Design and Synthesis of Inositol Phosphate-Based Probes

DPhil Dissertation

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Inositol phosphates play a fundamental role in many intracellular processes. Of particular importance is the role of phosphatidylinositol 3,4,5-trisphosphate [PtdIns(3,4,5)P_3] in the protein kinase B (PKB/Akt) signalling pathway. PtdIns(3,4,5)P_3 recruits PKB to the cell membrane through binding interactions with its pleckstrin homology (PH) domain. In several human cancers, this signalling pathway is upregulated, resulting in increased cell growth and proliferation. In order to investigate the therapeutic potential of the PtdIns(3,4,5)P_3–PH domain binding interaction, it is necessary to develop inositol phosphate-based probes.

This DPhil dissertation highlights the synthesis of a number of derivatives of the PtdIns(3,4,5)P_3 head group – inositol 1,3,4,5-tetrakisphosphate [Ins(1,3,4,5)P_4]. These derivatives incorporated phosphate isosteres at both the 3- and 5-positions of Ins(1,3,4,5)P_4, through the utilisation of novel protection and deprotection strategies. In addition, this dissertation highlights the efficient synthesis of the natural product inositol 1,3-bisphosphate [Ins(1,3)P_2] and our work towards the synthesis of inositol pyrophosphate derivatives.

Declarations

I, Áine Slowey, hereby certify that this dissertation, which is approximately 46,466 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

I was admitted as a probationary research student in October 2008 and as a candidate for the degree of Doctor of Philosophy in January 2010; the higher study for which this is a record was carried out in the University of Oxford between 2008 and 2012.

Date Signature of candidate

I hereby certify that the candidate has fulfilled the conditions of the Regulations appropriate for the degree of Doctor of Philosophy in the University of Oxford and that the candidate is qualified to submit this dissertation in application for that degree.

Date Signature of supervisor

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For my Mum

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Table of Contents

List of Abbreviations

Ac	acetyl
ADP	adenosine 5’-diphosphate
Akt	protein kinase B
ALK2	activin receptor-like kinase-2
All	allyl
aq.	aqueous
Arg	arginine
Asn	asparagine
Asp	aspartic acid
ATP	adenosine 5’-triphosphate
Atz	tetrazolylalanine
Bcr	breakpoint cluster region
BMP	bone morphogenetic protein
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
Bz	benzoyl
c	concentration
CAN	cerium(IV) ammonium nitrate
CE	cholesterol esterase
CK2	casein kinase 2
conc.	concentrated
COSY	correlation spectroscopy
CSA	camphorsulfonic acid

DABCO	1,4-diazabicyclo[2.2.2]octane
DAG	diacylglycerol
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	N,N′-dicyclohexylcarbodiimide
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIBAL	diisobutylaluminium hydride
DIPA	diisopropylamine
DIPEA	diisopropylethylamine
DMA	dimethyl acetamide
DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
ee	enantiomeric excess
EEA1	early endosomal antigen 1
EGF	epidermal growth factor
eq.	equivalent
Et	ethyl
FOP	fibrodysplasia ossificans progressiva
Glu	glutamic acid
His	histidine
HMBC	heteronuclear multiple bond coherence
HMPA	hexamethylphosphoramide
HOBT	hydroxybenzotriazole
HPLC	high performance liquid chromatography

HSQC	heteronuclear single quantum coherence
IGF	insulin-like growth factor
Ins(1,3,4)P_3	inositol 1,3,4-trisphosphate
Ins(1,3,4,5)P_4	inositol 1,3,4,5-tetrakisphosphate
Ins(1,4)P_2	inositol 1,4-bisphosphate
Ins(1,4,5)P_3	inositol 1,4,5-trisphosphate
IP6K	inositol hexakisphosphate kinase
IR	infra red spectroscopy
ITC	isothermal titration calorimetry
KHMDS	potassium bis(trimethylsilyl)amide
LPA	lysophosphatidic acid
Lys	lysine
*m*CPBA	3-chloroperoxybenzoic acid
MD	molecular dynamics
Me	methyl
MHCT	methyl hydrogen 2,3-*O*-cyclohexylidene tartarate
Ms	methanesulfonyl
mTOR	mammalian target of rapamycin
NAD/H	nicotinamide adenine dinucleotide
NF-κB	nuclear factor κ-light-chain-enhancer of activated B cells
NMR	nuclear magnetic resonance
PCC	pyridinium chlorochromate
PDB	protein data bank
PDK	phosphoinositide dependent kinase
PG	protecting group

PH	pleckstrin homology
Ph	phenyl
Phe	phenylalanine
PI3K	phosphoinositide 3-kinase
PKB	protein kinase B
PKC	protein kinase C
PLC	phospholipase C
PMB	4-methoxybenzyl
PP-InsP_4	diphosphoinositol tetrakisphosphate
PP-InsP_5	diphosphoinositol pentakisphosphate
PPIP5K	diphosphoinositol pentakisphosphate kinase
PPL	porcine pancreatic lipase
PPM	parts per million
Pr	propyl
PtdIns	phosphatidylinositol
PtdIns(3,4)P_2	phosphatidylinositol 3,4-bisphosphate
PtdIns(4,5)P_2	phosphatidylinositol 4,5-bisphosphate
PTEN	phosphatase and tensin homologue
PTSA	4-toluenesulfonic acid
RGM	repulsive guidance molecule
RT	room temperature
SEM	β-trimethylsilylethoxymethyl
Ser	serine
TBA	tetrabutylammonium
TBDMS	*tert*-butyldimethylsilyl

TBDPS	*tert*-butyldiphenylsilyl
TCA	trichloroacetimidate
TES	triethylsilyl
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetimidate
TGF-β	transforming growth factor-β
THF	tetrahydrofuran
Thr	threonine
TIPS	triisopropylsilyl
TLC	thin layer chromatography
Ts	4-toluenesulfonyl
TTBP	2,4,6-tri-*tert*-butylpyridine
Tyr	tyrosine
VEGF	vascular endothelial growth factor
VL	variable loop

Chapter 1:

Introduction

1. Introduction

1.1 Inositol Structure and Nomenclature

In 1850, an optically inactive cyclohexane hexol was isolated from heart muscle by Scherer, and named ‘inosit’, from the Greek for muscle or sinew.[1] This name, with the suffix ‘ol’ added in English and French, became a commonly used generic term for all cyclohexane hexols, until the elucidation of the nine possible stereoisomers of inositol by Bouveault in 1894 (Figure 1.1).[2]

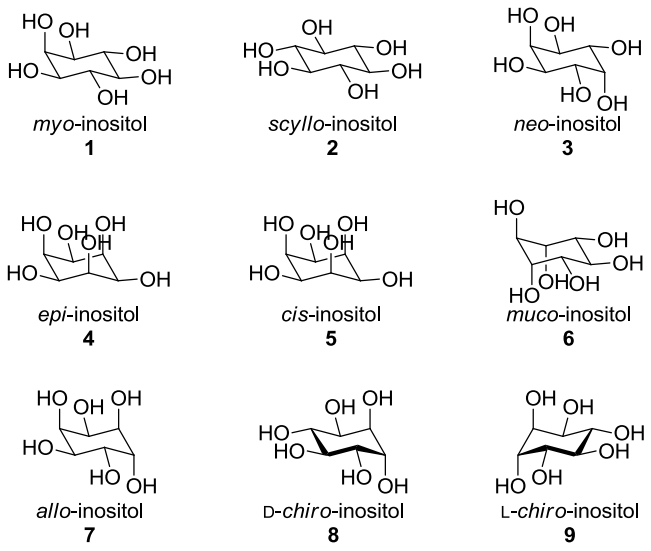


Figure 1.1. The nine stereoisomers of inositol.

Of these nine stereoisomers, only five have been found to naturally occur in biological systems (*myo*-inositol **1**, *scyllo*-inositol **2**, *neo*-inositol **3**, D-(+)-*chiro*-inositol **8** and L-(-)-*chiro*-inositol **9**). Of these, *myo*-inositol **1** is the most abundant, present in either free or combined form in all living species.[3] *myo*-Inositol **1**, with its five equatorial hydroxyl groups and one axial hydroxyl

group, contains an internal plane of symmetry and so is deemed a *meso* compound. Therefore, substitution of a phosphate or any other group on either side of this internal plane will disturb the symmetry of the molecule and render the resulting inositol derivative chiral.

As different substitutions across the plane of symmetry changed the relative priorities of the substituents, similar compounds often interchanged between traditional D- and L-numbering. In order to bring consistency to the numbering of *myo*-inositol derivatives, a relaxation of the lowest-locant rule was recommended, which allowed all biologically related *myo*-inositols to possess D-numbering.[4] However, even with this recommendation, the numbering of the ring carbons in *myo*-inositol can be confusing. Therefore, a useful mnemonic developed by Agranoff can be used to avoid uncertainty (Figure 1.2).[5-7]

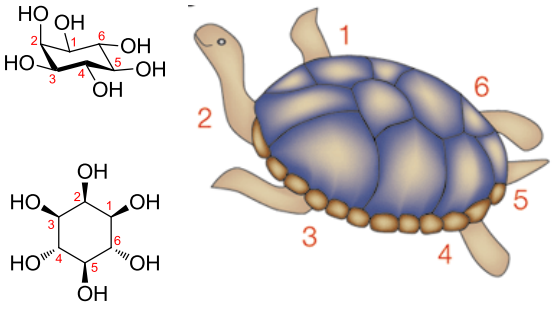


Figure 1.2. Agranoff's turtle indicating the numbering of *myo*-inositol **1**.[5-7] Reproduced with permission © American Society for Biochemistry and Molecular Biology.

With Agranoff's turtle, the turtle's head always represents the axial hydroxyl group at the 2-position. When using the D-numbering system, one counts anticlockwise. Therefore, as the head is always the 2-position, the front left

flipper becomes the 3-position, the rear left flipper becomes the 4-positon, the tail becomes the 5-positon, the rear right flipper becomes the 6-position and the front right flipper becomes the 1-position. If using the L-numbering system, one would count clockwise, and so the opposite numbering would be true.

1.2 History of *myo*-Inositol and the Phosphoinositides

Little progress regarding the purpose or biological functions of *myo*-inositol **1** was made until 1942, when inositol-containing phospholipids were discovered in the brain by Folch.[8] This discovery prompted various investigations into phospholipid signalling, further motivated by the discovery in 1953 that acetylcholine stimulated the incorporation of $^{32}PO_4^{2-}$ into phospholipids of the pigeon pancreas *in vitro*.[9] In a subsequent paper, Hokin and Hokin demonstrated that the specific phospholipids responsible for this effect were phosphatidylinositol (PtdIns) and phosphatidic acid.[10] Therefore, the phenomenon by which stimuli enhance the radioactive labelling of phosphatidylinositol has been generally termed the 'phospholipid effect' or more specifically, the 'PI effect'.

Considering the results of these studies, Durell and Garland proposed that acetylcholine stimulates the hydrolysis of one or more phospholipids, which might be the mechanism by which acetylcholine induces membrane depolarisation and triggers neural impulses.[11] Michell subsequently noted that the receptors that stimulated phosphoinositide turnover in the cell also activated Ca^{2+}-dependent processes inside the cell. This discovery led to

the proposal that receptor-stimulated turnover of inositol phospholipids resulted in increased intracellular Ca^{2+} levels, and could constitute a new signalling pathway.[12] This hypothesis was proven experimentally by Berridge in 1983, with the demonstration that phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] **10** was the principal substrate for the hydrolytic enzyme phospholipase C (PLC), rather than phosphatidylinositol, as previously thought.[13,14] Subsequently, it has been shown that activated phospholipase C cleaves PtdIns(4,5)P_2 **10** to give a water-soluble unit, inositol 1,4,5-triphosphate [Ins(1,4,5)P_3] **11**, and a lipid-soluble diacylglycerol (DAG) unit, which remains in the cell membrane.[15] Both of these molecules act as second messengers inside the cell: Ins(1,4,5)P_3 **11** acts at Ins(1,4,5)P_3 receptors (InsP_3Rs) to modulate the release of Ca^{2+} from intracellular stores (Figure 1.3), while DAG binds to, and activates, protein kinase C, promoting phosphorylation of target proteins in the cell. Additionally, DAG can be further metabolised to produce arachidonic acid, a starting point for the synthesis of prostaglandins.[3]

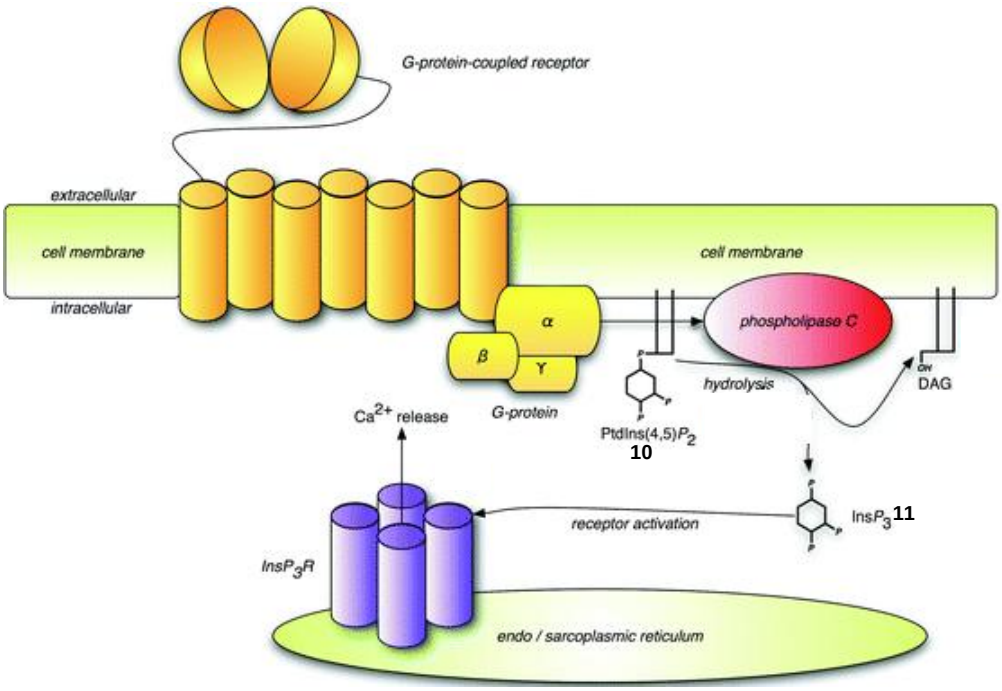


Figure 1.3. Schematic representation of the Ins(1,4,5)P_3 signalling cascade.[16]

The Ins(1,4,5)P_3 Ca^{2+} mobilising signal was found to be terminated by metabolism of Ins(1,4,5)P_3 **11** in one of two ways. A specific 5-phosphatase was found to remove the 5-position phosphate group of Ins(1,4,5)P_3 **11**, affording inositol 1,4-bisphosphate [Ins(1,4)P_2] **12**, which is not active as a Ca^{2+} mobilising agent. Additionally, a specific kinase, phosphoinositides 3-kinase (PI3K), was found to phosphorylate the 3-position of Ins(1,4,5)P_3 **11** to afford inositol 1,3,4,5-tetrakisphosphate [Ins(1,3,4,5)P_4] **13**,[3] the biological activity of which will be discussed in subsequent chapters. However, it was shown that these products were not the only inositol phosphates found to accumulate in cells upon appropriate stimulation.[17] This discovery indicated that PtdIns(4,5)P_2 **10** was at the centre of a complex network of inositol phosphates and phospholipids, strictly mediated by a variety of phospholipases, kinases and phosphatases. Mapping out this complex metabolic pathway required the isolation of a large number of differently

phosphorylated inositols. Ballou *et al.* successfully isolated Ins(1,4,5)P_3 **11** in 1961 by chemical hydrolysis of bovine brain phosphoinositides. However, the isolation process proved difficult and the large quantities required for biological studies were not available through this method.[18-20]

Therefore, the chemical synthesis of this substance and other inositol phosphates became necessary, and has been a focus of interest for the last thirty years. The various synthetic strategies used to synthesise these enantiomerically pure inositol phosphate derivatives will now be discussed. In this dissertation, normal bond thickness is used for racemic compounds or compounds where the stereochemistry is unknown. Solid and broken wedges are used to indicate absolute configuration in enantiomerically pure compounds.

1.3 Synthesis of Inositol Phosphates

As *myo*-inositol **1** is a *meso* compound, substitution of a phosphate or any other group either side of the plane of symmetry will render the derivative chiral. Traditionally, the synthesis of enantiomerically pure inositol phosphates has been achieved through either racemate formation by non-stereoselective attack on the *meso* starting material, followed by resolution to yield the desired pure enantiomers, or synthesis of the pure enantiomers directly from chiral non-racemic precursors without the need for resolution.[21] In the resolution approach, *myo*-inositol **1** is usually the *meso* starting material used, as it is relatively inexpensive and readily available. Hydroxyl groups are then protected with a variety of protecting groups and these are

differentially deprotected to expose the groups destined for phosphorylation. Either before or after phosphorylation, the resulting racemate is resolved into its constituent diastereomers, which can then be purified by chromatography or crystallisation.[21]

1.4 Resolution of Enantiomers

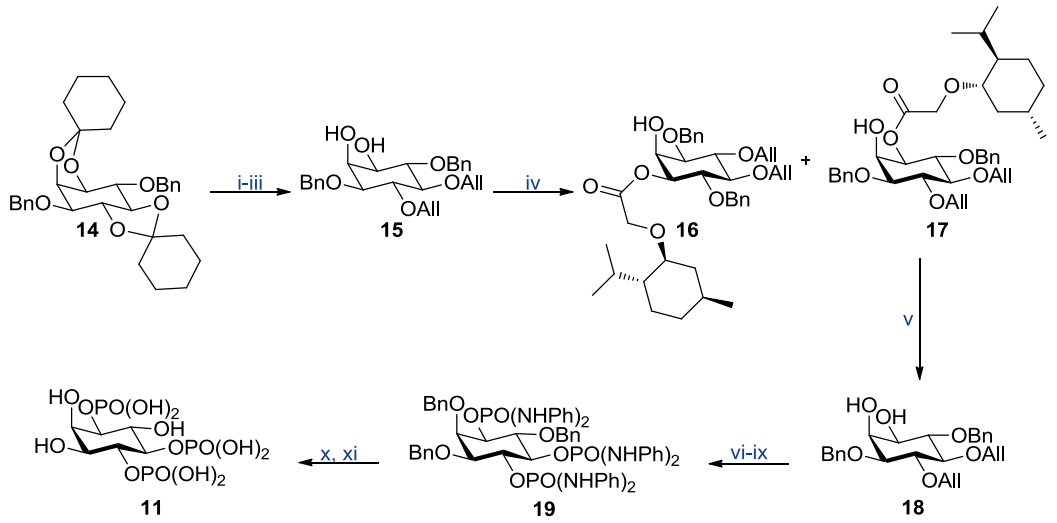
The most widely used resolution method for the synthesis of inositol phosphates involves the conversion of a partially-protected racemic or *meso* inositol derivative into a pair of diastereomeric esters, through reaction with a chiral enantiomerically pure acid or acid derivative. These diastereomeric esters can then be separated by normal chromatographic methods or selective crystallisation, and subsequent hydrolysis can afford the enantiomers of the starting racemic alcohol and the recovered chiral acid.

The diastereomeric purity of the separated esters can be accurately determined by HPLC or X-ray powder diffraction, which can be used to determine the absolute configuration of the individual enantiomeric alcohols derived from them.[3] Multiple variations of this general approach are known in the literature, some of which will now be discussed.

1.4.1 Menthoxyacetyl Esters

In the first published synthesis of Ins(1,4,5)P_3 **11**, Ozaki *et al.* found that they could resolve diol **15** by preparing a diastereomeric mixture of 1-α-menthoxyacetyl esters, synthesised in a regioselective manner by reaction

with the corresponding acid chloride. These diastereomers could then be separated efficiently through crystallisation and subsequent flash chromatography (Scheme 1.1).[22]



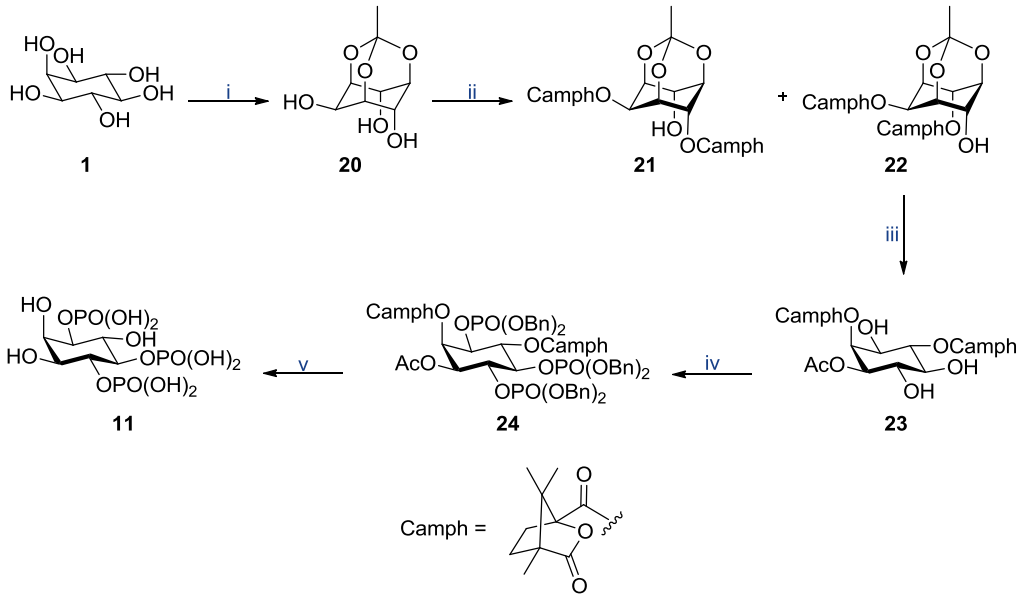
Scheme 1.1. Synthesis of Ins(1,4,5)P_3 **11** by Ozaki *et al.*[22] *Reagents and conditions*: i) TsOH·H_2O, ethylene glycol, $CHCl_3$, RT, 80%; ii) AllBr, NaH, DMF; iii) AcOH, H_2O, 90 °C, 88% over two steps; iv) 1-menthoxyacetylchloride, pyridine, 39%; v) NaOH (aq.), MeOH, 98%; vi) AllBr, NaOH, benzene, reflux, 76%; vii) BnCl, NaH, DMF, 98%; viii) $RhCl(PPh_3)_3$, DABCO then HCl, MeOH, 58%; ix) $(PhNH)_2POCl$, DMAP, pyridine, 41%; x) ^{i}AmONO, AcOH, pyridine; xi) H_2, Pd/C, MeOH.

Selective removal of the 4,5-cyclohexylidene group of starting material **14** was achieved upon treatment with ethylene glycol in the presence of 4-toluenesulfonic acid, subsequent allylation of the hydroxyl groups, and hydrolytic removal of the remaining cyclohexylidene afforded diol **15**. Reaction of diol **15** with (−)-menthoxyacetyl chloride afforded the desired (−)-menthoxyacetyl diastereomeric esters, which were separated by crystallisation. Alkaline hydrolysis of the desired diastereomer **17** then afforded the enantiomerically pure diol **18**. Protecting group manipulation and phosphorylation of the resulting triol by treatment with

dianilidophosphoric chloride gave rise to the desired phosphorylated product **19**, which upon deprotection afforded the natural product Ins(1,4,5)P_3 **11**.[22] This synthesis has subsequently prompted the use of this methodology in the synthesis of various inositol phosphates and phospholipids. [23-27]

1.4.2 (*S*)-Camphanoyl Esters

The use of camphanate esters is probably the most popular method of resolving racemic inositol derivatives, as (−)-camphanic chloride is a stable crystalline reagent, readily available in high enantiomeric purity, and the resulting camphanate esters are often highly crystalline.[3] Garrett *et al.* reported a rapid synthesis of chiral non-racemic Ins(1,4,5)P_3 **11** in five steps from *myo*-inositol, through the use of (−)-camphanate esters (Scheme 1.2). The orthoacetate **20** was prepared from *myo*-inositol **1** through treatment with triethyl orthoacetate and 4-toluenesulfonic acid in DMF. This orthoacetate was then treated with (−)-camphanoyl chloride and a sub-stoichiometric amount of DMAP, to yield the two diastereomeric dicamphanates. The orthoacetate functionality allowed these diesters to be readily crystallised, and so separation was possible without column chromatography. Isolation of the desired diastereomer **22** was achieved by crystallisation from ethyl acetate, which was subjected to acidic hydrolysis conditions to afford regiomeric acetate esters. The desired regioisomer **23** could then be crystallised directly from the mixture, and subsequent phosphorylation afforded the desired phosphate **24**. Deprotection of the protecting groups then afforded the desired natural product Ins(1,4,5)P_3 **11** (Scheme 1.2).[28]



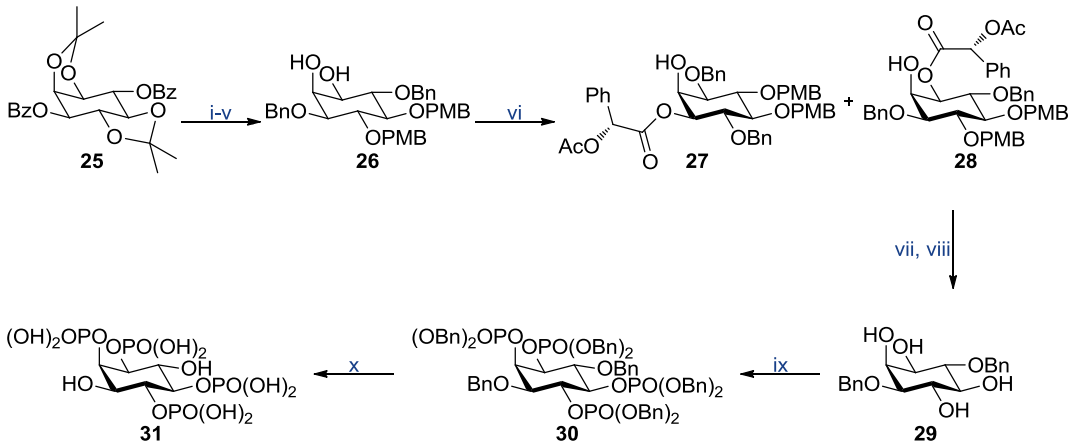
Scheme 1.2. Synthesis of Ins(1,4,5)P_3 **11** by Garrett *et al.*[28] *Reagents and conditions*: i) TsOH·H_2O, triethyl orthoacetate, DMF, 100 °C, 4 h, 65%; ii) (−)-camphanoyl chloride, DMAP, CH_2Cl_2, 0 °C→RT, 2 h, 37%; iii) 80% aq. TFA, 7 d, 25%; iv) (a) $(BnO)_2PN^iPr_2$, 1*H*-tetrazole, CH_2Cl_2, RT, 1 h, (b) *m*CPBA, CH_2Cl_2, −78 °C→RT, 72%; v) (a) H_2, Pd/C, MeOH, H_2O, RT, 12 h, (b) conc. NH_3 (aq.), 60 °C, 4 h.

Garrett's methodology has proved popular, and has been utilised in various synthetic routes to *myo*-inositol phosphates and phospholipids.[29-35] These include the synthesis of membrane-permeant analogues of Ins(3,4,5,6)P_4 by Roemer *et al.*[36] and the synthesis of biotinylated Ins(1,3,4,5)P_4 by Anraku *et al.*[37]

1.4.3 Acetylmandelic Esters

Mills and Potter chose to use the chiral auxiliary (*S*)-(+)-*O*-acetylmandelic acid to resolve the diastereomers formed in their synthesis of Ins(1,2,4,5)P_4 **31** (Scheme 1.3). *O*-Acetylmandelic acid has many benefits, as it is relatively inexpensive, 99% pure by GLC, and unlike enantiomers of the

commonly used camphanic chloride, both *R* and *S* isomers are cheaply available. Prior to its use in this synthetic route, *O*-acetylmandelic acid had not been used for the resolution of inositol derivatives. Fortunately, it was found to react with diol **26** exclusively at the equatorial hydroxyl group, and allowed the separation of the two resulting diastereomers by flash chromatography.[38]



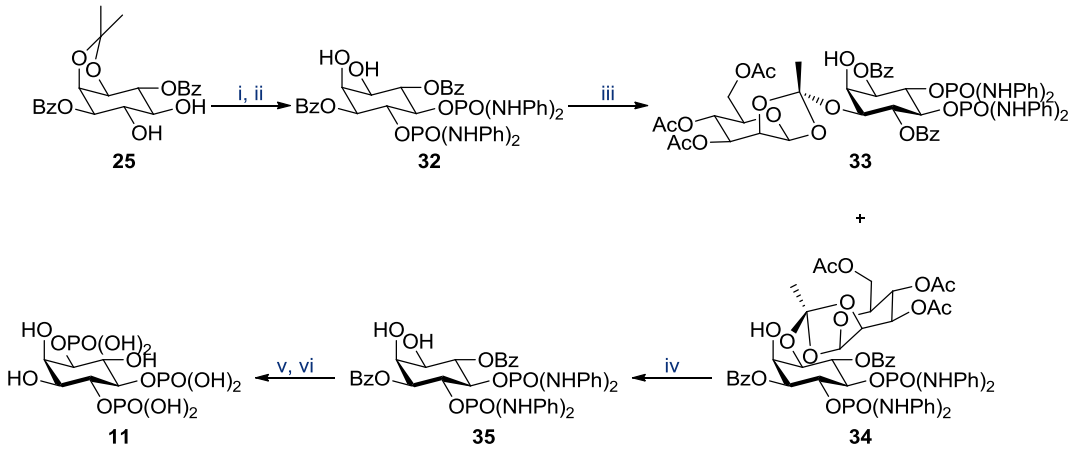
Scheme 1.3. Synthesis of Ins(1,2,4,5)P_4 **31** by Mills *et al.*[38] *Reagents and conditions*: i) NaOH, MeOH, reflux, 30 min, 82%; ii) BnBr, NaH, DMF, 2 h, RT, 91%; iii) ethylene glycol, CH_2Cl_2, TsOH, RT, 80%; iv) PMBCl, NaH, DMF, RT, 2 h, 80%; v) HCl, MeOH, 50 °C, 30 min, 90%; vi) DMAP, DCC, (*S*)-(+)-*O*-acetylmandelic acid, CH_2Cl_2, −20 °C, 37%; vii) NaOH, MeOH, reflux, 30 min, 99%; viii) HCl, EtOH, reflux, 4 h, 86%; ix) (a) $(BnO)_2PN^iPr_2$, 1*H*-tetrazole, CH_2Cl_2, RT, 10 min; (b) *m*CPBA, 0 °C, 30 min, 94%; x) Na, NH_3 (liq.).

Hydrolysis of the chiral auxiliary and the PMB protecting groups afforded alcohol **29**, and subsequent phosphorylation and deprotection afforded Ins(1,2,4,5)P_4 **31**, along with its enantiomer Ins(2,3,5,6)P_4.[38] Mills *et al.* also used this methodology in their synthesis of the enantiomers Ins(2,4,5)P_3 and Ins(2,5,6)P_3.[39] After the success of Mills *et al.* with the use of (*S*)-(+)-*O*-acetylmandelic acid as a chiral auxiliary, this methodology has been used in

a variety of syntheses of inositol phosphates and phospholipids,[40,41] such as the synthesis of caged Ins(1,3,4,5)P_4 and membrane-permeant 3-position phosphorylated phosphoinositides by Dinkel *et al.*[42,43]

1.4.4 Carbohydrate Orthoacetate Ester

Shvets *et al.* proposed the use of derivatives of carbohydrates as resolving agents. Suitably protected inositol racemates could be either transesterified or glycosylated by means of D-mannose orthoacetate or D-glucose orthoacetate, to give diastereomeric intermediates that could be separated by column chromatography or selective crystallisation (Scheme 1.4).[26,44,45]



Scheme 1.4. Synthesis of Ins(1,4,5)P_3 **11** by Stepanov *et al.*[45] *Reagents and conditions*: i) $(PhNH)_2POCl$, pyridine, 96%; ii) HCl (aq.); iii) D-mannose ethylorthoacetate; iv) HCl (aq.), 82%; v) $(PhNH)_2POCl$, pyridine, 45%; vi) (a) ^{i}AmONO, AcOH, pyridine, (b) H_2, Pd/C.

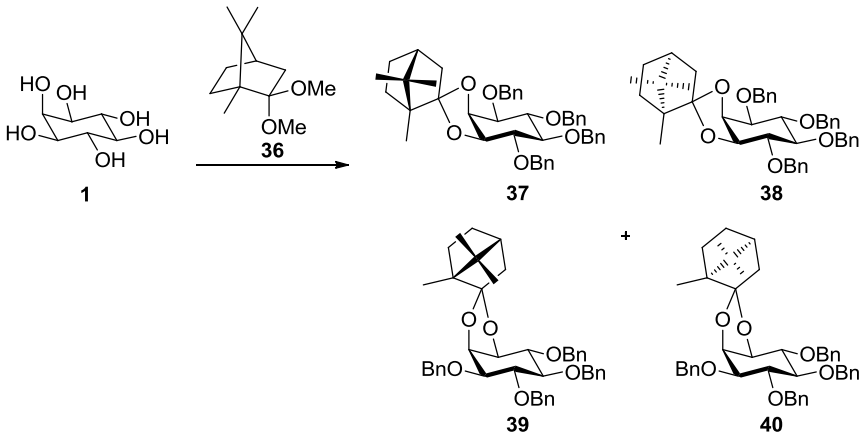
In this synthesis of Ins(1,4,5)P_3, the optical resolution of diol **32** was achieved through transesterification with D-mannose ethylorthoacetate, to

afford diastereomers **33** and **34**, which could be separated by preparative HPLC. Acidic hydrolysis of the D-mannose moiety afforded diol **35**, and phosphorylation with dianilidophosphoryl chloride and subsequent deprotection afforded the desired natural product Ins(1,4,5)P_3 **11**.

1.4.5 Camphor Dimethyl Acetal

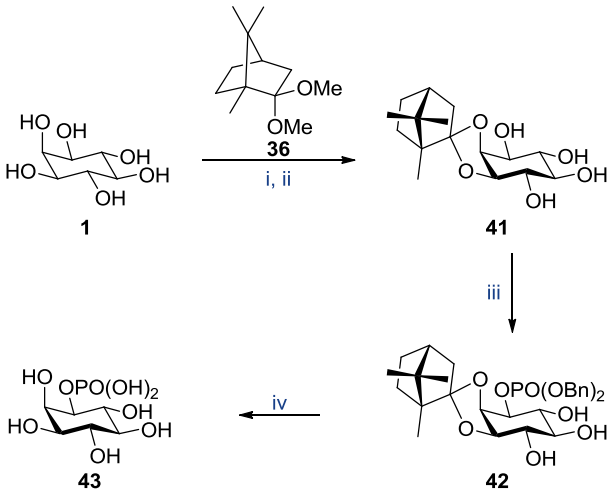
In the synthetic routes previously discussed, the initial reaction often involved the formation of cyclohexylidene or isopropylidene acetals of *myo*-inositol. Therefore, Salamończyk and Pietrusiewicz proposed the formation of D-camphor acetals, as they would simultaneously act as both protecting groups and chiral auxiliaries, eliminating the need for optical resolution at the later stage of synthesis.

These researchers investigated the reaction of *myo*-inositol **1** and D-camphor dimethyl acetal **36**, in order to obtain enantiomerically pure diastereomers of the *meso* starting material. Treatment of *myo*-inositol **1** with a two-fold excess of D-camphor dimethyl acetal **36** gave a complex mixture of isomeric mono and bisacetals, which upon partial hydrolysis, could be converted into four cis monoacetals which could then be isolated and identified individually as their tetra-*O*-benzyl derivatives **37**–**40** (Scheme 1.5).[46]



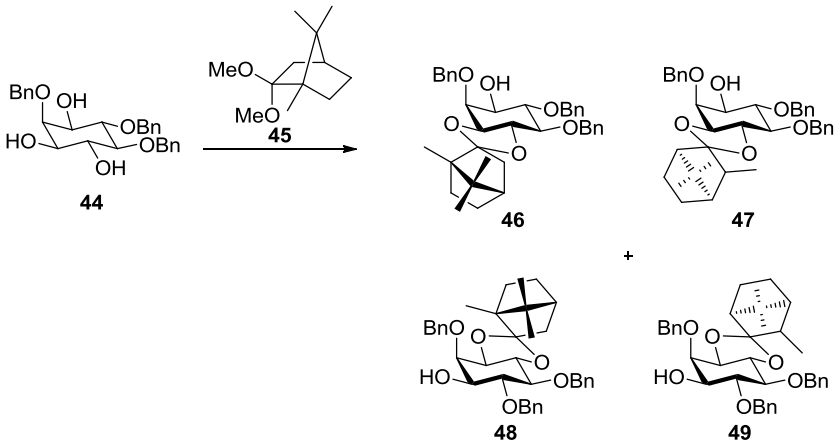
Scheme 1.5. Resolution of inositol diastereomers by formation of D-camphor acetal derivatives.[46]

Salamończyk *et al.* subsequently discovered that under carefully controlled hydrolytic conditions, precipitation-driven equilibration of the original complex mixture leads to the formation of a single product, which precipitates gradually from the reaction mixture (Scheme 1.6). This solid product **41** with acetal formation at the 2- and 3-position hydroxyl groups was obtained in 70% yield by simple filtration and was of sufficient purity to be used in the synthesis of enantiomerically pure inositol phosphates (Scheme 1.6). Selective phosphorylation of alcohol **41** at the 1-position hydroxyl group was then possible through treatment with dibenzyl phosphorchloridate, and isolation of the mono-phosphate afforded the desired product **42** in 23% yield. Deprotection of the benzyl protecting groups and D-camphor acetal afforded natural product D-Ins(1)*P* **43** (Scheme 1.6).[47] This methodology was also used in their expedient synthesis of Ins(1,4,5)P_3 **11** and Ins(1,4)P_2 **12**,[48] and by Bruzik *et al.* in their synthesis of phosphorthioate analogues of phosphatidylinositol phosphates.[49]



Scheme 1.6. Synthesis of D-Ins(1)*P* **43** by Salamończyk *et al.*[47] *Reagents and conditions*: i) DMSO, H_2SO_4; ii) $TsOH \cdot H_2O$, $CHCl_3$, MeOH, H_2O, 70%; iii) $(OBn)_2POCl$, pyridine, 23%; iv) (a) H_2, Pd/C, (b) AcOH (aq.), 88%.

Bruzik *et al.* further developed this methodology in their synthesis of various enantiomerically pure and regioselectively protected *myo*-inositols.[50] This methodology was applied by Painter *et al.* to their system of benzyl-protected inositol derivatives in their synthesis of 3-position phosphorylated *myo*-inositol phospholipids and derivatives (Scheme 1.7).[51]

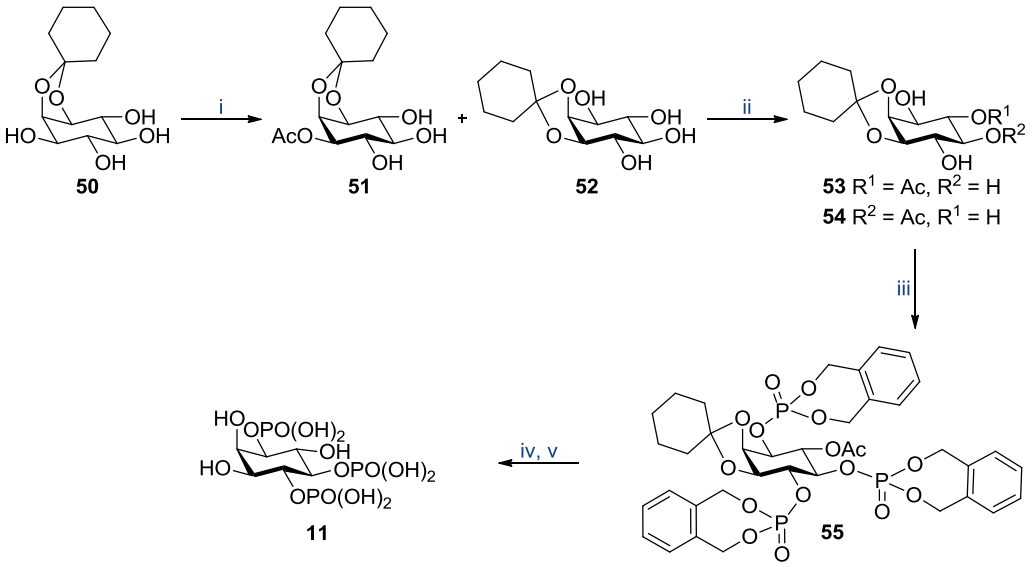


Scheme 1.7. Diastereomeric resolution of benzylated inositol derivative **44** by Painter *et al.*[51]

The desired product **46**, with camphor acetal formation on the 3- and 4-position hydroxyl groups, could be selectively isolated from the undesired diastereomers by silica gel column chromatography. This methodology has since been used by Conway *et al.* in their synthesis of inositol phosphate derivatives.[52-59]

1.4.6 Enzymatic Resolution

Some degree of success has been reported for the use of enzymes in the resolution of protected inositol diastereomers. Ling and Ozaki investigated the enzyme-catalysed enantioselective esterification of racemic cyclohexylidene-inositol derivatives in organic solvents. These researchers found that two lipases from *Pseudomonas species* (Amano Lipase P and Lipase CES) were effective in this resolution in 1,4-dioxane. Both enzymes selectively acetylated the 3-position hydroxyl group of the (−)-enantiomer, to afford the acetylated product **51** and alcohol **52** in 98% enantiomeric excess. This methodology was then applied to the synthesis of enantiomerically pure Ins(1,4,5)P_3 **11** (Scheme 1.8).[60]

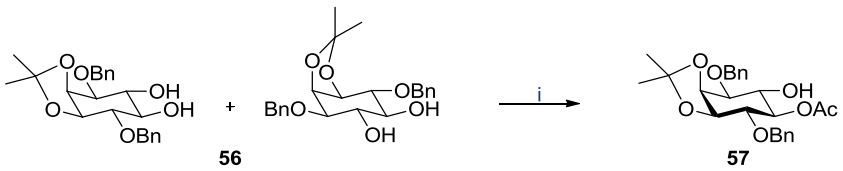


Scheme 1.8. Synthesis of Ins(1,4,5)P_3 **11** by Ling and Ozaki.[60] *Reagents and conditions*: i) Ac_2O, 1,4-dioxane, lipase, 50%; ii) Ac_2O, 4 Å molecular sieves, DMA, 74%; iii) $[C_6H_4(CH_2O)_2PNEt_2]$, 1*H*-tetrazole, CH_2Cl_2 then *m*CPBA, 48%; iv) H_2, Pd/C, H_2O, MeOH; v) conc. NH_4OH (aq.).

Treatment of alcohol **52** with acetic anhydride in the presence of 4 Å molecular sieves afforded a 1:1 mixture of 5- and 6-mono-*O*-acetylated products **53** and **54**. This mixture was subjected to standard phosphorylation conditions to afford both phosphorylated products, which are separable by silica gel column chromatography to afford the desired intermediate **55**. Removal of the remaining protecting groups then afforded the desired natural product Ins(1,4,5)P_3 **11** in >13% yield from *myo*-inositol.[60] This methodology has since been used by Watanabe in the synthesis of various inositol phospholipids.[61]

Cunha *et al.* have also recently reported the kinetic resolution of racemic *myo*-inositol derivatives using the lipase B of *Candida antarctica* (Novozym 435), which converted racemic diols into enantiomerically pure

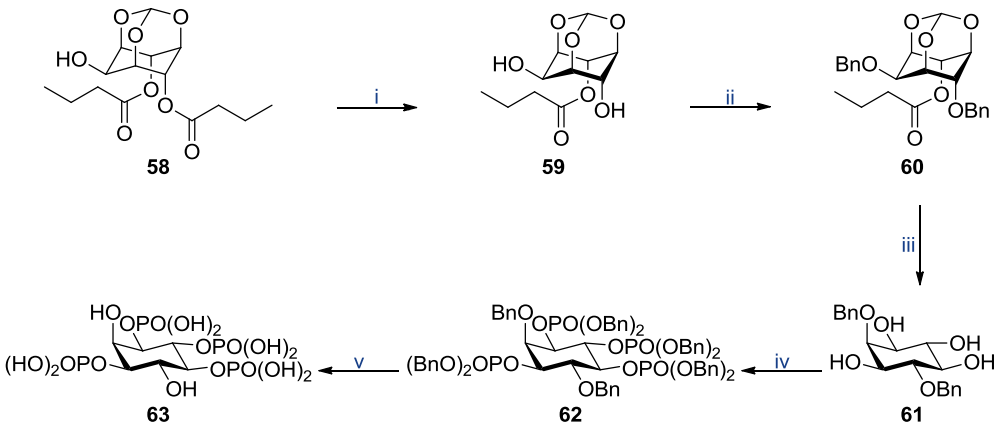
monoacetates.[62-64] This discovery was especially interesting as this type of bulky *myo*-inositol derivative with two types of protecting groups had not previously been employed as a substrate for lipases. The acylation of racemic diol **56** by lipase B of *Candida antarctica* was found to be highly regioselective, occurring exclusively at the 5-position hydroxyl group, to afford monoacetate **57** in 34% yield and >99% enantiomeric excess (Scheme 1.9).[62-64] This methodology was also used by Seo *et al.* in their synthesis of enantiomerically active regioisomers of *myo*-inositol mono- and bisphosphates,[65] and by Watanbe *et al.* in their synthesis of PtdIns(3,4,5)P_4.[66]



56 **57**

Scheme 1.9. Kinetic resolution of **56** by Cunha *et al.*[62] *Reagents and conditions*: i) Novozym 435, EtOAc, 48 h, 30 °C, 34%.

The use of esterases and lipases in the stereo- and regioselective hydrolysis of *meso* or racemic inositol derivatives has also been investigated, allowing the isolation of the enantiomerically active mono-ester. Baudin *et al.* proposed the enantioselective monodeacylation of a *meso*-orthoformate derivative of *myo*-inositol. After screening several orthoformate derivatives, these researchers found, when using dibutyrate **58**, pig liver esterase (PLE) yielded the (−)-butyrate **59** with high enantioselectivity (ee >95%) and in high yields (83%) (Scheme 1.10).[67]

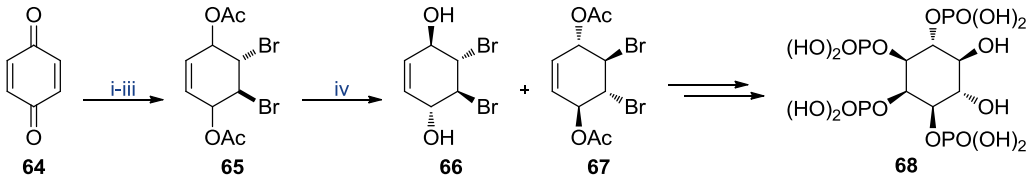


Scheme 1.10. Synthesis of Ins(1,3,4,6)P_4 **63** by Baudin *et al.*[67] *Reagents and conditions*: i) PLE, pH 6.8 buffer, 68%; ii) benzyl trichloroacetimidate, TfOH, CH_2Cl_2, cyclohexane, 62%; iii) NaOH, MeOH; iv) $(BnO)_2PN^iPr_2$, 1*H*-tetrazole, CH_3CN; (b) *m*CPBA, 98%; v) H_2, Pd/C, EtOH.

Subsequent benzylation of this mono-butyrate **59** with benzyl trichloroacetimidate afforded protected intermediate **60**, which upon acid hydrolysis of the orthoester and alkaline deacylation afforded enantiomerically pure triol **61**. Phosphorylation and deprotection then afforded the desired natural product Ins(1,3,5,6)P_4 **63**.[67] Gou *et al.* investigated cyclohexylidene *myo*-inositol derivatives in stereoselective deacylations using similar methodology. Cholesterol esterase (CE) and porcine pancreatic lipase (PPL) were found to deacylate the racemic butyrate derivative in high enantioselectivity, affording enantiomerically pure intermediates to be used in the synthesis of Ins(1,3,4)P_3 and Ins(1,3,4,5)P_4 **13**.[23]

To investigate the synthesis of inositol phosphate derivatives from conduritol B building blocks, Podeschwa *et al.* synthesised racemic diacetoxydibromocyclohex-5-ene in three steps from *p*-benzoquinone. The

desired enantiopure compounds were then obtained by hydrolysis of racemic diacetate with PPL (pig pancreas lipase, type II) in diethyl ether and phosphate buffer at pH 7. Hydrolysis stops after 50% conversion of the starting material, and gave excellent enantioselectivity (> 99% ee) for both the remaining resulting (−)-diol **66** and (+)-acetate **67**, which can be easily separated due to their different solubilities in dichloromethane (Scheme 1.11).[68]



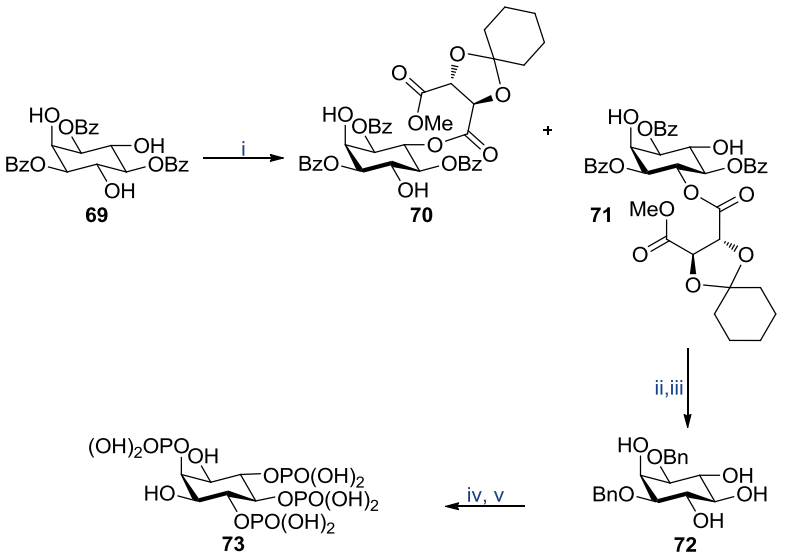
Scheme 1.11. Synthesis of Ins(1,2,3,6)P_4 **68** by Adelt *et al.*[69] *Reagents and conditions*: i) Br_2, $CHCl_3$, 0 °C, 98%; ii) $NaBH_4$, Et_2O, −20 °C→RT, 88%; iii) pyridine, acetic acid, 12 h, 68%; iv) PPL, pH 7 buffer, 4 d, 38% each.

From these chiral non-racemic starting materials, several symmetrical and non-symmetrical conduritol B derivatives can be synthesised, which have been used as starting materials for the synthesis of a number of *myo*-inositol polyphosphates by Adelt and co-workers (Scheme 1.11).[68-73]

1.4.7 Acylation with Tartarate Ester

Ozaki *et al.* found that methanesulfonyl mixed anhydrides of certain tartarate mono-ester derivatives, especially methyl hydrogen 2,3-*O*-cyclohexylidene tartarate (MHCT), were very efficient at enantioselective acylation of *meso*-1,3,5-tribenzoyl *myo*-inositol **69**. Reaction with the mixed anhydride of

MHCT gave rise to a 62% yield of D-1,3,4,5-tetra-acylated *myo*-inositol derivative **71** with 96% diastereomeric excess (Scheme 1.12).[74,75]



Scheme 1.12. Synthesis of Ins(2,4,5,6)P_4 **73** by Ozaki *et al.*[74,75] *Reagents and conditions*: i) MHCT, MsCl, *N*-methylmorpholine, DMAP, THF, 57%; ii) benzyl trichloroacetimidate, TfOH; iii) NaOMe; iv) $(OBn)_2PN^iPr_2$, 1*H*-tetrazole, CH_2Cl_2; v) H_2, Pd/C.

Conversion of this acylated intermediate to Ins(2,4,5,6)P_4 **73** was then possible through hydrolysis of the tartarate ester, phosphorylation and hydrogenolysis of the benzyl and benzoyl protecting groups.

1.5 Synthesis from Chiral Non-Racemic Starting Materials

An alternative to resolution of intermediates is to begin the synthetic sequence with chiral non-racemic starting materials. A number of synthetic routes have been developed using naturally occurring chiral non-racemic inositol, or inositol-like, derivatives as starting materials.

1.5.1 L-Quebrachitol and D-Pinitol

Several synthetic routes to *myo*-inositol phosphates have been reported starting from L-quebrachitol **74** and D-pinitol **75**, *chiro*-inositols available in abundance from plant sources (Figure 1.4).[21,76]

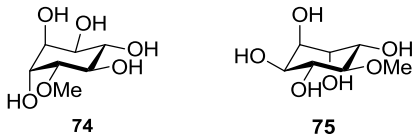
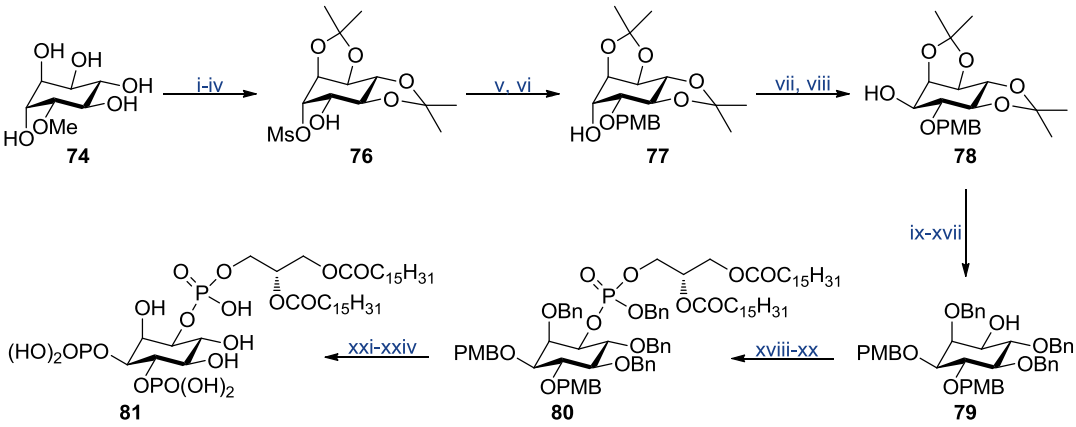


Figure 1.4. Structures of L-quebrachitol (L-2-*O*-methyl-*chiro*-inositol) **74** and D-pinitol (D-5-*O*-methyl-*chiro*-inositol) **75.**

Qiao *et al.* report the use of L-quebrachitol in the preparation of phosphatidylinositol polyphosphates (Scheme 1.13). Four of the five vicinal hydroxyl groups were protected as their acetonides. Subsequent mesylation of the remaining 3-position hydroxyl group, demethylation and concurrent removal of the acetonides with BBr_3 gave rise to a pentol with an intact mesyl group at the 3-position. Separation of the regioisomers formed after reprotection of the hydroxyl groups with 2-methoxypropene afforded intermediate **76**. The isolation of **76** allowed differential protection of the 4-position hydroxyl group, and gave access to PtdIns(3,4)P_2 **81** after inversion of the stereogenic centre at the 3-position. This inversion was achieved through an oxidation/reduction sequence after removal of the mesylate protecting group, to afford alcohol **78**. With the *myo*-inositol skeleton in place, protecting group manipulation allowed selective installation of the alkylated phosphate at the 1-position hydroxyl group, to afford the desired

phosphorylated product **80**. Subsequent phosphorylation and deprotection afforded the desired natural product PtdIns(3,4)P_2 **81** (Scheme 1.13).[77]

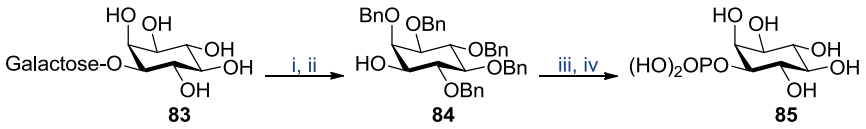


Scheme 1.13. Synthesis of PtdIns(3,4)P_2 **81** by Qiao *et al.*[77] *Reagents and conditions*: i) 2-methoxypropene, CSA, DMF, 60 °C, 4 h; ii) MsCl, NEt_3, CH_2Cl_2, 91% over two steps; iii) BBr_3, CH_2Cl_2, 0 °C, 8 h; iv) 2-methoxypropene, CSA, DMF, 60 °C, 4 h, 65% over two steps; v) NaH, PMBCl, DMF; vi) $LiAlH_4$, THF, 63% over two steps; vii) Swern oxidation; viii) $NaBH_4$, MeOH, 86%; ix) NaH, PMBCl, DMF; x) AcCl, MeOH, CH_2Cl_2, 80% from two steps; xi) BnBr, NaH, DMF; xii) conc. HCl, MeOH, 85% over two steps; xiii) Bu_2SnO, toluene, reflux; xiv) Allyl bromide, CsF, DMF, 18 h, RT; xv) BnBr, NaH, DMF, xvi) $RhCl(PPh_3)_3$, DABCO, EtOH, reflux; xvii) acetone, 1 M HCl, reflux, 82% over five steps; xviii) $BnOP(N^iPr_2)_2$, *i*-Pr_2NH, tetrazole; xix) diacylglycerol, tetrazole; xx) *t*-BuOOH; xxi) DDQ, CH_2Cl_2, H_2O; xxii) $(BnO)_2PN^iPr_2$, 1*H*-tetrazole; xxiii) *t*-BuOOH; xxiv) 20% $Pd(OH)_2$/C, *t*-BuOH.

Qiao *et al.* also applied this methodology to the synthesis of PtdIns(4,5)P_2 **10** and PtdIns(3,4,5)P_3 **82**.[77] Other synthetic routes to inositol phosphate derivatives from L-quebrachitol **74** and D-pinitol **75** have been reported, all involving an oxido-reductive inversion of one of the axial hydroxyl groups of the chiral starting material.[21,78]

1.5.2 Galactinol

The use of galactinol **83** as a chiral non-racemic starting material in the synthesis of inositol phosphates has also been reported. Ballou and Pizer converted this naturally occurring derivative to pentabenzyl inositol **84** (Scheme 1.14).



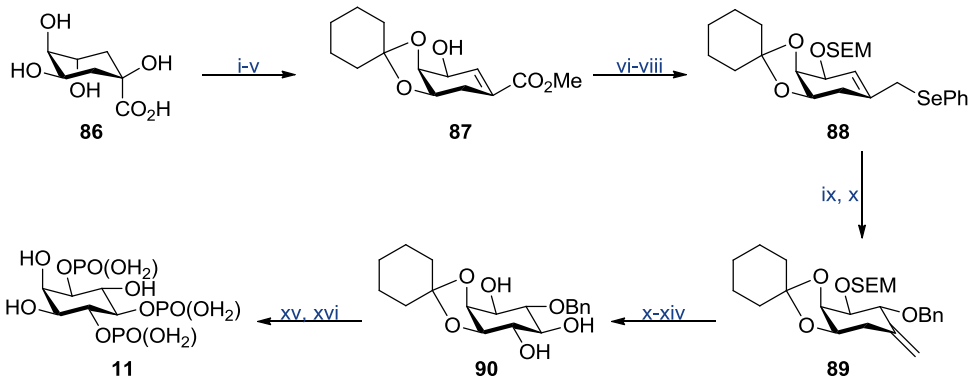
Scheme 1.14. Synthesis of Ins(3)*P* **85** by Ballou *et al.*[79] *Reagents and conditions:* i) (a) BnBr, DMF, Ag_2O; (b) BnCl, KOH, 140 °C; ii) HCl gas, reflux; iii) pyridine, diphenylphosphorochloridate, 25 °C, 18 h; iv) EtOH, Pd/C, H_2 gas, PtO_2.

This derivative **84** then underwent phosphorylation, and subsequent deprotection to afford Ins(3)*P* **85**.[79]

1.5.3 Quinic Acid

Quinic acid has also be used as a chiral non-racemic starting material in the synthesis of inositol phosphates. In the synthesis of Ins(1,4,5)P_3 **11** by Falck and Abdali (Scheme 1.15), quinic acid **86** was converted into a lactone by concurrent lactonisation and ketalisation using cyclohexanone and Amberlite IR-120 resin. The resulting tertiary alcohol underwent mesylation, and sequential lactone methanolysis, pyridinium chlorochromate (PCC) oxidation and triethylamine-induced mesylate elimination generated an

enone, which afforded ester **87** through hydride delivery exclusively from the less hindered β-face (Scheme 1.15).[80]



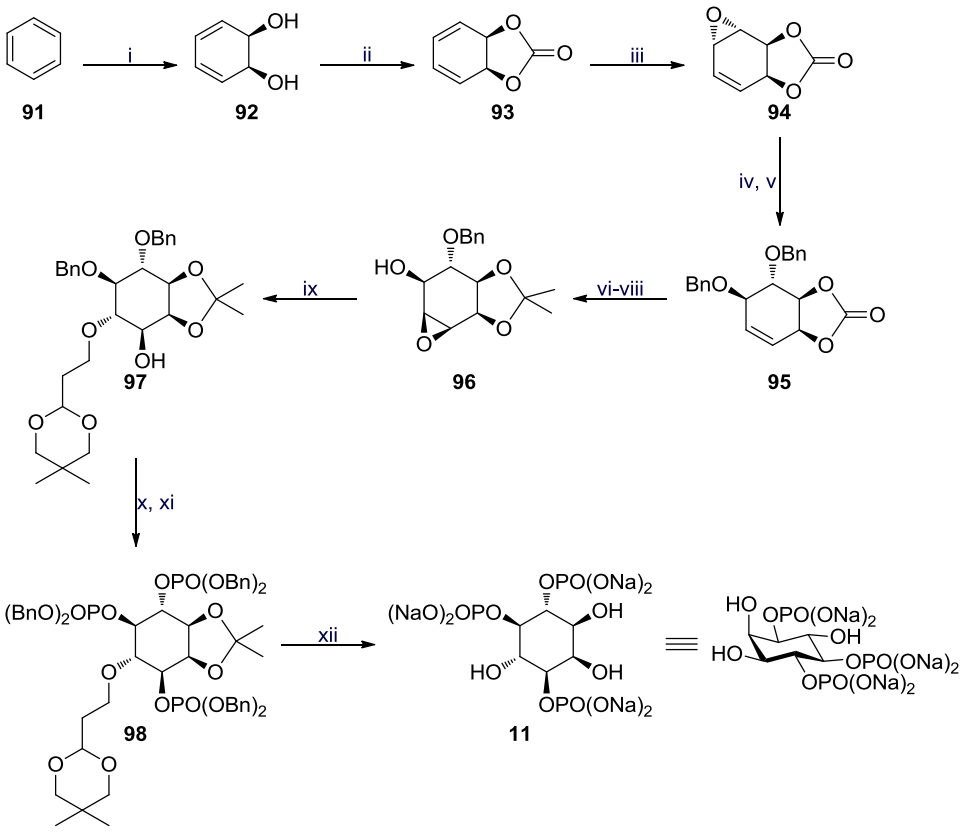
Scheme 1.15. Synthesis of Ins(1,4,5)P_3 **11** by Falck *et al.*[80] *Reagents and conditions*: i) $C_6H_{10}O$, Amberlite IR-120; ii) MsCl, NEt_3, 0 °C, 85% over two steps; iii) NaOMe, MeOH; iv) PCC, NEt_3; v) $NaBH_4$, 0 °C, 80% over three steps; vi) SEMCl, $(^iPr)_2NEt$; vii) DIBAL, toluene, −78 °C; viii) *N*-(PhSe)phth, PBu_3, −15 °C, 42% over three steps; ix) $NaIO_4$, 1,4-dioxane, pH 7 buffer, 0 °C; x) KH, BnBr, 85 % over two steps; xi) O_3, CH_2Cl_2, MeOH, −78 °C; xii) TBDMSOTf, NEt_3, 91% over two steps; xiii) BH_3, THF, 0 °C, H_2O_2; xiv) *n*-Bu_4NF, HMPA, 100 °C, 72% over two steps; xv) KH, THF, 60 °C, $[(BnO)_2PO]_2O$; xvi) H_2, 10% Pd/C, EtOH, AcOH, H_2O, 62% over two steps.

Ester **87** was transformed to phenyl selenide **88** by protection of the hydroxyl group as its β-trimethylsilylethoxymethyl (SEM) ether, reduction of the ester using diisobutylaluminium hydride (DIBAL) and selenylation of the resulting primary alcohol with *N*-(phenylseleno)phthalimide. Stereoselective *in situ* [2,3]-sigmatropic rearrangement of the allylic selenoxide derived from **88** and benzylation of the product evolved **89** as the sole product. Ozonolysis of **89** and conversion to the *tert*-butyldimethylsilyl (TBDMS) enol ether then proceeded with complete regiospecificity. Hydroboration of the enol ether followed by alkaline peroxide oxidation gave the desired *myo*-

inositol skeleton, and deprotection of the SEM and TBDMS protecting groups afforded triol **90**, which upon phosphorylation and deprotection afforded Ins(1,4,5)P_3 **11** as its hexasodium salt.[80]

1.5.4 Oxidation of Benzene

Ley and co-workers decided to introduce the stereogenic centres required for *myo*-inositol derivatives in a sequential fashion, starting with an aromatic precursor. Microbial oxidation of benzene by *Pseudomonas putida* gives rise to *cis*-1,2-dihydroxycyclohexa-3,5-diene **92**, which can be used in the synthesis of various inositol and other cyclitol natural products.[81-84] In the synthesis of Ins(1,4,5)P_3 **11**, this diol **92** is converted into a cyclic epoxycarbonate by treatment with dimethyl carbonate, followed by a stereoselective epoxidation with *m*-chloroperbenzoic acid (*m*CPBA) (Scheme 1.16). The epoxide was then regioselectively opened with benzyl alcohol in the presence of camphorsulfonic acid (CSA), and subsequent benzylation gave desired intermediate **95** with four of the required stereogenic centres established (Scheme 1.16).[82]



Scheme 1.16. Synthesis of Ins(1,4,5)P_3 **11** by Ley *et al.*[82] *Reagents and conditions*: i) *Pseudomonas putida*; ii) $(MeO)_2CO$, MeONa; iii) *m*CPBA, CH_2Cl_2, 0 °C→RT, 95%; iv) BnOH, CSA, CH_2Cl_2, 85%; v) BnBr, Ag_2O, DMF, 81%; vi) NEt_3, MeOH, H_2O; vii) *m*CPBA, pH 8, CH_2Cl_2; viii) 2,2-Dimethoxypropane, CSA, CH_2Cl_2, 72% over three steps; ix) sodium-2β-propoxy-5,5-dimethyl-1,3-dioxane, HMPA, THF, 95 °C, 43%; x) H_2, Pd/C, EtOH; xi) *n*-BuLi, DIPA, tetrabenzylpyrophosphate, THF, 62% over two steps; xii) H_2, Pd/C, EtOH then TFA, H_2O, 74%.

To set up the remaining hydroxyl substituents, they performed hydroxyl group directed epoxidation of the cyclohexene bond. Removal of the carbonate group followed by directed epoxidation and reprotection of the diol as the acetonide afforded epoxide **96** in good yields. Regioselective opening of the epoxide was then achieved using sodium-2β-propoxy-5,5-dimethyl-1,3-dioxane as a hydroxide equivalent, which afforded the desired *myo*-inositol skeleton **97**. Debenzylation and phosphorylation gave rise to

the desired phosphorylated product, and removal of the protecting groups afforded Ins(1,4,5)P_3 **11.**[82]

1.5.5 Peptide Desymmetrisation Catalysts

Miller and co-workers became interested in the application of catalytic asymmetric phosphorylation in the synthesis of inositol derivatives, and so developed two peptide catalysts **99** and **100** (Figure 1.5) to be used in the synthesis of a range of inositol phosphates and phospholipids.[85-90]

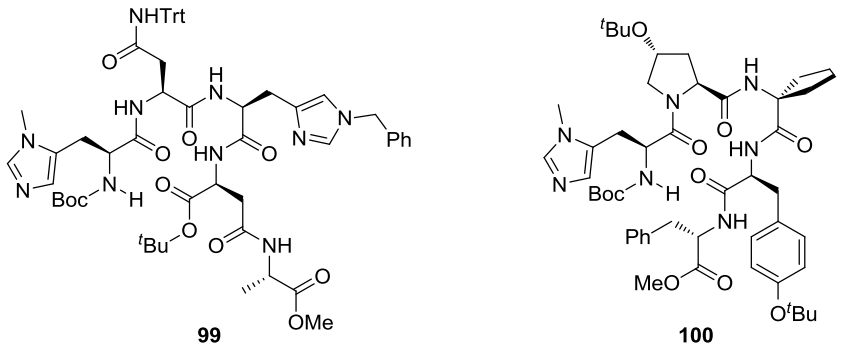
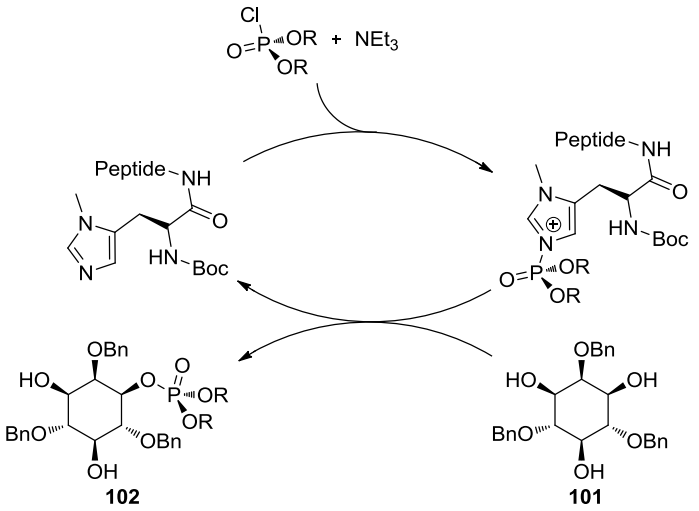


Figure 1.5. Structures of peptide catalysts involved in enantioselective phosphorylation reactions.[85-90]

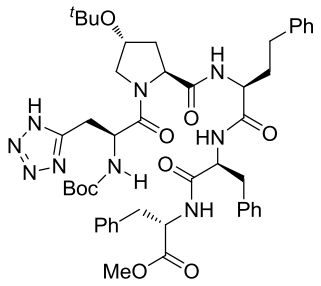
In the presence of diphenyl chlorophosphate and triethylamine, these peptide catalysts were found to phosphorylate the 1- or 3-position hydroxyl groups of triol **101** respectively, with high enantioselectivity and approximately 70% conversion (Scheme 1.17).



Scheme 1.17. Enantioselective phosphorylation of triol **101** by Miller's peptide catalysts **99** and **100.**[85-90]

This phosphorylation reaction was compatible with a range of protecting groups, such as benzyl, allyl, and 4-methoxybenzyl substituents, allowing the synthesis of a range of inositol phosphate derivatives.[85-90]

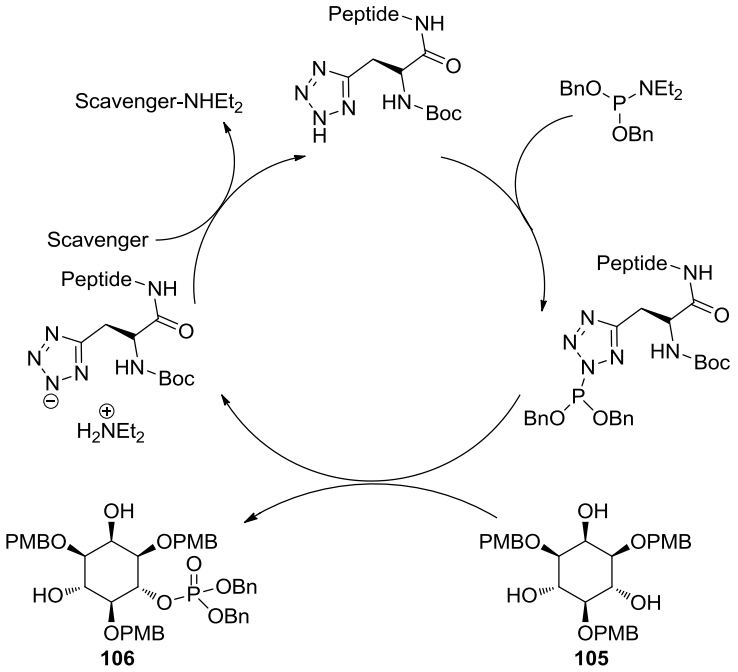
Furthermore, Miller *et al.* recently reported their successful asymmetric phosphorylation through catalytic P(III) phosphoramidite transfer and the enantioselective synthesis of D-Ins(6)*P* **103**.[91] This transformation was achieved through the use of a chiral non-racemic peptide phosphitylation catalyst based on a non-proteinogenic amino acid, tetrazolylalanine (Atz) (Figure 1.6).



104

Figure 1.6. Structure of peptide catalyst **104** involved in enantioselective P(III) phosphoramidite transfer.[91]

Miller and coworkers set out to explore their Atz-based peptide for its ability to distinguish between the 4- and 6-position hydroxyl groups of *myo*-inositol derivatives (Scheme 1.18). These experiments indicated that peptide **104** could catalyse the selective phosphorylation of the 6-position hydroxyl group, and as little as 5 mol% of the catalyst could be employed while maintaining excellent conversion of the product (15:85 *e.r.*).[91] From 1,3,5-tri-*O*-(4-methoxybenzyl)-*myo*-inositol **105**, the peptide-catalysed desymmetrisation proceeded in 71% yield and 15.4:84.6 *e.r.* The minor isomer then underwent kinetic resolution conditions to yield the desired product **106** in >1.9:98.1 *e.r.* (Scheme 1.18). The resulting enantiomerically pure monophosphate was then subjected to deprotonation with sodium metal and liquid ammonia to yield the disodium salt of D-Ins(6)*P* **103**.[91]

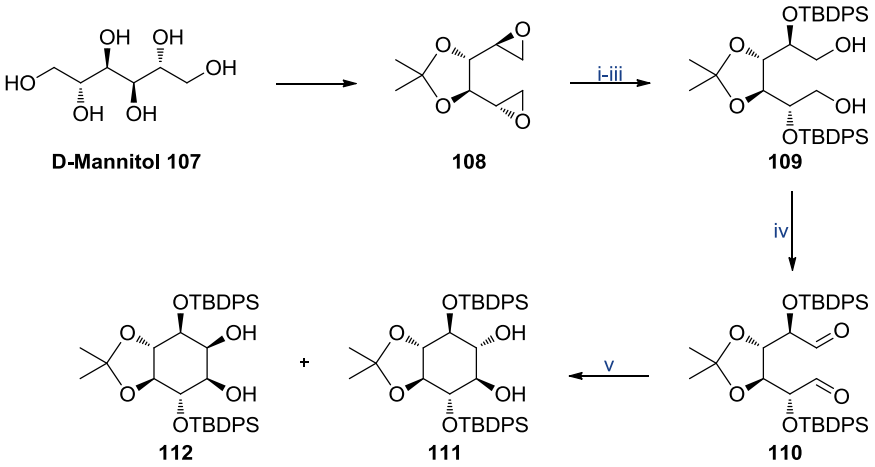


Scheme 1.18. Enantioselective phosphorylation of triol **105** by Miller peptide catalyst **104.**[91]

1.5.6 Pinacol Reaction

Chiara *et al.* proposed that an intramolecular pinacol coupling of a polyoxygenated 1,6-dialdehyde would allow for direct transformation to a *myo*-inositol ring, giving the carbocycle and the vicinal diol in a single step (Scheme 1.19). This observation was based on a highly efficient reductive coupling promoted by the versatile reagent samarium di-iodide, which has been shown to be a mild reagent for the high yielding, stereoselective reductive coupling of dialdehydes.[92] Starting with a C2-symmetric diepoxide prepared from D-mannitol, protecting-group manipulation and Swern oxidation gave dialdehyde **110**, which underwent a pinacol reaction in high yield and good *cis*-selectivity to give the expected non-C2-symmetric *myo*-inositol derivative **112** as the major product. Some undesired *scyllo*-inositol

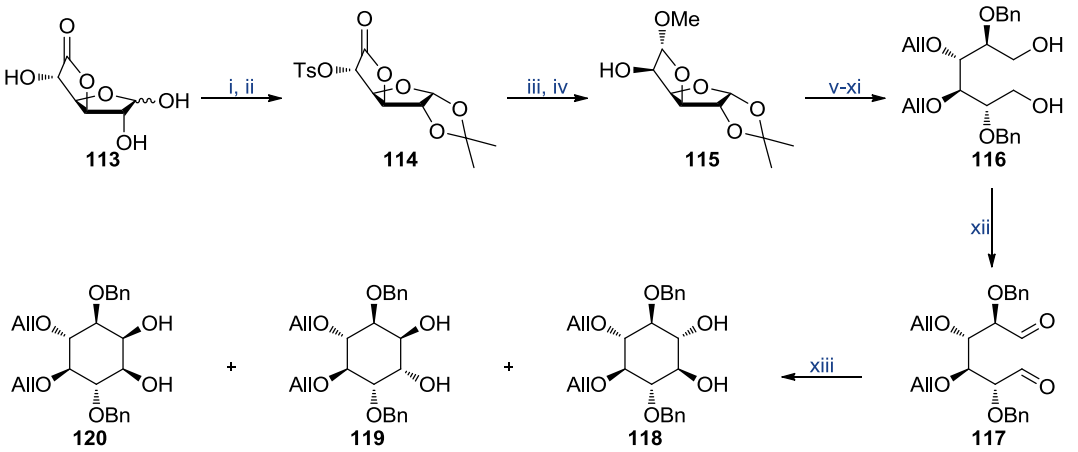
side product **111** was also formed, which could be separated from the *myo*-inositol product by column chromatography (Scheme 1.19).[92]



Scheme 1.19. Synthesis of enantiomerically pure inositol derivatives by Chiara *et al.*[92] *Reagents and conditions*: i) BnOH, NaH, DMF, 0 °C→RT, 24 h, 83%; ii) TBDPSCl, imidazole, DMAP, DMF, 0 °C→RT, 10 h, 96%; iii) H_2, Pd/C, ^{i}PrOH, EtOH, RT, 18 h, 87%; iv) $(COCl)_2$, DMSO, NEt_3, THF, −78 °C→RT, 3 h; v) SmI_2, ^{t}BuOH, THF, −50 °C→RT, 5 h, 86% over two steps.

Ozaki *et al.* also reported the use of a pinacol coupling reaction in their synthesis of *myo*-inositol derivative **120** from glucuronolactone **113** (Scheme 1.20). In this synthesis, optical resolution of the intermediates was unnecessary, but epimerisation of the 5-position (glucose numbering) was required. Conversion of glucuronolactone **113** to the 1,2-acetonide was followed by tosylation of the remaining free hydroxyl group. The lactone **114** was then reduced with diisobutylaluminium hydride (DIBAL) to the lactol, which was treated with potassium carbonate and methanol to invert the configuration at the 5-position through an oxirane intermediate. Protecting-group manipulation afforded the 1,6-diol **116**, which was subjected to Swern

oxidation conditions to give the dialdehyde. Using a low-valent titanium species, cyclisation of dialdehyde **117** gave the *myo*-inositol skeleton **120** as the major product, along with some *chiro*- and *scyllo*- inositol by-products **118** and **119** (Scheme 1.20).[75]

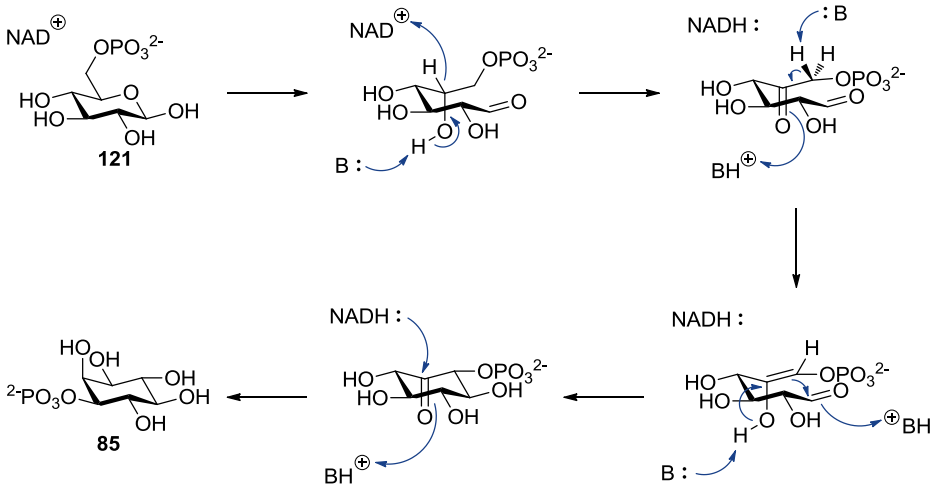


Scheme 1.20. Synthesis of enantiomerically pure *myo*-inositol derivatives **120** by Ozaki *et al.*[75] *Reagents and* conditions: i) TsOH, acetone, 64%; ii) TsCl, pyridine, 88%; iii) $^{i}Bu_2AlH$, 88%; iv) K_2CO_3, MeOH, 87%; v) HCl, MeOH, 100%; vi) BnCl, NaH, 73%; vii) aq. H_2SO_4, 91%; viii) $NaBH_4$, 75%; ix) TrCl, pyridine, 80%; x) AllBr, NaH, 93%; xi) aq. HCl, 91%; xii) $(COCl)_2$, DMSO, NEt_3; xiii) $TiCl_4$, Zn(Cu).

The *myo*-configuration can also be exclusively obtained through treatment of diol **116** with triphenylphosphine and 2,4,5-triiodoimidazole to give the olefin, which can then be oxidised with OsO_4 to afford the *myo*-inositol derivative in high yields. This intermediate can undergo protecting group manipulation, phosphorylation and deprotection to afford a wide range of *myo*-inositol phosphate derivatives.

1.5.7 Ferrier Rearrangement

Bender *et al.* were inspired by the biosynthetic conversion of glucose-6-phosphate to *myo*-inositol 3-phosphate by the enzyme *myo*-inositol-3-phosphate synthase, as the synthetic route contains an interesting sequence of chemical transformations, including a stereoselective intramolecular aldol reaction (Scheme 1.21).[93,94]

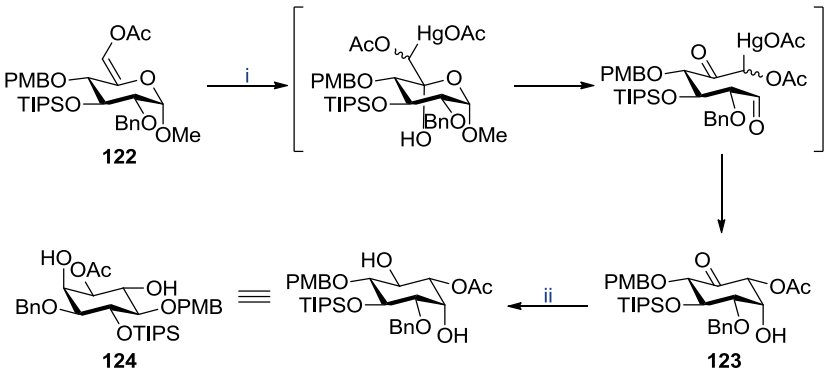


Scheme 1.21. Biosynthetic route to Ins(3)*P* **85** from glucose-6-phosphate **121**.[93]

At this time, the Ferrier reaction was well-established for the stereoselective conversion of unsubstituted enol ethers to the corresponding 6-deoxyinososes.[95] Bender *et al.* proposed that the Ferrier rearrangement could be used to generate a mercury enolate that could undergo the desired carbocyclisation process to provide the *myo*-inositol skeleton (Scheme 1.22). However, the absence of any literature precedent for the use of terminally substituted enol ethers in this reaction precluded any prediction

as to the stereochemical outcome of the intramolecular aldol reaction, particularly with respect to the stereogenic centre at the 2-position.[93]

The desired enol acetate for the Ferrier rearrangement proved to be readily accessible. The desired alcohol was prepared by conventional methods from methyl α-D-glucopyranoside, and was oxidised to the aldehyde, which allowed conversion to the enol acetate **122** with high selectivity for the (*Z*)-isomer. Oxymercuration resulted in the formation of an organomercurial intermediate, which did not cyclise to the product until excess chloride was added. When workup was performed immediately after chloride addition, diastereomeric α-mercurio ketones were formed from the (*Z*)-and (*E*)-enol acetates. However, both enol acetates appeared to give the axial 2-position hydroxyl group and equatorial 1-position acetate with strong diastereoselectivity. The desired product **123** was then easily isolated by chromatography as the pure (>97%) diastereomer. To complete the biomimetic sequence, product **123** was then efficiently converted to the enantiomerically pure *myo*-inositol derivative **124** by stereoselective hydroxyl-directed hydride reduction (Scheme 1.22).[93]

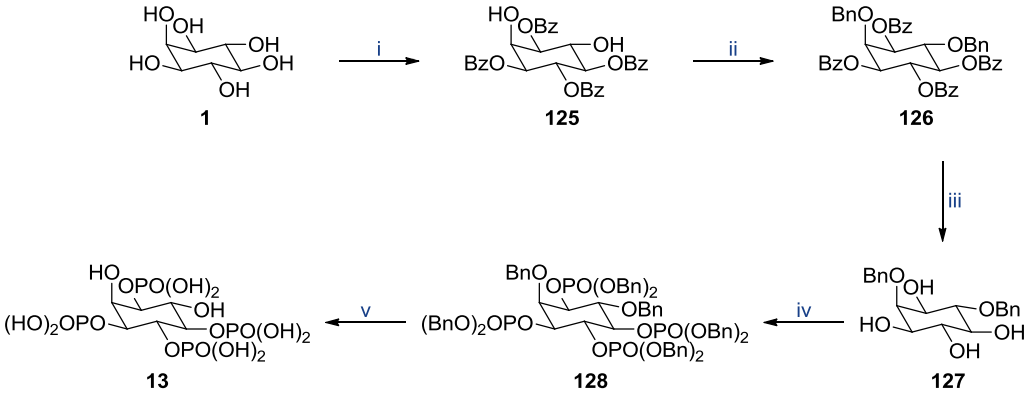


Scheme 1.22. Reaction mechanism of Ferrier rearrangement to afford *myo*-inositol derivative **125**.[93,96] *Reagents and conditions*: i) (a) $Hg(OAc)_2$, acetone/H_2O (3:2), (b) NaCl(aq), 35%; ii) $Me_4NBH(OAc)_3$, AcOH, MeCN, 89%.

This methodology has since been employed in the synthesis of a variety of inositol phosphates and phospholipids.[96-101]

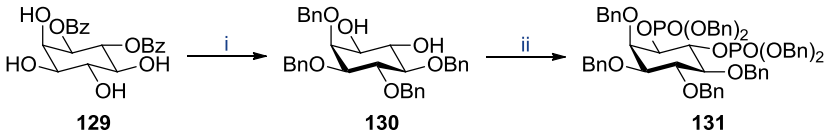
1.6 Alternative Methods to Synthesise Chiral Non-Racemic Inositol Phosphates

Some alternative methods for the synthesis of inositol phosphates have also been reported. Ozaki and co-workers found that when *myo*-inositol **1** was reacted with 3.5 equivalents of benzoyl chloride, a 37% yield of 1,3,4,5-tetrabenzoyl-*myo*-inositol **125** was obtained, along with lesser yields of seven other partially-protected derivatives. Benzylation with benzyl trichloroacetimidate and cleavage of the benzoyl groups resulted in the desired alcohol, and subsequent phosphorylation and hydrogenolysis afforded the desired natural product Ins(1,3,4,5)P_4 **13** (Scheme 1.23).[75]



Scheme 1.23. Synthesis of Ins(1,3,4,5)P_4 **13** by Ozaki *et al.*[75] *Reagents and* conditions: i) BzCl (3.5 eq.), 37%; ii) benzyl trichloroacetimidate, TfOH; iii) NaOMe; iv) (a) $(OBn)_2PN^iPr_2$, 1*H*-tetrazole, (b) *m*CPBA; v) H_2, 10% Pd/C.

Chung and co-workers have also synthesised a range of *myo*-inositol phosphates, through selective benzoylation of several *myo*-inositol derivatives and then subjecting these derivatives to benzoyl group migration conditions. The resulting mixture of regioisomers can then be separated by silica gel column chromatography, fractional crystallisation or preparative HPLC. The separated regioisomers can undergo protecting group manipulation, phosphorylation and deprotection to afford a range of inositol phosphates (Scheme 1.24).[102-107]



Scheme 1.24. Synthesis of Ins(1,6)P_2 derivative **131** by Chung *et al.*[106] *Reagents and* conditions: i) (a) BnBr, AgOTf, 2.6-di-t-butyl-pyridine; (b) NaOH, MeOH, reflux; ii) (a) $(OBn)_2PN^iPr_2$, 1*H*-tetrazole, (b) 30% H_2O_2.

Ins(1,2,6)P_3 can also be prepared with high enantiomeric purity (>98% ee) in large quantities by fermentation of phytic acid using baker's yeast.[108]

1.7 Summary

The diverse and complex cellular functions of inositol phosphates and phospholipids prompted the need for large-scale chemical preparation of these derivatives. Consequently, a wide variety of methods have been developed in the search for short and efficient synthetic routes to these enantiomerically pure products, a selection of which have been discussed.

Chapter 2:

Synthesis of 3-position Ins(1,3,4,5)P_4 derivatives

2. Synthesis of 3-position Ins(1,3,4,5)P_4 derivatives

2.1. Introduction to PKB Signalling Pathway

Protein kinase B (PKB or Akt) is a serine/threonine kinase which is involved in a multitude of downstream cellular responses. There are three known isoforms of PKB (PKBα, PKBβ and PKBγ), all of which have high sequence homology. All of these isoforms contain a pleckstrin homology (PH) domain, a kinase domain and a short carboxy terminal domain.[109]

PKB plays a pivotal role in cell growth, proliferation and survival, through its involvement in the phosphoinositide 3-kinase (PI3K) signalling pathway.[109] This pathway commences with the stimulation of cells by appropriate growth factors, such as epidermal growth factor (EGF), insulin-like growth factors (IGFs) and vascular endothelial growth factor (VEGF).[110] This stimulation leads to the recruitment of PI3K to the plasma membrane, where it phosphorylates PtdIns(4,5)P_2 **10** at its 3-position to generate PtdIns(3,4,5)P_3 **82** (Figure 2.1).[111]

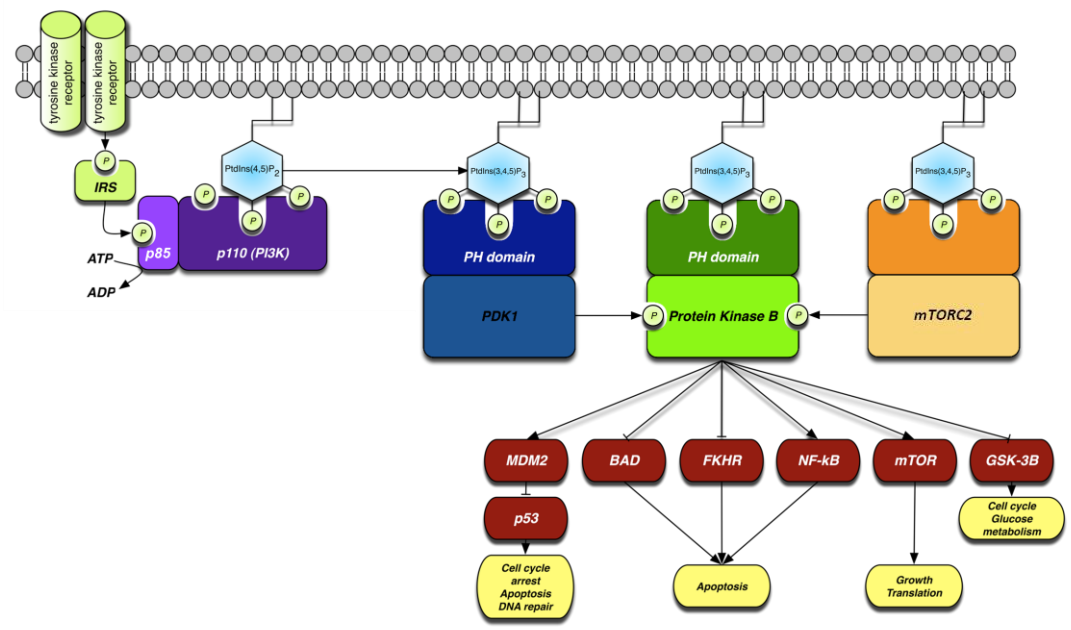


Figure 2.1. Outline of the PI3K signalling pathway and PKB activation of downstream events.[109,112,113]

PKB is then recruited to the plasma membrane by virtue of the interaction of its PH domain with PtdIns(3,4,5)P_3 **82**. Crystallographic studies of the isolated PH domain indicate that binding of PtdIns(3,4,5)P_3 **82** disrupts the hydrogen bonding network present in the ligand-binding pocket, resulting in a significant conformational change.[114]

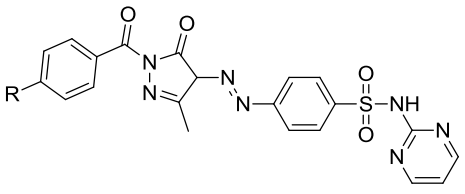
This conformational change exposes the activation loop of PKB, which allows phosphorylation of Thr308 by phosphoinositide-dependent kinase 1 (PDK1).[114,115] A second phosphorylation of Ser473 by mTORC2 activates PKB, which subsequently interacts with proteins such as the mammalian target of rapamycin (mTOR) and NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), leading to cell growth and proliferation through a multitude of downstream signalling effects (Figure 2.1).[113,115,116]

2.2. Anticancer Therapeutics Targeting PKB

The PKB signalling pathway experiences genomic aberrations such as mutation, amplification and rearrangement more frequently than any other pathway in human cancer, resulting in uncontrolled cell growth.[112,117] This signalling pathway is, therefore, an attractive target for the development of novel anticancer agents.

The central kinase domain of PKB became the most obvious target for the development of small-molecule inhibitors, based on the success of similar kinase domain inhibitors of c-kit and Bcr/Abl.[117] However, due to the high sequence homology in the ATP-binding pocket among several protein kinases in the AGC kinase family, these compounds displayed promiscuity, which led to off-target effects and toxicity.[117]

As the sequence identity of different PH domains is usually less than 30%, targeting the PKB PH domain offers the possibility of developing selective inhibitors and avoiding these off-target effects.[117] Several small-molecule PKB PH domain inhibitors have been identified through *in silico* structure-based drug design (Figure 2.2).[117-119]



132 R = Cl
133 R = H
134 R = *t*-Bu
135 R = NH_2
136 R = OH

Figure 2.2. Structures of PKB PH domain inhibitors.[118]

These compounds, based upon a central pyrimidinyl-benzenesulfonamide core showed promising *in vitro* activity, exhibiting PH domain-binding selectivity for PKB compared to other PH domain-containing proteins. However, *in vivo*, these compounds failed to achieve blood concentrations required to inhibit PKB, and so did not exhibit anti-tumour activity.[117]

Another approach to targeting the PKB PH domain involved the development of structurally modified phosphatidylinositol ether lipid analogues.[110,120,121] As the PH domain of PKB binds to PtdIns(3,4,5)P_3 **82** upon phosphorylation of the 3-position hydroxyl group of PtdIns(4,5)P_2 **10**,[122,123] Kozikowski and co-workers developed PtdIns analogues such as **137** lacking this hydroxyl group, thus preventing 3-position phosphorylation (Figure 2.3).[120,121]

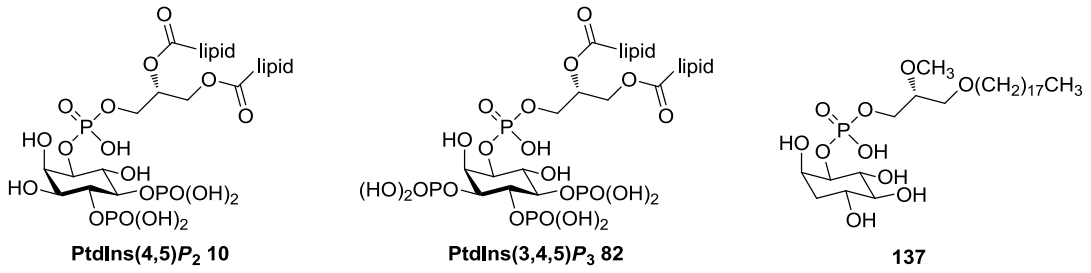


Figure 2.3. Structures of PtdIns(4,5)P_2, PtdIns(3,4,5)P_3 and Kozikowski analogue **137**.[120,121]

These compounds were found to inhibit PKB activation and phosphorylation of several downstream substrates, leading to a 20-30 fold increase of apoptosis in cancer cell lines with PKB over-expression.[110,117] However, it has yet to be determined if these compounds are effective *in vivo*, where they would presumably undergo extensive phosphorylation.

2.3. X-Ray Crystal Structure of PKB PH Domain

As the interaction between the PH domain of PKB and the 3-position phosphate of PtdIns(3,4,5)P_3 **82** is so crucial for PKB activation, we proposed to further probe the specific binding interactions present at this position. Analysis of the X-ray crystal structures obtained by Milburn *et al.* of the *apo* PKB PH domain and the PKB PH domain bound to Ins(1,3,4,5)P_4 **13,** the headgroup of PtdIns(3,4,5)P_3 **82**, provides structural evidence for the role of the 3-position phosphate in the conformational change observed in PKB (Figure 2.4 and Figure 2.5).[114]

Upon binding of Ins(1,3,4,5)P_4 **13**, Lys14 in the ligand-binding pocket moves 1.2 Å to bind the 3-position and 4-position phosphates, while Arg23 also moves 6.2 Å to make contact with the 1-position and 3-position phosphates (Figure 2.4).

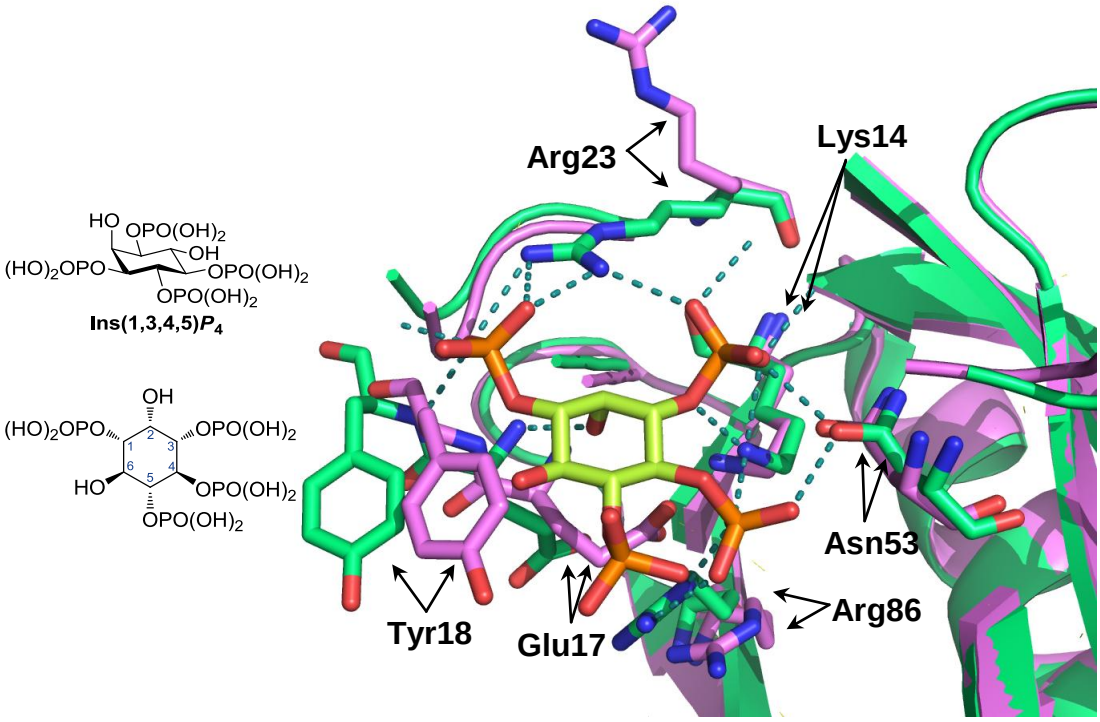


Figure 2.4. X-Ray crystal structures of *apo* PKB PH domain (lilac, PDB code: 1UNP) and PKB PH domain-Ins(1,3,4,5)P_4 complex (green, PDB code: 1UNQ), with important residues highlighted.[114]

These interactions, along with the 2.3 Å movement of Arg86 towards the 4-position phosphate, the repulsion of Glu17 from the ligand-binding site by the negatively charged phosphoinositides, and the 5.0 Å movement of Tyr18 to avoid steric interactions with the 5-position phosphate, contribute to the dramatic conformational change observed in the PKB PH domain upon binding of Ins(1,3,4,5)P_4 **13** (Figure 2.5). It is assumed that this conformational change occurs *in vivo* upon binding of PtdIns(3,4,5)P_3 to the PKB PH domain.

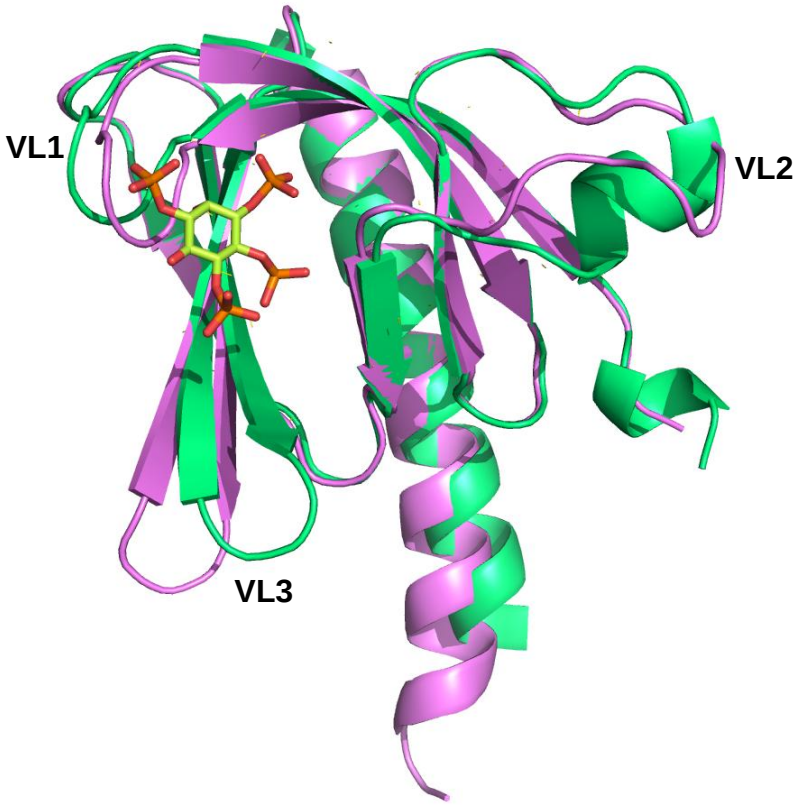


Figure 2.5. X-Ray crystal structure of *apo* PKB PH domain (lilac) and PKB PH domain-Ins(1,3,4,5)P_4 complex (green), showing ligand-induced conformational change.[114]

2.4. Synthetic Aims

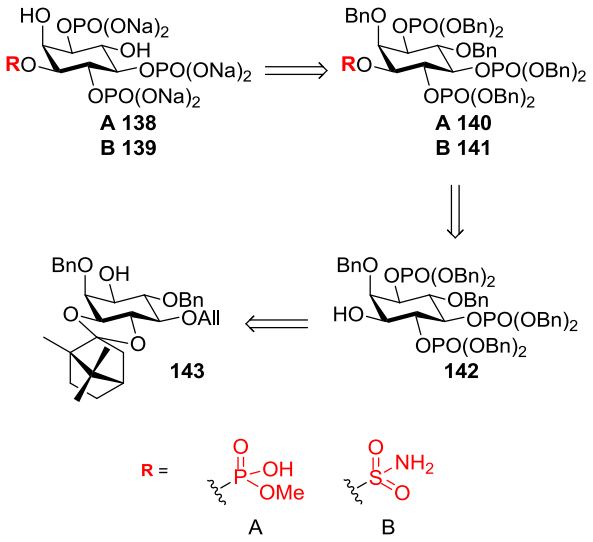
These crystal structures reveal the important hydrogen bonding interactions between the 3-position phosphate and Asn53, Arg23 and Lys14 in the ligand-binding pocket (Figure 2.4). In order to determine the role of these specific interactions in the activation of PKB, we proposed the installation of phosphate isosteres possessing differing hydrogen bonding and salt-bridge forming capabilities at this position.

As the PH domain of PKB binds to PtdIns(3,4,5)P_3 **82** and Ins(1,3,4,5)P_4 **13** with equal affinity *in vitro*,[124] we proposed the synthesis of analogues of

Ins(1,3,4,5)P_4 modified at the 3-position phosphate. In particular, we focused on the synthesis of sulfamate moiety **139**, which would allow increased hydrogen bonding interactions at this position, and methyl phosphate ester **138**, which would be mono-charged at physiological pH and so have decreased hydrogen bonding interactions and altered electrostatic interactions.

2.5. Retrosynthetic Analysis

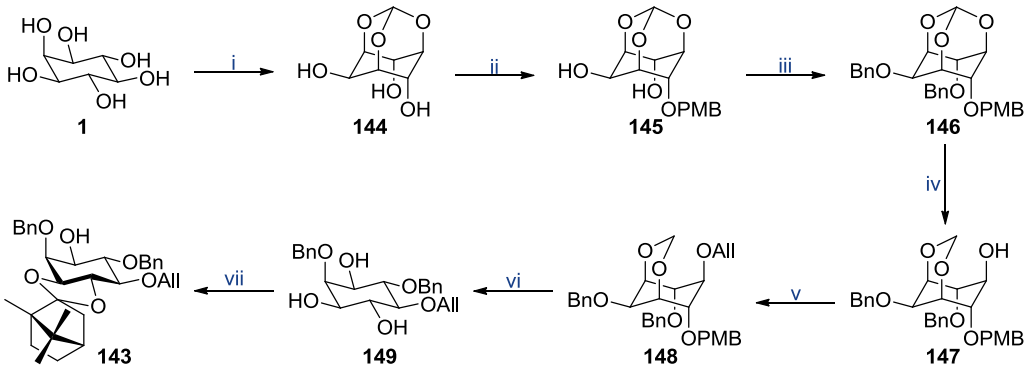
Our retrosynthetic analysis proposed a synthetic route to the desired intermediates **138** and **139**, which involved the initial synthesis of a key chiral, non-racemic intermediate **143** (Scheme 2.1).



Scheme 2.1. Retrosynthetic analysis for synthesis of 3-position derivatives **138** and **139**.

The synthetic route to this key intermediate **143** is well established within the Conway group, based on a synthetic route reported by Painter *et al.*[51,52] This route uses the relatively inexpensive starting material *myo*-inositol **1**,

and employs (*S*)-camphor as a chiral auxiliary in the resolution of the inositol intermediates (Scheme 2.2).



Scheme 2.2. Synthetic route to key intermediate **143**. *Reagents and conditions*: i) $(EtO)_3CH$ (3.0 eq.), $TsOH{\cdot}H_2O$ (0.3 eq.), DMF, 100 °C, 21 h, 71%; ii) NaH (1.1 eq.), PMBCl (1.1 eq.), DMF, 0 °C→RT, 24 h, 78%; iii) NaH (2.5 eq.), BnBr (2.5 eq.), DMF, 0 °C→RT, 24 h, 94%; iv) DIBAL (2.5 eq.), CH_2Cl_2, 0 °C→RT, 4 h, 95%; v) NaH (2.0 eq.), AllBr (2.0 eq.), DMF, 0 °C→RT, 24 h, 91%; vi) conc. HCl, MeOH, reflux, 5 h, 82%; vii) $TsOH{\cdot}H_2O$ (0.3 eq.), 150 (2.5 eq.), CH_2Cl_2, reflux, 16 h, 15%.

This synthetic route commences with the protection of *myo*-inositol **1** with triethylorthoformate, using synthetic and purification conditions reported by Billington *et al.*[125] Recrystallisation and flash column chromatography afforded the desired inositol orthoformate **144** in 71% yield.

This orthoformate **144** then underwent regioselective alkylation, as treatment with sodium hydride and 4-methoxybenzyl chloride (PMBCl) afforded intermediate **145** in 78% yield. The selectivity of this reaction is proposed to arise from the deprotonation of one of the hindered axial hydroxyl groups in preference to the less hindered equatorial hydroxyl group, due to the ability of the sodium to chelate between the two axial

hydroxyl groups (Figure 2.6).[125] It should be noted that this reaction is regioselective and not stereoselective, as mono-alkylation breaks the plane of symmetry of *meso* inositol orthoformate **144** and so produces PMB-protected inositol **145** as a racemate.

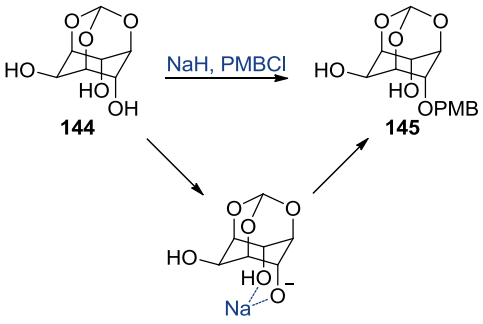


Figure 2.6. Regioselective PMB ether formation via favourable axial alkoxide formation.[125]

Perbenzylation of **145** by treatment with sodium hydride and benzyl bromide then gave the desired fully protected intermediate **146** in 94% yield. Treatment of intermediate **146** with diisobutylaluminium hydride (DIBAL) then selectively afforded alcohol **147** in excellent yield (Scheme 2.2).

To gain insight into the exceptional selectivity of this DIBAL reduction, Gilbert and co-workers investigated the reaction mechanism using deuterium-labelling experiments, where it was found that at least two equivalents of DIBAL are required for the reaction to proceed.[52,126,127] The first equivalent of DIBAL is thought to act as a Lewis acid, and coordinates preferentially to the most accessible oxygen of the orthoformate, the 5-position oxygen. Coordination to the 1-position or 3-position oxygens is unfavourable due to steric interactions with the equatorial benzyl group at the 2-position (Figure 2.7).

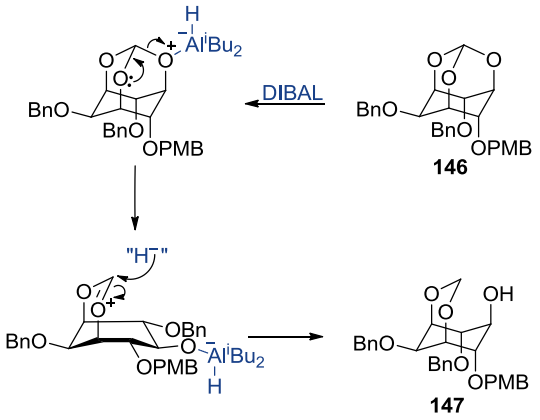


Figure 2.7. Proposed mechanism for the regioselective DIBAL reduction of orthoformate **146**.[52,126,127]

This coordination promotes formation of an oxacarbenium ion, which ring flips into a boat conformation to relieve unfavourable 1,3-diaxial interactions. Reduction of the oxacarbenium ion then occurs through reaction of a second equivalent of DIBAL to afford compound **147** in 95% yield, without the need for further purification (Figure 2.7).

Treatment of the resulting alcohol **147** with sodium hydride and allyl bromide gave the desired allyl-protected intermediate **148** in excellent yield (Scheme 2.2). Simultaneous deprotection of the methylene acetal and the PMB ether was then achieved by acid-catalysed methanolysis, to afford the racemic triol **149** in high yield.

As enantiomerically pure inositol intermediates are required for our synthetic route, a diastereomeric resolution of the racemic triol **149** was performed. This triol was reacted with 1-(*S*)-(−)-camphor dimethyl acetal **150**, which was synthesised in one step from commercially available 1-(*S*)-(−)-camphor.

This reaction protected the vicinal diol of triol **149** as the camphor acetal, resulting in a mixture of four possible diastereomers in a ratio of 1:1:1:1 (Figure 2.8).[51]

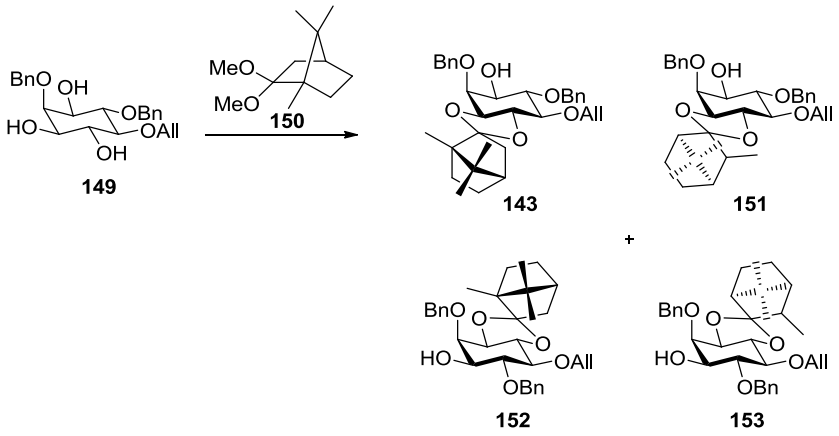
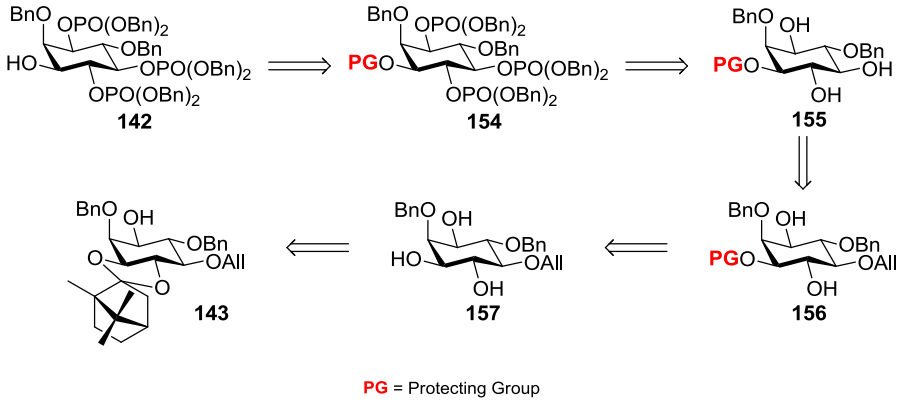


Figure 2.8. Diastereomeric resolution of inositol derivative **149.**[51]

Fortunately, the desired diastereomer **143** can be isolated from the other three diastereomers by careful silica gel column chromatography, which affords key intermediate **143** in moderate yield.

2.6. Synthetic Route to Intermediate 142

From the desired diastereomer **143**, our synthetic route for the synthesis of 3-position derivatives involved cleaving the camphor acetal, selectively protecting the 3-position hydroxyl group and deprotecting the 5-position allyl group (Scheme 2.3).



Scheme 2.3. Revised retrosynthetic analysis for synthesis of 3-position alcohol **142**.

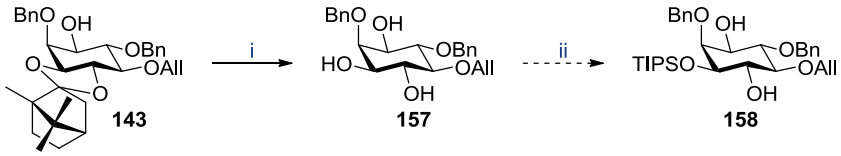
Phosphorylation of the subsequent triol **155** would afford the desired phosphorylated intermediate **154**, and deprotection of the 3-position protecting group would afford the free hydroxyl group at this position. This alcohol **142** would allow for the installation of phosphate isosteres to afford our desired compounds **138** and **139** (Scheme 2.3).

This protecting group strategy had been previously attempted by the Conway group, utilising both a PMB ether and a benzoyl ester to selectively protect the 3-position hydroxyl group.[53] However, efforts to deprotect these protecting groups to afford compound **142** proved unsuccessful. The harsh conditions required caused significant debenzylation of the phosphate groups, along with migration of the phosphate groups around the inositol ring.

As it had been previously demonstrated that a triisopropylsilyl (TIPS) protecting group could be removed from the 1-position hydroxyl group of 2,6-bis-*O*-benzyl-1-*O*-triisopropylsilyl 3,4,5-tris(dibenzylphosphate) *myo*-

inositol without promoting phosphate migration,[56] we proposed the installation of a TIPS protecting group on the 3-position hydroxyl group.

Deprotection of the camphor acetal by methanolysis proceeded in good yield, to afford triol **157**. The development of conditions for the selective protection of the 3-position hydroxyl group as a TIPS ether was then required (Scheme 2.4).



Scheme 2.4. Synthetic route to intermediate **157** and proposed synthesis of **158**. *Reagents and conditions*: i) CH_3COCl (0.6 eq.), CH_2Cl_2, MeOH, RT, 12 h, 71%; ii) see Table 2.1.

The regioselective protection of a single hydroxyl group in the presence of other hydroxyl groups can be achieved using stannylene acetal chemistry.[128,129] This reaction was optimised by the Conway group to allow selective protection of the 3-position hydroxyl group of inositol in the presence of the 1- and 4-position hydroxyl groups.[53]

This regioselective protection involves the initial formation of a stannylene acetal complex of the starting triol **157**, through reaction with dibutyltin oxide. When the reaction is heated under reflux in toluene or other non-polar solvent, mass spectrometry and ^{119}Sn NMR techniques have shown that the stannylene acetal exists as a dimer, with the tin atom five-coordinate and one of the oxygen atoms tri-coordinate (Figure 2.9).[129]

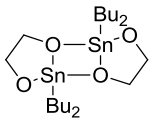


Figure 2.9. Representation of stannylene acetal in non-polar solvent.[129]

In these reactions, a Soxhlet apparatus containing 3 Å molecular sieves is used to remove the water generated in the reaction mixture. The preformed stannylene complex is then reacted with an alkylating agent, such as benzoyl chloride, at room temperature to give selective protection of the 3-position hydroxyl group.

These reactions can also be performed in polar solvents, where a trigonal bipyramidal intermediate is formed due to the coordination of a solvent molecule to the central tin atom (Figure 2.10).[129]

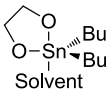


Figure 2.10. Representation of stannylene acetal in polar solvent.[129]

These reactions involve heating a solution of the appropriate diol, dibutyltin oxide, the desired alkylating agent, and an optional additive agent in acetonitrile under reflux, again using a Soxhlet apparatus containing 3 Å molecular sieves. In these trigonal bipyramidal complexes, the apical oxygen possesses a longer O-Sn bond, and so has greater reactivity, leading to the selectivity observed at the 3-position (Figure 2.11).

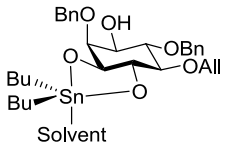


Figure 2.11. Representation of stannylene acetal of inositol derivative **157** in polar solvent.

Steric considerations may also play a part in the selectivity observed, as the 4-position hydroxyl group is less accessible due to its proximity to the equatorial 5-position allyl ether. Additives, such as caesium fluoride, tetrabutylammonium bromide (TBABr) and tetrabutylammonium iodide (TBAI), can be employed in the reaction mixture, as they replace the coordinating solvent and polarise the apical oxygen, increasing its reactivity.

2.7. Attempted Synthesis of Intermediate 158

Considering this stannylene acetal chemistry, we explored a range of conditions to selectively protect the 3-position hydroxyl group as a TIPS ether (Table 2.1).

Table 2.1 indicates that both one-pot procedures (entries 2 and 3) and two-step procedures were attempted, involving the pre-formation of the stannylene acetal in polar (entries 4-10) and non-polar (entry 1) solvents. A range of additives were also included in the reaction mixtures, such as imidazole (entries 2 and 3), TBAI (entries 5 and 7), TBABr (entry 7) and cesium fluoride (entry 5), in order to increase the reactivity of the stannylene acetal. Finally, 2,4,6-tri-*tert*-butylpyridine (TTBP) was added to the reaction

mixture (entry 10), to neutralise any acid that may be formed in the reaction mixture and cause degradation.

Despite multiple attempts, only limited amounts of the desired product **158** could be isolated from the reaction mixtures, with the results of these reactions inconsistent. It was, therefore, clear that an alternative, more robust synthetic route was required.

157 **158**

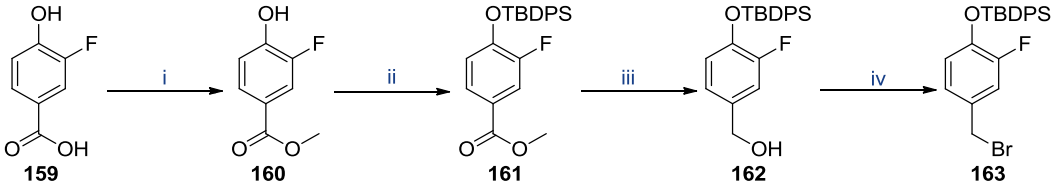
Entry	Conditions	Reaction Time	Yield
1	i. Bu_2SnO, toluene, reflux ii. TIPSOTf (2 eq.), DMF	48 h	**22%**
2	Bu_2SnO, TIPSOTf (1 eq.), imidazole (1 eq.), MeCN, reflux	42 h	**0%**
3	Bu_2SnO, TIPSCl (1 eq.), imidazole (1 eq.), MeCN, reflux	42 h	**0%**
4	i. Bu_2SnO, methanol, reflux ii. TIPSOTf (1.5 eq.), THF, 50 °C	48 h	**0%**
5	i. Bu_2SnO, methanol, reflux ii. TIPSCl (1 eq.), TBAI (0.5 eq.), CsF (0.5 eq.), THF, 50 °C	92 h	**0%**
6	i. Bu_2SnO, methanol, reflux ii. TIPSOTf (1 eq.), THF	22 h	**19%**
7	i. Bu_2SnO, methanol, reflux ii. TIPSOTf (3.5 eq.), TBAI (0.5 eq.), TBABr (0.5 eq.), THF, 50 °C	48 h	**7%**
8	i. Bu_2SnO, methanol, reflux ii. TIPSOTf (3 eq.), THF	20 h	**4%**
9	i. Bu_2SnO, methanol, reflux ii. TIPSOTf (1 eq.) syringe pump addition, DMF	24 h	**0%**
10	i. Bu_2SnO, methanol, reflux ii. TIPSOTf (1.5 eq.), TTBP (1.65 eq.), THF	48 h	0%

Table 2.1. Summary of reaction conditions for regioselective protection of triol **157** as TIPS ether **158**.

2.8. Synthesis of Key Intermediate 164

In 2009, Crich and co-workers reported the synthesis of a novel 4-(*tert*-butyldiphenylsiloxy)-3-fluorobenzyl protecting group, which could be deprotected under standard desilylation conditions.[130] This protecting group possessed the characteristics of a benzyl protecting group, which had been demonstrated to be highly compatible with stannylene acetal chemistry.[101] Therefore, we hypothesised that this protecting group could overcome the reactivity problems observed with TIPS protection of the 3-position hydroxyl group.

Following the conditions reported by Crich *et al.*, synthesis of the desired 4-(*tert*-butyldiphenylsiloxy)-3-fluorobenzyl bromide **163** commenced with the protection of 3-fluoro-4-hydroxybenzoic acid **159** with TBDPSCl, followed by reduction of the acid using lithium aluminium hydride.[130] However, in our hands, we found that the harsh conditions required to reduce the acid to the alcohol caused significant degradation of the TBDPS protected starting material. We, therefore, decided to first convert this acid to a methyl ester, which would allow milder reduction conditions to be employed (Scheme 2.5).



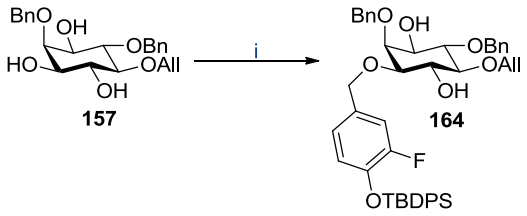
Scheme 2.5. Synthetic route to **163**. *Reagents and conditions*: i) conc. H_2SO_4, MeOH, reflux, 24 h, 80%; ii) TBDPSCl (2.5 eq.), NEt_3 (6 eq.), CH_2Cl_2, RT, 7 h, 85%; iii) $LiAlH_4$ (3 eq.), THF, RT, 4 h, 85%; iv) PPh_3 (1.5 eq.), CBr_4 (1.5 eq.), CH_2Cl_2, RT, 2 h, 69%.

Upon successful synthesis of alcohol **162**, Appel reaction conditions, involving the use of triphenylphosphine and carbon tetrabromide, were then employed to convert the primary alcohol to the desired bromide **163**. Some hydrolysis of the bromide **163** was observed during purification by column chromatography, however, the desired compound could still be isolated in significant yields.

The next challenge was to install the 4-(*tert*-butyldiphenylsiloxy)-3-fluorobenzyl protecting group on the 3-position hydroxyl group using stannylene acetal chemistry. We initially pre-formed the stannylene intermediate in methanol before adding the bromide **163** in THF, as these conditions had afforded consistent quantities of the desired TIPS-protected intermediate **158** in previous reactions. However, these conditions were found to be incompatible with our substrates, with only degradation products isolated.

As a one-pot reaction in acetonitrile was successful in the installation of a benzyl ether at the 3-position hydroxyl group of a similar substrate,[101] we proposed replicating these reaction conditions with our substrates.

Therefore, triol **157** was heated under reflux with dibutyltin oxide, bromide **163** and TBAI in acetonitrile for 48 h, using a Soxhlet apparatus containing 3 Å molecular sieves. These reaction conditions were found to be successful, and our desired intermediate **164** was isolated in 62% yield after purification by column chromatography (Scheme 2.6).



Scheme 2.6. Successful synthesis of **164**. *Reagents and conditions*: i) Bu_2SnO (1.1 eq.), TBAI (1 eq.), **163** (3 eq.), CH_3CN, reflux, 48 h, 62%.

Analysis of the crude ^1H NMR spectrum indicated that only negligible quantities of the 4-position protected intermediate were produced in the reaction mixture. As the analogous benzyl protection of the 3-position hydroxyl group occurs with the formation of ~17% of the 4-position protected intermediate,[101] it is possible that the increased steric bulk of the TBDPS group directs this protecting group towards the less sterically hindered 3-position, thus increasing the selectivity of this reaction.

2.9. Characterisation of Intermediate 164

At this point in the synthesis, it was important to confirm that the regiochemistry observed with the stannylene acetal chemistry was correct. Through the use of 1D and 2D ^1H and ^{13}C NMR spectroscopic techniques,

full assignment of intermediate **164** was achieved (Figure 2.12, Figure 2.13 and Figure 2.14).

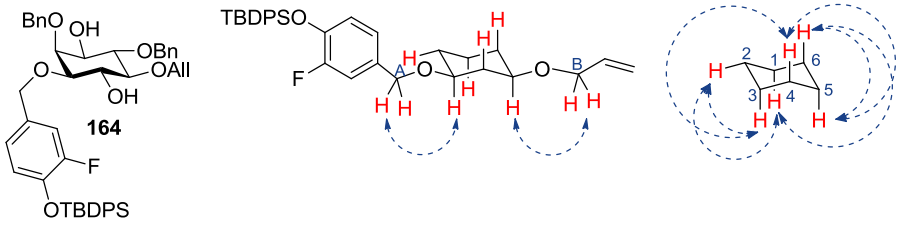


Figure 2.12. Diagram indicating couplings observed in NMR spectra of intermediate **164.**

The CH_2 of the allyl group (B, Figure 2.12) was easily identifiable in the COSY spectrum through its coupling to the distinctive CH of the allyl group. In an overlay of the HMBC and HSQC spectra, this CH_2 group was shown to couple to a ring proton, which was identified as H5 (Figure 2.12). Analysis of the COSY spectrum indicated that H5 coupled to two ring protons, identified as H6, due to its coupling to a benzylic CH_2 in the HSQC-HMBC overlay, and H4 (Figure 2.12).

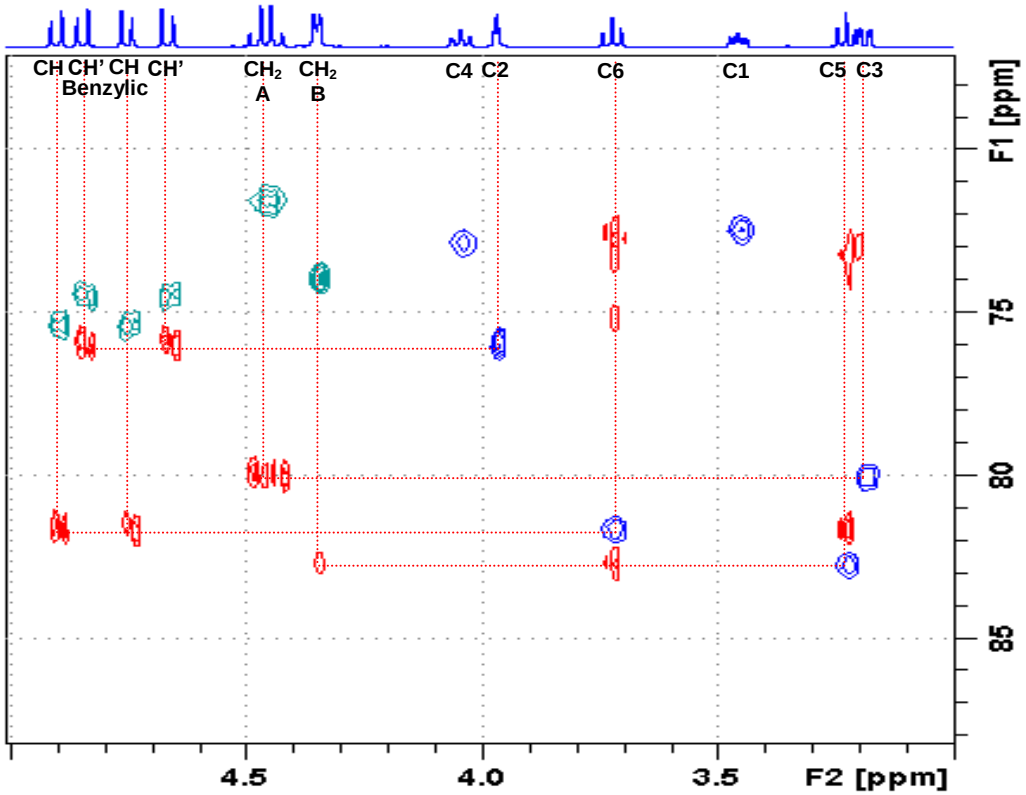


Figure 2.13. Overlay of HMBC and HSQC spectra showing regioisomer determination of intermediate **164**.

H6 was found to show strong correlation with another ring proton, identified as H1, which in turn coupled to another ring proton, identified as H2. H2 is easily identifiable due to its characteristic signal in the ^{1}H NMR spectrum, as it is the only proton that has two equatorial-axial couplings. This identification was further confirmed as this proton was also found to couple to a benzylic CH_2 in the HSQC-HMBC overlay (Figure 2.13).

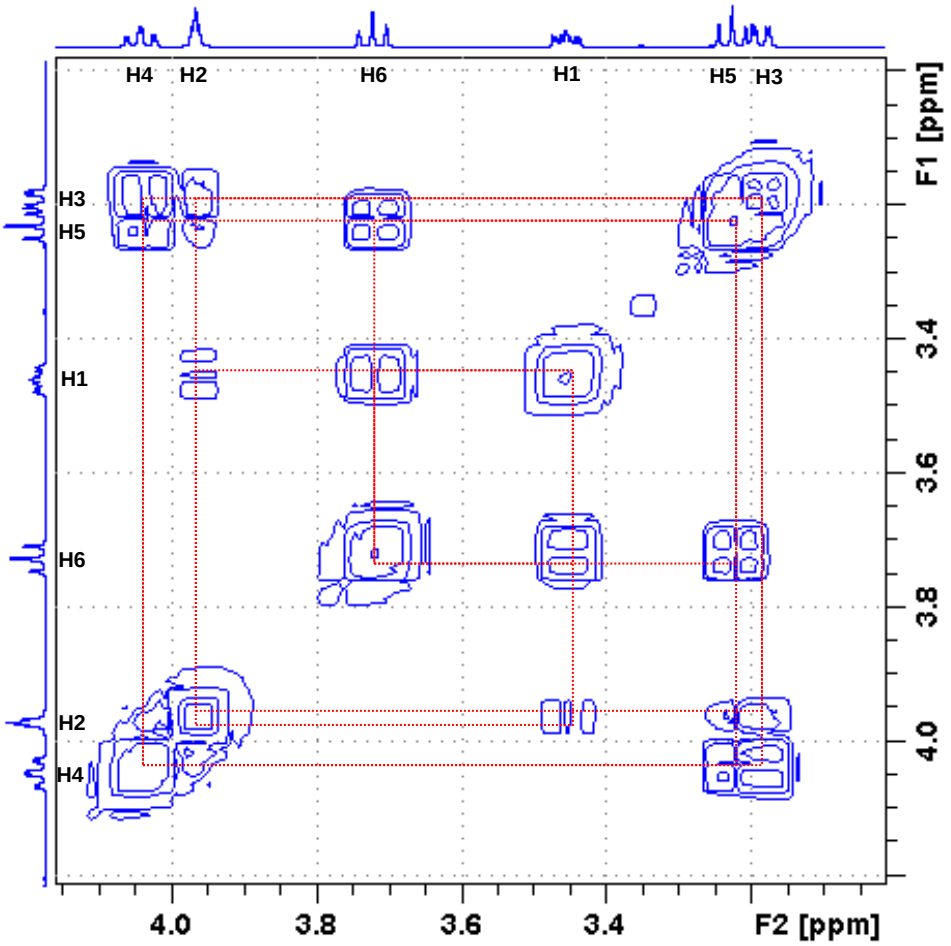
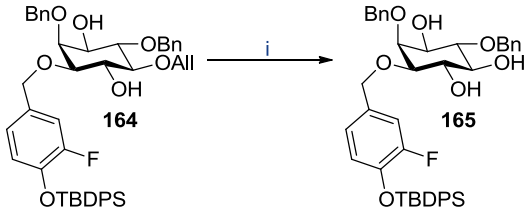


Figure 2.14. COSY spectrum showing regioisomer determination of intermediate **164**.

H2 and H4 both coupled to another ring proton, which was identified as H3. This ring proton showed coupling to a benzylic CH_2 in the HSQC-HMBC overlay which corresponded to the 4-(*tert*-butyldiphenylsiloxy)-3-fluorobenzyl protecting group (Figure 2.12). We could, therefore, conclude that this protecting group was successfully installed exclusively on the 3-position hydroxyl group.

2.10. Deprotection of Intermediate 166

After the successful synthesis and characterisation of intermediate **164**, the next step in our synthetic route was the deprotection of the allyl group on the 5-position.



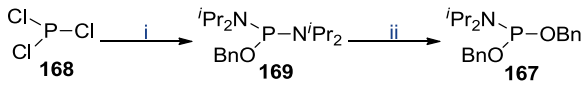
Scheme 2.7. Synthesis of intermediate **165**. *Reagents and conditions*: i) $PdCl_2$ (0.6 eq.), MeOH, RT, 12 h, 71%.

This transformation was achieved using conditions reported by Mandai and co-workers, which involved reacting the allyl ether **164** with palladium(II) chloride in methanol.[131] This reaction had been previously optimised in the Conway group for use on inositol substrates.[53] However, we found that reducing the number of equivalents of palladium(II) chloride along with subjecting the reaction mixture to an aqueous, rather than oxidative, work-up, further increased the yield of this reaction, and afforded intermediate **165** in 71% yield (Scheme 2.7).

At this point, it was necessary to install benzyl-protected phosphate groups to provide the desired trisphosphate **166**. We chose to follow a reliable method for the installation of these protected phosphates, which was to react the desired alcohol with bis(benzyloxy)-*N*,*N*-diisopropylamino phosphine **167** in the presence of catalytic 1*H*-tetrazole.[132] The resulting

inositol phosphine is subsequently oxidised with 3-chloroperoxybenzoic acid (*m*CPBA) to yield the desired benzylphosphate ester in high yields.

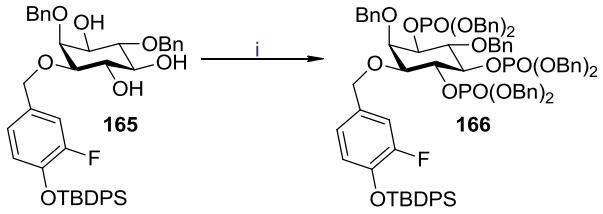
Therefore, the synthesis of bis(benzyloxy)-*N*,*N*-diisopropylamino phosphine **167** was attempted, starting from the commercially available phosphorus trichloride (Scheme 2.8).



Scheme 2.8. Synthetic route to phosphoramidite **167**. *Reagents and conditions*: i) (a) Pyridine (1.0 eq.), BnOH (1.0 eq.), Et_2O, −78 °C→RT, 3 h; (b) $^{i}Pr_2NH$ (4.1 eq.), −10 °C→RT, 12 h, 46%; ii) 1*H*-tetrazole (0.4 eq.), BnOH (1.0 eq.), CH_2Cl_2, RT, 2 h, 73%.

Phosphorus trichloride was reacted with one equivalent of benzyl alcohol followed by excess diisopropyl amine in diethyl ether to afford crude benzyloxy bis(*N*,*N*-diisopropylamino)phosphine **169**. This material was then reacted with one equivalent of benzyl alcohol and 1*H*-tetrazole in dichloromethane to give the desired bis(benzyloxy)-*N*,*N*-diisopropylamino phosphine **167** in 73% yield after purification by silica gel column chromatography (Scheme 2.8).

To achieve phosphorylation, triol **165** was reacted with excess bis(benzyloxy)-*N*,*N*-diisopropylamino phosphine **167** and catalytic 1*H*-tetrazole in dichloromethane at room temperature for 14 h. After this time, the reaction mixture was cooled to −78 °C and oxidised with *m*CPBA, to afford the desired trisphosphate **166** in 91% yield (Scheme 2.9)



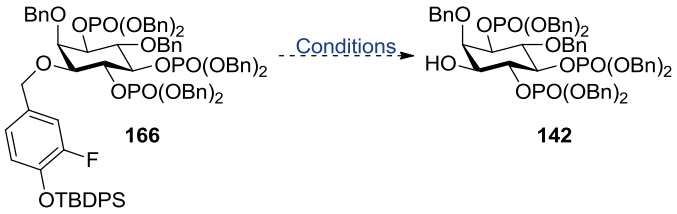
Scheme 2.9. Phosphorylation of intermediate **165**. *Reagents and conditions:* i) a) **167** (9.5 eq.), 1*H*-tetrazole (9.5 eq.), CH_2Cl_2, RT, 14 h; b) *m*CPBA (9.5 eq.), −78 °C→RT, 2 h, 91%.

After the successful synthesis of intermediate **166**, our next task was to deprotect the 4-(*tert*-butyldiphenylsiloxy)-3-fluorobenzyl protecting group to afford the free 3-position hydroxyl group.

We first replicated the conditions described by Crich *et al.* for this deprotection, by heating a solution of intermediate **166** and TBAF in DMF at 90 °C.[130] However, after 3 h, only deprotection of the TBDPS protecting group was observed, and prolonged reaction times caused complete degradation of the starting material **166**. Therefore, we investigated alternative conditions for the deprotection of the 4-(*tert*-butyldiphenylsiloxy)-3-fluorobenzyl protecting group, starting with one-pot procedures (Table 2.2).

Our initial attempts involved reacting intermediate **166** with TBAF, until complete TBDPS deprotection was observed by mass spectrometry and TLC analysis. Once *in situ* formation of intermediate **170** was achieved, a variety of acids (Table 2.2, entries 2-5) were added to the reaction mixture. It was hypothesised that these acids would promote cleavage of the resulting

4-hydroxy-3-fluorobenzyl protecting group; however, none of these reactions afforded desired compound **142** in a quantifiable yield.



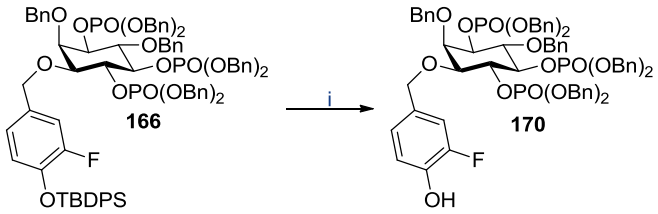
Entry	Conditions	Reaction Time	Result
1	TBAF (2.0 eq.), DMF, 90 °C	12 h	Desilylation product **170**
2	TBAF (3.0 eq.), THF $FeCl_3$ (2.0 eq.)	48 h	Desilylation product **170**
3	TBAF (3.0 eq.), THF acetic acid (conc.)	48 h	Desilylation product **170**
4	TBAF (3.0 eq.), THF hydrochloric acid (1.0 M)	48 h	Desilylation product **170**
5	TBAF (3.0 eq.), THF hydrochloric acid (conc.)	48 h	Desilylation product **170**

Table 2.2. Summary of reaction conditions for one-pot deprotection of 4-(*tert*-butyldiphenylsiloxy)-3-fluorobenzyl-protected intermediate **166.**

Due to the failure of these one-pot procedures, we next proposed isolating and purifying the 4-hydroxy-3-fluorobenzyl-protected intermediate **170**, before attempting various deprotection conditions to afford the desired intermediate **142**.

Deprotection of the TBDPS protecting group proceeded smoothly, as reaction of intermediate **166** with TBAF and subsequent purification by silica

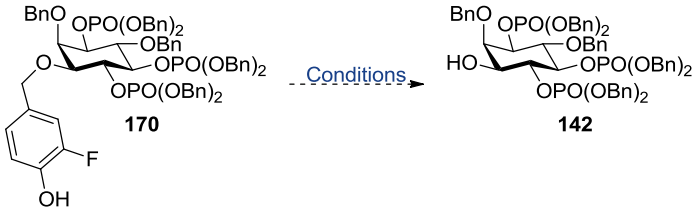
gel column chromatography afforded desired intermediate **170** in 86% yield (Scheme 2.10).



Scheme 2.10. Synthesis of key intermediate **170**. *Reagents and conditions*: i) TBAF (3.0 eq.), CH_2Cl_2, RT, 30 min, 86%.

After the successful synthesis of intermediate **170**, we next investigated several conditions for the deprotection of the 4-hydroxy-3-fluorobenzyl protecting group (Table 2.3).

Our initial attempts proved unsuccessful, with either no reaction (Table 2.3, entries 1-4) or degradation of the starting materials (Table 2.3, entries 5-7) observed. However, upon using trifluoroacetic acid to promote the cleavage of the 4-hydroxy-3-fluorobenzyl protecting group (Table 2.3, entry 8), the desired intermediate **142** was successfully isolated in 39% yield.



Entry	Conditions	Reaction Time	Result
1	Acetyl chloride (0.5 eq.), MeOH/CH_2Cl_2	48 h	Starting materials
2	Sodium methoxide (0.5 eq.), MeOH	24 h	Starting materials
3	Sodium methoxide (1.0 eq.), MeOH $FeCl_3.6H_2O$ (0.5 eq.)	24 h	Starting materials
4	$FeCl_3.6H_2O$ (0.5 eq.), Et_2O	24 h	Starting materials
5	Tin(IV) chloride (0.5 eq.), CH_2Cl_2	1 h	Degradation
6	Iodobenzene diacetate (0.5 eq.), CH_2Cl_2	1 h	Degradation
7	Iodotrimethylsilane (0.5 eq.), $CHCl_3$	1 h	Degradation
8	TFA (1.0 eq.), CH_2Cl_2	4 h	39% yield
9	TFA, $CHCl_3$, MeOH (5:5:1)	30 min	51% yield

Table 2.3. Summary of reaction conditions for deprotection of 4-hydroxy-3-fluorobenzyl-protected intermediate **170.**

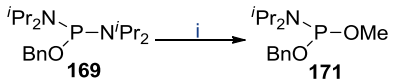
In an attempt to optimise these reaction conditions, it was found that trifluoroacetic acid, chloroform and methanol in a ratio of 5:5:1 promoted this deprotection in a shorter time, thus preventing excessive degradation of the starting materials and products (Table 2.3, entry 9). These improved conditions allowed the isolation of the desired intermediate **142** in 51% yield, with no evidence of phosphate migration around the inositol ring. Similar conditions have been used to deprotect a PMB ether in the presence of

benzyl-protected phosphate groups, and no phosphate migration was observed in that system either.[133]

2.11. Synthesis of Final Compounds 138 and 139

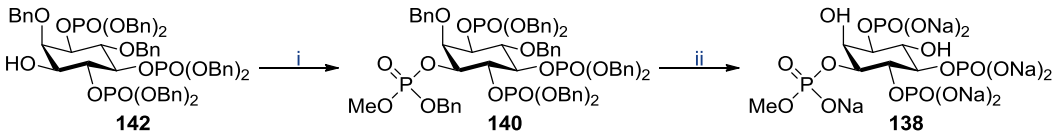
With a reliable synthesis of alcohol **142** in hand, we could now begin investigating the incorporation of isosteric phosphate replacements at the 3-position. The presence of the three protected phosphates in intermediate **142** could be problematic, as steric crowding around the inositol ring would likely limit the size of the functionality that could be installed at the free hydroxyl. As relief from this steric strain could occur *via* phosphate migration, it was very important that mild conditions were used to install these isosteres.

A methyl phosphate ester, previously selected because of its interesting electronic properties, would be an ideal isostere to install at the 3-position hydroxyl group, due to its relatively mild synthetic conditions. The installation of this isostere could be achieved by reaction of alcohol **142** with the appropriate phosphoramidite **171**, followed by oxidation which, upon global deprotection, would yield the desired methyl phosphate ester **140** (Scheme 2.12). Using modified phosphoramidite conditions to those already discussed,[132] phosphoramidite **171** was prepared from benzyloxy bis(*N*,*N*-diisopropylamino)phosphoramidite **169** upon treatment with methanol in the presence of 1*H*-tetrazole. These reaction conditions gave the desired phosphoramidite **171** in 30% yield (Scheme 2.11).



Scheme 2.11. Synthesis of phosphoramidite **171.** *Reagents and conditions*: i) 1*H*-tetrazole (0.4 eq.), MeOH (1.0 eq.), CH_2Cl_2, RT, 2 h, 30%.

Phosphoramidite **171** was then reacted with alcohol **142** in the presence of 1*H*-tetrazole at room temperature. Once consumption of the starting material was observed by TLC analysis, the reaction was cooled to −78 °C and oxidised using *m*CPBA. After warming to room temperature, work-up and purification by silica gel column chromatography afforded the desired methyl phosphate ester **140** in 73% yield as a mixture of diastereomers (Scheme 2.12). Crucially, no phosphate migration was observed by either ^{1}H or ^{31}P NMR spectroscopy.

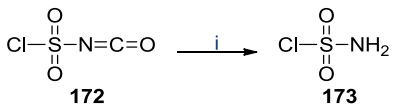


Scheme 2.12. Synthetic route to final compound **138.** *Reagents and conditions*: i) a) 1*H*-tetrazole (5.0 eq.), **171** (5.0 eq.), CH_2Cl_2, RT, 14 h; b) *m*CPBA (5.0 eq.), −78 °C→RT, 2 h, 73%; ii) $NaHCO_3$ (7.0 eq), Pd (black) (18.0 eq.), H_2, ^{t}BuOH, MeOH, RT, 12 h, 100%.

Our final step was the global deprotection of the benzyl protecting groups, which proceeded smoothly in 100% yield. Hydrogenolysis of protected intermediate **140** in the presence of palladium black and sodium bicarbonate afforded the desired final product **138** as the presumed heptasodium salt, without the need for further purification (Scheme 2.12).

Our second choice of phosphate isostere was a sulfamate group. This group can potentially act as both a hydrogen bond acceptor through the lone pairs of electrons on the oxygen and nitrogen atoms, and as a hydrogen bond donor through the hydrogens bonded to nitrogen. We decided to follow the sulfamoylation procedure reported by Okada and co-workers and widely used within the Conway group.[53,56,134] This method involves treating the desired alcohol with sulfamoyl chloride in dimethyl acetamide (DMA), which affords the desired sulfamate with excellent conversion and high isolated yields. The DMA in this case acts as both reaction solvent and base.

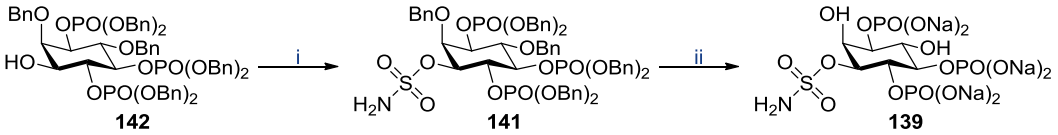
As sulfamoyl chloride **173** is highly unstable, it was freshly prepared from chlorosulfonyl isocyanate **172** by treatment with formic acid at 0 °C. This reaction gave the crude sulfamoyl chloride, which was used as a 2.5 M stock solution in dichloromethane without further purification (Scheme 2.13).



172 **173**

Scheme 2.13. Synthesis of sulfamoyl chloride **173**. *Reagents and conditions*: i. Formic acid, CH_2Cl_2, 0 °C, presumed 2.5 M solution in CH_2Cl_2.

A solution of the alcohol **142** in DMA was cooled to 0 °C and treated with the freshly prepared sulfamoyl chloride **173**. The reaction proceeded smoothly at room temperature, and TLC analysis confirmed that it had reached completion after 5 h. Subsequent work-up and purification by silica gel column chromatography afforded the desired sulfamate **141** in 82% yield (Scheme 2.14).



Scheme 2.14. Synthetic route to final compound **139.** *Reagents and conditions*: i) **173** (2.0 eq.), DMA, 0 °C→RT, 5 h, 82%; ii) $NaHCO_3$ (6.0 eq), Pd (black) (16.0 eq.), H_2, ^tBuOH, MeOH, H_2O, RT, 12 h, 100%.

Global deprotection of intermediate **141** by hydrogenolysis, using similar conditions to those used with intermediate **140**, successfully afforded the desired final compound **139** as the presumed hexasodium salt, again without the need for further purification.

2.12. Molecular Dynamics Simulations

After the successful synthesis of the two 3-position-modified Ins(1,3,4,5)P_4 derivatives, methyl phosphate ester **138** and sulfamate **139**, we decided to perform molecular dynamics (MD) simulations on these derivatives to investigate their binding interactions with the PKB PH domain. Our collaborators Dr Ian Gould and Sarah Rosen at Imperial College London carried out 150 ns simulations using Amber 11 on both methyl phosphate ester **138** and sulfamate **139**, with different affinities observed for both derivatives.

The simulated free energy of binding for methyl phosphate derivative **138** was calculated at −69.73 $kcal^{-1}$ mol^{-1}, similar to the free energy of binding calculated for Ins(1,3,4,5)P_4 (−71.97 $kcal^{-1}$ mol^{-1}). From analysis of the MD

simulations of this derivative, it was clear that the limited size of the methyl phosphate group is well tolerated within the tight binding site. Therefore, it is likely that derivative **138** binds to the PKB PH in a similar manner to that observed with Ins(1,3,4,5)P_4 in the X-ray crystal structure (Figure 2.15).

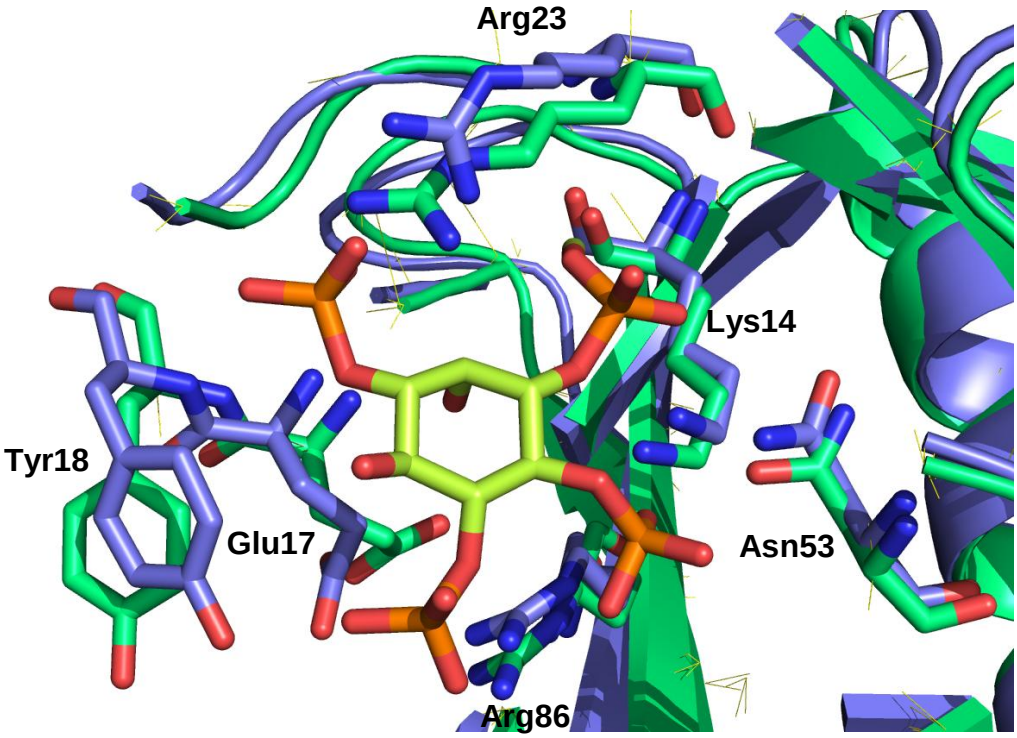


Figure 2.15. PyMOL representation of a 150 ns molecular dynamic simulation of derivative **138** using Amber 11, illustrating the PKB PH domain-Ins(1,3,4,5)P_4 complex (green), PKB PH domain-derivative **138** complex (blue) and derivative **138** (yellow).[114]

However, the simulated free energy of binding for sulfamate derivative **139** was calculated at −32.40 kcal^{-1} mol^{-1}, significantly less favourable than the free energy of binding calculated for Ins(1,3,4,5)P_4 (−71.97 kcal^{-1} mol^{-1}). The MD simulations of this derivative indicated the sulfamate group at the 3-position could not be tolerated within the binding site, completely disrupting

the hydrogen bonding interactions between the remaining phosphates and the PKB PH domain (Figure 2.16).

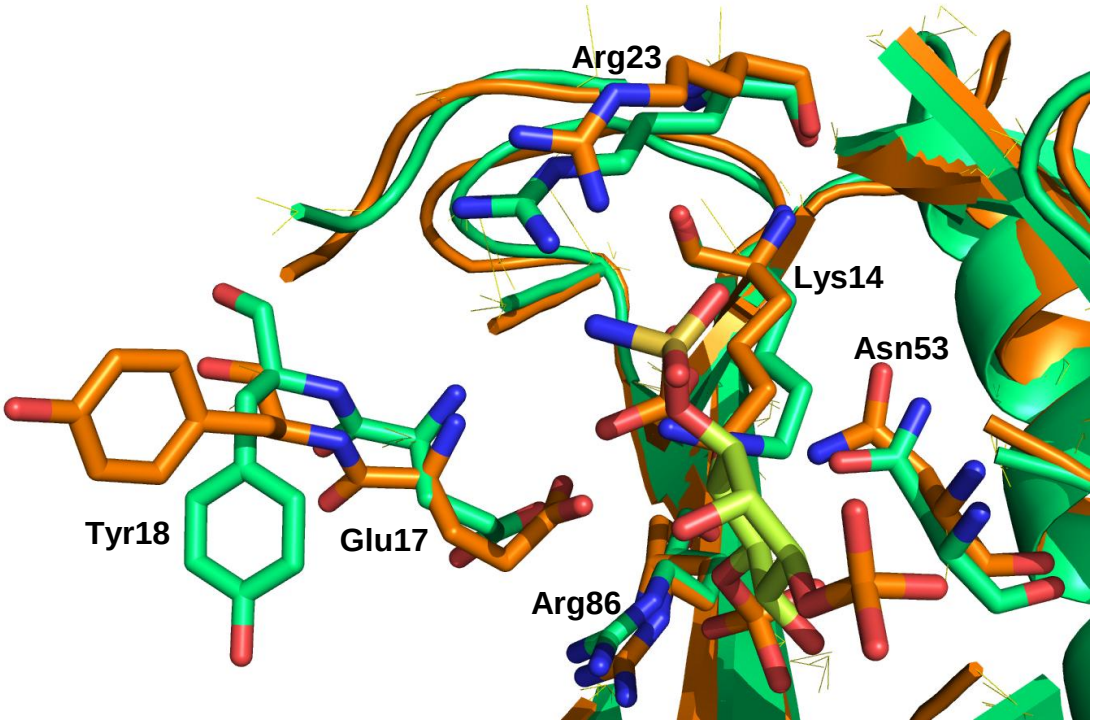


Figure 2.16. PyMOL representation of a 150 ns molecular dynamic simulation of derivative **139** using Amber 11, illustrating the PKB PH domain-Ins(1,3,4,5)P_4 complex (green), PKB PH domain-derivative **139** complex (orange) and derivative **139** (yellow).[114]

The biological activity of methyl phosphate ester **138** and sulfamate **139** has now been investigated in order to correlate their binding affinity to that calculated in the MD simulations (see Chapter 3).

2.13. Summary

In summary, the enantiomerically pure 3-position alcohol **142** was developed through novel applications of an unusual protecting group. The

utility of this approach was demonstrated by the successful synthesis of two 3-position-modified Ins(1,3,4,5)P_4 derivatives, methyl phosphate ester **138** and sulfamate **139**. MD simulations of these derivatives were carried, and their biological activity has been investigated (see Chapter 3).

Chapter 3:

Synthesis of 5-position Ins(1,3,4,5)P_4 derivatives

3. Synthesis of 5-position Ins(1,3,4,5)P_4 derivatives

3.1. Introduction to PKB PH Domain Variable Loop 3

After the successful synthesis and evaluation of 3-position Ins(1,3,4,5)P_4 derivatives, we proposed investigating other key interactions between Ins(1,3,4,5)P_4 and the PKB PH domain. The PH domain of PKB undergoes a significant conformational change upon binding to Ins(1,3,4,5)P_4 (Chapter 2). One of the most dramatic changes occurs at the variable loop 3 (VL3), which moves up to 7.8 Å towards the phosphoinositide-binding pocket (Figure 3.1).[114]

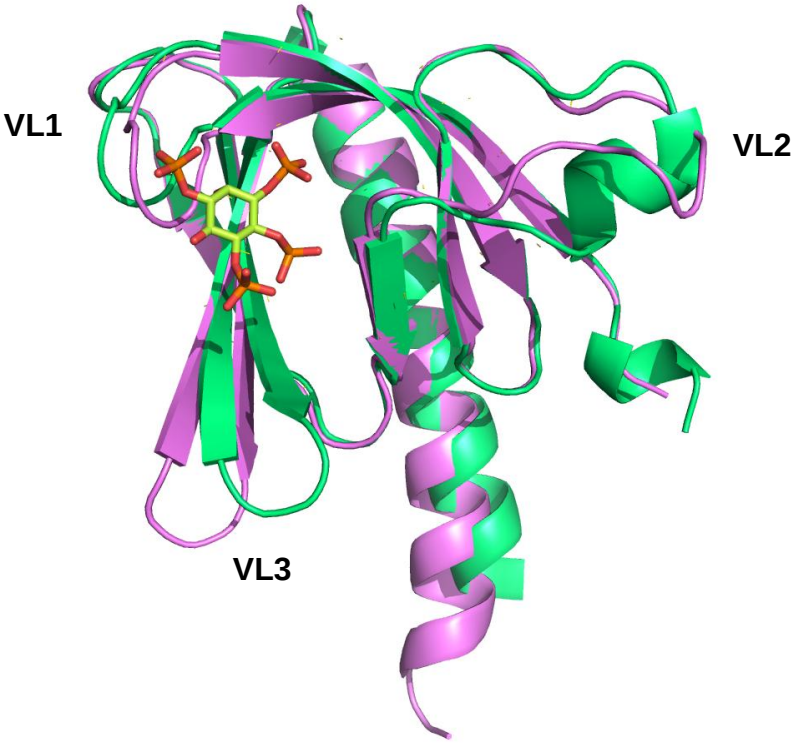


Figure 3.1. X-Ray crystal structures of *apo* PKB PH domain (lilac, PDB code: 1UNP) and PKB PH domain-Ins(1,3,4,5)P_4 complex (green, PDB code: 1UNQ), showing ligand-induced conformational change.[114]

It is this movement that prompts the dissociation of the PH and kinase domains of PKB, allowing phosphorylation and activation of PKB by PDK1.[135-137] This loop has, therefore, become a target for the development of PKB PH domain inhibitors. One such allosteric inhibitor, inhibitor VIII **174**, has been found to target VL3 through a ring-stacking interaction with Trp80 on the tip of loop 3, thereby locking PKB in its inactive conformation (Figure 3.2).[136-138]

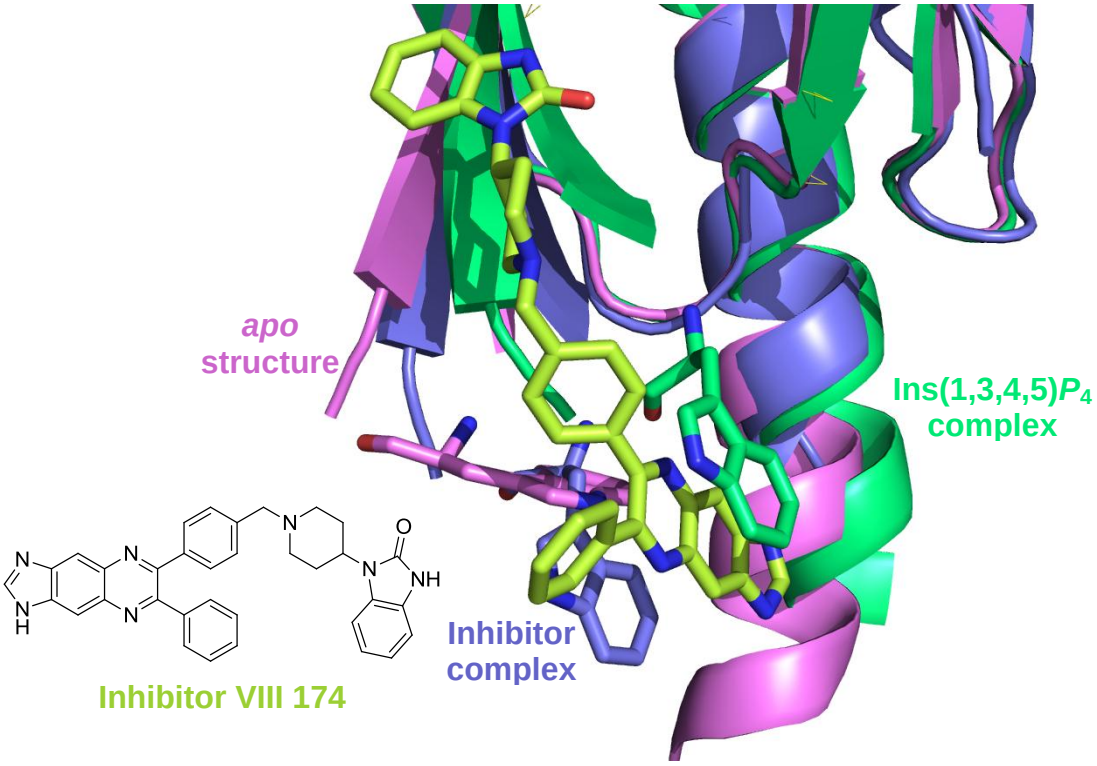


Figure 3.2. X-Ray crystal structure of *apo* PKB PH domain (lilac), PKB PH domain-Ins(1,3,4,5)P_4 complex (green), PKB PH domain-inhibitor VIII **174** complex (purple, PDB code: 3O96) and inhibitor VIII **174** (yellow), showing inhibitor-induced stabilisation of inactive conformation through ring-stacking interactions with Trp80 (sticks).[114,137]

An alternative strategy for the development of inhibitors could utilise the highly flexible nature of VL3. We noted that Gln79, also situated on loop 3,

moves 5.2 Å towards the 5-position phosphate of Ins(1,3,4,5)P_4 **13** upon ligand binding (Figure 3.3).

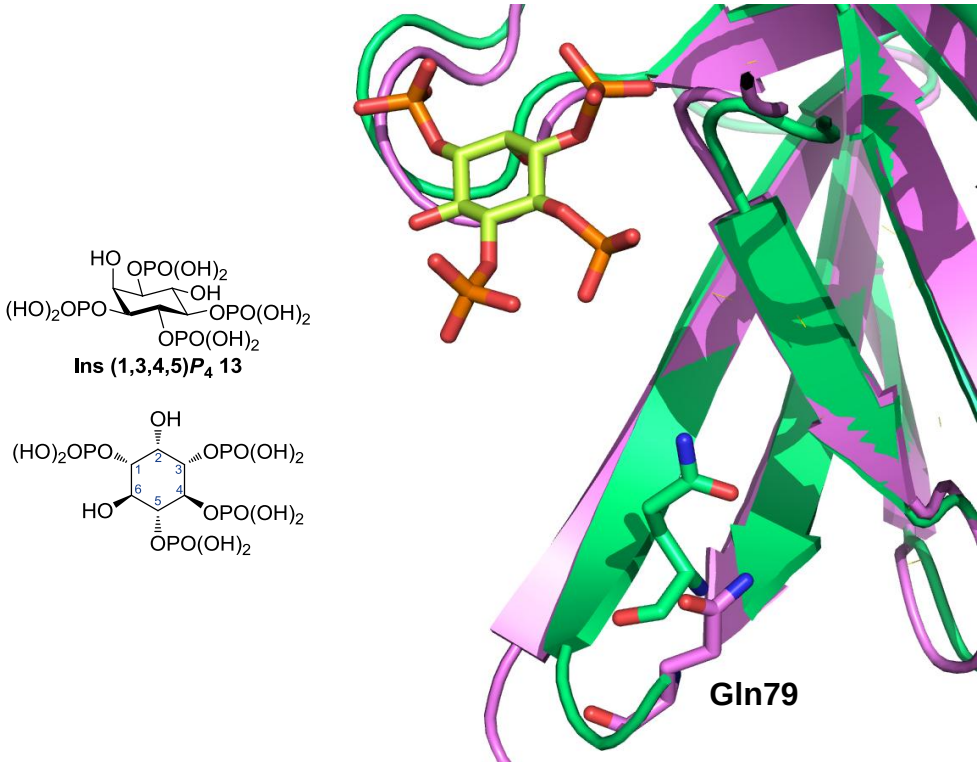


Figure 3.3. X-Ray crystal structure of *apo* PKB PH domain (lilac) and PKB PH domain-Ins(1,3,4,5)P_4 **13** complex (green), with Gln79 highlighted (sticks).[114]

Therefore, we postulated that derivatives of Ins(1,3,4,5)P_4 **13** with suitable modifications at the 5-position could form additional interactions with Gln79 compared to native Ins(1,3,4,5)P_4. We predict that these derivatives could then act as inhibitors, as their binding to the PKB PH domain would prevent its recruitment to the plasma membrane and binding to PtdIns(3,4,5)P_3, thus preventing the co-localisation of PKB and PDK1 at this site and subsequent phosphorylation.

3.2. Molecular Dynamics Simulations of Ins(1,3,4,5)P_4 Derivatives

Therefore, we proposed the development of derivatives of Ins(1,3,4,5)P_4 **13**, incorporating phosphate isosteres at the 5-position that could form hydrogen bonding interactions with Gln79. In collaboration with Dr Ian Gould and Sarah Rosen at Imperial College London, we proposed using molecular dynamics (MD) simulations to predict a series of Ins(1,3,4,5)P_4 derivatives that would form the desired interactions with Gln79.[139] Therefore, several potential 5-position isosteres were screened for both their synthetic potential and their ability to form the desired interactions *in silico*.

In the X-ray crystal structure of Ins(1,3,4,5)P_4 **13** complexed to the PKB PH domain (Figure 3.3), an 8.5 Å distance separates the 5-position phosphate and Gln79, thus preventing suitable hydrogen bonding interactions.[114] We, therefore, initially proposed extending the linker between the phosphate group and the inositol ring, thus allowing the 5-position phosphate to participate directly in H-bonding interactions with Gln79 and loop 3 (Figure 3.4).

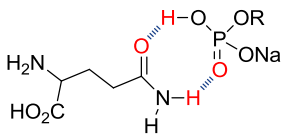


Figure 3.4. Proposed hydrogen bonding interactions between Gln79 and phosphate derivatives.

We first proposed the extended phosphate **175** (Figure 3.5) and, as carboxylate groups can form similar interactions to phosphate groups and are widely employed as a phosphate isosteres,[140] we proposed retaining the same linker length and investigating carboxylate derivative **176** (Figure 3.6).

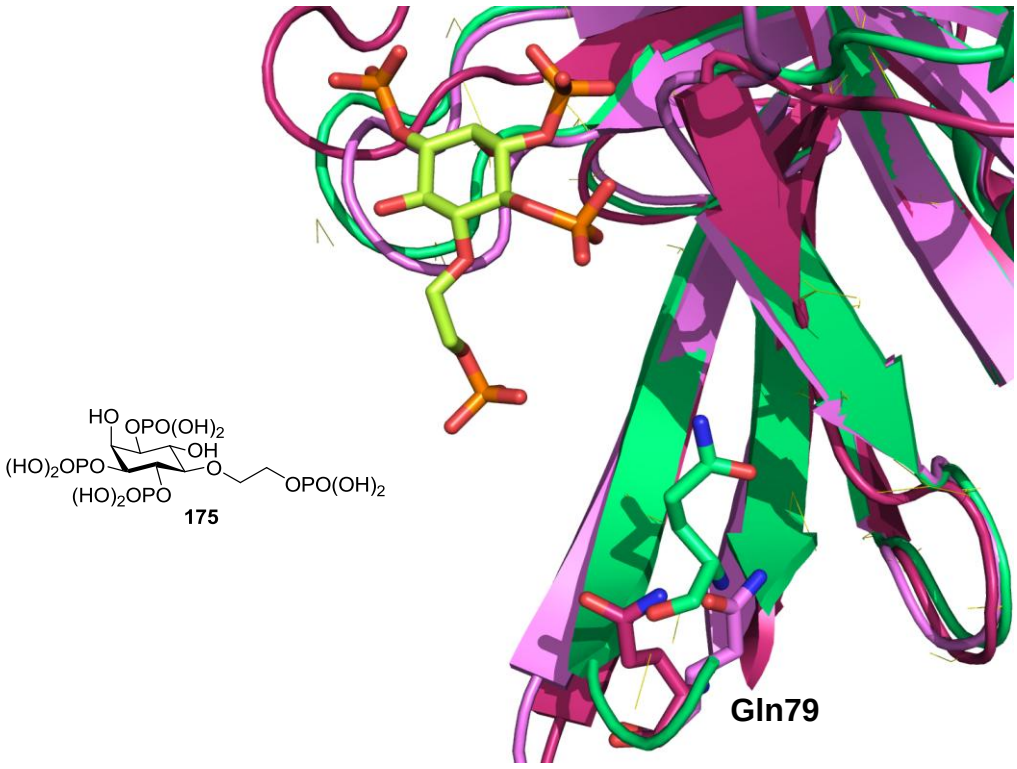


Figure 3.5. PyMOL representation of a 150 ns molecular dynamics simulation of derivative **175** using Amber 11, illustrating *apo* PKB PH domain (lilac), PKB PH domain-Ins(1,3,4,5)P_4 complex (green), PKB PH domain-derivative **175** complex (purple) and derivative **175** (yellow).[114]

Unfortunately, the simulated free energy of binding for these derivatives was calculated at −59.07 kcal^{-1} mol^{-1} for phosphate **175** and −59.12 kcal^{-1} mol^{-1} for carboxylate **176**, significantly less favourable than the free energy of binding calculated for Ins(1,3,4,5)P_4 **13** (−71.97 kcal^{-1} mol^{-1}). Analysis of the MD simulations of these derivatives indicated that loss of the hydrogen

bonding capabilities of the phosphate group at the 5-position greatly reduced the number of hydrogen bonding interactions between the inositol derivative and the PKB PH domain (Figure 3.5 and Figure 3.6), thus causing the dramatic decrease in binding affinity observed.

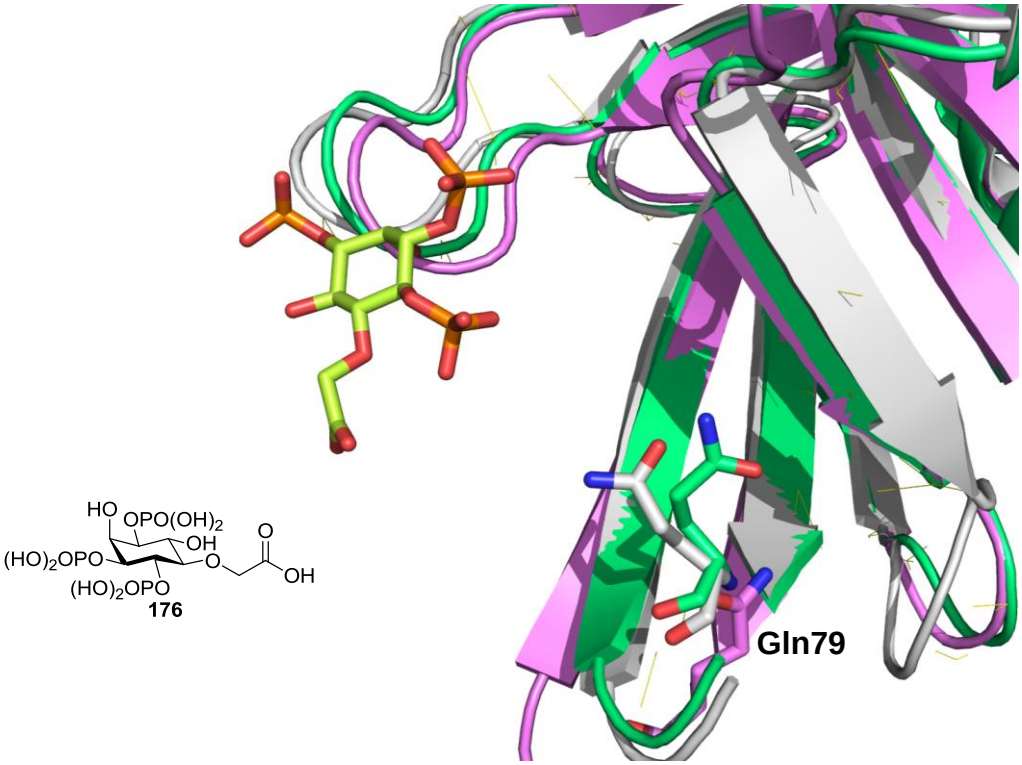


Figure 3.6. PyMOL representation of a 150 ns molecular dynamics simulation of derivative **176** using Amber 11, illustrating *apo* PKB PH domain (lilac), PKB PH domain-Ins(1,3,4,5)P_4 complex (green), PKB PH domain-derivative **176** complex (white) and derivative **176** (yellow).[114]

We, therefore, proposed incorporation of a pyrophosphate **177** (Figure 3.7) or triphosphate **178** (Figure 3.8) at this 5-position. We hypothesised that these derivatives would maintain the critical hydrogen bonding interactions of the phosphate at the 5-position, while extending the additional phosphate moiety towards loop 3 and Gln79. As derivatives with pyrophosphate groups

incorporated at the 5-position have previously been shown to act as inhibitors of PKB (see Chapter 5),[141] we were hopeful that these derivatives would show increased binding affinity with the PKB PH domain compared with Ins(1,3,4,5)P_4 **13**.

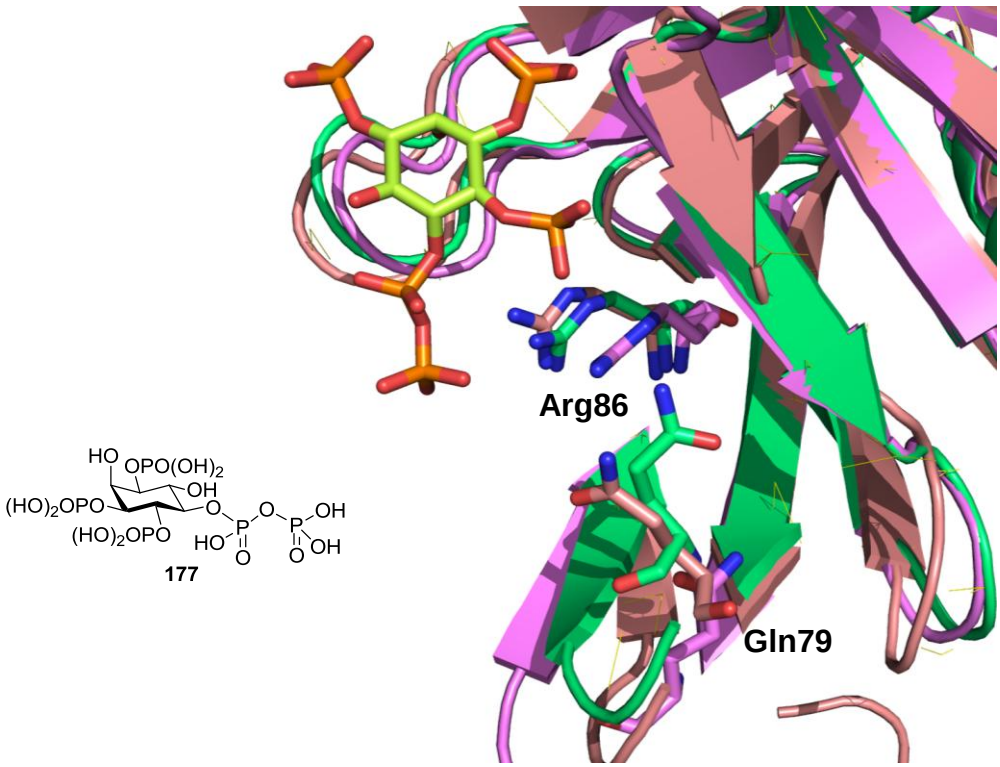


Figure 3.7. PyMOL representation of a 150 ns molecular dynamics simulation of derivative **177** using Amber 11, illustrating *apo* PKB PH domain (lilac), PKB PH domain-Ins(1,3,4,5)P_4 complex (green), PKB PH domain-derivative **177** complex (salmon) and derivative **177** (yellow).[114]

The simulated free energy of binding for these derivatives was calculated at −71.75 kcal^{-1} mol^{-1} for pyrophosphate **177** and −80.62 kcal^{-1} mol^{-1} for triphosphate **178**, more favourable than the values calculated for Ins(1,3,4,5)P_4 **13**. However, upon analysis of the MD simulations of these derivatives, the pyrophosphate and triphosphate groups were found to have

increased binding interactions with Arg86, previously seen to form strong interactions with the 4-position phosphate. In addition, these derivatives did not show any increased binding interactions with Gln79 as desired, with calculated distances of 8.8 Å and 8.9 Å separating the terminal phosphate and Gln79 for pyrophosphate **177** and triphosphate **178** respectively (Figure 3.7 and Figure 3.8).

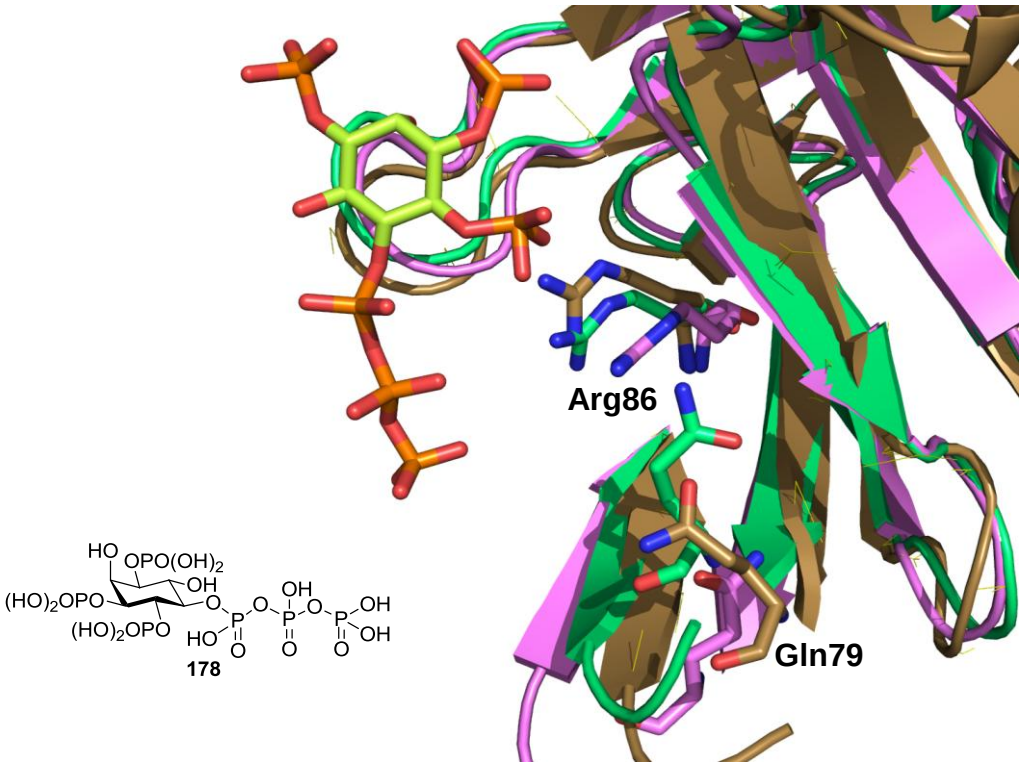


Figure 3.8. PyMOL representation of a 150 ns molecular dynamics simulation of derivative **178** using Amber 11, illustrating *apo* PKB PH domain (lilac), PKB PH domain-Ins(1,3,4,5)P_4 complex (green), PKB PH domain-derivative **178** complex (brown) and derivative **178** (yellow).[114]

To overcome the problems observed with increased binding of Arg86 with the pyrophosphate and triphosphate derivatives **177** and **178**, we proposed incorporating long-chain amide moieties on the 5-position phosphate to form

the desired hydrogen bonding interaction with Gln79, as amide groups are well documented to form hydrogen bonding interactions with glutamine residues (Figure 3.9).

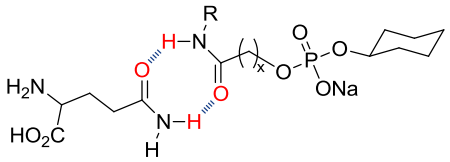


Figure 3.9. Proposed hydrogen bonding interactions between Gln79 and amide derivatives.

In order to probe the desired chain length between the amide group and 5-position phosphate, several derivatives with varying chain lengths were proposed and analysed. MD calculations indicated that amide derivative **179** had a simulated free energy of binding of −88.49 kcal^{-1} mol^{-1}, significantly more favourable than the values calculated for Ins(1,3,4,5)P_4 **13**. This derivative was also predicted to form the desired binding interactions with Gln79. This resulted in the 3.4 Å movement of Gln79 towards the inositol binding site compared to the Ins(1,3,4,5)P_4-bound complex. The movement of loop 3 also caused an unexpected increase in hydrogen bonding interactions between Asn54 and Trp80, as the distance between these residues was decreased from 12.5 Å in the Ins(1,3,4,5)P_4-bound complex to 7.7 Å. This additional interaction might help stabilise the new protein conformation, and could contribute to the increased binding affinity predicted for this derivative **179** (Figure 3.10).

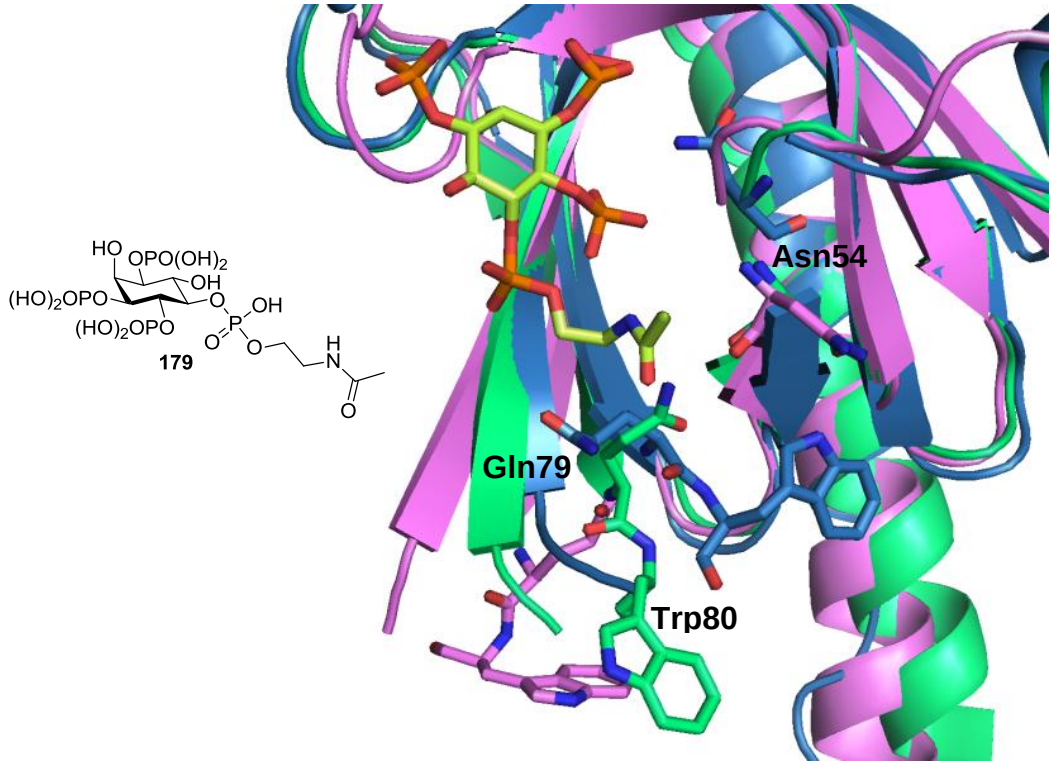
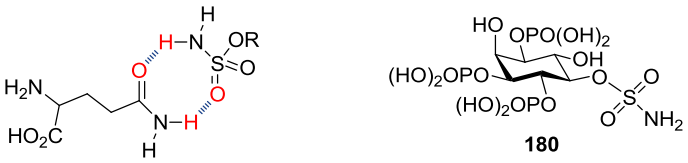


Figure 3.10. PyMOL representation of a 150 ns molecular dynamics simulation of derivative **179** using Amber 11, illustrating *apo* PKB PH domain (lilac), PKB PH domain-Ins(1,3,4,5)P_4 complex (green), PKB PH domain-amide **179** complex (blue) and amide **179** (yellow), showing ligand-induced hydrogen bonding to Gln79, and increased interactions between Asn54 and Trp80.[114]

The encouraging result observed with amide derivative **179** prompted us to propose the incorporation of other strong hydrogen bond donors and acceptors at the 5-position. As a phosphate isostere, sulfamate groups can form similar strong hydrogen bonding interactions with Gln79 (Figure 3.11).[140] As this isostere had already been successfully incorporated on the 3-position of Ins(1,3,4,5)P_4 (see Chapter 2), we proposed installing a sulfamate isostere on the 5-position.



180

Figure 3.11. Proposed hydrogen bonding interactions between Gln79 and sulfamate derivative **180**.

The sulfamate derivative **180** had been previously synthesised in the Conway group, and in fluorescence quenching experiments, found to have moderate binding to the PKB PH domain at concentrations above 10 μM.[53] However, this derivative was never investigated for its ability to bind Gln79 or other residues on loop 3, and so we felt that its synthesis would be of interest to this project.

Upon carrying out MD simulations of the sulfamate derivative **180**, the simulated free energy of binding was calculated at −71.96 $kcal^{-1}$ mol^{-1}. This value is less favourable than the values calculated for Ins(1,3,4,5)P_4 **13**, further reinforcing that loss of the phosphate group at the 5-position reduces the hydrogen bonding interactions with the PKB PH domain and decreases binding affinity. In addition, the sulfamate derivative **180** did not show any binding interaction with Gln79 as desired (Figure 3.12).

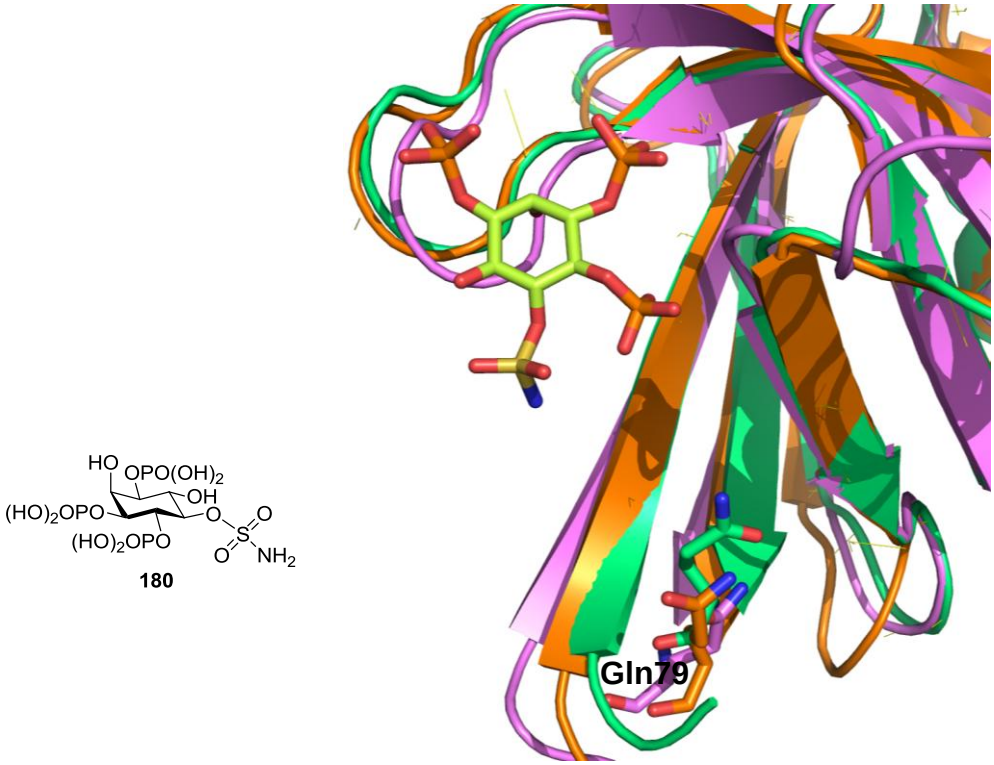
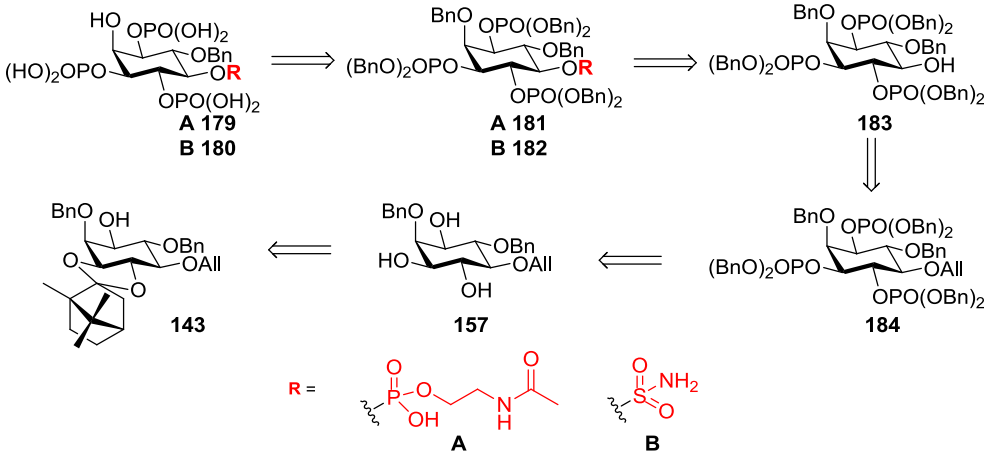


Figure 3.12. PyMOL representation of a 150 ns molecular dynamic simulation of derivative **180** using Amber 11, illustrating *apo* PKB PH domain (lilac), PKB PH domain-Ins(1,3,4,5)P_4 complex (green), PKB PH domain-sulfamate **180** complex (orange) and amide **180** (yellow).[114]

However, as the sulfamate derivative **180** had previously shown interesting biological testing results, we decided to attempt its synthesis to use as a comparison between the binding affinity data and the MD simulations.

3.3. Synthetic Aims

With the desired target compounds identified, retrosynthetic routes for the synthesis of desired products **179** and **180** were proposed (Scheme 3.1).

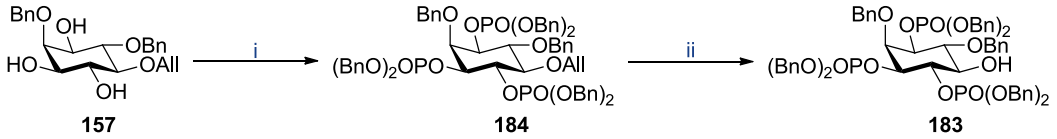


Scheme 3.1. Retrosynthetic analysis for synthesis of 5-position derivatives **179** and **180**.

The final compounds **179** and **180** could be afforded through hydrogenolysis of their benzyl-protected precursors. The protected intermediates **181** and **182** can both be synthesised through modification of alcohol **183**, which can be synthesised in two steps from triol **157**. The complex synthesis of triol **157** involving the resolution of the racemic *myo*-inositol starting materials through the use of a camphor auxiliary is described in Chapter 2.

3.4. Synthesis of Intermediate 183

Starting with triol **157**, we attempted to use similar phosphoramidite conditions to those described in Chapter 2 to achieve phosphorylation of this intermediate in high yield. This reaction proceeded smoothly, and phosphorylated intermediate **184** was isolated in 89% yield after oxidative work-up and purification by silica gel column chromatography (Scheme 3.2).



157 184 183

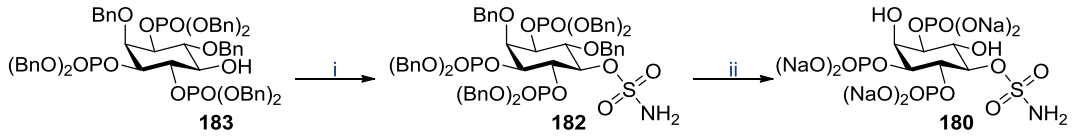
Scheme 3.2. Synthesis of key intermediate **183**. *Reagents and conditions*: i) a) **167** (7.5 eq.), 5-phenyl-1*H*-tetrazole (7.5 eq.), CH_2Cl_2, RT, 14 h; b) *m*CPBA (7.5 eq.), −78 °C→RT, 2 h, 89%; ii) $PdCl_2$ (1.0 eq.), MeOH, RT, 3 h, 67%.

The next step was to deprotect the allyl group at the 5-position, to afford the desired free hydroxyl group at this position. It was proposed to use the deallylation conditions that were optimised in the deallylation of intermediate **164** in Chapter 2, and so stirred the protected intermediate **184** with palladium(II) chloride at room temperature for three hours. After this time, complete conversion to the desired product **183** was observed, and purification by silica gel column chromatography afforded the desired alcohol **183** in excellent yield. Fortunately, no migration of the phosphate groups was observed by ^{1}H and ^{31}P NMR spectroscopy when using these reaction conditions.

3.5. Synthesis of Sulfamate Derivative 180

As the successful incorporation of the sulfamate isostere had been achieved at the 3-position, it was decided to attempt the synthesis of derivative **180** first. Sulfamoyl chloride **173** was freshly prepared from chlorosulfonyl isocyanate **173** as before (see Chapter 2), and reacted with alcohol **183** in DMA. Subsequent work-up and purification by silica gel column

chromatography afforded the desired sulfamate **182** in 74% yield (Scheme 3.3).

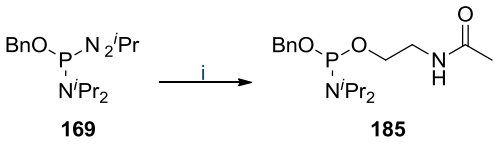


Scheme 3.3. Synthetic route to sulfamate derivative **180**. *Reagents and conditions*: i) **173** (2.0 eq.), DMA, 0 °C→RT, 5 h, 74%; ii) $NaHCO_3$ (6.0 eq), Pd (black) (16.0 eq.), H_2, ^tBuOH, MeOH, H_2O, RT, 12 h, 100%.

The resulting protected intermediate **182** underwent global deprotection, and hydrogenolysis in the presence of palladium black and sodium bicarbonate. This reaction afforded the desired final compound **180** as the sodium salt, in 100% yield and without the need for further purification.

3.6. Synthesis of Amide Derivative 179

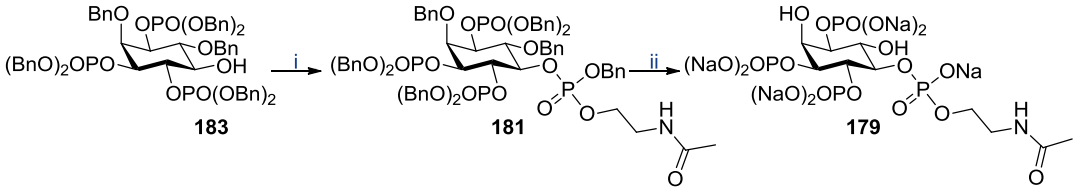
Following the successful synthesis of sulfamate **180**, the synthesis of desired 2-acetamidoethyl benzyl *N,N*-diisopropylphosphoramidite **185** was commenced. Similar to the conditions used for the preparation of phosphoramidite **171** in Chapter 2, a solution of commercially available *N*-acetylethanolamine was added dropwise to a solution of benzyloxy bis(*N*,*N*-diisopropylamino)phosphoramidite **169** and 1*H*-tetrazole (Scheme 3.4). This reaction was stirred at room temperature for 2 h, after which time, purification by silica gel column chromatography afforded the desired phosphoramidite **185** in 38% yield.



169 185

Scheme 3.4. Synthesis of phosphoramidite **185**. *Reagents and conditions*: i) 1*H*-tetrazole (0.4 eq.), *N*-acetylethanolamine (1.0 eq.), CH_2Cl_2, RT, 2 h, 38%.

Phosphoramidite **185** was reacted with the alcohol **183** in the presence of 1*H*-tetrazole at room temperature (Scheme 3.5). On consumption of the starting material, as monitored by TLC analysis, the reaction was cooled to −78 °C and the P(III) species oxidised using *m*CPBA. Subsequent work-up and purification by silica gel column chromatography afforded the desired phosphate ester **181** in 44% yield as a mixture of diastereomers (Scheme 3.5). No phosphate migration was observed during the reaction by either ^{1}H or ^{31}P NMR spectroscopy.



183 181 179

Scheme 3.5. Synthetic route to final compound **179**. *Reagents and conditions*: i) a) 1*H*-tetrazole (4.4 eq.), **185** (4.4 eq.), CH_2Cl_2, RT, 14 h; b) *m*CPBA (4.4 eq.), −78 °C→RT, 2 h, 44%; ii) $NaHCO_3$ (7.0 eq), Pd (black) (18.0 eq.), H_2, ^{t}BuOH, MeOH, RT, 12 h, 100%.

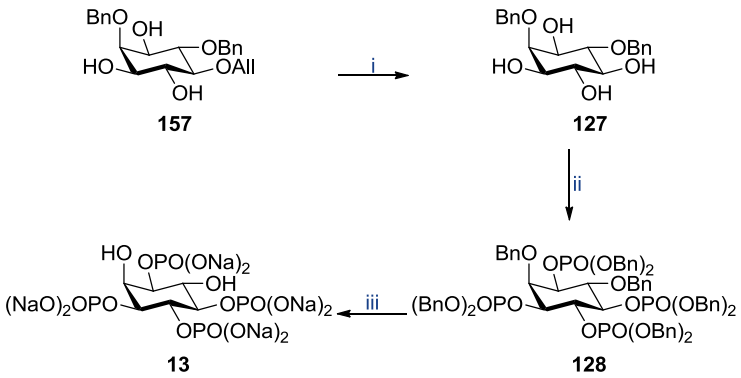
The final step involved global deprotection, under analogous conditions to those used for the sulfamate **182** above. Hydrogenolysis of protected intermediate **181** in the presence of palladium black and sodium bicarbonate afforded the desired final product **179** as the sodium salt, in 100% yield and without the need for further purification (Scheme 3.5).

3.7. Synthesis of Natural Product Ins(1,3,4,5)P_4 13

After the successful synthesis of target compounds **179** and **180**, the synthesis of the natural product Ins(1,3,4,5)P_4 **13** was proposed, using similar conditions to those employed in the synthesis of our final compounds. This compound could then be used as a positive control in the biological evaluation of **179** and **180**.

To synthesise Ins(1,3,4,5)P_4, the deallylation of triol **157** to afford alcohol **127** was proposed, which could then be phosphorylated and deprotected to afford Ins(1,3,4,5)P_4 **13** in significant quantities.

Therefore, triol **157** was subjected to the optimised deallylation conditions, and stirred with palladium(II) chloride in methanol for three hours. Complete deprotection of the starting material was observed, and subsequent work-up and purification by silica gel column chromatography afforded the desired alcohol **127** in 72% yield (Scheme 3.6)



Scheme 3.6. Synthetic route to natural product Ins(1,3,4,5)P_4 **13**. *Reagents and conditions*: i) $PdCl_2$ (0.6 eq.), MeOH, RT, 3 h, 72%; ii) a) **167** (10.0 eq.), 5-phenyl-1*H*-tetrazole (10.0 eq.), CH_2Cl_2, RT, 12 h; b) *m*CPBA (10.0 eq.), −78 °C→RT, 2 h, 58%; iii) $NaHCO_3$ (8.0 eq), Pd (black) (20.0 eq.), H_2, ^{t}BuOH, MeOH, RT, 12 h, 100%.

Alcohol **127** was then phosphorylated as described previously. Phosphoramidite **167** was freshly prepared, and stirred with alcohol **127** for 12 hours in the presence of 5-phenyl-1*H*-tetrazole. Oxidation by *m*CPBA at −78 °C and subsequent purification by silica gel column chromatography afforded the desired phosphorylated intermediate **128** in 58% yield (Scheme 3.6).

As before, the final step involved the global deprotection of the benzyl protecting groups. Hydrogenolysis of protected intermediate **128** in the presence of palladium black and sodium bicarbonate afforded the desired natural product **13** in 100% yield, without the need for further purification (Scheme 3.6).

3.8. Biological Evaluation of Inositol Phosphate Derivatives

The biological activity of 5-position Ins(1,3,4,5)P_4 derivatives **179** and **180** and 3-position Ins(1,3,4,5)P_4 derivatives **138** and **139** was investigated in a biochemical assay that was carried out by Mingxuan Wu and Dr Dorothea Fiedler at Princeton University. This assay used purified inactive PKB and activated PDK1. The two kinases were incubated in the presence of varying concentrations of each derivative, and inhibition of PKB phosphorylation at Thr308 was monitored using a phosphospecific antibody (Figure 3.13)

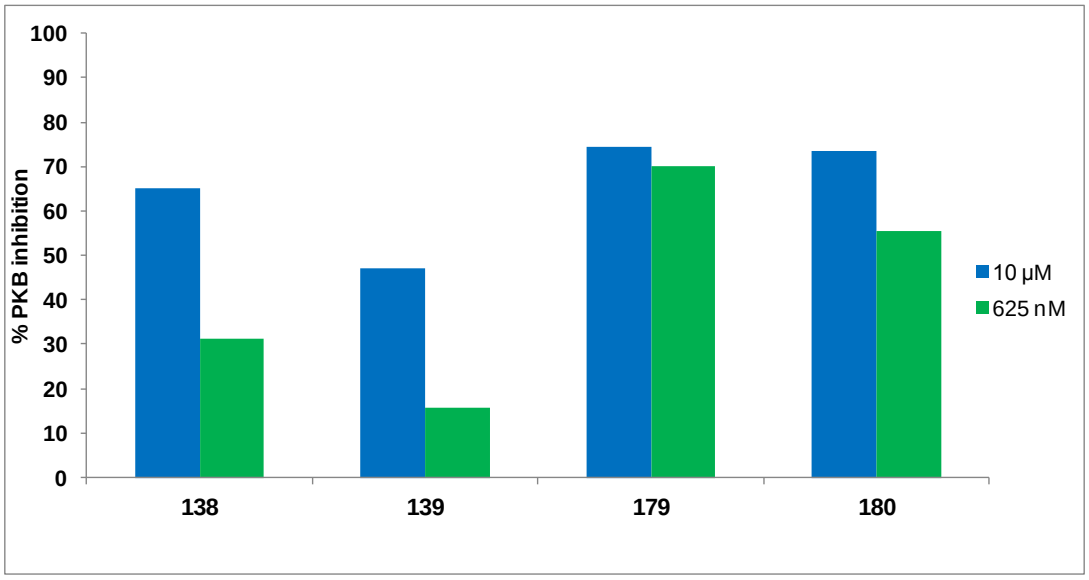


Figure 3.13. Percentage inhibition of PKB phosphorylation at Thr308 after incubating purified PKB with inositol phosphate derivative **138**, **139**, **179** and **180**.

From these preliminary results, it has been found that 5-position amide **179** demonstrated excellent inhibitory activity at both 10 µM and 625 nM concentrations. It is clear that using MD simulations to design inositol phosphate derivatives with high binding affinity with the PKB PH domain has been a successful approach, which will allow the synthesis of highly selective inositol phosphate derivatives in the future.

3.9. Summary

Several 5-position derivatives were proposed, which we hypothesised would form the desired hydrogen bonding interactions with Gln79, situated on variable loop 3 of the PKB PH domain. These derivatives were analysed in 150 ns molecular dynamic simulations, and the derivatives predicted to be most potent were selected for synthesis and biological evaluation. The synthesis of the enantiomerically pure 5-position alcohol **183** was achieved, which underwent successful modification to afford the two desired 5-position Ins(1,3,4,5)P_4 derivatives, phosphate ester **179** and sulfamate **180**. The successful synthesis of the natural product Ins(1,3,4,5)P_4 **13** was also achieved.

Chapter 4:

Synthesis and Biological Evaluation of Ins(1,3)P2

4. Synthesis and Biological Evaluation of Ins(1,3)P_2

4.1. Bone Morphogenetic Protein Signalling Pathway

In addition to playing a pivotal role in cell growth, proliferation and survival, (Chapter 2), the PI3K and PKB signalling pathway has been implicated in skeletal development and bone turnover, through its association with bone morphogenetic protein (BMP)-mediated osteoblast differentiation.[142-145] BMPs belong to the transforming growth factor-β (TGF-β) family, and were originally identified as factors that induced the formation of bone and cartilage when implanted at ectopic sites in rats.[146] Over 20 BMP ligands have been reported in the literature, all of which are recognised by two families of serine/threonine kinase receptors: type I receptors of about 50–55 kDa (BMPRII, ActRIIA and ActRIIB) and type II receptors of more than 70 kDa (ALK2, ALK3 and ALK6).[147-149]

Binding of the BMP ligands promotes the oligomerisation of type I and type II receptors, facilitated by members of the repulsive guidance molecule (RGM) family (Figure 4.1). Formation of these heteromers induces conformational changes of the receptor molecules, thus allowing the constitutively active type II receptors to phosphorylate type I receptors. These activated type I receptors then phosphorylate BMP-responsive regulatory Smad (R-Smad) effectors (Smad1, Smad5 and Smad8), which are anchored to the cell membrane through interactions with various cytoplasmic scaffolding proteins (Figure 4.1).[148,150-152]

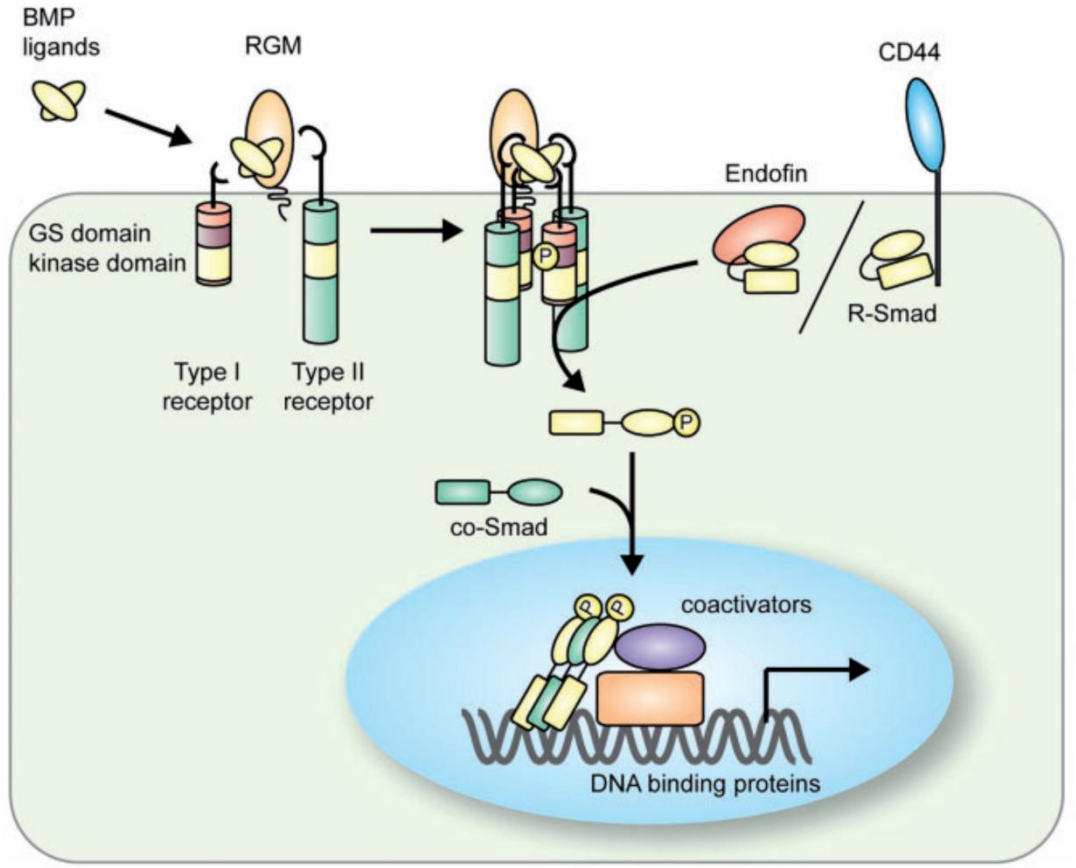


Figure 4.1. Outline of the BMP and Smad signal transduction pathway and regulation of downstream events.[148] Reproduced with permission © Oxford Journals.

These phosphorylated R-Smads then form a complex with Smad4, a common-partner Smad (co-Smad) that facilitates TGF-β signalling in mammals, and translocate to the nucleus, where they regulate transcription of target genes through direct binding to DNA, interaction with DNA-binding proteins and recruitment of transcriptional coactivators and/or corepressors (Figure 4.1).[148,150,151]

4.2. Introduction to Fibrodysplasia Ossificans Progressiva

Dysregulation of the BMP signalling pathway has been implicated in a number of skeletal and vascular abnormalities.[148] The most severe and disabling of these diseases is *fibrodysplasia ossificans progressiva* (FOP). FOP is a rare congenital disorder, resulting in progressive and widespread postnatal ossification of soft tissues, and no effective treatments are known.[153] FOP results in severe debilitation and reduced life expectancy due to joint fusion and restrictive lung disease with thoracic involvement.[153] In 2006, Shore *et al.* discovered that affected individuals harbour conserved mutations in the ACVR1 gene, which is thought to cause constitutive activation of the BMP type I receptor, activin receptor-like kinase-2 (ALK2).[153,154] Constitutive activation of ACVR1 upregulates BMP4 signalling, downregulates BMP antagonists, induces ectopic chondrogenesis and stimulates joint fusions.[154] Therefore, pharmacological tools that could selectively inhibit BMP signalling, but not TGF-β or other signal transduction pathways, could help to elucidate the role of BMP signalling in diverse physiological processes.

Yu *et al.* performed an *in vivo* screening approach to identify compounds that inhibited BMP signalling while selecting against those with nonspecific or undesirable biological effects.[150] Using this screening approach, they found that dorsomorphin **186** (Figure 4.2) acted as an inhibitor of BMP type I receptors ALK2, ALK3 and ALK6, and did not inhibit the function of ALK4, ALK5 and ALK7, the TGF-β type I receptors.[150]

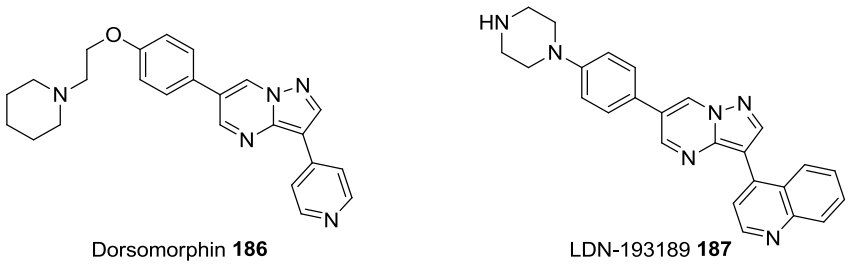


Figure 4.2. Structures of BMP receptor inhibitors dorsomorphin and LDN-193189.[150,153]

Optimisation of this inhibitor found that replacement of the pendent pyridine ring of dorsomorphin **186** with a 4-quinolinyl group improved potency, while replacement of the 2-(1-piperidinyl)ethoxy group with piperazine improved metabolic stability. The resulting compound, LDN-193189 **187** (Figure 4.2), was found to inhibit BMP4-mediated Smad1, Smad5 and Smad8 activation with greater potency than dorsomorphin while retaining 200-fold selectivity for the BMP signalling pathway over the TGF-β signalling pathway.[153] The clinical effectiveness of these compounds is now under investigation.[155]

4.3. Introduction to the FYVE Domain

After the discovery of dorsomorphin **186** and LDN-193189 **187** as inhibitors of BMP receptor-ligand interactions, we proposed that inhibition of other binding interactions involved in the BMP signalling pathway could also be investigated for their therapeutic potential. As discussed previously, several scaffolding proteins, such as endofin (endosome-associated FYVE-domain protein) and Hgs [a protein which was originally cloned as Hrs (hepatic growth factor-regulated tyrosine kinase substrate)], have been found to mediate BMP signal transduction through facilitating the interaction between

the R-Smads and the activated type I receptor, and the interaction between the activated R-Smads and Smad4.[151,156-158]

These proteins anchor to the internal surface of the phospholipid bilayer through specific interactions of their FYVE domains (small, cysteine-rich, zinc-binding domains named after their identification in the Fab1p, YOTB, Vac1p and EEA1 proteins), with phosphatidylinositol 3-phosphate [PtdIns(3)*P* **188**], which is found to be particularly abundant in membranes of early endosomes and other endocytic vesicles.[159-162] PtdIns(3)*P* production is regulated by the action of PI3K on phosphatidylinositol (PtdIns), implying that both FYVE domains and PH domains share general 3-phosphoinositide recognition principles.[159] We hypothesised that perturbing the binding interaction between PtdIns(3)*P* **188** and the FYVE domain could prevent recruitment of the scaffolding proteins to the cell membrane, thus inhibiting activation of the R-Smads and potentiation of the BMP signalling pathway. This hypothesis was reinforced upon the discovery that deletion of the FYVE domain of endofin significantly reduced its BMP transcriptional responses.[156]

4.4. Synthetic Aims

In order to probe the interaction of the FYVE domain with PtdIns(3)*P* **188**, we proposed to synthesise the headgroup of PtdIns(3)*P*, Ins(1,3)P_2 **189**. This soluble molecule would facilitate calculation of binding affinities to the FYVE domains of scaffolding proteins by isothermal titration calorimetry, and

aid crystallisation of these proteins complexed with the PtdIns(3)*P* headgroup.

Upon retrosynthetic analysis of the desired product, Ins(1,3)P_2, we proposed a novel synthetic route building on our previous experience of the synthesis of inositol polyphosphates. It was hypothesised that Ins(1,3)P_2 **189** could be afforded through hydrogenolysis of the benzyl-protected precursor **190** (Figure 4.3). This protected intermediate could be synthesised through phosphorylation of diol **191**, which could be afforded from the methanolysis of protected intermediate **192**. This desired intermediate could be synthesised by selective cleavage of inositol orthoformate **194** and subsequent benzylation of the resulting alcohol **193**. This desired inositol orthoformate **194** could be afforded from the perbenzylation of orthoformate **144**, which can be synthesised from *myo*-inositol in the same manner as described previously in Chapter 2.

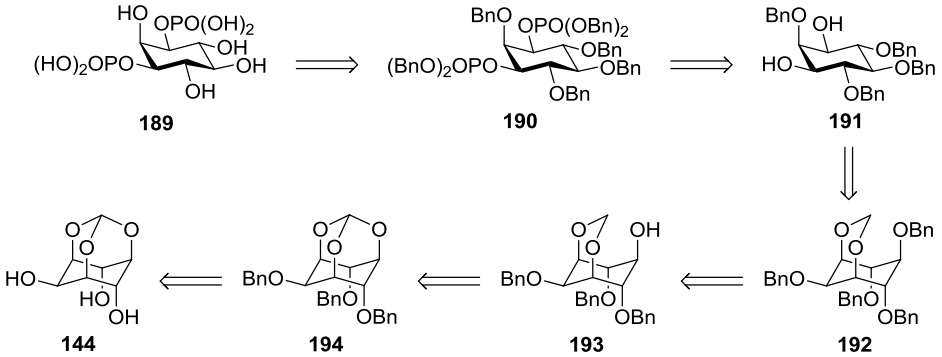
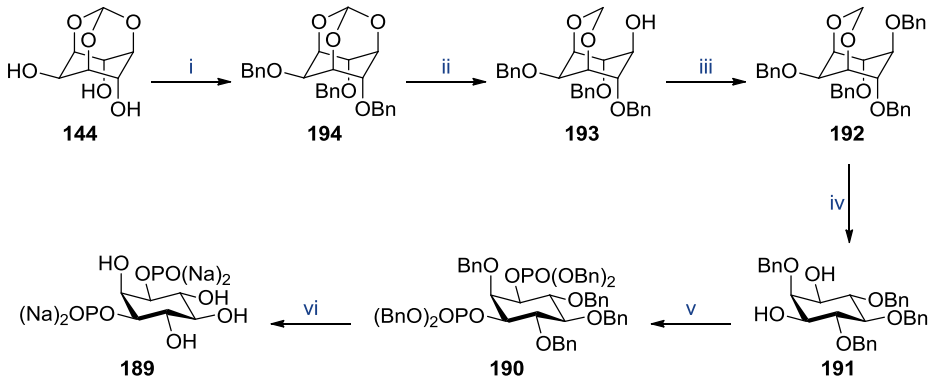


Figure 4.3. Retrosynthetic analysis for synthesis of Ins(1,3)P_2 **189**.

4.5. Synthesis of Ins(1,3)P_2

The synthesis of natural product Ins(1,3)P_2 **189** commenced with the synthesis of inositol orthoformate **144** from *myo*-inositol **1**, following conditions published by Billington *et al.* and discussed in Chapter 2.[125] Orthoformate **144** was then treated with sodium hydride and benzyl bromide at 0 °C, and perbenzylation afforded protected orthoformate **194** in 85% yield (Scheme 4.1).



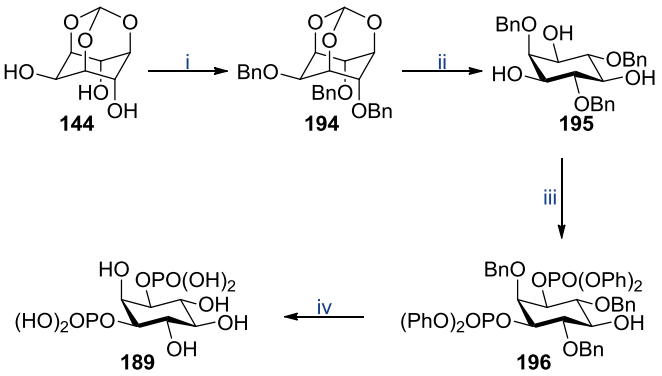
Scheme 4.1. Synthesis of Ins(1,3)P_2 **189**. *Reagents and conditions*: i) NaH (5.0 eq.), BnBr (5.0 eq.), DMF, 0 °C→RT, 12 h, 85%; ii) DIBAL (2.5 eq.), CH_2Cl_2, 0 °C→RT, 5 h, 96%; iii) NaH (2.0 eq.), BnBr (2.0 eq.), DMF, 0 °C→RT, 12 h, 93%; iv) conc. HCl (aq.), MeOH, reflux, 12 h, 77%; v) a) **167** (7.0 eq.), 1*H*-tetrazole (7.0 eq.), CH_2Cl_2, RT, 14 h; b) *m*CPBA (7.0 eq.), −78 °C→RT, 2 h, 77%; vi) $NaHCO_3$ (4.0 eq.), Pd black (16.0 eq.), H_2, ^{t}BuOH, H_2O, RT, 12 h, 100%.

Orthoformate **194** was subjected to the DIBAL reduction conditions discussed in Chapter 2, and selective cleavage of the orthoformate moiety afforded alcohol **193** in 96% yield. Subsequent benzylation of alcohol **193** with benzyl bromide in the presence of sodium hydride afforded the fully protected derivative **192** in 93% yield. Methanolysis of intermediate **192** in

the presence of concentrated hydrochloric acid then afforded the desired alcohol **191** in 77% yield. Phosphorylation of diol **191** was obtained using phosphoramidite chemistry as described earlier. Bis(benzyloxy)-*N*,*N*-diisopropylamino phosphine **167** was synthesised and immediately reacted with alcohol **191** in the presence of 1*H*-tetrazole in dichloromethane. Once consumption of the starting material was observed by TLC analysis, the reaction was cooled to −78 °C and oxidised using *m*CPBA. After warming the reaction mixture to room temperature, work-up and purification by silica gel column chromatography afforded the desired phosphorylated product **190** in 77% yield (Scheme 4.1). The final step involved the global deprotection of the benzyl protecting groups. Hydrogenolysis of protected intermediate **190** in the presence of palladium black and sodium bicarbonate successfully afforded the desired final product Ins(1,3)P_2 **189** in 100% yield as the sodium salt, without the need for further purification (Scheme 4.1).

4.6. Comparison to Other Synthetic Routes

Other synthetic routes to Ins(1,3)P_2 **189** have been reported, the earliest of which was published by Billington *et al.* in 1987.[3,163] This synthesis preceded the publication by Gilbert and co-workers of the selective DIBAL reduction of the orthoformate protecting group,[52,126,127] and so, although two steps shorter than the synthetic route discussed above, this synthetic route resulted in a lower overall yield (Scheme 4.2).

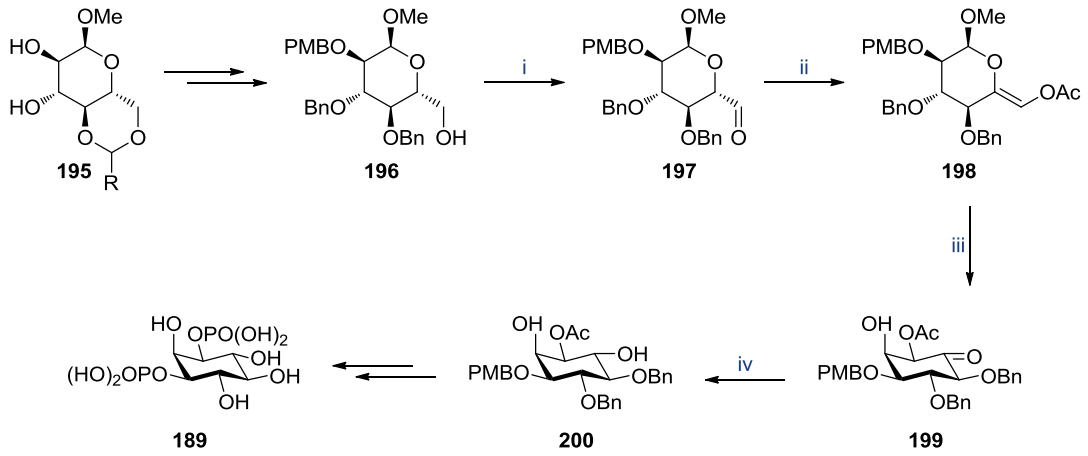


Scheme 4.2. Synthesis of Ins(1,3)P_2 **189** as reported by Billington *et al.*[3,163] *Reagents and conditions*: i) NaH (3.0 eq.), BnBr (4.0 eq.), DMF, RT; ii) 0.1 M HCl, MeOH, reflux, 15 min, 87%; iii) $(PhO)_2POCl$, CH_2Cl_2, NEt_3, DMAP, 46%; iv) Li in liquid NH_3, −78 °C, 100%.

In this synthesis, Billington and co-workers subject benzyl-protected intermediate **194** to acidic methanolysis conditions, which affords the corresponding triol **195**. They then attempt selective phosphorylation of the 1- and 3-positions in the presence of the 5-position hydroxyl group, using diphenyl chlorophosphate. This phosphorylation is achieved in 46% yield, which results in a 34% overall yield of Ins(1,3)P_2 **189** from orthoformate **144** (Scheme 4.2).[3,163] However, in contrast, our method involving selective cleavage of orthoformate **194** and phosphorylation of the 1- and 3-position hydroxyl groups using standard phosphoramidite chemistry allowed the successful synthesis of Ins(1,3)P_2 **189** in 45% overall yield from orthoformate **144** (Scheme 4.2).

The synthesis of Ins(1,3)P_2 **189** was also reported by Chen and co-workers in 1998, as part of their synthesis of PtdIns(3)*P* **188** and PtdIns(4)*P* derivatives.[99] Starting from methyl α-D-glucopyranoside **195**, protecting group manipulations and oxidation of the primary alcohol afforded

intermediate **198**, which was subjected to Ferrier rearrangement conditions (Scheme 4.3).



Scheme 4.3. Synthesis of Ins(1,3)P_2 **189** as reported by Chen *et al.*[99] *Reagents and conditions*: i) oxalyl chloride, DMSO, CH_2Cl_2, −78 °C, then NEt_3, 1 h; ii) K_2CO_3, Ac_2O, CH_3CN, reflux, 12 h, 85%; iii) $Hg(OAc)_2$, acetone–water (3:2), 45 min, followed by sat. NaCl solution, rt, 12 h, 77%; iv) $NaBH(OAc)_3$, CH_3CN, AcOH, 45 min, 81%.

This reaction generated the desired inositol ring **199**, the selective reduction of which afforded key intermediate **200**, allowing the synthesis of a range of phosphatidylinositol derivatives. However, in this route, the synthesis of Ins(1,3)P_2 **189** was only achieved in 6% overall yield from methyl α-D-glucopyranoside **195**.

4.7. Biological Evaluation of Ins(1,3)P_2

With its successful synthesis in hand, studies to elucidate the role of Ins(1,3)P_2 **189** in the BMP signalling pathway and validate Ins(1,3)P_2 as a therapeutically relevant target were undertaken. To establish the binding

affinity of Ins(1,3)P_2 for the FYVE domain of scaffolding protein endofin, isothermal titration calorimetry (ITC) was employed (Figure 4.4). This work was carried out by Dr Alex Bullock and Dr Eleanor Williams at the Oxford Structural Genomics Consortium.

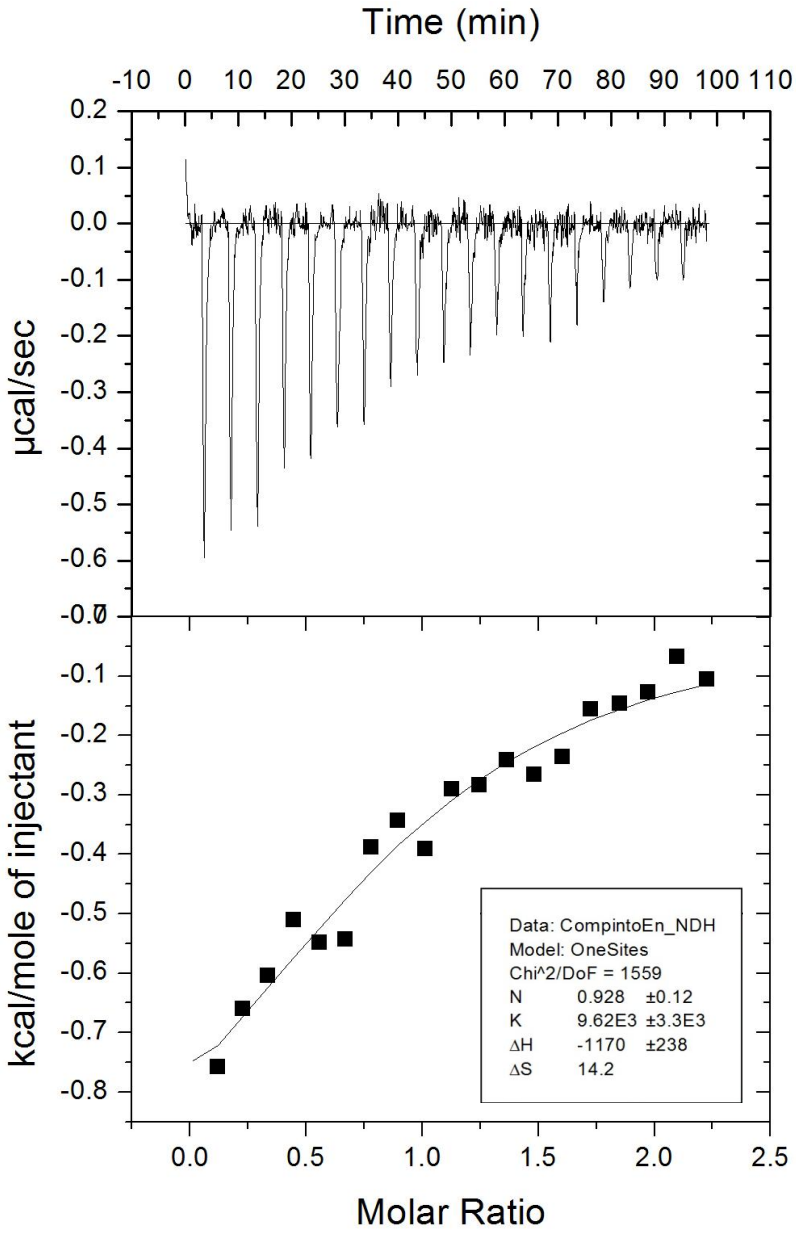


Figure 4.4. ITC data of Ins(1,3)P_2 **189** binding to the FYVE domain of endofin.

ITC established that Ins(1,3)P_2 **189** bound to the FYVE domain of endofin with a K_D value of 103 µM, a figure that is comparable to results published by Dumas *et al.*, who observed a K_D of 24 µM for Ins(1,3)P_2 **189** binding to the FYVE domain of EEA1.[164] However, these results suggest an important role for the phospholipid chain in recognition and overall affinity, as Sankaran *et al.* observed a considerably lower K_D value of 2.5 µM for PtdIns(3)*P* **188** binding to the analogous FYVE domain of Hgs.[165]

With these results in hand, our next aim was to obtain a crystal structure of Ins(1,3)P_2 **189** in complex with the FYVE domain of a scaffolding protein involved in the BMP signalling pathway. As initial attempts to crystallise Ins(1,3)P_2 **189** in complex with endofin proved unsuccessful, an alternative FYVE domain-containing protein, Hgs, was investigated, as successful crystallisation of this scaffolding protein was previously achieved in the absence of Ins(1,3)P_2 **189**.[166] To establish the binding affinity of Ins(1,3)P2 for the FYVE domain of Hgs, ITC was again employed (Figure 4.5).

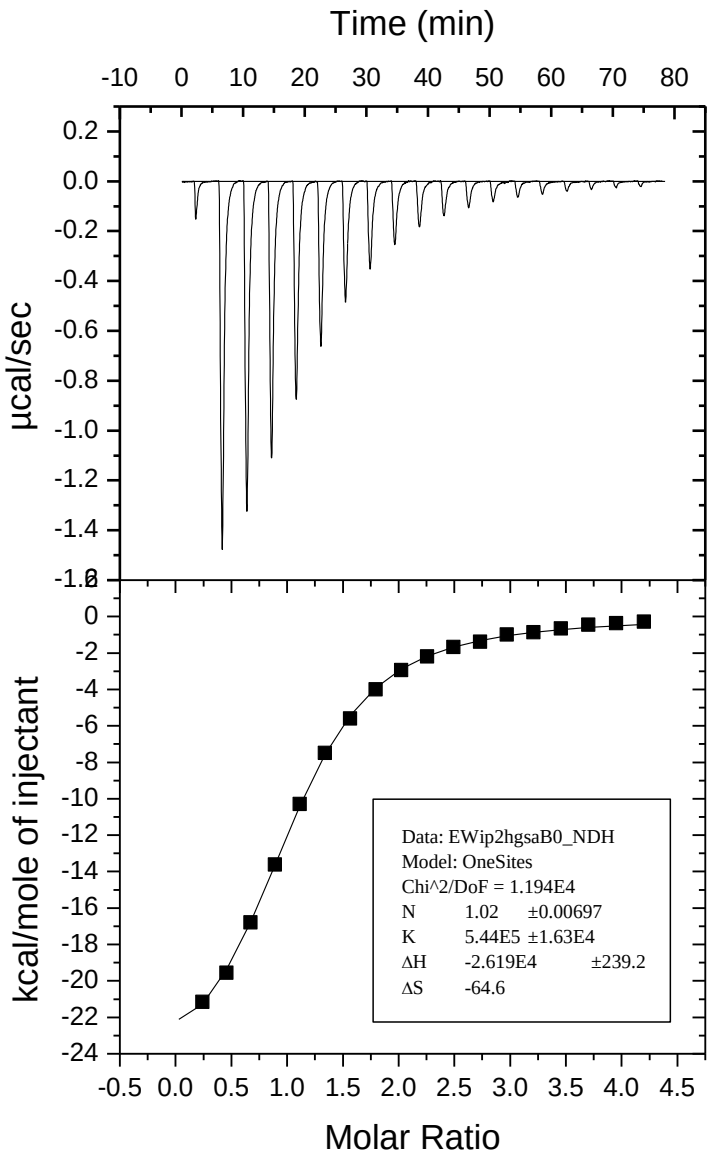


Figure 4.5. ITC data of Ins(1,3)P_2 **189** binding to the FYVE domain of Hgs.

This ITC study established that Ins(1,3)P_2 **189** bound to the FYVE domain of Hgs with a K_D value of 1.8 µM, indicating superior binding of Ins(1,3)P_2 **189** to the FYVE domain of Hgs over that of endofin. These results indicated that attempts to obtain an X-ray crystal structure with Ins(1,3)P_2 **189** and Hgs could be more successful. Hgs was found to crystallise well with Ins(1,3)P_2 **189**, and an X-ray crystal structure of the complex was solved (Figure 4.6).

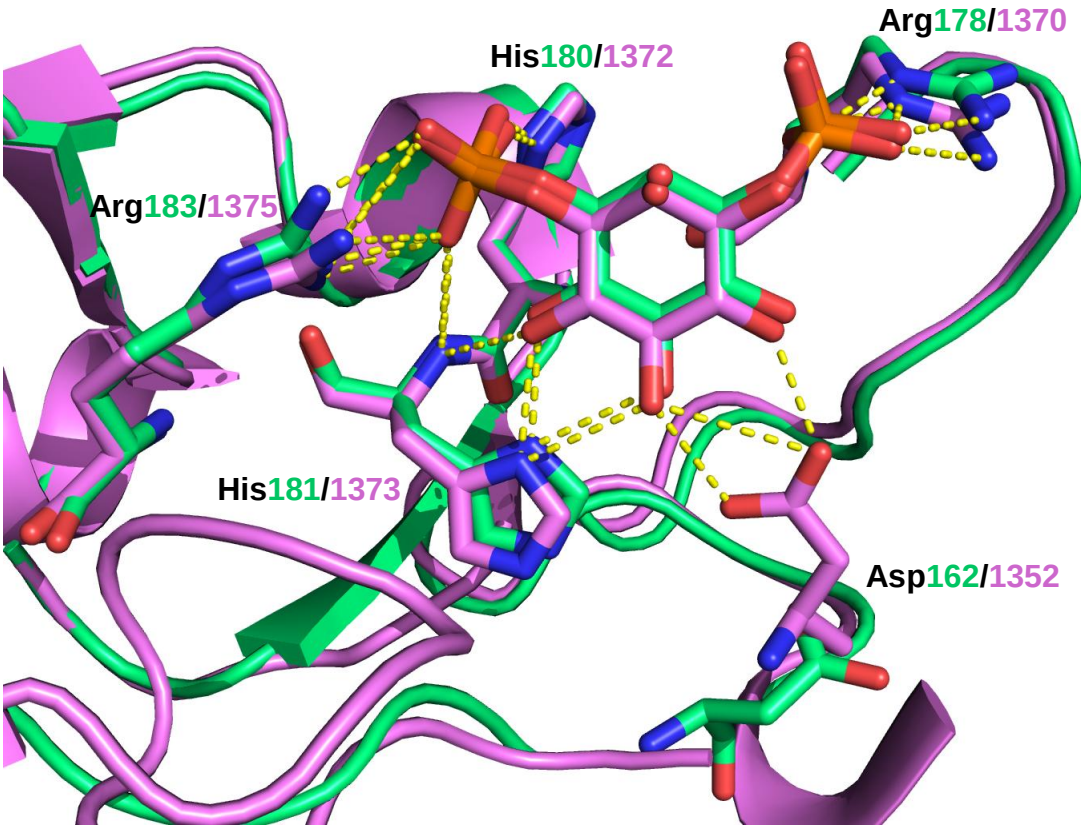


Figure 4.6. X-Ray crystal structures of Ins(1,3)P_2 **189** bound to FYVE domain of Hgs (green, PDB code: 4AVX) and Ins(1,3)P_2 **189** bound to FYVE domain of EEA1 (lilac, PDB code: 1JOC),[164] with key residues highlighted.

The 1.7 Å resolution of this X-ray crystal structure clearly shows that Ins(1,3)P_2 **189** binds to the FYVE domain of Hgs in a similar manner to that observed with Ins(1,3)P_2 **189** and the FYVE domain of EEA1 (Figure 4.6).[164,167] In this crystal structure, Arg178 forms hydrogen bonding interactions with the 1-position phosphate, while the 3-position phosphate occupies a shallow pocket derived from His180, His181 and Arg183. The 4- and 5-position hydroxyl groups are also found to participate in hydrogen bonding interactions with the imidazole side chain of His181 (Figure 4.6).

However, this crystal structure also shows a significant difference between the FYVE domains of EEA1 and Hgs upon binding to Ins(1,3)P_2 **189**. In the Hgs crystal structure, no interactions between the carboxylate oxygens of Asp162 and the 5- and 6-position hydroxyl groups of Ins(1,3)P_2 are observed, due to the distance of 7.2 Å between the 5-position hydroxyl group and Asp162 in this crystal structure (Figure 4.6). In the EEA1 crystal structure, a distance of 5.7 Å is observed between the 5-position hydroxyl group and Asp1352, thus allowing the 5- and 6-position hydroxyl groups to interact with the carboxylate oxygens of Asp1352.[164,167]

This difference in hydrogen bonding interactions between the 5- and 6-position hydroxyl groups and the conserved aspartic acid residue might be due to the absence of a short α-helical segment in the linker region of the Hgs FYVE domain compared to the EEA1 FYVE domain, caused by a three residue deletion in Hgs relative to the majority of FYVE domains.[164]

Therefore, probing this hydrogen bonding interaction may be a good starting place to investigate selectivity for Hgs over other FYVE domain-containing proteins, and design compounds that specifically inhibit the BMP signalling pathway.

4.8. Future Work

Based on the differences in binding of Ins(1,3)P_2 **189** to the FYVE domains of Hgs and EEA1, we proposed the synthesis of derivatives of Ins(1,3)P_2

189 with appropriate modifications, which could selectively bind with the Hgs FYVE domain.

The incorporation of bulky substituents on the 1-position phosphate or the 5- and 6-position hydroxyl groups could prevent binding of the Ins(1,3)P_2 derivative to other FYVE domain-containing proteins through disruption of the aspartic acid–hydroxyl group hydrogen bonding interactions; while incorporation of extended hydroxyl groups at the 5- and 6-positions could pick up additional hydrogen bonding interactions with Asp162, thus increasing the binding affinity of this derivative to the Hgs FYVE domain. The successful synthesis of these Ins(1,3)P_2 derivatives would then allow a full investigation of the critical hydrogen bonding interactions between Ins(1,3)P_2 and the Hgs FYVE domain.

4.9. Summary

In conclusion, the headgroup of PtdIns(3)*P*, Ins(1,3)P_2 **189**, was successfully synthesised in seven steps, starting from *myo*-inositol **1**. The synthesis of this natural product was achieved in 45% overall yield from the orthoformate **144**, which represents the highest yielding synthetic route to this derivative reported to date. ITC was undertaken to investigate the interaction of Ins(1,3)P_2 **189** with the FYVE domain of endofin, and an X-ray crystal structure was obtained of Ins(1,3)P_2 **189** bound to FYVE domain of Hgs. These results will now facilitate the synthesis and biological evaluation of derivatives of Ins(1,3)P_2.

Chapter 5:

Towards the Synthesis of PP-InsP_5 derivatives

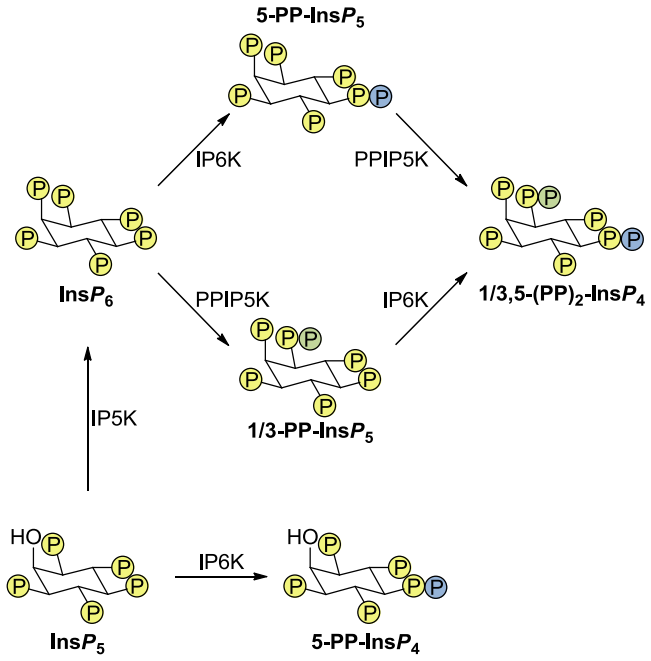
5. Towards the Synthesis of PP-InsP_5 derivatives

5.1. Introduction to Inositol Pyrophosphates

After the discovery of the inositol phosphates as important signalling molecules, it was widely believed that InsP_6 was the endpoint of *myo*-inositol metabolism. However, in the early 1990s, it became clear that this preconceived limit of six phosphates around the inositol ring was incorrect, due to the discovery of inositol pyrophosphates.[168-171] These pyrophosphates were first identified in 1993 and characterised as diphosphorylated and bisdiphosphorylated derivatives of InsP_5 and InsP_6: PP-InsP_5, PP-InsP_4 and $(PP)_2$-InsP_4.[172,173]

PP-InsP_5 (also known as InsP_7) and $(PP)_2$-InsP_4 (known as InsP_8) remain the best characterised naturally occurring inositol pyrophosphates.[172-176] Their biosynthesis is dependent on two classes of enzymes: the inositol hexakisphosphate kinases 1, 2 and 3 (IP6K1, IP6K2 and IP6K3)[177,178] and the diphosphoinositol pentakisphosphate kinases 1 and 2 (PPIP5K1 and PPIP5K2).[175,176] The biosynthesis of $(PP)_2$-InsP_4 involves two complementary metabolic pathways (Scheme 5.1). In the initial pathway, InsP_6 is phosphorylated by IP6K at the 5-position of the inositol ring to form a single isomer of PP-InsP_5.[177,178] This isomer is then further phosphorylated by PPIP5K to form $(PP)_2$-InsP_4.[175,176,179] In the alternate pathway, PPIP5K acts on InsP_6 to form an alternative isomer of PP-InsP_5, to which IP6K adds a phosphate at the 5-position. Although there is experimental evidence for

both metabolic pathways, it has been determined that the catalytic selectivity of the PPIP5K kinase domain for 5-PP-InsP_5 is 2.5-fold higher than the corresponding value for InsP_6,[176,180,181] indicating that the first metabolic pathway is kinetically dominant.



Scheme 5.1. Metabolic pathways involved in the synthesis of PP-InsP_5 and (PP)$_2$-InsP_4. Adapted from Shears *et al.*[182]

5.2. Inositol Pyrophosphates as Signalling Molecules

The free energy of hydrolysis of the diphosphate bond of PP-InsP_5 has been estimated computationally at 6.6 kcal mol^{-1}, higher than that of adenosine 5'-diphosphate (ADP) (6.4 kcal/mol) but lower than that of adenosine 5'-triphosphate (ATP) (7.3 kcal/mol).[172] In 1996, Voglmaier and co-workers

proposed that due to this latent energy, the inositol pyrophosphates could function as high-energy phosphate donors in a similar manner to ATP.[183]

This hypothesis was confirmed by Saiardi *et al.* using ^{32}P labelling studies to demonstrate that the β-phosphate of the pyrophosphate moiety is transferred to substrate proteins.[184,185] Unlike phosphate transfer from ATP, this phosphorylation is *independent* of protein kinase activity.[184] Protected by their negative charges from attack by water and other nucleophiles, phosphoric anhydrides are considered chemically stable *in vivo*. Therefore, phosphoryl transfer reactions allow the transmission of relatively large amounts of free energy, allowing the phosphate anhydrides to function as a cellular ‘‘currency of energy’’. Consequently, phosphoryl transfer reactions are coupled to thermodynamically unfavourable metabolic reactions, which makes the overall process thermodynamically favourable.[140,186] Saiardi *et al.* found that co-ordination of a magnesium ion is essential for phosphate transfer to occur.[184] The site of phosphorylation is a serine surrounded by acidic residues, and these acidic residues might play a role in coordinating the magnesium ions required for the reaction to take place.[184]

For phosphate transfer to occur, the substrate protein also has to be ‘primed’ by an initial casein kinase 2 (CK2)-dependent phosphorylation event. Therefore, the inositol pyrophosphate further phosphorylates the serine that is initially phosphorylated by CK2.[185,187] The discovery of this nonenzymatic diphosphorylation of serine proposed a novel idea concerning

the molecular action of inositol pyrophosphates and a novel mechanism of covalent modification (Figure 5.1, A).

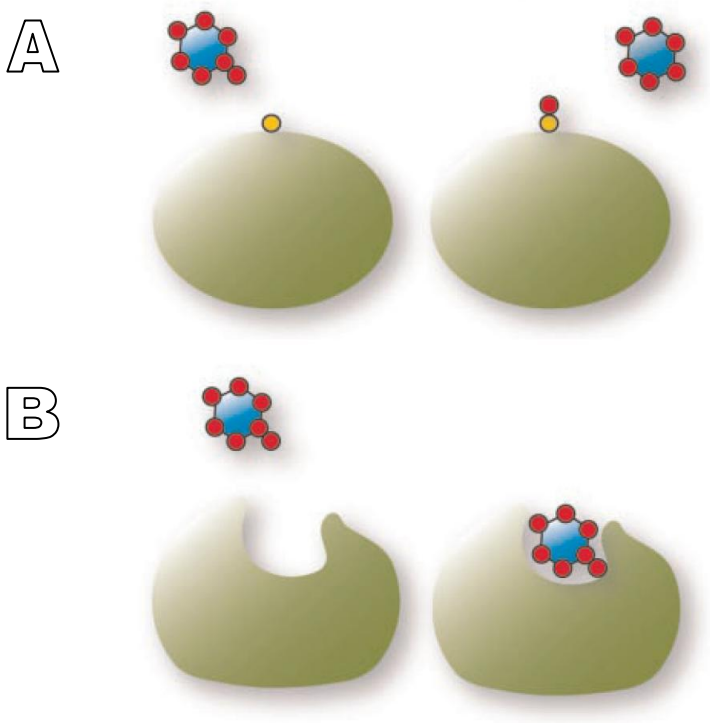


Figure 5.1. Proposed signalling pathways for inositol pyrophosphates: a) Nonenzymatic diphosphorylation, b) Protein-binding interactions.[188] Reproduced with permission © John Wiley & Sons, Inc.

While this phosphate transfer pathway is thought to mediate many of the cellular processes regulated by InsP_7 and InsP_8 in eukaryotes, such as apoptosis, vesicle trafficking, cytoskeletal dynamics, exocytosis, insulin signalling and neutrophil activation,[141,182,188,189] another signalling pathway has also been suggested for the inositol pyrophosphates, protein-binding interactions (Figure 5.1, B).

These protein-binding interactions were confirmed in 2003, when Luo and co-workers reported that 5-PP-InsP_5 bound to the PH domain of CRAC with affinities similar to that of Ins(1,3,4,5)P_4 **13**.[190] This evidence suggests that the competition between 5-PP-InsP_5 and PtdIns(3,4,5)P_3 for binding to this PH domain could regulate the intracellular distribution of CRAC, which is a key factor in determining the directionality of chemotaxis of *Dictyostelium*.[190] Another receptor for InsP_7 was also discovered by Lee *et al.*[179,191,192] In this work, 1/3-PP-InsP_5 was shown to bind to the Pho80/Pho85/Pho81 cyclin-dependent kinase/cyclin kinase inhibitor complex, augmenting the inhibitory activity of Pho81, so that it no longer hyperphosphorylates the transcription factor Pho4.[191,192]

Even so, a criticism of this proposed signalling pathway is that even the most abundant of the inositol pyrophosphates, PP-InsP_5, is present in cells at 20-fold lower levels than InsP_6.[174] Therefore, a receptor for one inositol pyrophosphate would have to be remarkably selective to prevent competition or inhibition from InsP_6, as even InsP6K and PPIP5K kinases recognise both InsP_6 and PP-InsP_5.[180] Gokhale *et al.* have recently discovered that the biosynthesis of these inositol pyrophosphates takes place in spatially-restricted compartments, allowing the generation of locally elevated levels of pyrophosphates, thus alleviating the problems associated with competitive binding of InsP_6.[180] It is also possible that co-localisation of the synthesis of the desired pyrophosphate with its protein target could facilitate signalling selectivity.[180] These proposals support the theory that inositol pyrophosphates act as ligands for a number of protein targets.

5.3. Inositol Pyrophosphates in PKB Binding Interactions

In 2010, Chakraborty and co-workers determined that 5-PP-InsP_5 also binds to the PH domain of PKB. When 5-PP-InsP_5 and PKB were preincubated together, 5-PP-InsP_5 was found to act as an inhibitor of PKB, by preventing its recruitment to the plasma membrane and subsequent phosphorylation and activation by PDK1 (Figure 5.2).[141]

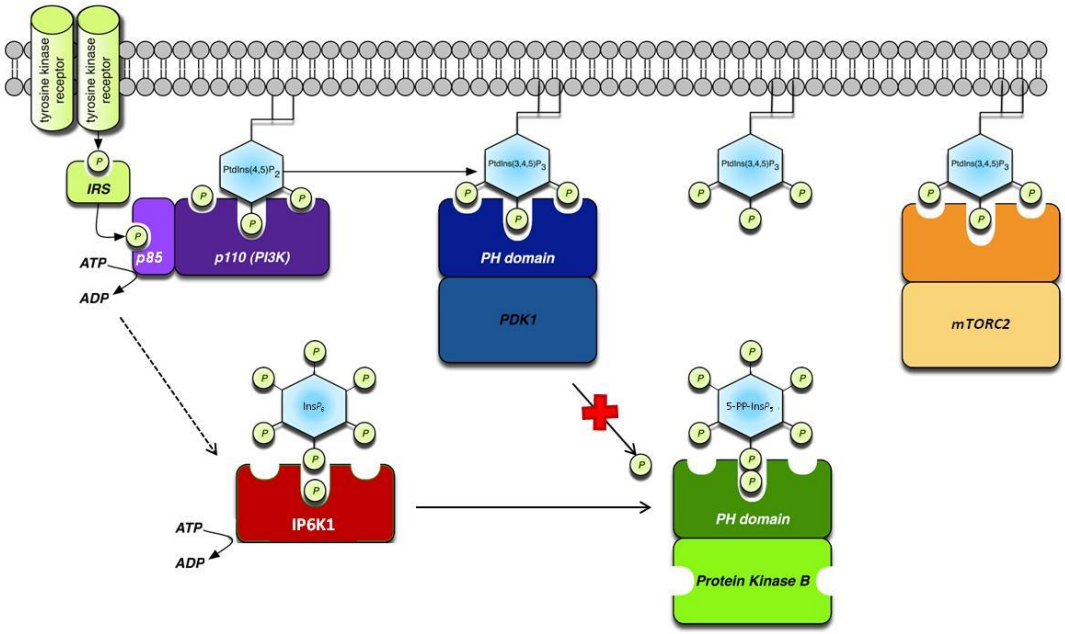


Figure 5.2. Proposed binding interactions of 5-PP-InsP_5 with PH domain of PKB.[109,193]

The 5-PP-InsP_5 regulation of PDK1 phosphorylation of PKB was found to be selective, as the catalytic activity of PDK1 towards artificial substrates was not affected by 5-PP-InsP_5. At a concentration of 1 µM, 5-PP-InsP_5 inhibited phosphorylation of PKB by 50%, whereas no inhibition was observed with InsP_6. Therefore, the pyrophosphate linkage in 5-PP-InsP_5 must differentiate it from InsP_6.[141]

Chakraborty and co-workers also found that signals resulting in an increase in PtdIns(3,4,5)P_3 levels led to a concurrent increase in 5-PP-InsP_5 levels, suggesting a dynamic control mechanism for PKB activation.[141,193] It is probable that relative localised concentrations of PtdIns(3,4,5)P_3 and 5-PP-InsP_5 directly influence the spatial and temporal status of PKB activation, and the stimulation of 5-PP-InsP_5 production by IP6K could be a downstream effect of PKB activation, making this a classic negative-feedback mechanism.[141,193]

5.4. Synthetic Aims

In order to probe these signalling pathways in greater detail and investigate the specific interactions of 5-PP-InsP_5 with the PKB PH domain, the synthesis of inositol pyrophosphates with phosphate and pyrophosphate isosteres incorporated at the 5-position was proposed. The first aim was to synthesis 5-PP-InsP_5 with a non-hydrolysable methylene diphosphonate linkage (Figure 5.3). The incorporation of this isostere would increase the hydrolytic stability of the pyrophosphate moiety, and prevent phosphate transfer to substrate proteins. This probe would therefore allow exclusive investigation of the protein-binding interactions of 5-PP-InsP_5.

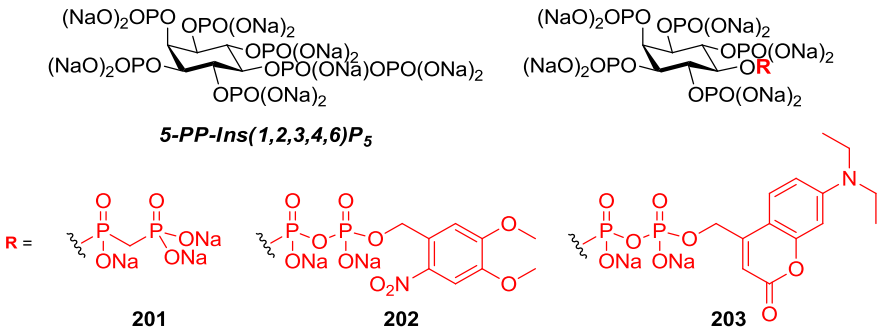
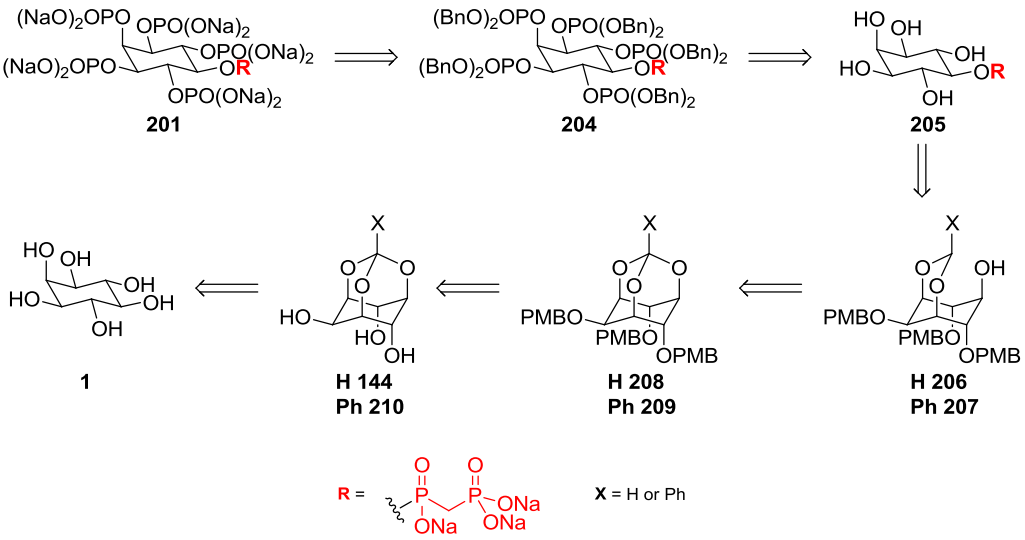


Figure 5.3. Primary synthetic targets, showing non-hydrolysable methylene diphosphonate moiety (**201**) along with pyrophosphate group protected with one-photon (**202**) and two-photon (**203**) photo-labile caging groups.

Secondly, the incorporation of a photo-labile caging group on the pyrophosphate linkage of 5-PP-InsP_5 was proposed (Figure 5.3). Irradiation of this compound with a light source of appropriate wavelength would allow complete spatial and temporal control of the release of the free pyrophosphate, facilitating the study of the role these pyrophosphates play in specific biological processes.

5.5. Retrosynthetic Analysis

It was initially decided to focus on the synthesis of the methylene diphosphonate derivative **201**, proposing a retrosynthesis which incorporated the methylene diphosphonate early in the synthetic route (Scheme 5.2).



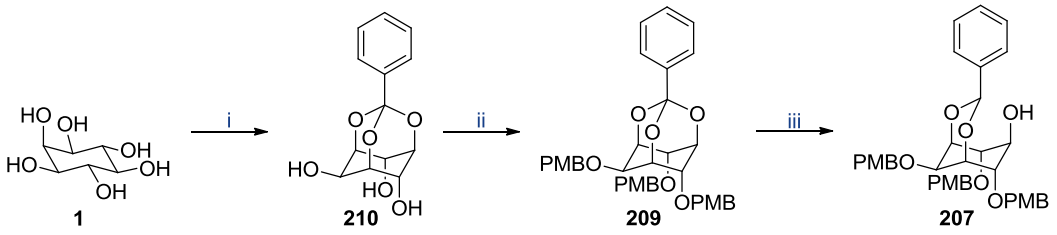
Scheme 5.2. Proposed retrosynthesis for 5-PP-InsP_5 methylene diphosphonate derivative **201**.

The viability of this synthetic route was investigated in preliminary test reactions. These reactions indicated that methylene protecting group **206** (Scheme 5.2, X=H) required harsh deprotection conditions, like refluxing hydrochloric acid in methanol, to participate in the reaction scheme, which were incompatible with benzyl-protected phosphate groups. Therefore, an orthobenzoate protecting group **210** (Scheme 5.2, X=Ph) was chosen due to its increased susceptibility to acid hydrolysis, and so enhanced compatibility with this synthetic route.[194]

5.6. Synthetic Route to Key Intermediate 207

The synthesis of alcohol **207** commenced with the protection of *myo*-inositol **1** using triethyl orthobenzoate. This reaction was initially attempted using DMF as the reaction solvent, replicating the conditions reported by Billington and co-workers for the formation of the orthoformate.[125] However, it was

found that the orthobenzoate reaction did not proceed as smoothly as its orthoformate analogue, with a large quantity of an additional product forming. Reports in the literature also alluded to complex reaction mixtures when using DMF as the solvent,[35,195] but these mixtures were not analysed. In our system, analysis by mass spectrometry and ^{1}H NMR spectroscopy identified the additional product as inositol orthoformate (**144**). This result has not been investigated further, but implies that DMF plays a role in the mechanism of this reaction in addition to that of a solvent. Additionally, the orthoformate side-product was not observed using DMSO as the reaction solvent. Therefore, DMSO became the solvent of choice, and the successful synthesis of inositol orthobenzoate was achieved following literature procedures (Scheme 5.3).[35,195,196]



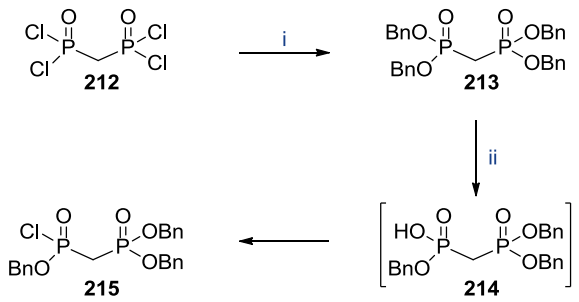
Scheme 5.3. Synthesis of alcohol **207**. *Reagents and conditions*: i) $C_6H_5C(OEt)_3$ (1.1 eq.), $TsOH{\cdot}H_2O$ (0.02 eq.), DMSO, 80 °C, 5 h, 48%; ii) NaH (5.0 eq.), PMBCl (5.0 eq.), DMF, 0 °C→RT, 48 h, 84%; iii) DIBAL (2.5 eq.), CH_2Cl_2, −40 °C, 5 h, 48%.

Treatment of inositol orthobenzoate **210** with sodium hydride and PMBCl proceeded smoothly (Scheme 5.3), with the protected orthobenzoate **209** precipitating from the reaction mixture at 0 °C. Isolation of this solid by filtration and subsequent washing with water and diethyl ether afforded the desired product **209** in 84% yield, without the need for further purification.

The next step was the reduction of the orthobenzoate protecting group to a benzylidene acetal, to afford the free 5-position hydroxyl group (Scheme 5.3). As discussed in Chapter 2, DIBAL is used for this transformation, as steric interactions ensure exclusive reduction at the 5-position hydroxyl group. However, in this system, it was found that the resulting benzylidene acetal **207** could also undergo DIBAL reduction to give a benzyl ether on either the 1- or 3-position hydroxyl group.[194] Analysis of this reaction showed that higher temperatures promoted the over-reduced product, although at low temperatures such as −78 °C, the reaction proved sluggish, and the longer reaction times required also promoted over-reduced product. Optimisation of the reaction conditions showed that a reaction time of less than 5 h at −40 °C limited the formation of the benzyl ether, and gave the desired benzylidene acetal **207** in 48% yield.

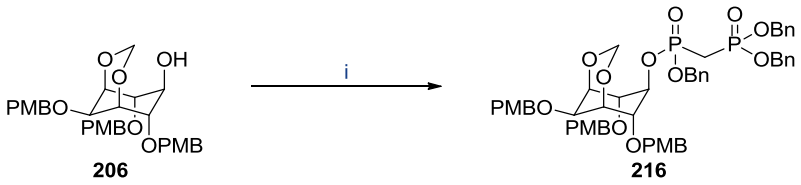
5.7. Attempted Synthesis of Intermediate 217

With a synthetic route to alcohol **207** in hand, our next challenge was to install the desired methylene diphosphonate moiety. Initial attempts involved the reaction of alcohol **207** with benzyl (bis(benzyloxy)phosphoryl)-methylphosphonochloridate **215**, which was synthesised in two steps from commercially available methylenediphosphonic dichloride **212** following literature procedures (Scheme 5.4).[197,198]



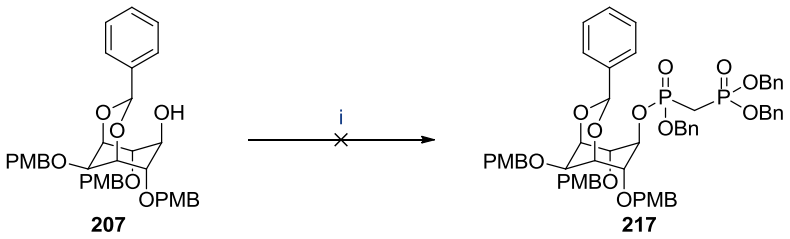
Scheme 5.4. Synthesis of intermediate **215**. *Reagents and conditions*: i) BnOH (5.0 eq.), 5-phenyl-1*H*-tetrazole (5.0 eq.), DIPEA (5.0 eq.), toluene, RT, 3 h, 75%; ii) quinuclidene (1.0 eq.), oxalyl chloride (2.0 eq.), DMF, toluene, reflux→0 °C, 5 h or a) DABCO (1.0 eq.), toluene, reflux, 2 h then 5% HCl solution; b) oxalyl chloride (5.0 eq.), DMF, toluene, RT, 2 h.

The reactivity of intermediate **215** was confirmed in reactions with 1,3-*O*-methylene-*myo*-inositol **206**, with the desired product isolated in low yields (Scheme 5.5).



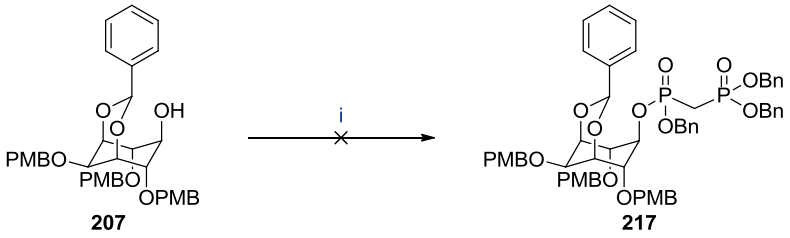
Scheme 5.5. Synthesis of **216**. *Reagents and conditions*: i) 5-phenyl-1*H*-tetrazole (2.0 eq.), 213 (3.2 eq.), DIPEA (10.0 eq.), toluene, RT→reflux, 2 h, 2%.

However, the analogous 1,3-*O*-benzylidene-*myo*-inositol **207** in this system appeared too unreactive to install the desired methylene diphosphonate in this manner, probably due to unfavourable steric interactions with the benzylidene acetal (Scheme 5.6).



Scheme 5.6. Attempted synthesis of **217**. *Reagents and conditions*: i) 5-Phenyl-1*H*-tetrazole (1.5 eq.), **215** (1.0 eq.), DIPEA (1.5 eq.), toluene, RT→70 °C, 15 h or KHMDS (15.0 eq.), **215** (1 eq.), THF, RT→50 °C→reflux, 24 h.

Therefore, it was proposed to install of the methylene diphosphonate group using the commercially available methylenediphosphonic dichloride **212**. Given the unreactive and hindered nature of alcohol **207**, it was hoped that, in a dilute system, only one equivalent of the alcohol would react with methylenediphosphonic dichloride. The reactive intermediate could then be quenched by the addition of anhydrous benzyl alcohol (Scheme 5.7).

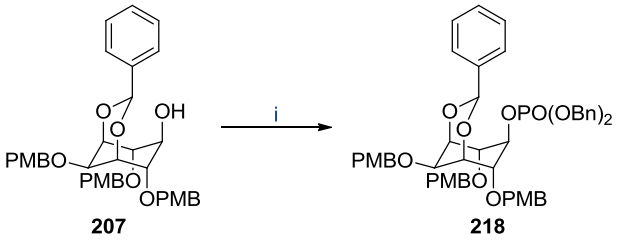


Scheme 5.7. Attempted synthesis of **217**. *Reagents and conditions*: i) a) KHMDS (15.0 eq.), **212** (1.2 eq.), THF, RT→reflux, 5 d; b) BnOH (6.0 eq.), KHMDS (10.0 eq.), RT, 4 h.

We found that reactions at low concentrations and low temperature afforded negligible amounts of the desired product **217**, with increased reaction times resulting in degradation of the starting materials. Increased concentration and temperature led to the formation of a complex mixture of products. This

result reinforced the idea that this ring system was too rigid, and the P(V) intermediate too bulky to be installed at this hindered position.

We proposed that the 5-position hydroxyl group could react with the smaller P(III) moiety and participate in the Michaelis–Arbuzov reaction, based on test reactions involving the reaction of alcohol **207** with phosphoramidite **218** and subsequent oxidation by *m*CPBA (Scheme 5.8).



207 **218**

Scheme 5.8. Synthesis of intermediate **218**. *Reagents and conditions*: i) a) 5-Phenyl-1*H*-tetrazole (3.0 eq.), **167** (3.0 eq.), CH_2Cl_2, RT, 12 h; b) *m*CPBA (4.0 eq.), −78 °C, 2 h, 92%.

Following literature precedent for the synthesis of diphosphate ester analogues,[199,200] it was proposed to isolate the P(III) intermediate **218** and react with dibenzyl (chloromethyl)phosphonate **219** (Figure 5.4).

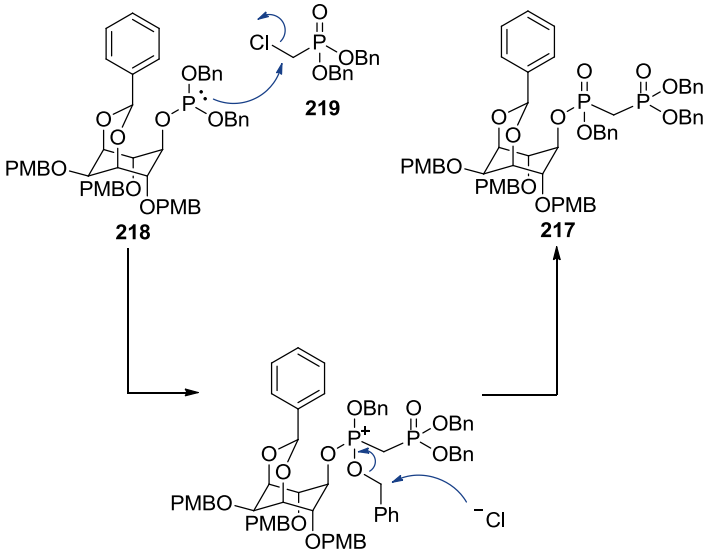
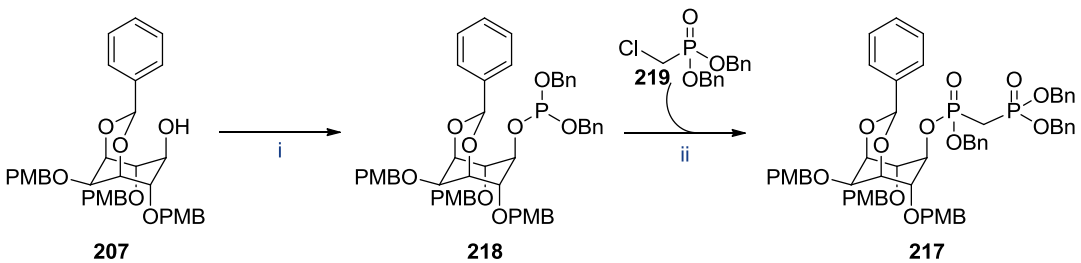


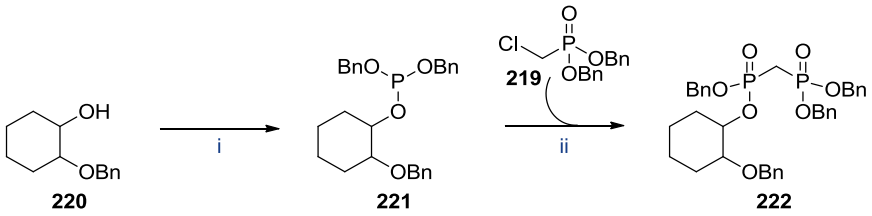
Figure 5.4. Proposed mechanism for synthesis of intermediate **217**.

Although expected to be unstable, the isolation of P(III) intermediate **218** proved relatively straightforward. The alcohol **207** was reacted with phosphoramidite **167** using our standard phosphorylation conditions, and the desired intermediate **218** was isolated in 90% yield after aqueous work-up (Scheme 5.9). This material was then reacted with dibenzyl (chloromethyl)phosphonate (**219**), although due to the reactivity of the benzyl chloride by-product compared to the starting material **218**, it was necessary to perform the reaction under reduced pressure, to remove the benzyl chloride *in vacuo*.



Scheme 5.9. Attempted synthesis of **217**. *Reagents and conditions*: i) 5-Phenyl-1*H*-tetrazole (3.5 eq.), **167** (3.5 eq.), CH_2Cl_2, RT, 12 h, 90%; ii) **219** (1.0 eq.), 140 °C, 9 mbar.

The reaction was heated to 140 °C at 9 mbar for 24 h, however, only negligible quantities of the desired product **217** were observed by NMR spectroscopic analysis, along with complex mixtures of degradation products. The reactions were repeated with longer reaction times and lower temperatures, but again proved unsuccessful.



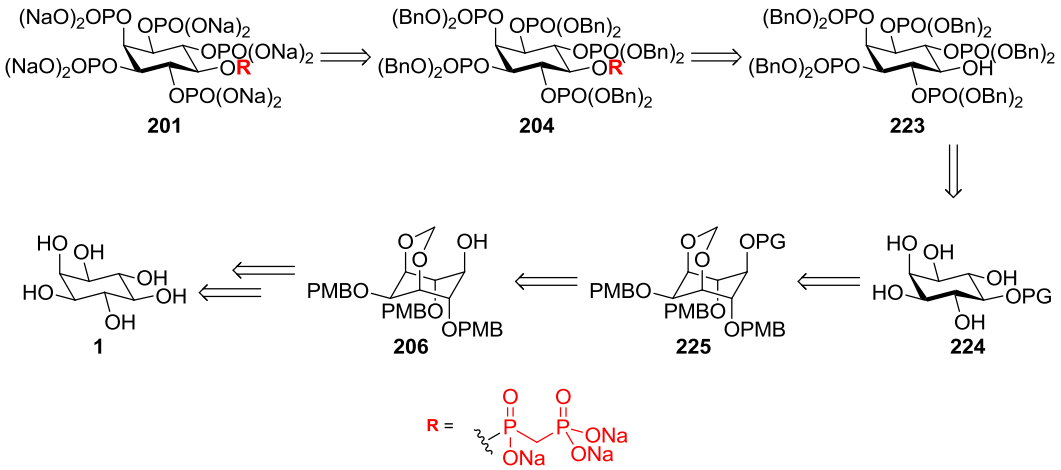
Scheme 5.10. Synthesis of **222**. *Reagents and conditions*: i) 5-Phenyl-1*H*-tetrazole (1.5 eq.), **167** (1.5 eq.), CH_2Cl_2, RT, 12 h, 90%; ii) **219** (1.0 eq.), 150 °C, 10 mbar, 2%.

2-(Benzyloxy)cyclohexanol **220** was used as a model system to test the feasibility of this synthetic route, which afforded the desired methylene diphosphonate in low yield (Scheme 5.10). Therefore, it could be concluded that 1,3-*O*-benzylidene-*myo*-inositol **207** was too sterically hindered to participate in this reaction.

5.8. Revised Retrosynthetic Route Towards Derivative 201

To overcome these reactivity problems, a revised retrosynthetic route was proposed. The incorporation of an orthogonal protecting group at the 5-position hydroxyl group would allow the installation of the methylene diphosphonate moiety later in the synthetic route. This should overcome the problems of steric bulk and rigidity that was observed with 1,3-*O*-

benzylidene-*myo*-inositol **207**, and also allow more divergent syntheses (Scheme 5.11).

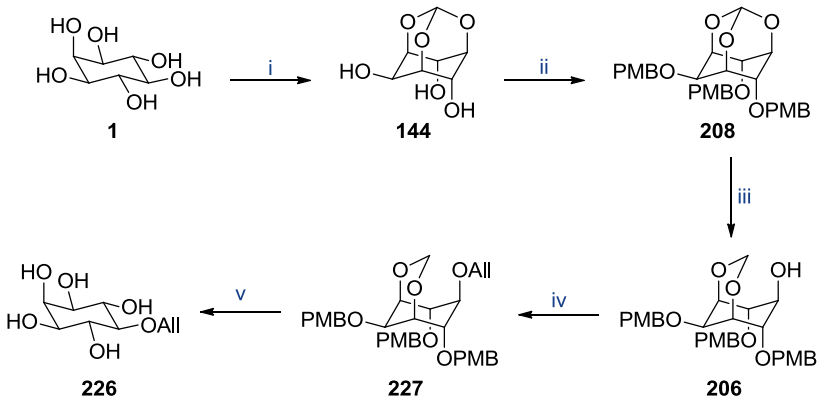


Scheme 5.11. Revised retrosynthesis for 5-PP-InsP_5 methylene diphosphonate derivative **201**.

The use of an allyl ether as the protecting group was initially proposed, as it had already demonstrated stability to the harshly acidic deprotection conditions required to remove both methylene and PMB protecting groups (see Chapter 2). This protecting group could be installed on the 5-position of 1,3-*O*-methylene-*myo*-inositol **206**, which can be synthesised in a high overall yield from *myo*-inositol **1**.

5.9. Attempted Synthesis of Intermediate 226

The synthesis of allyl-protected intermediate **227** proceeded smoothly, using similar methodology to that discussed above (Scheme 5.12).



Scheme 5.12. Synthesis of key intermediate **226**. *Reagents and conditions*: i) $(EtO)_3CH$ (3 eq.), $TsOH{\cdot}H_2O$ (0.3 eq.), DMF, 100 °C, 21 h, 71%; ii) NaH (6.0 eq.), PMBCl (5.0 eq.), DMF, 0 °C→RT, 12 h, 97%; iii) DIBAL (2.5 eq.), CH_2Cl_2, 0 °C→RT, 5 h, 97%; iv) NaH (2.5 eq.), allyl bromide (2.5 eq.), DMF, 0 °C→RT, 12 h, 53%; v) HCl, MeOH, reflux, 5 h.

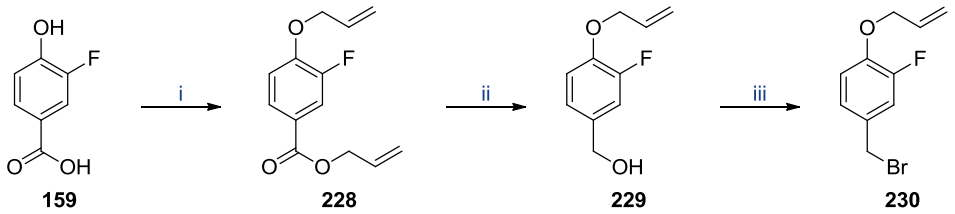
When intermediate **227** was subjected to the methanolysis conditions previously used to deprotect methylene and PMB protecting groups (see Chapter 2), an insoluble solid was obtained that could not be characterised. Reports in the literature implied that intermediate **226** could only be characterised as its per-*O*-acetyl derivative,[201] so we were confident that the insoluble solid obtained from the reaction was our desired product **226**. However, as this solid was found to be insoluble in both dichloromethane and acetonitrile, successful phosphorylation of **226** could not be achieved, and this synthetic route was abandoned.

5.10. Synthesis of Key Intermediate 232

After these solubility problems, it was postulated that an aromatic protecting group on the 5-position hydroxyl group of **226** would convey greater solubility, as Zhang *et al.* reported that 5-*O*-(4-methoxybenzyl)-*myo*-inositol

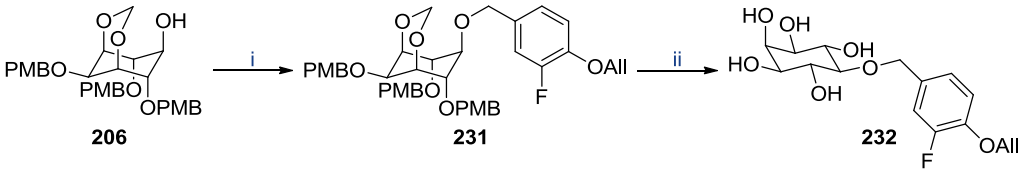
was soluble in organic solvents.[133] However, as a PMB protecting group at this position was not compatible with our synthetic route, and no other known protecting groups were found to possess the desired characteristics, we embarked on the design of a novel protecting group. The 4-*O*-allyl-3-fluorobenzyl protecting group was proposed for use in our synthetic route, as it had previously been demonstrated that the 4-hydroxy-3-fluorobenzyl protecting group could be deprotected under acidic conditions (see Chapter 2). It was hoped that the hydrochloric acid produced in the cleavage of allyl ethers using palladium(II) chloride would be sufficiently acidic to allow deprotection.

The desired 4-*O*-allyl-3-fluorobenzyl bromide **230** was successfully synthesised in three steps from commercially available starting materials (Scheme 5.13). Allyl protection of both the ester and the phenol was achieved using microwave irradiation, although longer reaction times and increased concentrations were necessary to prevent the reaction halting after mono-protection. It is interesting to note that the Claisen rearrangement product was not observed, probably due to the electron-withdrawing properties of the fluorine substituent.



Scheme 5.13. Synthesis of intermediate **230**. *Reagents and conditions*: i) K_2CO_3 (2.5 eq.), allyl bromide (4.0 eq.), DMF, MW, 100 °C, 1 h, 64%; ii) $LiAlH_4$ (3.0 eq.), THF, 0 °C→RT, 1 h, 67%; iii) PPh_3 (2.0 eq.), CBr_4 (2.0 eq.), CH_2Cl_2, RT, 5 h, 48%.

Reduction of the ester **228** to the alcohol **229** was achieved using lithium aluminium hydride, and reaction with carbon tetrabromide and triphenylphosphine afforded the aryl bromide **230**. The bromide **230** was then reacted with alcohol **206** using standard alkylating conditions, resulting in the successful synthesis of our desired product **231** in excellent yield (Scheme 5.14).



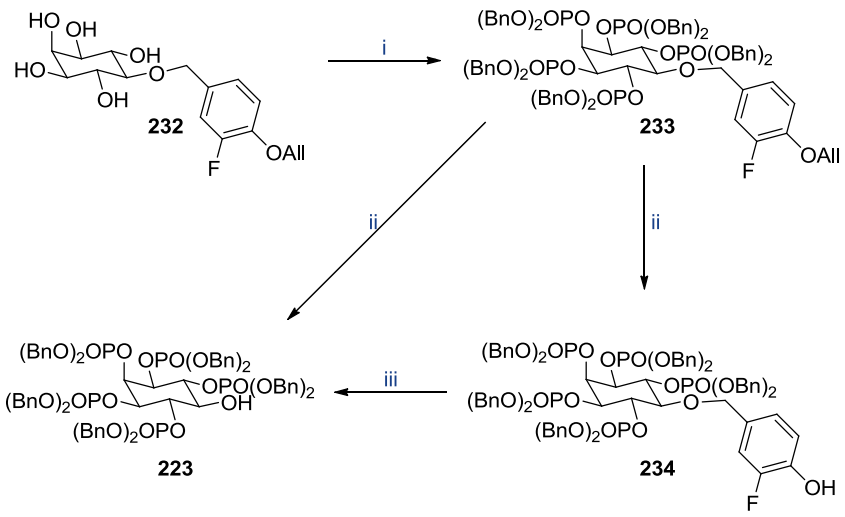
Scheme 5.14. Synthesis of key intermediate **232**. *Reagents and conditions*: i) NaH (2.0 eq.), **230** (2.0 eq.), TBAI (2.0 eq.), DMF, 0 °C→RT, 12 h, 79%; ii) HCl, MeOH, EtOAc, 50 °C, 12 h, 67%.

After some optimisation, it was discovered that methanolysis of the protected intermediate **231** at 50 °C for 12 h deprotected the methylene and PMB ethers without cleaving the 4-*O*-allyl-3-fluorobenzyl protecting group. Cooling the reaction mixture to 0 °C caused our desired product **232** to

precipitate out of solution, and filtration afforded our desired product in high yield without the need for further purification.

5.11. Synthesis of Key Intermediate 223

As the intermediate **232** was partially soluble in dichloromethane, phosphorylation could be achieved using standard phosphoramidite chemistry. This reaction reached completion after 12 h, and oxidation by *m*CPBA gave our desired phosphorylated product **233** in excellent yield (Scheme 5.15).



Scheme 5.15. Attempted synthesis of key intermediate **223**. *Reagents and conditions*: i) a) 1*H*-tetrazole (12.5 eq.), **167** (12.5 eq.), CH_2Cl_2, RT, 12 h; b) *m*CPBA (12.5 eq.), −78 °C, 15 min, 89%; ii) $PdCl_2$ (2.0 eq.), MeOH, RT→reflux, 24 h; iii) TFA, $CHCl_3$, MeOH, RT, 1 h.

We next attempted to deprotect the 4-*O*-allyl-3-fluorobenzyl protecting group of **233** using palladium(II) chloride in methanol. After extended reaction times (1-3 days) under reflux, some of the desired product **223** was

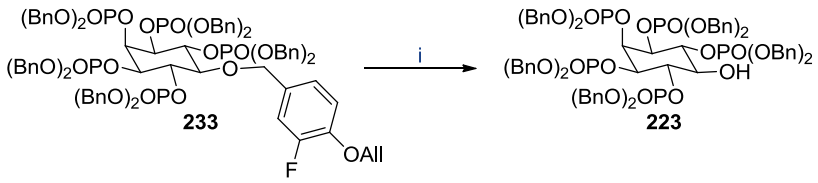
observed by mass spectrometry, although this could not be isolated. The major product in the crude reaction mixture, however, appeared to be the 4-hydroxy-3-fluorobenzyl-protected intermediate **234** (Scheme 5.15). Acidification of the reaction mixture with TFA did not push the reaction towards completion, and resulted in debenzylation of the phosphate groups.

Our next approach was a two-step deprotection, similar to the synthetic route developed for the deprotection of the 4-*O*-*tert*-butyldiphenylsiloxy-3-fluorobenzyl protecting group (see Chapter 2). It was proposed that deallylation with palladium(II) chloride would allow isolation of the 4-hydroxy-3-fluorobenzyl-protected intermediate **234**, which could be deprotected using the trifluoroacetic acid, chloroform and methanol conditions previously optimised. However, after working up the palladium(II) chloride reaction, NMR spectroscopic analysis indicated lower yields than expected of the 4-hydroxy-3-fluorobenzyl-protected intermediate **234**, due to the formation of side products during the reaction. However, analysis by mass spectrometry indicated that the desired alcohol **223** was also present in the reaction mixture, and so the crude reaction mixture was subjected to the acidic deprotection conditions described above.

The deprotection of the 4-hydroxy-3-fluorobenzyl protecting group in this system was much slower than that observed with the protected intermediate **170** in Chapter 2, possibly due to the more hindered nature of this substrate. As a consequence of the prolonged reaction times, significant debenzylation of the phosphate groups was observed, resulting in significantly lower yields

of the desired alcohol **223**. Purification of the desired alcohol **223** was also problematic. Repeated column chromatography only resulted in the isolation of small quantities of the desired alcohol **223**. It therefore became apparent that this route could not be used to prepare the large quantities of alcohol **223** required.

It was next proposed to investigate conditions that could deprotect the 4-*O*-allyl-3-fluorobenzyl group in one step. It was postulated that oxidative deprotection conditions, similar to those traditionally used to cleave PMB and other electron-rich aryl ethers could be successful in this system. Based on established conditions for the deprotection of PMB ethers[202-204] and recent reports of the deprotection of allyl ethers,[205] our protected intermediate **233** was stirred with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) at room temperature for two days. However, even after such prolonged reaction times, no reaction was observed. Our next attempt involved using ceric ammonium nitrate (CAN), also widely used to deprotect PMB ethers.[206] In a mixture of acetonitrile and water, our protected intermediate **233** was stirred with CAN at room temperature for 2.5 hours, after which time successful deprotection of the 4-*O*-allyl-3-fluorobenzyl group was observed by ^{1}H and ^{19}F NMR spectroscopic analysis (Scheme 5.16).

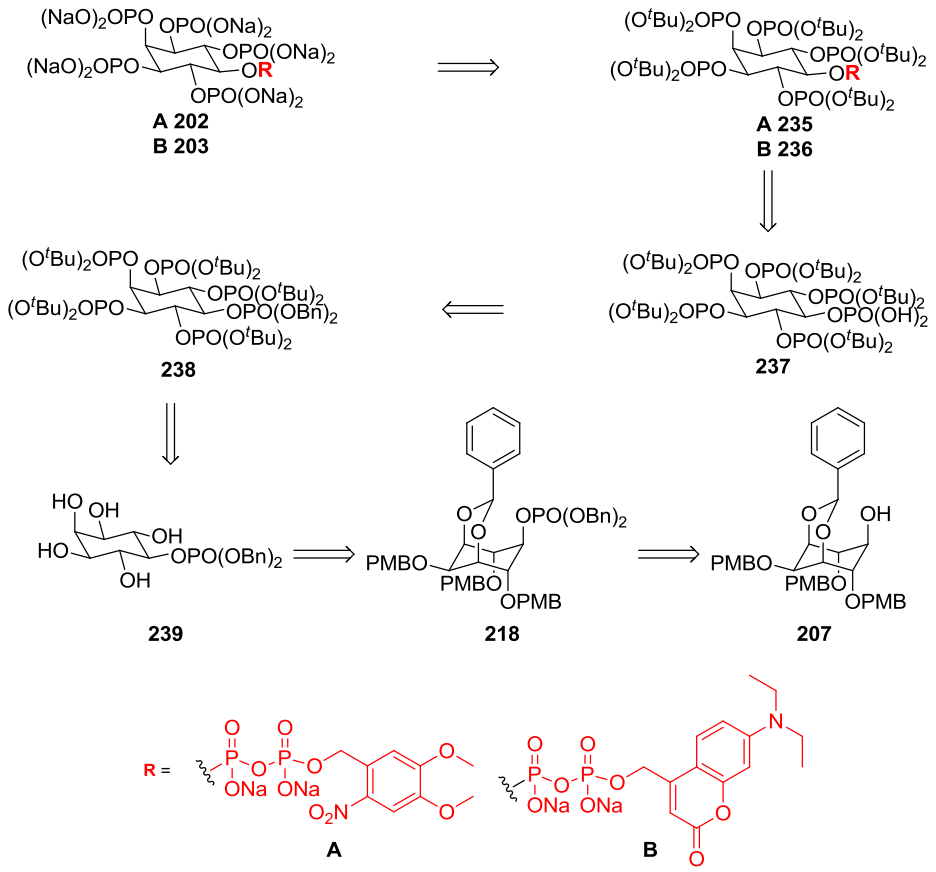


Scheme 5.16. Synthesis of key intermediate **223**. *Reagents and conditions*: i) CAN (10.0 eq.), CH_3CN, H_2O, RT, 2.5 h, 7%.

A minor amount of phosphate group debenzylation was observed, possibly due to the acidic nature of CAN. This mixture of products led to difficulties in purifying the desired alcohol **223**, and resulted in low yields of the isolated alcohol. However, careful optimisation of this reaction should avoid this problem, resulting in higher yields of the desired alcohol **223**, and a workable synthetic route to this key intermediate. This key intermediate can now be modified to incorporate the desired methylene diphosphonate moiety, along with other desired pyrophosphate mimics.

5.12. Attempted Synthesis of Intermediate 238

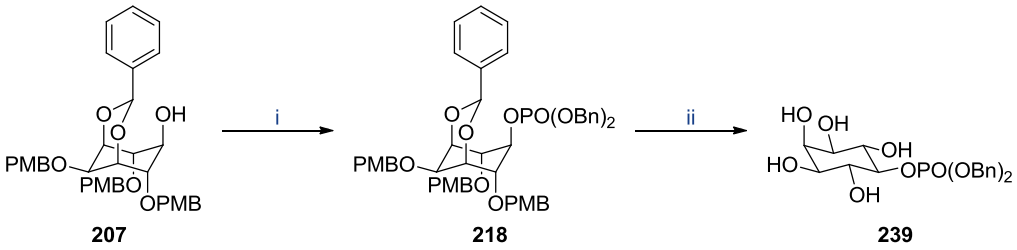
As the second aim of this project was the synthesis of caged pyrophosphate intermediates **202** and **203**, a revised retrosynthetic route for the synthesis of these intermediates was proposed (Scheme 5.17).



Scheme 5.17. Proposed retrosynthesis for caged 5-PP-InsP_5 derivatives **202** and **203**.

Our proposed route involved installation of the photolabile pyrophosphate linkages through coupling a free phosphate group at the 5-position with the corresponding photocaged phosphate group, following conditions reported by Geißler *et al.*[207,208] (Scheme 5.17). In order to obtain the free phosphate group selectively at the 5-position, an orthogonal protecting group was required for the remaining phosphates. A *tert*-butyl protecting group was chosen, due to its stability to hydrogenolysis and its facile removal in the presence of the desired photolabile caging groups.[208] These *tert*-butyl-protected phosphate groups could be installed on the free hydroxyl groups, which could be obtained from the deprotection of the benzylidene acetal and PMB protecting groups in the presence of a benzyl-protected phosphate

group at the 5-position. This phosphate group could be installed at this position by phosphorylation of the hydroxyl group using standard phosphoramidite chemistry, as discussed previously. The phosphorylation of intermediate **207** reached completion in 12 h at room temperature to afford the desired phosphorylated intermediate **218** in 92% yield (Scheme 5.18).



Scheme 5.18. Synthesis of intermediate **239**. *Reagents and conditions*: i) a) 5-phenyl-1*H*-tetrazole (3.0 eq.), **167** (3.0 eq.), CH_2Cl_2, RT, 12 h; b) *m*CPBA (4.0 eq.), −78 °C, 2 h, 92%; ii) TFA, $CHCl_3$, MeOH, −10 °C, 5 min, 99% yield.

Deprotection of the benzylidene acetal and PMB ethers required strongly acidic conditions. As it had previously been demonstrated that, for short periods of time, the phosphate benzyl groups were stable to a mixture of trifluoroacetic acid, chloroform and methanol (see Chapter 2), conditions which were reported to have deprotected PMB ethers,[133] we investigated whether these conditions could also cleave the benzylidene acetal.

The phosphorylated intermediate **218** was stirred in a mixture of trifluoroacetic acid, chloroform and methanol (5:5:1) at −10 °C for 5 minutes, after which time TLC and NMR spectroscopic analysis indicated that the PMB ethers and the benzylidene acetal had been cleaved. Concentration of the reaction mixture and washing of the solid with hexane and diethyl ether

afforded the desired phosphorylated intermediate **239** in excellent yield. The required *tert*-butyl protected phosphoramidite **240** was prepared in two steps from phosphorus trichloride, following literature procedures.[207] This phosphoramidite **240** was then reacted with phosphorylated intermediate **239** under standard phosphorylation conditions. Unfortunately, this reaction did not provide our desired product, and only degradation of the inositol starting material was observed. The *tert*-butyl-protected phosphoramidite **240** might be too bulky to react with such a sterically restricted intermediate, and the prolonged reaction times promote degradation of the starting material **239**. The result was even more surprising as phosphorylation of a similar intermediate **232** with a benzyl-protected phosphoramidite proceeded in 89% yield (Scheme 5.15).

It is clear that an alternative protecting group strategy is required for the synthesis of these desired caged intermediates. The phosphorylated intermediate **239** will be compatible with a wide range of phosphate protecting groups, and its robust synthetic route will be instrumental in the successful synthesis of the desired intermediates.

5.13. Summary

In conclusion, the key intermediate **223** was successfully synthesised. Optimisation of the deprotection conditions utilised in the synthesis of this product will allow greater yield of the intermediate, allowing the synthesis of a range of pyrophosphate mimics. In addition, the synthesis of intermediate

223 involved the development of a novel hydroxyl protecting group **230**. This group has been demonstrated to withstand conditions used to deprotect methylene and PMB ethers, and might be of benefit in future synthetic routes.

Progress was also made towards the synthesis of photolabile pyrophosphate derivatives. Phosphorylated intermediate **239** was successfully synthesised in five steps from *myo*-inositol **1**, which will play a crucial role in the synthesis of pyrophosphate derivatives **202** and **203**.

5.14. Future Work and Summary

This project has served to highlight the biological relevance and synthetic complexity of a range of inositol phosphates. Starting from a common precursor, *myo*-inositol, various synthetic manipulations have afforded natural and unnatural derivatives of Ins(1,3,4,5)P_4 and Ins(1,3)P_2, along with late-stage intermediates in the synthesis of inositol pyrophosphate derivatives.

In particular, this project has broadened the synthetic utility of the enantiomerically pure intermediate **157**. Resolved **157** was previously used by the Conway group in the synthesis of 1-, 4- and 5-position Ins(1,3,4,5)P_4 derivatives, and its utilisation in the successful synthesis of 3-position Ins(1,3,4,5)P_4 derivatives has allowed access to the four phosphorylated positions of Ins(1,3,4,5)P_4 through a common starting material. While the

diastereomeric resolution approach used in the synthesis of intermediate **157** may be lengthier than some of the alternative resolution approaches discussed, the highly reliable reactions involved allow the facile synthesis of large amounts of the racemic precursors, affording large quantities of the desired intermediate **157**.

This project has also highlighted the benefit of incorporating MD simulations into the design of phosphate isosteres. In particular, this approach has allowed us to explore the proposed binding interactions between each individual phosphate group and its unique binding site. The screening of proposed phosphate isosteres by this approach afforded us a greater understanding of the effects of subtle changes on the system as a whole, allowing the final selection of a phosphate isostere for the 5-position of Ins(1,3,4,5)P_4 that could not have been selected by any other method.

The use of MD simulations could become more important in future investigations of the interactions between inositol phosphates and their target proteins. In particular, MD simulations could be utilised in the investigation of binding interactions between inositol pyrophosphate derivatives and the PKB PH domain, along with other inositol pyrophosphate binding proteins. MD simulations could also be crucial in the selection of phosphate isosteres for the synthesis of Ins(1,3)P_2 derivatives. With the successful synthetic routes to a variety of key intermediates discussed, this project will facilitate the robust synthesis of a wide range of inositol phosphate derivatives.

Chapter 6:

Experimental

6. Experimental

6.1. Molecular Dynamic Simulations and Analysis

(Carried out by Sarah Rosen and Dr Ian Gould at Imperial College London)

Initial configuration - the X-ray crystal structure of the PKB PH domain in complex with Ins(1,3,4,5)P_4 [from the RCSB Protein Data Bank, accession code 1UNQ] provided the initial co-ordinates. The acetyl group bonded to the *N* terminus of the PH domain was deleted as it is sufficiently far from the inositol phosphate binding site, and is not present *in vivo*. Crystallographic water molecules present in the PDB were kept. Using the Leap module of Amber, the systems were charge-neutralised with Na^+ counter-ions. The systems were solvated with TIP3P water molecules within a truncated octahedral unit cell, with a minimum of 8 Å distance from the edge of the box to any protein or ligand atom.

MD protocol - the complexes were minimised in two stages. In the first stage, the water molecules and Na^+ counter-ions were energy-minimised, whilst harmonic restraints of 500 kcal mol^{-1} $Å^{-1}$ were applied to the protein and ligand; 500 steps of steepest descent and 500 steps of conjugate gradient minimisation were carried out. In the second stage of minimisation, the restraints were removed, and the entire system was minimised using 1500 steps of steepest descent and 2000 steps of conjugate gradient minimisation. In all simulations, periodic boundary conditions were applied. For the calculation of non-bonded interactions, the particle mesh Weald

method was applied with a direct space cut-off of 8 Å. Each minimised system was heated from 0 K to 300 K using an NVT ensemble for 50 ps. Following this, 50 ps of equilibration was carried out using the NPT ensemble at 1 bar. MD simulations of each system were then run for 150 ns using the NPT ensemble, with snapshots saved every 10 ps. For each simulation the SHAKE algorithm was applied to constrain all bonds involving hydrogen atoms, allowing a time-step of 2 fs to be used. In order to maintain the temperature during the simulations, the Langevin thermostat was used with a collision frequency of 1.0 ps^{-1}. Production MD simulations were performed with the GPU accelerated version of PMEMD in Amber11.

Free energy calculations - MM-PBSA (Molecular Mechanics-Poisson-Boltzmann Surface Area) calculations were implemented to calculate the free energy of binding for the inositol phosphate derivative–PKB PH domain interactions. Calculations were carried out using the Perl scripts in Amber. Each snapshot from the 150 ns MD simulation was stripped of water and counter-ions. The interaction energy and solvation free energy were calculated for the complex, receptor and ligand. Results were averaged to obtain an estimate of the binding free energy.

Hydrogen bond interactions - the Ptraj program within Amber 11 was used to analyse the hydrogen bonding interactions for each system. We specified a list of all potential hydrogen bond donors and acceptors, and analysed pair interactions for the duration of each 150 ns MD simulation. A

cut-off distance of 3.5 Å, and angle cut-off of 120° were specified for the pair interactions.

6.2. General Experimental Protocols

^{1}H NMR spectra were recorded on Bruker DPX250 (250 MHz); Bruker DPX400 (400 MHz); Bruker AV400 (400 MHz) or Bruker AV500 (500 MHz) spectrometers using deuterochloroform (unless indicated otherwise) as a reference for internal deuterium lock. The chemical shift data for each signal are given as δH in units of parts per million (ppm) relative to tetramethylsilane (TMS) where δH (TMS) = 0.00 ppm. The multiplicity of each signal is indicated by: s (singlet); br s (broad singlet); d (doublet); t (triplet); q (quartet); dd (doublet of doublets); ddd (doublet of doublet of doublets); dddd (doublet of doublet of doublet of doublets); ddt (doublet of doublet of triplets); sp (septet); or m (multiplet). The number of protons (n) for a given resonance signal is indicated by nH. Coupling constants (*J*) are quoted in Hz and are recorded to the nearest 0.1 Hz. Identical proton coupling constants (*J*) are averaged in each spectrum and reported to the nearest 0.1 Hz. The coupling constants are determined by analysis using Bruker TopSpin software.

^{13}C NMR spectra were recorded on a Bruker AV500 (126 MHz) spectrometer using the PENDANT or DEPT Q pulse sequences with broadband proton decoupling and internal deuterium lock. The chemical shift data for each signal are given as δC in units of parts per million (ppm) relative to tetramethylsilane (TMS) where δC (TMS) = 0.00 ppm. Where appropriate, coupling constants (*J*) are quoted in Hz and are recorded to the nearest 0.1 Hz. ^{1}H and ^{13}C NMR spectra were assigned using 2D NMR

experiments including COSY, HSQC, HMBC, DEPT-135, HMQC, and DEPT Q.

^{31}P NMR spectra were recorded on Bruker DRX500 (202 MHz) and Bruker DPX250 (161 MHz) spectrometers using broadband proton decoupling pulse sequences and deuterium internal lock. The chemical shift data for each signal are given as δP in units of parts per million (ppm) relative to 85% phosphoric acid as an external reference.

^{19}F NMR spectra were recorded on Bruker AV500 (470 MHz) and Bruker AV400 (376 MHz) spectrometers using a broadband proton decoupling pulse sequence and deuterium internal lock. The chemical shift data for each signal are given as δF in units of parts per million (ppm) relative to trichlorofluoromethane where δF = 0.00 ppm. Coupling constants (*J*) are quoted in Hz and are recorded to the nearest 0.1 Hz.

Mass spectra were acquired on either a Micromass LCT Premier spectrometer or Bruker MicroTOF spectrometer using electrospray ionisation, operating in positive or negative mode, from solutions of methanol, acetonitrile or water. *m/z* values are reported in Daltons and followed by their percentage abundance in parentheses.

Melting points were determined using a Kofler hot stage microscope and are uncorrected.

Microanalyses were obtained from the Elemental Analysis Service, London Metropolitan University, London.

Infrared Spectra were obtained either as: **a** thin film on sodium chloride discs; **b** potassium bromide disc; **c** thin film or solid sample using diamond ATR module as indicated. The spectra were recorded on a Bruker Tensor 27 spectrometer. Absorption maxima are reported in wavenumbers (cm^{-1}).

Specific Optical Rotations were measured using a Perkin Elmer Model 241 polarimeter, in cells with a path length of 1 dm. The light source was maintained at 589 nm. The concentration (*c*) is expressed in g/100 mL (equivalent to g/0.1 dm^3). Specific rotations are denoted $[\alpha]_D^T$ and are given in implied units of 10^{-1} deg cm^2 g^{-1} (T = ambient temperature in °C).

Analytical thin layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} aluminium-supported thin layer chromatography sheets. Visualisation was by absorption of UV light (λ_{max} 254 or 365 nm), or thermal development after dipping in one of: **a** ethanolic solution of phosphomolybdic acid (PMA); **b** aqueous solution of potassium permanganate, potassium carbonate and sodium hydroxide; **c** ethanolic solution of 4-anisaldehyde, sulfuric acid and acetic acid.

Flash Column chromatography was performed on a Biotage SP1 system using KP-Sil™ cartridges or carried out manually on Merck silica gel 60

(240-400 mesh), eluting with solvents as supplied, under a positive pressure of compressed air (unless otherwise stated).

Anhydrous solvents were obtained under the following conditions: dry *N*,*N*-dimethylformamide and methanol were purchased from SigmaAldrich UK in SureSeal™ bottles and used without further purification; acetonitrile, dichloromethane, diethyl ether, tetrahydrofuran and toluene were dried by passing them through a column of activated basic alumina according to the Grubbs' procedure;[209] all other solvents were used as supplied (analytical or HPLC grade) without purification.

Chemicals were purchased from Acros UK, Sigma Aldrich UK, Alfa Aesar UK, Fisher UK, Fluka UK, Fluorochem, Merck or TCI-Europe. All solvents and reagents were purified, when necessary, by standard techniques.[210] Where appropriate and if not stated otherwise, all non-aqueous reactions were performed in a flame-dried flask under an inert atmosphere of nitrogen or argon, using a double vacuum manifold with the inert gas passing through a bed of activated 4 Å molecular sieves and self indicating silica gel.

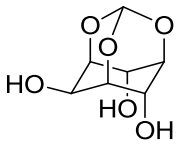
Lyophilisation using a Christ Alpha 2-4-LD lyophiliser was used to remove water from aqueous solutions.

Solvent was removed under reduced pressure using a Buchi™ rotary evaporator attached to a diaphragm pump. Brine refers to a saturated aqueous solution of sodium chloride. Hexane refers to a mixture of hexane

isomers and petroleum ether refers to the fraction of light petroleum ether boiling in the range 40-60°C.

6.3. Synthesis of 3-position InsP_4 derivatives

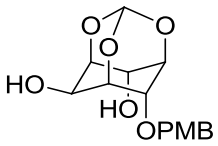
D-*myo*-Inositol 1,3,5-orthoformate 144



D-*myo*-Inositol **1** (20.00 g, 111.0 mmol, 1.0 eq.) and 4-toluenesulfonic acid monohydrate (6.30 g, 33.3 mmol, 0.3 eq.) were dissolved in dry *N,N*-dimethylformamide (250 mL). Triethyl orthoformate (49.40 g, 44.00 mL, 333.0 mmol, 3.0 eq.) was added and the reaction mixture was stirred at 100 °C for 21 h. When the reaction was deemed to be complete by TLC analysis, the mixture was allowed to cool to RT and the 4-toluenesulfonic acid was quenched by the addition of a saturated aqueous solution of sodium bicarbonate (50 mL). The precipitated sodium tosylate was filtered and washed with methanol (20 mL), and the filtrate concentrated under reduced pressure. Crystallisation from methanol gave the desired product (6.38 g) as a colourless solid. The mother liquor was then concentrated and adsorbed onto silica gel. Purification by silica gel chromatography, eluting with chloroform/methanol (90:10) afforded D-*myo*-inositol 1,3,5-orthoformate **144** (15.31 g total yield, 71%) as a colourless crystalline solid: R_f 0.51 (chloroform/methanol, 80:20); mp 270-272 °C (dec. *from chloroform/methanol*) (Lit.[125] 300-302 °C sealed tube); δ_H (400 MHz, DMSO-D_6) 5.49 (1H, s, equatorial O*H*), 5.45 (2H, d, *J* 0.8, 2 × axial O*H*), 5.34 (1H, d, *J* 6.0, 3-*H*), 4.28-4.26 (2H, m, 2 × inositol ring), 4.05 (1H, d, *J* 3.6, 1 ×

inositol ring), 4.01-4.00 (1H, m, 1 × inositol ring), 3.95-3.94 (1H, m, 1 × inositol ring). The data are in good agreement with literature values.[51,125]

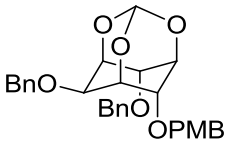
(±)-D-4-*O*-(4-Methoxybenzyl)-*myo*-inositol 1,3,5-orthoformate 145



D-*myo*-Inositol 1,3,5-orthoformate **144** (1.85 g, 9.8 mmol, 1.0 eq.) was dissolved in dry *N,N*-dimethylformamide (80 mL), and cooled to 0 °C. Sodium hydride (60% dispersion in mineral oil, 420 mg, 10.7 mmol, 1.1 eq.) was added portionwise over 1 h, ensuring that the temperature was maintained at 0 °C. The mixture was then warmed to RT and stirred for 2 h. The suspension was re-cooled to 0 °C and 4-methoxybenzyl chloride (1.67 g, 1.45 mL, 10.7 mmol, 1.1 eq.) was added dropwise over a period of 30 min. The mixture was warmed to RT, and stirred for 12 h. When the reaction was deemed to be complete by TLC analysis, the sodium hydride was quenched by the addition of H_2O (12 mL) and the mixture was concentrated under reduced pressure. The resulting residue was reconstituted with H_2O (10 mL) and ethyl acetate (40 mL), and the layers were separated. The aqueous phase was further extracted with ethyl acetate (3 × 40 mL). The combined organic layers were washed with brine (30 mL), dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The resulting oil was adsorbed onto silica gel, and purified by silica gel chromatography, eluting with hexane and ethyl acetate

(80:20, 75:25, 70:30, 60:40) to afford (±)-D-4-*O*-(4-methoxybenzyl)-*myo*-inositol 1,3,5-orthoformate **145** (2.34 g, 78%) as a colourless solid: R_f 0.28 (hexane/ethyl acetate, 80:20); mp 97-99 °C (*from hexane/ethyl acetate*) (Lit.[51] 99-100 °C); δ_H (400 MHz, $CDCl_3$) 7.24 (2H, d, *J* 8.5, Ar*H*), 6.90 (2H, d, *J* 8.5, Ar*H*), 5.44 (1H, s, 3-*H*), 4.62 (1H, d, *J* 11.3, OC$H_A$$H_B$), 4.58 (1H, d, *J* 11.3, OC$H_A$$H_B$), 4.44-4.39 (2H, m, 2 × inositol ring), 4.25-4.21 (3H, m, 3 × inositol ring), 4.07 (1H, d, *J* 1.5, 1 × inositol ring), 3.82 (3H, s, OCH_3), 3.77 (1H, s, O*H*), 3.21 (1H, s, O*H*). The data are in good agreement with literature values.[51]

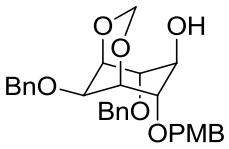
(±)-D-2,6-Bis-*O*-benzyl-4-*O*-(4-methoxybenzyl)-*myo*-inositol 1,3,5-orthoformate 146



Benzyl bromide (19.22 g, 13.37 mL, 112.4 mmol, 2.5 eq.) and dry *N,N*-dimethylformamide (150 mL) were added to a flask containing sodium hydride (60% dispersion in mineral oil, 4.50 g, 112.4 mmol, 2.5 eq.) that has been washed with petroleum ether, and cooled to 0 °C. A solution of (±)-D-4-*O*-(4-methoxybenzyl)-*myo*-inositol 1,3,5-orthoformate **145** (13.95 g, 44.9 mmol, 1.0 eq.) in dry *N,N*-dimethylformamide (100 mL) was added dropwise over a period of 30 min to the stirred suspension, maintaining the temperature at 0 °C. The reaction mixture was stirred for 30 min at 0 °C and then at RT for 12 h, after which time the reaction was deemed to be

complete by TLC analysis. The reaction was quenched by the addition of methanol (30 mL) and concentrated under reduced pressure. The resulting oil was reconstituted with ethyl acetate (80 mL) and H_2O (60 mL) and the layers were separated. The aqueous phase was further extracted with ethyl acetate (2 × 80 mL). The combined organic layers were washed with brine (30 mL), dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The resulting oil was adsorbed onto silica gel, and purified by silica gel chromatography, eluting with petroleum ether and ethyl acetate (80:20) to afford (±)-D-2,6-bis-*O*-benzyl-4-*O*-(4-methoxybenzyl)-*myo*-inositol 1,3,5-orthoformate **146** (20.74 g, 94%) as a colourless oil: R_f 0.44 (petroleum ether/ethyl acetate 80:20); δ_H (400 MHz, $CDCl_3$) 7.39-7.20 (10H, m, Ar*H*), 7.12 (2H, d, *J* 8.8, $OCH_2C_6H_4OCH_3$), 6.81 (2H, d, *J* 8.8, $OCH_2C_6H_4OCH_3$), 5.53 (1H, s, 3-*H*), 4.64 (2H, s, OCH_2Ph), 4.61 (1H, d, *J* 11.6, OCH_AH_BPh), 4.54 (1H, d, *J* 11.2, $OCH_{A'}H_{B'}C_6H_4OCH_3$), 4.47 (1H, d, *J* 11.6, OCH_AH_BPh), 4.43-4.40 (2H, m, $OCH_{A'}H_{B'}C_6H_4OCH_3$ and 1 × inositol ring), 4.33-4.27 (4H, m, 4 × inositol ring), 4.04-4.03 (1H, m, 1 × inositol ring), 3.81 (3H, s, OCH_3). The data are in good agreement with literature values.[51]

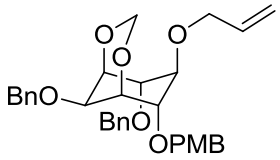
(±)-D-2,6-Bis-*O*-benzyl-4-*O*-(4-methoxybenzyl)-1,3-*O*-methylene-*myo*-inositol 147



(±)-D-2,6-Bis-*O*-benzyl-4-*O*-(4-methoxybenzyl)-*myo*-inositol 1,3,5-orthoformate **146** (23.12 g, 47.1 mmol, 1.0 eq.) was dissolved in dry dichloromethane (250 mL) and cooled to 0 °C. A solution of diisobutylaluminium hydride in hexanes (1.0 M, 118.00 mL, 117.8 mmol, 2.5 eq.) was added dropwise over a period of 30 min, maintaining the temperature at 0 °C. The mixture was then allowed to reach RT and stirred for 5 h, after which time the reaction was deemed to be complete by TLC analysis. The reaction mixture was cooled to 0 °C and quenched by the careful addition of H_2O (5 mL), sodium hydroxide (5 mL, 15% solution) and additional H_2O (12 mL). The resulting gel was dried by addition of magnesium sulfate, filtered, and concentrated under reduced pressure to afford (±)-D-2,6-bis-*O*-benzyl-4-*O*-(4-methoxybenzyl)-1,3-*O*-methylene-*myo*-inositol **147** (22.02 g, 95%) as a colourless oil: R_f 0.55 (petroleum ether/ethyl acetate 60:40); δ_H (400 MHz, $CDCl_3$) 7.34-7.26 (10H, m, Ar*H*), 7.20 (2H, d, *J* 8.6, $OCH_2C_6H_4OCH_3$), 6.83 (2H, d, *J* 8.6, $OCH_2C_6H_4OCH_3$), 5.56 (1H, d, *J* 4.8, 3-*H*), 4.68 (1H, d, *J* 11.9, OCH_AH_BPh), 4.67 (1H, d, *J* 4.8, 3-*H'*), 4.60 (1H, d, *J* 11.3, $OCH_{A'}H_{B'}C_6H_4OCH_3$), 4.61 (2H, s, OCH_2Ph), 4.58 (1H, d, *J* 11.9, OCH_AH_BPh), 4.51 (1H, d, *J* 11.3, $OCH_{A'}H_{B'}C_6H_4OCH_3$), 4.44-4.42 (2H, m, 2 × inositol ring), 4.29-4.28 (1H, m, 1 × inositol ring), 4.03-3.99 (2H, m, 2

× inositol ring), 3.99-3.94 (1H, m, 1 × inositol ring), 3.81 (3H, s, OCH_3), 2.97 (1H, d, *J* 10.3, OH). The data are in good agreement with literature values.[51]

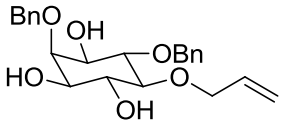
(±)-D-5-*O*-Allyl-2,6-bis-*O*-benzyl-4-*O*-(4-methoxybenzyl)-1,3-*O*-methylene-*myo*-inositol 148



Allyl bromide (10.82 g, 7.75 mL, 89.5 mmol, 2.0 eq.) and dry *N,N*-dimethylformamide (150 mL) were added to a flask containing sodium hydride (60% dispersion in mineral oil, 3.58 g, 89.5 mmol, 2.0 eq.) that has been washed with petroleum ether, and cooled to 0 °C. A solution of (±)-D-2,6-bis-*O*-benzyl-4-*O*-(4-methoxybenzyl)-1,3-*O*-methylene-*myo*-inositol **147** (22.02 g, 44.7 mmol, 1.0 eq.) in dry *N,N*-dimethylformamide (100 mL) was added dropwise over a period of 30 min to the stirred suspension, keeping the temperature constant at 0 °C. The reaction mixture was stirred for 30 min at 0 °C and then at RT for 12 h, after which time the reaction was deemed to be complete by TLC analysis. The residual sodium hydride was quenched by the addition of methanol (30 mL) and the reaction mixture concentrated under reduced pressure. The resulting oil was reconstituted with ethyl acetate (80 mL) and H_2O (30 mL) and the layers were separated. The aqueous phase was then further extracted with ethyl acetate (2 × 80 mL). The combined organic layers were then dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The resulting oil

was adsorbed onto silica gel, and purified by silica gel chromatography, eluting with petroleum ether and ethyl acetate (100:0, 80:20, 70:30, 60:40, 0:100) to afford (±)-D-5-*O*-allyl-2,6-bis-*O*-benzyl-4-*O*-(4-methoxybenzyl)-1,3-*O*-methylene-*myo*-inositol **148** (21.57 g, 91%) as a colourless oil: R_f 0.56 (petroleum ether/ethyl acetate 70:30); δ_H (400 MHz, $CDCl_3$) 7.36-7.31 (10H, m, Ar*H*), 7.25 (2H, d, *J* 8.7, $OCH_2C_6H_4OCH_3$), 6.87 (2H, d, *J* 8.7, $OCH_2C_6H_4OCH_3$), 5.89 (1H, dddd, *J* 17.2, 10.6, 5.4, 5.4, CH_X=CH_YH_Z), 5.23 (1H, dd, *J* 17.2, 1.7, CH_X=CH_YH_Z), 5.18 (1H, d, *J* 5.6, 3-*H*), 5.16 (1H, dd, *J* 10.6, 1.7, CH_X=CH_YH_Z), 4.83 (1H, d, *J* 5.6, 3-*H'*), 4.66 (1H, d, *J* 11.6, OCH_AH_BPh), 4.65 (2H, s, OCH_2Ph), 4.60 (1H, d, *J* 12.1, $OCH_{A'}H_{B'}C_6H_4OCH_3$), 4.59 (1H, d, *J* 11.6, OCH_AH_BPh), 4.53 (1H, d, *J* 12.1, $OCH_{A'}H_{B'}C_6H_4OCH_3$), 4.26-4.24 (2H, m, 2 × inositol ring), 4.14-4.12 (2H, dd, *J* 5.4, 1.3, OCH_2CH_X=CH_YH_Z), 3.91-3.87 (2H, m, 2 × inositol ring), 3.82-3.81 (1H, m, 1 × inositol ring), 3.81 (3H, s, OCH_3), 3.54-3.51 (1H, m, 1 × inositol ring). The data are in good agreement with literature values.[51]

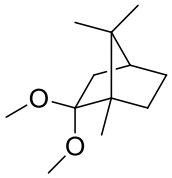
(±)-D-5-*O*-Allyl-2,6-bis-*O*-benzyl-*myo*-inositol 149



Concentrated hydrochloric acid (37%, 40.00 mL) was slowly added to a stirred solution of (±)-D-5-*O*-allyl-2,6-bis-*O*-benzyl-4-*O*-(4-methoxybenzyl)-1,3-*O*-methylene-*myo*-inositol **148** (21.57 g, 40.5 mmol, 1.0 eq.) in methanol (400 mL) and heated under reflux for 5 h, after which time the reaction was

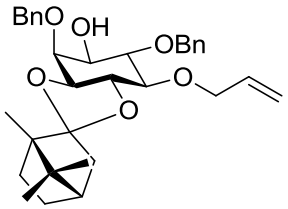
deemed to be complete by TLC analysis. The reaction was cooled to 0 °C and the hydrochloric acid was quenched by the addition of sodium hydrogen carbonate (60.22 g). The solid components were removed by filtration, and the filtrate was adsorbed onto silica gel. Purification by silica gel chromatography, eluting with ethyl acetate/petroleum ether (30:70, 40:60, 100:0) afforded (±)-D-5-*O*-allyl-2,6-bis-*O*-benzyl-*myo*-inositol **149** (13.22 g, 82%) as a colourless solid: R_f 0.52 (ethyl acetate); mp 110-112 °C (*from methanol)* (Lit.[51] 111-112 °C); δ_H (400 MHz, $CDCl_3$) 7.38-7.32 (10 H, m, Ar*H*), 5.99 (1H, dddd, *J* 17.1, 10.8, 5.6, 5.6, CH_X=CH$_Y$H$_Z$), 5.31 (1H, dd, *J* 17.1, 1.8, CH$_X$=CH_YH$_Z$), 5.20 (1H, dd, *J* 10.8, 1.8, CH$_X$=CH$_Y$$H_Z$), 4.92 (1H, d, *J* 11.2, OCH_AH$_B$Ph), 4.89 (1H, d, *J* 12.0, OC$H_{A'}$H$_{B'}$Ph, 4.78 (1H, d, *J* 11.2, OCH$_A$$H_B$Ph), 4.76 (1H, d, *J* 12.0, OCH$_{A'}$$H_{B'}$Ph), 4.38-4.33 (2H, m, OCH_2CH$_X$=CH$_Y$H$_Z$), 4.03-4.02 (1H, m, 1 × inositol ring), 3.84-3.71 (2H, m, 2 × inositol ring), 3.60-3.56 (1H, m, 1 × inositol ring), 3.49-3.45 (1H, m, 1 × inositol ring), 3.25-3.20 (1H, m, 1 × inositol ring), 2.54 (1H, br s, O*H*), 2.35-2.31 (2H, m, 2 × O*H*); *m/z* (ES$^+$) 423 ([M+Na]$^+$, 100%). The data are in good agreement with literature values.[51]

1-(*S*)-(−)-Camphor dimethylacetal 150



To a solution of 1-(*S*)-(−)-camphor (8.00 g, 52.5 mmol, 1.0 eq.) in petroleum ether (70 mL), Montmorillonite K-10 clay (15.00 g) and trimethylorthoformate (22.30 g, 23.00 mL, 210.2 mmol, 4.0 eq.) were added and stirred at RT for 6 days, with conversion monitored by ^{1}H NMR analysis. When the reaction was deemed to be complete, the mixture was filtered through a pad of Celite® and washed with petroleum ether (3 × 50 mL). The combined filtrates were concentrated under reduced pressure to yield 1-(*S*)-(−)-camphor dimethylacetal **150** (10.23 g, 97%) as a colourless oil. Analysis by ^{1}H NMR showed 83% conversion. δ_H (400 MHz, $CDCl_3$) 3.21 (3H, s, OC*H*$_3$), 3.15 (3H, s, OC*H*$_3$), 2.21-2.16 (1H, m, camphor ring), 1.76-1.63 (3H, m, camphor ring), 0.95 (3H, s, 1-C*H*$_3$), 0.90 (3H, s, 7-C*H*$_3$), 0.81 (3H, s, 7-C*H*$_3$). The data are in good agreement with literature values.[211] This material was used without further purification.

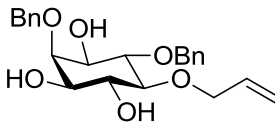
(−)-D-5-*O*-Allyl-2,6-bis-*O*-benzyl-3-*O*-*endo*,4-*O*-*exo*(L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidene)-*myo*-inositol 143



To a solution of (±)-D-5-*O*-allyl-2,6-bis-*O*-benzyl-*myo*-inositol **149** (4.01 g, 9.9 mmol, 1.0 eq.) in dichloromethane (50 mL), 1-(*S*)-(−)-camphor dimethylacetal **150** (83%, 5.96 g, 24.9 mmol, 2.5 eq.) and 4-toluenesulfonic acid monohydrate (569 mg, 2.9 mmol, 0.3 eq.) were added and heated under reflux for 16 h. When the reaction was deemed to be complete by TLC analysis, the solvent was concentrated under reduced pressure. The resulting oil was adsorbed onto silica gel, and purified by silica gel chromatography, eluting with petroleum ether and ethyl acetate (95:5, 94:6, 93:7, 92:8, 91:9, 90:10, 80:20) to give (−)-D-5-*O*-allyl-2,6-bis-*O*-benzyl-3-*O*-*endo*,4-*O*-*exo*(L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidene)-*myo*-inositol **143** (765 mg, 15%) as a colourless oil: R_f 0.70 (petroleum ether/ethyl acetate, 80:20); $[\alpha]_D^{25}$ −12.0 (*c* 0.6 in $CHCl_3$), [Lit.[51] $[\alpha]_D^{25}$ −11.7 (*c* 1.3 in $CHCl_3$)]; δ_H (400 MHz, $CDCl_3$) 7.42-7.26 (10H, m, Ar*H*), 5.98 (1H, dddd, *J* 16.9, 10.9, 5.6, 5.6, CH_X=CH_YH$_Z$), 5.33 (1H, dd, *J* 16.9, 1.6, CH$_X$=CH_YH$_Z$), 5.18 (1H, dd, *J* 10.9, 1.6, CH$_X$=CH$_Y$$H_Z$), 5.02 (1H, d, *J* 11.5, OCH_AH$_B$Ph), 4.94 (1H, d, *J* 11.0, OC$H_{A'}$H$_{B'}$Ph, 4.79 (1H, d, *J* 11.0, OCH$_{A'}$$H_{B'}$Ph), 4.69 (1H, d, *J* 11.5, OCH$_A$$H_B$Ph), 4.42 (1H, dd, *J* 11.7, 5.6 OCH_AH$_B$CH=CH$_2$), 4.23-4.18 (2H, m, OCH$_A$$H_B$CH=CH$_2$ and 1 × inositol ring), 3.99 (1H, dd, *J* 9.5, 9.5,

1 × inositol ring), 3.71-3.51 (3H, m, 3 × inositol ring), 3.30 (1H, dd, *J* 9.8, 1.5, 1 × inositol ring), 2.49 (1H, d, *J* 7.8, O*H*), 2.16 (1H, dt, *J* 13.4, 3.7, 1 × camphor ring), 1.96-1.90 (1H, m, 1 × camphor ring), 1.77-1.71 (2H, m, 2 × camphor ring), 1.48 (1H, d, *J* 13.3, 1 × camphor ring), 1.45-1.38 (1H, m, 1 × camphor ring), 1.28-1.21 (1H, m, 1 × camphor ring), 1.04 (3H, s, CH_3), 0.88 (3H, s, CH_3 camphor bridge), 0.87 (3H, s, CH_3 camphor bridge. The data are in good agreement with literature values.[51]

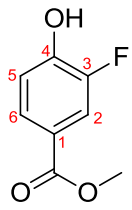
(−)-D-5-*O*-Allyl-2,6-bis-*O*-benzyl-*myo*-inositol 157



Acetyl chloride (64 mg, 57 µL, 0.8 mmol, 0.6 eq.) was added to a solution of (−)-D-5-*O*-allyl-2,6-bis-*O*-benzyl-3-*O*-*endo*,4-*O*-*exo*(L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidene)-*myo*-inositol **143** (709 mg, 1.3 mmol, 1.0 eq.) in dichloromethane (6 mL) and methanol (4 mL) and stirred at RT for 12 h. When the reaction was deemed to be complete by TLC analysis, the acid was quenched by the addition of triethylamine (0.50 mL) and the volatile components were removed under reduced pressure. The resulting oil was adsorbed onto silica gel, and purified by silica gel chromatography, eluting with chloroform and methanol (98:2) to give (−)-D-5-*O*-allyl-2,6-bis-*O*-benzyl-*myo*-inositol **157** (389 mg, 71%) as a colourless solid: R_f 0.33 (chloroform/methanol, 95:5); $[\alpha]_D^{25}$ −19.4 (*c* 1.0 in $CHCl_3$) [Lit.[53] $[\alpha]_D^{20}$ −17.6 (*c* 1.0 in $CHCl_3$)]; mp 110-112 °C (*from methanol)* (Lit.[51] 111-112 °C); δ_H

(400 MHz, $CDCl_3$) 7.38-7.30 (10 H, m, Ar*H*), 5.98 (1H, dddd, *J* 16.9, 10.9, 5.6, 5.6, CH_X=CH$_Y$H$_Z$), 5.31 (1H, dd, *J* 16.9, 1.3, CH$_X$=CH_YH$_Z$), 5.20 (1H, dd, *J* 10.9, 1.3, CH$_X$=CH$_Y$$H_Z$), 4.93 (1H, d, *J* 11.1, OCH_AH$_B$Ph), 4.89 (1H, d, *J* 11.5, OCH_AH$_B$Ph, 4.78 (1H, d, *J* 11.1, OCH$_A$$H_B$Ph), 4.75 (1H, d, *J* 11.5, OCH$_A$$H_B$Ph), 4.41-4.30 (2H, m, OCH_2CH$_X$=CH$_Y$H$_Z$), 4.03-4.02 (1H, m, 1 × inositol ring), 3.84-3.78 (1H, m, 1 × inositol ring), 3.76-3.71 (1H, m, 1 × inositol ring), 3.60-3.56 (1H, m, 1 × inositol ring), 3.50-3.45 (1H, m, 1 × inositol ring), 3.25-3.20 (1H, m, 1 × inositol ring), 2.53 (1H, d, *J* 2.1, O*H*), 2.34-2.31 (2H, m, 2 × O*H*)); *m/z* (ES$^+$) 423 ([M+Na]$^+$, 100%). The data are in good agreement with literature values.[51]

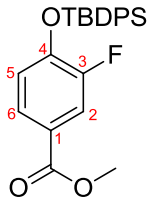
Methyl-3-fluoro-4-hydroxybenzoate 160



Aqueous concentrated sulfuric acid (15.00 mL, 281.4 mmol, 9.0 eq.) was added to a stirred solution of 3-fluoro-4-hydroxybenzoic acid **159** (5.00 g, 32.0 mmol, 1.0 eq.) in methanol (200 mL), and the reaction mixture was heated under reflux for 12 h. When the reaction was deemed to be complete by TLC analysis, the sulfuric acid was neutralised by the careful addition of saturated aqueous sodium bicarbonate solution (50 mL), and the reaction mixture was concentrated under reduced pressure. The resulting residue was reconstituted with H_2O (30 mL) and ethyl acetate (100 mL), and the

layers were separated. The aqueous phase was further extracted with ethyl acetate (2 × 30 mL), and the combined organic layers were washed with saturated aqueous sodium bicarbonate solution (20 mL) and brine (20 mL), dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The resulting residue was adsorbed onto silica gel, and purified by silica gel chromatography, eluting with petroleum ether and ethyl acetate (70:30) to afford methyl-3-fluoro-4-hydroxybenzoate **160** (4.63 g, 85%) as a pale yellow powder: R_f 0.66 (petroleum ether/ethyl acetate, 60:40); mp 99-101 °C (*from ethyl acetate*) (Lit.[212] 91-93 °C); δ_H 7.77 (2×1H, d, *J* 10.0, H-2, H-6), 7.04 (1H, dd, *J* 8.4, 8.4, H-5), 5.57 (1H, br s, O*H*), 3.90 (3H, s, OCH_3); δ_F (376 MHz, $CDCl_3$) −140.5. The data are in good agreement with literature values.[212]

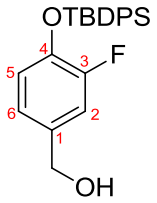
Methyl-4-(*tert*-butyldiphenylsiloxy)-3-fluorobenzoate 161



To a stirred solution of methyl-3-fluoro-4-hydroxybenzoate **160** (509 mg, 2.9 mmol, 1.0 eq.) and triethylamine (1.81 g, 2.50 mL, 17.6 mmol, 6.0 eq.) in dichloromethane (50 mL), *tert*-butyldiphenylsilyl chloride (2.02 g, 1.90 mL, 7.3 mmol, 2.5 eq.) was added dropwise over a period of 30 min. The reaction mixture was stirred at RT for 7 h, after which time the reaction was deemed to be complete by TLC analysis. Dichloromethane (30 mL) and H_2O

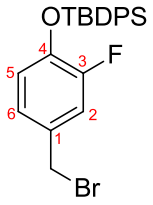
(20 mL) were added, and the resulting layers separated. The aqueous phase was further extracted with dichloromethane (2 × 15 mL), and the combined organic layers were washed with brine (20 mL), dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The resulting oil was adsorbed onto silica gel, and purified by silica gel chromatography, eluting with petroleum ether and ethyl acetate (100:0, 99:1) to afford *methyl-4-(tert-butyldiphenylsiloxy)-3-fluorobenzoate* **161** (1.04 g, 85%) as a colourless oil: R_f 0.89 (petroleum ether/ethyl acetate, 90:10); ν_{max} (thin film)/cm^{-1} 2933 (m), 2859 (m), 1724 (s), 1613 (m), 1516 (s), 1439 (m), 1302 (s), 1202 (m), 934 (m), 876 (w), 765 (w), 702 (m); δ_H (400 MHz, $CDCl_3$) 7.73-7.70 (5H, m, Ar*H*), 7.46-7.45 (2H, m, H-2, H-6), 7.44-7.36 (5H, m, Ar*H*), 6.62 (1H, dd, *J* 8.4, 8.4, H-5), 3.84 (3H, s, OC*H*$_3$), 1.14 (9H, s, *tert*-butyl C*H*$_3$); δ_C (126 MHz, $CDCl_3$) 165.9 (C=O), 153.2 (Ar C-3, d, *J* 246.2), 147.8 (Ar C-4, d, *J* 12.1), 135.3 (Ar CH), 131.8 (Ar C), 130.2 (Ar C-6), 129.0 (Ar CH), 128.0 (Ar CH), 125.2 (Ar C-2, d, *J* 3.1), 123.6 (Ar C-1, d, *J* 6.2), 120.9 (Ar C-5), 117.7 (Ar CH), 117.6 (Ar CH), 52.0 (OCH_3), 26.3 (*tert*-butyl CH_3), 19.6 (*tert*-butyl C); δ_F (376 MHz, $CDCl_3$) −131.2; *m/z* (ES^+) [Found: $(M+Na)^+$ 431.1445. $C_{24}H_{25}FO_3SiNa$ requires M^+ 431.1449], *m/z* (ES^+) 499 (100%), 449 ($[M+CH_3CN+H]^+$, 75%); Anal. calcd for $C_{24}H_{25}FO_3Si$: C 70.6, H 6.1; Found: C 70.7; H 6.1.

4-(*tert*-Butyldiphenylsiloxy)-3-fluorobenzyl alcohol 162



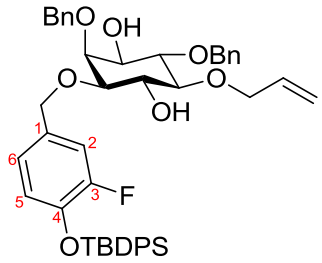
Lithium aluminium hydride (1.0 M in THF, 279 mg, 7.40 mL, 3.0 eq.) was added dropwise over a period of 30 min to a solution of methyl-4-(*tert*-butyldiphenylsiloxy)-3-fluorobenzoate **161** (1.00 g, 2.4 mmol, 1.0 eq.) in THF (60 mL) at 0 °C. This mixture was stirred at RT for 4 h, until the reaction was deemed to be complete by TLC analysis. The reaction mixture was cooled to 0 °C, and the lithium aluminium hydride was quenched by the careful addition of ethyl acetate (15 mL), followed by H_2O (5 mL). Magnesium sulfate was added to the cloudy suspension, and the suspension was filtered through Celite®. The filtrate was concentrated under reduced pressure, adsorbed onto silica gel, and purified by silica gel chromatography, eluting with petroleum ether and ethyl acetate (90:10) to afford 4-(*tert*-butyldiphenylsiloxy)-3-fluorobenzyl alcohol **162** (791 mg, 85%) as a colourless oil: R_f 0.15 (petroleum ether/ethyl acetate, 90:10); δ_H (400 MHz, $CDCl_3$) 7.73-7.71 (4H, m, Ar*H*), 7.42-7.35 (6H, m, Ar*H*), 7.06 (1H, dd, *J* 11.2, 2.0, H-2), 6.73 (1H, d, *J* 8.4, H-6), 6.59 (1H, dd, *J* 8.4, 8.4, H-5), 4.53 (2H, d, *J* 6.0, CH_2), 1.54 (1H, t, *J* 6.0, O*H*), 1.12 (9H, s, *tert*-butyl CH_3); δ_F (376 MHz, $CDCl_3$) −131.6; The data are in good agreement with literature values.[130]

4-(*tert*-Butyldiphenylsiloxy)-3-fluorobenzyl bromide 163



To a solution of 4-(*tert*-butyldiphenylsiloxy)-3-fluorobenzyl alcohol **162** (755 mg, 2.0 mmol, 1.0 eq.) and triphenylphosphine (791 mg, 3.0 mmol, 1.5 eq.) in dichloromethane (10 mL), carbon tetrabromide (988 mg, 3.0 mmol, 1.5 eq.) was added dropwise over a period of 30 min. The resulting solution was stirred for 1.5 h at RT, until the reaction was deemed to be complete by TLC analysis. The reaction mixture was concentrated under reduced pressure, adsorbed onto silica gel, and purified by silica gel chromatography, eluting with petroleum ether and ethyl acetate (99:1) to afford 4-(*tert*-butyldiphenylsiloxy)-3-fluorobenzyl bromide **163** (603 mg, 69%) as a colourless oil: R_f 0.86 (petroleum ether/ethyl acetate, 85:15); δ_H (400 MHz, $CDCl_3$) 7.73-7.70 (4H, m, Ar*H*), 7.47-7.36 (6H, m, Ar*H*), 7.08 (1H, dd, *J* 11.2, 2.4, H-2), 6.76 (1H, d, *J* 8.0, H-6), 6.55 (1H, dd, *J* 8.0, 8.4, H-5), 4.36 (2H, s, CH_2), 1.12 (9H, s, *tert*-butyl CH_3); δ_F (376 MHz, $CDCl_3$) −131.0. The data are in good agreement with literature values.[130]

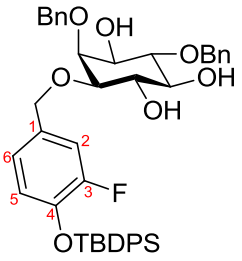
(−)-D-3-*O*-[4'-(*tert*-Butyldiphenylsiloxy)-3'-fluoro]benzyl-5-*O*-allyl-2,6-bis-*O*-benzyl-*myo*-inositol 164



To a solution of (−)-D-5-*O*-allyl-2,6-bis-*O*-benzyl-*myo*-inositol **157** (541 mg, 1.3 mmol, 1.0 eq.), dibutyltin oxide (369 mg, 1.5 mmol, 1.1 eq.) and tetrabutylammonium iodide (498 mg, 1.3 mmol, 1 eq.) in acetonitrile (60 mL), 4-(*tert*-butyldiphenylsiloxy)-3-fluorobenzyl bromide **163** (1.80 g, 4.0 mmol, 3.0 eq.) was added. The reaction was heated under reflux using a Soxhlet apparatus filled with 4 Å molecular sieves for 48 h, after which time the reaction was deemed to be complete by TLC analysis. The reaction mixture was concentrated under reduced pressure, the resulting residue reconstituted with H_2O (15 mL) and ethyl acetate (50 mL), and the layers separated. The aqueous phase was further extracted with ethyl acetate (2 × 30 mL), and the combined organic layers washed with brine (15 mL), dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The resulting residue was adsorbed onto silica gel, and purified by silica gel chromatography, eluting with petroleum ether and ethyl acetate (80:20) to afford (−)-D-*3-O-[4'-(tert-butyldiphenylsiloxy)-3'-fluoro]benzyl-5-O-allyl-2,6-bis-O-benzyl-myo-inositol* **164** (627 mg, 62%) as a colourless oil: R_f 0.67 (petroleum ether/ethyl acetate, 60:40); $[\alpha]_D^{25}$ −4.6 (*c* 0.2 in $CHCl_3$); ν_{max} (thin

film)/cm^{-1} 3451 (s), 2859 (m), 1586 (s), 1428 (m), 1299 (s), 1115 (s), 742 (m); δ_H (400 MHz, $CDCl_3$) 7.70-7.23 (4H, m, Ar*H*), 7.43-6.59 (16H, m, Ar*H*), 7.01 (1H, dd, *J* 11.2, 1.9, Ar H-2), 6.68 (1H, d, *J* 9.1, Ar H-6), 6.58 (1H, dd, *J* 8.4, 8.4, Ar H-5), 5.97 (1H, dddd, *J* 17.2, 10.9, 5.7, 5.7, CH_X=CH$_Y$H$_Z$), 5.29 (1H, dd, *J* 17.2, 1.5, CH$_X$=CH_YH$_Z$), 5.17 (1H, dd, *J* 10.9, 1.5, CH$_X$=CH$_Y$$H_Z$), 4.89 (1H, d, *J* 11.2, OCH_AH$_B$Ph), 4.84 (1H, d, *J* 11.4, OC$H_{A'}$H$_{B'}$Ph), 4.75 (1H, d, *J* 11.2, OCH$_A$$H_B$Ph), 4.66 (1H, d, *J* 11.4, OCH$_{A'}$$H_{B'}$Ph), 4.47, (1H, d, *J* 11.6, OCH_AH$_B$Ar), 4.43 (1H, d, *J* 11.6, OCH$_A$$H_B$Ar), 4.34 (2H, dd, *J* 5.7, 1.3, OCH_2CH$_X$=CH$_Y$H$_Z$), 4.03 (1H, dd, *J* 4.7, 1.9, inositol ring H-4), 3.97-3.96 (1H, m, inositol ring H-2), 3.72 (1H, dd, *J* 9.1, 9.1, inositol ring H-6), 3.25-3.23 (1H, m, inositol ring H-1), 3.22 (1H, dd, *J* 9.1, 9.1, inositol ring H-5), 3.17-3.19 (1H, m, inositol ring H-3), 2.44 (1H, d, *J* 1.9, 4-OH), 2.23 (1H, d, *J* 5.9, 1-OH), 1.12 (9H, s, *tert*-butyl CH_3); δ_C (126 MHz, $CDCl_3$) 153.6 (Ar C-3, d, *J* 244.9), 143.0 (Ar C-4, d, *J* 12.7), 138.4 (Ar C-1, d, *J* 8.8), 135.4 (Ar CH), 135.0 (*C*H=CH$_2$), 132.4 (Ar C), 130.0 (Ar C), 128.4 (Ar CH), 128.3 (Ar CH), 128.0 (Ar CH), 127.8 (Ar CH), 127.7 (Ar CH), 127.7 (Ar CH), 127.6 (Ar CH), 123.1 (Ar C-6, d, *J* 3.6), 121.2 (Ar C-5), 116.9 (Ar C-2), 82.8 (inositol ring C-5), 81.7 (inositol ring C-6), 76.0 (inositol ring C-2), 75.4 (O*C*H$_2$), 74.5 (O*C*H$_2$), 74.0 (O*C*H$_2$), 72.9 (inositol ring C-4), 72.5 (inositol ring C-1), 71.6 (Ar CH$_2$), 26.4 (*tert*-butyl CH$_3$), 19.6 (*tert*-butyl C); δ_F (376 MHz, $CDCl_3$) −131.4; *m/z* (ES$^+$) [Found: (M+Na)$^+$ 785.3246. $C_{46}H_{51}FO_7SiNa$ requires M^+ 785.3280], *m/z* (ES$^+$) 821 ([M+CH$_3$CN+NH$_4$]$^+$, 100%), 780 ([M+NH$_4$]$^+$, 60%); Anal. calcd for $C_{46}H_{51}FO_7Si$: C 72.1, H 6.7; Found: C 72.5; H 6.7.

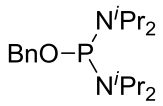
(+)-D-3-*O*-[4'-(*tert*-Butyldiphenylsiloxy)-3'-fluoro]benzyl-2,6-bis-*O*-benzyl-*myo*-inositol 165



Palladium(II) chloride (17 mg, 0.9 mmol, 0.6 eq.) was added to a solution of (−)-D-3-*O*-[4'-(*tert*-butyldiphenylsiloxy)-3'-fluoro]benzyl-5-*O*-allyl-2,6-bis-*O*-benzyl-*myo*-inositol **164** (119 mg, 0.1 mmol, 1.0 eq.) in methanol (10 mL) and stirred overnight at RT. When the reaction was deemed to be complete by TLC analysis, the reaction mixture was filtered through Celite®, washed with ethyl acetate and concentrated under reduced pressure. The resulting residue was reconstituted in H_2O (10 mL) and ethyl acetate (30 mL) and the phases were separated. The aqueous layer was re-extracted with ethyl acetate (3 × 15 mL) and the combined organic extracts were washed with brine (20 mL), dried over magnesium sulfate, filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography, eluting with petroleum ether and ethyl acetate (70:30, 60:40) to give *(+)-D-3-O-[4'-(tert-butyldiphenylsiloxy)-3'-fluoro]benzyl-2,6-bis-O-benzyl-myo-inositol* **165** (78 mg, 71%) as a colourless oil: R_f 0.11 (petroleum ether/ethyl acetate, 70:30); $[\alpha]_D^{25}$ +3.7 (*c* 0.6 in $CHCl_3$); ν_{max} (thin film)/cm^{-1} 3445 (m), 2928 (m), 1732 (m), 1517 (s), 1428 (m), 1297 (m), 1115 (s), 902 (w), 821 (w); δ_H (400 MHz, $CDCl_3$) 7.72-7.70 (4H, m, Ar*H*), 7.43-

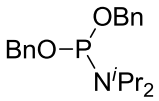
7.26 (16H, m, Ar*H*), 7.01 (1H, dd, *J* 11.0, 2.0, Ar H-2), 6.69 (1H, d, *J* 8.4, Ar H-6), 6.58 (1H, dd, *J* 8.4, 8.4, Ar H-5), 4.88-4.85 (3H, m, OC*H*$_2$Ph and OC*H*$_A$H$_B$Ph), 4.67 (1H, d, *J* 11.5, OCH$_A$*H*$_B$Ph), 4.48 (1H, d, *J* 11.6, OC*H*$_{A'}$H$_{B'}$Ar), 4.41 (1H, d, *J* 11.6, OCH$_{A'}$*H*$_{B'}$Ar) , 4.00-3.99 (1H, m, inositol ring H-2), 3.96 (1H, d, *J* 9.3, inositol ring H-4), 3.67 (1H, dd, *J* 9.3, 9.3, inositol ring H-6), 3.52-3.48 (1H, m, inositol ring H-1), 3.47-3.43 (1H, m, inositol ring H-5), 3.20 (1H, dd, *J* 9.7, 2.3, inositol ring H-3), 2.55 (1H, d, *J* 2.1, 5-O*H*), 2.49 (1H, d, *J* 2.1, 4-O*H*), 2.33 (1H, d, *J* 6.4, 1-O*H*), 1.13 (9H, s, *tert*-butyl C*H*$_3$); δ_C (126 MHz, $CDCl_3$) 153.4 (Ar C, d, *J* 245.6, Ar C-3), 143.2 (Ar C, d, *J* 7.2, Ar C-4), 138.4 (Ar C, d, *J* 20.9, Ar C-1), 138.5 (Ar C), 138.3 (Ar C), 135.4 (Ar CH), 132.4 (Ar C), 130.0 (Ar C), 128.7 (Ar CH), 128.5 (Ar CH), 128.4 (Ar CH), 128.0 (Ar CH), 127.0 (Ar CH), 127.8 (Ar CH), 127.7 (Ar CH), 123.2 (d, *J* 3.2, Ar C-6), 121.2 (Ar C-5), 115.8 (Ar C-2, d, *J* 18.3), 81.6 (inositol ring C-6), 80.1 (inositol ring C-3) 76.2 (inositol ring C-2), 75.0 (CH_2), 74.8 (CH_2), 74.7 (inositol ring C-5), 72.5 (inositol ring C-4), 72.4 (inositol ring C-1), 71.6 (CH_2), 26.4 (*tert*-butyl CH_3), 19.6 (*tert*-butyl C); δ_F (470 MHz, $CDCl_3$) −131.3; *m/z* (ES^+) [Found: $(M+Na)^+$ 745.2966. $C_{43}H_{47}FO_7SiNa$ requires M^+ 745.2967], *m/z* (ES^+) 745 ($[M+Na]^+$, 100%), 740 ($[M+NH_4]^+$, 20%); Anal. calcd for $C_{43}H_{47}FO_7Si$: C 71.4, H 6.5; Found: C 71.5; H 6.6.

Benzyloxy bis(*N,N*-diisopropyl)phosphoramidite 169



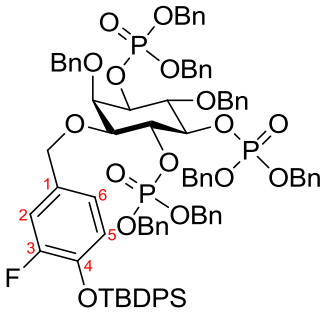
Anhydrous pyridine (16.39 g, 16.70 mL, 206.0 mmol, 1.0 eq.) was added to a stirred solution of phosphorus trichloride **168** (28.26 g, 18.00 mL, 206.0 mmol, 1.0 eq.) in anhydrous diethyl ether (200 mL) at −78 °C, and a white precipitate formed. A solution of benzyl alcohol (21.91 g, 21.00 mL, 206.0 mmol, 1.0 eq.) in dry diethyl ether (150 mL) was added dropwise over 1 h. The mixture was allowed to reach RT and stirred for 2 h. The resulting precipitate was filtered through a Schlenk filter under a blanket of nitrogen, and then cooled to -10 °C. Anhydrous *N,N*-diisopropylamine (87.55 g, 118 mL, 846.0 mmol, 4.1 eq.) was added portionwise over a period of 30 min, and the mixture stirred at RT overnight. The resulting precipitate was removed by filtration through Celite®, and the filtrate concentrated under reduced pressure to yield benzyloxy bis(*N,N*-diisopropyl)phosphoramidite **169** (33.09 g, 46%) as a yellow oil: δ_H (400 MHz, $CDCl_3$) 7.33-7.25 (5H, m, Ar*H*), 4.69 (2H, d, *J* 7.4, OCH_2Ph), 3.66-3.57 (4H, m, NC*H*$(CH_3)_2$), 1.22 (24H, dd, *J* 6.7, 5.2, NCH$(CH_3)_2$); δ_P (161 MHz, $CDCl_3$) 123.7. The data are in good agreement with literature values.[213]

Bis(benzyloxy)-*N,N*-diisopropylphosphoramidite 167



1*H*-Tetrazole (0.45 M in acetonitrile, 8.00 mL, 3.4 mmol, 0.4 eq.) was added to a stirred solution of benzyloxy bis(*N,N*-diisopropyl)phosphoramidite **169** (3.19 g, 8.5 mmol, 1.0 eq.) in dichloromethane (50 mL). Dry benzyl alcohol (0.92 g, 0.90 mL, 8.5 mmol, 1.0 eq.) was added dropwise over a period of 30 min. The resulting mixture was stirred at RT for 2 h. The solvent was removed under reduced pressure to give a colourless paste which was purified by silica gel chromatography, eluting with triethylamine, ethyl acetate and petroleum ether (5:5:90) to yield bis(benzyloxy)-*N,N*-diisopropylphosphoramidite **167** (2.29 g, 73%) as a colourless oil: δ_H (400 MHz, $CDCl_3$) 7.38-7.25 (10H, m, Ar*H*), 4.79 (2H, dd, *J* 12.6, 8.2, OCH_AH$_B$Ph), 4.71 (2H, dd, *J* 12.6, 8.2, OCH$_A$$H_B$Ph), 3.74-3.69 (2H, m, NC*H*(CH$_3$)$_2$), 1.22 (12H, d, *J* 6.8, NCH(CH_3)$_2$); δ_P (161 MHz, $CDCl_3$) 148.0. The data are in good agreement with the literature.[132]

(+)-D-3-*O*-[4'-(*tert*-Butyldiphenylsiloxy)-3'-fluoro]benzyl-2,6-bis-*O*-benzyl-*myo*-inositol 1,4,5-tris(dibenzylphosphate) 166



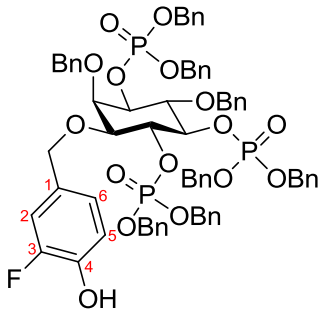
1*H*-Tetrazole (0.45 M in acetonitrile, 5.60 mL, 2.5 mmol, 7.5 eq.) was added to a solution of bis(benzyloxy)-*N,N*-diisopropylphosphoramidite **167** (1.22 g, 2.5 mmol, 7.5 eq.) in dichloromethane (30 mL) and the resulting mixture was stirred for 10 min. A solution of (+)-D-3-*O*-[4'-(*tert*-butyldiphenylsiloxy)-3'-fluoro]benzyl-2,6-bis-*O*-benzyl-*myo*-inositol **165** (240 mg, 0.3 mmol, 1.0 eq.) in dichloromethane (20 mL) was added *via* cannula, and the resulting mixture was stirred overnight. After 12 h, TLC analysis indicated that the reaction was incomplete, and a further solution of bis(benzyloxy)-*N,N*-diisopropylphosphoramidite **167** (325 mg, 0.7 mmol, 2.0 eq.) in dichloromethane (10 mL) was added *via* cannula, followed by 1*H*-tetrazole in acetonitrile (0.45 M, 1.46 mL, 0.7 mmol, 2.0 eq.). The resulting mixture was stirred for 2 h, after which time the reaction was deemed to be complete. The mixture was cooled to −78 °C and 3-chloroperoxybenzoic acid (assume 60%, 930 mg, 3.1 mmol, 9.5 eq.) was added. The mixture was allowed to reach RT and stirred for 2 h, after which time the reaction was deemed to be complete by TLC analysis. The 3-chloroperbenzoic acid was

quenched with a 10% aqueous solution of sodium metabisulfite (30 mL) and the resulting mixture was stirred for 15 min. The resulting organic and aqueous phases were separated, and the aqueous layer was re-extracted with dichloromethane (3 × 30 mL). The combined organic extracts were washed sequentially with saturated aqueous sodium bicarbonate solution (20 mL) and brine (20 mL), dried over magnesium sulfate, filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography, eluting with ethyl acetate and petroleum ether (30:70, 40:60, 60:40) to give *(+)-D-3-O-[4'-(tert-butyldiphenylsiloxy)-3'-fluoro]benzyl-2,6-bis-O-benzyl-myo-inositol 1,4,5-tris(dibenzylphosphate)* **166** (448 mg, 91%) as a colourless oil: R_f 0.11 (petroleum ether/ethyl acetate, 70:30); $[\alpha]_D^{25}$ +2.8 (*c* 0.8 in $CHCl_3$); ν_{max} (thin film)/cm^{-1} 3033 (m), 2932 (m), 1517 (s), 1455 (m), 1275 (s), 1216 (m), 1014 (s), 899 (m), 822 (w), 738 (m), 697 (m); δ_H (400 MHz, $CDCl_3$) 7.69 (4H, d, *J* 7.7, Ar*H*), 7.41-7.30 (9H, m, Ar*H*), 7.25-7.05 (35H, m, Ar*H*), 6.99-6.94 (3H, m, Ar*H* and Ar H-2), 6.60 (1H, d, *J* 8.5, Ar H-6), 6.49 (1H, dd, *J* 8.5, 8.5, Ar H-5), 5.04-5.01 (1H, m, OC$H_A$$H_B$Ph), 4.96-4.61 (14H, m, OC$H_A$$H_BPh, OCH_2$Ph and inositol ring H-4), 4.65-4.61 (2H, m, OCH_2Ph), 4.51 (1H, d, *J* 9.1, inositol ring H-5), 4.32-4.29 (2H, m, inositol ring H-2 and OC$H_A$$H_B$Ph), 4.24-4.19 (2H, m, inositol ring H-1 and OC$H_A$$H_B$Ph), 4.09 (1H, dd, *J* 9.5, 9.5, inositol ring H-6), 3.34 (1H, dd, *J* 9.8, 1.9, inositol ring H-3), 1.11 (9H, s, *tert*-butyl); δ_C (126 MHz, $CDCl_3$) 153.4 (Ar C, d, *J* 246.4, Ar C-3), 142.7 (Ar C, d, *J* 11.6, Ar C-4) 138.1 (Ar C), 139.0 (Ar C), 136.1 (Ar C), 136.0 (Ar C), 135.9 (Ar C), 135.8 (Ar C), 135.7 (Ar C), 135.5 (Ar C), 135.4 (Ar C), 135.4 (Ar CH), 135.4 (Ar CH), 132.4 (Ar CH), 130.9 (Ar CH), 130.1 (Ar CH), 129.9 (Ar CH), 128.5 (Ar

CH, d, *J* 4.7), 128.26 (Ar CH), 128.23 (Ar CH), 128.22 (Ar CH, d, *J* 1.9), 128.06 (Ar CH), 128.03 (Ar CH, d, *J* 3.6), 128.00 (Ar CH), 127.81 (Ar CH), 127.77 (Ar CH), 127.73 (Ar C-2, d, *J* 3.6), 127.69 (Ar CH), 127.5 (Ar CH), 127.1 (Ar CH), 122.9 (Ar CH, d, *J* 3.6, Ar C-6), 120.9 (Ar C-5), 115.6 (Ar CH), 115.5 (Ar CH), 79.0 (inositol ring C-5), 78.0 (inositol ring C-1), 77.9 (inositol ring C-6), 77.4 (inositol ring C-3), 77.2 (inositol ring C-4), 74.5 (inositol ring C-2), 71.0 (CH_2), 69.5 (CH_2), 69.47 (CH_2, d, *J* 3.6), 69.44 (CH_2), 69.34 (CH_2, d, *J* 4.1), 69.30 (CH_2), 69.1 (CH_2, d, *J* 4.5), 69.0 (CH_2, d, *J* 4.5), 26.4 (*tert*-butyl CH_3), 19.6 (*tert*-butyl C); δ_F (470 MHz, $CDCl_3$) −131.5; δ_P (202 MHz, $CDCl_3$) −0.4, −0.6, −0.9; *m/z* (ES^+) [Found: $(M+Na)^+$ 1525.48 (100.0%), 1526.48 (98.2%), 1527.48 (53.2%), 1528.49 (20.0%), 1529.49 (5.9%), 1530.49 (1.4%). $C_{85}H_{86}FO_{16}P_3Si$ requires M^+ 1525.47 (100.0%), 1526.48 (98.6%), 1527.48 (54.6%), 1528.48 (21.7%), 1529.48 (6.8%), 1530.48 (1.8%)]; *m/z* (ES^+) 1525 ($[M+Na]^+$, 100%), 1604 ($[M+NHEt_3]^+$, 80%), 1520 ($[M+NH_4+MeCN]^+$, 45%); Anal. calcd for $C_{85}H_{86}FO_{16}P_3Si$: C 67.9, H 5.8; Found: C 67.8; H 5.7.

*Due to resonance overlap in the ^{13}C NMR spectrum, some carbon environments have not been accounted for.

(−)-D-3-*O*-[4'-Hydroxy-3'-fluoro]benzyl-2,6-bis-*O*-benzyl-*myo*-inositol 1,4,5-tris(dibenzylphosphate) 170



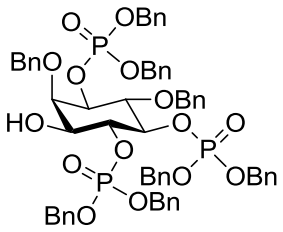
Tetra-*n*-butyl ammonium fluoride solution in tetrahydrofuran (1.0 M, 0.11 mL, 0.1 mmol, 3.0 eq.) was added to a stirred solution of (+)-D-3-*O*-[4'-(*tert*-butyldiphenylsiloxy)-3'-fluoro]benzyl-2,6-bis-*O*-benzyl-*myo*-inositol 1,4,5-tris(dibenzylphosphate) **166** (53 mg, 0.03 mmol, 1.0 eq.) in dichloromethane (5 mL) and stirred at RT for 2 h. After this time, the reaction was deemed to be complete by TLC analysis. The reaction mixture was neutralised by the addition of 1.0 M hydrochloric acid solution and the aqueous and organic layers were separated. The aqueous layer was re-extracted with dichloromethane (3 × 15 mL), and the combined organic extracts were washed sequentially with saturated aqueous sodium bicarbonate solution (10 mL) and brine (10 mL), dried over magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with ethyl acetate and petroleum ether (40:60, 60:40) to afford *(−)-D-3-O-[4'-hydroxy-3'-fluoro]benzyl-2,6-bis-O-benzyl-myo-inositol 1,4,5-tris(dibenzylphosphate)* **170** (23 mg, 53%) as a colourless oil: R_f 0.39 (petroleum ether/ethyl acetate, 50:50); $[\alpha]_D^{25}$ −1.3 (*c* 0.3 in $CHCl_3$);

ν_{max} (thin film)/cm^{-1} 3443 (s), 2961 (w), 1628 (w), 1522 (m), 1497 (m), 1455 (s), 1378 (m), 1261 (s), 1017 (s), 876 (w), 798 (m), 737 (m), 696 (m); δ_H (400 MHz, $CDCl_3$) 7.35 (2H, d, *J* 6.8, Ar*H*), 7.29-7.24 (6H, m, Ar*H*), 7.24-7.21 (5H, m, Ar*H*), 7.21-7.17 (15H, m, Ar*H*), 7.15-7.09 (10H, m, Ar*H*), 7.04 (1H, dd, *J* 11.4, 1.5, Ar H-2), 6.96 (2×1H, d, *J* 7.3, Ar*H* and Ar H-6), 6.89-6.82 (2H, m, Ar*H* and Ar H-5), 5.30 (1H, br s, O*H*), 5.05-4.73 (15H, m, OC*H*$_2$Ph and inositol ring H-4), 4.70-4.62 (2H, m, OC*H*$_2$Ph), 4.53 (1H, d, *J* 9.1, inositol ring H-5), 4.39 (1H, d, *J* 11.3, OC*H*$_2$Ph), 4.32-4.29 (2H, m, OC*H*$_2$Ph and inositol ring H-2), 4.25 (1H, ddd, *J* 9.7, 7.4, 2.3, inositol ring H-1), 4.10 (1H, dd, *J* 9.5, 9.5, inositol ring H-6), 3.41 (1H, dd, *J* 9.9, 2.0, inositol ring H-3); δ_C (126 MHz, $CDCl_3$) 151.7 (Ar C, d, *J* 245.3, Ar C-3), 143.1 (Ar C, d, *J* 12.5, Ar C-4) 138.1 (Ar C, d, *J* 7.5, Ar C-1), 136.0 (Ar C, d, *J* 4.1), 135.8 (Ar C, d, *J* 5.6), 135.7 (Ar C), 135.5 (Ar C), 128.5 (Ar CH, d, *J* 5.1), 128.2 (Ar CH, d, *J* 4.5), 128.0 (Ar CH), 127.8 (Ar CH, d, *J* 4.5), 127.6 (Ar CH, d, *J* 3.2), 127.5 (Ar CH), 127.1 (Ar CH), 124.1 (Ar CH), 124.0 (Ar CH), 116.9 (Ar C), 115.1 (Ar C), 115.0 (Ar CH), 78.9 (inositol ring C-1), 78.0 (inositol ring C-5), 77.9 (inositol ring C-4), 77.8 (inositol ring C-6), 77.2 (inositol ring C-3), 75.2 (CH_2), 74.9 (inositol ring C-2), 74.5 (CH_2), 71.2 (CH_2), 69.5 (CH_2), 69.4 (CH_2, d, *J* 4.1), 69.37 (CH_2, d, *J* 4.7), 69.35 (CH_2, d, *J* 5.7), 69.1 (CH_2, d, *J* 5.1); δ_F (376 MHz, $CDCl_3$) −139.3; δ_P (161 MHz, $CDCl_3$) −1.5, −1.8, −2.1; *m/z* (ES$^+$) [Found: (M+K)$^+$ 1303.33 (100.0%), 1304.33 (87.4%), 1305.33 (48.4%), 1306.34 (17.3%), 1307.34 (2.6%), 1308.34 (0.2%). $C_{69}H_{68}FO_{16}P_3K$ requires M^+ 1303.33 (100.0%), 1304.33 (76.0%), 1305.33 (39.0%), 1306.34 (15.0%), 1307.34 (4.6%), 1308.34 (1.1%)]; *m/z* (ES$^+$) 1287 ([M+Na]$^+$, 100%), 1366 ([M+NHEt$_3$]$^+$, 95%), 1282

([M+NH_4]$^+$, 40%), 1265 ([M+H]$^+$, 5%); Anal. calcd for $C_{69}H_{68}FO_{16}P_3$: C 65.5, H 5.4; Found: C 65.4; H 5.5.

*Due to resonance overlap in the ^{13}C NMR spectrum, some carbon environments have not been accounted for.

(−)-D-2,6-Bis-O-benzyl-*myo*-inositol 1,4,5-tris(dibenzylphosphate) 142

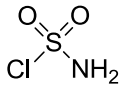


(−)-D-3-*O*-[4'-Hydroxy-3'-fluoro]benzyl-2,6-bis-*O*-benzyl-*myo*-inositol 1,4,5-tris(dibenzylphosphate) **170** (211 mg, 0.2 mmol, 1.0 eq.) was dissolved in a mixture of trifluoroacetic acid (10 mL), chloroform (10 mL) and methanol (2 mL) and stirred at RT for 5 minutes, after which time the reaction was deemed to be complete by TLC analysis. The reaction was quenched by addition of a saturated aqueous solution of sodium bicarbonate (5 mL), dichloromethane (30 mL) and H_2O (10 mL) were added, and the resulting layers separated. The aqueous phase was further extracted with dichloromethane (2 × 50 mL), and the combined organic layers were washed with brine (10 mL), dried over magnesium sulfate, filtered and concentrated under reduced pressure. The resulting oil was purified by silica gel chromatography, eluting with toluene and ethyl acetate (60:40, 40:60), to afford a colourless solid. Recrystallisation of this solid from acetonitrile afforded *(−)-D-2,6-bis-O-benzyl-myo-inositol 1,4,5-tris(dibenzylphosphate)*

142 (97 mg, 51%) as a colourless solid: R_f 0.29 (toluene/ethyl acetate, 30:70); mp 105-107 °C (*from acetonitrile*); $[\alpha]_D^{25}$ -24.6 (*c* 0.3 in $CHCl_3$); ν_{max} (thin film)/cm^{-1} 3333 (s), 3064 (m), 2916 (m), 2359 (w), 1497 (w), 1455 (m), 1379 (w), 1263 (s), 1215 (w), 1014 (s), 880 (w), 736 (m), 695 (m); δ_H (400 MHz, $CDCl_3$) 7.37-7.06 (36H, m, Ar*H*), 7.06-7.05 (2H, m, Ar*H*), 6.96-6.95 (2H, m, Ar*H*), 5.09-4.72 (15H, m, OCH_AH_BPh), 4.67-4.61 (2H, m, OC$H_A$$H_B$Ph and inositol ring H-4), 4.48 (1H, dd, *J* 9.4, 9.4, inositol ring H-5), 4.32-4.28 (1H, m, inositol ring H-1), 4.25 (1H, dd, *J* 2.5, 2.5, inositol ring H-2), 4.05 (1H, dd, *J* 9.4, 9.4, inositol ring H-6), 3.72 (1H, ddd, *J* 9.4, 3.9, 2.5, inositol ring H-3); δ_C (126 MHz, $CDCl_3$) 138.6 (Ar C), 138.0 (Ar C), 135.8 (Ar C, d, *J* 7.8), 135.7 (Ar C, d, *J* 7.1), 135.5 (Ar C, d, *J* 2.7), 135.4 (Ar C, d, *J* 2.7), 135.3 (Ar C, d, *J* 3.4), 135.3 (Ar C, d, *J* 2.6), 128.6 (Ar CH), 128.5 (Ar CH), 128.5 (Ar CH), 128.4 (Ar CH), 128.4 (Ar CH), 128.3 (Ar CH), 128.2 (Ar CH), 128.1 (Ar CH), 128.1 (Ar CH), 128.1 (Ar CH), 128.0 (Ar CH), 127.8 (Ar CH), 127.7 (Ar CH), 127.7 (Ar CH), 127.6 (Ar CH), 127.6 (Ar CH), 127.5 (Ar CH), 127.3 (Ar CH), 127.2 (Ar CH), 80.3 (CH, dd, *J* 5.6, 3.6, inositol ring C-4), 78.7 (inositol ring C-2), 78.6-78.5 (CH, m, inositol ring C-5), 77.8-77.7 (CH, m, inositol ring C-1), 77.6 (CH, d, *J* 6.5, inositol ring C-6), 75.9 (OCH_2), 74.5 (OCH_2), 71.2 (inositol ring C-3), 70.2 (d, *J* 5.7, OCH_2), 70.1 (d, *J* 5.5, OCH_2), 69.4 (d, *J* 5.6, OCH_2), 69.3 (d, *J* 5.1, OCH_2), 69.2 (d, *J* 5.1, OCH_2), 69.1 (d, *J* 4.1, OCH_2); δ_P (161 MHz, $CDCl_3$) −2.1, −0.1, −0.3; *m/z* (ES^+) [Found: $(M+Na)^+$ 1163.3234. $C_{62}H_{63}O_{15}P_3Na$ requires $M+Na^+$ 1163.3272]; *m/z* (ES^+) 1163 ($[M+Na]^+$, 100%), 1158 ($[M+NH_4]^+$, 5%); Anal. calcd for $C_{62}H_{63}O_{15}P_3$: C 65.3, H 5.6; Found: C 65.3; H 5.4.

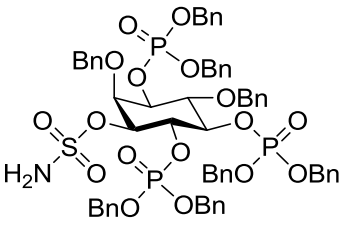
*Due to resonance overlap in the ^{13}C NMR spectrum, some carbon environments have not been accounted for.

Sulfamoyl chloride 173



Formic acid (1.70 g, 1.39 mL, 36.8 mmol, 1.1 eq.) was added portionwise over a period of 30 min to a solution of chlorosulfonyl isocyanate **172** (4.95 g, 3.05 mL, 35.0 mmol, 1.0 eq.) in dichloromethane (14 mL) at 0 °C. The mixture was stirred for 20 min at 0 °C under a stream of nitrogen gas, then warmed to RT and stirred for 13 h. This afforded a stock solution of sulfamoyl chloride **173** in dichloromethane (presumed 2.5 M), which was used without further purification.

(−)-D-2,6-Bis-*O*-benzyl-3-*O*-sulfamoyl-*myo*-inositol 1,4,5-tris(dibenzylphosphate) 141



To a solution of (−)-D-2,6-bis-O-benzyl-*myo*-inositol 1,4,5-tris(dibenzylphosphate) **142** (75 mg, 0.06 mmol, 1.0 eq.) in anhydrous

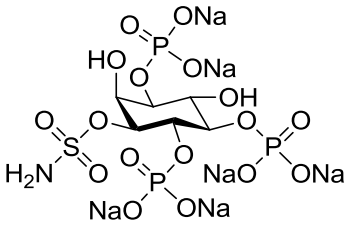
dimethylacetamide (1 mL) at 0 °C was added a solution of sulfamoyl chloride **173** in dichloromethane (presumed 2.5 M, 53 µL, 0.1 mmol, 2.0 eq.). The mixture was stirred at 0 °C for 10 min, then warmed to RT and stirred for 5 h, after which time TLC analysis indicated full consumption of the starting material. The mixture was diluted with H_2O (15 mL) and ethyl acetate (15 mL) and the layers were separated. The aqueous phase was further extracted with ethyl acetate (2 × 25 mL), and the combined organic layers were washed with brine (10 mL), dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The resulting oil was purified by silica gel chromatography, eluting with toluene and ethyl acetate (80:20, 60:40) to afford *(−)-D-2,6-bis-O-benzyl-3-O-sulfamoyl-myo-inositol 1,4,5-tris(dibenzylphosphate)* **141** (65 mg, 82%) as a colourless oil: R_f 0.61 (toluene/ethyl acetate, 40:60); $[\alpha]_D^{25}$ −4.8 (*c* 0.3 in $CHCl_3$); ν_{max} (thin film)/cm^{-1} 3193 (w), 3065 (m), 2956 (w), 2341 (w), 1956 (w), 1886 (w), 1813 (w), 1731 (w), 1587 (w), 1497 (m), 1455 (m), 1385 (s), 1262 (s), 1215 (w), 1189 (m), 1013 (s), 887 (w), 815 (m), 737 (s), 696 (s); δ_H (400 MHz, $CDCl_3$) 7.37-7.35 (2H, m, Ar*H*), 7.30-7.11 (34H, m, Ar*H*), 7.03-7.02 (2H, m, Ar*H*), 6.95 (2H, m, Ar*H*), 5.87 (2H, s, NH_2), 5.03-4.70 (16H, m, OCH_2 and inositol ring H-4), 4.62-4.58 (2H, m, OCH_2 and inositol ring H-2), 4.51 (1H, ddd, *J* 9.4, 9.4, 9.1, inositol ring H-5), 4.45 (1H, dd, *J* 9.4, 2.5, inositol ring H-1), 4.36 (1H, ddd, *J* 9.9, 8.0, 2.2, inositol ring H-3), 4.05 (1H, dd, *J* 9.4, 9.4, inositol ring H-6); δ_C (126 MHz, $CDCl_3$) 137.8 (Ar C), 135.7 (Ar C, d, *J* 7.5), 135.9 (Ar C, d, *J* 6.8), 135.5 (Ar C, d, *J* 7.1), 135.4-135.3 (Ar C, m), 128.59 (Ar CH), 128.56 (Ar CH), 128.54 (Ar CH), 128.51 (Ar CH), 128.4 (Ar CH), 128.3 (Ar CH), 128.28 (Ar CH), 128.20 (Ar CH), 128.17 (Ar CH), 128.11 (Ar

CH), 127.89 (Ar CH), 127.87 (Ar CH), 127.79 (Ar CH), 127.77 (Ar CH), 127.71 (Ar CH), 127.3 (Ar CH), 78.04 (inositol ring C-1), 78.01 (inositol ring C-5), 77.3* (inositol ring C-6), 77.2* (inositol ring C-4), 76.8* (inositol ring C-2), 76.7* (inositol ring C-3), 76.1 (OCH_2), 74.7 (OCH_2), 70.6 (d, *J* 5.7, OCH_2), 70.0 (d, *J* 5.1, OCH_2), 69.6 (d, *J* 5.7, OCH_2), 69.4 (d, *J* 5.2, OCH_2), 69.38 (d, *J* 5.6, OCH_2), 69.34 (d, *J* 5.0, OCH_2); δ_P (161 MHz, $CDCl_3$) −0.3, −0.4, −1.4; *m/z* (ES^+) [Found: $(M+Na)^+$ 1242.29 (100.0%), 1243.30 (68.1%), 1244.30 (30.8%), 1245.31 (9.2%), 1246.31 (1.9%). $C_{62}H_{64}NO_{17}P_3S$ requires $M+Na^+$ 1242.29 (100.0%), 1243.30 (68.1%), 1244.30 (30.8%), 1245.31 (9.2%), 1246.31 (1.9%)]; *m/z* (ES^+) 1242 ($[M+Na]^+$, 100%), 1237 ($[M+NH_4]^+$, 30%), 1220 ($[M+H]^+$, 2%); Anal. calcd for $C_{62}H_{64}NO_{17}P_3S$: C 61.0, H 5.3, N 1.1; Found: C 61.1; H 5.3, N 1.2.

*Signal was assigned from HSQC NMR spectrum.

**Due to resonance overlap in the ^{13}C NMR spectrum, some carbon environments have not been accounted for.

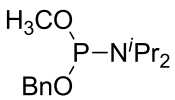
(−)-D-3-*O*-Sulfamoyl-*myo*-inositol 1,4,5-tris(phosphate) 139



To a stirred solution of (−)-D-2,6-bis-*O*-benzyl-3-*O*-sulfamoyl-*myo*-inositol 1,4,5-tris(dibenzylphosphate) **141** (50 mg, 41 μmol, 1.0 eq.) in a mixture of ^tBuOH (3 mL), MeOH (1 mL) and H_2O (0.5 mL) was added Pd black (70 mg,

656 µmol, 16.0 eq.) and sodium bicarbonate (20 mg, 248 µmol, 6.0 eq.). The mixture was stirred under 1 atm of H_2 at RT for 12 h. The suspension was filtered through Celite® and washed with diethyl ether. The Pd residues were then washed with distilled H_2O, and the filtrate lyophilised to give *(−)-D-3-O-sulfamoyl-myo-inositol 1,4,5-tris(phosphate)* **139** (28 mg, 100%) as a colourless solid: $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ −2.9 (*c* 0.7 in H_2O); ν_{max} (KBr disc)/cm^{-1} 3425.1 (s), 2961.2 (s), 2920.1 (s), 2838.9 (w), 2723.1 (m), 2359.9 (w), 1650.9 (m), 1459.9 (m), 1359.7 (s), 1102.6 (s), 974.9 (s), 808.4 (w), 603.8 (w), 538.8 (w); δ_H (400 MHz, D_2O) 4.51-4.50 (1H, m, 1 × inositol ring), 4.43-4.35 (2H, m, 2 × inositol ring), 3.92-3.82 (3H, m, 3 × inositol ring); δ_C (126 MHz, D_2O) 79.5 (inositol ring), 77.4 (inositol ring), 73.5 (CH, d, *J* 5.1, inositol ring), 73.3 (CH, dd, *J* 6.1, 6.1, inositol ring), 71.9 (CH, d, *J* 6.7, inositol ring), 69.6 (inositol ring); δ_P (161 MHz, D_2O) 5.2, 4.3, 3.3; *m/z* (ES^-) [Found: $(M-Na)^-$ 607.8373. $C_6H_{10}NNa_5O_{17}P_3S$ requires 607.8376]; *m/z* (ES^-) 338 (100%), 541 ($[M-4Na]^-$, 20%), 563 ($[M-3Na]^-$, 20%), 585 ($[M-2Na]^-$, 10%), 519 ($[M-5Na]^-$, 5%), 607 ($[M-Na]^-$, 2%).

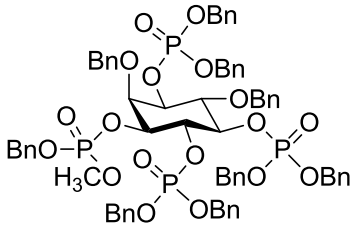
Benzyloxymethoxy-*N,N*-diisopropylphosphoramidite 171



1*H*-Tetrazole (0.45 M in acetonitrile, 2.50 mL, 0.4 eq.) was added to a stirred solution of benzyloxy bis(*N,N*-diisopropyl)diisopropylphosphoramidite **169** (1.08 g, 2.8 mmol, 1.0 eq.) in dichloromethane (30 mL). A solution of

anhydrous methanol (371 mg, 473 µL, 8.5 mmol, 1.0 eq.) in dichloromethane (20 mL) was added dropwise over a period of 30 min. The resulting mixture was stirred at RT for 2 h. The solvent was removed under reduced pressure to give a colourless paste which was purified by silica gel chromatography, eluting with triethylamine and petroleum ether (5:95) to give benzyloxy-methoxy-*N,N*-diisopropylphosphoramidite **171** (227 mg, 30%) as a colourless oil: δ_H (400 MHz, $CDCl_3$) 7.38-7.25 (5H, m, Ar*H*), 4.77 (4H, dd, *J* 12.6, 8.3, OC*H*$_A$H$_B$Ph), 4.68 (1H, dd, *J* 12.6, 8.3, OCH$_A$*H*$_B$Ph), 3.70-3.62 (2H, m, NC*H*$(CH_3)_2$), 3.45 (3H, d, *J* 13.1, OC*H*$_3$), 1.21 (6H, d, *J* 4.5, NCH(C*H*$_3$)$_2$), 1.20 (6H, d, *J* 4.5, NCH(C*H*$_3$)$_2$); δ_P (161 MHz, $CDCl_3$) 148.6. The data are in good agreement with literature values.[214]

(−)-D-2,6-Bis-*O*-benzyl-*myo*-inositol 3-(*O*-benzyl-*O*-methyl)phosphate-1,4,5-tris(dibenzylphosphate) 140



1*H*-Tetrazole (0.45 M solution in acetonitrile, 267 µL, 0.1 mmol, 3.0 eq.) was added to a solution of benzyloxymethoxy-*N,N*- diisopropylaminophosphine **171** (32 mg, 0.1 mmol, 3 eq.) in anhydrous dichloromethane (15 mL) and stirred at RT for 10 min. A solution of (−)-D-2,6-bis-O-benzyl-*myo*-inositol 1,4,5-tris(dibenzylphosphate) **142** (46 mg, 0.04 mmol, 1.0 eq.) in dichloromethane (15 mL) was then added to the flask *via* cannula, and the

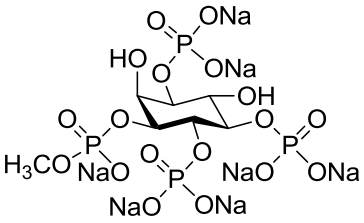
resulting mixture was stirred at RT. After 12 h, TLC analysis indicated that the reaction was incomplete, and a further two equivalents of benzyloxymethoxy-*N,N*-diisopropylaminophosphine **171** (21 mg, 0.08 mmol, 2.0 eq.) were added, followed by 1*H*-tetrazole (0.45 M in acetonitrile, 177 µL, 0.08 mmol, 2.0 eq.). The resulting mixture was stirred for 2 h. The mixture was cooled to −78 °C and 3-chloroperoxybenzoic acid (assume 60%, 57 mg, 0.2 mmol, 5.0 eq.) was added. The mixture was allowed to reach RT and stirred for 2 h, after which time the reaction was deemed to be complete by TLC analysis. The 3-chloroperbenzoic acid was quenched with a 10% aqueous solution of sodium metabisulfite (5 mL) and stirred for 15 min. The resulting organic and aqueous phases were separated, and the aqueous layer re-extracted with dichloromethane (3 × 20 mL). The combined organic extracts were washed with brine (10 mL), dried over magnesium sulfate, filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography, eluting with ethyl acetate and toluene (30:70, 20:30, 50:50, 30:20) to give *(−)-D-2,6-bis-O-benzyl-myo-inositol 3-(O-benzyl-O-methyl)phosphate-1,4,5-tris(dibenzylphosphate)* **140** (47 mg, 89%) as a colourless oil and a mixture of diastereomers: R_f 0.22 (toluene/ethyl acetate, 40:60); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ −3.5 (*c* 0.4 in $CHCl_3$); ν_{max} (thin film)/cm^{-1} 3484 (w), 3064 (w), 3033 (w), 2955 (w), 1956 (w), 1497 (m), 1455 (m), 1381 (w), 1278 (s), 1214 (m), 1015 (s), 874 (m), 737 (m), 696 (m); δ_H (400 MHz, $CDCl_3$) 7.36 (4H, d, *J* 6.7, Ar*H*, major and minor diastereomers), 7.31-7.10 (82H, m, Ar*H*, major and minor diastereomers), 6.97 (4H, d, *J* 7.1, Ar*H*, major and minor diastereomers), 5.10-4.72 (38H, O*C*H_2 and inositol ring H-4, major and minor

diastereomers), 4.66-4.63 (4H, m, OC*H*$_2$ and inositol ring H-2, major and minor diastereomers), 4.48 (1H, dd, *J* 18.4, 9.2, inositol ring H-5, major diastereomer), 4.44 (1H, dd, *J* 17.4, 9.2, inositol ring H-5, minor diastereomer), 4.35-4.28 (4H, m, inositol ring H-1 and H-3, major and minor diastereomers), 4.08 (1H, dd, *J* 9.2, 9.2, inositol ring H-6, major diastereomer), 4.07 (1H, dd, *J* 9.2, 9.2, inositol ring H-6, minor diastereomer), 3.57 (6H, d, *J* 11.3, OCH_3, major and minor diastereomers); δ_C (126 MHz, $CDCl_3$) 138.0 (d, *J* 3.8, Ar C), 136.1-135.9 (m, Ar C), 135.8-135.7 (m, Ar C), 135.6-135.4 (m, Ar C), 128.6 (Ar CH), 128.5 (Ar CH), 128.5 (Ar CH), 128.5 (Ar CH), 128.4 (Ar CH), 128.3 (Ar CH), 128.3 (Ar CH), 128.2 (Ar CH), 128.2 (Ar CH), 128.2 (Ar CH), 128.1 (Ar CH), 128.1 (Ar CH), 128.0 (Ar CH), 128.0 (Ar CH), 127.9 (Ar CH), 127.8 (Ar CH), 127.7 (Ar CH), 127.5 (Ar CH), 127.4 (Ar CH), 127.4 (Ar CH), 127.3 (Ar CH), 127.2 (Ar CH), 78.0 (inositol ring C-5), 77.7 (inositol ring C-6), 77.2* (inositol ring C-1), 77.1* (inositol ring C-2), 76.0 (inositol ring C-4), 75.8 (OCH_2), 75.5 (inositol ring C-3), 74.7 (OCH_2), 69.6 (d, *J* 4.9, OCH_2), 69.5 (d, *J* 5.3, OCH_2), 69.3 (d, *J* 5.5, OCH_2), 69.2 (d, *J* 5.2, OCH_2), 54.6 (d, *J* 5.9, CH_3); δ_P (202 MHz, $CDCl_3$) 0.4 (minor diastereomer), 0.2 (major diastereomer), -0.1 (major and minor diastereomers), -0.2 (major and minor diastereomers), -0.5 (major and minor diastereomers); *m/z* (ES^+) [Found: $(M+Na)^+$ 1347.3611. $C_{70}H_{72}NaO_{18}P_4$ requires $M+H^+$ 1347.3561]; *m/z* (ES^+) 1347 ($[M+Na]^+$, 100%), 1342 ($[M+NH_4]^+$, 30%), 1325 ($[M+H]^+$, 2%); Anal. calcd for $C_{70}H_{72}O_{18}P_4$: C 63.4, H 5.5; Found: C 63.3; H 5.3.

*Signal was assigned from HSQC NMR spectrum.

**Due to resonance overlap in the ^{13}C NMR spectrum, some carbon environments have not been accounted for.

(−)-D-*myo*-Inositol-1,4,5-trisphosphate-3-*O*-methylphosphate ester 138

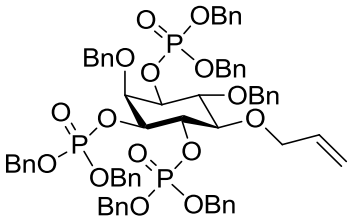


To a stirred solution of (−)-D-2,6-bis-*O*-benzyl-*myo*-inositol 3-(*O*-benzyl-*O*-methyl)phosphate-1,4,5-tris(dibenzylphosphate) **140** (44 mg, 33 μmol, 1.0 eq.) in a mixture of ^{t}BuOH (3 mL) and H_2O (0.5 mL) was added Pd black (64 mg, 602 μmol, 18.0 eq.) and sodium bicarbonate (19 mg, 231 μmol, 7.0 eq.). The mixture was stirred under 1 atm of H_2 at RT for 12 h. The suspension was filtered through Celite® and washed with diethyl ether. The Pd residues were then washed with distilled H_2O, and the filtrate lyophilised to give *(−)-D-myo-inositol-1,4,5-trisphosphate-3-O-methylphosphate ester* **138** (25 mg, 100%) as a colourless solid: $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ −4.1 (*c* 0.6 in H_2O); ν_{max} (solid)/cm^{-1} 3247.3 (s), 1645.1 (w), 1413.6 (s), 1038.4 (s), 869.2 (m), 786.5 (w); δ_H (400 MHz, D_2O) 4.47 (1H, s, 1 × inositol ring), 4.31 (1H, dd, *J* 18.9, 9.5, 1 × inositol ring), 3.99-3.95 (1H, m, 1 × inositol ring), 3.92-3.80 (3H, m, 3 × inositol ring), 3.58 (3H, d, *J* 10.8, OCH_3); δ_C (126 MHz, D_2O) 77.6 (inositol ring), 74.6 (inositol ring), 73.9 (inositol ring), 73.8 (inositol ring), 72.1 (inositol ring), 69.8 (inositol ring), 53.2 (d, *J* 5.5, CH_3); δ_P (161 MHz, D_2O)

5.0, 4.4, 3.0, 2.0; *m/z* (ES^+) [Found: $(M+H)^+$ 668.8266. $C_7H_{12}Na_7O_{18}P_4$ requires $M+H^+$ 668.8252]; *m/z* (ES^+) 167 (100%), 624 ($[M-2Na+3H]^+$, 50%), 646 ($[M-Na+2H]^+$, 40%), 602 ($[M-3Na+4H]^+$, 10%).

6.4. Synthesis of 5-position InsP_4 derivatives

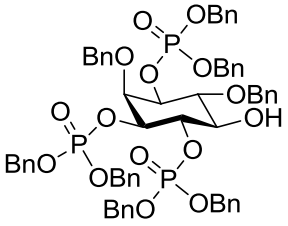
(+)-D-5-*O*-Allyl-2,6-bis-*O*-benzyl-*myo*-inositol 1,3,4-tris(dibenzylphosphate) 184



5-Phenyl-1*H*-tetrazole (1.14 g, 7.8 mmol, 7.5 eq.) was added to a solution of bis(benzyloxy)-*N,N*-diisopropylphosphoramidite **167** (2.70 g, 7.8 mmol, 7.5 eq.) in dichloromethane (30 mL) and the reaction was stirred for 10 min. A solution of (−)-D-5-*O*-allyl-2,6-bis-*O*-benzyl-*myo*-inositol **157** (418 mg, 1.04 mmol, 1.0 eq.) in dichloromethane (20 mL) was added *via* cannula, and the resulting mixture was stirred for 12 h, after which time the reaction was deemed to be complete by TLC analysis. The mixture was cooled to −78 °C and 3-chloroperoxybenzoic acid (assume 60%, 2.24 g, 7.8 mmol, 7.5 eq.) was added. The mixture was allowed to reach RT and stirred for 2 h, after which time the reaction was deemed to be complete by TLC analysis. The 3-chloroperbenzoic acid was quenched by the addition of a 10% aqueous solution of sodium metabisulfite (30 mL) and the reaction was stirred for 15 min. The resulting organic and aqueous phases were separated, and the aqueous layer was re-extracted with dichloromethane (3 × 30 mL). The combined organic extracts were washed sequentially with saturated aqueous sodium bicarbonate solution (20 mL) and brine (20 mL), dried over

magnesium sulfate, filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography, eluting with ethyl acetate and petroleum ether (40:60 to 80:20) to give (+)-D-5-*O*-allyl-2,6-bis-*O*-benzyl-*myo*-inositol 1,3,4-tris(dibenzylphosphate) **184** (1.09 g, 89%) as a colourless oil: R_f 0.58 (petroleum ether/ethyl acetate, 60:40); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ +2.2 (*c* 0.7 in H_2O), [Lit.[53] $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ +2.0 (*c* 1.0 in H_2O)]; δ_H (400 MHz, $CDCl_3$) 7.37-7.16 (40 H, m, Ar*H*), 5.85 (1H, dddd, *J* 16.8, 10.9, 5.6, 5.6, CH_X=C$H_Y H_Z$), 5.16 (1H, dd, *J* 16.8, 1.5, CH_X=C$H_Y H_Z$), 5.08-4.71 (18H, m, OCH_2Ph, CH_X=C$H_Y H_Z$ and inositol ring H-4), 4.61 (1H, dd, *J* 2.1, 2.1, inositol ring H-2), 4.32-4.22 (4H, m, OCH_2CH_X=C$H_Y H_Z$ and 2 × inositol ring H-1 and H-3), 4.01 (1H, dd, *J* 9.6, 9.6, inositol ring H-6), 3.32 (1H, dd, *J* 9.3, 9.3, inositol ring H-5); δ_P (162 MHz, $CDCl_3$) −1.2, −1.4, −1.9. The data are in good agreement with literature values.[53]

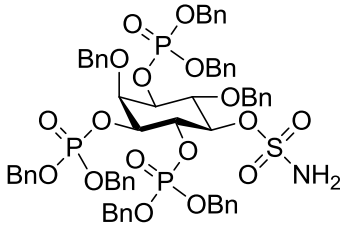
(+)-D-2,6-Bis-*O*-benzyl-*myo*-inositol 1,3,4-tris(dibenzylphosphate) 183



Palladium(II) chloride (30 mg, 0.2 mmol, 1.0 eq.) was added to a solution of (+)-D-5-*O*-allyl-2,6-bis-*O*-benzyl-*myo*-inositol 1,3,4-tris(dibenzylphosphate) **184** (206 mg, 0.2 mmol, 1.0 eq.) in methanol (15 mL) and the reaction was stirred for 3 h at RT. When the reaction was deemed to be complete by TLC analysis, the reaction mixture was filtered through Celite®, washed with ethyl

acetate and concentrated under reduced pressure. The residue was reconstituted in H_2O (10 mL) and ethyl acetate (30 mL) and the phases were separated. The aqueous layer was re-extracted with ethyl acetate (3 × 15 mL) and the combined organic extracts were washed with brine (20 mL), dried over magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with petroleum ether and ethyl acetate (70:30 to 20:80) to give (+)-D-2,6-bis-*O*-benzyl-*myo*-inositol 1,3,4-tris(dibenzylphosphate) **183** (133 mg, 67%) as a colourless oil: R_f 0.58 (petroleum ether/ethyl acetate, 50:50); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ +4.3 (*c* 0.6 in $CHCl_3$), [Lit.[53] $[\alpha]_D^{25}$ +4.3 (*c* 1.2 in $CHCl_3$)]; δ_H (400 MHz, $CDCl_3$) 7.38-7.14 (40H, m, Ar*H*), 5.07-5.01 (4H, m, OCH_2Ph), 4.99-4.86 (9H, m, OCH_2Ph), 4.76-4.66 (4H, m, OCH_2Ph and inositol ring H-4), 4.49-4.48 (1H, m, inositol ring H-2), 4.44 (1H, br s, O*H*), 4.29-4.23 (2H, m, 2 × inositol ring H-1 and H-3), 3.95 (1H, dd, *J* 9.4, 9.4, inositol ring H-6), 3.69 (1H, dd, *J* 8.8, 8.8, inositol ring H-5); δ_P (101 MHz, $CDCl_3$) 1.7, −0.5, −0.5. The data are in good agreement with literature values.[53]

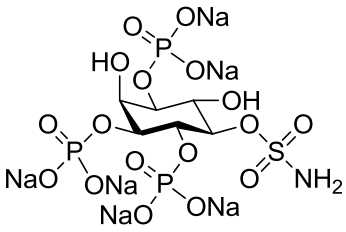
(+)-D-2,6-Bis-*O*-benzyl-5-*O*-sulfamoyl-*myo*-inositol 1,3,4-tris(dibenzylphosphate) 182



To a solution of (+)-D-2,6-bis-*O*-benzyl-*myo*-inositol 1,3,4-tris(dibenzylphosphate) **183** (80 mg, 0.07 mmol, 1.0 eq.) in anhydrous dimethylacetamide (1 mL) at 0 °C was added a solution of sulfamoyl chloride **173** in anhydrous dichloromethane (presumed 2.5 M, 56 µL, 0.1 mmol, 2.0 eq.). The mixture was stirred at 0 °C for 10 min, then warmed to RT and stirred for 5 h, after which time TLC analysis indicated consumption of the starting material. The mixture was diluted with H_2O (15 mL) and ethyl acetate (15 mL) and the layers were separated. The aqueous phase was further extracted with ethyl acetate (2 × 25 mL), and the combined organic layers were washed with brine (10 mL), dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The resulting oil was purified by silica gel chromatography, eluting with toluene and ethyl acetate (80:20, 60:40) to afford (+)-D-2,6-bis-*O*-benzyl-5-*O*-sulfamoyl-*myo*-inositol 1,3,4-tris(dibenzylphosphate) **182** (59 mg, 74%) as a colourless oil: R_f 0.63 (toluene/ethyl acetate, 40:60; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ +3.0 (*c* 0.2 in $CHCl_3$), [Lit.[53] $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ +2.6 (*c* 0.5 in $CHCl_3$)]; δ_H (400 MHz, $CDCl_3$) 7.50-7.45 (2H, m, Ar*H*), 7.32-7.26 (28H, m, Ar*H*), 7.21-7.14 (10H, m, Ar*H*), 5.94 (2H, s, NH_2), 5.11-4.99 (5H, m, OCH_2Ph), 4.96-4.73 (9H, m, OCH_2Ph + inositol ring

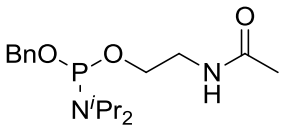
H-4), 4.75 (1H, d, *J* 11.4, OC*H*$_A$H$_B$Ph), 4.75-4.73 (1H, m, OCH$_A$*H*$_B$Ph), 4.68-4.61 (2H, m, 1 × OCH$_A$*H*$_B$Ph, inositol ring H-2), 4.61-4.54 (1H, m, inositol ring H-5), 4.22-4.14 (2H, m, 2 × inositol ring H-1 and H-3), 4.08-4.00 (1H, m, inositol ring H-6); δ_P (161 MHz, $CDCl_3$) −0.6, −0.6, −0.7. The data are in good agreement with literature values.[53]

(+)-D-5-*O*-Sulfamoyl-*myo*-inositol 1,3,4-tris(phosphate) 180



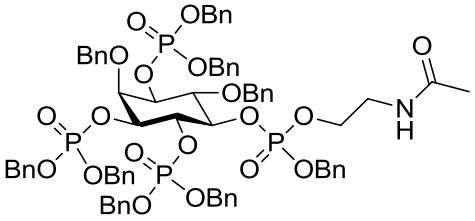
To a stirred solution of (+)-D-2,6-bis-*O*-benzyl-5-*O*-sulfamoyl-*myo*-inositol 1,3,4-tris(dibenzylphosphate) **182** (55 mg, 45 µmol, 1.0 eq.) in a mixture of ^{t}BuOH (3 mL), MeOH (1 mL) and H_2O (0.5 mL) was added Pd black (76 mg, 720 µmol, 16.0 eq.) and sodium bicarbonate (22 mg, 270 µmol, 6.0 eq.). The mixture was stirred at 1 atm of H_2 and at RT for 12 h. The suspension was filtered through Celite® and washed with diethyl ether. The Pd residues were then washed with distilled H_2O, and the filtrate was lyophilised to give (+)-D-5-*O*-sulfamoyl-*myo*-inositol 1,3,4-tris(phosphate) **180** (29 mg, 100%) as a colourless solid: $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ +3.4 (*c* 0.9 in H_2O), [Lit.[53] $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ +3.1 (*c* 0.2 in H_2O)]; δ_H (400 MHz, D_2O) 4.37-4.28 (3H, m, 3 × inositol ring), 3.89-3.85 (3H, m, 3 × inositol ring); δ_P (161 MHz, D_2O) 5.2, 4.2, 3.8. The data are in good agreement with literature values.[53]

2-Acetamidoethyl benzyl *N,N*-diisopropylphosphoramidite 185



Anhydrous 1*H*-tetrazole (3-4 wt% in acetonitrile, 3.30 mL, 0.4 eq.) was added to a stirred solution of benzyloxy bis(*N,N*-diisopropyl)diisopropylphosphoramidite **169** (1.05 g, 2.8 mmol, 1.0 eq.) in anhydrous dichloromethane (15 mL). A solution of *N*-acetylethanolamine (291 mg, 260 µL, 1.0 mmol, 1.0 eq.) in anhydrous dichloromethane (15 mL) was added dropwise over a period of 30 min. The resulting mixture was stirred at RT for 2 h, after which time the reaction was deemed to be complete by TLC analysis. The solvent was removed under reduced pressure to give a colourless paste which was purified by silica gel chromatography, eluting with triethylamine, petroleum ether and ethyl acetate (5:70:25, 5:60:35, 5:50;45) to give *2-acetamidoethyl benzyl N,N-diisopropylphosphoramidite* **185** (390 mg, 38%) as a colourless oil: δ_H (400 MHz, $CDCl_3$) 7.41-7.29 (5H, m, Ar*H*), 6.03 (1H, br s, N*H*), 4.85-4.64 (2H, m, OCH_2Ph), 3.83-3.63 (4H, m, OCH_2CH_2NH), 3.56-3.39 (2H, m, NC*H*$(CH_3)_2$), 1.86 (3H, s, CH_3), 1.27 (6H, d, *J* 1.3, NCH(CH_3)$_2$), 1.24 (6H, d, *J* 1.3, NCH(CH_3)$_2$); δ_P (101 MHz, $CDCl_3$) 149.2.

(−)-D-2,6-Bis-*O*-benzyl-*myo*-inositol 5-(*O*-benzyl-*O*-2'-acetamidoethyl) phosphate-1,3,4-tris(dibenzylphosphate) 181



Anhydrous 1*H*-tetrazole (3-4 wt% in acetonitrile, 1.50 mL, 0.5 mmol, 2.5 eq.) was added to a solution of 2-acetamidoethyl benzyl *N,N*-diisopropylphosphoramidite **185** (186 mg, 0.5 mmol, 2.5 eq.) in anhydrous dichloromethane (15 mL) and stirred at RT for 10 min. A solution of (+)-D-2,6-bis-*O*-benzyl-*myo*-inositol 1,3,4-tris(dibenzylphosphate) **183** (251 mg, 0.2 mmol, 1.0 eq.) in anhydrous dichloromethane (15 mL) was then added to the flask *via* cannula, and the resulting mixture was stirred at RT for 12 h. After 12 h, TLC analysis showed that the reaction was not complete, and a further two equivalents of 2-acetamidoethyl benzyl *N,N*-diisopropylphosphoramidite **185** (141 mg, 0.4 mmol, 1.9 eq.) was added, followed by 1*H*-tetrazole (3-4 wt% in acetonitrile, 1.20 mL, 0.4 mmol, 1.9 eq.). The resulting mixture was stirred for 2 h. The mixture was cooled to −78 °C and 3-chloroperoxybenzoic acid (assume 95%, 175 mg, 0.9 mmol, 4.4 eq.) was added. The mixture was allowed to reach RT and stirred for 2 h, after which time the reaction was deemed to be complete by TLC analysis. The 3-chloroperbenzoic acid was quenched by the addition of a 10% aqueous solution of sodium metabisulfite (5 mL) and stirred for 15 min. The resulting organic and aqueous phases were separated, and the

aqueous layer was re-extracted with dichloromethane (3 × 20 mL). The combined organic extracts were washed with brine (10 mL), dried over magnesium sulfate, filtered and concentrated under reduced pressure. The resulting residue was purified with silica gel chromatography, eluting with ethyl acetate and toluene (30:70, 20:30, 50:50, 30:20) to give (−)-D-2,6-bis-*O*-benzyl-*myo*-inositol *5-(O-benzyl-O-2'-acetamidoethyl)phosphate-1,3,4-tris(dibenzylphosphate)* **181** (128 mg, 44%) as a colourless oil in a mixture of diastereomers (major : minor, 1 : 0.7): R_f 0.11 (petroleum ether/ethyl acetate, 50:50); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ −1.0 (*c* 0.6 in $CHCl_3$); ν_{max} (thin film)/cm^{-1} 3484 (w), 3064 (w), 3033 (w), 1670 (m), 1455 (m), 1270 (s), 1009 (s), 736 (m), 696 (m); δ_H (500 MHz, $CDCl_3$) 7.59-7.03 (90H, m, Ar*H*, major and minor diastereomers), 5.16-5.13 (1H, m, OCH_2Ph, major diastereomer), 5.07-4.78 (34H, m, 12 × OCH_2Ph major diastereomer, 18 × OCH_2Ph minor diastereomer, 2 × N*H* major and minor diastereomers, 2 × inositol ring major and minor diastereomers), 4.76-4.71 (4H, m, 4 × OCH_2Ph major diastereomer), 4.67-4.66 (2H, m, 2 × inositol ring major and minor diastereomers), 4.55-4.47 (2H, m, 1 × OCH_2Ph major diastereomer, 1 × inositol ring major diastereomer), 4.36-4.32 (1H, m, 1 × inositol ring major diastereomer), 4.30-4.17 (4H, m, 1 × inositol ring major diastereomer, 3 × inositol ring minor diastereomer), 4.12 (1H, dd, *J* 9.3, 9.3, 1 × inositol ring minor diastereomer), 4.04 (1H, dd, *J* 9.5, 9.5, 1 × inositol ring major diastereomer), 4.06-3.95 (1H, m, 1 × CH_2 minor diastereomer), 3.90-3.85 (1H, m, 1 × CH_2 major diastereomer), 3.82-3.74 (2H, m, 2 × CH_2 major and minor diastereomers), 3.36-3.31 (1H, m, 1 × CH_2 major diastereomer), 3.23-3.17 (3H, m, 1 × CH_2 major diastereomer, 2 × CH_2 minor diastereomer),

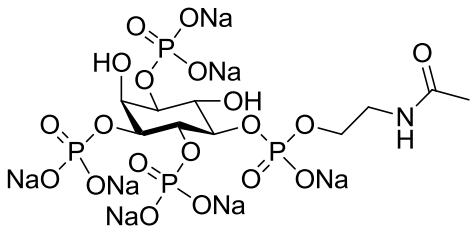
1.78 (3H, s, CH_3 minor diastereomer), 1.77 (3H, s, CH_3 major diastereomer); δ_C (126 MHz, $CDCl_3$) 170.7 (C=O, major diastereomer), 170.4 (C=O, minor diastereomer), 138.0 (Ar C), 137.8 (Ar C), 137.7 (Ar C), 135.8 (Ar C), 135.8 (Ar C), 135.7 (Ar C), 135.7 (Ar C), 135.6 (Ar C), 135.6 (Ar C), 135.6 (Ar C), 135.5 (Ar C), 135.4 (Ar C), 135.4 (Ar C), 135.3 (Ar C), 128.6 (Ar CH), 128.6 (Ar CH), 128.5 (Ar CH), 128.5 (Ar CH), 128.5 (Ar CH), 128.4 (Ar CH), 128.4 (Ar CH), 128.3 (Ar CH), 128.3 (Ar CH), 128.2 (Ar CH), 128.1 (Ar CH), 128.1 (Ar CH), 128.1 (Ar CH), 128.0 (Ar CH), 128.0 (Ar CH), 128.0 (Ar CH), 127.9 (Ar CH), 127.9 (Ar CH), 127.8 (Ar CH), 127.8 (Ar CH), 127.7 (Ar CH), 127.7 (Ar CH), 127.6 (Ar CH), 127.4 (Ar CH), 127.3 (Ar CH), 127.0 (Ar CH), 78.4 (inositol ring, major and minor diastereomers), 77.9 (inositol CH, major and minor diastereomers), 77.6 (inositol ring, d, *J* 6.7, major diastereomer), 77.5 (inositol ring, d, *J* 6.7, minor diastereomer), 76.7* (inositol ring, major and minor diastereomers), 76.2 (inositol ring, major and minor diastereomers), 75.9 (CH_2, major diastereomer), 75.8 (CH_2, minor diastereomer), 75.2 (inositol ring, major and minor diastereomers), 75.0 (CH_2, minor diastereomer), 74.6 (CH_2, major diastereomer), 70.0 (CH_2), 70.0 (CH_2), 70.0 (CH_2), 69.9 (CH_2), 69.9 (CH_2), 69.8 (CH_2), 69.8 (CH_2), 69.7 (CH_2), 69.7 (CH_2), 69.6 (CH_2), 69.6 (CH_2), 69.5 (CH_2), 69.5 (CH_2), 69.4 (CH_2), 69.4 (CH_2), 69.3 (CH_2), 67.6 (d, *J* 5.1, CH_2, minor diastereomer), 67.2 (d, *J* 5.1, CH_2, major diastereomer), 39.5 (CH_2, major diastereomer), 39.4 (CH_2, minor diastereomer), 22.7 (CH_3, minor diastereomer), 22.6 (CH_3, major diastereomer); δ_P (202 MHz, $CDCl_3$) −0.8 (major diastereomer), −1.3 (minor diastereomer), −1.5 (major diastereomer), −1.6 (minor diastereomer), −1.7 (major and minor diastereomers), −1.7 (major diastereomer), −2.1 (minor

diastereomer); *m/z* (ES^+) [Found: $(M+Na)^+$ 1418.3905. $C_{73}H_{77}NNaO_{19}P_4$ requires $M+Na^+$ 1418.3932]; *m/z* (ES^+) 1418 ($[M+Na]^+$, 100%), 1413 ($[M+NH_4]^+$, 80%), 1396 ($[M+H]^+$, 40%); Anal. calcd for $C_{73}H_{77}NO_{19}P_4$: C 62.8, H 5.6, N 1.0; Found: C 62.7; H 5.7, N 1.0.

*Signal was assigned from HSQC NMR spectrum.

**Due to resonance overlap in the ^{13}C NMR spectrum, some carbon environments have not been accounted for.

(+)-D-*myo*-Inositol-1,3,4-trisphosphate-5-*O*-acetamidoethylphosphate ester 179

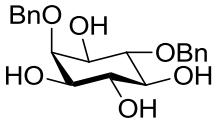


To a stirred solution of (−)-D-2,6-bis-*O*-benzyl-*myo*-inositol 5-(O-benzyl-O-2'-acetamidoethyl)phosphate-1,3,4-tris(dibenzylphosphate) **181** (71 mg, 51 µmol, 1.0 eq.) in a mixture of ^{t}BuOH (6 mL) and H_2O (1.5 mL) was added Pd black (106 mg, 979 µmol, 18.0 eq.) and sodium bicarbonate (29 mg, 353 µmol, 7.0 eq.). The mixture was stirred at 1 atm of H_2 at RT for 12 h. The suspension was filtered through Celite® and washed with diethyl ether. The Pd residues were then washed with distilled H_2O, and the filtrate was lyophilised to give *(+)-D-myo-inositol-1,3,4-trisphosphate-5-O-acetamidoethylphosphate ester* **179** (40 mg, 100%) as a colourless solid: $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ +3.5 (*c* 1.0 in H_2O); ν_{max} (solid)/cm^{-1} 2989.2 (s), 2361.8 (w), 1731.2

(s), 1498.8 (m), 1397.7 (m), 1372.3 (m), 1247.8 (m), 1207.1 (s), 1094.8 (s), 1034.0 (s), 977.4 (m), 837.1 (m), 810.8 (w), 771.0 (m), 750.0 (m), 705.6 (w), 667.3 (w); δ_H (400 MHz, D_2O) 4.46 (1H, br s, 1 × inositol ring), 4.32-4.28 (1H, m, 1 × inositol ring), 4.02-3.92 (4H, m, 2 × inositol ring and C*H*$_2$), 3.90-3.86 (1H, m, 1 × inositol ring), 3.84-3.80 (1H, m, 1 × inositol ring), 3.36-3.30 (2H, m, C*H*$_2$), 1.94 (3H, s, C*H*$_3$); δ_C (126 MHz, D_2O) 174.3 (C=O), 77.8 (inositol ring), 73.9 (inositol ring), 73.5 (inositol ring), 73.1 (inositol ring), 71.7 (inositol ring), 64.3 (inositol ring), 64.3 (CH_2), 40.1 (CH_2), 22.0 (*C*H$_3$); δ_P (202 MHz, D_2O) 5.0, 4.4, 3.5, 1.1; *m/z* (ES^-) [Found: $(M-3Na+2H)^-$ 671.9037. $C_{10}H_{18}NNa_4O_{19}P_4$ requires M^- 671.9020]; *m/z* (ES^-) 671 ($[M-3Na+2H]^-$, 100%), 649 ($[M-4Na+3H]^-$, 95%), 693($[M-2Na+H]^-$, 65%).

6.5. Synthesis of Ins(1,3,4,5)P_4

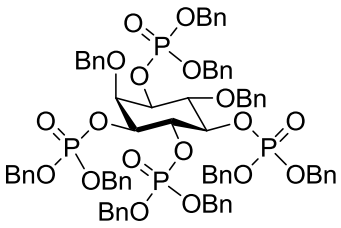
(−)-D-2,6-Bis-*O*-benzyl-*myo*-inositol 127



Palladium(II) chloride (26 mg, 0.1 mmol, 0.6 eq.) was added to a solution of (−)-D-5-*O*-allyl-2,6-bis-*O*-benzyl-*myo*-inositol **157** (101 mg, 0.2 mmol, 1.0 eq.) in methanol (15 mL) and stirred for 3 h at RT. When the reaction was deemed to be complete by TLC analysis, the reaction mixture was filtered through Celite®, washed with ethyl acetate and concentrated under reduced pressure. The resulting residue was reconstituted in H_2O (10 mL) and ethyl acetate (30 mL) and the phases separated. The aqueous layer was re-extracted with ethyl acetate (3 × 15 mL) and the combined organic extracts washed with brine (20 mL), dried over magnesium sulfate, filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography, eluting with petroleum ether and ethyl acetate (70:30 to 40:60) to give (−)-D-2,6-bis-*O*-benzyl-*myo*-inositol **127** (65 mg, 72%) as a colourless solid: R_f 0.46 (petroleum ether/ethyl acetate, 50:50); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ −27.4 (*c* 0.4 in $CHCl_3$), [Lit.[67] −29.2 (*c* 1.00 in EtOH)]; mp 141-143 °C (*from ethyl acetate)* (Lit.[67] 145-146 °C), δ_H (400 MHz, $CDCl_3$) 7.40-7.32 (10H, m, Ar*H*), 4.92-4.87 (3H, m, OCH_2Ph), 7.81 (1H, d, *J* 11.4, OCH_2Ph), 4.04 (1H, dd, *J* 2.5, 2.5, inositol ring H-2), 3.76 (1H, dd, *J* 9.4, 9.4, inositol ring H-4), 3.69-3.60 (2H, m, 2 × inositol ring H-6 and H-1), 3.51-3.42

(2H, m, 2 × inositol ring H-3 and H-5), 2.59 (1H, d, *J* 1.9, O*H*), 2.52 (1H, d, *J* 1.9, O*H*), 2.37 (1H, d, *J* 5.1, O*H*), 2.33 (1H, d, *J* 7.2, O*H*). The data are in good agreement with literature values.[67]

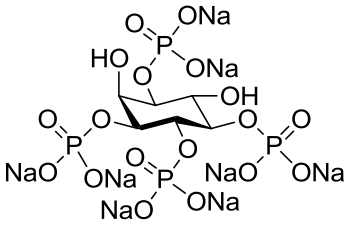
(−)-D-2,6-Bis-*O*-benzyl-*myo*-inositol 1,3,4,5-tetrakis(dibenzylphosphate) 128



5-Phenyl-1*H*-tetrazole (182 mg, 1.2 mmol, 10.0 eq.) was added to a solution of bis(benzyloxy)-*N,N*-diisopropylphosphoramidite **167** (431 mg, 1.2 mmol, 10.0 eq.) in dichloromethane (5 mL) and stirred for 10 min. A solution (−)-D-2,6-bis-*O*-benzyl-*myo*-inositol **127** (45 mg, 0.1 mmol, 1.0 eq.) in dichloromethane (5 mL) was added *via* cannula, and the resulting mixture was stirred for 12 h, after which time the reaction was deemed to be complete by TLC analysis. The mixture was cooled to −78 °C and 3-chloroperoxybenzoic acid (assume 60%, 352 mg, 1.2 mmol, 10.0 eq.) was added. The mixture was allowed to reach RT and stirred for 2 h, after which time the reaction was deemed to be complete by TLC analysis. The 3-chloroperbenzoic acid was quenched by the addition of a 10% aqueous solution of sodium metabisulfite (7 mL) and stirred for 15 min. The resulting organic and aqueous phases were separated, and the aqueous layer re-extracted with dichloromethane (3 × 30 mL). The combined organic extracts

were washed with saturated aqueous sodium bicarbonate solution (20 mL) and brine (20 mL), dried over magnesium sulfate, filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography, eluting with ethyl acetate and petroleum ether (40:60 to 80:20) to give (−)-D-2,6-bis-*O*-benzyl-*myo*-inositol 1,3,4,5-tetrakis(dibenzylphosphate) **128** (106 mg, 58%) as a colourless oil: R_f 0.67 (petroleum ether/ethyl acetate, 50:50); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ −3.6 (*c* 0.6 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 7.28-7.09 (50H, m, Ar*H*), 5.08-4.61 (22H, m, OC*H*$_2$Ph and 2 × inositol ring H-2 and H-4), 4.45 (1H, dd, *J* 18.5, 9.2, 1 × inositol ring), 4.33-4.20 (2H, m, 2 × inositol ring), 4.07 (1H, dd, *J* 9.5, 9.5, 1 × inositol ring); δ_P (162 MHz, $CDCl_3$) −1.2, −1.4, −1.6, −1.9. The data are in good agreement with literature values.[67]

(−)-D-*myo*-Inositol-1,3,4,5-trisphosphate 13

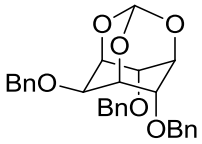


To a stirred solution of (−)-D-2,6-bis-*O*-benzyl-*myo*-inositol 1,3,4,5-tetrakis(dibenzylphosphate) **128** (85 mg, 0.06 mmol, 1.0 eq.) in a mixture of ^{t}BuOH (6 mL) and H_2O (1.5 mL) was added Pd black (130 mg, 1.2 mmol, 20.0 eq.) and sodium bicarbonate (41 mg, 0.5 mmol, 8.0 eq.). The mixture was stirred under 1 atm of H_2 at RT for 12 h. The suspension was filtered through Celite® and washed with diethyl ether. The Pd residues were then

washed with distilled H_2O, and the filtrate lyophilised to give (−)-D-*myo*-inositol-1,3,4,5-tetrakisphosphate **13** (48 mg, 100%) as a colourless solid: $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ −2.6 (*c* 0.9 in H_2O), [Lit.[67] −2.5 (*c* 1.0 in H_2O)]; δ_H (400 MHz, D_2O) 4.49 (1H, s, 1 × inositol ring), 4.29 (1H, dd, *J* 18.0, 8.8, 1 × inositol ring), 3.97 (1H, dd, *J* 7.6, 7.6 1 × inositol ring), 3.85 (3H, s, 3 × inositol ring); δ_P (162 MHz, D_2O) 4.6, 3.7, 3.4, 3.3. The data are in good agreement with literature values.[67]

6.6. Synthesis of Ins(1,3)P_2

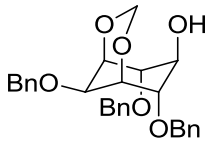
D-2,4,6-Tris-*O*-benzyl-*myo*-inositol 1,3,5-orthoformate 194



Benzyl bromide (4.48 g, 3.12 mL, 26.2 mmol, 5.0 eq.) and dry *N,N*-dimethylformamide (30 mL) were added to a flask containing sodium hydride washed with petroleum ether (60% dispersion in mineral oil, 1.05 g, 26.2 mmol, 5.0 eq.) and cooled to 0 °C. A solution of D-*myo*-inositol 1,3,5-orthoformate **144** (1.02 g, 5.2 mmol, 1.0 eq.) in dry *N,N*-dimethylformamide (30 mL) was added dropwise over a period of 30 min to the stirred suspension, maintaining the temperature at 0 °C. The reaction mixture was stirred for 30 min at 0 °C and then at RT overnight, after which time the reaction was deemed to be complete by TLC analysis. The reaction was quenched by the addition of methanol (10 mL) and concentrated under reduced pressure. The resulting oil was reconstituted with ethyl acetate (50 mL) and H_2O (30 mL) and the layers were separated. The aqueous phase was further extracted with ethyl acetate (2 × 50 mL). The combined organic layers were washed with brine (20 mL), dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The resulting oil was adsorbed onto silica gel, and purified by silica gel chromatography, eluting with petroleum ether and ethyl acetate (80:20 to 70:30) to afford D-2,4,6-tris-*O*-benzyl-*myo*-inositol 1,3,5-orthoformate **194** (2.09 g, 85%) as a colourless

solid: R_f 0.74 (petroleum ether/ethyl acetate 90:10); mp 97-99 °C (from ethyl acetate*)* (Lit.[125] 102-104 °C); δ_H (400 MHz, $CDCl_3$) 7.40-7.18 (15H, m, Ar*H*), 5.54 (1H, d, *J* 1.4, 3-*H*), 4.65 (2H, s, OCH_2Ph), 4.62 (2H, d, *J* 11.6, OC$H_A$$H_B$Ph), 4.48 (2H, d, *J* 11.6, OC$H_A$$H_B$Ph), 4.47-4.43 (1H, m, 1 × inositol ring), 4.34 (2H, dd, *J* 4.1, 4.1, 2 × inositol ring), 4.30-4.29 (2H, m, 2 × inositol ring), 4.06-4.05 (1H, m, 1 × inositol ring); *m/z* (ES^+) 483 ($[M+Na]^+$, 100%). The data are in good agreement with literature values.[125]

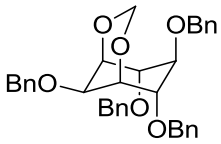
D-2,4,6-Tris-*O*-benzyl-1,3-*O*-methylene-*myo*-inositol 193



D-2,4,6-Tris-*O*-benzyl-*myo*-inositol 1,3,5-orthoformate **194** (1.07 g, 2.4 mmol, 1.0 eq.) was dissolved in dry dichloromethane (30 mL) and cooled to 0 °C. A solution of diisobutylaluminium hydride in hexanes (1.0 M, 6.00 mL, 5.9 mmol, 2.5 eq.) was added dropwise over a period of 30 min, maintaining the temperature at 0 °C. The mixture was then allowed to reach RT and stirred for 5 h, after which time the reaction was deemed to be complete by TLC analysis. The reaction mixture was cooled to 0 °C and quenched by the careful addition of H_2O (0.20 mL), sodium hydroxide (0.20 mL, 15% solution) and additional H_2O (0.60 mL). The resulting gel was dried by addition of magnesium sulfate, filtered, and concentrated under reduced pressure to afford D-2,4,6-tris-*O*-benzyl-1,3-*O*-methylene-*myo*-inositol **193** (1.03 g, 96%) as a colourless oil: R_f 0.44 (petroleum ether/ethyl

acetate 70:30); δ_H (400 MHz, $CDCl_3$) 7.37-7.26 (15H, m, Ar*H*), 5.58 (1H, d, *J* 4.8, 3-*H*), 4.69 (2H, d, *J* 11.7, OC$H_A$$H_B$Ph), 4.68 (1H, d, *J* 4.8, 3-*H'*), 4.62 (2H, s, OCH_2Ph), 4.58 (2H, d, *J* 11.7, OC$H_A$$H_B$Ph), 4.46-4.45 (2H, m, 2 × inositol ring), 4.32-4.31 (1H, m, 1 × inositol ring), 4.04-4.02 (2H, m, 2 × inositol ring), 4.00-3.97 (1H, m, 1 × inositol ring), 2.99 (1H, d, *J* 10.4, O*H*). The data are in good agreement with literature values.[127]

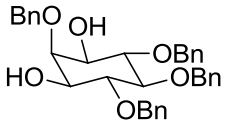
D-2,4,5,6-Tetrakis-*O*-benzyl-1,3-*O*-methylene-*myo*-inositol 192



Benzyl bromide (727 mg, 0.50 mL, 4.2 mmol, 2.0 eq.) and dry *N,N*-dimethylformamide (30 mL) were added to a flask containing sodium hydride washed with petroleum ether (60% dispersion in mineral oil, 170 mg, 4.2 mmol, 2.0 eq.) and cooled to 0 °C. A solution of D-2,4,6-tris-*O*-benzyl-1,3-*O*-methylene-*myo*-inositol **193** (985 mg, 2.1 mmol, 1.0 eq.) in dry *N,N*-dimethylformamide (30 mL) was added dropwise over a period of 30 min to the stirred suspension, maintaining the temperature at 0 °C. The reaction mixture was stirred for 30 min at 0 °C and then at RT for 12 h, after which time the reaction was deemed to be complete by TLC analysis. The reaction was quenched by the addition of methanol (10 mL) and concentrated under reduced pressure. The resulting oil was reconstituted with ethyl acetate (50 mL) and H_2O (30 mL) and the layers were separated. The aqueous phase was further extracted with ethyl acetate (2 × 50 mL).

The combined organic layers were washed with brine (20 mL), dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The resulting oil was adsorbed onto silica gel, and purified by silica gel chromatography, eluting with petroleum ether and ethyl acetate (80:20 to 70:30) to afford D-2,4,5,6-tetrakis-*O*-benzyl-1,3-*O*-methylene-*myo*-inositol **192** (1.09 g, 93%) as a colourless oil: R_f 0.73 (petroleum ether/ethyl acetate 70:30); δ_H (400 MHz, $CDCl_3$) 7.36-7.20 (20H, m, Ar*H*), 5.21 (1H, d, *J* 5.5, 3-*H*), 4.86 (1H, d, *J* 5.5, 3-*H'*), 4.64 (4H, s, OCH_2Ph), 4.62 (2H, d, *J* 11.7, OC$H_A$$H_B$Ph), 4.55 (2H, d, *J* 11.7, OC$H_A$$H_B$Ph), 4.28 (2H, s, 2 × inositol ring), 3.97 (2H, d, *J* 5.4, 2 × inositol ring), 3.86-3.85 (1H, m, 1 × inositol ring), 3.64 (1H, dd, *J* 5.5, 5.5, 1 × inositol ring). The data are in good agreement with literature values.[127]

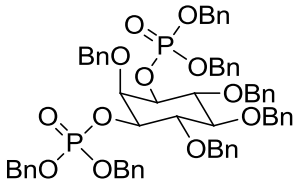
D-2,4,5,6-Tetrakis-*O*-benzyl-*myo*-inositol 191



Concentrated hydrochloric acid (37%, 2 mL) was slowly added to a stirred solution of D-2,4,5,6-tetrakis-*O*-benzyl-1,3-*O*-methylene-*myo*-inositol **192** (1.00 g, 1.8 mmol, 1.0 eq.) in methanol (40 mL) and heated at 80 °C for 4 h and 60 °C for 12 h. After this time the reaction was deemed to be complete by TLC analysis. The reaction was cooled to 0 °C and the hydrochloric acid was quenched by the addition of sodium hydrogen carbonate. The solid components were removed by filtration, and the filtrate was adsorbed onto

silica gel. Purification by silica gel chromatography, eluting with ethyl acetate/petroleum ether (10:90 to 40:60) afforded D-2,4,5,6-tetrakis-*O*-benzyl-*myo*-inositol **191** (794 mg, 77%) as a colourless oil: R_f 0.27 (petroleum ether/ethyl acetate 70:30); δ_H (400 MHz, $CDCl_3$) 7.40-7.27 (20H, m, Ar*H*), 4.94-4.89 (4H, m, OC*H*$_2$Ph), 4.81-4.77 (4H, m, OC*H*$_2$Ph), 4.01 (1H, dd, *J* 2.7, 2.7, 1 × inositol ring), 3.81 (2H, dd, *J* 9.4, 9.4, 2 × inositol ring), 3.60-3.56 (2H, m, 2 × inositol ring), 3.50 (1H, dd, *J* 9.2, 9.2, 1 × inositol ring), 2.28 (2H, d, *J* 5.6, O*H*). The data are in good agreement with literature values.[127]

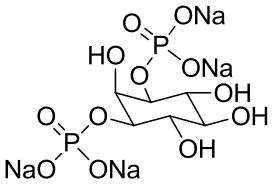
D-2,4,5,6-Tetrakis-*O*-benzyl-*myo*-inositol 1,3-bis(dibenzylphosphate) 190



1*H*-Tetrazole (0.45 M in acetonitrile, 1.00 mL, 0.4 mmol, 5.0 eq.) was added to a solution of bis(benzyloxy)-*N,N*-diisopropylphosphoramidite **167** (159 mg, 0.5 mmol, 5.0 eq.) in dichloromethane (10 mL) and stirred for 10 min. A solution of D-2,4,5,6-tetrakis-*O*-benzyl-*myo*-inositol **191** (50 mg, 0.09 mmol, 1.0 eq.) in dichloromethane (15 mL) was added *via* cannula, and the resulting mixture was stirred at RT. After 12 h, TLC analysis showed that the reaction was not complete, and a solution of bis(benzyloxy)-*N,N*-diisopropylphosphoramidite **167** (60 mg, 0.2 mmol, 2.0 eq.) in

dichloromethane (5 mL) was added, followed by 1*H*-tetrazole (0.45 M in acetonitrile, 0.40 mL, 0.2 mmol, 2.0 eq.) and stirred for 2 h. The mixture was cooled to −78 °C and 3-chloroperoxybenzoic acid (assume 60%, 186 mg, 0.6 mmol, 7.0 eq.) was added. The mixture was allowed to reach RT and stirred for 2 h, after which it was deemed to be complete by TLC analysis. The 3-chloroperbenzoic acid was quenched by the addition of a 10% aqueous solution of sodium metabisulfite and stirred for 15 min. The resulting organic and aqueous phases were separated, and the aqueous layer re-extracted with dichloromethane (3 × 30 mL). The combined organic extracts were washed with saturated aqueous sodium bicarbonate solution (20 mL) and brine (20 mL), dried over magnesium sulfate, filtered and concentrated under reduced pressure. The resulting residue was purified with silica gel chromatography, eluting with ethyl acetate and toluene (10:90 to 60:40) to give D-2,4,5,6-tetrakis-*O*-benzyl-*myo*-inositol 1,3-bis(dibenzylphosphate) **190** (93 mg, 77%) as a colourless oil: R_f 0.77 (toluene/ethyl acetate, 40:60); δ_H (400 MHz, $CDCl_3$) 7.33-7.16 (40H, m, Ar*H*), 4.98-4.87 (8H, m, OC*H*$_2$Ph), 4.85-4.77 (6H, m, OC*H*$_2$Ph), 4.73 (2H, s, OC*H*$_2$Ph), 4.49 (1H, dd, *J* 2.5, 2.5, 1 × inositol ring), 4.30 (2H, ddd, *J* 10.0, 7.9, 2.2, 2 × inositol ring), 4.03 (2H, dd, *J* 9.6, 9.6, 2 × inositol ring), 3.46 (1H, dd, *J* 9.3, 9.3, 1 × inositol ring), δ_P (202 MHz, $CDCl_3$) −1.4. The data are in good agreement with literature values.[215]

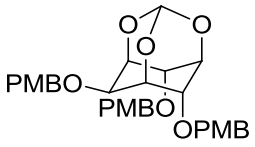
D-*myo*-Inositol-1,3-bisphosphate 189



To a stirred solution of D-2,4,5,6-tetrakis-*O*-benzyl-*myo*-inositol 1,3-bis(dibenzylphosphate) **190** (61 mg, 0.06 mmol, 1.0 eq.) in a mixture of ^{t}BuOH (4 mL) and H_2O (1 mL) was added Pd black (105 mg, 0.9 mmol, 16.0 eq.) and sodium bicarbonate (21 mg, 0.2 mol, 4.0 eq.). The mixture was stirred under 1 atm of H_2 at RT for 12 h. The suspension was filtered through Celite® and washed with diethyl ether. The Pd residues were then washed with distilled H_2O, and the filtrate lyophilised to give D-*myo*-inositol-1,3-bisphosphate **189** (29 mg, 100%) as a colourless solid: δ_H (400 MHz, D_2O) 4.22 (1H, dd, *J* 2.7, 2.7, 1 × inositol ring), 3.85 (2H, ddd, *J* 9.2, 9.2, 2.7, 2 × inositol ring), 3.66 (2H, dd, *J* 9.5. 9.5, 2 × inositol ring), 3.29 (1H, dd, *J* 9.3, 9.3, 1 × inositol ring); δ_P (161 MHz, D_2O) +5.3; *m/z* (ES^+) 130 (100%), 407 ($[M-Na+H]^+$, 20%), 429 ($[M+H]^+$, 10%). The data are in good agreement with the literature.[99]

6.7. Towards the synthesis of PP-InsP_5 derivatives

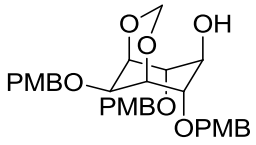
D-2,4,6-Tris-*O*-(4-methoxybenzyl)-*myo*-inositol 1,3,5-orthoformate 208



4-Methoxybenzyl bromide (5.02 g, 4.30 mL, 32.2 mmol, 5.0 eq.) and dry *N,N*-dimethylformamide (30 mL) were added to a flask containing sodium hydride washed with petroleum ether (60% dispersion in mineral oil, 1.54 g, 38.6 mmol, 6.0 eq.) and cooled to 0 °C. A solution of D-*myo*-inositol 1,3,5-orthoformate **144** (2.00 g, 6.4 mmol, 1.0 eq.) in dry *N,N*-dimethylformamide (30 mL) was added dropwise over a period of 30 min to the stirred suspension, maintaining the temperature at 0 °C. The reaction mixture was stirred for 30 min at 0 °C and then at RT for 12 h, after which time the reaction was deemed to be complete by TLC analysis. The reaction was quenched by the addition of methanol (10 mL) and concentrated under reduced pressure. The resulting oil was reconstituted with ethyl acetate (50 mL) and H_2O (30 mL) and the layers were separated. The aqueous phase was further extracted with ethyl acetate (2 × 50 mL). The combined organic layers were washed with brine (20 mL), dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The resulting oil was adsorbed onto silica gel, and purified by silica gel chromatography, eluting with petroleum ether and ethyl acetate (95:5 to 50:50) to afford D-2,4,6-tris-*O*-(4-methoxybenzyl)-*myo*-inositol 1,3,5-orthoformate **208** (3.46 g,

97%) as a colourless solid: R_f 0.39 (petroleum ether/ethyl acetate 70:30); mp 65-67 °C (from ethyl acetate*)* (Lit.[216] 47-48 °C); δ_H (400 MHz, $CDCl_3$) 7.29 (2H, d, *J* 8.5, $OCH_2C_6H_4OCH_3$), 7.11 (4H, d, *J* 8.5, $OCH_2C_6H_4OCH_3$), 6.85 (2H, d, *J* 8.6, $OCH_2C_6H_4OCH_3$), 6.81 (4H, d, *J* 8.6, $OCH_2C_6H_4OCH_3$), 5.52 (1H, d, *J* 4.8, 3-*H*), 4.59 (2H, s, $OCH_2C_6H_4OCH_3$), 4.53 (2H, d, *J* 11.1, $OCH_2C_6H_4OCH_3$), 4.39-4.41 (3H, m, $OCH_2C_6H_4OCH_3$ and 1 × inositol ring), 4.30 (2H, dd, *J* 3.6, 3.6, 2 × inositol ring), 4.24-4.23 (2H, m, 2 × inositol ring), 4.01-4.00 (1H, m, 1 × inositol ring), 3.81 (6H, s, OCH_3), 3.78 (3H, s, OCH_3); *m/z* (ES^+) 573 ($[M+Na]^+$, 100%). The data are in good agreement with literature values.[216]

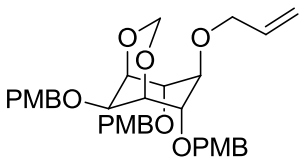
D-2,4,6-Tris-*O*-(4-methoxybenzyl)-1,3-*O*-methylene-*myo*-inositol 206



D-2,4,6-Tris-*O*-(4-methoxybenzyl)-*myo*-inositol 1,3,5-orthoformate **208** (1.12 g, 2.0 mmol, 1.0 eq.) was dissolved in dry dichloromethane (30 mL) and cooled to 0 °C. A solution of diisobutylaluminium hydride in hexanes (1.0 M, 5.10 mL, 5.1 mmol, 2.5 eq.) was added dropwise over a period of 30 min, maintaining the temperature at 0 °C. The mixture was then allowed to reach RT and stirred for 5 h, after which time the reaction was deemed to be complete by TLC analysis. The reaction mixture was cooled to 0 °C and quenched by the careful addition of H_2O (0.20 mL), sodium hydroxide (0.20 mL, 15% solution) and additional H_2O (0.50 mL). The resulting gel was

dried by addition of magnesium sulfate, filtered, and concentrated under reduced pressure to afford *D-2,4,6-tris-O-(4-methoxybenzyl)-myo-inositol 1,3-O-methylene* **206** (1.09 g, 97%) as a colourless oil: R_f 0.30 (petroleum ether/ethyl acetate 70:30); ν_{max} (thin film)/cm^{-1} 3557 (w), 2915 (m), 1612 (m), 1514 (w), 1464 (m), 1302 (s), 1211 (m), 1138 (m), 1033 (m), 732 (s); δ_H (400 MHz, $CDCl_3$) 7.25 (2H, d, *J* 8.5, $OCH_2C_6H_4OCH_3$), 7.19 (4H, d, *J* 8.5, $OCH_2C_6H_4OCH_3$), 6.84 (6H, d, *J* 8.8, $OCH_2C_6H_4OCH_3$), 5.53 (1H, d, *J* 4.8, 3-*H*), 4.66 (1H, d, *J* 4.8, 3-*H'*), 4.61-4.49 (6H, m, $OCH_2C_6H_4OCH_3$), 4.39 (2H, br s, 2 × inositol ring), 4.26-4.25 (1H, m, 1 × inositol ring), 3.99-3.98 (2H, m, 2 × inositol ring), 3.95-3.90 (1H, m, 1 × inositol ring), 3.81 (6H, s, OCH_3), 3.79 (3H, s, OCH_3), 2.96 (1H, d, *J* 10.3, OH); δ_C (126 MHz, $CDCl_3$) 159.2 (Ar C), 130.0 (Ar C), 129.5 (Ar CH), 129.2 (Ar CH), 113.8 (Ar CH), 113.7 (Ar CH), 85.6 (O_2CH_2), 80.7 (inositol ring), 72.7 (inositol ring), 71.6 (OCH_2Ar), 70.2 (OCH_2Ar), 69.7 (inositol ring), 69.5 (inositol ring), 55.2 (OCH_3); *m/z* (ES^+) [Found: $(M+Na)^+$ 575.2250. $C_{31}H_{36}O_9Na$ requires M^+ 575.2252], *m/z* (ES^+) 275 ($[M+Na]^+$, 100%); Anal. calcd for $C_{31}H_{36}O_9$: C 67.4, H 6.6; Found: C 67.5; H 6.7.

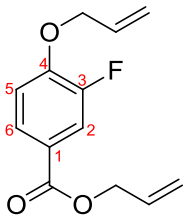
D-5-*O*-Allyl-2,4,6-tris-*O*-(4-methoxybenzyl)-1,3-*O*-methylene-*myo*-inositol 227



Allyl bromide (546 mg, 0.39 mL, 4.5 mmol, 2.5 eq.) and dry *N,N*-dimethylformamide (10 mL) were added to a flask containing sodium hydride washed with petroleum ether (60% dispersion in mineral oil, 181 mg, 4.5 mmol, 2.5 eq.) and cooled to 0 °C. A solution of D-2,4,6-tris-*O*-(4-methoxybenzyl)-*myo*-inositol 1,3-*O*-methylene **206** (1.00 g, 1.8 mmol, 1.0 eq.) in dry *N,N*-dimethylformamide (5 mL) was added dropwise over a period of 30 min to the stirred suspension, keeping the temperature constant at 0 °C. The reaction mixture was stirred for 30 min at 0 °C and then at RT for 12 h after which time the reaction was deemed to be complete by TLC analysis. The reaction was quenched by the addition of methanol (5 mL) and concentrated under reduced pressure. The resulting oil was reconstituted with ethyl acetate (30 mL) and H_2O (10 mL) and the layers were separated. The aqueous phase was then further extracted with ethyl acetate (2 × 40 mL). The combined organic layers were then dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The resulting oil was adsorbed onto silica gel, and purified by silica gel chromatography, eluting with petroleum ether and ethyl acetate (100:0, 80:20, 70:30, 60:40, 0:100) to afford *D-5-O-allyl-2,4,6-tris-O-(4-methoxybenzyl)-1,3-O-methylene-myo-inositol* **227** (569 mg, 53%) as a

colourless oil: R_f 0.46 (petroleum ether/ethyl acetate 70:30); ν_{max} (thin film)/cm^{-1} 2908 (w), 1612 (m), 1586 (w), 1512 (s), 1464 (w), 1366 (w), 1302 (m), 1247 (s), 1175 (m) 1082 (s), 1012 (m), 821 (s); δ_H (400 MHz, $CDCl_3$) 7.29 (2H, d, *J* 8.6, $OCH_2C_6H_4OCH_3$), 7.24 (4H, d, *J* 8.6, $OCH_2C_6H_4OCH_3$), 6.89-6.85 (6H, m, $OCH_2C_6H_4OCH_3$), 5.89 (1H, dddd, *J* 16.9, 11.1, 5.6, 5.6, CH_X=CH_YH_Z), 5.24 (1H, dd, *J* 16.9, 1.7, CH_X=CH_YH_Z), 5.17-5.15 (2H, m, CH_X=CH_YH_Z and 3-*H*), 4.81 (1H, d, *J* 5.5, 3-*H'*), 4.58 (2H, d, *J* 11.3, OCH_2), 4.57 (2H, s, OCH_2), 4.52 (2H, d, *J* 11.3, OCH_2), 4.20 (2H, s, inositol ring H-1 and H-3), 4.13 (2H, d, *J* 5.6, OCH_2CH_X=CH_YH_Z), 3.86 (2H, d, *J* 5.8, inositol ring H-4 and H-6), 3.81 (6H, s, OCH_3), 3.80 (3H, s, OCH_3), 3.77 (1H, dd, *J* 1.9, 1.9, inositol ring H-2), 3.52 (1H, dd, *J* 5.8, 5.8, inositol ring H-5); δ_C (126 MHz, $CDCl_3$) 159.3 (Ar C), 135.0 (CH=CH_2), 129.8 (Ar C), 129.7 (Ar CH), 129.5 (Ar CH), 129.4 (Ar CH), 116.9 (CH=CH_2), 113.8 (Ar CH), 113.8 (Ar CH), 85.4 (O_2CH_2), 81.6 (inositol ring C-4 and C-6), 80.3 (inositol ring C-5), 72.5 (OCH_2CH=CH_2), 72.1 (inositol ring C-1 and C-3), 71.4 (OCH_2), 70.6 (OCH_2), 69.8 (inositol ring C-2), 55.2 (OCH_3), 55.2 (OCH_3); *m/z* (ES^+) [Found: $(M+Na)^+$ 615.2566. $C_{34}H_{40}O_9Na$ requires M^+ 615.2565]; *m/z* (ES^+) 694 ($[M+NHEt_3]^+$, 100%), 615 ($[M+Na]^+$, 80%); Anal. calcd for $C_{34}H_{40}O_9$: C 68.9, H 6.8; Found: C 69.0; H 6.7.

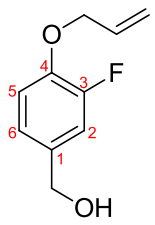
Allyl 4-*O*-allyl-3-fluorobenzoate 228



3-Fluoro-4-hydroxybenzoic acid **159** (1.49 g, 9.6 mmol, 1.0 eq.) and potassium carbonate (3.32 g, 24.0 mmol, 2.5 eq.) were sealed in a microwave reaction vial, to which dry *N,N*-dimethylformamide (10 mL) and allyl bromide (4.64 g, 3.30 mL, 38.4 mmol, 4.0 eq.) were added. The vial was irradiated at 100 °C for 1 h, after which time the reaction was deemed to be complete by TLC analysis. The potassium carbonate was filtered, and the filtrate diluted with ethyl acetate (20 mL) and H_2O (20 mL). The layers were separated, and the aqueous phase was further extracted with ethyl acetate (2 × 50 mL). The combined organic layers were dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The resulting oil was adsorbed onto Celite®, and purified by silica gel chromatography, eluting with petroleum ether and ethyl acetate (95:5 to 85:15) to afford *allyl 4-O-allyl-3-fluorobenzoate* **228** (1.45 g, 64%) as a colourless oil: R_f 0.58 (petroleum ether/ethyl acetate, 90:10); ν_{max} (thin film)/cm^{-1} 3583 (w), 2921 (w), 1712 (s), 1614 (m), 1512 (m), 1435 (m), 1276 (s), 1127 (m), 980 (m), 893 (m); δ_H (400 MHz, $CDCl_3$) 7.83-7.80 (1H, m, *H*-6), 7.78 (1H, dd, *J* 11.5, 2.0, *H*-2), 6.98 (1H, dd, *J* 8.3, 8.3, *H*-5), 6.10-5.99 (2H, m, 1 × CH_X=C$H_Y$$H_Z$, 1 × C$H_{X'}$=C$H_{Y'}$$H_{Z'}$), 5.45 (1H, dd, *J* 17.3, 1.3, CH_X=C$H_Y$$H_Z$), 5.40 (1H, dd, *J* 17.2, 1.2, $CH_{X'}$=C$H_{Y'}$$H_{Z'}$), 5.35 (1H, dd, *J* 10.5,

1.3, CH_X=$CH_Y$$H_Z$), 5.29 (1H, dd, *J* 10.4, 1.2, $CH_{X'}$=$CH_{Y'}$$H_{Z'}$) 4.80 (2H, d, *J* 5.6, 1.4, OCH_2), 4.68 (2H, d, *J* 5.3, 1.4, OCH_2); δ_C (126 MHz, $CDCl_3$) 165.9 (C=O), 151.9 (C-3, d, *J* 235.0), 153.6 (C-1), 132.1 (*C*H=CH_2), 132.0 (*C*H=CH_2), 126.5 (C-6, d, *J* 3.5), 123.1 (C-4, d, *J* 6.0), 118.7 (CH=*C*H_2), 118.3 (CH=*C*H_2), 117.4 (C-2, d, *J* 20.1), 113.8 (C-5), 69.9 (O*C*H_2), 65.6 (O*C*H_2); δ_F (376 MHz, $CDCl_3$) −133.6; *m/z* (ES^+) [Found: $(M+Na)^+$ 259.0739. $C_{13}H_{13}FO_3Na$ requires M^+ 259.0741], *m/z* (ES^+) 429 (100%); 259 ($[M+Na]^+$, 85%); Anal. calcd for $C_{13}H_{13}FO_3$: C 66.1, H 5.5; Found: C 66.2; H 5.4.

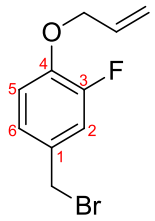
4-*O*-Allyl-3-fluorobenzyl alcohol 229



Lithium aluminium hydride (1 M in THF, 602 mg, 15.90 mL, 3.0 eq.) was added dropwise over a period of 30 min to a solution of allyl 4-*O*-allyl-3-fluorobenzoate **228** (1.25 g, 5.3 mmol, 1.0 eq.) in THF (50 mL) that was cooled to 0 °C. This mixture was stirred at RT for 1 h, by which time the reaction was deemed to be complete by TLC analysis. The reaction mixture was cooled to 0 °C, and quenched by the careful addition of ethyl acetate (15 mL), followed by H_2O (5 mL). Magnesium sulfate was added to the cloudy suspension, and the suspension was filtered through Celite®. The filtrate was concentrated under reduced pressure, adsorbed onto silica gel, and purified by silica gel chromatography, eluting with petroleum ether and

ethyl acetate (75:25 to 65:35) to afford *4-O-allyl-3-fluorobenzyl alcohol* **229** (646 mg, 67%) as a colourless oil: R_f 0.45 (petroleum ether/ethyl acetate, 70:30); ν_{max} (thin film)/cm^{-1} 2921 (w), 1648 (w), 1587 (m), 1459 (s), 1275 (m), 1219 (w), 1121 (m), 1015 (s), 872 (m), 805 (m); δ_H (400 MHz, $CDCl_3$) 7.12 (1H, d, *J* 11.8, *H*-2), 7.04 (1H, d, *J* 8.3, *H*-6), 6.95 (1H, dd, *J* 8.3, 8.3, *H*-5), 6.06 (1H, dddd, *J* 17.1, 10.7, 5.4, 5.4, CH_X=CH$_Y$H$_Z$), 5.43 (1H, d, *J* 17.1, CH$_X$=CH_YH$_Z$), 5.31 (1H, d, *J* 10.7, CH$_X$=CH$_Y$$H_Z$), 4.62-4.61 (4H, m, OC$H_2$), 1.65 (1H, dd, *J* 5.4, 5.4, O*H*); δ_C (126 MHz, $CDCl_3$) 152.7 (C3', d, *J* 246.2), 145.9 (C4', d, *J* 10.8), 134.4 (C1', d *J* 5.8), 132.8 (*C*H=CH$_2$), 122.6 (C6', d, *J* 3.6), 118.2 (CH=*C*H$_2$), 115.4 (C5', d, *J* 1.9), 115.1 (C2', d, *J* 18.9), 70.2 (O*C*H$_2$CH=CH$_2$), 64.5 (OCH$_2$); δ_F (376 MHz, $CDCl_3$) −133.7; *m/z* (ES$^+$) [Found: (M+Na)$^+$ 205.0635. $C_{10}H_{11}FO_2Na$ requires M^+ 205.0635], *m/z* (ES$^+$) 304 (100%); 413 (80%); 205 ([M+Na]$^+$, 65%); Anal. calcd for $C_{10}H_{11}FO_2$: C 65.9, H 6.1; Found: C 66.1; H 6.0.

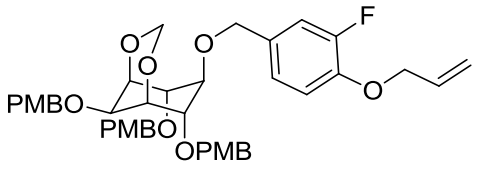
4-*O*-Allyl-3-fluorobenzyl bromide 230



To a solution of 4-*O*-allyl-3-fluorobenzyl alcohol **229** (560 mg, 13.1 mmol, 1.0 eq.) and triphenylphosphine (1.61 g, 6.1 mmol, 2.0 eq.) in dichloromethane (50 mL), carbon tetrabromide (2.03 g, 6.1 mmol, 2.0 eq.) was added portionwise over a period of 30 min and the resulting solution

was stirred for 5 h at RT, by which time the reaction was deemed to be complete by TLC analysis. The reaction mixture was concentrated under reduced pressure, adsorbed onto silica gel, and purified by silica gel chromatography, eluting with petroleum ether and ethyl acetate (95:5, 80:20, 70:30) to afford *4-O-allyl-3-fluorobenzyl bromide* **230** (362 mg, 48%) as a colourless oil: R_f 0.91 (petroleum ether/ethyl acetate, 90:10); ν_{max} (thin film)/cm^{-1} 3082 (m), 2924 (w), 1620 (s), 1514 (m), 1434 (w), 1384 (s), 1317 (m), 1225 (s), 1131 (m), 1014 (w), 958 (m), 879 (m), 715 (w); δ_H (400 MHz, $CDCl_3$) 7.14 (1H, dd, *J* 11.6, 2.2, *H*-2), 7.09-7.06 (1H, m, *H*-6), 6.91 (1H, dd, *J* 8.4, 8.4, *H*-5), 6.05 (1H, dddd, *J* 17.1, 10.7, 5.6, 5.6, CH_X=CH_YH_Z), 5.43 (1H, dd, *J* 17.1, 1.4, CH_X=CH_YH_Z), 5.32 (1H, dd, *J* 10.7, 1.4, CH_X=CH_YH_Z), 4.61 (2H, d, *J* 5.5, OCH_2), 4.44 (2H, s, OCH_2); δ_C (126 MHz, $CDCl_3$) 152.3 (C3', d, *J* 247.8), 146.7 (C4', d, *J* 10.6), 132.5 ($CH=CH_2$), 130.9 (C1', d *J* 6.5), 124.9 (C6', d, *J* 3.6), 118.3 ($CH=CH_2$), 117.0 (C2', d, *J* 19.0), 115.1 (C5', d, *J* 2.3), 70.1 ($OCH_2CH=CH_2$), 32.8 (OCH_2); δ_F (376 MHz, $CDCl_3$) −133.0; *m/z* (FI^+) [Found: $(^{79}M)^+$ 243.9900, $(^{81}M)^+$ 245.9874, $C_{10}H_{11}FOBr$ requires $^{79}M^+$ 243.9899, $^{81}M^+$ 245.9879]; *m/z* (FI^+) 243 ($[^{79}M]^+$, 100%), 245 ($[^{81}M]^+$, 95%); Anal. calcd for $C_{10}H_{10}FOBr$: C 49.0, H 4.1; Found: C 48.9; H 4.0.

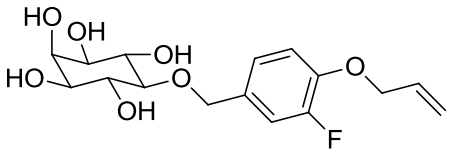
D-5-*O*-[4-*O*-Allyl-3-fluorobenzyl]-2,4,6-tris-*O*-(4-methoxybenzyl)-1,3-*O*-methylene-*myo*-inositol 231



Sodium hydride (60% dispersion in mineral oil, 41 mg, 1.0 mmol, 2.0 eq.) was added to a solution of D-2,4,6-tris-*O*-(4-methoxybenzyl)-1,3-*O*-methylene-*myo*-inositol **206** (290 mg, 0.5 mmol, 1.0 eq.) in dry *N,N*-dimethylformamide (15 mL) at 0 °C and stirred at RT for 1h. The solution was cooled to 0 °C and 4-*O*-allyl-3-fluorobenzyl bromide **230** (262 mg, 1.0 mmol, 2.0 eq.) and tetrabutylammonium iodide (382 mg, 1.0 mmol, 2.0 eq.) were added. The reaction was stirred at RT for 12 h, after which time the reaction was deemed to be complete by TLC analysis. The reaction was quenched by the addition of methanol (5 mL) and concentrated under reduced pressure. The resulting oil was reconstituted with ethyl acetate (50 mL) and H_2O (20 mL) and the layers were separated. The aqueous phase was then further extracted with ethyl acetate (2 × 50 mL). The combined organic layers were then dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The resulting oil was adsorbed onto silica gel, and purified by silica gel chromatography, eluting with petroleum ether and ethyl acetate (75:25 to 60:40) to afford *D-5-O-[4-O-allyl-3-fluorobenzyl]-2,4,6-tris-O-(4-methoxybenzyl)-1,3-O-methylene-myo-inositol* **231** (296 mg, 79%) as a pale yellow solid: R_f 0.41 (petroleum

ether/ethyl acetate 70:30), mp 96-98 °C (*from ethyl acetate)*; ν_{max} (thin film)/cm^{-1} 2929 (w), 1612 (m), 1514 (s), 1439 (m), 1274 (s), 1175 (w), 1082 (m), 1012 (m), 758 (w); δ_H (400 MHz, $CDCl_3$) 7.31-7.29 (2H, m, Ar*H*), 7.24-7.20 (4H, m, Ar*H*), 7.01 (1H, d, *J* 12.4, Ar*H*), 6.90-6.85 (8H, m, $OCH_2C_6H_4OCH_3$), 6.06 (1H, dddd, *J* 17.0, 10.5, 5.6, 5.6, CH_X=CH_YH_Z), 5.42 (1H, dd, *J* 17.0, 1.5, CH_X=CH_YH_Z), 5.30 (1H, dd, *J* 10.5, 1.65 CH_X=CH_YH_Z), 5.16 (1H, d, *J* 5.8, 3-*H*), 4.83 (1H, d, *J* 5.8, 3-*H'*), 4.60-4.58 (5H, m, OCH_2), 4.54 (3H, d, *J* 9.3, OCH_2), 4.46 (2H, d, *J* 11.4, OCH_2), 4.21 (2H, s, inositol ring H-1 and H-3), 3.90 (2H, d, *J* 6.0, inositol ring H-4 and H-6), 3.81 (6H, s, OCH_3), 3.80 (3H, s, OCH_3), 3.77-3.76 (1H, m, inositol ring H-2), 3.52 (1H, dd, *J* 6.0, 6.0, inositol ring H-5); δ_C (126 MHz, $CDCl_3$) 159.4 (Ar C), 159.3 (Ar C), 152.6 (Ar C-3, d, *J* 246.09), 145.9 (Ar C-4, d, *J* 10.5), 132.8 ($CH=CH_2$), 131.9 (Ar C-1, d *J* 5.8), 129.7 (Ar CH), 129.6 (Ar CH), 129.6 (Ar CH), 129.5 (Ar CH), 123.4 (Ar C-6, d, *J* 3.5), 118.1 ($CH=CH_2$), 115.9 (Ar C-2, d, *J* 18.8), 115.0 (Ar C-5, d, *J* 1.4), 113.9 (Ar CH), 113.8 (Ar CH), 113.7 (Ar CH), 85.5 (O_2CH_2), 81.6 (inositol ring C-4 and C-6), 80.3 (inositol ring C-5), 72.6 ($OCH_2CH=CH_2$), 72.0 (inositol ring C-1 and C-3), 71.3 (OCH_2), 70.6 (OCH_2), 70.2 (OCH_2), 69.8 (inositol ring C-2), 55.2 (OCH_3); δ_F (376 MHz, $CDCl_3$) −134.0; *m/z* (ES^+) [Found: $(M+Na)^+$ 739.2905. $C_{41}H_{45}FO_{10}Na$ requires M^+ 739.2889], *m/z* (ES^+) 739 ($[M+Na]^+$, 100%); Anal. calcd for $C_{41}H_{45}FO_{10}$: C 68.7, H 6.3; Found: C 68.7; H 6.4.

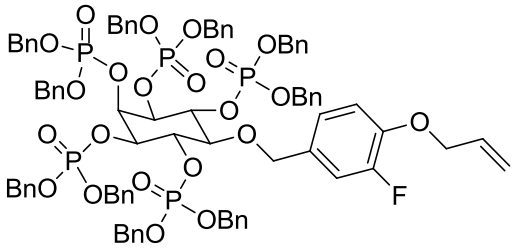
D-5-*O*-[4-*O*-Allyl-3-fluorobenzyl]-*myo*-inositol 232



Concentrated hydrochloric acid (37%, 10.00 mL) was slowly added to a stirred solution of D-5-*O*-[4-*O*-allyl-3-fluorobenzyl]-2,4,6-tris-*O*-(4-methoxybenzyl)-1,3-*O*-methylene-*myo*-inositol **231** (101 mg, 0.1 mmol, 1.0 eq.) in methanol (10 mL) and ethyl acetate (5 mL) and heated at 50 °C for 12 h, after which time the reaction was deemed to be complete by TLC analysis. The reaction was cooled to 0 °C and the precipitated product was filtered and washed with diethyl ether to afford *D-5-O-[4-O-allyl-3-fluorobenzyl]-myo-inositol* **232** (32 mg, 67%) as a colourless solid: ν_{max} (solid)/cm^{-1} 3164.5 (s), 2923.4 (w), 1518.4 (w), 1415.1 (m), 1370.6 (m), 1273.7 (w), 1245.9 (w), 1195.2 (w), 1145.6 (m), 1113.8 (s), 1044.5 (s), 1000.8 (s), 929.6 (w), 893.1 (m), 724.6 (m); mp 265-267 °C (*from methanol)*; δ_H (400 MHz, DMSO-D_6) 7.33 (1H, d, *J* 13.2, Ar H-2), 7.11-7.04 (2H, m, Ar H-5 and H-6), 6.03 (1H, dddd, *J* 17.2, 10.5, 5.2, 5.2, C*H*$_X$=CH$_Y$H$_Z$), 5.39 (1H, dd, *J* 17.2, 1.6, CH$_X$=C*H*$_Y$H$_Z$), 5.26 (1H, dd, *J* 10.5, 1.6, CH$_X$=CH$_Y$*H*$_Z$), 4.69 (1H, s, O*H*), 4.68 (3H, s, 1 × O*H* and OC*H*$_2$Ph), 4.61 (2H, d, *J* 5.2, OC*H*$_2$CH**$_X$**=CH$_Y$H$_Z$), 4.52 (1H, d, *J* 3.0, O*H*), 4.44 (2H, d, *J* 5.5, 2 × O*H*), 3.70-3.69 (1H, m, inositol ring H-2), 3.54-3.48 (2H, m, inositol ring H-4 and H-6), 3.17-3.13 (2H, m, inositol ring H-1 and H-3), 2.95 (1H, dd, *J* 9.1, 9.1, inositol ring H-5); δ_C (126 MHz, DMSO-D_6) 151.5 (Ar C-3, d, *J* 242.9), 144.8

(Ar C-4, d, *J* 10.5), 133.4 (*C*H=CH_2), 133.3 (Ar C-1), 123.3 (Ar C-6, d, *J* 3.3), 117.9 (CH=*C*H_2), 115.3 (Ar C-2, d, *J* 18.2), 114.7 (Ar C-5), 84.0 (inositol ring C-5), 72.5 (O*C*H_2Ar and inositol ring C-2), 72.4 (inositol ring C-4 and C-6), 71.9 (inositol ring C-1 and C-3), 69.2 (O*C*H_2CH=CH_2); δ_F (376 MHz, $CDCl_3$) −135.3; *m/z* (ES^+) [Found: $(M+Na)^+$ 367.1162. $C_{16}H_{21}FO_7Na$ requires M^+ 367.1164], *m/z* (ES^+) 413, (100%); 381, (86%); 367 ($[M+Na]^+$, 85%). Anal. calcd for $C_{16}H_{21}FO_7$: C 55.8, H 6.1; Found: C 55.7; H 6.2.

D-5-*O*-[4-*O*-Allyl-3-fluorobenzyl]-*myo*-inositol 1,2,3,4,6-pentakis(dibenzylphosphate) 233



Anhydrous 1*H*-tetrazole (3-4 wt% in acetonitrile, 14.30 mL, 4.9 mmol, 12.5 eq.) was added to a solution of bis(benzyloxy)-*N*,*N*-diisopropylphosphoramidite **167** (1.70 g, 4.9 mmol, 12.5 eq.) in anhydrous dichloromethane (40 mL) and stirred at RT for 10 min. A solution of D-5-*O*-[4-*O*-allyl-3-fluorobenzyl]-*myo*-inositol **232** (135 mg, 0.4 mmol, 1.0 eq.) in dichloromethane (20 mL) was then added to the flask via cannula, and the resulting mixture was stirred at RT for 12 h, after which time the reaction was deemed to be complete by TLC analysis. The mixture was cooled to −78 °C and 3-chloroperoxybenzoic acid (assume 95%, 890 mg, 4.9 mmol, 12.5 eq.) was added. The mixture was allowed to reach RT and stirred for

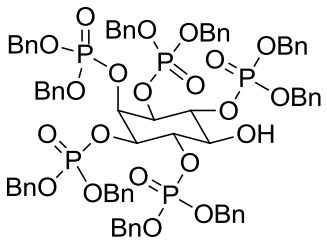
2 h, after which it was deemed to be complete by TLC analysis. The 3-chloroperbenzoic acid was quenched by the addition of a 10% aqueous solution of sodium metabisulfite (30 mL) and stirred for 15 min. The resulting organic and aqueous phases were separated, and the aqueous layer re-extracted with dichloromethane (3 × 50 mL). The combined organic extracts were washed with brine (10 mL), dried over magnesium sulfate, filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography, eluting with ethyl acetate and toluene (30:70, 20:30, 50:50, 30:20) to give *D-5-O-[4-O-allyl-3-fluorobenzyl]-myo-inositol 1,2,3,4,6-pentakis(dibenzylphosphate)* **233** (580 mg, 89%) as a colourless oil: R_f 0.64 (petroleum ether/ethyl acetate, 50:50); ν_{max} (thin film)/cm^{-1} 1456 (w), 1276 (s), 1018 (s), 740 (m), 696 (m); δ_H (400 MHz, $CDCl_3$) 7.30-7.13 (47H, m, Ar*H* and Ar H-2), 7.10 (1H, d, *J* 8.4, Ar H-6), 7.01 (4H, d, *J* 7.4, Ar*H*), 6.73 (1H, dd, *J* 8.4, 8.4, Ar H-5), 6.00 (1H, dddd, *J* 16.8, 10.9, 5.6, 5.6, CH_X=CH_YH$_Z$), 5.63 (1H, d, *J* 9.3, inositol ring H-2), 5.36 (1H, dd, *J* 16.8, 1.7, CH$_X$=CH_YH$_Z$), 5.24 (1H, d, *J* 10.9, 1.7 CH$_X$=CH$_Y$$H_Z$), 5.21-5.17 (2H, m, OC$H_2$), 5.11-4.99 (14H, m, inositol ring H-4, H-6 and OCH_2), 4.94-4.86 (4H, m, OCH_2), 4.74 (2H, s, OCH_2Ar), 4.69 (2H, dd, *J* 10.3, 10.3, OCH_2), 4.49 (2H, d, *J* 5.6, OCH_2CH$_X$=CH$_Y$H$_Z$), 4.36 (2H, dd, *J* 9.3, 9.3, inositol ring H-1 and H-3), 3.49 (1H, dd, *J* 9.4, 9.4, inositol ring H-5); δ_C (126 MHz, $CDCl_3$) 152.3 (Ar C-3, d, *J* 245.1), 145.6 (Ar C-4, d, *J* 10.7), 135.8 (Ar C), 135.8 (Ar C), 135.7 (Ar C), 135.7 (Ar C), 135.6 (Ar C), 135.5 (Ar C), 132.8 (*C*H=CH$_2$), 131.1 (Ar C-1, d, *J* 6.7), 128.5 (Ar CH), 128.4 (Ar CH), 128.4 (Ar CH), 128.3 (Ar CH), 128.3 (Ar CH), 128.3 (Ar CH), 128.2 (Ar CH), 128.2 (Ar CH), 128.1 (Ar CH), 127.9 (Ar CH), 127.9 (Ar CH), 127.7 (Ar CH),123.3 (Ar C-6, d, *J*

3.4), 118.0 (CH=*C*H_2), 115.6 (Ar CH), 115.4 (Ar CH), 114.8 (Ar C-5, d, *J* 1.9), 78.7 (inositol ring C-5), 76.8* (Ar C-2), 76.5* (inositol ring C-4 and C-6), 76.0 (inositol ring C-2), 73.8 (inositol ring C-1 and C-3), 73.5 (O*C*H_2Ar), 70.1 (O*C*H_2CH=CH_2), 70.0 (OCH_2), 69.8 (OCH_2), 69.8 (OCH_2), 69.8 (OCH_2), 69.7 (OCH_2), 69.6 (OCH_2), 69.6 (OCH_2), 69.4 (OCH_2), 69.3 (OCH_2); δ_P (202 MHz, $CDCl_3$) −0.7, −1.3, −2.5; δ_F (376 MHz, $CDCl_3$) −134.2; *m/z* (ES^+) [Found: (M+Na)+ 1667.4179. $C_{86}H_{86}FO_{22}P_5Na$ requires M^+ 1667.4175]; *m/z* (ES^+) 1667 ([M+Na]$^+$, 100%); Anal. calcd for $C_{86}H_{86}FO_{22}P_5$: C 62.8, H 5.3; Found: C 62.9; H 5.2.

*Signal was assigned from HSQC NMR spectrum.

**Due to resonance overlap in the ^{13}C NMR spectrum, some carbon environments have not been accounted for.

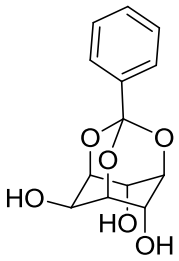
D-*myo*-Inositol 1,2,3,4,6-pentakis(dibenzylphosphate) 223



Cerium ammonium nitrate (99 mg, 0.2 mmol, 10.0 eq.) was added to a solution of D-5-*O*-[4-*O*-allyl-3-fluorobenzyl]-*myo*-inositol 1,2,3,4,6-pentakis(dibenzylphosphate) **233** (30 mg, 0.02 mmol, 1.0 eq.) in acetonitrile (2.40 mL) and H_2O (0.60 mL) and stirred at RT for 2.5 h. When the reaction was deemed to be complete by TLC analysis, the reaction was concentrated

under reduced pressure. The resulting oil was reconstituted with ethyl acetate (20 mL) and H_2O (10 mL) and the layers were separated. The aqueous phase was then further extracted with ethyl acetate (2 × 20 mL). The combined organic layers were then dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The resulting oil was purified by silica gel chromatography, eluting with toluene and ethyl acetate (90:10 to 70:30) to afford *D-myo-inositol 1,2,3,4,6-pentakis(dibenzylphosphate)* **223** (2 mg, 7%) as a colourless oil: Rf 0.49 (toluene/ethyl acetate, 50:50); δ_H (400 MHz, $CDCl_3$) 7.29-7.27 (4H, m, Ar*H*), 7.25-7.15 (42H, m, Ar*H*), 7.10 (4H, d, *J* 7.4, Ar*H*), 5.52 (1H, d, *J* 9.0, inositol ring H-2), 5.27 (1H, d, *J* 2.4, O*H*), 5.13-5.07 (5H, m, OCH_2), 5.06-5.00 (11H, m, OCH_2), 4.97-4.95 (4H, m, OCH_2), 4.84 (2H, d, *J* 17.7, 8.9, inositol ring H-4 and H-6), 4.41 (2H, dd, *J* 9.5, 9.5, inositol ring H-1 and H-3), 3.78 (1H, ddd, *J* 8.9, 8.9, 2.4, inositol ring H-5); δ_C (126 MHz, $CDCl_3$) 135.7 (Ar C), 135.6 (Ar C), 135.5 (Ar C), 135.5 (Ar C), 135.4 (Ar C), 128.5 (Ar CH), 128.4 (d, *J* 1.4, Ar CH), 128.4 (Ar CH), 128.3 (Ar CH), 128.2 (Ar CH), 128.1 (Ar CH), 127.9 (d, *J* 1.8, Ar CH), 127.8 (Ar CH), 127.8 (Ar CH), 127.7 (Ar CH), 78.2 (inositol ring C-4 and C-6), 76.4* (inositol ring C-2), 73.6 (inositol ring C-1 and C-3), 72.2 (inositol ring C-5), 70.0 (OCH_2), 69.9 (OCH_2), 69.9 (OCH_2), 69.8 (OCH_2), 69.7 (OCH_2), 69.7 (OCH_2), 69.6 (OCH_2); δ_P (202 MHz, $CDCl_3$) 1.1, −0.3, −1.2; *m/z* (ES^+) [Found: (M+Na)+ 1503.3551. $C_{76}H_{77}O_{21}P_5Na$ requires M^+ 1503.3538]; *m/z* (ES^+) 1503 ($[M+Na]^+$, 100%), 1498 ($[M+NH_4]^+$, 95%).

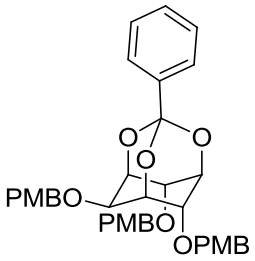
D-*myo*-Inositol 1,3,5-orthobenzoate 210



D-*myo*-Inositol **1** (1.01 g, 5.5 mmol, 1.0 eq.) and 4-toluenesulfonic acid monohydrate (25 mg, 0.1 mmol, 0.02 eq.) were dissolved in dry dimethyl sulfoxide (16 mL). Triethyl orthobenzoate (1.36 g, 1.40 mL, 6.1 mmol, 1.1 eq.) was added and the reaction mixture was stirred at 80 °C for 5 h. When the reaction was deemed to be complete by TLC analysis, the mixture was allowed to cool to RT and the 4-toluenesulfonic acid was quenched by the addition of triethylamine (1 mL). The mixture was concentrated under reduced pressure and then evaporated to dryness using a GeneVac® evaporation system. The resulting solid was dissolved in hot ethyl acetate and filtered through a short silica gel column. Concentration of the filtrate under reduced pressure and crystallisation afforded D-*myo*-inositol 1,3,5-orthobenzoate **210** (719 mg, 48%) as a colourless crystalline solid: R_f 0.58 (petroleum ether/ethyl acetate, 65:35); mp 215-217 °C (*from ethyl acetate*) (Lit.[196] 210-211 °C); δ_H (400 MHz, DMSO-D_6) 7.56-7.54 (2H, m, 3-Ar*H*), 7.37-7.34 (3H, m, 3-Ar*H*), 5.52 (2H, s, 2 × O*H*), 5.36 (1H, d, *J* 6.3, O*H*), 4.40 (2H, dd, *J* 3.6, 3.6, 2 × inositol ring), 4.22-4.20 (1H, m, 1 × inositol ring), 4.16-4.14 (2H, m, 2 × inositol ring), 4.08-4.07 (1H, m, 1 × inositol ring) *m/z*

(ES$^-$) 531 ([2M−H]$^-$, 100%), 265 ([M−H]$^-$, 80%). The data are in good agreement with literature values.[196]

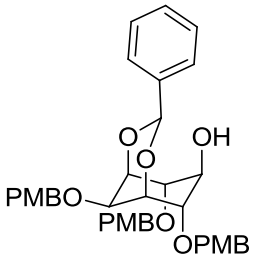
D-2,4,6-Tris-*O*-(4-methoxybenzyl)-*myo*-inositol 1,3,5-orthobenzoate 209



4-Methoxybenzyl chloride (877 mg, 0.76 mL, 5.6 mmol, 5.0 eq.) and dry *N,N*-dimethylformamide (5 mL) were added to a flask containing sodium hydride washed with petroleum ether (60% dispersion in mineral oil, 225 mg, 5.6 mmol, 5.0 eq.) and cooled to 0 °C. A solution of D-*myo*-inositol 1,3,5-orthobenzoate **210** (306 mg, 1.1 mmol, 1.0 eq.) in dry *N,N*-dimethylformamide (5 mL) was added dropwise over a period of 30 min to the stirred suspension, maintaining the temperature at 0 °C. The reaction mixture was stirred for 30 min at 0 °C and then at 60 °C for 48 h, after which time the reaction was deemed to be complete by TLC analysis. The reaction was cooled to 0 °C and the resulting precipitate was filtered and washed with H_2O (20 mL) and diethyl ether (20 mL) to afford D-*2,4,6-tris-O-(4-methoxybenzyl)-myo-inositol 1,3,5-orthobenzoate* **209** (604 mg, 84%) as a colourless solid: R_f 0.43 (petroleum ether/ethyl acetate 70:30); mp 151-153 °C (from *N,N*-dimethylformamide); ν_{max} (thin film)/cm^{-1} 2907 (w), 1609 (m), 1584 (w), 1511 (s), 1449 (m), 1334 (w), 1302 (w), 1246 (s), 1175 (m), 1137

(w), 1101 (s), 1073 (m), 1026 (m), 1003 (s), 984 (s), 916 (w), 851 (w), 817 (s), 759 (m), 697 (m), 619 (w); δ_H (400 MHz, $CDCl_3$) 7.65-7.63 (2H, m, 3-Ar*H*), 7.34-7.30 (5H, m, 3 × 3-Ar*H* and 2 × $OCH_2C_6H_4OCH_3$), 7.13 (4H, d, *J* 8.6, $OCH_2C_6H_4OCH_3$), 6.86-6.81 (6H, m, $OCH_2C_6H_4OCH_3$), 4.62 (2H, s, $OCH_2C_6H_4OCH_3$), 4.56 (2H, d, *J* 1.1, $OCH_AH_BC_6H_4OCH_3$), 4.51-4.50 (1H, m, inositol ring H-5), 4.44-4.41 (6H, m, *2* × $OCH_AH_BC_6H_4OCH_3$, inositol ring H-1, H-3, H-4 and H-6), 4.07 (1H, dd, *J* 1.5, 1.5, inositol ring H-2), 3.81 (3H, s, OCH_3), 3.78 (6H, s, OCH_3); δ_C (126 MHz, $CDCl_3$) 159.3 (Ar C), 159.2 (Ar C), 137.2 (Ar C), 130.1 (Ar CH), 129.8 (Ar CH), 129.6 (Ar CH), 129.3 (Ar CH), 129.2 (Ar CH), 127.9 (Ar CH), 125.3 (Ar CH), 114.0 (Ar CH), 113.8 (Ar CH), 113.7 (Ar CH), 107.8 ($O_3CC_6H_5$), 73.7 (inositol ring C-4 and C-6), 72.0 (inositol ring C-1 and C-3), 71.2 (CH_2), 70.7 (CH_2), 69.1 (inositol ring C-5), 65.7 (inositol ring C-2), 55.2 (OCH_3), 55.2 (OCH_3); *m/z* (ES^+) [Found: $(M+Na)^+$ 649.2409. $C_{37}H_{38}O_9Na$ requires M^+ 649.2408]; m/z (ES^+) 627 ($[M+H]^+$, 100%); 649 ($[M+Na]^+$, 95%); Anal. calcd for $C_{37}H_{38}O_9$: C 70.9, H 6.1; Found: C 71.0; H 5.9.

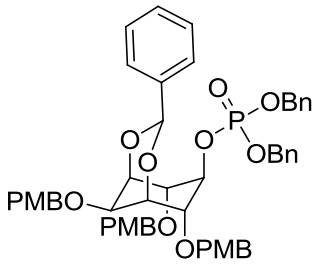
D-2,4,6-Tris-*O*-(4-methoxybenzyl)-1,3-*O*-benzylidene-*myo*-inositol 207



D-2,4,6-Tris-*O*-(4-methoxybenzyl)-*myo*-inositol 1,3,5-orthobenzoate **209** (1.12 g, 1.8 mmol, 1.0 eq.) was dissolved in dry dichloromethane (60 mL) and cooled to −40 °C. A solution of diisobutylaluminium hydride in hexanes (1.0 M, 4.46 mL, 4.5 mmol, 2.5 eq.) was added dropwise over a period of 30 min, and the mixture was stirred at −40 °C for 5 h, after which time the over-reduced product was observed by TLC analysis. The reaction mixture was quenched by the careful addition of H_2O (1.80 mL), sodium hydroxide (1.80 mL, 15% solution) and additional H_2O (4.50 mL). The resulting gel was dried by addition of magnesium sulfate, filtered, and concentrated under reduced pressure. The resulting residue was purified with silica gel chromatography, eluting with petroleum ether and ethyl acetate (80:20 to 40:60) to afford *D-2,4,6-tris-O-(4-methoxybenzyl)-myo-inositol 1,3-O-benzylidene* **207** (535 mg, 48%) as a colourless oil: R_f 0.53 (petroleum ether/ethyl acetate 70:30); ν_{max} (thin film)/cm^{-1} 2907 (w), 1612 (m), 1513 (s), 1461 (w), 1368 (w), 1302 (w), 1247 (s), 1175 (m), 1064 (s), 1032 (m), 997 (w), 820 (m), 761 (w), 700 (w); δ_H (400 MHz, $CDCl_3$) 7.54-7.52 (2H, m, 3-Ar*H*), 7.42-7.35 (5H, m, 3 × 3-Ar*H* and 2 × $OCH_2C_6H_4OCH_3$), 7.27 (4H, d, *J* 8.8, $OCH_2C_6H_4OCH_3$), 6.91-6.88 (6H, m, $OCH_2C_6H_4OCH_3$), 5.71 (1H, s, 3-

H), 4.69 (2H, d, *J* 11.5, OC*H*$_A$H$_B$C$_6$H$_4$OCH$_3$), 4.66 (2H, s, OC*H*$_2$C$_6$H$_4$OCH$_3$), 4.55 (2H, d, *J* 11.5, OCH$_A$*H*$_B$C$_6$H$_4$OCH$_3$), 4.38 (2H, d, *J* 2.3, inositol ring H-1 and H-3), 3.97 (1H, s, inositol ring H-4), 3.95 (1H, s, inositol ring H-6), 3.82 (3H, s, OC*H*$_3$), 3.81 (6H, s, OC*H*$_3$), 3.75 (1H, ddd, *J* 8.5, 8.5, 2.3, inositol ring H-5), 3.59 (1H, dd, *J* 2.3, 2.3, inositol ring H-2), 2.45 (1H, d, *J* 2.6, O*H*); δ_C (126 MHz, $CDCl_3$) 159.5 (Ar C), 159.3 (Ar C), 138.0 (Ar C), 129.9 (Ar CH), 129.7 (Ar CH), 129.6 (Ar CH), 129.5 (Ar CH), 129.47 (Ar CH), 129.43 (Ar CH), 129.2 (Ar CH), 128.4 (Ar CH), 126.6 (Ar CH), 126.3 (Ar CH), 114.2 (Ar CH), 114.0 (Ar CH), 113.9 (Ar CH), 113.7 (Ar CH), 113.7 (Ar CH), 92.8 (O$_2$*C*HC$_6$H$_5$), 80.9 (inositol ring C-4 and C-6), 73.7 (inositol ring C-1 and C-3), 73.6 (inositol ring C-5), 71.3 (CH_2), 70.4 (CH_2), 67.8 (inositol ring C-2), 55.3 (CH_3); *m/z* (ES^+) [Found: $(M+Na)^+$ 651.2566. $C_{37}H_{40}O_9Na$ requires M^+ 651.2565], *m/z* (ES^+) 688 (100%), 651 ($[M+Na]^+$, 85%); Anal. calcd for $C_{37}H_{40}O_9$: C 70.7, H 6.4; Found: C 70.6; H 6.3.

D-2,4,6-Tris-*O*-(4-methoxybenzyl)-1,3-*O*-benzylidene-*myo*-inositol 5-dibenzylphosphate 218

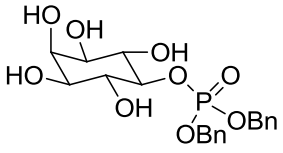


5-Phenyl-1*H*-tetrazole (397 mg, 2.7 mmol, 3.0 eq.) was added to a solution of bis(benzyloxy)-*N,N*-diisopropylphosphoramidite **167** (940 mg, 2.7 mmol, 3.0 eq.) in anhydrous dichloromethane (30 mL) and stirred for 10 min. A solution D-2,4,6-tris-*O*-(4-methoxybenzyl)-1,3-*O*-benzylidene-*myo*-inositol **207** (574 mg, 0.9 mmol, 1.0 eq.) in anhydrous dichloromethane (30 mL) was added *via* cannula, and the resulting mixture was stirred for 12 h, after which time the reaction was deemed to be complete by TLC analysis. The mixture was cooled to −78 °C and 3-chloroperoxybenzoic acid (assume 60%, 1.04 g, 3.6 mmol, 4.0 eq.) was added. The mixture was allowed to reach RT and stirred for 2 h, after which time the reaction was deemed to be complete by TLC analysis. The 3-chloroperbenzoic acid was quenched by the addition of a 10% aqueous solution of sodium metabisulfite (10 mL) and stirred for 15 min. The resulting organic and aqueous phases were separated, and the aqueous layer re-extracted with dichloromethane (3 × 30 mL). The combined organic extracts were washed with saturated aqueous sodium bicarbonate solution (20 mL) and brine (20 mL), dried over magnesium sulfate, filtered and concentrated under reduced pressure. The resulting

residue was purified by silica gel chromatography, eluting with ethyl acetate and petroleum ether (40:60 to 80:20) to give *D-2,4,6-tris-O-(4-methoxybenzyl)-1,3-O-benzylidene-myo-inositol 5-dibenzylphosphate* **218** (744 mg, 92%) as a colourless oil: R_f 0.50 (petroleum ether/ethyl acetate, 65:35); ν_{max} (thin film)/cm^{-1} 2957 (w), 1612 (m), 1514 (s), 1456 (m), 1370 (w), 1249 (s), 1175 (m), 1075 (s), 999 (s), 821 (m), 744 (m), 698 (m); δ_H (400 MHz, $CDCl_3$) 7.54-7.52 (2H, m, 3-Ar*H*), 7.40-7.37 (3H, m, 3-Ar*H*), 7.34-7.32 (2H, m, $OCH_2C_6H_4OCH_3$), 7.30-7.27 (6H, m, Ar*H*), 7.22-7.20 (8H, m, 4 × Ar*H* and 4 × $OCH_2C_6H_4OCH_3$), 6.87 (2H, d, *J* 8.6, $OCH_2C_6H_4OCH_3$), 6.78 (4H, d, *J* 8.6, $OCH_2C_6H_4OCH_3$), 5.78 (1H, s, 3-*H*), 4.99 (1H, d, *J* 11.9, OCH_AH_BPh), 4.97 (1H, d, *J* 11.8, $OCH_{A'}H_{B'}Ph$), 4.93 (1H, d, *J* 11.9, OCH_AH_BPh), 4.92 (1H, d, *J* 11.8, $OCH_{A'}H_{B'}Ph$), 4.63 (2H, s, $OCH_2C_6H_4OCH_3$), 4.59 (2H, d, *J* 11.1, $OCH_AH_BC_6H_4OCH_3$), 4.58-4.57 (1H, m, inositol ring H-5), 4.50 (2H, d, *J* 11.1, $OCH_AH_BC_6H_4OCH_3$), 4.36 (2H, d, *J* 2.2, inositol ring H-1 and H-3), 4.15 (1H, s, inositol ring H-4), 4.13 (1H, s, inositol ring H-6), 3.80 (3H, s, OCH_3), 3.75 (6H, s, OCH_3), 3.59 (1H, dd, *J* 2.2, 2.2, inositol ring H-2); δ_C (126 MHz, $CDCl_3$) 159.3 (Ar C), 159.3 (Ar C), 137.9 (Ar C), 135.9 (Ar C), 135.8 (Ar C), 129.8 (Ar C), 129.5 (Ar CH), 129.4 (Ar CH), 129.4 (Ar CH), 129.3 (Ar CH), 129.2 (Ar CH), 128.4 (Ar CH), 128.4 (Ar CH), 128.3 (Ar CH), 127.7 (Ar CH), 126.5 (Ar CH), 113.9 (Ar CH), 113.7 (Ar CH), 92.9 ($O_2CHC_6H_5$), 80.4 (d, *J* 3.8, inositol ring C-4 and C-6), 80.2 (d, *J* 6.7, inositol ring C-5), 73.1 (inositol ring C-1 and C-3), 71.1 (CH_2), 70.35 (CH_2), 69.3 (CH_2), 69.2 (CH_2), 37.4 (inositol ring C-2), 55.2 (CH_3), 55.2 (CH_3); δ_P (202 MHz, $CDCl_3$) −1.8; *m/z* (ES^+) [Found: $(M+Na)^+$ 911.3142.

$C_{51}H_{53}O_{12}P$ Na requires M^+ 911.3167], *m/z* (ES^+) 911 ($[M+Na]^+$, 100%); Anal. calcd for $C_{51}H_{53}O_{12}P$: C 68.9, H 6.0; Found: C 69.1; H 5.9.

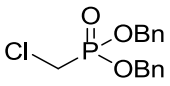
D-*myo*-Inositol 5-dibenzylphosphate 239



D-2,4,6-Tris-*O*-(4-methoxybenzyl)-1,3-*O*-benzylidene-*myo*-inositol 5-dibenzylphosphate **218** (320 mg, 0.3 mmol, 1.0 eq.) was dissolved in a mixture of trifluoroacetic acid (10 mL), chloroform (10 mL) and methanol (2 mL) and stirred at −10 °C for 5 min. When the reaction was deemed to be complete by TLC analysis, the reaction was concentrated under reduced pressure. The resulting solid was washed with hexane and diethyl ether to afford *D-myo-inositol 5-dibenzylphosphate* **239** (143 mg, 99%) as a colourless solid: mp 188-190 °C (*from acetonitrile*); ν_{max} (solid)/cm^{-1} 3409.7 (w), 3348.8 (w), 2365.0 (w), 1456.0 (w), 1230.2 (m), 1216.2 (m), 1115.4 (m), 1019.2 (s), 933.8 (m), 735.3 (s), 696.1 (m); δ_H (400 MHz, $CDCl_3$) 7.38-7.28 (10H, m, Ar*H*), 5.09 (4H, d, *J* 6.8, OC*H*$_2$Ph), 4.99 (2H, br s, O*H*), 4.66 (3H, br s, O*H*), 4.01 (1H, dd, *J* 18.1, 9.1, inositol ring H-5), 3.73-3.73 (1H, m, inositol ring H-2), 3.63 (2H, dd, *J* 9.4, 9.4, inositol ring H-4 and H-6), 3.24 (2H, dd, *J* 9.4, 2.1, inositol ring H-3 and H-1); δ_C (126 MHz, $CDCl_3$) 136.7 (Ar C), 136.6 (Ar C), 128.3 (Ar CH), 128.0 (Ar CH), 127.7 (Ar CH), 83.4 (d, *J* 6.8, inositol ring C-5), 72.4 (inositol ring C-2), 71.5 (inositol ring C-3 and C-1), 71.3 (d, *J* 3.2, inositol ring C-6 and C-4), 68.1 (OCH_2), 68.1 (OCH_2); δ_P

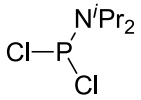
(161 MHz, $CDCl_3$) −1.2; *m/z* (ES^+) [Found: $(M+Na)^+$ 463.1129. $C_{20}H_{25}O_9PNa$ requires 463.1128]; *m/z* (ES^+) 463 ($[M+Na]^+$, 100%), 107 (100%), 191 (97%); Anal. calcd for $C_{20}H_{25}O_9P$: C 54.5, H 5.7; Found: C 54.6; H 5.8.

Dibenzyl (chloromethyl)phosphonate 219



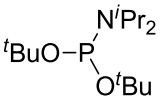
Anhydrous triethylamine (4.90 mL, 35.8 mmol, 3.0 eq.) was added dropwise over a period of 30 min to a solution of chloromethylphosphonic dichloride (2.00 g, 1.20 mL, 11.9 mmol, 1.0 eq.) in dry THF (100 mL) at 0 °C and stirred for 10 min. Benzyl alcohol (1.97 g, 2.70 mL, 26.3 mmol, 2.2 eq.) was added dropwise over a period of 30 min and the solution was stirred at 0 °C for 1 h and at RT for 4 h. The reaction was filtered and the filtrate was concentrated under reduced pressure. The resulting oil was adsorbed onto silica gel, and purified by silica gel chromatography, eluting with petroleum ether and ethyl acetate (93:7 to 40:60) to afford dibenzyl (chloromethyl)phosphonate **219** (2.25 g, 61%) as a colourless oil. R_f 0.33 (petroleum ether/ethyl acetate, 70:30); δ_H (400 MHz, $CDCl_3$) 7.40-7.32 (10H, m, Ar*H*), 5.16 (1H, d, *J* 11.6, OC$H_A$$H_B$Ph), 5.13 (1H, d, *J* 11.8, OC$H_{A'}$$H_{B'}$Ph), 5.10 (1H, d, *J* 11.6, OC$H_A$$H_B$Ph), 5.08 (1H, d, *J* 11.8, OC$H_{A'}$$H_{B'}$Ph), 3.49 (2H, d, *J* 10.3, CH_2Cl); δ_P (162 MHz, $CDCl_3$) 20.4. The data are in good agreement with literature values.[217]

_N,N_-Diisopropylphosphoramidodichloridite 241



Dry *N,N*-diisopropylamine (8.08 g, 11.20 mL, 80.2 mmol, 1.0 eq.) was added to a stirred solution of phosphorus trichloride (11.01 g, 7.00 mL, 80.2 mmol, 1.0 eq.) in dry diethyl ether (60 mL) at 0 °C and stirred at RT for 2 h. The resulting precipitate was filtered through Schlenk apparatus under a blanket of nitrogen, and the filtrate was concentrated under reduced pressure to yield *N,N*-diisopropylphosphoramidodichloridite **241** (5.06 g, 32%) as a yellow oil. δ_H (400 MHz, $CDCl_3$) 3.92-3.75 (2H, m, N[C*H*$(CH_3)_2]_2$), 1.22 (12H, d, *J* 6.7, N[CH(C*H*$_3)_2]_2$). δ_P (161 MHz, $CDCl_3$) 170.2. The data are in good agreement with literature values.[218]

Di-*tert*-butyl *N,N*-diisopropylphosphoramidite 240



A solution of *N,N*-diisopropylethylamine (12.02 g, 16.20 mL, 9.3 mmol, 2.5 eq.) and *tert*-butanol (8.35 g, 10.70 mL, 11.9 mmol, 3.0 eq.) in anhydrous diethyl ether (20 mL) was added dropwise over a period of 30 min to a stirred solution of *N,N*-diisopropylphosphoramidodichloridite **241** (7.54 g, 3.7 mmol, 1.0 eq.) in anhydrous diethyl ether (40 mL) at 0 °C, then

heated under reflux for 4 h. The resulting precipitate was filtered through Schlenk apparatus under a blanket of nitrogen, and the filtrate was concentrated under reduced pressure. Vacuum distillation at 135 °C and 20 mbar afforded di-*tert*-butyl *N,N*-diisopropylphosphoramidite **240** (8.80 g, 85%) as a colourless oil. δ_H (250 MHz, $CDCl_3$) 3.77-3.59 (2H, m, $N[CH(CH_3)_2]_2$), 1.42 (18H, s, 2 × $OC(CH_3)_3$) 1.22 (12H, d, *J* 6.7, $N[CH(CH_3)_2]_2$); δ_P (101 MHz, $CDCl_3$) 130.6. The data are in good agreement with the literature.[218]

Chapter 7:

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7. Bibliography

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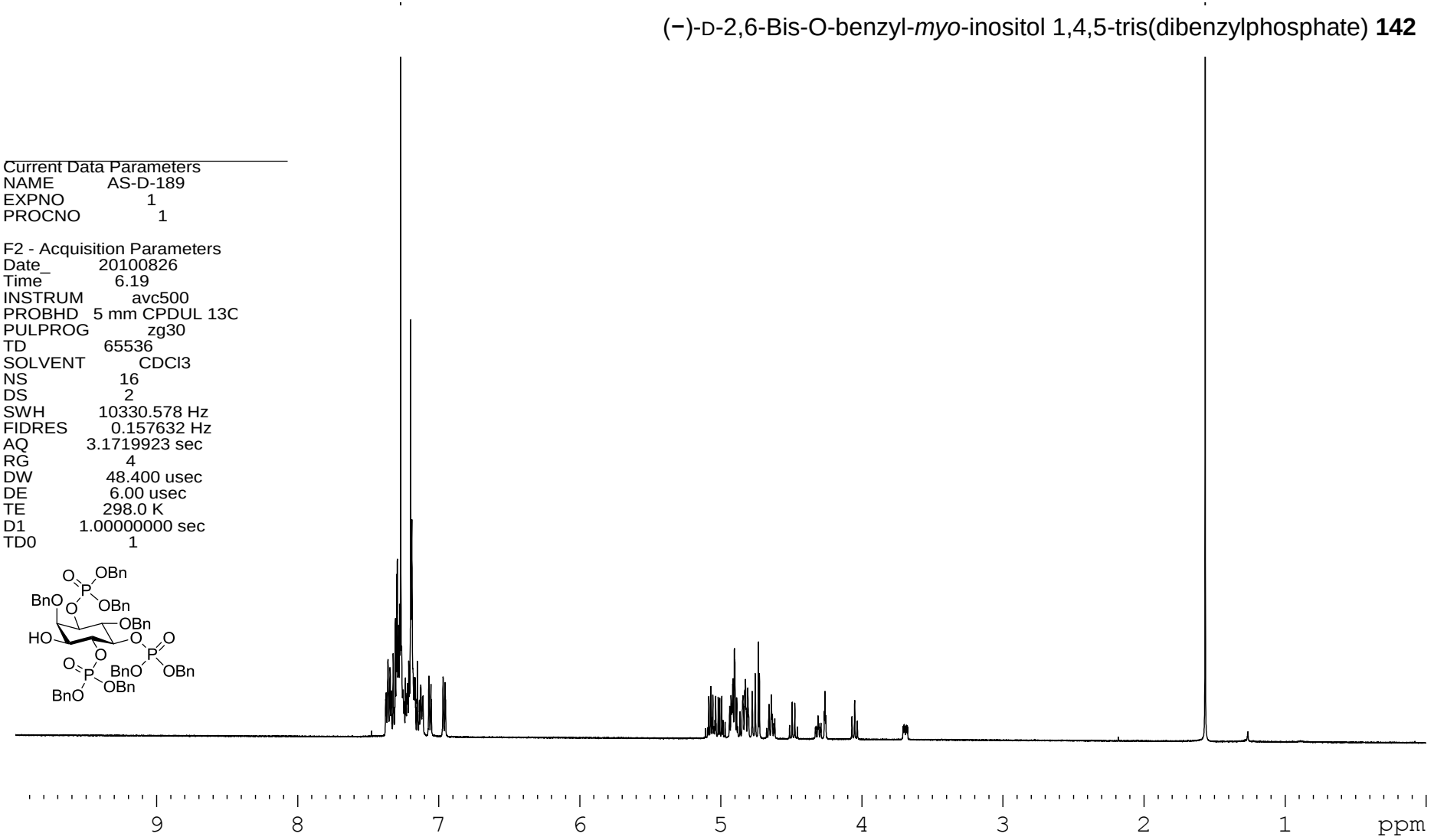
8. Appendix – Selected NMR Spectra

(−)-D-2,6-Bis-O-benzyl-*myo*-inositol 1,4,5-tris(dibenzylphosphate) **142**



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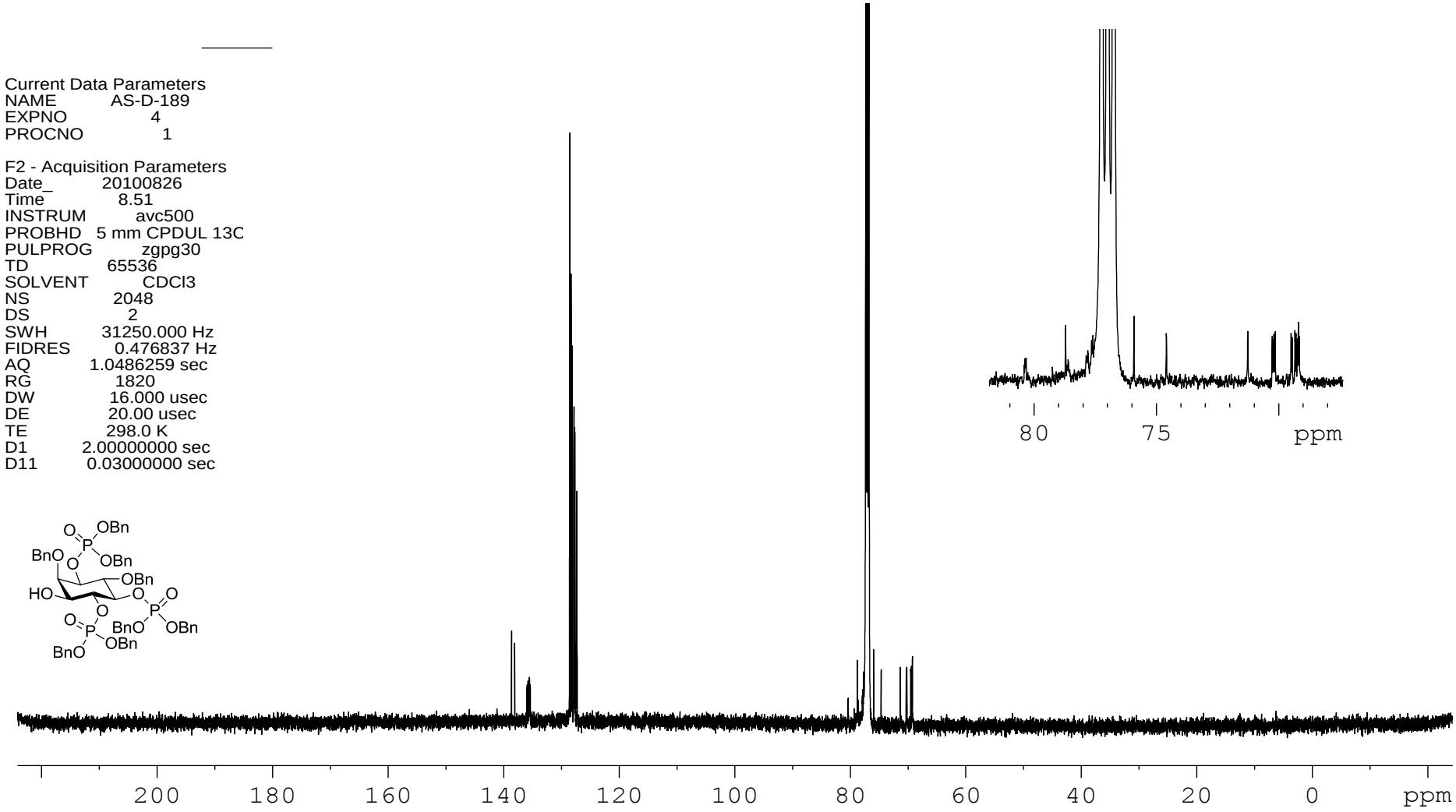
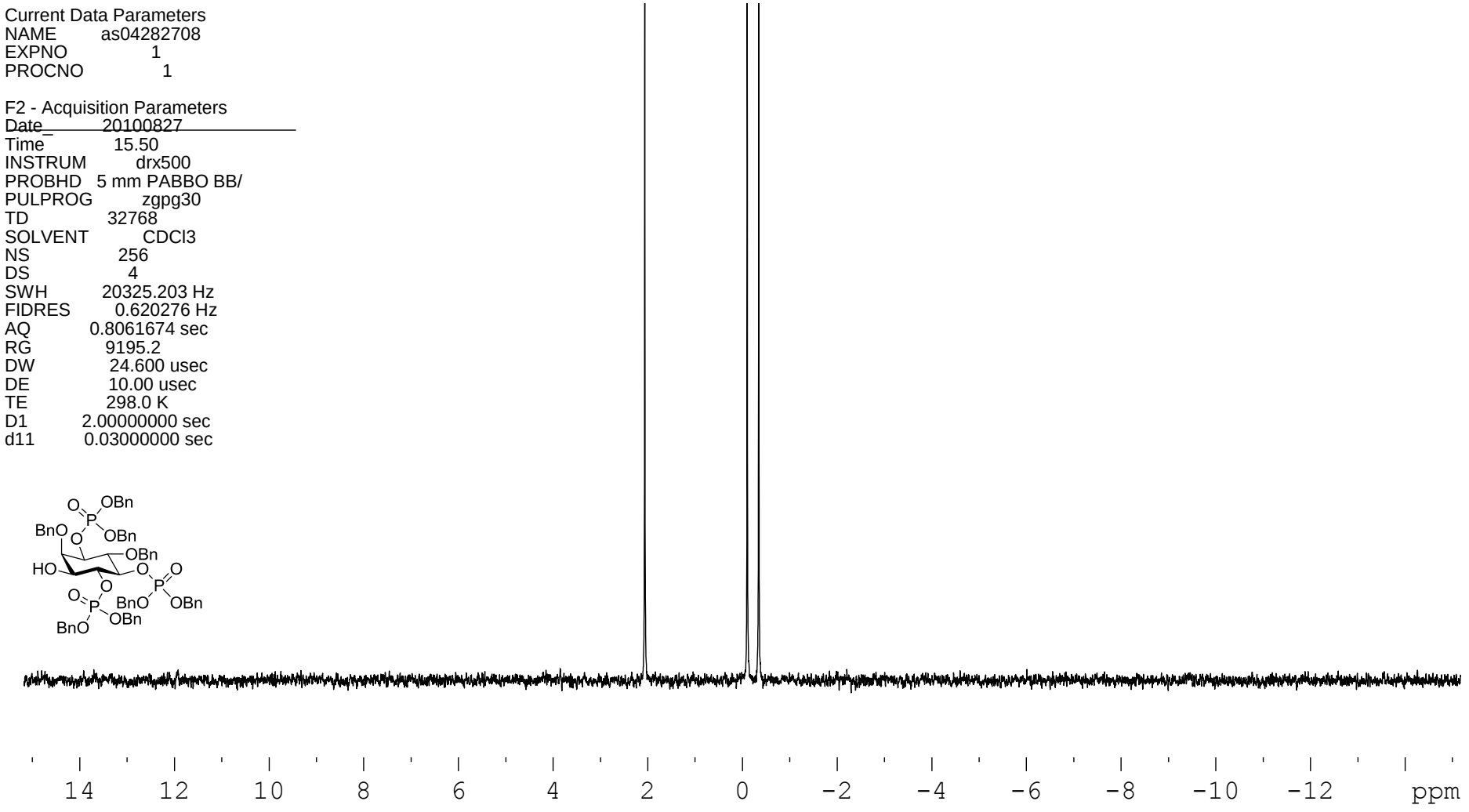
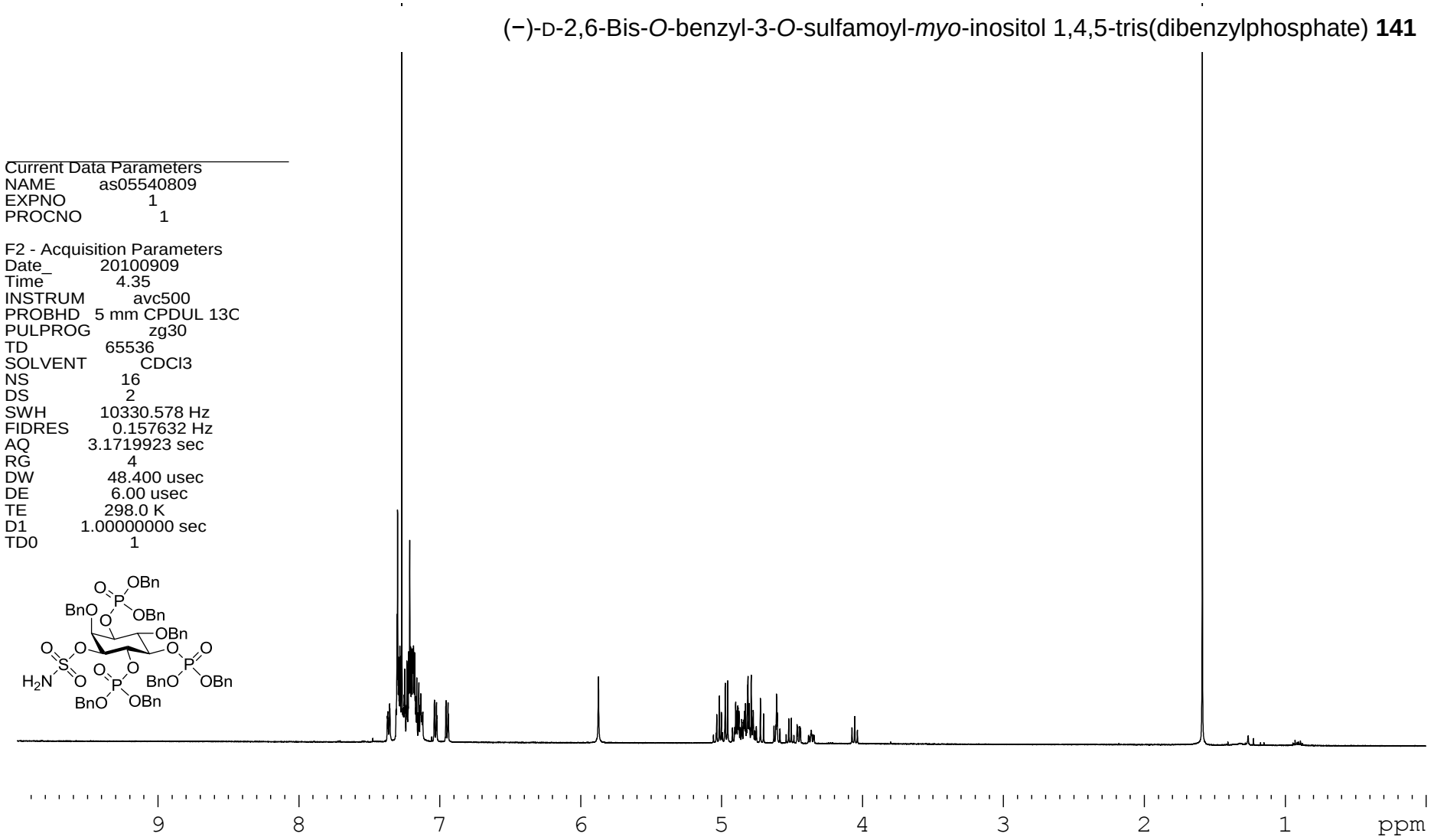


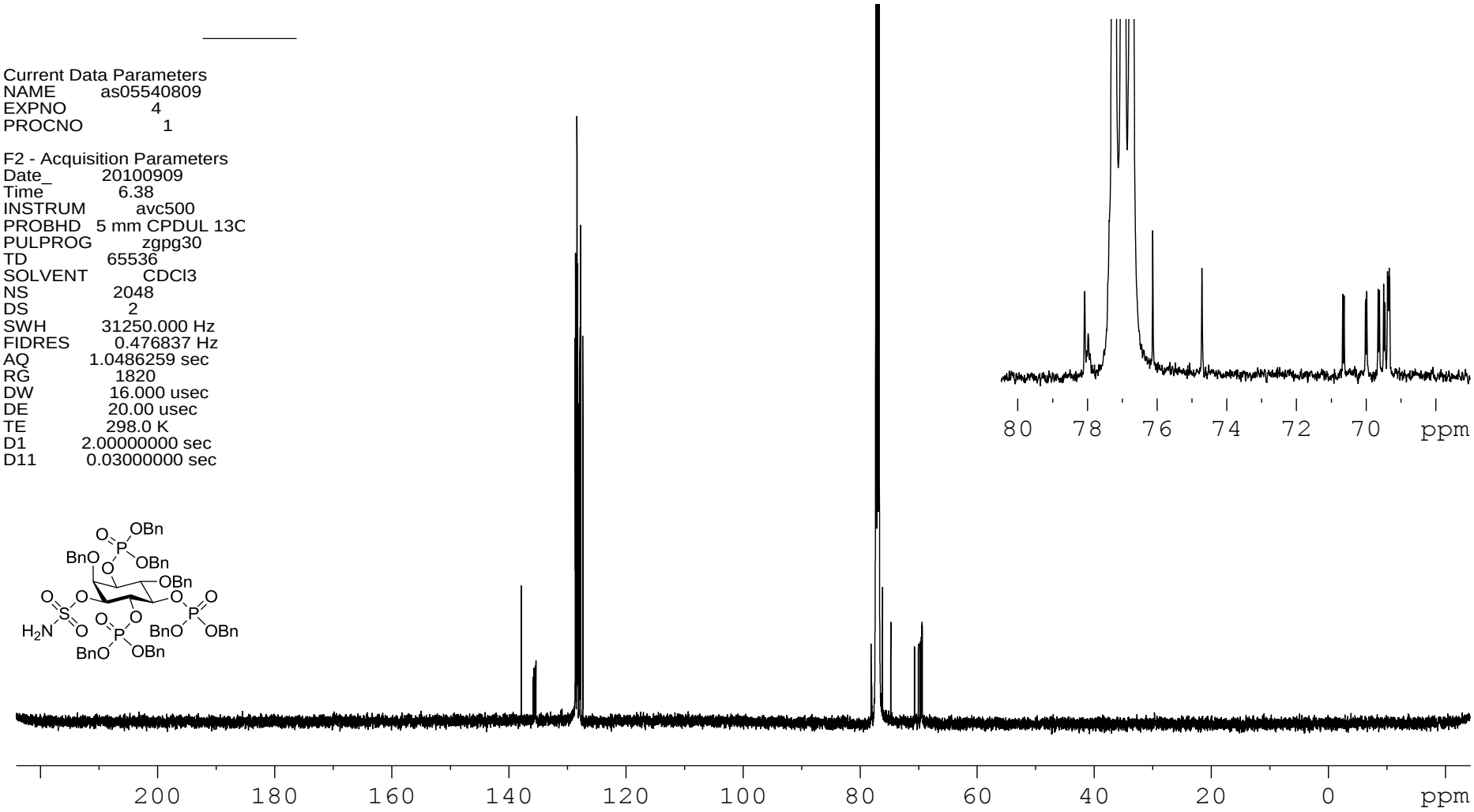
(−)-D-2,6-Bis-O-benzyl-*myo*-inositol 1,4,5-tris(dibenzylphosphate) **142**



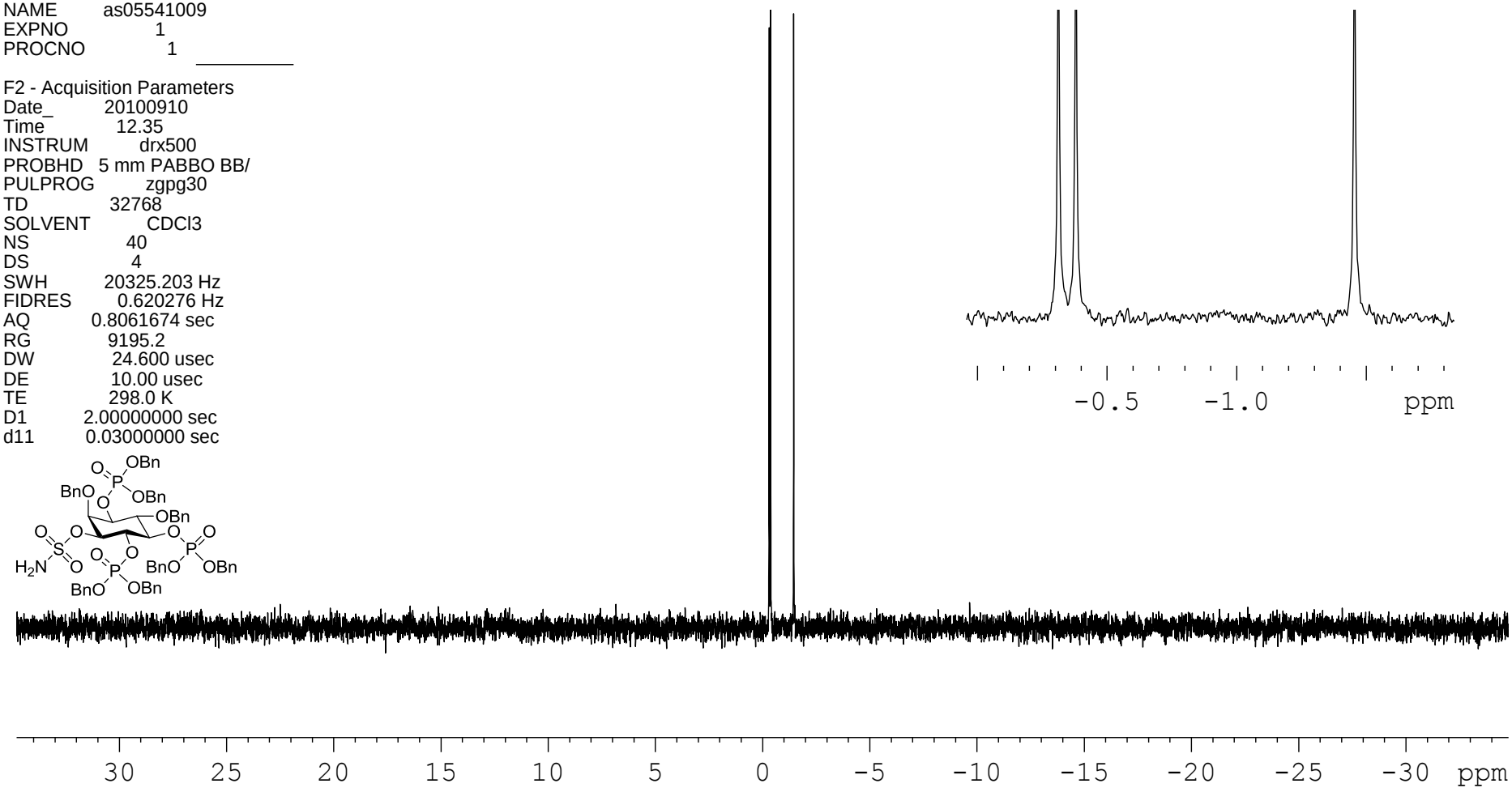
(−)-D-2,6-Bis-*O*-benzyl-3-*O*-sulfamoyl-*myo*-inositol 1,4,5-tris(dibenzylphosphate) **141**



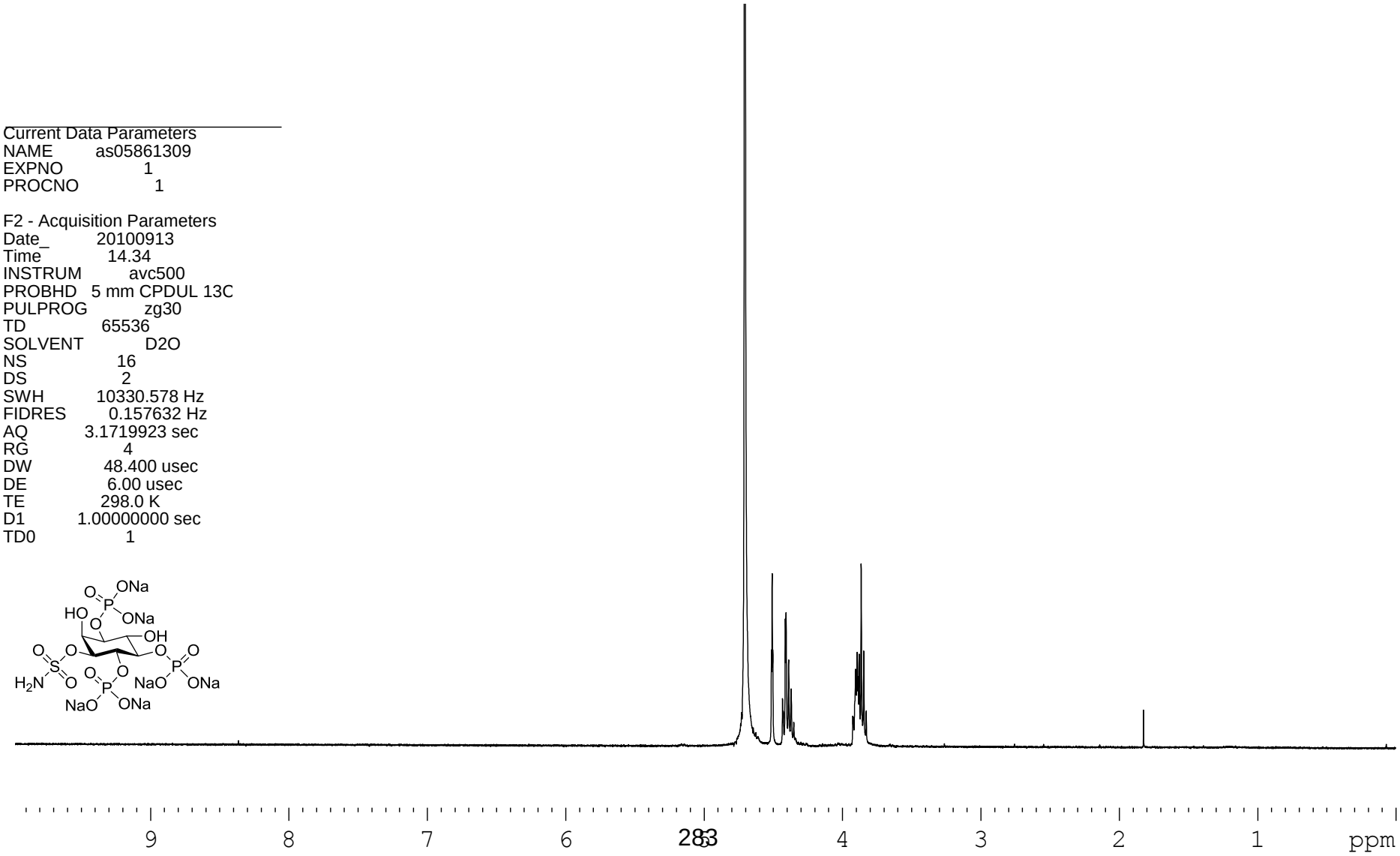
(−)-D-2,6-Bis-*O*-benzyl-3-*O*-sulfamoyl-*myo*-inositol 1,4,5-tris(dibenzylphosphate) **141**



(−)-D-2,6-Bis-*O*-benzyl-3-*O*-sulfamoyl-*myo*-inositol 1,4,5-tris(dibenzylphosphate) **141**



(−)-D-3-*O*-Sulfamoyl-*myo*-inositol 1,4,5-tris(phosphate) **139**



(−)-D-3-*O*-Sulfamoyl-*myo*-inositol 1,4,5-tris(phosphate) **139**

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EXPNO 2
PROCNO 1

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Date_ 20120824
Time 10.07
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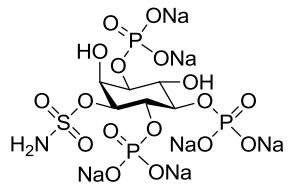
PROBHD 5 mm CPDUL 13C
PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 3072
DS 2
SWH 31250.000 Hz
FIDRES 0.476837 Hz
AQ 1.0486259 sec
RG 1820
DW 16.000 usec
DE 20.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec



(−)-D-3-*O*-Sulfamoyl-*myo*-inositol 1,4,5-tris(phosphate) **139**

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EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
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Time 14.11
INSTRUM drx500
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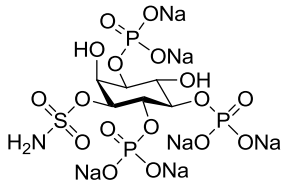
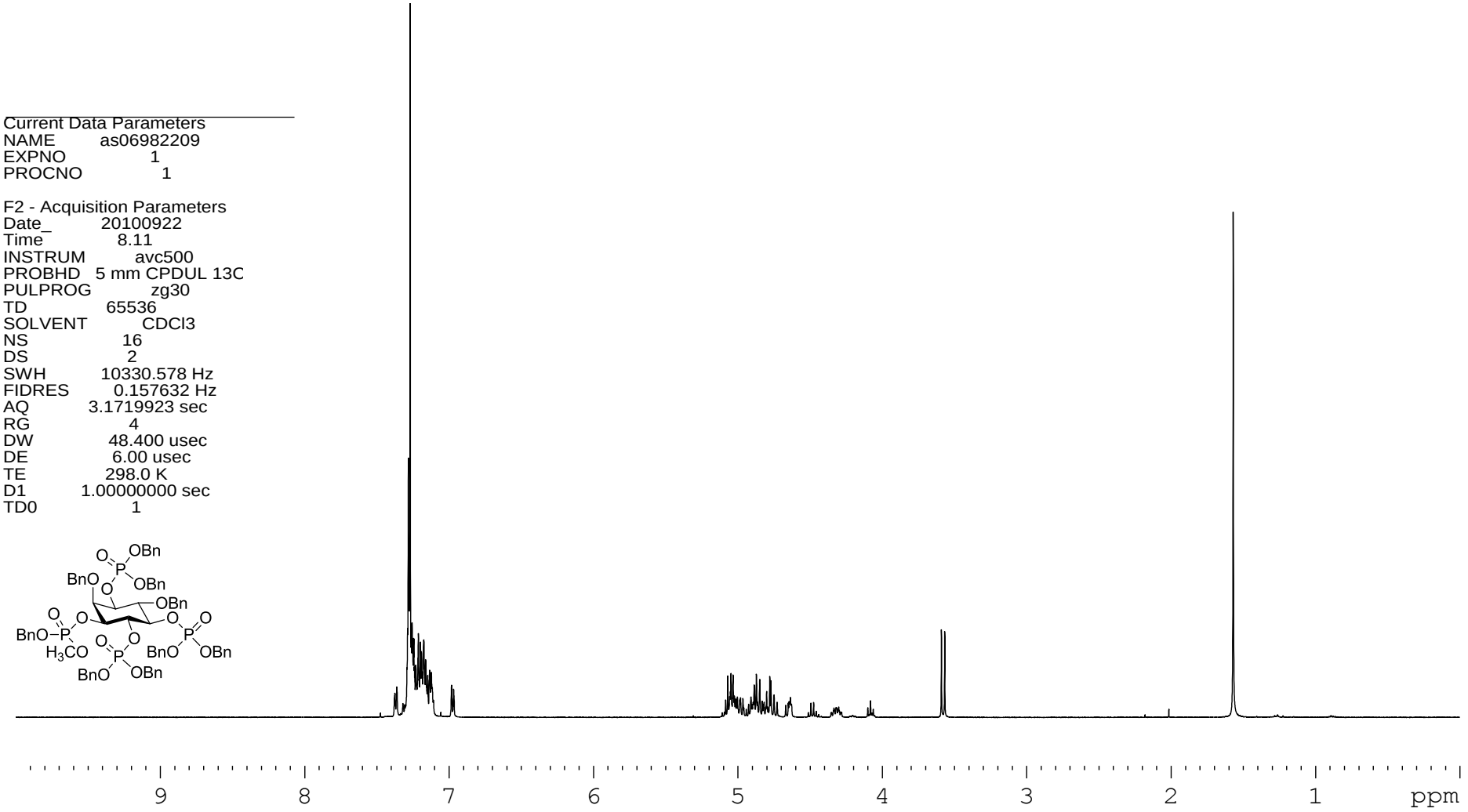
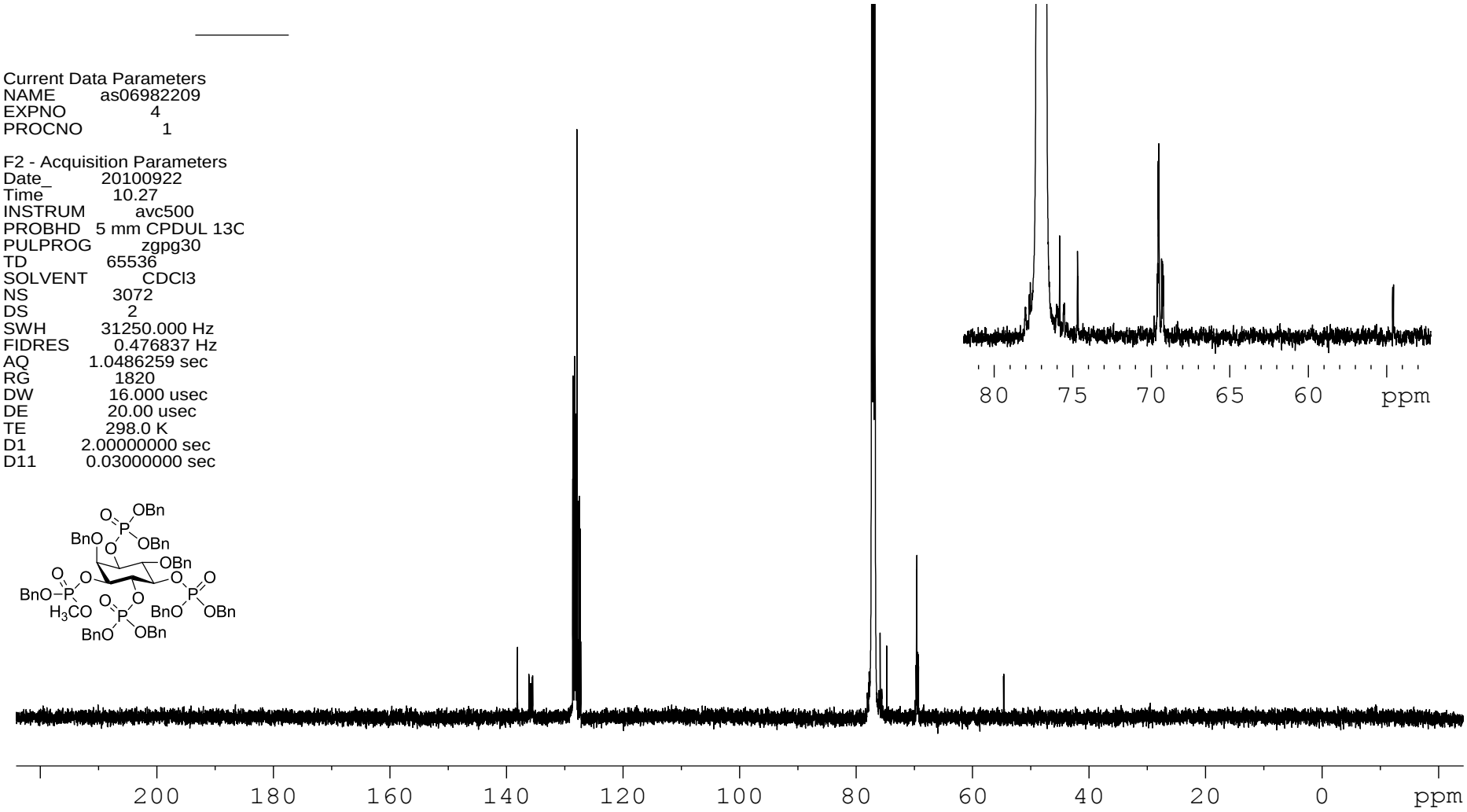
PULPROG zgpg30
TD 32768
SOLVENT D2O
NS 169
DS 4
SWH 10162.602 Hz
FIDRES 0.310138 Hz
AQ 1.6122848 sec
RG 14596.5
DW 49.200 usec
DE 10.00 usec
TE 298.1 K
D1 2.00000000 sec
d11 0.03000000 sec



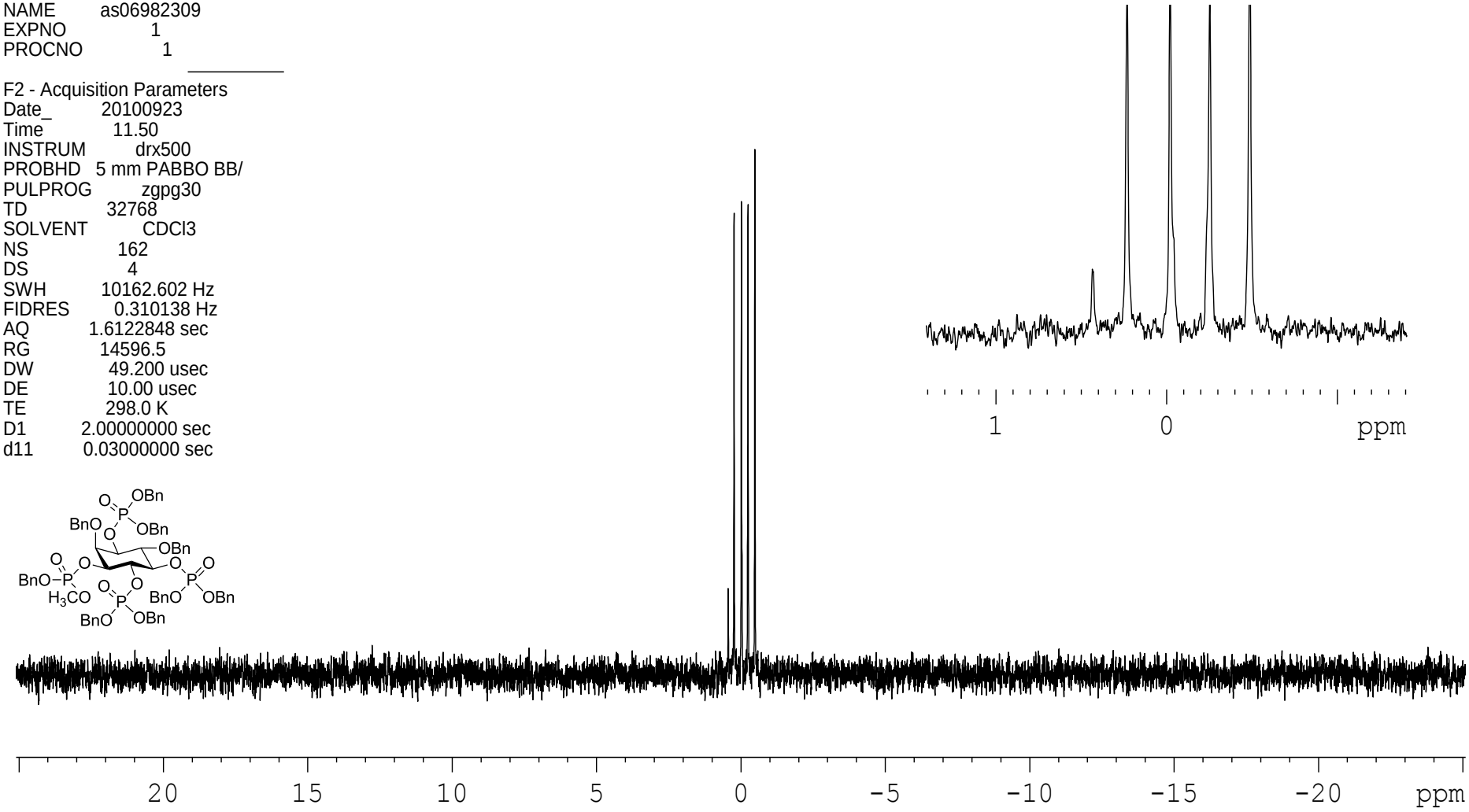
(−)-D-2,6-Bis-*O*-benzyl-*myo*-inositol 3-(*O*-benzyl-*O*-methyl)phosphate-1,4,5-tris(dibenzylphosphate) **140**



(−)-D-2,6-Bis-*O*-benzyl-*myo*-inositol 3-(*O*-benzyl-*O*-methyl)phosphate-1,4,5-tris(dibenzylphosphate) **140**



(−)-D-2,6-Bis-*O*-benzyl-*myo*-inositol 3-(*O*-benzyl-*O*-methyl)phosphate-1,4,5-tris(dibenzylphosphate) **140**



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PROCNO 1

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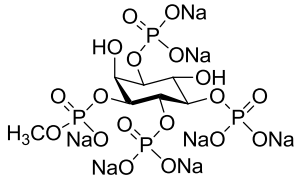
PULPROG zg0f2pr
TD 65536
SOLVENT D2O
NS 32
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1720407 sec
RG 406.4
DW 48.400 usec
DE 11.00 usec
TE 572.7 K
D1 2.00000000 sec
d11 0.03000000 sec

(−)-D-*myo*-Inositol-1,4,5-trisphosphate-3-*O*-methylphosphate ester **138**



(−)-D-*myo*-Inositol-1,4,5-trisphosphate-3-*O*-methylphosphate ester **138**

Current Data Parameters
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EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
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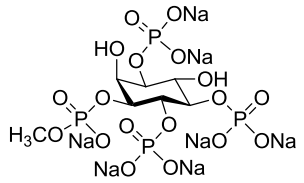
Time 12.35
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 3072
DS 2
SWH 31250.000 Hz
FIDRES 0.476837 Hz
AQ 1.0486259 sec
RG 1820
DW 16.000 usec
DE 20.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec



(−)-D-*myo*-Inositol-1,4,5-trisphosphate-3-*O*-methylphosphate ester **138**

Current Data Parameters
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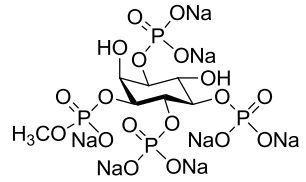
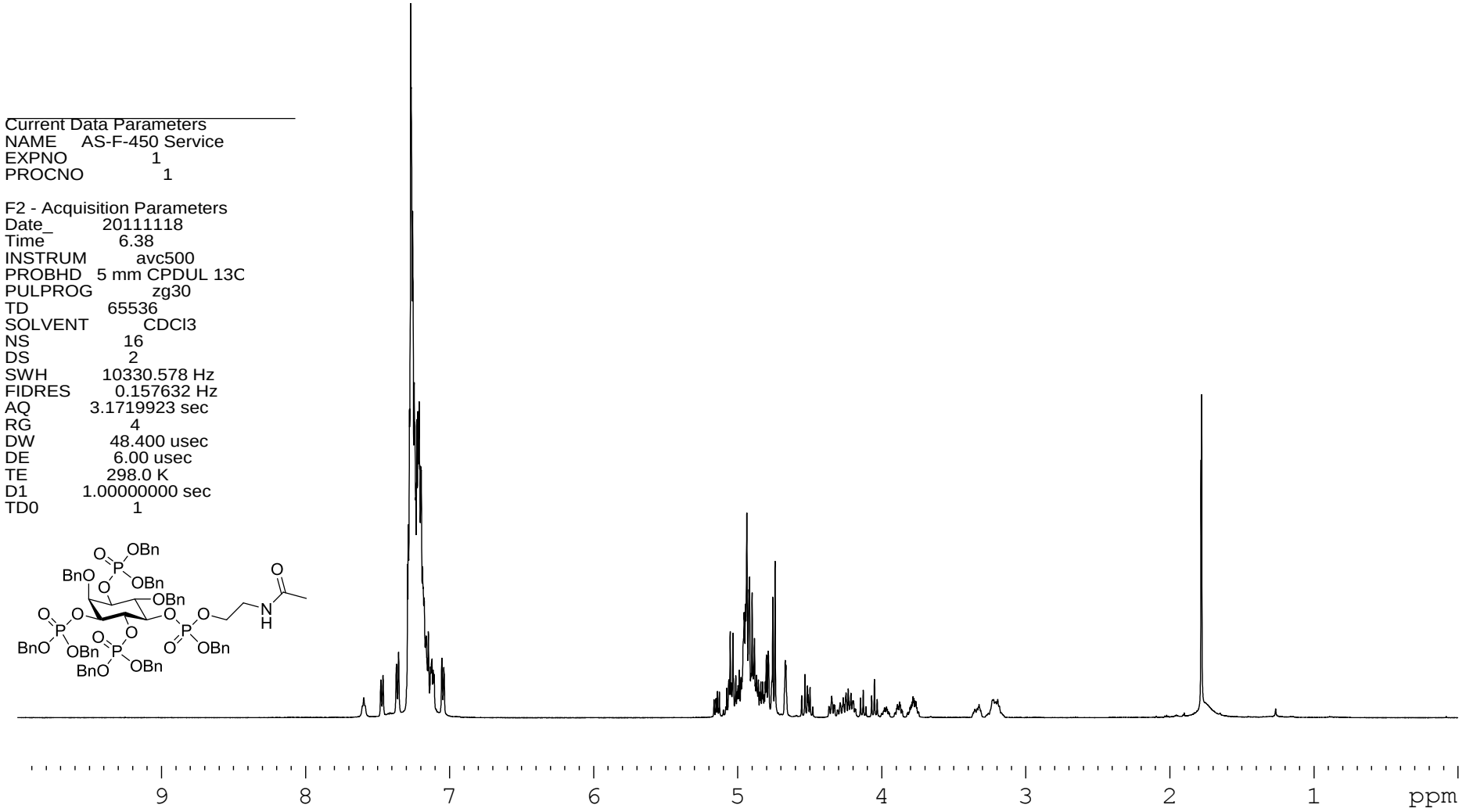
EXPNO 2
PROCNO 1

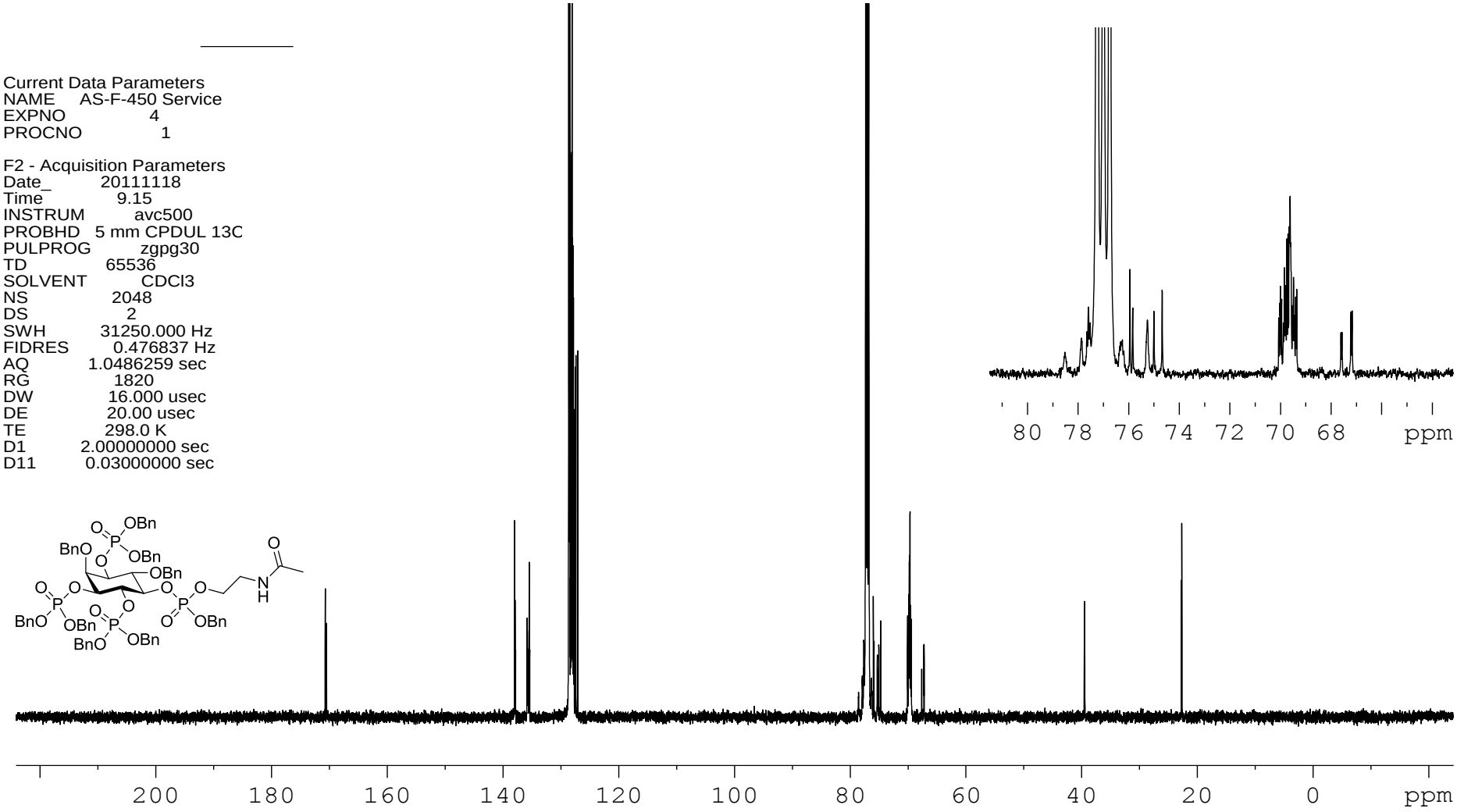
F2 - Acquisition Parameters
Date_ 20120821
Time 20.47
INSTRUM drx500
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 32768
SOLVENT D2O
NS 33
DS 4
SWH 20325.203 Hz
FIDRES 0.620276 Hz
AQ 0.8061674 sec
RG 10321.3
DW 24.600 usec
DE 10.00 usec
TE 298.0 K
D1 2.00000000 sec
d11 0.03000000 sec



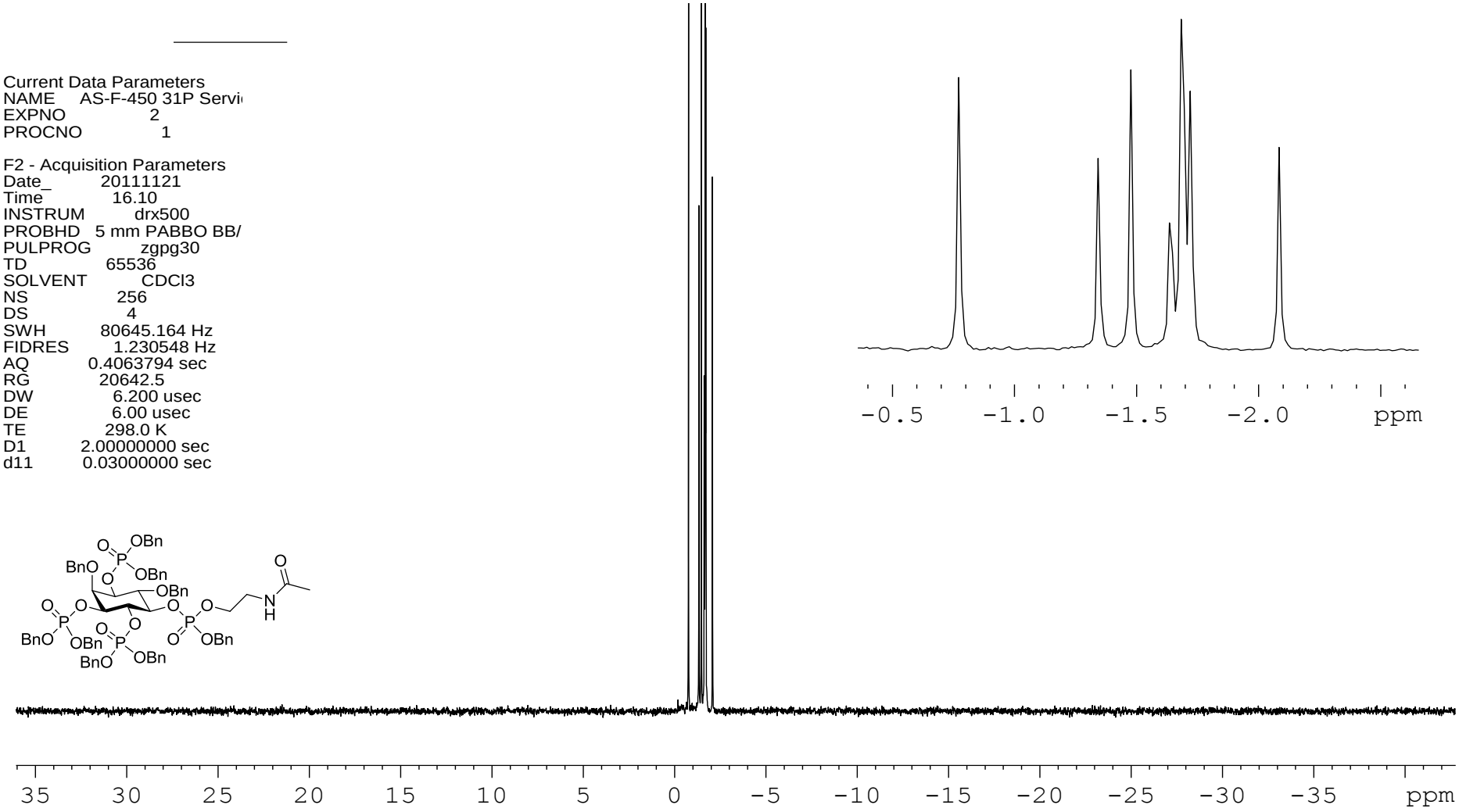
(−)-D-2,6-Bis-*O*-benzyl-*myo*-inositol 5-(*O*-benzyl-*O*-2'-acetamidoethyl) phosphate-1,3,4-tris(dibenzylphosphate) **181**



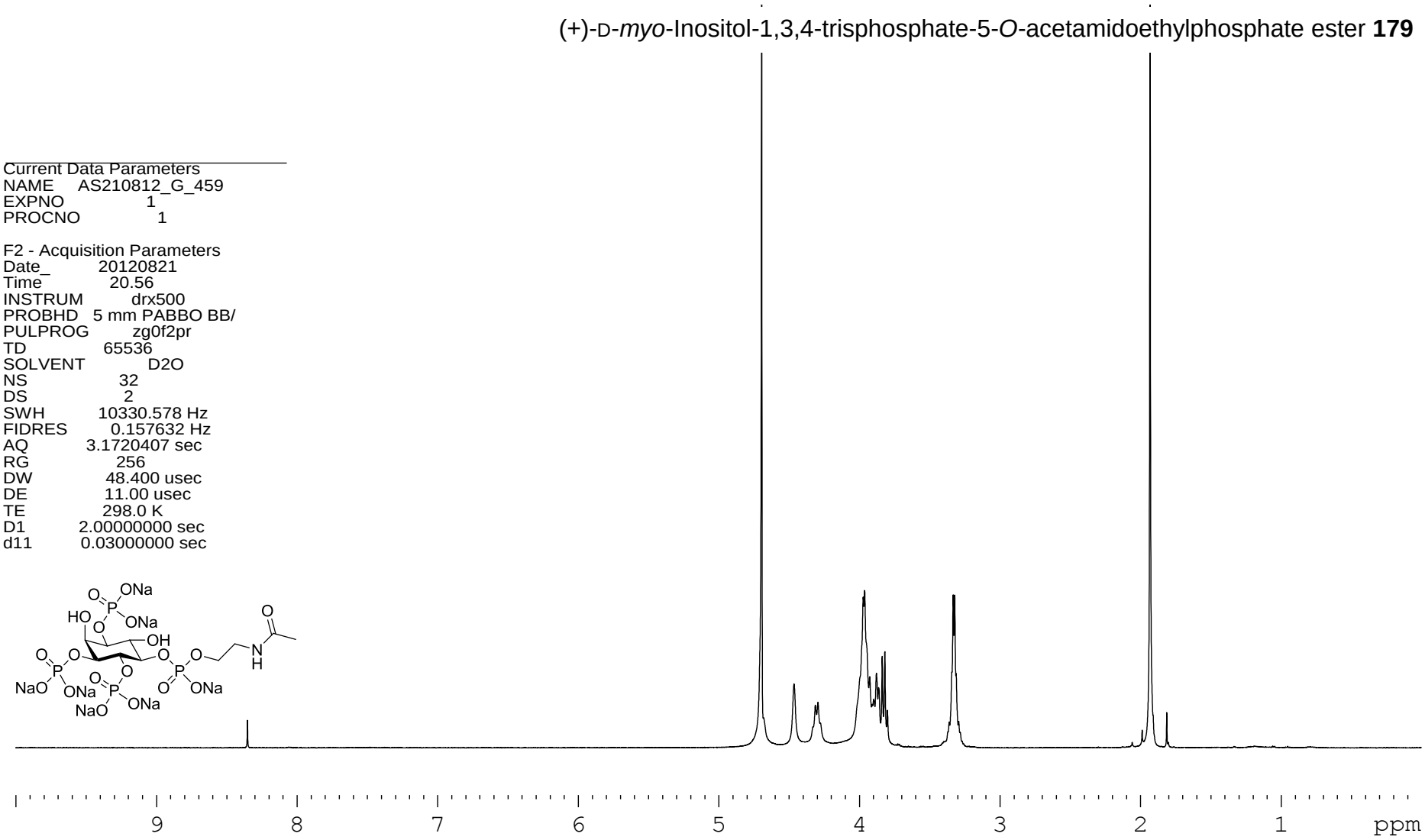
(−)-D-2,6-Bis-*O*-benzyl-*myo*-inositol 5-(*O*-benzyl-*O*-2'-acetamidoethyl) phosphate-1,3,4-tris(dibenzylphosphate) **181**



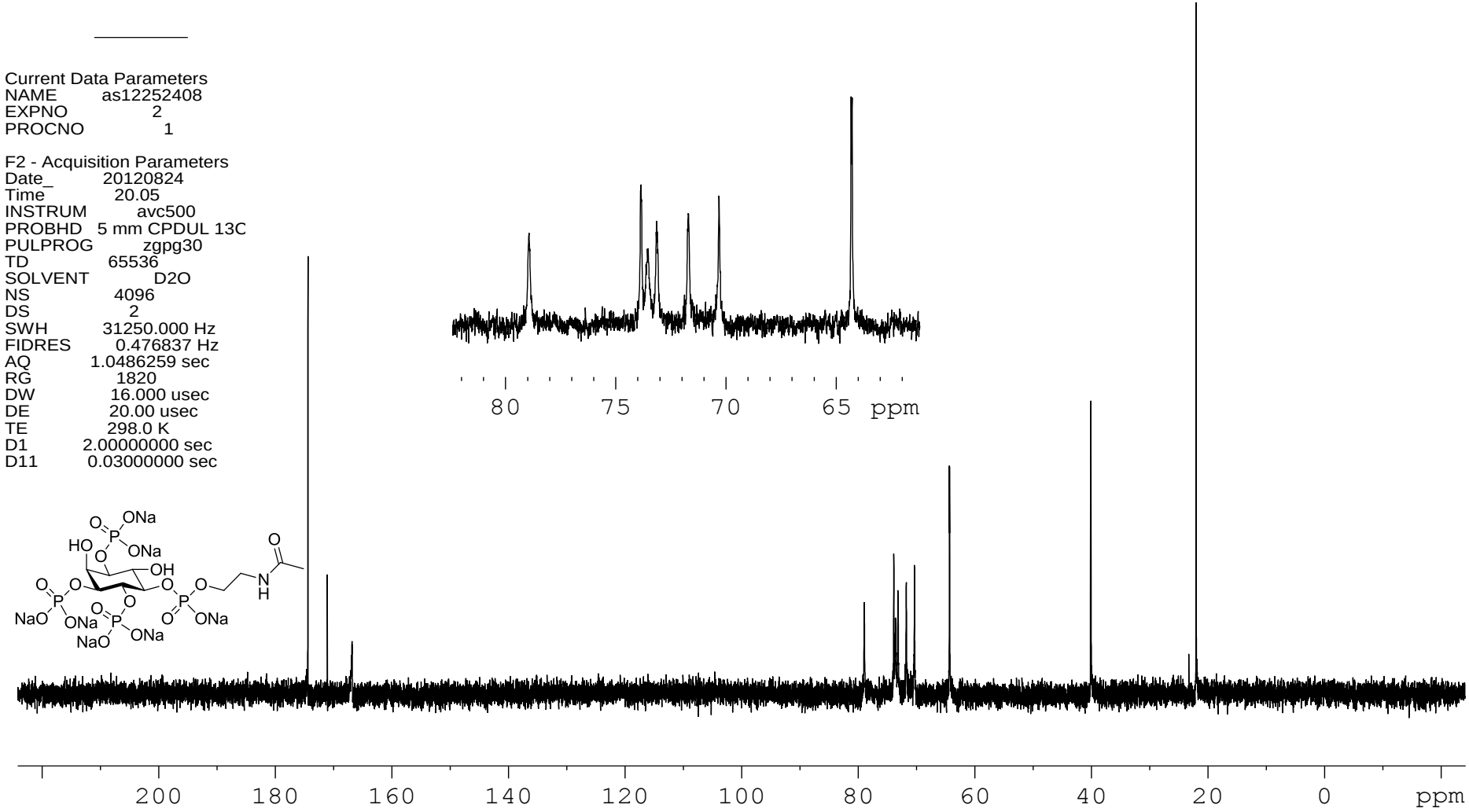
(−)-D-2,6-Bis-*O*-benzyl-*myo*-inositol 5-(*O*-benzyl-*O*-2'-acetamidoethyl) phosphate-1,3,4-tris(dibenzylphosphate) **181**



(+)-D-*myo*-Inositol-1,3,4-trisphosphate-5-*O*-acetamidoethylphosphate ester **179**



(+)-D-*myo*-Inositol-1,3,4-trisphosphate-5-*O*-acetamidoethylphosphate ester **179**



(+)-D-*myo*-Inositol-1,3,4-trisphosphate-5-*O*-acetamidoethylphosphate ester **179**

Current Data Parameters
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PROCNO 1

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Time 21.00
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PULPROG zgpg30
TD 32768
SOLVENT D2O
NS 64
DS 4
SWH 20325.203 Hz
FIDRES 0.620276 Hz
AQ 0.8061674 sec
RG 14596.5
DW 24.600 usec
DE 10.00 usec
TE 298.0 K
D1 2.00000000 sec
d11 0.03000000 sec