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Enzymatic Resolution of Racemic 1,2:5,6-di-*O*-Cyclohexylidene and 1,2:3,4-di-*O*-Cyclohexylidene-*myo*-Inositol

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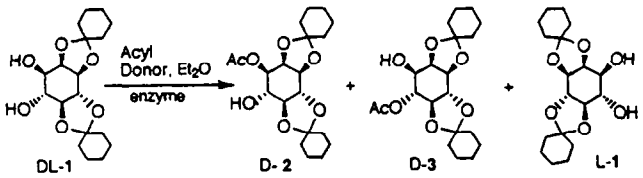
Abstract–Enzyme-catalyzed regio- and enantioselective esterification of racemic 1,2:5,6-di-*O*cyclohexylidene- and 1,2:3,4-di-*O*-cyclohexylidene-*myo*-inositol, which are key intermediates for syntheses of various naturally occurring *myo*-inositol phosphate derivatives, proceeded exclusively in organic solvent to give optically pure materials and selectively protected products in gram scale. Hydrolysis of mono-*O*-acetates of the corresponding racemic materials catalyzed by the same enzymes yielded complementary products. The present study provides a new and efficient method for obtaining optically pure *myo*-inositol derivatives.

Introduction

D-*myo*-Inositol 1,4,5-trisphosphate, which is derived from receptor-activated phospholipase C hydrolysis of phosphatidylinositol 4,5-bisphosphate, has been found to be a second messenger, which successively metabolizes to a number of inositol phosphate derivatives in cellular signaling system.[1] In order to clarify the individual function of these phosphates involved as metabolites in this system, chemical synthesis becomes imperative. One of the biggest problems in the synthesis of these phosphates is efficiently providing optically pure inositol intermediates.[2] Although some chiral synthesis of *myo*-inositol derivatives starts from other chiral materials,[3] *myo*-inositol derivatives are usually employed, which rely on the cumbersome procedures of conversion of the racemic inositol derivatives into a pair of diastereomeric isomers, followed by separation of the diastereomers.[4] Another method is the use of chiral HPLC column to separate individual enantiomer in small amount.[5] Enzyme catalyzed enantioselective hydrolysis was also used to resolve racemic inositol derivatives[6] or to yield optically active intermediate from *meso* inositol derivatives.[7] As part of our study on chemical synthesis of biologically active *myo*-inositol phosphate derivatives, we have succeeded in kinetic resolution of racemic 1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol (**1**) and 1,2:3,4-di-*O*-cyclohexylidene-*myo*-inositol (**4**) by enzyme-catalyzed selective esterification in organic solvent and hydrolysis.[8] In this paper, we would like to give a description in detail.

Enzymatic Esterification

Enzyme catalyzed selective esterification in organic solvent has been recently developed as a new method for obtaining optically active materials and proved to be a useful method for resolution of hydroxyl group containing compounds.[9] In our research, two important intermediates for synthesizing inositol phosphate derivatives,[4e,6a,10,11] racemic **1** and **4**, which are readily available by acetalization of *myo*-inositol with 1-ethoxycyclohexene in a single step,[10] were successfully resolved by the application of this method. The kinetic resolution of DL-**1** is shown in Scheme I. Acetic anhydride and some active acetates were used as acyl donors. Among several commercially available hydrolytic enzymes, a lipase from *Pseudomonas sp.* (Amano Lipase P) and a lipase from *Candida cyclindracea* (Amano Lipase AY) were active for acetylation of racemic **1**. The results are listed in Table 1. Lipase AY acetylated the hydroxyl group exclusively at C-4 position of D-enantiomer to give optically active material L-**1** and selectively protected product D-4-*O*-acetyl-1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol(D-**3**). Complete resolution was achieved when acetic anhydride was used. Phenyl and nitrophenyl acetates, which have not been commonly used as acyl donors for enzymatic esterification, also exhibited high activities for the reaction. Lipase P preferably acetylated the hydroxyl group at C-3 position of the same enantiomer. Optically pure D-3-acetylated 1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol (D-**2**) was obtained. Amano Lipase G, Lipase A, and PLE-A showed no activity for the resolution.

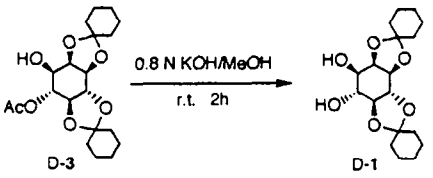


Scheme I.

Table 1. Enzymatic esterification of racemic **1**

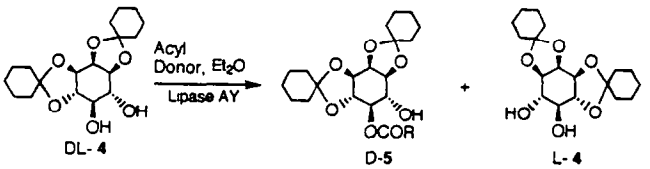
Lipase	Acyl Donor	Reaction Time	yield (e.e.) D-2	D-3	L-1
P	Ac_2O	23 h	25% (100%)		73% (36%)
P	$AcOCH_2CCl_3$	3 days	trace		
AY	$AcOCH_2CCl_3$	18 h		29% (100%)	63% (58%)
AY	$AcOCH{=}CH_2$	17 h		23% (100%)	70% (52%)
AY	$AcOC(CH_3){=}CH_2$	19 h		15% (100%)	67% (44%)
AY	AcOPh	19 h		38% (100%)	53% (92%)
AY	AcO-C6H4-NO2	17 h		40% (100%)	55% (84%)
AY	Ac_2O	16 h		48% (100%)	51% (98%)

The optical purities of the products were determined by HPLC analysis with the use of chiral column. It was found that the D- and L-enantiomers of racemic **1** were readily separated by the column of Chiralcel OD[12] (isopropanol/hexane=1:25). The retention time of D- and L-enantiomer was 15.0 and 19.0 min respectively (flow rate of eluent 0.8 ml/min). So the optical purities of the products were conveniently analyzed after the acetates were hydrolyzed (Scheme II). The absolute configuration was confirmed by comparing the specific rotation of L-**1** with that of the known L-1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol[6a] and referring to HPLC chart.



Scheme II.

The resolution of racemic **4** is shown in Scheme III. Some acid anhydrides with different chain length such as acetic anhydride, propionic anhydride, and hexanoic anhydride were used as acyl donors. Among the enzymes mentioned above, only Lipase AY was active for this reaction. It catalyzed the acylation of the hydroxyl group at C-5 position of D-enantiomer selectively to give optically pure D-5-*O*-acylated-1,2:3,4-di-*O*-cyclohexylidene-*myo*-inositol (D-**5**) and material L-**4**. Results are listed in Table 2. The optical purities of the products were determined by using the same HPLC column as above (isopropanol/-hexane=1:9). The retention time of L-**4** and D-**4** was 9.0 and 13.3 min respectively (flow rate of eluent 0.6 ml/min). The absolute configuration of the products was also determined by comparing the corresponding specific rotation with that of the known D- and L-1,2:3,4-di-*O*-cyclohexylidene-*myo*-inositol.[13]



Scheme III.

Table 2. Enzymatic esterification of racemic **4**

Acyl Donor	Reaction Time	Yield (e.e.) D-5	Yield (e.e.) L-4
acetic anhydride	4 h	50% (96%)	48% (100%)
propionic anhydride	24 h	54% (84%)	37% (100%)
hexanoic anhydride	24 h	50% (92%)	43% (100%)

Effect of solvent on enzymatic esterification was studied by carrying out the reaction of racemic **4** with acetic anhydride catalyzed by Lipase AY in different solvents. Results are listed in Table 3. Ethyl ether and benzene were fairly effective solvents for this resolution. Lipase AY lost its activity when water miscible solvents such as acetone, THF, and dioxane were used.[9c,14]

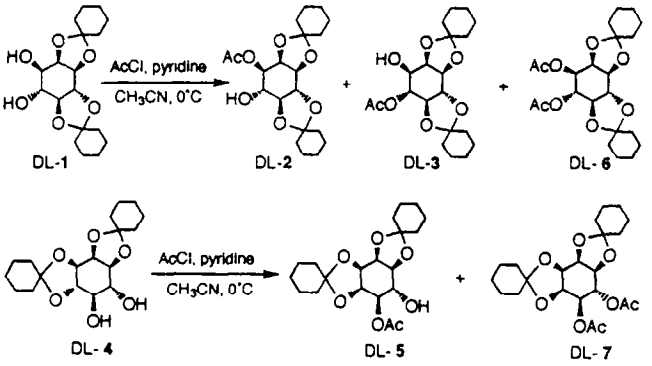
Table 3. Effect of solvent on the resolution

Solvent	Reaction Time	Yield (e.e.) D-5	Yield (e.e.) L-4
Et_2O	4 h	50% (96%)	48% (100%)
Benzene	10.5 h	44% (100%)	54% (88%)
AcOEt	30 h	13% (94%)	86% (18%)
Acetone	2 days	8%*	85% (0)
THF	24 h	No Reaction	
Dioxane	30 h	No Reaction	

Acetylated product was a mixture of 5- and 6-acetylated products, optical purity not determined.

Enzymatic Hydrolysis

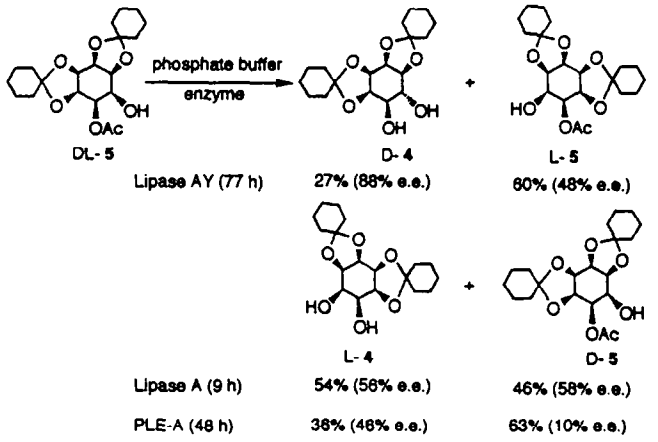
In order to resolve hydroxyl group containing material by enzymatic hydrolysis, the hydroxyl group is first acylated chemically. The preparation of acylated materials of 1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol (DL-**2**) and 1,2:3,4-di-*O*-cyclohexylidene-*myo*-inositol (DL-**5**) was outlined in Scheme IV. The synthesis of racemic **2** was straightforward. The hydroxyl group at C-3 position was predominantly acetylated to give DL-**2** in 51% yield with the recovery of starting material 39%. Only trace amounts of 4-acetylated and diacetylated products (DL-**3** and DL-**6**) were detected with TLC. The acetylation of **4** gave mono-acetylated product DL-**5** in 24% yield and di-acetylated product (DL-**7**) 73%.



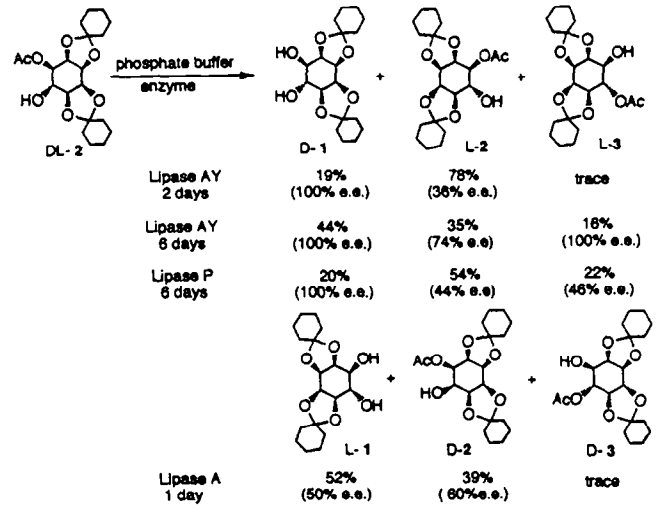
Scheme IV.

The mono-acetate DL-**2** and DL-**5** were subjected to enzyme-catalyzed hydrolysis. Lipase AY catalyzed the hydrolysis of D-enantiomer of racemic **5** which was the same enantiomer as it selected in esterification, to yield complementary products (Scheme V).[9d] The optical purities of the products were modest. Lipase A and PLE-A, both showed no activity in esterification, hydrolyzed L-enantiomer.

Lipase P and Lipase AY hydrolyzed D-enantiomer of racemic **2** exclusively to give optically pure D-**1** (Scheme VI). Some amount of acetyl group migrated product **3** was obtained during hydrolysis. A control experiment in the absence of enzyme was carried out and **3** was also obtained. This suggested that the migration of acetyl group was promoted by phosphate buffer.[15] The hydrolysis of D-**3** by Lipase AY should be much faster than migration. So optically pure L-**3** was obtained.



Scheme V.



Scheme VI.

The kinetic resolution of racemic 1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol (DL-**1**) and racemic 1,2:3,4-di-*O*-cyclohexylidene-*myo*-inositol (DL-**4**) was perfomed by enzyme catalyzed selective esterification in organic solvent and hydrolysis. Selectively protected products D-**2**, L-**2**, D-**3**, L-**3**, D-**5**, and L-**5** were obtained. All of these optically active materials are useful intermediates for synthesis of naturally occurring products. This provides a new and efficient method for resolution and selective protection of inositol derivatives.

Experimental

General procedures

All solvents and reagents used were reagent grade, and in case further purification was required, standard procedures[16] were followed. Thin layer chromatograms (TLC) were performed on precoated silica gel 60-F254 plates (E. Merck, Darmstadt). 300–200 Mesh silica gel (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 bath temperature of 40°C or lower. Elemental analyses were performed by the Advanced Center for the Chemical Analysis of Ehime University. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 270 MHz (JEOL GSX-270) with tetramethylsilane (δ 0 in CDCl3) as an internal standard. IR spectra were recorded on a Hitachi EPI G-3 spectrometer. Specific rotations were determined on a Union PM-101 digital polarimeter in a 1 cm cell. The melting points were recorded on Yanaco melting point apparatus and are uncorrected. High performance liquid chromatography (HPLC) was performed on a Shimazu chromatography system with the column of Chiralcel OD.

*General procedure for enzymatic esterification of racemic 1,2:5,6-di-O-cyclohexylidene-*myo*-inositol (DL-1)*

To a clear solution of racemic **1** (1.063 g, 3.125 mmol) in anhydrous diethyl ether (50 ml) were added enzyme powder (Lipase AY, 2.368 g) and acetic anhydride (1.20 ml, 12.730 mmol). The suspension was stirred at room temperature and the reaction was checked by TLC. Enzyme was filtered off after no more change was detected from TLC. The filtrate was washed successively with saturated $NaHCO_3$ solution and brine, and dried over anhydrous Na_2SO_4. Solvent was evaporated and residue was chromatographed ($Et_2O/CHCl_3$=1/3) to give:

4-*O*-Acetyl-1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol (D-**3**) 601.9 mg, 50%, R_f 0.50 ($Et_2O/CHCl_3$=1/3), m.p. 114–116°C (from Et_2O), $[\alpha]_D^{21}$ -14.0° (c 5.4, methanol), IR (nujol), 3500, 1715, 1210, 1160, 1100, 1080, 1050, 960, 900, 840, and 760 cm^{-1}, ^{1}H NMR ($CDCl_3$), δ 1.10–1.70 (m, 20H, cyclohexylidene), 2.10 (s, 3H, acetyl), 2.20 (s, 1H, OH), 3.50 (dd, 1H, J_{56}=10.7 Hz, J_{54}=8.5 Hz, H-5), 3.79 (t, 1H, J_{32}=J_{34}=3.4 Hz, H-3), 4.10 (dd, 1H, J_{65}=10.7 Hz, J_{61}=7 3 Hz, H-6), 4.28 (t, 1H, J_{12}=J_{16}=7 3 Hz, H-1), 4.35 (dd, 1H, J_{21}=7 3 Hz, J_{23}=3.4 Hz, H-2), and 5.08 (dd, 1H, J_{45}=8.5 Hz, J_{43}=3.4 Hz, H-4); Calcd for $C_{20}H_{30}O_7$: C, 62.80; H, 7.92%; Found: C, 63.22; H, 7.92%.

L-**1** 471.5 mg, 45%, R_f 0.11 ($Et_2O/CHCl_3$=1/3), m.p. 144–146°C (from Et_2O), $[\alpha]_D^{21}$ +19.3° (c 0.75, $CHCl_3$), (lit.[6a] $[\alpha]_D^{20}$ +18.4°, c 1.0, $CHCl_3$), IR (nujol), 3450, 3300, 1260, 1210, 1160, 1130, 1100, 1040, 990, 910, 890, 830, 760, and 740 cm^{-1}, ^{1}H NMR ($CDCl_3$), δ 1.30–1.80 (m, 20H, cyclohexylidene), 2.60 (s, 2H, OH), 3.40 (dd, 1H, J_{56}=10.4 Hz, J_{54}=8.9 Hz, H-5), 3.85 (dd, 1H, J_{32} =4.3 Hz, J_{34}=4.6 Hz, H-3), 3.94 (dd, 1H, J_{61}=7.9 Hz, J_{65}=10.4 Hz, H-6), 4.05 (dd, 1H, J_{45}=8.9 Hz, J_{43}=4.6 Hz, H-4), 4.32 (dd, 1H, J_{12}=6 4 Hz, J_{16}=7 9 Hz, H-l), and 4.48 (dd, 1H, J_{21}=6.4 Hz, J_{23}=4.3 Hz, H-2).

In the case where Lipase P was used for the resolution, DL-**1** 71.5 mg (0.210 mmol), Ac_2O 80 μl (0.849 mmol), Lipase P 140.9 mg. Products were:

3-*O*-Acetyl-1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol (D-**2**) 20.1 mg, 25%, R_f 0.31 ($Et_2O/CHCl_3$=1/3), m.p. 109–111°C (from Et_2O), $[\alpha]_D^{28}$ +16.6 (c 1.05, $CHCl_3$), IR

(nujol) 3450, 1740, 1230, 1170, 1100, 1060, 930, 900, 850, and 830 cm^{-1}, ^1H NMR (CD_3OD), δ 1.25–1.60 (m, 20H, cyclohexylidene), 2.00 (s, 3H, acetyl), 3.37 (dd, 1H, J_{56}=10.7 Hz, J_{54}=8.9 Hz, H-5), 3.72 (dd, 1H, J_{65}=10.7 Hz, J_{61}=8 2 Hz, H-6), 3.81 (dd, 1H, J_{45}=8.9 Hz, J_{43}=5.2' Hz, H-4), 4.25 (dd, 1H, J_{16}=8.2 Hz, J_{12}=6 4 Hz, H-1), 4.44 (dd, 1H, J_{21}=6.4 Hz, J_{23}=4.3 Hz, H-2), and 4.88 (dd, 1H, J_{34}=5.2 Hz, J_{32}=4.3 Hz, H-3); Calcd for $C_{20}H_{30}O_7$: C, 62.80; H, 7.92%; Found: C, 63.08; H, 7.98%.

L-**1** 52.4 mg, 73%.

*Hydrolysis of the acetate of 4-O-acetyl-1,2:5,6-di-O-cyclohexylidene-*myo*-inositol (D-3)*

D-**3** (60.7 mg, 0.159 mmol) was dissolved in 2.5 ml 0.8 N KOH in methanol and stirred at room temperature for 2 h. Diethyl ether was added to the reaction system, then washed with water and brine and dried over anhydrous Na_2SO_4. After solvent was evaporated, residue was subjected to chromatography (SiO_2, $Et_2O/CHCl_3$=1/3) to give D-**1** (47.1 mg, 87%). $[\alpha]_D^{24}$ -22.3° (c 0.74, $CHCl_3$), m.p. 144–145°C (from Et_2O).

The hydrolysis of D-**2** was carried out in the same way.

*Enzymatic esterification of racemic 1,2:3,4-di-O-cyclohexylidene-*myo*-inositol (DL-4)*

The same enzymatic esterification procedure was used for resolution of racemic **4** (41.6 mg, 0.136 mmol) by Lipase AY (95.9 mg) to give D-5-*O*-acetyl-1,2:3,4-di-*O*-cyclohexylidene-*myo*-inositol (D-**5**) (25.9 mg, 50%) and L-1,2:3,4-di-*O*-cyclohexylidene-*myo*-inositol (L-**4**) (22.3 mg, 48%).

D-**5** R_f 0.55 ($Et_2O/CHCl_3$=213), amorphous solid, $[\alpha]_D^{27}$ +1.4° (c 1.44, MeOH, 10 cm cell), IR (nujol) 3450, 1720, 1240, 1160, 1100, 1040, 980, 940, 860, and 780 cm^{-1}, ^1H NMR ($CDCl_3$), δ 1.20–1.80 (m, 20H, cyclohexylidene), 2.00 (s, 3H, acetyl), 3.70 (dd, 1H, J_{32}=3 7 Hz, J_{34}=10.1 Hz, H-3), 3.82 (t, 1H, J_{61}= J_{65}=2 7 Hz, H-6), 4.05 (dd, 1H, J_{43}=10.1 Hz, J_{45}=9.5 Hz, H-4), 4.27 (dd, lH, J_{12}=6.9 Hz, J_{16}=2.7 Hz, H-1), 4.52 (dd, 1H, J_{21}=6.9 Hz, J_{23}=3.7 Hz, H-2), and 4.55 (dd, 1H, J_{54}=9.5 Hz, J_{56}=2.7 Hz, H-5); Calcd for $C_{20}H_{30}O_7$: C, 62.80; H, 7.92%; Found: C, 63.28; H, 8.13%.

L-**4** R_f 0.16 ($Et_2O/CHCl_3$=2/3), m.p. 155–157°C (ether), $[\alpha]_D^{27}$ +5.3° (c 1.17, MeOH, 10 cm cell), (lit.[12] $[\alpha]_D^{20}$ +5.3°, c 0.87, $CHCl_3$, m.p. 153–154°C), IR (nujol) 3450, 3150, 1260, 1160, 1100, 1080, 1040, 1010, 916, 890, 840, and 780 cm^{-1}, ^1H NMR (CD_3OD), δ 1.15–1.65 (m, 20H, cyclohexylidene), 3.55 (dd, 1H, J_{54}=8.9 Hz, J_{56}=5.2 Hz, H-5), 3.62 (dd, 1H, J_{61}=4 9 Hz, J_{65}=5.2 Hz, H-6), 3.67 (dd, 1H, J_{34}=10.1 Hz, J_{32}=3 4 Hz, H-3), 3.84 (dd, 1H, J_{43}=10.1 Hz, J_{45}=8.9 Hz, H-4), 4.14 (dd, 1H, J_{12}=5 8 Hz, J_{16}=4 9 Hz, H-1), and 4.88 (dd, 1H, J_{21}=5.8 Hz, J_{23}=3.4 Hz, H-2).

The hydrolysis of D-**5** was carried out in the same way as D-**3** to give D-**4** quantitatively. m.p. 157–159°C (from ethyl acetate), $[\alpha]_D^{20}$ -5.0 (c 2.0, methanol), (lit.[12] $[\alpha]_D^{20}$ -5.0, c 1.26, $CHCl_3$, m.p. 154–155°C).

*Racemic 3-O-acetyl-1,2:5,6-di-O-cyclohexylide-*myo*-inositol (DL-2)*

To a clear solution of DL-**1** (593.5 mg, 1.743 mmol) in anhydrous acetonitrile (30 ml) were added acetic chloride (136 μl, 1.913 mmol) and pyridine (272 μl, 3.367 mmol). The mixture was stirred at 0°C for 5 h. Reaction was quenched by addition of water. Aqueous layer was extracted with ether. The combined organic layer was washed with saturated $NaHCO_3$ solution and brine and dried over anhydrous Na_2SO_4. After solvent was evaporated, the residue was chromatographed (SiO_2, $Et_2O/CHCl_3$=1/3) to give DL-**2** (342.2 mg, 51%) with the recovery of DL-**1** 233.5 mg, 39%.

*Racemic 5-O-acetyl-1,2:3,4-di-O-cyclohexylidene-*myo*-ino-sitol (DL-5)*

To a solution of racemic **4** (509.3 mg, 1.469 mmol) in anhydrous acetonitrile (30 ml) were added acetic chloride (215 μl, 3.024 mmol) and pyridine (468 μl, 5.794 mmol). The mixture was stirred at 0°C for 30 min. After general work up procedure, 5-*O*-acetyl-1,2:3,4-di-*O*-cyclohexylidene-*myo*-inositol (DL-**5**) (140.0 mg, 24%) and 5,6-di-*O*-acetyl-1,2:3,4-di-*O*-cyclohexylidene-*myo*-inositol (DL-**7**) (463.6 mg, 73%) were obtained.

7 R_f 0.73 ($Et_2O/CHCl_3$=1/3), oil, ^1H NMR ($CDCl_3$), δ 1.30–1.80 (m, 20H, cyclohexylidene), 2.08 (s, 3H, acetyl), 2.11 (s, 3H, acetyl), 3.78 (dd, 1H, J_{34}=10.1 Hz, J_{32}=3.4 Hz, H-3), 4.22 (dd, 1H, J_{43}=10.1 Hz, J_{45}=8.9 Hz, H-4), 4.33 (dd, 1H, J_{12}=6.6 Hz, J_{16}=3 4 Hz, H-1), 4.65 (dd, 1H, J_{21}=6.6 Hz, J_{23}=3 4 Hz, H-2), 5.05 (dd, 1H, J_{56}=3.1 Hz, J_{54}=8.9 Hz, H-5), 5.15 (dd, 1H, J_{61}=3-4 Hz, J_{65}=3.1 Hz, H-6).

Representative procedure of enzyme-catalyzed enantioselective hydrolysis

To a solution of racemic **2** (49.7 mg, 0.130 mmol) in acetone (1 ml) were added phosphate buffer (9 ml, 0.1 M KH_2PO_4/0.1 M Na_2HPO_4=4/6) and Lipase A (30.2 mg). The mixture was stirred at room temperature for one day. Products were extracted with ether. Ether layer was washed with water and brine and dried over anhydrous Na_2SO_4. After solvent was evaporated, the residue was chromatographed to give L-**1** (23.1 mg, 52%) and D-**2** (19.2 mg,39%).

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