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Enzyme Assisted Preparation of Enantiomerically Pure β-Adrenergic Blockers I. A Facile Screening Method for Suitable Biocatalysts

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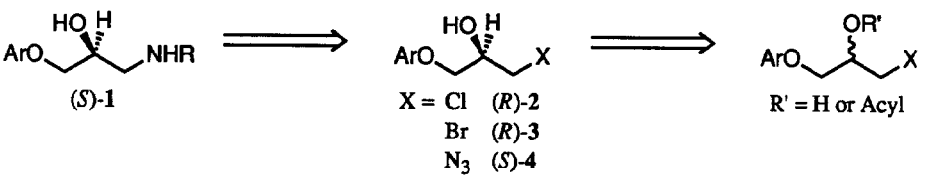
Abstract: The enantioselective preparation of potential precursors for β-adrenergic blockers was studied by enzymatic hydrolysis of the corresponding ester derivatives. Based on the facile separation of both substrates and products by HPLC on a chiral support a convenient screening method was developed for a rapid evaluation of the best suited esterhydrolase.

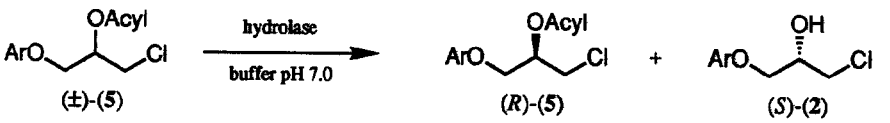
It is well established that the biological activities of β-adrenergic blockers of the general formula **1** reside largely in the (*S*)-enantiomers[1] of these molecules. Consequently, numerous attempts have been made to prepare these compounds in enantiomerically pure form, e.g. by asymmetric synthesis[2] or biotransformations[3].

(*S*)-**1** can be correlated retrosynthetically with a number of potential precursers, e.g. the corresponding α-chloro-, α-bromo- or α-azido-derivatives (Scheme 1), which in principle all could serve as starting materials for the title compounds.



Scheme 1

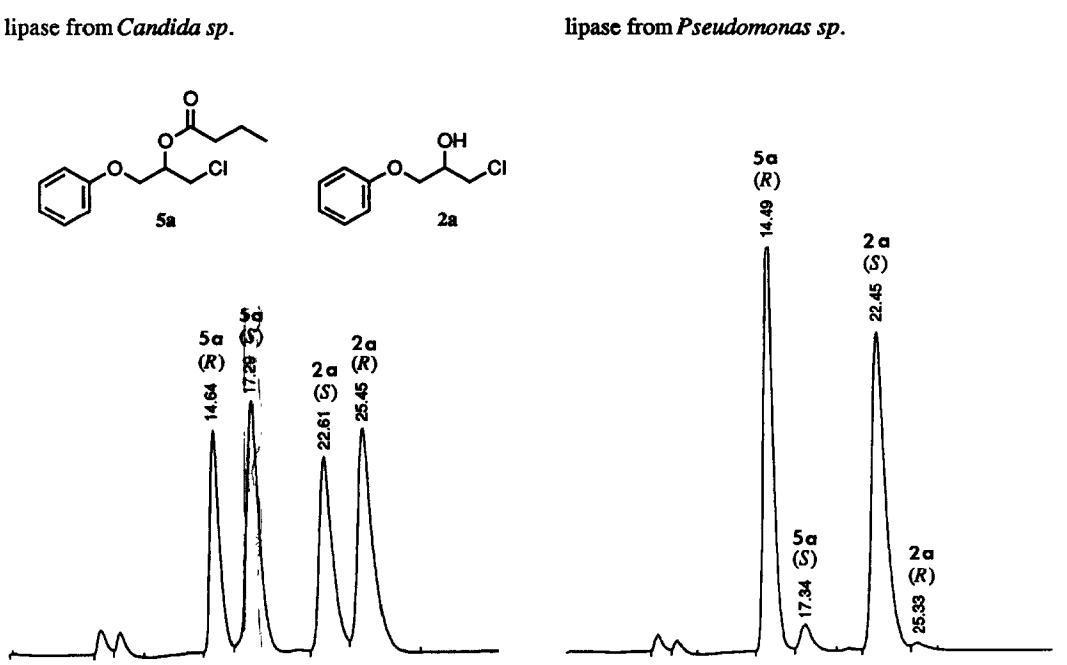
Esterhydrolases (Esterases, Lipases) are well known for their capabilty of enantiomer differentiation. In view of our previous experience[4] in this area and based on literature data[5], we felt that the enantioselective hydrolysis (Scheme 2) or synthesis of the corresponding esters[6] could well provide a facile route to the desired intermediates in optically pure form.



Scheme 2

For this purpose, however the most suitable
a) biocatalyst
b) intermediate
first had to be found.

Target orientated screening for suitable enzymes is usually a very time consuming and tedious enterprise. We have therefore decided to develop a facile screening system for this purpose based on the enantioselective separation of the reaction products by HPLC on chiral supports. Using a commerically available column (Chiralcel OB)[7] all products resulting from the enantioselective, enzymatic hydrolyses (Scheme 1) can be separated simultaneously. A direct determination of the achieved conversions, as well as configurations and enantiomeric purities of both substrates and products, can be achieved in one single experiment. The obtained data allow a rapid determination of the enantioselectivities[8] displayed by the employed biocatalysts and thus a rapid evaluation of their synthetic usefulness.

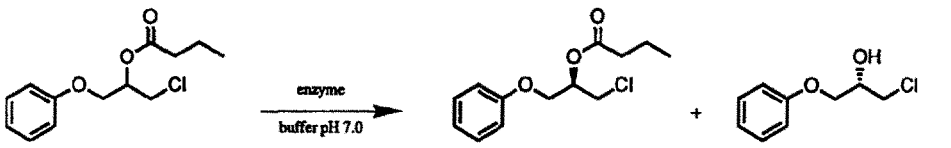


Scheme 3

The results obtained in the hydrolyses of 2-butyloxy-1-chloro-3-phenoxypropan [(±)-**5a**)] with a series of biocatalysts are summarized in Table 1 and exemplified in Scheme 3 for two lipases of widely different enantioselectivities.

Both from Table 1 and Scheme 3 it is obvious that the best suited biocatalysts are the lipases from *Mucor miehei* and *Pseudomonas sp.*. In view of the much higher specific activity the lipase from *Pseudomonas sp.* was chosen for further experiments.

Table 1



(±)-(5a) (*R*)-(5a) (*S*)-(2a)

enzyme	*t* (25%) [h]	products	% e.e.	conv.[7]	*E*[7]
lipase from *Aspergillus oryzae*	–	(±)-(2a)	–	–	–
lipase from *Aspergillus niger*	–	(±)-(2a)	–	–	–
lipase from *Aspergillus sojae*	12	(*R*)-(2a) (*S*)-(5a)	19.6 24.7	0.44	2
lipase from *Candida lipolytica*	74	(*R*)-(2a) (*S*)-(5a)	70.7 90.5	0.44	42
lipase from *Candida sp.*	2	(*S*)-(2a) (*R*)-(5a)	20.7 12.7	0.62	<2
lipase from *Chromobacterium viscosum*	<2	(*R*)-(2a) (*S*)-(5a)	66.9 75.6	0.47	14
lipase from *Humicola lanuginosa*	28	(*R*)-(2a) (*S*)-(5a)	61.0 94.0	0.39	60
lipase from *Geotrichium candidum*	12	(*R*)-(2a) (*S*)-(5a)	48.2 60.2	0.44	6
lipase from *Mucor miehei*	53	(*R*)-(2a) (*S*)-(5a)	44.4 98	0.31	>100
lipase from *Penicillium roquefortii*	–	(±)-(2a)	–	–	–
lipase from *Rhizopus delemar*	52	(*S*)-(2a) (*R*)-(5a)	20.9 12.7	0.62	2
lipase from *Porcine pancreas* (PPL)	19	(*R*)-(2a) (*S*)-(5a)	43.7 82	0.35	15
cholesterinesterase from *Pseudomonas fluorescens*	<1	(*R*)-(2a) (*S*)-(5a)	24.6 27.6	0.47	2
lipase from *Pseudomonas sp.*	<2	(*R*)-(2a) (*S*)-(5a)	86.2 96.3	0.47	>100
porcine liver esterase (PLE)	6	(*S*)-(2a) (*R*)-(5a)	14.0 1.5	0.90	1

In view of the convenient accessibility and synthetic usefulness the α-chloro-, α-bromo- and α-azido-intermediates were chosen for the following preparative studies.[6]

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