Organometallic Chemistry and Homogeneous Catalysis

Dr. Alexey Zazybin

Lecture N13

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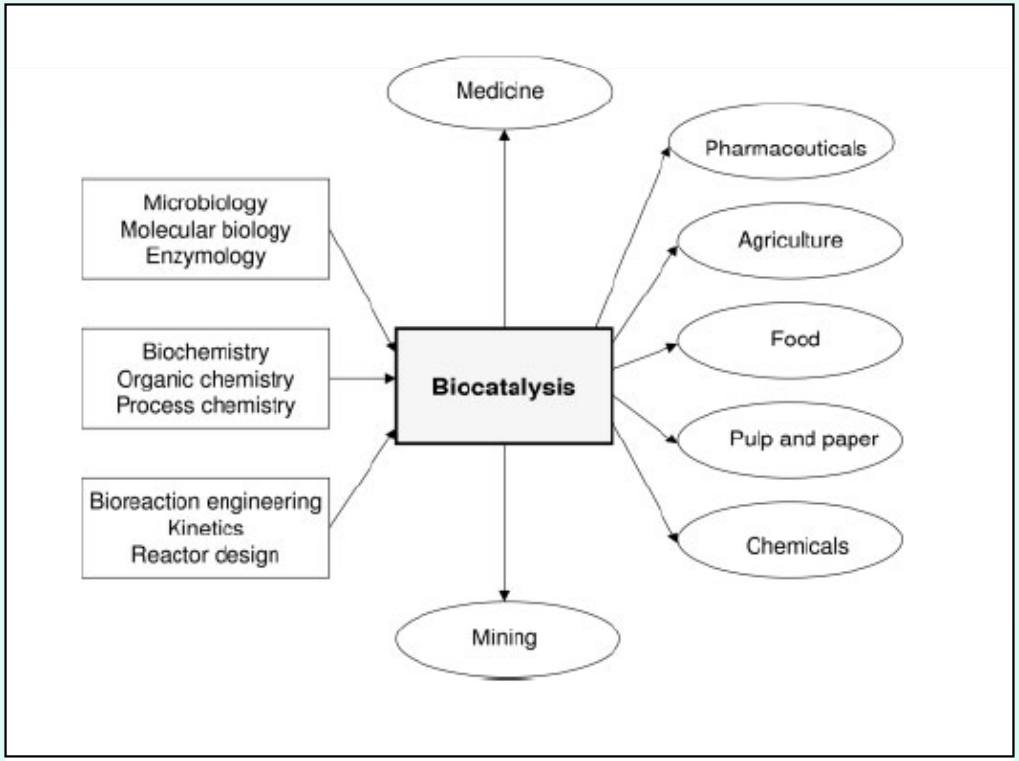
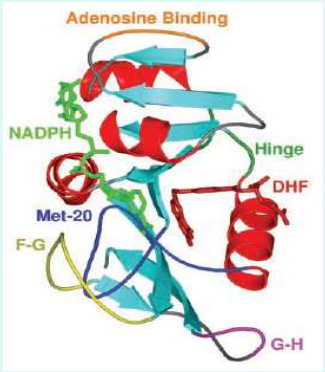
Enzime Catalysis



If 15-20 years ago enzymes were applied as a catalysts only in a few laboratories, now chemist (not majored in biocatalysis) are using enzymes and enzyme-containing cells both in laboratories and in industry

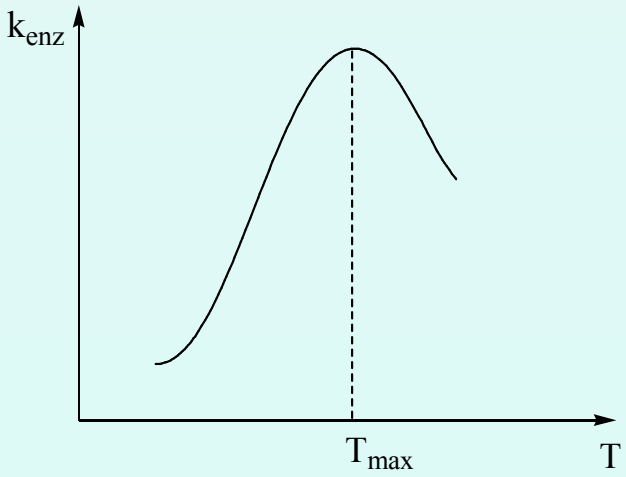


Enzyme catalysis allows:

1) To proceed reactions in mild conditions: close to room temperature, atmospheric pressure, close to neutral pH
2) To synthesize chiral molecules which can be used as syntones for the obtaining of drugs, nutrients and so on
3) Using less steps to synthesize compound which hard or impossible to obtain using traditional methods of organic synthesis.

Reaction conditions:

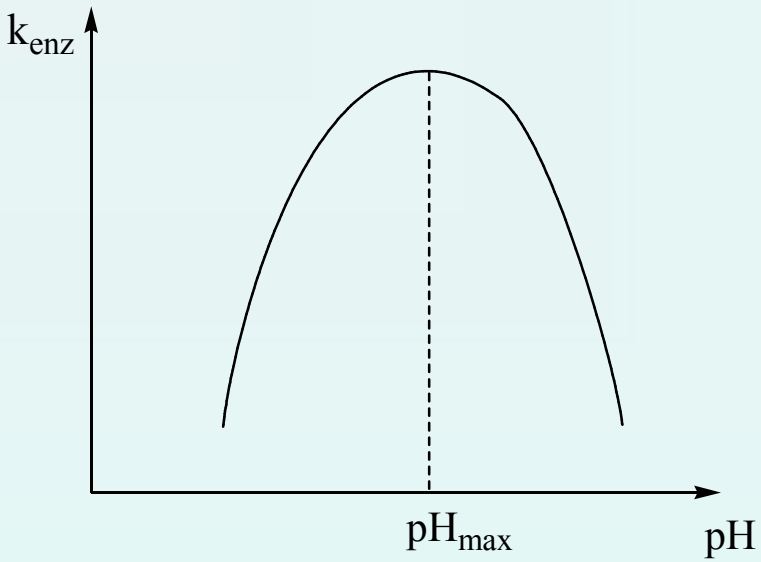
1) Temperature



For the most of enzymes optimal temperature range is 30-40^{0}C. Most enzymes undergo irreversable denaturation at 40-50^{0}C

Exception:
Adenylate kinase – active at 100^{0}C
Chymotrypsin – active at -45^{0}C
Horseradish peroxidase – active at -65^{0}C

2) pH



For the most of enzymes optimum of pH is in the range of 7-8. Even so some exceptions are possible:

Enzyme	Optimum of pH
Adenylate kinase	1
Pepsin	2
Chymotrypsin	7.8
Alkakine phosphatase	8-10

3) Solvent

a) Water – ideal solvent for the enzyme functioning

Disadvantages:
-many organic solvents are not soluble in water
-some of organic reactions (*i.e.*, esterification) hardly take place in water
-it is often hard to extract products (*i.e.,* amines, polyalcohols) from the reaction mixture

b) Associated solvents (glycerol, ethylene glycol, formamide) – these solvents do not cause denaturation of enzymes. Enzymes can dissolve in these solvents and can quite efficiently catalyze organic reactions

Disadvantages:
- problems with catalyst regeneration
-the same problem with extraction of products (*i.e.,* amines, polyalcohols) from the reaction mixture

c) DMSO, DMF, alcohols – enzymes are often soluble in these solvents, but in most cases they are inactive because of denaturation

d) Water-organic solvent (THF, 1,4-dioxane, CH_3CN) mixtures – in these solvents enzymes could be soluble or insoluble, but in both cases they demonstrate catalytic activity

e) Hydrophobic solvents (hexane, toluene) – enzymes are insoluble in these solvents, but they are active catalysts in these media and show new properties

New properties of enzymes in hydrophobic solvents:

-High thermostability

-Ability to catalyze reactions which are not possible in water

Considering insolubility of enzymes in hydrophobic solvents:

-Now it is easy to separate enzymes from the reaction mixture – “plus”

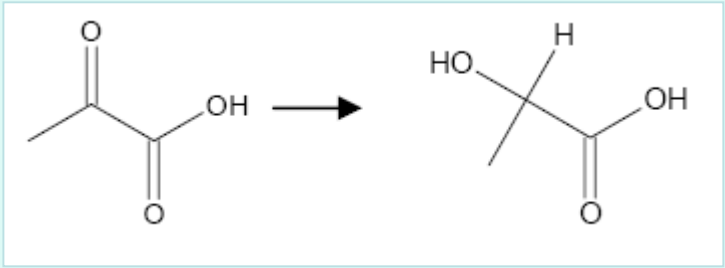
-Low efficiency (only surface molecules are active) – ‘minus’

Enzyme classification:

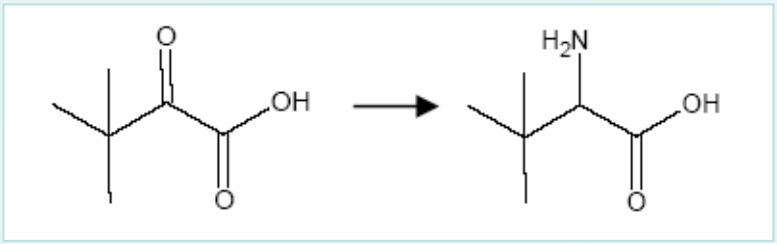
Class 1. Oxidoreductases – these referred to as *oxidases*, *reductases*, *hydrogenases*; common conversion is ketones/keto-acids to chiral alcohols

(a) EC 1.1.1.27 – lactate dehydrogenase

pyruvate + NADH + H^+ → lactate + NAD^+

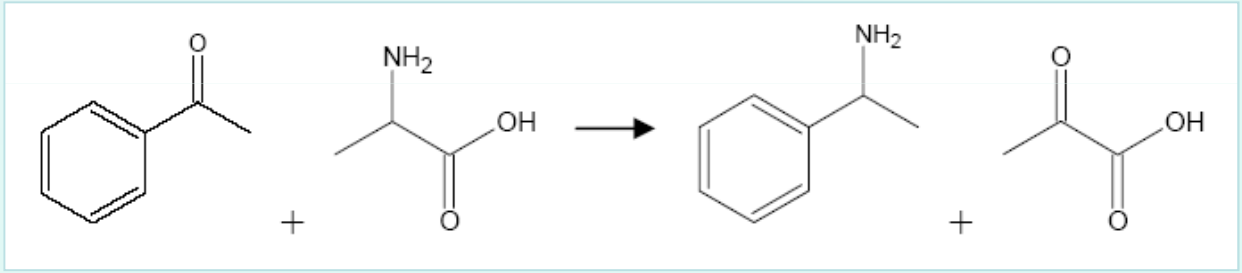


(b) EC 1.4.1.9 leucine dehydrogenase (reductive amination)
trimethylpyruvate + NADH + NH_4^+ → L-*tert*-leucine + NAD^+ + H_2O

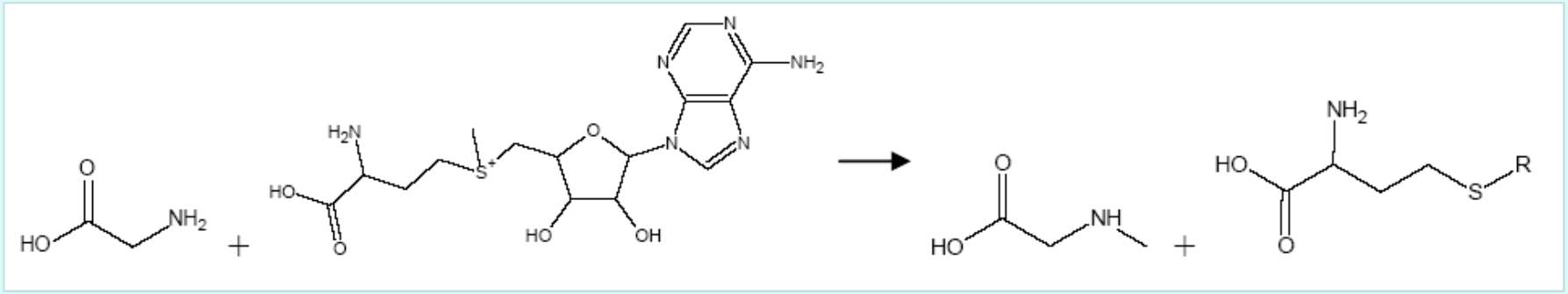


Class 2. Transferases – *aminotransferases* (transaminases)

(a) EC 2.6.1.x transaminase
acetophenone + L-alanine → phenylethylamine + pyruvate



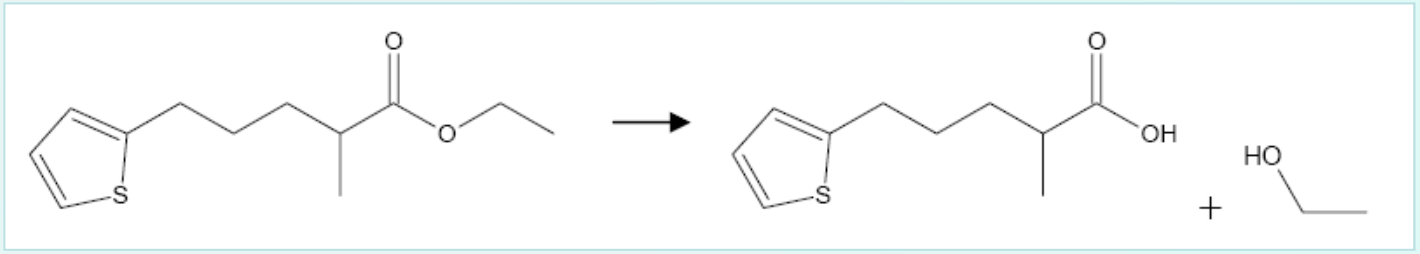
(b) EC 2.1.1.20 glycine-N-methyltransferase
glycine + S-adenosyl-methione → methlyamino-acetic acid + S-adenosyl-cysteine



Class 3. Hydrolases – *lipases*, *nitrilases, proteases*

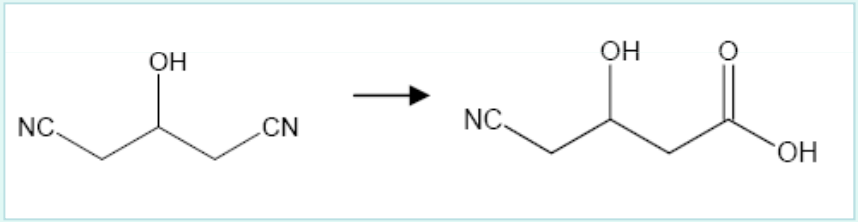
(a) EC 3.1.1.x lipase

ethyl 2-methyl-5-thien-2-ylpentanoate + H_2O → 2-methyl-5-thien-2-ylpentanoic acid + ethanol



(b) EC 3.5.5.1 nitrilase

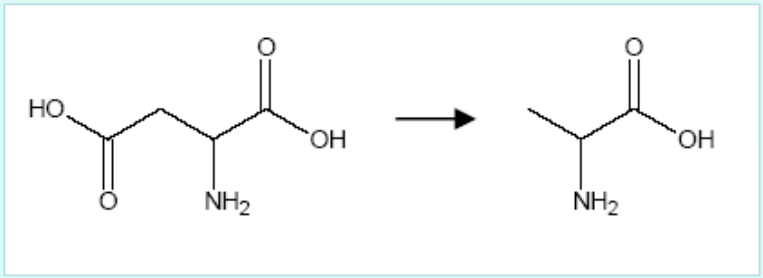
3-hydroxypentanedinitrile + $2H_2O$ → 4-cyano-3-hydroxybutanoic acid

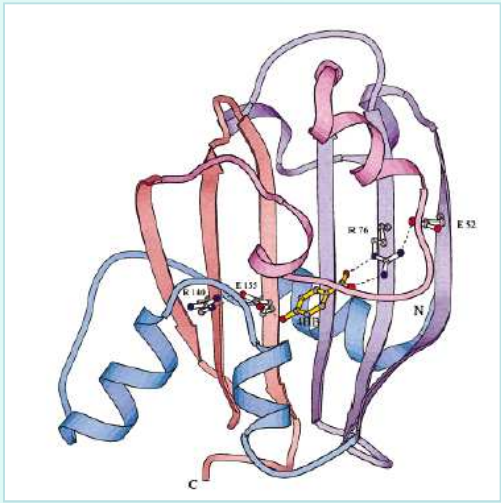


Class 4. Lyases

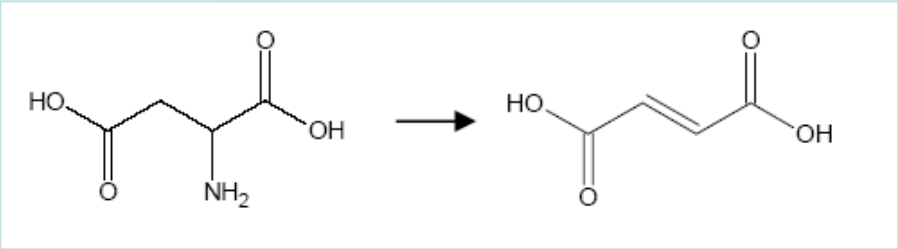
(a) EC 4.1.1.12 L-Asp-β-decarboxylase

aspartate → alanine + CO_2



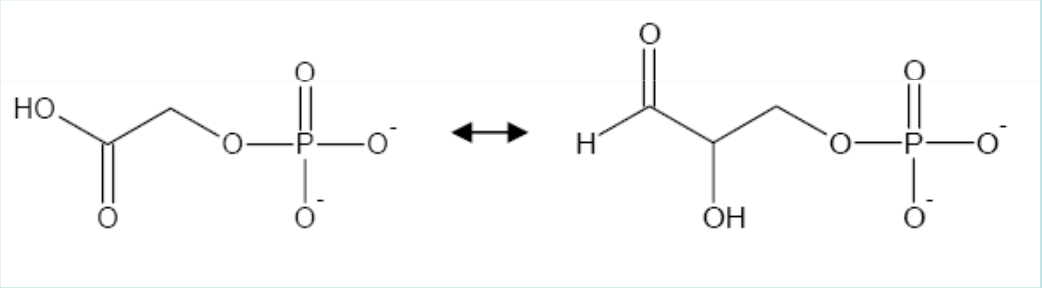


(b) EC 4.3.1.1 aspartate ammonia lyase (aspartase)
aspartate → fumarate + NH_3

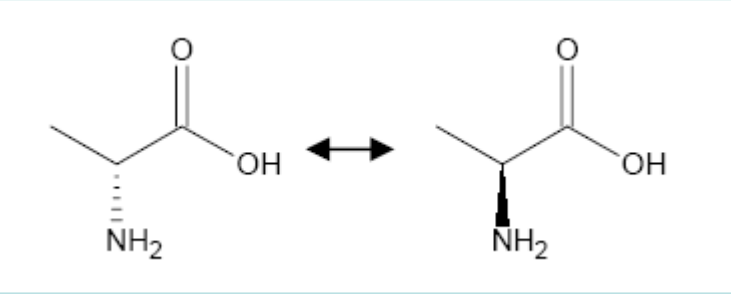


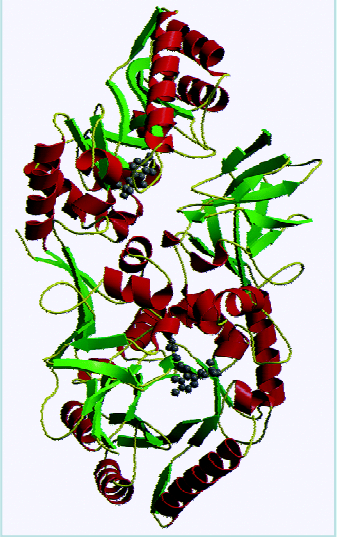
Class 5. Isomerases

(a) EC 5.3.1.1 triose phosphate isomerase
dihydroxyacetone phosphate → glyceraldehyde-3-phosphate



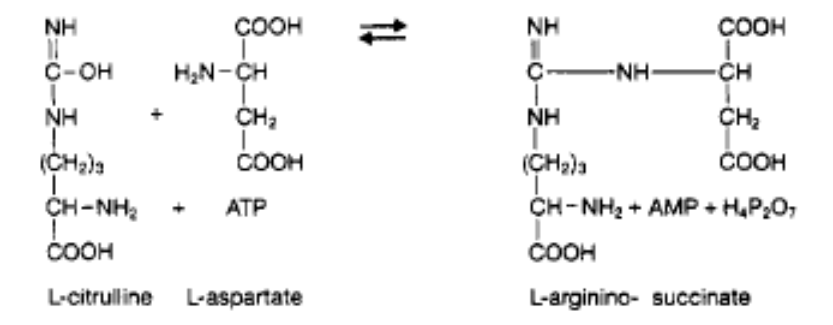
(b) EC 5.1.1.1 alanine racemase
L-alanine → D-alanine





Class 6. Ligases

L-citrulline + L-aspartate + ATP → L-arginino-succinate + AMP + $H_4P_2O_7$

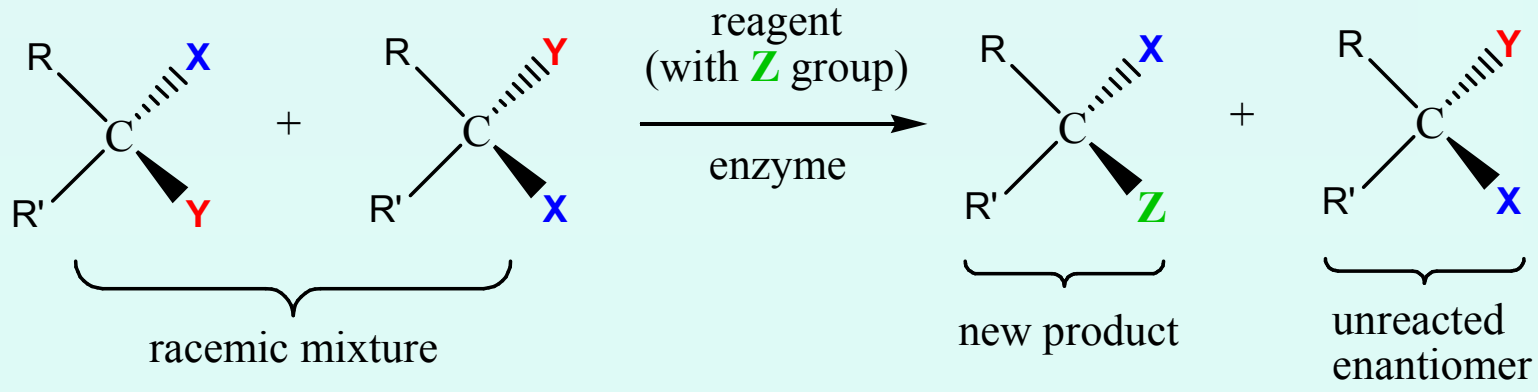


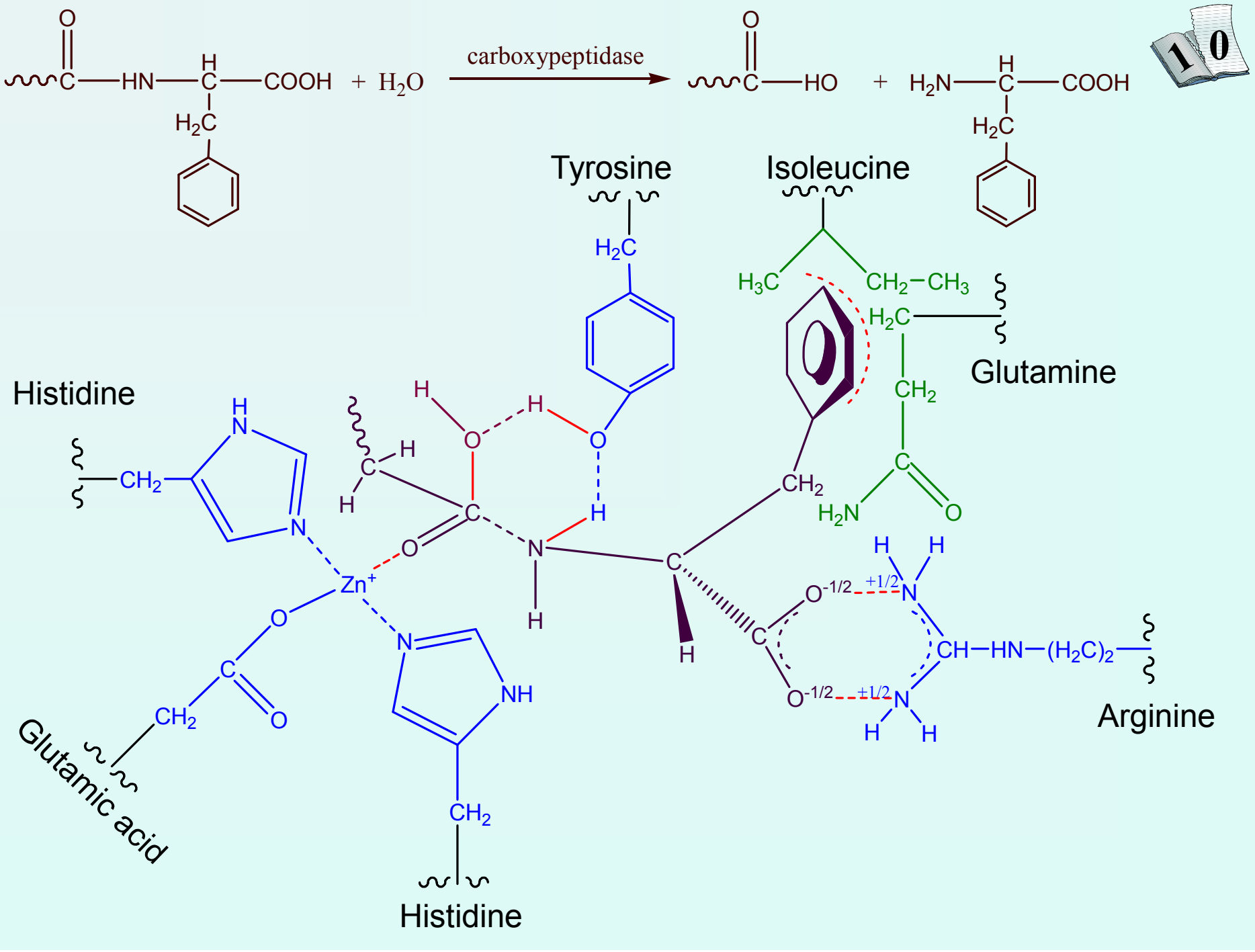
High stereoselectivity is a reason of enzyme application in at least 90% of cases

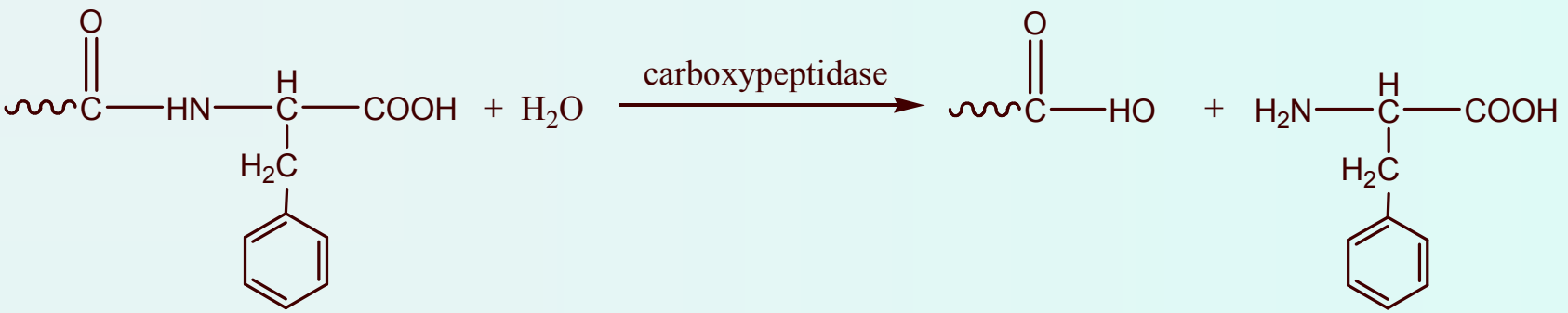
Enzymes can distinguish between substrates of 3 main types:

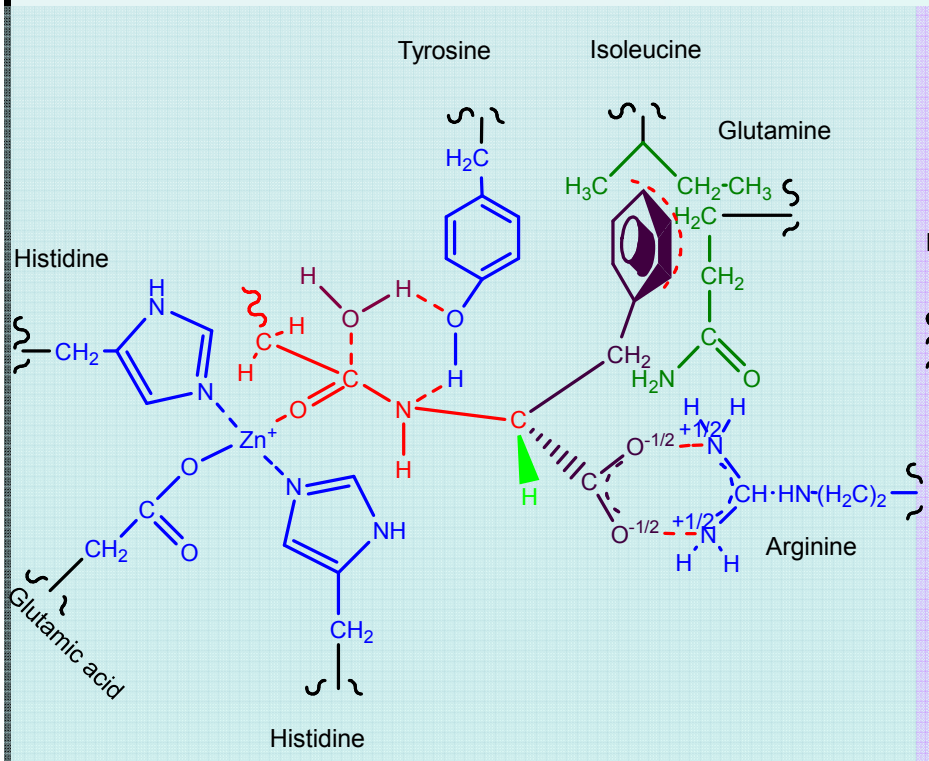
1. Enantiomers recognition

   Due to chirality the enzyme's active center can cause a transformation of only one of the enatiomers of racemic mixture

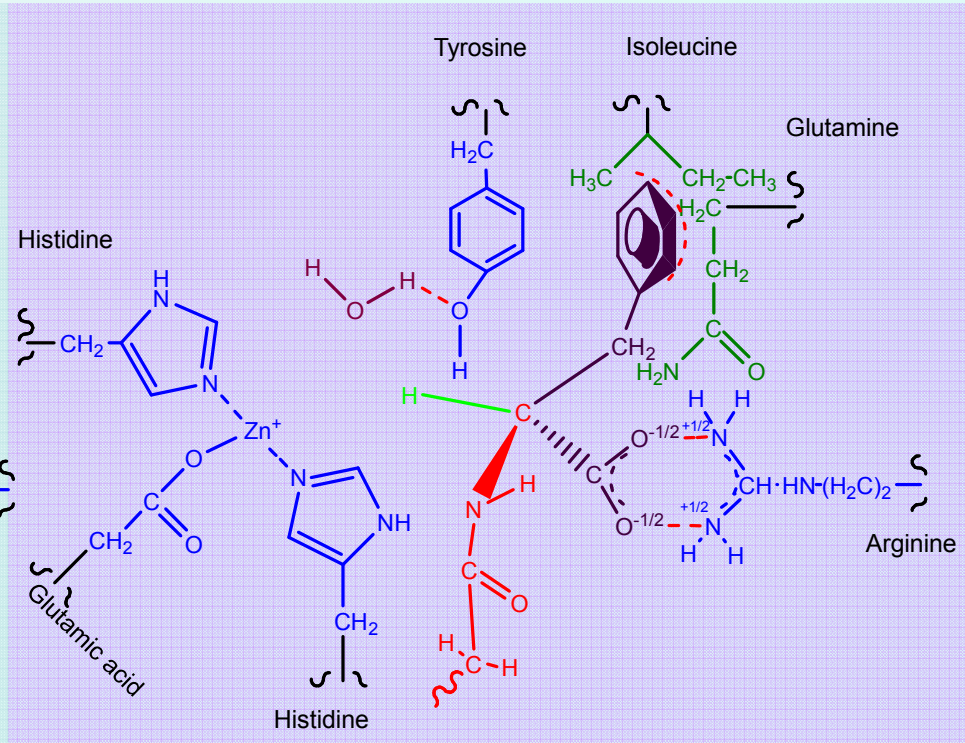



carboxypeptidase
Tyrosine
Isoleucine
Glutamine
Histidine
Glutamic acid
Histidine
Arginine



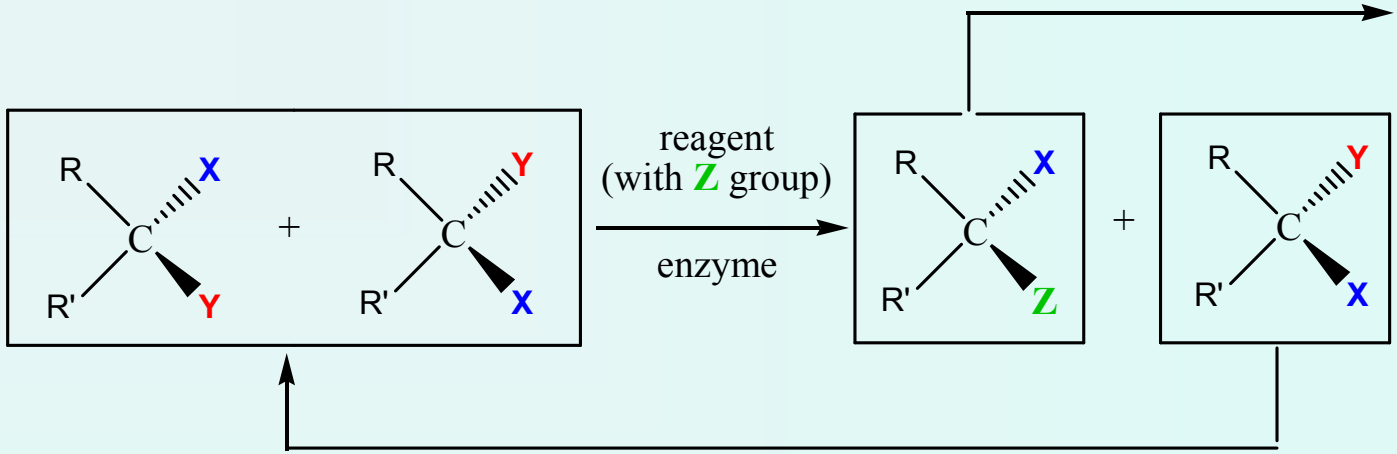


L-amino acid residue

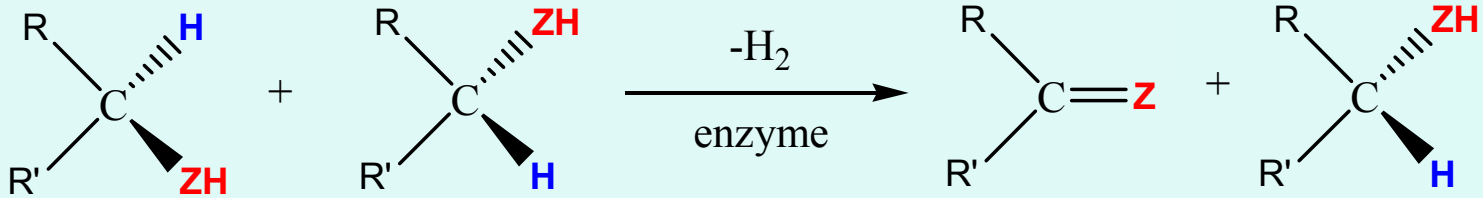


D-amino acid residue
Does not fit!

The final product can not reach more then 50% from the starting racemic mixture. That is why the residue is used as a starting material for the racemization:



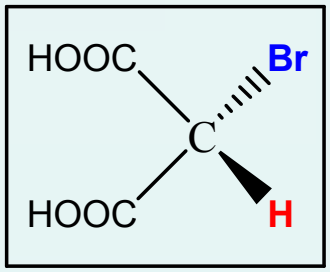
For this type of reaction usually class 3 of enzymes is used – Hydrolases.
In some cases class 1 (Oxidoreductases) also can be used:



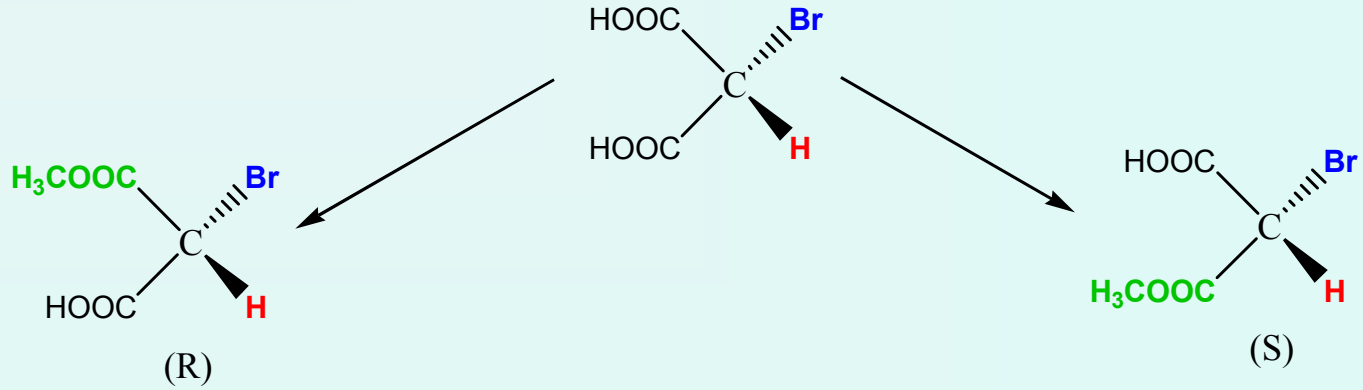
In this reaction chiral center of the oxidized substrate disappears.

2. Enantiotopic groups recognition (enzymatic asymmetrization)

Bromomalonic acid:

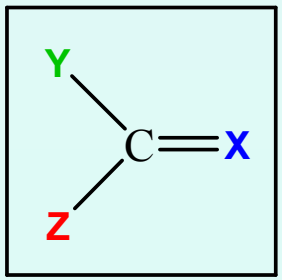


This compound is not chiral, but it is prochiral.
Conversion of one of the –COOH groups to some other group causes the formation of chiral product



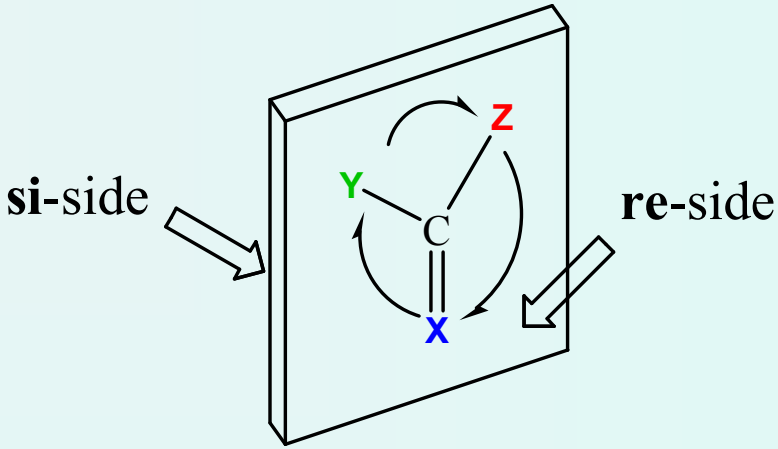
In contrast to the previous case, maximum yield is 100% (not 50%)

3. Differentiation of enantiotopic sides

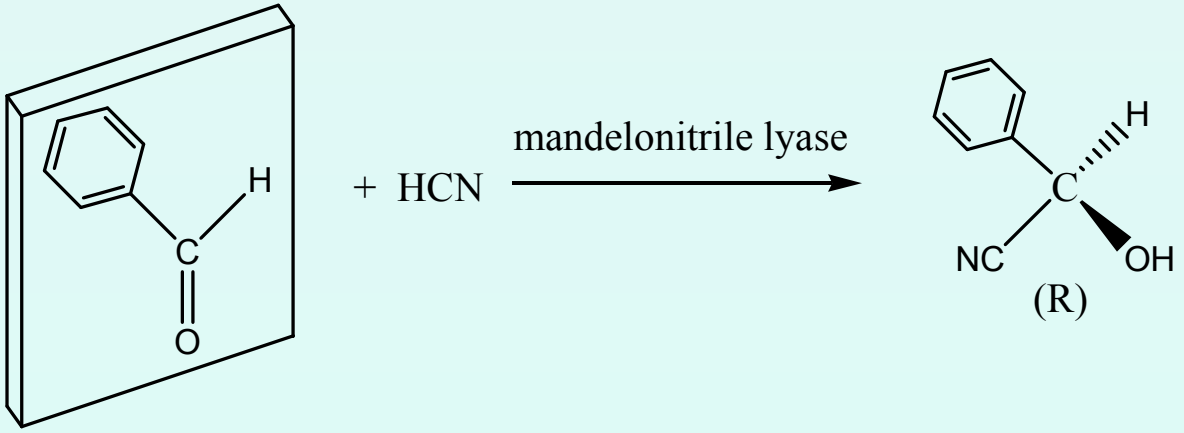


These molecules are also prochiral.
Addition of any molecule to the C=X bond causes the formation of chiral product

If to put groups in a row from highest priority to the lowest priority:
X > Y > Z we can differentiate to sides of the molecule:



For this type of reaction usually class 1 of enzymes is used - Oxidoreductases.
In some cases class 4 (lyases) also can be used:

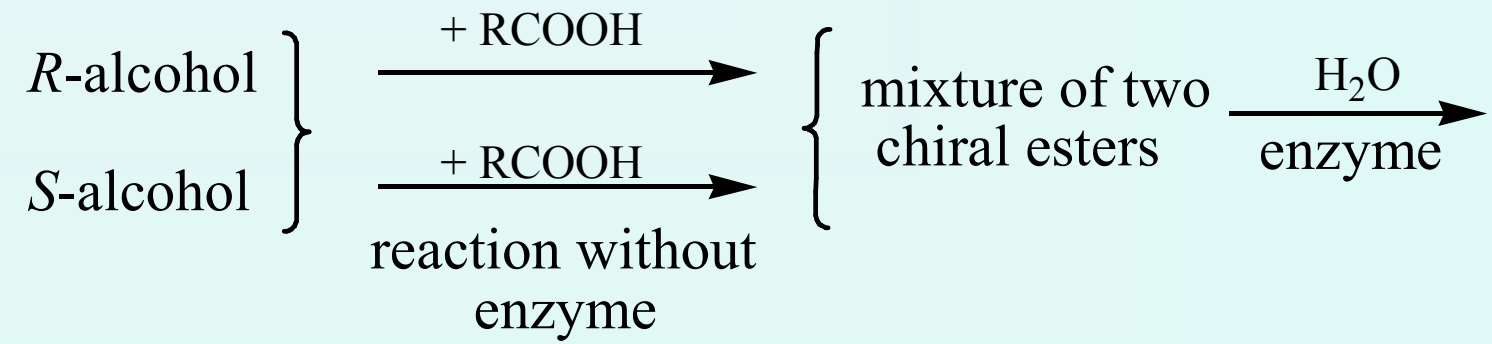


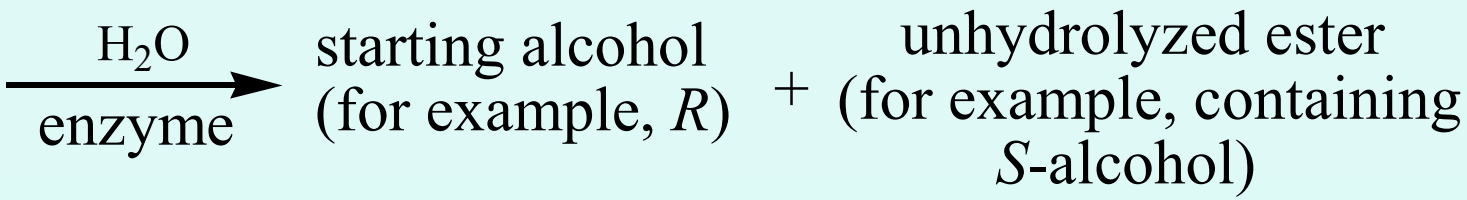
In water this reaction leads to the racemic mixture formation.
In EtOAc only *R*-isomer (selectivity 95%) can be obtained

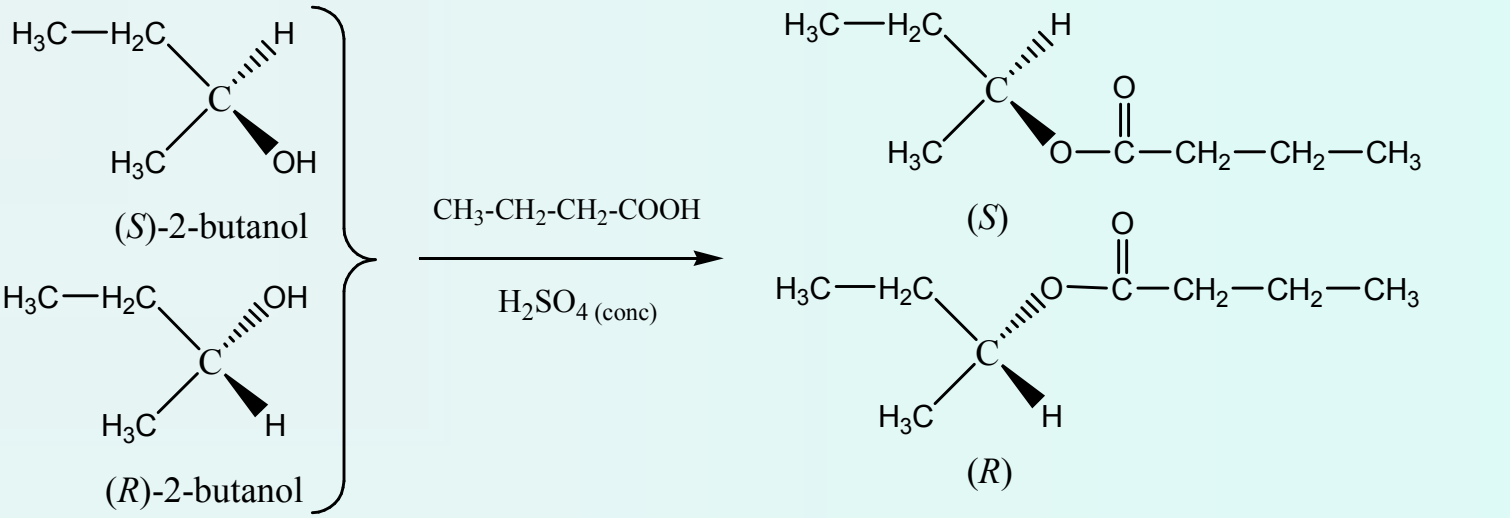
Application of hydrolases in organic synthesis

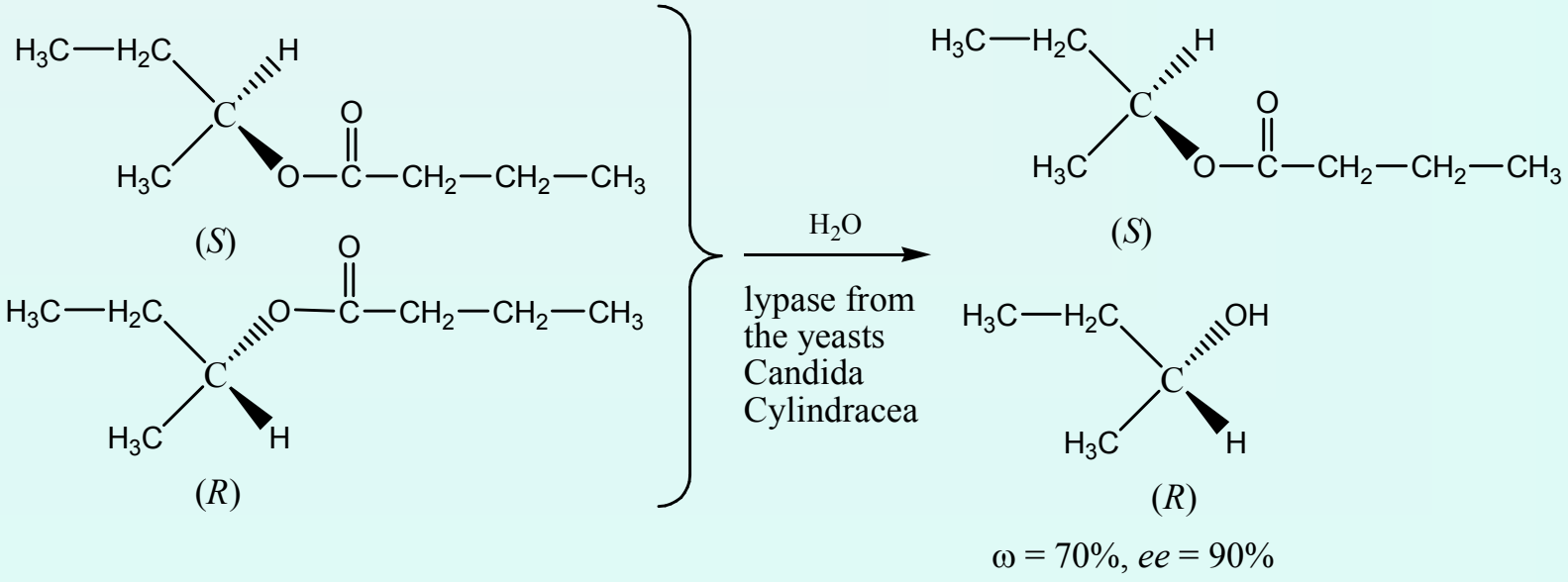
1. Separation of enantiomers

One of the most frequently used scheme of enantiomers separation consists of esterification with subsequent hydrolysis of only one of enantiomers:



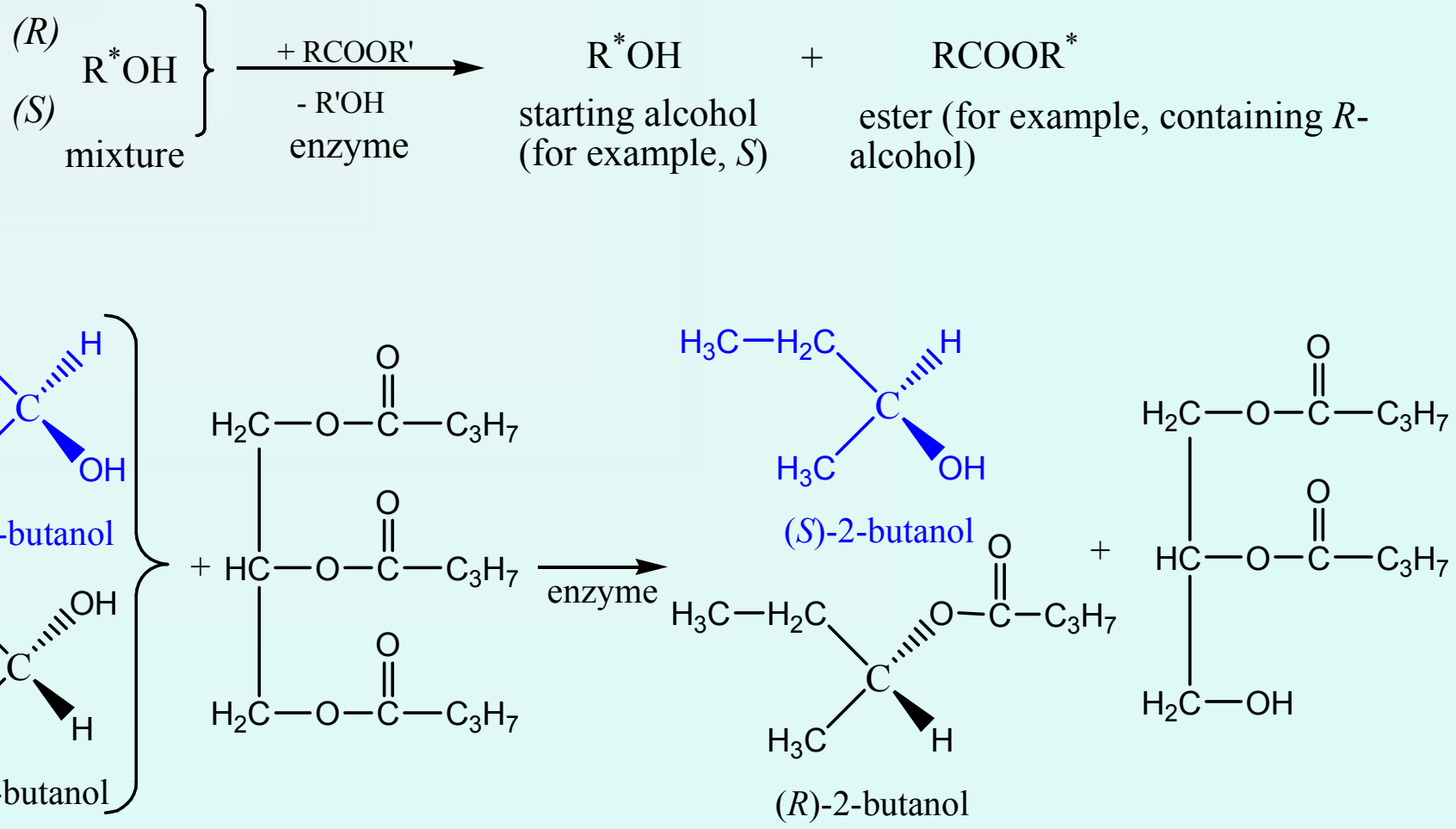






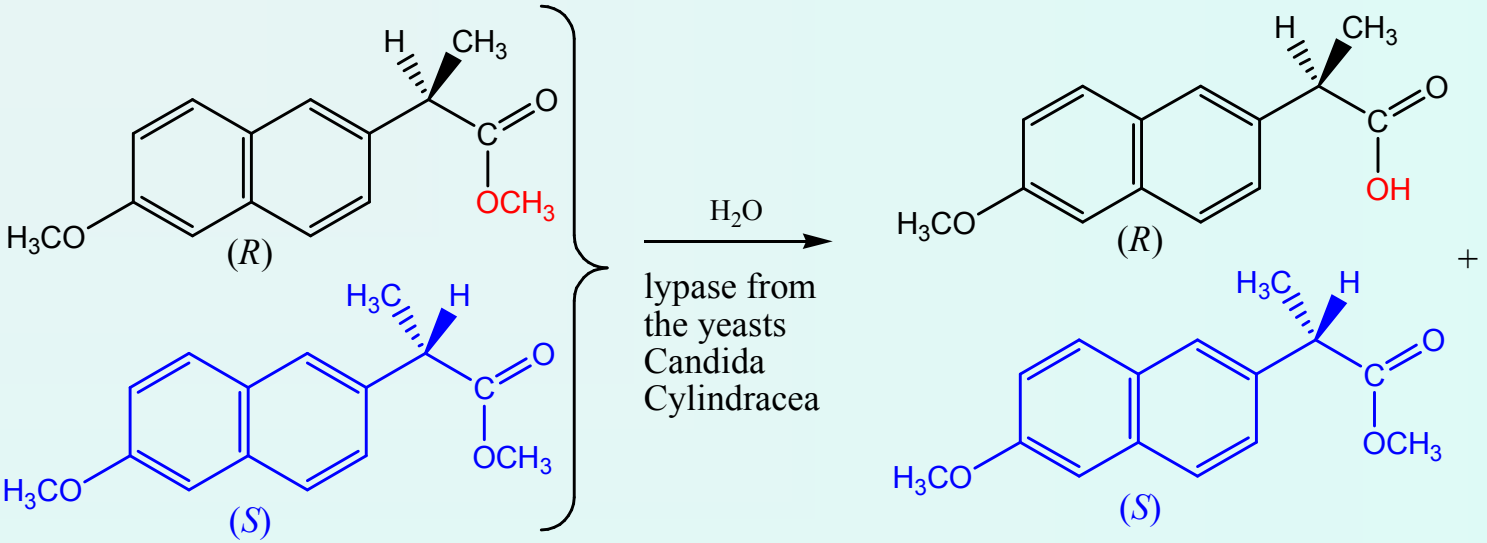
Disadvantage: 2 stages (chemical and enzymatic)

Using of water-free media possess to elaborate 1-stage scheme:

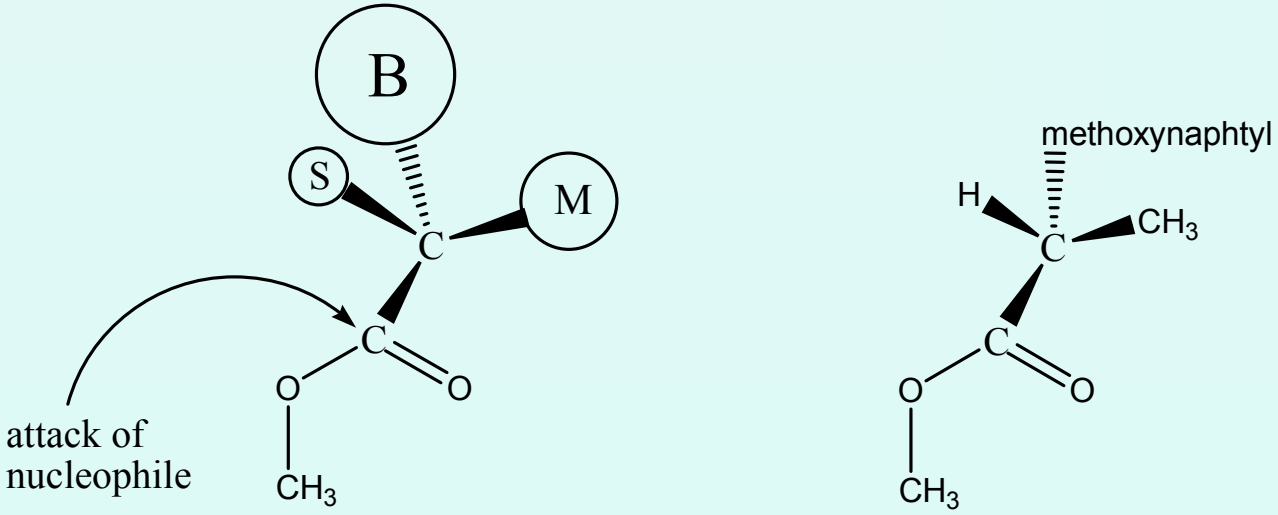


2. Hydrolysis of chiral esters

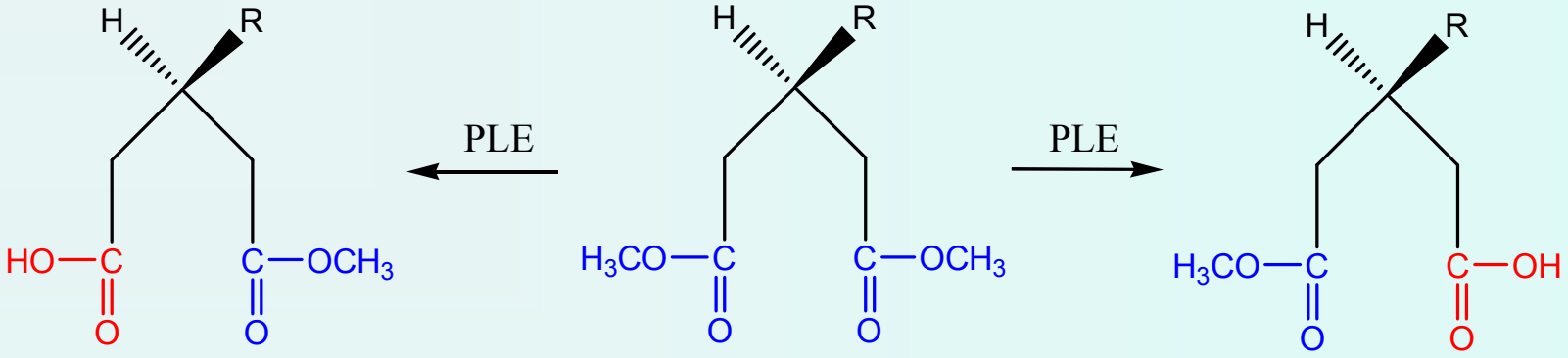
a) For the arthritis treatment Naproxen can be used. It is (*S*)-isomer of 2-(6-methoxynaphthalene-2-yl) propanoic acid :



In some cases it is possible to predict – what isomer will be hydrolyzed:



b) Other example – hydrolysis of prochiral esters of dicarboxylic acids with PLE - pig liver esterase

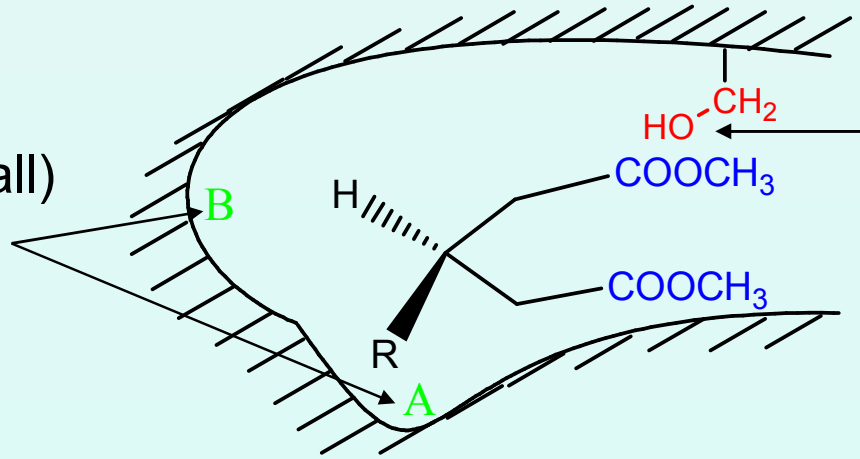


If R = CH_3, C_2H_5, - (*S*)-isomer can be obtained

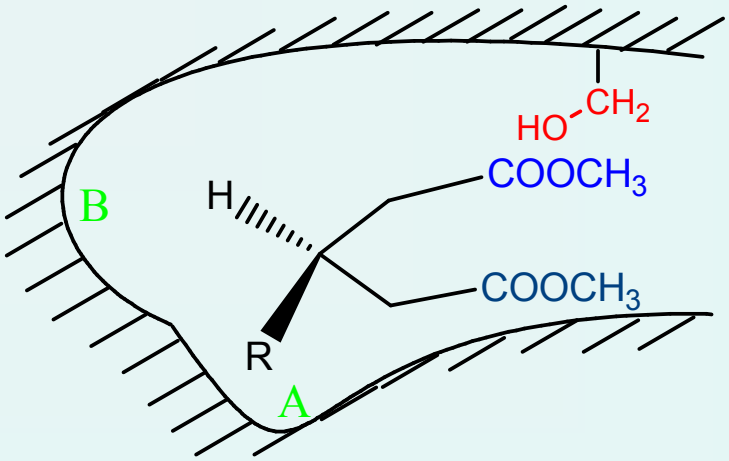
If R = Ph, CH_2Ph, - (*R*)-isomer can be synthesized

Structure of enzyme's active center:

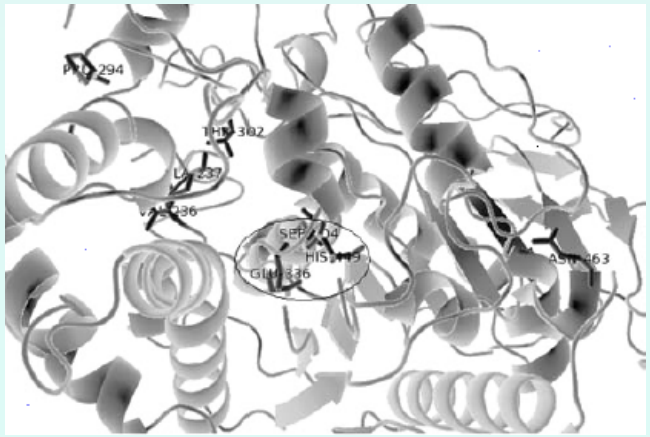
Pockets A (small) and B (large) are adsorption centers of PLE



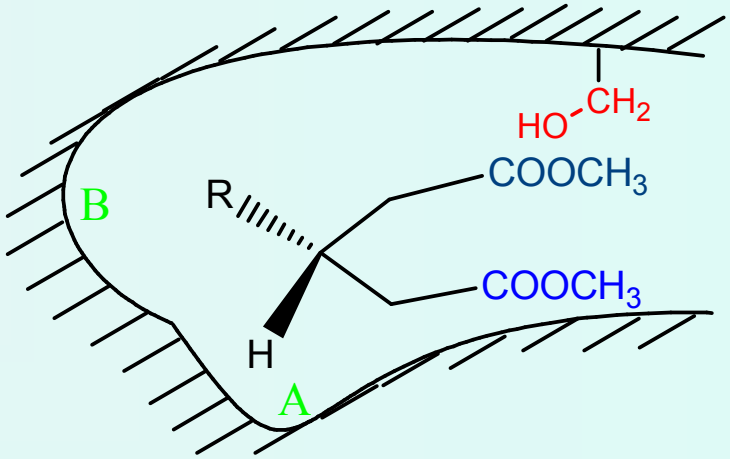
Serine's OH-group is the catalytic center of PLE



But if the size of R does not let it to locate in the pocket A, it locates in the pocket B (in that case (*R*)-group $COOCH_3$ is directed to serine.



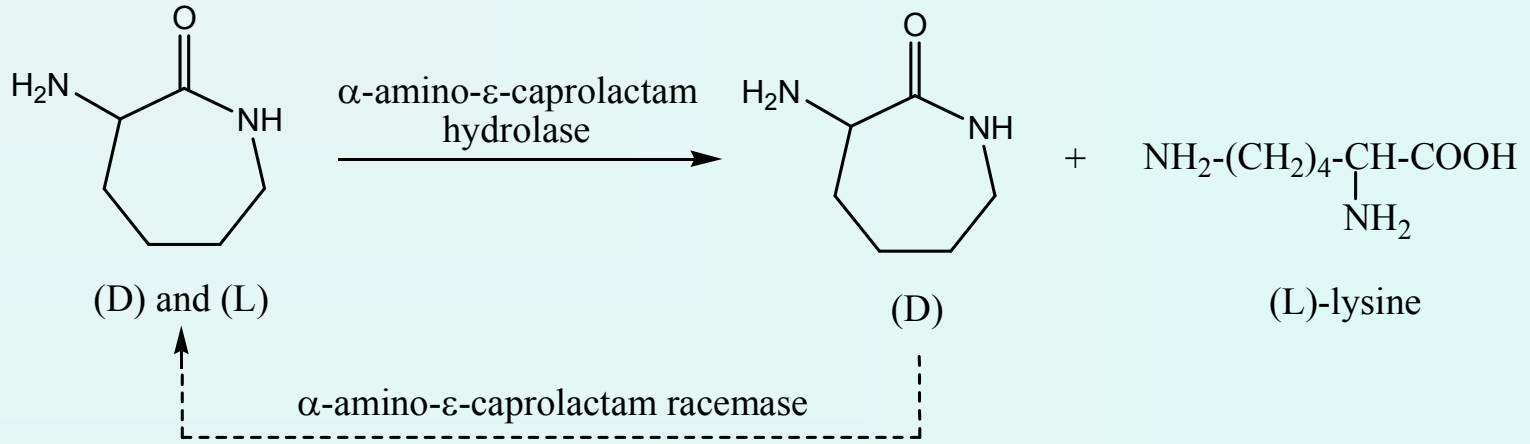
Thermodynamically for R is more favorable to locate in the pocket A (in that case (*S*)-group $COOCH_3$ is directed to serine.



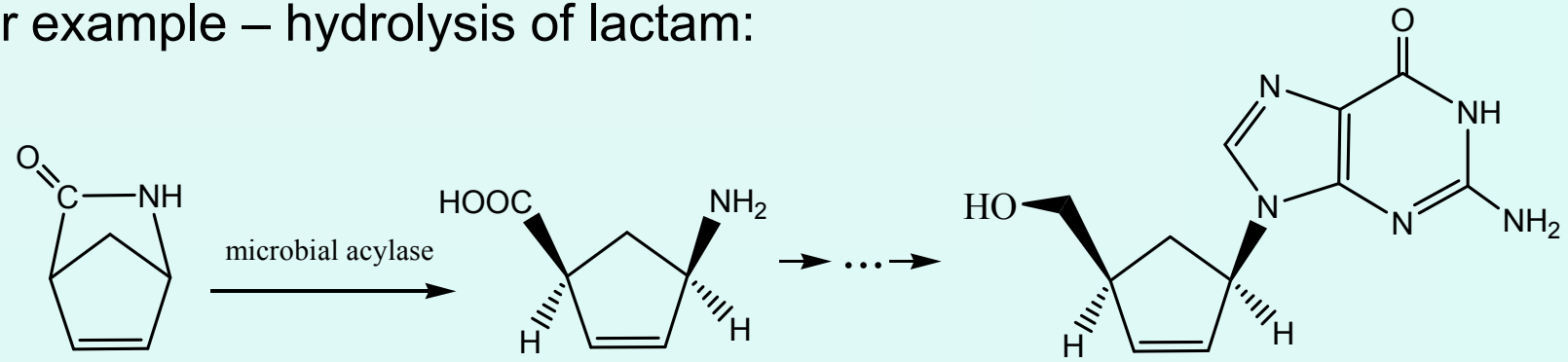
R	ω, %	*ee*, %
Me	97	79 (*R*)
Et	67	50 (*R*)
Ph	98	42 (*S*)
CH_2Ph	95	54 (*S*)

3. Hydrolysis of amides

Lysivit-C, the well-known anti-atherosclerosis supplement contains L-Lysine as a main component. It can be obtained by the selective hydrolysis of α-amino-ε-caprolactam (applied in industry, 450 M$ pa):



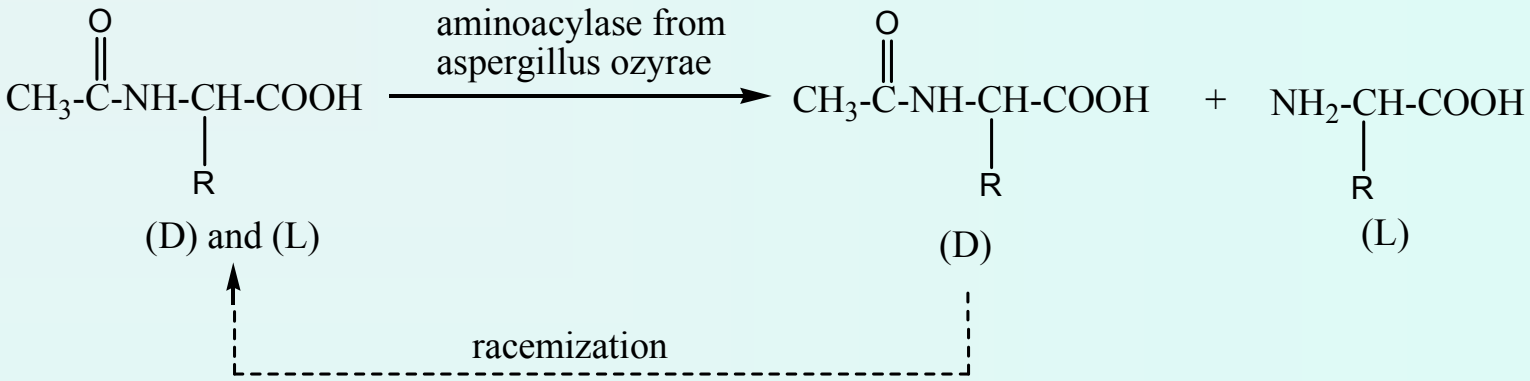
Other example – hydrolysis of lactam:



The resulting amino acid is a starting material for the synthesis of Carbovir (medicine for the AIDS)

Also very important example – synthesis of L-amino acids:

Hydrolysis of amide bond with subsequent racemization helps to reach high amounts of product:



Tanabe Seiyaki applied this scheme in industrial scale

4. Acylation of polyalcohols

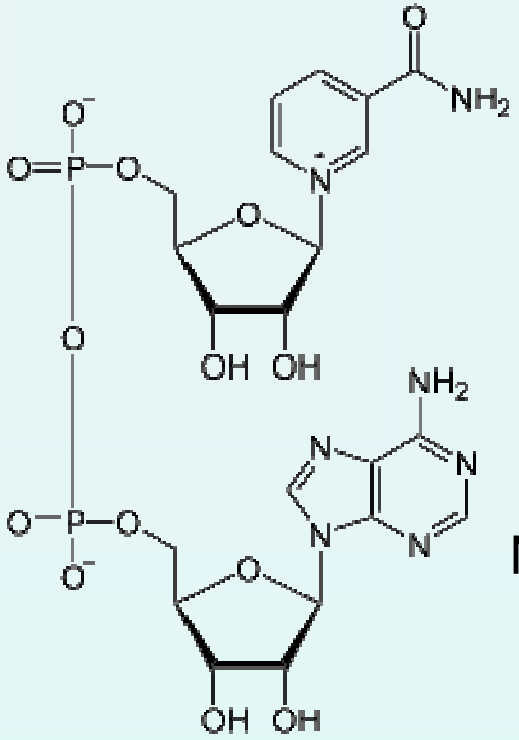
Acylation of *purine* that is currently being developed by Glaxo Welcome as an anti-leukaemic agent. Using an immobilized lipase from *Candida antarctica*, and vinyl acetate as acyl donor, a 99% conversion to the 5'-monoacetate is obtained, which renders the compound more soluble and thus, increases bioavailability.

This transformation is almost impossible to achieve by conventional chemical acetylation reagents because of their known preference for *N*-acylation. Regioselectivity in this process is remarkably high, less than 0.1% of 3-acetate and below 0.3% of 3,5-diacetate is formed.

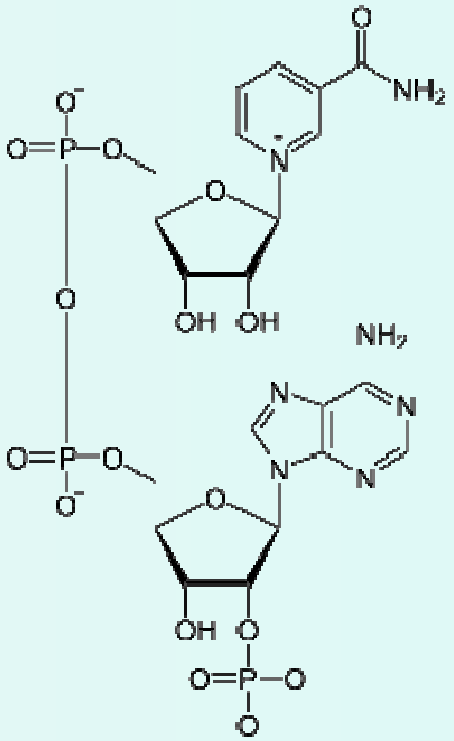
Application of oxidoreductases in organic synthesis

Some factors of using oxidoreductases:

1) Important factor – presence of coenzyme.
NAD - Nicotinamide Adenine Dinucleotide or NAD(P)
NAD^+ or $NAD(P)^+$ - oxidized form
NADH or NAD(P)H – reduced form



NAD^+



NAD(P)

If regeneration of coenzyme is not provided, the stoichiometric (to substrate) amount is needed. NAD(P) is a common substance but it is quite expensive. Usually other, conjugated redox reaction is used to regenerate co-factor.

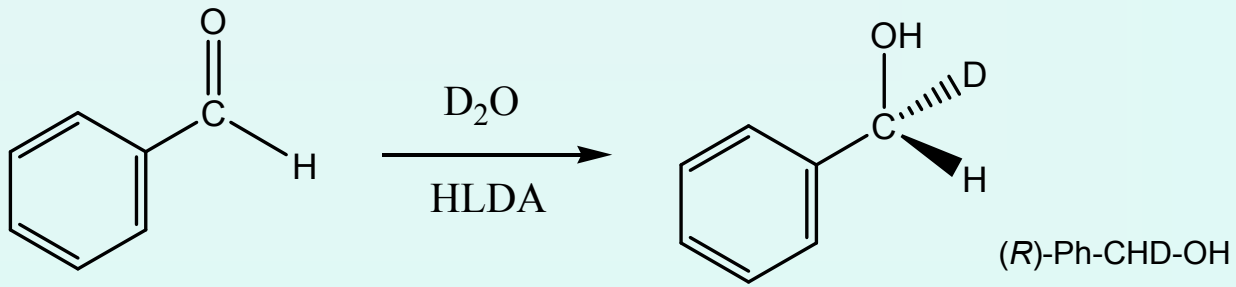
2) Oxidoreductases often are inhibited with the reaction products, that is why high concentrations (in compare to hydrolases) are needed.

1. Reduction

Reduction reactions is the widest group of reactions catalyzed by enzymes. In the German handbook "*Methoden der organischen Chemie*" there is an entire chapter devoted to the biocatalytic reduction. We will have a look on 2 of them:

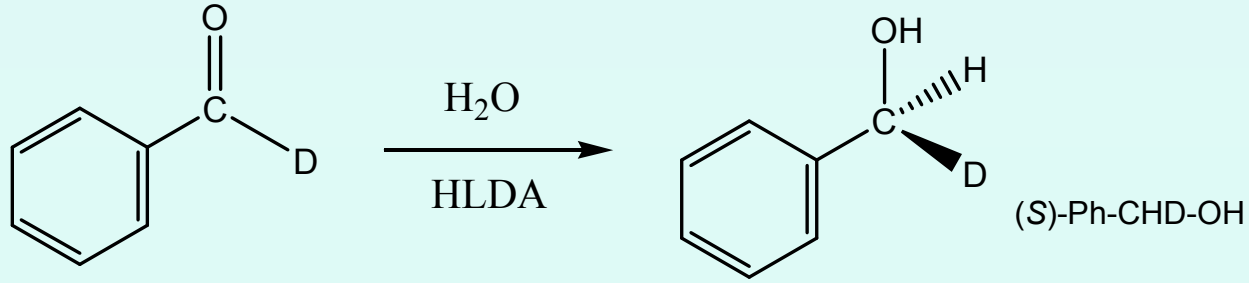
1) Synthesis of D and T- labeled chiral alcohols

Reduction of aldehydes in the presence of horse liver alcohol dehydrogenase (HLAD) in D_2O leads to the labeled alcohols obtaining



(*R*)-Ph-CHD-OH

How to get (*S*)-deuterated benzyl alcohol?



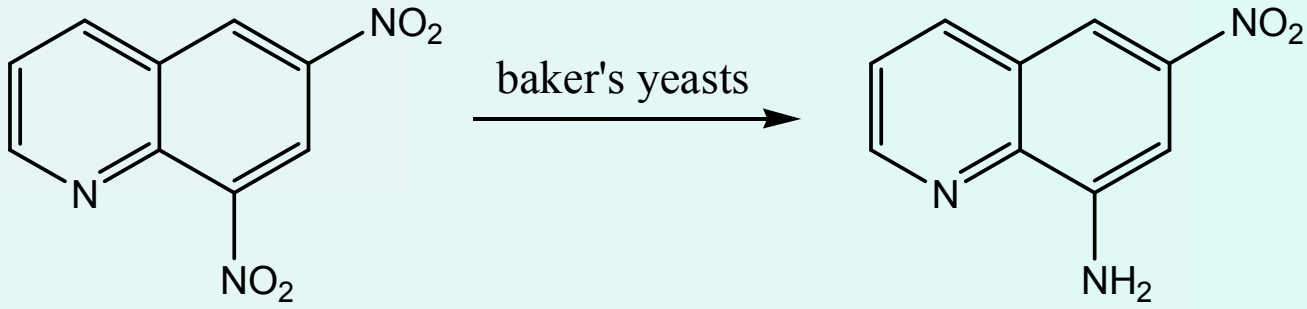
(*S*)-Ph-CHD-OH

ω = 95%

ee = 92%

2) Selective reduction of polynitrocompounds

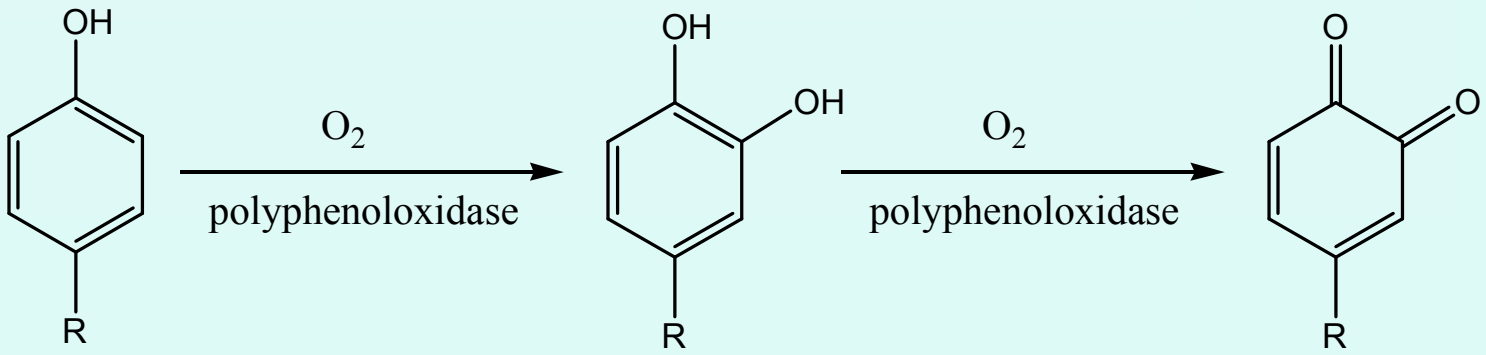
If there are 2 or more nitro-groups in the compound using enzymes it is possible to reduce selectively one of them:



2. Oxidation

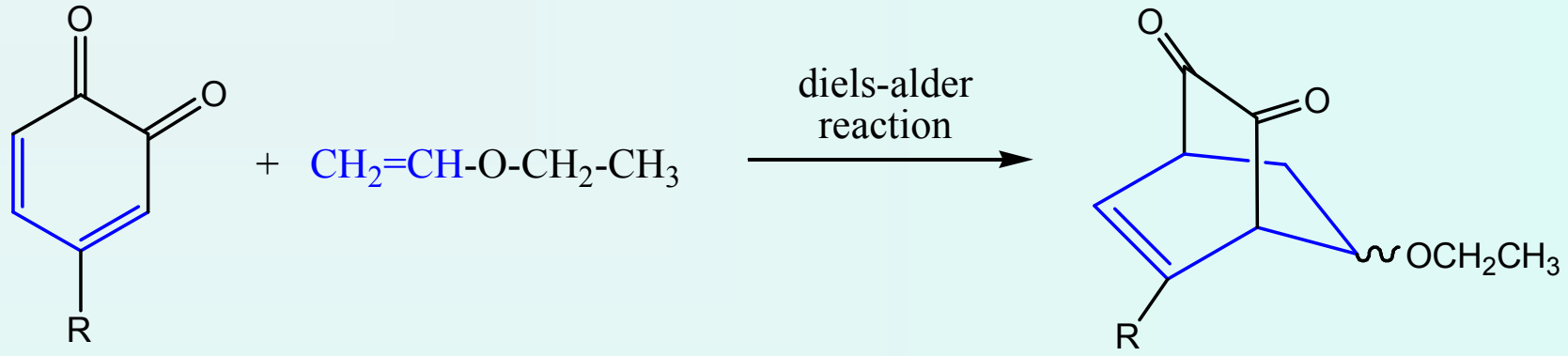
1) Oxidation of phenols

Enzyme polyphenoloxidase catalyzes oxidation of substituted phenols with oxygen to form catechols and ortho-quinones

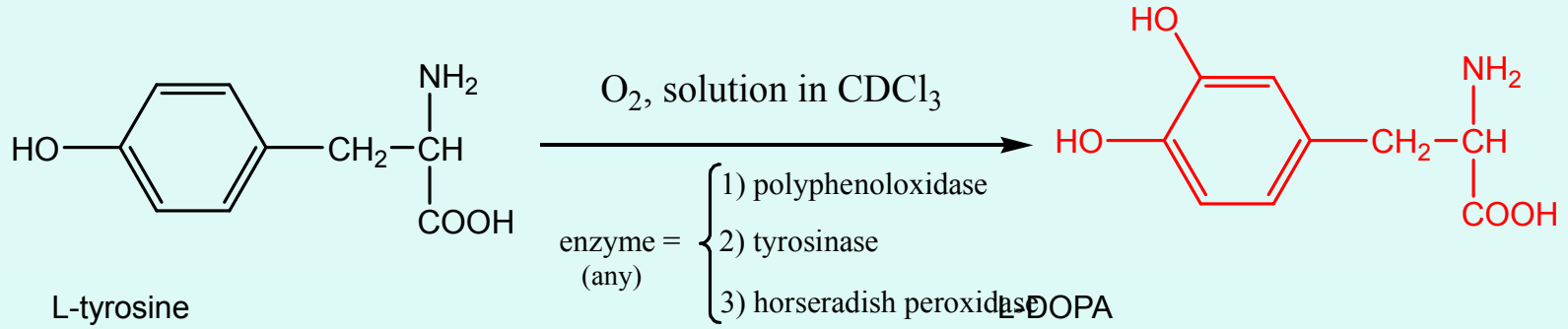


In water solution ortho-quinones are unstable and can be easily polymerized to form dark-colored resins.

To prevent polymerization traps-dienophiles can be used:

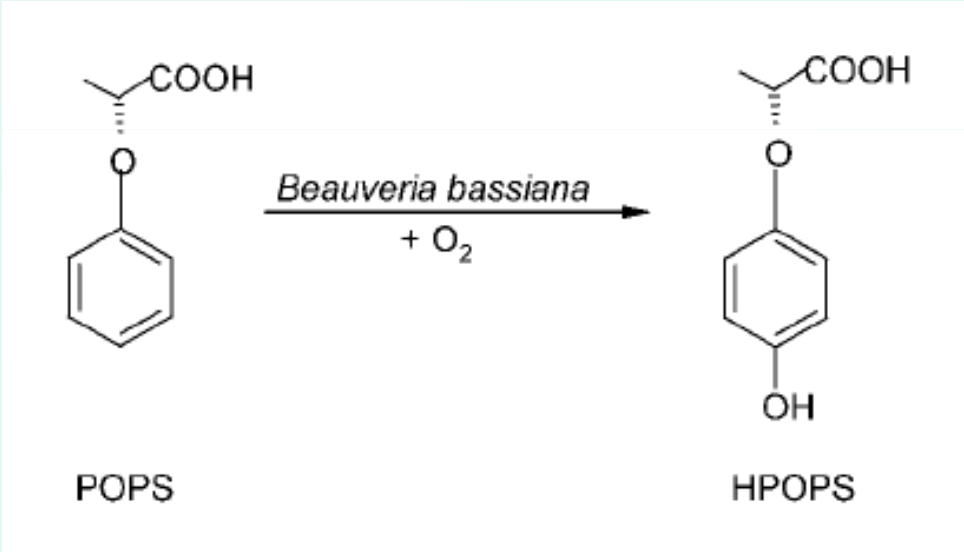


But more important is using of organic solvents where ortho-quinones undergo polymerization with lower rate and it is possible to transform them back to catechols using ascorbic acid as a reducing agent



4-Hydroxyphenoxypropionic Acid as herbicide intermediate

The hydroxylation of aromatics serves as an example for a sussessfull industrial production of intermediates in a technical scale. BASF Ludwigshafen produces isomerically pure (*R*)-2-(4-hydroxyphenoxy)-propionic acid (HPOPS) from (*R*)-2-phenoxypropionic acid (POPS) in a 100 m_3 fermenter for use as a herbicide intermediate:



Selectivity >99%, *ee* > 98%

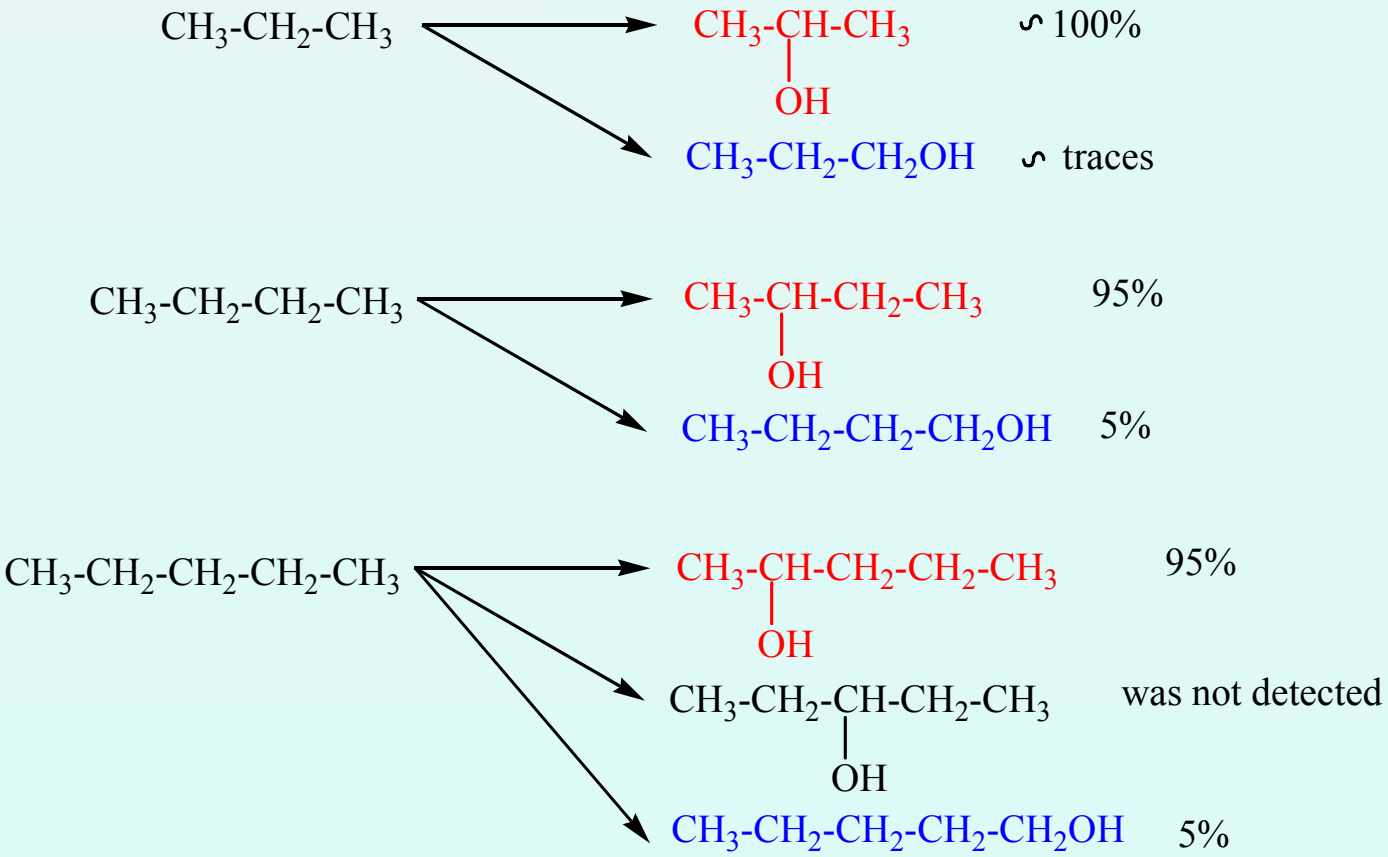
Today this hydroxylation of aromatics runs on a scale of >100 t/a.

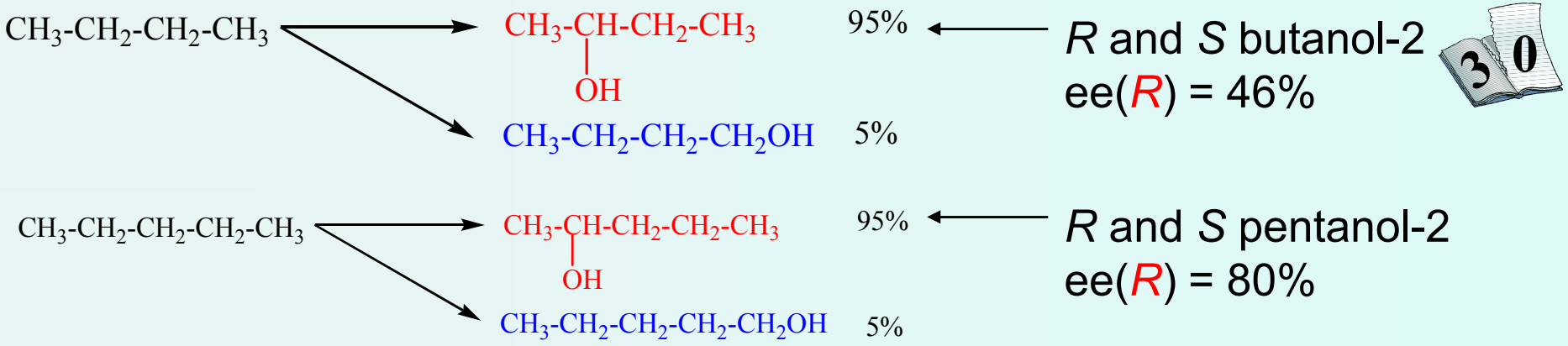
2) Oxidation of alkanes

Enzyme particulate methane monooxygenase (pMMO) catalyzes oxidation of alkanes (up to pentane) with oxygen to form alcohols:

$$CH_4 + NADH + H^+ + 1/2O_2 \longrightarrow CH_3OH + NAD^+ + H_2O$$

Only linear alkanes undergo oxidation with pMMO and predominatingly secondary alcohols with OH group at C2 carbon can be obtained:





What should be the structure of the enzyme active center to explain the following features of this reaction:

-Substrate selectivity (not all the alkanes are reactive, but only up to pentane)
-Regioselectivity (only C2 position)
-Enantioselectivity (only *R*)

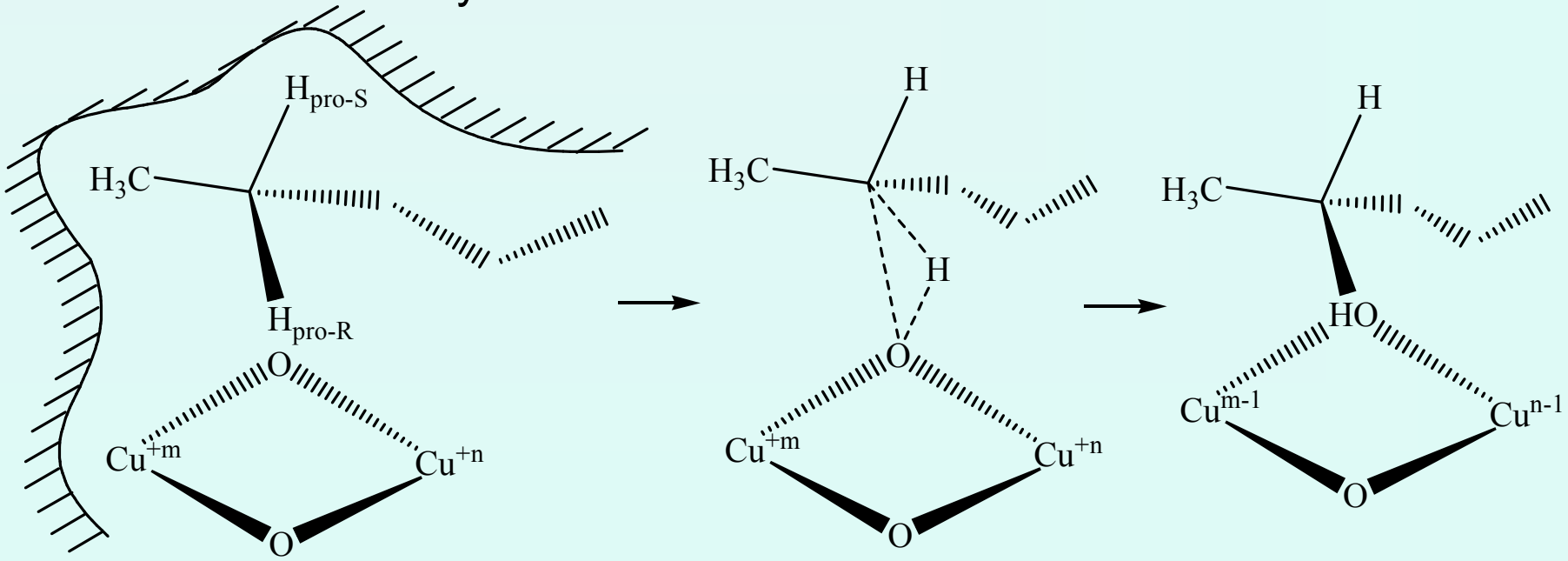
1. Restriction on the size of the alkane molecule means that catalyst's active center is not close to the surface and that it has relatively small size.

If active center is near the surface, may be alkane molecule does not need to penetrate entirely into the pocket of active center (only with 1-2 carbon atoms)

If the active center has enough size to dispose C6, C7 and higher alkenes it would be possible to detect at least traces of products.

2. Very small amount of C1 hydroxylation products and absence of C3 hydroxylation (in the case of pentane) means that inside the active center there is a small hydrophobic pocket, where only CH_3 group could be disposed (in this case H-atoms at C2 are oriented towards the catalytic center).

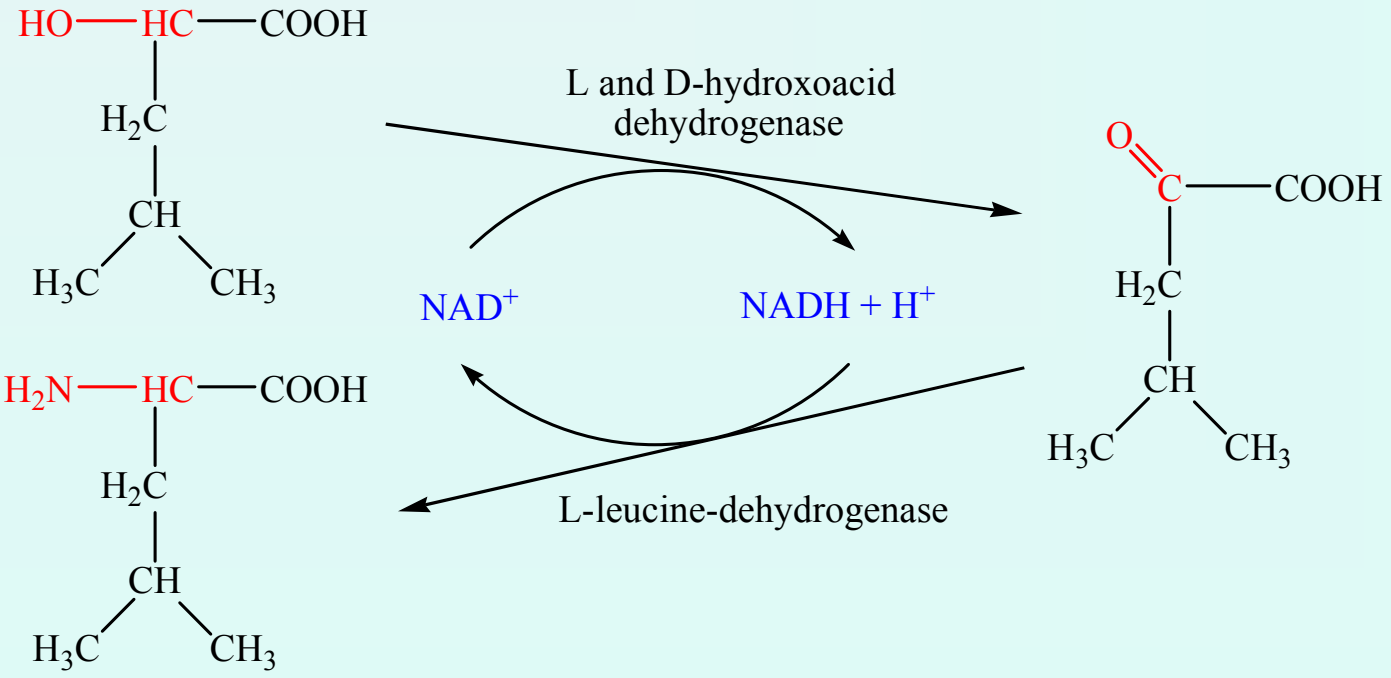
3. Preferential formation of *R*-isomer means that there is such kind of hydrophobic pocket and active center disposition that pro-*R* H-atom is directed towards catalytic center.



3. Conjugation of oxidation and reduction

New trends in application of enzyme catalysis in organic synthesis is using of cascade reactions – “one-pot” synthesis where the product of the first reaction can be used without separation as the starting material for the second reaction

Good example is a transformation of α-oxy carboxylic acids to α-amino acids:

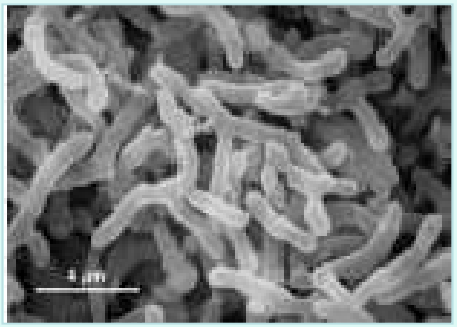


Very important that co-enzyme *self-regenerates* in this cascade process.

Application of lyases in organic synthesis

- **cleavage/formation of C-C, C-O, and C-N** or other bonds by other means than by hydrolysis or oxidation
- C-C bond formation - pivotal reactions in organic synthesis
- **regio- and enantiospecific** (100% yield and 100% e.e. possible)
- redox neutral reactions - **no cofactor regeneration**
- many lyases containing prosthetic groups - need **cofactor for activity**

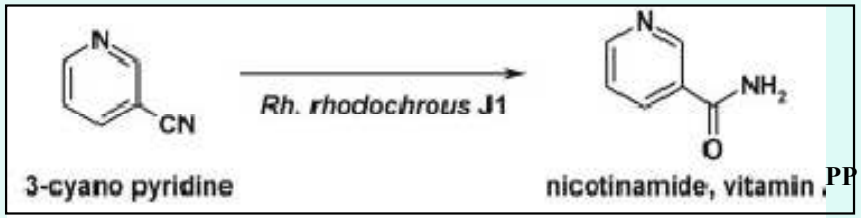
1. Nitrilehydratase



applications based on nitrilehydratase from *Rhodococcus rhodochrous* commercialized

SYNTHESIS OF AMIDES

Rh. rhodochrous J1 accepts aromatic and arylaliphatic nitriles for example synthesis of nicotinamide (Lonza):

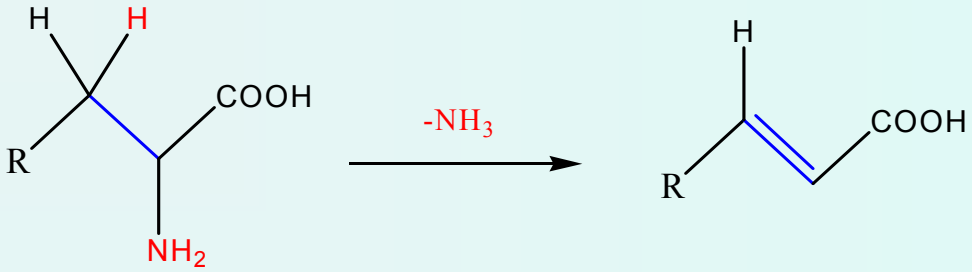


Large-scale production of commodity chemical acrylamide (Mitsubishi, Nitto Chemicals) and other amides (e.g., acetamide, isobutyramide, crotonamide, methacrylamide,)

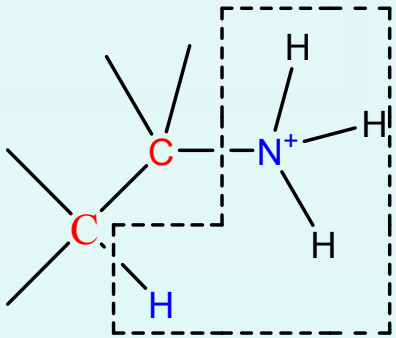
- simpler than chemical process (Cu catalyst)
- mild conditions (5°C), no polymerisation inhibitor needed
- high selectivity, productivity and higher product quality

2. Histidine ammonia lyase (HAL)

HAL releases ammonia or amino compounds with formation of double bond or ring. This process is an important step of metabolism.



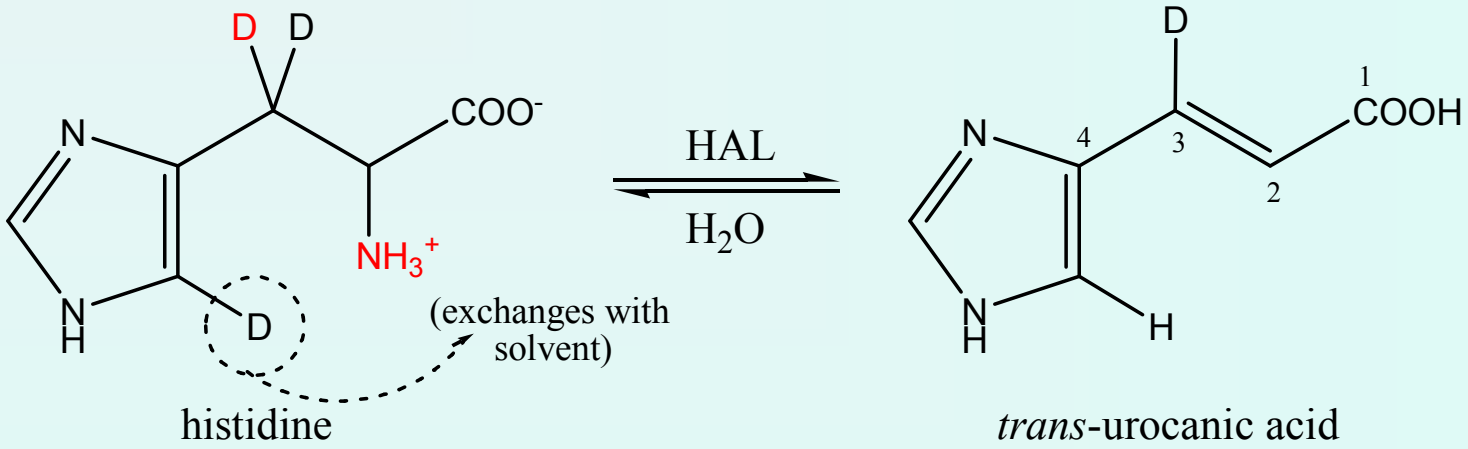
In general, NH_3 elimination is quite a difficult problem for the traditional organic synthesis



Addition of base eliminates H^+ from NH_3^+. Further NH_2- elimination – unfavorable process. This problem could be solved with methylation of amino group to $N(CH_3)_3^+$, which is a good “leaving” group in the Hoffmann elimination.

If not to use permethylation, the problem of β-atom H acidity increasing should be solved in some way (without H^+ elimination from NH_3^+).

HAL can be used to solve this problem. HAL catalyzes histidine conversion to the trans-urocanic acid:



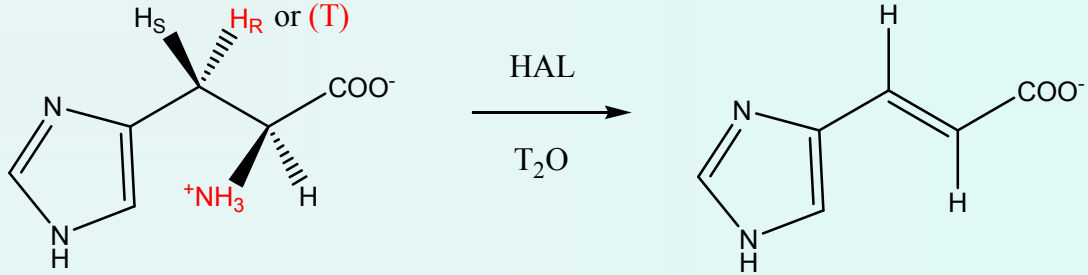
histidine — *trans*-urocanic acid

The reversibility of this reaction was confirmed with the labeled-atoms experiments:

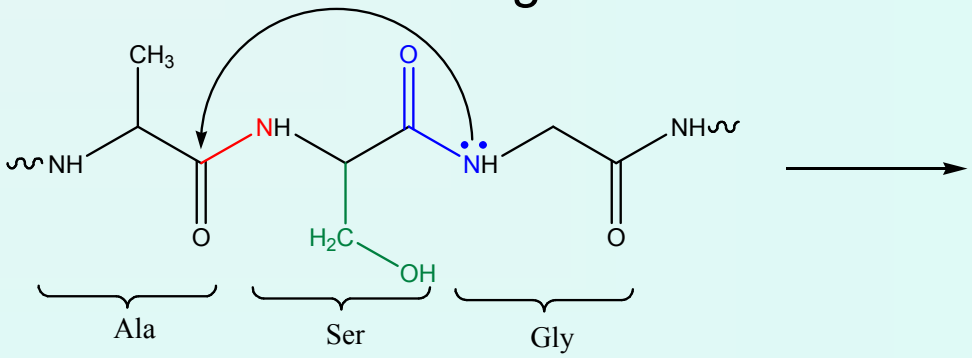
1) When [2-^{14}C] urocanic acid and histidine were stayed in the presence of HAL, [^{14}C]-histidine was detected

2) When HAL was stayed with NH_3 and urocanic acid, histidine was detected

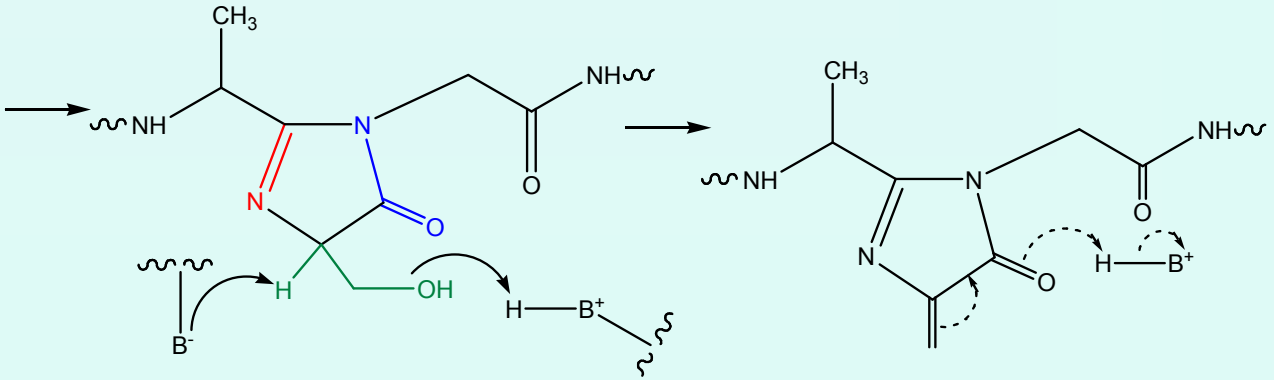
Interesting information gave the experiment where the reaction took place in T_2O. It was found that T-label could be detected only at the pro-R hydrogen position. In the molecule of urocanic acid T-label was not found (even so it is a reversible transformation):

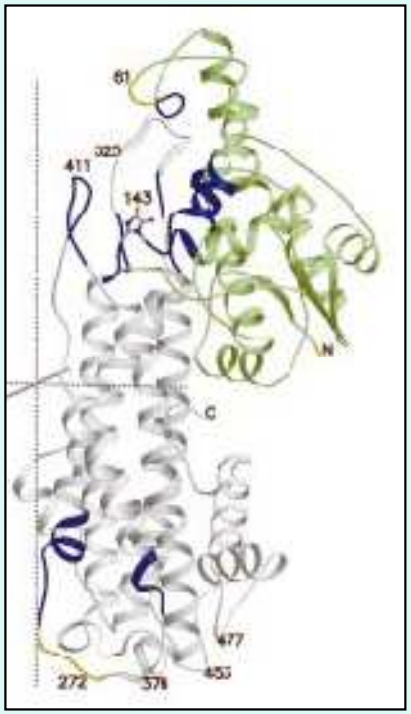


It means that H and NH_3^+ are eliminated according to the *anti*-elimination type



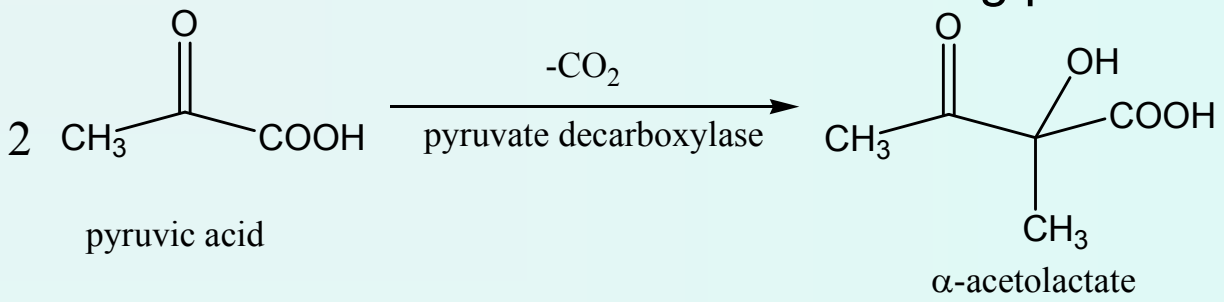
The structure of the HAL active center:




61
325
411
143
N
C
272
378
477
453

3. Decarboxylases

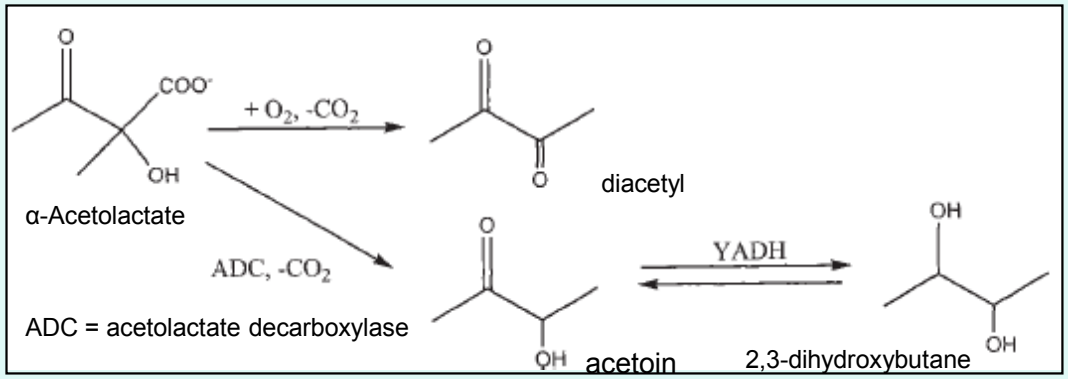
Pyruvate supplies the body with pyruvic acid, a natural compound that plays important roles in the manufacture and use of energy. Pyruvate is not an essential nutrient, since human's body makes all it needs. But it can be found in food: beer and red wine contain about 75 mg per serving.



pyruvic acid

α-acetolactate

α-Acetolactate can be oxidatively decarboxylated by oxygen to diacetyl:



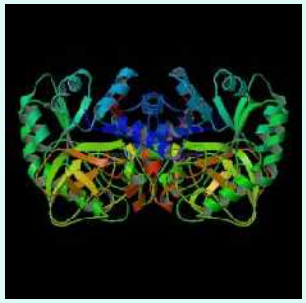
In brewery processes, the bad taste of diacetyl spoils the beer. By addition of ADC acetolactate is first decarboxylated to acetoin and then reduced to innocuous 2,3-dihydroxybutane by yeast alcohol dehydrogenase (YADH).

Application of isomerases in organic synthesis

Main application - interconversion of isomers - geometric or structural changes within a molecule (e.g., epimerases, racemases, cis-trans isomerases, tautomerases)

1. Racemases and Epimerases

- catalyze inversion of stereocenters
- usually act on stereocenter adjecent to carbonyl functionality
- reversibly cleave C-H bond by lowering pK_a of hydrogen by stabilisation of resulting anion
- epimerase - convert one diastereomer selectively to another diastereomer
- racemases - convert enantiomer to racemate



- Alanine racemase

Peptide transformation

Natural peptides consist of L-amino acids. But it is known that some bioactive peptides have insertions of D-amino acids. For example, opioid peptides (short sequences of amino acids which mimic the effect of opiates in the brain): deltorphines, dermorphines, some neuroactive tetrapeptides of mollusc and frogs.

frog *Phyllomedusa*

snail *Achatina fulica*

mussel *Mytilus edulis*

Synthesis of peptides like that is possible by two pathways:

1) addition of D-amino acid to a peptide growing chain (usually inconvenient because most of enzymes can insert only L-amino acids.

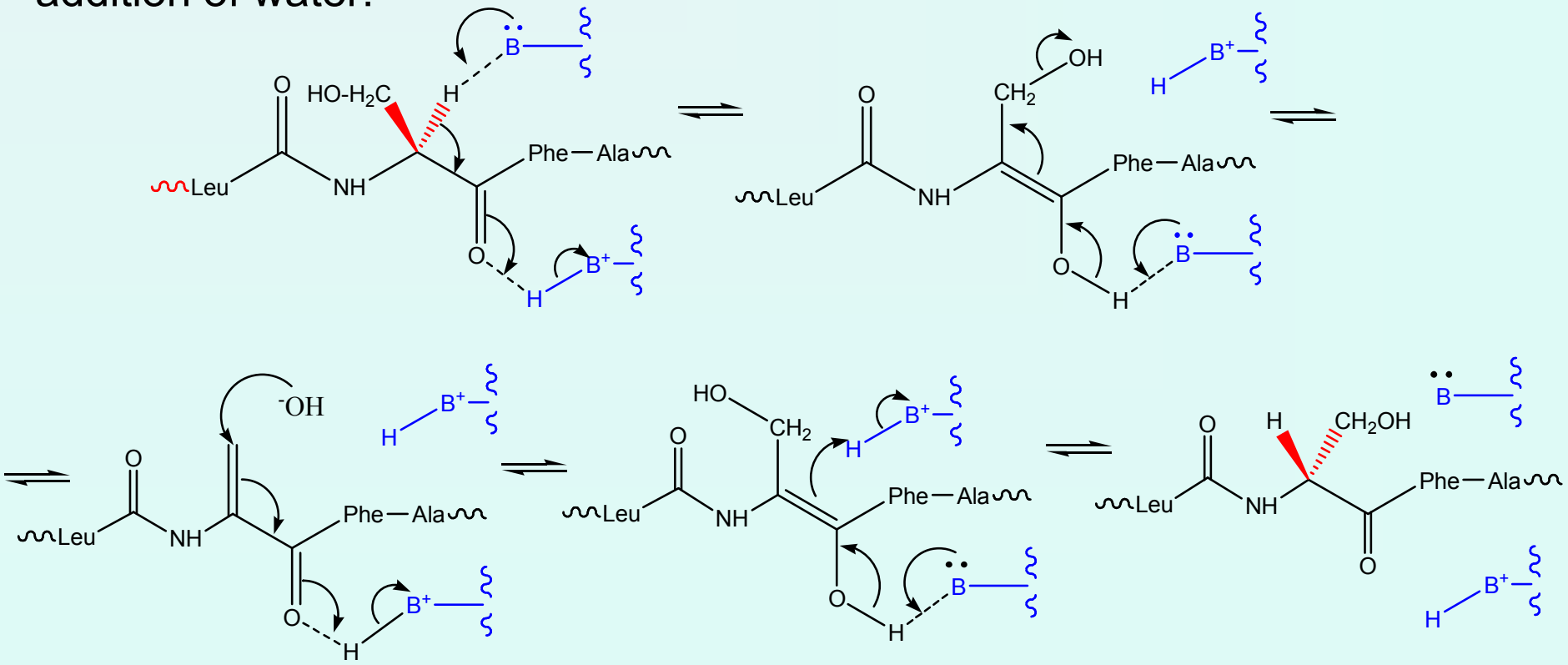
2) posttranslation epimerization – when enzyme is used to transfer L-amino acid fragment inside the chain to D-amino acid.

From spider *Agelenopsis aperta* toxin an enzyme which cause L→D amino acid transformation in the chain Leu-X-Phe-Ala was obtained

X is an amino acid which undergoes the transformation

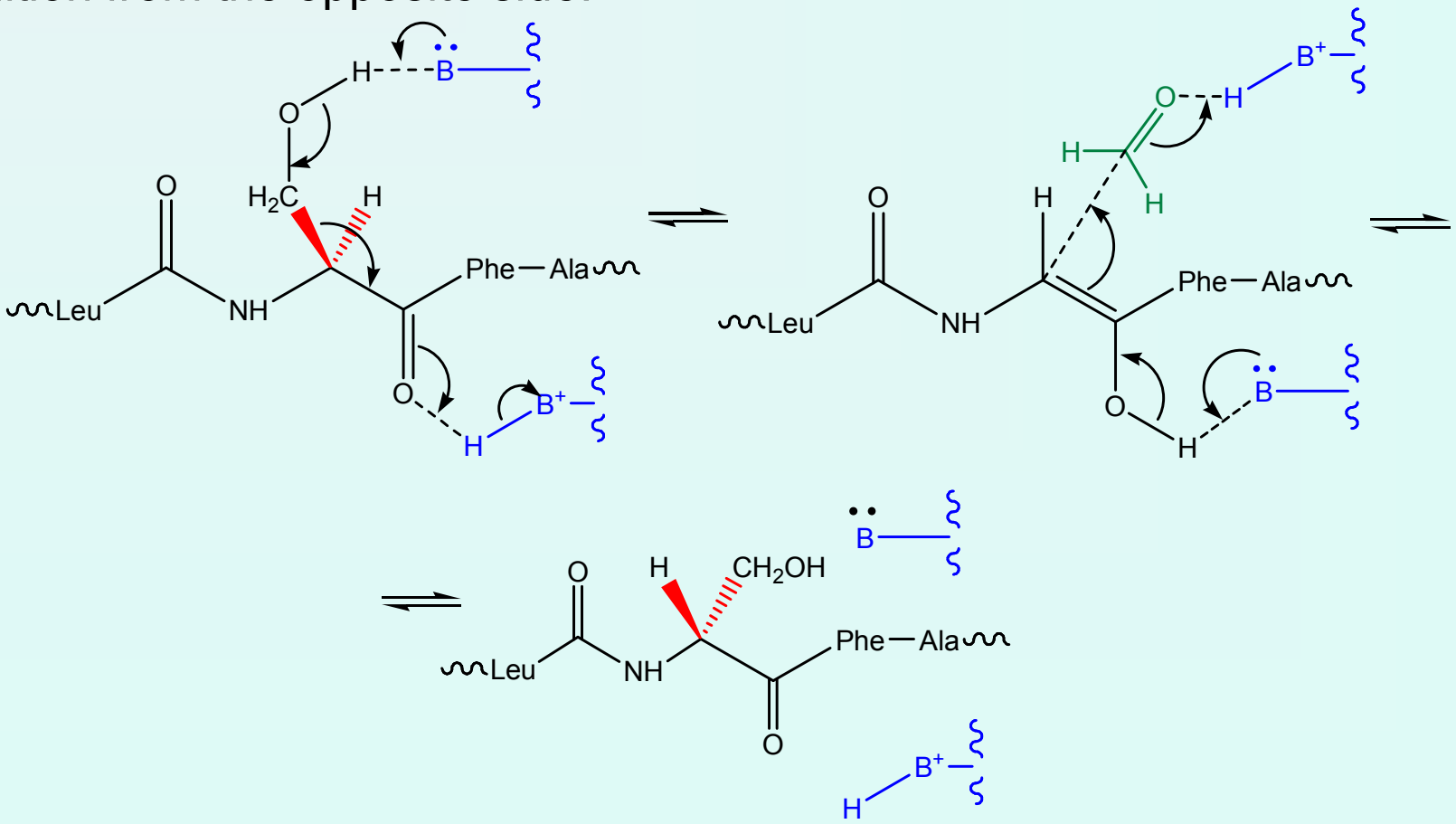
What is the mechanism of epimerization?

1) The first mechanism that was suggested is subsequent elimination and addition of water:



To check this mechanism the reaction was performed in H_2O^{18}. But the experiment showed that ^{18}O incorporation into the peptide molecule does not take place (the incorporation should take place at the step of OH^- addition to the C=C bond).

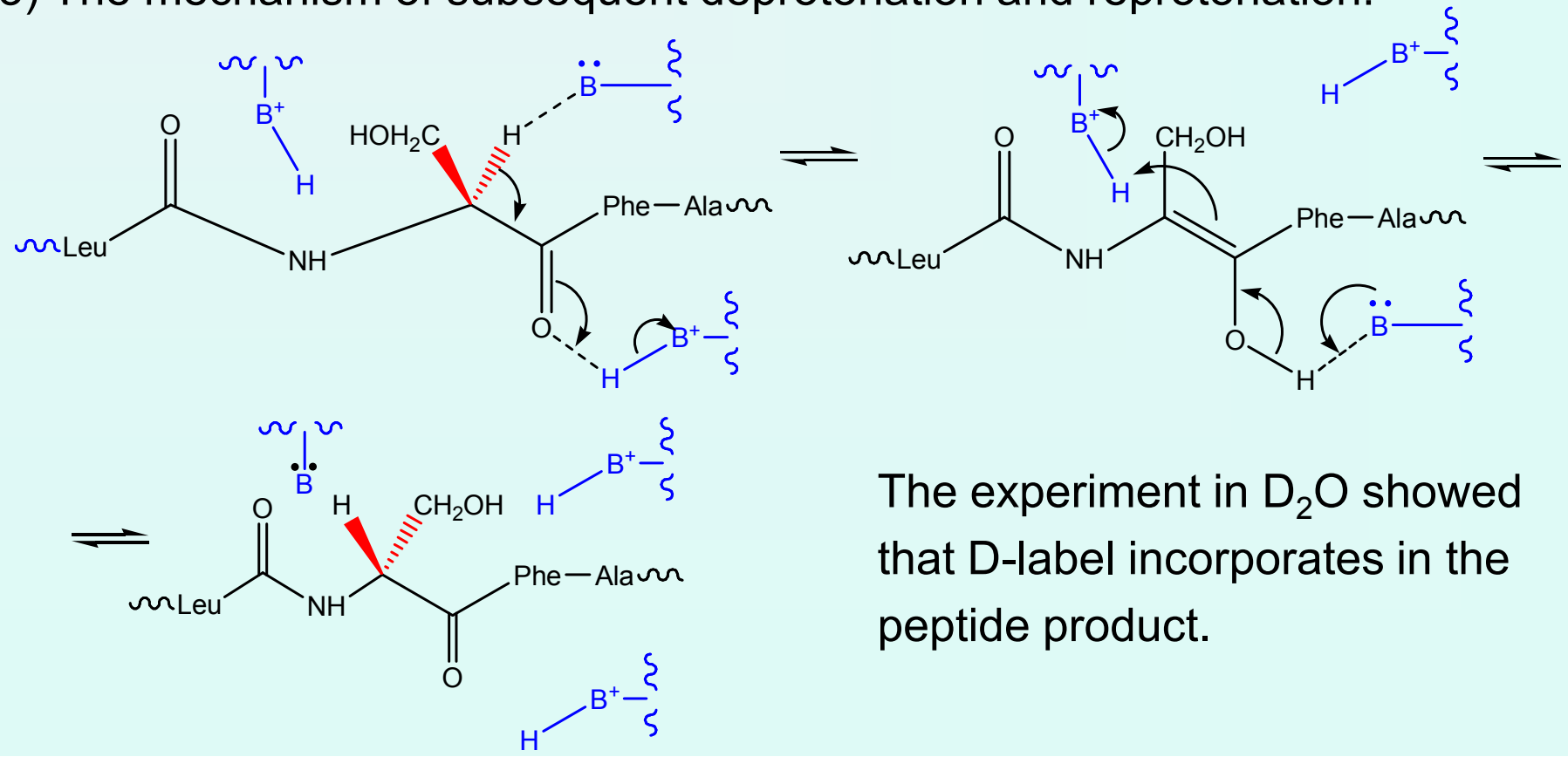
2) The second mechanism suggests formaldehyde formation and its addition from the opposite side:



To check this mechanism the reaction was performed in the presence of the formaldehyde trap - hydroxylamine. But the experiment showed that even at the NH_2OH concentration of 10 mmole/l the reaction takes place.

The most important evidence against these two mechanisms is that amino acids which do not contain OH-groups (like alanine, cysteine, O-methylserine) can also undergo the epimerization.

3) The mechanism of subsequent deprotonation and reprotonation:



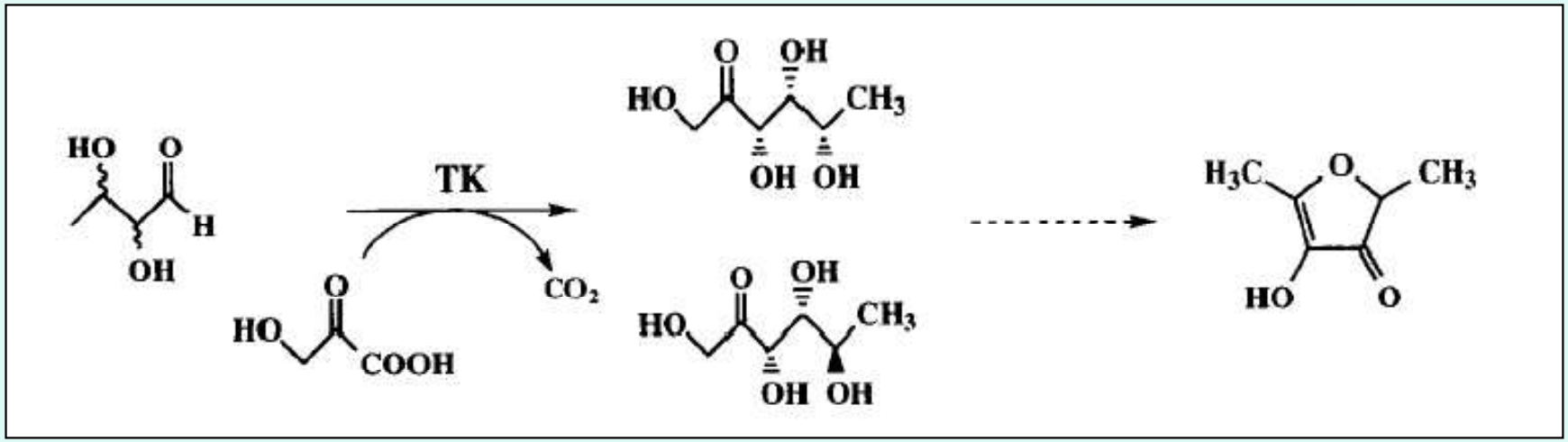
The experiment in D_2O showed that D-label incorporates in the peptide product.

Application of transferases in organic synthesis

Transketolase

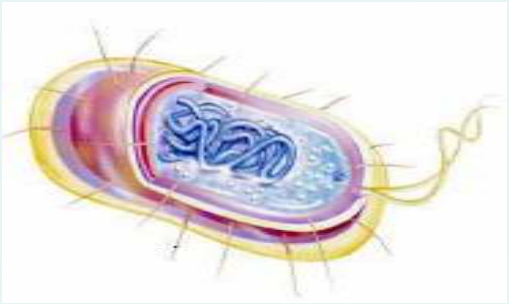
There is currently great interest in the use of biocatalysis for preparing flavor and fragrance components because of the desire to produce 'natural' molecules that can command a premium price as food additives.

Transketolase (TK) from spinach has been employed in a chemoenzymatic synthesis of 6-deoxy-L-sorbose, which is a known precursor of furaneol, a compound with caramel-like flavor



Whole cells vs. isolated enzyme

FERMENTATION



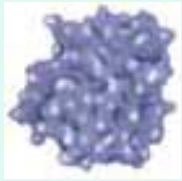
Advantages

- allow more enzymes and/or cosubstrate
- cheap

Disadvantages

- side-reactions from other enzymes
- low tolerance to organic solvents
- low productivity

ENZYMATIC PROCESSES



Advantages

- smaller reactors
- less contamination by other enzymes
- increased stability and life-time of immobilized enzyme
- higher productivity

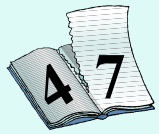
Disadvantages

- expensive
- addition of cofactors not economical
- not stable outside the cell

Advantages of Enzyme technology:

- very **efficient** catalysts
- high degree of **selectivity**
- **environmentaly friendly**
  sustainable
  fully biodegradable
  no toxic metals required
- operation at mild condition
  (0- 110°C, pH 2-12)
- catalyze **broad spectrum**
  of reactions
- **compatibility** with each other
- **less byproducts**
- **non-toxic, non-flamable**
- can be **reused** (immobilized)

Limitations of Enzyme technology:

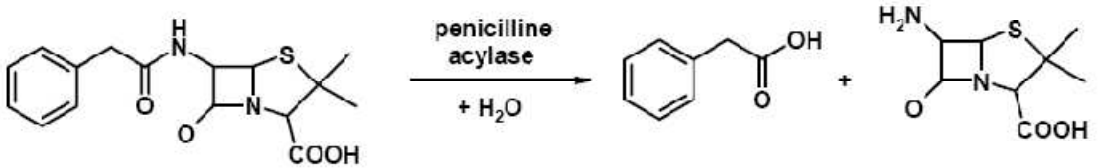


- **narrow** operation parameters
- highest **activity in water**
- coenzyme (**cofactor)** requirement
- prone to **inhibitions**
- **less stable**
- often **expensive**
- **allergies**

Enzyme price

To be applied in industry the price of enzyme should be 1-10% of the price of product.

Peniciline amidase: $1 / kg 6-Aminopenicillanic acid



penicillin G

6-amino penicillanic acid

Enzyme	Price (US$/kg)
Bacterial α-amylase	2.4
Amyloglucosidase	3.5
Glucose isomerase	11
Pullulanase	30
Lysozyme	60
Cellulase/hemicellulase	10
β-Glucanase	3
Fungal α-amylase	10
"Carbohydrase" for animal feeds	26

Enzyme stability

Usually to be applied in industry the half-life of enzyme should be at least 1-2 months.

Enzyme	Half-life reported (month)
Aspartase	24
Pen acylase	6
Fumarase	180
Transaminase	3
Glucose isomerase (60°C)	2
Proteases	2

Protein engineering and **directed evolution** techniques

- enhance stability of natural enzyme
- adapt it to the new operation conditions (e.g., non-aqueous environments)

Current use and future outlook

- most of enzyme processes introduced during **past 30 years** (>100)
- **1% of known 3,000 enzymes used** in large amounts
- **latest progress in research**
  - three-dimensional structure determination
  - detailed study of enzyme reaction meachanism
  - rational improvement of enzyme properties (e.g., stability, selectivity)
  - high throughput evolution techniques
- number of **enzyme processes expected to increase** further
  - optically pure fine chemicals and therapeutics
  - synthesis of antibiotics
  - paper production and recycling
  - selective glycosylation of peptide drugs
  - environmental technologies