BIOCATALYSTS FOR THE SYNTHESIS OF PHARMACOLOGICALLY ACTIVE COMPOUNDS

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BIOCATALYSIS

Pharmacologically active compounds are usually **polyfunctional** and/or **chiral** molecules. Their synthesis requires chemo- regio- and stereoselective transformations.

BIOCATALYSTS

MICROORGANISMS

Saccharomyces cerevisiae

- Ubiquitous
- Cheap
- Easy to use

ENZYMES

Hydrolases

- Wide range of substrate specificity
- Organic solvents
- Cofactors not required

TWO APPLICATIVE EXAMPLES

- BRIVARACETAM

Article

A New Chemoenzymatic Synthesis of the Chiral Key Intermediate of the Antiepileptic Brivaracetam

Samuele Ciceri [1,*], Paride Grisenti [2], Shahrzad Reza Elahi [1] and Patrizia Ferraboschi [1,*]

Molecules (**2018**), *23*, 2206

- PRAMIPEXOLE

Baker's yeast catalyzed preparation of a new enantiomerically pure synthon of (*S*)-pramipexole and its enantiomer (dexpramipexole)

Patrizia Ferraboschi [a,*], Samuele Ciceri [a], Pierangela Ciuffreda [b], Maria De Mieri [a,†], Diego Romano [c], Paride Grisenti [d]

Tetrahedron: Asymmetry (**2014**), *25*, 1239–1245

Seawater-Based Biocatalytic Strategy: Stereoselective Reductions of Ketones with Marine Yeasts

Immacolata Serra,[a] Benedetta Guidi,[b] Gaetan Burgaud,[c] Martina L. Contente,[a] Patrizia Ferraboschi,[b] Andrea Pinto,*[d] Concetta Compagno,[a] Francesco Molinari,[a] and Diego Romano*[a]

ChemCatChem (**2016**), *8*, 3254 – 3260

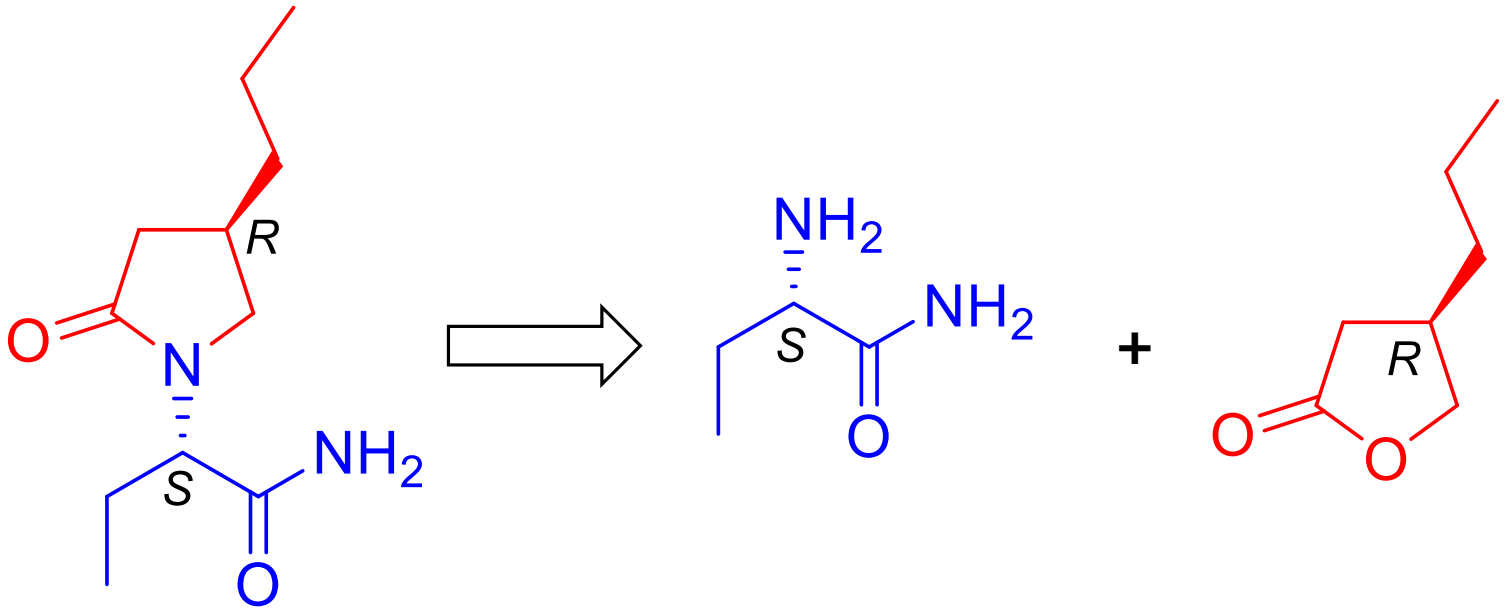
(*S*)-Pramipexole and Its Enantiomer, Dexpramipexole: A New Chemoenzymatic Synthesis and Crystallographic Investigation of Key Enantiomeric Intermediates

Samuele Ciceri [1,*], Patrizia Ferraboschi [1], Paride Grisenti [2], Shahrzad Reza Elahi [1], Carlo Castellano [3], Matteo Mori [4] and Fiorella Meneghetti [4,*]

Catalysts (**2020**), *10*, 941

BRIVARACETAM

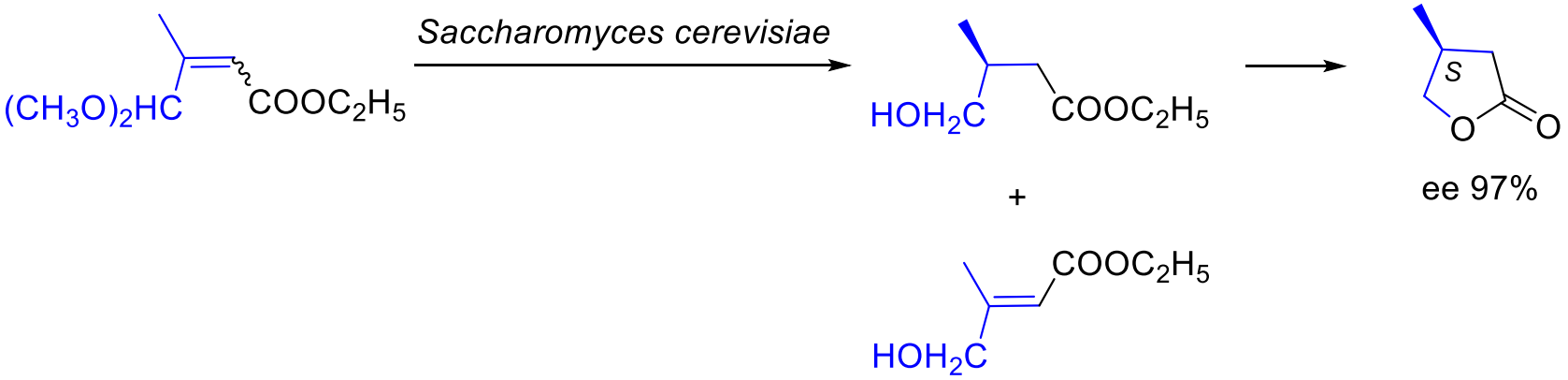
ANTICONVULSANT DRUG (2016)



Schule, A.; Merschaert, A.; Szczepaniak, C.; Marechal, C.; Carly, N.; O'Rourke, J.; Ates, C. *Org. Process Res. Dev.* **2016**, *20*, 1566-1575
Wang, P.; Li, P.; Wei, Q.; Liu, Y. *Processes to produce brivaracetam*. WO2016191435A1, 2016.

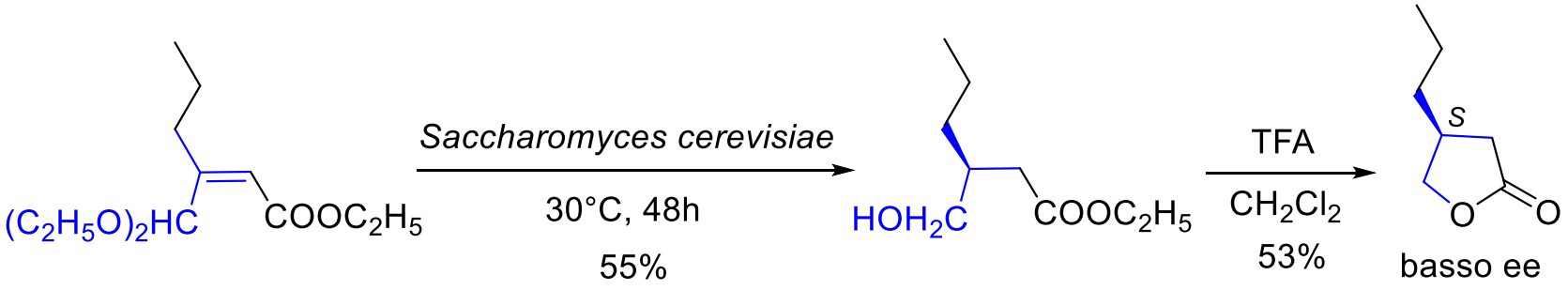
BRIVARACETAM

1. *Saccharomyces cerevisiae* (reduction of activated double bonds)



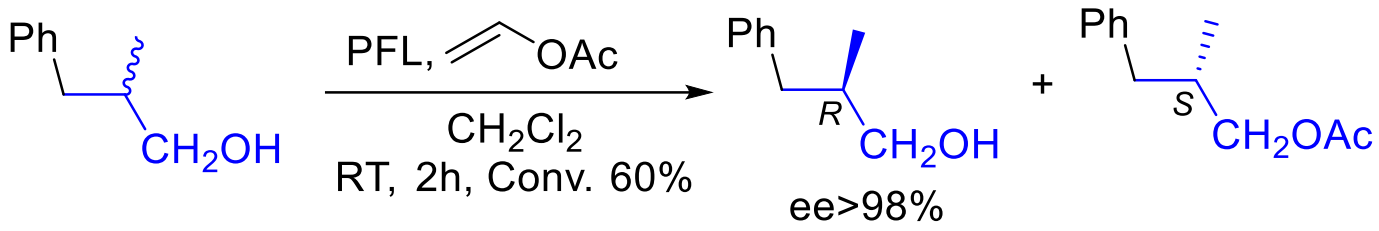
Leuenberger, H. G. W.; Boguth, W.; Barner, R.; Schmid, M.; Zell, R. *Helv. Chim. Acta* **1979**, *62*, 455-463.

Results

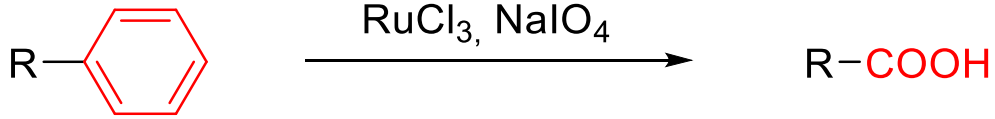


BRIVARACETAM

2. Lipases (kinetic resolution of alcohols)



Ferraboschi, P.; Casati, S.; De Grandi, S.; Grisenti, P.; Santaniello, E. *Biocatalysis* **1994**, *10*, 279-288

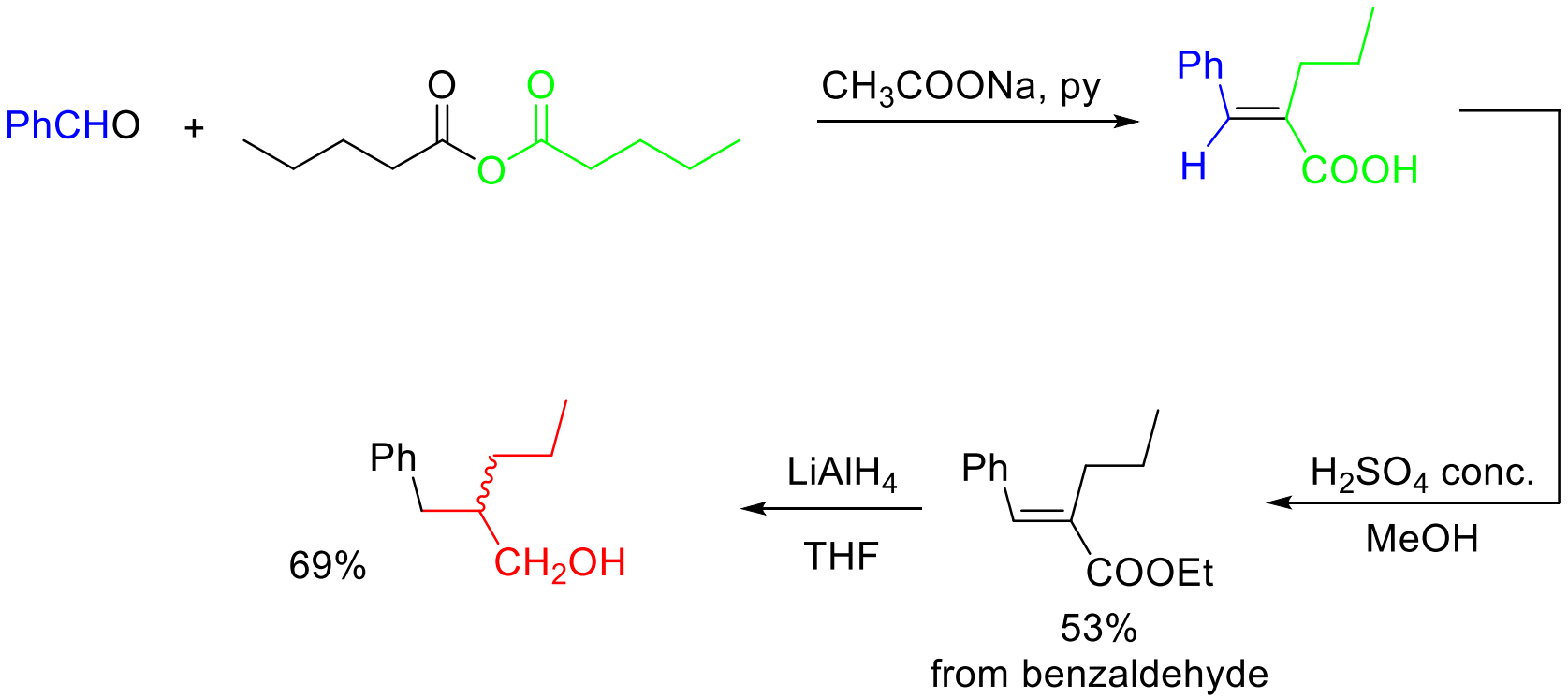


Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936–3938.

PFL = *Pseudomonas fluorescens* lipase

BRIVARACETAM

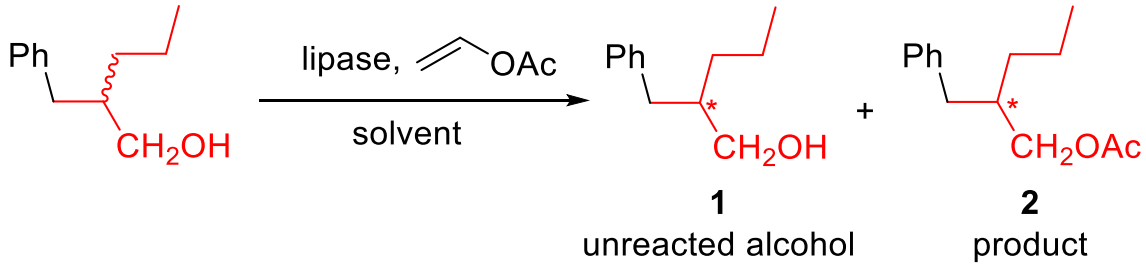
Synthesis of the racemic substrate of the lipase-catalyzed resolution



Ciceri, S.; Grisenti, P.; Reza Elahi, S.; Ferraboschi, P. *Molecules* **2018**, *23*, 2206.

BRIVARACETAM

KINETIC RESOLUTION



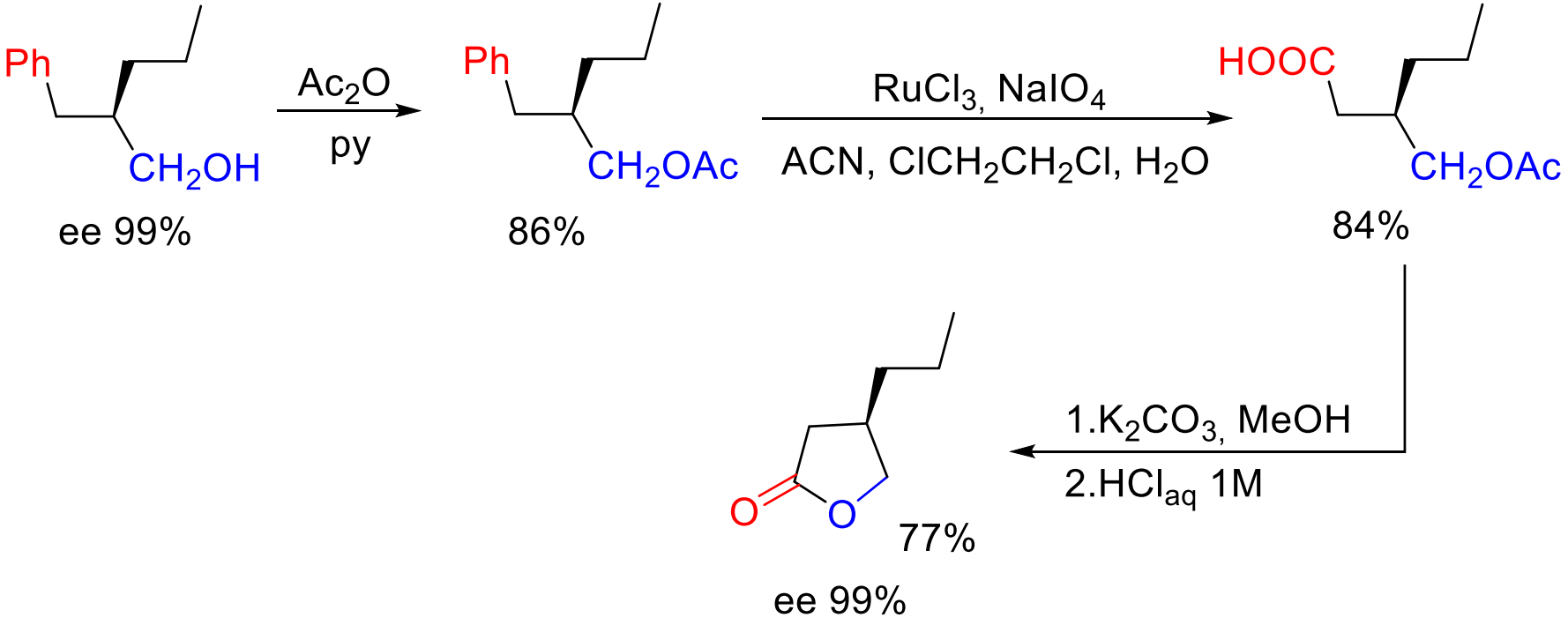
Entry	Lipase	Solvent	Time (h)	Conv. (%)*	ee*, config.	E
1	PFL	CH_2Cl_2	144	35	81[a], *S*	15
2	PFL	Toluene	48	62	99[b], *R*	20
3	CCL	Toluene	24	72	58[b], *S*	3
4	CAL-B Novozym®	Toluene	2	61	5[b], *R*	1
5	PPL	Toluene	192	71	53[b], *S*	2

[a] ee of **2**. [b] ee of **1**. * chiral HPLC analysis

Ciceri, S.; Grisenti, P.; Reza Elahi, S.; Ferraboschi, P. *Molecules* **2018**, *23*, 2206.

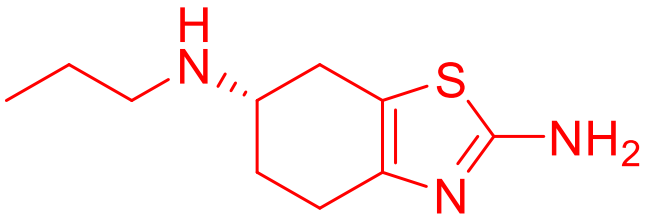
BRIVARACETAM

Accomplishment of the synthesis



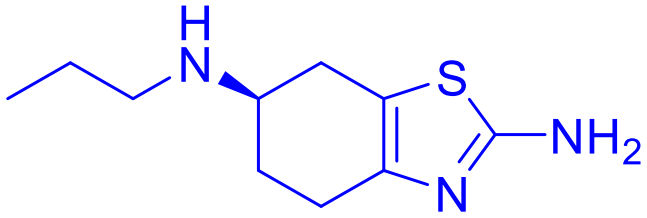
Ciceri, S.; Grisenti, P.; Reza Elahi, S.; Ferraboschi, P. *Molecules* **2018**, *23*, 2206.

PRAMIPEXOLE



(*S*)-pramipexole

In therapy as anti-Parkinson drug



(*R*)-pramipexole

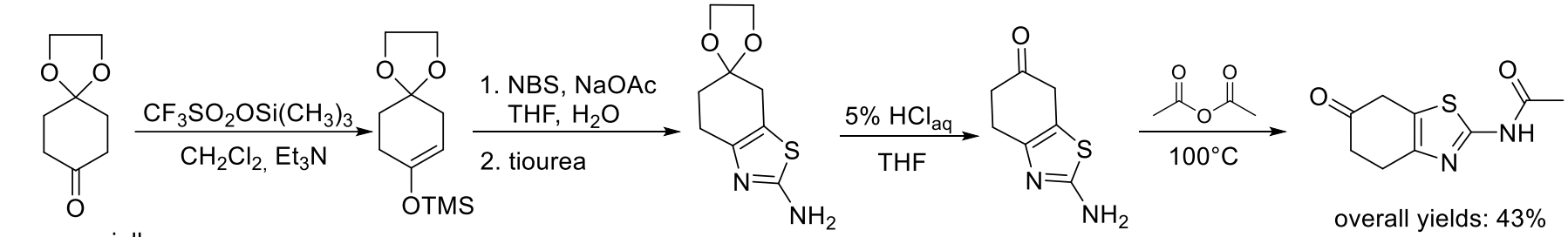
- Investigated for the management of ALS
- Under study for the treatment of eosinophil-associated disorders

Usually, they are resolved by fractional crystallization of a diastereomeric salt or by preparative chiral HPLC

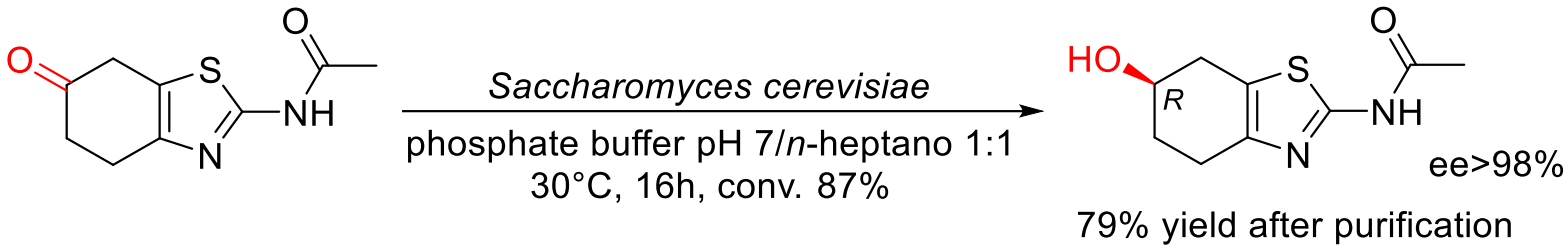
PRAMIPEXOLE

1. *Saccharomyces cerevisiae* (reduction of the carbonyl group)

Synthesis of the carbonyl compound



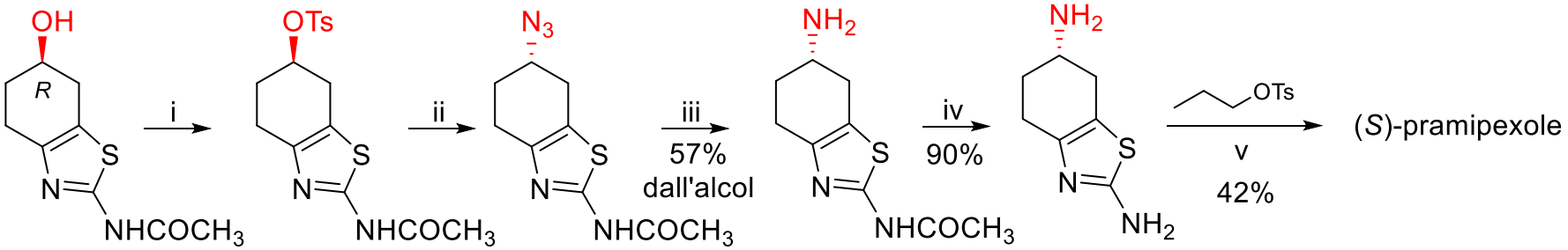
BIOREDUCTION



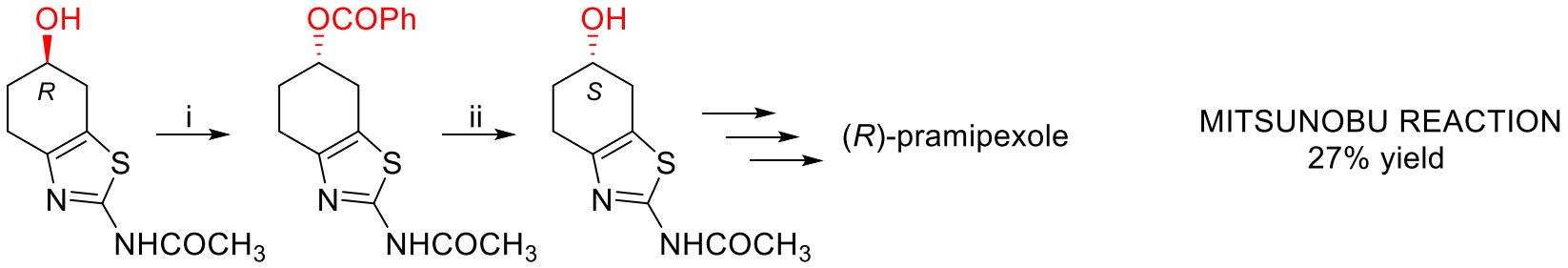
Ferraboschi, P.; Ciceri, S.; Ciuffreda, P.; De Mieri, M.; Romano, D.; Grisenti, P. *Tetrahedron: Asymmetry* **2014**, *25*, 1239-1245.

PRAMIPEXOLE

Accomplishment of the synthesis



i) TsCl, py; ii) NaN_3, DMF; iii) Ph_3P polymer bound, THF/H_2O; iv) 5% HCl, THF; v) $NEt(iPr)_2$, DMF, 1 M NaOH, 12 M HCl.



MITSUNOBU REACTION
27% yield

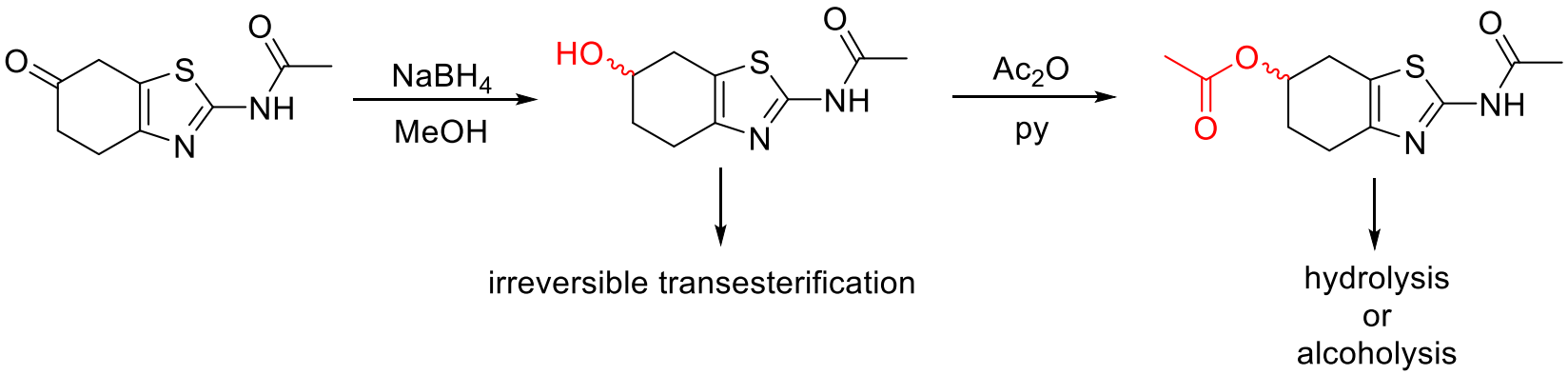
i) PhCOOH, DEAD, Ph_3P, DMF; ii) 1% NaOH, MeOH

Ferraboschi, P.; Ciceri, S.; Ciuffreda, P.; De Mieri, M.; Romano, D.; Grisenti, P. *Tetrahedron: Asymmetry* **2014**, *25*, 1239-1245.

PRAMIPEXOLE

2. Hydrolases (kinetic resolution of racemic alcohols)

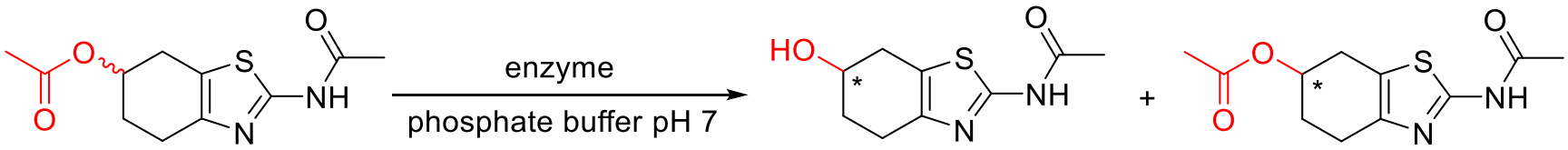
Synthesis of the racemic substrates of the hydrolase-catalyzed resolution



Ciceri, S.; Ferraboschi, P.; Grisenti, P.; Reza Elahi, S.; Castellano, C.; Mori, M.; Meneghetti, F. *Catalysts* **2020**, 10, 941.

PRAMIPEXOLE

HYDROLYSIS



Entry	Enzyme	Time (h)	Conv. (%)[a]	ee (%)[a]; configuration		E
				alcohol	acetate	
1	PPL	168	0	/	/	/
2	PFL	312	22	16; *R*	/	1.4
3	CAL-A Immobead 150	30	46	39; *R*	/	3.1
4	CAL-B Novozym®	120	54	/	31; *S*	2.3
5	CCL	120	64	/	38; *R*	2.1
6	Alcalase CLEA	96	30	37; *R*	/	2.5

[a] chiral HPLC.

ALCOHOLYSIS

Among the previously tested enzymes, only CAL B Novozym® was active (E=3.1, in absolute ethanol).

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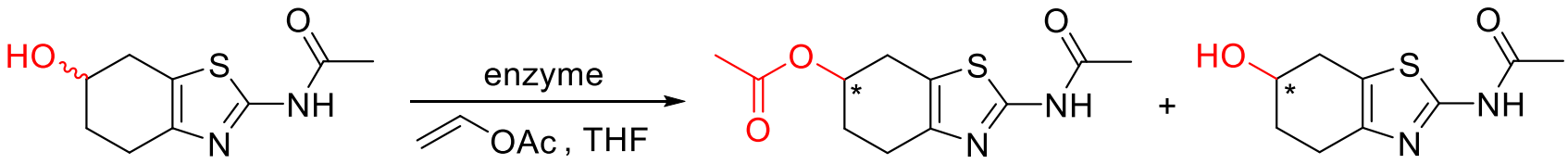
PRAMIPEXOLE

IRREVERSIBLE TRANSESTERIFICATION



Entry	Enzyme	Time (h)	Conv. (%)[a]	ee (%)[a]; configuratiom		E
				alcohol	acetate	
1	Alcalase CLEA	168	0	/	/	/
2	Lipase Amano F-AP15 from Rhizopus oryzae	168	0	/	/	/
3	PPL	168	< 5	/	*R*	/
4	Lipase from Aspergillus Niger	168	14	/	20; *R*	1.5
5	CAL-B Novozym®	7	60	28; *S*	/	1.9
6	CCL	168	20	/	41; *S*	2.6
7	PFL	73	20	/	68; *R*	6.2
8	CAL-A Immobead 150	168	23	/	68; *R*	6.4

[*] Chiral HPLC.

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PRAMIPEXOLE

IRREVERSIBLE TRANSESTERIFICATION
(solvent screening)

Solvent	Enzyme	Time (h)	Conv. (%)[a]	ee (%)[a]; Configuration		E
				alcohol	acetate	
Toluene	PFL	72	35	9; S	15; *R*	3.3
	CAL-A Immobead 150	16	62	10; *S*	6; *R*	1.2
	CCL	4	45	20; *R*	26; *S*	2.0
Methyl Isobutyl ketone	PFL	168	33	26; *S*	72; *R*	7.9
	CAL-A Immobead 150	23	64	91; *S*	61; *R*	12.5
	CCL	120	51	45; *R*	53; *S*	5.0
Acetone	PFL	168	/	/	/	/
	CAL-A Immobead 150	168	34	50; *S*	89; *R*	28.2
	CCL	168	/	/	/	/

[a] *chiral HPLC.*

The higher the hydrophilicity of the reaction medium, the lower the conversion rate and the higher the enantioselectivity.

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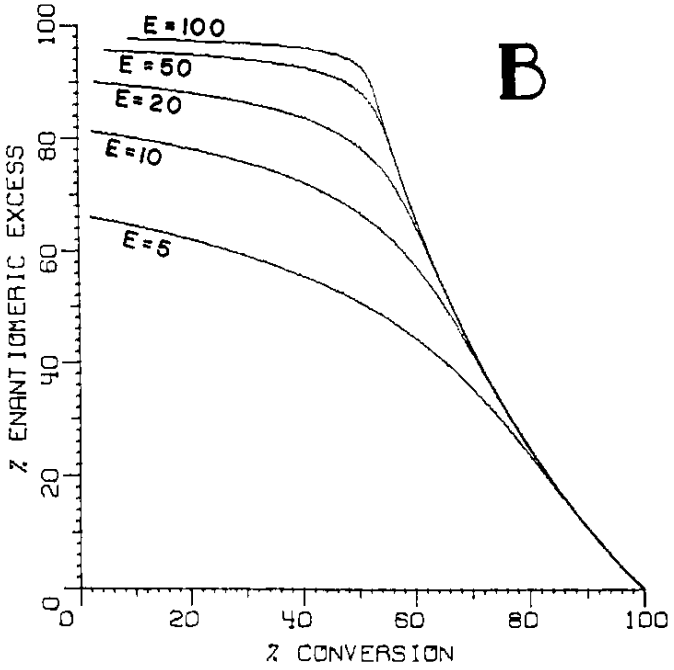
PRAMIPEXOLE

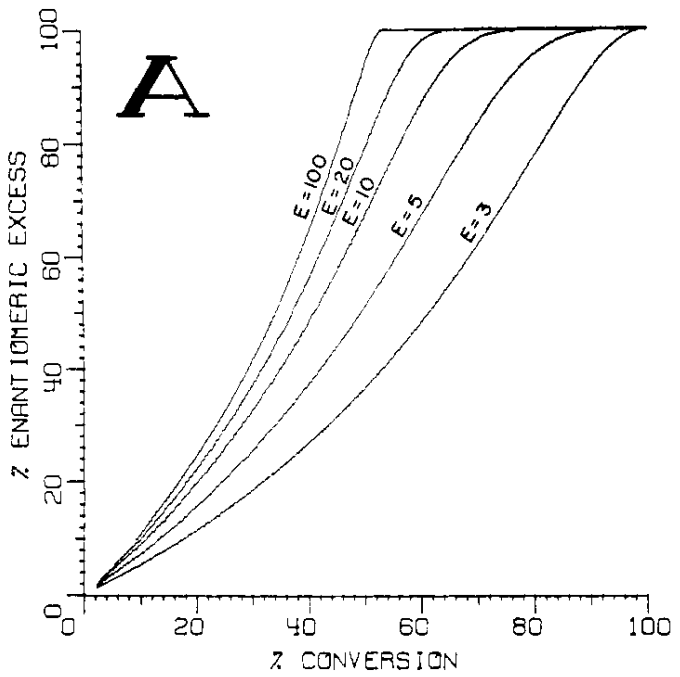
Product

$$E = \frac{\ln[1 - c(1 + ee_p)]}{\ln[1 - c(1 - ee_p)]}$$

Substrate

$$E = \frac{\ln[(1 - c)(1 - ee_s)]}{\ln[(1 - c)(1 + ee_s)]}$$





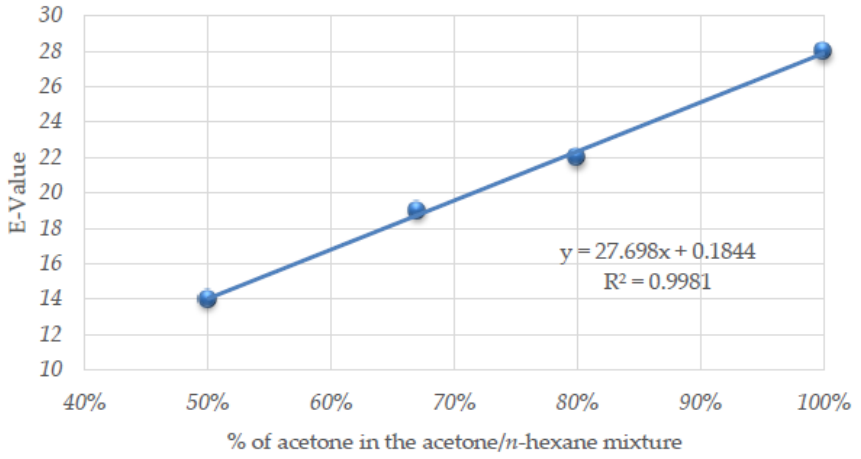
C.-S. Chen, Y. Fujimoto, G. Girdaukas, C. J. Sih, *J. Am. Chem. Soc.* **1982**, *104*, 7294.

PRAMIPEXOLE

IRREVERSIBLE TRANSESTERIFICATION
(CAL-A in acetone)

Entry	Solvent	Time (h)	Conv. (%)[a]	ee (%)[a]; Configuration		E
				alcohol	acetate	
1	Acetone + 0.1% H_2O	336	32	39; S	90; *R*	27.0
2	Acetone/*n*-hexane 1:1	32	69	>99; *S*	47; *R*	13.8
3	Acetone/*n*-hexane 2:1	95	50	75; *S*	79; *R*	19.0
4	Acetone/*n*-hexane 4:1	168	45	68; *S*	83; *R*	21.8
5	Acetone	168	34	50; *S*	89; *R*	28.2

[a] *chiral HPLC.*



linear correlation between the E value and the % of acetone in the reaction mixture.

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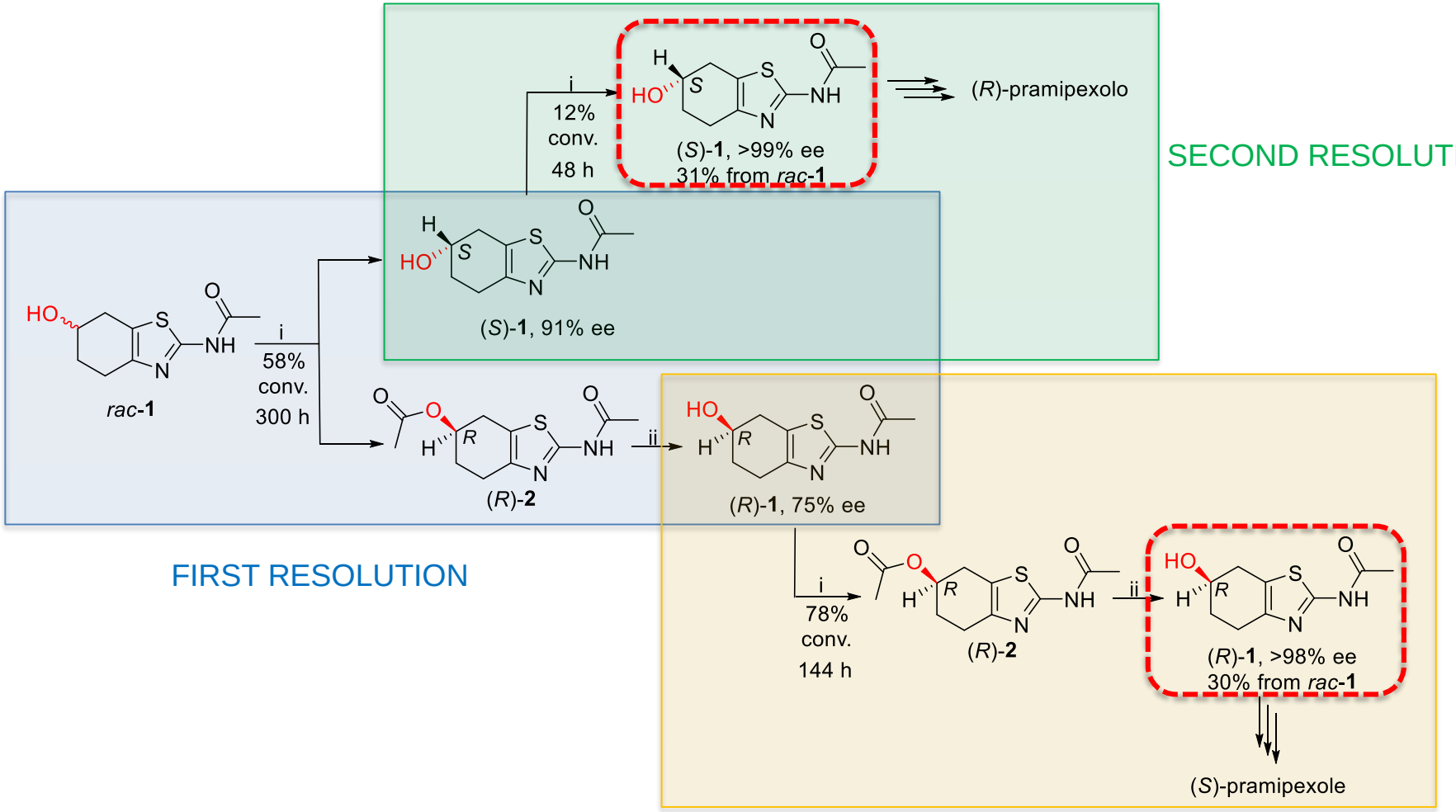
PRAMIPEXOLE



(**i**) CAL-A, vinyl acetate, acetone/*n*-hexane 4:1; (**ii**) 1% NaOH, methanol.

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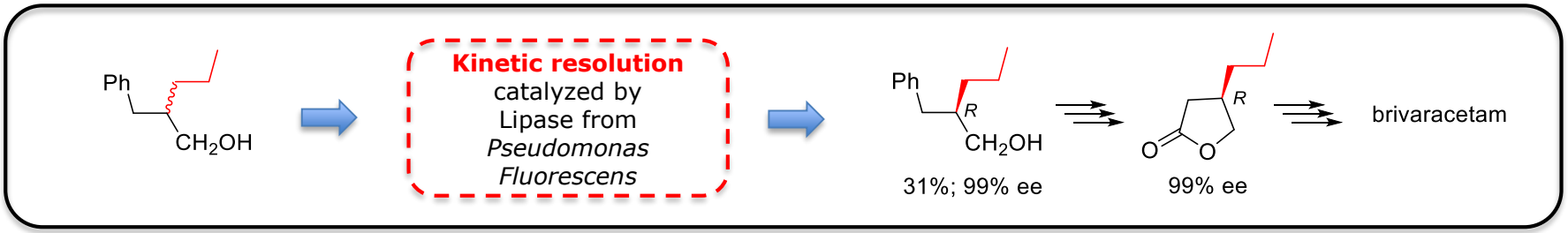
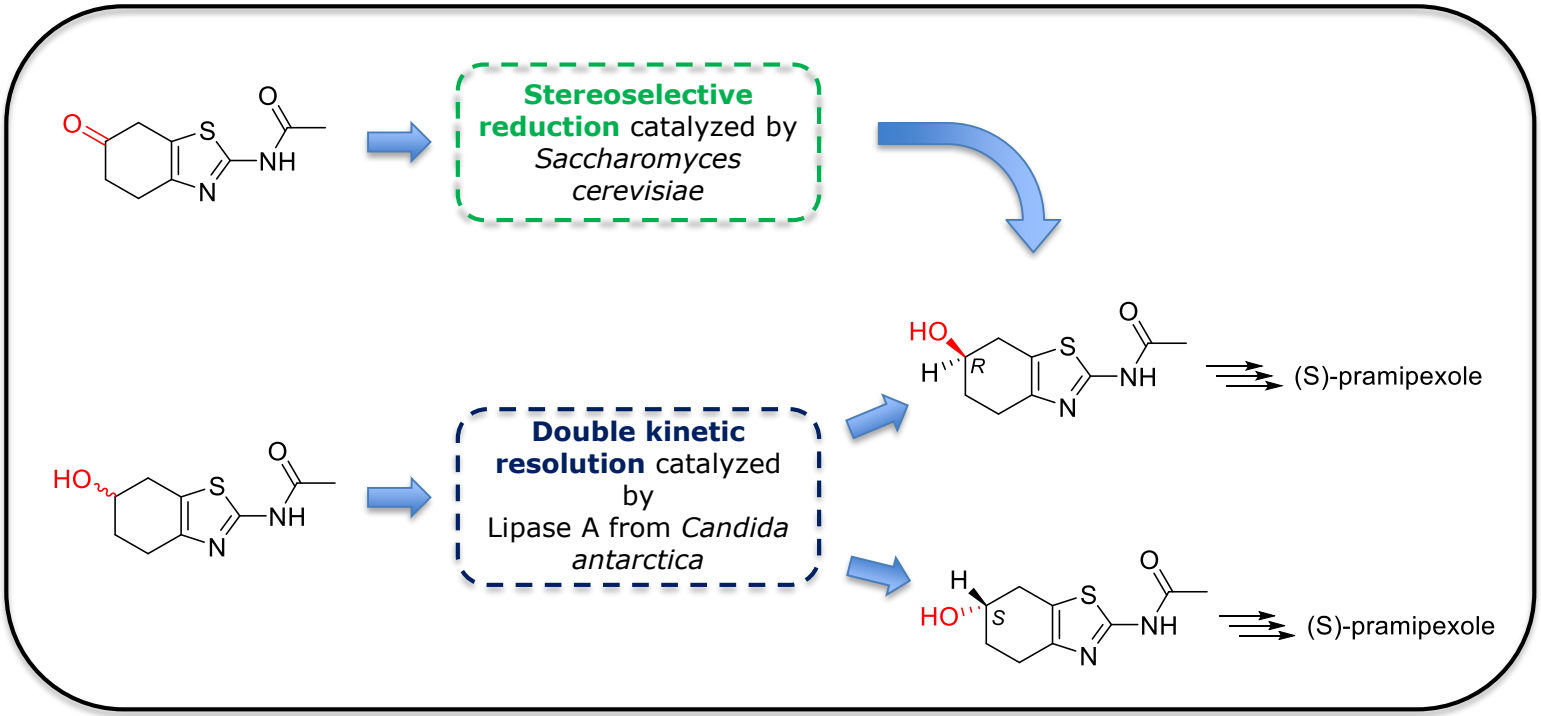
CONCLUSIONS





THANK YOU FOR YOUR ATTENTION