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A chemoenzymatic synthesis of D-*myo*-inositol 1,4,5-trisphosphate

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ABSTRACT

Enzyme-catalyzed esterification of racemic 2,3-*O*-cyclohexylidene-*myo*-inositol (DL-**1**) proceeded exclusively in 1,4-dioxane to give optically pure L-1-*O*-acetyl-2,3-*O*-cyclohexylidene-*myo*-inositol (L-**2**) and D-2,3-*O*-cyclohexylidene-*myo*-inositol (D-**1**). A new practical route has been developed for the synthesis of D-*myo*-inositol 1,4,5-trisphosphate starting from D-**1** via selective acylation of the 6-hydroxyl group.

INTRODUCTION

The chemical synthesis of D-*myo*-inositol 1,4,5-trisphosphate has attracted much attention because it has been found to act as a second messenger in the cell signaling system[1]. Several synthetic methods have been reported starting from *myo*-inositol[2]. These reports have focused mostly on the optical resolution of inositol derivatives and selective protection and deprotection of the six hydroxyl groups of inositol. The cumbersome optical resolution procedure, which consists of the formation of diastereomers of inositol derivatives and separation of them, along with tedious protection steps, usually results in a low overall yield of the final product. Herein we report a new route for the synthesis of D-*myo*-inositol 1,4,5-trisphosphate starting from 2,3-*O*-cyclohexylidene-*myo*-inositol (**1**) (Scheme 1)[3]. This method involves kinetic resolution of **1** by enzymatic esterification in an organic solvent.

RESULTS AND DISCUSSION

Enzymatic resolution.—Because **1**, prepared in a single step from inositol[4], is an important precursor for the synthesis of various naturally occurring *myo*-inositol derivatives[4,5], the development of an efficient procedure for optical resolution of **1** is imperative. There are, however, few methods for the resolution of **1**. Tsai and

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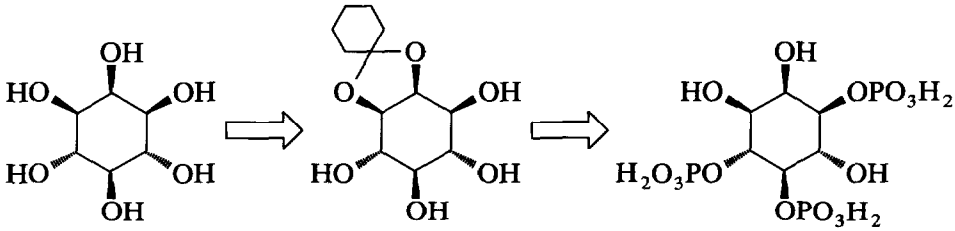


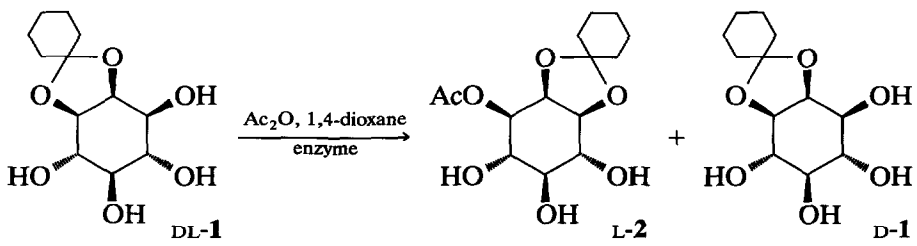
myo-inositol **1** **13** D-*myo*-inositol 1,4,5-triphosphate

Scheme 1.

co-workers developed a technique by preparing an optically active analogue, D-2,3-*O*-(1′*R*,2′*R*,4′*R*-1′,7′,7′-trimethyl[2.2.1]bicycloheptan-2′-ylidene)-*myo*-inositol, and used it in the same way as 2,3-*O*-cyclohexylidene-*myo*-inositol for the synthesis of various optically active *myo*-inositol derivatives[5b]. However, preparation of this analogue is difficult because the acetalization of inositol with D-camphor dimethyl acetal gives four diastereomers, whose separation is difficult. The yields of the products are also low.

We have investigated the resolution of racemic **1** by enzyme-catalyzed enantioselective esterification in organic solvents, as recently developed as a new method for obtaining optically active materials[6] (Scheme 2). Among commercially available hydrolytic enzymes, we found that two lipases from *Pseudomonas sp.* (Amano Lipase P and Lipase CES) were effective for the resolution in 1,4-dioxane. Representative results are listed in Table I. Lipase P and Lipase CES selectively acetylated the hydroxyl group at the C-1 position of the L enantiomer. Increasing the mass equivalent of Lipase P and the reaction time improved the optical purity of the product D-**1**. Lipase CES acetylated the L enantiomer exclusively and gave the optically pure 1-acetylated product L-**2** (49%, 98% ee) and D-**1** (49%, 100% ee). Amano Lipase AK, Lipase M, Lipase AY, and PLE-A were not active for the resolution. No reaction takes place in the absence of enzyme.

The recovery and reuse of enzyme were studied and the results are listed in Table II. The recovered enzyme was almost inactive (Runs 1 and 2). We consid-



Scheme 2.

TABLE I

Enzymatic resolution of racemic 2,3-mono-*O*-cyclohexylidene-*myo*-inositol (**1**)

Enzyme	Mass equiv	Ac_2O equiv	Reaction time (h)	Yield (%) (ee)	
				L-**2**	D-**1**
Lipase P	5	6	13	32 (84%)	67 (30%)
Lipase P	8	8	22	46 (90%)	53 (68%)
Lipase P	11	8	46	49 (80%)	43 (100%)
Lipase CES	10	6	46	49 (98%)	49 (100%)

ered that the water of hydration of the enzyme, which is essential for enzyme activity[6c,7], was extracted by the highly polar solvent (1,4-dioxane), and so a certain amount of water was added to soak the enzyme powder for hydration of the enzyme protein. After the excess of water had been evaporated and the enzyme dried under vacuum, the enzyme was reused under the same conditions as used for the first time (Run 3). Then enzyme was reactivated by hydration, thus indicating the importance of the water of hydration for enzyme activity in an anhydrous organic solvent.

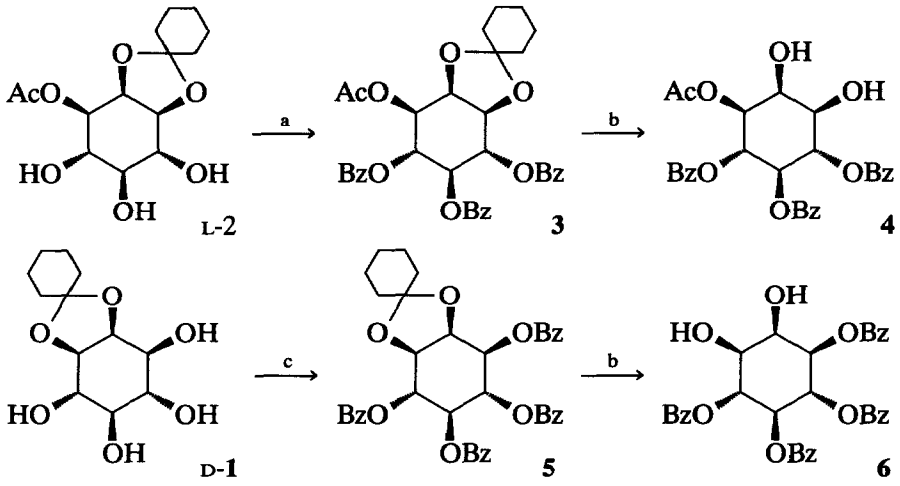
The absolute configuration of D-**1** was determined by its specific rotation[8]. The optical purity was determined by HPLC analysis with the use of a chiral column. The D and L enantiomers of racemic 1-*O*-acetyl-4,5,6-tri-*O*-benzoyl-*myo*-inositol (DL-**4**) and 1,4,5,6-tetra-*O*-benzoyl-*myo*-inositol (DL-**6**) were readily separated by a Chiralcel OD column (Daicel Chemical Industries, 1:5 2-propanol–hexane). The retention times of the D and L enantiomers were 7.8 and 10.5 min for **4** and 8.1 and 12.5 min for **6**, respectively (flow rate of eluent, 1.0 mL/min). Thus the optical purities of D-**1** and L-**2** were determined as the tetrabenzoylated derivative **6** and 1-*O*-acetyl-4,5,6-tri-*O*-benzoyl-L-*myo*-inositol (**4**) respectively (Scheme 3).

Synthesis of D-myo-*inositol 1,4,5-trisphosphate.*—We have previously reported the synthesis of D-*myo*-inositol 1,4,5-trisphosphate (**13**) from both 1,2:4,5-di-*O*-cyclohexylidene-*myo*-inositol[9] and 1,3,5-tri-*O*-benzoyl-*myo*-inositol[10]. Both 1,2:4,5-di-*O*-isopropylidene-*myo*-inositol (Gigg's group)[11] and 1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol (Vacca's[12] and Chen's[13] group), along with some other inositol derivatives[14] were generally used as starting materials for the synthesis. Common

TABLE II

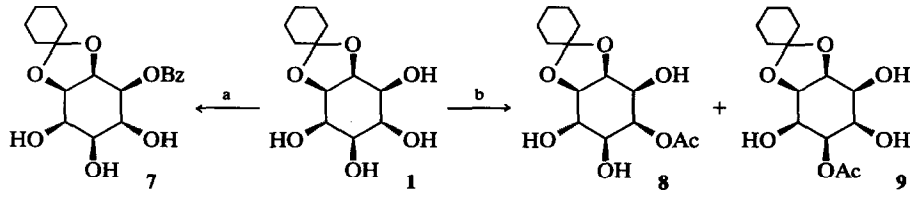
Recovery and reuse of Lipase CES

Run	Treament of enzyme before reuse	Reaction time (h)	Yield (%) (ee)	
			L-**2**	D-**1**
1	dried under vacuum for 15 min	72	trace reaction	
2	dried under vacuum for 3 h	48	trace reaction	
3	water added, soaked for 20 min, dried under vacuum for 3 h	68	44 (88%)	51 (46%)

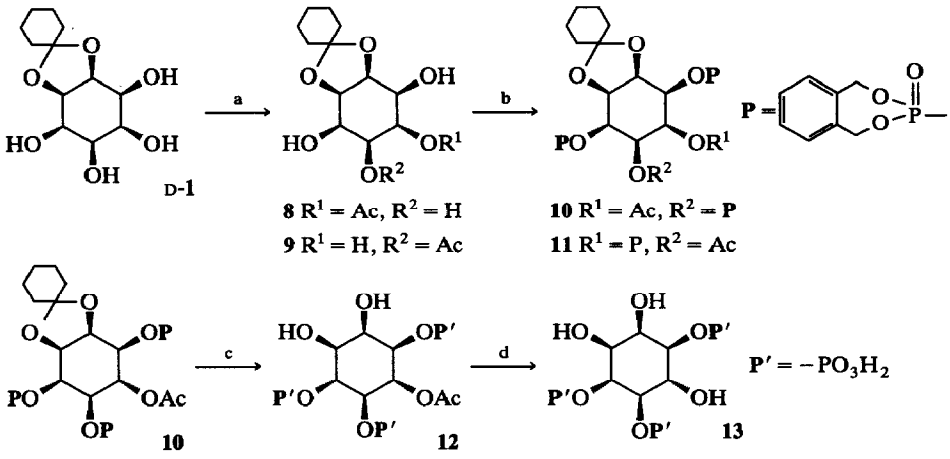


Scheme 3. (a) BzCl, 6 equiv; Et_3N, 6 equiv; DMAP, CH_2Cl_2, room temperature overnight. (b) 80% AcOH, reflux, 2 h. (c) BzCl, 8 equiv; Et_3N, 8 equiv; as for (a).

drawbacks of these methods are the low yield of starting materials and the multiple protection steps required in the procedures. These two factors led to a low yield of **13** in the synthesis. A new possibility was explored from readily available **1**. It could be selectively protected at the C-1 hydroxyl group by bulky acylating or silylating reagents[5a]. Independently, in our experiments, selective protection was attempted by direct acylation. Thus, benzoyl chloride, benzoic anhydride, acetyl chloride, and acetic anhydride were used as acylating reagents and the reaction was performed in such solvents as pyridine, DMF, and DMA at different temperatures (Scheme 4). Treatment of **1** with benzoyl chloride (2 equiv) in pyridine at room temperature mainly gave the 1-*O*-benzoylated product (47%). In contrast, when acetic anhydride (6 equiv) was used as acylating reagent in DMA at room temperature in the presence of powdered 4A molecular sieve, the 6- and 5-mono-*O*-acetylated products were obtained in ca. 1:1 ratio. The 6-acetylated product, whose hydroxyl groups at C-2, 3, and 6 positions are properly protected, is a key intermediate for the synthesis of inositol 1,4,5-trisphosphate. Thus the protection was accomplished in one step. No satisfactory results were obtained with benzoic anhydride and acetyl chloride.



Scheme 4. (a) BzCl, 2 equiv; pyridine. (b) AC_2O, 6 equiv; 4A molecular sieves, DMA.



Scheme 5. (a) Ac_2O, 4A molecular sieves, DMA. (b) ref 10. (c) H_2 Pd–C, H_2O–MeOH. (d) Concd NH_4OH.

The synthesis of **13** starting from optically pure D-**1** is shown in Scheme 5. The 6- and 5-acetylated products (**8** and **9**) were isolated as a mixture in a total yield of 74%. The mixture was subjected to phosphorylation using the method developed in this laboratory[15] to give the phosphorylated products **10** and **11**, which were separated by chromatography on silica gel. The yields of **10** and **11** were 41 and 48%, respectively, from the mixture of **8** and **9**. Removal of both *o*-xylylene and cyclohexylidene groups of **10** by catalytic hydrogenolysis, followed by hydrolysis of the acetyl group in concentrated ammonia solution at room temperature, and purification by cellulose chromatography afforded **13** quantitatively from **10**. Thus **13** was synthesized in an overall yield of 30% from D-**1**. Based on the yield of the starting material[4], the overall yield of **13** from *myo*-inositol was greater than 13%.

We have thus described the kinetic resolution of racemic **1** by enzymatic esterification in an organic solvent and synthesis of D-*myo*-inositol 1,4,5-trisphosphate from the optically pure D-**1**. The short steps in the procedure, along with the ready availability of the starting material provides a new and practical route for the synthesis of our target product. The application of the optically active materials for the synthesis of naturally occurring products is under investigation.

EXPERIMENTAL

General methods.—All solvents and reagents used were reagent grade, and where further purification was required, standard procedures were followed[16]. Thin-layer chromatograms (TLC) utilized precoated Silica Gel 60-F254 plates (E. Merck, Darmstadt). Silica gel (Wakogel C-300, 300–200 mesh) was used for silica gel chromatography, and the ratio of gel to compound was in the range of

30:1–100:1. Organic solvents were removed on a rotary evaporator under the vacuum of a water aspirator with a bath temperature of 40°C or less. Elemental analyses were performed by the Advanced Center for the Chemical Analysis of Ehime University. Nuclear magnetic resonance (^{1}H NMR and ^{31}P NMR) spectra were recorded at 270 MHz (Jeol GSX-270) with Me_4Si (δ 0 in $CDCl_3$) as the internal standard for ^{1}H NMR. IR spectra were recorded on a Hitachi EPI G-3 spectrometer. Specific rotations were measured on a Union PM-101 digital polarimeter in a 1-cm cell. The melting points were recorded on a Yanaco melting point apparatus and are uncorrected. High performance liquid chromatography (HPLC) was performed on a Shimadzu chromatography system with a Chiralcel OD column.

*Typical procedure for enzymatic resolution of racemic 2,3-O-cyclohexylidene-*myo*-inositol* (**1**).—To a solution of **1** (29.5 mg, 0.114 mmol) in anhyd 1,4-dioxane (3 mL) were added successively enzyme powder (Amano Lipase CES, 283.4 mg) and Ac_2O (65 μL, 0.690 mmol). The suspension was stirred for 46 h at room temperature. The enzyme was filtered and washed with MeOH. After air-drying, the enzyme was kept for reuse. The mixed filtrate was evaporated under diminished pressure by aspirator to dryness and residue was chromatographed (silica gel, 20:10:1 EtOAc–CH_2Cl_2–MeOH) to give L-1-*O*-acetyl-2,3-*O*-cyclohexylidene-*myo*-inositol (L-**2**) (16.8 mg, 49%) and D-2,3-*O*-cyclohexylidene-*myo*-inositol (D-**1**) (14.5 mg, 49%).

L-**2**: R_f 0.29 (20:10:3 EtOAc–CH_2Cl_2–MeOH); mp 150–152°C (from CH_2Cl_2); $[\alpha]_D^{21}$ +28.6° (*c* 1.75, MeOH, 98% ee); IR (Nujol) 3400, 1740, 1240, 1160, 1100, 1040, 940, 860, 770 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.30–1.78 (m, 10 H, cyclohexylidene), 2.18 (s, 3 H, acetyl), 3.38 (dd, 1 H, $J_{4,5}$ 10.1, $J_{5,6}$ 9.8 Hz, H-5), 3.62 (dd, 1 H, $J_{3,4}$ 7.3 Hz, H-4), 3.78 (dd, 1 H, $J_{1,6}$ 9.8 Hz, H-6), 4.05 (dd, 1 H, $J_{2,3}$ 4.4 Hz, H-3), 4.42 (dd, 1 H, $J_{1,2}$ 4.4 Hz, H-2), and 5.03 (dd, 1 H, H-1). Anal. Calcd for $C_{14}H_{22}O_7$: C, 55.61; H, 7.35. Found: C, 55.77; H, 7.61.

D-**1**: R_f 0.10 (20:10:3 EtOAc–CH_2Cl_2–MeOH); mp 188–189°C (from MeOH); $[\alpha]_D^{21}$ +36.9° (*c* 1.30, MeOH); (lit.[8] $[\alpha]_D^{20}$ +42.4° (*c* 0.33, EtOH); mp 172–174°C); ^{1}H NMR (CD_3OD): δ 1.35–1.78 (m, 10 H, cyclohexylidene), 3.11 (dd, 1 H, $J_{4,5}$ 10.1, $J_{5,6}$ 9.2 Hz, H-5), 3.52 (dd, 1 H, $J_{3,4}$ 7.3 Hz, H-4), 3.58 (dd, 1 H, $J_{1,6}$ 9.5, H-6), 3.66 (dd, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 3.92 (dd, 1 H, $J_{2,3}$ 4.9 Hz, H-3), and 4.36 (dd, 1 H, H-2).

When 1.08 g of racemic **1** was resolved in the same way, 593.2 mg of L-**2** (47%, 96% ee) and 434.1 mg of D-**1** (40%, 100% ee) were obtained.

*1-O-Acetyl-4,5,6-tri-O-benzoyl-2,3-O-cyclohexylidene-*myo*-inositol* (**3**).—To a solution of L-**2** (30.0 mg, 0.100 mmol) in 3 mL CH_2Cl_2 were added successively a catalytic amount of 4-dimethylaminopyridine, Et_3N (90 μL, 0.647 mmol), and BzCl (75 μL, 0.646 mmol). The mixture was stirred overnight at room temperature and the reaction was quenched by adding water. The aqueous layer was extracted with CH_2Cl_2. The combined organic layer was washed with satd $NaHCO_3$, brine, and dried over anhyd Na_2SO_4. After the solvent was evaporated, the residue was

subjected to silica gel chromatography (1:3 EtOAc–hexane) to give **3** (59.0 mg, 96%); R_f 0.26 (1:3 EtOAc–hexane); mp 190–191°C (from EtOAc); $[\alpha]_D^{21}$ −3.4° (*c* 2.95, $CHCl_3$, 98% ee); IR (Nujol) 1730, 1250, 1150, 1090, 900 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.50–1.80 (m, 10 H, cyclohexylidene), 2.04 (s, 3 H, acetyl), 4.50 (dd, 1 H, $J_{3,4}$ 7.0, $J_{2,3}$ 5.2 Hz, H-3), 4.67 (dd, 1 H, $J_{1,2}$ 4.0 Hz, H-2), 4.58 (dd, 1 H, $J_{1,6}$ 10.1 Hz, H-1), 5.61 (t, 1 H, $J_{4,5} = J_{5,6} = 9.2$ Hz, H-5), 5.81 (dd, 1 H, H-4), 6.04 (dd, 1 H, H-6), and 7.24–7.98 (m, 15 H, aromatic).

*1-O-Acetyl-4,5,6-tri-O-benzoyl-*myo*-inositol* (**4**).—Compound **3** (28.0 mg, 0.046 mmol) was suspended in 80% AcOH (10 mL) and was heated to reflux for 4 h. The mixture was dissolved in 10 mL of EtOAc and washed successively with water, satd $NaHCO_3$, and brine. The organic layer was dried over anhyd Na_2SO_4. The solvent was evaporated and the residue was chromatographed (silica gel, 2:1 EtOAc–hexane) to give **4** (18.7 mg, 77%); R_f 0.45 (2:1 EtOAc–hexane); mp 103–104°C (from EtOAc); $[\alpha]_D^{19}$ −21.3° (*c* 1.69, $CHCl_3$, 98% ee); IR (Nujol) 3450, 1720, 1260, 1090, 1020, 970, 900 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.00 (s, 3 H, acetyl), 3.38 (s, 1 H, OH), 3.47 (s, 1 H, OH), 4.04 (dd, 1 H, $J_{3,4}$ 10.1, $J_{2,3}$ 2.8 Hz, H-3), 4.41 (t, 1 H, $J_{1,2}$ 2.8 Hz, H-2), 5.31 (dd, 1 H, $J_{1,6}$ 10.1 Hz, H-1), 5.77 (t, 1 H, $J_{4,5} = J_{5,6} = 10.1$ Hz, H-5), 5.84 (dd, 1 H, H-4), 6.11 (t, 1 H, H-6), and 7.23–7.96 (m, 15 H, aromatic). Anal. Calcd for $C_{29}H_{26}O_{10}$: C, 65.16; H, 4.91%. Found: C, 65.19; H, 5.18%.

*1,4,5,6-Tetra-O-benzoyl-2,3-O-cyclohexylidene-*myo*-inositol* (**5**).—To a suspension of D-**1** (43.0 mg, 0.154 mmol) in 4 mL CH_2Cl_2 were added successively a catalytic amount of 4-dimethylaminopyridine, Et_3N (175 μL, 1.258 mmol), and BzCl (145 μL, 1.250 mmol). The mixture was stirred for 6 h at room temperature. The reaction was quenched by adding water. The aqueous layer was extracted with CH_2Cl_2. The combined organic layer was washed with satd $NaHCO_3$, brine, and dried over anhyd Na_2SO_4. After the solvent was evaporated, the residue was chromatographed (5:1 $CHCl_3$–hexane) to give **5** as crystals (102.1 mg, 98%); R_f 0.17 (5:1 $CHCl_3$–hexane); mp 242–243°C (from EtOAc); $[\alpha]_D^{21}$ −24.7° (*c* 2.19, $CHCl_3$, 94% ee); (physical data of antipode, lit.[17] $[\alpha]_D^{26}$ +29.3°, *c* 3.00, $CHCl_3$; mp 239–239.5°C); IR (Nujol) 1700, 1250, 1080, 1050, 680 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.22–1.80 (m, 10 H, cyclohexylidene), 4.57 (dd, 1 H, $J_{3,4}$ 7.2, $J_{2,3}$ 5.8 Hz, H-3), 4.83 (dd, 1 H, $J_{1,2}$ 4.0 Hz, H-2), 5.70 (t, 1H, $J_{5,6} = J_{4,5} = 9.0$ Hz, H-5), 5.73 (dd, 1 H, $J_{1,6}$ 9.9, H-1), 5.90 (dd, 1 H, H-4), 6.20 (dd, 1 H, H-6), 7.20–7.58 (m, 12 H, aromatic), and 7.80–8.08 (m, 8 H, aromatic).

*1,4,5,6-Tetra-O-benzoyl-*myo*-inositol* (**6**).—A suspension of **5** (70.0 mg, 0.104 mmol) in 80% AcOH (5 mL) was heated to reflux for 2 h. The mixture was dissolved in 10 mL EtOAc and washed successively with water, satd $NaHCO_3$, and brine. After the organic layer was dried over anhyd Na_2SO_4, the solvent was evaporated and the residue was chromatographed (silica gel, 1:2 EtOAc–hexane) to give **6** as crystals (60.6 mg, 98%); R_f 0.10 (1:2 EtOAc–hexane); mp 210–212°C (from EtOAc); $[\alpha]_D^{22}$ −30.6° (*c* 1.57, EtOAc, 94% ee); $[\alpha]_D^{20}$ −16.6° (*c* 1.57, $CHCl_3$, 94% ee); (physical data of antipode, lit.[17] $[\alpha]_D^{18}$ +19.8°, *c* 1.01, $CHCl_3$; mp 226–227°C); IR (Nujol) 3400, 1720, 1260, 1160, 1080, 1060, 1020 cm^{-1}; ^1H NMR

($CDCl_3$): δ 3.35 (s, 1 H, OH), 3.43 (d, 1 H, *J* 8.2 Hz, OH[C-3]), 4.13 (ddd, 1 H, $J_{3,4}$ 9.8, $J_{2,3}$ 2.4, $J_{H,OH}$ 8.2 Hz, H-3), 4.60 (m, 1 H, H-2), 5.47 (dd, 1 H, $J_{1,6}$ 10.4, $J_{1,2}$ 2.4 Hz, H-1), 5.86 (t, 1 H, $J_{4,5} = J_{5,6} = 9.8$ Hz, H-5), 5.94 (t, 1 H, H-4), 6.33 (dd, 1 H, H-6), and 7.23–8.00 (m, 20 H, aromatic). Anal. Calcd for $C_{34}H_{28}O_{10}$: C, 68.44; H, 4.74%. Found: C, 68.32; H, 4.94%.

*Racemic 1-O-benzoyl-2,3-O-cyclohexylidene-*myo*-inositol* (**7**).—To a solution of racemic **1** (54.0 mg, 0.208 mmol) in anhyd pyridine (2 mL) was added BzCl (48 μL, 0.414 mmol). The mixture was stirred at room temperature overnight, and was quenched by adding water. After the solvent was evaporated to dryness, the residue was subjected to silica gel chromatography (20:10:1 EtOAc–CH_2Cl_2–MeOH) to give **7** (35.5 mg, 47%); R_f 0.26 (ethyl (20:10:1 EtOAc–CH_2Cl_2–MeOH); mp 196–197°C (from MeOH); (lit.[17], mp 198–200°C for L-**7**); ^1H NMR (19:1 $CDCl_3$–CD_3OD): δ 1.23–1.80 (m, 10 H, cyclohexylidene), 3.40 (dd, 1 H, $J_{4,5}$ 10.1, $J_{5,6}$ 9.2 Hz, H-5), 3.70 (dd, 1 H, $J_{3,4}$ 7.3 Hz, H-4), 4.01 (t, 1 H, $J_{1,6}$ 9.2 Hz, H-6), 4.10 (dd, 1 H, $J_{2,3}$ 5.2 Hz, H-3), 4.54 (t, 1 H, $J_{1,2}$ 5.2 Hz, H-2), 5.24 (dd, 1 H, H-1), and 7.46–8.12 (m, 5 H, aromatic).

D-6-O-*Acetyl-2,3-*O*-cyclohexylidene-*myo*-inositol* (**8**) *and* D-*5-*O*-acetyl-2,3-*O*-cyclohexylidene-*myo*-inositol* (**9**).—To a solution of optically pure D-**1** (65.4 mg, 0.252 mmol) in 3 mL anhyd DMA were added powdered 4A molecular sieves (97.6 mg) and Ac_2O (240 μL, 2.546 mmol). The mixture was stirred at room temperature for 84 h. The molecular sieves were filtered off. After the solvent was evaporated, the residue was chromatographed (silica gel, 20:10:1 EtOAc–CH_2Cl_2–MeOH) to give **8** and **9** as a mixture (56.3 mg, 74%). Anal. Calcd for $C_{14}H_{22}O_7$: C, 55.61; H, 7.35%, Found: C, 55.27; H, 7.30%.

Compounds **8** and **9** were further purified by recrystallization from $CHCl_3$.

Compound **8**: R_f 0.31 (20:10:3 EtOAc–CH_2Cl_2–MeOH); mp 159–160°C (from $CHCl_3$–hexane); $[\alpha]_D^{21}$ + 21.9° (*c* 1.28, MeOH); IR (Nujol) 3450, 3350, 1700, 1240, 1200, 1150, 1100, 1080, 1020, 980, 900, 830, 700 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.18–1.80 (m, 10 H, cyclohexylidene), 2.08 (s, 3 H, acetyl), 3.42 (dd, 1 H, $J_{4,5}$ 8.9, $J_{5,6}$ 8.2 Hz, H-5), 3.86 (dd, 1 H, $J_{3,4}$ 7.8 Hz, H-4), 3.88 (dd, 1 H, $J_{1,6}$ 8.2, $J_{1,2}$ 4.4 Hz, H-1), 4.07 (dd, 1 H, $J_{2,3}$ 5.3 Hz, H-3), 4.42 (dd, 1 H, H-2), and 5.06 (t, 1 H, H-6).

Compound **9**: R_f 0.31 (20:10:3 EtOAc–CH_2Cl_2–MeOH); mp 171–173.5°C (from $CHCl_3$); $[\alpha]_D^{21}$ + 35.4° (*c* 1.32, MeOH); ^1H NMR ($CDCl_3$ + CD_3OD): δ 1.24–1.70 (m, 10 H, cyclohexylidene), 2.08 (s, 3 H, acetyl), 3.63 (dd, 1 H, $J_{4,5}$ 9.8, $J_{3,4}$ 7.0 Hz, H-4), 3.72 (m, 2 H, H-1, H-6), 4.00 (dd, 1 H, $J_{2,3}$ 5.2 Hz, H-3), 4.35 (m, 1 H, H-2), and 4.64 (m, 1 H, H-5).

D-*6-O-Acetyl-2,3-*O*-cyclohexylidene-1,4,5-tri-*O*-(o-xylene-α,α′-diyldioxyphosphoryl)-*myo*-inositol* (**10**) *and* D-*5-*O*-acetyl-2,3-*O*-cyclohexylidene-1,4,6-tri-*O*-(o-xylene-α,α′-diyldioxyphosphoryl)-*myo*-inositol* (**11**).—To a suspention of the mixture of **8** and **9** (45.3 mg, 0.150 mmol) in 4 mL CH_2Cl_2 were added *1H*-tetrazole (98.3 mg, 1.405 mmol) and o-xylene-α,α′-diyl *N,N*-diethylphosphoramidite (171.9 mg, 0.719 mmol) at room temperature. The suspension was stirred at room temperature for 1 h. Ion-exchanged water (200 μL, 11.1 mmol) was added and

stirring was continued for 15 min. The mixture was allowed to cool at −40°C and *m*-chloroperoxybenzoic acid (218.9 mg, 1.269 mmol) was added. The stirring was continued at room temperature for 30 min. The aqueous layer was extracted with EtOAc. The combined organic layer was washed with 10% Na_2SO_3, satd $NaHCO_3$, brine, and dried over anhyd Na_2SO_4. After the solvent was evaporated, the residue was chromatographed (silica gel, 1:4 acetone–$CHCl_3$) to afford **10** (51.8 mg, 41%) and **11** (60.6 mg, 48%).

Compound **10**: R_f 0.21 (4:1 $CHCl_3$–acetone); mp 126–128°C (from EtOAc); $[\alpha]_D^{19}$ −20.0° (*c* 2.30, $CHCl_3$); IR (Nujol) 1740, 1280, 1205, 1160, 1010, 930, 820, 760 cm^{-1}, ^1H NMR ($CDCl_3$), δ 1.43-1.92 (m, 10 H, cyclohexylidene), 2.26 (s, 3 H, acetyl), 4.30 (dd, 1 H, $J_{3,4}$ 7.3, $J_{2,3}$ 4.6 Hz, H-3), 4.69 (t, 1 H, $J_{1,2}$ 4.6 Hz, H-2), 4.75 (ddd, 1 H, $J_{5,6} = J_{4,5} = J_{H,P} = 9.8$ Hz, H-5), 4.86–5.60 (m, 14 H, methylene, H-1 and H-4 of inositol), 5.71 (t, 1 H, $J_{1,6}$ 9.8 Hz, H-6), and 7.26–7.39 (m, 12 H, aromatic); ^{31}P NMR ($CDCl_3$, external H_3PO_4): −1.91, −2.40, and −3.48. Anal. Calcd for $C_{38}H_{43}O_{16}P_3$: C, 53.77; H, 5.12%. Found: C, 53.45, H, 5.42%.

Compound **11**: R_f 0.38 (4:1 $CHCl_3$–acetone); $[\alpha]_D^{19}$ −30.7° (*c* 3.03, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.41–1.84 (m, 10 H, cyclohexylidene), 2.25 (s, 3 H, acetyl), 4.31 (dd, 1 H, $J_{3,4}$ 7.2, $J_{2,3}$ 4.7 Hz, H-3), 4.74–5.71 (m, 17 H, methylene and inositol), 5.71 (t, 1 H, $J_{1,6} = J_{5,6} = 9{,}8$ Hz, H-6), and 7.24–7.42 (m, 12 H, aromatic); ^{31}P NMR ($CDCl_3$, external H_3PO_4): 0.81, −1.11, and −3.04.

D-myo-*Inositol 1,4,5-triphosphate* (**13**).—To a suspension of **10** (46.0 mg, 0.054 mmol) in 4:1 MeOH–water was added 5% Pd–C (58.6 mg) at 0°C. The suspension was stirred at room temperature under H_2 overnight. The catalyst (Pd–C) was filtered off and the filtrate was evaporated to give crude **12**, which was dissolved in 5 mL concd NH_3 and the mixture was allowed to stir at room temperature overnight. After the NH_3 solution was evaporated off the remaining solid was purified by cellulose chromatography (cellulose powder, Whatman CC-41, 5:4:1 PrOH–NH_4OH–H_2O) to give **13** as its ammonium salt, which was treated with an H^+ cation-exchange column (Diaion SK 1B) to give the free acid, followed by transforming it into its pyridinium salt by adding some drops of pyridine into the free acid solution and evaporating to dryness. Finally, **13** was obtained as its sodium salt by passing the pyridinium salt through a Na^+ cation-exchange column (Dowex). After the solvent was evaporated and the residue was dried under vacuum, **13** was obtained as crystals (26.8 mg, quantitatively from **10**); R_f 0.29 (5:4:1 PrOH–NH_4OH–H_2O); mp > 270°C (from MeOH–water); $[\alpha]_D^{25}$ (ammonium salt) −9.4° (*c* 1.30, H_2O); (lit.[9b] $[a]_D^{23}$ −10.3°, *c* 1.80, H_2O); ^1H NMR (D_2O): δ 3.56 (dd, 1 H), 3.77 (dd, 1 H), 3.80–3.91 (m, 2 H), 4.05–4.18 (m, 2 H); ^{31}P NMR (D_2O, external H_3PO_4): 5.42, 4.52, and 3.82.

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