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A New Efficient Method for Resolution of *myo*-Inositol Derivatives by Enzyme Catalyzed Regio- and Enantio-selective Esterification in Organic Solvent

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Abstract: *Racemic 1,2 5,6-di-O-cyclohexylidene- and 1,2 3,4-di-O-cyclohexylidene-myo-inositol were resolved by enzyme catalyzed esterification in organic solvent*

D-*myo*-Inositol 1,4,5-triphosphate has been found to be a second messenger and metabolized to other inositol bis-, tris-, tetrakis, and pentakis-phosphate isomers in cellular signalling system [1] In order to clarify the individual function of these phosphates involved as metabolites in this system, chemical supply of them is necessary. One of the biggest problems in the synthesis of these phosphates is efficiently providing optically pure inositol intermediates.[2] Besides the preparation of optically active *myo*-inositol derivatives from other chiral materials,[3] many methods have been reported for this purpose from racemic or meso *myo*-inositol derivatives. Most of them are resolution by using different optically pure reagents,[4] 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. Another is the use of chiral HPLC column to separate individual enantiomer in small amount.[5] Recently enzyme catalyzed selective hydrolysis was used to resolve some racemic or meso inositol carboxylates.[6] In this method , inositol derivatives have to be first acylated and then the carboxylates subjected to enzyme catalyzed hydrolysis to give a desired enantiomer Usually the enantioselectivity is low and further purification such as recrystallization is necessary. 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 successful method for resolution of many kinds of alcohols.[7] But there is no report about the resolution of inositol derivatives by this method yet. Herein, we report the application of this method to obtain optically active inositol derivatives from racemic materials. Racemic 1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol (1) and 1,2:3,4-di-*O*-cyclohexylidene-*myo*-inositol (5) which are important intermediates for synthesizing inositol phosphate derivatives were chosen as substrates and acylated with acid anhydride catalyzed by hydrolytic enzymes in anhydrous organic solvent It has been found that the two enantiomers of racemic 1 and 5 could be separated completely

Scheme 1

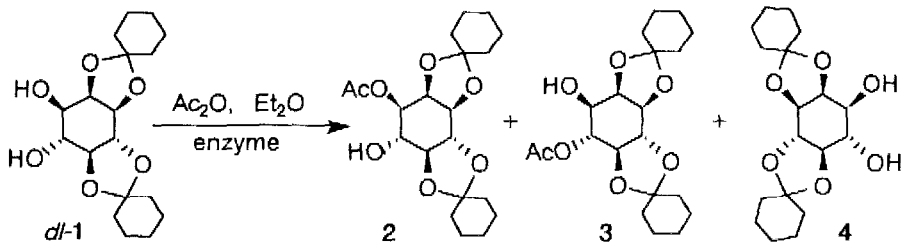

Table 1. The Resolution of Racemic 1,2:5,6-di-*O*-cyclohexylidene Inositol

enzyme	reaction time	Yield (D/L*)		
		2	3	4
Lipase P	23 h	25% (100:0)	trace	73% (32:68)
Lipase AY	16 h	trace	48% (100:0)	51% (1:99)

* The results of acetylated products 2 and 3 were determined by HPLC after the acetates were hydrolyzed.

Among several commercially available hydrolytic enzymes, a lipase from *Pseudomonas sp.* (Amano Lipase P) and a lipase from *Candida cyclindracea* (Amano Lipase AY) exhibited high regio- and enantio-preference in acetylation of racemic **1** (Scheme 1). In a typical experiment, to a solution of **1** (0.24 mmol) in anhydrous ethyl ether (3 ml), 150 mg of enzyme powder and 1 mmol of acetic anhydride were added. This suspension was stirred at room temperature. The reaction was monitored by TLC. The enzyme was filtered when no more reaction proceeded and products were separated by silica gel chromotography (ethyl ether/chloroform=1/3). The optical purity was analyzed by HPLC (Chiralcel OD column, isopropanol/hexane=1/25). The results are listed in Table 1. Lipase AY exclusively acetylates the hydroxyl group at C-4 position of D-enantiomer almost completely (48.4%) to give two optically active inositol intermediates **3** and **4**. The enantiomeric excess of **3** and **4** were 100% and 98% respectively. Lipase P preferably acetylates 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 (**2**) was obtained in the yield of 25% and diol **4** 73% (36% e.e.).

In order to determine optical purity and absolute configuration of **2** and **3**, the acetates were hydrolyzed in 0.5N KOH/MeOH (10 eq. room temperature 2h). After purification by silica gel chromotography, the optical purity of the resulting diol was determined by HPLC. The absolute configuration was confirmed by comparing its specific rotation value with that of the known D-(-)-1,2:5,6-di-O-cyclohexylidene *myo*-inositol and referring to HPLC.[6a, 8]

Racemic **5** could be also resolved in this way (Scheme 2). Lipase AY catalyzed the acylation of D-enantiomer of racemic material at 5-position exclusively to give two optically active inositol derivatives. Different acid anhydrides such as acetic anhydride, propionic anhydride, hexanoic anhydride and lauric anhydride have been used and Lipase AY was active for all of them. Two enantiomers of racemic **5** could be also resolved completely. The optical purity of the products was analyzed by HPLC in the same way as above.

Scheme 2

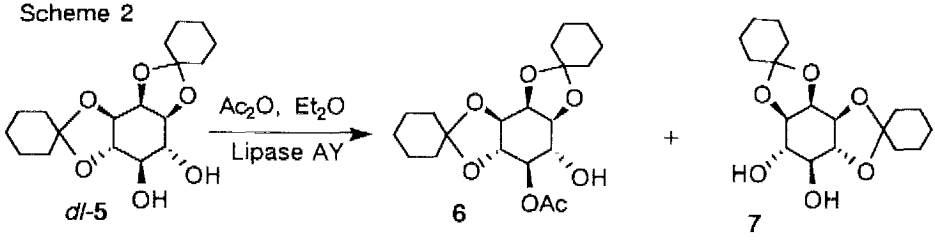

Table 2 The Effect of Solvent on the Resolution

Solvent	Reaction Time	Yield (D/L)	
		6	7
Et_2O	4 h	50% (98 2)	48% (0 100)
Benzene	10.5 h	44% (100 0)	54% (6.94)
AcOEt	30 h	13% (97:3)	86% (41 59)
Acetone	2 days	8%*	85% (50 50)
THF	24 h	No Reaction	
Dioxane	30 h	No Reaction	

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

The absolute configuration of **6** was determined by transforming it to the known D-(-)-1,2-*O*-cyclohexylidene *myo*-inositol and comparing their specific rotation.[8, 9] Then the absolute configuration of 7 was proved to be L-enantiomer by referring to HPLC. Strong solvent effect was observed. Ethyl ether and benzene were very effective solvents for this resolution. Lipase AY lost its activity when water soluble solvents such as acetone, THF, and dioxane were used (Table 2).

The optically pure inositol intermediates we have resolved are very useful for synthesizing D-*myo*-inositol 1,4,5-triphosphate and other different D- and L-inositol phosphate derivatives.[4d, 6a, 10]

In summary, it has been found that enzyme catalyzed esterification in organic solvent is also very convenient and effective for resolving inositol derivatives and optically active *myo*-inositol phosphates can be synthesized from the optically pure materials resolved by this method In addition to racemic 1 and 5, the resolution of other meso and racemic *myo*-inositol derivatives by this method and the effect of solvent on the resolution are under investigation.

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8. The specific rotation $[\alpha]_D^{28}$ of the hydrolyzed product of **2** was -23.9° (c 0 54, MeOH), lit.[7a], $[\alpha]_D^{20}$ -18°, (c 1.0, $CHCl_3$) $[\alpha]_D^{21}$ of 1,2-*O*-cyclohexylidene *myo*-inositol derived from **6** was -31° (c 0.51, EtOH), lit.[9] $[\alpha]_D^{20}$ -39.4°, (c 1 53, EtOH)

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