Biocatalysis

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Enzyme Science and Engineering
BBL433

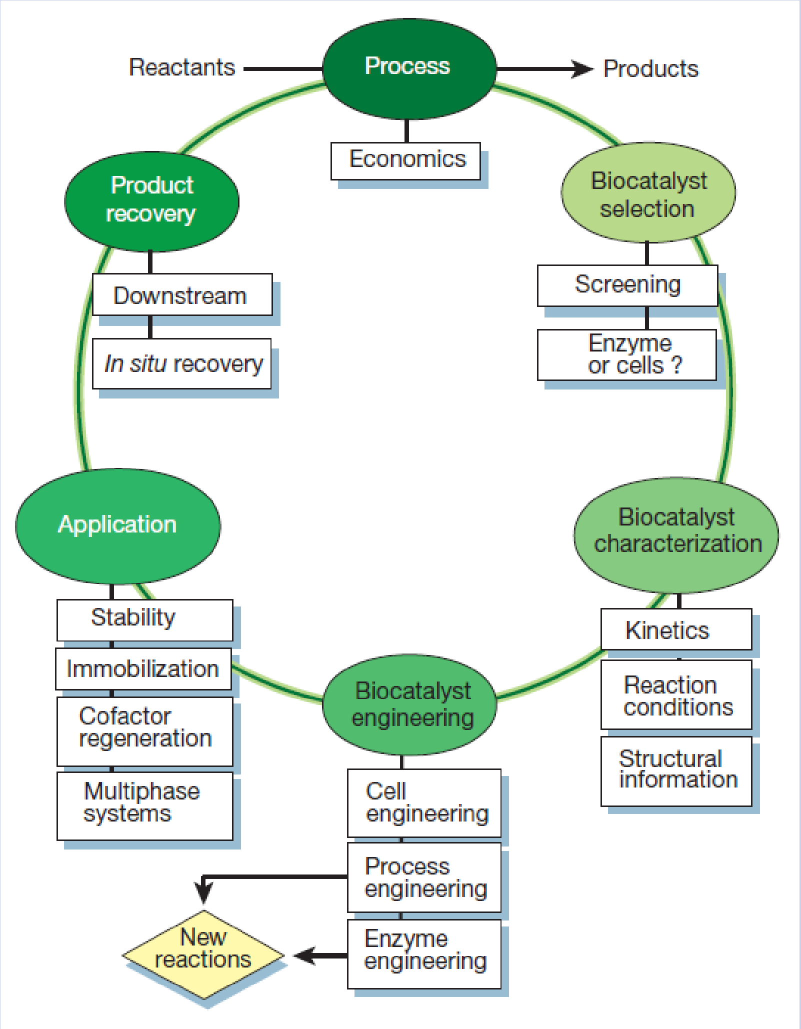


Figure 1 The biocatalysis cycle.

Applications

Enzyme for
- Fine chemicals

Bioethanol
Amino acids
Aspartame
High fructose syrup
Vitamin F/B12
Dextran

- Pharmaceutical compounds

E.g..,
L-Dopa
Cephalosporins etc

Advantages of Biocatalysis

- Enzymes have a very good selectivity :

  Stereoselectivity – enantioselectivity in most cases are > 99 %

  Chemo- and regioselectivity – react on one location over another similar group without the need for protection groups.
- Mild reaction conditions:

  aqueous solvent, room temperature, normal pressure, neutral pH
- Environmentally friendly: enzymes are biodegradable
- Fewer side reactions
- High efficiency
- Cheap and simple starting material can be used.

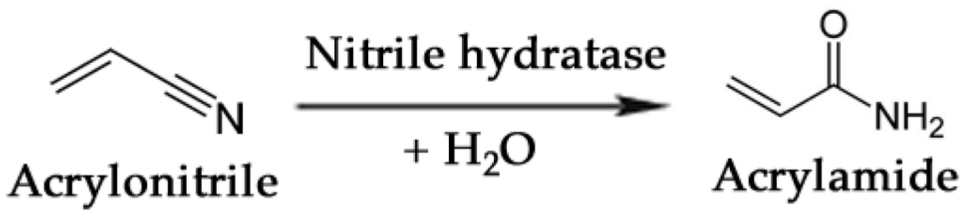
- Overall lower cost of production.

Table 1.1.1 Biocatalysis alignment with green chemistry.

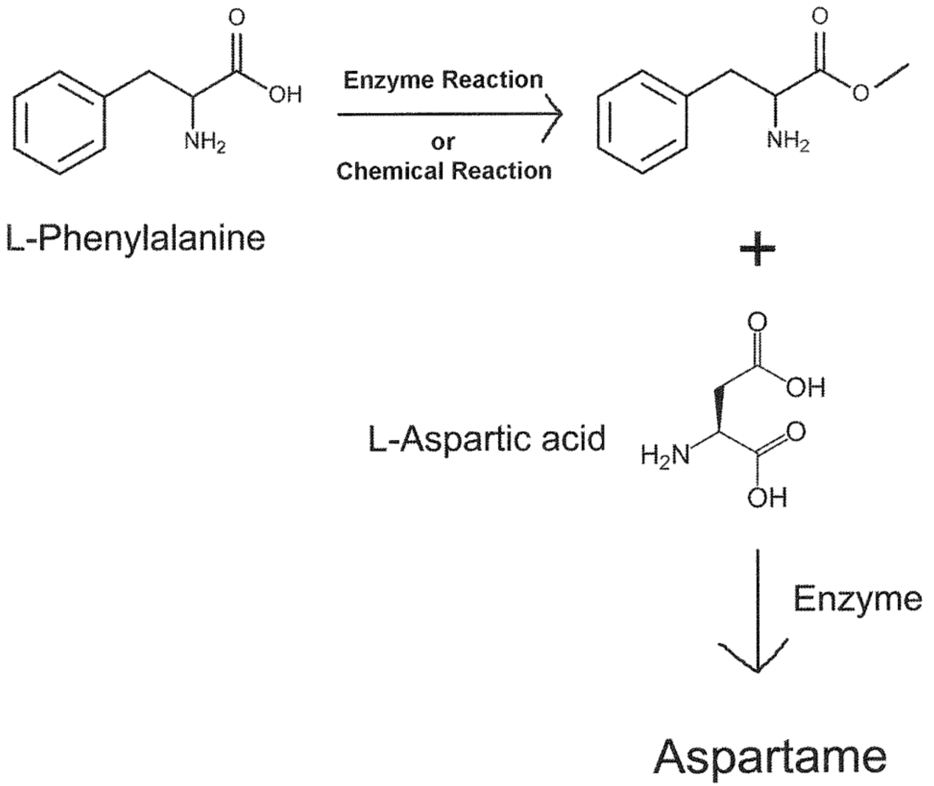
Green chemistry principle	Biocatalysis
1. Prevention (of waste)	Biocatalysis can enable new, more sustainable routes to APIs effectively reducing level of waste.
2. Atom economy	Biocatalysis often enables more efficient synthetic routes.
3. Less hazardous (less toxic reagents and intermediates) chemical syntheses	Generally low toxicity.
4. Designing safer (less toxic) chemicals	No impact.
5. Safer solvents and auxiliaries	Often performed in water; when solvents are used they are generally Class I or II.
6. Design for energy efficiency	Usually performed slightly above room temperature.
7. Use of renewable feedstocks	Biocatalysts are renewable.
8. Reduce derivatives (e.g., protecting groups)	Chemo-, regio-, enantio-selective nature of enzymes often obviates need for protecting groups.
9. Catalysis (preferred over stoichiometric reagents)	Catalytic.
10. Design for degradation (avoid environmental build-up)	No impact on design of products (although biocatalysts themselves are degradable in the environment).
11. Real-time analysis for pollution (and hazard) prevention	No impact.
12. Inherently safer chemistry for accident prevention	Biocatalysis is generally performed under mild conditions where risk of explosions/ run-away reactions is minimal.

Biocatalysis..recap

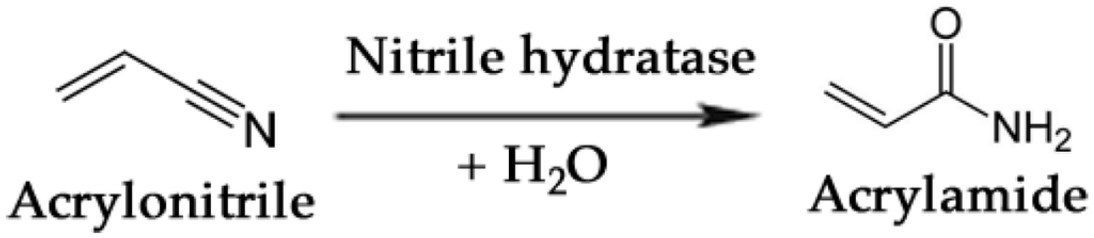
Case study 1: Production of acrylamide



Case study 2: Production of aspartame using Thermolysin



Case study 1: Production of acrylamide



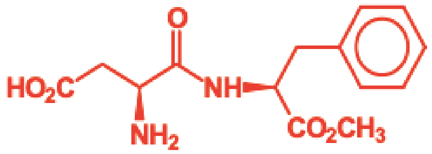
Nitrile hydratase from Rhodococcus is used with whole cell immbolization in polyacrylamide
3 % w/v substrate is used in a continuous reactor
1% w/v or 50000 U/L of enzyme used
Conversion of upto 0.02 w/v
4000 tones per year using enzymatic synthesis

Whole cell biocatalysis Vs Free Enzyme

Advantage/Disadvantage

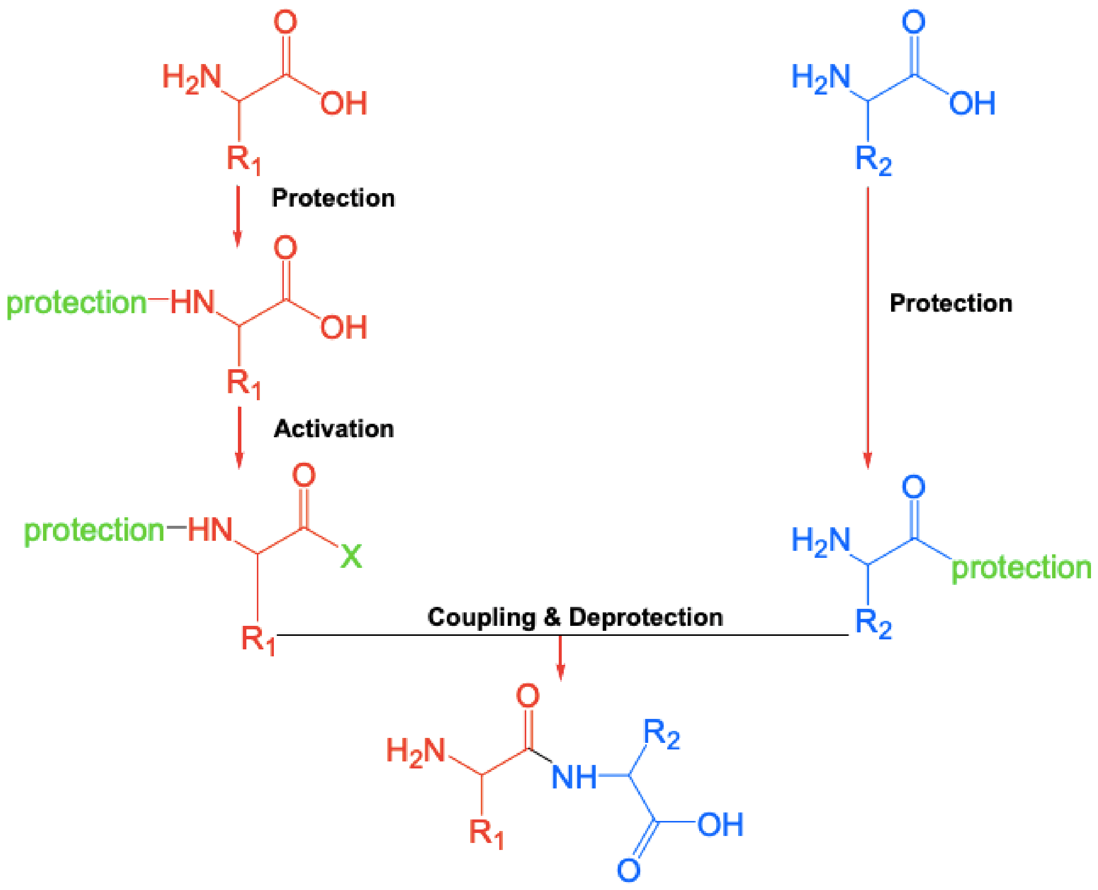
very low amidase activity which otherwise would produce unwanted acrylic acid from the acrylamide.

Case study 2: Production of aspartame



Aspartame (200)

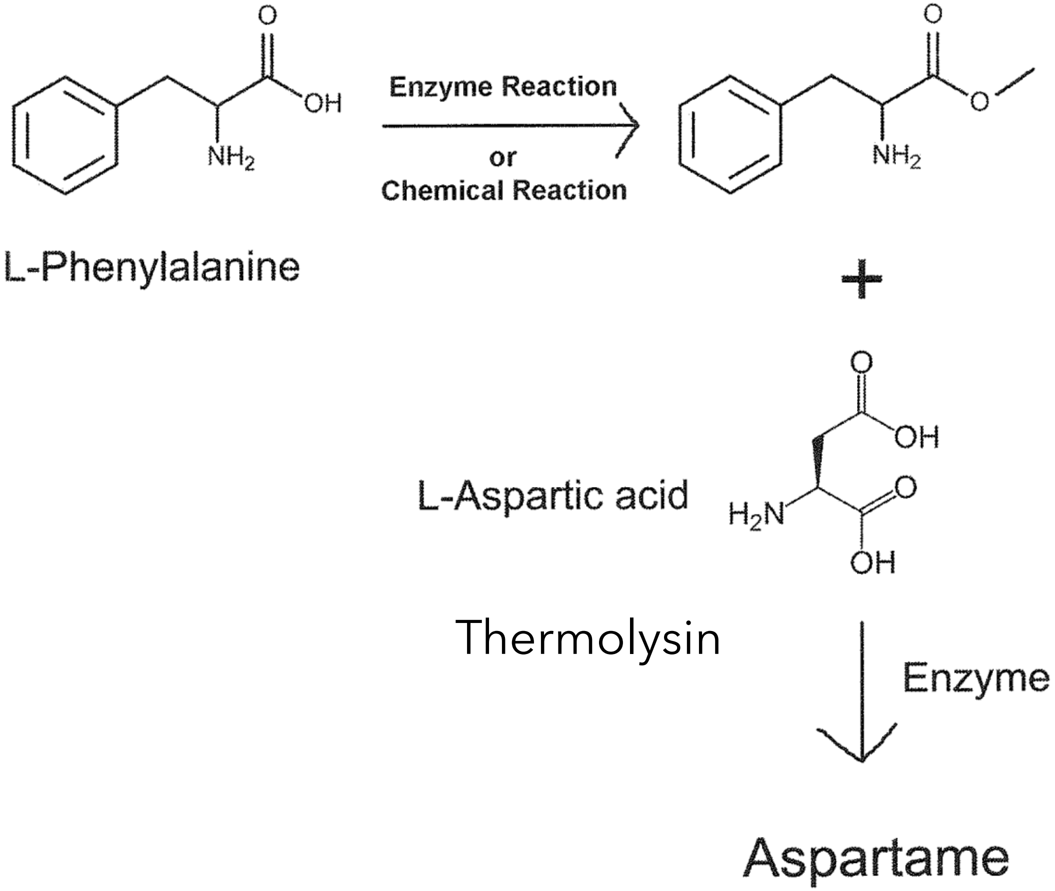
dipeptide of the natural amino acids L-aspartic acid an L-phenylalanine

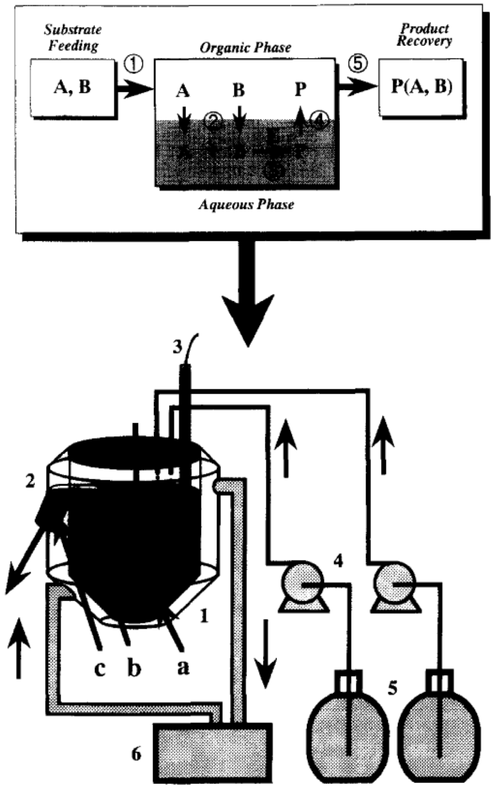


Chemical synthesis leads to production of β-derivative, Which is bitter in taste

Very poor yield

Case study 2: Production of aspartame using Thermolysin

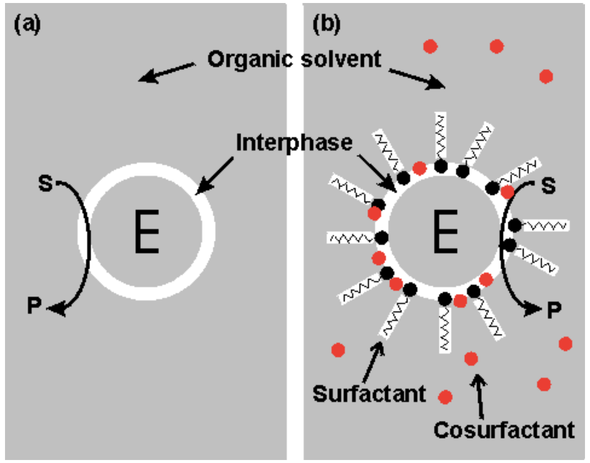


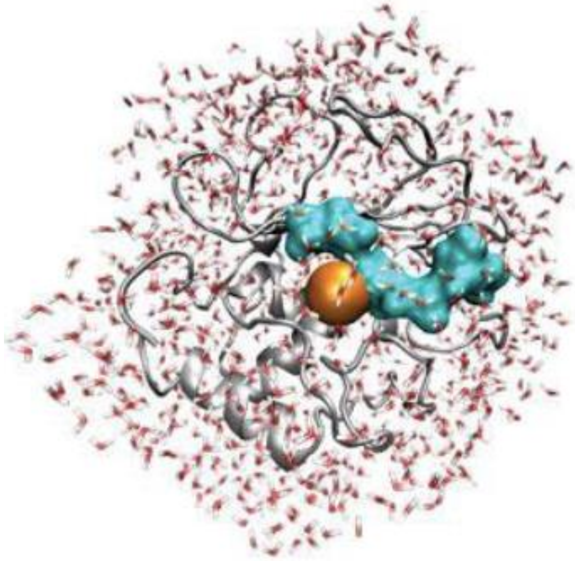


Non aqueous enzymology

Very low water!

Completely in organic solvent

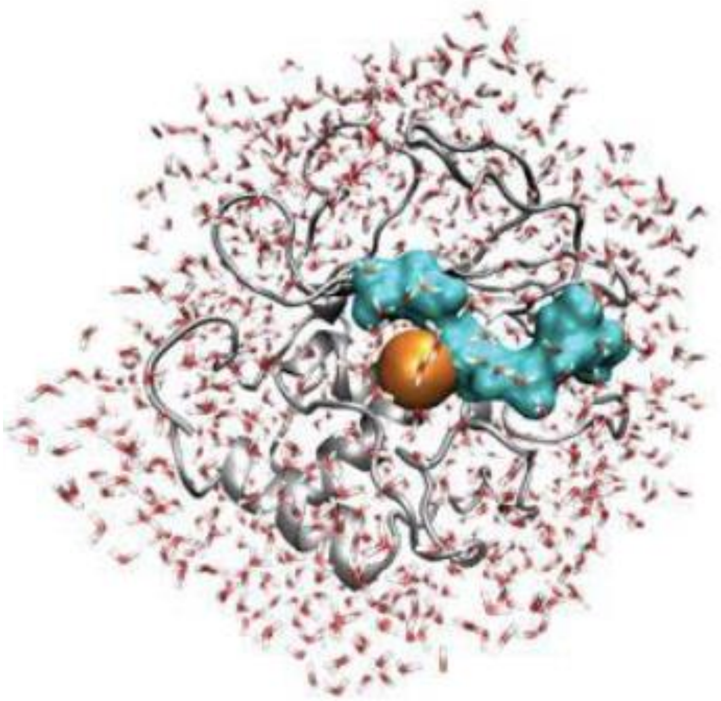




E.g.,

1. Monophasic organic media
2. Biphasic organic media (reverse micelles etc, lyophilized enzymes)
3. Ionic liquid/super ciritical liquids

Can enzyme work in the absence of H_20 ?



E.g., Alpha chymotrypsin requires 50 molecules of water per enzyme to remain catalytically active

Some enzymes require more water molecules e.g., Poluphenol oxidase require 3.5×10^7 water molecules

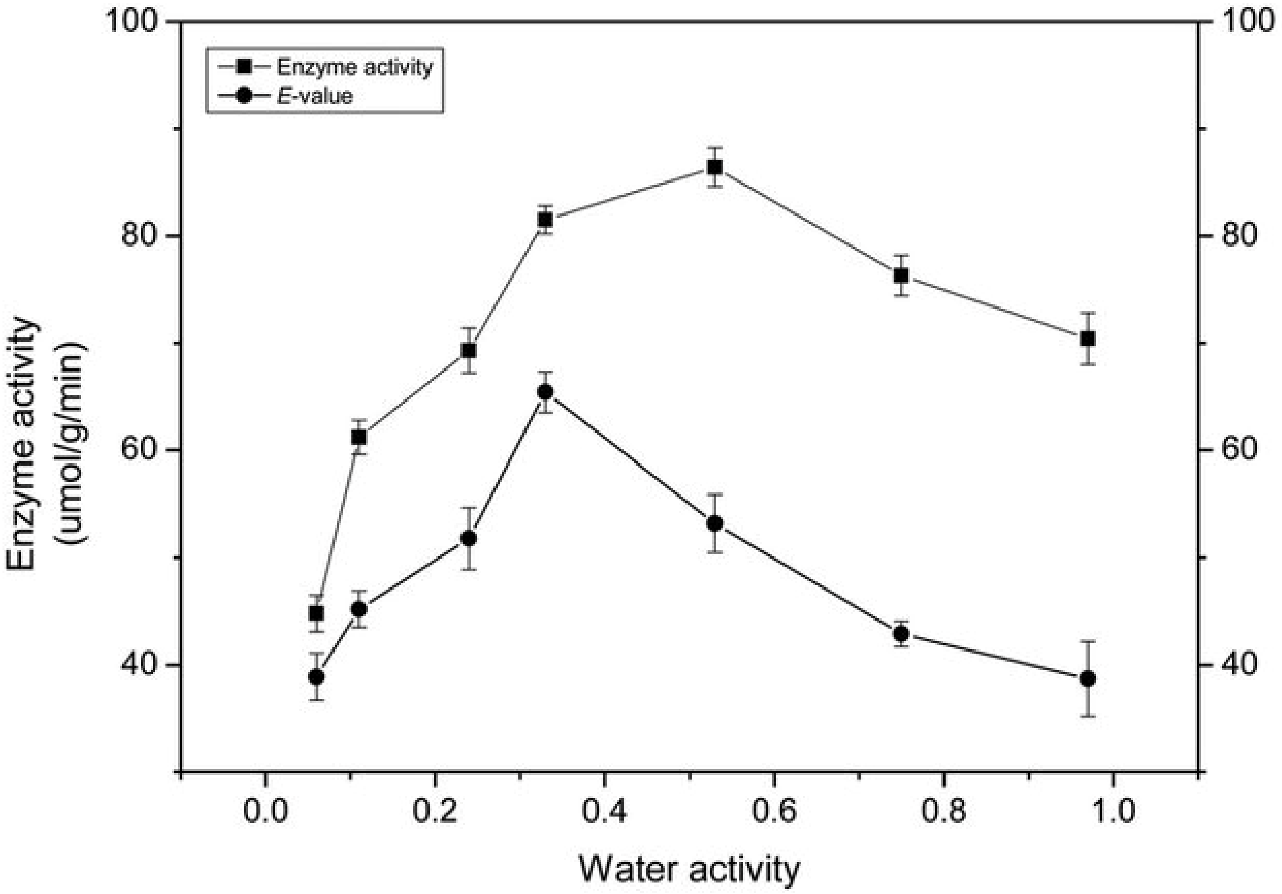
Mobility of enzyme side chains are reduced, increasing stiffness!

Advantages of enzyme reaction in organic solvent

1. **Increased solubility of non-polar substrates**
2. **Shifting thermodynamic equilibrium to favor synthesis over hydrolysis**
3. **Suppression of water dependent side reactions (e.g. hydrolysis of acid anhydrides and halides)**
4. **Alteration in substrate specificity**
5. **Immobilization by simple adsorption to macro-porous hydrophobic supports**
6. **Ease of recovery of the enzyme**
7. **Ease of product recovery from low b.p. solvents**
8. **Enhanced thermo-stability of enzymes**
9. **Elimination of microbial contamination**
10. **Use of enzymatic step directly in a multi-step chemical process**

Water activity

Water activity (***a**$_w$*) is the partial vapor pressure of water in a substance divided by the standard state partial vapor pressure of water.



Horseradish peroxidase catalyzed oxidation of p-anisidine in mono-phasic organic solvents

solvent	Reaction rate (μmol/mg E/min)
Toluene	60
Benzene	54
Hexane	75
Hexadecane	75
Cyclohexane	75
Chloroform	09
Ethyl acetate	45
Diethyl ether	105
Methanol	00

- Dioxane 00
- Dioxane +

  5% aq. buffer 08
- Dioxane +
- 15% aq. Buffer 33
- Dioxane +

  30% aq. Buffer 129

Properties of common organic solvents

Solvent											
	MTBE	Acetone	CH_3OH	THF	Hexane	DIPE	EA	C_2H_5OH	C_6H_{12}	Toluene	DMF
B.P. [^{0}C]	55.2	56.2	65.0	67.0	69.0	69.0	77.1	78.5	80.7	110.6	153
M[g/mol]	8.15	58.08	32.04	72.1	86.17	102.17	88.10	46.07	84.16	92.13	73.09
Ɛ[-]	4.5	20.7	32.63	7.58	1.89	3.23	6.02	24.3	2.02	2.38	36.7
Log P	1.15	-0.23	-0.76	0.49	3.5	1.9	0.68	-0.24	3.2	2.5	-1.0
µ[D]	1.23	2.70	1.71	1.74	0.0	1.24	1.83	1.4	0.0	0.30	3.24

Boiling point

Dielectric constant

Log P

Density

$$\text{Log } P = \log \{[A]_{water}/[A]_{n\text{-octanol}}$$

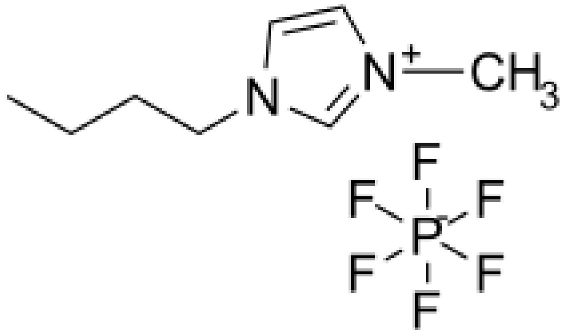
***n*-octanol is chosen as a reference because it mimics biological membranes with a hydrophobic tail and a hydrophilic head capable of hydrogen bonding**

Hexane has 0.001% solubility in water., Ethanol/Acetone has 100% solubility in water

Ionic liquids

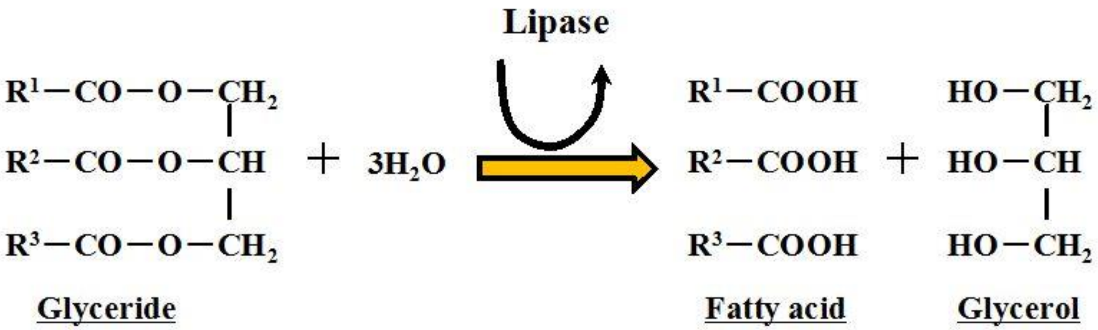
- Salts that do not crystalize at room temperature
- No vapour pressure
- Stable in room temperature
- Does not de-activate enzymes

E.g.,



The chemical structure of 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM]PF_6)

Lipase enzyme example



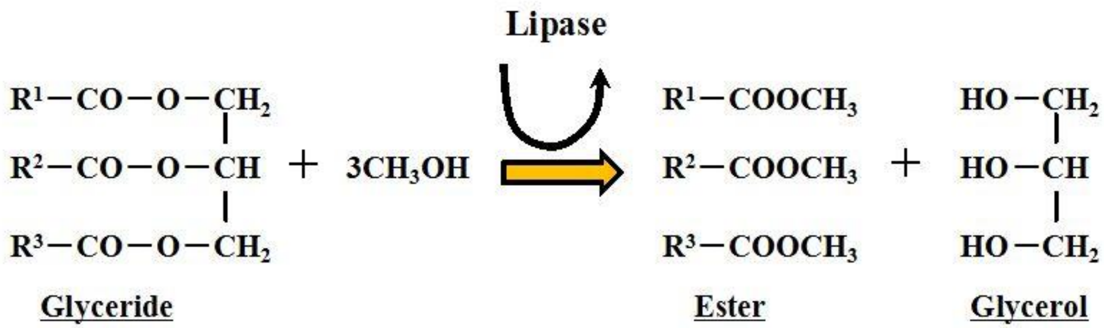
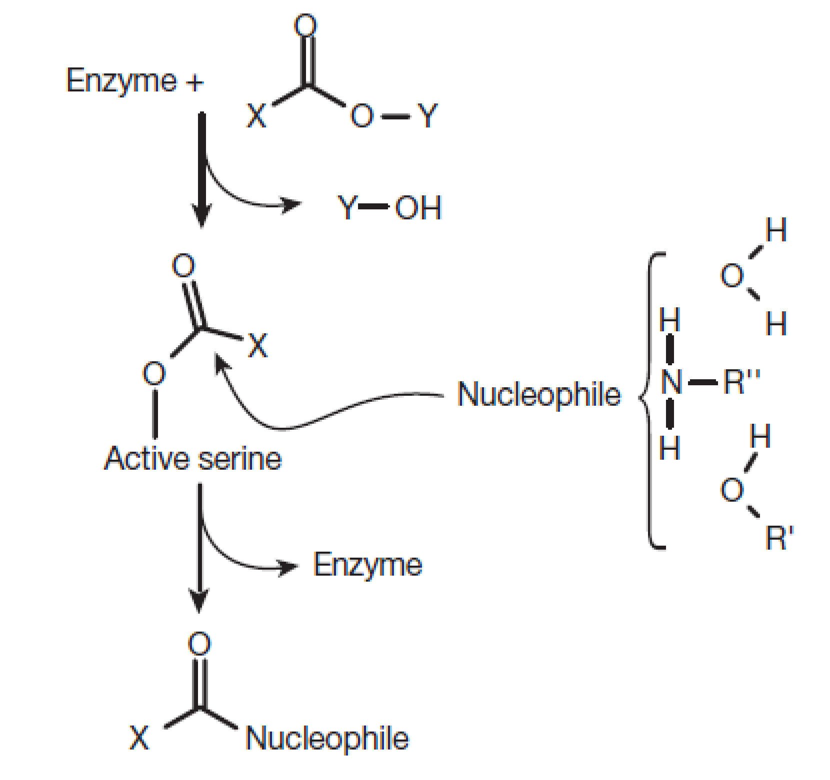
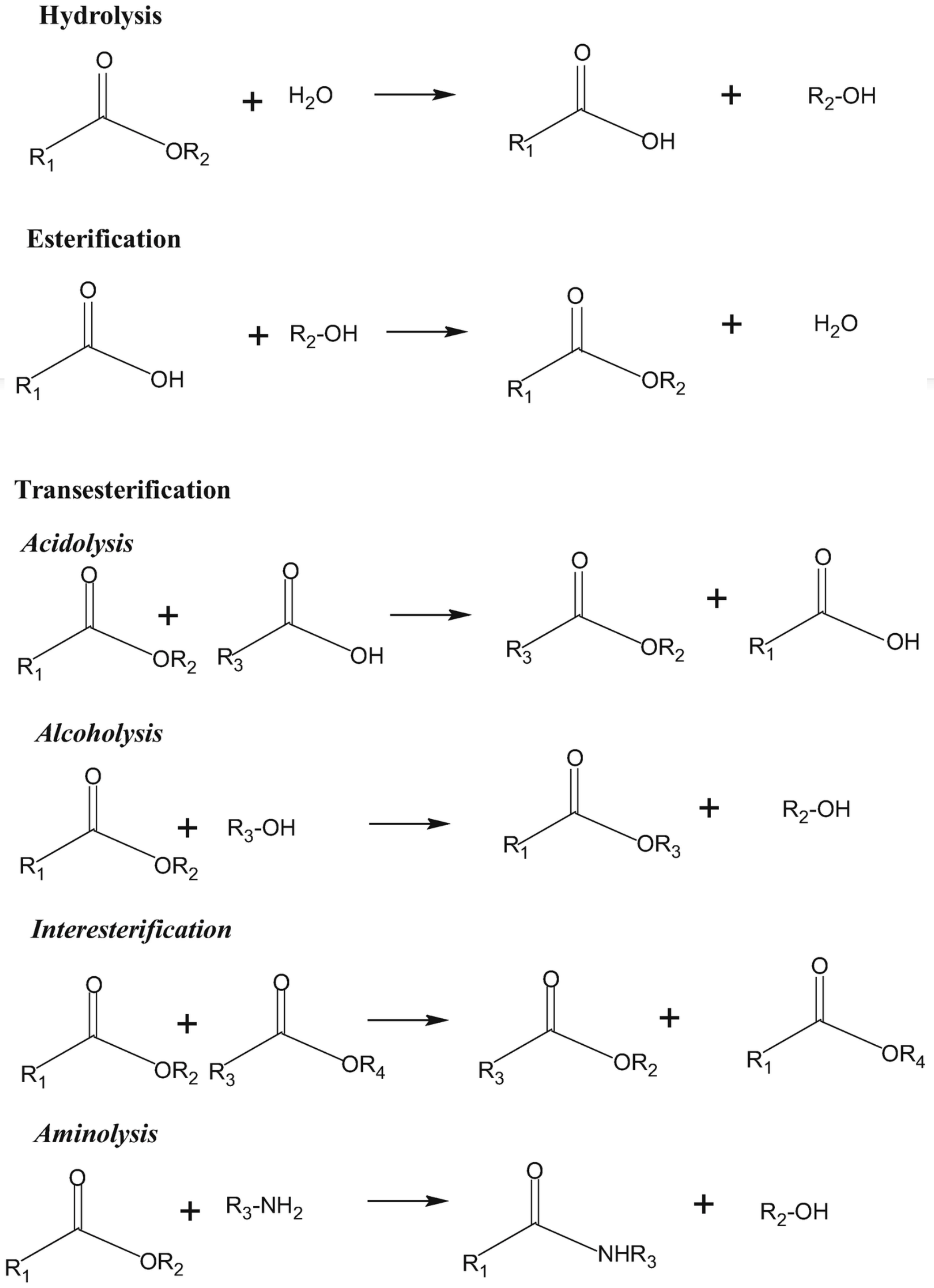


Figure 4 Reaction mechanism of lipase biocatalysis. The nucleophilic attack on carbonyl functionalities serves as target for reaction engineering approaches.

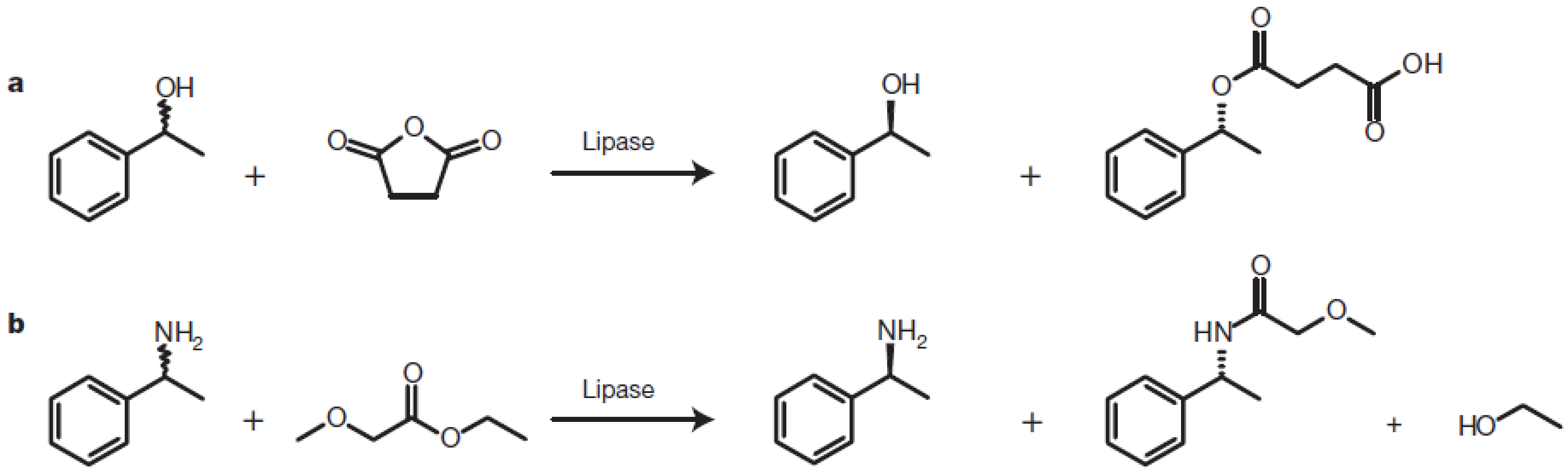


Applications of Lipase catalyzed trans/Inter-esterification reactions

- Cocoa Butter substitute
- Human milk fat substitute
- Structured lipids (MLM type)
- Low- calorie structured lipids (LML type)
- Biodiesel
- Monoglycerides

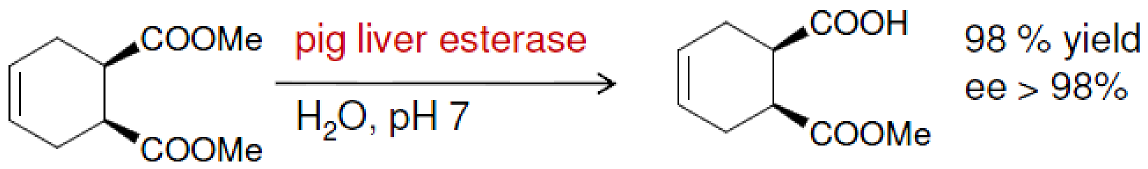


Kinetic resolution of alcohols/Amines

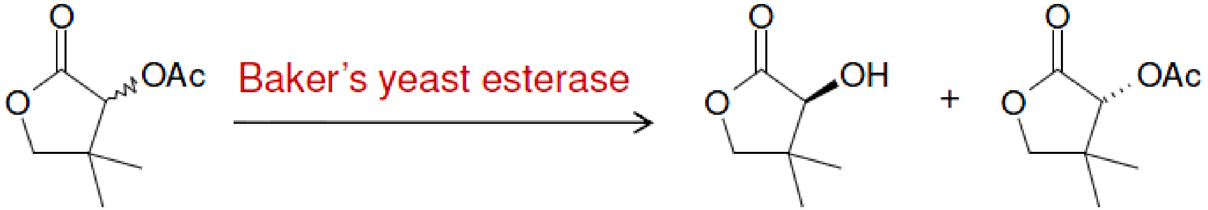


Biocatalysis in Organic Synthesis

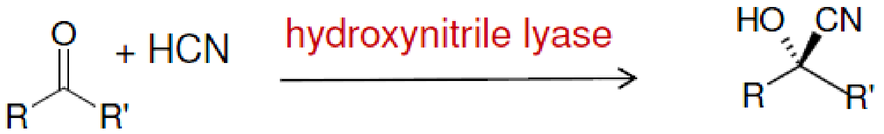
- There are lots of examples of application of enzymes outside living system to carry-out organic reactions.
  - Desymmetrization



  - Kinetic Resolution

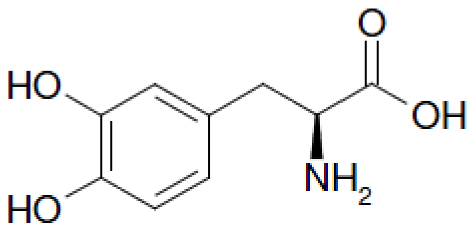


  - Asymmetric synthesis



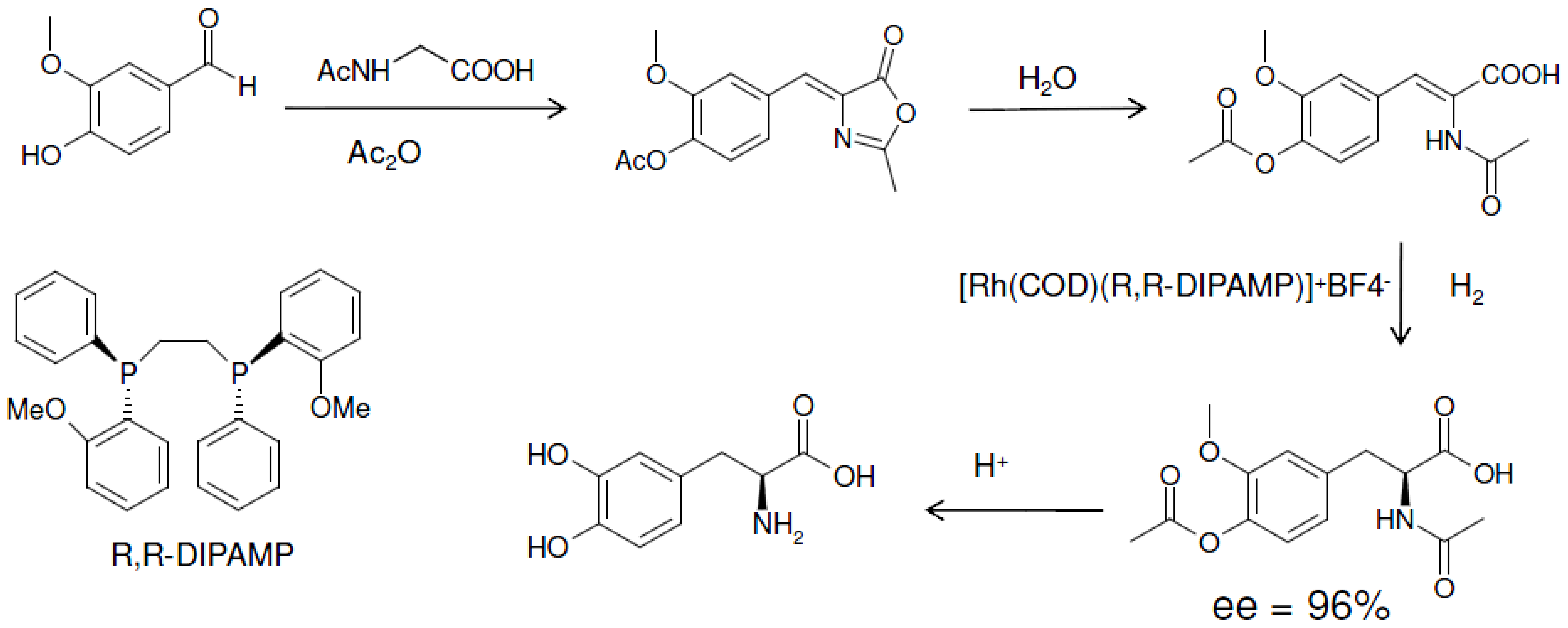
Faber, K. *Biotransformation in Organic Chemistry*. Springer, **2004**

L-Dopa



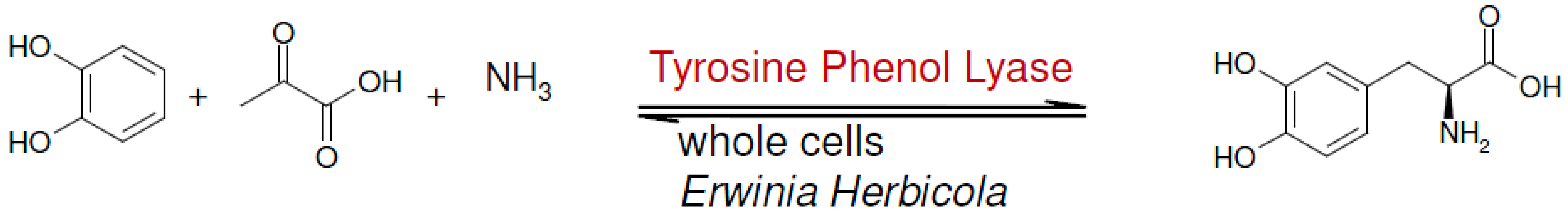
- Treatment for Parkinson's disease, which is caused by lack of Dopamine or its receptors in brain.
- L-Dopa is decarboxylated in vivo to become Dopamine.
- Since Dopamine cannot pass through blood brain barrier, L-Dopa is administered with dopadecarboxylase-inhibitors to maintain its form outside the brain.
- About 250 tons are produced per year.

L-Dopa Chemical Synthesis

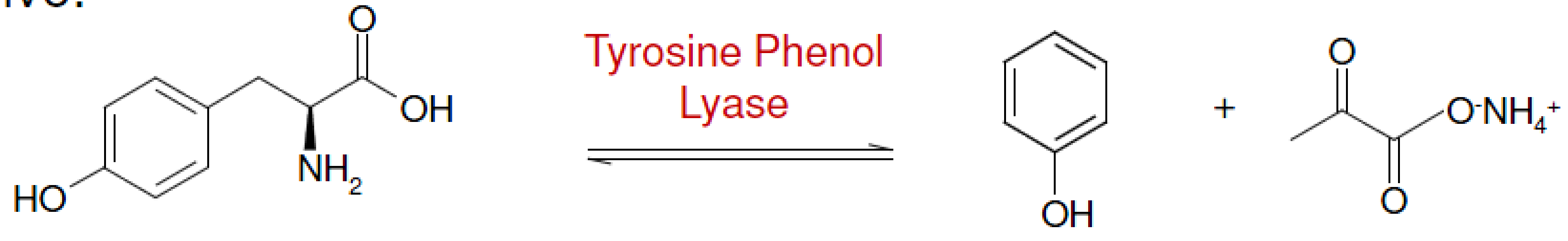


- The first use of asymmetric catalysis in pharmaceutical industry (1968)
- Developed by William Knowles, Noble Prize winner in Chemistry 2001 (together with Noyori and Sharpless) for asymmetric hydrogenation and oxidation reactions.

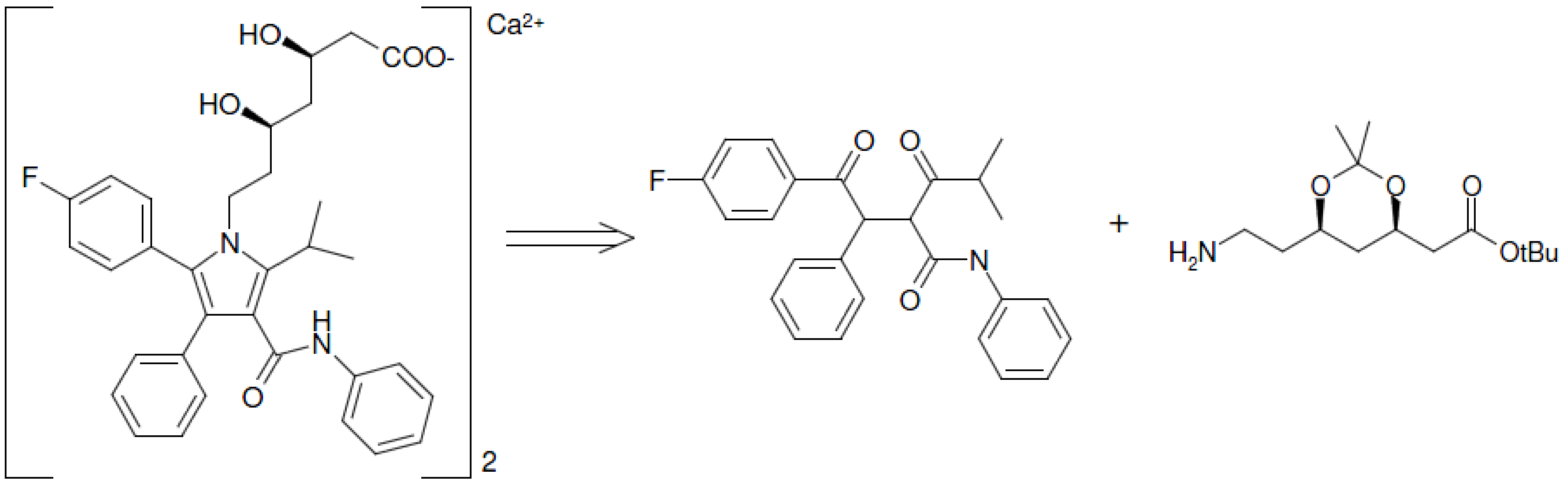
L-Dopa Enzymatic Synthesis



- The reaction are first used in 1993 by Ajinomoto Co. Ltd., Japan
- L-Dopa is insoluble in reaction medium and appears as crystalline precipitation in reaction.
- One of the most economical processes to date. Most of L-Dopa productions today are from this method.
- Tyrosine Phenol Lyase is enzyme that catalyzes breakdown of tyrosine in vivo.

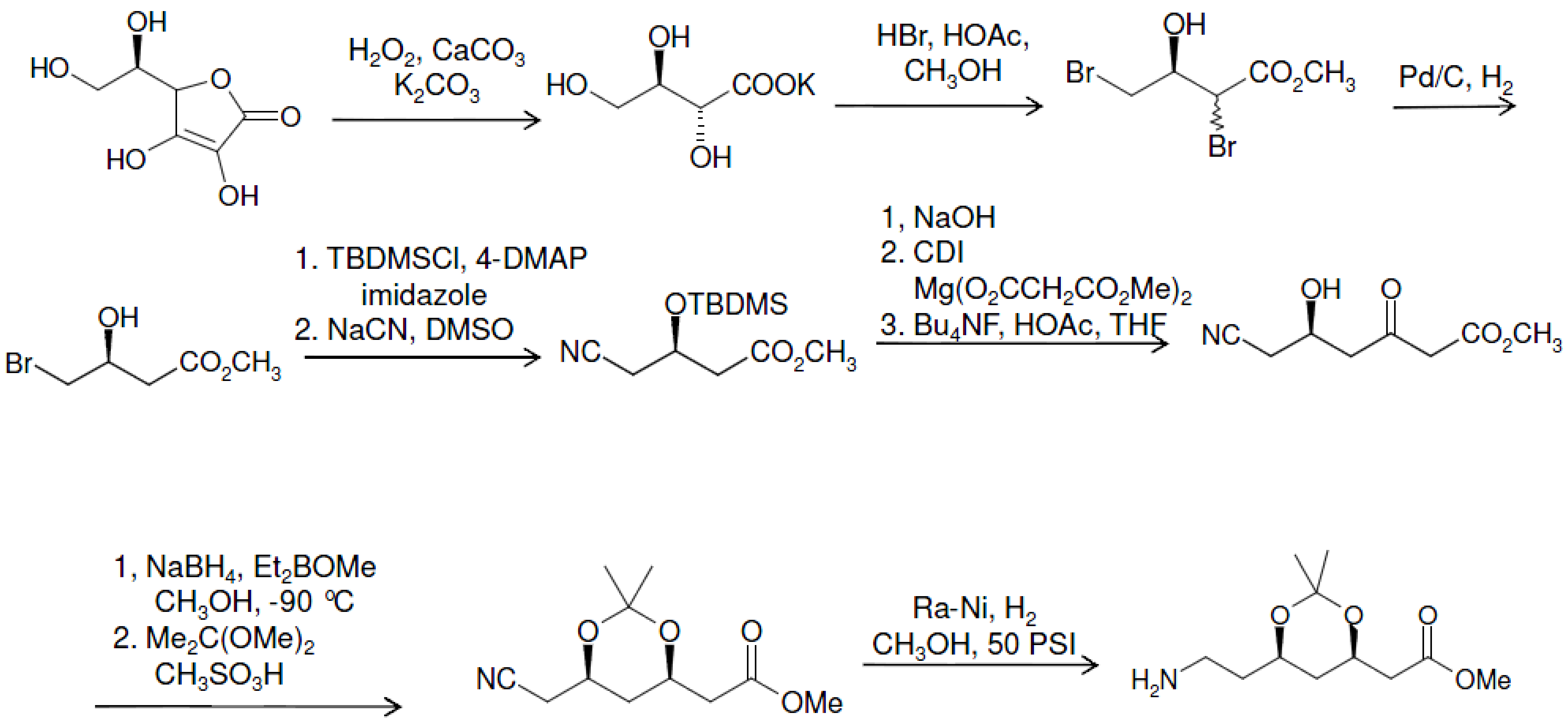


Atorvastatin Calcium (Lipitor)

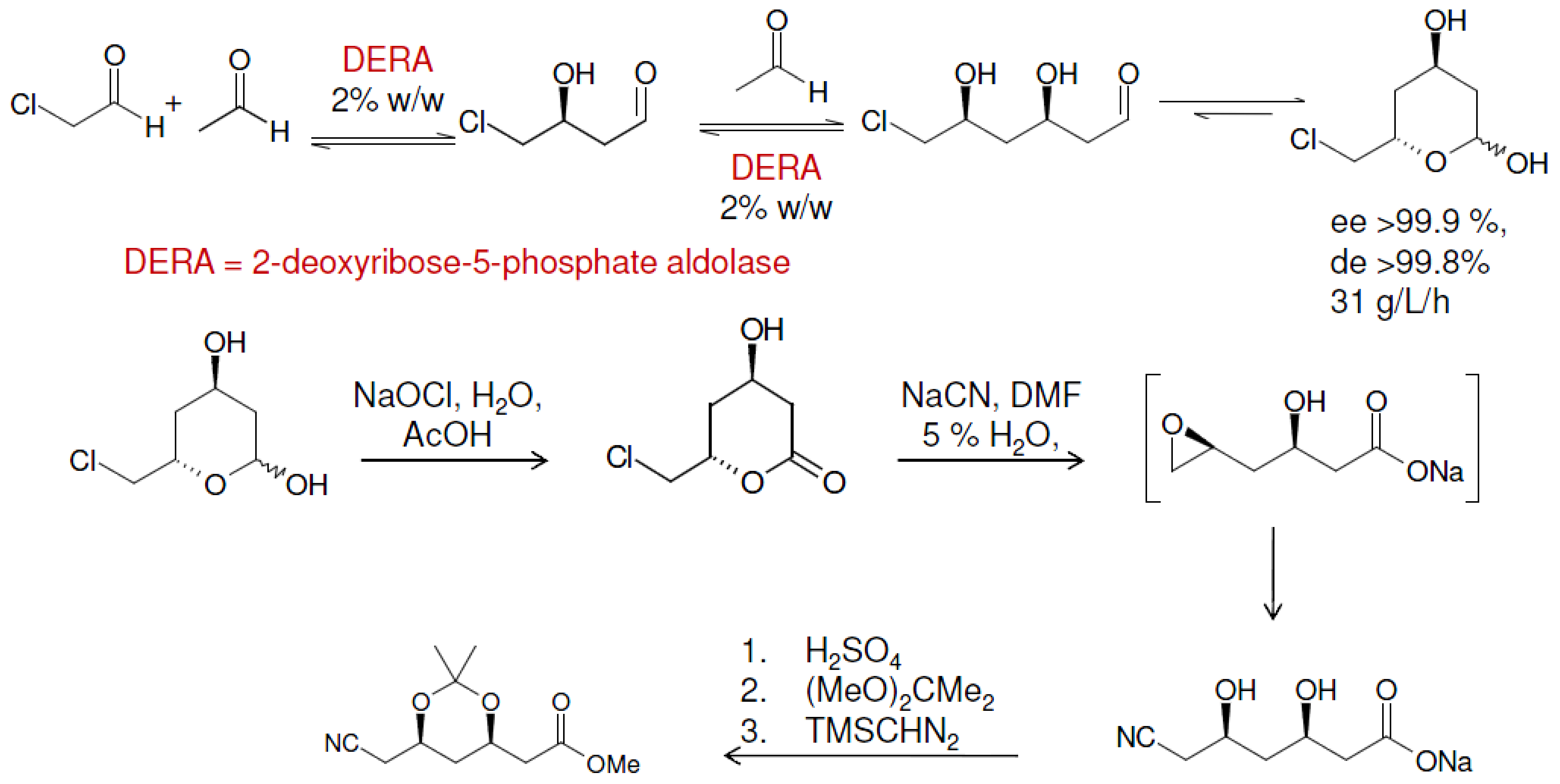


- Member of anti-cholesterol statin drugs
- Marketed by Pfizer company, world's best selling drugs since 2001
- Inhibit HMG CoA Reductase, which is the rate limiting step in cholesterol biosynthesis.
- Penta-substitute pyrrole is synthesized by Paal-Knorr pyrrole reactions.

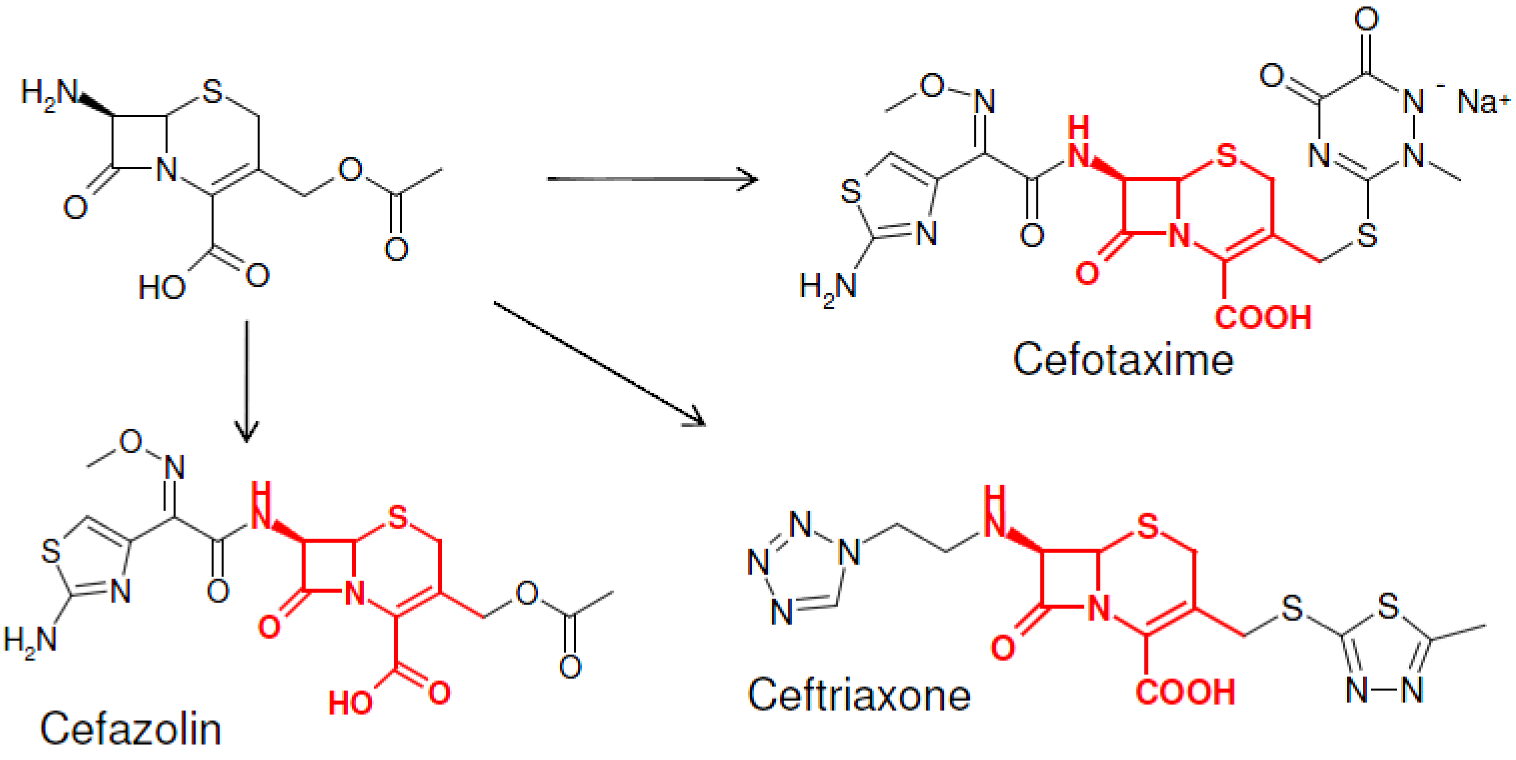
Chemical Synthesis



Chemoenzymatic Methods

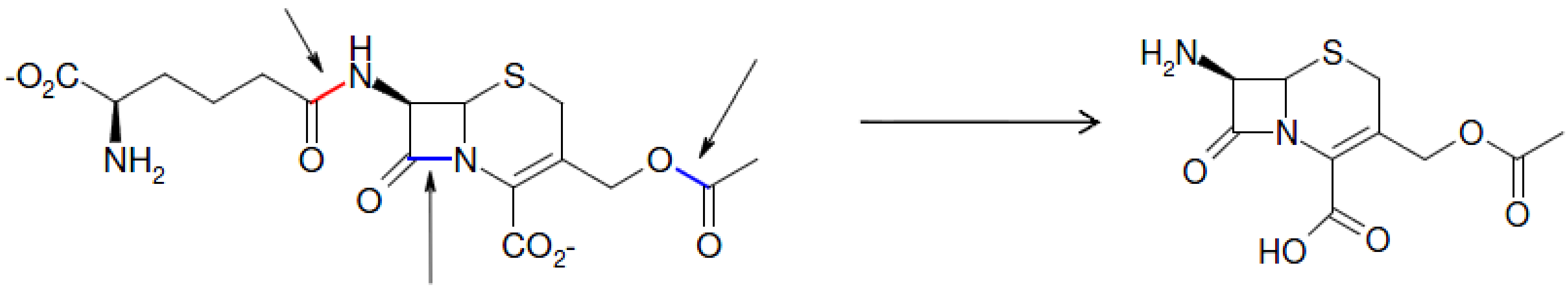


7-aminocephasporanic acid (7-ACA)



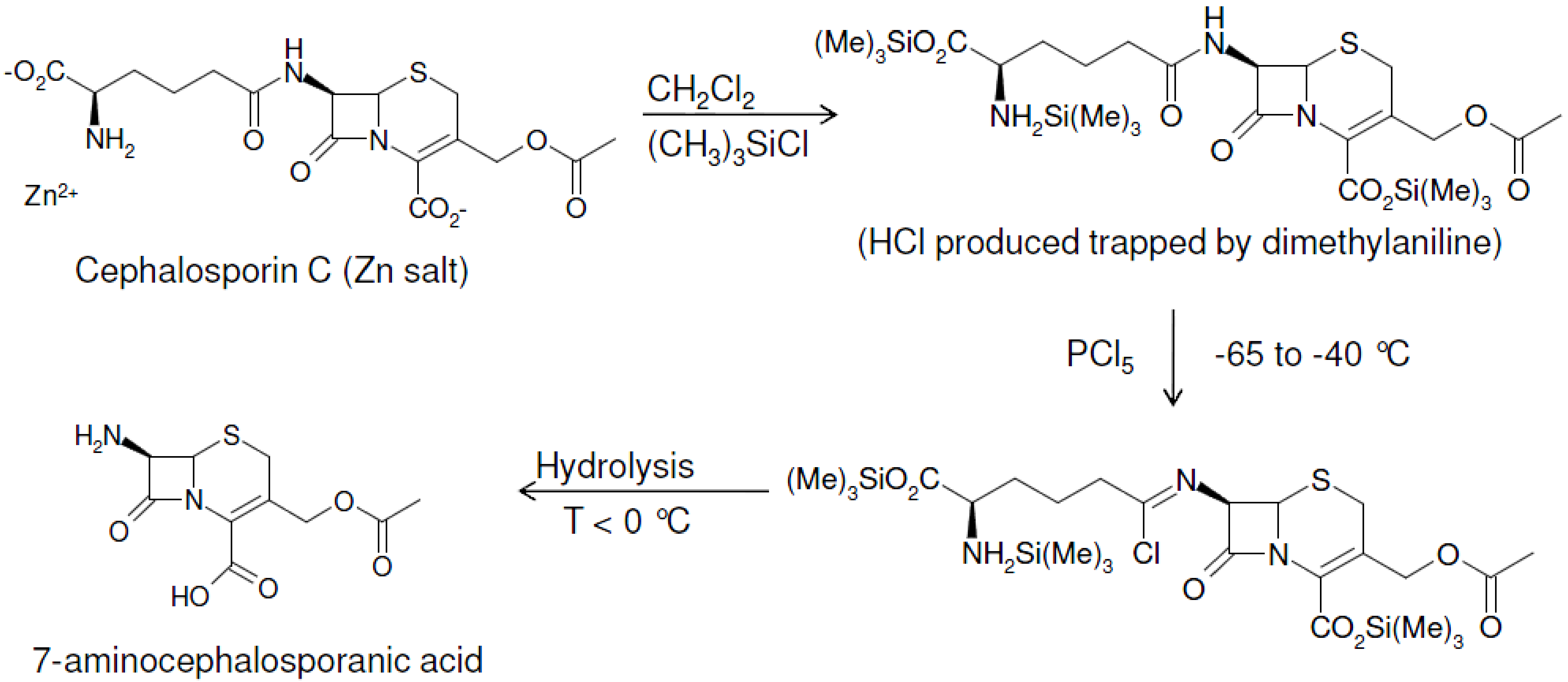
- Precursor to semi-synthetic penicillins.
- Annual synthesis of 7-ACA is 1000 tons/year

Transformation of 7-ACA

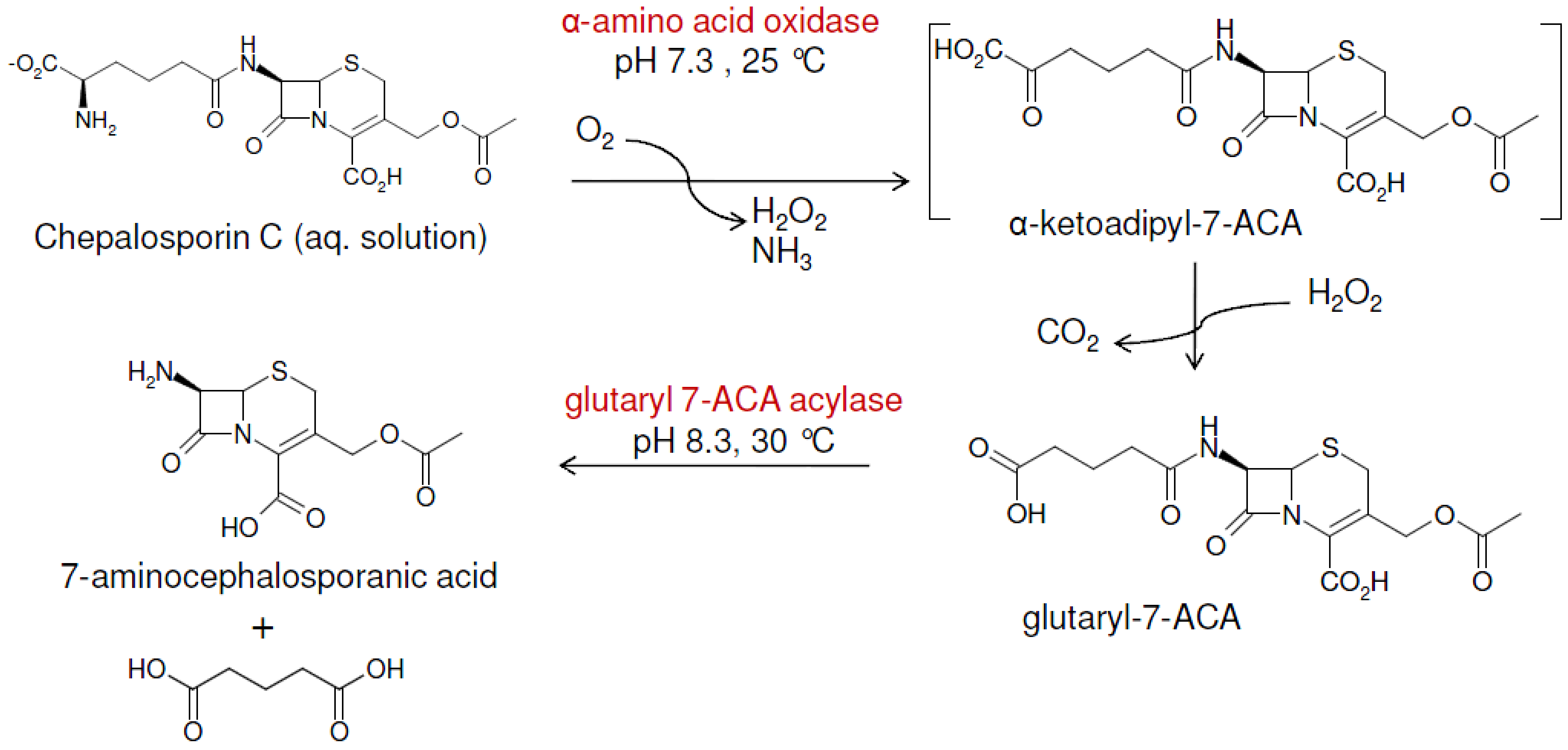


- Cephalosporin C is readily available by fermentation of *Cephalosporium acremonium* .
- Cephalosporin C exhibits weak antibiotic activity.
- Semi-synthetic penicillins show better antibiotic activity.
- Have to cleave the amide bond in the presence of more labile β-lactam amide bond.
- Chemical methods reaction are proved to be complicated.

7-ACA Chemical Synthesis



7-ACA Enzymatic Synthesis



Chemical vs Enzymatic Methods of 7-ACA

	Chemical	Enzymatic
Waste generated	31 tons/ton of ACA	0.3 tons/ ton of ACA
Heavy atom used	Zn	no heavy atom
Hazardous chemicals	PCl_5, TMSCl	no toxic chemicals
Solvents	dichloromethane	water
Volume of chemical used	equimolar	catalytic enzyme
Temperature	low T	room temperature
Equipment used	highly-corrosion-resistant alloy	stainless steel

- Biocatalytic method reduces raw material costs by factor of 4 in Hoeschst company, Germany.
- Environmental Protection Cost are reduced by 90%.

Table 1 Enzymes commonly used in organic synthesis

Enzymes	Reactions
Esterase, lipases	Ester hydrolysis, formation
Amidases (proteases, acylases)	Amide hydrolysis, formation
Dehydrogenases	Oxidoreduction of alcohols and ketones
Oxidases (mono- and dioxygenases)	Oxidation
Peroxidases	Oxidation, epoxidation, halohydration
Kinases	Phosphorylation (ATP-dependent)
Aldolases, transketolases	Aldol reaction (C–C bond)
Glycosidases, glycosyltransferases	Glycosidic bond formation
Phosphorylases, phosphatases	Formation and hydrolysis of phosphate
Sulphotransferases	Formation of sulphate esters
Transaminases	Amino acid synthesis (C–N bond)
Hydrolases	Hydrolysis
Isomerases, lyases, hydratases	Isomerization, addition, elimination, replacement

Questions?