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A convenient synthesis of D-*myo*-inositol 1,4,5-trisphosphate (Ins(1,4,5)P_3) and L-*myo*-inositol 1,4,5-trisphosphate (Ins(3,5,6)P_3)

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Abstract

An efficient synthesis of an optically active inositol derivative that is a precursor to D-*myo*-inositol 1,4,5-trisphosphate (Ins(1,4,5)P_3, (−)) is described. Crystallization of the diastereomers of (±)-1-*O*-[(+)-menthoxycarbonyl]-6-*O*-benzyl-2,3:4,5-di-*O*-isopropylidene-*myo*-inositol diastereomers from methanol gives only one diastereomer. Alkaline hydrolysis gives the useful inositol derivative (−)-6-*O*-benzyl-2,3:4,5-di-*O*-isopropylidene-*myo*-inositol. Likewise, crystallization of the diastereomers of (±)-3-*O*-[(−)-menthoxycarbonyl]-4-*O*-benzyl-1,2:5,6-di-*O*-isopropylidene-*myo*-inositol from methanol gave a pure compound which could be hydrolyzed to give (+)-4-*O*-benzyl-1,2:5,6-di-*O*-isopropylidene-*myo*-inositol, a precursor to D-*myo*-inositol 3,5,6-trisphosphate (Ins(3,5,6)P_3, (+)). The ease with which these enantiomerically pure inositol derivatives were isolated may facilitate the synthesis of more complex inositol phosphate derivatives such as D-*myo*-inositol 1,3,4,5-tetrakisphosphate.

Keywords: Phosphatidylinositol; D-*myo*-Inositol 1,4,5-trisphosphate

1. Introduction

In the last decade, inositol-based lipids have become even more important in biochemical pathways than initially proposed by Streb et al. [1] and Nishizuka [2]. As a result, the synthesis of analogs of the metabolites of the inositol signaling pathway (D-*myo*-inositol 1,4,5-trisphosphate (Ins(1,4,5)P_3, (−)-**1**, Fig. 1) in particular) has been the goal of various workers in the last decade (for recent reviews, see Potter and Lampe [3a] and Bruzik [3b]. The total synthesis of Ins(1,4,5)P_3 has been reported [4], but the lengthy path (11 steps starting from *myo*-inositol) depended upon the successful separation of inositol diastereomers by chiral column chromatography. Other workers [5–8] have also reported the preparation of intermediates that can be converted to Ins(1,4,5)P_3 in fewer steps, but extensive chromatography steps were necessary to separate the inositol enantiomers formed. An alternate procedure used lipases to separate inositol stereoisomers, but still

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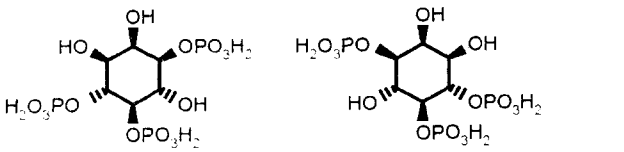


(-)-**1**: Ins (1,4,5)P_3 (+)-**1**: Ins (3,5,6)P_3

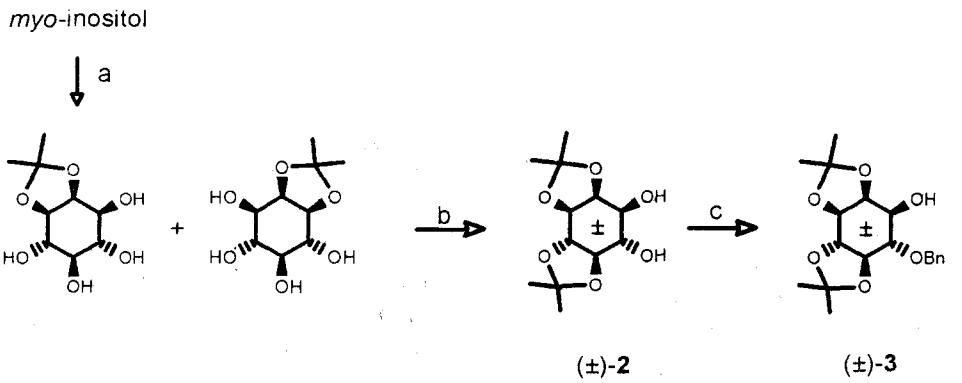
Fig. 1. Structures of D-*myo*-inositol 1,4,5-trisphosphate (Ins(1,4,5)P_3) and L-*myo*-inositol 1,4,5-trisphosphate (Ins(3,4,5)P_3).

required extensive use of silica gel chromatography to separate the products of the enzyme reaction [9]. A more convenient method of obtaining optically active inositol intermediates employs crystallization of inositol diastereomers [10]. We now report that the racemic compound (±)-6-*O*-benzyl-2,3:4,5-di-*O*-isopropylidene-*myo*-inositol ((±)-**3**), whose synthesis has previously been reported by Chung and Ryu [11], can be separated into its antipodes by forming the menthyl carbonate using (+)-menthyl chloroformate and crystallizing the product from MeOH. Alkaline hydrolysis of the crystallized products resulted in optically active (−)-**3**. The enantiomer of (−)-**3** can be prepared by reacting racemic (±)-**3** with (−)-menthyl chloroformate, crystallization of the product in methanol, and subsequent alkaline hydrolysis. These antipodes can be used as precursors to Ins(1,4,5)P_3 and Ins(3,5,6)P_3 ((−)-**1** and (+)-**1**) (Fig. 1). We also correct errors in the literature assignments [9,11] of the protons in the NMR spectra of (±)-6-*O*-benzyl-2,3:4,5-di-*O*-protected-*myo*-inositols.

2. Results and discussion

myo-Inositol was converted to (±)-2,3:4,5-di-*O*-isopropylidene-*myo*-inositol ((±)-**2**) (Scheme 1) according to the methods published by Gigg et al. [5], de la Pradilla et al. [6], or Noda and Keenan [12]. Despite the low yield of the product from *myo*-inositol (ca. 20–30%), the ease at which (±)-**2** was isolated makes this a convenient starting material for the synthesis of various inositol derivatives. Regioselective benzylation of the 6-hydroxy group was accomplished via an *O*-stannylated intermediate as previously reported [11] to give (±)-6-*O*-benzyl-2,3:4,5-di-*O*-isopropylidene-*myo*-inositol ((±)-**3**) in 60% yield, which is readily purified by flash chromatography (hexane–ethyl acetate 4:1) or by radial-band chromatography.

Although the ^{1}H NMR spectra of (±)-**3** (as well as of (−)-**3** and (+)-**3**) are identical to that of Chung and Ryu [11], based on our ^{1}H COSY NMR spectrum of (−)-**3** (Fig. 2), we conclude that the assignment of the protons published in their report is in error. Table 1 shows the ^{1}H assignments for (±)-6-*O*-benzyl-2,3:4,5-di-*O*-isopropylidene-*myo*-inositol ((±)-**3**) as reported in Ref. [11], (±)-6-*O*-benzyl-2,3:4,5-di-*O*-cyclohexylidene-*myo*-inositol ((−)-**10**) as reported in



Scheme 1. Synthesis of (±)-6-*O*-benzyl-2,3:4,5-di-*O*-isopropylidene-*myo*-inositol ((±)-**3**). Reagents: (a) *p*-TsOH/dimethoxypropane, Me_2SO; (b) *p*-TsOH, dimethoxypropane, acetone; (c) (i) di-*n*-butyltin oxide, MeOH, reflux, (ii) CsF, BnBr, DMF.

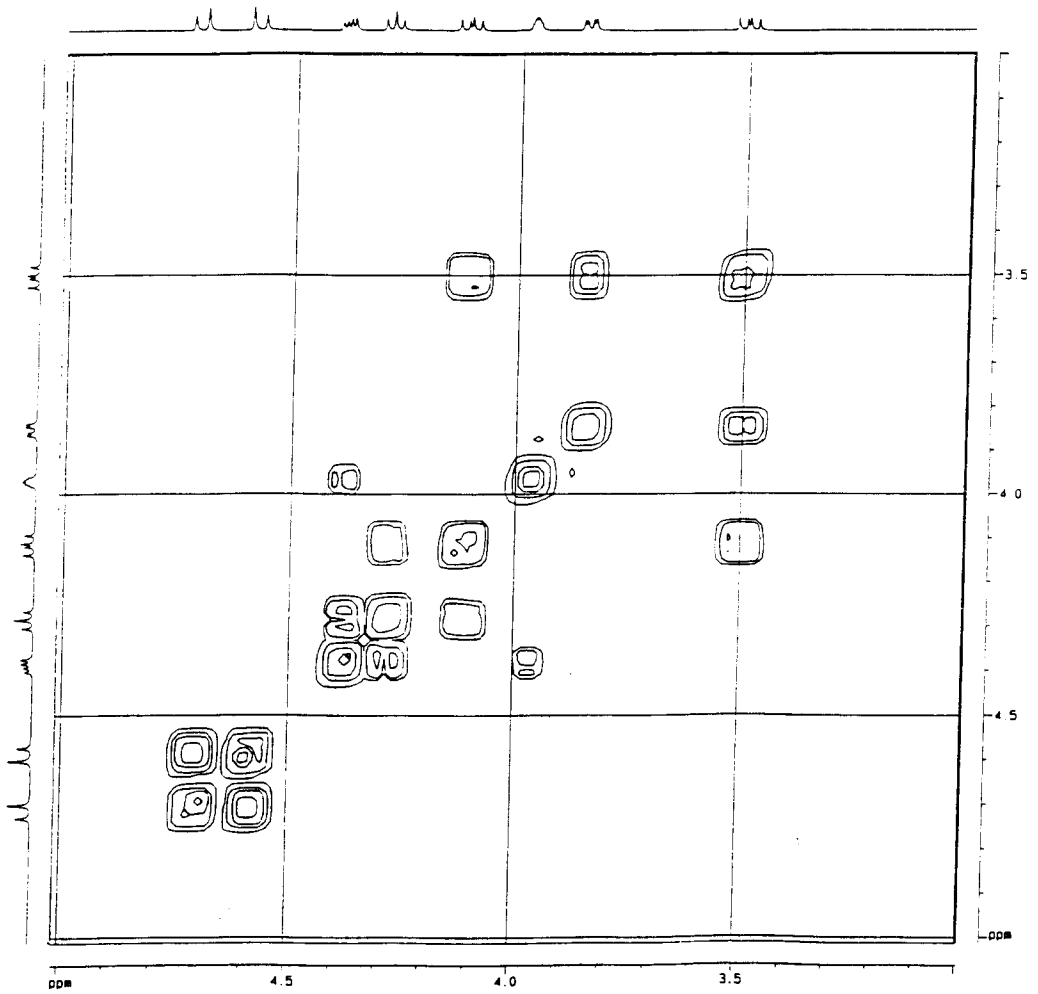
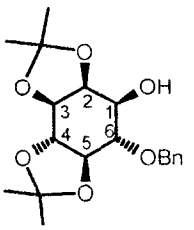


Fig. 2. ^{1}H COSY NMR spectrum of (−)-**3**.

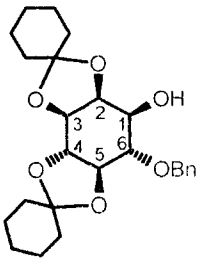
Ref. [9], and (−)-6-*O*-benzyl-2,3:4,5-di-*O*-isopropylidene-*myo*-inositol ((−)-**3**) based on our ^{1}H COSY NMR spectrum (Fig. 2). To facilitate comparison among these inositol derivatives, we have used a single numbering system for the inositol ring (Table 1). Guo et al. [9] used cyclohexylidene instead of isopropylidene protecting groups, but there is expected to be little difference between the ^{1}H spectrum of their compound ((−)-**10**) and ours ((−)-**3**) in the region between δ 3 and 5 ppm. However, their assignments of the inositol methine protons also differ from ours. In their report [9], the assignments for the H-1/H-3 and H-4/H-6 pairs are reversed, due presumably to the difficulty in correctly assigning the multiplet appearing between δ 4.06 and 4.07 ppm. The proper assignment of the H-1/H-3 and H-4/H-6 pairs in these molecules can be made by analyzing the ^{1}H NMR spectra of the corresponding acetate derivatives of (−)-**10** and (−)-**3** ((−)-**11** and **8**, respectively). The results are shown in Table 2. Introduction of an acetate at the C-1 position of (−)-**10** and (−)-**3** would result in a downfield shift of ~ 1.2 ppm of the H-1 signal [13]. In the ^{1}H NMR spectrum of **8**, the double doublet at 5.28 ppm (J = 3.8, 3.0 Hz) is assigned as H-1, and is consistent with the assignment of the multiplet at 4.03 ppm of the ^{1}H NMR spectrum of (−)-**3** as H-1. In the report by Guo et al., the double doublet at 5.30 ppm (J = 2.4, 5.9 Hz) in the ^{1}H NMR spectrum of (−)-**11** is assigned to H-2, which is inconsistent with their assignment of H-2 in the ^{1}H NMR spectrum of (−)-**10** (dd, 4.46 ppm, J = 3.6, 7.5 Hz). It is more likely that the H-1 signal in their compounds appears as a multiplet between 4.06 and 4.07 ppm in the ^{1}H NMR spectrum of compound (−)-**10**, and as a double doublet at 5.30 ppm (J = 2.4, 5.9 Hz) in compound (−)-**11**.

Reaction of (±)-**3** to form racemic carbonate (±)-**4** occurred in high yield using (+)-menthyl chloroformate and pyridine as the base. Although (+)-

Table 1
^{1}H NMR assignments of racemic (±)-**3**[a] [11], (−)-6-*O*-benzyl-2,3:4:5-di-*O*-cyclohexylidene-*myo*-inositol ((−)-**10**) [9], and (−)-**3**[b]



(-)-3

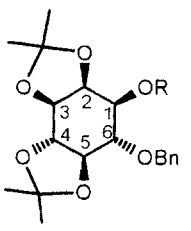


(-)-10
Guo et al. [9]

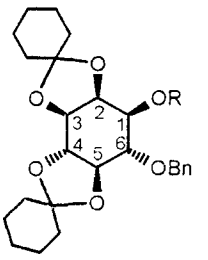
	Literature		This work
Proton	(±)-**3** [ref. 11]	(-)-**10** [ref. 9]	(-)-**3**
H-1	3.86 (dd, 7.8, 2.4 Hz)	4.38 (t, 3.6, 7.5 Hz)	4.03 (m)
H-2	3.98 (dd, 3.8 Hz)	4.46 (dd, 3.6, 7.5 Hz)	4.44 (dd, 3.6, 7.1 Hz)
H-3	4.39 (dd)	4.06-4.07 (m)	4.34 (t, 7.3 Hz)
H-4	4.29 (7.3 Hz)	3.93 (dd, 2, 7.7 Hz)	4.17 (dd, 7.9, 10.6 Hz)
H-5	3.51 (10.6, 7.5 Hz)	3.58 (dd, 7.8, 10.5 Hz)	3.56 (dd, 7.9, 10.4 Hz)
H-6	4.13 (dd)	4.23 (dd, 3.6, 7.5 Hz)	3.92 (dd, 2.4, 7.9 Hz)

[a]Compound (±)-**3** (abbreviated **13b** in Ref. [11]) was misnamed as (±)-4-*O*-benzyl-1,2:4:5-di-*O*-isopropylidene-*myo*-inositol by the authors [11]. In their numbering system, compound (±)-**3** should have been named (±)-4-*O*-benzyl-1,2:5:6-di-*O*-isopropylidene-*myo*-inositol. Our numbering system gives preference to the isomer that gives Ins(1,4,5)P_3, and therefore (±)-**3** is named (±)-6-*O*-benzyl-2,3:4:5-di-*O*-isopropylidene-*myo*-inositol.
[b]Based on the ^{1}H COSY NMR spectrum of (−)-**3** Fig. 2 and ^{1}H NMR spectra of **8**.

Table 2
^{1}H NMR assignments of H-1 and H-2 of (−)-**3**, **8**, (−)-**10** [9], and (−)-**11** [9]

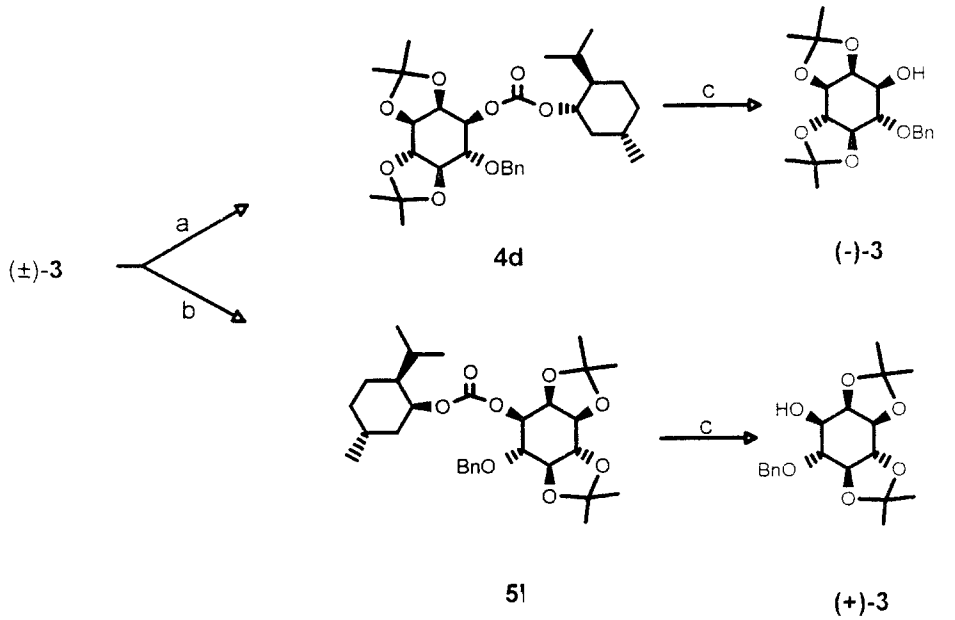


(-)-3: R = H
8: R = Ac



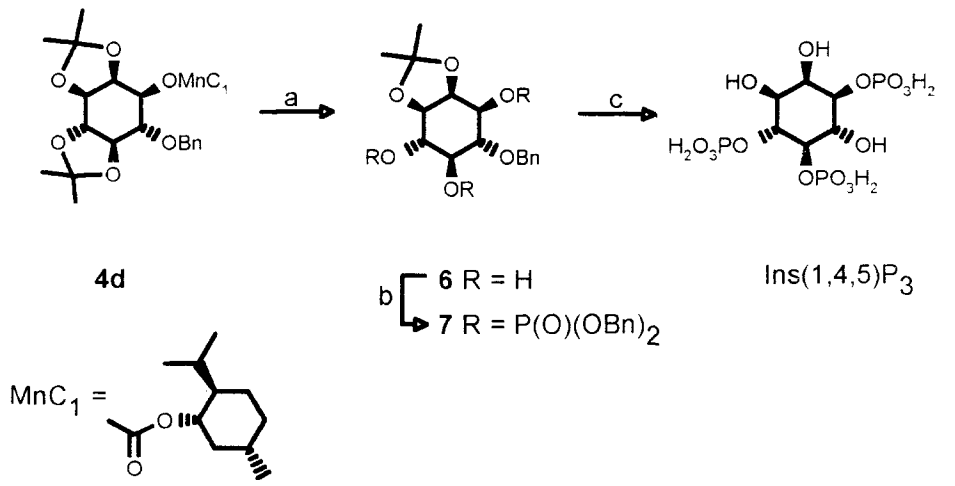
(-)-10: R = H
(-)-11: R = Ac
Guo et al. [9]

	This work		Literature	
Proton	(-)-**3**	**8**	(-)-**10** [9]	(-)-**11** [9]
H-1	4.03 (m)	5.28 (dd, 3.7, 2.9 Hz)	4.38 (t, 3.6, 7.5 Hz)	4.52 (dd, 4.2, 7.8 Hz)
H-2	4.44 (dd, 3.6, 7.1 Hz)	4.50 (dd, 6.9, 4.5 Hz)	4.46 (dd, 3.6, 7.5 Hz)	5.30 (dd, 2.4, 3.9 Hz)



Scheme 2. Resolution of (±)-**3** by fractional crystallization of menthyl carbonates. Reagents: (a) (i) (+)-menthyl chloroformate, pyridine, CH_2Cl_2, (ii) crystallize from MeOH; (b) (-)-menthyl chloroformate, pyridine, CH_2Cl_2, (ii) crystallize from MeOH; (c) KOH, MeOH.

menthyl chloroformate has an *ee* of only 97%, the low cost of this reagent makes it an economical alternative to camphanic acid derivatives. Once the racemic menthyl carbonate was formed, isolation of one diastereomer (**4d**) was accomplished easily by crystallization from methanol (Scheme 2) in 28% yield. Additional crystals of **4d** could be formed by cooling the supernatant to 0 °C, but these were not used for the remainder of the synthesis. Hydrolysis of **4d** with KOH in methanol gave (−)-**3** ($[\alpha]_D^{24}$ −17.4° (*c* 1.69, $CHCl_3$); lit. $[\alpha]_D$ −16.0° (*c* 0.9, $CHCl_3$)) [8] in quantitative yield after flash chromatography (hexane–ethyl acetate 4:1). Conversely, reaction of (±)-**3** with (−)-menthyl chloroformate followed by crystallization from methanol gave enantiomerically pure **5l**, the enantiomer of **4d**. Neither **4d** nor **5l** have



Scheme 3. Synthesis of Ins(1,4,5)P_3. Reagents: (a) (i) PPTS, ethylene glycol, CH_2Cl_2, (ii) K_2CO_3, MeOH; (b) (i) bis(benzyloxy)diisopropylaminophosphine, 1*H*-tetrazole, CH_2Cl_2, (ii) mCPBA, −40°C; (c) (i) Pd-C, H_2, EtOH-AcOH-H_2O.

an observable optical rotation when using the sodium D line. Alkaline hydrolysis of **5l** gave (+)-**3** ($[\alpha]_D^{24}$ +17.9° (*c* 1.69, $CHCl_3$)) in quantitative yield after isolation by chromatography. Conversion of enantiomerically pure (−)-**3** to the corresponding acetate derivative, **8**, was accomplished using acetic anhydride, DMAP, and pyridine in methylene chloride.

Selective hydrolysis of the *trans*-ketal protecting group in **4d** (Scheme 3) with pyridinium *p*-toluenesulfonate followed by alkaline hydrolysis of the menthyl carbonate in methanol gave triol **6** ($[\alpha]_D^{24}$ +21° (*c* 0.43, $CHCl_3$), $[\alpha]_D^{24}$ +8.6° (*c* 0.6, MeOH); lit. $[\alpha]_D$ +12° (*c* 0.6, MeOH)) [8] in 27% yield after purification by flash chromatography and recrystallization from hexane–ethyl acetate 1:1. The low yield of this reaction is due solely to undesired hydrolysis of the *cis*-ketal protecting group. This can be avoided in the future by shortening the time of the acidic hydrolysis step. Phosphitylation of triol **6** with bis(benzyloxy)diisopropylaminophosphine followed by in situ oxidation with mCPBA gave **7** in 92% yield after flash chromatography. Hydrogenolysis over palladium/charcoal gave Ins(1,4,5)P_3 in quantitative yield. When a similar scheme was followed using **5l** as the starting material, Ins(3,5,6)P_3 was obtained in 71% yield. Although no phosphate migration was seen by ^{1}H NMR spectroscopy, it may be prudent to conduct the hydrogenolysis reaction under neutral conditions in the future to preclude the possibility of migration.

3. Conclusion

The syntheses of (−)-**1** and (+)-**1** reported here are easily accomplished because of the facile separation of the two inositol diastereomers (**4d** from **4l** for the preparation of (−)-**1**, and **5l** from **5d** for the preparation of (+)-**1**) by crystallization, opening the possibility of large-scale production of these biologically useful compounds. Column chromatography was required in only two steps, a substantial reduction in the use of this laborious procedure in comparison to the synthetic schemes published by Aguilo et al. [8] and Guo et al. [9]. Even more interesting is the possibility of using **4d** and **5l** as starting materials for a wide variety of inositol phosphates and inositol lipids. These two compounds are similar to the key starting materials used by Guo et al. [9] in the synthesis of various 3-phosphorylated inositols.

4. Experimental

General methods.—Optical rotations were determined with a Jasco Model DIP polarimeter. ^{1}H (internally referenced to tetramethylsilane) and ^{31}P (externally referenced to 80% phosphoric acid) NMR spectra were taken on a Bruker APX 400-MHz NMR unless otherwise noted. ^{1}H COSY NMR spectra were recorded with 45° transverse pulses using the pulse program supplied by Bruker. Radial-band chromatography was performed on a Chromatotron (Harrison Research, Palo Alto, CA). Silica gel for flash and radial-band chromatography was purchased from EM Science (Gibbstown, NJ). Thin-layer chromatography was performed on aluminum plates of Silica Gel $60F_{254}$ (EM Science, Gibbstown, NJ) and visualized by fluorescence quenching using a mineral lamp, and/or by spraying with a 10% solution of sulfuric acid in ethanol followed by charring. Melting points are uncorrected. Elemental analysis was performed by Desert Analytics (Tuscon, AZ). (+)-Menthyl chloroformate and (−)-menthyl chloroformate were purchased from Aldrich Chemical and used without purification. CH_2Cl_2 and pyridine were dried by refluxing over calcium hydride and distilled prior to use. Me_2NCHO was dried over molecular sieve type 3A for 72 h prior to use. Solvents were ACS reagent grade and were used without additional purification. Racemic (±)-**2** was synthesized according to published procedures reported [5,6,11].

(±)-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 a 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 concentrated and the residue was placed under vacuum (> 12 Torr) for 3 h. Powdered caesium fluoride (2.22 g, 14.6 mmol) was added to the stannylidene derivative, and after an additional 2 h under vacuum, a solution of benzyl bromide (2.4 mL, 20.4 mmol) in dry Me_2NCHO (14.6 mL) was added. After the mixture was stirred under nitrogen for 4 h, it was diluted with water and extracted with CH_2Cl_2. The organic phases were pooled, dried ($MgSO_4$), filtered, and concentrated. Flash chromatography (Kieselgel 60, 38 × 2.5 cm, hexane–EtOAc 4:1) gave (±)-**3** [1] (1.66 g, 65% yield): R_f 0.35, hexane–EtOAc 4:1; ^{1}H NMR (200

[1] Isomeric 1-*O*-benzyl-2,3:4,5-di-*O*-isopropylidene-*myo*-inositol (R_f 0.17, hexane–EtOAc 4:1) was formed in 30% yield.

MHz, $CDCl_3$) δ 1.37 (s, 3 H, CH_3), 1.48 (s, 6 H, 2 × CH_3), 1.53 (s, 3 H, CH_3), 2.64 (d, 1 H, J = 1.6 Hz, OH), 3.56 (dd, 1 H, J = 10.4, 7.9 Hz, H-5), 3.92 (dd, 1 H, J = 7.9, 2.4 Hz, H-6), 4.03 (m, 1 H, H-1), 4.17 (dd, 1 H, J = 7.9, 10.6 Hz, H-4), 4.34 (t, J = 7.3 Hz, H-3), 4.44 (dd, 1 H, J = 7.1, 3.6 Hz, H-2), 4.64 (d, 1 H, J = 11.7 Hz, OCH_2), 4.78 (d, 1 H, J = 11.7 Hz, OCH_2), 7.26–7.36 (m, 5 H, C_6H_5); Anal. Calcd. for $C_{19}H_{26}O_6$: C, 65.13; H, 7.48. Found: C, 65.25; H, 7.50.

1-O-[(+)-Menthoxycarbonyl]-6-O-benzyl-2,3:4,5-di-O-isopropylidene-myo-inositol (**4d**).—A solution containing (±)-**3** (3.62 g, 10.3 mmol, dried by repeated 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 room temperature under nitrogen. 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 that produced colorless crystals upon trituration with methanol. Recrystallization from methanol gave **4d** (1.53 g, 28% yield): mp 128–131 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.72–1.08 (m, 14 H), 1.31 (s, 3 H, CH_3), 1.42 (s, 6 H, 2 × CH_3), 1.46 (s, 3 H, CH_3), 1.62–1.67 (m, 2 H), 1.89–2.07 (m, 2 H), 3.57 (dd, 2 H, J = 7.9, 10.7 Hz, H-5), 3.85 (dd, 1 H, J = 2.4, 7.8 Hz, H-6), 3.99 (dd, 1 H, J = 7.4, 10.0 Hz, H-4), 4.35 (t, 1 H, J = 7.3 Hz, H-3), 4.48 (dd, 1 H, J = 4.0, 6.9 Hz, H-2), 4.55 (m, 1 H, H-1′), 4.71 (s, 2 H, OCH_2), 5.12 (t, 1 H, J = 3.3 Hz, H-1), 7.22–7.32 (m, 5 H, C_6H_5).

(−)-6-O-Benzyl-2,3:4,5-di-O-isopropylidene-myo-inositol ((−)-**3**).—A mixture of **4d** (468 mg, 0.880 mmol) in KOH–methanol (50 mL, 25% w/v) was refluxed until TLC (hexane–ethyl acetate, 4:1) indicated that the reaction was complete. The solvent was removed in vacuo, and the resulting residue was suspended in a saturated aqueous ammonium chloride solution and extracted with CH_2Cl_2. The organic phases were pooled, dried ($MgSO_4$), filtered, and concentrated. Radial-band chromatography (elution with hexane–EtOAc 4:1) gave (−)-**3** (308 mg, quantitative yield): ([α]$_D^{24}$ −17.4° (*c* 1.69, $CHCl_3$); lit. [α]$_D$ −16.0° (*c* 0.9, $CHCl_3$)) [8]. The ^{1}H NMR spectrum of this compound was identical to that of (±)-**3**.

3-O-[(−)-Menthoxycarbonyl]-4-O-benzyl-1,2:5,6-di-O-isopropylidene-myo-inositol (**5l**).—Reaction of (±)-**3** (1.56 g, 4.5 mmol) with (−)-menthyl chloroformate (1.06 mL, 5.00 mmol) was performed in the same fashion as the synthesis of **4d** gave **5l** (670 mg, 28% yield): mp 130–131 °C; the ^{1}H NMR of this compound was identical to that of **4d**.

(+)-4-O-Benzyl-1,2:5,6-di-O-isopropylidene-myo-inositol ((+)-**3**).—The hydrolysis of **5l** (468 mg, 0.880 mmol) was identical to that described for **4d** to give (+)-**3** in quantitative yield: [α]$_D^{24}$ +17.9° (*c* 1.69, $CHCl_3$). The ^{1}H NMR spectrum of this compound was identical to that of (±)-**3**.

(+)-6-O-Benzyl-2,3-O-isopropylidene-myo-inositol (**6**).—A solution containing **4d** (532 mg, 1.00 mmol), PPTS (30 mg, 0.09 mmol), and ethylene glycol (10 mL of a 100 mM solution in CH_2Cl_2, 1.00 mmol) in CH_2Cl_2 (20 mL) was refluxed for 10 h and then washed with an aqueous NaCl solution (15% w/v, 2 × 5 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated. The residue was redissolved in methanol (10 mL) and refluxed with anhydrous potassium carbonate (138 mg, 1.00 mmol) for 1 h. After the solvent was removed under reduced pressure, the residue was resuspended in CH_2Cl_2 and filtered through Celite, and the eluant was concentrated. Recrystallization of the crude product from hexane–EtOAc 1:1 gave triol **6** (83.2 mg, 27% yield): [α]$_D^{24}$ +21° (*c* 0.43, $CHCl_3$); [α]$_D^{24}$ +8.6° (*c* 0.6, CH_3OH); lit. [α]$_D$ +12° (*c* 0.6, CH_3OH) [8]; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.36 (s, 3 H, CH_3), 1.55 (s, 3 H, CH_3), 2.51 (br s, 3 H, 3 × OH), 3.42 (m, 1 H), 3.67 (t, 1 H, J = 6.5 Hz), 3.82 (dd, 1 H, J = 9.9, 7.5 Hz), 3.93 (dd, 1 H, J = 8.3, 4.1 Hz), 4.05 (dd, 1 H, J = 7.2, 5.8 Hz), 4.35 (dd, 1 H, J = 5.7, 4.2 Hz), 4.81 (d, 1 H, J = 11.1 Hz, OCH_aH_b), 4.88 (d, 1 H, J = 11.4 Hz, OCH_aH_b), 7.30–7.39 (m, 5 H, C_6H_5).

(−)-6-O-Benzyl-2,3-O-isopropylidene-myo-inositol 1,4,5-tris(dibenzyl phosphate) (**7**).—A suspension of triol **6** (40 mg, 0.129 mmol), 1*H*-tetrazole (82 mg, 1.16 mmol), and bis(benzyloxy)diisopropylaminophosphine (1.55 mL of a 375 mM CH_2Cl_2 solution, 0.580 mmol) was stirred at room temperature until the 1*H*-tetrazole dissolved (30 min). Oxidation of the phosphite triester was accomplished by adding mCPBA (86 mg, 0.50 mmol assuming 50% purity) at −40°C. The mixture was allowed to warm to room temperature, diluted with CH_2Cl_2, and washed successively with an aqueous sodium sulfite solution (10% w/v), a saturated aqueous $NaHCO_3$ solution, water, and brine. The organic phase was dried ($MgSO_4$), filtered, and concentrated. Column chromatography (Kieselgel 60, 12 × 1.0 cm, hexane–EtOAc 1:1) gave **7** (113 mg, 92% yield): [α]$_D^{24}$ −5.23° (*c* 0.65, $CHCl_3$); ^{1}H NMR (200 MHz,

$CDCl_3$) δ 1.34 (s, 3 H, CH_3), 1.50 (s, 3 H, CH_3), 4.17 (dd, 1 H, $J = 2.3$, 7.6 Hz), 4.27 (t, 1 H, $J = 6.5$ Hz), 4.58–5.07 (m, 14 H), 7.09–7.30 (m, 35 H); ^{31}P ($CDCl_3$) δ −1.82, −1.77, −1.53.

D-myo-*Inositol 1,4,5-trisphosphate* ((−)-**1**).—A suspension of **7** (100 mg, 0.092 mmol), glacial acetic acid (0.60 mL, 0.011 mmol), and palladium/charcoal (10%, 10 mg) in 5 mL of EtOH–water (4:1) was stirred under hydrogen overnight. The mixture was centrifuged to remove the catalyst. After the solvent was removed under reduced pressure, the residue was redissolved in water (10 mL), stirred with NaOH (0.26 mL of a 2 M aqueous solution, 0.55 mmol), and precipitated with MeOH (0.50 mL). The precipitate was pelleted by centrifugation and washed with MeOH (2 × 0.50 mL) to give Ins(1,4,5)P_3 (39 mg, 0.092 mmol) as the hexasodium salt in quantitative yield: $[\alpha]_D^{24}$ −20° (*c* 0.05, H_2O, pH 9); lit. $[\alpha]_D$ −24° (*c* 0.5, H_2O, pH 9.3) [9]; ^1H NMR (D_2O) δ 3.60 (dd, 1 H, $J = 2.5$, 9.8 Hz), 3.72–3.97 (m, 3 H), 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* (**6a**).—Mild acid hydrolysis of **5l** (79 mg, 0.15 mmol) followed by alkaline hydrolysis, as described for **4d**, gave triol **6a** (25 mg, 68% yield): $[\alpha]_D^{24}$ +15.5° (*c* 1.04, $CHCl_3$). The ^1H NMR spectrum of this compound was identical to that of **6**.

D-myo-*Inositol-3,5,6-trisphosphate* ((+)-**1**).—Phosphitylation, oxidation, purification, and hydrogenolysis of **6a** (25 mg, 0.082 mmol) was performed as described for Ins(1,4,5)P_3 to give Ins(3,5,6)P_3 (6.0 mg, 0.011 mmol) as the hexasodium salt in 71% yield: $[\alpha]_D^{24}$ +17° (*c* 0.03, H_2O, pH 10); lit. $[\alpha]_D$ +35° (*c* 0.05, H_2O, pH 10.0) [7]; lit. $[\alpha]_D$ +27° (*c* 0.15, H_2O, pH 6.4) [14]; ^1H and ^{31}P NMR spectra were similar to those of (−)-**1**.

1-O-*Acetyl-6*-O-*benzyl-2,3:4,5-di*-O-*isopropylidene*-myo-*inositol* (**8**).—A solution containing (−)-**3** (466 mg, 1.33 mmol), acetic anhydride (162 mL, 1.70 mmol), pyridine (162 mL, 2.00 mmol), and DMAP (10 mg, 0.082 mmol) in CH_2Cl_2 (10 mL) was stirred at room temperature 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, concentrated, and the crude product was purified by column chromatography (Kieselgel 60, 8 × 2.5 cm, hexane–EtOAc 8:1) to give **8** (494 mg, 1.23 mmol) in 93% yield: ^1H NMR ($CDCl_3$) δ 1.34 (s, 3 H, CH_3), 1.42 (s, 6 H, CH_3), 1.45 (s, 3 H, CH_3), 2.10 (s, 3 H, CH_3), 3.61 (dd, 1 H, $J = 10.6$, 7.9 Hz, H-5), 3.82 (dd, 1 H, $J = 7.9$, 2.9 Hz, H-6), 4.02 (dd, 1 H, $J = 9.1$, 7.6 Hz, H-4), 4.37 (t, 1 H, $J = 7.2$ Hz, H-3), 4.50 (dd, 1 H, $J = 6.9$, 4.5 Hz, H-2), 4.74 (s, 2 H, OCH_2), 5.28 (dd, 1 H, $J = 3.7$, 2.9 Hz, H-1), 7.26–7.36 (m, 5 H, C_6H_5).

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