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Sustainable Biocatalytic Synthesis of β-Hydroxyl-α-Amino Acids on an Industrial Scale

Basel,

27.06.2019

β-Hydroxyl-α-Amino Acids – Important Building Blocks

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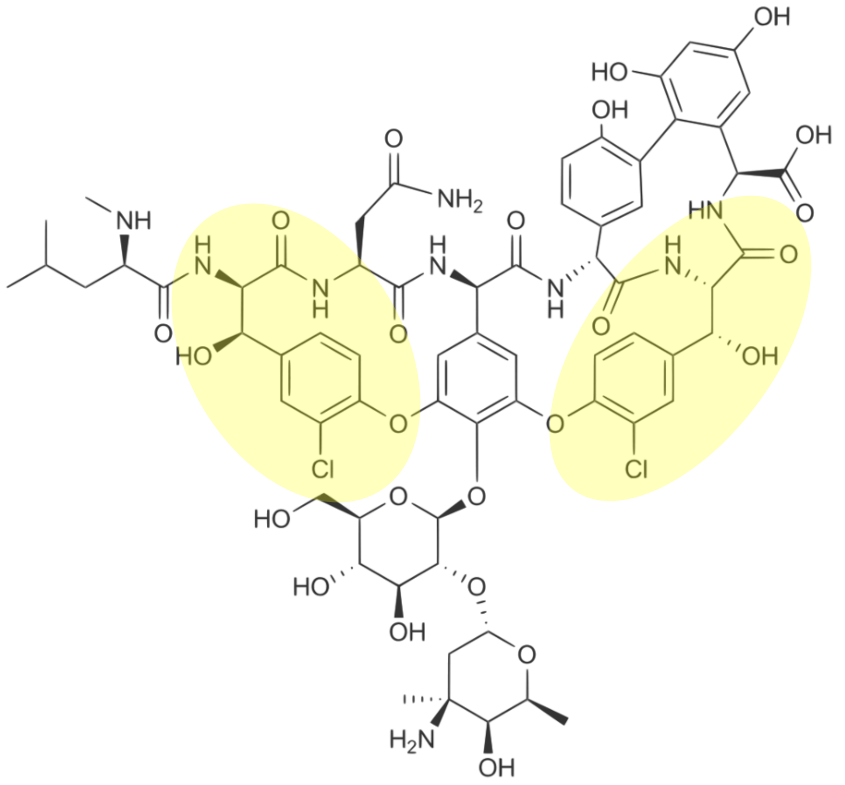
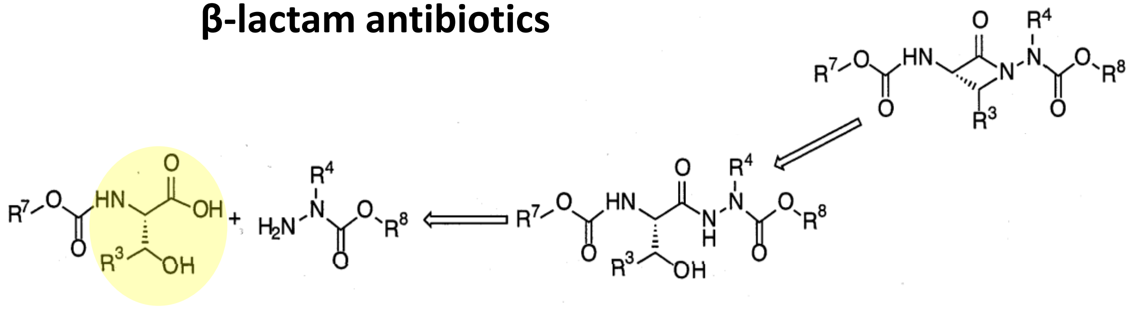
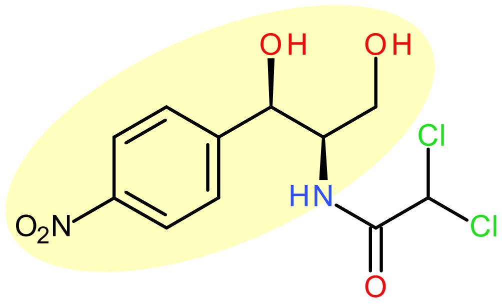


Vancomycine family antibiotics, e.g. Oritavancin, Teicoplanin

β-lactam antibiotics



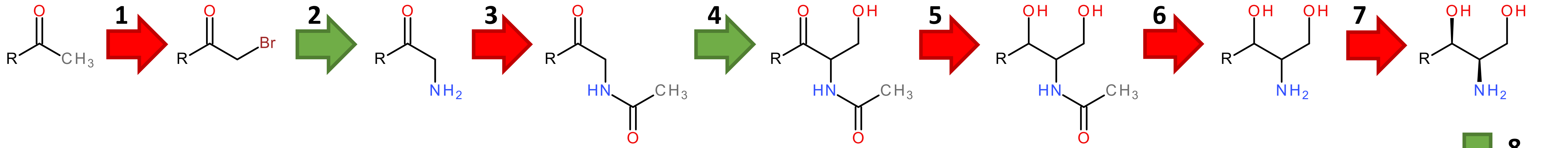
J. Org. Chem. 2002, 67, 8962-8969

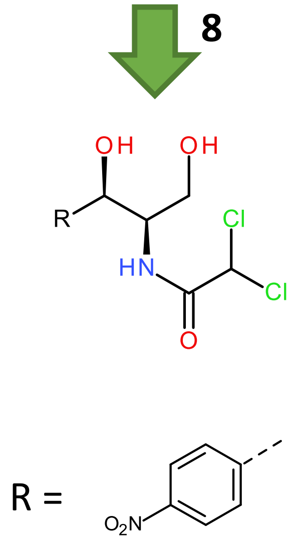


Chloramphenicol

e.g. Typhus treatment of penicillin resistant strains

Traditional Synthesis of Chloramphenicol



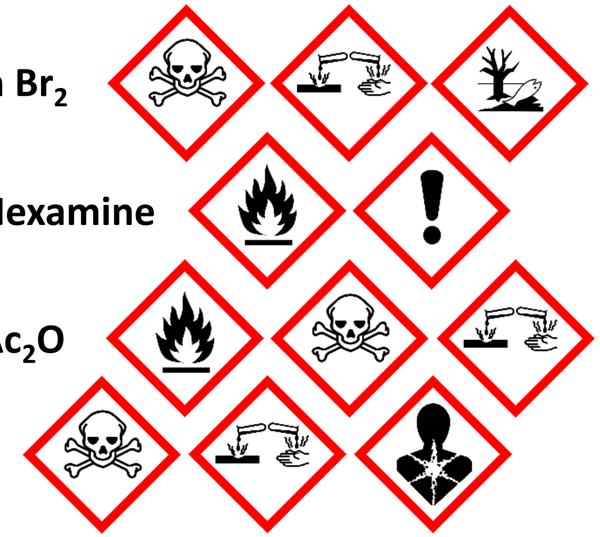


1. Bromination with Br_2

2. Amination with Hexamine

3. Protection with Ac_2O

4. Aldol Addition with Formaldehyde

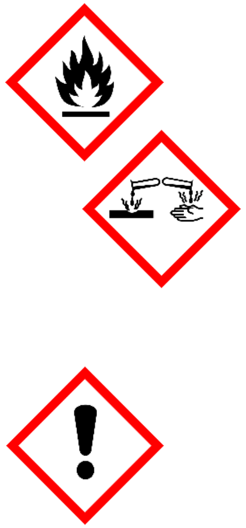


5. Reduction with $Al(i\text{-}PrO)_3$

6. Deprotection with HCl

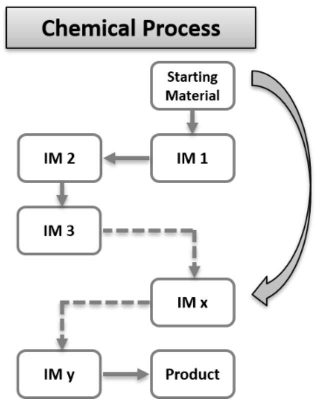
7. Resolution

8. Amidation



→ More efficient and sustainable route is desired

The Benefits of Enzyme Catalysis

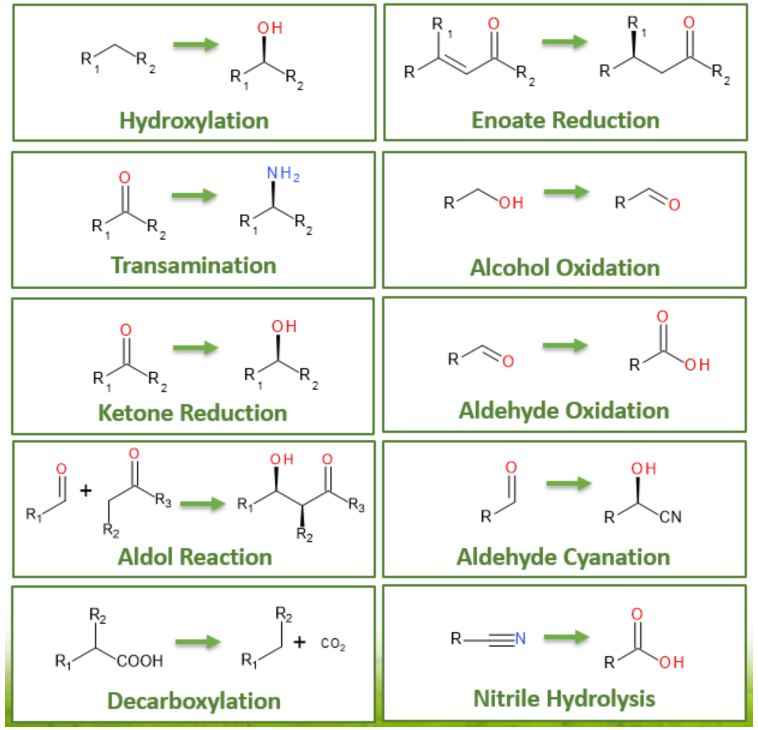


Shorter Synthetic Routes

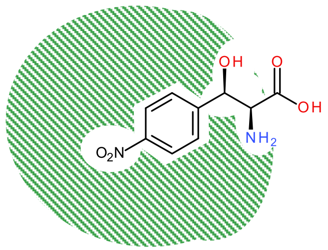
No need for protective group chemistry

No need for prior substrate activation

Very Diverse Reaction Set



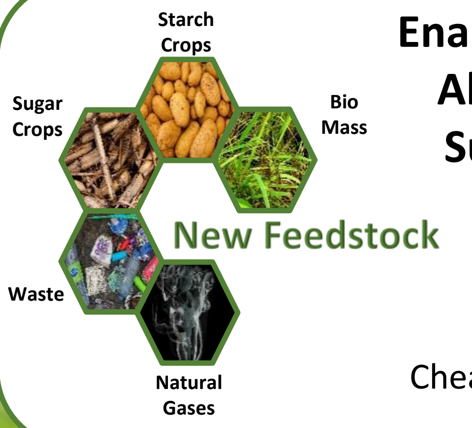
Extraordinary Selectivity



Stereo selectivity
Regio selectivity
Chemo selectivity

By specific substrate binding

Enabling use of Alternative Substrates



e.g.
Bio based
or
Cheaper materials

Mild Environment Friendly Reaction Conditions

e.g.
Ambient temperature

No heavy metals

Enzymatic Approach for Chloramphenicol

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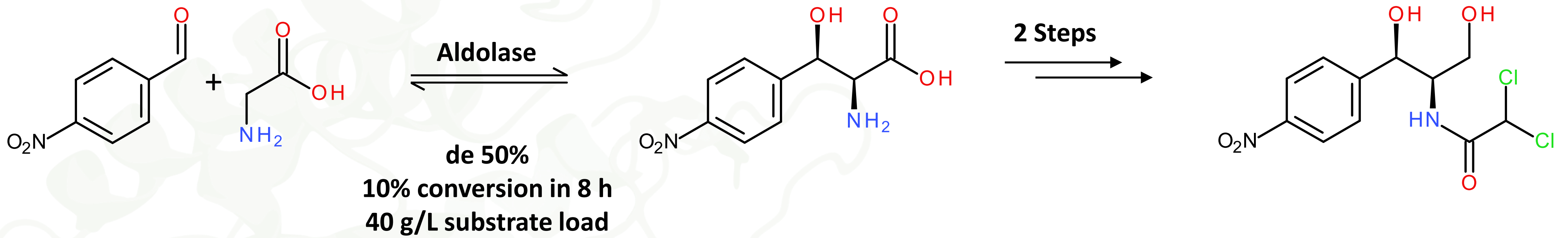


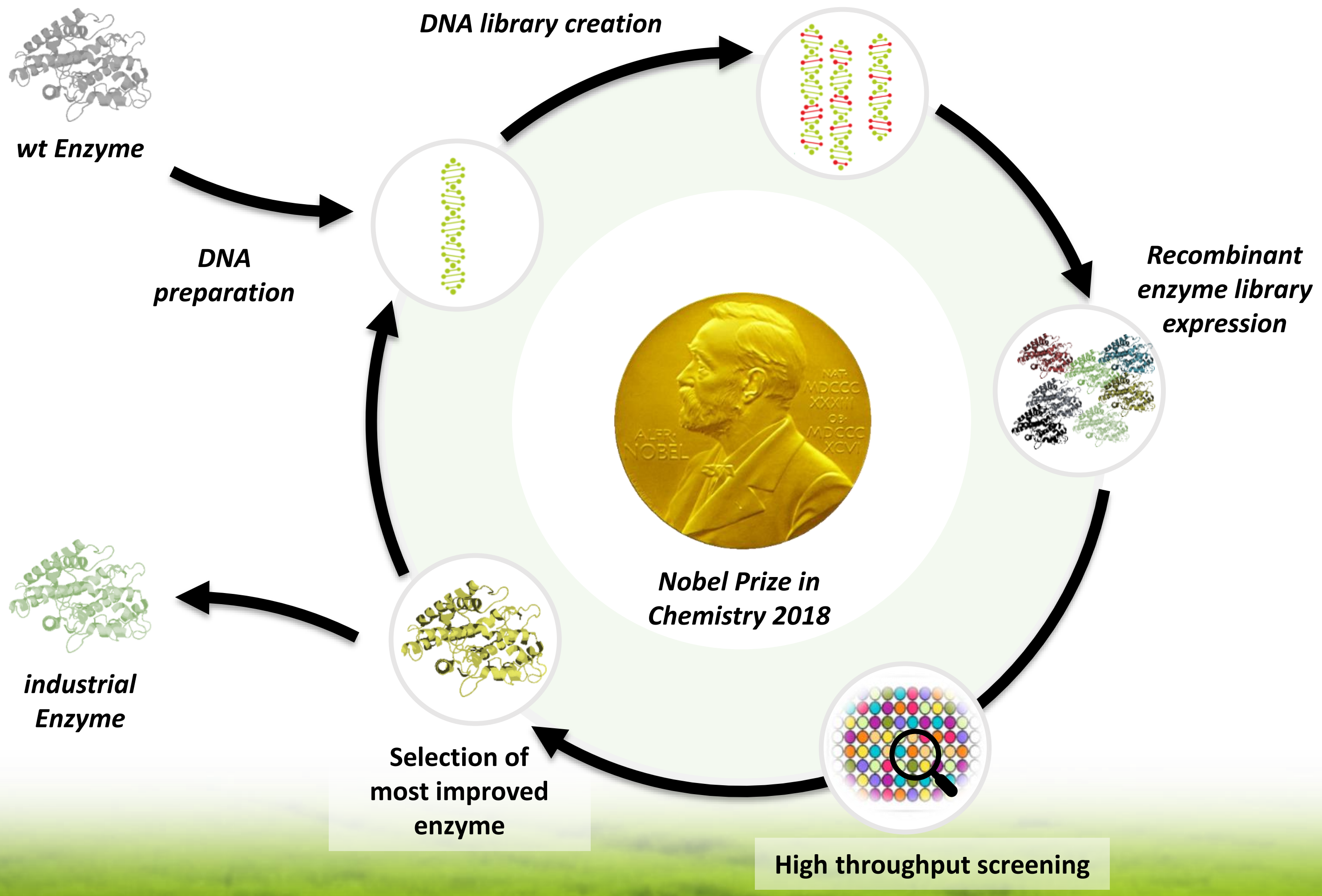
Problem:

Natural enzyme is industrially not applicable

Solution:

Enzyme engineering by directed evolution using BioEngine®

Common Directed Evolution Approaches



1. DNA Libraries by Error Prone PCR

+ Theoretically full sequence space coverage
- Library size 10^7 to 10^9 variants
  - → Screening effort often ca. 100k variants
  - → HPLC / GC screening not applicable
  - → Loss of information
- Subsequent site saturation recommended

2. Site Saturation Mutagenesis or DNA Shuffling

+ Smaller library sizes
+ HPLC / GC screening applicable
  - → Comprehensive reaction insights
- Superficial sequence space coverage
- Easily stagnates at local maxima

Directed Evolution Using BioEngine®

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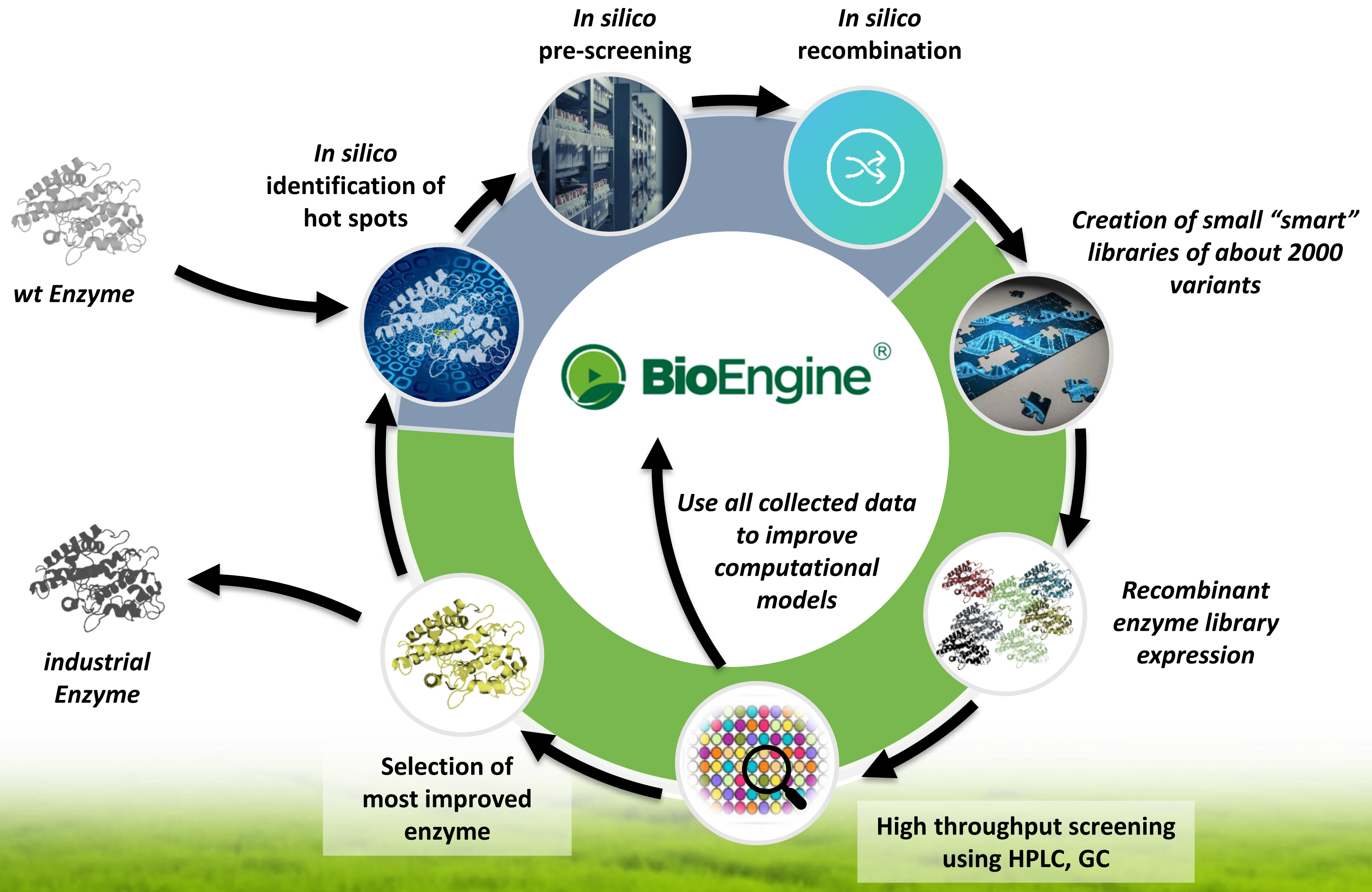

BioEngine®

- *In silico* **hot spot identification** covers full sequence space
- *In silico* enzyme **activity screening** (10,000 variants/day)
- *In silico* **stability screening** (1,000 variants/day)
- All screenings under **real process conditions**
- HPLC/GC analytics provide comprehensive reaction insights
- Only **4-5 weeks per round** of evolution
- Online improvement of *in silico* methods

Optimization of Aldolase for Industrial Application using BioEngine®

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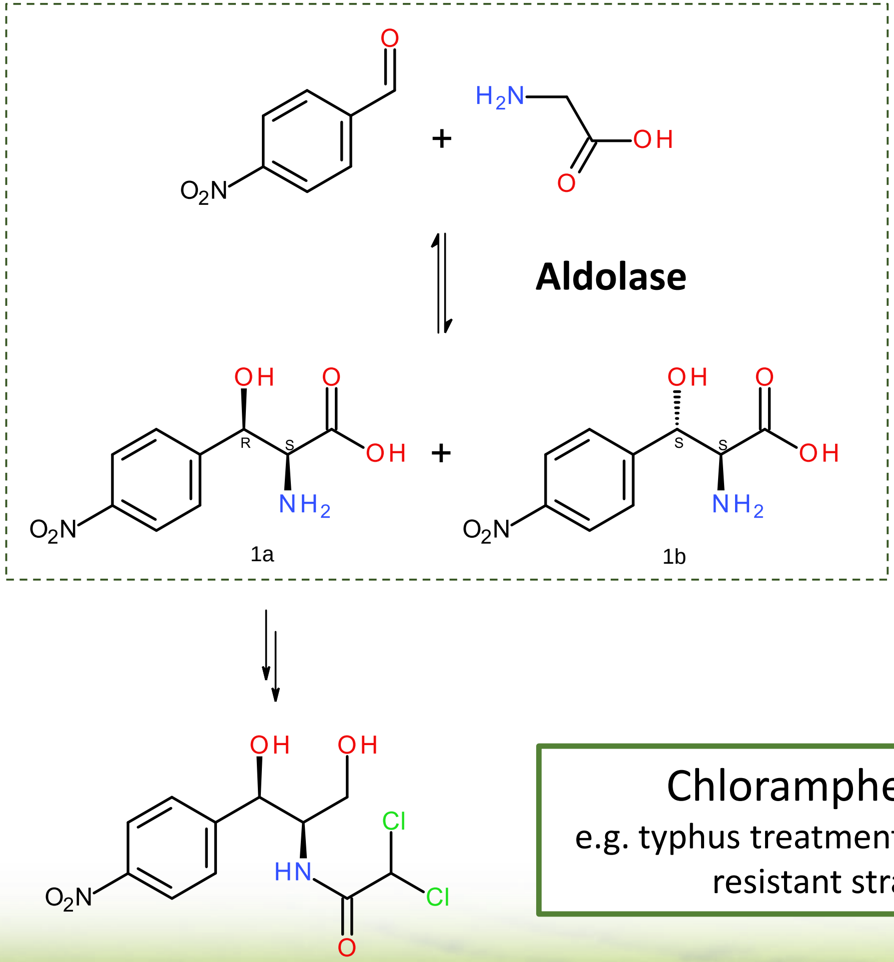

Wildtype Enzyme:

- de 50%
- 10% conversion in 8 h
- 40 g/L substrate load

BioEngine®

Industrial Enzyme:

- de >90%
- >80% conversion in 8 h
- 200 g/L substrate load

9 rounds of evolution

Chloramphenicol
e.g. typhus treatment of penicillin resistant strains

5 steps (60 %) shorter route compared to chemical synthesis

Patent application No.: PCT/CN2018/086227

Who is Enzymaster?

Enzymaster 酶赛 - Short Company Presentation

Enzymaster - Where Green Magic Happens

International consortium of founders

Zhenlin Lv
President

Yong Koy Bong, PhD
Chairman

Dr. Thomas Daußmann
EVP

Haibin Chen, PhD
VP

Enzymaster (Ningbo) Bio-Engineering Co., Ltd. in Ningbo (China):
Lab space: ca. 3000 m^2
Fermentation pilot plant: up to 1000 L
Employees: ca. 100 (60% in R&D and Tech)

Enzymaster Deutschland GmbH in Düsseldorf (Germany):
Employees: 4

Shanghai:
SJTU π-Supercomputer account
Computational enzyme engineering
500,000 CPU hours/year

Enzymaster - History

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2019
- Moved R&D to new space with ca. 3000 m^2.

2018
- Enzymaster Deutschland GmbH established.
- First international enzyme evolution project.

2017
- Headquarters with 1400 m^2 R&D space established.
- Round-A Financing completed.
- Awarded as HI-TECH enterprise.

2016
- Listed on Ningbo Equity Exchange Board.
- Technology commercialized at partner's site (> 10 mt scale).

2015
- BioEngine® established. First enzyme evolution project initiated.
- Scientific Advisory Board established.

2014
- Recipient of "Ningbo 3315 Programme" (Startup Team).
- Angel-Round Financing completed.

2013
- Enzymaster (Ningbo) Bio-Engineering Co., Ltd. established as sino-foreign Joint Venture

Setting up of BioEngine® platform (2013–2015)

Our Asset - Enzyme Directed Evolution

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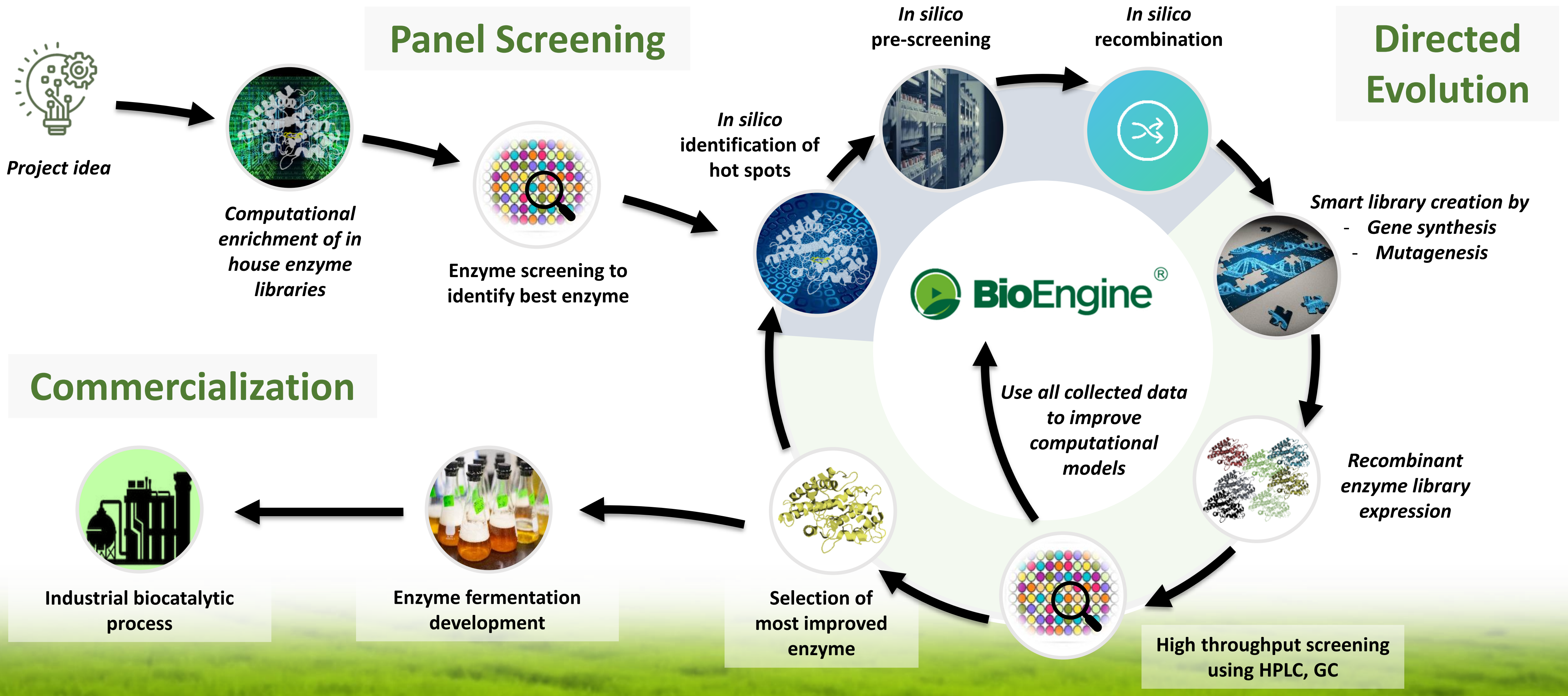
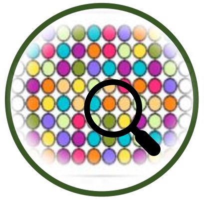


Enzymaster - Service Offer

Insufficient Enzyme

BioEngine®

Superior Industrial Enzyme



Enzyme panel screening

„Smart“ enzyme directed evolution

Enzyme preparation by fermentation

Enzyme catalytic process development

Custom biocatalytic manufacturing

Idea

R&D

Industrial process

Enzymasters’ Computational Resources

- In-house hardware:
  Graphics workstations
  Linux file server
  Linux GPU compute nodes

- SJTU π-Supercomputer account
  332 CPU Nodes 2x Xeon E5 (16 cores)
  20 fat Nodes 256GB RAM
  70 GPU Nodes (K20/40/80/P100)
  Usage of ~ 500,000 core hours/year

→ Extensive Lab & Computer Resources allow to perform up to 6 evolution projects in parallel

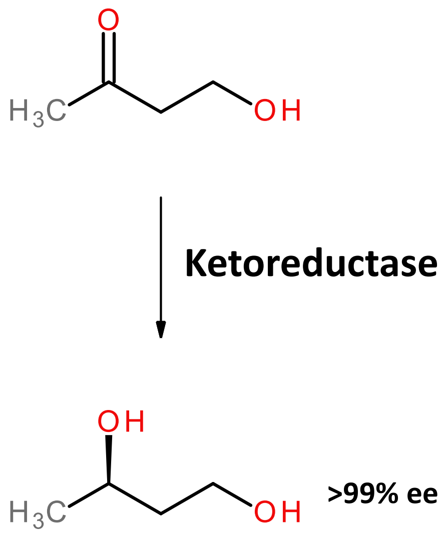
Enzymaster - Success Stories

Idea

R&D

Industrial process

BioEngine®



50 MT scale Production of (*R*)-1,3-Butanediol

Initial Enzyme:

- 48 h
- 57% conversion
- 60 g/L substrate load

BioEngine®

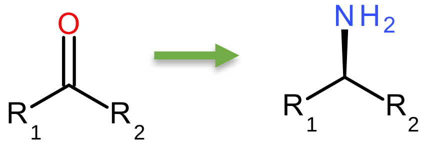
- **3 rounds of evolution**
- **3 months**

Industrial Enzyme:

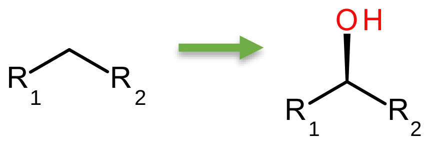
- 24 h
- 99% conversion
- 400 g/L substrate load

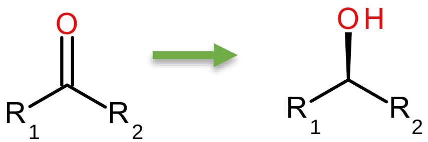
Patent application No.: 201811537509.4
Yet to be published

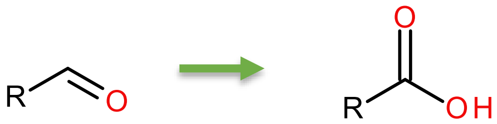
Key Reaction Types & Enzyme Classes

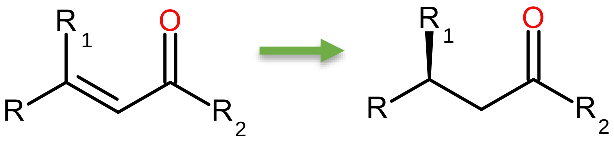

Transamination

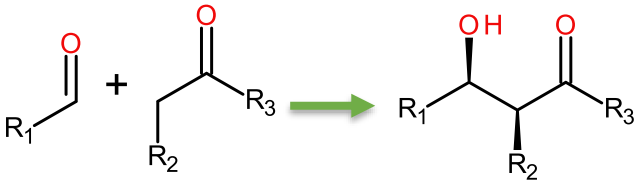

Alcohol Oxidation

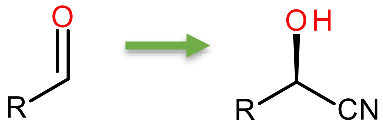

Hydroxylation

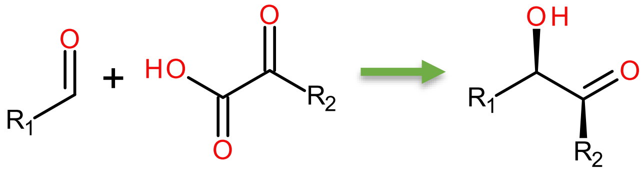

Ketone Reduction

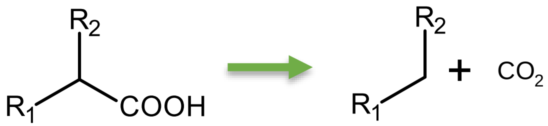

Aldehyde Oxidation

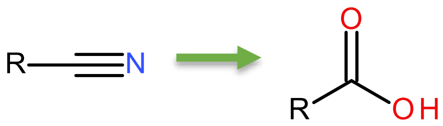

Enoate Reduction

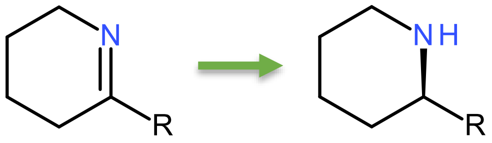

Aldol Reaction


Aldehyde Cyanation


Carboligation


Decarboxylation


Nitrile Hydrolysis


Imine Reduction

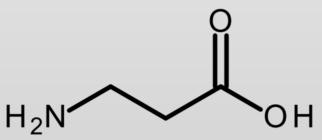
Key Enzyme Classes

- Aminotransferases
- Ketoreductases/ADHs
- Aldolases
- Esterases/Lipases
- Decarboxylases
- Oxynitrilases
- Nitrilases
- TPP-dep. Lyases

Enzymaster - Top Commercial Products

Enzymes developed for own productions are demonstrating our industrial competence

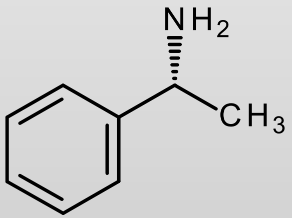
Building block and food supplement:
β-Alanine



Production Scale:
100 – 150 mt/month

Application:
Pharma and Food

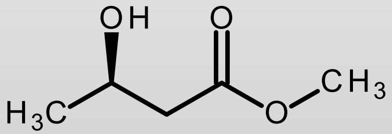
Chiral auxiliary:
(R)-α-Phenylethylamine
R-PEA



Production Scale:
1000 mt

Application:
Pharma

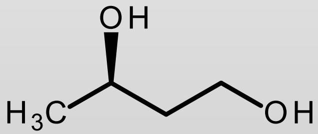
Chiral building block:
Methyl(R)-3-hydroxybutyrate
R-MHB



Production Scale:
Up to 100 mt

Application:
Pharma and Food

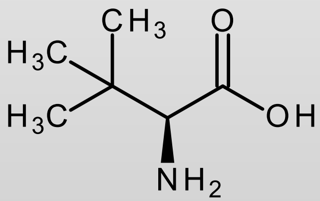
Chiral building block:
(R)-1,3-Butanediol
R-BDO



Production Scale:
Up to 50 mt

Application:
Pharma and Cosmetics

Chiral building block:
L-*tert*-Leucine



Production Scale:
Up to 10 mt

Application:
Pharma

Enzymaster - Further Commercial Products

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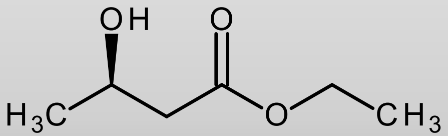
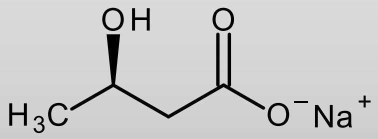
Ethyl (R)-3-Hydroxybutyrate R-EHB



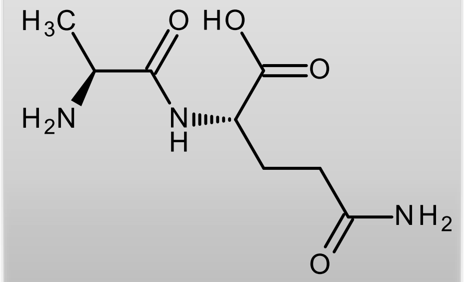
Food Supplement

(R)-3-Hydroxybutyric Acid, Sodium Salt R-BHB



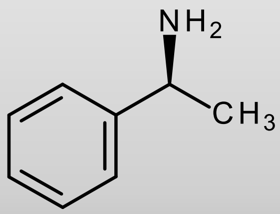
Food Supplement

Glutamine-S



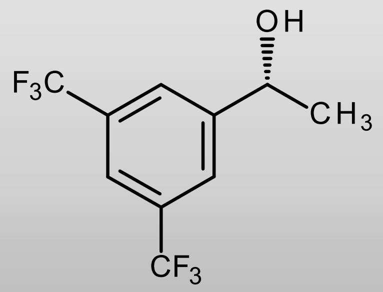
Food Supplement

(S)-α-Phenylethylamine



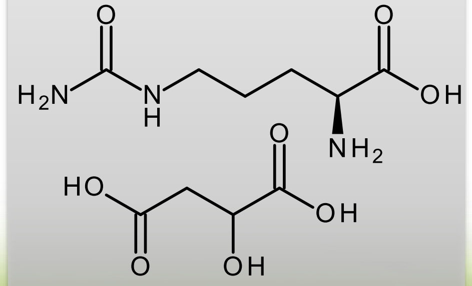
Chiral auxiliary

(R)-1-[3,5-Bis(trifluoro-methyl)phenyl]ethanol



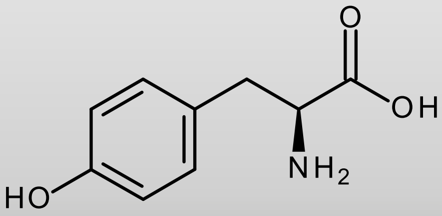
Aprepitant IM

L-Citrulline DL-Malate 2:1



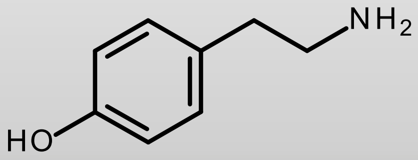
Food Supplement

L-Tyrosine



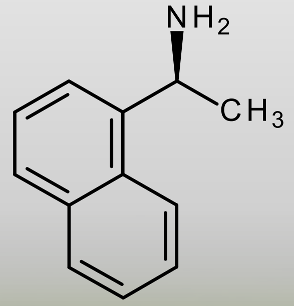
Food Supplement

Tyramine



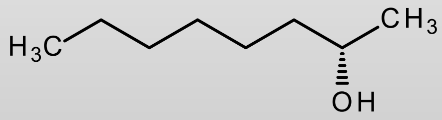
Food Supplement

(R)-α-Naphtylethyl-amine



Cinacalcet IM

(S)-2-Octanol



Chiral building block

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Green magic happens here

We aim to contribute to a better societal and environmental future

Proprietary Technology

Industrial Innovation

Environmental Impact