# Enzymatic Amine Synthesis

Professor Nicholas Turner

University of Manchester

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Centre of Excellence for Biocatalysis, Biotransformations and Biocatalytic Manufacture

# *Enzymatic Amine Synthesis*

**Nicholas J. Turner**

**School of Chemistry & Manchester Institute of Biotechnology, University of Manchester, UK**

**Next Generation of Biocatalysis for Industrial Chemical Synthesis**
**Brussels, BE**
**3rd December 2013**

MARIE CURIE ACTIONS
PEOPLE

SEVENTH FRAMEWORK PROGRAMME

# *A Network of EU Funded IB Projects*

BIONEXGEN

CHEM21

AMBIOCAS

KYROBIO

BIOTRAINS

BIO INTENSE

P4fifty

MARIE CURIE ACTIONS PEOPLE

suprabio
innovative bio solutions

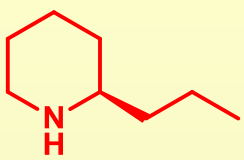
- **Enhanced collaborations with academic and industrial scientists across the EU.**
- **Providing a real focus for the development of industrially relevant technology.**
- **Training of the next generation of IB scientists for industry and public sector.**

# *Amines – Versatile Industrial Chemicals: $RNH_2$*

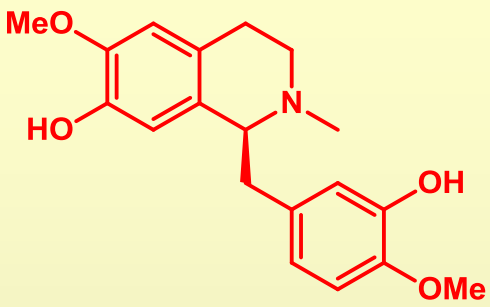
- **Intermediates for pharmaceuticals, agrochemicals**
- **Building blocks for polyamides (*e.g*. diamines)**
- **Solvents, Cleaning agents**
- **Wood treatment**
- **Personal care**
- **Water treatment**
- **Lubricants**
- **Disinfectants**

- **Traditionally manufactured using chemical process,**
- ***e.g*. reductive amination, addition of ammonia to olefins**
- **Requires high temp/pressure/pH/metal catalysts**

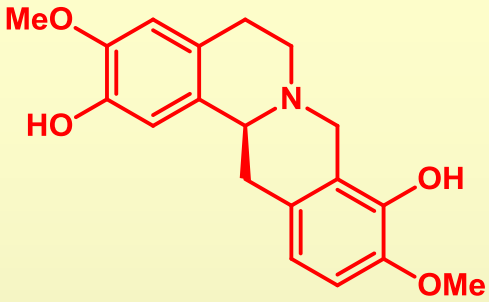
# *Alkaloids*



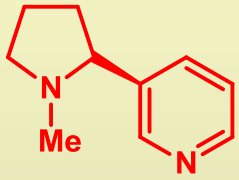

(*R*)-coniine
(hemlock)



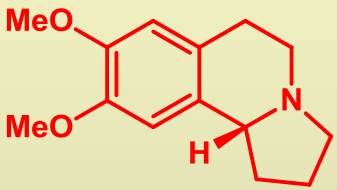

(*S*)-reticuline



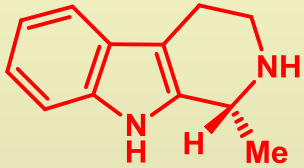

(*S*)-scoulerine



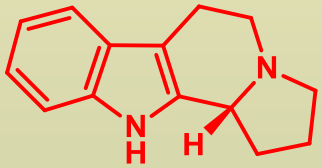

(*S*)-nicotine



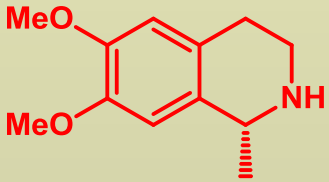

Crispine A
(anti-tumour)




(*R*)-Eleagnine
(chocolate, cocoa)



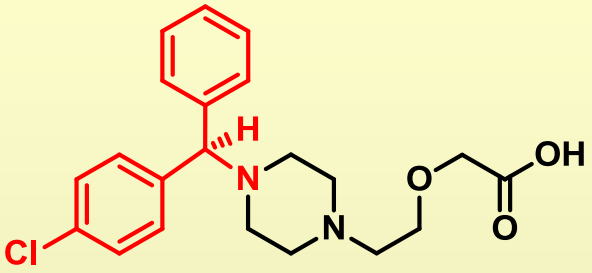

(*R*)-harmicine
(anti-leishmania)



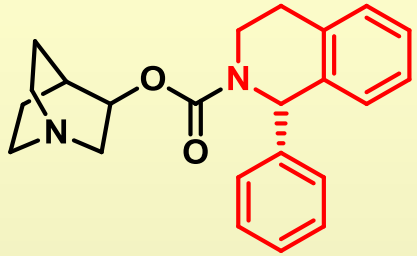

(*R*)-salsolidine

***Biosynthesis √ Total Synthesis √ Biocatalysis?***

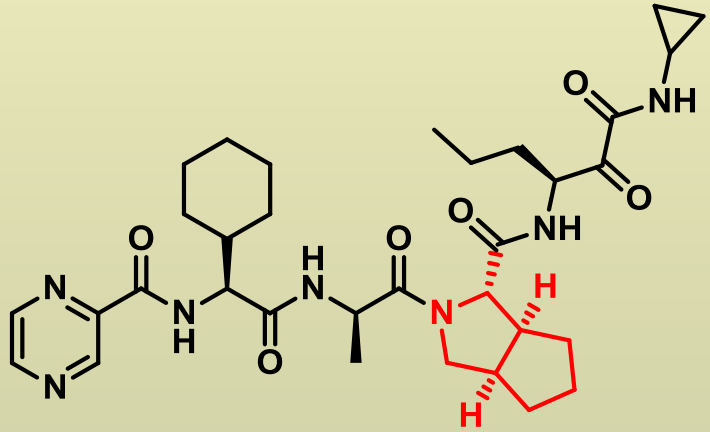
# *Synthetic APIs*




***Levocetirizine***

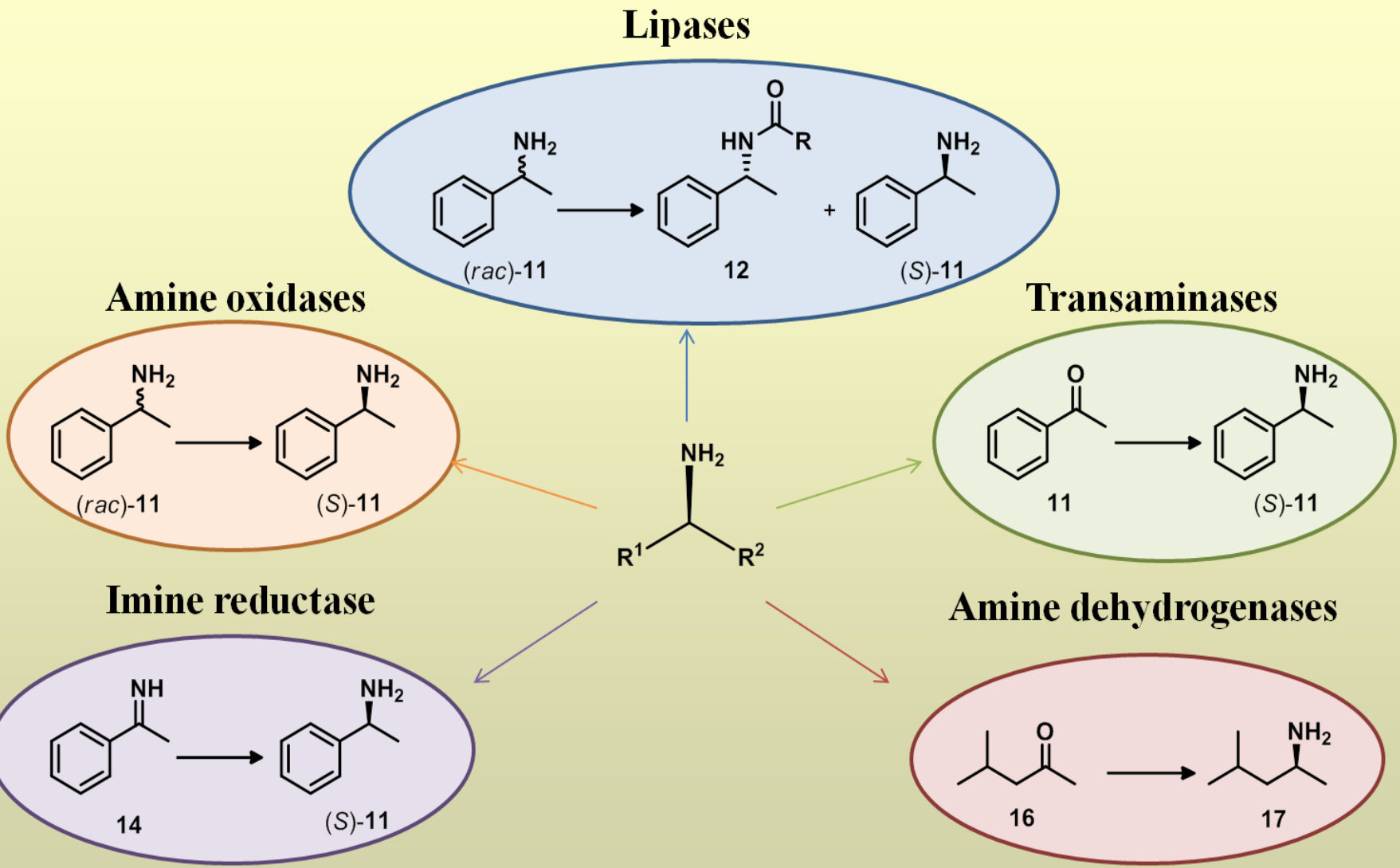



***Solifenacin***




***Telaprevir***

# *Biocatalysts for Amine Synthesis*



# *5 Major Themes in Biocatalysis*

- **Biocatalysts (enzymes/whole cells) can replace chemo-catalysts in synthetic routes (e.g. KREDs for ketone reduction).**

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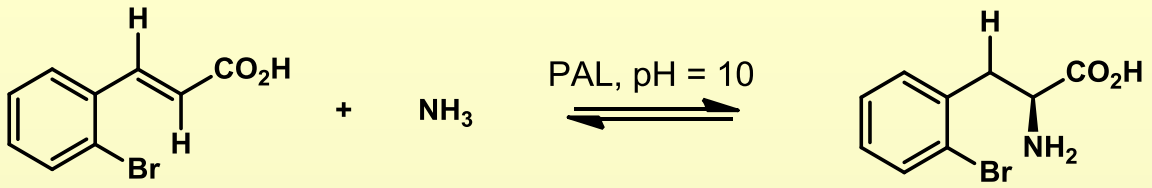
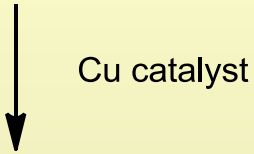
- **Biocatalysts can also enable new synthetic pathways which may be shorter, more efficient and more sustainable.**

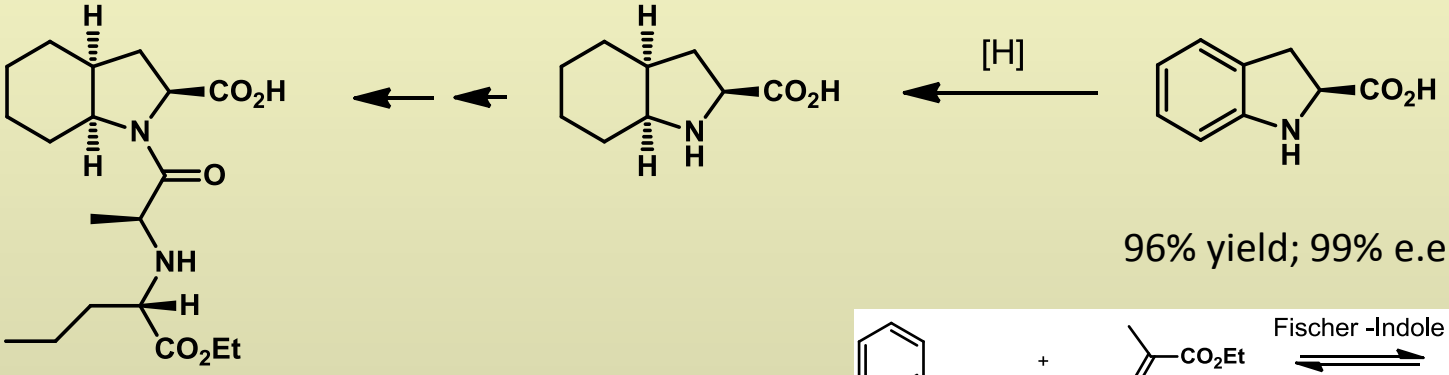
# *PAL mediated synthesis of perindopril*

**DSM**



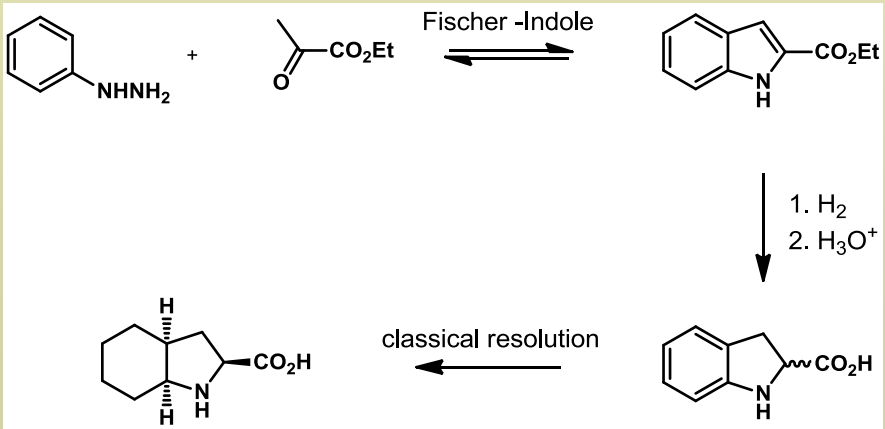




tonne-scale production




96% yield; 99% e.e.

Anti-hypertensive



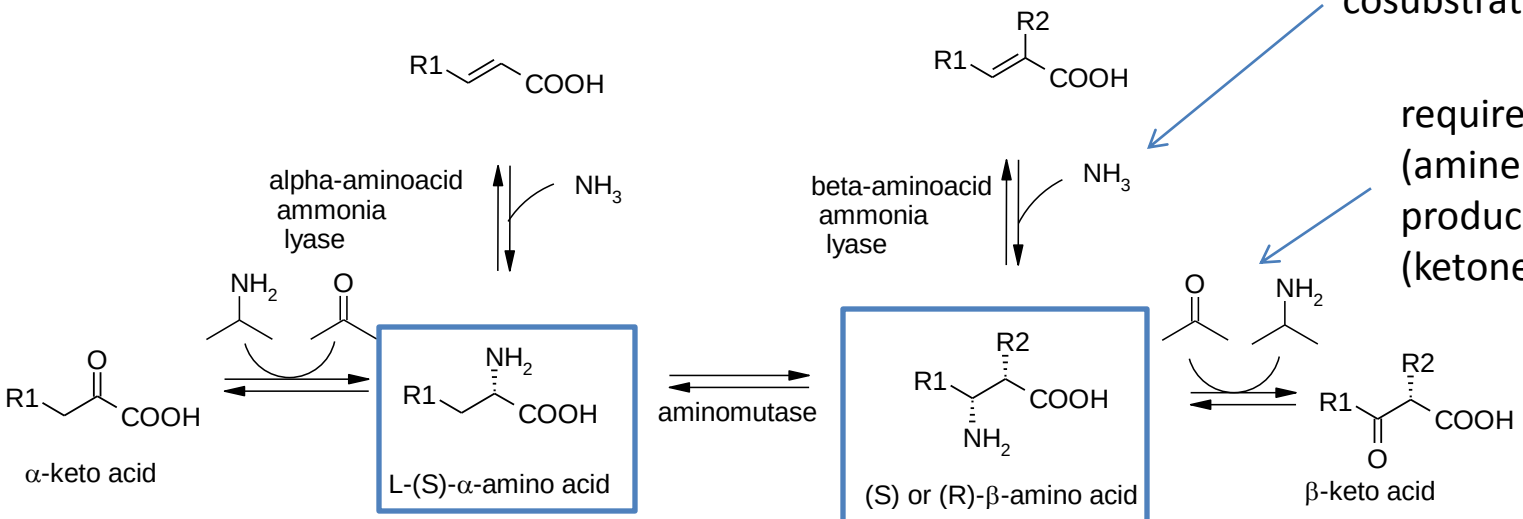

**B. de Lange *et al*., *ChemCatChem*, 2011, *3*, 289.**

# Formation and use of (beta)amino compounds

- ammonia lyases: direct addition of ammonia to double bond

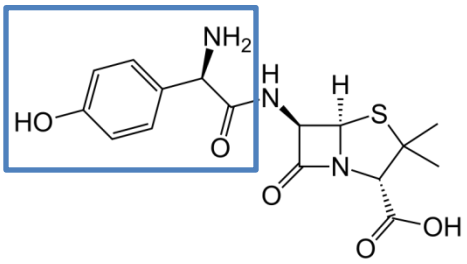
"self-sufficient: no cofactors or cosubstrates needed

requires cosubstrates (amine donor) and produces side-product (ketone)



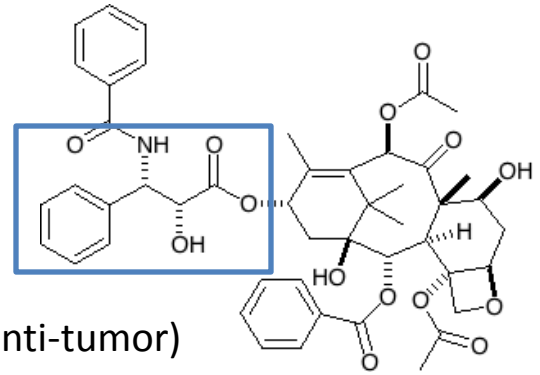

- aminomutases: 1,2-shift of amino group

- aminotransferases: conversion of ketone to amine



amoxycillin (antibiotic)

pharmaceutical bioactive compounds



taxol (anti-tumor)

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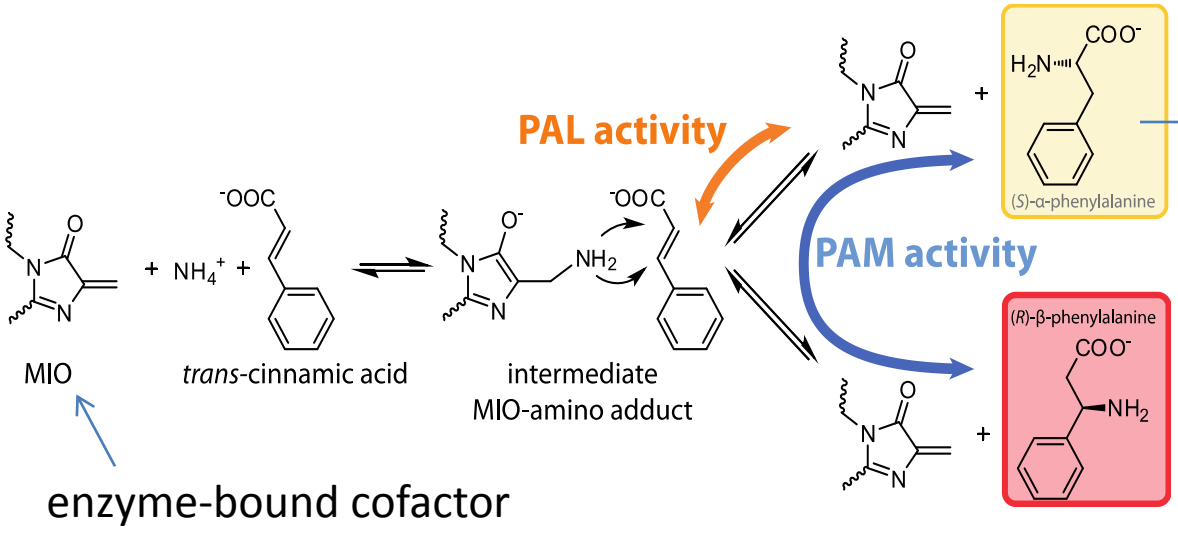
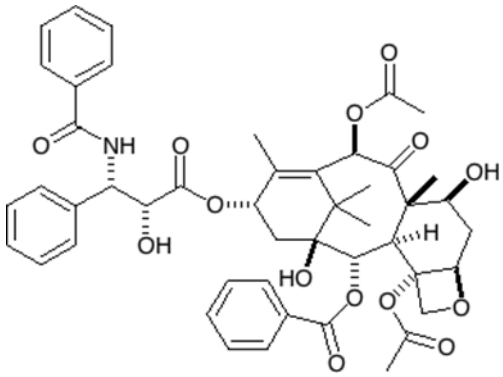
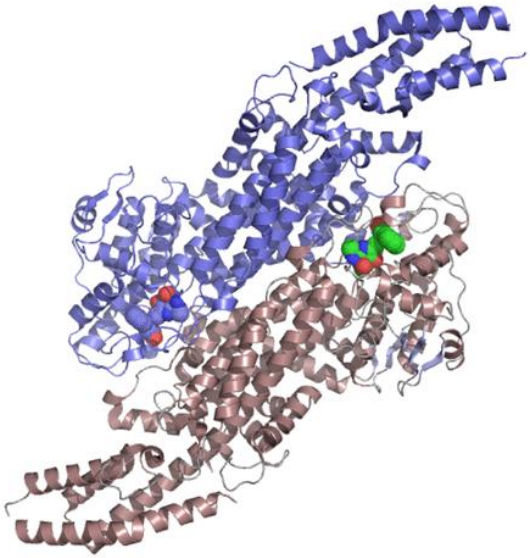
# TcPAM: phenylalanine aminomutase from *Taxus* (WP1)





- asymmetric amination of cinnamic acid & related compounds
- kinetic resolution of β-amino acids


- Heberling MM, Wu B, Bartsch S, Janssen DB. Curr Opin Chem Biol 2013, 17:250-60.
- Wu B, Szymański W, Wybenga GG, Heberling MM, Bartsch S, de Wildeman S, Poelarends GJ, Feringa BL, Dijkstra BW, Janssen DB. Angew Chem Int Ed Engl 2012, 51:482-6.
- Wu B, Szymański W, Heberling MM, Feringa BL, Janssen DB. Trends Biotechnol 2011, 29:352-62.
- Bartsch S, Wybenga GG, Wu B, Dijkstra BW, Janssen DB. ChemCatChem 2013, 5: 1797-1802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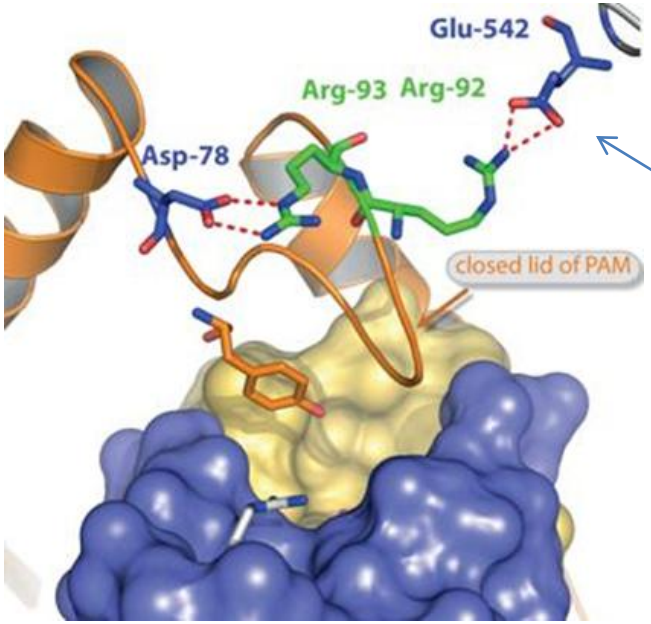
# Mutase → lyase engineering of *Tc*PAM (WP1)

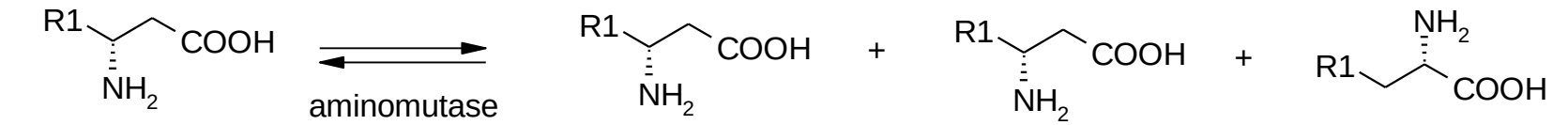



**active site (Tyr80) covered by a loop**

- restricted loop opening → mutase
- rapid loop opening → lyase (product escapes)

**molecular dynamics simulations**

- engineer rapid loop opening by R92S mutation
- 40-fold higher reaction rates
- resulting engineered enzyme: catalyzes kinetic resolution of β-amino acids, acts as a β-lyase




rac-β-amino acid

100 mg

*Tc*PAM-Arg92Ser

(S)-β-amino acid

48.4 mg

ee > 97%

yield = 48.8%

cinnamic acid

L-(S)-α-amino acid

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# *5 Major Themes in Biocatalysis*

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- **Biocatalysts can also enable new synthetic pathways which may be shorter, more efficient and more sustainable.**
- **Combining bio/bio- and bio/chemo-catalysis generates opportunities for the design of new synthetic routes.**

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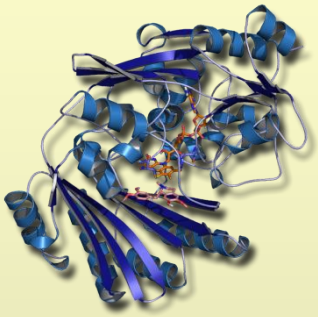
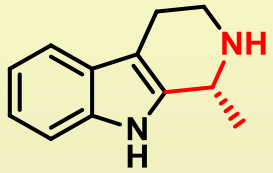
- **Biocatalysts can also enable new synthetic pathways which may be shorter, more efficient and more sustainable.**
- **Combining bio/bio- and bio/chemo-catalysis generates opportunities for the design of new synthetic routes.**
- **Need biocatalysts with broad substrate scope that are active and stable under the conditions of a chemical process (fit biocatalyst to process rather than vice-versa).**
- **Range of emerging technologies for biocatalyst development (directed evolution/rational engineering/pathway engineering).**

# *Design – Evolution - Synthesis*

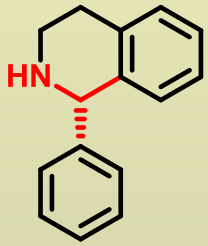
**Protein Design**






**Biocatalysts** → **Synthesis**

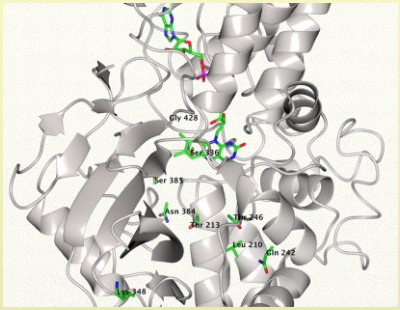
**Design features:**
- **Selectivity**
- **Specificity**
- **Stability**



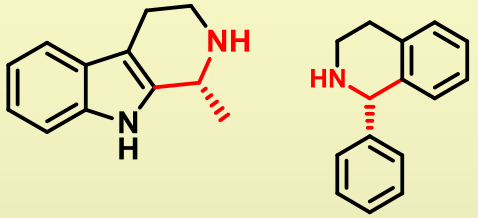

**Protein Evolution**

# *Synthetic Biology*

**Directed evolution and rational design**

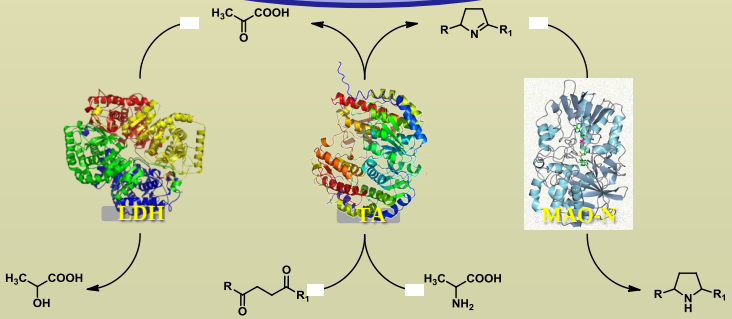


**Biocatalysis on synthetic substrates**

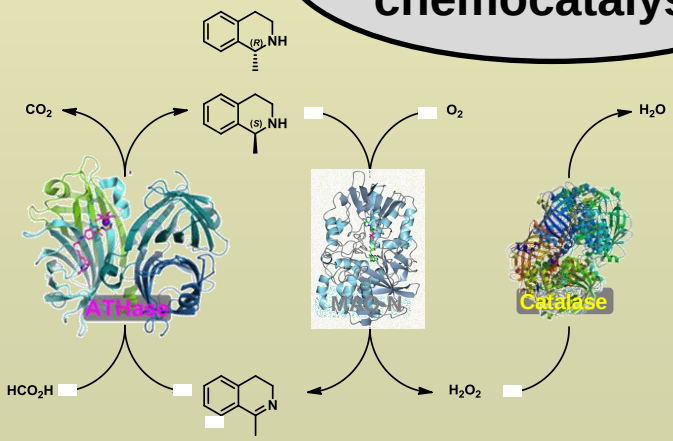



**Biocatalysts**

**Combination with other biocatalysts**




**Combination with chemocatalysts**



# *Challenges for Biocatalysis*

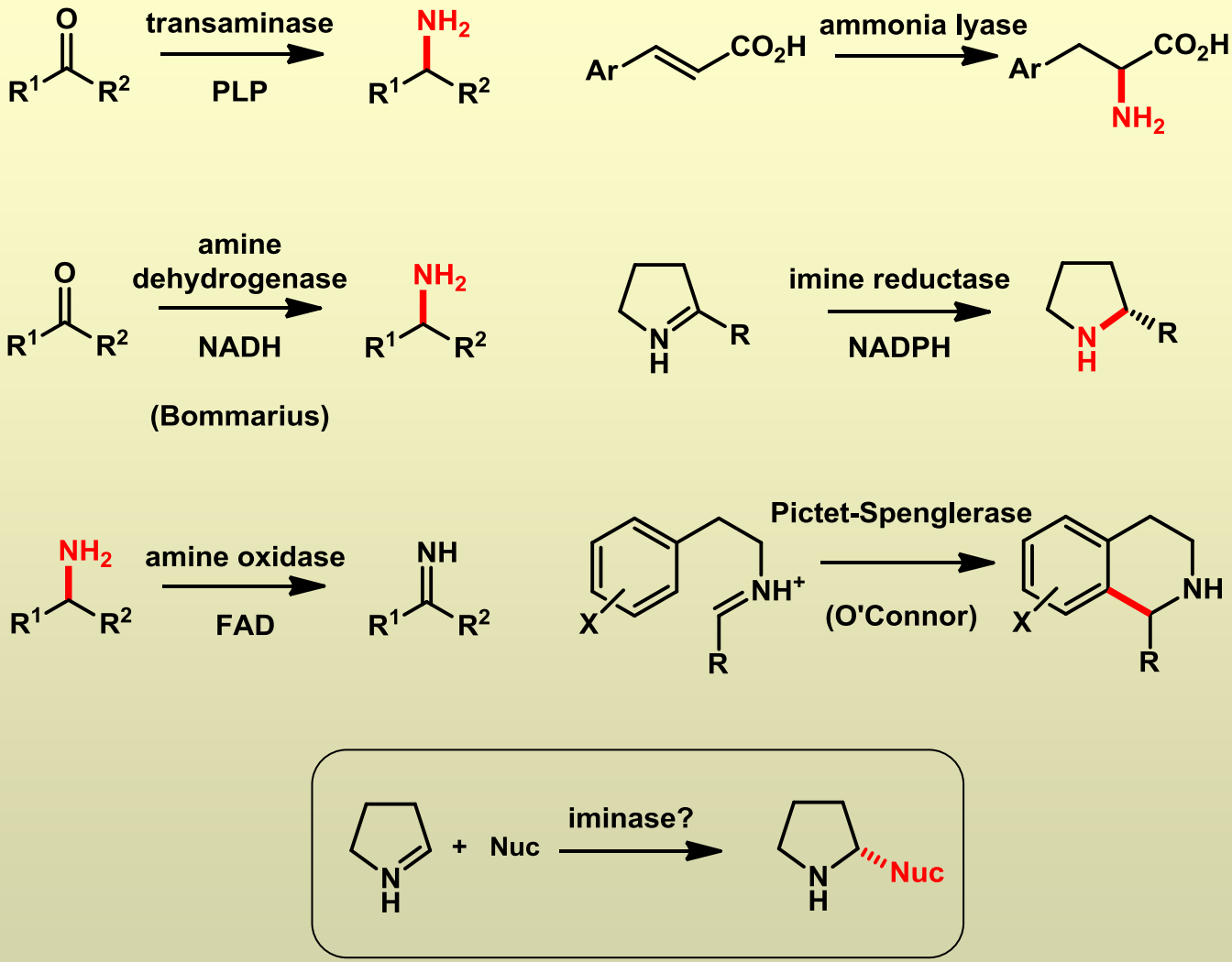
- **Can we design new & general synthetic routes to target classes (e.g. amino acids, alkaloids, terpenes etc.) based upon bio- and chemo-catalysis?**

- **Can we develop guidelines for route design for synthetic chemists (biocatalytic retro-synthesis)?**

- **Where are the gaps in biocatalysis – which reactions are currently not available (and would be desirable)?**

- **How do we significantly expand the biocatalysis toolbox?**

# *(Asymmetric) Biocatalytic Amine Toolbox 2013*




***Need new biocatalysts for (C-C) formation in addition to (C-N)***

# *Challenges for Biocatalysis*

commentary

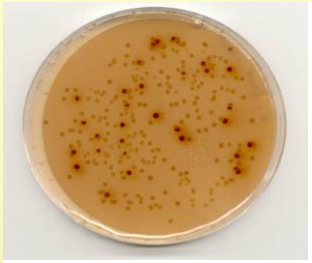
## Biocatalytic retrosynthesis

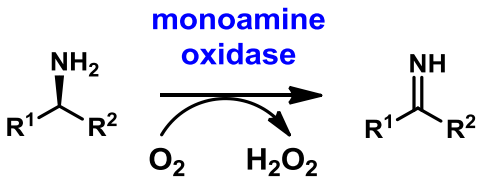
Nicholas J Turner & Elaine O'Reilly

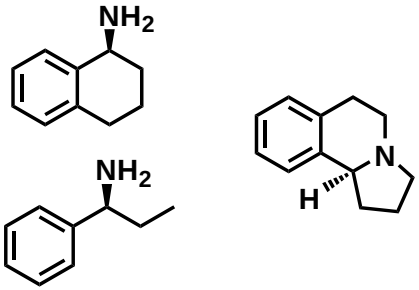
The recent development of a broad range of biocatalysts that can be applied in organic synthesis has brought into focus the need to rethink the way in which organic target molecules might be constructed in the future. To aid synthetic chemists in identifying where biocatalysts might be usefully applied, we propose that guidelines and rules for 'biocatalytic retrosynthesis' be developed and that this new approach be embedded in the future training and education of organic chemists.

***Nature Chem. Biol*., 2013, *9*, 285-288.**

# *Directed evolution of MAO-N*







**Wild-Type MAO-N**

**1st random library ca. 150,000 clones**

**D1**

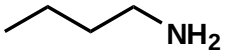
**2nd random library followed by 'hot-spot' libraries ca. 20,000 clones**

**D5**

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

**D6/D7**

**simple achiral $1^o$ amines**




**chiral $1^o$ amines**

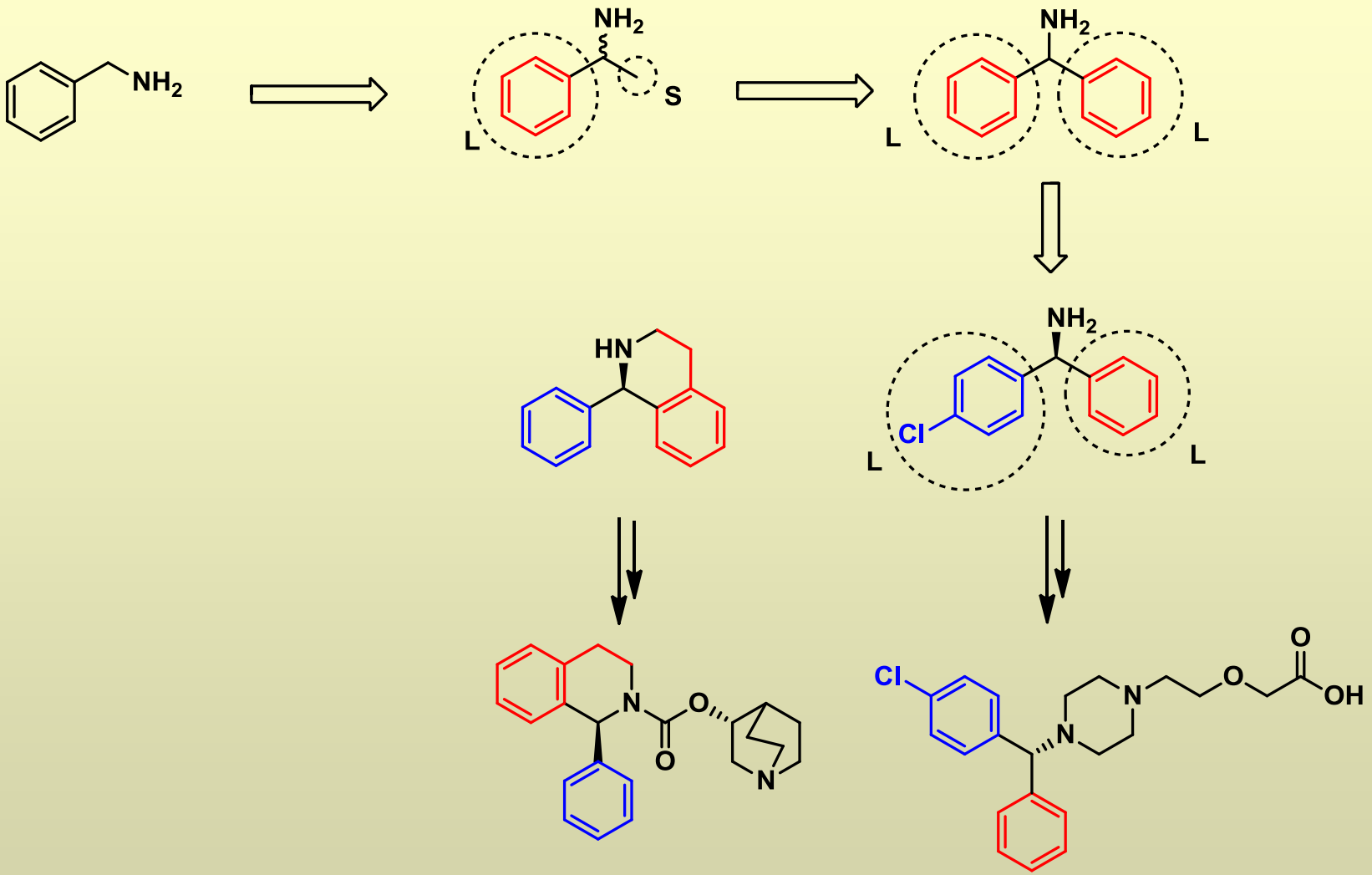
**chiral $1^o/2^o/3^o$ amines**

**broad spectrum (*S*)-selective amine oxidase**

**>$10^3$ improvement in $k_{cat}$**

**e.e. >98%**

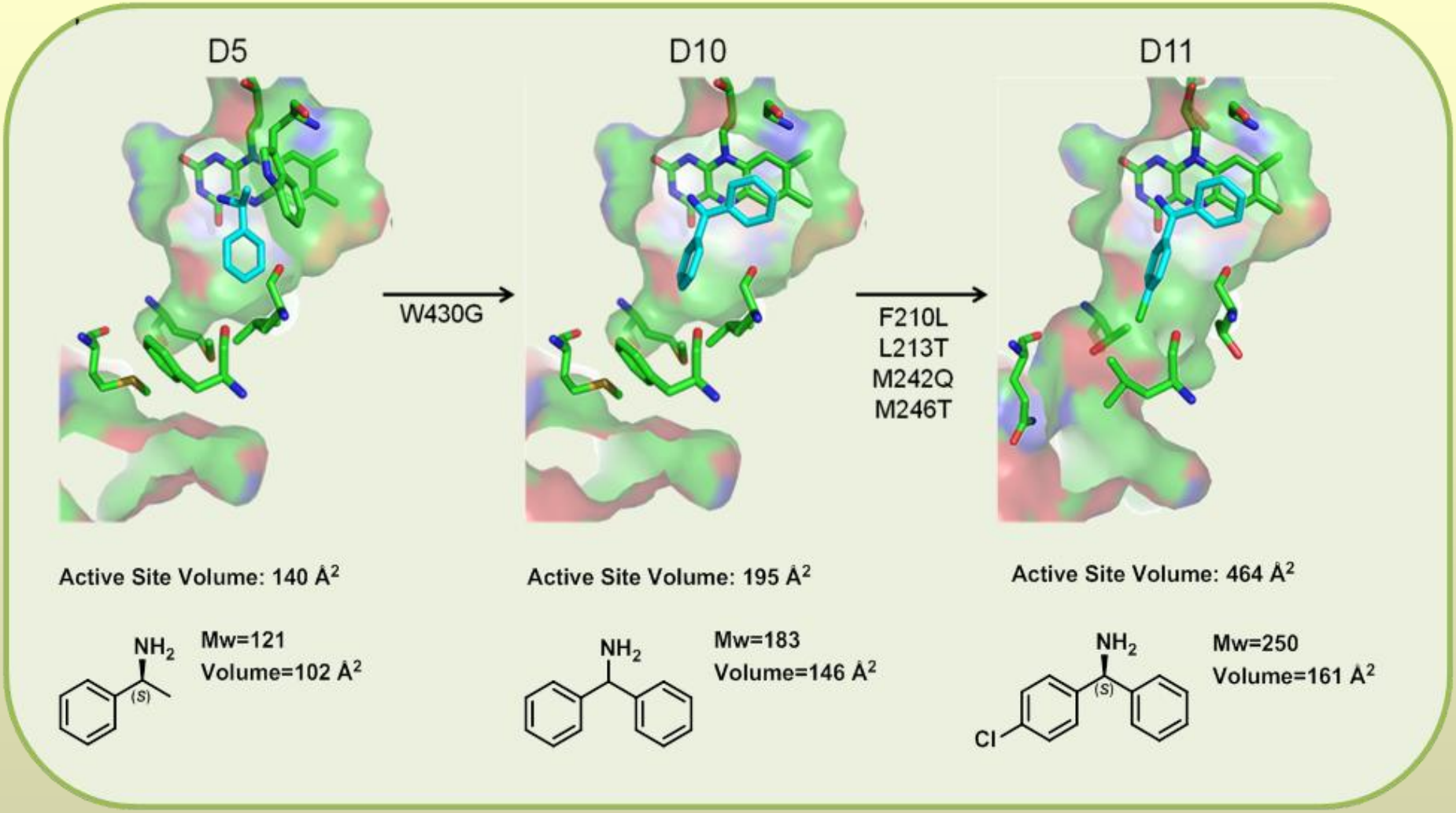
# *Engineering MAO-N for diarylaminomethanes*




**Solifenacin** **Levocitirizine**

D. Ghislieri A.P. Green, M. Pontini, *et al*., *J. Am. Chem. Soc*., 2013, *135*, 10863-10869.

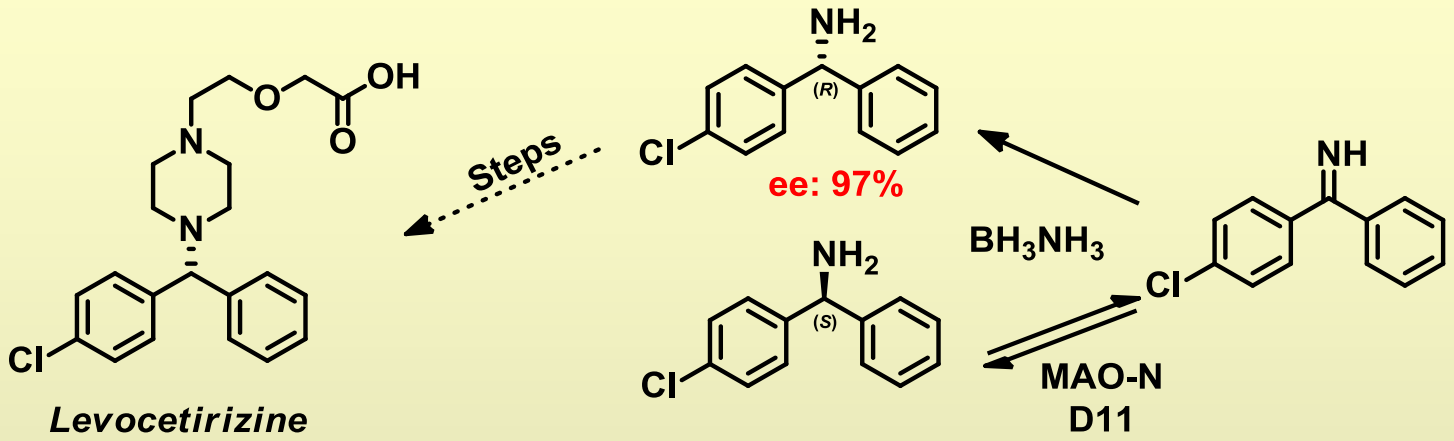
# *Rational engineering MAO-N*



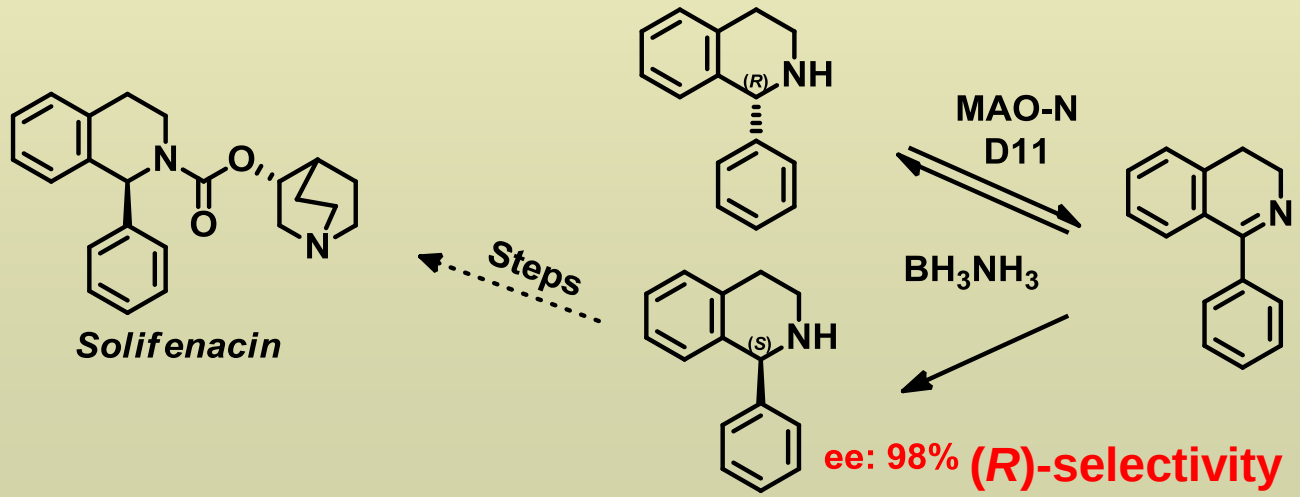

D. Ghislieri A.P. Green, M. Pontini, *et al*., *J. Am. Chem. Soc*., 2013, *135*, 10863-10869.

# *Deracemisation of API building blocks*

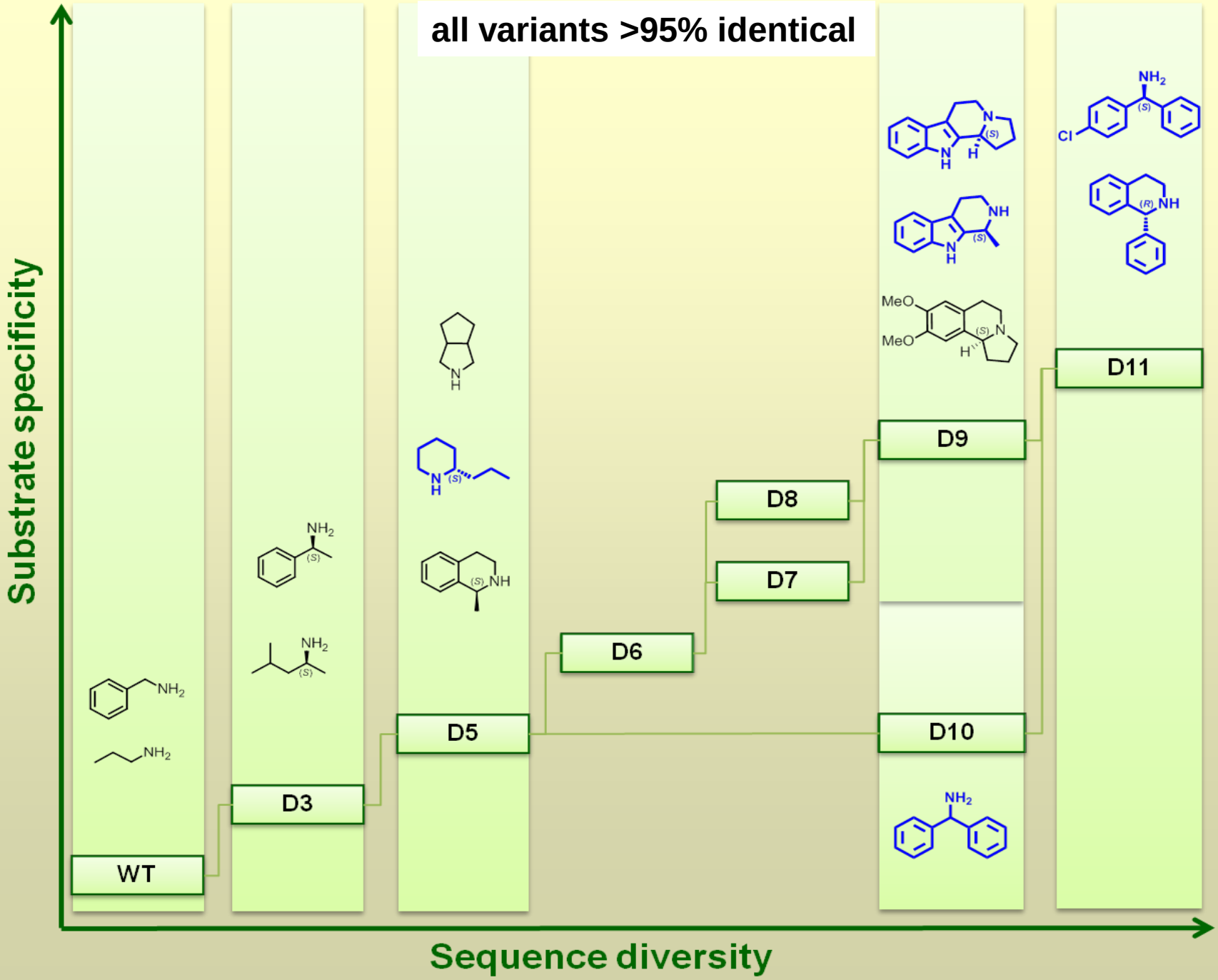
**4-chlorobenzhydrylamine:**



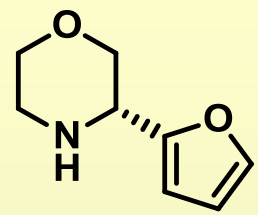

**1-phenyltetrahydroisoquinoline:**



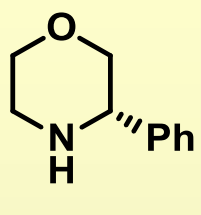

D. Ghislieri A.P. Green, M. Pontini, *et al*., *J. Am. Chem. Soc*., 2013, *135*, 10863-10869.


all variants >95% identical
Substrate specificity
Sequence diversity
WT
D3
D5
D6
D7
D8
D9
D10
D11

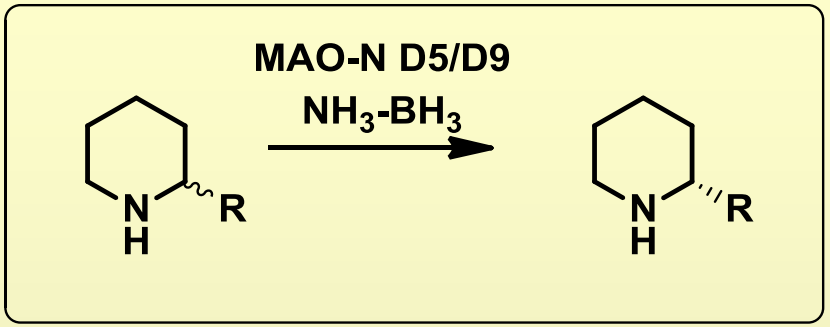
# Piperidines

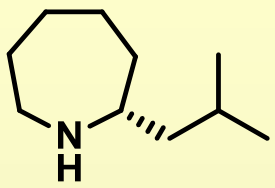


conv. 99%
ee: 99%

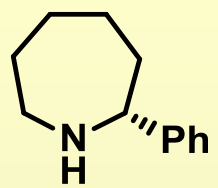


conv. 92%
ee: 99%

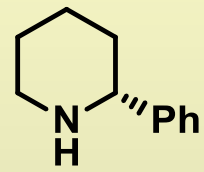


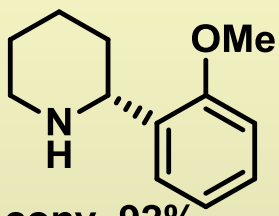
conv. 99%
ee: 99%



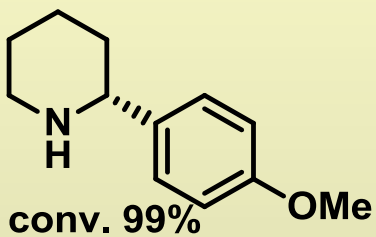
conv. 92%
ee: 99%



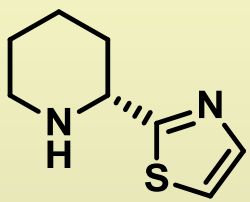
conv. 99%
ee: 99%



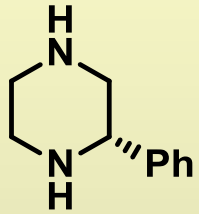
conv. 92%
ee: 85%



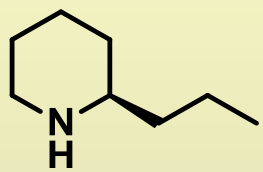
conv. 99%
ee: 99%



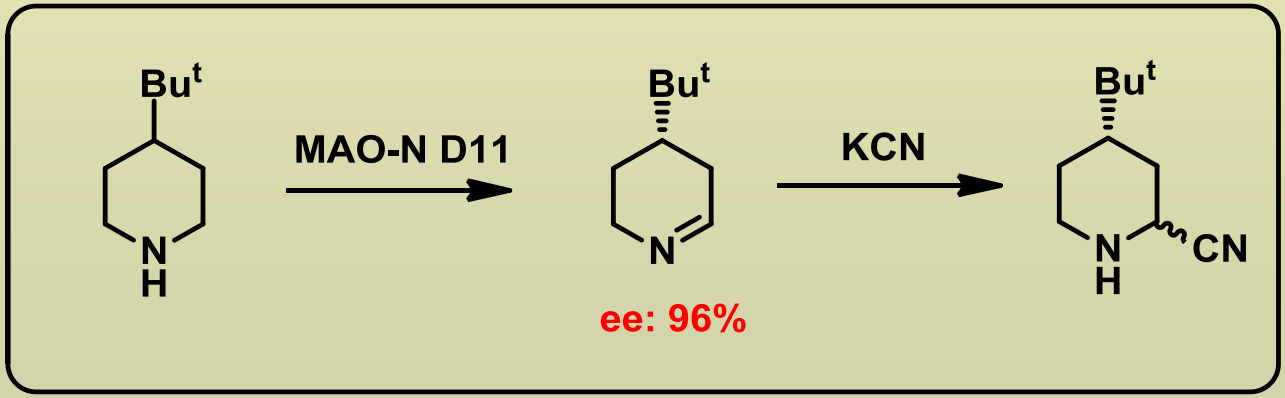
conv. 99%
ee: 99%



conv. 99%
ee: 99%

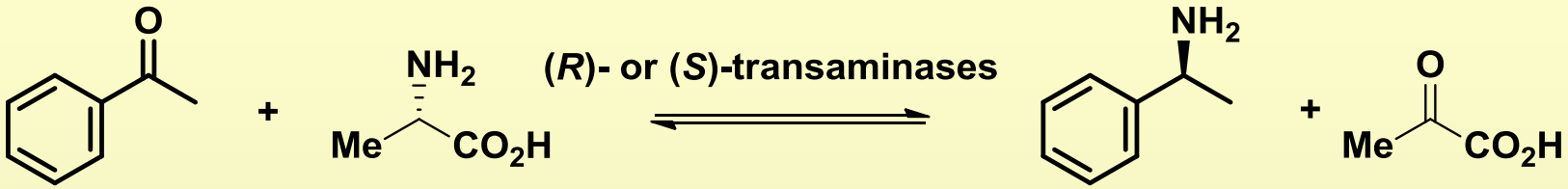


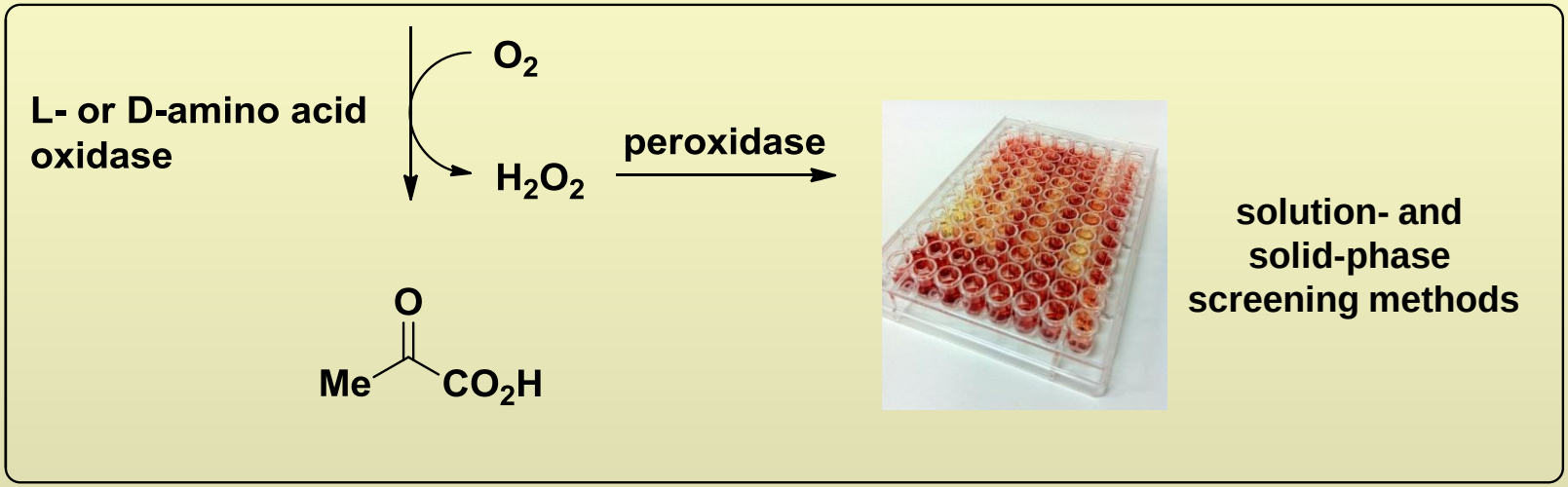
conv. 99%
ee: 88%

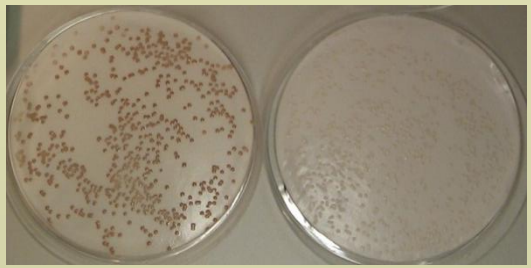



Marta Pontini and Bas Groenendaal

# *Transaminases*



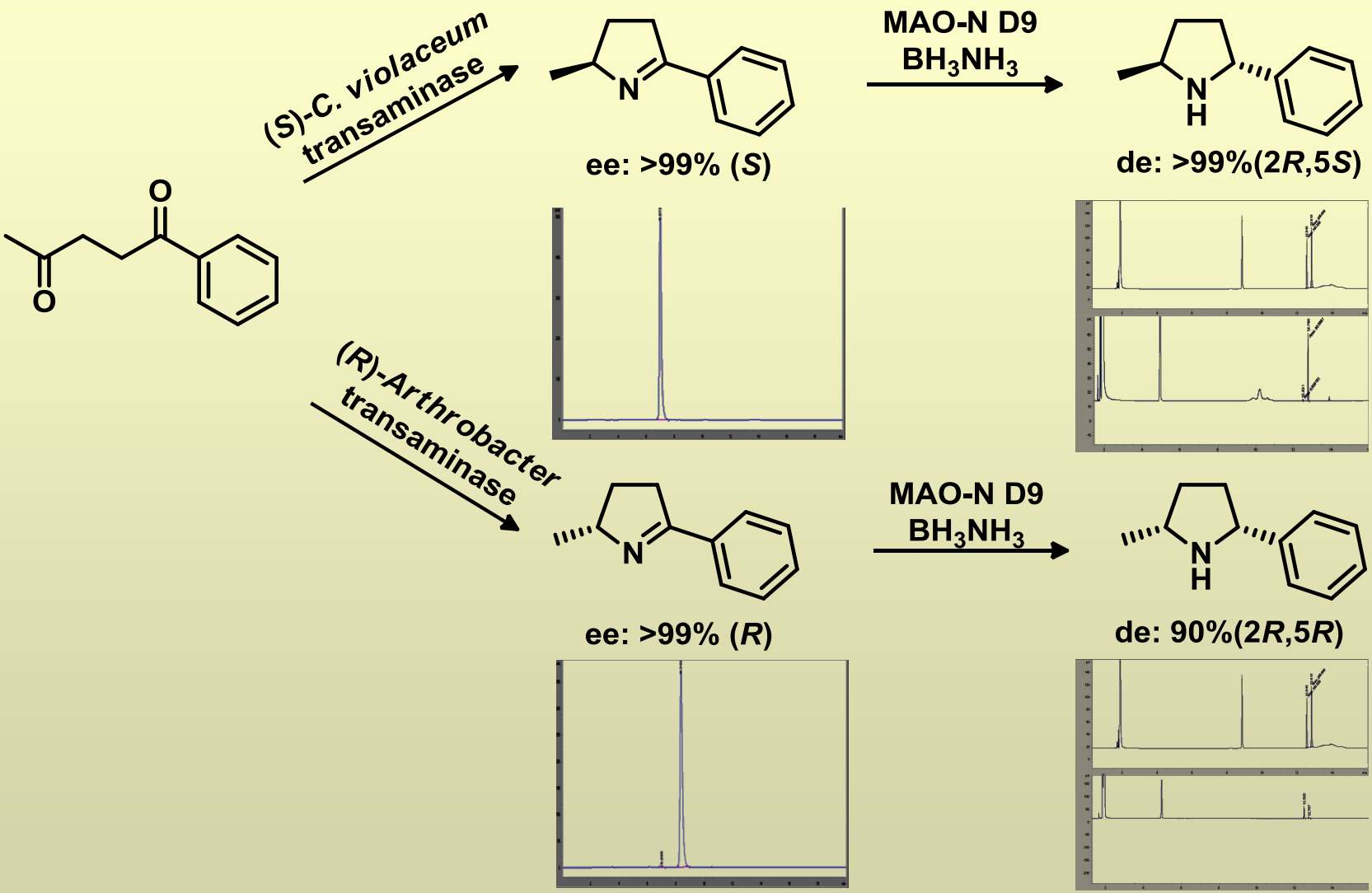



Jennifer Hopwood & Simon Willies

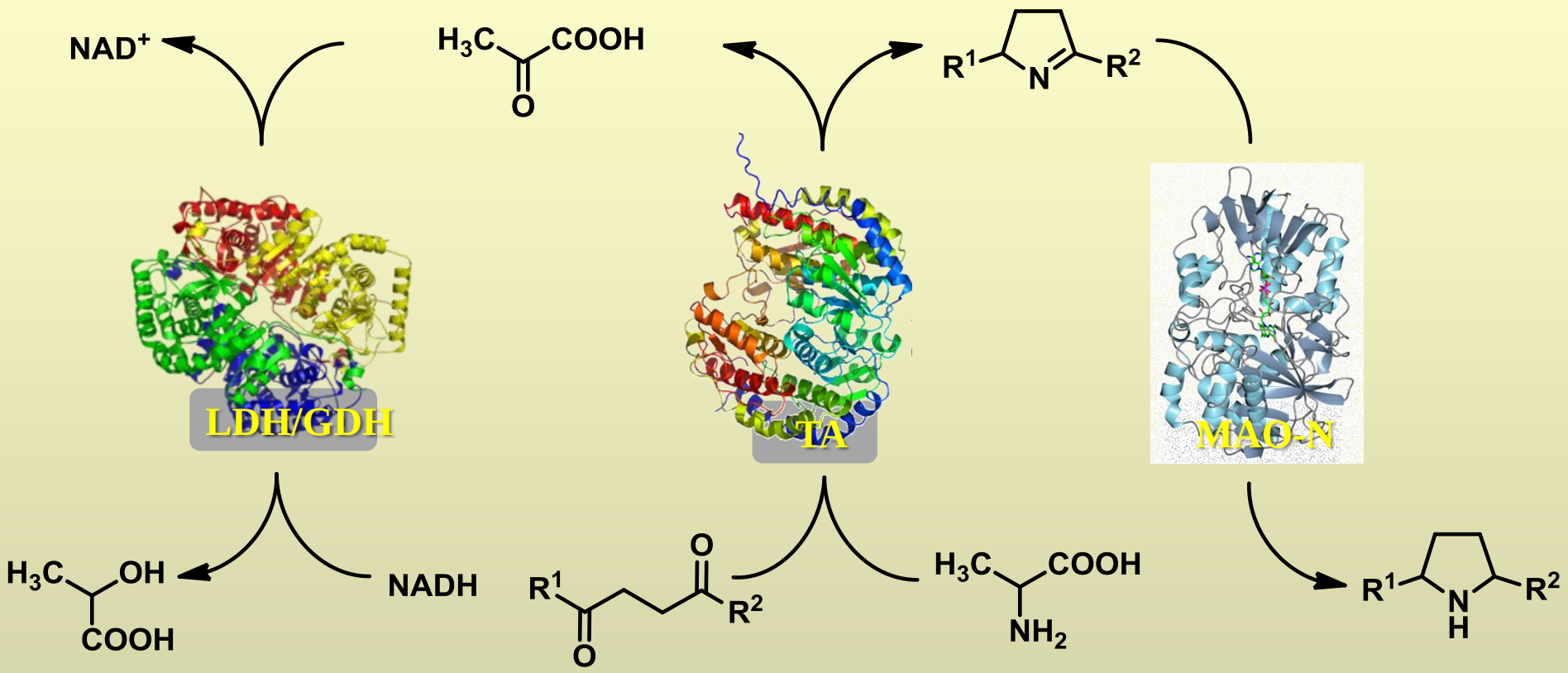
J.A. Hopwood, R.C. Lloyd and N.J. Turner, *Chem Commun*., 2011, *47*, 773.

# *MAO-N / ω-TA tandem reaction*




Elaine O'Reilly, Diego Ghislieri & Jennifer Hopwood (Dr. Reddy's)

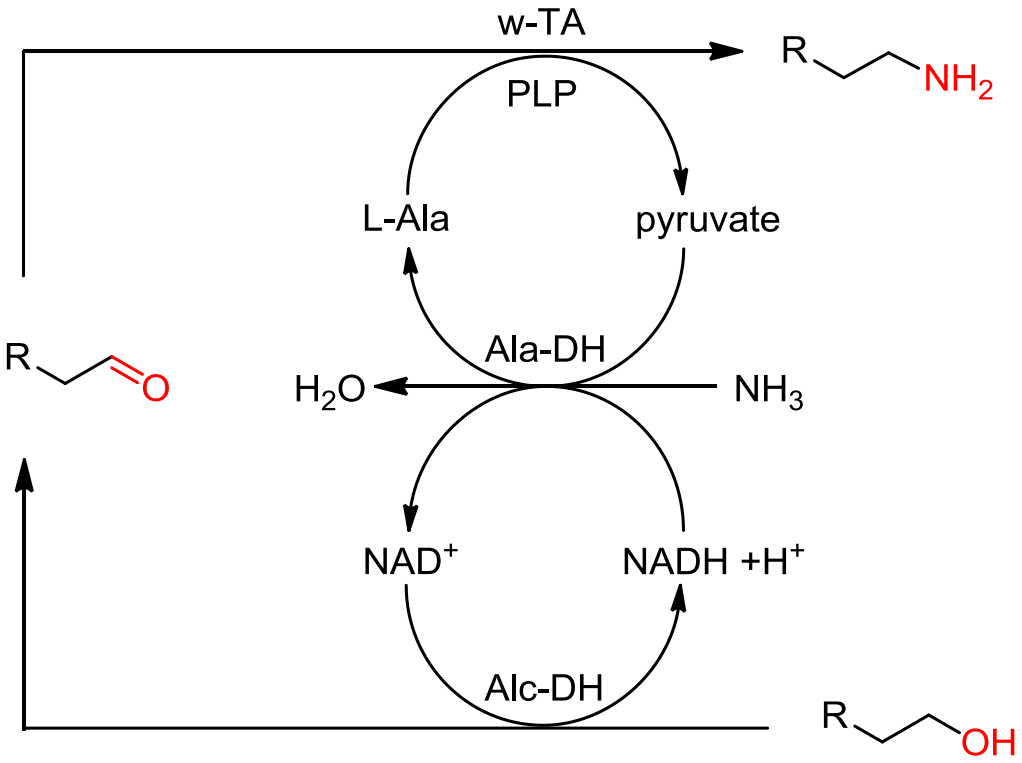
# *MAO-N / ω-TA tandem reaction*




Diego Ghislieri & Jennifer Hopwood

# Self-sufficient biocatalytic network for aliphatic amines (WP1)

1. alcohol to aldehyde by NAD-dependent alcohol dehydrogenase (AlcDH)
2. aldehyde amination by an ω-aminotransferase (requires amino donor)
3. regeneration of amino-donor and the NAD cofactor with NADH-deptendent AlaDH




Net reaction:
•alcohol + $NH_3$ → amine + $H_2O$

•enzyme discovery by database analysis

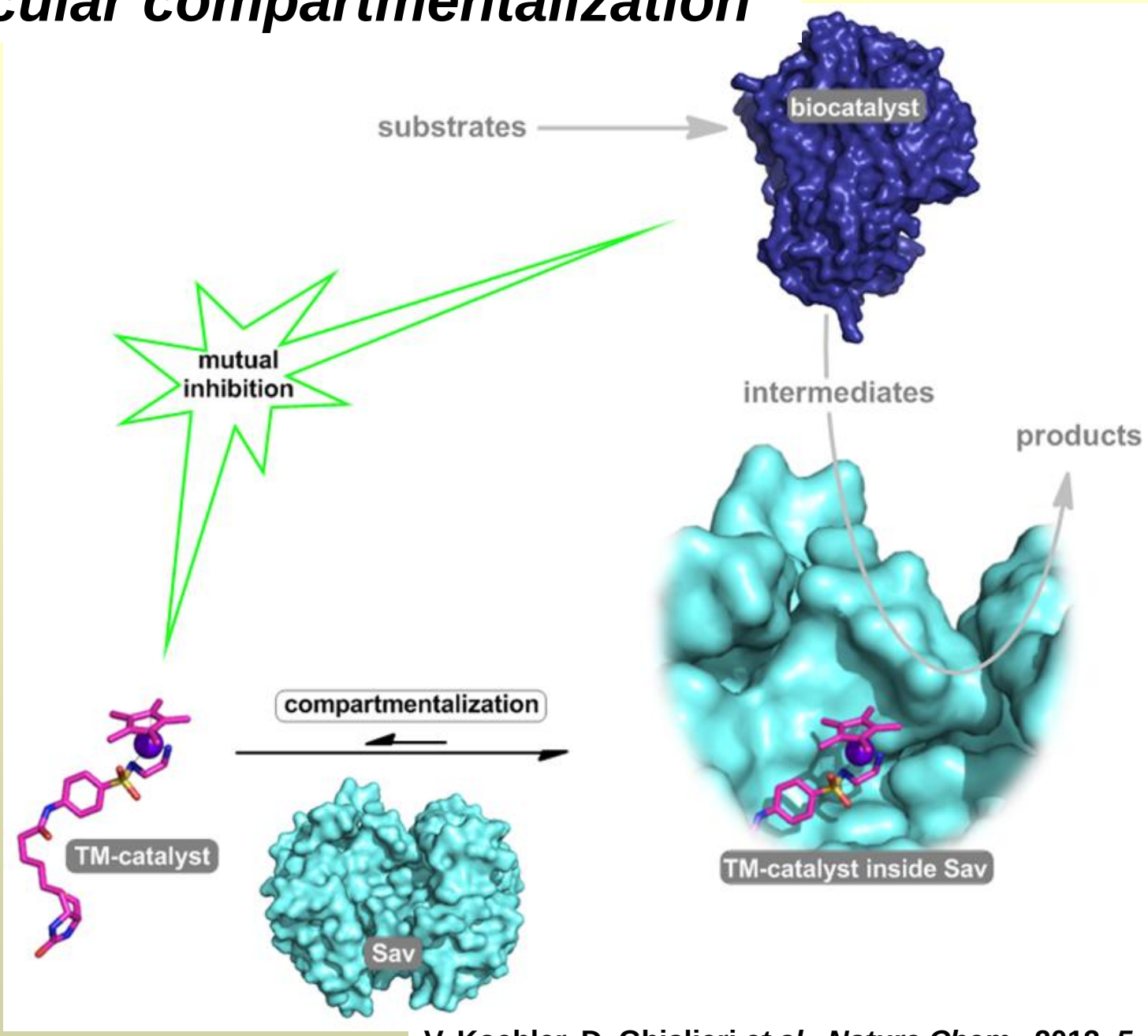
•different target amines

university of groningen

BIONEXGEN
the next generation of biocatalysts

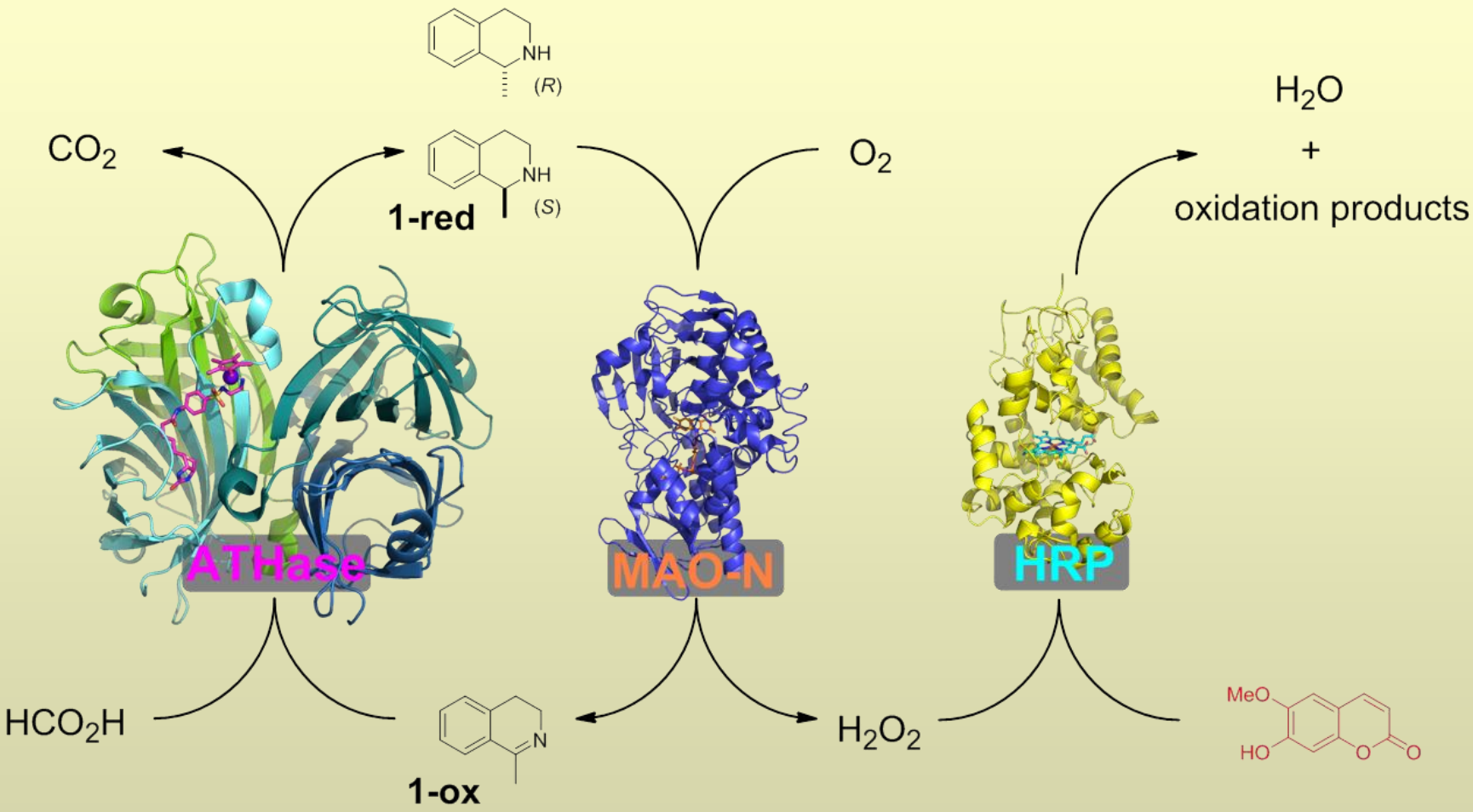
Cyntia Palacio, Univ. of Groningen
Collaboration with BASF

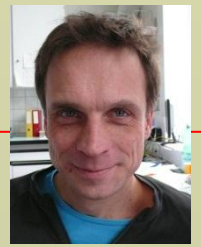
# *Molecular compartmentalization*




V. Koehler, D. Ghislieri *et al.*, *Nature Chem.*, 2013, *5*, 93-99.

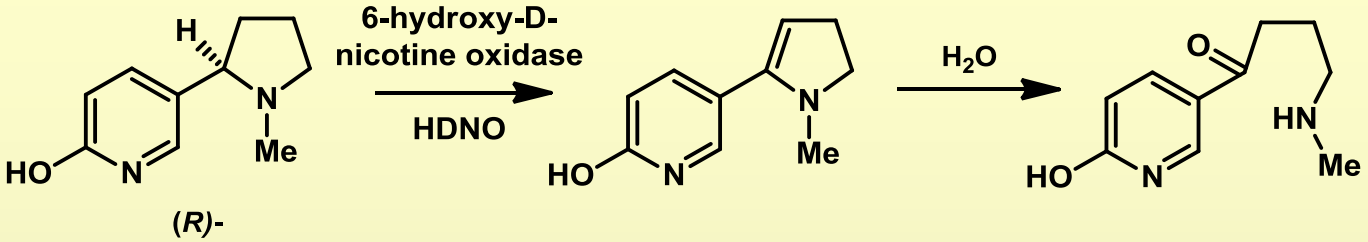
# *MAO-N / ATH tandem reaction*

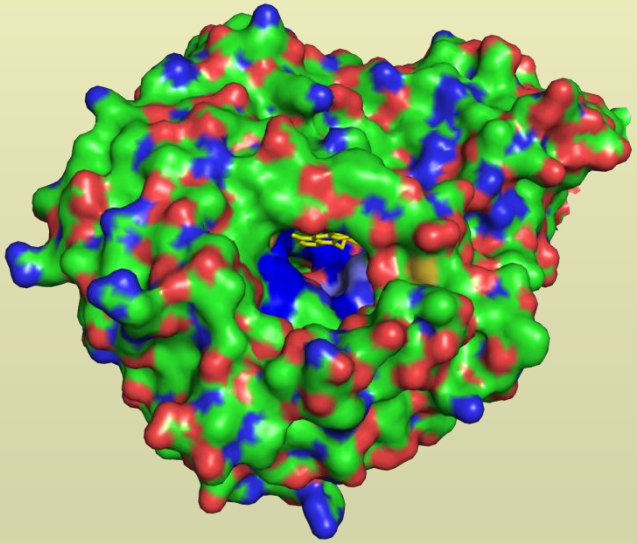


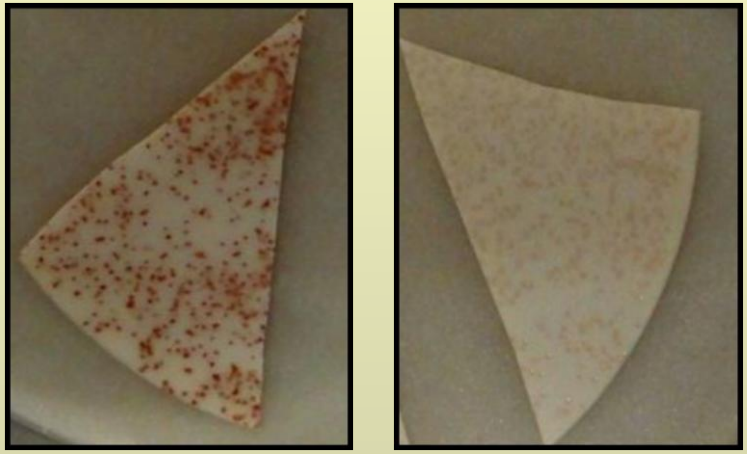



**In collaboration with Tom Ward, Valentin Koehler (University of Basel)**

# *(R)*-Selective amine oxidase



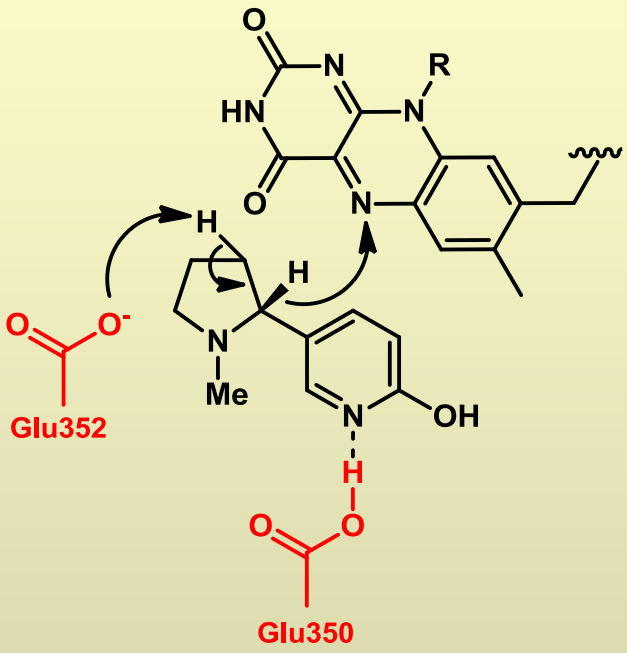



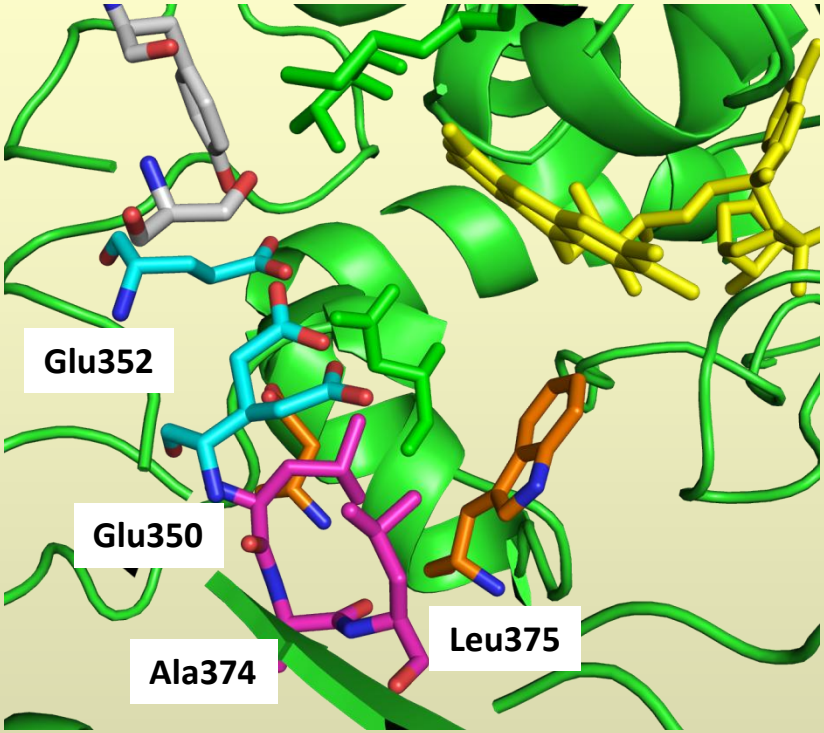


*A) 20 mM (R)-nicotine* *B) 20 mM (S)-nicotine*

G. Schulz *et al*., *J. Mol. Biol*., 2005, *352*, 418-428.

# *(R)-Selective amine oxidase*



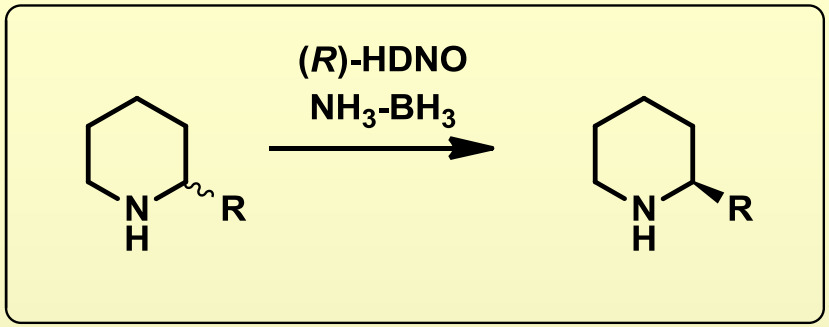

CASTing libraries

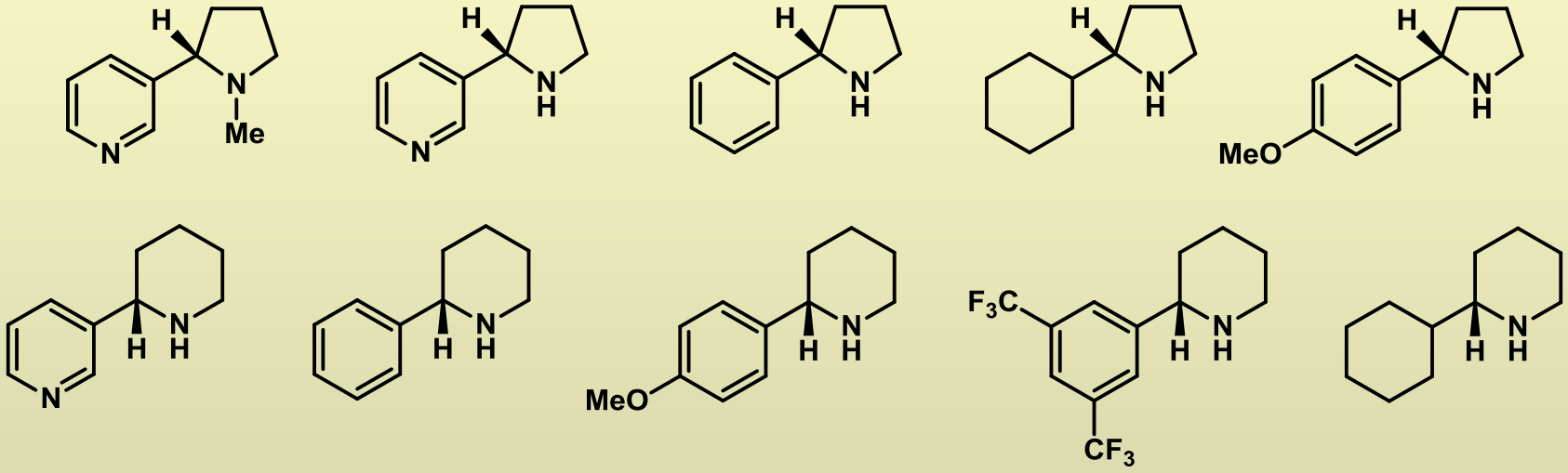
Ala374/Leu375: no hits

Glu350/Glu352: several hits including Leu350/Asp352

**Rachel Heath**

# *Substrate specificity of Leu350/Asp352 HDNO*

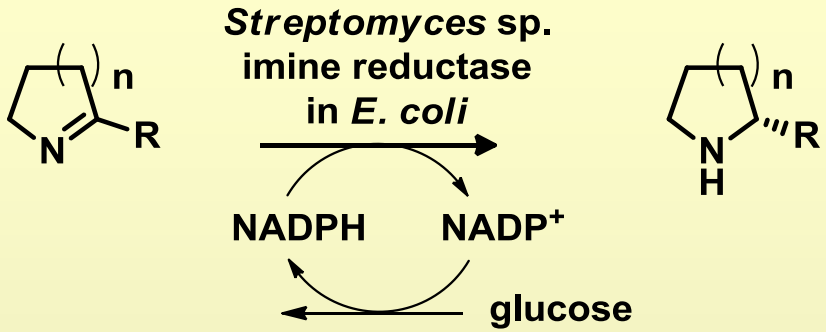


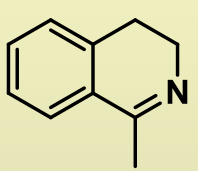

**all >95% e.e. and (*S*)-enantiomers**

R. Heath *et al*., *ChemCatChem*, 2013 (submitted).

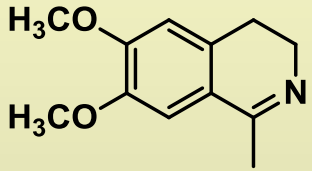
# *(S)-Imine reductase*



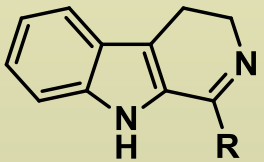

K. Mitsukura *et al*., *Org. Biomol. Chem.*, 2011, *8*, 4533.



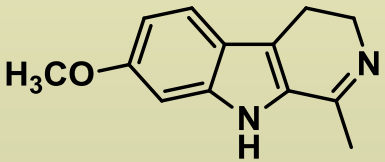

**7**




**9**




**11a: R= Me**
**11b: R= Et**
**11c: R= *i*Pr**
**11d: R= cyclohexyl**

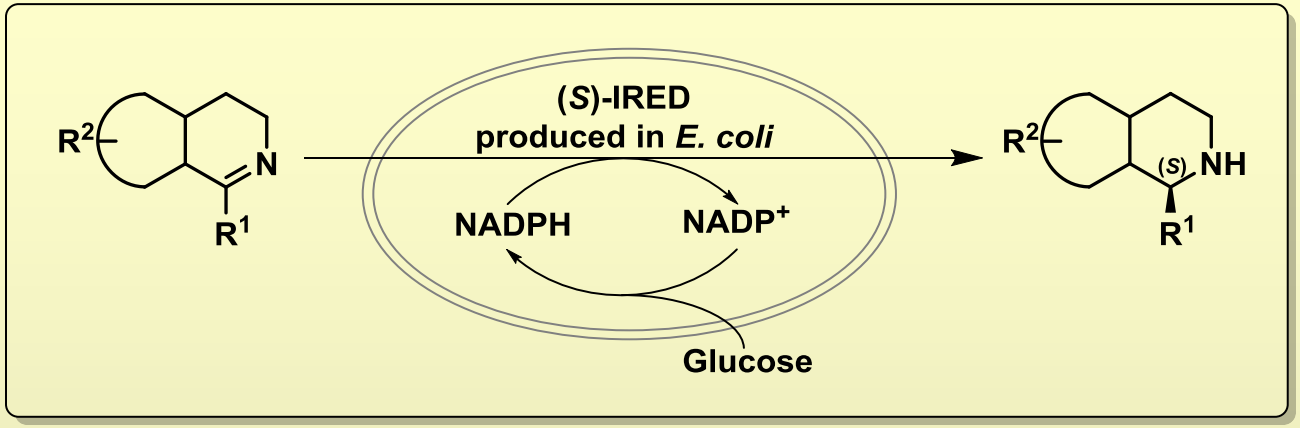


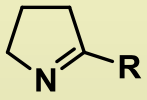

**13**

| | Conversion (1h) | Conversion (6h) | %ee (6h) | Product |
|---|---|---|---|---|
| **7** | 98.8 | 99.9 | 97.8 | (*S*) |
| **9** | 48.0 | 91.6 | >98 | (*S*) |
| **11a** | 99.4 | 99.6 | >98 | (*S*) |
| **11b** | 68.9 | 99.6 | 99.8 | (*S*) |
| **11c** | 13.9 | 49.7 | 98.4 | (*S*) |
| **11d** | 28.7 | 97.3 | 99.6 | (*S*) |
| **13** | 28.5 | 50.0 | 98.6 | (*S*) |

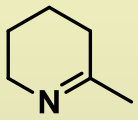
F. Leipold, S. Hussain, D. Ghislieri and N.J. Turner, *ChemCatChem*, 2013, *5*, in press.

# (S)-Imine reductase

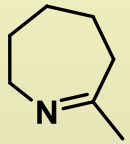


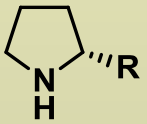

**1a:** R= Me
**1b:** R= Ph
**1c:** R= *p*-Methoxyphenyl
**1d:** R= *p*-Fluorophenyl



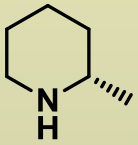

**3**



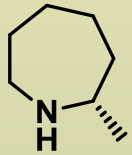

**5**




**2a:** R= Me
**2b:** R= Ph
**2c:** R= *p*-Methoxyphenyl
**2d:** R= *p*-Fluorophenyl




**4**

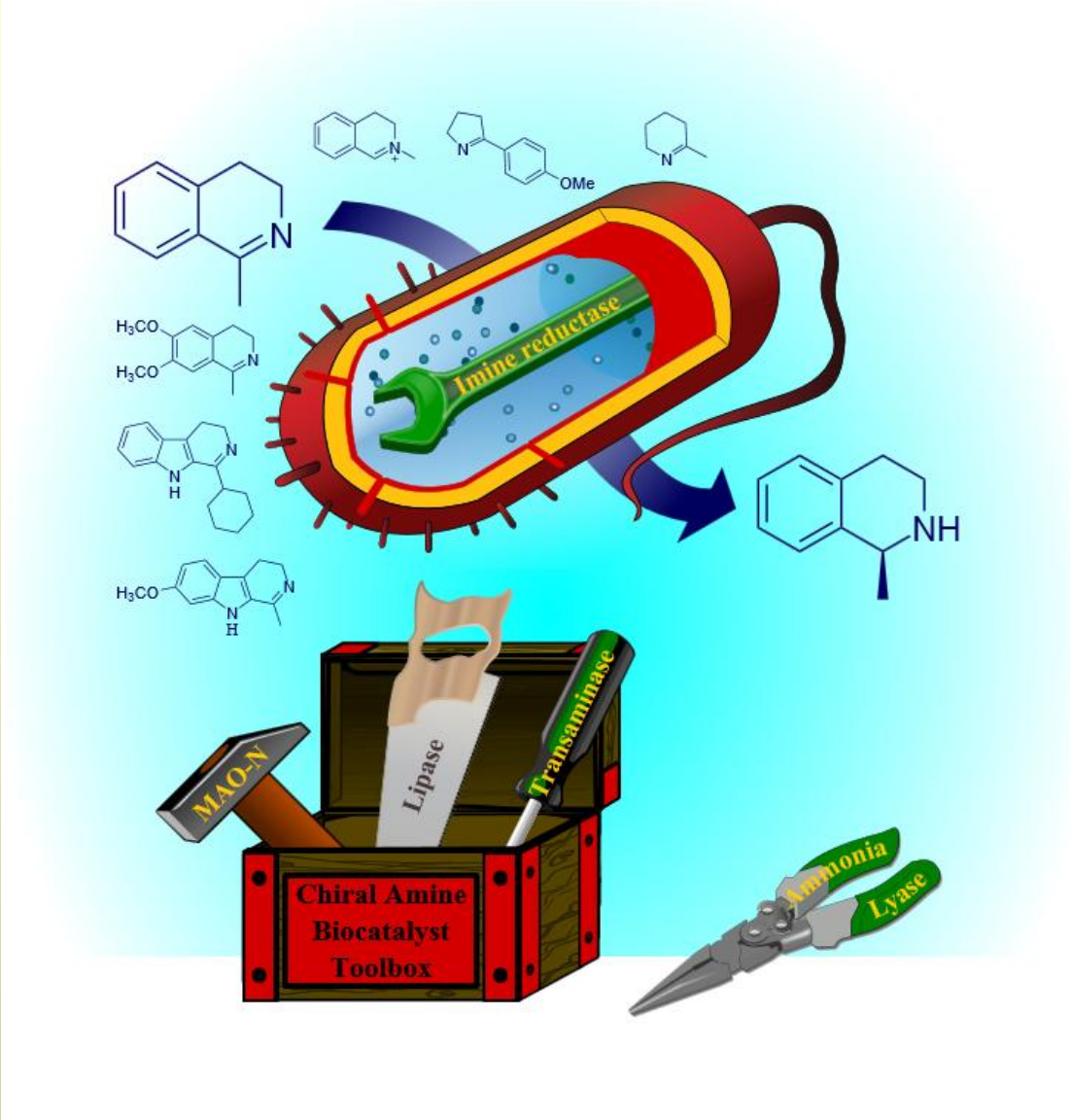



**6**

| | Conversion (6h) | Conversion (18h) | %ee (18h) | Product |
|---|---|---|---|---|
| **1a** | 57 | 55 | >95 | (*S*) |
| **1b** | 28 | 36 | 87 | (*R*) |
| **1c** | 14 | 17 | >98 | (*R*) |
| **1d** | 25 | 42 | 98 | (*R*) |
| **3** | >98 | >98 | >98 | (*S*) |
| **5** | >98 | >98 | >98 | (*S*) |

**F. Leipold, S. Hussain, D. Ghislieri and N.J. Turner, *ChemCatChem*, 2013, *5*, in press.**

# *IREDs – New Biocatalysts for the Toolbox*




F. Leipold, S. Hussain, D. Ghislieri and N.J. Turner, *ChemCatChem*, 2013, *5*, in press.

# *Discovery Biocatalysts Ltd*
# *(www.discovery-bc.co.uk)*

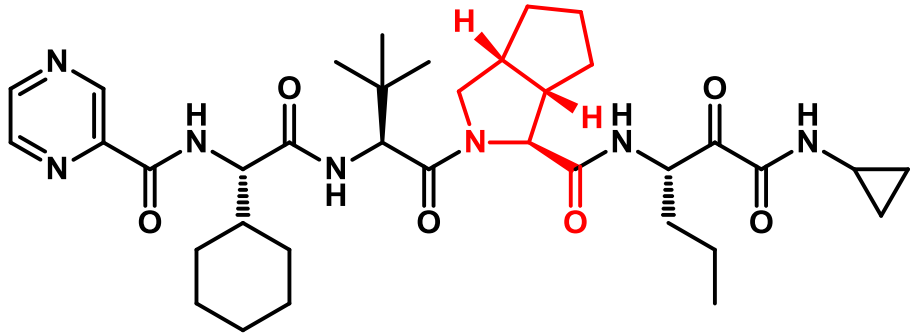

(*S*)-MAO-N kit available

(*R*)-HDNO and IRED Screening Kits will be made available soon through Discovery Biocatalysts.

Contact Gareth DeBoos.

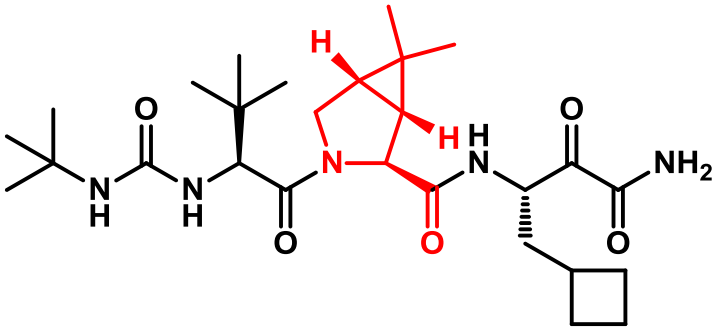
# *Hepatitis C viral protease inhibitors*

Telaprevir (Vertex - J&J)



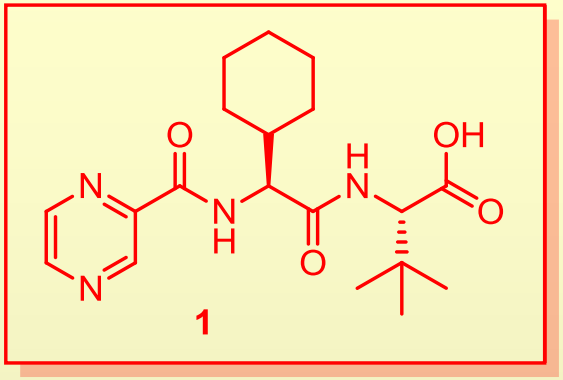
P. Revill et al., *Drugs Future* **2007**, 788

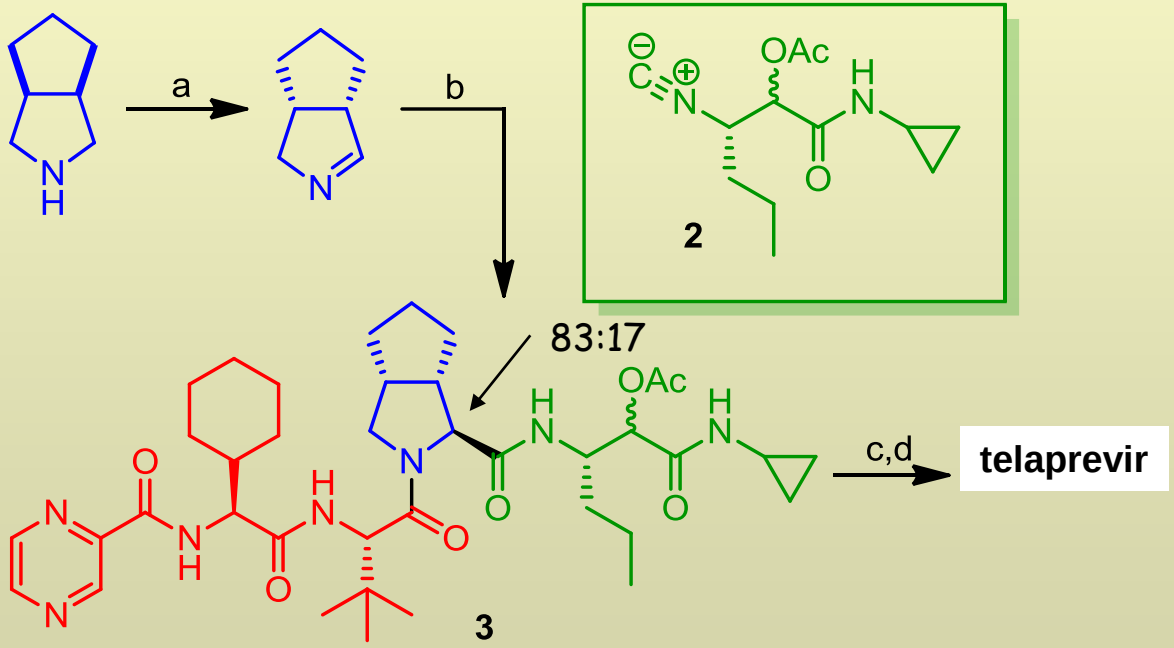
Boceprevir (Merck)



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

# *Multi-component synthesis of telaprevir*


Reagents and conditions: a) MAO-N, 100 mM $KPO_4$, 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.**

# *Acknowledgements*

**Amine biocatalysis at UniMan**:

Diego Ghislieri (BIOTRAINS)
Rachel Heath (BIONEXGEN)
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Friedemann Leipold (BIONEXGEN)
Shahed Hussain
Kirk Malone (BIONEGEN)
Simon Willies (AMBIOCAS)
Rehanna Aslam (AMBIOCAS)
James Galman (BIOINTENSE)
Anthony Green (CHEM21)
Jennifer Hopwood
Claire Doherty (SUPRABIO)
Francesco Mutti (BIONEXGEN)
Elaine O'Reilly (AMBIOCAS)
Beatrice Bechi (CHEM21)
Susanne Herter (CHEM21)
Peter Both (CHEM21)
Nick Weise (KYROBIO)
Sarah Lovelock
Ian Rowles
Mark Corbett (BIONEXGEN ) … and John Whittall (all of the above)

BIONEXGEN

**The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n° [266025].**

SEVENTH FRAMEWORK PROGRAMME