Biocatalysis

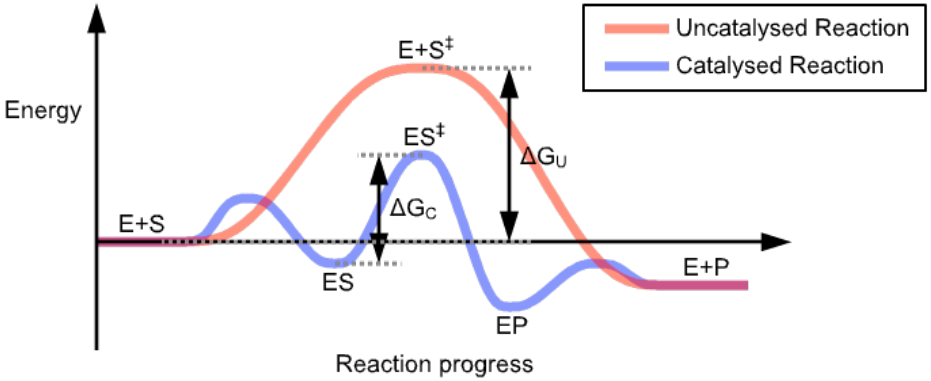
Enzymes are catalysts evolved in nature to achieve the speed and coordination of a multitude of chemical reaction necessary to develop and maintain life.

Enzymes are globular proteins which range from 62 (monomer of 4-oxalocrotonate tautomerase) to over 2 500 amino acid residues (animal fatty acid synthase), but only a small portion (~ 3-4 amino acids are directly involved in catalysis)

Classification

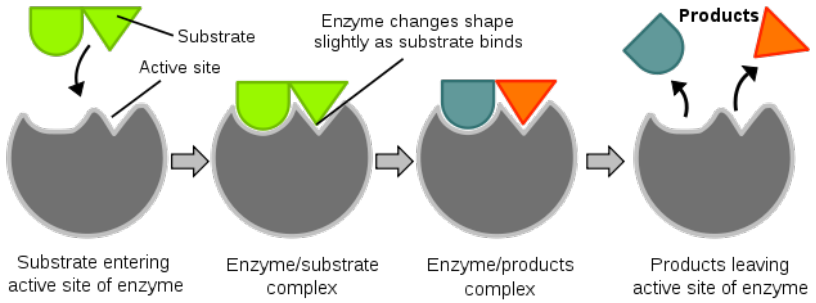
Oxidoreductases catalyze oxidation/reduction reactions
Transferases transfer a functional group (e.g. methyl group)
Hydrolases catalyze the hydrolysis of various bonds
Lyases cleave various bonds by means other than hydrolysis and oxidation
Isomerases catalyze isomerization changes within a single molecule
Ligases join two molecules with covalent bonds

Enzyme reaction



Induced fit

Model for the enzyme-substrate interaction introduced by Koshland



Mechanism of transitions state stabilization

Catalysis by bond strain

affinity of the enzyme to the transition state is greater than to the substrate itself → ground state destabilization effect

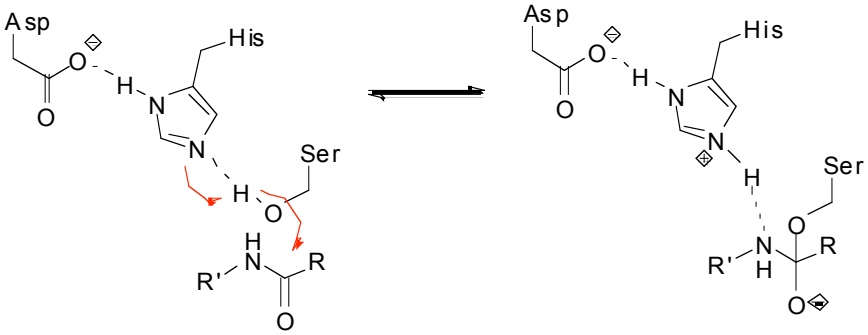
Catalysis by proximity and orientation

enzyme-substrate interactions align reactive groups and hold them close together → reduces the overall loss of entropy

Catalysis involving proton donors or acceptors (acid/base catalysis)

stabilization of developing charges in the transition state → activation of nuceophiles and electrophiles or stabilization of leaving groups

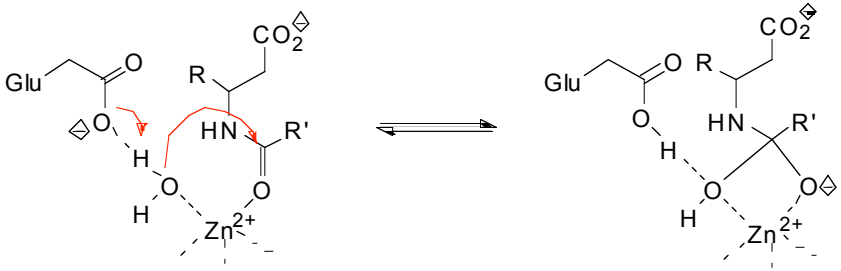
initial step of the serine protease catalytic mechanism:



Electrostatic catalysis

stabilization of charged transition states by forming ionic bonds with residues of the active site

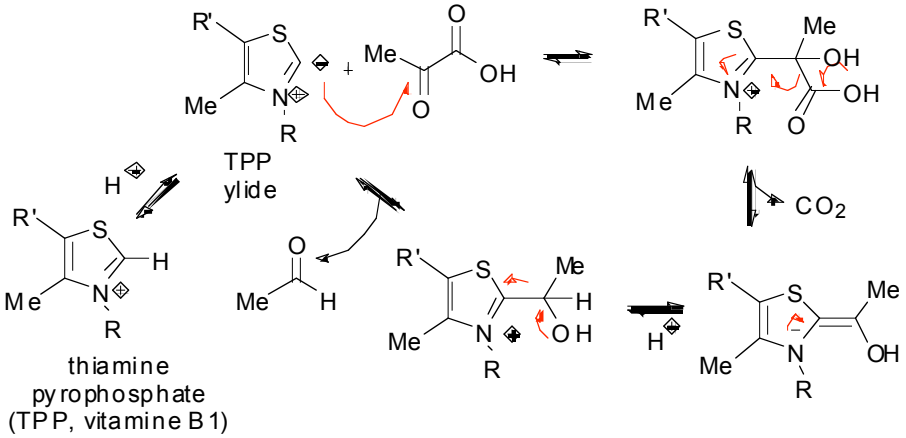
initial step of the carboxypeptidase catalytic mechanism:



Covalent catalysis

substrate is forming a transient covalent bond with a residue in the active site in order to reduce energy of later transitions states of the reaction

pyruvate decarboxylase mechanism



Advantages of biocatalysts and enzymes

- very high enantioselectivity
- very high regioselectivity
- transformation under mild conditions
- 'green chemistry' e.g. solvent often water

Disadvantages of biocatalysts and enzymes

- often low specific activity
- instability at extreme temperatures and pH values
- availability for selected reactions only
- long development time for new enzymes

advances in genomics, directed evolution, gene and genome shuffling and the exploration of Earth´s biodiversity aided by bioinformatics and high-throughput screening facilitate the discovery and optimization of enzymes

It is estimated that biocatalysis and biotransformations account for 30% of the chemical business by the year 2050

Biocatalysis

Biotransformations on an Industrial Scale

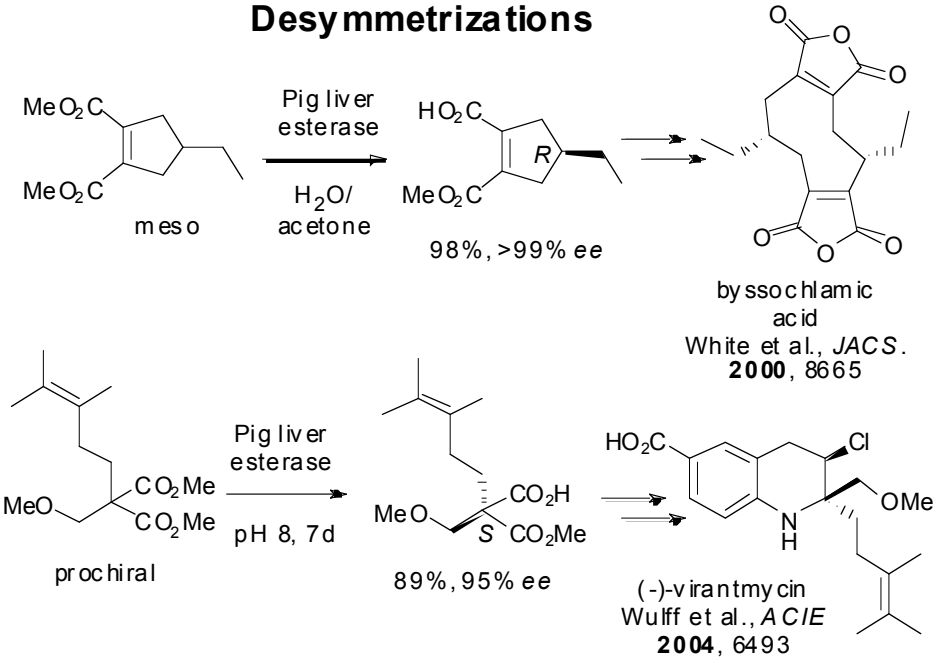
t/a	product	enzyme
> 1 000 000	high-fructose corn syrup	glucose isomerase
> 100 000	lactose-free milk	lactase
> 10 000	acrylamide	nitrilase
	cocoa butter	lipase
> 1 000	nicotinamide	nitrilase
	D-pantothenic acid	aldonolactonase
	(*S*)-chloropropionic acid	lipase
	6-aminopenillanic acid	penicillin amidase
	7-aminocephalosporanic acid	glutaryl amidase
	aspartame	thermolysin
	L-aspartate	aspartase
	D-phenylglycine	hydantoinase
	D-*p*-OH-phenylglycine	hydantoinase
> 100	ampicillin	penicillin amidase
	L-methionine, L-valine	aminoacylase
	L-carnitine	dehydrase/ hydroxylase
	L-DOPA	β-tyrosinase
	L-malic acid	fumarase
	(*S*)-methoxyisopropyl-amine	lipase
	(*R*)-mandelic acid	nitrilase
	L-alanine	L-aspartate-β-de-carboxylase

further applications:
baby foods, brewing industry, fruit juice, dairy industry, starch, paper, biofuels, detergents, rubber,....

Literature

- K. Drauz, H. Waldmann, *Enzyme Catalysis in Organic Synthesis*, Wiley-VCH, **2002**
- V. Gotor, I. Alfonso, E. Garcia-Urdiales, *Asymmetric Organic Synthesis with Enzymes*, Wiley-VCH, **2008**
- E. Garcia-Junceda, *Multi-Step Enzyme Catalysis*, Wiley-VCH, **2008**
- D. Enders, K.-E. Jaeger, *Asymmetric Synthesis with Chemical and Bioblogical Methods*, Wiley-VCH, **2007**
- A.S. Bommarius, B. R. Riebel, *Biocatalysis*, Wiley-VCH, **2004**
- G. Carrea, S. Riva, *Organic Synthesis with Enzymes in Non-Aqueous Media*, Wiley-VCH, **2008**

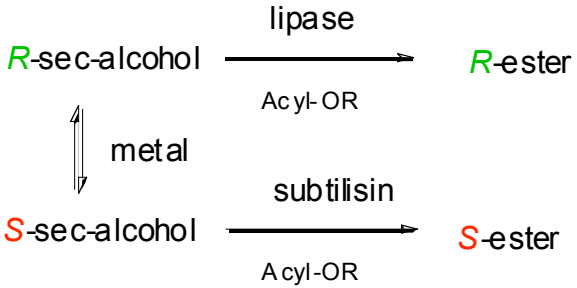
Desymmetrizations



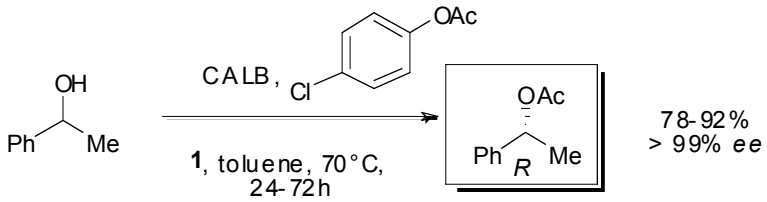
enantiotopos-differentiating hydrolysis

Dynamic kinetic resolutions

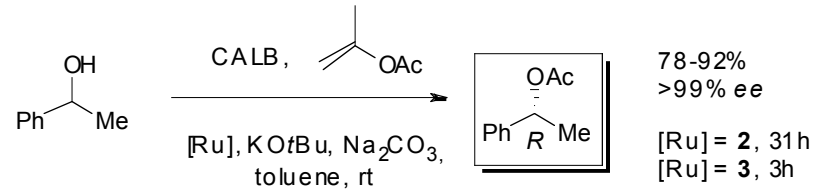
enzyme-metal combination

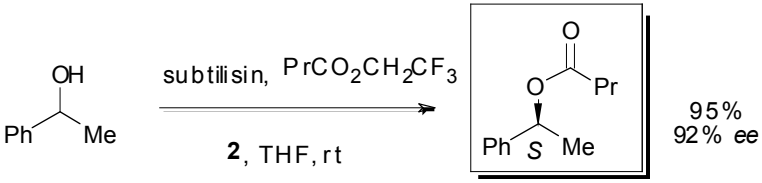


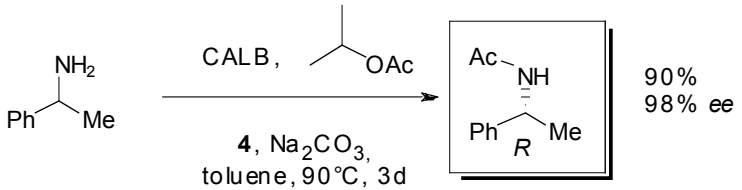
Ruthenium-catalyzed reactions



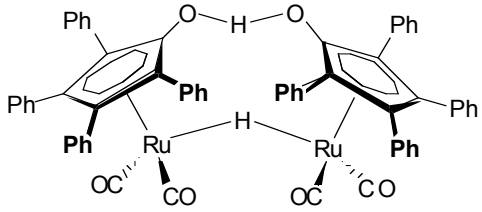
used for the production of *R*-phenylethanol by DSM



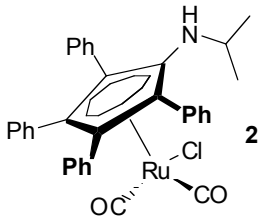


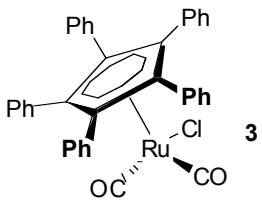


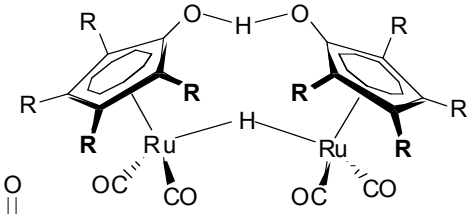
Ru-catalysts:

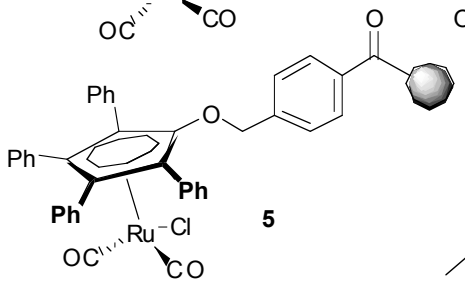


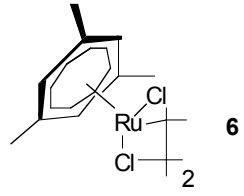
Shov's catalyst **1**





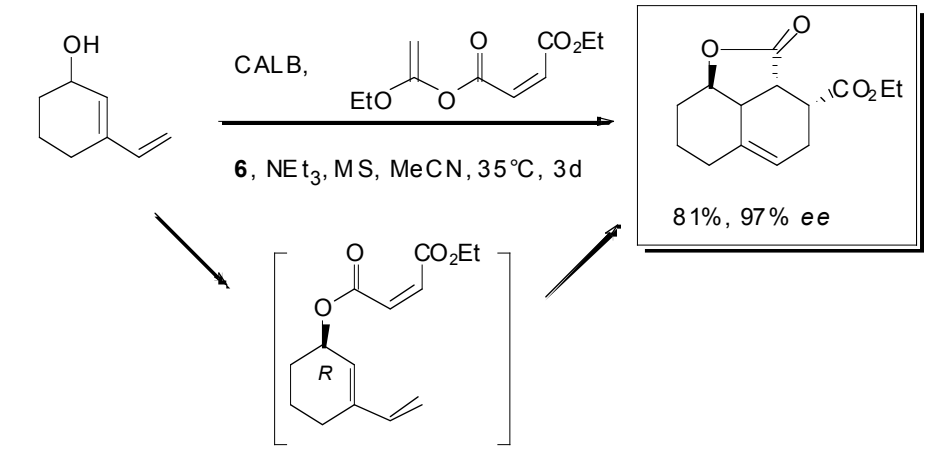




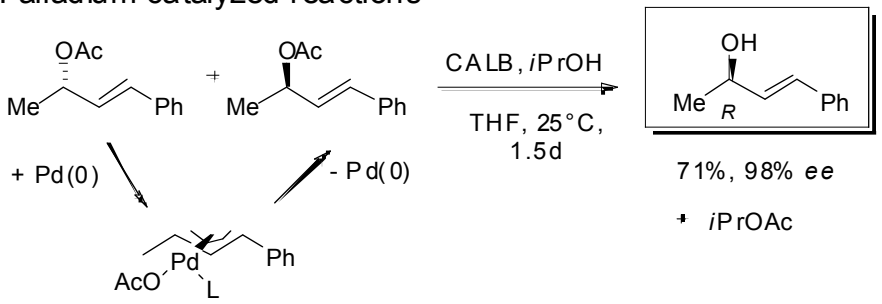


Biocatalysis

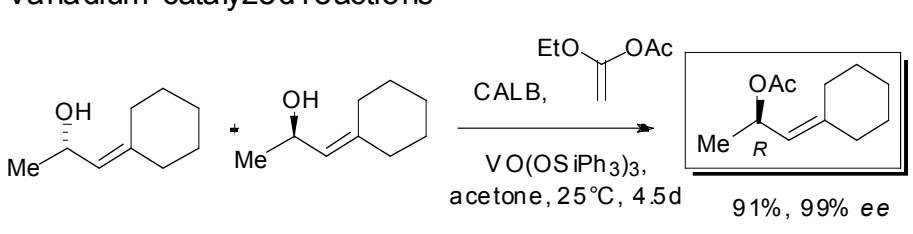
Tandem-DRK-Diels-Alder reaction



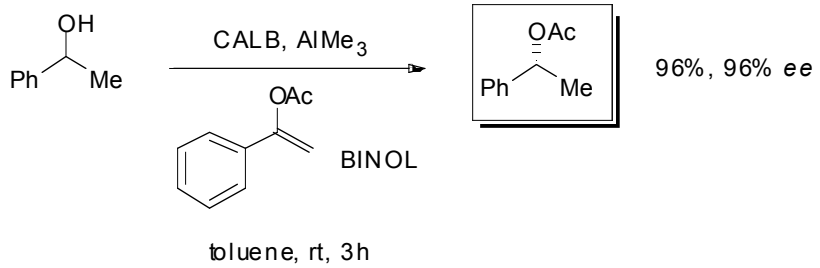
Palladium-catalyzed reactions



Vanadium-catalyzed reactions

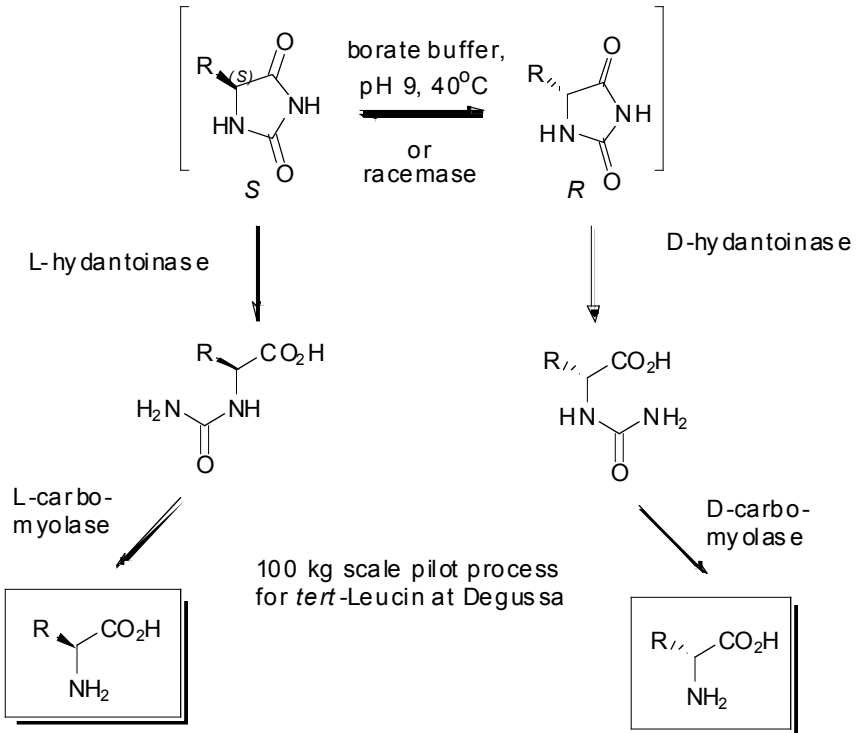


Meerwein-Ponndorf-Verley-Oppenauer reaction

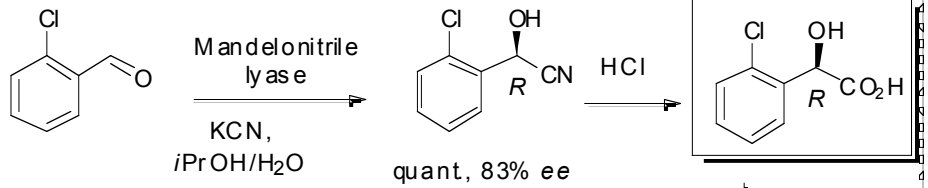


DRK with enzyme-base combination

Hydantoinase-carbamylase system

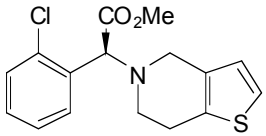


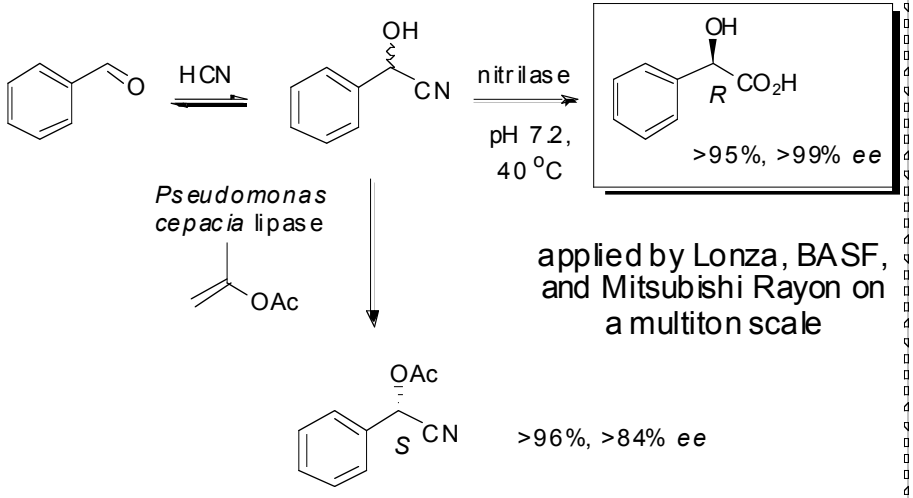
Cyanohydrine-mediated DRK



produced by DSM Chemie Linz, Nippon Shokubai, Clariant

antiplatelet clopidogrel (Plavix)





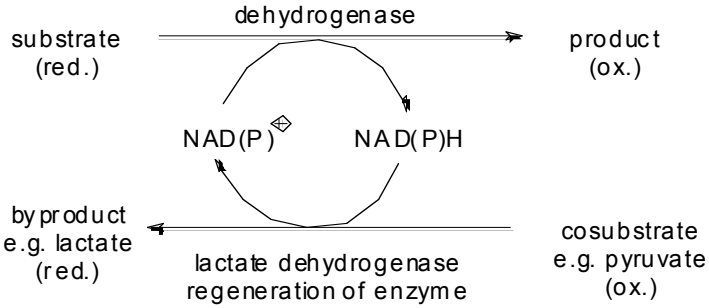
applied by Lonza, BASF, and Mitsubishi Rayon on a multiton scale

nonselective nitrile hydratase: *Rhodococcus rhodochrous J1*
- acrylamide production (Nitto process, > 20 000 t/a)
- nicotinamide synthesis (Lonza, 3000 t/a)

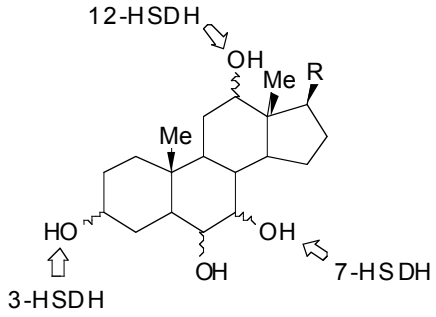
Oxidations

drawback: co-factor dependence of oxidases/reductases

solutions: - closed-loop systems with an additional enzyme for co-factor regeneration
- electrochemical co-factor recycling
- application of metals for regeneration
- living whole cells



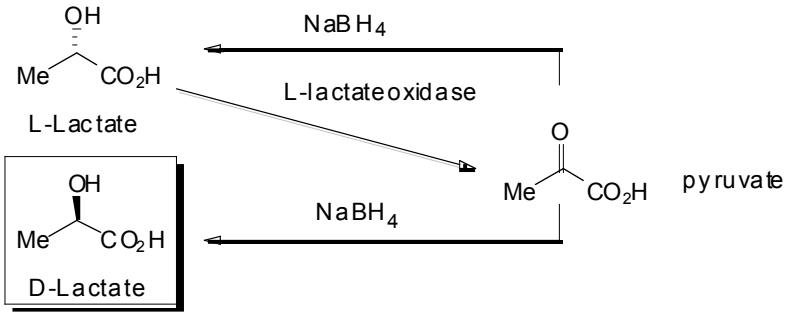
Oxidations of alcohols and amines



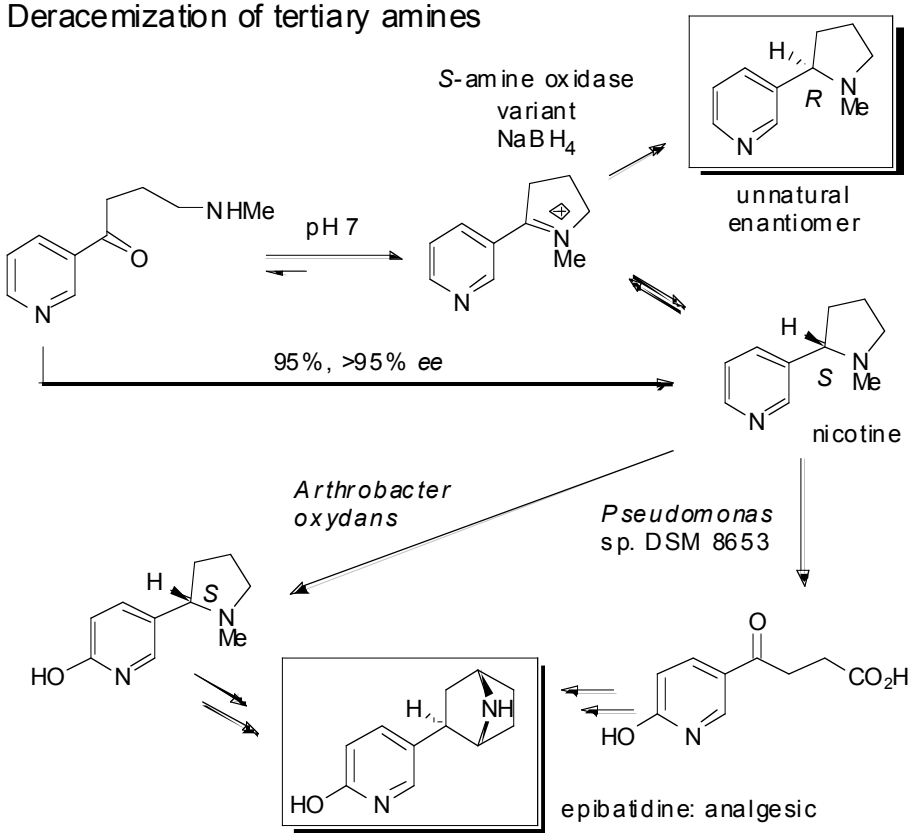
regioselective oxidations of bile acid depending on hydroxysteroid dehydrogenase used

Biocatalysis

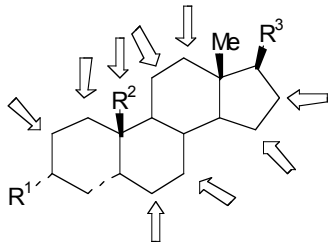
Deracemization of secondary alcohols



Deracemization of tertiary amines

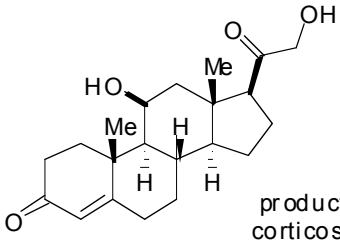


Oxygenation of nonactivated carbon centers

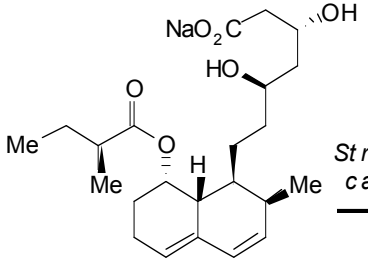


R^1 = O, OH
R^2 = H, Me

almost all C atoms at the steroid nucleus can be hydroxylated stereospecifically



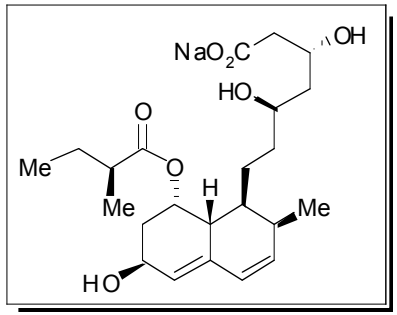
production of corticosterone



ML 236B
from *Penicillium citrinum*

Streptomyces carbophilus

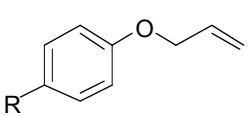
70%



pravastatin
(Pravachol)

produced by BMS and Sankyo Pharma
3.6 billion US Dollar annual market value

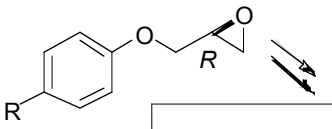
Epoxidation

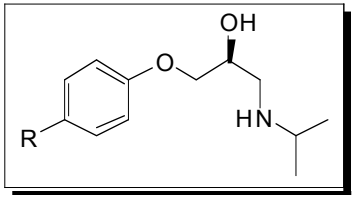


R = CH_2CH_2OMe

Rhodococcus equi

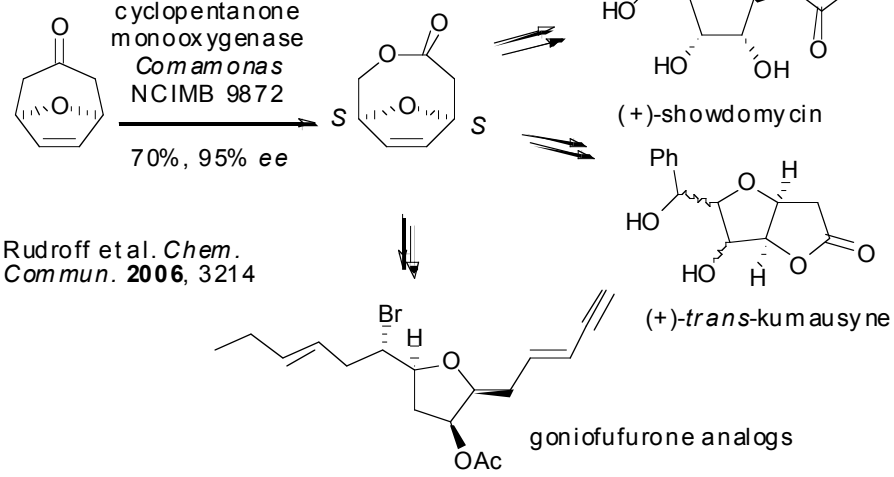
70%





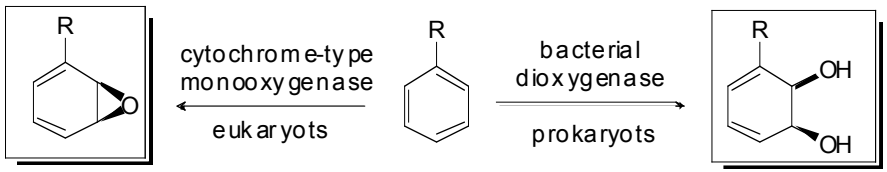
metoprolol
Lopresor/Toprol-XL
hypertension

Baeyer-Villiger-Oxidations

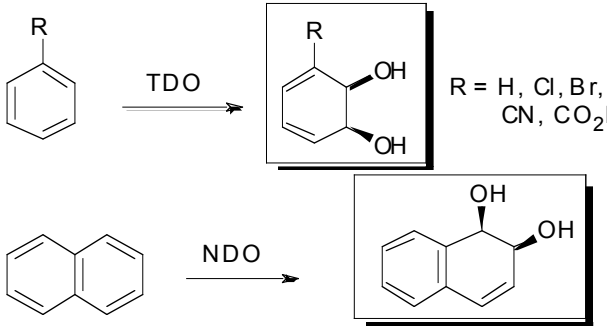


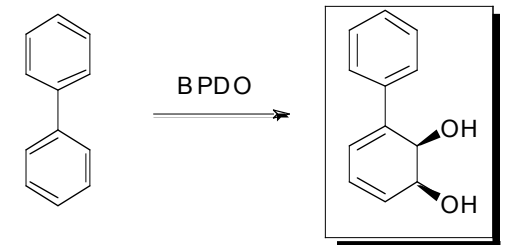
Rudroff et al. *Chem. Commun.* **2006**, 3214

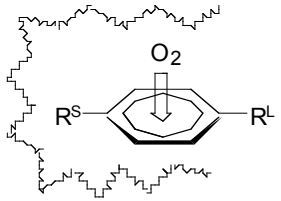
Aryl dihydroxylations



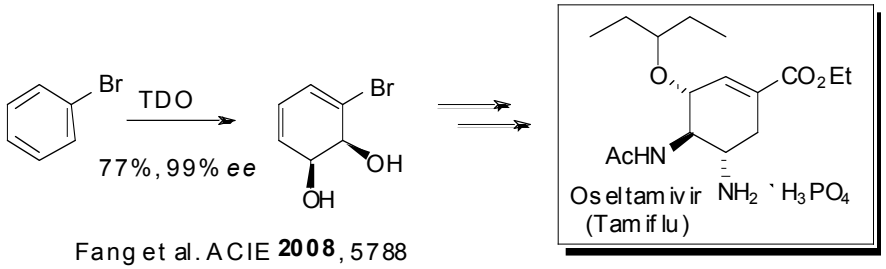
ortho and *meta* hydroxylation occurs using toluene (TDO, *Pseudomonas putida* F39/D), naphthalene (NDO, *P. putida* 119), or biphenyl dioxygenases (BPDO, *Sphingomonas yanoikuyae* B8/36)



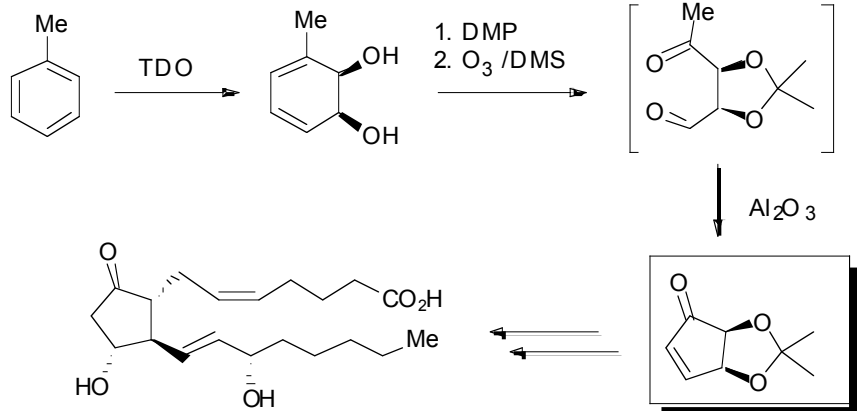




model for predicting the regio- and stereochemical course for the *cis* selective dihydroxylation reaction



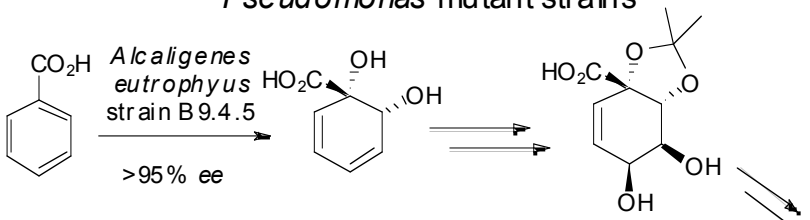
Fang et al. ACIE **2008**, 5788



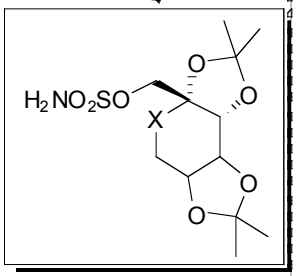
Hudlicky et al. *JACS* **1988**, 4735

Biocatalysis

ipso and *ortho* dioxygenations possible with *Ralstonia* and *Pseudomonas* mutant strains



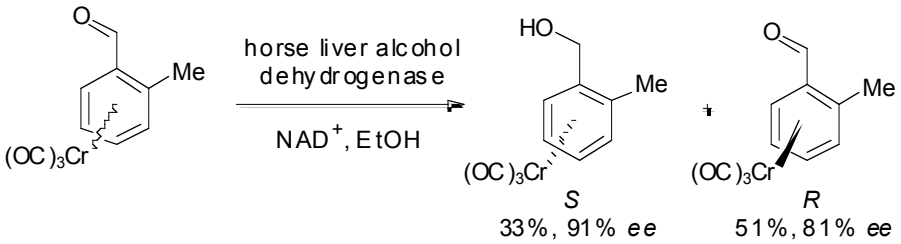
Parker et al. *Synlett* **2004**, 2095



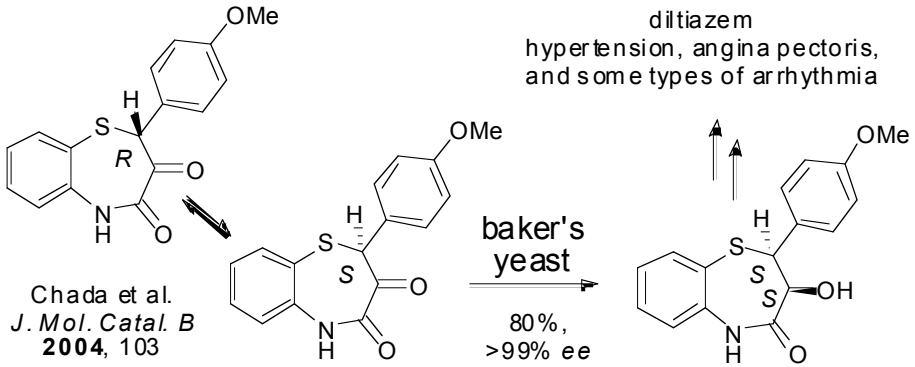
X = O: topiramate
anti-epilepsy, anti-migrane

Reductions

Reduction of aldehydes

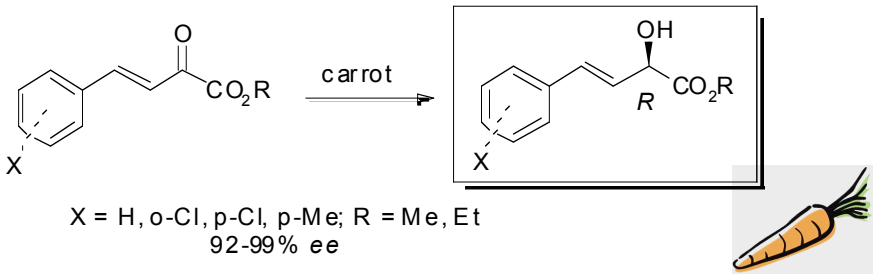


Reduction of ketones



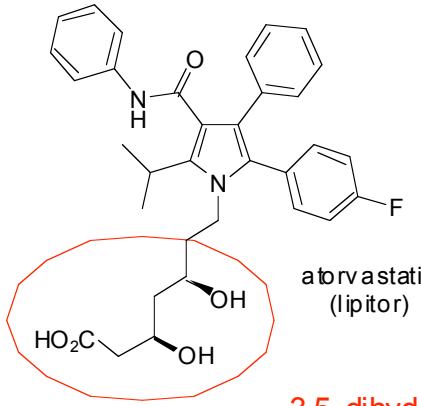
Chada et al.
J. Mol. Catal. B **2004**, 103

diltiazem
hypertension, angina pectoris, and some types of arrhythmia



X = H, o-Cl, p-Cl, p-Me; R = Me, Et
92-99% ee

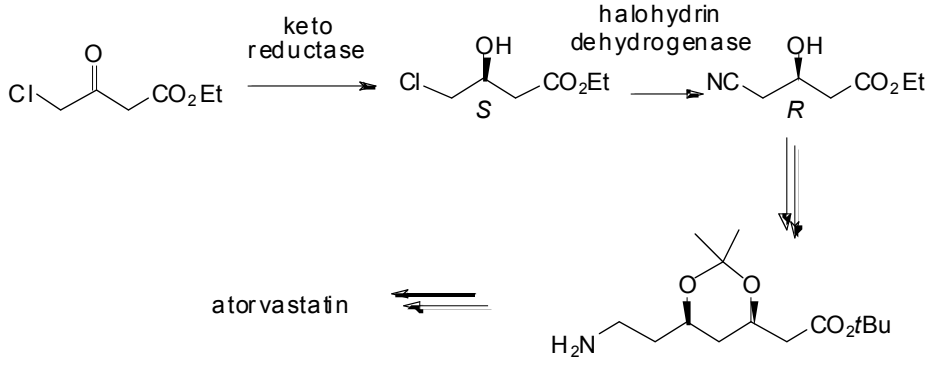
Formation of the 3,5-dihydroxy side chain in statins



atorvastatin
(lipitor)

produced by Pfizer
12.4 billion US Dollar **2008**

3,5-dihydroxy side chain common in all statins

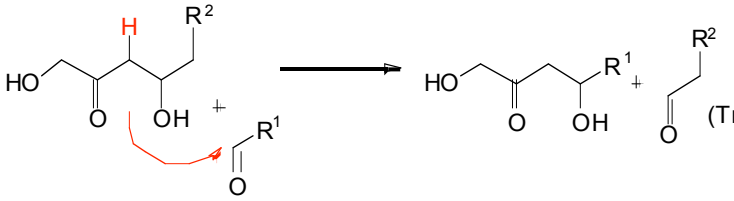


Carbon-Carbon coupling reactions

stereodivergent product generation possible using stereocomplementary enzymes

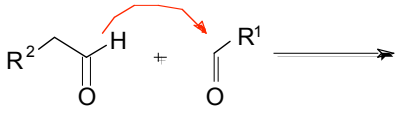
Aldolreactions

Aldolase
(Lyase class)

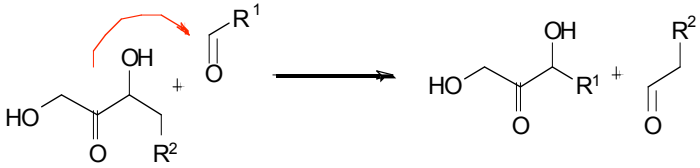


Aldolase/
Transaldolase
(Transferase class)

Thiamine diphosphate dependent conversions



Ketolase
(Lyase class)



Ketolase/
Transketolase
(Transferase class)

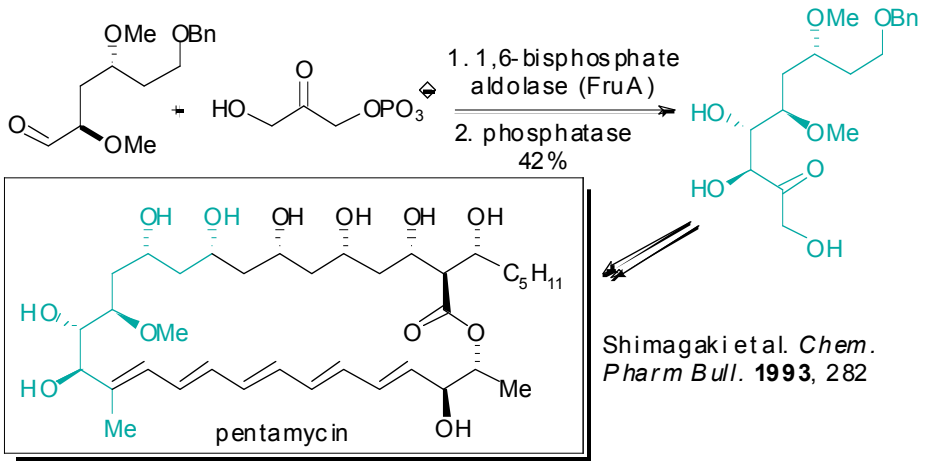
Enzyme classification dependent on the nucleophile:

1. pyruvate-dependent aldolase
2. dihydroxyacetone phosphate (DHAP)-dependend aldolase
3. acetaldehyde-dependent aldolase
4. glycine-dependent aldolase

dihydroxyacetone phosphate (DHAP)-dependend aldolase

-> generation of 2 stereocenters

1,6-bisphosphate aldolase (FruA)

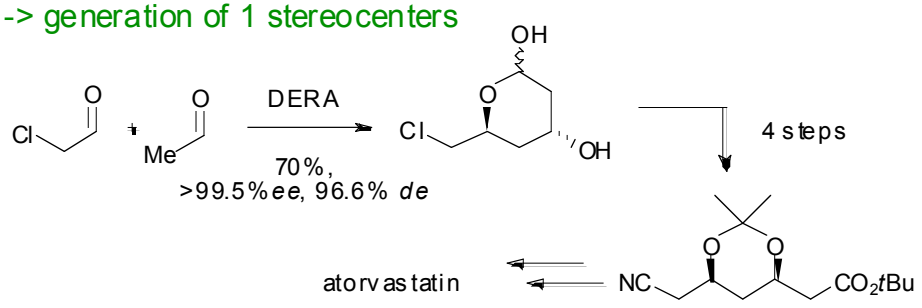


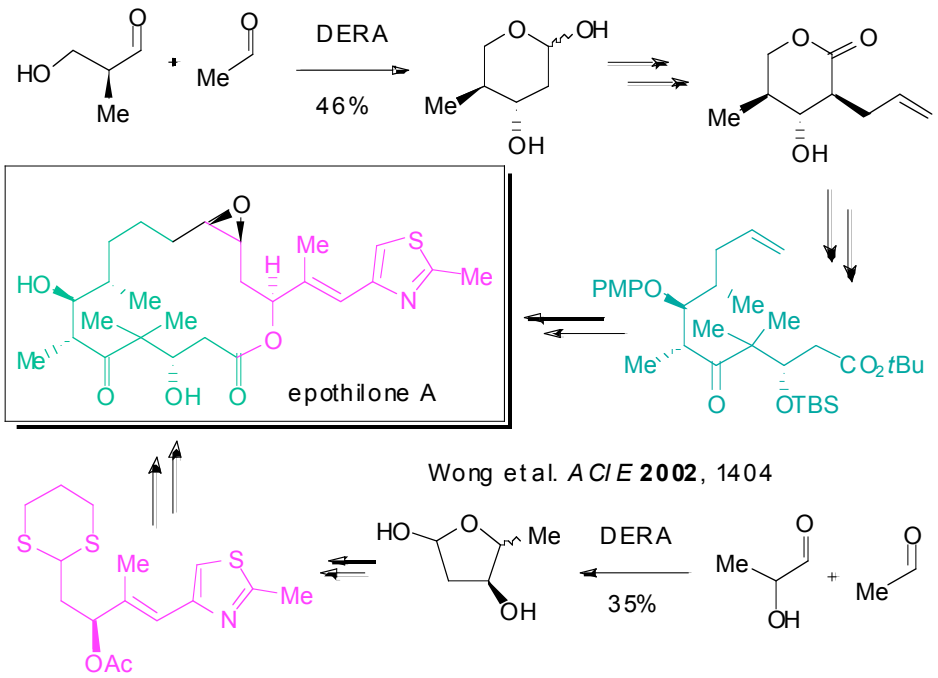
Shimagaki et al. *Chem. Pharm Bull.* **1993**, 282

acetaldehyde-dependent aldolase

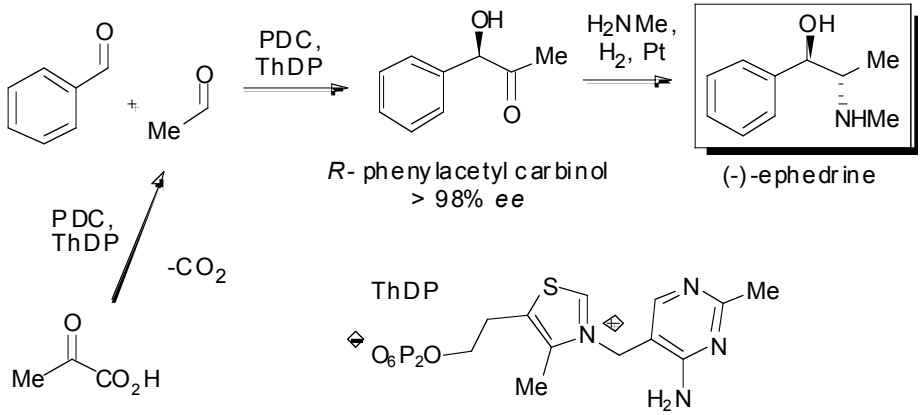
2-deoxyribose-5-phosphate aldolase (DERA)

-> generation of 1 stereocenters



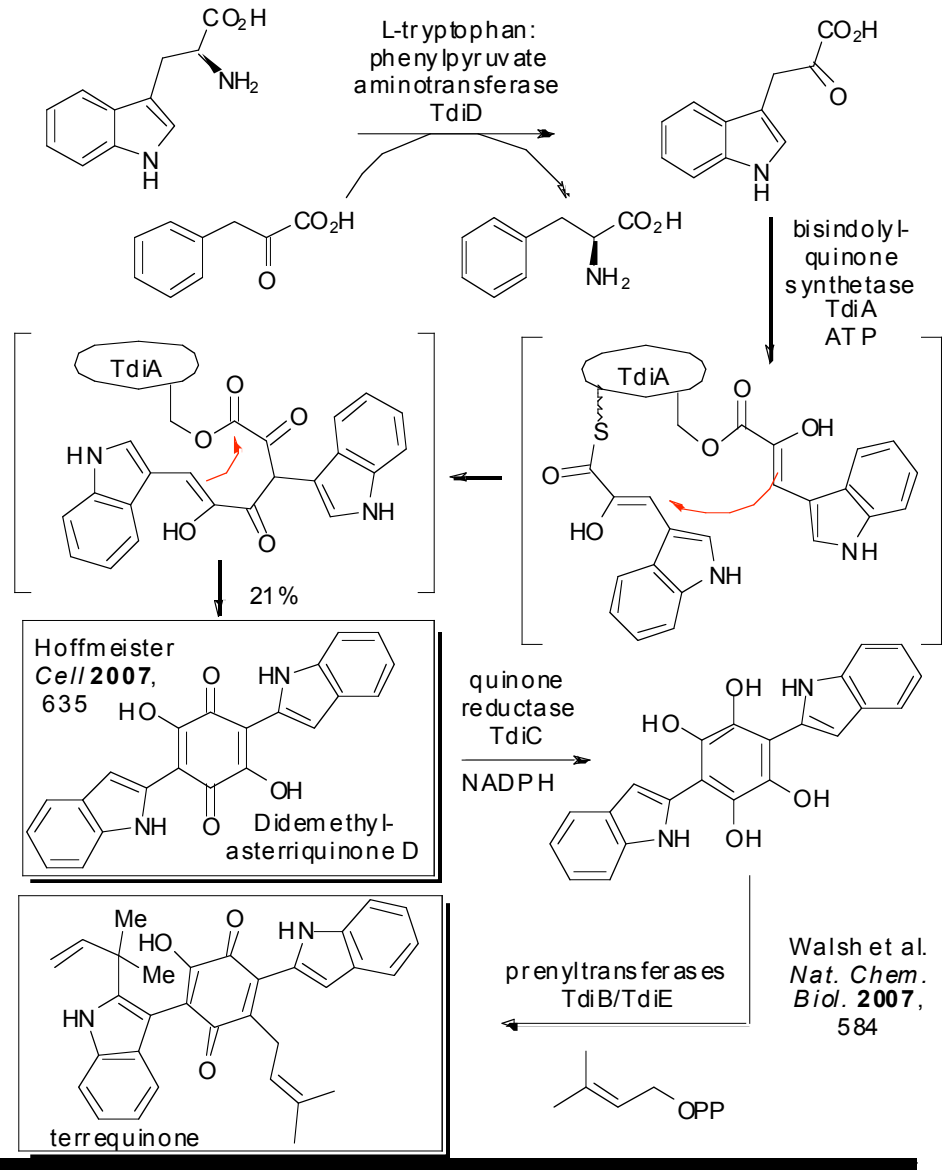


Pyruvate decarboxylase (PDC, thiamin diphosphate depended)



Total Synthesis of Natural Products

in vitro reconstitution of complete biosynthetic pathways



Biocatalysis