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Novel Substrates and Inhibitors of Inositol-Binding Enzymes

by Lawrence W. Leung

A dissertation submitted to the Graduate Faculty in Biochemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

1997

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This manuscript has been read and accepted for the Graduate Faculty in Biochemistry in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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Finally, I thank the members of my supervisory committee for reviewing this dissertation.

Abstract

Novel Substrates and Inhibitors of

Inositol-Binding Enzymes

by Lawrence W. Leung

Advisor: Professor Robert Bittman

An efficient synthesis of an optically active inositol derivative that is a precursor to D-*myo*-inositol 1,4,5-trisphosphate (D-IP₃) is described. Regioselective crystallization of inositol diastereomers **D4P** and **L4M** from methanol followed by alkaline hydrolysis gives the two enantiomers (-)-6-O-benzyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol and (+)-4-O-benzyl-1,2:5,6-di-O-isopropylidene-*myo*-inositol. The ease with which these enantiomerically pure inositol derivatives were isolated facilitates the synthesis of more complex inositol phosphate derivatives such as D-*myo*-inositol 1,3,4,5-tetrakisphosphate. The preparation of an activated inositol phosphate (**3HP**) and its use in the synthesis of a fluorescent analog of phosphatidylinositol and phosphatidylinositol 4,5-bisphosphate is also described.

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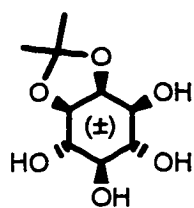
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List of Abbreviations

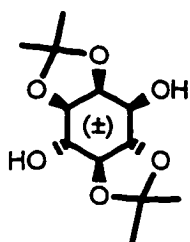
1. All, allyl
2. Bn, benzyl
3. BnBr, benzyl bromide
4. BnCl, benzyl chloride
5. CAN, ceric(IV) ammonium nitrate
6. DAG, 1,2-diacyl-*sn*-glycerol
7. DCC, 1,3-dicyclohexylcarbodiimide
8. DMAP, 4-dimethylaminopyridine
9. DME, 1,2-dimethoxyethane
10. DMF, *N,N*-dimethylformamide
11. Fraser-Reid reagent, dibenzyl-*N,N*-diisopropylphosphoramidite
12. LAH, lithium aluminum hydride
13. mCPBA, *meta*-chloroperoxybenzoic acid
14. MSNT, 1-(2-mesitylenesulfonyl)-3-nitro-1,2,4-triazole
15. NMR, nuclear magnetic resonance
16. NPCl, 5,5-dimethyl-2-oxo-2-chloro-1,3,2-dioxophosphorinan
17. *p*-TsOH, *p*-toluenesulfonic acid, monohydrate
18. PI, phosphatidylinositol
19. PI-PLC, phosphatidylinositol-specific phospholipase C
20. PIP₂, phosphatidylinositol 4,5-bisphosphate
21. PIP₃, phosphatidylinositol 3,4,5-trisphosphate

- 22. PKC, protein kinase C
- 23. PPTS, pyridinium *p*-toluenesulfonic acid
- 24. Prop, propenyl
- 25. py, pyridine
- 26. rt, room temperature
- 27. TBDMS, *tert*-butyldimethylsilyl
- 28. TBDMSCl, *tert*-butyldimethylsilyl chloride
- 29. THF, tetrahydrofuran

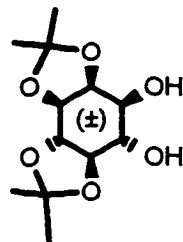
List of Structures



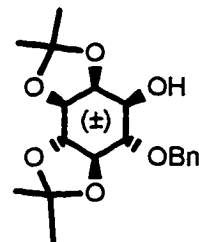
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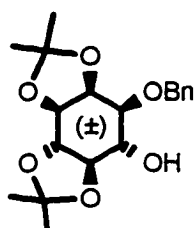
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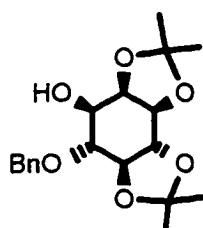
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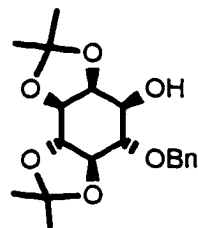
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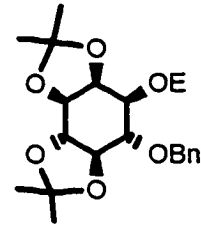
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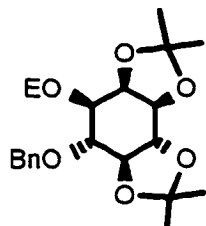
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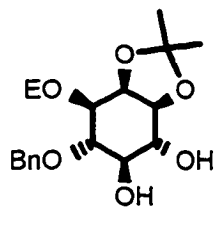
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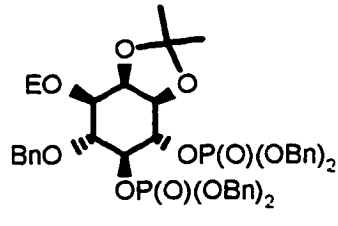
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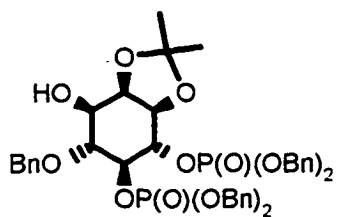
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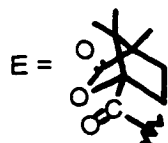
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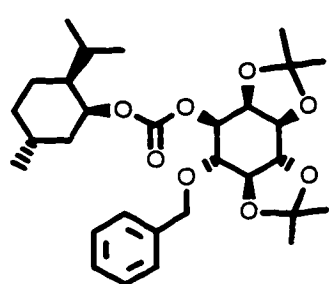
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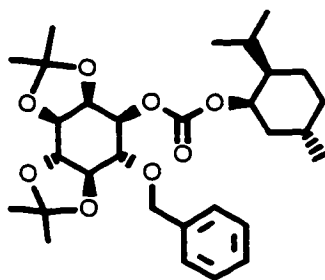
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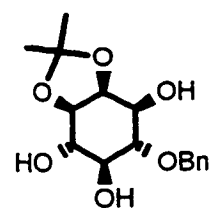
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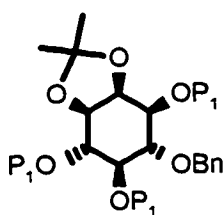
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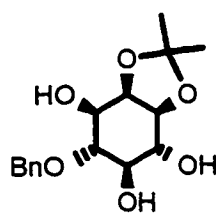
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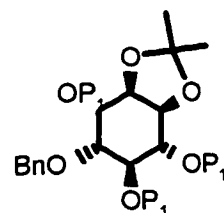
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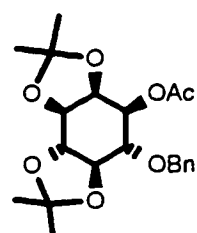
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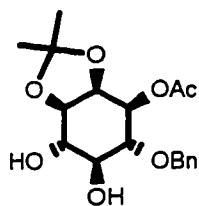
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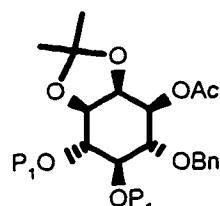
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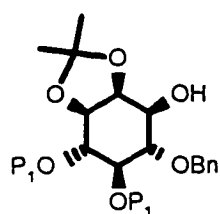
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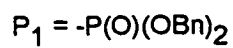
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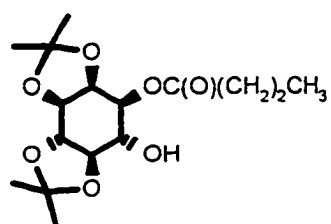


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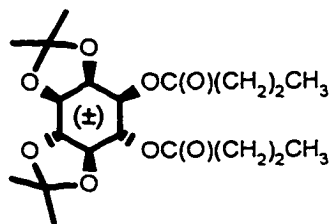


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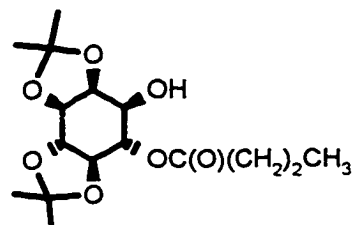




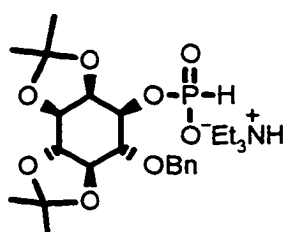
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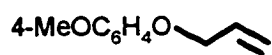
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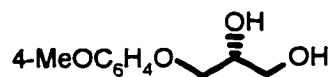
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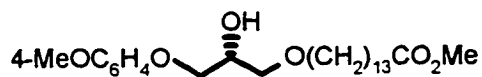
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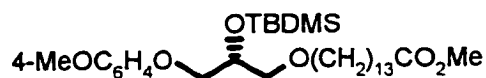
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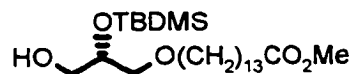
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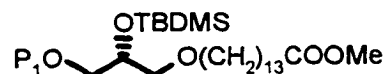
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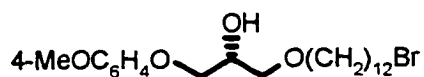
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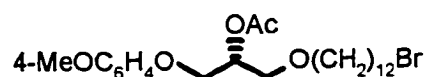
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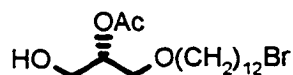
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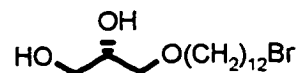
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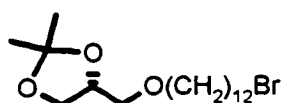
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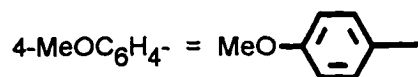
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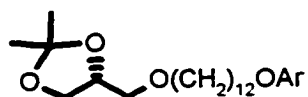


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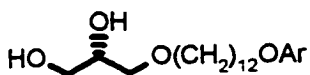


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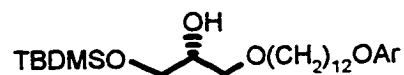




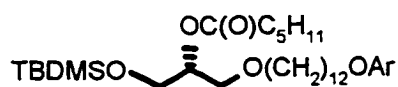
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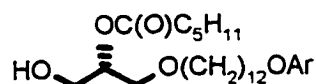
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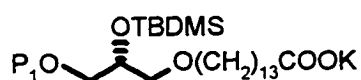
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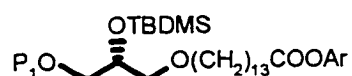
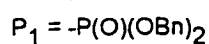
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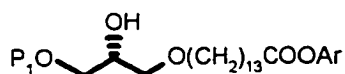
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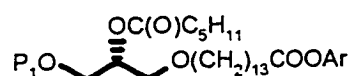
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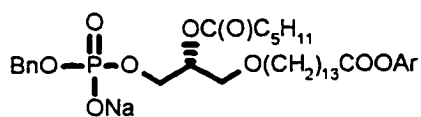
3.18



3.19

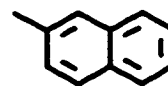


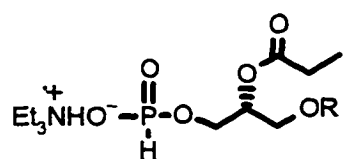
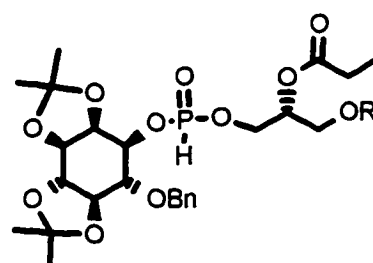
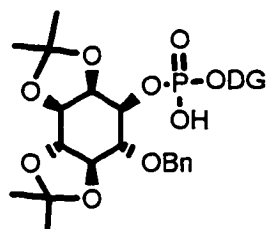
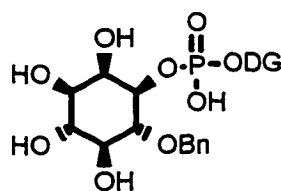
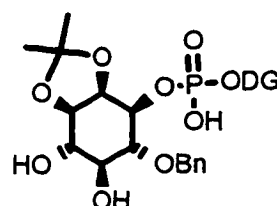
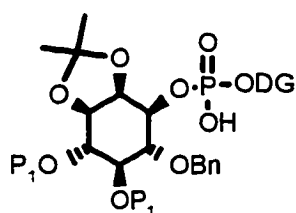
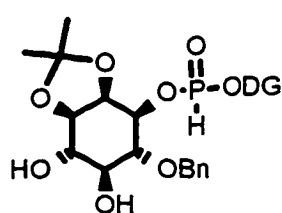
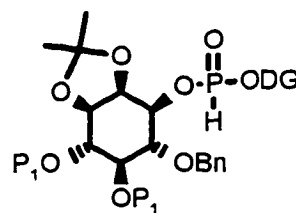
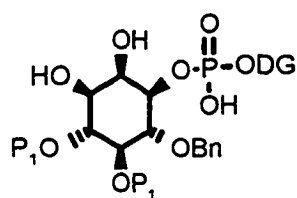
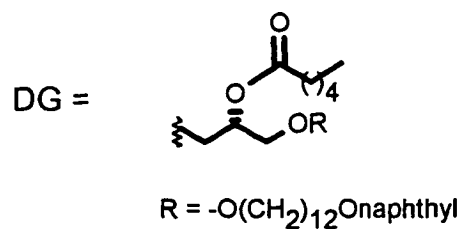
3.20



3.21

Ar =



**3.16HP****4.1****4.2****4.3a****4.3b****4.4**
 $P_1 = -P(O)(OBn)_2$
**4.5****4.6****4.7**

Chapter 1. Introduction

In the last decade, inositol based lipids have become even more important in biochemical pathways than initially proposed by Streb *et al.* (1983) and Nishizuka (1984). Their work centered on biochemical signal transduction involving phosphatidylinositol 4,5-bisphosphate (PIP₂) a substrate for a family of phosphatidylinositol phospholipase C (PI-PLC) isoenzymes. Hydrolysis of PIP₂ results in the formation of diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP₃), two intracellular second messengers. In addition to this canonical pathway of PIP₂ hydrolysis, a new inositol lipid, phosphatidylinositol 3,4,5-trisphosphate (PIP₃), is involved in a separate signaling pathway (reviewed by Cantley *et al.*, 1991). More recently, novel phosphatidylinositols have been discovered in membrane anchors for various membrane associated proteins (reviewed by Low, 1989, and Englund, 1993). In order to better understand the biochemical processes, we have undertaken the synthesis of analogs of these inositol lipids. These new substrates and inhibitors exhibit interesting and useful biochemical properties.

The early history of inositol-containing lipids

In contrast to the current rapid progress in this field, the initial discoveries into the chemistry and biochemistry of inositol-containing lipids proceeded very slowly. The biochemical significance of inositol lipids was not realized until

several years after their initial discovery (Folch and Wooley, 1942; Folch, 1949). Dawson (1954) observed that when guinea pig brain tissue was incubated with ^{32}P labeled orthophosphate, the radiolabel was incorporated into an inositol-containing lipid to a greater extent than into phosphatidylcholine or phosphatidylserine. A year later, it was noted that when acetylcholine was added to the incubation medium, the incorporation of radiolabel into the inositol-containing lipid was further enhanced (Hokin and Hokin, 1955). At the time, the structure of the inositol lipid was obscure, despite the fact that a structure had been proposed (albeit incorrectly) for the diphosphoinositide initially isolated by Folch. The successful determination of the structures of inositol lipids remained unclear until Dittmer and Dawson (1960) reexamined the brain extract used by Folch and determined that it contained three inositol lipids, including a new triphosphoinositide. Realizing that the acidic extract used by Folch would cause phosphate migration in this mixture of inositides, Brockerhoff and Ballou (1961) demonstrated that the phosphodiester must be between the glycerol backbone and the inositol 1-hydroxy position, and that the phosphate monoesters were at the 4- and 5-hydroxy positions in the di- and tri-phosphoinositide, respectively. A biochemical pathway in which sequential phosphorylation of the newly named phosphatidylinositol (PI) resulted in phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate was also shown. Previously, the biochemical synthesis of PI from inositol and

CDP-diacylglycerol was demonstrated (Agranoff *et al.*, 1958; Paulus and Kennedy, 1958).

Despite these advances, and the proposal of a metabolic cycle involving PI and phosphatidic acid (Hokin and Hokin, 1964; Durell and Garland, 1969), the role of inositol lipids in the cellular response to hormonal stimulation was not clear. Numerous hormones were shown to cause the release of calcium from intracellular stores, resulting in a rise in intracellular calcium ion concentration ($[Ca^{2+}]_i$) concomitant with their physiological effect. This prompted Michell (1975) to propose his "calcium gate" hypothesis in which hydrolysis of PI to inositol and DAG triggered the increase of $[Ca^{2+}]_i$. However, the hypothesis could not account for the experimental observation that the hormone concentrations that caused maximum physiological response (and increase in $[Ca^{2+}]_i$) did not cause the maximum PI hydrolysis. The finding that hydrolysis of PIP_2 (to DAG and IP_3) in iris smooth muscle in response to acetylcholine (Abdel-Latif *et al.*, 1977) eventually led to the revised hypothesis that PIP_2 hydrolysis was the event that triggered calcium mobilization. Moreover, it was shown that PIP_2 hydrolysis in rat hepatocytes in response to vasopressin occurred within 1 minute and preceded the rise in $[Ca^{2+}]_i$ (Kirk *et al.*, 1981).

The understanding of the role of the inositol lipids in transducing extracellular signals came when Streb *et al.* (1983) demonstrated that introduction of IP_3 to permeabilized pancreatic cells resulted in the release of intracellular calcium. At the same time, DAG, the lipophilic product of PIP_2

hydrolysis, was shown to activate protein kinase C (PKC) in conjunction with acidic phospholipids (Nishizuka, 1984). Thus, the link between the binding of cell surface hormone receptors and intracellular response was made.

Eventually, the intracellular store of calcium was identified as the endoplasmic reticulum, and the receptor for IP_3 was isolated from endoplasmic reticulum as well (for a review, see Clapham, 1995). Fueled by the interest in PIP_2 , investigators found several other inositol-containing lipids such as phosphatidylinositol 3-phosphate and glycosylphosphatidylinositol. The biochemical significance of these new lipids has been an area of intense research in the past few years.

Organic synthesis of inositol derivatives

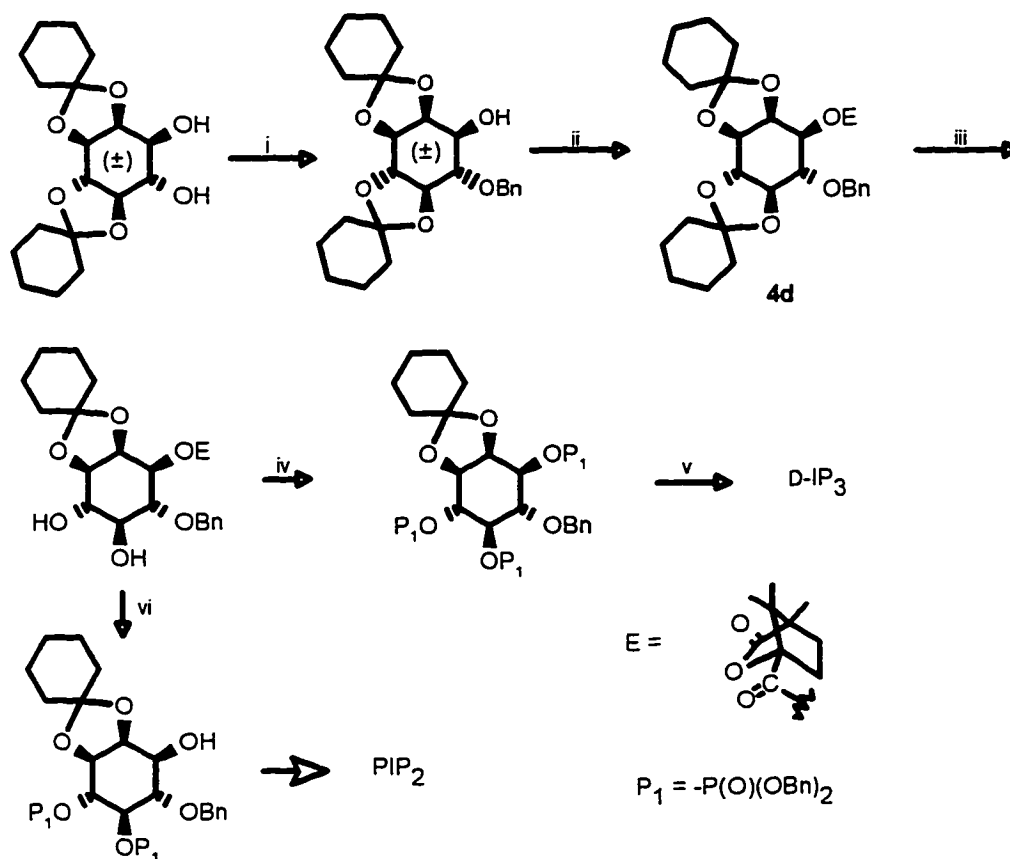
In order to better understand these biochemical processes in which inositol lipids are involved, the synthesis of analogs of these inositol lipids has undergone renewed interest. In particular, fluorescent analogs of PIP_2 would be useful in developing assays to measure enzyme kinetics, or in providing information on the mechanism of hydrolysis (Williams and Katan, 1996). Fluorescent PI analogs could be used to monitor vesicular traffic and the movement of PI-anchored membrane proteins. Modified PI and PIP_2 molecules could also be used as ligands in affinity chromatography to isolate new enzymes involved in cellular trafficking and metabolism. The synthesis of such analogs,

however, would rely upon the successful synthesis of protected inositol derivatives.

The practical synthesis of a suitably protected inositol head group has been the key stumbling block in the preparation of biologically interesting phosphatidylinositols. Although the total synthesis of PIP_2 was reported in 1988 (Dreef *et al.*, 1988), the lengthy path employed in the preparation of a penta-protected inositol derivative required the successful separation of diastereomers by column chromatography. Following a method to produce IP_3 (Ozaki *et al.*, 1986), Dreef *et al.* synthesized the non-phosphorylated inositol portion of PIP_2 , coupled it to a diacylglycerol and then selectively deprotected the 4- and 5- hydroxy positions of the inositol ring to allow phosphorylation.

Other workers have since synthesized a similar penta-protected inositol derivative that can be used in the preparation of PIP_2 or IP_3 . The most promising of these is the method of Vaca *et al.* (1987, Scheme 1.1) and modifications introduced by other natural product chemists. These methods use enzymes or chromatography to separate inositol diastereomers. However, the rapid and convenient synthesis of an enantiomerically pure penta-protected inositol derivative crucial in the synthesis of PIP_2 as well as IP_3 has remained elusive. One of the difficulties in the synthesis is the separation of inositol regio- and stereo-isomers. In particular, the formation of the desired ($\pm$)-2,3:4,5-di-O-cyclohexylidene-*myo*-inositol and its separation from other inositol di-O-cyclohexylidenes by differential crystallization is not easily reproducible.

Scheme 1.1: Synthesis of D-IP₃ (Vacca *et al.*, 1989) and an inositol head group

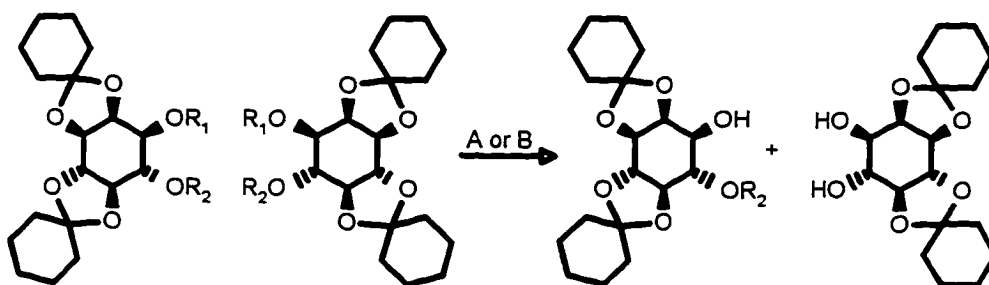


(i) (a) di-*n*-butyltin oxide/toluene, (b) CsF, BnBr/DMF; (ii) (a) (1*S*)-(-)-camphanic chloride, imidazole/CH₂Cl₂, (b) SiO₂ chromatography; (iii) HCl/EtOAc, MeOH; (iv) (a) LiOH/DME, H₂O, (b) KH/THF, (c) tetrabenzyl pyrophosphate; (v) 10% Pd/C, H₂/EtOH; (vi) (a) Fraser-Reid reagent, 1*H*-tetrazole/CH₂Cl₂, (b) mCPBA, (c) KOH.

Subsequent benzylation of the 6-hydroxy group of ($\pm$)-2,3:4,5-di-O-cyclohexylidene-*myo*-inositol proceeds with high yield, but the resolution of the racemates by esterification with (1*S*)-(-)-camphanic chloride followed by column chromatography is tedious because the two isomers migrate closely. A more convenient resolution method was developed by Guo *et al.* (1982, Scheme 1.2) in which the diol is regioselectively esterified with butyryl chloride and then deacylated enantioselectively with pig liver esterase. Although this approach provides a solution to the problem of separating inositol diastereomers, it still relies on the unreliably produced diol. The scheme also requires extensive use of silica gel chromatography in its early steps.

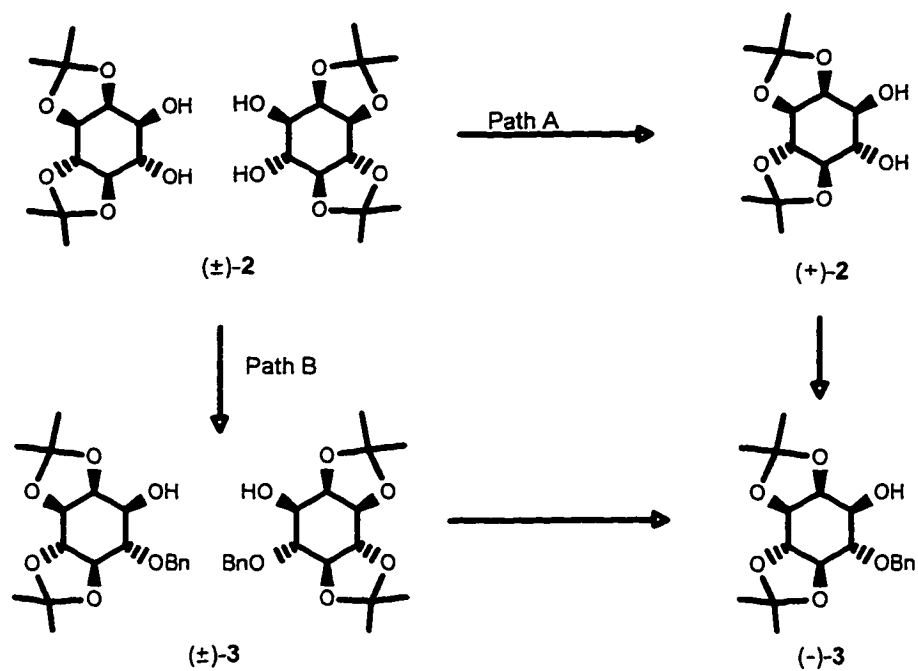
The synthesis of PI derivatives was also accomplished by Noda and Keenan (1990), who used a mixture of PI's (isolated from yeast) as the starting material, thus avoiding the problem of resolving racemic intermediates. As a model for reactions involving phosphatidylinositol, Noda and Keenan used *myo*-inositol and reported that ($\pm$)-2,3:4,5-di-O-isopropylidene-*myo*-inositol could be easily and reliably separated from its regioisomer(s) by differential crystallization. Subsequently, other research groups have reported modified procedures to synthesize the same diol. Although the separation of the enantiomers of this diol had not been attempted, the ease at which ($\pm$)-2,3:4,5-di-O-isopropylidene-*myo*-inositol can be synthesized relative to the corresponding cyclohexylidene derivative warranted its investigation.

Scheme 1.2: Resolution by enantioselective deacylation (Guo *et al.*, 1992)



Path A: $R_1 = R_2 = -COCH_3$, cholesterol esterase, Tween 80/ 0.1 M phosphate buffer, 168 h

Path B: $R_1 = H$, $R_2 = -COC_3H_7-n$, porcine pancreatic lipase/ 0.1 M phosphate buffer, 48 h

Scheme 1.3: General strategy to a PIP₂ head group

Eventually, a scheme to conveniently synthesize gram quantities of (-)-6-O-benzyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol ((-)-**3**) was developed (Scheme 1.3). This intermediate is a useful precursor for IP₃, PI, and PIP₂ syntheses. Another useful inositol derivative, (-)-4-O-*tert*-butyldimethylsilyl-2,3:5,6-di-O-isopropylidene-*myo*-inositol is prepared from a byproduct of one of the precursors of (±)-**3**, further enhancing the utility of this scheme.

These various inositol derivatives were used to prepare several different PI analogs.

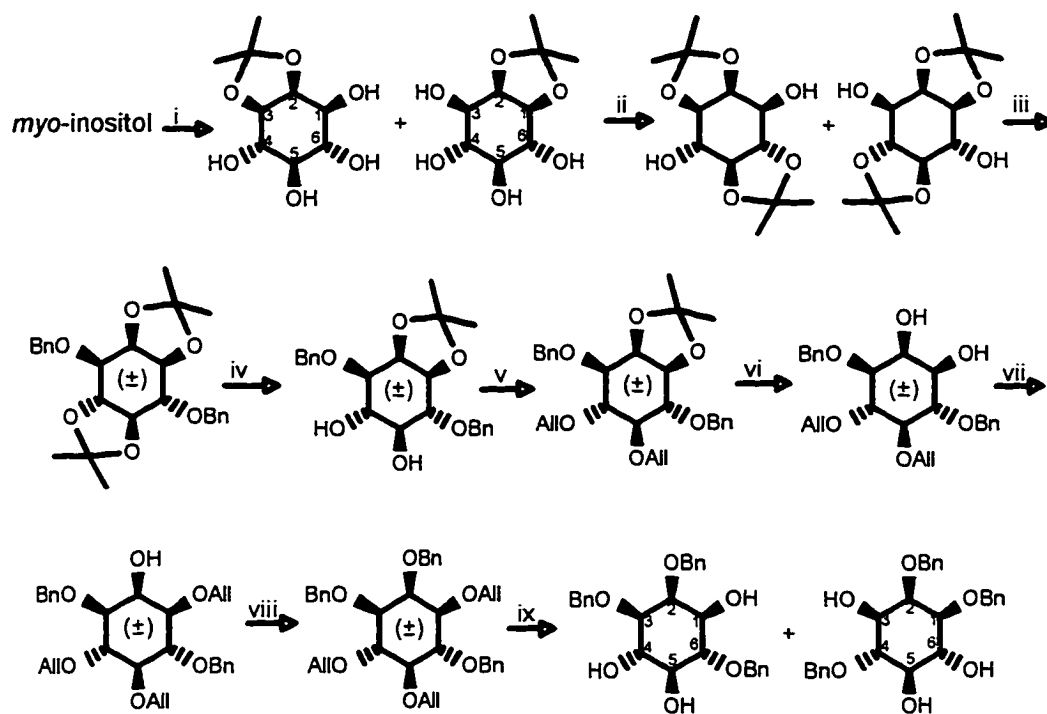
Chapter 2. Syntheses of Headgroups for PI and PIP₂

The synthesis of analogs of the metabolites of the inositol signaling pathway has been the goal of various workers in the last decade. Initial interest focused on phosphatidylinositol 4,5-bisphosphate, IP₃, and their analogs. Later, the discovery of other inositol lipids bearing phosphates at the C3-hydroxy position (such as phosphatidylinositol 3,4,5-trisphosphate) as well as glycosylphosphatidylinositol (a class of lipids used to anchor proteins to the plasma membrane) prompted the synthesis of a new set of lipid molecules as inhibitors and probes for the enzymes involved in these biochemical processes. Key to all these syntheses was the ability to produce large quantities of a suitably protected inositol molecule in a convenient fashion.

Previous syntheses of protected inositol derivatives

In one of the earliest efforts to synthesize a precursor to IP₃ (albeit in racemic form), Gigg *et al.* (1985, Scheme 2.1) used a diol initially isolated by Angyal *et al.* (1952). The nine-step synthesis relied upon the separation of (±)-2,3:5,6-di-*O*-isopropylidene-*myo*-inositol from a mixture of other di-*O*-isopropylidene-*myo*-inositol derivatives, and subsequent protection and deprotection steps to arrive at the racemic triol. In a similar synthesis, Ozaki *et al.* (1986) achieved the same triol (Scheme 2.2) in an optically active form following a synthetic route that used cyclohexylidene instead of isopropylidene protecting groups in the early steps. Cyclohexylidene derivatives of inositol

Scheme 2.1: Synthesis of an IP₃ precursor (Gigg *et al.*, 1985)



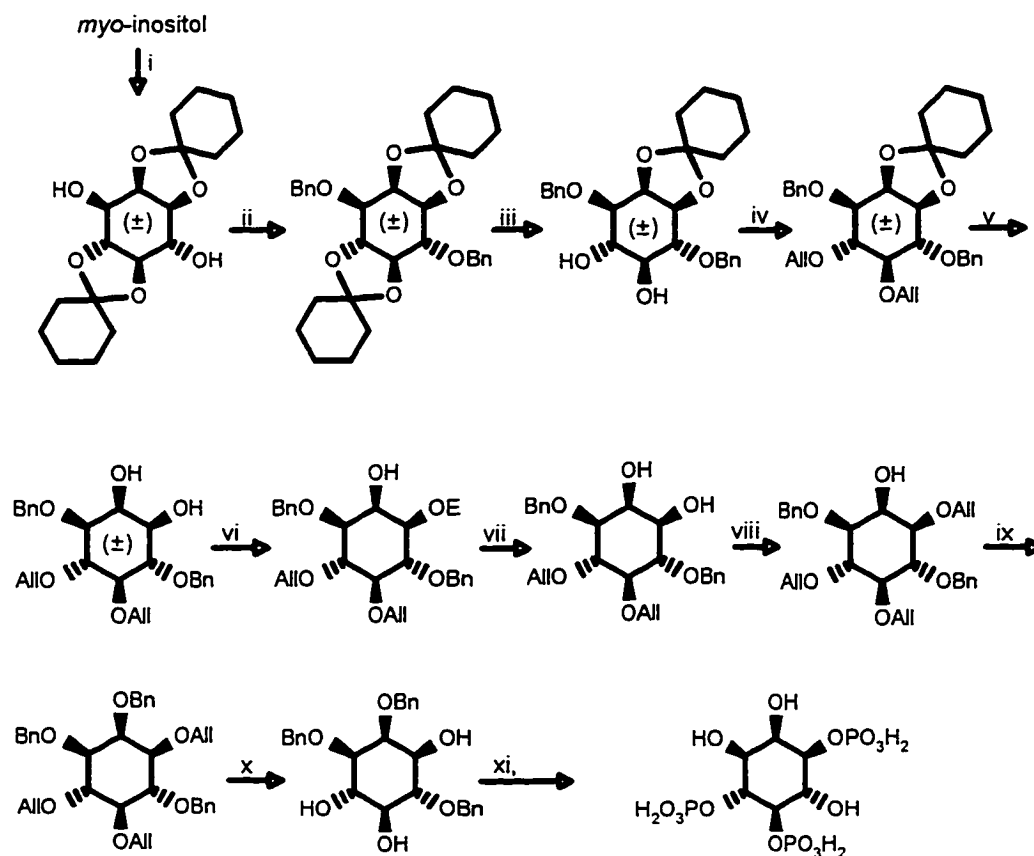
(i) *p*-TsOH/dimethoxypropane, DMSO; (ii) *p*-TsOH/acetone, dimethoxypropane;

(iii) BnBr, NaH/DMF; (iv) *p*-TsOH/acetone, H₂O; (v) AllylBr, NaH/DMF; (vi) AcOH/H₂O; (vii)

(a) di-*n*-butyltin oxide/MeOH, (b) AllylBr/DMF; (viii) BnBr, NaH/DMF; (ix) (a) KOBu-*t*/DMSO,

(b) H⁺. (Allyl = allyl, Bn = benzyl)

Scheme 2.2: Synthesis of D-IP₃ (Ozaki *et al.*, 1986)



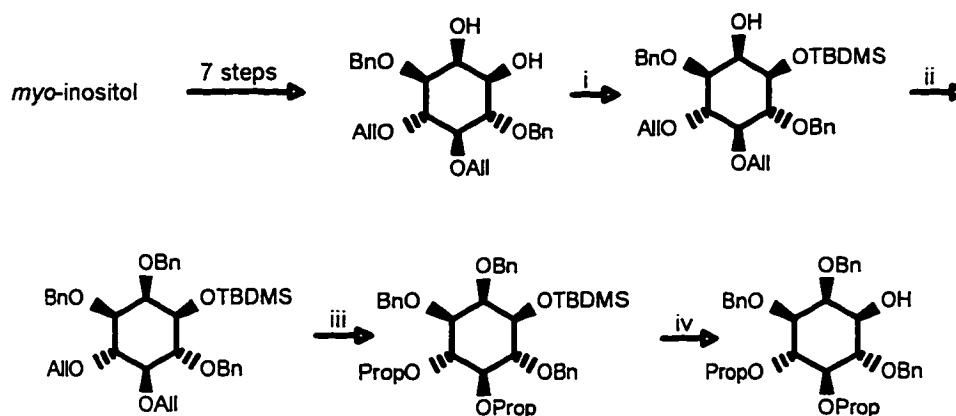
E = l-menthoxyacetyl

(i) *p*-TsOH/diethoxycyclohexane; (ii) BnCl, NaH/DMF; (iii) ethylene glycol, *p*-TsOH/CHCl₃; (iv) AlIBr, NaH/DMF; (v) AcOH/H₂O, 90 °C; (vi) (a) l-menthoxyacetyl chloride/py, (b) SiO₂ chromatography; (vii) NaOH/MeOH; (viii) AlIBr, NaOH/benzene; (ix) BnCl, NaH/DMF; (x) (a) RhCl(PPh₃)₃/DABCO, (b) HCl/MeOH; (xi) (PhNH)₂P(O)Cl, DMAP/py; (xii) (a) isoamyl nitrite/AcOH, py, Ac₂O, (b) H₂, 5% Pd-C/MeOH.

were also prepared by Angyal *et al.* (1961), who favored them over the isopropylidene derivatives because the former were isolatable in higher yield. Both of these schemes developed by Gigg and Ozaki could be modified to produce a head group suitable for the synthesis of PIP₂, but would require eleven synthetic steps starting from *myo*-inositol (Scheme 2.3). This approach was undertaken by Dreef *et al.* (1988) as part of the synthesis of PIP₂.

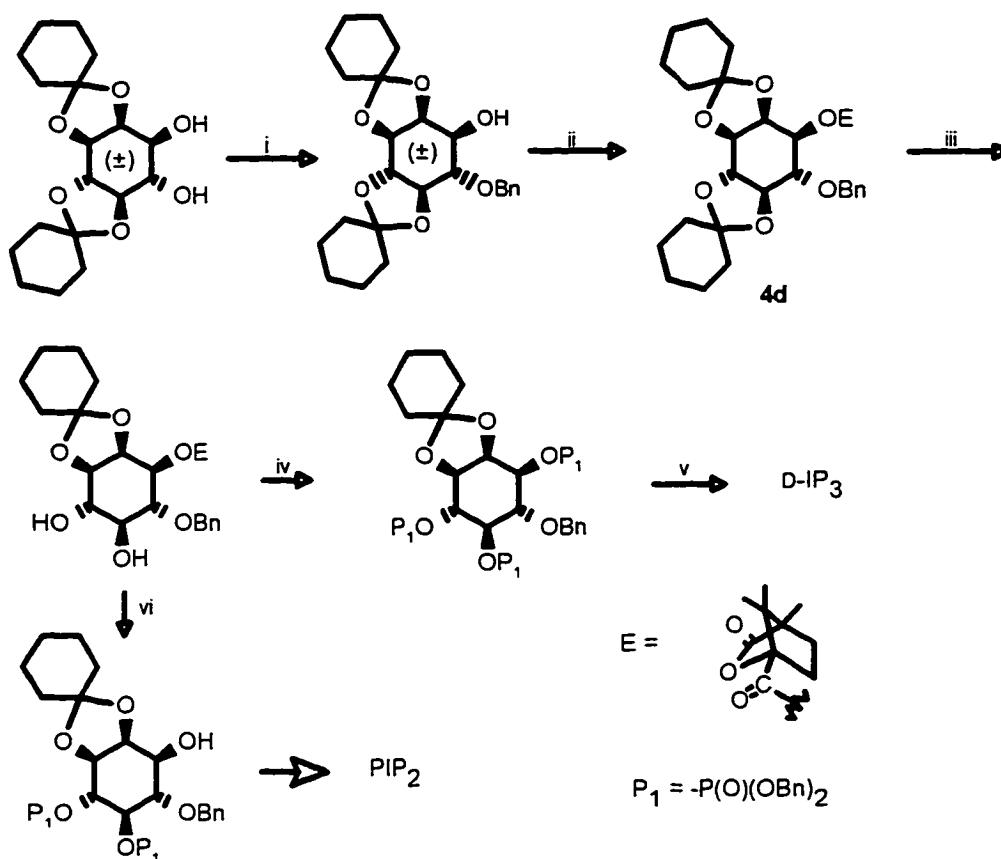
An easier route to IP₃, as well as a headgroup for PIP₂ (Scheme 2.4), began with (±)-6-*O*-benzyl-2,3:4,5-di-*O*-cyclohexylidene-*myo*-inositol, initially used by Garegg *et al.* (1984). Regioselective protection of the C6-hydroxy group of (±)-2,3:4,5-di-*O*-cyclohexylidene-*myo*-inositol was accomplished in two steps by first converting the diol to a di-*n*-butylstannylidene intermediate, which then reacted with benzyl bromide in the presence of cesium fluoride. The resulting penta-protected *myo*-inositol derivative was racemic, but Vacca *et al.* (1987) was able to separate the two enantiomers by converting them to diastereomers using (1*S*)-(-)-camphanic chloride and then separating them by column chromatography. The conversion of the diastereomer of interest (**4d**) to IP₃ was greatly facilitated by the fact that the C4- and C5-hydroxy groups can be deprotected without removing the acetal at the C2- and C3-hydroxy positions to give a 4,5-diol. Treatment with base resulted in a triol which was converted to IP₃. More importantly, isolation of the intermediate diol, phosphorylation, and alkaline hydrolysis of the camphanate would result in the formation of a molecule that could be coupled to a diacylglycerol to form a protected PIP₂.

Scheme 2.3: Synthesis of a PIP₂ head group (Dreef *et al.*, 1988)



- (i) *tert*-butyldimethylsilyl chloride/py; (ii) BnBr, NaH/DMF; (iii) 1,5-cyclooctadiene-*bis*[methyldiphenyl-phosphine]iridium hexafluorophosphate/1,2-dichloroethane; (iv) tetra-*n*-butylammonium fluoride.

Scheme 2.4: Synthesis of D-IP₃ (Vacca *et al.*, 1989) and an inositol head group



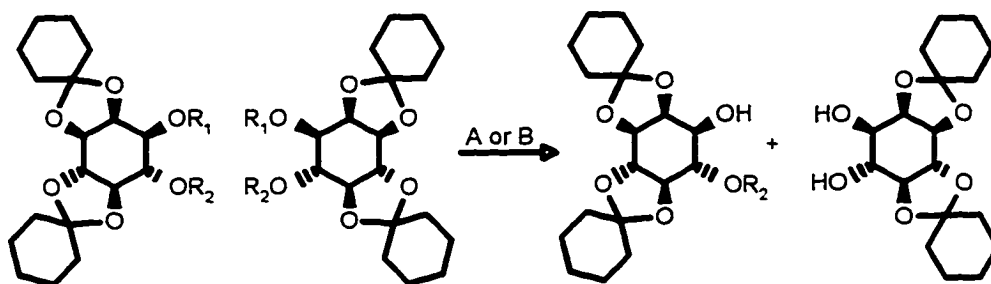
(i) (a) di-*n*-butyltin oxide/toluene, (b) CsF, BnBr/DMF; (ii) (a) (1*S*)-(-)-camphanyl chloride, imidazole/CH₂Cl₂, (b) SiO₂ chromatography; (iii) HCl/EtOAc, MeOH; (iv) (a) LiOH/DME, H₂O, (b) KH/THF, (c) tetrabenzyl pyrophosphate; (v) 10% Pd/C, H₂/EtOH; (vi) (a) Fraser-Reid reagent, 1*H*-tetrazole/CH₂Cl₂, (b) mCPBA, (c) KOH.

Disadvantages of previous syntheses of protected inositol derivatives

The major drawback of Vacca's route to IP_3 is the difficult separation of diastereomers by chromatography. One method of achieving the separation of enantiomers without resorting to chromatographic separation of diastereomers was by using enzymatic hydrolysis of racemic mixtures. Liu and Chen (1989) investigated the possibility of separating racemic *myo*-inositol esters by enantioselective enzymatic hydrolysis. Their initial findings demonstrated that $(\pm)$ -2,3:4,5-di-*O*-cyclohexylidene-*myo*-inositol could be converted to its corresponding racemic diacetate; only one enantiomer was shown to be hydrolyzed by cholesterol esterase over a one week period (Scheme 2.5, path A). The technique was later refined by converting the racemic diol to a monobutyrate (Guo *et al.*, 1992), which could be hydrolyzed enantiospecifically by the less costly porcine pancreatic lipase in a few hours (Scheme 2.5, path B). Separation of the enzyme products gave optically pure (96% ee) $(-)$ -6-*O*-butyryl-2,3:4,5-di-*O*-cyclohexylidene-*myo*-inositol, which was then converted to the same triol used by Vacca *et al.* (1987) to synthesize D- IP_3 .

Another disadvantage that both Scheme 2.4 and Scheme 2.5 suffer from is the reliance on $(\pm)$ -2,3:4,5-di-*O*-cyclohexylidene-*myo*-inositol. Several groups have reported difficulty in isolating this diol from the reaction mixture which contains two other inositol diols (Mereyala and Pannala, 1995; Angyal *et al.*, 1965). Surprisingly, no research group to date has employed the isopropylidene variant of $(\pm)$ -2,3:4,5-di-*O*-cyclohexylidene-*myo*-inositol to synthesize IP_3 or

Scheme 2.5: Resolution by enantioselective deacylation (Liu and Chen, 1989; Guo *et al.*, 1992)



Path A: $R_1 = R_2 = -COCH_3$, cholesterol esterase, Tween 80/ 0.1 M phosphate buffer, 168 h

Path B: $R_1 = H$, $R_2 = -COC_3H_7-n$, porcine pancreatic lipase/ 0.1 M phosphate buffer, 48 h

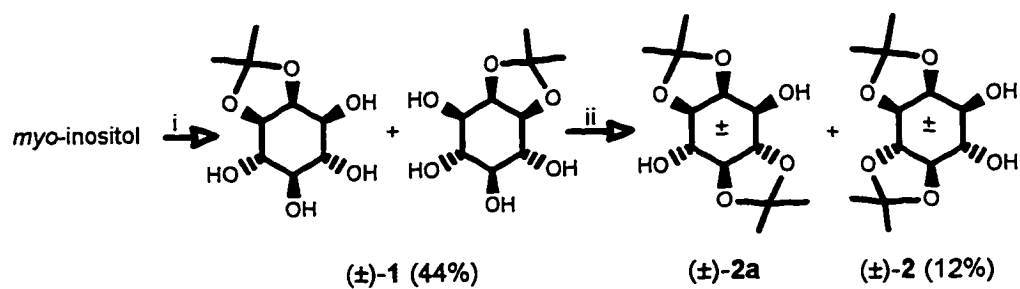
PIP₂, although one has recently reported a lengthy route to synthesize (-)-6-*O*-benzyl-2,3:4,5-di-*O*-isopropylidene-*myo*-inositol, which was then converted to IP₃ (Aguilo *et al.* 1992).

Isopropylidene derivatives of *myo*-inositol as possible starting materials for IP₃ and PIP₂

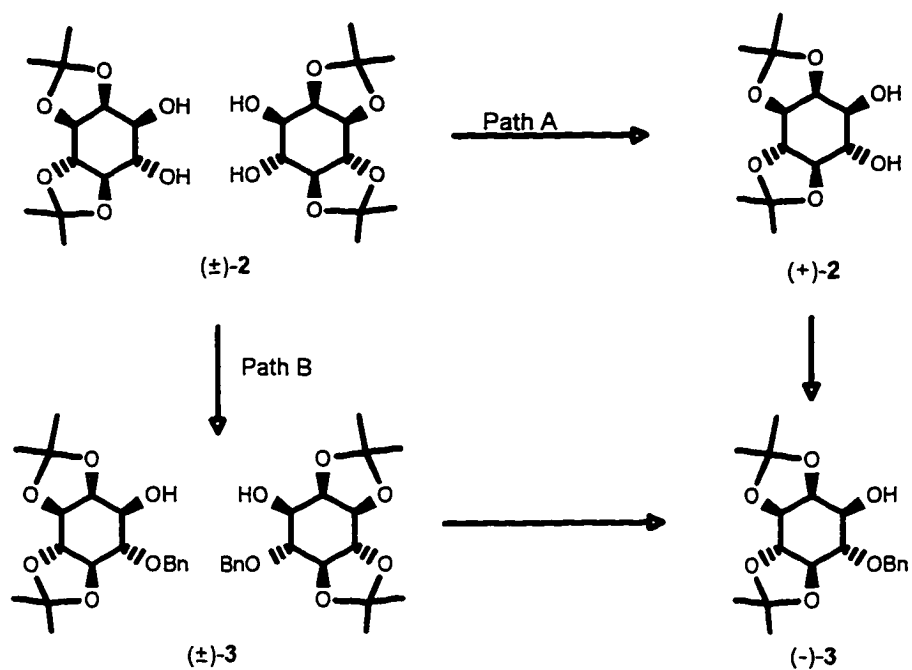
In their report of the synthesis of (±)-2,3:5,6-di-*O*-isopropylidene-*myo*-inositol ((±)-**2a**, Figure 1), Gigg *et al.* (1985) reported that the other di-*O*-isopropylidene-*myo*-inositols were formed, but not isolated. Noda and Keenan (1990) subsequently isolated (±)-2,3:4,5-di-*O*-isopropylidene-*myo*-inositol ((±)-**2**) from other inositol diols using fractional crystallization (Figure 1). Other workers have further improved upon the yield and ease of isolation of this diol (Desai *et al.*, 1994; Chung and Ryu, 1994; de la Pradilla *et al.*, 1990), but none reported the separation of the enantiomers of (±)-**2**.

The ease with which (±)-**2** can be synthesized relative to the corresponding cyclohexylidene derivative made it an attractive starting material for IP₃ and PIP₂ derivatives, but only if the separation of the enantiomers of the diol (or its monobenzyl derivative) could be accomplished. The published reports of Vacca *et al.* (1987) and Chen and co-workers (Liu and Chen, 1989; Guo *et al.*, 1992) demonstrated that this might be achieved by resolving the enantiomers of (±)-**2** (either enzymatically or by chromatographic separation of diastereomers, Scheme 2.6, path A) or the racemic monobenzylated derivative,

Figure 1: Synthesis and isolation of di-*O*-isopropylidene-*myo*-inositol (Noda and Keenan, 1990)



(i) *p*-TsOH/dimethoxypropane, DMSO; (ii) *p*-TsOH/dimethoxypropane, acetone.

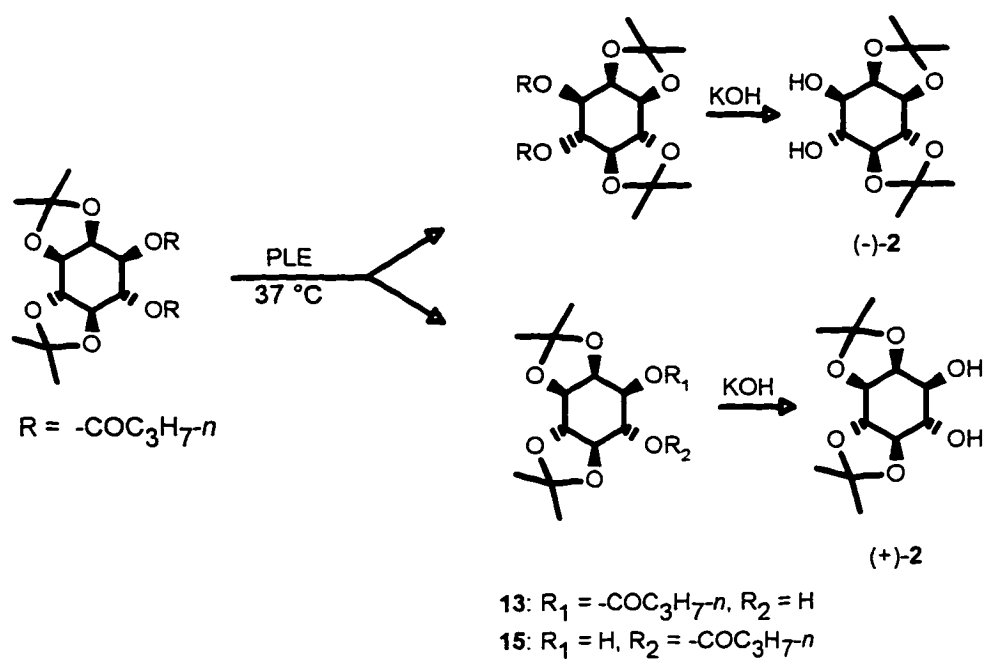
Scheme 2.6: General strategy to a PIP₂ head group

(±)-6-O-benzyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol ((±)-**3**) (by chromatographic separation of diastereomers, Scheme 2.6, path B). In either case, the goal was to discover a convenient method to prepare (-)-**3**.

myo-Inositol was converted to (±)-2,3:4,5-di-O-isopropylidene-*myo*-inositol ((±)-**2**) according to the methods published (Noda and Keenan, 1990; de la Pradilla *et al.*, 1990). In order to unambiguously distinguish (±)-**2** from (±)-2,3:5,6-di-O-isopropylidene-*myo*-inositol ((±)-**2a**, which is also formed), it was necessary to convert a small sample of each product to its corresponding diacetate and record their respective melting points (220 - 221 °C versus 158 - 159 °C) and ¹H NMR spectra.

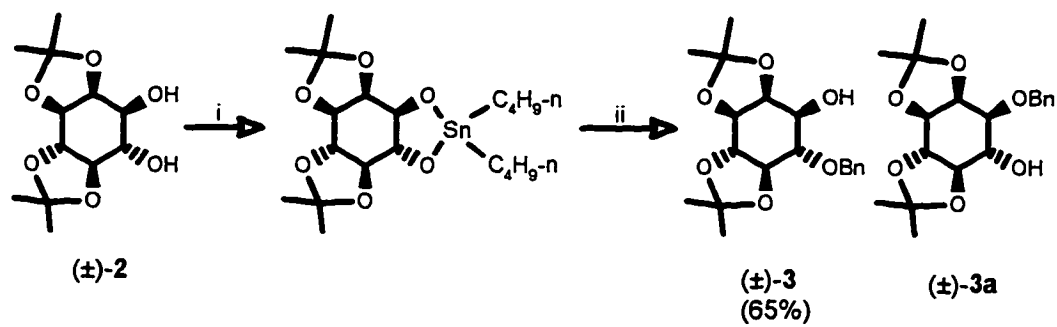
The diacetate of (±)-**2**, (±)-1,6-diacetyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol, proved not to be a substrate for either cholesterol esterase or pig liver esterase. Conversion of the diol to the corresponding bis-menthyl carbonates followed by silica gel chromatography, a technique that was partly successful in resolving di-O-cyclohexylidene-*myo*-inositols (Liu and Chen, 1989), failed to separate the two diastereomers. There was also no enzymatic hydrolysis of (±)-6-butyryl-2,3:4,5-di-O-isopropylidene-*myo*-inositol using pig liver esterase. However, conversion of (±)-**2** to (±)-1,6-dibutyryl-2,3:4,5-di-O-isopropylidene-*myo*-inositol and subsequent hydrolysis with pig liver esterase resulted in the formation of a mixture of monobutyrate (21% yield) and the starting material (41% yield) after 48 h at rt (Scheme 2.7). Saponification of the nonhydrolyzed starting material gave the diol (-)-**2** ($[\alpha]_D^{24} - 4.8^\circ$ (c 0.425, CHCl₃)),

Scheme 2.7: Resolution by enantioselective deacylation of ($\pm$)-14



whereas hydrolysis of the mixture of monobutyrate gave (+)-**2** ($[\alpha]_D^{24} +2.4^\circ$ (c 1.705, CHCl_3)). In view of the low yield of the monobutyrate, and the uncertainty of the enantiomeric excess indicated by the observed specific rotation measurements, the separation of the enantiomers of ($\pm$)-**3** was investigated.

Several methods were investigated to monobenzylate ($\pm$)-**2**, including phase-transfer conditions, Ag_2O in benzyl bromide, and thallos ethoxide with benzyl bromide. However, monobenzylation via a dibutylstannylene gave the best results (Figure 2). Conversion of ($\pm$)-**2** to the corresponding dibutylstannylene was accomplished by refluxing one equivalent of di-*n*-butyltin oxide with the diol in methanol until a clear solution formed, which took less than 1 h. After the solvent was removed *in vacuo*, powdered CsF (2 eq) was added to the solid residue and the mixture was left under vacuum for 3 h. A solution of benzyl bromide (2.5 eq) in DMF was added, and the mixture was stirred for 4 h. After an aqueous workup, the product ($\pm$)-**3** (60% yield) was separated from the undesired byproduct, ($\pm$)-1-*O*-benzyl-2,3:4,5-di-*O*-isopropylidene-*myo*-inositol (**3a**, 29% yield), by flash chromatography (elution with hexane/EtOAc 4:1). Acid hydrolysis of **3** and **3a** resulted in the known ($\pm$)-6-*O*-benzyl-*myo*-inositol (mp 165-167 °C, lit. (Garegg *et al.*, 1984) 165-167 °C) and ($\pm$)-1-*O*-benzyl-*myo*-inositol (mp 203-205 °C, lit. (*ibid.*) 203-206 °C), respectively.

Figure 2: Regioselective benzylation of ($\pm$)-2

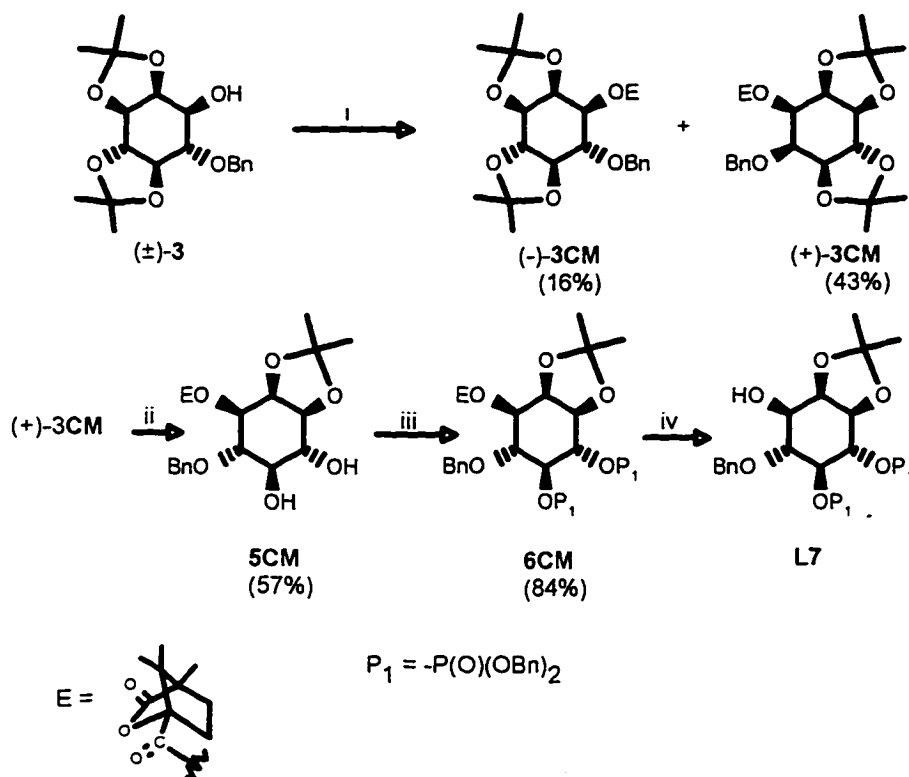
(i) di-*n*-butyltin oxide/MeOH reflux; (ii) CsF, BnBr/DMF.

Optical resolution of racemic isopropylidene-*myo*-inositol derivatives

Esterification of the C1-hydroxy position of ($\pm$)-**3** with (-)-camphanic acid with DCC and DMAP in CH_2Cl_2 gave a mixture of diastereomers ((+)-**3CM** and (-)-**3CM**) which could only be partially separated by column chromatography (Scheme 2.8). Alkaline hydrolysis of the isolated camphanates gave (+)-**3** ($[\alpha]_D^{24} +14^\circ$ (c 0.49, CHCl_3)) in 61% yield (from (+)-**3CM**) and (-)-**3** ($[\alpha]_D^{24} -20^\circ$ (c 0.32, CHCl_3); lit. (Aguilo *et al.*, 1992) $[\alpha]_D -16^\circ$ (c 0.9, CHCl_3)) in 56% yield (from (-)-**3CM**). To test the remainder of the synthetic scheme, the camphanate (+)-**3CM** was used instead of (-)-**3CM**. After mild acid hydrolysis of the *trans* 4,5-acetal, the conversion to a bis-dibenzylphosphate inositol derivative (**6CM**) was accomplished without difficulty. However, attempts to remove the camphanate ester with base (LiOH/MeOH , reflux) resulted in low yield due to phosphotriester hydrolysis. This drawback, in conjunction with the difficulty in isolating (-)-**3CM**, made this method of producing a PIP_2 headgroup unsuitable.

A more convenient method of isolating the two enantiomers involves using (+)- and (-)-menthyl chloroformate (Figure 3). Initially, it was observed that the reaction of 1.2 eq. of (-)-menthyl chloroformate with 1.0 eq. of ($\pm$)-**3** proceeded only partially, giving the expected monocarbonate in 47% yield, and the starting material (21% yield) with a specific rotation of -12.5° (c 1.02, CHCl_3), indicating that one enantiomer, (+)-**3**, was preferentially reacting to form the carbonate. Conversely, partial reaction between (+)-menthyl chloroformate and ($\pm$)-**3** was found to yield unreacted starting material enriched in (+)-**3**.

Scheme 2.8: Resolution of ($\pm$)-3 using camphanic chloride and subsequent conversion to L7 (model reaction)

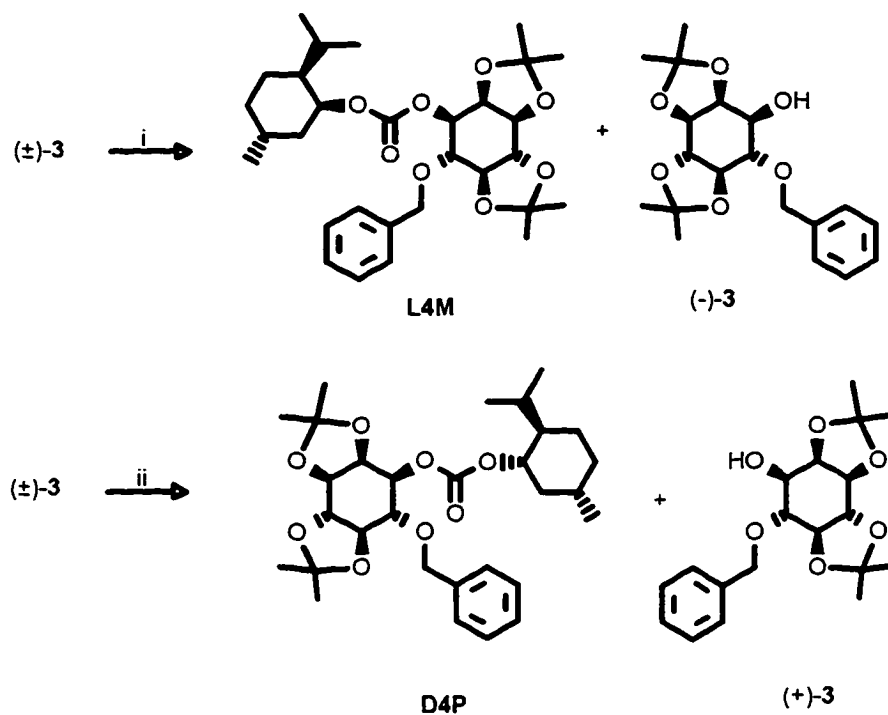


(i) (a) (1*S*)-(-)-camphanic chloride, DCC, DMAP/ CH_2Cl_2 ; (b) SiO_2 chromatography; (ii)

PPTS/ CH_2Cl_2 ; (iii) (a) Fraser-Reid reagent, 1*H*-tetrazole/ CH_2Cl_2 , (b) mCPBA; (iv)

$\text{K}_2\text{CO}_3/\text{MeOH}$.

Figure 3: Kinetic resolution of ($\pm$)-3 by formation of menthyl carbonates



(i) (a) (-)-menthyl chloroformate, py/CH₂Cl₂; (b) SiO₂ chromatography;

(ii) (a) (+)-menthyl chloroformate, py/CH₂Cl₂; (b) SiO₂ chromatography.

Despite this promising result, kinetic resolution of ($\pm$)-**3** did not prove to reliably produce significant amounts of enantiomerically (+)-**3** or (-)-**3**. Fortunately, a much simpler method was discovered (Figure 4). It was found that ($\pm$)-**3** could be quantitatively converted to the racemic carbonate by repeatedly evaporating it from pyridine or cyclohexane to effect removal of residual water. Crystallization of racemic carbonate **4P** (the product of ($\pm$)-**3** and (+)-menthyl chloroformate) in methanol gave enantiomerically pure **D4P**. Alkaline hydrolysis (refluxing KOH/MeOH) gave (-)-**3** ($[\alpha]_D^{24}$ -17.4° (c 1.69, CHCl₃)) in 29% yield from ($\pm$)-**3**. Crystallization of racemic **4M** (from ($\pm$)-**3** and (-)-menthyl chloroformate) gave enantiomerically pure **L4M**. Alkaline hydrolysis gave (+)-**3** ($[\alpha]_D^{24}$ +17.9° (c 0.71, CHCl₃)) in 26% yield from ($\pm$)-**3**. The absolute configuration of these structures was determined by converting **D4P** to (-)-*myo*-inositol 1,4,5-trisphosphate (D-IP₃, Figure 5) and **L4M** to (+)-*myo*-inositol 3,5,6-trisphosphate (L-IP₃, Figure 5). Further evidence that (-)-**3** and (+)-**3** have the structures shown was provided by Aguilo *et al.* (1992), who also synthesized (-)-**3** and converted it to D-IP₃.

With the ability to produce pure **D4P** in large quantities, methods for the introduction of dibenzyl phosphate groups at the C4- and C5- hydroxy positions and deprotection of the C1-hydroxy position were needed to complete the synthesis of the PIP₂ head group (Scheme 2.9). The difficulty encountered in removing the camphanate ester of **6CM** without phosphate triester hydrolysis indicated that the same problem would plague the deprotection of the menthyl

Figure 4: Resolution of ($\pm$)-3 by fractional crystallization of menthyl carbonates

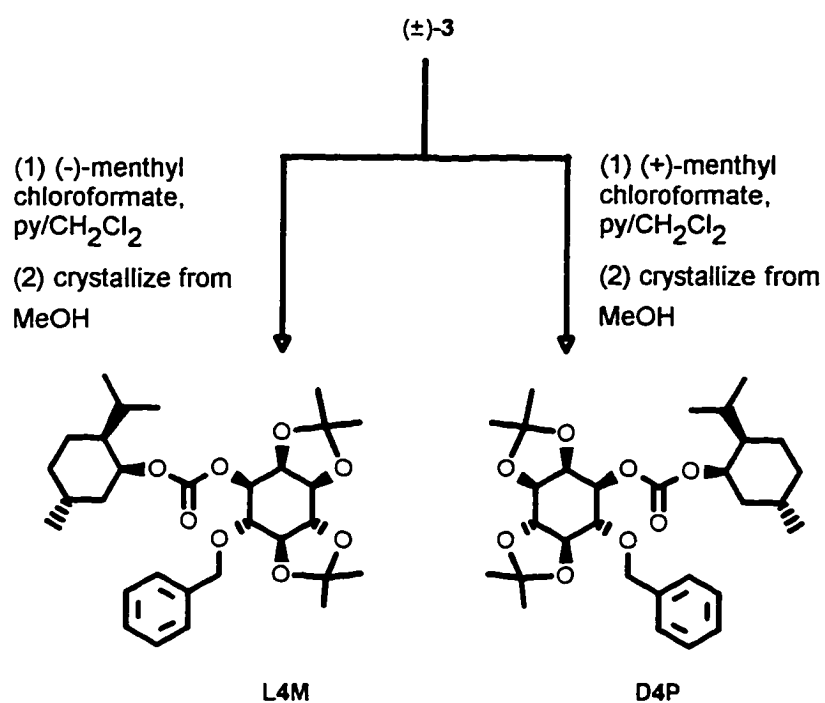
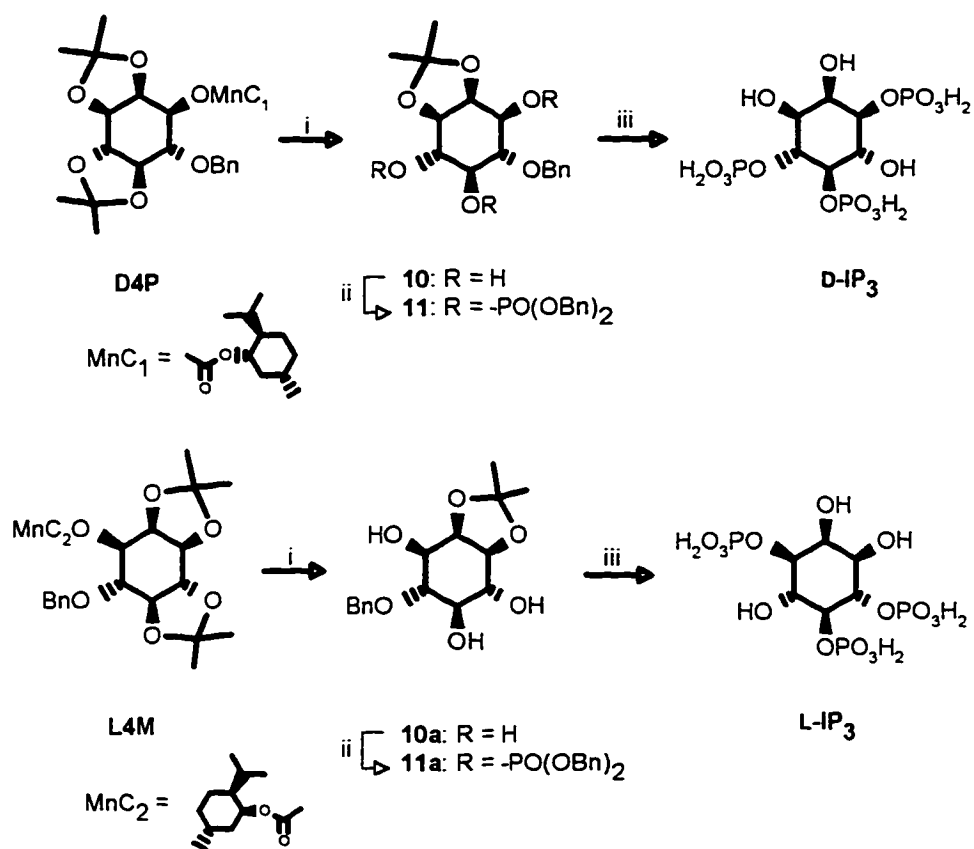


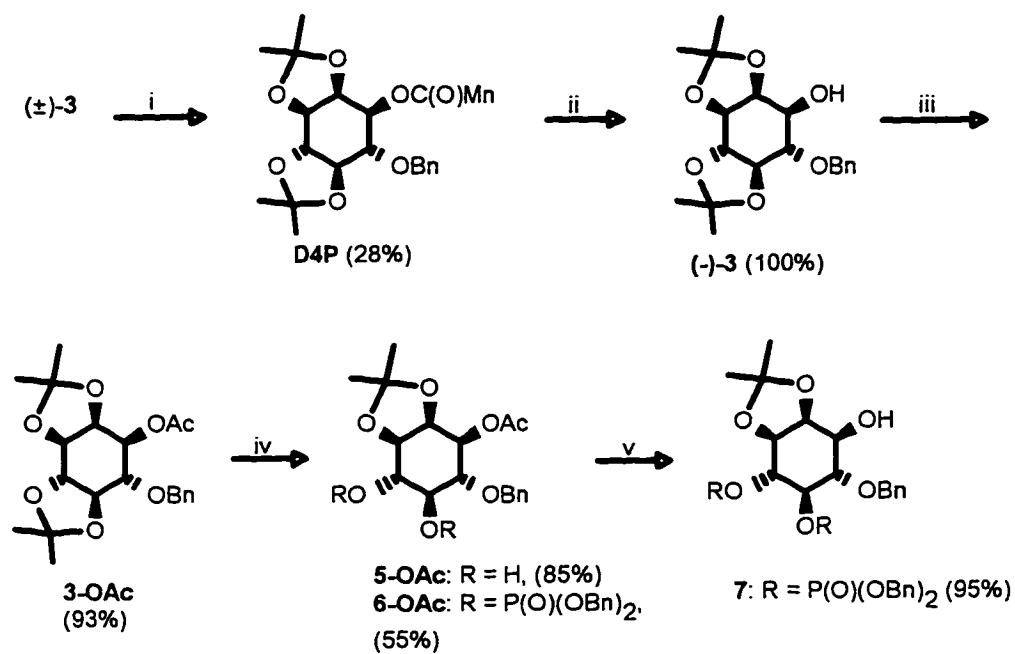
Figure 5: Synthesis of D-IP₃ and L-IP₃



(i) (a) PPTS/MeOH, (b) K₂CO₃/MeOH; (ii) (a) Fraser-Reid reagent, 1*H*-tetrazole, (b)

mCPBA, (c) SiO₂ chromatography; (iii) (a) Pd/C, H₂/EtOH, (b) AcOH.

Scheme 2.9: Synthesis of a PIP₂ head group



(i) (+)-menthyl chloroformate, py/CH₂Cl₂; (ii) KOH/MeOH; (iii) Ac₂O, DMAP, py/CH₂Cl₂; (iv)

PPTS, HO(CH₂)₂OH/CH₂Cl₂; (v) (a) Fraser-Reid reagent, 1*H*-tetrazole/CH₂Cl₂, (b)

mCPBA, (vi) K₂CO₃/MeOH.

carbonate if **D4P** was converted to the corresponding bis-dibenzylphosphate. Therefore, **D4P** was converted to **3-OAc** in the expectation that the acetate could be hydrolyzed under mild conditions. Regioselective removal of the 4,5-acetal, subsequent phosphitylation with Fraser-Reid reagent, and *in situ* oxidation occurred with reasonable yield. As expected, the removal of the acetate occurred easily without any loss of the phosphotriester groups to give **7**.

Synthesis of (±)-**2** and (±)-**3** (Figures 1 and 2)

(±)-2,3-O-Isopropylidene-myoinositol ((±)-**1**)

A mixture of *myo*-inositol (10.0 g, 55.5 mmol), 2,2-dimethoxypropane (17.0 mL, 138 mmol), and *p*-TsOH (100 mg, 0.526 mmol) in dry DMSO (30 mL) was stirred at 100 °C for 30 min, when a clear solution was formed. After the solution was cooled to rt, Et₃N (1 mL, 7.17 mmol), absolute EtOH (40 mL), and Et₂O (200 mL) were added. The mixture was stirred for 4 h at rt, which resulted in the formation of a white precipitate. The precipitate was isolated by filtration on a sintered glass funnel, and washed with Et₂O/MeOH (40 mL, 5:1), followed by ether, affording (±)-2,3-O-isopropylidene-*myo*-inositol (5.41 g, 24.6 mmol) in 44% yield.

(±)-2,3:4,5-di-O-Isopropylidene-myoinositol ((±)-**2**)

A mixture of (±)-**1** (4.35 g, 19.8 mmol), 2,2-dimethoxypropane (131 mL, 1.07 mol), and *p*-TsOH (414 mg, 1.43 mmol) was stirred at 55 °C for 2 h. The reaction was cooled to rt and Et₃N (500 μL, 3.50 mmol) was added, resulting

in a light brown mixture which was filtered and washed with CHCl_3 (100 mL). Evaporation of the filtrate on a rotary evaporator gave the products, which appeared as a dark brown residue.

Flash chromatography (Keiselgel 60, 25 x 2.5 cm; $\text{CHCl}_3/\text{MeOH}$ 15:1, 10:1) gave both ($\pm$)-1,2:4,5-di-O-isopropylidene-*myo*-inositol (($\pm$)-**2a**) and ($\pm$)-2,3:4,5-di-O-isopropylidene-*myo*-inositol (($\pm$)-**2**). Fractions containing both geometric isomers were pooled and the solvents were removed under reduced pressure. The resulting white solid was recrystallized with EtOAc/hexane (1:1) to give pure ($\pm$)-**2b**. The mother liquor was evaporated to give a white solid that was recrystallized (acetone/hexane 1:2) to give slender needles of ($\pm$)-**2** (616 mg, 2.54 mmol) in 12% yield.

Acetylation of ($\pm$)-**2** (616 mg, 2.54 mmol) with acetic anhydride (616 μL , 7.62 mmol), pyridine (719 μL , 7.62 mmol), and a catalytic amount of DMAP (~10 mg, 0.04 mmol) in CH_2Cl_2 (10 mL) gave ($\pm$)-1,6-diacetyl,2,3:4,5-di-O-isopropylidene-*myo*-inositol in quantitative yield. The diacetate of ($\pm$)-**2a**, ($\pm$)-3,6-diacetyl,2,3:5,6-di-O-isopropylidene-*myo*-inositol, was synthesized in a similar fashion.

($\pm$)-**2a**: mp 168-169 °C, lit. (Noda and Keenan, 1990) 171-173 °C; ^1H NMR (CDCl_3) δ 1.40 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 1.56 (s, 3H, CH_3), 2.45 (d, 1H D_2O exch., $J = 8.6$ Hz, OH), 2.62 (d, 1H D_2O exch., $J = 12.5$ Hz, OH), 3.28-3.39 (m, 1H, H-5), 3.84-3.97 (m, 2H), 3.99-4.11 (m, 2H), 4.49 (t, 1H, $J = 4.55$ Hz, H-2).

(±)-**2**: mp 160-163 °C, lit. (*ibid.*) 163-164 °C; (*R*, 0.52, CHCl₃/MeOH 9:1, v/v); ¹H NMR (CDCl₃) δ 1.39 (s, 3H, CH₃), 1.46 (s, 3H, CH₃), 1.48 (s, 3H, CH₃), 1.54 (s, 3H, CH₃), 2.61 (d, 1H D₂O exch., *J* = 8.5 Hz, OH), 2.88 (d, 1H D₂O exch., *J* = 3.0 Hz, OH), 3.33 (dd, 1H, *J* = 9.2 Hz, 10.6 Hz, H-5), 3.79-3.95 (m, 2H), 3.99-4.11 (m, 2H), 4.43 (t, 1H, *J* = 4.8 Hz, H-2).

(±)-3,4-Diacetyl,2,3:4,5-di-*O*-isopropylidene-*myo*-inositol: mp 150-157 °C, lit. (*ibid.*) 158 - 159 °C; ¹H NMR (CDCl₃) δ 1.29 (s, 3H, CH₃), 1.39 (s, 3H, CH₃), 1.40 (s, 3H, CH₃), 1.43 (s, 3H, CH₃), 2.05 (s, 3H, CH₃), 2.10 (s, 3H, CH₃), 3.57 (dd, 1H, *J* = 10.4, 8.6 Hz, H-5), 3.99 (dd, 1H, *J* = 10.6, 7.7 Hz, H-6), 4.33 (t, 1H, *J* = 7.2 Hz, H-1), 4.44 (dd, 1H, *J* = 6.6, 4.0 Hz, H-2), 5.15-5.27 (m, 2H, H-3, H-4).

(±)-3,6-Diacetyl,2,3:5,6-di-*O*-isopropylidene-*myo*-inositol: mp 216 - 221 °C, lit. (*ibid.*) 220 - 221 °C; ¹H NMR (CDCl₃) δ 1.26 (s, 3H, CH₃), 1.36 (s, 3H, CH₃), 1.41 (s, 3H, CH₃), 1.52 (s, 3H, CH₃), 2.07 (s, 3H, CH₃), 2.12 (s, 3H, CH₃), 3.43 (dd, 1H, *J* = 11.0, 9.5, H-5), 4.03-4.15 (m, 2H, H-1, H-4), 4.54 (t, 1H, *J* = 4.5 Hz, H-2), 5.06 (dd, 1H, *J* = 10.6, 4.2 Hz, H-3), 5.22 (dd, 1H, *J* = 11.0, 6.9 Hz, H-6).

(±)-6-*O*-Benzyl-2,3:4,5-di-*O*-isopropylidene-*myo*-inositol ((±)-**3**)

Di-*n*-butyltin oxide (1.90 g, 7.70 mmol) was added to solution of (±)-**2** (1.90 g, 7.30 mmol) in methanol (25 mL) to form a milky suspension, which was refluxed until a nearly clear solution was formed (45 min). The solvent was evaporated and the residue was placed under vacuum (>12 torr) for 3 h.

Powdered CsF (2.22 g, 14.6 mmol) was added to the stannylidene, and after an additional 2 h under vacuum, a solution of benzyl bromide (2.4 mL, 20.4 mmol) in DMF (14.6 mL) was added. After the mixture was stirred under N₂ for 4 h, it was diluted with water (250 mL) and extracted with CH₂Cl₂. The organic phases were pooled, dried (MgSO₄), filtered, and evaporated. Flash chromatography (Kieselgel 60, 38 x 2.5 cm, hexane/EtOAc 4:1) gave (±)-**3** (1.66 g, 4.75 mmol) in 65% yield.

¹H NMR (CDCl₃) δ 1.37 (s, 3H, CH₃), 1.48 (s, 6H, 2 x CH₃), 1.53 (s, 3H, CH₃), 2.64 (d, 1H, *J* = 1.6 Hz, OH), 3.56 (dd, 1H, *J* = 10.4, 7.9 Hz, H-5), 3.92 (dd, 1H, *J* = 7.9, 2.4 Hz, H-4), 4.03 (m, 1H, H-3), 4.17 (dd, 1H, *J* = 7.9, 10.6 Hz, H-6), 4.34 (t, *J* = 7.3 Hz, H-1), 4.44 (dd, 1H, *J* = 7.1, 3.6 Hz, H-2), 4.64 (d, 1H, *J* = 11.7 Hz, OCH₂), 4.78 (d, 1H, *J* = 11.7 Hz, OCH₂), 7.26 - 7.36 (m, 5H, C₆H₅). Anal. Calcd. for C₁₉H₂₆O₆ C, 65.13; H, 7.48. Found: C, 65.25; H, 7.50.

Resolution of (±)-**2** via enzymatic hydrolysis of di-butyrate (Scheme 2.7)

(±)-1,6-di-O-butyryl,2,3:4,5-di-O-isopropylidene-myo-inositol

A solution containing (±)-**2** (2.96 g, 11.4 mmol), pyridine (2.0 mL, 25 mmol), butyryl chloride (2.5 mL, 23.9 mmol), and DMAP (500 mg, 4.09 mmol) in 1,2-dichloroethane (100 mL) was stirred under N₂ at rt for 2 h. The reaction mixture solvent was filtered to removed the precipitated pyridinium hydrochloride, the solvent removed under reduced pressure. The residue was redissolved in hexane and washed with an aqueous saturated NaHCO₃ solution

and H₂O. Column chromatography (Kieselgel 60, 16 x 2.5 cm, hexane/ethyl acetate 4:1) gave the dibutyrate (±)-**14** (3.84 g, 9.64 mmol) in 85% yield.

Racemic **14** (371 mg, 0.925 mmol) in acetone (1 mL) was added dropwise to 0.1 M potassium phosphate buffer (pH 7.4, 20 mL) and stirred vigorously at 37 °C. Pig liver esterase was added (2200 units). After the mixture was stirred for 68 h, it was poured into a saturated aqueous NaHCO₃ solution (20 mL) and the products were extracted with CH₂Cl₂ (3 x 25 mL). The organic phases were pooled and washed with brine (25 mL), dried (MgSO₄), filtered, and evaporated to give a yellow liquid. Flash chromatography (Kieselgel 60, 28 x 2.5 cm, hexane/EtOAc 4:1, 1:1, 1:3) gave 151.6 mg (0.380 mmol) of unhydrolyzed **14** in 41% yield, a 65.7 mg (0.198 mmol) mixture of **13** and **15** (in a ratio of 85%:15% determined by ¹H NMR), in 21% combined yield, and 6.5 mg (0.0250 mmol) of (±)-**2** in 27% yield. Alkaline hydrolysis of the unhydrolyzed **14** in KOH/methanol (25% w/v, 5 mL) gave (-)-**2** (98.9 mg) in quantitative yield ($[\alpha]_D^{22} -4.8^\circ$ (c 0.425 CHCl₃)). Similar treatment of the mixture containing **13** and **15** gave (+)-**2** in quantitative yield ($[\alpha]_D^{22} +2.4^\circ$, (c 1.705 CHCl₃)).

Benzylation of (-)-**2** gave (+)-**3** ($[\alpha]_D^{22} +7.0^\circ$ (c 0.875 CHCl₃)), confirming that the configuration of the structures are correct as drawn.

Attempted resolution of ($\pm$)-2 via enzymatic hydrolysis of di-acetates

In a typical reaction, a solution of ($\pm$)-1,6-diacetyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol (1.58 g, 4.60 mmol) in dissolved acetone (4 mL) was added dropwise to a solution containing cholesterol esterase (49 mg, 233 units) and Tween 80 (700 μ L) in potassium phosphate buffer (150 mL, 250 mM, pH 7.5). The resulting suspension was stirred vigorously for 20 days at rt without any evidence of hydrolysis.

Other reaction conditions were attempted without success, and are outlined in the table below.

Amount of diacetate/solvent	Reaction mixture	Enzyme	Time and temp.
808.9 mg (2.37 mmol)/ 4 mL DMF	80 mL phosphate buffer + 400 μ L Tween 80	cholesterol esterase, 25 mg (120 units)	18 days @ 28 °C
1.58 g (4.60 mmol)/4 mL acetone	150 mL phosphate buffer + 700 μ L Tween 80	cholesterol esterase, 49 mg (233 units)	28 days @ 28 °C
287 mg (0.83 mmol)/2 mL acetone	20 mL phosphate buffer (100 mM, pH 7.0)	pig liver esterase ((NH ₄) ₂ SO ₄ suspension), 23 μ L (254 units)	12 days @ 32 °C

Resolution of ($\pm$)-**3** via camphanate esters (Scheme 2.8)

3-O-(-)-Camphanyl-4-O-benzyl-1,2,5,6-di-O-isopropylidene-myo-inositol
 ((+)-**3CM**) and *1-O-(-)-Camphanyl-6-O-benzyl-2,3,4,5-di-O-isopropylidene-*
myo-inositol ((-)-**3CM**)

A solution containing ($\pm$)-**3** (329 g, 0.938 mmol), (-)-camphanic acid (198 mg, 1.00 mmol), DCC (219 mg, 2.00 mmol), and DMAP (122 mg, 1.00 mmol) in 10 mL of CH_2Cl_2 was stirred overnight at rt under N_2 . The reaction mixture was filtered to remove the precipitate, washed with brine (3 x 3 mL), dried (MgSO_4), and filtered. Column chromatography (Kieselgel 60, $\text{CH}_2\text{Cl}_2/\text{EtOAc}$, 97:3) afforded (+)-**3CM** ($[\alpha]_D^{24} +6.0^\circ$ (c 0.48, CHCl_3)) in 43% yield (R_f 0.21; 213 mg, 0.401 mmol), (-)-**3CM** ($[\alpha]_D^{24} -18.8^\circ$ (c 0.348, CHCl_3)) in 16% yield (R_f 0.18; 79.4 mg, 0.149 mmol), and a mixture of the two diastereomers in 21% yield (101 mg, 0.190 mmol).

For the less polar diastereomer (+)-**3CM**: $^1\text{H NMR}$ (CDCl_3) δ 0.913 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 1.06 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 1.42 (br s, 6H, 2 x CH_3), 1.45 (s, 3H, CH_3), 1.67-2.01 (m, 2H, CH_2), 2.37-2.57 (m, 2H, CH_2), 3.53 (dd, 1H, $J = 8.4, 9.4$ Hz, H-5), 3.86-3.95 (m, 2H, H-4, H-3), 4.34 (dd, 1H, $J = 6.6$ Hz, 8.0 Hz, H-6), 4.60 (dd, 1H, $J = 4.5, 6.3$ Hz, H-2), 4.75 (d, 1H, $J = 12.0$ Hz, OCH_aH_b), 4.83 (d, 1H, $J = 11.9$ Hz, OCH_aH_b), 5.30 (t, 1H, $J = 4.3$ Hz, H-1), 7.27-7.36 (m, 5H, C_6H_5).

For the more polar diastereomer (-)-**3CM**: $^1\text{H NMR}$ (CDCl_3) δ 0.900 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 1.05 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.43 (br s, 6H, 2 x CH_3), 1.52 (s, 3H, CH_3), 1.61-2.05 (m, 2H, CH_2), 2.38-2.57 (m, 2H, CH_2), 3.55

(dd, 1H, $J = 8.9, 10.0$ Hz, H-5), 3.83-3.96 (m, 2H, H-4, H-3), 4.35 (dd, 1H, $J = 4.7, 6.5$ Hz, H-6), 4.60 (dd, 1H, $J = 4.8, 6.5$ Hz, H-2), 4.72 (d, 1H, $J = 12.0$ Hz, OCH_2H_b), 4.83 (d, 1H, $J = 11.8$ Hz, OCH_2H_b), 5.27 (t, 1H, $J = 4.6$ Hz, H-1), 7.26-7.34 (m, 5H, C_6H_5).

(-)-6-O-Benzyl-2,3:4,5-di-O-isopropylidene-myo-inositol ((-)-3)

A mixture of (-)-**3CM** (17.4 mg, 0.0328 mmol) in KOH/methanol (25% w/v) was stirred at rt for 30 min. The solvent was removed *in vacuo*, and the resulting residue was suspended in a saturated aqueous NH_4Cl solution (10 mL) and extracted with CH_2Cl_2 (3 x 5 mL). The organic phases were pooled, dried (MgSO_4), filtered, and evaporated. Column chromatography (Kieselgel 60, hexane/EtOAc 4:1) gave (-)-**3** ($[\alpha]_D^{24} -20^\circ$ (c 0.32, CHCl_3); lit. (Aguilo *et al.*, 1992) $[\alpha]_D -16^\circ$ (c 0.9, CHCl_3)) in 56% yield (6.4 mg, 0.018 mmol). The ^1H NMR spectrum of this compound was identical to that of the racemic material.

(+)-4-O-Benzyl-1,2:5,6-di-O-isopropylidene-myo-inositol ((+)-3)

A mixture of (+)-**3CM** (24.4 mg, 0.0460 mmol) in KOH/methanol (25% w/v) was stirred at rt for 30 min. The solvent was removed *in vacuo*, and the resulting residue suspended in a saturated aqueous NH_4Cl solution (10 mL) and extracted with CH_2Cl_2 (3 x 5 mL). The organic phases were pooled, dried (MgSO_4), filtered, and evaporated. Column chromatography (Kieselgel 60, hexane/EtOAc 4:1) gave (+)-**3** ($[\alpha]_D^{24} +14^\circ$ (c 0.49, CHCl_3)) in 61% yield (9.8 mg,

0.028 mmol). The ^1H NMR spectrum of this compound was identical to that of the racemic material.

3-O-Camphanyl-4-O-benzyl-1,2-O-isopropylidene-myo-inositol (**5CM**)

A solution of (+)-**3CM** (232.1 mg, 0.4374 mmol), ethylene glycol (4.00 mL of a 100 mM CH_2Cl_2 solution, 0.400 mmol), and *p*-TsOH (5 mg, 0.03 mmol) in CH_2Cl_2 (5 mL) was stirred at rt for 45 min, neutralized with Et_3N , diluted with CH_2Cl_2 (5 mL), and washed with a saturated aqueous NaHCO_3 solution (2 x 3 mL), water (3 mL), and brine (3 mL). The organic phase was dried (MgSO_4), filtered, and evaporated. Column chromatography (Kieselgel 60, 15 cm x 2.5 cm, elution with hexane/EtOAc 1:2) gave **5CM** (93.5 mg, 0.196 mmol) in 57% yield.

^1H NMR (CDCl_3) δ 0.968 (s, 3H, CH_3), 0.997 (s, 3H, CH_3), 1.10 (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 1.84-1.96 (m, 2H, CH_2), 2.36-2.45 (m, 2H), 2.60 (m, 2H, 2 x OH), 3.40 (t, 1H, $J = 9.3$ Hz, H-5), 3.72-3.78 (m, 2H), 4.04 (t, 1H, $J = 7.6$ Hz), 4.53 (t, 1H, $J = 4.7$ Hz, H-2), 4.72 (d, 1H, $J = 11.3$ Hz, OCH_2), 4.88 (d, 1H, $J = 11.3$ Hz, OCH_2), 5.14 (dd, 1H, $J = 9.1, 4.2$ Hz, H-6), 7.23-7.31 (m, 5H, C_6H_5).

3-O-Camphanyl-4-O-benzyl-1,2-O-isopropylidene-myo-inositol 5,6-bis(dibenzyl phosphate) (6CM)

A suspension of **5CM** (93.9 mg, 0.196 mmol), 1*H*-tetrazole (84.0 mg, 1.20 mmol), and Fraser-Reid reagent (1.50 mL of a 423 mM CH₂Cl₂ solution, 0.600 mmol) in CH₂Cl₂ (50 mL) was stirred at rt for 2 h. Oxidation of the reaction was accomplished by adding mCPBA (250 mg, 0.280 mmol assuming 50% purity) at -40 °C. The mixture was warmed to rt, then diluted with CH₂Cl₂, washed successively with an aqueous Na₂SO₃ solution (10% w/v, 2 x 10 mL), a saturated aqueous NaHCO₃ solution (2 x 8 mL), water (15 mL), and brine. The organic phase was dried (MgSO₄), filtered, and evaporated. Column chromatography (Kieselgel 60, hexane/EtOAc 3:1) gave the product (165 mg, 0.196 mmol) in 84% yield.

¹H NMR (CDCl₃) δ 0.930 (s, 3H, CH₃), 0.996 (s, 3H, CH₃), 1.07 (s, 3H, CH₃), 1.27 (s, 3H, CH₃), 1.47 (s, 3H, CH₃), 1.54-1.68 (m, 2H, CH₂), 1.86-1.93 (m, 2H, CH₂), 2.36-2.45 (m, 2H, CH₂), 4.10-4.16 (m, 1H, H-1), 4.49-4.85 (m, 5H), 4.93 (t, 1H, *J* = 8.1 Hz), 5.01 (d, 4H, *J* = 7.4 Hz, 2 x -P(O)(OCH₂H_b-C₆H₅)₂), 5.08 (d, 4H, *J* = 7.6 Hz, 2 x -P(O)(OCH_aH_b-C₆H₅)₂), 5.38-5.44 (m, 1H, H-3), 7.17-7.38 (m, 25H); ³¹P NMR δ -1.77, -1.60.

4-O-Benzyl-1,2-O-isopropylidene-myo-inositol 5,6-bis(dibenzyl phosphate) (L7)

A suspension of **6CM** (29.5 mg, 0.0289 mmol) and lithium hydroxide (6.1 mg, 0.1447 mmol) in MeOH (10 mL) was refluxed for 3 h. TLC (hexane/EtOAc 1:1) indicated the formation of several products, which were not isolated.

Resolution of ($\pm$)-**3** via menthylcarbonates (Scheme 2.9)

1-O-[(+)-Menthoxycarbonyl]-6-O-benzyl-2,3:4,5-di-O-isopropylidene-myo-inositol (**D4P**)

A solution containing ($\pm$)-**3** (3.62 g, 10.3 mmol, dried by evaporation from pyridine), (+)-menthyl chloroformate (2.43 mL, 11.3 mmol), and anhydrous pyridine (1.00 mL, 12.0 mmol) in CH_2Cl_2 (50 mL) was stirred overnight at rt under N_2 . The reaction mixture was poured into a saturated aqueous NaHCO_3 solution and extracted with CH_2Cl_2 . The organic phases were pooled, dried (MgSO_4), and filtered. The solvent was removed under reduced pressure to give a yellow oil which produced colorless crystals upon trituration with methanol. Recrystallization in methanol gave enantiomerically pure **D4P** (1.53 g, 2.87 mmol, 28% yield).

^1H NMR (CDCl_3) δ 0.724-1.08 (m, 14H), 1.31 (s, 3H, CH_3), 1.42 (s, 6H, 2 x CH_3), 1.46 (s, 3H, CH_3), 1.62-1.67 (m, 2H), 1.89-2.07 (m, 2H), 3.57 (dd, 2H, J = 10.7, 7.9 Hz, H-5), 3.85 (dd, 1H, J = 7.8, 2.4 Hz, H-4), 3.99 (dd, 1H, J = 10.0, 7.4 Hz, H-3), 4.35 (t, 1H, J = 7.3 Hz, H-6), 4.48 (dd, 1H, J = 4.0, 6.9, H-2), 4.55 (m, 1H, H-1'), 4.71 (s, 2H, OCH_2), 5.12 (t, 1H, J = 3.3 Hz, H-1), 7.22-7.32 (m, 5H, C_6H_5)

(-)-6-O-Benzyl-2,3:4,5-di-O-isopropylidene-myo-inositol ((-)-**3**)

A mixture of **D4P** (468.1 mg, 0.879 mmol) in KOH/methanol (50 mL, 25% w/v) was refluxed until TLC indicated that the reaction was complete. The

solvent was removed *in vacuo*, and the resulting residue was suspended in a saturated aqueous NH_4Cl solution (20 mL) and extracted with CH_2Cl_2 (3 x 5 mL). The organic phases were pooled, dried (MgSO_4), filtered, and evaporated. Column chromatography (Kieselgel 60, 8 x 2.5 cm, hexane/EtOAc 4:1) gave (-)-**3** ($[\alpha]_D^{24}$ -17.4° (c 1.69, CHCl_3); lit. (Aguilo *et al.*, 1992) $[\alpha]_D$ -16° (c 0.9, CHCl_3)) in quantitative yield. The ^1H NMR spectrum of this compound was identical to that of the racemic material.

1-O-Acetyl-6-O-benzyl-2,3:4,5-di-O-isopropylidene-myo-inositol (3-OAc)

A solution of (-)-**3** (466 mg, 1.33 mmol), acetic anhydride (162 μL , 1.70 mmol), pyridine (162 μL , 2.00 mmol), and DMAP (10 mg, 0.082 mmol) in CH_2Cl_2 (10 mL) was stirred at rt overnight. The solution was poured into brine and extracted with CH_2Cl_2 . The organic phases were pooled, washed with a saturated aqueous NaHCO_3 solution, water, and brine. The organic phase was dried (MgSO_4), filtered, evaporated, and the crude product was purified by column chromatography (Kieselgel 60, 8 x 2.5 cm, hexane/EtOAc 8:1) to give **3-OAc** (494.2 mg, 1.231 mmol) in 93% yield.

^1H NMR (CDCl_3) δ 1.34 (s, 3H, CH_3), 1.42 (s, 6H, CH_3), 1.45 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 3.61 (dd, 1H, J = 10.6, 7.9 Hz, H-5), 3.82 (dd, 1H, J = 7.9, 2.9 Hz, H-4), 4.02 (dd, 1H, J = 9.1, 7.6 Hz, H-3), 4.37 (t, 1H, J = 7.2 Hz, H-6), 4.50 (dd, 1H, J = 6.9, 4.5 Hz, H-2), 4.74 (s, 2H, OCH_2), 5.28 (dd, 1H, J = 3.7, 2.9 Hz, H-1), 7.26-7.36 (5H, m, C_6H_5).

1-O-Acetyl-6-O-benzyl-2,3-O-isopropylidene-myo-inositol (5-OAc)

A solution of **3-OAc** (110 mg, 0.280 mmol), ethylene glycol (2.80 mL of a 100 mM CH₂Cl₂ solution, 0.280 mmol), and PPTS (5 mg, 0.02 mmol) in CH₂Cl₂ (10 mL) was refluxed for 8 h and then poured into an aqueous NaCl solution (15% w/v, 10 mL). The aqueous phase was extracted with Et₂O and the organic phases were pooled, washed with saturated aqueous NaHCO₃ solution, water, and brine. The organic phase was dried (MgSO₄), filtered, and evaporated. Column chromatography (Kieselgel 60, 12 cm x 2.5 cm, eluting with hexane/EtOAc 4:1, 2:1, 1:1) gave **5-OAc** (84.0 mg, 0.238 mmol) in 85% yield.

¹H NMR (CDCl₃) δ 1.32 (s, 3H, CH₃), 1.51 (s, 3H, CH₃), 2.05 (s, 3H, CH₃), 3.45 (t, 1H, *J* = 8.5 Hz, H-5), 3.72-3.78 (m, 2H, H-4, H-6), 3.89-4.34 (br m, 2H, 2 x OH), 3.99 (t, 1H, *J* = 5.0 Hz, H-3), 4.37 (br s, 1H, H-2), 4.73 (d, 1H, *J* = 11.1 Hz, OCH₂), 4.78 (d, 1H, *J* = 11.5 Hz, OCH₂), 5.13 (br s, 1H, H-1), 7.30-7.31 (m, 5H, C₆H₅).

1-O-Acetyl-3-O-benzyl-2,3-O-isopropylidene-myo-inositol 4,5-bis(dibenzyl phosphate) (6-OAc)

A suspension of **5-OAc** (84.0 mg, 0.238 mmol), 1*H*-tetrazole (105.0 mg, 1.50 mmol), and Fraser-Reid reagent (2.00 mL of a 375 mM CH₂Cl₂ solution, 0.750 mmol) in CH₂Cl₂ (50 mL) was stirred at rt until the 1*H*-tetrazole dissolved (30 min). Oxidation of the reaction was accomplished by adding mCPBA (350 mg, 1.09 mmol assuming 50% purity) at -40 °C. After the mixture was allowed to warm to rt, the reaction was diluted with CH₂Cl₂, washed successively with an

aqueous Na_2SO_3 solution (10% w/v, 2 x 10 mL), a saturated aqueous NaHCO_3 solution (2 x 8 mL), water (15 mL), and brine. The organic phase was dried (MgSO_4), filtered and evaporated. Column chromatography (Kieselgel 60, 12 x 1.0 cm, hexane/EtOAc 2:1, 1:1), gave the product (115 mg, 0.132 mmol) in 55% yield.

^1H NMR (CDCl_3) δ 1.28 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 4.03 (dd, 1H, $J = 7.6, 5.6$ Hz), 4.30 (t, 1H, $J = 6.6$ Hz), 4.49 (dd, 1H, $J = 6.7, 3.8$ Hz), 4.57–4.70 (m, 2H), 4.74–5.17 (m, 10H), 5.29 (dd, 1H, $J = 7.6, 3.8$ Hz), 7.14–7.36 (m, 25H, 5 x $\text{-C}_6\text{H}_5$); ^{31}P NMR δ -1.54, -1.96.

6-O-Benzyl-2,3-O-isopropylidene-myo-inositol 4,5-bis(dibenzyl phosphate) (7)

A suspension of **6-OAc** (285.4 mg, 0.3271 mmol) and potassium carbonate (70.0 mg, 0.500 mmol) in MeOH (20 mL) was stirred at rt for 45 min, poured into an aqueous NaCl solution (15%, w/v), and extracted with CH_2Cl_2 . The pooled organic phases were washed with water (2 x 20 mL) and brine (20 mL), dried (MgSO_4), filtered, and evaporated. Column chromatography (Kieselgel 60, 10 x 2.5 cm, hexane/EtOAc 1:1;1:3) gave the product (257.2 mg, 0.3096 mmol) in 95% yield.

^1H NMR (CDCl_3) δ 1.28 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 2.48 (br s, 1H, OH), 4.00 (br s, 2H), 4.27 (t, 1H, $J = 6.0$ Hz), 4.41–4.44 (m, 1H), 4.49–4.62 (m, 2H), 4.74–5.10 (m, 10H), 7.22–7.28 (m, 25H, 5 x C_6H_5); ^{31}P NMR δ -1.66, -1.84.

Synthesis of D- and L- *myo*-inositol 1,4,5-trisphosphate (Figure 5)

(+)-6-O-Benzyl-2,3-O-isopropylidene *myo*-inositol (**10**)

A solution containing **D4P** (532 mg, 1.00 mmol), PPTS (30 mg, 0.09 mmol), and ethylene glycol (10 mL of a 100 mM solution in CH₂Cl₂, 1.000 mmol) in CH₂Cl₂ (20 mL) was refluxed for 10 h and then washed with an aqueous NaCl solution (15% w/v, 2 x 5 mL). The organic phase was dried (MgSO₄), filtered, and evaporated. The residue was redissolved in methanol (10 mL) and refluxed with anhydrous K₂CO₃ (138 mg, 1.00 mmol) for 1 h. The solvent was removed under reduced pressure. The residue was resuspended in CH₂Cl₂, filtered through Celite, and the eluant was evaporated. Recrystallization of the crude product (hexane/EtOAc 1:1) gave the triol (83.2 mg, 0.268 mmol) in 27% yield. $[\alpha]_D^{24} +21^\circ$ (c 0.43, CHCl₃); lit. (Aguilo *et al.*, 1992) $[\alpha]_D^{25} +15^\circ$ (c 0.6, CH₃OH).

¹H NMR (CDCl₃) δ 1.39 (s, 3H, CH₃), 1.55 (s, 3H, CH₃), 2.65 (br s, 3H, 3 x OH), 3.42 (dd, 1H, *J* = 9.9, 8.1 Hz), 3.67 (t, 1H, *J* = 7.9 Hz), 3.82 (dd, 1H, *J* = 9.8, 7.5 Hz), 3.94 (dd, 1H, *J* = 7.7, 4.1 Hz), 4.05 (m, 1H, H-2), 4.35 (dd, 1H, *J* = 5.6, 4.2 Hz), 4.73 (d, 1H, *J* = 11.1 Hz, OCH₂H_b), 4.78 (d, 1H, *J* = 11.5 Hz, OCH₂H_a), 7.30-7.31 (m, 5H, C₆H₅).

6-O-Benzyl-2,3-O-isopropylidene-*myo*-inositol 1,4,5-tris(dibenzyl phosphate) (**11**)

A suspension of the triol, **10**, (40.0 mg, 0.129 mmol), 1*H*-tetrazole (82.0 mg, 1.16 mmol), and Fraser-Reid reagent (1.55 mL of a 375 mM CH₂Cl₂ solution, 0.580 mmol) in CH₂Cl₂ (25 mL) was stirred at rt until the 1*H*-tetrazole dissolved

(30 min). Oxidation of the phosphite was accomplished by adding mCPBA (86 mg, 0.50 mmol assuming 50% purity) at $-40\text{ }^{\circ}\text{C}$. The mixture was allowed to warm to rt, then diluted with CH_2Cl_2 , washed successively with an aqueous Na_2SO_3 solution (10% w/v, 2 x 10 mL), a saturated aqueous NaHCO_3 solution (2 x 8 mL), water (15 mL), and brine. The organic phase was dried (MgSO_4), filtered, and evaporated. Column chromatography (Kieselgel 60, 12 x 1.0 cm, hexane/EtOAc 1:1) gave the product (113.4 mg, 0.119 mmol) in 92% yield. $[\alpha]_{\text{D}}^{24} -5.23^{\circ}$ (c 0.650, CHCl_3).

^1H NMR (200 MHz CDCl_3) δ 1.34 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 4.17 (dd, 1H, $J = 2.3, 7.6\text{ Hz}$), 4.27 (t, 1H, $J = 6.5\text{ Hz}$), 4.58-5.07 (m, 18H), 7.09-7.30 (m, 35H); ^{31}P (CDCl_3) δ -1.82, -1.77, -1.53.

D-myo-Inositol 1,4,5-trisphosphate (*D*-IP₃)

A suspension of **11** (100 mg, 0.092 mmol), acetic acid (0.60 mL, 0.011 mmol), and palladium/charcoal (10%, 10 mg) in EtOH/ H_2O (4:1) was stirred under H_2 overnight. The mixture was centrifuged to remove the catalyst. The solvent was removed under reduced pressure and the residue was redissolved in water (10 mL) and stirred with NaOH (0.260 mL of a 2 M aqueous solution, 0.552 mmol) and precipitated with MeOH (0.500 mL). The precipitate was pelleted by centrifugation and washed with MeOH (2 x 0.500 mL) to give *D*-IP₃ (39.1 mg, 0.092 mmol) as the hexasodium salt in quantitative yield. $[\alpha]_{\text{D}}^{24} -20^{\circ}$ (c 0.3, H_2O , pH 9.5), lit. (Guo *et al.*, 1992) $[\alpha]_{\text{D}} -20^{\circ}$ (c 0.5, H_2O , pH 9.5).

^1H NMR (D_2O) δ 3.60 (dd, 1H, J = 2.5, 9.8 Hz), 3.72-3.97 (m, 3H), 4.11-4.23 (m, 2 H); ^{31}P (CDCl_3) δ 2.19, 2.93, 4.00.

(-)-4-O-Benzyl-1,2-O-isopropylidene-myo-inositol (10a)

A solution containing **L4M** (79.4 mg, 0.150 mmol) and PPTS (10 mg, 0.03 mmol) in MeOH (12 mL) was refluxed until TLC indicated that the reaction was complete and KOH/MeOH (25% w/v, 1 mL) was added. After the solution was refluxed for 1h, the solvent was removed under reduced pressure and the residue was diluted with a saturated aqueous NH_4Cl solution (60 mL) and the product was extracted with CHCl_3 (3 x 25 mL). The organic phases were pooled, washed with brine (2 x 25 mL), dried (MgSO_4), filtered, and evaporated. Column chromatography (Kieselgel 60, hexane/EtOAc 1:1) gave the triol (25.4 mg, 0.082 mmol) in 68% yield. $[\alpha]_{\text{D}}^{24} +15.5^\circ$ (c 1.04, CHCl_3).

^1H NMR (CDCl_3) δ 1.36 (s, 3H, CH_3), 1.55 (s, 3H, CH_3), 2.45 (d, 1H, J = 5.0 Hz, OH), 2.72 (d, 2H, J = 11.7 Hz, 2 x OH), 3.42 (t, 1H, J = 8.9 Hz), 3.67 (t, 1H, J = 7.9 Hz), 3.82 (t, 1H, J = 8.4 Hz), 3.94 (m, 1H), 4.04 (m, 1H), 4.43 (dd, 1H, J = 5.6, 4.2 Hz), 4.78 (d, 1H, J = 11.1 Hz, OCH_2H_b), 4.88 (d, 1H, J = 11.5 Hz, OCH_2H_b), 7.30-7.31 (m, 5H, C_6H_5).

4-O-Benzyl-1,2-O-isopropylidene-myo-inositol 3,5,6-tris(dibenzyl phosphate)
(11a)

A suspension of **10a** (25.4 mg, 0.0818 mmol), 1*H*-tetrazole (52 mg, 0.74 mmol), and Fraser-Reid reagent (0.873 mL of a 423 mM CH₂Cl₂ solution, 0.370 mmol) in CH₂Cl₂ (10 mL) was stirred at rt until the 1*H*-tetrazole dissolved (30 min). Oxidation of the phosphite was accomplished by adding mCPBA (86 mg, 0.50 mmol assuming 50% purity) at -40 °C. The mixture was allowed to warm to rt, then diluted with CH₂Cl₂, washed successively with an aqueous Na₂SO₃ solution (10% w/v, 2 x 10 mL), a saturated aqueous NaHCO₃ solution (2 x 8 mL), water (15 mL), and brine. The organic phase was dried (MgSO₄), filtered, and evaporated. Column chromatography (Kieselgel 60, 12 x 1.0 cm, hexane/EtOAc 2:1, 1:1) gave the product (34.3 mg, 0.0555 mmol) in 68% yield. ¹H NMR (CDCl₃) δ 1.24 (s, 3H, CH₃), 1.50 (s, 3H, CH₃), 4.17-4.30 (m, 2H), 4.59-4.84 (m, 4H), 4.91-5.06 (m, 14H), 7.21-7.33 (m, 35H); ³¹P (CDCl₃) δ -2.55, -2.76, -2.92.

D-myo-Inositol 3,5,6-trisphosphate (L-IP₃)

A suspension of **11a** (17 mg, 0.016 mmol), acetic acid (0.60 mL, 0.011 mmol), and palladium/charcoal (10%, 10 mg) in EtOH/H₂O (4:1) was stirred under H₂ overnight. The mixture was filtered through glass wool and centrifuged to remove the catalyst. The solvent was removed under reduced pressure and the residue was redissolved in water (1 mL) and stirred with NaOH (0.050 mL of a 2 M aqueous solution, 0.100 mmol) and precipitated with MeOH (0.500 mL). The precipitate was pelleted by centrifugation and washed with MeOH (2 x 0.500

mL) to give L-IP₃ (6.0 mg, 0.011 mmol) as the hexasodium salt in 71% yield.

$[\alpha]_D^{24} +17^\circ$ (c 0.30, H₂O, pH 10), lit. (Guo *et al.*, 1992) $[\alpha]_D +20^\circ$ (c 0.5, H₂O, pH 9.5); ¹H NMR and ³¹P spectra were identical to that of D-IP₃.

Chapter 3. Fluorescent Diradyl Glycerol Derivatives

Fluorescent lipid derivatives have been used in the past to investigate the kinetic properties of lipases, protein-lipid interactions, and membrane fusion. Naphthyl-bearing lipid analogs have demonstrated success as a mimic of phosphatidylcholine (Hendrickson *et al.*, 1987, Figure 3.1) and to a lesser extent, phosphatidylinositol (Shashidhar *et al.*, 1991, Figure 3.2). The naphthyl group was particularly useful because of its relatively small size and low polarity. Other fluorophores that had been used in the past, such as 5-(dimethylamino)-1-naphthalenesulfonate (dansyl) and 7-nitro-2-oxa-1,3-diazol-4-yl (NBD) (Figure 3.3), were deemed unsuitable because of the possibility of excess membrane perturbation caused by their relatively high polarity. However, a fluorescent inositol lipid analog (Shashidhar *et al.*, 1991) was found to be a poor substrate for PI-PLC, which can be explained by the proximity of the naphthyl group to the inositol ring. Incorporation of the naphthyl group at the end of one of the alkyl chains would presumably circumvent this problem. This can be accomplished by esterification of 2-naphthol to a glycerol derivative bearing a carboxyl function at the terminus of one of its acyl or alkyl chains. Alternately, a terminal bromide can be used to form an ether bond with naphthol. In either case, it was desirable to introduce the fluorophore late in the synthetic schemes in order to allow for the possibility of incorporating numerous other reporter groups such as 9-anthrylvinyI or spin-labeled probes into these glycerol derivatives.

Figure 3.1: A naphthylvinyl-labeled glycerol ether analog of phosphatidylcholine (Hendrickson *et al.*, 1987)

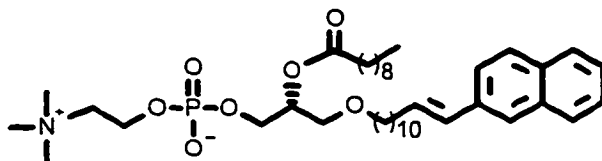


Figure 3.2: 2-Naphthyl-*myo*-inositol-1-phosphate, a fluorescent analog of phosphatidylinositol (Shashidhar *et al.*, 1991)

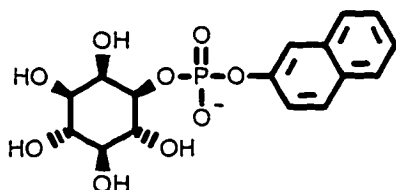
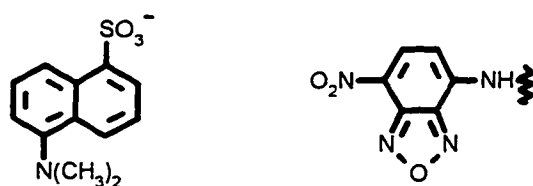


Figure 3.3: 5-(*N,N*-Dimethylamino)-1-naphthalenesulfonate (dansyl) and 7-nitro-2-oxa-1,3-diazol-4-yl (NBD) fluorescent probes

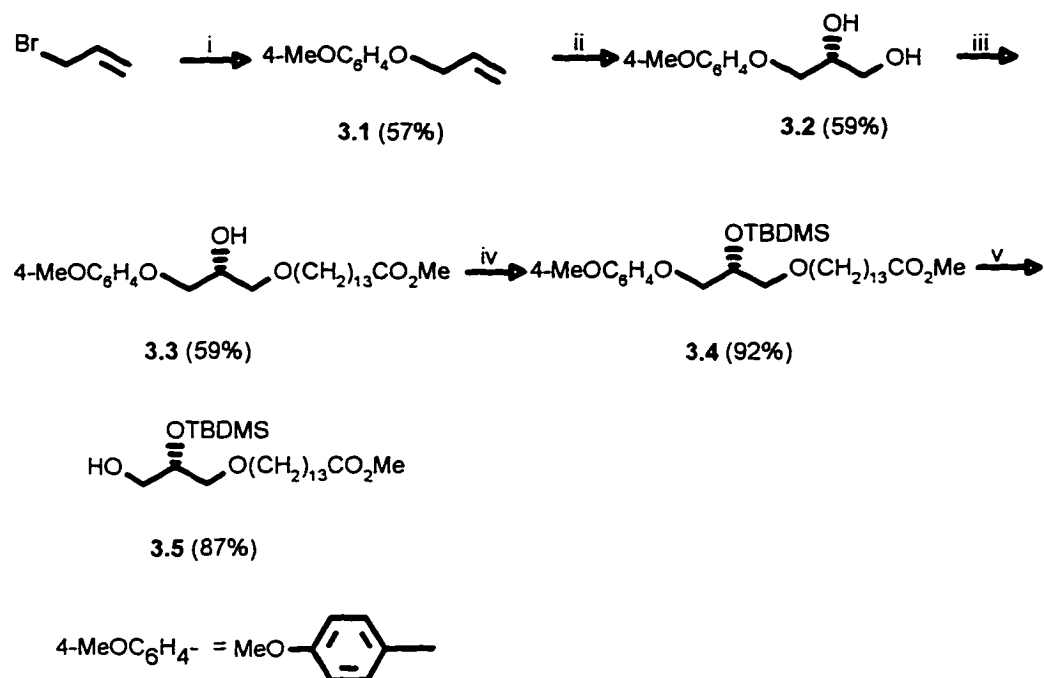


In contrast to the difficulties encountered with the formation of a PIP_2 head group, the synthesis of a fluorescent diradyl glycerol was fairly trouble-free. The large-scale synthesis of asymmetric diacylglycerols had already been described in detail (Vilch  ze and Bittman, 1994), and was easily modified to accommodate a *sn*-1 alkyl chain which terminated with an ester (Scheme 3.1) or a bromide (a good leaving group for a $\text{S}_{\text{N}}2$ reaction, Scheme 3.2) instead of a methyl group. In both schemes, the starting chiral diol **3.2** is converted to a dibutylstannylene which can be regioselectively 1-*O*-alkylated.

Incorporation of a fluorophore via an ester linkage

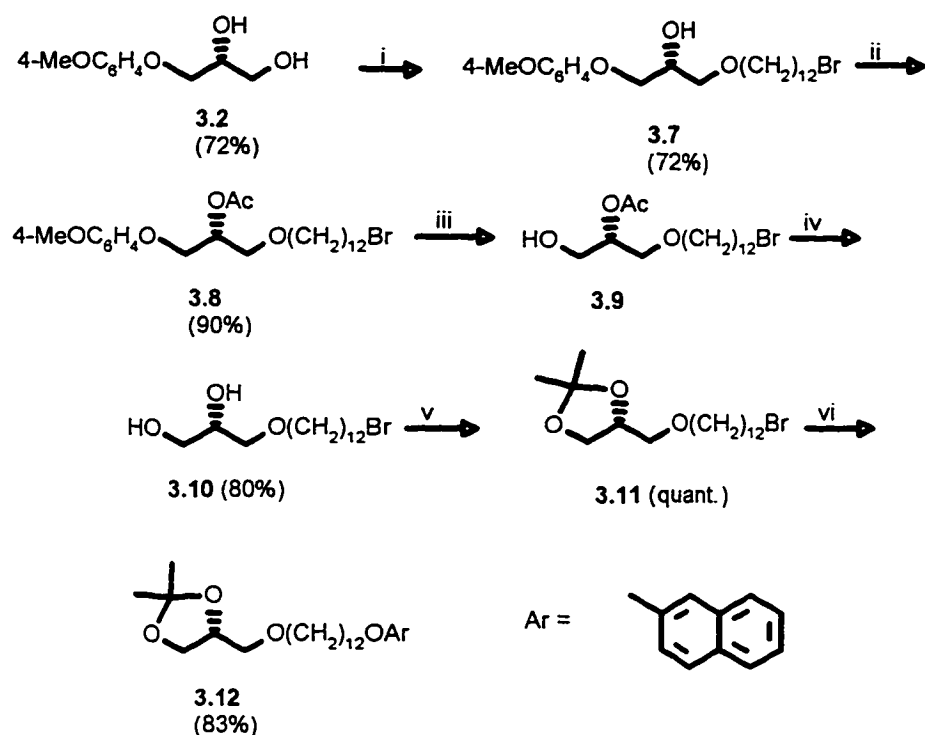
A protected alkylglycerol derivative, **3.5** (Scheme 3.1), had already been proposed as a suitable precursor for a tritiated analog of lyso-PI. Although this goal was not achieved, it was thought that this intermediate could find use in the synthesis of a fluorescent PIP_2 analog. Diol **3.2** was regioselectively alkylated (via the intermediate dibutylstannylene) with methyl 14-bromotetradecanoate (synthesis shown in Scheme 3.3) in 59% yield. Alkylation of the secondary hydroxy position also occurred (30% yield), but this more polar product was easily separated from the desired compound by column chromatography. The remaining hydroxyl group was protected as a *tert*-butyldimethylsilyl ether and the 4-methoxyphenyl group was removed with ceric (IV) ammonium nitrate (CAN). After introduction of a bis-dibenzylphosphate at the *sn*-3 position, formation of the naphthyl ester was accomplished by removal of the methyl ester using pig

Scheme 3.1: Synthesis of a diradyl glycerol precursor (3.5)



(i) 4-methoxyphenol, K₂CO₃, 18-crown-6/DMF; (ii) AD-mix α /*t*-BuOH:H₂O, 1:1; (iii) a) di-*n*-butyltin oxide/MeOH, (b) CsF, methyl 14-bromotetradecanoate/DMF; (iv) TBDMSCl, imidazole/DMF; (v) CAN/MeCN:H₂O, 4:1.

Scheme 3.2: Synthesis of a fluorescent monoalkyl *sn*-glycerol analog (3.12)

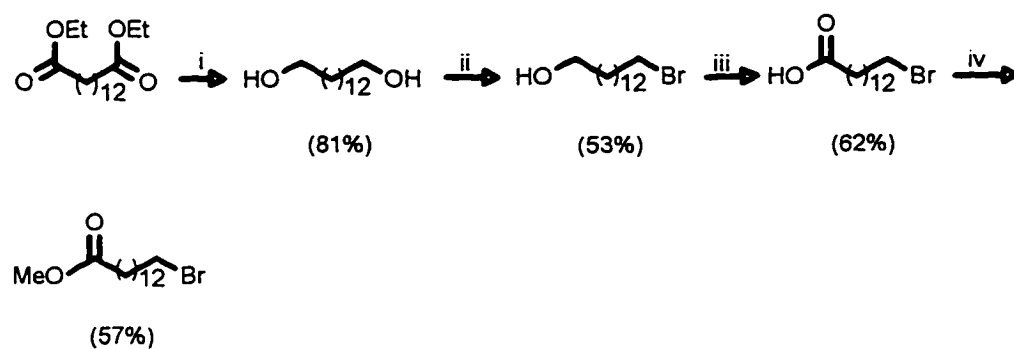


(i) (a) di-*n*-butyltin oxide/MeOH; (b) CsF, 1,12-dibromododecane/DMF; (i) Ac₂O,

DMAP/py; (iii) CAN/MeCN:H₂O, 3:1; (iv) K₂CO₃/MeOH; (v) 2,2-dimethoxypropane,

p-TsOH; (vi) 2-naphthol, K₂CO₃/DMF.

Scheme 3.3: Synthesis of methyl 14-bromotetradecanoate



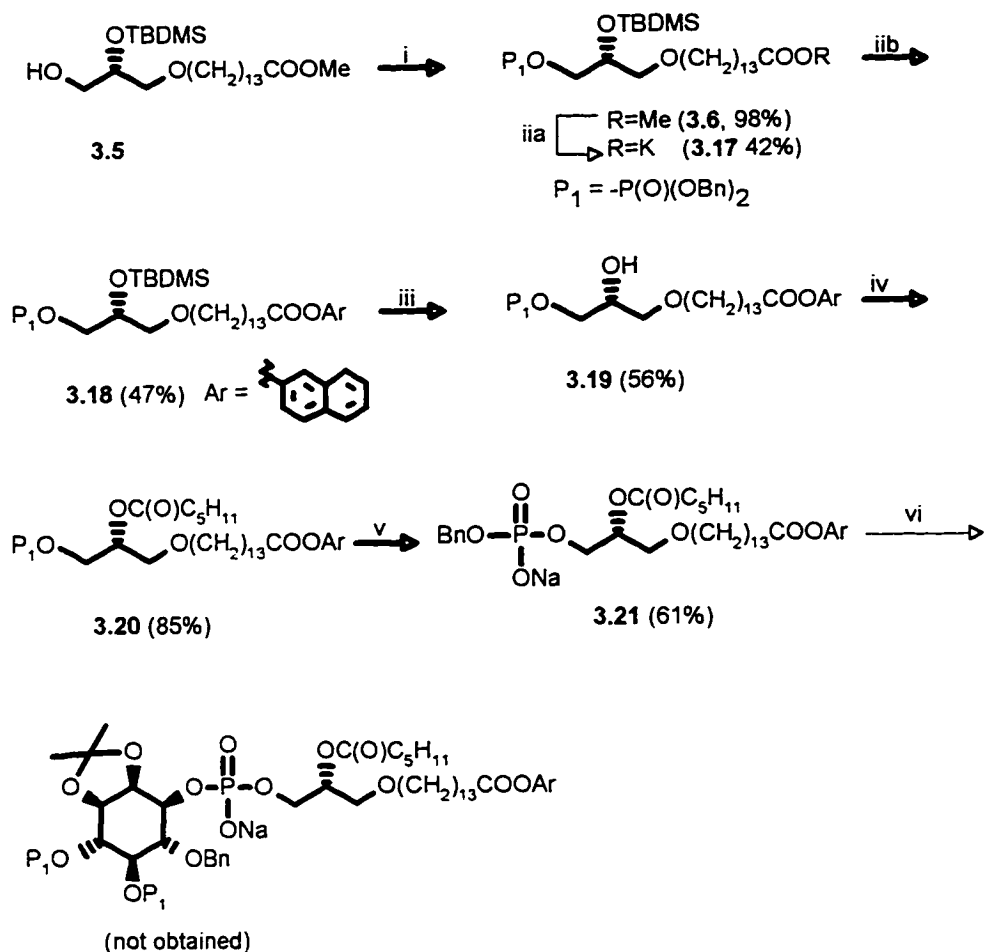
(i) LAH/THF; (ii) 48% aq. HBr/pet. ether; (iii) PDC/DMF; (iv) *p*-TsOH/MeOH, 2,2-dimethoxypropane.

liver esterase and esterification of the resulting carboxylic acid with 2-naphthol in the presence of DCC and DMAP. Mild acid treatment to remove the silyl ether and subsequent acylation with hexanoic anhydride resulted in a fully protected fluorescent derivative, **3.20** (Scheme 3.4). Removal of one of the benzyl groups with sodium iodide in refluxing methyl ethyl ketone (MEK) gave a compound that was thought to be suitable for coupling to an inositol derivative using 1-(2-mesitylene-sulfonyl)-3-nitro-1,2,4-triazole (MSNT). Disappointingly, the coupling of **3.21** with compound **7** (page 78) did not occur. Despite this setback, another method of forming a phosphodiester bond which uses *H*-phosphonates was investigated and is described in the subsequent chapter.

Incorporation of a fluorophore via an ether linkage

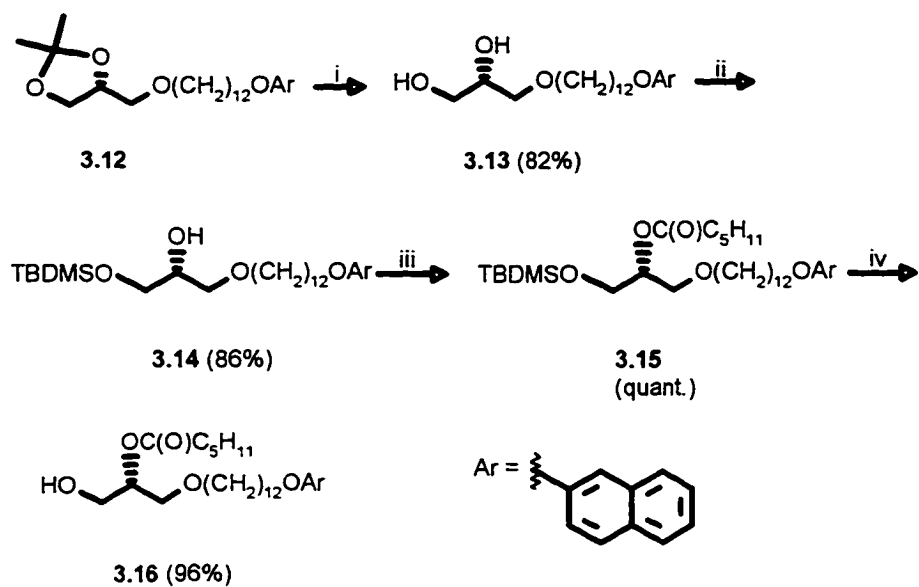
A simpler route to produce a fluorescent diradyl glycerol derivative is shown in Schemes 3.2 and 3.5. Instead of introducing the naphthol group as an ester, use of a less polar ether linkage was hypothesized to be less perturbing to the membrane. Monoalkylation of diol **3.2** to form **3.7** (Scheme 3.2) via a dibutylstannylene derivative with excess 1,12-dibromododecane was accomplished in moderate yield (75%); however, attempts at removal of the 4-methoxyphenyl group with CAN to give diol **3.10** resulted in a yield of only 25%. Acetylation of **3.7** to give the acetate, **3.8**, followed by removal of the aryl

Scheme 3.4: Failed attempt to synthesize a fluorescent PIP₂ analog



(i) (a) 1*H*-tetrazole, Fraser-Reid reagent/CH₂Cl₂; (b) mCPBA; (i) (a) pig liver esterase/
potassium phosphate buffer (pH 7.4); (b) Dowex 50X8; (c) 2-naphthol, DMAP,
DCC/CH₂Cl₂; (iii) 10% HCl/acetonitrile; (iv) caproic anhydride, py, DMAP/CH₂Cl₂; (v)
NaI/methyl ethyl ketone; (vi) **7** (see text), MSNT/py.

Scheme 3.5: Synthesis of 3.16, a fluorescent diradyl glycerol



(i) *p*-TsOH/CH₂Cl₂; (ii) TBDMSCl, imidazole/DMF; (iii) hexanoic anhydride, DMAP, py/CH₂Cl₂; (iv) HCl/EtOH.

ether with CAN afforded **3.9** in the anticipated high yield (86%), and subsequent removal of the acetate gave **3.10** in 80% yield. The resulting diol was converted to the 2,3-isopropylidene (**3.11**) and reacted with preformed 2-naphthyl-alkoxide to give **3.12**. Acid hydrolysis (Scheme 3.5) of the acetal restored the diol, which was then reacted with *tert*-butyldimethylsilyl chloride in a regioselective manner to give predominately the 3-O-silyl ether. Acylation with hexanoic anhydride followed by hydrolysis of the silyl ether gave **3.16** in good overall yield without acyl migration.

Synthesis of methyl 14-bromotetradecanoate

1,14-Tetradecanediol

Diethyl tetradecanoate (25.0 g, 79.5 mmol) in THF (100 mL) was added dropwise over 1.5 h to a suspension containing LAH (1.25 g, 0.33 mol) in THF (500 mL) at -10 °C. Once the addition was complete, the mixture was warmed to rt. After the mixture was stirred overnight, the reaction was refluxed for 2 h, cooled to rt, and quenched with EtOAc (100 mL). The solvents were removed by evaporation, and the resulting gray residue was suspended in a 2.5% aqueous HCl solution, extracted with CH₂Cl₂, dried (MgSO₄), filtered, and concentrated under reduced pressure. Recrystallization from benzene gave the diol (14.8 g, 64.1 mmol, mp 85-88 °C) in 81% yield.

14-Bromotetradecan-1-ol

A mixture of hydrobromic acid (166 mL of a 48% aqueous solution) and 1,14-tetradecanediol (14.8 g, 64.1 mmol) in petroleum ether (60-90 °C, 500 mL) was stirred overnight at 60 °C. The organic phase was removed and additional petroleum ether was added (350 mL). After the mixture was stirred for 5 h, the remaining petroleum ether was decanted, and the pooled organic phases were washed with a saturated aqueous NaHCO₃ solution (3 x 100 mL) and water. The organic phase was dried (MgSO₄), filtered, and evaporated. Crystallization from hexane gave the product (9.89 g, 34.1 mmol) in 53% yield.

¹H NMR (CDCl₃) δ 1.26 (br s, 20H, (CH₂)₁₀), 1.50-1.56 (m, 2H), 1.78-1.92 (m, 3H), 3.41 (t, 2H, *J* = 6.8 Hz, CH₂Br), 3.63 (t, 2H, *J* = 6.5 Hz, CH₂OH).

14-Bromotetradecanoic acid

A mixture containing 14-bromotetradecan-1-ol (9.90 g, 34.1 mmol) and pyridinium dichromate (PDC) (35.0 g, 93 mmol) in DMF (300 mL) was stirred at rt. After 48 h, the mixture was diluted with water (800 mL) and extracted with Et₂O. The organic phase was dried (MgSO₄), filtered, and evaporated to give a clear liquid. Flash chromatography (Kieselgel 60, 35 x 2.5 cm, gradient of hexane/EtOAc 2:1, then 1:3) afforded the acid in 62% yield (6.5 g, 21 mmol).

¹H NMR (CDCl₃) δ 1.22 (br s, 20H, (CH₂)₁₀), 1.55-1.86 (m, 2H), 2.29 (t, 2H, *J* = 7.4 Hz, CH₂CO₂H), 3.35 (t, 2H, *J* = 6.8 Hz, CH₂Br), 9.2 (br s, 1H, CO₂H).

Methyl 14-bromotetradecanoate

A solution of 14-bromotetradecan-1-oic acid (6.5 g, 21 mmol) and 2,2-dimethoxypropane (10 mL, 81 mmol) in MeOH (90 mL) was refluxed with a catalytic amount of *p*-TsOH (>200 mg) overnight. After neutralization with triethylamine (2 mL) the solvent was evaporated under reduced pressure. The residue was dissolved in diethyl ether (350 mL), washed with a saturated aqueous NaHCO₃ solution (3 x 80 mL), water (3 x 80 mL), brine (80 mL), dried (MgSO₄), and filtered. Flash chromatography (Kieselgel 60, 12 x 2.5 cm, gradient of hexane/EtOAc 30:1, then 20:1) gave the product (3.84 g, 12.0 mmol) in 57% yield.

¹H NMR (CDCl₃) δ 1.26 (br s, 20H, (CH₂)₁₀), 1.57-1.88 (br s, 2H), 2.30 (t, 2H, *J* = 7.5 Hz, CH₂CO₂H), 3.33-3.39 (m, 2H, CH₂Br), 3.67 (s, 3H, OCH₃).

Synthesis of a diradyl glycerol precursor from allyl bromide (Scheme 3.1)

1-(4'-Methoxyphenoxy)-3-propene (3.1)

A solution containing 4-methoxyphenol (15.9 g, 128 mmol), K₂CO₃ (31.8 g, 230 mmol), and 18-crown-6 (5.0 g, 20 mmol) in anhydrous DMF (100 mL) was stirred under N₂ at rt for 1 h before allyl bromide (12.2 mL, 141 mmol) was added. After the mixture was stirred overnight, the reaction mixture was filtered, and the DMF solution was extracted with hexane (4 x 100 mL). The organic phase was washed with water, dried (MgSO₄), filtered, and evaporated to give

the crude product. Vacuum distillation (119-123 °C/10 torr) gave pure 1-(4'-methoxyphenyloxy)-3-propene (12.0 g, 73.0 mmol) in 57% yield.

^1H NMR (CDCl_3) δ 3.74 (s, 3H, OCH_3), 4.47 (d, 2H, $J = 5.2$ Hz, $\text{CH}_2\text{OC}_6\text{H}_4$), 5.21-5.42 (m, 2H, CH_2), 5.96-6.07 (m, 1H, CH), 6.78-6.87 (m, 4H, C_6H_4).

3-O-(4'-Methoxyphenyl)-sn-glycerol (**3.2**)

Compound **3.1** (10 g, 61 mmol) was added to a mixture of AD-mix α (100 g) in *t*-BuOH/water (760 mL, 1:1, v/v), and stirred at 0 °C. After 24 h, sodium sulfite (112 g) was added, and the mixture was stirred for an additional 45 min at rt. The *t*-BuOH phase was removed and evaporated to give a brown residue that was pooled with the aqueous phase, extracted with chloroform (3 x 250 mL), dried (MgSO_4), filtered, and evaporated to give yellow flakes. Recrystallization from EtOAc/*i*-PrOH 9:1 gave **3.2** (7.1 g, 36 mmol) in 59% yield. HPLC and ^1H NMR of the corresponding bis-Mosher esters (synthesized and analyzed according to Vilchèze and Bittman, 1994) indicated 84% ee; mp 77-78 °C, lit. (Vilchèze and Bittman, 1994) 78 °C, (Takano *et al.*, 1990) 78 °C; $[\alpha]_{\text{D}}^{24}$ -6.23° (*c* 0.530, MeOH), lit. (Vilchèze and Bittman, 1994) $[\alpha]_{\text{D}}^{24}$ -7.58° (*c* 4.0, MeOH), (Takano *et al.* 1990) $[\alpha]_{\text{D}}^{24}$ -8.25° (*c* 1.15, MeOH).

^1H NMR (CDCl_3) δ 2.4 (br s, 1H, OH), 2.9 (br s, 1H, OH), 3.74 (s, 3H, OCH_3), 3.91-4.18 (m, 5H), 6.75-6.93 (m, 4H, C_6H_4).

1-O-(14'-Carbomethoxyltridecyl)-3-O-(4''-methoxyphenyl)-sn-glycerol (3.3)

A suspension containing **3.2** (2.10 g, 11.0 mmol) and di-*n*-butyltin oxide (2.74 g, 11.0 mmol) in methanol (30 mL) was refluxed until a clear solution was formed (1 h). The solvent was removed under reduced pressure, powdered CsF was added (3.16 g, 20.8 mmol), and the mixture was placed under vacuum for 3 h. A solution of methyl 14-bromotetradecanoate (3.512 g, 11.5 mmol) in DMF (20 mL) was added, and the resulting suspension was stirred under N₂ for 2 h at -40 °C, and then at 5 °C overnight. The reaction mixture was diluted with CH₂Cl₂ (200 mL) and washed with brine. The organic phase was dried (MgSO₄), filtered, and evaporated under reduced pressure. Column chromatography (Keiselgel 60; 20 x 2.5 cm, gradient of hexane/EtOAc, 20:1, then 2:1) gave the product **3.3** (2.84 g, 6.47 mmol) in 59% yield.

¹H NMR (CDCl₃) δ 1.26 (br s, 16H, (CH₂)₈), 1.5-1.61(m, 6H), 2.30 (t, 2H, *J* = 7.5 Hz, CH₂COO), 2.53 (d, 1H, *J* = 4.6 Hz, OH), 3.48 (t, 2H, *J* = 6.6 Hz, OCH₂(CH₂)₁₂), 3.58 (m, 2H, CH₂O), 3.66 (s, 3H, CO₂CH₃), 3.77 (s, 3H, C₆H₄OCH₃), 3.97 (m, 2H, CH₂OC₆H₄), 4.07-4.19 (m, 1H, CHOH), 6.79-6.89 (m, 4H, C₆H₄).

1-O-(14'-Carbomethoxyltridecyl)-2-O-tert-butyldimethylsilyl-3-O-(4'-methoxyphenyl)-sn-glycerol (3.4)

A solution of **3.3** (2.5 g, 5.7 mmol), *tert*-butyldimethylsilyl chloride (953 mg, 6.33 mmol), and imidazole (465 mg, 6.83 mmol) in DMF (10 mL) was stirred

at rt under N₂ overnight. The solvent was evaporated under reduced pressure, and the crude product was redissolved in CH₂Cl₂ and washed successively with a saturated aqueous NaHCO₃ solution, water, and brine. The organic phase was dried (MgSO₄), filtered, and evaporated to give **3.4** (2.89 g, 5.24 mmol) in 92% yield.

¹H NMR (CDCl₃) δ 0.079 (s, 3H, SiCH₃), 0.094 (s, 3H, 0.094, SiCH₃), 0.875 (s, 9H, SiC(CH₃)₃), 1.23 (br s, 16H, (CH₂)₈), 1.51-1.70 (m, 6H), 2.27 (t, 2H, *J* = 7.4 Hz, CH₂COO), 3.42 (t, 2H, *J* = 6.7 Hz, OCH₂(CH₂)₁₂), 3.43-3.52 (m, 2H, CH₂O), 3.68 (s, 3H, CO₂CH₃), 3.73 (s, 3H, C₆H₄OCH₃), 3.81 (dd, 1 H, *J* = 6.5, 9.4 Hz, CH₃H_bOC₆H₄), 3.97 (dd, 1 H, *J* = 4.2, 9.6, CH_aH_bOC₆H₄), 4.06-4.17 (m, 1H, CHOTBDMS), 6.76-6.84 (m, 4H, C₆H₄).

1-O-(14'-Carbomethoxytridecyl)-2-O-tert-butyltrimethylsilyl-sn-glycerol (3.5)

A suspension containing **3.4** (1.05 mg, 2.00 mmol) and CAN (2.63 g, 4.80 mmol) in acetonitrile/water (12.5 mL, 4:1) was stirred at 0 °C for 6 min. The suspension was poured into a saturated aqueous NaHCO₃ solution (100 mL) and extracted with EtOAc (3 x 50 mL). The organic phases were pooled and washed with brine, dried (Na₂SO₄), filtered, and evaporated under reduced pressure. Column chromatography (Kieselgel 60, 42 x 2.5 cm, hexane/EtOAc 8:1) gave **3.5** (610 mg, 1.38 mmol) in 87% yield.

¹H NMR (CDCl₃) δ 0.066 (s, 6H, Si(CH₃)₂), 0.865 (s, 9H, SiC(CH₃)₃), 1.23 (br s, 16H, (CH₂)₈), 1.52-1.72 (m, 6H), 2.17 (d, 1H, *J* = 6.3 Hz, OH), 2.27 (t, 2H, *J*

= 7.6 Hz, CH₂CO₂Me), 3.39 (t, 2H, J = 5.2 Hz, OCH₂(CH₁₂)), 3.28-3.50 (m, 2H, CH₂O(CH₂)₁₃), 3.56-3.59 (m, 2H, CH₂OH), 3.63 (s, 3H, CO₂CH₃), 3.71-3.88 (m, 1H, CH-OTBDMS).

Synthesis of a fluorescent monoalkyl *sn*-glycerol analog (**3.12**) (Scheme 3.2)

1-O-(12'-Bromododecyl)-3-O-(4''-methoxyphenyl)-*sn*-glycerol (**3.7**)

A mixture of diol **3.2** (2.10 g, 11.0 mmol) and di-*n*-butyltin oxide (2.74 g, 11.0 mmol) in methanol (30 mL) was refluxed until a clear solution was formed (30 min). The solvent was removed under reduced pressure to produce a colorless syrup. Repeated addition of cyclohexane (20 mL) followed by evaporation resulted in a solid foam that was further dried under vacuum for 3 h. Powdered CsF (2.20 g, 14.6 mmol) was added to a portion of the product (3.10 g, 7.31 mmol), and the mixture was suspended with DMF (130 mL) containing 1,12-dibromododecane (9.80 g, 30.0 mmol). After the mixture was stirred overnight, the solvent was removed under reduced pressure. The resulting residue was suspended in brine and extracted with CH₂Cl₂. The organic phase was dried (MgSO₄), filtered, and evaporated. Column chromatography (Kieselgel 60, 29 x 5 cm, gradient of hexane/EtOAc 99:1, then 82.5:17.5) gave **3.7** (2.35 g, 5.27 mmol) in 72% yield.

¹H NMR (CDCl₃) δ 1.24 (br s, 16H, (CH₂)₈), 1.56 (m, 2H), 1.82 (m, 2H), 2.61 (br s, 1H, OH), 3.39 (t, 2H, J = 6.8 Hz, CH₂Br), 3.46 (t, 2H, J = 6.7 Hz,

OCH₂(CH₂)₁₁Br), 3.52 (dd, 1H, $J = 9.8, 5.2$ Hz, CH₃H_bO(CH₂)₁₂Br), 3.59 (dd, 1H, $J_{b,a} = 9.8, 4.4$ Hz, CH_aH_bO(CH₂)₁₂Br), 3.74 (s, 3H, CH₃OC₆H₄), 3.95 (dd, 2H, $J = 4.5, 6.3$ Hz, C₆H₄OCH₂), 4.15 (m, 1H, CH₂CHOHCH₂), 6.83 (m, 4H, C₆H₄).

1-O-(12'-Bromododecyl)-2-O-acetyl-3-O-(4"-methoxyphenyl)-sn-glycerol (3.8)

The entire sample of **3.7** (2.35 g, 5.27 mmol) was dissolved in CH₂Cl₂ (100 mL) containing pyridine (1.0 mL, 12 mmol), acetic anhydride (1.0 mL, 11 mmol), and DMAP (10 mg, 0.08 mmol). After the mixture was stirred for 72 h, the reaction was poured into an aqueous saturated NaHCO₃ solution (50 mL) and extracted with CH₂Cl₂ (3 x 50 mL). The pooled organic phases were washed with brine, dried (MgSO₄), filtered, and evaporated to give acetate **3.8** (2.30 g, 4.27 mmol) in 90% yield. The product was used without further characterization.

1-O-(12'-Bromododecyl)-2-O-acetyl-sn-glycerol (3.9)

A suspension of **3.8** (2.30 g, 4.72 mmol) and CAN (6.20 g, 11.3 mmol) in MeCN/water (60 mL, 45:15) was stirred at 0 °C for 30 min, and then diluted with brine (100 mL). The MeCN was removed by evaporation under reduced pressure, and the mixture was extracted with CH₂Cl₂ (5 x 100 mL). The pooled organic phases were washed with a saturated aqueous NaHCO₃ solution (3 x 100 mL), dried (MgSO₄), filtered, and evaporated. Column chromatography (Kieselgel 60, 31 x 2.5 cm, hexane/EtOAc 3:1) gave crude **3.9** (1.54 g, 4.04 mmol if pure), which was used without further purification or characterization.

1-O-(12'-bromododecyl)-sn-glycerol (3.10)

Crude **3.9** (1.54 g, 4.04 mmol if pure) was dissolved in methanol (15 mL) and stirred with K_2CO_3 (200 mg) for 20 min. After the solvent was evaporated, a black syrup formed, which was dissolved in CH_2Cl_2 . Elution with hexane/EtOAc (300 mL, 3:1) through a sintered glass funnel packed with silica gel, and evaporation of the eluant gave **3.10** (1.282 g, 3.779 mmol) in 72% yield from **3.7**.

^1H NMR ($CDCl_3$) δ 1.23 (s, 14H, $(CH_2)_7$), 1.53 (m, 2H), 1.77 (m, 4H), 2.45 (s, 1H, OH), 2.81 (s, 1H, OH), 3.37 (t, 2H, $J = 6.9$ Hz, CH_2Br), 3.42 (t, 2H, $J = 6.6$ Hz, $OCH_2(CH_2)_{11}Br$), 3.47 (dd, 2H, $J = 4.4, 1.0$ Hz, $CH_2O(CH_2)_{12}Br$), 3.63 (m, 2H, $HOCH_2$), 3.82 (m, 1H, $CHOH$).

1-O-(12'-Bromododecyl)-2,3-isopropylidene-sn-glycerol (3.11)

A solution of **3.10** (1.282 g, 3.779 mmol) in 2,2-dimethoxypropane (25 mL) containing *p*-TsOH (200 mg) was stirred for 1 h, and then neutralized with Et_3N (5 mL) and evaporated under reduced pressure. The crude product was eluted with hexane/EtOAc (2:1) through a sintered glass funnel packed with silica gel. Evaporation of the solvent under reduced pressure gave **3.11** (1.43 g, 3.77 mmol) in quantitative yield.

^1H NMR ($CDCl_3$) δ 1.23 (s, 16H, $(CH_2)_8$), 1.32 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.52 (m, 2H), 1.81 (m, 2H), 3.36 (t, 2H, $J = 6.8$ Hz, CH_2Br), 3.42 (m, 3H,

$\text{OCH}_2(\text{CH}_2)_{11}\text{Br}$ and $\text{CH}_2\text{H}_b\text{O}(\text{CH}_2)_{12}\text{Br}$, 3.47 (dd, 1H, $J = 9.9, 5.6$ Hz, $\text{CH}_2\text{H}_b\text{O}(\text{CH}_2)_{12}\text{Br}$), 3.68 (dd, 1H, $J = 8.2, 6.4$ Hz, OCH_cH_d), 4.02 (dd, 1H, $J = 8.2, 6.4$ Hz, OCH_cH_d), 4.22 (m, 1H, CHO).

1-O-(12'-(2''-O-Naphthyl)-dodecyl)-2,3-isopropylidene-sn-glycerol (3.12)

A solution of 2-naphthol (1.15 g, 8.00 mmol) and K_2CO_3 (2.21 g, 16.0 mmol) in DMF (50 mL) was stirred for 1 h, and then added to **3.11** (1.43 g, 3.77 mmol). The solution was stirred under N_2 at rt for 72 h. The solvent was evaporated, and the residue diluted with brine (75 mL) and extracted with CH_2Cl_2 (3 x 25 mL). The organic phases were pooled, dried (MgSO_4), filtered, and evaporated. Column chromatography (Kieselgel 60, 23 x 2.5 cm, hexane/EtOAc 9:1) gave **3.12** (1.51 g, 3.41 mmol) in 83% yield.

^1H NMR (CDCl_3) δ 1.26 (s, 16H, $(\text{CH}_2)_8$), 1.35 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.53 (m, 2H), 1.83 (m, 2H), 3.39 (dd, 1H, $J = 11.0, 5.6$ Hz, $\text{CH}_2\text{H}_b\text{O}(\text{CH}_2)_{12}\text{O-naphthyl}$), 3.44 (m, 2H, $\text{OCH}_2(\text{CH}_2)_{11}\text{O-naphthyl}$), 3.50 (dd, 1H, $J = 9.9, 5.6$ Hz, $\text{CH}_2\text{H}_b\text{O}(\text{CH}_2)_{12}\text{O-naphthyl}$), 3.71 (dd, 1H, $J = 8.1, 6.4$ Hz, $\text{OCH}_c\text{H}_d\text{CHOCH}_2\text{O}(\text{CH}_2)_{12}$), 4.04 (t, 2H, $J = 6.5$ Hz, $\text{CH}_2\text{O-naphthyl}$), 4.05 (dd, 1H, $J = 8.1, 6.4$ Hz, $\text{OCH}_c\text{H}_d\text{CHOHCH}_2\text{O}(\text{CH}_2)_{12}$), 4.25 (m, 1H, CHO), 7.10-7.41 (m, 4H, naphthyl), 7.68-7.76 (m, 3H, naphthyl).

Synthesis of a fluorescent diradyl *sn*-glycerol analog (3.16) (Scheme 3.5)

1-O-(12'-(2''-O-Naphthyl)-dodecyl)-sn-glycerol (3.13)

A solution of **3.12** (1.51 g, 3.41 mmol) and *p*-TsOH (50 mg) in CH₂Cl₂ (50 mL) was refluxed overnight, and then neutralized with excess Et₃N. The solvents were removed under reduced pressure to give a yellow residue. Column chromatography (Kieselgel 60, hexane/EtOAc 1:1) gave **3.13** (1.12 g, 2.78 mmol) in 82% yield.

¹H NMR (CDCl₃) δ 1.26 (s, 16H, (CH₂)₈), 1.54 (m, 2H), 1.81 (m, 2H), 2.21 (br s, 1H, OH), 2.6 (br s, 1H, OH), 3.38 (dd, 1H, *J* = 6.6, 11.0 Hz, CH₂H₅O(CH₂)₁₂), 3.43 (t, 2H, *J* = 6.5 Hz, OCH₂(CH₂)₁₁), 3.51 (dd, 1H, *J* = 7.1, 12.8 Hz, CH₂H₅O(CH₂)₁₂), 3.65 (m, 2H, HOCH₂), 3.87 (m, 1H, CHO), 4.04 (t, 2H, *J* = 6.5 Hz, CH₂Onaphthyl), 7.1-7.4 (m, 4H, naphthyl), 7.67-7.75 (m, 3H, naphthyl).

1-O-(12'-(2''-O-Naphthyl)-dodecyl)-3-O-tert-butyldimethylsilyl-sn-glycerol (3.14)

A mixture of **3.13** (1.12 g, 2.78 mmol) and imidazole (227 mg, 3.34 mmol) was rendered anhydrous by repeatedly dissolving and evaporating from cyclohexane (3 x 25 mL) and redissolved in DMF (100 mL). The solution was cooled to 0 °C and *tert*-butyldimethylsilyl chloride was added (461 mg, 3.05 mmol). After the solution was stirred for 45 h at rt, the solvent was evaporated under reduced pressure to give a residue that was redissolved in CH₂Cl₂ (60 mL) and washed with a saturated aqueous NaHCO₃ solution (2 x 15 mL), water (15 mL), and brine (15 mL). The organic phase was dried (MgSO₄), filtered, and

evaporated. Column chromatography (Kieselgel 60, hexane/EtOAc 1:1) gave **3.14** (1.23 g, 2.38 mmol) in 86% yield.

^1H NMR (CDCl_3) δ 0.051 (s, 6H, $\text{Si}(\text{CH}_3)_2$), 0.879 (s, 9H, $\text{SiC}(\text{CH}_3)_3$), 1.26 (s, 16H, $(\text{CH}_2)_8$), 1.50 (m, 2H), 1.82 (m, 2H), 2.48 (d, 1H, $J = 4.9$ Hz, OH), 3.42 (t, 2H, $J = 6.6$ Hz, $\text{OCH}_2(\text{CH}_2)_{11}$), 3.45 (m, 2H, $\text{CH}_2\text{O}(\text{CH}_2)_{12}$), 3.59 (dd, 1H, $J = 6.2$, 10.2 Hz, $\text{TBDMSOCH}_2\text{H}_b\text{O}$), 3.65 (dd, 1H, $J = 4.6$, 10.2 Hz, $\text{TBDMSOCH}_2\text{H}_b\text{O}$), 3.78 (m, 1H, CHO), 4.04 (t, 2H, $J = 6.6$ Hz, $\text{CH}_2\text{O-naphthyl}$), 7.10-7.43 (m, 4H, naphthyl), 7.68-7.75 (m, 3H, naphthyl).

1-O-(12'-(2''-O-Naphthyl)-dodecyl)-2-O-hexanoyl-3-O-tert-butyltrimethylsilyl-sn-glycerol (3.15)

A solution of **3.14** (1.23 g, 2.38 mmol), DMAP (100 mg, 0.8 mmol), and hexanoic anhydride (0.83 mL, 3.6 mmol) in pyridine (10 mL) was stirred under N_2 at rt overnight. The solvent was evaporated, and the residue was dissolved in Et_2O (60 mL) and washed with a saturated NaHCO_3 solution (2 x 15 mL), water (15 mL), and brine (15 mL). The organic phase was dried (MgSO_4), filtered, and evaporated. Column chromatography (Kieselgel 60, hexane/EtOAc 10:1) gave **3.15** (1.44 g, 2.40 mmol) in quantitative yield.

^1H NMR (CDCl_3) δ 0.0234 (s, 6H, $\text{Si}(\text{CH}_3)_2$), 0.857 (br s, 12H, $\text{SiC}(\text{CH}_3)_3$ and $\omega\text{-CH}_3$), 1.25 (s, 18H, $(\text{CH}_2)_8$ and CH_2CH_3), 1.39-1.56 (m, 6H), 1.82 (m, 2H), 2.29 (t, 2H, $J = 7.5$ Hz, CH_2CO), 3.37 (dt, 1H, $J = 2.5$, 6.5 Hz, $\text{OCH}_2\text{H}_b(\text{CH}_2)_{11}$), 3.43 (dt, 1H, $J = 2.5$, 6.5 Hz, $\text{OCH}_2\text{H}_b(\text{CH}_2)_{11}$), 3.53 (d, 2H, $J = 4.9$ Hz,

TBDMSOCH₂), 3.67 (dd, 1H, $J = 5.4, 10.8$, CH_cH_dO(CH₂)₁₂), 3.73 (dd, 1H, $J = 5.1, 10.2$ Hz, CH_cH_dO(CH₂)₁₂), 4.04 (t, 2H, $J = 6.5$ Hz, CH₂O-naphthyl), 4.98 (m, 1H, CHO), 7.10-7.44 (m, 4H, naphthyl), 7.67-7.75 (m, 3H, naphthyl).

1-O-(12'-(2''-O-Naphthyl)-dodecyl)-2-O-hexanoyl-sn-glycerol (3.16)

A suspension of **3.15** (0.580 g, 0.943 mmol) and aqueous HCl (5 mL, 10% v/v) in EtOH (50 mL, 95%) was stirred at rt for 1 h, and then diluted with brine (200 mL). After the volatiles were removed under reduced pressure, the aqueous suspension was extracted with CH₂Cl₂ (3 x 50 mL). The organic phases were pooled and washed with an aqueous saturated NaHCO₃ solution, water, brine, dried (MgSO₄), filtered, and evaporated to give **3.16** (452.7 mg, 0.904 mmol) in 96% yield. (The product was repeatedly dissolved in DMF and evaporated to remove all traces of *tert*-butyldimethylsilyl alcohol.)

¹H NMR (CDCl₃) δ 0.857 (t, 3H, $J = 6.3$ Hz, ω -CH₃), 1.26 (s, 18H, (CH₂)₈ and CH₂-CH₃), 1.42-1.59 (m, 6H), 1.80 (m, 2H, CH₂CH₂O-naphthyl), 2.33 (t, 2H, $J = 7.4$ Hz, CH₂CO₂), 3.42 (t, 1H, $J = 6.5$ Hz, OCH_aH_b(CH₂)₁₁), 3.43 (t, 1H, $J = 6.5$ Hz, OCH_aH_b(CH₂)₁₁), 3.56 (dd, 1H, $J = 5.0, 10.5$, CH_cH_dO(CH₂)₁₂), 3.63 (dd, 1H, $J = 5.1, 11.1$ Hz, CH_cH_dO(CH₂)₁₂), 3.78 (t, 2H, $J = 4.6$ Hz, HOCH₂), 4.04 (t, 2H, $J = 6.5$ Hz, CH₂O-naphthyl), 4.98 (m, 1H, HOCH₂CHO), 7.03-7.44 (m, 4H, naphthyl), 7.67-7.75 (m, 3H, naphthyl).

Attempted synthesis of a naphthyl-labeled PIP₂ using MSNT

Di-O-Benzyl O-[1'-(14"-carboxymethoxytridecyl)-2'-O-tert-butyldimethylsilyl-sn-glycerol] phosphate (3.6)

A mixture of **3.5** (222 mg, 0.500 mmol), 1*H*-tetrazole (105 mg, 1.50 mmol), and Fraser-Reid reagent (1.77 mL of a 423.4 mM CH₂Cl₂ solution, 0.750 mmol) in CH₂Cl₂ (5 mL) was stirred under nitrogen at rt for 2 h (all the 1*H*-tetrazole was dissolved at this point). The reaction was cooled to -40 °C, and mCPBA (345 mg, 1.00 mmol assuming 50% purity) was added. After the solution was warmed to rt, the solution was diluted with CH₂Cl₂ (20 mL) and washed successively with aqueous Na₂SO₃ solution (2 x 10 mL, 10% w/v), a saturated aqueous NaHCO₃ solution (2 x 7 mL), water (1 x 7 mL), and brine. The organic phase was dried (MgSO₄), filtered, and evaporated to a syrup. Flash chromatography (Kieselgel 60, 9 x 2.5 cm, hexane/EtOAc 4:1) gave **3.6** (347 mg, 0.492 mmol) in 98% yield. ¹H NMR (CDCl₃) δ 0.024 (s, 6H, Si(CH₃)₂), 0.832 (s, 9H, SiC(CH₃)₃), 1.21 (s, 16H, (CH₂)₈), 1.48-1.58 (m, 6H), 2.27 (t, 2H, *J* = 7.5 Hz, CH₂CO₂CH₃), 3.30-3.37 (m, 4H), 3.62 (s, 3H, CO₂CH₃), 3.89-4.04 (m, 3H), 5.00 (d, 2H, *J* = 7.8 Hz, 2 x CH₂H_bC₆H₅), 5.01 (d, 2H, *J* = 7.9 Hz, 2 x CH_aH_bC₆H₅), 7.22-7.30 (m, 10H, 2 x C₆H₅); ³¹P NMR (CDCl₃) δ -0.697.

Di-O-Benzyl O-[1'(14"-Carbo-(2'''-naphthoxytridecyl)-2'-O-tert-butyldimethylsilyl-sn-glycerol] phosphate (3.18)

A suspension of **3.6** (217 mg, 0.308 mmol) and crude pig liver esterase (200 mg) in potassium phosphate buffer (0.10 M, pH 7.0, 12 mL) was stirred at 37 °C for 72 h. The mixture was extracted with EtOAc (50 mL), dried (MgSO₄),

filtered, and evaporated to a residue. Flash chromatography (Kieselgel 60, hexane/EtOAc 1:3) gave the crude product (182 mg), which was converted to the free acid by eluting through a Dowex 50X8 column with THF/H₂O (25 mL, 10:1 v/v). After the solvents were evaporated as a pyridine azeotrope, the free acid was dissolved in dry CH₂Cl₂ (10 mL) and stirred with 2-naphthol (39.6 mg, 0.275 mmol), DMAP (33.6 mg, 0.275 mmol), and DCC (375 μ L of a 1 M CH₂Cl₂ solution, 0.375 mmol) under N₂. After 14 h, the mixture was filtered and evaporated. Flash chromatography (Kieselgel 60, 14 x 2.5 cm, hexane/EtOAc 4:1) was used to isolate the product (96.5 mg, 0.118 mmol) in 47% yield. ¹H NMR (CDCl₃) δ 0.024 (s, 6H, Si(CH₃)₂), 0.831 (s, 9H, SiC(CH₃)₃), 1.22 (s, 16H, (CH₂)₈), 1.40-1.75 (m, 6H), 2.57 (t, 2H, *J* = 7.4 Hz, CH₂CO₂CH₃), 3.30-3.37 (m, 4H), 3.63-4.02 (m, 3H), 4.99 (s, 2H, 2 x CH₂H_bC₆H₅), 5.03 (s, 2H, 2 x CH_aH_bC₆H₅), 7.07-7.83 (m, 17H, 2 x C₆H₅, naphthyl); ³¹P NMR (CDCl₃) δ -0.764.

Di-O-Benzyl O-[1'-(14"-carbo-(2"-naphthoxytridecyl)-sn-glycerol] phosphate
(**3.19**)

A mixture of **3.18** (58.5 mg, 0.0714 mmol) in a solution of 10% HCl/MeCN (5 mL) was stirred at 0 °C for 2.5 h and then at rt for 30 min. The mixture was poured into a saturated aqueous NaHCO₃ solution (10 mL) and extracted with EtOAc (3 x 5 mL). The organic phases were dried (MgSO₄), filtered, and evaporated to an oily residue which was purified by flash chromatography (Kieselgel 60, gradient of hexane/EtOAc 4:1, then 1:1) to give **3.19** (28.3 mg,

0.0402 mmol) in 56% yield. ^1H NMR (CDCl_3) δ 1.25 (s, 16H, $(\text{CH}_2)_8$), 1.47-1.80 (m, 6H), 2.45 (br s, 1H, OH), 2.58 (t, 2H, $J = 7.5$ Hz, $\text{CH}_2\text{CO}_2\text{CH}_3$), 3.34-3.40 (m, 4H), 3.86-3.91 (m, 1H, CHOH), 3.97-4.06 (m, 2H), 5.01 (s, 2H, 2 x $\text{CH}_2\text{H}_b\text{C}_6\text{H}_5$), 5.05 (s, 2H, 2 x $\text{CH}_2\text{H}_b\text{C}_6\text{H}_5$), 7.16-7.52 (m, 14H, 2 x C_6H_5 , naphthyl); 7.75-7.84 (m, 3H, naphthyl); ^{31}P NMR (CDCl_3) δ -0.0966.

Di-O-Benzyl O-[1'-(14''-carbo-(2'''-naphthoxytridecyl)-2'-O-hexanoyl-sn-glycerol] phosphate (3.20)

A solution containing **3.19** (28.3 mg, 0.0402 mmol), caproic anhydride (18.5 μL , 0.080 mmol), pyridine (16.2 μL , 0.200 mmol), and DMAP (10 mg, 0.08 mmol) in CH_2Cl_2 (10 mL) was stirred under N_2 overnight. The reaction was washed with an aqueous saturated NaHCO_3 solution (2 x 3 mL), water (1 x 3 mL), and brine, dried (MgSO_4), filtered, and evaporated. Flash chromatography (Kieselgel 60, 12 x 1.0 cm, hexane/EtOAc 2:1) gave **3.20** (27.9 mg, 0.0344 mmol) in 85% yield. ^1H NMR (CDCl_3) δ 0.838 (t, 3H, $J = 6.5$ Hz, $\omega\text{-CH}_3$), 1.25 (s, 18H, $(\text{CH}_2)_8$, CH_2CH_3), 1.44-1.76 (m, 10H), 2.24 (t, 2H, $J = 7.4$ Hz, CO_2CH_2), 2.57 (t, 2H, $J = 7.4$ Hz, CH_2CO_2), 3.21-3.63 (m, 4H), 4.08-4.16 (m, 2H), 5.01 (s, 2H, 2 x $\text{CH}_2\text{H}_b\text{C}_6\text{H}_5$), 5.05 (s, 2H, 2 x $\text{CH}_2\text{H}_b\text{C}_6\text{H}_5$), 5.10 (m, 1H, $\text{CHOCOC}_5\text{H}_{11}$), 7.16-7.51 (m, 14H, 2 x C_6H_5 , naphthyl); 7.74-7.84 (m, 3H, naphthyl); ^{31}P NMR (CDCl_3) δ -0.787.

O-Benzyl O-[1'-(14''-carbo-(2'''-naphthoxytridecyl)-2'-O-hexanoyl-sn-glycerol] phosphate (3.21)

A solution of **3.20** (27.9 mg, 0.0344 mmol) and NaI (4.5 mg, 0.030 mmol) in dry methyl ethyl ketone (8 mL) was gently refluxed for 1 h. The solvent was evaporated under reduced pressure to give a yellow residue that was purified by column chromatography (CM52, eluting with a gradient of CHCl₃/MeOH 100:0 to 80:20) to afford the desired product (15.3 mg, 0.0215 mmol) in 61 % yield. ¹H NMR (CDCl₃) δ 0.810 (t, 3H, *J* = 7.3 Hz, ω-CH₃), 1.20 (s, 18H, (CH₂)₈, CH₂CH₃), 1.34-1.46 (m, 6H), 1.72-1.77 (m, 4H), 2.13 (t, 2H, *J* = 7.4 Hz, CO₂CH₂), 2.57 (t, 2H, *J* = 7.5 Hz, CH₂CO₂), 3.21-3.37 (m, 4H), 3.85 (m, 2H), 4.86 (m, 2H, 2 x CH₂C₆H₅), 5.05 (m, 1H, CHOCOC₅H₁₁), 7.16-7.51 (m, 9H, C₆H₅, naphthyl); 7.74-7.84 (m, 3H, naphthyl); ³¹P NMR (CDCl₃) δ 0.517.

For the coupling reaction, **3.21** (15.3 mg, 0.0215 mmol) and **7** (20.5 mg, 0.0261 mmol) were rendered anhydrous by evaporation from pyridine (3 x 3 mL) and redissolved in the same solvent. MSNT (15.9 mg, 0.0538 mmol) was added, and the reaction mixture was stirred under N₂ at rt. After 3 h, TLC did not reveal any new products, and the starting materials were still present.

Chapter 4: Synthesis of Naphthyl-labeled PI and PIP₂ Using H-Phosphonates

One of the driving forces advancing the progress of organophosphorus chemistry in the past thirty years has been the need to chemically synthesize polyoligonucleotides. Various techniques to form a phosphodiester bond between ribonucleotides and deoxyribonucleotides have been developed, and this same chemistry has been adapted to the synthesis of phospholipids. There are two broad categories for forming the phosphate ester: phosphodiester methods and phosphotriester methods.

Phosphodiester approach

In this method, a hydroxyl group is phosphorylated and after activation, is coupled to the hydroxyl group of a second molecule to form the phosphate bond directly. The phosphodiester approach suffers from several drawbacks, such as the formation of side-products such as pyrophosphates and phosphotriesters. In nucleotide synthetic chemistry, separation of the nucleotide starting material and the phosphodiester product by chromatography using low capacity adsorbents is a time-consuming but essential step to achieve high yields.

Phosphotriester approach

To address the shortcomings of the phosphodiester methods, Letsinger *et al.* (1975) developed an approach in which a protected phosphate was coupled

to a free hydroxyl group, resulting in a phosphotriester. The relatively non-polar product can be isolated by flash chromatography on silica gel, and side products are virtually eliminated. This method is now the preferred technique to produce relatively large quantities of polynucleotides. In particular, the *H*-phosphonate is especially useful because of the speed of the reactions and the ease of workup.

***H*-Phosphonate**

Hall *et al.* (1957) first reported the possibility of using esters of phosphonic acid to synthesize phosphodiester bonds, but were unable to bring this idea to fruition due to the lack of a convenient oxidation step. Subsequent workers (Garegg *et al.*, 1985; Froehler and Matteucci, 1986) synthesized a di-deoxyribonucleotide by reacting a nucleotide bearing a 3'-*H*-phosphonate with a nucleoside containing a 5'-hydroxyl in the presence of a condensing reagent (diphenylchlorophosphate or pivaloyl chloride). Oxidation of the *H*-phosphonate triester to the phosphodiester was accomplished using a mixture of I_2 /water/pyridine. This work was extended to include the formation of phospholipids by Lindh and Stawinski (1989). Some useful features of this method are short reaction times, high yields, and the ability to synthesize modified phosphate esters by selecting different oxidizing agents (such as sulfur and selenium).

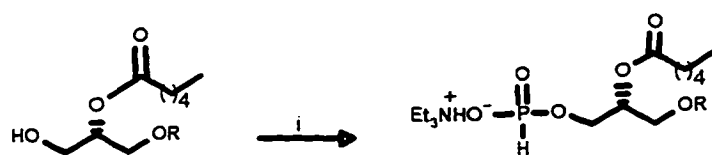
Coupling of inositol and diradyl glycerol molecules

With the syntheses of various fluorescent diradylglycerols and an inositol bis-phosphate analog completed, the next step in the scheme was the formation of the phosphodiester between the lipid and inositol molecules. Unfortunately, reaction between the inositol derivative, **7**, and the phosphodiester, **3.21**, using MSNT in pyridine did not result in the formation of a phosphodiester product (Scheme 3.5). This failure could be attributed to the steric bulk of the bis-dibenzylphosphorylated inositol. It was therefore decided to form the phosphate bond between an inositol derivative and the fluorescent diradylglycerol before introduction of the bis-dibenzylphosphates, an approach Dreef *et al.* (1988) used in their synthesis of PIP₂. Instead of the phosphoramidite method used by Dreef *et al.* (1988), the *H*-phosphonate approach was used. Previously, Garigapati and Roberts (1993) had demonstrated that phosphatidylinositol derivatives could be synthesized in high yield using this technique. In their work, the coupling agent, 5,5-dimethyl-2-oxo-2-chloro-1,3,2-dioxophosphorinan (NPCl) was used instead of pivaloyl chloride to avoid the possibility of pivalate formation. The *H*-phosphonate approach has a distinct advantage over the phosphoramidite method in that the intermediates in the former method are much more stable. *H*-Phosphonate monoesters can be stored at -20 °C for several months without any decomposition, and are easier to synthesize than the corresponding phosphoramidite.

Conversion of the glycerol derivative, **3.16**, to its corresponding *H*-phosphonate, **3.16HP** (Scheme 4.1), proceeded according to the procedure of Lindh and Stawinski with the exceptions that CH_2Cl_2 was used instead of toluene, and the reaction was quenched by the addition of 0.1 M TEAB buffer (pH 8.0) instead of water. The latter modification was made because the reaction time of the subsequent coupling to the inositol derivative, (-)-**3**, increases from 1 h to several days if the acid is used instead of the salt. Even so, the yield of the coupling reaction was only fair (37%). The yield was improved to 64% by converting (-)-**3** to its corresponding *H*-phosphonate and coupling it to **3.16** to give **4.1**. This sequence had the extra advantage of minimizing the formation of a symmetric *H*-phosphonate triester in the initial step.

Oxidation of **4.1** to **4.2** (Scheme 4.2) and selective deprotection of the 4,5-isopropylidene gave **4.3**, which was purified using CM52 chromatography eluting with a $\text{CHCl}_3/\text{MeOH}$ gradient (Comfurius and Zwaal, 1977). However, phosphorylation using Fraser-Reid reagent occurred with poor yield, and resulted in a large number of impurities that migrated closely with the product (**4.4**).

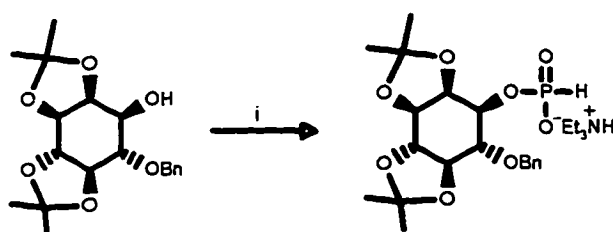
Scheme 4.1: Formation of a phosphodiester bond via the *H*-phosphonate method



3.16

R = -O(CH₂)₁₂Onaphthyl

3.16HP



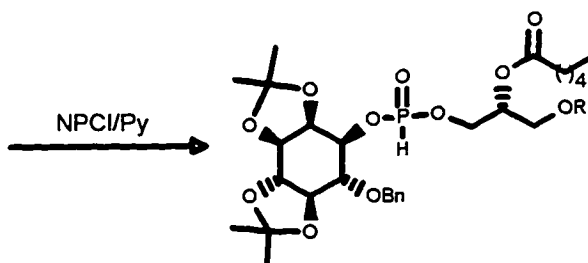
(-)-3

3HP

(i) (a) PCl₃, imidazole, Et₃N/CH₂Cl₂; (b) Et₃NH⁺HCO₃⁻.

Path A: **3.16HP** + **(-)-3**

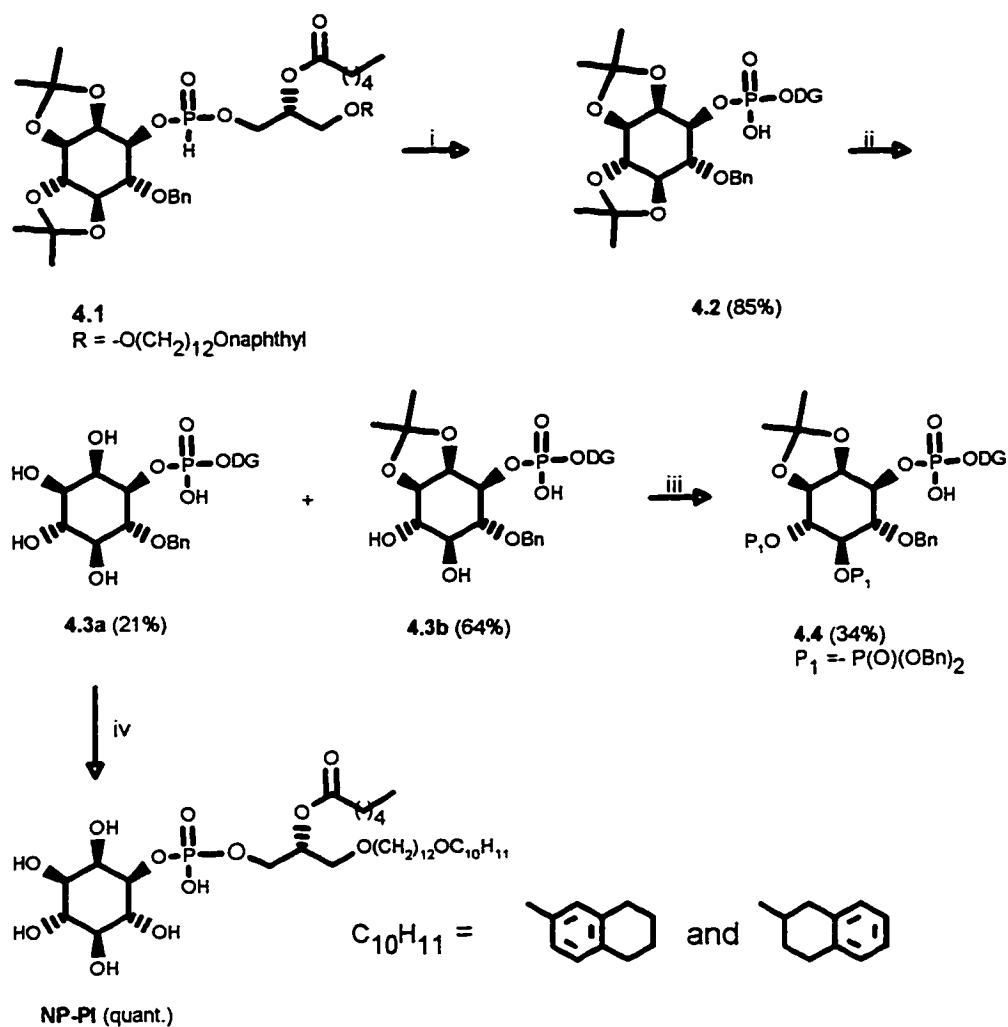
Path B: **(-)-3HP** + **3.16**



4.1

(Path A: 32%, Path B: 64%)

Scheme 4.2: Synthesis of a fluorescent phosphatidylinositol analog (NP-PI)



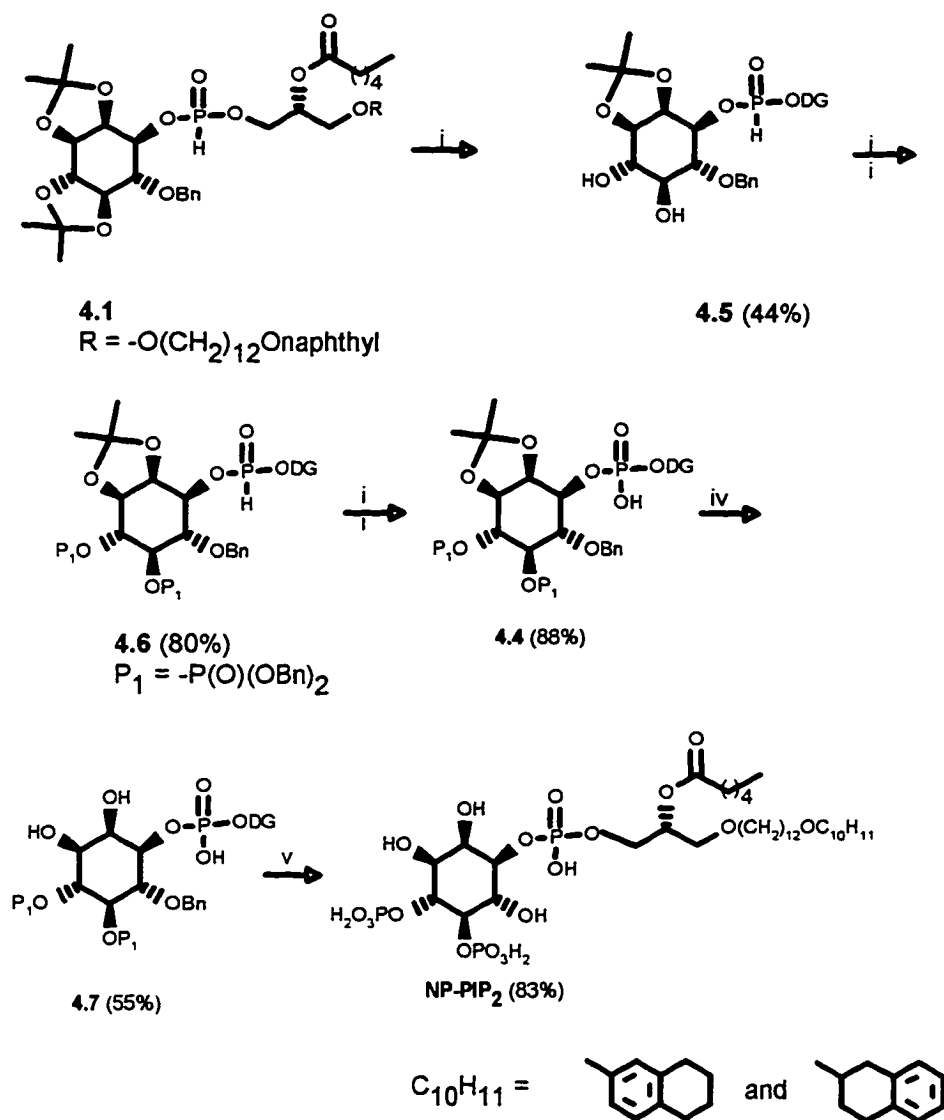
(i) $\text{I}_2/\text{py}/\text{H}_2\text{O}$; (ii) PPTS/ CHCl_3 ; (iii) a) 1*H*-tetrazole, Fraser-Reid reagent/ CH_2Cl_2 ; (b)

mCPBA; (iv) 10% Pd/C, H_2 .

An alternate route in which the *H*-phosphonate was oxidized after introduction of the bisdibenzylphosphates proved to give the same product (**4.4**) in greater overall yield starting from **4.1** (Scheme 4.3). Selective deprotection of the *trans*-isopropylidene group of **4.1** to give the diol, **4.5**, followed by phosphorylation and *in situ* oxidation with mCPBA gave the corresponding bis-dibenzylphosphate (**4.6**) in good yield. Surprisingly, the *H*-phosphonate did not oxidize under these conditions. A second oxidation with I₂/water/pyridine was required, opening the possibility of selectively introducing a modified phosphodiester (*i.e.* oxidation with elemental sulfur to produce a phosphorothioate) into this molecule without altering the inositol 4- and 5-phosphates. Acidic hydrolysis of the remaining isopropylidene group and hydrogenolysis of the benzyl functions was required to give the final product.

Unfortunately, catalytic hydrogenolysis of the the benzyl protection group using palladium (10% on charcoal) (Aldrich Chemical Co., Milwaukee, WI) and one atmosphere of hydrogen gas at room temperature also resulted in the unanticipated partial reduction of the naphthyl ring. Previously, catalytic hydrogenation of naphthalene ethers using Raney nickel as the catalyst had been shown to require high pressures of hydrogen (250 atm) and elevated temperatures (Stork, 1946; Musser and Adkins, 1937). Hydrogenation of the naphthyl ring at lower pressures and temperature is known to require a more powerful catalyst such as platinum on charcoal (Stork, 1946). Nevertheless, the results of HR-FABMS show four hydrogens atoms were added to the naphthyl

Scheme 4.3: Synthesis of a fluorescent phosphatidylinositol bis-4,5-phosphate analog (NP-PIP₂)



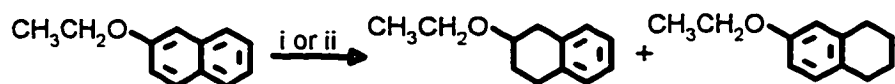
(i) *p*-TsOH, ethylene glycol/CH₂Cl₂; (ii) (a) Fraser-Reid reagent/CH₂Cl₂; (b) mCPBA;

(iii) I₂/py/H₂O; (iv) HCl/CH₂Cl₂; (v) 10% Pd/C, H₂.

ring under the relatively mild conditions using palladium on charcoal in both phosphatidylinositol (**NP-PI**) and phosphatidylinositol 4,5-bisphosphate derivatives (**NP-PIP₂**). Unfortunately, a conclusive interpretation of the ¹H spectra of these two derivatives was not possible.

To confirm the unexpected finding that a naphthoxy ether can undergo catalytic hydrogenation under mild conditions using palladium/charcoal as a catalyst, the hydrogenation of 2-ethoxynaphthalene was attempted (Figure 4.1). In this model reaction, 2-ethoxynaphthalene was subjected to catalytic hydrogenation under conditions similar to those used for the deprotection of **4.7** to synthesize **NP-PIP₂** (the solvent, EtOH/water, did not dissolve ethoxy-2-naphthalene). Sodium dihydrogen phosphate (4 eq.) was also added to make the solvent acidic, as would have been the case when the phosphate groups of **NP-PIP₂** were deprotected. After the reaction mixture was stirred for 72 h under one atm of hydrogen, a mixture of products had formed. Analysis by GC/MS revealed the presence of two 2-ethoxytetrahydronaphthalene derivatives and some unreacted starting material in a nearly 1:1:1 ratio. The ¹H spectrum of this product mixture also confirmed this result.

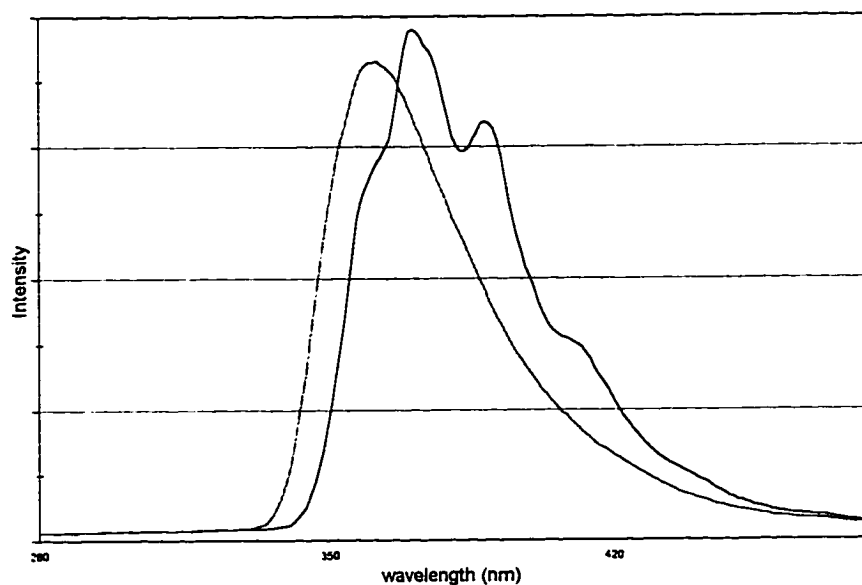
When the model reaction was repeated using CH₂Cl₂/MeOH 4:1 as the solvent, and 4 eq. of phosphoric acid, only 10% of the starting material remained after 72 h. The fluorescent spectra of the products are distinct from the spectrum of 2-ethoxynaphthalene (Figure 4.2). Based on the results of the model reaction, it is likely that the products of the hydrogenolysis

Figure 4.1: Hydrogenation of 2-ethoxynaphthalene

(i) H_2 , Pd/C, $\text{NaH}_2\text{PO}_4/\text{EtOH}$ (4:1 v/v) (34% starting material left after 72 h); (ii) H_2 , Pd/C,

$\text{H}_3\text{PO}_4/\text{CH}_2\text{Cl}_2$, MeOH (4:1 v/v) (10% starting material left after 72 h).

Figure 4.2 : Fluorescent spectra of 2-ethoxy-naphthalene vs. reduced products (ethoxytetrahydronaphthalenes)



solid lines: 2-ethoxynaphthalene (46.6 mM in hexane)

dashed lines: ethoxytetrahydronaphthalenes (45.0 mM in hexane)

Conditions : Excitation at 285 nm, 1 mm excitation slits; 1 mm emission slits.

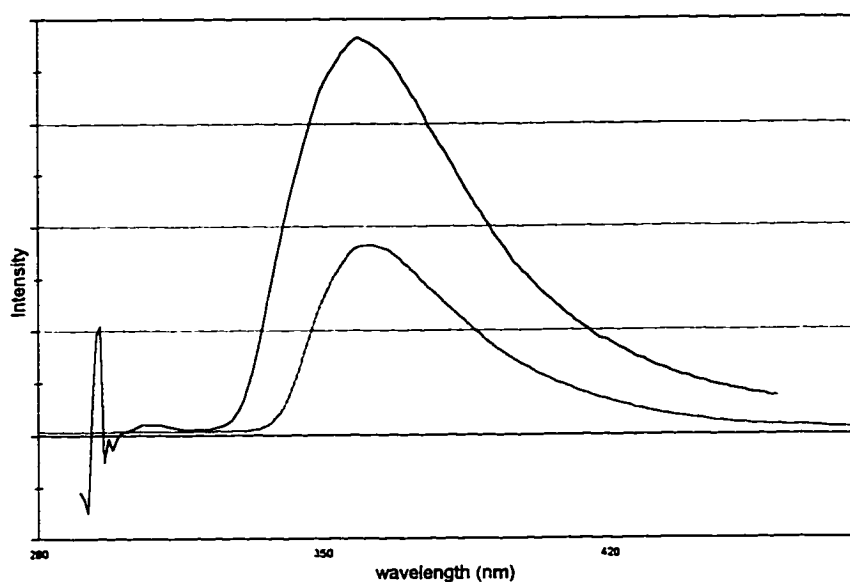
of **4.3a** are 1-phospho-*myo*-inosityl-1'-O-(12''-(2'''-O-1''',2''',3''',4'''-tetrahydronaphthyl)-dodecyl))-2''-O-hexanoyl-*sn*-glycerol and 1-phospho-*myo*-inosityl-1'-O-(12''-(2'''-O-6''',7''',8''',9'''-tetrahydronaphthyl)-dodecyl))-2''-O-hexanoyl-*sn*-glycerol (**NP-PI**, page 84), and that the products of the hydrogenolysis of **4.7** are 4,5-bisphosphoryl-1-phospho-*myo*-inosityl-1'-O-(12''-(2'''-O-1''',2''',3''',4'''-tetrahydronaphthyl)-dodecyl))-2'-O-hexanoyl-*sn*-glycerol and 4,5-bisphosphoryl-1-phospho-*myo*-inosityl-1'-O-(12''-(2'''-O-6''',7''',8''',9'''-tetrahydronaphthyl)-dodecyl))-2'-O-hexanoyl-*sn*-glycerol (**NP-PIP₂**, page 86). The fluorescent spectrum of **NP-PIP₂** is similar to that of the reduced 2-ethoxynaphthalene derivatives (Figure 4.3). Analysis of this product by HPLC shows an elution profile (Figure 4.4) similar to that for a radiolabeled PIP₂ reported by Low (1990).

Synthesis of a naphthyl-labeled phosphatidylinositol analog (NP-PI) (Scheme 4.2)

6-O-Benzyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol H-phosphonate (**3HP**)

Imidazole (687.0 mg, 10.09 mmol) was dried by repeatedly suspending and evaporating from cyclohexane three times. The imidazole was resuspended in CH₂Cl₂ (20 mL) and stirred under N₂ at 0 °C. To this mixture was added dropwise PCl₃ (194.2 μL, 2.23 mmol) in 5 mL of CH₂Cl₂, followed by Et₃N (804.6 μL, 5.77 mmol) in 5 mL of CH₂Cl₂. The reaction was cooled to -15 °C and stirred for 10 min. A solution of (-)-**3** (260.0 mg, 0.7420 mmol) in CH₂Cl₂ (20 mL) was

Figure 4.3: Fluorescent spectra of ethoxytetrahydronaphthalenes vs. NP-PIP₂ (see Scheme 4.3 for structure)

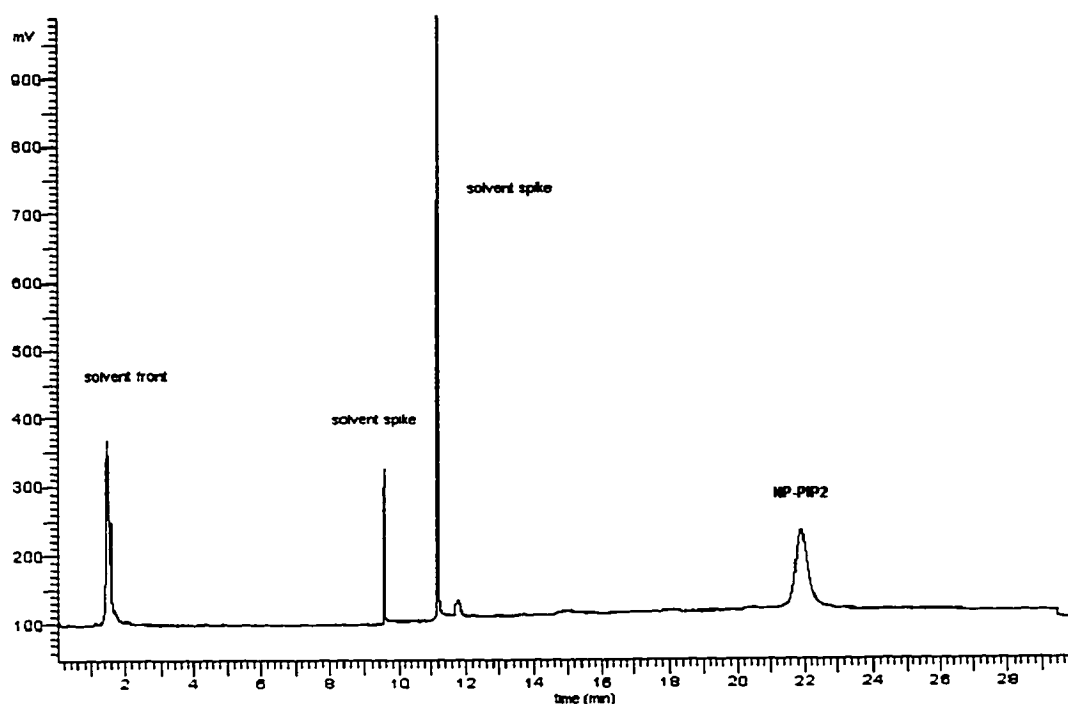


Upper curve: **NP-PIP₂** (14.7 mM in water)

Lower curve: ethoxytetrahydronaphthalenes (45.0 mM in hexane)

Conditions : Excitation at 285 nm, 1 mm excitation slits; 1 mm emission slits.

Figure 4.4: HPLC chromatogram of NP-PIP₂ (see Scheme 4.3 for structure)



Conditions: Alltech Econosphere NH₂; 4.6 x 250 mm. Solvent A: CHCl₃/MeOH/water 20:9:1 (v/v/v). Solvent B: solvent A with ammonium acetate (0.6 M NH₄OAc). Elution method: (1) 100 % solvent A, 2.0 mL/min for 2.5 min; (2) 100% solvent A to 100% solvent B in a linear gradient, 2.0 mL/min for 13 min; (3) 100% solvent B, 2.0 mL/min for 11 min. Detection at 280 nm.

added dropwise over 60 min. After the addition was complete, the reaction was stirred for an additional 20 min at $-15\text{ }^{\circ}\text{C}$ and then poured into TEAB buffer (80 mL, 0.1 M, pH 8). The phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (2 x 30 mL). The organic phases were pooled and washed with water (2 x 25 mL), dried (MgSO_4), filtered, and evaporated to give **3HP** (257.0 mg, 0.4975 mmol, triethylammonium salt) in 67% yield.

^1H NMR (CDCl_3) δ 1.27 (t, 9H, $J = 7.3\text{ Hz}$, 3 x CH_3), 1.33 (s, 3H, CH_3), 1.42 (s, 6H, CH_3), 1.53 (s, 3H, CH_3), 2.98 (q, 6H, 7.3 Hz , 3 x CH_2), 3.52 (dd, 1H, $J = 10.6, 8.2\text{ Hz}$, H-5), 4.00 (dd, 1H, $J = 8.2, 4.0\text{ Hz}$, H-4), 4.10 (dd, 1H, $J = 10.5, 7.9\text{ Hz}$, H-3), 4.30 (t, 1H $J = 7.2\text{ Hz}$, H-6), 4.48 (dd, 1H, $J = 6.3, 4.1\text{ Hz}$, H-2), 4.60 (dt, 1H, $J = 4.1, 6.3\text{ Hz}$, H-1), 4.74 (d, 1H, $J = 11.9\text{ Hz}$, OCH_aH_b), 4.81 (d, 1H, $J = 11.9\text{ Hz}$, OCH_aH_b), 7.03 (d, 1H, $J = 634.7\text{ Hz}$, PH), 7.22-7.37 (5H, m, C_6H_5). ^{31}P NMR (CDCl_3) δ 6.293.

6-O-Benzyl-2,3:4,5-di-O-isopropylidene-1-phospho-myo-inositol-1'-O-(12''-(2'''-O-naphthyl)-dodecyl))-2''-O-hexanoyl-sn-glycerol (**4.2**) (Scheme 4.2)

A mixture of **3HP** (266.6 mg, 0.520 mmol) and **3.16** (311.3 mg, 0.622 mmol) was rendered anhydrous by repeated evaporation from pyridine (3 x 2 mL) and redissolved in pyridine (6 mL). To this solution was added NPCl (256.7 mg, 0.560 mmol). After the mixture was stirred under N_2 for 15 min, a solution containing iodine (264.0 mg, 1.04 mmol) in pyridine/water (98:2 v/v, 1 mL) was added, and the mixture was stirred for an additional 5 min at rt. An aqueous

solution of sodium bisulfite (5% w/v) was added dropwise until the the brown solution became colorless. Solid sodium bicarbonate (100 mg) was added, and the solution was evaporated under reduced pressure. The residue was suspended in CHCl_3 and filtered through glass wool, and the resulting eluant was evaporated. Purification of the crude product by column chromatography (CM52, eluting with CHCl_3 , $\text{CHCl}_3/\text{MeOH}$ 90:10) gave pure **4.2** (453.2 mg, 0.485 mmol, 85 % yield, R_f 0.56; $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$ 65:25:4). The fraction eluting in pure chloroform contained mostly unreacted **3.16** and some **4.2** (50 mg total mass of crude products).

^1H NMR (CDCl_3) δ 0.845 (t, 3H, J = 6.6 Hz, $\omega\text{-CH}_3$), 1.25 (s, 18H), 1.38 (s, 3H), 1.45 (s, 6H), 1.54 (s, 3H), 1.4-1.7 (m, 6H), 1.7-2.0 (m, 2H), 2.25 (t, 2H, J = 7.4 Hz, CH_2CO_2), 3.28-3.41 (m, 2H), 3.49-3.63 (m, 3H), 3.94-4.00 (m, 1H), 4.05-4.08 (m, 5H), 4.56 (t, 1H, J = 6.4 Hz), 4.48-4.54 (m, 2H), 4.78 (dd, 2H, J = 11.8, 26.4 Hz), 5.14 (m, 1H), 7.08-7.43 (m, 9H), 7.61-7.73 (m, 3H). ^{31}P NMR (CDCl_3) δ 0.199.

6-O-Benzyl-2,3-O-isopropylidene-1-phospho-myo-inosityl-1'-O-(12''-(2'''-O-naphthyl)-dodecyl))-2'-O-hexanoyl-sn-glycerol (4.3a) and *6-O-Benzyl-1-phospho-myo-inosityl-1'-O-(12''-(2'''-O-naphthyl)-dodecyl))-2'-O-hexanoyl-sn-glycerol (4.3b)* (Scheme 4.2)

A solution containing **4.2** (365.8 mg, 0.3912 mmol), PPTS (50 mg, 0.200 mmol), and ethylene glycol (21.7 μL , 0.390 mmol) in 40 mL of chloroform was refluxed for 14 h, and then stirred at rt overnight. The reaction was quenched

with the addition of 1 mL of triethylamine and evaporated under reduced pressure. Column chromatography (CM52, CHCl₃/MeOH 100:0 to 60:40) gave **4.3a** (222.8 mg, 0.2487 mmol; R_f 0.42, CHCl₃/MeOH/H₂O, 65:25:4) in 64% yield and **4.3b** (73.5 mg, 0.0834 mmol; R_f 0.26, CHCl₃/MeOH/H₂O, 65:25:4) in 21% yield.

1-Phospho-myo-inositol-1'-O-(12''-(2'''-O-1''',2''',3''',4'''-tetrahydronaphthyl)-dodecyl))-2''-O-hexanoyl-sn- glycerol and 1-phospho-myo-inositol-1'-O-(12''-(2'''-O-6''',7''',8''',9'''-tetrahydronaphthyl)-dodecyl))-2''-O-hexanoyl-sn-glycerol (NP-PI) (Scheme 4.2)

A mixture of **4.3a** (22.6 mg, 0.0257 mmol) and 10% Pd/C (20 mg) in 10 mL of ethanol (95%) was stirred under hydrogen at rt overnight. The catalyst was removed by filtration through glass wool and the solvent was evaporated under reduced pressure to give **NP-PI** as the sodium salt (20.1 mg, quantitative yield; R_f 0.19, CHCl₃/MeOH/H₂O, 65:25:4). ³¹P NMR (CDCl₃/CD₃OD/D₂O 100:15:1) δ -0.948. FABMS [M-H]: Calcd. for C₃₇H₆₂O₁₃P: 745.39. Found 745.32.

Synthesis of a naphthyl-labeled phosphatidylinositol 4,5-bisphosphate analog (NP-PIP₂) (Schemes 4.2 and 4.3)

(oxidation of H-phosphonate prior to phosphorylation of 4- and 5-hydroxy positions, Scheme 4.2)

6-O-Benzyl-4,5-bisdibenzylphosphoryl-2,3-O-isopropylidene-1-phospho-myo-inositol-1'-O-(12''-(2'''-O-naphthyl)-dodecyl))-2'-O-hexanoyl-sn-glycerol (4.4)

A solution of **4.3b** (222.8 mg, 0.2487 mmol), 1*H*-tetrazole (104.5 mg, 1.492 mmol), and Fraser-Reid reagent (1.99 mL of a 375 mM CH₂Cl₂ solution, 0.7461 mmol) in CH₂Cl₂ (10 mL) was stirred at rt for 2 h, and then cooled to -40 °C. *m*CBPA (343.3 mg, 1.000 mmol assuming 50% purity) was added, and the reaction was allowed to warm to rt. The reaction mixture was diluted with CHCl₃ (50 mL) and was washed successively with aqueous Na₂SO₃ solution (10% w/v, 2 x 20 mL), a saturated aqueous NaHCO₃ solution (2 x 15 mL), water (15 mL), and brine. The organic phase was dried (MgSO₄), filtered, and evaporated. Column chromatography (Kieselgel 60, 8 x 2.5 cm, CHCl₃/MeOH 9:1) gave the product **4.4** (98.3 mg, 0.08330 mmol) in 34% yield.

¹H NMR (CDCl₃) δ 0.815 (t, 3H, *J* = 6.1 Hz, ω-CH₃), 1.23 (br s, 18H, (CH₂)₈, -CH₂CH₃), 1.32-1.48 (m, 12H), 1.77-1.84 (m, 2H), 2.22 (t, 2H, *J* = 7.5 Hz, C(O)CH₂), 3.27-3.52 (m, 4H, CH₂OCH₂), 4.00-4.10 (m, 4H, POCH₂, -CH₂Onaphthyl), 4.26-4.39 (m, 1H), 4.64-5.26 (m, 16H), 7.08-7.70 (m, 29H, 5 x C₆H₅, naphthyl), 7.66-7.74 (m, 3 H, naphthyl); ³¹P -1.87, -2.27, -3.43.

(oxidation of H-phosphonate after phosphorylation of 4- and 5-hydroxy positions, Schemes 4.1 and 4.3)

1-O-(12'-(2''-O-Naphthyl)-dodecyl)-2-O-hexanoyl-sn-glycerol H-phosphonate (3.16HP)

Imidazole (753.0 mg, 11.06 mmol) was dried by repeatedly suspending and evaporating from cyclohexane three times. The imidazole was resuspended in CH_2Cl_2 (10 mL) and stirred under N_2 at 0 °C. To this mixture was added a solution of PCl_3 (212.9 μL , 2.44 mmol) in 2.5 mL of CH_2Cl_2 dropwise, followed by Et_3N (881 μL , 6.32 mmol) in CH_2Cl_2 (2.5 mL). The reaction was cooled to -15 °C and stirred for 10 min. A solution of **3.16** (452.7 mg, 0.9040 mmol) in CH_2Cl_2 (10 mL) was added dropwise over 60 min. After the addition was complete, the reaction was stirred for an additional 20 min at -15 °C and then poured into TEAB buffer (40 mL, 0.1 M, pH 8). The phases were separated, and the aqueous phase extracted with CHCl_3 (3 x 10 mL). The organic phases were pooled and washed with water (2 x 15 mL), dried (MgSO_4), filtered, and evaporated to give **3.16HP** (722.5 mg, crude product).

^1H NMR (CDCl_3) δ 0.843 (t, 3H, J = 6.6 Hz, $\omega\text{-CH}_3$), 1.25 (br s, 18H, $(\text{CH}_2)_8$, $-\text{CH}_2\text{CH}_3$), 1.30-1.40 (m, 9H, $\text{N}(\text{CH}_2\text{CH}_3)_3$), 1.41-1.57 (m, 6H), 1.73-1.84 (m, 2H), 2.29 (t, 2H, J = 7.4 Hz, $\text{C}(\text{O})\text{CH}_2$), 2.84-3.04 (m, 6H, $\text{N}(\text{CH}_2\text{CH}_3)_3$), 3.33 (dd, 2H, J = 6.4, 11.9 Hz, $-\text{OCH}_2$), 3.54 (d, 2H, 5.1 Hz, $-\text{CH}_2\text{O}$), 3.93-4.06 (m, 4H, POCH_2 , $-\text{CH}_2\text{Onaphthyl}$), 5.09-5.14 (m, 1H, CHO), 6.82 (d, 1H, J = 619 Hz, PH), 7.08-7.42 (m, 4H, naphthyl), 7.66-7.74 (m, 3 H, naphthyl); ^{31}P 5.28.

O-(6-*O*-Benzyl-2,3:4,5-di-*O*-isopropylidene-*myo*-inositol) *O*-(1'-*O*-(12''-(2'''-*O*-naphthyl)-dodecyl))-2'-*O*-hexanoyl-*sn*-glycerol) H-phosphonate (**4.1**)

A) 3.16HP and (-)-3 (Scheme 4.1, path (A))

A mixture of crude **3.16HP** (655.2 mg, 0.984 mmol if pure) and (-)-**3** (400.0 mg, 1.1412 mmol) was rendered anhydrous by repeated evaporation from pyridine (2 x 5 mL), and then dissolved in the same solvent (10 mL). To this solution was added NPCl (553 mg, 3.00 mmol) and the mixture was stirred at rt under N₂ overnight. The reaction was quenched by adding water (1 mL), and the solvents were evaporated under reduced pressure. Column chromatography (Kieselgel 60, eluting with hexane/EtOAc 3:1) gave the product (274.4 mg, 0.3059 mmol) in 32% yield.

B) 3HP and 3.16 (Scheme 4.1, path (B))

A mixture of **3HP** (987.33 mg, 1.919 mmol) and **3.16** (1.481 g, 2.958 mmol) was rendered anhydrous by repeated evaporation from pyridine (2 x 5 mL), and then dissolved in the same solvent (10 mL). To this solution was added NPCl (823 mg, 5.00 mmol) and the mixture was stirred at rt under N₂ for 72 h. The reaction was then diluted with ice-cold TEAB buffer (100 mL, 0.1 M, pH 7.5) and extracted with CHCl₃ (4 x 25 mL). The organic phase was dried (MgSO₄), filtered, and evaporated under reduced pressure. Column chromatography (Kieselgel 60, 14 x 2.5 cm, eluting with hexane/EtOAc 4:1, then 2:1) gave the product, **4.1** (1.679 g, 1.872 mmol) in 64% yield.

¹H NMR (CDCl₃) δ 0.845 (t, 3H, *J* = 6.5 Hz, ω-CH₃), 1.22 (br s, 18H, (CH₂)₈, -CH₂CH₃), 1.32-1.44 (m, 6H), 1.48 (s, 3H, CH₃), 1.51 (s, 6H, 2 x CH₃), 1.57 (s, 3H, CH₃), 1.61-1.84 (m, 2H), 2.27 (t, 2H, *J* = 7.4 Hz, C(O)CH₂),

3.28-3.58 (m, 5H, $-\text{CH}_2\text{OCH}_2$, H-5), 3.93-4.13 (m, 4H, POCH_2 , $-\text{CH}_2\text{Onaphthyl}$), 4.26-4.42 (m, 4H), 5.07-5.12 (m, 1H, CHO), 6.94 (d, 1H, $J = 700$ Hz, PH), 7.08-7.42 (m, 4H, naphthyl), 7.52-7.73 (m, 3 H, naphthyl); ^{31}P 8.89, 9.23.

O-(6-*O*-Benzyl-2,3-*O*-isopropylidene-my α -inositol) *O*-(1'-*O*-(12''-(2'''-*O*-naphthyl)-dodecyl))-2'-*O*-hexanoyl-sn-glycerol) H-phosphonate (**4.5**) (Scheme 4.3)

A solution containing **4.1** (1.679 g, 1.872 mmol), *p*-TsOH (10 mg, 0.04 mmol), and ethylene glycol (18.7 mL of a 100 mM CH_2Cl_2 solution, 1.87 mmol) in CH_2Cl_2 (50 mL) was refluxed for 30 min, neutralized with excess Et_3N (200 μL , 1.5 mmol), and evaporated. Radial band chromatography (elution with hexane/EtOAc 1:3) gave the title compound (710.0 mg, 0.8284 mmol) in 44% yield.

^1H NMR (CDCl_3) δ 0.853 (t, 3H, $J = 6.3$ Hz, $\omega\text{-CH}_3$), 1.24 (br s, 18H, $(\text{CH}_2)_8$, $-\text{CH}_2\text{CH}_3$), 1.33-1.60 (m, 12H), 1.74-1.84 (m, 2H, $\text{CH}_2\text{CH}_2\text{Onaphthyl}$), 2.26-2.35 (m, 4H, $\text{C}(\text{O})\text{CH}_2$, 2 x OH), 3.30-3.51 (m, 5H, CH_2OCH_2 , H-5), 3.73-3.81 (m, 2H), 3.97-4.06 (m, 4H, POCH_2 , $-\text{CH}_2\text{Onaphthyl}$), 4.12-4.26 (m, 1H), 4.36-4.46 (m, 1H), 4.65-4.96 (m, 3H), 4.91-5.09 (m, 1H, CHO), 6.86 (d, 1H, $J = 701$ Hz, PH), 7.09-7.43 (m, 9H, benzyl, naphthyl), 7.66-7.74 (m, 3 H, naphthyl); ^{31}P 8.28, 9.57.

O-(6-*O*-Benzyl-4,5-bisdibenzylphosphoryl-2,3-*O*-isopropylidene-my α -inositol) *O*-(1'-*O*-(12''-(2'''-*O*-naphthyl)-dodecyl))-2'-*O*-hexanoyl-sn-glycerol) H-phosphonate (**4.6**) (Scheme 4.3)

A solution of **4.5** (710.0 mg, 0.8284 mmol), 1*H*-tetrazole (350.3 mg, 4.920 mmol), and Fraser-Reid reagent (6.63 mL of a 375 mM CH₂Cl₂ solution, 2.49 mmol) in CH₂Cl₂ (50 mL) was stirred at rt for 2 h, and then cooled to -40 °C. *m*CBPA (1.13 g, 4.92 mmol assuming 50% purity) was added and the reaction was allowed to warm to rt. The reaction mixture was diluted with CHCl₃ (50 mL) and was washed successively with an aqueous Na₂SO₃ solution (10% w/v, 2 x 20 mL), a saturated aqueous NaHCO₃ solution (2 x 15 mL), water (15 mL), and brine. The organic phase was dried (MgSO₄), filtered, and evaporated. Radial band chromatography (eluting with hexane/EtOAc 1:1, then 1:3), gave the product (917.6 mg, 0.6661 mmol) in 80% yield. This compound was used without further characterization.

6-O-Benzyl-4,5-bisdibenzylphosphoryl-2,3-O-isopropylidene-1-phospho-myo-inositol-1'-O-(12''-(2'''-O-naphthyl)-dodecyl))-2'-O-hexanoyl-sn-glycerol (4.4)

A solution containing **4.6** (327.9 mg, 0.238 mmol) and I₂ (66.4 mg, 0.2618 mmol) in pyridine/water (98:2, 6 mL) was stirred at rt. After 20 min, the reaction was diluted with a solution of aqueous NaHSO₃ (10% w/v, 50 mL) and extracted with CH₂Cl₂. The organic phase was dried (MgSO₄), filtered, and evaporated. Column chromatography (CM52, eluting with CHCl₃/MeOH 100:0, 98:2, then 95:5 in a stepwise gradient) gave the product (292.5 mg, 0.2100 mmol) in 88% yield.

^1H NMR (CDCl_3) δ 0.815 (t, 3H, $J = 6.1$ Hz, $\omega\text{-CH}_3$), 1.23 (br s, 18H, $(\text{CH}_2)_8$, $-\text{CH}_2\text{CH}_3$), 1.32-1.48 (m, 12H), 1.77-1.84 (m, 2H), 2.22 (t, 2H, $J = 7.5$ Hz, $\text{C}(\text{O})\text{CH}_2$), 3.27-3.52 (m, 4H, CH_2OCH_2), 4.00-4.10 (m, 4H, POCH_2 , $-\text{CH}_2\text{Onaphthyl}$), 4.26-4.39 (m, 1H), 4.64-5.26 (m, 16H), 7.08-7.70 (m, 29H, 5 x C_6H_5 , naphthyl), 7.66-7.74 (m, 3 H, naphthyl); ^{31}P -1.87, -2.27, -3.43.

6-O-Benzyl-4,5-bisdibenzylphosphoryl-1-phospho-myo-inositol-1'-O-(12''-(2'''-O-naphthyl)-dodecyl))-2'-O-hexanoyl-sn-glycerol (4.7)

A mixture containing **4.4** (292.5 mg, 0.2100 mmol) and aqueous HCl (37% w/w, 100 μL , 1.21 mmol) in CH_2Cl_2 (20 mL) was stirred at rt for 1h. The reaction was quenched by the addition of pyridine (121.3 μL , 1.5 mmol), and to this mixture was added hexane (6.5 mL) to precipitate the pyridinium hydrochloride. After filtration, the solvents were evaporated, and the product was purified by column chromatography (CM52, eluting with $\text{CHCl}_3/\text{MeOH}$ 100:0, 95:5, 90:10 then 80:20 in a stepwise gradient) to give the title compound (155.9 mg, 0.1152 mmol) in 55% yield.

^1H NMR (CDCl_3) δ 0.704 (t, 3H, $J = 6.8$ Hz, $\omega\text{-CH}_3$), 1.16-1.28 (br s, 18H, $(\text{CH}_2)_8$, $-\text{CH}_2\text{CH}_3$), 1.34-1.58 (m, 6H), 1.88-1.93 (m, 2H), 2.13-2.25 (m, 2H), 3.11-3.18 (m, 2H), 3.64 (m, 4H), 3.83-3.86 (m, 1H), 4.14 (t, 2H, $J = 6.5$ Hz, $\text{CH}_2\text{Onaphthyl}$), 4.34-4.36 (m, 2H), 4.55 (1H, m), 4.68-5.11 (m, 14H), 6.73-7.34 (m, 29H, 5 x C_6H_5 , naphthyl), 7.63-7.84 (m, 3 H, naphthyl); ^{31}P -0.0435, 0.227, 2.49.

4,5-Bisphosphoryl-1-phospho-myo-inositol-1'-O-(12''-(2'''-O-1''',2''',3''',4'''-tetrahydronaphthyl)-dodecyl))-2'-O-hexanoyl-sn-glycerol and 4,5-bisphosphoryl-1-phospho-myo-inositol-1'-O-(12''-(2'''-O-6''',7''',8''',9'''-tetrahydronaphthyl)-dodecyl))-2'-O-hexanoyl-sn-glycerol (NP-PIP₂)

A suspension containing **4.7** (155.9 mg, 0.1152 mmol) and 10% Pd-C (160 mg) in EtOH/water (25 mL, 4:1) was stirred under H₂ for 24 h. The catalyst was removed by centrifugation, and the solvents were evaporated to give the title compound (84.0 mg, 0.0931 mmol) in 83% yield. ³¹P (CD₃OD/D₂O 12:1) -0.759, 4.19, 4.77. HRFABMS [M+Na]: Calcd. for C₃₇H₆₅O₁₉P₃Na: 929.3231. Found 929.3198.

HPLC analysis was performed by dissolving the title compound (4.0 mg) in a solution of CHCl₃/MeOH/H₂O (1 mL, 20:9:1, v/v/v, solvent A) and applying an aliquot (50 µL) at a flow rate of 1.8 mL/min to a 4.6 mm x 250 mm Econosphere NH₂ column (5 µM spherical silica gel with an *n*-propylamine bonded phase; Alltech Associated, Deerfield, IL) equilibrated with solvent A. The column was eluted with 5 mL of solvent A at a flow rate of 1.8 mL/min, followed by 26 mL of a linear gradient of 100% solvent A to 100% 0.6 M NH₄OAc in solvent A (solvent B) at 1.8 mL/min, and finally with 20.8 mL of solvent B at a flow rate of 1.8 mL/min. Ultraviolet absorbance of the eluant (Figure 4.3) at 280 nm was measured using a Perkin Elmer LC-235 diode array detector.

Chapter 5: Synthesis and *In Vitro* activity of *myo*-inositol 4-(hexadecyloxy)-3-(*S*)-methoxybutanephosphonate (C4-PI) against various cancer cell lines

Introduction

Since the discovery of the canonical phosphatidylinositol (PI) signaling pathway (Berridge, 1993; Nishizuka, 1984), the synthesis of analogs of the lipid and inositol components of this system has been undertaken by several different research groups (Potter and Lampe, 1995; Bhamare *et al.*, 1996). Substrate analogs that can inhibit PI-specific phospholipase C (PI-PLC) isoforms are of special interest in that they can be used to study the mechanism and function of this key enzyme (Powis *et al.*, 1992; Pratt *et al.*, 1992). Isosteric substitution of the phosphate group with a phosphonate results in a nonhydrolyzable linkage. A series of phosphonate substrate analogs was synthesized by Shashidhar *et al.* (1990a, 1990b) and tested as inhibitors of *B. cereus* PI-PLC, but these compounds showed only modest inhibition of the enzyme. Naturally occurring compounds such as U-73122 (Mostafapour *et al.*, 1991) and Ro09-1450 (Aoki *et al.*, 1991) as well as the synthetic compounds hexadecylphosphocholine (miltefosine, Pawelczyk and Lowenstein, 1993) and ET-18-OCH₃ (edelfosine, Powis *et al.*, 1992) inhibit mammalian isoforms of PI-PLC at much lower concentrations. Powis *et al.* (1992) reported that edelfosine selectively inhibited mammalian PI-PLC (IC₅₀ of 0.4 - 1.0 μ M), and Überall *et al.* (1991) found that miltefosine inhibited inositol phosphate formation.

Edelfosine and miltefosine are antitumor ether lipids. Edelfosine has recently completed phase II clinical investigation, and is being marketed, whereas miltefosine and its topical formulation Miltex are marketed in Germany for topical treatment of subcutaneous breast cancer metastases. The mechanism by which edelfosine and other ether lipids exert their antineoplastic activity is still uncertain, but may involve several factors. Of particular interest is the possibility that antitumor ether lipids inhibit key events in biochemical signal transduction, including inhibition of both PI-PLC (Überall *et al.*, 1991) and phosphatidylinositol-3-kinase (PI-3-kinase, Berggren *et al.*, 1993). PI-3-kinase was inhibited by both edelfosine and 1-O-octadecyl-2-O-methoxy-*rac*-glycero-3-*rac*-inositol (IP-10); the latter differs from edelfosine in that it contains an inositol headgroup instead of choline. The inositol ether lipid IP-10 was a substrate for the kinase, and probably is also a substrate for PI-PLC, which precludes its use as an inhibitor. IP-10 was not as effective as edelfosine in killing the leukemic cell lines HL60 and K562 and the ovarian cancer cell lines BG1 and BG3 (Nosedá *et al.*, 1987). Edelfosine inhibited the activation of *Raf-1* in cultured cell lines, and thereby blocked signal transduction via the mitogen-activated protein kinase cascade (Zhou *et al.*, 1996).

Chemistry

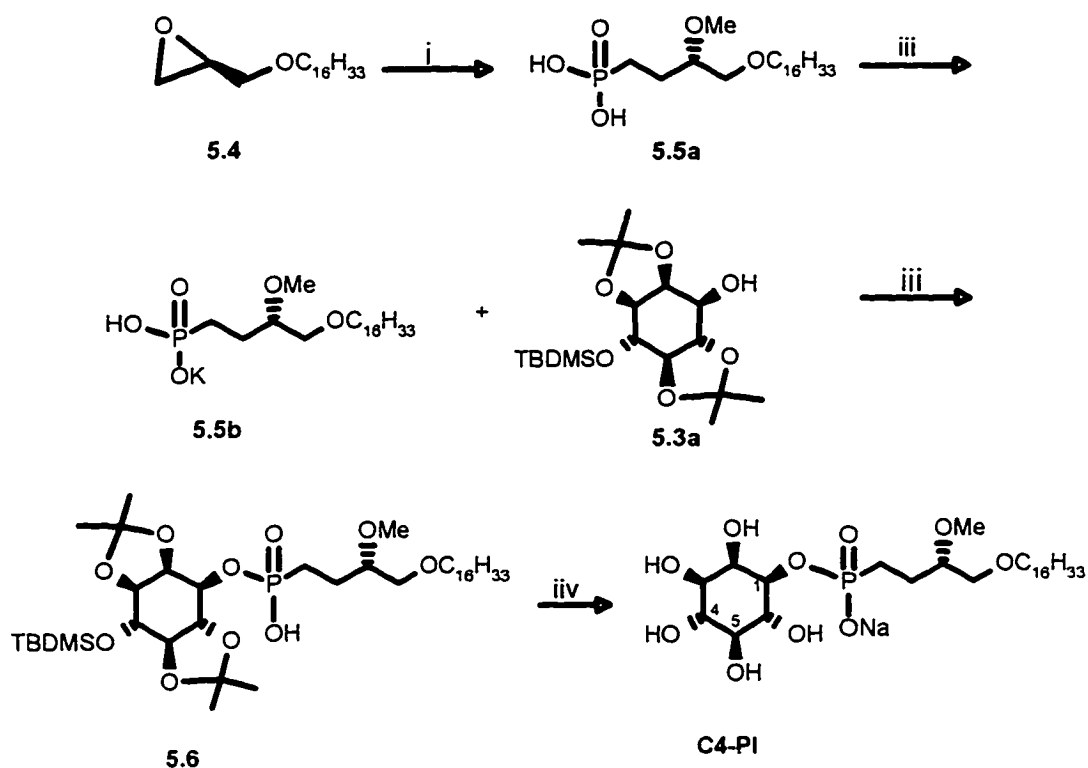
Although the inositol phosphonates synthesized by Shashidhar *et al.* (1990a) were poor inhibitors of bacterial PI-PLC, substitution of the hydrophobic

portion of the analogs with a group that more closely resembles diacylglycerol may enhance their ability to inhibit the enzyme. Furthermore, the compounds were made using a racemic protected inositol derivative; however, it is known that only D-*myo*-inositol lipids are substrates for PI-PLC (Shashidhar *et al.*, 1990b). Nosedá *et al.* (1987) hypothesized that an inositol analog of edelfosine (edelfosine was later shown to inhibit PI-PLC, Powis *et al.*, 1992) might exhibit antitumor properties. The synthesis of such a molecule (**C4-PI**) is shown in Scheme 5.1.

The method published by Martin *et al.* (1994) to synthesize a suitably penta-protected inositol derivative was used without modification. Reaction of the starting racemic inositol diol (only one enantiomer is shown in Scheme 5.2) with (1*S*)-(-)-camphanic chloride resulted in regioselective acylation of the 1-hydroxy group. After the 4-hydroxy group was protected as the *tert*-butyldimethylsilyl ether, column chromatography afforded separation of the two enantiomers (elution with CH₂Cl₂/acetone, 98:2). Alkaline hydrolysis of the products gave the two enantiomers **5.3a** and **5.3b**.

The synthetic scheme used previously to obtain 2'-(trimethylammonio)-ethyl 4-(hexadecyloxy)-3(*S*)-methoxybutanephosphonate (an isosteric phosphonate analog of edelfosine (Bittman *et al.*, 1993; Bittman *et al.*, 1994; see Figure 5.1) was modified to complete the synthesis of D-*myo*-inositol 4-(hexadecyloxy)-3(*S*)-methoxybutane-phosphonate (**C4-PI**, Scheme 5.1) and L-*myo*-inositol 4-(hexadecyloxy)- 3(*S*)-methoxybutane-phosphonate (**5.8**,

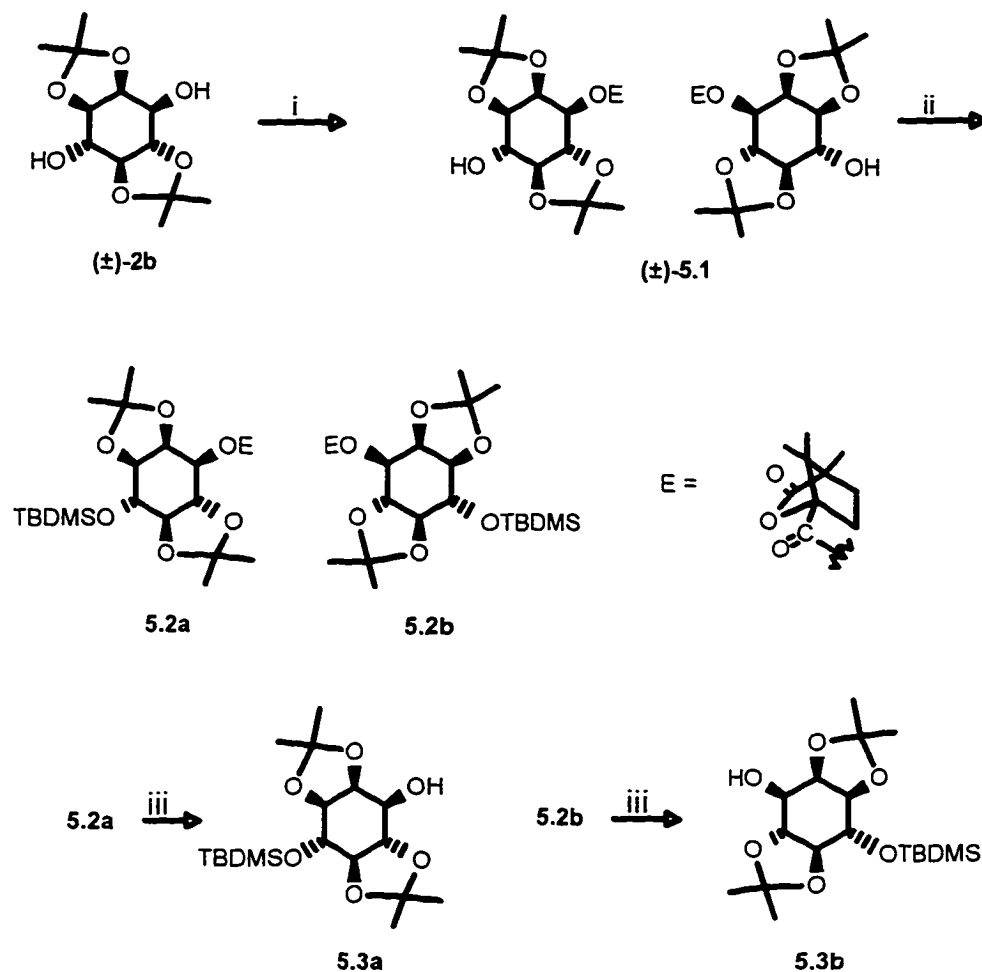
Scheme 5.1: Synthesis of D-*myo*-inositol 4-(hexadecyloxy)-3(*S*)-methoxy-butanephosphonate (C4-PI)



(i) see Scheme I of *J. Med. Chem.* **1994**, 37, 425-430; (ii) aqueous KOH (to pH 4.5);

(iii) MSNT/ py, rt, 1 h; (iv) PPTS/MeOH, reflux, 4 d.

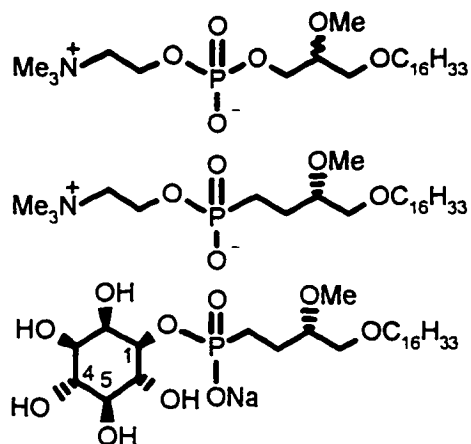
Scheme 5.2: Synthesis of 4-*O*-*tert*-butyldimethylsilyl-2,3:5,6-di-*O*-isopropylidene-*myo*-inositol (5.3a) and 6-*O*-*tert*-butyldimethylsilyl-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol (5.3b)



(i) (–)-camphanyl chloride, imidazole/DMF; (ii) TBDMS chloride, imidazole/DMF;

(iii) KOH/MeOH.

Figure 5.1: Structures of some antitumor ether lipids and their isosteric phosphonate analogs



1-O-hexadecyl-2-O-methyl-3-*rac*-glycerophosphocholine (top)

2'-(trimethylammonio)ethyl 4-(hexadecyloxy)-3(*S*)-methoxybutanephosphonate (middle)

D-*myo*-inositol 4-(hexadecyloxy)-3(*S*)-methoxybutanephosphonate (sodium salt) (C4-PI)
bottom)

Scheme 5.3). Synthesis of the phosphonic acid **5.5a** proceeded according to the method published previously (Bittman *et al.*, 1993). Briefly, ring opening of hexadecyl (*R*)-2-oxiranylmethyl ether (**5.4**) with lithium dimethyl methanephosphonate in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ at low temperature gave dimethyl 4-(hexadecyloxy)-3(*S*)-hydroxybutanephosphonate. O-Methylation and subsequent ester hydrolysis of the resulting dimethyl 4-(hexadecyloxy)-3(*S*)-methoxy-butanephosphonate (using trimethylsilyl bromide followed by THF/water) gave the crude phosphonic acid **5.5a**. Recrystallization from cyclohexane gave the pure product.

The key step in the syntheses of the inositol phosphonates prepared by Pratt *et al.* (1992) and Shashidhar *et al.* (1990a) was the formation of the phosphonate ester between a suitably protected inositol derivative and a phosphonic acid group using trichloroacetonitrile in pyridine. However, this method did not give the desired product using **5.5a** (a phosphonic acid) and **5.3a** (an inositol derivative). To overcome this difficulty, the phosphonic acid **5.5a** was converted to the monopotassium salt **5.5b** which was then coupled to the protected inositol derivative **5.3a** using MSNT in pyridine at room temperature. The reaction proceeded swiftly, but resulted in only a moderate yield. Since the phosphonate linkage is acid-labile (Shashidhar *et al.*, 1990a), deprotection of the inositol protecting groups (isopropylidene and *tert*-butyldimethylsilyl ether) with strong acids such as hydrofluoric acid (as had been done by Martin *et al.*, 1994) was precluded. Instead, deprotection of the acid-labile groups was

accomplished using PPTS in moderate yield (Mori *et al.*, 1993). The diastereomer of C4-PI (**5.8**, Scheme 5.3) was synthesized in a similar fashion using **5.3b** (the enantiomer of **5.3a**) in the coupling reaction with butanephosphonate **5.5b**. However, the biological properties of **5.8** were not investigated.

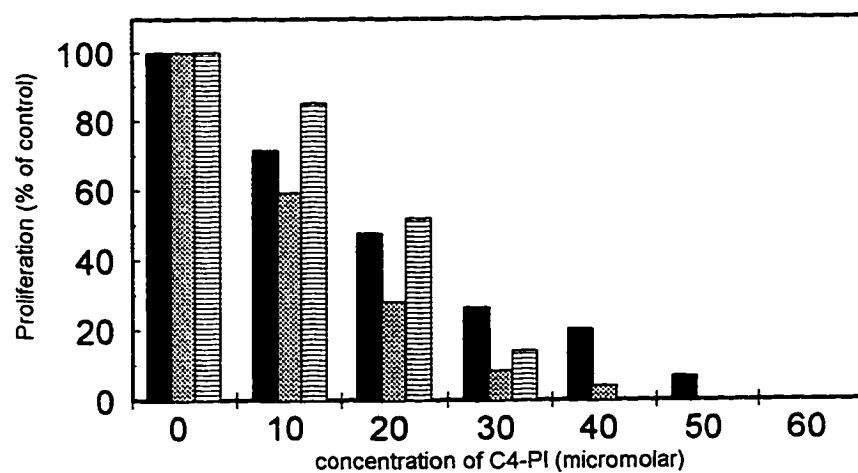
Biological Results

C4-PI inhibited the proliferation of all eight epithelial cell lines examined (Figure 5.2 and Table 5.1); however, the breast cell lines MCF-7 and MDA-MB-468 and to a lesser extent the neuroblastoma cell SK-N-SH were more sensitive to C4-PI than the other cell lines. The proliferation of MDA-MB-468 and MCF-7 was completely inhibited at 40 and 50 μ M C4-PI, respectively, whereas SK-N-SH proliferation was completely inhibited by 60 μ M.

Discussion

Various tumor cell lines have been shown to have different sensitivity toward antitumor ether lipids. Recently, edelfosine has been shown to be effective in killing breast cancer cells by *in vitro* purging of bone marrow (Dietzfelbinger *et al.*, 1993). It is likely that many factors are responsible for determining activity. In this study, MCF-7 (hormone sensitive) and MDA-MB-468 (hormone insensitive), two breast cancer cell lines, are more susceptible to the

Figure 5.2: Inhibition of proliferation by C4-PI



Effects of C4-PI on SK-N-SH (black boxes), MCF-7 (gray boxes), and MDA-MB-468 (horizontally striped boxes) on cell proliferation.

Table 5.1: Effect of C4-PI on Growth of Various Tumor Cell Lines

<u>Proliferation (% of control)</u>					
Conc. (μM)	A549 (Lung)	SK-N-MC (Neuroblastoma)	Ovcar-3 (Ovary)	A498 (Kidney)	DU 145 (Prostate)
0	100	100	100	100	100
10	101.9 $\pm$ 5.6	74.6 $\pm$ 8.5	103.2 $\pm$ 5.6	74.6 $\pm$ 8.5	89.5 $\pm$ 6.2
20	91.0 $\pm$ 3.7	68.9 $\pm$ 10.6	98.9 $\pm$ 13.2	59.3 $\pm$ 8.3	81.8 $\pm$ 8.3
30	76.8 $\pm$ 4.7	58.3 $\pm$ 15.0	78.6 $\pm$ 9.8	45.2 $\pm$ 3.8	50.8 $\pm$ 8.0
40	69.7 $\pm$ 2.2	48.7 $\pm$ 8.0	72.8 $\pm$ 7.3	23.2 $\pm$ 3.5	49.6 $\pm$ 9.1
50	63.8 $\pm$ 6.4	27.9 $\pm$ 3.3	67.7 $\pm$ 6.7	61.1 $\pm$ 1.6	49.2 $\pm$ 7.6
60	45.4 $\pm$ 4.7	11.1 $\pm$ 9.9	69.3 $\pm$ 11.1	14.7 $\pm$ 2.3	50.7 $\pm$ 4.5

anti-proliferative action of C4-PI than several other cancer cell lines. Although the reasons for the relative sensitivity of these cells are not yet known, the relative importance of PI signaling pathways in the growth of these cell lines may be responsible in part. One possible explanation of the enhanced susceptibility of MDA-468 cells may be the finding that more than one-half of the total PI-PLC γ -1 in those cells is associated with the plasma membrane (Todderud *et al.* 1992). Bae and Rhee have recently reported that C4-PI inhibits PI-PLC γ -1 *in vitro*; at 30 μ M of C4-PI approximately 50% of enzyme activity was inhibited. In light of the findings by Powis *et al.* (1992) and Pawelczyk and Lowenstein (1993) regarding the inhibition of PI-PLC by edelfosine and miltefosine, respectively, it would be interesting to examine the level of membrane-associated PI-PLC in the various cell lines that have been tested.

Since it is well established that the preferred substrate for the several isoforms of mammalian PI-PLC is phosphatidylinositol 4,5-bisphosphate (PIP₂) and not PI (Peterson *et al.*, 1992; Wilson *et al.*, 1984), it may be worthwhile to undertake the synthesis and evaluation of the 4,5-bisphosphate derivative of C4-PI.

Experimental Procedures

4-(Hexadecyloxy)-3(S)-methoxybutanephosphonic acid was provided by Dr. H.-S. Byun and was purified by recrystallization from cyclohexane. Cancer cells were obtained and cultured according to the protocols of ATCC (Rockville,

MD). Biological assay methods have been described elsewhere (Lu *et al.*, 1994). C4-PI was dissolved in water to give a stock solution of 30 mM.

Synthesis of D-*myo*-inositol 4-(hexadecyloxy)-3(S)-methoxybutane phosphonate (C4-PI)

(±)-3-O-Camphanoyl-2,3:5,6-di-O-isopropylidene *myo*-inositol (**5.1**, Scheme 5.2)

A solution of (±)-**2b** (739.0 mg, 2.840 mmol) and imidazole (190.6 mg, 2.80 mmol) in anhydrous DMF (10 mL) was cooled to 0 °C while stirring under N₂. (1*S*)-(-)-Camphanic chloride (535.2 mg, 2.47 mmol) in DMF (5 mL) was added dropwise through an addition funnel over 3 h. After the addition was complete, the reaction was stirred for an additional 3 h at 0 °C, and then for 18 h at rt. The mixture was poured into water (250 mL) and extracted with Et₂O (3 x 50 mL). The organic phases were pooled, washed with water and dried (MgSO₄). Filtration and evaporation of the solvent gave a crude product that was purified by flash chromatography (Kieselgel 60, hex/EtOAc 1:1, 1:2) to give the racemic mixture **5.1** (363 mg, 0.820 mmol) in 29% yield. A small amount of the undesired (±)-6-camphanyl-2,3:5,6-di-O-isopropylidene *myo*-inositol (90 mg) was also isolated.

¹H NMR (CDCl₃) : (δ) 0.97-1.06 (m, 3H), 1.13 (s, 6H), 1.31 (s, 3H), 1.45 (s, 3H), 1.47 (s, 3H), 1.52 (s, 3H), 1.58-1.73 (m, 1H), 1.89-2.18 (m, 2H), 2.45-2.57 (m, 1H), 2.80 (d, 1H, *J* = 3.0 Hz, OH), 3.43 (t, 1H, *J* = 10.1 Hz), 3.87-3.98 (m, 1H), 4.03-4.13 (m, 2H), 4.63-4.72 (m, 1H), 5.22 (dd, 1H, *J* = 10.5, 3.6 Hz).

3-O-Camphanoyl-6-O-tert-butyldimethylsilyl-2,3:5,6-di-O-isopropylidene myo-inositol (5.2a) and *1-O-Camphanoyl-4-O-tert-butyldimethylsilyl-2,3:5,6-di-O-isopropylidene myo-inositol (5.2b)*

tert-Butyldimethylsilyl chloride (137.9 mg, 0.910 mmol) was added to a cooled solution (0 °C) of **5.1** (363 mg, 0.824 mmol) and imidazole (67.3 mg, 0.989 mmol) in 5 mL of DMF. The solution was stirred at 0 °C under N₂ for 2 h, and then allowed to warm to rt overnight. The reaction was poured into water (80 mL) and extracted with Et₂O (4 x 30 mL). The combined organic phases were washed with a saturated aqueous NaHCO₃ solution (15 mL), water (2 x 15 mL), and brine (15 mL), and the organic phase was dried (MgSO₄), filtered, and concentrated. The title compounds were separated by flash chromatography (Kieselgel 60, CH₂Cl₂/acetone 97:3) to give **5.2a** (190 mg, 0.342 mmol) in 42% yield, and **5.2b** (195 mg, 0.351 mmol) in 43% yield.

5.2a : ¹H NMR (CDCl₃) (δ) 0.109 (s, 6H), 0.910 (s, 9H), 0.962 (s, 3H), 1.12 (s, 6H), 1.29 (s, 3H), 1.40 (s, 3H), 1.43 (s, 3H), 1.49 (s, 3H), 1.64-1.77 (m, 1H), 1.90-2.17 (m, 2H), 2.46-2.56 (m, 1H), 3.34 (t, 1H, *J* = 10.0 Hz), 3.80 (dd, 1H, *J* = 10.4, 6.4 Hz), 3.91-4.07 (m, 2H), 4.65 (t, 1H, *J* = 4.4 Hz), 5.15 (dd, 1H, *J* = 10.5, 4.2 Hz)

5.2b : ¹H NMR (CDCl₃) (δ) 0.110 (s, 6H), 0.909 (s, 9H), 0.997 (s, 3H), 1.06 (s, 3H), 1.13 (s, 3H), 1.28 (s, 3H), 1.40 (s, 3H), 1.42 (s, 3H), 1.47 (s, 3H), 1.62-1.76 (m, 1H), 1.87-2.17 (m, 2H), 2.41-2.56 (m, 1H), 3.34 (t, 1H, 10.0 Hz), 3.81 (dd, 2H, *J* = 10.5, 3.3 Hz), 3.96-4.06 (m, 2H), 4.60 (t, 1H, *J* = 4.7 Hz), 5.16 (dd, 1H, *J* = 10.5, 4.4 Hz).

4-O-tert-Butyldimethylsilyl-2,3:5,6-di-O-isopropylidene-myo-inositol (5.3a)

A mixture of **5.2a** (195 mg, 0.351 mmol) in KOH/methanol (5 mL, 25% w/v) was refluxed until a clear solution was obtained (>1 h). The solvent was evaporated, and the residue was suspended in a saturated aqueous ammonium chloride solution (30 mL) and extracted with CHCl₃ (3 x 20 mL). The organic phase was washed with water and brine, dried (MgSO₄), filtered, and concentrated under reduced pressure. Flash chromatography (Keiselgel 60, hexane/EtOAc 2:1) gave the product (70 mg, 0.186 mmol) in 46% yield. $[\alpha]^{24}_{\text{D}} +34.0^{\circ}$ (c 0.35, CHCl₃).

¹H NMR (CDCl₃) (δ) 0.0694-0.154 (m, 6H), 0.905 (s, 9H), 1.36 (s, 3H), 1.41 (s, 3H), 1.44 (s, 3H), 1.51 (s, 3H), 2.80 (s, 1H), 3.25 (t, 1H, *J* = 10.0 Hz), 3.62-3.84 (m, 3H), 3.95-4.05 (m, 2H), 4.43 (t, 1H, *J* = 4.7 Hz).

6-O-tert-Butyldimethylsilyl-1,2:4,5-di-O-isopropylidene-myo-inositol (5.3b)

A mixture of **5.2b** (256.3 mg, 0.685 mmol) in KOH/methanol (5 mL, 25% w/v) was stirred for 30 min. The solvent was evaporated, and the residue was suspended in a saturated aqueous ammonium chloride solution (30 mL) and extracted with CHCl₃ (3 x 20 mL). The organic phase was washed with water and brine, dried (MgSO₄), filtered, and concentrated under reduced pressure. Flash chromatography (Keiselgel 60, hexane/EtOAc 2:1) gave the product (187.0 mg, 0.497 mmol) in 73% yield. $[\alpha]^{24}_{\text{D}} -21.6^{\circ}$ (c 1.02, CHCl₃). The ¹H NMR spectrum of this compound is identical to that of **5.3a**.

4-O-(tert-Butyldimethylsilyl)-2,3:5,6-di-O-isopropylidene-myo-inositol
4-(Hexadecyloxy)-3(S)-methoxybutane-phosphonate (5.6, Scheme 5.1)

The potassium salt of 4-(hexadecyloxy)-3(S)-methoxybutane phosphonate was prepared by suspending the phosphonic acid **5.5a** (100 mg, 0.255 mmol) in water (15 mL) and adding aqueous KOH (~1.5 mL, 100 mM) until the mixture reached a pH of approximately 4.5 (pH paper). This slightly turbid aqueous solution was extracted twice with CHCl₃/MeOH (2:1, 150 mL). After evaporation of the organic phase, the monopotassium salt (**5.5b**) was isolated in quantitative yield. A mixture of the inositol derivative **5.3a** (93.8 mg, 0.250 mmol, *R_f* 0.40 (CHCl₃/MeOH/H₂O 65:25:4, solvent A), $[\alpha]_D^{24} +34.0^\circ$ (c 0.345, CHCl₃)) and the butanephosphonate **5.5b** (110.2 mg, 0.256 mmol) was rendered anhydrous by repeated evaporation from anhydrous pyridine, and then redissolved in the same solvent (5 mL). MSNT (187 mg, 0.630 mmol) was added, and the reaction was stirred at rt for 1 h (a white precipitate formed within 10 min). The reaction was quenched by adding water (300 μ L) and allowed to stir for an additional 15 min. After removal of the solvent under reduced pressure as a toluene azeotrope, the resulting residue was redissolved with CHCl₃ (80 mL) and washed with brine (3 $\times$ 20 mL). The organic phase was dried (MgSO₄), filtered, and evaporated. Column chromatography (CM52, 1.5 $\times$ 15 cm, CHCl₃/MeOH 95:5 to 90:10) gave **5.6** (72 mg, 0.095 mmol) in 38% yield. *R_f* 0.51 (CHCl₃/MeOH/H₂O 64:25:4; solvent B). $[\alpha]_D^{24} +0.6^\circ$ (c 0.690, CHCl₃).

^1H NMR (CDCl_3) δ 0.061 (s, 6H), 0.863 (s, 12H), 1.22 (s, 26H), 1.36-1.98 (m, 16H), 3.11 (t, 1H, $J = 6.8$ Hz), 3.35 (br s, 8H), 3.57-3.94 (m, 4H), 4.51 (t, 1H, $J = 9.7$ Hz); ^{31}P NMR (CDCl_3) δ 28.78.

D-myo-Inositol 4-(hexadecyloxy)-3(S)-methoxybutane phosphonate (**C4-PI**)

A solution containing **5.6** (45.2 mg, 59.0 mmol) and PPTS (10 mg, 0.04 mmol) in MeOH (10 mL) was refluxed for 4 days, cooled to rt, and then neutralized with excess solid NaHCO_3 (50 mg). The suspension was filtered through a plug of glass wool and evaporated to give a white solid. Column chromatography (CM52, 1.5×15 cm, CHCl_3 /MeOH 80%, 60%, 50%) gave the product (20.8 mg, 35.4 mmol) as the monosodium salt in 60% yield. R_f 0.20 (solvent B). $[\alpha]_D^{24}$ -1.6° (c 0.27, H_2O with cyclohexylamine, pH >10 by pH paper).

^1H NMR (200 MHz; CDCl_3) δ 0.88 (t, 3H, $J = 6.6$ Hz), 1.25 (s, 26H), 1.4-1.7 (m, 2H), 1.7-2.0 (4H), 3.21 (t, 1H, $J = 9.2$ Hz), 3.35-3.44 (m, 15H), 3.64 (t, 1H, $J = 9.4$ Hz), 3.75 (t, 1H, $J = 9.4$ Hz), 4.12 (1H, m); ^{31}P NMR (CDCl_3) δ 31.80; HRFABMS $[\text{M} + 1]^+$: calcd for $\text{C}_{27}\text{H}_{55}\text{O}_{10}\text{PNa}$ 593.3343, found 593.3403.

Synthesis of the diastereomer of C4-PI (Scheme 5.3)

4-O-(tert-Butyldimethylsilyl)-1,2:4,5-di-O-isopropylidene-myoinositol
4-(hexadecyloxy)-3(S)-methoxybutane- phosphonate (**5.7**)

A mixture of the inositol derivative **5.3b** (186.9 mg, 0.500 mmol, R_f 0.40 (solvent A), $[\alpha]^{24}_D$ -21.6° (c 0.205, CHCl_3)) and the butanephosphonate **5.5b** (110.2 mg, 0.255 mmol) was rendered anhydrous by repeated evaporation from anhydrous pyridine (3 x 2 mL) and then redissolved in 3 mL of the same solvent. MSNT (185 mg, 0.625 mmol) was added, and the reaction was stirred at rt overnight (a white precipitate formed within 10 min). The reaction was quenched by adding water (1 mL) and the mixture was stirred for an additional 10 min. After removal of the solvent under reduced pressure as a toluene azeotrope, the resulting residue was purified by column chromatography (CM52, 1.5 x 15 cm, $\text{CHCl}_3/\text{MeOH}$ 100:0 to 0:100) to give **5.7** (62.0 mg, 0.081 mmol) in 31% yield.

L-myo-Inositol 4-(Hexadecyloxy)-3(S)-methoxybutane-phosphonate (**5.8**)

A solution containing **5.7** (23.0 mg, 0.030 mmol) and PPTS (10 mg, 0.04 mmol) in methanol (10 mL) was refluxed for 3 days and then neutralized with excess solid NaHCO_3 (50 mg). The mixture was filtered through glass wool and evaporated under reduced pressure. Column chromatography (CM52 15 x 1.5 cm, $\text{CHCl}_3/\text{MeOH}$, 100:0 to 50:50) gave crude **5.8**, which was then recrystallized with acetone/MeOH (4:1, v/v) to give the pure product (10.8 mg, 0.018 mmol) in 61% yield. ($[\alpha]^{24}_D$ +9.2° (c 0.40, H_2O with cyclohexylamine, pH >10 by pH paper). HRFABMS $[M + 1]^+$: calcd for $\text{C}_{27}\text{H}_{55}\text{O}_{10}\text{PNa}$ 593.3343, found 593.3412.

Appendix: General Experimental Procedures

Optical rotations were determined with a Jasco Model DIP polarimeter. ^1H (internally referenced to tetramethylsilane) and ^{31}P (externally referenced to 80% H_3PO_4) NMR spectra were taken on a Bruker APX 400 NMR unless otherwise noted. ^1H COSY spectra were recorded using the 45° transverse pulse program provided by Bruker. Radial band chromatography was performed on a Chromatotron (TC Research, Palo Alto, CA). Silica gel for flash chromatography was purchased from EM Science. Thin layer chromatography was performed on aluminum plates of silica gel 60 F 254 (EM Science; Gibbstown, NJ) and visualized by fluorescence quenching using a mineral lamp, and/or by spraying with an ammonium molybdate solution described by Clarke and Dawson (1981) followed by charring. Melting points were measured on a Laboratory Devices Mel-Temp and are uncorrected. Elemental analysis was performed by Desert Analytics (Tucson, AZ). (+)-Menthyl chloroformate and (-)-menthyl chloroformate were purchased from Aldrich Chemical Co. (Milwaukee, WI) and used without purification. Enzymes were purchased from Sigma Chemical Co. (St. Louis, MO). CH_2Cl_2 , Et_3N , and pyridine were dried by refluxing over CaH_2 and distilled prior to use. PCl_3 was distilled prior to use. DMSO was dried over activated molecular sieves 3A. Solvents were ACS reagent grade and used without additional purification.

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