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Note

A convenient chemoenzymatic synthesis of D-and L-*myo*-inositol 1,4,5,6-tetrakisphosphate

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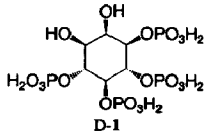
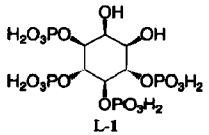
Previously we reported a practical procedure for the preparation of optically active D- and L-1,2-*O*-cyclohexylidene-*myo*-inositol by enzymatic esterification in an anhydrous organic solvent [1]. Now we report the use of both optically active materials for a convenient synthesis of D- and L-*myo*-inositol 1,4,5,6-tetrakisphosphate (D-**1** and L-**1**) [2]. The former was recently prepared by Bruzik and co-workers [3].



D-1



L-1

As previously reported, Lipase CES (a lipase from *Pseudomonas sp.*)-catalyzed esterification of racemic 1,2-*O*-cyclohexylidene-*myo*-inositol gave D-3-*O*-acetyl-1,2-*O*-cyclohexylidene-*myo*-inositol (D-**3**) and L-1,2-*O*-cyclohexylidene-*myo*-inositol (L-**2**). The acetyl group of D-**3** was hydrolyzed in concentrated aqueous ammonia solution to give D-**2** (Scheme 1), D-**2** and L-**2** were used for synthesis of L-**1** and D-**1**, respectively.

The synthesis of D-**1** from optically pure L-**2** is shown in Scheme 2. The phosphitylation of L-**2** proceeded smoothly by reacting with dibenzyl *N*,*N*-diisopropylphosphoramidite in the presence of 1*H*-tetrazole followed by oxidation to give fully protected D-*myo*-inositol 1,4,5,6-tetrakisphosphate (D-**4**). Debenzylation of the phosphoric ester was performed by catalytic hydrogenolysis. Hydrolysis of the cyclohexylidene group was catalyzed by acidic phosphate liberated during hydrogenolysis. After being purified by cellulose chromatography (5:4:1 PrOH–aq NH_3–H_2O) and on an ion-exchange column, the final product (D-**1**) was obtained

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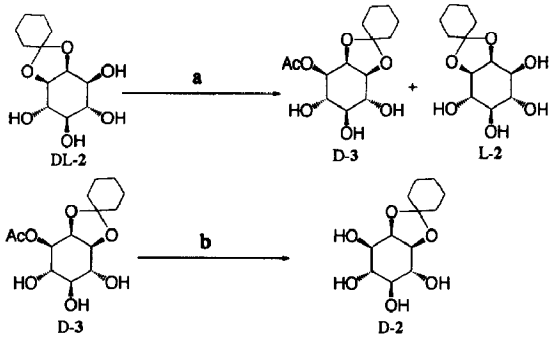


Scheme 1. a, Enzyme, Ac_2O, 1,4-dioxane. b, Concentrated aq NH_3.

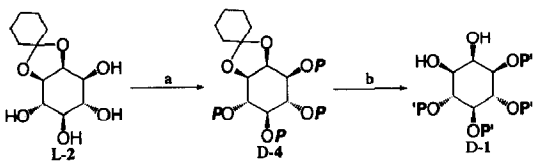
as its sodium salt. The product gave satisfactory NMR spectra in accord with reported ones. The L-enantiomer was obtained from D-**2** in the same way.

The absolute configurations of our starting materials (D-and L-**2**) were determined by comparing their specific rotations with reported values. The configuration was further confirmed by transforming L-**2** into L-3,4,5,6-*O*-tetrabenzoyl-1,2-*O*-cyclohexylidene-*myo*-inositol and comparing its specific rotation with that of D-3,4,5,6-*O*-tetrakisbenzoyl-1,2-*O*-cyclohexylidene-*myo*-inositol derived from L-quebrachitol [1]. The absolute configurations of our final products were attributed without doubt, based on the configurations of the starting materials.

This report provides a short practical route for the synthesis of both enantiomers of *myo*-inositol 1,4,5,6-tetrakisphosphate. For the L-enantiomer, this is the first synthetic report.

1. Experimental

General methods.—All solvents and reagents used were reagent grade. Where further purification was required, standard procedures were followed [4]. Thin-layer chromatography (TLC) were performed on precoated plates of Silica Gel 60-F254 (E. Merck, Darmstadt). Silica gel (300–200 mesh, Wakogel C-300) was used for silica gel chromatography, and the ratio of silica 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 lower. Elemental analyses were performed by the Advanced Center for the Chemical Analysis of



Scheme 2. a, $(BnO)_2PN^iPr_2$, mCPBA. b, H_2, 5% Pd–C. *P*, $(BnO)_2PO$; P', PO_3HNa.

Ehime University. ^1H NMR, ^{13}C NMR, and ^{31}P NMR spectra were recorded at 270.16, 67.94, and 109.36 MHz (Jeol GSX-270), respectively. Tetramethylsilane (δ 0 in $CDCl_3$) was used as the internal standard for ^1H and ^{13}C NMR. Phosphoric acid was used as the external standard for ^{31}P NMR. Specific rotations were measured with 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.

D- *and* L-*1,2*-O-*Cyclohexylidene*-myo-*inositol* (D-**2**, *and* L-**2**).—Compounds L-**2** and D-**3** were obtained by previously reported [1] enzymatic resolution of racemic 1,2-*O*-cyclohexylidene-*myo*-inositol. For hydrolysis of the acetyl group of D-**3**, material D-**3** was dissolved in concentrated NH_4OH and stirred at room temperature for 30 min. The solvent was evaporated and the residue was recrystallized from MeOH to give D-**2**.

L-**2**: R_f 0.10 (20:10:3 EtOAc–CH_2Cl_2–MeOH); mp 188–189° C (from MeOH); $[\alpha]_D^{21}$ +36.9° (*c* 1.3, MeOH, 100% ee [5]); (lit. [6] $[\alpha]_D^{20}$ +42.4°, *c* 0.33, EtOH, mp 172–174° C); ^1H NMR (CD_3OD): δ 1.35–1.78 (m, 10 H, cyclohexylidene), 3.11 (dd, 1 H, $J_{5,6}$ 10.1 Hz, H-5), 3.52 (dd, 1 H, H-6), 3.58 (dd, 1 H, $J_{4,5}$ 9.2 Hz, H-4), 3.66 (dd, 1 H, $J_{3,4}$ 9.5 Hz, H-3), 3.92 (dd, 1 H, $J_{1,6}$ 7.3, $J_{1,2}$ 4.9 Hz, H-1), 4.36 (dd, 1 H, $J_{2,3}$ 4.0 Hz, H-2).

D-**2**: mp 188–190° C (from MeOH); $[\alpha]_D^{22}$ −36.0° (*c* 1.05, MeOH, 96% ee [5]); (lit. [6] $[\alpha]_D^{20}$ −39.4°, *c* 1.55, EtOH, mp 173–174° C).

D- *and* L-*2,3*-O-*Cyclohexylidene-1,4,5,6-tetra*-O-*(bisbenzyloxyphosphoryl)*-myo-*inositol* (D-**4**, *and* L-**4**).—To a suspension of D-**2** (102.1 mg, 0.393 mmol) in 5 mL of CH_2Cl_2 were added 1*H*-tetrazole (334.6 mg, 4.779 mmol) and bisbenzyl *N,N*-diisopropyl-phosphoramidite (838.2 mg, 2.430 mmol) at room temperature. The suspension was stirred at room temperature for 1 h. The mixture was cooled to −40° C and *m*-chloroperoxybenzoic acid (546.3 mg, 3.167 mmol) was added. The stirring was continued at room temperature for 1 h. The aqueous layer was extracted with EtOAc. The combined organic layer was washed with 10% Na_2SO_3, satd $NaHCO_3$ solution, brine, and dried over anhyd Na_2SO_4. The solvent was evaporated and the residue was chromatographed (silica gel, 1:1 EtOAc–hexane) to afford L-**4** (408.2 mg, 80%) as an oil.

D-**4** Was obtained in the same way from L-**2** (72%).

D-**4**: R_f 0.4 (20:10:1 EtOAc–CH_2Cl_2–MeOH); $[\alpha]_D^{23}$ −8.7° (*c* 5.77, CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 1.20–1.80 (m, 10 H, cyclohexylidene), 4.26 (dd, 1 H, *J* 6.1, *J* 5.8 Hz), 4.66 (m, 1 H), 4.76 (m, 2 H), 4.90–5.15 (complex, 18 H), 7.20 (m, 40 H, aromatic), ^{31}P NMR ($CDCl_3$, external H_3PO_4): −0.62, −0.52, −0.46, and 0.06. Anal. Calcd for $C_{68}H_{72}O_{18}P_4$: C, 62.76; H, 5.59%. Found: C, 62.38, H, 5.63%.

L-**4**: $[\alpha]_D^{25}$ +8.7° (*c* 6.80, CH_2Cl_2).

D-myo-*Inositol 1,4,5,6-tetrakisphosphate* (D-**1**).—To a suspension of D-**4** (136.0 mg, 0.105 mmol) in 4:1 MeOH–H_2O was added 5% Pd–C (140.0 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 D-**1**. The crude product was purified by cellulose chromatography (cellulose powder, Whatman CC-41, 5:4:1 PrOH–aq NH_3–H_2O) to give D-**1** as its ammonium salt, which was passed through a H^+-cation-exchange column (Diaion SK 1B) to give the free

acid, followed by transformation into its pyridinium salt by adding some drops of pyridine into the free acid solution and evaporating to dryness. Finally D-**1** was obtained as the sodium salt by passing the pyridinium salt through a Na^+-cation-exchange column (Dowex). The solvent was evaporated and the residue was dried under vacuum to give D-**1** (62.0 mg, quantitative); R_f 0.30 (5:4:1 PrOH–aq NH_3–H_2O); mp > 270° C, $[\alpha]_D^{24}$ −10.2° (*c* 2.46, H_2O, pH 10.7, adjusted by addition of cyclohexylamine); (lit. [3] $[\alpha]_D$ −6.2°, *c* 2.15, H_2O, pH 9.5); ^1H NMR (D_2O, pD 6.8): δ 3.56 (dd, 1 H, $J_{3,4}$ 9.5 Hz, H-3), 3.92 (ddd, 1 H, $J_{1,6} = J_{H,P} = 9.5$, $J_{1,2}$ 2.8 Hz, H-1), 3.94 (ddd, 1 H, $J_{5,6}$-$J_{H,P} = 9.5$ Hz, H-5), 4.05 (dd, 1 H, $J_{2,3}$ 2.8 Hz, H-2), 4.14 (ddd, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 4.25 (ddd, 1 H, H-6); ^{31}P NMR (D_2O, pD 6.8, external H_3PO_4): 0.83 (1P), 1.75 (1P), and 1.85 (2P); ^{13}C NMR (D_2O, pD 7.6): 71.33 (s, 1C), 71.88 (s, 1C), 75.28 (m, 1C), 77.05 (m, 2C), 78.33 (m, 1C). Anal. Calcd for $C_6H_{12}Na_4O_{18}P_4 \cdot H_2O$: C, 11.54; H, 2.59%. Found: C, 11.55, H, 2.63%.

L-**1**: mp > 270° C, $[\alpha]_D^{24}$ +9.8° (*c* 1.43, H_2O, pH 11.1, adjusted by addition of cyclohexylamine).

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