Studies on the Synthesis of Highly Functionalized Molecules through Chemo-Enzymatic Methodology

March, 2000
Koichi MITSUKURA

The Graduate School of Natural Science and Technology
(Doctor Course)
Okayama University
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Chapter 1.

Thiacrown Ether Additive Effect on Stereo- and Regio-selectivity in the Lipase-catalyzed Reaction; Preparation of Optically Active Hydroxyalkanenitriles
1-1-1. Introduction

Lipases are the most frequently used enzymes in organic synthesis because of their stability, availability and their acceptance of a broad range of substrates. The synthetic value of lipases has been well recognized because their reactions proceed efficiently and selectively under mild conditions. Since only a limited number of lipase-catalyzed reactions are applicable for practical optical resolution, several methods were recommended to improve their reaction performance: optimization of reaction conditions, modification of substrate, selection of non-aqueous media, and use of additive that regulates lipase reactivity. Crown ethers are promising candidates as additives and are known to be as complexing agents for several proteins. Reinhoudt et al. reported that serine proteases were activated by crown ethers, especially by 18-crown-6. Some crown ethers are known to be potential to enhance both the enantioselectivity and reaction rate in the lipase-catalyzed hydrolysis of 2-cyano-1-methylethyl acetate (1a). In particular, 1,4,8,11-tetrathiacyclotetradecane (4) was confirmed as the best additive among 35 types of crown ethers and their acyclic analogues tested. To determine the scope and limitation of this thiacrown ether additive effect in lipase-catalyzed reaction for preparing optically active 3-hydroxyalkanenitriles, ten acetates of hydroxyalkanenitriles were examined as substrates. It was found that the enantioselectivity of this reaction greatly depended on the nature of the substrate when 5 mol% of thiacrown ether 4 was added. Among the tested substrates, this thiacrown ether offered the highest enantioselectivity in the lipase-catalyzed hydrolysis of 1-(cyanomethyl)propyl acetate (1b). This remarkable additive effect was applied to the synthesis of the optically pure attractant pheromone of ant Myrmica scabrinodis (A), (R)-3-octanol (5).

1-1-2. Effect of thiacrown ether additive on PCL-catalyzed hydrolysis of various 3-hydroxyalkanenitriles.

Acetates 1 as model substrates and PCL as the enzyme were chosen, because 3-hydroxyalkanenitriles are useful chiral building blocks, and PCL is applicable to various substrates. Racemic 3-hydroxyalkanenitriles, (±)-2, were prepared through two different pathways, one of which was the reaction of potassium cyanide with oxiranes. Three 3-hydroxyalkanenitriles, 2a, 2b, and 2e, were thus prepared in 54-74% yields. This method is very convenient for preparing corresponding 3-hydroxyalkanenitriles from oxiranes, though limited types of oxiranes are commercially available. The second method is nucleophilic addition of lithioacetonitrile to aldehydes. Seven acetates, 1c, 1d, 1f, 1g, 1h, 1i, and 1j, were prepared through this path, though the reaction required expensive butyl lithium as a lithiation reagent and should be carried out very carefully.
under argon atmosphere. The alcohols obtained were converted to the corresponding acetates, 1b-1j, in almost quantitative yields by treatment with acetyl chloride in the presence of pyridine as a base.

The hydrolysis of (±)-1 was carried out in a non-buffered aqueous solution to exclude any effect of complexation between this thiacrown ether 4 and metal cation (Eq. 1). When 2-cyano-1-methylethyl acetate (1a) was employed as a substrate, the optimum value was obtained in the presence of 5 mol% and 33 mol% of the thiacrown ether to the substrate. Therefore, it was decided to use 5 mol% of thiacrown ether towards the substrate. The reaction was typically carried out as follows: 12.5 mL of an aqueous lipase solution was added to a solution of (±)-1 (1.25 mmol), together with a crown ether 4 additive (5 mol% toward the substrate) in 1.3 mL of acetone. The resulting mixture was stirred at 35 °C, and the reaction was stopped when the mole ratio of acetate 1 and alcohol 2 became equal. The alcohol (R)-2 produced and remaining ester (S)-1 were extracted with ethyl acetate and separated by silica-gel TLC (hexane / ethyl acetate = 2:1). The enantiomeric excess of the remaining acetate (S)-1 was determined by capillary GLC analysis using chiral stationary phase (Chiraldex G-Ta). The enantiomeric excess of alcohol (R)-2 was also determined by the GLC analysis of the corresponding acetate. Because this reaction is a kinetic resolution of the racemic substrate, the optical purities of the produced alcohol and remaining acetate depend on the reaction conversion. The effect of this crown ether on enantioselectivity was evaluated by comparison of E values, which were calculated by the equation proposed by Sih. The relative rate was calculated from the percentage conversion per reaction time. These results obtained for the reaction at 35 °C are summarized in Table 1, Fig 1a, and Fig 1b.

There were marked differences of the additive effect on the enantioselectivity among the substrates employed (Fig. 1a), while thiacrown ether only slightly influenced the reaction rate (Fig. 1b). The most significant enhancement in enantioselectivity was observed when the reaction was carried out using 1-(cyanomethyl)propyl acetate (1b: R=Et) as the substrate (Entry 4). Although the hydrolysis of 1b proceeded with modest enantioselectivity (E=53) without the additive (Entry 3), the addition of 5 mol% thiacrown ether 4 to the substrate significantly enhanced enantioselectivity, reaching a higher level for practical use (E= >700) (Entry 4).
The enantioselectivities of the reactions of 1h and 1i were similarly enhanced about 2-3 times by the addition of thiacrown 4 (Entries 15-18). On the contrary, no marked enhancement in enantioselectivity was observed when acetates, 1e (R=n-Bu), 1f (R=n-Oct), and 1j (R=phenethyl) were used (Entries 9-12, 19, and 20). With substrates 1a, 1d, and 1g, the enantioselectivity was slightly enhanced (Fig. 1a). These results obviously suggest that this crown ether additive cannot change the original stereochemistry of the product, but does enhance its potential ability to a level at which the reaction can be used practically.

Figures 2a and 2b show results of the hydrolysis of acetate 1a under different temperature conditions. Addition of only 5 mol% of thiacrown ether accelerated the hydrolysis rate of both enantiomers with a similar magnitude of increase at each temperature. It is quite interesting that the hydrolysis proceeded even at 0 °C and reached 27% conversion after 24 h in the presence of thiacrown ether 4, though the hydrolysis conversion after 24 h reaction was only 3% in the absence of the crown ether (Fig. 2a).

These reactions were conducted in non-buffered aqueous media; the reaction rate gradually lowered in the reaction course and E values obtained were markedly reduced at over 40% conversion. This would be a result of inactivation of enzyme by lower pH or a reverse reaction. Figure 3 show results of the hydrolysis of acetate (±)-1b both in the presence and absence of thiacrown ether 4 at 35°C; pH values of both the solutions reached 3.4–3.5 after 20 hours because the reactions were carried out under lower pH conditions.

Fig. 2. (a) Reaction course of the hydrolysis of (R)-1a in the presence of thiacrown ether 4 (b) Reaction course of the hydrolysis of (S)-1a in the presence of thiacrown ether 4
conditions of no pH control. The reaction was dramatically accelerated by the crown ether additive as shown in Figure 3. The hydrolysis did not stop after 30 hours when 5 mol% of 4 was present, while the reaction stopped after 25 h in the absence of thiacrown ether 4. It should be emphasized that the enzymatic reaction proceeded in the pH range of 3.4-3.5 in the presence of thiacrown ether 4. Addition of thiacrown ether clearly had a strong influence on the lipase activity. Why does the crown ether modify lipase performance?

Crown ethers bind metal cations with varying strength depending on structural variations. The lipase solution employed was confirmed to contain the following alkali and alkaline earth metal cations: Na⁺, 6.5 x 10⁻⁴ mol/L; K⁺, 4.6 x 10⁻⁴ mol/L; Mg²⁺, 6 x 10⁻⁴ mol/L; and Ca²⁺, 6.3 x 10⁻⁴ mol/L. The amounts are believed to be too small to affect the enzyme reactivity, and it is well known that thiacrown ether does not possess strong affinity with these alkali metal cations. Some metal salts were recently reported to influence the catalytic activities of enzymes, but in that case crown ether-metal cation complexation may not be involved. Recently Reinhoudt and his colleagues reported that 18-crown-6 activated some enzymes; in that case, 18-crown-6 might modify the conformation of the enzyme by trapping water molecules at the active site or the enzyme surface.

The following mechanism has been proposed for enhanced enantiomolecivity by thiacrown ether additive when lipophilic thiacrown ethers remain at the entrance of the lipase, they may modify the local conformation of the lipase: this increases fitness of the substrate introduced to the active site of the enzyme, traps the product alcohol, and accelerates the diffusion of the alcohol into the bulk water phase. C-NMR spectra supports the possibility that thiacrown ether can trap the alcohol. When thiacrown ether 4 was added to a CDCl₃ solution of the product alcohols, 2b and 2f, large C-NMR spectral changes were observed at the carbon signals at 3-position of both products (Fig 4), while no significant induced chemical shift change was observed for substrate 1b or 1f (Table 2). An induced chemical shift of nitrile carbon for 2f was -0.24 ppm, while no significant induced chemical shift was observed for that of 2b (Δδ = +0.002 ppm). These results may suggest that nitriles 2b and 2f interact with thiacrown ether in
different fashions. Because the enzymatic reaction was performed in water, a $^{13}$C NMR experiment was also carried out in D$_2$O. Due to the low solubility of the thiacrown ether 4 in D$_2$O, however, no spectral change was detected in the $^{13}$C NMR signal of the monoacetate. To confirm this hypothesis, the conformational change of the enzyme by thiacrown ether should be investigated, though the change may be too small to be seen using the present analytical instrument. It has been reported that the conformational change in the protein is reflected in the CD spectra. However, no significant spectral change was observed when the CD spectra of the PCL aqueous solution were measured in the presence of 5 ~ 33 mol% of thiacrown ether 4. Although the mechanism of this enhancement has not yet been elucidated completely, these results suggest an interesting application in organic synthesis, especially for the synthesis of chiral natural products.


As described the additive effect of thiacrown ether in PCL-catalyzed reaction led to the highly efficient optical resolution of racemic 1b; this provided 3-hydroxypentanenitrile 2b in an optically pure form (>99%ee). Applying this, the facile synthesis of the attractive pheromone of the ant Myrmica scabrinodis (A) was accomplished using (R)-2b as a starting material (Scheme 1).

An aqueous lipase solution (100 mL: including ca. 700 mg of PCL) was added to a solution of (±)-1b (1.41 g, 9.99 mmol), together with thiacrown ether 4 (5 mol % toward the substrate) in 10 mL of acetone. The resulting mixture was stirred at 35 °C, and the reaction was stopped when ester and alcohol reached to equal molar ratios which were monitored by gas chromatography analysis. Alcohol (R)-2b produced and ester (S)-1b remaining were extracted with ethyl acetate and separated by silica gel flash column chromatography (hexane / ethyl acetate=2:1); this gave (R)-2b (4.94 mmol, 49%) and (S)-1b (4.55 mmol, 46%). The enantiomeric excess of these compounds was confirmed as >99 %ee by GLC analysis using chiral capillary column. (R)-2b was converted to t-butyldimethylsilyl ether (R)-6b in 97% yield. Carbon elongation of (R)-6b was successfully achieved through the Wittig reaction protocol; (R)-6b was treated with disobutylaluminim hydride (DIBAL) at -78°C, giving the corresponding aldehyde 7, which was used without isolation for subsequent Wittig reaction; silyl ether (R)-8 was thus obtained in 77% overall yield from (R)-2b. Finally, hydrogenation of (R)-8 released the target molecule (R)-5 ([α]$_D^{23}$)
9.5° (c0.96, CHCl₃), lit.36 -9.7°), the attractant pheromone of the ant Myrmica scabrinodis (A), in 88% yield (66% overall yield from (R)-2b). The antipode of this pheromone, (S)-5, was also synthesized from (S)-1b through the same reaction sequences in 51% overall yield.

It should be emphasized that the key step of this synthesis is a pollution-free chemical reaction using a natural enzymatic system. Lipase-catalyzed reactions are particularly useful even for a large-scale preparative organic synthesis. In particular, Pseudomonas cepacia lipase is commercially available and not very expensive; hence, the present protocol can undoubtedly allow us to evolve a smarter and more convenient synthesis of pheromone compounds.

1-2-1. Introduction

Lipase-catalyzed regioselective acetylation or deacetylation is known to be useful means of preparing partially acetylated compounds.2a,16 It was recently reported a simple preparation of 4-hydroxy-3-alkyl-2-butenyl acetate (10) via lipase-catalyzed reaction, though a prolonged reaction time was required to perform at 0 °C and realize good regioselectivity.17 In addition, lipase-catalyzed reactions in a buffer solution proceeded so rapidly that diol was formed. This caused significant drop in the chemical yield of the desired monoacetate 10.

Reinhoudt et al. reported that serine proteases were activated by crown ethers, especially by 18-crown-6.8 Itoh et al. also found that some crown ethers had potentiality to enhance both the enantioselectivity and reaction rate in the lipase-catalyzed hydrolysis of 2-cyano-1-methylethyl acetate.1 In particular, 1,4,8,11-tetrathiacyclotetradecane (4) was confirmed as the best additive among 35 types of crown ethers and their acyclic analogues tested.1 In this chapter 1-2., It is described that the reaction rate of partial hydrolysis of diacetate 9 catalyzed by Candida rugosa lipase (CRL) or Pseudomonas cepacia (PCL) was greatly improved by addition of catalytic amounts of thiocrown ether 4; the reaction efficiency of the PCL- and CRL-catalyzed reaction was thus greatly improved (Eq. 2 and Fig. 5).
1-2-2. Buffer Free Highly Regioselective Partial Hydrolysis of 4-Acetoxy-2-methyl-2-butene Acetate

Five types of lipases were chosen and their activity was tested in the presence of thiacrown ether 4 in the hydrolysis of diacetate 9 as a model compound. The hydrolysis was carried out in a non-buffered aqueous solution to exclude any effect of complexation between crown ether and metal cation.

\[
\begin{align*}
\text{Lipase} & \quad \text{H}_2\text{O-acetone=10:1} \\
\text{35°C} & \quad \text{~C:} \\
\text{4} & \quad \text{(Eq. 2)}
\end{align*}
\]

\[
\begin{align*}
\text{HO-CH}_3\text{OAc} & \quad \text{AcO-CH}_3\text{OAc} \\
\text{10} & \quad \text{11}
\end{align*}
\]

The resulting monoacetates, a mixture of 10 and 11, were converted to the corresponding t-butyldimethylsilyl ether (13 and 14) and the regioselectivity of the reaction was determined by capillary GLC analysis. The enzymes employed catalyzed hydrolysis of 9 at the sterically hindered position to afford monoacetate 10 preferentially, though their reactivity was not satisfactory in the absence of additive. Addition of thiacrown ether 5 greatly enhanced the reaction rate in most cases especially in CRL- or PCL-catalyzed reaction (Table 4 and Fig. 5).

It should be emphasized that there were clearly differences in the additive effect depending on origin of the enzymes (Fig. 5). When the CRL-catalyzed reaction was carried out in the presence of 5 mol% of thiacrown ether (see Fig. 5), the rate was accelerated more than 13 fold and the regioselectivity was slightly increased up to 87% with a high 81% reproducible yield (Entry 3 in Table 4). The reaction rate was even more accelerated in the presence of 33 mol% of thiacrown and reached 27 times as compared with that in the absence of thiacrown ether (Entry 4 in Table 4). Addition of thiacrown ether also greatly improved the rate of PCL-catalyzed reaction (Entries 7 and 8). Like the CRL-catalyzed reaction, a larger amount of thiacrown ether further accelerated PCL-catalyzed reaction (Entry 8).
and CRL-catalyzed reaction was thus greatly during the isolation process, because presence of a large amount of thiacrown ether makes it chromatography. f) Enzymes lose their reactivity under the conditions employed, hence the observed during the isolation process. d) The rate was calculated from percentage conversion reaction stopped at the indicated hydrolysis ratio.

c) Due to the hydrophilicity of the diol produced, significant loss was observed (Sigma Type II). c) The value in parenthesis is a GLC yield. Significant loss was observed (Sigma Type II). c) Due to the hydrophilicity of the diol produced, significant loss was while the regioselectivity remained at the level recorded with 5 mol% additive concentration. Presence of a large amount of thiacrown ether did not inhibit the enzymatic reaction, while too excessive amount of the ether caused acceleration of further hydrolysis of monoacetate to diol; a significant drop in the yield of monoacetate was thus observed (Entry 9). 20

Because substrate 9 is easily hydrolyzable, diacetate 9 was naturally hydrolyzed to give 10 and 11 non-regioselectively in a buffer solution even at pH 6.5–7.0 at room temperature. Lipase-catalyzed reactions in a buffer solution proceeded so rapidly that they formed diol 12; this caused a significant drop in the chemical yield of the monoacetate (Entries 1 and 5). The efficiency of the PCL- and CRL-catalyzed reaction was thus greatly improved by addition of thiacrown ether 4; this makes the present reaction particularly important for obtaining the monoacetate 2 practically.

PPL-catalyzed reaction, in contrast, was not modified by thiacrown ether additive even when 33 mol% of 4 was used (Entry 12). The regioselectivity was not changed in lipase F or AL-catalyzed reactions, though the reaction rate was similarly improved (Entries 14 and 16). Our employment of this crown ether additive cannot change the original stereochemistry of the product, but does enhance its potential ability to a level at which the reaction can be used practically.

Why does the crown ether modify lipase performance? We currently assume that two factors are involved in the reaction. 4 One is the interaction between the thiacrown ether and the enzyme. The employed crown ether may interact with certain sites of the lipase, thereby modifying its local conformation, activating it, and causing the change in stereoselectivity of the enzymatic reaction. 4 This factor seems to have strong probability because the additive activity of thiacrown 4 depends on the origin of the enzymes employed and a great change was observed in CRL-catalyzed reaction.

Table 4. Results of Lipase-catalyzed Partial Hydrolysis of Diacetate 9

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme b)</th>
<th>Thiacrown</th>
<th>Time a)</th>
<th>Yield of 19:11 (Ratio 19:12)</th>
<th>Yield of 9 (Recovered)</th>
<th>Yield of 12 a)</th>
<th>Relative Rate a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CRL 0 (pH 7.2)</td>
<td>0.5</td>
<td>61</td>
<td>86:20</td>
<td>0</td>
<td>6</td>
<td>122</td>
</tr>
<tr>
<td>2</td>
<td>CRL 0</td>
<td>20</td>
<td>87</td>
<td>74:26</td>
<td>4</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>CRL 5 mol%</td>
<td>1.5</td>
<td>81</td>
<td>87:13</td>
<td>8</td>
<td>0</td>
<td>54</td>
</tr>
<tr>
<td>4</td>
<td>CRL 33 mol%</td>
<td>0.8</td>
<td>85</td>
<td>86:14</td>
<td>0</td>
<td>0</td>
<td>106</td>
</tr>
<tr>
<td>5</td>
<td>PCL 0 (pH 7.2)</td>
<td>0.5</td>
<td>65</td>
<td>80:20</td>
<td>0</td>
<td>5</td>
<td>130</td>
</tr>
<tr>
<td>6</td>
<td>PCL 0</td>
<td>2.0</td>
<td>74</td>
<td>96:4</td>
<td>3</td>
<td>0</td>
<td>37</td>
</tr>
<tr>
<td>7</td>
<td>PCL 5 mol%</td>
<td>1.0</td>
<td>87</td>
<td>98:2</td>
<td>6</td>
<td>0</td>
<td>87</td>
</tr>
<tr>
<td>8</td>
<td>PCL 33 mol%</td>
<td>0.7</td>
<td>89</td>
<td>95:5</td>
<td>0</td>
<td>0</td>
<td>127</td>
</tr>
<tr>
<td>9</td>
<td>PCL 100 mol%</td>
<td>0.7</td>
<td>75*</td>
<td>86:14</td>
<td>0</td>
<td>0</td>
<td>130</td>
</tr>
<tr>
<td>10</td>
<td>PPL 0</td>
<td>144</td>
<td>51</td>
<td>94:6</td>
<td>49</td>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td>11</td>
<td>PPL 5 mol%</td>
<td>120</td>
<td>14*</td>
<td>94:6</td>
<td>73</td>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td>12</td>
<td>PPL 33 mol%</td>
<td>144</td>
<td>40*</td>
<td>91:9</td>
<td>39</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>13</td>
<td>AL 0</td>
<td>10</td>
<td>20</td>
<td>88:12</td>
<td>50</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>14</td>
<td>AL 5 mol%</td>
<td>12</td>
<td>61</td>
<td>94:6</td>
<td>6</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>15</td>
<td>F 0</td>
<td>144</td>
<td>33*</td>
<td>81:19</td>
<td>47</td>
<td>0</td>
<td>0.02</td>
</tr>
<tr>
<td>16</td>
<td>F 5 mol%</td>
<td>24</td>
<td>50</td>
<td>86:14</td>
<td>50</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

a) The reaction was conducted in a mixed solvent of deionized water and acetone (water : acetone=10:1). b) PCL (Amano: Lipase PS); CRL (Meito : Lipase OF); AL: Acromobacter sp. lipase (Meito); F: Porcine pancreatic lipase (Amano); PPL: Porcine pancreatic lipase (Sigma Type II). c) Due to the hydrophilicity of the diol produced, significant loss was observed during the isolation process. d) The rate was calculated from percentage conversion per reaction time. e) The value in parenthesis is a GLC yield. Significant loss was observed during the isolation process, because presence of a large amount of thiacrown ether makes it difficult to isolate the product from the reaction mixture by silica gel flash column chromatography. f) Enzymes lose their reactivity under the conditions employed, hence the reaction stopped at the indicated hydrolysis ratio.
Crystal structures of CRL show that it has two conformations that differ mainly in the orientation of a helical surface loop. Thiacrown ether additive may convert the closed form of CRL to the open form which can enhance regioselectivity, as suggested by the results of CRL-catalyzed reaction after treatment of 2-propanol or sodium deoxycholate. The origin of 18-crown-6 activation was suggested by Reinhoudt et al. to be that the crown ether may modify the conformational change of the enzyme by trapping water molecules at the active site or enzyme surface. It is assumed that the interaction mechanism of 18-crown-6 and thiacrown are different. The former is hydrophilic but thiacrown ether is a very hydrophobic compound and therefore unable to catch the water; rather, thiacrown ether may interact with the enzyme directly.

The second factor is complexation of crown ether with substrate or products, 10 or 11. Crown ethers bind neutral organic molecules in the crystal states and non-polar media. Thiacrown ether may bind alcohol/ester molecules in the course of the reaction, so that chemical equilibrium of the hydrolysis reaction occurring near the active site could be modified. When thiacrown ether was added to a CDCl$_3$ solution of the product alcohol, 10 or 11, large $^{13}$C NMR spectral changes were observed at the carbonyl carbon signals of both products (Fig. 6), while no significant induced chemical shift was observed for substrate 9. Induced chemical shift of a compound by thiacrown ether seems to provide a good guideline for considering additive effect in the present reaction. Thiacrown ether was suggested to make the product unsuitable for the reverse reaction by trapping it, thus accelerating diffusion of the alcohol into the bulk water phase.

In summary, addition of a catalytic amount of thiacrown ether realized highly regioselective and efficient lipase-catalyzed reaction in a buffer free medium. This type of regioselective partial hydrolysis of diacetate 9 is impossible by a chemical reaction such as alkaline hydrolysis. The amount of employed thiacrown ether was only 5 mol% to the substrate; this corresponded to 250 times crown 4 based on the lipase molecule. Obviously this work represents not only a significant advance in the manner of preparation of partially acetylated compounds but also provides a new aspect in application of enzymatic reaction to organic synthesis.
1-3-1. Introduction

The value of an enzymatic reaction in organic synthesis is extensively increased by its environmentally friendly aspect.\(^2\) Since only a limited number of lipase-catalyzed reactions is applicable for practical optical resolution, development of an efficient strategy to improve their reaction performance is desirable.\(^1\) It has been reported that thiacrown ethers, especially 1,4,8,11-tetraathiacyclotetradecane, have the potential to enhance both the enantioselectivity and reaction rate in the lipase-catalyzed reaction as a chapter 1-1. (Figure 7).\(^1\)

Extensive studies have been made to disclose the factors deciding the stereoselectivity of lipases.\(^2,26,27\) Among these, "the transition-state model" proposed by Ema \textit{et al.} \(^27\) in which the authors rationalized the enantioselectivity of lipases towards secondary alcohol is especially attractive. They showed the usefulness of their theory to realize successful kinetic resolution of a large secondary alcohol,\(^28\) and in fact, we previously demonstrated that a lipase-catalyzed reaction was applicable even for the reaction of extremely bulky compounds such as \(\alpha\)-(tributylstannyl)alkanols.\(^29\) The finding of thiacrown ether modification of the lipase-catalyzed reaction could not be explained by the traditional "binding step model" in which the enantioselectivity originated from the fitness of the substrate towards the active site of the enzyme. Therefore, It was decided to investigate the detail of stereoselectivity on diastereoselectivity using 3-hydroxy-2-methyl or 3-hydroxy-2-methylalkanenitriles as model compounds from the standpoint of the regulating effects of thiacrown ethers on stereoselectivity of the lipase-catalyzed reaction.

In this chapter the results of the thiacrown ether effect on diastereoselectivity of the lipase-catalyzed hydrolysis of acetate of 3-hydroxy-2-alkyl-alkanenitriles will be reported, and the differences of the origin between diastereoselectivity and enantioselectivity in the lipase-catalyzed reaction are also discussed. Based on these results on diastereoselectivity and enantioselectivity of the lipase-catalyzed reaction, a double enzymatic reaction method to isolate one enantiomer from four enantiomeric isomers of 3-hydroxy-2-alkylalkanenitriles has been demonstrated.

1-3-2. Kinetic aspect of the thiacrown ether modified lipase-catalyzed reaction.
Before the study of thiacrown ether effect on diastereoselectivity using lipase-catalyzed reaction of 3-hydroxy-2-alkylalkanenitriles as model compounds, the kinetic parameters of *Pseudomonas cepacia* lipase (PCL)-catalyzed hydrolysis of 1-cyanobutan-2-yl acetate (1b) was measured because the most drastic modification was recorded when various types of acetates of 2-hydroxyalkanenitriles were submitted to the lipase-catalyzed reaction in the presence of thiacrown ether (Eq. 3).

Enantiomerically pure acetates *(R)-1b* and *(S)-1b* were reacted with the lipase-catalysis, and the progress of the reaction was monitored by gas chromatography to obtain the initial rate \(v_o\). The hydrolysis of 1b was done in a non-buffered aqueous solution to exclude any effect of complexation between the thiacrown ether 4 and metal cation (Eq. 3). The reaction was typically done as follows: 12.5 mL of an aqueous lipase solution was added to a solution of acetate 1b, together with a crown ether 4 additive (5 mol% toward the substrate) in 1.25 mL of acetone. The resulting mixture was stirred at 35 °C. The concentration of the acetate \([S_o]\) was systematically changed, and the plot of \(v_o\) against \([S_o]\) afforded a typical saturation curve. The apparent \(V_{max}\) and \(K_m\) were obtained by the nonlinear-squares method applied to the Michaelis-Menten type of equation (Table 6).

### Table 6. Kinetic parameters for the PCL-catalyzed hydrolysis of 1-cyanobutan-2-yl acetate 1b in the presence of thiacrown ether 4

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate (mol. amount)</th>
<th>(V_{max}) (mM min(^{-1})mg lipase(^{-1}))</th>
<th>(K_m) (M)</th>
<th>(V_{max}/K_m) (min(^{-1})mg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>(R)-1b</em> 0</td>
<td>(5.6\pm2.0\times10^{-4})</td>
<td>(5.7\pm2.5\times10^{-2})</td>
<td>(1.0\times10^{6})</td>
</tr>
<tr>
<td>2</td>
<td><em>(R)-1b</em> 0.05</td>
<td>(2.2\pm0.4\times10^{-5})</td>
<td>(2.3\pm0.6\times10^{-2})</td>
<td>(1.0\times10^{5})</td>
</tr>
<tr>
<td>3</td>
<td><em>(S)-1b</em> 0</td>
<td>(4.7\pm2.8\times10^{-5})</td>
<td>(3.7\pm2.1\times10^{-2})</td>
<td>(1.3\times10^{6})</td>
</tr>
<tr>
<td>4</td>
<td><em>(S)-1b</em> 0.05</td>
<td>(9.9\pm7.2\times10^{-5})</td>
<td>(9.5\pm7.0\times10^{-2})</td>
<td>(1.0\times10^{6})</td>
</tr>
</tbody>
</table>

a) Conditions: lipase (typically, 3-10 mg and 150-300 mg for *(R)- and *(S)-enantiomers, respectively), optically pure (>99% ee) acetate (typically, ca. 0.05-0.4 M) and deionized water (5.0 mL) and acetone (35 °C). Because of the heterogeneous reaction, the nonlinear least-squares method was applied to the Michaelis-Menten type of equations: \(v_o=V_{max}(E)mg[S_o]/(K_m + [S_o])\), where \(V_{max}\) is normalized by the weight of lipase \((E)mg\).

The table 6 clearly shows that the thiacrown ether modification on enantioselectivity in the lipase-catalyzed reaction originated from the differences of \(V_{max}\) and not from \(K_m\). Thiacrown ether significantly accelerated the reaction using both enantiomers, and the most important fact is that the difference in \(V_{max}\) values of *(R)-1b* between that of *(S)-1b* drastically enlarged by 20-fold when the reactions were done in the presence of 5 mol% of thiacrown ether 4 (Entries 2 and 4), while it was 10-fold in the absence of thiacrown ether (Entries 1 and 3). No significant difference in \(K_m\) was observed between *(R)-1b* and *(S)-1b* (Entries 1 and 3) in the absence of thiacrown ether, and the difference was expanded only slightly when the reactions done in the presence of 5 mol% of thiacrown ether 4 (Entries 2 and 4). It is well known that \(K_m\) of the lipase-catalyzed reaction is generally large, indicating that the binding of lipases to the substrates is
weak. A very interesting point is that \( \text{Km} \) were increased by addition of thiacrown ether; the additive compound weakened the binding force of the enzyme to the substrate. These experimental results strongly support the transition-state model proposed by Ema et al.\(^\text{27}\) in that the ability of lipases to discriminate between the enantiomers at the transition-state is high, while the ability to recognize the chirality in the binding step is poor. Thiacrown ether may modify the transition state and expand the difference of \( \text{Vmax} \) between the enantiomers, increasing the enantioselectivity, though there still remains another possibility to explain the origin of the thiacrown ether modification. The value of the catalytic efficiency (\( \text{Vmax}/\text{Km} \)) on \((S)-1b\) was slightly reduced by addition of thiacrown ether (Entry 4), while no difference was observed for \((R)-1b\) (Entries 1 and 2), so that increased enantioselectivity may arise from the enantioselective inhibition of \((S)-1b\) in the presence of the thiacrown ether near the active site.

1.3.3. Thiacrown ether effect on diastereoselectivity of the lipase-catalyzed reaction.

The origin of stereoselectivity of lipase-catalyzed reaction seems to be explained by the transition-state model as described above, however, another question remains concerning the origin of stereoselectivity for diastereomers, whether a neighborhood functional group of the hydrolysis point does or does not affect the stereochemistry of an enzymatic reaction.

We investigated this point using 3-hydroxy-2-methyl or 3-hydroxy-2-ethylalkanenitriles, \(15a\) (\(R^1 = \text{Et}, R^2 = \text{Me}\)), \(15b\) (\(R^1, R^2 = \text{Et}\)), and \(15c\) (\(R^1, R^2 = \text{Me}\)), as model substrates from the standpoint of modifying the property of the thiacrown ether towards diastereoselectivity (Eq. 4). Racemic 3-hydroxy-2-methyl- or 3-hydroxy-2-ethylalkanenitriles, \((\pm)-15a-15c\), were prepared through nucleophilic addition of lithiopropionitrile or lithiobutyronitrile to aldehydes.\(^\text{28}\) The alcohols obtained were converted to the corresponding acetates, \(16a-16c\), in almost quantitative yields by treatment with acetyl chloride in the presence of pyridine as base. The hydrolysis was done using two types of lipases, lipase AL from \(Achromobacter\) sp (Meito)\(^\text{22}\) and PCL (Amano). Because the starting acetate was a mixture of four enantiomers, the reaction was stopped when the hydrolysis ratio reached about 25% conversion. The initial rate was calculated from the percentage conversion per reaction time. Because this reaction is a kinetic resolution of a racemic substrate, the optical purity of the producing alcohol and remaining acetate depends on the reaction conversion. The effect of this thiacrown ether on enantioselectivity was evaluated by comparison of \(E\) values, which were calculated by the equation proposed by Sih et al.\(^\text{12}\) The alcohol \(16\) produced and remaining ester \(15\) were extracted with ethyl acetate and separated by silica-gel flash column chromatography (hexane/ethyl acetate = 5:1) and the diastereomeric excess (de) of alcohol \(16\) produced was measured by capillary GC analysis. The enantiomeric excess of alcohol \((anti-16a, syn-16b, anti-16c, and syn-16c\) was measured by capillary GC analysis using the chiral stationary phase (Chiralcal G-Ta) of the corresponding acetate. The enantiomeric excess (ee) of the remaining acetate \((anti-15a, syn-15a, anti-15b, and syn-15b) was also measured by GC analysis. Unfortunately, we could not measured enantioselectivity of \(15c\) by GC analysis at an early stage of the reaction because signals responsible for the four enantiomers are too close to calculate
the ee of each isomer with sufficient reliability. The results are summarized in Table 7.

\[
\text{Lipase} \quad \text{H}_2\text{O-} \text{Acetone (10:1)} \quad \text{anti-16} \quad \text{syn-16} \quad (4)
\]

There were significant differences on the additive effect on the reaction rate for lipase AL-catalyzed reaction of 15a and 15b with slightly increased diastereoselectivity (Entries 2 and 4), while no acceleration was observed for 15c (Entry 10). With both substrates 15a and 15b, the enantioselectivity towards anti-isomers was slightly lowered by addition of thiocrown ether, while that of syn isomers was increased (Entries 2 and 4). On the other hand, thiocrown ether slightly accelerated the reaction rate of PCL-catalyzed reaction of 15c, but slightly reduced the diastereoselectivity (Entry 8).

Although measurement of kinetic parameters for these compounds was tried, unfortunately, this was unsuccessful because separation of the isomers 15a and 15b was impossible by preparative means of silica-gel thin-layer chromatography or column chromatography.

A great increase of enantioselectivity was caused by thiocrown ether for acetate of 3-hydroxypentanenitrile 1b as described earlier (Table 6), while no marked increase in diastereoselectivity by the addition of the thiocrown ether was found for any of the acetates. These results obviously suggest that diastereoselectivity and enantioselectivity are controlled by different origins. It may suggest that the thiocrown ether-modified conformational change of lipase protein is so small that it does not reflect diastereo-favoritism, though it is high enough to cause modification of the transition state.

Table 7. Thiacrown ether additive effect on the lipase-catalyzed reaction

<table>
<thead>
<tr>
<th>Entry</th>
<th>R1</th>
<th>R2</th>
<th>Lipase</th>
<th>mol. amount (h)</th>
<th>Time (h)</th>
<th>Rate (%)</th>
<th>%Conv. (%)</th>
<th>%De (%)</th>
<th>%Ee (%)</th>
<th>E value (%)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Et</td>
<td>Me</td>
<td>PCL</td>
<td>0</td>
<td>5</td>
<td>5.2</td>
<td>26</td>
<td>72</td>
<td>&gt;99</td>
<td>&gt;243</td>
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<tr>
<td>2</td>
<td>Et</td>
<td>Me</td>
<td>PCL</td>
<td>0.05</td>
<td>5</td>
<td>5.1</td>
<td>25</td>
<td>72</td>
<td>&gt;99</td>
<td>&gt;285</td>
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<tr>
<td>3</td>
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<td>AL</td>
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<td>24</td>
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<td>21</td>
<td>86</td>
<td>&gt;99</td>
<td>&gt;501</td>
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<tr>
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<td>94</td>
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<td>AL</td>
<td>0.05</td>
<td>72</td>
<td>0.3</td>
<td>21</td>
<td>61</td>
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</tr>
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</table>

a) Rate shows % conv. per reaction time (h) and was determined by capillary GC (MS) analysis. b) anti-16 was obtained as a major isomer in all reactions tested. c) Determined by capillary GC analysis using chiral stationary phase (Chiralcel OD-T). Optical purity of the product shows >99% ee when no isomer was detected by GC analysis. d) E\(^{11}\) = \(\text{E}^{11} = \text{E}(\text{ee}_{16}/\text{ee}_{15}+\text{ee}_{15})\), E\(^{11}\) \(<\text{E}(\text{ee}_{16}/\text{ee}_{15}+\text{ee}_{15})\) \(<\text{E}(\text{ee}_{16}/\text{ee}_{15}+\text{ee}_{15})\). e) It was impossible to determine %ee of 16 by GC analysis because the four signals responsible for each enantiomer were too close to allow calculation of the enantiomeric excess with reliability.
The high enantioselectivity and broad substrate specificity is quite an important property of the lipase catalyzed reaction because these factors are usually difficult to make compatible with each other in other enzymatic reaction systems. It was found, however, that diastereoselectivity and enantioselectivity of lipase-catalyzed reaction have different origins. This makes it desirable to form a strategy to obtain optically pure α-alkyl-β-hydroxyalkanenitriles by lipase-catalyzed optical resolution of racemic diastereo-mixture. 3-Hydroxy-2-methylpentanenitrile 16a and 3-hydroxy-2-methylbutanenitrile 16b\(^{33}\) was chosen as model substrates to demonstrate such strategy of preparing both diastereo- and enantiomeric pure compounds by a lipase-catalyzed reaction, because they can be used as building blocks for the synthesis of sex attractant pheromones.\(^{34,35}\)

It is reasonable to hypothesize that a double enzymatic reaction system provides a useful means to resolve 3-hydroxy-2-methylalkanenitriles; the first stage is evaluation of an enzyme that has high diastereoselectivity, and the second is to identify an enzyme that reacts specifically with one enantiomer. Synthesis of anti-alkanenitrile 15a (R\(^1\)=Et, R\(^2\)=Me) was accomplished through this reaction protocol as shown in Scheme 1. Lipase AL-catalyzed hydrolysis of (±)-15a gave anti-16a in 26% yield with 86% de, and this was then treated with PCL in the presence of vinyl acetate as acyl donor in diisopropyl ether to afford (2S,3R)-15a in optically pure form (>99% ee) with 96% de (Scheme 2, upper equation). It was difficult, however, to find an enzyme that hydrolyze acetate 15c (R\(^1\), R\(^2\)= Me) with sufficient anti diastereoselectivity; lipase AL-catalyzed hydrolysis of (±)-16c (R\(^1\),R\(^2\)= Me) gave anti-16c with the highest diastereoselectivity (60% de). Using the same strategy, (2S,3R)-15c was obtained in optically pure form (>99% ee), though diastereoselectivity was insufficient (65% de) (Scheme 2, middle equation). Incidentally, another enzyme combination was required to prepare (2R,3S)-15c because the hydrolysis reaction stopped at about 30% conversion when lipase AL was used. The best lipase providing (2R,3S)-15c was lipase OF; acetate anti-15c was obtained with 58% de and this was further treated with Hemi-cellulase (from Aspergillus sp.) to afford (2R,3S)-15c with >99% ee with 86% de (Scheme 2, lower equation). Based on the GC analysis of the reaction course, it was found that (25,3R)-15c(anti) was initially hydrolyzed by lipase AL or OF, (2R,3R)-15c(syn) was next consumed, the third isomer hydrolyzed was (2S,3S)-15c(syn), and (2R,3S)-15c(anti) was the last isomer hydrolyzed by the lipase.
Kinetic study of lipase-catalyzed hydrolysis of acetate 3-hydroxyalkanenitriles in the presence or absence of thiacrown ether suggested that diastereoselectivity and enantioselectivity should be viewed from different mechanistic aspects. A double lipase catalyzed reaction was therefore useful for resolving 3-hydroxy-2-methylalkanenitriles by the lipase-catalyzed reaction. The reactions are particularly beneficial even for large scale preparative organic synthesis, because the key step of this synthesis is an environmentally friendly enzymatic reaction, and particularly of note is that all lipases used are commercially available and not expensive. Hence this protocol can undoubtedly allow us to evolve a smarter and more convenient synthesis of optically active building blocks for organic synthesis.

Summary

Both reaction rate and enantioselectivity in *Pseudomonas cepacia* lipase (PCL)-catalyzed hydrolysis of 3-hydroxyalkanenitrile acetates were significantly changed by the addition of catalytic amounts of thiacrown ether (1,4,8,11-tetrathiacyclotetradecane). Although the reaction rate of various nitriles was accelerated, the enantioselectivity greatly depended on the nature of the substrate. Among ten substrates tested, thiacrown ether offered the highest enantioselectivity in PCL-catalyzed hydrolysis of 1-(cyanomethyl)propyl acetate. Forty or more times this crown ether, molarity based on the enzyme, was required to attain an acceptably high reaction rate and enantioselectivity. Applying this method, the optically pure attractant pheromone of ant *Myrmica scabrinodis* (A), (R)-3-octanol and its (S)-isomer were successfully synthesized in good overall yields.

Reaction efficiency of the lipase-catalyzed partial hydrolysis of 4-acetoxy-2-methyl-2-butenyl acetate was greatly enhanced by addition of 5 mol% of thiacrown ether to the substrate with particularly remarkable improvement especially observed in the CRL- and PCL-catalyzed reactions. Simple preparation of 4-hydroxy-2-methyl-2-butenyl acetate has thus been achieved through buffer free lipase-catalyzed hydrolysis reaction.

The additive effect on diastereoselectivity towards the lipase-catalyzed hydrolysis of acetates of 3-hydroxy-2-methyl- or 3-hydroxy-2-ethylalkanenitriles has been investigated. Diastereoselectivity was not influenced by thiacrown ether additives, although a significant modification of enantioselectivity was observed. Origin of the diastereoselectivity of the lipase-catalyzed reaction was thus evidently different from that of
enantioselectivity. Based on these results, an easy preparation of optically active 3-hydroxy-2-methylpentanenitrile and 3-hydroxy-2-methylbutanenitrile have been demonstrated through lipase-catalyzed reaction by a double enzymatic reaction strategy.

**Experimental Section**

**General Procedures**

Reagents and solvents were purchased from common commercial sources and were used as received or purified by distillation from appropriate drying agents. Reactions requiring anhydrous conditions were run under an atmosphere of dry argon. Silica gel (Wako gel C-300, 300E) was used for column chromatography and silica gel (Wako gel B-5F) for thin layer chromatography. $^1$H NMR, $^{13}$C NMR spectra were recorded on Varian VXR-200 (200MHz) and VXR-500 (500MHz) spectrometer, and chemical shifts are expressed in ppm downfield from tetramethylsilane (TMS) in CDCl$_3$ as an internal reference. IR spectra were obtained on JASCO A-102 and FTIR-230 spectrometers. The regioselectivity was determined by capillary gas chromatography (Chiraldex G-TA, φ 0.25mm Å x 20m, 100-150°C, He).

**Materials.** Crown ethers were purchased from Aldrich and used without further purification. *Pseudomonas cepacia* lipase was provided by Amano Pharmaceutical Co., Ltd. (Japan).

**Chapter1-1.**

**3-Hydroxypentanenitrile (2b).** To a solution of 1,2-epoxybutane (10.8 g, 150 mmol) in ethanol (22 mL) under argon atmosphere at 50-60 °C was added 32% KCN aq. solution (150 mmol, 21 mL) and 30% CH$_3$CO$_2$H aq. solution (150 mmol, 21 mL) dropwise at the same time. The mixture was
stirred for 3 h, keeping pH value at around 7. After being cooled to room temperature, the mixture was extracted with Et₂O. The combined organic layers were dried over MgSO₄, filtered, and concentrated by evaporator. Kugelrohr distillation afforded 2b (11.0 g, 111.0 mmol) as a colorless oil in 74% yield: Rf 0.29 (hexane/ethyl acetate=2:1); bp 145 °C/ 45 Torr (Kugelrohr); ¹H NMR (200 MHz, δ, CDCl₃, J=Hz) 0.95 (3H, t, J=7.4), 1.59 (2H, dq, J=7.5, 7.4), 2.51 (2H, dd, J=17.0, 5.3), 2.90 (1H, s, OH), 3.8-3.9 (1H, m); ¹³C NMR (50 MHz, ppm, CDCl₃, J=Hz) 9.56, 25.51, 29.33, 68.81, 117.87; IR (neat, cm⁻¹) 3400, 2900, 2250, 1460, 1405, 1105, 980. Anal. Calcd for C₇H₁₂NO: C, 60.58; H, 9.15; N, 14.13. Found: C, 60.39; H, 9.38; N, 13.28.

(R)-2b (>99%ee): [α] D -0.8 (c. 1.51, CHCl₃) ; (S)-2b (>99%ee) : [α] D +1.6 (c.0.66, CHCl₃). Using the same procedure, γ-hydroxyheptanenitrile 2e was also prepared from the corresponding epoxide.

Caution: Potassium cyanide is very toxic so that the reaction must be carried out in a well ventilated area.

3-Hydroxy-4-pentenenitrile (2g). To a solution of diisopropyl amine (3.04 g, 30.04 mmol) in THF (30 mL) under argon atmosphere at -10 °C was added a solution of n-BuLi in hexane (1.62 M, 17.0 mL, 27.54 mmol). The mixture was stirred at 10 °C for 1h and cooled to -78°C and then a solution of CH₃CN (1.23 g, 29.96 mmol) in THF (15 mL) was added. The mixture was stirred at -78 °C for 1 h, then a solution of propionaldehyde (1.40 g, 24.97 mmol) in THF (15 mL) was added, and the mixture was again stirred at -78 °C for 3 h. The reaction was quenched with saturated aqueous NH₄Cl and treated with aqueous 2M HCl. The mixture was extracted with Et₂O, and the combined organic layers were dried over MgSO₄, filtered, and concentrated by evaporator. The obtained yellow oily residue was purified by flash column chromatography (gradient elution hexane/ethyl acetate=8:1 to 4:1) to give an alcohol 2g as a colorless oil (2.23 g, 23.0 mmol) in 92% yield: Rf 0.27 (hexane/ethyl acetate=2:1); bp 150 °C/42 Torr (Kugelrohr); ¹H NMR (200 MHz, δ, CDCl₃, J=Hz) 2.58 (2H, dd, J=16.7, 5.9), 3.03 (1H, brs, OH), 4.43 (1H, dt, J=16.4, 5.3), 5.26 (1H, dd, J=10.4, 1.3), 5.37 (1H, dt, J=16.4, 1.3), 5.89 (1H, ddd, J=16.4, 10.4, 5.9); ¹³C NMR (50 MHz, ppm, CDCl₃) 25.87, 68.34, 117.26, 117.40, 137.21; IR (neat, cm⁻¹) 3410, 2880, 2240, 1410, 1120, 930. Anal. Calcd for C₇H₁₄NO: C, 61.84; H, 7.27; N, 14.42. Found: C, 61.77; H, 7.34; N, 14.24.

Using the same procedure, hydroxyalkanenitriles, 2c-2j, were also prepared from the corresponding aldehydes.

3-Hydroxyhexanenitrile (2c). Rf 0.17 (hexane/ethyl acetate=4:1); bp 160 °C/ 43 Torr (Kugelrohr); ¹H NMR (200 MHz, δ, CDCl₃, J=Hz) 0.93 (3H, t, J=7.1), 1.36-1.59 (4H, m), 2.45 (1H, dd, J=16.6, 6.4), 2.56 (1H, dd, J=16.6, 4.9), 2.84 (1H, brs, OH), 3.93 (1H, dt, J=17.4, 5.8); ¹³C NMR (50 MHz, ppm, CDCl₃) 13.64, 18.51, 25.98, 38.45, 67.27, 117.83; IR (neat, cm⁻¹) 3400, 2950, 2240, 1460, 1415, 1120, 1015. Anal. Calcd for C₇H₁₄NO: C, 66.11; H, 10.30; N, 11.01. Found: C, 65.91; H, 10.32; N, 11.00.

3-Hydroxyhexanenitrile (2c). Rf 0.27 (hexane/ethyl acetate=3:1); bp 108 °C/ 2 Torr (Kugelrohr); ¹H NMR (200 MHz, δ, CDCl₃, J=Hz) 0.89 (3H, t, J=6.9), 1.29-1.36 (4H, m), 1.51-1.58 (2H, m), 2.50 (2H, dd, J=16.7,4.9), 3.93 (1H, dt, J=17.4, 5.8); ¹³C NMR (50 MHz, ppm, CDCl₃) 25.87, 68.34, 117.26, 117.40, 137.21; IR (neat, cm⁻¹) 3410, 2880, 2240, 1410, 1120, 930. Anal. Calcd for C₇H₁₄NO: C, 61.84; H, 7.27; N, 14.42. Found: C, 61.77; H, 7.34; N, 14.24.
2.81 (1H, brs, OH), 3.90 (1H, m); $^{13}$C NMR (50 MHz, ppm, CDCl$_3$) 13.81, 22.29, 25.95, 27.39, 36.06, 67.51, 117.86; IR (neat, cm$^{-1}$) 3420, 2925, 2240, 1465, 1410, 1120, 1020.

3-Hydroxy-4-methylpentanenitrile (2d).

Rf 0.40 (hexane/ethyl acetate=2:1); bp 115 °C/4 Torr (Kugelrohr); $^1$H NMR (200 MHz, δ, CDCl$_3$, J=Hz) 0.94 (3H, t, J=7.4, 7.3), 1.79 (1H, dq, J=13.3, 6.7), 2.50 (1H, d, J=3.1), 2.53 (1H, d, J=1.4), 2.63-2.77 (1H, brs, OH), 3.63-3.71 (1H, m); $^{13}$C NMR (50 MHz, ppm, CDCl$_3$) 17.24, 18.40, 23.52, 33.16, 72.42, 118.28; IR (neat, cm$^{-1}$) 3460, 2970, 2250, 1470, 1420, 1130, 1060.

Anal. Calcd for C$_6$H$_{11}$NO: C, 63.69; H, 9.80; N, 12.38. Found: C, 63.30; H, 9.81; N, 11.98.

3-Hydroxy undecanenitrile (2f). Rf 0.25 (hexane/ethyl acetate=1:1); bp 115 °C/3.5 Torr (Kugelrohr); $^1$H NMR (200 MHz, δ, CDCl$_3$, J=Hz) 0.86 (3H, t, J=6.5), 1.23 (12H, s), 1.55 (2H, t, J=6.4), 2.51 (2H, m), 2.78 (1H, brs, OH), 3.8-4.0 (1H, m); $^{13}$C NMR (50 MHz, ppm, CDCl$_3$) 14.01, 22.57, 25.31, 26.01, 29.12, 29.25, 29.36, 31.74, 36.45, 67.64, 117.79; IR (neat, cm$^{-1}$) 3455, 2925, 2250, 1450, 1410, 1120, 1075. Anal. Calcd for C$_{11}$H$_{21}$NO: C, 72.08; H, 11.55; N, 7.64. Found: C, 73.91; H, 11.64; N, 7.23.

3-Hydroxy-3-cyclohexylpropanenitrile (2h).

Rf 0.54 (hexane/ethyl acetate=2:1); bp 115 °C/2.8 Torr (Kugelrohr); $^1$H NMR (200 MHz, δ, CDCl$_3$, J=Hz) 0.88-1.33 (4H, m), 1.37-1.58 (1H, m), 1.58-1.95 (6H, m), 2.43 (1H, s, OH), 2.48 (1H, dd, J=16.9, 6.9), 2.58 (1H, dd, J=16.9, 4.9), 3.62-3.71 (1H, m); $^{13}$C NMR (50 MHz, ppm, CDCl$_3$) 25.31, 25.61, 25.77, 26.03, 27.77, 28.81, 42.74, 71.83, 118.24; IR (neat, cm$^{-1}$) 3455, 2925, 2250, 1450, 1060, 1040. Anal. Calcd for C$_{11}$H$_{15}$NO: C, 70.55; H, 9.87; N, 9.14. Found: C, 70.70; H, 10.07; N, 9.05.

3-Hydroxy-3-phenylpropanenitrile (2i).

Rf 0.32 (hexane/ethyl acetate=2:1); bp 115 °C/2.8 Torr (Kugelrohr); $^1$H NMR (200 MHz, δ, CDCl$_3$, J=Hz) 2.50-2.53 (1H, brs, OH), 2.74 (2H, d, J=6.2), 5.02 (1H, t, J=6.2), 7.38 (5H, s); $^{13}$C NMR (50 MHz, ppm, CDCl$_3$, J=Hz) 27.88, 70.02, 117.29, 125.48, 128.78, 128.88, 140.97; IR (neat, cm$^{-1}$) 3440, 2900, 2250, 1500, 1450, 1060, 760, 700.

3-Hydroxy-3-phenylpentanenitrile (2j).

Rf 0.29 (hexane/ethyl acetate=2:1); bp 115 °C/2.5 Torr (Kugelrohr); $^1$H NMR (200 MHz, δ, CDCl$_3$, J=Hz) 1.83-1.95 (2H, m), 2.48-2.62 (3H, m), 2.65-2.82 (2H, m), 3.93 (1H, dt, J=13.2, 5.7), 7.17-7.34 (5H, m); $^{13}$C NMR (50 MHz, ppm, CDCl$_3$) 26.21, 31.52, 37.85, 66.82, 117.65, 126.17, 128.32, 128.54, 140.67; IR (neat, cm$^{-1}$) 3420, 2910, 2240, 1590, 1490, 1450, 1410, 1050, 750, 700.

1-(Cyanomethyl)prop-2-enyl acetate (1g). To 3-hydroxy-4-pentenenitrile (2g) solution (963 mg, 9.92 mmol) in CH$_2$Cl$_2$ (20 mL) and pyridine (1.0 mL) was added a CH$_2$Cl$_2$ solution (20 mL) of acetylchloride (1.56 g, 19.9 mmol) at 0 °C and the solution was stirred at room temperature for 3 h. The reaction was quenched by the addition of crushed ice, extracted with CH$_2$Cl$_2$, dried over MgSO$_4$ and concentrated by evaporator. The crude product was purified by flash column chromatography (hexane/ethyl acetate=10:1) to provide acetate 2g (1.24 g, 8.91 mmol) in 90% yield. Rf 0.53 (hexane/ethyl acetate=2:1); bp 155 °C/45 Torr (Kugelrohr); $^1$H NMR (200 MHz, δ, CDCl$_3$, J=Hz) 0.93 (3H, t, J=7.4), 1.6-1.9 (2H, m), 2.07 (3H, s), 2.57 (2H, dd, J=17.2, 5.4), 2.63 (2H, dd, J=17.2, 5.4), 4.8-5.0 (1H, m);
\[ ^{13} \text{C NMR (50 MHz, ppm, CDCl}_3 \] 9.27, 20.76, 22.34, 26.18, 69.78, 116.23; IR (neat, cm\(^{-1}\)) 2950, 2200, 2250, 1702, 1220, 1050. Anal. Calcld for C\(_7\)H\(_{11}\)N\(_2\): C, 60.42; H, 6.52; N, 10.07. Found: C, 60.36; H, 6.59; N, 9.91.

Using the same procedure, 1-alkyl-2-cyano acetates 1b-1j were also prepared from the corresponding hydroxalkanenitriles.

I-(Cyanomethyl)propyl acetate (1b). Rf 0.53 (hexane/ethyl acetate=2:1); bp 155 °C/ 45 Torr (Kugelrohr); \(^1\)H NMR (200 MHz, \(\delta\), CDCl\(_3\), J=Hz) 0.93 (3H, t, J=7.4), 1.6-1.9 (2H, m), 2.07 (3H, s), 2.57 (1H, J=17.2, 5.4), 2.63 (1H, dd, J=17.2, 5.4), 4.8-5.0 (1H, m); \(^{13}\)C NMR (50 MHz, ppm, CDCl\(_3\)) 9.27, 20.76, 22.34, 26.18, 69.78, 116.23, 170.19; IR (neat, cm\(^{-1}\)) 2950, 2200, 1720, 1220, 1050. Anal. Calcld for C\(_7\)H\(_{11}\)N\(_2\): C, 59.56; H, 7.85; N, 9.92. Found: C, 58.89; H, 8.14; N, 9.87.

(R)-1b (>99% ee): \([\alpha]_{25}^{20} +79.2 (c=1.765, \text{CH}_3\text{OH})\)

(S)-1b (>99% ee): \([\alpha]_{25}^{20} -80.0 (c=1.050, \text{CHCl}_3)\)

l-(Cyanomethyl)butyl acetate (1e). Rf 0.41 (hexane/ethyl acetate=2:1); bp 172 °C/ 43 Torr (Kugelrohr); \(^1\)H NMR (200 MHz, \(\delta\), CDCl\(_3\), J=Hz) 0.92 (3H, t, J=6.6), 1.26-1.34 (2H, m), 1.61-1.73 (2H, m), 2.07 (3H, s), 2.56 (1H, J=16.9, 5.0), 2.70 (1H, dd, J=16.9, 5.0), 4.95 (1H, J=5.4, 5.3); \(^{13}\)C NMR (50 MHz, ppm, CDCl\(_3\)) 13.49, 18.24, 20.78, 22.78, 35.09, 68.33, 116.27, 170.18; IR (neat, cm\(^{-1}\)) 2950, 2240, 1740, 1225, 1025. Anal. Calcld for C\(_8\)H\(_{13}\)N\(_2\): C, 61.91; H, 8.44; N, 9.03. Found: C, 61.21; H, 8.48; N, 8.98.

1-(Cyanomethyl)-2-methylpropyl acetate (1d). Rf 0.39 (hexane/ethyl acetate=4:1); bp 122 °C/ 3.5 Torr (Kugelrohr); \(^1\)H NMR (200 MHz, \(\delta\), CDCl\(_3\), J=Hz) 0.95 (6H, d, J=6.9), 2.03 (1H, dd, J=13.6, 6.8), 2.10 (3H, s), 2.59 (1H, dd, J=17.1, 5.8), 2.64 (1H, dd, J=17.1, 4.0), 4.74-4.83 (1H, m); \(^{13}\)C NMR (50 MHz, ppm, CDCl\(_3\)) 17.68, 18.13, 20.61, 20.75, 30.85, 72.96, 116.48, 170.25; IR (neat, cm\(^{-1}\)) 2970, 2250, 1745, 1375, 1235, 1030. Anal. Calcld for C\(_9\)H\(_{15}\)N\(_2\): C, 63.88; H, 8.93; N, 8.28. Found: C, 63.75; H, 8.94; N, 8.28.

1-(Cyanomethyl)nonyl acetate (1f). Rf 0.50 (hexane/ethyl acetate=4:1); bp 158 °C/ 2.5 Torr (Kugelrohr); \(^1\)H NMR (200 MHz, \(\delta\), CDCl\(_3\), J=Hz) 0.86 (3H, t, J=6.5), 1.25 (12H, s), 1.66-1.76 (2H, m), 2.08 (3H, s), 2.57 (1H, dd, J=17.0, 5.2), 2.70 (1H, dd, J=17.0, 5.2), 4.96 (1H, J=5.4, 5.3); \(^{13}\)C NMR (50 MHz, ppm, CDCl\(_3\)) 14.00, 20.85, 22.54, 22.80, 29.12, 29.06, 29.24, 31.70, 33.07, 68.65, 116.28, 170.24; IR (neat, cm\(^{-1}\)) 2920, 2240, 1740, 1460, 1415, 1370, 1230, 1025. Anal. Calcld for C\(_{13}\)H\(_{23}\)N\(_2\): C, 69.19; H, 10.29; N, 6.22. Found: C, 69.19; H, 10.28; N, 6.23.

2-Cyano-1-cyclohexylethyl acetate (1h). Rf 0.48 (hexane/ethyl acetate=4:1); bp 166 °C/ 3.2 Torr (Kugelrohr); \(^1\)H NMR (200 MHz, \(\delta\), CDCl\(_3\), J=Hz) 0.85-1.41 (5H, m), 1.63-1.80 (6H, m), 2.10 (3H, s), 2.60 (1H, dd, J=17.1, 5.7), 2.70 (1H, dd, J=17.1, 5.0), 4.75-4.84 (1H, m); \(^{13}\)C NMR

2-Cyano-2-phenylethyl acetate (1i). Rf 0.59 (hexane/ethyl acetate=2:1); mp 115-117 °C; 1H NMR (200 MHz, δ, CDCl₃) 2.15 (3H, s), 2.89 (2H, d, J=5.8), 5.97 (1H, t, J=6.1), 7.39 (5H, s); 13C NMR (50 MHz, ppm, CDCl₃) 20.86, 25.49, 70.39, 115.98, 126.06, 128.89, 128.99, 137.11, 169.55; IR (neat, cm⁻¹) 2940, 2250, 1740, 1490, 1450, 1240, 1050, 760, 710.

1-(Cyanomethyl)-3-phenylpropyl acetate (1j). Rf 0.54 (hexane/ethyl acetate=2:1); bp 182 °C/2.8 Torr (Kugelrohr); 1H NMR (200 MHz, δ, CDCl₃) 1.98-2.05 (2H, m), 2.08 (3H, s), 2.66 (4H, ddd, J=31.8, 17.0, 5.1), 5.00 (1H, dt, J=13.5, 5.0), 7.14-7.33 (5H, m); 13C NMR (50 MHz, ppm, CDCl₃) 20.78, 22.90, 31.30, 34.59, 68.04, 116.12, 126.28, 128.20, 128.54, 140.04, 170.19; IR (neat, cm⁻¹) 2920, 2240, 1740, 1490, 1450, 1240, 1050, 760, 710.

Lipase-catalyzed hydrolysis. A typical example is described below: To an acetone solution (10 mL) of ester (±)-lb (1.41 g, 10.0 mmol) and thiacrown ether 4 (134 mg, 0.5 mmol), lipase PS aqueous solution (100 mL) was added and the resulting mixture was incubated at 35°C. The progress of the reaction was monitored by GLC analysis using Quadlex MS (<1.25 x 25 M). The reaction was stopped by the addition of small pieces of ice, then the mixture was extracted with ethyl acetate, dried over MgSO₄ and concentrated to dryness. The product 2b and remaining substrate (-)-1b were separated by silica-gel TLC (hexane/ethyl acetate= 2:1). The lipase solution was prepared in the following way: A boltex shaking suspension of lipase PS (705 mg) in deionized water (110 mL) was centrifuged at 3000 rpm for 5 min at room temperature, then 100 mL of the supernatant was immediately used as the enzyme solution. GLC analysis for determination of % ee of (±)-1b was carried out using a capillary column on chiral phase; Chiraldex G-TA, φ0.25 mm x 20 m; Carrier gas: He 40 mL/min; Temp (°C): 100 or 130, Inlet pressure: 1.35 kg/cm²; Amount 400 ng; Detection: FID. The results of GLC analyses of racemic 1a-1h are summarized in Table 3.

The attractive pheromone of the ant Myrmica scabrinodis (A) : (R)-3-Octanol (5). To a solution of (R)-lb (1.03 g, 10.4 mmol, >99%ee) in dry DMF (20 mL) was added a DMF (15 mL) solution of t-butyldimethylsilylchloride (2.74 g, 19.2 mmol) and imidazole (1.23 g, 18.1 mmol) in DMF (5 mL) at 0 °C. After stirring of the mixture for 5 h at room temperature, the reaction was quenched by crushed ice and extracted with ether. The organic layer was washed with water, dried over anhydrous MgSO₄ and concentrated by evaporator to dryness. Silica gel column chromatography (hexane-ethyl acetate=10:1) gave 6b (2.14 g, 10.06 mmol) in 97% yield as a colorless liquid. Under argon atmosphere, to a CH₂Cl₂ (10 mL) solution of 6b (346 mg, 1.6 mmol) was added DIBAL (1.6 mmol, 1.2 mL as 1.5 M toluene solution) at -78°C and the resulting mixture was stirred for 4 h at the same temperature. The reaction was quenched by 5 mL of water containing 1 g of SiO₂ powder. After further stirred for 15 min. at room temperature, silica gel was filtered off through a glass sintered filter. The resulting solution was extracted with CH₂Cl₂, dried over anhydrous...
Na$_2$SO$_4$ and concentrated by evaporator to give aldehyde (R)-7 (315 mg) which was immediately used for further Wittig reaction. To a solution of propyltriphenylphosphonium bromide (578 mg, 1.5 mmol) in ether (5 mL) was added n-BuLi (1.5 mmol as 1.6 M hexane solution) at 0 °C to form an orange solution. To this mixture was added an ether (5 mL) solution of (R)-7 (315 mg) at 0°C and the whole was stirred for 4 h at room temperature. The reaction was quenched by the addition of aqueous NH$_4$Cl solution and extracted with ether. The organic layer was dried over MgSO$_4$, and evaporated to give the crude oil. Silica gel flash column chromatography (hexane-ether=20:1) gave silyl ether (R)-8 (280 mg, 1.23 mmol) in 77% yield from 6b. Silyl ether (R)-8 (114 mg, 0.56 mmol) was hydrogenated using PtO$_2$ (12 mg) as catalyst under H$_2$ (1 atm) in ethanol (3.0 mL) at room temperature for 2 days. Silica gel flash column chromatography (hexane-ethyl acetate=50:1 to 20:1) gave (R)-5 (64.2 mg, 0.49 mmol) in 88% yield.

(R)-(E/Z)-6-(t-Butyldimethylsilyloxy)-3-octene (8). Rf 0.85 (hexane/ethyl acetate=10:1); bp. 80 °C/1 Torr (Kugelrohr) ; [a]$_D^{23}$ +16.1 (c0.84, CHCl$_3$); $^1$H NMR (200 MHz, δ, CDCl$_3$, J=Hz) 0.88-0.97 (6H, m), 1.16-1.50 (10H, m), 1.52 (1H, s, OH), 3.51-3.54 (1H, m); $^{13}$C NMR (50 MHz, ppm, CDCl$_3$) 9.87, 14.04, 22.64, 25.33, 30.11, 31.91, 36.90, 73.34; IR (neat, cm$^{-1}$) 3350, 2940, 2960, 1460, 1340, 1250.

(S)-5 ([a]$_D^{23}$+8.8(c.0.490, CHCl$_3$)) was prepared by procedure described above. The spectroscopic data were the same as (R)-5.

NMR Binding Experiments. $^{13}$C NMR binding studies (Table 2 and Fig. 4) were carried out with a Varian VXR-200 spectrometer (SC-NMR Laboratory of Okayama University). The substrate was dissolved in CDCl$_3$ at a concentration of 0.05 mol/L in the presence of thiacrown ether 4.

(R)-3-Octanol (5).$^{26}$ Rf 0.19 (hexane/ethyl acetate=10:1); bp. 88 °C/30 Torr (Kugelrohr); [a]$_D^{23}$+9.5 (c 0.960, CHCl$_3$), lit. $^{26}$-9.7; $^1$H NMR (200 MHz, δ, CDCl$_3$, J=Hz) 0.88-0.97 (6H, m), 1.16-1.50 (10H, m), 1.52 (1H, s, OH), 3.51-3.54 (1H, m); $^{13}$C NMR (50 MHz, ppm, CDCl$_3$) 9.87, 14.04, 22.64, 25.33, 30.11, 31.91, 36.90, 73.34; IR (neat, cm$^{-1}$) 3350, 2940, 2960, 1460, 1340, 1250. Anal. Calcd for C$_{14}$H$_{26}$O$_2$: C, 69.35; H, 12.47. Found: C, 69.00; H, 12.50.

(R)-3-Octanol (5).$^{26}$ Rf 0.19 (hexane/ethyl acetate=10:1); bp. 88 °C/30 Torr (Kugelrohr); [a]$_D^{23}$+9.5 (c 0.960, CHCl$_3$), lit. $^{26}$-9.7; $^1$H NMR (200 MHz, δ, CDCl$_3$, J=Hz) 0.88-0.97 (6H, m), 1.16-1.50 (10H, m), 1.52 (1H, s, OH), 3.51-3.54 (1H, m); $^{13}$C NMR (50 MHz, ppm, CDCl$_3$) 9.87, 14.04, 22.64, 25.33, 30.11, 31.91, 36.90, 73.34; IR (neat, cm$^{-1}$) 3350, 2940, 2960, 1460, 1340, 1250. Anal. Calcd for C$_{14}$H$_{26}$O$_2$: C, 69.35; H, 12.47. Found: C, 69.00; H, 12.50.
Table 2. Chemical shift changes of 50MHz 13C NMR spectra (in 0.05 M CDCl3) of 1b, 1f, 2b and 2f in the presence of thiacyclon after 4 (2 mol eq.)

<table>
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<tr>
<th>Compound</th>
<th>Amount of 4</th>
<th>C5 ppm</th>
<th>C7 ppm</th>
<th>C4 ppm</th>
<th>C2 ppm</th>
<th>C3 ppm</th>
<th>C1(CN) ppm</th>
<th>C6(CO) ppm</th>
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<tbody>
<tr>
<td>1b (OAc)</td>
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<td>9.26</td>
<td>20.76</td>
<td>22.34</td>
<td>26.18</td>
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<td>116.23</td>
<td>170.19</td>
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<tr>
<td>1b (OAc)</td>
<td>2.0 eq.</td>
<td>9.35</td>
<td>20.85</td>
<td>22.42</td>
<td>26.24</td>
<td>69.84</td>
<td>116.23</td>
<td>170.27</td>
</tr>
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<td>2b (OH)</td>
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<td>***</td>
<td>25.50</td>
<td>29.33</td>
<td>68.81</td>
<td>117.86</td>
<td>***</td>
</tr>
<tr>
<td>2b (OH)</td>
<td>2 eq.</td>
<td>9.61</td>
<td>***</td>
<td>25.64</td>
<td>29.50</td>
<td>69.09</td>
<td>117.88</td>
<td>***</td>
</tr>
<tr>
<td>1f (OAc)</td>
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<td>1f (OAc)</td>
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<td>2f (OH)</td>
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<td>2f (OH)</td>
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<td>25.32</td>
<td>36.54</td>
<td>67.81</td>
<td>117.55</td>
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Table 3. Results of GLC analysis and [α]D values of Acetates 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>[α]D (c in CHCl3)</th>
<th>GLC</th>
<th>GLC k</th>
<th>GLC α</th>
<th>GLC Column Temp</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-1a</td>
<td>Me</td>
<td>[α]D 0 = +46.9 (c1.15)</td>
<td>91%ee</td>
<td>5.2</td>
<td>1.1</td>
<td>100</td>
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<tr>
<td>(S)-1a</td>
<td>Me</td>
<td>[α]D 0 = -51.4 (c1.23)</td>
<td>94%ee</td>
<td>9.4</td>
<td>2.8</td>
<td>2.5</td>
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<tr>
<td>(R)-1b</td>
<td>Et</td>
<td>[α]D 0 = +60.0 (c1.81)</td>
<td>&gt;99%ee</td>
<td>7.3</td>
<td>2.7</td>
<td>100</td>
</tr>
<tr>
<td>(S)-1b</td>
<td>Et</td>
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<td>94%ee</td>
<td>11.2</td>
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<tr>
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<td>n-Pr</td>
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<td>n-Pr</td>
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<td>52%ee</td>
<td>15.3</td>
<td>6.6</td>
<td>1.6</td>
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<td>i-Pr</td>
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<td>4.1</td>
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<td>19.0</td>
<td>8.5</td>
<td>2.1</td>
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<td>n-Bu</td>
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<td>17.2</td>
<td>7.6</td>
<td>100</td>
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<tr>
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<td>25.4</td>
<td>11.7</td>
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<td>26.7</td>
<td>12.4</td>
<td>130</td>
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<tr>
<td>(S)-1f</td>
<td>n-Oct</td>
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<td>20%ee</td>
<td>35.1</td>
<td>16.5</td>
<td>1.3</td>
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<td>(R)-1g</td>
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<td>73% ee</td>
<td>6.3</td>
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<td>100</td>
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<td>(S)-1g</td>
<td>Vinyl</td>
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<td>67%ee</td>
<td>8.3</td>
<td>3.2</td>
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<td>(R)-1h</td>
<td>c-Hex</td>
<td>[α]D 0 = -31.4 (c0.52)</td>
<td>99% ee</td>
<td>13.6</td>
<td>5.5</td>
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<tr>
<td>(S)-1h</td>
<td>c-Hex</td>
<td>[α]D 0 = +33.3 (c0.74)</td>
<td>82% ee</td>
<td>16.6</td>
<td>7.3</td>
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<tr>
<td>(R)-1i</td>
<td>Ph</td>
<td>[α]D 0 = -67.3 (c0.65)</td>
<td>&gt;99% ee</td>
<td>15.8</td>
<td>6.9</td>
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<tr>
<td>(S)-1i</td>
<td>Ph</td>
<td>[α]D 0 = -72.8 (c0.64)</td>
<td>&gt;99% ee</td>
<td>17.2</td>
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<tr>
<td>(R)-1j</td>
<td>PhCH2CH2</td>
<td>[α]D 0 = -7.7 (c0.81)</td>
<td>59% ee</td>
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<td>7.4</td>
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<tr>
<td>(S)-1j</td>
<td>PhCH2CH2</td>
<td>[α]D 0 = +17.9 (c1.03)</td>
<td>34% ee</td>
<td>19.0</td>
<td>8.5</td>
<td>1.1</td>
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</table>

a) GLC analysis for determination of % ee of (+)-I was carried out using a capillary column on chiral phase; Chiraldex G-TA (ø0.25 x 20 M).
Chapter 1-2.

Lipase-catalyzed Partial Hydrolysis of Diacetate (9). To deionized water (2.4 mL) was added PCL powder (75 mg) and this was suspended vigorously for 5 minutes. The mixture was centrifuged at 3000 rpm for 5 minutes and the supernatant (1.2 mL) was used for the enzymatic reaction. The lipase solution (1.2 mL) was added to diacetate 9 (75 mg, 0.403 mmol) together with thiacrown 4 (5.4 mg, 5 mol% toward a substrate) in acetone solvent (0.12 mL). The mixture was stirred at 35°C for 0.7 h. The progress of the reaction was monitored by GLC analysis using Quadlex MS (0.25 mm x 25 m). After consumption of the starting material 9, the reaction was quenched with crushed ice and NaCl and the mixture was immediately extracted with CH2Cl2. The organic layer was dried over MgSO4. The crude product was purified by silica gel flash chromatography (hexane/AcOEt=7:1) and gave illonoacetate, a mixture of 10 and 11, (51.50 mg, 0.357 mmol) in 89% yield. The ratio of 10 and 11 was determined as 95:5 by capillary GLC analysis using Chiraldex G-TA (0.25 mm x 20 m, He) as tert-Butyldimethylsilyl ether 12 and 13. Monoacetate 10 and 11 were isolated by silica gel thin layer chromatography (TLC), though triplicate TLC separation was needed.

4-Acetoxy-2-methyl-2-butenyl acetate (9). Rf 0.2 (hexane: ethyl acetate=6:1); bp. 105 °C/ 2 Torr (kugelrohr); 1H NMR (200 MHz, δ, CDCl3, J=Hz) 1.69 (3H, s) 2.02 (3H, s) 2.05 (3H, s) 4.45 (2H, s) 4.59 (2H, d, J=6.8 Hz) 5.57 (1H, tq, J=6.92 Hz, 1.32 Hz); 13C NMR (50 MHz, ppm, CDCl3) 14.04, 20.82, 60.49, 68.43, 121.58, 135.68, 170.80, and 170.55; IR (neat, cm⁻¹) 2930, 1730, 1430, 1220, and 950.

4-Hydroxy-3-methyl-2-butenyl acetate (10). Rf 0.5 (hexane: ethyl acetate=1:1); bp. 122 °C/ 15 Torr (Kugelrohr); 1H NMR (200 MHz, δ, CDCl3, J=Hz) 1.67 (3H, s) 2.00 (3H, s) 2.16 (1H, brs, OH) 3.98 (2H, s) 4.58 (2H, d, J=6.8 Hz) 5.52-5.57 (1H, m); 13C NMR (50 MHz, ppm, CDCl3) 13.59, 20.78, 60.80, 67.10, 118.02, 140.79, and 171.12; IR (neat, cm⁻¹) 3400, 2900, 2850, 1720, 1240, and 950; Anal. Calcd for C7H12O3: C, 58.32; H, 8.39. Found: C, 57.82; H, 8.40.

4-Hydroxy-2-methyl-2-butenyl acetate (11). Rf 0.5 (hexane: ethyl acetate=1:1); bp. 125 °C/ 15 Torr (Kugelrohr); 1H NMR (200 MHz, δ, CDCl3, J=Hz) 1.60 (1H, brs, OH) 1.67 (3H, s) 2.06 (3H, s) 4.17 (2H, d, J=6.8 Hz) 4.45 (2H, s) 5.64 (1H, t, J=6.6 Hz); 13C NMR (50 MHz, ppm, CDCl3) 13.97, 20.87, 58.83, 68.98, 121.01, 133.12, and 171.12; IR (neat, cm⁻¹) 3400, 2950, 2850, 1730, 1230, and 780; Anal. Calcd for C5H12O3: C, 58.32; H, 8.39. Found: C, 58.02; H, 8.56.

2-Methyl-2-buten-1,4-diol (12). Rf 0.3 (CH2Cl2: methanol = 10:1); Bp. 86 °C/ 5 Torr (Kugelrohr); 1H NMR (200 MHz, δ, CDCl3, J=Hz) 1.65 (3H, s) 3.23 (2H, brs, OH) 3.97 (2H, d) 4.16 (2H, d, J=6.8 Hz) 5.61 (2H, dq, J=6.8Hz, 1.4 Hz); 13C NMR (50 MHz, ppm, CDCl3) 13.63, 58.69, 67.38, 123.26, and 137.99; IR (neat, cm⁻¹) 3369, 2919, 1675, 1458, and 997.

4-t-Butyldimethylsilyloxy-3-methyl-2-butenyl acetate (13). Rf 0.7 (hexane: ethyl acetate=3:1); Bp. 90 °C/ 2.5 Torr (Kugelrohr); 1H NMR (200 MHz, δ, CDCl3, J=Hz) 0.054 (6H, s) 0.90 (9H, s) 1.65 (3H, s) 2.04 (3H, d) 4.02 (2H, s) 4.62 (2H, d, J=7.3 Hz) 5.60 (1H, dq, J=7.1 Hz, 1.5 Hz); 13C NMR (50 MHz, ppm, CDCl3) 13.53, 18.34, 20.96, 25.86, 60.88, 67.37, 117.35.
IR (neat, cm⁻¹) 2950, 2850, 1740, 1230, and 960;
Found: C, 60.64; H, 10.44.

4-t-Butyldimethylsilyloxy-2-methyl-2-butenyl acetate (14). Rf 0.7 (hexane: ethyl acetate=1:1); bp. 90 °C/2.5 Torr (Kugelrohr);
¹H NMR (200 MHz, CDCl₃, J=Hz) 0.057 (6H, s) 0.89 (9H, s) 1.65 (3H, s) 2.06 (3H, s) 4.22 (2H, d, J=6.0 Hz) 4.45 (2H, s) 5.58 (1H, t, J=6.2 Hz);
¹³C NMR (50 MHz, ppm, CDCl₃) 14.11, 18.34, 20.90, 25.92, 59.77, 69.28, 128.53, 131.05, and 170.80; IR (neat, cm⁻¹) 2950, 2850, 1740, 1240, and 840;
Found: C, 60.64; H, 10.44.

Results of GLC analysis: ChiralDEX G-TA, l/J 0.25 mm x 20 m,
Carrier gas: He 40 mU/min. Temp: 100°C, Inlet pressure: 1.35 Kg/cm², Amount 400 ng.
Detection: FID; t-BuMe₂Si ether 13: Rt =22.8 min., t-BuMe₂Si ether 14: Rtₛ =25.8 min.

NMR Binding Experiments. ¹³C NMR studies were carried out with a Varian VXR-200 spectrometer. Thiacrown ether 4 was dissolved in CDCl₃ at a concentration of 0.05 mol/L and the results are summarized in Table 5.

Chapter1-3.

Kinetic measurement. A typical example is as follows: An aqueous solution of lipase PS (5.25 mL, which includes 3.7 mg of the enzyme)’, optically pure acetate 1b (typically, about 0.01-1.0 M)(1 M= 1 mol dm⁻³) in acetone (0.50 mL), and thiacrown ether 4 (7.4 mg) was stirred at 200 rpm in a test tube with a rubber stopper in a water bath at 35°C. At an appropriate time, samples (50-100 μL) were withdrawn and filtered through a cellulose acetate membrane filter, and eluted with ethyl acetate. The filtrate was analyzed by gas chromatography (“Quadrex”bonded fused silica methyl silicone, φ 0.25 mm x 25 m, N₂) to obtain the conversion; v= 2.44 x10⁻³ (mmol/min.)/ 0.0037 g(E)= 6.6 x 10⁻⁴ mol dm⁻³ mg (E)⁻¹ min⁻¹ Five data points were routinely collected to measure the initial rate (vᵢ) at each substrate concentration [Sᵢ]. Plot of vᵢ against [Sᵢ] afforded a saturation curve, and the apparent Vmax and Km values were obtained by the nonlinear least-squares method applied to the Michaelis-Menten type of equation as follows: vᵢ= Vmax(E)mg[Sᵢ]/(Km + [Sᵢ]), where Vmax is normalized by the weight of lipase (E)mg.

Preparation of the lipase solution: A typical example is as follows: A Boltex shaking suspension of PCL (51.8 mg) in deionized water (7.35 mL) was centrifuged at 3000 rpm for 5 min at room temperature, then 5.25 mL of the supernatant was immediately used as the enzyme solution. Because the enzyme content in PCL used here was about 10% by weight and the remainder was mostly amorphous inorganic compounds (celite), the content of the enzyme was thus estimated as 3.7 mg; this (3.7 mg/ g PCL) was used as the enzyme amount when the kinetic parameters were calculated because it was used by PCL having the same lot number in all experiments.

(±)-3-Hydroxy-2-methylpentanenitrile (16a). To a solution of diisopropyl amine (3.04 g, 30.0 mmol) in THF (30.0 mL) under argon atmosphere at -10 °C was added a solution of n-BuLi in hexane (1.62 M, 17.0 mL, 27.5 mmol). The mixture was stirred at 10 °C for 1 h and cooled to -78°C and then a solution of propionitrile (1.23 g, 30.0 mmol) in THF (15.0 mL) was added. The mixture was stirred at -78 °C for 1 h, then a
solution of propionaldehyde (1.40 g, 25.0 mmol) in THF (15.0 mL) was added, and the mixture was again stirred at -78 °C for 3 h. The reaction was quenched with saturated aqueous NH₄Cl and treated with aqueous 2M HCl. The mixture was extracted with Et₂O, and the combined organic layers were dried over MgSO₄, filtered, and concentrated by evaporator. The obtained yellow oily residue was purified by flash column chromatography (gradient elution hexane/ethyl acetate=8:1 to 4:1) to give an alcohol 16a as a colorless oil (2.23 g, 23.0 mmol) in 92% yield: Rf 0.29 (hexane/ethyl acetate=2:1); bp 95 °C/4 Torr (Kugelrohr); ¹H NMR (200 MHz, δ, CDCl₃, J= Hz) 0.99 (3H, t, J= 7.2), 1.36 (3H, d, J= 7.2), 1.83 (2H, dq, J= 7.4, 4.3), 3.52 (1H, ddd, J= 7.2, 5.7, 4.3 ); ¹³C NMR (50 MHz, ppm, CDCl₃) 9.85, 14.59, 20.08, 32.50, 120.92; IR (neat, cm⁻¹) 3459, 2973, 2246, 1143, 977; Found: C, 63.43; H, 9.88; N, 12.41%. Calcd for C₈H₁₃NO: C, 63.69; H, 9.80; N, 12.38%.

Using the same procedure, hydroxyalkanenitriles, 16b-16c, were also prepared from the corresponding aldehydes. (±)-3-Hydroxy-2-ethylpentanenitrile (16b). Rf 0.40 (hexane/ethyl acetate=2:1); bp 102 °C/3 Torr (Kugelrohr); ¹H NMR (200 MHz, δ, CDCl₃, J= Hz) 1.01 (3H, t, J= 7.5), 1.11 (3H, t, J= 7.5), 1.25 (1H, s), 1.59-1.90 (4H, m), 2.54 (1H, ddd, J= 9.6, 5.7, 3.9 ) 3.61(1H, dt, J= 6.5, 3.9); ¹³C NMR (50 MHz, ppm, CDCl₃) 9.95, 11.94, 22.57, 28.57, 40.86, and 119.96; IR (neat, cm⁻¹) 3495, 2972, 2243, 1744, 1470, 1374, 1231, 1022; Found: C, 61.73; H, 8.39; N, 9.00%. Calcd for C₇H₁₃NO: C, 61.91; H, 8.44; N, 9.03%. Using the same procedure, acetates 15a, 15b, and 15c were also prepared from the corresponding hydroxyalkanenitriles.

(±)-2-Cyanopentan-3-yl acetate (15a). To 2-methyl-3-hydroxypentanenitrile (16a) solution (963 mg, 9.92 mmol) in dichloromethane (CH₂Cl₂) (20.0 mL) and pyridine (1.0 mL) was added a CH₂Cl₂ solution (20 mL) of acetylchloride (1.56 g, 19.9 mmol) at 0 °C and the solution was stirred at room temperature for 3 h. The reaction was quenched by the addition of crushed ice, extracted with CH₂Cl₂, dried over MgSO₄ and concentrated by evaporator. The crude product was purified by flash column chromatography (hexane/ethyl acetate=10:1) to provide acetate 15a (1.24 g, 8.91 mmol) in 90% yield: Rf 0.55 (hexane/ethyl acetate=2:1); bp 100 °C/3 Torr (Kugelrohr); ¹H NMR (200 MHz, δ, CDCl₃, J= Hz) 0.92 (3H, t, J= 7.4), 1.29 (3H, d, J= 7.0), 1.73 (2H, dq, J= 9.7, 7.5), 2.12 (3H, s), 2.88 (1H, dq, J=7.2, 4.13), 4.86 (1H, ddd, J= 7.4, 5.9, 4.2); ¹³C NMR (50 MHz, ppm, CDCl₃) 9.52, 14.47, 20.74, 25.40, 29.85, 73.94, 119.80, 170.40; IR (neat, cm⁻¹) 2977, 2247, 1743, 1458, 1387, 1235; Found: C, 61.73; H, 8.39; N, 9.00%. Calcd for C₈H₁₃NO₂: C, 61.91; H, 8.44; N, 9.03%. Using the same procedure, acetates 15a, 15b, and 15c were also prepared from the corresponding hydroxyalkanenitriles.

(±)-4-Cyanohexan-3-yl acetate (15b). Rf 0.68 (hexane/ethyl acetate=2:1); bp 113 °C/6 Torr (Kugelrohr); ¹H NMR (200 MHz, δ, CDCl₃, J= Hz) 0.93 (3H, t, J= 7.6), 1.09 (3H, t, J=7.4), 1.57-1.83 (4H, m), 2.12 (3H, s), 2.68 (1H, ddd, J=8.8, 6.4, 3.9), 4.94 (1H, ddd, J= 7.5, 5.9, 3.9); ¹³C NMR (50 MHz, ppm, CDCl₃) 9.59, 11.81, 20.81, 22.48, 25.79, 37.98, 72.82, 119.01, 170.39; IR (neat, cm⁻¹) 2972, 2243, 1744, 1463, 1374, 1231, 1022;
Found: C, 63.50; H, 8.95; N, 8.32%. Calcd for C₂₉H₅₀N₂: C, 63.88; H, 8.93; N, 8.28%.

(±)-3-Cyanobutan-2-yl acetate (15c). Rf 0.55 (hexane/ethyl acetate=2:1); bp 95 °C/6Tor (Kugelrohr); ¹H NMR (200 MHz, δ, CDCl₃, J= Hz) 1.32 (3H, d, J= 7.2), 1.37 (3H, d, J= 6.4), 2.11 (3H, s), 2.82 (1H, dq, J= 7.3, 4.7), 4.97 (1H, dq, J= 6.4, 4.6); ¹³C NMR (50 MHz, ppm, CDCl₃) 14.16, 17.90, 20.85, 31.46, 69.61, 119.79, 170.06; IR (neat, cm⁻¹) 2988, 2246, 1742, 1453, 1378, 1238, 1038.

Lipase-catalyzed hydrolysis for the experiment in Table 7. A typical example is described below: To an acetone solution (10.0 mL) of ester (±)-15a (1.41 g, 10.0 mmol) and thiacrown ether 4 (0.134 g, 0.50 mmol), lipase PS aqueous solution (100 mL) was added and the resulting mixture was incubated at 35°C. The progress of the reaction was monitored by GLC analysis using Quadrex MS (φ 0.25 mm x 25 m). The reaction was stopped by the addition of small pieces of ice, then the mixture was extracted with ethyl acetate, dried over MgSO₄ and concentrated by evaporator. The product 16a and remaining substrate (-)-15a were separated by silica-gel flash column chromatography (hexane/ethyl acetate=10:1 to 2:1). The lipase solution was prepared in the following way: A Boltex shaking suspension of PCL (0.705 g) in deionized water (110 mL) was centrifuged at 3000 rpm for 5 min at room temperature, then 100 mL of the supernatant was immediately used as the enzyme solution. GLC analysis for measurement of % ee of (±)-15a was done using a capillary column on chiral phase; Chiraldex G-TA, φ0.25 mm x 20 m; Carrier gas: He 40 mL min⁻¹; Temp (°C); 100 or 130, Inlet pressure; 1.35 kg cm⁻²; Amount 400 ng; Detection; FID. The results of GC analyses of racemic 15a and 15b are summarized as follows. anti-15a: tᵣ=7.04 min (2S,3R) and 12.86 min (2R,3S), syn-1b: tᵣ=8.62 min and 9.86 min; anti-15b: tᵣ=9.20 min (2S,3R) and 12.88 min (2R,3S), syn-1c: tᵣ=10.35 min and 10.72 min; anti-15c: tᵣ=5.00 min (2S,3R) and 7.89 min (2R,3S), syn-15c: tᵣ=5.25. Two signals due to syn-isomers ((2R,3R)-15c and (2S,3S)-15c) completely overlapped. A slight overlap of the large signal (tᵣ=5.25) corresponded to two syn-isomers with the signal of (2S,3R)-15c (tᵣ=5.20) was occurred, so that it was difficult to calculate the %ee of anti-15c precisely. Fortunately, It was succeeded in measuring the %ee of anti-15c after the lipase-catalyzed reaction because the process reduced the content of the syn-isomers included in the product at a level that did not affect any result for calculating the %ee of anti-15c.

Preparation of optically active 3-hydroxy-2-methylalkanenitriles through lipase-catalyzed reaction by a double enzymatic reaction strategy. (2S,3R)-2-Cyanopentan-3-yl acetate (15a). To a suspension of lipase AL (4.04 g) in deionized water (260 mL) was added an acetone (26 mL) solution of racemic acetate 15a (8.08 g, 52.1 mmol) and this was stirred at 35°C for 160 h. The reaction was monitored by GC analysis and stopped by crushed ice and NaCl when reaction conversion reached about 25%. The mixture was extracted with ethyl acetate, dried over MgSO₄, and concentrated by evaporator. Silica-gel flash column chromatography (hexane/ethyl acetate=10:1) gave the product 16a (1.49 g, 13.2 mmol, 25%) and the unreacted acetate 15a (4.05 g, 26.1 mmol, 50%). Capillary GC analysis showed that a diastereomeric ratio of 16a was 86% anti selectivity. A mixture of 16a
(1.06 g, 9.40 mmol, 86% de), lipase PS (530 mg), and vinyl acetate (1.23 g, 14.3 mmol) in 25.0 mL of diisopropyl ether was stirred at RT for 41 h. The mixture was filtered through a glass sintered filter with a celite pad to remove the lipase and washed with CH₂Cl₂. The filtrate was concentrated by evaporator and chromatographed on silica-gel (hexane/ethyl acetate = 7:1) and gave acetate 15a (864 mg, 5.57 mmol, 59%) and alcohol 16a (430 mg, 3.80 mmol, 41%). Capillary GC analysis using chiral column (G-Ta) showed that the enantiomeric excess of 15a: [α]D 59 +49.5 (c 1.01, CHCl₃) obtained was >99% ee with 96% de (anti).

The stereochemistry of 16a produced by the lipase AL-catalyzed reaction was analyzed as reference compounds as follows (Scheme 2): To a DMF (13.0 mL) solution of NaH (239 mg, 5.99 mmol) was added a DMF (2.0 mL) solution of 16a: [α]D 5 0.5 (c 0.88, CHCl₃) (0.452 g, 3.99 mmol) and benzylbromide (819 mg, 4.79 mmol) at 0°C and the mixture was stirred for 0.5 h at RT. Silica gel flash column chromatography of the product gave 548 mg (2.70 mmol) of 3-benzyloxy-2-methylpentanenitrile in 68% yield. The nitrile (460 mg, 2.26 mmol) was dissolved in CH₂Cl₂ (10 mL) and then treated with DIBAH (3.45 mmol in 1.5 M toluene) at -78°C for 1 h. The reaction was quenched by NH₄Cl aqueous solution and acidified by 2 M HCl, and the mixture was extracted with CH₂Cl₂. The combined organic layers were concentrated by evaporator and silica gel flash column chromatography (hexane/ethyl acetate = 50:1 to 20:1) gave 204 mg (0.994 mmol) of 3-benzyloxy-2-methylpentanal (17): [α]D 23 -38.7 (c 1.36, CHCl₃) in 44% yield. Comparing the spectrum data of 1H and 13C-NMR analysis of 17 with those of reference, 33 aldehyde 17 was confirmed to have anti configuration.

The absolute configuration of 15a obtained by the lipase-catalyzed reaction was identified as follows: acetate 15a was treated with 1.0 eq. of lithium hydroxide (LiOH·H₂O) in a mixed solvent (THF-MeOH-H₂O = 3:1:1) at RT for 18 h to provide alcohol 16a: [α]D 23 +0.5 (c 0.88, CHCl₃) which was treated with 6 M HCl under reflux conditions for 72 h to give 3-hydroxy-2-methylpentanoic acid: [α]D 0 -7.0 (c 1.59, CHCl₃). This acid was then reacted with t-butyldimethylsilylechloride in the presence of imidazole as base in DMF at RT for 6 h to give the t-butyldimethylsilylester which was then treated with LiOH·H₂O in a mixed solvent of THF-MeOH-H₂O (3:1:1) at RT and afforded 3-(t-butyldimethylsilyloxy)-2-methylpentanoic acid (18): [α]D 0 -10.4 (c 1.98, CHCl₃) in 27% yield (two steps). Comparing the value of the optical rotation with the authentic sample (2R,3S)-18: +10.0 (c 1.11, CHCl₃), the configuration of the 2-position and 3-position of the starting nitrile 15a was assigned as 2S,3R.

(2S,3R)-3-Benzylloxy-2-methylpentanal (17). 3H NMR (200 MHz, δ, CDCl₃, J = Hz) 0.96 (3H, t, J = 7.4), 1.08 (3H, d, J = 7.1), 1.55-1.75 (2H, m) 2.68 (1H, dq, J = 6.9, 2.2), 3.67-3.71 (1H, m) 4.48 (1H, d, AB, J = 11.5) 4.60 (1H, d, AB, J = 11.5) 7.26-7.36 (5H, m), 9.76 (1H, d, J = 2.1); 13C NMR (50 MHz, ppm, CDCl₃) 8.83, 10.12, 23.41, 48.94, 71.53, 80.42, 127.71, 127.84, 128.38, 138.18, 204.67; IR (neat, cm⁻¹) 2969, 2873, 1724, 1458, 1070, 743. These spectra were identical with those in reference. 34
(2S,3R)-3-(t-Butyldimethylsilyloxy)-2-methylpentanoic acid (18). 3H NMR (200 MHz, 8, CDCl3, J = Hz) 0.08 (3H, s), 0.09 (3H, s) 0.85 (9H, s) 0.91 (3H, t, J = 5.4) 1.18 (3H, d, J = 7.0) 1.49-1.64 (2H, m) 2.67 (1H, dq, J = 7.2, 5.4) 3.78-3.85 (1H, m) 0.85 (9H, s) 0.91 (3H, t, J = 5.4) 1.18 (3H, d, J = 7.0) 1.49-1.64 (2H, m) 2.67 (1H, dq, J = 7.2, 5.4) 3.78-3.85 (1H, m) 11.15 (1H, s); 13C NMR (50 MHz, ppm, CDCl3) 8.90, 13.51, 15.23, 25.73, 26.98, 44.30, 75.09, 178.66; IR (neat, cm⁻¹) 3104, 2954, 1710, 1465, 1255, 1119, 1013, 839

Scheme 3

Measurement of the diastereo-favoritism of the lipase-catalyzed reaction. Acetate 16c produced by the lipase-catalyzed reaction was converted to the authentic sample, i.e. 5-hydroxy-4-methylhexan-3-one (21) as shown in Scheme 4.

To N,N-dimethylformamide (DMF) (26.0 mL) solution of 16c (1.35 g, 13.6 mmol, 87% de) and TBDMSI (2.47 g, 16.4 mmol) was added a DMF (4.0 mL) solution of imidazole (1.39 g, 20.4 mmol) at 0°C and the mixture was stirred at RT for 6 h. The reaction mixture was extracted with ether and the combined organic layer was dried over MgSO4 and concentrated by evaporator. Silica gel flash column chromatography (hexane/ethyl acetate = 100:1) gave TBDMS ether 19 (2.88 g, 13.8 mmol) in 99% yield. A dichloromethane (8.0 mL) solution of 19 (274 mg, 1.28 mmol) was added to 1.50 mmol of diisobutyraluminum hydride (DIBAH, 1.5 M in toluene) dropwise at -78°C and the reaction mixture was stirred at the same temperature for 1.5 h. The reaction was quenched by addition of 2 M HCl and the mixture was extracted with ether. The combined organic layer was dried over MgSO4 and concentrated by evaporator to give a crude oil which was used in the next reaction without purification. The crude oil was dissolved in 6.0 mL of THF and was treated with ethylmagnesium bromide (2.6 mmol, 0.65 M in THF) at -78°C for 1 h. The reaction was quenched by addition of NH4Cl saturated aqueous solution and acidified by 2 M HCl. The mixture was extracted with ether, and the combined organic layer was dried and concentrated by evaporator. Silica gel flash column chromatography (hexane/ethyl acetate = 20:1) gave alcohol 20 (280 mg, 1.14 mmol) in 89% yield. To a suspension of solution of RuCl3·H2O (6.8 mg, 0.032 mmol) and NaIO4 (103 mg, 0.481 mmol) in 5 mL of mixed solvent (CH3CN:H2O = 2:3) was added a CCl4 (2.0 mL) solution of 20 (81 mg, 0.329 mmol) at RT and the mixture was stirred for 24 h at RT. The reaction was quenched by addition of 2-propanol and was extracted with CH2Cl2. The combined organic layer was washed with brine, NaHCO3 saturated aqueous solution, and finally with water. The organic layer was dried (MgSO4) and concentrated by evaporator to dryness to give an oily product. This was dissolved in THF (2.0 mL) and treated with tetrabutylammonium fluoride (TBAF) (1.0 M in THF)
at 0°C for 3 h. The mixture was extracted with ether, dried (MgSO₄), and concentrated by evaporator. Silica gel flash column chromatography (hexane/ethyl acetate= 10:1) gave 5-hydroxy-4-methylhexan-3-one (21) ³⁵ (22 mg, 0.169 mmol) in 51% yield (two steps from 20). This hydroxy ketone was composed of two isomers. ³¹H NMR analysis of 21 showed that the major isomer was the anti isomer because the coupling constant (J value) of H₂-H₅ was 7.22 Hz, while that of the minor isomer was 3.25 Hz.

<table>
<thead>
<tr>
<th>1) LiOH·H₂O</th>
<th>2) TBDMSCl, Im, DMF, RT</th>
<th>3) OTBDMS</th>
<th>DIBAH</th>
<th>CH₂Cl₂, -78°C</th>
<th>4) OTBDMS-CHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2R,3S)-15c</td>
<td>Y = 99%</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(2S,3R)-3-Cyanobutan-2-yl acetate (15c). Using the same protocol described above, (2S,3R)-15c was obtained,[β]D +10.5 (c 1.30, CHCl₃) 65% de (anti), >99% ee. Preparation of (2R,3S)-15c was accomplished using two different types of enzymes as described in Scheme 2.

(2R,3S)-3-Hydroxy-2-methylbutanenitrile (16c). To a suspension of lipase OF (4.34 g) in de-ionized water (180 mL) was added an acetone (15 mL) solution of acetate (±)-16c (7.24 g, 51.3 mmol) and thiocrown ether 4 (0.040 g, 0.15 mmol, 0.5 mol%) and the mixture was stirred at 35°C for 36 h. The reaction mixture was extracted with CH₂Cl₂ and concentrated by evaporator. Silica gel flash column chromatography (hexane/ethyl acetate= 7:1 to 2:1) gave alcohol 16c (3.90 g, 35.1 mmol, 68%) and acetate 15c (0.869 g, 6.16 mmol, 12%). Diastereomeric excess of 15c was measured by capillary GC analysis as 58% de with >99% ee. Acetate 15c (1.24 g, 8.78 mmol) was then hydrolyzed by hemi-cellulase (Amano) (744 mg) in 15 mL of 0.1 M phosphate buffer (pH 7.2) at 35°C for 137 h. Silica gel flash column chromatography of the extract gave alcohol 16c (456 mg, 4.60 mmol, 52%) and acetate 15c (534 mg, 3.78 mmol, 43%) unreacted. Capillary GC analysis using chiral column (G-TA) showed that the enantiomeric excess of 15c was >99% ee with 86% de (anti). [α]D⁻¹ 11.8 (c 1.27, CHCl₃).

(4S,5R)-5-hydroxy-4-methylhexan-3-one (21) ³⁵ (85% de). ¹H NMR (200 MHz, δ, CDCl₃, J= Hz) 1.06 (3H, t, J= 7.3), 1.11 (3H, d, J= 7.4), 1.20 (3H, d, J= 6.3), 1.25 (1H, s), 2.55 (2H, d, J= 7.3), 2.50-2.62 (1H, m) 3.91 (1H, m)*. *The spin decoupling test showed that the coupling constant of C-5 proton (Jₕ₅₋₆) of the major isomer was 7.22 Hz, while that of the minor one is 3.25 Hz; ¹³C NMR(50 MHz, ppm, CDCl₃) 7.52, 14.15, 20.90, 52.57, 35.76, 69.62, 216.55; IR (neat, cm⁻¹) 3315, 2929, 2858, 1726, 1471, 1257, 1007, 837.
References and Notes


(9) (a) Fujiwhara, M.; Mori, K. *Agric. Biol. Chem.* 1986, 50, 2925. (b) Cammaerts, M.-C.; Mori, K. *Physiol. Entomol.* 1987, 12, 381.


(19) Crown ethers bind metal cations with varying strength depending on structural variations. The lipase solution employed was confirmed to contain the following alkali and alkaline earth metal cations: Na+, 6.5x10⁻⁴ mol/L; K+, 4.6x10⁻⁴ mol/L; Mg²⁺, 6x10⁻⁴ mol/L; Ca²⁺, 6.3x10⁻⁴ mol/L.

(20) Marked formation of diol was observed (18% at 1.0 h reaction) when the reaction was carried out in the presence of 300 mol% of thiacrown ether to the substrate, while the starting diacetate still remained (2.3%) at that time. Because thiacrown ether was a very hydrophobic compound and insoluble in water, the state of the reaction mixture became very sluggish.


(24) Because the enzymatic reaction was performed in water, a ¹³C NMR experiment was also carried out in D₂O as solvent. Due to very low
solubility of the thiacrown ether 4 in D,O, however, no spectral change was detected in the 13C NMR signal of the monoacetate.


(31) The binding energy for (R)-1a calculated from RTlnKm is >0.13 Kcal mol⁻¹.

(32) Preliminary experiments revealed that lipase AL gave the alcohol with the highest diastereoselectivity among 28 types of lipases tested.
Chapter 2.

Synthesis of Various Difluorocyclopropane Derivatives Using Chemo-Enzymatic Reaction

2-1-1. Introduction

The difluoromethylene group is well known as an isoelectronic and isosteric substitute for oxygen in phosphate analogues and geminal difluorinated compounds thus mimic the tetrahedral transition states related to the hydrolytic action of proteases and esterases; this caused enzyme inhibition to occur when the nucleophilic hydroxyl group is part of the active site of the enzyme. The utility of cyclopropane derivatives in the construction of a variety of cyclic and acyclic organic compounds has been amply demonstrated. Substitution of two fluorine atoms on the cyclopropane ring is expected to alter both chemical reactivity and biological activity due to the strong electron-withdrawing nature of fluorine atom. These make efficient methods for the synthesis of a suitably functionalized building block for chiral difluorocyclopropane even more necessary. Here, the first successful synthesis of an optically pure difluorocyclopropane building block through lipase-catalyzed asymmetric hydrolysis of the corresponding prochiral diacetate was described.

For the strategy of this synthesis, it was decided to use lipase-catalyzed hydrolysis protocol. The synthetic value of lipase has been well recognized because the reaction proceeds efficiently and selectively under mild conditions.
2-1-2. Efficient Synthesis of Enantiopure 1,2-Bis(hydroxymethyl)-3,3-difluorocyclopropane Derivatives through Lipase-Catalyzed Reaction

Prochiral diacetate was prepared as follows; easily available dibenzyl ether of (Z)-2-butendiol was subjected to Taguchi’s difluorocyclopropanation using cis-addition of difluorocarbene derived from sodium difluoroacetate in diglyme at 180 °C, followed by debenzylation and acetylation to afford the desired diacetate 23 for 3 steps in overall 81 % yield (Scheme 5). The asymmetric hydrolysis of 23 was typically carried out as follows: to a phosphate buffer solution (10 ml, 0.1 M at pH 7.2) was added 23 (1.0 mmol) and lipase QL (50 wt% towards the substrate) and the mixture was stirred at 35 °C (Eq. 5). The alcohol 22 produced was extracted with ethyl acetate and purified by silica gel flash column chromatography (hexane / ethyl acetate = 5:1 to 2:1).

Twenty-eight commercially available lipases were screened for their activity but only five were found to have hydrolyzed acetate 23 to afford monoacetate 22 with more than 60% ee; lipase QL (Meito) from Alcaligenes sp. provided the corresponding monoacetate 22 in the highest enantiomeric excess (Table 5, Entry 1). Lipase TL and PCL also gave 22 with good enantiomeric excess (Entries 2 and 3). In contrast, no reliable results were obtained when 23 was subjected to the reaction of PPL, though it once gave 22 with 96% ee (Entry 5). Because the PPL-catalyzed reaction proceeded very slowly, partial racemization of the product apparently occurred.

Table 8. Asymmetrization of prochiral diacetate 23 through lipase-catalyzed hydrolysis

<table>
<thead>
<tr>
<th>Entry</th>
<th>Liapse</th>
<th>Time (h)</th>
<th>%ee of 22 (Yield)</th>
<th>ee(%) of 22 (c in CHCl3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>QL</td>
<td>5</td>
<td>&gt;99 % (81%)</td>
<td>+15.5 (c 1.2)</td>
</tr>
<tr>
<td>2</td>
<td>TL</td>
<td>6</td>
<td>90 % (58%)</td>
<td>+15.4 (c 1.39)</td>
</tr>
<tr>
<td>3</td>
<td>PCL</td>
<td>8</td>
<td>85 % (97%)</td>
<td>+12.5 (c 1.22)</td>
</tr>
<tr>
<td>4</td>
<td>AL</td>
<td>48</td>
<td>85 % (75%)</td>
<td>+12.5 (c 1.20)</td>
</tr>
<tr>
<td>5</td>
<td>PPL</td>
<td>168</td>
<td>62 % (53%)</td>
<td>+10.0 (c 1.00)</td>
</tr>
</tbody>
</table>

The reaction was carried in 0.1M postassium phosphate buffer at pH 7.2 and enantiomeric excess was determined by capillary GC analysis using Chiralix-GTa (ø 0.25 mm x 20 M, He, 70 °C or 100°C). QL: Alcaligenes sp. TL: Pseudomonas sp. PCL: Pseudomonas cepacia. AL: Achromobacter sp. PPL: Porcine pancreatic lipase.

Diacetate of (trans)-1,2-bis(hydroxymethyl)-3,3-difluorocyclopropane 24 is not prochiral but racemic form, so that optical resolution of (±)-24 was performed using lipase-catalyzed reaction (Eq. 6). In this reaction, the best result was recorded when (±)-24 was reacted with lipase SL.
(Pseudomonas cepacia SL-25, Meito), and diacetate (-)-24 remaining was obtained with >99% ee (E value of the reaction was 11).11

\[
\text{Lipase SL} \quad \text{pH 7.2, 35°C, 1h} \quad E = 11
\]

In summary, the synthesis of difluorocyclopropane building blocks 22 and 24 with extremely high optical purity was succeeded through lipase-catalyzed reaction. Lipase-catalyzed reactions are particularly useful even for large-scale preparative organic synthesis. The present protocol will undoubtedly allow us to evolve a smarter and more convenient synthesis of chiral difluorocyclopropane derivatives.

2-2-1. Introduction

The substitution of two fluorine atoms on the cyclopropane ring is expected to alter both its chemical reactivity and biological activity due to the strong electron-withdrawing nature of the fluorine atom, and this makes it possible to create new molecules that would exhibit a unique biological activity or functionality.4 It is interesting in the special properties of the difluorocyclopropanes5,15 and very recently accomplished the first synthesis of optically pure 1,1-difluoro-2,3-(bishydroxymethyl)cyclopropane.13,14

It was postulated that the bisdifluorocyclopropanes (trans,trans) and (cis,cis)-31 would become unique sources of important organic compounds, such as a liquid crystal, monomer part for the synthesis of a unique polymer, and building blocks for the synthesis of difluoromethylene compounds. However, neither report concerning this idea or the synthesis of the bisdifluorocyclopropane derivatives have been reported so far, hence, It was
decided to attempt the synthesis of several types of bisdifluorocyclopropanes. In this chapter, the first synthesis of these bisdifluorocyclopropane derivatives, (trans,trans)-31 and (cis,cis)-31, and the optically active bisdifluorocyclopropanes (+)-(trans,trans)-31 and (-)-(trans,trans)-31 has been accomplished through an enzymatic-chemical hybrid reaction methodology.

2-2-2. Synthesis of Optically Active Bisdifluorocyclopropanes through a Chemo-Enzymatic Reaction Strategy

The target bisdifluorocyclopropane derivatives, (trans,trans)-31 and (cis,cis)-31, should be derived from the (E)- or (Z)-3-tributylstannyl-2-propene-1-ol (35). The value of an enzymatic reaction in organic synthesis is extensively increased by its environmentally friendly nature. The lipase-catalyzed reaction was used for isolating the tributylstannyl alcohols, (E)-35 and (Z)-35, from a stereomixture (Scheme 6). Several lipases stereoselectively hydrolyzed acetate 36, and Pseudomonas cepacia lipase (PCL) was found to be the best enzyme that smoothly hydrolyzed the acetate 36 to provide the (E)-olefin 35 with perfect selectivity. The pure (Z)-isomer 35 was obtained as the unreacted acetate 36 by the PCL-catalyzed reaction when the reaction time was prolonged. This is a very convenient method to obtain the pure isomers of (E)-35 and (Z)-35 in the laboratory, though there exists several means to stereoselectively prepare (E)-35 using transition-metal chemistry. The hydroxyl group of (E)-35 was protected as the benzyl ether and treated with copper (II) nitrate hydrate in THF at room temperature to produce the corresponding diene (E,E)-29 in 74% yield (Scheme 6, Method A). On the other hand, it was prepared diene (E,E)-29 with another protocol for synthesizing a large amount of diol 28 (Scheme 6, Method B).

Bisdifluorocyclopropanes (trans,trans)-31 was directly synthesized in 74% yield from (E,E)-29 using 5 eq. of difluorocarbene which was produced by the thermolysis of sodium chlorodifluoroacetate. The benzyl protecting group was essential in achieving difluorocyclopropanation with sufficient
yield. A significant drop in the chemical yield of the desired bisdifluorocyclopropane was observed when the reaction was carried out using the diacetate as a substrate. Bisdifluorocyclopropane (cis,cis)-31 was also synthesized from (Z)-35 using the same procedure (Scheme 6, Method A). The diacetate of (trans,trans)-2,2,5,5-tetrafluoro-1,6-bis(hydroxymethyl)bicyclopropane (32) is a 1:1:2 mixture of (1S,3R,4R,6S)-32, (1R,3S,4S,6R)-32, and meso-(1R,3S,4R,6S)-32. Optical resolution of (+)-(trans,trans)-32 was very successfully achieved by the lipase SL-catalyzed reaction (Scheme 7).

The first synthesis of the optically active bisdifluorocyclopropane was accomplished very simply and efficiently. The stereochemistry of (+)-(trans,trans)-31 was assigned as (1S,3R,4R,6S), and (-)-(trans,trans)-32 was (1R,3S,4S,6R), based on the CD exciton chirality method using the 9-anthracenecarboxylate derivative 34 (Fig. 8). The CD spectrum of the bis(9-anthracene)carbonyl ester 34, which was derived from (+)-(trans,trans)-31, exhibited positive chirality on the Cotton effect [387.2 nm and 364.0 nm (ΔE=1.20), CH3CN], while negative chirality on the Cotton effect [387.4 nm and 366.0 nm (ΔE=3.05), CH3CN] was observed by the 9-anthracene carboxylate derivative 34 derived from (-)-(trans,trans)-32. These observed Cotton effects were corresponded to E2 absorption of the anthracene group (E2, λmax=375 nm (ε=28756). X-ray crystallographic analysis of the dibenzyl ether 30, which was derived from (trans,trans)-meso-33, was successful and the stereochemistry of the lipase-catalyzed reaction was thus fully confirmed (Fig. 9).
In conclusion, it has been demonstrated the first synthesis of bisdifluorocyclopropane derivatives via a chemo-enzymatic reaction methodology. Results of the CD spectroscopic analysis showed that these bisdifluorocyclopropanes, \((\text{trans,trans})-31\), exist with a helical shape configuration; this seems to suggest that a unique helical shape polymeric compound may be produced from \((\text{trans,trans})-31\) as a monomer unit.

2-3-1. Introduction

To clarify the special property of gem-difluorocyclopropanes and accomplished the first synthesis of several types new gem-difluorocyclopropanes in optically pure form was accomplished.\(^{13,24}\) As anticipated by the nature of fluorine atom, the shape of the difluorinated analogue of bicyclopropane 1,6-bis(hydroxymethyl)-2,2,5,5-tetrafluorobicyclopropane (31) was a little bit different from the simple bicyclopropane 37.

Figure 10 shows the results of the optimized structure of a gem-fluorinated bicyclopropane by MO (PM3) calculation.\(^{25}\) Calculation suggested a kinked form of two difluorocyclopropane groups for compound 31, while no such twisted form was suggested for bicyclopropane 37. This was confirmed by results of the CD spectroscopic analysis of optically active 31 in that large CD spectral change on the Cotton effect was observed.\(^{24}\) Even more interesting by, the computational chemistry suggests a highly helical shape for oligo-gem-difluorocyclopropanes, such as pentakis-gem-difluorocyclopropane 38, as shown in Figure 10. Oligo- and poly-gem-difluorocyclopropanes are challenging targets for synthetic organic chemists. In this chapter, it has been reported our initial results of synthesizing bis- and oligo-gem-difluorocyclopropane derivatives\(^{27}\) through the olefin metathesis reaction strategy.\(^{26}\)
2-3-2. Synthesis of Bis- and Oligo-gem-difluorocyclopropanes Using Olefin Metathesis Reaction

First, the olefin metathesis reaction of 1-benzyloxymethyl-2,2-difluoro-3-vinylcyclopropane (40a) was investigated as a model compound using the Grubbs catalyst, \((\text{PCy}_3)_2\text{Cl}_2\text{Ru=CHPh} \) (39) \(^{26}\) (Scheme 8).

![Scheme 8. Synthesis of bis-gem-Difluorocyclopropanes](image)

The desired coupling products 40a would be obtained without difficulty because wide range functional group tolerance has been reported for the reaction.\(^{26a}\) However, desired compound 41a was obtained in poor yield (6 %) in the presence of 5 mol % of the catalyst, though the stereochemistry of the newly formed olefinic part exhibited perfect (E)-selectivity. Four solvent systems were tested: dichloromethane (\(\text{CH}_2\text{Cl}_2\)), toluene, benzene, and tetrahydrofuran (THF), and the desired product 41a was obtained only when the reaction was carried out in \(\text{CH}_2\text{Cl}_2\) at room temperature, and significant decomposition of both substrate and the catalyst was observed under elevated temperature conditions. Increasing the amount of the catalyst caused no enhancement of the chemical yield, the yield remained at a range of 5-7% even in the presence of 1.0 equivalent of the catalyst, and a significant amount of unidentified purple solid was produced.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Conditions (^a) (Method)</th>
<th>Product (Stereochemistry) (^b)</th>
<th>Yield (%)</th>
</tr>
</thead>
</table>
| 1     | \[\begin{array}{c}
        \text{BnO} \quad \text{H} \\
        \text{F, F}
      \end{array}\] 40a | RT, 72 h (B) | \[\begin{array}{c}
        \text{BnO} \quad \text{H} \\
        \text{OBn}
      \end{array}\] 41a (E only) | 35% |
| 2     | \[\begin{array}{c}
        \text{BnO} \quad \text{H} \\
        \text{F, F}
      \end{array}\] 40b | RT, 60 h (A) | \[\begin{array}{c}
        \text{BnO} \quad \text{H} \\
        \text{OBn}
      \end{array}\] 41b (E:Z=5:1) | 16% |
| 3     | \[\begin{array}{c}
        \text{BnO} \quad \text{H} \\
        \text{F, F}
      \end{array}\] 40c | RT, 12 h (A) | \[\begin{array}{c}
        \text{BnO} \quad \text{H} \\
        \text{OBn}
      \end{array}\] 41c (E only) | 9% |
| 4     | \[\begin{array}{c}
        \text{BnO} \quad \text{H} \\
        \text{F, F}
      \end{array}\] 42a | RT, 24 h (A) | \[\begin{array}{c}
        \text{BnO} \quad \text{H} \\
        \text{OBn}
      \end{array}\] 43a (E:Z=4:1) | 80% |
| 5     | \[\begin{array}{c}
        \text{BnO} \quad \text{H} \\
        \text{F, F}
      \end{array}\] 42b | RT, 24 h (A) | \[\begin{array}{c}
        \text{BnO} \quad \text{H} \\
        \text{OBn}
      \end{array}\] 43b (E:Z=5:1) | 68% |
| 6     | \[\begin{array}{c}
        \text{BnO} \quad \text{H} \\
        \text{F, F}
      \end{array}\] 42e | RT, 60 h (A) | \[\begin{array}{c}
        \text{BnO} \quad \text{H} \\
        \text{OBn}
      \end{array}\] 43c (E:Z=5:1) | 36% |

\(^a\) The reaction was carried out in \(\text{CH}_2\text{Cl}_2\) in the presence of 15 mol% or 100 mol% of the catalyst. \(^b\) The ratio of the stereochemistry of the olefinic part was determined by \(^1\)H NMR analysis. Method A: The reaction was carried out in the presence of whole amount of the catalyst (15 mol%). Method B: Five additions of 20 mol% of the catalyst solution were made of 12 h intervals.
A total of 100 mol% of the catalyst was used. Thus, 20 mol% of the catalyst as dichloromethane solution was added dropwise five times at 12 h intervals (Method B) ; this increased the chemical yield and the desired coupling product 41a was obtained in 35% yield (Table 5, Entry 1). Fortunately, the olefin metathesis reaction of allylic ether 42a proceeded smoothly to give the coupling product 43a in efficient yield of 80% using 15 mol% of the catalyst (Method A, Entry 4). Six types of bis- and oligo-gem-difluorinated cyclopropane derivatives were thus synthesized for the first time (Table 5). In both types of substrates 40 and 42, bisdifluorocyclopropanes 40c and 42c provided the coupling products 41c and 43c in low yields (Entries 3 and 6), respectively. This seemed to be due to the strong chelating effect of the difluorocyclopropane groups towards ruthenium which caused the death of the catalyst by complexation, and a significant amount of unidentified purple solid was produced on the reaction.

It is postulated that decomposition of a metallacyclobutane intermediate like 45 produces ethylene gas and regenerates the Ruthenium carben complex 46 to complete the catalytic cycle (Figure 11). Because the coupling product 41a was indeed obtained, though the yield was insufficient, the step to form the key metallacyclobutene 47 and subsequent decomposition might occur smoothly, and it would release 41a and carben complex 44. In the present reaction, however, the retro decomposition of metallacyclobutene 45 to vinylcyclopropane 40a seems to occur due to the strong electron withdrawing nature of difluoromethylene moiety and this stopped the catalytic cycle.

All of the products, 41a-41c, 43a-43c, obtained possess olefinic parts between difluorocyclopropane moieties, so that it can be converted them to hybrid types of difluorocyclopropanes.

In conclusion, It was demonstrated by the first synthesis of bis- and oligo-gem-difluorocyclopropane derivatives via olefin metathesis reaction methodology. This reaction is applicable for the synthesis of an optically active one because the synthesis of the starting materials 40 and 42 in optically pure form has been already achieved. It would be need to improve the reaction efficiency by changing the catalyst and synthesizing hybrid types of poly-gem-difluorocyclopropanes.
Summary

Efficient synthesis of chiral difluorocyclopropane building block has been accomplished; prochiral diacetate of 1,2-bis(hydroxymethyl)-3,3-difluorocyclopropane was converted to the corresponding monoacetate through *Alcaligenes* sp. lipase-catalyzed hydrolysis with >99% enantiomeric excess.

The first synthesis of bisdifluorocyclopropane derivatives has been accomplished via a chemo-enzymatic reaction strategy; (E)- and (Z)-3-tributylstannyl-2-propenols were prepared and their conversion to the (trans,trans)- and (cis,cis)-bisdifluorocyclopropanes was successful. The subsequent lipase-catalyzed reaction efficiently afforded optically active (trans,trans)-2,2,5,5-tetrafluoro-1,6-bis(hydroxymethyl)bicyclopropane.

Synthesis of six types of novel bis- and oligo-gem-difluorocyclopropanes has been accomplished through the olefin metathesis reaction protocol. Since newly produced gem-difluorocyclopropanes possess an olefinic part between the two difluorocyclopropane moieties, further modification was possible; synthesis of a novel hybrid compound which has an oxirane ring between two difluorocyclopropane rings in their molecules has been demonstrated.

Experimental Section

General Procedures

Reagents and solvents were purchased from common commercial sources and were used as received or purified by distillation from appropriate drying agents. Reactions requiring anhydrous conditions were run under an atmosphere of dry argon. Silica gel (Wako gel C-300E) was used for column chromatography and silica gel (Wako gel B-5F) for thin layer chromatography. ^1H NMR, ^19F NMR, ^13C NMR spectra and were recorded on Varian VXR-200 (200MHz) and VXR-500 (500MHz) spectrometer, and chemical shifts are expressed in ppm downfield from tetramethylsilane (TMS) in CDCl3 or hexafluorobenzene (C6F6) as an internal reference. IR spectra were obtained on FT/IR-230 spectrometers. Optical rotation was measured with JASCO DIP-101 digital polarimeter. The regioselectivity was determined by capillary gas chromatography (MS, φ 0.25mm Å x 20m, 100-250°C, He). The optically purity was determined by capillary gas chromatography (Chiraldex G-TA, φ 0.25mm Å x 20m, 70-100°C, He).
Chapter 2-1.

Preparation of 1,2-Bis(acetoxymethyl)-3,3-difluorocyclopropanes (cis)-1,2-Bis(benzyloxy methyl)-3,3-difluorocyclopropane (56).

To a suspension of NaNH (Oily (60%), 4.41g, 110 mmol) in dry DMF (50 mL) was added cis-2-Buten-1,4-diol 54 (4.41 g, 50 mmol) in dry DMF (70 mL) at 0°C under argon atmosphere, and the mixture was stirred at same temperature for 0.5h. After being added this suspension benzyl chloride (12.0 mL, 105 mmol), the mixture was stirred at room temperature for 8h. The reaction was quenched by addition of crushed ice, extracted with ethyl acetate. Silica gel flash column chromatography, (gradient elution hexane / ethyl acetate = 80:1 to 10:1) gave dibenzyl ether 55 (13.5 g, 50 mmol) in 100% yield. A solution of 55 (10.73g, 40 mmol) in dry diglyme (40 mL) was heated to 180°C. A solution of sodium chlorodifluoroacetate (30.49g, 200 mmol) in dry diglyme (80 mL) was added at same temperature over a period of 5h. After keeping the reaction at 180°C for additional 1h, and cooling to room temperature, the reaction mixture was poured into ice water, the aqueous solution was extracted with hexane and ethyl acetate, and the combined organic layers were washed with water, dried over MgSO₄ and the solvents were concentrated under reduced pressure. Silica gel flash column chromatography, (gradient elution hexane / ethyl acetate = 10:1 to 10:1) gave 56 (12.0 g, 37.7 mmol) in 94% yield. : Rf 0.36 (hexane / ethyl acetate=10:1); bp 298, 24.01 (t, J₆₋₇=10.7), 57.80 (d, J₆₋₇=5.9), 113.22 (dd, J₆₋₇=290.2, 282.7), 170.60; ¹⁹F NMR (188 MHz, δ, CDCl₃, J=Hz) 10.32 (d, J₆₋₇=164.8), 36.29 (dt, J₆₋₇=165.1, J₆₋₇=12.1); IR (neat, cm⁻¹) 3032, 2866, 1469, 1367, 1282, 1192, 1086, 1028, 912, 849, 742, 700. Anal. Calcd for C₁₉H₂₀F₂O₂: C, 77.74; H, 6.33 Found: C, 73.63; H, 6.45

Preparation of (cis)-1,2-Bis(acetoxymethyl)-3,3-difluorocyclopropane (23). A solution of difluorocyclopropane 56 (6.02 g, 18.9 mmol) in methanol (20 mL) was stirred at room temperature using palladium carbon (1.20 g, 20 wt%) under H₂ (1 atm) for 24h. The crude products were filtrated with glass sintered filter on celite, and concentrated by evaporation of the solvent to afford as colorless oil. To a crude diol 57 solution in dichloromethane (30 mL) and pyridine (4.6 mL, 56.9 mmol) was added a CCI₂ solution (10 mL) of acetyl chloride (3.27 g, 41.7 mmol) at 0°C and the solution was stirred at room temperature for 3h. The reaction was quenched by addition of crushed ice, extracted with ethyl acetate, dried over MgSO₄ and concentrated. The crude product was purified by flash column chromatography (hexane / ethyl acetate= 10:1) to give an diacetate 23 (3.62 g, 16.3 mmol) for 2 steps in 86% yield: Rf 0.50 (hexane/ ethyl acetate=2:1); bp 122 °C/ 5 Torr (Kugelrohr); ¹H NMR (200 MHz, δ, CDCl₃, J=Hz) 2.03-2.18 (2H, m), 2.06 (6H, s), 4.17-4.38 (4H, m); ¹³C NMR (50 MHz, δ, CDCl₃, J=Hz) 20.68, 3.50-3.69 (4H, m), 4.49 (4H, ABq, J=11.8), 113.22 (dd, J₆₋₇=290.2, 282.7), 170.60; ¹⁹F NMR (188 MHz, δ, CDCl₃, J=Hz) 10.32 (d, J₆₋₇=164.8), 36.29 (dt, J₆₋₇=165.1, J₆₋₇=12.1); IR (neat, cm⁻¹) 2968, 1743, 1479, 1377, 1234, 1118, 1038, 837. Anal. Calcd for C₉H₁₂F₂O₄: C, 48.65; H, 5.44 Found: C, 48.77; H, 5.72.
[(1R,3S)-3-Hydroxymethyl-2,2-difluorocyclopropyl]methyl acetate (22). To a buffer solution (pH 7.2) (100 mL) was added Lipase QL powder (1.11 g, Meito Sangyo). The lipase solution was added to a diacetate 23 (2.22 g, 9.99 mmol). The mixture was stirred at room temperature for 4 h. The reaction was quenched with a crushed ice and NaCl. The crude product was extracted with ethyl acetate. The organic layer was dried over MgSO\(_4\) and concentrated. Silica gel flash column chromatography, (gradient elution hexane / ethyl acetate = 4:1 to 2:1) gave a monoacetate (22) (1.66 g, 9.21 mmol, 92%). Rf 0.22 (hexane/ethyl acetate=2:1); bp 108 °C/6.5 Torr (Kugelrohr);\(^1\)H NMR (200 MHz, CDCl\(_3\), J=Hz) 1.95-2.10 (2H, m), 2.06 (3H, s), 2.48 (3H, s), 3.67-3.90 (2H, m), 4.12-4.36 (2H, m); \(^1\)C NMR (50 MHz, CDCl\(_3\), J=Hz) 20.76, 24.00 (t, J\(_{CF}\)=0.2), 27.63 (t, J\(_{CF}\)=0.1), 55.91 (d, J\(_{CF}\)=5.5), 58.06 (d, J\(_{CF}\)=5.6), 113.71 (dd, J\(_{CF}\)=290.7, 282.1), 170.94; \(^1\)H NMR (500 MHz, CDCl\(_3\), J=Hz) 58 (7.9), 60 (7.9); IR (neat, cm\(^{-1}\)) 3432, 2964, 1738, 1478, 1374, 1241, 1186, 1037, 907, 833, 719. Anal. Calcd for C\(_9\)H\(_{12}\)F\(_2\)O\(_4\): C 46.67; H 5.60. Found: C 47.05; H 5.93. Analysis by GPC for determination of enantiomeric excess of monoacetate was carried out using a capillary column on chiral phase; Chiraldex G-TA, l/J 0.25 mm x 20 m; Carrier gas: He 40 mL/min; Temp (°C); 100, Inlet pressure; 1.35kg/cm\(^2\); Amount; 400ng; Detection; FID. The results of GC analysis of 22: tR of (1R, 3S)-22; 9.5 min., (1S, 3R)-22, 11.3 min. (R)- and (S)-\(\alpha\)-methoxy-\(\alpha\)-(trifluoromethyl)-phenylacetic acid (MTPA) demonstrated negative chemical shift differences (\(\Delta\delta = \delta_\text{R} - \delta_\text{S}\)) for protons on C-1 and C-2 shown below. The optimized structure by semiempirical (PM3) calculation of (S)-MTPA ester of (1R, 2S)-22 agreed with these results (Fig. 12). We are now attempting to confirm this by X-ray crystallographic analysis of (S)-(+)\-6-methoxy-\(\alpha\)-methyl-2-naphthalenecacetate of 22. However, single crystals suitable for X-ray diffraction have not yet been obtained.

**Preparation of (E)-1,4-dibenzyloxy-2-butenediene (60).**

A solution of 2-Butyne-1,4-diol 58 (17.2 g, 200 mmol) in dry THF (80 mL) was added dropwise with stirring to a solution of lithium aluminum hydride (11.4 g, 300 mmol) in dry ether (150 mL). The mixture was refluxed for 36h
under argon atmosphere. After cooling to room temperature, the mixture was diluted with THF (200 mL) and vigorously stirred at 0°C, decomposed with 6M-HCl (20 mL) (added dropwise until the grey slurry just turned white) excess amount of lithium aluminum hydride). After being allowed to stand for some time, the organic layers were decanted, added 6M-HCl (5 mL) and stirred for 5 minutes. The organic layer collected was concentrated to afford (E)-2-Buten-1,4-diol 59 (11.9 g, 135.1 mmol). To a suspension of NaH (Oily 60%, 11.9g, 297.2 mmo! in dry DMF (80 mL) was added a diol 59 (11.9 g, 135.1 mmol) in dry DMF (80 mL) at 0°C under argon atmosphere, stirred at same temperature for 0.5h. After being added this suspension benzyl chloride (11.2g, 88 mmol), the mixture was stirred at room temperature for 22h. The reaction was quenched by addition of crushed ice, extracted with ether. Silica gel flash column chromatography, (hexane / ethyl acetate = 10:1) gave dibenzyl ether 60 (32.6 g, 121.5 mmol) for 2 steps in 61% yield (Scheme 9). Rf 0.27 (hexane/ ethyl acetate=1:1); 1H NMR (200 MHz, 8, CDCl3, J=Hz) 4.04 (4H, dd, J=2.9, 1.2), 4.52 (4H, s), 5.88 (2H, dd, J=3.5, 2.9, 1.3), 6.29 (2H, dd, J=20.7, 9.0, 2.9), 7.20-7.38 (10H, m); 13C NMR (50 MHz, ppm, CDCl3) 70.06, 72.14, 127.54, 127.66, 128.32, 129.44, 138.17.

Using the same procedure, (trans)-1,2-Bis(acetoxy methyl)-3,3-difluorocyclopropane 24 was also prepared from the corresponding dibenzyl ether 61.

(trans)-1,2-Bis(benzylxoy methyl)-3,3-difluorocyclopropane (61). Rf 0.33 (hexane/ ethyl acetate=10:1); 1H NMR (200 MHz, 8, CDCl3, J=Hz) 1.66-1.80 (2H, m), 3.45-3.70 (4H, m), 4.53 (4H, ABq, J=11.9), 7.20-7.40 (10H, m); 13C NMR (50 MHz, 8, CDCl3, J=Hz) 26.45 (4H, ABq, J=11.9), 70.00, 72.45, 113.95 (t, J= 295.27); 19F NMR (188 MHz, 8, CDCl3, J=Hz) 23.64; IR (neat, cm-1) 3032, 2864, 1477, 1367, 1261, 1194, 1099, 1032, 741.

(trans)-1,2-Bis(hydroxymethyl)-3,3-difluorocyclopropane (62). Rf 0.14 (hexane/ ethyl acetate=1:1); bp 100 °C/ 2 Torr (Kugelrohr); 1H NMR (200 MHz, 8, CDCl3, J=Hz) 1.65-1.88 (2H, m), 3.27 (2H, s), 3.50-3.66 (1H, m), 3.80-3.93 (1H, m); 13C NMR (50 MHz, ppm, CDC13) 29.29 (t, J= 286.5), 59.21 (t, J= 286.5), 114.40 (t, J=295.27); 19F NMR (188 MHz, 8, CDCl3, J=Hz) 23.56; IR (neat, cm-1) 3334, 2898, 1477, 1367, 1244, 1175, 1113, 1026, 931, 830.

Lipase-catalyzed Reaction

To a buffer solution (pH 7.2) (182 mL) was added Lipase SL powder (2.02 g, Meito Sangyo). The lipase solution was added to a diacetate 24 (8.07 g, 36.32 mmol). The mixture was stirred at room temperature for 0.5h. The reaction was quenched with a crushed ice and NaCl. The crude product was
extracted with ethyl acetate. The organic layer was dried over MgSO₄ and concentrated. Silica gel flash column chromatography, (gradient elution hexane / ethyl acetate = 7:1 to 1:1) gave monoacetate (25) (3.63 g, 20.15 mmol, 560%) and diacetate (24) (3.05 g, 13.73 mmol, 38%).

\[(\text{S},\text{S})-25\] (51% ee) \([\alpha]_D^{24} +14.9\) (c. 2.41, CHCl₃) as a diacetate 24 (IR,3R)-24 (>99% ee) \([\alpha]_D^{24} -9.6\) (c. 1.4, CHCl₃)

\[(\text{S},\text{S})\)-3-Hydroxymethyl-2,2-difluorocyclopropyl]methyl acetate (25). \(R_f 0.23\) (hexane / ethyl acetate=2:1); \(\text{H} NMR (200 MHz, \text{CDCl}_3, \delta=Hz) 1.64-1.88 (2H, m), 2.03 (3H, s), 3.02 (1H, s), 3.65 (1H, dd, \(J=6.3, 2.0\)), 4.00 (1H, dd, \(J=13.2, 6.6\)), 4.12-4.16 (1H, m); \(\text{C} NMR (50 MHz, \text{CDCl}_3, \delta=Hz) 20.65, 25.16 (d, \(J_c=5.5\)), 60.85 (d, \(J_c=10.9\)), 114.56 (t, \(J_c=286.3\)), 171.16; \(\text{F} NMR (188 MHz, \text{CDCl}_3, \delta=Hz) 22.35 (dd, \(J_F=164.3, J_H=12.2\)), 23.86(dd, \(J_F=164.3, J_H=13.2\)); IR (neat, cm⁻¹) 3432, 2964, 1738, 1478, 1374, 1241, 1186, 1115, 1037, 907, 833, 719.

Chapter 2-2.

**Preparation of 2,4-hexadiyn-1,6-diol (27).**
A mixture of propargyl alcohol (26) (22.42 g, 400 mmol), pyridine (8 g), methanol (40 mL) and cuprous chloride (8.34 g, 80 mmol) was stirred at room temperature for 22 h. The mixture was diluted with ethyl acetate (100 mL), neutralized with saturated ammonium chloride solution. After stirring at room temperature for 1 h, the organic layers were decanted and washed with saturated sodium carbonate solution, filtered with suction using Buchner funnel on celite to give 2,4-hexadiyn-1,6-diol 27 (10.05 g, 91.3 mmol) in 46% yield.

**Preparation of 2,4-hexadiene-1,6-diol (28).**
A solution of diol 27 (4.40 g, 40 mmol) in dry THF (50 mL) was added dropwise with stirring to a solution of lithium aluminum hydride (4.55 g, 120 mmol) in dry THF (150 mL). The mixture was refluxed for 22 h under argon atmosphere. After cooling to room temperature, the mixture was diluted with diethyl ether (100 mL) and vigorously stirred at 0°C. Excess amount of lithium aluminum hydride was decomposed with 6M-HCl (6 mL).
(added dropwise until the grey slurry just turned white). After being allowed to stand for some time, the organic layers were decanted, added 6M-HCl (8 mL) and stirred for 5 minutes. The crude product was filtered with glass sintered filter. The filtrate and organic layer were combined and concentrated to afford (E,E)-2,4-hexadiene-1,6-diol 28 (3.91 g, 34.2 mmol) in 86% yield.

(E,E)-1,6-dibenzyloxy-2,4-hexadiene (29). To a suspension of NaH (Oily (60%), 4.2 g, 105 mmol) in dry DMF (50 mL) was added a diol 28 (4.0 g, 35 mmol) in dry DMF (20 mL) at 0°C under argon atmosphere, stirred at same temperature for 0.5h. After being added this suspension benzyl chloride (1.12 g, 88 mmol) , the mixture was stirred at room temperature for 8h. The reaction was quenched by addition of crushed ice, extracted with ethyl acetate. Silica gel flash column chromatography, (gradient elution hexane / ethyl acetate = 80:1 to 10:1) gave diene 29 (9.52 g, 32 mmol) in 92% yield. Rf 0.28 (hexane/ethyl acetate = 10:1); IH NMR (200 MHz, ii, CDCl3, J=Hz) 4.05 (4H-1, d, 1=5.6), 4.50 (4H, s), 5.80 (2H, ddd, 1=11.5,8.9, 5.7), 6.29 (2H, ddd, J=20.7, 9.0, 2.9), 7.20-7.38 (10H-1, m); 13C NMR (50 MHz, ppm, CDCl3) 70.15, 71.99, 127.52, 127.65, 128.29, 129.88, 131.91, 138.16; IR (neat, cm-I) 3029, 2850, 1625, 1495, 1453, 1253, 1167, 1139, 1029, 972, 740, 699.

(trans,trans)-1,6-Bis(benzyloxymethyl)-2,2,5,5-tetrafluorobicyclopropane (30). A solution of diene 29 (6.23 g, 21.2 mmol) in dry diglyme (20 mL) was heated to 180°C. A solution of sodium chlorodifluoroacetate (32.3 g, 212 mmol) in dry diglyme (65 mL) was added at same temperature over a period of 5h. After keeping the reaction at 180°C for additional 1h, and cooling to room temperature, the reaction mixture was poured into ice water, the aqueous solution was extracted with hexane and ethyl acetate, and the combined organic layers were washed with water, dried over MgSO4 and the solvents were concentrated under reduced pressure. Silica gel flash column chromatography, (gradient elution hexane / ethyl acetate = 40:1 to 5:1) gave 30 (6.2 g, 15.7 mmol) as colorless liquid in 74% yield.

meso-(30). Rf 0.22 (hexane/ethyl acetate=10:1); mp 105-106 °C; 1H NMR (200 MHz, δ, CDCl3, J=Hz) 1.21-1.41 (2H, m), 1.80 (2H, ddd, J=13.7, 13.6, 6.8), 3.51 (2H, ddd, J=19.3, 10.9, 1.6), 3.56-3.69 (2H, m), 4.52 (4H, ABq, J=12.0), 7.24-7.40 (10H, m); 13C NMR (50 MHz, δ, CDCl3, J=Hz) 23.46 (dt, J驴F=11.2, 3.8), 28.02 (t, J=9.8), 66.16 (d, J=4.5), 72.58, 113.88 (ddd, J驴F=290.3, 287.2, 2.8), 127.60, 127.76, 128.43, 137.76; 19F NMR (188 MHz, δ, CDCl3, J=Hz) 22.61 (dd, J驴F=161.2, J驴F=13.2), 24.60 (dd, J驴F=161.1, J驴F=13.6) ; IR (neat, cm-I) 3062, 3033, 2891, 1461, 1366, 1303, 1253, 1167, 1139, 1014, 1029, 972, 742, 705. Anal. Calcd for C22H22F4O2: C, 67.00; H, 5.62 Pound: C, 66.83; H, 5.75
dl·(30). Rf 0.20 (hexane/ethyl acetate=10:1); mp 82 °C;H NMR (200 MHz, δ, CDCl3) 1.39-1.56 (2H, m), 1.57-1.79 (2H, m), 3.53 (4H, d, J=7.1), 4.51 (4H, ABq, J=11.9), 7.22-7.40 (10H, m); 13C NMR (50 MHz, δ, CDCl3, J=Hz) 22.89 (dt, J驴F=11.0, 3.7), 27.54 (t, J=9.7), 66.08 (d, J=4.1), 72.56, 114.05 (dd, J驴F=290.5, 286.7), 127.58, 127.77, 128.44, 137.76; 19F NMR (188 MHz, δ, CDCl3, J=Hz) 23.88 (dd, J驴F=160.2, J驴F=12.0), 25.20 (dd, J驴F=160.7, J驴F=12.6). (IR, 35, 4S, 6R)- 30: [αI]D²⁺=+30.2 (c.1.12, CHCl₃)
trans,trans)-1,6-Bis(hydroxymethyl)-2,2,5,5-
tetrafluorobicyclopropane (31). A solution of dibenzyl ether 30 (7.06 g, 17.9 mmol) in methanol (80 mL) was stirred at room temperature using palladium carbon (2.83 g, 40 wt%) under H₂ (1 atm) for 55 h. The crude products were filtrated with glass sintered filter on celite, concentrated to afford diol 31 as colorless oil.

meso-(31). RF 0.20 (hexane/ethyl acetate=1:1); mp 88-89 °C; ¹H NMR (200 MHz, δ, Aceton-d₆, J=Hz) 1.40-1.60 (2H, m), 1.87 (2H, ddd, J=14.4, 14.3, 6.6), 2.96 (2H, s), 3.48-3.82 (4H, m); ¹³C NMR (50 MHz, δ, Aceton-d₆, J=Hz) 23.46 (dt, J₅₋₁=13.7, 2.2), 30.73 (d, J=2.5), 58.78 (d, J=5.5), 115.57 (ddd, J₅₋₁=289.2, 285.9, 3.0); ¹⁹F NMR (188 MHz, δ, Aceton-d₆, J=Hz) 23.55 (dd, J₉₋₁=160.1, J₉₋₁=13.6), 26.7 (dd, J₉₋₁=160.2, J₉₋₁=14.3); IR (neat, cm⁻¹) 3321, 2923, 1452, 1250, 1127, 965, 890.

Anal. Calcd for C₈H₁₀F₄O₂: C, 44.87; H, 4.71. Found: C, 44.69; H, 5.68.

dl-(31). RF 0.10 (hexane/ethyl acetate=1:1); bp 140 °C/6.5 Torr (Kugelrohr); ¹H NMR (200 MHz, δ, CDCl₃, J=Hz) 1.41-1.78 (4H, m), 2.16 (2H, s), 3.65-3.85 (4H, m); ¹³C NMR (50 MHz, δ, CDCl₃, J=Hz) 23.46 (dt, J₅₋₁=13.7, 2.2), 30.73 (d, J=2.5), 58.78 (d, J=5.5), 115.57 (ddd, J₅₋₁=289.2, 285.9, 3.0); ¹⁹F NMR (188 MHz, δ, CDCl₃, J=Hz) 23.55 (dd, J₉₋₁=160.1, J₉₋₁=13.6); IR (neat, cm⁻¹) 3321, 2923, 1452, 1250, 1127, 965, 890. Anal. Calcd for C₆H₁₀F₄O₂: C, 44.87; H, 4.71 Found: C, 44.69; H, 5.68.

dl-(32). RF 0.63 (hexane/ethyl acetate=1:1); mp 68-69 °C; ¹H NMR (200 MHz, δ, CDCl₃, J=Hz) 1.41-1.78 (4H, m), 2.16 (2H, s), 3.65-3.85 (4H, m); ¹³C NMR (50 MHz, δ, CDCl₃, J=Hz) 23.46 (dt, J₅₋₁=13.7, 2.2), 30.73 (d, J=2.5), 58.78 (d, J=5.5), 115.57 (ddd, J₅₋₁=289.2, 285.9, 3.0); ¹⁹F NMR (188 MHz, δ, CDCl₃, J=Hz) 23.55 (dd, J₉₋₁=160.1, J₉₋₁=13.6); IR (neat, cm⁻¹) 3321, 2923, 1452, 1250, 1127, 965, 890. Anal. Calcd for C₁₂H₁₄F₄O₄: C, 48.33; H, 4.73. Found: C, 49.06; H, 5.75.

trans,trans)-1,6-Bis(acetoxymethyl)-2,2,5,5-
tetrafluorobicyclopropane (32). To a crude diol solution in dichloromethane (80 mL) and pyridine (5.54 mL, 71.6 mmol) was added a CH₂Cl₂ solution (10 mL) of acetyl chloride (4.21 g, 53.7 mmol) at 0°C. The reaction was quenched by addition of crushed ice, extracted with ethyl acetate, dried over MgSO₄ and concentrated. The crude product was purified by flash column chromatography (hexane/ethyl acetate=10:1) to give a diacetate 32 (4.65 g, 15.6 mmol) for 2 steps in 87% yield.

meso-(32). RF 0.63 (hexane/ethyl acetate=10:1): mp 68-69 °C; ¹H NMR (200 MHz, δ, CDCl₃, J=Hz) 1.32-1.48 (2H, m), 1.88 (2H, ddd, J=13.8, 13.4, 7.8), 2.06 (6H, s), 4.00-4.25 (4H, m); ¹³C NMR (50 MHz, δ, CDCl₃, J=Hz) 23.89 (dt, J₅₋₁=11.0, 3.7), 27.16 (t, J=4.6), 60.29 (d, J=4.6), 113.37 (ddd, J₅₋₁=290.3, 286.9, 2.4), 170.81; ¹⁹F NMR (188 MHz, δ, CDCl₃, J=Hz) 22.51 (dd, J₉₋₁=160.1, J₉₋₁=13.2); 13C NMR (50 MHz, δ, CDCl₃, J=Hz) 23.89 (dt, J₅₋₁=11.0, 3.7), 27.16 (t, J=4.6), 60.29 (d, J=4.6), 113.37 (ddd, J₅₋₁=290.3, 286.9, 2.4), 170.81; IR (neat, cm⁻¹) 2950, 1741, 1465, 1371, 1235, 1036, 742. Anal. Calcd for C₁₂H₁₄F₄O₄: C, 48.33; H, 4.73 Found: C, 49.06; H, 5.75.

dl-(32). RF 0.63 (hexane/ethyl acetate=10:1); bp 138 °C/ 7 Torr (Kugelrohr); ¹H NMR (200 MHz, δ, CDCl₃, J=Hz) 1.39-1.56 (2H, m), 1.57-1.79 (2H, m), 3.53 (4H, d, J=7.1), 4.51 (4H, ABq, J=11.9), 7.22-7.40 (10H, m); ¹³C NMR (50 MHz, δ, CDCl₃, J=Hz) 22.89 (dt, J₅₋₁=11.0, 3.7), 27.54 (t, J=9.7), 66.08 (d, J=4.1), 72.56, 114.05 (dd, J₅₋₁=290.5, 286.7), 127.58, 127.77, 128.44, 137.76; ¹⁹F NMR (188 MHz, δ, CDCl₃, J=Hz) 22.89 (dt, J₉₋₁=162.4, J₉₋₁=13.2); IR (neat, cm⁻¹) 2950, 1741, 1465, 1371, 1235, 1036, 742. Anal. Calcd for C₁₂H₁₄F₄O₄: C, 48.33; H, 4.73 Found: C, 49.06; H, 5.75.
Lipase-Catalyzed Reaction

To a buffer solution (pH 7.2) (76 mL) was added Lipase SL powder (1.13 g, Meito Sangyo). The lipase solution was added to diacetate 32 (2.26 g, 7.59 mmol). The mixture was stirred at room temperature for 2.5 h. The reaction was quenched with a crushed ice and NaCl. The crude product was extracted with ethyl acetate. The organic layer was dried over MgSO\textsubscript{4} and concentrated. Silica gel flash column chromatography, (gradient elution hexane / ethyl acetate = 7: 1 to 1: 1) gave monoacetate 31 (907 mg, 3.54 mmol, 47%), diol 30 (399 mg, 1.85 mmol, 24%) and diacetate 32 (454 mg, 1.52 mmol, 20%).

(lS, 3R, 4R, 6S)-30: $[\alpha]_D^20 = +31.5$ (c. 0.75, CHCl\textsubscript{3}) as a diacetate 32

(lR, 3S, 4S, 6R)-32: $[\alpha]_D^20 = -30.3$ (c. 1.11, CHCl\textsubscript{3})

(trans, trans)-1, 6-Bis(acetoxymethyl)-2,2,5,5-tetrafluorobicyclopropane meso-(33). Rf 0.26 (hexane/ethyl acetate=2:1); bp 145 °C/5 Torr (Kugelrohr); $^1$H NMR (200 MHz, $\delta$, CDCl\textsubscript{3}, J=Hz) 1.28-1.50 (2H, m), 1.68-1.88 (2H, m), 2.06 (3H, s), 2.56 (1H, s), 3.58-3.83 (2H, m), 4.00-4.24 (2H, m); $^13$C NMR (50 MHz, $\delta$, CDCl\textsubscript{3}, J=Hz) 20.57, 22.96 (dt, $J_{CF}$=11.1, 4.1), 24.06 (dt, J=11.1, 4.9), 27.02 (t, J=10.5), 30.19 (t, J=10.1), 59.18 (d, J=5.5), 60.38 (d, J=5.0), 113.64 (ddd, J=298.7, 291.4, 2.1), 113.64 (ddd, J=289.7, 286.8, 2.7), 171.03; $^1$F NMR (188 MHz, $\delta$, CDCl\textsubscript{3}, J=Hz) 22.08 (dd, J=218.6, 162.6, 119.9), 24.54 (dd, J=162.8, J\textsubscript{CF}=13.2) dR (neat, cm\textsuperscript{-1}) 3423, 3033, 2961, 2894, 1739, 1462, 1378, 1241, 1173, 1035, 865, 715. Anal. Calcd for C\textsubscript{31}H\textsubscript{21}F\textsubscript{4}O\textsubscript{3}: C, 46.88; H, 4.72 Found: C, 46.24; H, 5.14.

dl-(33). Rf 0.26 (hexane/ethyl acetate=2:1); $^1$H NMR (200 MHz, $\delta$, CDCl\textsubscript{3}, J=Hz) 1.48-1.84 (4H, m), 2.02 (1H, s), 2.06 (3H, s), 3.69 (2H, dt, J=6.8, 1.6), 4.05-4.15 (2H, m); $^1$C NMR (50 MHz, $\delta$, CDCl\textsubscript{3}, J=Hz) 20.68, 22.33 (dt, J=11.0, 3.0), 23.11 (dt, J\textsubscript{CF}=10.8, 4.2), 26.40 (t, J\textsubscript{CF}=11.4), 29.42 (t, J=9.8), 59.22 (d, J\textsubscript{CF}=5.1), 60.38 (d, J\textsubscript{CF}=3.7), 113.61 (t, J\textsubscript{CF}=288.3), 113.94 (dd, J\textsubscript{CF}=290.0, 286.0), 171.03; $^1$F NMR (188 MHz, $\delta$, CDCl\textsubscript{3}, J=Hz) 23.10 (dd, J\textsubscript{CF}=162.1, J\textsubscript{CF}=12.1), 24.50 (dd, J=31.37, 10.2), 25.26 (dd, J\textsubscript{CF}=161.9, J\textsubscript{CF}=13.2).

(IR, 3S, 4S, 6R)-33: $[\alpha]_D^22=-51.9$ (c.0.74, CHCl\textsubscript{3}) (>99% ee)

The monooacetate was identified to meso isomer, the diol was converted to diacetate with acetyl chloride, and GPC analysis for determination of enantiomeric excess of the diacetate was carried out using a capillary column on chiral phase; Chiraldex G-TA, $\phi$ 0.25 mm x 20 m; Carrier gas: He 40 mL/min; Temp (°C): 100, Inlet pressure; 1.35 kg/cm\textsuperscript{2}; Amount: 400ng; Detection: FID. The results of GC analysis of 32: tR of (lS, 3R, 4R, 6S)-32; 26.2 min., (IR, 3S, 4S, 6R)-32., 25.2 min., and (trans, trans)-meso-32; 38.6 min

(trans, trans)-1, 6-Bis[(9-anthracene-carbonyl)methyl]-2,2,5,5-tetrafluorobicyclopropane (34). $^1$H NMR (200 MHz, $\delta$, CDCl\textsubscript{3}, J=Hz) 1.70-2.10 (2H+2H, m), 4.48-4.76 (4H, m), 7.38-7.58 (8H, m), 7.85-8.10 (4H, m), 8.52 (2H, s)
Preparation of vinylcyclopropanes 40a-c and allyl ether 42a-c

Scheme 10

1) 3,4-Dihydro-2H-pyran
   PPTs, CH₂Cl₂, RT

2) BnBr, NaN₃, DMF, RT

3) p-TsOH·H₂O, MeOH, RT

Y=82% (2 steps)

[(1SR,3SR)-3-(benzyloxymethyl)-2,2-difluorocyclopropyl]methanol (49a). To a solution of 25 (679 mg, 3.77 mmol, >99%ee) and a solution of 3,4-Dihydro-2H-pyran (DHP, 381 mg, 4.53 mmol) in dichloromethane (10 mL) was added pyridinium p-toluenesulfonate (PPTs, 95 mg, 0.678 mmol) at 0°C. The solution was stirred at room temperature for 15h. The reaction was quenched by saturated sodium hydrogen carbonate solution, extracted with ethyl acetate. The organic layer was dried over MgSO₄ and concentrated. A solution of 48a in methanol (10 mL) was added K₂CO₃ (782 mg, 5.66 mmol) at room temperature. After being stirred for 17h at the same temperature, the crude product was purified by silica gel flash column chromatography (hexane / ethyl acetate = 2:1), to afford 49a (680 mg, 2.98 mmol) in 96% yield (Scheme 10): Rf 0.26 (hexane / ethyl acetate = 2:1); ¹H NMR (200 MHz, δ, CDCl₃, J=Hz) 1.40-1.62 (4H, m), 1.62-1.88 (4H, m), 2.60 (1H, t, J=2.2); ¹³C NMR (50 MHz, δ, CDCl₃, J=Hz) 19.44, 19.70, 25.34, 25.38, 26.87 (t, J_C=10.5), 27.13 (t, J_C=10.4), 28.99 (t, J_C=10.4), 29.20 (t, J_C=10.5), 30.55, 59.40, 59.49, 62.74, 63.05, 64.11, 64.18, 98.63, 99.54, 115.03 (t, J_C=286.6), 115.08 (t, J_C=286.7); ¹⁹F NMR (188 MHz, δ, CDCl₃, J=Hz) 22.97 (dd, J_F=163.5, J_F=12.9), 23.61 (t, J_F=8.1), 24.41 (dd, J_F=163.5, J_F=13.2); IR (neat, cm⁻¹) 3423, 2946, 1478, 1379, 1261, 1185, 1126, 1030, 903, 874, 812, 757, 723.

To a suspension of NaH (Oily (60%), 186 mg, 4.65 mmol) in dry DMF (6 mL) was added 48a (689 mg, 3.10 mmol) in dry DMF (2 mL) at 0°C under argon atmosphere, stirred at same temperature for 0.5h. After being added this suspension benzyl bromide (636 mg, 3.72 mmol) in dry DMF (2 mL), the mixture was stirred at room temperature for 12h, and then concentrated. The resulting oily product was dissolved in methanol (10 mL) added p-toluene sulfonic acid monohydrate (p-TsOH·H₂O, 54 mg, 0.314 mmol) at room temperature, then stirred same temperature. After 3h, methanol was removed by evaporation. The crude product was purified by silica gel flash column chromatography (hexane / ethyl acetate = 4:1), to afford 49a (680 mg, 2.98 mmol) in 96% yield (Scheme 10): Rf 0.26 (hexane / ethyl acetate = 2:1); ¹H NMR (200 MHz, δ, CDCl₃, J=Hz) 1.40-1.62 (4H, m), 1.62-1.88 (4H, m), 2.60 (1H, t, J=2.2); ¹³C NMR (50 MHz, δ, CDCl₃, J=Hz) 19.44, 19.70, 25.34, 25.38, 26.87 (t, J_C=10.5), 27.13 (t, J_C=10.4), 28.99 (t, J_C=10.4), 29.20 (t, J_C=10.5), 30.55, 59.40, 59.49, 62.74, 63.05, 64.11, 64.18, 98.63, 99.54, 115.03 (t, J_C=286.6), 115.08 (t, J_C=286.7); ¹⁹F NMR (188 MHz, δ, CDCl₃, J=Hz) 22.97 (dd, J_F=163.5, J_F=12.9), 23.61 (t, J_F=8.1), 24.41 (dd, J_F=163.5, J_F=13.2); IR (neat, cm⁻¹) 3423, 2946, 1478, 1379, 1261, 1185, 1126, 1030, 903, 874, 812, 757, 723.
(trans,trans)-[(RS,RS,4RS,5RS)-4-(Benzyloxyethyl)-2,2,5,6-tetrafluoro-1,3,4,6-bis(methanoxymethyl) methanol (50).] Rf 0.22 (hexane/ethyl acetate = 2:1); mp 49 °C; [α]D +13.0 (c 1.92, CHCl3), (>99% ee).

Using the same procedure, vinylcyclopropanes 49b and 50 were also prepared from the corresponding monoacetate 22 and 33.

IHNMR (200 MHz, 8, CDCl3, J=Hz) 2.00-2.15 (2H, m), 2.89 (1H, s), 3.43-3.85 (4H, m), 4.54 (2H, ABq, J=11.9), 7.25-7.43 (5H, m); 13C NMR (188 MHz, δ, CDCl3, J=Hz) 21.72 (dd, JF=162.1, JH=12.2), 23.39 (dd, JF=181.3, JH=11.5), 25.06 (dd, JF=162.4, JH=14.2); IR (neat, cm−1) 3366, 3043, 2945, 1745, 1361, 1281, 1184, 1149, 1031, 907, 875, 817.

(trans)-(1SR,3RS)-1-Benzyloxyethyl-2,2-difluoro-3-vinylcyclopropane (40a). The benzyloxy alcohol 49a (300 mg, 1.31 mmol) was dissolved in dry dichloromethane (8 mL) and PDC (590 mg, 1.57 mmol) was added at room temperature. After 24h of stirring, the mixture was filtered through a short column of Florisil (hexane / ether = 4:1), and the solvents were evaporated. A suspension of phosphonium salt (56 mg, 1.38 mmol) in dry THF (10 mL) was treated with t-BuOK (154 mg, 1.37 mmol) at 0°C and then added a solution of aldehyde 52 in dry THF (3 mL). The yellow reaction mixture was stirred at room temperature for 5h. The crude product was purified by silica gel flash column chromatography (hexane / ether = 4:1), to afford difluorovinylcyclopropane 40a (173 mg, 0.771 mmol) for two steps in 59% yield (Scheme 10). Using the same procedure, difluorovinylcyclopropane 40b was also prepared from the corresponding benzyloxy alcohol 49b.
(cis)-(1SR,3SR)-1-Benzzyloxymethyl-2,2-difluoro-3-vinylcyclopropane (49b). Rf 0.53 (hexane/ethyl acetate = 10:1); 1H NMR (200 MHz, δ, CDCl3, J=Hz) 1.86 (1H, dddd, J= 13.9, 13.9, 6.9, 1.6), 2.05 (1H, dt, J= 13.0, 7.5), 3.46-3.73 (2H, m), 4.54 (2H, ABq, J=12.0), 5.20 (1H, d, J=1.7), 5.36 (1H, dd, J=29.9, 1.5), 5.56 (1H, ddt, J=9.1, 3.3, 1.6) 7.24-7.40 (5H, m); 13C NMR (50 MHz, δ, CDCl3, J=Hz) 29.41 (t, JCF=10.0), 30.98 (t, JCF=11.5), 66.30 (d, JCF=4.7), 72.54, 114.68 (dd, JCF=290.9, 288.5), 117.92, 127.70, 127.78, 128.45, 131.19 (d, JCF=3.1), 137.85; 19F NMR (188 MHz, δ, CDCl3, J=Hz) 24.10 (dd, JIF=160.9, JHF=12.2), 25.73 (dd, JIF=159.0, JHF=13.2); IR (neat, em-I) 3030, 2867, 1641, 1468, 1411, 740, 698.

The benzyloxy alcohol 50 (760 mg, 2.50 mmol) was dissolved in dry dichloromethane (10 mL) and PDC (1.41 g, 3.75 mmol) was added at room temperature. After 9 h of stirring, the mixture was filtered through a short column of Florisil (hexane/ether = 4:1), and concentrated. A suspension of phosphonium salt (1.21 g, 2.99 mmol) in dry THF (7 mL) was treated with t-BuOK (365 mg, 3.25 mmol) at 0°C and then added a solution of aldehyde 52 in dry THF (3 mL). The yellow reaction mixture was stirred at room temperature for 5 h. The crude product was purified by silica gel flash column chromatography (gradient solution hexane/ethyl acetate = 100:1 to 10:1) to afford 40c (230 mg, 0.766 mmol) in 31% yield for two steps.

(eis)-(1SR,3SR)-1-Benzzyloxymethyl-2,2-difluoro-3-vinylcyclopropane (40b). Rf 0.41 (hexane/ethyl acetate = 10:1); 1H NMR (200 MHz, δ, CDCl3, J=Hz) 2.00-2.15 (1H, m), 2.29-2.48 (1H, m), 3.58-3.66 (2H, m), 4.52 (2H, ABq, J=12.0), 5.26 (2H, d, J=17.7), 5.50 (1H, dd, J=17.3, 8.0), 7.15-7.40 (5H, m); 13C NMR (50 MHz, δ, CDCl3, J=Hz) 27.23 (t, JCF=9.9), 29.30 (t, JCF=12.1), 63.48 (d, JCF=5.1), 72.54, 114.68 (dd, JCF=290.9, 288.5), 117.92, 127.78, 128.45, 131.19 (d, JCF=3.1), 137.85; 19F NMR (188 MHz, δ, CDCl3, J=Hz) 24.10 (dd, JIF=160.9, JHF=12.2), 25.73 (dd, JIF=159.0, JHF=13.2); IR (neat, cm-1) 3030, 2867, 1641, 1468, 1411, 740, 698.

Scheme 11

The benzyloxy alcohol 50 (760 mg, 2.50 mmol) was dissolved in dry dichloromethane (10 mL) and PDC (1.41 g, 3.75 mmol) was added at room temperature. After 9h of stirring, the mixture was filtered through a short column of Florisil (hexane / ether = 4:1), and concentrated. A suspension of phosphonium salt (1.21 g, 2.99 mmol) in dry THF (7 mL) was treated with t-BuOK (365 mg, 3.25 mmol) at 0°C and then added a solution of aldehyde 52 in dry THF (3 mL). The yellow reaction mixture was stirred at room temperature for 5h. The crude product was purified by silica gel flash column chromatography (gradient solution hexane / ethyl acetate = 100:1 to 10:1) to afford 40c (230 mg, 0.766 mmol) in 31% yield for two steps.

(eis)-(1SR,3SR,4RS,6SR)-2,2,5,5-tetrafluoro-1-Benzzyloxymethyl-6-vinylcyclopropane (40c).
(trans)-(1SR,3SR)-1-Allyloxymethyl-3-benzyloxymethyl-2,2-difluorocyclopropane (42a). To a suspension of NaH (Oily, 600 mg, 132 mg) in dry DMF (2 mL) was added a benzyloxy alcohol 49a (502 mg, 2.20 mmol) in dry THF (7.5 mL) at room temperature under argon atmosphere, stirred at 80°C for 10 minutes. After being added at same temperature this suspension allyl bromide (200 mg, 1.62 mmol) in dry THF (2.5 mL), the mixture was stirred at room temperature for 10 h. The reaction was quenched by addition of crushed ice, extracted with ether. Silica gel flash column chromatography, (gradient elution hexane/ethyl acetate = 50:1) gave allyl ether 42a (573 mg, 2.14 mmol) in 97% yield (Scheme 9): R\textsubscript{f} 0.58 (hexane/ethyl acetate=4:1); \textsuperscript{1}H NMR (200 MHz, \text{\text{CDCl}}_3, J=Hz) 1.56-1.84 (2H, m), 3.45-3.69 (4H, m), 4.00 (2H, ABq, J=12.0), 5.22 (1H, dt, J=5.9, 1.4), 5.24 (1H, ddd, J=30.4, 2.9, 1.3), 5.90 (1H, dddd, J=22.3, 10.7, 5.5, 1.0) 7.25-7.48 (5H, m); \textsuperscript{13}C NMR (50 MHz, \text{\text{CDCl}}_3, J=Hz) 26.47 (t, J\textsubscript{CF}=10.5), 65.95, 71.44, 72.52, 114.80 (t, J\textsubscript{CF}=286.6), 117.33, 127.68, 127.74, 128.43, 134.39, 137.89; \textsuperscript{19}F NMR (188 MHz, \text{\text{CDCl}}_3, J=Hz) 23.53 (t, J\textsubscript{PF}=7.5); IR (neat, cm\textsuperscript{-1}) 3029, 2867, 1641, 1455, 1253, 1096, 983, 917, 784, 700.

Using the same procedure, allyl ethers 42b and 42c were also prepared from the corresponding benzyloxy alcohols 49b and 50.

(cis)-(1RS,3SR)-1-Allyloxymethyl-3-benzyloxymethyl-2,2-difluorocyclopropane (42b). Rf 0.64 (hexane/ethyl acetate=4:1); \textsuperscript{1}H NMR (200 MHz, \text{\text{CDCl}}_3) 1.86-2.12 (2H, m), 3.43-3.72 (4H, m), 3.85-4.08 (2H, m), 4.52 (2H, ABq, J=11.8), 5.17 (1H, d, J=1.5), 5.27 (1H, dt, J=17.5, 1.5), 5.88 (1H, ddd, J=22.5, 10.6, 5.9) 7.20-7.45 (5H, m); \textsuperscript{13}C NMR (50 MHz, \text{\text{CDCl}}_3) 24.95 (t, J\textsubscript{CF}=10.5), 63.4 (t, J\textsubscript{CF}=5.7), 71.58, 72.75, 114.18 (d, J\textsubscript{CF}=289.7, 283.2), 117.41, 127.78, 128.43, 134.32, 137.79; \textsuperscript{19}F NMR (188 MHz, \text{\text{CDCl}}_3, J=Hz) 10.97 (d, J\textsubscript{PF}=161.8), 36.47 (dt, J\textsubscript{PF}=161.4, J\textsubscript{PF}=12.6); IR (neat, cm\textsuperscript{-1}) 3028, 2864, 1705, 1477, 1367, 1262, 1194, 1085, 1026, 925, 845, 741, 703.

Olefin Metathesis (Method A)

1,4-Bis(1RS,3SR)-3-benzyloxymethyl-2,2-difluorocyclopropyl methyloxy)-(E)-2-butene (43b). To a solution of (26.8 mg, 0.10 mmol) in dry dichloromethane (1.0 mL) was added Grubb's reagent (12.3 mg, 0.02 mol).
Octen added a solution of Grubb's reagent (16.5 mg, dichloromethane (Method B) 2866, 1710, 1602, 1476, 1367, 1283, 1189, 1088, 741. MHz, acetate. Silica gel flash column chromatography, (hexane/ethyl acetate = 10:1) gave (17.5 mg, 0.034 mmol) in 68% yield. Ref 0.28 (hexane/ethyl acetate=4:1); 1H NMR (200 MHz, δ, CDCl₃, J=Hz) 1.89-2.10 (4H, m), 3.45-3.72 (8H, m), 3.85-4.12 (4H, m), 4.51 (4H, ABq, J=11.6), 5.69 (1H, t, J=3.9)(minor), 5.77 (1H, t, J=2.7)(major), 7.09-7.45 (10H, m); 13C NMR (50 MHz, δ, CDCl₃, J=Hz) 24.95 (t, J=10.3), 63.49 (t, J=4.9), 70.44, 72.75, 114.16 (dd, J=289.8, 282.8), 128.51, 128.59, 129.24, 130.06, 138.64; 19F NMR (188 MHz, δ, CDCl₃, J=Hz) 10.88 (d, J=162.1)(minor), 10.98 (d, J=161.8)(major), 36.45 (dt, J=162.1, J=12.2); IR (neat, cm⁻¹) 3029, 2866, 1710, 1602, 1476, 1367, 1283, 1189, 1088, 741.

(Method B)

(E)-(1SR,3RS,6RS,8SR)-2,2,7,7-tetrafluoro-1,8-bisbenzoyloxymethyl-1,3,6,8-bismethano-4-Octen (41b). To a solution of 40a (22.4 mg, 0.10 mmol) in dry dichloromethane (1.0 mL) was added a solution of Grubb's reagent (16.5 mg, 0.020 mmol, 20 mol%) in dry dichloromethane (0.2 mL) at room temperature under argon atmosphere every twelve hours, stirred for 72h. The crude product was purified by silica gel thin layer chromatography (hexane/ethyl acetate = 10:1), to afford 41a (7.3 mg, 0.0174 mmol) in 35% yield. Ref 0.58 (hexane/ethyl acetate=4:1); 1H NMR (200 MHz, δ, CDCl₃, J=Hz) 1.56-1.84 (2H, m), 3.45-3.69 (4H, m), 4.00 (2H, dt, J=5.4, 1.4), 4.54 (2H, ABq, J=12.0), 5.22 (1H, dt, J=5.9, 1.4), 5.24 (1H, ddd, J=30.4, 2.9, 1.3), 5.90 (1H, dddd, J=22.3, 10.7, 5.5, 1.0) 7.25-7.48 (5H, m); 13C NMR (50 MHz, δ, CDCl₃, J=Hz) 26.47 (t, J=10.5), 65.95, 71.44, 72.52, 114.80 (t, J=286.6), 117.33, 127.68, 127.74, 128.43, 134.39, 137.89; 19F NMR (188 MHz, δ, CDCl₃, J=Hz) 23.53 (t, J=7.5); IR (neat, cm⁻¹) 3027, 2865, 1767, 1481, 1367, 1262, 1194, 1022, 929, 743, 703.  

(E)-(1SR,3SR,6RS,9SR,11RS,12RS,14SR)-2,2,5,5,10,10,13,13-Octafluoro-1,3,4,6,9,11,12,14-tetramethano-1,14-bisbenzoyloxymethyl-7-tetradecane (41c). Ref 0.28 (hexane/ethyl acetate=10:1); 1H NMR (200 MHz, δ, CDCl₃, J=Hz) 1.78-2.64 (2H, m), 3.46-3.67 (4H, m), 4.43-4.58 (4H, m), 5.42 (1H, dd, J=11.9, 5.0), 7.20-7.45 (10H, m); IR (neat, cm⁻¹) 3034, 2865, 1464, 1258, 1180, 1083, 1021, 743, 699.  

(E)-(1SR,3RS,4RS,9SR,11RS,12RS,14SR)-2,2,5,5,10,10,13,13-Octafluoro-1,3,4,6,9,11,12,14-tetramethano-1,14-bisbenzoyloxymethyl-7-tetradecane (41c). Ref 0.28 (hexane/ethyl acetate=10:1); 1H NMR (200 MHz, δ, CDCl₃, J=Hz) 1.25-1.50 (4H, m), 1.83 (2H, ddd, J=13.9, 13.8 (6.8), 3.44-3.70 (4H, m), 4.53 (4H, ABq, J=12.0), 5.38 (2H, dd, J=4.8, 2.4), 7.25-7.40 (10H, m); 19F NMR (188 MHz, δ, CDCl₃, J=Hz) 22.58 (dd, J=160.7, J=12.2), 25.02 (dd, J=161.4, J=13.9); 13C NMR (50 MHz, δ, CDCl₃, J=Hz) 23.45 (dt, J=12.7, 2.7), 26.89 (dt, J=10.0, 4.0), 28.20 (t, J=10.5), 31.61 (t, J=10.8), 66.12 (d, J=4.5), 112.94 (m), 125.70, 127.62, 127.81, 128.46, 137.73; IR (neat, cm⁻¹) 3032, 2866, 1457, 1255, 1172, 1094, 1031, 742, 706.  

1,4-Bis(1SR,3SR)-3-benzoyloxymethyl-2,2-difluorocyclopropyl methyloxy-[(E)-2-buten e (43a). Ref 0.22 (hexane/ethyl acetate=4:1); 1H NMR (200 MHz, δ, CDCl₃) 1.58-1.80 (4H, m), 3.42-3.68 (8H, m), 3.99 (4H, tt, J=3.0, 1.4)(major), 4.06 (t, J=3.1)(minor), 4.53 (4H, ABq, J=12.0), 5.71
(tt, J=3.6,1.1)(minor), 5.79 (2H, tt, J=2.8,1.3), 7.24-7.40 (10H, m); 13C NMR (50 MHz, δ, CDCl₃, J=Hz) 26.43 (t, J_c=10.5), 66.01, 72.53, 114.76 (t, J_c=286.5), 127.68, 127.75, 128.43, 129.26, 137.87; 19F NMR (188 MHz, δ, CDCl₃) 23.49; IR (neat, cm⁻¹) 3025, 2865, 1729, 1477, 1369, 1262, 1193, 1102, 743.

1,4-Bis[(1SR,3RS,4RS,6SR)-6-benzyloxymethyl-2,2,3,3-tetrafluoro-1,3-4,6-bismethano]methoxy-(E)-2-butenes (43c). Rf 0.33 (hexane/ethyl acetate=4:1); 1H NMR (200 MHz, δ, CDCl₃, J=Hz) 1.16-1.35 (2H, m), 1.65-1.90 (2H, m), 3.35-3.70 (8H, m), 3.87-4.23 (4H, m), 4.53 (4H, ABq, J=12.1), 5.72 (2H, t, J=4.2)(minor), 5.80 (1H, J=2.3), 7.10-7.45 (10H, m); 13C NMR (50 MHz, δ, CDCl₃, J=Hz) 23.28 (m), 27.84, 66.17 (d, J_c=4.7), 70.35, 72.62, 113.88 (t, J_c=289.1), 128.42, 128.58, 129.25, 130.00, 137.71; 19F NMR (188 MHz, δ, CDCl₃, J=Hz) 22.51 (dd, J_F=164.1, 12.2), 24.49 (dd, J_F=160.9, J_F=12.2); IR (neat, cm⁻¹) 3028, 2924, 2860, 1705, 1614, 1458, 1367, 1300, 1255, 1175, 1101, 1021, 740, 704.

References and Notes


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(6) For the review in this field see: T. Itoh, Y. Takagi, and H. Tsukube, J. Molecular Catalysis B: Enzymatic 1997, 3, 261.


(9) The absolute configuration of 22 was a tentative one. We assume it to be (1R, 2S) based on the results of Mosher's modified method by


(16) 3-Tributylstanny-2-propen-1-ol was prepared as a mixture of (E)-2 and (Z)-2 (ca. 1:1) by the rapid addition of tributyltin hydride with propargyl alcohol in the presence of AIBN as a radical initiator.

(17) Lipase SL (Pseudomonas cepacia SL-25), lipase PS (Pseudomonas cepacia), and AL (Achromobacter sp.) preferably gave trans-2 with more than 80% selectivity and the best selectivity was recorded when the reaction was catalyzed by PCL.


(20) Analysis by GPC for determination of % ee of 5 was carried out using a capillary column on a chiral phase; Chiralaldex G-TA, φ0.25 mm x 20 m; Carrier gas: He 40 mL/min; Temp (°C); 100, Inlet pressure; 1.35 kg/cm²; Amount; 400 ng; Detection; FID. The results of GC analyses of 5: tR of (+)-(trans,trans)-5; 26.2 min., (-)-(trans,trans)-5; 25.2 min., and (trans,trans)-meso-5; 38.6 min.

(21) Crystal and refinement data for 8: C22H22F4O2, formula weight = 394.41, monoclinic, space group P2₁, a= 8.6979 Å, b= 6.0163 , c= 19.3163 Å, V= 1005.5700Å³, Z=2, dcalc= 1.30 g cm⁻³, R(Rw)=0.082 for 1142 diffraction data with l>3.00σ (l) and 253 valiable.


(25) MacSpartan Plus was employed for MO (PM3) calculation.


(28) It was essential to use the catalyst as CH$_2$Cl$_2$ solution. No increase of the chemical yield was observed when the catalyst powder was added to the reaction mixture in a five fold portion.


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