Sustainable Production of Advanced Intermediates and API's Using Bio- and Homogeneous Catalysis

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Outline

1. DSM - Company profile and history
2. API Synthesis in drug development – Technology leadership
3. API synthesis examples@DSM
    a. Almorexant (Catalytic Asymmetric Hydrogenation)
    b. Statins (Aldolase)
4. Acknowledgements

Company Profile and History

Successful transformation from Coal mining to a global Life Sciences & Material Sciences company

Coal Mining	Commodity Chemicals	Specialty Chemicals	Life Sciences & Materials Sciences

Driving focused growth!

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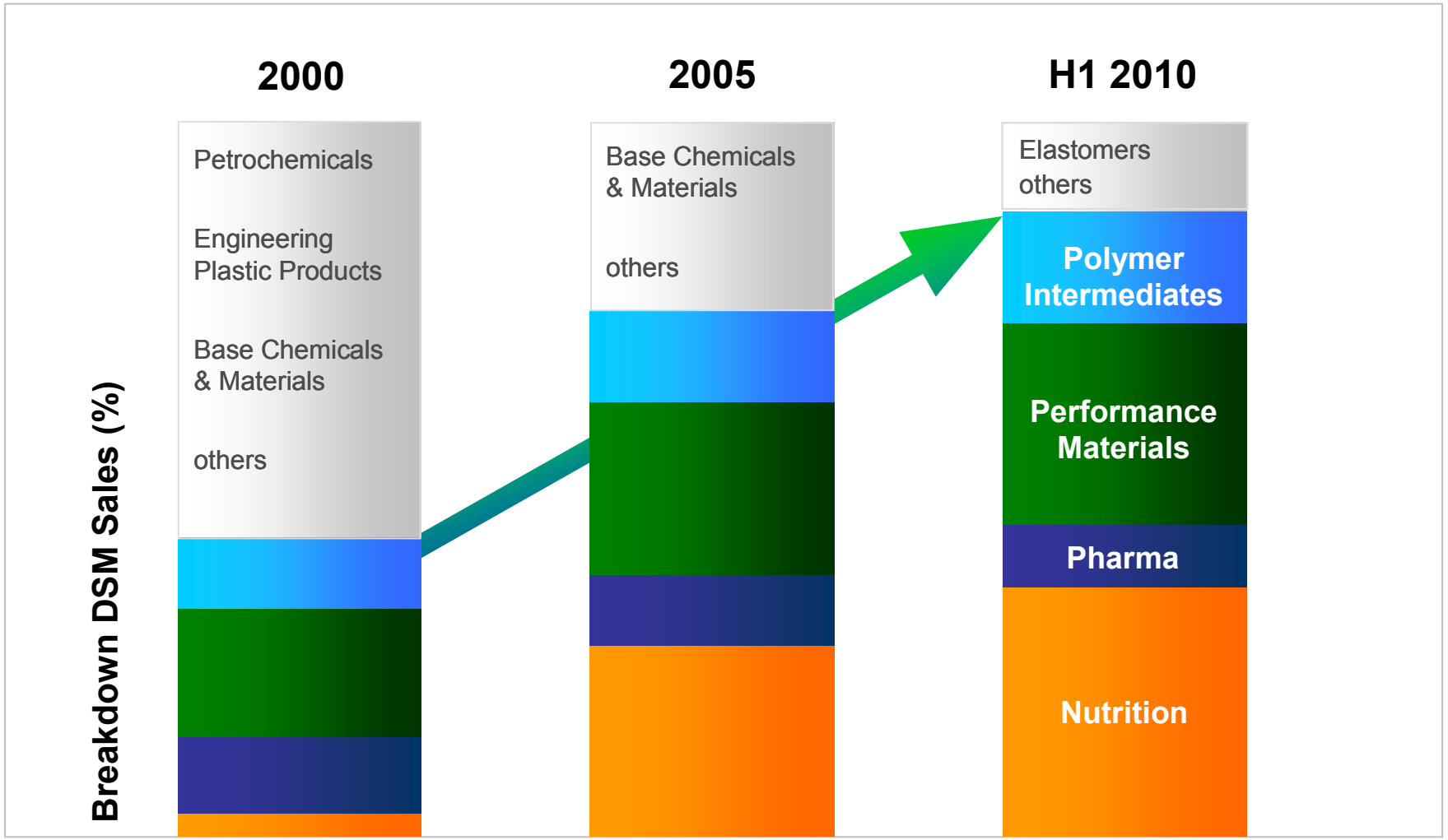
DSM transformation of last 10 years



Annual sales in 2011 was ~9 billion Euro. End 2011, ~22.000 Employees

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Current organization

LIFE SCIENCES

Nutrition

- DSM Nutritional Products
- DSM Food Specialties

Pharma

- DSM Pharma-ceutical Products
- DSM Sinochem Pharmaceuticals

EBA

MATERIAL SCIENCES

Performance Materials

- DSM Resins
- DSM Engineering Plastics
- DSM Dyneema

Polymer Intermediates

- DSM Fibre Intermediates

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DSM Pharmaceutical Products (DPP)

Global provider of custom manufacturing and development services

Pharma Chemicals

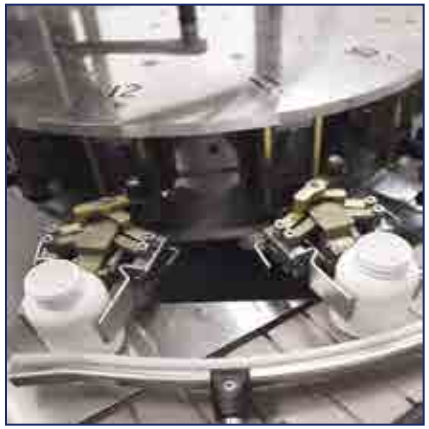
Primary manufacturing of APIs and Intermediates

Biologics

Custom manufacturing of biopharmaceutical ingredients

BioSolutions

Microbial fermentation and associated product recovery



Dosage Forms

Secondary manufacturing of sterile injectables, non-sterile liquids, and oral dosage forms

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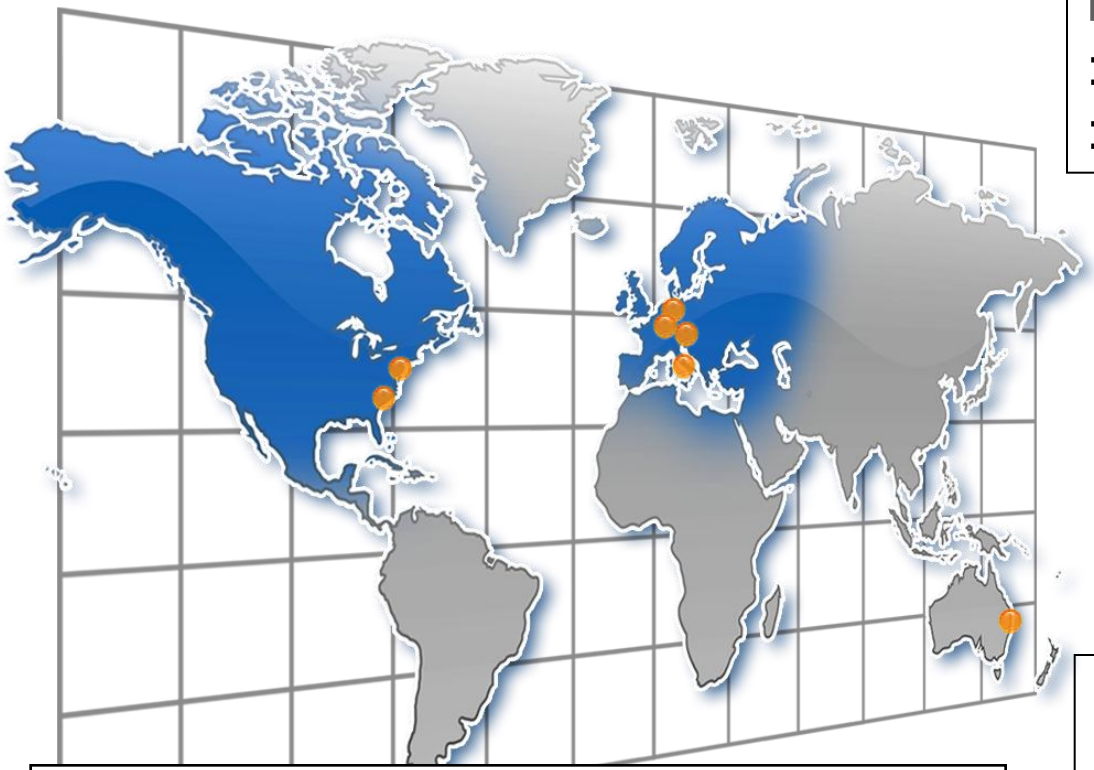
Global Presence of DPP



Biosolutions:
::Capua, Italy
::Delft, The Netherlands

Pharma Chemicals:
::Linz, Austria
::Venlo, Netherlands
::Regensburg,Germany
::Geleen, Netherlands

Biologics:
::Groningen, Netherlands
::Brisbane, Australia

Dosage Forms:
::Greenville NC,
::Parsippany, NJ (DPP Head Quarters)

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Sustainability

"Walking on two legs"

DSM: Leader in Sustainable Manufacturing

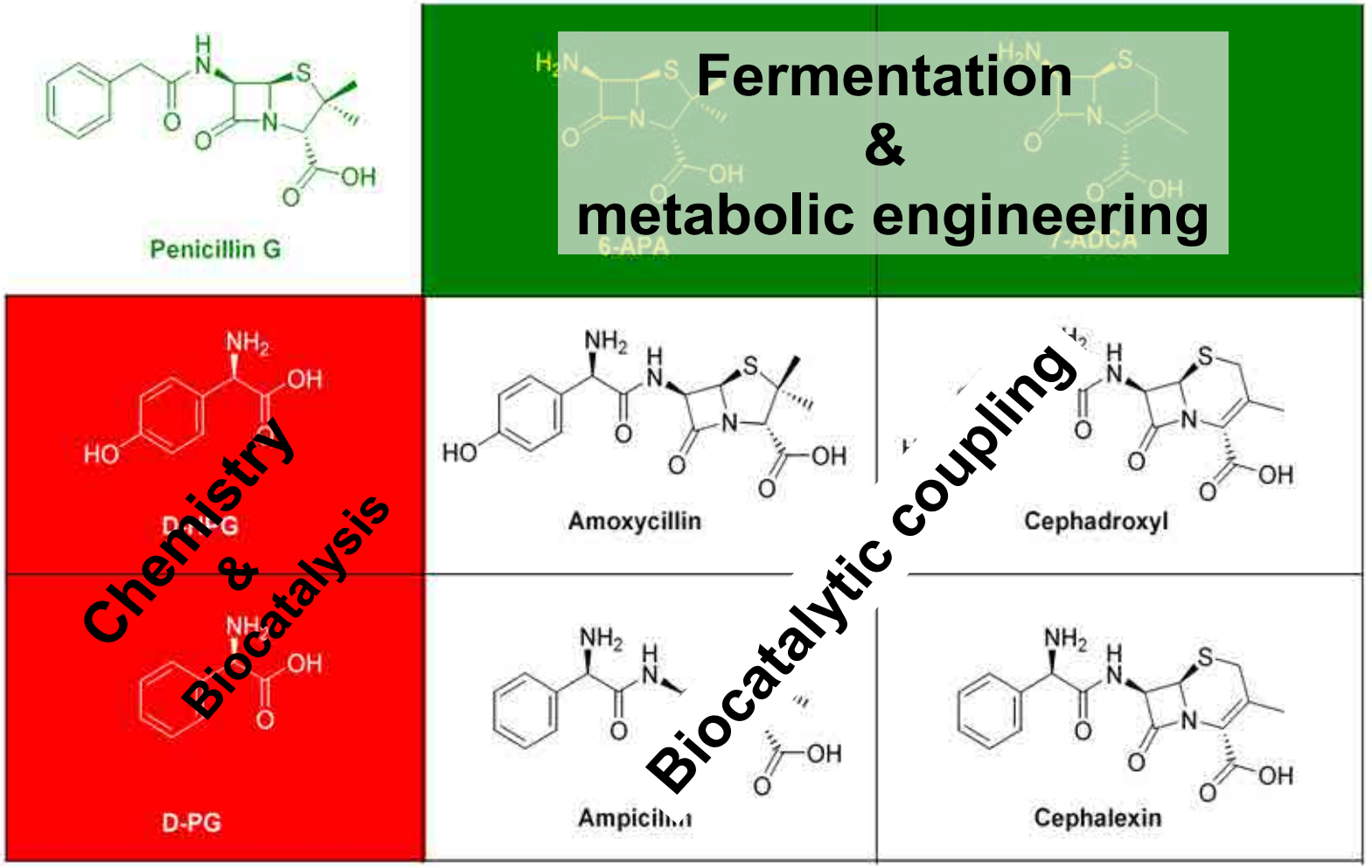
Dow Jones Sustainability Indexes
Member 2008/09

Process Technology
Integrated Process - Low Energy *and* Energy Integration

Renewable Raw Materials

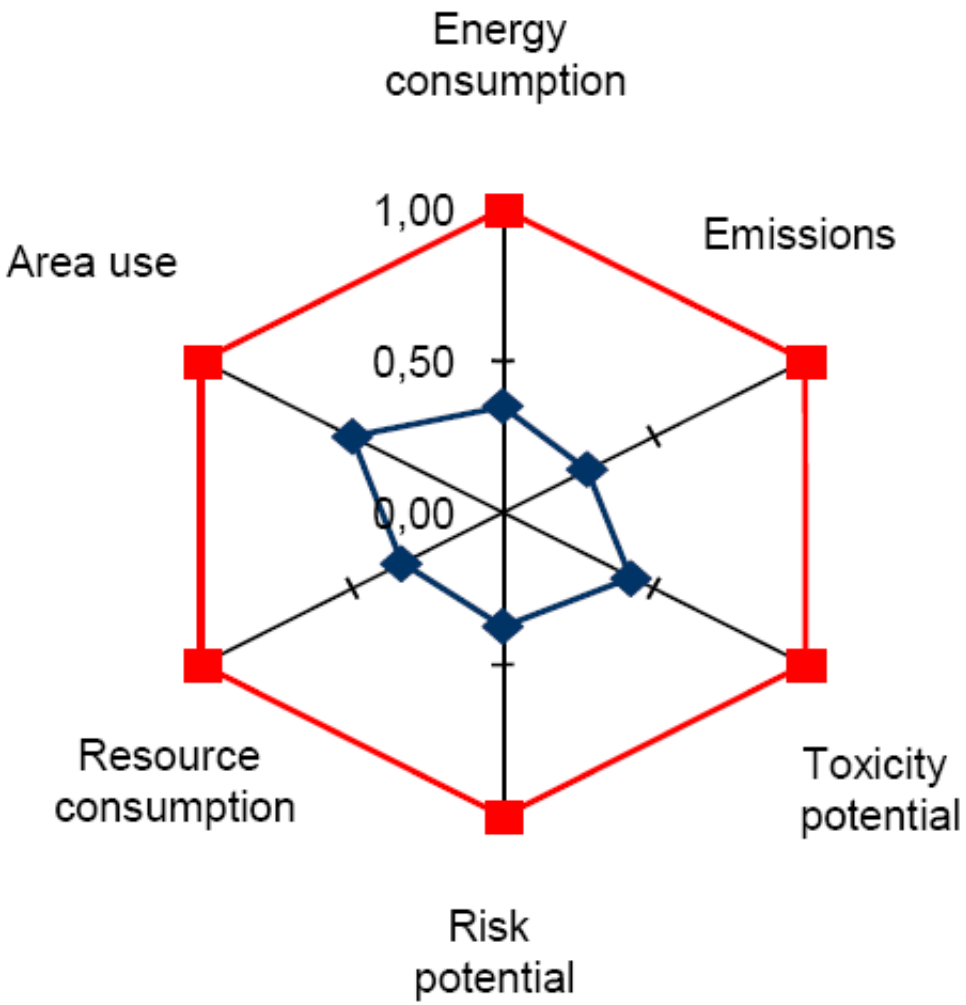
DSM Sinochem Pharmaceuticals

Long history in sustainable production of antibiotics



Sustainability

Comparison of pure chemical route vs “walking on two legs” route



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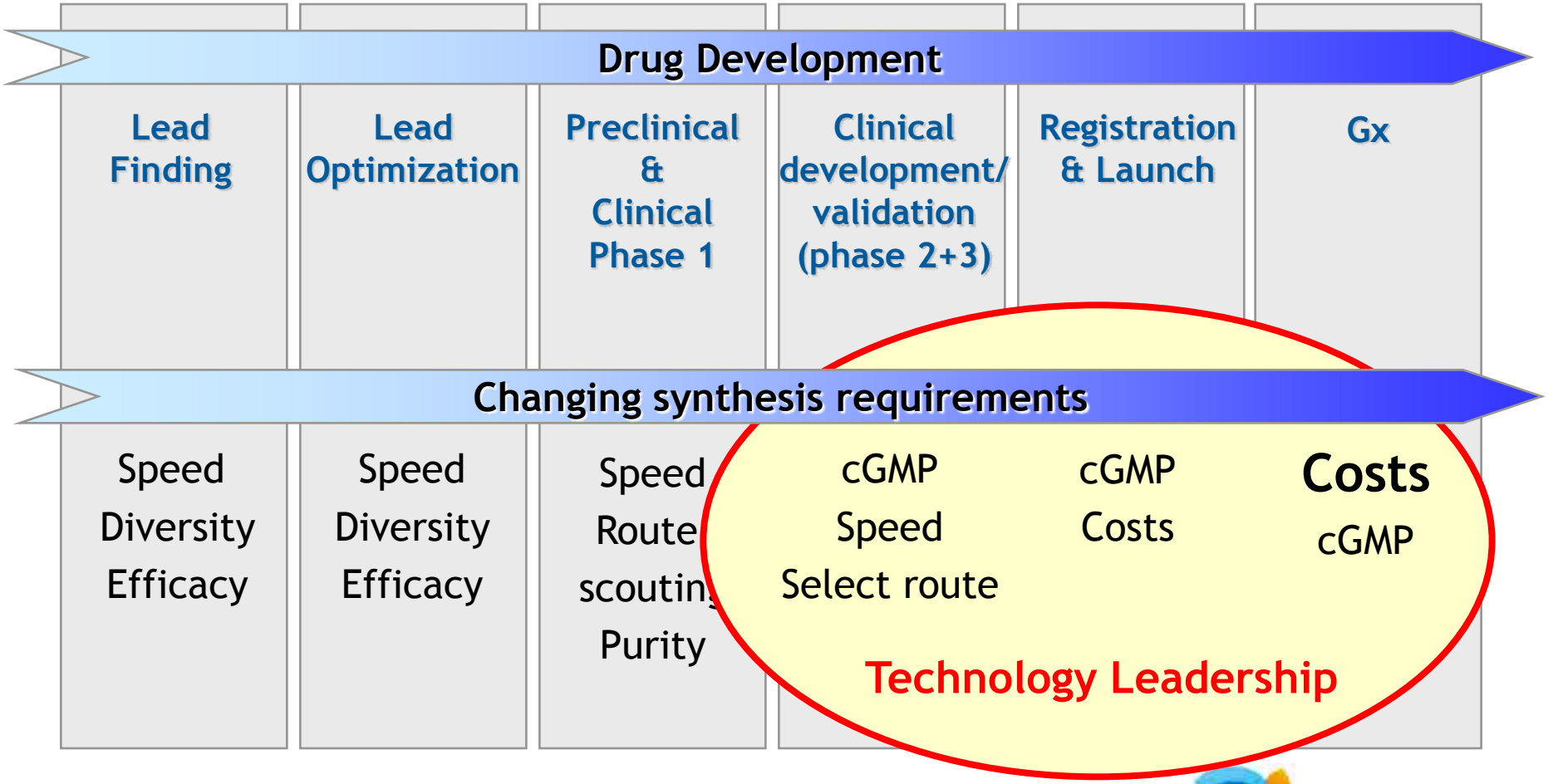
API synthesis in drug development

API synthesis in drug development



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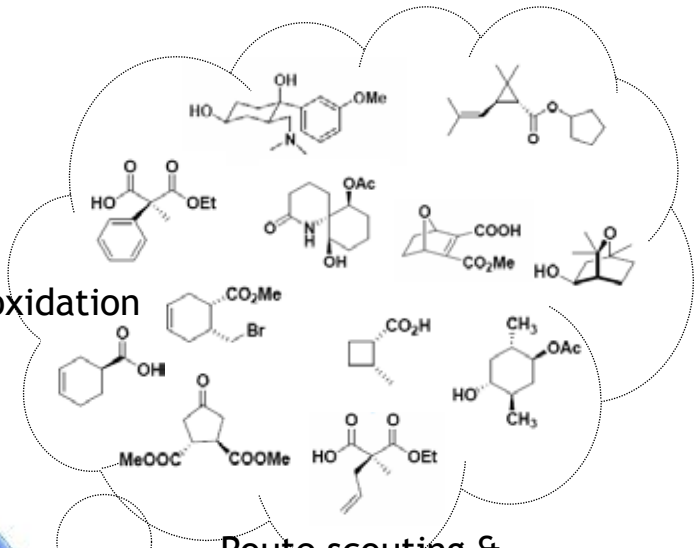
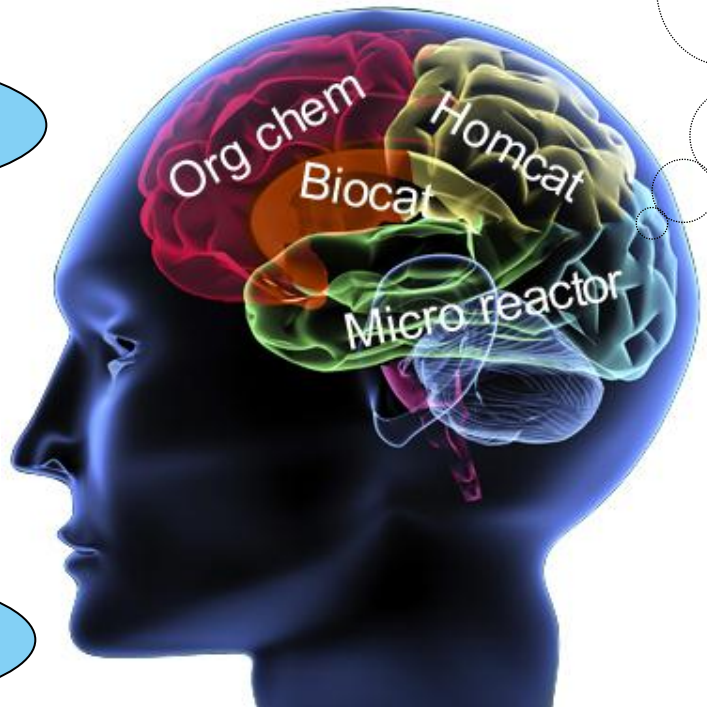
Technology Leadership

General organic chemistry and process R&D

Catalytic oxidation



Homogeneous catalysis



Route scouting & retrosynthesis

Chirality, amino acids & peptides

Microreactor technology

Flow chemistry

Biocatalysis

Fermentation & downstream processing

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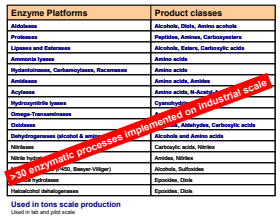
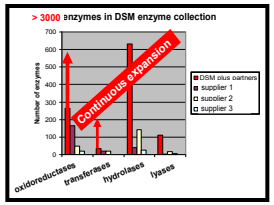
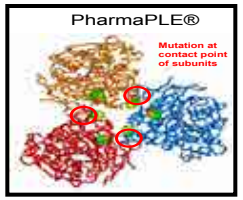
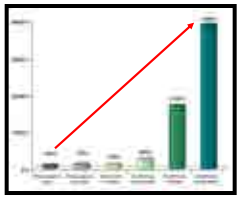
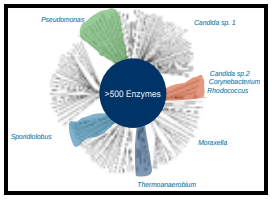
Industrial Biocatalysis



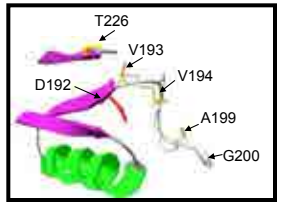








- Broadest collection of off the shelf enzymes (>3000)
- Excellent expression and enzyme design capabilities
- Secure supply of enzyme at any scale (with FTO!)
- Unmet track record (also regulatory) in large scale implementation (>30 processes industrialized)
- Critical mass for all important expertises
- Open innovation approach provides access to huge network



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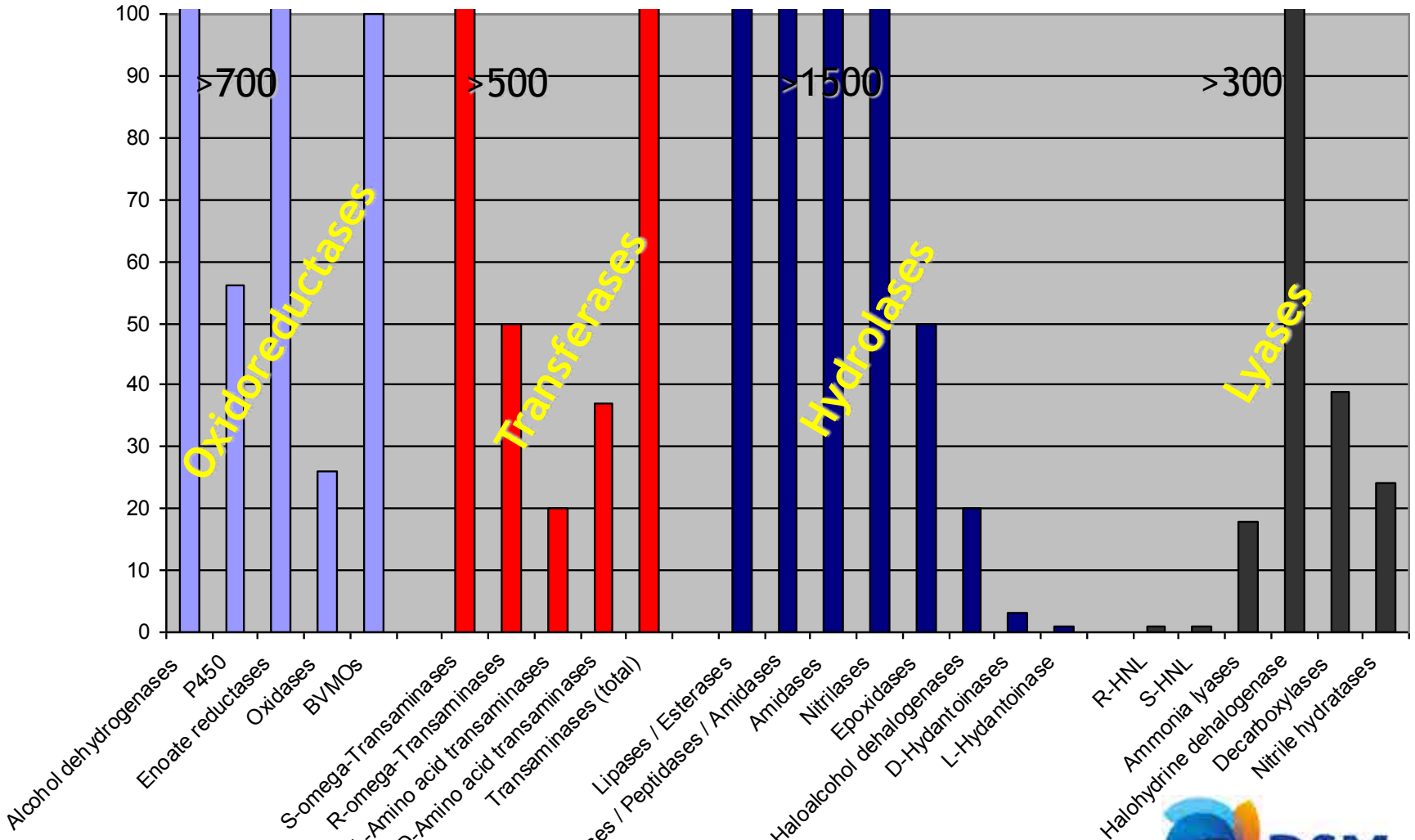
DSM Enzyme collection

> 3000 enzymes ready for screening



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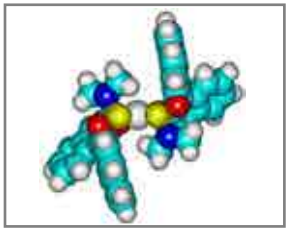
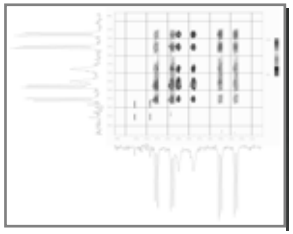
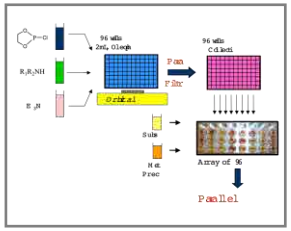
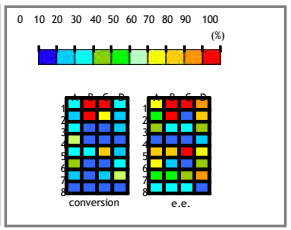
Homogeneous Catalysis



- Screening for the right catalyst from our HomCat Platforms using HTS
- Custom made Monophos® catalysts for asymmetric reduction
- Aromatic substitutions: C-C couplings and amine synthesis
- Rapid identification of the best catalytic system using all available catalysts: proprietary ones AND commercially available ones
- HomCat process development and full scale implementation

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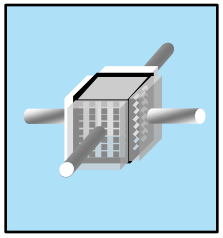
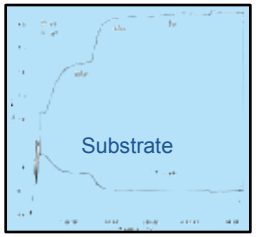
Flow Chemistry - Micro Reactors

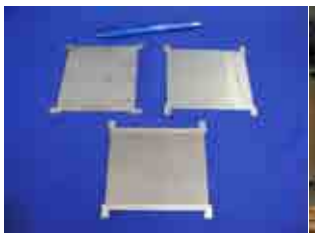
Process Intensification

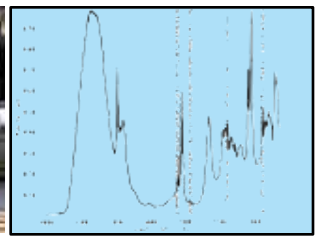
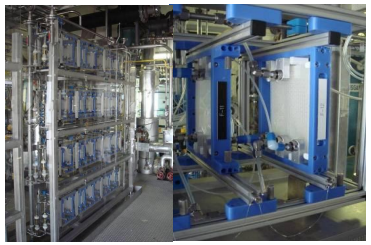






- Flow process development from lab to pilot to full-scale plant
  …in a variety of reactor concepts
- Integration of reaction and work- up
- Rapid reaction optimization and numbering up
  …using various hazardous reagents
  …under cGMP conditions





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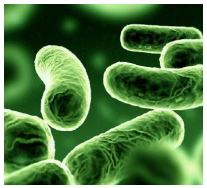
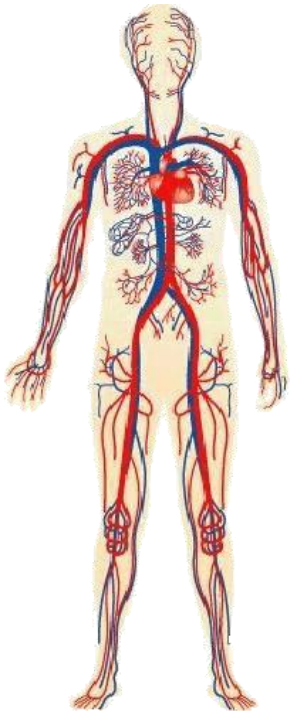
Micro process technology

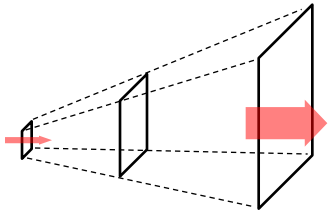
Learning from nature



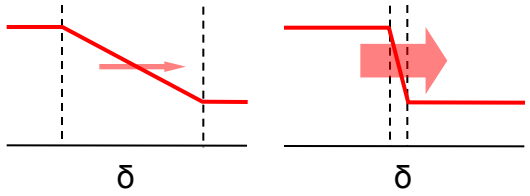
Evolution

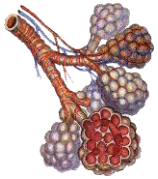


Enhanced transfer by increased transfer area



Enhanced transfer by decreased transfer distance



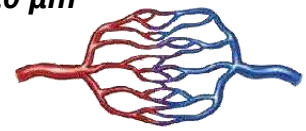


Pulmonary vesicle ***400 µm***

15000m²/m³

Capillary vains ***10 µm***

400.000 m2/m3



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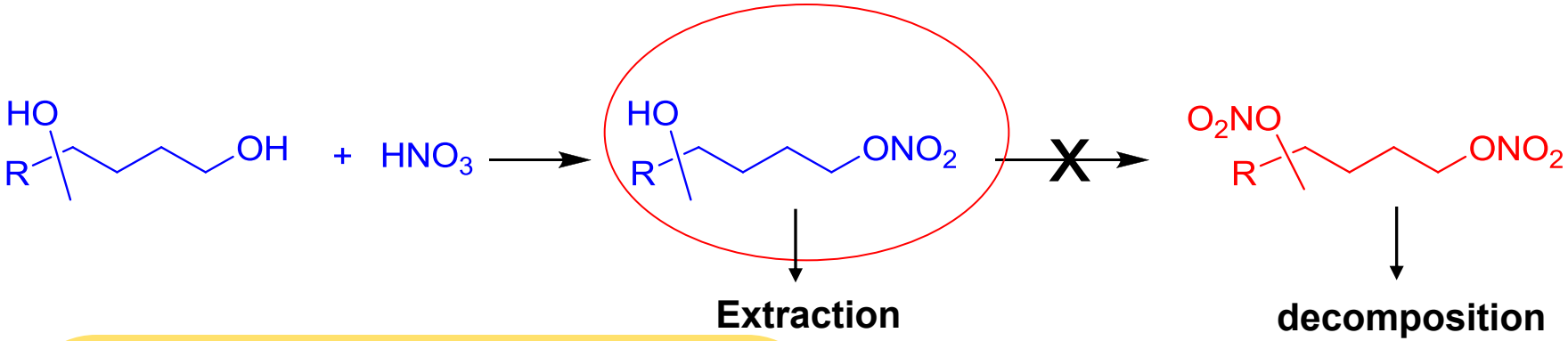
Example: Micro reactor in production

Objective: Manufacture the desired quality and yield at full GMP level.



Process typicals

- Batch operation at low temperature (2 °C)
- Exothermic (nitration, neutralization)
- HNO_3 (high concentration, excess)
- Danger (runaway, explosive)
- Low productivity (diluted)
- Unstable product

Business needs

- **Intrinsic safety**
- **High productivity**
- **High selectivity**
- **GMP production**

Challenge: Selectively get mono-nitro product

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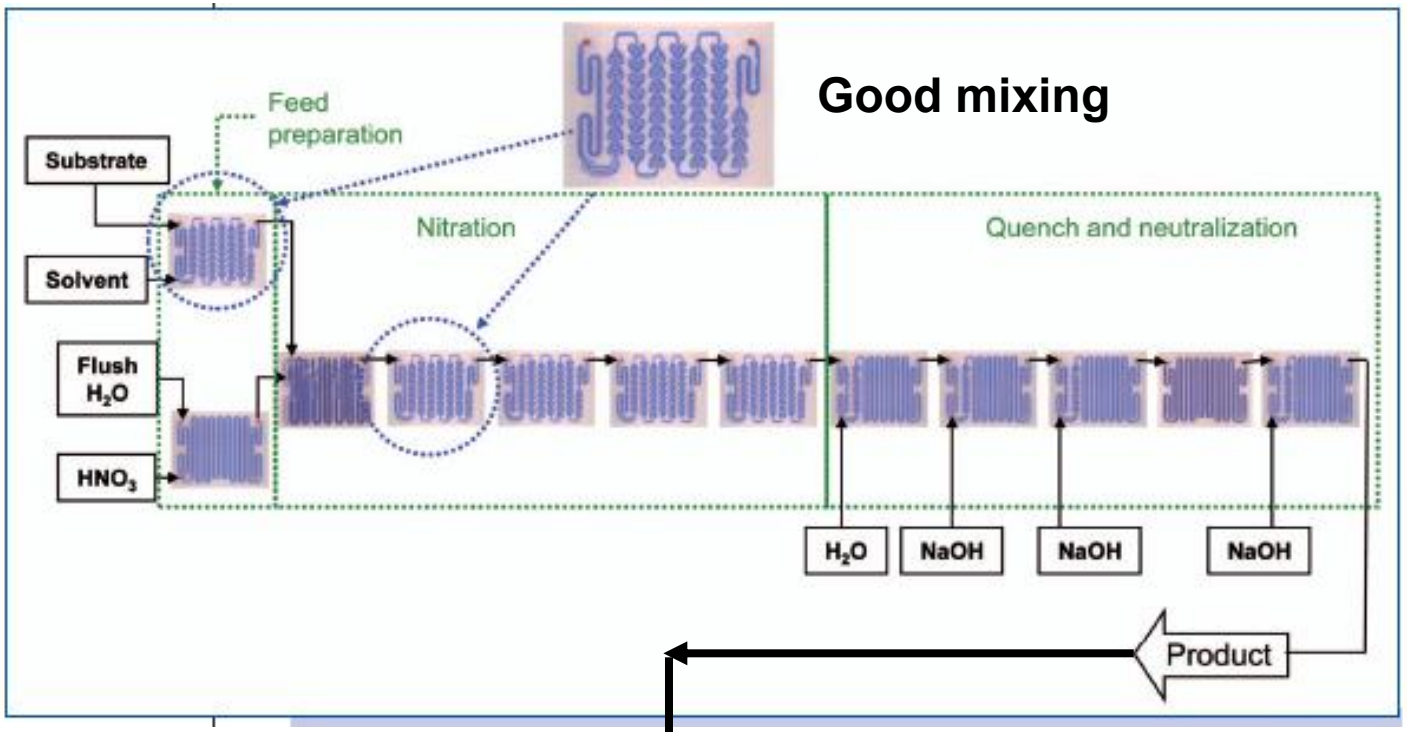
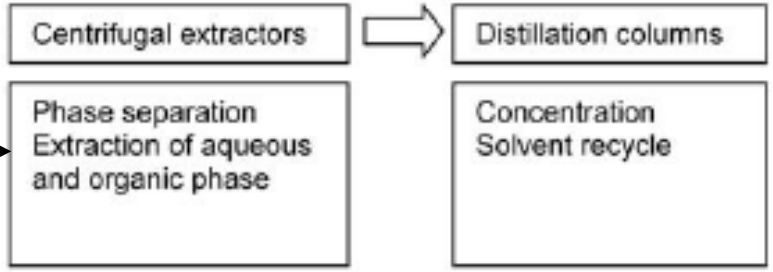
Micro reactor in production



Downstream equipment:

- continuous extraction columns
- centrifugal extractors
- distillation



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Micro reactor in production

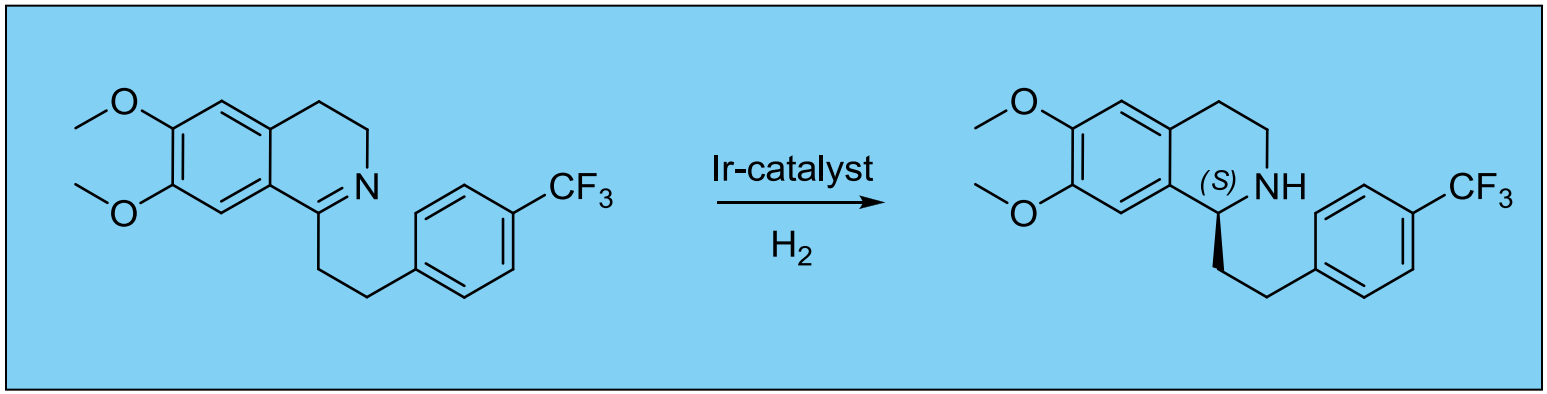
Scale-up to 800 T/a

2 towers with 4 lines each

96 micro reactors in total

API Synthesis Examples @DSM

3a. Almorexant
Catalytic asymmetric hydrogenation



Key step for Almorexant – Actelion Pharmaceuticals
Insomnia treatment
Clinical phase III

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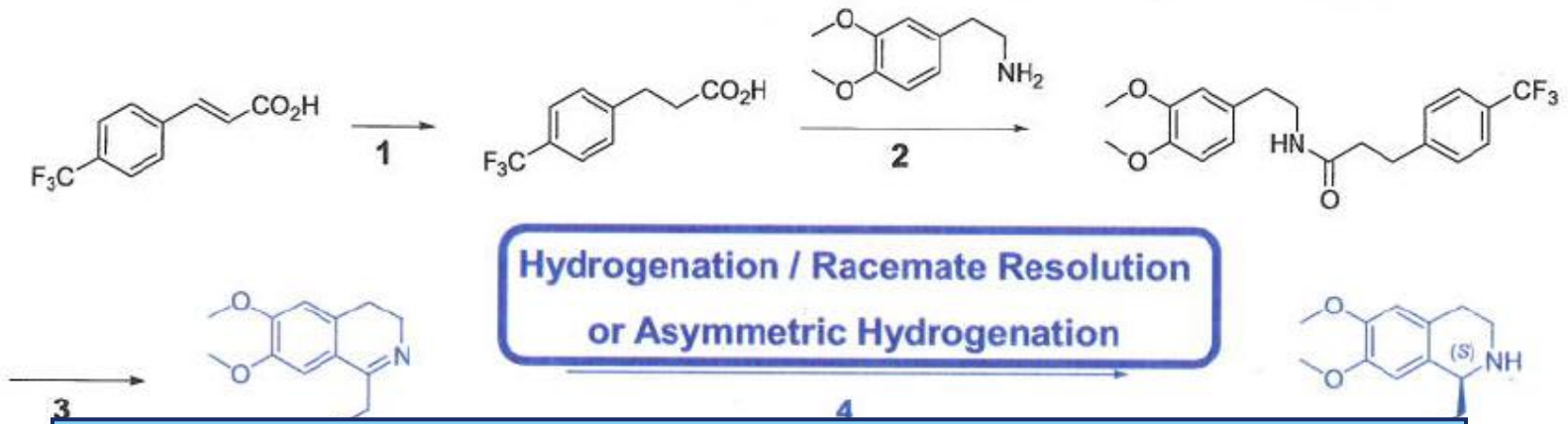
Synthesis overview

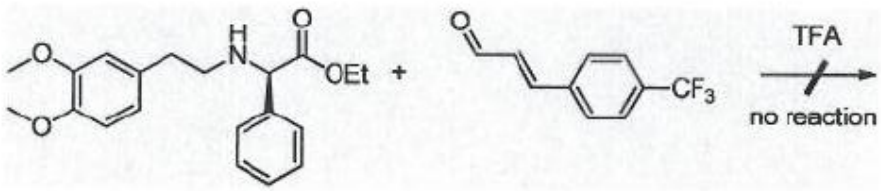


3 Options considered:

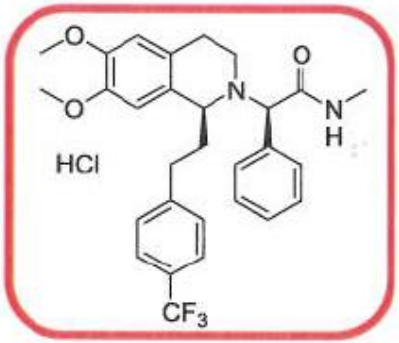
Asymmetric transfer Hydrogenation (Noyori *et al*)

Asymmetric Hydrogenation

Dynamic Kinetic Resolution (similar work from Blacker *et al*)



almorexant



Typical Asymmetric Hydrogenation@DSM

Initial Screening

few ligands, solvent, T, P
conv. and e.e. > 20%

Endeavour

- 8 vessels (5 ml)
- Indep. P (<33 bar) and T (<200°C)
- Overhead stirring
- On line monitoring of gas uptake

High Throughput Screening

many ligands
conv. and e.e. > 90%

A 96 Premex

- 96 vessels (2.5 ml)
- Pressure up till 100 bar
- Stirring by magnetic bar

Optimization

the best ligand
conv. and e.e. > 95%

Standard autoclave
100mL, optimal stirring

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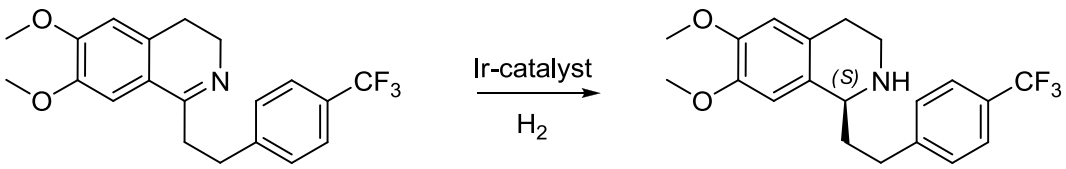
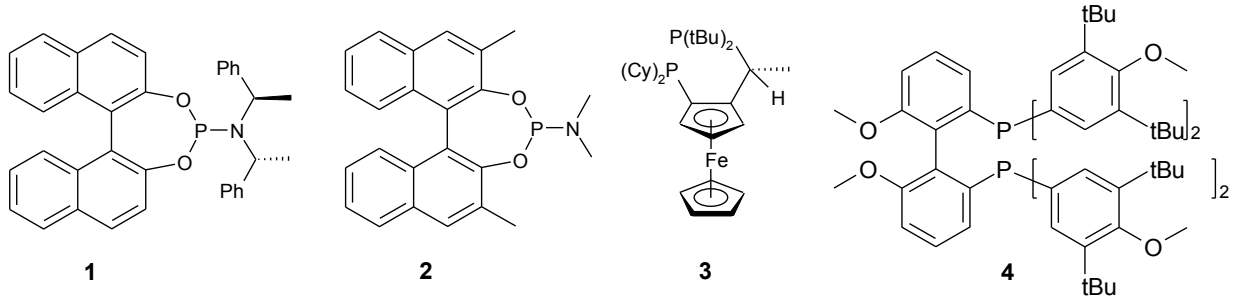
Asymmetric hydrogenation step

Condition screen with 4 representative ligands (High Throughput)





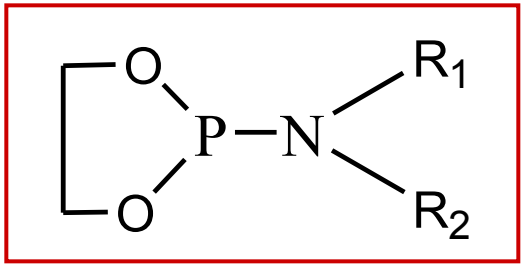
1 2 3 4

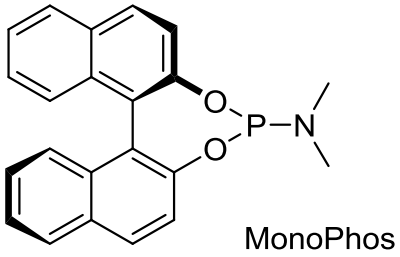
A: $[Ir(COD)Cl]_2$
B: $Ir(COD)_2BF_4$

Pht. = Phthalimide

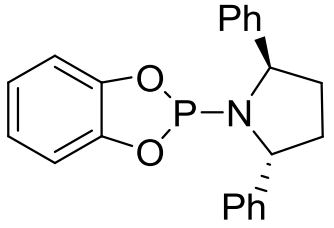
ee	EtOAc			MIBK			DCM			IPA		
	none	Pht.	I_2	none	Pht.	I_2	none	Pht.	I_2	none	Pht.	I_2
4B	17	16	1	3	3	1	3	4	0	1	43	1
3B	1	1	-25	22	20	-22	31	31	-40	8	8	-26
2B	31	30	28	28	32	29	53	40	-11	20	25	17
1B	-44	-41	-44	-28	-24	-27	-33	-27	-24	-26	-12	-47
4A	10	10	0	-2	-1	0	6	1	12	22	15	6
3A	67	66	76	28	34	35	15	18	36	31	41	49
2A	64	62	51	35	34	45	27	17	32	-2	-2	43
1A	-56	-66	-49	-54	-55	-31	-57	-57	-26	-27	-27	-51

Chiral phosphoramidite ligands

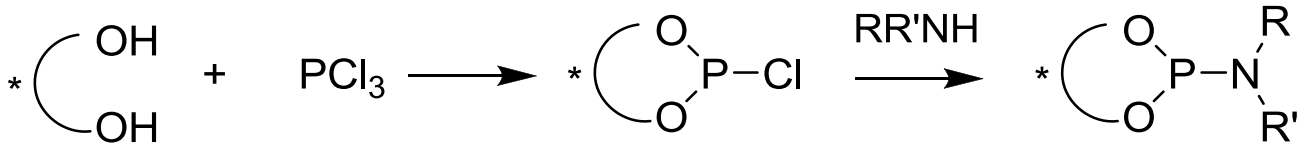




MonoPhos



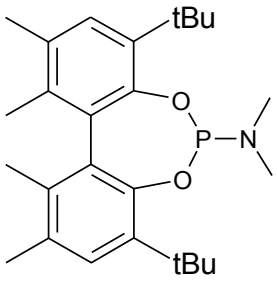
- Monodentate ligands
- Much easier to prepare than bisphosphines



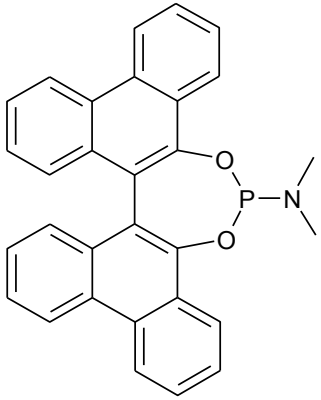
- Chirality from diol and/or amine
- Modular ligand: diversity from the diol and/or amine part
- Till 2000 not known for asymmetric hydrogenation

DSM and University of Groningen collaboration, see e.g.: *Adv. Synth. Cat.* **2003**, *345*, 308; *Org. Proc. Res. Dev,* **2007**, *11*, 585; *Org. Proc. Res. Dev,* **2010**, *14*, 568.

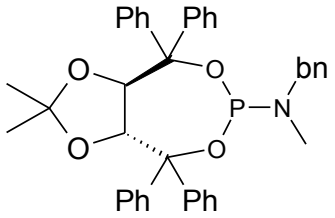
Further exploration MonoPhos family



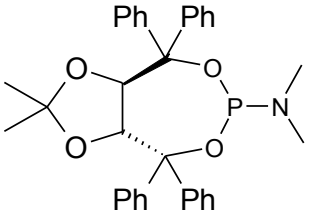
3% ee



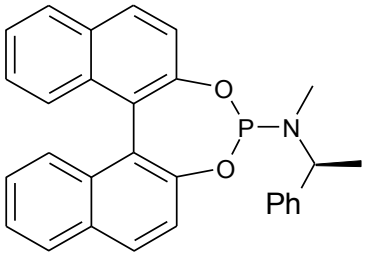
29% ee



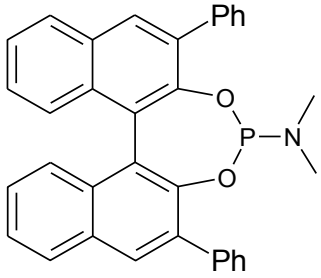
17% ee



10% ee



32% ee



79% ee

➔ **Zenith of ee for MonoPhos library**

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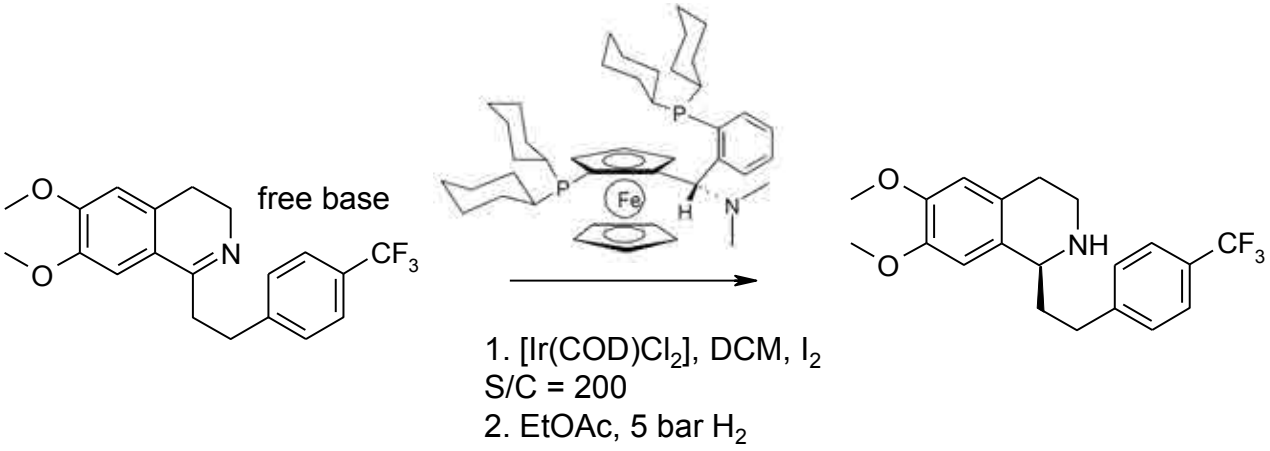
Further exploration JosiPhos lead

28 Solvias Ligands: Josiphos, Walphos, Mandyphos, and Taniaphos types $[Ir(COD)Cl]_2$ and I_2 in EtOAc

Taniaphos Ligand *SL-T002-2* gave the best ee



➔ Process was selected for scale up and piloting (1 m^3)

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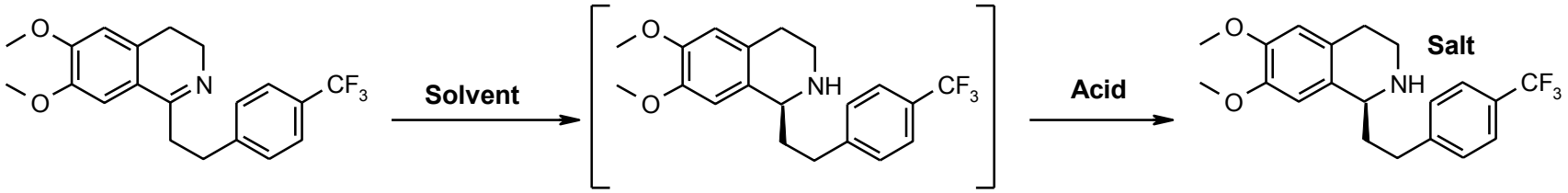
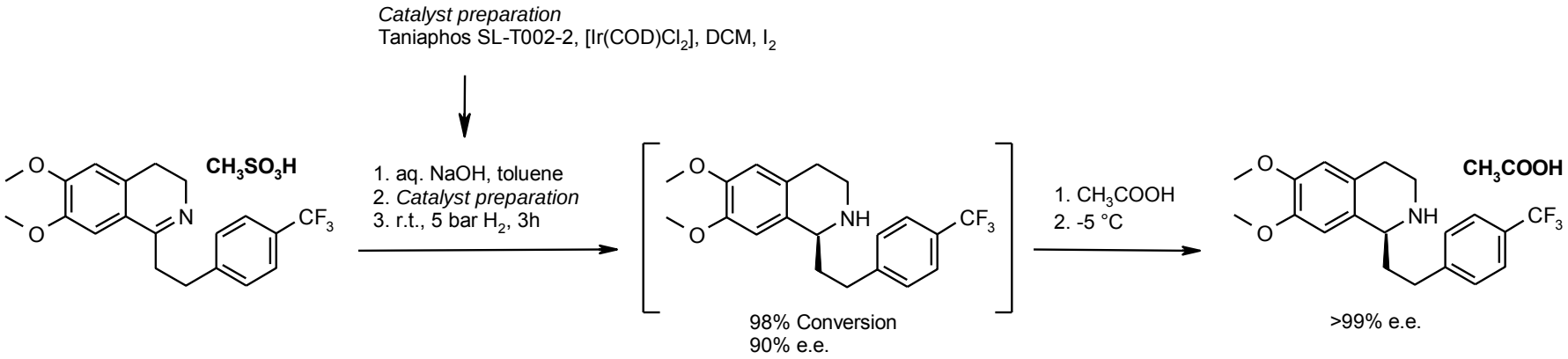
Process optimization



Solvent	Acid	ee of Salt	ee of ML
EtOAc	$MeSO_3H$	87%	68%
Toluene	$MeSO_3H$	93%	30%
Toluene	TsOH	-	-
Toluene	CH_3CO_2H	99.7%	65%
Toluene / Heptane	CH_3CO_2H	93%	12%
Toluene / Heptane	HCO_2H	88-93%	72-77%

All solvents gave similar ee's in IPC (83-90%)

Full scale protocol



Achievements

- Commercially viable S/C was reached on 6 m^3 scale
- Acetate salt
  - single crystallization
  - non-corrosive
- Robust crystallization procedure
- Isolated yield was increased (compared to Noyori)

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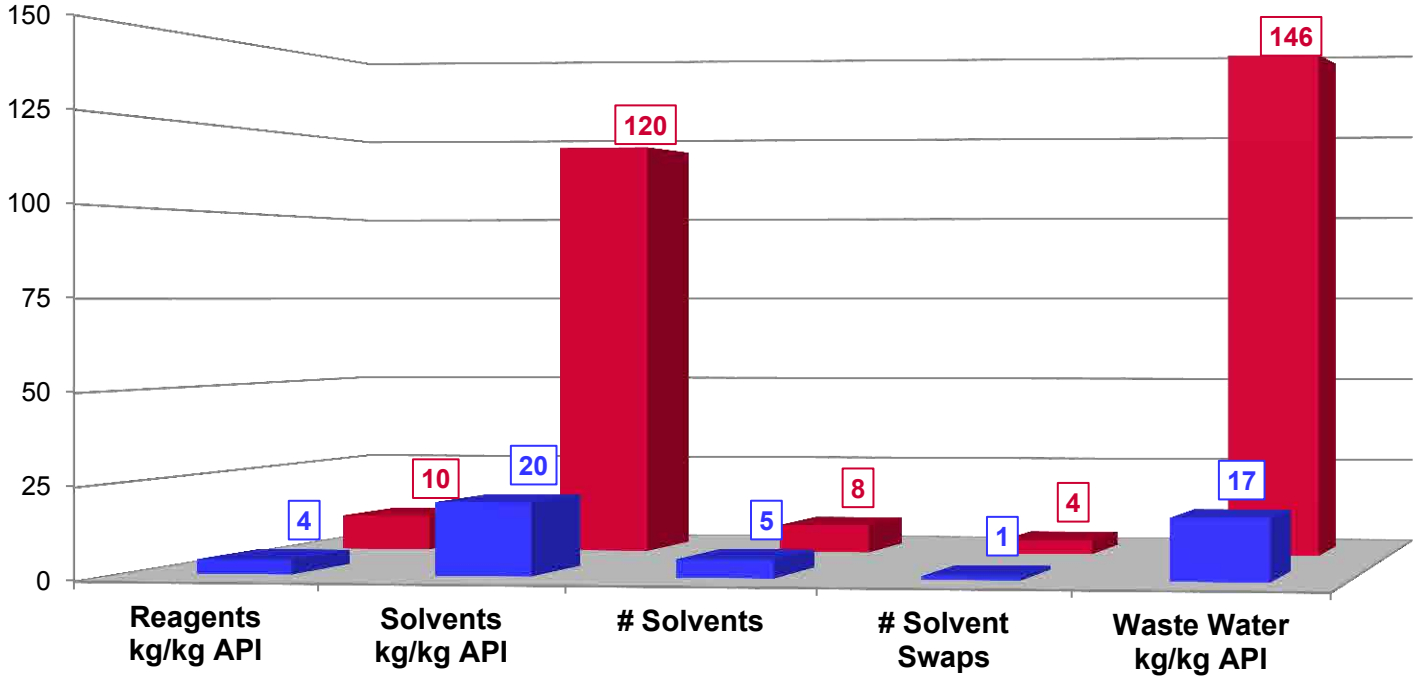
Waste reduction



Phase I (ATH)

Phase III (AH)

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Almorexant - Summary

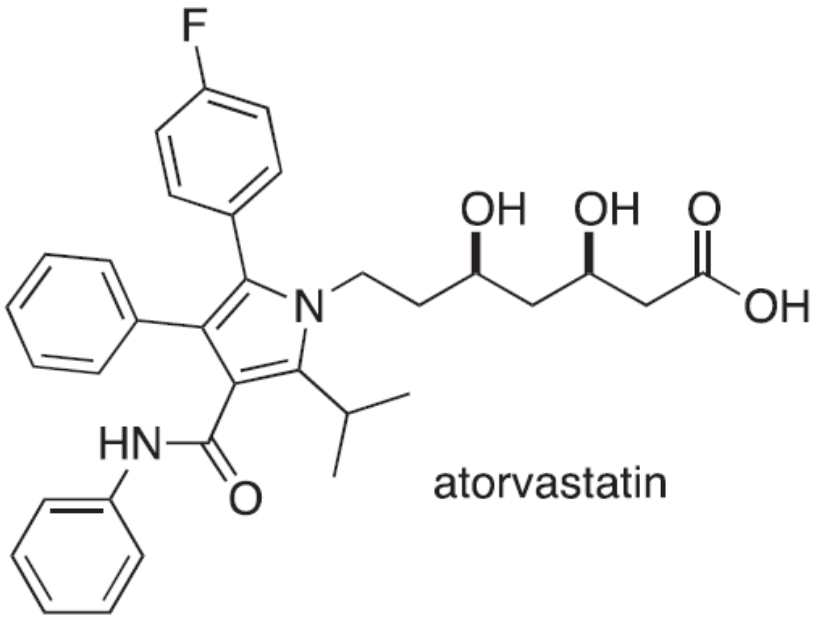
Catalytic asymmetric hydrogenation step replaces 5 steps for 1
(3 steps for the chiral resolving agent)

Applied at multi ton scale

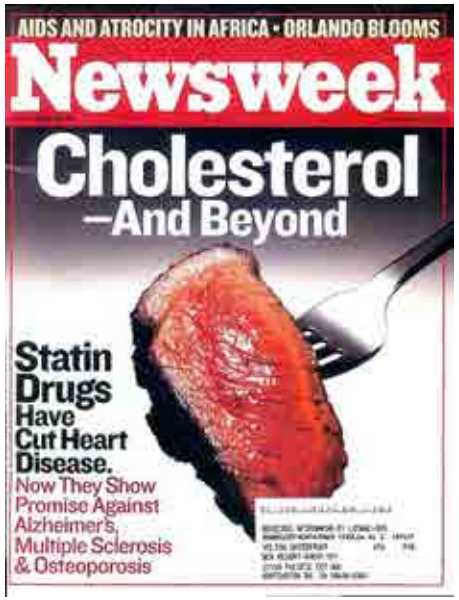
Unfortunately the drug development has been abandoned

3b. Statins by Aldolase technology

3b. Statins, e.g. Atorvastatin (Lipitor)



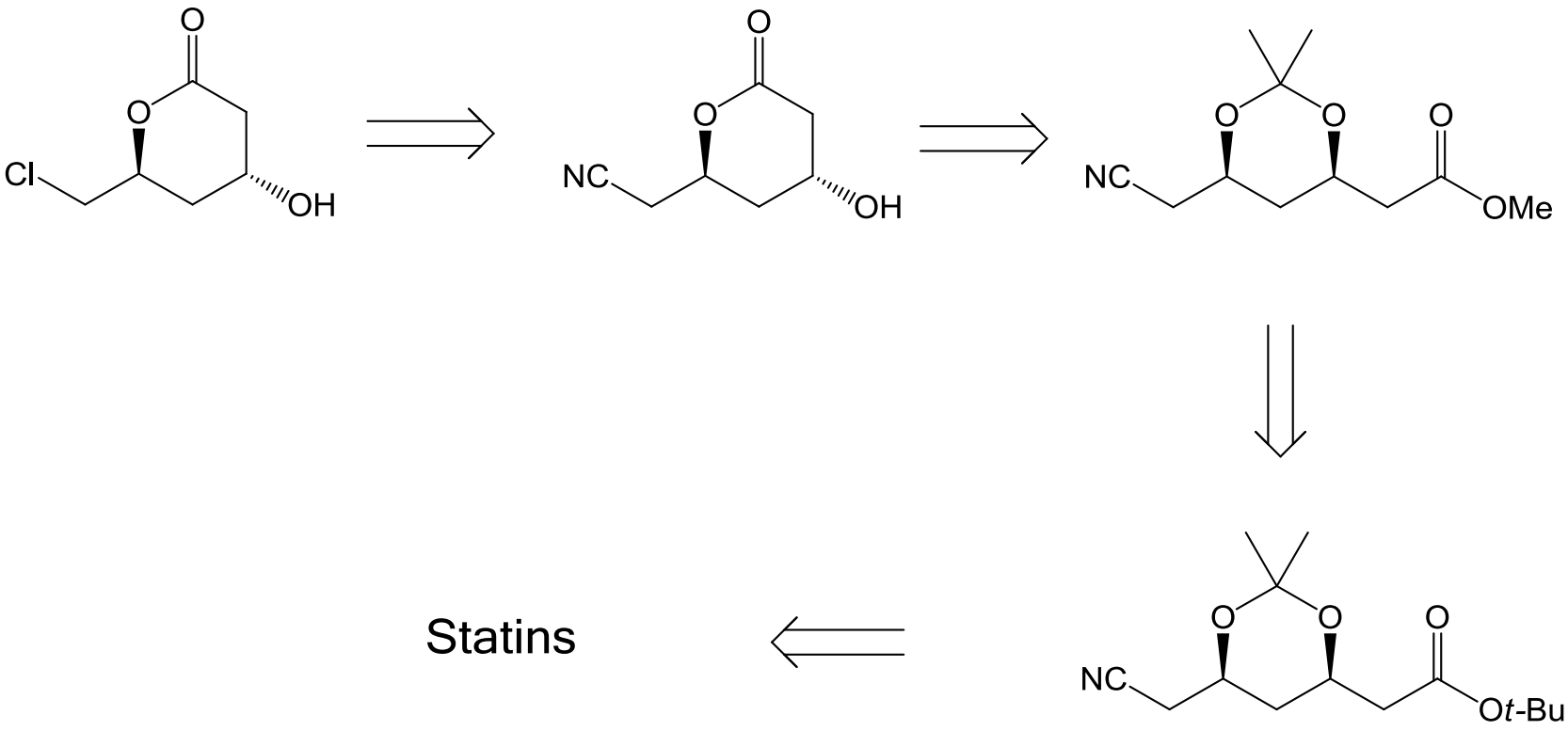
atorvastatin



Best selling drug for last 5 years (fighting obesity)
Patent expired in 2012
Several 'second at market' analogues

Lowest cost route required

Retrosynthesis



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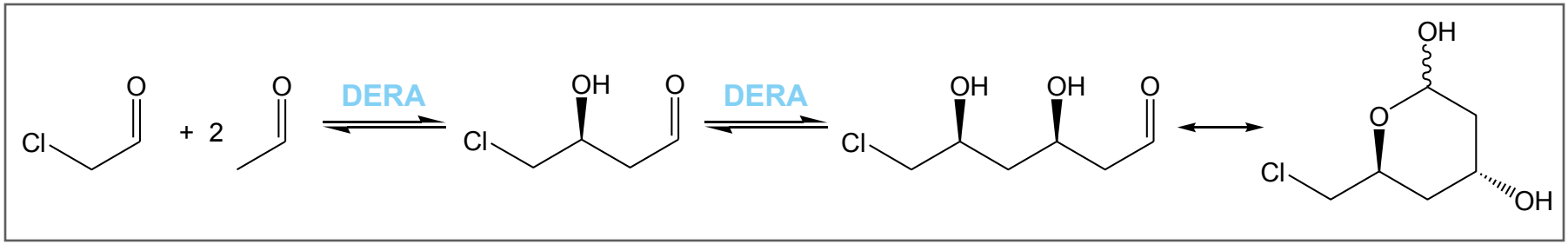
DERA Catalyzed Tandem Aldol Reaction

Enzymatic synthesis of a deoxysugar



Published information

- Low product concentration: 1-2 (w)%
- Low enzyme stability
- Work-up not suitable for scale-up
- By-product formation is ‘black-box’

Wong *et al.* (1995) *J.Am.Chem.Soc.*, **117**, 3333

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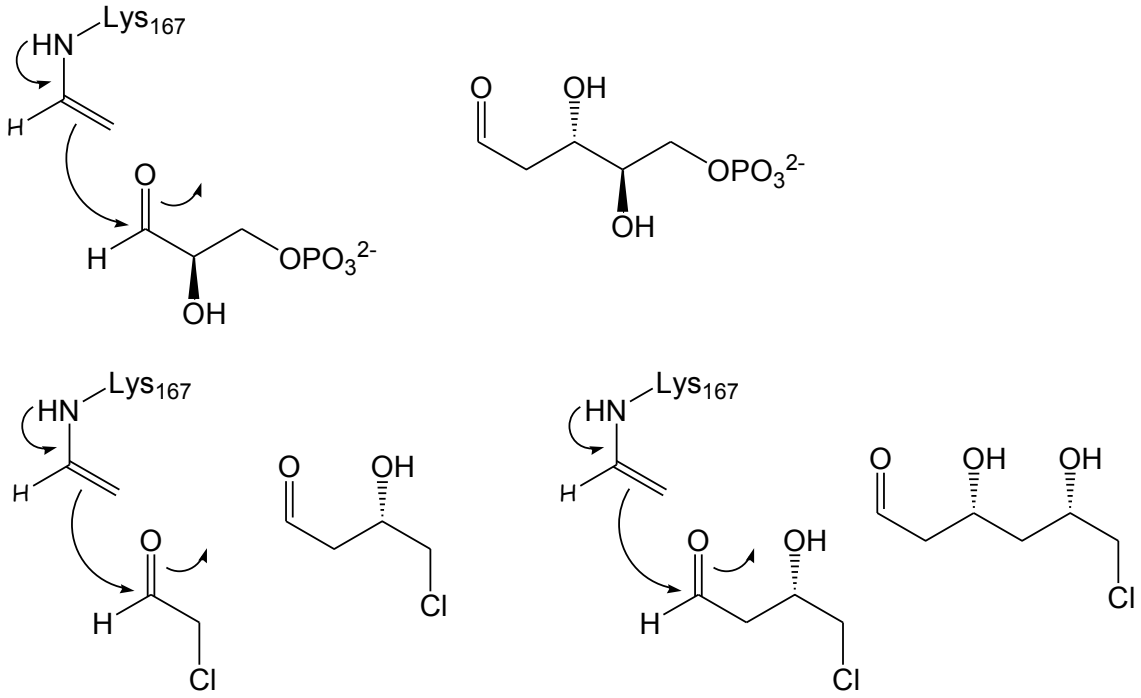
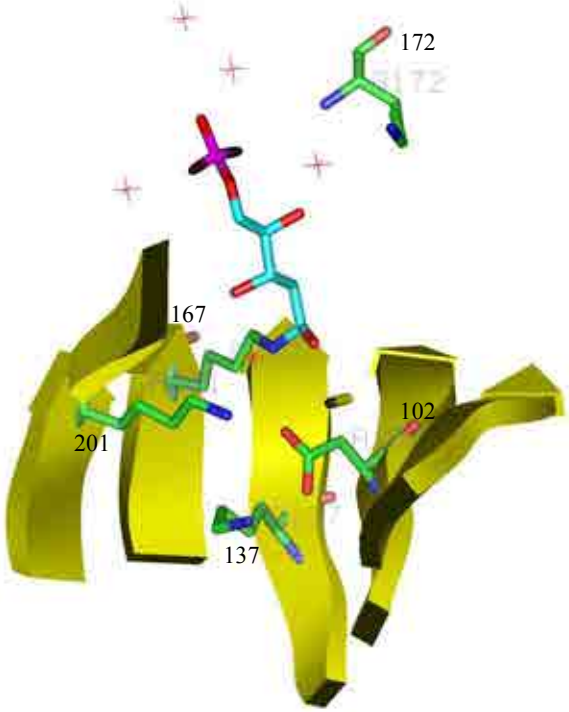
DERA is Type I Aldolase: Mechanism

Active site with Lys_{172}, Lys_{201}, **Lys_{167}**, Lys_{137}, Cys_{47} and Asp_{102}

Lys_{167} with carbinolamine intermediate





Heine *et al.* (2001) Science **294**, 269 - thanks to Jan Metske van der Laan

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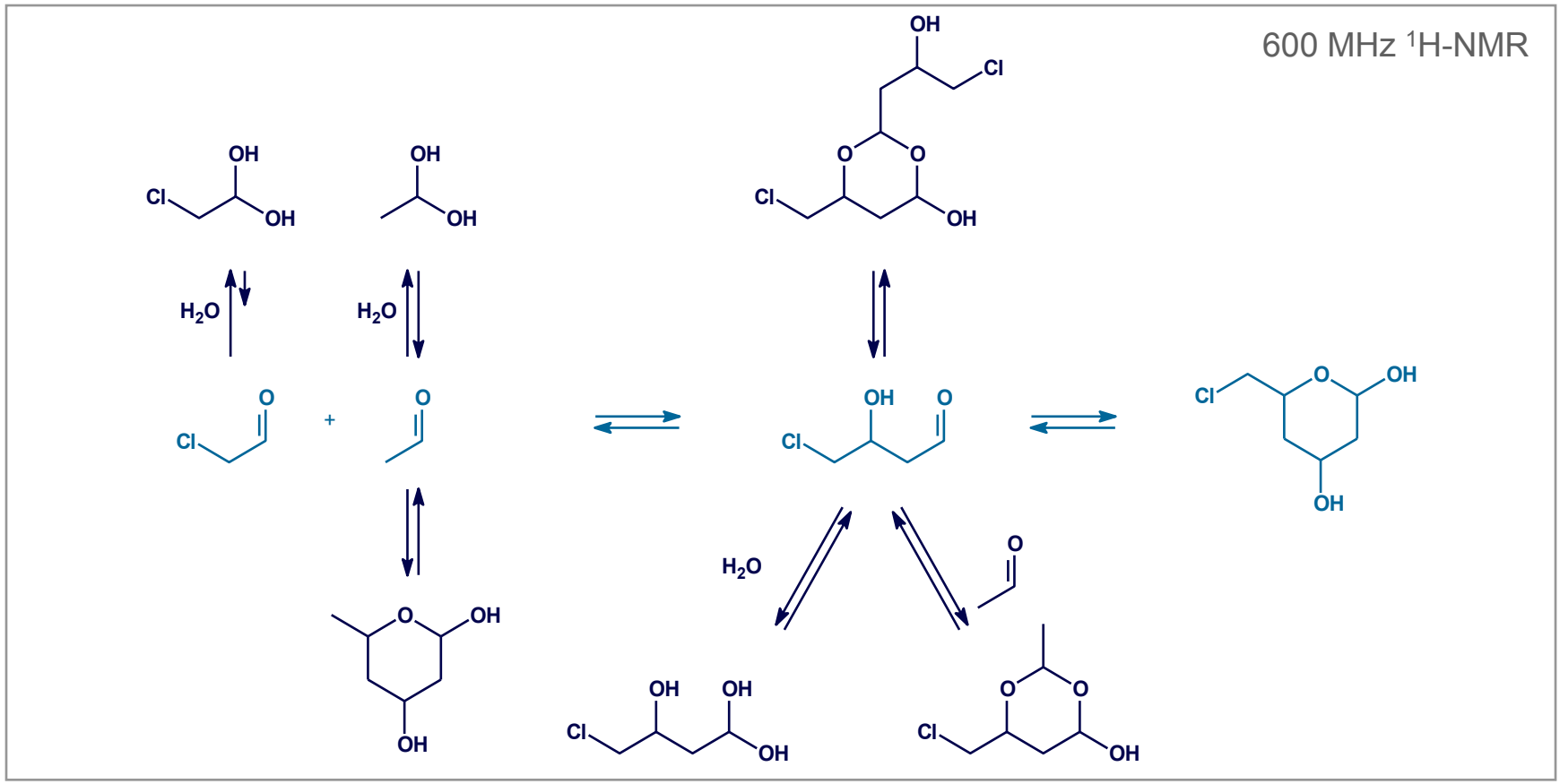
Reaction Intermediates and Products



Reaction is far more complicated than expected

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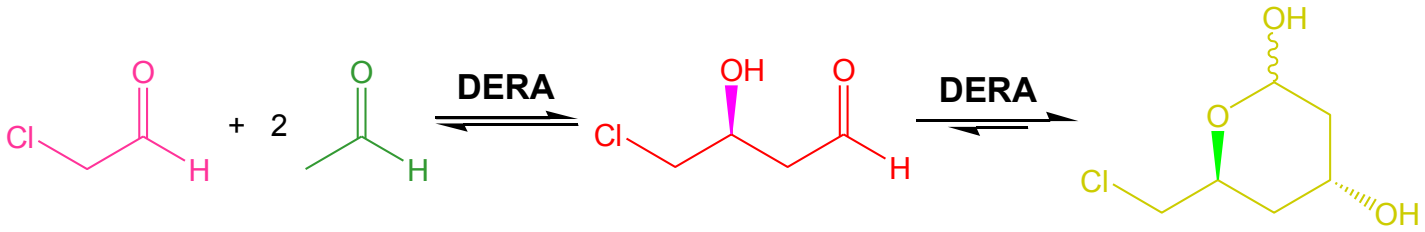
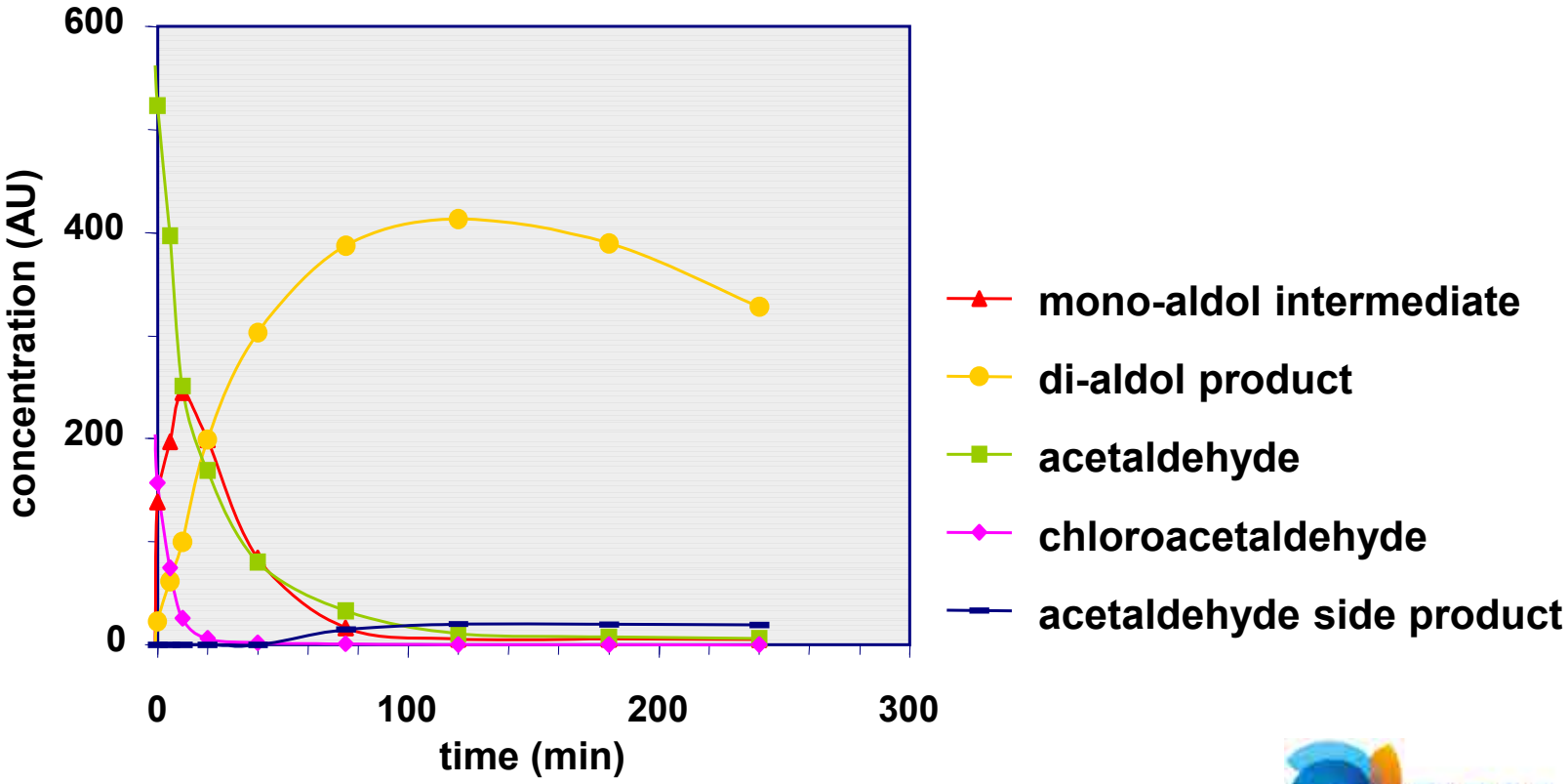
Typical Batch Reaction: 600 MHz ^{1}H-NMR





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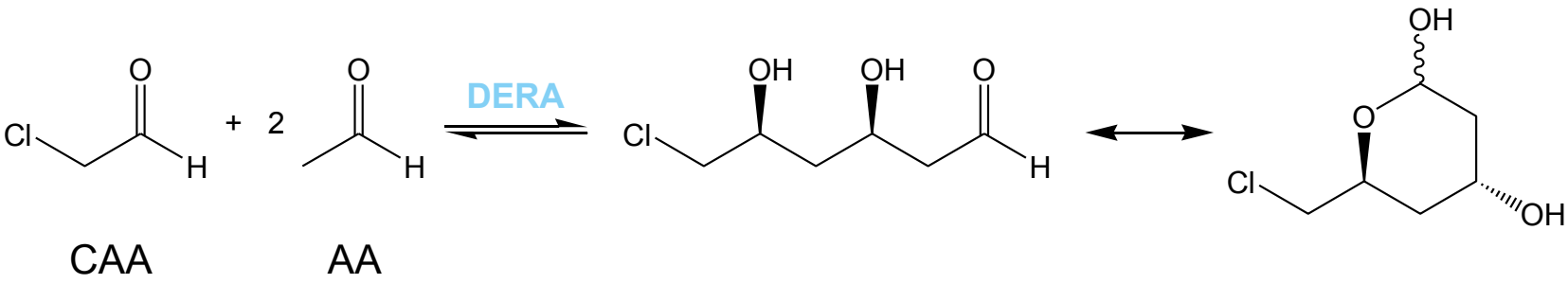
Drawbacks of DERA Reactions



CAA AA

Facts on DERA:

- Low activity for acceptor substrates
- Inactivation by aldehyde substrates
- Therefore: large amounts of enzyme needed
- However, an efficient process is feasible (WO 03006656 to DSM)

→ Directed evolution to improve DERA

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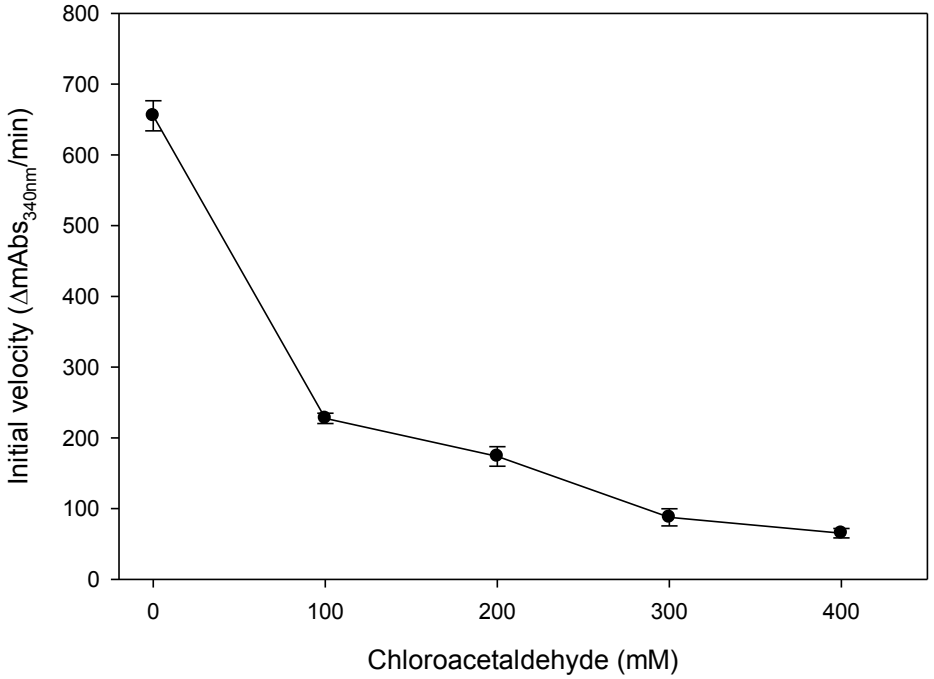
DERA Limitations:

Acceptance of Chloroacetaldehyde and inactivation



Chloro-acetaldehyde (mM)	Remaining activity of DERA after 5 min exposure to indicated concentration of chloroacetaldehyde
0 mM	100%
100 mM	34.7 ± 0.45
200 mM	26.1 ± 0.87
300 mM	13.4 ± 1.07
400 mM	9.9 ± 0.61

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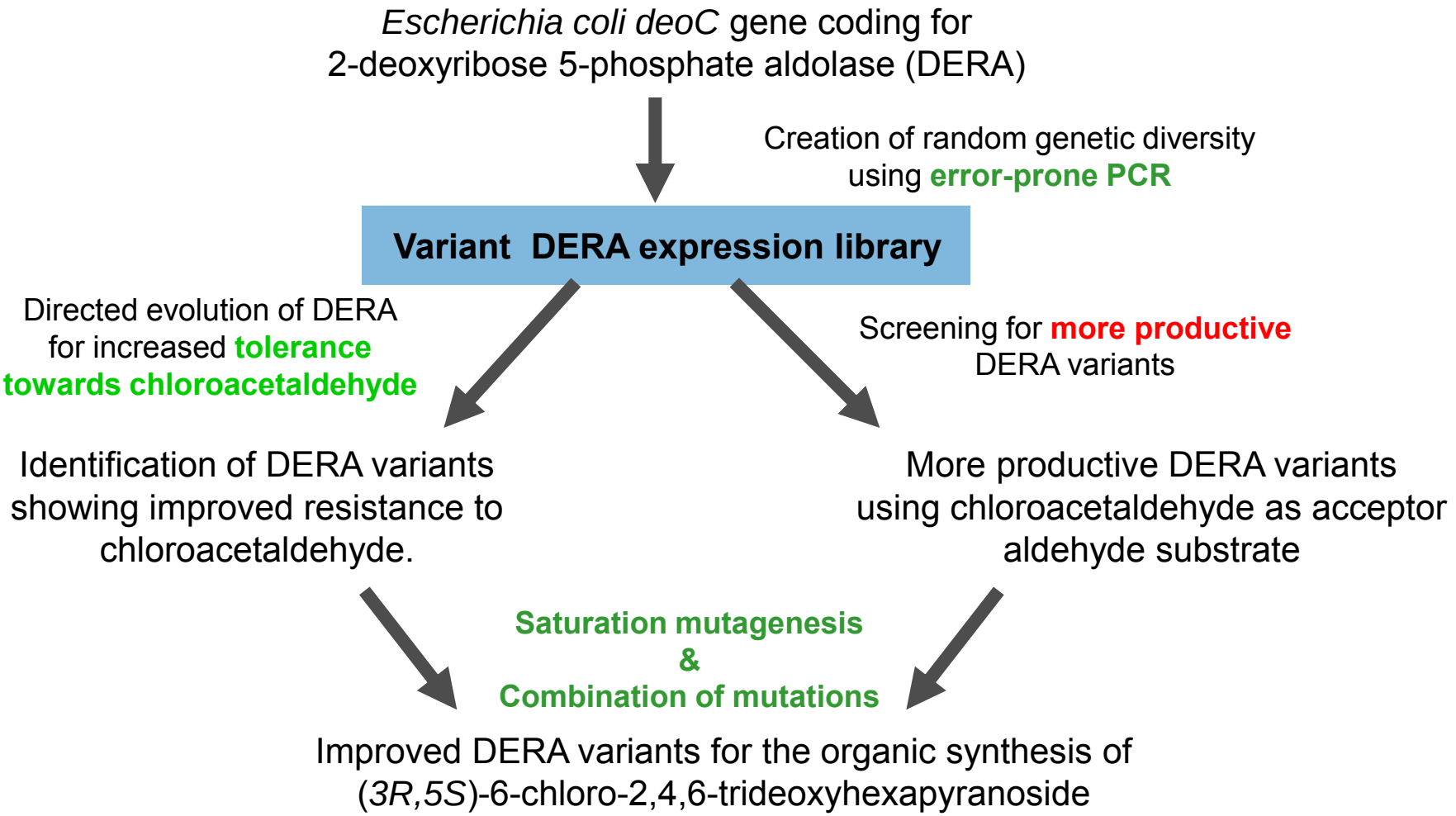
Directed Evolution Strategy for DERA



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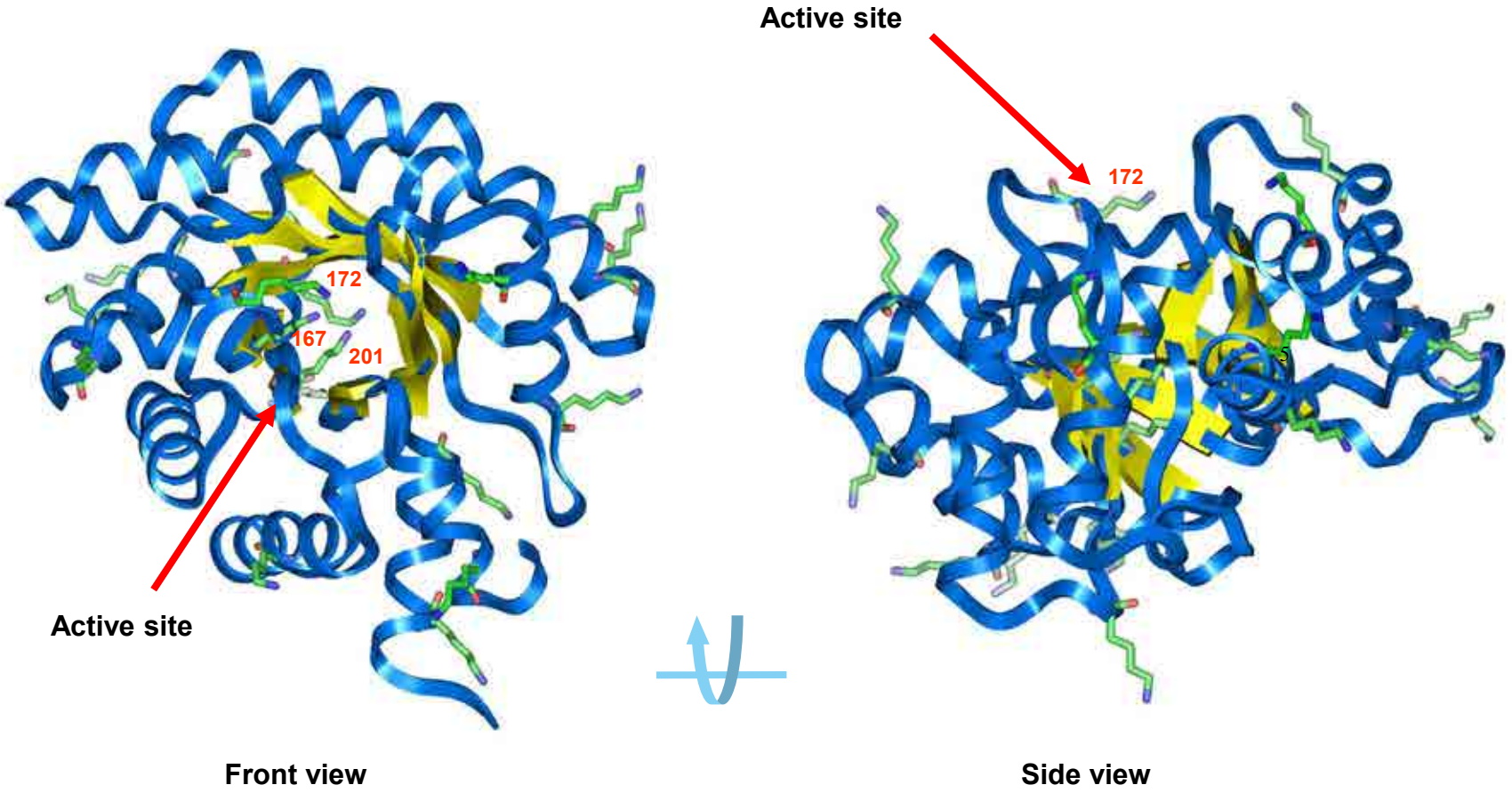
DERA 3D structure (ribbon presentation)



Lysine residues are shown (18 in total)

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Improvement of tolerance to CAA

Variant DERA expression library
(obtained through error-prone PCR)

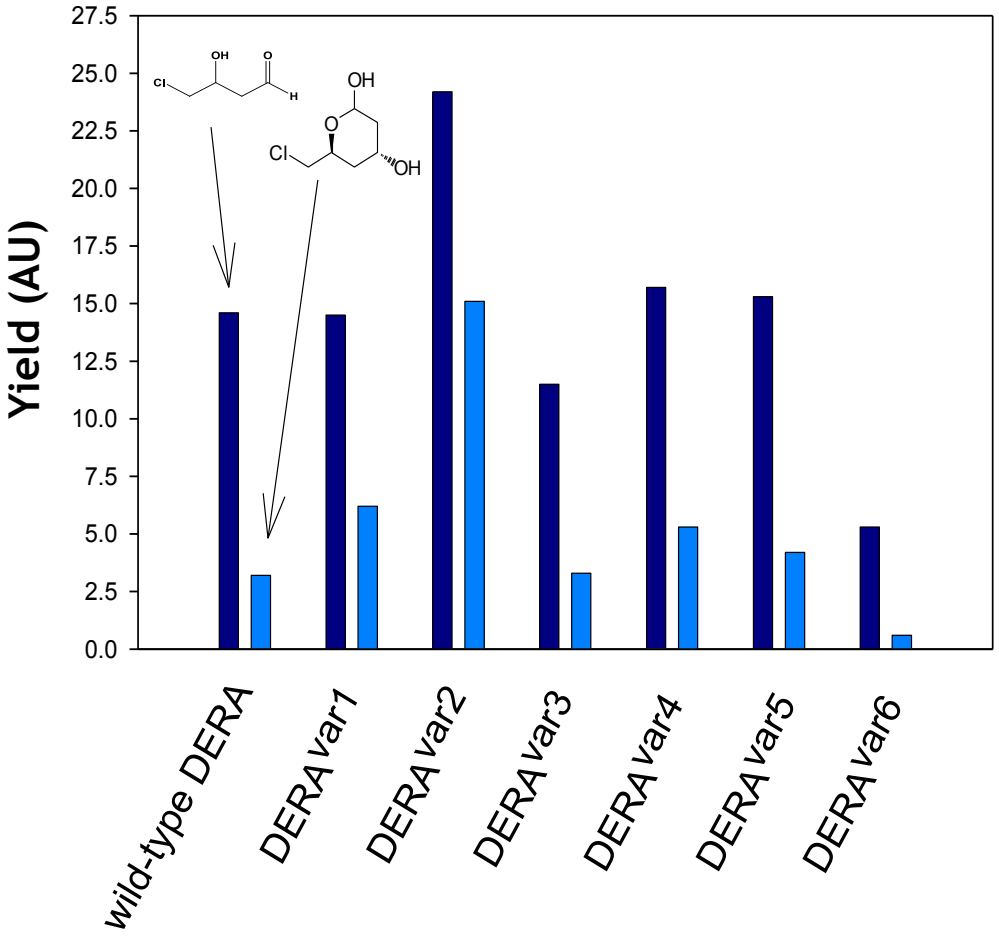
Screening of 10,000 randomly chosen
DERA variant clones

Identification of 63 stability screening hits

2 rounds of recombination
(screening of 3,000 clones per round)

Isolation of 10 stability screening hits

Improvement of tolerance to CAA



Synthesis conditions:

- 200 mM CAA, 465 mM AA
- 0.1 M $NaHCO_3$ (pH 7.2), RT, 16 h.
- GC-Analysis for (*S*)-4-chloro-3-hydroxybutyraldehyde ("monoaldol") and (*3S,5R*)-6-chloro-2,4,6-trideoxy-hexapyranoside ("acetal")
- Yields are reported in Arbitrary Units

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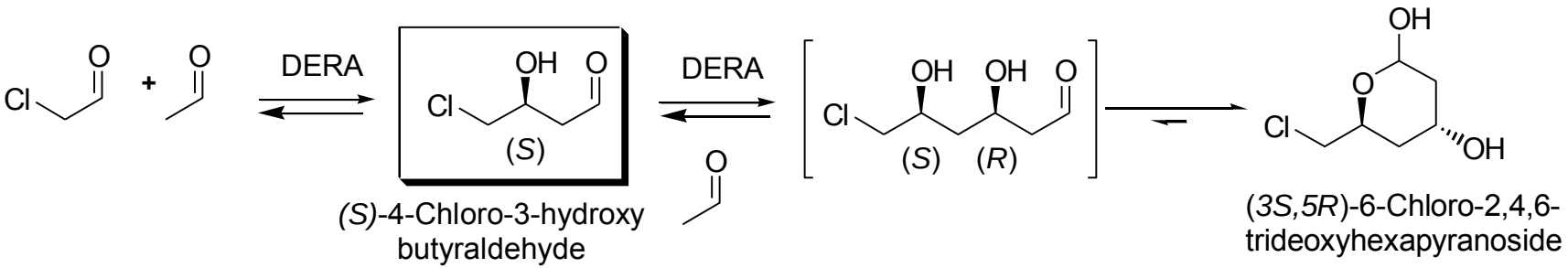
Screening for variant DERA's with Improved Productivity



(S)-4-Chloro-3-hydroxy butyraldehyde

(*3S,5R*)-6-Chloro-2,4,6-trideoxyhexapyranoside

Variant DERA expression library

(obtained trough error-prone PCR)

Screening of 3,000 randomly chosen DERA variant clones using high-throughput GC/MS

Identification of 9 productivity screening hits

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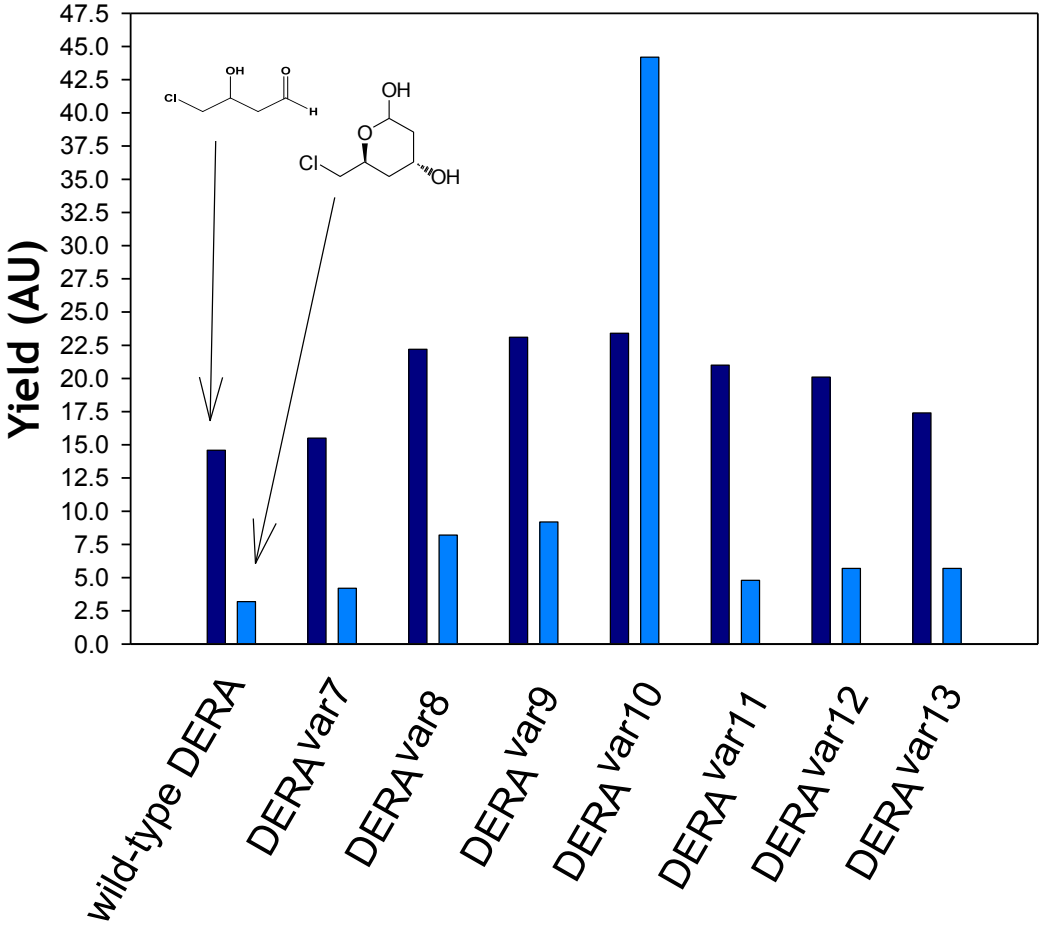
Screening for Improved Productivity



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- Yields are reported in Arbitrary Units

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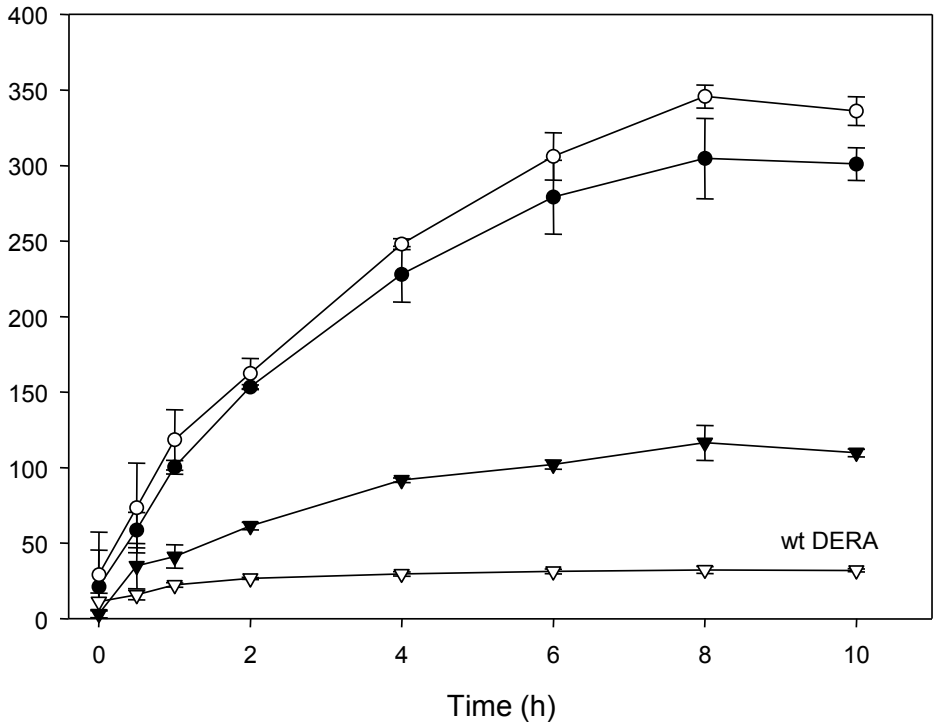
Combination of Beneficial Mutations

- Most productive variants from the two screenings combined
- Test with constant DERA amounts:



F200I / ext. C-terminus

F200I / ΔY259

F200I

→ Mutations from both screenings are synergistic

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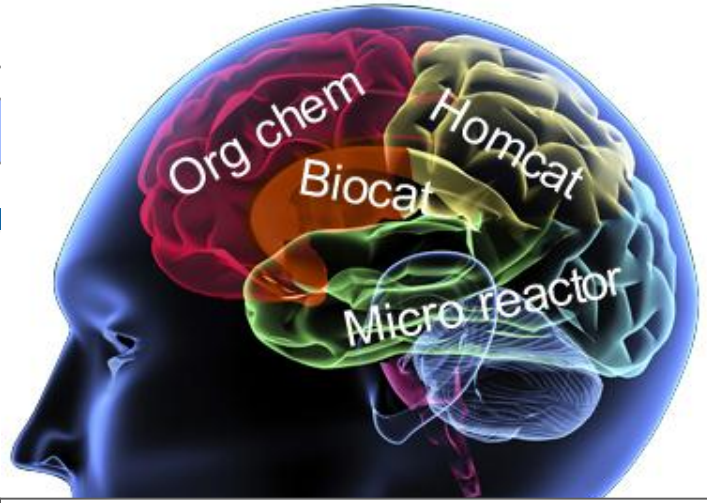
Statin intermediate - Summary

DERA enzyme (Aldolase) in our opinion the lowest cost technology to produce chiral building block for Statins

Subsequent reactions to Statins also performed (and improved)

Better impurity profile than other technologies

DSM's route scouting and manufacturing services for API synthesis



Desk Studies

Route Scouting

P

ion

h

InnoSyn®

InnoSyn® route scouting services

focused & flexible, targeting pre-clinical & phase-I

IP approach: newly generated IP for the customer

DSM Pharma Chemicals

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4. Acknowledgements

Homogeneous Catalysis

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Gerard Verzijl
Lizette vd Vondervoort
Hans de Vries

Biocatalysis

Daniel Mink
Martin Schurmann
Michael Wolberg
… …
… …

R&D and production sites in Venlo and Linz

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