# CHEM-E4109
# MODERN METHODS IN BIOCATALYSIS

*chapter #4: aminations & deaminations*

11.3.2022

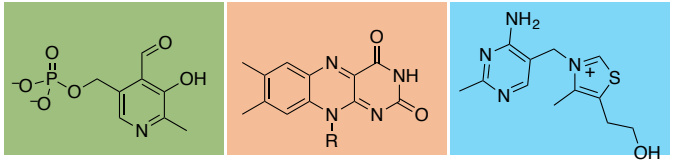
Jan Deska
Bioorganic
Chemistry

# TODAY'S MENU

Nitrogen-containing organic molecules of particular importance in e.g. pharma or agrochemical products

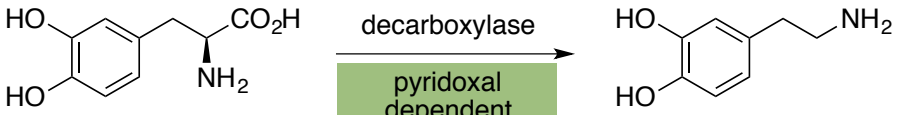
- oxidative enzymes can be exploited to prepare chiral amines
- focus on transferases
- similar to oxidoreductases: control of equilibria crucial for productive aminations

# Amino acid metabolism



e.g. metabolic fate of Dopa

- L-Dopa used in Parkinson treatment
- source of dopamine (and other neurotransmitters)




L-Dopa dopamine

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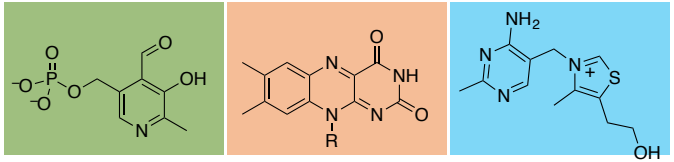
# Amino acid metabolism



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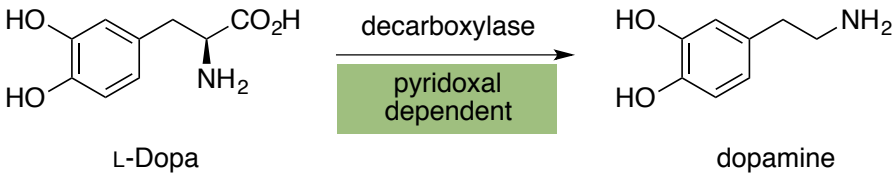


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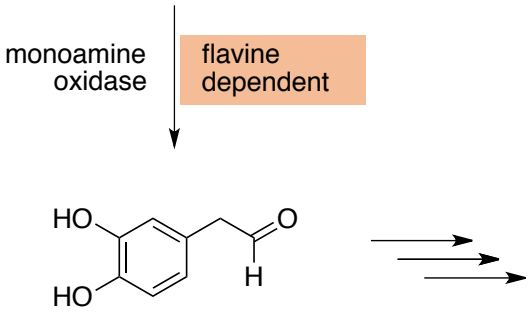


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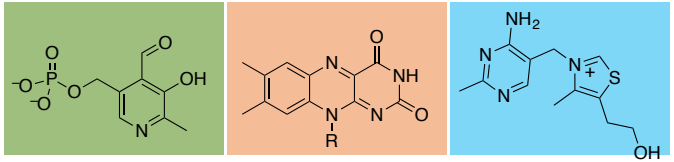

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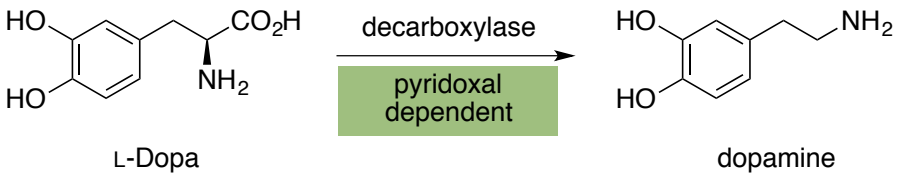
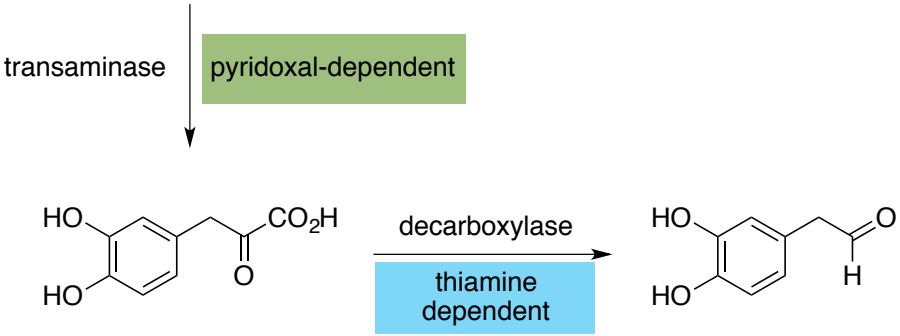



L-Dopa dopamine

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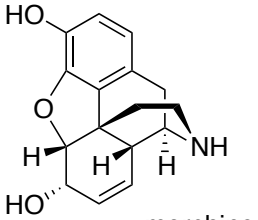

morphine

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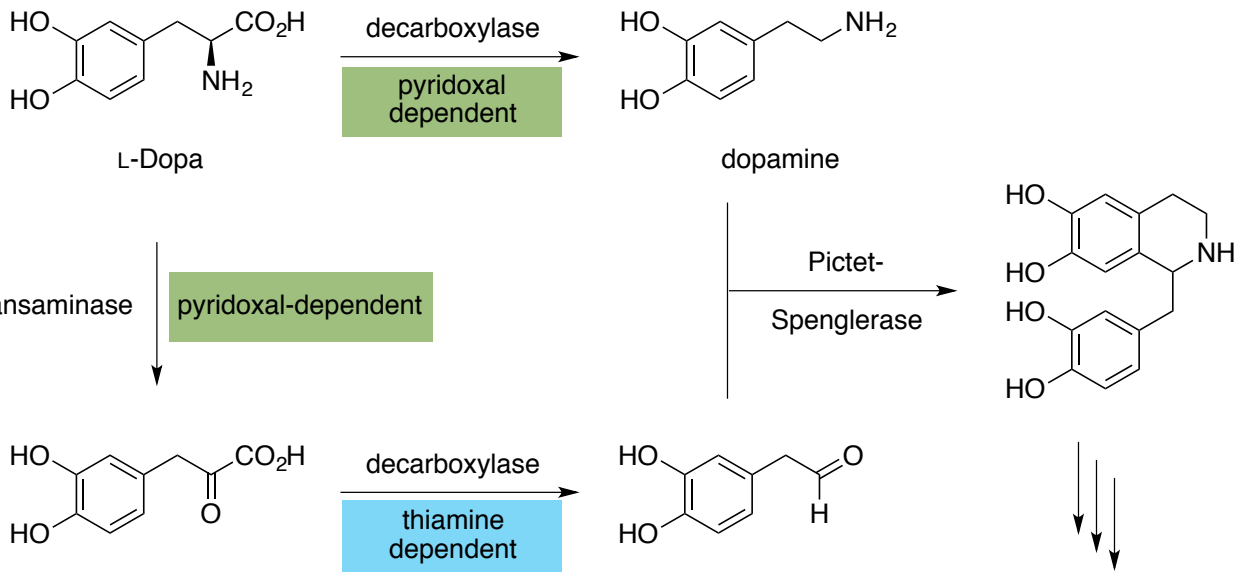
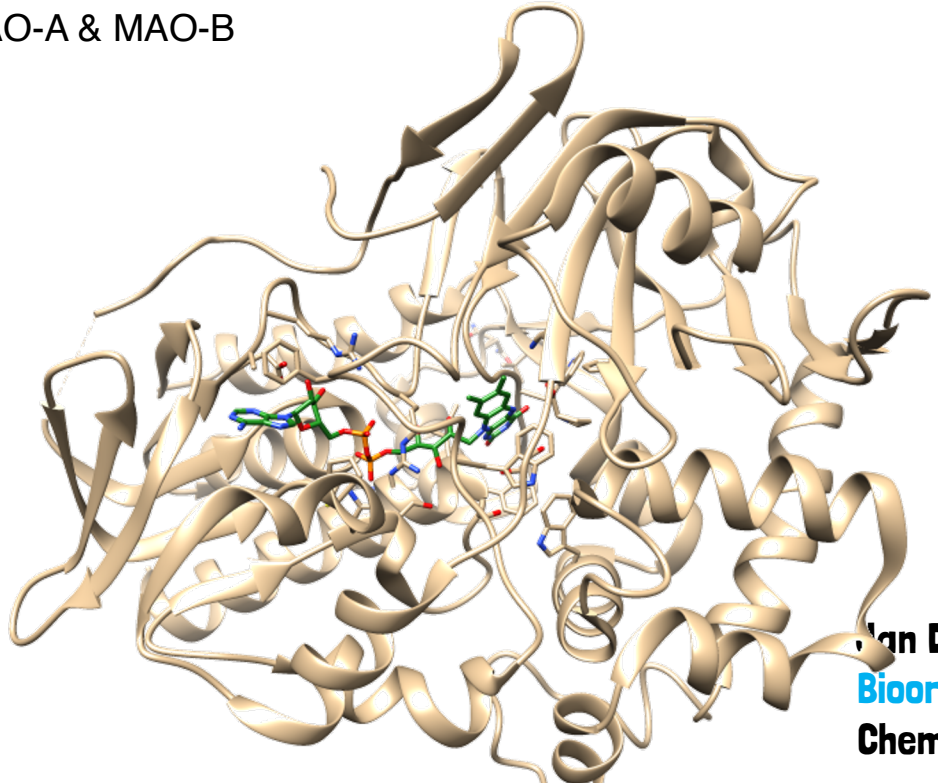


# Monoamine oxidases

Monoamine oxidase N or MAO-N (*Aspergillus niger*)

- homo-tetramer, subunits à 56 kDa, 495 amino acids
- MAO-N 24% homologeous to human MAO-A & MAO-B
- flavoprotein

- catalyzes oxidation of amines to imines
- poor activity on non-natural substrates, but evolved proteins proved to accept a variety of synthetic amines



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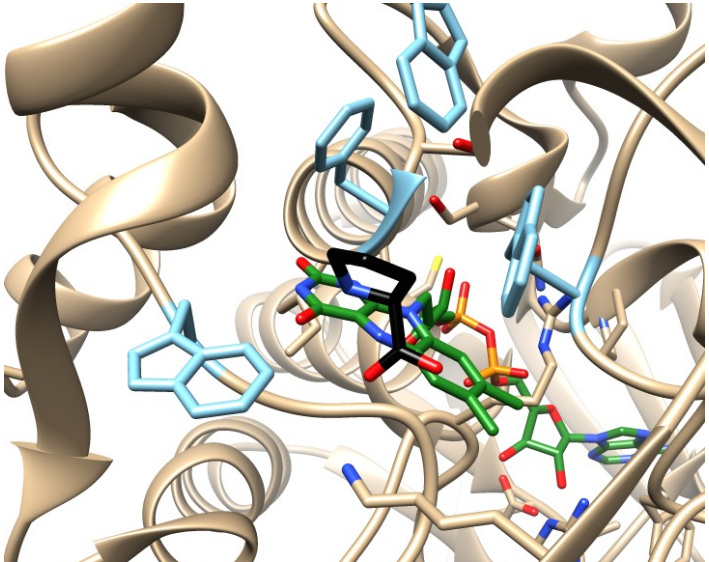
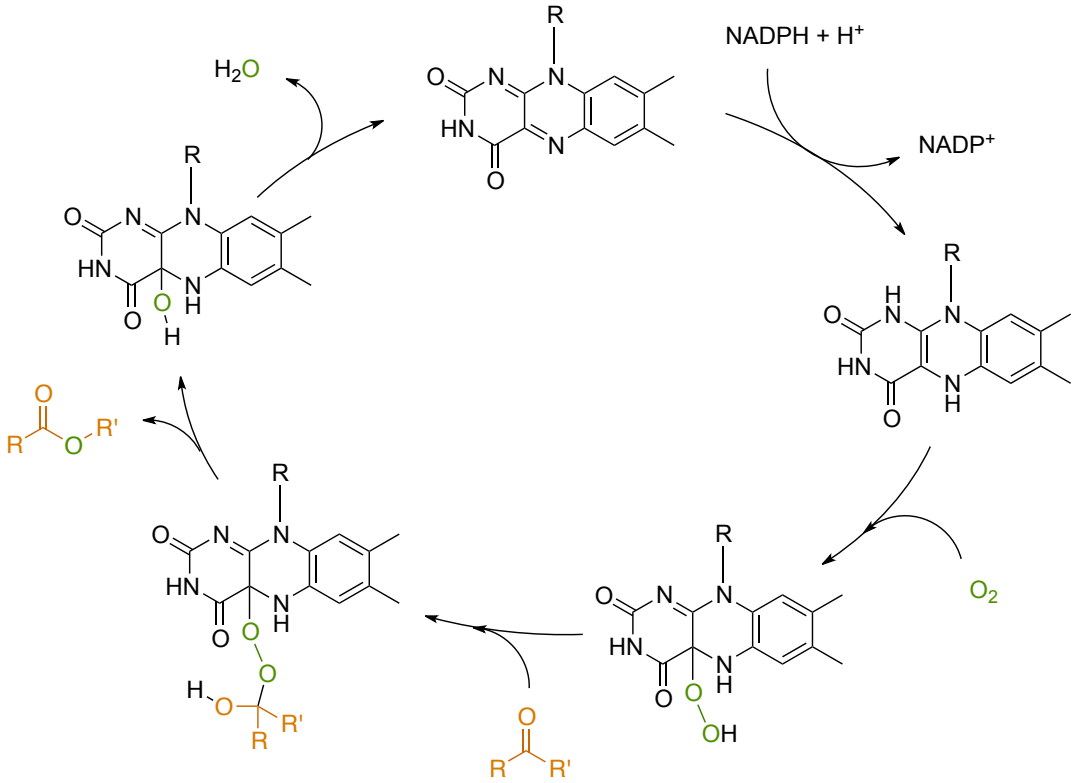
- aromatic cage aligns substrate on top of the FAD

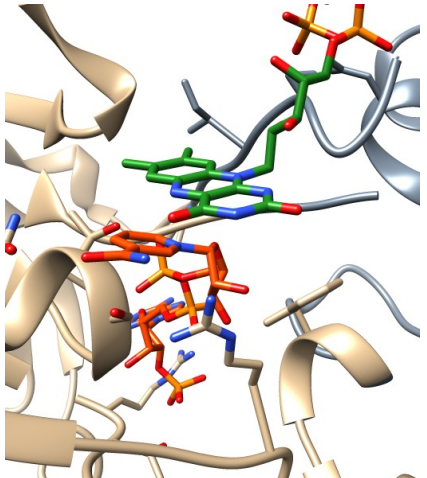


# Recap: Baeyer–Villiger monooxygenases

Mechanism




Kamerbeek, Janssen, van Berkel, Fraaije, *Adv. Synth. Catal.* **2003**, *345*, 667-678.



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# Recap: Baeyer–Villiger monooxygenases

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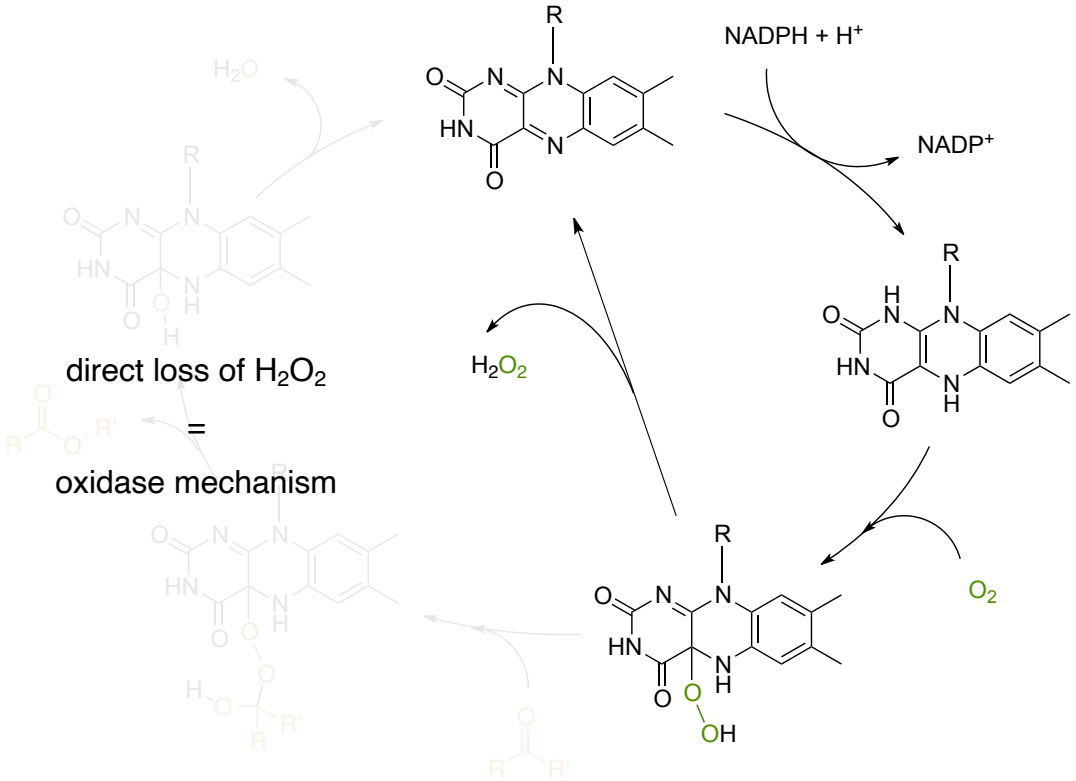


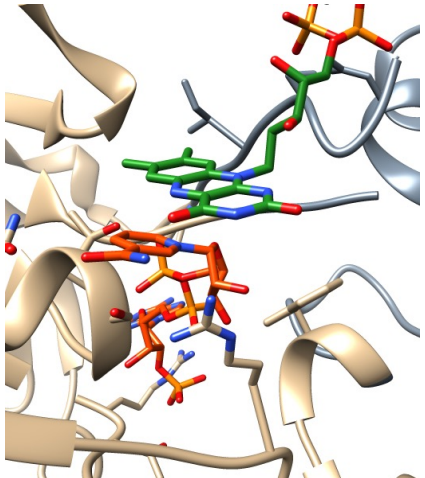

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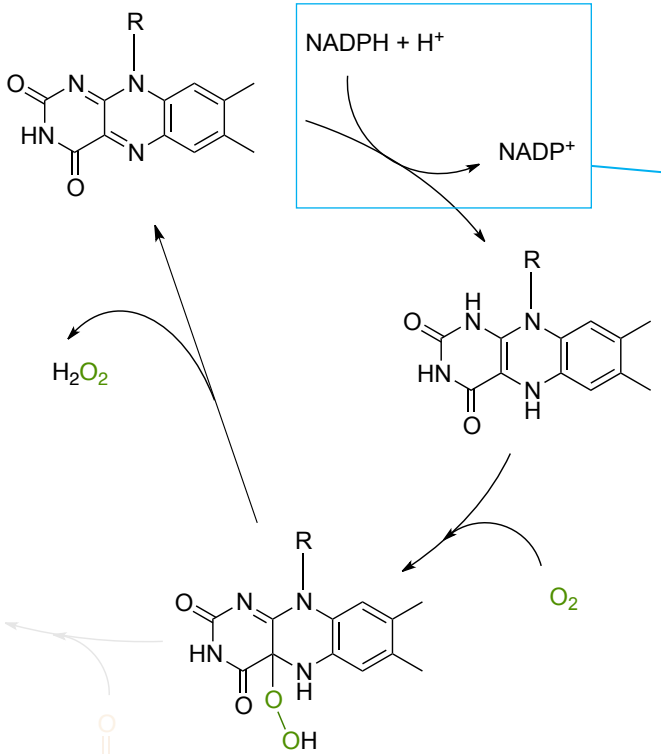
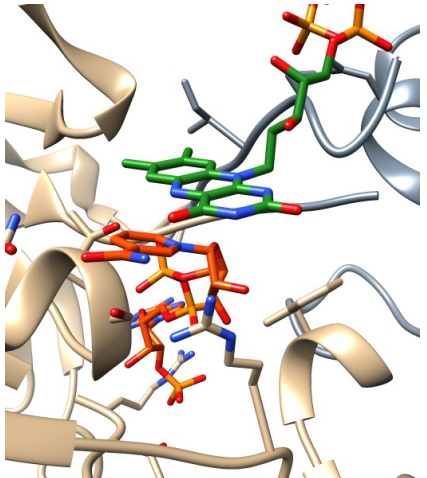



direct loss of $H_2O_2$

=

oxidase mechanism



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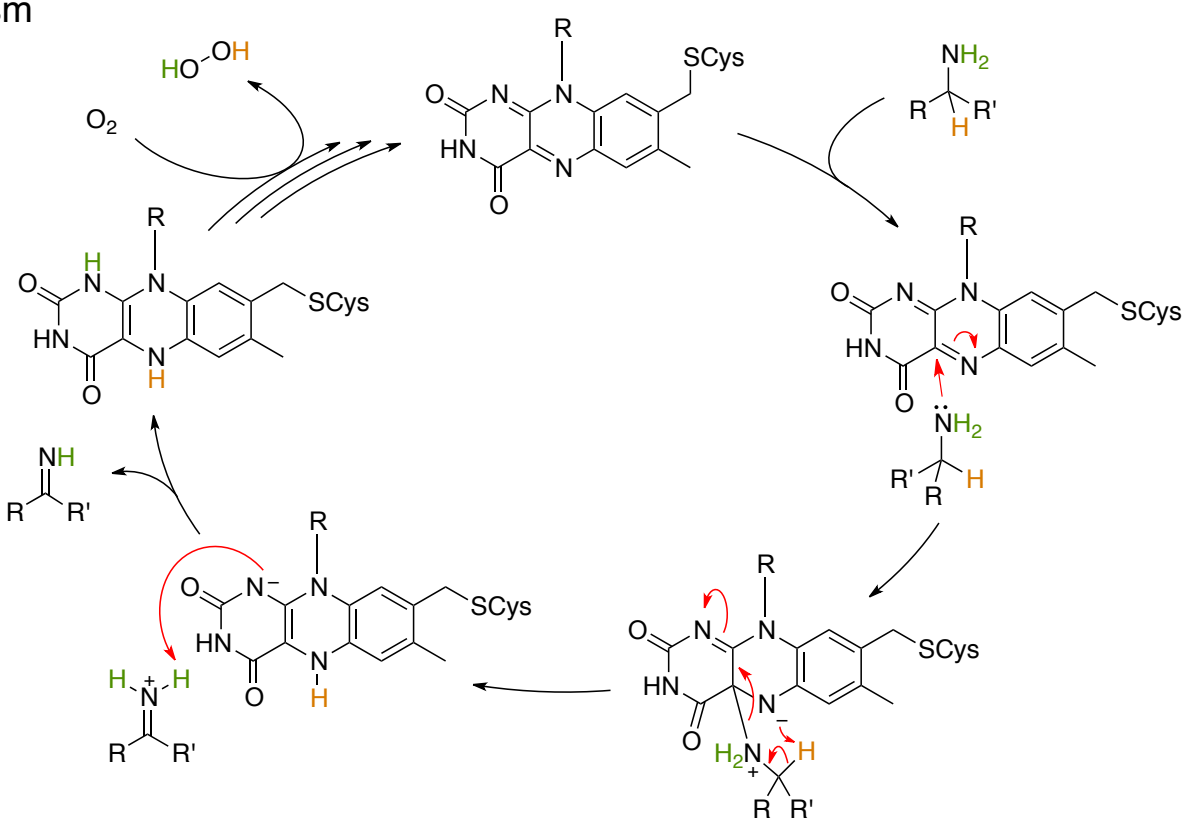


Abad, Zenn, Kästner, *J. Am. Chem. Soc.* **2013**, *117*, 14238-14246.

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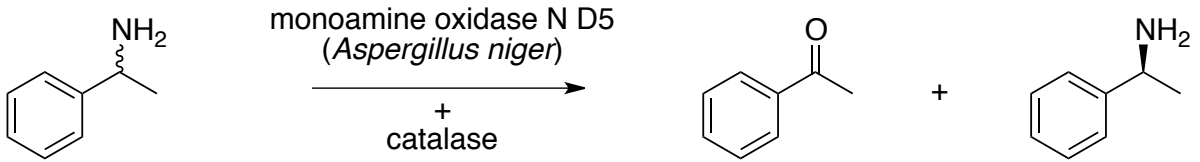
# Asymmetric transformations

kinetic resolutions of primary and secondary, branched amines




*rac*

99% *ee*

Atkin, Reiss, Koehler, Bailey, Hart, Turkenburg, Turner, Brzozowski, Grogan, *J. Mol. Biol.* **2018**, *53*, 1218-1231.

# Asymmetric transformations

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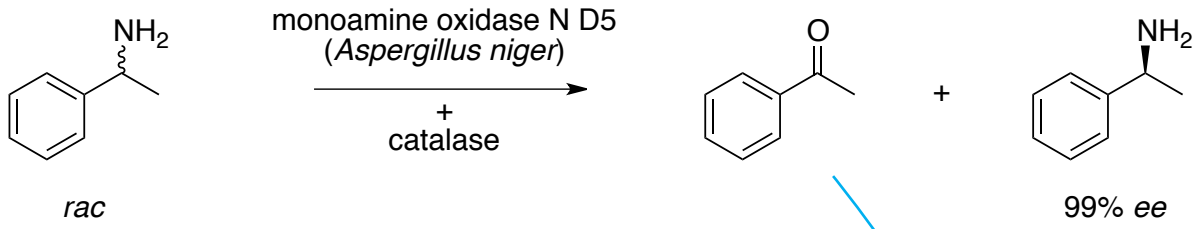
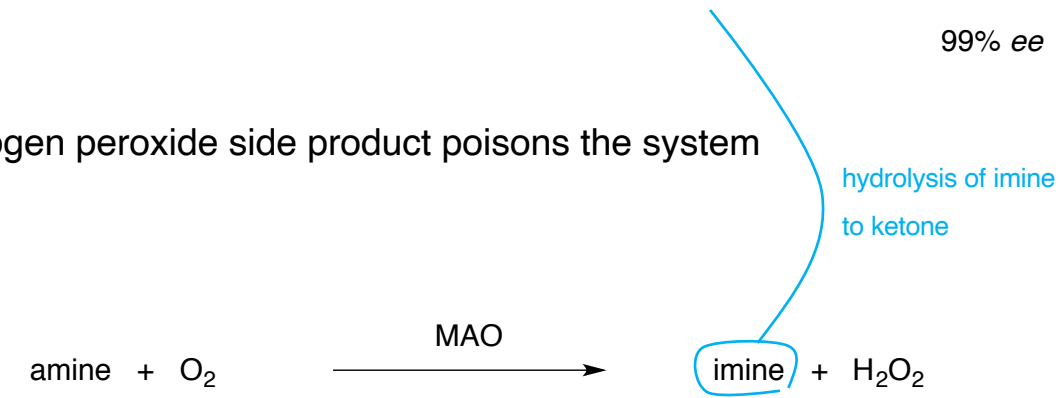



- hydrogen peroxide side product poisons the system

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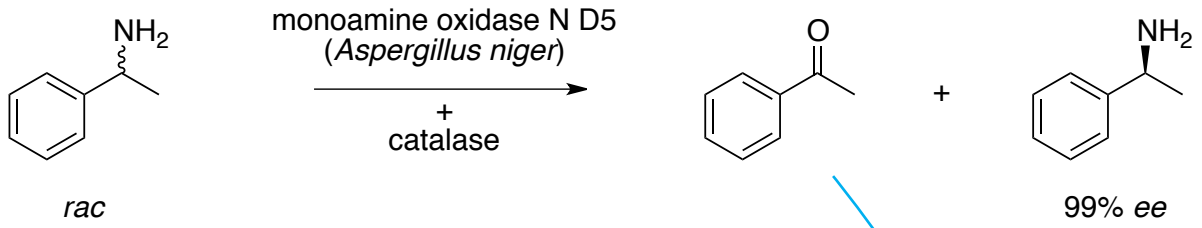
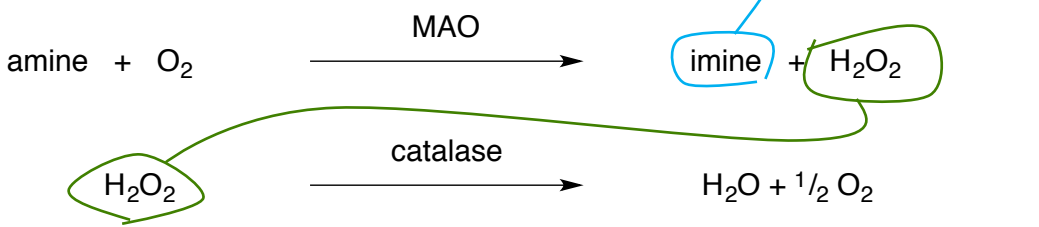



- hydrogen peroxide side product poisons the system
- catalase (heme enzyme) disproportionates $H_2O_2$

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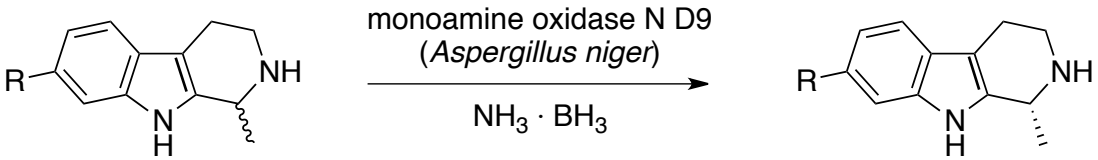
# Asymmetric transformations

deracemization

- by non-enzymatic re-reduction of imine




*rac*

R = H: eleagnine
OMe: leptaflorin

>90% yield, 99% *ee*

- in-situ formed achiral imine is reduced by $BH_3$ in a racemic fashion
- $H_2O_2$ is destroyed by borane
- over time, the non reactive enantiomer accumulates, without any side products

= high yields & high ee

Ghislieri, Green, Pontini, Willies, Frank, Grogan, Turner, *J. Am. Chem. Soc.* **2013**, *135*, 10863-10869.

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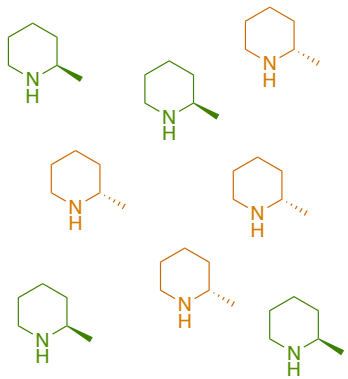

4 : 4



Ghislieri, Green, Pontini, Willies, Frank, Grogan, Turner, *J. Am. Chem. Soc.* **2013**, *135*, 10863-10869.

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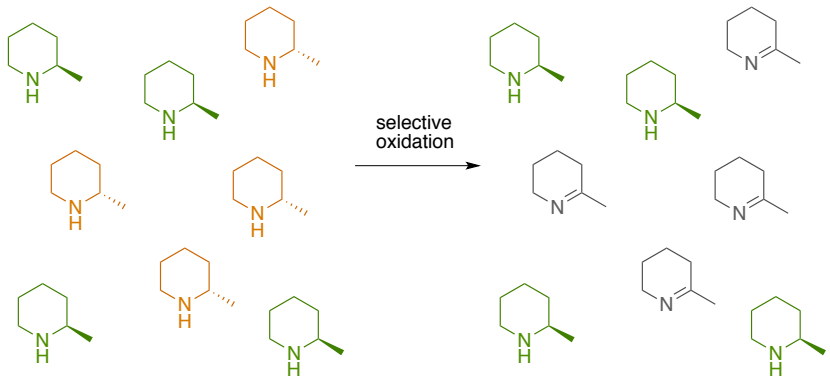


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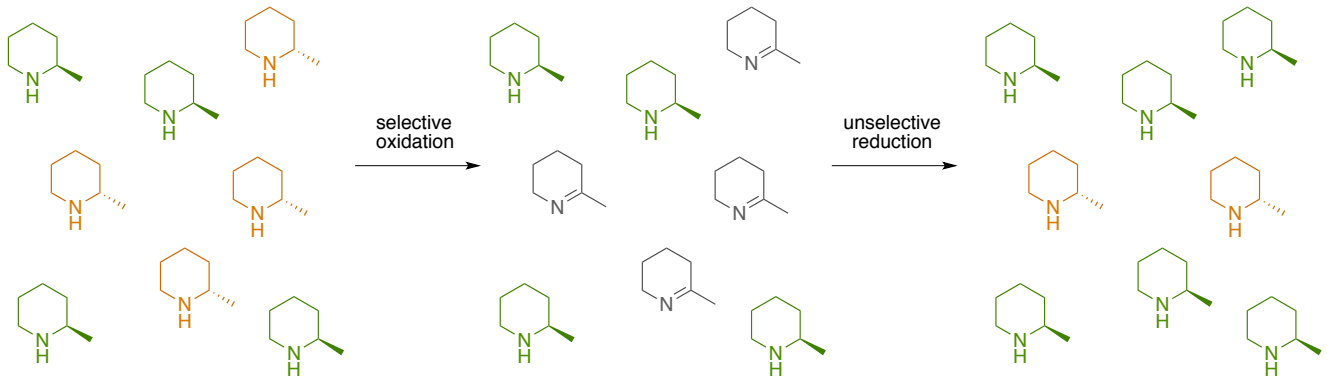


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6 : 2

Ghislieri, Green, Pontini, Willies, Frank, Grogan, Turner, *J. Am. Chem. Soc.* **2013**, *135*, 10863-10869.

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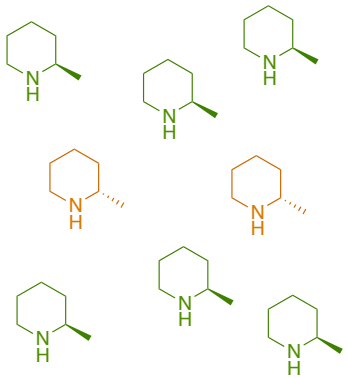

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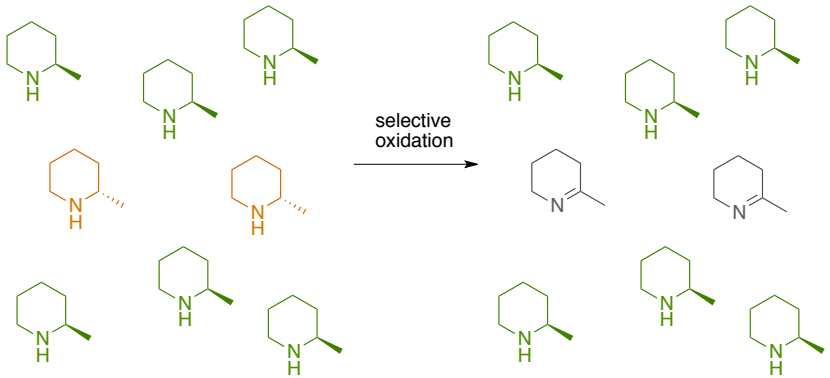


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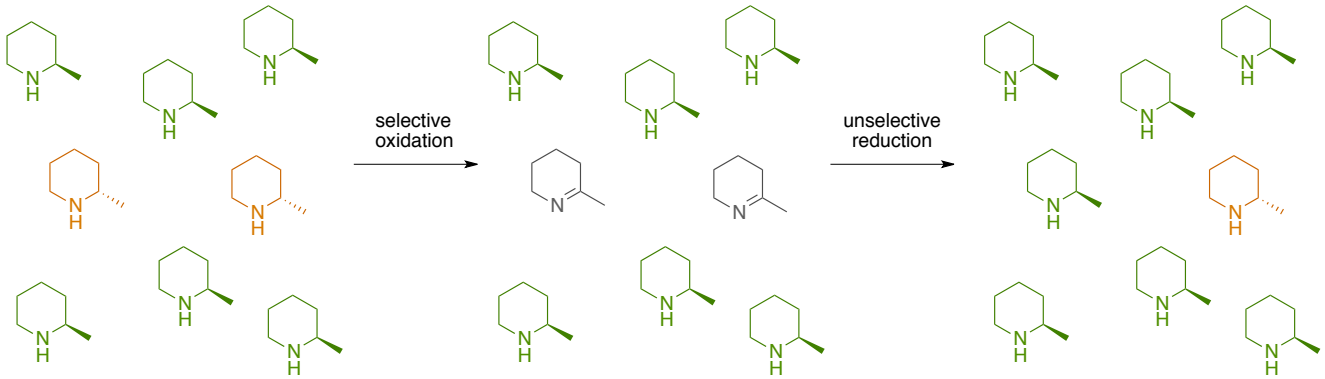


6 : 2

7 : 1

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# Asymmetric transformations

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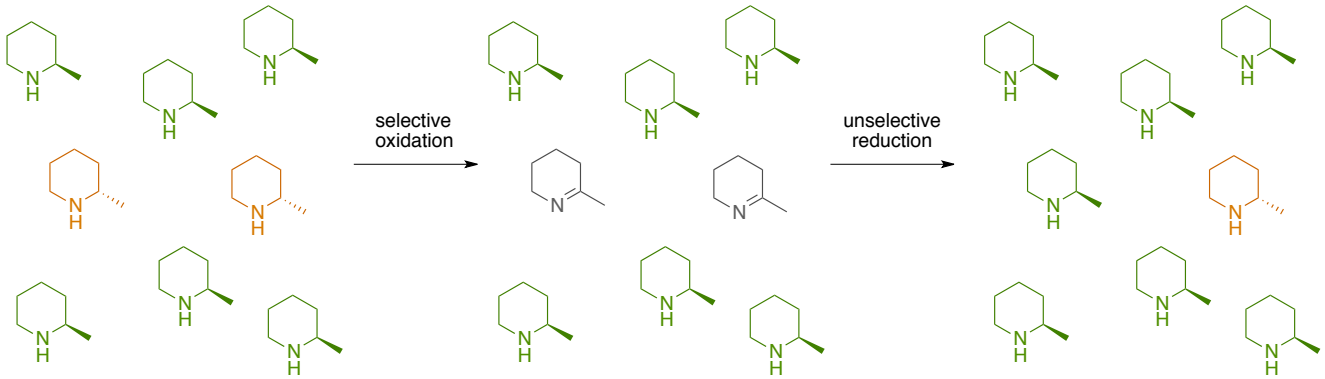



6 : 2

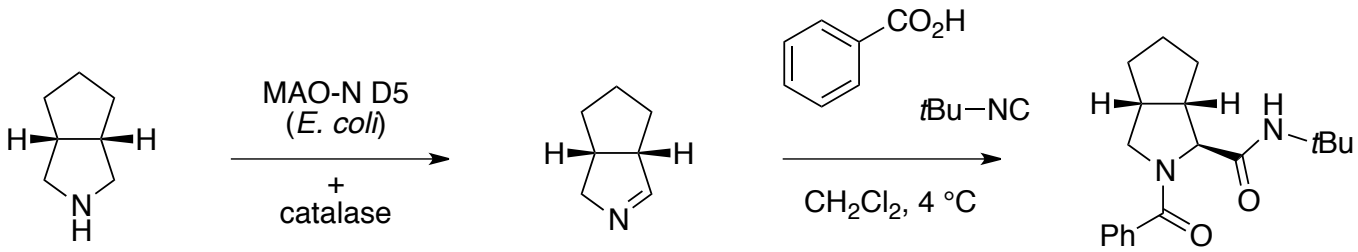
7 : 1

continuously repeated until only one enantiomer remains

Ghislieri, Green, Pontini, Willies, Frank, Grogan, Turner, *J. Am. Chem. Soc.* **2013**, *135*, 10863-10869.

# Asymmetric transformations

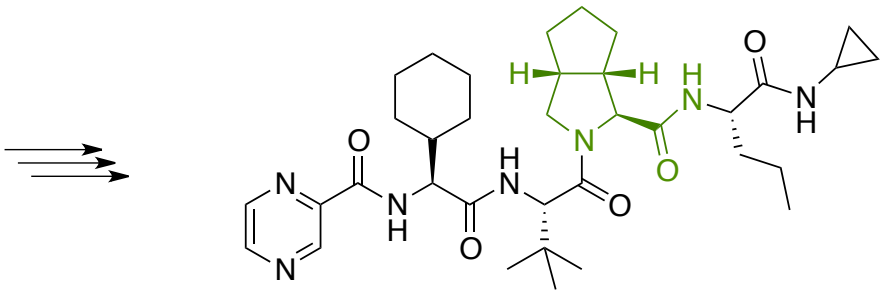
desymmetrization




*meso*
(achiral)

83% yield, 94% *ee*

80% yield, dr = 93:7



telaprevir ("Incivek" or "Incivo")
anti hepatitis C

Znabet, Ruijter, de Kanter, Köhler, Helliwell, Turner, Orru, *Angew. Chem. Int. Ed.* **2010**, *49*, 5289-5292.

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# EC 2: TRANSFERASES

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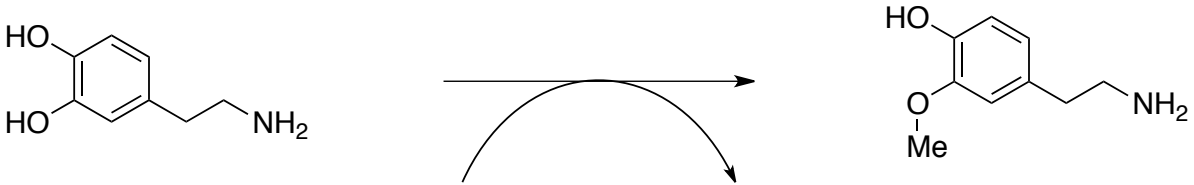
# Transferases - features

enzymes of class EC 2 catalyze the transfer of functional groups

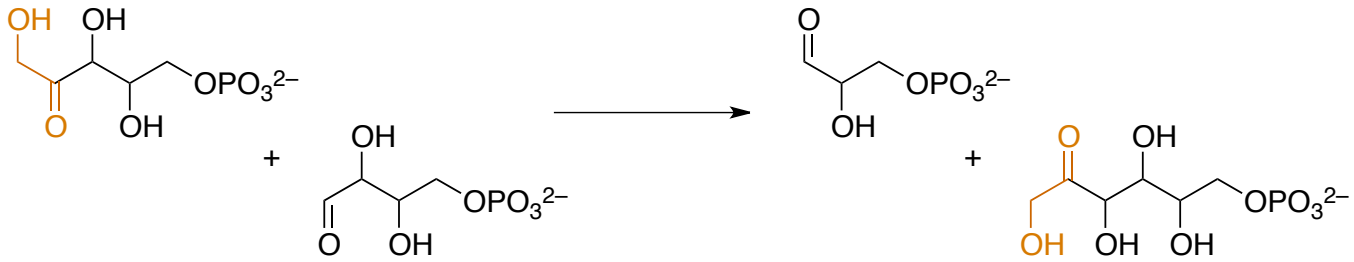
from one donor substrate to an acceptor substrate

methyltransferases




S-adenosylmethionine S-adenosylhomocysteine

transketolases

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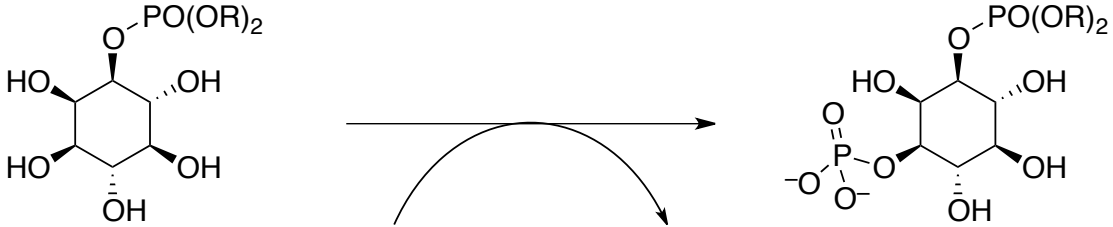
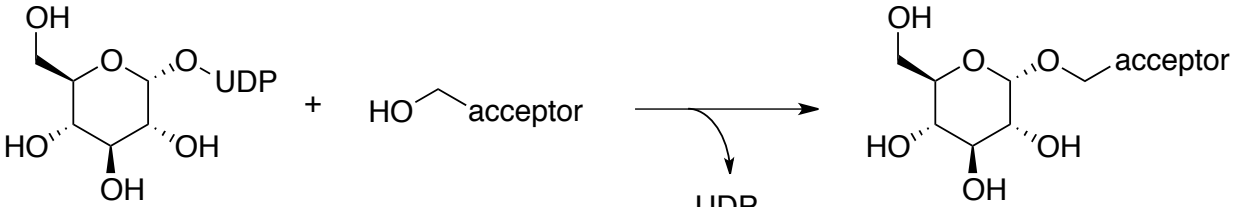
kinases




adenosintriphosphate adenosindiphosphate

glycosyltransferases

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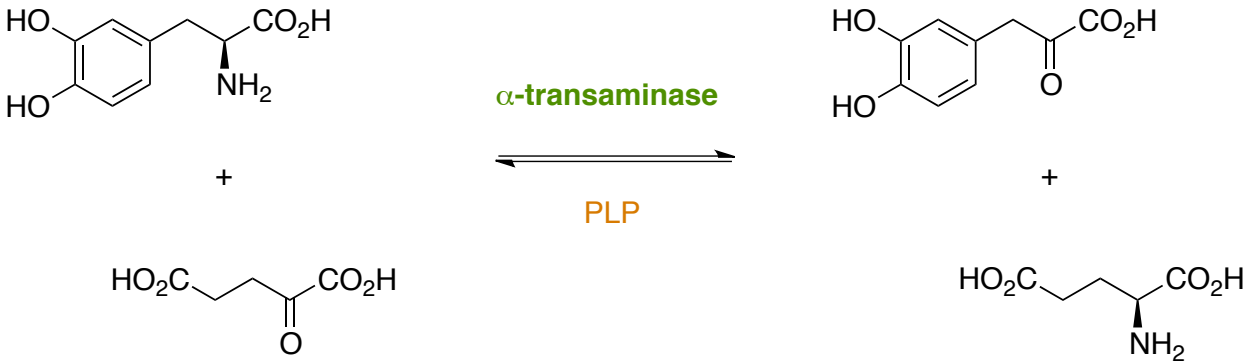
# Transaminases

exchange of amino groups between amino acids and α-ketocarboxylic acids



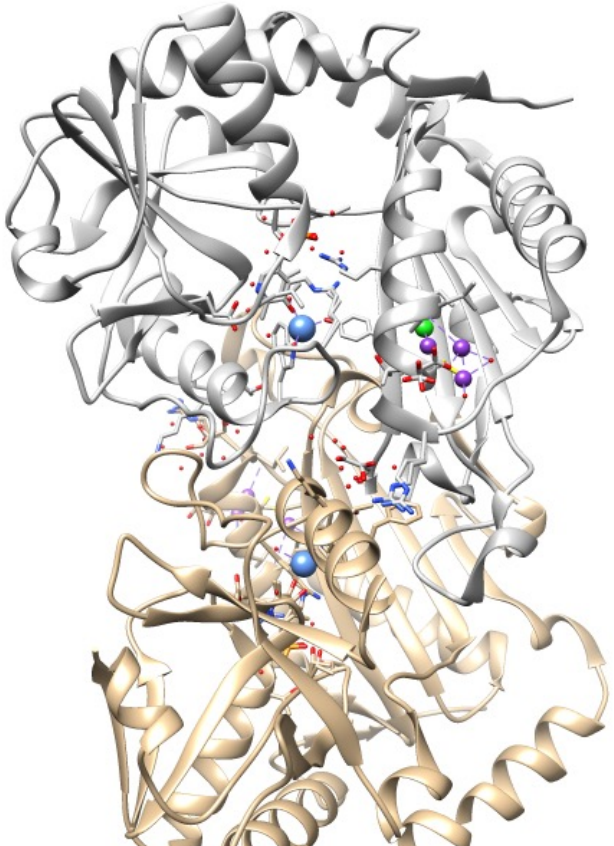

- α-transaminases important for biosynthesis of non-proteinogenic amino acids
- also involved in the breakdown of amino acids

= synthetically more interesting: ω-transaminases (do not require carboxylate substrate)

# ω-Transaminases

transaminase from *Aspergillus fumigatus*

- homo-dimer, subunits à 326 amino acids
- pyridoxal-dependent
- contains structural potassium (purple) and catalytic sodium (blue) ions
- catalyzes aminations and deaminations

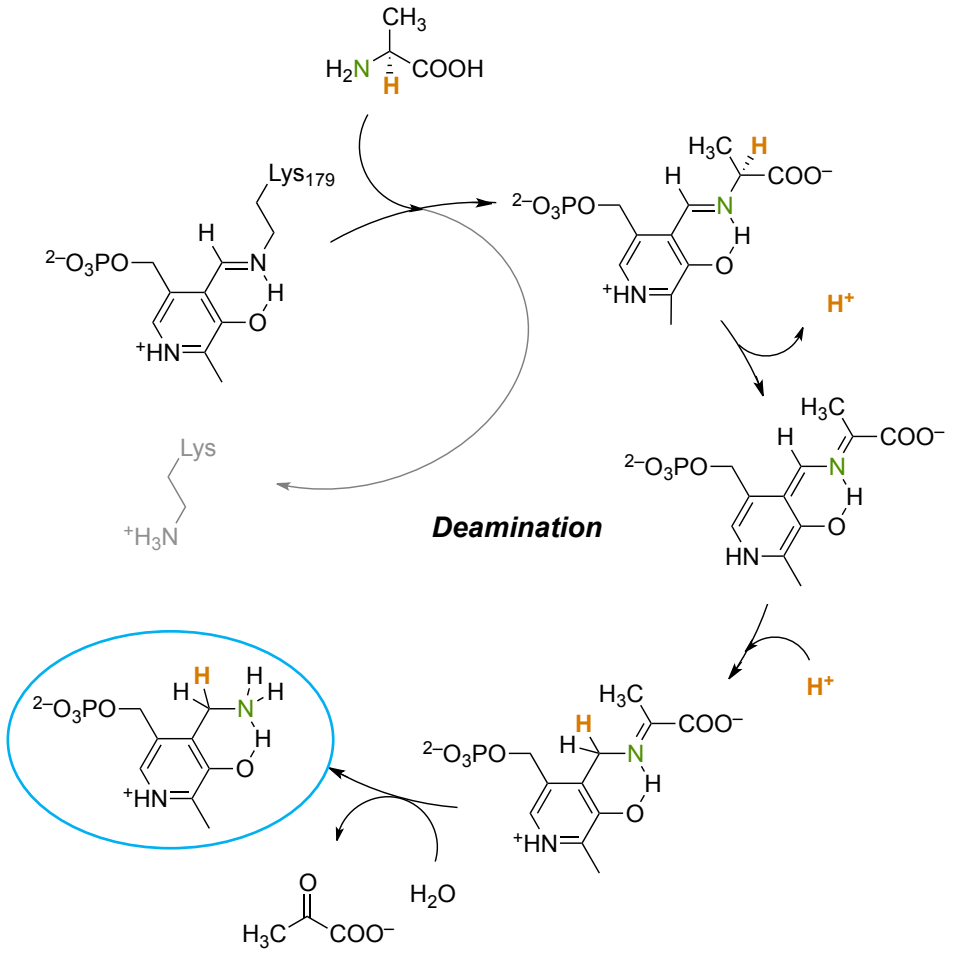


# ω-Transaminases

mechanism

- deamination of donor (Ala or Glu)

  + amination of substrate (ketone)

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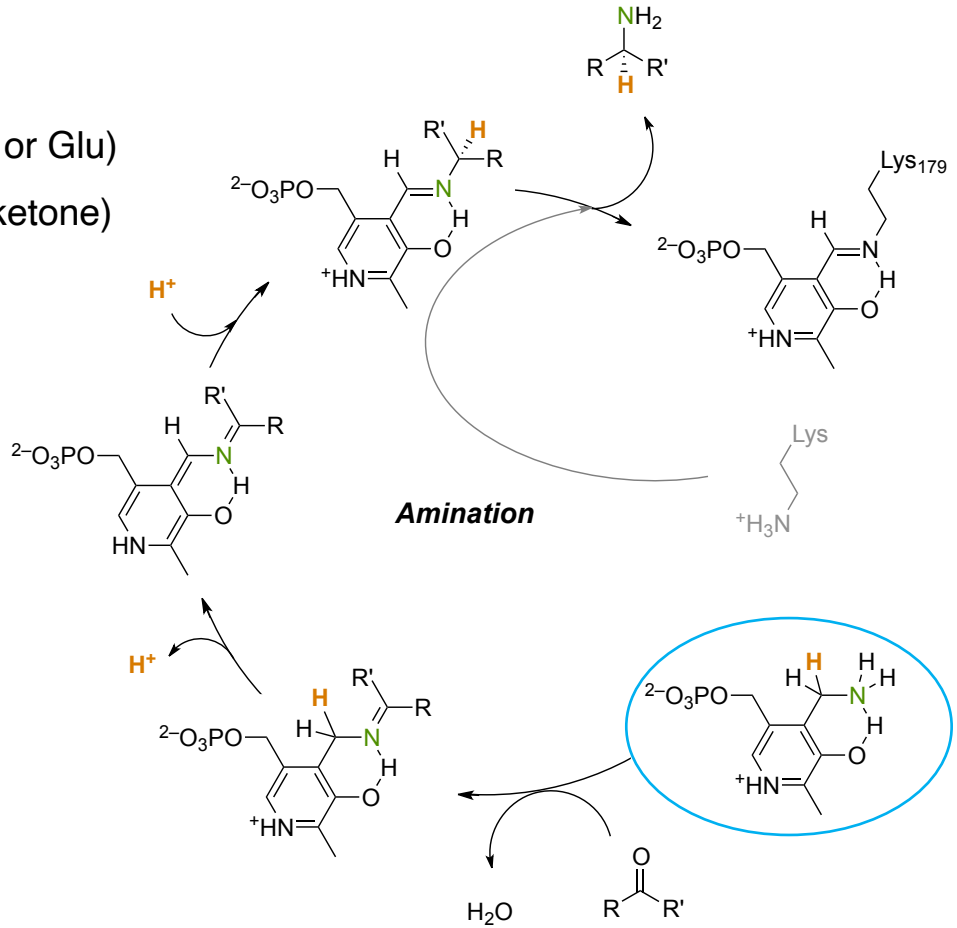


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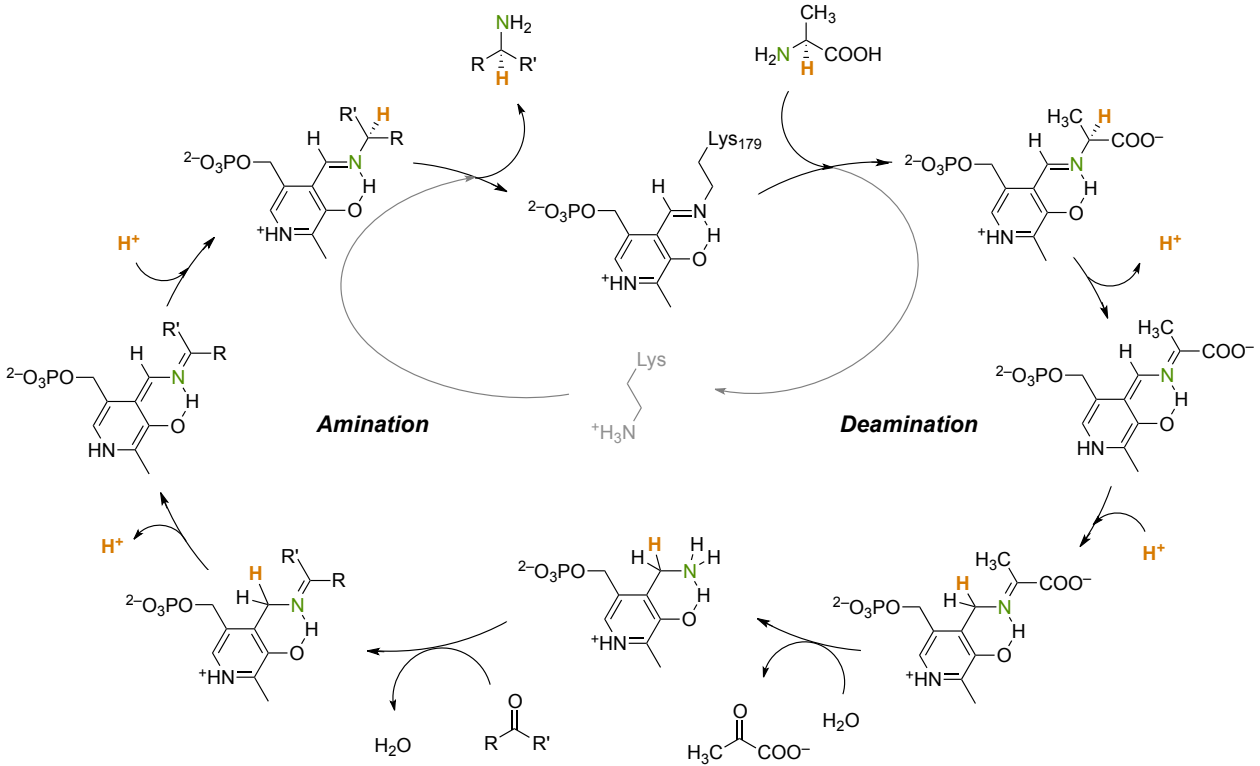


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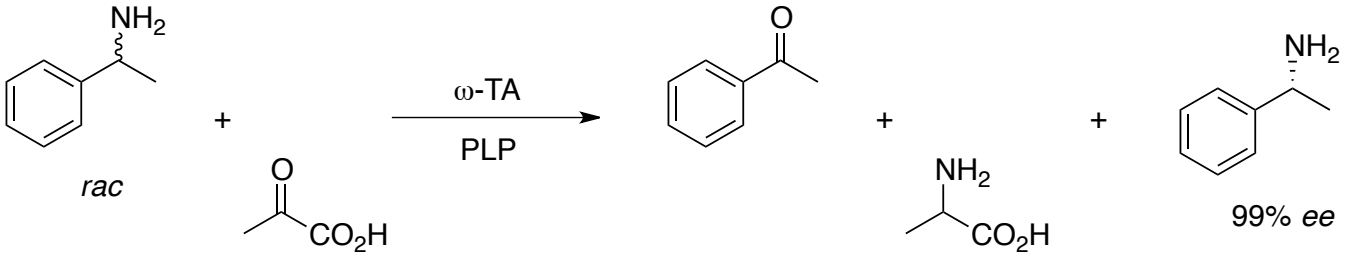
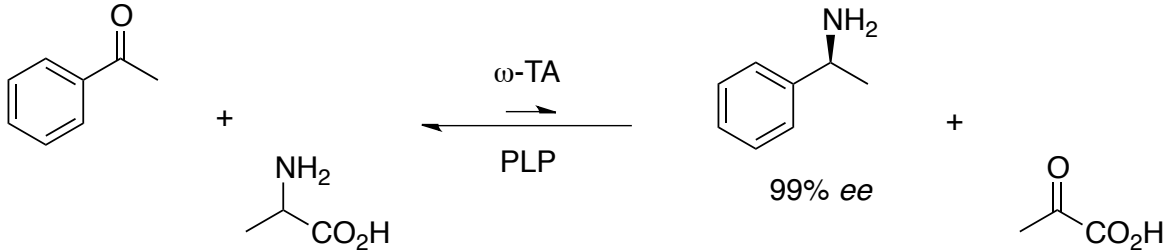
# ω-Transaminases

concepts for the application of ω-TAs

- enantiodiscriminative deamination = kinetic resolution




- asymmetric amination = enantioselective synthesis



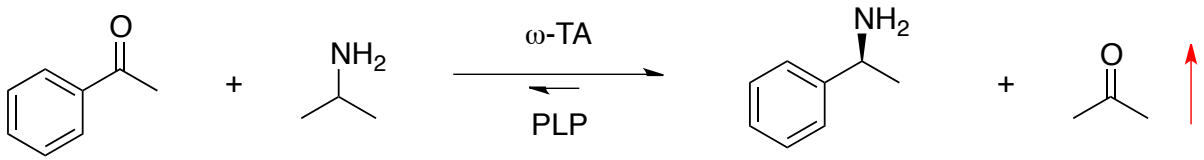

unfavourable equilibrium!

Jan Deska
Bioorganic
Chemistry

# ω-Transaminases

equilibrium shift towards the desired amine products

volatile co-products



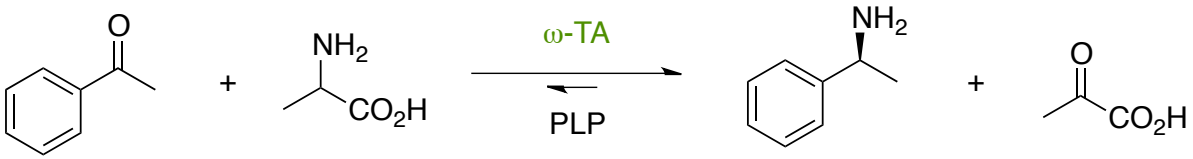

- isopropylamine is deaminated to acetone
- acetone is less soluble in water, thus can be removed by slight vacuum, elevated temperature or $N_2$-purge

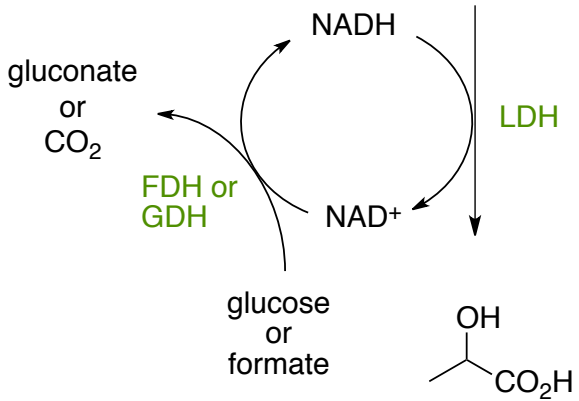
– requires relatively high concentrations of isopropylamine

# ω-Transaminases

equilibrium shift towards the desired amine products

enzyme-coupled pyruvate removal



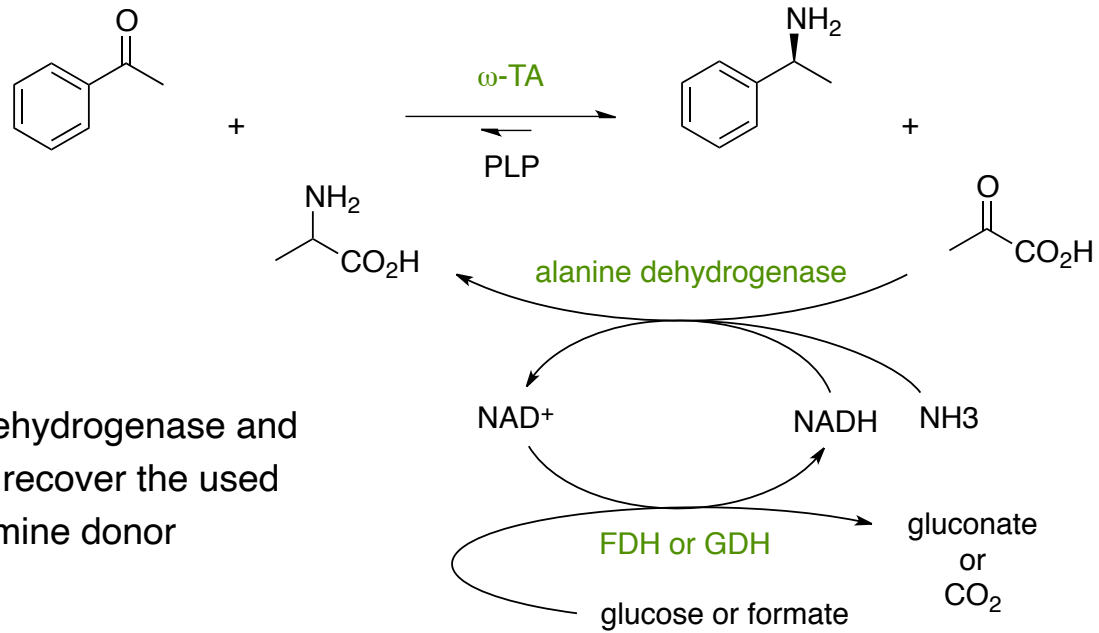

- lactate dehydrogenase (LDH) removes pyruvate

- requires cofactor recycling systems such as GDH and FDH to provide sufficient NADH

# ω-Transaminases

equilibrium shift towards the desired amine products

enzyme-coupled alanine recycling

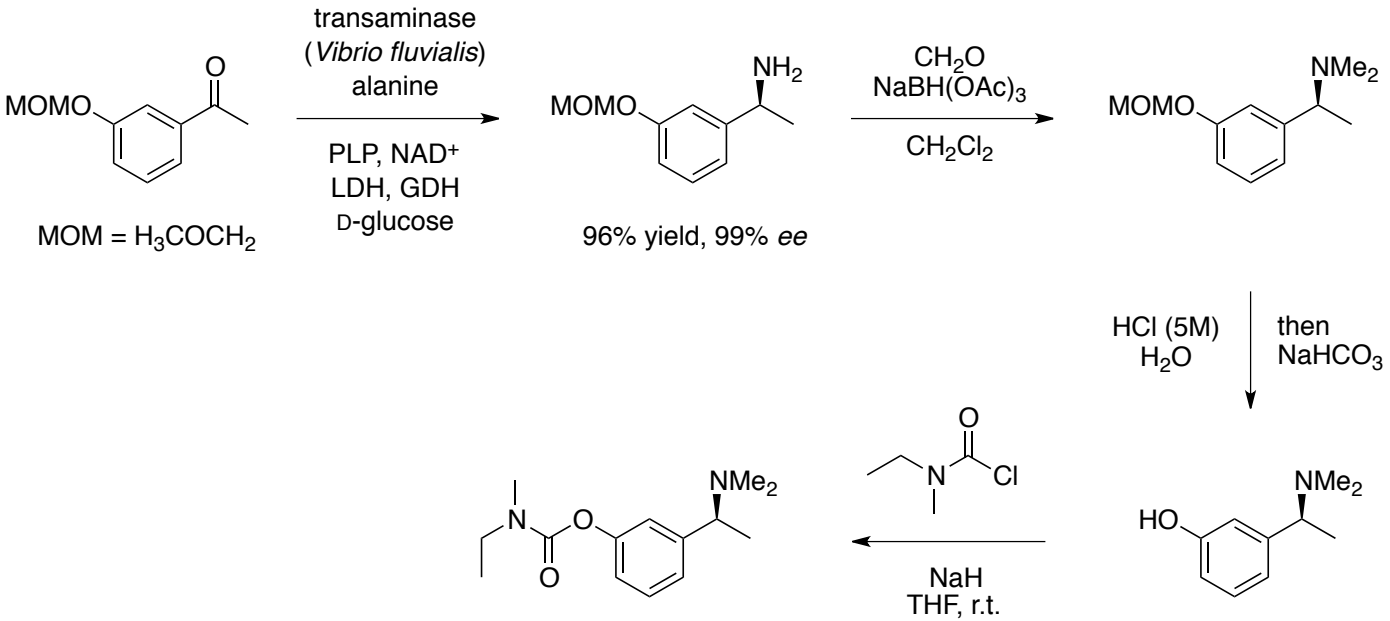



- alanine dehydrogenase and ammonia recover the used alanine amine donor

- relatively high price of AlaDH

# ω-Transaminases

synthetic applications

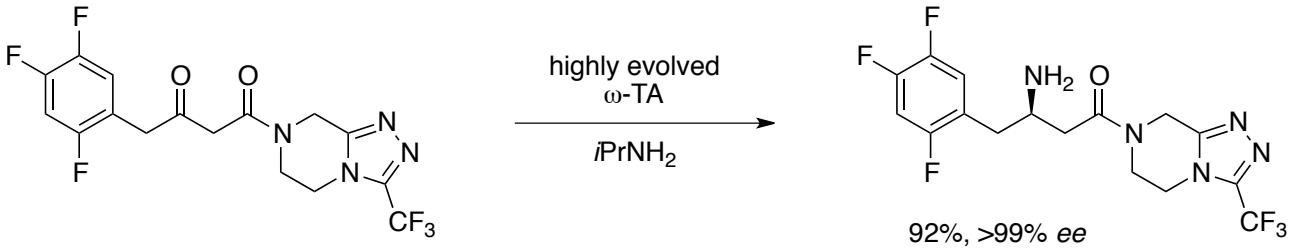



rivastigmine ("Exelon")
treatment of dementia, Alzheimer's and Parkinson's

Fuchs, Koszelewski, Tauber, Kroutil, Faber, *Chem. Commun.* **2010**, *46*, 5500-5502.

# ω-Transaminases

synthetic applications




sitagliptin
treatment of diabetes mellitus II

- late-stage biotransformation
- up to 200 g/L
- original industrial process: ruthenium-catalyzed enamine reduction

Savile, Janey, Mundorff, Moore, Tam, Jarvis, Colbeck, Krebber, Fleitz, Brands, Devine, Huisman, Hughes, *Science* **2010**, *329*, 305-309.

# SUMMARY

amine oxidases

- niche biocatalysts with some quite unique applications
- flavin-dependent mechanism with similarities to Baeyer-Villiger monooxygenases

transaminases

- blockbuster enzyme class
- very developed, first industrial applications
- PLP-dependent mechanism that relies on a series of redox processes
- yet, not classified as oxidoreductase