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Lipase action on some non-triglyceride substrates

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Abstract

Approaches for improving methodologies of kinetic resolution of enantiomers as well as of regioselective protection of functional groups of complex chiral molecules involving lipase-catalytic reactions are highlighted. Decyclization of hemiacetals by lipase as well as exclusive pathways of lipase-catalyzed derivatization of prostanoids are brought out. Lipase-triggered cascade-reactions are noticed.
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1. Introduction

Several textbooks [1–6] and analytical review papers [7–21] have been published to discuss various aspects of the use of lipases as tools in organic synthesis. Application of lipases in synthesis as well as in medicine, industrial chemical processes, etc. with emphasis on the structure of lipases and mechanism of lipase-catalyzed reactions have been discussed [7]. Lipase-supported synthesis of biologically active compounds [8] and pharmaceuticals [9], use of preparative biotransformations in organic chemistry [10–12], lipase-catalyzed ester synthesis [13], use of lipases in racemic resolutions [14] and polymer synthesis [15] have been analyzed in detail. Some interesting papers for synthetic chemists on certain more general problems in lipase-catalysis have been published as well. These are focused on:

1. activity of lipases in organic solvents versus water [16];
2. interfacial activation of lipases [17];
3. possible multiplicity of catalytic sites and functions of an enzyme molecule [18]; and
4. commercial scale biocatalysis: pros and cons [19].

Molecular recognition and mechanism for some reactions catalyzed by different hydrolases [20] has been discussed. A review about molecular modeling for biocatalysis has been published [21].

However, there are some features in the use of lipases in synthetic and natural product chemistry which have not enough been brought out yet. This paper is concentrated on the fields of our specific experience in the use of lipases in preparative synthesis of physiologically active compounds.

Lipases have been defined as fat-splitting enzymes able to act on the interface between two phases. However, they usually retain some residual activity also in bulk aqueous phase hydrolyzing esters in their molecular solutions. Lipases maintain their catalytic activity in low-water media (organic phase, supercritical

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fluids, ionic liquids) and tolerate a wide variety of artificial substrates.

The conventional functions of lipases involve acyl transfer resulting in:

1. hydrolysis of esters;
2. ester synthesis;
3. transesterification;
4. amidation, etc.

Recently there have been revealed also some unconventional functions of lipases, viz. decyclization of hemiacetals followed by trapping of the open-chain hydroxyaldehyde upon acetylation [22] and catalysis of elimination of acetic acid from acetyl-β-ketol moiety [23].

Lipases usually recognize chiral substrates and nucleophiles in stereoselective manner. Lipases may bear also non-specific catalytic sites that probably could be responsible of lower enantioselectivity in certain cases as well as for any of the unconventional functions.

Thus, tolerance of lipases to different media and artificial substrates, ability to work as phase-transfer agent on the interfaces of a multiphase system, high stereoselectivity of catalytic performance along with an intriguing versatility makes lipases the biocatalysts of choice as well as of challenge for synthetic chemist.

2. Lipases as tools for organic synthesis

General principles of the use of a lipase as a stereoselective biocatalyst in organic synthesis have been comprehensively presented in several works [1–6].

In addition to this, we should like to propose a list of promising approaches to the solving of the practical problems in lipase-catalyzed preparative synthesis as well as in natural product chemistry. Some complications of the use of lipase-catalytic steps in synthesis are indicated.

2.1. Two roles of organic synthesis

Organic synthesis allows to gain useful compounds and also is a tool for generating new knowledge. The goal of every synthetic chemist is to gain the target compound—in high yield, as pure as required and as cheap as one can. Organic synthesis in its second (but not secondary) role allows us to verify the structures of natural products, hypothetic metabolic pathways, etc. Normally these two roles are integrated: preparative synthetic research using lipases provides interesting novel results for the further studies of the catalytic properties of the enzyme.

2.2. Features of a lipase-catalytic process important for synthesis

When using lipase-catalysis we need high reaction velocity and high selectivity corresponding to the goal. Usually the following types of selectivity of lipase-catalyzed reactions have been stressed.

1. Substrate selectivity
   The ability of the enzyme to distinguish and act on a subset of compounds within a larger group of chemically related compounds [19].
2. Functional group selectivity
   The ability of the enzyme to act on one functional group selectively in the presence of other equally reactive or more reactive functional groups in the molecule [19].
3. Stereoselectivity
   The ability of the enzyme to act on a single enantiomer or diastereomer selectively—this is the most important feature of lipase-catalysis allowing kinetic resolution of enantiomers and diastereomers.
4. Regioselectivity
   The ability of the enzyme to act on one location in a molecule selectively allowing regioselective protection and/or deprotection of complex molecules–polyols, etc.
5. Chemoselectivity
   The ability of the enzyme to produce a certain product selectively upon acting on a functional group; in some cases more than one chemically different alternative products can be formed upon acting on one certain functional group (for example, hemiacetal ester versus hydroxyaldehyde ester [22]).

In general, measures having been used to achieve high reaction velocity and selectivity are usually subdivided into:

1. prognosticating, screening, monitoring and
2. engineering, which includes one or more of following fields:

- substrate engineering;
- medium engineering;
- lipase engineering;
- process engineering (mixing conditions, microwave promotion, etc.).

2.3. Approaches for enhancing velocity, enantioselectivity and yield of lipase-catalyzed reactions

2.3.1. Prognosticating, screening and monitoring of a lipase-catalytic process

An approach for rapid screening in order to find out a proper hydrolase for a certain substrate from 100 or more enzymes has been elaborated [24].

Regarding to substrate selectivity, the accessibility of a certain ester substrate to a lipase in water medium can be prognosticated by using quantum chemical (QC) method [25] for calculation of localization parameters of frontier orbitals.

Methods of different level have been used to prognosticate substrate selectivity, enantiopreference and enantioselectivity of lipase-catalyzed reactions. Empiric rules allow us to predict configurations of chiral substrates and nucleophiles preferred by lipases [26]. Simplified models of the "box model" type describing the active site geometry allow to design synthetically equivalent substrates and nucleophiles of improved suitability to the active site [27,28].

Molecular dynamic simulations provide in some cases good predictions of enantiopreference and enantioselectivity (*E*-values) for kinetic resolution of enantiomers [29–33].

Aside of the traditional (titrimetric, chromatographic) methods for monitoring progress of a lipase-catalyzed reaction—^{1}H NMR spectroscopy has been shown to be useful for monitoring the changes in the quantities of all components in the system [34,35] as well as enantiopurity (by adding chiral shift reagent) of those allowing to calculate *E*-value. However, using of chiral shift reagents for investigation of an ester and an alcohol together in one sample may result in complications due to very different complex formation ability.

2.3.2. Use of irreversible reactions

Parameters controlling enantioselectivity of lipase in organic synthesis have been discussed in detail [36].

Use of vinyl and isopropenyl acetates and other achiral activated esters for enantioselective transesterification is well documented [1–6]. Synthesis of vinyl esters of arylaliphatic chiral carboxylic acids for using in resolution aimed at a significant enhancement of reaction rate and enantioselectivity has been reported [37]. A new irreversible procedure using orthoesters has been developed recently to improve the efficiency of kinetic resolution [38]. The use of irreversible reactions usually gives also higher chemical yields in kinetic resolutions.

2.3.3. Use of supercritical fluids as reaction media

Supercritical fluids and especially supercritical carbon dioxide ($SCCO_2$) are considered to be the media of great appeal for industrial biocatalytic applications. $SCCO_2$ is a valuable low-water medium because of its natural origin, nontoxicity, nonflammability, low environmental impact, availability at low cost as well as high mass transfer and diffusion rates. Attempts to set up continuous operations as well as integrated processes of enzymatic reaction and the downstream resolution of products and unreacted substrates have been made [39,40].

Use of $SCCO_2$ as a reaction medium made possible to hydrolyze esters that are completely inaccessible to lipases in water [41]. However, the use of $SCCO_2$ as a medium for enzymatic resolution even for the gram scale application requires a rather complicated and tedious optimization of the process due to a large number of partition equilibria in the system.

$SCCO_2$ as medium for lipase-catalyzed reactions may be really the one of choice for industrial applications [42] but it seems that it cannot compete with the use of organic solvents as low-water media on laboratory scale.

2.3.4. Use of ionic liquids as media for lipase-catalyzed reactions

Room-temperature ionic liquids are attracting growing interest as alternative media for lipase-catalytic reactions [43,44]. Lipase-catalyzed transesterifications in ionic liquids may proceed with up to 25 times higher enantioselectivity than in conventional organic solvents [43]. Lipase-catalyzed alcoholysis, ammonolysis and perhydrolysis show higher reaction rates in ionic liquids than those in organic solvents in several cases. Ionic liquids are environmentally benign since

they have no detectable vapor pressure being of interest also for industry as replacements for volatile organic solvents.

Ionic liquids seem to be promising tools for improving activity, selectivity and stability of lipases as asymmetric catalysts.

2.3.5. Use of ingredients enhancing activity and enantioselectivity of enzyme-catalyzed reactions in organic media

The effects of additives on the lipase-catalyzed reactions has been reviewed recently [45]. Size of the molecule as a parameter for solvent effect on *Candida antarctica* lipase B (CALB) enantioselectivity has been investigated [46].

The importance of the influence of salts in determining the activity and stability of enzymes in aqueous medium is well documented. The catalytic performance of enzymes in organic solvents can be improved by the incorporation of carbohydrates, polymers as well as organic buffers into the dry catalyst. A dramatic enhancement in the activity of protease subtilisin observed in organic solvents was brought about by the presence of simple non-buffering salts in the lyophilized enzyme [47]. Assistance in maintaining the native structure of the enzyme in organic media could be provided by highly polar salt matrix.

By addition of metal ion-containing water, a marked enhancement of the enantioselectivity for the lipase-catalyzed esterification of substituted α-phenoxycarboxylic acids was observed. The mechanism has been investigated using individual enantiomers [48].

An interesting approach has been proposed to enhance enzymatic (for *Pseudomonas cepacia* lipase (PCL) and protease *Subtilisin carlsberg*) enantioselectivity in organic solvents: forming salts of substrate molecules with bulky counterions in order to exacerbate steric differences of substrate enantiomers. Significant enhancement of E (four to eight times) was achieved for some amino- and hydroxyacids in acetonitrile, tetrahydrofuran for PCL [49].

2.3.6. Optimum water level in low-water media

For ester synthesis and transesterification reactions [36] water activity should be maintained on an optimum level [45]. The water activity has to be high enough for maintaining lipase activity. On the other hand, water activity should be as low as possible since water acts as competing nucleophile for acyl-enzyme thus suppressing the expected acyl transfer. It is concluded that the rates of lipase-catalyzed reactions in organic media are usually strongly dependent on the water activity in the reaction medium. The direction of the influence of the water activity on the enzyme enantioselectivity seems to depend on the structure of the substrate as well as other parameters of the process [36].

A practical procedure using salt hydrates to buffer optimal water level during lipase-catalyzed synthesis in organic media has been recommended [50].

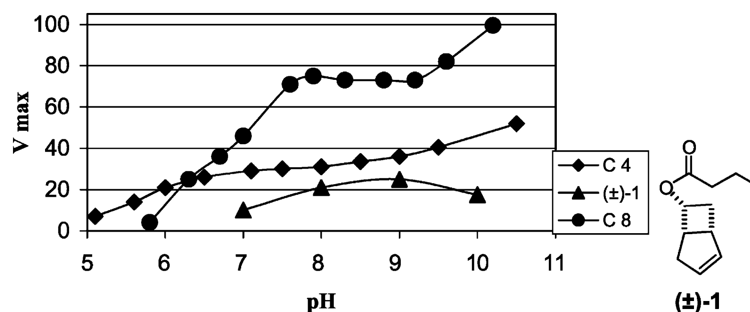
2.3.7. pH-dependence of velocity and enantioselectivity of lipase-catalyzed reactions

The pH-dependence of reaction rate has been discussed in comparison of lipases and esterases based on structural investigation of these two families [51]. Maximum activity of lipases in water has been observed typically at pH values above 8. A statistically significant, enhanced occurrence of non-polar residues close to the surface, clustering around the active site of lipases has been detected. Lid opening can further strengthen the pattern. The active site of lipases is suggested to display negative potential in the pH-range associated with their pH-optimum.

Comparison of *Thermomyces* (*Humicola*) *lanuginosa* lipase (HLL)-catalyzed hydrolysis rates of tributyrin with those of trioctanoin as well as of an artificial substrate (±)-**1** (Scheme 1) [52] suggests the following issues:

1. hydrolysis of trioctanoin is of higher pH-sensitivity than the hydrolysis of other substrates;
2. maximum rate of hydrolysis for (±)-**1** was observed at pH 8.5–9.0; and
3. the hydrolysis rate for native substrates was observed to increase along with increasing value of pH of reaction medium while rate of hydrolysis of (±)-**1** clearly decreased from pH 9 to 10.

Enantioselectivity of HLL-catalyzed hydrolysis of (±)-**1** was determined showing almost linear decrease starting from $E = 200$ at pH 7.0 down to $E = 33$ at pH 10.



Scheme 1. Relationship between maximum hydrolysis rate[a,b,c] and pH of the reaction medium [52]: (a) hydrolysis kinetics has been recorded on a pH-stat; (b) substrates, tributyrin (C_4), trioctanoin (C_8) and 6-endo-butanoyloxybicyclo[3.2.0]hept-2-ene (±)-(**1**); (c) hydrolysis was catalyzed by *Thermomyces* (*Humicola*) *lanuginosa* lipase (HLL).

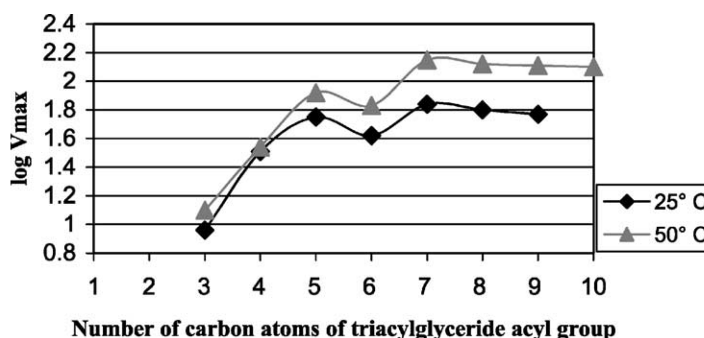
2.3.8. Influence of acyl chain length on enantioselectivity and rate of lipase-catalyzed reactions

The enantioselectivity of CALB-catalyzed transesterification is strongly influenced by the acyl chain length of the achiral acyl donor applied. The longest of the acyl donors studied afforded the highest enantioselectivity over a wide temperature range [53].

The reaction velocity of HLL-catalyzed hydrolysis of different triacylglycerides was recorded (Scheme 2) at 25 and 50 °C [52]. It is noteworthy that rate of HLL-catalyzed hydrolysis of tributyrin almost does not depend on temperature within the range of 25–50 °C. Higher vibrational energy accelerating the reaction could be expected to prevent partially formation of hydrogen bonds essential for functioning of the catalytic machinery of HLL that evidently results in parity between pros and cons in the case of tributyrin.

Hydrolysis rate is shown (Scheme 2) to depend dramatically on the length of acyl chain within the range of 2–5 carbon atoms. Our experience suggests that similar relationships could be expected also for the hydrolysis of a number of artificial substrates. Despite of their higher reactivity, the substrates with acyl chains longer than C_4 are inconvenient to be used because of the problems (their surfactant properties in water medium) arising on work-up of the product.

Application of vinyl acetate instead of longer-chain vinyl esters for lipase-catalyzed transesterification in organic media is clearly preferred because of the simplicity of handling. Low boiling point of vinyl acetate as well as that of acetaldehyde and acetic acid both forming unavoidably in the system allow to isolate all the above components by evaporation at low temperature under reduced pressure on rotary evaporator. Moreover, the hydrolysis of vinyl acetate does not lead to the formation of alcohol, a putative competitive nucleophilic agent, in the reaction mixture. In spite of some drawbacks of the use of vinyl acetate—related to effects resulting from acetaldehyde action on the



Scheme 2. Relationship between the reaction velocity and the acyl chain length of triglycerides at 25 and 50 °C.

lipase or reaction products observed in some cases—these advantages above together with possibility to use inexpensive vinyl acetate as a reaction medium seem to compensate the expected relatively lower reaction rate. Possible limitations in the use of the bulk vinyl acetate as reaction medium may arise from the low solubility of any of the reaction components in it or the need of making use of certain medium effects afforded by prevalence of other solvents (on reaction rate, enantioselectivity, etc.).

A serious disadvantage of the use of vinyl esters with longer achiral (expectedly, also with chiral) acyl chains for transesterification is assumed to be related to disproportional in comparison to that of complex alcohols enhancement of competitiveness of water as nucleophile. Thus, water that is always present in the system to maintain lipase activity reacts preferably and, as a consequence, expected acyl transfer by lipase is diminished markedly. Moreover, a significant amount of a long-chain fatty acid complicating the product isolation is produced. In conclusion, application of a longer acyl chain that may potentially result in higher *E*-values appears to be seriously diminished by disadvantages. Therefore, vinyl and isopropenyl acetates are the most widely used acyl donors.

2.3.9. Mixing conditions: ultrasonication, microwave promotion, isolation of products

Rate of lipase-catalyzed hydrolysis of esters in water medium depends on the area of interface between lipophilic and water phases. Interface area, in turn, depends on the conditions of mixing used to emulsify the system. The agitation method may also have an influence on the outcome of the lipase-catalyzed reaction in organic solvents [54]. Used pre-stirring of enzyme preparations for 18 h with magnet bars caused serious damage to the catalyst resulting in significant inactivation compared to pre-shaking during the same time. This applies to both free and immobilized enzyme. Thus, the use of a shaker or an over-head stirrer is recommended.

Moreover, a suggestion not to use any kind of agitation in the case of some immobilized enzymes (Novozym 435) has been given [52].

2.3.9.1. Microwave promotion of lipase-catalyzed reactions: ultrasonication. Porcine pancreatic lipase has been demonstrated to catalyze enantioselective acylation of 1,2,3,4-tetrahydro-1-naphtol, 1-indanol and menthol in organic media under microwave irradiation. Reaction rates and enantioselectivities were enhanced up to 14- and 3–9-fold, respectively. Almost the same effect has been obtained by using ultrasonication [55].

Microwave promotion has been shown to enhance both rate and selectivity of lipase-catalyzed reactions under solvent-free conditions [56], as well as to activate lipases included in natural seed matrix (!) probably by changing microstructure of seed matrix [57].

2.3.9.2. Extractive separation of products of lipase-catalyzed reaction. In kinetic resolution of secondary alcohols upon lipase-catalyzed transesterification the target ester has to be separated from the less-favored to lipase enantiomer of starting alcohol. Usually this resolution has been performed by column chromatography that is disfavored for technology. In order to overcome this, an interesting approach has been proposed recently. A long-chain perfluorinated acyl has been transferred lipase-catalytically to the favored enantiomer of alcohol thus producing labeled product. This allows extractive separation of the fluorinated ester and nonfluorinated alcohol between a fluorous and an organic solvent. The best biphasic system for this purpose was found to be methanol/*n*-perfluorohexane [58].

2.3.10. Improving the yield of kinetic resolution: dynamic resolution

Theoretically, lipase-catalyzed kinetic resolution can afford target enantiomer in maximum yield of up to 50%. In order to achieve a higher yield, the initial lipase-catalyzed step affording enantiopure product corresponding to the favored enantiomer has been followed (in the case of a substrate or a nucleophile bearing one stereogenic center) either by:

1. racemization of less reactive enantiomer in situ or stepwise (by using spontaneous [59], chemically catalyzed or enzyme-catalyzed [60] reactions); this approach, dynamic resolution, has been discussed in several reviews [61,62];
2. inversion of the configuration of less reactive enantiomer (example, a one-pot two-step process

integrating lipase-catalyzed acylation with following inversion of unfavored secondary alcohol enantiomer upon Mitsunobu esterification [63]).

2.3.11. Lipase engineering

Different approaches and methods allow to enhance the rate and enantioselectivity of the catalytic action of lipase. These approaches could be subdivided as follows.

A. Specific [64] and non-specific [65] pre-treatment of lipases.
B. Chemical modification of lipases [66].
C. Site-directed mutagenesis [67,68].
D. Directed evolution [69].

Several studies on lipase protein engineering [70], methods for improving of hydrolases' catalytic performance [71], etc. are available.

Non-specific pre-treatment of enzymes, for instance, with 2-propanol [65], is not aimed at preparing lipase selective for a certain substrate structure. Specific pre-treatment is, in reverse, oriented on overcoming of low catalytic activity and moderate enantioselectivity of a lipase towards a certain substrate by molecular imprinting of the enzyme. "Substrate matching" strategy based on lipase "memory" as a new efficient strategy for enhancing the lipase enantioselectivity has been proposed [64].

3. Dramatic effects in rate and enantioselectivity, switched enantiopreference within homologs

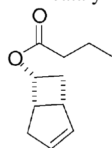
Lipase-catalyzed hydrolysis of esters of 6-endo-hydroxybicyclo[3.2.0]hept-2-ene, a chiral precursor of prostanoids as been studied by several groups [72–75]. The above extensive kinetic resolution study has resulted in recording of excellent to poor reaction rates and enantioselectivities depending on lipase and acyl group used.

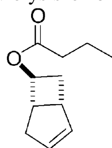
Regarding this family of ester substrates a kinetic study using high-purity porcine pancreatic lipase (PPL) was carried out. The study resulted in observing of some exciting phenomena [75] (see Table 1).

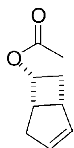
3.1. A superlative reaction rate for an artificial substrate

The racemic endo-butyrate (±)-**1** appeared to be an excellent substrate for PPL. The rate of its lipase-catalyzed hydrolysis was comparable to the rate of the hydrolysis of fast triacylglycerol substrate tributyrin (Table 1, runs 1–4) [75]. The reproducibility of the experiment has been carefully checked up through a number of parallel trials using different preparations of high-purity PPL as well as substrate. An exciting issue remaining unclear has been regarding the product partition in the heterogeneous system.

Table 1
PPL-catalyzed hydrolysis of bicyclic substrates and tributyrin







Run no.	Substrate, ratio, amount (g)	Lipase ($\mu g\,ml^{-1}$)	Colipase ($\mu g\,ml^{-1}$)	NaTDC, 4 mM	k_{cat} (s^{-1})	$k_i \times 10^3$ (s^{-1})
1	(±)-**1** (0.2)	0.2	–	–	3870	2.1
2	(±)-**1** (0.2)	0.5	1.5	+	3560	0.22
3	Tributyrin	0.2	–	–	5100	1.1
4	Tributyrin	0.2	1.5	+	4900	<0.001
5	(±)-**2** (0.2)	20	–	–	<4	
6	(±)-**2** (0.2)	200	–	–	<5	
7	(±)-**1** + (±)-**2** 1/1 (0.2)	1.0	–	–	420	5.7
8	(±)-**1** + (±)-**2** 1/1 (0.2)	1.0	2.2	+	400	0.71
9	(±)-**3** (0.2)	80	–	–	<9	

This resulted in an unusual aggregation of the product to form most probably solid phase including all of the enantiomerically highly pure heptenol. In order to extract the product with Et_2O from the reaction mixture the multiphase system had to be vigorously stirred during some hours [52]. These results suggested that the conformational flexibility, typical of triacylglycerides, is not a prerequisite of native-level fitness of an artificial substrate to PPL.

*3.1.1. Properties of homologous (±)-**3** and diastereomeric (±)-**2***

Homologous to (±)-**1** endo-acetate (±)-**3** was cleaved by PPL by a rate 400 times lower than that of (±)-**1**. Diastereomer of (±)-**1**, exo-butyrate (±)-**2** was about 10^3 times less reactive than (±)-**1**; (±)-**2** was found to inhibit the hydrolysis of (±)-**1** (Table 1, runs 7 and 8).

Structural basis of the substrate selectivity of pancreatic lipases have been highlighted [76]. However, probably MD simulation study could aid to explanation of the above case by adding an additional dimension to arguable structural considerations.

3.2. Switched enantiopreference for 2-phenoxyalkanoic acid ester homologs

Ethyl 2-phenoxyalkanoate substrates **4–9** (Table 2) with increasing acyl chain lengths displayed a gradual change in enantioselectivity with (*R*)-enantiomer preference for **4** switching to (*S*) preference for the substrates with longer acyl chains. Substrate **5** is close to the switch and displayed low hydrolysis rate along with very low enantioselectivity with already small (*S*)-enantiomer enantiopreference [77].

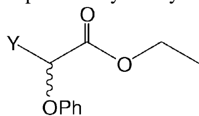
The highest *E*-value and a high initial rate were observed for substrate **7**. When a mutant of HLL was used where the tryptophan residue in the lid of the lipase had been replaced with a glycine (HLL-W89G), the initial rate towards **7** increased twofold but the *E*-value remained virtually the same (Table 2). The use of *Rhizomucor miehei* lipase (RML) showed a dramatic increase in the initial rate compared to that of HLL for substrate **7** [77]. It is not yet clear why such a significant change took place, since the two lipases mentioned usually behave similarly.

4. Substrate selectivity: weak substrates for lipase-catalyzed hydrolysis in aqueous medium

Hydrolytic action of lipases towards various substrates in water is continuously of interest because of allowing to investigate (in vitro) behavior of model systems simulating the processes occurring in vivo.

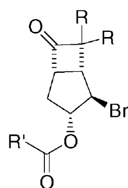
A number of esters, putative artificial substrates for lipase-catalyzed hydrolysis, have been reported to be almost inactive or very poorly hydrolyzed in aqueous medium. An evident common feature for these esters is the presence of certain structural fragments, mainly polar functional groups, in their molecule. The members of the first group [25,78] of inactive esters (**10–13**)

Table 2
Lipase-catalyzed hydrolysis of ethyl 2-phenoxyalkanoates **4–9**



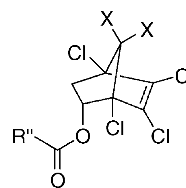
Substrate	Y (see graphics)	Lipase	Initial rate (μmol mg^{-1} min^{-1})	Enantioselectivity (*E*-value)	Enantiopreference
4	CH_3	HLL	190	13	*R*
5	CH_2CH_3	HLL	4.5	1.5	*S*
6	$(CH_2)_2CH_3$	HLL	31	14	*S*
7	$(CH_2)_4CH_3$	HLL	180	57	*S*
		HLL-W89G	360	69	
		RML	2600	30	
8	$(CH_2)_5CH_3$	HLL	90	30	*S*
9	$(CH_2)_7CH_3$	HLL	25	20	*S*

bear keto and bromo functional groups. The members of the second group [79] (**14–16**) bear at least four chloro-substituents each. The members of the third group [52] bear halogen and multiple bond functional groups (**17–18**). And the fourth group [80] of our example esters are derivatives of 1,2-aminoalcohols (**19–20**).



No.	R'	R
10	CH_3	H
11	CH_3	CH_3
12	CH_3	Ph
13	C_3H_7	H

tum chemical investigations were carried out. Frontier orbital localization was studied because of the supposed crucial manner of ester–H_2O interaction leading to the formation of suspension-like systems instead of emulsions needed for lipophilic phase recognition by lipase [25].

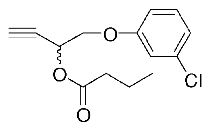


No.	R''	X
14	CH_3	H
15	CH_3	Cl
16	CH_3	OCH_3

Some of esters containing aromaticity [77] or a bromine atom, etc. [25] (and bicyclic carbon skeleton, see Table 3) have been shown to undergo lipase-catalyzed hydrolytic kinetic resolution. Some of above problematic esters have been hydrolyzed upon lipase-catalysis using water-immiscible cosolvent (**17**; Et_2O) [25] or in low-water medium (**13**; $SCCO_2$) [41].

In order to illustrate the results the comparison of four similar compounds is given in Table 3.

It was concluded that for weak substrates the highest occupied molecular orbital (HOMO) was located on a restricted part of the molecule apart from the reaction center that probably leads to the formation



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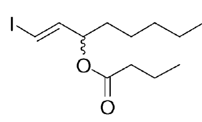

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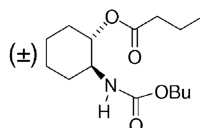

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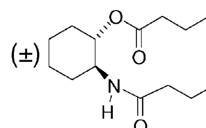

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of a system (in water) inaccessible to lipase. For the reactive esters the HOMO was delocalized on the whole molecule. Regularity in HOMO energetic values was also observed [25].

The occurrence of interaction between above inactive in water medium esters and water molecules that is unfavorable for the ability of lipase to recognize the interface was suggested because their hydrolysis has been successfully performed in modified, low-water media (see also [81,82]).

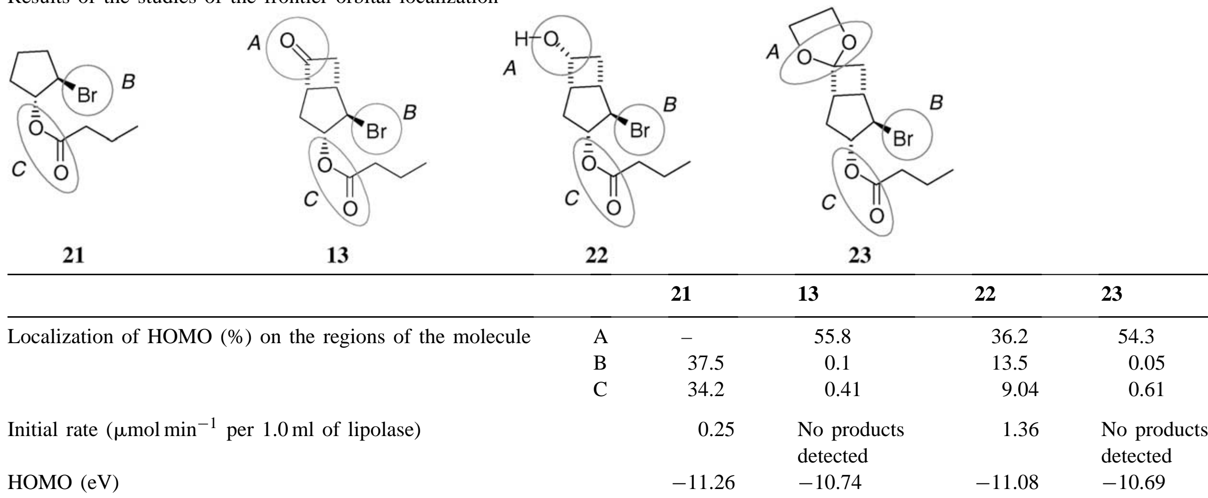
Kinetic resolution of the parent alcohols of inactive ester substrates has been usually performed using lipase-catalyzed transesterification in organic media (for aminoalcohols, see [83]).

In order to elaborate a method for prognosticating the inaccessibility to lipase of putative substrates quan-

5. Preparation of enantiomers of required enantiomeric excess: sequential resolution

Several pairs of enantiomers have been reported to have not only opposite sign of optical rotation but also opposite biological effects.

Table 3
Results of the studies of the frontier orbital localization



		21	13	22	23
Localization of HOMO (%) on the regions of the molecule	A	–	55.8	36.2	54.3
	B	37.5	0.1	13.5	0.05
	C	34.2	0.41	9.04	0.61
Initial rate (μmol min^{-1} per 1.0 ml of lipolase)		0.25	No products detected	1.36	No products detected
HOMO (eV)		−11.26	−10.74	−11.08	−10.69

A good example is Japanese beetle pheromone: (*R*)-enantiomer attracts male beetles while (*S*)-enantiomer interferes, even as a 1% impurity [84]. The same effect is known also for some active compounds of medicines including prostanoids (for example, for cloprostenol—a highly potent luteolytic agent used in veterinary medicine).

According to the regulatory guidelines the determination of physiological effect of each enantiomer of a drug candidate is required. The problem seems to remain: how to estimate the level of enantiomeric excess of a novel compound required for adequate testing. Probably it could be solved by simultaneous preliminary testing of each enantiomer with different exactly determined enantiomeric excess, perhaps of 98% e.e. and 99.8% e.e. If difference in physiological activity of samples is observed, a sample of next level of enantiomeric excess (99.98%) should be tested.

Highly pure enantiomers of physiologically active compounds can be prepared by using enzymatic or chemical processes with repeated chiral recognition (with multiple enantioselection).

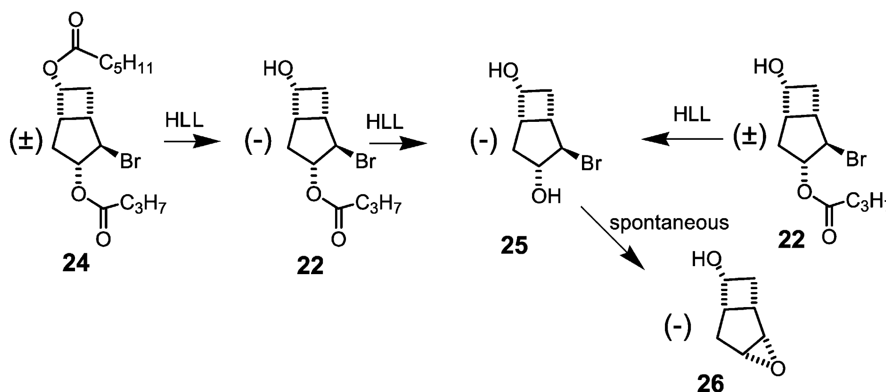
Resolution of racemic complex polyol compounds (for instance, prostanoids) of up to 99.98% e.e. or even higher may be performed by using chemical, for instance, mandelic acid derivatization approach [85,86] in one-pot. Enantiomers of a monoatomic alcohol may be resolved (up to very high enantiomeric purity) by using the same approach as sequential, stepwise, by oligomer synthesis followed by diastereomer resolution.

High enantiomeric excess may be achieved by using of a sequential lipase-catalyzed kinetic resolution [36].

For complex polyhydroxycarboxylic acids (example, F-type prostanoids) realization of highly selective enantiorecognition mode (tested by using both individual enantiomers of cloprostenol) with CALB has been observed [23]. The selectivity exceeded the detection level for ^{1}H NMR.

The problem of determination of very high enantiomeric excess of a compound is probably more complicated to resolve than the problem of preparation of samples of super-high enantiomeric purity. Lipase-catalyzed kinetic resolution has been used to aid the determination of high enantiomeric purity by concentrating the minor enantiomer in order to lower, by using a tested process e.e. of the sample to 98% or less being the detection level for the most of suitable methods [87].

For lipase-catalyzed kinetic resolution of carboxylic acids and alcohols of simple structure the most usual problem to be solved by sequential resolution is to improve good, moderate and even fair enantioselectivity of process in order to gain a product with e.e. at least 98%. In sequential resolution the substrate has to go through the active site of the enzyme at least twice



Scheme 3. HLL-catalyzed doubly enantioselective kinetic resolution. A lipase-triggered cascade of reactions resulting in epoxide (**26**) formation.

during the process resulting in a high total apparent enantioselectivity.

Double enantioselection has been used for demethoxycarbonylation of possible precursors for carbacyclin synthesis [88]. Highly efficient double enantioselection by lipase has been used for transesterification of (*R*,*S*)-carboxylic acid vinyl esters with (*R*,*S*)-1-phenylethanol [89]. In this process CALB showed excellent enantioselectivity ($E > 100$) toward (*R*,*S*)-1-phenylethanol and moderate enantioselectivity ($E \approx 10$) toward carboxylic acid vinyl esters. Also CALB-catalyzed doubly enantioselective aminolysis reactions have been reported [90]. Preparation of *S*-2-ethylhexyl-*para*-methoxycinnamate by lipase-catalyzed sequential kinetic resolution has been described [91].

Occurrence of double enantioselection by HLL has been shown for hydrolysis of a bicyclic bis-acylated bromodiol. Resulting bromodiol (**25**) was found to contain the minor enantiomer less than the detection level (Scheme 3) [92].

The importance of use of highly enantiopure compounds as medicines is sometimes considered to be arguable because of racemization of a number of chiral compounds is occurring in the living organism. This may be true only in the case of simple compounds bearing one stereogenic center. For complex physiologically highly active molecules like prostanoids bearing up to five stereogenic centers racemization of one (or more) stereogenic center could be considered as normal (due!) metabolic inactivation.

6. Cascade-reactions triggered by lipase: low apparent chemoselectivity

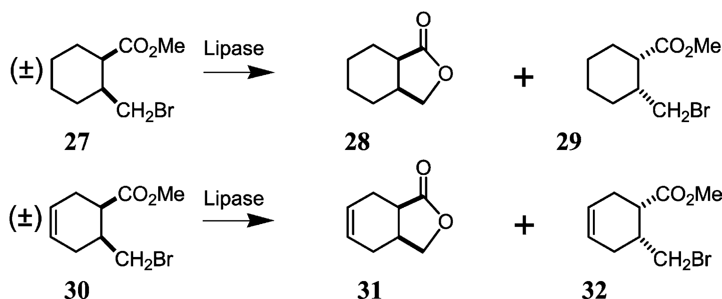
Lipase-catalyzed reactions used for kinetic resolution may result, either by hydrolysis or transesterification, in generation of activated species or in exposition (sterically or by cleavage of protecting groups) of functional groups of a substrate to intra- or intermolecular attacks.

Both, enzymatic and spontaneous cascade-reactions have been reported (Scheme 3) [92]. Whereas the enzymatic cascade-reactions may be called in some cases as sequential kinetic resolution yielding a product of enhanced enantiomeric excess [92], the spontaneous cascade-reactions triggered by initial lipase-catalyzed reaction more likely cause the low overall apparent chemoselectivity of the process.

Also racemization of some compounds to be resolved upon lipase-catalyzed amidation has been observed during the process. This was found to be a novel turnover-related racemization [93] in the CALB-catalyzed acylation of α-aminonitriles, for which a plausible mechanism has been proposed.

6.1. Dehydrohalogenation: ring-closure reactions

Recently, an interesting enzyme-triggered enantio-convergent cascade-reaction [94] as well as an epoxide hydrolase-catalyzed diastereo-convergent hydrolysis of a haloalkyl oxirane which triggers spontaneous ring closure affording the product in stereoselective



Scheme 4. Lipase-catalyzed hydrolysis triggers spontaneous lactonization.

fashion has been described [95]. In these processes the halohydrin, which is unstable in the environment where it has been formed, was produced by an epoxide hydrolase. The similar result has been observed upon lipase-catalyzed hydrolysis of bromoesters [92,96].

6.1.1. Epoxidation

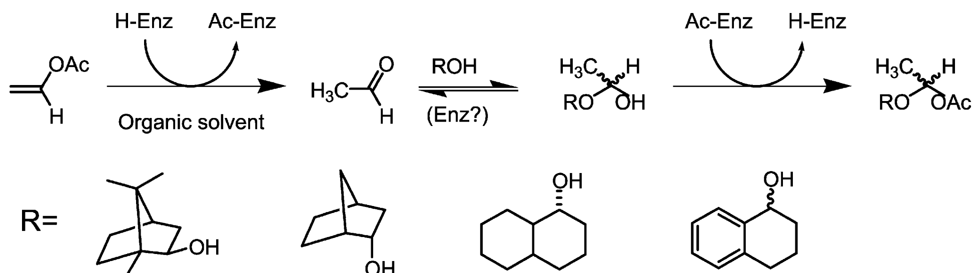
A three-step reaction sequence pictured on Scheme 3 consisting of two lipase-catalytic hydrolysis reactions proceeding in strict order have been reported to afford bromodiol **25**. This compound is unstable in aqueous medium at pH 7 [52] undergoing dehydrohalogenation within minutes on pH-stat. Spontaneous dehydrohalogenation was found to occur faster than enzymatic hydrolysis of monoester **22**. Consequently, final ratio of bromodiol **25** and hydroxyepoxide **26** in the crude product may be determined by the distribution of bromodiol **25** between bulk lipophilic phase and the interface between the phases where it could be exposed to attack by water molecule. Perhaps, rate of this reaction could depend strongly also on mixing conditions. Bromodiol **25** could be expected to be stable in the lipophilic phase, because after completing the lipase-catalyzed hydrolysis the ratio of compounds **25**/**26** appeared to be as high as ∼3/1.

6.1.2. Lactonization

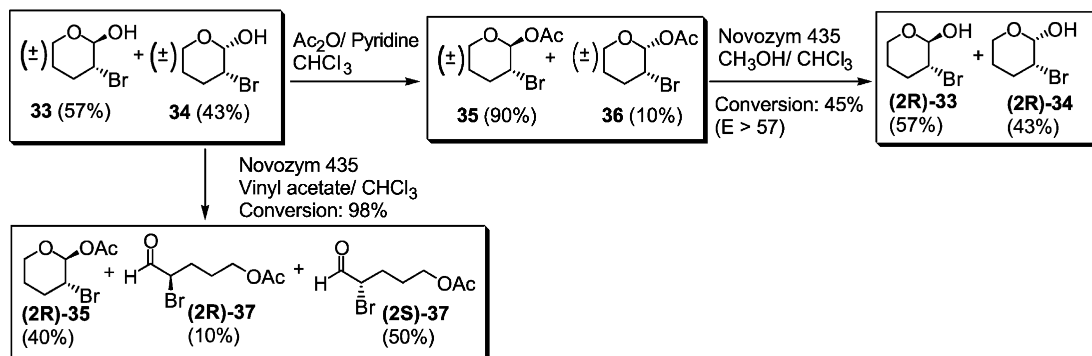
An interesting spontaneous ring-closure reaction of bromocarboxylic acids resulting in lactones has been described to occur under hydrolytic conditions at pH 7: the process has been triggered by initial pig liver esterase (PLE) catalyzed hydrolysis of bromoesters **27** and **30**, respectively (Scheme 4). All the products (**28**, **31**) and unreactive bromoesters (**29**, **32**) were gained enantimerically pure (e.e. = 97%) [96].

6.2. Lipase-catalyzed aldehyde generation and cascade-reactions

Some sterically hindered secondary alcohols (Scheme 5) react slowly to yield hemiacetal esters as mixtures of diastereomers under conditions of lipase-catalytic transesterification in organic media [97]. Their formation can be explained by the reaction of the alcohol with acetaldehyde ("spontaneous cascade-reaction") that is produced by the



Scheme 5. Formation of hemiacetal esters [97]: a two-step cascade of reactions triggered by the initial lipase-catalyzed aldehyde generation.



Scheme 6. Chemical and lipase-catalyzed acetylation (deacetylation) of rac-2-bromo-5-hydroxypentanal.

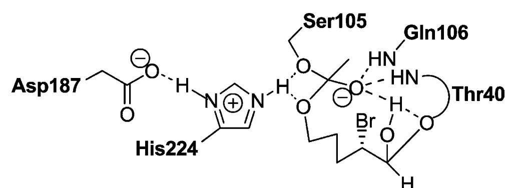
lipase-catalyzed splitting of vinyl acetate and subsequent acetylation of the resulting hemiacetal by the lipase ("enzymatic cascade-reaction").

Based on some recent results (Scheme 6) the enzymatic nature of cleavage of hemiacetal (Scheme 5) could be suggested with certain confidence [22].

Lipase-catalyzed decyclization of hemiacetals of α-bromo-ω-hydroxypentanal (Scheme 6) as well as α-bromo-ω-hydroxybutanal (Scheme 7) is either enantioselective or not depending on steric complementary of respective *trans*-hemiacetals to CALB [22].

During the enzymatic acetylation of the C_5-aldehyde (Scheme 6) 2*S*-enantiomer was stabilized by the lipase as the open-chain isomer followed by trapping upon acetylation while the 2*R*-enantiomer afforded preferentially the cyclic *trans*-acetate (2*R*)-**35**. CALB-catalyzed acetylation of C_4-aldehyde resulted in an almost racemic mixture of open-chain acetates.

The activation energies of the decyclization of hemiacetal range between 7 and 9.5 kcal∗mol^{-1}. Hemiacetal decyclization has been expected to occur upon transhemiacetalization with the hydroxyl group of Thr40 of CALB (Scheme 8).

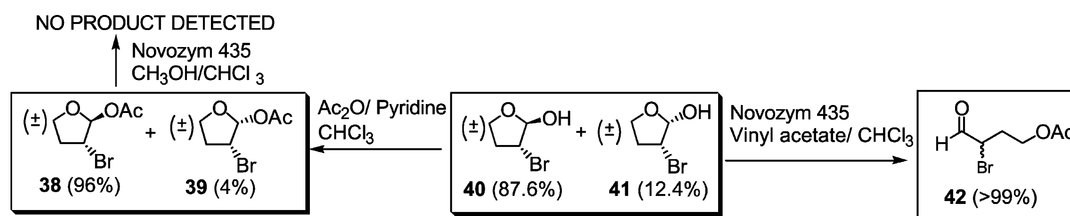


Scheme 8. Probable tetrahedral intermediate of CALB-catalytic ω-acetylation of (2*S*)-2-bromo-5-hydroxypentanal.

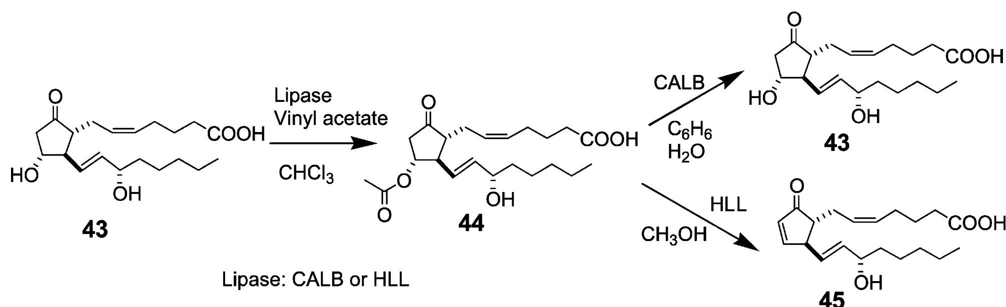
The activation of deoxymonosaccharides by lipase upon decyclization undoubtedly leads to occurrence of cascade-reactions under proper conditions.

6.3. A lipase-catalyzed unexpected elimination reaction: reversibility of acetylation of a β-ketol moiety depends on lipase and reaction medium

Exclusive pathways have been described showing possibility to gain different products upon lipase-catalyzed reactions depending on lipase and reaction medium (Scheme 9) [23].



Scheme 7. Chemical and lipase-catalyzed acetylation of *rac*-2-bromo-4-hydroxybutanal.



Scheme 9. HLL-catalyzed elimination of acetic acid from 11-acetyl-PGE_2.

Elimination of acetic acid from 11-acetyl-PGE_2 (**44**) (Scheme 9) has been shown to be not a spontaneous reaction but catalyzed by HLL while CALB allows to deprotect 11-OH resulting in (starting) PGE_2 (**43**) upon hydrolytic cleavage of ester. The elimination reaction has been supposed to occur via a mechanism devoid of covalent binding of the substrate to the enzyme [23]. The 11-acetylation of **43** has been catalyzed by both of the above lipases. 11-Acetyl-PGE_2 has been shown to be sufficiently stable (the rate of spontaneous degradation (to PGA_2) was estimated to be ~2% per 24 h) for handling under the conditions used [52].

7. Lipase-catalyzed reactions of isomeric forms of hydroxyaldehydes: dynamic kinetic resolution

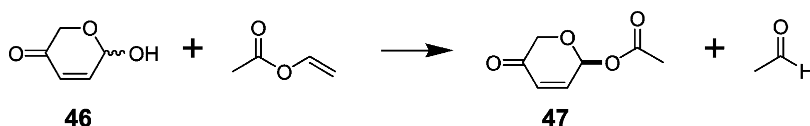
Examples of the enantioselective acetylation of a hemiacetal (**33**) and of the enantioselective deacetylation of corresponding hemiacetal esters have been shown in Schemes 6–8. As well as a lipase-catalyzed decyclization of hemiacetals followed by trapping upon acetylation. The latter process was observed to occur with low enantioselectivity or almost without enantioselection. Some examples should be referred to in this context.

A very interesting example [59,61] of dynamic kinetic resolution involving spontaneous racemization of a cyclic hemiacetal and enantioselective lipase-catalyzed acetylation of the proper hemiacetal enantiomer has been described (Scheme 10).

6-Acetoxy-2H-pyran-3(6H)-one **47** has been obtained in near quantitative yield and of moderate (76%) excess of the (*S*)-enantiomer. Taking into account the structural features of hemiacetal **46** the enantiopurity of the hemiacetal ester gained seems surprisingly high giving evidence of probable occurrence of enantiorecognition in mode based on non-steric interactions in this case. No lipase-catalytic acetylation of open-chain form corresponding to hydroxyaldehyde **46** has been reported.

Enzyme-catalyzed asymmetric hydrolysis of both cyclic [98] and acyclic [99] hemiacetal esters has been described. A method for resolution of dialkyl acetals of chiral aldehydes has also been suggested [100].

Significant efforts have been aimed at development of lipase-catalytic regioselective protection and deprotection methods for saccharides. Esterification of methyl glycoside mixtures by lipase-catalysis has been investigated [101]; *Pseudomonas cepacia* lipase showed high substrate specificity for the esterification of methyl α-D-galactopyranoside. Methods for the



Scheme 10. Dynamic kinetic resolution of hemiacetals.

regioselective acylation of some α-D-galactopyranoside derivatives have been proposed [102].

Methods for lipase-catalytic regioselective acylation of disaccharides have been described [103]. Peracetylated α-D-galactopyranose has been hydrolyzed (catalyzed with *Candida cylindracea* lipase (CCL)) regioselectively at the 4-position under neutral pH and at 6-position under acidic conditions [104].

Lipase-catalyzed synthesis of *N*-acetylated hydroxylated amines [105], arylaliphatic glycolipids [106] and α-butylglycoside lactate [107], representatives of selective reactions of important classes of natural products has been reported. An enzymatic approach to combine ring-opening polymerization of ε-caprolactone and regioselective acylation of methyl glycopyranosides has been investigated [108].

A review on biocatalytic selective modifications of different types of nucleosides [109] as well as an interesting report on the synthesis of acyclic nucleosides that have significant antiviral activity are available [110].

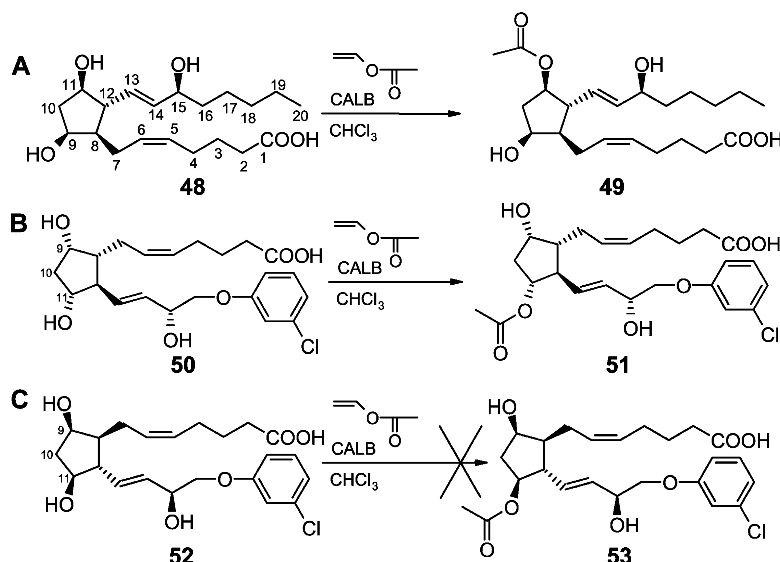
8. Lipase-catalyzed derivatization of prostanoids

A number of prostanoids are continuously used in biological, pharmacological and medical research. The simplest approach to the synthesis of several prostanoids involves interconversions starting from $PGF_{2\alpha}$, PGE_2, etc. Prostanoids are (poly)hydroxycarboxylic acids of complex structure. Some of them are both acid- and base-sensitive compounds. Consequently, methodologies of chemo- and regioselective treatment of hydroxyl groups of the parent PG are of crucial importance. The choice of synthetic methods as well as of protecting groups should be in accordance with the stability requirements for target compounds.

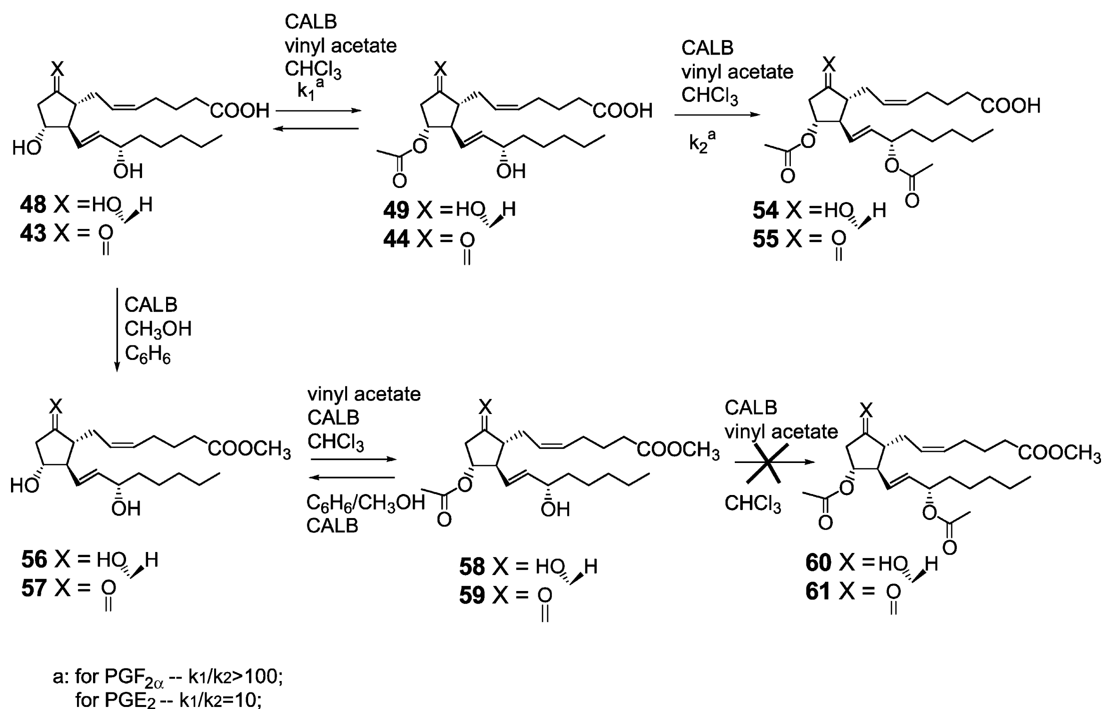
Lipases have been described [23] to allow chemo-, regio- and stereoselective protection and deprotection of functional groups of several prostanoids in many cases. However, there have also been described lipase-catalyzed reactions of prostanoids that are not chemo- and regioselective or even lead to unexpected results (see Scheme 9).

Lipase-catalyzed acetylation of prostanoids has been shown by CALB-catalyzed acylation of both individual enantiomers of cloprostenol **50** and **52** (Scheme 11) to be a highly stereo(enantio)selective process.

CALB-catalyzed acetylation of (+)-cloprostenol (**50**) as well as natural $PGF_{2\alpha}$ (**48**) was found to occur highly regioselectively: difference in the rates of acetylation of 11-OH and 15-OH group has been found to be at least 10^2.



Scheme 11. The enantio- and regioselective acetylation of prostanoids of F series catalyzed by CALB: (A) acetylation of natural $PGF_{2\alpha}$ (**48**); (B) acetylation of (+)-cloprostenol (**50**); (C) no 11-acetate of (−)-cloprostenol (**53**) was obtained upon lipase-catalyzed acetylation.



Scheme 12. CALB-catalyzed derivatization of $PGF_{2\alpha}$ and PGE_2.

Regioselectivity of acetylation of PGE_2 (**43**) was observed to be significantly lower compared to F-type prostanoids **48** and **50** (Scheme 12).

Prostaglandin E_1 is very slowly acetylated by CALB. Some exclusive pathways for lipase-catalyzed derivatization of prostanoids (Scheme 12) have been found [23,52].

1. Unlike to the 15-OH group in the free carboxylic acids **44** and **49** the 15-OH group of 11-acetyl-PGE_2 methyl ester (**59**) as well as that of 11-acetyl-$PGF_{2\alpha}$ methyl ester (**58**) were found to be inaccessible to CALB (substrate-assisted catalysis could be expected to occur in the case of free carboxylic acid).
2. 11-Acetyl-PGE_2 (**44**) has been deacetylated by CALB in wet benzene affording parent PGE_2 (**43**), while treatment of **44** by HLL in methanol yielded exclusively PGA_2 (**45**) (see Scheme 9).

The results of studies of lipase-catalyzed derivatization of prostanoids suggest the interaction between the carboxyl group of a PG and functional groups of a lipase to be decisive in determining modes of recognition and subsequent binding of PG and a lipase.

Elimination reaction catalyzed by HLL (Scheme 9) could be related to “strong aid” of “oxy-anion hole” supporting tendency to enolization of the cyclopentanone moiety of 11-acetyl-PGE_2 thus enforcing elimination.

Evidence of non-steric interactions occurring during molecular recognition between several polyfunctional alcoholic nucleophiles, other than PGs, and CALB has been discussed [111,112].

Regarding to eicosanoid-protein recognition features the interesting recent results should be referred to: in the research of cyclooxygenase mechanisms [113] there has been determined the structure of PGHS-1 at 3 Å resolution with arachidonic acid bound in a chemically productive conformation [114]. The role of each amino acid residue has become clearly evident.

The active site structure (geometry) of several lipases is known from the X-ray difractometric studies. Thus, modeling of certain binding modes for prostanoids in lipase active site could give rather

plausible results by using computational methods (and additionally, taking also into account the above guidelines of empiric origin).

However, a PG/lipase molecular recognition is always a very complicated process depending on a number of partial interactions and therefore any predictions of functional group selectivity, chemoselectivity and even regio- as well as diastereoselectivity should be treated carefully.

9. Lipases and retrosynthetic pathways for natural products

Based on recent results, some first steps of the retrosynthetic schemes for several compounds isolated from natural sources can be clearly identified as hydrolase-catalytic. That may have been followed (preceded in retrosynthetic scheme) by some spontaneous (cascade) reactions: by those initiated by non-specific components and factors of natural environment—H_2O, O_2, sunlight, thermal activation, etc.

The richest natural sources of different prostanoids are corals: horny coral *Plexaura homomalla* [115–117], soft coral *Lobophyton depressum* [118], etc. It seems noteworthy that just 11-acetylated derivatives of F-type prostanoids as well as 15-acetylated A-type prostaglandins and their esters have been the most prevailing components of the prostanoid fractions isolated from the corals. These natural derivatives of prostanoids have been shown above (Schemes 9 and 11 and [23]) to be easily gained upon one to three lipase-catalytic reactions starting from $PGF_{2\alpha}$ and PGE_2 [119], respectively.

From soft coral *Lobophyton depressum* (15*S*)-11-acetyl-$PGF_{2\alpha}$ (**49**) and its methyl ester (**53**), which are characteristic products of lipase-catalyzed acetylation (Scheme 11) as well as 18-acetoxy-11-acetyl-$PGF_{2\alpha}$ and also corresponding methyl ester have been isolated [118]. The 18-acetoxy-derivatives of $PGF_{2\alpha}$ may have been formed upon one to three lipase-catalytic steps and enzyme-catalyzed functionalization of 17, 18 double bond (characteristic for PG-s of three series), since isolation not of a diastereomeric mixture but a single compound has been reported.

The similar evidences have been observed also for sterols isolated from soft corals [120].

As a conclusion, lipase-catalyzed reactions have been used successfully in semisynthesis of prostanoids [23] and sterols [121].

In addition, the best of pronounced examples suitable for demonstrating the steric scope of synthetic use of lipases in natural product chemistry could be selective acylation of 10-deacetylbaccatin III, a compound of taxol family [122], which consists of compounds isolated from *Taxus brevifolia* (Scheme 13).

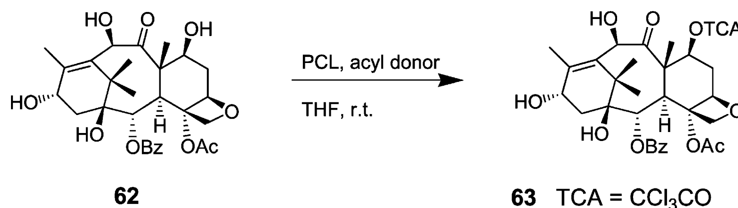
10. Inhibition of lipases

In previous paragraphs the main concern has been how to enhance the rate of lipase-catalyzed reactions and how to do it as selectively as possible. For this, several factors inhibiting the lipase-catalytic reactions have been identified and their influence has been tried to diminish.

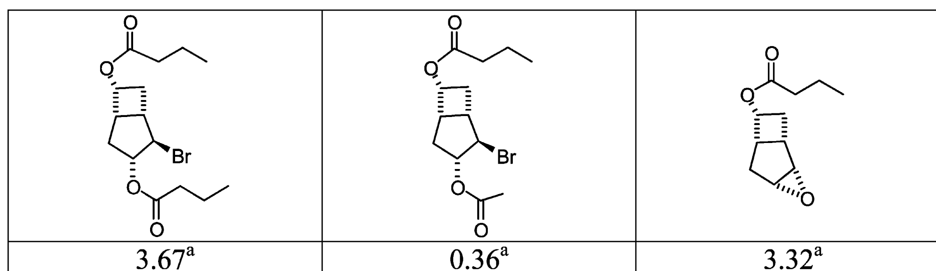
On the other hand, knowledge of the inhibiting factors allow to control lipase-catalytic changes occurring in several biochemical systems including those which occur in vivo. Digestive process could be an example where lipase inhibitors are currently used as drugs for treating obesity.

Probably the most important role of inhibitors is to be used in the study of structural and mechanistic properties of lipases.

Both of the fields have been reviewed well [123,124]. In the current minireview several inacces-



Scheme 13. PCL-catalyzed reaction of 10-deacetylbaccatin III [122].



Scheme 14. Cyclobutanol ester substrates. Non-steric retarding of the hydrolysis by remote structural change: (a) initial rate of HLL-catalyzed hydrolysis (μmol min^{-1} per 1.0 ml of lipolase).

sible to lipases esters have been referred to, which have been shown to interact with water in an especial manner making the interface between lipophilic and water phases unrecognizable to lipases. In addition, the stereoisomeric contaminants (more hydrophilic, weak substrates) may occupy prevalently surface of the lipophilic phase making it less recognizable to a lipase thus causing an inhibitory effect (Table 1) [75].

Structural change in the substrate molecule apart from the reaction center may lead to significant inhibition of the hydrolysis as it has been observed for bicyclic substrates (Scheme 14) [25].

This inhibitory effect illustrated (Scheme 14) has been assumed to arise from possibility of two orientations of the bipolar substrate molecules on interface between water and the substrate [25].

Recent studies of lipase inhibitors mainly concentrate on synthesis of 2-oxo amide triacylglyceride analogues [125] and the determination of their inhibitory effect on pancreatic [126] and gastric [127] lipases [128]. To inhibit lipases, also usual inhibitors of serine-hydrolases are valid: alkylphosphonates, sulfonyl fluorides, etc. For instance, lipoprotein lipase has been efficiently inhibited by alkanesulfonyl fluorides [129]. Interesting inhibitory effect of quercetin on *Candida rugosa* lipase has been reported [130].

11. Summary

Lipases have been established as valuable asymmetric catalysts in organic synthesis. They allow to perform a number of stereo- and regioselective transformations in the synthesis of physiologically active compounds. Especially valuable is the contribution of the lipases to semisynthesis of the complex natural products.

Lipase-catalyzed reactions have been described to trigger cascade-reactions of different products. Lipase-catalyzed aldehyde generation starting from deoxysugars triggering cascade-reactions seems undoubtedly to be an issue for further study.

Acknowledgements

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References

[1] K. Faber, Biotransformations in Organic Chemistry, fourth ed., Springer, Heidelberg, 2000.
[2] U.T. Bornscheuer, R.J. Kazlauskas, Hydrolases in Organic Synthesis, Wiley-VCH, Weinheim, 1999.
[3] A.M.P. Koskinen, A.M. Klibanov, Enzymatic Reactions in Organic Media, Kluwer Academic Publishers, Dordrecht, 1995.
[4] P. Berglund, K. Hult, Biocatalytic synthesis of enantiopure compounds using lipases, in: R.N. Patel (Ed.), Stereoselective Biocatalysis, Marcel Dekker, New York, 2000, Chapter 21, pp. 633–657.
[5] S.M. Roberts, N.J. Turner, A.J. Willetts, M.K. Turner, Introduction to Biocatalysis Using Enzymes and Micro-Organisms, Cambridge University Press, Cambridge, 1995.
[6] L. Poppe, L. Novak, Selective Biocatalysis, Verlag Chemie, Weinheim, 1992.
[7] R.D. Schmid, R. Verger, Angew. Chem. Int. Ed. 37 (1998) 1608.

[8] F. Theil, Chem. Rev. 95 (1995) 2203.
[9] A. Zaks, D.R. Dodds, DDT 2 (1997) 513.
[10] S.M. Roberts, J. Chem. Soc., Perkin Trans. 1 (1998) 157.
[11] S.M. Roberts, J. Chem. Soc., Perkin Trans. 1 (1999) 1.
[12] W.A. Loughlin, Bioresource Technol. 74 (2000) 49.
[13] A.R.M. Yahya, W.A. Anderson, M. Moo-Young, Enzyme Microbial Technol. 23 (1998) 438.
[14] R.V. Muralidhar, R. Marchant, P. Nigam, J. Chem. Technol. Biotechnol. 76 (2001) 3.
[15] R.A. Gross, A. Kumar, B. Kalra, Chem. Rev. 101 (2001) 2097.
[16] A.M. Klibanov, TIBTECH 15 (1997) 97.
[17] R. Verger, TIBTECH 15 (1997) 32.
[18] G. Dodson, A. Wlodawer, TIBS 23 (1998) 347.
[19] J.D. Rozzell, Bioorg. Med. Chem. 7 (1999) 2253.
[20] M. Holmquist, Curr. Protein Peptide Sci. 1 (2000) 209.
[21] R.J. Kazlauskas, Curr. Opin. Chem. Biol. 4 (2000) 81.
[22] L. Villo, A. Metsala, O. Parve, T. Pehk, Tetrahedron Lett. 43 (2002) 3203.
[23] O. Parve, I. Järving, I. Martin, A. Metsala, I. Vallikivi, M. Aidnik, T. Pehk, N. Samel, Bioorg. Med. Chem. Lett. 9 (1999) 1853.
[24] M. Baumann, B.H. Hauer, U.T. Bornscheuer, Tetrahedron: Asymmetry 11 (2000) 4781.
[25] O. Parve, I. Vallikivi, A. Metsala, Ü. Lille, V. Tõugu, P. Sikk, T. Käämbre, H. Vija, T. Pehk, Tetrahedron 53 (1997) 4889.
[26] R.J. Kazlauskas, A.N. Weissfloch, A.T. Rappaport, L.A. Cuccia, J. Org. Chem. 56 (1991) 2656.
[27] I. Borreguero, J.V. Sinisterra, A. Rumbero, J.A. Hermoso, M. Martinez-Ripoll, A.R. Alcantara, Tetrahedron 55 (1999) 14961.
[28] K. Lemke, M. Lemke, F. Theil, J. Org. Chem. 62 (1997) 6268.
[29] C. Orrenius, F. Hæffner, D. Rotticci, N. Öhrner, T. Norin, K. Hult, Biocatal. Biotrans. 16 (1998) 1.
[30] F. Hæffner, T. Norin, K. Hult, Biophys. J. 74 (1998) 1251.
[31] S. Raza, L. Fransson, K. Hult, Protein Sci. 10 (2001) 329.
[32] A. Tafi, A. Van Almsick, F. Corelli, M. Crusco, K.E. Laumen, M.P. Schneider, M. Botta, J. Org. Chem. 65 (2000) 3659.
[33] M. Botta, E. Cernia, F. Corelli, F. Manetti, S. Soro, BBA 1296 (1996) 121.
[34] H.K. Weber, H. Weber, R.J. Kazlauskas, Tetrahedron: Asymmetry 10 (1999) 2635.
[35] L. Brecker, D.W. Ribbons, TIBTECH 18 (2000) 197.
[36] P. Berglund, Biomol. Eng. 18 (2001) 13.
[37] H. Yang, E. Henke, U.T. Bornscheuer, J. Org. Chem. 64 (1999) 1709.
[38] R. Morrone, M. Piatelli, G. Nicolosi, Eur. J. Org. Chem. (2001) 1441.
[39] T. Dumont, D. Barth, M. Perrut, J. Supercrit. Fluids 6 (1993) 85.
[40] Z. Knez, V. Rizner, M. Habulin, D. Bauman, J. Am. Oil Chem. Soc. 72 (1995) 1345.
[41] O. Parve, I. Vallikivi, L. Lahe, A. Metsala, Ü. Lille, V. Tõugu, H. Vija, T. Pehk, Bioorg. Med. Chem. Lett. 7 (1997) 811.
[42] M. Perrut, High Pressure Biotechnol. 224 (1992) 401.
[43] K.-W. Kim, B. Song, M.-Y. Choi, M.-J. Kim, Org. Lett. 3 (2001) 1507.
[44] R.M. Lau, F. Van Rantwijk, K.R. Seddon, R.A. Sheldon, Org. Lett. 2 (2000) 4189.
[45] F. Theil, Tetrahedron 56 (2000) 2905.
[46] J. Ottosson, L. Fransson, J.W. King, K. Hult, BBA 1594 (2002) 325.
[47] Y.L. Khmelnitsky, S.H. Welch, D.S. Clark, J.S. Dordick, J. Am. Chem. Soc. 116 (1994) 2647.
[48] T. Okamoto, S. Ueji, Chem. Commun. (1999) 939.
[49] T. Ke, A.M. Klibanov, J. Am. Chem. Soc. 121 (1999) 3334.
[50] L. Kvittingen, B. Sjursnes, T. Anthonsen, P. Halling, Tetrahedron 48 (1992) 2793.
[51] P. Fojan, P.H. Jonson, M.T.N. Petersen, S.B. Petersen, Biochimie 82 (2000) 1033.
[52] Our unpublished results.
[53] J. Ottosson, K. Hult, J. Mol. Catal. B: Enzym. 11 (2001) 1025.
[54] L. Kvittingen, B. Sjursnes, P. Halling, T. Anthonsen, Tetrahedron 48 (1992) 5259.
[55] G. Lin, W.Y. Lin, Tetrahedron Lett. 39 (1998) 4333.
[56] J.-R. Carrillo-Munoz, D. Bouvet, E. Guibe-Jampel, A. Loupy, A. Petit, J. Org. Chem. 61 (1996) 7746.
[57] C.T. Ponne, A.C. Möller, L.M.M. Tijskens, P.V. Bartels, M.M.T. Meijer, J. Agric. Food Chem. 44 (1996) 2818.
[58] B. Hungerhoff, H. Sonnenschein, F. Theil, Angew. Chem. Int. Ed. 40 (2001) 2492.
[59] M. van den Heuvel, A.D. Cuiper, H. van der Deen, R.M. Kellogg, B.L. Feringa, Tetrahedron Lett. 38 (1997) 1655.
[60] U.T. Strauss, K. Faber, Tetrahedron: Asymmetry 10 (1999) 4079.
[61] M.T. El Gihani, J.M.J. Williams, Curr. Opin. Chem. Biol. 3 (1999) 11.
[62] R. Azerad, D. Buisson, Curr. Opin. Biotechnol. 11 (2000) 565.
[63] E. Vänttinen, L.T. Kanerva, Tetrahedron: Asymmetry 6 (1995) 1779.
[64] D. Lee, Y.K. Choi, M.-J. Kim, Org. Lett. 2 (2000) 2553.
[65] A. Cipiciani, M. Cittadini, F. Fringuelli, Tetrahedron 54 (1998) 7883.
[66] G. DeSantis, J.B. Jones, Curr. Opin. Biotechnol. 10 (1999) 324.
[67] A. Magnusson, K. Hult, M. Holmquist, J. Am. Chem. Soc. 123 (2001) 4354.
[68] F. Manetti, D. Mileto, F. Corelli, S. Soro, C. Palocci, E. Cernia, I. D'Acquarica, M. Lotti, L. Alberghina, M. Botta, BBA 1543 (2000) 146.
[69] R.J. Kazlauskas, H.K. Weber, Curr. Opin. Chem. Biol. 2 (1998) 121.
[70] A. Svendsen, BBA 1543 (2000) 223.
[71] P. Villeneuve, J.M. Muderhwa, J. Graille, M.J. Haas, J. Mol. Catal. B: Enzym. 9 (2000) 113.
[72] I.C. Cotterill, E.L.A. Macfarlane, S.M. Roberts, J. Chem. Soc., Perkin Trans. 1 (1988) 3387.
[73] N. Klempier, K. Faber, H. Griengl, Biotech. Lett. 2 (1989) 685.

[74] N. Klempier, P. Geymayer, P. Stadler, K. Faber, H. Griengl, Tetrahedron: Asymmetry 1 (1990) 111.
[75] O. Parve, A. Pals, V. Kadarpik, Ü. Lille, P. Sikk, A. Lõokene, T. Välimäe, Bioorg. Med. Chem. Lett. 3 (1993) 357.
[76] F. Carriere, C. Withers-Martinez, H. van Tilbeurgh, A. Roussel, C. Cambillau, R. Verger, BBA 1376 (1998) 417.
[77] P. Berglund, I. Vallikivi, L. Fransson, H. Dannacher, M. Holmquist, M. Martinelle, F. Björkling, O. Parve, K. Hult, Tetrahedron: Asymmetry 10 (1999) 4191.
[78] J. Spreitz, R. Grobbauer, H. Griengl, K. Faber, Braumschweig (1990) 93.
[79] B. Berger, C.G. Rabiller, K. Königsberger, K. Faber, H. Griengl, Tetrahedron: Asymmetry 1 (1990) 541.
[80] H. Hönig, P. Seufer-Wasserthal, Synthesis (1990) 1137.
[81] D.B. Berkowitz, R.E. Hartung, S. Choi, Tetrahedron: Asymmetry 10 (1999) 4513.
[82] P. Ciuffreda, S. Casati, E. Santaniello, Tetrahedron 56 (2000) 317.
[83] A. Maestro, C. Astorga, V. Gotor, Tetrahedron: Asymmetry 8 (1997) 3153.
[84] J.H. Tumlinson, M.G. Kleinn, R. Doolittle, T.T. Latt, A.T. Proveaux, Science 197 (1977) 789.
[85] O. Parve, M. Aidnik, Ü. Lille, I. Martin, I. Vallikivi, L. Vares, T. Pehk, Tetrahedron: Asymmetry 9 (1998) 885.
[86] R. Aav, O. Parve, T. Pehk, A. Claesson, I. Martin, Tetrahedron: Asymmetry 10 (1999) 3033.
[87] G. Caron, G.W.-M. Tseng, R.J. Kazlauskas, Tetrahedron: Asymmetry 5 (1994) 83.
[88] M. Node, T. Inoue, M. Araki, D. Nakamura, K. Nishide, Tetrahedron: Asymmetry 9 (1998) 157.
[89] H. Yang, E. Henke, U.T. Bornscheuer, Tetrahedron: Asymmetry 10 (1999) 957.
[90] V.M. Sánchez, F. Rebolledo, V. Gotor, J. Org. Chem. 64 (1999) 1464.
[91] M. Majeriæ, V. Šunjiæ, Tetrahedron: Asymmetry 7 (1996) 815.
[92] O. Parve, A. Pals, V. Kadarpik, L. Lahe, Ü. Lille, P. Sikk, A. Lõokene, T. Välimäe, Bioorg. Med. Chem. Lett. 3 (1993) 359.
[93] P. Lopez-Serrano, J.A. Jongejan, F. van Rantwijk, R.A. Sheldon, Tetrahedron: Asymmetry 12 (2001) 219.
[94] S.F. Mayer, A. Steinreiber, R.V.A. Orru, K. Faber, Tetrahedron: Asymmetry 12 (2001) 41.
[95] A. Steinreiber, K. Edegger, S.F. Mayer, K. Faber, Tetrahedron: Asymmetry 12 (2001) 2067.
[96] E.J. Toone, J.B. Jones, Tetrahedron: Asymmetry 2 (1991) 207.
[97] H.-E. Högberg, M. Lindmark, D. Isaksson, K. Sjödin, M.C.R. Franssen, H. Jongejan, J.B.P.A. Wijnberg, A. de Groot, Tetrahedron Lett. 41 (2000) 3193.
[98] O. Parve, I. Vallikivi, L. Lahe, P. Sikk, T. Käämbre, Ü. Lille, Proc. Estonian Acad. Sci. Chem. 46 (1997) 186.
[99] R. Chenevert, M. Desjardins, R. Gagnon, Chem. Lett. (1990) 33.
[100] G. Egri, E. Fogassi, L. Novak, L. Poppe, Tetrahedron: Asymmetry 8 (1997) 547.
[101] A. Cordova, K. Hult, T. Iversen, Biotechnol. Lett. 19 (1997) 15.
[102] J.J. Gridley, A.J. Hacking, H.M.I. Osborn, D.G. Spackman, Tetrahedron 54 (1998) 14925.
[103] B. La Ferla, L. Lay, G. Russo, L. Panza, Tetrahedron: Asymmetry 11 (2000) 3647.
[104] A. Bastida, R. Fernandez-Lafuente, G. Fernandez-Lorente, J.M. Guisan, G. Pagani, M. Terreni, Bioorg. Med. Chem. Lett. 9 (1999) 633.
[105] T. Maugard, M. Remaud-Simeon, D. Petre, P. Monsan, J. Mol. Catal. B: Enzym. 5 (1998) 13.
[106] R.T. Otto, H. Scheib, U.T. Bornscheuer, J. Pleiss, C. Syldatk, R.D. Schmid, J. Mol. Catal. B: Enzym. 8 (2000) 201.
[107] M.-P. Bousquet, R.M. Willemot, P. Monsan, E. Boures, Biotecnol. Bioeng. 62 (1999) 225.
[108] A. Cordova, T. Iversen, K. Hult, Macromolecules 31 (1998) 1040.
[109] M. Ferrero, V. Gotor, Chem. Rev. 100 (2000) 4319.
[110] A. Boto, R. Hernandez, E. Suarez, Tetrahedron Lett. 42 (2001) 9167.
[111] D. Rotticci, F. Haeffner, C. Orrenius, T. Norin, K. Hult, J. Mol. Catal. B: Enzym. 5 (1998) 267.
[112] D. Rotticci, C. Orrenius, K. Hult, T. Norin, Tetrahedron: Asymmetry 8 (1997) 359.
[113] L.J. Marnett, Curr. Opin. Chem. Biol. 4 (2000) 545.
[114] M.G. Malkowski, S.L. Ginell, W.L. Smith, R.M. Garavito, Science 289 (2000) 1933.
[115] W.P. Schneider, R.A. Morge, B.E. Henson, J. Am. Chem. Soc. 99 (1977) 6062.
[116] W.P. Schneider, G.L. Bundy, F.H. Lincoln, E.G. Daniels, J.E. Pike, J. Am. Chem. Soc. 99 (1977) 1222.
[117] J.C. Coll, Chem. Rev. 92 (1992) 613.
[118] S. Carmely, Y. Kashman, Y. Loya, Y. Benayahu, Tetrahedron Lett. 21 (1980) 875.
[119] K. Valmsen, I. Järving, W.E. Boeglin, K. Varvas, R. Koljak, T. Pehk, A.R. Brash, N. Samel, PNAS 98 (2001) 7700.
[120] R. Koljak, A. Lopp, T. Pehk, K. Varvas, A.-M. Müürisepp, I. Järving, N. Samel, Tetrahedron 54 (1998) 179.
[121] R. Jäälaid, I. Järving, T. Pehk, O. Parve, Ü. Lille, Nat. Prod. Lett. 15 (2001) 221.
[122] D. Lee, K.-C. Kim, M.-J. Kim, Tetrahedron Lett. 39 (1998) 9039.
[123] Y. Gargouri, S. Ransac, R. Verger, BBA 1344 (1997) 6.
[124] S. Patkar, F. Björkling, Lipases—their structure, biochemistry and application, in: P. Nolley, S.B. Petersen (Eds.), Lipase Inhibitors, Cambridge University Press, Cambridge, 1994 (Chapter 10).
[125] A. Chiou, R. Verger, G. Kokotos, Lipids 36 (2001) 535.
[126] A. Chiou, T. Markidis, V. Constantinou-Kokotou, R. Verger, G. Kokotos, Org. Lett. 2 (2000) 347.
[127] S. Kotsovolou, A. Chiou, R. Verger, G. Kokotos, J. Org. Chem. 66 (2001) 962.
[128] G. Kokotos, R. Verger, A. Chiou, Chem. Eur. J. 6 (2000) 4211.
[129] G. Kokotos, S. Kotsovolou, V. Constantinou-Kokotou, G. Wu, G. Olivecrona, Bioorg. Med. Chem. Lett. 10 (2000) 2803.
[130] M.T. Gatto, S. Falcocchio, E. Grippa, G. Mazzanti, L. Battinelli, G. Nicolosi, D. Lambusta, L. Saso, Bioorg. Med. Chem. 10 (2002) 269.