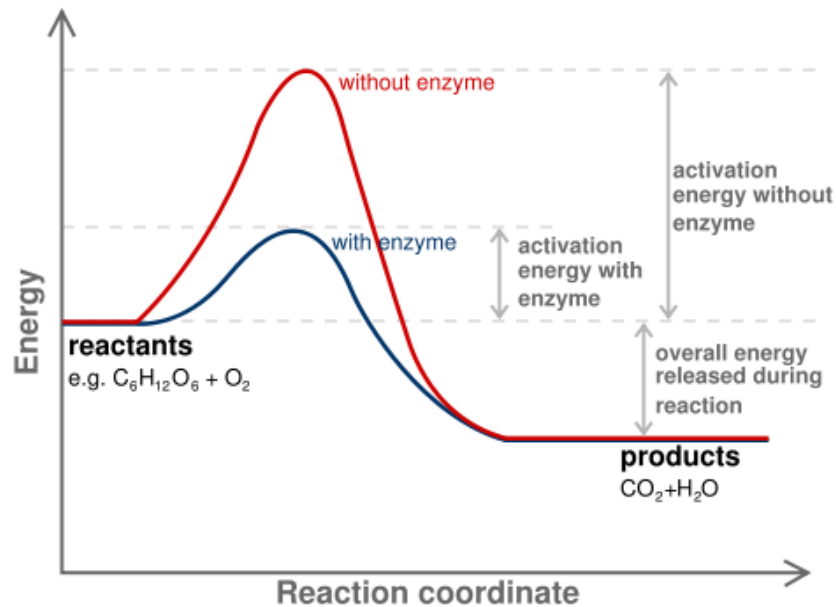


Use of Biocatalysis in the Pharmaceutical Industry

Andy Tjeng
Monday Night Seminar

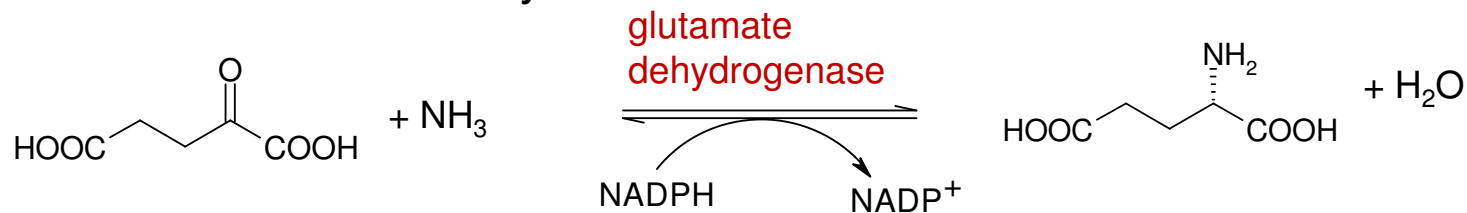
Biocatalysis

- The use of biological entity, either whole cells or isolated enzymes, for synthetic transformation.
- Enzymes are catalytic proteins that catalyze all reactions in living organism.
- Enzymes activate reactions in the active sites of the molecule, and lower the activation barrier of reactions. Enzymes are very efficient catalyst, with rate enhancement of reaction about 10^6 to 10^{17}

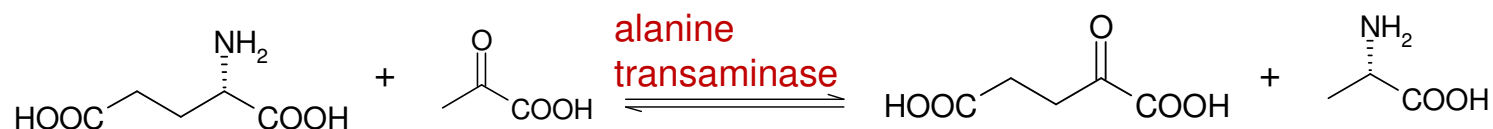


Types of Enzymatic Reactions

1. Oxidoreductase – catalyze oxidation-reduction reactions



2. Transferases – transfer of groups



3. Hydrolases – formation / breakdown of esters, amides, lactones, etc

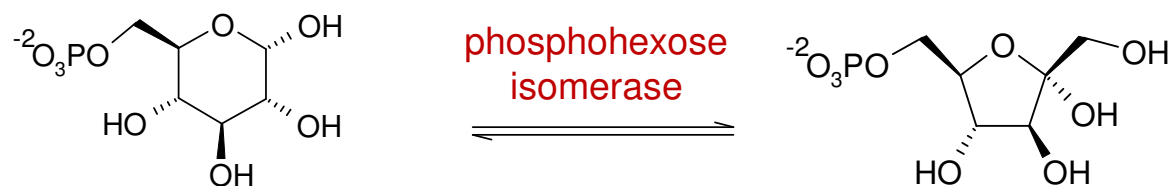


Types of Enzymatic Reactions

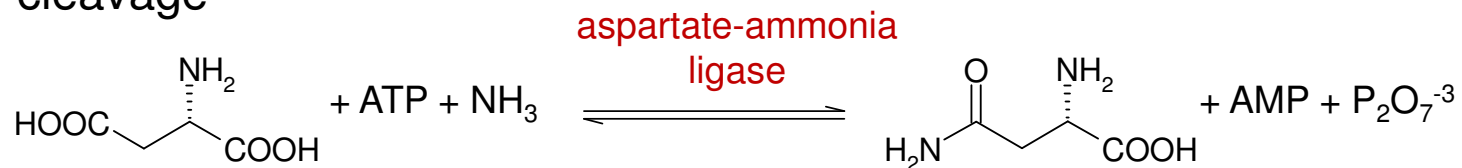
4. Lyases – addition-elimination on C=C, C=N, C=O bonds



5. Isomerases – isomerization such as racematization, epimerization



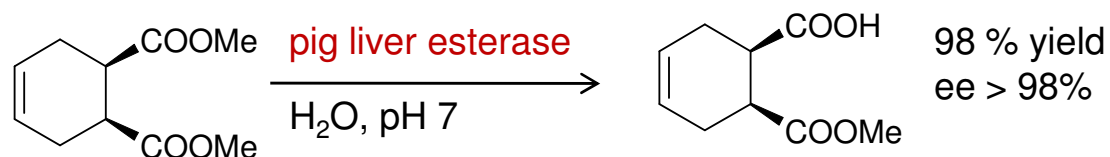
6. Ligases – formation-cleavage of C-O, C-C, C-N bonds with ATP cleavage



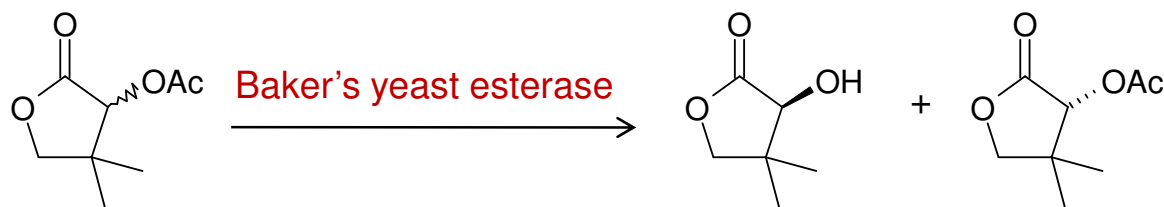
Biocatalysis in Organic Synthesis

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

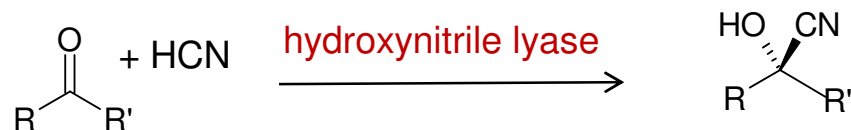
- Desymmetrization



- Kinetic Resolution

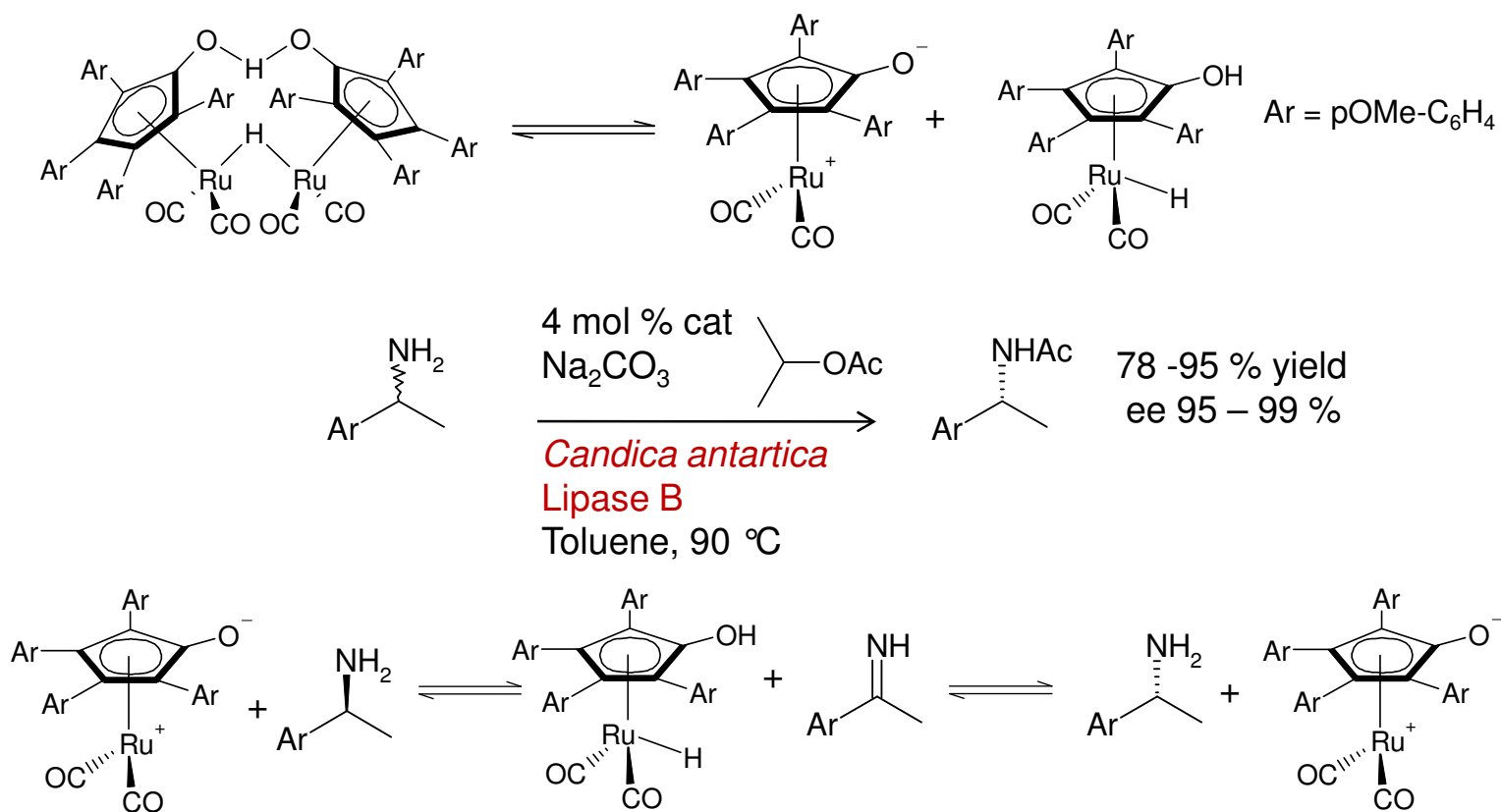


- Asymmetric synthesis



Biocatalysis in Organic Synthesis

- Recent example: Lipase-Ruthenium combination of dynamic kinetic resolution.



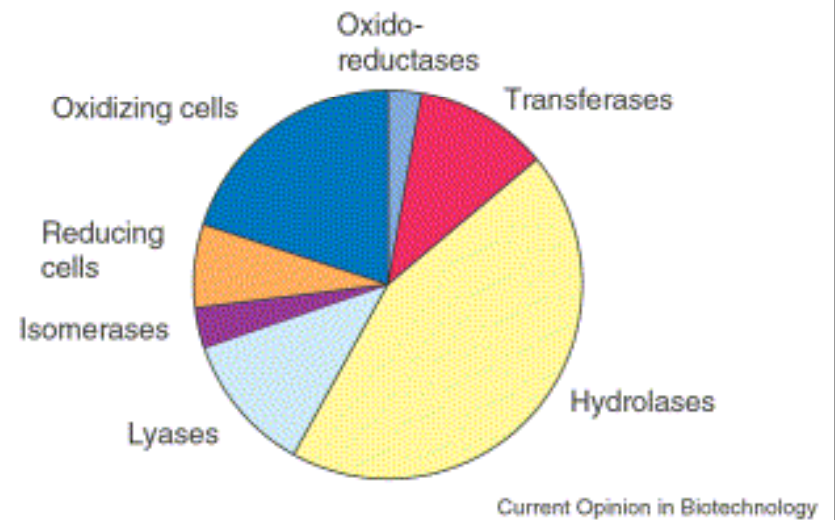
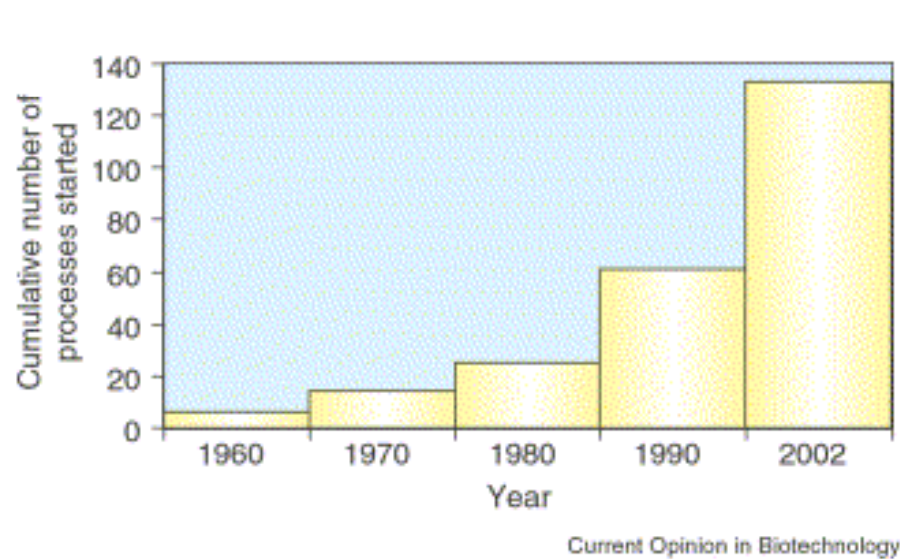
Advantages of Biocatalysis

- 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.

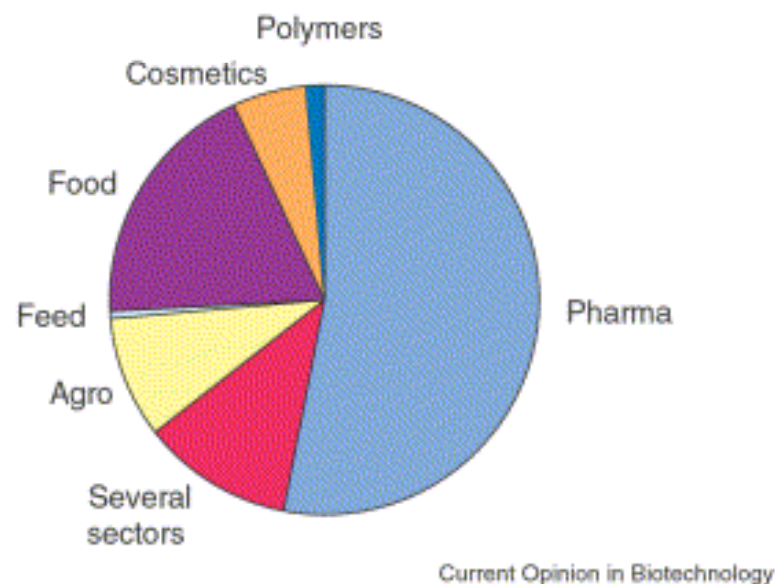
Biocatalysis in Industry

- There is increasing number of biocatalysis used in industrial scale over the past decades.
- In 2002, there are 134 industrial biocatalysis transformation (production > 100 kg/year)



Biocatalysis in Pharmaceutical Industry

- Chiral compounds production has become more and more important, especially in pharmaceutical industry.
- Application of biocatalysis in pharmaceutical industry is the major part among industrial biocatalysis.
- Even so, in many cases, biocatalysis is still under-utilized. It is used only when chemical methods are failed.



Misconceptions about Biocatalysis

- 'Enzymes are too expensive.'

The price of enzymes are about the same with other chiral catalyst.

Enzyme	Approx. price in \$/kg	Catalyst	Approx. price in \$/kg
Lactic dehydrogenase	100,000	BINAP	40,000
Porcine liver esterase	15,000	ChiraPhos	10,000
Penicillin amidase	10,000	Platinum	12,000
Aspartase	10,000	Sharpless	10,000
Trypsin	5000	Pd(Diphos) ₂	5000
Lipase	5000	Rh(PPh ₃) ₃ Cl	2000
Glucose isomerase	500	Jacobsen	1000
Detergent protease	250	Chirald	500
Glucosylase	100	Raney nickel	30

- 'Productivity are too low'

Majority of industrial enzymes give products concentration of > 100 g/L, and productivity of 100 t/a to > 10,000 t/a

Misconceptions about Biocatalysis

- ‘Enzymes are too unstable.’

immobilized enzymes have shown stability toward reuse.

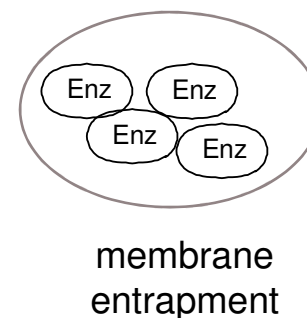
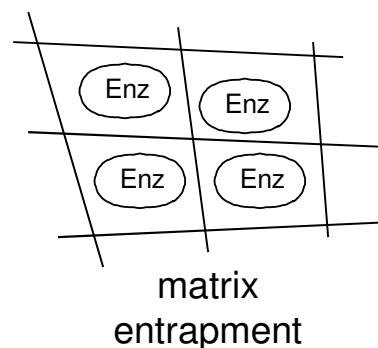
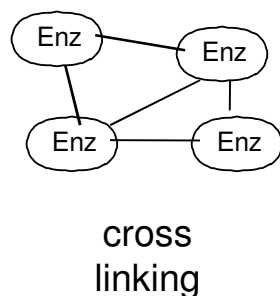
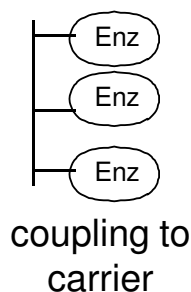
Biocatalyst	Reported half-life
Aspartase	6 months – 2 years
Fumarase	180 days
Penicilin amidase	> 6 months
Lactase	90 days
Protease	> 60 days
Tryptophan Synthase	50 days

- ‘Enzymes act on natural substrate only.’

Enzyme-substrate recognition's is not ‘lock and key’ but ‘induced fit’.

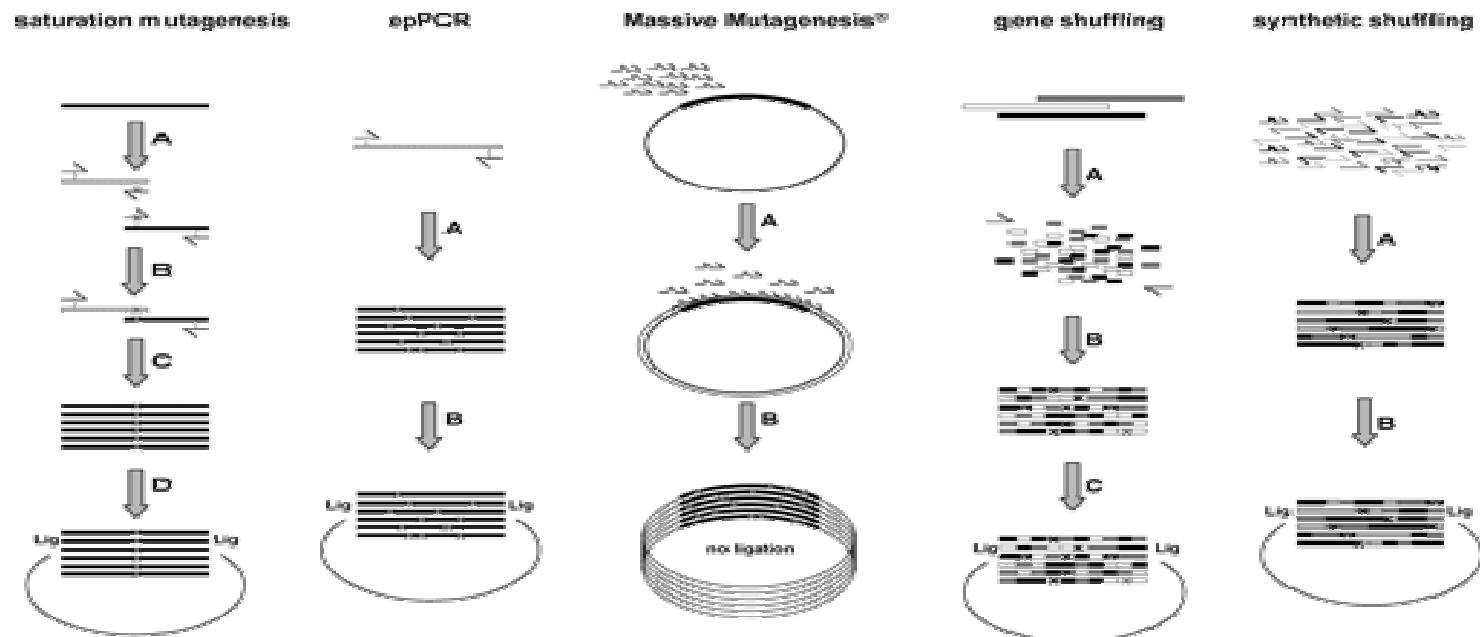
Immobilized Enzymes

- Immobilized enzymes are enzymes that physically localized in a certain region of space with retention of their catalytic activity.
- Advantages:.
 - Increase stability.
 - Easy separation from reaction mixture
 - Repeated or continuous use.
 - Prevention of protein contamination on product.
- Methods of enzymes immobilization:



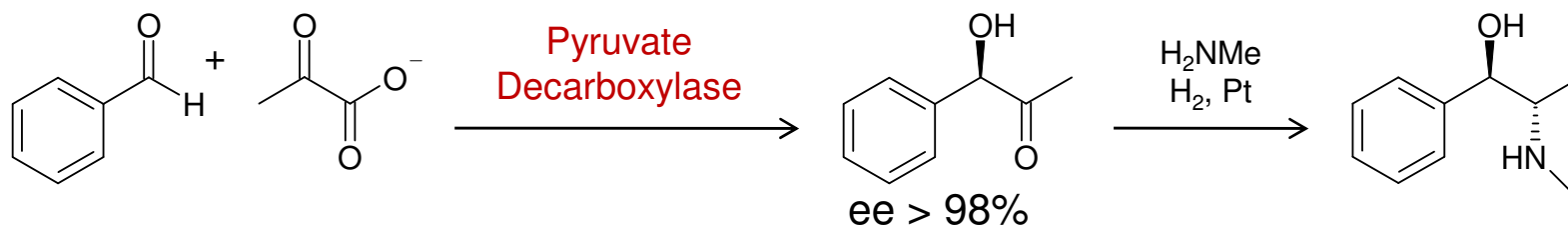
Genetic Engineering

- Natural enzymes usually do not perform efficiently under industrial conditions.
- Directed evolution is methods developed to provide enzymes library followed by high throughput screening and selection. Enzymes for industrial purpose are generated by this process.

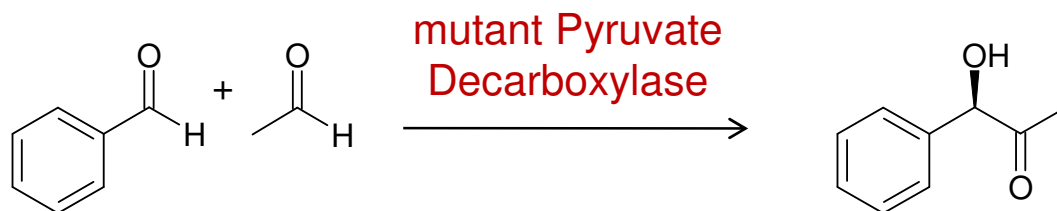


Case Studies

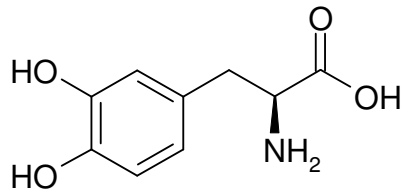
R-PAC (Phenyl Acetyl Carbinol)



- Precursor for ephedrine, which is used for treatment of asthma
- First example of biocatalysis for pharmaceutical compounds (as early as 1932)
- The mutant enzyme can accept acetaldehyde as substrate, therefore lower starting material costs. Productivity are also increased to 100 g/L.

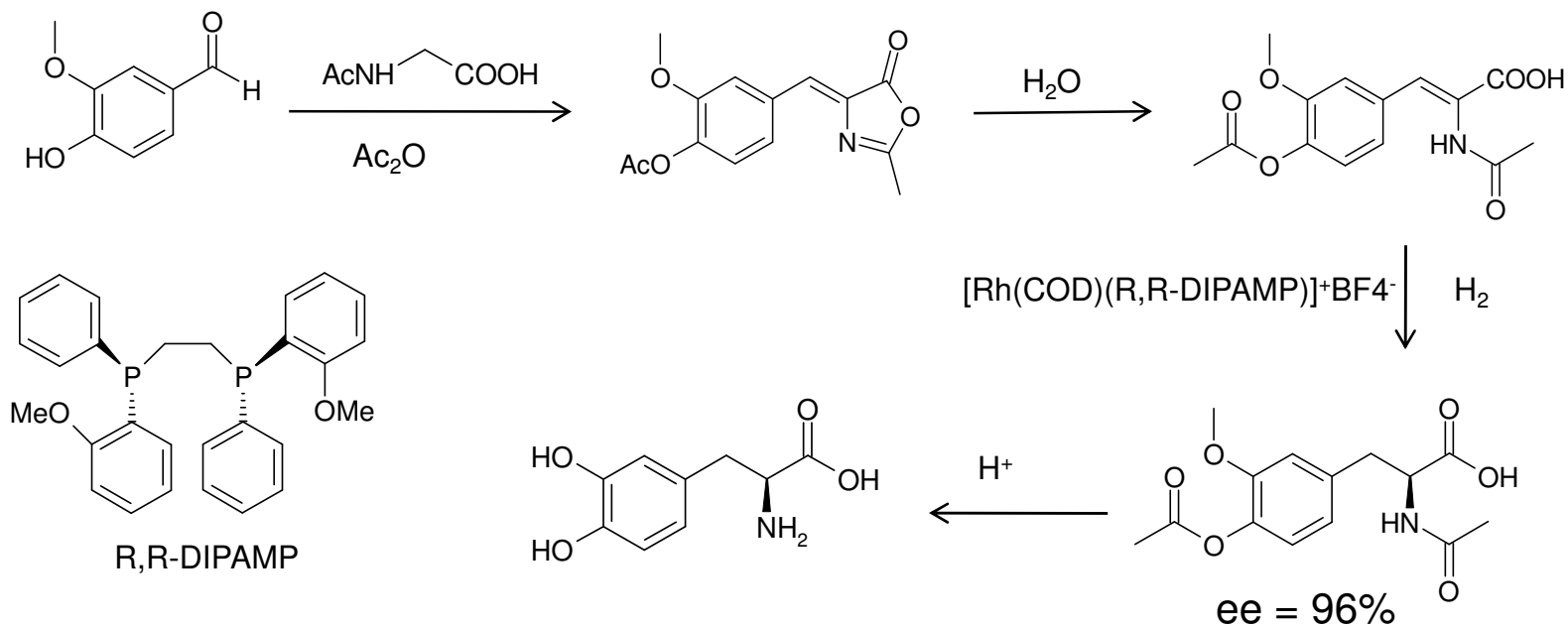


L-Dopa



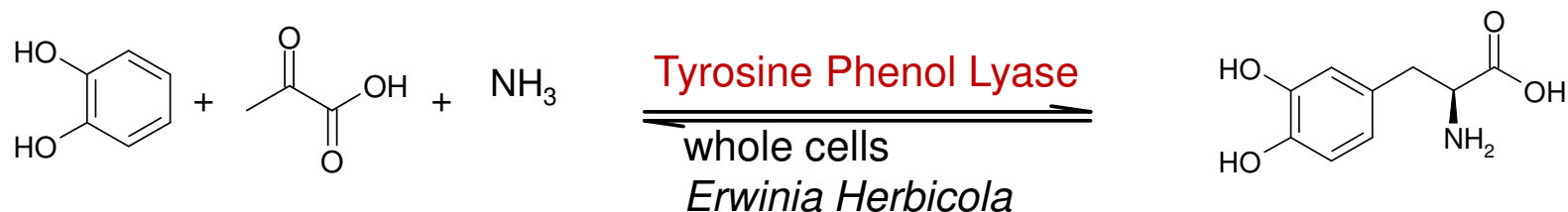
- Treatment for Parkinson's disease, which is caused by lack of Dopamine or its receptors in brain.
- L-Dopa is decarboxylated in vivo to become Dopamine.
- Since Dopamine cannot pass through blood brain barrier, L-Dopa is administered with dopadecarboxylase-inhibitors to maintain its form outside the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

L-Dopa Chemical Synthesis

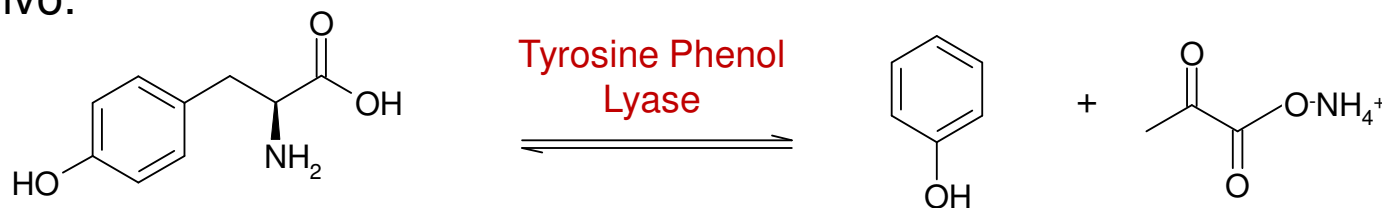


- The first use of asymmetric catalysis in pharmaceutical industry (1968)
- Developed by William Knowles, Noble Prize winner in Chemistry 2001 (together with Noyori and Sharpless) for asymmetric hydrogenation and oxidation reactions.

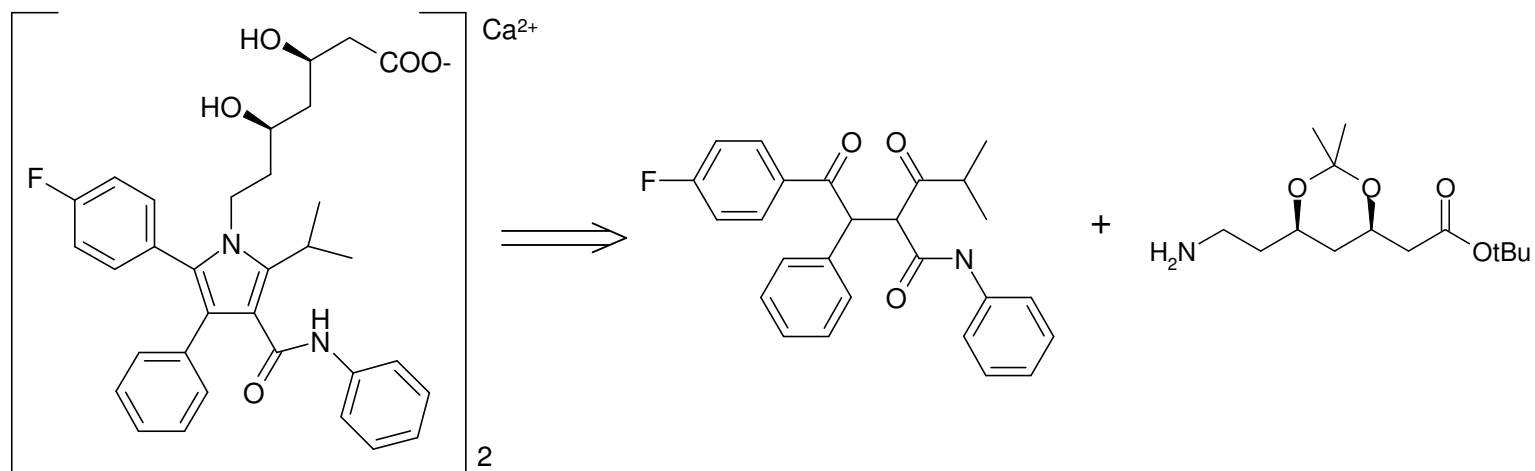
L-Dopa Enzymatic Synthesis



- 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.

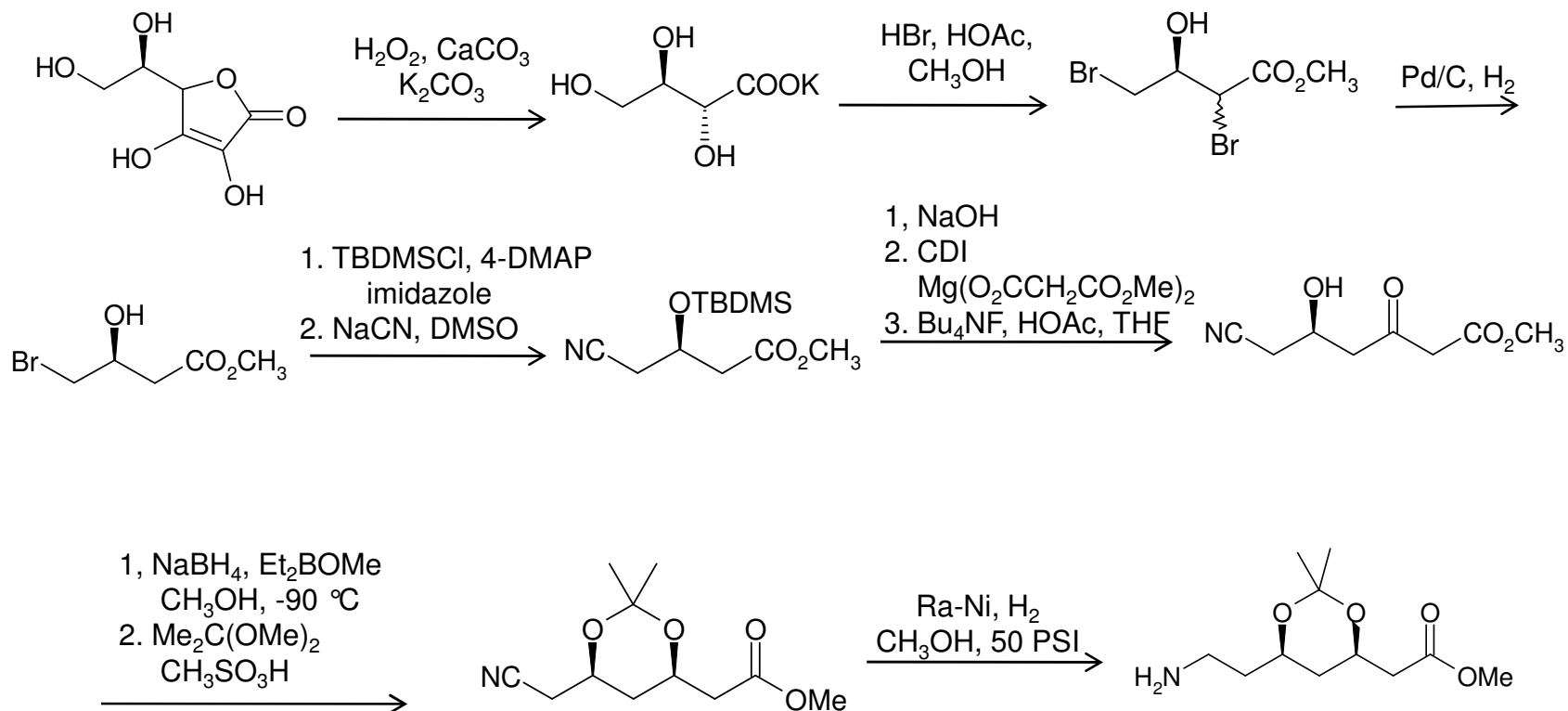


Atorvastatin Calcium (Lipitor)

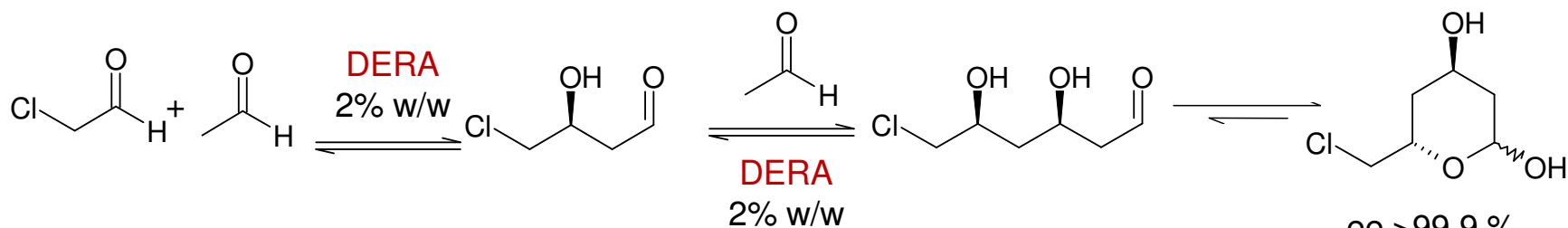


- Member of anti-cholesterol statin drugs
- Marketed by Pfizer company, world's best selling drugs since 2001
- Inhibit HMG CoA Reductase, which is the rate limiting step in cholesterol biosynthesis.
- Penta-substitute pyrrole is synthesized by Paal-Knorr pyrrole reactions.

Chemical Synthesis

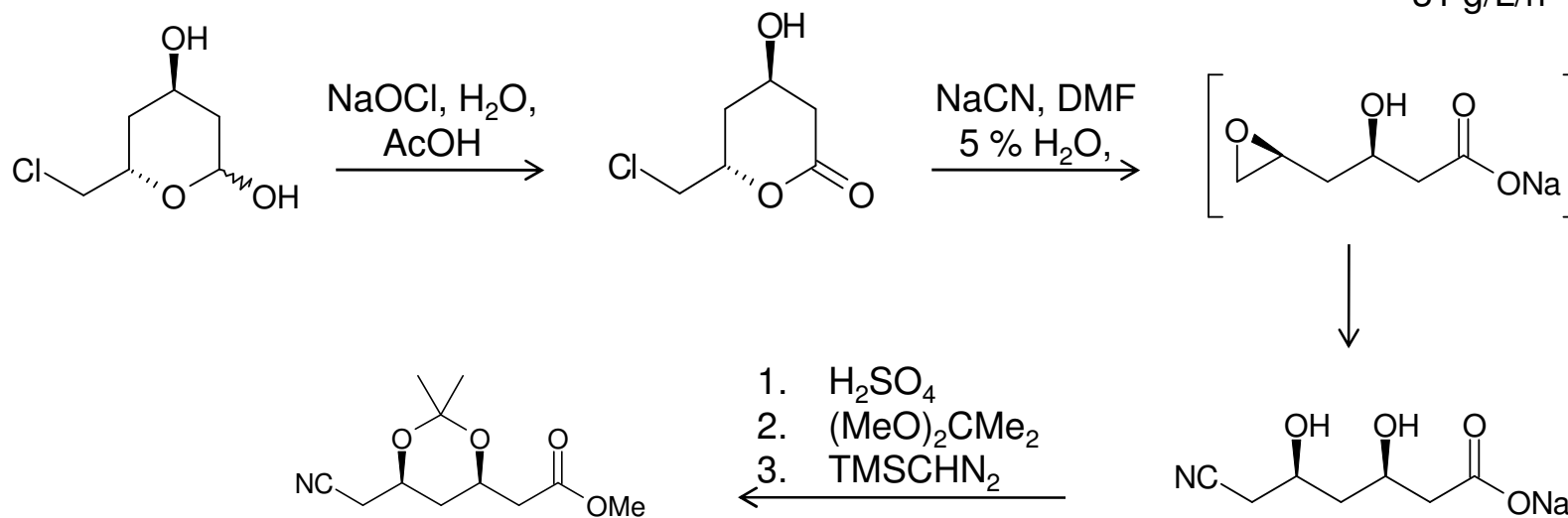


Chemoenzymatic Methods

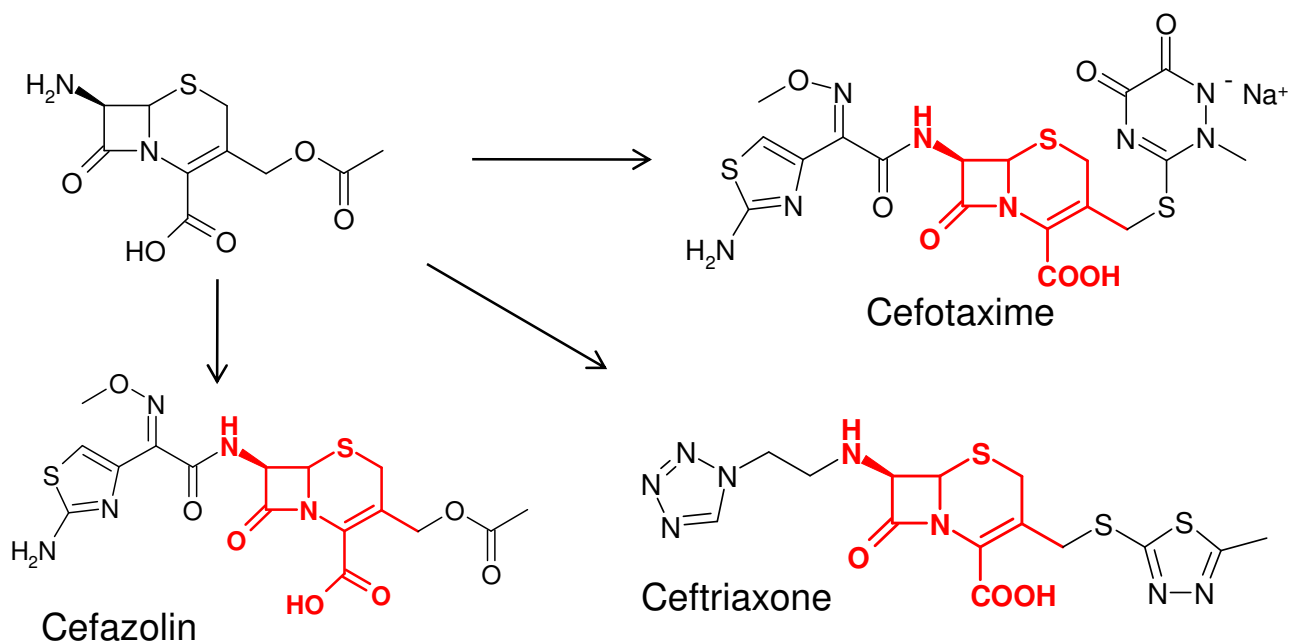


DERA = 2-deoxyribose-5-phosphate aldolase

ee >99.9 %,
de >99.8%
31 g/L/h

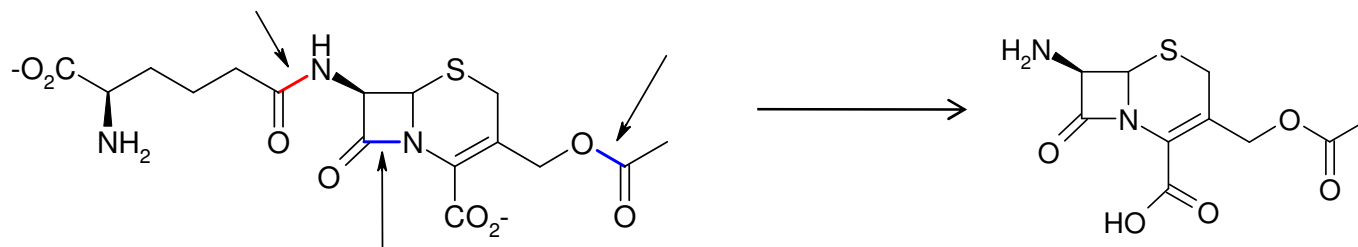


7-aminocephasporanic acid (7-ACA)



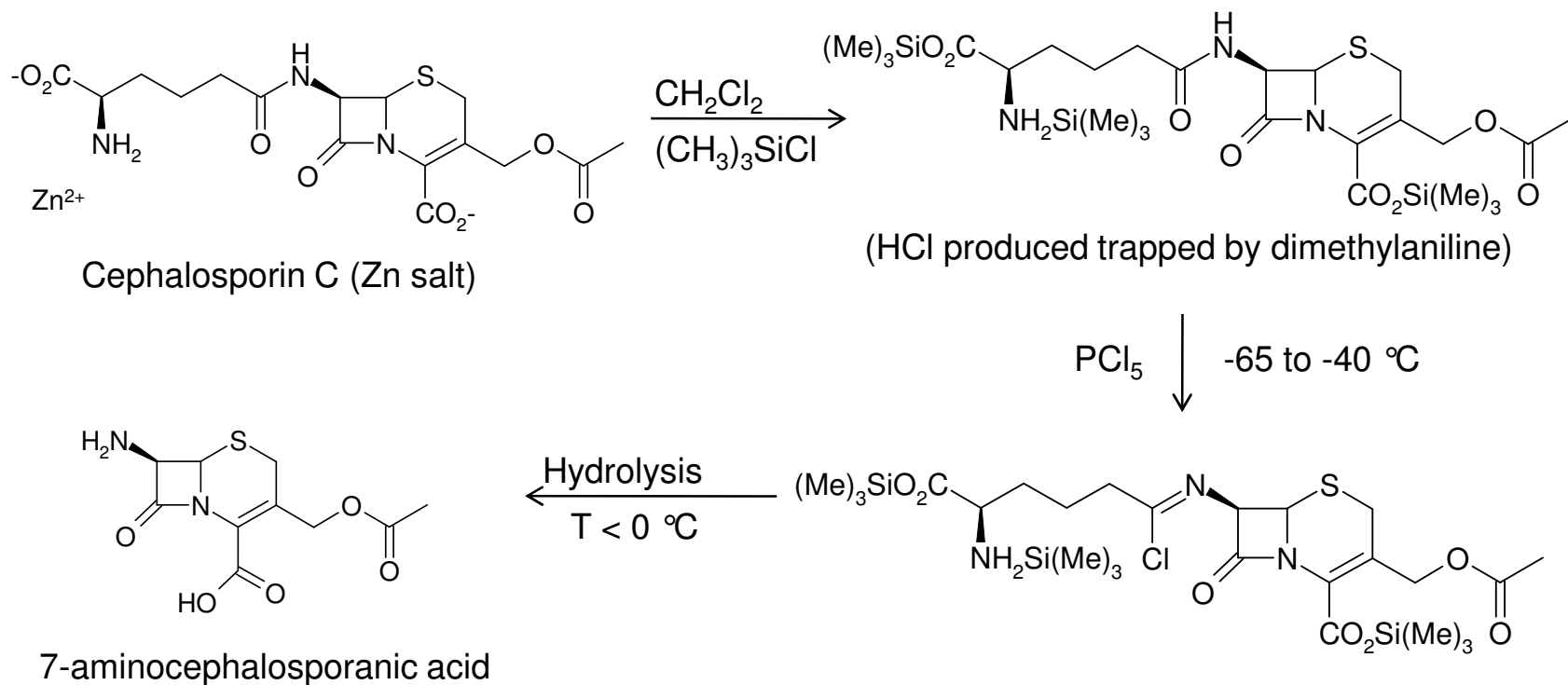
- Precursor to semi-synthetic penicillins.
- Annual synthesis of 7-ACA is 1000 tons/year

Transformation of 7-ACA

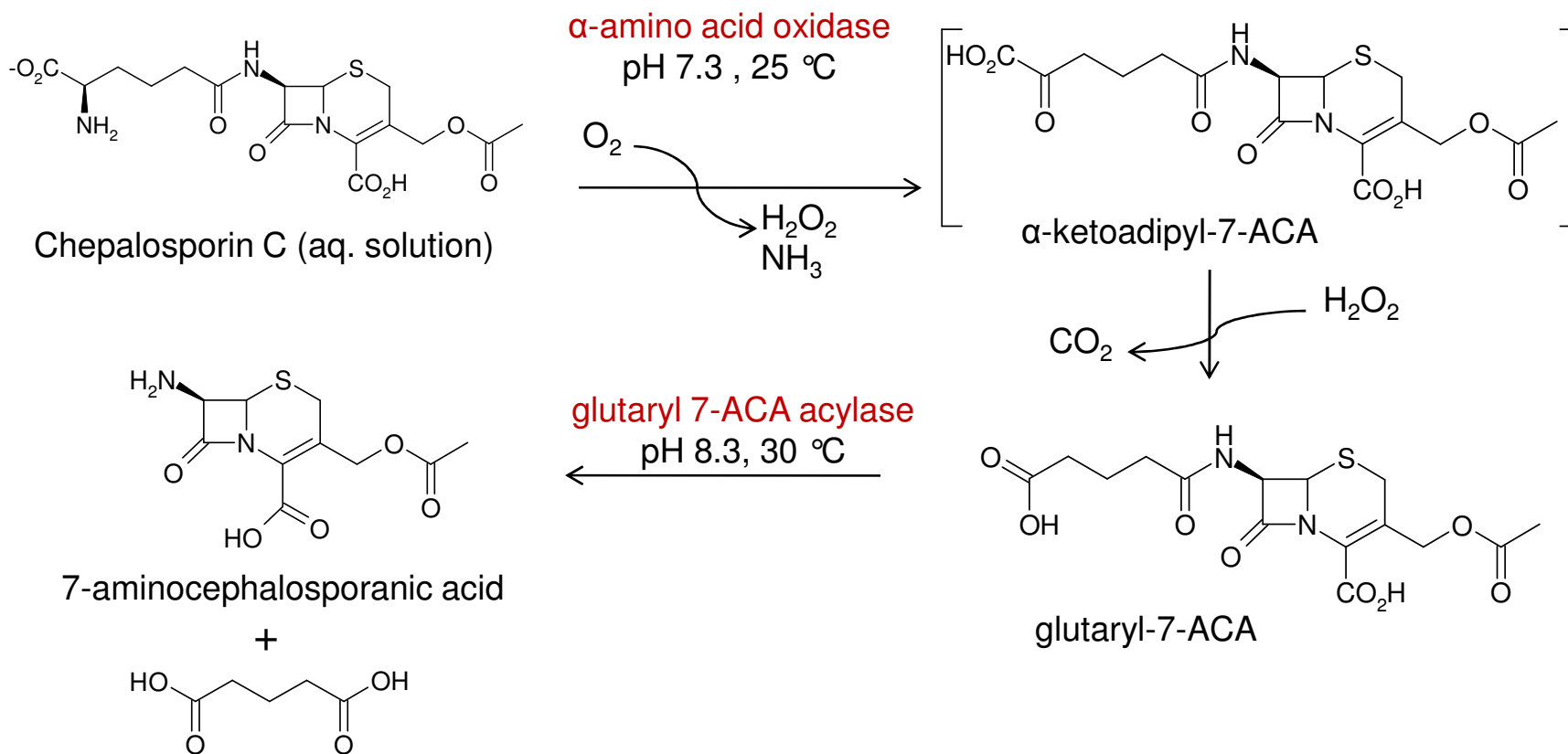


- Cephalosporin C is readily available by fermentation of *Cephalosporium acremonium*.
- Cephalosporin C exhibits weak antibiotic activity.
- Semi-synthetic penicillins show better antibiotic activity.
- Have to cleave the amide bond in the presence of more labile β -lactam amide bond.
- Chemical methods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

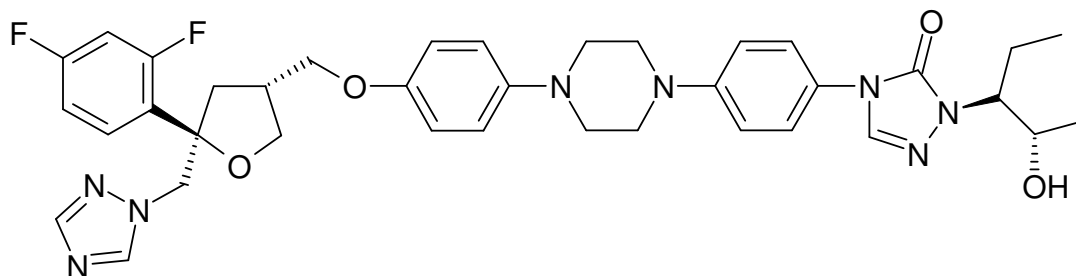
- Biocatalytic method reduces raw material costs by factor of 4 in Hoeschst company, Germany.
- Environmental Protection Cost are reduced by 90%.

Enzymatic Methods

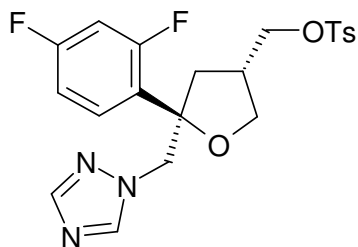
- Chemicals method has been obsolete and changed by enzymatic methods due to environmental concerns.
- One-step enzymatic procedure currently result in very low yield, but it is under development.



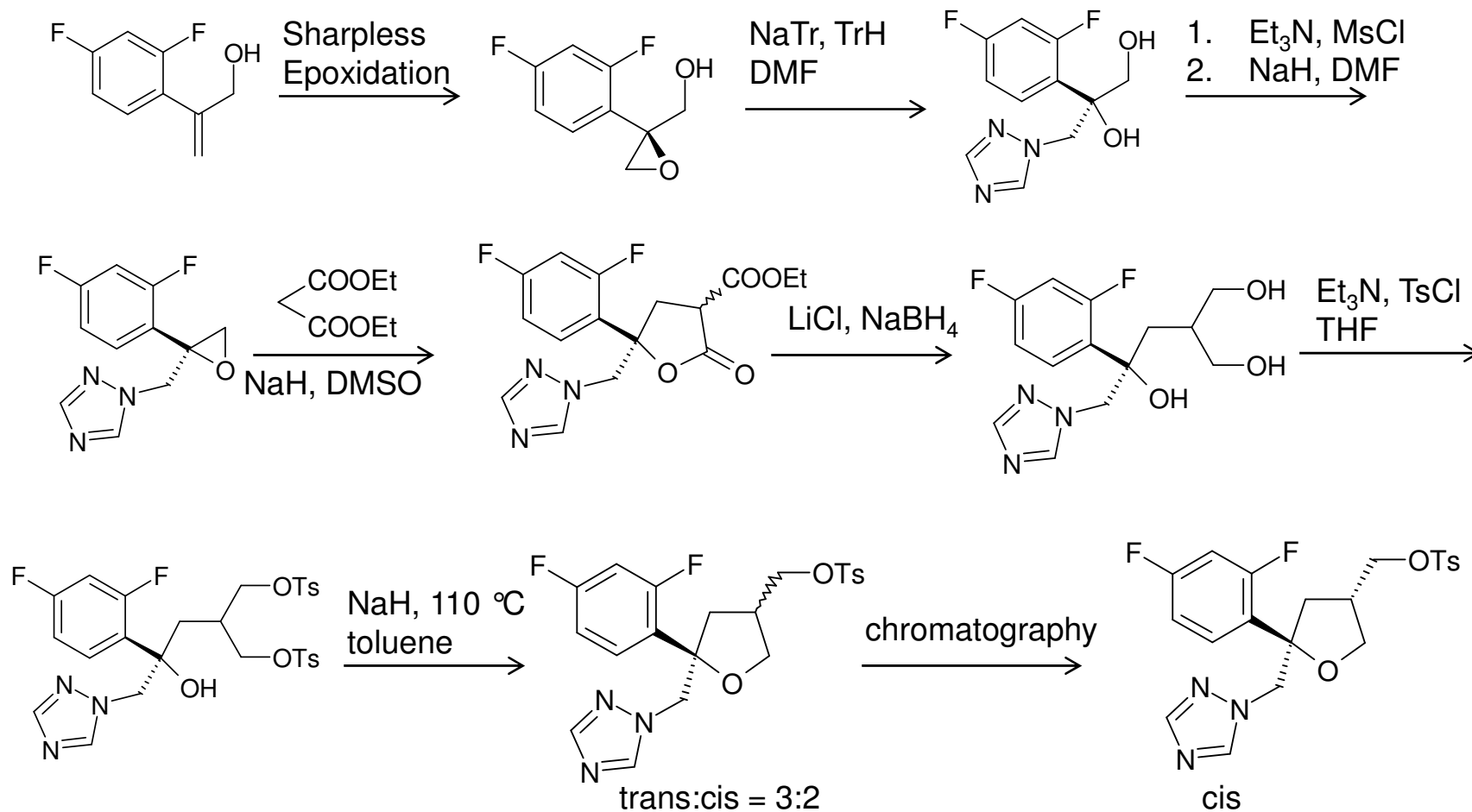
Posaconazole



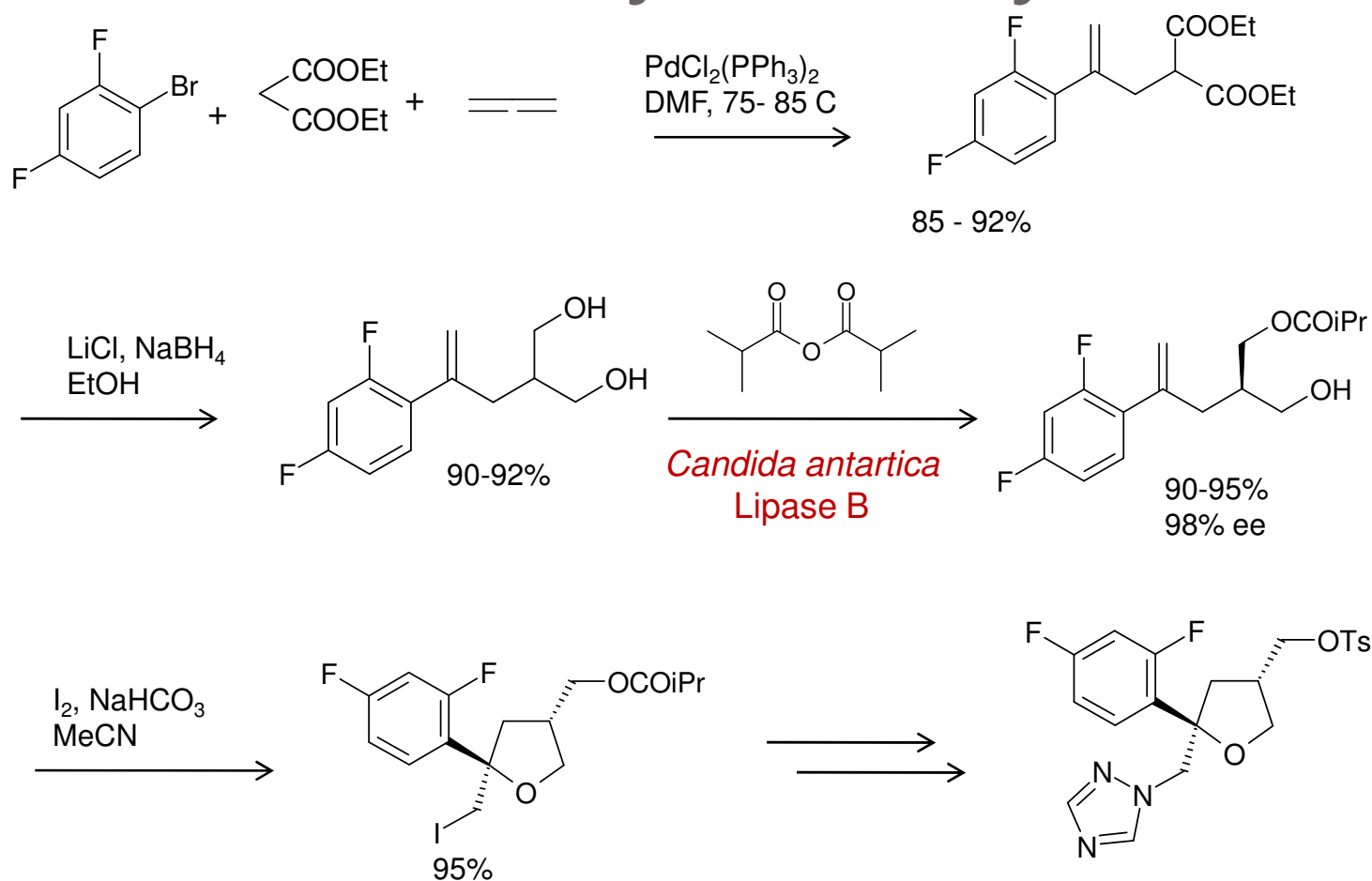
- Broad-spectrum orally active azole-antifungal for treatment of systemic *Candida* and *Aspergillus* infections.
- Developed by Schering-Plough.



Chemical Synthesis



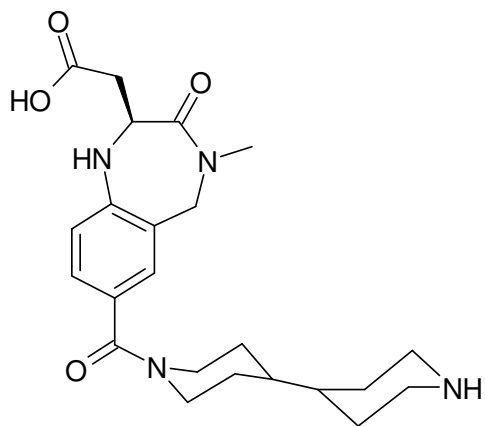
Chemoenzymatic Synthesis



Comparison of Chemical vs Chemoenzymatic Methods

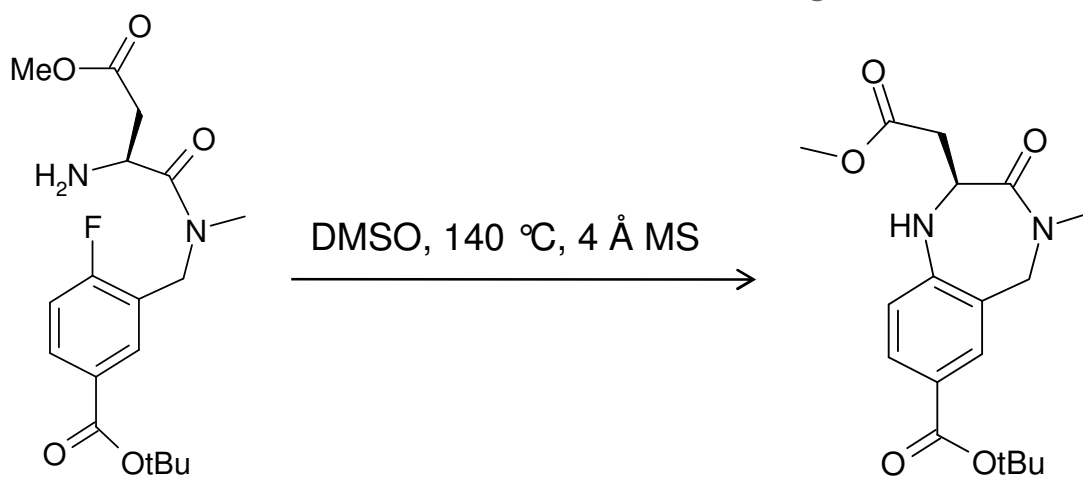
- Chemical method using Sharpless epoxidation consists of 12 steps which provided only 6% overall yield.
- Chemical method suffers from poor diastereomeric control in cyclization, and required chromatography to separate isomers.
- Chemoenzymatic method with enzymatic desymmetrization consists of 7 steps from inexpensive starting material, with only 1 isolated intermediate give overall 35% yield.

Lotrafiban



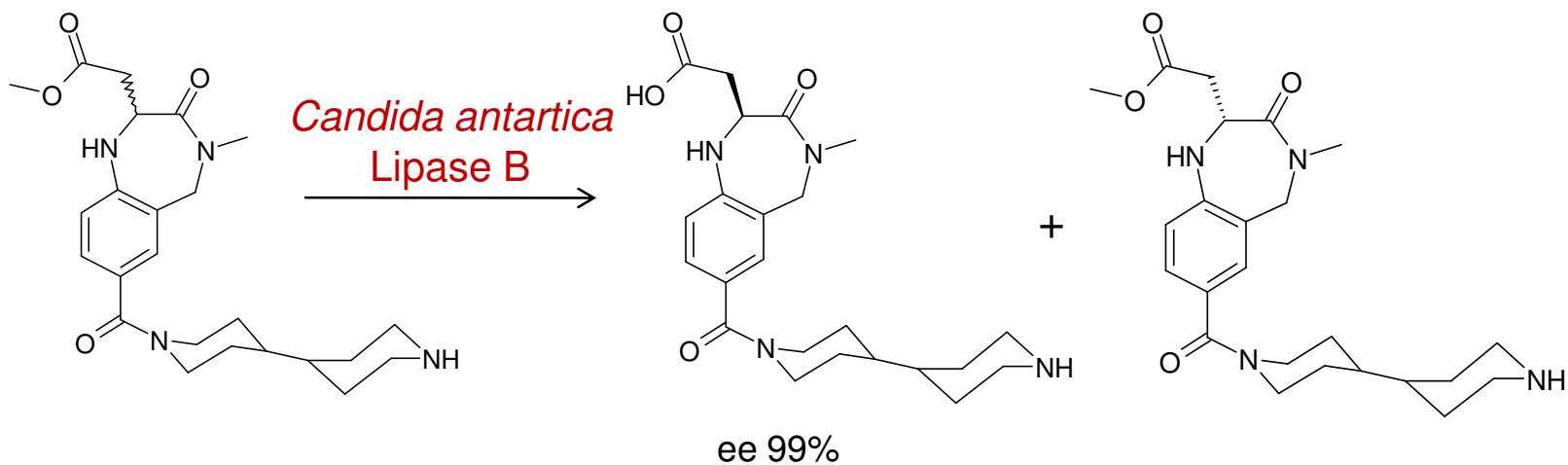
- Potent nonpeptidic glycoprotein IIb/IIIa fibrinogen receptor antagonist, inhibits platelet aggregation for thrombotic events.
- However, the clinical program was discontinued during phase III.

Chemical Synthesis



- Reaction yield is ~40%, but in small scale the reaction gives 100% ee
- While scaling-up, the enantiomer purity drops to ee of 91%, which is unacceptable for pharmaceutical.
- Attempt to increase the ee by crystallization failed and prevention of partial racemization cannot be solved.

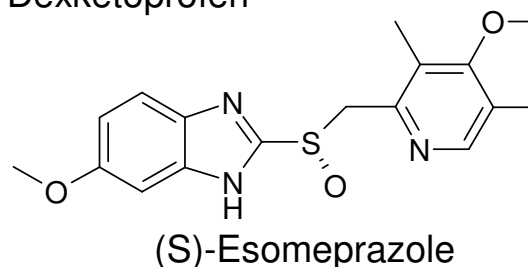
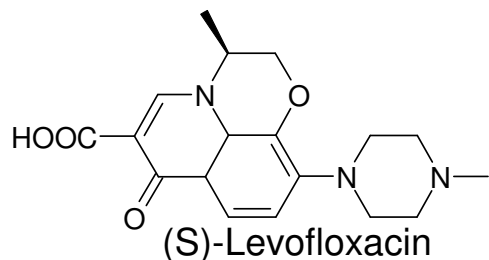
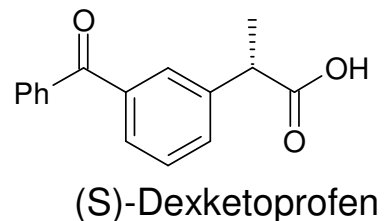
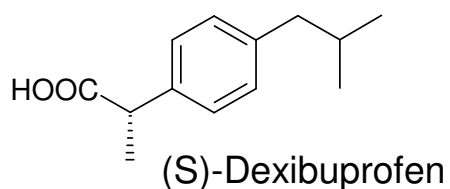
Enzymatic Resolution



- Product are easily separated.
- The unwanted enantiomer can be racemized which give yield of 84% (overall 42%)
- The immobilized enzyme can be reused multiple times.

Chiral Switches

- Chiral drugs that have been marketed as mixtures of enantiomers, but have been redeveloped as single enantiomers.
- Some techniques used to prepare the enantiomers:
 - Crystallization of diastomeric salts
 - Chiral pool
 - Enzymatic resolutions
- Examples of drugs that have undergone chiral switch by enzymatic kinetic resolutions:



Conclusion

- Biocatalysis has become part of synthetic chemistry, and its application will increase in the future.
- Introduction of biocatalysis in synthetic route enable us to create more economical process, with shorter routes, increased selectivity, and more environmentally friendly reactions.
- Instead of competing with conventional organic methods, biocatalysis can complement chemical methods to give better transformations.
- As there are only small numbers of enzymes that has been used in synthetic transformations, there's a huge opportunity of applications of biocatalysis synthetic transformations.

Thank you

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