MANCHESTER 1824
The University of Manchester

FAPESP
Brazilian ChemComm Symposium – Chemistry and Sustainable Energy
05/11/2012 - FAPESP - São Paulo

CoEBio3
Centre of Excellence for Biocatalysis, Biotransformations and Biocatalytic Manufacture

Design and Evolution of New Biocatalysts for Organic Synthesis

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Biocatalysis in Synthesis

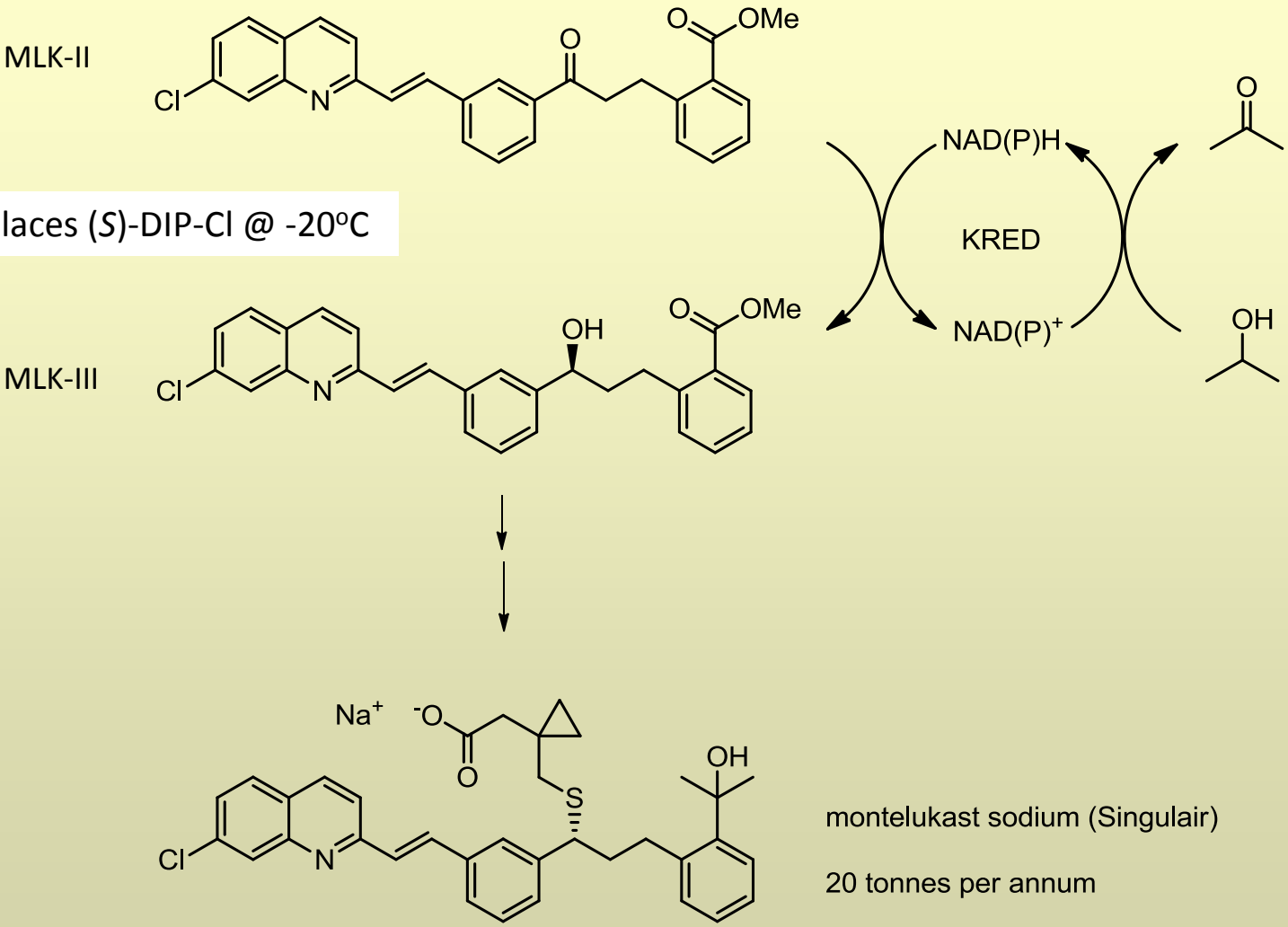
- **Biocatalysts can replace chemo-catalysts in synthetic routes (e.g. KRED for ketone reduction).**

- **Biocatalysts can also enable new synthetic pathways which may be shorter and more efficient.**

- **Combining bio- and chemo-catalysis generates further opportunities for new synthetic routes.**

- **Need biocatalysts with broad substrate scope that are active and stable under the conditions of a chemical process.**

Montelukast



D. Rozzell and J. Liang, *Speciality Chemicals Magazine*, 2008, 36.

Montelukast

Comparison of biocatalytic and (*S*)-DIP-Cl process metrics for MLK-II to MLK-III

Parameter	Biocatalytic Process	(S)-DIP-Cl Process
Ketone Concentration	100g/L	100g/L
Catalytic/Stoichiometric	**Catalytic**	1.8 eq DIP-Cl
Temperature	**45 °C**	-25 °C
Conversion	99.3%	Not provided
Product Isolation	Direct filtration	Extraction with high dilution
Enantiomeric Excess	>99.9%	99.2% (after recryst.)
Solvent/MLK-III (L/Kg)	**6**	30-50
Solvents Used	IPA, H_2O, toluene	DCM, THF
Other Waste Generation	Biodegradable enzyme, cofactor	Non-biodegradable borate salts Other inorganics, 3.6 eq. pinene

D. Rozzell and J. Liang, *Speciality Chemicals Magazine*, 2008, 36.

Biocatalysis in Synthesis

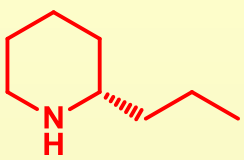
- **Can we design new & general synthetic routes to target classes (e.g. amino acids, alkaloids, terpenes etc.) based upon bio- and chemo-catalysis?**

- **Can we develop guidelines for route design for synthetic chemists (retro-biocatalysis).**

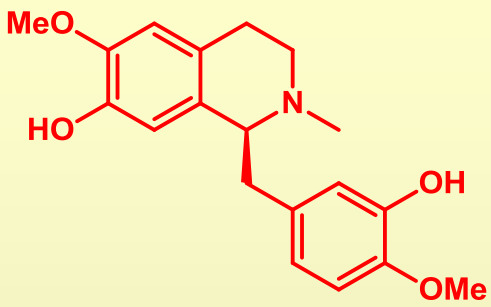
- **Where are the gaps in biocatalysis – which reactions are currently not available?**

- **How can we expand the biocatalysis toolbox?**

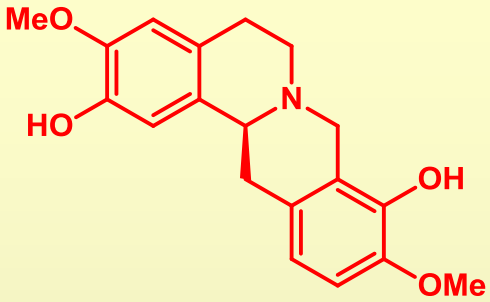
Alkaloids



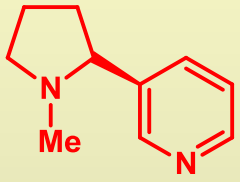
(R)-coniine
(hemlock)



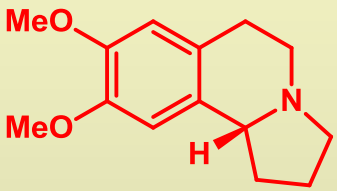
(S)-reticuline



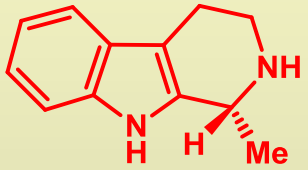
(S)-scoulerine



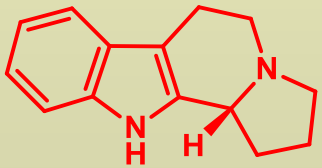
(S)-nicotine



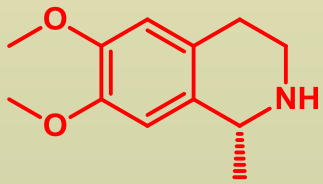
Crispine A
(anti-tumour)



(R)-Eleagnine
(chocolate, cocoa)



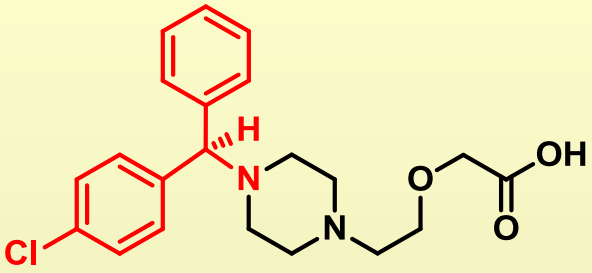
(R)-harmicine
(anti-leishmania)



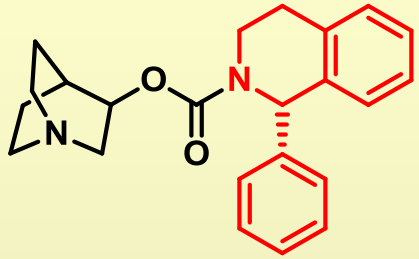
(R)-salsolidine

Biosynthesis √ Total Synthesis √ Biocatalysis?

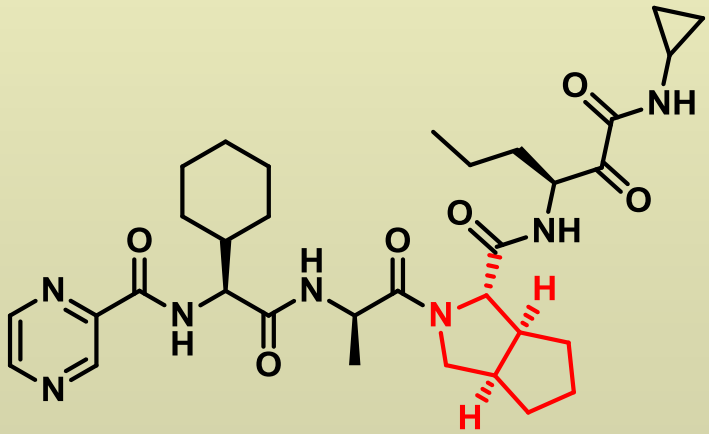
Synthetic APIs



Levocetirizine



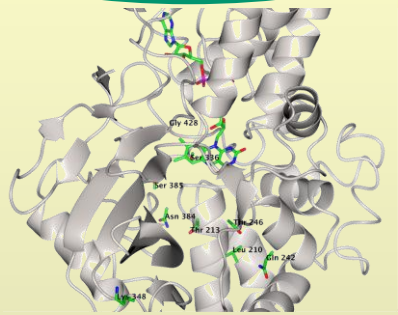
Solifenacin



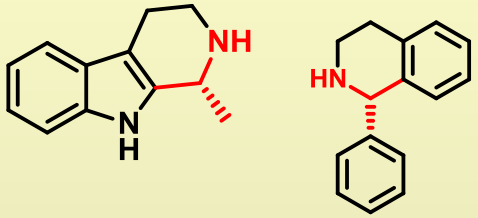
Telaprevir

Synthetic Biology

Directed evolution and rational design

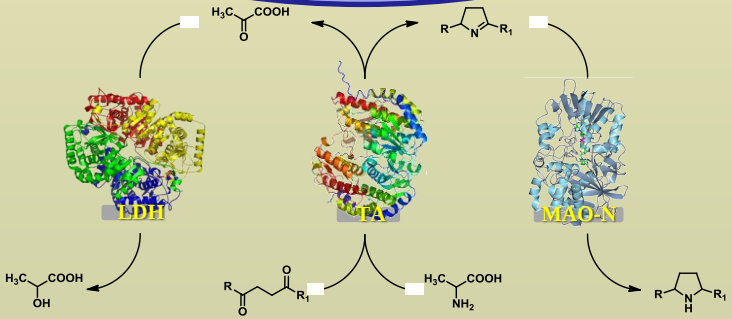


Biocatalysis on synthetic substrates

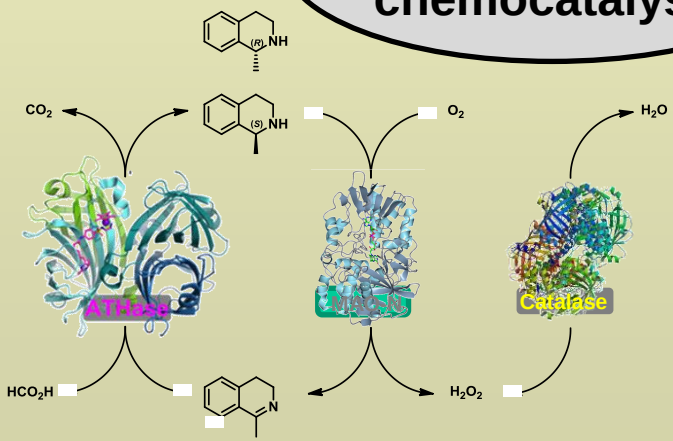


Biocatalysts

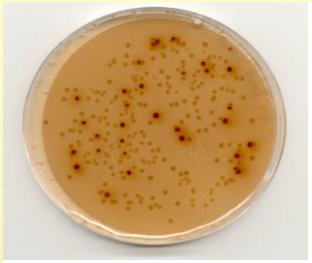
Combination with other biocatalysts

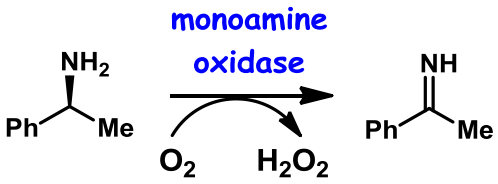


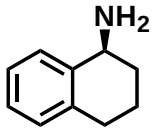
Combination with chemocatalysts

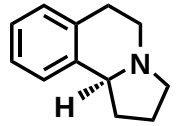


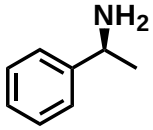
Directed evolution of MAO-N

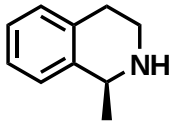


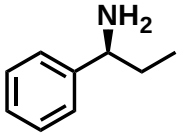












Wild-Type MAO-N

1st random library ca. 150,000 clones

D1

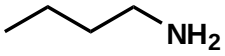
2nd random library followed by 'hot-spot' libraries ca. 20,000 clones

D5

'active-site' libraries ca. 10,000 clones

D6/D7

simple achiral 1° amines



chiral 1° amines

chiral 1°/2°/3° amines

broad spectrum (*S*)-selective amine oxidase

>10^3 improvement in k_{cat}
e.e >98%

MAO-N D8 D11

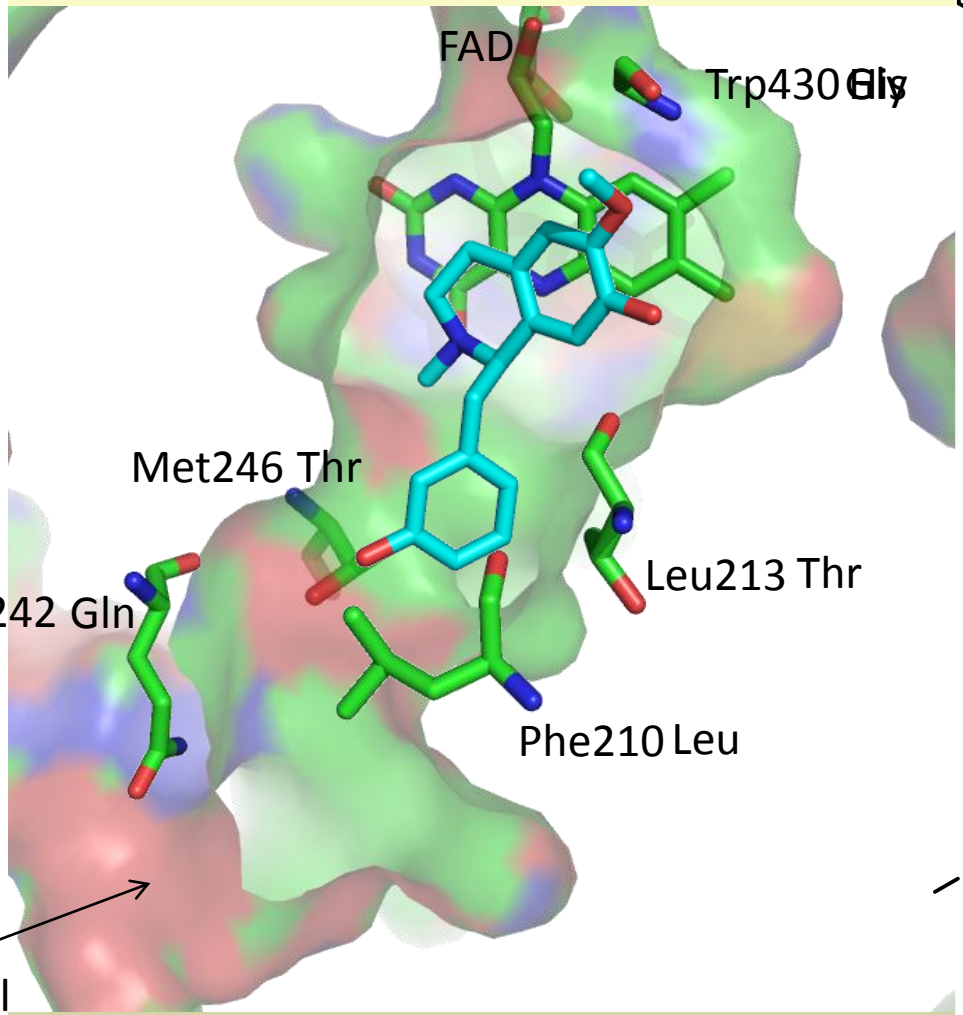
Active Site Volume

D5 - 140Å

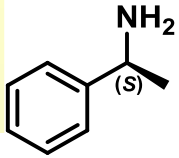
D6 - 169Å

D9 - 409Å

D11 - 464Å

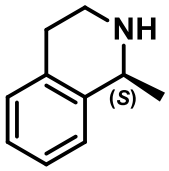


α-Methylbenzylamine



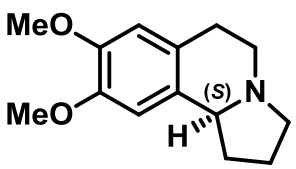
M_w=121
Volume= 102Å

Methyl-tetrahydroisoquinoline



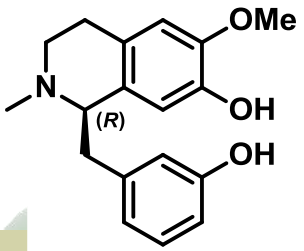
M_w=147
Volume=122Å

Crispine A



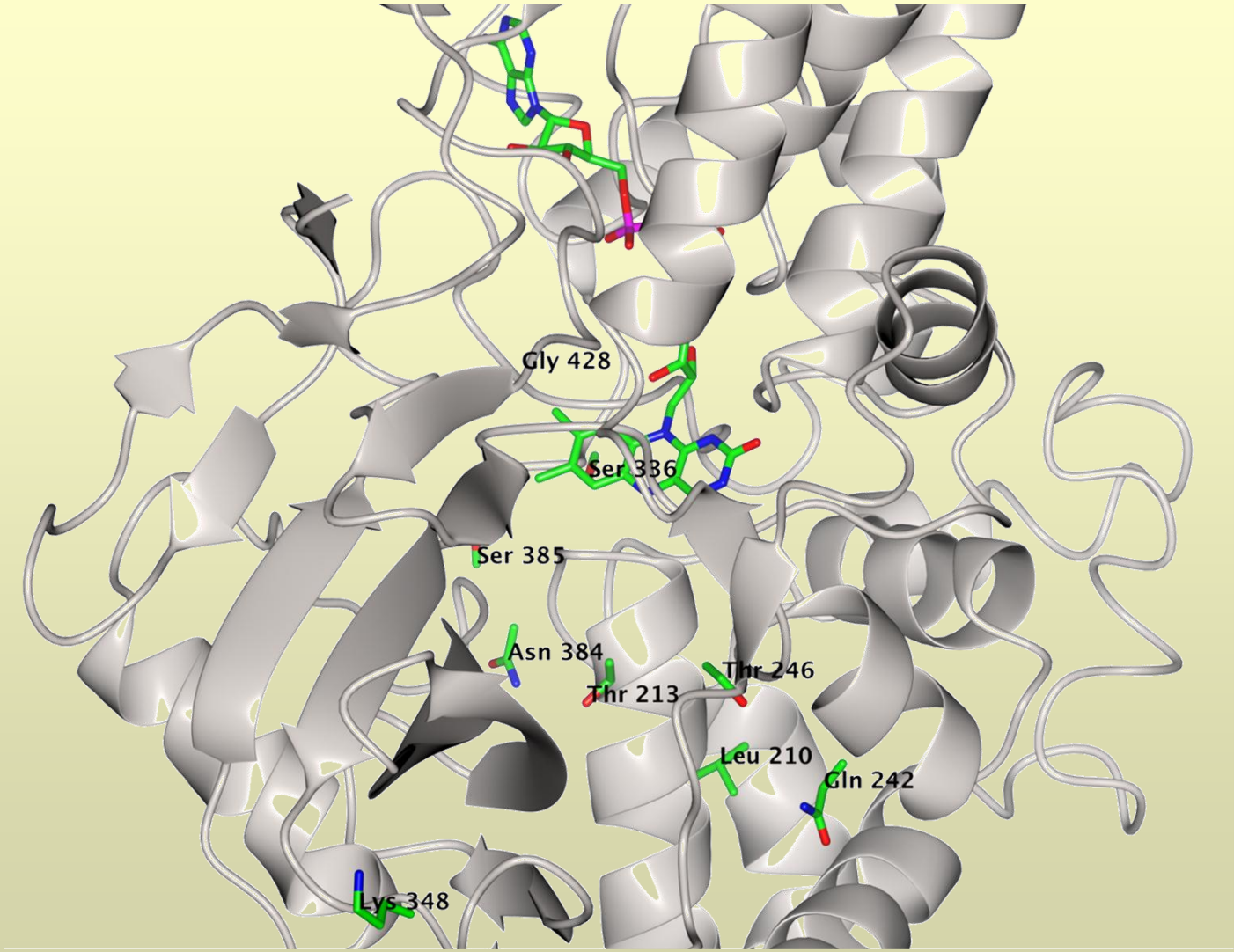
M_w=233
Volume=183Å

Benzylisoquinoline

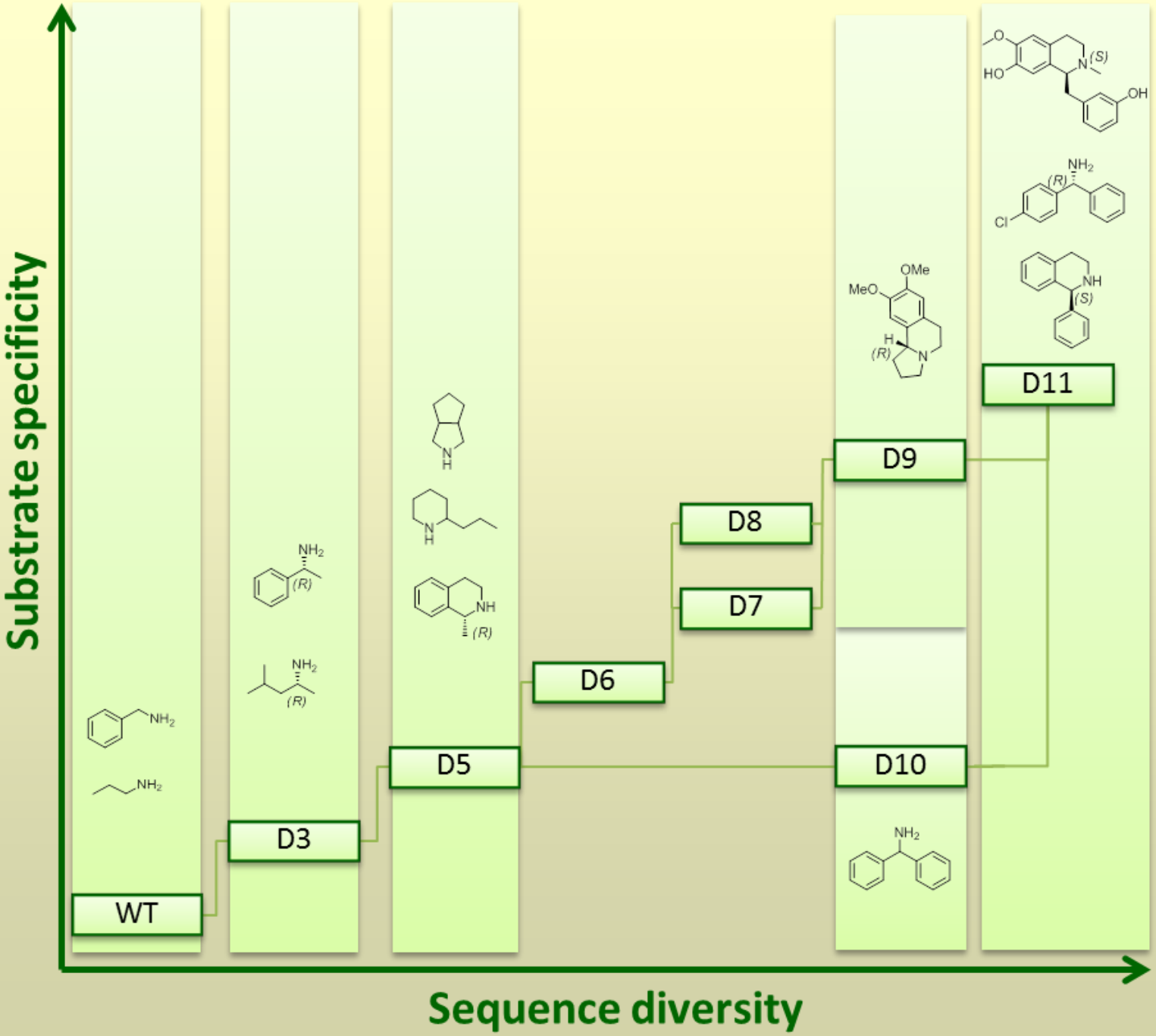


M_w=299
Volume=232Å

MAO-N D11 crystal structure

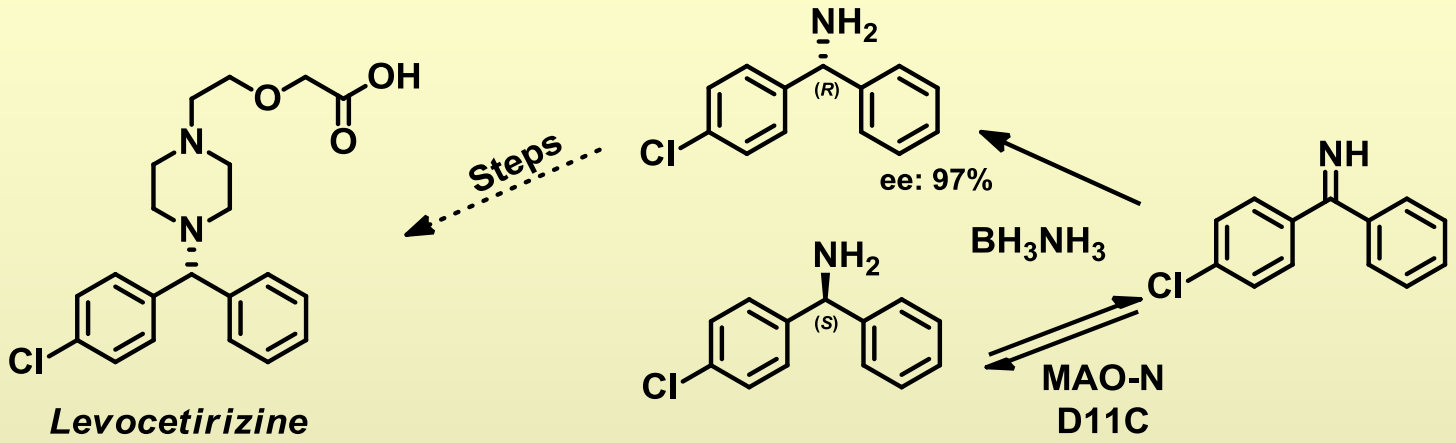


In collaboration with Gideon Grogan and Annika Frank (University of York)

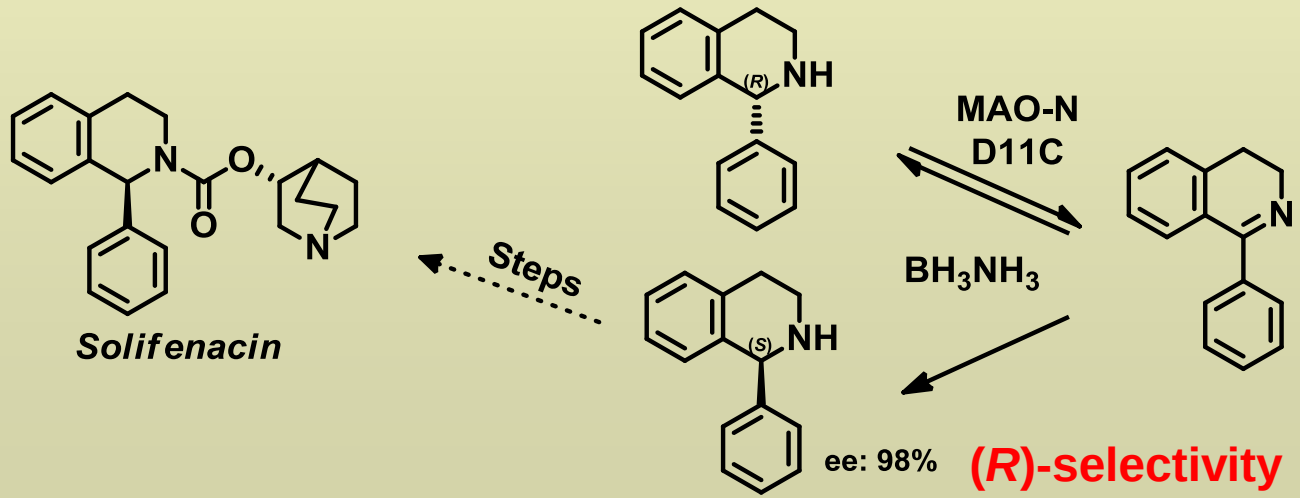

Substrate specificity
Sequence diversity
WT
D3
D5
D6
D7
D8
D9
D10
D11
NH2
(R)
NH
(S)
OMe
MeO
HO
OH
Cl

Deracemisation of API building blocks

4-chlorobenzhydrylamine:

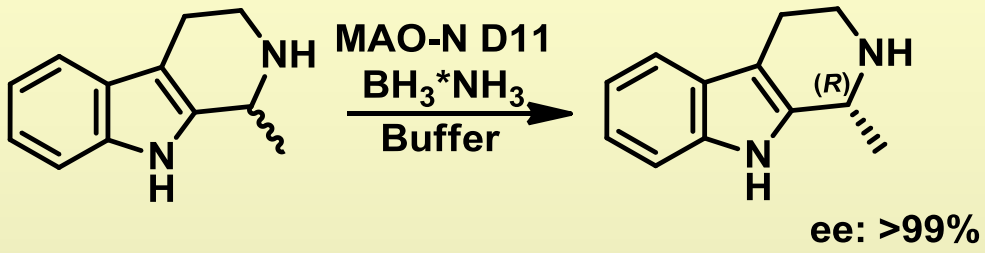


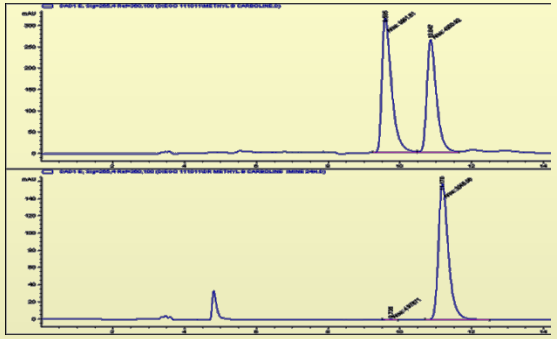
1-phenyltetrahydroisoquinoline:



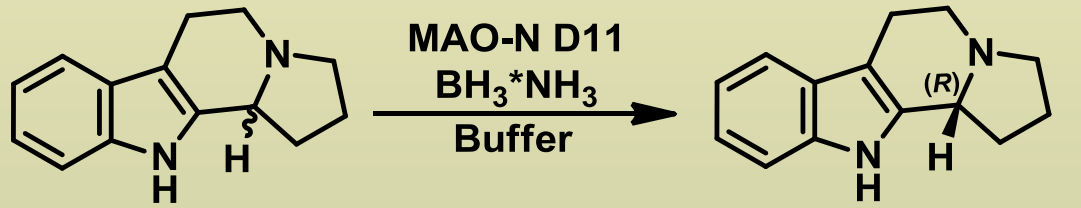
Deracemisation of tetrahydro-β-carbolines

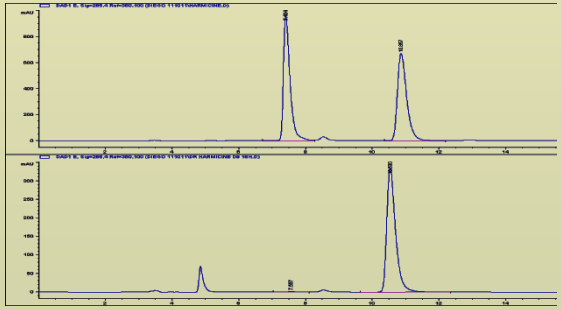
Eleagnine:



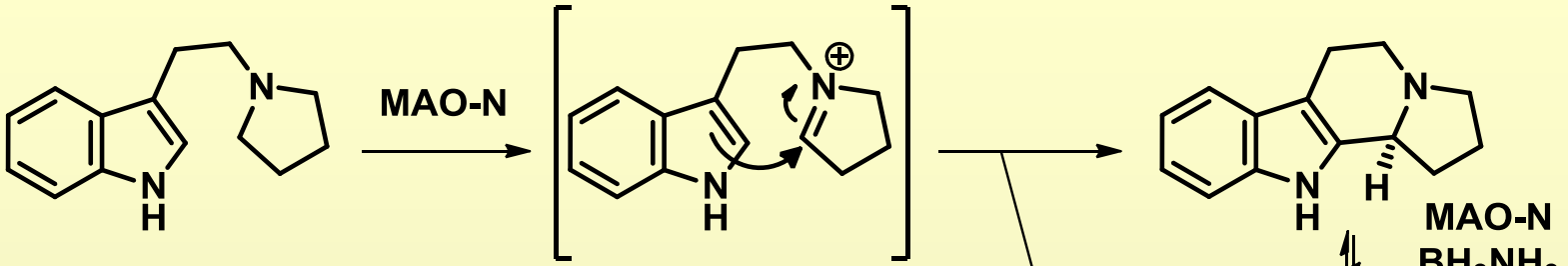


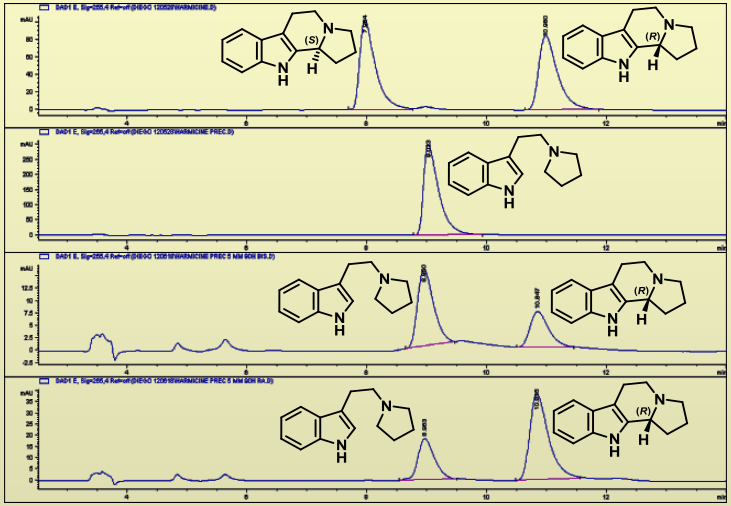
Harmicine:





4 reactions: 1 x C-C; 2 x Ox; 1 x Red

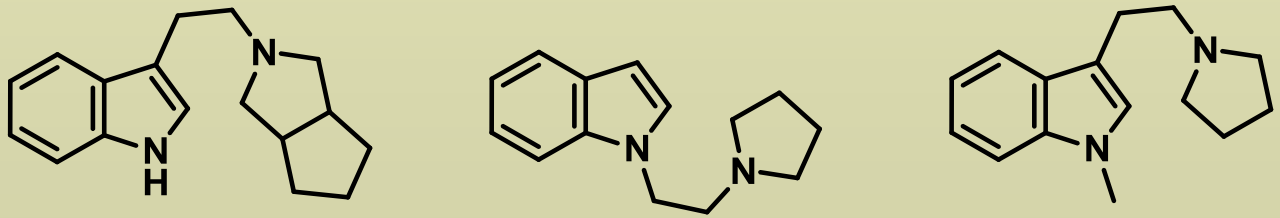




Harmicine:

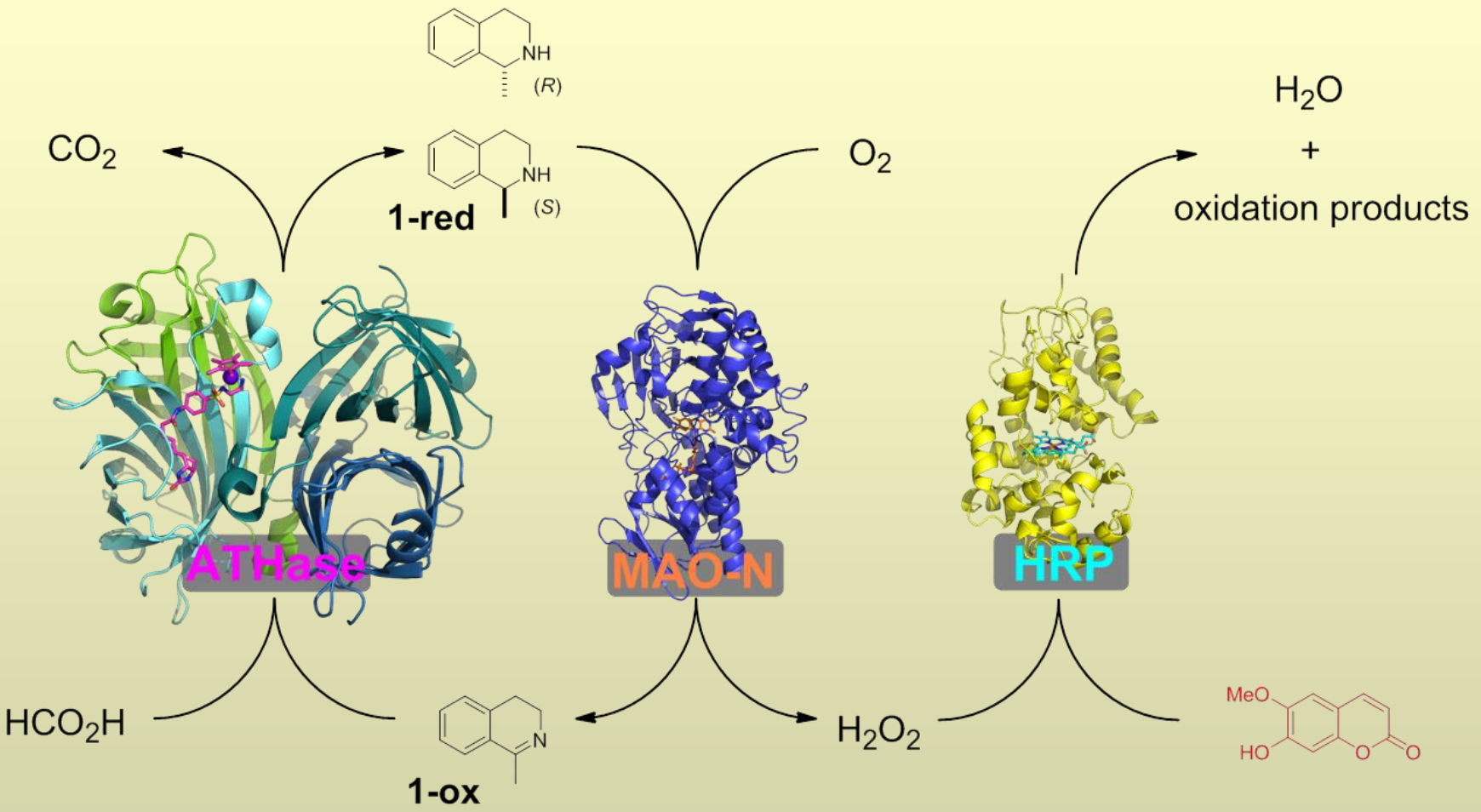
Conv.: 72%

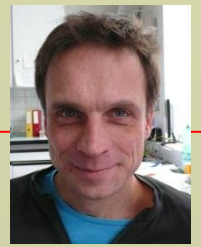
ee: >99%



Diego Ghislieri with Anthony Green and Marta Pontini

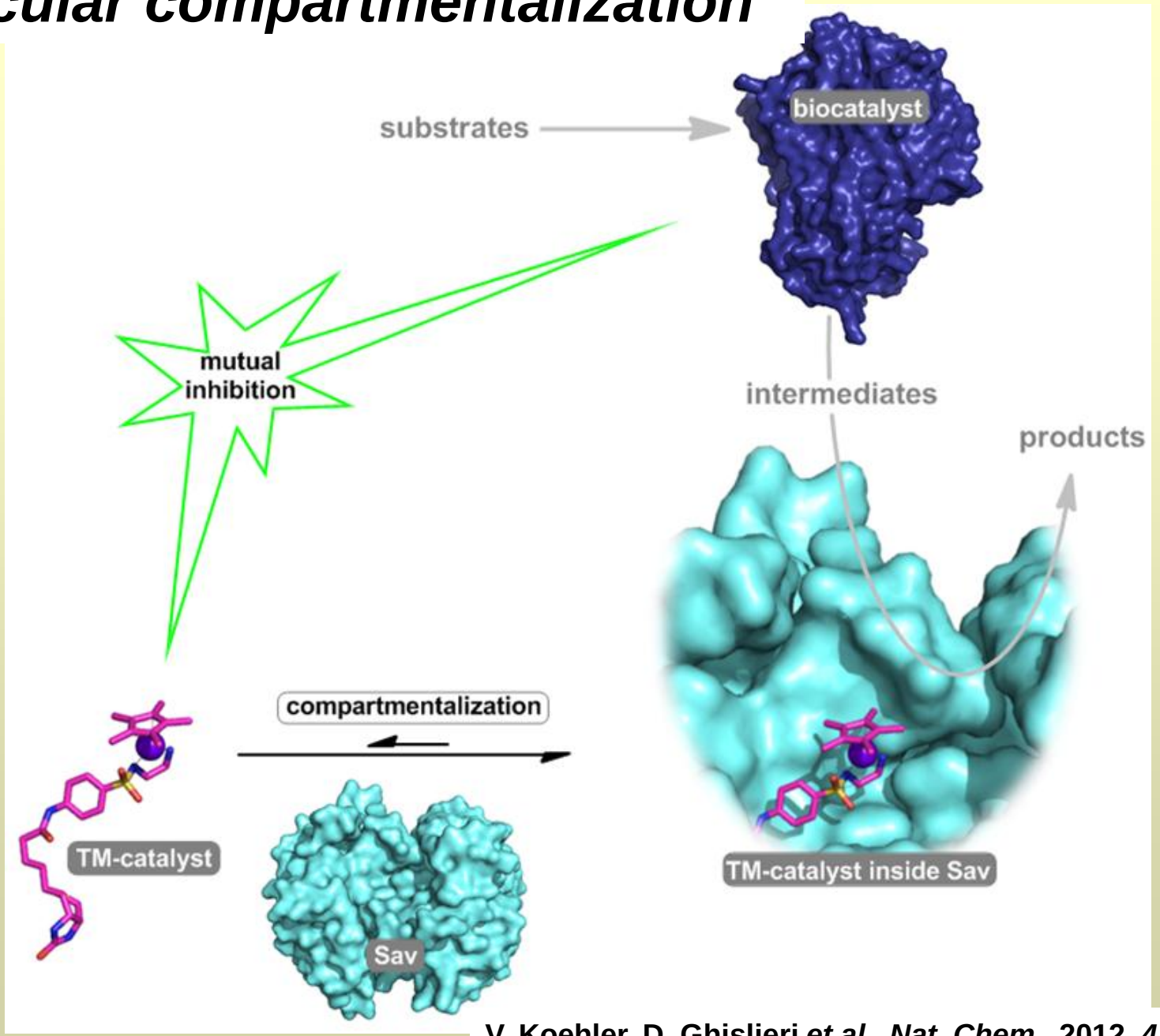
MAO-N / ATH tandem reaction





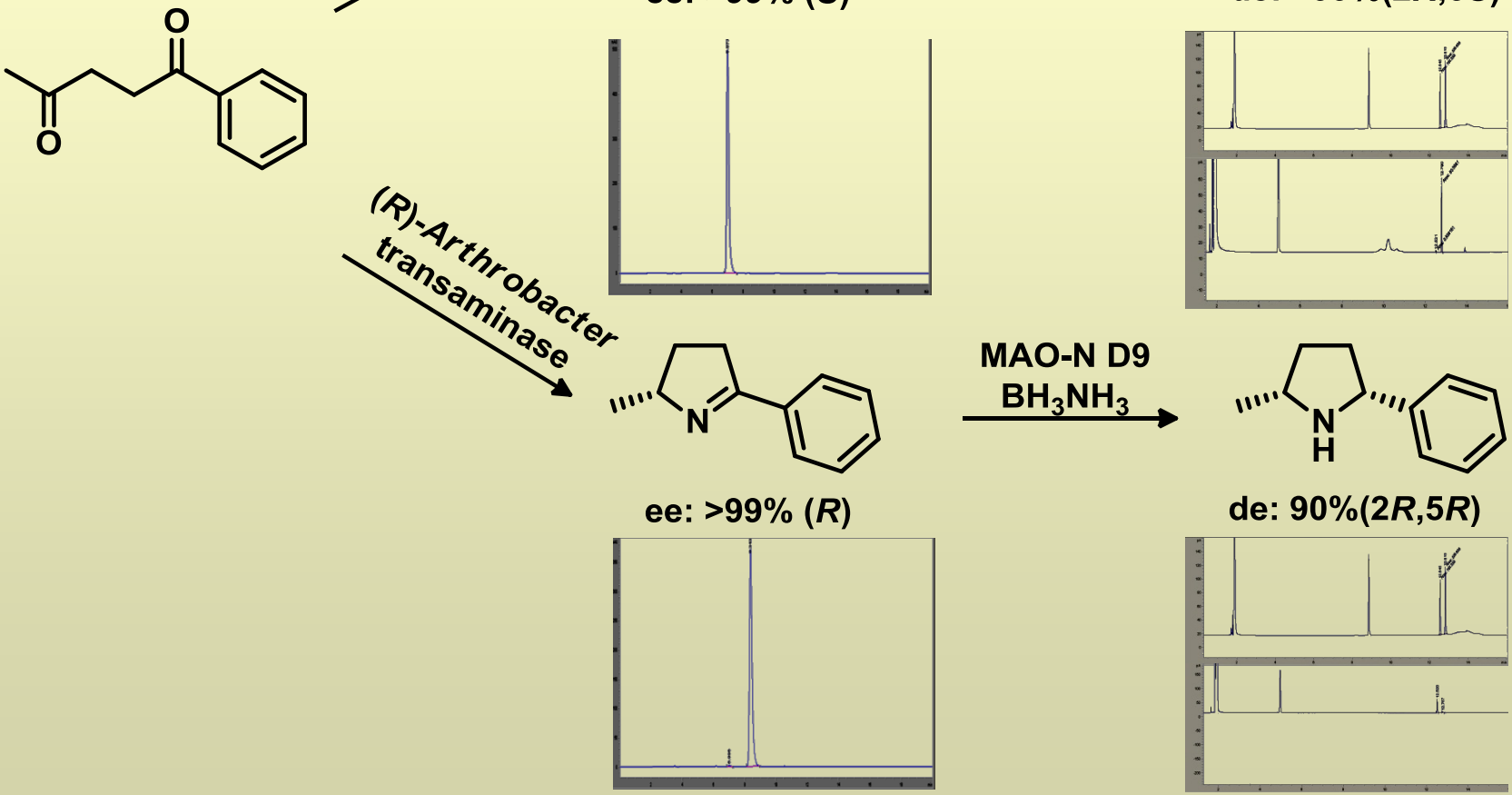
In collaboration with Tom Ward, Valentin Koehler (University of Basel)

Molecular compartmentalization



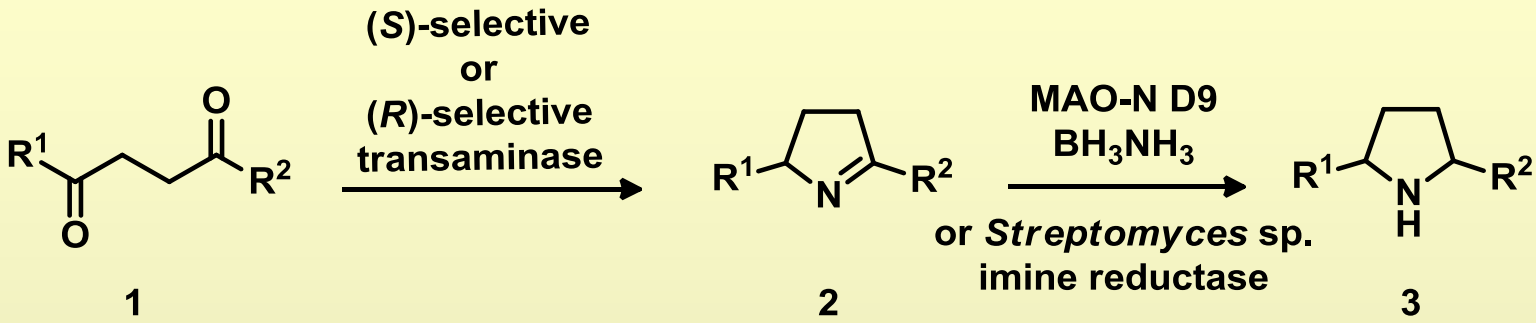
V. Koehler, D. Ghislieri *et al.*, *Nat. Chem.*, 2012, *4*, in press.

MAO-N / ω-TA tandem reaction



Diego Ghislieri & Jennifer Hopwood

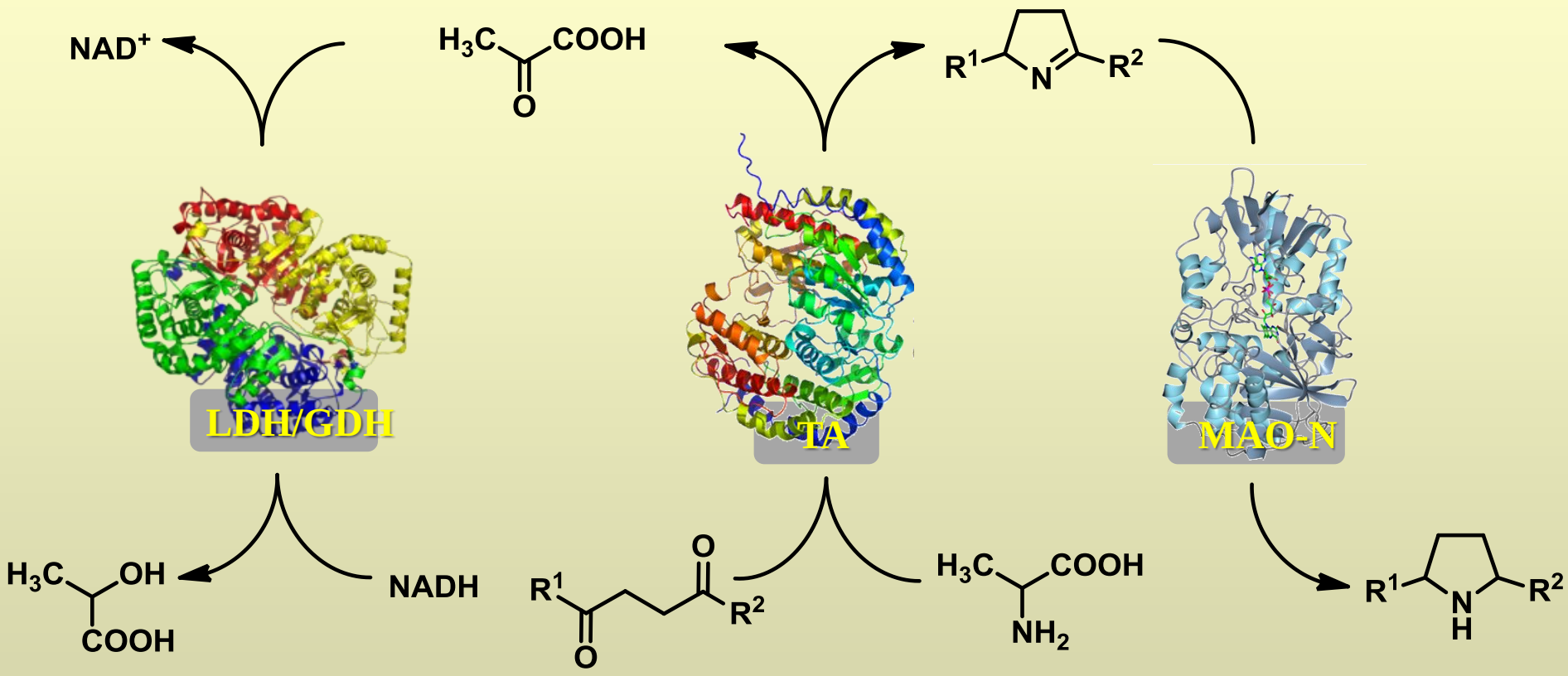
MAO-N / ω-TA tandem reaction



R^1	R^2	Transaminase	ee 2 (%)	de 3 (%)
Me	Bn	*(S)-C. violaceum*	>99 (*S*)	80 (2*R*,5*S*)
Me	Bn	*(R)-Arthrobacter*	>99 (*R*)	99 (2*R*,5*R*)
Et	Ph	*(S)-C. violaceum*	>99 (*S*)	94 (2*R*,5*S*)
Me	Me	*(S)-C. violaceum*	>99 (*S*)	99 (imine reductase)
Me	Me	*(R)-Arthrobacter*	>99 (*R*)	99 (imine reductase)

Diego Ghislieri & Jennifer Hopwood

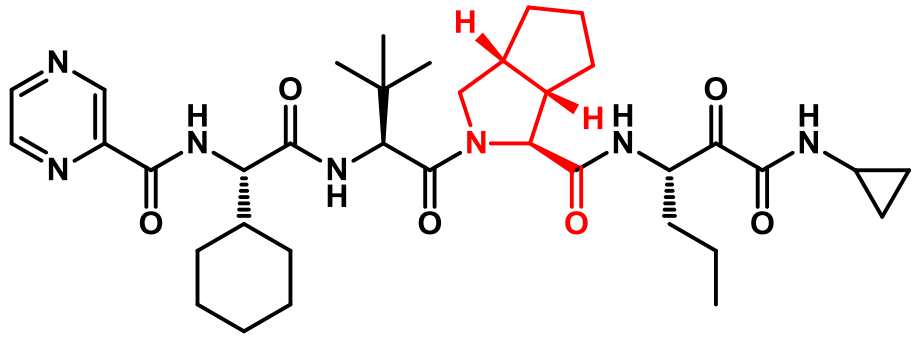
MAO-N / ω-TA tandem reaction



Diego Ghislieri & Jennifer Hopwood

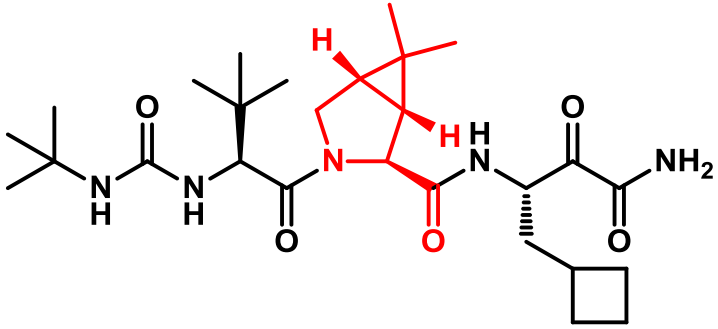
Hepatitis C viral protease inhibitors

Telaprevir (Vertex - Phase III)



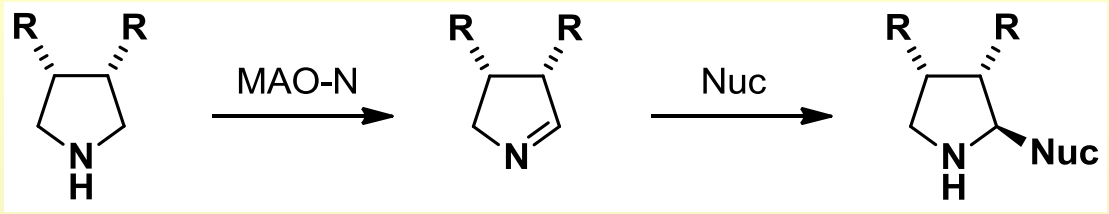
P. Revill et al., *Drugs Future* **2007**, 788

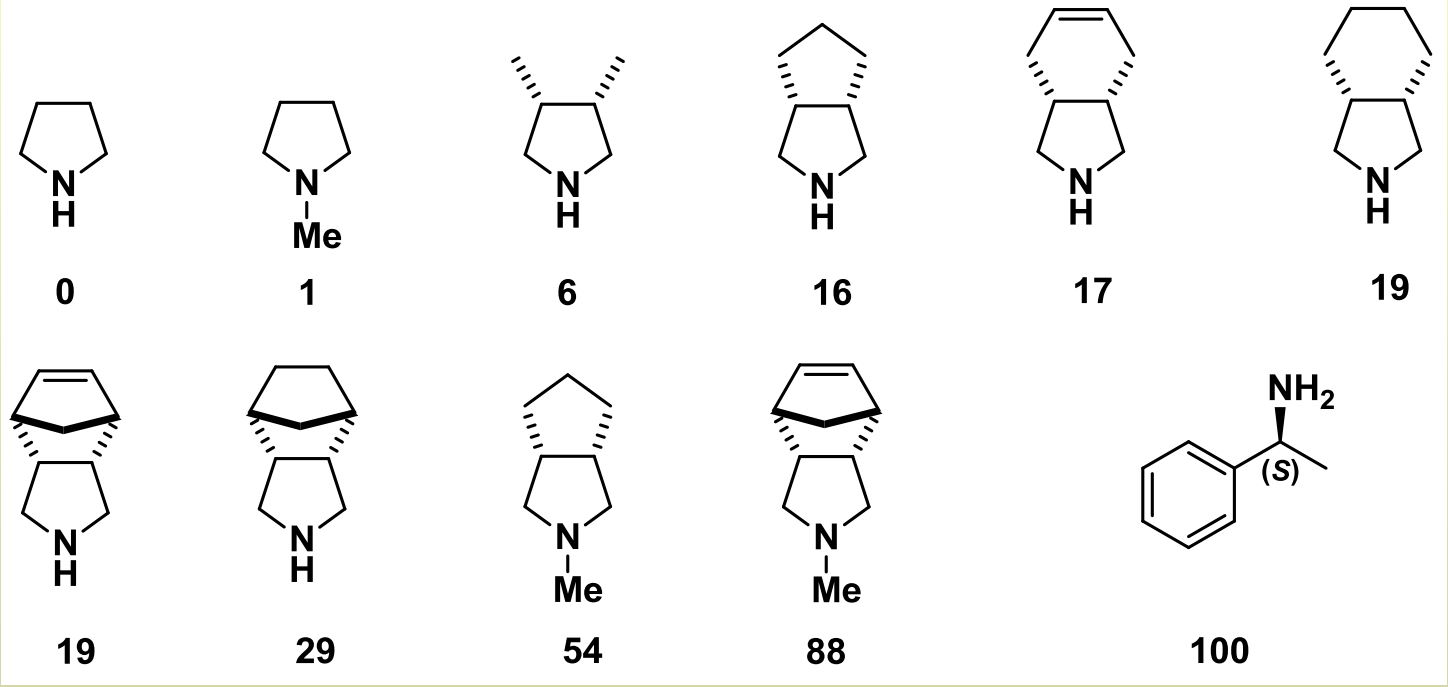
SCH 503034 (Schering-Plough - Phase III)



F. G. Njoroge et al., *Acc. Chem. Res.* **2008**, 50

Desymmetrisation of symmetrical amines





e.e.'s = >98%

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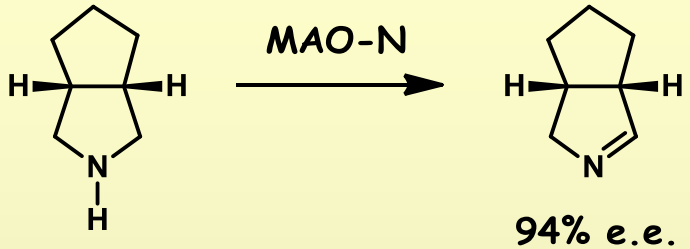
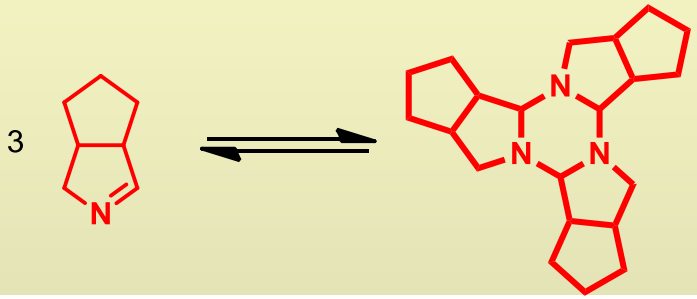
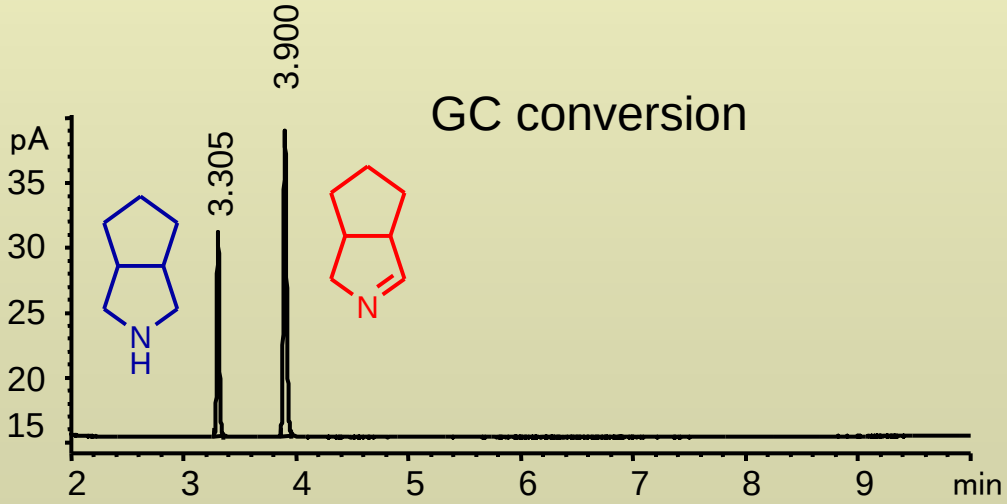
biocatalytic oxidation

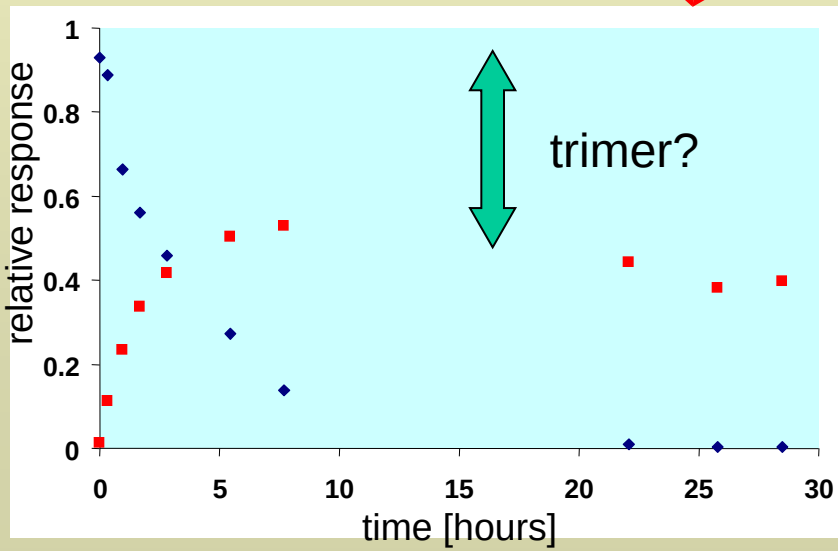
Valentin Koehler





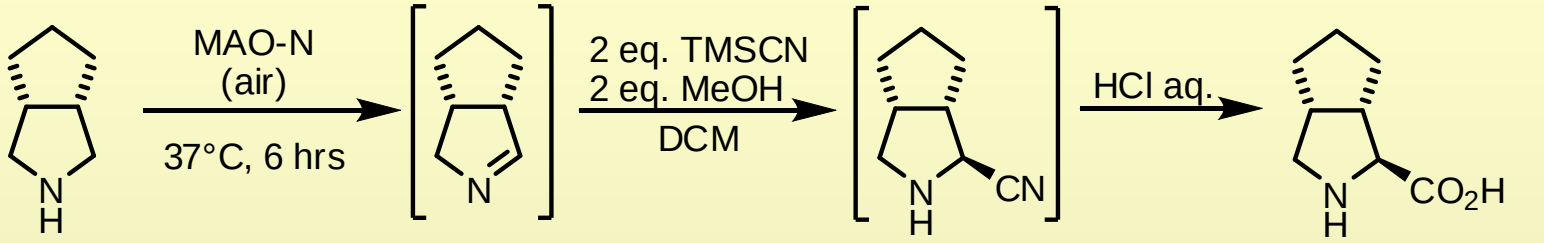
GC conversion



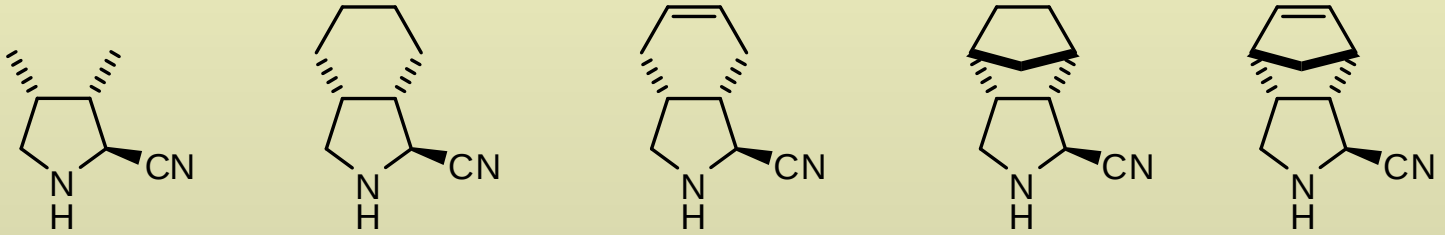


biotransformation: 50 mM, wet cells (100g/L)
after **5hr 30 min**

Synthesis of bicyclic amino acid



51% from amine
dr 96 : 4; 94 % e.e.
crystallise
dr 150:1; 99% e.e.



dr: 86 : 14 | 96 : 4 | 90 : 10 | > 99 : 1 | > 99 : 1

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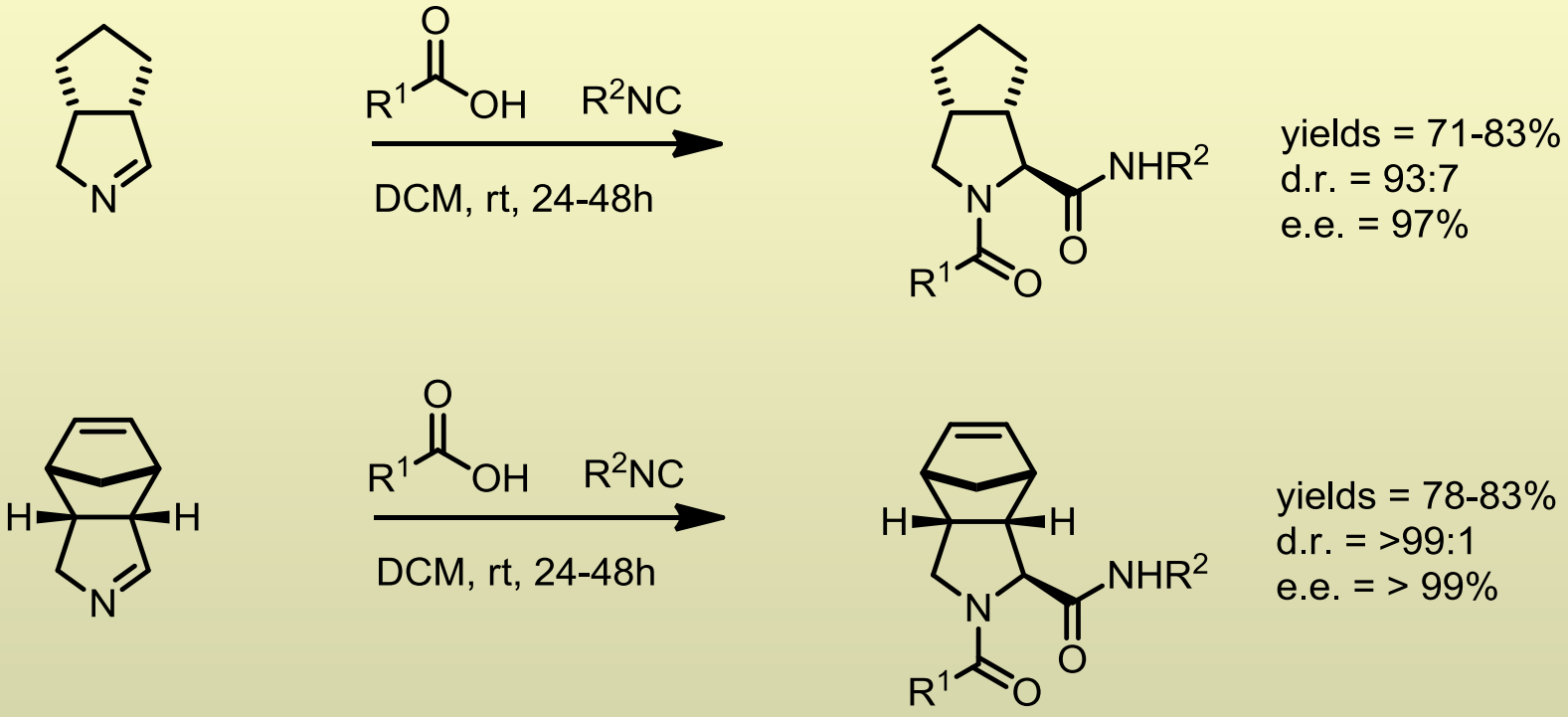
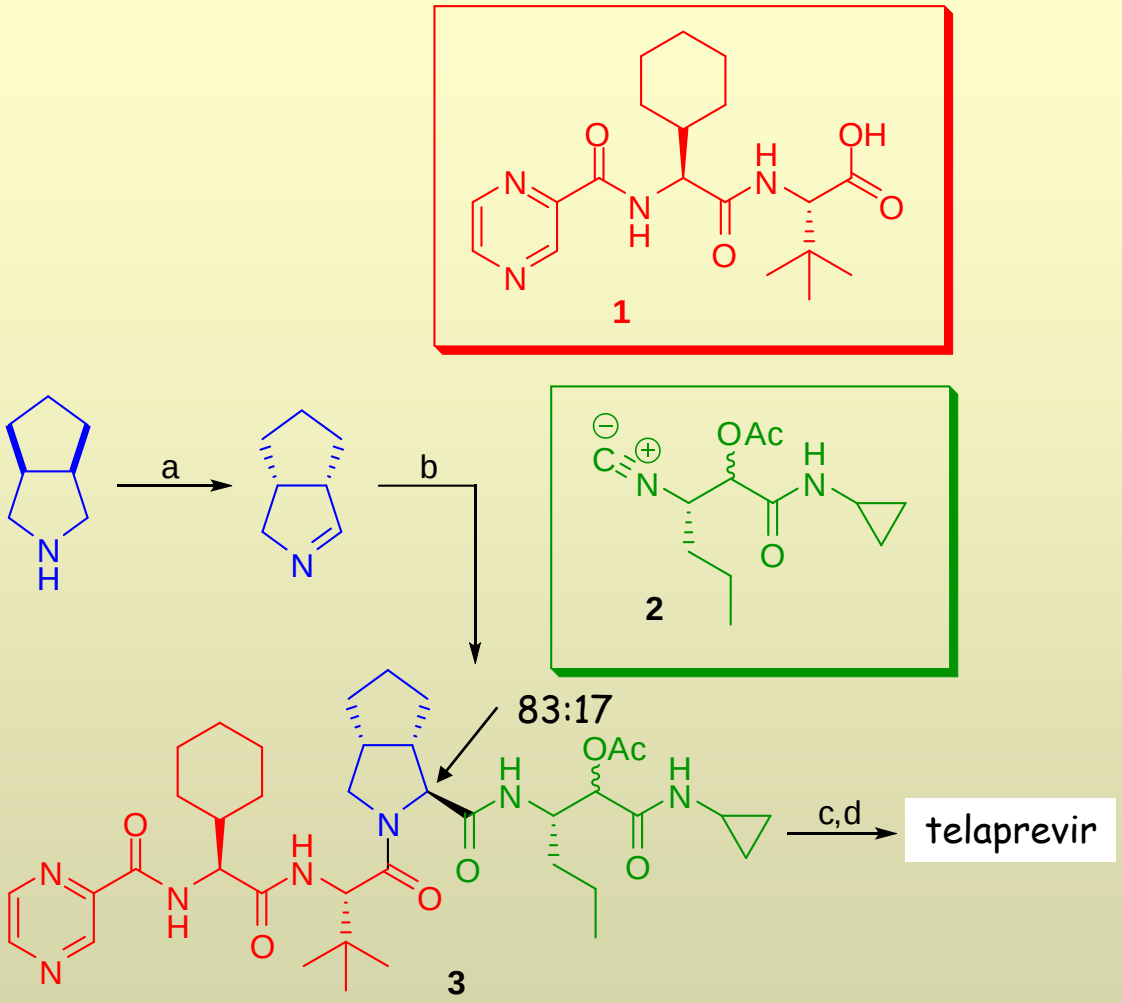
Multi-component reactions

with Romano Orru (Amsterdam)



A. Znabet, R. Orru, N.J. Turner *et al*., *Angew. Chem. Int. Ed*., 2010, *49*, 5289.

Multi-component synthesis of telaprevir



Reagents and conditions: a) MAO-N, 100 mM KPO4, pH = 8.0, 37 °C, then: b) **1**,**2**, CH_2Cl_2, 50%; c) K_2CO_3, MeOH; d) Dess-Martin, CH_2Cl_2, 50% over 2 steps.

A. Znabet, R. Orru, N.J. Turner *et al*., *Chem. Commun*., 2010, 7918.

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Galactose oxidase:
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Ammonia lyase:
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Lignin degradation/biorefinery:
Mark Corbett, Emma Fellows, Lucy Heap, Chris Spencer, Claire Doherty

Carboxylic acid reductase et al:
Katharina Hugentobler, Andy Hill

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