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

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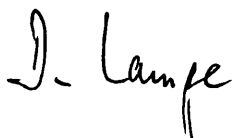

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ABSTRACT

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 sulphoxidation of the phosphite furnished the fully protected 1-phosphorothioate analogue of Ins(1,4,5)P_3. Deblocking gave DL-*myo*-inositol 4,5-bisphosphate 1-phosphorothioate [Ins(1,4,5)P_3-1S].

A fluorescent chromophore was selectively attached to the 1-position of this Ins(1,4,5)P_3 analogue. The precursor DL-2,3,6-tri-*O*-benzyl-4,5-bis-[di-(2-cyanoethoxy)phospho]-*myo*-inositol was used to synthesise [^{35}S]-labelled Ins(1,4,5)P_3-1S 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 (-)-(ω)-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 Ca^{2+}-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, sulphoxidised and de-protected 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)P_3 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-*scyllo*-inositol. Successive removal of protecting groups gave 1-*O*-allyl-3,6-di-*O*-benzyl-*scyllo*-inositol and 1,4-di-*O*-benzyl-*scyllo*-inositol which were phosphorylated and deblocked yielding *scyllo*-inositol 1,4,5-trisphosphate and *scyllo*-inositol 1,2,4,5-tetrakisphosphate, respectively. These compounds were highly potent agonists in Ca^{2+}-mobilisation. Thiophosphorylation and deprotection of 1,4-di-*O*-benzyl-*scyllo*-inositol gave *scyllo*-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-*scyllo*-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-[(-)-ω-camphanoyl]-1-*O*-*p*-methoxybenzyl-*myo*-inositol were synthesised and separated by crystallisation.

Finally, methods to substitute the Ins(1,4,5)P_3 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.

Dedication

Diese Arbeit ist meinen Eltern in Dankbarkeit gewidmet.

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Publications

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

- Dethard Lampe and Barry V L Potter "Synthesis of *myo*-inositol 1-phosphorothioate 4,5-bisphosphate: Preparation of a fluorescently labelled *myo*-inositol 1,4,5-trisphosphate analogue", J. Chem. Soc., Chem. Commun. 1990, 1500-1501.

- Dethard Lampe, Stephen J Mills and Barry V L Potter "Total Synthesis of the Second Messenger Analogue D-*myo*-Inositol 1-Phosphorothioate 4,5-Bisphosphate: Optical Resolution of DL-1-*O*-Allyl-2,3,6-Tri-*O*-Benzyl-*myo*-inositol and Fluorescent Labelling of *myo*-Inositol 1,4,5-Trisphosphate", J. Chem. Soc. Perkin Trans. 1, 1992, 2899-2906.

- Dethard Lampe and Barry V L Potter "Synthesis of 2-Fluoro-2-Deoxy-*myo*-Inositol 1,4,5-Trisphosphate and *scyllo*-Inositol 1,2,4-Trisphosphate, Novel Analogues of the Second Messenger *myo*-Inositol 1,4,5-Trisphosphate", Tetrahedron Lett., 1993, *34*, 2365-2368.

- Dethard Lampe, Changsheng Liu and Barry V L Potter "Synthesis of Novel Inositol Polyphosphate Analogues Modified at the 1-Position", Phosphorus, Sulfur, and Silicon, 1993, *77*, 317.

Abbreviations

All	allyl
ADP	adenosine 5´-diphosphate
AMP	adenosine 5´-monophosphate
ATP	adenosine 5´-triphosphate
Bn	benzyl
Bn-OMe	*p*-methoxybenzyl
Bu^tOK	potassium *tert*-butoxide
Bu^tOOH	*tert*-butyl hydroperoxide
Bz	benzoyl
cAMP	adenosine 3´,5´-cyclic monophosphate
Camph	camphanate ester
COSY	correlated spectroscopy
mCPBA	*meta*-chloroperbenzoic acid
DABCO	1,4 diazabicyclo[2.2.2]octane
DAG	1,2-diacylglycerol
DAST	diethylaminosulphur trifluoride
DMAP	4-(dimethylamino)pyridine
DMSO	dimethylsulphoxide
DMF	dimethylformamide
DNA	deoxyribonucleic acid
E.C.	Enzyme Catalogue
GPI	glycosyl-phosphatidylinositol
GPI-PLC	GPI-specific PLC
G-protein	GTP-binding protein
GroPIns(4,5)P_2	glycerophospho-*myo*-inositol 4,5-bisphosphate
GTP	guanosine 5´-triphosphate

HPLC	high performance liquid chromatography
IANBD	*N*-[{2-(iodoacetoxy)ethyl}-*N*-methyl]amino-7-nitro-2,1,3-benzoxadiazole
$Ins(1,4,5)P_3$	1D-*myo*-inositol-1,4,5-trisphosphate
$Ins(1,3,4,5)P_4$	1D-*myo*-inositol-1,3,4,5-tetrakisphosphate
$Ins(1,4,5)P_3$-1S	1D-*myo*-inositol-4,5-bisphosphate-1-phosphorothioate
$Ins(1,4,5)PS_3$	1D-*myo*-inositol-1,4,5-trisphosphorothioate
IR	infra-red
MEM	2-methoxyethoxymethyl
NMR	nuclear magnetic resonance
Ph	phenyl
PIM	putative insulin mediator
PLC	phospholipase C
PMB	*para*-methoxybenzyl
PKC	protein kinase C
PtdIns	phosphatidylinositol
$PtdIns(4,5)P_2$	phosphatidylinositol-4,5-bisphosphate
PtdIns-PLC	phosphatidylinositol-specific PLC
PTSA	*para*-toluenesulphonic acid monohydrate
RNA	ribonucleic acid
TBPP	tetrabenzyl pyrophosphate
TEAB	triethylammonium bicarbonate
TIPS	1,1,3,3-tetraisopropyldisiloxane
tlc	thin layer chromatography
TPA	12-*O*-tetradecanoylphorbol-13-acetate
UV	ultra-violet
VSG	variant surface glycoprotein

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GENERAL SECTION

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].

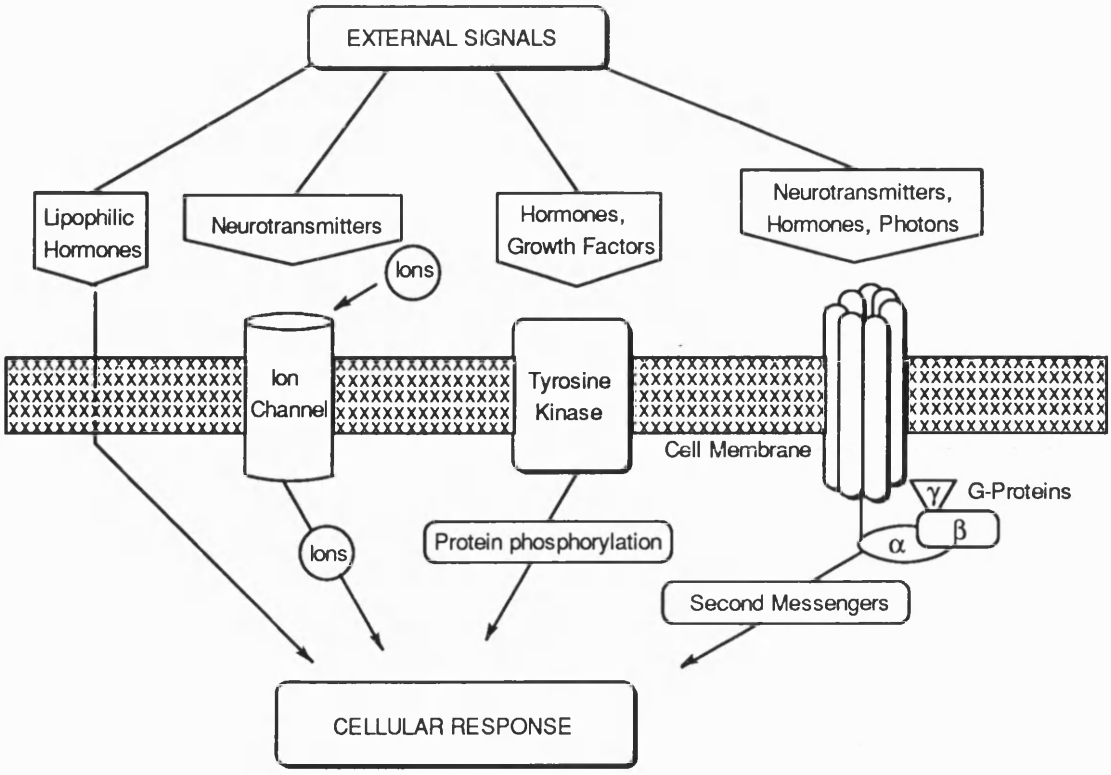


Figure 1.1 Getting the message across the cell membrane

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 α, β and γ in order of decreasing mass. On activation, guanosine diphosphate (GDP) bound to the α-subunit is replaced by guanosine triphosphate (GTP) and the $\beta\gamma$ subunit dissociates from the activated α-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)P_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 α subunit then hydrolyses GTP to GDP, the α-

GDP subunit recombines with the βγ subunit and the G-protein returns to its basal state.

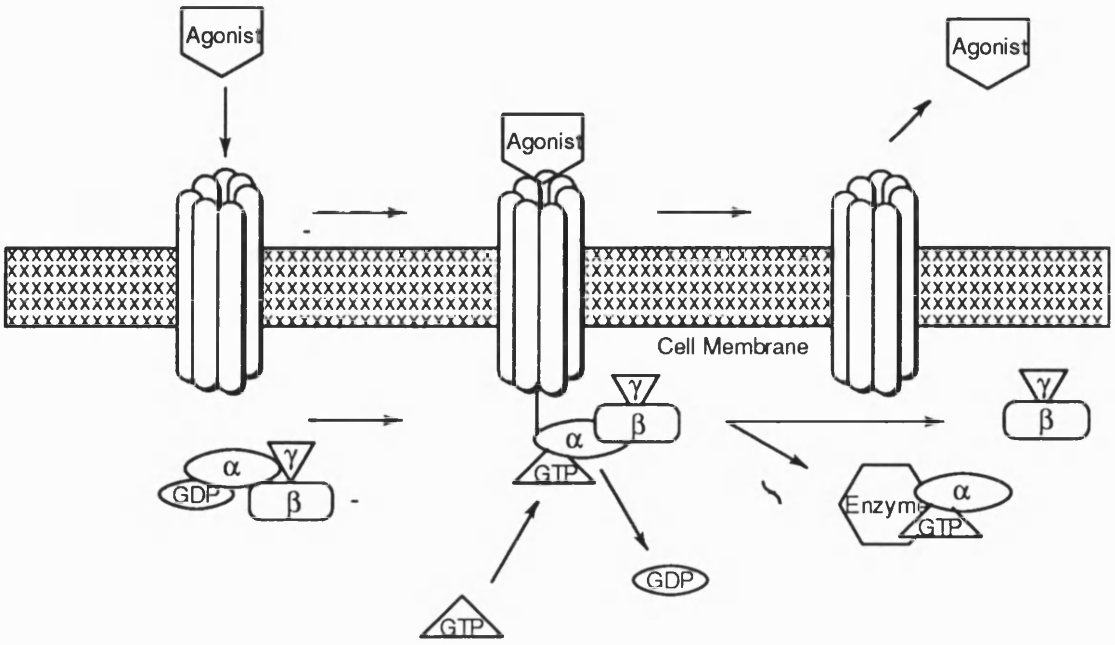


Figure 1.2 GTP-binding proteins in signal transduction

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)P_3] (in combination with Ca^{2+} 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 tranduction mechanism mediated by G-protein coupled receptors can be summarised as:

external signal (hormone, neurotransmitter)
↓
receptor
↓
transducer (G-protein)
↓
amplifier (*e.g.* adenylate cyclase, guanylate cyclase, PtdIns-PLC)
↓
second messenger (cAMP, cGMP, DAG and Ins(1,4,5)P_3)
↓
intracellular effector
↓
cellular response (growth, secretion, etc.)

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 Ca^{2+} 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) [6,7].
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_2C_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 R_2C_2 complex into a R_2 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 specifities for cAMP *versus* cGMP exist).

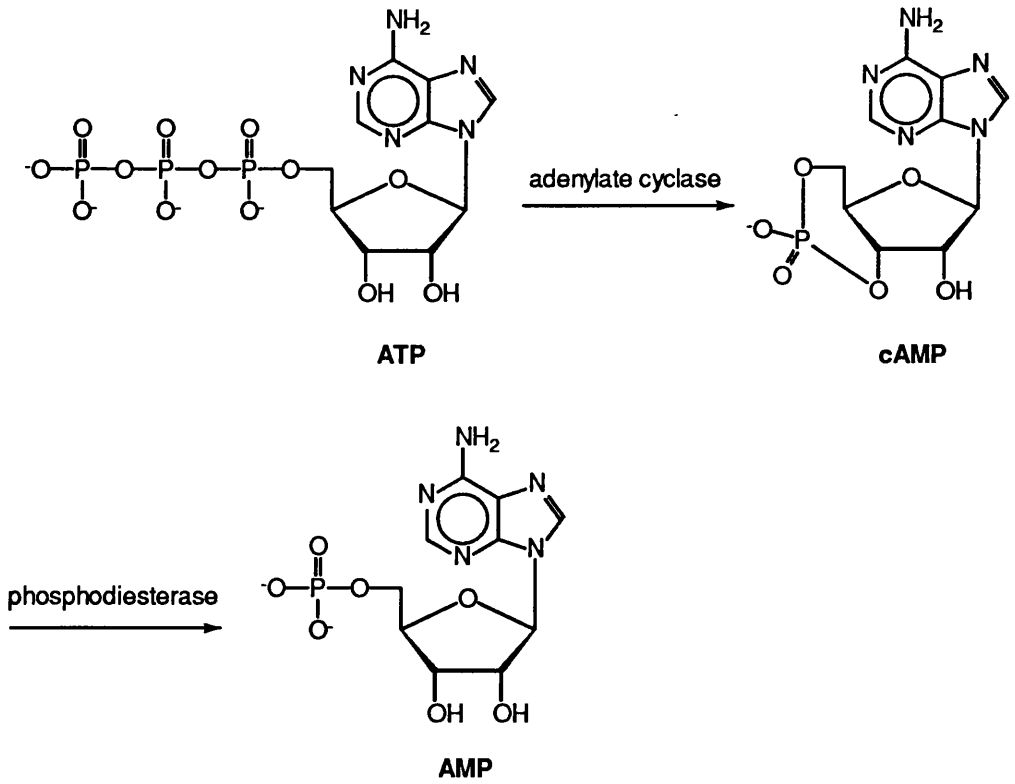
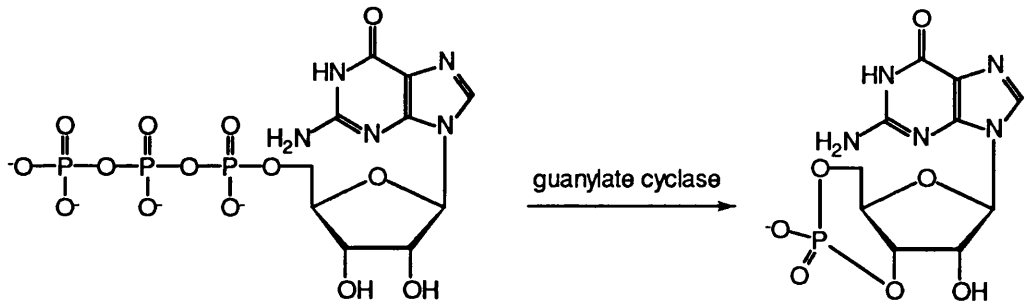


Figure 1.3 Formation and deactivation of cAMP

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[1,9]. In contrast to cAMP, whose levels are low before activation of the signal tranduction 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 [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.



GTP cGMP

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].

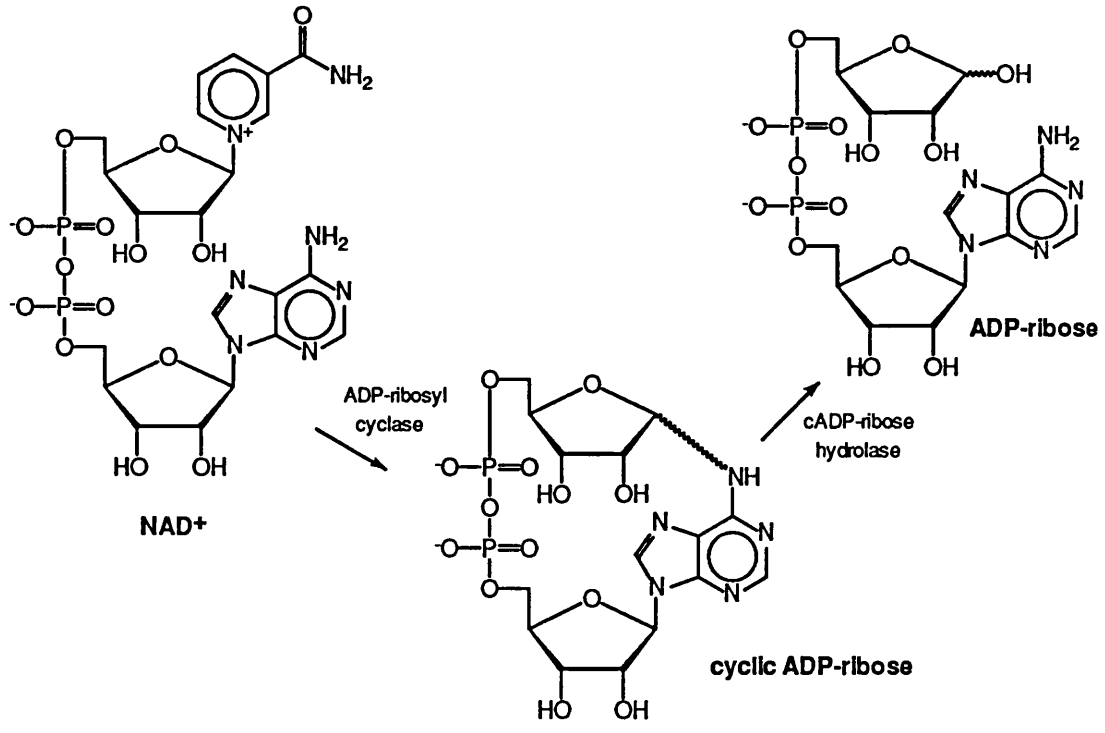
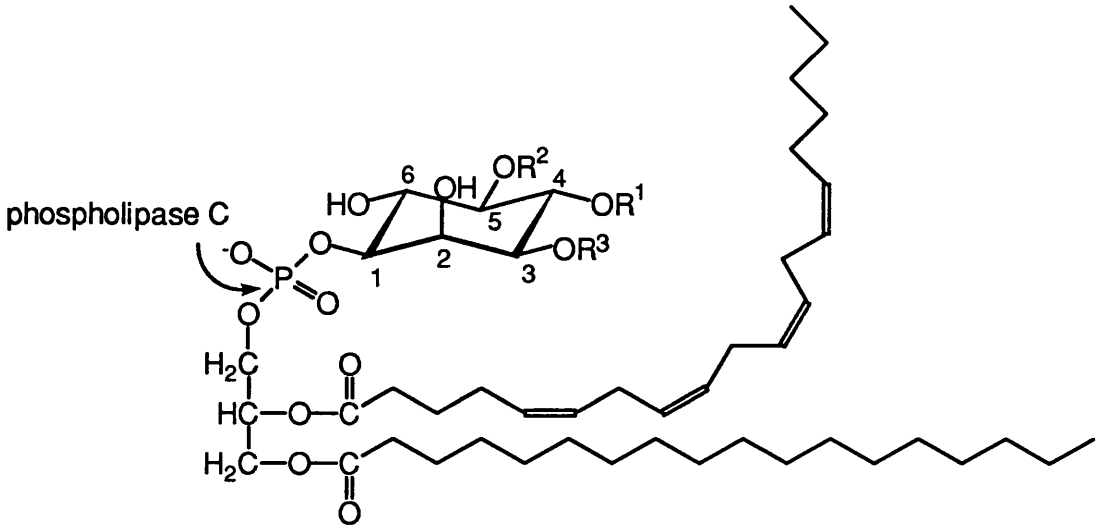


Figure 1.5 Formation and deactivation of cADP-ribose

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)P_2] **3**. Recently, the presence of phosphatidylinositol 3-phosphate [PtdIns(3)P][15] **4** and phosphatidylinositol 3,4,5-trisphosphate [PtdIns(3,4,5)P_3][16] **5** has also been demonstrated. Together, these inositol phospholipids constitute less than 10 % of the total phospholipid of animal cells.



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)P_2

4: $R^1 = R^2 = H$, $R^3 = PO_3^{2-}$ PtdIns(3)P

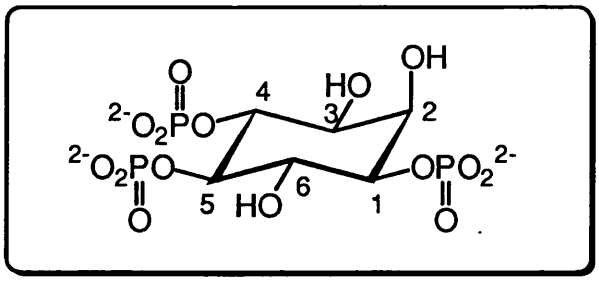
5: $R^1 = R^2 = R^3 = PO_3^{2-}$ PtdIns(3,4,5)P_3

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 $^{32}P_i$ 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-*myo*-inositol 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].



D-*myo*-Inositol 1,4,5-trisphosphate **6**

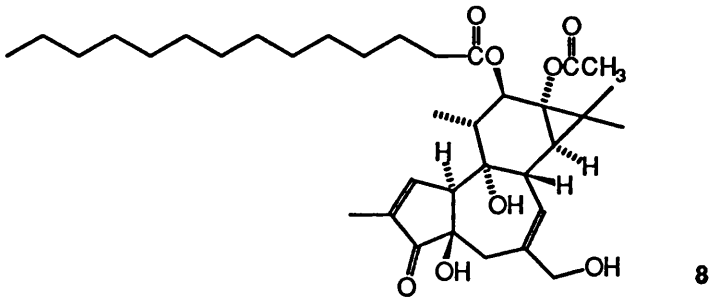
Diacylglycerol

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.

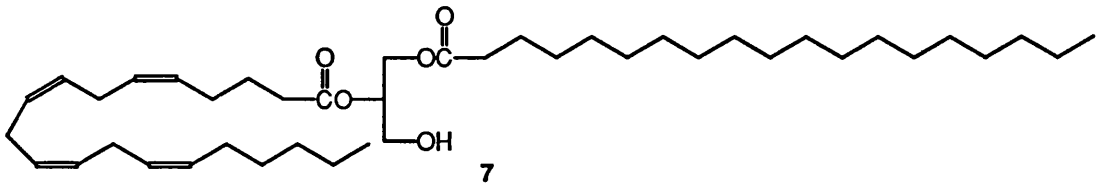
Protein kinase C [23,24], of which there are several subspecies [25], was found to be highly stereospecific [26]. For activation, the diacylglycerol has to be in the 1,2-*sn*-configuration (*sn* stands for *s*tereospecific *n*umbering [27]), both 2,3-*sn*- and 1,3-DAG are inactive. It also seems to be 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) **8**, with molecular structures similar to DAG, can activate PKC both *in vitro* and *in vivo* [28].



8

12-*O*-Tetradecanoylphorbol 13-Acetate (TPA)



7

1,2-Diacylglycerol (DAG)

Figure 1.7 Diacylglycerol and Phorbol ester

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 promotors 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 A_2, giving monoacylglycerol and the eicosanoid precursor arachidonic acid, which is used in prostaglandin synthesis [29].

1.4 The Ins(1,4,5)P_3 Receptor

The Ca^{2+} mobilising property of Ins(1,4,5)P_3 is mediated by an endoplasmic reticular receptor specific for D-Ins(1,4,5)P_3. Specific binding sites for Ins(1,4,5)P_3 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)P_3 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)P_3 is required to effect Ca^{2+} 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 [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 [39], seven [36] or eight [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 [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 [40-43]. Binding to the purified receptor protein was found to be unaffected by calcium [34], suggesting the involvement of a distinct calcium binding protein conferring calcium sensitivity to the receptor [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 [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 [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 [46] and chronic ethanol consumption [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-cyclohexanehexol 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.

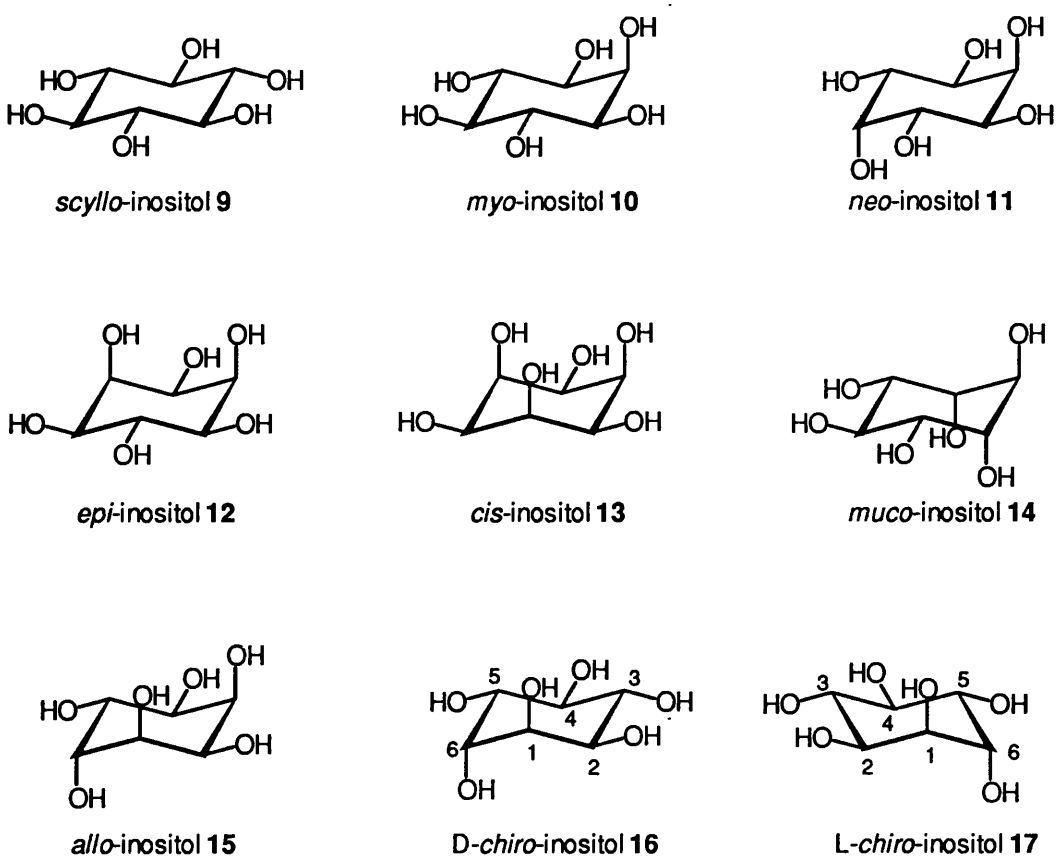


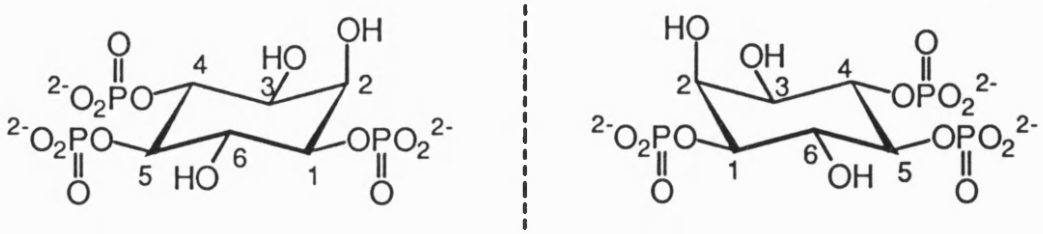
Figure 1.8 The nine inositol stereoisomers

The IUPAC commission has issued rules for the nomenclature of cyclitols [48], the most important of which are summarised here:

- the *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-*myo*-inositol),

- 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 ester 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-*myo*-inositol 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-*myo*-inositol 1-phosphate, respectively.



D-*myo*-Ins(1,4,5)P_3 **L-*myo*-Ins(1,4,5)P_3**

Figure 1.9 The enantiomers of Ins(1,4,5)P_3

Recent recommendations for the numbering of atoms in *myo*-inositol [49] propose that, as all naturally occuring 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, *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. *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].

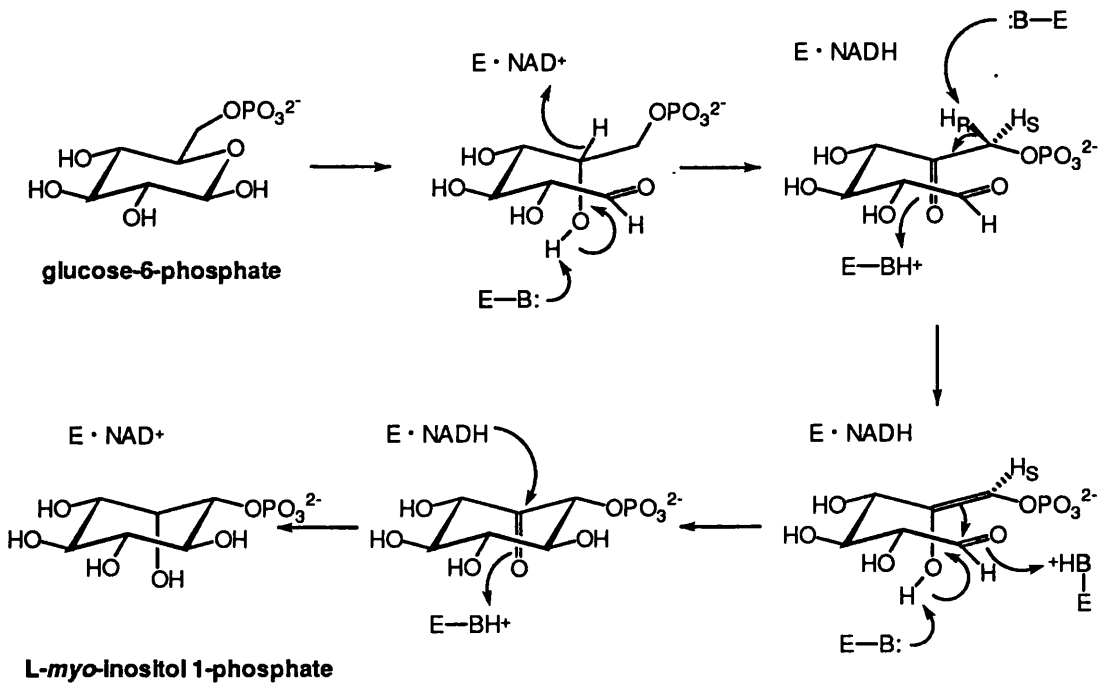


Figure 1.10 Biosynthesis of L-*myo*-inositol 1-phosphate

L-Ins(1)P is then hydrolysed to free *myo*-inositol by the enzyme inositol monophosphatase.

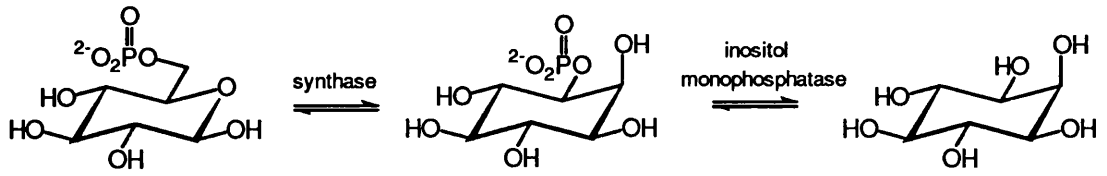


Figure 1.11 *De novo* synthesis of *myo*-inositol

1.6.2 Inositol monophosphatase

Inositol monophosphatase (E.C. 3.1.3.25) [54-57] not only catalyses the dephosphorylation of D- and L-*myo*-inositol 1-phosphate but also D- and L-*myo*-inositol 4-phosphate and *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.

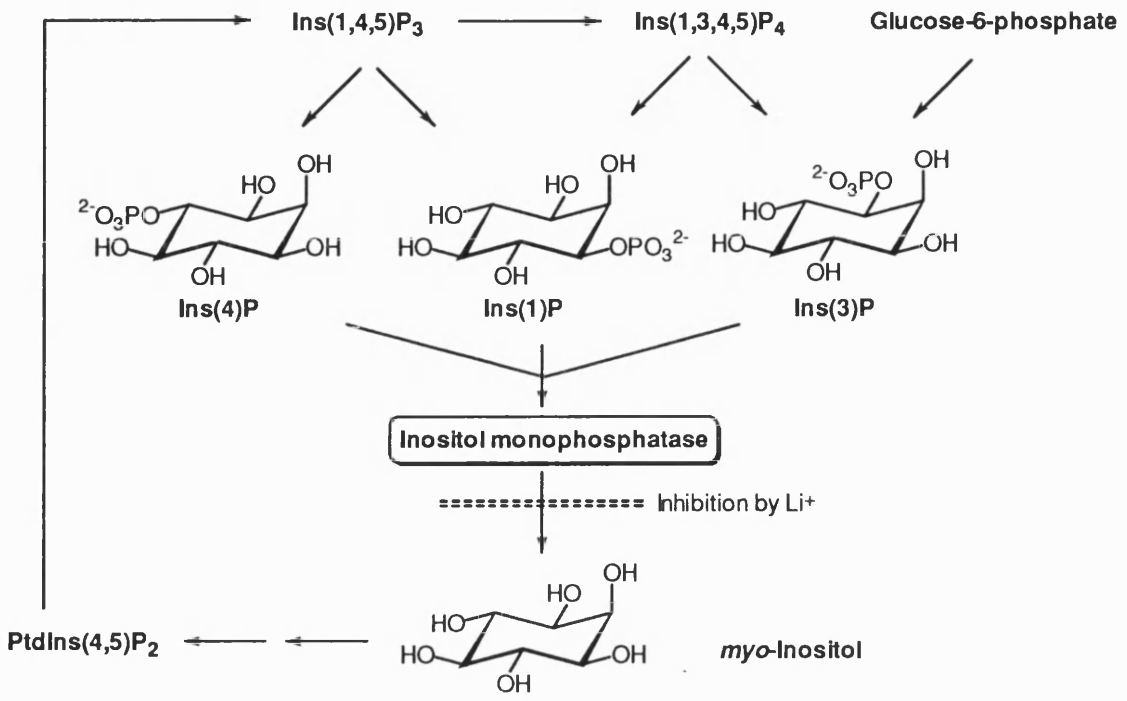


Figure 1.12 Inositol monophosphatase, a key enzyme in the inositol phosphate cycle

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)P_2-phosphatase [62] and Ins(1,3,4)P_3-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 [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 α-hydroxyl groups in the 2- and 6-position play very different roles in the hydrolysis of Ins(1)P by inositol monophosphatase. The 2-hydroxyl group is important for recognition of the substrate by the enzyme [2-deoxy-Ins(1)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-Ins(1)P **21** was synthesised and found to be a potent inhibitor [68], indicating that the 3- and 5-hydroxyl groups are not necessary for enzyme recognition.

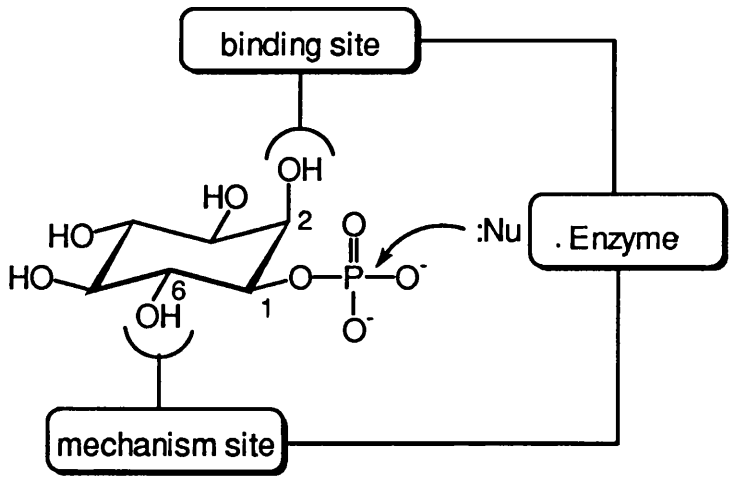


Figure 1.13 Inositol monophosphatase: enzyme-substrate interaction

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)[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-methylenephosphonate 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

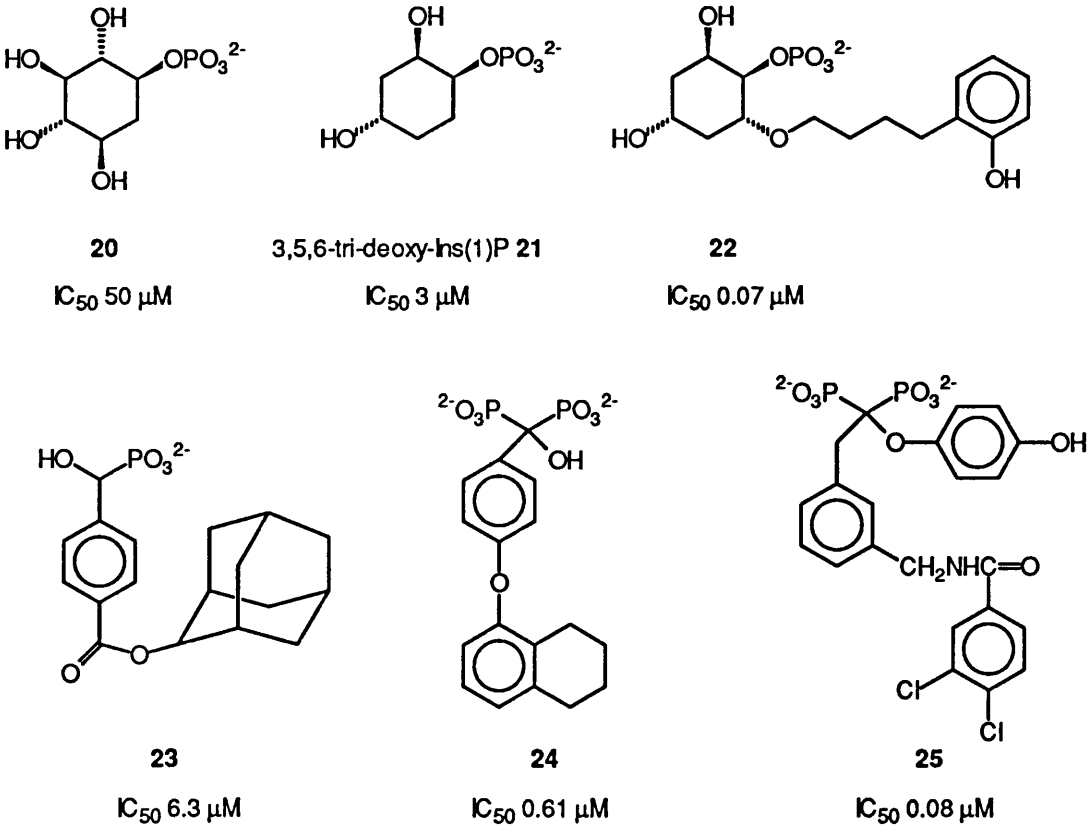


Figure 1.14 Inhibitors of Inositol Monophosphatase

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)P_3: 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 PtdIns synthetase (E.C. 2.7.8.11) can then fuse CMP-phosphatidate and *myo*-inositol to form PtdIns **1**, releasing CMP in the process. PtdIns is phosphorylated stepwise by specific kinases first to PtdIns(4)P **2** and then to PtdIns(4,5)P_2, which can be used once more for signalling.

Once Ins(1,4,5)P_3 has mobilised Ca^{2+} 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)P_3: a 3-kinase and a 5-phosphatase, producing Ins(1,3,4,5)P_4 and Ins(1,4)P_2, respectively.

1.6.4 Ins(1,4,5)P_3 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 specifities with respect to Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4. Type 1 5-phosphatase, a 60 kDa protein, has apparent K_m values of 3 and 0.8 µM for Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4, respectively, and hydrolyses Ins(1,4,5)P_3 *ca.* 12 times faster than Ins(1,3,4,5)P_4. The substrate of the type 2 phosphatase, a 160 kDa protein, seems to be primarily Ins(1,4,5)P_3 (K_m 18 µM), the K_m of this enzyme for Ins(1,3,4,5)P_4 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 Ins(1,4,5)P_3 / Ins(1,3,4,5)P_4 5-phosphatase is the enzyme most affected [77].

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 [78]. Ins(1,4)P_2 is then further dephosphorylated to Ins(1)P and/or Ins(4)P and finally to inositol which is used for the re-synthesis of phosphoinositides.

1.6.5 Ins(1,4,5)P_3 3-kinase

The existance of a second pathway of Ins(1,4,5)P_3 metabolism was first suggested by the discovery of Ins(1,3,4)P_3 [79], which was found to be derived from Ins(1,3,4,5)P_4 via a 5-dephosphorylation. The source of 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 Ins(1,4,5)P_3 3-kinase activity was then demonstrated in various tissues [80] and the enzyme has been purified from rat brain [81]. 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 Ca^{2+}, which is intriguing since it suggests that the Ins(1,4,5)P_3-induced rise of free cytosolic calcium acts as a regulator to promote the formation of Ins(1,3,4,5)P_4 at the expense of Ins(1,4,5)P_3.

The physiological significance of Ins(1,3,4,5)P_4 [82] is not quite clear yet. Binding sites for this molecule as distinct from those for Ins(1,4,5)P_3 have been identified in a variety of tissues [83-86]. In contrast to the Ins(1,4,5)P_3 binding sites they seem to be associated with plasma membranes rather than the ER membrane [87]. Suggestions [88,89] 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.

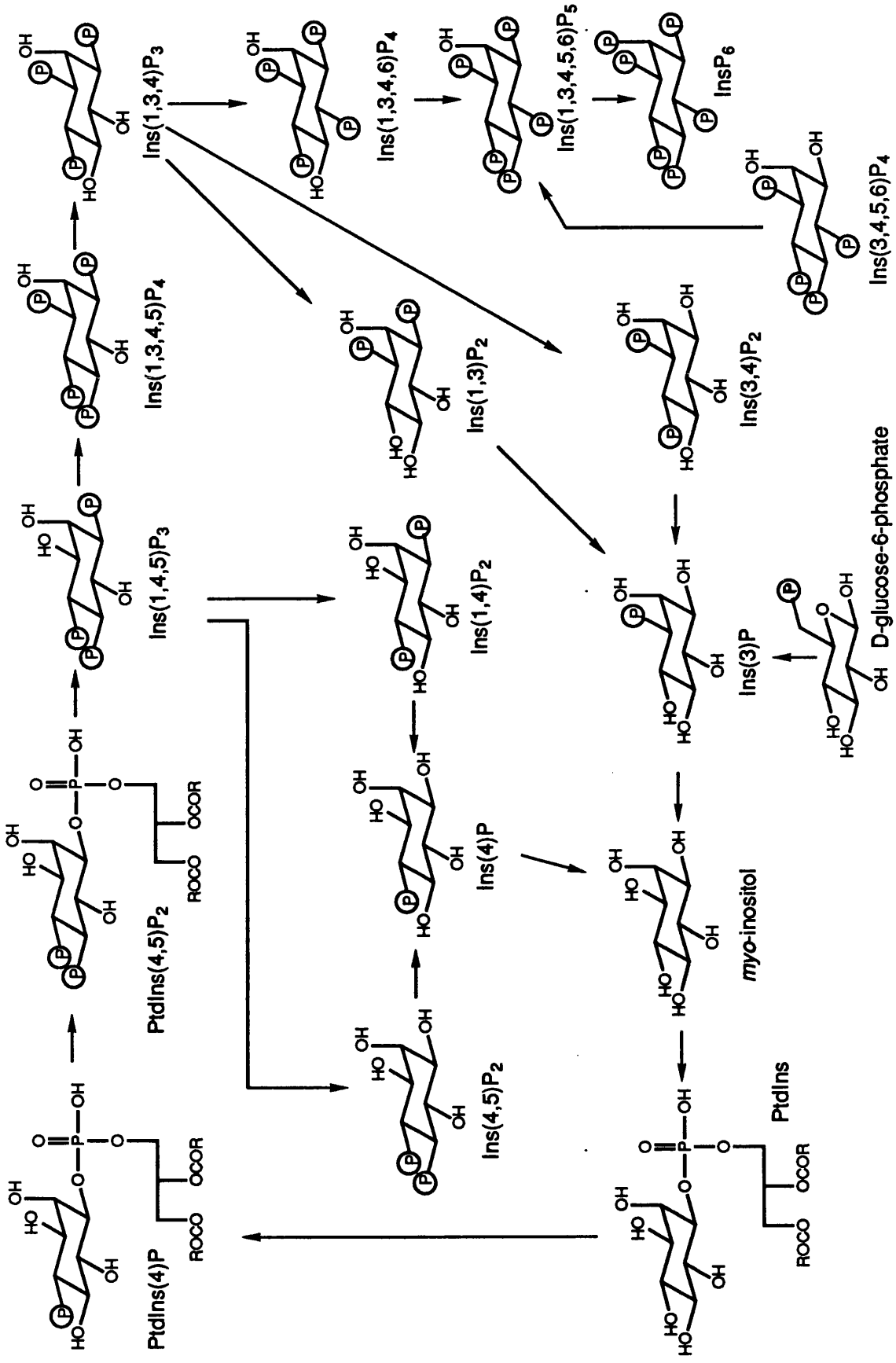


Figure 1.15 The Inositol Phosphate Cycle

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 *in vitro* [113].

The discovery of GPI anchors and putative insulin mediators has attracted the interest of bioorganic chemists [114-118].

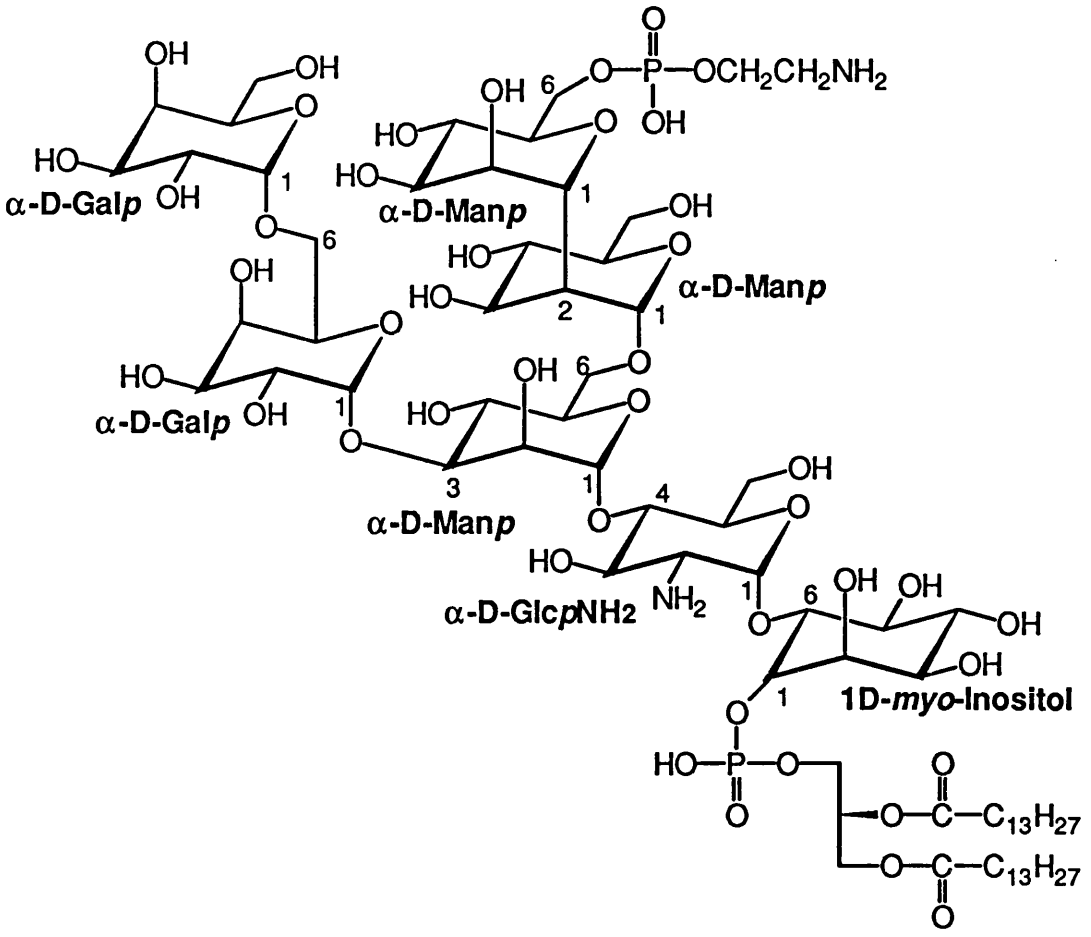


Figure 1.17 Structure of the GPI anchor of the variant surface glycoprotein of *Trypanosoma brucei*

Efforts to synthesise the GPI anchor of the variant surface glycoprotein (VSG) of the parasitic protozoan *Trypanosoma brucei* [119] (Figure 1.17) have recently been successful [116]. 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)P_3 in 1983. Two review articles [122,123] and two books [124,125] summarise current developments 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.

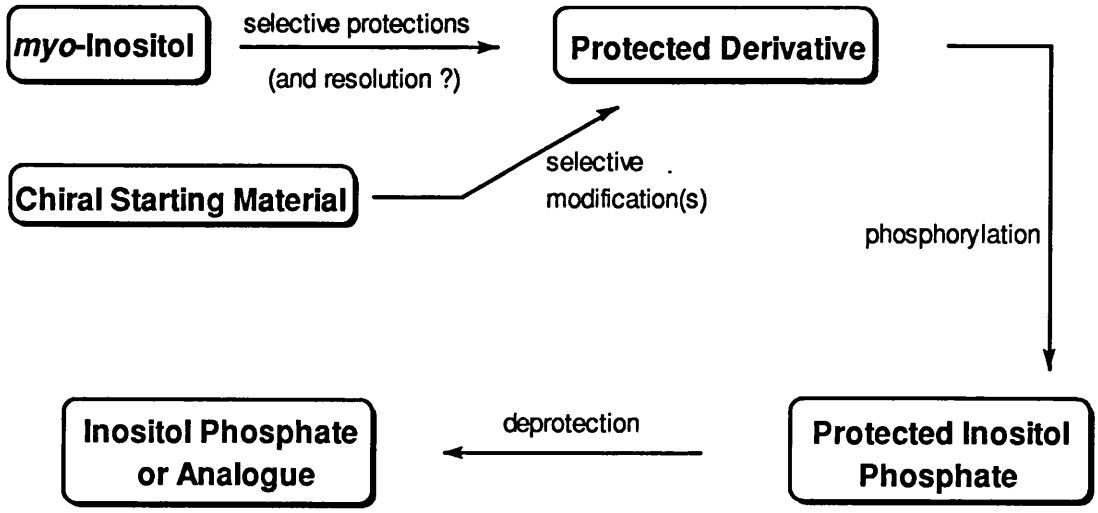


Figure 2.1 Synthesis of Inositol Phosphates and Analogues

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 + $PhCH_2Cl$, powdered KOH, 130-140°C), but there are milder options, e.g. use of sodium hydride, $PhCH_2Br$ 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].

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 $[(Ph_3P)_3RhCl]$[138] or $PdCl_2(PhCN)_2$ (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 $SmCl_3$-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-*O*-cyclohexylidene-*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*-butyldiphenylsilyl 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-*myo*-inositol was obtained by Gigg *et al.* by converting the di-*O*-isopropylidene acetals into the dibenzoates. 3,6-Di-*O*-benzoyl-1,2:4,5-di-*O*-isopropylidene-*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 [149]. The same authors also succeeded in the isolation of 3,4-di-*O*-benzoyl-1,2:5,6-di-*O*-isopropylidene-*myo*-inositol by fractional crystallisation of a mixture of the soluble dibenzoates (giving access to 1,2:5,6-di-*O*-isopropylidene-*myo*-inositol) and in the preparation of 1,2:3,4-di-*O*-isopropylidene-*myo*-inositol *via* the 5,6-di-*O*-allyl ether [155].

The 1,2-mono-acetals are obtainable by hydrolysis of the di-acetals under mild acidic conditions, since the *trans*-acetals are less stable than the 1,2-*cis*-acetal [150]. Direct syntheses of 1,2-cyclohexylidene-*myo*-inositol from *myo*-inositol using 1,1-dimethoxycyclohexane and a strongly acidic resin-bound sulphonic acid catalyst (45-50 % yield) [156] or cyclohexanone / PTSA in DMF / toluene followed by *in situ* ethanolysis of the *trans*-acetals (93 % yield) [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_4 [157]. Reaction of *myo*-inositol with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (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 *myo*-inositol gave selectively the 3,4-siloxane on treatment with TIPS-Cl [157]. A number of enantiomerically pure regioselectively protected inositol derivatives have been synthesised by 1,2-acetalisation of *myo*-inositol with D-camphor dimethyl acetal, protection of the 3,4-position with TIPS and various further protection/deprotection steps [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 ($TiCl_4$) 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.

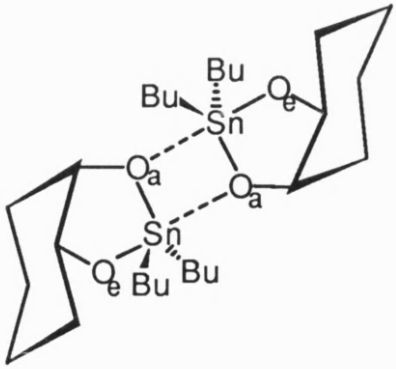


Figure 2.2 The trigonal bipyramidal structure of sugar-stannylene complexes

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*-hydroxyl 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 diffent 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 pentaco-ordinate complex.

2.2 The Synthesis of Optically Active Inositol Derivatives

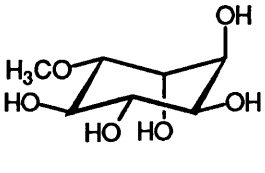
Chiral starting materials

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 occuring compounds such as L-quebrachitol (1L-2-*O*-methyl-*chiro*-inositol) **26** [in the synthesis of L-1-deoxy-1-fluoro-*myo*-inositol [170], D-Ins(1)P [171], L-Ins(1)P [172], L-Ins(1,4,5)P_3 [173], L-Ins(2,4,5)P_3 and L-*chiro*-Ins(1,3,5)P_3 [174], a number of 3-modified D-*myo*-inositol analogues [175], D-3-deoxy-Ins(1,4,5)P_3 and D-3-deoxy-Ins(1,5,6)P_3 [176], as well as in the synthesis of L-*chiro*-Ins(2,3,5)P_3 [177]]. Other chiral starting materials which have been used are D-pinitol (1D-3-*O*-methyl-*chiro*-inositol) **27** [in the synthesis of D-1-deoxy-1-fluoro-*myo*-inositol and D-1,5-dideoxy-1,5-difluoro-*neo*-inositol [178], D-Ins(1,4,5)P_3 [173], D-Ins(2,4,5)P_3 and D-*chiro*-Ins(1,3,5)P_3 [174]], (-)-quinic acid **28** [in the synthesis of D-Ins(1,4,5)P_3 [179] and the 5-methylenephosphonate analogue thereof [180]], D-glucurono-6,3-lactone **29** [in the synthesis of precursors of D-Ins(1,4,5)P_3 [181]] and D-galactose **30** [in the synthesis of D-6-deoxy-Ins(1,4,5)P_3, D-6-deoxy-Ins(1,2-cyclic-4,5)P_3 and D-6-deoxy-Ins(1,5)P_2 *via* Ferrier rearrangement [182]]. Methyl α-D-glucopyranoside **31** has been employed in a biomimetic synthesis of enantiomerically pure D-*myo*-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].

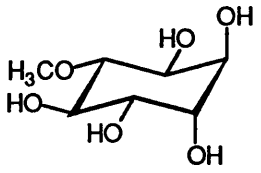
Enzymes in the synthesis of optically active inositol derivatives

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)P_3 [185,187], D-Ins(1,3,4)P_3 [186,187] and D-Ins(1,3,4,5)P_4 [159,186,187] as well as L-Ins(1,3,4,5)P_4 [159] have been synthesised. A new method involving enzymes is the enzyme-catalysed selective esterification of *myo*-inositol

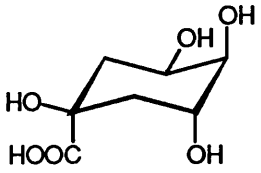
derivatives, which has been applied to resolve racemic 1,2:5,6- and 1,2:3,4-di-*O*-cyclohexylidene-*myo*-inositol [189].



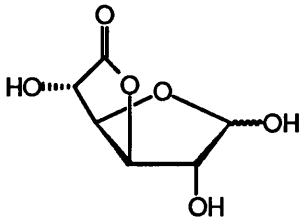
L-quebrachitol **26**



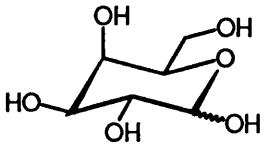
D-pinitol **27**



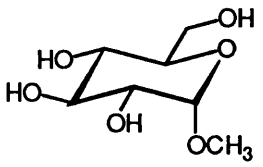
(-)-quinic acid **28**



D-glucurono-6,3-lactone **29**



D-galactose **30**



methyl-α-D-glucopyranoside **31**

Figure 2.3 Chiral starting materials in the synthesis of inositol phosphates

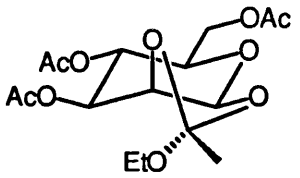
Chiral HPLC-columns

Chiral HPLC-columns have been used to resolve 4,5-di-*O*-benzyl-1,6-di-*O*-PMB-*myo*-inositol in the synthesis of Ins(1,3,4)P_3 [190] and 1,3,4,5-tetra-*O*-benzoyl-*myo*-inositol in the synthesis of Ins(1,3,4,5)P_4 [148].

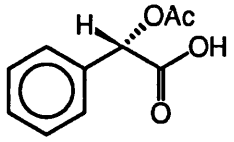
Resolving Agents

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], *l*-(+)-*O*-acetyl-mandelic acid **33** [in the synthesis of 1D- and 1L-4-*O*-benzyl-*myo*-inositol [192]], *S*-(-)- camphanic acid chloride **34** and its *R*-(+)-isomer, D- and L-camphor dimethyl acetals **35** [in the synthesis of Ins(1,4,5)P_3 and Ins(1,4)P_2 [193,194], where the camphor acetal has also been used as protecting group for the 1,2-position], *l*-menthyl chloroformate **36** [185], *l*-menthoxy acetylchloride **37** [in the synthesis of Ins(1,4,5)P_3 [195-197]

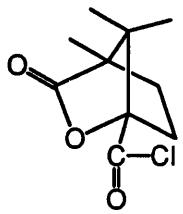
and Ins(1,3,4,5)P_4 [197] and *R*-(+)-1-phenylethyl isocyanate **38** [in the synthesis of D-Ins(1,3,4,5)P_4 [159]]. Optical resolution *via* formation camphanate esters seems to be the most generally useful method available at the present time, both *S*-(-)- and *R*-(+)-camphanic acid chloride (stable crystalline reagents, which are commercially available in high optical purity) have been employed to obtain optically active inositol derivatives.



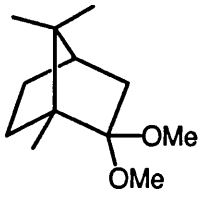
3,4,6-tri-O-acetyl-1,2-O-ethylorthoacetyl-β-D-mannopyranose **32**



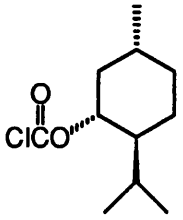
l-(+)-*O*-acetylmandelic acid **33**



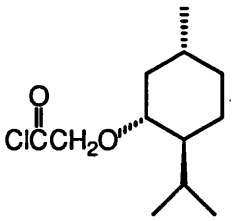
(1S)-(-)-camphanic chloride **34**



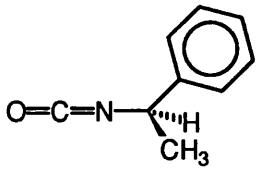
(1R)-camphor dimethylacetal **35**



l-menthyl chloroformate **36**



l-menthoxyacetyl chloride **37**



R-(+)-1-phenylethyl isocyanate **38**

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 inositols; 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: $(RO)_{3-x}P(O)Cl_x$, 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 $[(RO)_3P\text{-}O\text{-}P(O)Cl_2]^+Cl^-$.

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 $POCl_3$ 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 *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 phosphochloridates have also been employed as phosphorylating agents. Bis(*para*-nitrobenzyl) phosphorochloridate and bis(*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 phenyl-, *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 neccessary 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)P_3. The possibility that undesired cyclic phosphodiesters are formed [which is often encountered when P(V) reagents are used] is reduced.

Phosphorochloridites

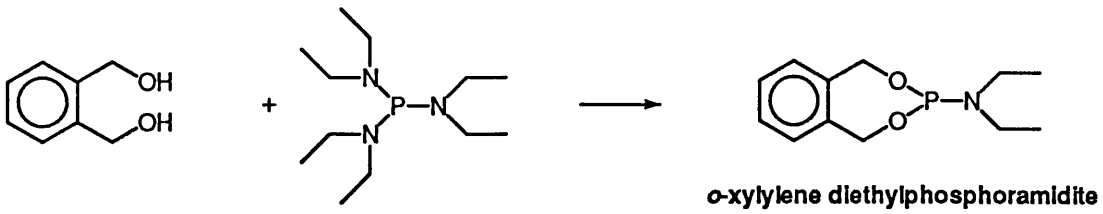
Phosphorochloridites (RR´PCl) have the disadvantage of being unstable towards moisture. In spite of this they have been widely used and have also been employed in the synthesis of several inositol phosphates. Dimethoxy chlorophosphine in the presence of the base *N,N*-diisopropylethylamine was used in the synthesis of Ins(1,4,5)P_3, Ins(1,3,4,5)P_4 and Ins(1,4,5,6)P_4 [222]. Bis(2-cyanoethoxy)chlorophosphine was for example applied in the preparation of Ins(1,3,4)P_3 [223] and Ins(1,4,5)P_3 [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)P_3. 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-benzodioxaphosphepane, 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.



o-xylylene diethylphosphoramidite

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 *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-*myo*-inositol were prepared in a one-pot reaction from this derivative [237].

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 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

[243,244]. The advantage of employing DTNB is that degradation of the phosphorothioate prior to analysis is not neccessary, 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 (RuO_2-$NaIO_4$) and stereoselectively reduced with sodium borohydride to give L-1,2:5,6-di-*O*-cyclohexylidene-4-*O*-methyl-*myo*-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 benzoylation of the remaining free hydroxyl group afforded the 1-silyl pentabenzoate. Deprotection of the silyl group with PTSA-acetic acid, phosphorylation with *o*-xylylene diethylphosphoramidite / 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-*myo*-inositol 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-*myo*-inositol (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 *scyllo*-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 methylate 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-*myo*-inositol, 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-*myo*-inositol, which was resolved using S-(-)-camphanic acid chloride [238]. Reaction of 1,2-*O*-cyclohexylidene-*myo*-inositol with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPS) gave selectively the 3,4-disiloxane, which could be selectively benzoylated 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*-cyclohexylidene-3,4-disiloxane-*myo*-inositol 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-*myo*-inositol, 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-cyclohexylidene or di-isopropylidene acetals of *myo*-inositol [249,250]. 1,2,3,4-Tetra-*O*-benzyl-*myo*-inositol [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-(-)-ω-camphanates [217].

myo-Inositol 1,5-bisphosphate

Both enantiomers of Ins(1,5)P_2 [254] have been prepared by phosphitylation of D- and L-1,2,4,6-tetra-*O*-benzyl-*myo*-inositol with *N,N*-diethylamino bis(2-cyanoethoxy) phosphine, followed by oxidation and hydrogenolysis. Resolution of the tetrabenzyl compounds (obtained by selective benzylation of 2,4,6-tri-*O*-

benzyl-*myo*-inositol 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 *myo*-inositol as starting material was reported by Ozaki *et al.* in 1986 [195] (Figure 2.6). DL-1,2:4,5-di-*O*-cyclohexylidene-*myo*-inositol **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-*myo*-inositol **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 [224] also employed 2,3,6-tri-*O*-benzyl-*myo*-inositol **47**, obtained by a similar route [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 *t*BuOOH 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 sulphoxidise the trisphosphite, giving the trisphosphorothioate analogue of Ins(1,4,5)P_3 [236].

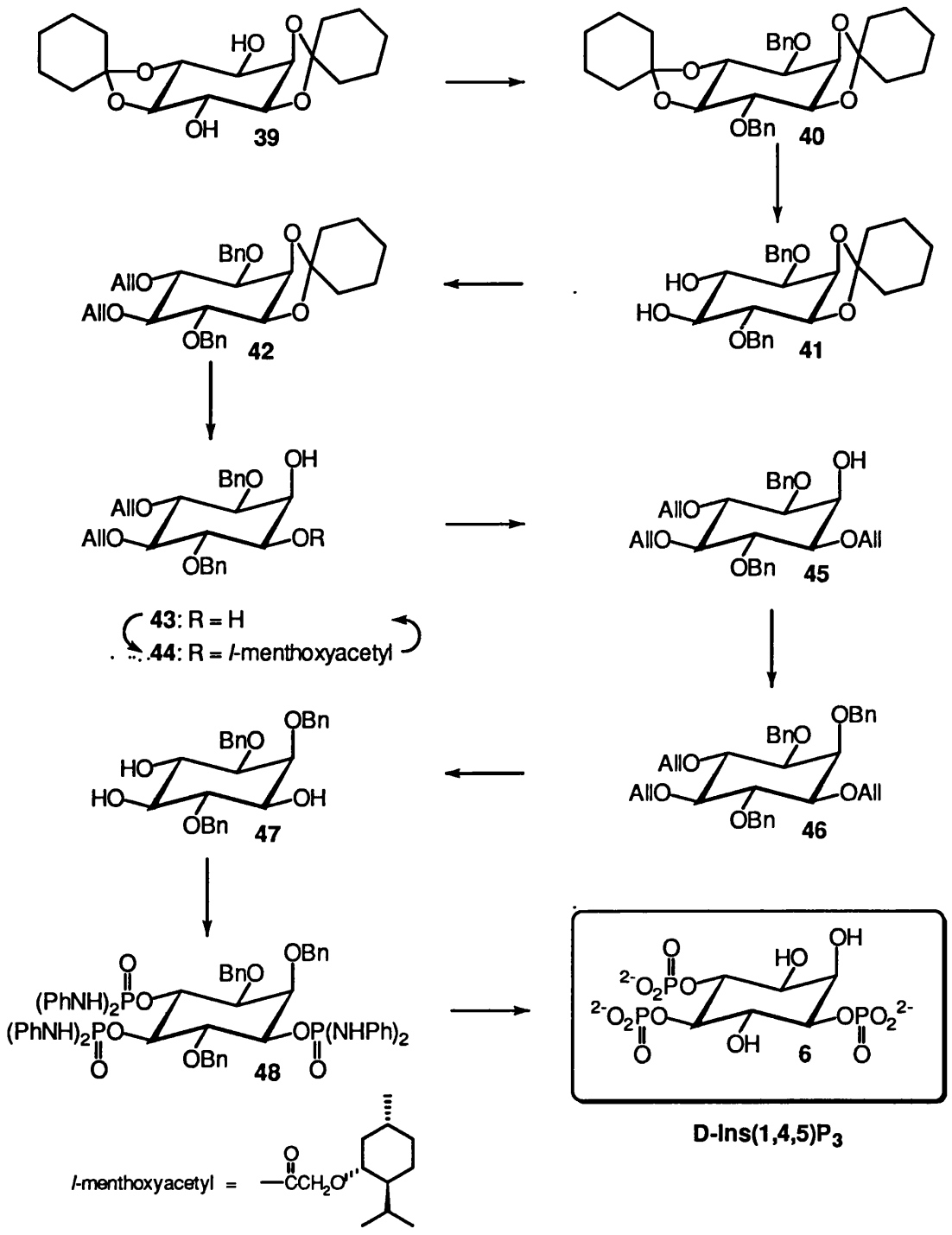


Figure 2.6 The first synthesis of D-*myo*-inositol 1,4,5-trisphosphate

Optically active D-Ins(1,4,5)P_3 was synthesised by using chiral 1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol [185,187], which could be prepared either by conversion into the diastereomeric di-*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*-cyclohexylidene-*myo*-inositol and the mono- or diacylated enantiomer (depending on enzyme and substrate), which on chemical deacylation afford 1D-(+)-2,3:4,5-di-*O*-cyclohexylidene-*myo*-inositol. 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*-cyclohexylidene-6-*O*-benzyl-*myo*-inositol. 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)P_3.
A short synthesis of racemic Ins(1,4,5)P_3 starting from 1,4-di-*O*-benzoyl-*myo*-inositol 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)P_3 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)P_3 [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-*myo*-inositol **56**. Debenzylation and phosphorylation with TBPP afforded **57**. Hydrogenolysis and treatment with moist trifluoroacetic acid afforded racemic Ins(1,4,5)P_3. This method has been modified to give D- and L-Ins(1,4,5)P_3 [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)P_3. This

approach has also been used to give analogues of inositol and Ins(1,4,5)P_3 modified at the 6-position.

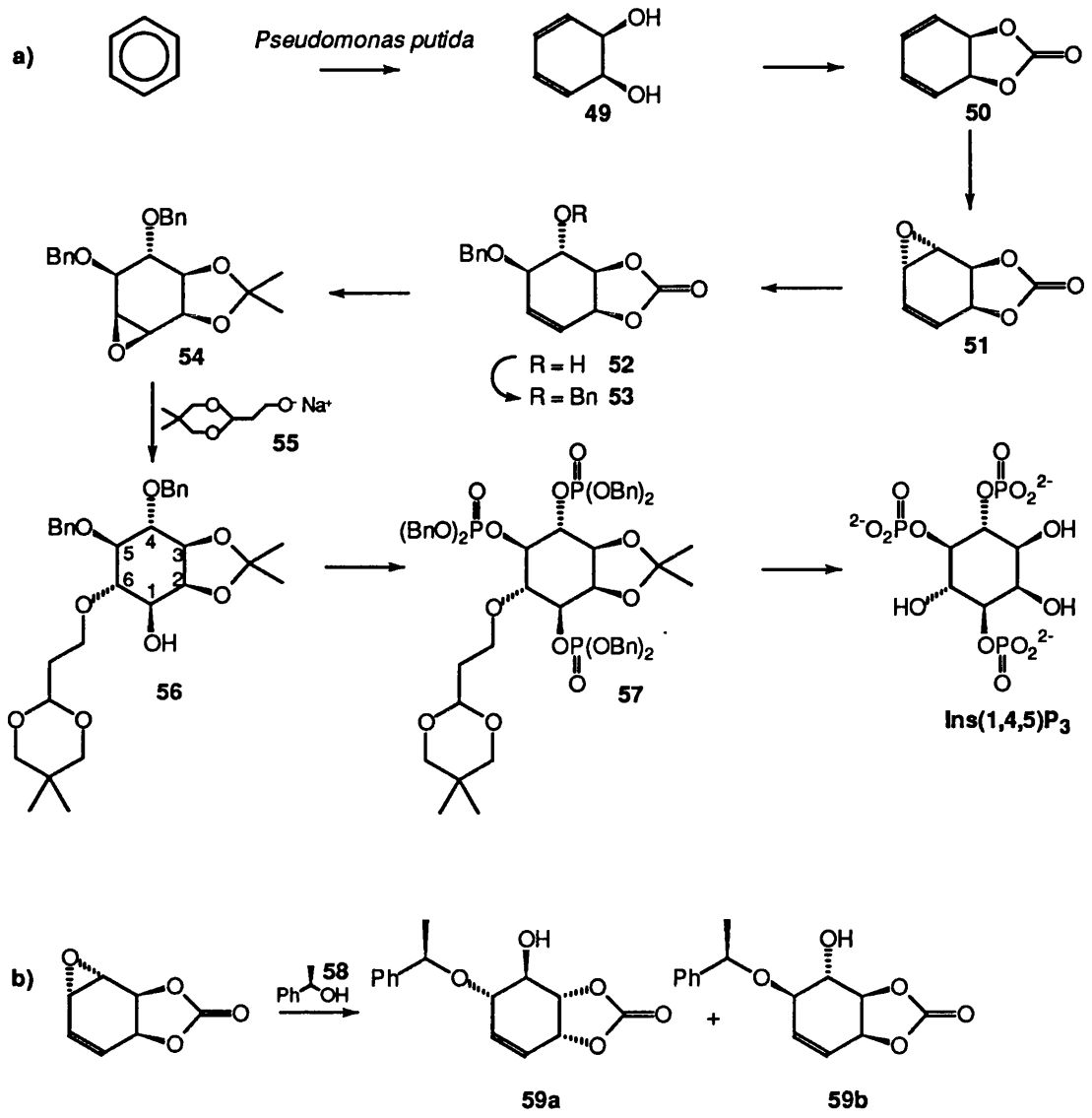


Figure 2.7 Synthesis of Ins(1,4,5)P_3 *via* the microbial oxidation of benzene

Benzene was also the starting material in another synthesis of racemic Ins(1,4,5)P_3 [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)P_3.

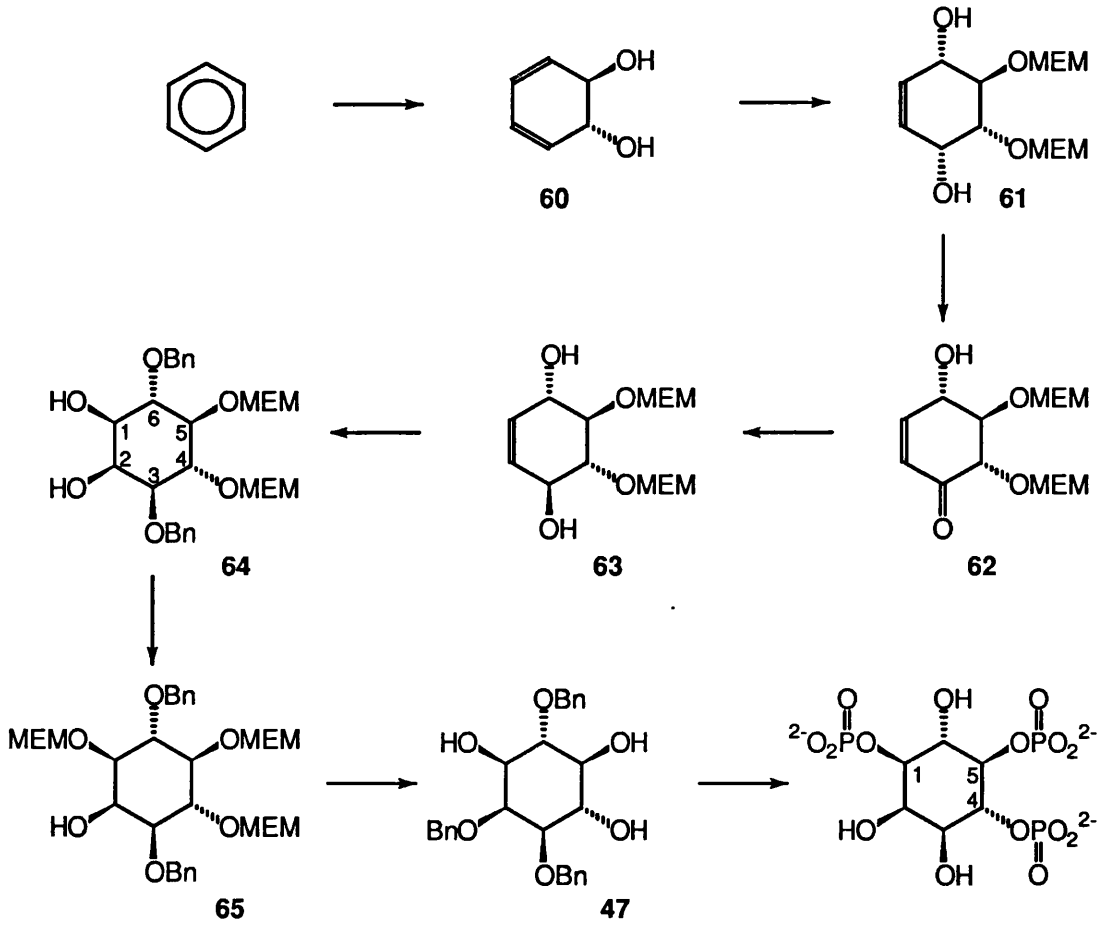


Figure 2.8 Synthesis of Ins(1,4,5)P_3 from benzene *via trans*-1,2-dihydroxy-cyclohexa-3,5-diene

Chiral starting materials have also been employed in the synthesis of Ins(1,4,5)P_3. 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 [illegible] regiospecifity. Hydroboration of the enol ether followed by alkaline peroxide

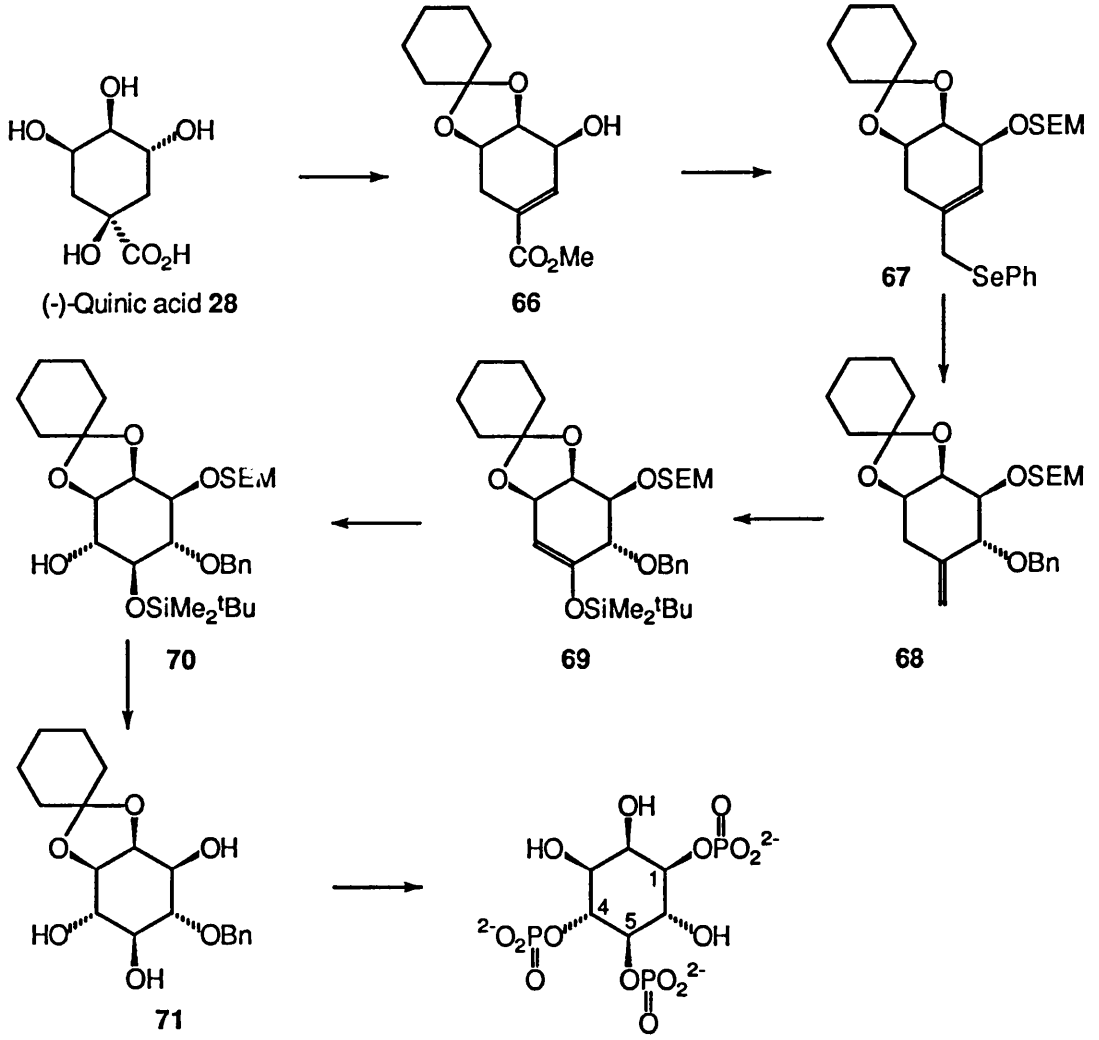


Figure 2.9 Synthesis of D-Ins(1,4,5)P_3 starting from (-)-quinic acid

oxidation furnished the alcohol **70**, which on desilylation gave the known D-2,3-*O*-cyclohexylidene-6-*O*-benzyl-*myo*-inositol **71**. Phosphorylation with TBPP and hydrogenolysis afforded D-Ins(1,4,5)P_3 [179].

Another synthesis of D- and L-Ins(1,4,5)P_3 made use of D-pinitol **27** and L-quebrachitol **26**, respectively [173]. The naturally occuring starting materials were demethylated to give D- and L-*chiro*-inositol **16** and **17**, respectively. Formation of the more stable *cis,cis*-dicyclohexylidene 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

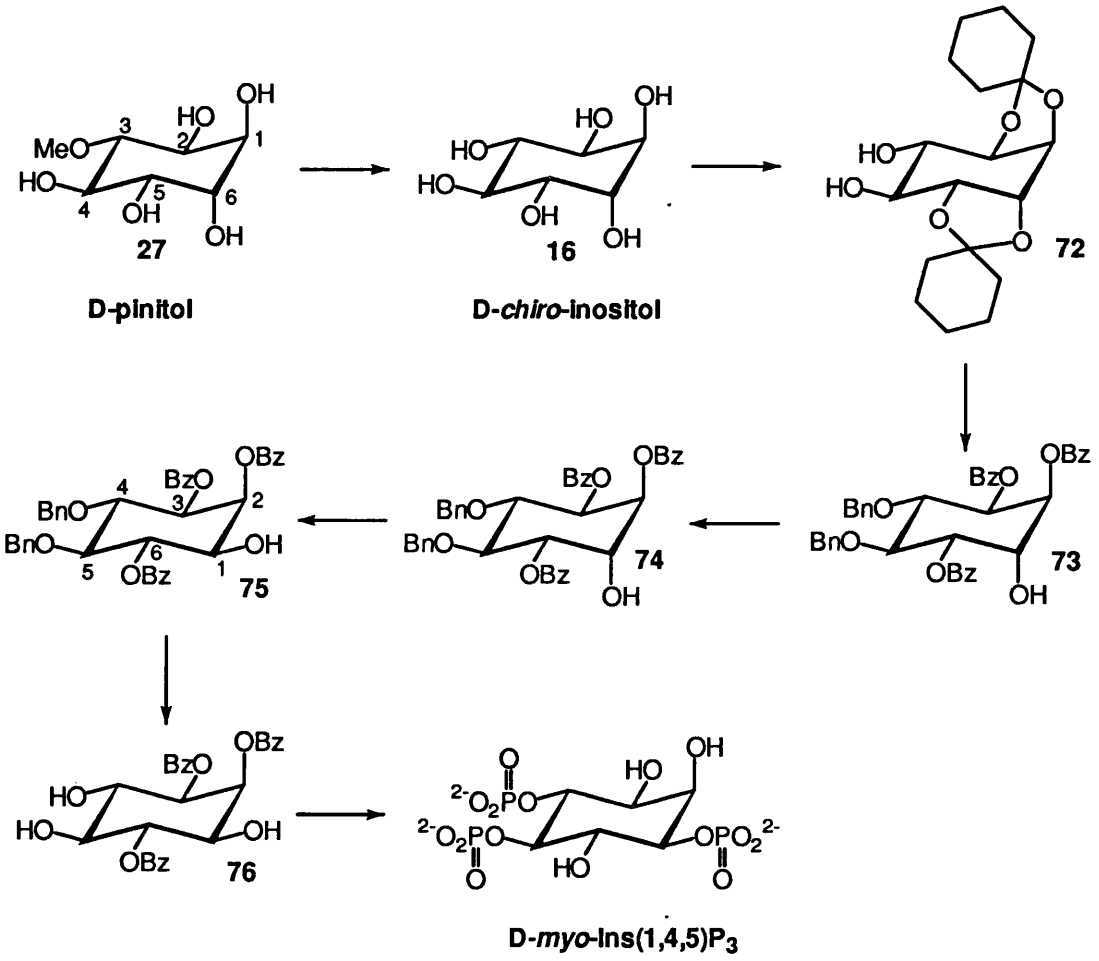


Figure 2.10 Synthesis of D-Ins(1,4,5)P_3 from D-pinitol

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-*myo*-inositol **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)P_3, respectively.

Several other preparations of racemic [169] and optically active [191,197,217,219,227,230,258] Ins(1,4,5)P_3 have been published, including the synthesis of [^{3}H]-labelled D- and L-Ins(1,4,5)P_3 [259].

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

D-Ins(1:2,4,5)P_3 has been synthesised by treatment of D-3,6-di-*O*-benzyl-4,5-bis(dibenzylphospho)-*myo*-inositol with *N*-methyl-pyridinium phosphodichloridate and subsequent deprotection [258]. It was also possible to obtain D-Ins(1:2,4,5)P_3 from D-Ins(1,4,5)P_3 by the action of water soluble 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide [260].

myo-Inositol 2,4,5-trisphosphate

Racemic Ins(2,4,5)P_3 has been prepared from 3,6-di-*O*-benzyl-4,5-di-*O*-allyl-*myo*-inositol [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)P_3 [216,217].

Another synthesis of Ins(2,4,5)P_3 using the benzyl-protected 2,4,5-triol of *myo*-inositol (derived from benzylation of 3,6-di-*O*-benzyl-2,4,5-di-*O*-MEM-*myo*-inositol, 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)P_3 [261] by resolution of a suitably protected precursor with *l*-menthoxyacetyl chloride and stepwise phosphorylation to give the fully protected precursor of D-Ins(2,4,5)P_3 was not concluded. However, the same authors [258] succeeded in the preparation of D-Ins(2,4,5)P_3 by phosphorylating 1,2-*O*-cyclohexylidene-3,6-di-*O*-benzyl-*myo*-inositol with

TBPP, removal of the acetal protecting group and resolution of this intermediate *via* the diastereomeric *l*-menthoxyacetic esters. Silylation of the 1-position, phosphorylation of the 2-position and hydrogenolysis afforded D-Ins(2,4,5)P_3

D- and L-Ins(2,4,5)P_3 were also prepared from L- and D-1,3,4-tri-*O*-benzoyl-*myo*-inositol, 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-*myo*-inositol.

myo-Inositol 1,3,4-trisphosphate

Several syntheses of racemic [217,223] and optically active [186,187,190] Ins(1,3,4)P_3 have been reported:

1,6-Di-*O*-*para*-methoxybenzyl-4,5-di-*O*-benzyl-*myo*-inositol (derived from both 1,2:3,4- and 1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol) was resolved either by use of a chiral column or conversion into the diastereomeric *l*-menthoxyacetic 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-*myo*-inositol. Phosphorylation with TBPP and hydrogenolysis afforded D-Ins(1,3,4)P_3 [190].

Optically active 1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol, obtained either by an enzymatic process or by conversion into the di-*l*-menthylcarbonates [185] was used in the synthesis of D-Ins(1,3,4)P_3 [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-*myo*-inositol, which was phosphorylated and hydrogenolysed to yield D-Ins(1,3,4)P_3.

2.5.4 Inositol Tetrakisphosphates

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

The first synthesis of Ins(1,3,4,5)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-*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 benzoylation of *myo*-inositol with 3.5 molar equivalents of benzoyl chloride in pyridine. *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 *o*-xylylene diethylphosphoramidite. Oxidation with *m*CPBA and hydrogenolysis afforded Ins(1,3,4,5)P_4. Resolution of the tetrabenzoate was accomplished by means of chiral column chromatography, thus giving access to optically active Ins(1,3,4,5)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-*myo*-inositol, obtained in modest yield by direct benzoylation of *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-Ins(1,3,4,5)P_3 [230].

D-Ins(1,3,4,5)P_3 was also prepared from D-1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol [186,187], which can be obtained by chemical or enzymatic means [185].

Further syntheses of racemic [222] and optically active [159,197,262] Ins(1,3,4,5)P_3 have been published.

myo-Inositol 1,2,4,5-tetrakisphosphate

Racemic Ins(1,2,4,5)P_4 has been prepared from 1,4-dibenzoyl-*myo*-inositol by phosphitylation with dimethoxychlorophosphite 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_4 as the octalithium salt.
In another method [253] 3,6-di-*O*-benzyl-4,5-di-*O*-(2-methoxyethoxy-methyl)-*myo*-inositol, obtained from benzene *via* conduritol B derivatives was employed. Cleavage of the MEM ethers afforded 3,6-di-*O*-benzyl-*myo*-inositol, which was phosphorylated with TBPP / sodium hydride and hydrogenolysed to give Ins(1,2,4,5)P_4.

myo-Inositol 1,3,4,6-tetrakisphosphate

The *meso*-compound Ins(1,3,4,6)P_4 was obtained by treatment of *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 *o*-xylylene diethylphosphoramidite. Oxidation and hydrogenolysis afforded Ins(1,3,4,6)P_4 [157].

myo-Inositol 1,4,5,6-tetrakisphosphate

Racemic Ins(1,4,5,6)P_4 [222] was synthesised from the 1,2-cyclohexylidene acetal of *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

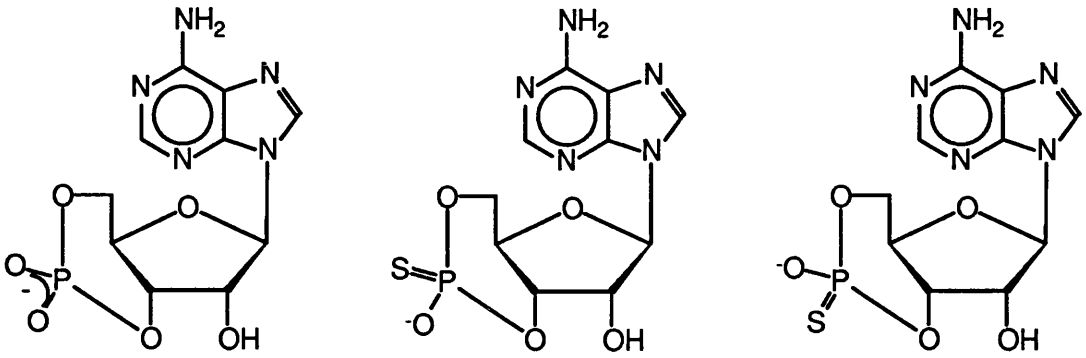
Phosphorothioates are analogues of phosphates in which a non-bridging oxygen atom has been exchanged for sulphur.

Figure 3.1 Phosphate *vs.* Phosphorothioate

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 substitition 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 π-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 occuring phosphates are potential inhibitors of phosphatase enzymes. The phosphorothioate analogues of cAMP, (*S*p)- and (*R*p)-cAMPS (the *S*p-analogue carrying an axial and the *R*p-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 (*R*p)-cGMPS was found to antagonise cGMP-dependent protein kinase [266].



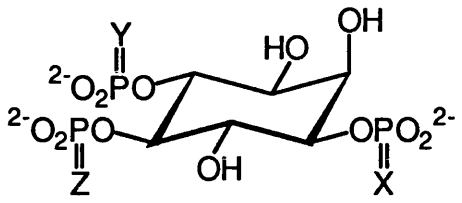
cAMP (*S*p)-cAMPS (*R*p)-cAMPS

Figure 3.2 AMP and AMPS

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 existance 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)P_3 analogue was *myo*-inositol 1,4,5-trisphosphorothioate [Ins(1,4,5)PS_3] **77** [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)P_3 binding site in the cerebellum [270]. Ins(1,4,5)PS_3 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)P_3 it causes sustained calcium release from intracellular stores [272].



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)P_3

Ins(1,4,5)PS_3 has now been supplemented by Ins(1,4,5)P_3-5S **78** [209-211] and Ins(1,4,5)P_3-4,5S_2 **79** [273]. Both compounds are also 5-phosphatase resistant and very similar to Ins(1,4,5)PS_3 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-[^{35}S]Ins(1,4,5)PS_3 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)P_3 receptor and the other one (which is showing only a low affinity for Ins(1,4,5)P_3) 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 sp^3 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-hydroxyproprionitrile and 1*H*-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-*myo*-inositol 1:2-cyclic-phosphorothioate in quantitative yield [280].

3.1.3 Phosphorothioate Analogues of Inositol Bisphosphates

myo-Inositol 1,4-bisphosphorothioate

DL-1,2:4,5-Di-*O*-isopropylidene-*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 *myo*-inositol 1,4-bisphosphorothioate [250].

myo-Inositol 4,5-bisphosphorothioate

The starting material for the preparation was 1,2,3,4-tetra-*O*-benzyl-*myo*-inositol which was phosphitylated with diisopropylamino(2-cyanoethoxy)chlorophosphine forming the bisphosphoroamidite 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

myo-Inositol 1,4,5-trisphosphorothioate

Myo-Inositol 1,4,5-trisphosphorothioate **77** [224,236] was the first Ins(1,4,5)P_3 analogue synthesised and has proved to be a valuable instrument in the elucidation of the biochemical role of Ins(1,4,5)P_3. Ins(1,4,5)P_3 was synthesised from the protected precursor DL-1,2,4-tri-*O*-benzyl-*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-*myo*-inositol has been reported [234]. Phosphitylation with bisbenzyldiisopropylaminophosphine in the presence of 1-*H* tetrazole in a CH_2Cl_2 - CH_3CN 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)P_3-5S **78** [209,211] followed the same route as the Ins(1,4,5)P_3 synthesis reported starting with 2,3,6-tri-*O*-benzyl-4,5-*O*-isopropylidene-*myo*-inositol 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 phosphitylated with bisbenzyldiisopropylaminophosphine. 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-*myo*-inositol followed by acid hydrolysis of the 4,5-acetal) was phosphitylated to form the 4,5-bisphosphite using either bis(2-cyanoethoxy)diisopropylaminophosphine or bisbenzyldiisopropylaminophosphine. 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)P_3 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 phenacetyl 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 phosphite triester with Bu^{t}OOH afforded the fully benzyl-protected inositol 1-phosphorothioate 4,5-bisphosphate **84**. Deblocking with sodium in liquid ammonia gave **80** in 74% yield.

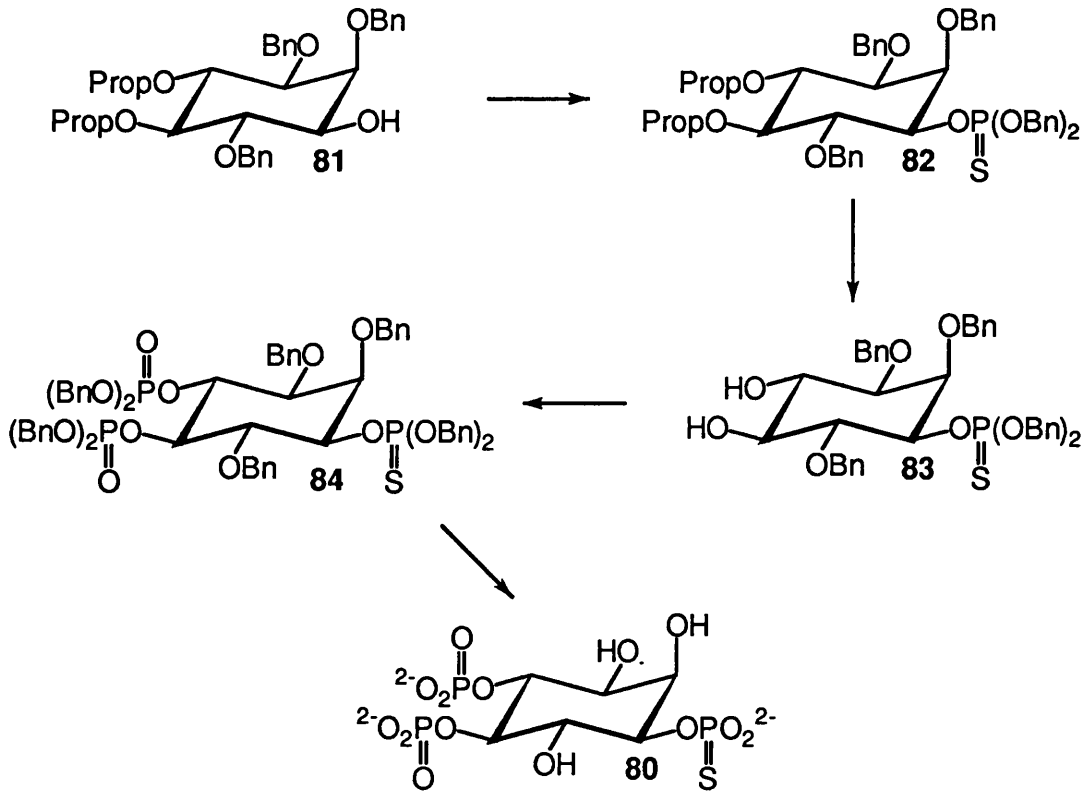


Figure 3.4 Synthesis of Ins(1,4,5)P_3-1S (Dreef et al.)

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[(dibenzoxy)phospho]-*myo*-inositol with bisbenzyldiisopropylaminophosphine in the presence of 1*H*-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-phosphorothio-ate 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-*myo*-inositol (DPPsI) have been described by two groups [281-283].
In the first synthesis [281,282] 1D-2,3:5,6-di-*O*-cyclohexylidene-4-*O*-methoxy-methyl-*myo*-inositol was phosphitylated with $ClP(OCH_3)N(Pr^i)_2$ and then converted directly to the protected 1,2-dipalmitoyl-*sn*-glycero-3-thiophospho-*myo*-inositol 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 methoxyphosphine to produce the phosphoramidite which was then condensed with 1D-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol 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-*myo*-inositol [283].

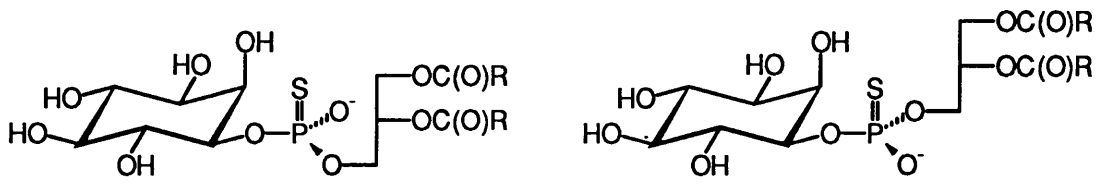


Figure 3.5 (R_P)- and (S_P)-1,2-dipalmitoyl-*sn*-glycero-3-thiophospho-*myo*-inositol

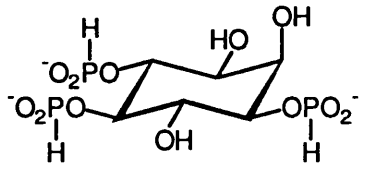
(R_P)- and (S_P)-1,2-dipalmitoyl-*sn*-glycero-3-thiophospho-*myo*-inositol have been used to examine the stereochemical mechanism by which phosphatidylinositol-specific PLC´s cleave phosphatidylinositol (PtdIns) [281,282]. In contrast to PtdIns itself, these phosphorothioate diesters are chiral at the phosphorus atom, thus making the determination of enzyme stereospecifity possible. It was found that the R_P 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

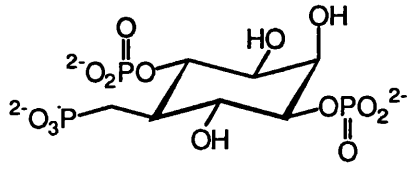
3.2.1 Phosphonates

One of the first Ins(1,4,5)P_3 analogues was DL-*myo*-inositol 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-*myo*-inositol and subsequent anionic debenzylation. However, no biological activity has been reported for this compound.

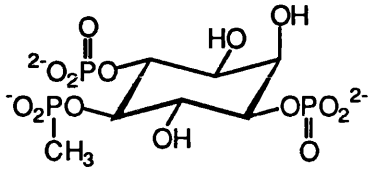
The 5-methylenephosphonate analogue of Ins(1,4,5)P_3 **86** has been prepared from (-)-quinic acid [180] and shown to be a long-lived agonist of calcium mobilisation.



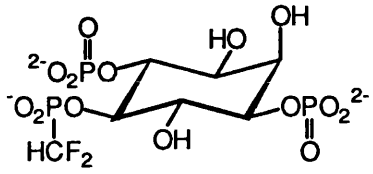
DL-Ins(1,4,5)$_3$-tris-1-H-phosphonate **85**



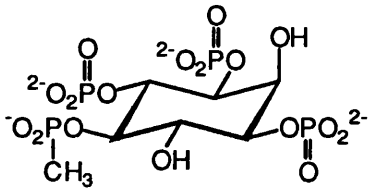
D-Ins(1,4,5)P_3-5-methylenephosphonate **86**



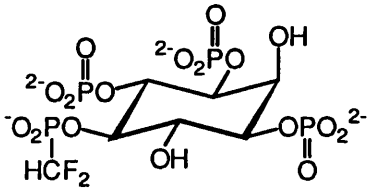
Ins(1,4,5)P_3-5-methylphosphonate **87**



Ins(1,4,5)P_3-5-(difluoromethyl)phosphonate **88**



Ins(1,3,4,5)P_4-5-methylphosphonate **89**



Ins(1,3,4,5)P_4-5-(difluoromethyl)phosphonate **90**

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 phosphonylating agents *bis*[6-(trifluoromethyl)benzotriazol-1-yl]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-bismethylphosphonate and *myo*-inositol 1,4,5-trismethylphosphonate 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 phosphonylating 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].

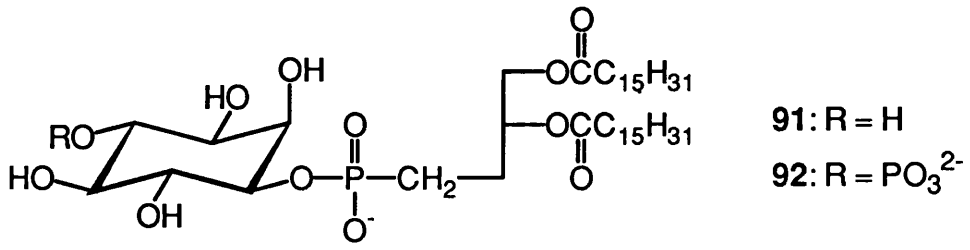


Figure 3.7 Methylenephosphonate Analogues of PtdIns and PtdIns(4)P

3.2.2 Sulphates, Sulphonamides and Carboxylates

The preparation of racemic sulphonamide, sulphate and carboxymethyl analogues of Ins(1,4,5)P_3 has been described [289,294]. However, none of these compounds showed any affinity for the Ins(1,4,5)P_3 receptor.

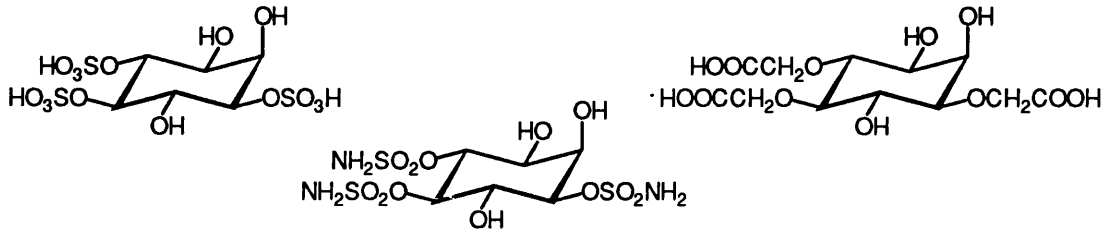


Figure 3.8 The trissulphate, trissulphonamide and triscarboxylate analogues of Ins(1,4,5)P_3

3.3 Fluorinated Analogues

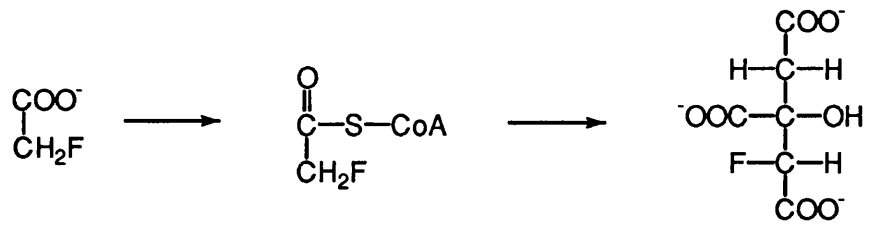
Of the 10 million compounds that had been registered in *Chemical Abstracts* by February 1990, 6.2 % possess a carbon-fluorine bond. Every year more than 50000 novel organofluorine compounds are synthesised, the number of papers published in this area of research has reached 5000 *per annum* [295]. These 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 shorty 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 Fe^{2+} ion. The high electonegativity of fluorine results in a strong interaction with the Fe^{2+}, 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.



Fluoroacetate Fluoroacetyl-Coenzyme A Fluorocitrate

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-fluoro-uracil 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 sulphydryl group of the enzyme) and the second step (addition of methylene-tetrahydrofolate - the donor of the C_1 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.

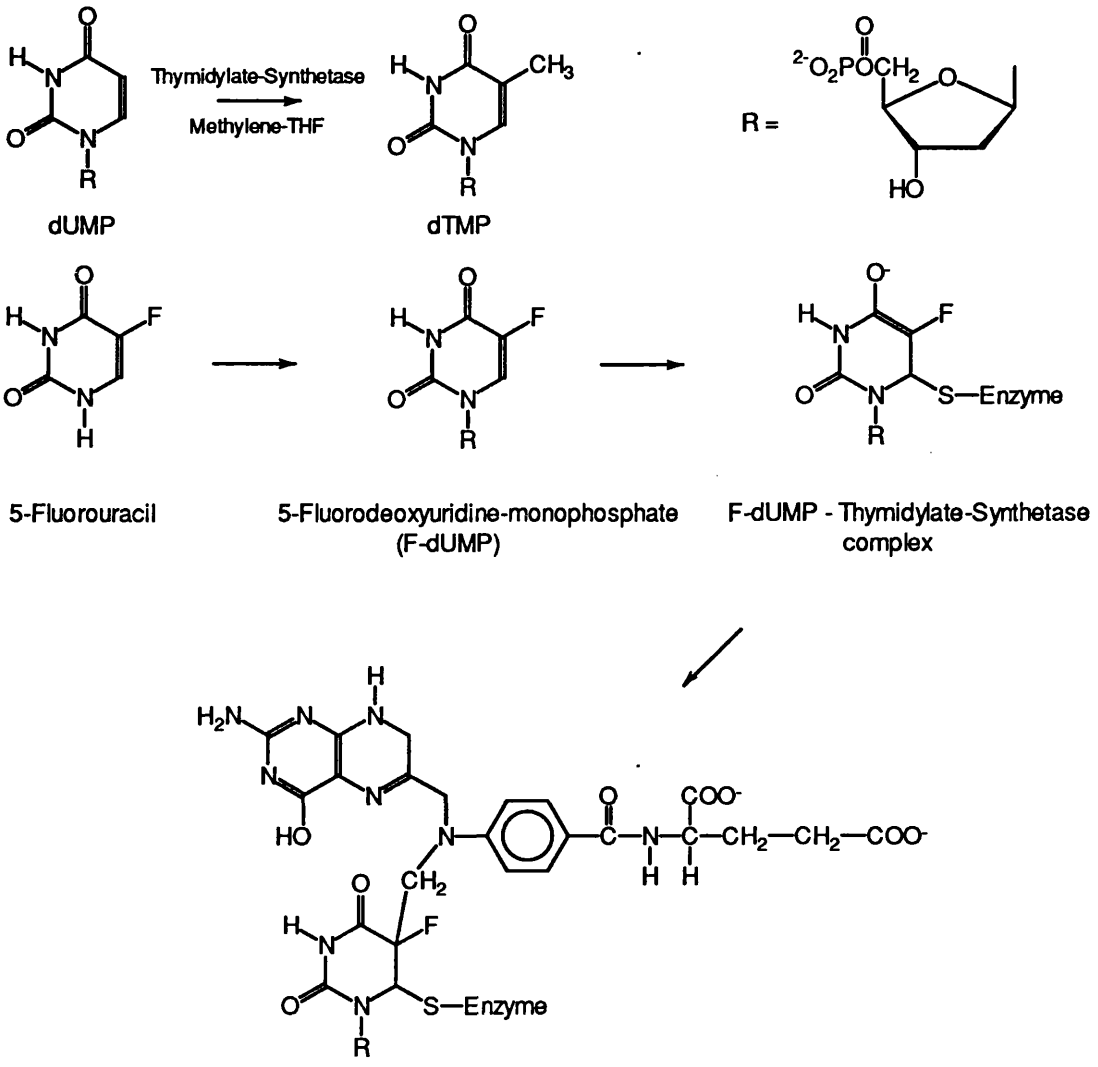


Figure 3.10 Inhibition of Thymidylate Synthetase by F-dUMP

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 [$(C_2H_5)_2NSF_3$, DAST) [301] 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 Ins(1,4,5)P_3 signalling system. Analogues of *myo*-inositol, which serve as substrates for PtdIns synthetase, may be incorporated into the cellular phosholipid and inhibit the formation of Ins(1,4,5)P_3 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-*myo*-inositol

Different starting materials have been employed in the syntheses of 1D-1-deoxy-1-fluoro-*myo*-inositol. Kozikowski *et al.* [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-*O*-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-*O*-methyl-*myo*-inositol with inversion of configuration on the fluorination site. Demethylation of this compound with BBr_3 afforded 1D-1-deoxy-1-fluoro-*myo*-inositol.
The same group had earlier reported a 10-step synthesis of this fluorinated isostere from *myo*-inositol [170,308], which involved fluorination at the 2-position of a suitably protected *myo*-inositol derivative and the optical resolution of the resulting protected fluoro-*scyllo*-inositol. Recovery of the *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 *myo*-inositol configuration) and afforded 1D- and 1L-1-deoxy-1-fluoro-*myo*-inositol after deprotection of the respective enantiomeric intermediates.
A similar synthesis also starting from *myo*-inositol has been reported by Offer *et al.* [309,310]. Here, the problems of recovery of the *myo*-inositol configuration and optical resolution were addressed efficiently in a single conversion, *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 *myo*-inositol.
An efficient synthesis of 1L-1-deoxy-1-fluoro-*myo*-inositol (= 1D-3-deoxy-3-fluoro-*myo*-inositol) in two steps was possible by direct fluorination of L-

quebrachitol (L-3-*O*-methyl-*chiro*-inositol). Treatment with DAST resulted in selective fluorination of the 1-position (which proceeded with inversion to give the *myo*-inositol configuration) and subsequent demethylation with boron tribromide afforded the fluoro-inositol analogue [170]. This compound is capable of entering into the PtdIns cycle and block the eventual production of both Ins(1,3,4,5)P_4 and Ins(1,3,4)P_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-*myo*-inositol was found to inhibit growth of NIH 3T3 cells even in the presence of *myo*-inositol [178].

3-Deoxy-3-fluoro-*myo*-inositol inhibits cell growth in the presence of *myo*-inositol on NIH 3T3 cells [178], although the medium used contained relatively high levels of *myo*-inositol compared to other growth media. 3-Deoxy-3-fluoro-*myo*-inositol competitively blocks the uptake of *myo*-inositol which suggests that the 3-fluoro derivative acts as a substrate for the PtdIns synthase enzyme incorporating *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 Ins(1,4,5)P_3 [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-*scyllo*-inositol

1-Deoxy-1-fluoro-*scyllo*-inositol was the first fluorinated analogue of inositol prepared [311]. DL-1-*O*-Benzoyl-3,4,5,6-tetra-*O*-benzyl-*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 *scyllo*-inositol derivative, which was then deprotected by mild hydrolysis followed by hydrogenolysis to give the title compound.
1-Deoxy-1-fluoro-*scyllo*-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-*scyllo*-inosose with sodium borotritide which produced mainly 1,3,4,5,6-penta-*O*-benzyl-*myo*-[2-^{3}H]-inositol. Reaction of the tritium labelled inositol with DAST and deprotection gave 1-deoxy-1-fluoro-*scyllo*-[1-^{3}H]-inositol [312].
The synthesis of 1-deoxy-1-fluoro-*scyllo*-inositol has also been reported by another group [307].

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-*myo*-inositol

A synthesis of [^{3}H]-labelled and unlabelled 2-deoxy-2-fluoro-*myo*-inositol 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-*myo*-inositol derivative from 1,3,4,5,6-penta-*O*-benzyl-*myo*-inositol 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-*myo*-inositol, and deprotection with dry HBr gave 2-deoxy-2-fluoro-*myo*-inositol in 51% yield.
Oxidation of 1,3,4,5,6-penta-*O*-benzyl-*myo*-inositol with Jones´ reagent (sodium dichromate / sulphuric acid) and reduction of the inosose with $NaBH_3T$ followed by treatment with DAST gave some 1,3,4,5,6-penta-*O*-benzyl-2-deoxy-2-fluoro-*myo*-[2-^{3}H]-inositol (with predominant formation of the *scyllo*-inositol analogue, see above). This compound was then deprotected as before to yield 2-deoxy-2-fluoro-*myo*-[2-^{3}H]-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-*myo*-inositol

1-*O*-Allyl-3,6-di-*O*-benzyl-4,5-*O*-isopropylidene-*myo*-inositol 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-*myo*-inositol compound [307].

2-*C*-Fluoromethyl-*myo*-inositol

DL-1-*O*-Benzoyl-2-oxiranyl-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol was converted into DL-2-*C*-fluoromethyl-3,4,5,6-tetra-*O*-benzyl-*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-*myo*-inositol

3-*O*-Benzyl-1,2:5,6-di-*O*-cyclohexylidene-*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 occuring backside attack of F^- more difficult and therefore disfavours the product of inversion. Deprotection of the major product furnished racemic 4-deoxy-4-fluoro-*myo*-inositol in 87% yield. Attempted deprotection of the minor *epi*-inositol product led to decomposition products [313].

5-Deoxy-5-fluoro-*myo*-inositol

Several methods of synthesis of 5-deoxy-5-fluoro-*myo*-inositol have been published. The starting material for the first method [303] was 1,4,6-tri-*O*-benzyl-2,3-*O*-cyclohexylidene-*neo*-inositol. Reaction with DAST and DMAP in toluene produced the 5-fluoro-*myo*-inositol derivative due to inversion at C-5 in 61% yield followed by deprotection which produced the 5-deoxy-5-fluoro-*myo*-inositol.
6-*O*-Benzyl-1,2:3,4-di-*O*-cyclohexylidene-*myo*-inositol was employed as starting material for the preparation of 5-deoxy-5-fluoro-*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-*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-*myo*-inositol [309,310] .

5-Deoxy-5,5-difluoro-*myo*-inositol

Oxidation of 1,4,6-tri-*O*-benzyl-2,3-*O*-cyclohexylidene-*myo*-inositol 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 [303].

A number of 2-, 3-, 4-, and 5-modified inositol analogues have been evaluated as substrates and inhibitors of PtdIns synthetase [315]. 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-*myo*-inositol, 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 neccessary 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-*myo*-inositol 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*-isopropylidene-*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)P_3] **103** [306].

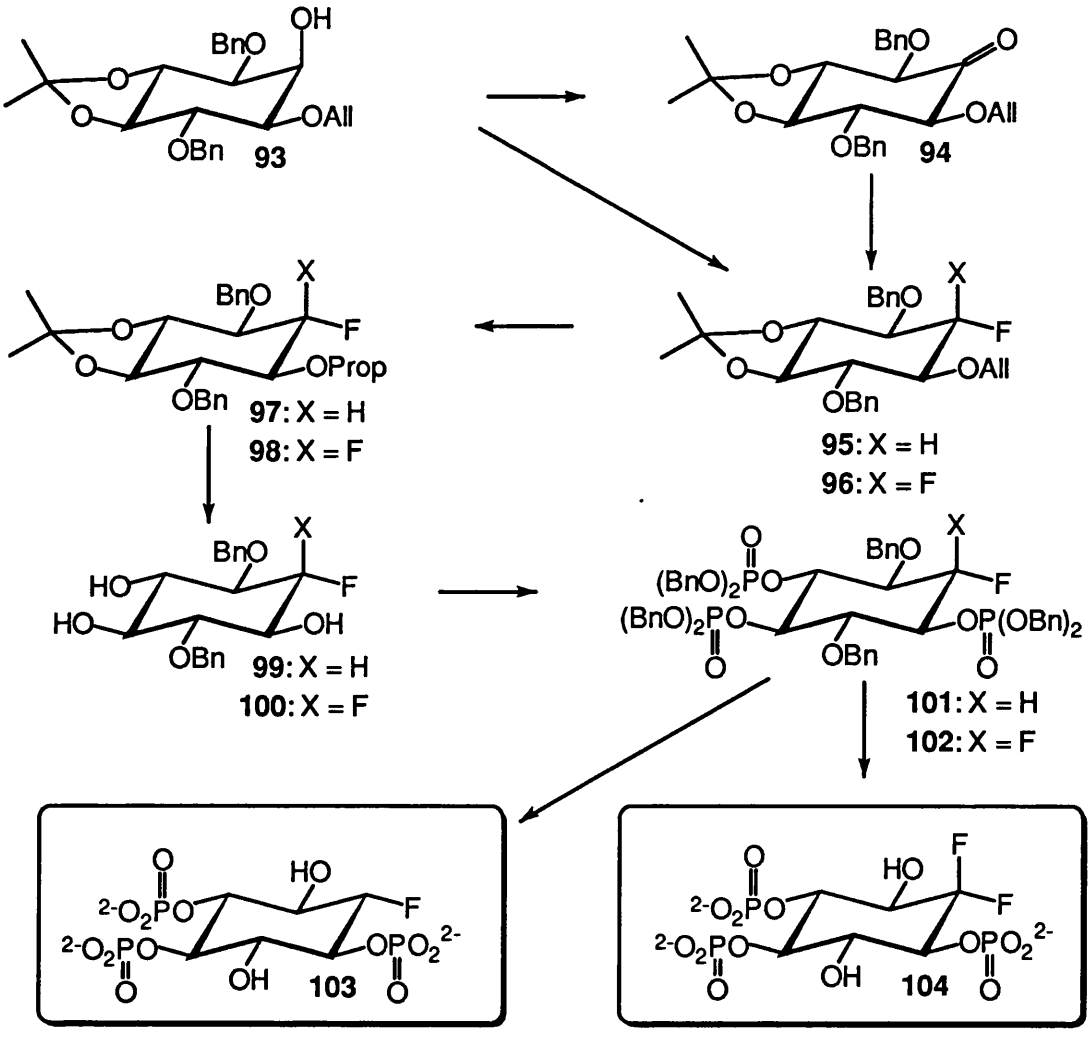


Figure 3.11 Synthesis of 2-F-*scyllo*-Ins(1,4,5)P_3 and 2,2-F_2-Ins(1,4,5)P_3

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 *scyllo*-inositol derivatives respectively, which could be separated by chromatography [168]. When the *scyllo*-epimer was treated with DAST, fluorination surprisingly occured with retention of configuration yielding the same derivative which was obtained from the *myo*-epimer.

2-Deoxy-2,2-difluoro-*myo*-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-*scyllo*-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-*scyllo*-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-*myo*-inositol by chromatographic separation of the 1-*O*-[(-)-ω-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-*scyllo*-Ins(1,4,5)P_3 and 2-deoxy-2,2-difluoro-*myo*-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-Ins(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-*myo*-inositol 1,4,5-trisphosphate

L-Quebrachitol was converted in two steps into D-3-deoxy-3-fluoro-*myo*-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-*scyllo*-inositol 1,3,4-trisphosphate

Reaction of 1,3,4-tri-*O*-allyl-5,6-di-*O*-benzyl-*myo*-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-*scyllo*-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-*scyllo*-inositol 1,3,4-trisphosphate [318].

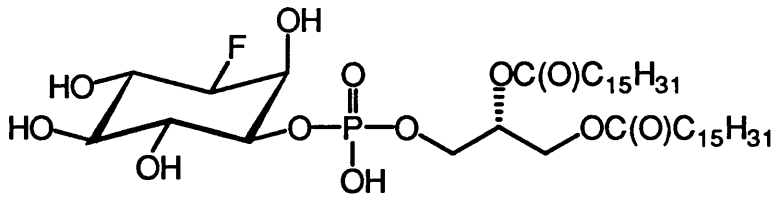
2-Deoxy-2,2-difluoro-*myo*-inositol 1,3,4-phosphate

1,3,4-Tri-*O*-allyl-5,6-di-*O*-benzyl-*myo*-inositol was also used to prepare 2-deoxy-2,2-difluoro-*myo*-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-*myo*-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 [311]. 2-Deoxy-2-fluoro-1-phosphatidyl-*scyllo*-inositol was obtained by condensation of 2-fluoro-2-deoxy-3,4,5,6-tetra-*O*-benzyl-*scyllo*-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-*myo*-inositol has been reported [319]. 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

4. Structure-Activity Studies on Ins(1,4,5)P_3 Analogues

4.1 Structural Requirements for Calcium Release

The Ins(1,4,5)P_3 receptor shows great stereo- and regiospecifity and structural requirements for Ins(1,4,5)P_3 analogues, in order to effect Ca^{2+}-release, are quite strict.

Whereas Ins(1:2,4,5)P_3, Ins(4,5)P_2 and GroPIns(4,5)P_2 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)P_3, respectively, most other natural inositol phosphates including Ins(1)P, Ins(1:2)P, Ins(1,4)P_2 [22], Ins(1,3,4)P_3 [269,324], Ins(1,3,4,5,6)P_5 [94,325] and InsP_6 (phytic acid) [269] are ineffective as agonists at the Ins(1,4,5)P_3 receptor.

The stereospecifity of the receptor is demonstrated by the fact that L-Ins(1,4,5)P_3, [= D-Ins(3,5,6)P_3] 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)P_3 [324,327].

Modifications at the 1-position

A number of semisynthetic 1-substituted Ins(1,4,5)P_3 analogues [323], able to release Ca^{2+}, 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)P_3 and a photoaffintity analogue derived therefrom [328] were able to effect Ca^{2+} release, both being *ca.* 8-fold weaker than Ins(1,4,5)P_3. Receptor binding of these derivatives was equally good, and the photoaffinity derivatives were successfully used to label Ins(1,4,5)P_3 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)P_3 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].

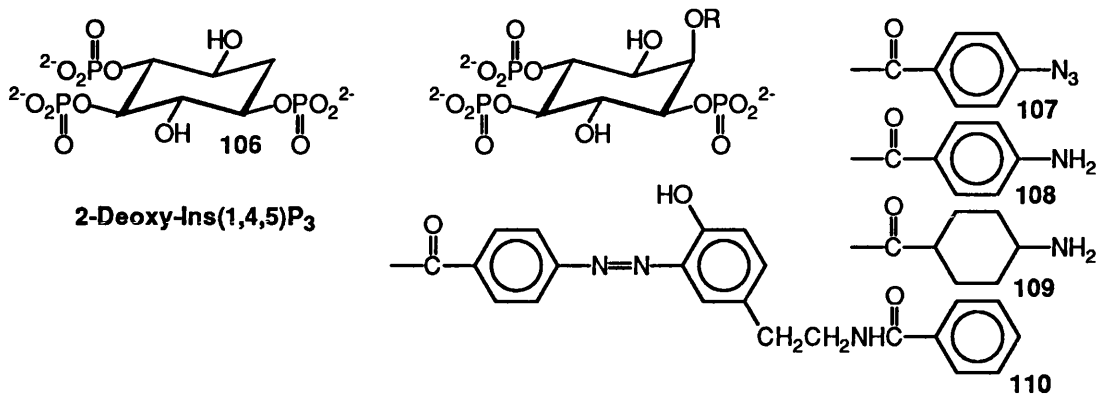


Figure 4.1 Ins(1,4,5)P_3 analogues modified at the 2-position

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 stereospecifity 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-*scyllo*-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-*scyllo*-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-*scyllo*-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.

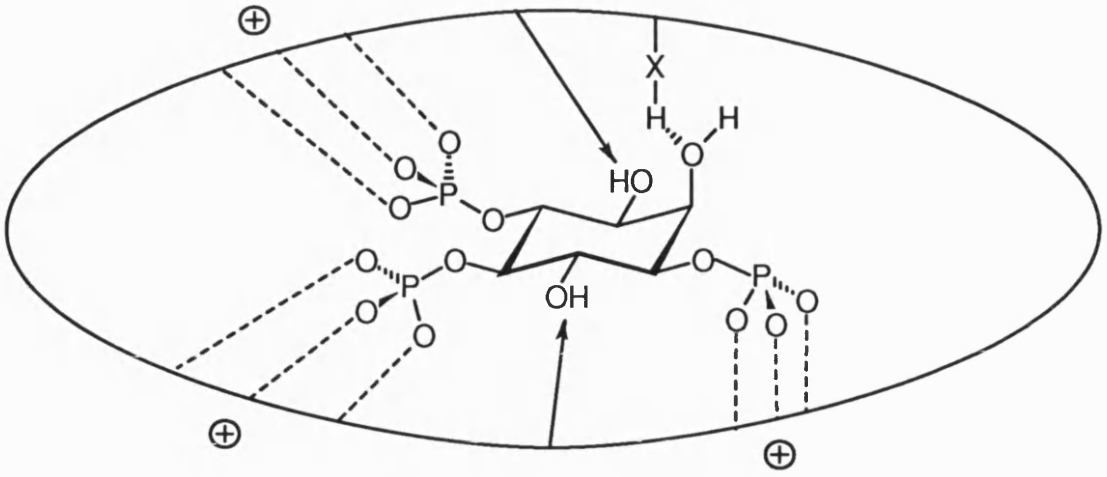


Figure 4.2 Putative interaction of the 2-hydroxyl group with the Ins(1,4,5)P_3 receptor

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 [^{3}H]-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)P_3 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)P_3 (EC_{50} 6.4 μM) [182].

DL-Ins(1,4,5,6)P_4 exhibited no Ca^{2+} releasing activity and did not appear to influence the Ins(1,4,5)P_3 mediated calcium release at concentrations up to 10 μM [94].

Other 6-modified Ins(1,4,5)P_3 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)P_3 was, however, evaluated by another group [324] and found to mobilise calcium with an EC_{50} of 65 μM [Ins(1,4,5)P_3 0.3 μM].

Multiple Modifications

The unnatural inositol phosphates Ins(2)P [338], Ins(2,4)P_2, Ins(1,2,6)P_3 [339], Ins(1,3,5)P_3 [324] and 3-deoxy-Ins(1,5,6)P_3 [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)P_3] was initially reported as being equipotent to Ins(1,4,5)P_3 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)P_3 is only a weak agonist more than an order of magnitude less potent than Ins(1,4,5)P_3 [EC_{50} 3.6 μM, cf. Ins(1,4,5)P_3 0.21 μM]. It was also demonstrated that treatment of Ins(1:2,4,5)P_3 with acid affords a mixture of Ins(1,4,5)P_3 and Ins(2,4,5)P_3 with receptor binding and calcium release properties similar to Ins(1,4,5)P_3, and it may therefore well be possible that the Ins(1:2,4,5)P_3 used in the initial experiments was contaminated with Ins(1,4,5)P_3 due to decomposition.

Synthetic Ins(2,4,5)P_3 was found to effect calcium release, being some 12-68 fold less potent than Ins(1,4,5)P_3 [320-322,324,342]. Tegge *et al.* [174,342] obtained

the same EC_{50} value [4 μM; Ins(1,4,5)P_3 0.13 μM] for D-Ins(2,4,5)P_3 and for D-*chiro*-inositol 1,3,4-trisphosphate, an analogue of Ins(2,4,5)P_3 with an axial 1-hydroxyl group. It thus appears that the difference between the two compounds, an equatorial *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 [174,342], this is another example for the stereospecificity of the Ins(1,4,5)P_3 receptor.

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

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

The racemic trissulphate, trissulphonamide, triscarboxymethyl and trismethylphosphonate analogues of Ins(1,4,5)P_3 did not show any biological activity [288,289], neither did the 4,5-dimethylenephosphonate analogue of DL-Ins(4,5)P_2 [345].

There has been a report by Schultz *et al.* [346] that *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 *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 [324]. If these results were to be confirmed, it would imply that the vacuolar receptor of *Neurospora crassa* is different from mammalian receptors, which seems remarkable since 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.

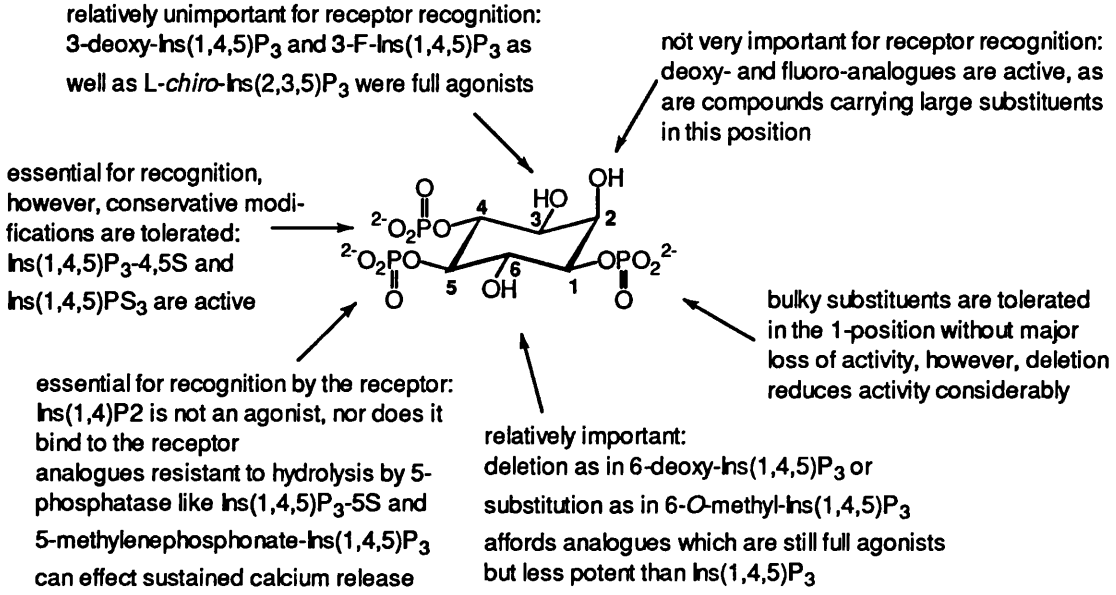


Figure 4.3 Structure-activity relationship for the Ins(1,4,5)P_3-receptor

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 Ins(1,3,4,6)P_4 and Ins(1,4,5)P_3 compete for the same receptor site [94,347]. As 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 Ins(1,3,4,6)P_4 to the 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.

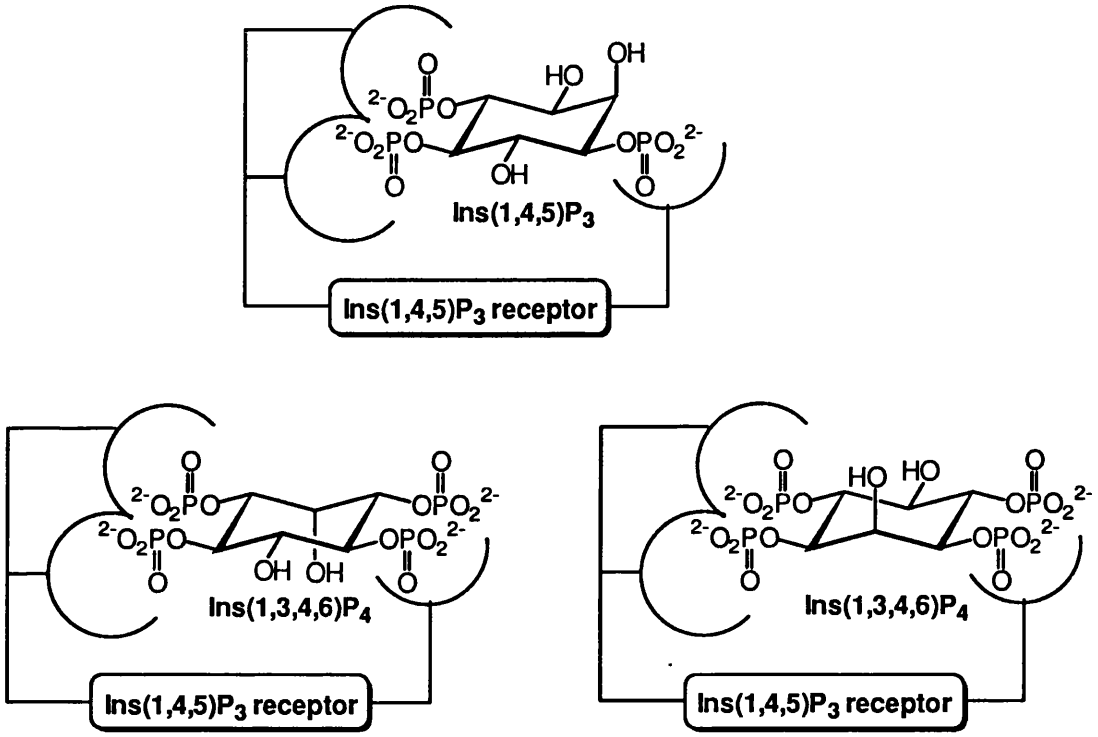


Figure 4.4 Alternative binding conformations of Ins(1,3,4,6)P_4

Both of these alternative binding conformations of Ins(1,3,4,6)P_4 show an additional 2-phosphate moiety in the now equatorial 2-position. As discussed for analogues of 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 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 [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 [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 µM and 171 µM, 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 [285,286] and L-*chiro*-Ins(2,3,5)PS_3 [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 [32,349-354] and decavanadate [22,355-357]; neither of them, however, show specifity for the Ins(1,4,5)P_3-receptor [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 [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 [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 [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-trissulphate, 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 specifity 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)P_3 3-kinase

The specifity of 3-kinase is in some respects greater than that of the Ins(1,4,5)P_3 receptor itself [272], Ins(1,4,5)P_3 and Ins(1,3,4,5)P_3 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)P_2 and Ins(2,4,5)P_3 are both very poor substrates for this enzyme [362,363], and Ins(1:2,4,5)P_3 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)P_3-5S [210,364] and Ins(1,4,5)P_3-4,5-S_2 [348] are substrates for 3-kinase, an additional phosphoro-thioate substitution in the 1-position is not tolerated: Ins(1,4,5)PS_3 is not a substrate for 3-kinase [268,365].

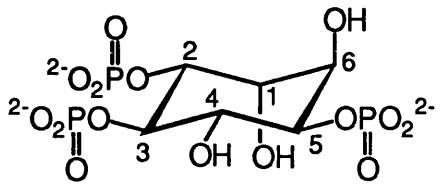
Modifications at the 2-position

DL-2-deoxy-Ins(1,4,5)P_3 was shown to be recognised well by the kinase [320], the apparent K_i in calcium-free medium was 1.6 μM [Ins(1,4,5)P_3 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)P_3. This analogue surprisingly showed an even greater affinity to the 3-kinase (K_i 0.36 μM) than Ins(1,4,5)P_3 in calcium free medium. However, no data indicating whether these analogues act as substrates or inhibitors were given.

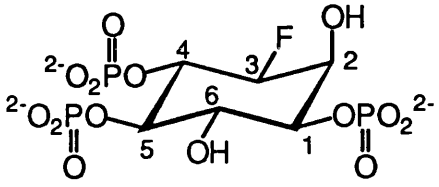
Both DL-2-deoxy-2-fluoro-*scyllo*-Ins(1,4,5)P_3 and DL-2-deoxy-2,2-difluoro-Ins(1,4,5)P_3 appeared to be substrates for 3-kinase [305]. After resolution of the racemic *gem*-difluoride, however, the substrate properties of D- and L-2-deoxy-2,2-difluoro-Ins(1,4,5)P_3 were found to be quite different. Whereas the D-isomer was a substrate for 3-kinase (apparent K_i = 10.2 μM), the L-isomer was a potent inhibitor of this enzyme (K_i 11.9 μM) [316]. The reason for this inhibitory activity is not yet known, however, L-2,2-F_2-Ins(1,4,5)P_3 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 Ins(1,4,5)P_3 analogues, both D-3-deoxy-3-fluoro-Ins(1,4,5)P_3 [176,317] and L-*chiro*-Ins(2,3,5)P_3 [177,332], a D-Ins(1,4,5)P_3 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].



L-*chiro*-Ins(2,3,5)P_3



D-3-F-(1,4,5)P_3

Figure 4.5 3-position modified Ins(1,4,5)P_3 analogues

Ins(1,3,4,5)P_4, the product of Ins(1,4,5)P_3 phosphorylation by 3-kinase, is a weak inhibitor of the enzyme (IC_{50} 90 µM). However, based upon the IC_{50} data it seems unlikely that sufficient Ins(1,3,4,5)P_4 can accumulate to affect the rate of Ins(1,4,5)P_3 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 Ins(1,4,5)P_3 [Ins(1,4,5)P_3-4,5S] was attenuated in comparison to Ins(1,4,5)P_3-5S (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)P_3-5S was a substrate for the 3-kinase with K_m = 5 μM [348], but phosphorylation of this compound occured considerably more slowly than that of the natural substrate [*ca.* 15 % the rate of Ins(1,4,5)P_3]. The 5-methylenephosphonate analogue of Ins(1,4,5)P_3, 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)P_3 is one of the few compounds that is recognised by the highly selective 3-kinase [182]. The kinetics of its metabolism indicate that is a substrate for this enzyme, and the phosphorylation of Ins(1,4,5)P_3 is inhibited competitively by 6-deoxy-Ins(1,4,5)P_3 with an apparent K_i of 5.7 μM [K_m for Ins(1,4,5)P_3, 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)P_3 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)P_3 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 [^{3}H]-Ins(1,4,5)P_3 phosphorylation (IC_{50} 327 μM) [324].

Summary

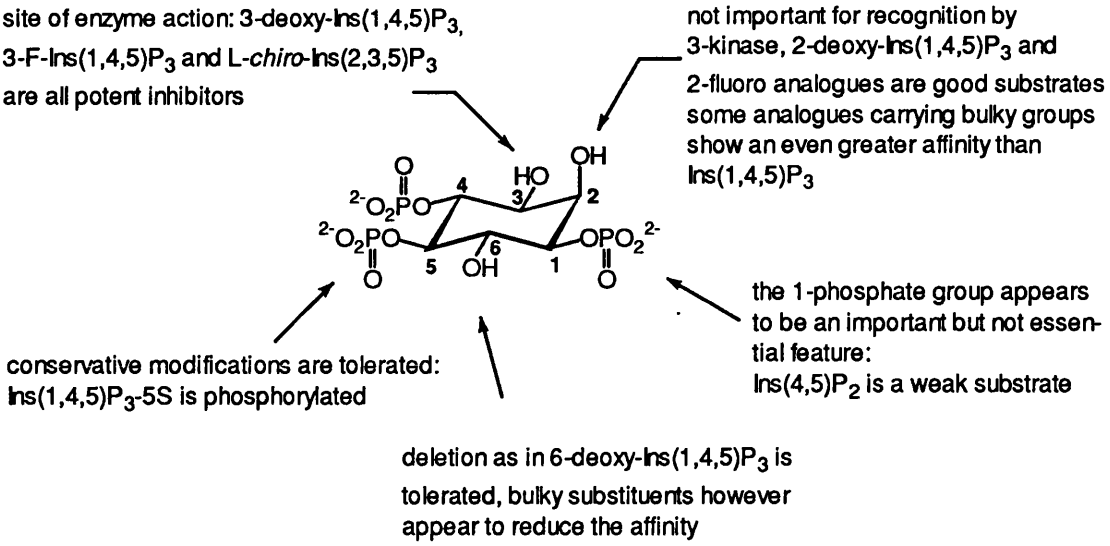


Figure 4.6 Structure-recognition relationship for 3-kinase

4.5 Structure-Recognition Studies on Ins(1,4,5)P_3 5-phosphatase

In contrast to the Ins(1,4,5)P_3 receptor and the Ins(1,4,5)P_3 3-kinase, the Ins(1,4,5)P_3 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)P_2 is a very poor substrate (K_m = 215 μM) [348].

Modifications at the 2-position

A number of racemic Ins(1,4,5)P_3 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-Ins(1,4,5)P_3 and most of the other 2-modified analogues showed an even greater affinity for 5-phosphatase than Ins(1,4,5)P_3.
2-Deoxy-2-fluoro-*scyllo*-Ins(1,4,5)P_3 was found to be well recognised by the phosphatase but to be a weaker substrate than Ins(1,4,5)P_3 [305]
The 5-phosphatase substrate properties of D- and L-2-deoxy-2,2-difluoro-Ins(1,4,5)P_3 are quite different. Whereas D-2-deoxy-2,2-difluoro-Ins(1,4,5)P_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 μM) [316]. Since 5-phosphatase is known to be specific for the D-isomer of Ins(1,4,5)P_3, and analogues in the L-conformation are usually not recognised very well by this enzyme [cf. L-Ins(1,4,5)P_3, K_i = 124 μM [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 μM [D-Ins(1,4,5)P_3: K_i = 13.1 μM].

Thus, it may be concluded that the 2-hydroxyl group may be involved in substrate recognition by the Ins(1,4,5)P_3 5-phosphatase, altough it does not appear to be an essential feature.

Modifications at the 3-position

3-Deoxy-Ins(1,4,5)P_3 was found to be a good substrate, binding with a slightly higher affinity than Ins(1,4,5)P_3 itself.
3-Deoxy-3-fluoro-Ins(1,4,5)P_3 was a substrate for erythrocyte 5-phosphatase and inhibited the dephosphorylation of [^{3}H]-Ins(1,4,5)P_3 with an apparent K_i of 3.9 μM [333].
L-*chiro*-Ins(2,3,5)P_3, which can be visualised as Ins(1,4,5)P_3 with an inverted 3-hydroxyl group, was found to be a very potent inhibitor of 5-phosphatase (K_i 7.7 μM) [177,332,333]. This is somewhat surprising, since the modification of the stereochemisty 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)P_3 in solution and / or bound to 5-phosphatase is sufficiently different from Ins(1,4,5)P_3 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)P_3 or;
b) the inhibition may be the result of non-productive binding of L-*chiro*-Ins(2,3,5)P_3 in an inverted and rotated mode. In this arrangement the analogue would mimic four elements of Ins(1,4,5)P_3 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)P_3 in this mode mimics an equatorial 2-phosphate group of an Ins(1,4,5)P_3 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)P_3 [Ins(2,4,5)P_3 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)P_3 now mimics the axial 6-hydroxyl group of an Ins(1,4,5)P_3 analogue. As discussed below (Modifications at the 6-position), the equatorial 6-hydroxyl group of Ins(1,4,5)P_3 may play an important role in the mechanism of 5-phosphatase catalysed hydrolysis and the inhibitory properties of L-*chiro*-Ins(2,3,5)P_3 may possibly be ascribed to the pseudo axial 6-hydroxyl group in the inverted binding conformation b).

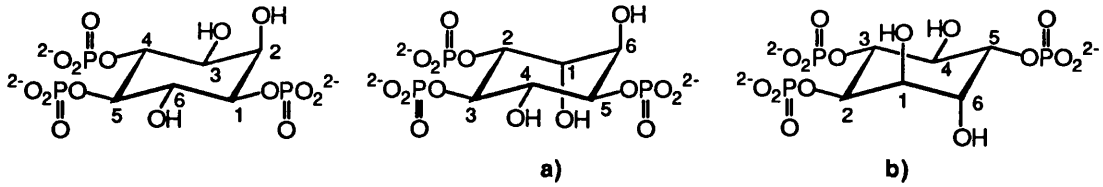


Figure 4.7 Alternative binding conformations of L-*chiro*-Ins(2,3,5)P_3 to 5-phosphatase

Modifications at the 5-position

It has been demonstrated by the 5-phosphorothioate analogue Ins(1,4,5)P_3-5S, that modification at the 5-position creates a potent inhibitor of the enzyme Ins(1,4,5)P_3-5-phosphatase (K_i 6.8 μM) [210,211].
The 5-methylenephosphonate analogue of D-Ins(1,4,5)P_3 is another long-lived agonist of calcium mobilisation [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)P_3 have been published, however no details on interactions of these 5-modified Ins(1,4,5)P_3 analogues with the 5-phosphatase have been reported to date.

Modifications at the 6-position

D-6-deoxy-Ins(1,4,5)P_3 [182] inhibited the dephosphorylation of [^{32}P]-Ins(1,4,5)P_3 by erythrocyte 5-phosphatase with a K_i of 76 μM [K_m for Ins(1,4,5)P_3 40 μM]. D-6-deoxy-Ins(1,4,5)P_3 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)P_3 and the approximately 4-fold lower affinity of DL-6-methoxy-Ins(1,4,5)P_3 for the aortic smooth muscle 5-phosphatase [324] underline the non-selectivety 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 [324]. It is possible that the role of the 6-hydroxyl group of Ins(1,4,5)P_3 is similar to the role that the 6-hydroxyl group of Ins(1)P plays in the dephosphorylation of this compound by inositol monophosphatase, *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)PS_3 was resistant to degradation by 5-phosphatase [270]. DL-Cyclohexane 1,2,4-trisphosphate was a weak competitive inhibitor of 5-

phosphatase (K_i 124 μM), and no inorganic phosphate release due to hydrolysis by the enzyme was detected [324].

Summary

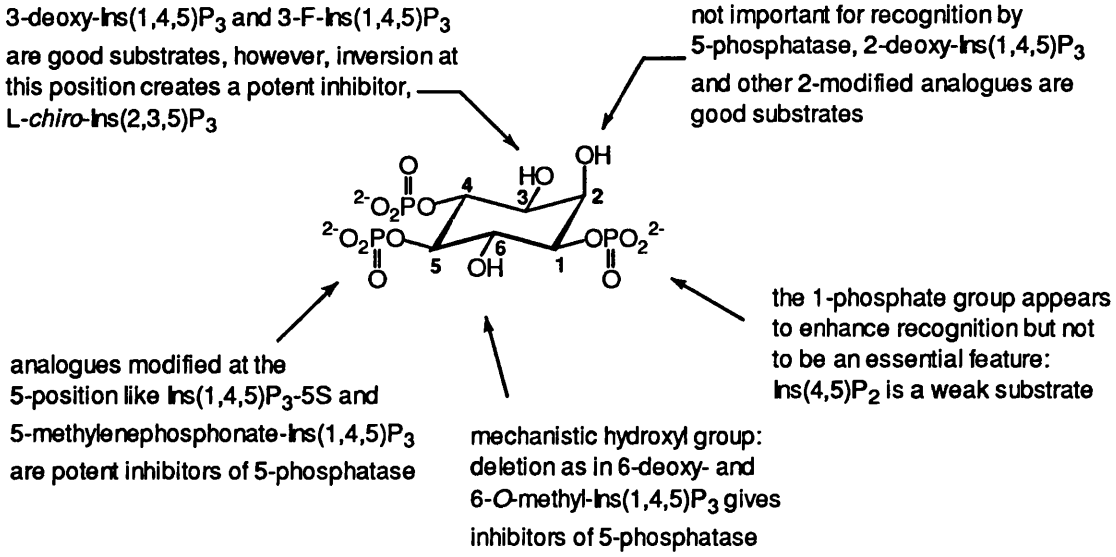


Figure 4.8 Structure-recognition relationship for 5-phosphatase

RESULTS AND DISCUSSION

5 Aims of this work

As outlined in the General Section, there is currently great interest in the second messenger D-*myo*-inositol 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-*myo*-inositol 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)P_3, *myo*-inositol 4,5-bisphosphate 1-phosphorothioate Ins(1,4,5)P_3-1S, 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)P_3. It was also thought that this method might be used to link Ins(1,4,5)P_3 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)P_3-1S was to be employed in the preparation of [^{35}S]-Ins(1,4,5)P_3-1S, a novel radioligand for the Ins(1,4,5)P_3 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)P_3 and L-Ins(1,4,5)P_3 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 Ca^{2+}.

The next objective was the preparation of analogues of Ins(1,4,5)P_3 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 mimick the phosphate arrangement of the known partial agonist Ins(1,3,4,6)P_4 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)P_4 3-phosphatase inhibitor, *myo*-inositol 1,4,5-trisphosphate-3-phosphorothioate, Ins(1,3,4,5)P_4-3S was devised. A key step included the optical resolution of a valuable intermediate in the synthesis of 3-position modified Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 analogues, DL-3-*O*-allyl-6-*O*-benzyl-1-*O*-*p*-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)P_3-3S.

Methods to replace the Ins(1,4,5)P_3 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 -CH_2-COOH group linked directly to an inositol ring carbon should be possible. Sterically, the -CH_2-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.

6 Synthesis of DL-*myo*-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)PS_3 and the 5-phosphorothioate Ins(1,4,5)P_3-5S 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 [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-*myo*-inositol was synthesised essentially according to the procedure of Gigg *et al.* [149,168].

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-*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-*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 *N,N*-dimethylformamide) gave 3,6-di-*O*-benzyl-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol **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 **124** was allylated. Selective allylation of the tetrol was accomplished by first forming a tin-complex [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 [168]) to the reaction mixture prior to the addition of allyl bromide [167]. The role of caesium fluoride in this reaction is discussed in Chapter 2.1.5.

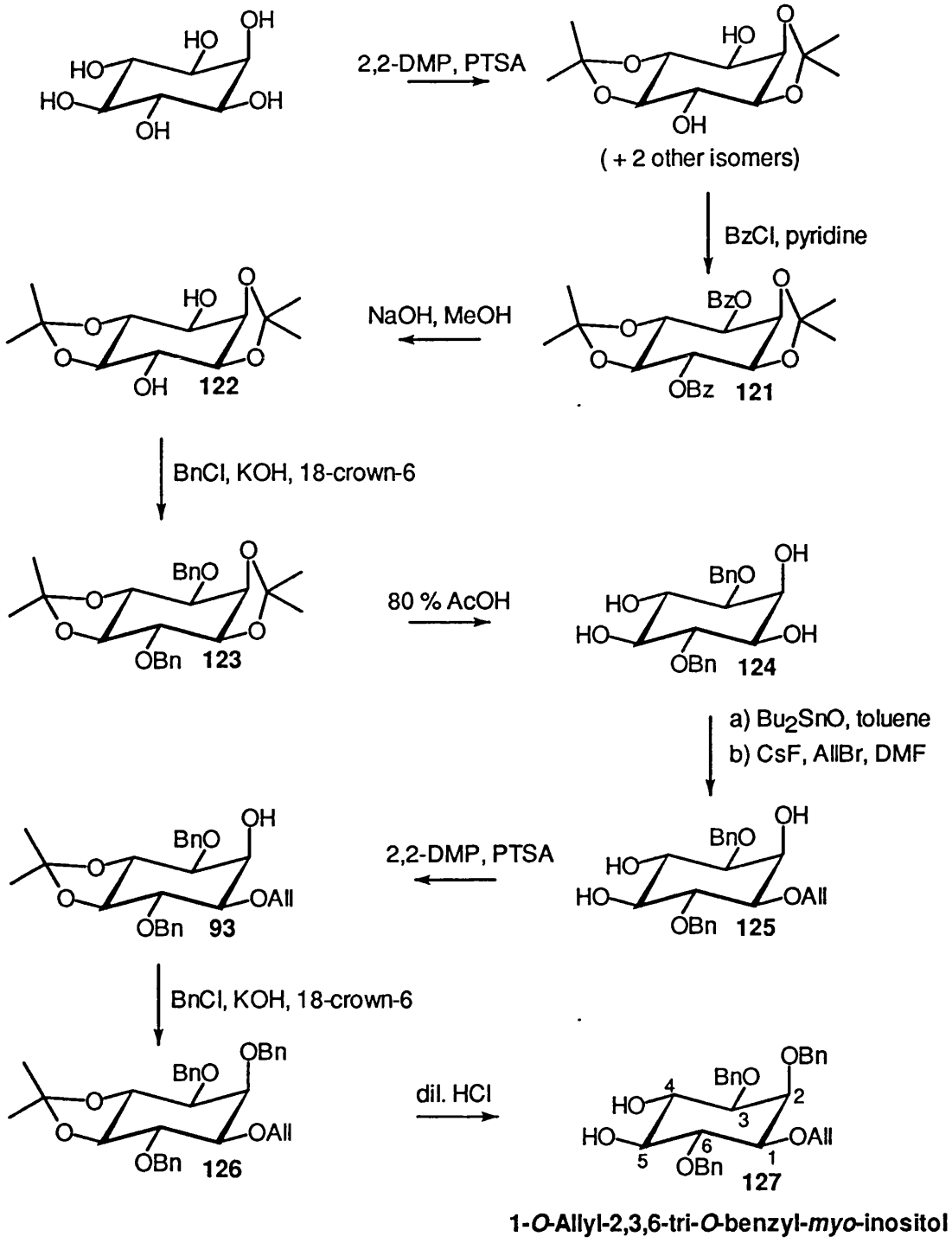
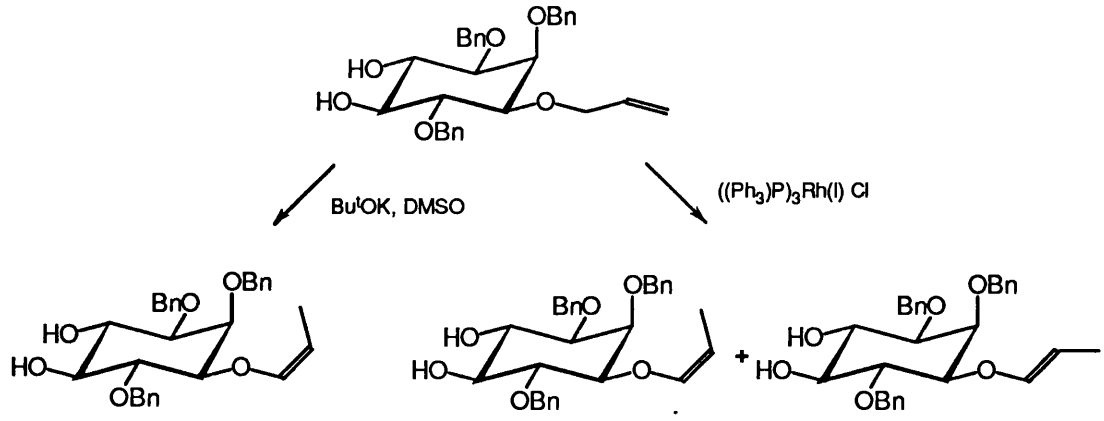


Figure 6.1 Synthesis of 1-*O*-Allyl-2,3,6-tri-*O*-benzyl-*myo*-inositol

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-*myo*-inositol **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 (I) 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^{t}OK) in DMSO [137].



cis / trans ca. 5:1

Figure 6.2 Isomerisation of the 1-*O*-allyl group

Yields were found to be higher when Bu^{t}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 Wilkinsons´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=C*H*-CH_3 and the *C*H=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 C*H*=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 δ = 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: δ = 122.56 ppm.

The strong p_π-d_π 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 dialkoxy(dialkylamino)phosphine. In this class of compounds the p_π-d_π 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.

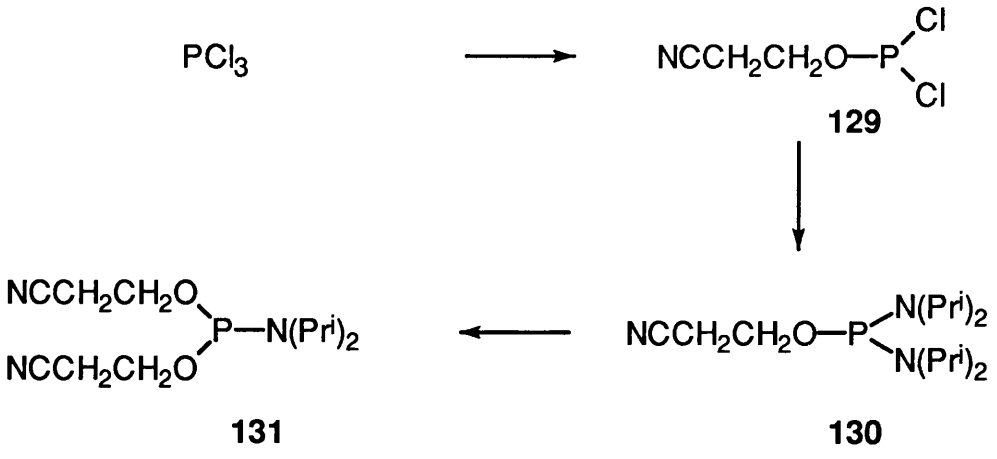


Figure 6.3 Synthesis of bis(2-cyanoethoxy)(diisopropylamino)phosphine

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-*myo*-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*-diisopropylaminophosphine [226] and 1*H*-tetrazole in dry dichloromethane to give the bisphosphite triester. ^{31}P-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^tOOH) [236] to the cooled reaction mixture. The ^{31}P-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.

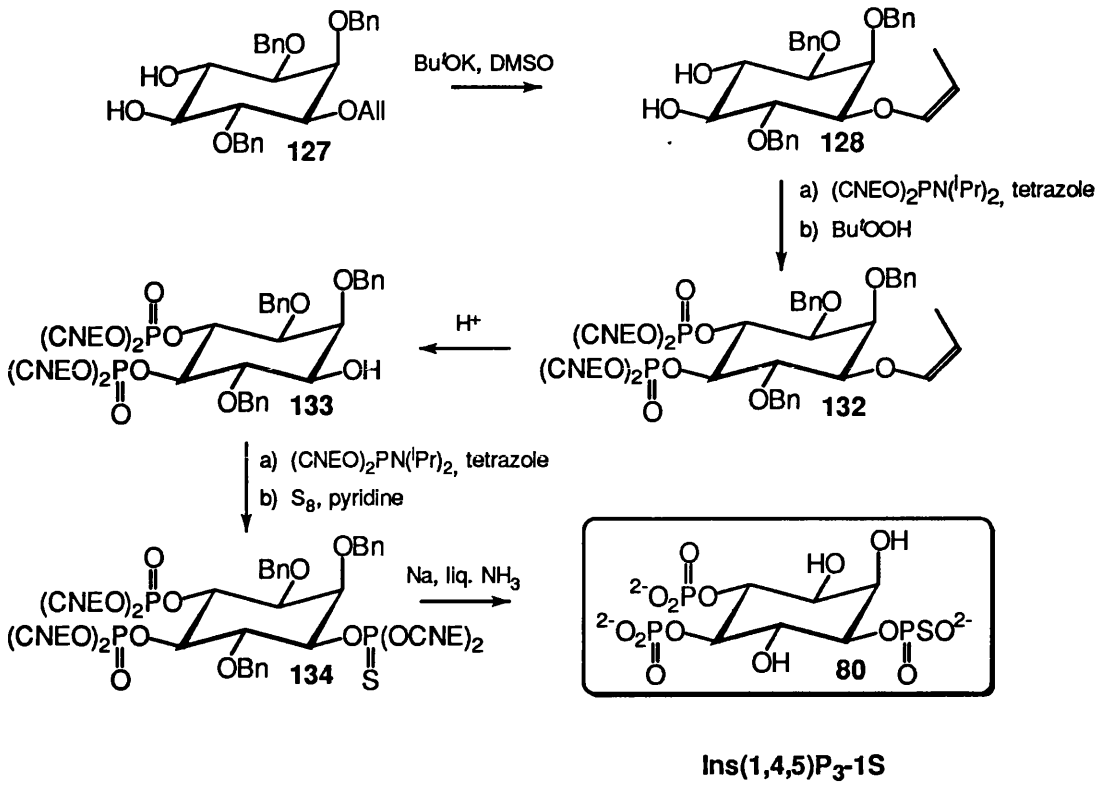


Figure 6.4 Synthesis of DL-*myo*-inositol 4,5-bisphosphate 1-phosphorothioate

The prop-1-enyl ether was cleaved by acidic hydrolysis with methanolic hydrochloric acid [140] or $HgO\text{-}HgCl_2$ [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 $HgO\text{-}HgCl_2$ 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 ^1H-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).

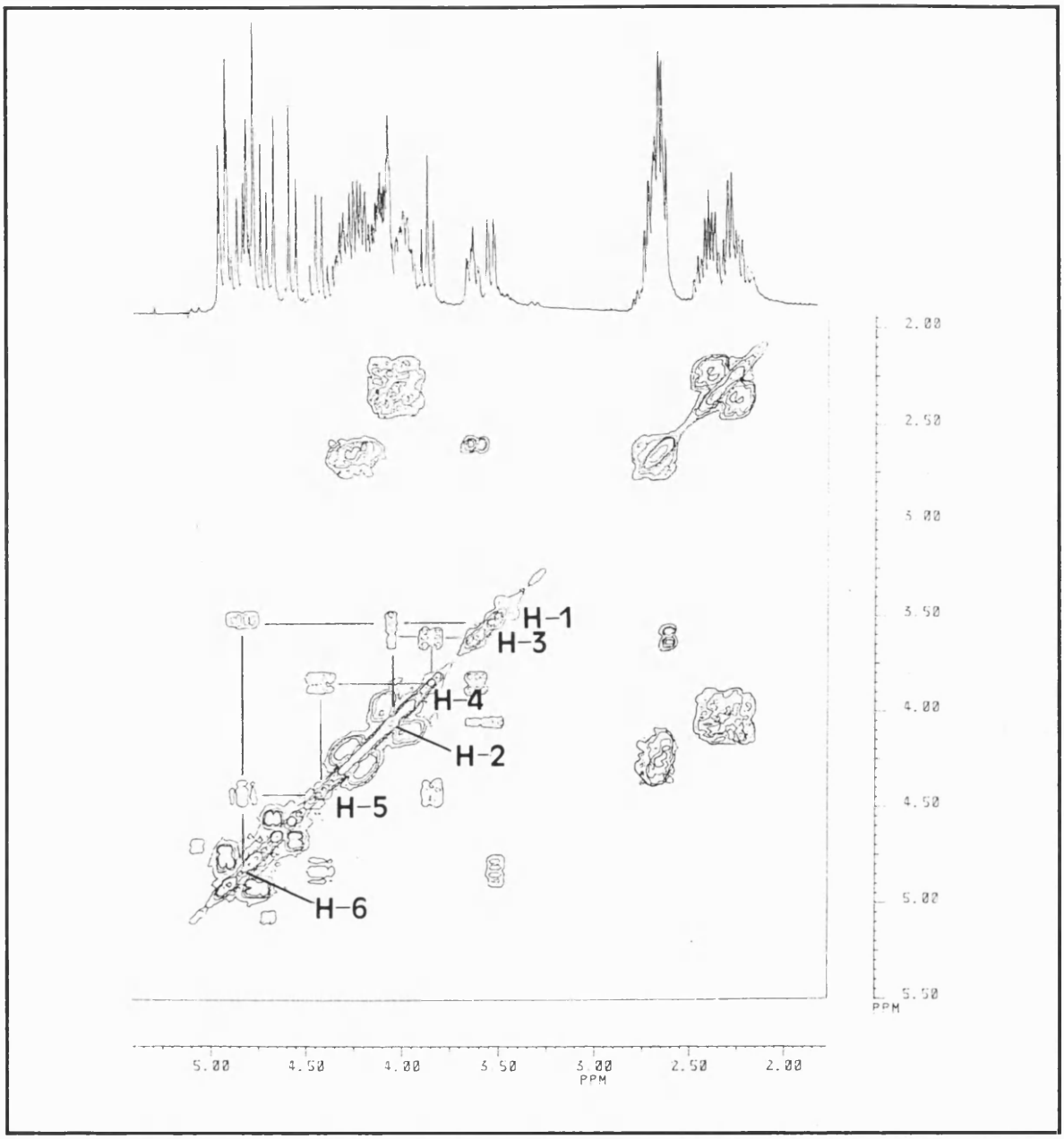


Figure 6.5 COSY spectrum of 133

The free hydroxyl group was then phosphitylated and the phosphite sulphoxidised with an excess of sulphur in pyridine [233] to give DL-2,3,6-tri-*O*-benzyl-4,5-bis[di(2-cyanoethoxy)phospho]-1-[bis(2-cyanoethoxy)thiophospho]-*myo*-inositol. ^{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 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}P-NMR spectroscopy (Figure 6.6) showed that the product possessed a single phosphorothioate group (δ_P 42.1 ppm) and two phosphate groups (δ_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).

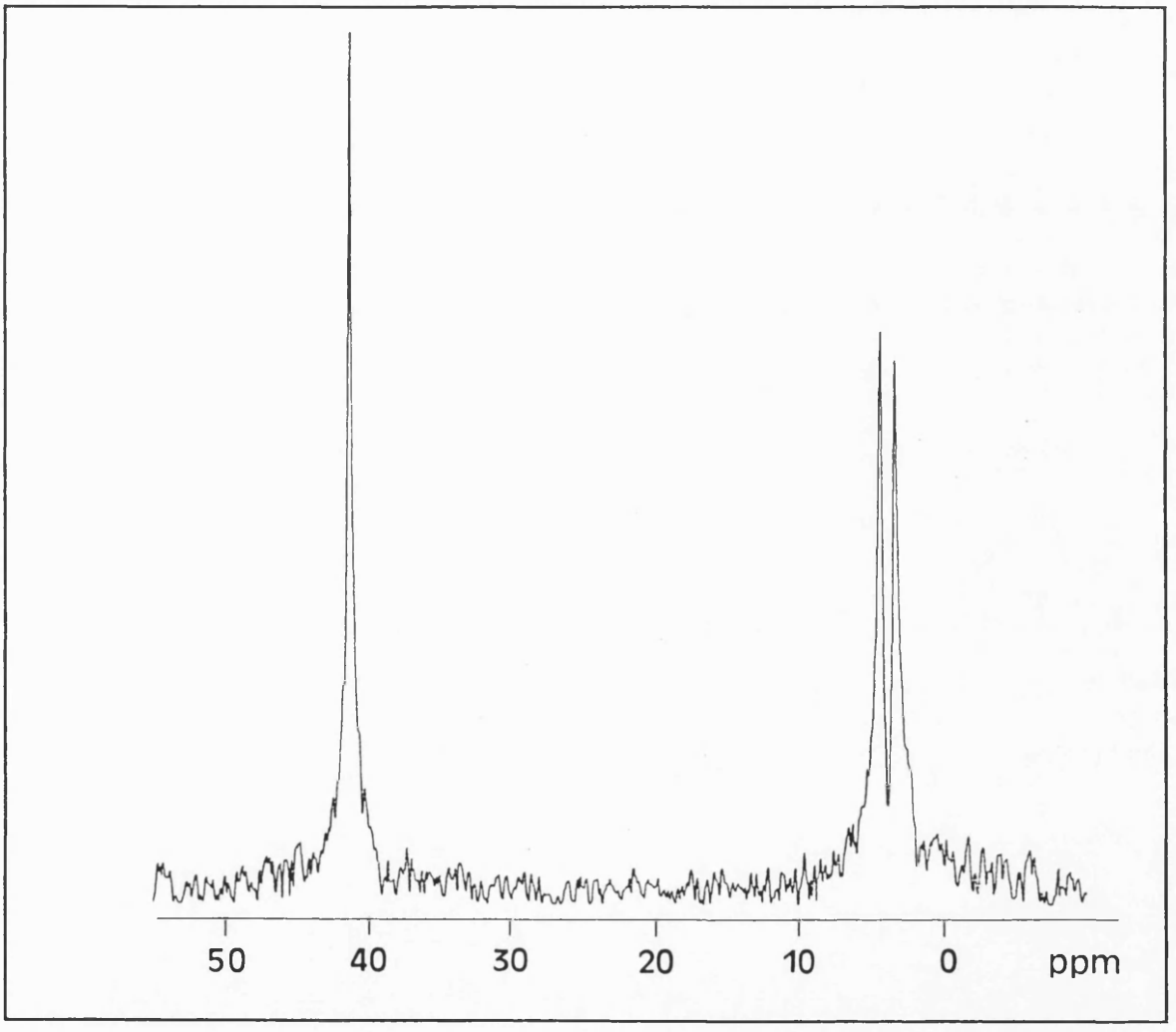


Figure 6.6 ^{31}P-NMR spectrum of 80

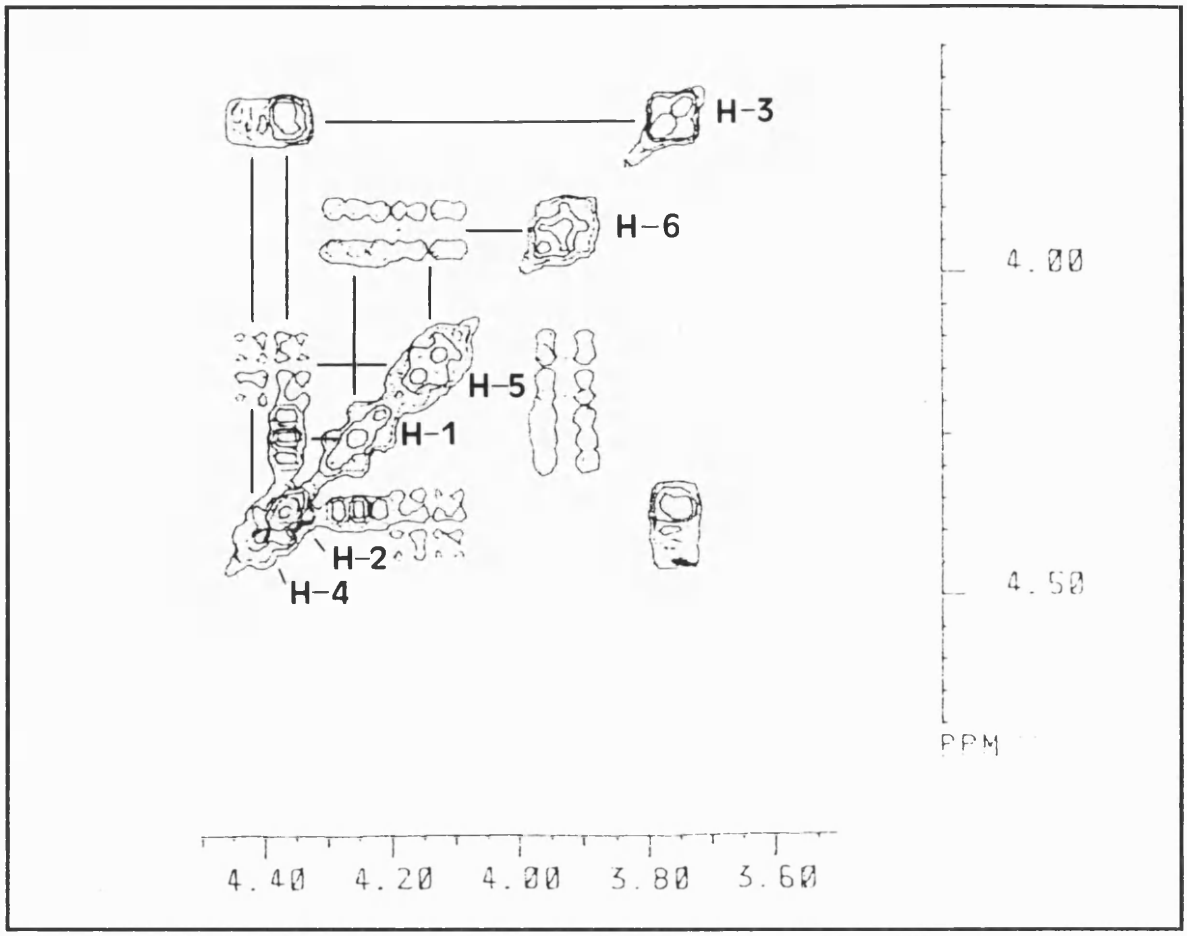


Figure 6.7 COSY spectrum of 80

6.4 [^{35}S]-DL-*myo*-inositol 4,5-bisphosphate 1-phosphorothioate

The synthesis of the metabolically stable radioligand D-[^{35}S(U)]-*myo*-inositol 1,4,5-trisphosphorothioate [^{35}S]-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 Ins(1,4,5)P_3 receptor and another site that displayed only low affinity for Ins(1,4,5)P_3.

In cooperation with NEN-DuPont, racemic 1,2,4-tri-*O*-benzyl-5,6-bis[di(2-cyanoethoxy)phospho]-*myo*-inositol was used to synthesise the radiolabelled DL-[^{35}S]-Ins(1,4,5)P_3-1S, which is a novel radioligand for the 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.

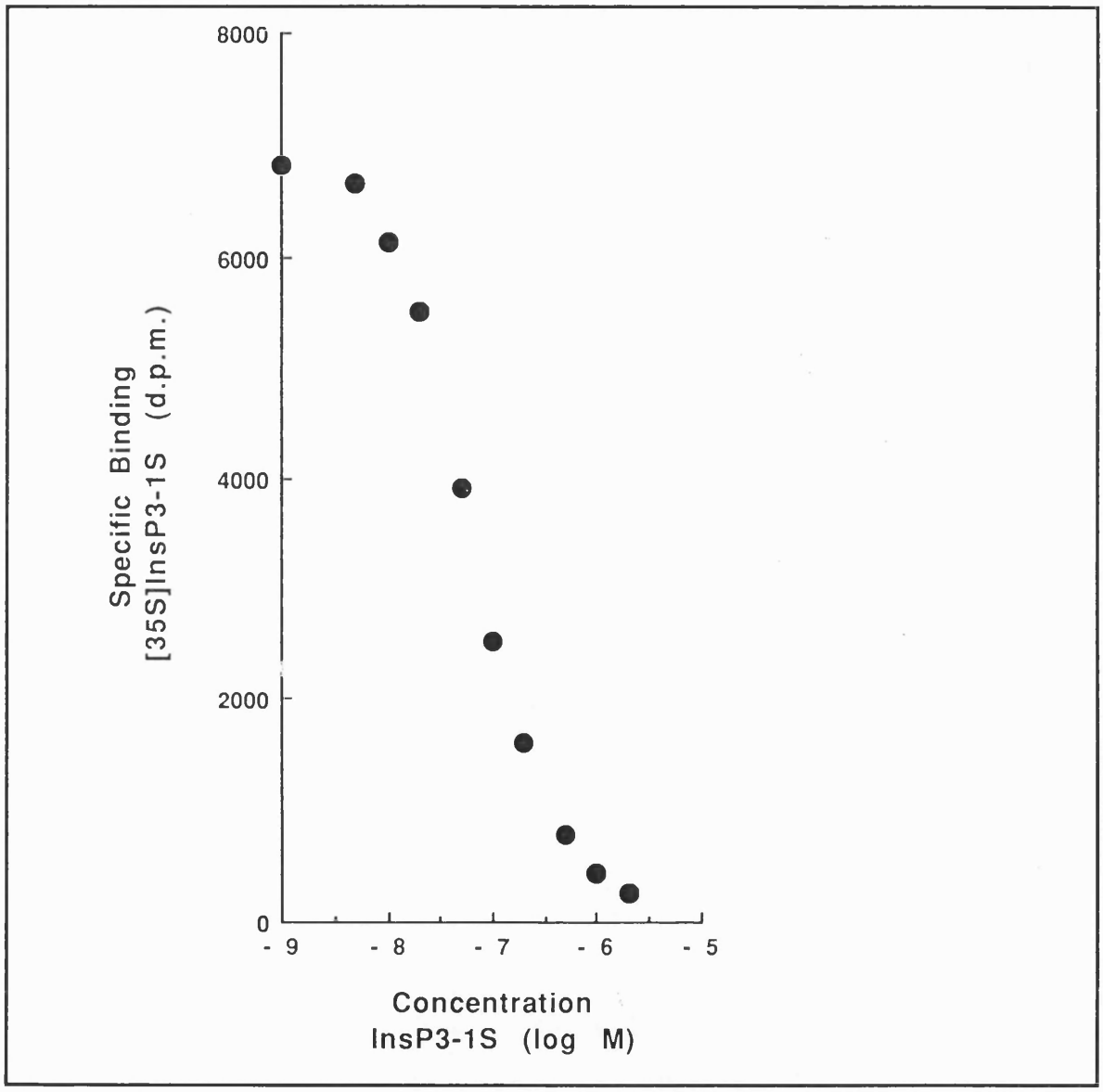


Figure 6.8 Displacement of [^{35}S]-Ins(1,4,5)P_3-1S by Ins(1,4,5)P_3-1S

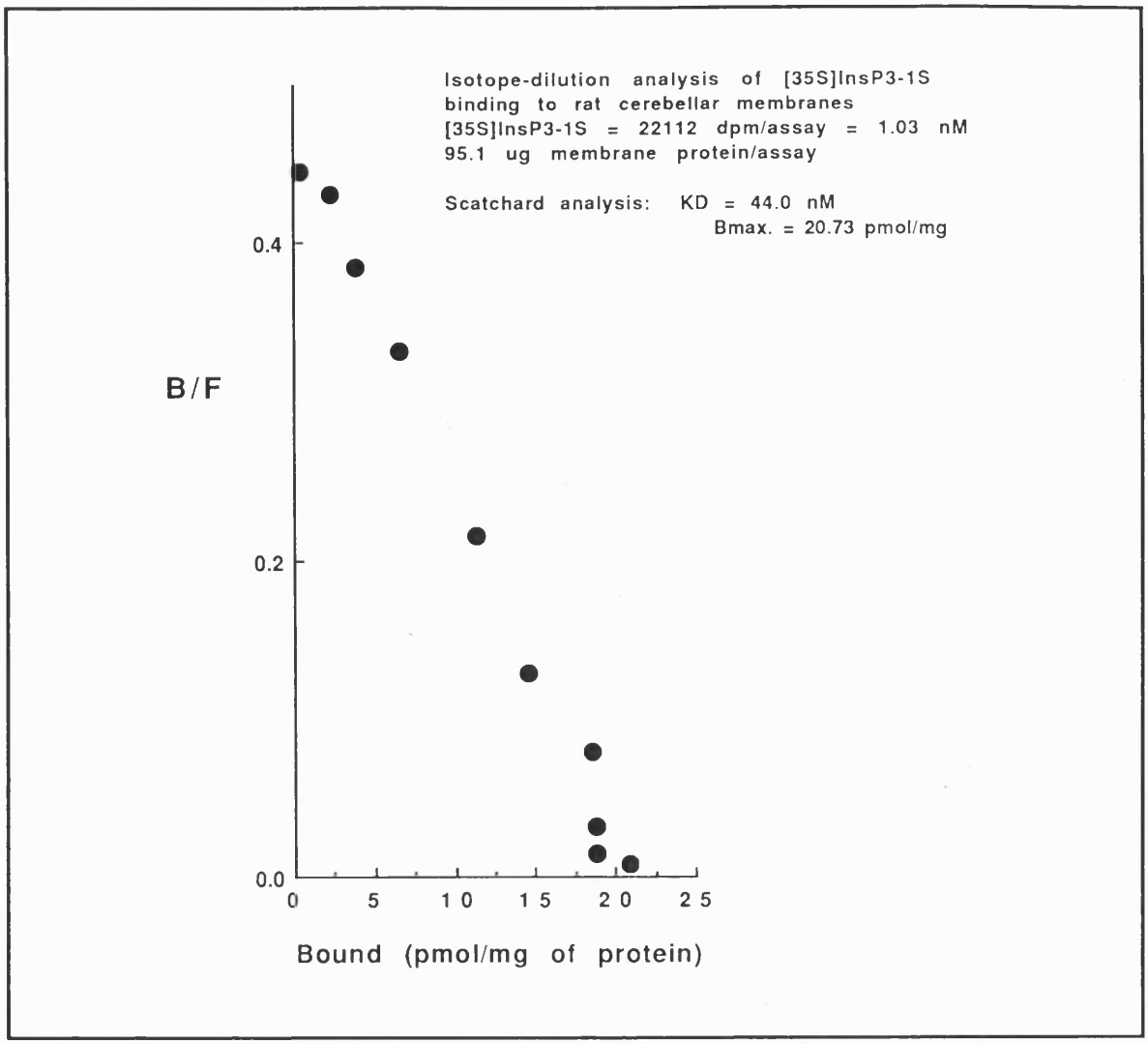


Figure 6.9 Scatchard transformation of [^{35}S]-Ins(1,4,5)P_3-1S binding data

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 [^{35}S]-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. [^{35}S]-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 [^{35}S]-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.

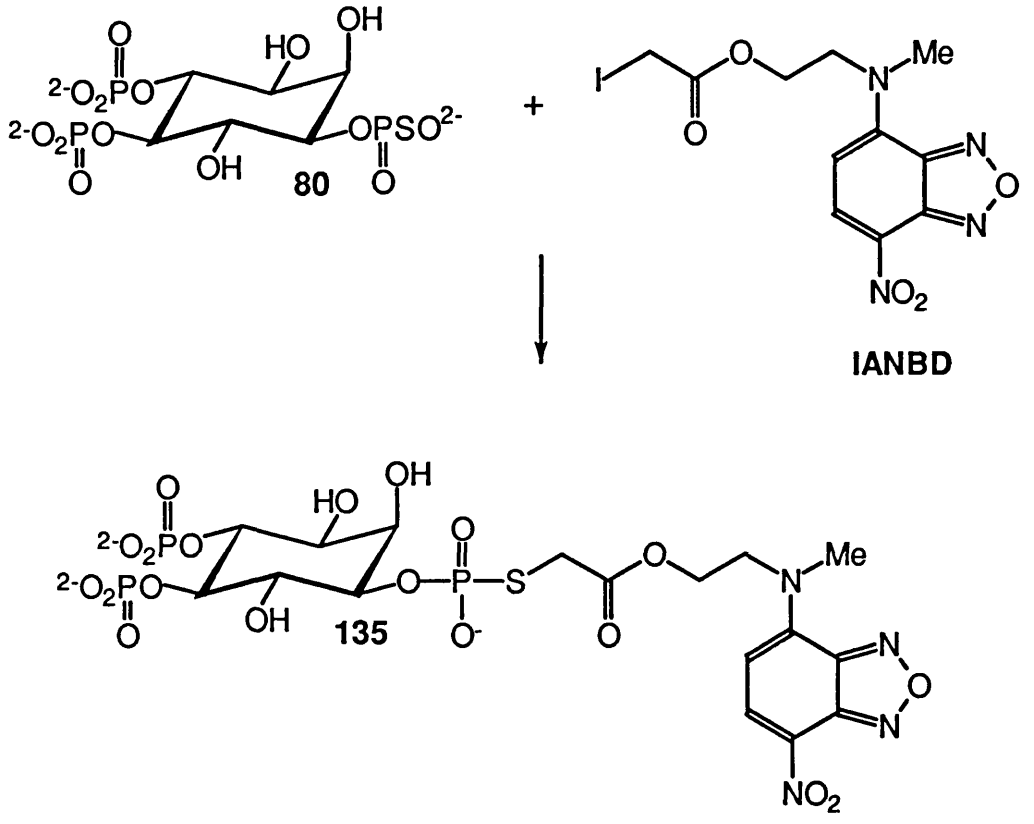


Figure 6.10 Synthesis of fluorescently labelled DL-Ins(1,4,5)P_3-1S

Nitrobenzoxadiazole (NBD) derivatives, such as the iodoacetate *N*-{2-(iodoacetoxy)ethyl]-*N*-methyl}amino-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 [376]. Such a probe appeared ideal to study the interactions of a fluorescently tagged Ins(1,4,5)P_3 with the intracellular receptor and the metabolic enzymes 5-phosphatase and 3-kinase.

Reaction of the phosphorothioate analogue DL-Ins(1,4,5)P_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 *ca.* 800 mmol dm^{-3} TEAB (yield 57 % of a dark orange glass). ^{31}P-NMR spectroscopy (Figure 6.11) clearly showed that the signal for the 1-phosphorothioate group (δ_P 42.1 ppm) of **80** had disappeared. Instead, a new signal at δ_P = 19.9 ppm was found for **135**.

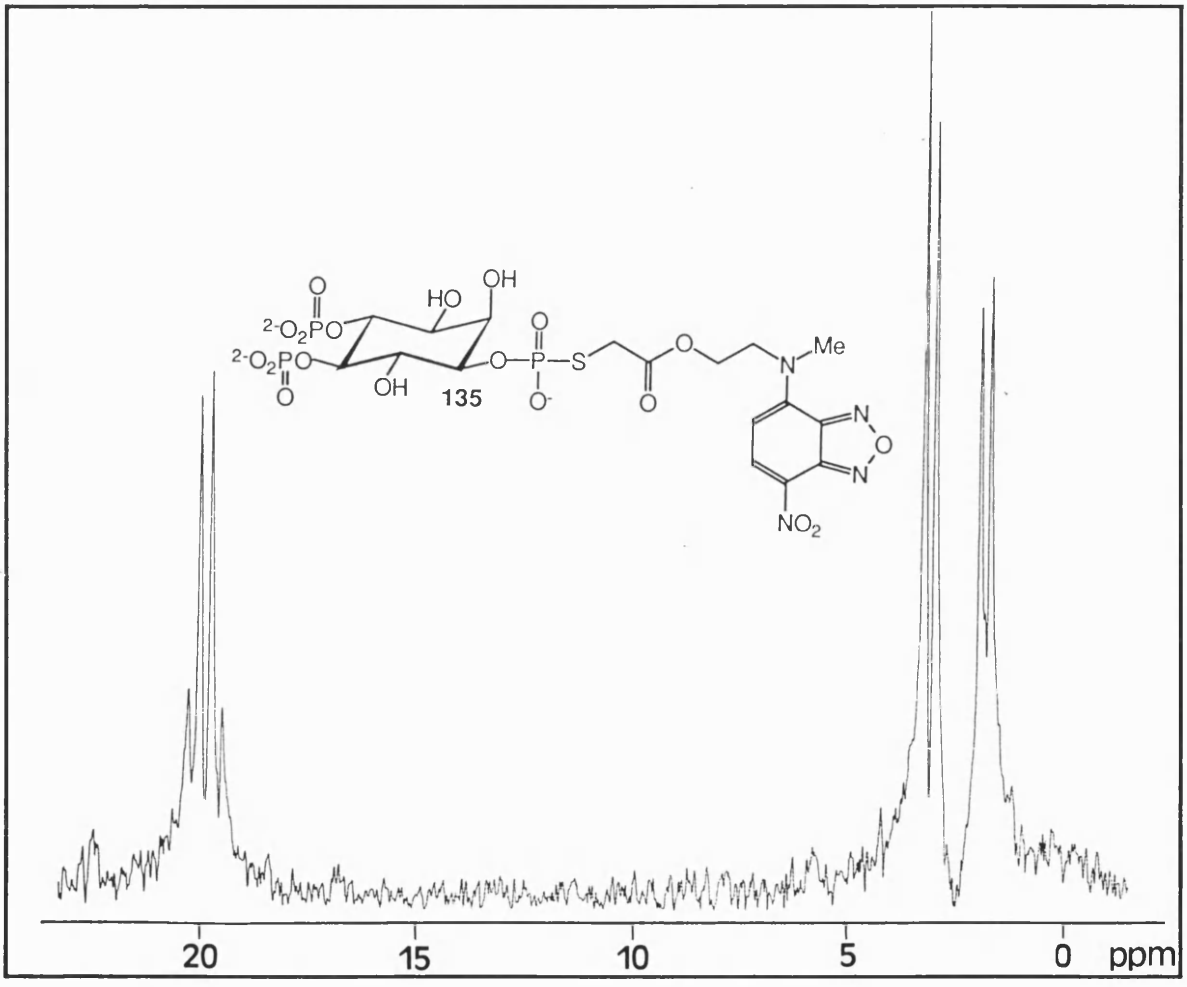


Figure 6.11 ^{1}H-coupled ^{31}P-NMR spectrum of 135

Chemical shifts of about 20 ppm in the ^{31}P-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.

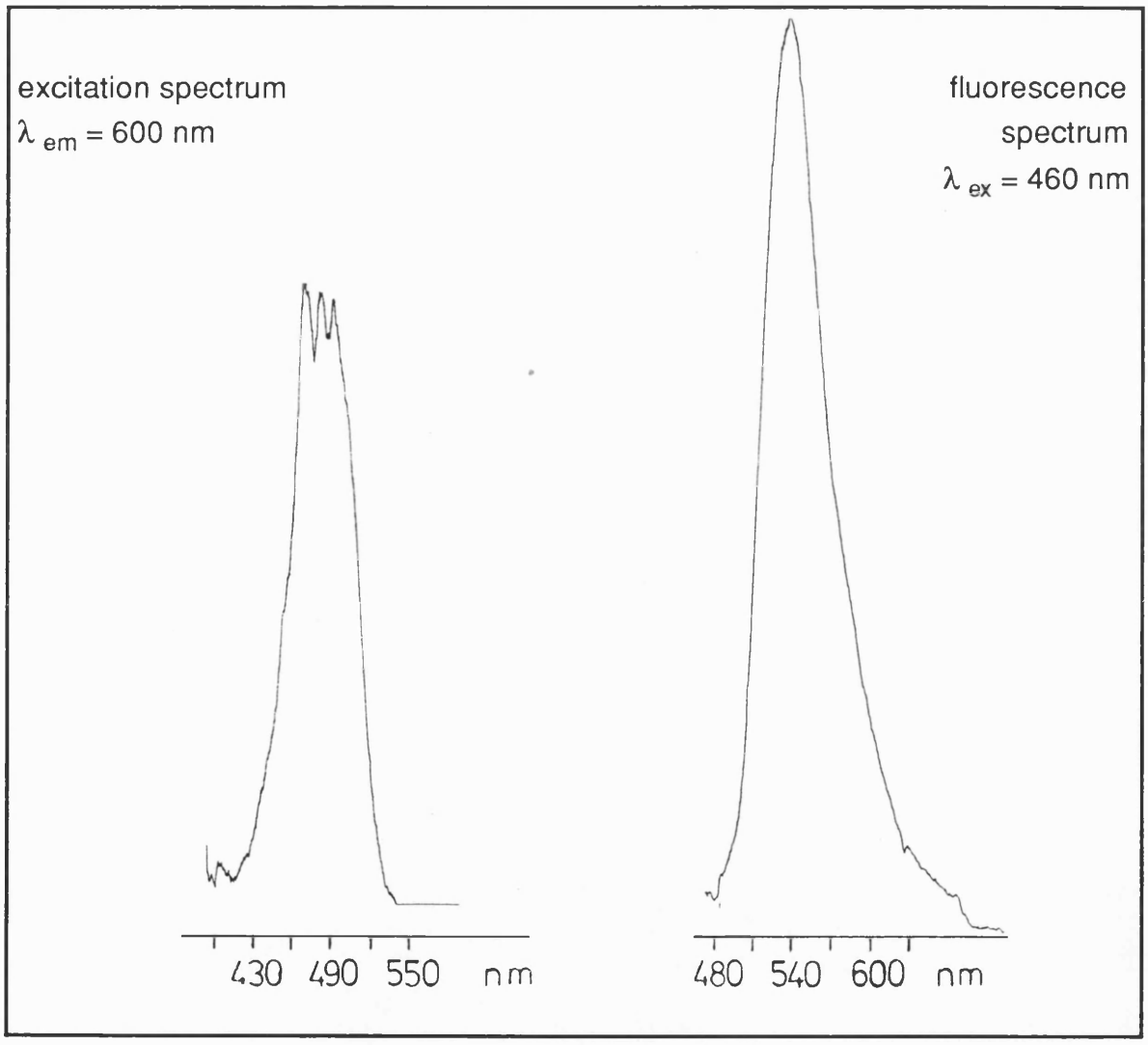


Figure 6.12 UV fluorescence spectrum of 135

Compound **135** was potent at mobilising ATP-sequestered intracellular Ca^{2+} from permeabilised cells and was recognised by the Ins(1,4,5)P_3 receptor with surprisingly high affinity [only *ca.* 6 fold less than Ins(1,4,5)P_3] in spite of the bulky additional group. The synthesis of **135** thus provides the first example of a biologically active Ins(1,4,5)P_3 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)P_3 analogues with relatively bulky groups in this position (see Chapter 4.1) were also almost equipotent to Ins(1,4,5)P_3 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 enviromentally 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 Ca^{2+}-release studies on DL-Ins(1,4,5)P_3-1S **80** and the fluorescent probe **135** (IP_3-1S-NBD). The assays were performed on electrically permeabilised SH-SY5Y neuroblastoma cells as described in [364], except that the cells were permeabilised using 3 pulses of a 3 µF capacitor rather then 12 pulses. The EC_{50} 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)P_3, which has EC_{50} = 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 $Ins(1,4,5)P_3$.

The kinetics of the Ca^{2+}-release were consistent with $Ins(1,4,5)P_3$-1S being a substrate for $Ins(1,4,5)P_3$ 5-phosphatase and 3-kinase.

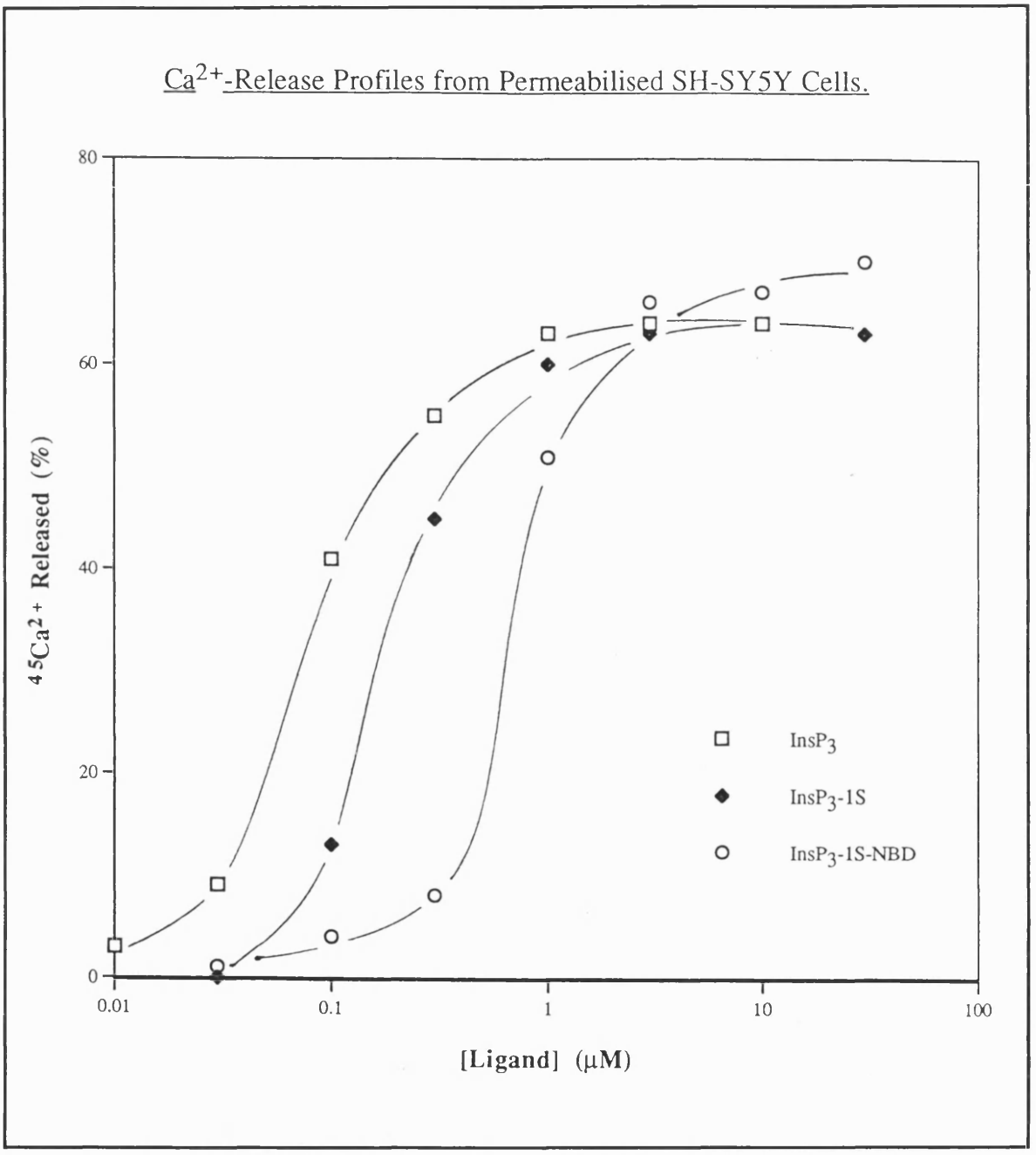


Figure 6.13

The Ca^{2+} mobilising properties of Ins(1,4,5)P_3-1S have also been studied in platelets. For this assay, rabbit platelets were isolated and washed according to Murphy *et al.* [377]. The platelets (10^9 / 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 $^{45}Ca^{2+}$ for 60 min and stimulated with the inositol phosphate analogue for 3 min at r.t. The remaining cell-associated $^{45}Ca^{2+}$ was then determined by rapid filtration. The results for Ins(1,4,5)P_3-1S are shown in Figure 6.14.

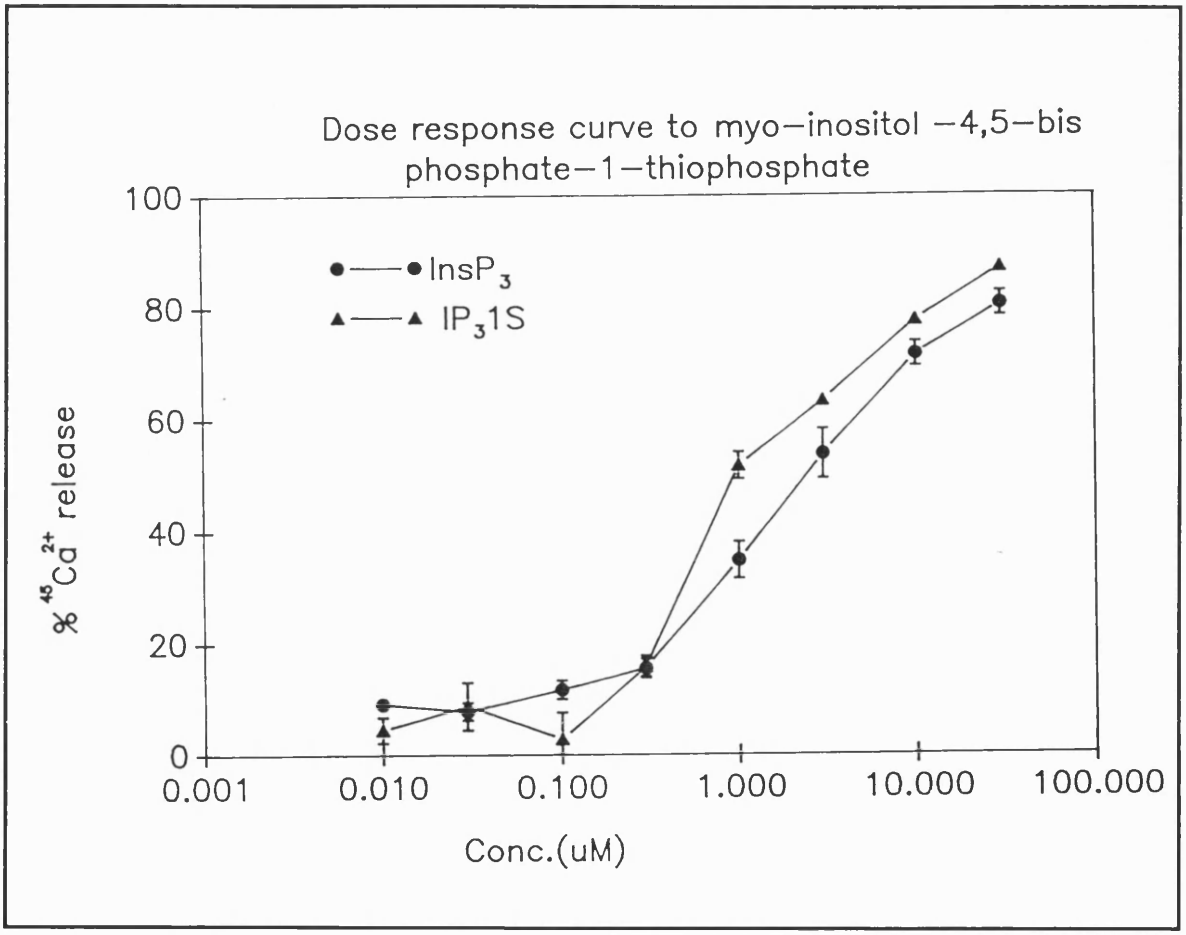


Figure 6.14 Ca^{2+} mobilisation of Ins(1,4,5)P_3 and Ins(1,4,5)P_3-1S in platelets

The results of binding studies are shown in Figure 6.15. [^{3}H]-Ins(1,4,5)P_3 was displaced from cerebellar membranes by adding either Ins(1,4,5)P_3 or

$Ins(1,4,5)P_3$-1S. The IC_{50} values obtained in this isotope dilution experiment were 22.7 nM and 37.6 nM for $Ins(1,4,5)P_3$ and $Ins(1,4,5)P_3$-1S, respectively.

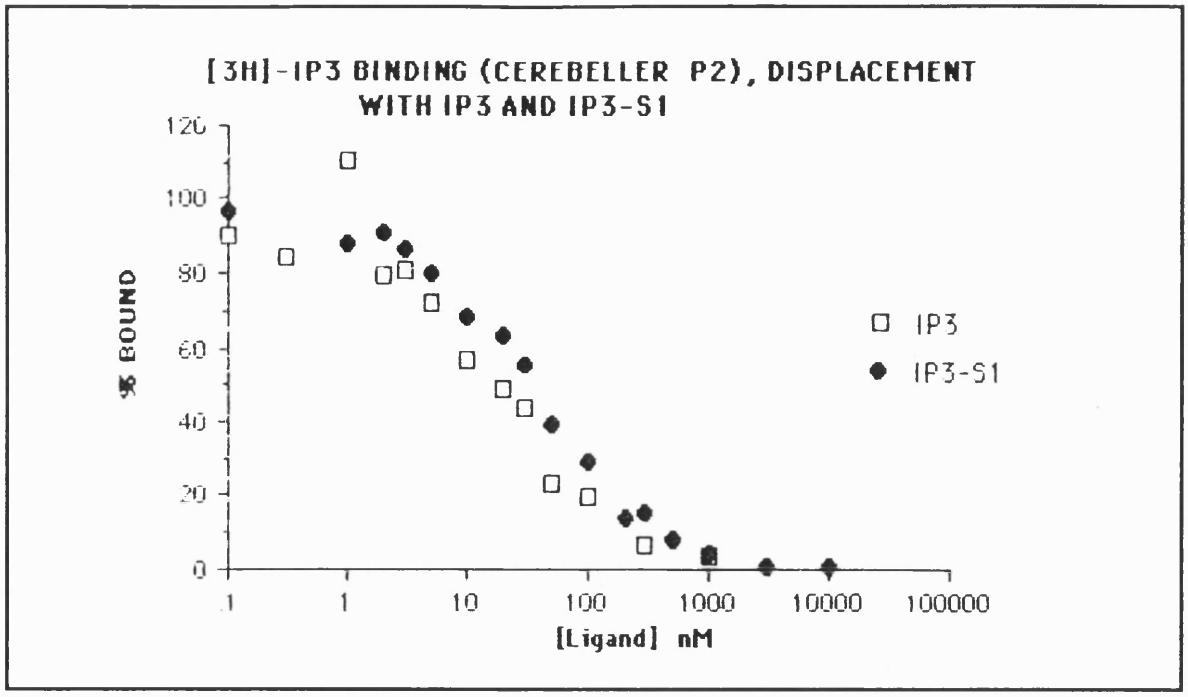


Figure 6.15

7 Synthesis of D-Ins(1,4,5)P_3-1S

7.1 Optical Resolution of 1-*O*-Allyl-2,3,6-tri-*O*-benzyl-*myo*-inositol

Myo-inositol, a *meso*-compound, was used as starting material in the synthesis of Ins(1,4,5)P_3-1S, 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)P_3, it was neccessary to resolve a precursor to synthesise 1D-Ins(1,4,5)P_3-1S.

Thus racemic 1-*O*-allyl-2,3,6-tri-*O*-benzyl-*myo*-inositol **127** was converted into its 4,5-bis-(-)-ω-camphanate ester using (-)-camphanic acid chloride / pyridine. The camphanate method was chosen because (-)-camphanic 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 (+)-camphanic acid chloride now commercially available, the diastereoisomers which can not be crystallised from (-)-camphanate mixtures are now also accessible, making this route even more generally applicable. However, (+)-camphanic acid chloride is markedly more expensive than (-)-camphanic 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 ^{1}H-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-

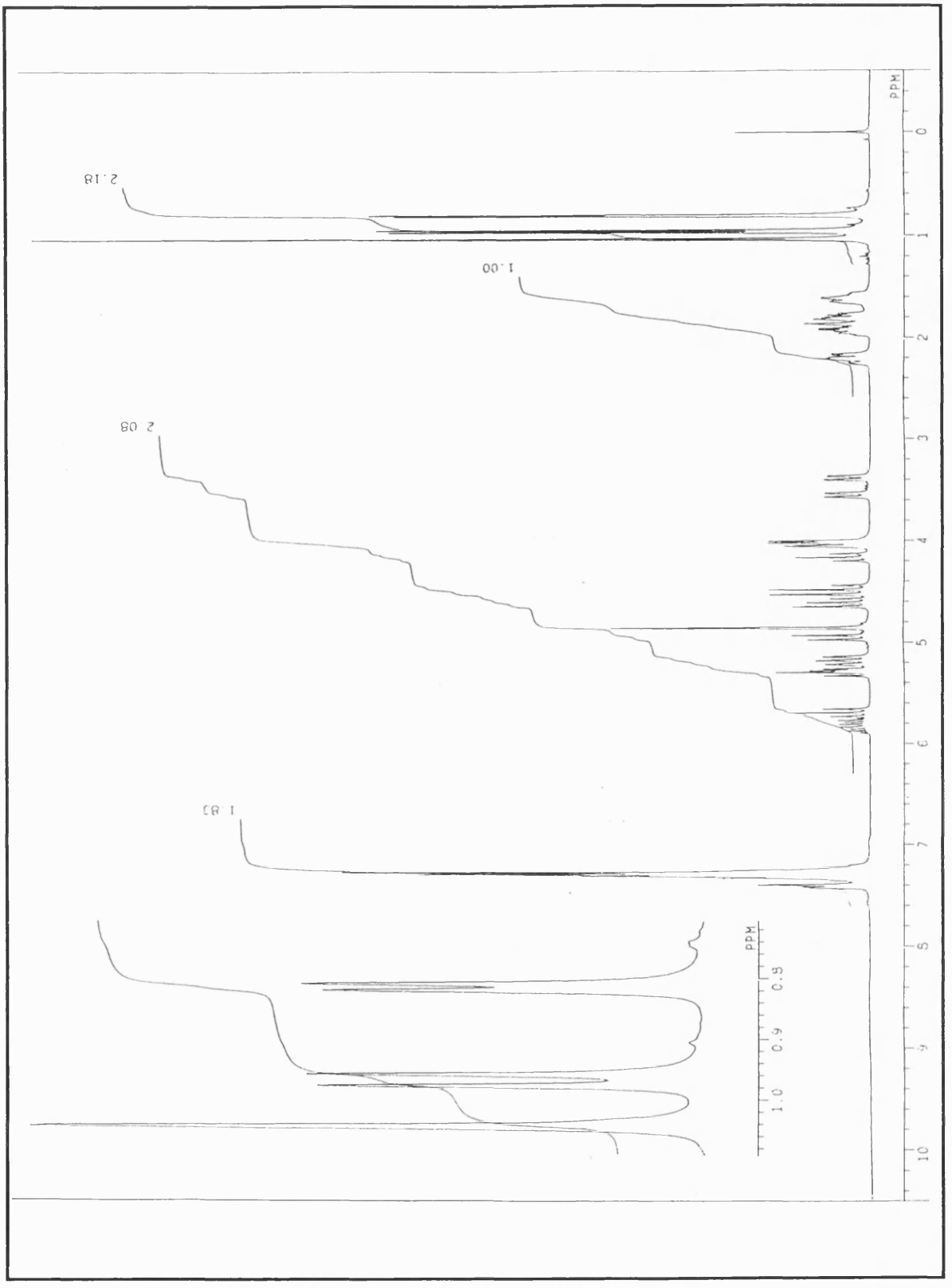


Figure 7.1 ^{1}H-NMR of 136

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-*myo*-inositol **146** [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-*myo*-inositol **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 [380].

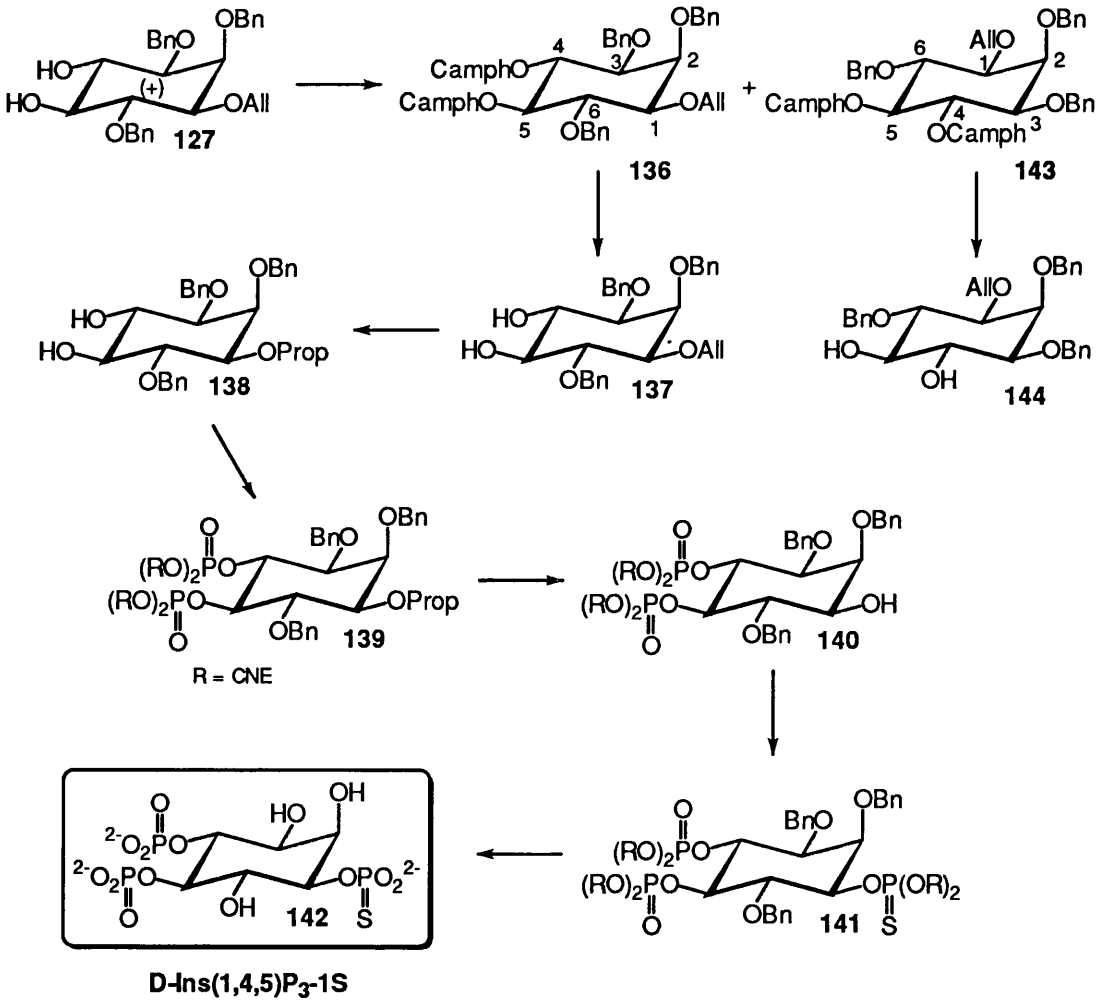


Figure 7.2 Optical resolution of 127 and synthesis of D-Ins(1,4,5)P_3-1S

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_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^{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 1D-*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_3 are currently being evaluated. The optically active precursor **140** is now being applied in the preparation of radiolabelled [^{35}S]-D-Ins(1,4,5)P_3-1S in cooperation with NEN-DuPont.

8 Synthesis of Novel Ins(1,4,5)P_3 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)P_3 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)PS_3 [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)P_3 and D-Ins(1,3,4,5)P_4, which are the natural substrates of this enzyme, but also synthetic inositol phosphates such as L-Ins(1,4,5)P_3, Ins(1,3,5)P_3 and DL-Ins(2,4,5)P_3 [324]. Unlike D-Ins(1,4,5)P_3, DL-Ins(2,4,5)P_3 and DL-Ins(1,3,4,5)P_4 however, L-Ins(1,4,5)P_3 [= D-Ins(3,5,6)P_3] and Ins(1,3,5)P_3 are competitive inhibitors of this enzyme with K_i values of 39 and 45 μM, respectively. In another study using human erythrocyte 5-phosphatase L-Ins(1,4,5)P_3 has also been found to inhibit the enzyme with a K_i of 124 μM [271].

Cooke *et al.* [271] observed that the trisphoshorothioate analogue of Ins(1,4,5)P_3, DL-Ins(1,4,5)PS_3, acted as a potent competitive inhibitor of 5-phosphatase (K_i 6 μM, cf. Ins(1,4,5)P_3: K_m = 40 μM). Thus the substitution of phosphate groups by phosphorothioate moieties resulted in increased binding properties (i.e. decrease of the K_i value) of the analogue. Racemic Ins(1,4,5)PS_3 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)P_3, we reasoned that phosphorothioate analogues of the competitive non calcium-mobilising, but weak 5-phosphatase inhibitors L-Ins(1,4,5)P_3 and Ins(1,3,5)P_3 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-*myo*-inositol 1,4,5-trisphosphorothioate

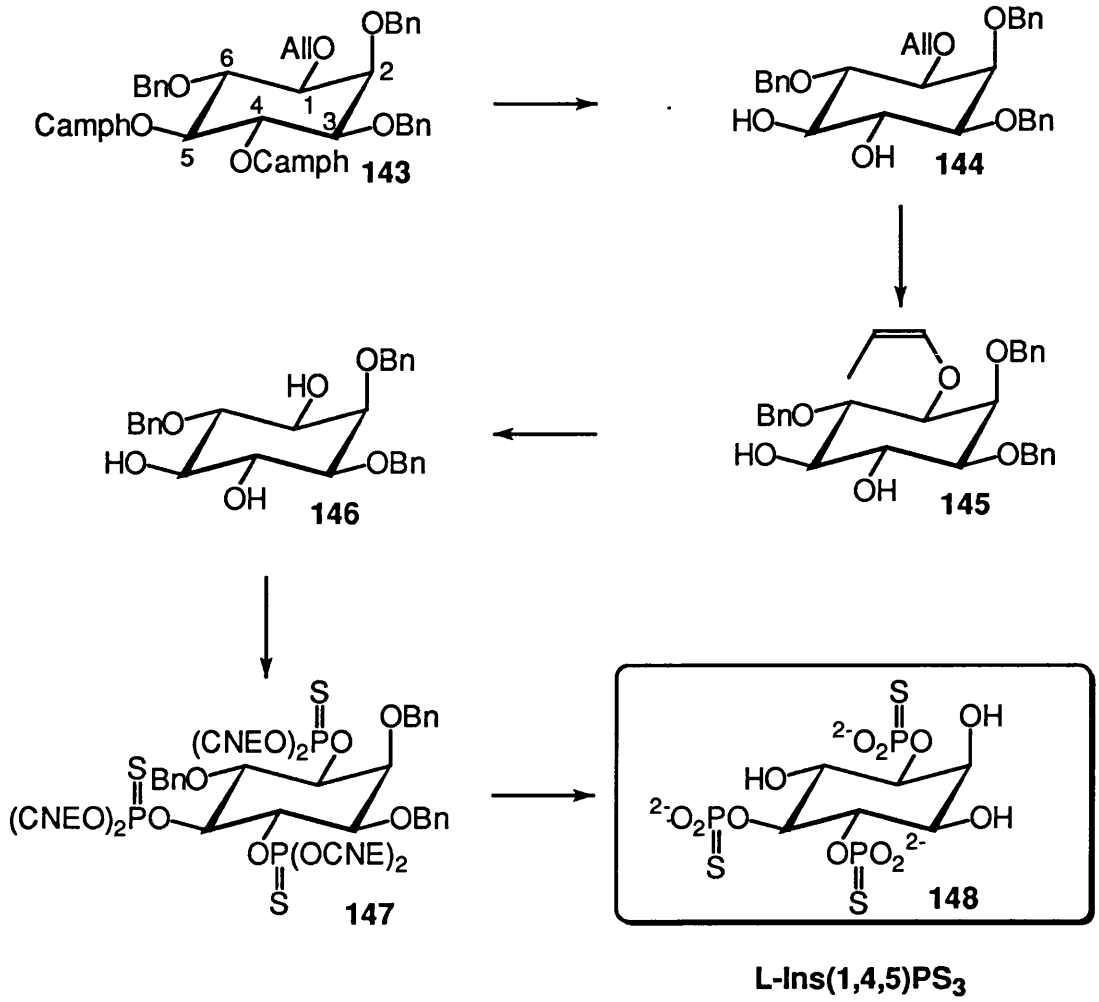


Figure 8.1 Synthesis of L-Ins(1,4,5)P_3

The L-enantiomer of 1-*O*-allyl-2,3,6-tri-*O*-benzyl-*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-*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)thiophospho]-*myo*-inositol **147**. Deblocking with sodium / liquid ammonia furnished 1L-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 [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.

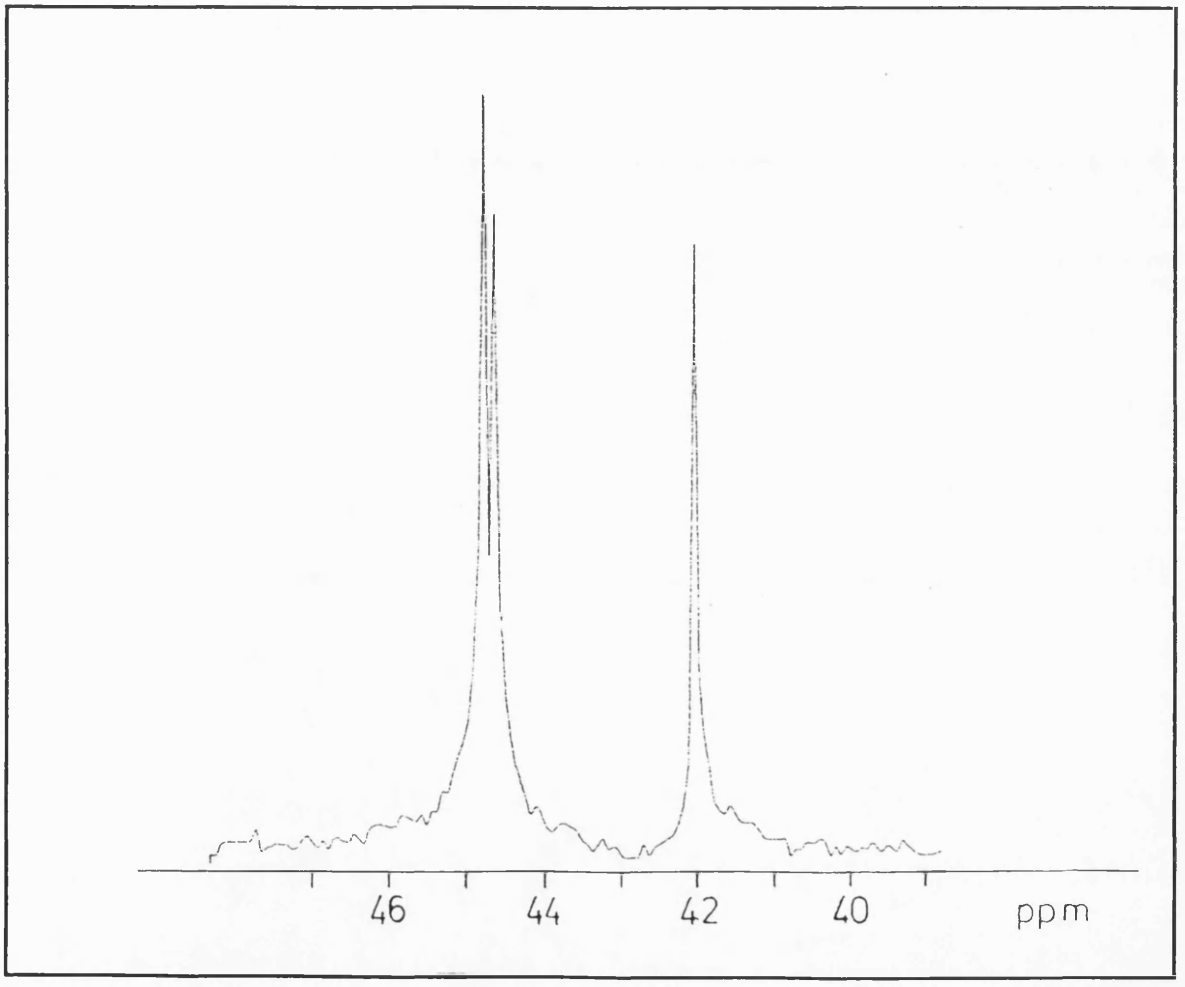
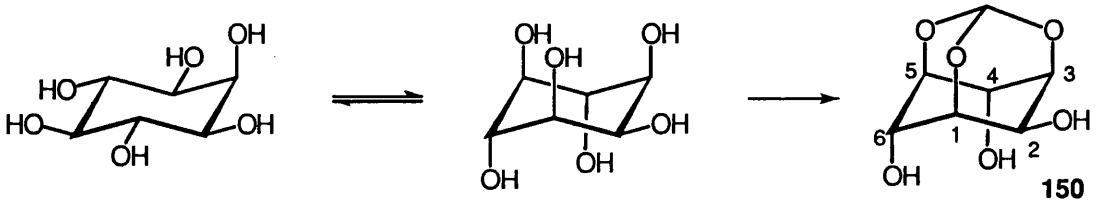


Figure 8.2 ^{31}P-NMR spectrum of 148

8.2 Synthesis of 2,4,6-tri-*O*-butyryl-*myo*-inositol

Myo-inositol was used as starting material to prepare 2,4,6-tri-*O*-butyryl-*myo*-inositol, 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.



150

Figure 8.3 Synthesis of the orthoformate ester of *myo*-inositol

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 and 0.28, 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

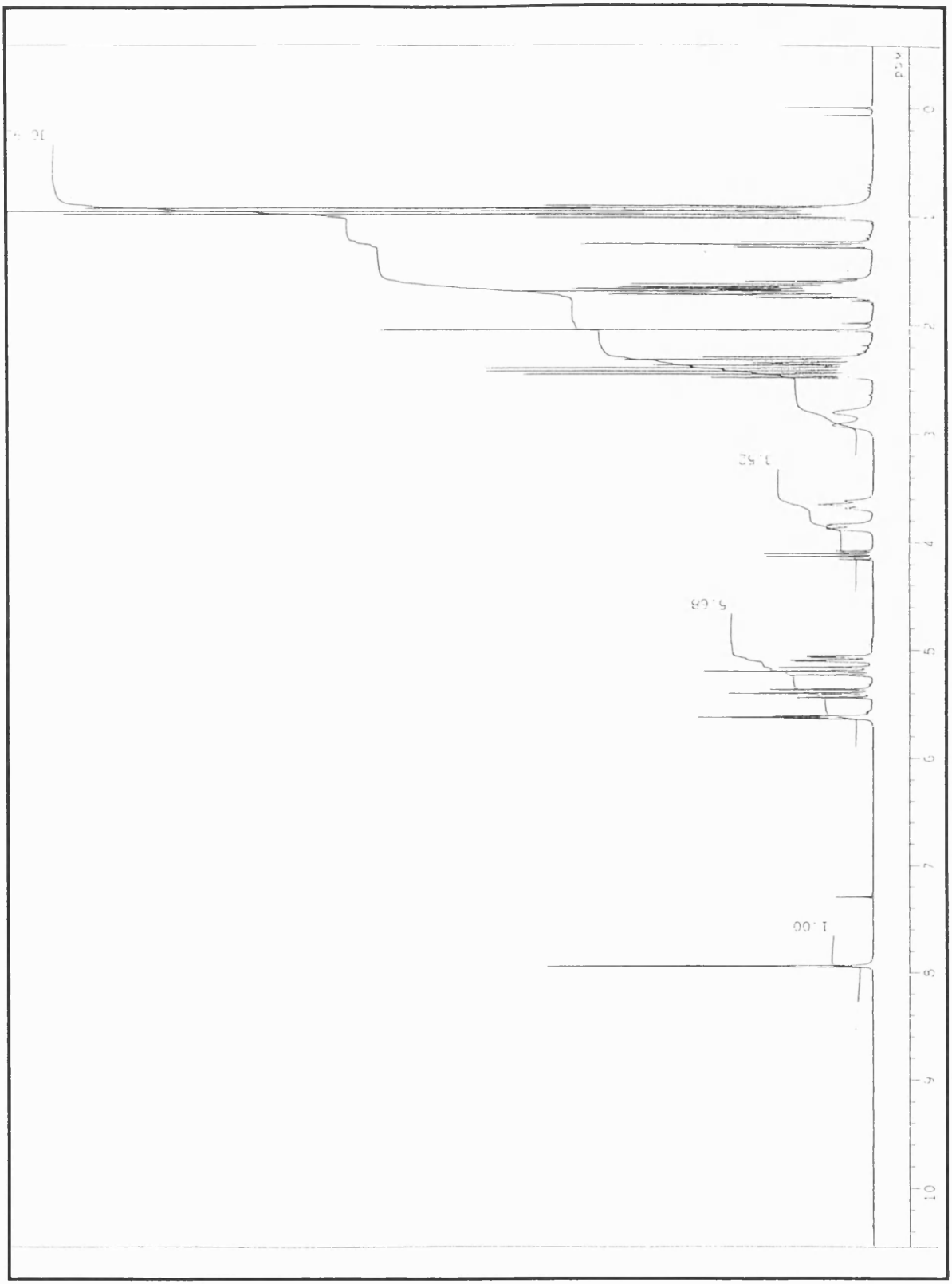


Figure 8.4 ^{1}H-NMR spectrum of 153

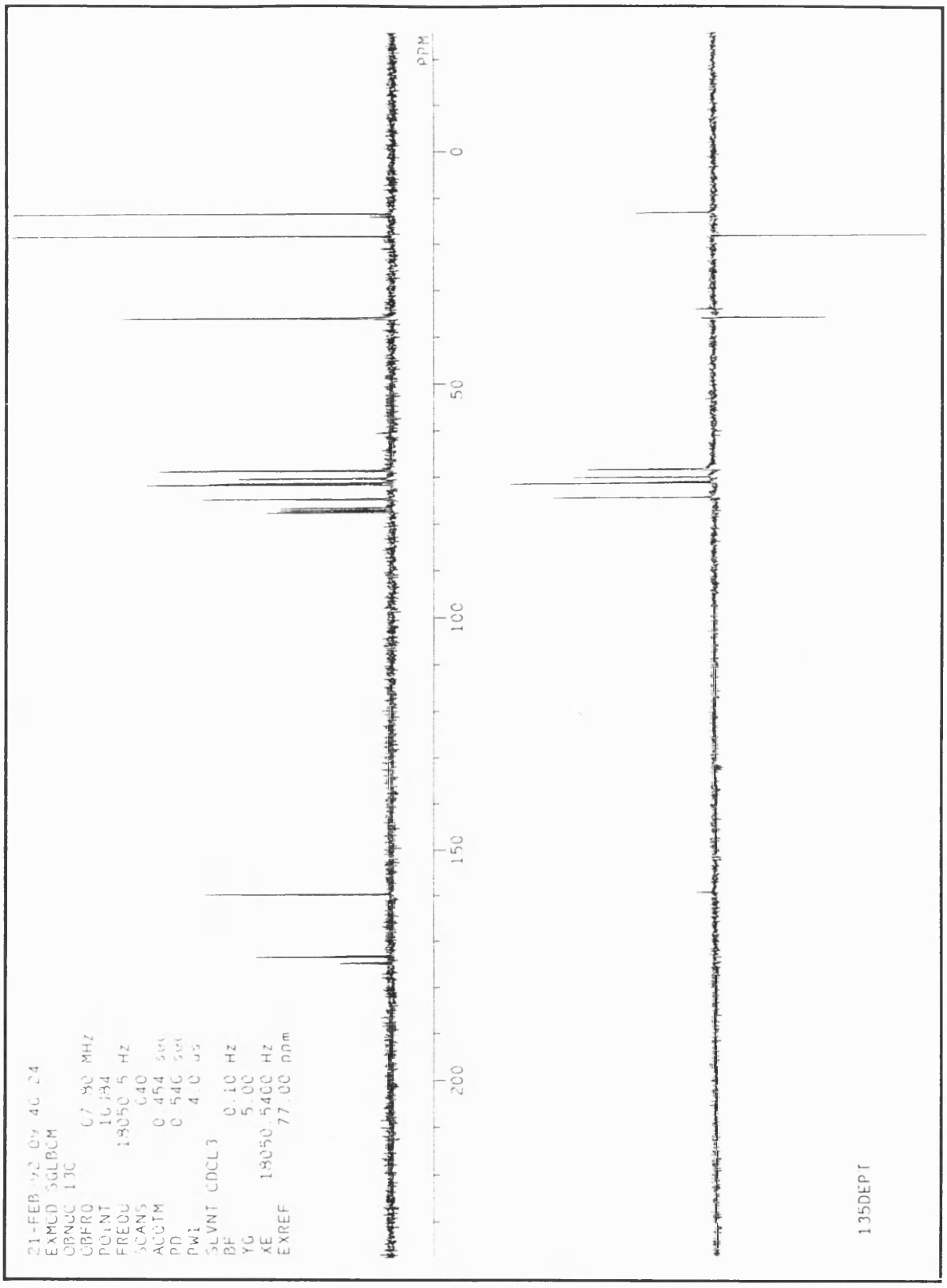


Figure 8.5 ^{13}C-NMR spectrum of 153

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_2O 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.

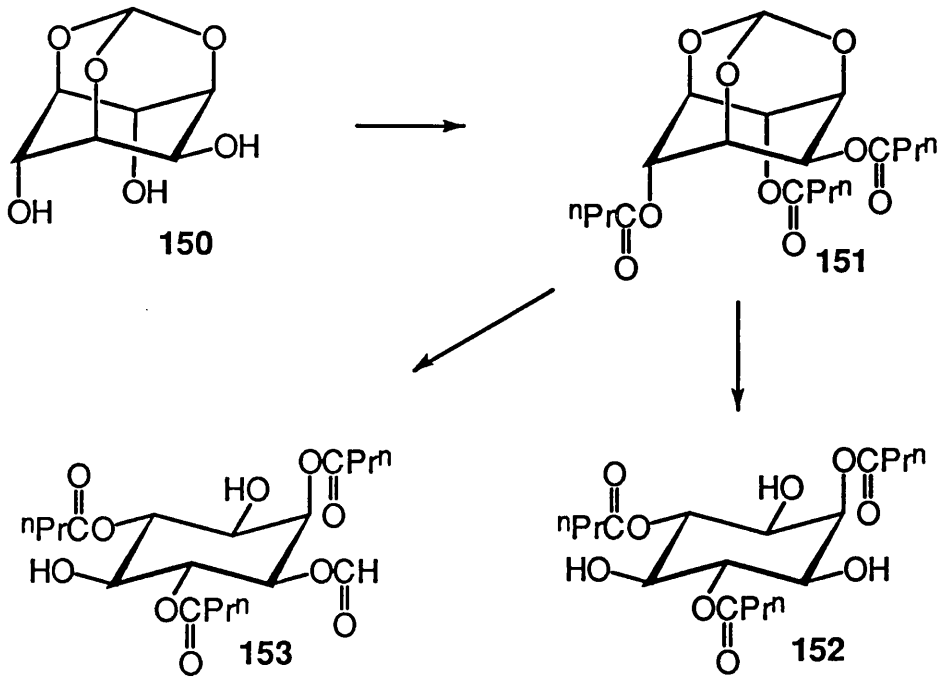


Figure 8.6 Preparation of 2,4,6-tri-*O*-butyryl-*myo*-inositol

The CI-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.

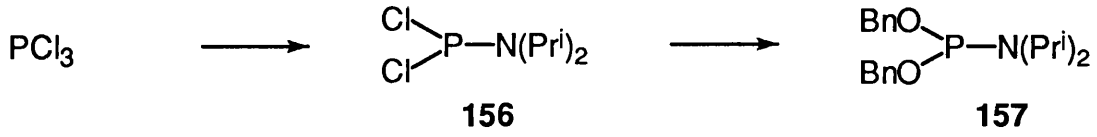
152 was thus digested with porcine liver esterase (Sigma) in 0.1 M phosphate buffer, pH 7. However, even after several days of incubation, no deacylation product could be detected by tlc or NMR and the project was abandoned.

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 PCl_3 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.



156 **157**

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)PS_3:

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-*myo*-inositol. 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)PS_3 **159** as the triethylammonium salt after ion-exchange chromatography. ^{31}P-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 $^3J_{H\text{-}P}$ coupling constants were also observed in the ^{1}H-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). The

^{13}C-NMR spectrum of **159** showed $^{2}J_{C\text{-}O\text{-}P}$ 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).

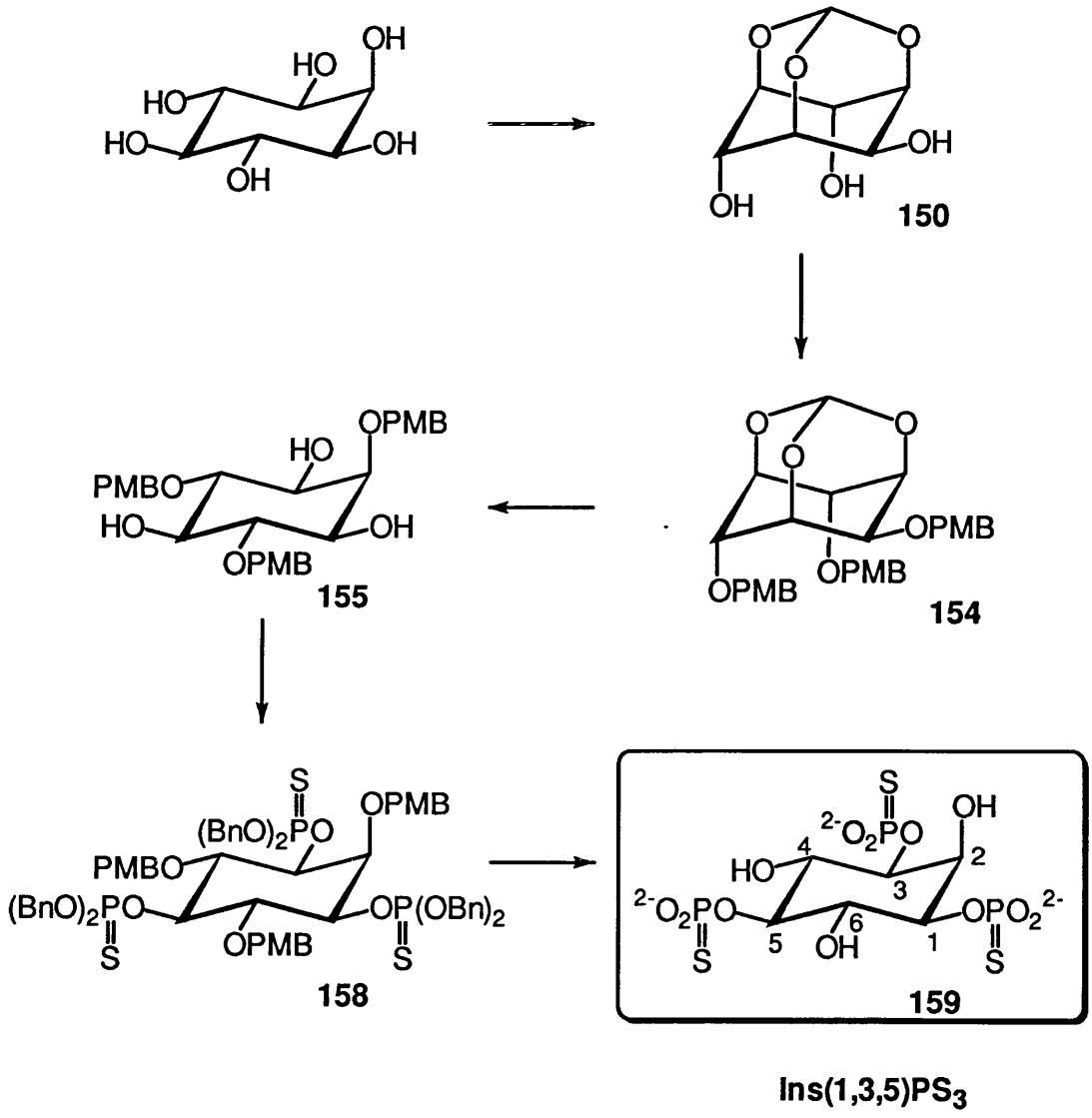


Figure 8.8 Synthesis of *myo*-inositol 1,3,5-trisphosphorothioate

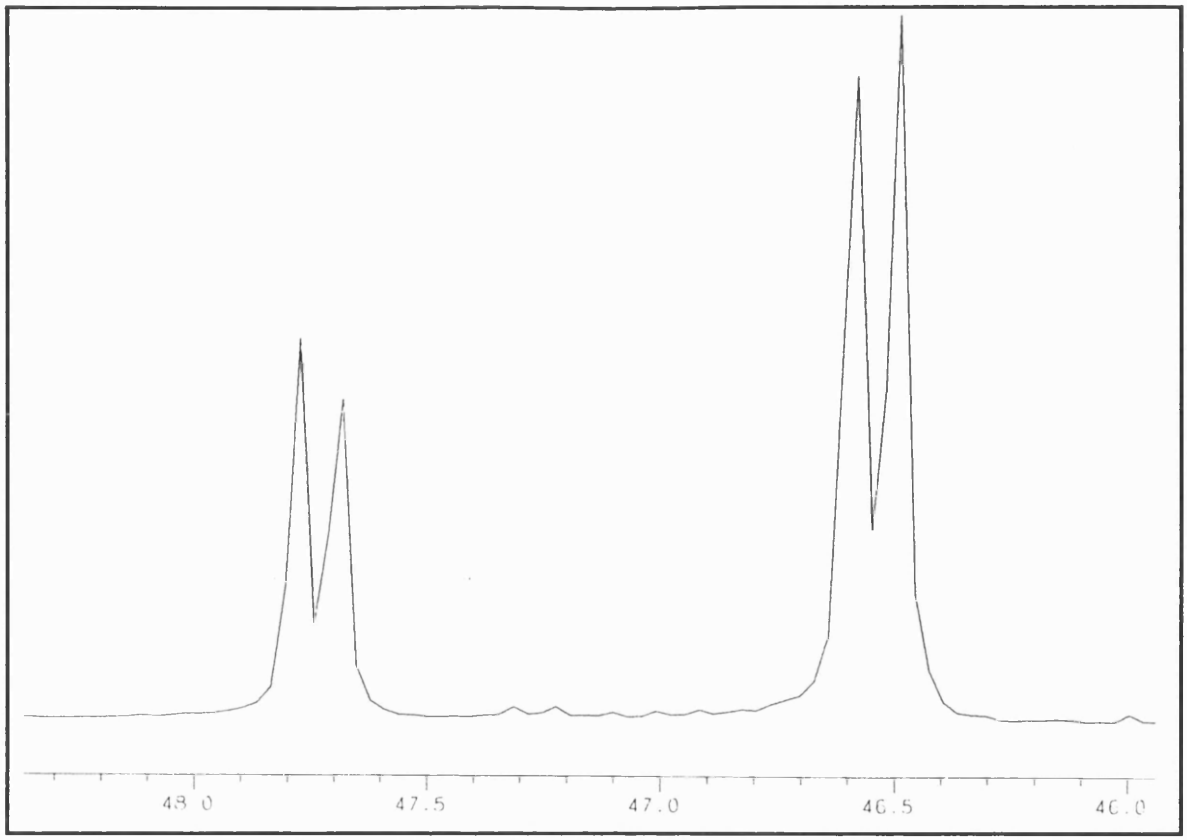


Figure 8.9 ^{31}P-NMR spectrum (^{1}H-coupled) of 159

8.5 Pharmacology

The potential 5-phosphatase inhibitors L-*myo*-inositol 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)P_3 5-phosphatase from human erythrocyte membranes, with submicromolar K_i values of 0.43 μM and 0.50 μM, 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 abilites. 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)PS_3 did not release any calcium in this system.

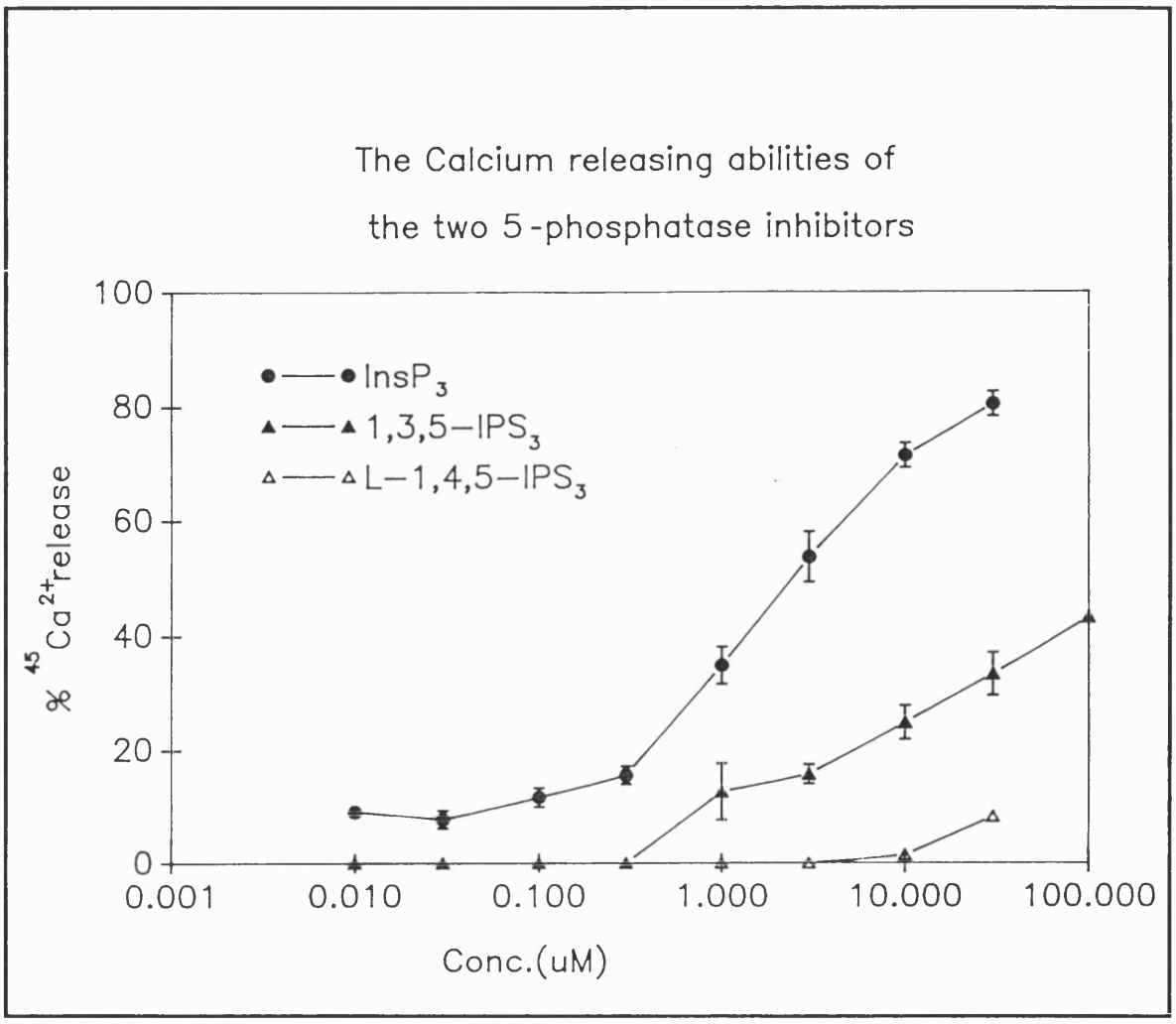


Figure 8.10 Calcium mobilisation of 148 and 159 in platelets

Both compounds were also assayed for their interactions with Ins(1,4,5)P_3 3-kinase. However, whereas L-Ins(1,4,5)PS_3 is recognised surprisingly well by the enzyme (apparent K_i = 1.46 μM), Ins(1,3,5)PS_3 shows hardly any interaction (apparent K_i 247 μM). Thus, whilst both L-Ins(1,4,5)PS_3 and Ins(1,3,5)PS_3 are potent 5-phosphatase inhibitors, Ins(1,3,5)PS_3 has the advantage of being selective for this enzyme.

The pharmacological data of 5-phosphatase inhibitors are summarised below:

inhibitor / substrate	5-phosphatase	3-kinase	Ins(1,4,5)P_3-receptor
D-2,3-diphosphoglycerate	350 or 978 μM	inhibitor	no interaction
L-*chiro*-Ins(1,4,6)P_3	44 μM	no interaction	no interaction
L-*chiro*-Ins(1,4,6)PS_3	0.3 μM	no interaction	no interaction
Ins(1,3,5)P_3	45 μM	no interaction	no interaction
Ins(1,3,5)PS_3 **159**	0.5 μM	no interaction	no interaction
DL-Ins(1,4,5)PS_3	1.7 μM	inhibitor	agonist
DL-Ins(1,4,5)P_3-4,5S	1.4 μM	substrate	agonist
DL-Ins(1,4,5)P_3-5S	6.8 μM	substrate	agonist
L-Ins(1,4,5)P_3	39 or 124 μM	40.3 μM	no interaction
L-Ins(1,4,5)PS_3 **148**	0.43 μM	1.46 μM	no interaction
D-Ins(1,4,5)P_3, K_m	19 μM	substrate	agonist

9 The Synthesis of *Scyllo*-Inositol Analogues

Modified analogues of Ins(1,4,5)P_3 are of great current interest as potential Ins(1,4,5)P_3 receptor antagonists and enzyme inhibitors. We chose to pursue *scyllo*-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)P_3 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 *scyllo*-Ins(1,2,4)P_3, the *scyllo*-analogue of Ins(1,4,5)P_3. In addition to the preparation of *scyllo*-Ins(1,2,4)P_3, the synthesis of *scyllo*-Ins(1,2,4,5)P_4 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)P_4, which has been shown to act as a partial agonist at the Ins(1,4,5)P_3 receptor. We were therefore interested in these *scyllo*-inositol analogues to find out more about structural requirements for compounds acting as partial agonists.

9.1 Synthesis of 1-*O*-Allyl-3,6-di-*O*-benzyl-*scyllo*-inositol

Thus, 1-*O*-allyl-3,6-di-*O*-benzyl-4,5-*O*-isopropylidene-*myo*-inositol **93**, an intermediate with a free 2-hydroxyl group used to synthesise 1-*O*-allyl-2,3,6-tri-*O*-benzyl-*myo*-inositol, was employed to generate *scyllo*-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 scyllo-inositol derivative **161**. ^{1}H-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 $J_1 = J_2 = 8.8$. 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.

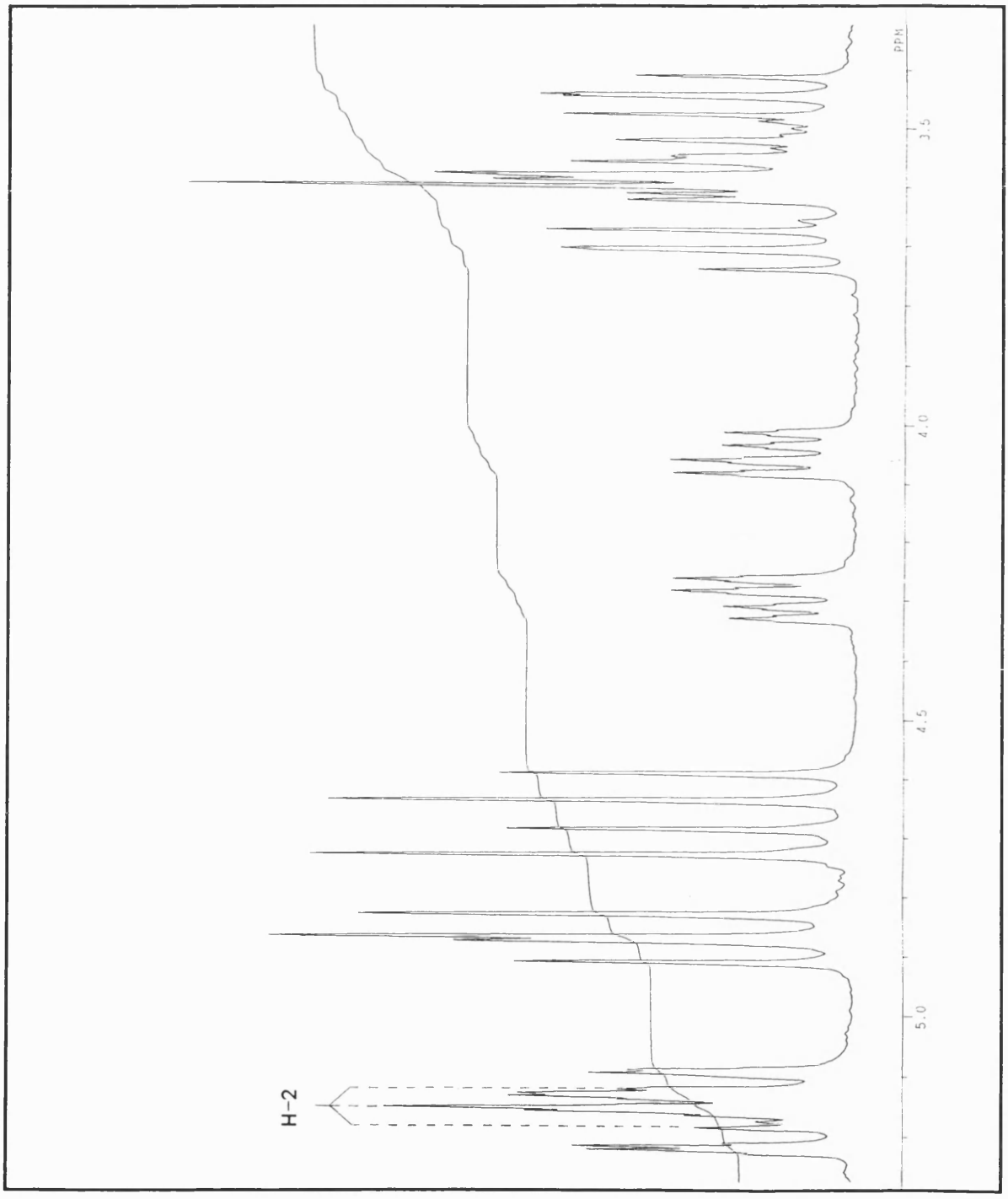


Figure 9.1 ^{1}H-NMR spectrum of 161 (expansion 3.4 ppm to 5.3 ppm)

Removal of acetate and isopropylidene protecting groups by saponification followed by acid hydrolysis gave 1-*O*-allyl-3,6-tri-*O*-benzyl-*scyllo*-inositol **164** either *via* **162** or **163**.

The synthesis of 1-*O*-allyl-3,6-di-*O*-benzyl-4,5-*O*-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-*O*-allyl-3,6-di-*O*-benzyl-4,5-*O*-isopropylidene-*myo*-inositol **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

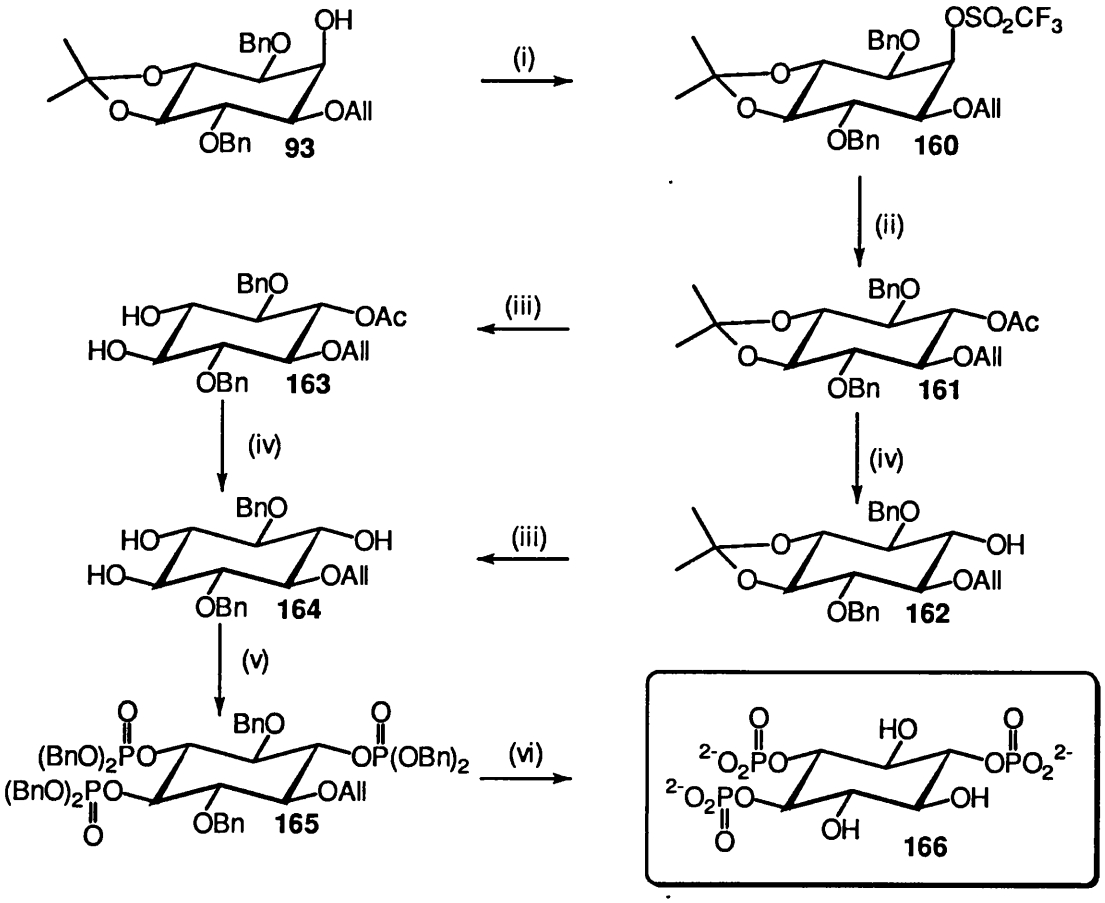


Figure 9.2 Synthesis of DL-*scyllo*-inositol 1,2,4-trisphosphate 166

Phosphitylation of **164** with bis(benzyloxy)(diisopropylamino)phosphine / 1*H*-tetrazole followed by oxidation with Bu^{t}OOH furnished **165**. The ^{1}H-coupled ^{31}P-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 ^{1}H-NMR could not be observed due to overlapping signals from the CH_2Ph and CH_2CH=CH_2 protons.

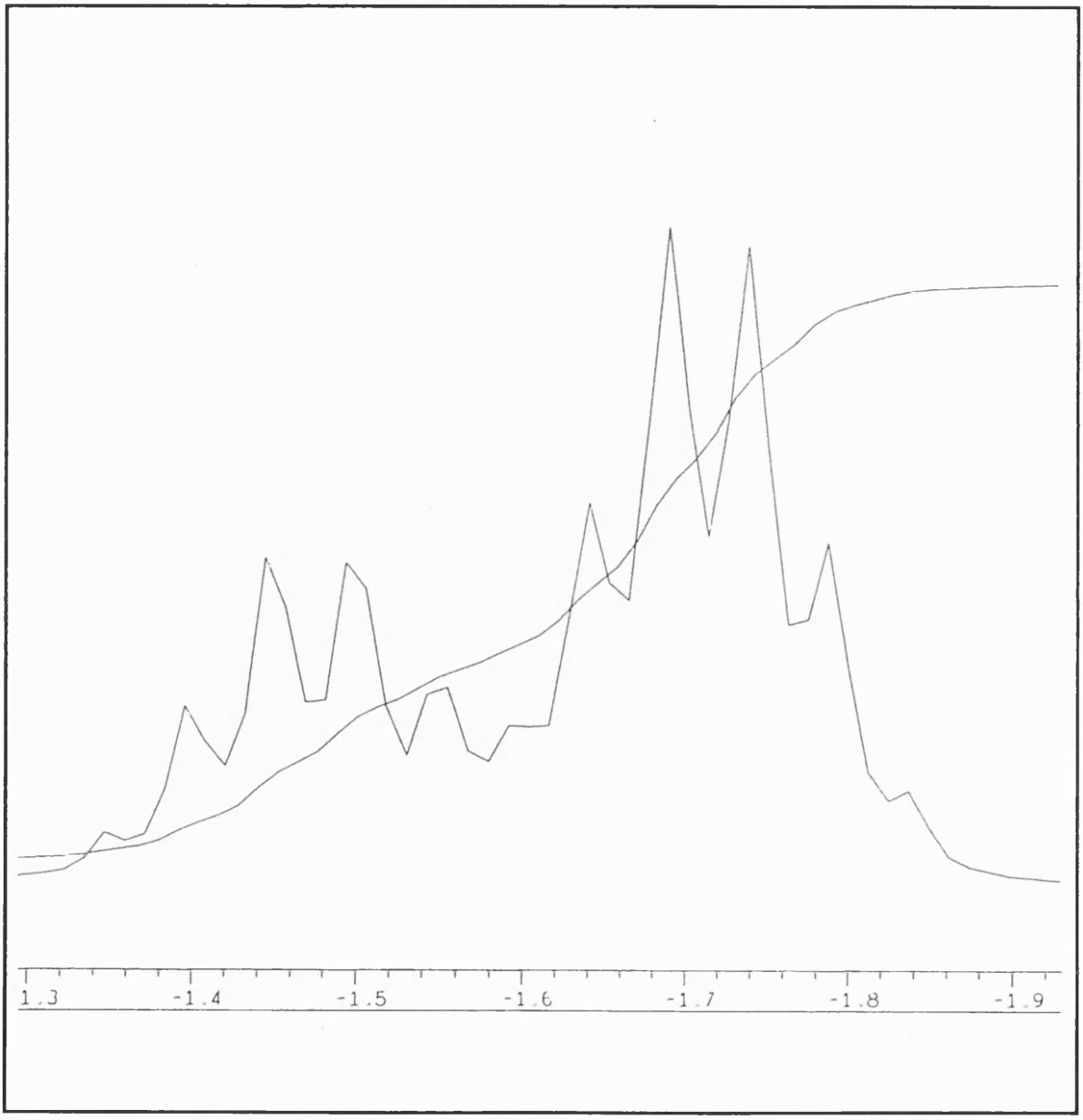


Figure 9.3 ^{1}H-coupled ^{31}P-NMR spectrum of 165

Treatment with sodium-liquid ammonia deblocked both the benzyl and allyl [143] 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 μM [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-*O*-Allyl-3,6-di-*O*-benzyl-*scyllo*-inositol **164** was isomerised to the *cis*-prop-1-enyl derivative **167** using Bu^{t}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.

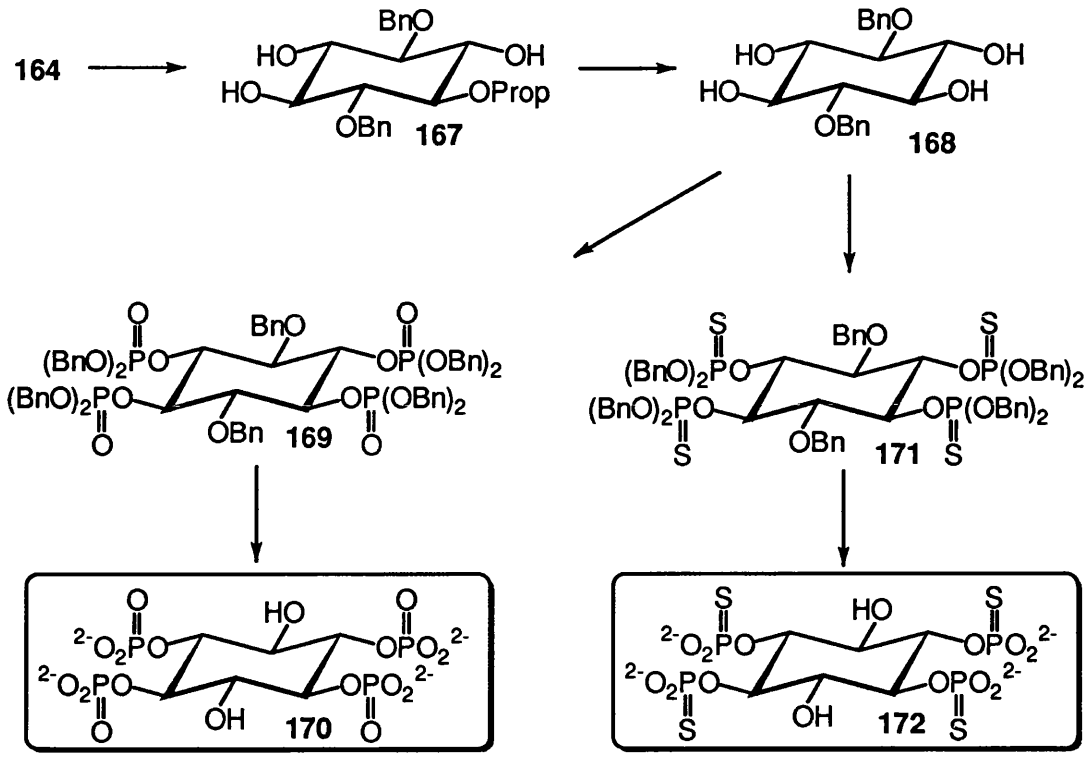


Figure 9.4 Synthesis of *scyllo*-inositol 1,2,4,5-tetrakisphosphate 170 and *scyllo*-inositol 1,2,4,5-tetrakisphosphorothioate 172

9.5 Pharmacology

DL-*Scyllo*-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)P_3 with EC_{50} = 130 nM.

The dose response curves obtained using T-cells (Figure 9.5) and rabbit platelets (Figure 9.6) are shown below.

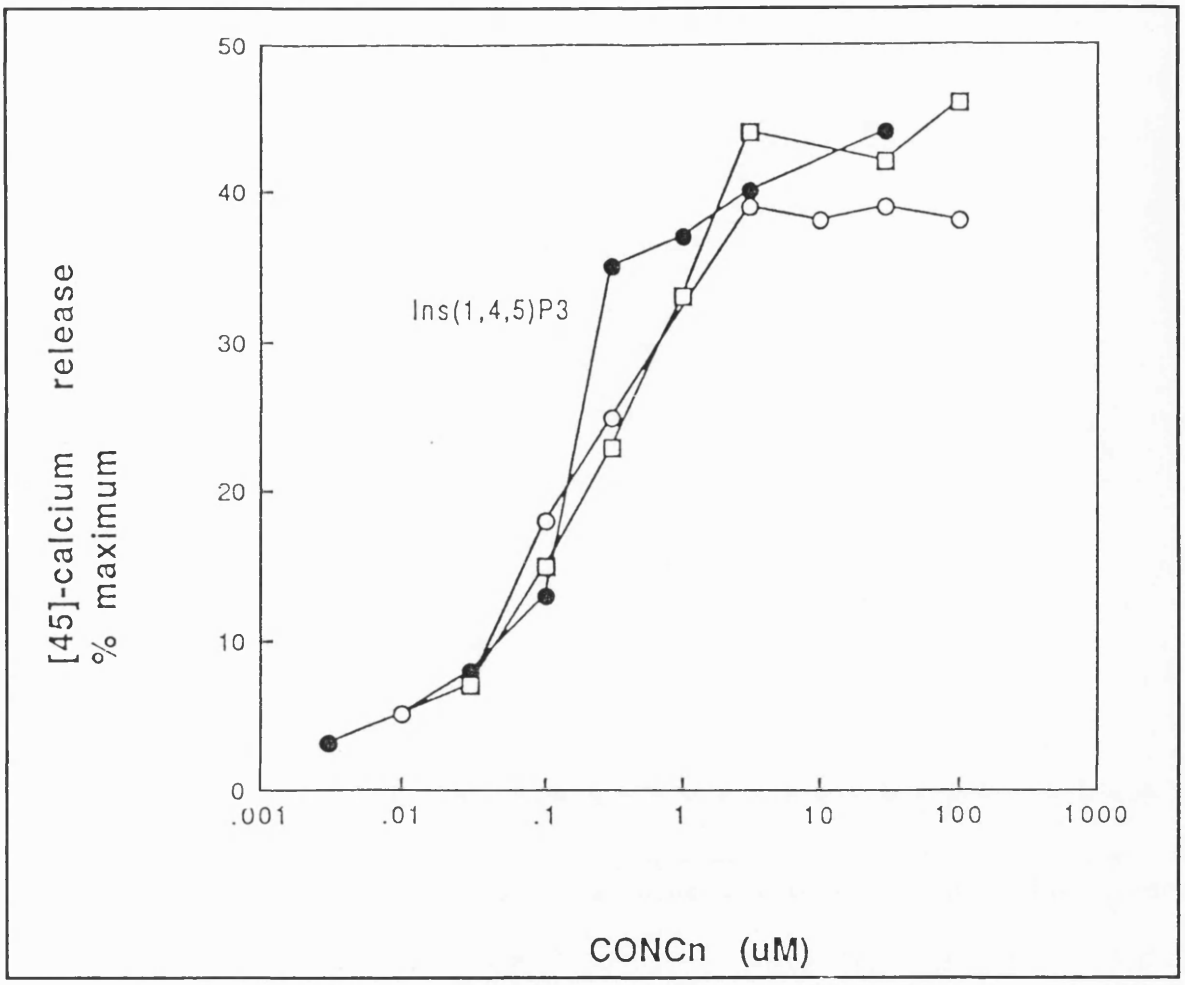


Figure 9.5 Calcium mobilisation of DL-*scyllo*-inositol 1,2,4-trisphosphate (□) and DL-2-deoxy-2-fluoro-*myo*-inositol 1,4,5-trisphosphate (o) in T-cells

The fact that *scyllo*-inositol 1,2,4-trisphosphate is of a potency similar to Ins(1,4,5)P_3 is not surprising since other 2-modified Ins(1,4,5)P_3 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)P_3]. In each case, the modified compound in racemic form is *ca.* 2.5 times less active than D-Ins(1,4,5)P_3. Assuming that the L-isomers are not biologically active, the differences in activity between 2-modified analogues and Ins(1,4,5)P_3 are indeed minute, indicating that the 2-hydroxyl group does not contribute significantly towards receptor binding.

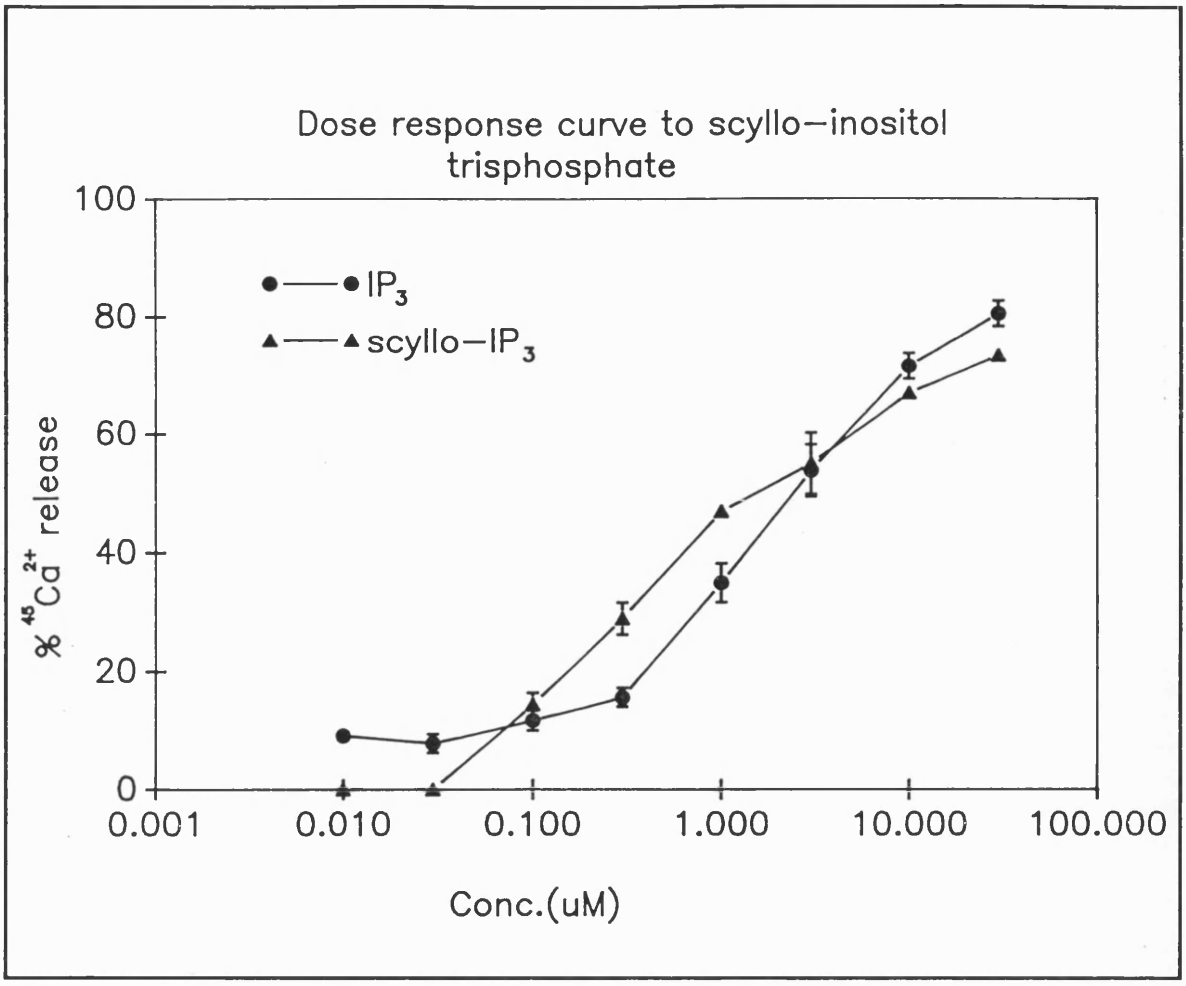


Figure 9.6 Ca^{2+} mobilisation of *scyllo*-Ins(1,2,4)P_3 in platelets

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 μM) and a moderately potent inhibitor of 5-phosphatase (K_i 24.2 μM).

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}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 [^{3}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). The fact that unlike Ins(1,3,4,6)P_4, *scyllo*-Ins(1,2,4,5)P_4 does not show any partional 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:

		scyllo-Ins(1,2,4,5)P_4	*scyllo*-Ins(1,2,4,5)PS_4
Binding	IC_{50}	14.37 ± 2.41 nM	430.58 ± 46.44 nM
	K_i	10.82 ± 2.00 nM	312.77 ± 34.60 nM
	slope	0.852 ± 0.037	1.007 ± 0.078
Ca^{2+} release	EC_{50}	82.56 ± 18.79 nM	1.606 ± 0.056 μM
	slope	1.068 ± 0.021	0.7019 ± 0.048

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.

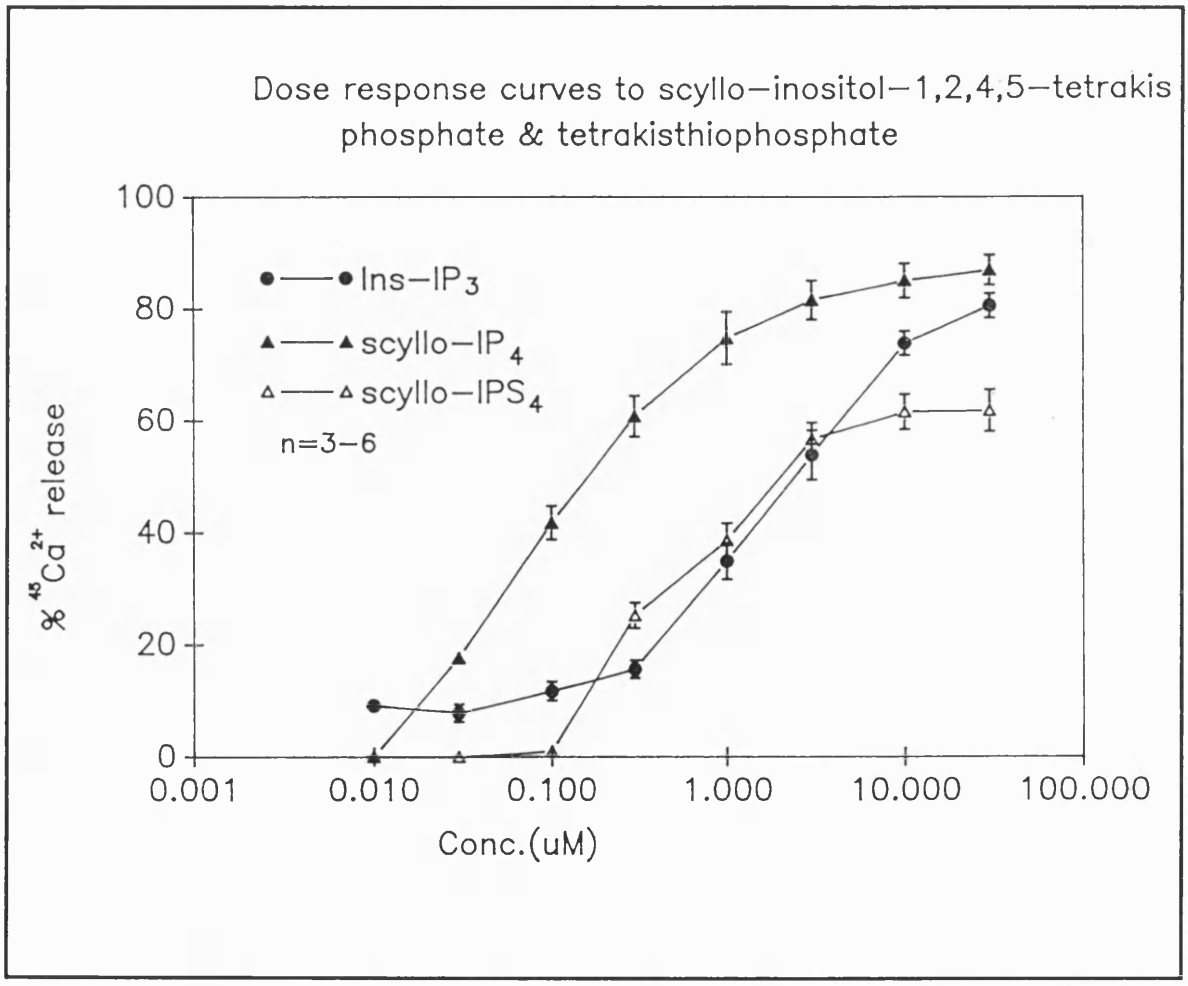


Figure 9.7 Calcium mobilisation of *scyllo*-Ins(1,2,4,5)P_4 and *scyllo*-Ins(1,2,4,5)PS_4 in platelets

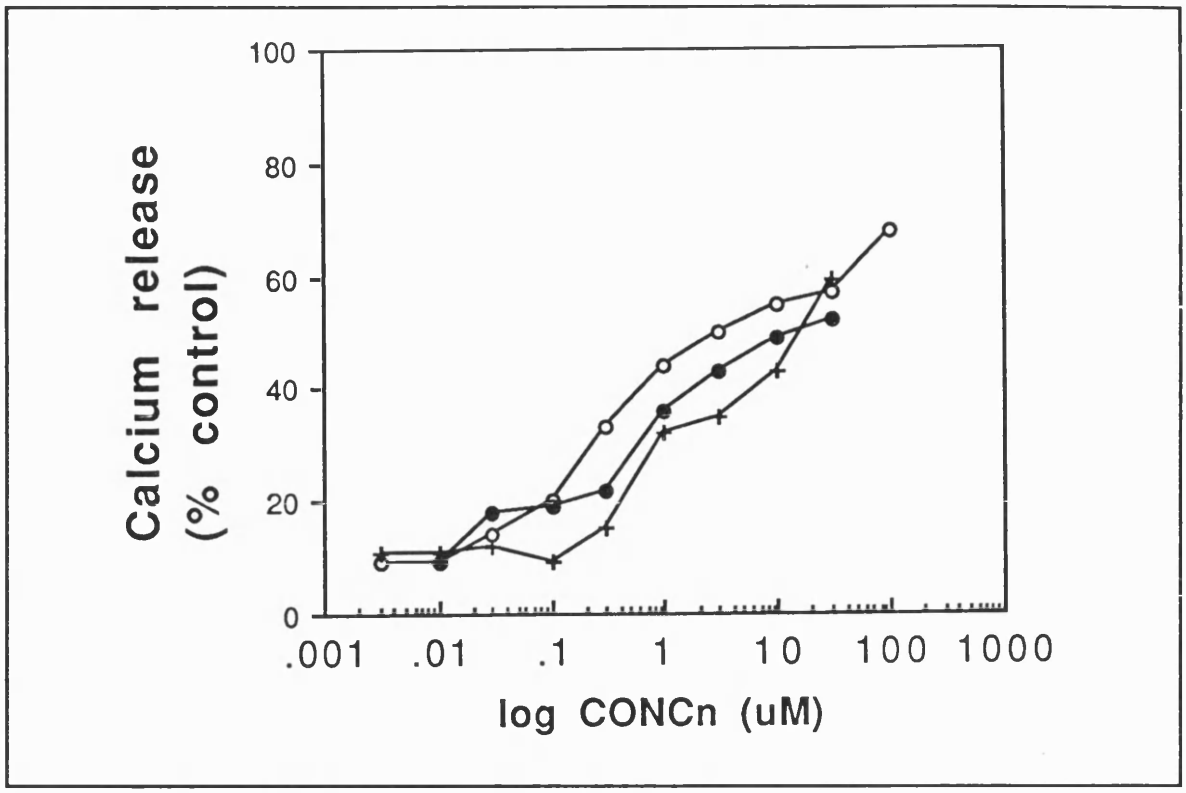


Figure 9.8 Calcium mobilisation of Ins(1,4,5)P_3 (o), *scyllo*-Ins(1,2,4,5)P_4 (•) and *scyllo*-Ins(1,2,4,5)PS_4 (+) in T-cells

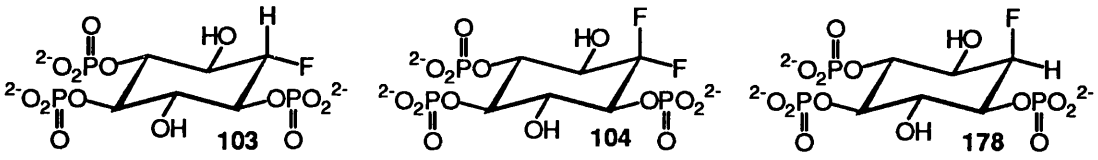
The interactions of these Ins(1,4,5)P_3 analogues with 5-phosphatase and 3-kinase are currently being investigated.

10 DL-2-Deoxy-2-fluoro-*myo*-inositol 1,4,5-trisphosphate

10.1 The Synthesis of DL-2-Deoxy-2-fluoro-Ins(1,4,5)P_3

A number of fluorinated analogues of Ins(1,4,5)P_3 have been prepared, including the 2-fluoro analogues 2-deoxy-2-fluoro-*scyllo*-Ins(1,4,5)P_3 **103** and 2-deoxy-2,2-difluoro-Ins(1,4,5)P_3 **104** (see Chapter 3.3).

The synthesis of 2-deoxy-2-fluoro-Ins(1,4,5)P_3 [2-F-Ins(1,4,5)P_3], the 2-fluoro analogue most similar to 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 Ins(1,4,5)P_3 even closer like 2-F-Ins(1,4,5)P_3 may show improved characteristics as compared to the former analogues.



2-F-*scyllo*-Ins(1,4,5)P_3 **2,2-F_2-Ins(1,4,5)P_3** **2-F-*myo*-Ins(1,4,5)P_3**

Figure 10.1 Analogues of 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 Ins(1,4,5)P_3 (see above), was chosen as the starting material to synthesise 2-F-Ins(1,4,5)P_3. An earlier attempt to synthesise 2-F-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.

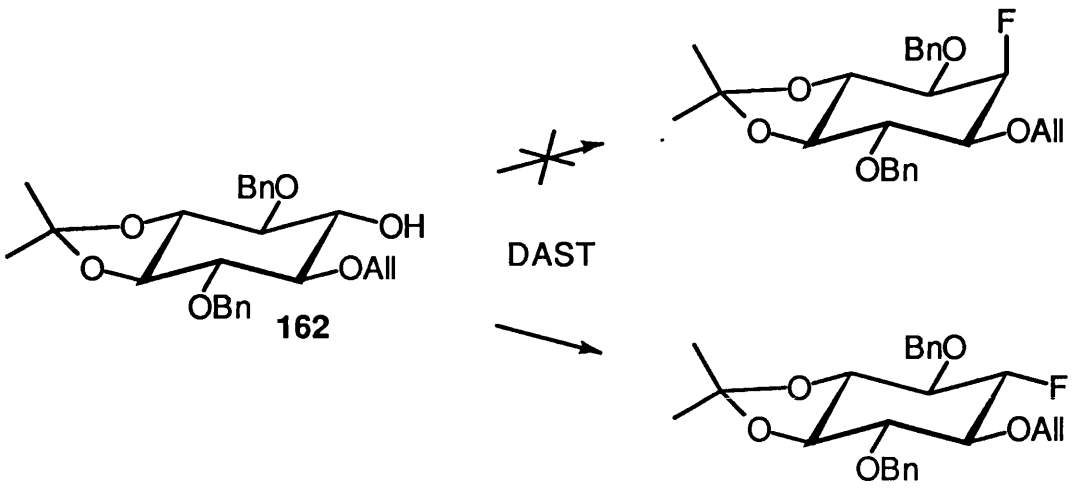


Figure 10.2 Retention of configuration in the DAST fluorination of 162

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 Bu^{t}OK / 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 $^2J_{HF}$ = 50.0 Hz and $^3J_{HF}$ = 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 $^2J_{HF}$ and $^3J_{HF}$ coupling constants measured are in good agreement with those reported for 2-deoxy-2-fluoro-*myo*-inositol [312], where $^2J_{HF}$ and $^3J_{HF}$ 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 $^2J_{HF}$ (50.4 Hz), but $^3J_{HF}$ 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 ^3J 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.

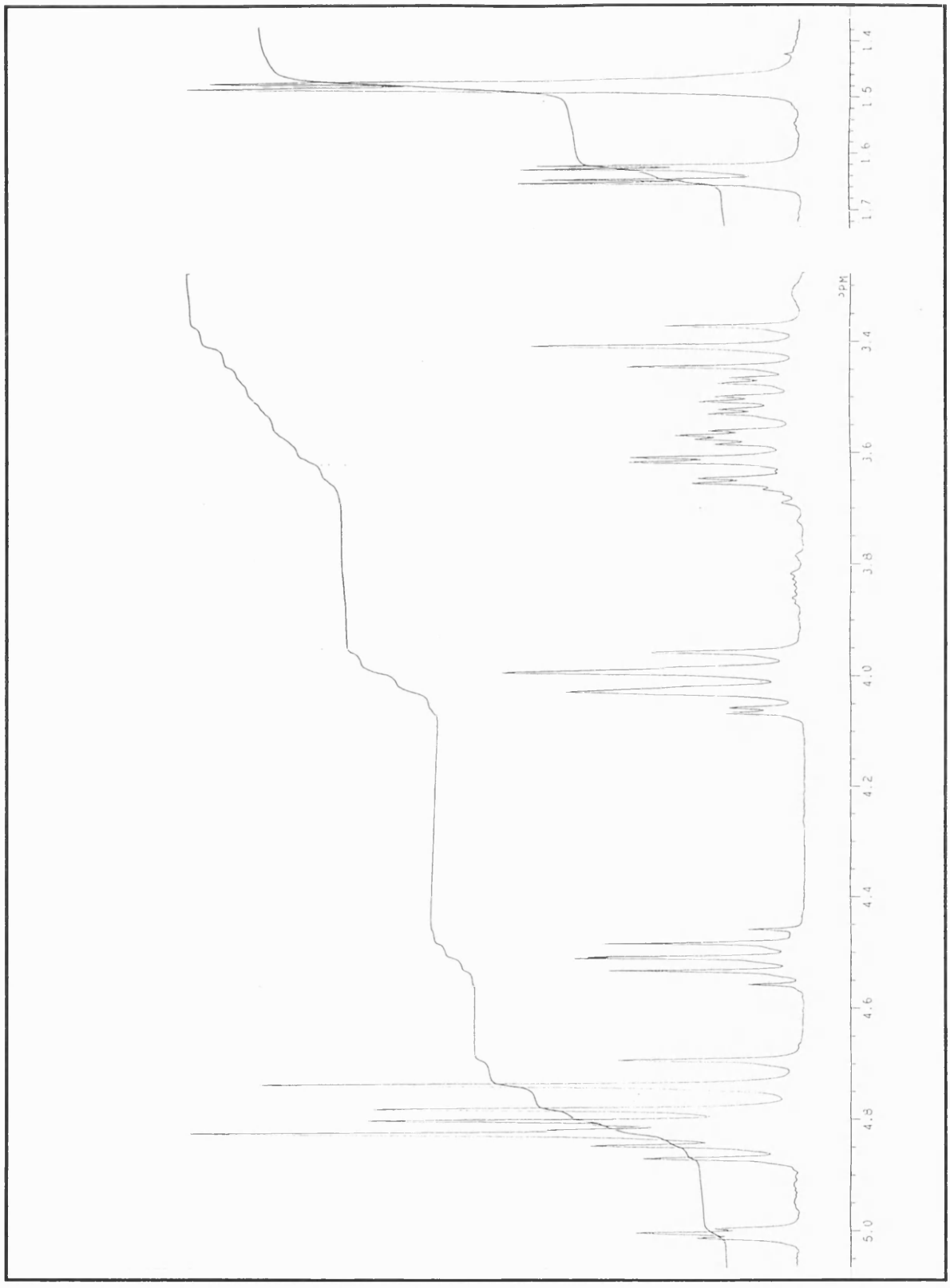


Figure 10.3 ^{1}H-NMR spectrum of 175 (expansion 1.4 - 5.1 ppm)

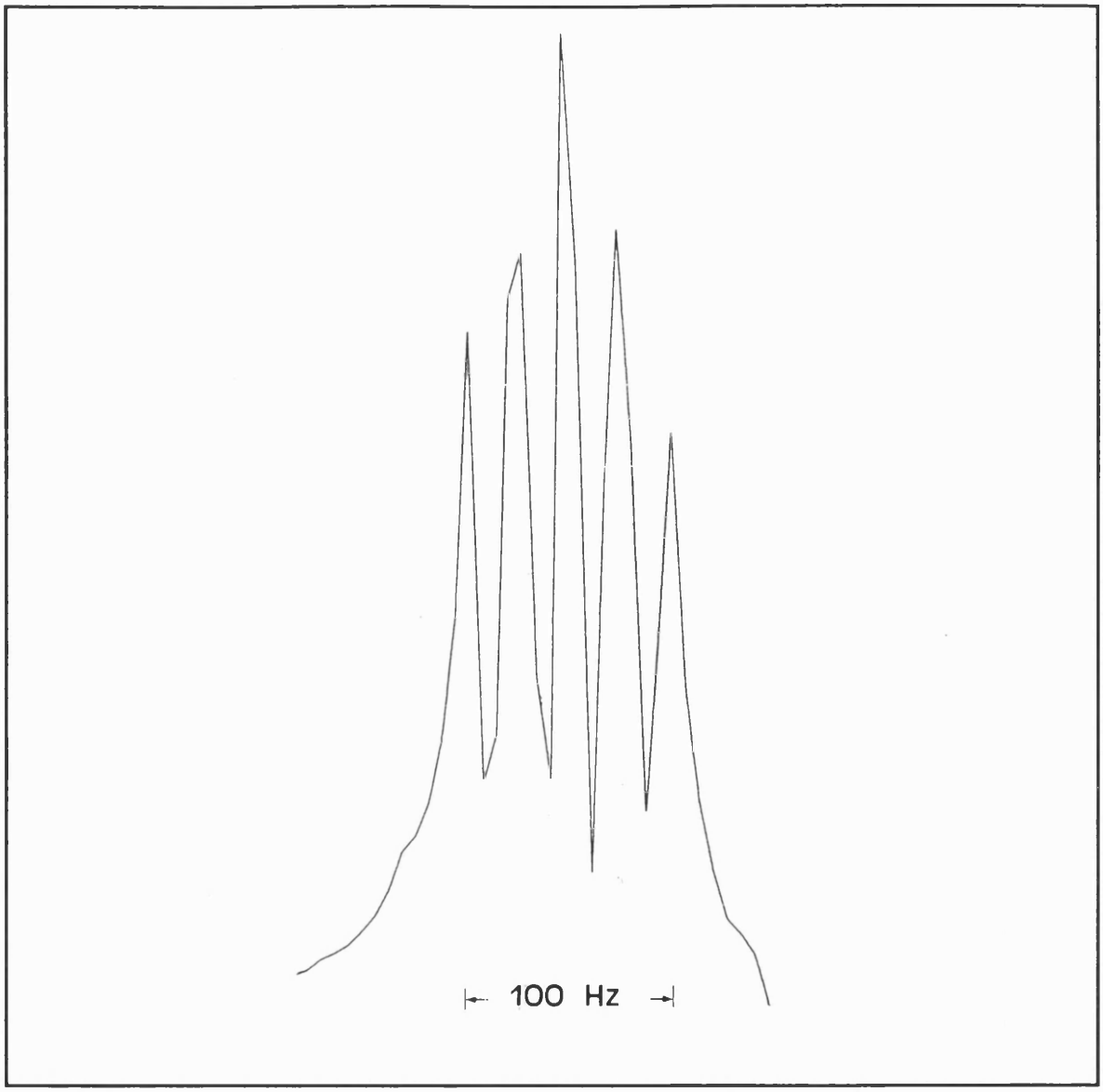


Figure 10.4 ^{19}F-NMR spectrum of 175 (signal at -210.21 ppm)

Removal of the acid labile propenyl and isopropylidene protecting groups gave **176**, which was phosphitylated with bis(benzyloxy)(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-*scyllo*-Ins(1,4,5)P_3 underwent slow defluorination ($t_{½} \approx$ 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**.

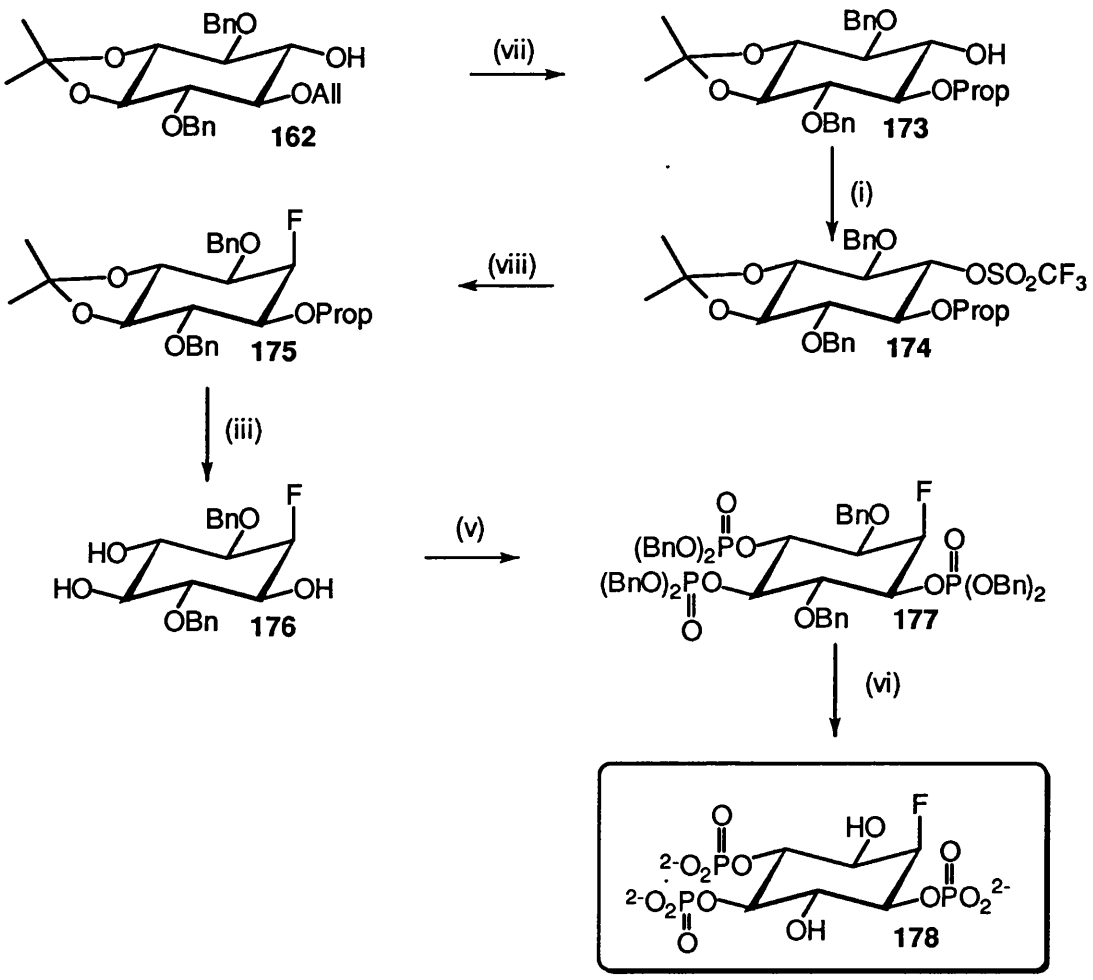


Figure 10.5 Synthesis of DL-2-deoxy-2-fluoro-Ins(1,4,5)P_3 178

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 $J_{2\text{-H,F}}$ 51.3 Hz and $J_{1(3)\text{-H, F}}$ 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.

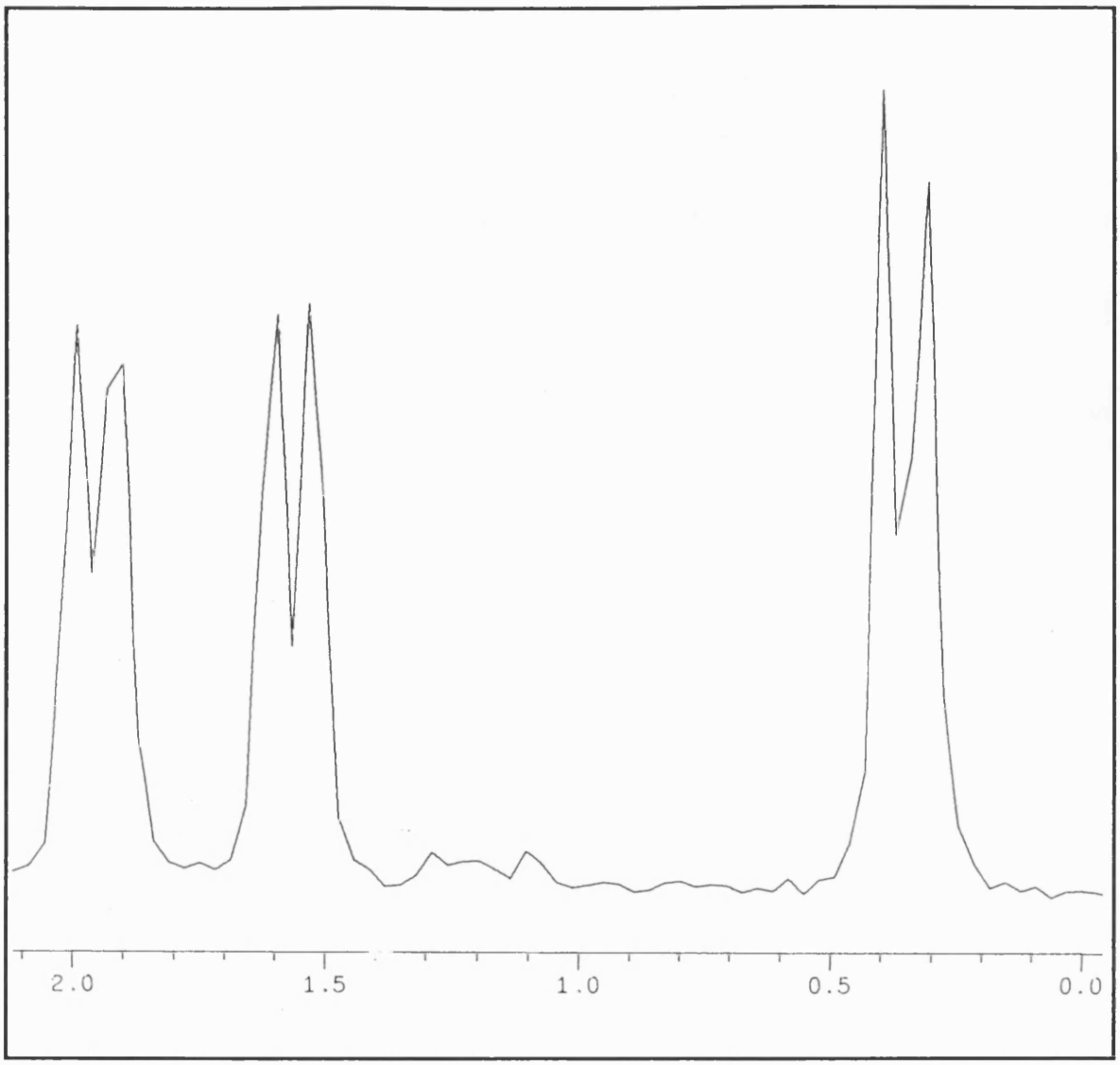


Figure 10.6 ^{1}H-coupled ^{31}P-NMR spectrum of 178

10.2 Pharmacology

The new fluorinated Ins(1,4,5)P_3 analogue **178** is now being evaluated, and preliminary results have shown it to be equipotent to Ins(1,4,5)P_3 in mobilising sequestered calcium assuming that only the D-isomer of **178** displays biological activity [EC_{50} of **178** = 0.10 μM, EC_{50} of D-Ins(1,4,5)P_3 = 0.05 μM]. Comparison of the calcium mobilising properties of **178** with those of 2F-*scyllo*-Ins(1,4,5)P_3 **103** (EC_{50} = 0.77 μM) and 2,2-F_2-Ins(1,4,5)P_3 **104** (EC_{50} = 0.41 μM) show that

the closer structural similarity of **178** to Ins(1,4,5)P_3 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)P_3 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)P_3.

178 is also a good substrate for 3-kinase, acting as a potent competitive inhibitor of the phosphorylation of [^{3}H]-Ins(1,4,5)P_3 by this enzyme (apparent K_i = 3.0 µM; for comparison: 2-F-*scyllo*-Ins(1,4,5)P_3 **103** and 2,2-F_2-Ins(1,4,5)P_3 **104** have K_i values of 8.8 µM and 11.0 µM, respectively).

Interactions of racemic 2-F-*myo*-Ins(1,4,5)P_3 with 5-phosphatase have also been studied, and the fluorinated derivative was found to be a moderately potent competitive inhibitor of this enzyme (K_i 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)P_3 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)P_3 analogues are summarised and compared to D-Ins(1,4,5)P_3 (= D-**6**) below:

	DL-103	**DL-104**	**L-104**	**D-104**	**DL-178**	**D-6**
EC_{50}	0.77	0.41	53	0.21	0.10	0.05
5-phosphatase, K_i	0.7 (S)	26.0 (I)	19 (I)	60 (S)	14.4 (I)	16-40
3-kinase, K_i	8.8 (S)	11.0 (S)	11.9 (I)	10.2 (S)	3.0 (S)	0.85-3.2

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

Figure 10.7 Pharmacological activity of fluorinated Ins(1,4,5)P_3 analogues

11 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-*myo*-inositol

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

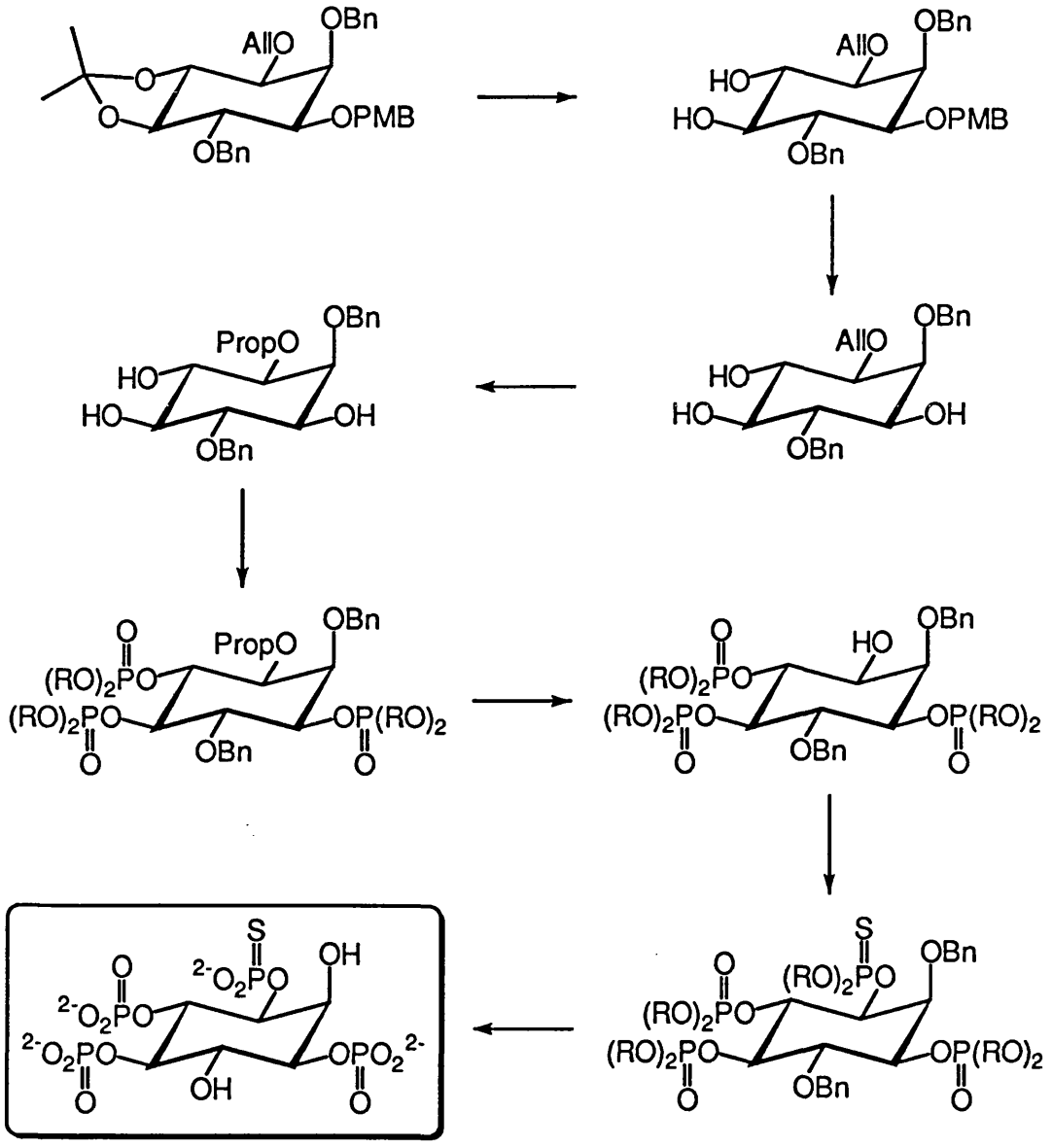


Figure 11.1 Route to Ins(1,4,5)P_3-3S from 3-*O*-allyl-2,6-di-*O*-benzyl-4,5-di-*O*-isopropylidene-1-*O*-*p*-methoxybenzyl-*myo*-inositol

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)P_3 and of Ins(1,3,4,5)P_4, 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)P_3 and Ins(1,3,4,5)P_4 analogues, including the derivative we were most interested in, *i.e.* the 3-phosphorothioate analogue of D-Ins(1,3,4,5)P_4 (Figure 11.1), which would be resistant to hydrolysis by Ins(1,3,4,5)P_4 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-*myo*-inositol (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-*myo*-inositol, DL-3-*O*-allyl-2-*O*-benzyl-4,5-di-*O*-isopropylidene-1-*O*-*p*-methoxybenzyl-*myo*-inositol **184** was synthesised by a 5-step route from DL-1:2,4:5-di-*O*-isopropylidene-*myo*-inositol **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-*myo*-inositol **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-*myo*-inositol 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-*myo*-inositol **179** was obtained in 63 % yield and only minor amounts of DL-3,6-di-*O*-allyl-1,2:4,5-di-*O*-

isopropylidene-*myo*-inositol **180** and DL-6-*O*-allyl-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol (not isolated) were formed.

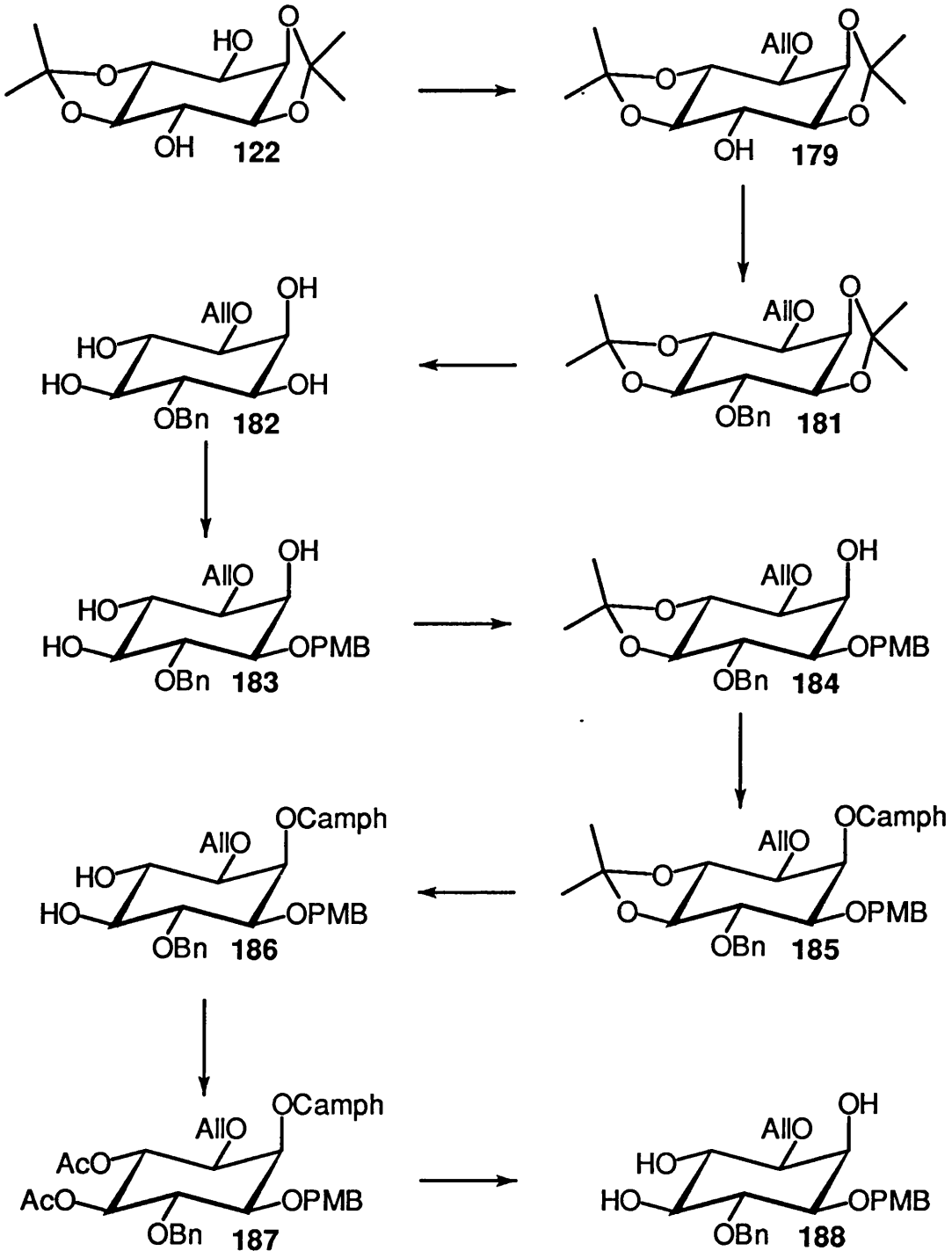


Figure 11.2 Optical resolution of DL-4,5-di-*O*-acetyl-3-*O*-allyl-6-*O*-benzyl-1-*O*-*p*-methoxybenzyl-*myo*-inositol

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-*myo*-inositol **181** thus obtained was then converted into DL-1-*O*-allyl-4-*O*-benzyl-*myo*-inositol **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-*myo*-inositol **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-*myo*-inositol **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-*myo*-inositol **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 ^{1}H-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-*myo*-inositol **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, R_f 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 $[\alpha]_D^{27}$ was found to be +19.0° (c = 4 in $CHCl_3$). The ^{1}H-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-*myo*-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 $[\alpha]_D^{20}$ = +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.

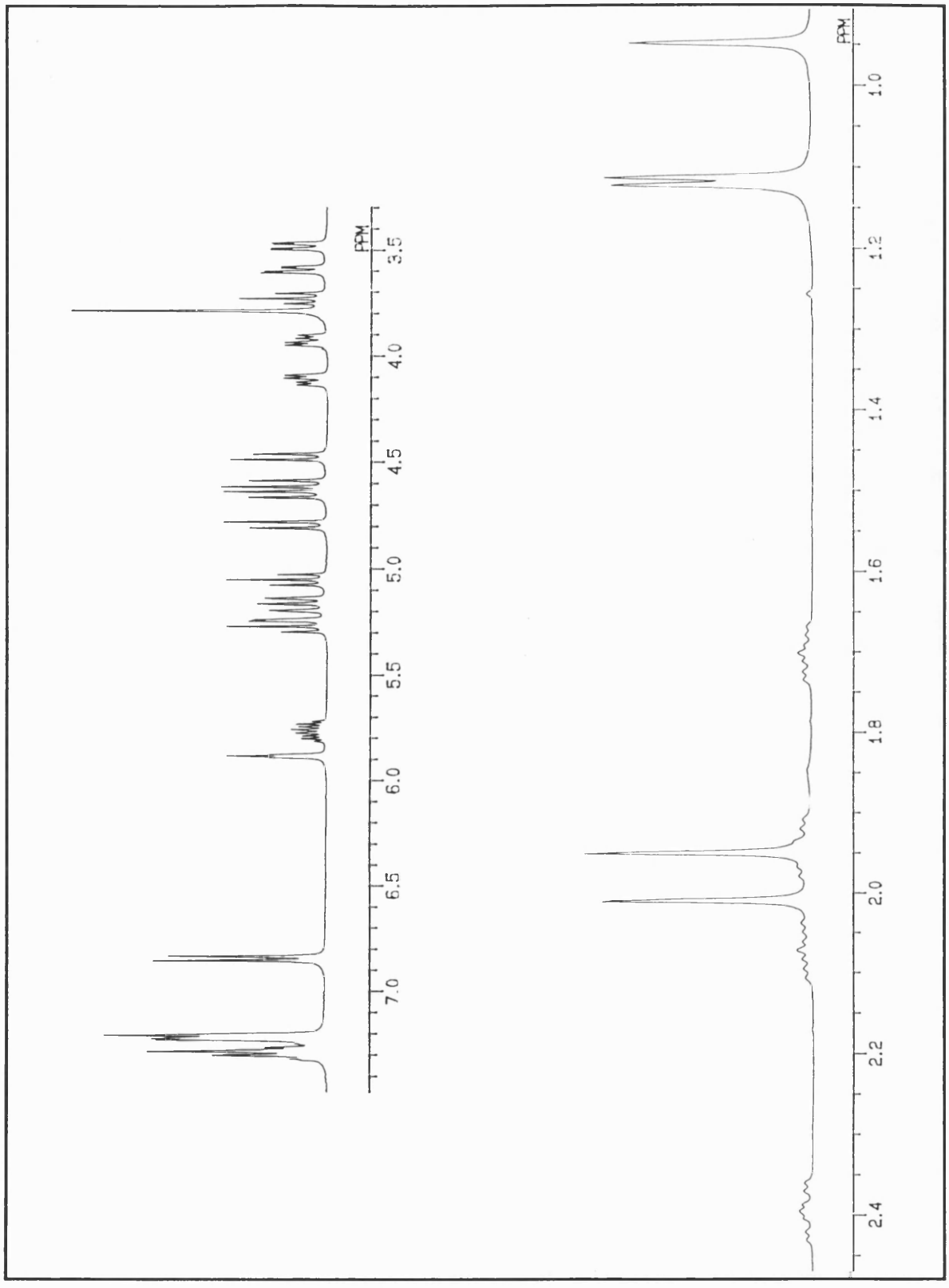


Figure 11.3 ^{1}H-NMR spectrum of (+)-187

12 Novel ring-fused inositol- lactones and *O*-methylene carboxylates

A synthesis of the racemic triscarboxymethyl analogue of Ins(1,4,5)P_3 has been reported by Westerduin *et al.* [289]. This isosteric compound did, however, not shown any affinity for the Ins(1,4,5)P_3 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)P_3 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)P_3 to show biological activity. Comparison of the pharmacological properties of the 1-*O*-methylenecarboxylate analogue with those of Ins(1,4,5)P_3 and Ins(4,5)P_2 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.

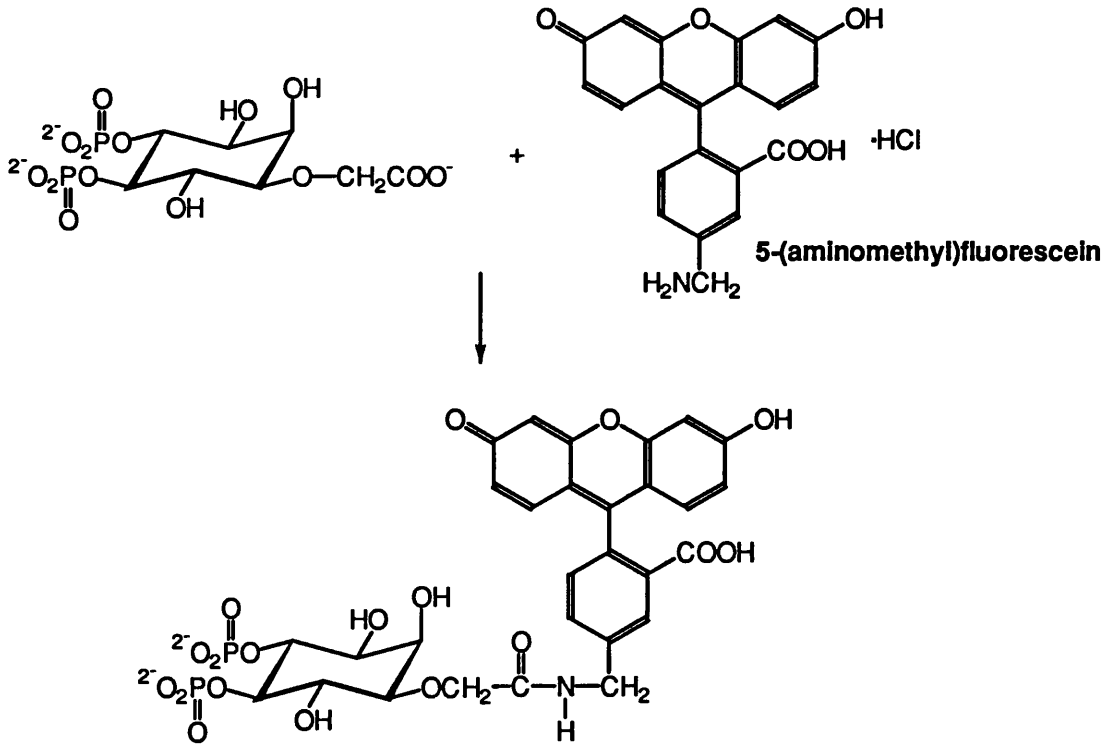


Figure 12.1 Introduction of fluorescent reporter groups into the 1-position of Ins(1,4,5)P_3 analogues *via* amide links

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*-methylenecarboxylate 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)P_3 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.

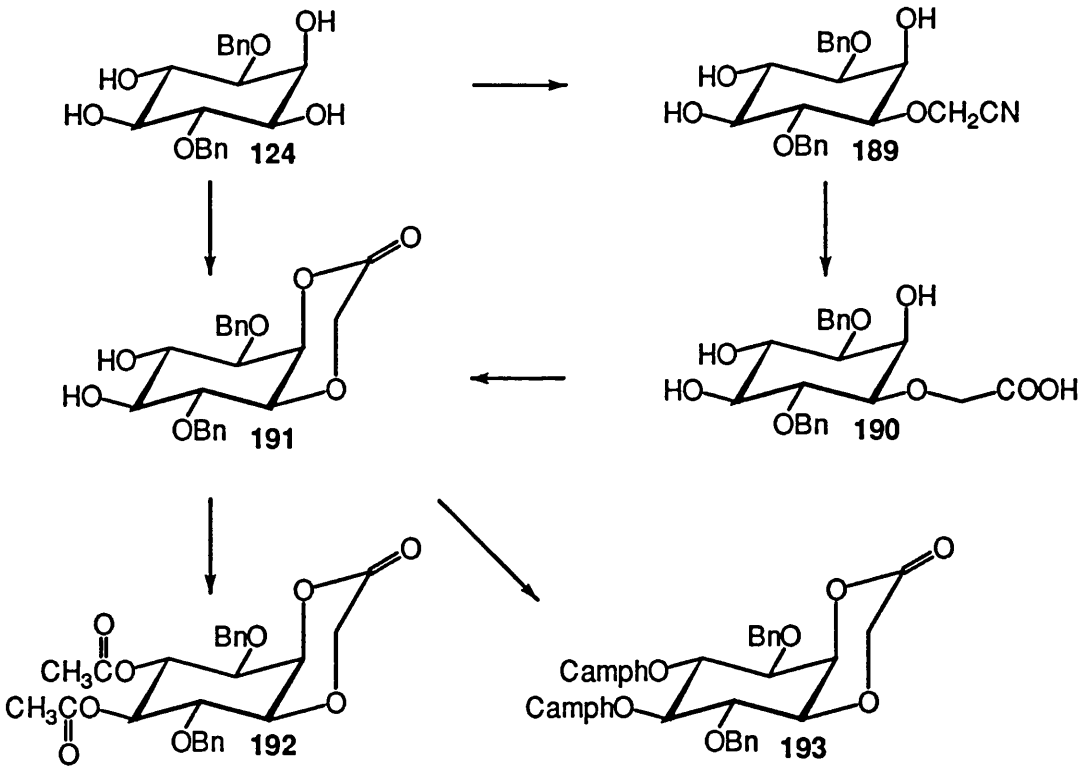


Figure 12.2

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 *C*H_2CN and at 116.21 ppm (s) for *C*N, and in the ^{1}H-NMR spectrum the AB-system at 3.83 and 3.89 ppm was assigned to the CH_2CN group. The ν(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}-H_{ax} 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}-H_{eq} 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-*myo*-inositol **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*-trifluoromethylsulphoxy-*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 $-CH_2-COOH$ moiety might be a better phosphate surrogate than the $-O-CH_2-COOH$ group obtained by oxidative cleavage of allyl ethers. Like compounds with an $-O-CH_2-COOH$ 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 *chiro*-inositol triflates, with potassium benzoate and 18-crown-6 catalyst in DMF at 80°C giving 85 % elimination product *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-*scyllo*-inositol **195** (yield 67 %, Figure 13.2), the substitution product. That this compound is indeed in the *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.

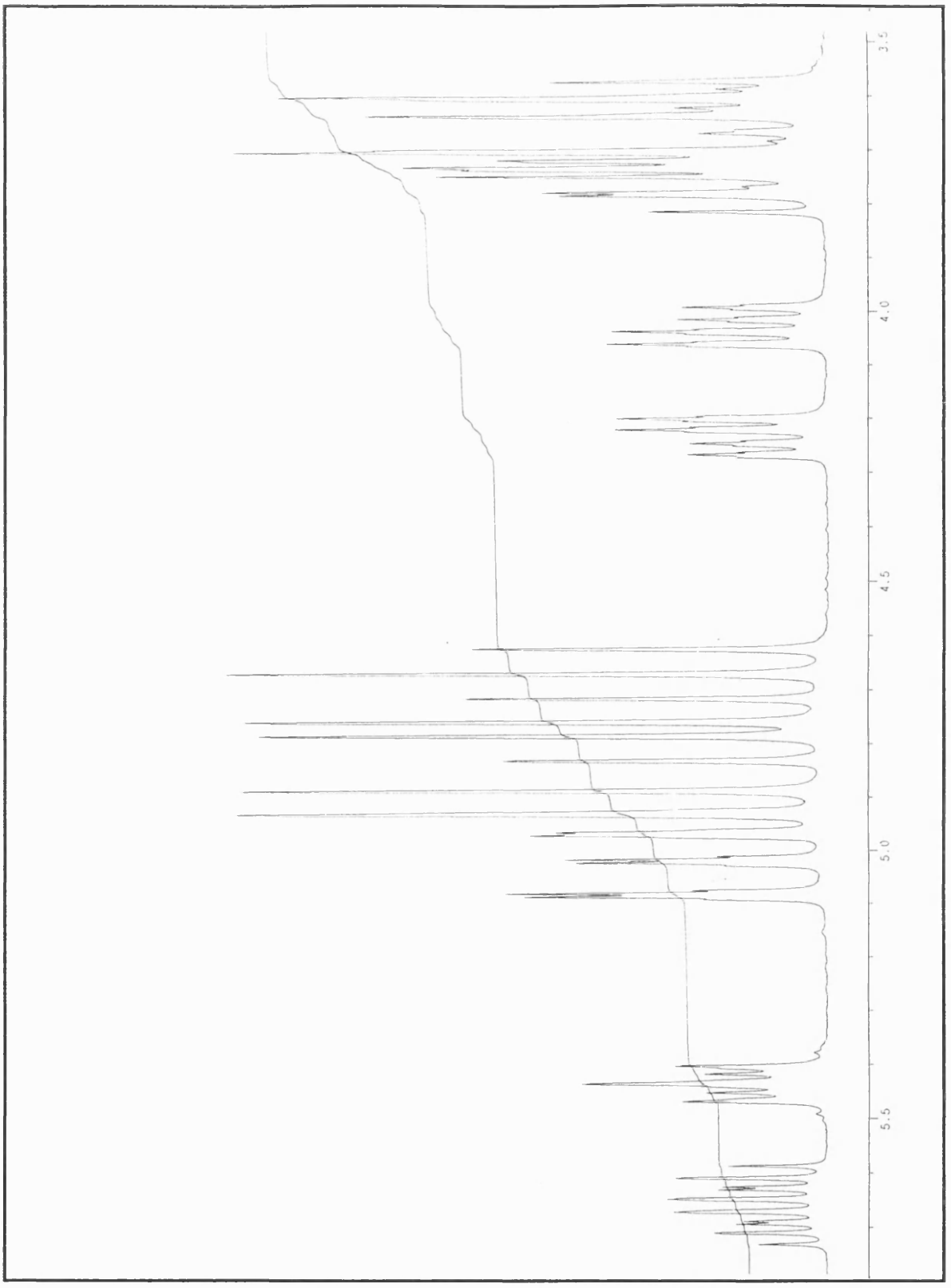


Figure 13.1 ^{1}H-NMR spectrum of 195 (expansion 3.5 to 5.8 ppm)

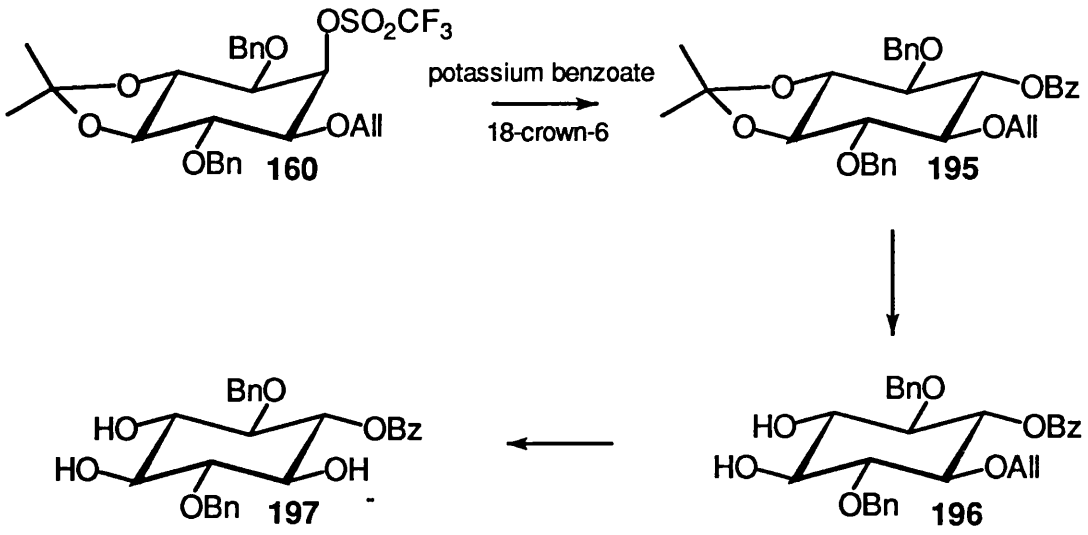


Figure 13.2

195 was then converted into the diol **196** by acid hydrolysis of the 4,5-*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}H-NMR spectra (see Figure 13.4 and 13.5). It was also evident from the ^{1}H-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 deducted:

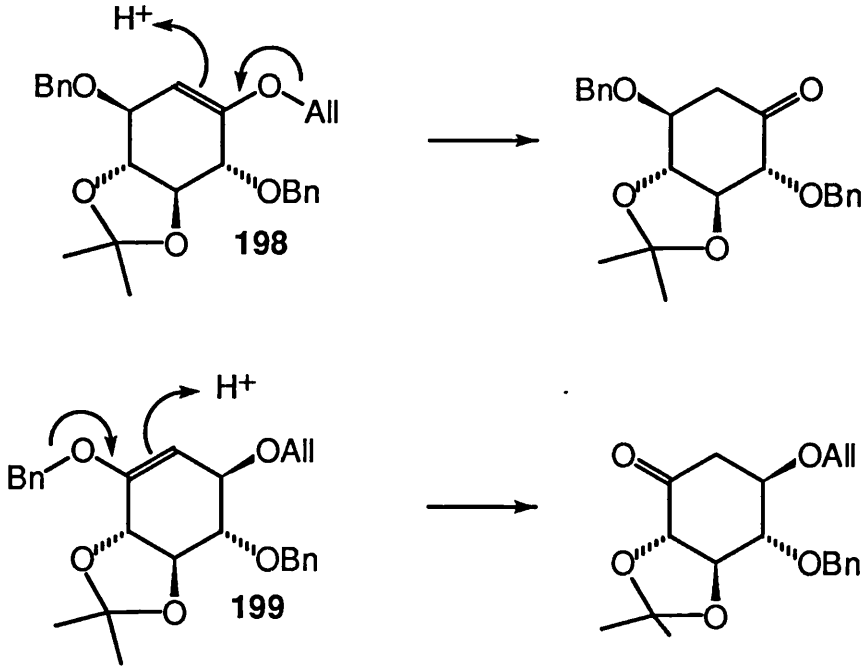


Figure 13.3

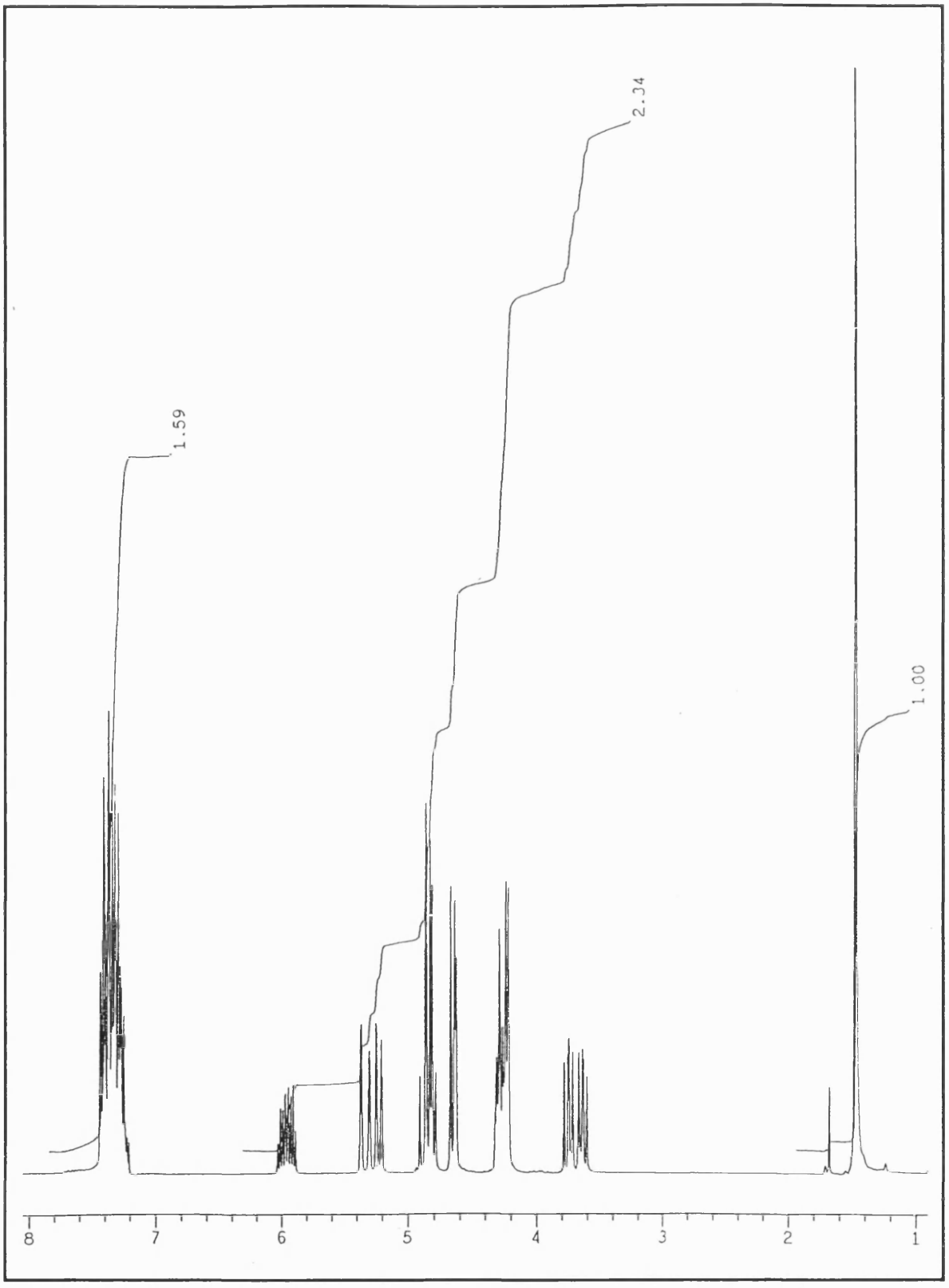


Figure 13.4 ^{1}H-NMR spectrum of 198

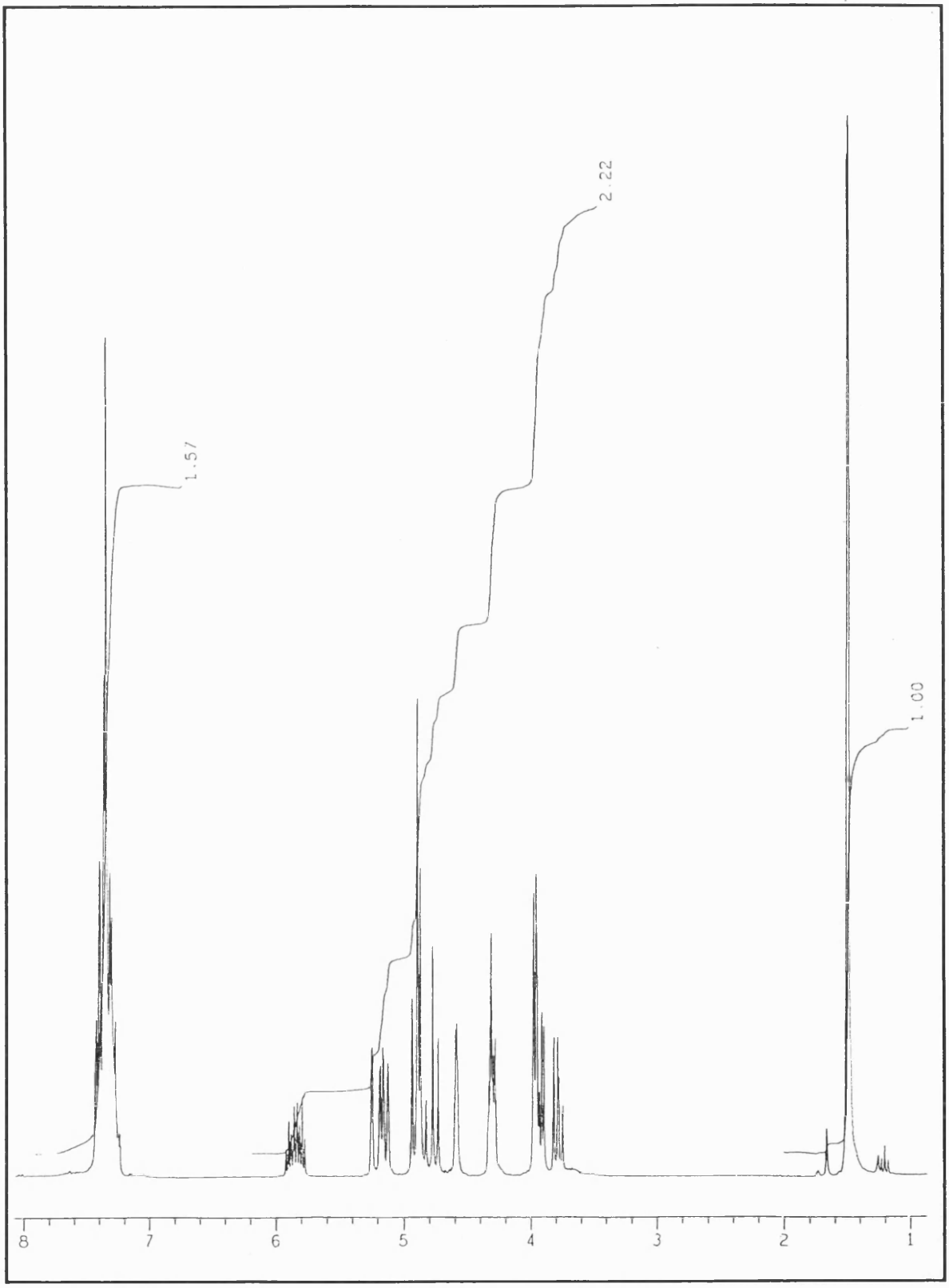


Figure 13.5 ^{1}H-NMR spectrum of 199

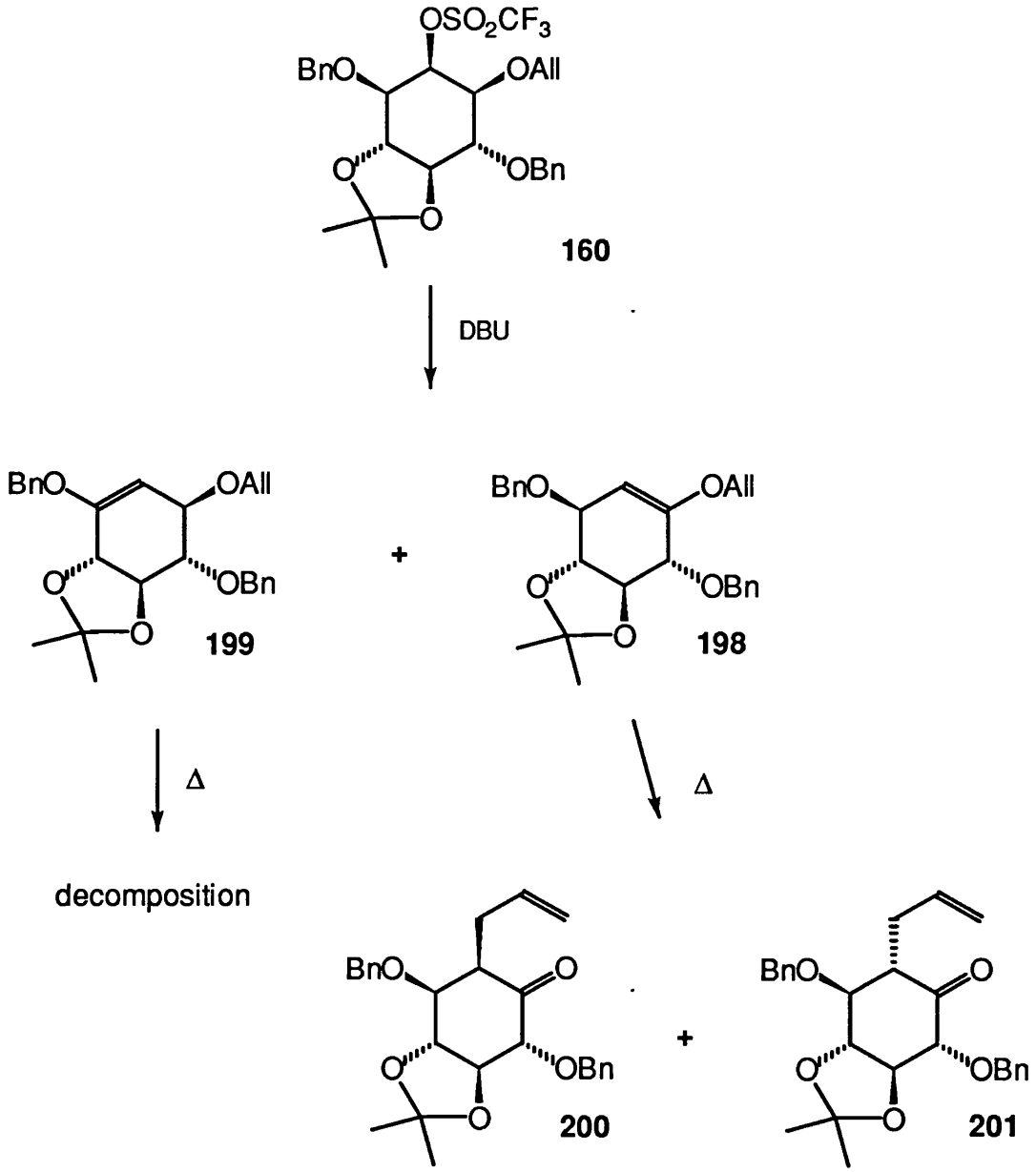


Figure 13.6 Elimination and Claisen rearrangement

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) [389], who obtained this compound by DAST fluorination of 1-*O*-allyl-3,6-di-*O*-benzyl-4,5-*O*-isopropylidene-*myo*-inositol **93**.

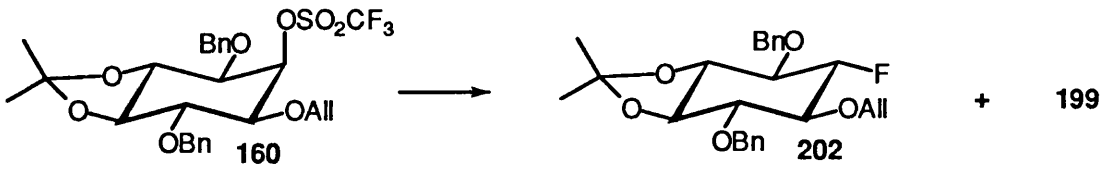


Figure 13.7 Reaction of 160 with tetrabutylammonium fluoride

The assignment of the *scyllo*-configuration to the main product **202** is based on the ^{1}H-NMR signal for C-2-H at 4.50 ppm, which appears as a dt with $J_{H\text{-}C\text{-}F}$ = 49.3 Hz and $J_{H\text{-}C1(3)\text{-}H}$ = 7.9 Hz. The large 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 $^3J_{H\text{-}F}$ 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. $^3J_{H\text{-}F}$ coupling constants in a 2-fluoro derivative with *myo*-inositol configuration can be expected to be much larger (≈ 29 Hz, see Chapter 10.1).

Claisen rearrangement:

When the product obtained by heating **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- (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 (δ_C 203.69 and 205.31), and it thus appears safe to conclude that the reaction mixture contained DL-2-deoxy-2-*C*-

allyl-3,6-di-*O*-benzyl-4,5-*O*-isopropylidene-*myo*-1-inosose **200** and DL-2-deoxy-2-*C*-allyl-3,6-di-*O*-benzyl-4,5-*O*-isopropylidene-*scyllo*-inosose **201**.

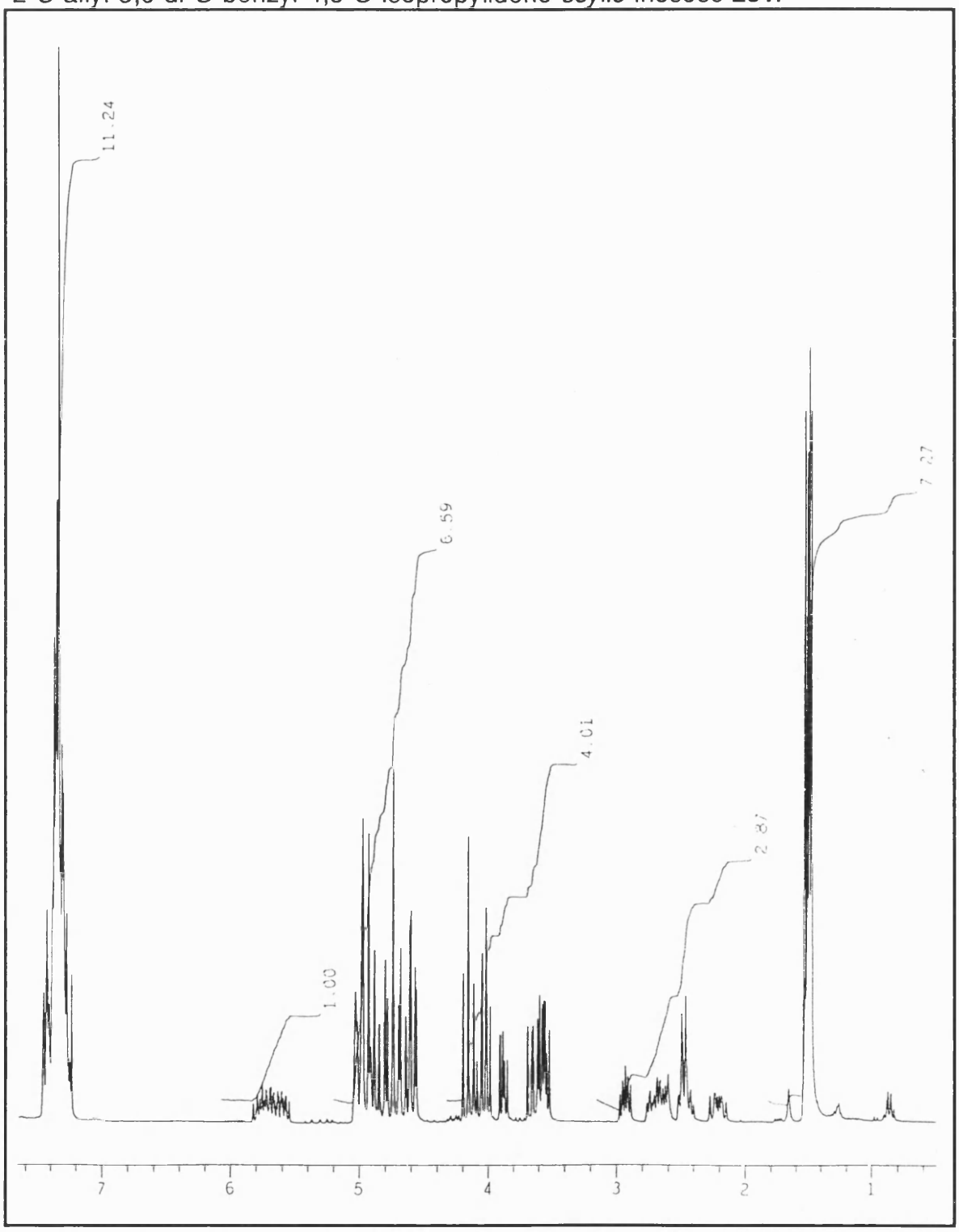


Figure 13.8 ^{1}H-NMR of the Claisen rearrangement product (200 and 201)

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.

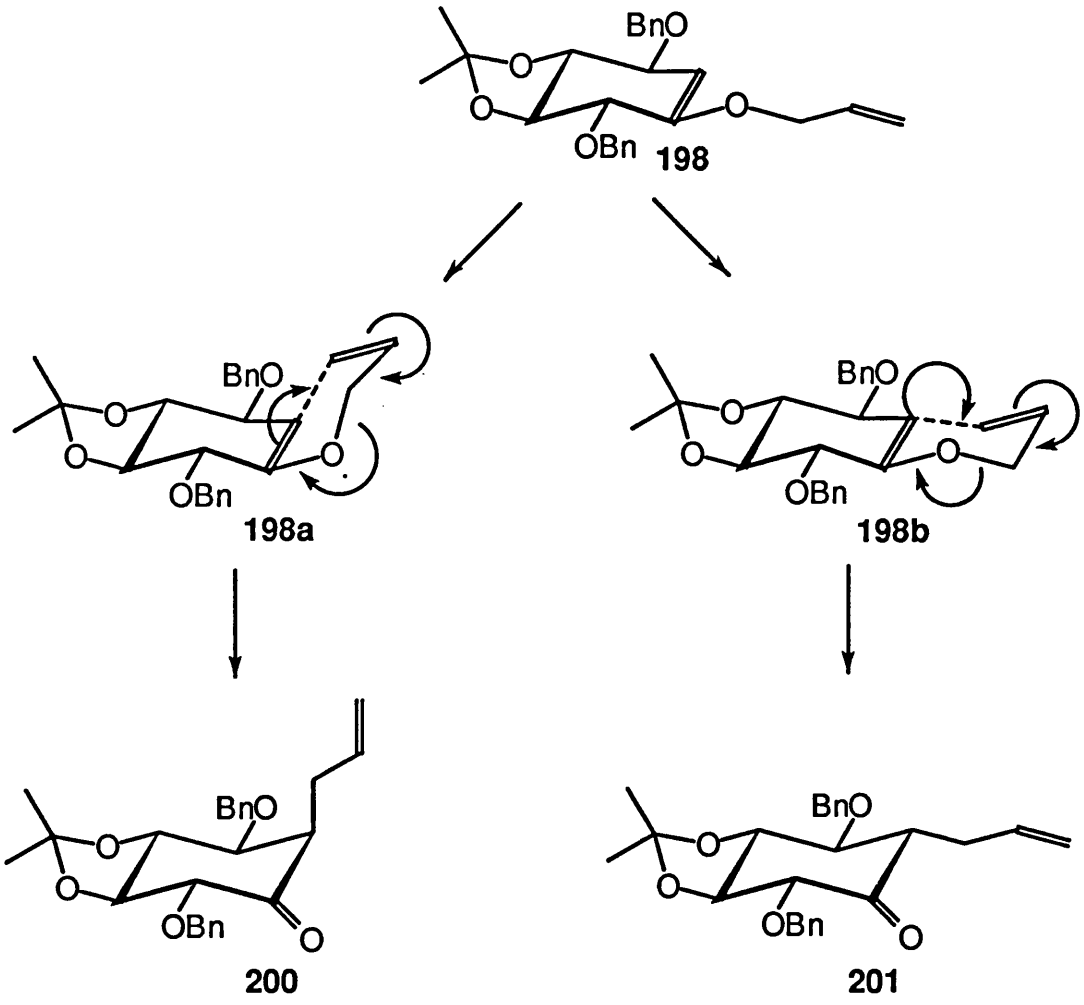


Figure 13.9 Possible transition states in the Claisen rearrangement of 198

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**.

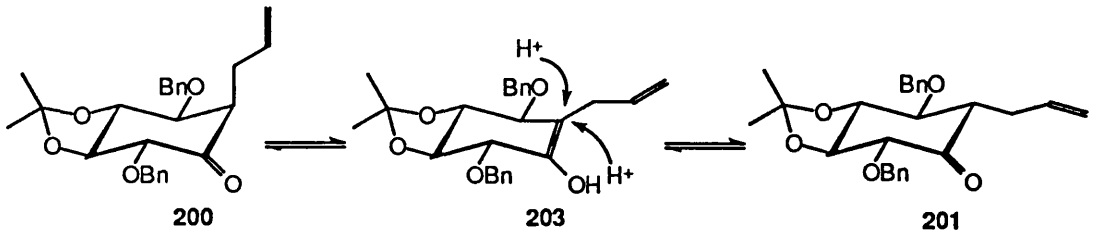


Figure 13.10 Possible epimerisation of 200 and 201 via enol 203

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-*myo*-inositol system **204** or into the axial 1-OH of the 6-deoxy-6-*C*-allyl-*chiro*-inositol system **205**. The same conversions performed on **201** would afford the *C*-allyl-*scyllo*-inositol derivative **206** and the 1-deoxy-1-*C*-allyl-*myo*-inositol **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.

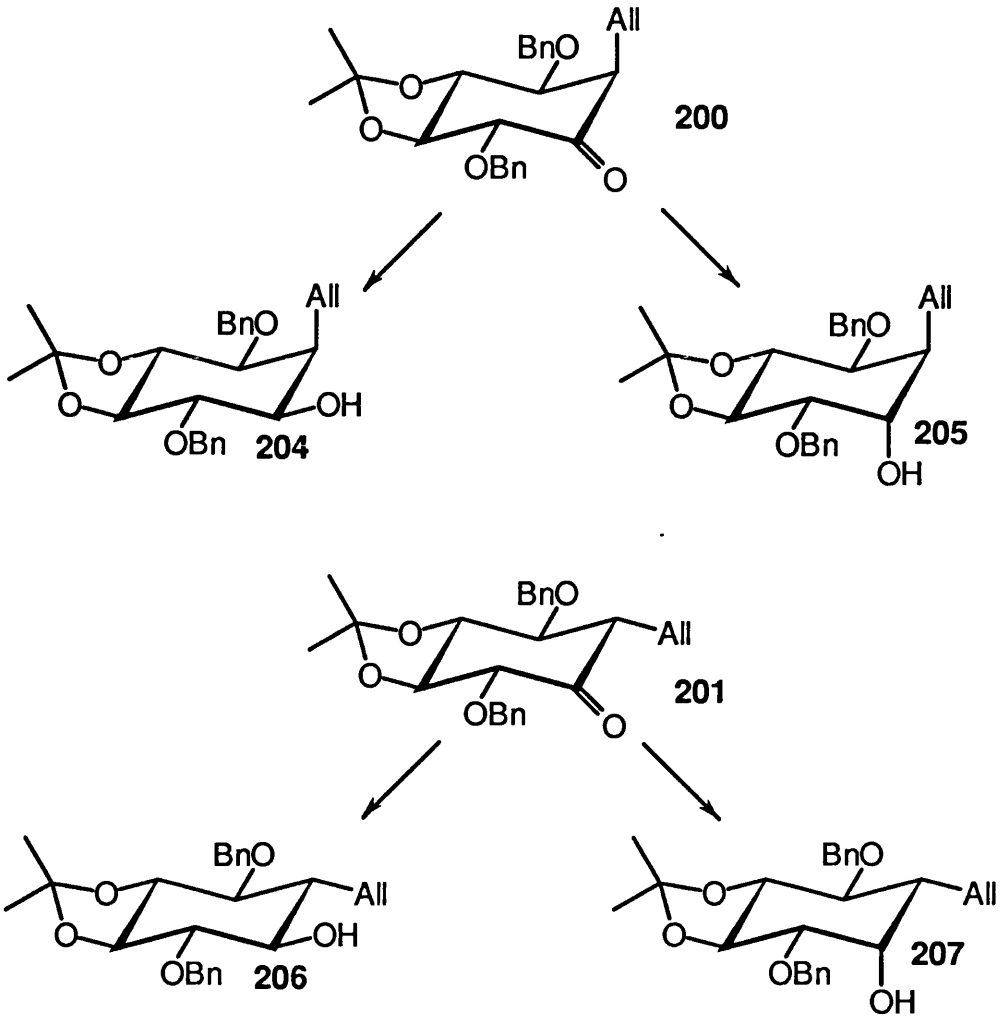


Figure 13.11 Derivatives accessible by selective reduction of 200 and 201

14 Outlook

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.

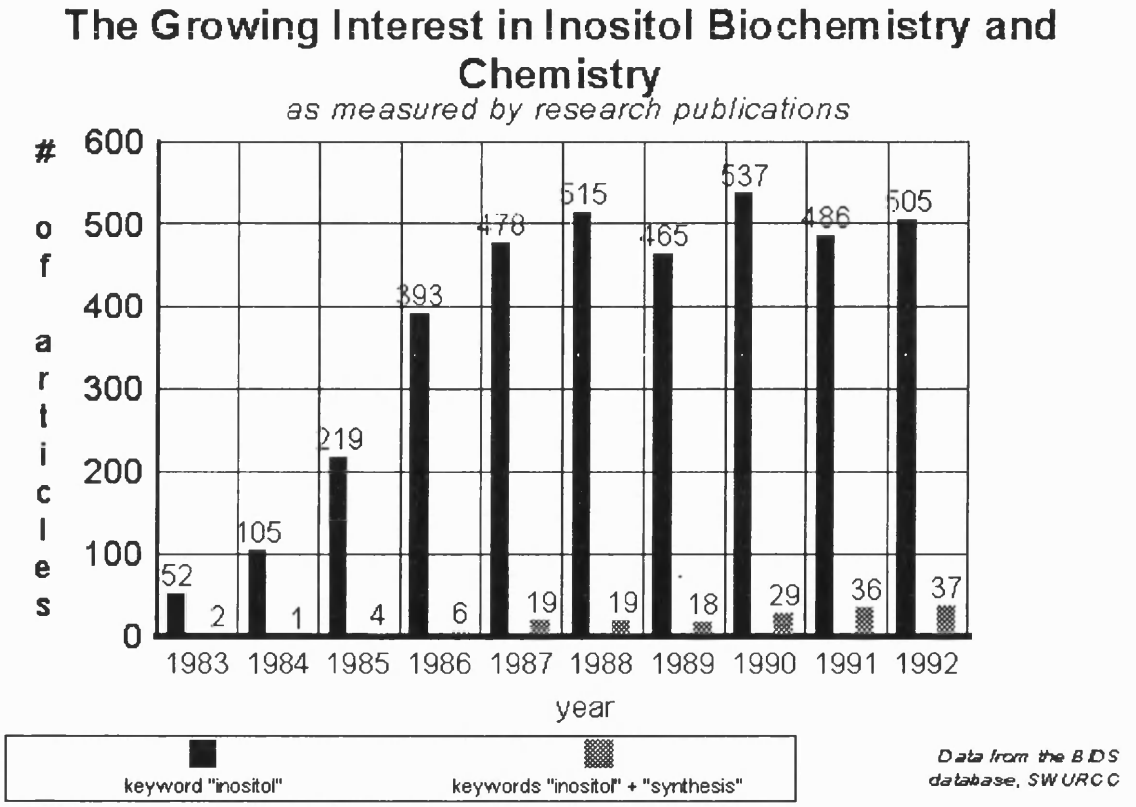


Figure 14.1 Publications on inositol research

Since 1983, the important role that inositol phosphates play in signal transduction has become increasingly clear. However, whilst the function of $Ins(1,4,5)P_3$ 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 Ins(1,4,5)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 ζ 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 Ins(1,4,5)P_3 signalling pathway:

Analogues of Ins(1,4,5)P_3 and Ins(1,3,4,5)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 Ins(1,4,5)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 Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 binding sites. The use of `caged´ Ins(1,4,5)P_3 [392] has allowed the study of calcium mobilisation in intact cell preparations. The first lipophilic Ins(1,4,5)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 Ins(1,4,5)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 Ins(1,4,5)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 Ins(1,4,5)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)P_3 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)P_3. 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 de-ionised 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 F_{254}, Art. no. 5554 and Merck hptlc plates silica 60 F_{254}, 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.* [395] and was carried out using Sorbsil C60 silica gel.

^{1}H- and ^{13}C-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-NMR and ^{19}F-NMR spectra were recorded on a Jeol FX-90Q spectrometer. ^{31}P- and ^{19}F-NMR chemical shifts were measured in ppm relative to external 85 % H_3PO_4 and internal $CFCl_3$, respectively. *J* 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. H_2SO_4), a hydroquinone solution (0.5 g hydroquinone dissolved in 100 ml water and a drop of conc. H_2SO_4) 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. H_2SO_4 (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 sulphydryl 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-*myo*-inositol 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-*p*-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 benzoyl 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)
δ_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)
δ_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-*myo*-inositol **123** (25.7 g, 58 mmol, 98 %).

m.p. 151-153°C (Lit. [149]: 153-155°C)
δ_H ($CDCl_3$, 300 MHz): 1.32, 1.39, 1.45, 1.48 (12 H, 4 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, C*H*$_2$Ph), 4.79, 4.88 (2 H, AB, J_{AB} 12.6, C*H*$_2$Ph), 7.22-7.42 (10 H, m, CH_2*Ph*)

16.4 DL-1,4-Di-*O*-benzyl-*myo*-inositol 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-*myo*-inositol **124** (53 g, 147 mmol, 95%).

m.p. 203-204°C (Lit. [149,396]: 205-207°C, 203-204°C)
δ_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, C*H*$_2$Ph), 4.67 (1 H, d, J 6.8, D_2O ex, OH), 4.76, 4.79 (2 H, AB, J_{AB} 11.4, C*H*$_2$Ph), 4.76 (1 H, d, J 5.0, D_2O ex, OH), 4.79 (1 H, d, J 4.6, D_2O ex, OH), 4.83 (1 H, d, J 5.0, D_2O ex, OH), 7.23-7.44 (10 H, m, CH_2*Ph*)

16.5 DL-1-*O*-Allyl-3,6-di-*O*-benzyl-*myo*-inositol 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-*myo*-inositol **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 $NaHCO_3$ 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. [168]: 107-108°C)
tlc (ether) R_f 0.3
δ_H ($CDCl_3$, 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, J_{AB} 12.6, J 5.7, 1.4, 1.4, C*H*$_2$-CH=CH$_2$), 4.24 (1 H, dd, J 2.8, 2.8, C-2-H), 4.69, 4.74 (2 H, AB, J_{AB} 11.7, C*H*$_2$Ph), 4.74, 4.93 (2 H, AB, J 11.2, C*H*$_2$Ph), 5.19 (1 H, ddt, J 10.4, 1.4, 1.4, *cis*-CH$_2$-CH=C*H*$_2$), 5.29 (1 H, ddt, J 17.2, 1.6, 1.6, *trans*-CH$_2$-CH=C*H*$_2$), 5.92 (1 H, ddt, J 17.2, 10.4, 5.7, CH$_2$-C*H*=CH$_2$), 7.25-7.40 (10 H, m, CH$_2$*Ph*)

16.6 DL-1-*O*-Allyl-3,6-di-*O*-benzyl-4,5-*O*-isopropylidene-*myo*-inositol 93

This compound was prepared according to the method of Gigg *et al.* [168].
DL-1-*O*-Allyl-3,6-di-*O*-benzyl-*myo*-inositol **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 (R_f 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-*myo*-inositol **93** (3.27 g, 7.4 mmol, 74 %).

m.p. 102-103°C (Lit. [168]: 103-105°C)
δ_H ($CDCl_3$, 300 MHz) 1.46 (3 H, s, CH_3), 1.47 (3 H, s, CH_3), 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, C*H*$_2$-CH=CH$_2$), 4.22 (1 H, dd, J 2.9, 2.9, C-2-H), 4.72, 4.86 (2 H, AB, J_{AB} 11.6, C*H*$_2$Ph), 4.76, 4.88 (2 H, AB, J_{AB} 12.0, C*H*$_2$Ph), 5.17 (1 H, ddt, J 10.25, 1.4, 1.4, *cis*-CH$_2$-CH=C*H*$_2$), 5.26 (1 H, ddt, J 17.2, 1.65, 1.65, *trans*-CH$_2$-CH=C*H*$_2$), 5.90 (1 H, ddt, J 17.2, 10.25, 5.5, CH$_2$-C*H*=CH$_2$), 7.28-7.41 (10 H, m, CH$_2$*Ph*)

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

DL-1-*O*-Allyl-3,6-di-*O*-benzyl-4,5-*O*-isopropylidene-*myo*-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 ($MgSO_4$) 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): R_f 0.9
δ_H ($CDCl_3$, 300 MHz) 1.46 (6 H, s, 2 CH_3), 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, C*H*$_2$-CH=CH$_2$), 4.18 (1 H, dd, J 2.7, 2.7, C-2-H), 4.56, 4.67 (2 H, AB, J_{AB} 11.8, C*H*$_2$Ph), 4.71, 4.85 (2 H, AB, J_{AB} 11.8, C*H*$_2$Ph), 4.76, 4.88 (2 H, AB, J_{AB} 11.9, C*H*$_2$Ph), 5.17 (1 H, ddt, J 10.25, 1.4, 1.4, *cis*-CH$_2$-CH=C*H*$_2$), 5.26 (1 H, ddt, J 17.2, 1.65, 1.65, *trans*-CH$_2$-CH=C*H*$_2$), 5.90 (1 H, ddt, J 17.2, 10.25, 5.5, CH$_2$-C*H*=CH$_2$), 7.28-7.41 (15 H, m, CH$_2$*Ph*)

16.8 DL-1-*O*-Allyl-2,3,6-tri-*O*-benzyl-*myo*-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 $NaHCO_3$ 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')
δ_H ($CDCl_3$, 270 MHz) 2.56, 2.60 (2 H, 2 br s, D_2O 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, $CH_2CH=CH_2$), 4.55, 4.61 (2 H, AB, J_{AB} 11.7, CH_2Ph), 4.75, 4.96 (2 H, AB, J_{AB} 11.9, CH_2Ph), 4.79, 4.89 (2 H, AB, J_{AB} 11.2, CH_2Ph), 5.19 (1 H, ddt, J 10.4, 1.4, 1.4, *cis*-$CH_2CH=CH_2$), 5.31 (1 H, ddt, J 17.2, 1.6, 1.6, *trans*-$CH_2CH=CH_2$), 5.91 (1 H, ddt, J 17.2, 10.4, 5.3, $CH_2CH=CH_2$), 7.25-7.42 (15 H, m, CH_2Ph);
δ_C ($CDCl_3$, 68 MHz) 71.45, 72.26, 74.02, 75.35 (4 t, $CH_2CH=CH_2$ and CH_2Ph), 72.20, 73.53, 74.60, 79.95, 80.76, 80.83 (6 d, inositol ring C), 116.73 (t, $CH_2CH=CH_2$), 127.37, 127.63, 127.70, 127.79, 127.96, 128.12, 128.38, 128.44 (8 d, CH_2Ph), 134.67 (d, $CH_2CH=CH_2$), 137.85, 138.76 (2 s, CH_2Ph);
m/z (70 eV EI) 399 [(M - C_7H_7)$^+$, 2 %], 307, 181 (5), 131 (10), 109 (5), 91 [(C_7H_7)$^+$, 100];
m/z (CI, 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 %).

δ_H ($CDCl_3$, 300 MHz) 1.56 (0.5 H, dd, J 6.8, 1.55, *trans*-CH=CH-CH_3), 1.68 (2.5 H, dd, J 6.8, 1.6, *cis*-CH=CH-CH_3), 2.67 (1 H, d, J 2.0, D_2O ex, OH), 2.70 (1 H, d, J 1.8, D_2O 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=C*H*-CH_3 and 3 CH_2Ph), 6.07-6.15 (1 H, m, C*H*=CH-CH_3), 7.25-7.42 (15 H, m, CH_2*Ph*);

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 (R_f 0.78) to a single product (R_f 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 $C_{30}H_{34}O_6$ (490.60): C, 73.45; H, 6.99 %.
δ_H ($CDCl_3$, 270 MHz) 1.67 (3 H, dd, J 6.9, 1.65, CH=CH-C*H*$_3$), 2.76 (1 H, d, J 2.4, D_2O ex, OH), 2.79 (1 H, d, J 2.0, D_2O ex, OH), 3.21 (1 H, dd, J 9.7, 2.4, C-3-H), 3.42 (1 H, ddd, J 9.2, 9.2, 2.2, D_2O shake gives dd, 9.2, 9.2, 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, D_2O shake gives dd, J 9.5, 9.5, C-4-H), 4.07 (1 H, dd, J 2.4, 2.4, C-2-H), 4.49 (1 H, dq, J 6.7, 6.7, CH=C*H*-CH_3), 4.49, 4.56 (2 H, AB, J_{AB} 11.6, CH_2Ph), 4.70, 4.88 (2 H, AB, J_{AB} 11.6, CH_2Ph), 4.77, 4.88 (2 H, AB, J_{AB} 11.6, CH_2Ph), 6.08 (1 H, dd, J 6.2, 1.65, C*H*=CH-CH_3), 7.23-7.42 (15 H, m, CH_2*Ph*);
δ_C ($CDCl_3$, 68 MHz) 9.44 (q, CH=CH-*C*H_3), 74.44, 75.35 (2 t, *C*H_2Ph), 72.07, 74.24, 74.96, 79.43, 80.28, 83.23 (6 d, inositol ring C), 100.97 (d, CH=*C*H-CH_3), 127.50, 127.66, 127.79, 128.18, 128.28, 128.41, 128.48 (7 d, CH_2*Ph*), 137.60, 138.43 (2 s, CH_2*Ph*), 145.34 (d, *C*H=CH-CH_3);
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 [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**.

δ_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 [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. [226] 64 %).

b.p. 120°C / 0.8 hPa (Lit. [226] 117°C / 0.5 Torr)
δ_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 [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 chromatogaphy of the crude material (eluting with ether / pentane 3:1) gave **131** (72 g, 265 mmol, 68 %, Lit. [226] 81 %).

δ_H ($CDCl_3$; 300 MHz) 1.14 (12 H, d, J 6.8, $CH(CH_3)_2$), 2.62-2.57 (4 H, t, J 6.2, OCH_2CH_2CN), 3.47-3.65 (2H, m, $CH(CH_3)_2$), 3.68-3.93 (4 H, m, OCH_2CH_2CN)

δ_P ($CDCl_3$; 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 $C_{42}H_{48}N_4O_{12}P_2$ (862.81): C, 58.47; H, 5.61; N, 6.49 %.
δ_H ($CDCl_3$, 270 MHz) 1.63 (3 H, dd, J 6.8, 1.6, CH_2=CH-CH_3), 2.16-2.39 (4 H, m, CH_2CH_2CN), 2.65-2.69 (4 H, m, CH_2CH_2CN), 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, CH_2CH_2CN, C-2-H, C-6-H), 4.38-4.99 (9 H, m, CH_2Ph, C-4-H, C-5-H, CH=C*H*-CH_3), 6.06 (1 H, dd, J 6.8, 1.6, C*H*=CH-CH_3), 7.26-7.40 (15 H, m, CH_2*Ph*);
δ_C ($CDCl_3$, 68 MHz) 9.34 (q, CH=CH-CH_3), 18.97, 19.10, 19.23, 19.33 (4 t,

CH_2CH_2CN), 62.11, 62.70 (2 t, CH_2CH_2CN), 71.97, 74.70 (2 t, CH_2Ph), 73.76, 74.83, 78.10, 78.52, 79.60, 82.48 (6 d, inositol ring C), 102.04 (d, CH=*C*H-CH_3) 116.60, 116.83, 116.93 (3 s, CN), 126.85, 127.31, 127.60, 127.70, 127.92, 128.12, 128.22, 128.34, 128.51 (9 d, CH_2*Ph*), 137.01, 137.85, 138.21 (3 s, CH_2*Ph*), 144.66 (d, *C*H=CH-CH_3);
δ_P ($CDCl_3$, 36 MHz) -3.57, -3.70;
m/z (+ve ion FAB) 863 [($M+H^+$), 1.3 %], 771 (0.3), 181 (6), 144 (8), 91 [$(C_7H_7)^+$, 100];
m/z (-ve ion FAB) 808 [$(M - CH_2CH_2CN)^-$, 55 %], 718 (12), 203 (100), 150 (85), 97 (70);
Accurate mass calc for $C_{42}H_{49}O_{12}N_4P_2$ $(M+H)^+$ 863.2822, found 863.2822

132 (mixture of *cis*- and *trans*-isomers):
δ_H ($CDCl_3$, 270 MHz) 1.51 (0.5 H, dd, J 6.8, 1.6, *trans*-CH_2=CH-CH_3), 1.63 (2.5 H, dd, J 6.8, 1.6, *cis*-CH_2=CH-CH_3), 2.10-2.45 (4 H, m, CH_2CH_2CN), 2.56-2.76 (4 H, m, CH_2CH_2CN), 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, CH_2CH_2CN, C-2-H, C-6-H), 4.38-4.99 (9 H, m, CH_2Ph, C-4-H, C-5-H, CH=C*H*-CH_3), 6.03-6.13 (1 H, m, C*H*=CH-CH_3), 7.26-7.40 (15 H, m, CH_2*Ph*);

16.15 DL-1,2,4-Tri-*O*-benzyl-5,6-bis[di(2-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 (R_f 0.76) into a single product (R_f

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_H ($CDCl_3$, 300 MHz) 2.14-2.46 (4 H, m, CH_2CH_2CN), 2.60-2.72 (5 H, m, CH_2CH_2CN, OH, OH D_2O 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, D_2O 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), 3.90-4.37 (8 H, m, CH_2CH_2CN), 4.43 (1 H, q, J 9.1, C-5-H), 4.56, 4,67 (2 H, AB, J_{AB} 11.3, CH_2Ph), 4.75, 4,93 (2 H, AB, J_{AB} 11.6, CH_2Ph), 4.79, 4,92 (2 H, AB, J_{AB} 11.4, CH_2Ph), 4.83 (1 H, q, J 9.3, C-6-H), 7.22-7.41 (15 H, m, CH_2Ph);

δ_C ($CDCl_3$, 68 MHz) 18.65, 18.78, 18.84 (3 t, CH_2CH_2CN), 62.02, 62.08, 62.66 (3 t, CH_2CH_2CN), 71.97, 74.05, 74.86 (3 t, CH_2Ph), 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, CH_2Ph), 136.91, 137.85, 138.04 (3 s, CH_2Ph);
δ_P ($CDCl_3$, 36 MHz) -5.52, -5.65;

m/z (+ve ion FAB) 823 [$(M+H)^+$, 1.3 %], 222 (8), 181 (6), 144 (8), 91 [$(C_7H_7)^+$, 100];
m/z (-ve ion FAB) 768 [$(M - CH_2CH_2CN)^-$, 45 %], 203 (100), 150 (95), 97 (80);
Accurate mass calc for $C_{39}H_{45}O_{12}N_4P_2$ $(M+H)^+$ 823.2509, found 823.2509

16.16 DL-2,3,6-Tri-*O*-benzyl-4,5-bis[di(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 %).

δ_H ($CDCl_3$, 270 MHz) 2.03-2.47 (6 H, m, CH_2CH_2CN), 2.49-2.79 (6 H, m, CH_2CH_2CN), 3.61 (1 H, dd, J 9.9, 1.8, C-3-H), 3.82-4.46 (14 H, m, CH_2CH_2CN, 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, J_{AB} 11.3, CH_2Ph), 4.87 (2 H, AB, CH_2Ph), 4.85 (1 H, q, J 8.6, C-4-H), 4.84, 4.92 (2 H, AB, J_{AB} 11.7, CH_2Ph), 7.27-7.44 (15 H, m, CH_2Ph)
δ_C ($CDCl_3$, 68 MHz) 18.78, 18.91, 19.14, 19.20, 19.27 (5 t, CH_2CH_2CN), 62.08, 62.18, 62.24, 62.37, 62.44, 62.53, 62.63, 62.79, 62.83 (9 t, CH_2CH_2CN), 72.33, 74.34, 77.26 (3 t, CH_2Ph), 74.63, 75.25, 78.04, 78.36, 78.46, 79.17 (6 d, inositol ring C), 116.41, 116.51, 116.57, 116.83, 116.96 (5 s, CN), 126.40, 127.60, 127.76, 128.05, 128.18, 128.31, 128.48 (7 d, CH_2Ph), 136.86, 137.72, 137.85 (3 s, CH_2Ph);
δ_P ($CDCl_3$, 36 MHz) 66.83, -2.83, -3.10;
m/z (+ve ion FAB) 1025 [$(M+H)^+$, 1.3 %], 181 (6), 144 (10), 91 [$(C_7H_7)^+$, 100].
m/z (-ve ion FAB) 970 [$(M - CH_2CH_2CN)^-$, 45 %], 219 (30), 203 (100), 150 (90), 97 (80);
Accurate mass calc for $C_{45}H_{52}O_{14}N_6P_3S$ $(M+H)^+$ 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_H (D_2O, 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_P (D_2O, pH 8, 36 MHz) 42.13, 4.58, 3.50;
m/z (+ve ion FAB) 538 [$(M+Et_3NH)^+$, 10%], 436 (M^+, 3), 102 (Et_3NH^+, 100)
Accurate mass calc for $C_{12}H_{31}O_{14}NP_3S$ $(M+Et_3NH)^+$ 538.0678 found 538.0678

16.18 Fluorescent Labelling of Ins(1,4,5)P_3-1S

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_P (D_2O, 36 MHz, ^1H-coupled) 1.72 (d, J 8.5, OPO_3^{2-}), 3.01 (d, J 8.5, OPO_3^{2-}), 19.86 (q, J 9.1, $OPSO_2^-$);
m/z (+ve ion FAB) 815 [$(M+Et_3NH)^+$, 3 %], (799 [$(M+Et_3NH - O)^+$, 5], 714 (M^+, 12), 698 [$(M - O)^+$, 14], 596 [$(Ins(1,4,5)P_3S\text{-}CH_2CO_2 + Et_3NH)^+$, 27], 102 ($Et_3NH^+$, 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-camphanic 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 (R_f 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° (c = 5 in $CHCl_3$);
Found C, 70.4; H 6.73. Calc for $C_{50}H_{58}O_{12}$ (851.00): C, 70.57; H, 6.87 %.
δ_H ($CDCl_3$, 300 MHz) 0.75 (6 H, 2 s, CH_3), 0.91 (3 H, s, CH_3), 0.95 (3 H, s, CH_3), 1.03 (6 H, s, 2 CH_3), 1.59-1.67 (2 H, m, CH_2), 1.77-1.88 (4 H, m, 2 CH_2), 2.27-2.39 (2 H, m, CH_2), 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, C*H*$_2$CH=CH$_2$), 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, C*H*$_2$Ph), 4.85, 4.87 (2 H, AB, J_{AB} 12.1, C*H*$_2$Ph), 4.62, 5.02 (2 H, AB, J_{AB} 11.3, C*H*$_2$Ph), 5.18 (1 H, ddt, J 10.4, 1.4, 1.4, *cis*-CH$_2$CH=C*H*$_2$), 5.27 (1 H, ddt, J 17.2, 1.6, 1.6, *trans*-CH$_2$CH=C*H*$_2$), 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$_2$C*H*=CH$_2$), 7.20-7.42 (15 H, m, CH$_2$*Ph*);
δ_C ($CDCl_3$, 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, *C*H$_2$CH=CH$_2$ and *C*H$_2$Ph), 72.82, 73.37, 74.08, 78.07, 78.23, 80.18 (6 d, inositol ring C), 91.05 (s), 117.45 (t, CH$_2$CH=*C*H$_2$), 126.85, 127.24, 127.34, 127.708, 127.83, 127.96, 128.18, 128.28, 128.39 (9 d, CH$_2$*Ph*), 134.12 (d, CH$_2$*C*H=CH$_2$), 137.04, 138.17 (3 s, CH$_2$*Ph*), 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-*myo*-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 (R_f 0.79) to a single product (R_f 0.59). After cooling, the solution was neutralised with solid CO_2. 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 $C_{30}H_{34}O_6$ (490.60): C, 73.45; H, 6.99 %.
$[\alpha]_D^{18}$ = +21.5° (c = 4 in $CHCl_3$), (Lit. [168]: $[\alpha]_D$ for the enantiomer - 20.5 °)
δ_H ($CDCl_3$, 270 MHz) 2.56, 2.60 (2 H, 2 br s, D_2O 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, CH_2CH=CH_2), 4.55, 4.61 (2 H, AB, J_{AB} 11.7, CH_2Ph), 4.75, 4.96 (2 H, AB, J_{AB} 11.9, CH_2Ph), 4.79, 4.89 (2 H, AB, J_{AB} 11.2, CH_2Ph), 5.19 (1 H, ddt, J 10.4, 1.4, 1.4, *cis*-CH_2CH=CH_2), 5.31 (1 H, ddt, J 17.2, 1.6, 1.6, *trans*-CH_2CH=CH_2), 5.91 (1 H, ddt, J 17.2, 10.4, 5.3, CH_2C*H*=CH_2), 7.25-7.42 (15 H, m, CH_2*Ph*);
δ_C ($CDCl_3$, 68 MHz) 71.45, 72.26, 74.02, 75.35 (4 t, *C*H_2CH=CH_2 and *C*H_2Ph), 72.20, 73.53, 74.60, 79.95, 80.76, 80.83 (6 d, inositol ring C), 116.73 (t, CH_2CH=*C*H_2), 127.37, 127.63, 127.70, 127.79, 127.96, 128.12, 128.38, 128.44 (8 d, CH_2*Ph*), 134.67 (d, CH_2*C*H=CH_2), 137.85, 138.76 (2 s, CH_2*Ph*);
m/z (70 eV EI) 399 [(M - C_7H_7)$^+$, 2 %], 307, 181 (5), 131 (10), 109 (5), 91 [(C_7H_7)$^+$, 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 $C_{30}H_{34}O_6$ (490.60): C, 73.45; H, 6.99 %.
$[\alpha]_D^{11}$ = +40.6° (c = 4 in $CHCl_3$)

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-enyl)-*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 $C_{42}H_{48}N_4O_{12}P_2$ (862.81) C, 58.47; H, 5.61; N, 6.49 %.
$[\alpha]_D^{19}$ = +11.5° (c = 3.5 in $CHCl_3$)
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 µmol) 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.

$[\alpha]_D^{15}$ = + 8.5° (c = 4.5 in $CHCl_3$, Lit. [379] $[\alpha]_D$ = +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.

$[\alpha]_D^{16}$ = +10.5° (c = 2.5 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 µmol) was deprotected as for the racemic compound to give pure **142** (21.5 µmol, 44 %) after ion-exchange chromatography.

$[\alpha]_D^{21}$ = -42.7° (c = 0.19 in H_2O, pH = 9.4)
Mass spectra and NMR data were identical to **80**.

18 Synthesis of 1L-*myo*-Inositol 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);
$[\alpha]_D^{18}$ = -25.0° (c = 4.2 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);

δ_C ($CDCl_3$, 68 MHz) 16.28, 16.44, 16.54 (3 q), 28.70, 31.10 (2 t), 53.58, 53.74, 54.56, 54.62 (4 s), 71.42, 71.97, 72.26, 74.79 (4 t, $CH_2CH=CH_2$ and CH_2Ph) 72.26, 73.56, 74.21 77.81, 78.49, 80.02 (6 d, inositol ring C), 90.66 (s), 117.16 (t, $CH_2CH=CH_2$), 126.95, 127.42, 127.53, 127.66, 127.86, 128.12, 128.31 (7 d, CH_2Ph), 134.21 (t, $CH_2CH=CH_2$), 137.23, 138.17, 138.24 (3 s, CH_2Ph), 166.65, 177.87, 178.00 (3 s);
m/z as **136**

18.2 1L-(-)-1-*O*-Allyl-2,3,6-tri-*O*-benzyl-*myo*-inositol 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 in $CHCl_3$, 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 in $CHCl_3$)
Mass spectra and NMR data were identical to **128**.

18.4 1D-(-)-1,2,4-tri-*O*-benzyl-*myo*-inositol 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 $CHCl_3$, Lit. [168] -9.0°, Lit. [396] +15.5° for the enantiomer)
δ_H ($CDCl_3$, 400 MHz) 2.3-2.7 (3 H, br s, D_2O 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, C*H*$_2$Ph), 4.83, 4.87 (2 H, AB, J_{AB} 11.5, C*H*$_2$Ph), 4.72, 4.92 (2 H, AB, J_{AB} 11.3, C*H*$_2$Ph), 7.28-7.33 (15 H, m, CH_2*Ph*)
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 (R_f 0.24) into a product (R_f 0.38) and ^{31}P-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 triphosphite into a new product (R_f 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 $CHCl_3$)
δ_H ($CDCl_3$, 270 MHz) 1.95-2.41 (4 H, m, CH_2C*H*$_2$CN), 2.58-2.62 (2 H, m, CH_2C*H*$_2$CN), 2.73-2.82 (6 H, m, CH_2C*H*$_2$CN), 3.64 (1 H, dd, J 9.9, 2.8, C-3-H), 3.68-3.89 (2 H, m, C*H*$_2$$CH_2$CN), 3.98-4.55 (14 H, m, C*H*$_2$$CH_2$CN, C-2-H, C-6-H, C-5-H, C-1-H), 4.60, 4.78 (2 H, AB, J_{AB} 11.7, C*H*$_2$Ph), 4.88 (2 H, AB, C*H*$_2$Ph),

4.81, 4.95 (2 H, AB, J_{AB} 11.6, CH_2Ph), 5.19 (1 H, q, J 9.5, C-4-H), 7.34-7.43 (15 H, m, CH_2*Ph*)

δ_C ($CDCl_3$, 68 MHz) 18.36, 18.52, 18.81, 18.94, 19.23, 19.40, 19.53 (7 t, CH_2*C*H_2CN), 62.44, 62.63, 62.73, 62.79, 62.86, 62.99, 63.09 (7 t, *C*$H_2$$CH_2$CN), 72.16, 73.92, 75.22 (3 t, *C*H_2Ph), 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, CH_2*Ph*), 137.17, 137.88, 138.21 (3 s, CH_2*Ph*)

δ_P ($CDCl_3$, 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 [$(M - CH_2CH_2CN)^-$, 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 (D_2O, 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 (D_2O; 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

19.1 (1 R, 3 S, 5 S, 6 R, 7 S, 8 S, 9 S)-2,4,10-trioxatricyclo [3.3.1.1^{3,7}] decane-6,8,9-triol [= *myo*-Inositol orthoformate] 150

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 *myo*-inositol (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 N_2 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 hydrogencarbonate solution, stirred for 15 min at r.t., diluted with water (1 litre) and extracted 3 times with 250 ml of $CHCl_3$. 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,
δ_H (CD_3OD, 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)
δ_C (CD_3OD, 68 MHz) 61.33 (d), 69.35 (2 d), 70.84 (d), 76.25 (2 d), 104.21 (d, HCOOO)
m/z (CI, 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, 4.1 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 (R_f 0.00) into a single product (R_f 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. $NaHCO_3$ 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 $COCH_2$), 4.31-4.33 (2 H, m, C-1-H, C-3-H), 4.55 (1 H, tq, 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 (CI, 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

ex, OH), 3.26 (2 H, d, J 5.9, D_2O ex, 2 OH), 3.56 (1 H, brd dd, J 9.7, 9.7, D_2O ex gives dd, J 9.5, 9.5, C-1-H), 3.75-3.79 (2 H, m, D_2O 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)

δ_C ($CDCl_3$, 68 MHz) 13.30 (q, butyrate CH_3), 18.13 (t, butyrate CH_2CH_3), 35.91 (t, butyrate $COCH_2$), 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 - $COCH_2CH_2CH_3$)$^+$, 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-*myo*-inositol 153:

δ_H ($CDCl_3$, 270 MHz) 0.93, 0.96, 0.99 (9 H, 3 t, J 7.3, 3 butyrate CH_3), 1.56-1.75 (6 H, m, butyrate CH_2CH_3), 2.32, 2.40, 2.45 (6 H, 3 t, J 7.5, butyrate $COCH_2$), 2.80, 2.92 (2 H, 2 brd s, D_2O ex, 2 OH), 3.65 (1 H, brd dd, D_2O ex gives dd, J 9.8, 9.8, C-5-H), 3.85 (1 H, brd d, J 9.2, D_2O 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)

δ_C ($CDCl_3$, 68 MHz) 13.36 (q, butyrate CH_3), 13.43 (2 q, 2 butyrate CH_3), 18.29 (2 t, 2 butyrate CH_2CH_3), 18.36 (t, butyrate CH_2CH_3), 35.94 (t, butyrate $COCH_2$), 36.00 (t, butyrate $COCH_2$), 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 (CI, Isobutane) 419 [(M+H)$^+$, 5 %], 401 [(M - OH)$^+$, 60], 331 [(M - OH - $COCH_2CH_2CH_3$)$^+$, 31), 89 (45), 71 (100)

19.4 2,4,6-Tri-*O*-*p*-methoxybenzyl-*myo*-inositol 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 (R_f 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 C*H*$_2$Ph, C-2-H), 4.58 (2 H, s, C*H*$_2$Ph), 5.51 (1 H, s, O_3CH), 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, *C*H_2PhOMe), 73.66 (d, inositol ring C), 103.08 (d, O_3CH), 113.62 (d, 6 *Ph*OMe C), 129.12 (d, 4 *Ph*OMe C), 129.58 (d, 2 *Ph*OMe C), 134.57 (s, 3 *Ph*OMe C), 159.16 (s, 3 *Ph*OMe C)
m/z (+ve ion FAB) 551 [(M + H)$^+$, 2 %], 429 [(M - $CH_2C_6H_4OCH_3$)$^+$, 6], 121 [($CH_2C_6H_4OCH_3$)$^+$, 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-*myo*-inositol 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_2O ex, 2 OH), 2.66 (1 H, brd s, D_2O 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, C*H*$_2$Ph), 4.76 (4 H, s, 2 C*H*$_2$Ph), 6.87 (4 H, d, J 8.6, CH_2*Ph*OMe),

6.89 (2 H, d, J 8.6, CH_2*Ph*OMe), 7.24 (2 H, d, J 8.6, CH_2*Ph*OMe), 7.29 (4 H, d, J 8.6, CH_2*Ph*OMe)
δ_C ($CDCl_3$, 68 MHz) 55.14 (q, OCH_3), 72.33 (d, 2 C, inositol ring C), 74.47 (t, *C*H_2Ph), 74.79, 78.95 (2 d, 2 C, inositol ring C), 81.48 (d, 2 C, inositol ring C), 113.78 (d, 2 *Ph*OMe C), 113.85 (d, 4 *Ph*OMe C), 129.45 (d, 2 *Ph*OMe C), 129.61 (d, 4 *Ph*OMe C), 130.58 (s, *Ph*OMe), 159.22 (s, *Ph*OMe)
m/z (+ve ion FAB) 539 [$(M-H)^+$, 1.5 %], 419 (5), 121 [$(CH_2C_6H_4OCH_3)^+$, 100]
m/z (-ve ion FAB) 706 (36 %), 693 [$(M + NBA)^-$, 57], 539 [$(M -H)^-$, 100], 419 [$(M - CH_2C_6H_4OCH_3)^-$, 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 PCl_3 (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)
δ_P ($CDCl_3$; 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 CH_2Cl_2 (250 ml) at -78°C. Benzyl alcohol (51.7 ml, 54.1 g, 0.5 mol) in dry CH_2Cl_2 (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 %).

δ_P ($CDCl_3$; 36 MHz) 145.24

19.8 2,4,6-Tri-*O*-*p*-methoxybenzyl-1,3,5-tris[bis(benzyl)thiophospho]-*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. Tlc (ethyl acetate / hexane 2:1) showed one product (R_f 0.81). The solution was partitioned between chloroform and sat. aqueous $NaHCO_3$, the organic phase was dried over magnesium sulphate and the solvents evaporated. The residue was chromatographed on silica gel to give to give **158** (835 mg, 61 %) as a syrup.

δ_H ($CDCl_3$, 270 MHz) 3.70 (6 H, 2 s, OCH_3), 3.76 (3 H, s, OCH_3), 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 C*H*$_2$Ph, C-2-H, C-5-H), 6.71 (4 H, d, J 8.6, CH_2*Ph*OMe), 6.80 (2 H, d, J 8.8, CH_2*Ph*OMe), 6.98 (4 H, dd, J 7.6, 1.9, CH_2*Ph*OMe), 7.11-7.35 (32 H, m, CH_2*Ph*OMe and CH_2*Ph*).
δ_C ($CDCl_3$, 68 MHz) 54.16 (q, OCH_3), 68.56, 68.73, 68.89, 69.02, 72.71, 74.14 (6 t, *C*H_2Ph), 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 *Ph*OMe C), 112.54 (d, 2 *Ph*OMe C), 126.89, 126.95, 127.21, 127.27, 127.34, 127.43, 127.56, 127.66, 127.76, 128.05 (10 d, CH_2*Ph* and *Ph*OMe), 129.35 (s, 2 *Ph*OMe C), 129.67 (s, 1 *Ph*OMe C), 134.54, 134.67, 134.80, 134.93 (4 s, CH_2*Ph*), 157.73 (s, *Ph*OMe C)
δ_P ($CDCl_3$, 36 MHz)
m/z (+ve ion FAB) 1370 [$(M + H)^+$, 0.17 %], 1247 (0.3), 121 [$(CH_2C_6H_4OCH_3)^+$, 100], 91 [$(CH_2C_6H_5)^+$, 37]
m/z (-ve ion FAB) 1277 [$(M - C_7H_7)^-$, 10 %), 293 [$(OP(S)(OCH_2C_6H_5)_2^-$, 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%).

δ_H (D_2O, 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)
δ_C (D_2O, 68 MHz) 69.63 (1 C, d, C-2), 70.83 (2 C, d, C-4, C-6), 74.29 (2 C, dd, J $_{C\text{-}O\text{-}P}$ 6.6, C-1, C-3), 78.41 (1 C, dd, J $_{C\text{-}O\text{-}P}$ 6.6, C-5)
δ_P (D_2O, 36 MHz) 47.74 (1 P, d, J 10.1, 5-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 [$(PSO_2)^-$, 40]
Accurate mass calc for $C_6H_{14}O_{12}P_3S_3$ $(M - H)^-$ 466.886, found 466.886;

20 Synthesis of *scyllo*-Inositol 1,2,4-trisphosphate

20.1 DL-1-*O*-Allyl-3,6-di-*O*-benzyl-4,5-*O*-isopropylidene-2-*O*-trifluoromethylsulphoxy-*myo*-inositol 160

A solution of 1-*O*-Allyl-3,6-di-*O*-benzyl-4,5-*O*-isopropylidene-*myo*-inositol **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 (R_f 0.39) to a product (R_f 0.62). The reaction was quenched with water (10 ml), diluted with dichloromethane (50 ml) and washed successively with sat. $NaHCO_3$, 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 ($CDCl_3$; 270 MHz) 1.45 (3 H, s, CH_3), 1.49 (3 H, s, CH_3), 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, C*H*$_2$-CH=CH_2), 4.78 (2 H, AB, J_{AB} 12.5, C*H*$_2$Ph), 4.76, 4.88 (2 H, AB, J_{AB} 11.5, C*H*$_2$Ph), 5.23 (1 H, t, J 2.9, C-2-H), 5.17-5.32 (2 H, m, CH_2-CH=C*H*$_2$), 5.99 (1 H, ddt, J 17.3, 10.35, 5.9, CH_2-C*H*=CH_2), 7.25-7.37 (10 H, m, CH_2*Ph*);
δ_C ($CDCl_3$; 68 MHz) 26.87 (q, CH_3), 71.86, 73.06, 73.54 (3 t, 2 *C*H_2Ph and *C*H_2CH=CH_2), 73.57, 76.88, 77.70, 78.08, 78.93, 85.84 (6 d, 6 inositol ring C), 112.40 (s, $(CH_3)_2$*C*), 118.21 (t, CH_2CH=*C*H_2), 125.28 (CF_3), 127.52, 127.58, 127.78, 128.23, 128.36, 128.59, 129.01 (7 d, CH_2*Ph*), 133.78 (d, CH_2*C*H=CH_2), 137.15, 138.28 (2 s, CH_2*Ph*);
m/z (+ve ion FAB) 573 [(M + H)$^+$, 2 %], 502 [(M - HCF_3)$^+$, 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_f 0.62) into a product (R_f 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 $C_{28}H_{34}O_7$ (482.57): C, 69.69; H, 7.10 %.

δ_H ($CDCl_3$; 270 MHz) 1.47 (6 H, s, 2 CH_3), 2.03 (3 H, s, $COCH_3$), 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, C*H*$_2$CH=CH_2), 4.61, 4.85 (2 H, AB, J_{AB} 12.4, C*H*$_2$Ph), 4.71, 4.88 (2 H, AB, J_{AB} 11.7, C*H*$_2$Ph), 5.08-5.22 (2 H, m, CH_2CH=C*H*$_2$), 5.15 (1 H, t, J 8.8, C-2-H), 5.81 (1 H, ddt, CH_2C*H*=CH_2, J 17.2, 10.4, 5.6), 7.24-7.39 (10 H, m, CH_2*Ph*)
δ_C ($CDCl_3$; 68 MHz) 20.92 (q, $COCH_3$), 26.95 (2 q, CH_3), 72.07, 72.98, 74.11 (3 t, *C*H_2Ph and *C*H_2CH=CH_2), 74.66, 76.77, 78.56, 78.88, 79.34, 81.61 (6 d,

inositol ring C), 112.58 (s, $(CH_3)_2C$), 116.77 (t, $CH_2CH=CH_2$), 127.44, 127.50, 127.70, 128.18, 128.22 (5 d, CH_2Ph), 134.67 (d, $CH_2CH=CH_2$), 138.30 (s, CH_2Ph), 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-*scyllo*-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 (R_f 0.70) into a product (R_f 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. [168] 105-106°C)
Found: C, 70.7; H, 7.26. Calc. for $C_{26}H_{32}O_6$ (440:54): C; 70.89; H, 7.32 %.
δ_H ($CDCl_3$; 270 MHz) 1.46 (6 H, s, 2 CH_3), 2.73 (1 H, d, J 2.4, D_2O 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, J_{AB} 12.5, ^3J 5.8, ^4J 1.5, $CH_2CH=CH_2$), 4.72, 4.90 (2 H, AB, J_{AB} 11.7, CH_2Ph), 4.72, 4.93 (2 H, AB, J_{AB} 11.7, CH_2Ph), 5.16 (1 H, ddt, J 10.3, 1.4, 1.4, *cis*-CH_2-$CH=CH_2$), 5.26 (1 H, ddt, J 17.2, 1.5, 1.5, *trans*-CH_2-$CH=CH_2$), 5.94 (1 H, ddt, J 17.2, 10.4, 5.7, CH_2-$CH=CH_2$), 7.24-7.40 (10 H, m, CH_2Ph)
δ_C ($CDCl_3$; 68 MHz) 26.92 (q, CH_3) 72.65, 72.95, 74.37 (3 t, CH_2Ph and $CH_2CH=CH_2$), 75.90, 78.43, 78.49, 79.11, 79.37, 83.39 (6 d, inositol ring C), 112.29 (s, $(CH_3)_2C$), 116.96 (t, $CH_2CH=CH_2$), 127.44, 127.60, 127.66, 127.83, 128.22, 128.31 (6 d, CH_2Ph), 134.99 (d, $CH_2CH=CH_2$), 138.27, 138.43 (2 s, CH_2Ph)
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-*scyllo*-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 (R_f 0.85) into a product (R_f 0.44). The mixture was allowed to cool and neutralised with $NaHCO_3$. 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 $C_{25}H_{30}O_7$ (442.51): C, 67.86; H, 6.83 %.
δ_H ($CDCl_3$; 270 MHz) 2.00 (3 H, s, $COCH_3$), 2.87 (1 H, d, J 2.4, D_2O ex, OH), 3.03 (1 H, d, J 2.4, D_2O 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_{AB} 12.5, ^3J 5.7, ^4J 1.5, $CH_2CH=CH_2$), 4.67, 4.76 (2 H, AB, J_{AB} 11.6, CH_2Ph), 4.76, 4.87 (2 H, AB, J_{AB} 11.1, CH_2Ph), 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*-$CH_2CH=CH_2$), 5.22 (1 H, ddt, J 17.2, 1.5, 1.5, *trans*-$CH_2CH=CH_2$), 5.84 (1 H, ddt, J 17.2, 10.4, 5.7, $CH_2CH=CH_2$), 7.24-7.37 (10 H, m, CH_2Ph)
δ_C ($CDCl_3$; 68 MHz) 20.98 (q, $COCH_3$), 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 CH_2Ph and $CH_2CH=CH_2$), 116.96 (t, $CH_2CH=CH_2$), 127.70, 127.76, 127.89, 127.99, 128.44, 128.51 (6 d, CH_2Ph), 134.46 (d, $CH_2CH=CH_2$), 138.11, 138.21 (2 s, CH_2Ph), 169.90 (s, CO);
m/z (+ve ion FAB) 443 [(M + H)$^+$, 4 %], 91 (100)
m/z (-ve ion FAB) 883 [(2 M - H)$^-$, 19 %], 553 [(M + NBA)$^-$, 100], 441 [(M - H)$^-$, 50]

20.5 DL-1-*O*-Allyl-3,6-di-*O*-benzyl-*scyllo*-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 (R_f 0.63) into a product (R_f 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]-*scyllo*-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 %.

δ_H ($CDCl_3$; 270 MHz): 3.58 (1 H, dd, J 8.4, 8.4, C-H), 3.67 (1 H, dd, J 8.4, 8.4, C-H), 3.73 (1 H, dd, J 8.4, 8.4, C-H), 4.12-4.26 (2 H, m, C*H*$_2$CH=CH$_2$), 4.55-5.15 (21 H, m, 8 C*H*$_2$Ph, $CH_2CH=CH_2$, 3 C-H), 5.78 (1 H, ddt, J 17.2, 10.4, 5.5, $CH_2CH=CH_2$), 6.97-7.35 (40 H, m, 8 CH_2*Ph*).
δ_C ($CDCl_3$; 68 MHz): 69.18, 69.28, 69.38, 69.44 (4 t, 4 PO-*C*H$_2$Ph), 73.56, 73.79, 74.31 (3 t, 2 *C*H$_2$Ph and *C*H$_2$CH=CH$_2$), 77.71, 78.14, 78.98, 79.08, 79.43, 79.66 (6 d, 6 inositol ring C), 117.09 (t, $CH_2CH=CH_2$), 127.11, 127.24, 127.79, 127.99, 128.09, 128.25 (6 d, CH_2*Ph*), 134.25 (d, $CH_2CH=CH_2$), 135.71, 137.85, 137.91 (3 s, CH_2*Ph*)
δ_P ($CDCl_3$, 162 MHz, ^{1}H-decoupled): -1.75 (2 P), -1.52 (1 P)
δ_P ($CDCl_3$, 162 MHz, ^{1}H-coupled): -1.72 (q, 2 P, J 7.9), -1.47 (q, 1 P, J 7.9)
m/z (+ve ion FAB) 1181 (1 %), 1090 [(M - C_7H_7)$^+$, 0.3], 181 (12), 91 (100)
m/z (-ve ion FAB) 1088 [(M - C_7H_7 - 2 H)$^-$, 7 %), 998 (2.5), 277 (100), 187 (16)

20.7 DL-*scyllo*-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%).

δ_H (D_2O, pH=8, 270 MHz) 3.48-3.52 (2 H, m, 2 C-H), 3.58 (1 H, dd, J 9.3, C-H), 3.84-4.07 (3 H, m, 3 C-H)
δ_C (D_2O, pH=8, 68 MHz)
δ_P (D_2O, pH=8, 162 MHz, ^{1}H-decoupled) 0.50 (1 P), 0.72 (2 P)
m/z (+ve ion FAB) 623 [(M + 2 Et_3NH)$^+$, 1.1 %], 522 [(M + Et_3NH)$^+$, 1.4], 102 (Et_3NH^+, 100)
m/z (-ve ion FAB) 839 [(2 M)$^-$, 15 %], 419 (M$^-$, 100), 97 (10)
Accurate mass calc for $C_6H_{14}O_{15}P_3$ (M - H)$^-$ 418.9546, found 418.9550;

21 Synthesis of *scyllo*-Inositol 1,2,4,5-tetrakisphosphate and *scyllo*-Inositol 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 (R_f 0.67) into a product (R_f 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 %.
δ_H (DMSO; 270 MHz) 1.53 (3 H, d, J 6.8, CH_3), 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=C*H*-CH_3), 4.66, 4.75 (2 H, AB, J_{AB} 11.2, CH_2Ph), 4.79, 4.79 (2 H, AB, J_{AB} 12.1, CH_2Ph), 5.04 (1 H, br s, D_2O ex, OH), 5.13 (1 H, br s, D_2O ex, OH), 5.26 (1 H, d, J 5.3, D_2O ex, OH), 6.21 (1 H, dd, J 6.4, 1.5, C*H*=CH-CH_3), 7.21-7.45 (10 H, m, CH_2*Ph*)
δ_C (DMSO; 68 MHz) 9.24 (q, CH=CH-CH_3), 72.43, 73.50, 73.79, 81.45, 82.52, 85.05 (6 d, 6 inositol ring C), 73.60, 73.92 (2 t, *C*H_2Ph), 96.24 (d, CH=C*H*-CH_3), 126.89, 127.05, 127.47, 127.54, 127.80 (5 d, CH_2*Ph*), 139.05, 139.47 (2 s, CH_2*Ph*), 148.13 (d, C*H*=CH-CH_3)
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 (R_f 0.73) into a product (R_f 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 %.

δ_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, C*H*$_2$Ph), 4.96, 4.97 (2 H, AB d, C*H*$_2$Ph), 7.21-7.41 (10 H, m, CH_2*Ph*)
δ_C (d_6-DMSO; 68 MHz) 73.79 (2 t, 2 *C*H_2Ph), 74.27 (4 d, 4 C-OH), 82.90 (2 d, 2 C-O-benzyl), 127.14 (2 d, CH_2*Ph*), 127.69, 128.05 (2 x 2 d, CH_2*Ph*), 139.89 (2 s, CH_2*Ph*).

21.3 3,6-Di-*O*-benzyl-1,2,4,5-tetrakis[di(benzyl)phospho]-*scyllo*-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_f 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 %.
δ_H ($CDCl_3$; 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, C*H*$_2$Ph), 7.06-7.30 (60 H, m, CH_2*Ph*)
δ_C ($CDCl_3$; 68 MHz): 69.51, 73.14 (2 t, *C*H_2Ph), 77.55, 79.73 (2 d, 6 inositol ring C), 127.37, 127.47, 127.86, 128.12, 128.25, 128.35 (6 d, CH_2*Ph*), 135.55, 135.64, 135.77, 137.53 (4 s, CH_2*Ph*)
δ_P ($CDCl_3$; 36 MHz; ^{1}H-decoupled): -3.71
m/z (+ve ion FAB) 1401 [$(M+H)^+$, 0.5 %], 1310 [$(M+H - C_7H_7)^+$, 0.2]
m/z (-ve ion FAB) 1309 [$(M - C_7H_7)^-$, 2.3 %], 1218 [$(M - 2\ C_7H_7)^-$, 0.6], 277 [$(OP(O)(OC_7H_7)_2)^-$, 100]

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_H (D_2O; 270 MHz): 3.48 (2 H, dd, J 9.3, 9.3, C-3,6-H), 3.87 (4 H, ddd, J 12.2, 9.3, 9.3, C-1,2,4,5-H)
δ_C (D_2O; 100 MHz): 73.61 (2 C), 77.09 (4 C)
δ_P (D_2O; 36 MHz; ^{1}H-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 $C_6H_{15}O_{18}P_4$ $(M - H)^-$ 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 ^{31}P-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 $C_{76}H_{76}O_{14}P_4S_4$ (1465.57): C, 62.29; H, 5.23 %.
δ_H ($CDCl_3$; 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_2Ph), 7.08-7.23 (60 H, m, CH_2*Ph*)
δ_C ($CDCl_3$; 68 MHz): 70.03, 72.59 (2 t, CH_2Ph), 77.97, 79.92 (2 d, inositol ring C), 127.43, 127.99, 128.05, 128.12, 128.22, 128.34 (6 d, CH_2*Ph*), 135.56, 135.64, 135.71, 137.53 (4 s, CH_2*Ph*)
δ_P ($CDCl_3$, 36 MHz; ^{1}H-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 [$(M - C_7H_7)^-$, 9 %], 1282 [$(M - 2\ C_7H_7)^-$, 3], 1249 [$(M - 2\ OC_7H_7)^-$, 4), 293 [$OP(S)(OC_7H_7)_2^-$, 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_2O; 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_2O; 68 MHz): 80.41 (2 C), 84.97 (4 C)
δ_P (D_2O; 36 MHz): 43.48 (d, J 11.0)
m/z (-ve ion FAB) 563 [$(M-H)^-$, 95 %], 529 (18), 188 (47), 154 (32), 113 (28), 95 [$(PSO_2)^-$, 100]
Accurate mass calc for $C_6H_{15}O_{14}P_4S_4$ $(M - H)^-$ 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 (R_f 0.53) to a product (R_f 0.67). The reaction was quenched with water (10 ml), diluted with dichloromethane (50 ml) and washed successively with sat. $NaHCO_3$, 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 - HCF_3), 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*-(*cis*-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 (R_f 0.67) to a product (R_f 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 $C_{26}H_{31}O_5F$ (442.53): C, 70.57; H, 7.06 %.
δ_H ($CDCl_3$; 270 MHz) 1.47 (3 H, s, CH_3), 1.48 (3 H, s, CH_3), 1.64 (3 H, dd, J 7.0, 1.65, OCH=CH-CH_3), 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=C*H*-CH_3), 4.72, 4.84 (2 H, AB, J_{AB} 12.3, CH_2Ph), 4.77, 4.82 (2 H, AB, J_{AB} 12.0, CH_2Ph), 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, OC*H*=CH-CH_3), 7.21-7.66 (10 H, m, CH_2*Ph*)
δ_C ($CDCl_3$; 68 MHz) 9.24 (q, OCH=CH-*C*H_3), 26.82, 26.92 (2 q, C(*C*H_3)$_2$), 71.52, 73.37 (2 t, 2 *C*H_2Ph), 74.74 (dd, $J_{C\text{-}C\text{-}F}$ 15.4, C-1(3)), 76.74, 76.81, 78.95 (3 d,

22 Synthesis of 2-deoxy-2-fluoro-*myo*-inositol 1,4,5-trisphosphate

22.1 DL-3,6-Di-*O*-benzyl-4,5-isopropylidene-1-*O*-(*cis*-prop-1-enyl)-*scyllo*-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 (R_f 0.59) into a product (R_f 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 %.
δ_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-C*H*$_3$), 2.69 (1 H, d, J 2.4, D_2O ex, OH), 3.52-3.75 (6 H, m, 6 C-H), 4.37 (1 H, dq, J 6.8, 6.8, OCH=C*H*-CH_3), 4.71, 4.93 (2 H, AB, J_{AB} 11.9, C*H*$_2$Ph), 4.74, 4.82 (2 H, AB, J_{AB} 11.7, C*H*$_2$Ph), 6.18 (1 H, dd, J 6.8, 1.65, OC*H*=CH-CH_3), 7.28-7.39 (m, 10 H, CH_2*Ph*)

δ_C ($CDCl_3$; 68 MHz) 8.99 (q, OCH=CH-*C*H_3), 26.63 (q, CH_3) 72.40, 72.62, (2 t, *C*H_2Ph), 75.22, 77.75, 78.04, 78.10, 78.43, 85.82 (6 d, inositol ring C), 99.38 (d, OCH=*C*H-CH_3), 112.16 (s, $(CH_3)_2$*C*), 127.18, 127.37, 127.54, 127.89, 128.05 (5 d, CH_2*Ph*), 137.92 (s, CH_2*Ph*), 147.00 (d, O*C*H=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, $J_{C\text{-}C\text{-}F}$ 17.6, C-3(1)), 90.24 (dd, $J_{C\text{-}F}$ 187.3, C-2), 103.18 (d, OCH=*C*H-CH_3), 112.26 (s, *C*$(CH_3)_2$), 127.50, 127.60, 127.73, 127.86, 128.18, 128.41 (6 d, CH_2*Ph*), 137.52, 138.24 (2 s, CH_2*Ph*), 144.95 (d, O*C*H=CH-CH_3)

δ_F ($CDCl_3$ with reference to $CFCl_3$; 84 MHz) -210.21 (dt, $J_{2\text{-}H\text{-}F}$ 51.3, $J_{1(3)\text{-}H\text{-}F}$ 29.3)

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 [$(M - CH{=}CH\text{-}CH_3)^-$, 14], 172 (69), 133 (56)

22.4 DL-3,6-di-*O*-benzyl-2-deoxy-2-fluoro-*myo*-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 $C_{20}H_{23}O_5F$ (362.40): C, 66.29; H, 6.40 %.

δ_H (d_6-DMSO; 270 MHz) 3.21-3.59 (5 H, m, 5 C-H), 4.67 (2 H, AB, CH_2Ph), 4.78, 4.82 (2 H, AB, J_{AB} 11.7, CH_2Ph), 4.85 (1 H, d, J 54.0, C-2-H), 5.09 (1 H, d, J 5.1, D_2O ex, OH), 5.16 (1 H, d, J 5.0, D_2O ex, OH), 5.32 (1 H, d, J 6.0, D_2O ex, OH), 7.21-7.45 (10 H, m, CH_2*Ph*)

δ_C (d_6-DMSO; 68 MHz) 69.62 (dd, $J_{C\text{-}C\text{-}F}$ 15.4, C-1(3)), 71.29 (t, *C*H_2Ph), 72.14 (d, inositol ring C), 73.73 (t, *C*H_2Ph), 74.37 (d, inositol ring C), 77.88 (dd, $J_{C\text{-}C\text{-}F}$ 17.7, C-3(1)), 81.38 (d, inositol ring C), 92.23 (dd, $J_{C\text{-}F}$ 178.5, C-2), 126.92, 127.21, 127.41, 127.47, 127.80, 128.02 (6 d, CH_2*Ph*), 138.76, 139.50 (2 s, CH_2*Ph*)

δ_F (d_6-DMSO with reference to $CFCl_3$; 84 MHz) - 212.6 (dt, $J_{2\text{-}H,F}$ 51.3, $J_{1(3)\text{-}H,\ F}$ 29.3)

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 %.
δ_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 C*H*$_2$Ph, 3 C-H), 6.95-7.33 (40 H, m, 8 CH_2*Ph*)
δ_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*)
δ_P ($CDCl_3$; 36 MHz; ^{1}H-decoupled) -1.61 (1 P), -1.95 (2 P)
δ_F (DMSO with reference to $CFCl_3$; 84 MHz) -213.1 (dt, $J_{2\text{-}H,F}$ 51.3, $J_{1(3)\text{-}H,\ F}$ 29.3)
m/z (+ve ion FAB) 1142 [(M - H)$^+$, 4.7 %], 1052 [(M - CH_2Ph)$^+$, 1.4], 181 (13), 91 (100)
m/z (-ve ion FAB) 1049 (13 %), 1015 (1), 959 (2.5), 277 {[OP(O)(OCH_2Ph)$_2$]$^-$, 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; ^{1}H-coupled) 1.96 (1 P, J 10.1), 1.56 (1 P, J 6.7), 0.37 (1 P, J 10.1)
δ_F (D_2O with reference to $CFCl_3$; 84 MHz) -211.8 (dt, $J_{2\text{-H,F}}$ 51.3, $J_{1(3)\text{-H, F}}$ 36.7)
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 $C_6H_{13}O_{14}FP_3$ $(M - H)^-$ 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-*myo*-inositol

23.1 DL-3-*O*-Allyl-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol 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-*myo*-inositol **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-*myo*-inositol **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_2O ex, OH), 3.81-4.05 (4 H, m, 4 C-H), 4.25, 4.33 (2 H, AB, d, J_{AB} 12.9, ^3J 6.0, $CH_2CH=CH_2$), 4.48 (1 H, t, J 4.4, C-2-H), 5.23 (1 H, d, J 10.3, $CH_2CH=CH_2$), 5.32 (1 H, d, J 17.2, $CH_2CH=CH_2$), 5.98 (1 H, ddt, J 16.9, 10.5, 5.7, $CH_2CH=CH_2$)
δ_C ($CDCl_3$; 68 MHz) 25.69, 26.66, 26.76, 27.99 (4 q, 4 CH_3), 70.94 (t, $CH_2CH=CH_2$), 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, $CH_2CH=CH_2$), 134.41 (d, $CH_2CH=CH_2$)
m/z (EI) 285 (6), 227 (1.4) 113 (100)
m/z (CI, 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-*myo*-inositol 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-*myo*-inositol **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, $CH_2CH=CH_2$), 4.47 (1 H, dd, J 4.6, 4.6, C-2-H), 5.17-5.36 (4 H, m, 2 $CH_2CH=CH_2$), 5.87-6.03 (2 H, m, 2 $CH_2CH=CH_2$).
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, $CH_2CH=CH_2$ and CH_2Ph), 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

$CH_2CH=CH_2$), 134.51, 134.61 (2 d, 2 $CH_2CH=CH_2$)

m/z (EI) 340 [M^+, 1.4 %], 325 (5), 113 (72) 41 (100)
m/z (CI, 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-*myo*-inositol 181

DL-3-*O*-Allyl-1,2:4,5-di-*O*-isopropylidine-*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 (R_f 0.34) into a product (R_f 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 $C_{22}H_{30}O_6$ (390.48): C, 72.66; H, 7.09 %.

δ_H ($CDCl_3$; 270 MHz) 1.36, 1.39, 1.44, 1.46 (12 H, 4 s, 4 CH_3), 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, J_{AB} 13.0, J 6.0, $CH_2CH=CH_2$), 4.45 (1 H, t, J 4.5, C-2-H), 4.83 (2 H, AB, CH_2Ph), 5.22 (1 H, dd, J 17.2, 1.2, $CH_2CH=CH_2$), 5.31 (1 H, dd, J 10.3, 0.8, $CH_2CH=CH_2$), 5.97 (1 H, ddt, J 16.8, 10.9, 6.4, $CH_2CH=CH_2$), 7.24-7.42 (5 H, m, CH_2Ph)
δ_C ($CDCl_3$; 68 MHz) 25.82, 26.86, 26.92, 27.76 (4 q, CH_3), 71.00, 71.88 (2 t, $CH_2CH=CH_2$, CH_2Ph), 74.63, 76.25, 76.81, 78.72, 79.82, 81.02 (6 d, inositol ring C), 109.79, 111.97 (2 q, $C(CH_3)_2$), 117.97 (t, $CH_2CH=CH_2$), 127.34, 127.83, 128.05 (3 d, CH_2Ph), 134.54 (d, $CH_2CH=CH_2$), 138.11 (s, CH_2Ph)

m/z (CI, 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-*myo*-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 (R_f 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 $C_{16}H_{22}O_6$ (310.35): C, 61.92; H, 7.15 %.

δ_H (d_6-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, D_2O shake gives t, J 9.1, C-5-H), 3.31 (1 H, m, D_2O 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, D_2O shake gives t, J 9.4, C-6-H), 3.89-3.94 (1 H, m, D_2O shake gives t, J 2.5, C-2-H), 4.00-4.17 (2 H, m, $CH_2CH=CH_2$), 4.64-4.83 (6 H, m, D_2O shake gives 2 H, AB s at 4.72, CH_2Ph and 4 OH), 5.15 (1 H, dd, J 10.4, 1.5, $CH_2CH=CH_2$), 5.32 (1 H, dd, J 17.2, 1.65, $CH_2CH=CH_2$), 5.92 (1 H, ddt, J 17.2, 10.4, 5.5, $CH_2CH=CH_2$), 7.24-7.44 (5 H, m, CH_2Ph)

δ_C (d_6-DMSO; 68 MHz) 69.96, 71.58, 72.30, 75.18, 79.59, 81.93 (6 d, inositol ring C), 70.1, 73.72 (2 t, $CH_2CH=CH_2$, CH_2Ph), 116.02 (t, $CH_2CH=CH_2$), 127.05, 127.66, 127.97 (3 d, CH_2Ph), 136.26 (d, $CH_2CH=CH_2$), 140.05 (s, CH_2Ph)

m/z (70 eV): 310 (M^+, 6 %), 149 (12), 113 (20), 91 (100), 86 (38), 55 (28), 41 [$(C_3H_5)^+$, 57]
m/z (CI, Iso-butane): 310 (M^+, 14 %), 219 [(M - C_7H_7), 22], 131 (98), 107 [$(OC_7H_7)^+$, 32], 91 [$(C_7H_7)^+$, 100], 69 (47)

23.5 DL-3-*O*-Allyl-6-*O*-benzyl-1-*O*-*p*-methoxybenzyl-*myo*-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 %.

δ_H ($CDCl_3$; 270 MHz) 2.83 (1 H, brd s, D_2O 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_2O 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, $CH_2CH=CH_2$), 4.15 (1 H, dd, J 2.7, 2.7, C-2-H), 4.61 (2 H, AB, CH_2Ph), 4.80, 4.91 (2 H, AB, J 11.2, CH_2Ph), 5.17 (1 H, dd, J 10.3, 1.3, $CH_2CH=CH_2$), 5.26 (1 H, dd, J 17.2, 1.5, $CH_2CH=CH_2$), 5.91 (1 H, ddt, J 17.2, 10.4, 5.7, $CH_2CH=CH_2$), 6.84 (2 H, d, J 8.8, CH_2Ph), 7.23-7.37 (7 H, m, CH_2Ph)

δ_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, $CH_2CH=CH_2$, CH_2Ph), 113.68 (d, CH_2Ph), 117.64 (t, $CH_2CH=CH_2$), 127.40, 127.76, 128.22, 129.35, 129.81 (5 d, CH_2Ph), 134.48 (d, $CH_2CH=CH_2$), 138.72 (s, CH_2Ph), 159.16 (s, C-OCH_3)

m/z (EI): 339 (44 %), 309 (14), 137 (77), 121 (100), 91 (63)
m/z (CI, 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-*myo*-inositol 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 (R_f 0.12) into a product (R_f 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_2O 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, $CH_2CH{=}CH_2$), 4.20 (1 H, dd, J 3.1, 3.1, C-2-H), 4.65, 4.69 (2 H, AB, J 11.35, CH_2Ph), 4.76, 4.91 (2 H, AB, J 11.6, CH_2Ph), 5.18 (1 H, dd, J 10.4, 1.5, $CH_2CH{=}CH_2$), 5.28 (1 H, ddt, J 17.3, 1.6, 1.6, $CH_2CH{=}CH_2$), 5.94 (1 H, ddt, J 17.2, 10.4, 5.7, $CH_2CH{=}CH_2$), 6.84 (2 H, d, J 8.8, CH_2Ph), 7.23-7.41 (7 H, m, CH_2Ph)

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, $CH_2CH{=}CH_2$ and 2 CH_2Ph), 111.51 (s, $C(CH_3)_2$), 113.68 (d, CH_2PhOMe), 117.45 (t, $CH_2CH{=}CH_2$), 127.28, 127.53, 128.09, 129.48, 129.77 (5 d, CH_2Ph and CH_2PhOMe), 134.44 (d, $CH_2CH{=}CH_2$), 138.66 (s, CH_2Ph), 159.22 (s, $C\text{-}OCH_3$)

m/z (+ve ion FAB) 469 [$(M-H)^+$, 3.6 %], 379 (4.9), 349 (3.7), 121 [$(C_7H_6OCH_3)^+$, 100), 91 (24)
m/z (-ve ion FAB) 622 [(M+NBA)$^-$, 66 %], 469 [$(M-H)^-$, 90], 429 (37), 349 [$(M - C_7H_6OCH_3)^-$, 100], 334 (44), 182 (70), 167 (47), 93 (40)

23.7 DL-3-*O*-Allyl-6-*O*-benzyl-2-*O*-[(-)-ω-camphanoyl]-4,5-*O*-isopropylidene-1-*O*-*p*-methoxybenzyl-*myo*-inositol 185

A mixture of **184** (470 mg, 1 mmol), (1*S*)-(-)-camphanic 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 (R_f 0.44) into a product (R_f 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_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, C*H*$_2$-CH=CH_2), 4.50-4.65 (4 H, 2 AB, overlapping, J 11.7, 2 C*H*$_2$Ph), 4.74-4.92 (4 H, 2 AB, overlapping, J 11.7, 2 C*H*$_2$Ph), 5.17 (2 H, ddt, J 10.3, 1.4, 1.4, *cis*-CH_2-CH=C*H*$_2$), 5.28 (2 H, ddt, J 17.3, 1.6, 1.6, *trans*-CH_2-CH=C*H*$_2$), 5.80-5.94 (4 H, m, CH_2-C*H*=CH_2 and C-2-H), 6.83 (4 H, dd, J 8.7, 3.6, CH_2*Ph*OMe), 7.21-7.40 (14 H, m, CH_2*Ph* and CH_2*Ph*OMe)

23.8 DL-3-*O*-Allyl-6-*O*-benzyl-2-*O*-[(-)-ω-camphanoyl]-1-*O*-*p*-methoxybenzyl-*myo*-inositol 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 (R_f 0.58) into a product (R_f 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 %).

δ_H ($CDCl_3$; 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_2O 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, $CH_2CH=CH_2$), 4.44-4.66 (4 H, m, 2 CH_2Ph), 4.71-4.94 (4 H, m, 2 CH_2Ph), 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 $CH_2CH=CH_2$ and 2 C-2-H), 6.83, 6.84 (4 H, 2 d, J 8.8, CH_2Ph), 7.20-7.33 (14 H, m, CH_2Ph),
δ_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, $CH_2CH=CH_2$ and CH_2Ph), 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_2Ph), 117.89, 117.98 (2 t, $CH_2CH=CH_2$), 127.75, 127.99, 128.09, 128.38, 128.41, 129.74, 129.81 (7 d, CH_2Ph), 134.05 (d, $CH_2CH=CH_2$), 138.39, 138.45 (2 s, CH_2Ph), 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_7H_7)$^+$, 4.8], 489 [(M - $C_7H_6OCH_3$)$^+$, 2), 121 [($C_7H_6OCH_3$)$^+$, 100), 91 (10)
m/z (-ve ion FAB) 763 [(M+NBA)$^-$, 22 %], 656 [(M+NBA - C_7H_7O)$^-$, 13], 609 [(M-H)$^-$, 14], 197 [(CamphO)$^-$, 100]

23.9 (+)-4,5-Di-*O*-acetyl-3-*O*-allyl-6-*O*-benzyl-2-*O*-[(-)-ω-camphanoyl]-1-*O*-*p*-methoxybenzyl-*myo*-inositol 187

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_f = 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 %);

δ_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, C*H*$_2$CH=CH_2), 4.48, 4.65 (2 H, AB, J_{AB} 10.5, C*H*$_2$Ph), 4.61, 4.80 (2 H, AB, J_{AB} 11.2, C*H*$_2$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=C*H*$_2$), 5.22 (1 H, dd, J 17.6, 1.5 *trans*-CH_2-CH=C*H*$_2$), 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_2C*H*=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*)
δ_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, *C*H_2CH=CH_2 and 2 *C*H_2Ph), 91.29 (s, Camph), 113.83 (d, CH_2*Ph*), 117.52 (t, CH_2CH=*C*H_2), 127.74, 127.98, 128.33, 129.86 (4 d, CH_2*Ph*),

129.26 (s), 133.66 (d, $CH_2CH=CH_2$), 137.98 (s, CH_2Ph), 159.45 (s, C-OCH_3), 166.51, 169.79, 169.99, 178.05 (4 s, carbonyl)

m/z (+ve ion FAB) 693 [$(M-H)^+$, 4 %], 603 [$(M-C_7H_7)^+$, 10], 121 (100), 91 (34)
m/z (-ve ion FAB) 847 [$(M+NBA)^-$, 40 %], 740 [$(M+NBA - C_7H_7O)^-$, 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 CO_2. 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)
$[\alpha]_D^{20}$ = +19.0° (c = 4 in $CHCl_3$),
Found: C, 67.0; H, 7.02. Calc for $C_{24}H_{30}O_7$ (430.50): C, 66.96; H, 7.02 %.
m/z (+ve ion FAB) 429 (3 %), 400 (10), 339 (8), 309 (5), 121 [$(C_7H_6OCH_3)^+$, 100], 91 (8)
m/z (-ve ion FAB) 583 [$(M+NBA)^-$, 100 %], 429 [$(M-H)^-$, 85], 322 [$(M - C_7H_7O)^-$, 30], 309 [$(M - C_7H_6OCH_3)^-$, 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) R_f 0.58
m.p. 141-142°C
Found C, 66.2; H, 6.34; N, 3.53. Calc. for $C_{22}H_{25}O_6N$ (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, C*H*$_2$Ph), 4.65 (1 H, dd, J 3.7, 3.7, C-2-H), 4.81, 4.92 (2 H, AB, J_{AB} 11.2, C*H*$_2$Ph), 7.29-7.38 (10 H, m, CH_2*Ph*)

δ_C ($CDCl_3$, 68 MHz) 56.24 (t, *C*H_2CN), 67.37, 71.84, 74.41, 78.91, 80.24, 80.37 (6 d, inositol ring C), 72.43, 75.44 (2 t, *C*H_2Ph), 116.21 (*C*N), 127.83, 127.96, 128.18, 128.48, 128.60 (5 d, CH_2*Ph*), 137.27, 138.27 (2 s, CH_2*Ph*),

m/z (70 eV EI) 399 $(M)^+$, 308 [$(M - C_7H_7)^+$, 15 %], 149 (10), 107 [$(C_7H_7O)^+$, 20], 91 [$(C_7H_7)^+$, 100]
m/z (CI, 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-*myo*-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) R_f 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_2COOH), 4.28 (1 H, s, C-2-H), 4.59, 4.66 (2 H, AB, J_{AB} 12.1, CH_2Ph), 4.76, 4.79 (2 H, AB, J_{AB} 11.4, CH_2Ph), 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_2COOH), 70.94, 74.08 (2 t, CH_2Ph), 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-*myo*-inositol **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. H_2SO_4 (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) R_f 0.69
m.p. 153-155°C
Found: C, 65.80; H, 5.91. Calc. for $C_{22}H_{24}O_7$ (400.43): C, 65.99; H, 6.04 %.

δ_H ($CDCl_3$, 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, CH_2COOR), 4.61, 4.68 (2 H, AB, J_{AB} 11.2, CH_2Ph), 4.74 (2 H, AB, CH_2Ph), 4.88 (1 H, dd, J 3.3, 3.3, C-2-H), 7.25-7.32 (10 H, m, CH_2Ph)

δ_C ($CDCl_3$, 68 MHz) 61.01 (t, CH_2CO), 70.54, 71.13, 73.69, 74.82, 75.63, 76.54, (6 d, inositol ring C), 72.23, 73.53 (2 t, CH_2Ph), 131.52, 131.59, 131.75, 131.95, 132.01 (5 d, CH_2Ph) 136.81 (s, CH_2Ph), 166.68 (s, CO)

ν_{max}/cm^{-1} (KBr-disc): 1735 (s, carbonyl)

m/z (low eV EI) 400 [$(M)^+$, 10%], 318 (15), 310 (20), 309 [$(M - C_7H_7)^+$, 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_2COOR), 4.63, 4.68 (2 H, AB, J_{AB} 12.1, CH_2Ph), 4.65, 4.70 (2 H, AB, J_{AB} 11.7, CH_2Ph), 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_2Ph)

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_2COOR), 72.56, 75.83 (2 t, CH_2Ph), 127.76, 127.83, 127.96, 128.18, 128.51, 128.57, 129.74 (7 d, CH_2Ph), 137.04 (s, CH_2Ph), 166.29, 169.57, 169.90 (3 s, CO)

m/z (+ve ion FAB) 969 [$(2M+H)^+$, 1 %], 485 [$(M+H)^+$, 8], 377 [$(M-C_7H_7O)^+$, 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-*O*-benzyl-4,5-di-*O*-[(-)-ω-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-(-)-camphanic 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° (c = 4 in $CHCl_3$)
Found C, 66.7; H, 6.44. Calc. for $C_{42}H_{48}O_{13}$ (760.84): C 66.30, H 6.36 %.
δ_H ($CDCl_3$, 400 MHz) 0.81, 0.84, 0.95, 0.98, 1.04, 1.06 (18 H, 6 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_2COOR), 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, C*H*$_2$Ph), 4.69, 4.87 (2 H, AB, J 11.2, C*H*$_2$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*)

δ_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, *C*H_2COOR and 2 *C*H_2Ph), 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-*myo*-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)
$[a]_D^{23}$ = + 96° (c = 0.1 in DMF)
Found: C, 62.9; H, 6.50. Calc. for $C_{22}H_{26}O_8$ (418.43): C, 63.15; H, 6.26 %.
Spectroscopical data were identical to **190**.

25 Elimination / Claisen Rearrangement

25.1 DL-1-*O*-Allyl-2-*O*-benzoyl-3,6-di-*O*-benzyl-4,5-*O*-isopropylidene-*scyllo*-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 (SiO_2, 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 $C_{33}H_{36}O_7$ (544.64): C, 72.77; H, 6.66 %.
δ_H ($CDCl_3$; 270 MHz) 1.48 (6 H, s, 2 CH_3), 3.57-3.81 (5 H, m, 5 C-H), 4.03, 4.23 (2 H, AB, ddd, J_{AB} 12.4, J 5.9, 1.65, 1.65, C*H*$_2$CH=CH_2), 4.65, 4.80 (2 H, AB, J_{AB} 12.5, C*H*$_2$Ph), 4.74, 4.91 (2 H, AB, J_{AB} 11.7, C*H*$_2$Ph), 4.95 (1 H, dq, J 10.8, 1.65, CH_2CH=C*H*$_2$), 5.05 (1 H, dq, J 17.2, 1.65, CH_2CH=C*H*$_2$), 5.43 (1 H, dd, J 8.9, 8.9, C-2-H), 5.66 (1 H, ddt, J 16.9, 10.6, 5.7, CH_2C*H*=CH_2), 7.11-7.60 (13 H, m, CH_2*Ph* and C(O)*Ph*), 8.02 (2 H, dd, J 7.15, 1.3, C(O)*Ph*)
δ_C ($CDCl_3$; 68 MHz) 26.95 (q, C(*C*H_3)$_2$), 71.88, 72.95, 74.08 (3 t, 2 *C*H_2Ph and *C*H_2CH=CH_2), 75.35, 76.42, 78.59, 78.88, 79.40, 81.44 (6 d, 6 inositol ring C), 112.55 (s, (CH_3)$_2$*C*), 117.06 (t, CH_2CH=*C*H_2), 127.28, 127.44, 127.63, 128.02, 128.18, 128.25, 129.71 (7 d, CH_2*Ph*), 132.92 (d, CH_2*Ph*), 134.54 (d, CH_2*C*H=CH_2), 137.98, 138.30 (2 s, CH_2*Ph*), 165.32 (s, C=O);
m/z (+ve ion FAB) 545 [(M + H)$^+$, 7 %], 487 [(M - OCH_2CH=CH_2)$^+$, 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-*scyllo*-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 (R_f 0.64) into a product (R_f 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 $C_{30}H_{32}O_7$ (504.58): C, 71.41; H, 6.39 %.

δ_H ($CDCl_3$; 270 MHz) 3.11 (1 H, brd s, D_2O ex, OH), 3.31 (1 H, brd s, D_2O ex, OH), 3.42-3.65 (5 H, m, 5 C-H), 4.06, 4.22 (2 H, AB, ddd, J_{AB} 12.3, J 6.0, 1.65, 1.65, C*H*$_2$CH=CH$_2$), 4.67, 4.70 (2 H, AB, J 11.5, C*H*$_2$Ph), 4.79, 4.90 (2 H, AB, J 11.2, C*H*$_2$Ph), 4.97 (1 H, dq, J 10.3, 1.65, CH$_2$CH=C*H*$_2$), 5.05 (1 H, dq, J 17.2, 1.65, CH$_2$CH=C*H*$_2$), 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, CH$_2$C*H*=CH$_2$), 7.13-7.61 (13 H, m, CH$_2$*Ph* and C(O)*Ph*), 8.04 (2 H, dd, J 7.0, 1.5, C(O)*Ph*))

δ_C ($CDCl_3$; 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 *C*H$_2$Ph and *C*H$_2$CH=CH$_2$), 117.35 (t, CH$_2$CH=*C*H$_2$), 127.70, 127.89, 128.02, 128.31, 128.44, 128.54, 129.71 (7 d, CH$_2$*Ph* and C(O)*Ph*)), 133.11 (d, C(O)*Ph*), 134.48 (d, CH$_2$*C*H=CH$_2$), 137.82, 138.34 (2 s, CH$_2$*Ph*), 165.55 (s, C=O);

m/z (+ve ion FAB) 505 [(M + H)$^+$, 7 %], 413 [(M - CH$_2$Ph)$^+$, 1], 397 [(M - OCH$_2$Ph)$^+$, 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 (R_f 0.47) into a product (R_f 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 (R_f 0.30). The suspension was cooled, neutralised with aqueous sodium hydrogen carbonate and extracted with ethyl acetate (2 x

100 ml). 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_2O ex gives dd, J 9.3, 9.3, C-H), 4.54, 4.82 (2 H, AB, J_{AB} 11.5, C*H*$_2$Ph), 4.84 (2 H, AB, C*H*$_2$Ph), 5.10 (1 H, dd, J 9.7, 9.7, C-2-H), 5.14 (1 H, brd s, D_2O ex, OH), 5.31 (1 H, brd s, D_2O ex, OH), 5.38 (1 H, d, J 6.0, D_2O 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 *C*H$_2$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, C*H*$_2$CH=CH$_2$ and 2 C-H), 4.63 (1 H, s,

C-H), 4.65, 4.83 (2 H, AB, J 11.7, C*H*$_2$Ph), 4.81, 4.87 (2 H, AB, J 11.7, C*H*$_2$Ph), 5.22 (1 H, dq, J 10.4, 1.5, $CH_2CH=CH_2$), 5.33 (1 H, dq, J 17.2, 1.5, $CH_2CH=CH_2$), 5.96 (1 H, ddt, J17.2, 10.4, 5.3, $CH_2CH=CH_2$), 7.24-7.43 (10 H, m, CH_2*Ph*)

δ_C ($CDCl_3$; 68 MHz) 27.02 (q, $C(CH_3)_2$), 68.79, 71.45, 72.82 (3 t, 2 CH_2Ph and $CH_2CH=CH_2$), 75.41, 77.23, 79.04, 80.11, 98.34 (5 d, 5 inositol ring C), 111.71 (s, $(CH_3)_2C$), 117.45 (t, $CH_2CH=CH_2$), 127.37, 127.47, 127.73, 128.09, 128.25 (5 d, CH_2*Ph*), 132.63 (d, $CH_2CH=CH_2$), 138.37 (s, CH_2*Ph*), 154.55 (s, inositol ring C);

m/z (+ve ion FAB) 421 [$(M - H)^+$, 0.7 %], 365 [$(M - OCH_2CH=CH_2)^+$, 0.6], 315 [$(M - OCH_2Ph)^+$, 1.8], 131 (20), 91 (100)

m/z (-ve ion FAB) 575 [$(M + NBA)^-$, 20 %], 437 (31), 381 [$(M - CH_2CH=CH_2)^-$, 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 $C_{26}H_{30}O_5$ (422.52): C, 73.91; H, 7.16 %.

δ_H ($CDCl_3$; 270 MHz) 1.49, 1.50 (6 H, 2 s, 2 CH_3), 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, C*H*$_2$-$CH=CH_2$), 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, J_{AB} 11.9, C*H*$_2$Ph), 4.86, 4.91 (2 H, AB, J_{AB} 12.1, C*H*$_2$Ph), 5.14 (1 H, dq, J 10.3, 1.65, $CH_2CH=CH_2$), 5.21 (1 H, dq, J 17.2, 1.65, $CH_2CH=CH_2$), 5.84 (1 H, ddt, J 17.1, 10.4, 5.7, $CH_2CH=CH_2$), 7.24-7.41 (10 H, m, CH_2*Ph*);

δ_C ($CDCl_3$; 68 MHz) 26.66 (q, $C(CH_3)_2$), 69.96, 72.14 (2 t, CH_2Ph and $CH_2CH=CH_2$), 74.02, 80.86, 81.23, 81.54 (4 d, 4 inositol ring C), 94.81 (d, C-2), 112.32 (s, $(CH_3)_2C$), 116.90 (t, $CH_2CH=CH_2$), 127.43, 127.53, 127.76, 127.92, 128.18, 128.44 (6 d, CH_2*Ph*), 134.77 (d, $CH_2CH=CH_2$), 135.94 (s, CH_2*Ph*), 138.30 (s, CH_2*Ph*), 153.84 (s, C-3);

m/z (+ve ion FAB) 423 [$(M + H)^+$, 1 %], 365 [$(M - OCH_2CH=CH_2)^+$, 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-*myo*-1-inosose 200 and DL-2-Deoxy-2-*C*-allyl-3,6-di-*O*-benzyl-4,5-*O*-isopropylidene-*scyllo*-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 (R_f 0.54) into what appeared to be a single product (R_f 0.45). Yield 200 mg (100 %).

δ_H ($CDCl_3$; 270 MHz) 1.48, 1.49, 1.51, 1.53 (12 H, 4 s, 4 CH_3), 2.14-2.97 (6 H, m, C*H*$_2$CH=CH$_2$, C-1-H), 3.51-4.19 (8 H, m, C-3,4,5,6-H), 4.55-5.03 (12 H, m, 4 C*H*$_2$Ph, CH$_2$CH=C*H*$_2$), 5.54-5.82 (2 H, m, CH$_2$C*H*=CH$_2$), 7.24-7.63 (20 H, m, CH$_2$*Ph*)

δ_C ($CDCl_3$; 68 MHz) 26.99 (q, CH_3), 29.03, 30.36 (2 t, *C*H$_2$CH=CH$_2$), 52.67, 52.97 (2 d, C-1), 71.71, 72.20, 72.36, 72.62 (4 t, *C*H$_2$Ph), 74.34, 75.18, 77.29, 78.65, 80.44, 81.57, 82.32 (7 d, inositol ring C), 112.68, 112.74 (2 s, *C*(CH$_3$)$_2$), 117.25, 117.54 (2 t, CH$_2$CH=*C*H$_2$), 127.57, 127.73, 127.99, 128.28, 128.35 (5 d, CH$_2$*Ph*), 134.57, 135.22 (2 d, CH$_2$*C*H=CH$_2$), 137.20, 137.36, 137.65, 137.98 (4 s, CH$_2$*Ph*), 203.69, 205.31 (2 s, C=O)

m/z (+ ive ion FAB) 423 [(M+H)$^+$, 8 %], 181 (9), 91 (100)
ν_{max}/cm^{-1} (KBr disk) 1715 (C=O)

25.6 DL-1-*O*-Allyl-3,6-di-*O*-benzyl-2-deoxy-2-fluoro-4,5-*O*-isopropylidene-*scyllo*-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, C*H*$_2$-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, C*H*$_2$Ph), 4.74, 4.82 (2 H, AB, J_{AB} 11.9, C*H*$_2$Ph), 5.13 (1 H, ddt, J 10.3, 1.2, 1.2, CH_2-CH=C*H*$_2$), 5.23 (1 H, ddt, J 17.2, 1.5, 1.5, CH_2-CH=C*H*$_2$), 5.89 (1 H, ddt, J 16.9, 10.5, 5.9, CH_2-C*H*=CH_2), 7.23-7.39 (10 H, m, CH_2*Ph*);
δ_C ($CDCl_3$; 68 MHz) 27.64 (q, C(*C*H_3)$_2$), 73.37, 73.82, 74.96 (3 t, 2 *C*H_2Ph and *C*H_2CH=CH_2), 77.75, 77.85, 78.07 (3 d), 79.22 [dd, $J_{C\text{-}C\text{-}F}$ 15.4, C-1(3)], 82.99 [dd, $J_{C\text{-}C\text{-}F}$ 17.6, C-3(1)], 98.33 (dd, $J_{C\text{-}F}$ 185.1, C-2), 113.52 (s, *C*(CH_3)$_2$), 117.84 (t, CH_2CH=*C*H_2), 128.25, 128.31, 128.38, 128.44, 128.51, 129.00 (6 d, CH_2*Ph*), 135.48 (d, CH_2*C*H=CH_2), 138.73, 139.02 (2 s, CH_2*Ph*),
δ_F ($CDCl_3$ with reference to $CFCl_3$; 84 MHz) 193.9 (dd, J 51.3, 14.7)
m/z (+ve ion FAB) 443 [(M + H)$^+$, 7 %], 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**.

26 References

1. D.J. Takemoto, J.M. Cunnick, *Cellular Signalling*, **1990**, *2*, 99-104.
2. E.J. Neer, D.E. Clapham, *Nature (London)*, **1988**, *333*, 129-134.
3. A.G. Gilman, *Annu. Rev. Biochem.*, **1987**, *56*, 615-649.
4. L. Stryer, H.R. Bourne, *Annu. Rev. Cell. Biol.*, **1986**, *2*, 391-419.
5. S. Ringer, *J. Physiol.*, **1883**, *4*, 29-42.
6. T.W. Rall, E.W. Sutherland, *J. Biol. Chem.*, **1958**, *232*, 1065-1076.
7. E.W. Sutherland, T.W. Rall, *J. Biol. Chem.*, **1958**, *232*, 1077-1091.
8. A. Levitzki, *Science*, **1988**, *241*, 800-806.
9. L. Stryer, *Ann. Rev. Neurosci.*, **1986**, *9*, 87-119.
10. E.E. Fersenko, S.S. Kolesnikov, A.L. Lyubarsky, *Nature (London)*, **1985**, *313*, 310-313.
11. D.L. Clapper, T.F. Walseth, P.J. Dargie, H.C. Lee, *J. Biol. Chem.*, **1987**, *262*, 9561-9568.
12. H.C. Lee, T.F. Walseth, G.T. Bratt, R.N. Hayes, D.L. Clapper, *J. Biol. Chem.*, **1989**, *264*, 1608-1615.
13. M.R. Hellmich, F. Strumwasser, *Cell Regul.*, **1991**, *2*, 193-202.
14. A. Galione, *Trends Pharmacol. Sci.*, **1992**, *13*, 304-306.
15. M. Whitman, C.P. Downes, M. Keeler, T. Keller, L. Cantley, *Nature (London)*, **1988**, *332*, 644-646.
16. A.E. Traynor-Kaplan, A.L. Harris, B.L. Thompson, P. Taylor, L.A. Sklar, *Nature (London)*, **1988**, *334*, 353-356.
17. M.R. Hokin, L.E. Hokin, *J. Biol. Chem.*, **1953**, *203*, 967-977.
18. L.E. Hokin, M.R. Hokin, *J. Biol. Chem.*, **1958**, *233*, 805-810.
19. E.G. Lapetina, R.H. Michell, *FEBS Lett.*, **1973**, *31*, 1-10.
20. R.H. Michell, *Biochim. Biophys. Acta*, **1975**, *415*, 81-147.
21. R.H. Michell, C.J. Kirk, L.M. Jones, C.P. Downes, J.A. Creba, *Phil. Trans. R. Soc. Lond. Ser. B*, **1981**, *296*, 123-137.
22. H. Streb, R.F. Irvine, M.J. Berridge, I. Schulz, *Nature (London)*, **1983**, *306*, 67-69.
23. U. Kikkawa, Y. Nishizuka, *Annu. Rev. Cell. Biol.*, **1986**, *2*, 149-178.
24. Y. Nishizuka, *Science*, **1986**, *233*, 305-312.
25. Y. Nishizuka, *Nature (London)*, **1988**, *334*, 661-665.

26. B.R. Ganong, C.R. Loomis, Y.A. Hannun, R.M. Bell, *Proc. Natl. Acad. Sci. USA*, **1986**, *83*, 1184-1188.

27. IUPAC-IUB Commission on Biochemical Nomenclature, *Biochim. Biophys. Acta*, **1968**, *152*, 1-9.

28. M. Castagna, Y. Takai, K. Kaibuchi, K. Sano, U. Kikkawa, Y. Nishizuka, *J. Biol. Chem.*, **1982**, *257*, 7847-7851.

29. W.L. Smith, *Biochem. J.*, **1989**, *259*, 315-324.

30. A. Spät, P.G. Bradford, J.S. McKinney, R.P. Rubin, J.W. Putney Jnr., *Nature (London)*, **1986**, *319*, 514-516.

31. A. Spät, A. Fabiato, R.P. Rubin, *Biochem. J.*, **1986**, *233*, 929-932.

32. P.F. Worley, J.M. Baraban, S. Supattapone, V.S. Wilson, S.H. Snyder, *J. Biol. Chem.*, **1987**, *262*, 12132-12136.

33. P.F. Worley, J.M. Baraban, J.S. Colvin, S.H. Snyder, *Nature (London)*, **1987**, *325*, 159-161.

34. S. Supattapone, P.F. Worley, J.M. Baraban, S.H. Snyder, *J. Biol. Chem.*, **1988**, *263*, 1530-1534.

35. T. Meyer, D. Holowka, L. Stryer, *Science*, **1988**, *240*, 653-656.

36. T. Furuichi, S. Yoshikawa, A. Miyawaki, K. Wada, N. Maeda, K. Mikoshiba, *Nature (London)*, **1989**, *342*, 32-38.

37. G.A. Mignery, C.L. Newton, B.T. Archer III, T.C. Südhof, *J. Biol. Chem.*, **1990**, *265*, 12679-12685.

38. C.D. Ferris, R.L. Huganir, S. Supattapone, S.H. Snyder, *Nature (London)*, **1989**, *342*, 87-89.

39. S. Yoshikawa, T. Tanimura, A. Miyawaki, M. Nakamura, M. Yuzaki, T. Furuichi, K. Mikoshiba, *J. Biol. Chem.*, **1992**, *267*, 16613-16619.

40. T. Meyer, L. Stryer, *Proc. Natl. Acad. Sci. USA*, **1988**, *85*, 5051-5055.

41. T.J. Rink, *Trends Neurosci.*, **1989**, *12*, 43-46.

42. M.J. Berridge, *J. Biol. Chem.*, **1990**, *265*, 9583-9586.

43. O.H. Petersen, M. Wakui, *J. Membr. Biol.*, **1990**, *118*, 93-105.

44. S.K. Danoff, S. Supattapone, S.H. Snyder, *Biochem. J.*, **1988**, *254*, 701-705.

45. S. Supattapone, S.K. Danoff, A.B. Theibert, S.K. Joseph, J. Steiner, S.H. Snyder, *Proc. Natl. Acad. Sci. USA*, **1988**, *85*, 8747-8750.

46. L.T. Young, S.J. Kish, P.P. Li, J.J. Warsh, *Neurosci. Lett.*, **1988**, *94*, 198-202.

47. T.L. Smith, *Life Sciences*, **1987**, *41*, 2863-2868.

48. IUPAC-IUB, *Pure Appl. Chem.*, **1974**, *37*, 285-297.

49. NC-IUB, *Biochem. J.*, **1989**, *258*, 1-2.

50. P.W. Majerus, *Annu. Rev. Biochem.*, **1992**, *61*, 225-250.

51. L.A. Mauck, Y.-H. Wong, W.R. Sherman, *Biochemistry*, **1980**, *19*, 3623-3629.

52. T. Maeda, F. Eisenberg Jnr., *J. Biol. Chem.*, **1980**, *255*, 8458-8461.

53. T.F. Donahue, S.A. Henry, *J. Biol. Chem.*, **1981**, *256*, 7077-7085.

54. K. Takimoto, M. Okada, Y. Matsuda, H. Nakagawa, *J. Biochem. (Tokyo)*, **1985**, *98*, 363-370.

55. P.V. Attwood, J.-B. Ducep, M.-C. Chanal, *Biochem. J.*, **1988**, *253*, 387-394.

56. J.L. Meek, T.J. Rice, E. Anton, *Biochem. Biophys. Res. Commun.*, **1988**, *156*, 143-148.

57. N.S. Gee, C.I. Ragan, K.J. Watling, S. Aspley, R.G. Jackson, G.G. Reid, D. Gani, J.K. Shute, *Biochem. J.*, **1988**, *249*, 883-889.

58. J.H. Allison, M.A. Stewart, *Nature (London),New Biol.*, **1971**, *233*, 267-268.

59. W.R. Sherman, L.Y. Munsell, B.G. Gish, M.P. Honchar, *J. Neurochem.*, **1985**, *44*, 798-807.

60. A.H. Drummond, *Trends Pharmacol. Sci.*, **1987**, *8*, 129-133.

61. S.R. Nahorski, S. Jenkinson, R.A.J. Challiss, *Biochem. Soc. Trans.*, **1992**, *20*, 430-434.

62. D.J. Storey, S.B. Shears, C.J. Kirk, R.H. Michell, *Nature (London)*, **1984**, *312*, 374-376.

63. G.M. Burgess, J.S. McKinney, R.F. Irvine, J.W. Putney Jnr., *Biochem. J.*, **1985**, *232*, 237-243.

64. I. Batty, S.R. Nahorski, *Biochem. J.*, **1987**, *247*, 797-800.

65. S. Avissar, G. Schreiber, A. Danon, R.H. Belmaker, *Nature (London)*, **1988**, *331*, 440-442.

66. A.H. Drummond, *Nature (London)*, **1988**, *331*, 388.

67. R. Baker, J.J. Kulagowski, D.C. Billington, P.D. Leeson, I.C. Lennon, N.J. Liverton, *J. Chem. Soc., Chem. Commun.*, **1989**, 1383-1385.

68. R. Baker, P.D. Leeson, N.J. Liverton, J.J. Kulagowski, *J.Chem.Soc., Chem. Commun.*, **1990**, 462-464.

69. R. Baker, C. Carrick, P.D. Leeson, I.C. Lennon, N.J. Liverton, *J.Chem.Soc., Chem. Commun.*, **1991**, 298-300.

70. A.M. MacLeod, R. Baker, M. Hudson, K. James, M.B. Roe, M. Knowles, G. MacAllister, *Med. Chem. Res.*, **1992**, *2*, 96-101.

71. S.R. Fletcher, R. Baker, P.D. Leeson, M. Teall, E.A. Harley, C.I. Ragan, *Bioorg. Med. Chem. Lett.*, **1992**, *2*, 627-630.

72. R. Baker, J.J. Kulagowski, P.D. Leeson, N.J. Liverton, *IUPAC - Trends in Medicinal Chemistry '90*, Ed. S. Sarel, R. Mechoulam, I. Agranat, *Blackwell Scientific Publications* **1992**, 199-205.

73. S.R. Fletcher, R. Baker, T. Ladduwahetty, A. Sharpe, M. Teall, J.R. Atack, *Bioorg. Med. Chem. Lett.*, **1993**, *3*, 141-146.

74. C.A. Hansen, R.A. Johanson, M.T. Williamson, J.R. Williamson, *J. Biol. Chem.*, **1987**, *262*, 17319-17326.

75. C. Erneux, M. Lemos, B. Verjans, P. Vanderhaeghen, A. Delvaux, J.E. Dumont, *Eur. J. Biochem.*, **1989**, *181*, 317-322.

76. O. Attree, I.M. Olivos, I. Okabe, L.C. Bailey, D.L. Nelson, R.A. Lewis, R.R. McInnes, R.L. Nussbaum, *Nature (London)*, **1992**, *358*, 239-242.

77. K.E. Nye, G.A. Riley, A.J. Pinching, *Clin. exp. Immunol.*, **1992**, *89*, 89-93.

78. G.W. Mayr, *Biochem. J.*, **1989**, *259*, 463-470.

79. R.F. Irvine, A.J. Letcher, D.J. Lander, C.P. Downes, *Biochem. J.*, **1984**, *223*, 237-243.

80. R.F. Irvine, A.J. Letcher, J.P. Heslop, M.J. Berridge, *Nature (London)*, **1986**, *320*, 631-634.

81. R.A. Johanson, C.A. Hansen, J.R. Williamson, *J. Biol. Chem.*, **1988**, *263*, 7465-7471.

82. I.R. Batty, S.R. Nahorski, R.F. Irvine, *Biochem. J.*, **1985**, *232*, 211-215.

83. P.G. Bradford, R.F. Irvine, *Biochem. Biophys. Res. Commun.*, **1987**, *149*, 680-685.

84. A.B. Theibert, V.A. Estevez, R.J. Mourey, J.F. Marecek, R.K. Barrow, G.D. Prestwich, S.H. Snyder, *J. Biol. Chem.*, **1992**, *267*, 9071-9079.

85. P. Enyedi, G.H. Williams, *J. Biol. Chem.*, **1988**, *263*, 7940-7942.

86. P.J. Cullen, R.F. Irvine, *Biochem. J.*, **1992**, *288*, 149-154.

87. A.H. Guse, E. Roth, F. Emmrich, *Biochem. J.*, **1992**, *288*, 489-495.

88. R.F. Irvine, R.M. Moor, *Biochem. Biophys. Res. Commun.*, **1987**, *146*, 284-290.

89. R.F. Irvine, R.M. Moor, W.K. Pollock, P.M. Smith, K.A. Wreggett, *Phil. Trans. R. Soc. Lond. Ser. B*, **1988**, *320*, 281-298.

90. C.P. Downes, P.T. Hawkins, R.F. Irvine, *Biochem. J.*, **1986**, *238*, 501-506.

91. C. Doughney, M.A. McPherson, R.L. Dormer, *Biochem. J.*, **1988**, *251*, 927-929.

92. T.M. Connolly, V.S. Bansal, T.E. Bross, R.F. Irvine, P.W. Majerus, *J. Biol. Chem.*, **1987**, *262*, 2146-2149.

93. S.B. Shears, J.B. Parry, E.K.Y. Tang, R.F. Irvine, R.H. Michell, C.J. Kirk, *Biochem. J.*, **1987**, *246*, 139-147.

94. D.J. Gawler, B.V.L. Potter, R. Gigg, S.R. Nahorski, *Biochem. J.*, **1991**, *276*, 163-167.

95. E. Pfeffer, *Pringheims Jb. Wis. Bot.*, **1872**, *8*, 429-475.

96. G.R. Bartlett, *Am. Zool.*, **1980**, *20*, 103-114.

97. R.E. Isaacks, D.R. Harkness, *Am. Zool.*, **1980**, *20*, 115-129.

98. D. Carpenter, M.R. Hanley, P.T. Hawkins, T.R. Jackson, L.R. Stephens, M. Vallejo, *Biochem. Soc. Trans.*, **1989**, *17*, 3-5.

99. M. Vallejo, T. Jackson, S. Lightman, M.R. Hanley, *Nature (London)*, **1987**, *330*, 656-658.

100. F.S. Menniti, K.G. Oliver, K. Nogimori, J.F. Obie, S.B. Shears, J.W. Putney Jnr., *J. Biol. Chem.*, **1990**, *265*, 11167-11176.

101. F.S. Menniti, K.G. Oliver, J.W. Putney Jnr., S.B. Shears, *Trends Biochem. Sci.*, **1993**, *18*, 53-56.

102. L.R. Stephens, R.F. Irvine, *Nature (London)*, **1990**, *346*, 580-583.

103. G.N. Europe-Finner, B. Gammon, C.A. Wood, P.C. Newell, *J. Cell. Sci.*, **1989**, *93*, 585-592.

104. F.S. Menniti, R.N. Miller, J.W. Putney Jnr., S.B. Shears, *J. Biol. Chem.*, **1993**, *268*, 3850-3856.

105. L. Stephens, T. Radenberg, U. Thiel, G. Vogel, K.-H. Khoo, A. Dell, T.R. Jackson, P.T. Hawkins, G.W. Mayr, *J. Biol. Chem.*, **1993**, *268*, 4009-4015.

106. M.A.J. Ferguson, A.F. Williams, *Annu. Rev. Biochem.*, **1988**, *57*, 285-320.

107. M.G. Low, M.A.J. Ferguson, A.H. Futerman, I. Silman, *Trends Biochem. Sci.*, **1986**, *11*, 212-215.

108. M.G. Low, *Biochem. J.*, **1987**, *244*, 1-13.

109. M.G. Low, A.R. Saltiel, *Science*, **1988**, *239*, 268-275.

110. T.L. Doering, W.J. Masterson, G.W. Hart, P.T. Englund, *J. Biol. Chem.*, **1990**, *265*, 611 614.

111. W.L. Roberts, S. Santikarn, V.N. Reinhold, T.L. Rosenberry, *J. Biol. Chem.*, **1988**, *263*, 18776-18784.

112. S.W. Homans, M.A.J. Ferguson, R.A. Dwek, T.W. Rademacher, R. Anand, A.F. Williams, *Nature (London)*, **1988**, *333*, 269-272.

113. M.P. Czech, J.K. Klarlund, K.A. Yagaloff, A.P. Bradford, R.F. Lewis, *J. Biol. Chem.*, **1988**, *263*, 11017-11020.

114. W.K. Berlin, W.S. Zhang, T.Y. Shen, *Tetrahedron*, **1991**, *47*, 1-20.

115. A. Zapata, M. Martin-Lomas, *Carbohydr. Res.*, **1992**, *234*, 93-106.

116. C. Murakata, T. Ogawa, *Tetrahedron Lett.*, **1991**, *32*, 671-674.

117. R. Plourde, M. D'Alarcao, *Tetrahedron Lett.*, **1990**, *31*, 2693-2696.

118. R. Verduyn, C.J.J. Elie, C.E. Dreef, G.A. van der Marel, J.H. van Boom, *Recl. Trav. Chim. Pays-Bas*, **1990**, *109*, 591-593.

119. M.A.J. Ferguson, S.W. Homans, R.A. Dwek, T.W. Rademacher, *Science*, **1988**, *239*, 753-759.

120. T. Posternak, *The cyclitols*, Holden-Day, Inc., San Francisco, **1965**.

121. D. J. Cosgrove, *Inositol Phosphates, their Chemistry, Biochemistry and Physiology*, Elsevier, Amsterdam, **1980**.

122. D.C. Billington, *Chem. Soc. Rev.*, **1989**, *18*, 83-122.

123. B.V.L. Potter, *Nat. Prod. Reps.*, **1990**, *7*, 1-24.

124. *Inositol Phosphates and Derivatives - Synthesis, Biochemistry and Therapeutic Potential*, American Chemical Society, Washington, DC, **1991**.

125. D.C. Billington, *The inositol phosphates: chemical synthesis and biological significance*, 1st ed., VCH Verlagsgesellschaft, Weinheim, New York, Basel, Cambridge, **1993**.

126. *Carbohydr. Res.*, **1992**, *234*.

127. S. Czernecki, C. Georgoulis, C. Provelenghiou, *Tetrahedron Lett.*, **1976**, *17*, 3535.

128. A.E. Jackson, R.A.W. Johnstone, *Synthesis*, **1976**, 685-687.

129. A.M. Felix, E.P. Heimer, T.J. Lambros, C. Tzougraki, J. Meienhofer, *J. Org. Chem.*, **1978**, *43*, 4194-4196.

130. B. ElAmin, G.M. Anantharamaiah, G.P. Royer, G.E. Means, *J. Org. Chem.*, **1979**, *44*, 3442-3444.

131. T. Bieg, W. Szeja, *Synthesis*, **1985**, 76-77.

132. J.N. BeMiller, R.E. Wing, C.Y. Meyers, *J. Org. Chem.*, **1968**, *33*, 4293-4296.

133. R. Allerton, H.G. Fletcher Jnr., *J. Am. Chem. Soc.*, **1954**, *76*, 1757-1760.

134. J. Arris, J. Baddiley, J.G. Buchanan, E.M. Thain, *J. Chem. Soc.*, **1956**, 4968-4973.

135. Y. Oikawa, T. Yoshioka, O. Yonemitsu, *Tetrahedron Lett.*, **1982**, *23*, 885-888.

136. J. Gigg, R. Gigg, S. Payne, R. Conant, *J.Chem.Soc., Perkin Trans. 1*, **1987**, 423-429.

137. C.C. Price, W.H. Snyder, *J. Am. Chem. Soc.*, **1961**, *83*, 1773.

138. E.J. Corey, J.W. Suggs, *J. Org. Chem.*, **1973**, *38*, 3224.

139. J.J. Oltvoort, C.A.A. van Boeckel, J.H. De Koning, J.H. van Boom, *Synthesis*, **1981**, 305-308.

140. J. Cunningham, R. Gigg, C.D. Warren, *Tetrahedron Lett.*, **1964**, 1191-1196.

141. R. Gigg, C.D. Warren, *J.Chem.Soc., Chem. Commun.*, **1968**, 1903-1911.

142. C.M. Stevens, R. Watanabe, *J. Am. Chem. Soc.*, **1950**, *72*, 725-727.

143. S.W. Baldwin, J.C. Tomesch, *J. Org. Chem.*, **1974**, *39*, 2382-2385.

144. B. Espanet, E. Dunach, J. Périchon, *Tetrahedron Lett.*, **1992**, *33*, 2485-2488.

145. S.M. Kadam, S.K. Nayak, A. Banerji, *Tetrahedron Lett.*, **1992**, *33*, 5129-5132.

146. P.A. Manthorpe, R. Gigg, *Meth. Carbohydr. Chem.*, **1980**, *III*, 305-311.

147. K.S. Bruzik, M.D. Tsai, *J. Am. Chem. Soc.*, **1992**, *114*, 6361-6374.

148. Y. Watanabe, T. Shinohara, T. Fujimoto, S. Ozaki, *Chem. Pharm. Bull.*, **1990**, *38*, 562-563.

149. J. Gigg, R. Gigg, S. Payne, R. Conant, *Carbohydr. Res.*, **1985**, *142*, 132-134.

150. S.J. Angyal, M.E. Tate, S.D. Gero, *J. Chem. Soc.*, **1961**, 4116-4122.

151. S.J. Angyal, G.C. Irving, D. Rutherford, M.E. Tate, *J. Chem. Soc.*, **1965**, 6662-6664.

152. D.J.R. Massy, P. Wyss, *Helv. Chim. Acta*, **1990**, *73*, 1037-1057.

153. R. Gigg, C.D. Warren, *J. Chem. Soc. (C)*, **1969**, 2367-2371.

154. D.E. Kiely, G.J. Abruscato, V. Baburao, *Carbohydr. Res.*, **1974**, *34*, 307-313.

155. J. Gigg, R. Gigg, S. Payne, R. Conant, *J.Chem.Soc., Perkin Trans. 1*, **1987**, 2411-2414.

156. C. Jiang, D.C. Baker, *J. Carbohydr. Chem.*, **1986**, *5*, 615-620.

157. Y. Watanabe, M. Mitani, T. Morita, S. Ozaki, *J.Chem.Soc., Chem. Commun.*, **1989**, 482-483.

158. H.W Lee,, Y. Kishi, *J. Org. Chem.*, **1985**, *50*, 4402-4404.

159. G. Baudin, B.I. Glänzer, K.S. Swaminathan, A. Vasella, *Helv. Chim. Acta*, **1988**, *71*, 1367-1378.

160. D.C. Billington, R. Baker, *J.Chem.Soc., Chem. Commun.*, **1987**, 1011-1013.

161. D.C. Billington, R. Baker, J.J. Kulagowski, I.M. Mawer, J.P. Vacca, S.J. DeSolms, J.R. Huff, *J.Chem.Soc., Perkin Trans. 1*, **1989**, 1423-1429.

162. I.H. Gilbert, A.B. Holmes, R.C. Young, *Tetrahedron Lett.*, **1990**, *31*, 2633-2534.

163. I.H. Gilbert, A.B. Holmes, M.J. Pestchanker, R.C. Young, *Carbohydr. Res.*, **1992**, *234*, 117-130.

164. S. David, S. Hanessian, *Tetrahedron*, **1985**, *41*, 643-663.

165. J. Gigg, R. Gigg, S. Payne, R. Conant, *J.Chem.Soc., Perkin Trans. 1*, **1987**, 423-429.

166. J. Alais, A. Veyrières, *J.Chem.Soc., Perkin Trans. 1*, **1981**, 377-381.

167. N. Nagashima, M. Ohno, *Chem. Lett.*, **1987**, 141-144.

168. J. Gigg, R. Gigg, S. Payne, R. Conant, *J.Chem.Soc., Perkin Trans. 1*, **1987**, 1757-1762.

169. K.L. Yu, B. Fraser-Reid, *Tetrahedron Lett.*, **1988**, *29*, 979-982.

170. A.P. Kozikowski, A.H. Fauq, J.M. Rusnak, *Tetrahedron Lett.*, **1989**, *30*, 3365-3368.

171. T. Akiyama, N. Takechi, S. Ozaki, *Tetrahedron Lett.*, **1990**, *31*, 1433-1434.

172. D. Mercier, J.E.G. Barnett, S.D. Gero, *Tetrahedron*, **1969**, *25*, 5681-5687.

173. W. Tegge, C.E. Ballou, *Proc. Natl. Acad. Sci. USA*, **1989**, *86*, 94-98.

174. W. Tegge, G.V. Denis, C.E. Ballou, *Carbohydr. Res.*, **1991**, *217*, 107-116.

175. A.P. Kozikowski, A.H. Fauq, G. Powis, D.C. Melder, *Med. Chem. Res.*, **1991**, *1*, 277-282.

176. M.J. Seewald, I.A. Akosy, G. Powis, A.H. Fauq, A.P. Kozikowski, *J.Chem.Soc., Chem. Commun.*, **1990**, 1638-1639.

177. C. Liu, S.R. Nahorski, B.V.L. Potter, *Carbohydr. Res.*, **1992**, *234*, 107-115.

178. A.P. Kozikowski, A.H. Fauq, G. Powis, D.C. Melder, *J. Am. Chem. Soc.*, **1990**, *112*, 4528 4531.

179. J.R. Falck, P. Yadagiri, *J. Org. Chem.*, **1989**, *54*, 5851-5852.

180. J.R. Falck, A. Abdali, S.J. Wittenberger, *J.Chem.Soc., Chem. Commun.*, **1990**, 953-955.

181. Y. Watanabe, M. Mitani, S. Ozaki, *Chem. Lett.*, **1987**, 123-126.

182. S.T. Safrany, R.J.H. Wojcikiewicz, J. Strupish, S.R. Nahorski, D. Dubreuil, J. Cleophax, S.D. Gero, B.V.L. Potter, *FEBS Lett.*, **1991**, *278*, 252-256.

183. S.L. Bender, R.J. Budhu, *J. Am. Chem. Soc.*, **1991**, *113*, 9883-9885.

184. V.A. Estevez, G.D. Prestwich, *J. Am. Chem. Soc.*, **1991**, *113*, 9885-9887.

185. Y.-C. Liu, C.-S. Chen, *Tetrahedron Lett.*, **1989**, *30*, 1617-1620.

186. D.-M. Gou, C.-S. Chen, *Tetrahedron Lett.*, **1992**, *33*, 721-724.

187. D.-M. Gou, Y.-C. Liu, C.-S. Chen, *Carbohydr. Res.*, **1992**, *234*, 51-64.

188. H. Hönig, P. Seufer-Wasserthal, A.E. Stütz, E. Zenz, *Tetrahedron Lett.*, **1989**, *30*, 811-812.

189. L. Ling, Y. Watanabe, T. Akiyama, S. Ozaki, *Tetrahedron Lett.*, **1992**, *33*, 1911-1914.

190. S. Ozaki, M. Kohno, H. Nakahira, M. Bunya, Y. Watanabe, *Chem. Lett.*, **1988**, 77-80.

191. A.E. Stepanov, O.B. Runova, G. Schlewer, B. Spiess, V.I. Shvets, *Tetrahedron Lett.*, **1989**, *30*, 5125-5128.

192. P.J. Garegg, B. Lindberg, I. Kvarnström, S.C.T. Svensson, *Carbohydr. Res.*, **1985**, *139*,

209-215.

193. K.S. Bruzik, G.M. Salamonczyk, *Carbohydr. Res.*, **1989**, *195*, 67-73.

194. G.M. Salamonczyk, K.M. Pietrusiewicz, *Tetrahedron Lett.*, **1991**, *32*, 6167-6170.

195. S. Ozaki, Y. Watanabe, T. Ogasawara, Y. Kondo, N. Shiotani, H. Nishii, T. Matsuki, *Tetrahedron Lett.*, **1986**, *27*, 3157-3160.

196. S. Ozaki, Y. Kondo, N. Shiotani, T. Ogasawara, Y. Watanabe, *J.Chem.Soc., Perkin Trans. 1*, **1992**, 729-737.

197. C.E. Dreef, R.J. Tuinman, C.J.J. Elie, G.A. van der Marel, J.H. van Boom, *Recl. Trav. Chim. Pays-Bas*, **1988**, *107*, 395-397.

198. H. Schiff, *Liebigs Ann. Chem.*, **1857**, *102*, 334-339.

199. M. Yoshikawa, T. Kato, T. Takenishi, *Tetrahedron Lett.*, **1967**, 5065-5068.

200. T. Desai, A. Fernandez-Mayoralas, J. Gigg, R. Gigg, S. Payne, *Carbohydr. Res.*, **1992**, *234*, 157-175.

201. E. Fischer, E. Pfähler, *Ber. Deut. Chem. Ges.*, **1920**, *53*, 1606-1621.

202. P. Brigl, H. Müller, *Ber. Deut. Chem. Ges.*, **1939**, *72*, 2121-2130.

203. B.M. Iselin, *J. Am. Chem. Soc.*, **1949**, *71*, 3822-3825.

204. J. Baddiley, A.R. Todd, *J. Chem. Soc.*, **1947**, 648-651.

205. J. Baddiley, V.M. Clark, J.J. Michalski, A.R. Todd, *J. Chem. Soc.*, **1949**, 815-821.

206. B. Jastorff, H. Hettler, *Tetrahedron Lett.*, **1969**, 2543-2544.

207. R.P. Glinski, A.B. Ash, C.L. Stevens, M.B. Sporn, H.M. Lazarus, *J. Org. Chem.*, **1971**, *36*, 245.

208. F. Eckstein, K.H. Scheit, *Angew. Chem. Int. Ed. Engl.*, **1967**, *6*, 362.

209. A.M. Cooke, N.J. Noble, S. Payne, R. Gigg, B.V.L. Potter, *J.Chem.Soc., Chem. Commun.*, **1989**, 269-271.

210. A.M. Cooke, N.J. Noble, R. Gigg, A.L. Willcocks, J. Strupish, S.R. Nahorski, B.V.L. Potter, *Biochem. Soc. Trans.*, **1988**, *16*, 992-993.

211. N.J. Noble, A.M. Cooke, B.V.L. Potter, *Carbohydr. Res.*, **1992**, *234*, 177-187.

212. M. Rubinstein, A. Patchornik, *Tetrahedron*, **1975**, *31*, 2107.

213. D.J. Cosgrove, *Carbohydr. Res.*, **1975**, *40*, 380-384.

214. H.G. Khorana, A.R. Todd, *J. Chem. Soc.*, **1953**, 2257-2260.

215. Y. Watanabe, H. Nahahira, M. Bunya, S. Ozaki, *Tetrahedron Lett.*, **1987**, *28*, 4179-4180.

216. S.J. DeSolms, J.P. Vacca, J.R. Huff, *Tetrahedron Lett.*, **1987**, *28*, 4503-4506.

217. J.P. Vacca, S.J. DeSolms, J.R. Huff, D.C. Billington, R. Baker, J.J. Kulagowski, I.M. Mawer, *Tetrahedron*, **1989**, *45*, 5679-5702.

218. P.M. Chouinard, P.A. Bartlett, *J. Org. Chem.*, **1986**, *51*, 75-78.

219. J.P. Vacca, S.J. DeSolms, J.R. Huff, *J. Am. Chem. Soc.*, **1987**, *109*, 3478-3479.

220. F. Cramer, H. Schaller, H.A. Staab, *Chem. Ber.*, **1961**, *94*, 1612-1621.

221. D.E. Hoard, D.G. Ott, *J. Am. Chem. Soc.*, **1965**, *87*, 1785-1788.

222. J.L. Meek, F. Davidson, F.W. Hobbs Jr, *J. Am. Chem. Soc.*, **1988**, *110*, 2317-2318.

223. C.E. Dreef, G.A. van der Marel, J.H. van Boom, *Recl. Trav. Chim. Pays-Bas*, **1987**, *106*, 161-162.

224. A.M. Cooke, B.V.L. Potter, *Tetrahedron Lett.*, **1987**, *28*, 2305-2308.

225. E. Uhlmann, J. Engels, *Tetrahedron Lett.*, **1986**, *27*, 1023-1026.

226. W. Bannwarth, A. Trzeciak, *Helv. Chim. Acta*, **1987**, *70*, 175-186.

227. C.B. Reese, J.G. Ward, *Tetrahedron Lett.*, **1987**, *28*, 2309-2312.

228. Y. Watanabe, A. Oka, Y. Shimizu, S. Ozaki, *Tetrahedron Lett.*, **1990**, *31*, 2613-2616.

229. Y. Watanabe, Y. Komoda, K. Ebisuya, S. Ozaki, *Tetrahedron Lett.*, **1990**, *31*, 255-256.

230. Y. Watanabe, T. Fujimoto, T. Shinohara, S. Ozaki, *J.Chem.Soc., Chem. Commun.*, **1991**, 428-429.

231. T. Akiyama, N. Takechi, S. Ozaki, K. Shiota, *Bull. Chem. Soc. Jpn.*, **1992**, *65*, 366-372.

232. S. Ozaki, Y. Watanabe, T. Ogasawara, M. Hirata, T. Kanematsu, *Carbohydr. Res.*, **1992**, *234*, 189-206.

233. P.M.J. Burgers, F. Eckstein, *Tetrahedron Lett.*, **1978**, 3835-3838.

234. C.E. Dreef, G.W. Mayr, J.-P. Jansze, H.C.P.F. Roelen, G.A. van der Marel, J.H. van Boom, *Bioorg. Med. Chem. Lett.*, **1991**, *1*, 239-242.

235. F. Eckstein, *Angew. Chem.*, **1983**, *95*, 431-514, *Angew. Chem. Int. Ed. Engl. 22*, 423-506

236. A.M. Cooke, R. Gigg, B.V.L. Potter, *J.Chem.Soc., Chem. Commun.*, **1987**, 1525-1526.

237. C.E. Dreef, C.M. Dreef-Tromps, G.A. van der Marel, J.H. van Boom, *Synlett*, **1990**, 481-483.

238. D.C. Billington, R. Baker, J.J. Kulagowski, I.M. Mawer, *J.Chem.Soc., Chem. Commun.*, **1987**, 314-316.

239. A.P. Briggs, *J. Biol. Chem.*, **1922**, *53*, 13-16.

240. J.L. Meek, F. Nicoletti, *J. Chromatogr.*, **1986**, *351*, 303-311.

241. J.L. Meek, *Proc. Natl. Acad. Sci. USA*, **1986**, *83*, 4162-4166.

242. G.W. Mayr, *Biochem. J.*, **1988**, *254*, 585-591.

243. G.L. Ellman, *Arch. Biochem. Biophys.*, **1959**, *82*, 70.

244. A.F.S.A. Habeeb, *Meth. Enzym.*, **1979**, *25*, 457-464.

245. G.M. Salamonczyk, K.M. Pietrusiewicz, *Tetrahedron Lett.*, **1991**, *32*, 4031-4032.

246. J.G. Molotkovsky, L.D. Bergelson, *Tetrahedron Lett.*, **1971**, *50*, 4791-4794.

247. T. Metschies, C. Schultz, B. Jastorff, *Tetrahedron Lett.*, **1988**, *29*, 3921-3922.

248. A.E. Stepanov, V.I. Shvets, *Chem. Phys. Lipids*, **1979**, *25*, 247-263.

249. S.J. Angyal, M.E. Tate, *J. Chem. Soc.*, **1961**, 4122-4128.

250. M.R. Hamblin, J.S. Flora, B.V.L. Potter, *Biochem. J.*, **1987**, *246*, 771-774.

251. M.R. Hamblin, B.V.L. Potter, R. Gigg, *Biochem. Soc. Trans.*, **1987**, *15*, 415-416.

252. M.R. Hamblin, B.V.L. Potter, R. Gigg, *J.Chem.Soc., Chem. Commun.*, **1987**, 626-627.

253. H.A.J. Carless, K. Busia, *Tetrahedron Lett.*, **1990**, *31*, 3449-3452.

254. P. Westerduin, H.A.M. Willems, C.A.A. van Boeckel, *Tetrahedron Lett.*, **1990**, *31*, 6915-6918.

255. S.V. Ley, F. Sternfeld, *Tetrahedron Lett.*, **1988**, *29*, 5305-5308.

256. S.V. Ley, M. Parra, A.J. Redgrave, F. Sternfeld, *Tetrahedron*, **1990**, *46*, 4995-5026.

257. H.A.J. Carless, K. Busia, *Tetrahedron Lett.*, **1990**, *31*, 1617-1620.

258. Y. Watanabe, T. Ogasawara, H. Nakahira, T. Matsuki, S. Ozaki, *Tetrahedron Lett.*, **1988**, *29*, 5259-5262.

259. J.F. Marecek, G.D. Prestwich, *J. Labelled Compd. Radiopharm.*, **1989**, *27*, 917-925.

260. R.J. Auchus, S.L. Kaiser, P.W. Majerus, *Proc. Natl. Acad. Sci. USA*, **1987**, *84*, 1206-1209.

261. Y. Watanabe, T. Ogasawara, N. Shiotani, S. Ozaki, *Tetrahedron Lett.*, **1987**, *28*, 2607-2610.

262. S. Ozaki, Y. Kondo, H. Nakahira, S. Yamaoka, Y. Watanabe, *Tetrahedron Lett.*, **1987**, *28*, 4691-4694.

263. H. Teichmann, G. Hilgetag, *Angew. Chem. Int. Ed. Engl.*, **1967**, *6*, 1013-1023.

264. A.W. Murray, M.R. Atkinson, *Biochemistry*, **1968**, *7*, 4023-4029

265. W.R.G. Dostmann, S.S. Taylor, H.-G. Genieser, B. Jastorff, S.O. Döskeland, D. Ögreid, *J.*

Biol. Chem., **1990**, *265*, 10484-10491.

266. E. Butt, M. van Bemmelen, L. Fischer, U. Walter, B. Jastorff, *FEBS Lett.*, **1990**, *263*, 47-50.

267. F. Eckstein, *Annu. Rev. Biochem.*, **1985**, *54*, 367-402.

268. C.W. Taylor, M.J. Berridge, A.M. Cooke, B.V.L. Potter, *Biochem. Soc. Trans.*, **1988**, *16*, 995-996.

269. J. Strupish, A.M. Cooke, B.V.L. Potter, R. Gigg, S.R. Nahorski, *Biochem. J.*, **1988**, *253*, 901-905.

270. A.L. Willcocks, B.V.L. Potter, A.M. Cooke, S.R. Nahorski, *Eur. J. Pharmacol.*, **1988**, *155*, 181-183.

271. A.M. Cooke, S.R. Nahorski, B.V.L. Potter, *FEBS Lett.*, **1989**, *242*, 373-377.

272. S.R. Nahorski, B.V.L. Potter, *Trends Pharmacol. Sci.*, **1989**, *10*, 139-144.

273. N.J. Noble, D. Dubreuil, B.V.L. Potter, *Bioorg. Med. Chem. Lett.*, **1992**, *2*, 471-476.

274. R.A.J. Challiss, E.R. Chilvers, A.L. Willcocks, S.R. Nahorski, *Biochem. J.*, **1990**, *265*, 421 427.

275. R.A.J. Challiss, S.M. Smith, B.V.L. Potter, S.R. Nahorski, *FEBS Lett.*, **1991**, *281*, 101-104.

276. R.G. Pearson, *J. Am. Chem. Soc.*, **1963**, *85*, 3533-3539.

277. G.R. Baker, D.C. Billington, D. Gani, *Tetrahedron*, **1991**, *47*, 3895-3908.

278. G.R. Baker, D.C. Billington, D. Gani, *Bioorg. Med. Chem. Lett.*, **1991**, *1*, 17-20.

279. A.M. Cooke, L. James, S.R. Nahorski, B.V.L. Potter, *Phosphorus,Sulfur,and Silicon*, **1990**, *51/52*, 19-22.

280. C. Schultz, T. Metschies, B. Jastorff, *Tetrahedron Lett.*, **1988**, *29*, 3919-3920.

281. G. Lin, M.D. Tsai, *J. Am. Chem. Soc.*, **1989**, *111*, 3099-3101.

282. G. Lin, F. Bennett, M.D. Tsai, *Biochemistry*, **1990**, *29*, 2747-2757.

283. G.M. Salamonczyk, K.S. Bruzik, *Tetrahedron Lett.*, **1990**, *31*, 2015-2016.

284. C.E. Dreef, G.A. van der Marel, J.H. van Boom, *Recl. Trav. Chim. Pays-Bas*, **1987**, *106*, 512-513.

285. C.E. Dreef, W. Schiebler, G.A. van der Marel, J.H. van Boom, *Tetrahedron Lett.*, **1991**, *32*, 6021-6024.

286. C.E. Dreef, J.-P. Jansze, C.J.J. Elie, G.A. van der Marel, J.H. van Boom, *Carbohydr. Res.*, **1992**, *234*, 37-50.

287. C.E. Dreef, R.J. Tuinman, A.W.M. Lefeber, C.J.J. Elie, G.A. van der Marel, J.H. van Boom, *Tetrahedron*, **1991**, *47*, 4709-4722.

288. H.A.M. Willems, G.H. Veeneman, P. Westerduin, *Tetrahedron Lett.*, **1992**, *33*, 2075-2078.

289. P. Westerduin, H.A.M. Willems, C.A.A. van Boeckel, *Carbohydr. Res.*, **1992**, *234*, 131-140.

290. S.S. Yang, T.R. Beattie, P.L. Durette, T.F. Gallagher, T.Y. Shen, *U. S. Pat.*, **1985**, *4515722*.

291. C.E. Dreef, C.J.J. Elie, G.A. van der Marel, J.H. van Boom, *Tetrahedron Lett.*, **1991**, *32*, 955-958.

292. C.E. Dreef, M. Douwes, C.J.J. Elie, G.A. van der Marel, J.H. van Boom, *Synthesis*, **1991**, 443-447.

293. M.S. Shashidhar, J.F.W. Keana, J.J. Volwerk, O.H. Griffith, *Chem. Phys. Lipids*, **1990**, *53*, 103-113.

294. P. Westerduin, H.A.M. Willems, C.A.A. van Boeckel, *Tetrahedron Lett.*, **1990**, *31*, 6919 6922.

295. J.A. Wilkinson, *Chem. Rev.*, **1992**, *92*, 505-519.

296. P.J. Card, *J. Carbohydr. Chem.*, **1985**, *4*, 451-487.

297. M. Schlosser, *Tetrahedron*, **1978**, *34*, 3-17.

298. J.T. Welch, *Tetrahedron*, **1987**, *43*, 3123-3197.

299. C. Heidelberger, N.K. Chaudhuri, P. Danneberg, D. Mooren, L. Griesbach, R.R. Duschinsky, R.J. Schnitzer, E. Pleven, J. Scheiner, *Nature (London)*, **1957**, *179*, 663-666.

300. R.R. Duschinsky, E. Pleven, C. Heidelberger, *J. Am. Chem. Soc.*, **1957**, *79*, 4559.

301. W.J. Middleton, *J. Org. Chem.*, **1975**, *40*, 574-578.

302. T.H. Ji, I. Ji, *Anal. Biochem.*, **1982**, *121*, 286-289.

303. C. Jiang, D.J.A. Schedler, P.E. Morris Jnr., A.A. Zayed, D.C. Baker, *Carbohydr. Res.*, **1990**, *207*, 277-285.

304. D.A. Sawyer, B.V.L. Potter, *Bioorg. Med. Chem. Lett.*, **1991**, *1*, 705-710.

305. S.T. Safrany, D. Sawyer, R.J.H. Wojcikiewicz, S.R. Nahorski, B.V.L. Potter, *FEBS Lett.*, **1990**, *276*, 91-94.

306. J.F. Marecek, G.D. Prestwich, *Tetrahedron Lett.*, **1989**, *30*, 5401-5404.

307. D.A. Sawyer, B.V.L. Potter, *J. Chem. Soc., Perkin Trans. 1*, **1992**, 923-932.

308. A.P. Kozikowski, Y. Xia, J.M. Rusnak, *J. Chem. Soc., Chem. Commun.*, **1988**, 1301-1303.

309. J.L. Offer, G.A. Smith, J.C. Metcalfe, *Biochem. Soc. Trans.*, **1990**, *18*, 590-591.

310. J.L. Offer, J.C. Metcalfe, G.A. Smith, *J. Chem. Soc., Chem. Commun.*, **1990**, 1312-1313.

311. S.S. Yang, T.R. Beattie, T.Y. Shen, *Tetrahedron Lett.*, **1982**, *23*, 5517-5520.

312. G. Lowe, F. McPhee, *J. Chem. Soc., Perkin Trans. 1*, **1991**, 1249-1253.

313. C. Jiang, J.D. Moyer, D.C. Baker, *J. Carbohydr. Chem.*, **1987**, *6*, 319-355.

314. S.S. Yang, J.M. Min, T.R. Beattie, *Synth. Commun.*, **1988**, *18*, 899-905.

315. J.D. Moyer, O. Reizes, A. Surender, C. Jiang, N. Malinowski, D.C. Baker, *Mol. Pharmacol.*, **1988**, *33*, 683-689.

316. S.T. Safrany, D.A. Sawyer, S.R. Nahorski, B.V.L. Potter, *Chirality*, **1992**, *4*, 415-422.

317. A.P. Kozikowski, A.H. Fauq, I.A. Akosy, M.J. Seewald, G. Powis, *J. Am. Chem. Soc.*, **1990**, *112*, 7403-7404.

318. M.F. Boehm, G.D. Prestwich, *Tetrahedron Lett.*, **1988**, *29*, 5217-5220.

319. A.P. Kozikowski, W. Tückmantel, G. Powis, *Angew. Chem.*, **1992**, *104*, 1408-1410, *Angew. Chem. Int. Ed. Engl., 31*, 1381-1383.

320. M. Hirata, Y. Watanabe, T. Ishimatsu, T. Ikebe, Y. Kimura, K. Yamaguchi, S. Ozaki, T. Koga, *J. Biol. Chem.*, **1989**, *264*, 20303-20308.

321. A.L. Willcocks, J. Strupish, R.F. Irvine, S.R. Nahorski, *Biochem. J.*, **1989**, *257*, 297-300.

322. D.L. Nunn, C.W. Taylor, *Biochem. J.*, **1990**, *270*, 227-232.

323. V. Henne, G.W. Mayr, B. Grabowski, B. Koppitz, H.D. Söling, *Eur. J. Biochem.*, **1988**, *174*, 95-101.

324. M.A. Polokoff, G.H. Bencen, J.P. Vacca, S.J. DeSolms, S.D. Young, J.R. Huff, *J. Biol. Chem.*, **1988**, *263*, 11922-11927.

325. S.K. Joseph, C.A. Hansen, J.R. Williamson, *Mol. Pharmacol.*, **1989**, *36*, 391-397.

326. C.W. Taylor, M.J. Berridge, K.D. Brown, A.M. Cooke, B.V.L. Potter, *Biochem. Biophys. Res. Commun.*, **1988**, *150*, 626-632.

327. A.L. Willcocks, A.M. Cooke, B.V.L. Potter, S.R. Nahorski, *Biochem. Biophys. Res. Commun.*, **1987**, *146*, 1071-1078.

328. G.D. Prestwich, J.F. Marecek, R.J. Mourey, A.B. Theibert, C.D. Ferris, S.K. Danoff, S.H. Snyder, *J. Am. Chem. Soc.*, **1991**, *113*, 1822-1825.

329. R.J. Mourey, V.A. Estevez, J.F. Marecek, R.K. Barrow, G.D. Prestwich, S.H. Snyder, *Biochemistry*, **1993**, *32*, 1719-1726.

330. S.J. Mills, S.T. Safrany, R.A. Wilcox, S.R. Nahorski, B.V.L. Potter, *Bioorg. Med. Chem. Lett.*, **1993**, in press.

331. R.A. Wilcox, S.R. Nahorski, D.A. Sawyer, C. Liu, B.V.L. Potter, *Carbohydr. Res.*, **1992**, *234*, 237-246.

332. C. Liu, S.R. Nahorski, B.V.L. Potter, *J.Chem.Soc., Chem. Commun.*, **1991**, 1014-1016.

333. S.T. Safrany, R.A. Wilcox, C. Liu, B.V.L. Potter, S.R. Nahorski, *Eur. J. Pharmacol.*, **1992**,

226, 265-272.

334. H.A.J. Carless, K. Busia, *Carbohydr. Res.*, **1992**, *234*, 207-215.

335. R.A.J. Challiss, A.L. Willcocks, B. Mulloy, B.V.L. Potter, S.R. Nahorski, *Biochem. J.*, **1991**, *274*, 861-867.

336. S.V. Ley, M. Parra, A.J. Redgrave, F. Sternfeld, A. Vidal, *Tetrahedron Lett.*, **1989**, *30*, 3557-3560.

337. S.V. Ley, *Pure Appl. Chem.*, **1990**, *62*, 2031-2034.

338. G.M. Burgess, R.F. Irvine, M.J. Berridge, J.S. McKinney, J.W. Putney, Jnr., **1984**, *224*, 741-746.

339. K.S. Authi, T.O. Gustafsson, N. Crawford, *Thrombosis and Haemostasis*, **1989**, *62*, 250.

340. D.B. Wilson, T.M. Connolly, T.E. Bross, P.W. Majerus, W.R. Sherman, A.N. Tyler, L.J. Rubin, J.E. Brown, *J. Biol. Chem.*, **1985**, *260*, 13496-13501.

341. R.F. Irvine, A.J. Letcher, D.J. Lander, M.J. Berridge, *Biochem. J.*, **1986**, *240*, 301-304.

342. G.V. Denis, C.E. Ballou, *Cell Calcium*, **1991**, *12*, 395-401.

343. A.P. Kozikowski, A.H. Fauq, G. Powis, P. Kurian, F.T. Crews, *J.Chem.Soc., Chem. Commun.*, **1992**, 362-364.

344. M. Poitras, S. Bernier, G. Boulay, A. Fournier, G. Guillemette, *Eur. J. Pharmacol.*, **1993**, *244*, 203-210.

345. J.P. Lyssikatos, M.D. Bednarski, *Bioorg. Med. Chem. Lett.*, **1993**, *3*, 685-688.

346. C. Schultz, G. Gebauer, T. Metschies, L. Rensing, B. Jastorff, *Biochem. Biophys. Res. Commun.*, **1990**, *166*, 1319-1327.

347. I. Ivorra, R. Gigg, R.F. Irvine, I. Parker, *Biochem. J.*, **1991**, *273*, 317-321.

348. S. T. Safrany, *Ph.D. Thesis*, University of Leicester, **1993**.

349. T.K. Ghosh, P.S. Eis, J.M. Mullaney, C.L. Ebert, D.L. Gill, *J. Biol. Chem.*, **1988**, *263*, 11075-11079.

350. G. Guillemette, S. LaMontagne, G. Boulay, B. Mouillac, *Mol. Pharmacol.*, **1989**, *35*, 339-344.

351. M.A. Tones, M.D. Bootman, B.F. Higgins, D.A. Lane, G.F. Pay, *FEBS Lett.*, **1989**, *252*, 105-108.

352. T.D. Hill, P.-O. Berggren, A.L. Boynton, *Biochem. Biophys. Res. Commun.*, **1987**, *149*, 897-901.

353. T. Nilsson, J. Zwiller, A.L. Boynton, P.-O. Berggren, *FEBS Lett.*, **1988**, *229*, 211-214.

354. P.J. Cullen, J.G. Comerford, A.P. Dawson, *FEBS Lett.*, **1988**, *228*, 57-59.

355. K.J. Föhr, J. Scott, G. Ahnert-Hilger, M. Gratzl, *Biochem. J.*, **1989**, *262*, 83-89.

356. J. Strupish, R.J.H. Wojcikiewicz, R.A.J. Challiss, S.T. Safrany, A.L. Willcocks, B.V.L. Potter, S.R. Nahorski, *Biochem. J.*, **1991**, *227*, 294.

357. M. Bencherif, R.J. Lukas, *Neurosci. Lett.*, **1992**, *134*, 157-160.

358. R.A.J. Challiss, S.T. Safrany, B.V.L. Potter, S.R. Nahorski, *Biochem. Soc. Trans.*, **1991**, *19*, 888-893.

359. G. Cornelius, G. Gebauer, D. Techel, *Biochem. Biophys. Res. Commun.*, **1989**, *162*, 852-856.

360. L. Petterson, B. Hedman, I. Andersson, N. Ngri, *Chem. Scr.*, **1983**, *22*, 254-264.

361. A.S. Campbell, G.R.J. Thatcher, *Bioorg. Med. Chem. Lett.*, **1992**, *2*, 655-658.

362. A.J. Morris, K.J. Murray, P.J. England, C.P. Downes, R.H. Michell, *Biochem. J.*, **1988**, *251*, 157-163.

363. S.H. Ryu, S.Y. Lee, K.-Y. Lee, S.G. Rhee, *FASEB J.*, **1987**, *1*, 388-393

364. S.T. Safrany, R.J.H. Wojcikiewicz, J. Strupish, J. McBain, A.M. Cooke, B.V.L. Potter, S.R. Nahorski, *Mol. Pharmacol.*, **1991**, *39*, 754-761.

365. C.W. Taylor, M.J. Berridge, A.M. Cooke, B.V.L. Potter, *Biochem. J.*, **1989**, *259*, 645.

366. M. Hirata, F. Yanaga, T. Koga, T. Ogasawara, Y. Watanabe, S. Ozaki, *J. Biol. Chem.*, **1990**, *265*, 8404-8407.

367. R. Schäfer, M. Nehls-Sahabandu, B. Grabowski, M. Dehlinger-Kremer, I. Schulz, G.W. Mayr, *Biochem. J.*, **1990**, *272*, 817-825.

368. A.N. Jina, J. Ralph, C.E. Ballou, *Biochemistry*, **1990**, *29*, 5203-5209.

369. M. Hirata, Y. Watanabe, T. Ishimatsu, F. Yanaga, T. Koga, S. Ozaki, *Biochem. Biophys. Res. Commun.*, **1990**, *168*, 379-386.

370. Fluka Catalogue, **1993**

371. P.A. Gent, R. Gigg, *J. Chem. Soc., Chem. Commun.*, **1974**, 277-278.

372. N.D. Sinha, J. Biernat, H. Köster, *Tetrahedron Lett.*, **1983**, *24*, 5843-5846.

373. A.D. Barone, J.Y. Tang, M.H. Caruthers, *Nucleic Acids Res.*, **1984**, *12*, 4051-4061.

374. R. Cosstick, L.W. McLaughlin, F. Eckstein, *Nucleic Acids Res.*, **1984**, *12*, 1791-1810.

375. P. Somerharju, K.W.A. Wirtz, *Chem. Phys. Lipids*, **1982**, *30*, 81-91.

376. P.B. Ghosh, M.W Whitehouse, *Biochem. J.*, **1968**, *108*, 155-156.

377. C.T. Murphy, M. Elmore, S. Kellie, J. Westwick, *Biochem. J.*, **1991**, *278*, 255-261.

378. T. Desai, J. Gigg, R. Gigg, S. Payne, *Carbohydr. Res.*, **1992**, *225*, 209-228.

379. T. Desai, J. Gigg, R. Gigg, S. Payne, S. Penades, *Carbohydr. Res.*, **1992**, *234*, 1-21.

380. T. Desai, A. Fernandez-Mayoralas, J. Gigg, R. Gigg, C. Jaramillo, S. Payne, S. Penades, N. Schnetz in *ACS Symp. Ser. 463, Inositol Phosphates and Derivatives, Preparation of Optically Active myo-Inositol Derivatives as Intermediates for the Synthesis of Inositol Phosphates*, (Ed.: A.B. Reitz), American Chemical Society, Washington,DC, **1991**, pp.86-102.

381. C. Liu, S.T. Safrany, S.R. Nahorski, B.V.L. Potter, *Bioorg. Med. Chem. Lett.*, **1992**, *2*, 1523-1528

382. A.M. Cooke, *Ph.D. Thesis*, University of Leicester, **1991**

383. T. Tanaka, S. Tamatsukuri, M. Ikehara, *Tetrahedron Lett.*, **1986**, *27*, 199-202.

384. S.T. Safrany, R.A. Wilcox, C. Liu, D. Dubreuil, B.V.L. Potter, S.R. Nahorski, *Mol. Pharmacol.*, **1993**, *43*, 499-503

385. R. Harrison, H.G.Jr. Fletcher, *J. Org. Chem.*, **1965**, *30*, 2317-2321.

386. B. Kohne, K. Praefcke, G. Mann, *Chimia*, **1988**, *42*, 139-141.

387. J. Fattah, J.M. Twyman, C.M. Dobson, *Magn. Reson. Chem.*, **1992**, *30*, 606-615.

388. H. Oediger, F. Möller, K. Eiter, *Synthesis*, **1972**, 591-598.

389. D.A. Sawyer, *Ph.D. Thesis*, University of Leicester, **1993**.

390. P.T. Hawkins, T.R. Jackson, L.R. Stephens, *Nature (London)*, **1992**, *358*, 157-159.

391. H. Nakanishi, K.A. Brewer, J.H. Exton, *J. Biol. Chem.*, **1993**, *268*, 13-16.

392. J.W. Walker, A.V. Somlyo, Y.E. Goldman, A.P. Somlyo, D.R. Trentham, *Nature (London)*, **1987**, *327*, 249-252.

393. C. Schultz, R.Y. Tsien, *FASEB J.*, **1992**, *6*, A1924.

394. S. Krishnamurthy, H.C. Brown, *J. Am. Chem. Soc.*, **1976**, *98*, 3383-3384

395. W.C. Still, M. Kahn, A. Mitra, *J. Org. Chem.*, **1978**, *43*, 2923-2925.

396. A.I. Lyutik, V.N. Krylova, S.P. Kozlova, B.A. Klyashchitskii, V.I. Shvets, R.P. Estigneeva, E.S. Zhdanovich, *Zh. Obshzh. Khim.*, **1971**, *41*, 2747-2753.