CHEM-E4109

MODERN METHODS IN BIOCATALYSIS

chapter #8: ester, amide & epoxide hydrolysis

25.3.2022

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TODAY'S MENU

last chapter on specific enzyme classes

- overview on hydrolases
- focus on serine hydrolases
  - ✓ return of the catalytic triade
  - ✓ stereochemical considerations and strategies
  - ✓ industrial applications

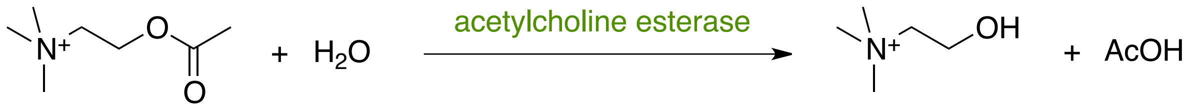
- brief look on epoxide hydrolases

Hydrolases - features

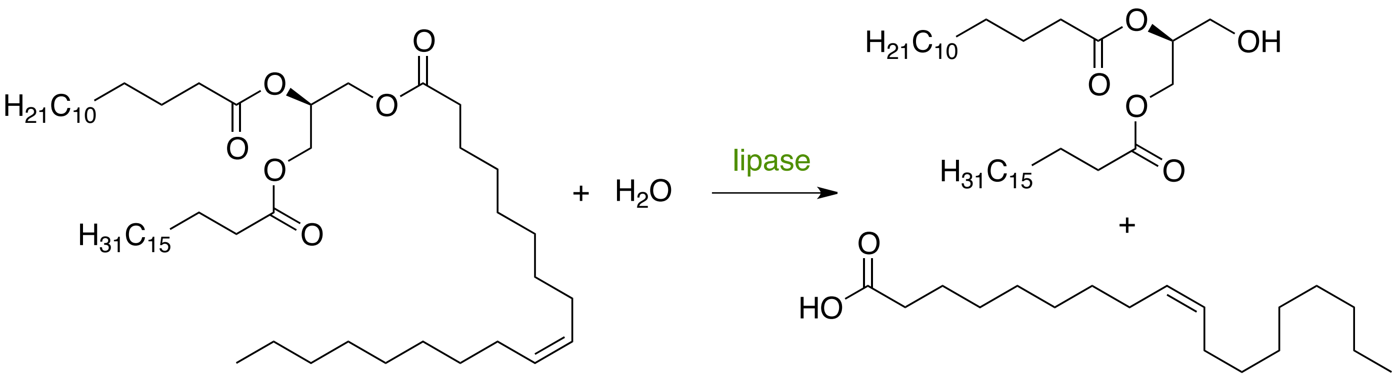
enzymes of class EC 3 catalyze the hydrolytic cleavage of chemical bonds

... and no surprise, sometimes they catalyze dehydrative couplings

ester hydrolase (esterase)



lipase



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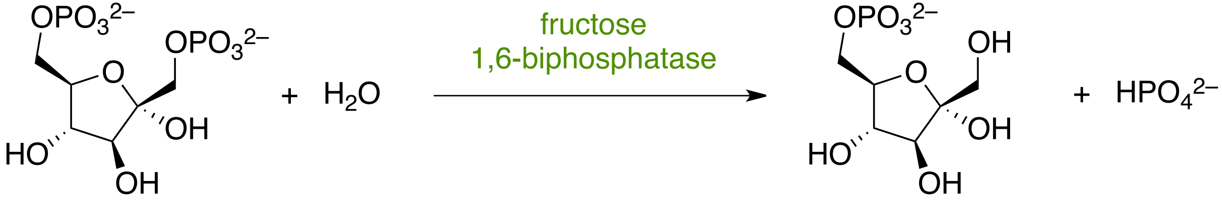
Hydrolases – features

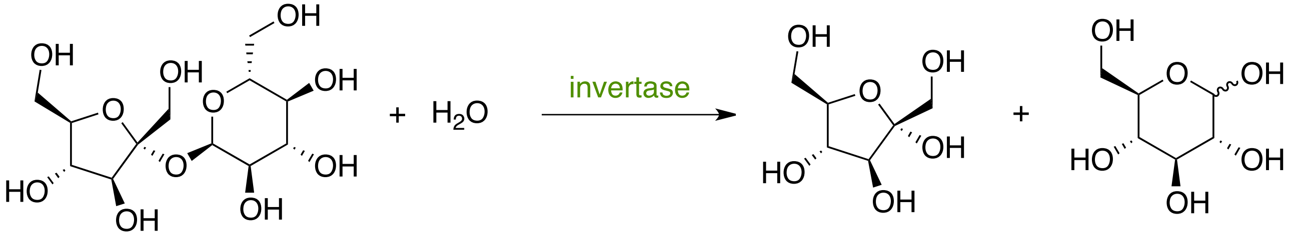
enzymes of class EC 3 catalyze the hydrolytic cleavage of chemical bonds

... and no surprise, sometimes they catalyze dehydrative couplings

phosphatase



glycosidase



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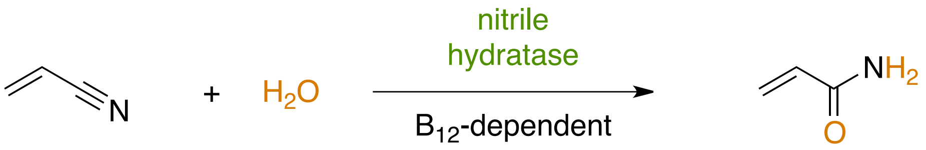
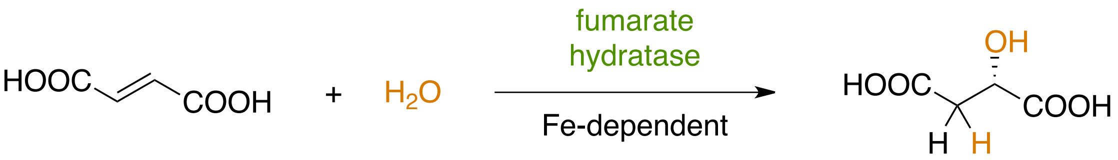
Hydrolases vs hydratases

not to be confused with hydratases (EC 4 = lyases), that catalyse addition of water without bond cleavage

nitrile hydratase



fumarate hydratase



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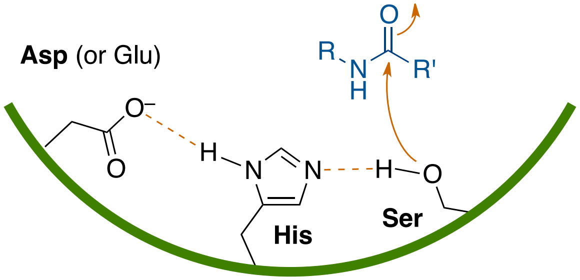
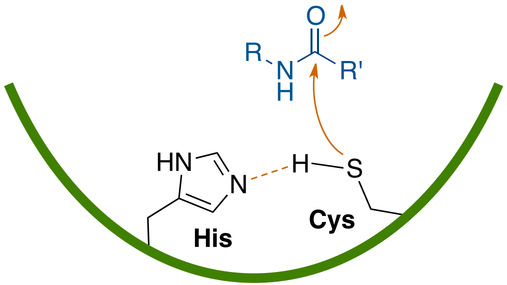
Four major protease families

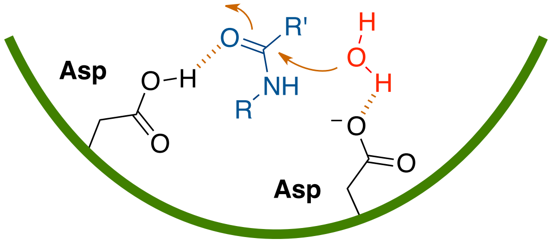
serine hydrolases



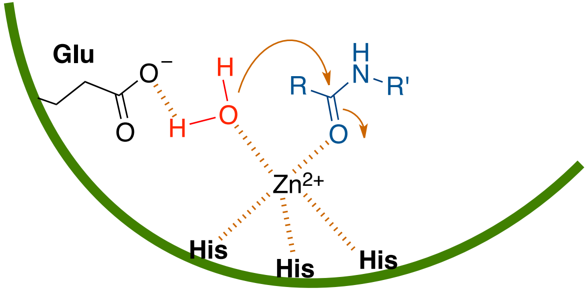
cysteine hydrolases



aspartate hydrolases



metalloproteases



Neitzel, *Nature Education* **2010**, *3*, 21

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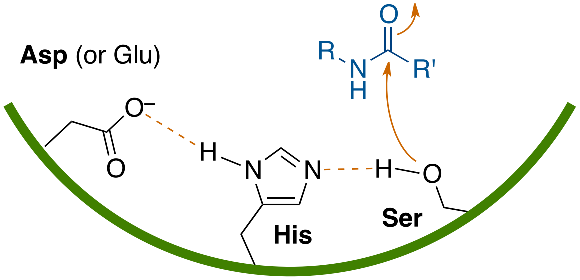
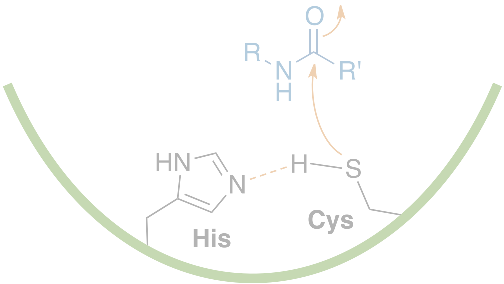
Four major protease families

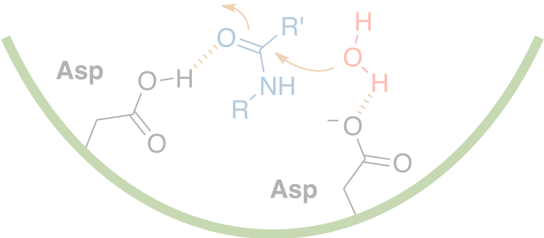
serine hydrolases



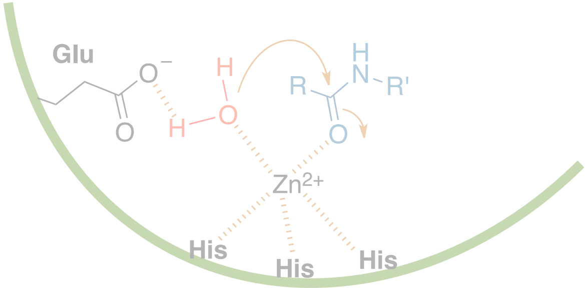
cysteine hydrolases



aspartate hydrolases



metalloproteases



Neitzel, *Nature Education* **2010**, *3*, 21

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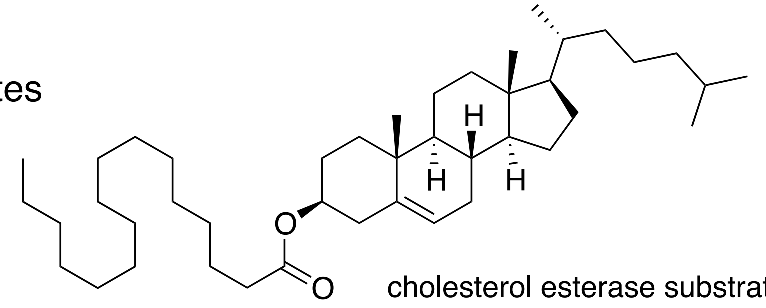
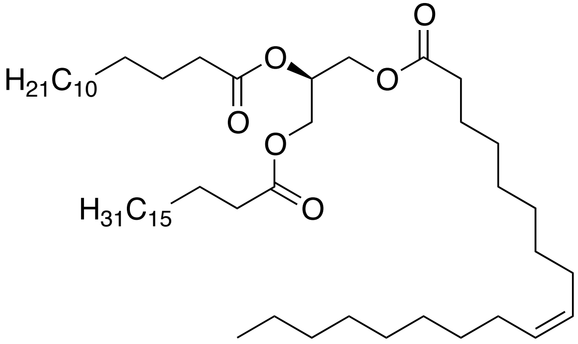
Serine hydrolases

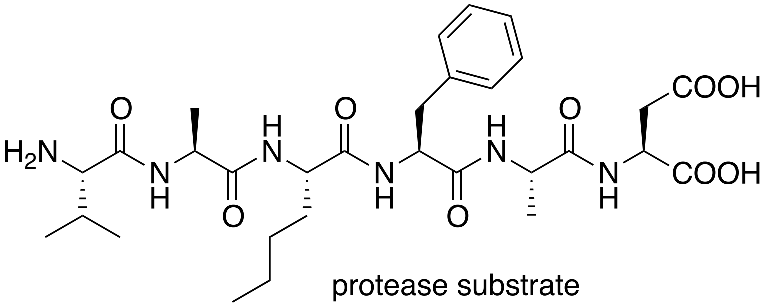
Often highly lipophilic substrates



cholesterol esterase substrate



lipase substrate



protease substrate

✓ enzymes relatively robust and tolerant towards organic reaction media

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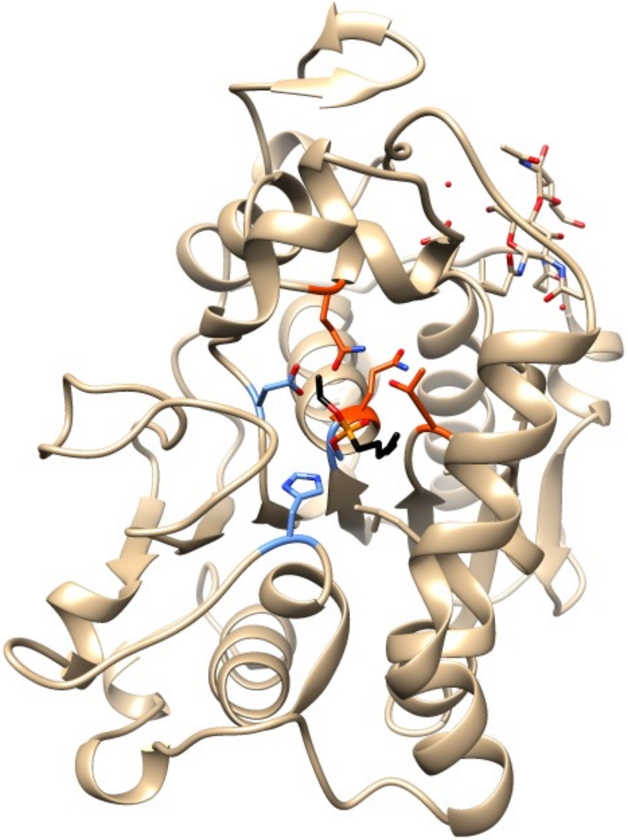
Lipases – the organic chemist's favourite biocatalysts

Lipase, isoform B (*Candida antarctica*)

- monomer, glycoprotein, 317 amino acids + 2 NAG
- no cofactors required
- no structural or catalytic metal ions
- highly active even under non-natural conditions
- tolerates anhydrous unpolar solvents such as heptane, toluene, MTBE, molten salts, ...



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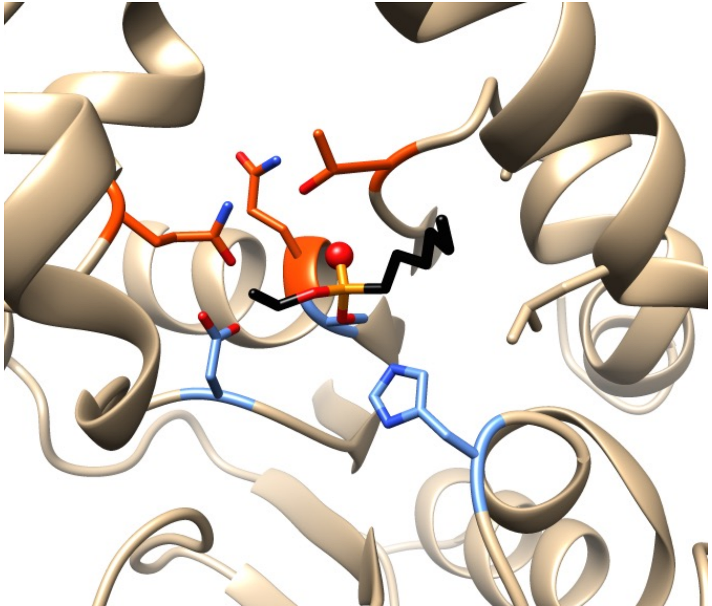
Lipases – the organic chemist's favourite biocatalysts

Lipase, isoform B (*Candida antarctica*)

- monomer, glycoprotein, 317 amino acids + 2 NAG
- no cofactors required
- no structural or catalytic metal ions
- catalyzes ester hydrolysis, amide hydrolysis, amide & ester formation, perhydrolysis, transesterification,...
- catalytic activation based on so-called catalytic triade consisting of serine, histidine and aspartate (blue)
- stabilization of charged intermediated through so-called oxyanion hole based on H-bond donors (Thr, Gly, Gln,...) (orange)



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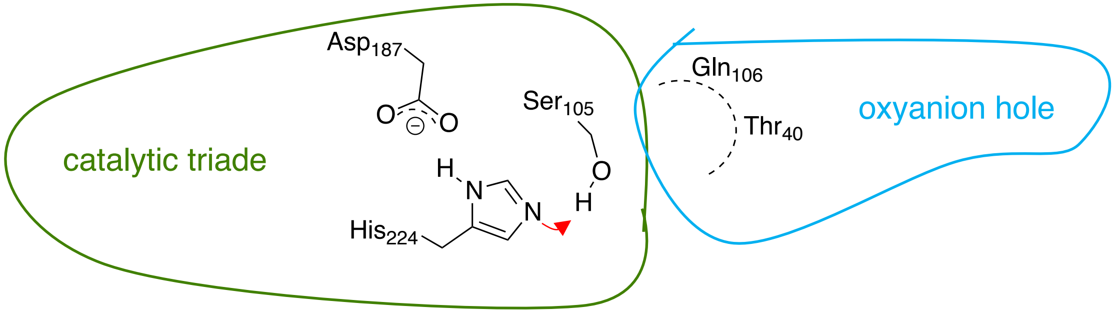
Serine hydrolases – mechanism



Asp-His combination acidifies serine:
formation of alkoxide

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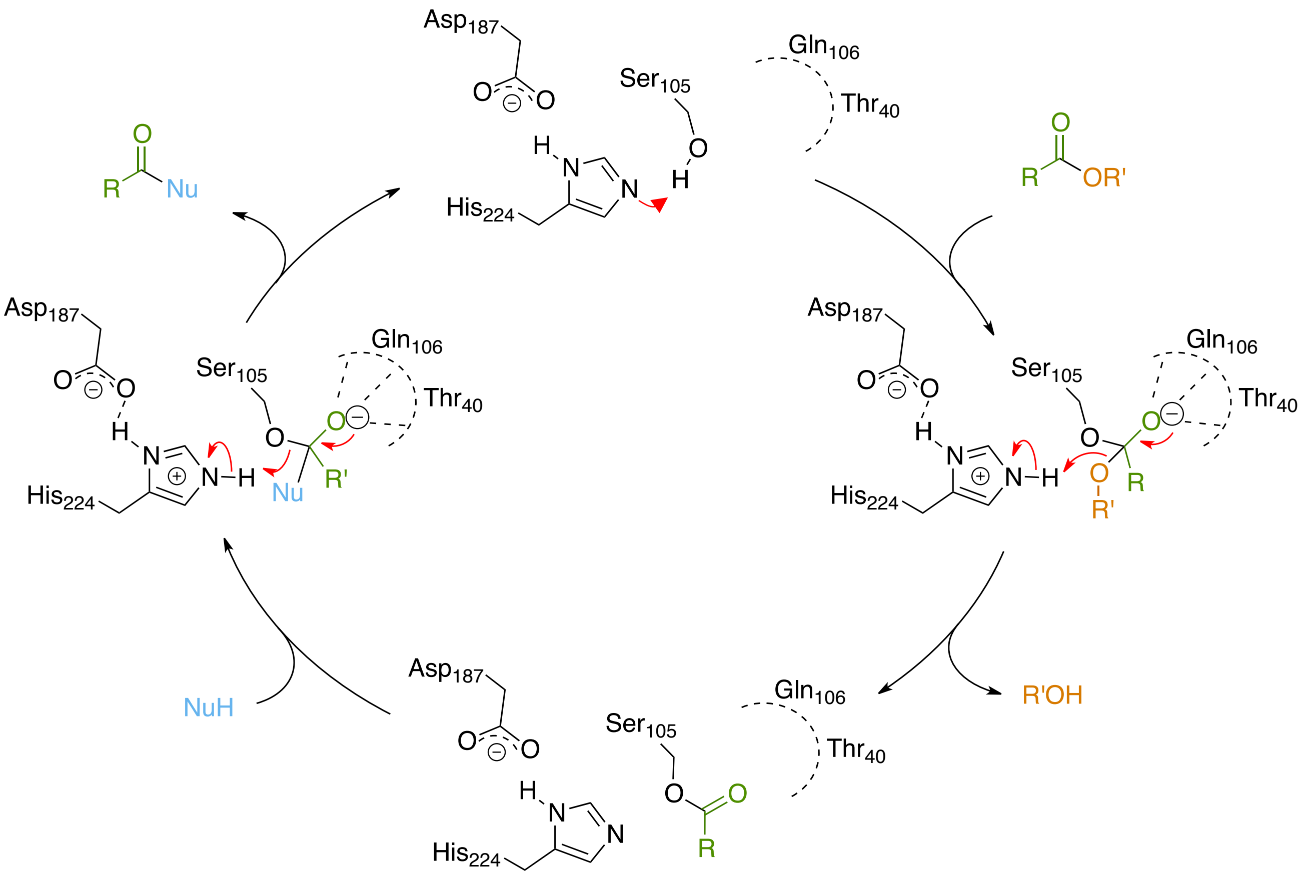
Serine hydrolases – mechanism



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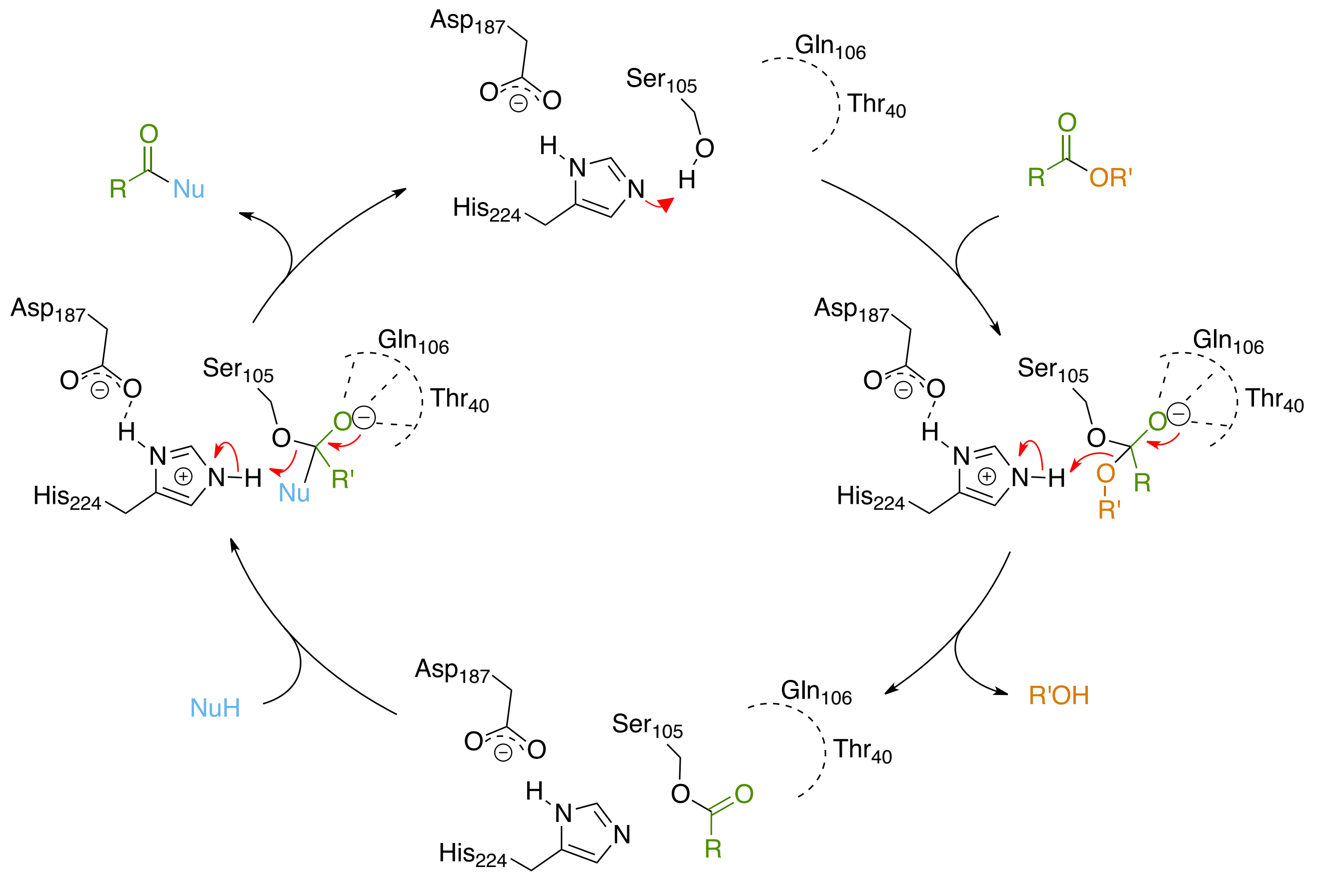
Serine hydrolases – mechanism



enantiodiscrimination of the nucleophile

enantiodiscrimination of the carboxyl donor

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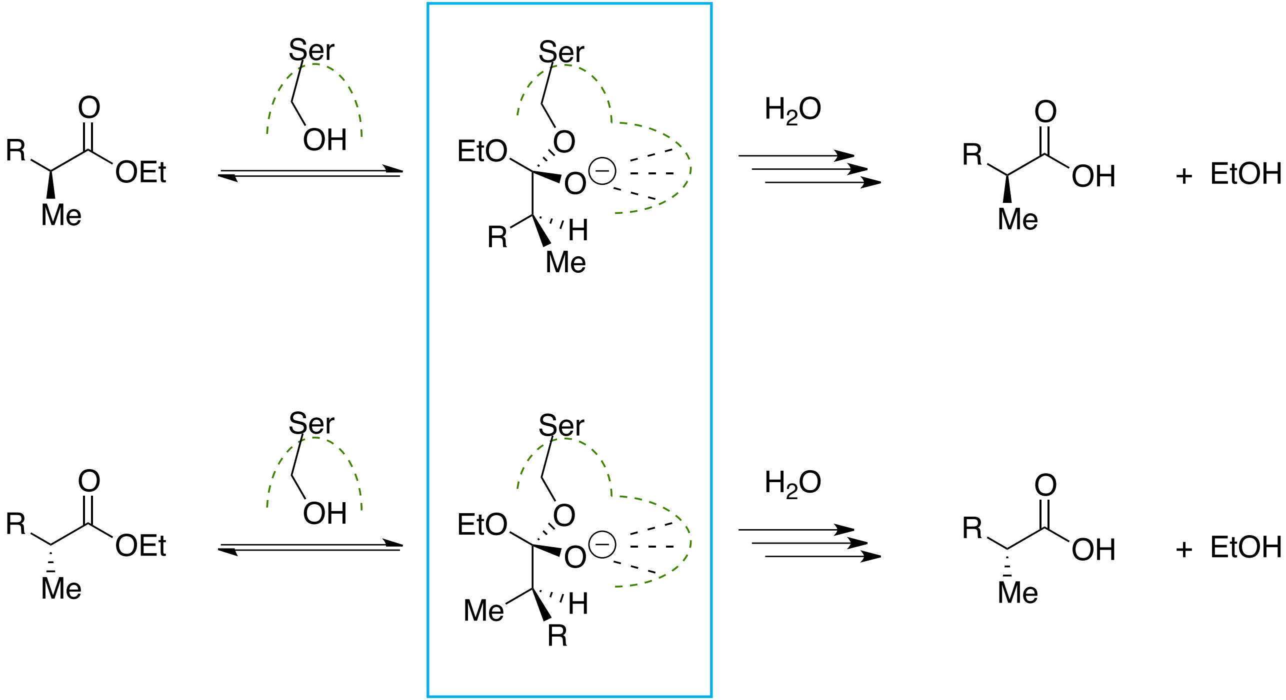
Serine hydrolases – mechanism

First tetrahedral intermediate: electrophile differentiation



diastereomeric intermediates

- ✓ if kinetics between (*R*) and (*S*) differ enough:
- ✓ kinetic resolution of esters, amides, and acids

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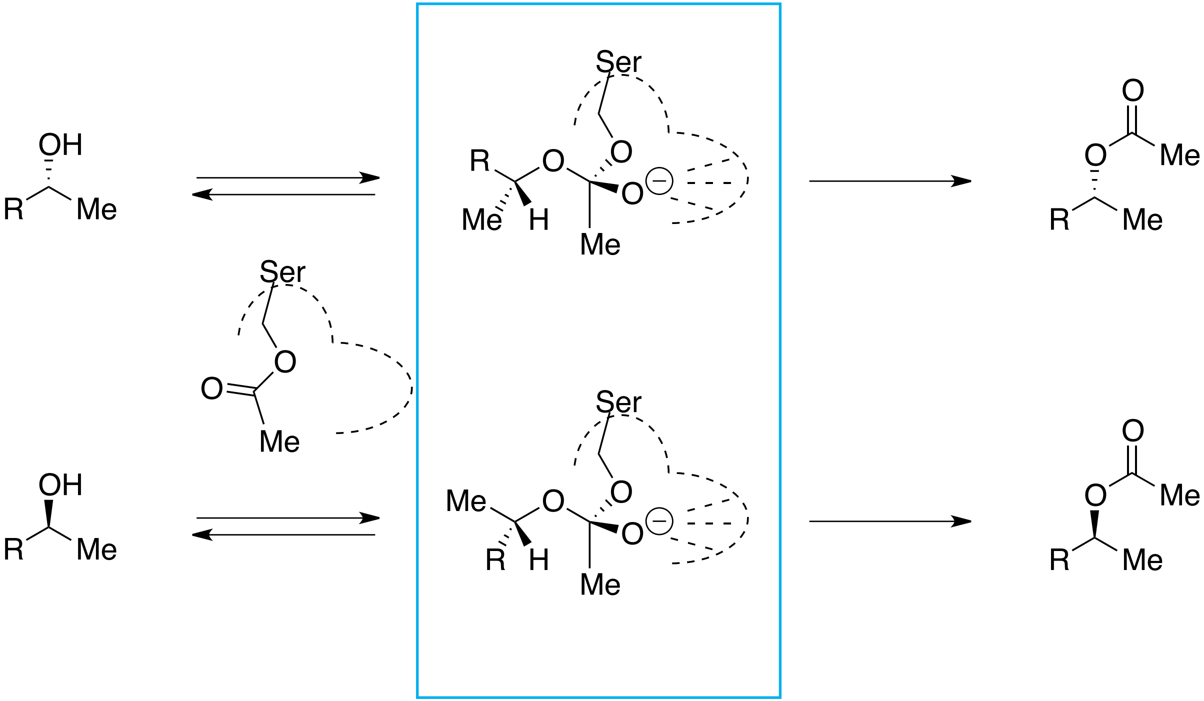
Serine hydrolases – mechanism

Second tetrahedral intermediate: nucleophile differentiation



diastereomeric intermediates

- ✓ if kinetics between (*R*) and (*S*) differ enough:
- ✓ kinetic resolution of alcohols and amines

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Lipases as Swiss Army knives

like no other class of enzymes, lipases fulfil the majority of requirements for the implication in synthetic-chemical processes

- very cheap and readily available (due to its use in washing powders etc.)
- very robust and tolerant to organic solvents (remember: lipases natively interact at the oil/water interface and hence don't bother greasy organics)
- low structural limitations and often wide substrate scope (bulky native substrates)
- surprisingly high stereoselectivities (considering the promiscous handling of non-natural substrates)
- activity on various chemical entities featuring diverse functional groups (amines, alcohols, thiols, and hydroperoxides; esters, amides, and carboxylic acids)
- (plus, some entirely out-of-the box activities such as Michael additions and Baeyer-Villiger oxidations)

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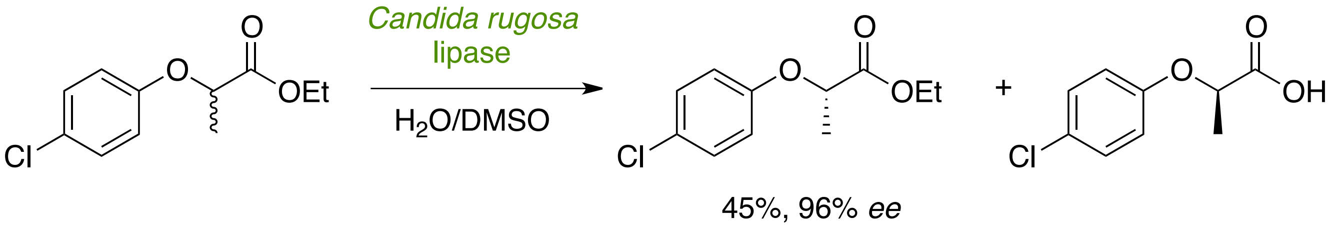
Stereoselective reactions

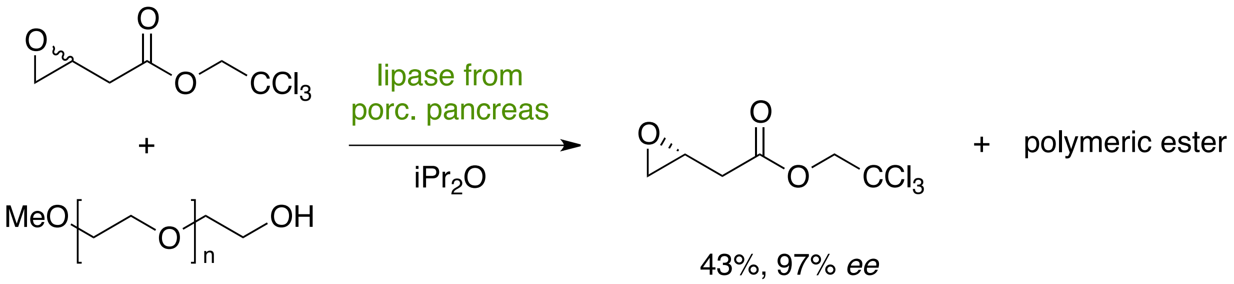
resolution of carboxylates

- stereodifferentiation on the first tetrahedral intermediate
- ester hydrolysis or transesterification more suitable than acid esterification

hydrolytic resolution



resolution via transesterification



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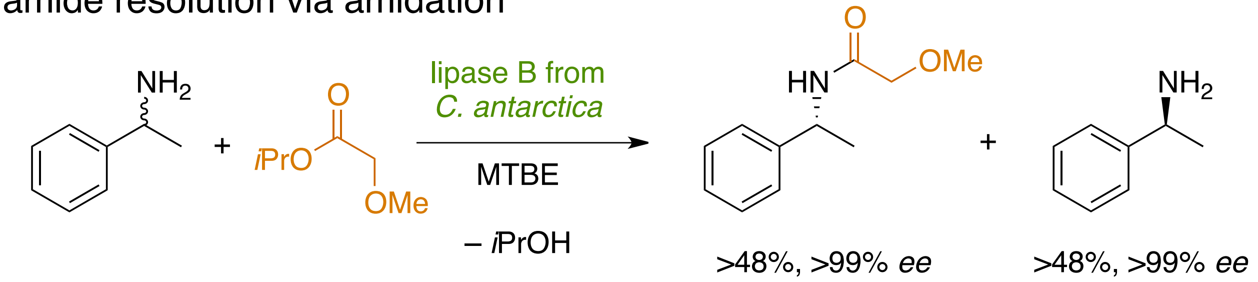
Stereoselective reactions

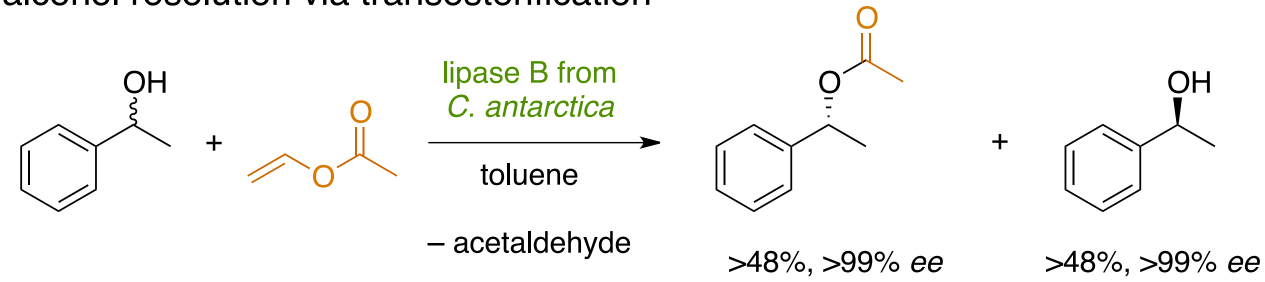
resolution of alcohols and amines

- stereodifferentiation on the second tetrahedral intermediate
- both ester/amide hydrolysis or (trans)esterification suitable, but...

amide resolution via amidation



alcohol resolution via transesterification



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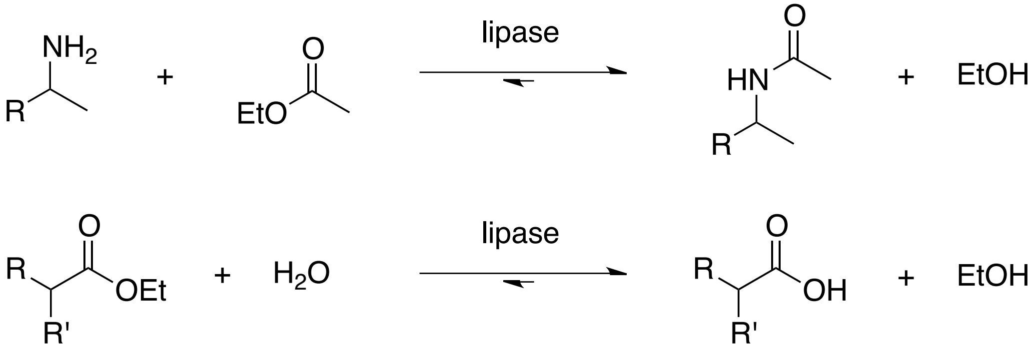
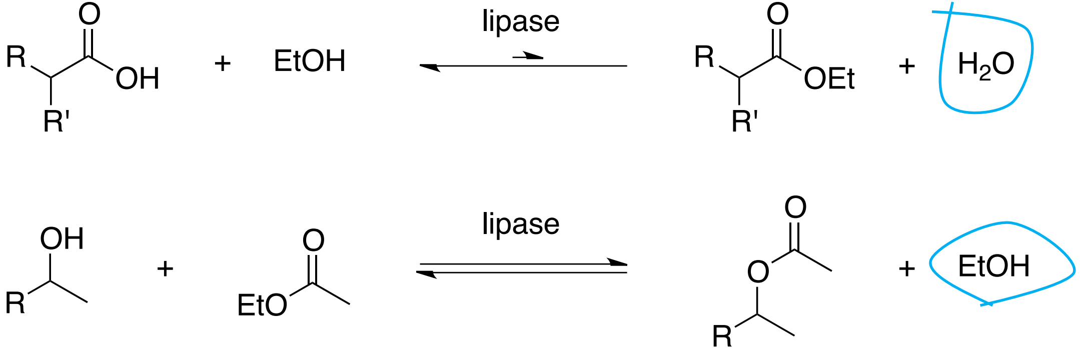
Reversibility in lipase-catalyzed reactions

sometimes the reaction equilibrium doesn't favour the desired product



but:



water removal
by desiccants

specialized acyl
donors required

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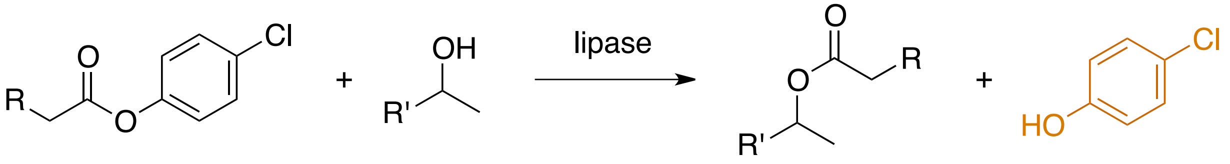
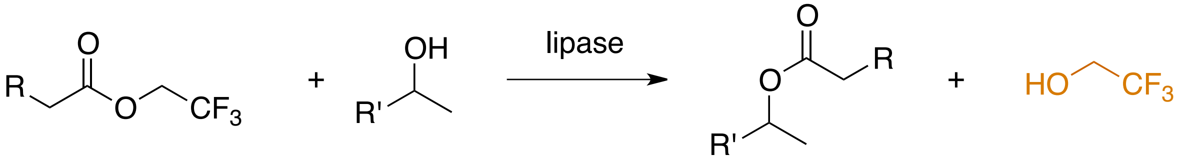
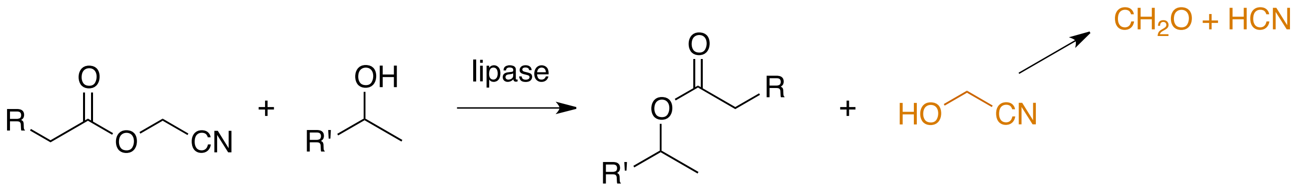
Reversibility in lipase-catalyzed reactions

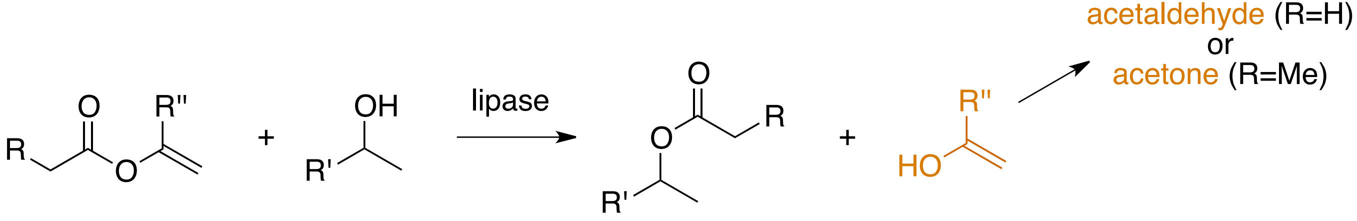
donors releasing poor nucleophiles





donors releasing instable entities to colapse to non-nucleophilic compounds





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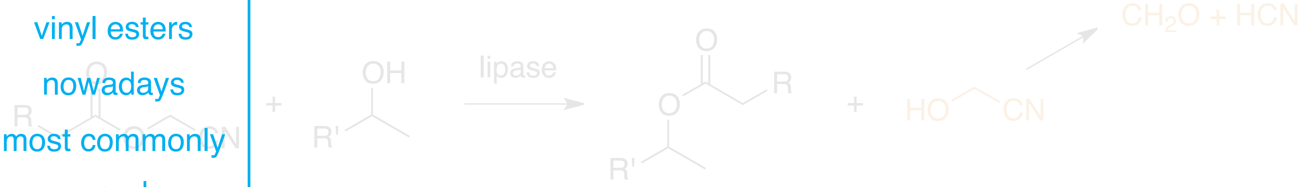
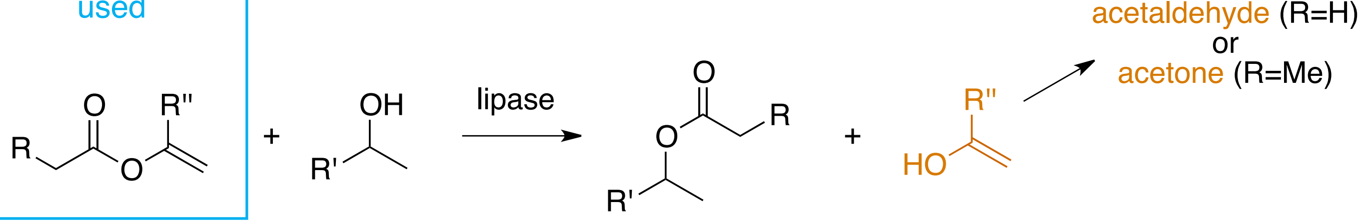
Reversibility in lipase-catalyzed reactions

donors releasing poor nucleophiles

donors releasing instable entities to colapse to non-nucleophilic compounds



vinyl esters nowadays most commonly used



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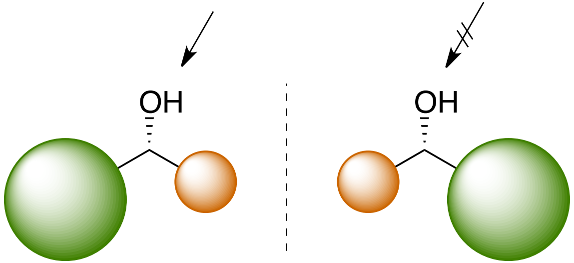
Kazlauskas' rules

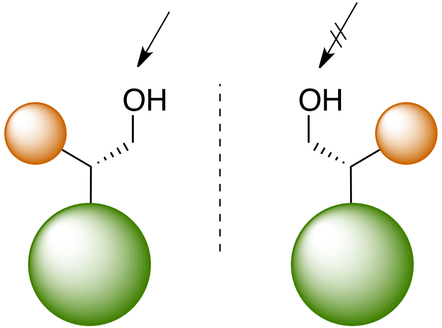
Kazlauskas proposed an empirical guideline to predict selectivities based on the sterics of the substituents

fast reacting enantiomer



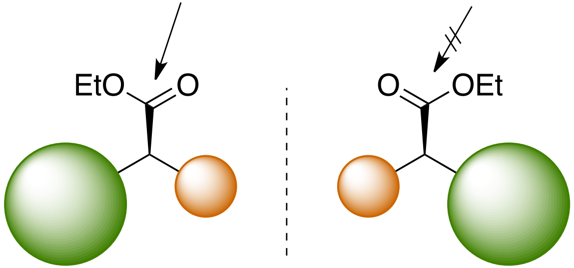
secondary alcohols (and amines)

fast reacting enantiomer



primary alcohols (and amines)

fast reacting enantiomer



α-chiral ester (and amides/acids)

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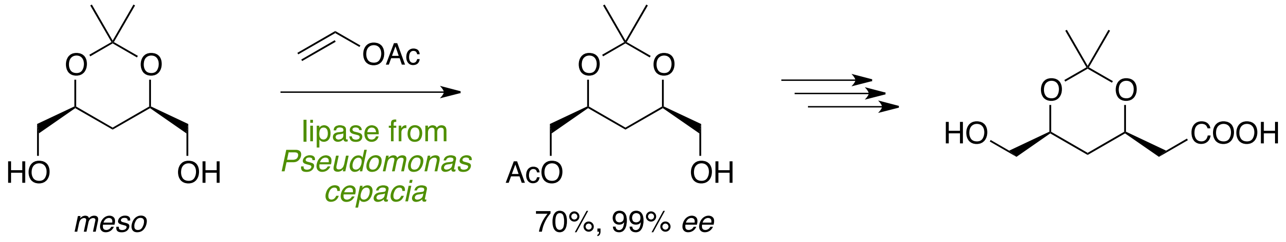
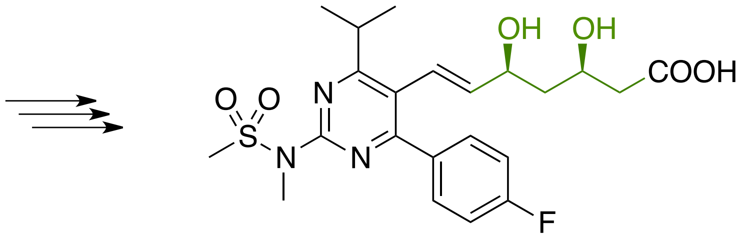
Overcoming yield limitations in kinetic resolutions

enantioselective desymmetrization of prochiral or *meso* difunctional compounds





rosuvastatin
(cholesterol lowerer)

Miyazawa, Yoshida, *Chem. Lett.* **1993**, 1529.

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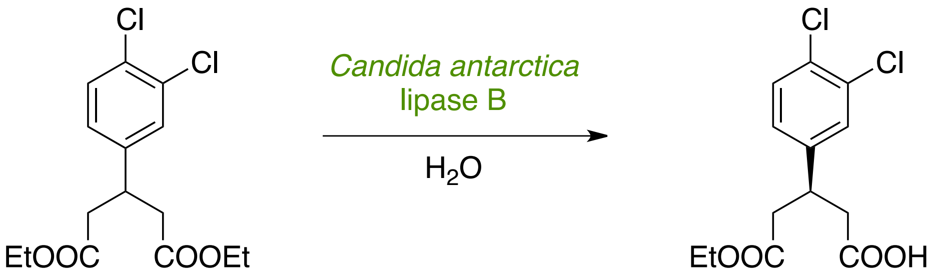
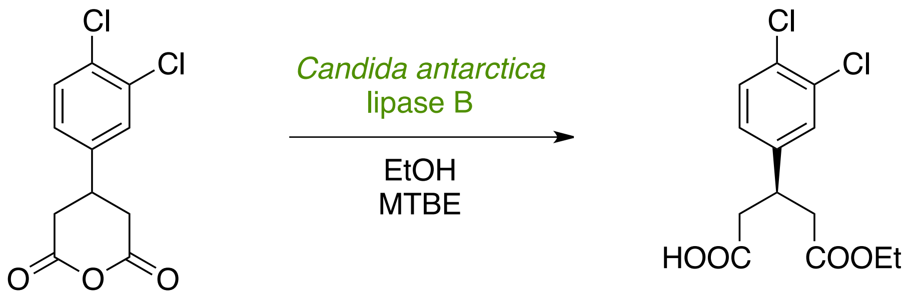
Stereochemistry of desymmetrizations

via de-esterification



82%, 99% *ee* (*S*)

via esterification



98%, 71% *ee* (*R*)

Homann, Vail, Morgan, Sabesan, Levy, Dodds, Zaks, *Adv. Synth. Catal.* **2001**, *343*, 744.

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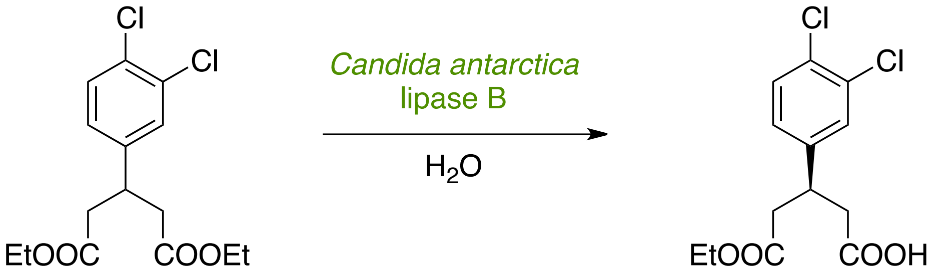
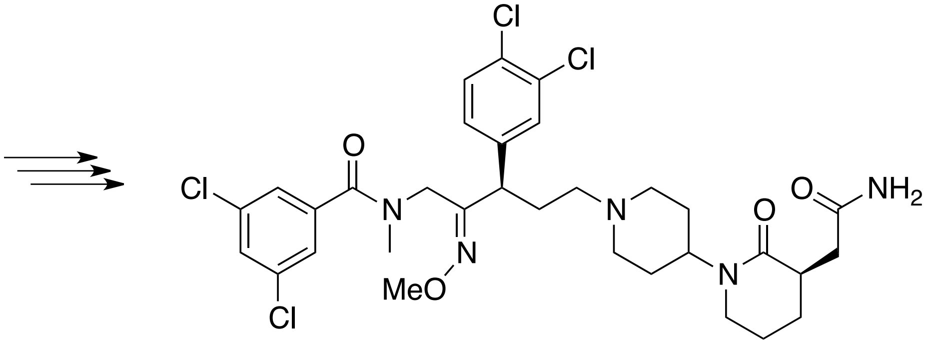
Stereochemistry of desymmetrizations

via de-esterification



82%, 99% *ee* (*S*)

9.7 kg



SCH 206272

(Neurokin receptor antagonist,
Schering-Plough)

Homann, Vail, Morgan, Sabesan, Levy, Dodds, Zaks, *Adv. Synth. Catal.* **2001**, *343*, 744.

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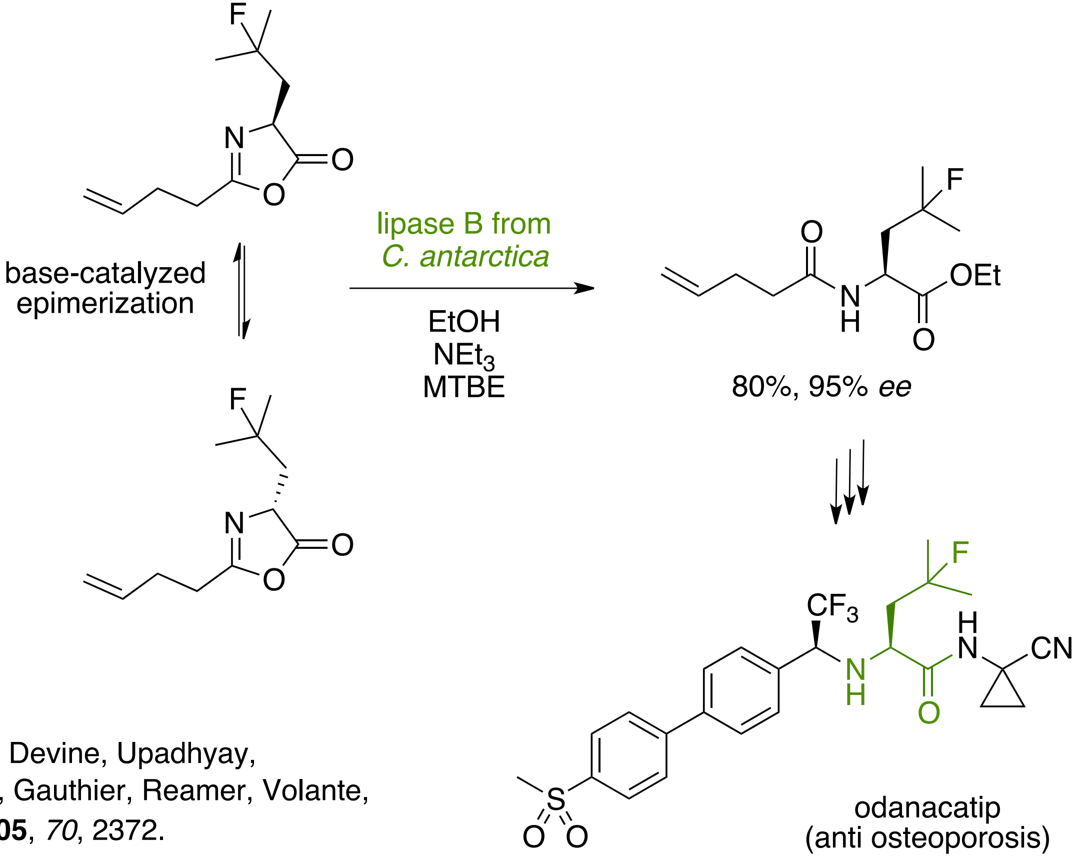
Overcoming yield limitations in kinetic resolutions

dynamic kinetic resolution of configurationally labile compounds



Limanto, Shafiee, Devine, Upadhyay, Desmond, Foster, Gauthier, Reamer, Volante, *J. Org. Chem.* **2005**, *70*, 2372.

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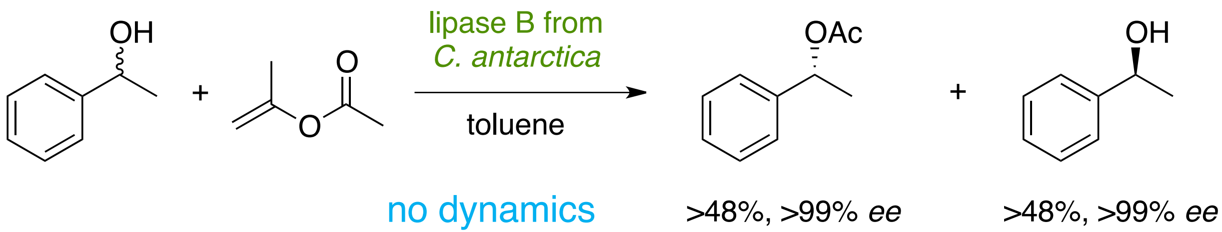
Overcoming yield limitations in kinetic resolutions

dynamic kinetic resolution of configurationally inert compounds?



Martín-Matute, Edin, Bogár, Kaynak, Bäckvall, *J. Am. Chem. Soc.* **2005**, *127*, 8817.

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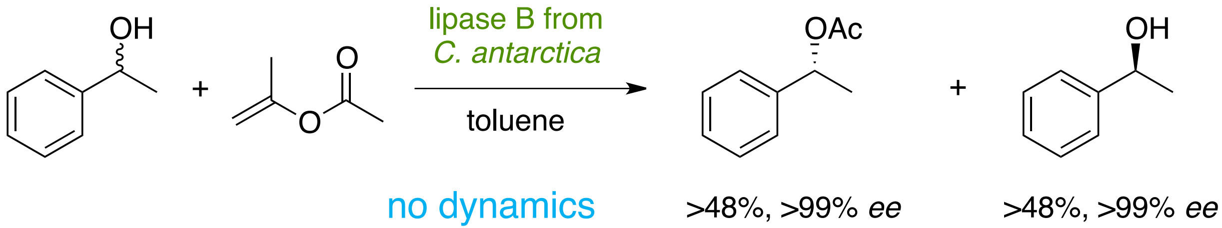
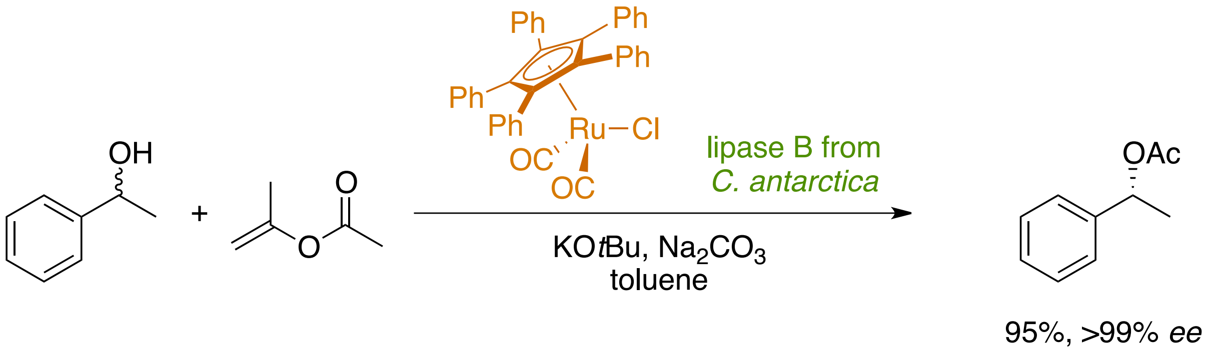
Overcoming yield limitations in kinetic resolutions

dynamic kinetic resolution of configurationally inert compounds?



but:



Martín-Matute, Edin, Bogár, Kaynak, Bäckvall, *J. Am. Chem. Soc.* **2005**, *127*, 8817.

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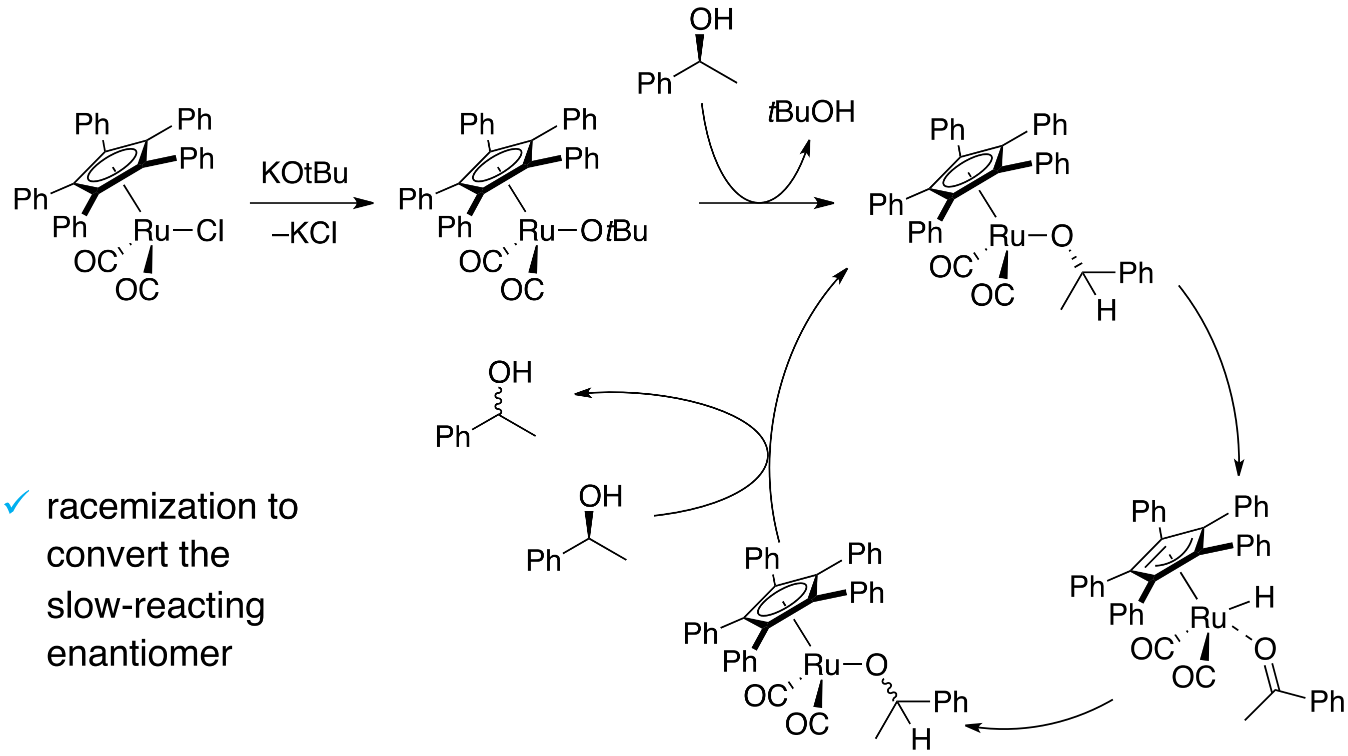
Overcoming yield limitations in kinetic resolutions

role of the ruthenium cocatalyst



✓ racemization to convert the slow-reacting enantiomer

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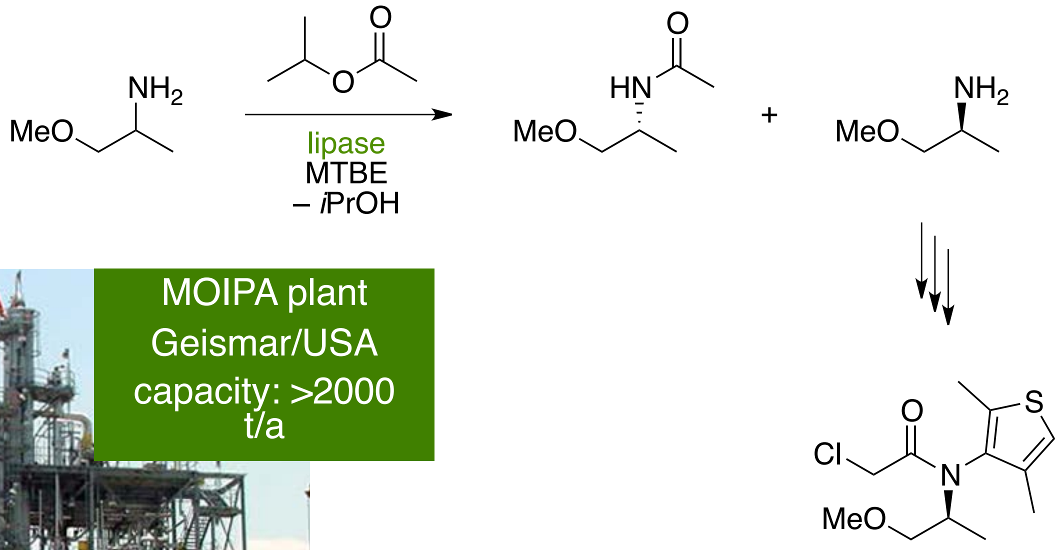
Martín-Matute, Edin, Bogár, Kaynak, Bäckvall, *J. Am. Chem. Soc.* **2005**, *127*, 8817.

Industrial applications

Outlook® (herbicide, BASF)

- synthesis of key amine by kinetic resolution



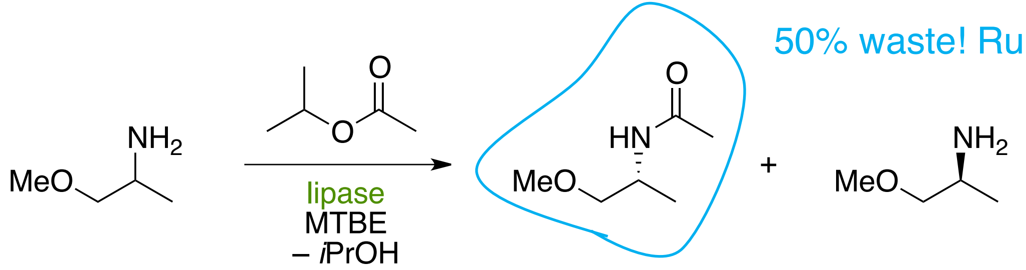
Outlook

MOIPA plant
Geismar/USA
capacity: >2000 t/a

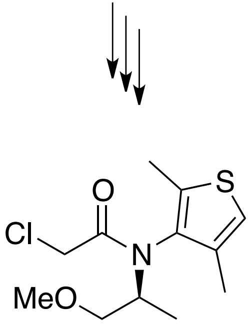
Industrial applications

Outlook® (herbicide, BASF)

- synthesis of key amine by kinetic resolution



50% waste! Ru-based DKR too expensive



Outlook

MOIPA plant
Geismar/USA
capacity: >2000 t/a

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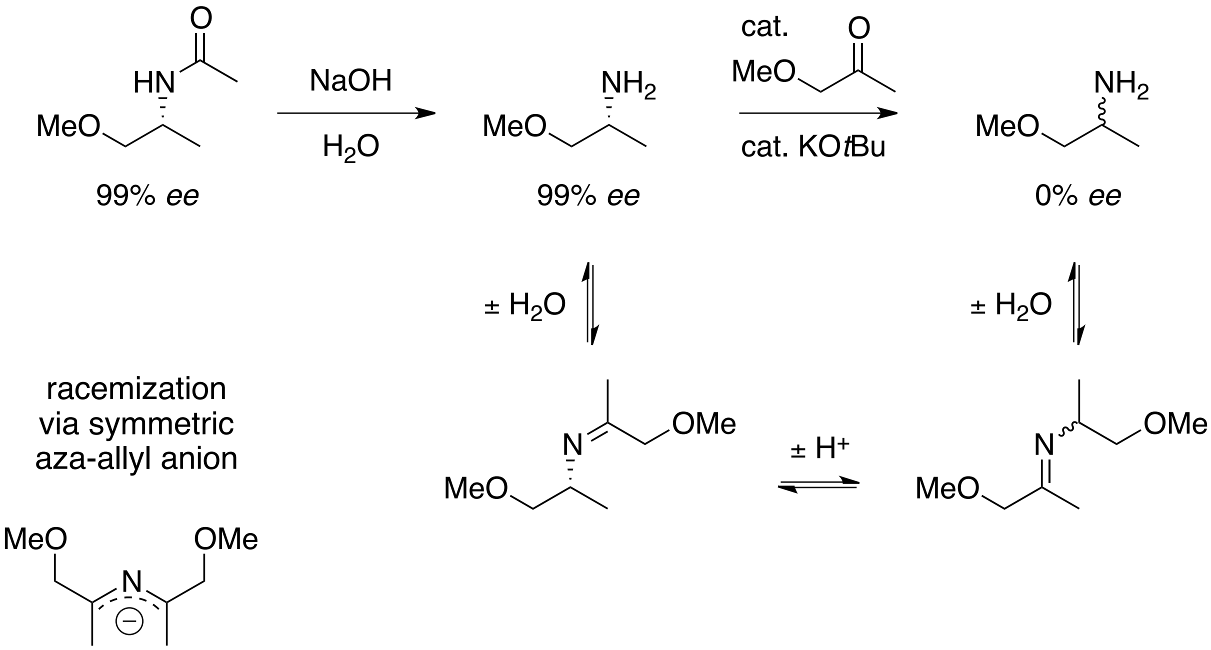
Industrial applications

Outlook® (herbicide, BASF)

distomer recycling!



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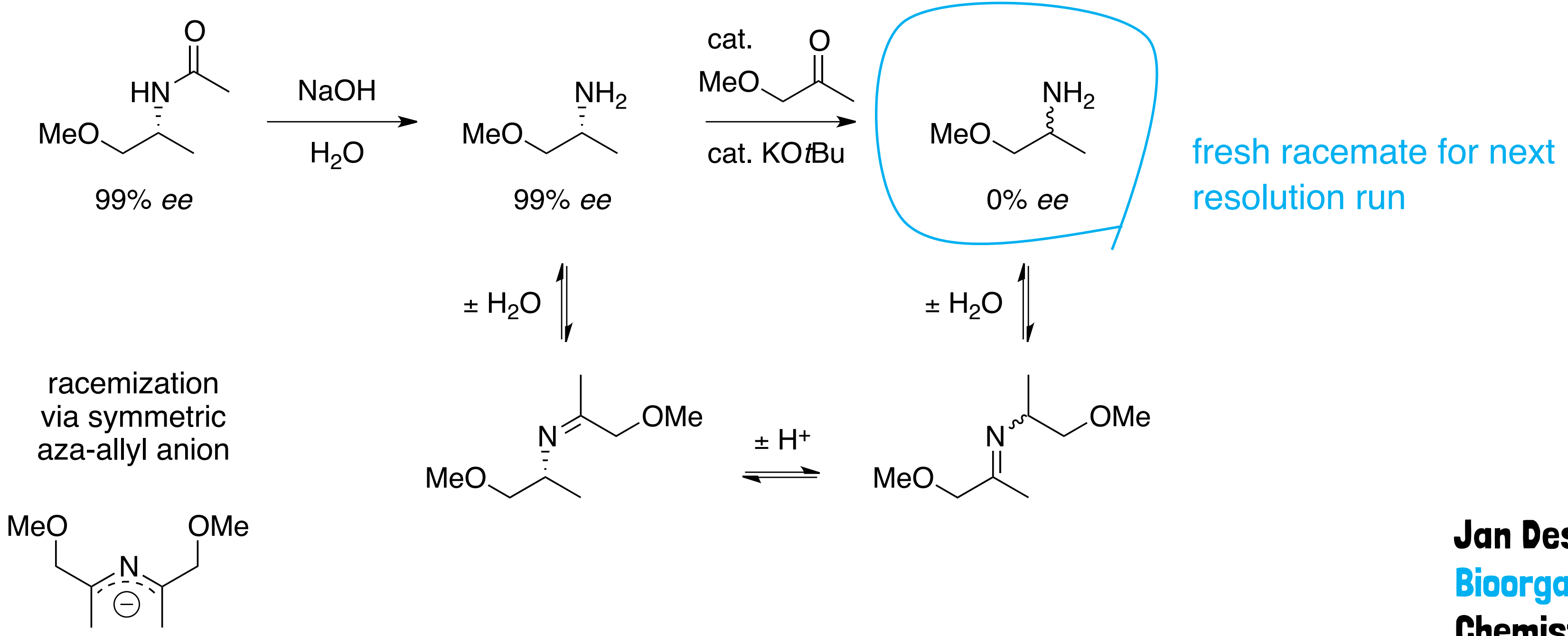
Industrial applications

Outlook® (herbicide, BASF)

distomer recycling!



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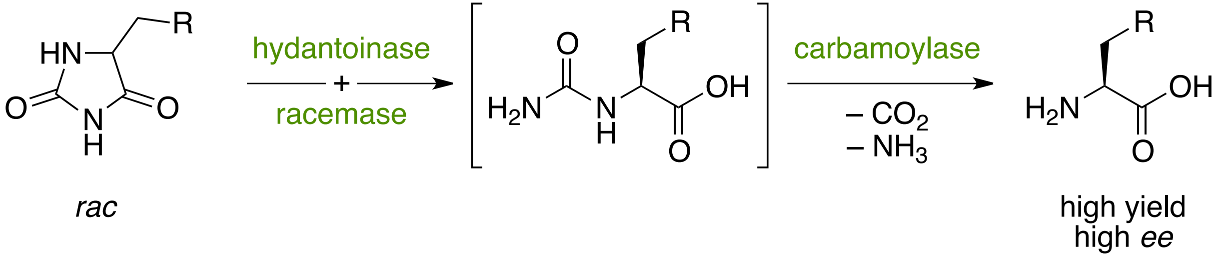
Industrial applications

Hydantoin process (Ajinomoto, Evonik, and others)

- synthesis of amino acids via dynamic kinetic resolution
- 2 hydrolases + 1 isomerase



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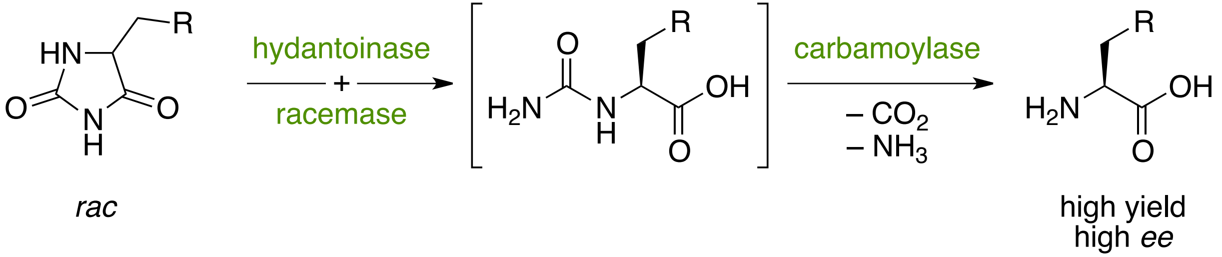

- hydantoins configurationally labile at pH > 8, but racemase more suitable
- optimized D- and L-selective hydantoinases available
- large scale processes for many natural and non-natural amino acids

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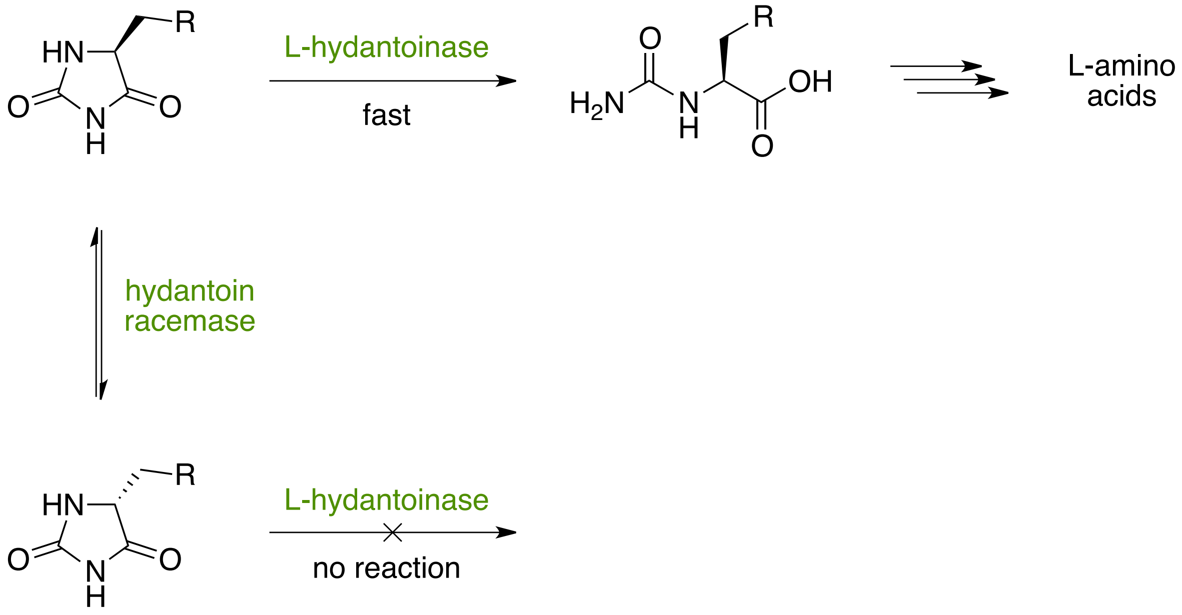
Industrial applications

Hydantoin process (Ajinomoto, Evonik, and others)

- close-up on the dynamic process:



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Industrial applications

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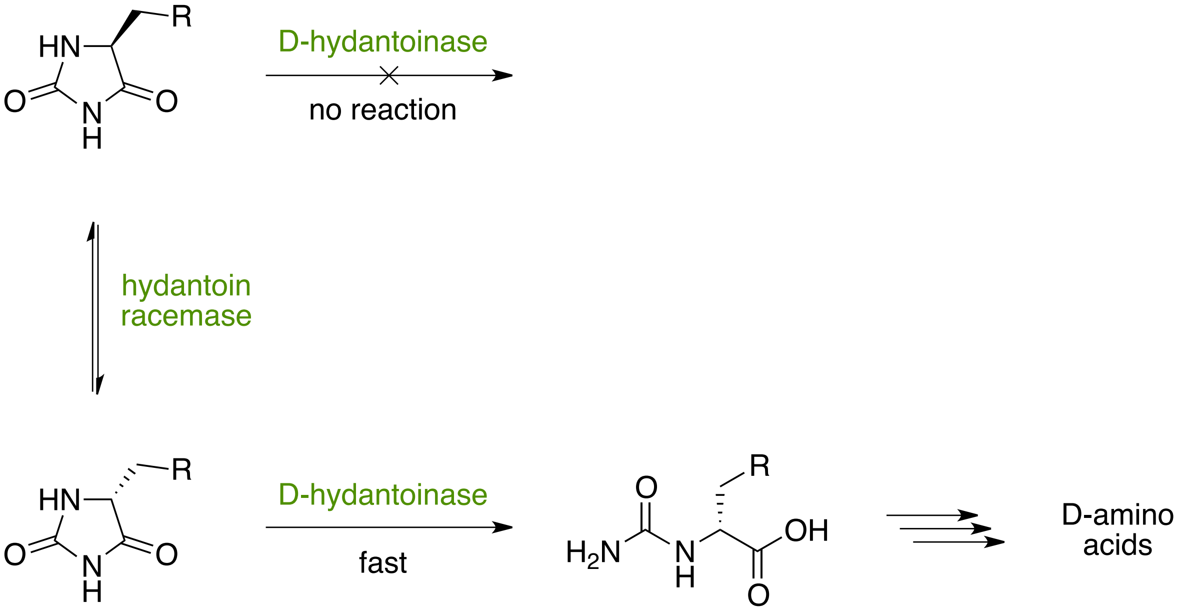

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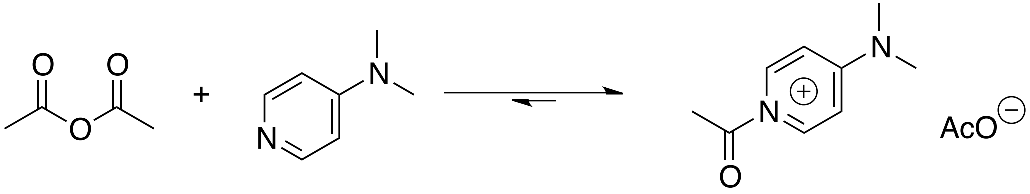
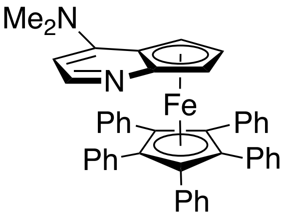
Biomimetic developments

formation of chiral acyl donors

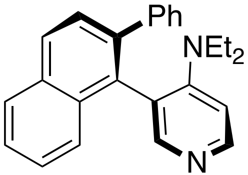
- Steglich catalyst as source for "hot" acyl donor



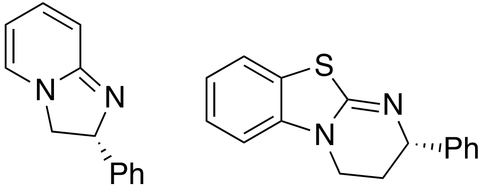
- chiral variations of Steglich's catalyst



Fu, 1999



Spivey, 2003



Birman, 2005 & 2008

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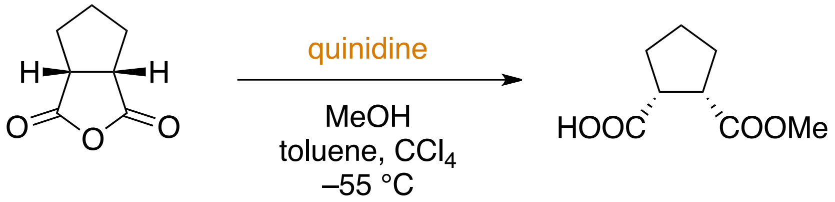
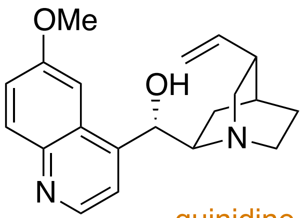
Biomimetic developments

formation of chiral acyl donors

Cinchona-catalyzed *meso*-anhydride alcoholysis (Bolm, 2000)

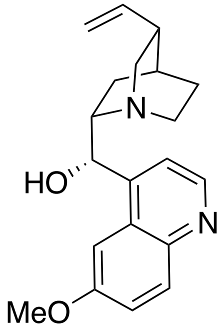


95%, 97% *ee*



quinidine

opposite enantiomer with:



quinine

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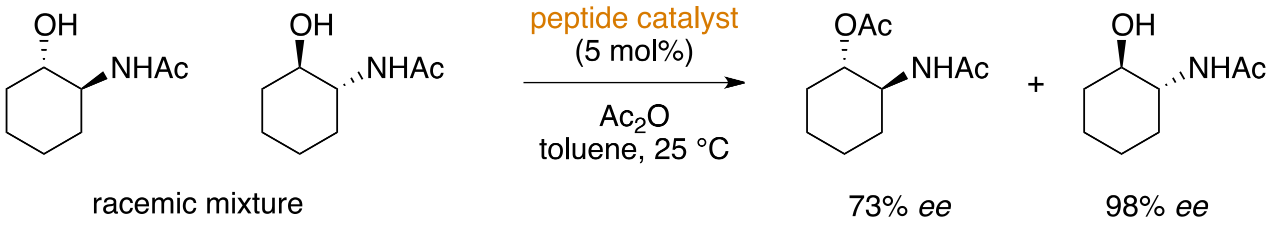
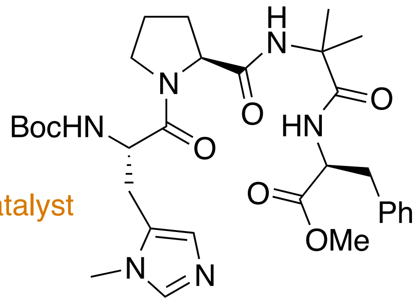
Bolm, Schiffers, Dinter, Gerlach, *J. Org. Chem.* **2000**, *65*, 6984.

Biomimetic developments

formation of chiral acyl donors

- small proteins: Miller's peptide catalysts





peptide catalyst

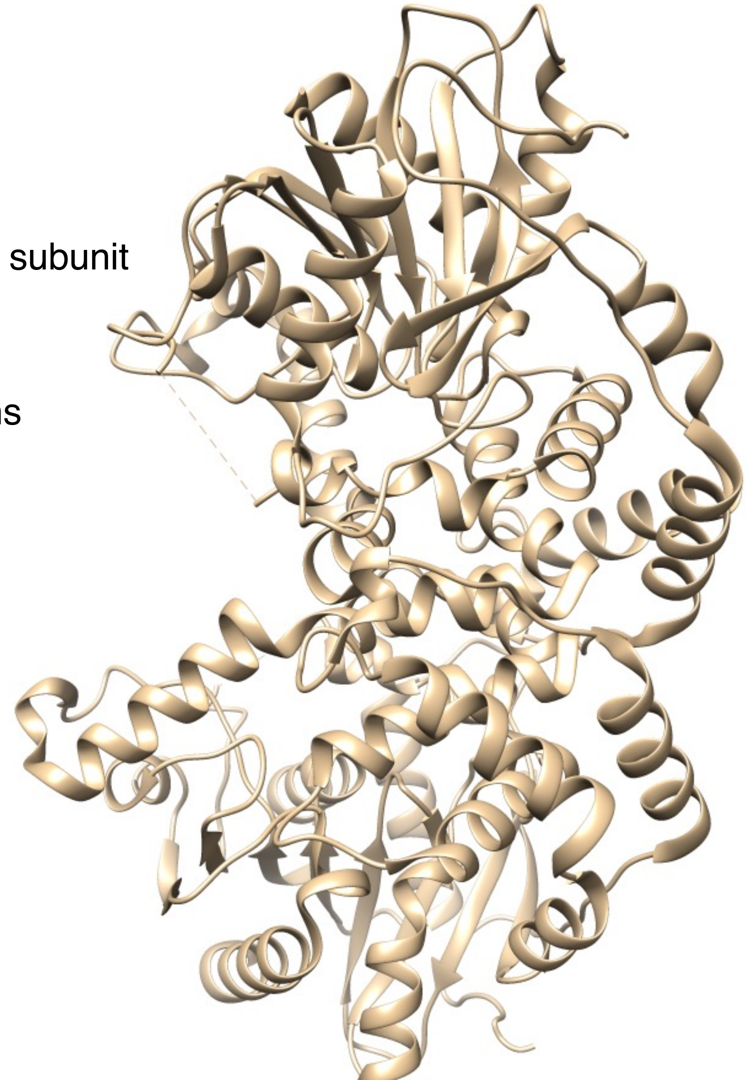
here: NHAc as H-bond donor required. Octapeptide also works with non-H-bonding substrates

Miller, Copeland, Papioannou, Horstmann, Ruel, *J. Am. Chem. Soc.* **1998**, *120*, 1629.

Epoxide hydrolases

epoxide hydrolase (*Aspergillus niger*)

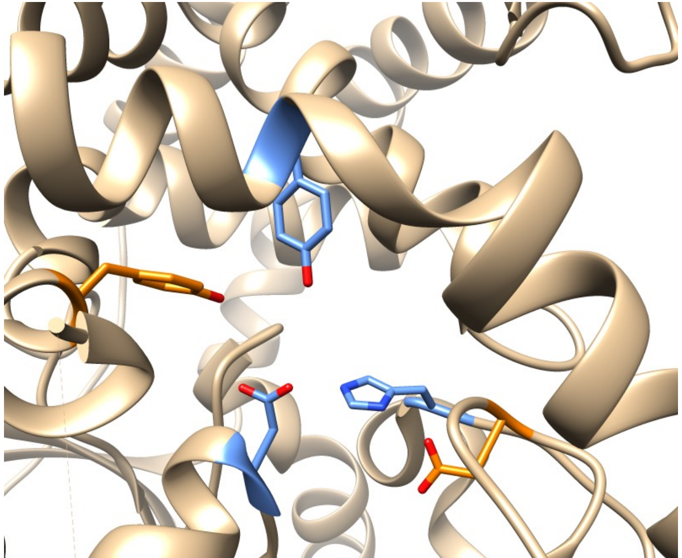
- homo dimer, 394 amino acids per subunit
- no cofactors required
- no structural or catalytic metal ions
- catalyzes hydrolysis of oxirans



Epoxide hydrolases

epoxide hydrolase (*Aspergillus niger*)

- homo dimer, 394 amino acids per subunit
- no cofactors required
- no structural or catalytic metal ions
- catalyzes hydrolysis of oxirans
- catalytic triade based on tyrosine, histidine and aspartate (blue)
- assisted by H-donors and acceptors (orange: tyrosine and aspartate)



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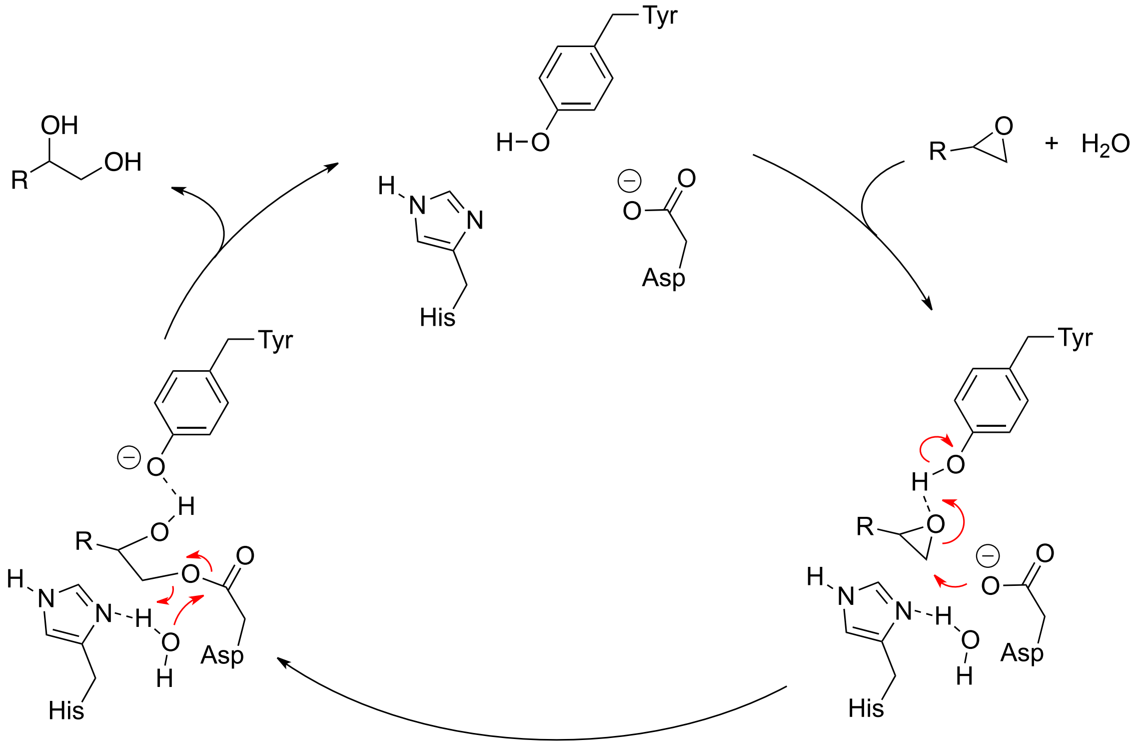
Epoxide hydrolases – mechanism



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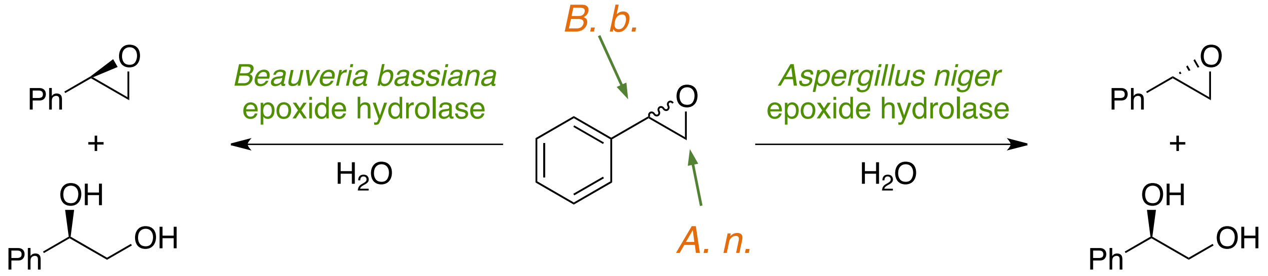
Epoxide hydrolases - synthetic applications

kinetic resolutions



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Epoxide hydrolases – synthetic applications

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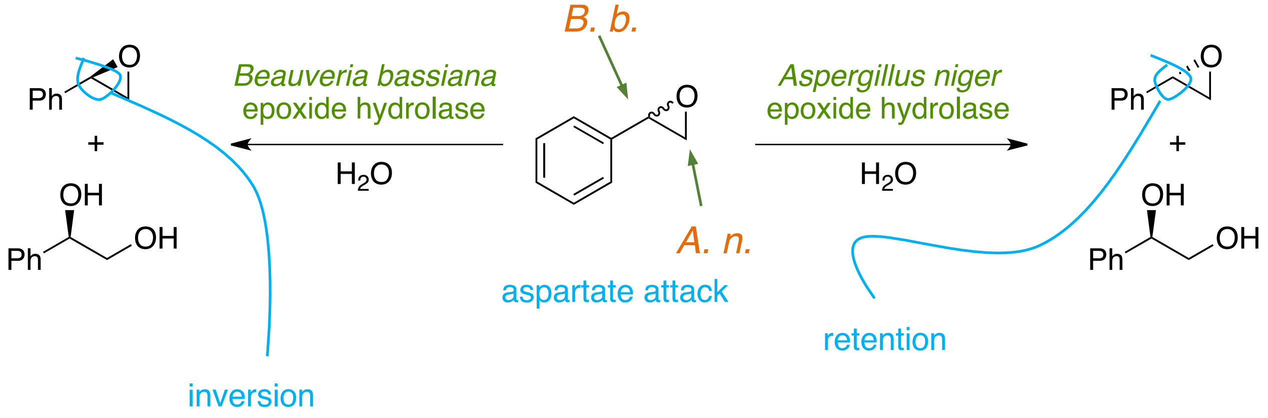

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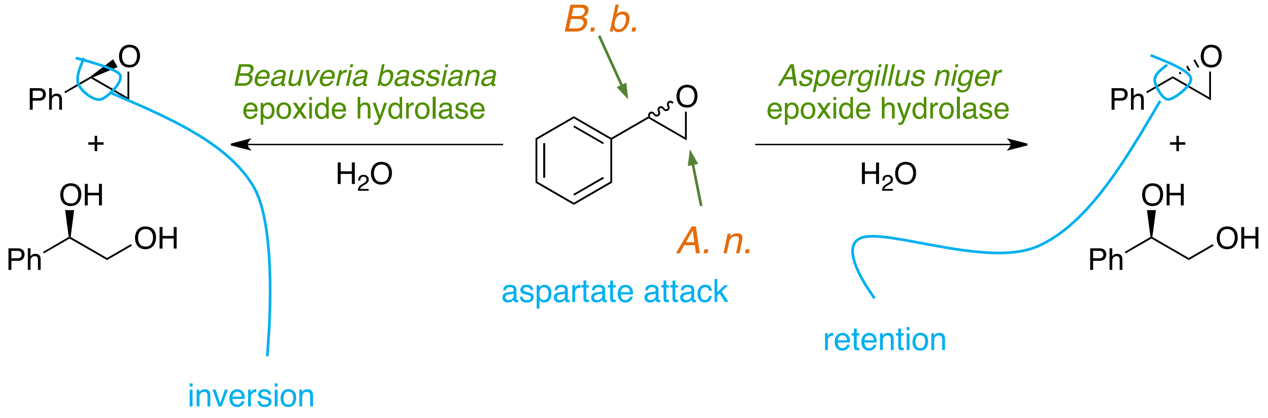
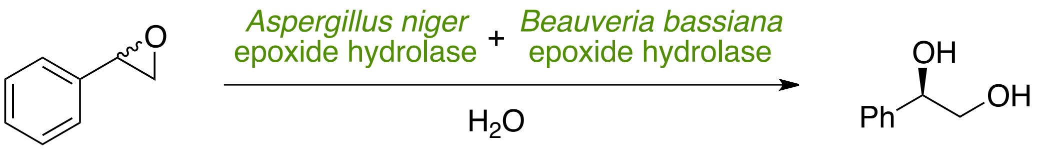


enantioconvergent hydrolysis



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SUMMARY

Lipase catalysis

- super versatile multi-tool
- mechanism based on catalytic triade
- most established application type of any of the biocatalyst families
- basis for various industrial applications

Epoxide hydrolysis

- slightly different catalytic triad
- much less applied, but beautiful niche applications