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Systematic study of lipase-catalyzed resolution of propranolol precursors

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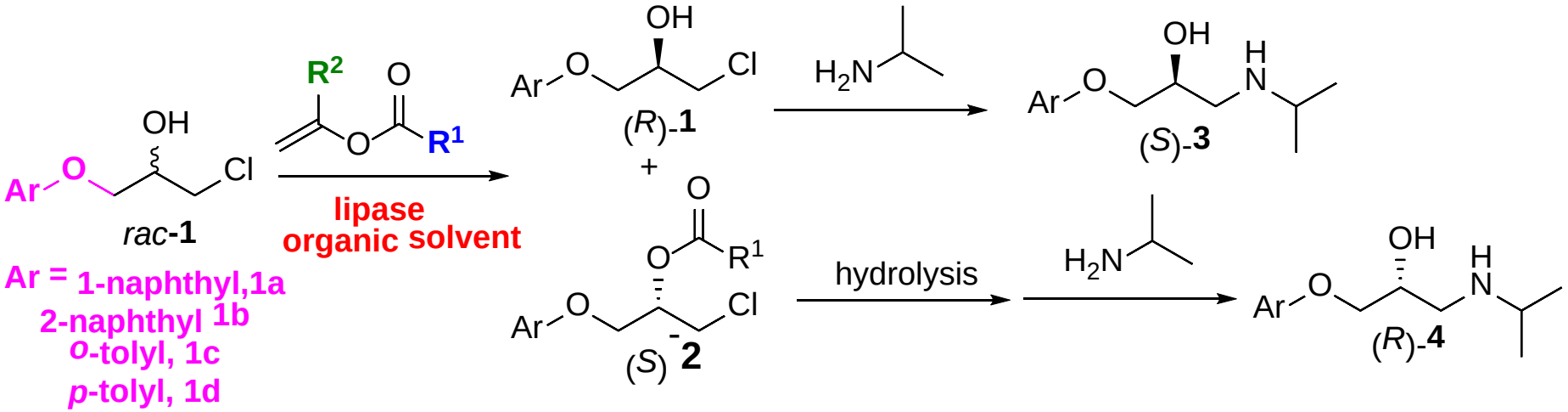
Graphical Abstract



Ar = 1-naphthyl,1a
2-naphthyl 1b
o-tolyl, 1c
p-tolyl, 1d

R^1 = Me, Et, Pr, $-CH_2Cl$, $-(CH_2)_{10}CH_3$

R^2 = H, Me

Abstract: Propranolol ((*R*,*S*)-1-isopropylamino-3-(1-naphthoxy)-2-propanol), is a well-known **beta-adrenergic blocking agent** used for treatment of **arterial hypertension** and other **cardiovascular disorders**, is commercially available as a racemic mixture. However, it is also well proven that **mainly the (*S*)-enantiomer has the desired therapeutic effect**; therefore, many stereoselective synthetic protocols for the preparation of the (*S*)-eutomer can be found in literature, mediated by an enzymatic resolution of the chemically-prepared racemate. Generally speaking, the **resolution should preferentially be carried on a precursor of the desired target drug** such as the racemic aryloxyhalohydrines, easily prepared by opening epychlorhydrine with an aromatic alcohol.

In this communication we present the **kinetic resolution** of **aryloxyhalohydrines** (precursors of propranolol and other beta-adrenergic blockers) by **lipase-catalyzed stereoselective transesterification with enol esters**. A ***factorial design of experiments*** was undertaken to assess **best reaction conditions** (temperature, solvent, acyl donor, …) for the efficient **separation of enantiomers**, **both of them useful for therapeutic purposes**; hence, besides the previously antihypertensive activity of (*S*)-propranolol, the correspondent (*R*)-antipode displays a stronger antiarrhythmic and membrane-stabilizing effect, and it is also useful as a vaginal contraceptive. Through this stereoselective enzymatic acylation, the correspondent halohydrine ester and remnant alcohol can be easily separated and efficiently transformed into both enantiomers of propranolol.

Keywords: propranolol; lipase; kinetic resolution, transterification; enantiomers

Introduction (1/4)

Hypertension, or elevated blood pressure, is one of the most common risk factor for coronary artery disease, heart failure, stroke, and renal failure. Approximately 50 million Americans have a systolic or diastolic blood pressure above 140/90 mm Hg (the onset of hypertension) and most commonly appears during the fourth, fifth, and sixth decades of life [1].

Hypertension is the main avoidable cause of premature death worldwide [2], and its treatment has become an important public health challenge in both economically developing and developed countries. According to a recent study [3], the global occurrence of hypertension is foreseen to hover around 40% in all adults, leading to a 5.2% increase in the overall prevalence between 2000 and 2010. This figure results of computing together a 2.6% decrease in high-income countries and a 7.7% increase in low/middle–income countries.

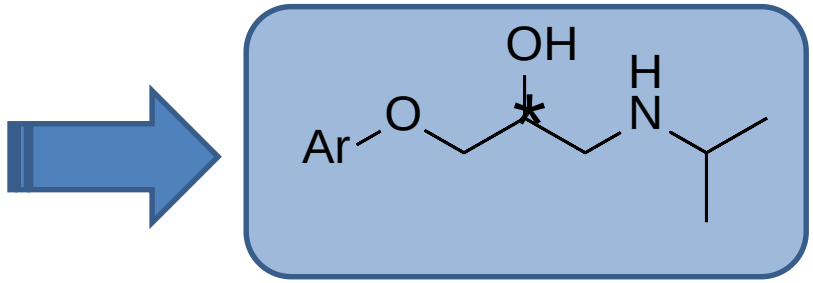
[1] Mancia, G.; Fagard, R., *et al.* 2013 ESH/ESC Guidelines for the management of arterial hypertension. *Eur. Heart J.* **2013**, *34 (28),* 2159-2219.
[2] Whelton, P. K.; Carey, R. M.; *et al.* 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: executive summary a report of the American College of Cardiology/American Heart Association task force on clinical practice guidelines. *Hypertension* **2018**, 71 (6), 1269-1324.
[3] Mathews, J. Global Antihypertensive Drugs Market US$ 23.1 Billion by 2023. https://www.linkedin.com/pulse/global-antihypertensive-drugs-market-us-231-billion-2023-mathews/

Introduction (2/4)

Today, a large number of drugs are currently available to treat hypertension [4], based on different mechanisms of action :

i. diuretics,
ii. sympatholytic drugs (centrally acting drugs, ganglionic blocker drugs, adrenergic neuron blocking drugs, β-adrenergic blocking drugs, α-adrenergic blocking drugs and mixed α/β-adrenergic blocking drugs),
iii. vasodilators (arterial or arterial and venous),
iv. calcium channel blockers,
v. angiotensin-converting enzyme inhibitors
vi. angiotensin receptor antagonists

One of the most archetypical compounds for treating hypertension are those **β-blockers** possessing the **aryloxypropanolamine** structure.



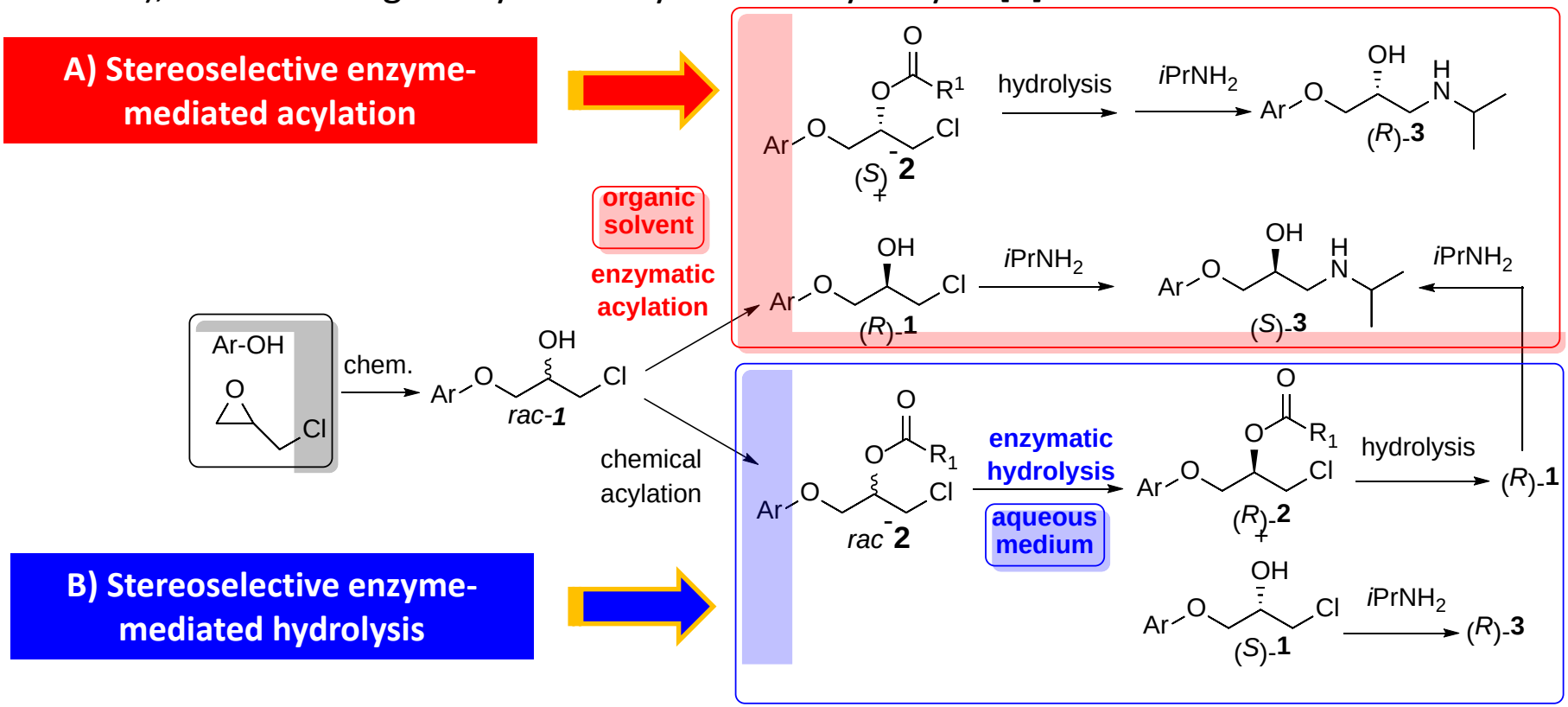
It is well-known that the **(*S*)-enantiomer of β-blockers are more potent antagonists than the corresponding (*R*)-antipodes** [5].

[4] Lemke, T. L.; Williams, D. A., *Foye's Principles of Medicinal Chemistry*. Wolters Kluwer Health, **2012**. ISBN: 978-1609133450

[5] Agustian, J.; Kamaruddin, A. H.; Bhatia, S., Single enantiomeric beta-blockers The existing technologies. *Process Biochem*. **2010**, *45 (10)*, 1587-1604.

Introduction (3/4)

Different chemoenzymatic procedures for preparing enantiopure version of these drugs, starting from racemic halohydrines (prepared by opening epychlorhydrine with an aromatic alcohol), rather through enzymatic acylation or hydrolysis [6]



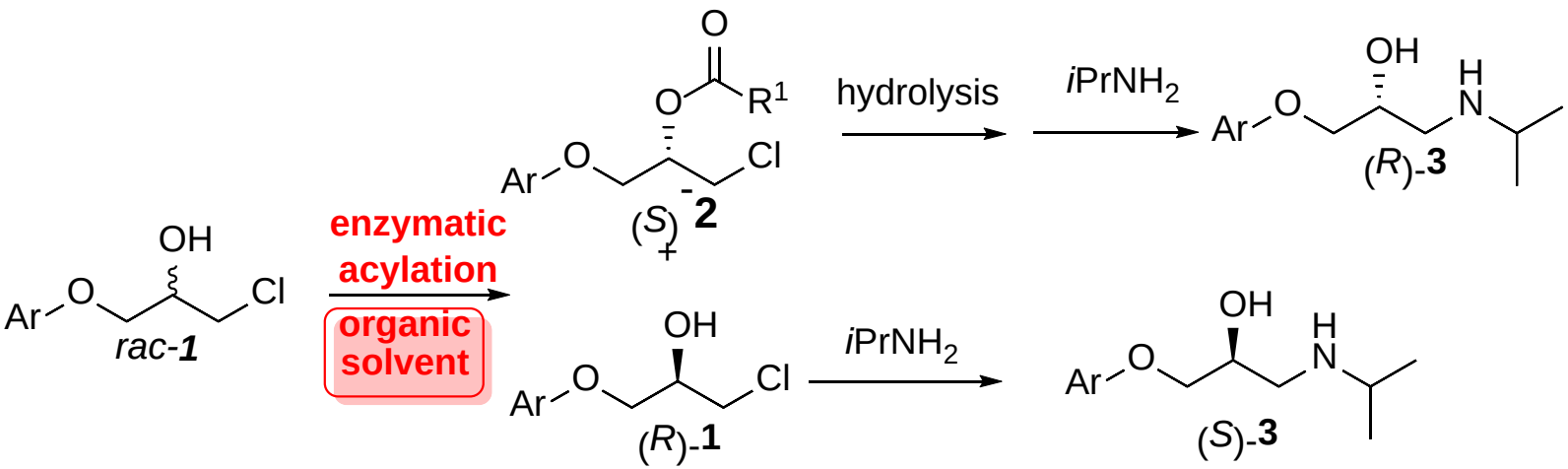
[6] Hoyos, P.; Pace, V.; Alcántara, A. R., *Chiral Building Blocks for Drugs Synthesis via Biotransformations*. In Asymmetric Synthesis of Drugs and Natural Products, Nag, A., Ed. CRC Press: Boca Raton, Florida, **2018**; pp 346-448.

Introduction (4/4)

Some comments on the resolution:

- Only moderate resolutions have been described using propranolol as substrate [7]
- Enzymatic acylation is preferred because the stereoselective discrimination is carried out in an earlier step.
- While hydrolysis worked faster than transesterification, the ease of workup and isolated yields are in favour of the latter [6]

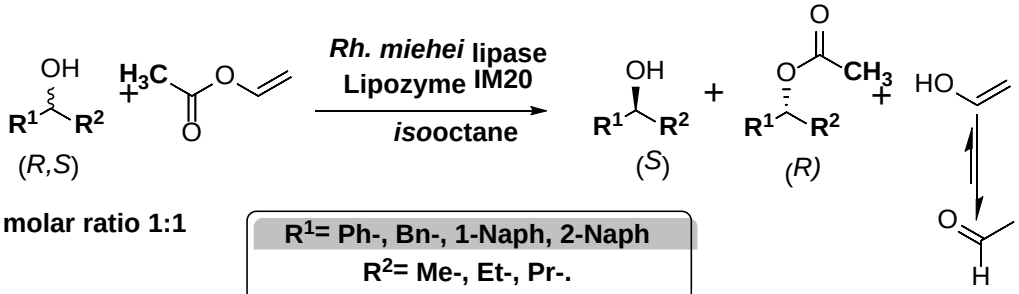
FOCUS ON ACYLATION: Reaction to optimize



[7] Barbosa, O.; Ariza, C.; Ortiz, C.; Torres, R., Kinetic resolution of (R/S)-propranolol (1-isopropylamino-3-(1-naphtoxy)-2-propanolol) catalyzed by immobilized preparations of *Candida antarctica* lipase B (CAL-B). *New. Biotech.* **2010**, 27 (6), 844-850..

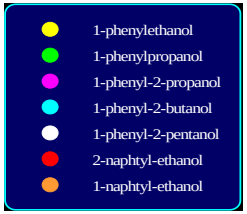
Results and discussion (1/8)

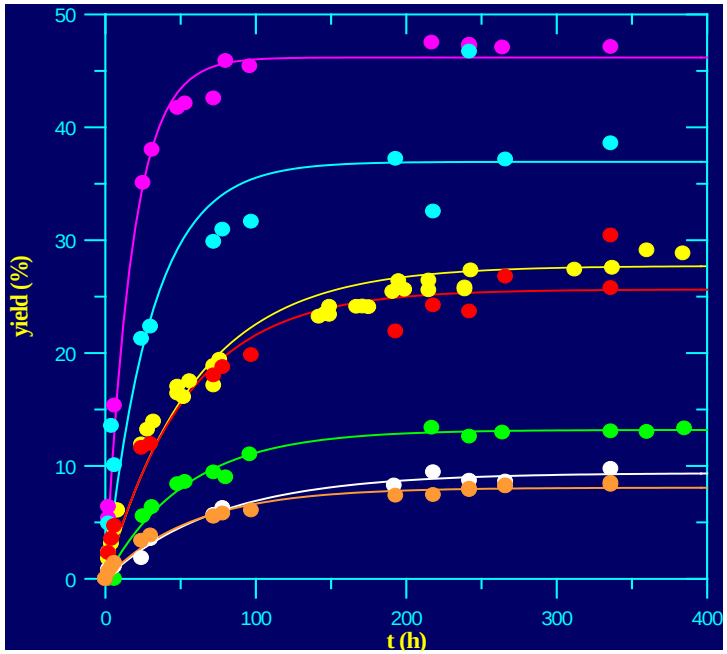
TEST REACTION: Secondary alcohols resolution



molar ratio 1:1

R^1= Ph-, Bn-, 1-Naph, 2-Naph
R^2= Me-, Et-, Pr-.





EXPERIMENTAL DESIGN [8]: To check influential variables

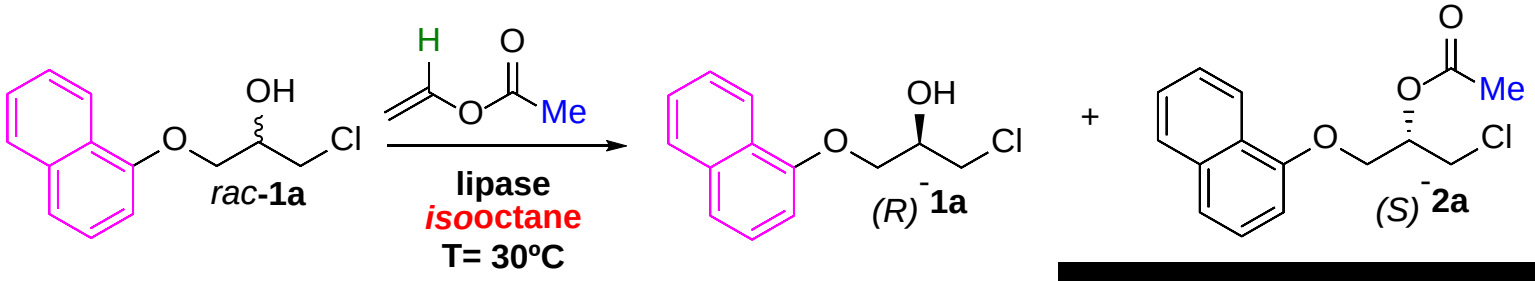
FACTOR	VARIABLE	MAXIMUM (+)	CENTRAL POINT (C. P.)	MINIMUM (-)
X_A	Solvent Log P	4,5	2,03	-0,4
X_B	Molar ratio Acyl donor/alcohol	5/1	3/1	1/1
X_C	Temperature (°C)	46	25	4
X_D	Catalyst amount (mg)	250	150	100

R^1	R^2	CONV. (336 h)	Vo (mM/h)	ees (%)	eep (%)	E	EF
Ph-	Me-	28	3.4	37	97	58	0,97
Ph-	Et-	13	1.8	15	>99	>100	0,99
Bn-	Me-	47	18.8	92	>99	>100	0,99
Bn-	Et-	39	8.7	75	>99	29	0,99
Bn-	Pr-	10	0.9	13	>99	15	0,99
2-Naph-	Me-	28	3.4	35	90	27	0,90
1-Naph	Me-	8	1.0	7	79	10	0,79

[8] De Fuentes, I. E. Ph. D. Thesis, Complutense University of Madrid, *unpublished data*

Results and discussion (2/8)

Test reaction: use of vinyl acetate and isooctane (according to the previous optimization)



Lipases tested:

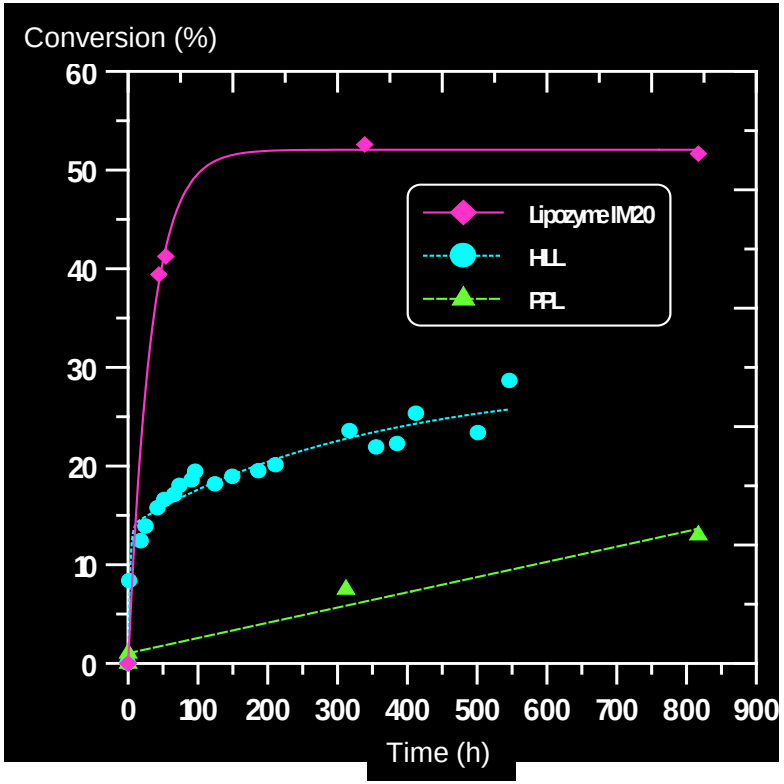
- Immobilized lipase from *Rhizomucor miehei* (Lipozyme IM20)
- Crude lipase from *Humicola lanuginosa* (HLL, recently renamed *Thermomyces laguginosus*)
- Crude lipase from Pig Pancreas (PPL)

Biocat.	Prot.[a] (mg)	t (h)	χ (%)	2	ee 2 (%)	1	ee 1 (%)	E_4[b]	EF_3[c]
HLL	106	547	30	*S*-(+)	>99	*R*-(-)	32	>100	0,73
PPL	106	817	8	*S*-(+)	65	*R*-(-)	3,1	4,5	0,36
IM20	15	340	53	*S*-(+)	>99	*R*-(-)	78	>100	0,71

[a] Protein amount (Biuret).

[b] Enantiomeric ratio (product), $E = [\ln [1-c(1+ee_p)]]/[\ln [1-c(1-ee_p)]]$

[c] Enantiomeric factor EF = (ees) / [c/ (1-c)]

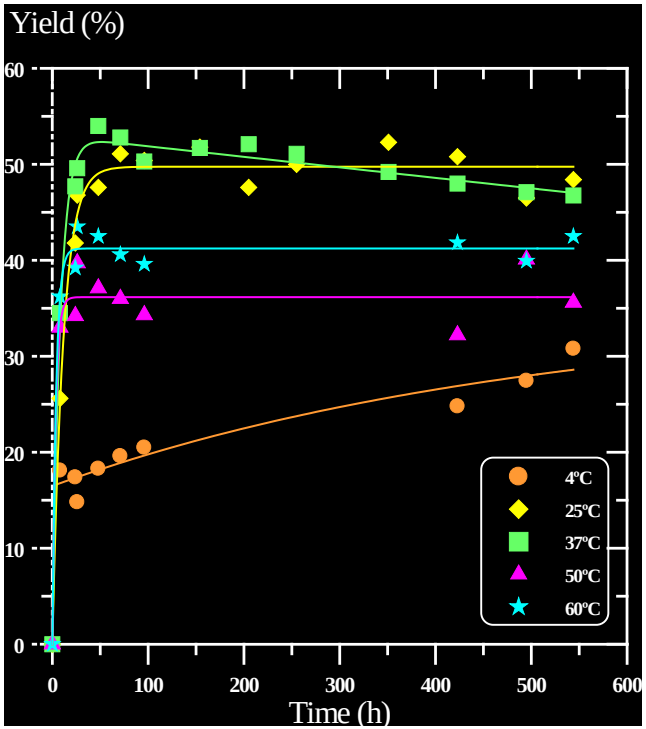


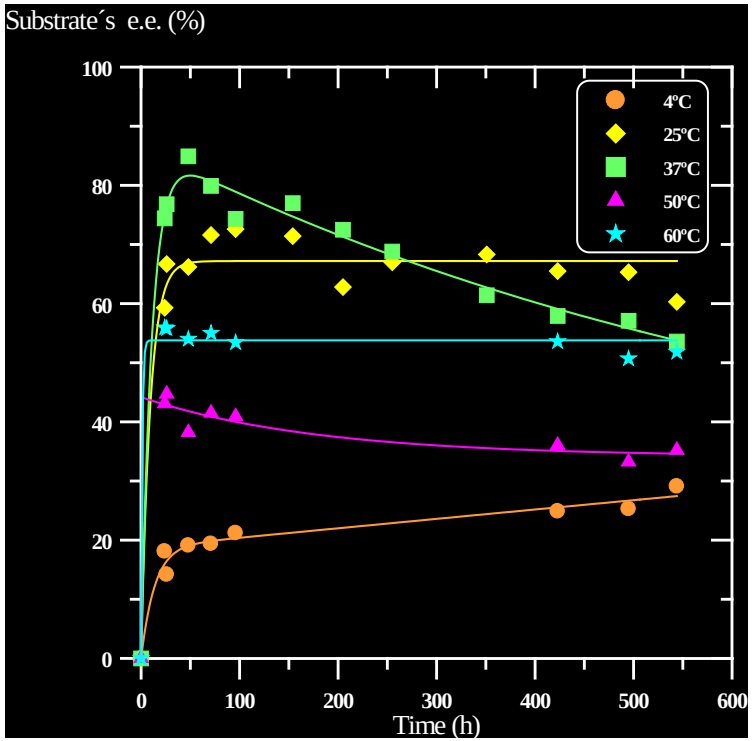
Conversion and enantiomeric excess followed by HPLC (chiral column Chiralcel-OD)

Best biocatalyst: Lipozyme IM20

Results and discussion (3/8)

Reaction optimization: TEMPERATURE



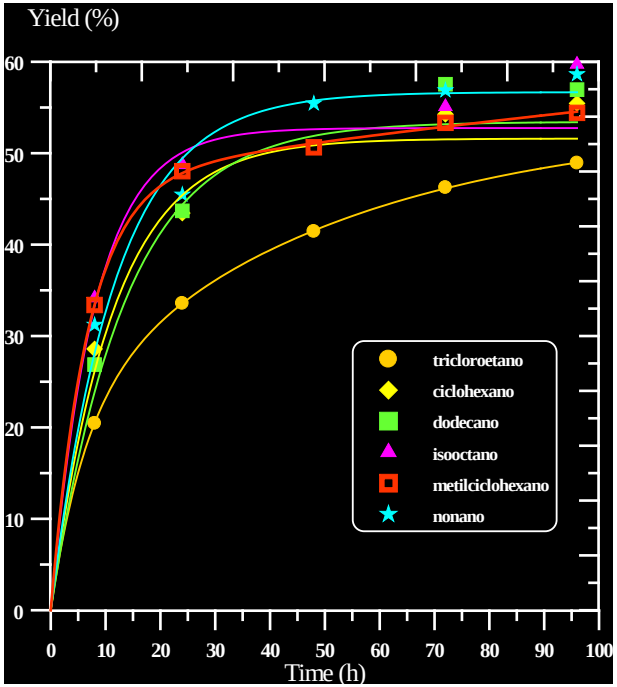


Best temperature: 37º C

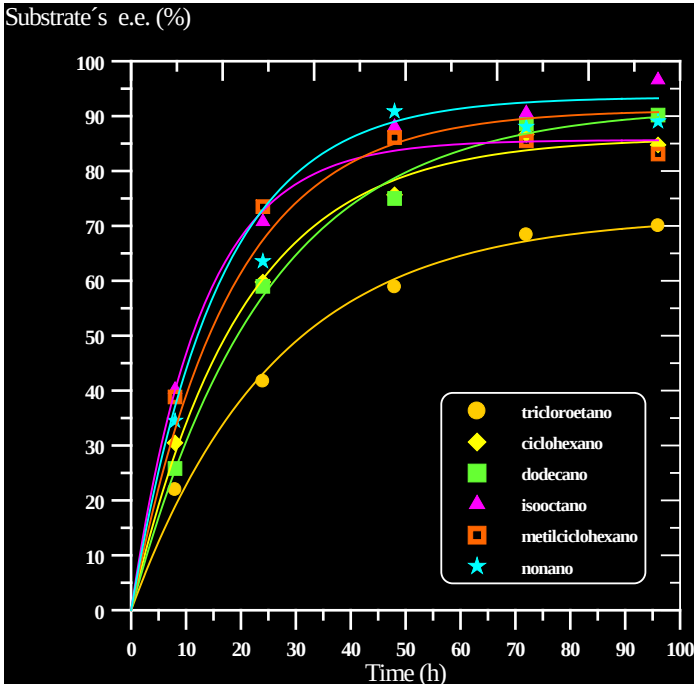
REACTION TIME 24 h.	T (°C)	c (%)	e.e of R-1a (%)	E	EF
	4	17	18	18	0.88
	25	42	59	18	0.81
	37	48	74	20	0.80
	50	34	43	17	0.83
	60	39	56	27	0.88

Results and discussion (4/8)

Reaction optimization: SOLVENT



Best solvent: *iso*octane

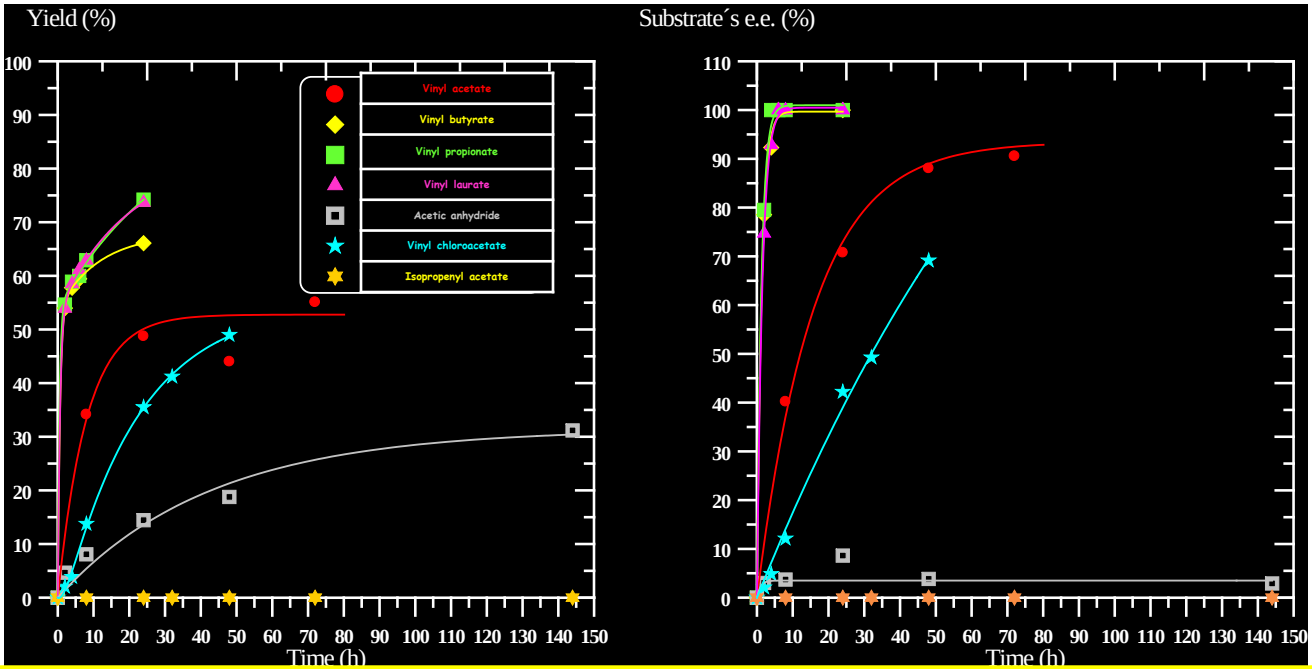


REACTION TIME 24 h.

solvent	logP	c (%)	e.e of R-1a (%)	E	EF
1,1,1-trichloroetane	2.5	34	42	15	0.81
Cyclohexane	3.2	43	60	16	0.80
Methylcyclohexane	3.7	48	73	19	0.79
isooctane	4.5	49	71	14	0.74
Nonane	5.1	45	64	16	0.78
dodecane	6.6	43	59	15	0.78

Results and discussion (5/8)

Reaction optimization: Acyl donor

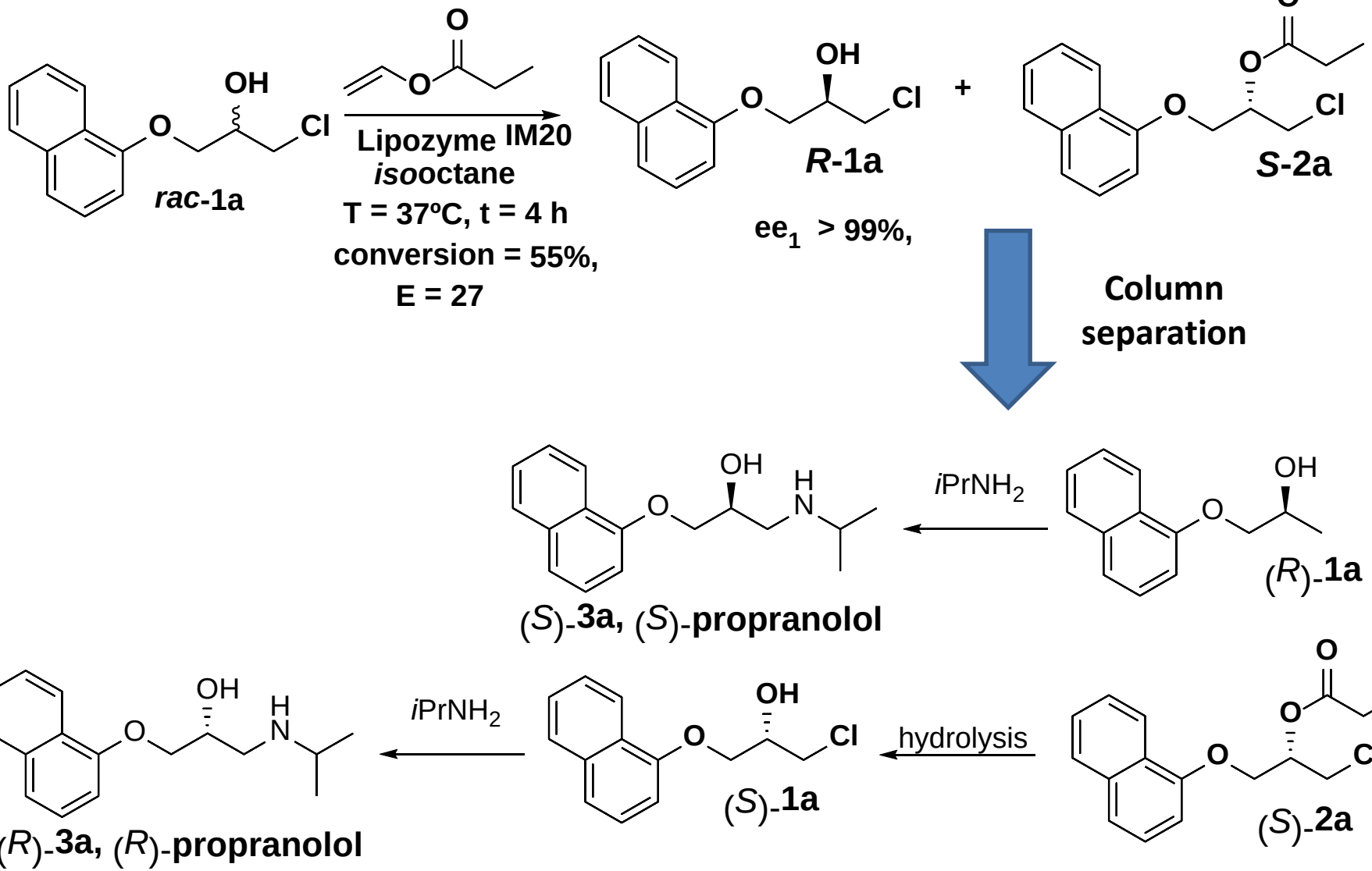


Best acyl donor:
Vinyl propionate

Acyl donor	T(h)	CONV. (%)	ees (%)	E	EF
Acetic anhydride	24	14	9	12	0.74
Isopropenyl acetate	144	-----	----	----	----
Vinyl chloroacetate	48	49	69	12	0.72
Vinyl acetate	24	59	71	14	0.74
Vinyl propionate	4	59	>99	>100	----
Vinyl butyrate	6	62	>99	>100	----
Vinyl laurate	6	8	>99	>100	----

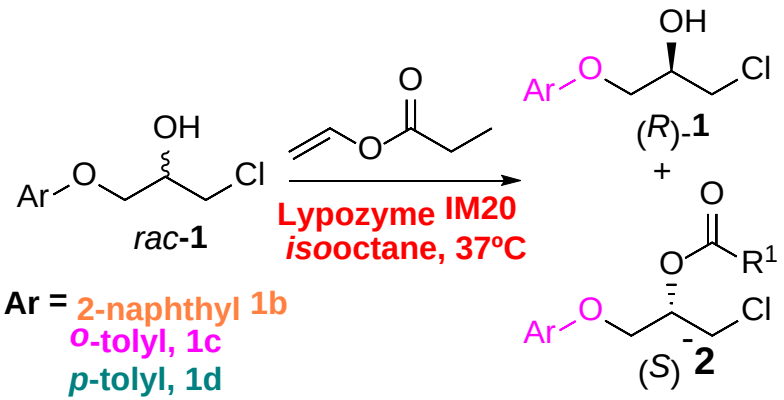
Best solvent:
***iso*octane**

Results and discussion (6/8)

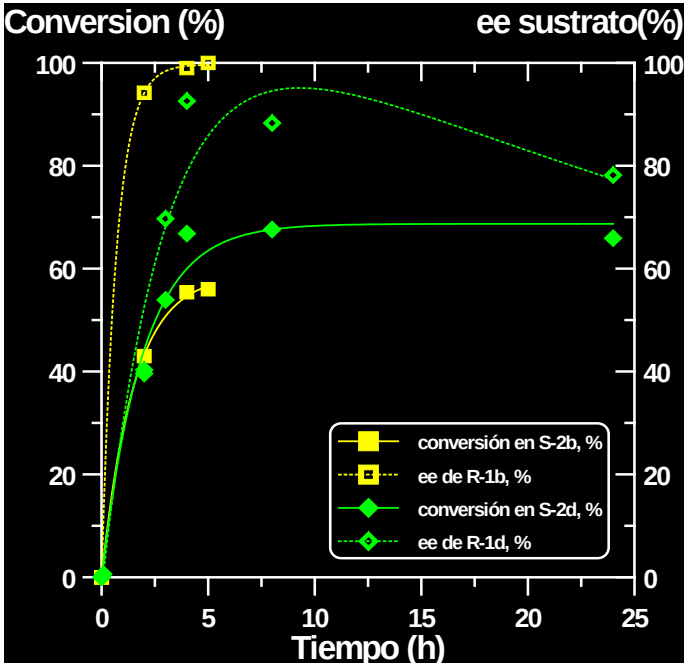


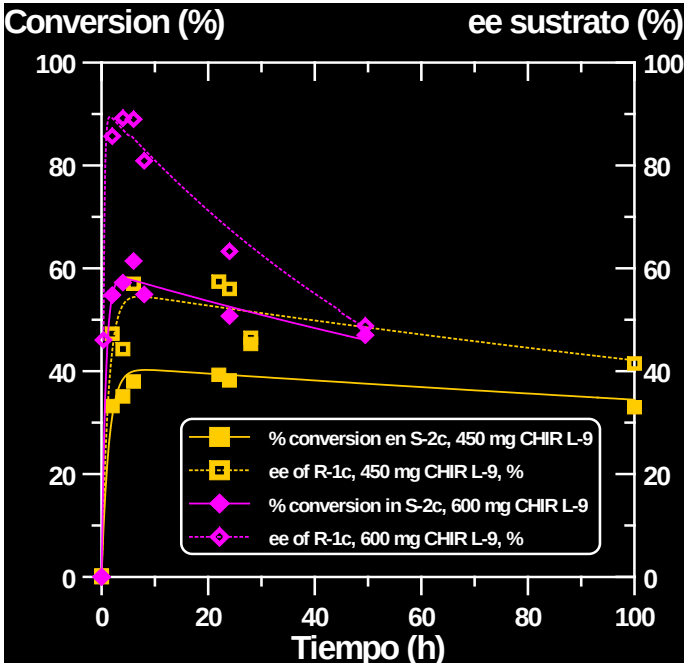
Results and discussion (7/8)

Other substrates, best exp. conditions



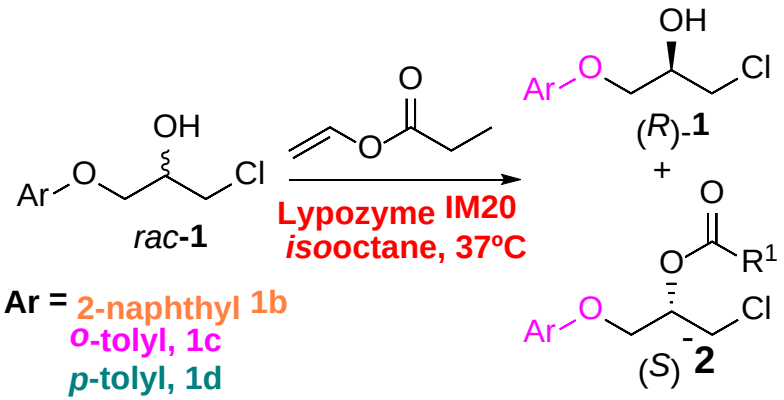
Ar = 2-naphthyl 1b
o-tolyl, 1c
p-tolyl, 1d





Results and discussion (8/8)

Other substrates, best exp. conditions



Ar = 2-naphthyl 1b
o-tolyl, 1c
p-tolyl, 1d

Substrate	t (h)	Biocat, (mg)	Conversion (%)	ee subst.R(-)	E
1b	5	450	56	> 99	41
1c	22	450	39	44	29
1c	4	600	37	89	15
1d	3	450	63	>99	18

Conclusions

- ✓ **Optimization of the kinetic resolution** of **aryloxyhalohydrines** (precursors of propranolol and other beta-adrenergic blockers) by **lipase-catalyzed stereoselective transesterification with enol esters**.

- ✓ A previous ***factorial design of experiments*** was undertaken to assess **best reaction conditions** (temperature, solvent, acyl donor, …)

- ✓ Best conditions for **acylation of racemic 1-chloro-3-(naphthalen-1-yloxy)propan-2-ol** (***propranolol precursor***)
  - Catalysts: Lipozyme IM20
  - T=37ºC
  - Acyl donor: vinyl propionate
  - Solvent: *iso*octane
  - CONVERSION: 55% ee_s > 99%

- ✓ **Easy column separation and straightforward synthesis of both enantiomers of beta-blockers**, useful for therapeutic purposes.

- ✓ Similar results were obtained in the stereoselective enzymatic acylation of other halohydrines, showing the applicability of the resolution procedure

Acknowledgments

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