Biocatalysis: key-element in the synthesis of bio-active (natural) products

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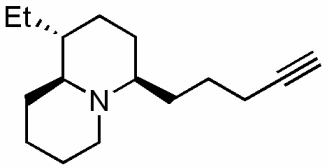
Outline of presentation

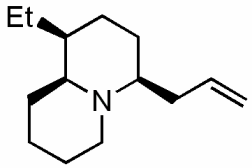
- **General Introduction**
- **Historic background information** on industrial amino acid resolution
  (T Sonke *et al*, in *Stereoselective Biocatalysis*; Ed RN Patel, Marcel Dekker, NY,Basel **2000,** Chapter 2)
- **New developments** in industrial biocatalysis (Schoemaker *et al*, *Science*, **2003**, *299*,1694; "Dispelling the Myths-Biocatalysis in Industrial Synthesis")
- **Unsaturated amino acids** as building blocks in organic synthesis using a combination of biocatalysis, homogeneous catalysis and classical organic synthesis (see J. Kaiser *et al*, *Org Biomol Chem*, **2005**, *3*, 3435)
- **Nitrile converting enzymes** in organic synthesis
  (MKS Vink *et al*, *Biotechnol J*, **2006**, *1*, 569-573)
- **Bioredox reactions** (Mang *et al*, *Angew Chemie Int Ed*, **2006**, *45*, 5201, and A Tuynman *et al*, *J Biol Chem*, **2000**, 3025)
- **Expanding the scope of C-C bond forming enzymes**
  (S Jennewein *et al*, *Biotechnology J*, **2006**, *1*, 537-538 and
  T van Herk *et al*, *J Org Chem*, **2006**, *71*, 6244-6247)
- **Conclusions**

Enzyme platforms
Natural products Chiral building blocks

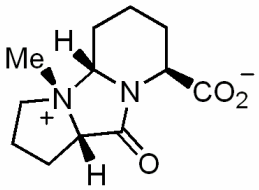
Aminopeptidases and Amidases



quinolizidine 233A

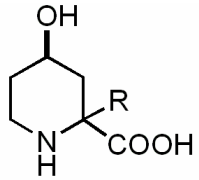


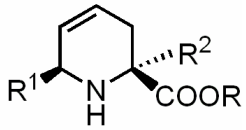
quinolizidine 207I



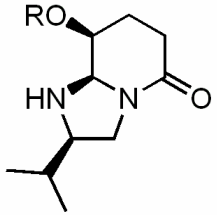
(–)-dysibetaine PP

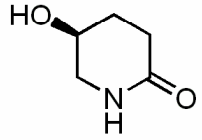
Aminopeptidases and Amidases



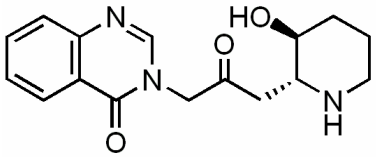


Hydroxynitrile lyases

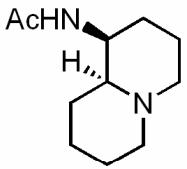




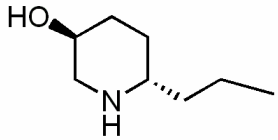
Aminopeptidases/ amidases or Hydroxynitrile lyases



febrifugine

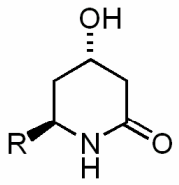


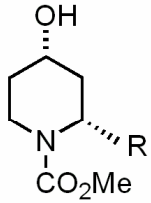
(+)-epiquinamide



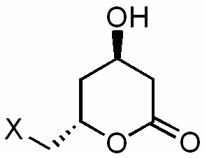
pseudoconhydrine

Nitrilases

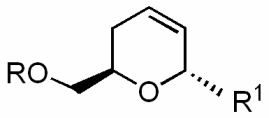




Aldolases



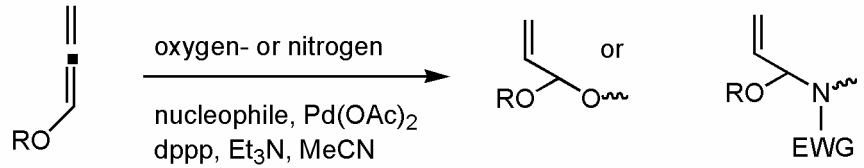
Lipases



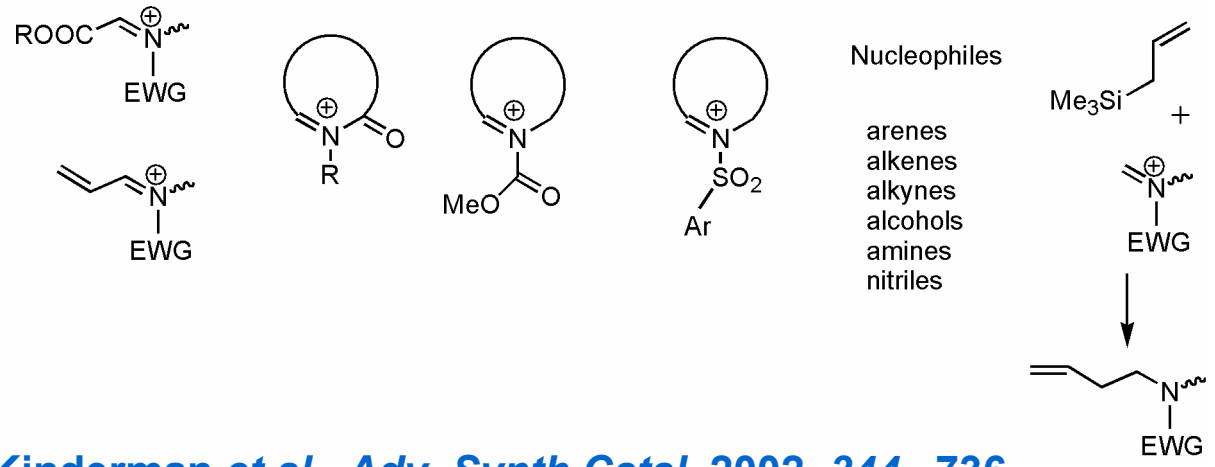
Introduction to the chemical methodology

1. Ruthenium catalyzed C-C bond formation

2. Oxy- or amido-palladation



3. N-acyl or N-sulfonyl iminium ion chemistry



Cf Sape Kinderman *et al*, *Adv. Synth.Catal.* 2002, *344*, 736 and references cited

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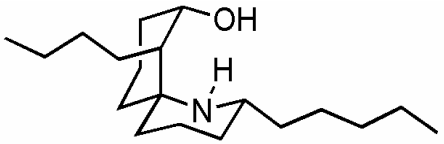
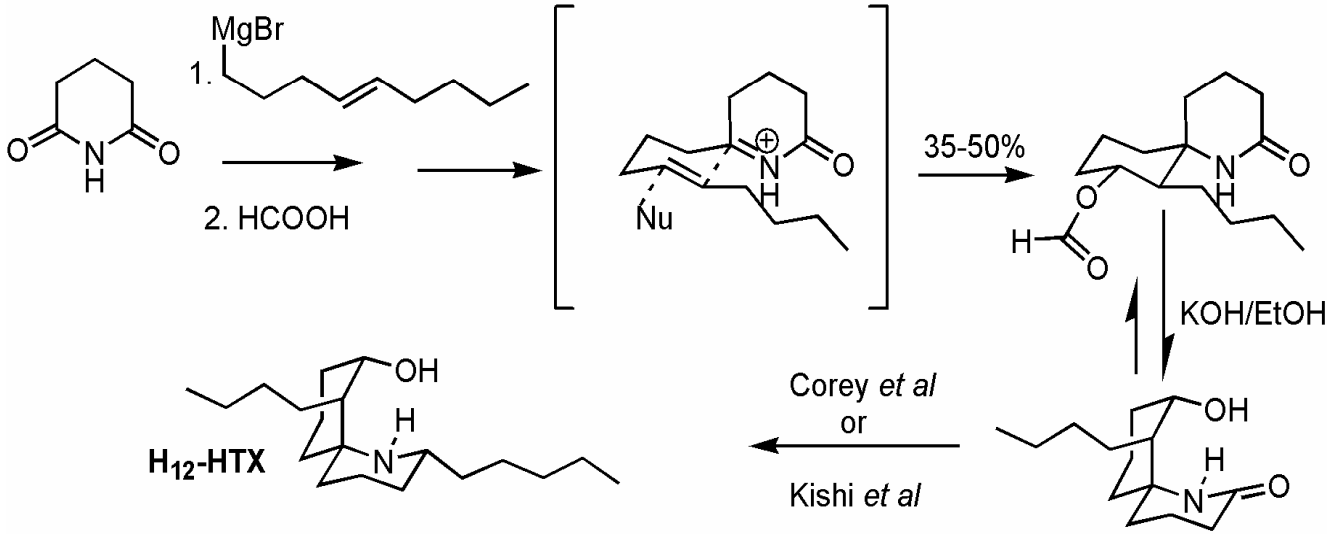
Control of relative stereochemistry via N-acyl iminium ion intermediates. Synthesis of perhydrohistrionicotoxin



Perhydrohistrionicotoxin (H_{12}-HTX)

The histrionicotoxins constitute a class of alkaloids isolated from the Colombian poison arrow frog *Dendrobates histrionicus*

"The name histrionicotoxin is misleading, since these alkaloids have relatively low toxicity They have been widely used in research as non competitive blockers of nicotinic receptor/ channels" **J.W. Daly *et al*, *J Nat Prod*, 2005,** *68*,1556

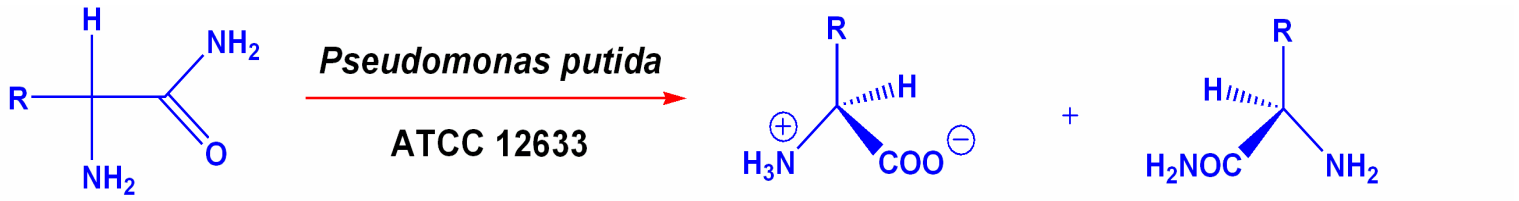


HE Schoemaker, WN Speckamp, *Tetrahedron Letters* **1978**, 4841

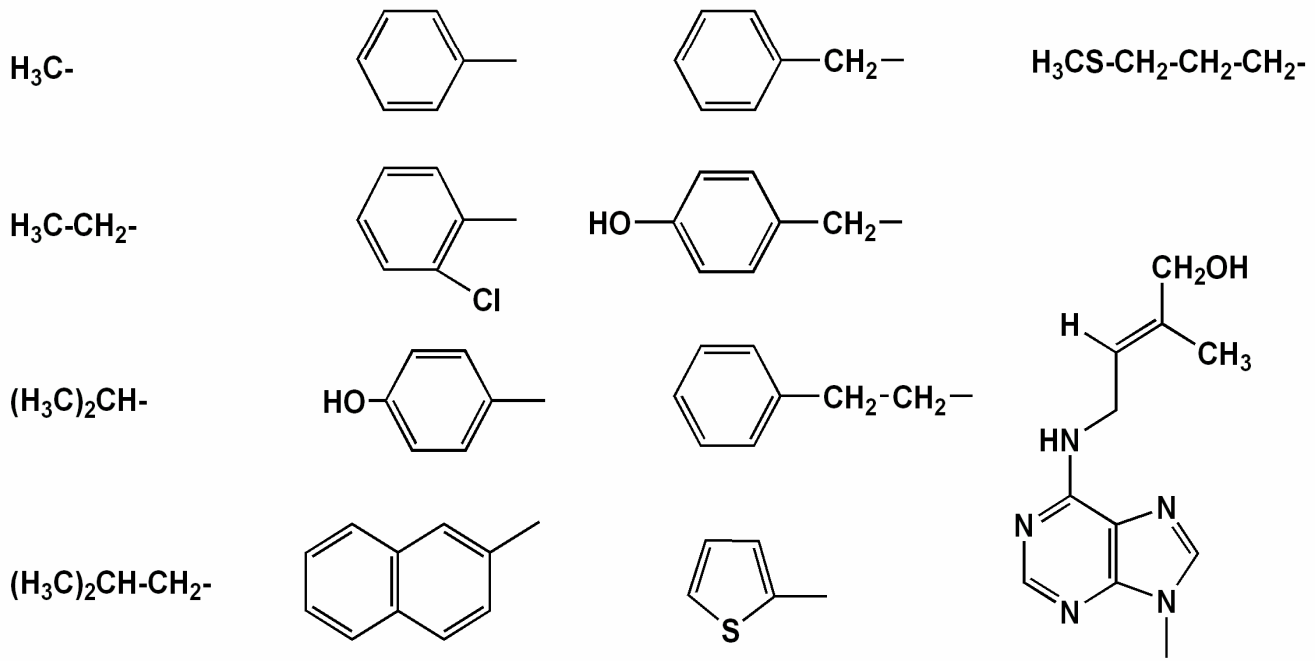
See also: DA Evans, EW Thomas, *ibid* **1979**, 411

See for a recent review on N-acyliminium ion chemistry:
BE Maryanoff *et al*, *Chem. Rev.*, **2004**, *104*, 1431-1628 (Johnson and Johnson, USA)

Aminopeptidase with broad substrate tolerance

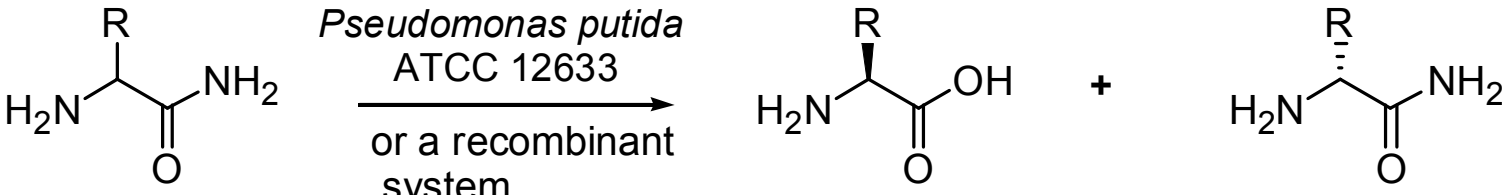


R



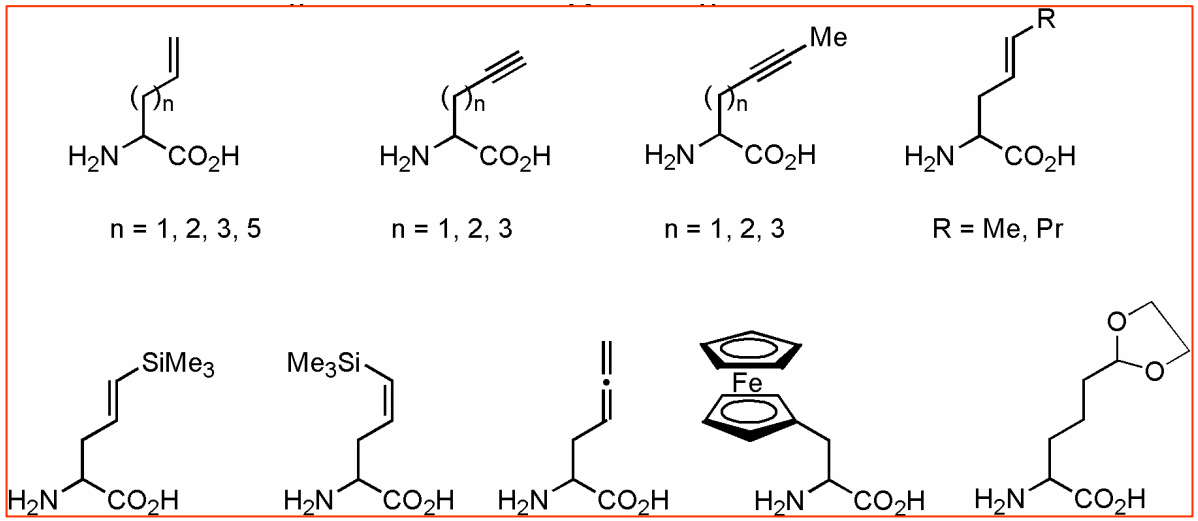
Cf HE Schoemaker, WHJ Boesten *et al*, *Acta Chem Scand* **1996**, *50*, 225

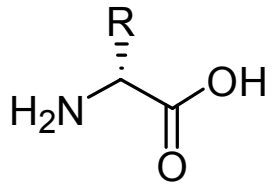
Resolution of unsaturated amino acid amides



separation,
then *Rhodococcus erythropolis*
NCIMB 11540

US patent 4705572
Boesten, Cals
(DSM, 1987)





LB Wolf, T Sonke, KCMF Tjen, B Kaptein, QB Broxterman, HE Schoemaker, FPJT Rutjes, *Adv. Synth. Catal*. **2001**, *343*, 662

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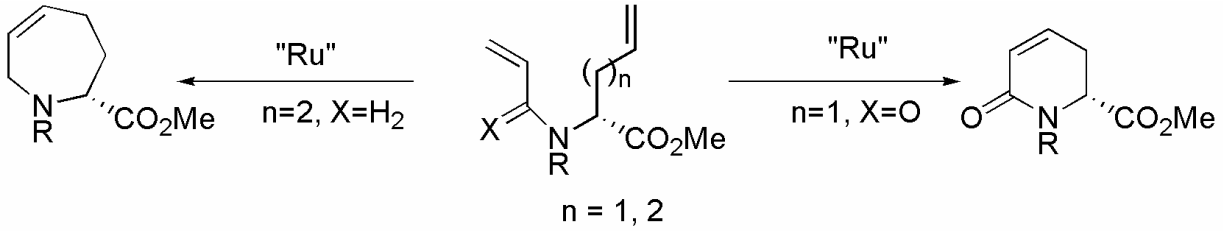
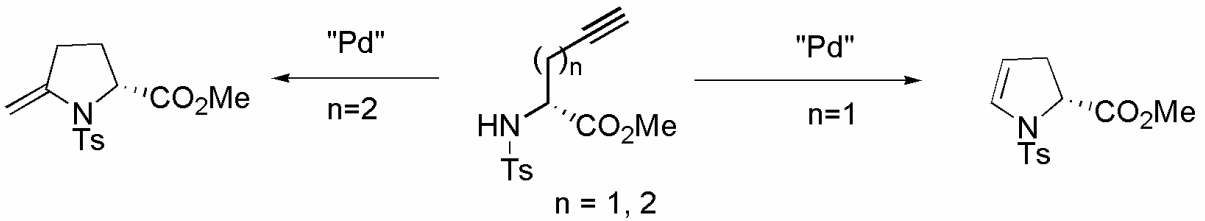
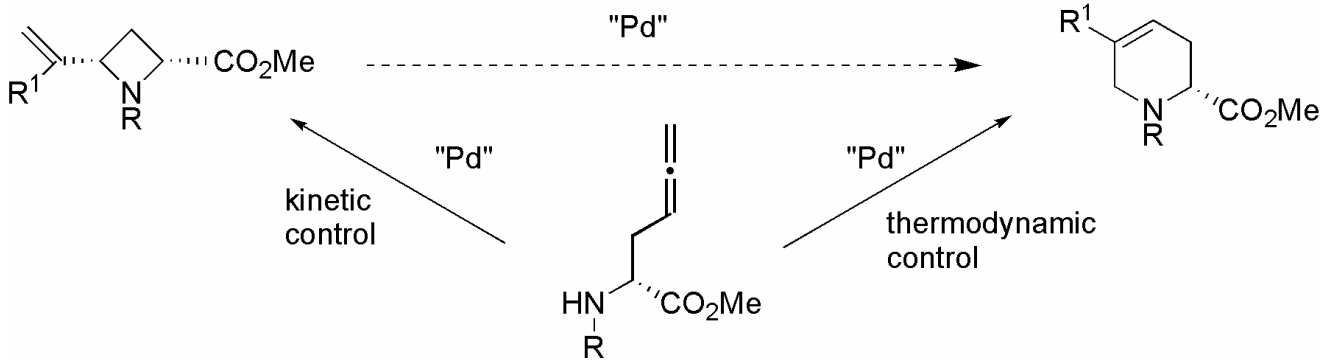
Synthesis of conformationally restricted cyclic amino acids



Rutjes, Schoemaker, *Tetrahedron Lett.* **1997**, *38*, 677; see also: *Adv.Synth.Catal*. **2002**, *344*, 736



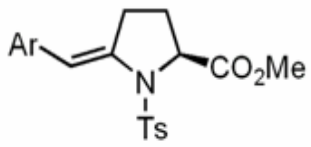
Wolf *et al*, *Tetrahedron Lett.* **1998**, *39*, 5081; *Adv.Synth.Catal*. **2002**, *344*, 70



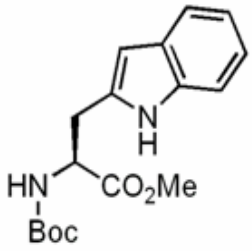
Rutjes, Tjen, Wolf, Karstens, Schoemaker, Hiemstra, *Organic Lett.* **1999**, *1*, 717

See also: Rutjes, Wolf, Schoemaker, *JCS Perkin Trans I*, **2000**, 4197

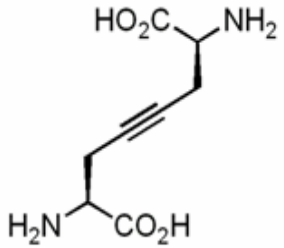
Applications of Acetylenic Amino Acids



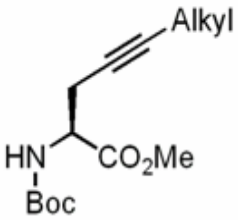
Adv Synth Catal **2002**, *344*, 70



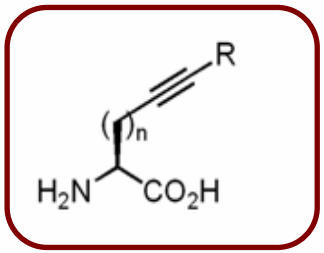
Org Lett **2003**, *5*, 1717

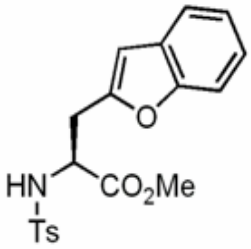


J Org Chem **2001**, *66*, 3584

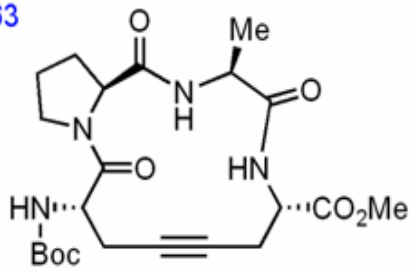


Amino Acids **2003**, *24*, 263

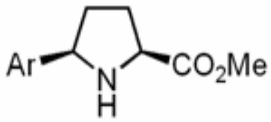




Adv Synth Catal **2004**, *346*, 823



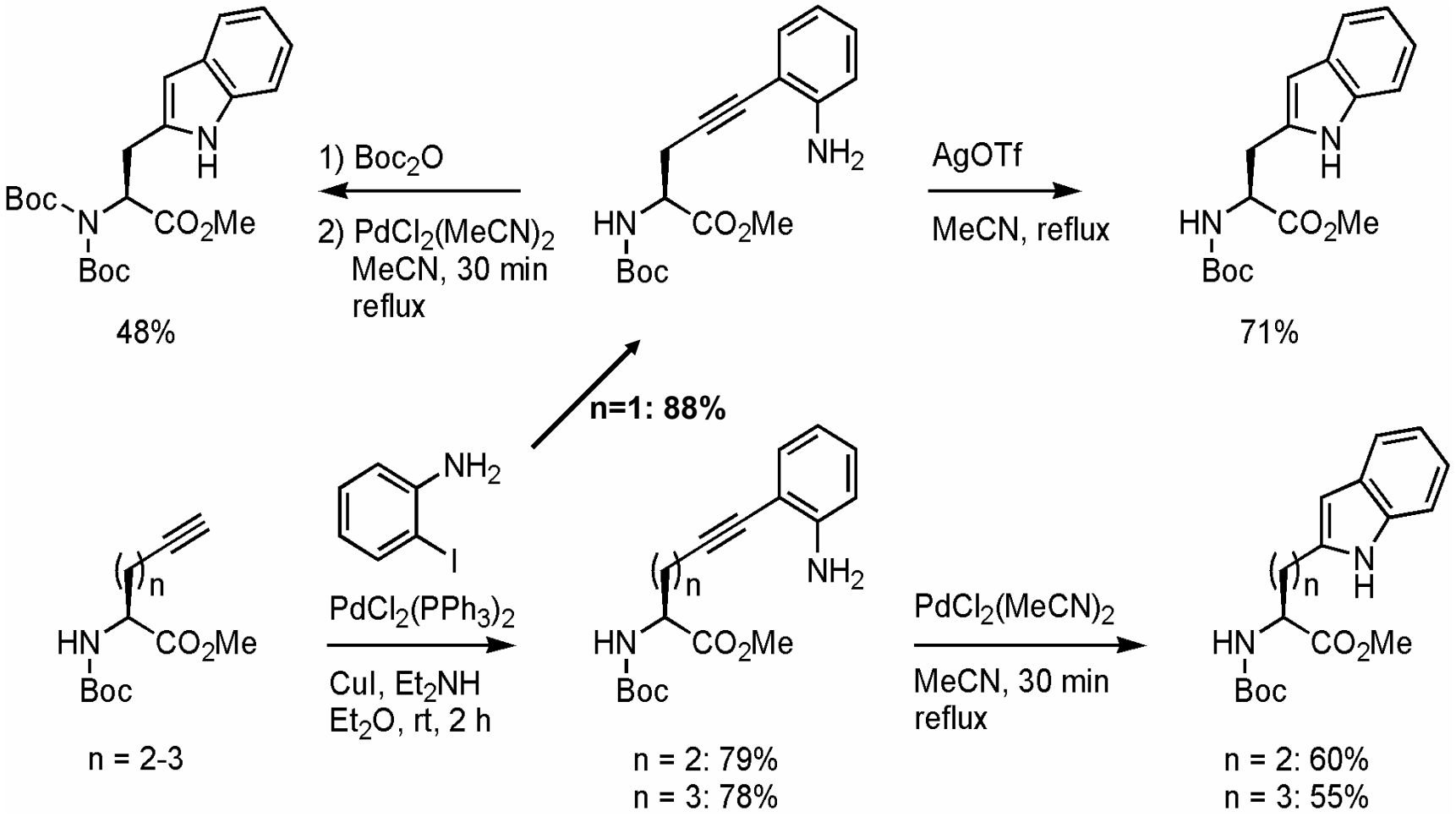
Tetrahedron Lett **2004**, *45*, 4379



J Org Chem **2005**, *70*, 1791

For applications of unsaturated amino acids, see: J Kaiser, SS Kinderman, BCJ van Esseveldt, FL van Delft, HE Schoemaker, RH Blaauw, FPJT Rutjes *Org Biomol Chem* **2005**, *3*, 3435–3467.

Isotryptophan Analogues



BCJ van Esseveldt, FL van Delft, R de Gelder, FPJT Rutjes., *Org. Lett*. **2003**, *5*, 1717

BCJ van Esseveldt, FL van Delft, JMM Smits, R de Gelder, HE Schoemaker, FPJT Rutjes *Adv. Synth. Catal*, **2004**, *346*, 823-834

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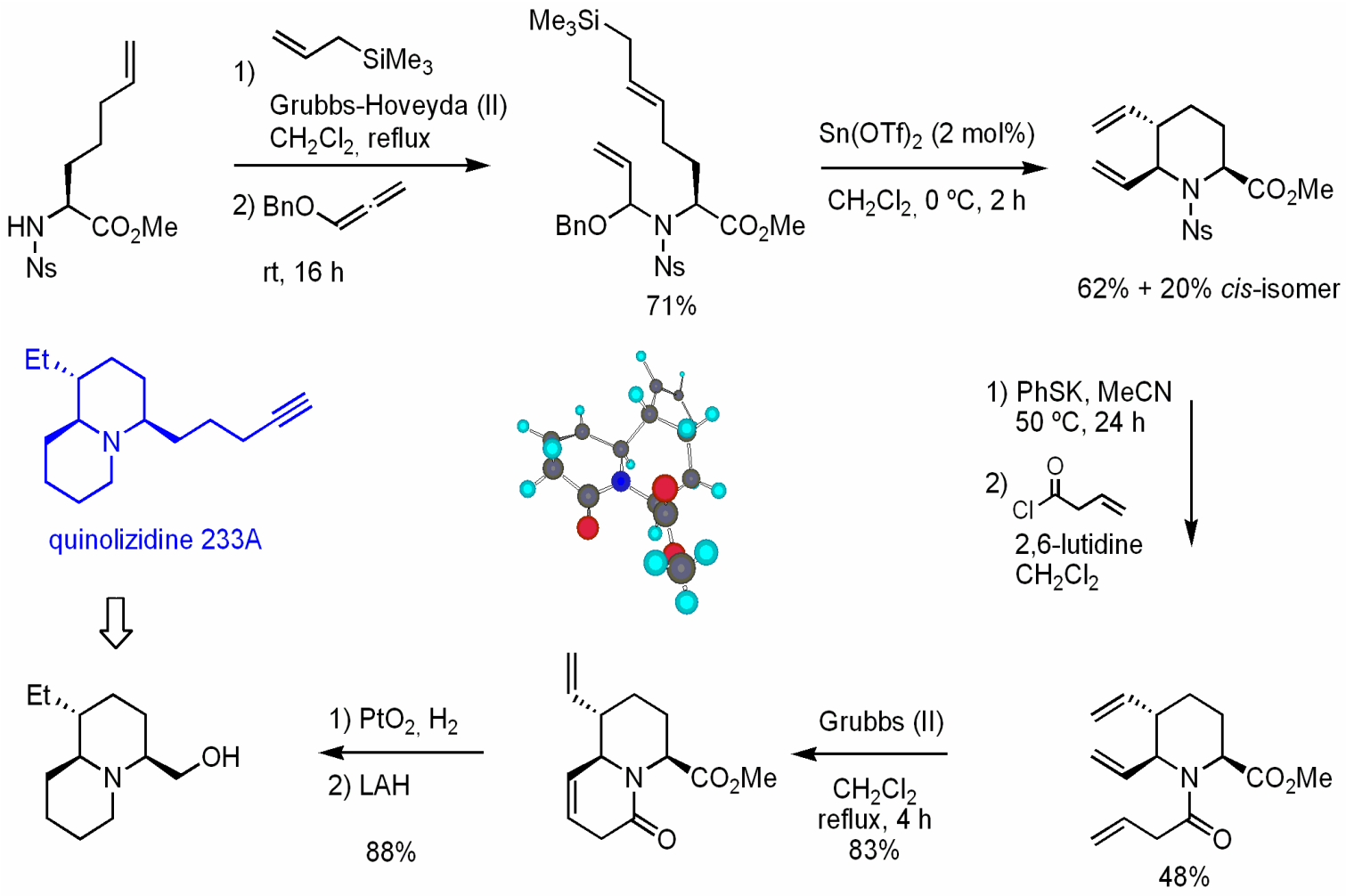
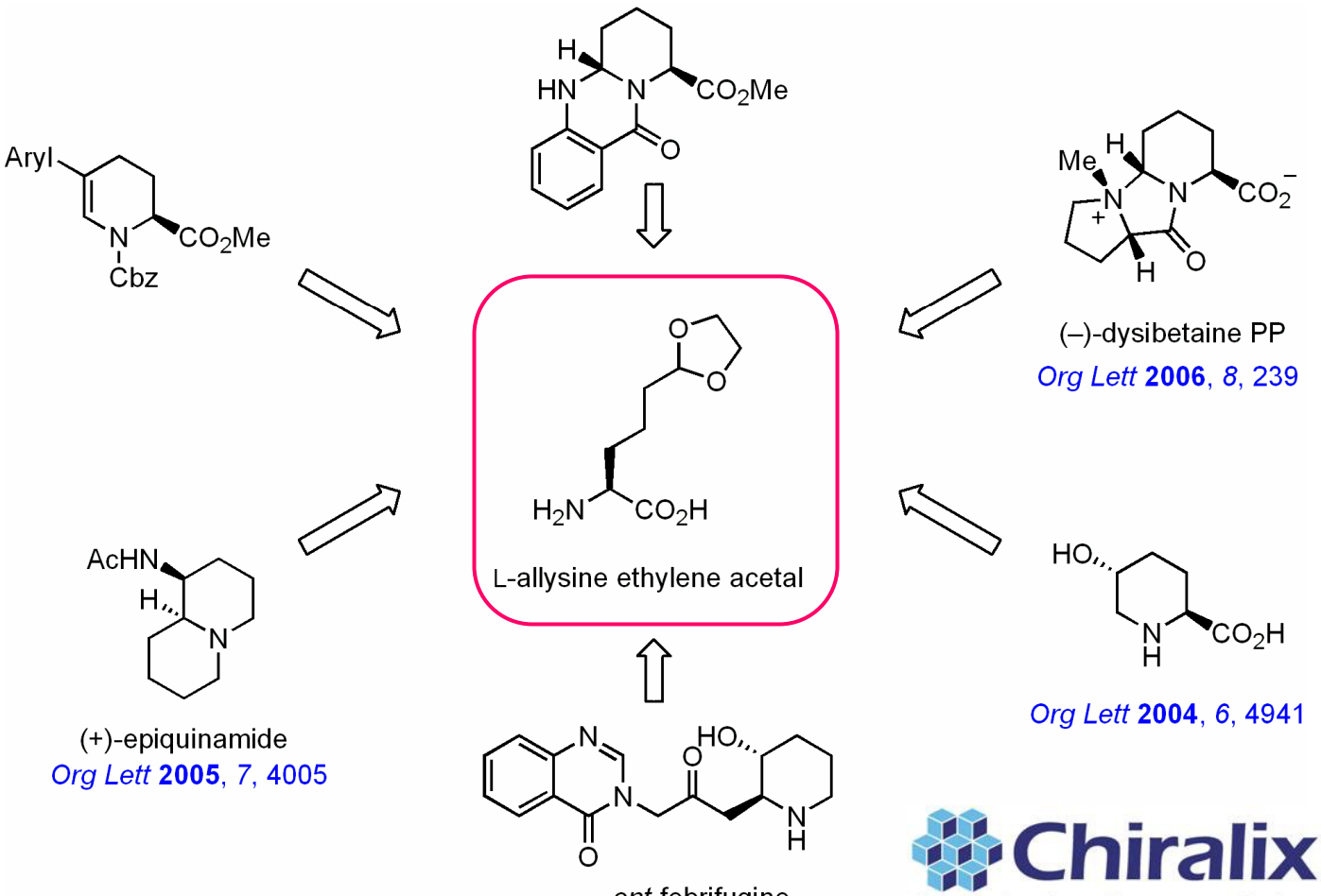
Formal Synthesis of Quinolizidine 233A



SS Kinderman, R de Gelder, JH van Maarseveen, HE Schoemaker, H Hiemstra, FPJT Rutjes, *J. Amer. Chem. Soc.* **2004**, *126*, 4100-4101.

Applications of L-Allysine Ethylene Acetal



(–)-dysibetaine PP
Org Lett **2006**, *8*, 239

(+)-epiquinamide
Org Lett **2005**, *7*, 4005

Org Lett **2004**, *6*, 4941

ent-febrifugine

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L-allysine ethylene acetal; one of the RESCOM *ChiraliTree*® products

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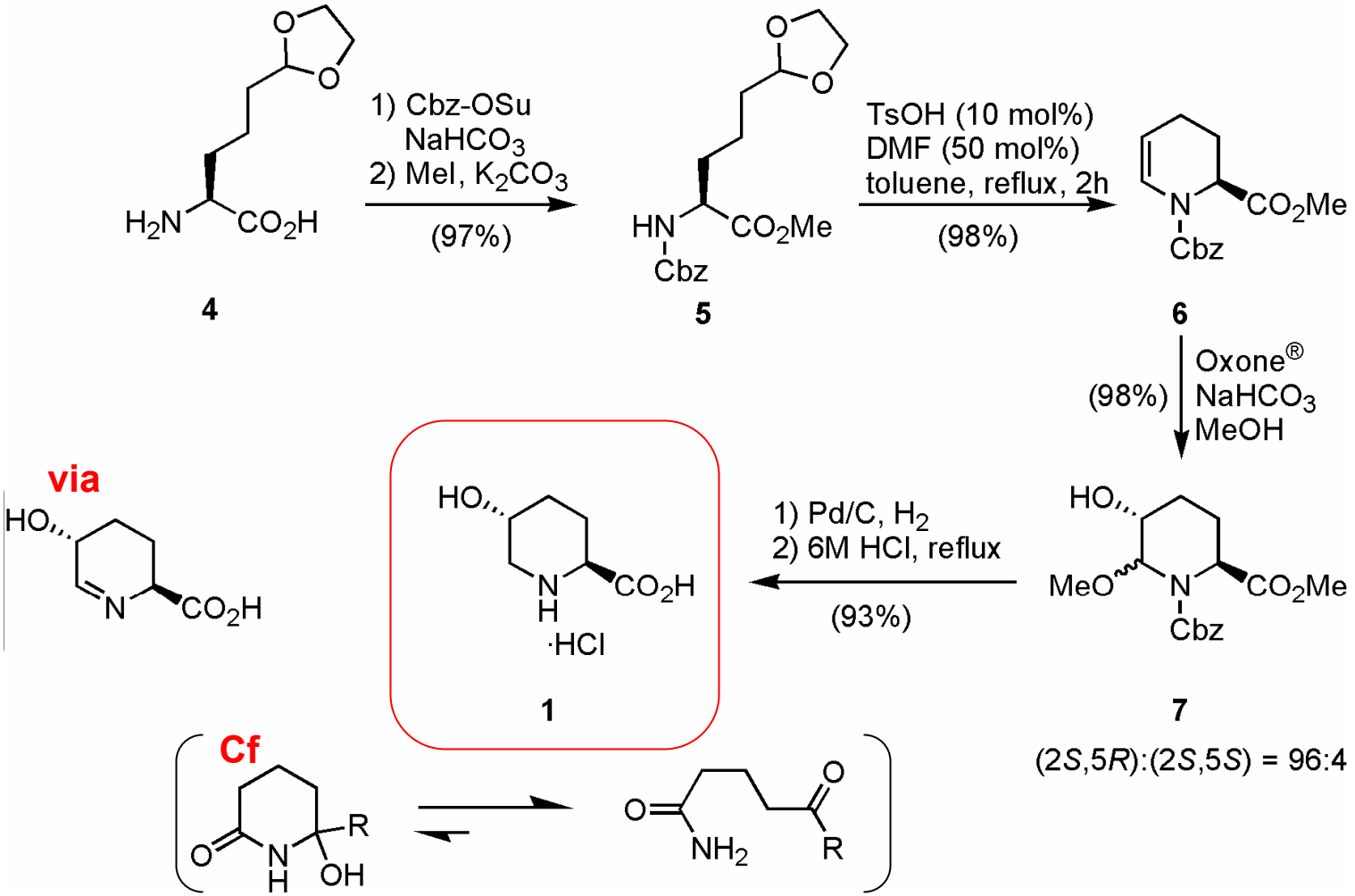
Synthesis of 5-hydroxy pipecolic acid



P Botman, HE Schoemaker, QB Broxterman, RH Blaauw, FL van Delft, FPJT Rutjes, Organic Letters **2004**, *6*, 4941-4944

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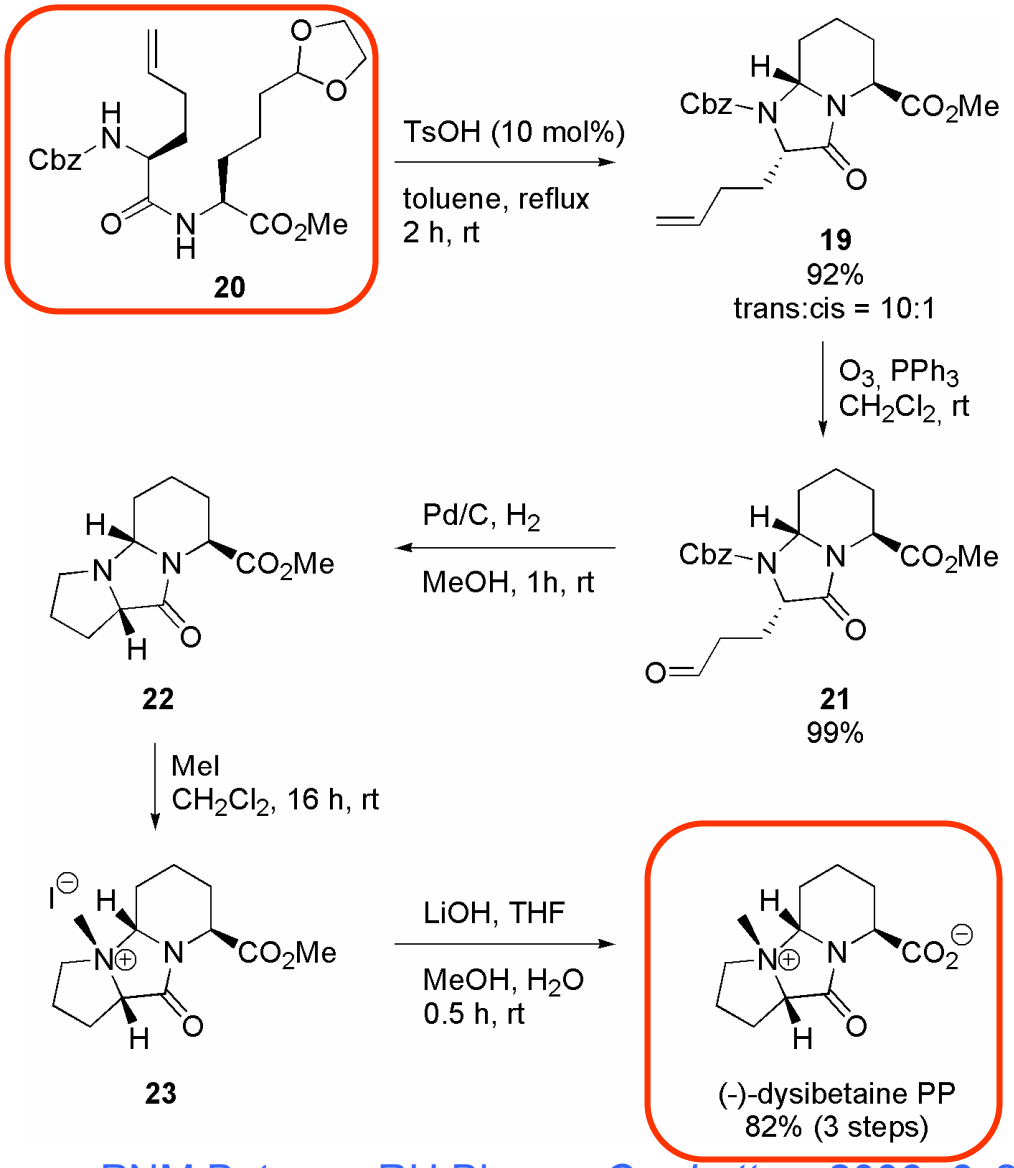
Synthesis of (-)-dysibetaine PP starting from homoallylglycine and allysine ethylene acetal



(-)dysibetainePP has been isolated from the Micronesian Sponge *Dysidea herbacea*

Sakai *et al*, J.Org.Chem 2004, *69*, 1180-1185

DR IJzendoorn, PNM Botman, RH Blaauw, *Org Letters* **2006**, *8*, 239.

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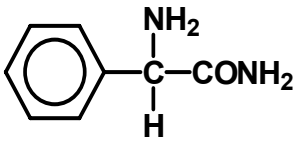
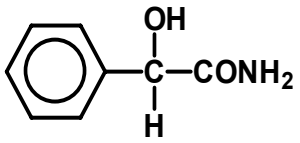
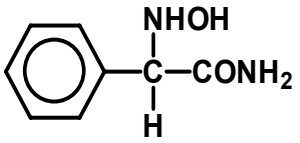
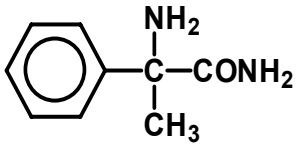
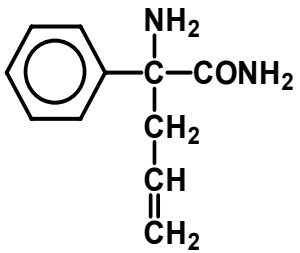
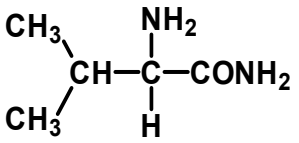
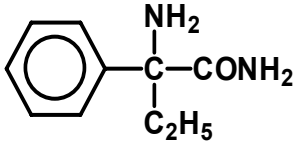
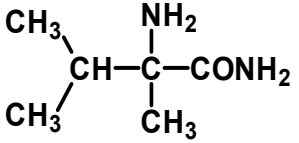
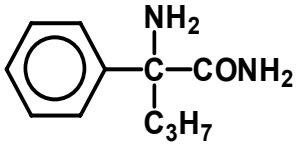
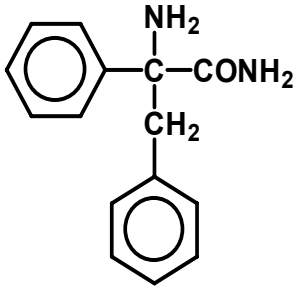
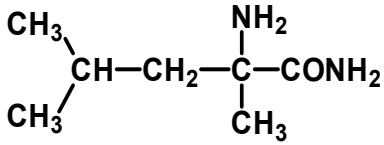
Ochrobactrum anthropi NCIMB 40321 cells: broad spectrum L-specific amidase activity

Substrate	Act.	Substrate	Act.	Substrate	Act.
 DL-phenylglycine amide	100	 DL-mandelic acid amide	5	 DL-*N*-hydroxyphenylglycine amide	25
 DL-α-methylphenylglycine amide	2	 DL-α-allylphenylglycine amide	4	 DL-valine amide	25
 DL-α-ethylphenylglycine amide	4			 DL-α-methylvaline amide	5
 DL-α-n-propylphenylglycine amide	1	 DL-α-benzylphenylglycine amide	0	 DL-α-methylleucine amide	15

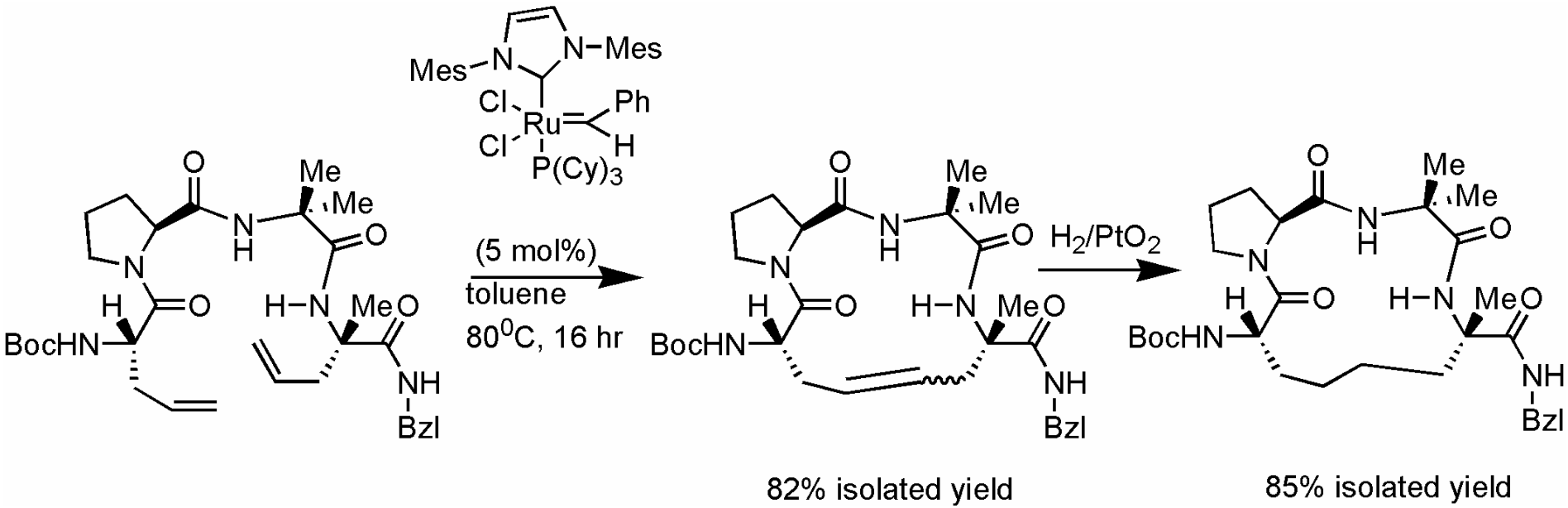
Whole cell transformations WJJ vd Tweel *et al*, *Appl Microbiol Biotechnol* **1993**, *39*, 296

Single enzyme activity *Sonke et al*, *Appl Environm Biotechnol* **2005**, *71*, 7961

DSM Unlimited.

Ring-closing metathesis reaction on β-turn mimic

Type II β-turn mimic: Boc-**L-Alg**-L-Pro-Aib-**L-Mag**-NH-CH_2Ph



- Reaction with Grubbs catalyst at higher temperature
- Inseparable mixture of *cis* en *trans* isomers
- Quantitative hydrogenation of the double bond

B Kaptein, QB Broxterman, HE Schoemaker, FPJT Rutjes, JJN Veerman, J Kamphuis, C Peggion, F Formaggio, C Toniolo *Tetrahedron* **2001**, *57*, 6567

with University of Padova, Italy

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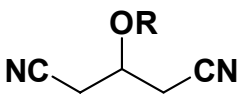
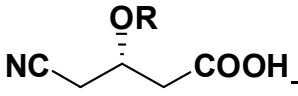
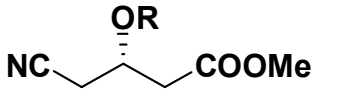
100% yield, 100% ee concepts
Desymmetrization of prochiral dinitriles

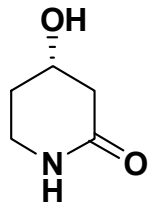


nitrilase activity of

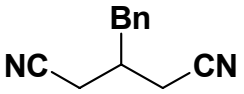
Rhodococcus erythropolis
NCIMB 11540





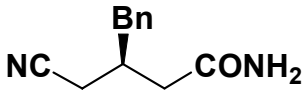


R=H yield 30%, ee 13%
R=Bz yield 21%, ee 63%
R=Bn yield 70%, ee 96%

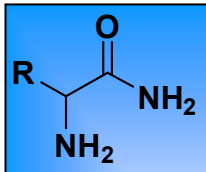


nitrile hydratase activity of

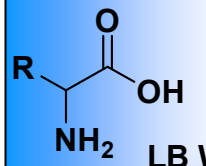
Rhodococcus erythropolis
NCIMB 11540



yield 67%
ee 98%



amidase activity of
Rhodococcus erythropolis
NCIMB 11540

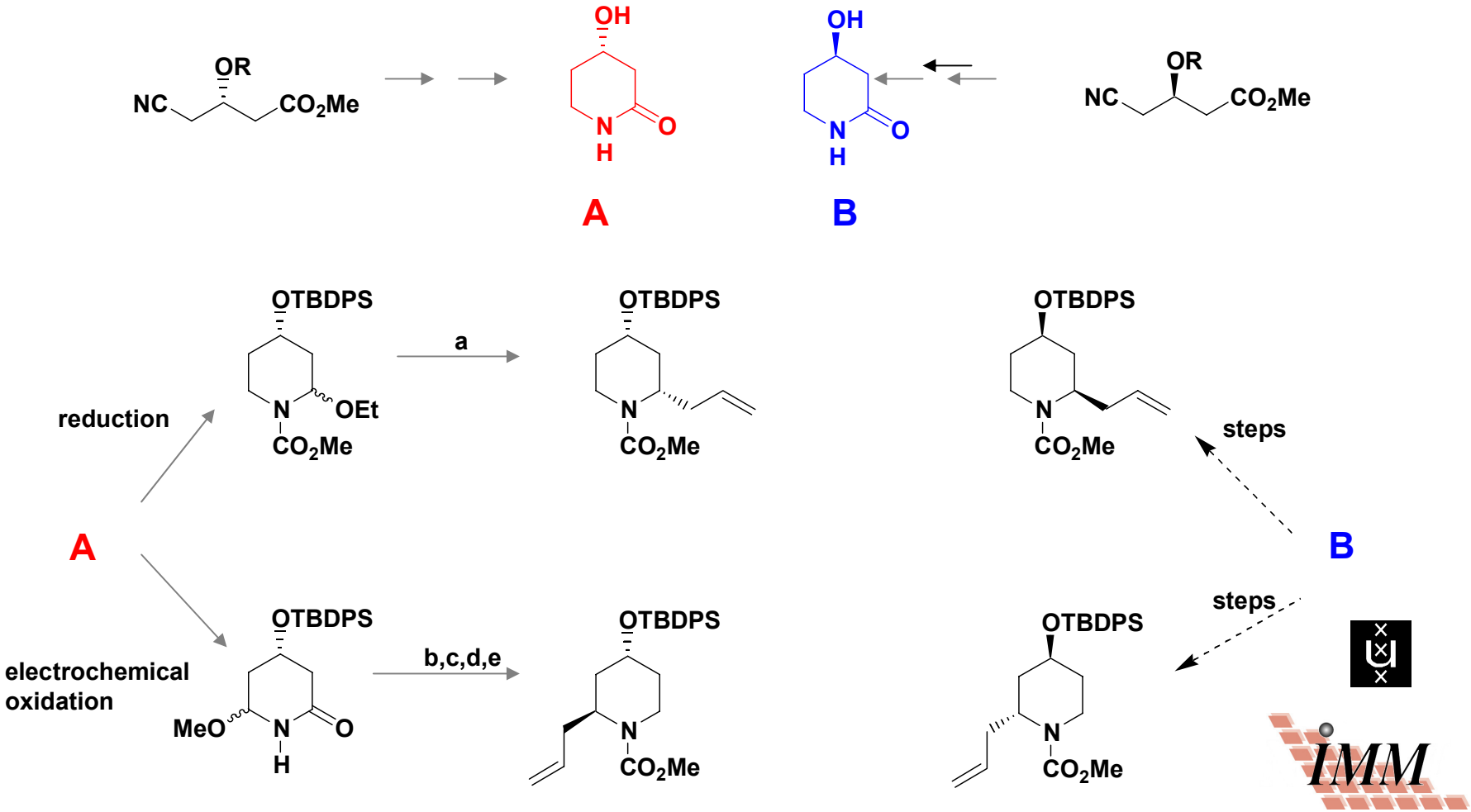


LB Wolf *et al*,
Adv.Synth.Catal.
343, 662 (2001)

WHJ Boesten, MJH Cals
US Patent 4705572 (1987)

cf : Turner and coworkers, Tetr. Asymm. (1992), *3*, 1547
Ohta and coworkers, Chem. Lett. (1991), 1823

A stereodivergent approach to substituted 4-hydroxypiperidines



MKS Vink, CA Schortinghuis, J Luten, JH van Maarseveen, HE Schoemaker, H Hiemstra, FPJT Rutjes *J. Org. Chem*. **2002**, *67*, 7869

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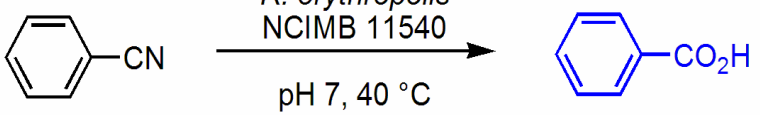
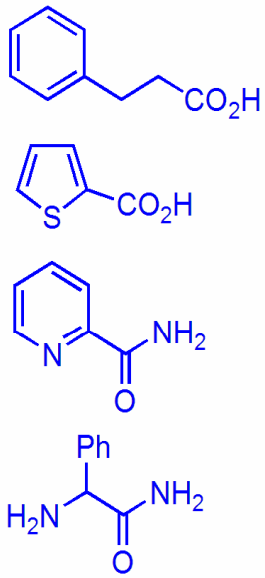
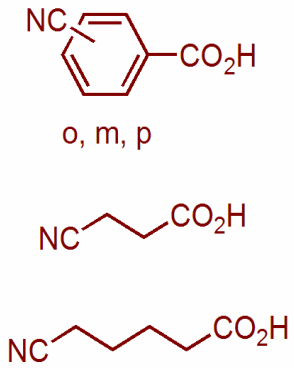
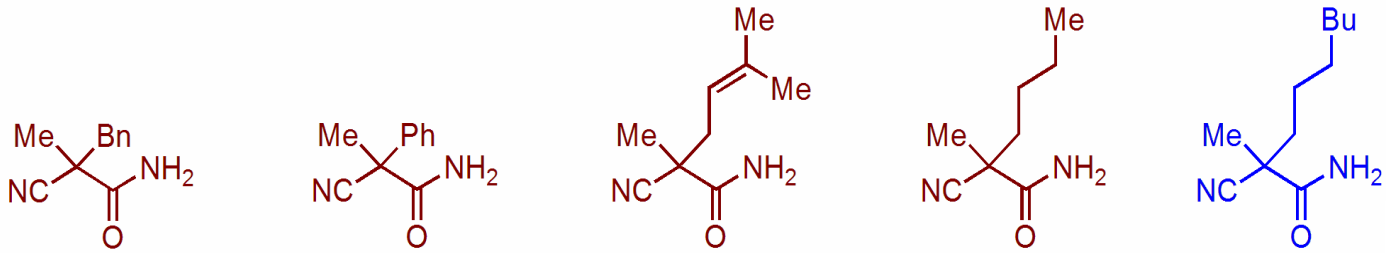
Monohydrolysis Leads to Desymmetrization





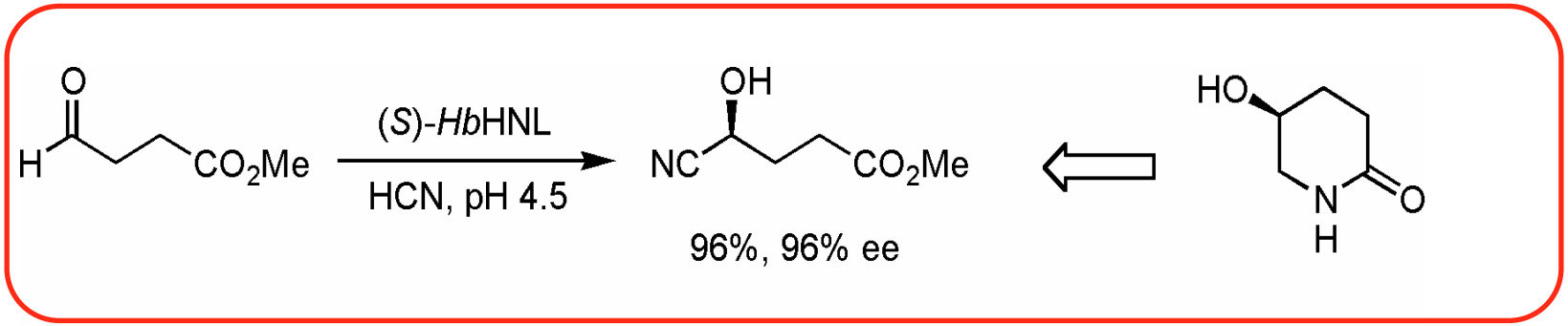


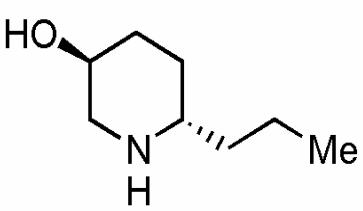


MKS Vink, R Wijtmans, RJF van den Berg, CA Schortinghuis, C Reisinger, H Schwab, HE Schoemaker, FPJT Rutjes, *Biotechnology Journal*, **2006,** *1*, 569-573

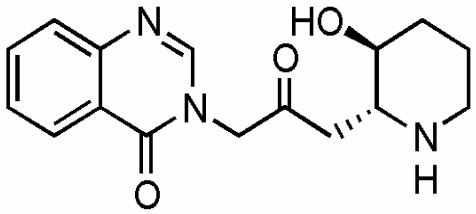
Unlimited. DSM

Towards Constrained β-Amino Alcohols





pseudoconhydrine



febrifugine
exhibits antimalarial activity

MKS Vink, Ph.D. Thesis
University of Amsterdam
31 October 2003

For other applications, see:
MKS Vink, CA Schortinghuis, A Mackova-Zabelinskaja, M Fechter, P Pöchlauer, AMCF Castelijns, JH van Maarseveen, H Hiemstra, H Griengl, HE Schoemaker, FPJT Rutjes
Adv. Synth. Catal. **2002**, *345*, 483

Chiralix innovative drug discovery solutions

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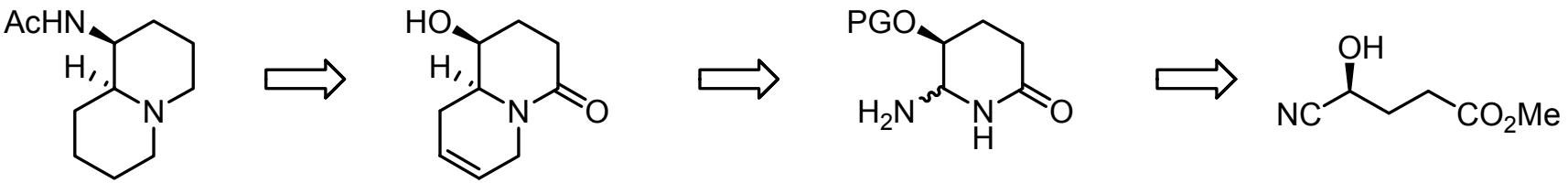
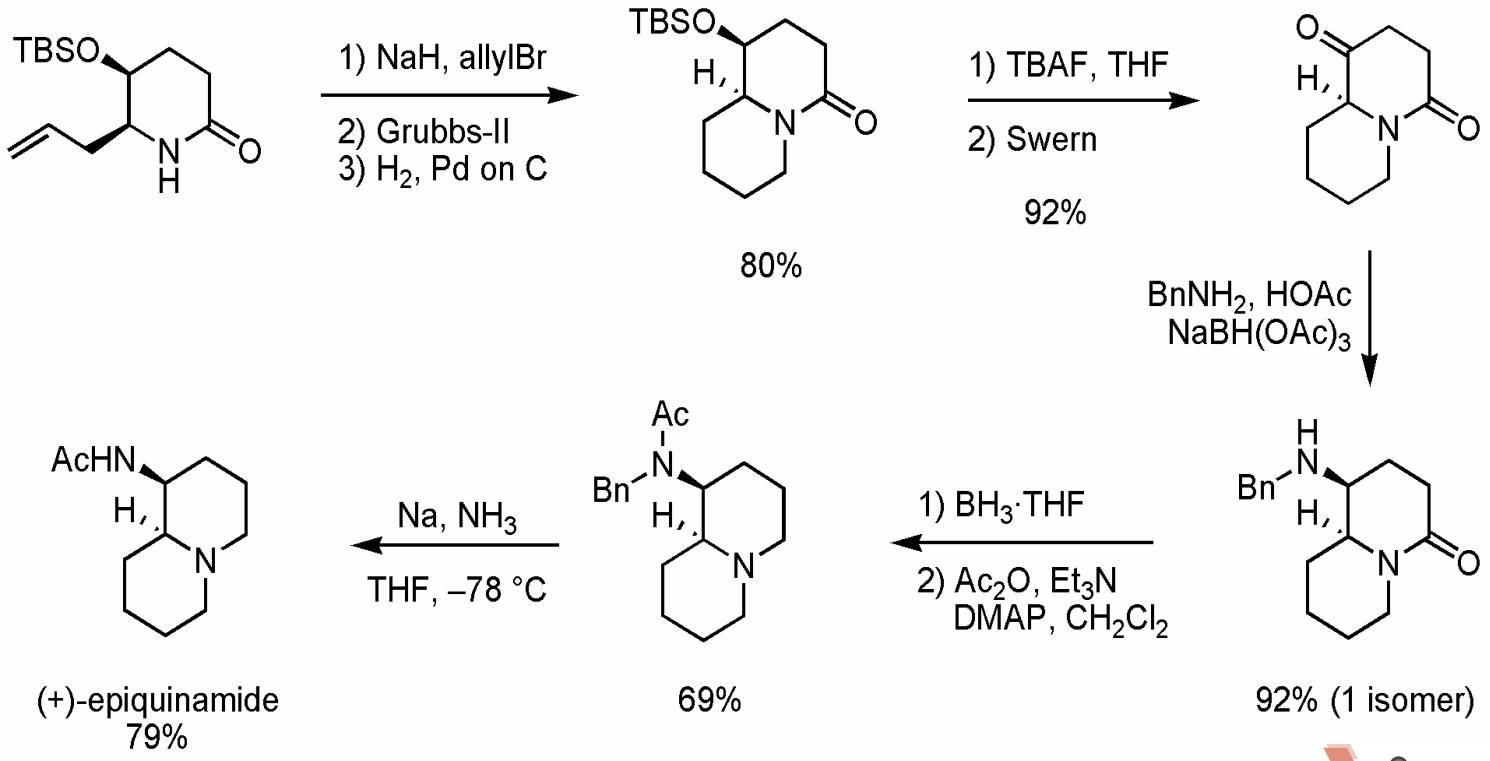
Synthesis of Epiquinamide

- Isolated by Daly from the Ecuadorian frog *Epipedobates tricolor* in 2003
- Represents a new structural class of nicotinic agonists
- Lead for the development of new therapeutics and pharmacological probes for nicotinic receptors.
- Amount of 240 µg was obtained from 183 frogs
- Absolute configuration recently established by Chiralix



RW Fitch, HM Garraffo, TF Spande, HJC Yeh, JW Daly, *J Nat Prod* **2003,** *66,* 1345.

Total Synthesis of (+)-Epiquinamide



- Both enantiomers have been prepared
- Absolute configuration is determined (collaboration with Daly, NIH)
- Series of analogues is being prepared to establish SAR

R Wijtmans; for a different pathway, see:
MA Wijdeven, PNM Botman, R Wijtmans,HE Schoemaker, FPJT Rutjes, RH Blaauw, *Org Lett* **2005**, *7*, 4005.

IMM

Chiralix
innovative drug discovery solutions

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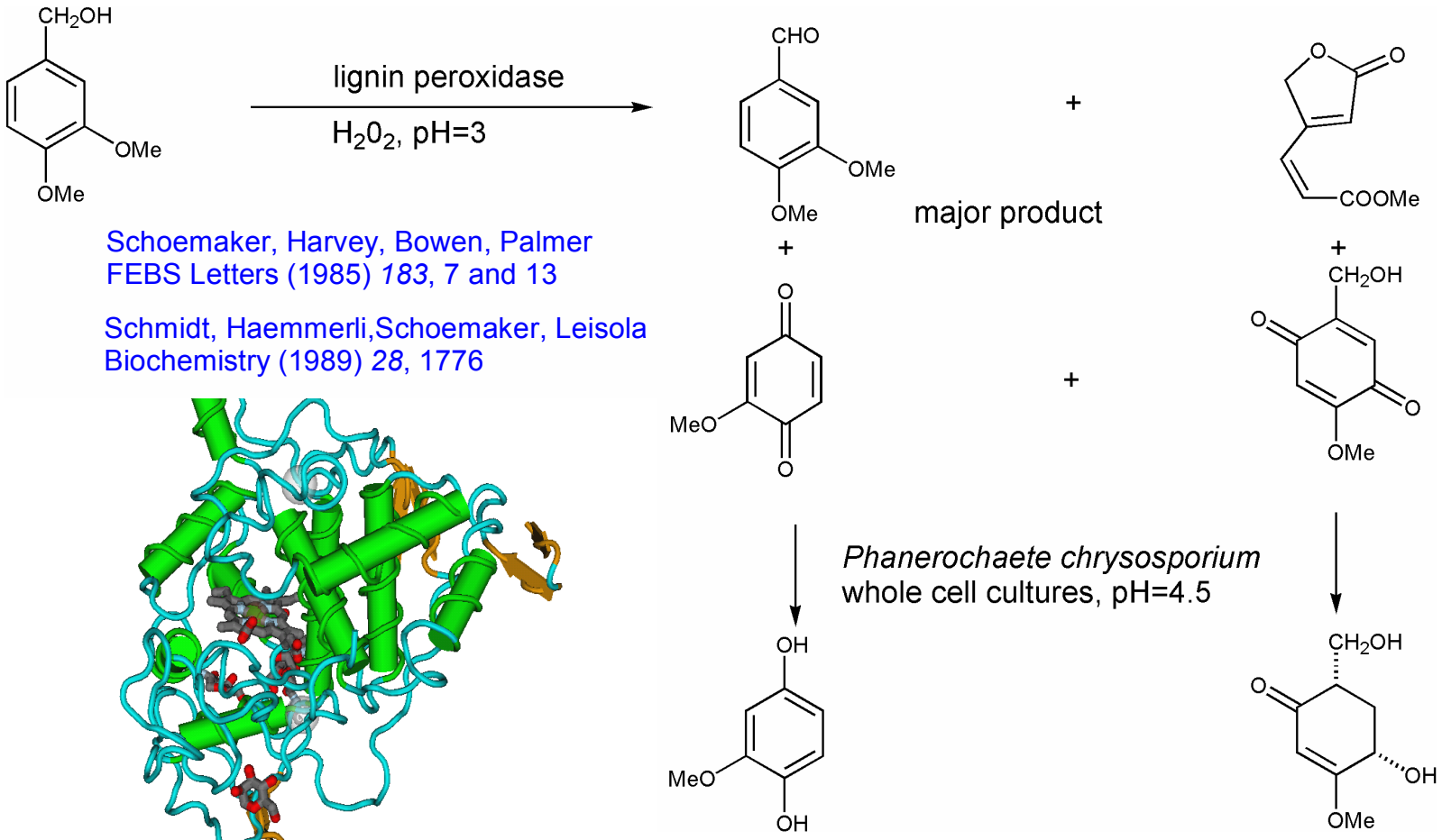
Redox enzymes involved in lignin catabolism



Schoemaker, Harvey, Bowen, Palmer
FEBS Letters (1985) *183*, 7 and 13

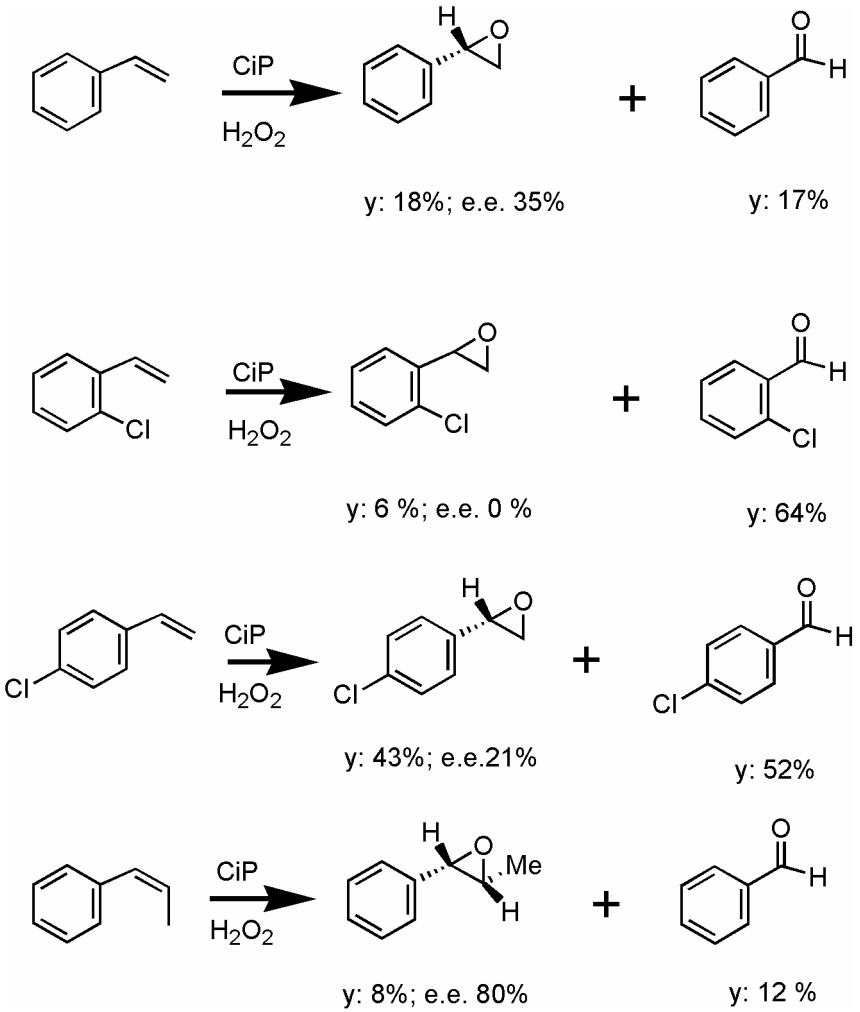
Schmidt, Haemmerli,Schoemaker, Leisola
Biochemistry (1989) *28*, 1776

Piontek, Glumoff, Winterhalter,
FEBS Letters (1993) *315*, 119

Tuor, Schoemaker, Leisola, Schmidt
Appl. Microbiol. Biotechnol. (1993) *38*, 674

Unlimited. DSM

Enantioselective epoxidation and C-C bond cleavage catalyzed by *Coprinus cinereus* peroxidase



Coprinus cinereus peroxidase (CiP)

Generous gift from NOVO Nordisk

CiP Crystal structure determination based on LiP crystal structure

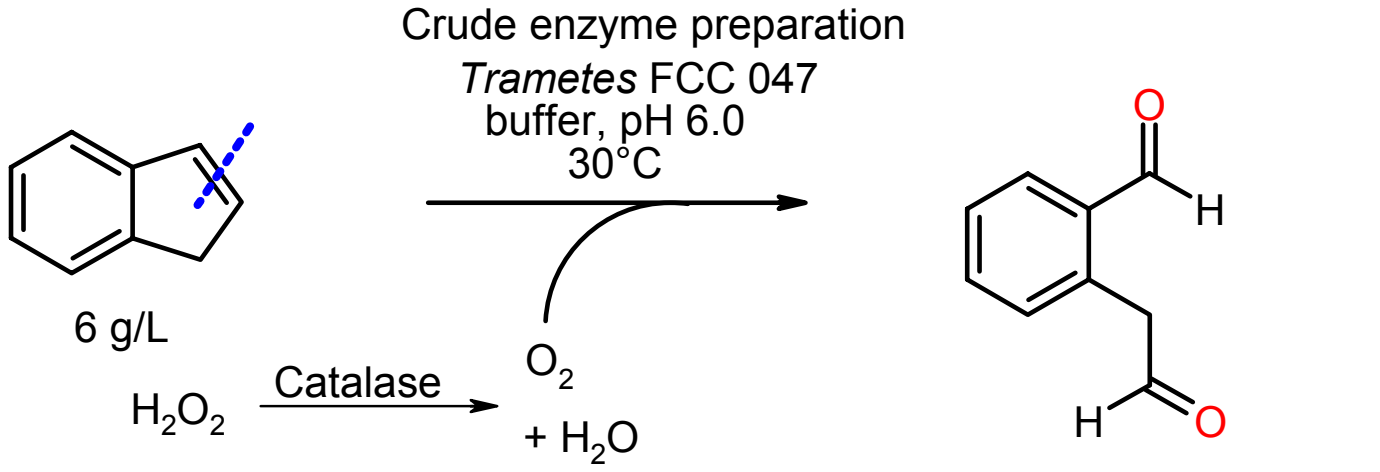
Petersen *et al*, FEBS Lett **1994**, *339*, 291-296

Lip and CiP: Enzyme mechanisms totally different

Mutants may be obtained via directed evolution techniques, see Cherry *et al*, Nature Biotechnology (**1999**), 4, 379-384

A Tuynman, J.L. Spelberg, I.M. Kooter, H.E. Schoemaker and R. Wever, J. *Biol Chem*, **2000**, *5*, 3025-3030

An Enzymatic equivalent to reductive Ozonization by Lyophilized cells of Trametes hirsuta GFCC047



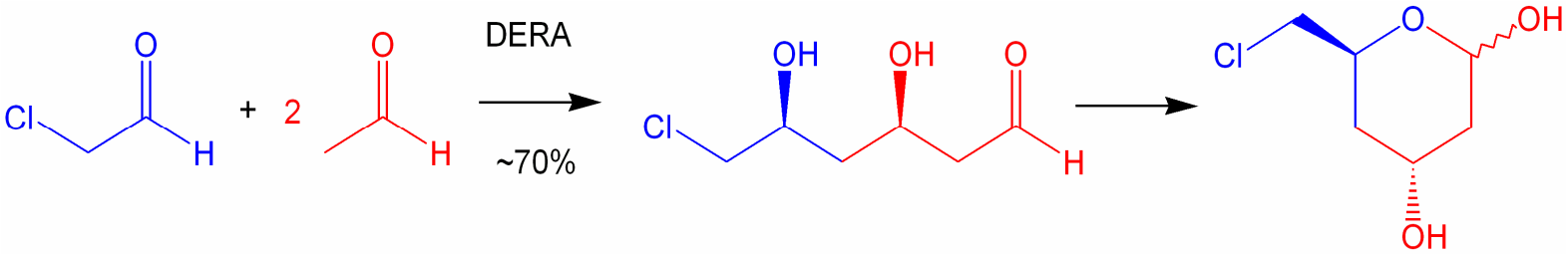
71% conversion,
60% chemoselectivity

Cf H Mang, J Gross, M Lara, C Goessler, HE Schoemaker GM Guebitz and W Kroutil, Angewandte Chemie, *Int Ed*, **2006**, *45*, 5201-5203

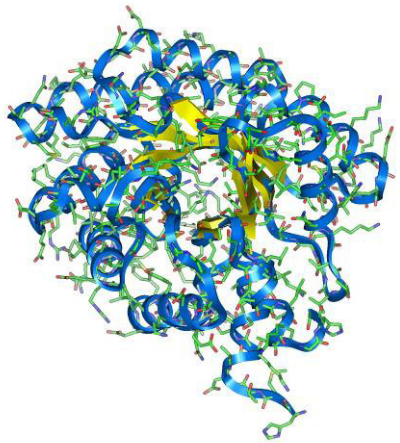
Professor Wolfgang Kroutil and coworkers,

Department of Chemistry, Organic and Bioorganic Chemistry,
Research Centre Applied Biocatalysis, University of Graz (Austria)

DERA catalyzed C-C bond formation



- 2-Deoxyribose-5-phosphate aldolase (DERA, EC 4.1.2.4) catalyzes the aldol reaction of acetaldehyde and D-glyceraldehyde-3-phosphate to form 2-deoxyribose-5-phosphate.
- DERA is a unique aldolase in that it can use two aldehydes both as the aldol donor and acceptor
- DERA can do two consecutive aldol condensations resulting in 2,4-dideoxyhexoses. These products cyclize to a stable hemiacetal

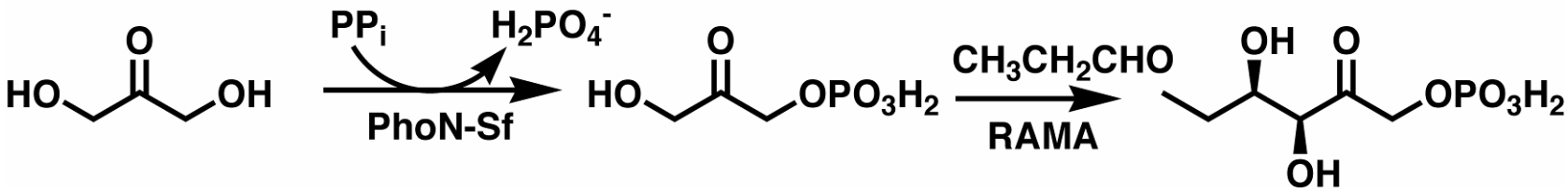


Cf HJ Gijsen and C-H Wong, J.Amer.Chem Soc (1995), 117, 2947
A Heine, G Desantis, JG Lutz, M. Mitchell, C-H Wong IA Wilson. (2001) Science,294, 369

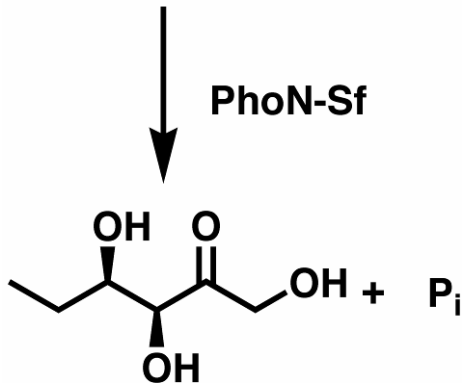
See for directed evolution of the DERA enzyme and a novel application:
S Jennewein, M Schürmann, M Wolberg, I Hilker, R Luiten, M Wubbolts and D Mink *Biotechnology J.* 2006, *1*, 537-548

Expanding the scope of aldolase catalyzed reactions

- Can we phosphorylate dihydroxyacetone with a bacterial acid phosphatase using PP_i (pyrophosphate) as cheap phosphate donor?
- Yes, phosphorylation is possible, product identical to commercially available DHAP
- Instead of isolating DHAP, can we use it in a one-pot aldolase catalyzed reaction (with rabbit muscle aldolase) ?



- Aldol adduct still phosphorylated, but subsequently dephosphorylated by PhoN-Sf (Efficient cascade reaction)



Teunie van Herk, AF Hartog, HE Schoemaker, R Wever
J. Org. Chem, **2006** , *71*, 6244-6247

Conclusions

- **Biocatalysis is an established technology and a valuable addition to the synthetic toolbox**

  See: HE Schoemaker, D Mink, MG Wubbolts
  Science, **2003**, *299*, 1694 Catalysis Viewpoint:
  Dispelling the Myths--Biocatalysis in Industrial Synthesis

- **Synergy with transition metal catalysis leads to a broad variety of enantiopure multifunctional building blocks and natural products**

  E.g.: J Kaiser, SS Kinderman, BCJ van Esseveldt, FL van Delft,
  HE Schoemaker, RH Blaauw, FPJT Rutjes
  Org Biomol Chem **2005**, *3*, 3435–3467.

- **Collaboration between industry and academia leeds to synergistic results**

- **Both CHIRALIX and DSM (RESCOM *ChiraliTree*®) provide many opportunities for using chirality in drug discovery**