

NOVEL SYNTHESIS OF MYO-INOSITOL PHOSPHATES

Field of the Invention

This invention is directed to the synthesis of phosphate-based inositol derivatives by rapid, economic and efficient processes.

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Background of the Invention

Many signaling molecules, such as hormones and neurotransmitters, exert effects on their target cell by binding to a receptor in the cell's membranes. Following binding, presence of the signaling molecule on its receptor is translated into an effect within the target cell by one or more molecules located within the membrane. The compound responsible for transducing the effect of many signaling molecules is believed to be phosphatidylinositol 4,5-bisphosphate ("PIP₂").

Hydrolysis of PIP₂, following binding of a signalling molecule to its receptor, results in the formation of D-*myo*-inositol 1,4,5-trisphosphate ("D-IP₃") and diacylglycerol. D-IP₃ causes calcium to be released from intracellular, nonmitochondrial stores; increased intracellular calcium levels, in turn, result in a variety of responses within the cell, such as muscle contraction, cell aggregation and thromboxane B₂ release. Moreover, diacylglycerol, together with phosphatidylserine and calcium, activates protein kinase C. PKC is well known to phosphorylate a number of proteins involved in the regulation of cell metabolism.

Accordingly, D-IP₃ and other phosphate-based inositol derivatives are useful in a variety of ways, including elucidative study of the pathways and mechanisms by which external signals affect intracellular processes. For example, isolation and identification of intracellular D-IP₃ receptors is helpful in illuminating the role of calcium mobilization in various physiological and pathological processes, as well as for the development of pharmacological agents capable of modulating the effects of calcium mobilization.

Synthesis of D-IP₃ has previously been reported (see S. Ozaki et al., 1986). However, Ozaki's process requires separation of several inositol diastereomers by column chromatography, a costly, inefficient and commercially impracticable process requiring silica gel that costs about \$150/kilogram. Although others (see J. Gigg et al., 1985; R. de la Pradilla et al., 1990; N. Noda et al., 1990; Chen et al., U.S. Patent No. 5,260,472) have reported D-IP₃ synthesis via fewer intermediates than arise in Ozaki's process, their

- 2 -

methods still require multiple chromatographic separations, and/or reactants that are not commercially available.

This invention provides rapid, convenient and economical synthesis of the enantiomerically pure inositol derivatives D-IP₃ and L-IP₃, employing minimal use of chromatography. Reaction intermediates are generally isolated by crystallization, and the reagents used are all commercially available, at relatively low cost.

Moreover, one of the intermediates made during the synthetic process can be converted into a useful precursor for the synthesis of inositol-based lipids and inositol analogs. The inositol-*H*-phosphonate 3-HP provided can be used to prepare various phosphatidylinositols.

SUMMARY OF THE INVENTION

This invention provides rapid, convenient and economical synthesis of 1-O-[(+)-menthoxycarbonyl]-6-O-benzyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol ("D4P"), D-*myo*-inositol 1,4,5-trisphosphate ("D-IP₃"), inositol 6-O-benzyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol *H*-phosphonate ("3-HP") and L-*myo*-inositol 1,4,5-trisphosphate ("L-IP₃"). The syntheses require fewer chromatographic isolation steps than do previously known reaction schemes.

D4P is synthesized according to the practice of this invention from D-*myo*-inositol via the intermediates (±)-2,3:4,5-di-O-isopropylidene-*myo*-inositol ("(±)-2"), (±)-6-O-benzyl-2,3:4,5-di-O-isopropylidene ("(±)-3") and 1-O-[(+)-menthoxycarbonyl]-6-O-benzyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol ("4P"), following which D4P is isolated from the racemic 4P by crystallization with methanol. D-IP₃ is made from D4P via the intermediates (+)-6-O-benzyl-2,3-O-isopropylidene *myo*-inositol and 6-O-benzyl-2,3-O-isopropylidene-*myo*-inositol 1,4,5-tris(dibenzyl phosphate). Chromatography is employed during this synthesis only for the isolation of 6-O-benzyl-2,3-O-isopropylidene-*myo*-inositol 1,4,5-tris(dibenzyl phosphate).

3-HP is made from D-4P via the intermediate (-)-6-O-benzyl-2,3-O-isopropylidene-*myo*-inositol. L-IP₃ is made via the compounds (±)-6-O-benzyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol ((±)-3), 3-O-[(+)-menthoxycarbonyl]-4-O-benzyl-1,2:5,6-di-O-isopropylidene-*myo*-inositol (4M), 4-O-benzyl-1,2-O-isopropylidene-*myo*-inositol 3,5,6-

- 3 -

tris(dibenzyl phosphate) and 4-O-benzyl-1,2-O-isopropylidene-*myo*-inositol 3,5,6-tris(dibenzyl phosphate). Chromatography is employed during this synthesis only for the isolation of 4-O-benzyl-1,2-O-isopropylidene-*myo*-inositol 3,5,6-tris(dibenzyl phosphate).

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DETAILED DESCRIPTION OF THE INVENTION

Following are abbreviations, and the full names of chemical compounds designated by the abbreviations, which may be found in this application: 1-O-[(+)-menthoxy carbonyl]-6-O-benzyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol: D4P; (±)-2,3:4,5-di-O-isopropylidene-*myo*-inositol: (±)-2; (±)-6-O-benzyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol: (±)-3; *rac*-1-O-[(+)-menthoxy carbonyl]-6-O-benzyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol: 4P; D-*myo*-inositol 1,4,5-trisphosphate: D-IP₃; *m*-chloroperoxybenzoic acid: mCPBA; inositol 6-O-benzyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol *H*-phosphonate: 3-HP; L-*myo*-inositol 1,4,5-trisphosphate: L-IP₃; and, 3-O-[(+)-menthoxy carbonyl]-4-O-benzyl-1,2:5,6-di-O-isopropylidene-*myo*-inositol: 4M.

This invention provides in general the synthesis of phosphoinositol derivatives, using *H*-phosphonates as intermediates and (+)-menthyl chloroformate to separate the various inositol derivatives. More specifically, a method is described of preparing D-1-O-[(+)-menthoxy carbonyl]-6-O-benzyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol ("D-4P") (see scheme 1, hereinbelow). Also provided herein is a method of preparing enantiomerically pure D-*myo*-inositol 1,4,5-trisphosphate ("D-IP₃") (see scheme 1, hereinbelow).

The first step in the process of preparing D-4P involves conversion of *myo*-inositol to (±)-2,3:4,5-di-O-isopropylidene-*myo*-inositol ("(±)-2") by acetonation of *myo*-inositol so as to give (±)-2,3-O-isopropylidene-*myo*-inositol, and acetonation of this product so as to give (±)-2,3:4,5-di-O-isopropylidene-*myo*-inositol. Next, this (±)-2 is converted to (±)-6-O-benzyl-2,3:4,5-di-O-isopropylidene ("(±)-3") by stannylation of (±)-2,3:4,5-di-O-isopropylidene-*myo*-inositol, followed by benzylation of this product so as to give (±)-6-benzyl-2,3:4,5-di-O-isopropylidene. Benzylation is accomplished by a number of means well known to ordinarily skilled artisans including, without limitation: reaction using cesium fluoride in benzyl bromide; sonication with silver oxide in benzyl bromide; phase transfer catalysis using sodium hydroxide in benzyl bromide; and, reaction with thallous ethoxide

- 4 -

in benzyl bromide. Preferably, benzylation is by reaction with cesium fluoride and benzyl bromide.

(±)-3 is then converted to *rac*-1-O-[(+)-menthoxycarbonyl]-6-O-benzyl-2,3:4,5-di-
5 O-isopropylidene-*myo*-inositol ("4P"), for example, by acylation of (±)-3 with menthyl chloroformate. D-4P is isolated from the racemate 4-P product, for example, using methanol. Proton and phosphorous NMR spectra of these compounds, as well as the other compounds made herein, have been conducted by standard means and are consistent with the predicted structures (for the spectra of the compound (±)-3, see Chung
10 and Ryu, 1994)).

The first step in the process of preparing enantiomerically pure D-*myo*-inositol 1,4,5-trisphosphate ("D-IP₃") (see scheme 1, hereinbelow) involves conversion of (+)-6-O-benzyl-2,3-O-isopropylidene-*myo*-inositol by hydrolysis of the D4P, e.g., with pyridinium *p*-toluenesulfonate, so as to give 1-O-[(+)-menthoxycarbonyl]-6-O-benzyl-2,3-O-isopropylidene-*myo*-inositol. This product is subjected to alkaline hydrolysis so as to give (+)-6-O-benzyl-2,3-O-isopropylidene-*myo*-inositol. The product of this step is then converted to 6-O-benzyl-2,3-O-isopropylidene-*myo*-inositol 1,4,5-tris(dibenzyl phosphite) by first phosphorylating (+)-6-O-benzyl-2,3-O-isopropylidene-*myo*-inositol so as to give 6-
20 O-benzyl-2,3-O-isopropylidene-*myo*-inositol 1,4,5-tris(dibenzyl phosphite). Phosphorylation can be by any means generally accepted in the art for attaching phosphorous atoms to compounds; preferably, this phosphorylation step is conducted with *N,N*-diisopropyl-di-benzyl phosphoramidite.

25 Following phosphorylation, the phosphite is oxidized *in situ*, e.g., with *m*-chloroperoxybenzoic acid ("mCPBA"), so as to give 6-O-benzyl-2,3-O-isopropylidene-*myo*-inositol 1,4,5-tris(dibenzyl phosphate). 6-O-Benzyl-2,3-O-isopropylidene-*myo*-inositol 1,4,5-tris(dibenzyl phosphate) is isolated from the resulting phosphate, for example, using SiO₂ chromatography. D-IP₃ is then formed by hydrogenolysis of 6-O-benzyl-2,3-O-isopropylidene-*myo*-inositol 1,4,5-tris(dibenzyl phosphate), for example,
30 using palladium on charcoal, so as to give 2,3-O-isopropylidene-*myo*-inositol 1,4,5-trisphosphate. Acidification of this product, e.g., with acetic acid, gives D-*myo*-inositol 1,4,5-trisphosphate.

- 5 -

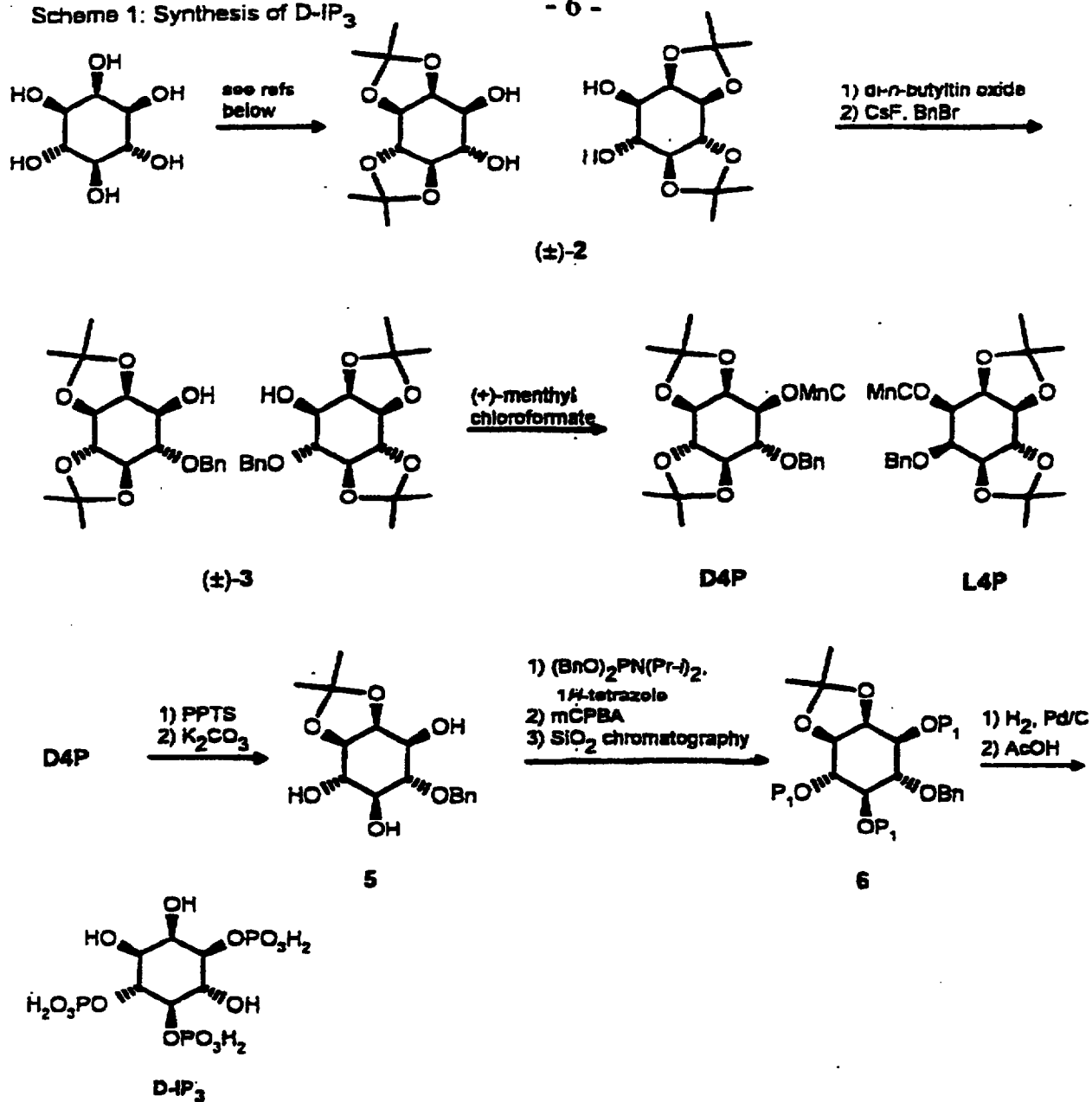
Further provided herein is a method of preparing 6-O-benzyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol *H*-phosphonate ("3-HP") (see scheme 3, hereinbelow). This method involves hydrolysis of D4P with potassium carbonate in methanol so as to give (-)-6-O-benzyl-2,3-O-isopropylidene-*myo*-inositol ("(-)-3"), followed by conversion of the product to 3-HP using PCl_3 and imidazole.

Still further provided herein is a method of preparing enantiomerically pure L-*myo*-inositol 1,4,5-trisphosphate (L-IP₃) (see scheme 2 hereinbelow). First, (±)-6-O-benzyl-2,3:4,5-di-O-isopropylidene ("(±)-3") is converted to *rac*-3-O-[(*-*)-menthoxycarbonyl]-4-O-benzyl-1,2:5,6-di-O-isopropylidene-*myo*-inositol ("4M"), preferably, but not necessarily, by acylation with (*-*)-menthyl chloroformate. L4M is crystallized from the product of this reaction, e.g., with methanol. It is then converted to 4-O-benzyl-1,2-O-isopropylidene-*myo*-inositol 3,5,6-tris(dibenzyl phosphate) by phosphorylation of 4-O-benzyl-1,2-O-isopropylidene-*myo*-inositol, e.g., with *N,N*-diisopropyl-dibenzyl phosphoramidite so as to give 4-O-benzyl-1,2-O-isopropylidene-*myo*-inositol 3,5,6-tris(dibenzyl phosphite). *In situ* oxidation of the product, e.g., with *m*-chloroperoxybenzoic acid, gives 4-O-benzyl-1,2-O-isopropylidene-*myo*-inositol 3,5,6-tris(dibenzyl phosphate), which is then isolated by a number of means, including SiO_2 chromatography. L-*myo*-inositol 1,4,5-trisphosphate is then formed by hydrogenolysis of 4-O-benzyl-1,2-O-isopropylidene-*myo*-inositol 3,5,6-tris(dibenzyl phosphate), e.g., using palladium on charcoal, so as to give 1,2-O-isopropylidene-*myo*-inositol 3,5,6-trisphosphate. This product is then acidified with acetic acid so as to give L-*myo*-inositol 3,5,6-trisphosphate.

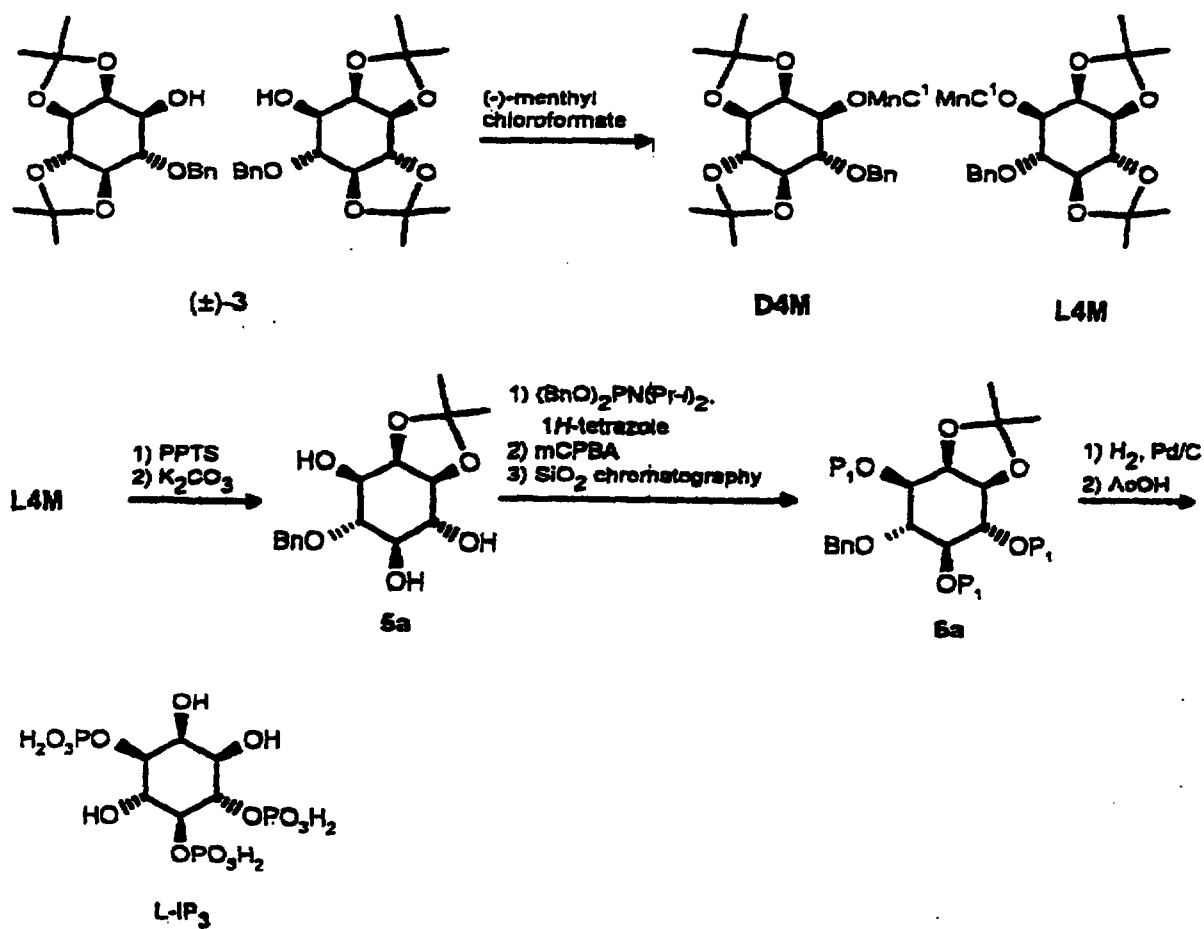
This invention will be better understood from the following examples. However, those of ordinary skill in the art will readily understand that the examples are merely illustrative of the invention as defined in the claims which follow thereafter. The description given herein includes such modifications to its compounds, reactants, reaction schemes and other provisions, that are obvious to ordinarily skilled artisans given the teachings of this invention.

Scheme 1: Synthesis of D-IP₃

- 6 -

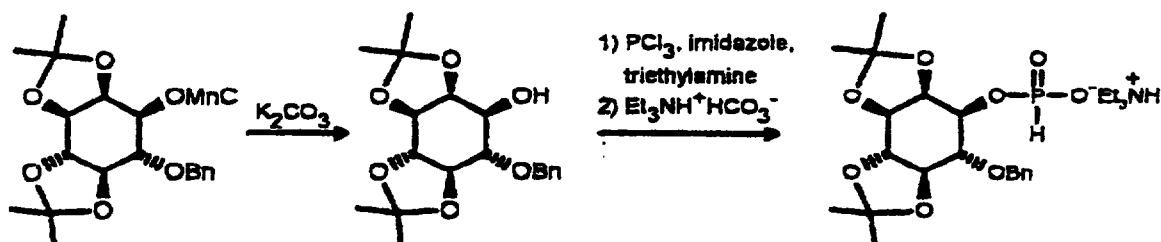


- 7 -

Scheme 2: Synthesis of L-IP₃

- 8 -

Scheme 3: Synthesis of 3HP

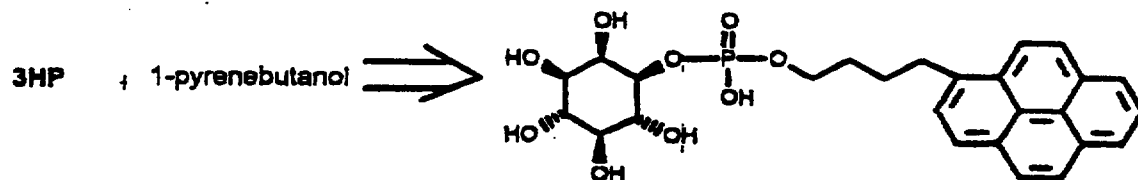


D4P (from scheme 1)

 $(-)-3$

3HP

MnC = (+)-menthyl
 Bn = $CH_2C_6H_5$



4-O-(1'-pyrene-1-butyl)phosphoryl D-myo-inositol

- 9 -

EXAMPLES

Example 1

Chromatography

All flash and SiO₂ chromatography was conducted using Kieselgel™ 60 (EM Science). All compounds made were judged to be pure using thin layer chromatography (Kieselgel™ 60, impregnated on aluminum sheets).

Example 2

Synthesis of D4P

10 Myo-inositol was converted (see scheme 1) to (±)-2,3:4,5-di-O-isopropylidene-myoinositol according to the methods of Gigg et al. or de la Pradilla et al. (the contents of which are incorporated herein by reference). Regioselective monobenylation of (±)-2 was accomplished via an O-stannylated intermediate to give (±)-6-O-benzyl-2,3:4,5-di-O-isopropylidene-myoinositol ("(±)-3"), readily purified by flash chromatography
15 (hexane:ethyl acetate 4:1), in 60% yield. (±)-3 was converted to racemic 4P using (+)-menthyl chloroformate.

D4P (mp 128-131°C.) was isolated from the racemic 4P by recrystallization in methanol.

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Example 3

Synthesis of D-IP₃

Selective hydrolysis of the transketal of D4P (see scheme 1) with pyridinium *p*-toluenesulfonate, followed by alkaline hydrolysis of the menthyl carbonate in methanol,
25 gave the triol (+)-6-O-benzyl-2,3-O-isopropylidene-myoinositol (5, $[\alpha]_{24}^D +21^\circ$ (c 0.43, CHCl₃); lit (de la Pradilla et al.) $[\alpha]_{24}^D +15^\circ$ (c 0.6, CH₃OH)) in 40% yield after purification by flash chromatography. The triol was also crystallized in 32% yield from the crude reaction mixture using hexane:ethyl acetate (4:1). Phosphitylation of the triol with *N,N*-diisopropylidibenzyl phosphoramidite (Fraser-Reid reagent), followed by *in situ* oxidation
30 with mCPBA, gave 6-O-benzyl-2,3-O-isopropylidene-myoinositol in 92% yield, after purification by flash chromatography. Hydrogenolysis gave 6-O-benzyl-2,3-O-isopropylidene-myoinositol over palladium on charcoal, followed by acidification with acetic acid, gave D-IP₃ in quantitative yield ($[\alpha]_{24}^D -20^\circ$ (c 0.05, H₂O, pH 9); lit. (Liu et al.): ($[\alpha]_{24}^D -30^\circ$ (c 0.5, H₂O, pH 9.5)).

- 10 -

Example 4**Synthesis of L-IP₃**

Reaction of (±)-3, prepared as described in Example 1 hereinabove, with (-)-menthyl chloroformate (see scheme 2), followed by crystallization with methanol, gave enantiomerically pure L4M (mp 130-131°C.), the enantiomer of D4P. Following the same scheme as set forth in Example 3 hereinabove, but using L4M as the starting material instead of D4P (see scheme 2), gave L-IP₃ in similar yield ($[\alpha]_{24^\circ\text{C}}^{\text{D}} +17^\circ$ (c 0.30, H₂O, pH 10)).

10 Example 5**Synthesis of 3-HP**

Hydrolysis of D4P with potassium carbonate (see scheme 3) in methanol gave (-)-3 ($[\alpha]_{24^\circ\text{C}}^{\text{D}} -17.4^\circ$ (c 1.69, CHCl₃); lit. (Chen et al.): ($[\alpha]_{24^\circ\text{C}}^{\text{D}} -16^\circ$ (c 0.9, CHCl₃)) in quantitative yield after column chromatography (hexane:ethyl acetate 4:1). Conversion to the corresponding H-phosphonate with PCl₃ and imidazole gave the product 3-HP in 70% yield. 3-HP was a colorless liquid that was stable for several months at -20°C.

- 11 -



What is claimed is:

1. A method of preparing 1-O-[(+)-menthoxycarbonyl]-6-O-benzyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol which comprises:
 - 5 (a) converting D-*myo*-inositol to (±)-2,3:4,5-di-O-isopropylidene-*myo*-inositol by the steps of:
 - (i) acetonation of *myo*-inositol so as to give (±)-2,3-O-isopropylidene-*myo*-inositol; and,
 - (ii) acetonation of the product of step (i) so as to give to (±)-2,3:4,5-di-O-isopropylidene-*myo*-inositol;
 - 10 (b) converting the product of step (a) to (±)-6-O-benzyl-2,3:4,5-di-O-isopropylidene by the steps of:
 - (i) stannylation of (±)-2,3:4,5-di-O-isopropylidene-*myo*-inositol; and,
 - (ii) benzylation of the product of step (i) so as to give (±)-6-O-benzyl-2,3:4,5-di-O-isopropylidene *myo*-inositol;
 - 15 (c) converting the product of step (b) to 1-O-[(+)-menthoxycarbonyl]-6-O-benzyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol; and,
 - (d) isolation of 1-O-[(+)-menthoxycarbonyl]-6-O-benzyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol from the product of step (c).
- 20 2. The method of claim 1, wherein the benzylation of step (b)(ii) is by reaction with cesium fluoride and benzyl bromide.
3. The method of claim 1, wherein the conversion of step (c) is by acylation with (+)-menthyl chloroformate.
- 25 4. The method of claim 1, wherein 1-O-[(+)-menthoxycarbonyl]-6-O-benzyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol is recrystallized in step (d) with methanol
- 30 5. A method of preparing 6-O-benzyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol *H*-phosphonate which comprises the steps of:
 - (a) hydrolysis of the 1-O-[(+)-menthoxycarbonyl]-6-O-benzyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol of claim 1 so as to give (-)-6-O-benzyl-2,3:4,5-O-isopropylidene-*myo*-inositol; and,



- 12 -

(b) converting the product of step (a) to 6-O-benzyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol *H*-phosphonate by reaction with PCl_3 and imidazole.

- 5 6. The method of claim 5, wherein the hydrolysis of step (a) is with potassium carbonate in methanol.
7. The method of claim 5, wherein (-)-6-O-benzyl-2,3:4,5-O-isopropylidene-*myo*-inositol is converted by reaction with PCl_3 and imidazole
- 10 8. A method of preparing enantiomerically pure D-*myo*-inositol 1,4,5-trisphosphate which comprises:
- (a) converting D-*myo*-inositol to ($\pm$)-2,3:4,5-di-O-isopropylidene-*myo*-inositol by the steps of:
- 15 (i) acetonation of *myo*-inositol so as to give ($\pm$)-2,3-O-isopropylidene-*myo*-inositol; and,
- (ii) acetonation of the product of step (i) so as to give to ($\pm$)-2,3:4,5-di-O-isopropylidene-*myo*-inositol;
- (b) converting the product of step (a) to ($\pm$)-6-O-benzyl-2,3:4,5-di-O-isopropylidene by the steps of:
- 20 (i) stannylation of ($\pm$)-2,3:4,5-di-O-isopropylidene-*myo*-inositol; and,
- (ii) benzylation of the product of step (i) so as to give ($\pm$)-6-O-benzyl-2,3:4,5-di-O-isopropylidene *myo*-inositol;
- (c) converting the product of step (b) to 1-O-[(+)-menthoxy carbonyl]-6-O-benzyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol;
- 25 (d) isolation of 1-O-[(+)-menthoxy carbonyl]-6-O-benzyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol from the product of step (c);
- (e) converting 1-O-[(+)-menthoxy carbonyl]-6-O-benzyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol to (+)-6-O-benzyl-2,3-O-isopropylidene *myo*-inositol by the steps of:
- 30 (i) hydrolysis of 1-O-[(+)-menthoxy carbonyl]-6-O-benzyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol with pyridinium *p*-toluenesulfonate so as to give 1-O-[(+)-menthoxy carbonyl]-6-O-benzyl-2,3-O-isopropylidene-*myo*-inositol; and,
- (ii) alkaline hydrolysis of the product of step (i) so as to give (+)-6-O-benzyl-2,3-O-isopropylidene-*myo*-inositol;



- 13 -

- (f) converting the product of step (e) to (-)-6-O-benzyl-2,3-O-isopropylidene-*myo*-inositol 1,4,5-tris(dibenzyl phosphate) by the steps of:
- (i) phosphitylation of the product of step (a) so as to give (-)-6-O-benzyl-2,3-O-isopropylidene-*myo*-inositol 1,4,5-tris(dibenzyl phosphite);
 - 5 (ii) *in situ* oxidation of the product of step (i) so as to give (-)-6-O-benzyl-2,3-O-isopropylidene-*myo*-inositol 1,4,5-tris(dibenzyl phosphate); and,
 - (iii) isolation of (-)-6-O-benzyl-2,3-O-isopropylidene-*myo*-inositol 1,4,5-tris(dibenzyl phosphate) from the product of step (ii) using SiO₂ chromatography; and,
 - 10 (g) converting the product of step (f) to D-*myo*-inositol 1,4,5-trisphosphate by the steps of:
 - (i) hydrogenolysis of the product of step (b) so as to give D-2,3-O-isopropylidene-*myo*-inositol 1,4,5-trisphosphate; and,
 - (ii) acidification of the product of step (ii) so as to give D-*myo*-inositol 1,4,5-
 - 15 trisphosphate.
9. The method of claim 8, wherein the phosphorylation of step (f)(i) is by reaction with with *N,N*-diisopropyl-di-benzyl phosphoramidite.
- 20 10. The method of claim 8, wherein the *in situ* oxidation of step (f)(ii) is with *m*-chloroperoxybenzoic acid ("mCPBA").
11. The method of claim 8, wherein the hydrogenolysis of step (g)(i) is with palladium on charcoal.
- 25 12. The method of claim 8, wherein the acidification of step (g)(ii) is with acetic acid.
13. The method of claim 8, wherein the benzylation of step (b)(ii) is by reaction with cesium fluoride and benzyl bromide.
- 30 14. The method of claim 8, wherein the conversion of step (c) is by acylation with (+)-menthyl chloroformate.
15. The method of claim 8, wherein 1-O-[(+)-menthoxycarbonyl]-6-O-benzyl-2,3:4,5-di-O-isopropylidene-*myo*-inositol is recrystallized in step (d) with methanol.
- 35



16. A method of preparing enantiomerically pure L-myo-inositol 1,4,5-trisphosphate which comprises the steps of:
- 5 (a) hydrolysis of 1-O-[(+)-menthoxy carbonyl]-6-O-benzyl-2,3:4,5-di-O-isopropylidene-myo-inositol so as to give (-)-6-O-benzyl-2,3:4,5-O-isopropylidene-myo-inositol;
- (b) converting the product of step (a) to ($\pm$)-6-O-benzyl-2,3:4,5-di-O-isopropylidene-myo-inositol *H*-phosphonate by reaction with PCl_3 and imidazole;
- 10 (c) acylation of ($\pm$)-6-O-benzyl-2,3:4,5-di-O-isopropylidene with (-)-menthyl chloroformate so as to give 3-O-[(+)-menthoxy carbonyl]-4-O-benzyl-1,2:5,6-di-O-isopropylidene-myo-inositol;
- (d) crystallization of 3-O-[(+)-menthoxy carbonyl]-4-O-benzyl-1,2:5,6-di-O-isopropylidene-myo-inositol from the product of step (c);
- 15 (e) converting 3-O-[(+)-menthoxy carbonyl]-4-O-benzyl-1,2:5,6-di-O-isopropylidene-myo-inositol to +4-O-benzyl-1,2-O-isopropylidene-myo-inositol 3,5,6-tris(dibenzyl phosphate) by the steps of:
- (i) phosphitylation of 3-O-[(+)-menthoxy carbonyl]-4-O-benzyl-1,2:5,6-di-O-isopropylidene-myo-inositol so as to give 4-O-benzyl-1,2-O-isopropylidene-myo-inositol 3,5,6-tris(dibenzyl phosphite); and,
- 20 (ii) *in situ* oxidation of the product of step (i) so as to give +4-O-benzyl-1,2-O-isopropylidene-myo-inositol 3,5,6-tris(dibenzyl phosphate);
- (f) isolation of +4-O-benzyl-1,2-O-isopropylidene-myo-inositol 3,5,6-tris(dibenzyl phosphate) from the product of step (e);
- 25 (g) hydrogenolysis of +4-O-benzyl-1,2-O-isopropylidene-myo-inositol 3,5,6-tris(dibenzyl phosphate) so as to give L-1,2-O-isopropylidene-myo-inositol 3,5,6-trisphosphate; and,
- (h) acidification of L-1,2-O-isopropylidene-myo-inositol 3,5,6-trisphosphate so as to give L-myo-inositol 3,5,6-trisphosphate.
- 30 17. The method of claim 16, wherein 3-O-[(+)-menthoxy carbonyl]-4-O-benzyl-1,2:5,6-di-O-isopropylidene-myo-inositol is recrystallized in step (b) using methanol.
18. The method of claim 16, wherein the phosphitylation of step (c)(i) is with *N,N*-diisopropyl-dibenzyl phosphoramidite.

- 15 -



19. The method of claim 16, wherein the *in situ* oxidation of step (c)(ii) is with *m*-chloroperoxybenzoic acid.
20. The method of claim 16, wherein 4-O-benzyl-1,2-O-isopropylidene-*myo*-inositol
5 3,5,6-tris(dibenzyl phosphate) is isolated in step (d) is using SiO₂ chromatography
21. The method of claim 16, wherein the hydrogenolysis of step (d) is using
10 palladium on charcoal.
22. The method of claim 16, wherein the acidification of step (f) is with acetic acid.
23. The method of claim 16, wherein the hydrolysis of step (a) is with potassium
15 carbonate in methanol.