Univerzita Karlova
Přírodovědecká fakulta

Studijní program: Medicinální chemie
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Příprava derivátů *myo*-inositolu
Preparation of *myo*-inositol derivatives

Bakalářská práce

Vedoucí závěrečné práce/Školitel: Mgr. Martin Kamlar, Ph.D.

Praha, 2022

Prohlášení:

Prehlasujem, že som záverečnú prácu spracoval samostatne a že som uviedol všetky použité informačné zdroje a literatúru. Táto práca ani jej podstatná časť nebola predložena k získaniu iného alebo rovnakého akademického titulu.

V Praze, 19.5.2022

Podpis

Abstrakt

Fosfatidylinositoly sú látky vyskutujúce sa v prírode so širokou bioaktivitou potrebnou pre prežitie a život bunky. Mechanizmus pôsobenia a úloha fosphatidylinositol 4,5-bisfosfátu nie je zatiaľ jasná. Táto bakalárska práca sa venuje syntéze derivátu selektívne chráneného *myo*-inositolu, ktorý bude využitelný ako prekurzor k syntéze derivátov fosfatidylinositolu 4,5-bisfosfátu.

Klíčová slova: *Inositol, chránící skupiny, regioizomery*

Abstract

Phosphoinositides are naturally occurring molecules with vast range of biological activity important for cell life and survival. The exact role and mechanism of phosphatidylinositol 4,5-bisphosphate's actions are not yet elucidated. This bachelor's thesis is dedicated to the synthesis of selectively protected *myo*-inositol derivative suitable as precursor for synthesis of phosphatidylinositol 4,5-bisphosphate derivatives.

Key words: *Inositol, protective groups, regioisomers*

Content

List of used abbreviations

10-CSA – 10-camphorsulfonic acid

ADP – adenosine diphosphate

AMPA – α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

ARF – ADP ribosylation factor

All – allyl

Bn – benzyl

BOM – benzyloxymethyl

CAN – ceric ammonium nitrate

CBZ – carboxybenzyl

DAG – 1,2-diacylglycerol

DDQ – 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone

DMSO – dimethyl sulfoxide

DMF – *N,N*-dimethylformamide

GTP – guanosine triphosphate

IP3 – inositol 1,4,5-trisphosphate

NMDA – *N*-methyl-D-aspartate

NMP – *N*-Methyl-2-pyrrolidone

PI(3,4)P_2 – phosphatidylinositol 3,4-bisphosphate

PI(3,4,5)P_3 – phosphatidylinositol 3,4,5-trisphosphate

PI(3,5)P_2 – phosphatidylinositol 3,5-bisphosphate

PI(4,5)P_2 – phosphatidylinositol 4,5-bisphosphate

PI3K – phosphoinositide 3-kinase

PI4K – phosphatidylinositol 4-kinase

PI3P – phosphatidylinositol 3-phosphate

PI4P – phosphatidylinositol 4-phosphate

PI5P – phosphatidylinositol 5-phosphate

PIP5K – phosphatidylinositol-4-phosphate 5-kinase

PLC – protein lipase C

PMB – *para*-methoxybenzyl

pMeOCl – *p*-methoxybenzyl chloride

pTSA – *p*-toluenesulfonic acid

Py – Pyridine

TBAF – Tetrabutylammonium fluoride

TBAI – Tetrabutylammonium iodide

TEA – Triethylamine

TLC – Thin Layer Chromatography

TMSCl – Trimethylsilyl chloride

TMSOTf – Trimethylsilyl trifluoromethanesulfonate

TrCl – Trityl chloride

Trityl – Triphenylmethyl

1. Introduction

The form of life as we know it is based on four important molecules: proteins, carbohydrates, nucleic acids and lipids. The most abundant monosaccharide unit is undoubtedly D-glucose[1]. Various combinations of monosaccharides forming a wide variety of molecules are necessary for cellular processes and survival. The known variety and the range of biological functions of saccharides expanded after the year 1850 when inositol was first isolated.

Inositol is a cyclohexanehexol sugar with nine possible conformations. However, only *myo*-inositol is prevalent in the life kingdom. The structural heterogenity of *myo*-inositol unit– the possibility of formation of various stereo- and regioisomers is associated with the wide range of complex molecules containing *myo*-inositol. The common forms of *myo*-inositol are inositol phosphates, phosphatidylinositols, phosphoinositides (phosphorylated forms of phosphatidylinositols) and glycosylphosphatidylinositols.[2]

Phosphoinositides serve as important modulators of cellular processes and occur in 7 forms; PIP3 (phosphatidylinositol 3-phosphate), PIP4, PIP5, PI(3,4)P_2 (phosphatidylinositol 3,4-bisphosphate), PI(3,5)P_2, PI(4,5)P_2 and PI(3,4,5)P_3 (phosphatidylinositol 3,4,5-trisphosphate). They take part in vast amount of processes, such as protein recruitation, plasma membrane signaling and membrane transport[3]. Interestingly, even process such as long-term memory potentiation rely on phosphoinositides[4]. Their metabolism and biosynthesis are controlled by enzymes that are evolutionary conserved. Defect in such processes is implicated in various diseases such as Alzheimer's disease[3].

Phosphatidylinositol 4,5-bisphosphate (PI(4,5)P_2) is a minor phosphoinositide found at cell's membranes. Despite its low abundance it is rich in participation in essential processes such as vesicular transport,[3] cytoskeleton dynamics (polymerization and branching of F-actin)[5] and transporter activity.[3] Its role in cell nucleus was recently found but its function and mechanism is not yet elucidated.[6] Synthesis of labeled PI(4,5)P_2 derivative would provide essential tool for elucidation of its actions on cell nucleus.

2. Theoretical introduction

2.1 Introduction to inositols

2.1.1 Stereochemistry

Inositol is a six-carbon carbocyclic sugar, chemically cyclohexane-1,2,3,4,5,6-hexol. The secondary hydroxyl groups are subject to axial/equatorial isomerism owing to the chair conformation of the cyclohexane backbone. The various combinations of the axial/equatorial arrangement of the six secondary hydroxyl groups lead to formation of 64 steoreoisomers, however due to symmetry reasons only nine are real (Figure 1).

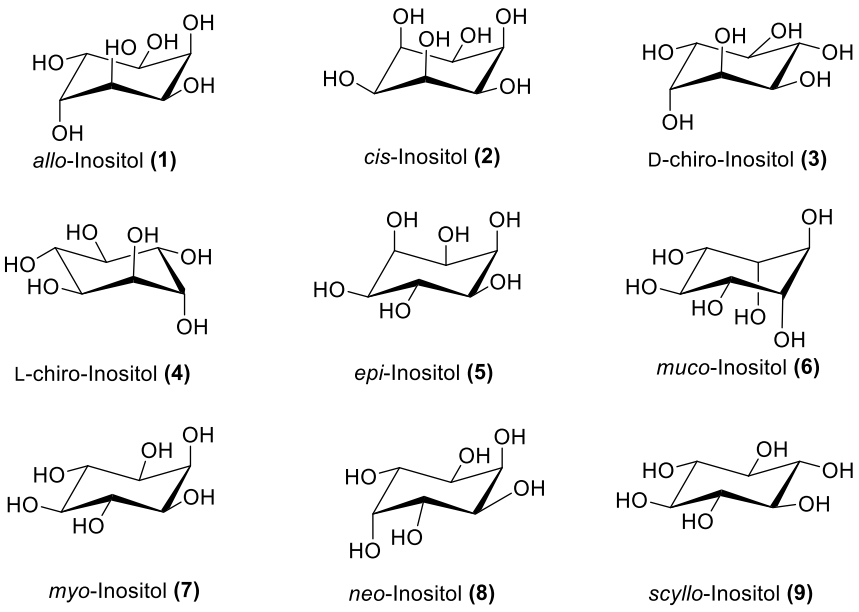
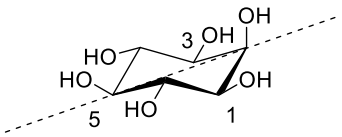


Figure 1 Stereoisomers of inositol

Scyllo-, *myo*-, *epi*-, *neo*-, *cis*- and *muco*- isomers have plane of symmetry, thus are optically inactive *meso* compounds. *Chiro*- inositol is optically active, forming two enantiomers D-*chiro* and L-*chiro* inositol. The *allo*- isomer was found to be subject of conformational interconversion at room temperature, leading to formation of its enantiomer in 50/50 ratio thus forming racemic mixture. Only six isomers were found in nature: *myo*-, *muco*-, *neo*-, D-*chiro*-, L-*chiro*-, and *scyllo*-. Figure 2 illustrates the plane of symmetry of important isomer *myo*-inositol along with carbon atom numbering. The plane of symmetry splits the molecule into two non-superimposable mirror images, meaning *myo*-inositol is

prochiral molecule - any modification that disturbs the symmetry leads to a chiral molecule. For example, substitution at C-6, C-1, C-4 or C-3 leads to a chiral molecule whereas substitution at C-5 and C-2 leaves the plane of symmetry intact, thus the molecule will stay achiral. The hydroxyl pair at C-6, C-1 and C-4, C-3 are enantiotopic, meaning that substitution of one hydroxyl, for example hydroxyl at C-6, leads to enantiomer of compound that would result from substitution of hydroxyl at C-1. This enantiotopic sites are chemically equivalent and are readily distinguished by enzymatic processes.[2]

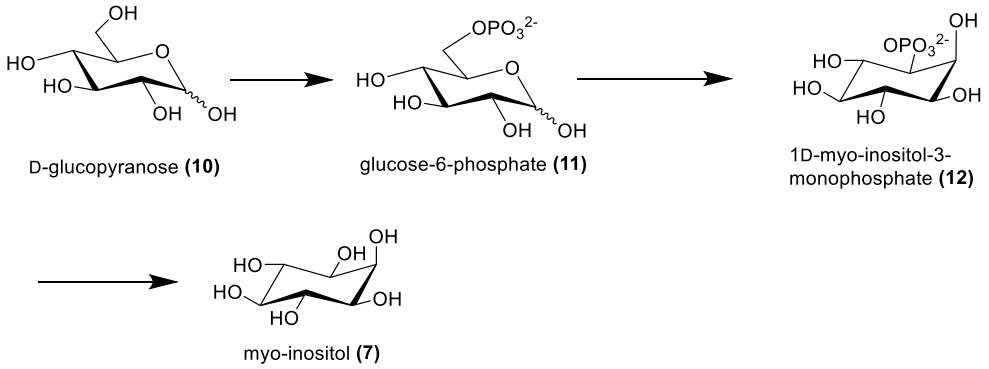


(7)

Figure 2 Plane of symmetry of *myo*-inositol

2.1.2 *Myo*-inositol

The myo-inositol is the most abundant isomer in the nature ranging from plants to yeast. It is known that myo-inositol derives directly from D-glucose. The biosynthesis process involves the cyclization of D-glucose-6-phosphate into 1D-myo-inositol-3-monophosphate by the action of inositol-3-phosphate synthase and subsequent release of the free myo-inositol (Scheme 1). Human body produces more than 4 g of inositol daily. Phosphorylation of the inositol unit produces two major families of molecules; inositol phosphates and phosphoinositides(phosphorylated forms of phosphatidylinositol). These molecules are required for various structural and functional processes such as membrane formation, membrane trafficking and signaling.[2] Absence of inositol in the cell leads to cell death. Altered inositol levels are associated with various neurological diseases. Inositol phospholipids in brain are crucial for neurological processes; they produce two second-messengers; IP_3 and DAG on receptor stimulation.[7] Defect of this pathway has been implicated in the pathogenesis of bipolar disorder and Alzheimer's disease. It is not surprising that inositol also plays a key role in osmoregulation. Kidney cells rely on inositol along with sodium to compensate the change in osmolarity caused by the elimination of water.[8] The osmoregulatory effects are also seen in the brain.[7]



Scheme 1 Biosynthesis of myo-inositol

Because of *myo*-inositol having plane of symmetry which splits the molecule into mirror image halves, confusion arises about carbon atom numbering. Carbon-1 could be assigned to either of the two enantiotopic carbons. IUPAC recommends (1976) that if the *myo*-inositol molecule is viewed in the vertical (Fischer-Tollens) projection with carbon-1 at the top and carbon-2 and carbon-3 at the front edge and if the hydroxyl or any substituent at the lowest numbered chiral center projects to the right then the configuration is assigned D. If it projects to the left the configuration is assigned L. However horizontal projection is used mostly. In that case if the substituent on the lowest-numbered chiral center is above the plane of the cycle and the numbering is counterclockwise, the resulting configuration is D. If the numbering is clockwise, the resulting configuration is L (Figure 3). 1-D or 1-L assignation is used to indicate that carbon-1 is the chiral center used to define the configuration.[2]

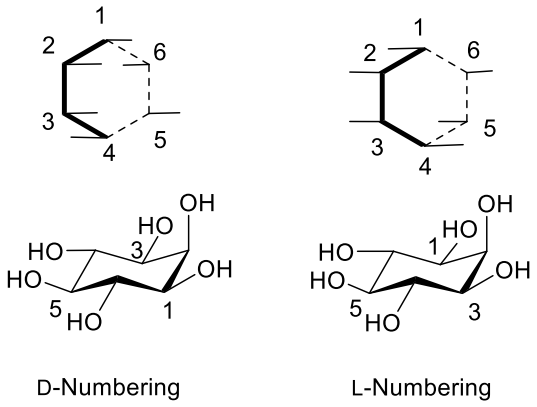
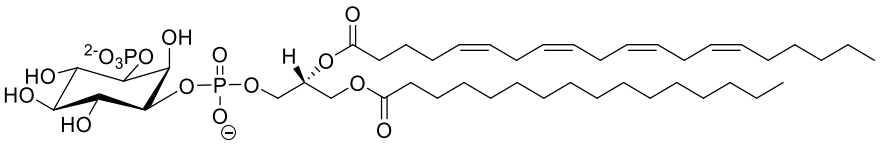


Figure 3 Numbering of *myo*-inositol

2.2 Phosphoinositides

2.2.1 Phosphatidylinositol 3-phosphate

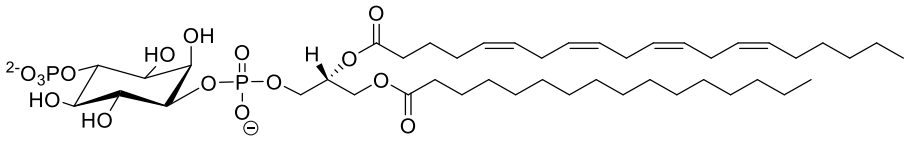


(13)

Figure 4 Structure of phosphatidylinositol 3-phosphate

Phosphatidylinositol 3-phosphate (PI3P) is a lipid which is predominantly found localized at early endosomes and helps to recruit a range of proteins involved in endosomal trafficking. PI3P generation can be also found at the autophagosomal membrane, indicating its further participation in the regulation of autophagy. Furthermore, PI3P is important to neuron function, for example modulation of levels of cell surface receptors for neurotransmitters and control of the autophagy, which further results in neurodegeneration. PI3P is found to be deficient in humans suffering with Alzheimer's disease. In endosomes PI3P is generated by the action of the class III phosphoinositide 3-kinases (PI3Ks). In the plasma membrane class II PI3Ks generate PI3P. Its generation via dephosphorylation of other phosphoinositides is known, mainly form phosphatidylinositol 3,4-bisphosphate and from phosphatidylinositol 3,5-bisphosphate.[3]

2.2.2 Phosphatidylinositol 4-phosphate



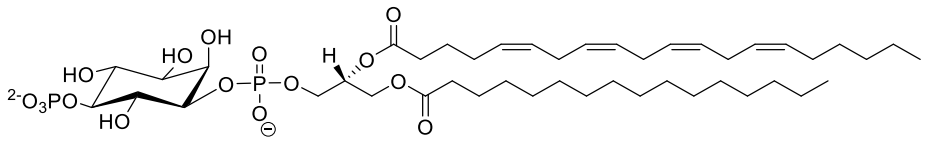
(14)

Figure 5 Structure of phosphatidylinositol 4-phosphate

Phosphatidylinositol 4-phosphate (PI4P) is a lipid which can be found at Golgi complex and in the plasma membrane. Its main regulatory functions are coordination of vesicle trafficking, lipid homeostasis and membrane biogenesis.[3] PI4P in the Golgi apparatus functions as protein binder; it binds effector proteins and GTP-binding protein ARF (ADP ribosylation factor). This complex then recruits proteins that need to be carried to the cell membrane.[9] In the plasma membrane PI4P maintains levels of phosphatidylinositol 4,5-

bisphosphate, thus is required for proper PLC signaling. Its biosynthesis is catalyzed by phosphatidylinositol 4-kinase (PI4K) families of enzymes. Phosphatidylinositol 4-kinase alpha (PI4KA) generates PI4P in the plasma membrane whereas phosphatidylinositol 4-kinase beta (PI4KB) generates PI4P in the Golgi apparatus.[3]

2.2.3 Phosphatidylinositol 5-phosphate

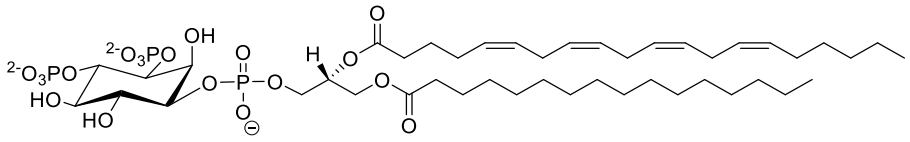


(15)

Figure 6 Structure of phosphatidylinositol 5-phosphate

Phosphatidylinositol 5-phosphate(PI5P) is the most recently discovered phosphoinositide, owing to its difficult detection with common analytical techniques.[3] PI5P was detected in all mammalian cells, notably at the cytoplasmic membrane. Smaller fractions found were in the nucleus.[10] Its suggested role in nucleus is regulation of chromatin function and transcription. PI5P might also take part in regulation of endosomal trafficking owing to its effect of stimulation of myotubularin which was found *in vitro*. However, the mechanism is yet unclear. Another implied role for PI5P is role in brain development via its effects on Notch proteins, which are key regulators of neurogenesis. Its biosynthesis is yet uncertain. Its generation could be done by phosphatase activity of enzymes myotubularin family, producing PI5P from phosphatidylinositol 4,5-bisphosphate.[3]

2.2.4 Phosphatidylinositol 3,4-bisphosphate



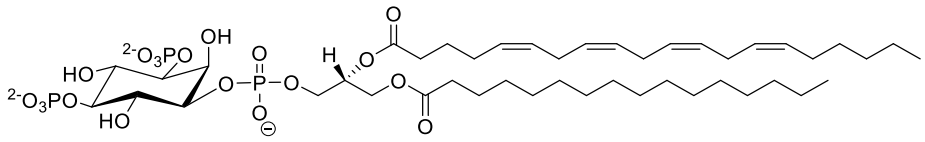
(16)

Figure 7 Structure of Phosphatidylinositol 3,4-bisphosphate

Phosphatidyl inositol 3,4-bisphosphate ($PI(3,4)P_2$) is a lipid that is mainly found in the plasma membrane and in the early endosomes.[3] It has been found that $PI(3,4)P_2$ has role in the formation of adherens junctions, endocytosis and exocytosis. Several proteins of the endocytic

pathways bind PI(3,4)P_2 with low affinity, resulting in recruitment of that proteins to plasma membrane, thus affecting the progression of endocytosis. Furthermore it was shown that PLC-mediated PI(3,4)P hydrolysis is necessary for exocytosis to take place.[11] PI(3,4)P_2 is also contributing to nervous system function with the role in neurite initiation and dendrite morphogenesis. It is biosynthesized by dephosphorylation of the phosphatidyl inositol 3,4,5-trisphosphate by the phosphoinositide 5-phosphatase. In the early endosomal system its generation is proposed to be the result of class II PI3K activity on PI4P.[3]

2.2.5 Phosphatidylinositol 3,5-bisphosphate

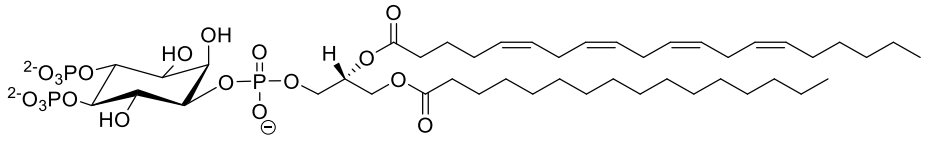


(17)

Figure 8 Structure of phosphatidylinositol 3,5-bisphosphate

Phospthatidylinositol 3,5-bisphosphate (PI(3,5)P_2) is a lipid that is mainly found in late endosomes and lysosomal membranes. In yeast its level increase in response to osmotic stress, whereas in mammalian cells its levels increase by the stimulation with growth factors or phagocytosis. One of its proposed roles in the late endosomes is regulation of the function of two-pore channels on the lysosomal membrane, thus maintaining membrane homeostasis. Its significance is further supported by neuronal excitotoxicity effect caused by its decreased levels, which negatively affect NMDA (*N*-methyl-D-aspartate)-induced voltage channels. Its biosynthesis is catalyzed by the action of PI3P-5 kinase enzyme families.[3]

2.2.6 Phosphatidylinositol 4,5-bisphosphate



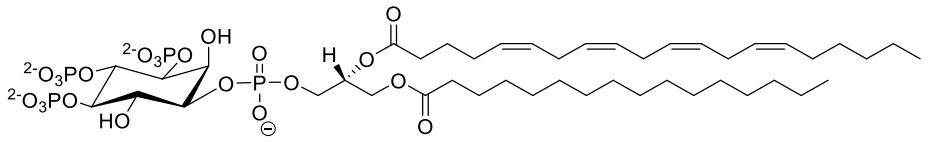
(18)

Figure 9 Structure of phosphatidylinositol 4,5-bisphosphate

Phosphatidylinositol 4,5-bisphosphate (PI(4,5)P_2) is a minor phospholipid found at cell's membranes. It is mainly found in the plasma membrane, where it functions as a

substrate for wide range of signaling proteins. One of its earliest known functions was the role of substrate for phospholipase C which in turn generated inositol 1,4,5 triphosphate (IP_3) and diacylglycerol (DAG), thus giving rise to second messenger pathway. PI(4,5)P_2 has important and well known role in PI(4,5)P_2 signaling cascade cycle, PI3K signaling and PI5P metabolism. It interacts with proteins that regulate intracellular processes such as vesicular transport,[3] cytoskeleton dynamics (polymerization and branching of F-actin)[5] and transporter activity. [3] Its role in cell nucleus was recently found but its function and mechanism is not yet elucidated.[6] PI(4,5)P_2 is generated via the action of phosphatidylinositol 4-phosphate 5-kinase (PIP5K) on PI4P.[3]

2.2.7 Phosphatidylinositol 3,4,5-trisphosphate



(19)

Figure 10 Structure of phosphatidylinositol 3,4,5-trisphosphate

Phosphatidylinositol 3,4,5-trisphosphate (PI(3,4,5)P_3) is a very low abundance phospholipid found at the cellular plasma membrane. Number of proteins bind PI(3,4,5)P_3 via pleckstrin homology domain. It functions as an activator of several intracellular signaling pathways and is able to control cell division and cell size. Moreover it has a crucial role at the synaptic terminal in the hippocampal neurons,[3] where it regulates and strengthens AMPA expression, thus being necessary for long-term potentiation..[4] PI(3,4,5)P3 further interacts with proteins to mediate synaptic plasticity and to maintain long-term potentiation, without such interaction memory consolidation is weakened.[12] Increased levels of PI(3,4,5)P_3 were shown to increase oligodendrocyte differentiation and support myelination. It is generated by the action of class I phosphoinositide 3-kinase enzyme families on PI(4,5)P_2.[3]

2.3 Synthesis of phosphoinositides

2.3.1 Synthesis of PI3P, PI(3,4)P_2 and PI(3,4,5)P_3 from *myo*-inositol

A described route for the synthesis of enantiometrically pure PI3P[13] starts with single enantiomer of biacetal (+)–**(20)** which was prepared by enzymatic method. The method consisted of enantioselective hydrolysis of 4-butyryl ester of (±)–**(20)** by porcine pancreatic lipase in biphasic system, yielding enantiomer (+)–**(20)**. The substrate then undergoes various selective protection and deprotection steps to couple phosphoramidite derivatives with C-1 and C-3 uncovered hydroxyls one at a time. The route for $PI(3,4)P_2$ and $PI(3,4,5)P_3$ synthesis starts with the enantiomer (-)–**(20)**, which is then subjected to similar transformations with common reagents to yield the desired products. For this reason only the synthesis of PI3P will be shown step by step whereas synthesis of $PI(3,4)P_2$ and $PI(3,4,5)P_3$ will be shown briefly.

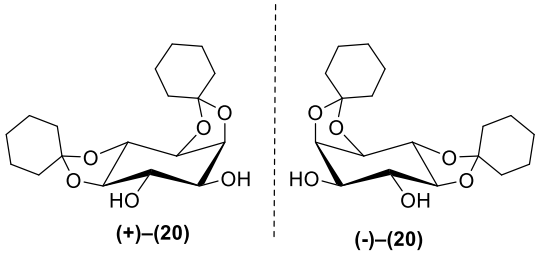
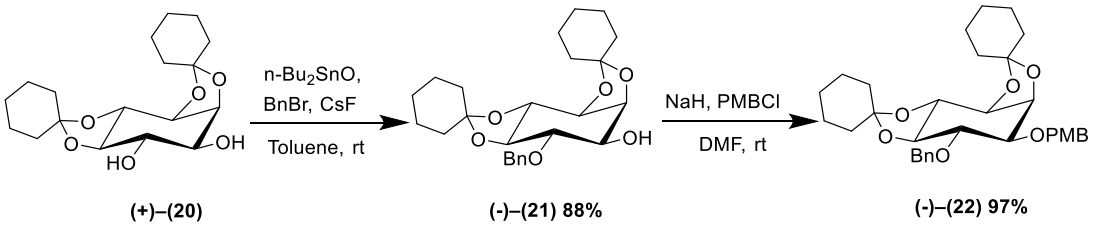


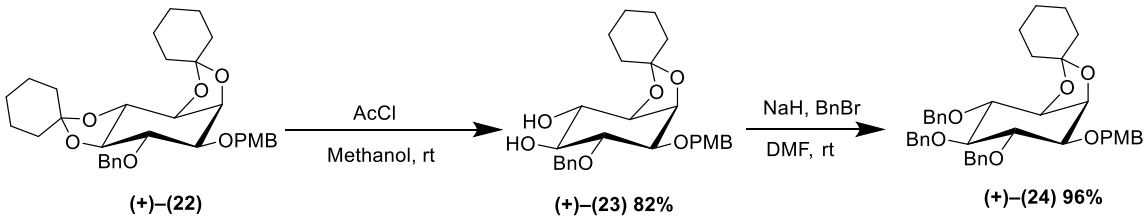
Figure 11 Precursor for syntheses of phosphoinositides

The PI3P synthesis route starts with reaction of (+)–**(20)** with di-*n*-butyltin oxide to form stannylene acetal. The stannylene acetal then regioselectively reacts with benzyl bromide to afford (-)–**(21).** The remaining free hydroxyl is protected with PMB group using PMBCl under basic conditions, affording (-)–**(22)**. See Scheme 2.



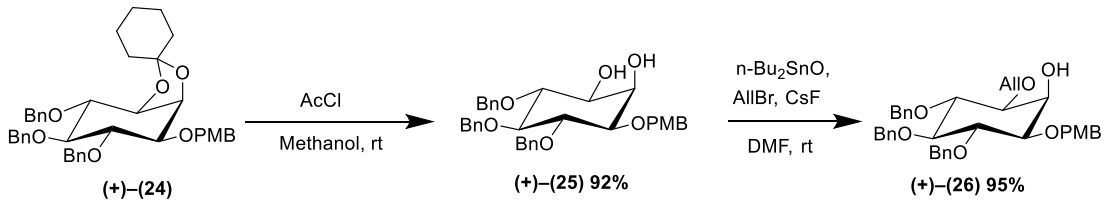
Scheme 2 Benzyl and *para*-methoxybenzyl protection

Then the *trans*-5,6-cyclohexylidene ketal was selectively hydrolyzed by acetyl chloride in methanol. The reaction mixture was carefully monitored using TLC, as the extended exposure resulted in hydrolysis of *cis*-1,2 cyclohexylidene ketal. The free C-5 and C-6 hydroxyls were subsequently protected with benzyl group with standard conditions (Scheme 3).



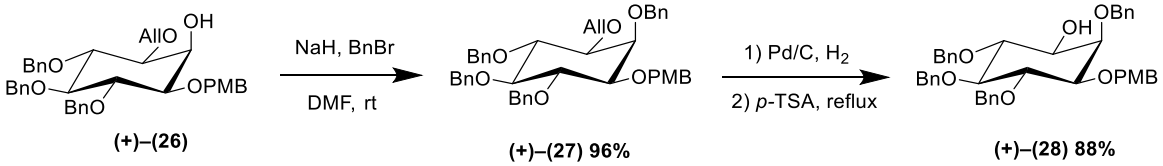
Scheme 3 Selective ketal hydrolysis and bisbenzylation

The *trans*-1,2-cyclohexylidene ketal of intermediate **(24)** was hydrolyzed with acetyl chloride in methanol. Then, highly regioselective allylation was achieved by formation of stannylene acetal with dibutyltin oxide and subsequent allylation with allyl bromide (AllBr), yielding compound **(26)**. The high regioselectivity is explained by the high steric hindrance of the axial C-2 hydroxyl, which the equatorial C-3 hydroxyl lacks (Scheme 4).



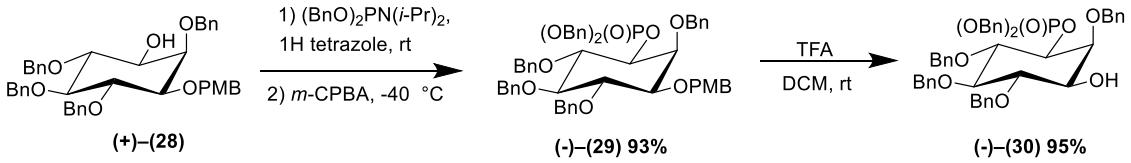
Scheme 4 Ketal hydrolysis and stereoselective allylation

The remaining C-2 hydroxyl is additionally benzylated with standard conditions. The product **(27)** underwent deallylation with 10% Pd/C and subsequent reflux with pTSA yielding free hydroxyl at C-3 **(28)**. (Scheme 5)



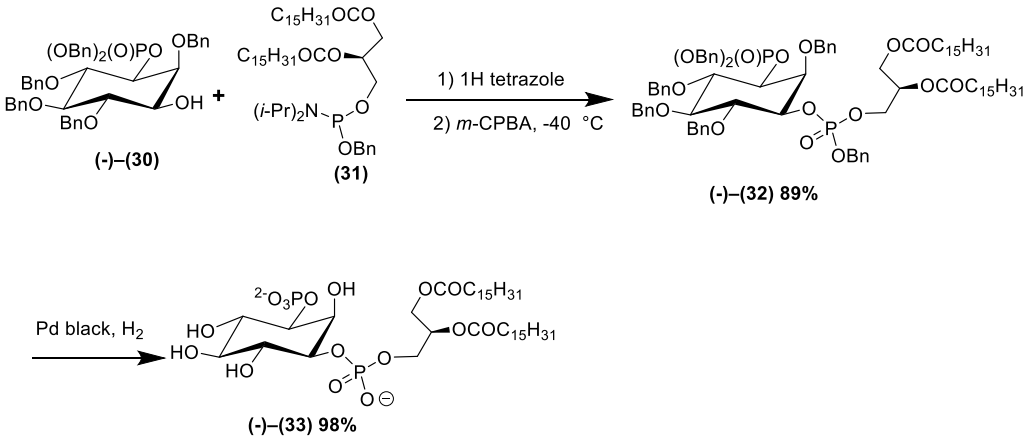
Scheme 5 Benzylation and deallylation

Phosphorylation of **(28)** with dibenzyl *N*,*N*-diisopropylphosphoramidite and subsequent treatment with *m*-CPBA afforded the corresponding 3-(dibenzyl phosphate) **(29),** which underwent PMB deprotection using 10% TFA in CH_2Cl_2 (Scheme 6).



Scheme 6 Phosphate introduction and PMB deprotection

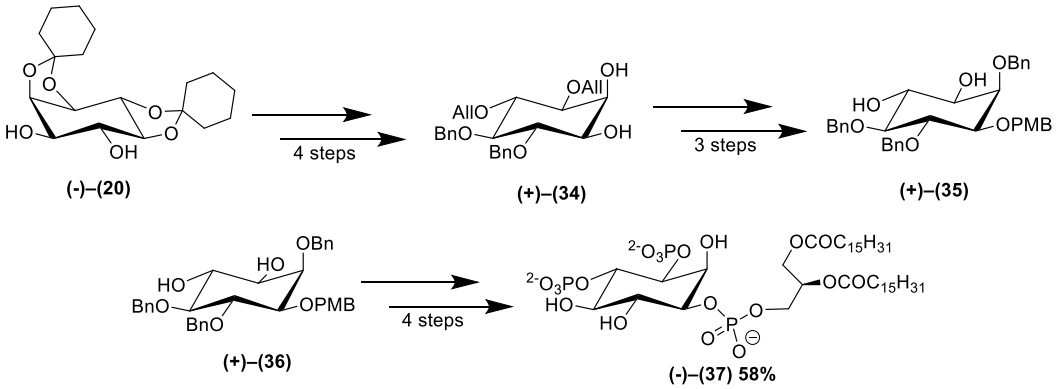
The free C-1 hydroxyl of compound **(30)** was coupled with 1,2-di-*O*-palmitoyl-sn-glycerol 3-(benzyl *N*,*N*-diisopropylphosphoramidite) **(31)** gave the perbenzylated derivative which was in turn debenzylated using Pd black H_2 hydrogenolysis to afford the final PI3P with an overall yield from (+)–**(22)** was 39%. (Scheme 7).



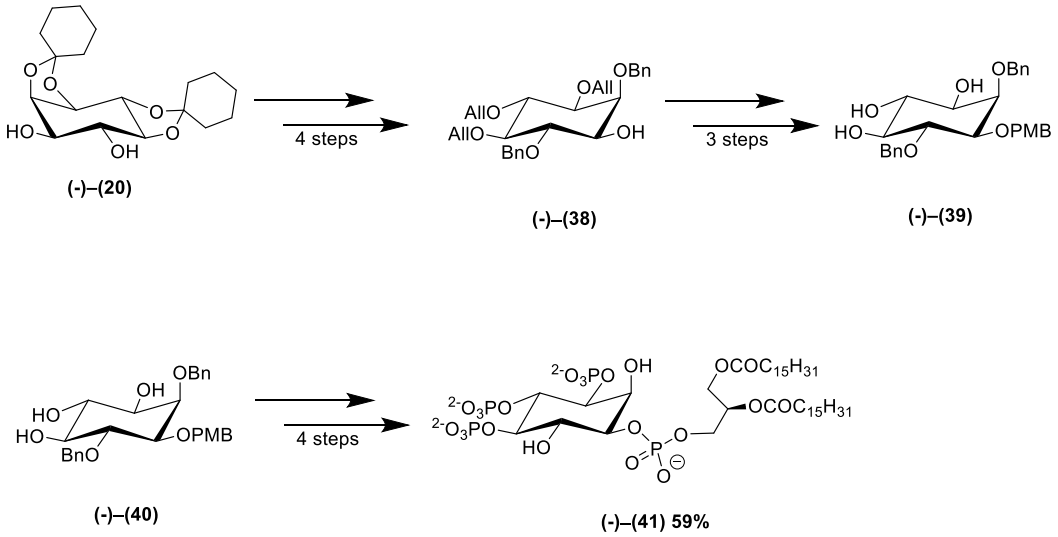
Scheme 7 Coupling with phosphoramidite derivative and hydrogenolysis

The route for synthesis of PI(3,4)P_2 starts with (-)-**(22)**, which is then transformed into compound **(34)** by four steps; allylation, selective *trans* ketal hydrolysis, benzylation and *cis* ketal hydrolysis. The compound **(34)** is then transformed into compound **(35)** by four steps: regioselective PMB protection with stannylene acetal, benzylation and deallylation. The free C-3 and C-4 hydroxyls are then coupled with phosphoramidite derivative to yield 3,4-bis-(dibenzyl phosphate). After that the PMB is cleaved and the resulting free hydroxyl is coupled with **(31)**. The last step is benzyl cleavage by hydrogenolysis. The overall yield from (-)–**(22)** was 58% (Scheme 8).

The route for synthesis of PI(3,4,5)P_3 paralleled the route for synthesis PI(3,4)P_2 (Scheme 9). The overall yield from (-)–**(22)** was 59%.



Scheme 8 PI(3,4)P_2 synthesis intermediates



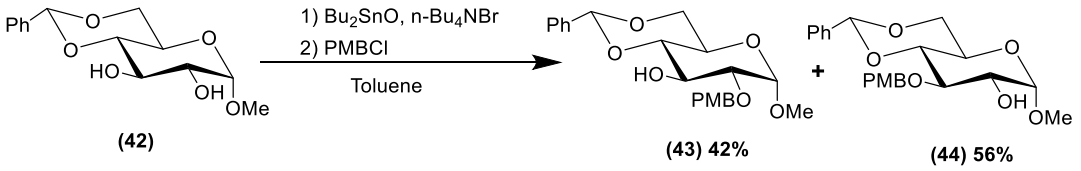
Scheme 9 PI(3,4,5)P_3 synthesis intermediates

2.3.2 Synthesis of PI3P and PI4P from glucose derivative

Another way to prepare the orthogonally protected *myo*-inositol derivative uses derivative of glucose, methyl 4,6-*O*-benzylidene-*α*-D-glucopyranoside **(42)** as a starting material. The method is based on selective protection and deprotection steps with the key reaction Ferrier carbocyclization. Preparation of enantiomerically pure PI3P and PI4P by this method was described.[14] The desired hydroxyl (at C2 or C3) is protected with PMB group, which is easily and selectively deprotected. The protection yields two intermediates, first with C2 and second with C3 protected hydroxyls, which act as a precursor for PI3P and PI4P, respectively. The intermediates undergo various protection/deprotection steps, oxidation and

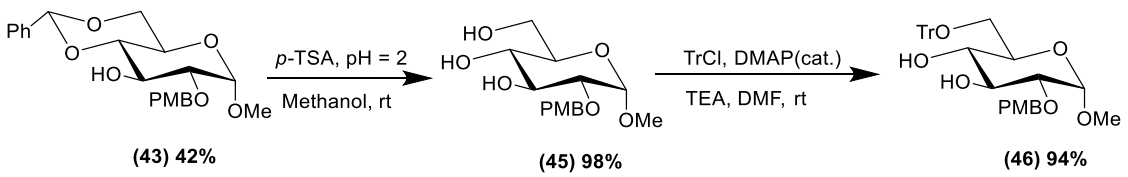
enolization to yield desired 5-enopyranoside. The key reaction, Ferrier carbocyclization, is used to convert the 5-enopyranoside into enantiomerically pure inositol derivative intermediate. The intermediate then further undergoes various protection/deprotection transformations to give free uncovered hydroxyls which are then coupled with phosphoramidite derivatives to yield the desired product. Only the route for synthesis of PI3P will be described, as the route for PI4P remains the same, only with different starting compound **(44)**.

The initial step of the PI3P synthesis is hydroxyl protection with *p*-methoxybenzyl group (PMB). First, **(42)** is refluxed with dibutyltin oxide (Bu_2SnO) to form stannylene acetal in an attempt to protect hydroxyl at C2 regioselectively. Then the intermediate is refluxed with PMBCl. The outcome is two products, both C2 (PI3P precursor) and C3 (PI4P precursor) protected intermediates (Scheme 10).



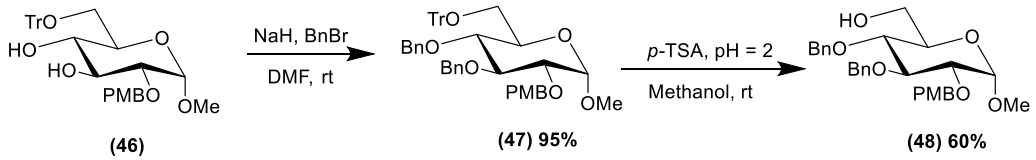
Scheme 10 Protection with PMB

Next, the benzylidene group is cleaved under mild acidic conditions (pH = 2) with *para*-toluenesulfonic acid (pTSA), resulting in deprotection of the C-6 and C-4 hydroxyls **(45)**. The C-6 hydroxyl is primary and sterically unhindered, which can be used in its selective protection with bulky groups such as trityl chloride (TrCl). The free secondary hydroxyls at C-4 and C-3 are relatively sterically hindered, thus their protection is rather unfavorable (Scheme 11).



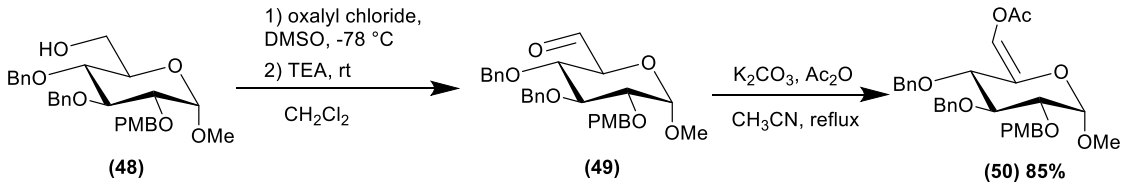
Scheme 11 Benzylidene hydrolysis and selective protection of primary hydroxyl

The remaining free secondary hydroxyls **(46)** are protected with the benzyl group using benzyl bromide (BnBr) under basic conditions, yielding a fully protected glucopyranose intermediate **(47)**. The trityl group is readily hydrolyzed using pTSA yielding intermediate **(48)**. See Scheme 12.



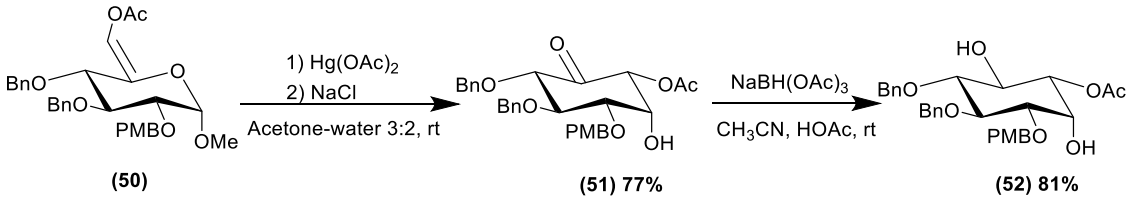
Scheme 12 Benzylation and detritylation

After the detritylation the primary hydroxyl is oxidized to aldehyde using mild oxidation procedure such as Swern oxidation. The resulting aldehyde **(49)** is then reacted with acetic anhydride (Ac_2O) under basic conditions to form enolacetate **(50)**, which is isolated as a *Z* isomer (Scheme 13).



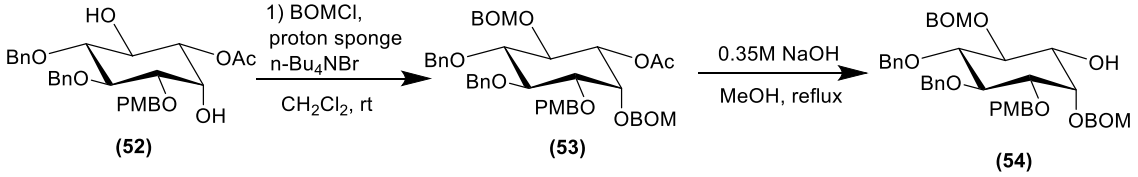
Scheme 13 Oxidation to aldehyde and subsequent transformation to enolacetate

Enolacetate **(50)** is then converted to the corresponding *myo*-inosose derivative using the Ferrier carbocyclization reaction. Soluble mercury (II) salt is used to form mercurial-aldehyde intermediate which then yields *myo*-inosose derivative **(51)** upon cyclization induced by the addition of NaCl. The inosose carbonyl is then highly stereoselectively reduced with triacetoxyborohydride ($NaBH(OAc)_3$) to yield the *myo*-inositol derivative **(52)** (Scheme 14).



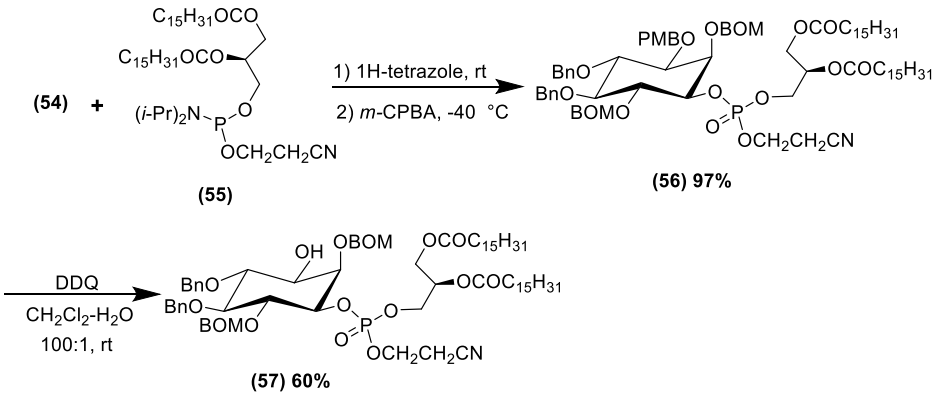
Scheme 14 Conversion into *myo*-inositol derivative by Ferrier carbocyclization and stereoselective reduction

The remaining free hydroxyl groups are then carefully protected with benzyl chloromethyl ether (BOMCl) and a non-nucleophilic base such as proton sponges to avoid acyl migration, yielding **(53)** with no detectable acyl migration. The acetyl ester is then hydrolyzed using NaOH yielding **(54)** (Scheme 15).



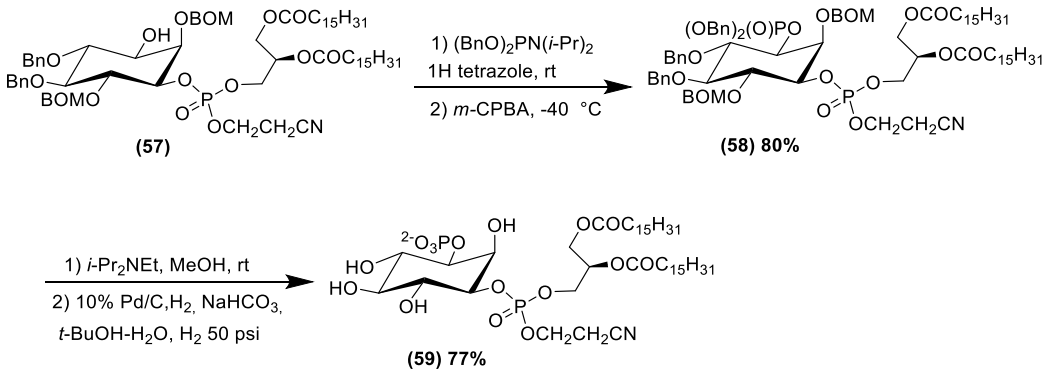
Scheme 15 Protection with BOM groups and ester hydrolysis

The *myo*-inositol derivative with free hydroxyl **(54)** was then coupled with (cyanoethyl)phosphoramidite derivative **(55)** to give compound **(56).** The PMB group was then deprotected with either DDQ or CAN (Scheme 16), the latter resulting in higher yields.



Scheme 16 Coupling with phosphoramidite derivative and PMB deprotection

Then another phosphate group is introduced using phosphoramidite derivative (BnO)2PN(*i*-Pr)$_2$ yielding an intermediate **(58)**. Subsequent removal of cyanoethyl group of **(58)** with diisopropylethyl amine (*i*-Pr$_2$NEt) followed by hydrogenation with Pd/C, H_2 (Scheme 17) gave the desired dipalmitoyl diester product PI3P **(59)**. The route remains the same for the synthesis of PI4P witch C3 protected hydroxyl (with PMB).



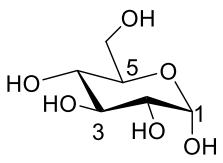
Scheme 17 Phosphate introduction with subsequent deprotection

2.4 Overview of carbohydrate chemistry

2.2.1 Hydroxyl group reactivity

In general, the reactivity of glucopyranose hydroxyl groups is influenced by the neighboring functionality, acid-base conditions, and axial/equatorial orientation in case of secondary hydroxyls. Ensuring that only selected hydroxyl undergoes reaction is often achieved by blocking the other hydroxyls with protecting groups. The utilization of subtle hydroxyl group reactivity differences can be done by carefully controlling the reaction conditions and by choosing the right protecting group.

The anomeric hydroxyl at C-1 (1) (Figure 12) is the most acidic hydroxyl owing to the negative inductive effect of ring oxygen. Under acidic conditions the (1) hydroxyl tends to be protonated, followed by elimination of water and yielding formation of the reactive oxocarbenium ion (Figure 13).



(60)

Figure 12 Numbering of glucopyranose carbons

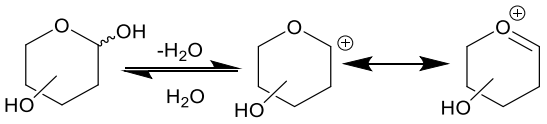


Figure 13 Formation of oxocarbenium ion

Under basic conditions and in general the most reactive is primary hydroxyl at position (6), which can be explained by low steric hindrance. Anomeric position (1) is the most reactive of secondary hydroxyls, followed by hydroxyl at position (2), which in turn, is followed by the (3) and (4) hydroxyls.

Axial/equatorial orientation of hydroxyls further influences their reactivity; axial hydroxyls generally react more slowly than those in equatorial orientation. It has been found that under under basic conditions, hydroxyl (2) shows enhanced reactivity when anomeric (protected) hydroxyl is *cis* (Figure 14).[15]

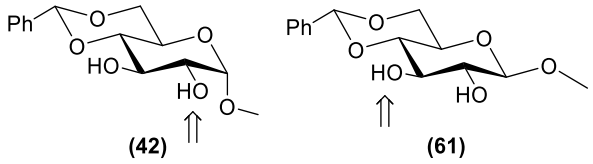


Figure 14 Influence of anomeric center orientation on preferred benzylation site

2.2.2 Glycosidation

In synthetic carbohydrate chemistry the reactive anomeric hydroxyl is often removed by substitution with alkoxy or aryloxy group, forming glycoside. The classical approach to glycoside formation is the Fischer glycosidation. The reaction is often performed using a solution/suspension of the carbohydrate in alcohol (Figure 15). This reaction is an equilibrium process and often leads to a mixture of furanose and pyranose ring forms and their anomers. Usually, the most thermodynamically stable product is pyranose with α anomer, due to the anomeric effect.

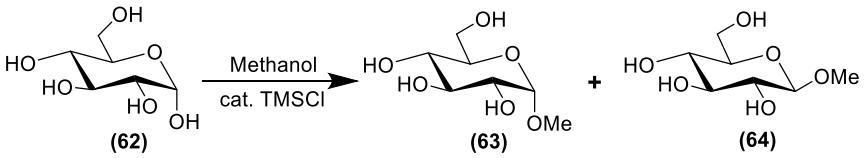


Figure 15 Classical Fischer glycosidation

The well-known alternative to the Fischer glycosidation is the Koenigs-Knorr reaction – substitution reaction of a protected glycosyl halide with an alcohol to give a glycoside.[15]

2.2.3 Ethers

Simple alkyl groups offer a reliable hydroxyl protection resistant to a wide pH range, nucleophiles, oxidizing and reducing reagents, organometallic and alkylating reagents. This resistance makes it difficult to deprotect, often requiring more vigorous conditions which can lead to unwanted products. Therefore, groups that can be relatively easily and selectively deprotected are used. For example benzyl, allyl, silyl ethers and their derivatives.

Typical conditions for the benzyl introduction are benzyl halide in DMF or THF, with a base such as NaH or Ag_2O. The deprotection can be selectively done using Pd-catalyzed hydrogenolysis or by oxidation with ozone. It has been mentioned that electron-donating phenyl substituents increase lability towards oxidizing reagents. For example, *para*-methoxybenzyl (PMB) can be selectively deprotected over benzyl using an one-electron oxidant such as DDQ or CAN.

Bulky benzyl derivatives, such as trityl, protect mainly the least hindered (primary) hydroxyl due to steric hindrance. However, with excess of tritylating agent tritylation of secondary hydroxyls can occur. The cleavage of trityl group can be done under mild protic conditions.

Allyl ethers are known to be easily introduced even under mild conditions that leave base-sensitive groups intact. Such method is indirect allyl introduction via the Pd-mediated allyl carbonate decarboxylation.

The most popular silyl protecting groups are trimethylsilyl (TMS) and bulky *tert*-butyldimethylsilyl (TBDMS). The latter can be used similarly as trityl to protect the least hindered hydroxyl. Their disadvantage is the tendency to migrate to proximal hydroxyl under basic conditions. Silyl ethers are selectively cleaved by fluoride ion donor, such as tetrabutylammonium fluoride (TBAF).[15]

2.2.4 Acetals

Acetal formation offers 1,2- or 1,3-diol acid-sensitive protection. Common reagents include benzaldehyde, acetone and cyclohexanone forming benzylidene, isopropylidene and cyclohexylidene acetals, respectively.

The classical approach to acetal formation includes acid-catalyzed condensation of acetone/benzaldehyde with the removal of water. The reactions are subject to thermodynamic control which means that the most stable molecules are the major products. For example, benzylidenes protect 1,3- pair because of the greater stability of dioxanes over dioxolanes. Isopropylidenes protect 1,2- pair because the potentially formed dioxane would suffer from 1,3- diaxial interactions (Figure 16), absent in dioxolanes.

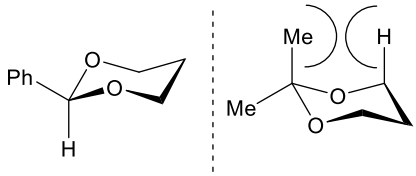


Figure 16 1,3- Diaxial interaction present in isopropylidene acetal

Alternative to direct condensation with carbonyls is transacetalization with their dimethoxy acetals. The methanol can be distilled off under reduced pressure further driving the equilibrium process to completion.[15]

2.2.5 Ferrier carbocyclization

The Ferrier carbocyclization reaction is an important carbohydrate-based transformation of 5-enopyranosides into enantiometrically pure carbocyclic compounds. The reaction is typically catalyzed with mercury(II) ions in aqueous acetone (Figure 17).

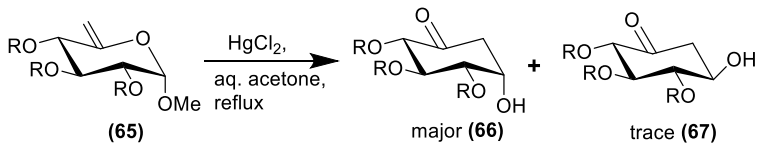


Figure 17 Ferrier carbocyclization

The proposed mechanism can be seen in Figure 18. The first step is oxymercuration of the double bond **(65),** yielding mercurial-hemiacetal **(68)**. Then the elimination of methanol

yields in the formation of a mercurial-aldehyde derivative **(69)**. After that intramolecular aldol-type cyclization of **(69)** results in carbocyclic products **(66), (67)**.

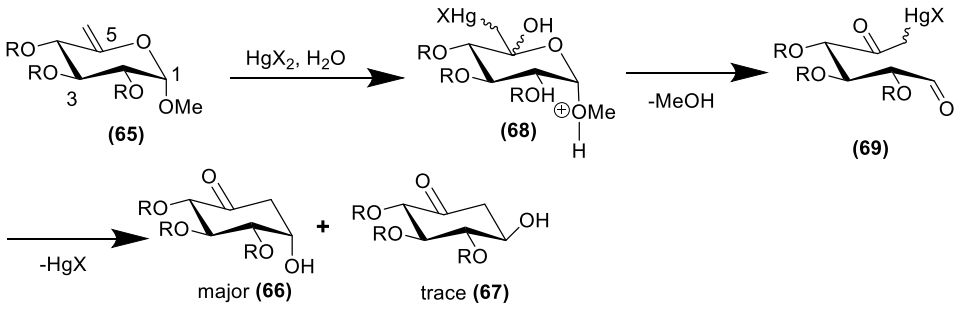


Figure 18 Mechanism of Ferrier carbocyclization

There is a strong correlation between the resulting cyclohexanone derivative orientation of the C-5 hydroxyl group and the substituent at C-3 of the 5-enopyranoside. The newly formed hydroxyl group at C-5 is transrelated to C-3 substituent, meaning that the upward directed C-3 substituent results in downward directed C-5 hydroxyl group and vice versa.

The enol-acetate version of Ferrier carbocyclization is effective in preparing enantiometrically pure *myo*-inositol derivatives.[16]

3. Aims of the work

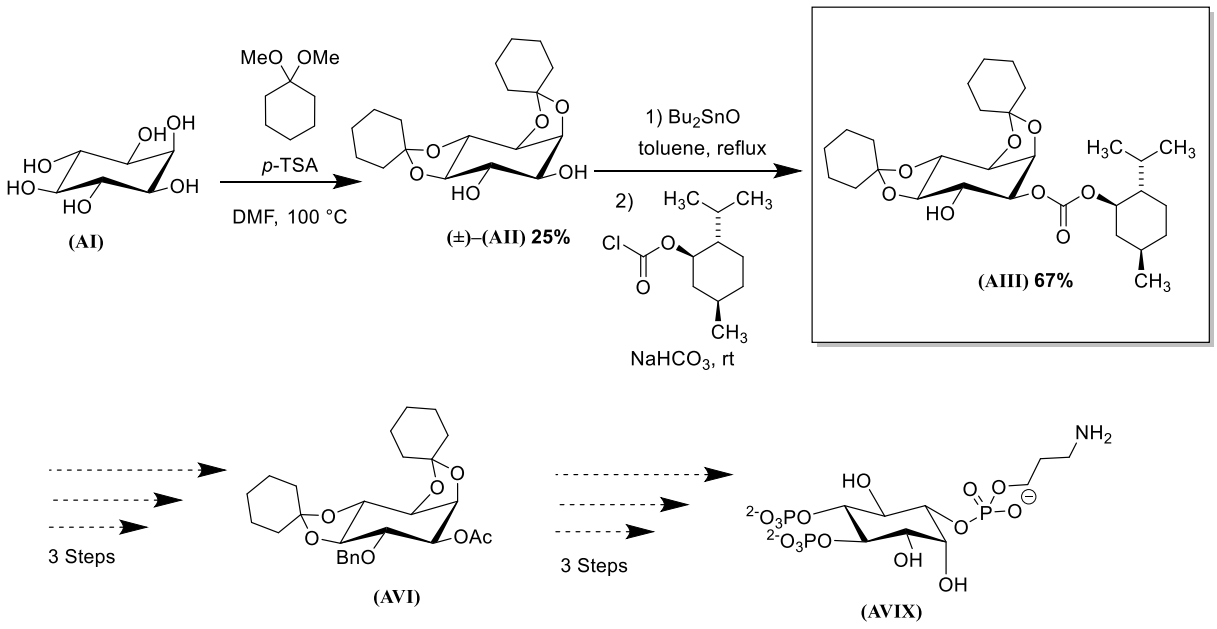
This bachelor's thesis is dedicated to synthesizing selected inositol derivatives as a part of our research. Particularly, the main goal of the work was to elaborate the synthesis of selectively protected inositol unit suitable for the preparation of phosphatidylinositol 4,5-bisphosphate derivatives. The goal can be divided into specific aims:

A) Preparation of selectively protected inositol unit from *myo*-inositol as a starting material
B) Preparation of selectively protected inositol unit from widely available D-glucopyranose as a starting material

4. Results and discussion

4.1 Synthetic approach using myo-inositol as a starting material

The first approach to the synthesis of orthogonally protected myo-inositol derivative suitable for preparation of phosphatidylinositol 4,5-bisphosphate derivatives uses *myo*-inositol **(AI)** as a starting material. The approach was inspired by described synthetic route for phosphatidylinositol 3-phosphates (PIP3s)[13]. According to the proposed scheme of synthesis (Scheme 18) the first step is double protection of the *meso* compound *myo*-inositol **(AI)** with cyclohexylidene ketal functionality. The resulting (±)–1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol **(AII)** product has no plane of symmetry, thus it exists as a mixture of two enantiomers (Figure 11). Separation of the two enantiomers is needed as the desired phosphatidylinositol has defined stereochemistry. The procedure to separate them with the chiral resolution has been described[17]. In the next step the enantiomers are subjected to chiral resolution with regioselective introduction of chiral derivatizing agent menthyl chloroformate. It is worth noting that the separation of the two enantiomers was done with enzymatic processes by the authors of the PIP3 synthetic route[13]. The desired enantiomer is then separated by crystallization and benzylated[17]. Afterward, the menthyl moiety is cleaved and the resulting free hydroxyl is acetylated, followed by further deprotection steps and coupling with phosphoradimite derivatives (Scheme 18).



Scheme 18 Synthetic approach using *myo*-inositol as a starting material

The compound (±)-1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol **(AII)** was synthesized by reported procedure[18] by the reaction of cyclohexanone dimethyl ketal with *myo*-inositol and a catalytic amount of pTSA. DMF was used as a solvent, and the reaction was heated to 100 °C. The reaction was monitored using TLC every 24 h with additions of cyclohexanone dimethyl ketal (0.23 equiv.) and pTSA (0.026 equiv.) until the starting material disappeared. The reaction took 72 hours to complete. As a result of the reaction three major products corresponding to regioisomers of di-*O*-cyclohexylidene-*myo*-inositol. Their retardation factors were similar, R_f = 0.73, 0.54 (unwanted), 0.59 (desired). One unwanted regioisomer was partially crystallized from the mixture (acetone:hexane, 1:10). The desired regioisomer was isolated using silica gel column chromatography with the yield 26% (the reported yield 23%)[18].

The noted reaction time in the reported procedure was 4 hours contrary to the experiment (72 hours). The reaction was done three times and either experiment was not able to reach complete conversion of starting material in 4 hours. The necessity for a longer reaction time and/or addition of reagents could be explained by the presence of water, which plays a role in the acetal equilibration. Furthermore, acetals are known to be susceptible to hydrolysis under acidic conditions. However, the reported procedure did not mention any dry reagents nor inert atmosphere. To further increase the total yield and drive the equilibrium forward one would assume that methanol could be removed by applying reduced pressure to the reaction mixture. However, this could require excess and/or additions of cyclohexanone dimethyl ketal as difference of boiling points of methanol and cyclohexanone dimethyl ketal is not too big; 64, 83 °C, respectively. Acidic conditions, elevated temperature, and prolonged-time could also decompose DMF to carbon monoxide and dimethylamine[19], thus quenching the acid catalyst. This could be bypassed by using NMP solvent, which is known to be more resistant to such decompositions[20].

The reaction was also done using huge excess of cyclohexanone in place of cyclohexanone dimethyl ketal, and with a toluene additive. The reaction was kept under reflux (150 °C) with Dean-Stark apparatus. The reaction took even more time to complete (4 days) and required the addition of acid catalyst each day, resulting in more difficult product separation. The long reaction time could also be explained by DMF decomposition, which quenches the acid catalyst.

The successfully prepared compound **(AII)** was then subject to chiral resolution. The first step was stannylene acetal formation done by reaction of **(AII)** with dibutyltin oxide (1.2 equiv.) in toluene solvent under reflux with Dean-Stark apparatus. After 24 hours menthyl

chloroformate (1.3 equiv.) was added and the product (±) **(AIII)** was isolated as mixture of enantiomers using column chromatography. According to procedure[17], the two enantiomers could be separated by crystallization. The reported solvent used was petroleum ether. Similar non-polar solvents tested solvents were aliphatics (hexane, heptane), aromatics (toluene, dry toluene) and ethers (methyl *tert*-butyl ether). Polar solvents such as alcohols (methanol, ethanol, *n*-butanol, isopropanol) of various purities (absolute ethanol, HPLC ethanol) and aqueous ethanol were also tested. However, after prolonged and repeated experiments the crystals could not be obtained. The result of crystallizations was either amorphous precipitate or a solid uncrystallized oil-like mixture. However, after one week of crystallization from aqueous ethanol some crystals appeared. Unfortunately, the crystals were not the desired product but unidentified compound which had missing acetal NMR signal, indicating hydrolysis took place. Thus, this approach was abandoned as the results from necessary chiral resolution did not match those reported in literature.

4.2 Synthetic approach using D-glucopyranoside as a starting material

The structural similarity of D-glucopyranoside **(BI)** with *myo*-inositol **(AI)** can be employed in the second approach (Figure 19). The approach was inspired by described synthetic route for phosphatidylinositol 3-phosphates (PIP3s) using Methyl 4,6-*O*-benzylidene-*α*-D-glucopyranoside as a starting material[14]. The benefit of this approach consists of using an already chiral precursor, meaning there is no need for chiral resolution. However, the necessity of converting D-glucopyranoside intermediate into *myo*-inositol derivative requires steps such as enolization and further protections/deprotections. Thus, the number of chemical transformations vastly outnumber the first approach (Scheme 19).

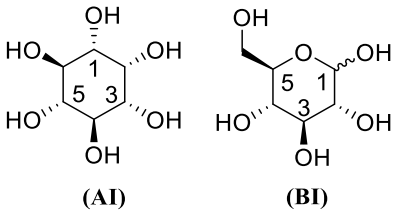


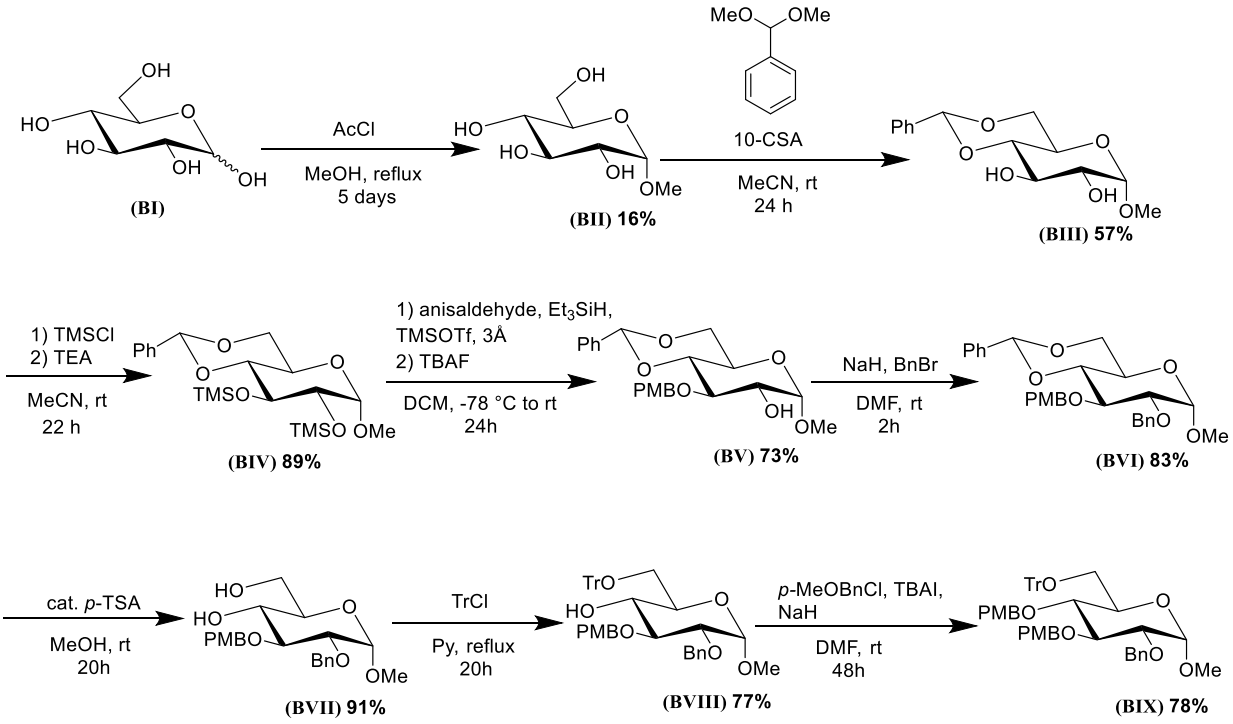
Figure 19 Structural similarity between D-glucopyranoside and *myo*-inositol

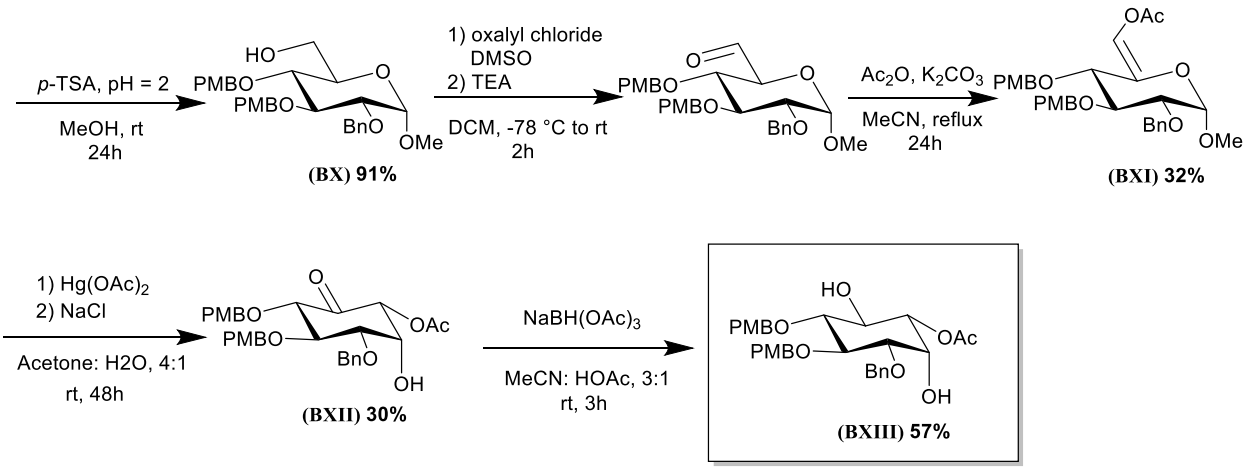
The synthesis of protected intermediate **(BIII)** started with the formation of methyl *α*-D-glucopyranoside **(BII)**. Anhydrous D-glucose reacted with methanol and acetyl chloride

under 7-day long reflux to afford **(BII).** The purification with column chromatography was not sufficient, the product was further purified using crystallization. The reaction gives four products: α, β furanosides and pyranosides. The former are kinetic products, and the latter are thermodynamic products. Thus, the reaction needs more time to reach equilibrium. The **(BII)** (α anomer) i**s** the most stable product due to the anomeric effect. The yield of α anomer **(BII)** was 16%.

Crystals of pure **(BII)** then reacted with benzaldehyde dimethyl acetal in MeCN solvent and camphor-10-sulphonic acid as a catalyst affording **(BIII)** with 57% yield (the reported yield 42%)[21].

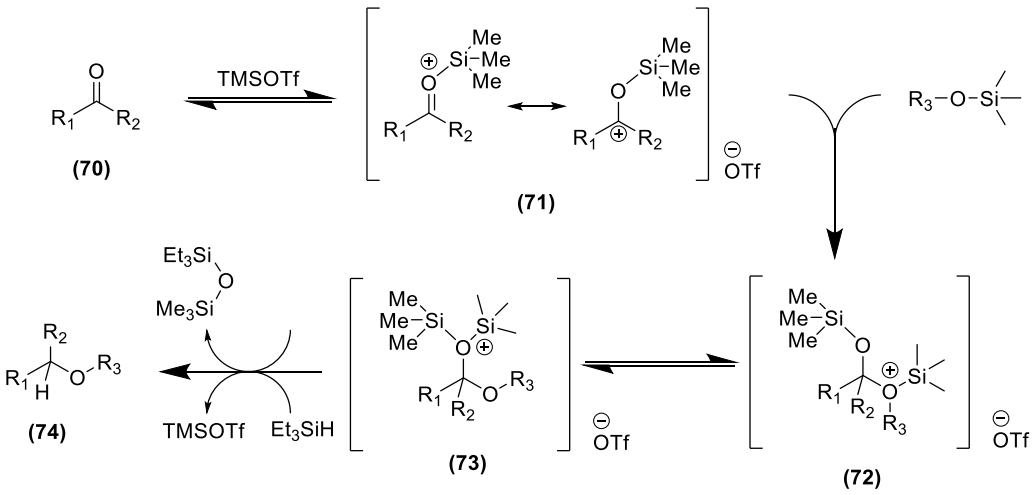
Next, the free hydroxyl groups of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside **(BIII)** were protected with trimethylsilyl (TMS) group by the reaction of **(BIII)** with the excess of TMSCl in MeCN solvent and TEA as a base. The reaction proceeded through as nucleophilic substitution with a high yield, 89% (the reported yield 74%)[22] affording **(BIV)**. The higher yield obtained compared to the literature can be explained by the fact that **(BIV)** was prepared from pure form of **(BIII)** directly, whereas in the literature it was prepared from **(BII)** in one-pot synthesis with no further purification of the intermediate **(BIII)**.





Scheme 19 Synthetic approach using D-glucopyranoside as a starting material

Next *para*-methoxybenzyl (PMB) group was introduced using the regioselective reductive etherification at C-3 hydroxyl group. The regioselectivity of C-3 hydroxyl over C-2 hydroxyl can be explained by steric effects and by decreased nucleophilicity of C-2 hydroxyl, caused by the anomeric inductive effect. The transformation was afforded by the reaction of compound **(BIV)** with triethylsilane (Et_3SiH) as a mild reducing agent, anisaldehyde as a PMB moiety donor, TMSOTf as a catalyst and DCM as a solvent. Molecular sieves were also added. The reaction was kept at -78 °C under argon atmosphere. After the PMB introduction was performed (observed disappearance of starting material using TLC analysis), TBAF was added to cleave the TMS group at the less reactive C-2 hydroxyl and left to react overnight. The process afforded **(BV)** with 53–73% (the reported yield 91%)[22] yields. Usage of dry and purified DCM resulted in increased yield, however the yield reported in literature was still not reached. The yield could be possibly further improved by using freshly distilled anisaldehyde. According to the proposed mechanism (Scheme 20)[23] the catalyst acts as a Lewis acid forming intermediate **(71)** with carbonyl. The intermediate is then attacked by trimethylsilyl ether nucleophile forming intermediate **(72)** which then rearranges to **(73)**. Subsequent hydride attack with silyl ether elimination and catalyst regeneration yields the desired product **(74)**.



Scheme 20 Proposed mechanism for TMSOTf activated triethylsilane-reductive etherification[23]

The C-2 hydroxyl was protected with benzyl group using the standard benzylation procedure; reaction of **(BV)** with benzyl bromide under basic conditions (60% NaH in mineral oil suspension) and dry DMF as a solvent. After 2 hours the reaction afforded **(BVI)** with 78–83% yields. The benzylation reaction generally proceeds with good yields, further yield improvement could be done by adding TBAI to generate iodide ions[24] which would generate the more reactive benzyl iodide.

Next, the 4,6-*O*-benzylidene acetal of **(BVI)** was hydrolyzed with catalytic amount of pTSA. The used solvent was methanol, in which the substrate **(BVI)** formed suspension. As the reaction proceeded the suspension was becoming clear solution. The reaction product **(BVII)** has two free hydroxyl groups and is thus more soluble in polar protic solvents. The product was isolated with 91–95% yields, which corresponds to the yields reported on similar substrates[14,25]. Dry conditions were not needed and would be in fact detrimental, as acetal hydrolysis requires water molecules.

The intermediate **(BVII)** has two free hydroxyls with different chemical reactivity as one is primary and the other secondary. The secondary hydroxyl is more sterically hindered and thus its relative reactivity towards bulky electrophiles is reduced in contrast to the primary hydroxyl. This difference of relative reactivity was employed in selective tritylation of the primary hydroxyl. The compound **(BVII)** reacted with trityl chloride in pyridine and reflux under argon atmosphere, affording compound **(BVIII)** with 81–85% yields, which corresponds to the yields reported on similar substrates[26]. The yield could be increased by helping the formation of trityl cation with silver salts or trifluoroacetic anhydride, although this approach is mostly used for secondary hydroxyls[27].

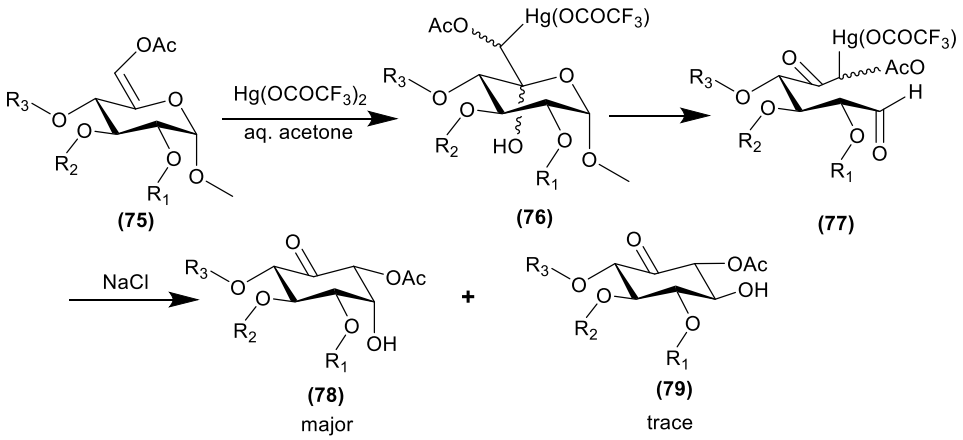
The remaining free hydroxyl of compound **(BVIII)** was then protected with PMB group using PMBCl and TBAI in dry DMF as a solvent and NaH as a base, under argon atmosphere. The TBAI was used to generate iodide ions[24], which convert PMBCl into more reactive PMBI. The reaction yielded **(BIX)** with 78–84% yields.

The trityl group of **(BIX)** was then hydrolyzed using acidic conditions. To the suspension of **(BIX)** in methanol pTSA was added to decrease the pH value to 2-3. As the reaction proceeded the suspension was becoming clear solution. The reaction afforded **(BX)** in 91% yield. The reaction on similar substrate was reported with 92% yield[28]. The yield is satisfactory, however it has been noted that 4-methoxytrityl (MMT) is easier to hydrolyze, although this approach is used mostly in polynucleotide synthesis[29].

Then the free primary hydroxyl of **(BX)** was oxidized to aldehyde using Swern oxidation method. The reaction was done using the standard procedure; slow addition of DMSO to the solution of oxalyl chloride in dry DCM at -78 °C and argon atmosphere. Then slow addition of solution of **(BX)** in dry DCM and after 15 minutes slow addition of TEA. The reaction mixture was then subject to classical work-up procedure and the product mixture **(BXI)** was used directly in the next transformation without purification. The vacuum dried mixture **(BXI)** was dissolved in suspension of K_2CO_3 in dry MeCN. Then acetanhydride was added and the reaction was refluxed under argon atmosphere overnight. The reaction afforded **(BXII)** (*Z*-isomer) in 32% yield. This two-step transformation on similar substrate was reported with 85% yield[14]. The low yield could be caused by decomposition of the aldehyde intermediate **(BXI)** and should have been used directly to the next step without storage. The yield could be increased by using oxidation which would yield cleaner intermediate **(BXI)** after work-up, such as Dess-Martin oxidation.

The acetyl-5-enopyranoside **(BXII)** was then transformed into *myo*-inosose derivative **(BXIII)** by the Ferrier carbocyclization reaction. The **(BXII)** was dissolved in the acetone:water, 4:1 solution and mercury(II) acetate was added. After formation of the mercurial intermediate (and disappearance of the starting material on TLC), a NaCl solution was added to induce the cyclization. The reaction afforded **(BXIII)** in 30% yield. The glucopyranoside enol-acetate version of Ferrier carbocyclization is known to afford enantiometrically pure *myo*-inosose derivatives. The reaction is also known for low yields with common mercury(II) salts, higher yields (50–80%) are obtainable with more expensive salts such as mercury(II) trifluoroacetate or palladium salts[16]. The proposed mechanism is shown at (Scheme 21). First, reaction between enol-acetate glucopyranoside derivative **(75)** and mercury(II) salt results in an oxy-mercuration intermediate **(76)**, which is then

transformed into the key intermediate **(77)**. The intermediate then cyclizes upon addition of NaCl salt to afford the desired *myo*-inosose derivative **(78)**.



Scheme 21 Enol-acetate version of Ferrier carbocyclization mechanism[16]

The keto functionality of **(BXIII)** was then reduced to alcohol with sodium triacetoxyborohydride in dry MeCN/HOAc solvent solution. This type of reduction is known for stereoselective *anti*-reduction[30]. The reaction proceeded through transition state shown at Figure 20 and afforded **(BXIV)** with 57% yield. The yield could be increased by using more expensive tetramethylammonium triacetoxyborohydride.

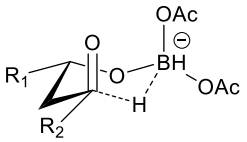


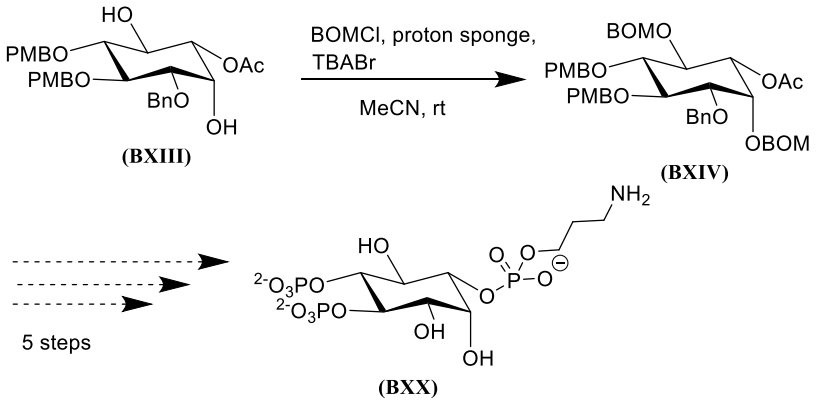
Figure 20 Transition state showing the reason for the reported stereoselectivity[30]

The total yield of intermediate **(BXIII)** from D-glucopyranoside by 13 step synthesis is 0.15%. The synthesis was not optimized. Ferrier carbocyclization, oxidation and subsequent enol-acetate formation, *myo*-inosose reduction are the key reactions to optimize for the higher total yield. Each would provide higher yield using more expensive reagents.

4.3 Future prospectives

The future transformations leading to phosphatidylinositol 4,5 bisphosphate derivative (Scheme 22) would involve protection of the two free hydroxyls with BOM protecting group under conditions mild enough to limit acyl migration, affording **(BXIV)**. After that the acetyl

ester would be hydrolyzed under basic conditions and the resulting free hydroxyl coupled with phosphoramidite derivative. Selective deprotection of PMB groups would be done with DDQ and the resulting free hydroxyls would be coupled with another phosphoramidite derivative. The final step would be broad benzyl/BOM deprotection with Pd/C and CBZ deprotection with TFA affording **(BXX)**.



Scheme 22 Transformations leading to phosphatidylinositol 4,5-bisphosphate

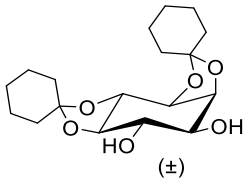
5. Experimental procedure

5.1 General procedure and chemicals

All chemicals used in this work were obtained from commercially available sources (Sigma-Aldrich, Fluorochem, Acros organics, Lach-Ner). Hexane, ethyl acetate, chloroform, DCM and ether were purified by distillation. Dry DCM was prepared by reflux with calcium hydride and subsequent distillation. All other solvents and reagents were not further purified/dried. The thin layer chromatography (TLC) analysis was done using Kieselgel 60 F 254 plates from Merck. Spots at the developed TLC plates were analyzed using AMC solution (prepared by dissolving 10 g of ceriuim(IV) sulfate dihydrate and 25 g of phosphotungstic acid in 1 liter of 1.2M sulfuric acid solution), vaniline solution (prepared by dissolving 12 g of vaniline and 2.5 ml of concentrated sulfuric acid in 200 ml of absolute ethanol) and UV lamp (NU – 6KL, $\lambda = 254$ nm) with subsequent activation using heat produced by heat gun. Mobile phase composition is given individually. Concentration in vacuo was done using rotary evaporator Büchi Rotavapor R-200. Substances were vacuum dried using oil pump Vacuubrand RZ 2. NMR spectra were measured using Bruker AVANCE III Spectrometer, ^{1}H at 400 MHz, ^{13}C at 100 MHz. Deuterated chloroform was used as a solvent, ^{1}H chemical shifts were referenced to the $CDCl_3$ residual peak (δ 7.26) and ^{13}C were referenced to $CDCl_3$ (δ 77.16). Molecular mass was determined by HRMS technique using Q-TOP COMPACT BRUKER machine. The ionization method was ESI. IR spectra were measured by Nicolet Avatar 370 FTIR machine. Silica gel column chromatography was done with Fluka 60 (40-63 μm) silica gel. The melting point was determined using the Büchi Melting Point B-545. Specific rotation was determined using the AUTOMATIC POLARIMETER Autopol III (Rudolph research, Flanders, New Jersey). The pH was measured using universal pH indicator strips manufactured by Lach-Ner.

5.2 Synthesis of intermediates

(±) 1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol (AII)



(±)

The compound was prepared by modified described procedure[18].

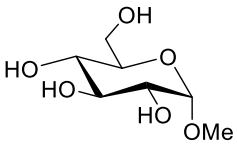
To a suspension of *myo*-inositol (5 g, 27,7 mmol) in DMF (30 ml) cyclohexanone dimethyl ketal (11.1 ml, 2.6 equiv.) and pTSA (monohydrate, 0.14 g, 0.026 equiv.) were added. The mixture was heated to 100 °C for one day. After that duration the reaction mixture changed color to orange, unreacted inositol was still observed as suspension and its presence was confirmed by analysis using TLC (Hexane: EtOAc, 2:1). pTSA (monohydrate, 0.14 g) and cyclohexanone dimethyl ketal (1 ml) were added. Next day there was no suspension, but unreacted inositol was still observed using TLC. pTSA (monohydrate 0.14 g) and cyclohexanone dimethyl ketal (1 ml) were added. Next day no unreacted inositol was seen, the reaction was quenched with TEA (3 ml). The mixture was then vacuum filtered with sintered glass and the filtrate was evaporated in vacuo, resulting in thick orange oil. The oil was dissolved in minimal amount of acetone (7 ml) and hexane was then added slowly with strong stirring until precipitation was observed. Then drop of acetone was added to re-dissolve the suspension and hexane (70 ml) was carefully added on top of the solution forming two layers. Next day the precipitate (byproduct) was filtered off and the filtrate was concentrated in vacuo, yielding orange oil. The oil was purified using silica gel column chromatography (Hexane: EtOAc, 3:1 → 7:3), yielding white amorphous solid, 2.45 g, yield: 25.9%, R_f = 0.59 (TLC $CHCl_3$: Acetone, 2:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.50 (dd, *J* = 6.4, 4.2 Hz, 1H), 4.34 (dd, *J* = 8.1, 6.3 Hz, 1H), 4.09-4.04 (m, 1H), 3.96 (dd, *J* = 10.4, 8.1 Hz, 1H), 3.87 (dd, *J* = 4.6 Hz, 4.6 Hz, 1H), 3.41 (dd, *J* = 10.4, 8.9 Hz, 1H), 1.73-1.25 (m, 20H) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 113.2, 111.3, 78.2, 77.9, 76.5, 75.7, 74.7, 73.5, 37.2, 36.7, 36.5, 34.3, 25.1, 25.1, 24.0, 23.7(2C), 23.6 ppm

The spectra are consistent with the literature[17,31].

Methyl *α*-D-glucopyranoside (BII)

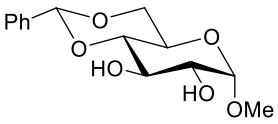


The compound was prepared by described procedure[32]. Dry anhydrous glucose **(BI)** (5 g, 27.75 mmol) was dissolved in methanol (40 ml, 32 equiv.). To the stirring suspension acetyl chloride (1.6 ml, 0.8 equiv.) was added dropwise. The mixture was refluxed at 80 °C for 7 days. After the noted duration the solution was concentrated in vacuo yielding brownish oil. The oil was purified using silica gel column chromatography (DCM: Methanol, 7:1) affording 2.32 g of white crystalline solid, which was then further purified by crystallization to yield 0.88 g of **(BII)**, 16.3% yield. R_f = 0.51 (TLC DCM: MeOH, 4.5:1).

^{1}H NMR (400 MHz, methanol-d4) δ 4.69 (d, *J* = 3.7 Hz, 1H), 3.83 (dd, *J* = 11.8, 2.4 Hz, 1H), 3.67 (t, *J* = 5.8 Hz, 1H), 3.66 (dd, *J* = 9.3, 5.8 Hz, 1H), 3.57-3.52 (m, 1H), 3.42 (s, 3H), 3.42-3.33 (m, 1H), 3.32-3.27 (1H, m) ppm

^{13}C NMR (100 MHz, methanol-d4) δ 101.2, 75.0, 73.5, 73.5, 71.8, 62.7, 55.5 ppm
The spectra are consistent with the literature[33].

Methyl 4,6-*O*-benzylidene-*α*-D-glucopyranoside (BIII)

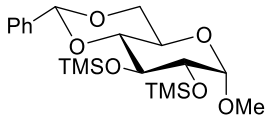


The compound was prepared by described procedure[21]. The **(BII)** (1.00 g, 5.15 mmol) were dissolved in dry acetonitrile (52 ml), then camphor-10-sulfonic acid was added (359 mg, 0.3 equiv.) and stirred for 15 minutes. After that benzaldehyde dimethylketal was added dropwise (1.16 ml, 1.5 mmol). After 24 hours, TEA (0.55 ml, 0.77 equiv.) was added, and the reaction was concentrated in vacuo. After that the residue was dissolved in DCM (50 ml) and washed (50 ml) with saturated sodium bicarbonate solution, distilled water and brine. The organic layer was then dried using anhydrous sodium sulfate and evaporated in vacuo. The crystalline residue was then purified using silica gel column chromatography (Hexane: EtOAc, 5:1 → 1:4) yielding 0.83 g of **(BIII)** as colorless solid, 57% yield, R_f = 0.25 (TLC (Hexane: EtOAc, 1:3).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51-7.47 (m, 2H), 7.38-7.34 (m, 3H), 5.53 (s, 1H), 4.81 (d, *J* = 3.9 Hz, 1H), 4.30 (dd, *J* = 9.8, 4.5 Hz, 1H), 3.94 (t, *J* = 9.2 Hz, 1H), 3.88 – 3.69 (m, 2H), 3.63 (m, 1H), 3.50 (t, *J* = 9.3 Hz, 1H), 3.47 (s, 3H) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 137.2, 129.1, 128.5 (2C), 126.4 (2C), 102.1, 99.7, 81.0, 73.0, 72.0, 69.0, 62.5, 55.7 ppm
The spectra are consistent with the literature[21].

Methyl 2,3-di-*O*-trimethylsilyl-4,6-*O*-benzylidene-*α*-D-glucopyranoside (BIV)



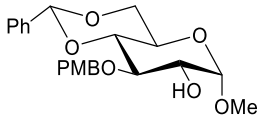
The compound was prepared by modified described procedure[22].
Vacuum dried **(BIII)** (5 g, 0.017 mol) was dissolved in 85 ml of MeCN. Then TEA (24 ml, 10 equiv.) was added, and the flask was purged with argon gas. The mixture was left to stir for 10 minutes and then TMSCl (9 ml, 4 equiv.) was added. The reaction was left to react overnight. After the noted duration disappearance of the starting material was seen on TLC (Hexane: EtOAc, 2:1). The reaction mixture was then diluted with 150 ml of DCM and washed two times with distilled water and brine. The organic DCM layer was dried with anhydrous sodium sulfate and then evaporated using rotary evaporator yielding reddish-brown crystalline residue. The residue was dissolved in the minimal amount of absolute ethanol and left to crystallize. Next day 2.79 g of colorless crystals was isolated. The mother liquor was purified using silica gel column chromatography (Hexane: EtOAc, 7:1 → 4:1), yielding 3.97 g of white solid. Total mass of isolated product was 6.76 g, 89% yield, R_f = 0.83 (TLC Hexane: EtOAc, 2:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50-7.46(m, 2H), 7.38-7.32(m, 3H), 5.50(s, 1H), 4.62(d, *J* = 3.7 Hz, 1H), 4.26(dd, *J* = 9.9, 4.6 Hz, 1H), 3.95(t, *J* = 8.9 Hz, 1H), 3.80(m, 1H), 3.70(t, *J* = 10.2 Hz, 1H), 3.61(dd, *J* = 8.8, 3.8 Hz, 1H), 3.41(s, 3H), 3.40(t, *J* = 9.2 Hz,1H), 0.16(s, 9H), 0.09(s, 9H) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 137.5, 129.0, 128.3(2C), 126.3(2C), 101.8, 101.2, 82.3, 74.3, 71.9, 69.3, 62.5, 55.5, 0.8(3C), 0.5(3C) ppm

The spectra are consistent with the literature[34].

Methyl 3-*O*(*p*-methoxybenzyl)-4,6-*O*-benzylidene-*α*-D-glucopyranoside (BV)



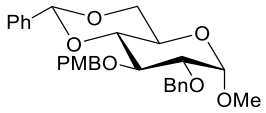
The compound was prepared by described procedure[22]. The **(BIV)** (4 g, 4.7 mmol) was dissolved in DCM (80 ml) under argon atmosphere with stirring. Then 4 g of 3Å molecular sieves, Et_3SiH (1.8 ml, 1.2 equiv.) and anisaldehyde (1.37 ml, 1.2 equiv.) were added. After 20 minutes of stirring the solution was cooled to -78 °C with ethanol/dry ice cooling bath. After that TMSOTf (0.18 ml, 0.1 equiv.) was added slowly. After 2 hours small amounts of starting material were still observed using TLC. After one hour the TLC analysis showed no starting material present. The reaction mixture was left to heat to room temperature and then TBAF (1M THF solution, 18.8 ml, 2 equiv.) was added. The reaction was left to react overnight. Then the reaction mixture was filtered through celite and concentrated in vacuo. The residue was then purified using silica gel column chromatography (Hexane: EtOAc, 3:1 → 1:2), yielding 2.75 g of amorphous solid, R_f = 0.32 (TLC Hexane: EtOAc, 1:1). Yield: 73%.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51-7.26(m, 7H), 6.84(m,2H), 5.57(s, 1H), 4.89 (d, J = 11.2 Hz, 1H), 4.71 (d, J = 11.2 Hz, 1H), 4.81(d, J = 3.8 Hz, 1H), 4.29(dd, J = 9.7, 4.4 Hz, 1H), 3.89-3.68(m, 7H), 3.62(t, J = 9.1 Hz, 1H), 3.44(s, 1H) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 159.4, 137.5, 130.5, 129.8(2C), 128.9, 128.3(2C), 126.1(2C), 113.9(2C), 101.3, 99.9, 82.2, 78.6, 74.6, 72.5, 69.2, 62.8, 55.4, 55.3 ppm

The spectra are consistent with the literature[35].

Methyl 2-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-4,6-*O*-benzylidene-*α*-D-glucopyranoside (BVI)



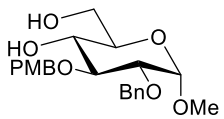
The compound was prepared by modified described procedure[14]. Flask with vacuum dried **(BV)** (2.77 g, 2.48 mmol) was filled with argon gas. Then dry DMF (15 ml) was added. Then NaH (60% mineral oil suspension, 0.55 g, 2 equiv.) was slowly added. The solution was left to stir for 15 minutes. After benzyl bromide (1.64 ml, 2 equiv.) was slowly added. After 2 hours no starting material was observed using TLC. The reaction was quenched with 5 ml of ethanol and diluted with 100 ml of DCM followed by washing two times with distilled water (100 ml) and brine (100 ml). The organic DCM layer was then dried with anhydrous sodium sulfate and concentrated in vacuo. The concentrated residue was purified using silica gel column chromatography (Hexane: EtOAc, 6:1 → 4:1) yielding white amorphous solid, 2.80 g, 83% yield. R_f = 0.63 (TLC Hexane: EtOAc, 2:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51-7.28 (m, 12H), 6.84(d, *J* = 8.6 Hz, 2H), 5.54 (s, 1H), 4.88-4.66 (m, 4H), 4.58 (d, *J* = 3.7 Hz, 1H), 4.26 (dd, *J* = 10.1, 4.7 Hz, 1H), 4.02 (t, *J* = 9.3 Hz, 1H), 3.85-3.79 (m, 1H), 3.79 (s, 3H), 3.71 (t, *J* = 10.2 Hz, 1H), 3.58 (t, *J* = 9.5 Hz, 1H), 3.53 (dd, , *J* = 9.2, 3.6 Hz, 1H), 3.40 (s, 3H) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 159.2, 138.3, 137.5, 131.0, 129.8(2C), 129.0, 128.5(2C), 128.4(2C), 128.2(2C), 128.0, 126.2(2C), 113.9(2C), 101.3, 99.4, 82.3, 79.3, 78.4, 75.2, 73.9, 69.2, 62.44, 55.5, 55.4 ppm

The spectra are consistent with the literature[36].

Methyl 2-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-*α*-D-glucopyranoside (BVII)



The compound was prepared by modified described procedure[25]. To a flask with vacuum dried **(BVI)** (1.52 g, 3.09 mmol) was added methanol (23 ml), forming white suspension. Then catalytic amount of pTSA (monohydrate, 60 mg, 0.1 equiv.) was added. The reaction

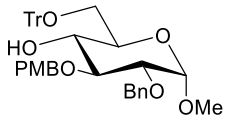
was left to react overnight. After that time the suspension disappeared yielding clear solution. No starting material was observed using TLC. The reaction was quenched with sodium bicarbonate (500 mg) and concentrated in vacuo. The residue was then purified using silica gel column chromatography (Hexane: EtOAc, 3:1 → 1:3), yielding colorless oil, 1.14 g, 91.3%. R_f = 0.28 (TLC Hexane: EtOAc, 1:3).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-6.80 (m, 9H), 4.95-4.57(m, 4H), 3.80 (s, 3H), 3.82-3.69 (m, 3H), 3.63-3.58 (m, 2H), 3.50-3.44 (m, 2H), 3.38 (s, 3H) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 159.5, 138.1, 130.9, 129.7(2C), 128.6(2C), 128.2(2C), 128.2, 114.2(2C), 98.3, 80.9, 79.9, 75.1, 73.3, 70.8, 70., 62.7, 55.4, 55.4 ppm

The spectra are consistent with the literature[36].

Methyl 2-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-6-triphenylmethyl-*α*-D-glucopyranoside (BVIII)



The compound was prepared by modified described procedure[37]. To a vacuum dried **(BVII)** (1.41 g 3.488 mmol) pyridine (25 ml) and TrCl (0.97 g, 1 equiv.) were added. The reaction mixture was heated to 100 °C and left to react overnight. After the noted duration no starting material was observed using TLC. The reaction was cooled to 0 °C using water/ice bath and 5 ml of distilled water was added. The mixture was stirred for 15 min and diluted with 100 ml of Et_2O. The pyridine was extracted from the diluted mixture by washing with 1M HCl, the completion of extraction was determined by observation of low pH of aqueous phase. The organic Et_2O layer was then additionally washed with sodium bicarbonate solution (50 ml), distilled water (50 ml) and brine (50 ml). Then the organic Et_2O layer was dried using anhydrous sodium sulfate and concentrated in vacuo. The residue was purified using silica gel column chromatography (Hexane: EtOAc, 5:1 → 4:1), white foamy solid, 1.71 g, 77.7% yield, R_f = 0.21 (TLC Hexane: EtOAc, 5:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46-7.18 (m, 22H), 6.90-6.84 (m, 2H), 4.89 (d, J = 11.0 Hz, 1H), 4.79 (d, J = 12.1 Hz, 1H), 4.71 (d, J = 11.5 Hz, 1H), 4.69 (d, J = 11.0 Hz, 1H), 3.75 (t, J

= 9.2 Hz, 1H), 3.79 (s, 3H), 3.55-3.49(m, 3H), 3.41 (s, 3H), 3.38 (d, *J* = 3.6 Hz, 1H), 3.35-3.24 (m, 2H) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 159.5, 143.9(3C), 138.3, 130.3, 129.8(2C), 128.8(6C), 128.6(2C), 128.0, 128.1(2C), 127.9(6C), 127.1(3C), 114.0(2C), 98.0, 86.8, 81.4, 79.8, 75.3, 73.2, 71.6, 70.1, 63.9, 55.4, 55.2 ppm

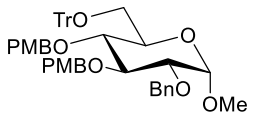
IR (KBr) νmax 3487, 3059, 3030, 2929, 2906, 2835, 1612, 1512, 1448, 1361, 1302, 1246, 1155, 1028 cm^{-1};

HRMS (m/z) for $C_{41}H_{42}O_7$ (M + Na) calcd: 669.2823 found: 669.2831

Melting point: 55.3 °C

$[\alpha]_D$ = + 54 ° (c = 0.63, Acetone)

Methyl 2-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl)-6-triphenylmethyl-*α*-D-glucopyranoside (BIX)



The compound was prepared by modified described procedure[26]. To a vacuum dried **(BVIII)** (1.00 g, 1.55 mmol) under argon atmosphere dry DMF (10 ml) was added, forming solution. Subsequently NaH (60% mineral oil suspension, 0.13 g, 2 equiv.) was added. The mixture was stirred for 15 minutes and after that *p*MeOBnCl (0.42 ml, 2 equiv.) and TBAI (1.17 g, 2 equiv.) were added. The solution was left to stir for 48 hours. After the noted duration no starting material was observed using TLC. The reaction was quenched with 5 ml of methanol and left to stir for 15 minutes. The mixture was subsequently diluted with DCM (100 ml) and washed two times with distilled water (50 ml) and brine (50 ml). Concentration in vacuo yielded yellowish oily residue. The residue was purified using silica gel column chromatography (Hexane: EtOAc, 5:1 → 3:1), yielding yellowish oil, 0.94 g, 78,9%. R_f = 0.36 (TLC Hexane: EtOAc, 4:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49-7.21 (m, 22H), 6.91-6.86 (m, 6H), 4.91-4.55 (m, 6H), 4.23 (d, *J* = 10.1, 1H), 3.95 (t, *J* = 8.6 Hz, 1H), 3.81 (s, 3H), 3.79 (s, 3H), 3.66 – 3.54 (m, 2H), 3.51-3.36 (m, 2H), 3.45 (s, 3H), 3.17 (dd, *J* = 10.0, 4.9, 1H) ppm

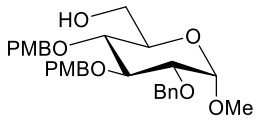
^{13}C NMR (100 MHz, $CDCl_3$) δ 159.3, 159.2, 144.1(3C), 138.5, 131.1, 129.9(2C), 129.8(2C), 128.9(6C), 128.5(2C), 128.1(2C), 128.0, 127.9, 127.8(6C), 127.0(3C), 113.9(2C), 113.7(2C), 98.0, 86.4, 82.1, 80.3, 77.9, 75.7, 74.7, 73.4, 70.4, 62.7, 55.4, 55.3, 55.0 ppm

IR (KBr) νmax 3059, 3030, 3005, 2929, 2835, 1734, 1612, 1512, 1448, 1358, 1302, 1246, 1178, 1159, 1034 cm^{-1};

HRMS (m/z) for $C_{49}H_{50}O_8$ (M + Na) calcd: 789.3398 found: 789.3405

$[\alpha]_D$ = +25 ° (c = 0.98, Acetone)

Methyl 2-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl)-*α*-D-glucopyranoside (BX)



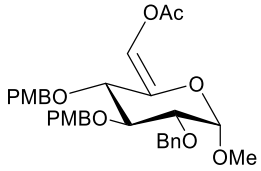
The compound was prepared by modified described procedure[14]. To a **(BIX)** (1.45 g, 1.89 mmol) methanol (50 ml) was added. The mixture was stirred for 10 minutes, forming white suspension. Then pTSA (monohydrate, 80 mg) was added by small portions (20 mg 0.05 equiv.) to reduce the pH value to 2-3 and the reaction was stirred under argon atmosphere. After 2 hours the reaction was monitored using TLC, resulting in observation of the starting material and product. TLC after next 24 hours resulted in observation of complete disappearance of the starting material thus the reaction was considered done. $NaHCO_3$ (500 mg) was added to the colorless reaction mixture and stirred for 15 minutes. After that the mixture was concentrated in vacuo and purified using silica gel column chromatography (Hexane: EtOAc, 3:1 → 1.5:1), yielding yellowish oil, 0.91 g, yield = 91.9%. R_f = 0.39 (TLC Hexane: EtOAc, 1:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36-7.20(m, 9H), 6.88-6.84(m, 4H), 4.92-4.56(m, 6H), 4.55 (d, J = 3.4 Hz, 1H), 3.97 (t, J = 9.3 Hz, 1H), 3.80(s, 3H), 3.82-3.74 (m, 2H), 3.79(s, 3H), 3.67-3.63(m, 1H), 3.49-3.43(m, 2H), 3.35(s, 3H) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 159.5, 159.3, 138.3, 131.1, 130.4, 129.8(2C), 129.7(2C), 128.6(2C),128.2(2C), 128.0, 114.0(2C), 113.9(2C), 98.3, 81.8, 80.1, 77.3, 75.5, 74.7, 73.5, 70.7, 62.1, 55.4, 55.4, 55.3 ppm

The spectra are consistent with the literature[38].

Methyl (*Z*)-2-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl)-6-O-acetyl-α-D-glucos-5-enopyranoside (BXI)



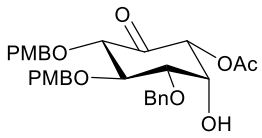
The compound was prepared by modified described procedure[14,39]. Vacuum dried **(BX)** (0.91 g, 1.735 mmol) was dissolved in dry DCM (1 ml). Then DCM (20 ml) was added to separate flask. The flask with pure DCM (20 ml) was cooled down to -78 °C using dry ice/ethanol bath. After that oxalyl chloride (0.21 ml, 1.4 equiv.) was added dropwise. Then dry DMSO (0.197 ml, 1.6 equiv.) was very slowly added dropwise. The mixture was left to stir for 15 minutes. After that **(BX)** was added dropwise and the mixture was left to stir for 1 hour. Then TEA (0.73 ml, 3 equiv.) was added dropwise. After the addition and 5 minutes of stirring the mixture was allowed to reach room temperature and the reaction was subsequently left to react for 1 hour. Then the reaction was quenched with 10 ml of saturated $NaHCO_3$ solution and stirred for 5 minutes. Smell of dimethyl sulfide was observed, indicating that reaction took place. After that the mixture was diluted with DCM (50 ml) and washed with (25 ml) $NaHCO_3$, distilled water and brine. The organic DCM phase was then dried using anhydrous sodium sulfate, evaporated in vacuo and further dried in vacuo. The yellowish oily residue was then dissolved in dry MeCN (28 ml). Anhydrous K_2CO_3 (2.4 g, 10 equiv.) was added, and the mixture was stirred for 5 minutes. After that Ac_2O (1.64 ml, 10 equiv.) was added slowly and the apparatus was flushed several times with argon. The reaction was heated to 95 °C for one day under argon atmosphere. After that the reaction mixture was diluted with DCM (50 ml) and washed 3x with saturated $NaHCO_3$ solution, distilled water and brine. After that the organic DCM layer was dried using anhydrous sodium sulfate and then evaporated in vacuo. Then silica gel column chromatography (Hexane: EtOAc, 4:1 → 3:1) was done affording 320 mg of yellowish oil. Yield: 32.6%. R_f = 0.28 (TLC Hexane: EtOAc, 3:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37-7.24(m, 9H), 7.16 (d, *J* = 1.5 Hz, 1H), 6.89-6.84(m, 4H), 4.86-4.60(m, 7H), 3.95-3.90(m, 2H), 3.81(s, 3H), 3.80(s, 3H), 3.58-3.54(m, 1H), 3.46(s, 3H), 2.15(s, 3H) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 167.4, 159.4, 159.3, 138.1, 135.2, 130.8, 129.9, 129.7(4C), 128.6(2C), 128.2(2C), 128.1, 123.1, 114.0(2C), 113.9(2C), 99.9, 81.1, 79.1, 77.6, 75.5, 74.2, 73.8, 56.3, 55.4, 55.3, 20.7 ppm

The spectra are consistent with the literature[38].

(1*R*,2*R*,3*S*,4*R*,5*S*)-3-benzyloxy-2-hydroxy-4,5-bis(*p*-methoxybenzyloxy)-6-oxocyclohexylacetate (BXII)



The compound was prepared by described procedure[40].To a vacuum dried **(BXI)** (151 mg, 0.267 mmol) acetone (5.12 ml) and distilled water (1.28 ml) were added, forming clear solution. Then mercury(II) acetate (0.127 mg, 1.5 equiv.) was added, suddenly the reaction mixture turned to yellow color. The mixture was left to stir for 24 hours in argon atmosphere and the reaction mixture was monitored using TLC. Small amount of starting material was observed. After 24 more hours of reaction the TLC analysis still showed small amount of starting material. The mixture was cooled down to 0 °C using water/ice bath and brine (0.8 ml) was added, the mixture turned to greyish color. The mixture was left to stir for 2 hours at room temperature. The mixture was diluted with DCM (50 ml) and washed with $Na_2S_2O_3$ solution (15 ml) and water (15 ml). Then the organic layer was dried using anhydrous sodium sulfate and evaporated in vacuo. The resulting oil was purified using silica gel column chromatography (Hexane: EtOAc, 2:1→ 1:1), yielding 45 mg of white amorphous solid, yield = 30.8%, R_f = 0.48 (TLC Hexane: EtOAc, 1:1).

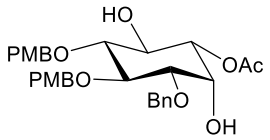
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34-7.21 (m, 9H), 6.87-6.83 (m, 4H), 5.16 (dd, *J* = 2.8, 1.2 Hz, 1H), 4.84 (d, J = 10.8 Hz, 1H), 4.82 (d, J = 10.3 Hz, 1H), 4.77 (d, J = 11.4 Hz, 1H), 4.73(d, J = 11.5 Hz, 1H), 4.72 (d, J = 11.4 Hz, 1H), 4.47 (d, *J* = 11.0 Hz, 1H), 4.32 (t, *J* = 2.7

Hz, 1H), 4.11 (dd, *J* = 9.4, 1.1 Hz, 1H), 4.04 (t, *J* = 9.2 Hz, 1H), 3.84 (d, *J* = 2.5 Hz, 1H), 3.81(s, 3H), 3.80(s, 3H), 2.23(s, 3H) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 197.9, 169.8, 159.4, 159.3, 137.3, 130.4, 129.9(2C), 129.7(2C), 129.5, 128.6(2C), 128.2, 128.0(2C), 113.8(2C), 113.7(2C), 83.2, 81.5, 78.9, 75.8, 74.9, 73.4, 73.2, 69.4, 55.3, 55.2, 20.5 ppm

The spectra are consistent with the literature[38].

1-*O*-acetyl-3-*O*-benzyl-4,5-di-*O*-(*p*-methoxybenzyl)-*myo*-inositol (BXIII)



The compound was prepared by described procedure[14].To a vacuum dried **(BXII)** (35 mg, 0.063 mmol) was added dry MeCN (1.23 ml). The mixture was stirred producing clear solution and cooled down to 0 °C with the ice/water bath. Subsequently $NaBH(OAc)_3$ (134.8 mg, 10 equiv.) and glacial acetic acid (0.33 ml) were added, producing clear yellowish solution. The solution was stirred at the room temperature for one hour. The reaction mixture was then analysed using TLC (Hexane: EtOAc, 1:1), resulting in observation of the new major spot and unreacted starting material. The reaction was left to react for one more hour. No starting material was observed using TLC afterwards. Saturated solution of Rochelle salt (0.8 ml) was slowly added, producing white suspension. This suspension was stirred for 30 minutes and was diluted with DCM (25 ml) afterwards. After that the mixture was washed (10 ml) with saturated $NaHCO_3$ solution, distilled water and brine. Then the organic DCM phase was dried using anhydrous sodium sulfate and evaporated in vacuo subsequently. The resulting yellowish oil was purified using silica gel column chromatography (Hexane: EtOAc, 3:1→ 1:1.5), yielding 20 mg of white amorphous solid, yield = 57.2%, R_f = 0.21 (TLC Hexane: EtOAc, 1:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36-7.24 (m, 9H), 6.89-6.85 (m, 4H), 4.89 (d, *J* = 11.0 Hz, 1H), 4.85 (d, *J* = 10.4 Hz, 1H), 4.79 (d, *J* = 10.4 Hz, 1H) 4.74-4.65 (m, 4H), 4.28 (t, *J* = 2.8 Hz, 1H), 4.08 (dd, *J* = 10.3, 9.3 Hz, 1H), 3.91 (t, *J* = 9.5 Hz, 1H), 3.81 (s, 3H), 3.80 (s, 3H), 3.55 (dd, *J* = 9.5, 2.7 Hz, 1H), 3.34 (t, *J* = 9.4 Hz, 1H), 2.16 (s, 3H) ppm

^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 159.5, 159.3, 137.5, 130.7, 130.6, 129.7(2C), 129.7(2C), 128.7 (2C), 128.2, 127.9 (2C), 114.1 (2C), 113.9 (2C), 82.6, 80.8, 80.3, 75.6, 75.3, 73.1, 73.0, 70.3, 67.9, 55.4, 55.4, 21.2 ppm

$[\alpha]_D$ = -3 ° (c = 0.70, $CHCl_3$)

The spectra and optical rotation are consistent with the literature[38].

6. Conclusion

Two synthetic approaches to synthesize selectively protected *myo*-inositol unit derivative were elaborated.

The synthetic approach using achiral *myo*-inositol started with cyclohexylidene acetal protection. Due to the loss of plane of symmetry the reaction afforded mixture of regioisomers, each with its enantiomer. The enantiomers of the desired regioisomer were converted into diasteromers using chiral derivatizating agent. The two diastereomers could not be separated by crystallization using various techniques and solvents. Despite having less reaction steps, this approach was abandoned.

The intermediate **(BXIII)** was successfully synthesized in 13 steps starting from already chiral and widely available D-glucopyranose. This approach vastly outnumbered the first approach in number of steps. The key reaction of this approach was Ferrier carbocyclization, which converted D-glucopyranose intermediate into *myo*-inositol derivative.

The aims of the work were fulfilled.

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Foremost, I would like to express my sincere gratitude to my supervisor Mgr. Martin Kamlar, Ph.D. for his continuous guidance, broad knowledge, support, and essential lab tips. I would also like to thank Assoc. Prof. RNDr. Jan Veselý, Ph.D. for invaluable chemical insights, making the thesis possible and accepting me to his prestige group. My big thank also goes to fellow lab mates for their help with lab matters, especially to Mgr. Ladislav Lóška.

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Finally my deepest gratitude to my loving and supporting parents.

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