Biocatalysis II

Hydrolytic reactions

Hydrolytic transformations involving amide-, ester bonds are the easiest to perform

Proteins used for these reactions are proteases, esterases or lipases.

A favourite class of enzymes are the hydrolases:

•No cofactors needed to recycle

•Broad substrate acceptance

•Large number of readily available enzymes

Reverse reactions have been studied as well in solvent systems with low water activity

Other types of reactions of hydrolases

Formation/Cleavage of phosphate esters

Epoxides

Nitriles

Organo-halides

Are a bit more tricky to achieve.

Biocatalysis II

Mechanistic and Kinetic aspects

The mechanism of amide- and ester hydrolyzing enzymes is very similar to that describing chemical hydrolysis by a base.
Active site acts as nucleophil
Attacks the carbonyl group at the substrate

The nucleophile can be
The –OH group of Serine
The COOH group of Aspartic acid
The –SH group of Cysteine

Best studied mechanism is the serine hydrolase (Ser,His,Asp)

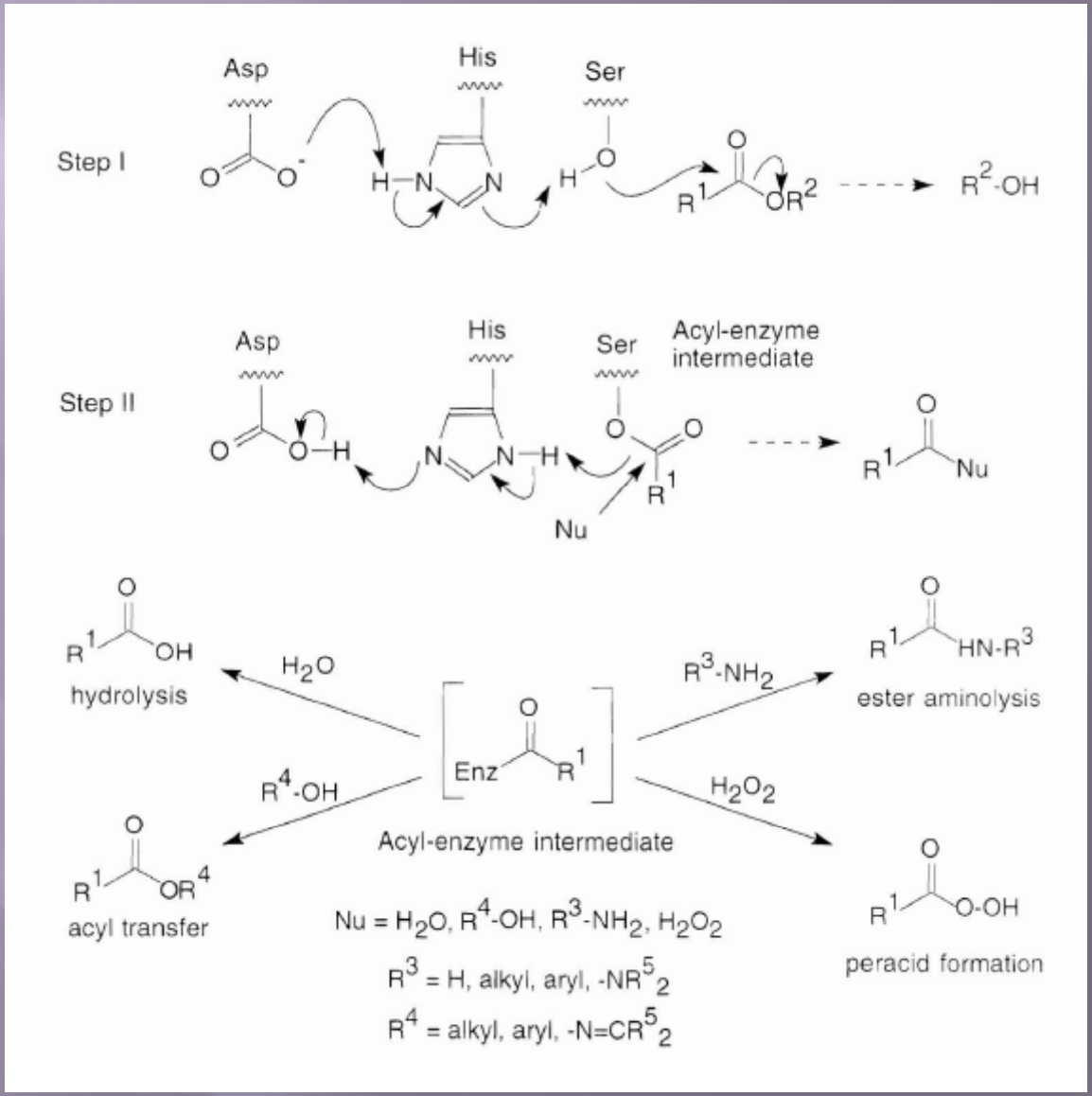
Biocatalysis II

- When operating in low water activity environment:
  - Any other niucleophile can compete with the water for the acyl-enzyme intermediate, giving rise to some synthetically useful transformations.
  - Attack of another alcohol R4-OH leads to a different ester R1-CO-OR4
  - Enzymatic acyl transfer reaction (interesterification).
  - Incoming amine R3-NH2 results in the formation of an N-substituted amide R1-CO-NH-R3.
  - Enzymatic aminolysis of esters.
  - Peracids are formed when H2O2 is used as the nucleophile.
  - Hydrazinolysis results in Hydrazides
  - Thiols, would form thioesters, are unreactive.

During all these reactions the chirality in the substrate is reckognized by the enzyme, which causes a preference for one of the two possible stereoisomeres.
The value of this discrimination is a crucial parameter → Selectivityof the reaction.
Governed by the reaction kinetics

Biocatalysis II

The serine hydrolase reaction



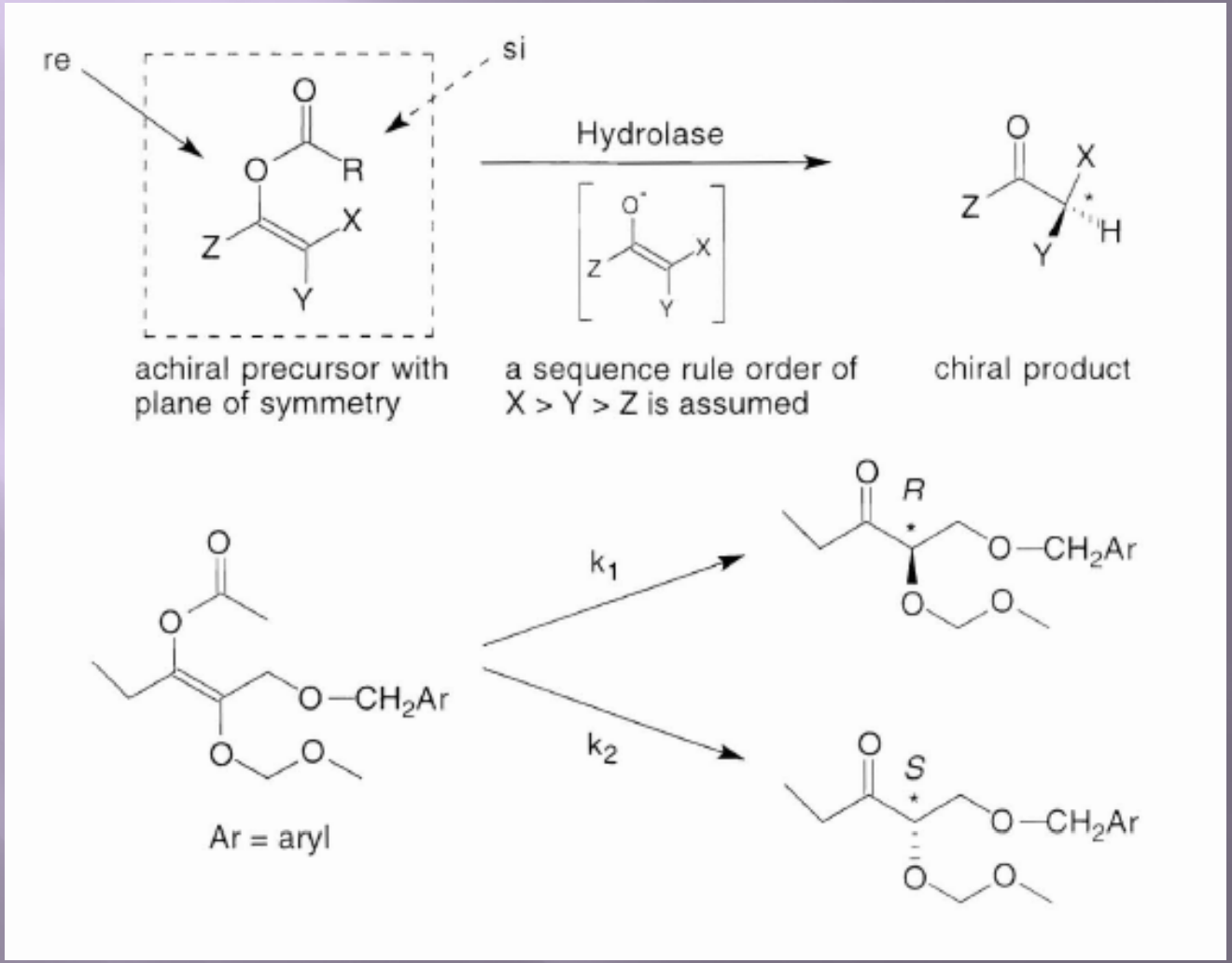
Biocatalysis II

- Enantiophase Differentiation:

  Hydrolases can distinguish between the two enantiomeric phases of achiral substrates such as enol esters possessing a plane of symmetry within the molecule.
  The nucleophilic attack occurs predominantly from one side, leading to an unsymmetric enolization of the unstable free enol towards one preferred side within the active site.
  This causes a new center of chirality during the reaction in the product.

Biocatalysis II

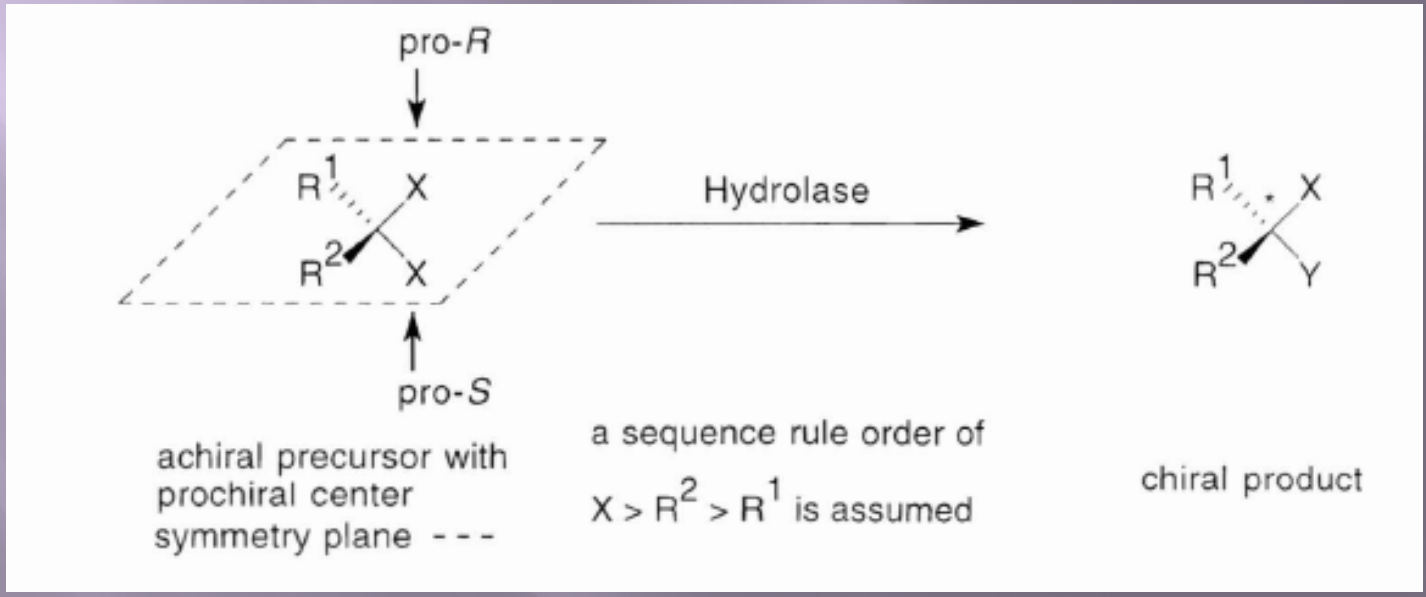
Enantiophase Differentiation



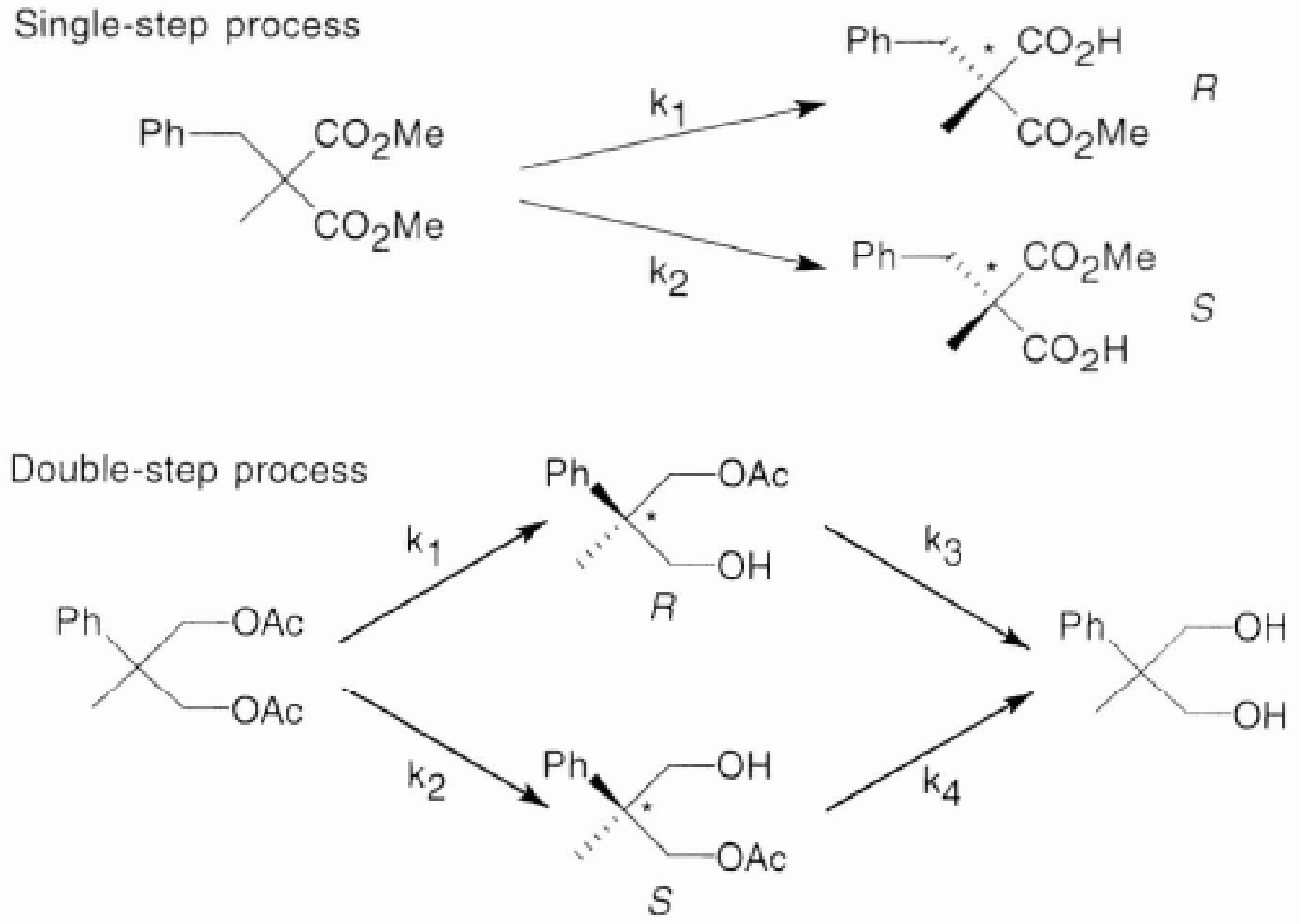
Biocatalysis II

Enantiotopos differentiation:

Prochiral substrates with two chemically identical but enantiotopic reactive groups X (pro-R and pro-S)
Enzymatic hydrolysis will result in a chiral discrimination between the two while transforming group X to Y.
Resulting in a chiral product.During the reaction the plane of symmetry is broken.



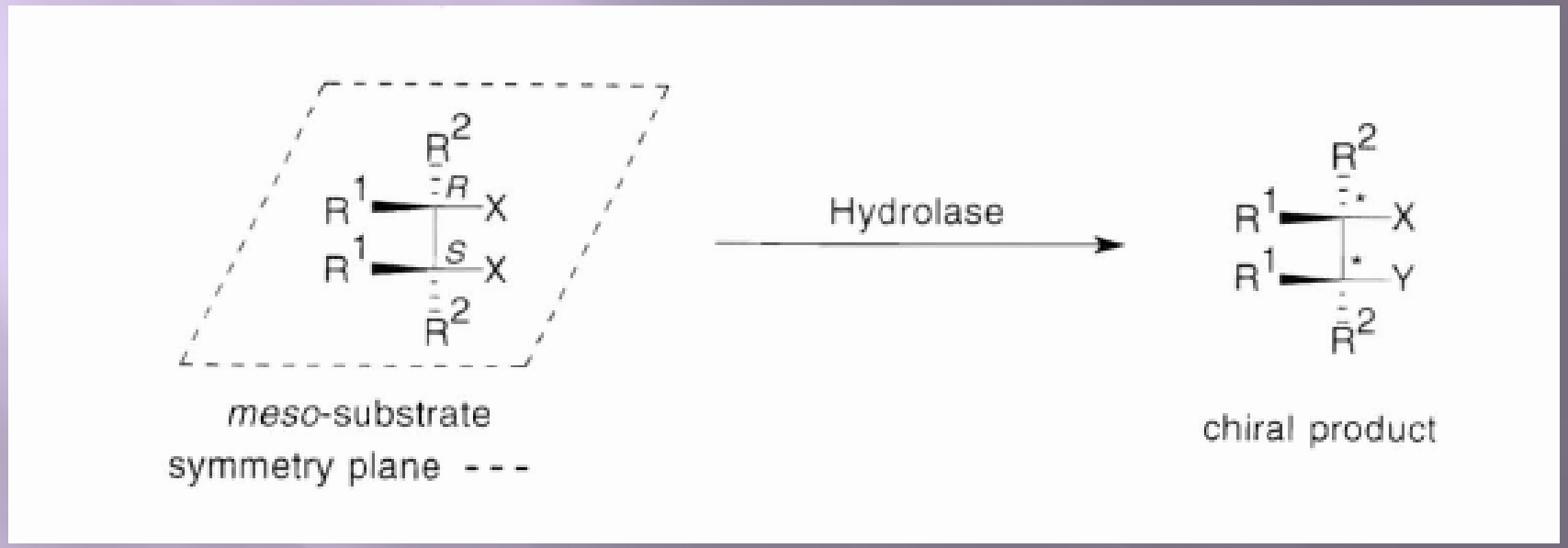
Biocatalysis II



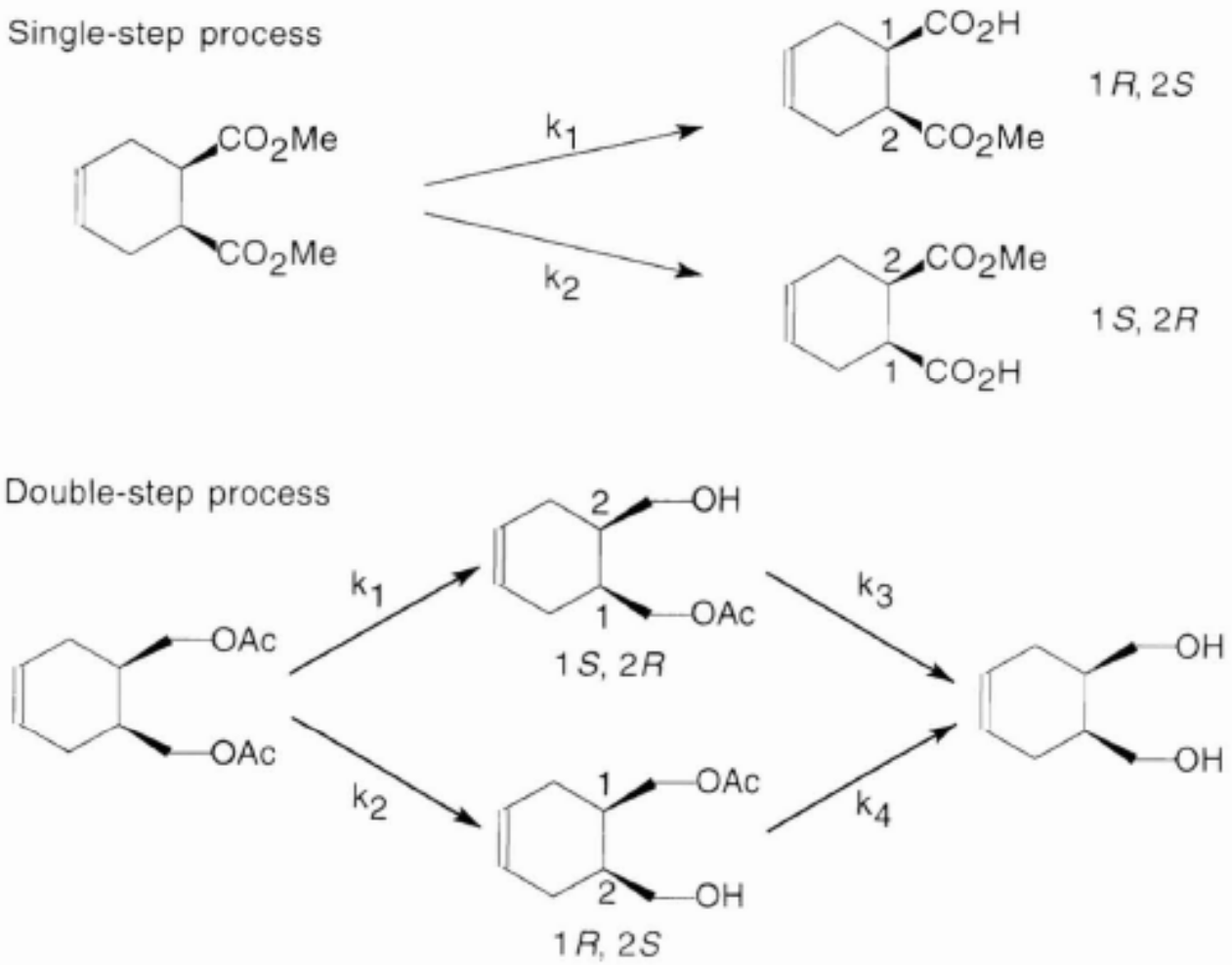
Biocatalysis II

- R,S configuration of substrate
  - 2 chemically identical groups X, psoitioned on carbon atoms of opposite (r,S)-configuration. *Meso* substrate
  - Reacts at different rates
  - This yields an optically active product due to the the transformation of one of the reactive groups from X to Y.
  - Leading to the destruction of the planbe of symmetry in the substrate molecule.
  - Open chain or cyclic cis-meso-diesters have been transformed into chiral monoesters.
  - The theoretical yield of chiral product from a single step reaction based on an
  - enantioface- or
  - enantiotopos-differentiation or
  - a desymmetrization of a meso compound is always 100%.

Biocatalysis II



Biocatalysis II



Desymmetrization of *meso-substrates*

The two chemically identical groups X, positioned on carbon atoms of opposite (R,S)-configuration in a meso-substrate, can react at different rates in a hydrolase-catalyzed reaction.

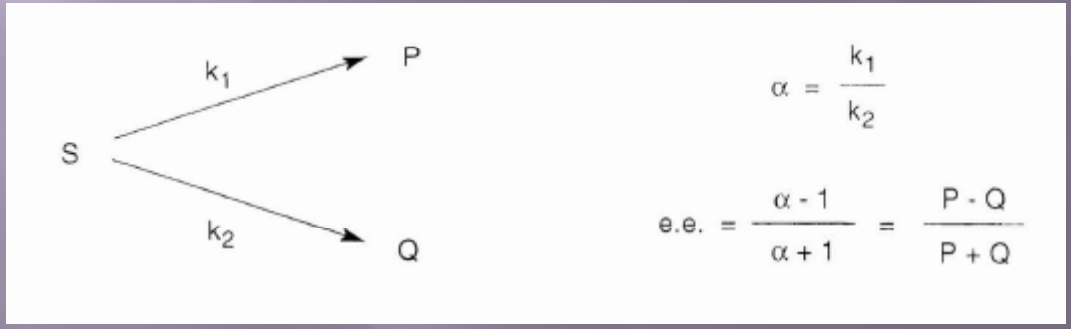
As a result, the optically inactive *meso-substrate is transformed into an optically active* product due to the transformation of one of the reactive groups from X into Y

going in hand with the destruction of the plane of symmetry within the substrate.

Numerous open-chain or cyclic *cis-meso-diesters have been transformed into chiral monoesters by this technique*

Biocatalysis II

•Since hydrolytic reactions are performed in an aqueous environment, they are virtually completely irreversible.
•The kinetics of all single step reactions is very simple: a prochiral or a meso substrate S is transformed into two enantiomeric products P and Q at different rates.æ
•Rate constant: apparent first order rate constant k1 and k2.
•The selectivity (alpha) is only governed by the ratio of k1/k2.
•is *independent of the conversion and therefore remains constant throughout the reaction*
•The optical purity of the product (e.e.) is *not dependent on the extent of the conversion.*
•The selectivity observed in such a reaction can *not be improved by stopping the reaction at different extents of conversion*
•Changing the 'environment' of the system



changing the 'environment' of the system

Parameters to be modified:

substrate modification,

Choice of another enzyme,

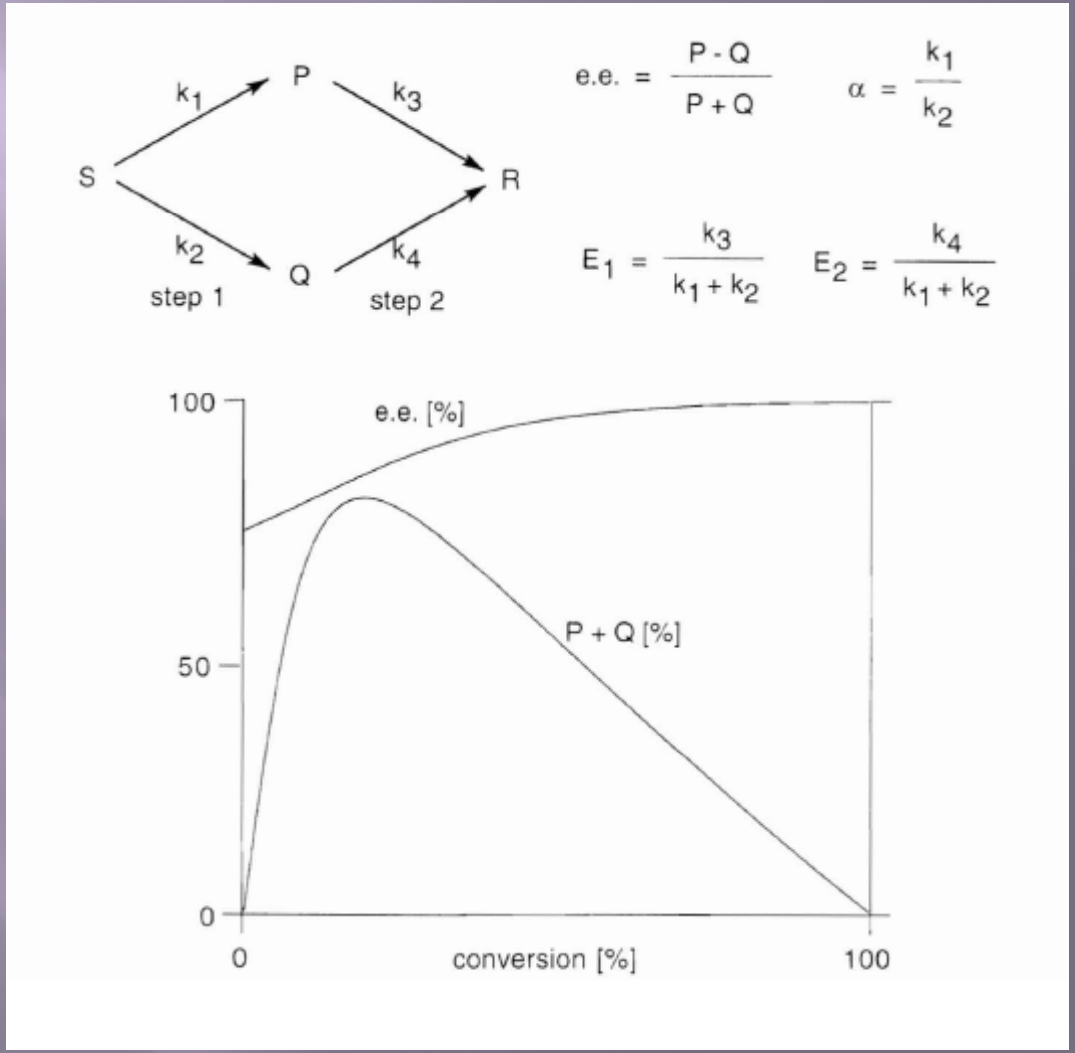
the addition of organic cosolvents,

variations in temperature

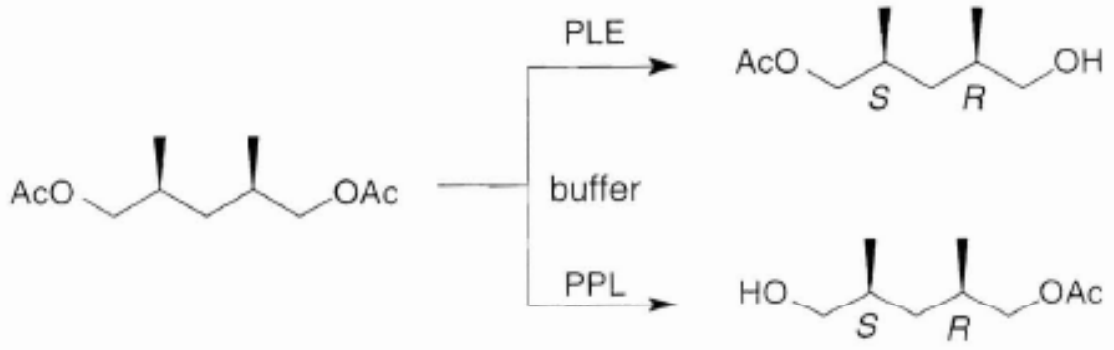
Variation of pH

Biocatalysis II

Double step kinetics

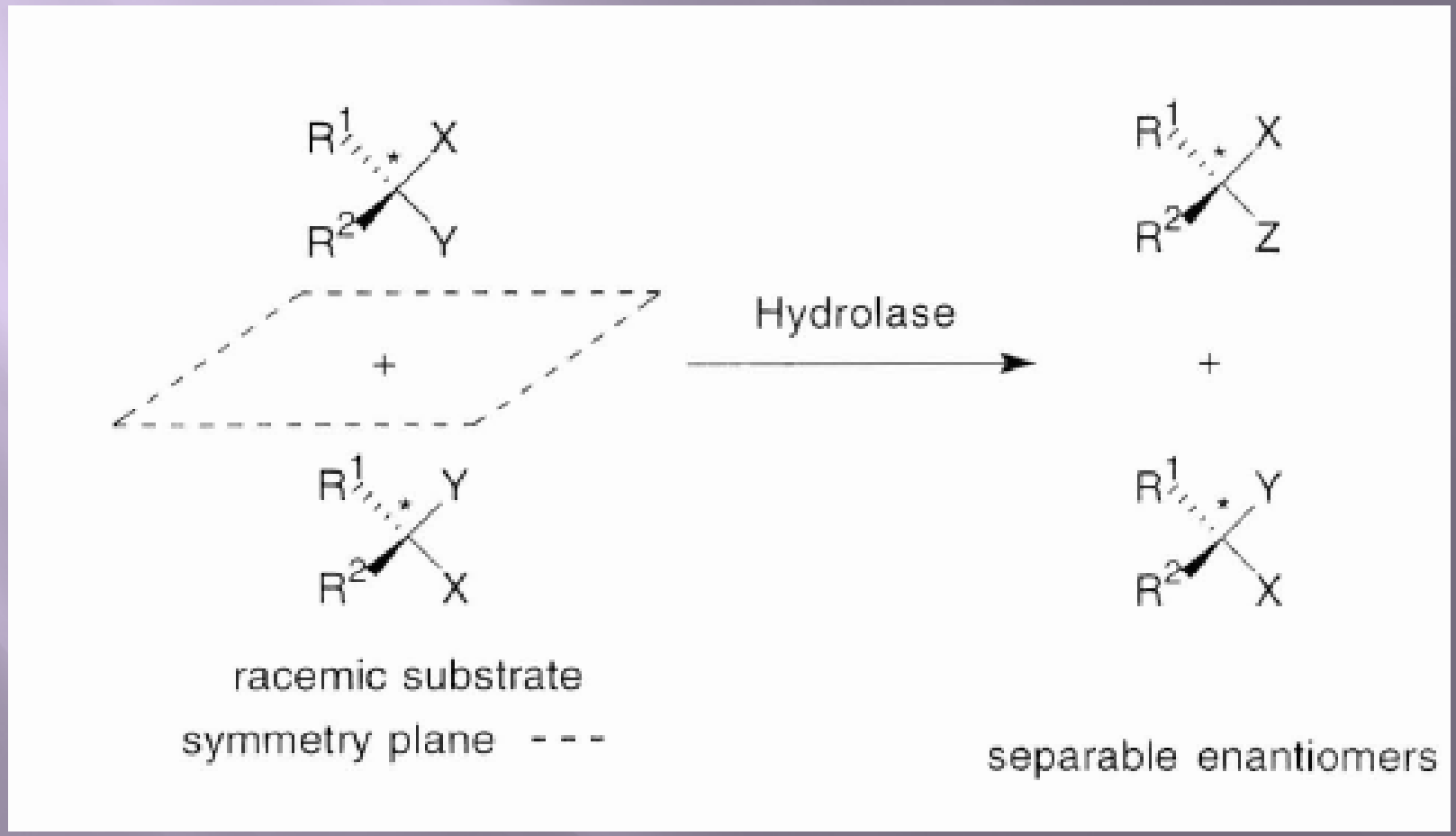


Desymmetrization of a meso-diacetate



Enzyme	Stereochemical Preference	Kinetic Constants		
		α	E_1	E_2
PLE	pro-*R*	2.47	0.22	0.60
PPL	pro-*S*	15.6	0.04	0.18

Enantiomer Differentiation



In practice, however, most cases of enzymatic resolution of a racemic 'Jbstrate do not show this ideal situation, i.e. in which one enantiomer is rapidly converted and the other not at all.

What one observes in these cases, is not a complete standstill of the cacti on at 50% conversion but a marked slowdown in reaction rate at around this point.

In these numerous cases one encounters some crucial dependencies:
•The velocity of the transformation of each enantiomer varies with the degree of conversion, since the ratio of the two substrate enantiomers does not remain constant during the reaction.
• Therefore, the optical purity of both substrate (e.e.s) and product (e.ep) becomes *a funtion of the extent of conversion.*

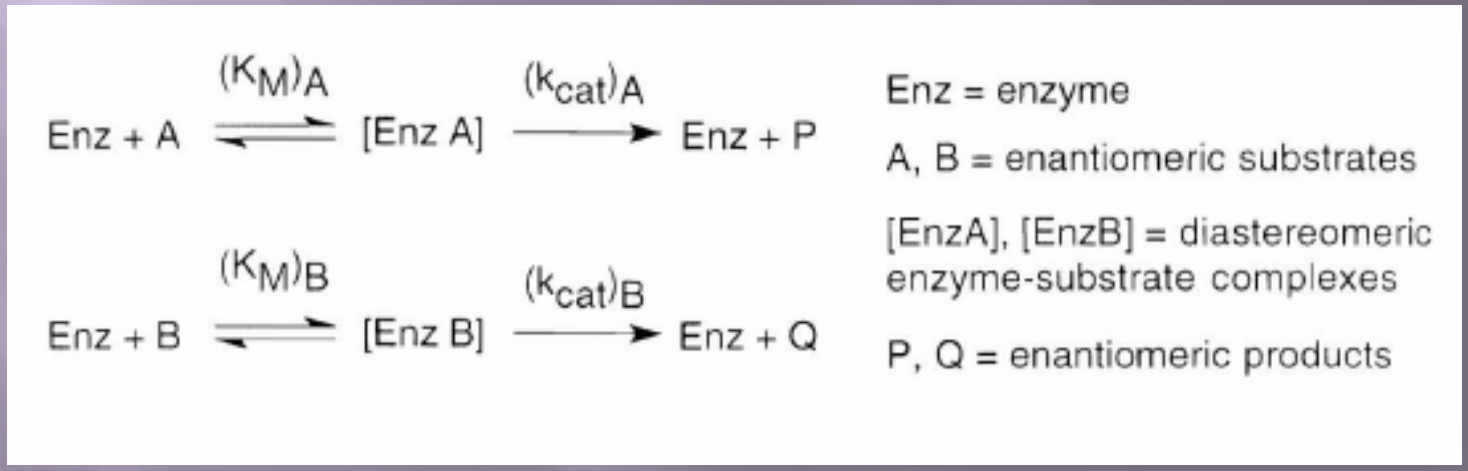
$$\text{Enantiomeric Ratio} \quad E = \frac{v_A}{v_B} = \frac{\left(\frac{k_{cat}}{K_M}\right)_A}{\left(\frac{k_{cat}}{K_M}\right)_B} \qquad \Delta\Delta G^{\neq} = -RT \ln E$$

Irreversible reaction

Hydrolytic reactions are considered as being completely irreversible due to the high 'concentration' of water in the aqueous environment (55.5 mol/l)

In the absence of enzyme inhibition Michaelis-Menten kinetics effectively describe the reaction in which two enantiomeric substrates (A and B) are transformed by an enzyme (Enz) into the corresponding enantiomeric products (P and Q)

Enzymatic kinetic resolution (irreversible reaction)



The dependence of the selectivity and the conversion of the reaction is

for the product:

$$E = \frac{\ln[1-c(1+e.e._P)]}{\ln[1-c(1-e.e._P)]}$$

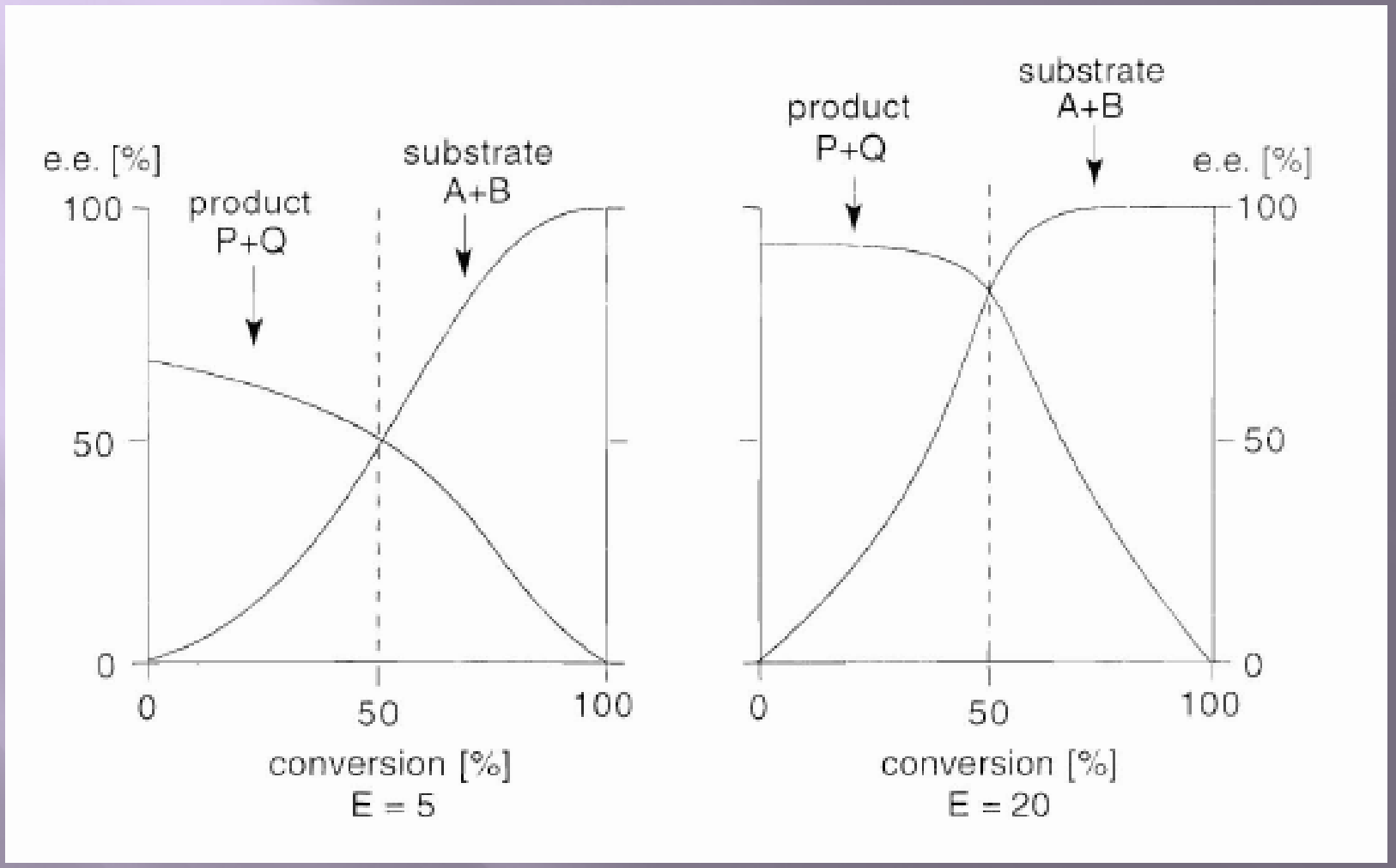
for the substrate:

$$E = \frac{\ln[(1-c)(1-e.e._S)]}{\ln[(1-c)(1+e.e._S)]}$$

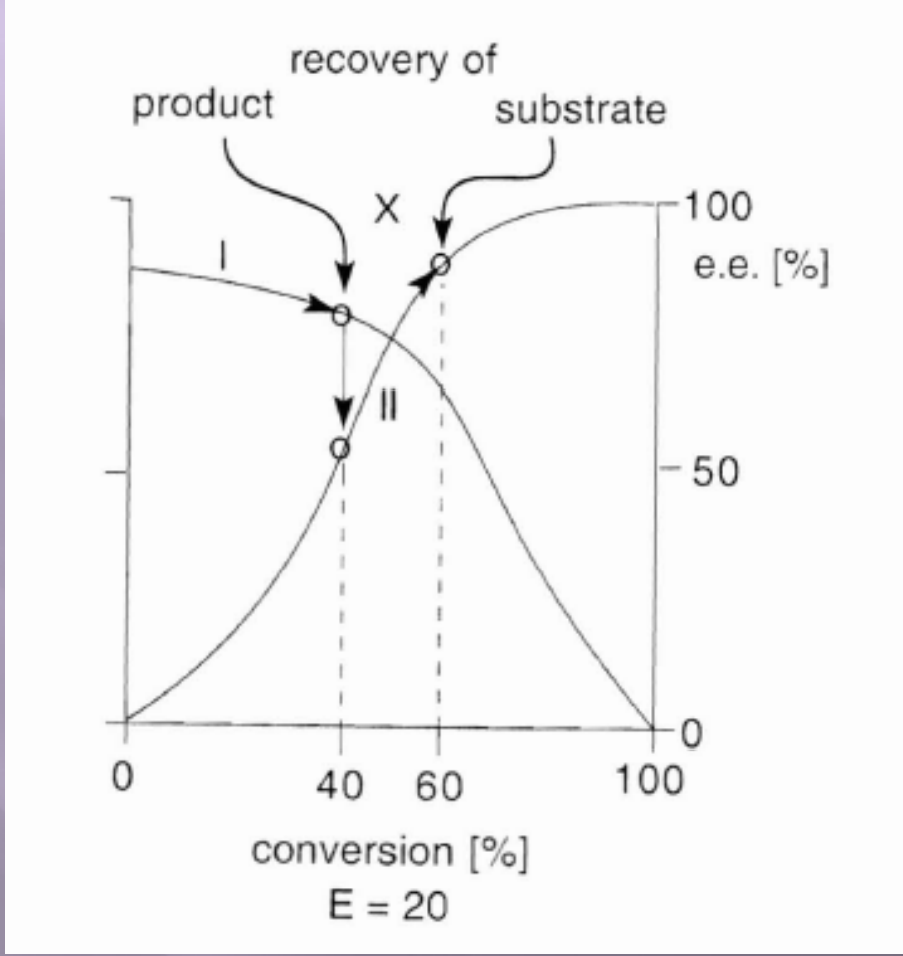
c = conversion, e.e. = enantiomeric excess of substrate (S) or product (P):
E = Enantiomeric Ratio.

relative quantities in contrast to the conversion, which is an *absolute Quantity*

$$E = \frac{\ln\dfrac{[e.e._P(1-e.e._S)]}{(e.e._P+e.e._S)}}{\ln\dfrac{[e.e._P(1+e.e._S)]}{(e.e._P+e.e._S)}}$$


e.e. [%]
100
50
0
product
P+Q
substrate
A+B
0
50
100
conversion [%]
E = 5
product
P+Q
substrate
A+B
e.e. [%]
100
50
0
0
50
100
conversion [%]
E = 20

Two-step enzymatic resolution



Reversible reaction

for the product

$$E = \frac{\ln [1-(1+K)c(1+e.e._P)]}{\ln [1-(1+K)c(1-e.e._P)]}$$

for the substrate:

$$E = \frac{\ln [1-(1+K)(c+e.e._S\{1-c\})]}{\ln [1-(1+K)(c-e.e._S\{1-c\})]}$$

c = conversion, e.e. = enantiomeric excess of substrate (S) or product (P),
E = Enantiomeric Ratio, K = equilibrium constant of the reaction.

Reversible reaction

$$\text{Enz} + \text{A} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [\text{Enz A}] \underset{k_4}{\overset{k_3}{\rightleftharpoons}} \text{Enz} + \text{P}$$

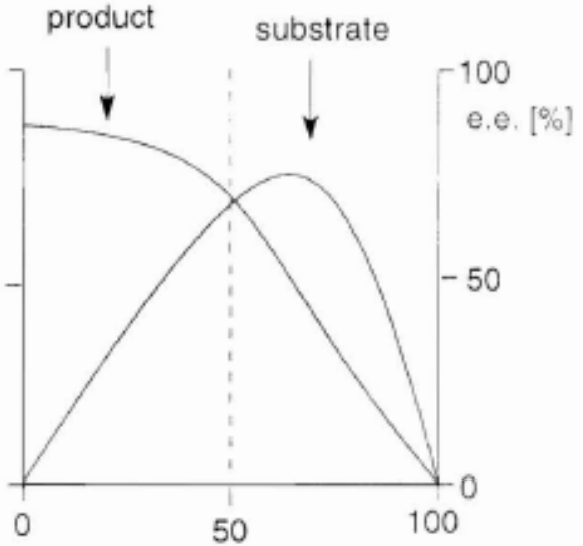
$$\text{Enz} + \text{B} \underset{k_6}{\overset{k_5}{\rightleftharpoons}} [\text{Enz B}] \underset{k_8}{\overset{k_7}{\rightleftharpoons}} \text{Enz} + \text{Q}$$

Enz = enzyme, A and B = enantiomeric substrates

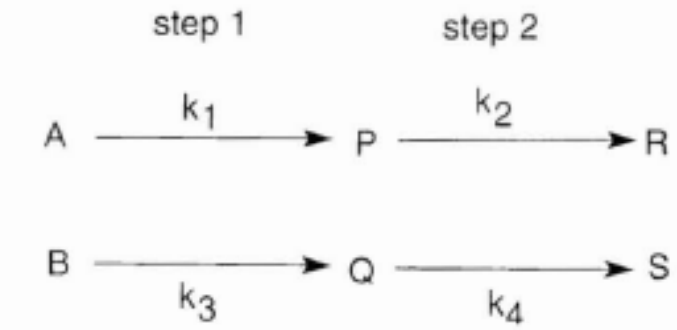
P and Q = enantiomeric products

k_1 through k_8 = rate constants

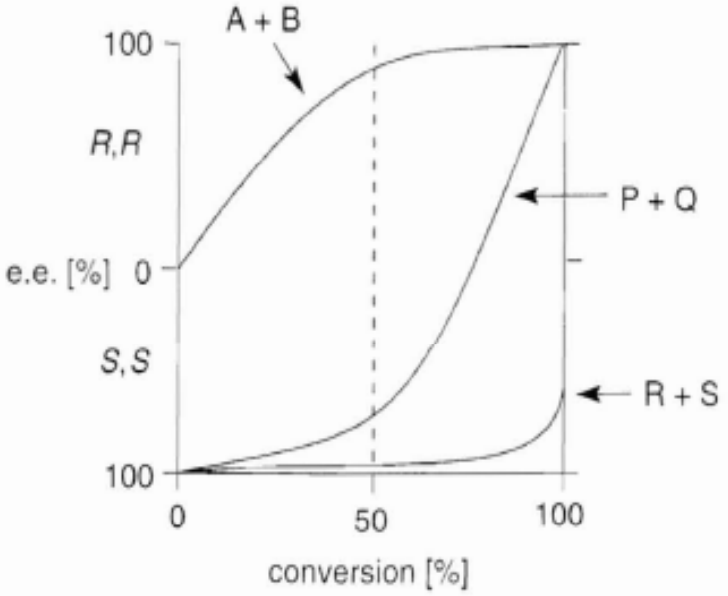
K = equilibrium constant



Sequential enzymatic kinetic resolution



A, B = enantiomeric starting material (diesters)
P, Q = enantiomeric intermediate products (monoesters)
R, S = enantiomeric final products (diols)
k_1 through k_4 = relative rate constants



Sequential enzymatic kinetic resolution

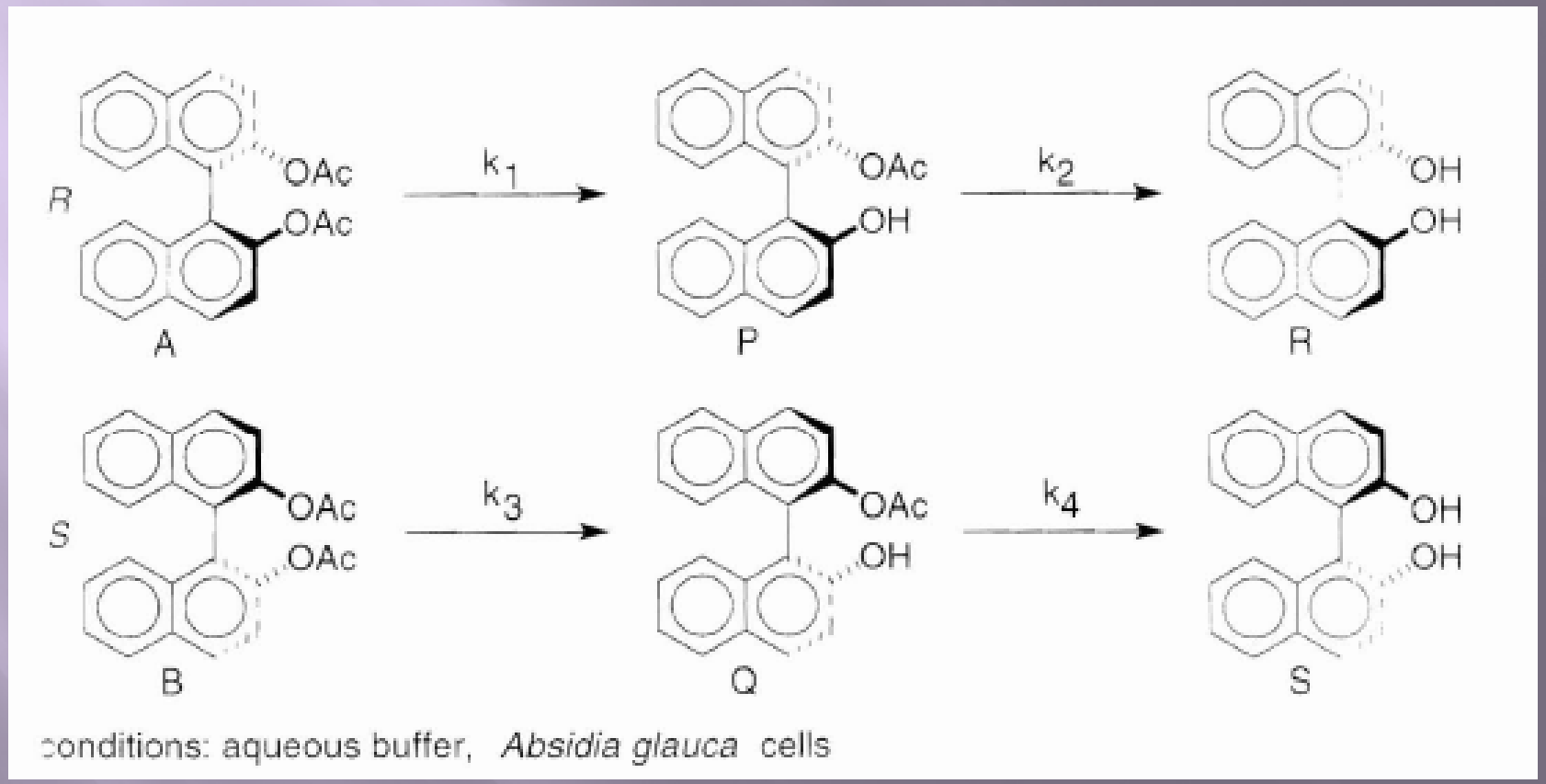
It has been shown that the maximum overall selectivity (Etot) of a sequential kinetic resolution can be related to the individual selectivities (E1, E2) of each of the steps

Etot represents the enantioselectivity that a hypothetical single-step resolution would need to yield the enantiomeric purity of the two-step resolution.

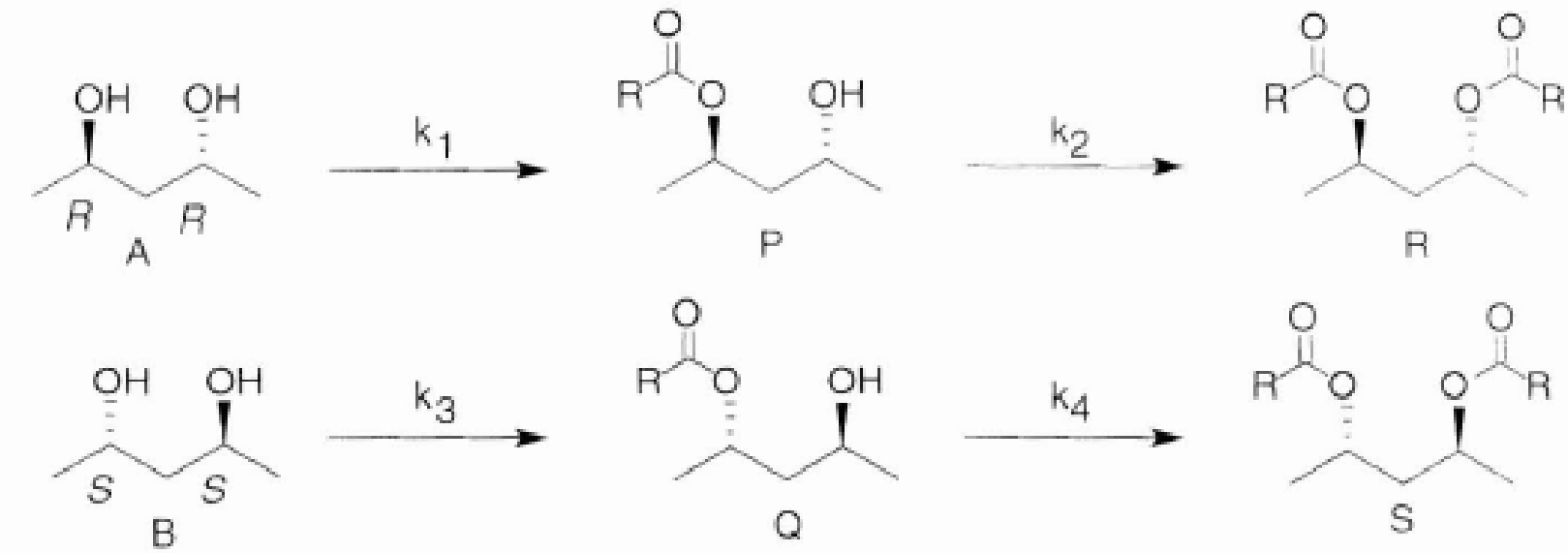
$$E_{tot} \approx \frac{E_1 \bullet E_2}{2}$$

This technique has been proven to be highly flexible. It was shown to work successfully not only in a hydrolytic reaction using cholesterol esterase or microbial cells, but also in the reverse esterification direction in an organic solvent catalyzed by a *Pseudomonas sp. lipase .*

Sequential enzymatic resolution by hydrolysis and esterification

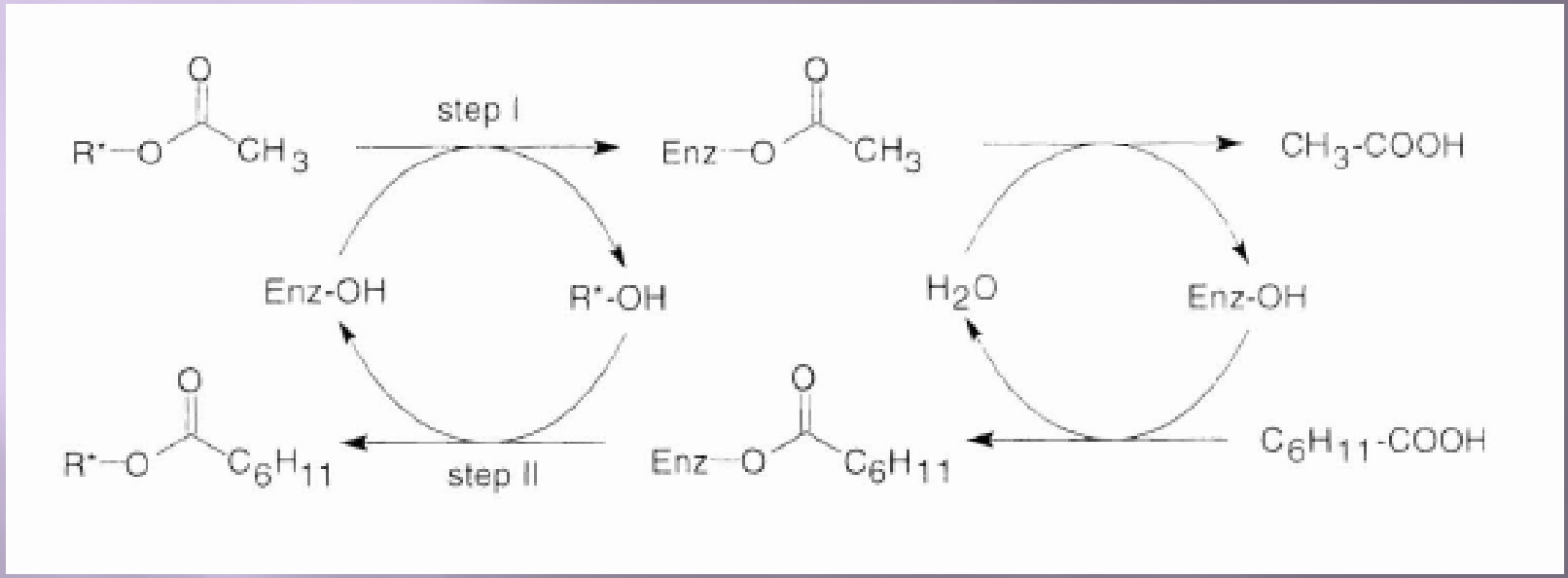


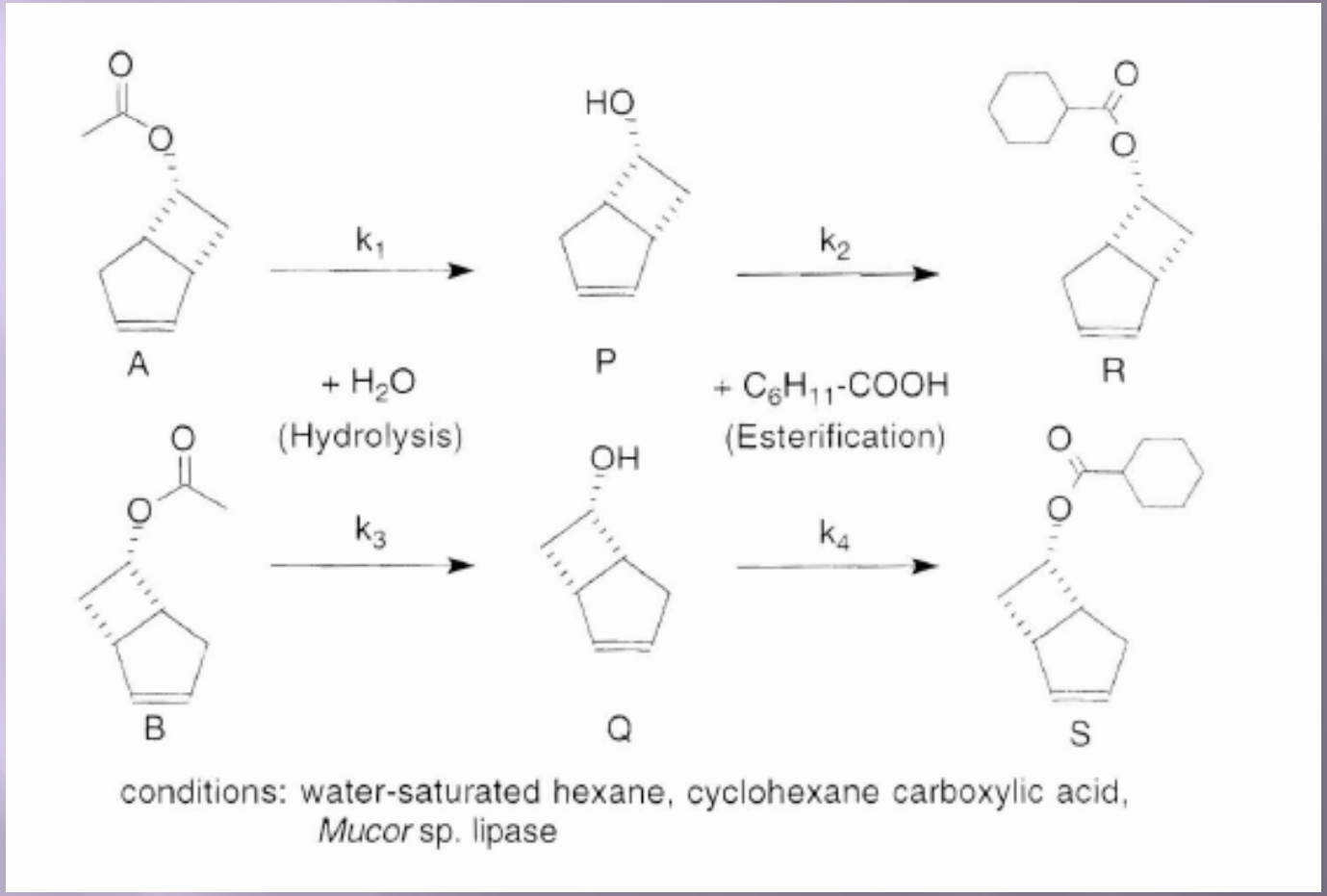
Sequential enzymatic resolution by hydrolysis and esterification



R = C_5H_{11}; conditions: *i*-octane, hexanoic acid, *Pseudomonas* sp. lipase

Mechanism of sequential enzymatic kinetic resolution via hydrolysis-esterification





The racemic starting acetate A and B is hydrolyzed to give alcohols P and Q in an organic medium containing a minimum amount of water, which in turn, by the action of the same lipase, are re-esterified with cyclohexanoic acid present in the mixture, As a consequence, the alcohol moiety of the substrate has to enter the active site of the lipase twice during the course of its transformation into the final product ester Rand S, An apparent selectivity of Etot = 400 was achieved in this way

In-situ inversion.

The final product of In-situ inversion.
of a kinetic resolution of a racemate is a mixture of enantiomeric product and substrate.

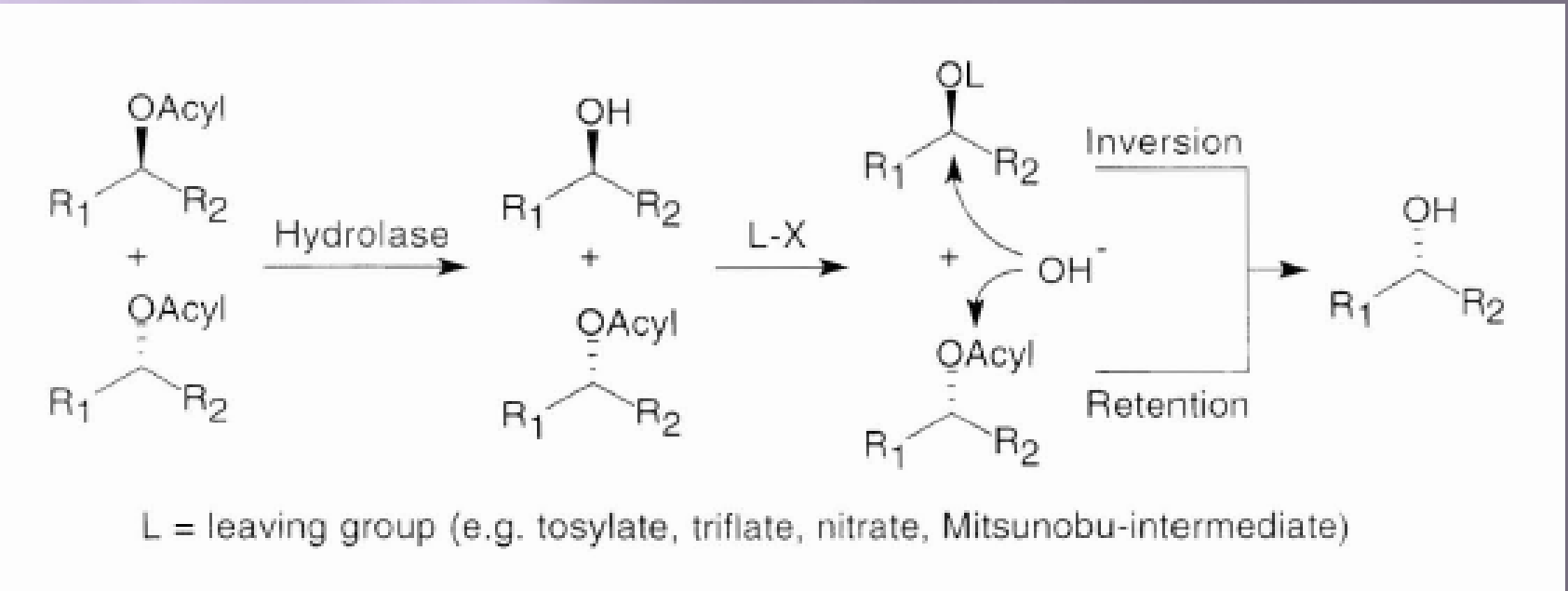
Separating them by physical or chemical means is often tedious and might pose a serious drawback to commercial applications, especially if the mixture comprises an alcohol and an ester.

However, if the molecule has only a single center of chirality, the alcohol may be chemically inverted into its enantiomer *be/ore separating the products*

Introduction of a good leaving group (L, e.g rosylate, tritlate, nitrate, Mitsunobu-intermediate) yields an activated esler, which can be hydrolyzed with *inversion of configuration, while the stereochemistry of the remaining carboxylic acid substrate ester is retained during hydrolysis.*

Result, a *single enantiomer is obtained as the final product*

In-situ inversion.



L = leaving group (e.g. tosylate, triflate, nitrate, Mitsunobu-intermediate)

Review questions:

Why are hydrolases so interestinng?

What is the mechanism of hydrolases?

What happens in organic media?

Eantioface- vs enantiotopos differentiation?

What happens to a meso substrate?

What is kinetic resolution?