Biocatalysis III

Dynamic resolution

Is a more elegant approach. It has a classical resolution and additionally the the reaction is carried out at conditions where the enantiomers are in rapid equilibrium.

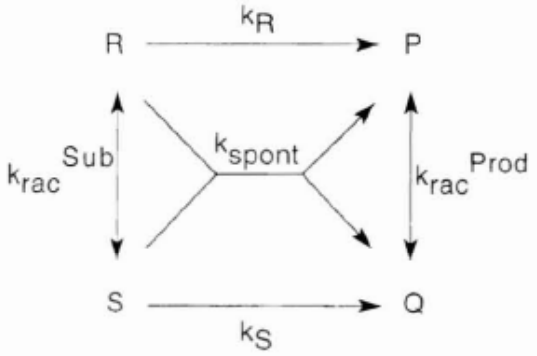
Since one enantiomer is rapidly depleted it is consantly supplied by the racemisation equilibrium.

For this to occur several reactions have to run simultaneously:

- *The enzyme has to display high specificity for the enantiomeric substrates R/S (kr>>ks or ks>>kr).*
- *Spontaneous hydrolysis (kspont) should be a minimum since it would yield racemic product.*
- *Racemisation of the substrate should occur at an equal or higher rate compared to the biocatalytic reaction in order to provide a sufficient amount of the well-fitting substrate enantiomer from the poor-fitting counterpart.*
- *Racemisation of the product should be minimal*

Biocatalysis III

Figure 2.9. Kinetic resolution with in-situ racemization

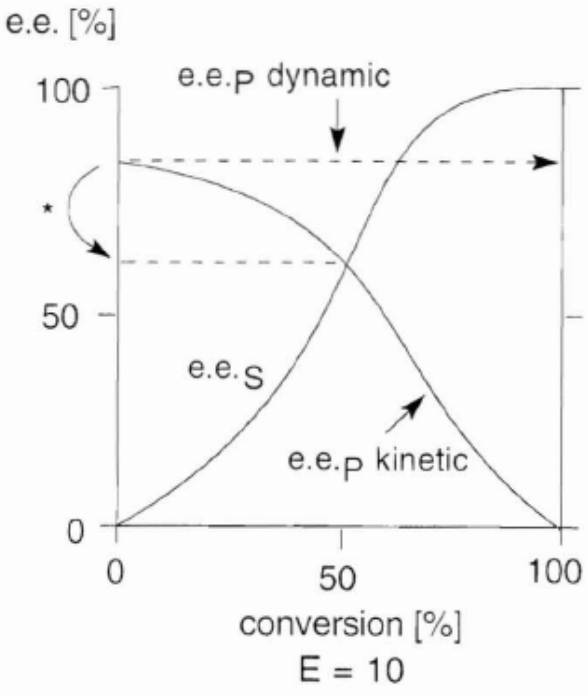


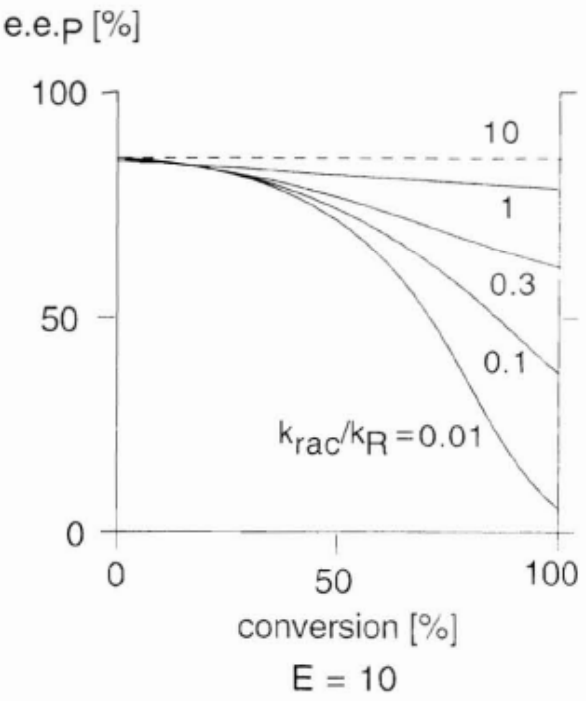
k_R, k_S = enzymatic hydrolysis of enantiomers R, S

k_{rac}^{Sub}, k_{rac}^{Prod} = racemization of substrate, product

k_{spont} = spontaneous hydrolysis

* depletion of e.e.$_P$ in kinetic resolution





Biocatalysis III

•Hydrolysis of the amide bond

•The hydrolysis of the carboxamide bond is naturally linked to the chemistry of amino acids and peptides. The three amino acids dominating the world market are glutamic acid, lysine, and D,L methionine.
•They are produced by fermentation but also synthetic routes as well. Most of them are produced enzymatically.

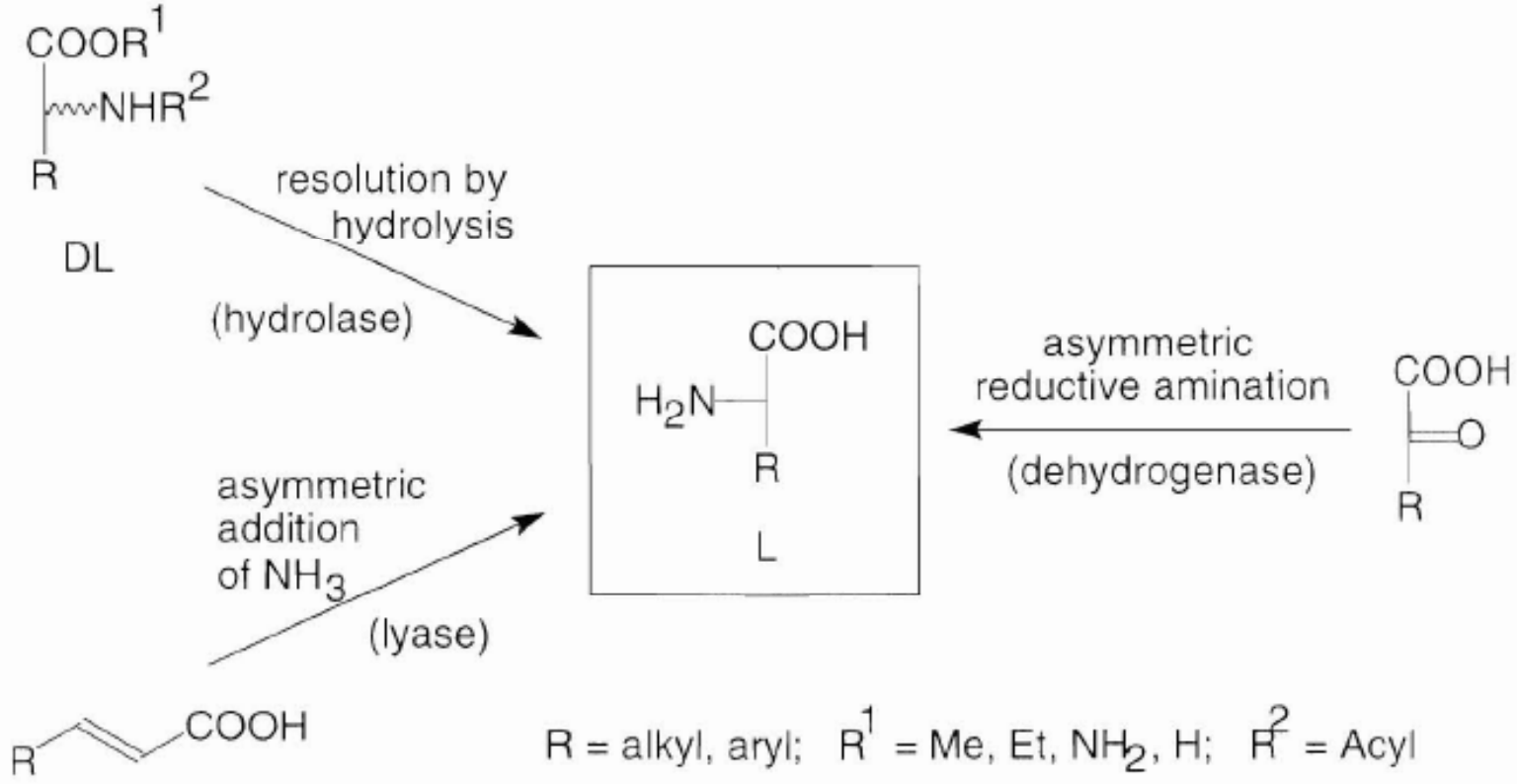
World production of amino acids using enzymatic processes

Amino acid	Amount [t/a]
L-alanine	250
L-aspartic acid	5000*
L-2,4-dihydroxyphenylalanine	200
L-methionine	240
L-phenylalanine	5000*
L-tryptophane	200
L-valine	150
D-phenylglycine	1000
D-*p*-hydroxyphenylglycine	1000

* Including the demand for the manufacture of the low-calorie sweetener aspartame.

Biocatalysis III

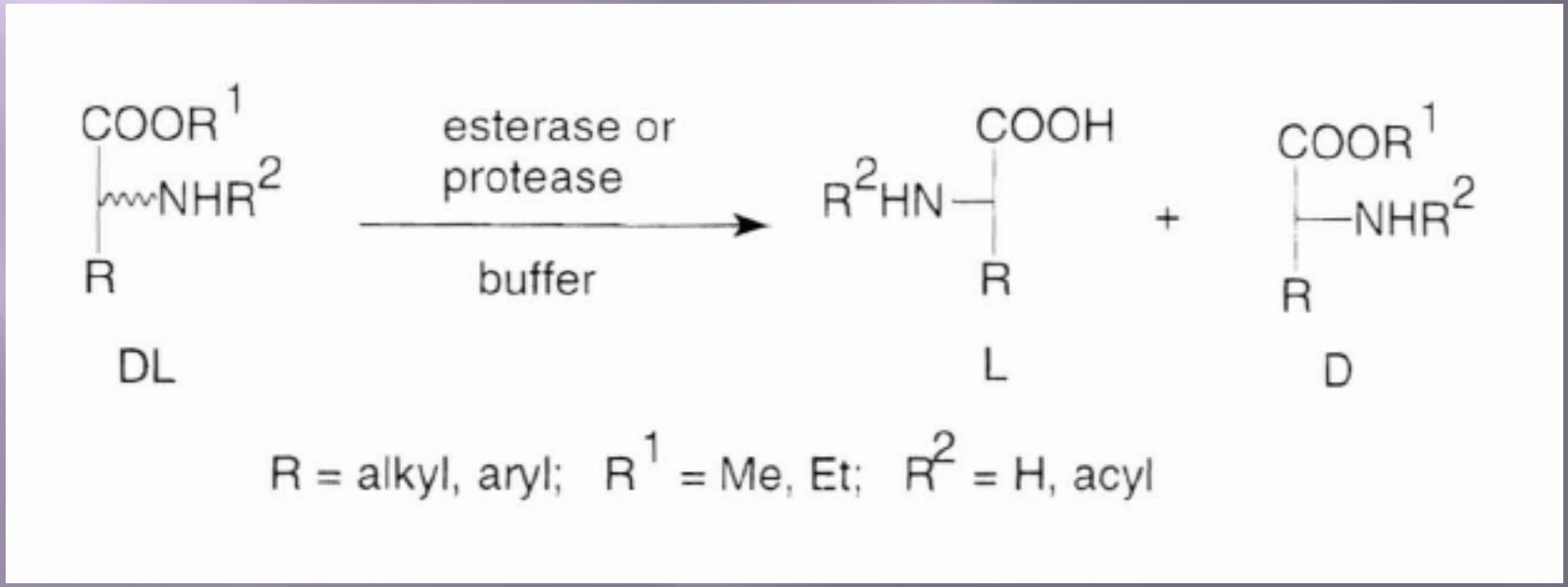
Scheme 2.10. Important enzymatic routes to enantiomerically pure α-amino acids



Biocatalysis II

- Enantiophase Differentiation:

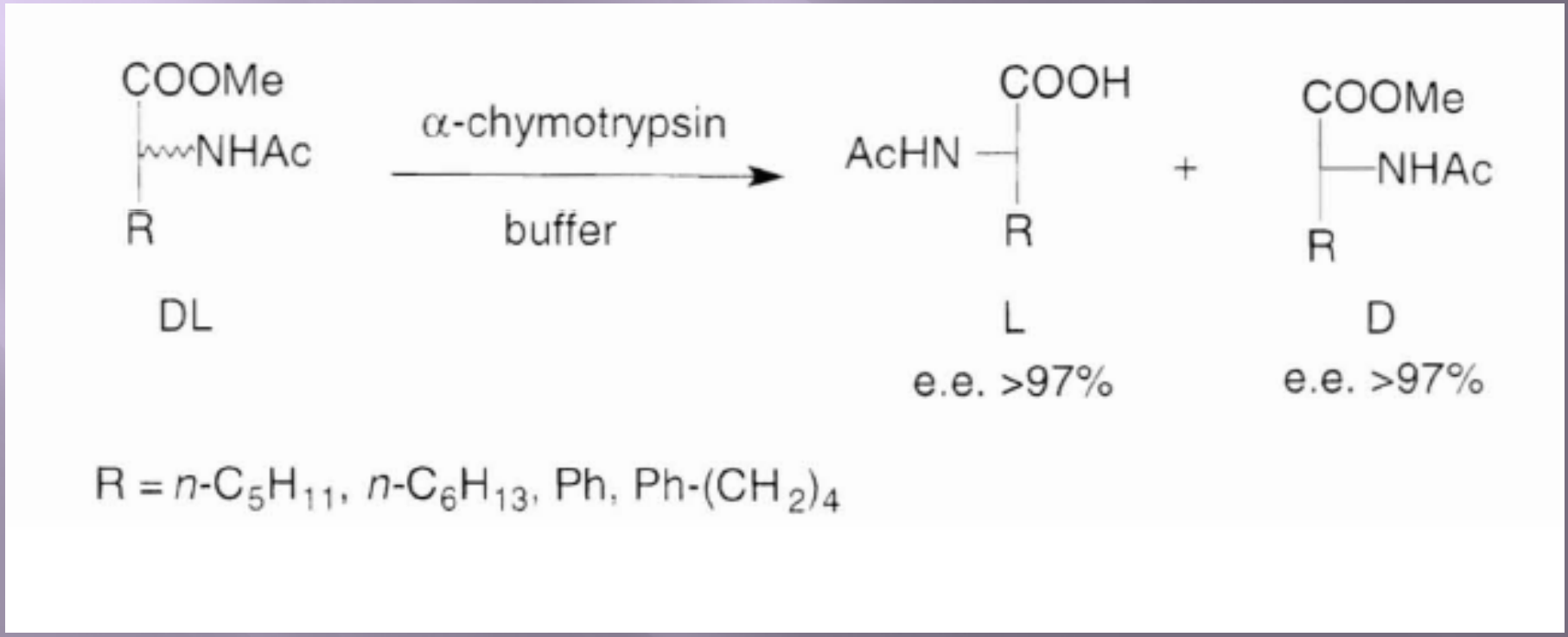
Hydrolases can distinguish between the two enantiomeric phases of achiral substrates such as enol esters possessing a plane of symmetry within the molecule.
The nucleophilic attack occurs predominantly from one side, leading to an unsymmetric enolization of the unstable free enol towards one preferred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



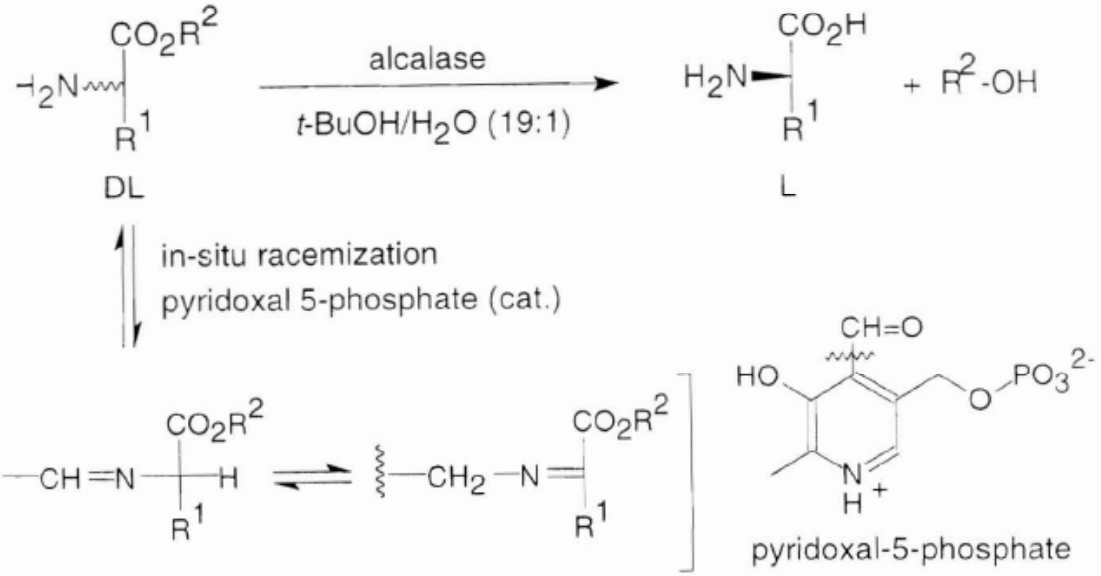
Biocatalysis III

The amino group of the substrate may be either free or (better) protected by an acyl functionality, preferably an acetyl-, benzoyl-, or the *ler!butyloxycarbonyl-(Boc)-group in order to avoid possible side-reactions such as ring-closure with the formation of diketopiperazines.*

Numerous enzymes have been used to hydrolyze N-acyl amino acid esters. the most versatile and thus very popular catalyst is ex-chymotrypsin isolated from bovine pancreas



Scheme 2.13. Dynamic resolution of amino acid esters



R^1	R^2		Product yield [%]	e.e. [%]
Ph-CH_2-	Ph-CH_2-	L-Phe	92	98
Ph-CH_2-	*n*-Bu-	L-Phe	92	98
4-Hydroxyphenyl-CH_2-	Ph-CH_2-	L-Tyr	95	97
4-Hydroxyphenyl-CH_2-	*n*-Pr-	L-Tyr	95	97
$(CH_3)_2CH$-CH_2-	Ph-CH_2-	L-Leu	87	93
n-Bu-	Ph-CH_2-	L-NorLeu	87	90
Et-	Ph-CH_2-	L-NorVal	87	91

Amidase Method

L-Amino acid amides are hydrolyzed enantioselectively by amino acid amidases

aminopeptidases

Sources:
mammalian kidney
pancreas

Plants: rice, arabidopsis

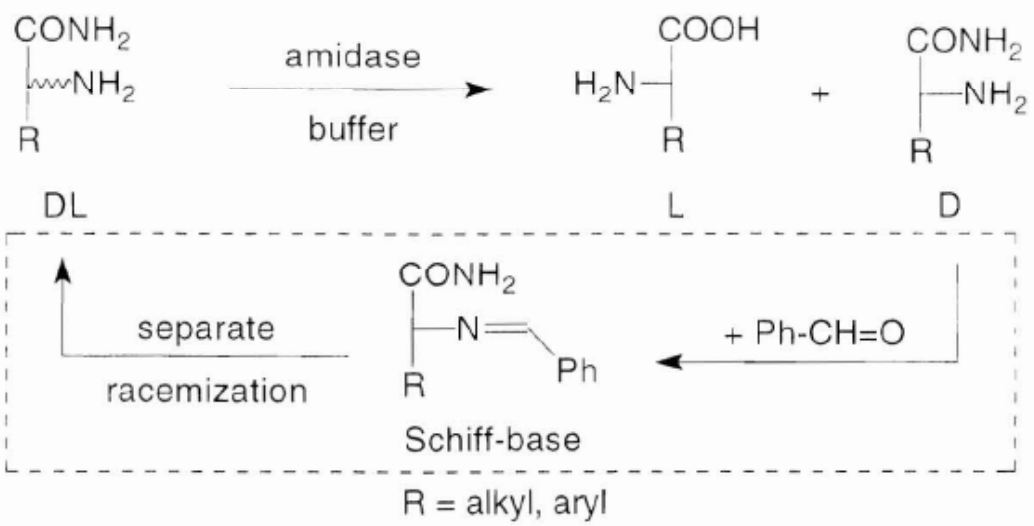
different microorganisms :
Pseudomonas sp.,
Aspergillus sp.,
Rhodococcus sp

Amidase Method

Again, the L-amino acids thus formed are separated from the unreacted D-amino acid amide by the difference in solubility in various solvents at various pH.

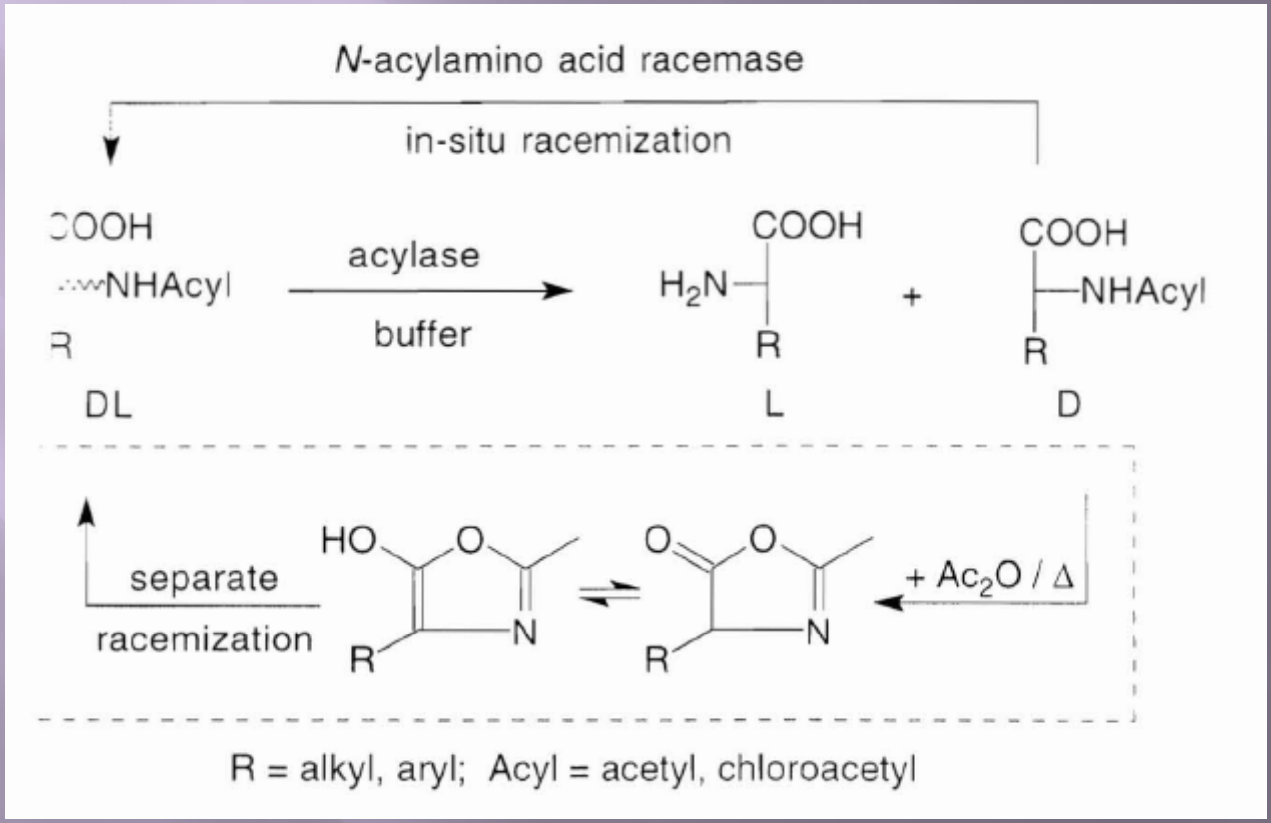
Amino acid amides are less susceptible to spontaneous chemical hydrolysis in the aqueous environment than the corresponding esters, the products which are obtained by this method are often of higher optical purities

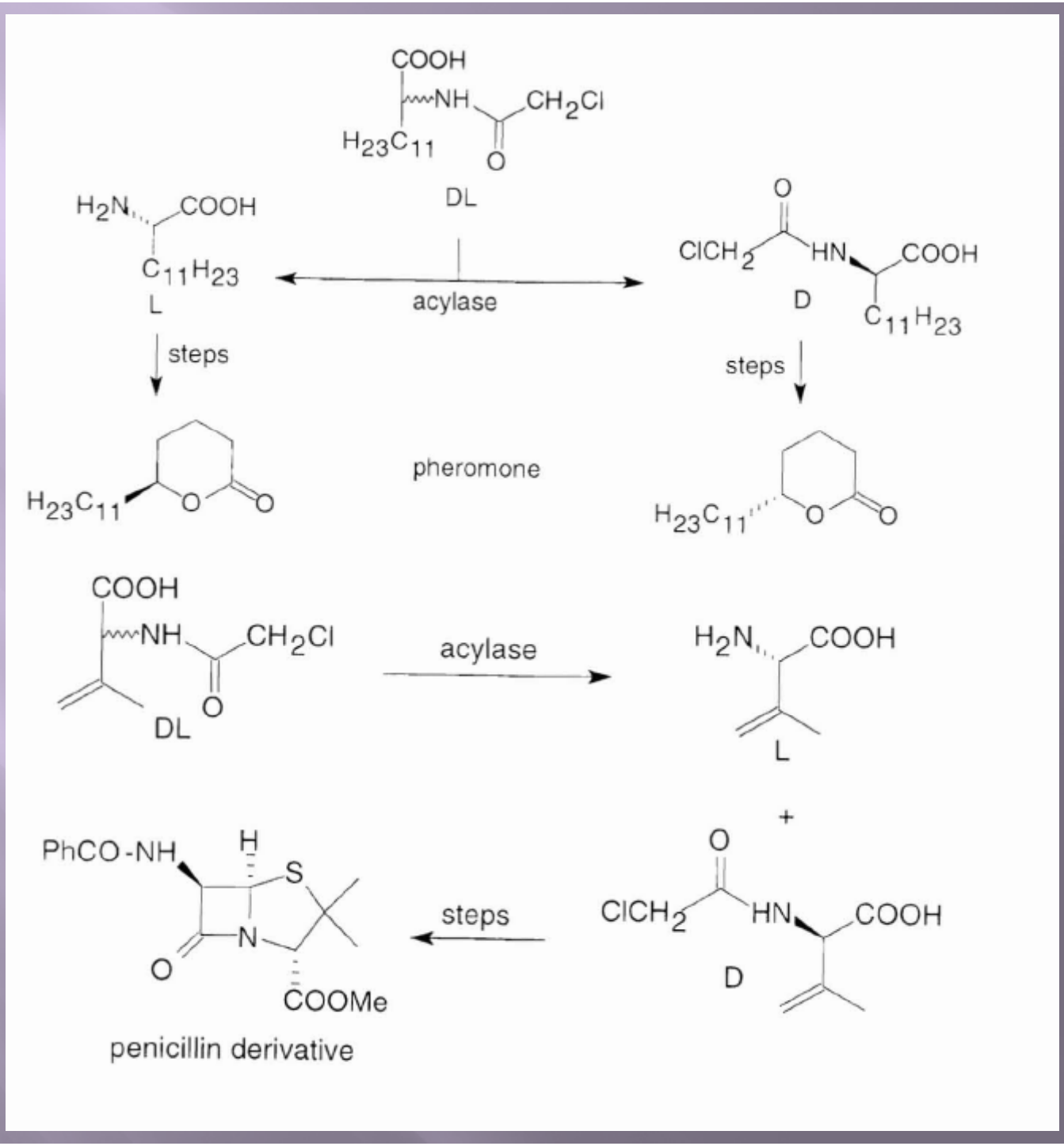
Scheme 2.14. Enzymatic resolution of amino acid amides via the amidase-method



Acylase-Method

Enzymatic resolution of *N-acyl amino acids via the acylase-method*




COOH
NH
CH2Cl
H23C11
O
DL
H2N
COOH
C11H23
L
acylase
ClCH2
HN
COOH
D
C11H23
steps
steps
H23C11
O
O
pheromone
H23C11
O
O
COOH
NH
CH2Cl
O
DL
acylase
H2N
COOH
L
+
PhCO-NH
H
S
N
O
COOMe
steps
ClCH2
HN
COOH
D
penicillin derivative

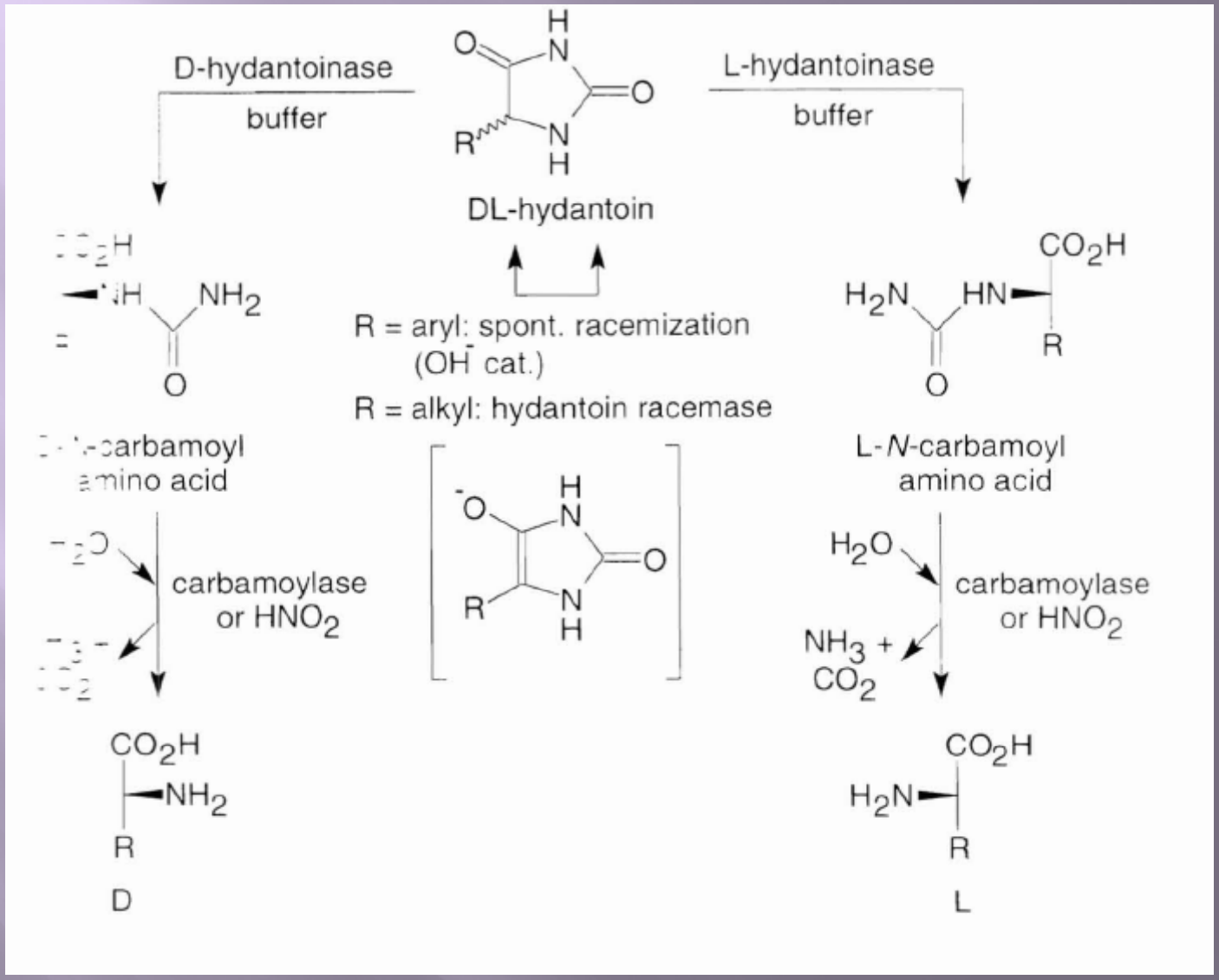
Hydantoinase-Method

S-Substituted hydantoins may be obtained easily in racemic form from cheap starting materials such as an aldehyde, hydrogen cyanide and ammonium carbonate.

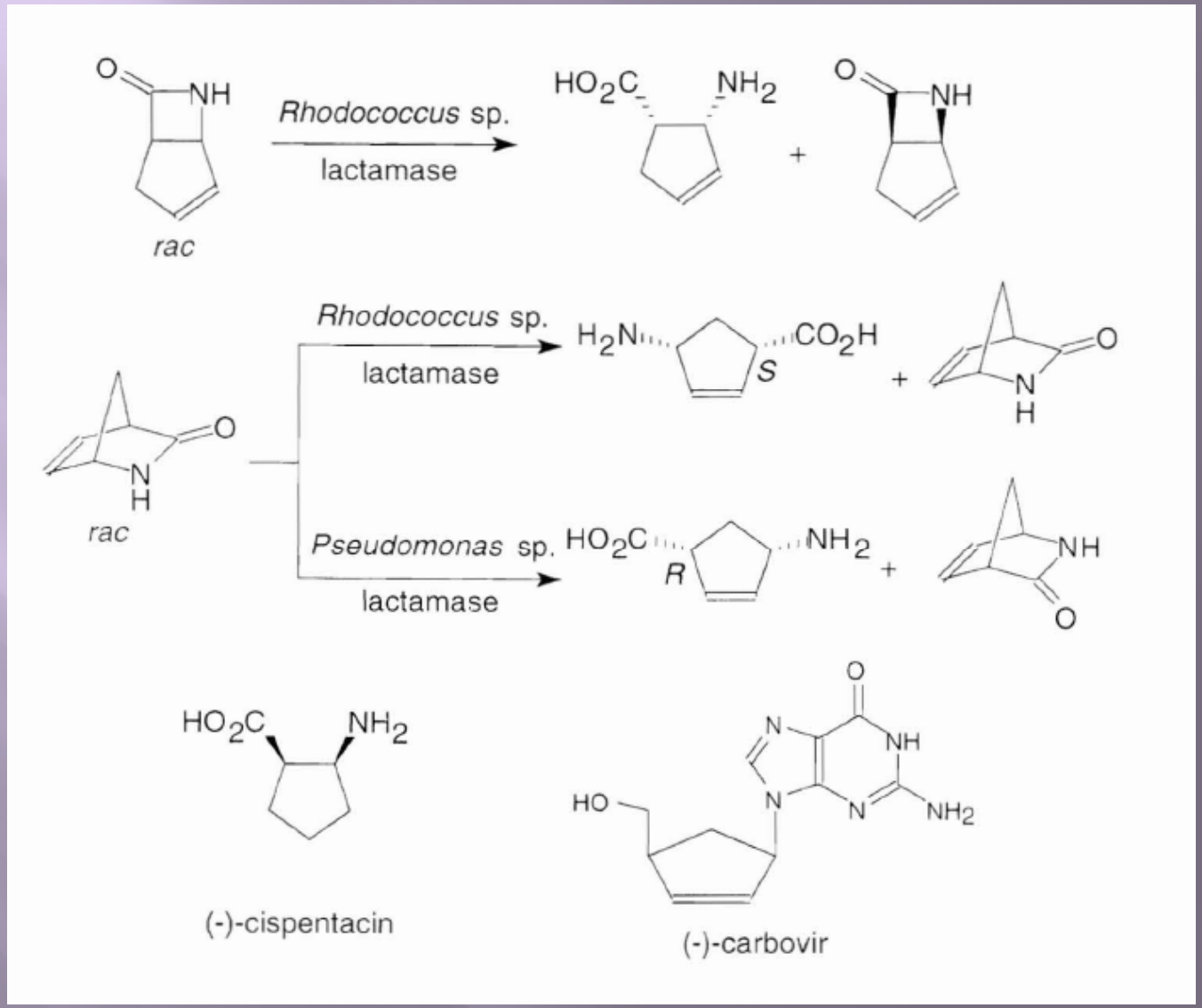
Hydantoinases from different microbial origins catalyze the hydrolytic ring-opening to form the corresponding N-carbamoyl-α-amino acids.

In nature, these enzymes are responsible for the cleavage of dihydropyrimidines occurring in pyrimidine catabolism, therefore the name 'dihydro-pyrimidinases', which is less often used, would be more apt.

Enzymatic resolution of N-acyl amino acids via the hydantoinase-method



Enzymatic resolution of bicyclic β-and γ-lactams via the lactamase-method



Esterases and Proteases

As a rule of thumb, when acting on nonnatural carboxylic esters, most proteases seem to retain a preference for the hydrolysis of that enantiomer which mimics the configuration of an L-amino acid more closely.

The following general rules can be applied for the construction of substrates for esterases and proteases:

For both esters of the general type 1 and II, the center of chirality (as indicated with an asterisk [*]) should be located as close as possible to the site of the reaction (that is the carbonyl group of the ester) to ensure an optiIllal chiral recognition. Thus, C(-substituted carboxylates and esters of secondary alcohols are usually more selectively hydrolyzed than their β–substituted counterparts and esters of chiral primary alcohols, respectively.

Both substituents R1 and R2 can be alkyl-or aryl-groups, but they should differ in size and polarity to aid the chiral recognition process or the enzyme. They may also be joined together to form cyclic structures.

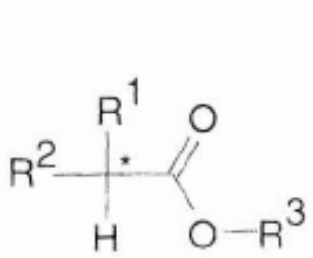
Esterases and Proteases

Polar or charged groups, such as -COOH, -CONH2 or -NH2, which are heavily hydrated in an aqueous environment should be absent since esterases (and in particular lipases) do not accept highly polar hydrophilic substrates. If such groups are required, they should be protected with an appropriate lipophilic unit.

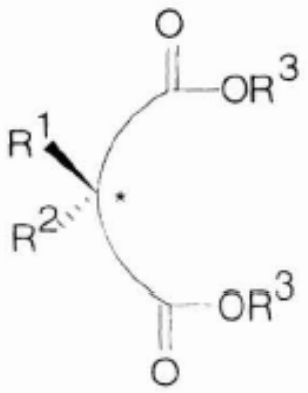
The alcohol moieties (R3) of type-I esters should be as short as possible. preferably methyl or ethyl. If neccessary, the reaction rate of ester hydrolysis may be enhanced by linking electron-withdrawing groups to the alcohol moiety to give methoxymethyl, cyanomethyl, or 2-haloethyl esters. On the contrary, carboxylates bearing long-chain alcohols are usually hydrolyzed at reduced reaction rates with esterases and proteases.

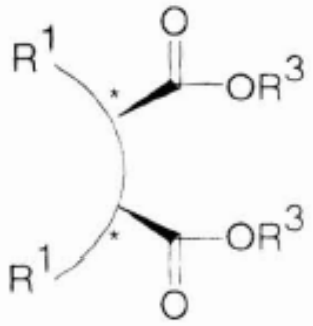
The same considerations are applicable to acylates of type II, where acetates or propionates are the preferred acyl moieties. Increasing the carbonyl reactivity of the substrate ester by adding electron-withdrawing substituents such as halogen (leading to (α–haloacetates) is a frequently used method to enhance the reaction rate in enzyme-catalyzed ester hydrolysis

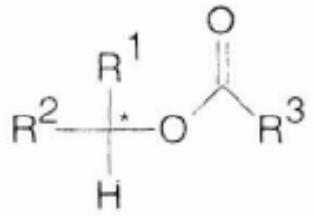
Types of substrates for esterases and proteases



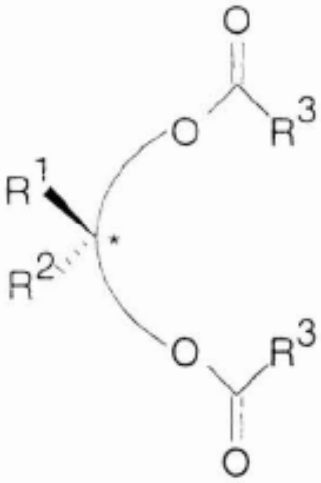
Type I



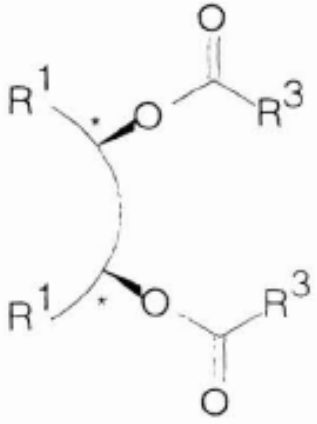




Type II



prochiral substrates



meso-forms

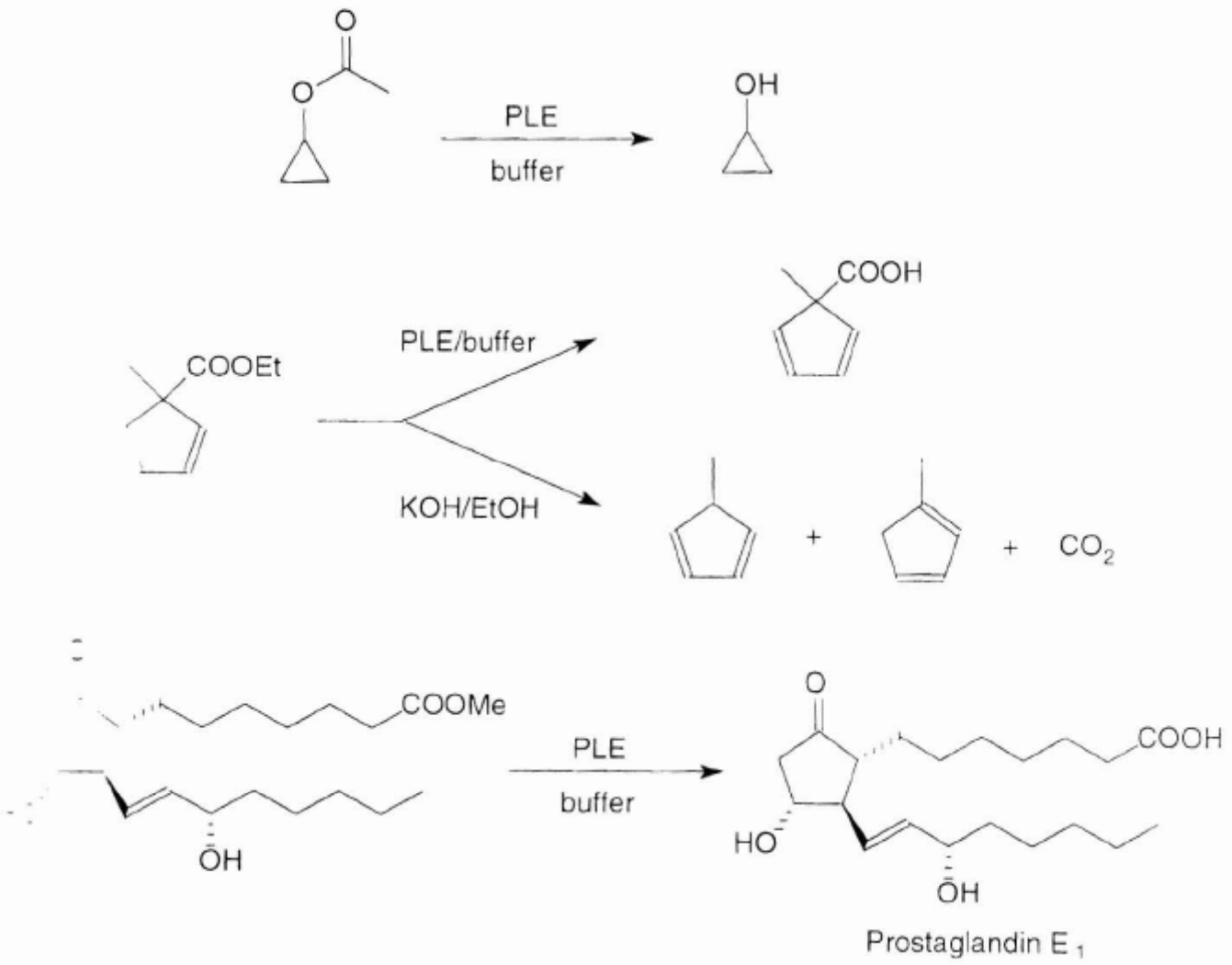
R^1, R^2 = alkyl, aryl; R^3 = Me, Et; * = center of (pro)chirality

Pig Liver Esterase and α-Chymotrypsin

Mild hydrolysis.
Acetates of primary and secondary alcohols sLich as cyclopropyl acetate [190] and methyl or ethyl carboxylates (such as the labile cyclopentadiene ester) can be selectively hydrolyzed under mild conditions using PLE, avoiding decomposition reactions which would occur during a chemical hydrolysis under acid-or base-catalysis

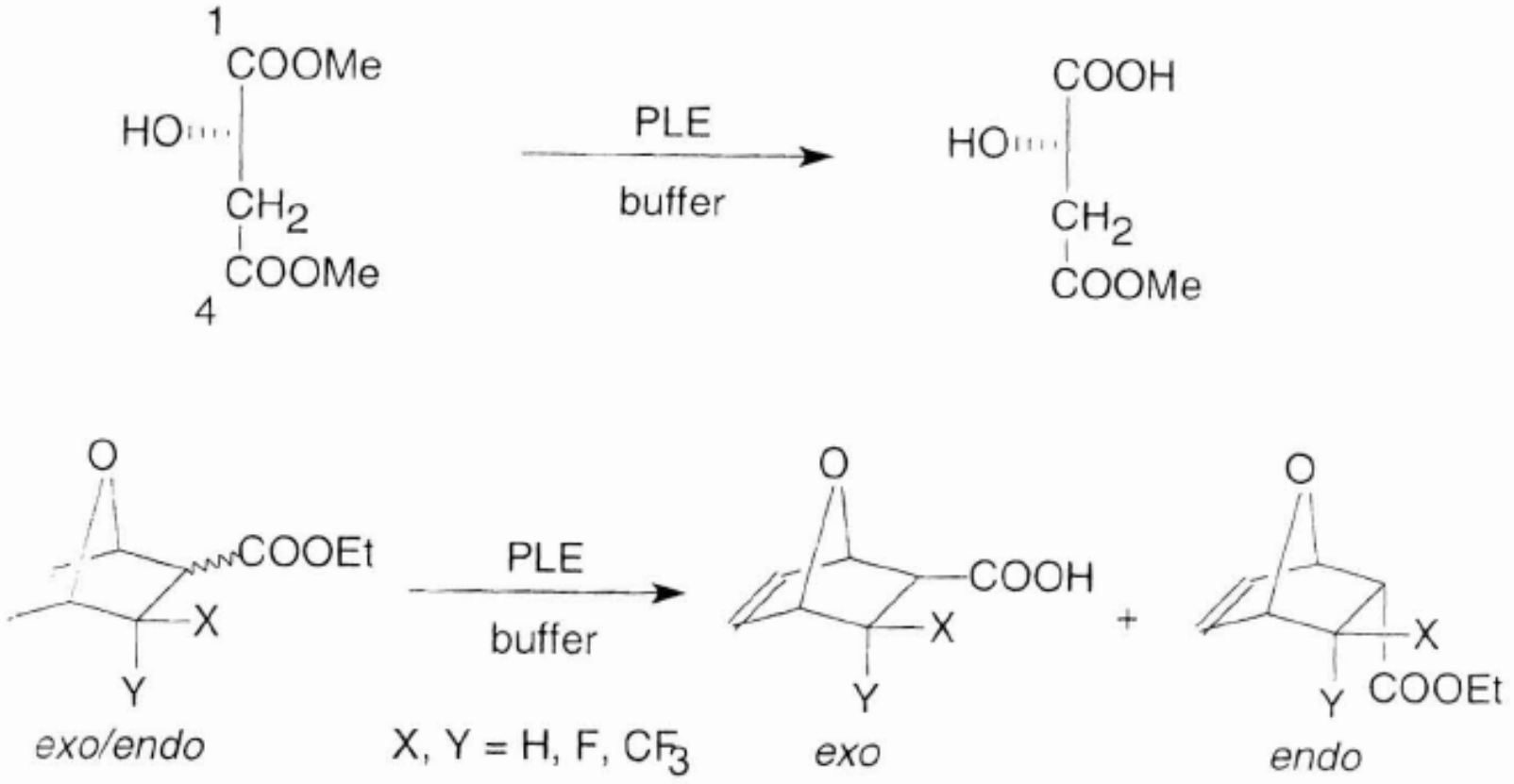
Regio-and diastereoselective hydrolysis.
Regiospecific hydrolysis of dimethyl malate at the 1-position can be effected with PLE as the catalyst. Similarly, hydrolysis of an *exo-/endo-mixture of diethyl dicarboxylates with a bicyclo[2.2.1]heptane framework occurs only on the less hindered exo-position leaving the endo-ester untouched, thus allowing a facile separation of the two positional isomers in a diastereomeric mixture*

Scheme 2.20. Mild ester hydrolysis by porcine liver esterase



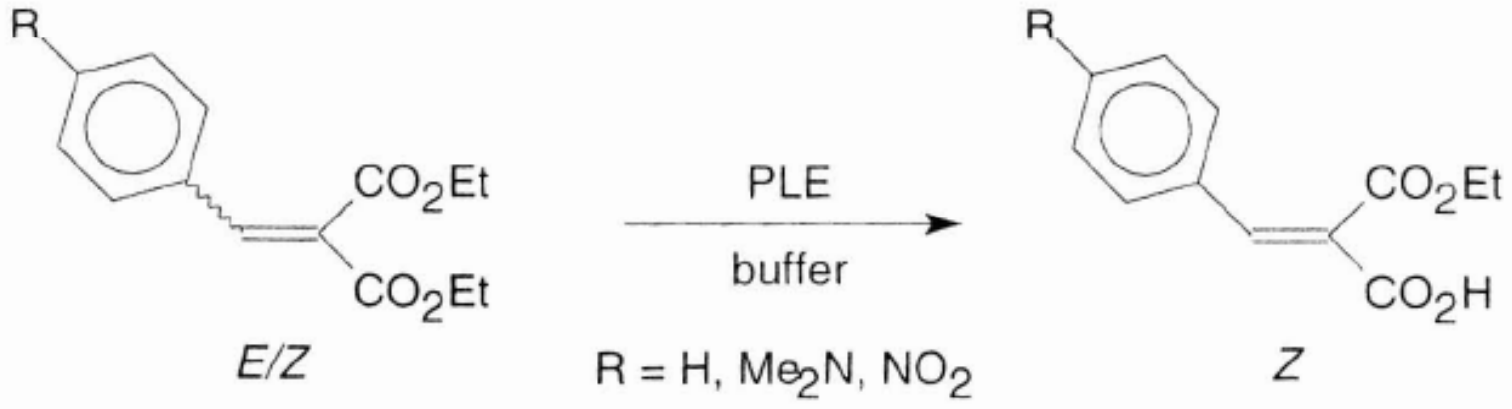
Prostaglandin E_1

Scheme **2.21.** Regio- and diastereoselective ester hydrolysis by porcine liver esterase

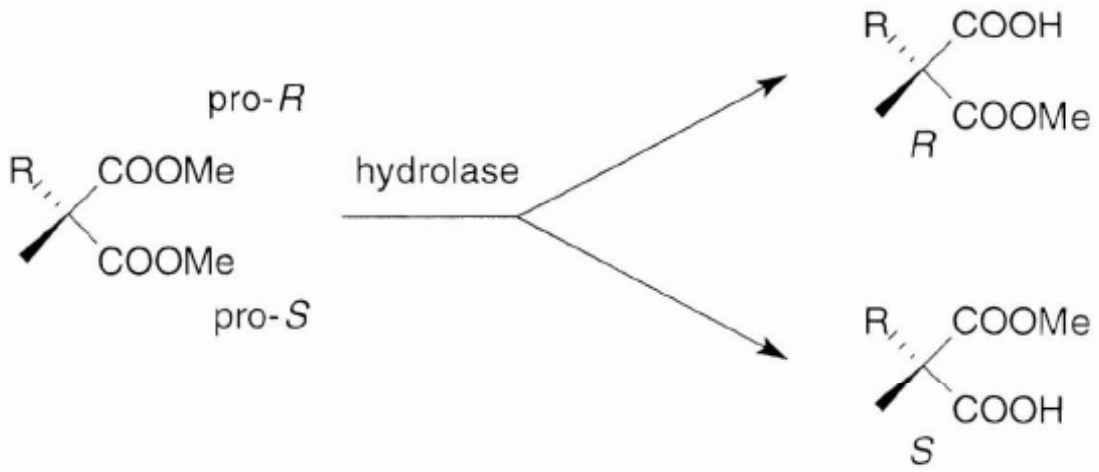


Separation of *E/Z*-isomers. With *E/Z*-diastereotopic diesters bearing an aromatic side chain, PLE selectively hydrolyzes the ester group in the more accessible *trans*-position to the phenyl ring, regardless of the *p*-substituent [195] (Scheme 2.22). In analogy to the hydrolysis of dicarboxylates (Scheme 2.3) the reaction stops at the monoester stage with no diacid being formed. Other hydrolytic enzymes (proteases and lipases) were less selective in this case.

Scheme 2.22. Regioselective hydrolysis of *E/Z*-diastereotopic diesters by porcine liver esterase



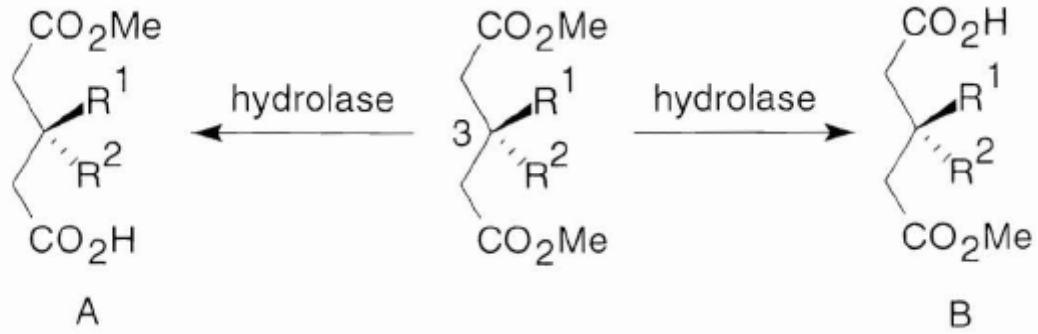
Scheme 2.23. Desymmetrization of prochiral malonates by porcine liver esterase and α-chymotrypsin



Enzyme	R	Configuration	e.e. [%]
PLE*	Ph-	*S*	86
PLE	C_2H_5-	*S*	73
PLE	*n*-C_3H_7-	*S*	52
PLE	*n*-C_4H_9-	*S*	58
PLE	*n*-C_5H_{11}-	*R*	46
PLE	*n*-C_6H_{13}-	*R*	87
PLE	*n*-C_7H_{15}-	*R*	88
PLE	*p*-MeO-C_6H_4-CH_2-	*R*	82
PLE	*t*-Bu-O-CH_2-	*R*	96
α-chymotrypsin	Ph-CH_2-	*R*	~100

The ethyl ester was used.

Scheme 2.25. Desymmetrization of prochiral glutarates

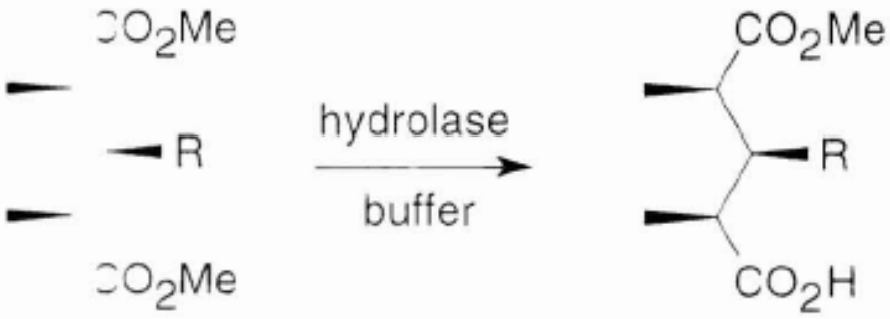


A B

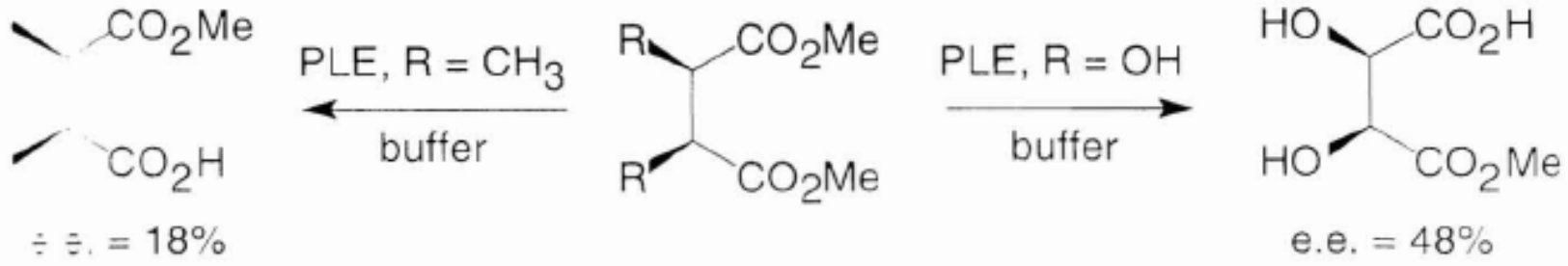
Hydrolase	R^1	R^2	Product	e.e. [%]
α-chymotrypsin*	AcNH-	H	B	79
α-chymotrypsin	Ph-CH_2-O-	H	B	84
α-chymotrypsin*	HO-	H	B	85
α-chymotrypsin	CH_3OCH_2O-	H	B	93
PLE	AcNH-	H	B	93
PLE	CH_3-	H	B	90
PLE	HO-	H	A	12
PLE	Ph-CH_2-CH=CH-CH_2-	H	A	88
PLE	*t*-Bu-CO-NH-	H	A	93
PLE	HO-	CH_3	A	99
Acinetobacter sp.*	HO-	H	B	>95
Arthrobacter sp.*	HO-	H	A	>95

* The corresponding ethyl esters were used.

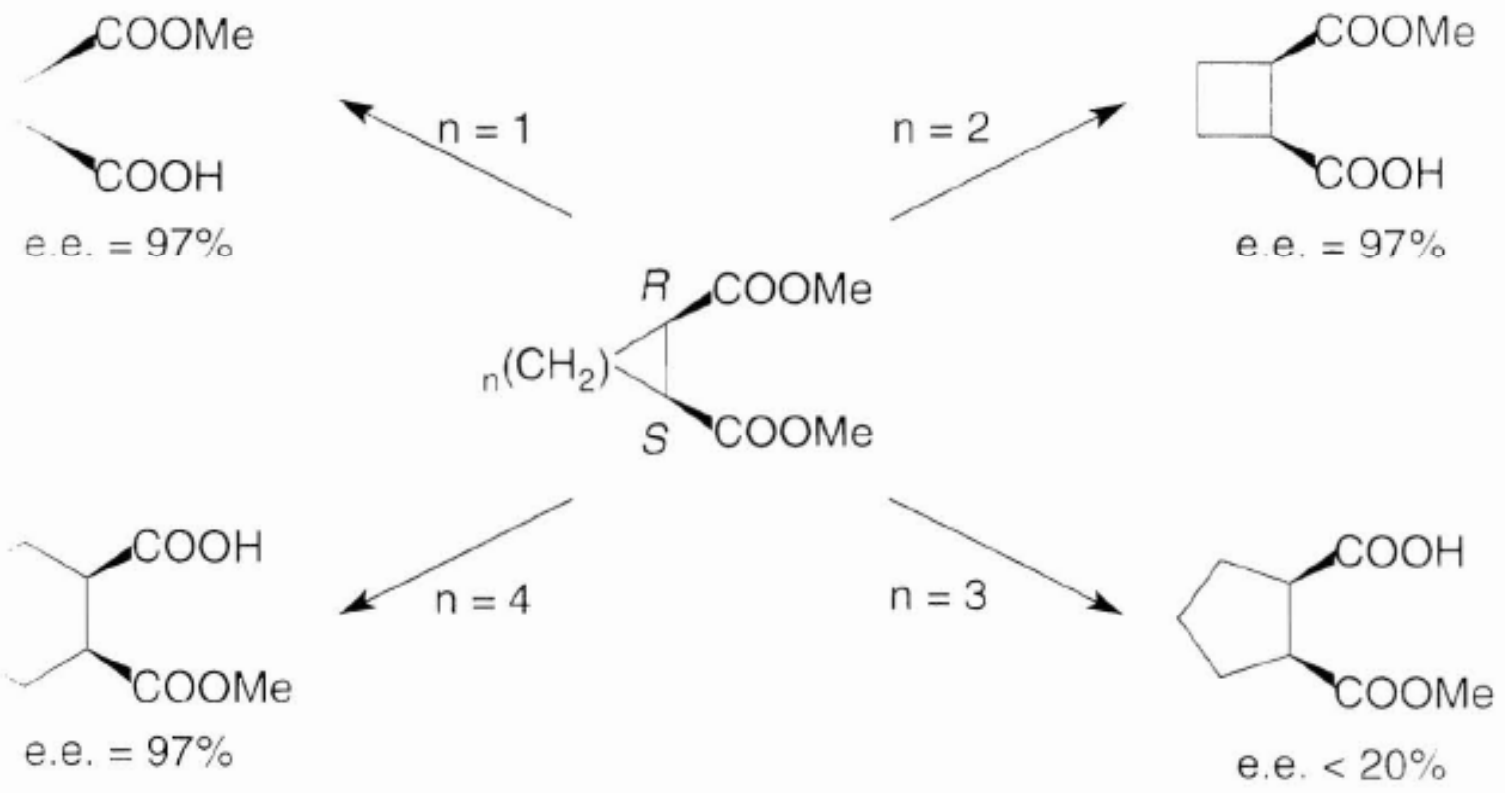
Scheme 2.26. Desymmetrization of acyclic *meso*-dicarboxylates by α-chymotrypsin and porcine liver esterase



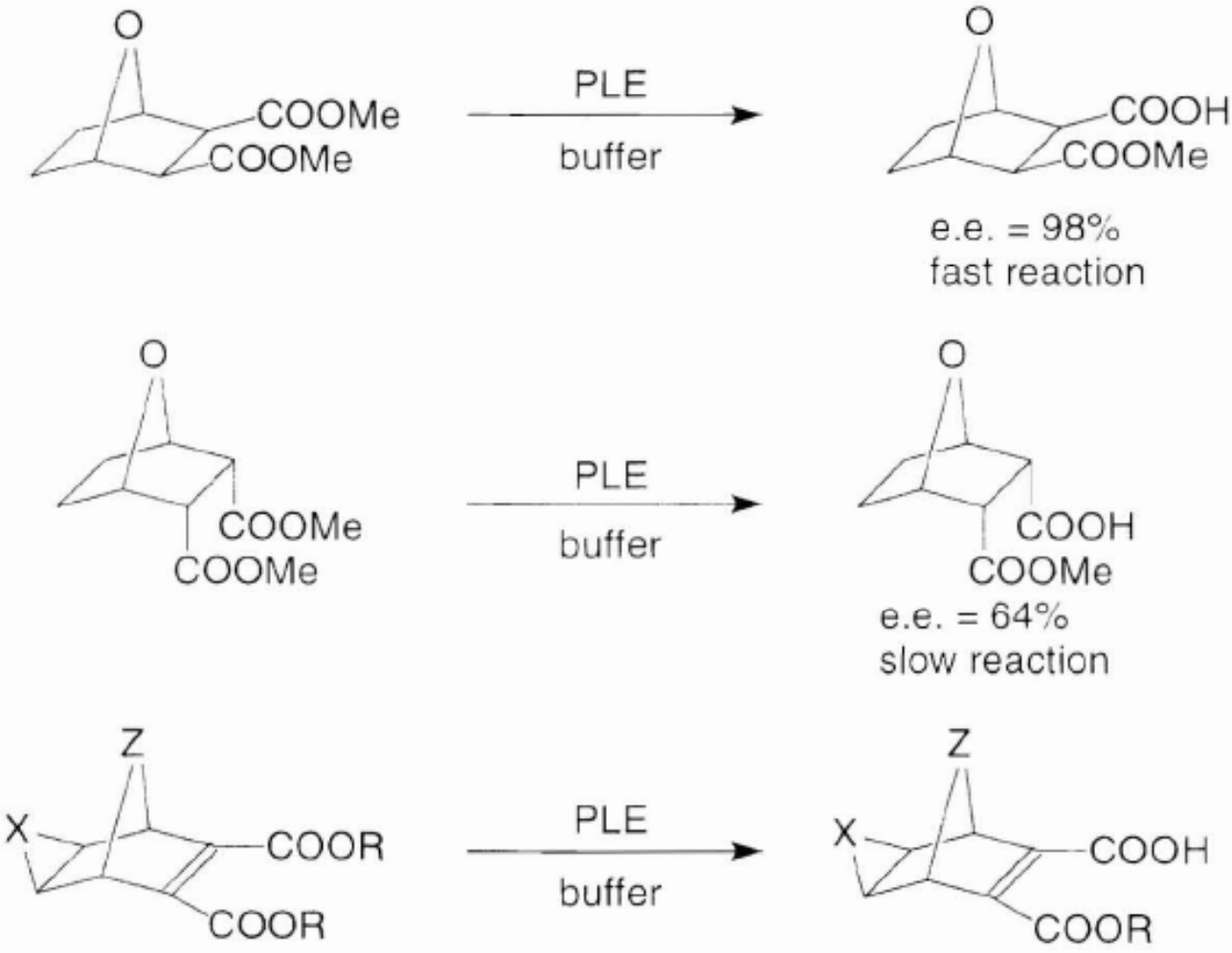
R	enzyme	e.e. [%]
H	PLE	64
OH	PLE	98
H	α-chymotrypsin	77



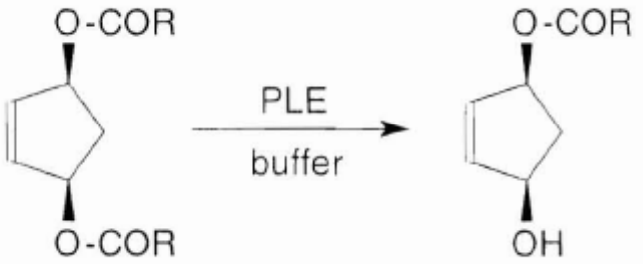
Scheme 2.27. Desymmetrization of cyclic *meso*-1,2-dicarboxylates by porcine esterase



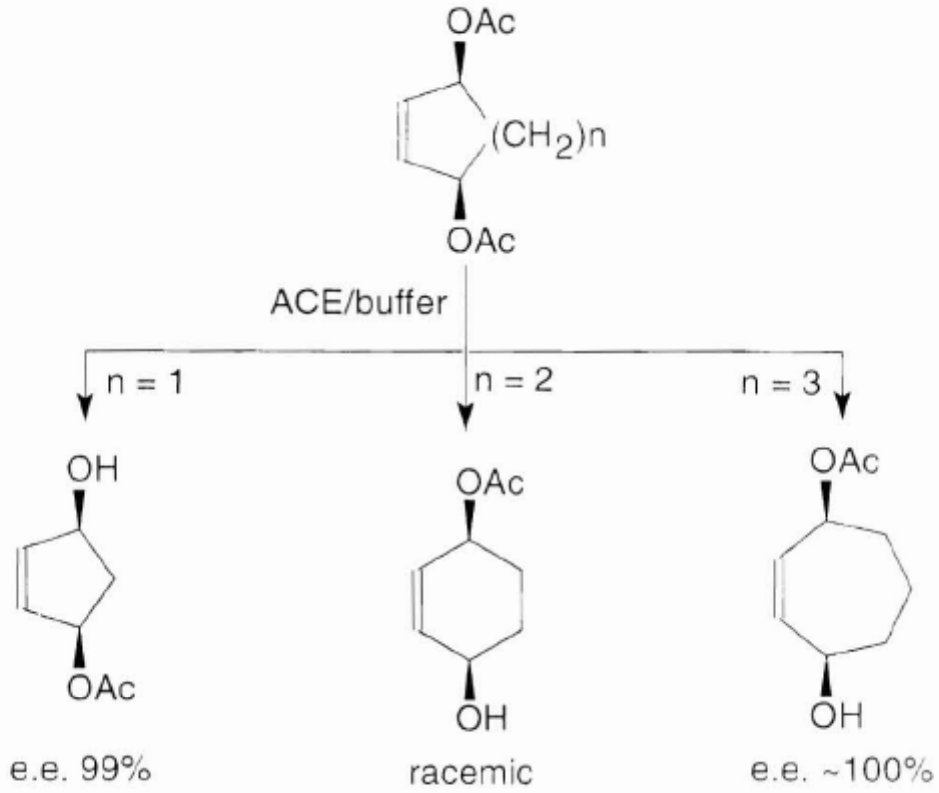
Scheme 2.28. Desymmetrization of polycyclic *meso*-1,2-dicarboxylates by porcine liver esterase



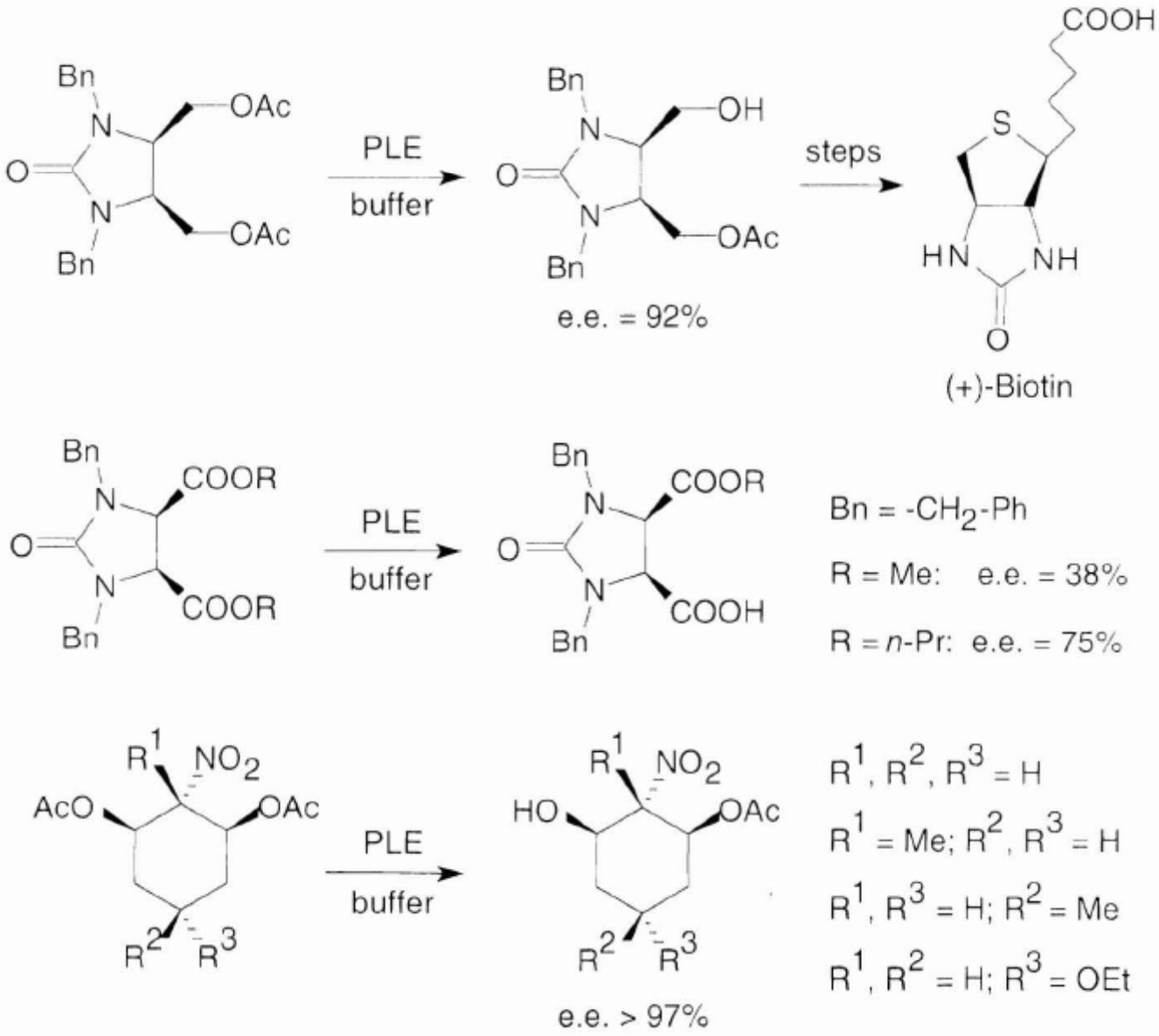
Scheme 2.29. Desymmetrization of cyclic *meso*-diacetates by porcine liver esterase and acetylcholine esterase



R	e.e. [%]
Me	86
Et	66
n-Pr	33

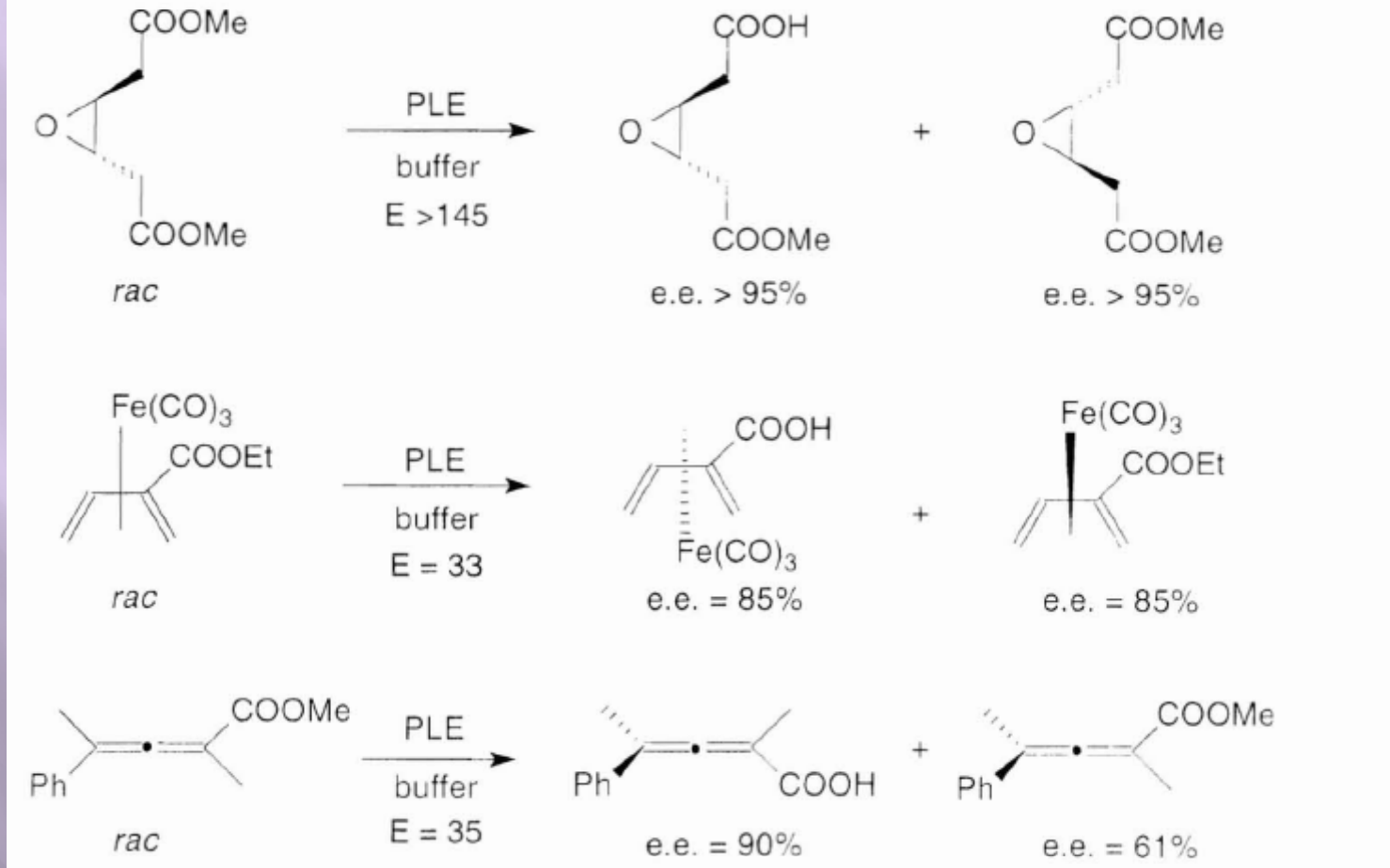


Scheme 2.30. Desymmetrization of *N*-containing cyclic *meso*-diesters by porcine liver esterase

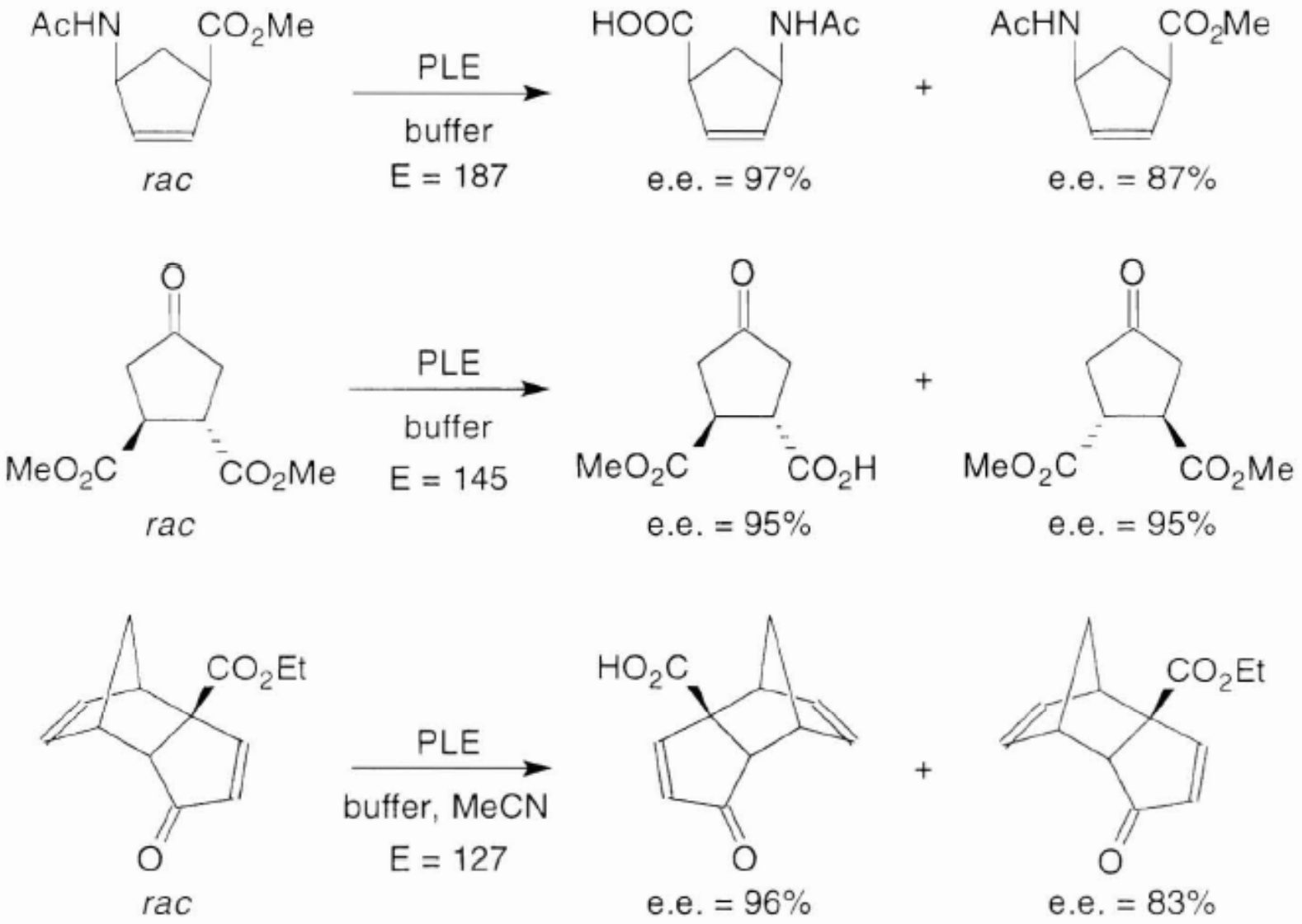


Resolution of racemic esters

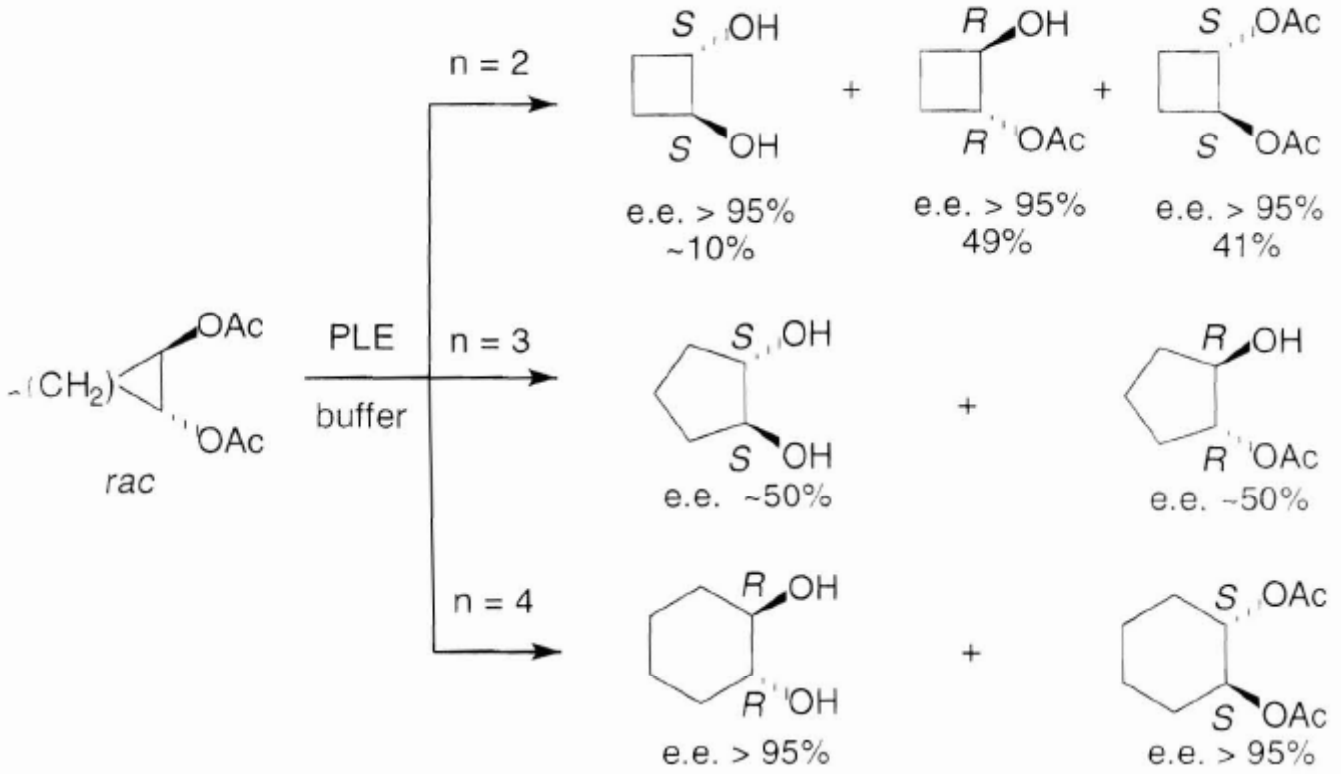
Scheme 2.31. Resolution of acyclic carboxylic esters by porcine liver esterase



Scheme 2.32. Resolution of cyclic carboxylic esters by porcine liver esterase



Scheme 2.33. Resolution of cyclic *trans*-1,2-diacetates by porcine liver esterase



Review questions:

Describe repeated resolution and why is it desired

What is dynamic resolution?

What is the esterase method

Which enzymes are being used mainly?

What is the lactamase method?

Esterases and proteases? Types of substrates for their reactions?

Describe desymmetrization of acyclic meso-dicarboxylates?