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International Year of CHEMISTRY 2011

POLITECNICO DI MILANO

Dip. Chimica, Materiali e Ingegneria Chimica "G. Natta"

6th October 2011





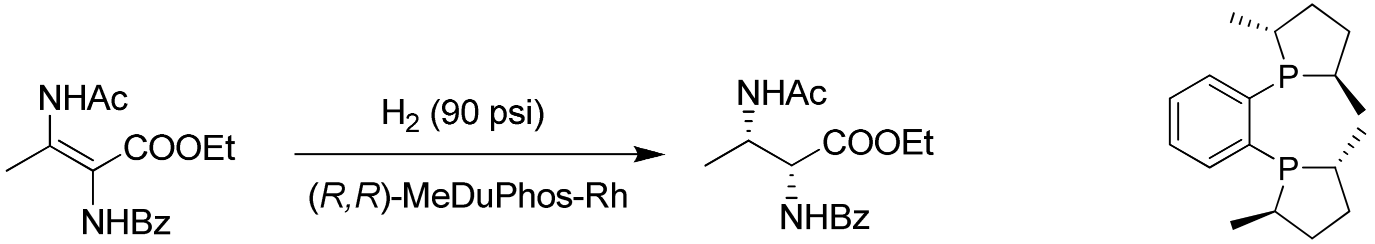
PRODUCTIVITY ENHANCEMENT OF BIOCATALYSED ENANTIOSELECTIVE C=C REDUCTIONS

THE BIOREDUCTION OF ALPHABROCINE
FROM WHOLE CELLS TO ISOLATED ENZYMES

Fabio Parmeggiani

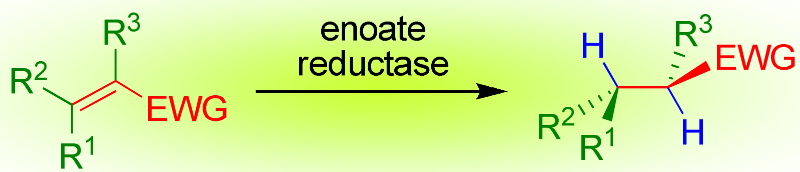
Enantioselective C=C reductions

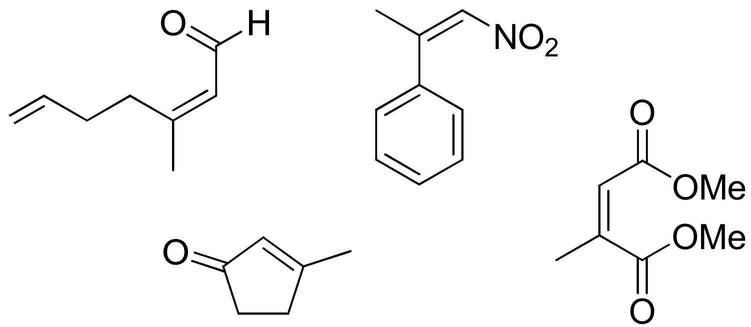
The asymmetric hydrogenation of prochiral C=C bonds is one of the most exploited strategies for the creation of up to two stereogenic centres.



(*R,R*)-MeDuPhos

Biocatalysis offers a highly stereoselective complementary or alternative variant: **enoate reductases** catalyse the reduction of C=C bonds activated with electron-withdrawing groups.





Baker's yeast in organic synthesis

Most of these reactions on preparative scale have been carried out with several microbial cells systems, particularly **Saccharomyces cerevisiae** (**baker's yeast**, b.y.).

Advantages:

- nonpathogenic and very simple to grow and use (without the help of microbiologists)
- extremely inexpensive (~1 €/kg)
- enzymes and cofactors are protected within the cell
- reaction setup is simple and practical

Baker's yeast in organic synthesis

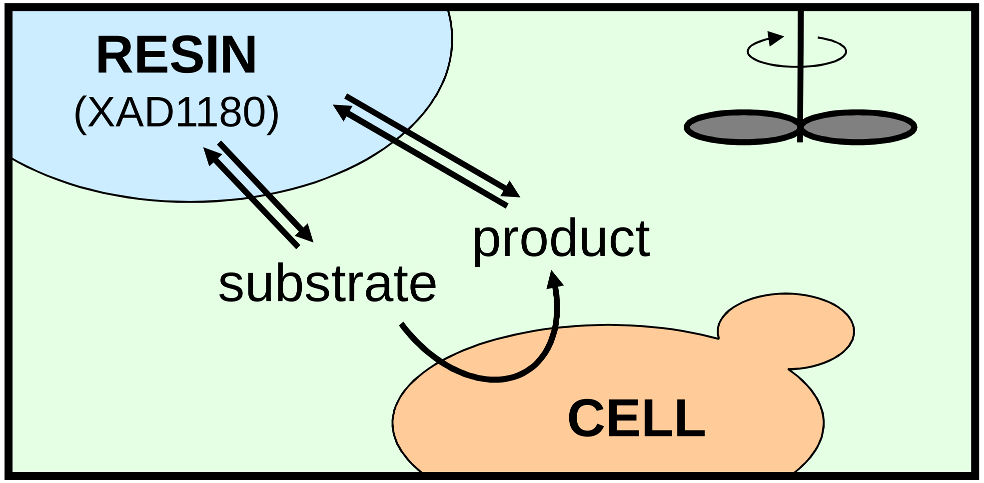
Several drawbacks hinder the industrial scale application:

- Huge amounts of biomass and water to deal with (product recovery is rather troublesome)
- Need for a chromatographic separation downstream
- Poor tolerance towards high concentration of substrates
- Usually non-quantitative conversions, often with side-activities (e.g. carbonyl reduction or ester hydrolysis) due to the presence of many other enzymes besides enoate reductases

Overall, these factors result in a **low productivity**, usually **< 0.5 g L^{-1} d^{-1}**

In situ substrate-feeding-product-removal (SFPR)

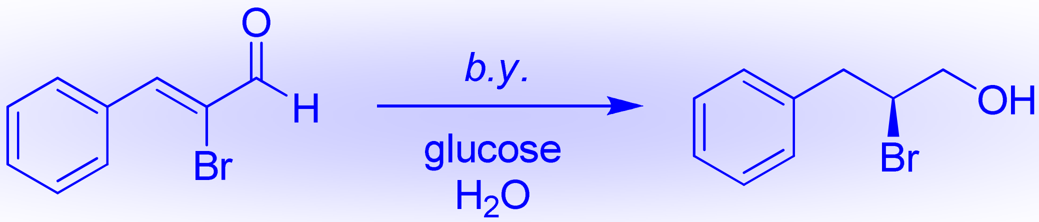
The substrate, typically lipophilic, is adsorbed on a **hydrophobic aromatic resin** (styrene-divinylbenzene copolymer). Therefore, it is released in the aqueous medium at very low concentrations and the product is readily removed as well.



Advantages:

- low concentrations minimize inhibition and toxic effects
- the recovery of reaction products is easier (solid phase extraction)

Baker's yeast mediated reduction of alphabrocine



First reported in 1977 - it was observed that the *ee* of the product is strongly dependent on the substrate concentration.

With the application of the SFPR strategy high yields and excellent *ee*s can be obtained.

Entry	Loading (gL^{-1})	Time (h)	Biocatalyst	Resin/substrate ratio	Yield (%)	*ee* (%)	Productivity ($gL^{-1}d^{-1}$)
1	0.1	48	b.y.	-	>90	91	<0.01
2	5.0	48	b.y.	-	>90	68	1.89
3	5.0	48	b.y.	1	91	99	2.26

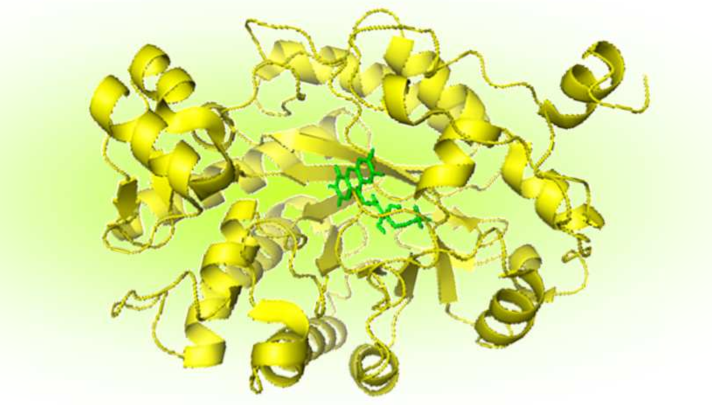
The productivity (defined as the product of loading and yield of the desired enantiomer, divided by the reaction time) is still too low!

Yeast enoate reductases: Old Yellow Enzymes

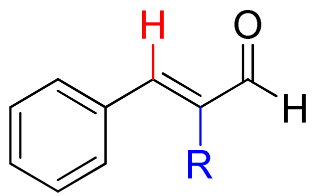
Discovered in **1933** (Warburg and Christian)

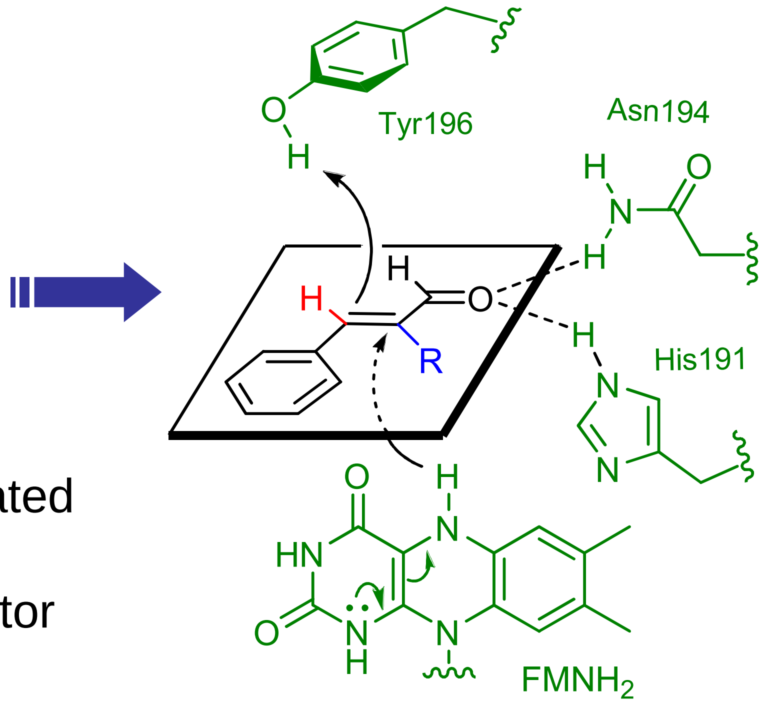
Family of closely similar **flavoproteins**:

- **OYE1** and **OYE2** in *S. pastorianus*
- **OYE2** and **OYE3** in *S. cerevisiae*
- many homologs in superior organisms

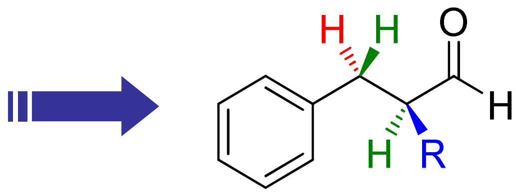


Mechanism:





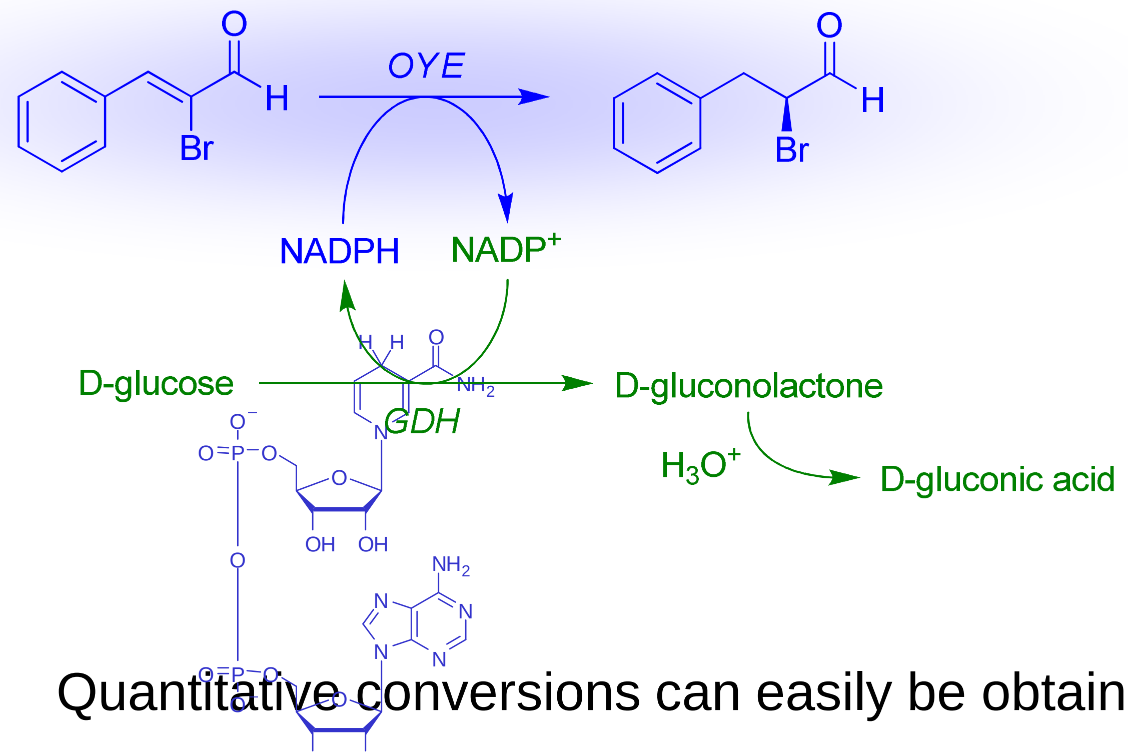
STEREOSPECIFIC ANTI-ADDITION

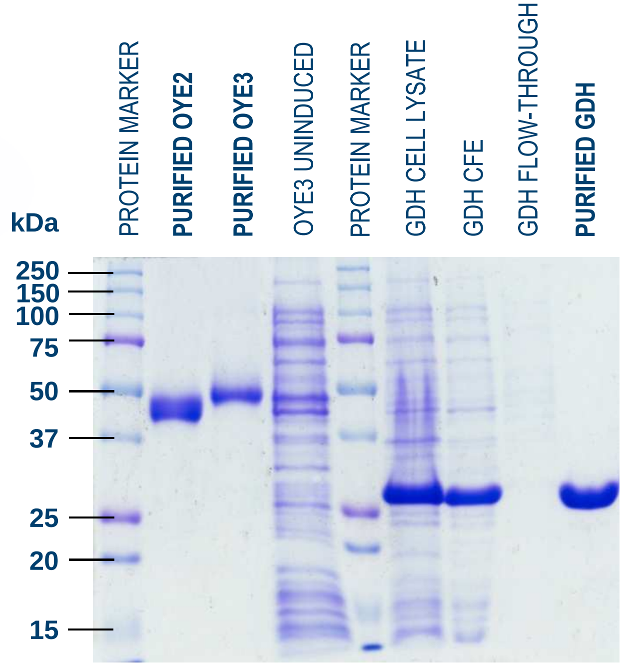


FMNH$_2$ is regenerated at the expense of NAD(P)H cofactor

α-hydride from the bottom
β-proton from the top

OYE mediated reduction of alphabrocine

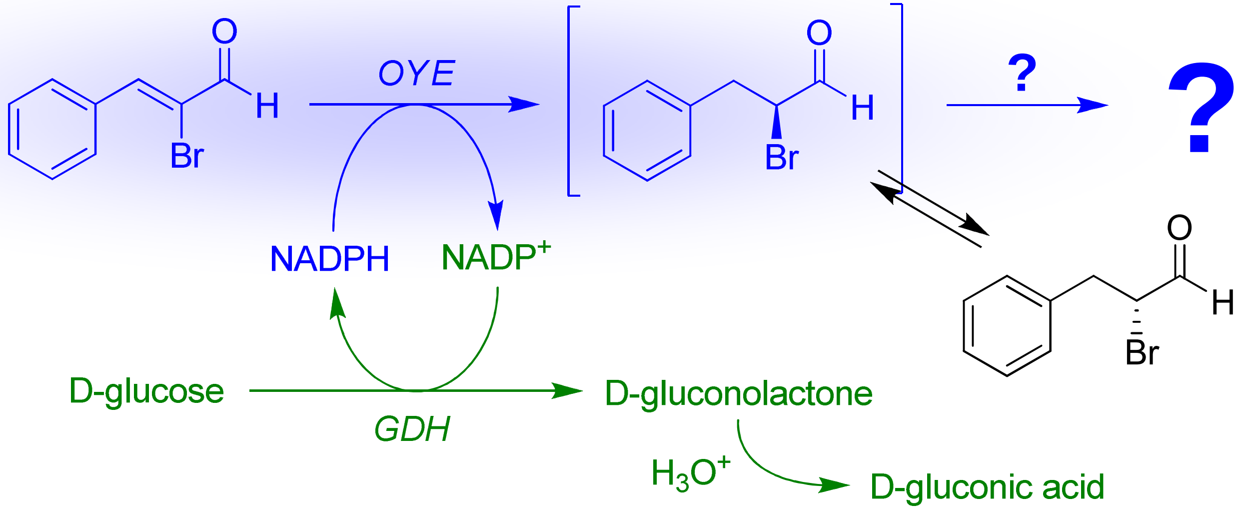




Quantitative conversions can easily be obtained, but *ees* are quite low.

Entry	Loading (gL^{-1})	Time (h)	Biocatalyst	Resin/substrate ratio	Yield (%)	*ee* (%)	Productivity ($gL^{-1}d^{-1}$)
1	1.0	6	OYE1	-	100	55	3.10
2	1.0	6	OYE2	-	100	64	3.28
3	1.0	6	OYE3	-	100	36	2.72

OYE mediated reduction of alphabrocine

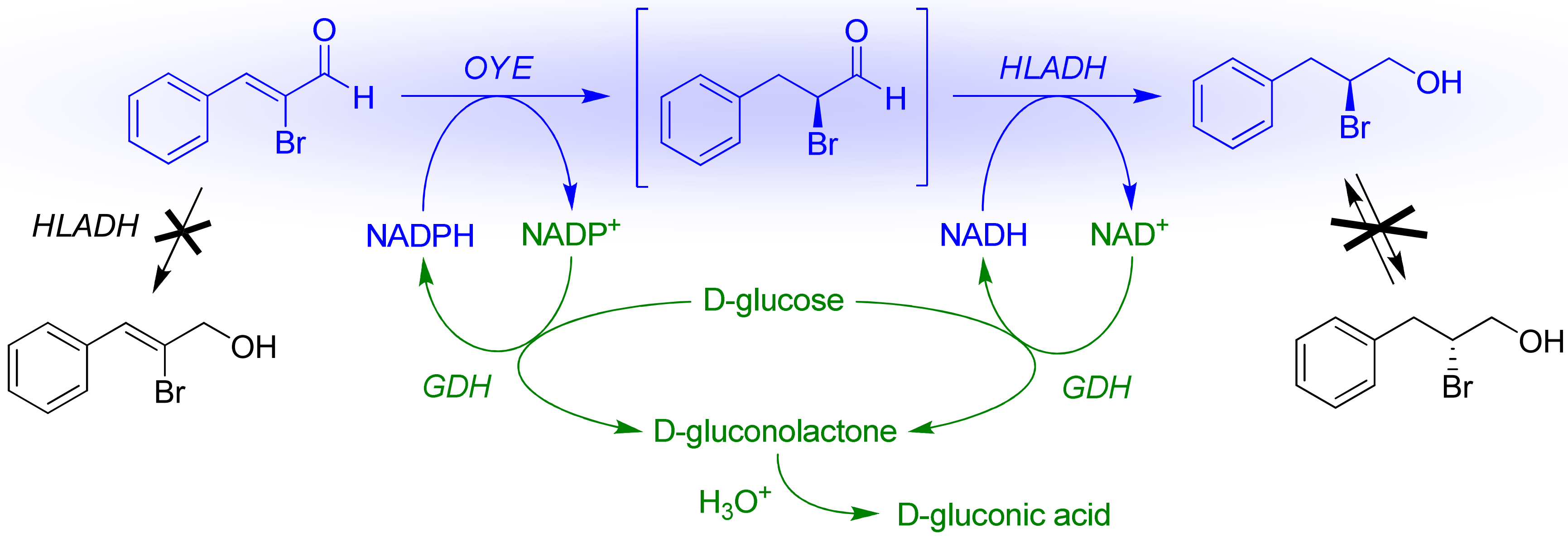


One more step is needed to preserve the optical purity of the aldehyde…

Disappointingly, even the application of the SFPR strategy does not provide a large improvement: the saturated aldehyde racemizes easily.

Entry	Loading (gL^{-1})	Time (h)	Biocatalyst	Resin/substrate ratio	Yield (%)	*ee* (%)	Productivity ($gL^{-1}d^{-1}$)
1	5.0	6	OYE1	1	100	74	17.40
2	5.0	6	OYE2	1	100	81	18.10
3	5.0	6	OYE3	1	100	62	16.20

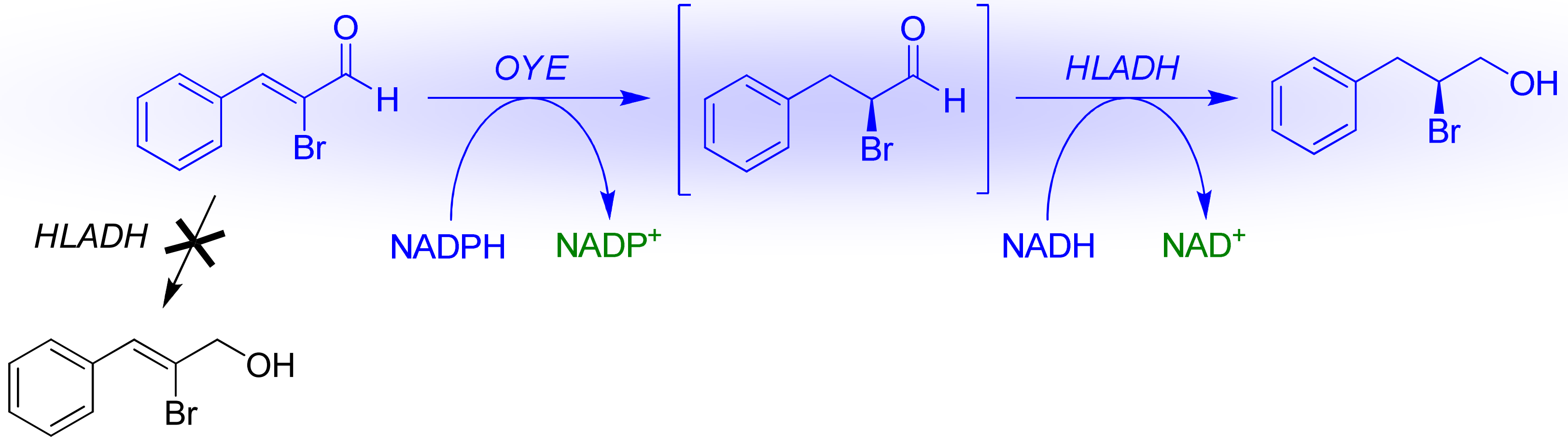
OYE-ADH mediated reduction of alphabrocine



A screening of commercial ADHs allowed to select the **horse liver alcohol dehydrogenase (HLADH)** for being extremely chemoselective.

Entry	Loading (gL^{-1})	Time (h)	Biocatalyst	Resin/substrate ratio	Yield (%)	*ee* (%)	Productivity ($gL^{-1}d^{-1}$)
1	5.0	6	OYE1+ADH	1	100	95	19.50
2	5.0	6	OYE2+ADH	1	100	98	19.80
3	5.0	6	OYE3+ADH	1	100	95	19.50

OYE-ADH mediated reduction of alphabrocine



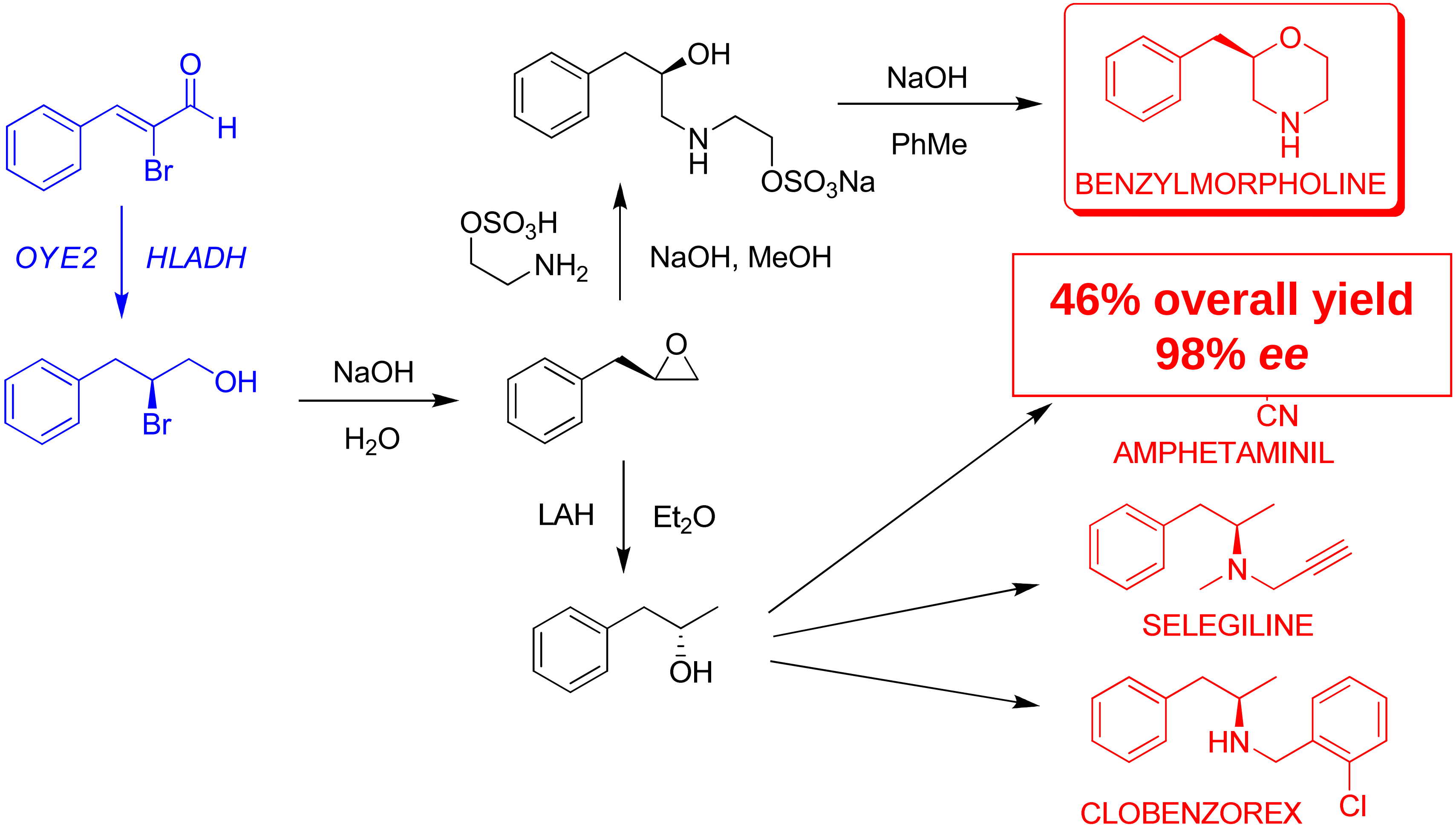
Productivity enhancement

Entry	Loading (gL^{-1})	Time (h)	Biocatalyst	Resin/substrate ratio	Yield (%)	*ee* (%)	Productivity ($gL^{-1}d^{-1}$)
1	10.0	6	OYE2+ADH	1	100	98	39.60
2	20.0	6	OYE2+ADH	1	100	97	78.80
3	30.0	6	OYE2+ADH	1	100	98	118.80
4	40.0	6	OYE2+ADH	1	69	97	108.74
5	50.0	6	OYE2+ADH	1	36	98	75.24

Over **50-fold improvement** with respect to the b.y. based process!

Applications

Examples of synthetic applications of the bioreduction of alphabrocine to the preparation of optically active chiral pharmaceuticals:



Acknowledgements

POLITECNICO DI MILANO

Prof. Claudio Fuganti
Prof. Elisabetta Brenna
Dr. Francesco G. Gatti

Istituto di Chimica del Riconoscimento Molecolare

Dr. Daniela Monti

ETH
Department of Biosystems Science and Engineering

Prof. Sven Panke
Christian Femmer

THANK YOU FOR YOUR ATTENTION