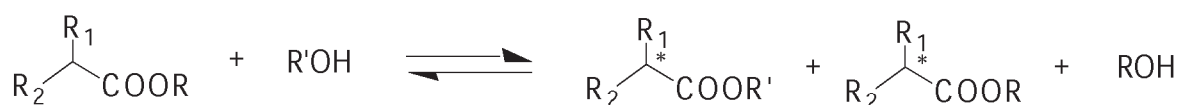
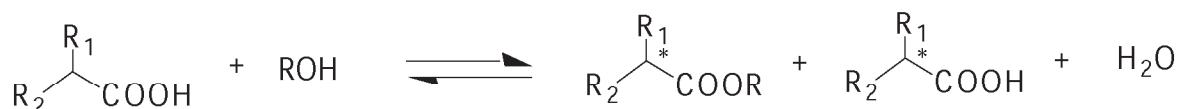
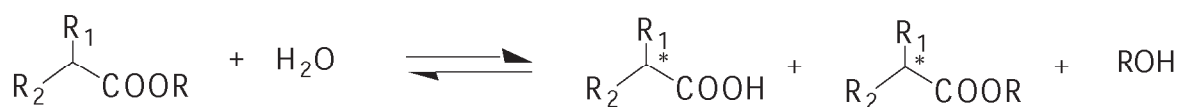
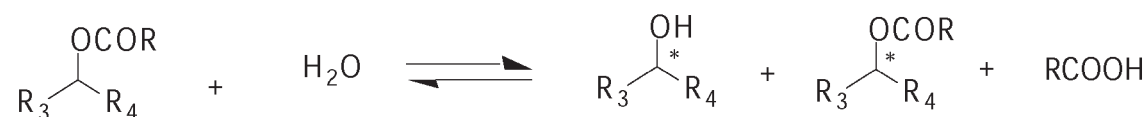


Examples of reaction types that are candidates for enzyme mediation:

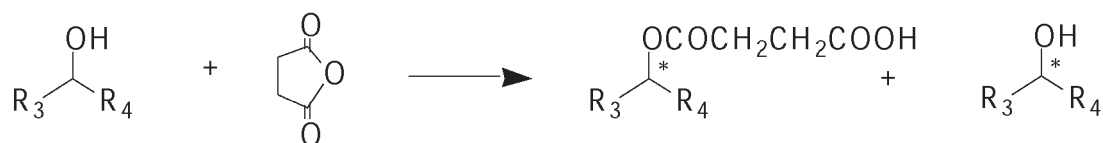
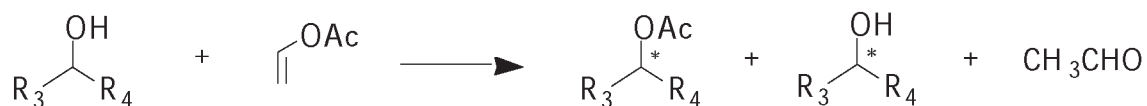
1) Resolution of acids by hydrolysis and esterification



2) Resolution of alcohols by hydrolysis and esterification (transesterification)

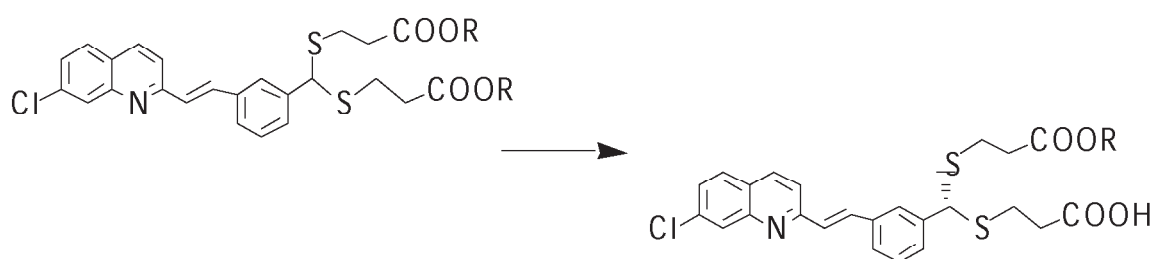


3) Resolution of alcohols by irreversible transesterification

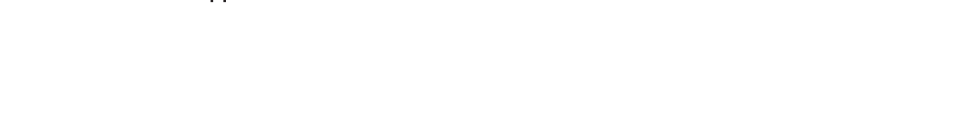
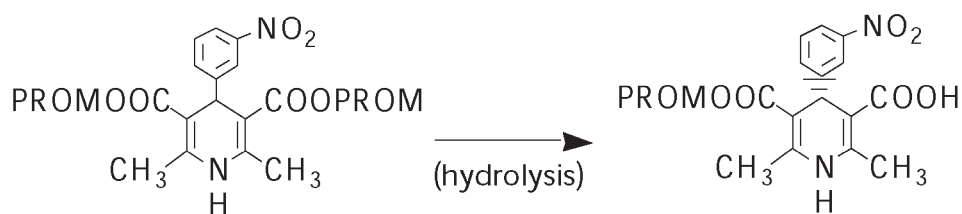
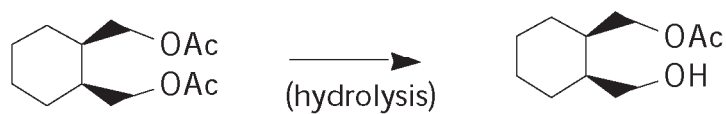
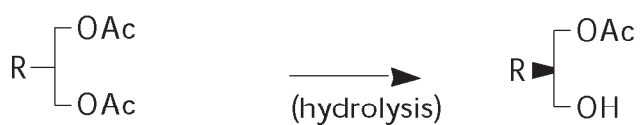
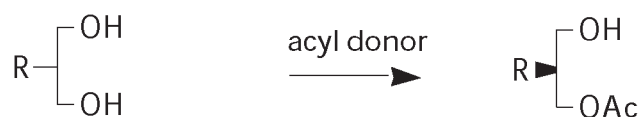


acyl donor : $\begin{array}{c} OAc \\ | \\ CH_2 \\ | \\ CH \end{array}$, $\begin{array}{c} OAc \\ | \\ C \\ // \\ C \end{array}$, Ac_2O , $AcOCH_2CCl_3$ etc.

4) Asymmetric synthesis of acids by hydrolysis

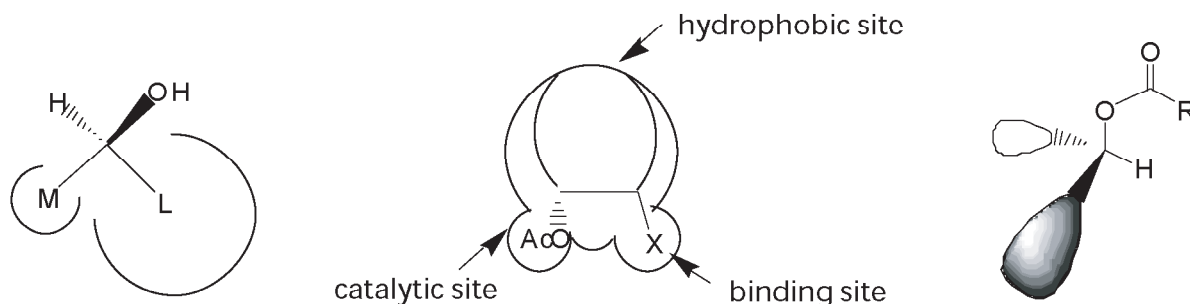


5) Asymmetric synthesis of alcohols by transesterification and hydrolysis of prochiral compounds and meso compounds



Hydrolytic Model for Lipase PS in Order to Predict Enantioselectivity of Secondary Alcohols

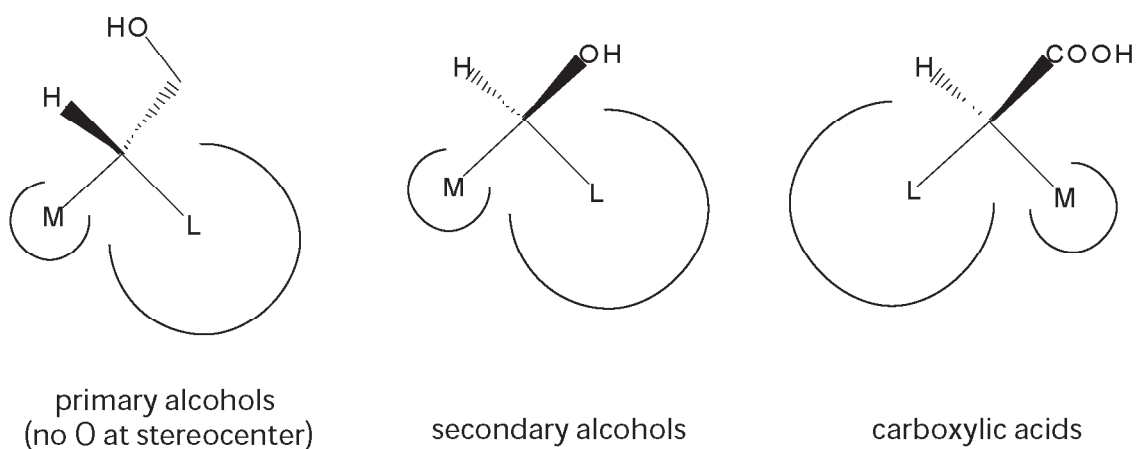
Many lipases including lipase PS show a preference for the R -configuration. Models of hydrolysis of esters of secondary alcohols are described.



R. J. Kazlauskas et al., *J. Org. Chem.*, 56, 2656 (1991).
 K. Sakai et al., *Tetrahedron: Asymmetry* 1, 395 (1990). *ibid.*, 2, 733 (1991).
 M. P. Schneider et al., *Pure & appl. Chem.*, 64, 1165 (1992).

Enantiopreference of Lipases toward Primary, Secondary Alcohols and Carboxylic Acids

These rules are based on the size of the substituents at the stereocenter and proposed for substrate mapping studies by Prof. Dr. Kazlauskas.



J. Org. Chem., 60, 6959 (1995). *J. Org. Chem.*, 56, 2656 (1991). *Biocatalysis*, 9, 209 (1994).

Determination of the Enantioselectivity

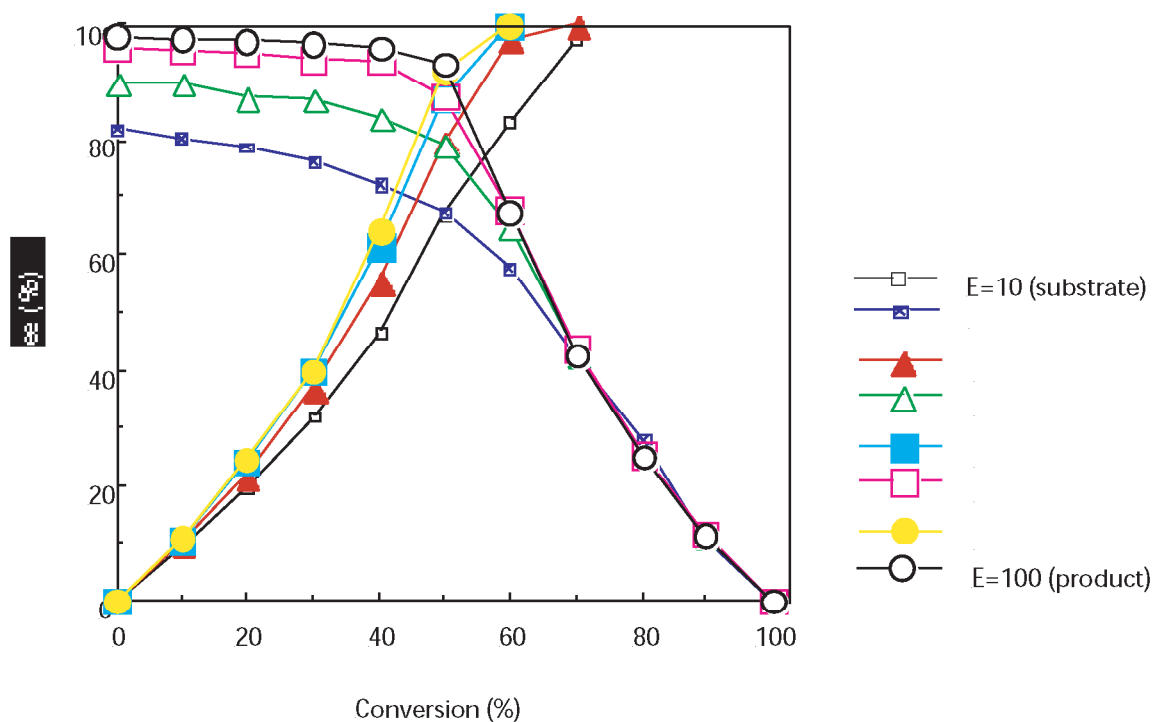
Enantiomeric excess

$$\text{Enantiomeric excess (\% ee)} = \frac{[S] - [R]}{[S] + [R]} \times 100$$

E-value

$$\text{E-value: } E = \frac{\ln[(1-c)(1-ee(S))]}{\ln[(1-c)(1+ee(S))]} = \frac{\ln[1-c(1+ee(P))]}{\ln[1-c(1-ee(P))]} \quad c: \text{ conversion}$$

C. J. Sih et al., J. Am. Chem. Soc., 104, 7294 (1982).



Simulation curve from E value

Factors influencing improvements or alterations of enzyme specificity

- | | |
|----------------------------------|---|
| 1. Enzymes | origin
concentration
enzyme type - powdered, immobilized and lyophilized
prepared condition - pH, buffer etc
mutation - site directed mutagenesis, random mutagenesis |
| 2. Substrates | structure
concentration |
| 3. Reaction Type | hydrolysis
esterification
transesterification |
| 4. Reagent | acyl donor etc
concentration |
| 5. Effect of Reaction Media | organic solvents
buffer
bilayer |
| 6. Effect of Water Concentration | |
| 7. Effect of Additive | |
| 8. Effect of Temperature | |
| 9. Effect of pH | |

The above factors each need optimization for a particular aspect of enzyme performance. To assist in the choice of the reaction conditions, some published applications using Amano lipase are given in the following pages. If you have any questions, please contact Amano.