Biocatalytic Routes to Enantiomerically Pure Chiral Amines

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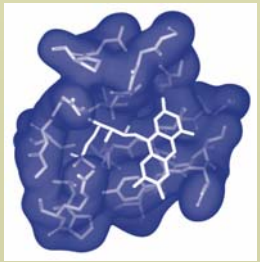

CoEBio3
Centre of Excellence for Biocatalysis, Biotransformations and Biocatalytic Manufacture

RSC Advances in Biocatalysis
University College London
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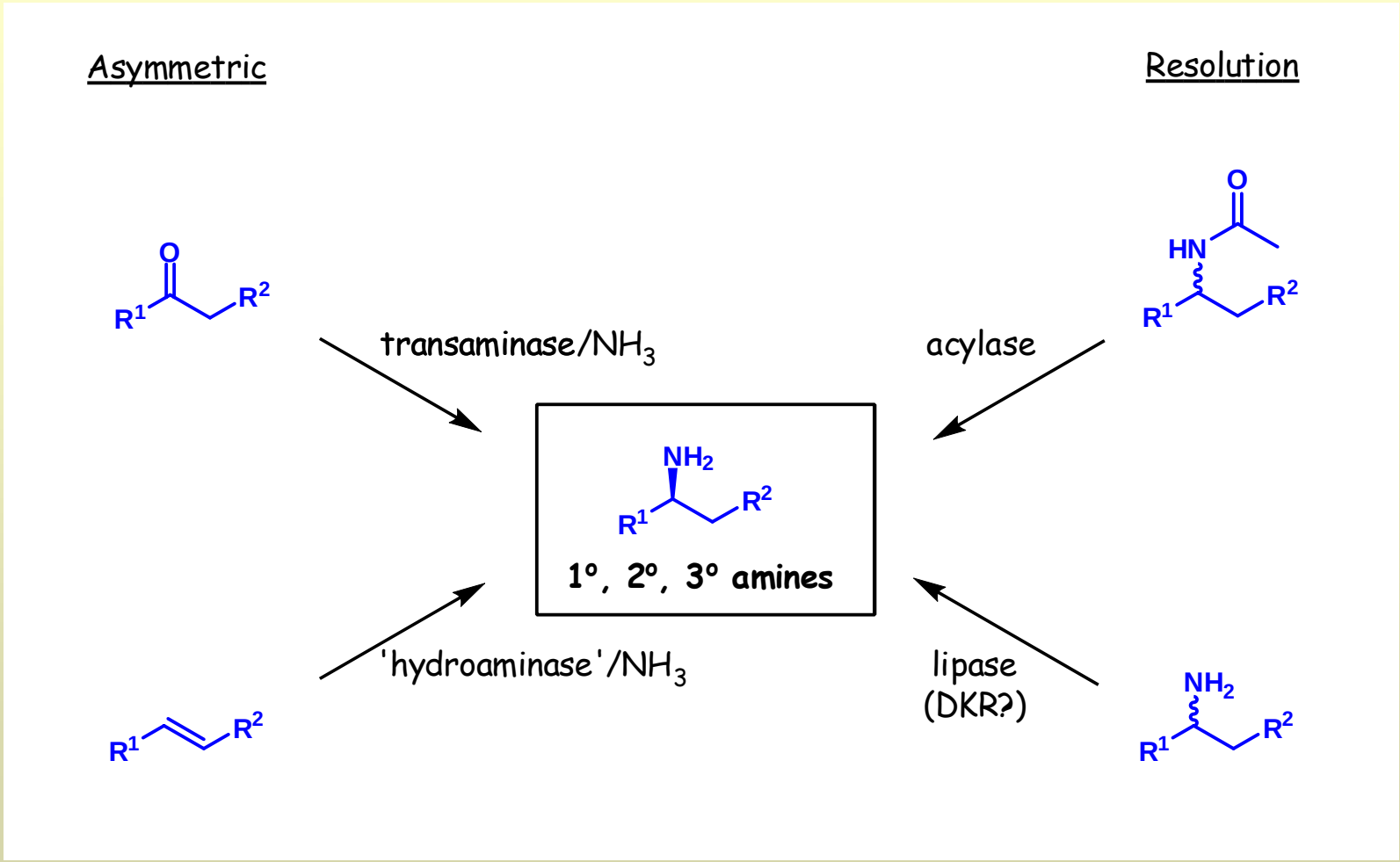
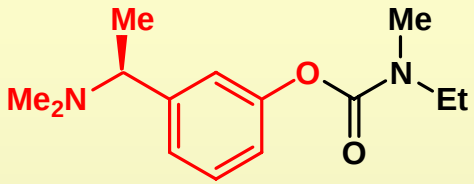
MANCHESTER 1824

Biocatalytic routes to chiral amines

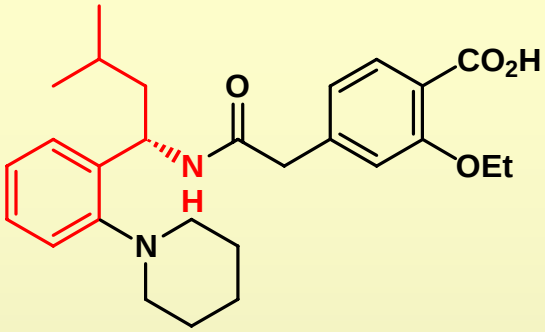


N.J. Turner and R. Carr, 'Biocatalytic routes to non-racemic chiral amines' in *Biocatalysis in the Pharmaceutical and Biotechnology Industries*, 2007, 743-755, CRC Press.
[also N.J. Turner and M.D. Truppo, 2009, Wiley, in press]

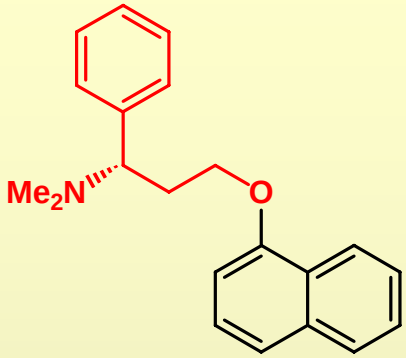
Drugs in development containing chiral amines



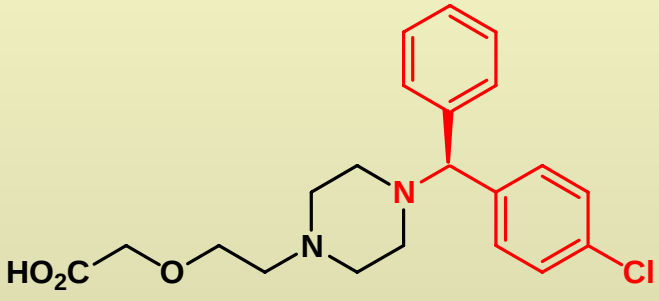
(S)-rivastigmine



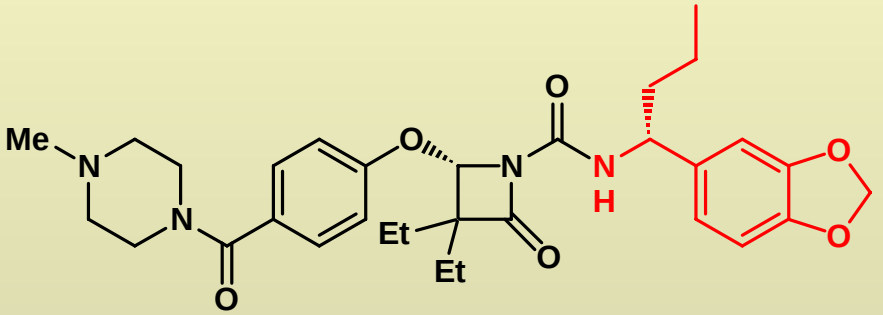
(S)-repaglinide



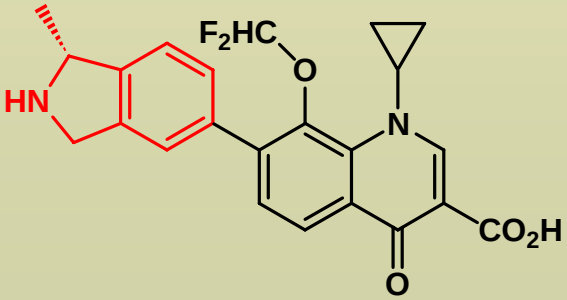
(S)-dapoxetine (Phase II)



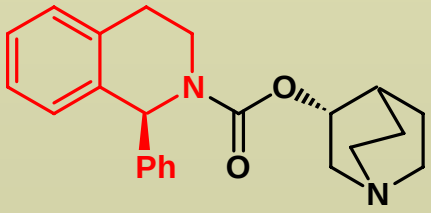
(R)-levocetirizine



(S)-DMP 777 (Phase II)

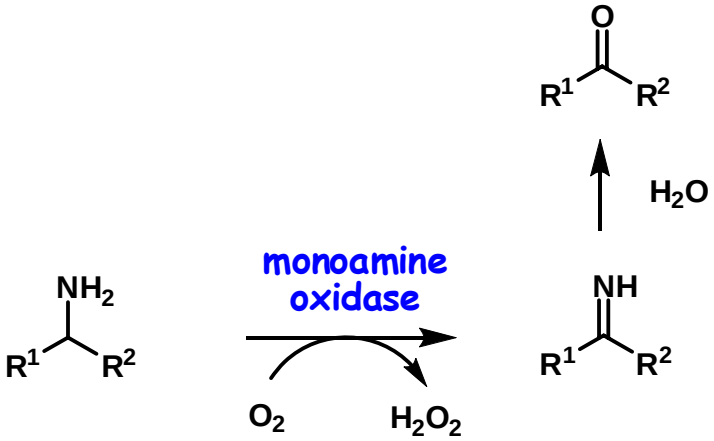


(R)-garenoxacin (Phase III)



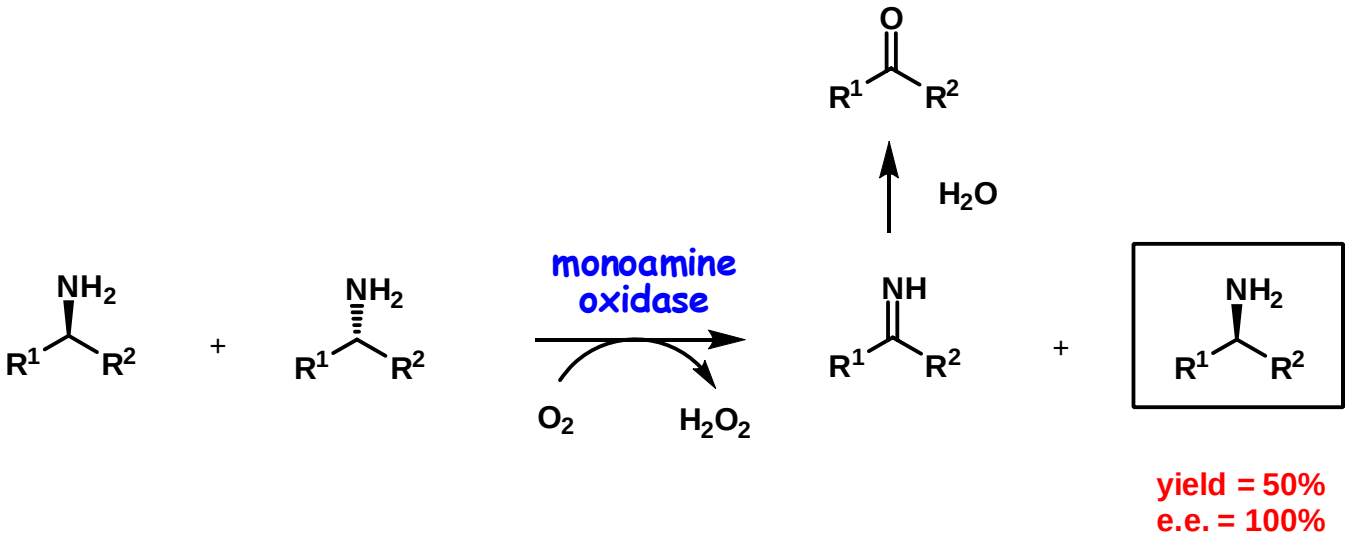
(S)-solifenacin (Phase III)

Monoamine oxidase



Monoamine oxidase

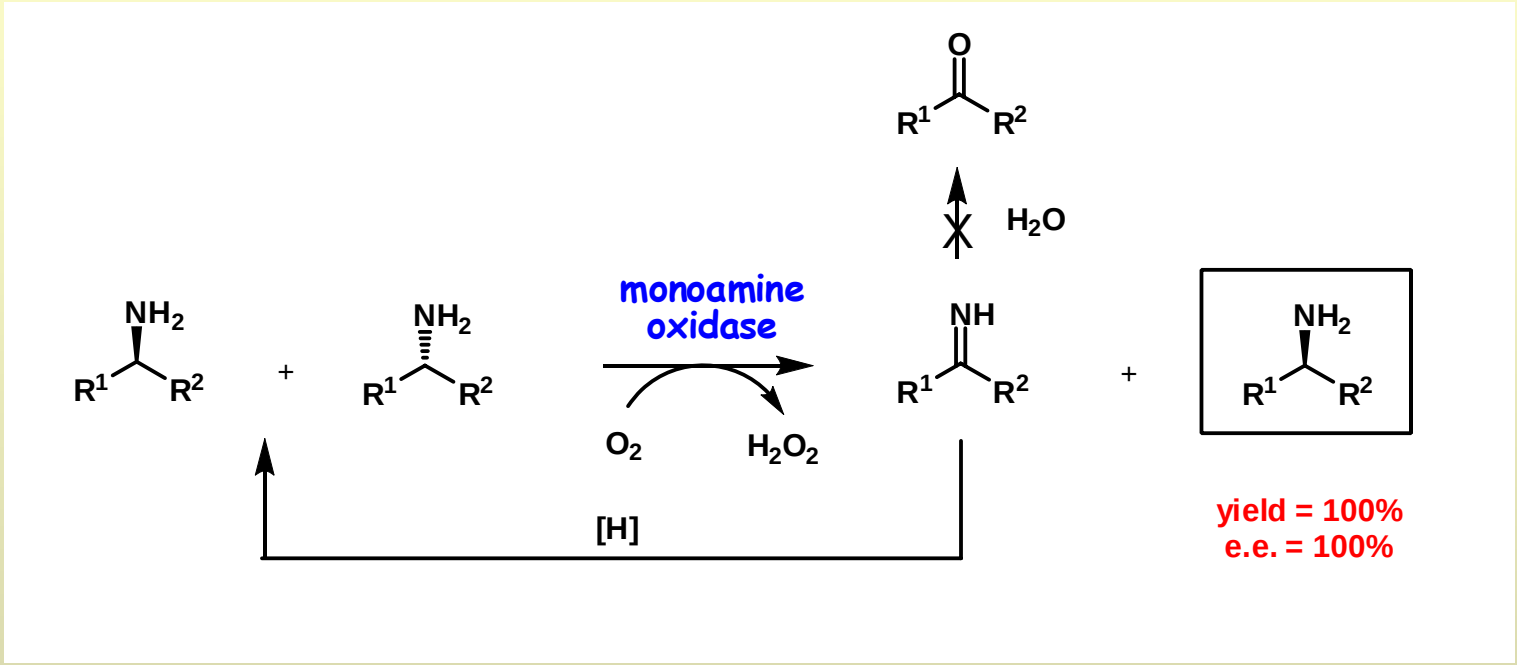
Kinetic resolution



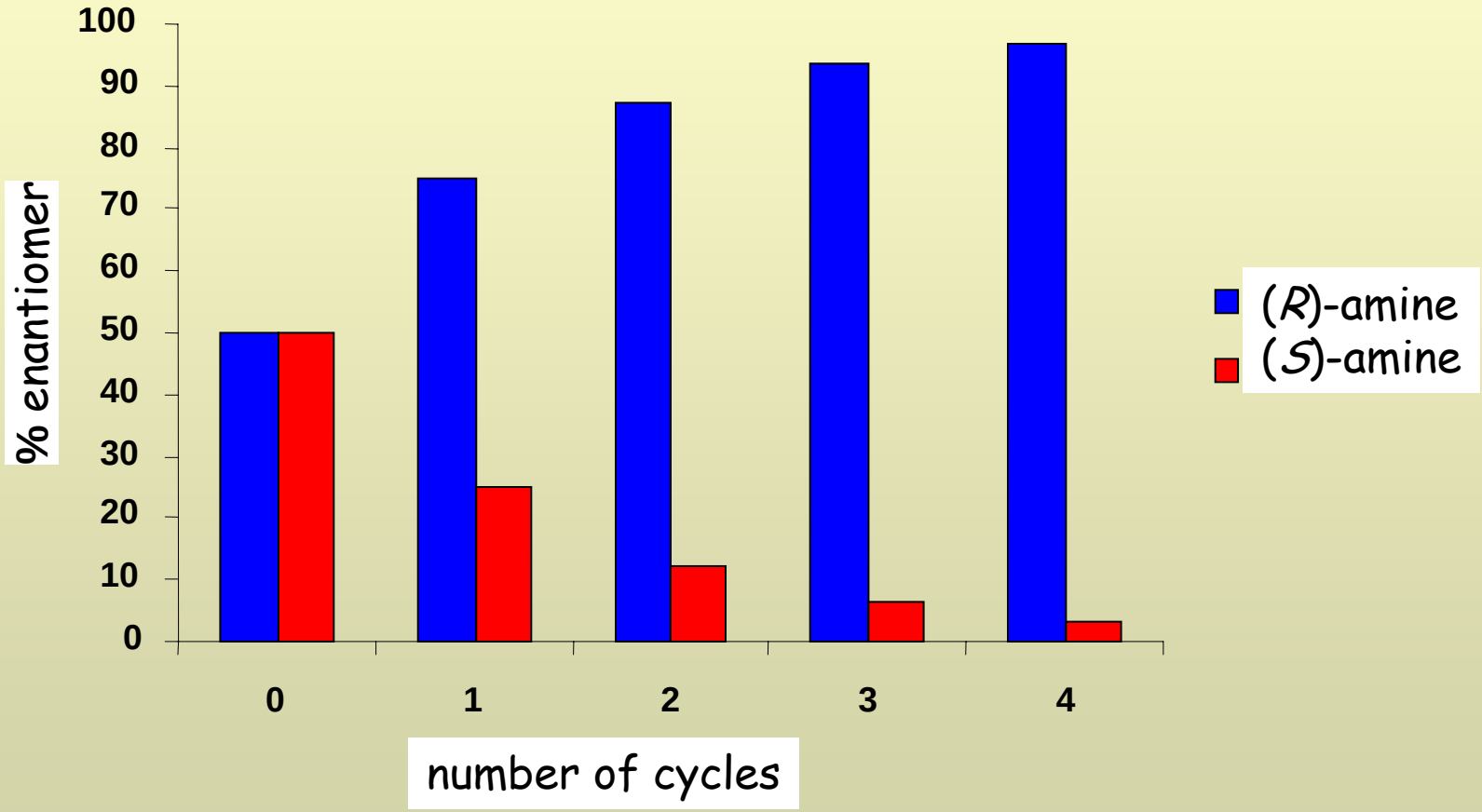
yield = 50%
e.e. = 100%

Monoamine oxidase

Deracemisation



Deracemisation



Alternative reducing agents

cyanoborohydride

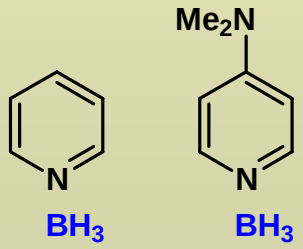
(*S*)-piperazine-2-carboxylic acid

Chem. Commun., 2002, 246.

amine-boranes

NH_3:BH_3

$tBuNH_2$:BH_3



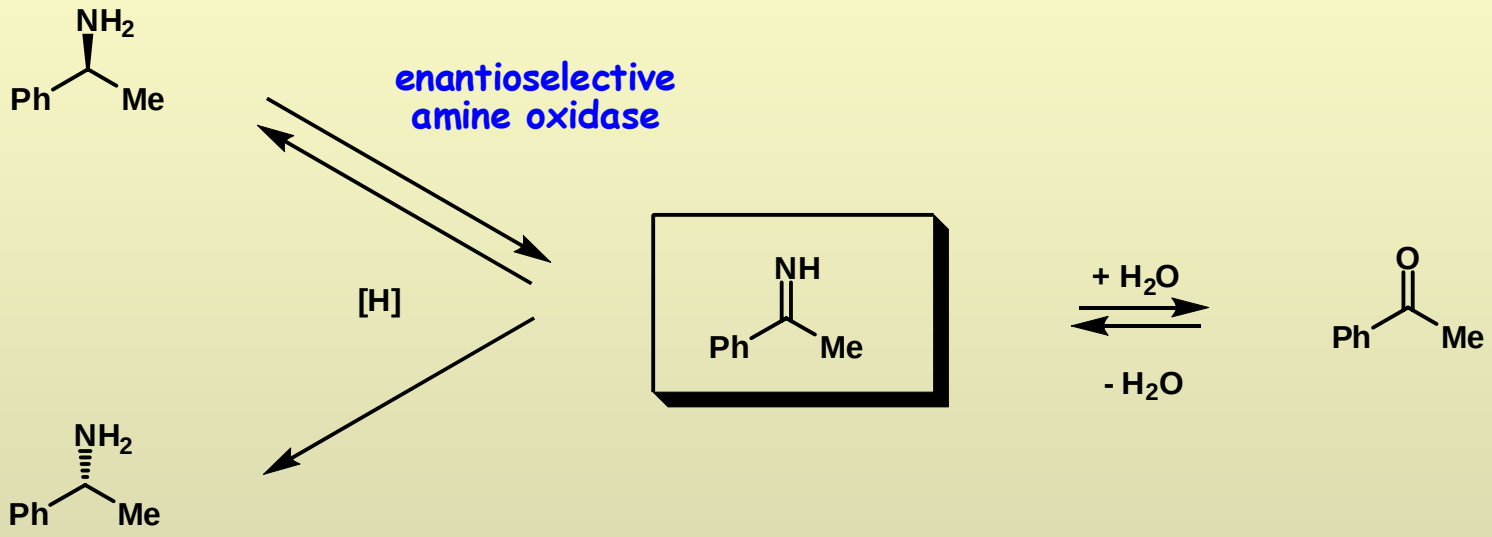
catalytic transfer hydrogenation

	time/min	yield/%	e.e./%
50 mM HCO_2NH_4, [H]	120	68	99
1M HCO_2NH_4, [H]	120	82	99
1M HCO_2NH_4, Pd/C	80	99	99

Tetrahedron Lett., 2002, *42*, 707.

Deracemisation of racemic chiral amines

L-(S)-α-methylbenzylamine (L-AMBA)

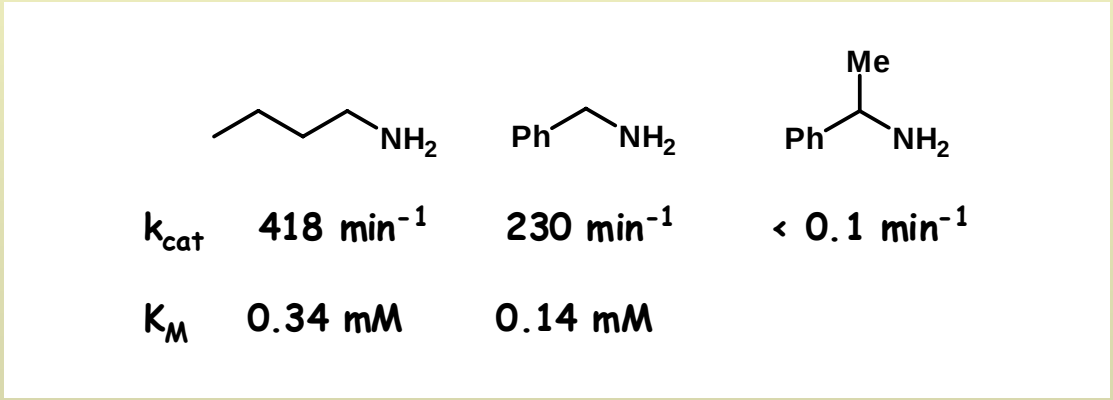


D-(R)-α-methylbenzylamine

Selection of amine oxidase for evolution

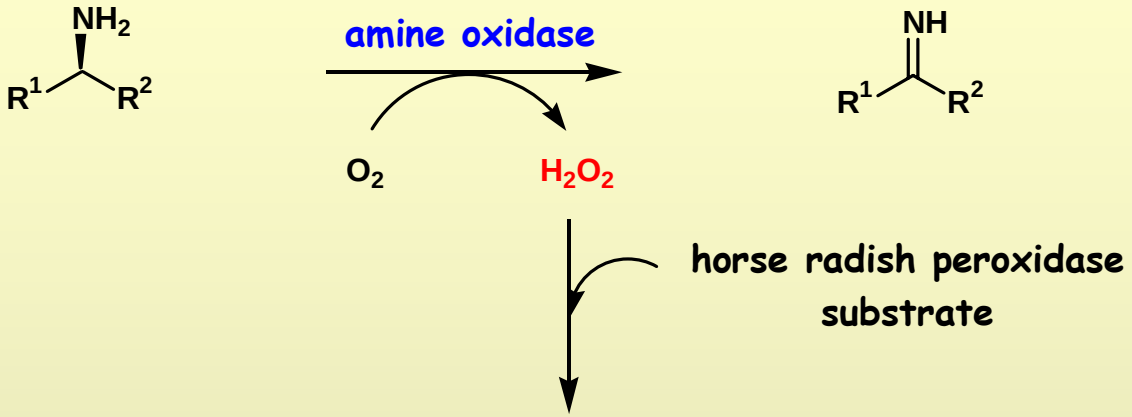
- Monoamine oxidase from *Aspergillus niger* (MAO-N) cloned and expressed in *E. coli*.

- High catalytic activity but narrow substrate specificity with no evidence of enantioselectivity

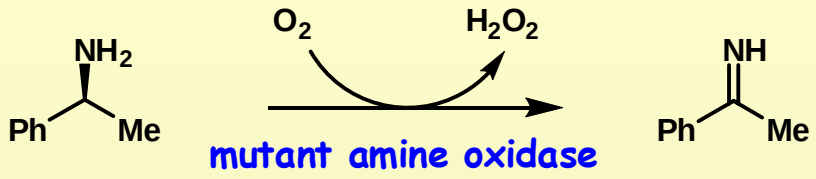


- Need to evolve enzyme with much broader substrate specificity high catalytic activity and high enantioselectivity

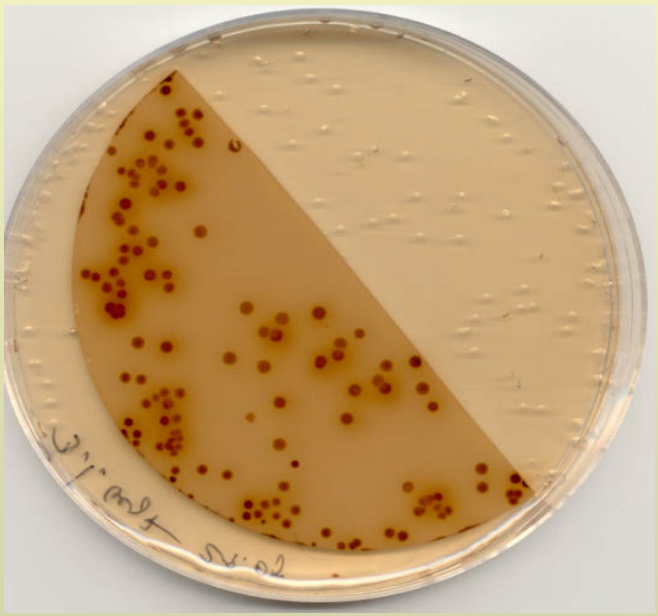
A colorimetric screen for oxidase activity

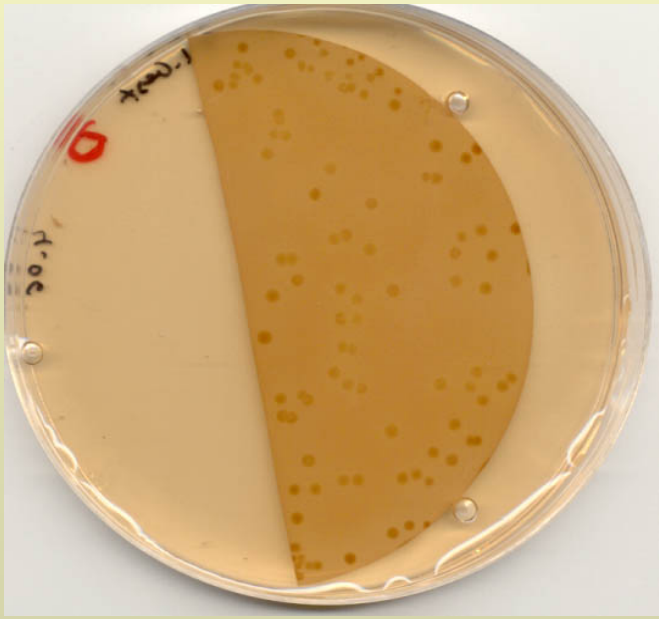


Screening against single enantiomers



L-(*S*)-α-methylbenzylamine



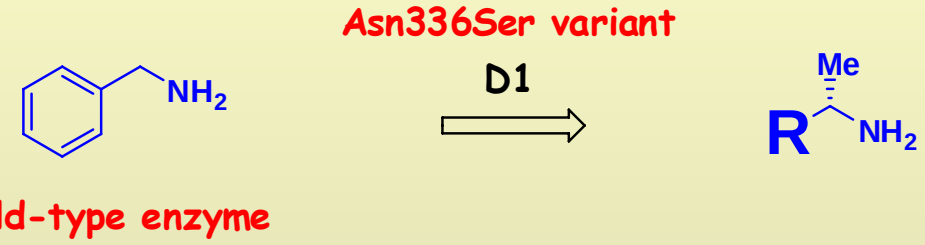


Single clone assayed against (*S*)-enantiomer Same clone assayed against (*R*)-enantiomer

R. Carr, M. Alexeeva and N.J. Turner, *Org. Biomol. Chem.*, 2003, *1*, 4133.

Asn336Ser variant is 50x more active and has broader substrate specificity

Screened library of 150,00 variants



wild-type enzyme

best substrates are simple achiral amines (*e.g.* amylamine, benzylamine)

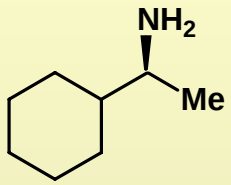
(S)-selective

>95% e.e

N.J. Turner *et al.*, *Angew. Chem. Int. Ed.*, 2002, *41*, 3177.
Angew. Chem. Int. Ed., 2003, *42*, 4807.

Substrate specificity of Asn336Ser mutant

Alicyclic primary amines (rel. rates)



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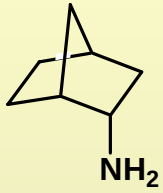

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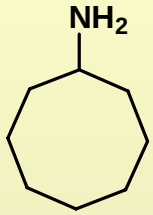

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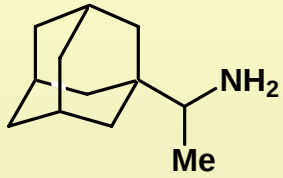

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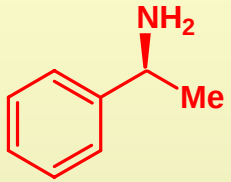

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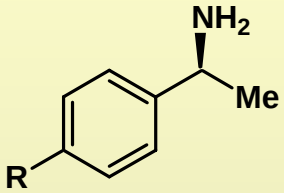
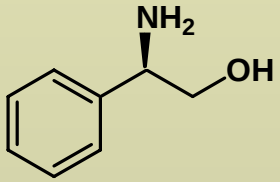


R = F: 100
R = NO_2: 64
R = Cl : 30
R = MeO: 13
R = Br : 10



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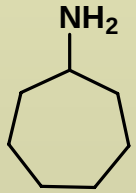

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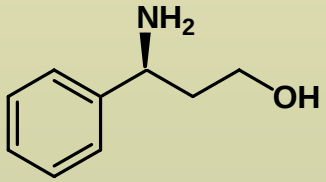

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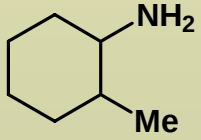

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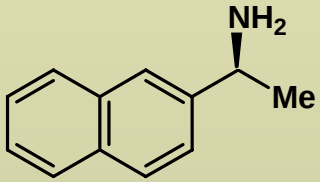
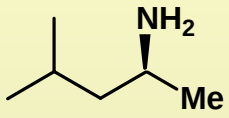


14

Substrate specificity of Asn336Ser mutant

Acyclic primary amines (rel. rates)



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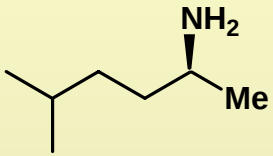

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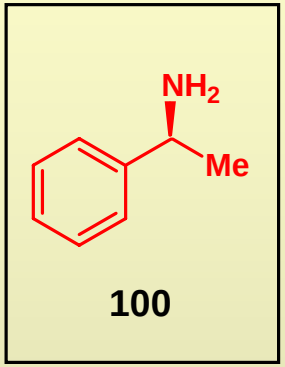

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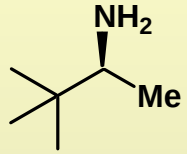

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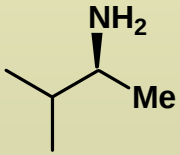

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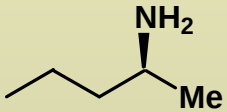

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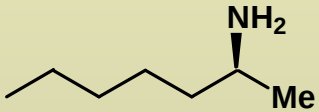

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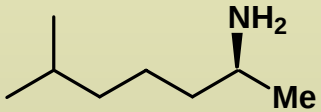

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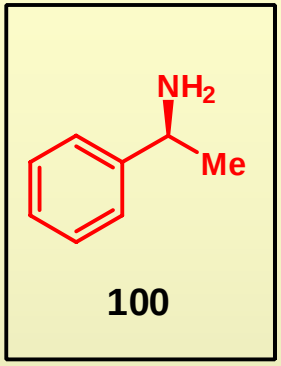
Substrate specificity of Asn336Ser mutant



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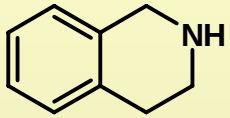

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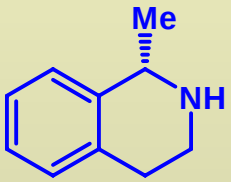

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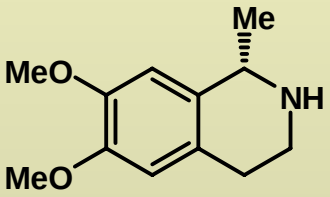

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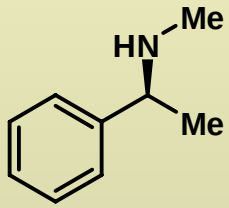
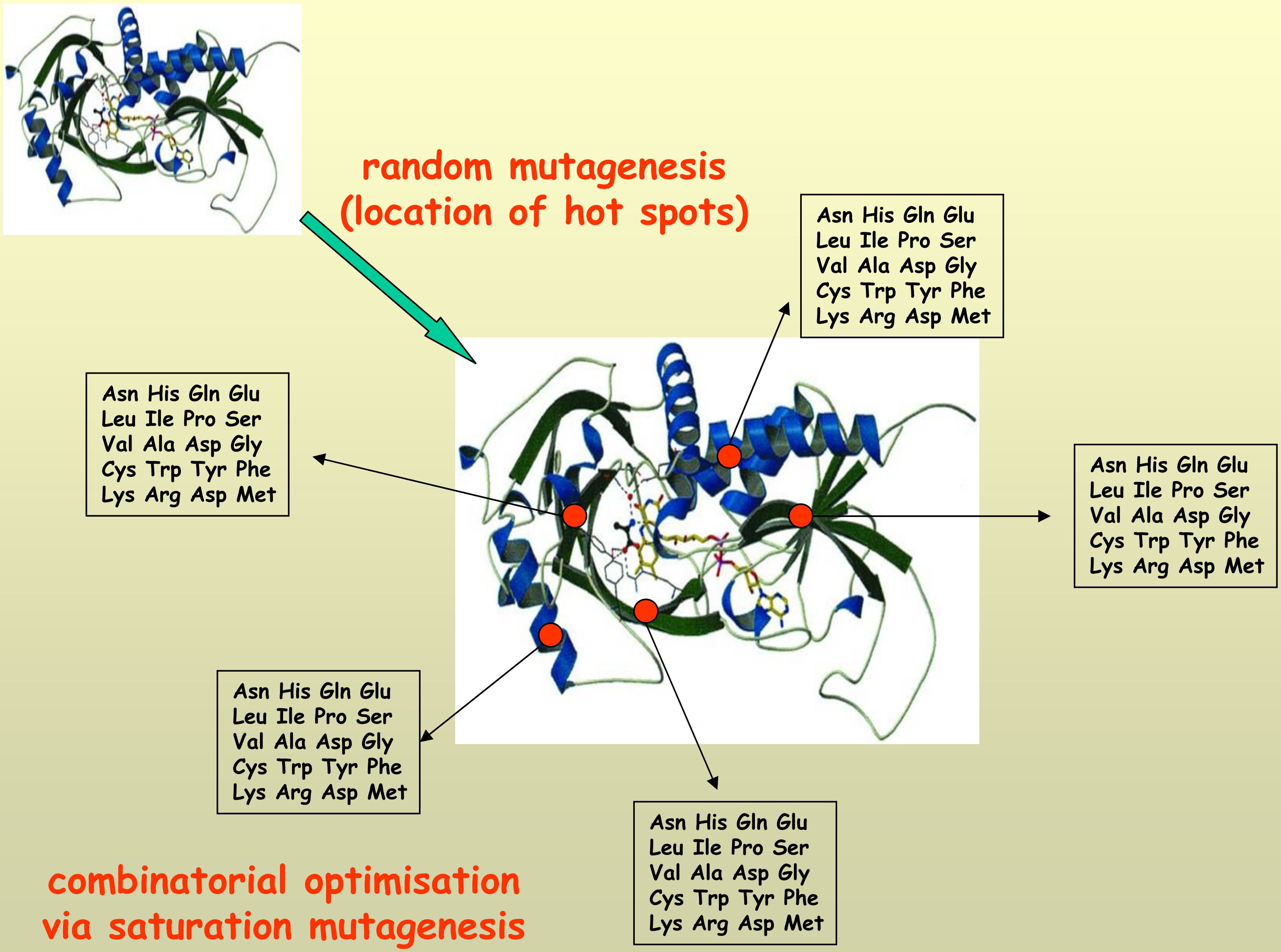


6

N.J. Turner *et al.*, *Angew. Chem. Int. Ed.*, 2002, *41*, 3177.
Angew. Chem. Int. Ed., 2003, *42*, 4807.

Strategy for directed evolution



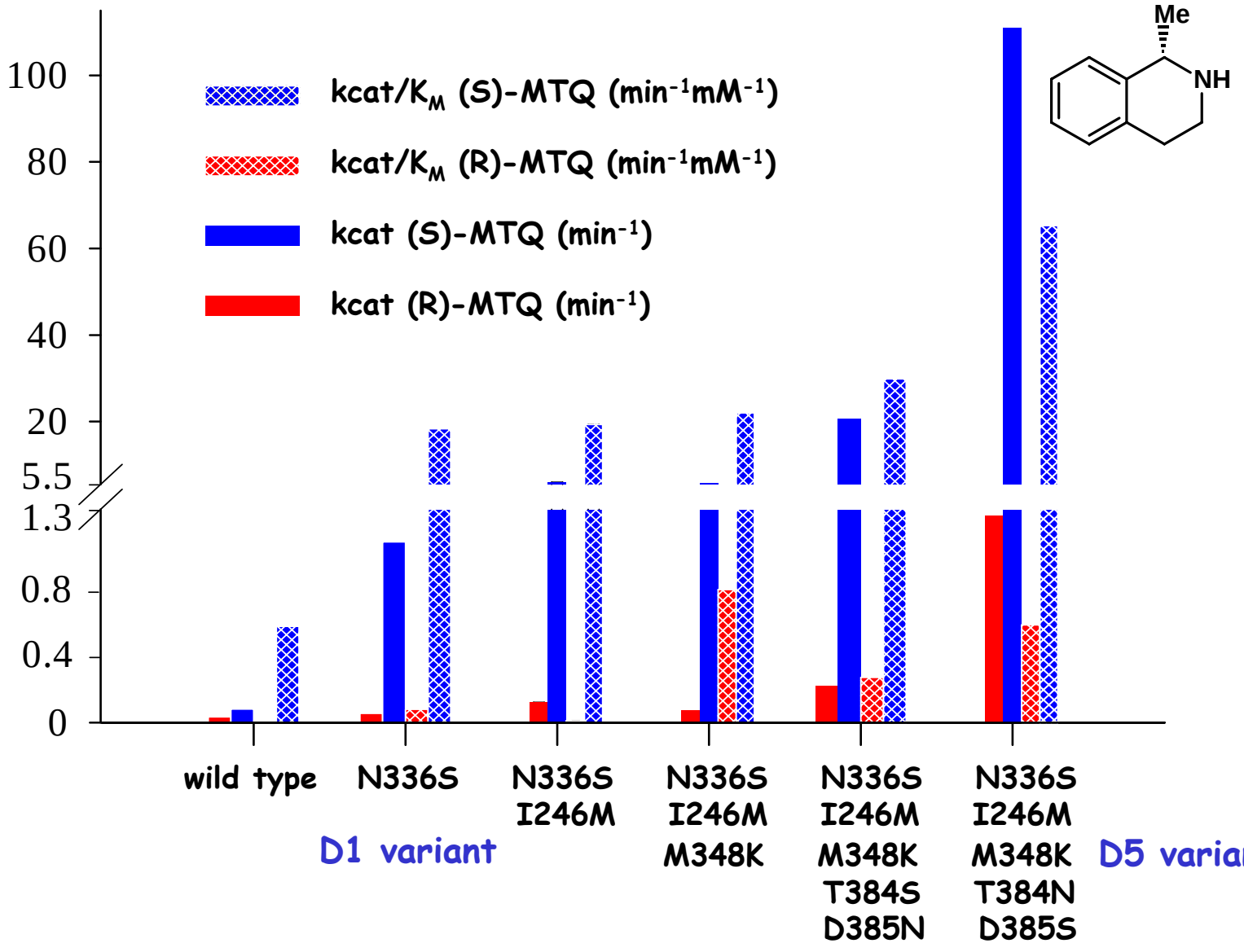
Further rounds of directed evolution

Residue	MAO-N wild-type	Optimal change	MAO-B
246	Ile	Met	Gln
259	Arg	Arg	Arg
260	Arg	Arg	Arg
289	Ala	Val	Val
336	Asn	Ser	Ile
348	Met	Lys	Lys
384	Thr	Asn	Phe
385	Asp	Ser	Ile

Reuben Carr

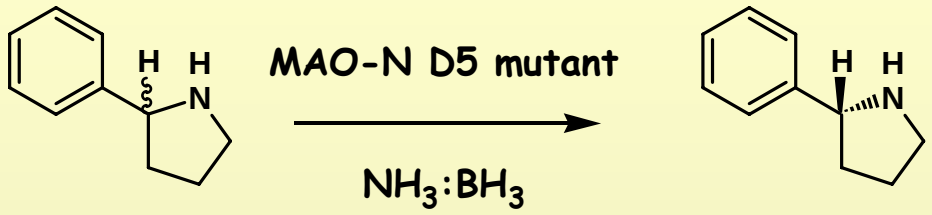
N.J. Turner *et al.*, *Angew. Chem. Int. Ed.*, 2002, *41*, 3177. *Angew. Chem. Int. Ed.*, 2003, *42*, 4807.

Progressive improvement in k_{cat}



kcat (S)-MTQ	0.08	1.1	6.0	5.7	20.6	110.8	(1385 fold)

Preparative deracemisation of (±)-2-PP

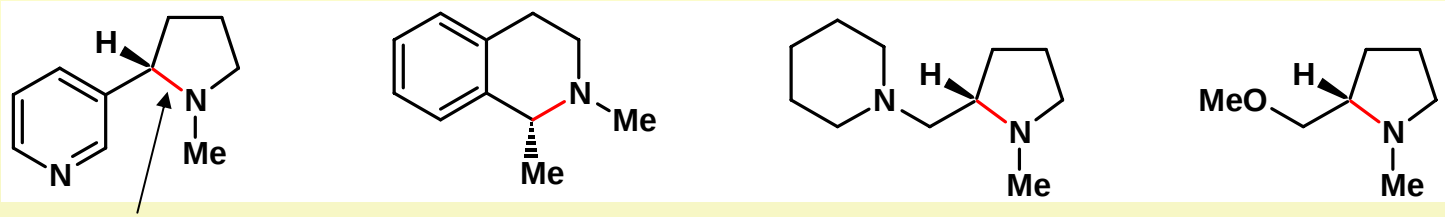


conv. >95%
e.e. >95%

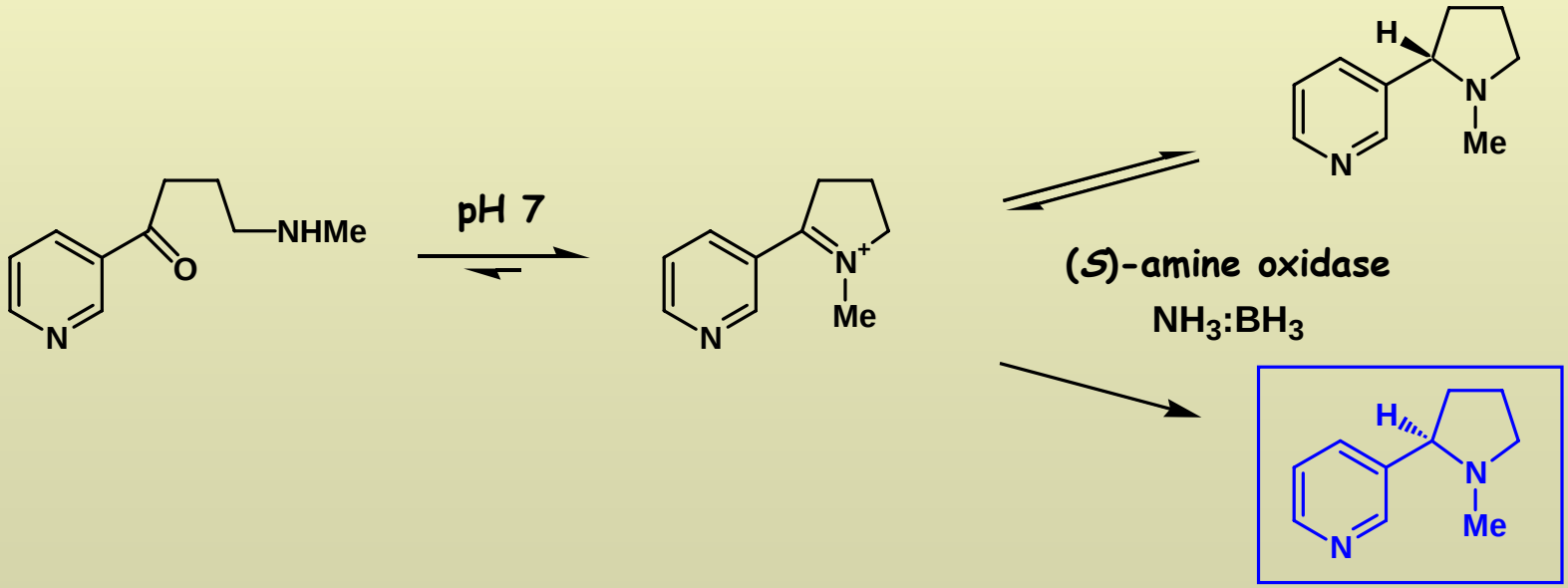
- 100 mM (±)-2-PP (14.7 gL^{-1})
- 250 mM NH_3:BH_3
- MAO-N immobilised on Eupergit
- after t = 48h, e.e. >95%
- work up adjust pH >10 then extract into MTBE
- yield = 83%

R. Carr, M. Alexeeva, M.J. Dawson, V. Gotor-Fernández, C.E. Humphrey and N.J. Turner
ChemBioChem, 2005, *6*, 1312.

3° amines are substrates



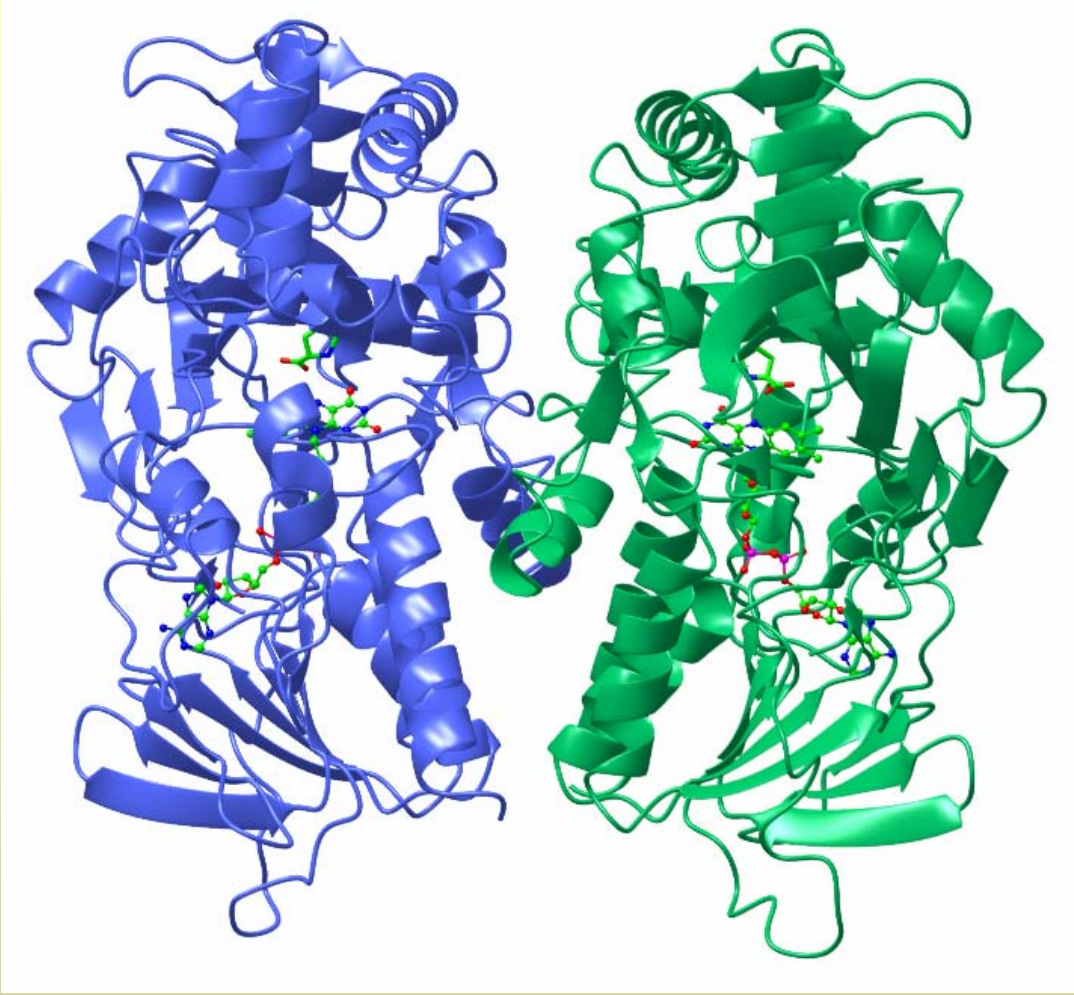
catalytic asymmetric reductive amination



24h; 95%; 98% e.e.

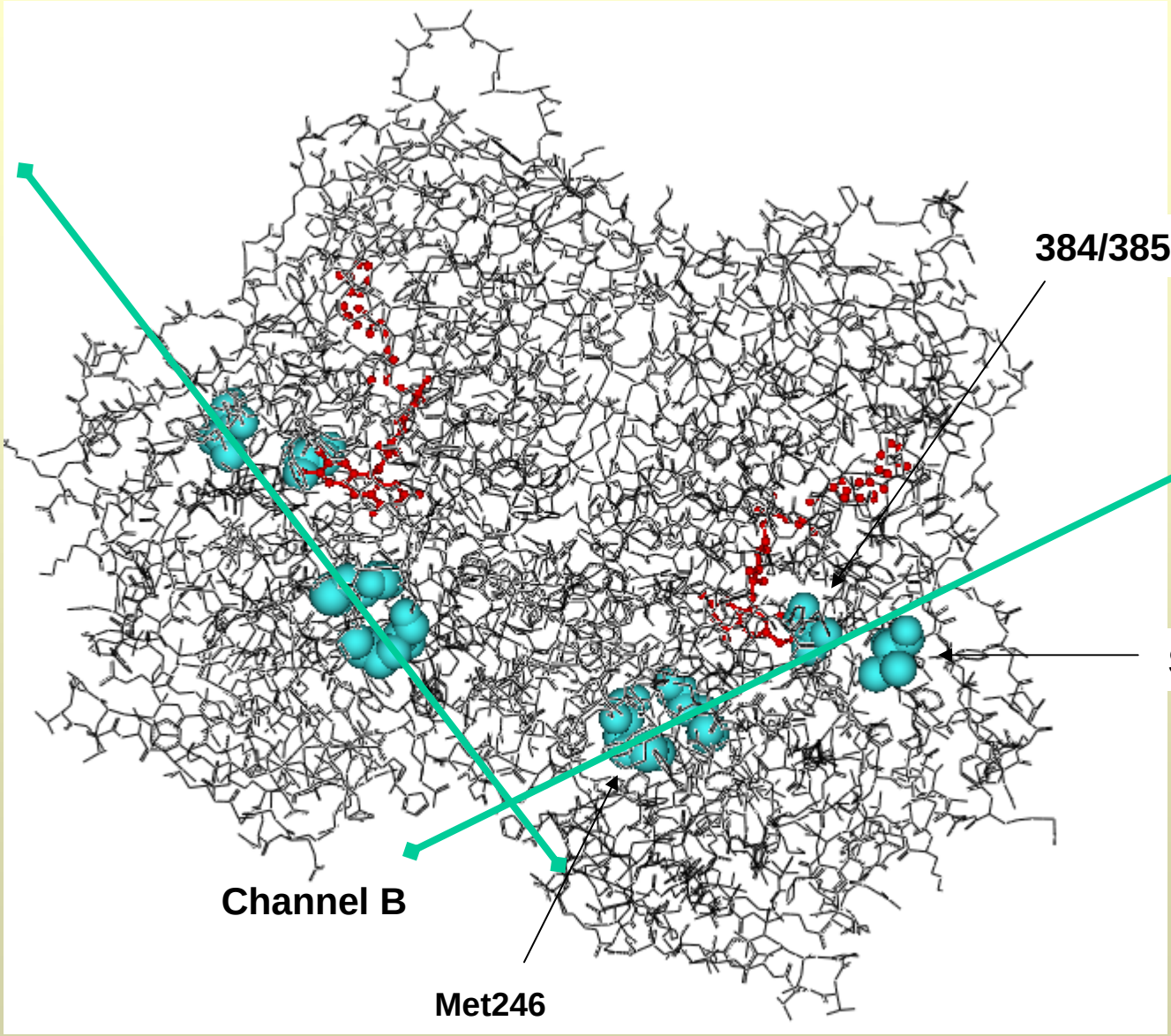
C.J. Dunsmore, R. Carr, T. Fleming and N.J. Turner, *J. Am. Chem. Soc.*, 2006, *128*, 2224.

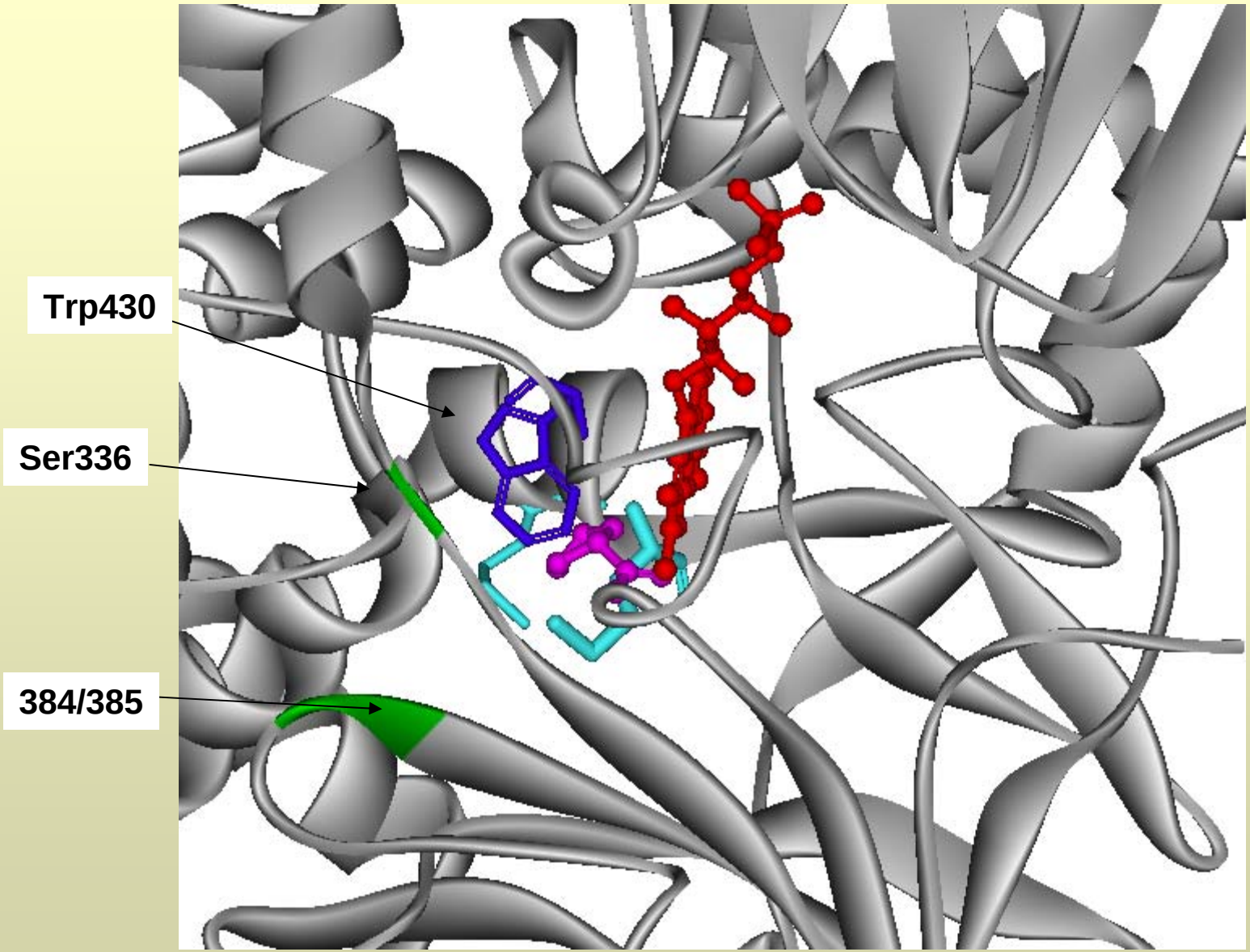
1.8 A structure of MAO-N (D5)



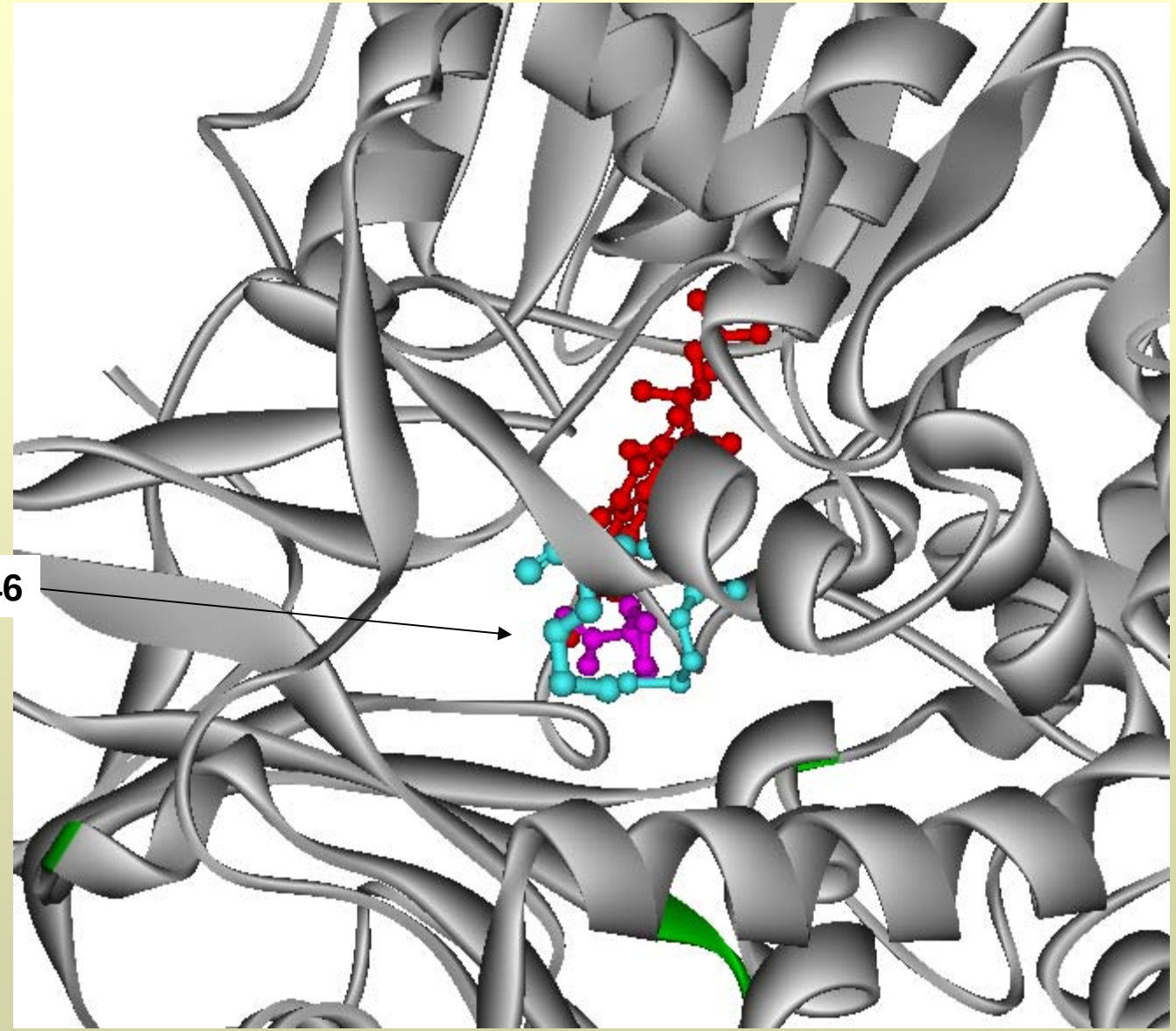
K. Atkin, G. Grogan, N.J. Turner et al., *J. Mol. Biol.*, 2008, *384*, 1218.

Channels pass through active-site of MAO-N





Channel A



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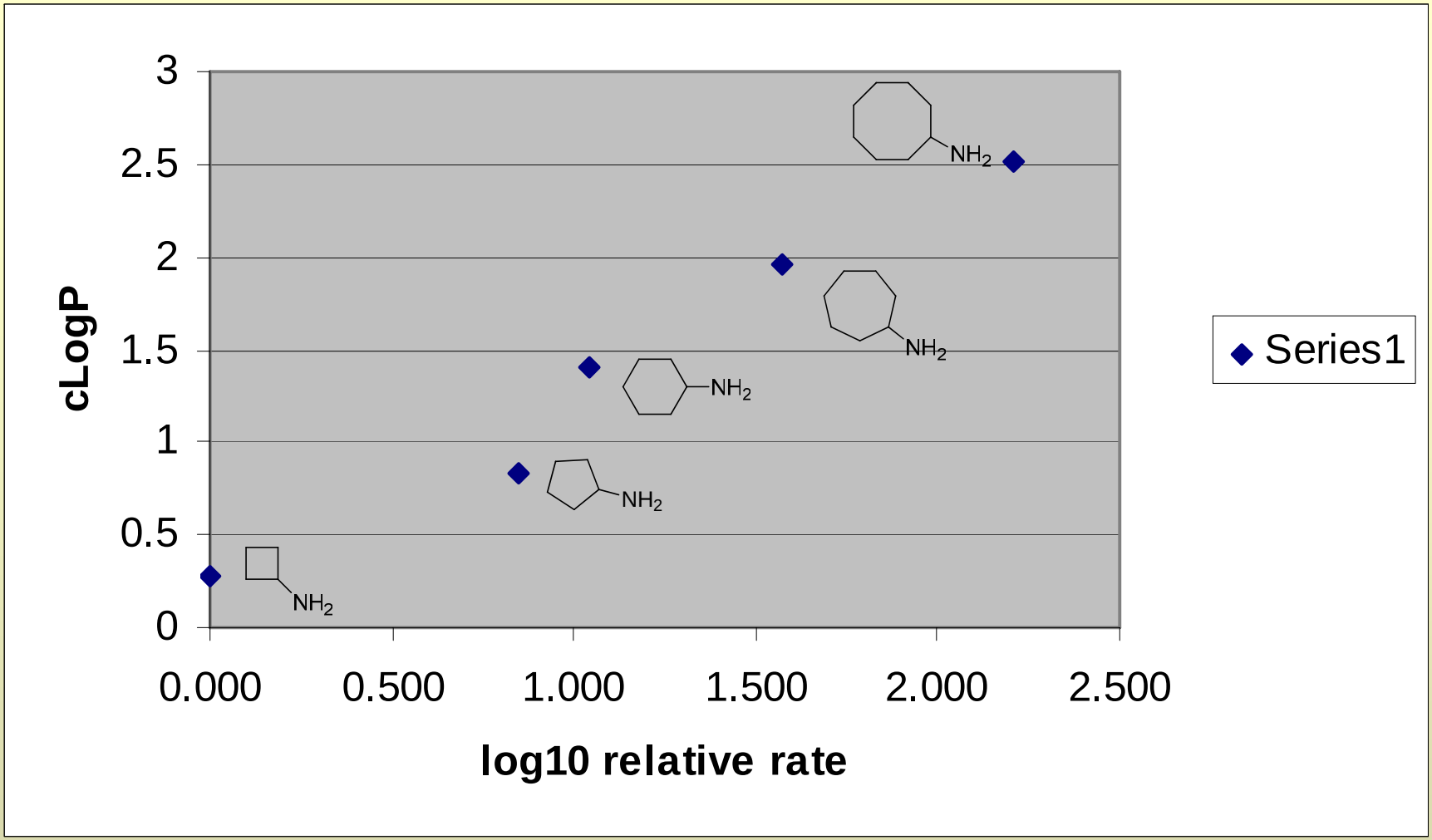
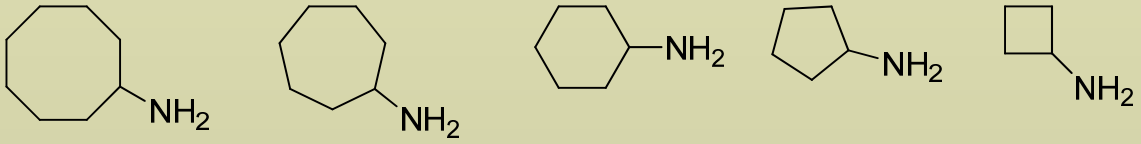
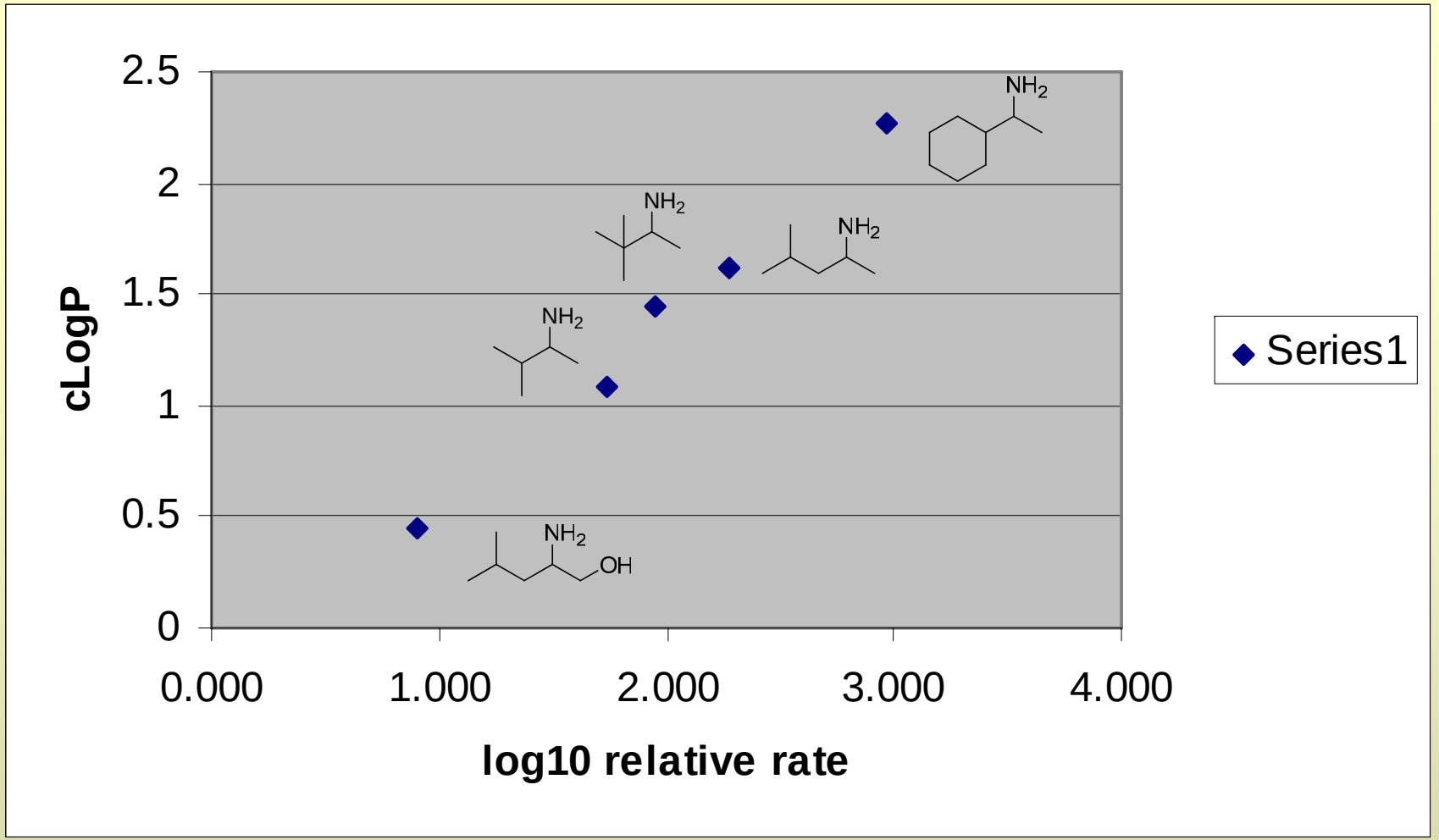
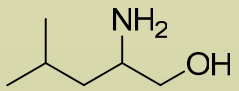
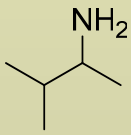


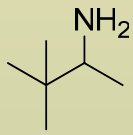


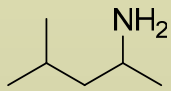
161	37	11	7	0	Relative rates of oxidation for Asn336Ser cf α-MeBzamine
2.52	1.96	1.40	0.83	0.27	cLogP

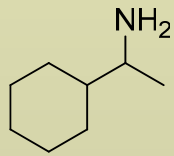








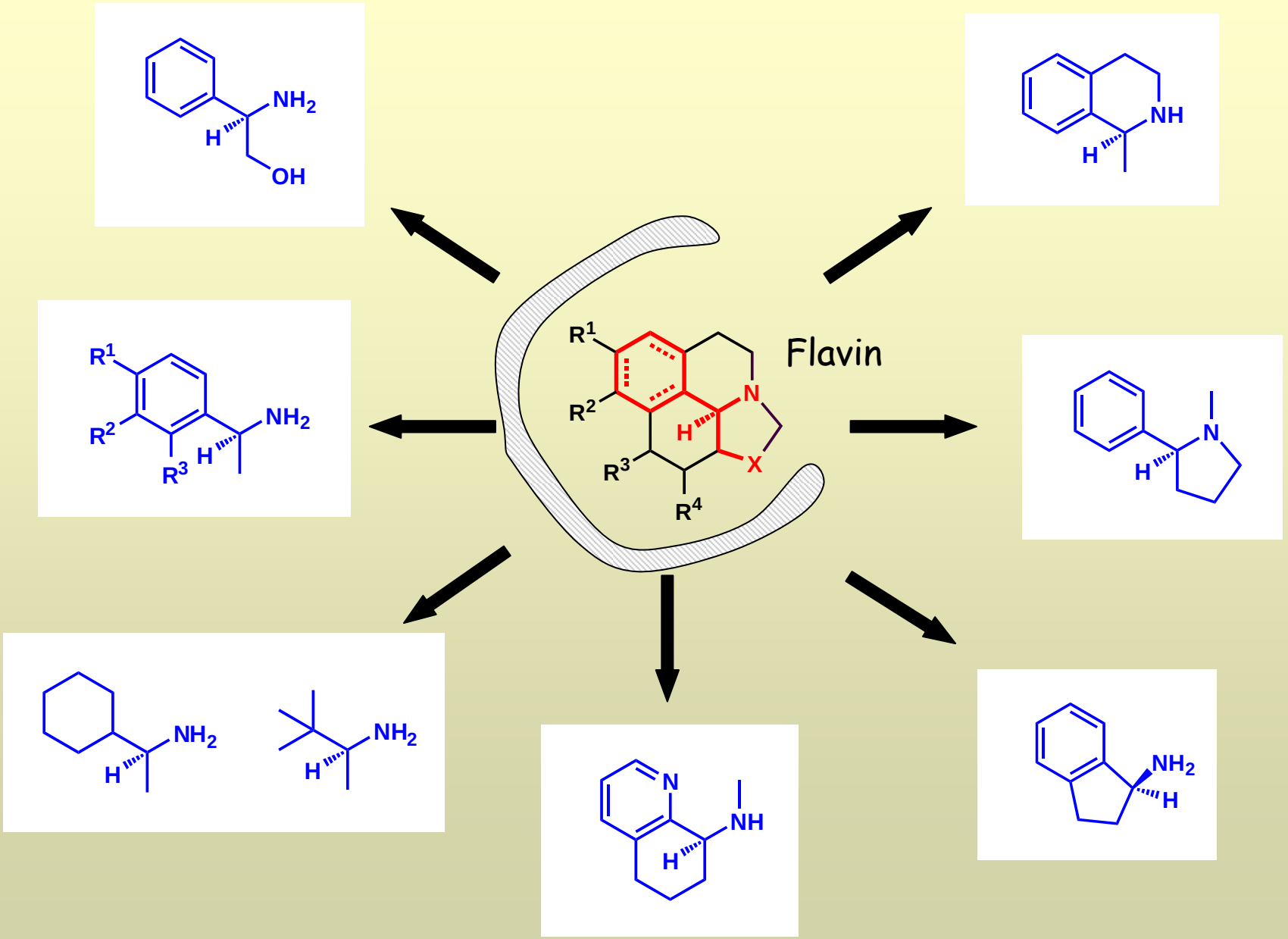




8	54	89	190	915	Relative rates of oxidation for Asn336Ser cf α-MeBzamine
0.45	1.09	1.44	1.62	2.27	cLogP

Applications in Synthesis

Desk-top model for chemists

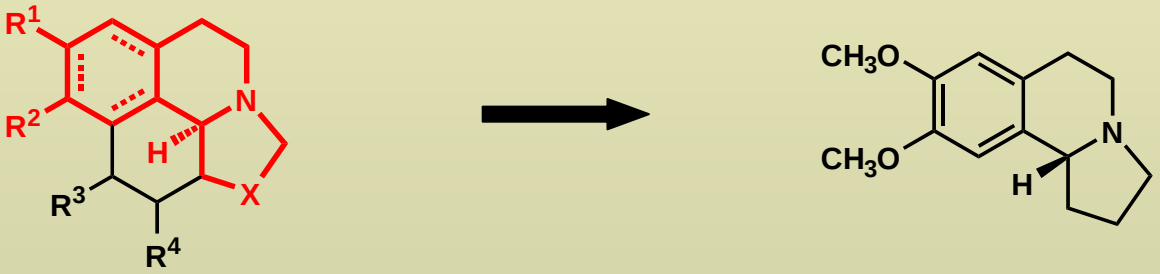


K.R. Bailey, A.J. Ellis, R. Reiss, T.J. Snape and N.J. Turner, *Chem Commun.*, 2007, 3640-3642.

Synthesis of (+)-crispine A

Welted Thistle
(*Carduus crispus*)

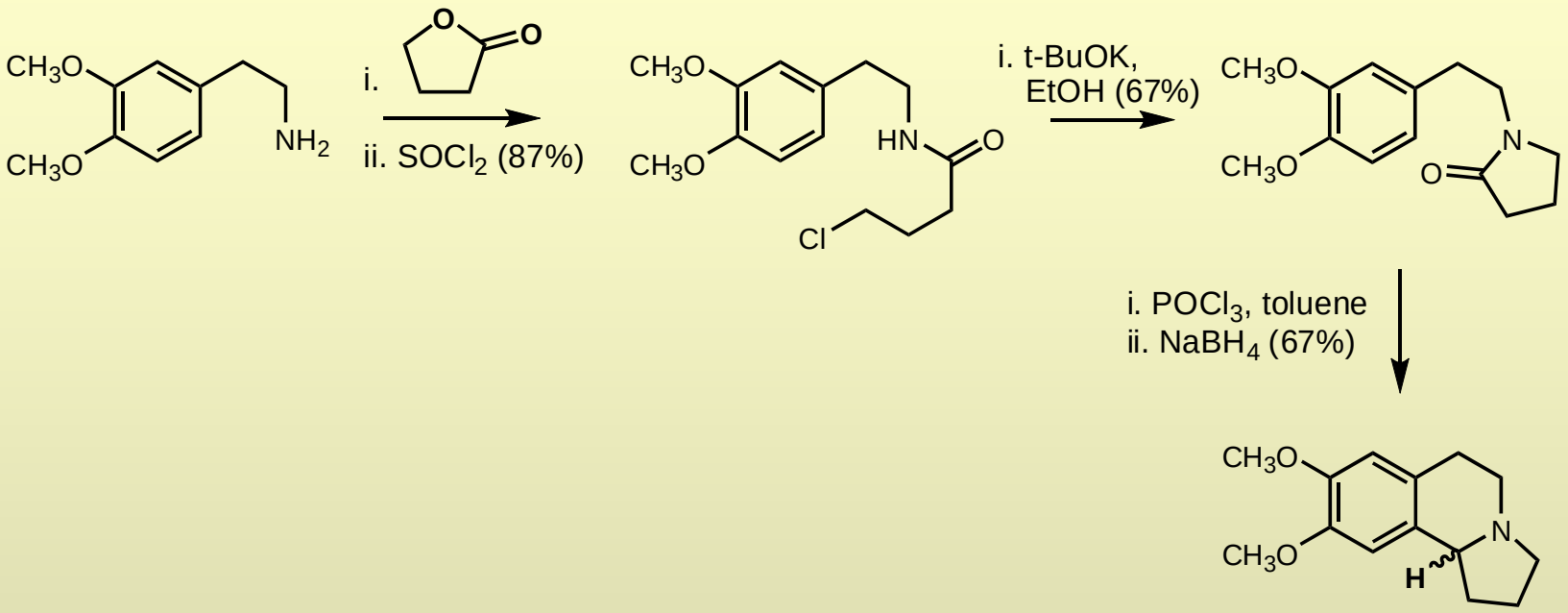
- Extracts widely used in folk medicine for treatment of bronchitis, gastroenteritis, rheumatism
- Crispine A has anti-tumour activity



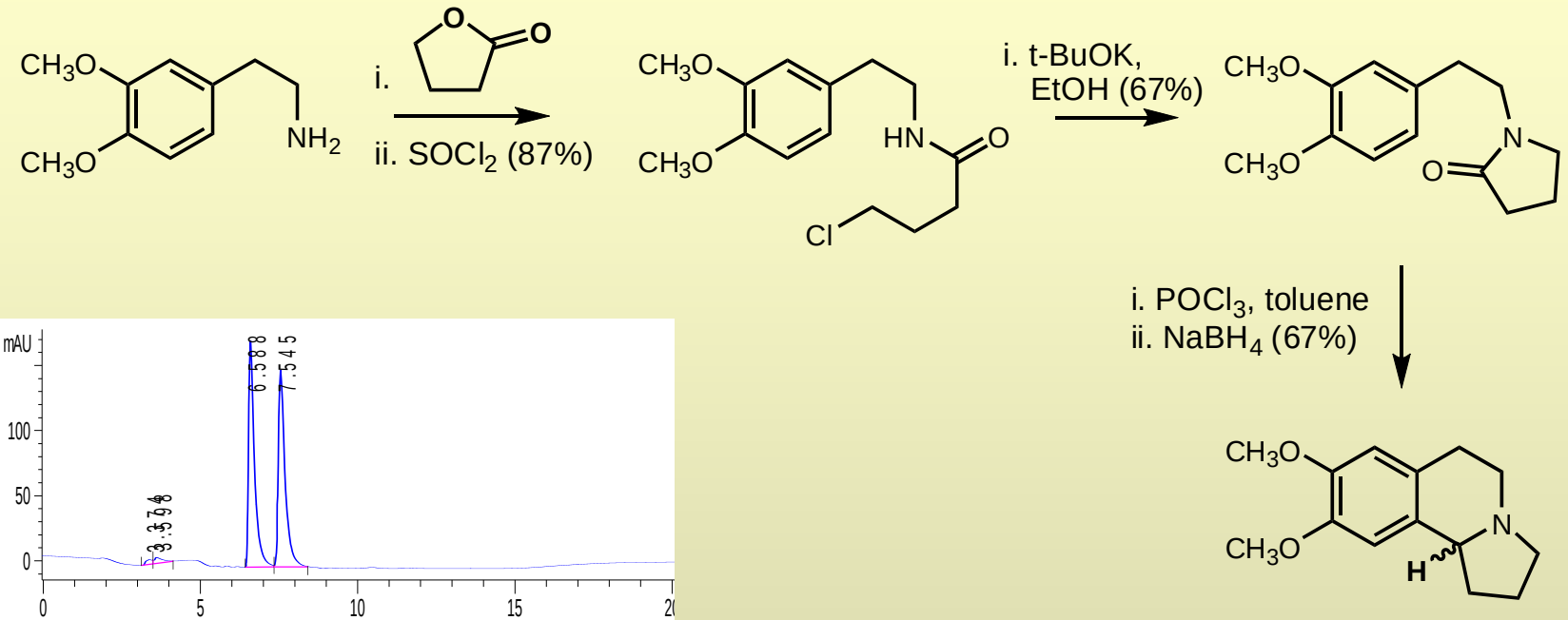
Crispine A

K.R. Bailey, A.J. Ellis, R. Reiss, T.J. Snape and N.J. Turner, *Chem Commun.*, 2007, 3640-3642.

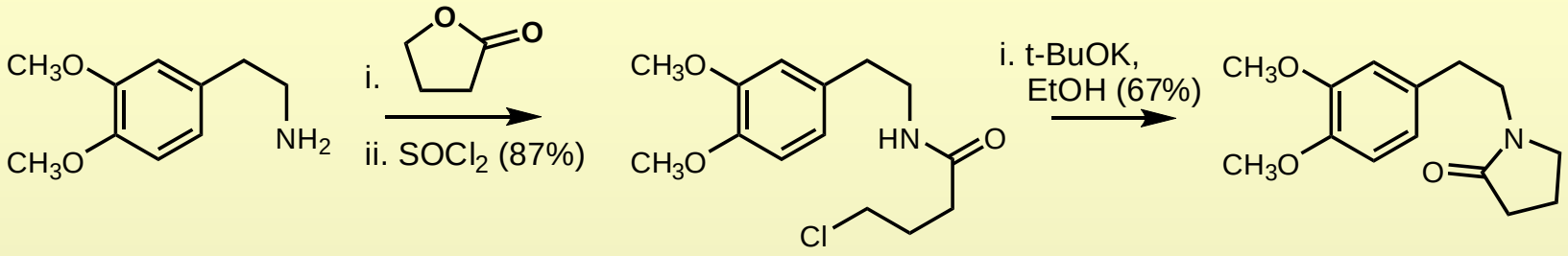
Synthesis of (+)-crispine A

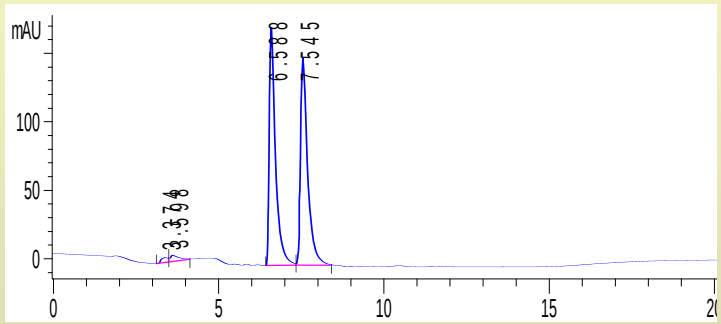


Synthesis of (+)-crispine A

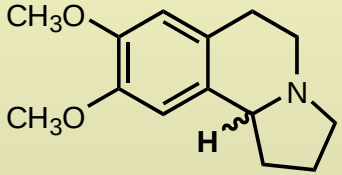


Synthesis of (+)-crispine A



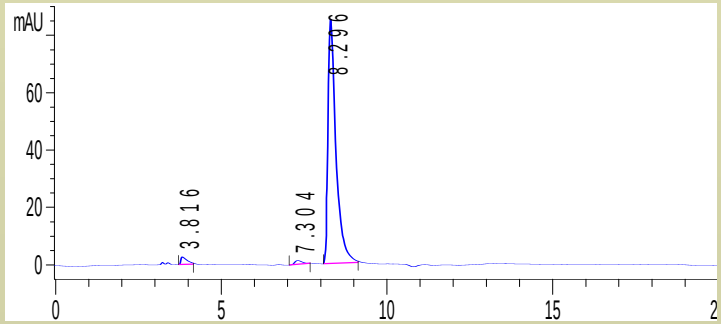


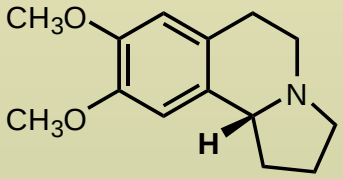
i. $POCl_3$, toluene
ii. $NaBH_4$ (67%)



MAO-N
NH_3:BH_3

MAO-N
NH_3:BH_3





yield = 95%
e.e. = 99%

Acknowledgements

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Ingenza
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Kate Atkin, Gideon Grogan (York)

AstraZeneca, BASF, Codexis, Contract Chemicals, Croda, DowPharma, Excelsyn, GlaxoSmithKline, Ingenza, Merck, Novacta, Novo-Nordisk, Shell

BBSRC, EPSRC & DTI

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