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An efficient chemoenzymic access to optically active *myo*-inositol polyphosphates

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ABSTRACT

The 1,4,5-tris-, 1,3,4-tris-, and 1,3,4,5-tetrakis-phosphates of 1D-*myo*-inositol have been prepared in their enantiomerically pure forms from the two enantiomers of 1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol. A facile enzymic preparation is also described of these chiral intermediates through enantiospecific deacylation of the corresponding racemic esters.

INTRODUCTION

The important role of Ins(1,4,5)P_3 (1D-*myo*-inositol 1,4,5-trisphosphate) as a second messenger in transmembrane signal transduction is well documented[1]. Once released from its membrane-associated precursor, Ins(1,4,5)P_3 undergoes a rapid turnover by two discrete mechanisms mediated by a 5-phosphatase and a 3-kinase, respectively, leading to several polyphosphate derivatives[2]. These molecules are involved in an intricate metabolic pathway and the physiological function of some of their metabolites remains to be assessed. As part of our interest in the enzymology and regulation of the cellular metabolism of Ins(1,4,5)P_3, efficient routes of synthesis to *myo*-inositol polyphosphates have been developed[3]. The strategy entailed a facile biocatalytic route to optically active *myo*-inositol derivatives as useful precursors to the target molecules. In this account, the merit of this synthetic approach is illustrated by the preparations of Ins(1,4,5)P_3 (ref. 4), Ins(1,3,4)P_3 (1D-*myo*-inositol 1,3,4-trisphosphate[5]), and Ins(1,3,4,5)P_4 (1D-*myo*-inositol 1,3,4,5-tetrakisphosphate[6]) in multigram quantities from the optical antipodes of 1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol (**1**) (Scheme 1).

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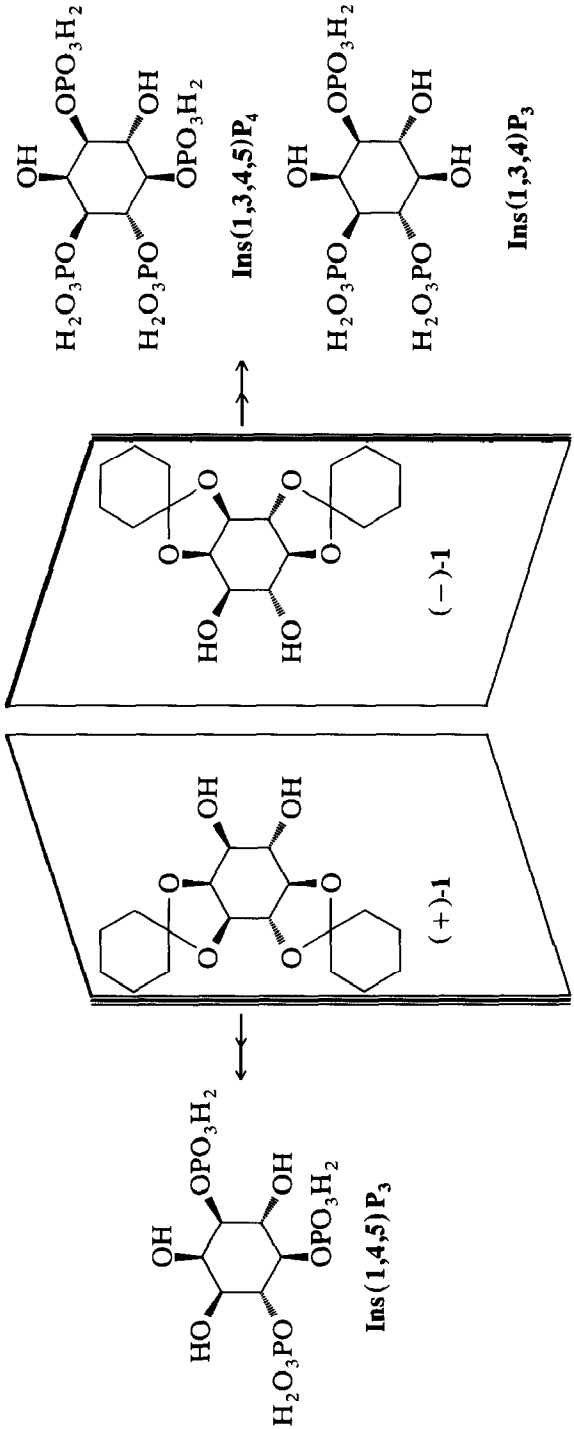
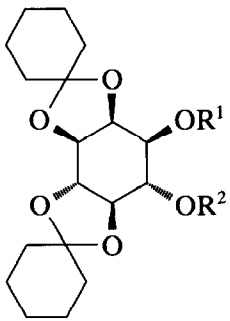


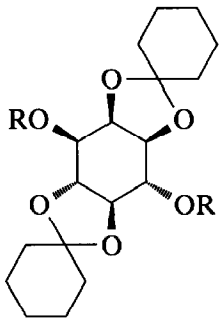
Scheme 1.

RESULTS AND DISCUSSION

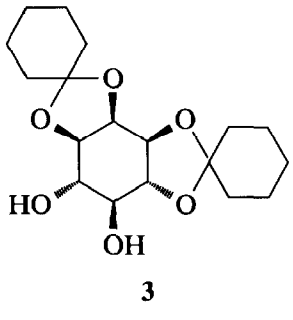
Enzymic preparation of the chiral precursors.—Treatments[7] of *myo*-inositol with 1-ethoxycyclohexene yielded a mixture of racemic 1,2:5,6- (**1**), 1,2:4,5- (**2**), and 1,2:3,4-di-*O*-cyclohexylidene-*myo*-inositol (**3**) in the ratios 1.2:1:0.5. These compounds could be separated readily from each other by a combination of flash-column chromatography and recrystallization, and biocatalytic resolution was applied to the major products **1** and **2**.



1 $R^1 = R^2 = H$
4 $R^1 = R^2 = C(O)Me$
6 $R^1 = R^2 = C(O)Pr$
8 $R^1 = H$, $R^2 = C(O)Me$
9 $R^1 = H$, $R^2 = C(O)Pr$



2 R = H
5 R = C(O)Me
7 R = C(O)Pr



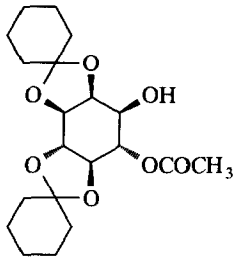
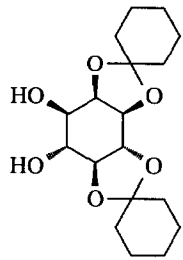
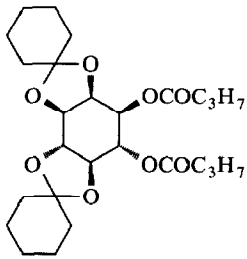
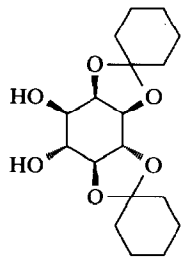
3

(only one enantiomeric form is shown)

In initial experiments, the diacetates and dibutyrates (**4–7**) of **1** and **2** were exposed to various commercial esterases, lipases, and proteases (esterases included pig liver esterase and cholesterol esterase; lipases included crude lipase preparations from porcine pancreas, *Candida cylindracea, Aspergillus niger, Geotrichum candidum, Humicola lanuginosa, Mucor meihei, Pseudomonas* sp., *Rhizopus niveus* and *Rhizopus oryzae*; and proteases included chymotrypsin, thermolysin, and proteases from *Aspergillus oryzae, Aspergillus sojae, Aspergillus satoi, Rhizopus* sp., and *Streptomyces caeapitosus*). Whereas the diesters (**4** and **6**) of **1** were deacylated by virtually all of the enzymes tested, the esters (**5** and **7**) of **2** were resistant. Different degrees of regio- and enantio-selectivity towards **4** and **6** were noted with different enzymes, of which cholesterol esterase (CE) and porcine pancreatic lipase (PPL) showed high degrees of stereochemical discrimination. Sequential enzymic hydrolysis of **4** with CE furnished nearly equal proportions of the 3-acetate **8** and the diol **1** with enantiomeric excess (ee) of 0.86 and 0.85, respectively (Table I). In contrast, when the dibutyrate **6** was treated with CE or PPL, optically active **1** (ee > 0.98) accompanied by minute quantities of the

TABLE I

Deacylation [a] of the 3,4-diacetate **4** and the 3,4-dibutyrate **6** with cholesterol esterase (CE) and porcine pancreatic lipase (PPL)

Substrate	Enzyme		Major products	
4 (1 g)	CE (120 U, 168 h)			
			(−)-**8**	(−)-**1**
		ee	0.86	0.85
		Recovery	345 mg	412 mg
6 (1 g)				
			(−)-**6**	(−)-**1**
	CE (120 U, 168 h)	ee	0.12	> 0.98
		Recovery	850 mg	95 mg
	PPL (500 mg, 168 h)	ee	0.13	> 0.98
		Recovery	870 mg	82 mg

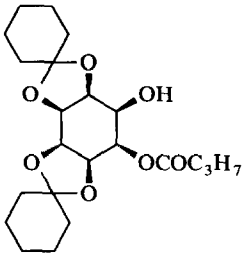
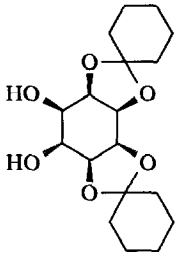
[a] See Experimental.

3-acetate (ee not determined) were obtained in low yields. The size of the acyl substituent affected not only the stereochemical outcome but also the product distribution. Presumably, this outcome resulted from a difference in the relative rates of deacylation for the mono- and di-acylates[8]. Thus, for the 3,4-diacetate **4**, the two consecutive rates of hydrolysis had the same order of magnitude, whereas the rate for the 3,4-dibutyrate **6** was much lower than that for the 3-butyrate **9**, probably due to steric hindrance.

Although these deacylations were highly stereoselective, the reactions were sluggish due to poor aqueous solubility and steric hindrance. Consequently, an excess of enzyme and prolonged incubation were necessary for satisfactory conversions. In order to circumvent this problem, the enantiospecific hydrolysis of the 3-butyrate **9**, a compound with minimized steric congestion and improved solubility, was examined. Compound **9** was obtained in fair yields via stannylidene-

TABLE II

Hydrolysis [a] of **9** with cholesterol esterase (CE) and porcine pancreatic lipase (PPL)

(±)-**9** (1 g) ⟶  + 

Enzyme (amount)	Conversion (%) [a] (time)	ee(S)	ee(P)	E [b]
CE (120 U)	48 (44 h)	0.86	0.93	79
PPL (500 mg)	52 (48 h)	0.95	0.88	58

[a] See experimental. [b] The enantiomeric ratio (E) is calculated from

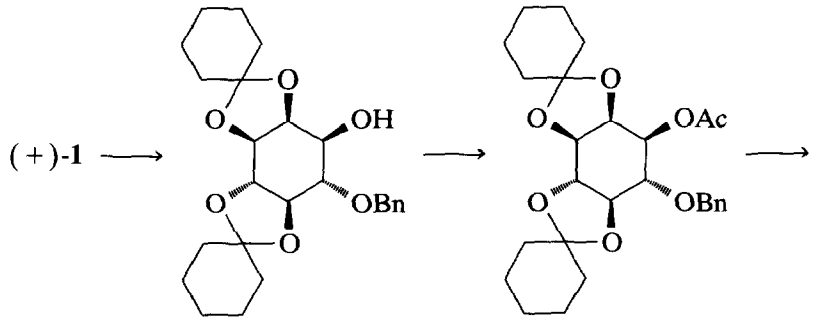
$$E = \ln\{1-C[1+ee(P)]\}/\ln\{1-C[1-ee(P)]\}$$

where C is the conversion and ee(P) is the ee value of the product (see ref. 9).

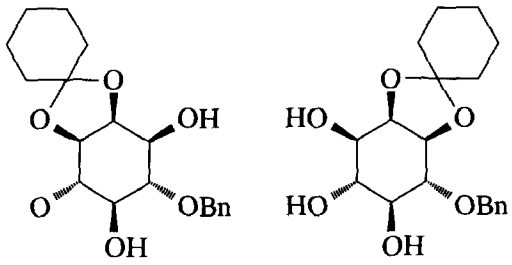
activated regioselective acylation[10] of **1**. As anticipated, both CE and PPL readily hydrolyzed **9** to afford optically active **1** with satisfactory enantiomeric purity (Table II). Although **9** is a monobutyrate of a vicinal diol, no acyl migration took place during the enzymic reactions. Even though PPL showed slightly lower antipodal differentiation compared to CE, it was preferred because of its low cost and ready availability. Accordingly, both enantiomers of **1** with optical purity > 95% were obtained in good yields after recrystallization.

Alternatively, chiral **1** could be obtained by chromatography of the corresponding dimenthoxycarbonyl diastereomers, followed by alkaline hydrolysis[3a]. This chemical process, although tedious, is especially useful when small quantities of chiral **1** are needed.

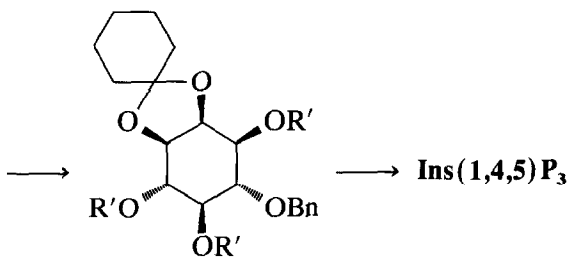
*Synthesis of Ins(1,4,5)P_3 from (+)-***1**.—The utility of (+)-**1** as a chiral precursor is demonstrated by the straightforward synthesis of Ins(1,4,5)P_3. Thus, stannylidene-activated regioselective benzylation[10] of (+)-**1** afforded (−)-**10** in good yield. Acid-catalyzed hydrolysis of the *trans*-ketal of **10** failed to yield the desired triol **12** in good yield, but gave an equimolar mixture of (+)-**12** and **12a**. The latter compound apparently resulted from acid-catalyzed migration of the cyclohexylidene group. In order to circumvent this problem, (−)-**10** was acetylated to give (−)-**11**, methanolysis of which removed the *trans*-4,5-*O*-cyclohexylidene ring and saponification of the product furnished (+)-**12**. Phosphorylation of the triol (+)-**12** by the phosphoramidite method[11], followed by debenzylation and removal of the cyclohexylidene group, gave Ins(1,4,5)P_3 [45% from (+)-**1**].



(−)-10 (−)-11



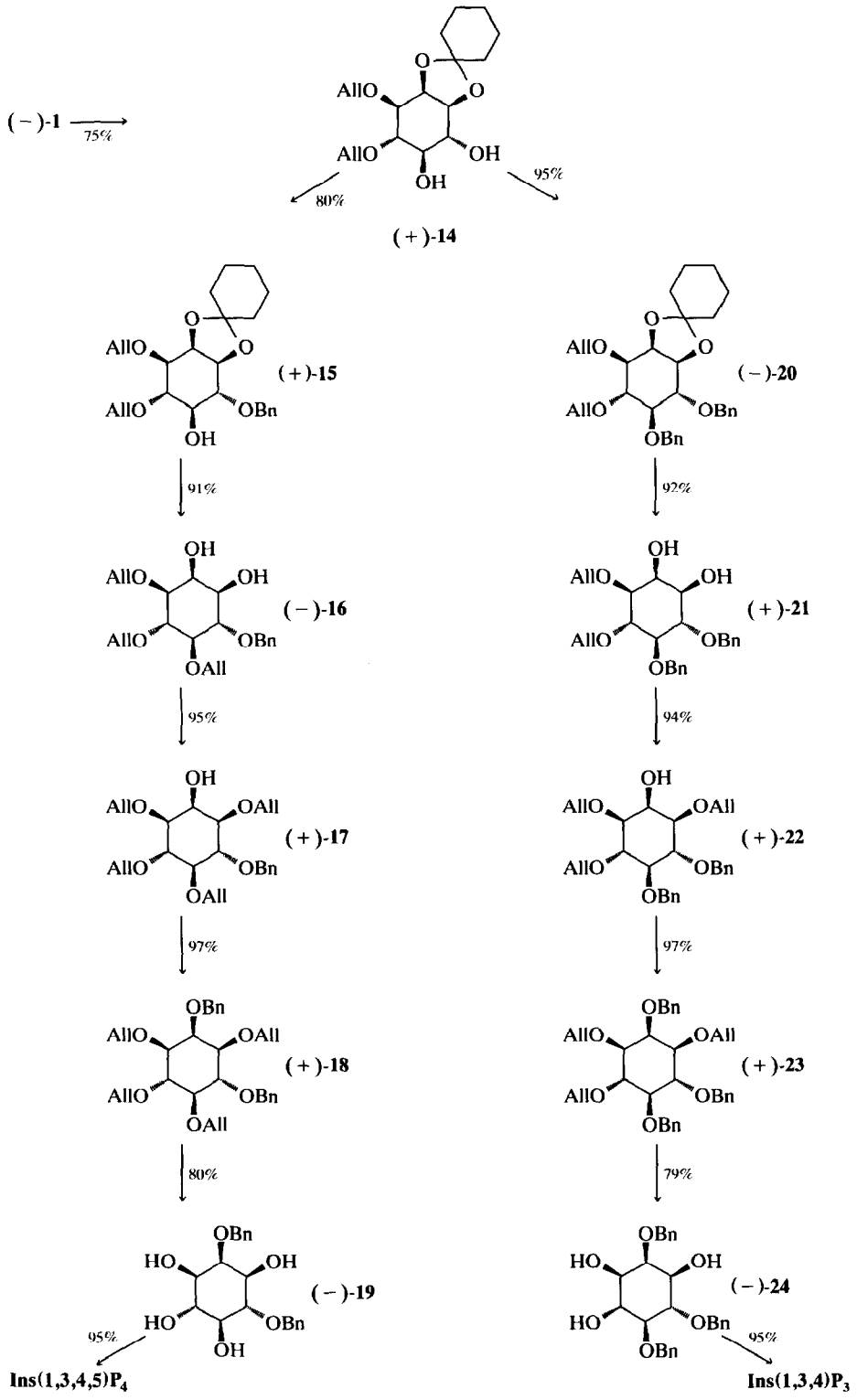
(+)-12 (+)-12a



(−)-13

$R' = P(O)(OBn)_2$

Synthesis of Ins(1,3,4)P_3 and Ins(1,3,4,5)P_4 from (−)-1.—Whereas (+)-**1** is a useful intermediate for the synthesis of Ins(1,4,5)P_3, retrosynthetic analysis indicated that (−)-**1** could be used to prepare Ins(1,3,4)P_3 and Ins(1,3,4,5)P_4. Thus, allylation of (−)-**1** and then selective removal of the *trans*-5,6-*O*-cyclohexylidene group afforded the key intermediate (+)-**14**; 6-*O*-benzylation of (+)-**14** led to Ins(1,3,4,5)P_4, whereas 5,6-di-*O*-benzylation led to Ins(1,3,4)P_3.


(−)-1
75%
AllO
O
O
AllO
OH
OH
95%
80%
(+)-14
(+)-15
AllO
OBn
(−)-20
91%
92%
(−)-16
OAll
(+)-21
95%
94%
(+)-17
(+)-22
97%
97%
(+)-18
(+)-23
80%
79%
HO
(−)-19
(−)-24
95%
95%
Ins(1,3,4,5)P4
Ins(1,3,4)P3

6-*O*-Benzylation of (+)-**14** via the corresponding *O*-stannylene acetal yielded (+)-**15** and allylation of HO-5 followed by removal of the 1,2-*O*-cyclohexylidene group gave (−)-**16**. 1-*O*-Allylation of (−)-**16** followed by 2-*O*-benzylation furnished the fully protected derivative (+)-**18**. Deallylation of (+)-**18** with 10% Pd/C and *p*-toluenesulfonic acid provided the tetrol (−)-**19**, phosphorylation and then debenzylation of which gave Ins(1,3,4,5)P_4 [38% from (−)-**1**].

Di-*O*-benzylation of (+)-**14** followed by acid hydrolysis of the cyclohexylidene group gave the diol (−)-**20**. The subsequent transformations were similar to those for the conversion of **16** into Ins(1,3,4,5)P_4. Thus, Ins(1,3,4)P_3 {hexapotassium salt, $[\alpha]_D$ +14° (*c* 2, H_2O, pH 8.2)} was obtained (60%) in 6 steps from **14**.

The above $[\alpha]_D$ value for Ins(1,3,4)P_3 does not accord with that (−6°) recorded[5] for the hexa-ammonium salt. No additional information concerning chiral Ins(1,3,4)P_3 appears to be available in the literature, but two lines of evidence support our chiral assignment: (*a*) the conversion of the common intermediate (+)-**14** into Ins(1,3,4,5)P_4; and (*b*) the $[\alpha]_D$ value [−25.4° (*c* 0.5, $CHCl_3$)] for the immediate precursor (−)-2,5,6-tri-*O*-benzyl-*myo*-inositol (**24**), which is similar to that (−27°) reported in the literature[12].

The syntheses described above demonstrate the versatility of the synthesis strategy. With both enantiomers of **1** on hand, virtually all of the *myo*-inositol polyphosphates can be prepared. This methodology is being developed to give analogues of these bioactive molecules which may serve as biochemical probes to study the cellular metabolism of IP_3.

EXPERIMENTAL

General methods.—The ^1H and ^{31}P NMR spectra were recorded with a Bruker AM-300 spectrometer. Optical rotations were determined at 23° with a Rudolph Autopol III polarimeter. HPLC was performed using a Model 501 pump (Waters Associates) equipped with a Rheodyne injector and a Model 481 UV/Vis detector (Waters Associates). Cholesterol esterase (CE) and crude porcine pancreatic lipase (PPL) powder (Type II) were purchased from Sigma Chemical Co., and the enzyme units were defined accordingly. Racemic **1**–**3** were prepared according to the procedure reported by Garegg et al.[7].

*(±)-6-O-Butyryl-1,2:4,5-di-O-cyclohexylidene-*myo*-inositol* (**9**).—A mixture of (±)-**1** (15 g, 44 mmol), Bu_2SnO (12 g, 48.4 mmol), and toluene (100 mL) was boiled under reflux with azeotropic removal of water for 2.5 h, then concentrated to dryness under reduced pressure. To the residue were added *N*,*N*-dimethylformamide (80 mL), CsF (13.8 g, 88 mmol), and butyryl chloride (4.8 mL, 46 mmol) at −41°. The mixture was stirred at 23° overnight, then diluted with CH_2Cl_2 (200 mL), washed with satd aq NaCl, dried (Na_2SO_4), and concentrated. Column chromatography (hexane–ether, 20:1 → 1:1) of the residue gave **9** (syrup, 10.9 g, 60%). ^1H NMR data ($CDCl_3$): δ 0.9 (t, 3 H, *J* 6 Hz, Me), 1.0–2.5 (m, 24 H, 12 CH_2), 3.0–3.2 (bs, 1 H, OH), 3.4–3.6 (m, 1 H, H-5), 3.7–4.1 (m, 2 H, H-1,3), 4.2–4.7 (m, 2 H, H-4,6), 4.8–5.0 (m, 1 H, H-2).

Biocatalytic resolution.—(*a*) A solution of the substrate (1 g) in *N*,*N*-dimethylformamide (7 mL) was introduced dropwise into 0.1 M potassium phosphate buffer (pH, 7.4, 70 mL) with vigorous stirring. The solution was homogenized to give a fine suspension. The reaction was initiated by adding the indicated amount of enzyme (Tables I and II), the mixture was stirred vigorously at 23°, and the reaction was monitored by TLC. The reaction was terminated by extracting the solution with EtOAc (2 × 70 mL), and the combined extracts were dried and concentrated. Column chromatography (hexane–EtOAc, 15:1 → 4:1) of the reaction was followed by saponification of the product (M NaOH, MeOH, 23°, 3 h) to yield **1** for the determination of the optical purity.

(*b*) Racemic **9** (15 g) was suspended in the buffer solution (2 L) containing 7 g of crude PPL powder as in (*a*). The mixture was stirred vigorously at 23° for 48 h, then extracted with EtOAc (2 × 2 L), and the combined extracts were dried (Na_2SO_4) and concentrated. Column chromatography of the residue afforded (−)-**1** (5.6 g, 88% ee), $[\alpha]_D$ −16° (*c* 1, $CHCl_3$), and (−)-**9** (6.5 g, 95% ee), $[\alpha]_D$ −14° (*c* 0.45, $CHCl_3$).

Determination of enantiomeric purity.—The antipodal alcohols, **1**, **8** and **9**, were each esterified with (*S*)-(−)-2-methoxy-2-(trifluoromethyl)phenylacetyl chloride and the resulting diastereomers were analyzed by HPLC using two columns (each 4.6 mm × 25 cm) of silica gel (10 μm) in tandem and elution with hexane–ether (5:1) at 1 mL/min. The retention times (min) were as follows: diester of **1**, (+) 16, (−) 22.5; monoester of **8**, (−) 15.5, (+) 17.2; monoester of **9**, (−) 17.6, (+) 23.3.

*(−)-6-*O*-Benzyl-2,3:4,5-di-*O*-cyclohexylidene-*myo*-inositol* (**10**).—The procedure for regioselective benzylation of (+)-**1** (5.8 g, 16.6 mmol) was identical to that described for **9** except that butyryl chloride was replaced by an equal amount of benzyl bromide (4.5 mL, 38 mmol), to afford (−)-**10** (syrup, 6.1 g, 84%), $[\alpha]_D$ −4° (*c* 1.6, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 1.44–1.75 (m, 20 H, 10 CH_2), 2.65 (d, 1 H, *J* 1.5 Hz, OH), 3.58 (dd, 1 H, *J* 7.8 and 10.5 Hz, H-5), 3.93 (dd, 1 H, *J* 2 and 7.8 Hz, H-4), 4.06–4.07 (m, 1 H, H-3), 4.23 (dd, 1 H, *J* 7.6 and 10.5 Hz, H-6), 4.38 (t, 1 H, *J* 3.6 and 7.5 Hz, H-1), 4.46 (dd, 1 H, *J* 3.6 and 7.5 Hz, H-2), 4.75 (q, 2 H, *J* 11.8 and 35 Hz, $OCH_2C_6H_5$), 7.28–7.43 (m, 5 H, C_6H_5).

*(−)-1-*O*-Acetyl-6-*O*-benzyl-2,3:4,5-di-*O*-cyclohexylidene-*myo*-inositol* (**11**).—A solution of (−)-**10** (6 g, 14 mmol) in CH_2Cl_2 (50 mL) was treated with acetic anhydride (4.5 mL, 42 mmol), triethylamine (12 mL, 126 mmol), and 4-dimethylaminopyridine (122 mg, 1 mmol) at 23° for 4 h. The mixture was washed with aq $NaHCO_3$ and water, dried, and concentrated. Column chromatography (hexane–ether 15:1) of the residue gave **11** (syrup, 6.6 g, 99%), $[\alpha]_D$ −15° (*c* 0.4, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 1.42–1.68 (m, 20 H, 10 CH_2), 2.14 (s, 3 H, Ac), 3.63 (dd, 1 H, *J* 7.8 and 11.4 Hz, H-5), 3.81 (dd, 1 H, *J* 2.4 and 7.8 Hz, H-3), 4.04 (dd, 1 H, *J* 7.8 and 10.8 Hz, H-6), 4.39 (t, 1 H, *J* 7.3 Hz, H-4), 4.52 (dd, 1 H, *J* 4.2 and 7.8 Hz, H-1), 4.78 (s, 2 H, $OCH_2C_6H_5$), 5.30 (dd, 1 H, *J* 2.4 and 3.9 Hz, H-2), 7.28–7.38 (m, 5 H, C_6H_5).

*(+)-6-*O*-Benzyl-2,3-*O*-cyclohexylidene-*myo*-inositol* (**12**).—A solution of (−)-**11**

(6 g, 12.6 mmol) in CH_2Cl_2–MeOH (1 : 1, 150 mL) was stirred with acetyl chloride (0.5 mL, 6 mmol) at 23° for 40 min. Triethylamine (1.7 mL, 12 mmol) was added, the solution was concentrated, the residue was treated with methanolic M KOH (15 mL) for 1 h at 23°, and the solvent was evaporated under reduced pressure. Column chromatography (CH_2Cl_2–ether–EtOH, 1:8:1) of the residue yielded (+)-**12** (amorphous, 3.2 g, 72%), $[\alpha]_D$ +24° (*c* 2, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 1.35–1.61 (m, 10 H, 5 CH_2), 3.10–3.18 (m, 1 H, H-5), 3.34–3.50 (m, 2 H, H-1,4), 3.68–3.74 (m, 1 H, H-6), 3.85 (t, 1 H, *J* 6.2 Hz, H-3), 4.18 (t, 1 H, *J* 9 Hz, H-2), 4.74 (q, 2 H, *J* 12 and 15 Hz, $OCH_2C_6H_5$), 4.87 (d, 1 H, *J* 3 Hz, OH), 4.96 (s, 1 H, OH), 4.98 (d, 1 H, *J* 2 Hz, OH), 7.22–7.42 (m, 5 H, C_6H_5).

Anal. Calcd for $C_{19}H_{26}O_6$: C, 65.13; H, 7.48. Found: C, 65.26; H, 7.45.

*(−)-6-O-Benzyl-2,3-O-cyclohexylidene-*myo*-inositol 1,4,5-tris(dibenzyl phosphate)* (**13**).—A mixture of tetrazole (3.6 g, 51 mmol), dibenzyl *N,N*-di-isopropylphosphoramidite (17.8 g, 51 mol), and CH_2Cl_2 (100 mL) was stirred under Ar at 23° for 1 h, and (+)-**12** (3 g, 8.6 mmol) was added in one portion. The solution was kept under the same conditions for another 12 h, cooled to −40°, then treated with triethylamine (14 mL, 100 mmol) and *m*-chloroperoxybenzoic acid (50% purity, 17.8 g, 51 mmol). The mixture was stirred at −40° for 30 min, then allowed to attain room temperature, diluted with CH_2Cl_2 (100 mL), washed with aq Na_2SO_3, aq $NaHCO_3$, and water, dried, and concentrated. Column chromatography (hexane–EtOAc, 20 : 1 → 2 : 1) of the residue furnished (−)-**13** (syrup, 9.4 g, 97%), $[\alpha]_D$ −6.0° (*c* 1.1, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 1.34–1.78 (m, 10 H, 5 CH_2), 4.18–4.22 (m, 1 H, H-5), 4.26–4.33 (m, 1 H, H-4), 4.63–4.71 (m, 3 H, H-1,3,6), 4.77–4.89 (m, 3 H, H-2, $OCH_2C_6H_5$), 4.91–5.17 (m, 12 H, $OCH_2C_6H_5$), 7.13–7.34 (m, 35 H, 7 C_6H_5).

*1D-*myo*-Inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3]*.—A solution of (−)-**13** (9 g, 8 mmol) in aq 90% EtOH was shaken under H_2 (50 psi) in the presence of 10% Pd/C (4.5 g) for 5 h, then filtered, and concentrated. A solution of the residue in acetic acid–water (1 : 1, 8 mL) was stirred at 23° for 4 h, EtOH (70 mL) was added, and the solution was concentrated to dryness. The residue was triturated with CH_2Cl_2–ether (1 : 1), the resulting white precipitate was dissolved in the minimum amount of water, and M KOH (6 equiv) was added. The solution was diluted with EtOH (80 mL), and the precipitate was dissolved in water (10 mL) and lyophilized to afford Ins(1,4,5)P_3 as the hexapotassium salt (5.05 g, 98%), $[\alpha]_D$ −24° (c 0.5, H_2O, pH 9.3). NMR data: ^1H (D_2O), δ 3.56 (dd, 1 H, *J* 3 and 9.3 Hz, H-3), 3.66–3.79 (m, 3 H, H-1,5,6), 3.95–4.04 (m, 1 H, H-4), 4.19 (bs, 1 H, H-2); ^{31}P (D_2O, external H_3PO_4), δ 2.86, 4.49, and 4.67.

*(+)-3,4-Di-O-allyl-1,2-O-cyclohexylidene-*myo*-inositol* (**14**).—A solution of (−)-**1** (6.7 g, 19.7 mmol) in *N,N*-dimethylformamide (50 mL) was treated with NaH (2.36 g, 78.8 mmol) and allyl bromide (6 mL, 69 mmol) for 3 h at 23°. The excess of NaH was destroyed with MeOH, the mixture was concentrated, and a solution of the residue in CH_2Cl_2 (150 mL) was washed with water, dried, and concentrated. Column chromatography (hexane–ether, 15 : 1) of the residue gave (+)-3,4-di-*O*-al-

lyl-1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol (syrup, 7.6 g, 92%), $[\alpha]_D$ +6.5° (*c* 0.4, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 1.38–1.76 (m, 10 H, 5 CH_2), 3.44 (dd, 1 H, *J* 7.5 and 11.4 Hz, H-5), 3.65 (t, 1 H, *J* 3 Hz, H-6), 3.77 (dd, 1 H, *J* 3 and 6.3 Hz, H-1), 4.06–4.25 (m, 5 H, H-2 and 2 OCH_2), 4.31 (t, 1 H, *J* 9 Hz, H-3), 4.37 (dd, 1 H, *J* 3.6 Hz, H-4), 5.15–5.21 (m, 2 H, CH_2=C), 5.26–5.35 (m, 2 H, CH_2=C), 5.83–5.89 (m, 2 H, 2 C=CH).

The foregoing compound was selectively hydrolyzed, as described for **12**, to yield **14** (syrup, 5.1 g, 82%), $[\alpha]_D$ +1.6° (*c* 1.9, $CHCl_3$). ^1H NMR data ($CDCl_3$), δ 1.36–1.78 (m, 10 H, 5 CH_2), 3.24–3.35 (m, 3 H, H-1 and 2 OH), 3.55–3.62 (m, 2 H, H-2,5), 3.72 (dd, 1 H, *J* 7.8 and 9.3 Hz, H-4), 3.97 (dd, 1 H, *J* 6 and 9.3 Hz, H-3), 4.19–4.22 (m, 2 H, OCH_2), 4.26 (m, 1 H, H-6), 4.37–4.44 (m, 2 H, OCH_2), 5.15–5.22 (m, 2 H, CH_2=C), 5.26–5.34 (m, 2 H, CH_2=C), 5.86–6.04 (m, 2 H, 2 C=CH).

(+)-3,4-Di-O-*allyl-6*-O-*benzyl-1,2*-O-*cyclohexylidene*-myo-*inositol* (**15**).—Regioselective benzylation of (+)-**14** (3 g, 7 mmol), as described for **10**, furnished (+)-**15** (syrup, 3.04 g, 80%), $[\alpha]_D$ +14° (*c* 1.5, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 1.46–1.81 (m, 10 H, 5 CH_2), 2.75 (bs, 1 H, OH), 3.46–3.49 (m, 1 H, H-5), 3.60–3.73 (m, 3 H, H-4 and CH_2), 4.16 (dd, 1 H, *J* 5.4 and 6 Hz, H-3), 4.22–4.30 (m, 3 H, H-6 and CH_2), 4.34–4.43 (m, 1 H, H-1), 4.40–4.43 (m, 1 H, H-2), 4.86 (q, 2 H, *J* 11.7 and 68 Hz, $OCH_2C_6H_5$), 5.18–5.24 (m, 2 H, CH_2=C), 5.29–5.37 (m, 2 H, CH_2=C), 5.90–6.05 (m, 2 H, 2 C=CH), 7.24–7.42 (m, 5 H, C_6H_5).

(−)-3,4,5-Tri-O-*allyl-6*-O-*benzyl*-myo-*inositol* (**16**).—Allylation of (+)-**15** (2.95 g, 6.9 mmol) with allyl bromide, as described for **14**, gave (−)-3,4,5-tri-*O*-allyl-6-*O*-benzyl-1,2-*O*-cyclohexylidene-*myo*-inositol (syrup, 3.18 g, 98%), $[\alpha]_D$ −9.2° (*c* 1.5, $CHCl_3$). ^1H NMR data ($CDCl_3$); δ 1.45–1.76 (m, 10 H, 5 CH_2), 3.23 (dd, 1 H, *J* 6.6 and 8.4 Hz, H-5), 3.54 (dd, 1 H, *J* 3.6 and 7.7 Hz, H-1), 3.66–3.73 (m, 2 H, H-3,4), 4.09 (dd, 1 H, *J* 5.4 and 7.0 Hz, H-6), 4.21–4.38 (m, 7 H, H-2 and 3 OCH_2), 4.81 (q, 2 H, *J* 7.4 and 36 Hz, $OCH_2C_6H_5$), 5.13–5.34 (m, 6 H, 3 CH_2=C), 5.89–6.04 (m, 3 H, 3 C=CH), 7.24–7.42 (m, 5 H, C_6H_5).

The foregoing compound (3.18 g, 6.77 mmol) was treated with acetyl chloride (0.2 mL, 2.6 mmol) in CH_2Cl_2–MeOH (1:1, 75 mL) at 23° for 1 h, and the solution was then concentrated. Column chromatography (hexane–EtOAc, 20:1 → 2:1) of the residue gave (−)-**16** (syrup, 2.45 g, 93%), $[\alpha]_D$ −10° (*c* 2, $CHCl_3$). ^1H NMR data ($CDCl_3$), δ 2.66 (d, 1 H, *J* 3.6 Hz, OH), 2.72 (d, 1 H, *J* 1.2 Hz, OH), 3.22–3.29 (m, 2 H, H-4,5), 3.40–3.51 (m, 1 H, H-3), 3.67–3.76 (m, 2 H, H-1,6), 4.12–4.19 (m, 3 H, H-2 and OCH_2), 4.21–4.41 (m, 4 H, 2 OCH_2), 4.84 (q, 2 H, *J* 7.5 and 3.6 Hz, $OCH_2C_6H_5$), 5.13–5.34 (m, 6 H, 3CH_2=C), 5.86–6.04 (m, 3 H, 3 C=CH), 7.27–7.40 (m, 5 H, C_6H_5).

(+)-1,3,4,5-Tetra-O-*allyl-6*-O-*benzyl*-myo-*inositol* (**17**).—Regioselective allylation of (−)-**16** (2.4 g, 6.2 mmol) with Bu_2SnO and CsF, as described for **9**, yielded (+)-**17** (syrup, 2.5 g, 95%), $[\alpha]_D$ +11° (*c* 3.1, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 2.48 (bs, 1 H, OH), 3.10–3.27 (m, 3 H, H-3,4,5), 3.66–3.75 (m, 1 H, H-6), 3.83 (t, 1 H, *J* 9.6 Hz, H-1), 4.16–4.20 (m, 5 H, H-2 and 2 OCH_2), 4.28–4.32 (m, 4 H, 2

OCH_2), 4.81 (q, 2 H, *J* 10.8 and 13 Hz, $OCH_2C_6H_5$), 5.12–5.21 (m, 4 H, 4 C=CH), 5.23–5.34 (m, 4 H, 2 CH_2=C), 5.87–6.04 (m, 4 H, 2 CH_2=C), 7.25–7.39 (m, 5 H, C_6H_5).

(+)-1,3,4,5-Tetra-O-*allyl-2,6-di*-O-*benzyl*-myo-*inositol* (**18**).—A solution of (+)-**17** (2.5 g, 5.8 mmol) in *N*,*N*-dimethylformamide (20 mL) was treated with NaH (0.349 g, 11.8 mmol) and benzyl bromide (1.1 mL, 8.7 mmol) for 8 h at 23°. Excess of NaH was destroyed with MeOH, the mixture was concentrated, and a solution of the residue in CH_2Cl_2 (100 mL) was washed with water, dried, and concentrated. Column chromatography (hexane–ether, 20:1 → 5:1) of the residue gave (+)-**18** (syrup, 3 g, 97%), $[\alpha]_D$ +0.8° (*c* 1.1, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 3.11–3.24 (m, 3 H, H-3,4,5), 3.76–3.95 (m, 3 H, H-1,2,6), 4.04–4.09 (m, 4 H, 2 OCH_2), 4.24–4.35 (m, 4 H, 2 OCH_2), 4.73–4.84 (m, 4 H, 2 $OCH_2C_6H_5$), 5.10–5.17 (m, 4 H, 2 CH_2=C), 5.21–5.32 (m, 4 H, 2 CH_2=C), 5.81–6.02 (m, 4 H, 4 C=CH), 7.21–7.43 (m, 10 H, 2 C_6H_5).

(−)-2,6-Di-O-*benzyl*-myo-*inositol* (**19**).—A mixture of (+)-**18** (3 g, 5.76 mmol), 10% Pd/C, *p*-toluenesulfonic acid (500 mg, 2.6 mmol), and MeOH–water (5:1, 90 mL) was stirred under reflux for 2 h, then filtered, and concentrated. Column chromatography (ether–EtOH, 10:1) of the residue furnished (−)-**19** (amorphous, 1.65 g, 80%), $[\alpha]_D$ −29° (*c* 0.65, EtOH). ^1H NMR data (CD_3OD): δ 3.24–3.30 (m, 1 H, H-5), 3.38 (dd, 1 H, *J* 2.4 Hz, H-1), 3.51–3.69 (m, 3 H, H-3,4,6), 3.85–3.86 (m, 1 H, H-2), 4.77–4.87 (m, 4 H, 2 $CH_2C_6H_5$), 7.18–7.41 (m, 10 H, 2 C_6H_5).

Anal. Calcd for $C_{27}H_{30}O_6$: C, 66.65; H, 6.71. Found: C, 66.71; H, 6.79.

*1*D-myo-*Inositol 1,3,4,5-tetrakisphosphate [Ins(1,3,4,5)P$_4$]*.—The tetrol (−)-**19** (1.6 g, 4.4 mmol) was phosphorylated with dibenzyl *N*,*N*-di-isopropylphosphoramidite, tetrazole, and *m*-chloroperoxybenzoic acid, as described for **13**, to yield 1D-*myo*-inositol 1,3,4,5-tetrakis(dibenzyl phosphate) (syrup, 6 g, 96%), $[\alpha]_D$ −3.5° (*c* 2.9, $CHCl_3$). ^1H NMR data ($CDCl_3$), δ 4.03–4.10 (m, 1 H, H-5), 4.20–4.31 (m, 2 H, H-1,3), 4.40–4.49 (m, 1 H, H-6), 4.60–5.07 (m, 22 H, H-2,4 and 10 $CH_2C_6H_5$), 6.94–7.38 (m, 50 H, 10 C_6H_5).

A mixture of the foregoing product (6 g, 5.3 mmol) and 10% Pd/C (3 g) in aq 60% EtOH was shaken under H_2 (50 psi) for 24 h, then filtered, and concentrated. The residue was dissolved in the minimum amount of water, M KOH (8 equiv) was added, and the solution was diluted with EtOH (200 mL). The precipitate was dissolved in water (20 mL) and lyophilized to afford Ins(1,3,4,5)P_4 as the octapotassium salt (3.4 g, 99%), $[\alpha]_D$ −3.5° (*c* 5.5, H_2O, pH 8.4). NMR data: ^1H (D_2O), δ 3.69–3.91 (m, 4 H, H-1,3,5,6), 4.14–4.23 (q, 1 H, *J* 9 and 18 Hz, H-4), 4.32 (bs, 1 H, H-2); ^{31}P (D_2O, external H_3PO_4), 2.94, 4.58, 4.73, and 5.27.

(−)-3,4-Di-O-*allyl-5,6-di*-O-*benzyl-1,2*-O-*cyclohexylidene*-myo-*inositol* (**20**).—Conventional benzylation of (+)-**14** (2 g, 8.8 mmol), as described for **18**, gave (−)-**20** (syrup, 2.9 g, 95%), $[\alpha]_D$ −4.5° (*c* 1.2, $CHCl_3$). ^1H NMR data ($CDCl_3$), δ 1.54–1.76 (m, 10 H, 5 CH_2), 3.36 (dd, 1 H, *J* 9 and 9.9 Hz, H-4), 3.58 (dd, 1 H, *J* 3.9 and 8.4 Hz, H-3), 3.76 (dd, 2 H, *J* 9 and 9.3 Hz, H-1,5), 4.14 (dd, 1 H, *J* 5.4 and 7.8 Hz, H-6), 4.22–4.32 (m, 4 H, 2 OCH_2), 4.36–4.39 (m, 1 H, H-2), 4.73–4.89 (m, 4

H, 2 OC$H_2C_6H_5$), 5.13–5.21 (m, 2 H, CH_2=C), 5.24–5.34 (m, 2 H, CH_2=C), 5.88–6.04 (m, 2 H, 2 C=CH), 7.23–7.39 (m, 10 H, 2 C_6H_5).

*(+)-3,4-Di-*O*-allyl-5,6-di-*O*-benzyl-*myo*-inositol* (**21**).—Removal of the cyclohexylidene group of (−)-**20** (2.6 g, 5 mmol), as described for **16**, gave (+)-**21** (syrup, 2 g, 92%), $[\alpha]_D$ +0.5° (*c* 1.2, $CHCl_3$). ^{1}H NMR data ($CDCl_3$): δ 2.61 (d, 1 H, *J* 4.2 Hz, OH), 2.67 (s, 1 H, OH), 3.31 (dd, 1 H, *J* 3 and 10.2 Hz, H-5), 3.40–3.50 (m, 2 H, H-3,4), 3.76–3.83 (m, 2 H, H-1,6), 4.15–4.20 (m, 3 H, H-2, and OCH_2), 4.28–4.41 (m, 2 H, OCH_2), 4.78 (dd, 2 H, *J* 11 and 23 Hz, OC$H_2C_6H_5$), 4.94 (t, 2 H, 10 Hz, OC$H_2C_6H_5$), 5.15–5.36 (m, 4 H, 2 CH_2=C), 5.88–6.06 (m, 2 H, 2 C=CH), 7.27–7.37 (m, 10 H, 2C_6H_5).

*(+)-1,3,4-Tri-*O*-allyl-5,6-di-*O*-benzyl-*myo*-inositol* (**22**).—Regioselective allylation of (+)-**21** (2 g, 4.5 mmol) with Bu_2SnO and CsF, as described for **9**, yielded (+)-**22** (syrup, 2.1 g, 94%), $[\alpha]_D$ +23.8° (*c* 3.3, $CHCl_3$). ^{1}H NMR data ($CDCl_3$), δ 2.50 (s, 1 H, OH), 3.25–3.34 (m, 2 H, H-3,5), 3.39 (t, 1 H, *J* 12 Hz, H-1), 3.81 (t, 1 H, *J* 9.6 Hz, H-6), 3.91 (t, 1 H, *J* 9.6 Hz, H-4), 4.20–4.22 (m, 5 H, H-2, 2 OCH_2), 4.32–4.37 (m, 2 H, OCH_2), 4.78–4.89 (m, 4 H, 2 OC$H_2C_6H_5$), 5.13–5.36 (m, 6 H, 3 CH_2=C), 5.89–6.05 (m, 3 H, 3 CH=C), 7.25–7.37 (m, 10 H, 2 C_6H_5).

*(+)-1,3,4-Tri-*O*-allyl-2,5,6-tri-*O*-benzyl-*myo*-inositol* (**23**).—Conventional benzylation of (+)-**22** (2 g, 4.2 mmol), as described for **18**, gave (+)-**23** (syrup, 2.3 g, 97%), $[\alpha]_D$ +15.5° (*c* 0.8, $CHCl_3$). ^{1}H NMR data ($CDCl_3$), δ 3.18–3.27 (m, 2 H, H-3,5), 3.38 (t, 1 H, *J* 9 Hz, H-1), 3.89 (t, 1 H, *J* 9 Hz, H-6), 3.97–4.01 (m, 2 H, H-2,4), 4.03–4.11 (m, 4 H, 2 OCH_2), 4.25–4.41 (m, 2 H, OCH_2), 4.76–4.90 (m, 6 H, 3 OC$H_2C_6H_5$), 5.10–5.34 (m, 6 H, 3 CH_2=C), 5.84–6.04 (m, 3 H, 3 CH=C), 7.22–7.44 (m, 15 H, 3 C_6H_5).

(−)-2,5,6-Tri-O-*benzyl*-*myo*-*inositol* (**24**).—Deallylation of (+)-**23** (2.3 g, 4 mmol), as described for **19**, yielded (−)-**24** (1.43 g, 79%), $[\alpha]_D$ −25° (*c* 0.5, $CHCl_3$). ^{1}H NMR data ($CDCl_3$): δ 2.39 (d, 1 H, *J* 3 Hz, OH), 2.49 (bs, 1 H, OH), 2.64 (bs, 1 H, OH), 3.32 (t, 1 H, *J* 9 Hz, H-5), 3.44 (d, 1 H, *J* 9 Hz, H-3), 3.57 (d, 1 H, *J* 9 Hz, H-1), 3.74–3.85 (m, 2 H, H-4,6), 3.97–3.99 (m, 1 H, H-2), 4.74–4.93 (m, 6 H, 3 OC$H_2C_6H_5$), 7.24–7.35 (m, 15 H, 3 C_6H_5).

Anal. Calcd for $C_{27}H_{30}O_6$: C, 71.98; H, 6.71. Found: C, 71.79; H, 6.88.

*1*D-*myo-Inositol 1,3,4-trisphosphate [Ins(1,3,4)P$_3$]*.—The triol (−)-**24** (1.4 g, 3.1 mmol) was phosphorylated and debenzylated, as described for Ins(1,3,4,5)P_4, to afford Ins(1,3,4)P_3 (1.9 g, 95%), $[\alpha]_D$ +13.6° (*c* 2, H_2O, pH 8.2). NMR data: ^{1}H (D_2O), δ 3.38 (t, 1 H, *J* 9 Hz, H-5), 3.64 (t, 1 H, *J* 9 Hz, H-6), 3.73–3.82 (m, 2 H, H-1,3), 3.99 (dd, 1 H, *J* 9 Hz, H-4), 4.30 (m, 1 H, H-2); ^{31}P (D_2O, external H_3PO_4), δ 3.28, 4.16, and 4.87.

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