 116.73 (t, CH₂CH=CH₂), 127.37, 127.63, 127.70, 127.79, 127.96, 128.12, 128.38, 128.44 (8 d, CH₂Ph), 134.67 (d, CH₂CH=CH₂), 137.85, 138.76 (2 s, CH₂Ph);

m/z (70 eV El) 399 [(M - C₇H₇)+, 2 %], 307, 181 (5), 131 (10), 109 (5), 91 [(C₇H₇)+, 100];
m/z (CI, Isobutane) 491 (M+H)+, 399 (10), 309, 181 (20), 131 (20), 107 (100), 91 (80), 69 (20)

17.3 1D-(+)-2,3,6-Tri-O-benzyl-1-O-(cis-prop-1-enyl)-myo-inositol 138

137 (840 mg, 1.71 mmol) and freshly sublimed potassium tert-butoxide (778 mg, 6.84 mmol) in dry DMSO (30 ml) was stirred for 3 h at 50°C. The solution was worked up as described for the racemic compound to give 138 (815 mg, 1.66 mmol, 97%).

m.p. 116-118°C (from ethanol / water)

Found: C, 73.3; H, 7.02. Calc for C₃₀H₃₄O₆ (490.60): C, 73.45; H, 6.99 %.

[α]D¹¹ = +40.6° (c = 4 in CHCl₃)
Mass spectra and NMR data were identical to 128.

17.4 1D-(+)-2,3,6-Tri-O-benzyl-4,5-bis[di(2-cyanoethoxy)phospho]-1-O-(cis-prop-1-eny1)-myo-inositol 139

To a mixture of 138 (348 mg, 0.71 mmol) and tetrazole (615 mg, 8.8 mmol) in dry dichloromethane (20 ml) was added bis(2-cyanoethoxy)diisopropylamino phosphine (1.9 g, 7.1 mmol). After stirring at r. t. for 1 h, water in THF (10% v/v) was added and the solution was stirred for another 30 min. 2,6-Lutidine (0.5 ml) and tert-butyl hydroperoxide (5 ml) was then added and stirring continued overnight. Work-up as for 132 gave 139 (485 mg, 0.57 mmol, 80%).

m.p. 131-132°C (from ethanol)

Found: C, 58.6; H, 5.65; N, 6.51. Calc for C₄₂H₄₈N₄O₁₂P₂ (862.81) C, 58.47; H, 5.61; N, 6.49 %.

[α]D₁₉ = +11.5° (c = 3.5 in CHCl₃)

Mass spectra and NMR data were identical to 132.

17.5 1D-(+)-2,3,6-Tri-O-benzyl-4,5-di-[bis-(2-cyanoethoxy)phospho]-myo-inositol 140

139 (200 mg, 232 pmol) was heated under reflux with 10 ml 1 M HCl:methanol (1:5) and work-up as for the racemic compound gave 140 (183 mg, 96 %) as a syrup.

[α]D₁⁵ = + 8.5° (c = 4.5 in CHCl₃, Lit. [379] [α]D = +5)

Mass spectra and NMR data were identical to 133.

17.6 1D-(+)-2,3,6-Tri-O-benzyl-4,5-bis[di(2-cyanoethoxy)phospho]-1-[bis-(2-cyanoethoxy)thiophospho]-myo-inositol 141

Compound 140 (152 mg, 185 μmol) was phosphitylated and sulphoxidised analogously to the racemic compound to give 141 (140 mg, 137 μmol) after column chromatography.
[a]_D^{16} = +10.5^\circ \ (c = 2.5 \ \text{in CHCl}_3) \\
Mass spectra and NMR data were identical to 134.

17.7 1D-(−)-myo-Inositol 4,5-bisphosphate 1-phosphorothioate 142

141 (50.6 mg, 49.4 pmol) was deprotected as for the racemic compound to give pure 142 (21.5 pmol, 44 %) after ion-exchange chromatography.

[a]_D^{21} = -42.7^\circ \ (c = 0.19 \ \text{in H}_2\text{O, pH = 9.4}) \\
Mass spectra and NMR data were identical to 80.

18 Synthesis of 1L-myoinositol 1,4,5-trisphosphorothioate

18.1 1L-(−)-1-O-Allyl-2,3,6-tri-O-benzyl-4,5-di-O-[(−)-ω-camphanoyl]-myo-inositol 143

The mother liquor left from the crystallisation of 136 was kept at -20°C for several days when a solid had formed at the bottom of the flask. The supernatant was filtered off and the solid dissolved in hot ether. After leaving the solution in the fridge for two days crystals had formed which were collected to give 143 (2.4 g, 2.8 mmol, 80 %).

m.p. 174-177°C (from ethyl acetate / hexane); 
[a]_D^{18} = -25.0^\circ \ (c = 4.2 \ \text{in CHCl}_3); 
Found: C, 70.7; H 6.82. Calc for C_{50}H_{58}O_{12} (851.00): C, 70.57; H, 6.87 %.

δ_H (CDCl_3, 270 MHz) 0.81 (3 H, s, CH_3), 0.82 (3 H, s, CH_3), 0.96 (3 H, s, CH_3), 0.98 (3 H, s, CH_3), 1.04 (6 H, s, 2 CH_3), 1.57-1.65 (2 H, m, CH_2), 1.76-1.98 (4 H, m, 2 CH_2), 2.16-2.26 (2 H, m, CH_2), 3.39 (1 H, dd, J 9.9, 2.0, C-3-H), 3.56 (1 H, dd, J 9.9, 2.0, C-1-H), 4.01 (2 H, d, J 5.3, CH_2CH=CH_2), 4.05 (1 H, dd, J 1.5, 1.5, C-2-H), 4.16 (1H, dd, J 9.8, 9.8, C-6-H) 4.47, 4.54 (2 H, AB, J_{AB} 11.7, CH_2Ph), 4.63, 4.95 (2 H, AB, J_{AB} 11.2, CH_2Ph), 4.86, 4.86 (2 H, AB, J_{AB} 12.4, CH_2Ph), 5.18 (1 H, ddt, J 10.4, 1.4, 1.4, cis-CH_2CH=CH_2), 5.27 (1 H, ddt, J 17.2, 1.6, 1.6, trans-CH_2CH=CH_2), 5.34 (1 H, dd, J 9.6, 9.6, C-5-H), 5.76 (1 H, dd, J 9.9, 9.9, C-4-H), 5.83 (1 H, ddt, J 17.2, 10.4, 5.7, CH_2CH=CH_2), 7.22-7.42 (15 H, m, CH_2Ph);
\[ \delta^C (\text{CDCl}_3, 68 \text{ MHz}) 16.28, 16.44, 16.54 (3 \text{ q}), 28.70, 31.10 (2 \text{ t}), 53.58, 53.74, 54.56, 54.62 (4 \text{ s}), 71.42, 71.97, 72.26, 74.79 (4 \text{ t}, \text{CH}_2\text{CH}=\text{CH}_2 \text{ and } \text{CH}_2\text{Ph}) 72.26, 73.56, 74.21 77.81, 78.49, 80.02 (6 \text{ d}, \text{inositol ring C}), 90.66 (\text{s}), 117.16 (\text{t}, \text{CH}_2\text{CH}=\text{CH}_2), 126.95, 127.42, 127.53, 127.66, 128.13, 128.31 (7 \text{ d}, \text{CH}_2\text{Ph}), 134.21 (\text{t}, \text{CH}_2\text{CH}=\text{CH}_2), 137.23, 138.17, 138.24 (3 \text{ s}, \text{CH}_2\text{Ph}), 166.65, 177.87, 178.00 (3 \text{ s}); \]

\[ m/z \text{ as } 136 \]

18.2 1L-(-)-1-0-Allyl-2,3,6-tri-O-benzyl-myoinositol 144

The (-)-biscamphanate 143 (2.37 g, 2.79 mmol) in 200 ml methanol containing 2.4 g NaOH was heated under reflux for 1 h. Work-up as for 137 gave 144 (1.36 g, 2.78 mmol, 100%).

m.p. 96-98°C (from ethanol, Lit. [168]: 96-98°C)

Found: C, 73.5; H, 6.94. Calc for C_{30}H_{34}O_{6} (490.60): C, 73.45; H, 6.99%.

\[ [\alpha]_D^{19} = -21.9° (c = 4.3 \text{ in CHCl}_3, \text{Lit. [168]}: [\alpha]_D = 20.5°) \]

Mass spectra and NMR data were identical to 127.

18.3 1L-(-)-2,3,6-Tri-O-benzyl-1-O-(cis-prop-1-enyl)-myo-Inositol 145

144 (546 mg, 1.11 mmol) and freshly sublimed potassium tert-butoxide (505 mg, 4.44 mmol) in dry DMSO (20 ml) was stirred for 3 h at 50°C. The solution was worked up as described for the racemic compound to give 145 (536 mg, 1.09 mmol, 98%).

m.p. 117-119°C (from ethyl acetate / hexane)

Found: C, 73.6; H, 6.98. Calc for C_{30}H_{34}O_{6} (490.60): C, 73.45; H, 6.99%.

\[ [\alpha]_D^{19} = -41.1° (c = 4.3 \text{ in CHCl}_3) \]

Mass spectra and NMR data were identical to 128.

18.4 1D-(-)-1,2,4-tri-O-benzyl-myoinositol 146

145 (413 mg, 843 μmol) was heated under reflux for 30 min after which tlc (ether) showed complete conversion of the starting material (R_f 0.90) to a single product (R_f 0.59). The solution was allowed to cool and an excess of NaHCO_3
was added. The solvent was evaporated and the residue was extracted with chloroform, dried over magnesium sulphate and the solvent evaporated to give 146 (329 mg, 731 µmol, 87%).

m.p. 116-118°C (from ethanol / water, Lit. [168] 118-120°C, Lit. [396] 117-119°C for the enantiomer)
\[\alpha\]_D^{18} = -10.1° (c = 2.5 in CHCl₃, Lit. [168] -9.0°, Lit. [396] +15.5° for the enantiomer)

δ_H (CDCl₃, 400 MHz) 2.3-2.7 (3 H, br s, D₂O ex, 3 OH), 3.27 (1 H, dd, J 9.7, 2.6, C-1-H), 3.46 (1 H, dd, J 9.1, 9.1, C-5-H), 3.52 (1 H, dd, J 9.5, 2.6, C-3-H), 3.68 (1 H, dd, J 9.2, 9.2, C-4-H), 4.01 (1 H, dd, J 9.5, 9.5, C-6-H), 4.07 (1 H, dd, J 2.6, 2.6, C-2-H), 4.58, 4.68 (2 H, AB, J_AB 11.7, CH₂Ph), 4.83, 4.87 (2 H, AB, J_AB 11.5, CH₂Ph), 4.72, 4.92 (2 H, AB, J_AB 11.3, CH₂Ph), 7.28-7.33 (15 H, m, CH₃Ph)

m/z (+ve ion FAB) 901 [(2M+H)+, 2 %], 499 [(M-H)+, 9], 181 (20), 91 (100)

m/z (-ve ion FAB) 603 [(M+NBA)-, 100 %], 449 [(M-H)-, 70]

18.5 1L-(−)-2,3,6-tri-O-benzyl-1,4,5-[bis(2-cyanoethoxy)thiophospho]-myo-inositol 147

Bis(2-cyanoethoxy)(diisopropylamino)phosphine (1.27 g, 4.74 mmol) was added to a solution of 146 (142 mg, 316 µmol) and tetrazole (399 mg, 5.69 mmol) in dichloromethane (15 ml). The mixture was stirred at r.t. for 1 h, after which tlc (chloroform / acetone 9:1) showed complete conversion of the starting material (Rᵋ 0.24) into a product (Rᵋ 0.38) and 3¹P-NMR showed three phosphite triester peaks at 141.13, 140.53 and 139.65 ppm. Dry pyridine (5 ml) and sulphur (500 mg, 15.6 mmol) was added and the suspension stirred overnight. Tlc showed conversion of the triphosphate into a new product (Rᵋ 0.37). The solvent was evaporated and the residue chromatographed on silica gel to give 147 (332 mg, 314 µmol, 99 %) as a syrup.

\[\alpha\]_D^{17} = -15.9° (c = 3.5 in CHCl₃)

δ_H (CDCl₃, 270 MHz) 1.95-2.41 (4 H, m, CH₂CH₂CN), 2.58-2.62 (2 H, m, CH₂CH₂CN), 2.73-2.82 (6 H, m, CH₂CH₂CN), 3.64 (1 H, dd, J 9.9, 2.8, C-3-H), 3.68-3.89 (2 H, m, CH₂CH₂CN), 3.98-4.55 (14 H, m, CH₂CH₂CN, C-2-H, C-6-H, C-5-H, C-1-H), 4.60, 4.78 (2 H, AB, J_AB 11.7, CH₂Ph), 4.88 (2 H, AB, CH₂Ph), 4.92-5.00 (2 H, AB, C₆H₅CON), 7.28-7.31 (15 H, m, CH₃Ph), 10.41-10.49 (1 H, br s, COO⁻).
4.81, 4.95 (2 H, AB, J_AB 11.6, CH₂Ph), 5.19 (1 H, q, J 9.5, C-4-H), 7.34-7.43 (15 H, m, CH₂Ph)

δ_C (CDCl₃, 68 MHz) 18.36, 18.52, 18.81, 19.23, 19.40, 19.53 (7 t, CH₂CH₂CN), 62.44, 62.63, 62.73, 62.79, 62.86, 62.99, 63.09 (7 t, CH₂CH₂CN), 72.16, 73.92, 75.22 (3 t, CH₂Ph), 74.57, 77.42, 77.78, 78.69, 78.78, 79.63 (6 d, inositol ring C), 116.38, 116.44, 116.60, 116.73, 116.96, 117.03 (6 s, CN), 125.88, 127.24, 127.53, 127.73, 127.83, 127.99, 128.44, 128.60 (8 d, CH₂Ph), 137.17, 137.88, 138.21 (3 s, CH₂Ph)

δ_P (CDCl₃, 36 MHz) 66.70, 66.43, 66.25

m/z (+ve ion FAB) 1057 [(M+H)+, 5.4 %], 91 (100)

m/z (-ve ion FAB) 1001 [(M - CH₂CH₂CN)-, 30 %], 219 (100)

18.6 1L-myo-Inositol 1,4,5-trisphosphorothioate 148

Ammonia was condensed into a three neck flask at -78°C. An excess of sodium was added to dry the liquid ammonia which was then distilled into a second three neck flask and kept at -78°C. Sodium was added until the solution remained blue. 147 (150 mg, 142 µmol) was dissolved in dry dioxane (1 ml) and added to the sodium-liquid ammonia mixture. After stirring for 15 min the reaction was quenched by adding ethanol to the mixture (the colour of the solution turned white). The ammonia was evaporated and the crude product was purified by ion-exchange chromatography on DEAE Sephadex A-25 eluting with a gradient of triethylammonium bicarbonate buffer (0.1 M to 1 M), pH 8.0. The triethylammonium salt of 148 eluted at a buffer concentration of ca. 800 mM. Yield 129 µmol (91 %).

δ_H (D₂O, pH 8; 400 MHz) 3.76 (1 H, dd, J 9.5, 2.8, C-3-H), 3.98 (1 H, dd, J 9.5, 9.5, C-6-H), 4.16-4.34 (2 H, m, C-1,5-H), 4.39 (1 H, dd J 2.8, 2.8, C-2-H), 4.54 (1 H, m, C-4-H).

δ_P (D₂O; pH 8; 36 MHz) 44.82, 44.69, 42.06

m/z (-ve ion FAB) 467 [(M-H)-, 100 %]
19 Synthesis of myo-Inositol 1,3,5-trisphosphorothioate


This compound was prepared according to the method of Billington et al. [161]. Purification by flash chromatography was performed according to Baudin et al. [159].

To a suspension of myoinositol (50 g, 278 mmol) and toluene-p-sulphonic acid (13.83 g, 73 mmol) in dry DMF (700 ml), triethyl orthoformate (83 ml, 500 mmol) was added dropwise under N₂ at 140°C. The solution was stirred for 3 h at 140°C and then allowed to cool. DMF was distilled off at reduced pressure at 70° to 80°C. The residue was treated with 100 ml of 10 % sodium hydrogen carbonate solution, stirred for 15 min at r.t., diluted with water (1 litre) and extracted 3 times with 250 ml of CHCl₃. The aqueous phase was lyophilised, the residue treated with methanol (2 litres) at 50°C, and the suspension filtered. Methanol was evaporated under vacuum. Flash chromatography with acetonitrile (crude product dissolved in water) gave 40.47 g (77 %) of 150.

m.p. 297-300°C (Lit. [161,158] 300-302°C, δₜ (CD₃OD, 270 MHz) 4.06-4.09 (2 H, m), 4.12-4.17 (2 H, m), 4.40-4.44 (2 H, m), 5.41 (1 H, d, J 1.3 Hz, OOOCH)
δₑ (CD₃OD, 68 MHz) 61.33 (d), 69.35 (2 d), 70.84 (d), 76.25 (2 d), 104.21 (d, HCOOO)
m/z (Cl, Isobutane) 191 [(M+H)⁺, 80 %], 173 [(M - OH)⁺, 30], 73 (100)

19.2 2,4,6-Tri-O-butyryl-myo-inositol orthoformate 151

A mixture of 10 (1.9 g, 10 mmol), DMAP (50 mg, 0.41 mmol), butyric anhydride (8 ml, 0.1 mol) and dry pyridine (50 ml) was stirred at r.t. for 1 h, after which tlc (hexane / ethyl acetate 3:1) showed complete conversion of the starting material (Rᵢ 0.00) into a single product (Rᵢ 0.66). Methanol (50 ml) was added to the solution and stirring continued for another 30 min. The solvents were evaporated and the residue taken up in chloroform (50 ml). The solution was washed successively with 1 M HCl, sat. NaHCO₃ solution (2 x 50 ml) and water (50 ml each) and then dried over magnesium sulphate. Evaporation of the solvent gave
a syrup which was recrystallised from petrol ether (b.p. 60-80°C) to give 151 (3.58 g, 89 %).

m.p. 68-68.5°C
Found: C, 57.0; H, 7.10. Calc for C_{19}H_{28}O_9 (400.43): 56.99; H, 7.05 %.

δ_H (CDCl_3, 270 MHz) 0.97 (6 H, t, J 7.4, 2 butyrate CH_3), 0.99 (3 H, t, J 7.4, butyrate CH_3), 1.70 (6 H, tq, J 7.5, 7.5, 3 butyrate CH_2CH_3), 2.21-2.40 (4 H, m, 2 butyrate COCH_2), 2.45 (2 H, t, J 7.4, butyrate COCH_2), 2.51-2.58 (4 H, m, 2 butyrate CH_2CH_3), 4.55 (1 H, t, J 1.7, 1.7, C-5-H), 5.17 (1 H, d, J 1.5, C-2-H), 5.54 (2 H, t, J 3.8, C-4-H, C-6-H), 5.60 (1 H, d, J 1.1, HCOOO)

δ_C (CDCl_3, 68 MHz) 13.56 (3 q, butyrate CH_3), 18.16 (2 t, butyrate CH_2CH_3), 18.29 (t, butyrate CH_2CH_3), 35.84 (2 t, butyrate COCH_2), 35.94 (t, butyrate COCH_2), 62.83 (d, C-5), 66.72 (2 d, C-1, C-3), 67.43 (2 d, C-4, C-6), 69.15 (d, C-2), 103.05 (d, HCOOO), 171.81 (2 s, CO), 173.14 (s, CO)

m/z (Cl, Isobutane) 401 [(M+H^+), 75 %], 71 (100)

19.3 2,4,6-Tri-O-butyryl-myo-inositol 152 and DL-2,4,6-Tri-O-butyryl-1-O-formyl-myo-inositol 153

A solution of 151 (400 mg, 1 mmol) in 80% trifluoroacetic acid (10 ml) was stirred for 4 h at r.t. after which tlc (ether / hexane 2:1) showed conversion of the starting material (R_f 0.74) into a major and a minor product (R_f 0.12 and 0.28, respectively). The solvents were evaporated at 45°C and the residue partitioned between chloroform and sat. NaHCO_3 solution. The organic layer was dried over magnesium sulphate, the solvent evaporated and the product mixture separated by flash chromatography (petrol ether / ether 1:1) to give 152 (246 mg, 63 %) and 153 (59 mg, 14 %).

DL-2,4,6-Tri-O-butyryl-myo-inositol 152:

m.p. 112-114°C (from ethyl acetate / hexane)
Found: C, 55.1; H, 7.83. Calc for C_{18}H_{30}O_9 (390.43): C, 55.37; H, 7.74 %.

δ_H (CDCl_3, 270 MHz) 0.95 (6 H, t, J 7.5, 2 butyrate CH_3), 0.98 (3 H, t, J 7.5, butyrate CH_3), 1.59-1.75 (6 H, m, 3 butyrate CH_2CH_3), 2.37 (4 H, t, J 7.3, butyrate COCH_2), 2.47 (2 H, t, J 7.4, butyrate COCH_2), 3.08 (1 H, d, J 7.0, D_2O

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ex, OH), 3.26 (2 H, d, J 5.9, D₂O ex, 2 OH), 3.56 (1 H, brd dd, J 9.7, 9.7, D₂O ex gives dd, J 9.5, 9.5, C-1-H), 3.75-3.79 (2 H, m, D₂O ex gives dd, J 10.0, 2.7, C-3-H, C-5-H), 5.14 (2 H, dd, J 9.9, 9.9, C-4-H, C-6-H), 5.49 (1 H, dd, J 2.8, 2.8, C-2-H)

δC (CDCl₃, 68 MHz) 13.30 (q, butyrate CH₃), 18.13 (t, butyrate CH₂CH₃), 35.91 (t, butyrate COCH₂), 68.66 (2 d, C-1, C-3), 71.23 (d, C-5), 73.50 (d, C-2), 74.70 (2 d, C-4, C-6), 174.27 (s, butyrate CO), 174.53 (2 s, butyrate CO)
m/z (+ve ion FAB) 781 [(2 M + H)+, 1.2 %], 391 [(M+H)+, 9], 373 [(M - OH)+, 100], 303 [(M - OH - COCH₂CH₂CH₃)+, 37]
m/z (-ve ion FAB) 543 [(M + NBA)-, 54 %], 389 [(M - H)-, 78], 122 (45), 87 (100)

DL-2,4,6-Tri-O-butyryl-1-O-formyl-myoinositol 153:

δH (CDCl₃, 270 MHz) 0.93, 0.96, 0.99 (9 H, 3 t, J 7.3, 3 butyrate CH₃), 1.56-1.75 (6 H, m, butyrate CH₂CH₃), 2.32, 2.40, 2.45 (6 H, 3 t, J 7.5, butyrate COCH₂), 2.80, 2.92 (2 H, 2 brd s, D₂O ex, 2 OH), 3.65 (1 H, brd dd, D₂O ex gives dd, J 9.8, 9.8, C-5-H), 3.85 (1 H, brd d, J 9.2, D₂O ex gives dd, J 10.1, 2.9, C-3(1)-H), 5.08 (1 H, dd, J 10.4, 2.7, C-1(3)-H), 5.20 (1 H, dd, J 10.0, 10.0, C-6(4)-H), 5.40 (1 H, dd, J 10.1, 10.1, C-4(6)-H), 5.62 (dd, 1 H, J 2.8, 2.8, C-2-H), 7.94 (1 H, s, OCOH)

δC (CDCl₃, 68 MHz) 13.36 (q, butyrate CH₃), 13.43 (2 q, 2 butyrate CH₃), 18.29 (2 t, 2 butyrate CH₂CH₃), 18.36 (t, butyrate CH₂CH₃), 35.94 (t, butyrate COCH₂), 36.00 (t, butyrate COCH₂), 68.57, 68.63, 70.29, 71.32, 71.55, 74.66 (6 d, inositol ring C), 159.61 (d, formyl CO), 173.11, 173.30, 174.66 (3 s, butyrate CO)
m/z (Cl, Isobutane) 419 [(M+H)+, 5 %], 401 [(M - OH)+, 60], 331 [(M - OH - COCH₂CH₂CH₃)+, 31], 89 (45), 71 (100)

19.4 2,4,6-Tri-O-p-methoxybenzyl-myoinositol orthoformate 154

NaH (5.33 g of a 60 % dispersion, 113 mmol) was added to a stirred solution of 149 (5 g, 26 mmol) in anhydrous DMF (250 ml) at r.t. The mixture was stirred for 20 min, after which p-methoxybenzyl chloride (17.6 ml, 20.36 g, 130 mmol) was added. The suspension was stirred overnight, after which tlc (hexane / ethyl acetate 1:1) showed a product (Rf 0.41). The reaction was quenched with water (10 ml), the solvents were evaporated and the residue partitioned between chloroform (200 ml) and water (50 ml). The organic phase was washed with
brine, dried over magnesium sulphate and evaporated to give a syrup which was chromatographed on silica gel (eluent ether / hexane 2:1) to give 154 (11.2 g, 20.3 mmol, 78 %)

m.p. 47-48°C (from ethanol / water)
Found: C, 67.4; H, 6.13. Calc for C_{31}H_{34}O_{9} (550.61): C, 67.62; H, 6.22 %.

δ_{H} (CDCl_{3}, 270 MHz) 3.76 (3 H, s, OCH_{3}), 3.79 (6 H, s, 2 OCH_{3}), 4.00 (1 H, brd s, C-5-H), 4.24 (2 H, brd s, 2 C-H), 4.29 (2 H, brd s, 2 C-H), 4.35 - 4.54 (5 H, m, 2 CH_{2}Ph, C-2-H), 4.58 (2 H, s, CH_{2}Ph), 5.51 (1 H, s, O_{3}CH), 6.80 (4 H, d, J 8.4, arom.), 6.83 (2 H, d, J 8.6, arom.), 7.10 (4 H, d, J 8.2, arom.), 7.28 (2 H, d, J 8.4, arom.)

δ_{C} (CDCl_{3}, 68 MHz) 55.07 (q, OCH_{3}), 66.65 (d, 2 inositol ring C), 68.08 (d, inositol ring C), 70.48 (d, 2 inositol ring C), 71.00 (t, CH_{2}PhOMe), 73.66 (d, inositol ring C), 103.08 (d, O_{3}CH), 113.62 (d, 6 PhOMe C), 129.12 (d, 4 PhOMe C), 129.58 (d, 2 PhOMe C), 134.57 (s, 3 PhOMe C), 159.16 (s, 3 PhOMe C)

m/z (+ve ion FAB) 551 [(M + H)^{+}, 2 %], 429 [(M - CH_{2}C_{6}H_{4}OCH_{3})^{+}, 6], 121 [(CH_{2}C_{6}H_{4}OCH_{3})^{+}, 100]

m/z (-ve ion FAB) 703 [(M - NBA)^{-}, 25 %], 535 (30), 322 (55), 188 (100)

19.5 2,4,6-Tri-O-p-methoxybenzyl-myoinositol 155

154 (5.6 g, 10.2 mmol) was heated under reflux in a mixture of 2M HCl (10 ml) and methanol (200 ml) for 30 min, when tlc (hexane / ethyl acetate 1:2) showed conversion of the starting material (R_{f} 0.67) into a product (R_{f} 0.35). The solution was cooled to r.t. and adjusted to pH 8 with ammonia solution. The solvents were evaporated and the residue extracted with ethyl acetate (2 x 200 ml). The combined extracts were evaporated and the crude product was chromatographed on silica gel using ether → ether / ethyl acetate 1:1 as eluent to give 155 (4.7 g, 8.7 mmol, 85 %).

m.p. 110-112°C (from ethyl acetate / hexane)
Found: C, 66.7; H, 6.77. Calc for C_{30}H_{36}O_{9} (540.61): C, 66.65; H, 6.71 %.

δ_{H} (CDCl_{3}, 270 MHz) 2.45 (2 H, d, J 5.7, D_{2}O ex, 2 OH), 2.66 (1 H, brd s, D_{2}O ex, OH), 3.40 - 3.55 (3 H, m, C-1,3,5-H), 3.61 (2 H, dd, J 9.25, 9.25, C-4,6-H), 3.77 (6 H, s, 2 OCH_{3}), 3.80 (3 H, s, OCH_{3}), 3.90 (1 H, dd, J 3.0, 3.0, C-2-H), 4.73 (2 H, s, CH_{2}Ph), 4.76 (4 H, s, 2 CH_{2}Ph), 6.87 (4 H, d, J 8.6, CH_{2}PhOMe),
6.89 (2 H, d, J 8.6, CH₂PhOMe), 7.24 (2 H, d, J 8.6, CH₂PhOMe), 7.29 (4 H, d, J 8.6, CH₂PhOMe)

δC (CDCl₃, 68 MHz) 55.14 (q, OCH₃), 72.33 (d, 2 C, inositol ring C), 74.47 (t, CH₂Ph), 74.79, 78.95 (2 d, 2 C, inositol ring C), 81.48 (d, 2 C, inositol ring C), 113.78 (d, 2 PhOMe C), 113.85 (d, 4 PhOMe C), 129.45 (d, 2 PhOMe C), 129.61 (d, 4 PhOMe C), 130.58 (s, PhOMe), 159.22 (s, PhOMe)

m/z (+ve ion FAB) 539 [(M-H)+, 1.5 %], 419 (5), 121 [(CH₂C₆H₄OCH₃)+, 100]

m/z (-ve ion FAB) 706 (36 %), 693 [(M + NBA)-, 57], 539 [(M -H)-, 100], 419 [(M -CH₂C₆H₄OCH₃)-, 38]

19.6 Diisopropylaminodichlorophosphine 156

This compound was prepared according to the method of Tanaka et al. [382].

A solution of diisopropylamine (350.5 ml, 2.5 mol) in dry ether (350 ml) was added dropwise over 1 h to a stirred solution of PCl₃ (109 ml, 1.25 mol) in dry ether (350 ml) at -78°C. After the addition the mixture was stirred a further 1 h at -78°C and then allowed to warm to room temperature. The precipitate was filtered and the solvent was evaporated. The crude product was distilled at reduced pressure to give 156 (158 g, 0.78 mol, 63 %).

b.p. 52°C / 0.6 mbar (Lit. [382] 72-73°C / 7 mmHg)

δP (CDCl₃; 36 MHz) 166.44

19.7 Bis(benzyloxy)(diisopropylamino)phosphine 157

156 (50.5 g, 0.25 mol) and triethylamine (69.7 ml, 50.6 g, 0.5 mol) were stirred in dry CH₂Cl₂ (250 ml) at -78°C. Benzyl alcohol (51.7 ml, 54.1 g, 0.5 mol) in dry CH₂Cl₂ (35 ml) was added dropwise over a period of 30 min. Cooling was removed and stirring continued for a further 4 h. The precipitate was filtered and the solvent was evaporated. The crude product was chromatographed on silica gel (hexane / triethylamine 10:1) to give 157 (72.1 g, 0.21 mol, 83 %).

δP (CDCl₃; 36 MHz) 145.24
19.8 2,4,6-Tri-O-p-methoxybenzyl-1,3,5-tris[bis(benzyl)thiophosphoryl]-myo-inositol 158

A mixture of 155 (541 mg, 1 mmol) and tetrazole (1.26 g, 18 mmol) was stirred at r.t. in dichloromethane (30 ml). 157 (6.2 g, 15 mmol) was added and stirring continued for 1 h. Pyridine (5 ml) and sulphur (500 mg) were added and stirring continued overnight. Tic (ethyl acetate / hexane 2:1) showed one product (Rf 0.81). The solution was partitioned between chloroform and sat. aqueous NaHCO₃, the organic phase was dried over magnesium sulphate and the solvents evaporated. The residue was chromatographed on silica gel to give 158 (835 mg, 61 %) as a syrup.

δH (CDCl₃, 270 MHz) 3.70 (6 H, 2 s, OCH₃), 3.76 (3 H, s, OCH₃), 4.07 (2 H, dd, J 9.5, 9.5, C-4,6-H), 4.43 (2 H, ddd, J 10.3, 10.3, 2.2, C-1,3-H), 4.60-5.10 (20 H, m, 9 CH₂Ph, C-2-H, C-5-H), 6.71 (4 H, d, J 8.6, CH₂PhOMe), 6.80 (2 H, d, J 8.8, CH₂PhOMe), 6.98 (4 H, dd, J 7.6, 1.9, CH₂PhOMe), 7.11-7.35 (32 H, m, CH₂PhOMe and CH₂Ph).

δC (CDCl₃, 68 MHz) 54.16 (q, OCH₃), 68.56, 68.73, 68.89, 69.02, 72.71, 74.14 (6 t, CH₂Ph), 76.12 (d, 1 inositol ring C), 76.54 (d, 2 inositol ring C), 76.70 (d, 2 inositol ring C), 78.91 (d, 1 inositol ring C), 112.38 (d, 4 PhOMe C), 112.54 (d, 2 PhOMe C), 126.89, 126.95, 127.21, 127.27, 127.34, 127.43, 127.56, 127.66, 127.76, 128.05 (10 d, CH₂Ph and PhOMe), 129.35 (s, 2 PhOMe C), 129.67 (s, 1 PhOMe C), 134.54, 134.67, 134.80, 134.93 (4 s, CH₂Ph), 157.73 (s, PhOMe C)

δP (CDCl₃, 36 MHz)

m/z (+ve ion FAB) 1370 [(M + H)+, 0.17 %], 1247 (0.3), 121 [(CH₂C₆H₄OCH₃)+, 100], 91 [(CH₂C₆H₅)+, 37]

m/z (-ve ion FAB) 1277 [(M - C₇H₇)-, 10 %], 293 [(OP(S)(OCH₂C₆H₅)₂]⁻, 100], 95 (55)

19.9 Myo-inositol-1,3,5-trisphosphorothioate 159

Ammonia was condensed into a three neck flask at -78°C. An excess of sodium was added to dry the liquid ammonia which was then distilled into a second three neck flask and kept at -78°C. Sodium was added until the solution remained blue. Compound 158 (237 mg, 173 µmol) was dissolved in dry dioxane (2 ml) and added to the sodium in liquid ammonia. After stirring for 15 min the
reaction was quenched with ethanol. Ammonia and ethanol were evaporated and the crude product was purified by ion-exchange chromatography on Pharmacia Q Sepharose Fast Flow eluting with a gradient of triethylammonium bicarbonate buffer (0.1 M to 1 M), pH 8.0. The triethylammonium salt of 159 eluted between 450 mM and 530 mM. Yield: 157 μmol (91%).

δH (D2O, 270 MHz) 3.87 (2 H, dd, J 9.3, 9.3, C-4-H, C-6-H), 4.08 (1 H, ddd, J 10.3, 9.2, 9.2, C-5-H), 4.19 (2 H, ddd, J 10.3, 10.3, 2.5, C-1-H, C-3-H), 4.62 (1 H, brd s, C-2-H)

δC (D2O, 68 MHz) 69.63 (1 C, d, C-2), 70.83 (2 C, d, C-4, C-6), 74.29 (2 C, dd, J C-O-P 6.6, C-1, C-3), 78.41 (1 C, dd, J C-O-P 6.6, C-5)

δP (D2O, 36 MHz) 47.74 (1 P, d, J 10.1, 1-P), 46.55 (2 P, d, J 10.1, 1-P, 3-P)

m/z (-ve ion FAB) 466 [100 %, (M - H)⁻], 432 (15), 238 (15), 113 (17), 95 [(PSO₂)⁻] 40

Accurate mass calc for C₆H₁₄O₁₂P₃S₃ (M - H)⁻ 466.886, found 466.886;

20 Synthesis of scylo-Inositol 1,2,4-trisphosphate

20.1 DL-1-O- Allyl-3,6-di-O-benzyl-4,5-O-isopropylidene-2-O-trifluoro-methylsulphoxy-myoinositol 160

A solution of 1-O-Allyl-3,6-di-O-benzyl-4,5-O-isopropylidene-myoinositol 93 (8.604 g, 19.5 mmol) and dry pyridine (10 ml) in dry dichloromethane (50 ml) was cooled to -78°C. Trifluoromethylsulphonic anhydride (6 ml, 10 g, 35.4 mmol) was added dropwise to the mixture. After the addition, cooling was removed and stirring continued for 2 h at r.t., after which tlc (petrol / ether 1:1) showed complete conversion of the starting material (Rf 0.39) to a product (Rf 0.62). The reaction was quenched with water (10 ml), diluted with dichloromethane (50 ml) and washed successively with sat. NaHCO₃, water and brine (100 ml each). The organic layer was dried over magnesium sulphate and the solvents evaporated

in vacuo. Toluene was added to the orange syrup in order to remove the remaining pyridine, which was then evaporated to give 160 as a yellow solid in quantitative yield.
δ_h (CDCl₃; 270 MHz) 1.45 (3 H, s, CH₃), 1.49 (3 H, s, CH₃), 3.39 (1 H, t, J 10.1, C-5-H), 3.43 (1 H, dd, J 8.8, 3.1, C-3(1)-H), 3.68 (1 H, dd, J 10.5, 2.8, C-1(3)-H), 3.86 (1 H, dd, J 9.9, 9.0, C-4(6)-H), 3.88 (1 H, dd, J 10.4, 9.3, C-6(4)-H), 4.10-4.25 (2 H, m, CH₂CH=CH₂), 4.78 (2 H, AB, J_AB 12.5, CH₂Ph), 4.76, 4.88 (2 H, AB, J_AB 11.5, CH₂Ph), 5.23 (1 H, t, J 2.9, C-2-H), 5.17-5.32 (2 H, m, CH₂-CH=CH₂), 5.99 (1 H, ddt, J 17.3, 10.35, 5.9, CH₂-C=CH₂), 7.25-7.37 (10 H, m, CH₂Ph);

δ_c (CDCl₃; 68 MHz) 26.87 (q, CH₃), 71.86, 73.06, 73.54 (3 t, 2 CH₂Ph and CH₂CH=CH₂), 73.57, 76.88, 77.70, 78.08, 78.93, 85.84 (6 d, 6 inositol ring C), 112.40 (s, (CH₃)₂C), 118.21 (t, CH₂CH=CH₂), 125.28 (CF₃), 127.52, 127.58, 127.78, 128.23, 128.36, 128.59, 129.01 (7 d, CH₂Ph), 133.78 (d, CH₂CH=CH₂), 137.15, 138.28 (2 s, CH₂Ph);

m/z (+ve ion FAB) 573 [(M + H)+, 2 %], 502 [(M - HCF₃)+, 13], 91 (100)

20.2 DL-2-O-Acetyl-1-O-allyl-3,6-di-O-benzyl-4,5-isopropylidene-scyllo-inositol 161

A mixture of crude 160 (5.73 g, 10 mmol) and caesium acetate (2.78 g, 15 mmol) in dry DMF (150 ml) was stirred at r.t. for 2 h after which tlc (petrol / ether 1:1) showed conversion of the starting material (R, 0.62) into a product (Rf 0.70). The solution was concentrated _in vacuo_ and the residue was partitioned between water and chloroform (100 ml each). The organic layer was dried over magnesium sulphate and evaporated. The crude product was purified by chromatography (ether / petrol 1:1) to give 161 (4.39 g, 9.1 mmol, 91 %).

m.p. 108-110°C (from hexane)

Found: C, 69.5; H, 7.07. Calc. for C₂₈H₃₄O₇ (482.57): C, 69.69; H, 7.10 %.

δ_h (CDCl₃; 270 MHz) 1.47 (6 H, s, 2 CH₃), 2.03 (3 H, s, COCH₃), 3.45 (1 H, dd, J 9.3, 8.2, C-H), 3.49-3.63 (3 H, m, C-H), 3.71 (1 H, dd, J 9.2, 8.2, C-H), 4.05, 4.29 (2 H, AB, dt, J_AB 12.6, J 5.7, 1.5, CH₂CH=CH₂), 4.61, 4.85 (2 H, AB, J_AB 12.4, CH₂Ph), 4.71, 4.88 (2 H, AB, J_AB 11.7, CH₂Ph), 5.08-5.22 (2 H, m, CH₂CH=CH₂), 5.15 (1 H, t, J 8.8, C-2-H), 5.81 (1 H, ddt, CH₂CH=CH₂, J 17.2, 10.4, 5.6), 7.24-7.39 (10 H, m, CH₂Ph)

δ_c (CDCl₃; 68 MHz) 20.92 (q, COCH₃), 26.95 (2 q, CH₃), 72.07, 72.98, 74.11 (3 t, CH₂Ph and CH₂CH=CH₂), 74.66, 76.77, 78.56, 78.88, 79.34, 81.61 (6 d,
inositol ring C), 112.58 (s, (CH₃)₂C), 116.77 (t, CH₂CH=CH₂), 127.44, 127.50, 127.70, 128.18, 128.22 (s, CH₂Ph), 134.67 (d, CH₂CH=CH₂), 138.30 (s, CH₂Ph), 169.70 (s, CO);
m/z (+ve ion FAB) 483 [(M + H)⁺, 4.5 %], 91 (100)
m/z (-ve ion FAB) 635 [(M + NBA)⁻, 60 %], 485 (50), 212 (100)

20.3 DL-1-O- Allyl-3,6-di-O-benzyl-4,5-O-isopropylidene-scy³lo-inositol 162

A mixture of 161 (3.19 g, 6.6 mmol) in methanol (50 ml) and 1 M aqueous sodium hydroxide (6 ml) was heated under reflux for 30 min after which tlc (petrol / ether 1:1) showed complete conversion of the starting material (Rf 0.70) into a product (Rf 0.63). The solution was allowed to cool and neutralised with solid carbon dioxide. The solvent was evaporated and the residue partitioned between water and chloroform. The organic phase was dried over magnesium sulphate and evaporated to give 162 as a white solid (2.46 g, 5.6 mmol, 85 %).

m.p. 101-103°C (from hexane, Lit. [¹⁶¹] 105-106°C)
Found: C, 70.7; H, 7.26. Calc. for C₂₆H₃₂O₆ (440.54): C; 70.89; H, 7.32 %.
δₜ (CDCl₃; 270 MHz) 1.46 (6 H, s, 2 CH₃), 2.73 (1 H, d, J 2.4, D₂O ex, OH), 3.39 (1 H, t, J 8.5, C-H), 3.51-3.64 (5 H, m, 5 C-H), 4.28, 4.39 (2 H, AB, dt, JₐB 12.5, 3J 5.8, 4J 1.5, CH₂CH=CH₂), 4.72, 4.90 (2 H, AB, JₐB 11.7, CH₂Ph), 4.72, 4.93 (2 H, AB, JₐB 11.7, CH₂Ph), 5.16 (1 H, ddt, J 10.3, 1.4, 1.4, cis-CH₂=CH=CH₂), 5.26 (1 H, ddt, J 17.2, 1.5, 1.5, trans-CH₂=CH=CH₂), 5.94 (1 H, ddt, J 17.2, 10.4, 5.7, CH₂-CH=CH₂), 7.24-7.40 (10 H, m, CH₂Ph)
δₙ (CDCl₃; 68 MHz) 26.92 (q, CH₃) 72.65, 72.95, 74.37 (3 t, CH₂Ph and CH₂CH=CH₂), 75.90, 78.43, 78.49, 79.11, 79.37, 83.39 (6 d, inositol ring C), 112.29 (s, (CH₃)₂C), 116.96 (t, CH₂CH=CH₂), 127.44, 127.60, 127.66, 127.83, 128.22, 128.31 (6 d, CH₂Ph), 134.99 (d, CH₂CH=CH₂), 138.27, 138.43 (2 s, CH₂Ph)
m/z (+ve ion FAB) 441 [(M + H)⁺, 8 %], 91 (100)
m/z (-ve ion FAB) 593 [(M + NBA)⁻, 100 %], 440 (M⁻, 30)

20.4 DL-2-O-Acetyl-1-O-allyl-3,6-di-O-benzyl-scy³lo-inositol 163

A solution of 161 (3.57 g, 7.4 mmol) in methanol / 1 M HCl 9:1 (100 ml) was heated under reflux for 20 min after which tlc (ethyl acetate / hexane 1:1)
showed complete conversion of the starting material (Rf 0.85) into a product (Rf 0.44). The mixture was allowed to cool and neutralised with NaHCO₃. The solution was concentrated and then partitioned between water and chloroform (100 ml each). The organic phase was dried over magnesium sulphate and evaporated to give 163 (2.7 g, 6.1 mmol, 83%).

m.p. 142-143°C (from ethyl acetate / hexane)

Found: C, 67.8; H, 6.80. Calc. for C<sub>25</sub>H<sub>30</sub>O<sub>7</sub> (442.51): C, 67.86; H, 6.83 %.

1H NMR (CDCl₃; 270 MHz) 2.00 (3 H, s, COCH₃), 2.87 (1 H, d, J 2.4, D₂O ex, OH), 3.03 (1 H, d, J 2.4, D₂O ex, OH), 3.32-3.57 (5 H, m, C-1,3,4,5,6-H) 4.09, 4.28 (2 H, AB, dt, J<sub>AB</sub> 12.5, 3J 5.7, 4J 1.5, CH₂CH=CH₂), 4.67, 4.76 (2 H, AB, J<sub>AB</sub> 11.6, CH₂Ph), 4.76, 4.87 (2 H, AB, J<sub>AB</sub> 11.1, CH₂Ph), 5.10 (1 H, dd, J 9.7, 9.7, C-2-H), 5.14 (1 H, ddt, J 10.4, 1.3, 1.3, cis-CH₂CH=CH₂), 5.22 (1 H, ddt, J 17.2, 1.5, 1.5, trans-CH₂CH=CH₂), 5.84 (1 H, ddt, J 17.2, 10.4, 5.7, CH₂CH=CH₂), 7.24-7.37 (10 H, m, CH₂Ph)

13C NMR (CDCl₃; 68 MHz) 20.98 (q, COCH₃), 73.66 (2 d, 2 inositol ring C), 73.76, 79.98, 80.34, 81.80 (4 d, inositol ring C), 74.15, 74.70, 75.38 (3 t, 2 CH₂Ph and CH₂CH=CH₂), 116.96 (t, CH₂CH=CH₂), 127.70, 127.76, 127.89, 127.99, 128.44, 128.51 (6 d, CH₂Ph), 134.46 (d, CH₂CH=CH₂), 138.11, 138.21 (2 s, CH₂Ph), 169.90 (s, CO);

m/z (+ve ion FAB) 443 [(M + H)<sup>+</sup>, 4 %], 91 (100)

m/z (-ve ion FAB) 883 [(2 M - H)<sup>-</sup>, 19 %], 553 [(M + NBA)<sup>-</sup>, 100], 441 [(M - H)<sup>-</sup>, 50]

20.5  DL-1-O-Allyl-3,6-di-O-benzyl-scyll/o-inositol 164

a) A solution of 162 (1.414 g, 3.21 mmol) in 80 % acetic acid (50 ml) was heated under reflux for 15 min, cooled and diluted with water (50 ml). After keeping the mixture at - 20°C overnight the precipitate was collected to give 164 (1.155 g, 2.88 mmol, 90%).

b) A mixture of 163 (2.24 g, 5.06 mmol) in methanol (100 ml) and 1 M aqueous sodium hydroxide (12 ml) was heated under reflux for 30 min after which tlc (ethyl acetate / hexane 2:1) showed complete conversion of the starting material (Rf 0.63) into a product (Rf 0.40). The solution was cooled and neutralised with solid carbon dioxide. The solvent was evaporated and the residue partitioned
between water and ethyl acetate. The organic phase was dried over magnesium sulphate and evaporated to give 164 as a white solid (1.76 g, 4.4 mmol, 87 %).

m.p. 180°C (from ethyl acetate)

Found: C, 69.0; H, 7.05. Calc, for C_{23}H_{28}O_6 (400.47): C; 68.98; H, 7.05 %.

δ_H (d_6-DMSO; 270 MHz) 3.05-3.21 (5 H, m, 5 C-H), 3.32-3.37 (1 H, m, D_2O ex gives dd, J 9.0, 9.0, C-H), 4.20, 4.32 (2 H, AB, brd d, J_{AB} 12.8, 3J 5.5, CH_2CH=CH_2), 4.71, 4.81 (2 H, AB, J_{AB} 11.4, CH_2Ph), 4.79 (2 H, AB, J_{AB} 11.5, CH_2Ph), 5.00 (1 H, d, J 3.7, D_2O ex, OH), 5.04-5.06 (2 H, m, D_2O ex gives 1 H, d, J 10.4, cis-CH_2CH=CH_2, OH), 5.13 (1 H, d, J 5.5, D_2O ex, OH), 5.21 (1 H, ddt, J 17.4, 1.65, 1.65, trans-CH_2CH=CH_2), 5.94 (1 H, ddt, J 17.2, 10.4, 5.5, CH_2CH=CH_2), 7.23-7.45 (10 H, m, CH_2Ph)

δ_C (d_6-DMSO; 68 MHz) 73.40, 73.82, 74.21 (3 t, CH_2Ph and CH_2CH=CH_2), 73.75, 73.92, 74.34, 82.48, 82.54, 82.90 (6 d, inositol ring C), 115.60 (t, CH_2CH=CH_2), 127.11, 127.21, 127.60, 127.69, 127.99, 128.08 (6 d, CH_2Ph), 136.42 (d, CH_2CH=CH_2), 139.53, 139.79 (2 s, CH_2Ph)

m/z (+ve ion FAB) 401 [(M + H)+, 4 %], 91 (100)

m/z (-ve ion FAB) 553 [(M + NBA)-, 80 %], 399 [(M - H)-, 100]

20.6 DL-1-O- Allyl-3,6-di-O-benzyl-2,4,5-tris[di(benzyl)phospho]-scylo-inositol 165

A solution of bis(benzyloxy)(diisopropylamino)phosphine 157 (19.9 g, 26 mmol) in dichloromethane (50 ml) was added to a solution of 164 (1 g, 2.5 mmol) and tetrazole (3.15 g, 45 mmol) in dichloromethane (50 ml). The mixture was stirred at r.t. for 1 h. Water (2 ml) in THF (20 ml) was added and the mixture was stirred for further 30 min. 2,6-Lutidine (2 ml) followed by tert-butyl hydroperoxide (20 ml) was then added and stirring continued overnight. The solution was washed with saturated aqueous sodium hydrogen carbonate (2 x 100 ml) and dried over magnesium sulphate. The solvents were evaporated and the residue chromatographed on silica gel with 0 % to 100 % ethyl acetate in hexane. The product was recrystallised from ethanol to give 165 (1.294 g, 1.5 mmol, 58 %).

tlc (ethyl acetate / hexane 1:1) R_f 0.38

m.p. 103-105°C

Found: C, 66.1; H, 5.72. Calc, for C_{65}H_{67}O_{15}P_3 (1181.16): C, 66.10; H, 5.72 %.
\[ \delta_H (\text{CDCl}_3; 270 \text{ MHz}): 3.58 (1 \text{ H, dd, J 8.4, 8.4, C-H}), 3.67 (1 \text{ H, dd, J 8.4, 8.4, C-H}), 3.73 (1 \text{ H, dd, J 8.4, 8.4, C-H}), 4.12-4.26 (2 \text{ H, m, CH}_2\text{CH}=\text{CH}_2), 4.55-5.15 (21 \text{ H, m, 8 CH}_2\text{Ph, CH}_2\text{CH}=\text{CH}_2, 3 \text{ C-H}), 5.78 (1 \text{ H, ddt, J 17.2, 10.4, 5.5, CH}_2\text{CH}=\text{CH}_2), 6.97-7.35 (40 \text{ H, m, 8 CH}_2\text{Ph}). \]

\[ \delta_C (\text{CDCl}_3; 68 \text{ MHz}): 69.18, 69.28, 69.38, 69.44 (4 \text{ t, 4 PO-CH}_2\text{Ph}), 73.56, 73.79, 74.31 (3 \text{ t, 2 CH}_2\text{Ph and CH}_2\text{CH}=\text{CH}_2), 77.71, 78.14, 78.98, 79.08, 79.43, 79.66 (6 \text{ d, 6 inositol ring C}), 117.09 (\text{ t, CH}_2\text{CH}=\text{CH}_2), 127.11, 127.24, 127.79, 127.99, 128.09, 128.25 (6 \text{ d, CH}_2\text{Ph}), 134.25 (\text{ d, CH}_2\text{CH}=\text{CH}_2), 135.71, 137.85, 137.91 (3 \text{ s, CH}_2\text{Ph}). \]

\[ \delta_P (\text{CDCl}_3, 162 \text{ MHz, } ^1\text{H-decoupled}): -1.75 (2 \text{ P), -1.52 (1 P}) \]

\[ \delta_P (\text{CDCl}_3, 162 \text{ MHz, } ^1\text{H-coupled): -1.72 (q, 2 P, J 7.9), -1.47 (q, 1 P, J 7.9)} \]

\[ m/z (+\text{ve ion FAB}) 1181 (1 \%), 1090 [(M - C_7\text{H}_7)^+, 0.3], 181 (12), 91 (100) \]

\[ m/z (-\text{ve ion FAB}) 1088 [(M - C_7\text{H}_7 - 2 \text{ H})^-, 7 \%], 998 (2.5), 277 (100), 187 (16) \]

**20.7 DL-scyll-o-inositol 1,2,4-trisphosphate 166**

Ammonia was condensed into a three neck flask at -78°C. An excess of sodium was added to dry the liquid ammonia which was then distilled into a second three neck flask and kept at -78°C. Sodium was added until the solution remained blue. Compound 165 (311 mg, 263 μmol) was dissolved in dry dioxane (2 ml) and added to the sodium-liquid ammonia mixture. After stirring for 15 min the reaction was quenched with ethanol. Ammonia and ethanol were evaporated and the crude product was purified by ion-exchange chromatography on Pharmacia Q Sepharose Fast Flow eluting with a gradient of triethylammonium bicarbonate buffers (0.1 M to 1 M), pH 8.0. The triethylammonium salt of 166 eluted at between 320 mM and 460 mM. Yield 240 μmol (91%).

\[ \delta_H (\text{D}_2\text{O, pH=8, 270 MHz}) 3.48-3.52 (2 \text{ H, m, 2 C-H}), 3.58 (1 \text{ H, dd, J 9.3, C-H}), 3.84-4.07 (3 \text{ H, m, 3 C-H}) \]

\[ \delta_C (\text{D}_2\text{O}, \text{pH}=8, 68 \text{ MHz}) \]

\[ \delta_P (\text{D}_2\text{O, pH=8, 162 MHz, } ^1\text{H-decoupled}) 0.50 (1 \text{ P), 0.72 (2 P}) \]

\[ m/z (+\text{ve ion FAB}) 623 [(M + 2 \text{Et}_3\text{NH})^+, 1.1 \%), 522 [(M + \text{Et}_3\text{NH})^+, 1.4], 102 (\text{Et}_3\text{NH}^+, 100) \]

\[ m/z (-\text{ve ion FAB}) 839 [(2 \text{ M})^-, 15 \%], 419 (\text{M}^-, 100), 97 (10) \]

*Accurate mass calc for C_{68}H_{140}O_{15}P_3 (M - H)^- 418.9546, found 418.9550;*
21 Synthesis of scyllo-Inositol 1,2,4,5-tetrakisphosphate and scyllo-Insitol 1,2,4,5-tetrakisphosphorothioate

21.1 DL-3,6-Di-O-benzyl-1-O-(cis-prop-1-enyl)-scyllo-inositol 167

A solution of 164 (443 mg, 1.106 mmol) and freshly sublimed potassium tert-butoxide (570 mg, 5 mmol) in dry DMSO (20 ml) was stirred for 3 h at 50°C after which tlc (ethyl acetate / hexane 2:1) showed complete conversion of the starting material (Rf 0.67) into a product (Rf 0.73). Water (50 ml) was added and the mixture was extracted with ether (2 x 50 ml). The organic phase was dried over magnesium sulphate and evaporated and the crude product was purified by flash chromatography (ether / hexane 1:1) to give 167 (430 mg, 97%).

m.p. 140°C (from ethanol / water)
Found: C, 68.6; H, 7.13. Calc. for C_{23}H_{28}O_6 (400.47): C; 68.98; H, 7.05 %.
δH (DMSO; 270 MHz) 1.53 (3 H, d, J 6.8, CH₃), 3.07-3.15 (1 H, m, C-H), 3.22-3.29 (3 H, m, C-H), 3.41-3.49 (2 H, m, C-H), 4.18 (1 H, dq, J 6.6, CH=CH-CH₃), 4.66, 4.75 (2 H, AB, J_{AB} 11.2, CH₂Ph), 4.79, 4.79 (2 H, AB, J_{AB} 12.1, CH₂Ph), 5.04 (1 H, br s, D₂O ex, OH), 5.13 (1 H, br s, D₂O ex, OH), 5.26 (1 H, d, J 5.3, D₂O ex, OH), 6.21 (1 H, dd, J 6.4, 1.5, CH=CH-CH₃), 7.21-7.45 (10 H, m, CH₂Ph)
δC (DMSO; 68 MHz) 9.24 (q, CH=CH-CH₃), 72.43, 73.50, 73.79, 81.45, 82.52, 85.05 (6 d, 6 inositol ring C), 73.60, 73.92 (2 t, CH₂Ph), 96.24 (d, CH=CH-CH₃), 126.89, 127.05, 127.47, 127.54, 127.80 (5 d, CH₂Ph), 139.05, 139.47 (2 s, CH₂Ph), 148.13 (d, CH=CH-CH₃)
m/z (+ve ion FAB) 401 [(M + H)+, 4 %], 181 (8), 91 (100)
m/z (-ve ion FAB) 799 [(2 M-H)-, 13 %], 566 (49), 553 [(M + NBA)-, 77], 399 [(M - H)-, 100], 341 (38)

21.2 1,4-Di-O-Benzyl-scyllo-inositol 168

167 (190 mg, 474 μmol) was heated under reflux in a mixture of methanol / 1 M HCl (5:1) for 15 min after which tlc (ethyl acetate / hexane 2:1) showed complete conversion of the starting material (Rf 0.73) into a product (Rf 0.43). The solvent was evaporated and the residue collected to give 168 (124 mg, 344 μmol, 73 %).
m.p. 309-310°C (from ethanol) with a phase transition between 240-260°C

Found: C, 66.6; H, 6.71. Calc. for C_{20}H_{24}O_{6} (360.41): C, 66.65; H, 6.71 %.

H (d_{6}-DMSO; 270 MHz) 3.03-3.07 (2 H, m, C-1,4-H), 3.18-3.21 (4 H, m, C-2,3,5,6-H), 4.76 (2 H, AB s, CH₂Ph), 4.96, 4.97 (2 H, AB d, CH₂Ph), 7.21-7.41 (10 H, m, CH₂Ph)

C (d_{6}-DMSO; 68 MHz) 73.79 (2 t, 2 CH₂Ph), 74.27 (4 d, 4 C-OH), 82.90 (2 d, 2 C-O-benzyl), 127.14 (2 d, CH₂Ph), 127.69, 128.05 (2 x 2 d, CH₂Ph), 139.89 (2 s, CH₂Ph).

21.3 3,6-Di-O-benzyl-1,2,4,5-tetrakis[di(benzyl)phospho]-scylllo-inositol

169

168 (307 mg, 852 µmol) and tetrazole (1.19 g, 17 mmol) were stirred at r.t. in dry dimethylformamide (15 ml). 157 was added to the mixture and stirring continued for 1 h, after which ^{31}P-NMR showed a single peak at 141 ppm. 2,6-Lutidine (2 ml) followed by tert-butyl hydroperoxide (6 ml) was then added and stirring was continued overnight. The solution was partitioned between chloroform and saturated aqueous sodium hydrogen carbonate (50 ml each). The organic phase was dried over magnesium sulphate, the solvents were evaporated and the residue chromatographed on silica gel with 0 % to 100 % ethyl acetate in hexane. The product was recrystallised from ethanol to give 169 (885 mg, 632 mmol, 74 %).

tlc (hexane / ethyl acetate 1:1) R_{t} 0.46

m.p. 110-111°C (from methanol)

Found: C, 65.2; H, 5.45. Calc. for C_{76}H_{76}O_{18}P_{4} (1401.32): C, 65.14; H, 5.47 %.

H (CDCl₃; 270 MHz): 4.01 (2 H, t, C-3,6-H), 4.66 (4 H, brd s, C-1,2,4,5-H), 4.73-5.02 (10 H, m, CH₂Ph), 7.06-7.30 (60 H, m, CH₂Ph)

C (CDCl₃; 68 MHz): 69.51, 73.14 (2 t, CH₂Ph), 77.55, 79.73 (2 d, 6 inositol ring C), 127.37, 127.47, 127.86, 128.12, 128.25, 128.35 (6 d, CH₂Ph), 135.55, 135.64, 135.77, 137.53 (4 s, CH₂Ph)

P (CDCl₃; 36 MHz; ^{1}H-decoupled): -3.71

m/z (+ve ion FAB) 1401 [(M+H)^{+}, 0.5 %], 1310 [(M+H - C_{7}H_{7})^{+}, 0.2]
m/z (-ve ion FAB) 1309 [(M - C_{7}H_{7})^{-}, 2.3 %], 1218 [(M - 2 C_{7}H_{7})^{-}, 0.6], 277 [(OP(O)(OC_{7}H_{7}))^{-}, 100]

217
21.4 Scyllo-inositol 1,2,4,5-tetrakisphosphate 170

Ammonia was condensed into a three neck flask at -78°C. An excess of sodium was added to dry the liquid ammonia which was then distilled into a second three neck flask and kept at -78°C. Sodium was added until the solution remained blue. Compound 169 (252 mg, 180 µmol) was dissolved in dry dioxane (4 ml) and added to the sodium-liquid ammonia mixture. After stirring for 15 min the reaction was quenched with ethanol. Ammonia and ethanol were evaporated and the crude product was purified by ion-exchange chromatography on Pharmacia Q Sepharose Fast Flow eluting with a gradient of triethylammonium bicarbonate buffers (0.1 M to 1 M), pH 8.0. The triethylammonium salt of 170 eluted between 560 mM and 650 mM. Yield 74 µmol (41 %).

δ_C (D_2O; 100 MHz): 73.61 (2 C), 77.09 (4 C)
δ_P (D_2O; 36 MHz; ¹H-coupled): 2.83 (d, J 8.3)
m/z (+ve ion FAB) 804 (1 %), 703 (2.5), 102 (100)
m/z (-ve ion FAB) 998 (4.5 %), 555 (4.5), 499 [(M - H)^+], 100,
Accurate mass calc for C₆H₁₅O₁₈P₄ (M - H)^+ 498.9209, found 498.9191.

21.5 3,6-Di-O-benzyl-1,2,4,5-tetrakis[di(benzyl)thiophosphoro]-scyllo-inositol 171

168 (522 mg, 1.45 mmol) and tetrazole (2.02 g, 29 mmol) were stirred at r.t. in dry dimethylformamide (25 ml). 157 was added to the mixture and stirring continued for 1 h when ³¹P-NMR showed a single peak at 141 ppm. Pyridine and an excess of sulphur was then added and stirring continued for 10 min. The solution was separated between chloroform and saturated aqueous sodium hydrogen carbonate (50 ml each). The organic phase was dried over magnesium sulphate, the solvents were evaporated and the residue chromatographed on silica gel (hexane / ether 3:1). The product was recrystallised from ethanol to give 171 (1.8 g, 1.23 mmol, 85 %).

tlc (hexane / ether 1:1) R_f 0.63
m.p. 91-93°C (from ethyl acetate / ethanol)
Found: C, 61.9; H 5.23. Calc. for C_{76}H_{76}O_{14}P_{4}S_{4} (1465.57): C, 62.29; H, 5.23 %.

δ_{H} (CDCl\textsubscript{3}; 270 MHz): 4.15 (2 H, t, C-3,6-H), 4.58 (4 H, brd, C-1,2,4,5-H), 4.75-5.23 (10 H, m, CH\textsubscript{2}Ph), 7.08-7.23 (60 H, m, CH\textsubscript{2}Ph)

δ_{C} (CDCl\textsubscript{3}; 68 MHz): 70.03, 72.59 (2 t, CH\textsubscript{2}Ph), 77.97, 79.92 (2 d, inositol ring C), 127.43, 127.99, 128.05, 128.12, 128.22, 128.34 (6 d, CH\textsubscript{2}Ph), 135.56, 135.64, 135.71, 137.53 (4 s, CH\textsubscript{2}Ph)

δ_{P} (CDCl\textsubscript{3}, 36 MHz; 1H-decoupled): 67.77

m/z (+ve ion FAB) 1465 [M+, 0.1 %], 1035 (0.1), 945 (0.2), 91 (100)

m/z (-ve ion FAB) 1373 [(M - C\textsubscript{7}H\textsubscript{7})\textsuperscript{-}, 9 %], 1282 [(M - 2 C\textsubscript{7}H\textsubscript{7})\textsuperscript{-}, 3], 1249 [(M - 2 OC\textsubscript{7}H\textsubscript{7})\textsuperscript{-}, 4], 293 [OP(S)(OC\textsubscript{7}H\textsubscript{7})\textsubscript{2}\textsuperscript{-}, 100]

21.6 Scyllo-inositol 1,2,4,5-tetrakisphosphorothioate 172

Ammonia was condensed into a three neck flask at -78°C. An excess of sodium was added to dry the liquid ammonia which was then distilled into a second three neck flask and kept at -78°C. Sodium was added until the solution remained blue. 171 (205 mg, 140 µmol) was dissolved in dry dioxane (3 ml) and added to the sodium-liquid ammonia mixture. After stirring for 15 min the reaction was quenched with ethanol. Ammonia and ethanol were evaporated and the crude product was purified by ion-exchange chromatography on Pharmacia Q Sepharose Fast Flow eluting with a gradient of triethylammonium bicarbonate buffers (0.1 M to 1 M), pH 8.0. The triethylammonium salt of 172 eluted between 850 mM and 950 mM. Yield: 25 µmol (18 %)

δ_{H} (D\textsubscript{2}O; 270 MHz): 3.46 (2 H, dd, J 9.1, 9.1, C-3,6-H), 3.83 (4 H, ddd, J 11.8, 9.1, 9.1, C-1,2,4,5-H)

δ_{C} (D\textsubscript{2}O; 68 MHz): 80.41 (2 C), 84.97 (4 C)

δ_{P} (D\textsubscript{2}O; 36 MHz): 43.48 (d, J 11.0)

m/z (-ve ion FAB) 563 [(M-H)\textsuperscript{-}, 95 %], 529 (18), 188 (47), 154 (32), 113 (28), 95 [(PSO\textsubscript{2})\textsuperscript{2-}, 100]

Accurate mass calc for C\textsubscript{6}H\textsubscript{15}O\textsubscript{14}P\textsubscript{4}S\textsubscript{4} (M - H)\textsuperscript{-} 562.8295, found 562.8273.
removed and stirring continued for 2 h at r.t., after which tlc (ether / petrol ether b.p. 40-60°C 1:1) showed conversion of the starting material (Rf 0.53) to a product (Rf 0.67). The reaction was quenched with water (10 ml), diluted with dichloromethane (50 ml) and washed successively with sat. NaHCO₃, water and brine. The organic layer was dried over magnesium sulphate and the solvents evaporated in vacuo. Toluene was added to the orange syrup in order to remove the remaining pyridine, which was then evaporated to give the title compound as a yellow solid in quantitative yield.

\[ m/z \] (+ve ion FAB) 863 (1.8 %), 650 (1.5), 502 [(M - HCF₃), 1.6], 212 (85), 91 (100)

\[ m/z \] (-ve ion FAB) 931 (1%), 149 (100)

22.3 DL-3,6-Di-O-benzyl-2-deoxy-2-fluoro-4,5-isopropylidene-1-O-(c/s-prop-1-enyl)-myo-inositol 175

Tetrabutylammonium fluoride (15 ml of a 1.1 M solution in THF) was added to crude 174 (2.15 g, 3.75 mmol) in THF (20 ml) at r.t. The colour of the reaction mixture turned to red / brown and tlc (ether / petrol ether b.p. 40-60°C) showed conversion of the starting material (Rf 0.67) to a product (Rf 0.56). Stirring was continued for a further 1 hour. The solvent was evaporated and the brown residue taken up in chloroform. After washing with water, the organic phase was dried over magnesium sulphate and evaporated and the crude product purified by flash chromatography (hexane / ether 3:1) to give 175 (1.143 g, 2.58 mmol, 69 %).

m.p. 70-72°C (from ethanol)
Found: C, 70.4; H, 7.03. Calc. for C₂₆H₃₁O₅F (442.53): C, 70.57; H, 7.06 %.

\[ \delta_H \] (CDCl₃; 270 MHz) 1.47 (3 H, s, CH₃), 1.48 (3 H, s, CH₃), 1.64 (3 H, dd, J 7.0, 1.65, OCH=CH-CH₃), 3.41 (1 H, t, J 9.6, C-H), 3.54 (1 H, ddd, J 29.5, 8.8, 2.4, C-1(3)-H), 3.59 (1 H, ddd, J 23.3, 10.4, 2.2, C-3(1)-H), 3.95-4.07 (2 H, m, 2 C-H), 4.50 (1 H, dq, J 6.8, 6.8, OCH=CH-CH₃), 4.72, 4.84 (2 H, AB, JAB 12.3, CH₂Ph), 4.77, 4.82 (2 H, AB, JAB 12.0, CH₂Ph), 4.91 (1 H, dt, J 50.0, 2.4, C-2-H), 6.01 (1 H, ddd, J 7.0, 1.65, 0.7, OCH=CH-CH₃), 7.21-7.66 (10 H, m, CH₂Ph)

\[ \delta_C \] (CDCl₃; 68 MHz) 9.24 (q, OCH=CH-CH₃), 26.82, 26.92 (2 q, C(CH₃)₂), 71.52, 73.37 (2 t, CH₂Ph), 74.74 (dd, JC-C-F 15.4, C-1(3)), 76.74, 76.81, 78.95 (3 d,
22 Synthesis of 2-deoxy-2-fluoro-myoinositol 1,4,5-trisphosphate

22.1 DL-3,6-Di-O-benzyl-4,5-isopropylidene-1-O-(cis-prop-1-enyl)-scylo-inositol 173

A solution of 162 (3.16 g, 7.2 mmol) and freshly sublimed potassium tert-butoxide (4 g, 3.27 mmol) in dry DMSO (50 ml) was stirred for 2 h at 55°C after which tlc (hexane / ether 1:1) showed complete conversion of the starting material (Rf 0.59) into a product (Rf 0.65). Water (100 ml) was added and the mixture was extracted with ether (2 x 100 ml). The organic phase was dried over magnesium sulphate and evaporated and the crude product purified by flash chromatography (hexane / ether 3:1) to give 173 (3.03 g, 95 %).

m.p. 109-110°C (from hexane)

Found: C, 70.7; H, 7.29. Calc. for C_{26}H_{32}O_{6} (440.54): C; 70.89; H, 7.32 %.

$\delta_{H}$ (CDCl$_3$; 270 MHz) 1.46 (6 H, s, 2 CH$_3$), 1.61 (3 H, dd, J 6.8, 1.65, OCH=CH-CH$_3$), 2.69 (1 H, d, J 2.4, D$_2$O ex, OH), 3.52-3.75 (6 H, m, 6 C-H), 4.37 (1 H, dq, J 6.8, 6.8, OCH=CH-CH$_3$), 4.71, 4.93 (2 H, AB, J$_{AB}$ 11.9, CH$_2$Ph), 4.74, 4.82 (2 H, AB, J$_{AB}$ 11.7, CH$_2$Ph), 6.18 (1 H, dd, J 6.8, 1.65, OCH=CH-CH$_3$), 7.28-7.39 (m, 10 H, CH$_2$Ph)

$\delta_{C}$ (CDCl$_3$; 68 MHz) 8.99 (q, OCH=CH-CH$_3$), 26.63 (q, CH$_3$) 72.40, 72.62, (2 t, CH$_2$Ph), 75.22, 77.75, 78.04, 78.10, 78.43, 85.82 (6 d, inositol ring C), 99.38 (d, OCH=CH-CH$_3$), 112.16 (s, (CH$_3$)$_2$O), 127.18, 127.37, 127.54, 127.89, 128.05 (5 d, CH$_2$Ph), 137.92 (s, CH$_2$Ph), 147.00 (d, OCH=CH-CH$_3$)

m/z (+ve ion FAB) 441 [(M + H)$^+$, 3%], 91 (100)

m/z (-ve ion FAB) 593 [(M + NBA)$^-$, 100%], 399 [(M - CH=CH-CH$_3$)$^-$, 66], 291 (31), 105 (38)

22.2 DL-3,6-Di-O-benzyl-4,5-isopropylidene-1-O-(cis-prop-1-enyl)-2-O-trifluoromethylsulphoxy-scyllo-inositol 174

A mixture of 173 (1.654 g, 3.75 mmol) and dry pyridine (10 ml) in dry dichloromethane (50 ml) was cooled to -78°C. Triflic anhydride (1.8 ml, 3 g, 10.62 mmol) was added dropwise to this mixture. After the addition cooling was...
inositol ring C), 81.86 (dd, J_C-C-F 17.6, C-3(1)), 90.24 (dd, J_C-F 187.3, C-2),
103.18 (d, OCH=CH-CH₃), 112.26 (s, C(CH₃)₂), 127.50, 127.60, 127.73, 127.86,
128.18, 128.41 (6 d, CH₂Ph), 137.52, 138.24 (2 s, CH₂Ph), 144.95 (d, OCH=CH-CH₃)
δ_F (CDCl₃ with reference to CFCl₃; 84 MHz) -210.21 (dt, J₂-h-F 51.3, J₁(3)-h-F
m/z (+ve ion FAB) 443 [(M+H)+, 6 %], 351 (3), 91 (100)
m/z (-ve ion FAB) 595 [(M + NBA)-, 100 %], 488 (20), 401 [(M - CH=CH-CH₃)-,
22.4 DL-3,6-di-O-benzyl-2-deoxy-2-fluoro-my-o-inositol 176

175 (801 mg, 1.81 mmol) was heated under reflux in 60 ml of a mixture of
methanol and 1 M HCl (5:1) for 30 min. The reaction was allowed to cool and
neutralised with an excess of sodium bicarbonate. The solvents were
evaporated and the title compound extracted with ethyl acetate to give 176 (451
mg, 1.24 mmol, 69 %).
m.p. 193-194°C (from ethanol)
Found: C, 66.0; H, 6.40. Calc. for C₂₀H₂₃O₅F (362.40): C, 66.29; H, 6.40 %.
δ_H (d₆-DMSO; 270 MHz) 3.21-3.59 (5 H, m, 5 C-H), 4.67 (2 H, AB, CH₂Ph), 4.78,
4.82 (2 H, AB, J_AB 11.7, CH₂Ph), 4.85 (1 H, d, J 54.0, C-2-H), 5.09 (1 H, d, J 5.1,
D₂O ex, OH), 5.16 (1 H, d, J 5.0, D₂O ex, OH), 5.32 (1 H, d, J 6.0, D₂O ex, OH),
7.21-7.45 (10 H, m, CH₂Ph)
δ_C (d₆-DMSO; 68 MHz) 69.62 (dd, J_C-C-F 15.4, C-1(3)), 71.29 (t, CH₂Ph), 72.14
(d, inositol ring C), 73.73 (t, CH₂Ph), 74.37 (d, inositol ring C), 77.88 (dd, J_C-C-F
17.7, C-3(1)), 81.38 (d, inositol ring C), 92.23 (dd, J_C-F 178.5, C-2), 126.92,
127.21, 127.41, 127.47, 127.80, 128.02 (6 d, CH₂Ph), 138.76, 139.50 (2 s, 
CH₂Ph)
δ_F (d₆-DMSO with reference to CFCl₃; 84 MHz) - 212.6 (dt, J₂-H,F 51.3, J₁(3)-H,F
m/z (+ve ion FAB) 361 [(M- H)+, 8 %], 91 (100)
m/z (-ve ion FAB) 528 (70 %), 515 [(M + NBA)-, 100], 470 (20), 408 (23), 361 [(M
- H)-, 71], 322 (50)
22.5 DL-3,6-di-O-benzyl-2-deoxy-2-fluoro-1,4,5-tris[di(benzyl)phospho]-myo-inositol 177

176 (185 mg, 510 µmol) and tetrazole (763 mg, 10.9 mmol) were stirred at r.t. in dichloromethane (30 ml). 157 was added and stirring continued for 1 h, after which $^{31}$P-NMR showed three signals at 141.74, 141.60 and 141.47 ppm. The mixture was cooled to -78°C and 2,6-lutidine (2 ml) and then tert-BuOOH (5 ml, 80 % in tert-butyl alcohol) was added to the mixture. The mixture was allowed to warm to r.t. and stirring continued for 1 h. The solution was partitioned between chloroform and sat. aqueous NaHCO$_3$, the organic phase was dried over magnesium sulphate and the solvents were evaporated. The residue was chromatographed on silica gel to give 177 (441 mg, 386 µmol, 76 %).

m.p. 129-131°C

Found: C, 65.2; H, 5.48. Calc. for C$_{62}$H$_{62}$O$_{14}$FP$_3$ (1143.09): C, 65.15; H, 5.47 %.

$\delta_H$ (CDCl$_3$; 270 MHz) 3.50 (1 H, ddd, J 27.0, 9.8, 1.5, C-3-H), 3.98 (1 H, t, J 9.6, C-6-H), 4.28 (1 H, ddt, J 27.0, 9.0, 1.5, C-1-H), 4.50-5.07 (19 H, m, 8 CH$_2$Ph, 3 C-H), 6.95-7.33 (40 H, m, 8 CH$_2$Ph)

$\delta_C$ (CDCl$_3$; 68 MHz) 69.25, 69.48, 72.07, 74.76 (4 t), 75.54 (dd, J$_{C\text{-}C\text{-}F}$ 17.6, C-3), 76.13 (ddd, J$_{C\text{-}C\text{-}F}$ 17.6, J$_{C\text{-}O\text{-}P}$ 5.2, C-1), 76.99, 77.19 (2 dd, J$_{C\text{-}O\text{-}P}$ 5.2, C-4 and C-5), 78.23 (d, C-6), 87.12 (dd, J$_{C\text{-}F}$ 185.1, C-2), 127.11, 127.21, 127.73, 128.05, 128.22, 128.38, 128.44, 128.54 (8 d, CH$_2$Ph), 135.71, 135.97, 136.81, 137.78 (4 s, CH$_2$Ph)

$\delta_P$ (CDCl$_3$; 36 MHz; $^1$H-decoupled) -1.61 (1 P), -1.95 (2 P)

$\delta_F$ (DMSO with reference to CFCl$_3$; 84 MHz) -213.1 (dt, J$_{2\text{-}H\text{-}F}$ 51.3, J$_{1\text{(3)}\text{-}H\text{-}F}$ 29.3)

$m/z$ (+ve ion FAB) 1142 [(M - H)$^+$, 4.7 %], 1052 [(M - CH$_2$Ph)$^+$, 1.4], 181 (13), 91 (100)

$m/z$ (-ve ion FAB) 1049 (13 %), 1015 (1), 959 (2.5), 277 ([OP(O)(OCH$_2$Ph)$_2$]$^-$, 100), 187 (14)

22.6 DL-2-deoxy-2-fluoro-myo-inositol 1,4,5-trisphosphate 178

Ammonia was condensed into a three neck flask at -78°C. An excess of sodium was added to dry the liquid ammonia which was then distilled into a second three neck flask and kept at -78°C. Sodium was added until the solution
remained blue. Compound 177 (79 mg, 69 µmol) was dissolved in dry dioxane (2 ml) and added to the sodium-liquid ammonia mixture. After stirring for 15 min the reaction was quenched with ethanol. Ammonia and ethanol were evaporated, the crude product dissolved in water and the solution adjusted to pH 7 with Dowex 50-W ion exchanger (H+-form). Purification by ion-exchange chromatography on Pharmacia Q Sepharose Fast Flow eluting with a gradient of triethylammonium bicarbonate buffer (0.1 M to 1 M), pH 8.0, gave the triethylammonium salt of 178 (eluting between 320 mM and 460 mM). Yield 28.3 µmol (41%).

δ_H (D_2O; 270 MHz) 3.86 (1 H, ddd, J 28.5, 9.9, 2.0, C-3-H), 3.90 (1 H, t, J 9.5, C-6-H), 4.09 (1 H, q, J 9.2, C-4(5)-H), 4.14 (1 H, ddt, J 27.5, 8.4, 1.7, C-1-H), 4.29 (1 H, q, J 9.2, C-5(4)-H), 5.10 (1 H, dt, J 51.8, 1.5, C-2-H)

δ_P (D_2O, 36 MHz; 1H-coupled) 1.96 (1 P, J 10.1), 1.56 (1 P, J 6.7), 0.37 (1 P, J 10.1)

m/z (-ve ion FAB) 421 [(M - H)-, 100 %], 401 (6), 339 (14), 325 (13), 311 (19), 272 (10), 93 (10)

Accurate mass calc for C_6H_{13}O_{14}F_3 (M - H)^- 420.950, found 420.950;

23 Optical Resolution of DL-4,5-Di-O-Acetyl-3-O-allyl-6-O-benzyl-1-O-para-methoxybenzyl-myoinositol

23.1 DL-3-O- Allyl-1,2:4,5-di-O-isopropylidene-myoinositol 179

Allyl bromide (4.8 ml, 6.74 g, 55.7 mmol) was added dropwise to a stirred suspension of DL-1,2:4,5-di-O-isopropylidene-myoinositol 3 (13.02 g, 50 mmol), BaO (15.35 g, 100 mmol) and Ba(OH)_2 x 8 H_2O (1.98 g, 6.25 mmol) in dimethylformamide (250 ml) at r.t. Stirring was continued for 60 h after which tlc (hexane / ethyl acetate 1:1) showed conversion of the starting material (R_f 0.19) into two minor products (R_f 0.70 and 0.47) and a major product (R_f 0.44). The mixture was diluted with methanol and neutralised with acetic acid / water 1:1. The solvents were evaporated and the residue was chromatographed on silica gel (petrol ether / ethyl acetate 1:1) to give DL-3-O-allyl-1,2:4,5-di-O-
isopropylidene-myoinositol 179 (9.46 g, 31.5 mmol, 63%).

m.p. 128-130°C (from petrol ether b.p. 60-80°C) (Lit.$^{[136]}$: 127-129°C)
Found: C, 60.1; H, 8.15. Calc for C$_{15}$H$_{24}$O$_6$ (300.35): C, 60.0; H, 8.05 %.

δ$_H$ (CDCl$_3$; 270 MHz) 1.39, 1.45, 1.47, 1.55 (12 H, 4 s, 4 CH$_3$), 3.31 (1 H, dd, C-5-H, J 10.3, 9.5), 3.51 (1 H, br s, D$_2$O ex, OH), 3.81-4.05 (4 H, m, 4 C-H), 4.25, 4.33 (2 H, AB, d, J$_{AB}$ 12.9, 3 J 6.0, CH$_2$CH=CH$_2$), 4.48 (1 H, t, J 4.4, C-2-H), 5.23 (1 H, d, J 10.3, CH$_2$CH=CH$_2$), 5.32 (1 H, d, J 17.2, CH$_2$CH=CH$_2$), 5.98 (1 H, ddt, J 16.9, 10.5, 5.7, CH$_2$H=CH$_2$

δ$_C$ (CDCl$_3$; 68 MHz) 25.69, 26.66, 26.76, 27.99 (4 q, 4 CH$_3$), 70.94 (t, CH$_2$CH=CH$_2$), 74.02, 74.60, 76.09, 76.81, 78.33, 81.54 (6 d, 6 inositol ring C), 109.99, 112.16 (2 s, 2 C(CH$_3$)$_2$), 117.97 (t, CH$_2$CH=CH$_2$), 134.41 (d, CH$_2$CH=CH$_2$)

m/z (El) 285 (6), 227 (1.4) 113 (100)

m/z (Cl, Iso-butane) 301 (100), 285 (6), 243 (35), 225 (7), 185 (14), 167 (20), 113 (41)

23.2 DL-3,6-Di-O-allyl-1,2:4,5-di-O-isopropylidene-myoinositol 180:

A second product (R$_f$ 0.70) was also isolated from the reaction mixture obtained in experiment 23.1. This product was identified as DL-3,6-di-O-allyl-1,2:4,5-di-O-isopropylidene-myoinositol 180 (Yield 730 mg, 2.1 mmol, 4 %). The 6-O-allyl isomer was not isolated.

m.p. 81-83°C (Lit.$^{[136]}$ 85-87°C)
Found: C, 63.8; H, 8.37. Calc for C$_{18}$H$_{28}$O$_6$ (340.42): C, 63.51; H, 8.29 %.

δ$_H$ (CDCl$_3$; 270 MHz) 1.38, 1.44, 1.46, 1.55 (12 H, 4 s, 4 CH$_3$), 3.34 (1 H, dd, J 10.6, 9.3, C-5-H), 3.66 (1 H, dd, J 10.6, 6.4, C-3(1)-H), 3.80 (1 H, dd, J 10.2, 4.1, C-1(3)-H), 3.98 (1 H, dd, J 9.7, 9.7, C-4(6)-H), 4.09 (1 H, dd, J 6.4, 5.1, C-6(4)-H), 4.20-4.37 (4 H, m, CH$_2$CH=CH$_2$), 4.47 (1 H, dd, J 4.6, 4.6, C-2-H), 5.17-5.36 (4 H, m, 2 CH$_2$CH=CH$_2$), 5.87-6.03 (2 H, m, 2 CH$_2$CH=CH$_2$).

δ$_C$ (CDCl$_3$; 68 MHz) 25.75, 26.79, 26.86, 27.89 (4 q, 4 CH$_3$), 70.97, 71.06 (2 t, CH$_2$CH=CH$_2$ and CH$_2$Ph), 74.60, 76.25, 76.68, 78.40, 79.95, 81.18 (6 d, 6 inositol ring C), 109.76, 111.90 (2 s, 2 C(CH$_3$)$_2$), 117.12, 117.93 (2 t, 2
CH₂CH=CH₂), 134.51, 134.61 (2 d, 2 CH₂CH=CH₂)

m/z (El) 340 [M⁺, 1.4 %], 325 (5), 113 (72) 41 (100)
m/z (Cl, Iso-butane) 341 [(M+H)⁺, 100], 325 (7), 283 (34), 225 (20), 167 (28), 113 (26)

23.3 DL-3-O-allyl-6-O-benzyl-1,2:4,5-di-O-isopropylidene-\textit{myo}-inositol 181

DL-3-O-allyl-1,2:4,5-di-O-isopropylidine-\textit{myo}-inositol 179 (21.6 g, 71.9 mmol), 18-crown-6 (954 mg, 3.6 mmol), potassium hydroxide (48 g) and benzyl chloride (102 ml) were heated with vigorous stirring to 120°C, at which point the reaction became exothermic. Heating was removed, and after 2 h the reaction mixture had cooled to room temperature. Tlc (hexane / ethyl acetate 1:1) showed conversion of the starting material (Rf 0.34) into a product (Rf 0.55). Toluene (85 ml) was added and the mixture was washed with brine, until the aqueous phase remained neutral. The toluene layer was dried over magnesium sulphate and evaporated at 95°C (high vacuum) to remove remaining solvent. The product was recrystallised from methanol to give 181 (25.7 g, 65.8 mmol, 91.5 %).

m.p. 122-123°C

Found: C, 72.83; H, 7.22. Calc for C₂₂H₃₀O₆ (390.48): C, 72.66; H, 7.09 %.

δH (CDCl₃; 270 MHz) 1.36, 1.39, 1.44, 1.46 (12 H, 4 s, 4 CH₃), 3.39 (1 H, dd, J 10.4, 9.3, C-5-H), 3.68 (1 H, dd, J 10.4, 6.5, C-H), 3.79 (1 H, dd, J 10.1, 4.1, C-H), 3.96 (1 H, t, J 9.7, C-H), 4.13 (1 H, t, J 5.6, C-H), 4.23, 4.32 (2 H, AB, d, JAB 13.0, 4.5, CH₂CH=CH₂), 4.45 (1 H, t, J 4.5, C-2-H), 4.83 (2 H, AB, CH₂Ph), 5.22 (1 H, dd, J 17.2, 12, CH₂CH=CH₂), 5.31 (1 H, dd, J 10.3, 0.8, CH₂CH=CH₂), 5.97 (1 H, ddt, J 16.8, 10.9, 6.4, CH₂CH=CH₂), 7.24-7.42 (5 H, m, CH₂Ph)

δC (CDCl₃; 68 MHz) 25.82, 26.86, 26.92, 27.76 (4 q, CH₃), 71.00, 71.88 (2 t, CH₂CH=CH₂, CH₂Ph), 74.63, 76.25, 76.81, 78.72, 79.82, 81.02 (6 d, inositol ring C), 109.79, 111.97 (2 q, C(CH₃)₂), 117.97 (t, CH₂CH=CH₂), 127.34, 127.83, 128.05 (3 d, CH₂Ph), 134.54 (d, CH₂CH=CH₂), 138.11 (s, CH₂Ph)

m/z (Cl, Iso-butane) 391 [(M+H)⁺, 80], 333 (50), 275 (11), 217 (16), 131 (49), 91 (100)
23.4 DL-1-O-Allyl-4-O-benzyl-my-inositol 182

181 (24.3 g, 62.2 mmol) was heated under reflux in a mixture of methanol (360 ml) and 1 M HCl (40 ml) for 30 min, after which tlc (ethyl acetate) showed conversion of the starting material into a product (Rf 0.28). The mixture was cooled, neutralised with triethylamine and the solvent was evaporated. The product was extracted with ethyl acetate and the solvent evaporated to give 182 (16.8 g, 54.1 mmol, 87%).

m.p. 152-153°C (from ethyl acetate)

Found: C, 61.9; H, 7.21. Calc for C₁₆H₂₂O₆ (310.35): C, 61.92; H, 7.15 %.

δₜ (d₆-DMSO; 270 MHz) 3.01 (1 H, dd, J 9.8, 2.4, C-1-H), 3.14 (1 H, dt, J 9.0, 4.8, D₂O shake gives t, J 9.1, C-5-H), 3.31 (1 H, m, D₂O shake gives dd, J 9.8, 2.5, C-3-H), 3.44 (1 H, t, J 9.2, C-4-H), 3.56 (1 H, dt, J 9.3, 4.8, D₂O shake gives t, J 9.4, C-6-H), 3.89-3.94 (1 H, m, D₂O shake gives dd, J 9.8, 2.5, C-2-H), 4.64-4.83 (6 H, m, D₂O shake gives 2 H, AB s at 4.72, CH₂Ph and 4 OH), 5.15 (1 H, dd, J 10.4, 1.5, CH₂CH=CH₂), 5.32 (1 H, dd, J 17.2, 1.65, CH₂CH=CH₂), 5.92 (1 H, ddt, J 17.2, 10.4, 5.5, CH₂CH=CH₂), 7.24-7.44 (5 H, m, CH₂Ph)

δₙ (d₆-DMSO; 68 MHz) 69.96, 71.58, 72.30, 75.18, 79.59, 81.93 (6 d, inositol ring C), 70.31, 73.72 (2 t, CH₂CH=CH₂, CH₂Ph), 116.02 (t, CH₂CH=CH₂), 127.05, 127.66, 127.97 (3 d, CH₂Ph), 136.26 (d, CH₂CH=CH₂), 140.05 (s, CH₂Ph)

m/z (70 eV): 310 (M⁺, 6 %), 149 (12), 113 (20), 91 (100), 86 (38), 55 (28), 41 [(C₃H₅)⁺, 57]

m/z (Cl, Iso-butane): 310 (M⁺, 14 %), 219 [(M - C₇H₇)⁺, 22], 131 (98), 107 [(OC₇H₇)⁺, 32], 91 [(C₇H₇)⁺, 100], 69 (47)

23.5 DL-3-O-Allyl-6-O-benzyl-1-O-p-methoxybenzyl-my-inositol 183

182 (21.7 g, 70 mmol) and dibutyltin oxide (26.1 g, 105 mmol) in toluene were heated under reflux for 3 h in a Dean and Stark apparatus. The reaction mixture was cooled and the toluene evaporated to give a syrup which was dried under vacuum for 2 h. Caesium fluoride (21.3 g, 140 mmol) was added to the syrup.
and dried for a further hour. Dry DMF (200 ml) was added to the syrup and \( p \)-methoxybenzyl chloride (11 g, 9.5 ml, 70 mmol) at r.t. After 24 h at r.t. tlc (ethyl acetate) showed conversion of the starting material (R\(_f\) 0.35) into a major product (R\(_f\) 0.51) and several less polar by-products (R\(_f\) 0.65, 0.77 and 0.89). The solvents were evaporated under reduced pressure and the residue was taken up in dichloromethane. The suspension was washed with water and 1 M HCl and the insoluble tin derivatives were removed by filtration through Celite. The solution was dried over magnesium sulphate, filtered and evaporated. The crude product was chromatographed on silica gel (ethyl acetate) to give 183. Yield 24.3 g (81 %).

m.p. 133-135°C (from ethyl acetate)
Found: C, 67.0; H, 7.08. Calc for \( C_{24}H_{30}O_7 \) (430.50): C, 66.96; H, 7.02 %.

\( \delta \)\(_H\) (CDCl\(_3\); 270 MHz) 2.83 (1 H, brd s, D\(_2\)O ex, OH), 3.10 (1 H, dd, J 9.6, 2.65, C-3(1)-H), 3.35 (1 H, dd, J 9.6, 2.6, C-1(3)-H), 3.40 (1 H, dd, J 9.3, 9.3, C-H), 3.4-3.5 (2 H, brd, D\(_2\)O ex, 2 OH), 3.77 (3 H, s, OCH\(_3\)), 3.79 (1 H, dd, J 9.3, 9.3, C-H), 3.90 (1 H, dd, J 9.4, 9.4, C-H), 4.05-4.19 (2 H, m, CH\(_2\)CH=CH\(_2\)), 4.15 (1 H, dd, J 2.7, 2.7, C-2-H), 4.61 (2 H, AB, CH\(_2\)Ph), 4.80, 4.91 (2 H, AB, J 11.2, CH\(_2\)Ph), 5.17 (1 H, dd, J 10.3, 1.3, CH\(_2\)CH=CH\(_2\)), 5.26 (1 H, dd, J 17.2, 1.5, CH\(_2\)CH=CH\(_2\)), 5.91 (1 H, ddt, J 17.2, 10.4, 5.7, CH\(_2\)CH=CH\(_2\)), 6.84 (2 H, d, J 8.8, CH\(_2\)Ph), 7.23-7.37 (7 H, m, CH\(_2\)Ph)

\( \delta \)\(_C\) (CDCl\(_3\); 68 MHz) 55.07 (OCH\(_3\)), 66.91, 71.65, 74.28, 78.69, 79.30, 80.44 (6 d, inositol ring C), 71.16, 72.00, 75.25 (3 t, CH\(_2\)CH=CH\(_2\), CH\(_2\)Ph), 113.68 (d, CH\(_2\)Ph), 117.64 (t, CH\(_2\)CH=CH\(_2\)), 127.40, 127.76, 128.22, 129.35, 129.81 (5 d, CH\(_2\)Ph), 134.48 (d, CH\(_2\)CH=CH\(_2\)), 138.72 (s, CH\(_2\)Ph), 159.16 (s, C-OCH\(_3\))

\( m/\text{z} \) (El): 339 (44 %), 309 (14), 137 (77), 121 (100), 91 (63)
\( m/\text{z} \) (Cl, Iso-butane): 429, 339 (11 %), 309 (7), 137 (14), 121 (100)

### 23.6 DL-3-O-Allyl-6-O-benzyl-4,5-O-isopropylidene-1-O-p-methoxybenzyl-myoinositol 184

A mixture of 183 (24 g, 55.7 mmol), \( p \)-toluenesulphonic acid monohydrate (2.25 g, 11.8 mmol) and 2-methoxypropene (19 ml, 14.3 g, 198.4 mmol) in dry DMF
(400 ml) was stirred overnight when tlc (ether) showed complete conversion of the starting material (Rf 0.12) into a product (Rf 0.67). Triethylamine (10 ml) was added and the solvents were evaporated. The resulting syrup was taken up in dichloromethane, washed with water and brine and dried over magnesium sulphate. The solvent was evaporated and the crude product purified by flash chromatography (petrol ether / ether 2:3) to give 184 (21.9 g, 46.5 mmol, 84%).

m.p. 87-89°C (from petrol ether b.p. 60-80°C)
Found: C, 69.1; H, 7.39. Calc for C_{27}H_{34}O_{7} (470.56): C, 68.92; H, 7.28 %.

δ_{H} (CDCl_{3}; 270 MHz) 1.43, 1.45 (6 H, 2 s, 2 CH_{3}), 2.68 (1 H, brd s, D_{2}O ex, 1 OH), 3.38 (1 H, dd, J 9.7, 9.7, C-H), 3.44 (1 H, dd, J 8.4, 3.1, C-3(1)-H), 3.50 (1 H, dd, J 10.3, 3.1, C-1(3)-H), 3.78 (3 H, s, OCH_{3}), 3.96 (1 H, dd, J 8.6, 8.4, C-H), 4.02 (1 H, dd, J 9.7, 9.7, C-H), 4.15, 4.26 (2 H, AB, dt, J_{AB} 12.8, J 5.8, 1.5, CH_{2}CH=CH_{2}), 4.20 (1 H, dd, J 3.1, 3.1, C-2-H), 4.65, 4.69 (2 H, AB, J 11.35, CH_{2}Ph), 4.76, 4.91 (2 H, AB, J 11.6, CH_{2}Ph), 5.18 (1 H, dd, J 10.4, 1.5, CH_{2}CH=CH_{2}), 5.28 (1 H, ddt, J 17.3, 1.6, 1.6, CH_{2}CH=CH_{2}), 5.94 (1 H, ddt, J 17.2, 10.4, 5.7, CH_{2}CH=CH_{2}), 6.84 (2 H, d, J 8.8, CH_{2}Ph), 7.23-7.41 (7 H, m, CH_{2}Ph)

δ_{C} (CDCl_{3}; 68 MHz) 26.79, 26.89 (2 q, 2 CH_{3}), 55.07 (q, OCH_{3}), 69.38, 75.96, 76.71, 78.33, 79.21, 80.41 (6 d, 6 inositol ring C), 70.58, 72.85, 73.21 (3 t, CH_{2}CH=CH_{2} and 2 CH_{2}Ph), 111.51 (s, C(CH_{3})_{2}), 113.68 (d, CH_{2}PhOme), 117.45 (t, CH_{2}CH=CH_{2}), 127.28, 127.53, 128.09, 129.48, 129.77 (5 d, CH_{2}Ph and CH_{2}PhOme), 134.44 (d, CH_{2}CH=CH_{2}), 138.66 (s, CH_{2}Ph), 159.22 (s, C-OCH_{3})

m/z (+ve ion FAB) 469 [(M-H)^{+}, 3.6 %], 379 (4.9), 349 (3.7), 121 [(C_{7}H_{6}OCH_{3})^{+}, 100], 91 (24)
m/z (-ve ion FAB) 622 [(M+NBA)^{-}, 66 %], 469 [(M-H)^{-}, 90], 429 (37), 349 [(M - C_{7}H_{6}OCH_{3})^{-}, 100], 334 (44), 182 (70), 167 (47), 93 (40)
23.7 DL-3-O-Asyl-6-O-benzyl-2-O-[(S)-\(\omega\)-camphanoyl]-4,5-O-isopropyldene-1-O-p-methoxybenzyl-my-o-inositol 185

A mixture of 184 (470 mg, 1 mmol), (1S)-(S)-camphonic chloride (433 mg, 2 mmol) and DMAP (10 mg, 0.08 mmol) in dry pyridine (5 ml) was stirred at r.t. overnight, after which tlc (hexane / ethyl acetate 1:1) showed conversion of the starting material (Rf 0.44) into a product (Rf 0.52). By hptlc there was no visible separation of the product spots. The solution was cooled in an ice-bath, water (0.5 ml) was added and stirring was continued for 10 min. Ether (50 ml) and dichloromethane (25 ml) were added and the organic phase was washed successively with saturated aqueous potassium chloride solution, ice-cold 1 M HCl, saturated aqueous potassium chloride solution and saturated aqueous sodium hydrogen carbonate solution (100 ml each) and then dried over magnesium sulphate. Evaporation of the solvents gave a syrup which was co-evaporated with toluene, then taken up in ether. Petrol ether b.p. 60-80°C was added and the solution was kept in the fridge overnight. The crystals were filtered off to give 185 as a 1 : 1 mixture of the two diastereoisomers. Yield (616 mg, 0.95 mmol, 95 %). Efforts to separate the diastereoisomers by recrystallisation from methanol were also unsuccessful.

m.p. 136-137°C (from ether / petrol ether)

m.p. 135-136°C (from methanol)

Found: C, 68.0; H, 7.13. Calc for C\(_{37}\)H\(_{46}\)O\(_{10}\) (650.77): C, 68.29; H, 7.12 %.

δ\(\text{H}\) (CDCl\(_3\); 270 MHz) 0.83 (3 H, s, CH\(_3\)), 0.90 (3 H, s, CH\(_3\)), 0.94 (3 H, s, CH\(_3\)), 0.98 (3 H, s, CH\(_3\)), 1.08 (3 H, s, CH\(_3\)), 1.08 (3 H, s, CH\(_3\)), 1.45 (6 H, s, CH\(_3\)), 1.48 (6 H, s, 2 CH\(_3\)), 1.62-1.71 (2 H, m, CH\(_2\)), 1.82-2.07 (4 H, m, 2 CH\(_2\)), 2.23-2.42 (2 H, m, CH\(_2\)), 3.40-3.89 (10 H, m, C-H), overlapping with 3.79 (3 H, s, OCH\(_3\)), 3.80 (3 H, s, OCH\(_3\)), 4.10-4.14 (4 H, m, CH\(_2\)-CH=CH\(_2\)), 4.50-4.65 (4 H, 2 AB, overlapping, J 11.7, 2 CH\(_2\)Ph), 4.74-4.92 (4 H, 2 AB, overlapping, J 11.7, 2 CH\(_2\)Ph), 5.17 (2 H, ddt, J 10.3, 1.4, 1.4, cis-CH\(_2\)-CH=CH\(_2\), 5.28 (2 H, ddt, J 17.3, 1.6, 1.6, trans-CH\(_2\)-CH=CH\(_2\)), 5.80-5.94 (4 H, m, CH\(_2\)-CH=CH\(_2\) and C-2-H)), 6.83 (4 H, dd, J 8.7, 3.6, CH\(_2\)PhOMe), 7.21-7.40 (14 H, m, CH\(_2\)Ph and CH\(_2\)PhOMe)
23.8 DL-3-O-allyl-6-O-benzyl-2-O-[(−)-ω-camphanoyl]-1-O-phenoxy-benzyl-myoinositol 186

185 (6.1 g, 9.5 mmol) was heated under reflux in a mixture of methanol (108 ml) and 1 M HCl (12 ml) for 30 min, when tlc (hexane / ethyl acetate 1:1) showed conversion of the starting material (Rf 0.58) into a product (Rf 0.11). Examination by hptlc (ethyl acetate / hexane 1:1) revealed two product spots only after the hptlc plate had been developed three times. The reaction mixture was cooled, neutralised with triethylamine and the solvent was evaporated. The residue was taken up in hot ether, filtered and kept at -20°C overnight. Crystals appeared, however, on filtration at r.t. the compound turned into an oil. Attempts to recrystallise from methanol and separate the diastereoisomers by flash chromatography (chloroform / acetone 10:1) were also unsuccessful. Yield of 186 4.8 g, (7.9 mmol, 83 %).

$\delta_1$ (CDCl$_3$; 270 MHz) 0.86, 0.92, 0.98, 1.02, (12 H, 4 s, 4 CH$_3$), 1.08 (6 H, s, 2 CH$_3$), 1.55-1.72 (2 H, m, Camph-CH$_2$), 1.82-2.1 (4 H, m, Camph-CH$_2$), 2.28-2.46 (2 H, m, Camph-CH$_2$), 3.2-3.87 (14 H, m, D$_2$O ex gives 10 H, m, 10 inositol ring H and 4 OH), 3.78 (6 H, s, 2 OCH$_3$), 3.99-4.22 (4 H, m, CH$_2$CH=CH$_2$), 4.44-4.66 (4 H, m, 2 CH$_2$Ph), 4.71-4.94 (4 H, m, 2 CH$_2$Ph), 5.18 (2 H, brd d, J 10.4, cis-CH$_2$-CH=CH$_2$), 5.28 (2 H, brd d, J 17.0, trans-CH$_2$-CH=CH$_2$), 5.82-5.92 (4 H, m, 2 CH$_2$CH=CH$_2$ and 2 C-2-H), 6.83, 6.84 (4 H, 2 d, J 8.8, CH$_2$Ph), 7.20-7.33 (14 H, m, CH$_2$Ph).

$\delta_C$ (CDCl$_3$; 68 MHz) 9.68 (q, 2 Camph-CH$_3$), 16.43, 16.48, 16.72, 16.79 (4 q, 4 Camph-CH$_3$), 28.95, 30.67 (2 t, Camph), 54.33, 54.91 (2 s, Camph), 55.25 (q, OCH$_3$), 70.90, 71.97, 75.23, 75.32 (4 t, CH$_2$CH=CH$_2$ and CH$_2$Ph), 67.71, 67.77, 72.20, 72.35, 73.94, 76.68, 76.74, 77.55, 77.72, 79.94, 80.34 (11 d, inositol ring C), 91.32 (s, Camph), 113.72, 113.75 (2 d, CH$_2$Ph), 117.89, 117.98 (2 t, CH$_2$CH=CH$_2$), 127.75, 127.99, 128.09, 128.38, 128.41, 129.74, 129.81 (7 d, CH$_2$Ph), 134.05 (d, CH$_2$CH=CH$_2$), 138.39, 138.45 (2 s, CH$_2$Ph), 159.35 (s, C-OCH$_3$), 166.62, 169.69, 178.10, 178.22 (4 s, carbonyl).

$m/z$ (+ve ion FAB) 609 [(M-H)$^+$, 1.5 %], 519 [(M-C$_7$H$_7$)$^+$, 4.8], 489 [(M - C$_7$H$_6$OCH$_3$)$^+$, 2], 121 [(C$_7$H$_6$OCH$_3$)$^+$, 100], 91 (10)

$m/z$ (-ve ion FAB) 763 [(M+NBA)$^-$, 22 %], 656 [(M+NBA - C$_7$H$_7$O)$^-$, 13], 609 [(M-H)$^-$, 14], 197 [(CamphO)$^-$, 100]
A mixture of 186 (2.07 g, 3.39 mmol), DMAP (186 mg, 1.37 mmol), acetic anhydride (13.8 mg, 12.9 ml, 136.5 mmol) and pyridine (30 ml) was kept for 2 h at r.t. when tlc (ethyl acetate / hexane 1:1) showed complete conversion of the starting material into a product. Examination by hptlc revealed two product spots ($R_t = 0.58$ and 0.63). The product was precipitated with ice water and taken up in chloroform. The solution was washed several times with water and dried over magnesium sulphate. Evaporation of the solvents gave a syrup which was recrystallised from ethyl acetate / hexane to give a 1:1 mixture of the two diastereoisomers (2.07 g, 2.98 mmol, 88 %). The mixture was taken up in hot ether and left overnight at 4°C, the crystals formed were found to be a 2:1 mixture of the less polar isomer : the more polar isomer. Further recrystallisations gave the pure (+)-isomer.

m.p. 142-143°C
$[\alpha]_D^{27} = +19.0°$ (c = 4 in CHCl$_3$);
Found: C, 65.4; H, 6.54. Calc for C$_{38}$H$_{46}$O$_{12}$ (694.78): C, 65.69; H, 6.67 %);

$\delta_H$ (CDCl$_3$; 400 MHz) 0.95 (3 H, s, CH$_3$), 1.115 (3 H, s, CH$_3$), 1.125 (3 H, s, CH$_3$), 1.66-1.73 (1 H, m, Camph-CH$_2$), 1.84-2.11 (2 H, m, Camph-CH$_2$), overlapping with 1.95 (3 H, s, C(O)CH$_3$), 2.01 (3 H, s, C(O)CH$_3$), 2.36-2.43 (1 H, m, Camph-CH$_2$), 3.48 (1 H, dd, J 9.8, 2.7, C-1(3)-H), 3.60 (1 H, dd, J 9.5, 2.7, C-3(1)-H), 3.73 (1 H, dd, J 9.8, 9.6, C-6-H), 3.79 (3 H, s, OCH$_3$), 3.93, 4.12 (2 H, AB, d, J$_{AB}$ 12.9, J 5.4, CH$_2$CH=CH$_2$), 4.48, 4.65 (2 H, AB, J$_{AB}$ 10.5, CH$_2$Ph), 4.61, 4.80 (2 H, AB, J$_{AB}$ 11.2, CH$_2$Ph), 5.05 (1 H, dd, J 10.0, 10.0, C-5(4)-H), 5.15 (1 H, dd, J 10.7, 1.5, cis-CH$_2$-CH=CH$_2$), 5.22 (1 H, dd, J 17.6, 1.5 trans-CH$_2$-CH=CH$_2$), 5.27 (1 H, dd, J 10.3, 10.3, C-4(5)-H), 5.77 (1 H, ddt, J 17.1, 10.25, 5.1, CH$_2$CH=CH$_2$), 5.89 (1 H, dd, J 2.7, 2.7, C-2-H), 6.85 (2 H, d, J 8.8, CH$_2$Ph), 7.20-7.33 (7 H, m, CH$_2$Ph).

$\delta_C$ (CDCl$_3$; 100 MHz) 9.69, 16.39, 16.79 (3 q, 3 Camph-CH$_3$), 20.70, 20.74 (2 q, 2 acetyl-CH$_3$), 29.04, 30.72 (2 t, Camph), 54.46, 54.92 (2 s, Camph), 55.27 (q, OCH$_3$), 67.65, 71.18, 72.02, 74.78, 77.45, 78.04 (6 d, 6 inositol ring C), 71.09, 72.18, 75.26 (3 t, CH$_3$CH=CH$_2$ and 2 CH$_2$Ph), 91.29 (s, Camph), 113.83 (d, CH$_2$Ph), 117.52 (t, CH$_2$CH=CH$_2$), 127.74, 127.98, 128.33, 129.86 (4 d, CH$_2$Ph), 232
129.26 (s), 133.66 (d, CH₂CH=CH₂), 137.98 (s, CH₂Ph), 159.45 (s, C-OCH₃), 166.51, 169.79, 169.99, 178.05 (4 s, carbonyl)

m/z (+ve ion FAB) 693 [(M-H)+, 4 %], 603 [(M-C₇H₇)+, 10], 121 (100), 91 (34)
m/z (-ve ion FAB) 847 [(M+NBA)+, 40 %], 740 [(M+NBA - C₇H₇O)-, 23], 197 [(CamphO)-, 100]

23.10 (+)-3-O- Allyl-6-O-benzyl-1-O-p-methoxybenzyl- myo-inositol 188

187 (2.48 g, 3.57 mmol) was dissolved in 100 ml methanol containing 4 g NaOH. The solution was heated under reflux for 30 min when tlc (ether) showed complete conversion of the starting material into a single product. After cooling, the solution was neutralised with solid CO₂. Water (100 ml) was added and the solution extracted twice with 100 ml chloroform each. The organic layers were dried over magnesium sulphate and the solvent evaporated to give 188 (1.48 g, 3.43 mmol, 96 %).

m.p. 157-158°C (from ethyl acetate)
[α]D²⁰ = +19.0° (c = 4 in CHCl₃),
Found: C, 67.0; H, 7.02. Calc for C₂₄H₃₀O₇ (430.50): C, 66.96; H, 7.02 %.
m/z (+ve ion FAB) 429 (3 %), 400 (10), 339 (8), 309 (5), 121 [(C₇H₆OCH₃)+, 100], 91 (8)
m/z (-ve ion FAB) 583 [(M+NBA)+, 100 %], 429 [(M-H)+, 85], 322 [(M - C₇H₇O)+, 30], 309 [(M - C₇H₆OCH₃)+, 20]
NMR data were identical to 183.

24  Lactonisation

24.1  DL-3,6-Di-O-benzyl-1-O-cyanomethylene- myo-inositol 189

A mixture of DL-1,4-di-O-benzyl- myo-inositol 124 (8 g, 22.2 mmol) and dibutyltin oxide (6 g, 24 mmol) in toluene (240 ml) was heated under reflux in a Dean and Stark apparatus for 4 h. The solution was evaporated to dryness in vacuo. Caesium fluoride (16.9 g, 111 mmol) was added to the resulting white solid and the mixture was dried in vacuo for 2 h. The mixture was then suspended in dry
DMF (300 ml) under nitrogen, and bromoacetonitrile (3.36 g, 1.95 ml, 28 mmol) was added. After stirring for 20 h at room temperature the solvent was evaporated. Water (150 ml), 2 M HCl (20 ml) as well as ethyl acetate (300 ml) were added to the residue, which was then filtered through Celite to remove tin compounds. The organic layers were dried over magnesium sulphate, evaporated to dryness and the product was purified by flash chromatography using ether / ethyl acetate (2:1) as eluent to give 189 (5.1 g, 12.8 mmol, 58%).

tlc (ether / ethyl acetate 1:1) Rf 0.58
m.p. 141-142°C
Found C, 66.2; H, 6.34; N, 3.53. Calc. for C_{22}H_{25}O_{6}N (399.45): C, 66.15; H, 6.31; N, 3.51 %.

δ_H (CDCl_3, 270 MHz) 2.90-3.10 (3 H, brd, OH), 3.22 (1 H, dd, J 9.4, 2.2, C-3(1)-H), 3.36 (1 H, dd, J 9.4, 2.2, C-1(3)-H), 3.41 (1 H, dd, J 9.4, 9.4, C-H), 3.84 (1 H, dd, J 9.5, 9.5, C-H), 3.83, 3.89 (2 H, AB, J_{AB} 9.5, CH_2CN), 4.24 (1 H, m, C-H), 4.31, 4.38 (2 H, AB, J_{AB} 15.8, CH_2Ph), 4.65 (1 H, dd, J 3.7, 3.7, C-2-H), 4.81, 4.92 (2 H, AB, J_{AB} 11.2, CH_2Ph), 7.29-7.38 (10 H, m, CH_2Ph)

δ_C (CDCl_3, 68 MHz) 56.24 (t, CH_2CN), 67.37, 71.84, 74.41, 78.91, 80.24, 80.37 (6 d, inositol ring C), 72.43, 75.44 (2 t, CH_2Ph), 116.21 (CN), 127.83, 127.96, 128.18, 128.48, 128.60 (5 d, CH_2Ph), 137.27, 138.27 (2 s, CH_2Ph),

m/z (70 eV El) 399 (M^+), 308 [(M - C_7H_7)^+], 149 (10), 107 [(C_7H_7O)^+, 20], 91 [(C_7H_7)^+, 100]

m/z (Cl, Isobutane) 442 [(M + C_3H_7)^+, 5 %], 400 [(M + H)^+, 25], 308 [(M - C_7H_7)^+, 20], 181 (40), 107 (55), 91 (100)

24.2 DL-3,6-Di-O-benzyl-1-O-carboxymethylene-my-o-inositol 190

189 (3.39 g, 8.5 mmol) was dissolved in a solution of NaOH (680 mg, 17 mmol) in 25 ml ethanol. The mixture was heated under reflux until no more ammonia was formed (4 h). Water was then added and the solution was acidified with hydrochloric acid to a pH of 3. The mixture was extracted with ethyl acetate (2 x 25 ml), the organic fractions were dried (magnesium sulphate) and the solvent was evaporated. Recrystallisation of the crude product from ethyl acetate /
ethanol (10:1) gave 190 (2.86 g, 6.85 mmol, 81 %)

tlc (ether / ethyl acetate 1:1) Rf 0.03
m.p. 185-187°C

Found: C, 62.9; H, 6.50. Calc. for C_{22}H_{26}O_{8} (418.43): C, 63.15; H, 6.26 %.

δ_{H} (d_{6}-DMSO, 270 MHz) 3.10 (1 H, dd, J 9.9, 2.2, C-3(1)-H), 3.16 (1 H, dd, J 9.9, 9.9, C-H), 3.27 (1 H, dd, J 9.9, 2.2, C-1(3)-H), 3.38 (1 H, brd, OH), 3.59 (1 H, dd, J 9.5, 9.5, C-H), 3.62 (1 H, dd, J 9.5, 9.5, C-H), 4.16, 4.21 (2 H, AB, J_{AB} 16.7, CH_{2}COOH), 4.28 (1 H, s, C-2-H), 4.59, 4.66 (2 H, AB, J_{AB} 12.1, CH_{2}Ph), 4.76, 4.79 (2 H, AB, J_{AB} 11.4, CH_{2}Ph), 4.86 (1 H, brd, OH), 4.95 (1 H, brd, OH), 7.22-7.44 (10 H, m, CH_{2}Ph), 12.6 (1 H, brd, COOH)

δ_{C} (d_{6}-DMSO, 68 MHz) 66.49, 72.04, 74.99, 79.34, 80.76, 81.06 (6 d, inositol ring C), 67.27 (t, CH_{2}COOH), 70.94, 74.08 (2 t, CH_{2}Ph), 126.95, 127.01, 127.47, 127.63, 127.83 (5 d, CH_{2}Ph), 139.02, 139.44 (2 s, CH_{2}Ph), 172.20 (s, COOH)

m/z (+ve ion FAB) 418 (M+, 100 %), 289 (35), 247 (90), 91 (85)
m/z (-ve ion FAB) 570 [(M+NBA - H)-, 60%], 417 [(M - H)-, 100), 326 (25), 272 (30), 167 (42)

ν_{max}/cm^{-1} (KBr-disc): 1720 (s, carbonyl)

24.3 DL-3,6-di-O-benzyl-(1-O-methylene-2-lacto)-myo-inositol 191

a) A mixture of DL-1,4-Di-O-benzyl-myosinositol 124 (4 g, 11.1 mmol) and dibutyltin oxide (3 g, 12 mmol) in toluene (120 ml) was heated under reflux in a Dean and Stark apparatus overnight. The solution was evaporated to dryness in vacuo. Caesium fluoride (8.44 g, 55.5 mmol) was added to the resulting white solid and the mixture was dried in vacuo for 2 h. The mixture was suspended in dry DMF (200 ml) under nitrogen and ethyl bromoacetate (6 g, 4 ml, 36 mmol) was added. The reaction was stirred at 80°C for 8 h, the solvent was evaporated and the residue was taken up in ethyl acetate. After washing with 1 M HCl and sat. KCl solution the mixture was stirred for 30 min with a sat. NaHCO_{3} solution and the precipitated tin derivatives were removed by filtration through Celite. The solvent was evaporated and the residue was chromatographed on silica gel.
eluting with ether / ethyl acetate to give 191 (700 mg, 1.75 mmol, 16%).

b) A mixture of 190 (1 g, 2.39 mmol), toluene (160 ml) and conc. H₂SO₄ (20 µl) was heated under reflux for 1 h in a Dean and Stark apparatus. The solvent was evaporated and the crude product taken up in ethyl acetate. The insoluble solid was filtered and the solvent was evaporated. Recrystallisation from ethyl acetate / hexane gave 191 (881 mg, 2.20 mmol, 92 %)

tlc (ether / ethyl acetate 1:1) Rₖ 0.69
m.p. 153-155°C
Found: C, 65.80; H, 5.91. Calc. for C₂₂H₂₄O₇ (400.43): C, 65.99; H, 6.04 %.

δₜ (CDCl₃, 270 MHz) 3.03-3.18 (1 H, brd, OH), 3.12-3.26 (1 H, brd, OH), 3.78-3.86 (2 H, m, C-3-H, C-5-H), 3.94 (1 H, dd, J 5.6, 5.6, C-6-H), 4.07 (1 H, dd, J 6.0, 3.0, C-1-H), 4.20-4.13 (1 H, m, C-4-H), 4.02, 4.30 (2 H, AB, J_AB 17.6, CH₂COOR), 4.61, 4.68 (2 H, AB, J_AB 11.2, CH₂Ph), 4.74 (2 H, AB, CH₂Ph), 4.88 (1 H, dd, J 3.3, 3.3, C-2-H), 7.25-7.32 (10 H, m, CH₂Ph)

δₜ (CDCl₃, 68 MHz) 61.01 (t, CH₂CO), 70.54, 71.13, 73.69, 74.82, 75.63, 76.54, (6 d, inositol ring C), 72.23, 73.53 (2 t, CH₂Ph), 131.52, 131.59, 131.75, 131.95, 132.01 (5 d, CH₂Ph) 136.81 (s, CH₂Ph), 166.68 (s, CO)

v_max/cm⁻¹ (KBr-disc): 1735 (s, carbonyl)

m/z (low eV El) 400 [(M)+, 10%], 318 (15), 310 (20), 309 [(M - C₇H₇)+, 100], 203 (20)

24.4 DL-4,5-Di-O-acetyl-3,6-di-O-benzyl-(1-O-methylene-2-lacto)-myo-inositol 192

A mixture of 191 (727 mg, 1.74 mmol), DMAP (86 mg, 0.7 mmol), acetic anhydride (7.1 mg, 6.6 ml, 70 mmol) and pyridine (15 ml) was stirred for 5 h at 50°C. The product was precipitated with ice water and taken up in chloroform. The solution was washed several times with water and dried over magnesium sulphate. Evaporation of the solvents gave a syrup which was recrystallised from ethyl acetate / hexane to give 192 (708 mg, 1.46 mmol, 84 %).
m.p. 159-163°C
Found C, 64.7; H, 5.78. Calc. for C_{26}H_{28}O_{9} (484.51): C, 64.46; H, 5.83 %.

δ_{H} (CDCl_{3}, 270 MHz) 1.99 (6 H, 2 s, CH_{3}), 3.69 (1 H, dd, J 8.4, 3.3, C-3(1)-H, ),
3.88 (1 H, dd, J 8.4, 8.4, C-6-H), 3.95 (1 H, dd, J 8.4, 3.1, C-1(3)-H), 4.04, 4.26
(2 H, AB, J_{AB} 17.6, CH_{2}COOR), 4.63, 4.68 (2 H, AB, J_{AB} 12.1, CH_{2}Ph), 4.65,
4.70 (2 H, AB, J_{AB} 11.7, CH_{2}Ph), 4.82 (1 H, dd, J 3.1, 3.1, C-2-H), 5.09 (1 H, dd,
J 9.0, 9.0, C-5-H), 5.39 (1 H, dd, J 9.0, 9.0, C-4-H), 7.28-7.36 (10 H, m, CH_{2}Ph)

δ_{C} (CDCl_{3}, 68 MHz) 20.69, 20.76 (2 q, OCOCH_{3}), 66.91, 71.23, 72.52, 76.61,
78.30, 81.12 (6 d, inositol ring C), 67.85 (t, CH_{2}COOR), 72.56, 75.83 (2 t,
CH_{2}Ph), 127.76, 127.83, 127.96, 128.18, 128.51, 128.57, 129.74 (7 d, CH_{2}Ph),
137.04 (s, CH_{2}Ph), 166.29, 169.57, 169.90 (3 s, CO)

m/z (+ve ion FAB) 969 [(2M+H)+, 1 %], 485 [(M+H)+, 8], 377 [(M-C_{7}H_{7}O)+, 5],
181 (20), 91 (100)
m/z (-ve ion FAB) 650 (70%), 637 [(M+NBA)-, 100], 530 (50), 483 [(M-H)-, 30]

ν_{max}/cm^{-1} (KBr-disc): 1770 and 1740 (s, carbonyl)

24.5 (-)-3,6-Di-0-benzyl-4,5-di-0-[(+-)-o-camphanoyl]-(1-O-methylene-
2-lacto)-myo-inositol 193

A mixture of 191 (3.07 g, 7.67 mmol), DMAP (175 mg, 1.43 mmol) and S-(+-)-
camphamic chloride (5 g, 23.15 mmol) in dry pyridine (60 ml) was stirred at r.t. for
2 h, after which tlc (ethyl acetate / hexane 1:1) showed complete conversion of
the starting material (R_{f} 0.32) into a product (R_{f} 0.81). Water (1 ml) was added
and the solution stirred for another 30 min. The mixture was then partitioned
between water and ether (50 ml each) and the ether layer was washed with
water and sat. NaHCO_{3} solution. The organic phase was then dried over
magnesium sulphate and the solvent evaporated to give a mixture of the two
diastereomers of 193 as a syrup (5.43 g, 7.13 mmol, 93 %). Recrystallisation
from methanol gave one diastereoisomer (1.83 g, 2.4 mmol, 62 % of the
diastereoisomer).
m.p. 133°C
$[\alpha]_D^{24} = -39.6^\circ$ (c = 4 in CHCl$_3$)

Found C, 66.7; H, 6.44. Calc. for C$_{42}$H$_{48}$O$_3$ (760.84): C 66.30, H 6.36 %.

$\delta_H$ (CDCl$_3$, 400 MHz) 0.81, 0.84, 0.95, 0.98, 1.04, 1.06 (18 H, s, 6 CH$_3$), 1.57-1.67 (2 H, m, Camph-CH$_2$), 1.78-1.95 (4 H, m, Camph-CH$_2$), 2.17-2.27 (2 H, m, Camph-CH$_2$), 3.43 (1 H, dd, J 9.8, 2.5, C-3(1)-H), 3.62 (1 H, dd, J 10.0, 2.7, C-1(3)-H), 4.12, 4.30 (2 H, AB, J 16.8, CH$_2$COOR), 4.17 (1 H, dd, J 9.8, 9.8, C-6-H), 4.38 (1 H, dd, J 2.6, 2.6, C-2-H), 4.57, 4.70 (2 H, AB, J 11.7, CH$_2$Ph), 4.69, 4.87 (2 H, AB, J 11.2, CH$_2$Ph), 5.28 (1 H, dd, J 9.5, 9.5, C-5-H), 5.66 (1 H, dd, J 9.8, 9.8, C-4-H), 7.22-7.33 (10 H, m, CH$_2$Ph)

$\delta_C$ (CDCl$_3$, 100 MHz) 9.60, 16.40, 16.53, 16.64, (4 q, Camph-CH$_3$), 28.75, 28.79, 31.20 (2 t, Camph), 52.14, 53.66, 53.99, 54.65, 54.74 (5 s, Camph), 66.64, 72.84, 73.84, 76.79, 78.47, 81.78 (6 d, 6 inositol ring C), 68.23, 72.84, 73.85 (3 t, CH$_2$COOR and 2 CH$_2$Ph), 90.67, 90.73 (2 s, Camph), 126.96, 127.45, 127.65, 127.93, 128.29, 128.47 (6 d, CH$_2$Ph) 137.18, 138.13 (s, CH$_2$Ph), 166.64, 166.75, 171.56, 177.92, 178.14 (5 s, 5 CO)

24.6 (+)-3,6-Di-O-benzyl-1-O-carboxymethylene-myco-inositol 194

193 (400 mg, 526 μmol) was dissolved in a solution of NaOH (0.5 g) in 50 ml methanol. The mixture was heated under reflux for 30 min. Water was then added and the solution was acidified with hydrochloric acid to a pH of 3. The mixture was extracted with ethyl acetate (2 x 25 ml), the organic fractions were dried (magnesium sulphate) and the solvent was evaporated. Recrystallisation of the crude product from ethyl acetate / ethanol (10:1) gave 194 (197 mg, 473 mmol, 90 %)

m.p. 308-310°C (with a phase transition between 240 and 255°C)
$[\alpha]_D^{23} = + 96^\circ$ (c = 0.1 in DMF)

Found: C, 62.9; H, 6.50. Calc. for C$_{22}$H$_{26}$O$_3$ (418.43): C, 63.15; H, 6.26 %.

Spectroscopical data were identical to 190.
25.1 DL-1-O-Allyl-2-O-benzoyl-3,6-di-O-benzyl-4,5-O-isopropylidene-
scylo-inositol 195

A solution of 160 (6.06 g, 10.6 mmol), potassium benzoate (5.13 g, 32 mmol) and 18-crown-6 (1.32 g, 5 mmol) in DMF (250 ml) was heated at 80°C for 4 h. The mixture was cooled, quenched with water (100 ml) and extracted with ether (2 x 200 ml). The combined organic layers were washed with brine, dried over magnesium sulphate and the solvent was evaporated. Flash chromatography (SiO₂, hexane / ether 3:1) gave 195 (3.9 g, 7.1 mmol, 67 %).

m.p. 141-142°C (ethanol)
Found: C, 72.7; H, 6.62. Calc. for C₃₃H₃₆O₇ (544.64): C, 72.77; H, 6.66 %.

1H (CDCl₃; 270 MHz) 1.48 (6 H, s, 2 CH₃), 3.57-3.81 (5 H, m, 5 C-H), 4.03, 4.23 (2 H, AB, ddd, JAB 12.4, J 5.9, 1.65, 1.65, CH₂CH=CH₂), 4.65, 4.80 (2 H, AB, JAB 12.5, CH₂Ph), 4.74, 4.91 (2 H, AB, JAB 11.7, CH₂Ph), 4.95 (1 H, dq, J 10.8, 1.65, CH₂CH=CH₂), 5.05 (1 H, dq, J 17.2, 1.65, CH₂CH=CH₂), 5.33 (1 H, dd, J 8.9, 8.9, C-2-H), 5.66 (1 H, ddt, J 16.9, 10.6, 5.7, CH₂CH=CH₂), 7.11-7.60 (13 H, m, CH₂Ph and C(0)Ph), 8.02 (2 H, dd, J 7.15, 1.3, C(O)Ph)

δC (CDCl₃; 68 MHz) 26.95 (q, C(CH₃)₂), 71.88, 72.95, 74.08 (3 t, 2 CH₂Ph and CH₂CH=CH₂), 75.35, 76.42, 78.59, 78.88, 79.40, 81.44 (6 d, 6 inositol ring C), 112.55 (s, (CH₃)₂C), 117.06 (t, CH₂CH=CH₂), 127.28, 127.44, 127.63, 128.02, 128.18, 128.25, 129.71 (7 d, CH₂Ph), 132.92 (d, CH₂Ph), 134.54 (d, CH₂CH=CH₂), 137.98, 138.30 (2 s, CH₂Ph), 165.32 (s, C=O);

m/z (+ve ion FAB) 545 [(M + H)+, 7 %], 487 [(M - OCH₂CH=CH₂)+, 15], 91 (100)
m/z (-ve ion FAB) 697 [(M + NBA)-, 100 %], 560 (42), 485 (55), 440 (55), 322 (60), 290 (97), 274 (85)

25.2 DL-1-O-Allyl-2-O-benzoyl-3,6-di-O-benzyl-scylo-inositol 196

195 (990 mg, 1.82 mmol) was heated under reflux in 80 % acetic acid (50 ml) for 30 min after which tlc (ethyl acetate / hexane 1:1) showed complete conversion of the starting material (R₁ 0.64) into a product (R₁ 0.38). The reaction mixture was allowed to cool and diluted with water (50 ml). The solution was stored at -
20°C overnight, the precipitate was collected and recrystallised from ethyl acetate / hexane. Yield 813 mg (1.61 mmol, 89 %).

m.p. 158-159°C (ethyl acetate / hexane)
Found: C, 71.6; H, 6.38. Calc. for C₃₀H₃₂O₇ (504.58): C, 71.41; H, 6.39 %.

δₜ (CDCl₃; 270 MHz) 3.11 (1 H, brd s, D₂O ex, OH), 3.31 (1 H, brd s, D₂O ex, OH), 3.42-3.65 (5 H, m, 5 C-H), 4.06, 4.22 (2 H, AB, ddd, JₜAB 12.3, J 6.0, 1.65, 1.65, CH₂CH=CH₂), 4.67, 4.70 (2 H, AB, J 11.5, CH₂Ph), 4.79, 4.90 (2 H, AB, J 11.2, CH₂Ph), 4.97 (1 H, dq, J 10.3, 1.65, CH₂CH=CH₂), 5.05 (1 H, dq, J 17.2, 1.65, CH₂CH=CH₂), 5.39 (1 H, dd, J 9.3, 9.3, C-2-H), 5.68 (1 H, ddt, J 17.2, 10.4, 5.9, CH₂CH=CH₂), 7.13-7.61 (13 H, m, CH₂Ph and C(O)Ph), 8.04 (2 H, dd, J 7.0, 1.5, C(O)Ph)

δₜ (CDCl₃; 68 MHz) 73.79, 73.92, 74.47, 79.86, 80.31, 81.90 (6 d, 6 inositol ring C), 74.28, 74.73, 75.44 (3 t, 2 CH₂Ph and CH₂CH=CH₂), 117.35 (t, CH₂CH=CH₂), 127.70, 127.89, 128.02, 128.31, 128.44, 128.54, 129.71 (7 d, CH₂Ph and C(O)Ph), 133.11 (d, C(O)Ph), 134.48 (d, CH₂CH=CH₂), 137.82, 138.34 (2 s, CH₂Ph), 165.55 (s, C=O);

m/z (+ve ion FAB) 505 [(M + H)+, 7 %], 413 [(M - CH₂Ph)+, 1], 397 [(M - OCH₂Ph)+, 3], 181 (10), 105 (31), 91 (100)
m/z (-ve ion FAB) 1007 [(2 M - H)-, 19 %], 670 (64), 657 [(M + NBA)-, 100], 550 (41), 503 [(M - H)-, 46]

25.3 DL-2-O-Benzoyl-1,4-di-O-benzyl-scyllo-inositol 197

196 (2.64 g, 5.23 mmol) and DABCO (118 mg, 1.08 mmol) were heated with stirring in a mixture of ethanol, toluene and water (7:3:1, 22 ml). When the reaction mixture had reached reflux temperature, tris(triphenylphosphine)-rhodium(I) chloride (334 mg, 364 μmol) was added and heating under reflux continued for 30 min. Tlc (ethyl acetate / hexane 1:1) showed conversion of the starting material (Rₜ 0.47) into a product (Rₜ 0.53). 1 M HCl (20 ml) was then added and after heating was continued for further 30 min, tlc (ethyl acetate / hexane 1:1) showed a product (Rₜ 0.30). The suspension was cooled, neutralised with aqueous sodium hydrogen carbonate and extracted with ethyl acetate (2 x
Evaporation of the solvent gave 197 (1.72 g, 3.71 mmol, 71 %)
m.p. 226-228°C (from ethyl acetate)
Found: C, 69.9; H, 6.14. Calc. for C_{27}H_{28}O_{7} (464.52): C, 69.81; H, 6.08 %.

δ_{H} (d_{6}-DMSO; 270 MHz) 3.26-3.53 (4 H, m, 4 C-H), 3.63 (1 H, ddd, J 9.3, 9.3, 6.0, D_{2}O ex gives dd, J 9.3, 9.3, C-H), 4.54, 4.82 (2 H, AB, J_{AB} 11.5, CH_{2}Ph), 4.84 (2 H, AB, CH_{2}Ph), 5.10 (1 H, dd, J 9.7, 9.7, C-2-H), 5.14 (1 H, brd s, D_{2}O ex, OH), 5.31 (1 H, brd s, D_{2}O ex, OH), 5.38 (1 H, d, J 6.0, D_{2}O ex, OH), 7.09-7.67 (13 H, m, Ph), 8.00 (2 H, dd, J 7.1, 1.5, Ph)

δ_{C} (d_{6}-DMSO; 68 MHz) 71.73, 74.00, 74.42, 75.52, 80.36, 82.76 (6 d, 6 inositol ring C), 73.72, 73.92 (2 t, 2 CH_{2}Ph), 127.14, 127.18, 127.44, 127.69, 127.91, 127.99, 128.62, 129.40 (8 d, CH_{2}Ph and C(O)Ph), 130.50 (s, C(O)Ph), 133.10 (d, C(O)Ph), 138.88, 139.68 (2 s, CH_{2}Ph), 165.45 (s, C=O);

m/z (+ve ion FAB) 462 [(M - 2 H)^{+}, 4 %], 413 (13)
m/z (-ve ion FAB) 355 (100 %), 342, 235

25.4 Elimination

160 (5.7 g, 10 mmol) was dissolved in dry dichloromethane (100 ml) and 1,8-diazabicyclo[5.4.0]undec-7-ene (1.52 g, 1.49 ml, 10 mmol) was added to this solution. The reaction mixture was stirred for 24 h after which tlc (petrol ether b.p. 60-80°C / ether 2:1) showed complete conversion of the starting material (R_{f} 0.35) into two major products (198, R_{f} 0.54 and 199, R_{f} 0.46). The solvent was evaporated and the residue separated by flash chromatography (petrol ether b.p. 40-60°C / ether 4:1) to give 198 (1.52 g, 3.6 mmol, 36 %) and 199 (1.39 g, 3.3 mmol, 33 %).

198:

m.p. 39-41°C (from ethanol)
Found: C, 74.0; H, 7.18. Calc. for C_{26}H_{30}O_{5} (422.52): C, 73.91; H, 7.16 %.

δ_{H} (CDCl_{3}; 270 MHz) 1.46 (6 H, s, 2 CH_{3}), 3.63 (1 H, dd, J 9.9, 7.7, C-H), 3.73 (1 H, dd, J 9.7, 8.2, C-H), 4.22-4.31 (4 H, m, CH_{2}CH=CH_{2} and 2 C-H), 4.63 (1 H, s,
C-H), 4.65, 4.83 (2 H, AB, J 11.7, CH2Ph), 4.81, 4.87 (2 H, AB, J 11.7, CH2Ph),
5.22 (1 H, dq, J 10.4, 1.5, CH2CH=CH2), 5.33 (1 H, dq, J 17.2, 1.5,
CH2CH=CH2), 5.96 (1 H, ddt, J17.2, 10.4, 5.3, CH2CH=CH2), 7.24-7.43 (10 H,
m, CH2Ph)

δc (CDCl3; 68 MHz) 27.02 (q, C(CH3)2), 68.79, 71.45, 72.82 (3 t, 2 CH2Ph and
CH2CH=CH2), 75.41, 77.23, 79.04, 80.11, 98.34 (5 d, 5 inositol ring C), 111.71
(s, (CH3)2O), 117.45 (t, CH2CH=CH2), 127.37, 127.47, 127.73, 128.09, 128.25 (5
d, CH2Ph), 132.63 (d, CH2CH=CH2), 138.37 (s, CH2Ph), 154.55 (s, inositol ring
C);

m/z (+ve ion FAB) 421 [(M - H)+, 0.7 %], 365 [(M - OCH2CH=CH2)+, 0.6], 315 [(M
-OCH2Ph)+, 1.8], 131 (20), 91 (100)

m/z (-ve ion FAB) 575 [(M + NBA)-, 20 %], 437 (31), 381 [(M - CH2CH=CH2)-,
10], 273 (37), 215 (50), 124 (60), 109 (100)

199:

m.p. 71-73°C (from ethanol)

Found: C, 73.7; H, 7.02. Calc. for C26H30O5 (422.52): C, 73.91; H, 7.16 %.

δH (CDCl3; 270 MHz) 1.49, 1.50 (6 H, 2 s, 2 CH3), 3.78 (1 H, dd, J 10.5, 8.5, C-
H), 3.92 (1 H, dd, J 10.6, 5.0, C-H), 3.96 (2 H, ddd, J 5.7, 1.5, 1.5, CH2-
CH=CH2), 4.27-4.33 (2 H, m, 2 C-H), 4.58 (1 H, q, J 1.8, C-H)

4.75, 4.90 (2 H, AB, JAB 11.9, CH2Ph), 4.86, 4.91 (2 H, AB, JAB 12.1, CH2Ph),
5.14 (1 H, dq, J 10.3, 1.65, CH2CH=CH2), 5.21 (1 H, dq, J 17.2, 1.65,
CH2CH=CH2), 5.84 (1 H, ddt, J 17.1, 10.4, 5.7, CH2CH=CH2), 7.24-7.41 (10 H,
m, CH2Ph);

δc (CDCl3; 68 MHz) 26.66 (q, C(CH3)2), 69.96, 72.14 (2 t, CH2Ph and
CH2CH=CH2), 74.02, 80.86, 81.23, 81.54 (4 d, 4 inositol ring C), 94.81 (d, C-2),
112.32 (8, (CH3)2O), 116.90 (t, CH2CH=CH2), 127.43, 127.53, 127.76, 127.92,
128.18, 128.44 (6 d, CH2Ph), 134.77 (d, CH2CH=CH2), 135.94 (s, CH2Ph),
138.30 (s, CH2Ph), 153.84 (s, C-3);

m/z (+ve ion FAB) 423 [(M + H)+, 1 %], 365 [(M - OCH2CH=CH2)+, 1], 91 (100)

m/z (-ve ion FAB) 422 (M-, 2 %), 149 (100), 133 (75)
25.5 DL-2-Deoxy-2-C-allyl-3,6-di-O-benzyl-4,5-O-isopropylidene-myco-1-inosose 200 and DL-2-Deoxy-2-C-allyl-3,6-di-O-benzyl-4,5-O-isopropylidene-scyllio-inosose 201

198 (200 mg, 473 μmol) was heated at 200°C for 15 min. After cooling, tlc (petrol ether b.p. 60-80°C / ether 2:1) showed complete conversion of the starting material (Rf 0.54) into what appeared to be a single product (Rf 0.45). Yield 200 mg (100%).

\[ \delta_H (\text{CDCl}_3; 270 \text{ MHz}) 1.48, 1.49, 1.51, 1.53 (12 \text{ H, 4 s, 4 CH}_3), 2.14-2.97 (6 \text{ H, m, } \text{CH}_2\text{CH}=\text{CH}_2, \text{C-1-H}), 3.51-4.19 (8 \text{ H, m, C-3,4,5,6-H}), 4.55-5.03 (12 \text{ H, m, 4 CH}_2\text{Ph, CH}_2\text{CH}=\text{CH}_2), 5.54-5.82 (2 \text{ H, m, CH}_2\text{CH}=\text{CH}_2), 7.24-7.63 (20 \text{ H, m, CH}_2\text{Ph}) \]

\[ \delta_C (\text{CDCl}_3; 68 \text{ MHz}) 26.99 (q, \text{CH}_3), 29.03, 30.36 (2 \text{ t, CH}_2\text{CH}=\text{CH}_2), 52.67, 52.97 (2 \text{ d, C-1}), 71.71, 72.20, 72.36, 72.62 (4 \text{ t, CH}_2\text{Ph}), 74.34, 75.18, 77.29, 78.65, 80.44, 81.57, 82.32 (7 \text{ d, inositol ring C}), 112.68, 112.74 (2 \text{ s, C(CH}_3)_2), 117.25, 117.54 (2 \text{ t, CH}_2\text{CH}=\text{CH}_2), 127.57, 127.73, 127.99, 128.28, 128.35 (5 \text{ d, CH}_2\text{Ph}), 134.57, 135.22 (2 \text{ d, CH}_2\text{CH}=\text{CH}_2), 137.20, 137.36, 137.65, 137.98 (4 \text{ s, CH}_2\text{Ph}), 203.69, 205.31 (2 \text{ s, C=O}) \]

\[ m/z (+ \text{ive ion FAB}) 423 [(\text{M+H})^+, 8 \text{ %}], 181 (9), 91 (100) \]

\[ \nu_{\text{max}} \text{ cm}^{-1} (\text{KBr disk}) 1715 (\text{C=O}) \]

25.6 DL-1-O-Allyl-3,6-di-O-benzyl-2-deoxy-2-fluoro-4,5-O-isopropylidene-scyllio-inositol 202

Tetrabutylammonium fluoride (3 ml of a 1.1 M solution in THF) was added to 160 (1.11 g, 1.95 mmol) in THF (20 ml) at r.t. The colour of the reaction mixture immediately turned to red / brown. Stirring was continued for a further 30 min, the solvent was evaporated and the brown residue taken up in chloroform. After washing with water, the organic phase was dried over magnesium sulfate, evaporated and the crude product purified by flash chromatography (hexane / ether 3:1) to give 202 (595 mg, 1.34 mmol, 69 %) and 199 (60 mg, 0.14 mmol, 7 %).
m.p. 111-112°C (from hexane)
Found: C, 70.4; H, 7.04. Calc. for C_{26}H_{31}O_5F (442.53): C, 70.57; H, 7.06 %.

δ_H (CDCl_3; 270 MHz) 1.42 (s, 6 H, 2 CH_3), 3.41-3.63 (4 H, m, 4 C-H), 3.71 (1 H, ddd, J 12.8, 9.7, 7.9, C-1-H or C-3-H), 4.24 (2 H, ddd, J 5.9, 1.5, 1.5, CH_2-CH=CH_2), 4.50 (1 H, dt, J 49.3, 7.9, C-2-H), 4.70, 4.85 (2 H, AB, J_AB 11.7, CH_2Ph), 4.74, 4.82 (2 H, AB, J_AB 11.9, CH_2Ph), 5.13 (1 H, ddt, J 10.3, 1.2, 1.2, CH_2-CH=CH_2), 5.23 (1 H, ddt, J 17.2, 1.5, 1.5, CH_2-CH=CH_2), 5.89 (1 H, ddt, J 16.9, 10.5, 5.9, CH_2-CH=CH_2), 7.23-7.39 (10 H, m, CH_2Ph);

δ_C (CDCl_3; 68 MHz) 27.64 (q, C(CH_3)_2), 73.37, 73.82, 74.96 (3 t, 2 CH_2Ph and CH_2CH=CH_2), 77.75, 77.85, 78.07 (3 d), 79.22 [dd, J_C-C-F 15.4, C-1(3)], 82.99 [dd, J_C-C-F 17.6, 6 CH_2Ph), 98.33 (dd, J_C-F 185.1, C-2), 113.52 (s, C(CH_3)_2), 117.84 (t, CH_2CH=CH_2), 122.85, 128.31, 128.38, 128.44, 128.51, 129.00 (6 d, CH_2Ph), 135.48 (d, CH_2CH=CH_2), 138.73, 139.02 (2 s, CH_2Ph),

δ_F (CDCl_3 with reference to CFCI_3; 84 MHz) 193.9 (dd, J 51.3, 14.7)

m/z (+ve ion FAB) 443 [(M + H)^+], 181 (8), 91 (100)

m/z (-ve ion FAB) 595 [(M + NBA)^-], 50 %], 442 (M^+, 29), 182 (72), 167 (100), 93 (85)

The minor product 199 was identical to 199 obtained by the DBU elimination reaction performed on 160.
References


209-215.


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386. B. Kohne, K. Praefcke, G. Mann, Chimia, 1988, 42, 139-141.


