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Enhancement of Selectivity and Reactivity of Lipases by Additives

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Contents

1. Introduction

Lipases have been established as valuable catalysts in organic synthesis in order to perform regio- and stereoselective transformations. Lipases catalyse the hydrolysis of carboxylic esters and acyl transfer onto hydroxy and amino groups with the formation of carboxylic esters and amides, respectively. These techniques offer a facile access to non-racemic building blocks[1] and selectively protected intermediates.[1h,2]

As typical induced-fit enzymes, lipases accept, as well as their natural substrates, triacyl glycerols, a broad range of substrates with an amazing structural diversity. One cannot expect, however, that a lipase always transforms a non-natural substrate in an optimal manner. Therefore, different, more or less empirical, strategies have been developed to improve reactivity and/or selectivity.

The substrate structure and the origin of the lipase mainly determine reactivity and selectivity in a lipase-catalysed reaction. Hence, the first step of an envisaged lipase-catalysed kinetic resolution or desymmetrisation of a given substrate comprises rapid screening of available lipases. There is no simple relationship, however, between structural parameters of a given substrate, the origin of the lipase and the outcome of the reaction. On the other hand, models have been developed in order to explain and predict the behaviour of certain lipases towards structural properties of substrates with regard to reactivity and selectivity,[3] which may help to optimise the reaction by modifying the substrate structure.

Lipase activity and selectivity are strongly influenced by the medium used for the desired reaction. In order to synthesise non-racemic alcohols or carboxylic esters it is possible to hydrolyse an ester or esterify an alcohol. Hydrolytic reactions can be carried out in pure buffered aqueous solutions and homogeneous or heterogeneous mixtures of organic solvents with aqueous buffers. The formation of carboxylic esters or amides is achieved in practically anhydrous organic solvents. In this case, the hydrophobicity of the solvent and the water activity have a major influence on the reaction.[4] Furthermore, the acyl donor has an influence on reactivity and selectivity.

Having identified a suitable lipase and a useful medium for a given substrate the outcome of the reaction may still not be satisfying. Fortunately, it has been demonstrated that an additional fine-tuning of the reaction conditions is achievable by using certain additives in the reaction mixture, which may have a beneficial influence on the microenvironment of the enzymes and hence on their selectivity and/or reactivity. From a synthetic organic chemist's point of view treatment of the reaction mixture with an additive is a convenient way to improve the outcome of the reaction.

Only a few systematic investigations have been undertaken on the influence of additives on lipase-catalysed reactions. Additives have been used successfully, however, in an

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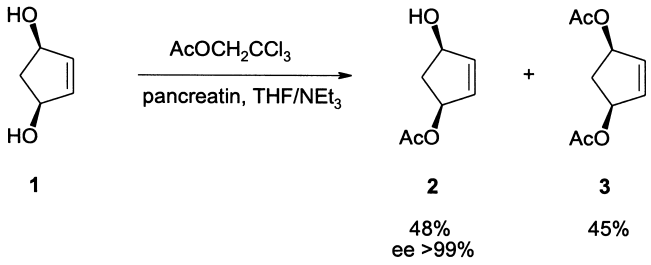


Scheme 1.

Table 1. Desymmetrisation of the *meso*-diol **1** with vinyl acetate in the presence of pancreatin

Entry	Solvent	Time (h)[a]	Yield (%)	e.e. (%)
1	THF/NEt_3	2.5	65	>99
2	THF	2.5	60	>99
3	Vinyl acetate/NEt_3	2.5	57	>99
4	Vinyl acetate	24	55	72

[a] Time required for the complete conversion of **1** into **2** and **3**.

empirical manner. These applications will be covered in this review.

2. Amines

2.1. Amines in esterifications

The beneficial influence of triethylamine on a lipase-catalysed reaction was observed for the first time, to the best of the author's knowledge, in his former laboratory in Berlin-Adlershof when investigating the enantioselective transesterification of the *meso*-diol **1** with 2,2,2-trichloroethyl acetate in the presence of pancreatin, a crude preparation of porcine pancreatic lipase (Scheme 1).[5]

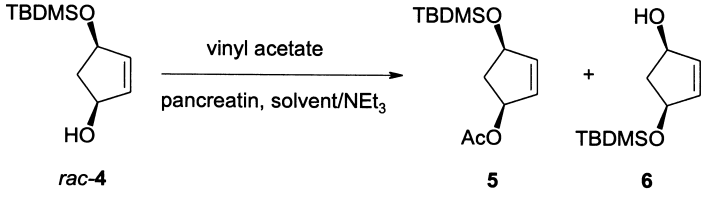
Whilst seeking a suitable solvent in which to dissolve the polar diol **1**, the author and his co-workers found that this was soluble in pyridine and tetrahydrofuran. When a solution of **1** in tetrahydrofuran was treated with 2,2,2-trichloroethyl acetate and pancreatin no conversion was observed within 24 h. Replacing the tetrahydrofuran by pyridine, however, showed a moderate conversion into the monoacetate **2** and the diacetate **3**. From these results it was concluded that a base might be necessary to promote the desired conversion. In order to avoid the use of pyridine, it was found that a combination of tetrahydrofuran and another base smoothly converted **1** into the almost enantiomerically pure monoacetate **2** and the *meso*-diacetate **3**. Among the various bases tested, namely pyridine, imidazole, 4-*N*,*N*-dimethylaminopyridine and triethylamine, the latter turned out to be superior with regard to reaction rate, yield and enantiomeric excess. The amount of triethylamine used was 0.7 equiv. vis-à-vis the substrate and this base has the advantage that it is easily removed by evaporation.

Changing the acyl donor from 2,2,2-trichloroethyl acetate to vinyl acetate for the same reaction (Scheme 1) gave slightly different results.[6] Table 1 summarises the key observations, namely that when vinyl acetate is utilised as the acylating agent triethylamine is not necessary to promote the reaction in tetrahydrofuran (cf. entries 1 and 2), whereas if neat vinyl acetate was used as solvent the reaction benefits from the presence of triethylamine in a significant manner by increasing the rate of conversion and the enantiomeric excess (e.e.) of the monoacetate **2** (entries 3 and 4).

Transesterification of the diol **1** with vinyl acetate in tetrahydrofuran in the presence of the non-commercially available lipase from *Mucor* sp. requires triethylamine as an additive in order to afford the monoacetate **2** in 85% yield and 94% e.e.[7]

In contrast to this work, however, for the transesterification of the *meso*-diol **1** with vinyl acetate in the presence of the lipase from *Mucor miehei* (Lipozyme®) triethylamine did not have any beneficial influence.[8]

For the kinetic resolution of the silylether *rac*-**4** Curran and co-workers[9] used pancreatin in different solvents in the



Solvent	Time (h)	Yield 5	ee 5	Yield 6	ee 6
THF/NEt_3	26	48%	98%	46%	96%
t-BuOMe/NEt_3	7	48%	98%	47%	98%
c-hexane/NEt_3	2	44%	98%	48%	97%

Scheme 2.

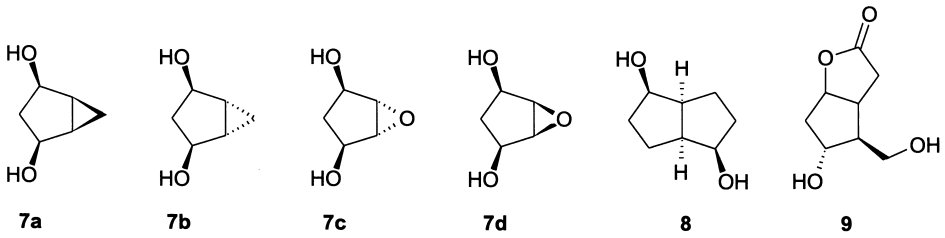
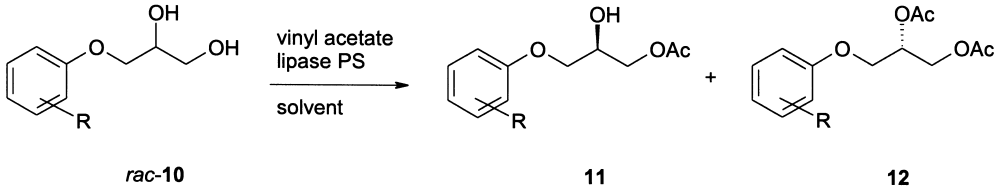


Figure 1.



Scheme 3.

Table 2. Influence of triethylamine on the kinetic resolution of *rac*-**10**

Entry	R	Solvent	Time (h)	Conversion	E^a	Reference
1	H	THF/NEt_3	92	0.52	23	13
2	H	THF	53	0.49	>100	14
3	2-OMe	THF/NEt_3	44	0.42	27	13
4	2-OMe	THF	100	0.23	48	13
5	3-*t*-Bu	THF/NEt_3	73	0.35	14	13
6	3-*t*-Bu	THF	100	0.39	>100	13
7	4-OMe	THF/NEt_3	28	0.51	>100	13
8	4-OMe	THF	100	0.49	>100	13

[a] Calculated according to Ref. 15.

presence of triethylamine to achieve a very high enantiomer selectivity (Scheme 2).

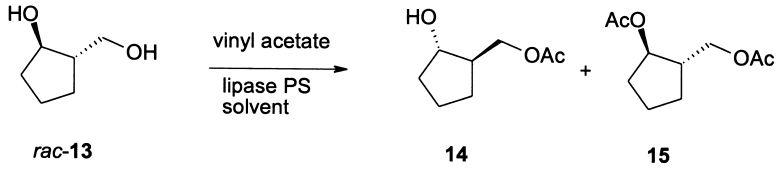
In recent years, the author and his-coworkers have used the solvent system tetrahydrofuran/triethylamine as standard conditions for the desymmetrisation of a variety of *meso*-diols **7a**–**d**[10] and for the kinetic resolution of diols **8**[11] and **9**[12] (Fig. 1).

For the *meso*-diols **7a**–**d**, vinyl acetate was used in the presence of different lipases such pancreatin, lipase from *Pseudomonas cepacia* (lipase PS from Amano), a mixture of the lipases A and B from *Candida antarctica* (lipase SP 382 from Novo Nordisk), *Mucor* sp. and *Yarrowia* sp. H 181. The C_2-symmetric diol **8** was resolved using 2,2,2-trichloroethyl acetate in the presence of pancreatin. The 1,3-diol **9**, a prostaglandin building block, could be resolved by transesterification with vinyl acetate in the presence of lipase from *Pseudomonas cepacia*.

The sequential kinetic resolution of 3-aryloxy-propane-1,2-diols (Scheme 3), under the influence of triethylamine as the additive, shows contradictory results (Table 2).[13]

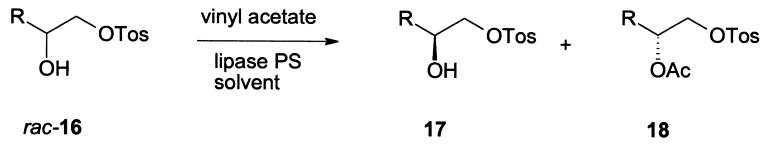
The results given in Table 2 indicate that triethylamine does not have a unique influence on the enantiomer selectivity. In most cases (entries 2–6) the presence of triethylamine decreases the selectivity. Only in the case of the 4-methoxy derivative (entries 7 and 8) did the *E*-value not change. The same is true for the rate of conversion. There is a slower conversion for the unsubstituted derivative in the presence of triethylamine (entries 1 and 2). On the other hand, there is a significantly higher rate for the 2- and 4-methoxy derivatives in the presence of triethylamine, which, for the 2-methoxy derivative, is accompanied by a decrease of selectivity (entries 3 and 4). Comparison of these results clearly shows that the effect of triethylamine depends on the substrate structure.

The lipase PS-catalysed sequential kinetic resolution of the cyclic 1,3-diol *rac*-**13** into **14** and **15** showed similar results (Scheme 4),[16] with the addition of triethylamine decreasing the selectivity without having an influence on the rate of conversion.



Solvent	Time	Conversion	*E*
THF/NEt_3	100 h	0.50	27
THF	100 h	0.49	>100

Scheme 4.



rac-**16** **17** **18**

Scheme 5.

Table 3. Kinetic resolution of *rac*-**16** under the influence of triethylamine

Entry	R	Solvent	*E*
1	$CH{=}CH_2$	*t*-BuOMe	50
2	$CH{=}CH_2$	*t*-BuOMe/NEt_3	>200
3	Me	*t*-BuOMe	111
4	Me	*t*-BuOMe/NEt_3	145
5	CH_2Cl	*t*-BuOMe	39
6	CH_2Cl	*t*-BuOMe/NEt_3	>200
7	Et	*t*-BuOMe	7
8	Et	*t*-BuOMe/NEt_3	60

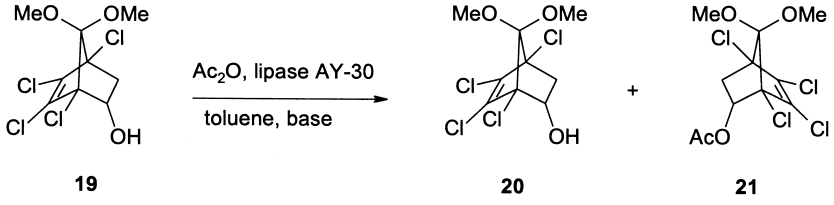
For the kinetic resolution of the 1,2-diol monotosylates *rac*-**16** into the alcohol **17** and the acetate **18** using vinyl acetate in the presence of lipase PS, both the reaction rate and the enantiomer selectivity were increased by adding 0.1 equiv. of triethylamine (Scheme 5, Table 3).[17]

The results shown in Table 3 confirm that in all cases triethylamine improves the selectivity of the reaction. The authors attribute the beneficial effect of triethylamine to the acid-trapping properties of the base towards traces of sulphonic acid that could be responsible for an enzyme-deactivation. They found that the kinetic resolution of other types of secondary alcohols was not influenced by triethylamine.

The bicyclic alcohol **19** was resolved by esterification with carboxylic acid anhydrides in the presence of lipases from *Candida cylindracea* (AY-30) in toluene (Scheme 6).[18] In this case, 1 equiv. of carboxylic acid is formed and if this acid is neutralised by mild bases such as 2,6-lutidine and potassium hydrogencarbonate the selectivity of the reaction increases remarkably. This effect indicates that trapping of the free acid is responsible for an enhancement of the enantiomer selectivity.

Researchers from Glaxo Wellcome[19] found that, for the kinetic resolution of *trans*-2-methoxycyclohexanol with vinyl acetate in the presence of lipase from *Candida antarctica* (CAL), triethylamine in cyclohexane improves the outcome of the reaction assuming that traces of acid liberated from vinyl acetate are trapped by the base (Scheme 7). The alcohol (1*S*,2*S*)-**22** is a precursor for tricyclic β-lactam antibiotics.

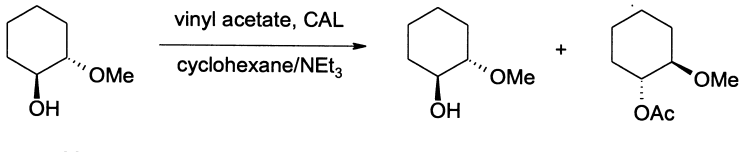
An extraordinary increase in the reaction rate was observed by Ogasawara et al.[20] for the desymmetrisation of the tricyclic *meso*-diol **24** into the enantiomerically pure monoacetate **25** by addition of 10% (vol.) of triethylamine to the reaction mixture (Scheme 8). Using the lipase from *Pseudomonas aeruginosa* (lipase LIP) the reaction time to achieve complete conversion was diminished from 10 days (without triethylamine) to 3 h in the presence of the base without any loss of selectivity! This corresponds to an increase of the reaction rate by two orders-of-magnitude.



19 **20** **21**

Base	Conversion	*E*
none	0.54	19
2,6-lutidine	0.47	180
$KHCO_3$	0.45	240

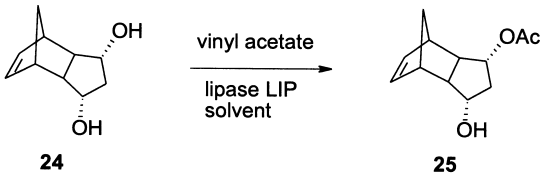
Scheme 6.



rac-**22** (1*S*,2*S*)-**22** (1*R*,2*R*)-**23**

yield 35%
ee 99%

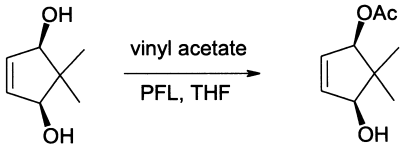
Scheme 7.



24 25

Solvent	Time	Yield	ee
THF	10 d	92%	>99%
THF/NEt_3	3 h	100%	>99%

Scheme 8.



26 27

Conditions	Time (d)	Yield	ee
PFL	7	50%	92%
PFL/sawdust	7	100%	95%
PFL/sawdust/NEt_3	7	93%	99%

Scheme 9.

The e.e. of the monoacetate **27**, a building block for chrysanthemic acids, could be improved by treatment of sawdust-supported lipase from *Pseudomonas fluorescens* (PFL) with triethylamine (Scheme 9).[21]

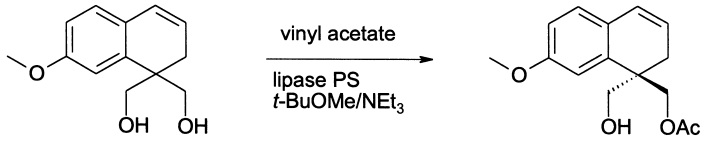
The enantiotopic selectivity for the desymmetrisation of the prochiral diol **28** into the (*R*)-monoacetate **29** was slightly increased from 85 to 93% e.e. in the presence of a catalytic amount of triethylamine with vinyl acetate using lipase PS immobilised on Hyflo Super Cell® as the catalyst (Scheme 10).[22]

Turner and co-workers[23] found a remarkable increase in the enantioselectivity for the dynamic kinetic resolution of oxazolin-5(4*H*)-ones *rac*-**30** by alcoholysis in the presence of lipase from *Mucor miehei* (renamed as *Rhizomucor miehei*) on a solid support (Lipozyme®) (Scheme 11). Compared with the reaction in pure toluene, the e.e. of the product (*S*)-**31** increased significantly by adding triethylamine to the solvent irrespective of the alcohol used. The best results were obtained with *n*-butanol as the nucleophile yielding (*S*)-**31** (R=*t*-Bu) in 67% yield and greater than 99% e.e. (Table 4).

A study by Parker et al.[24] on the influence of triethylamine on the dynamic kinetic resolution of *rac*-**30** (R=phenyl) with *n*-butanol in the presence *Candida antarctica* lipase B in different solvents having different thermodynamic water activities focused on reactivity and selectivity and revealed interesting results. Selected key data are summarised in Table 5.

In general, in the absence of triethylamine an increased thermodynamic water activity led to decreasing initial rates and enantiomeric excesses of the product. Addition of a catalytic amount of triethylamine to the reaction mixture compensates the negative influence of higher degrees of water activity enhancing the initial rate and enantioselectivity. Furthermore, the authors found when conducting the reaction in solvents with high water activity the corresponding acid of the ester **31** was formed as a side product and this acid inhibits the lipase. This effect is reversible simply by adding triethylamine. It is likely that one of the functions of triethylamine is the formation of ion pairs with acidic impurities or side products thereby protecting the lipase from deactivation.

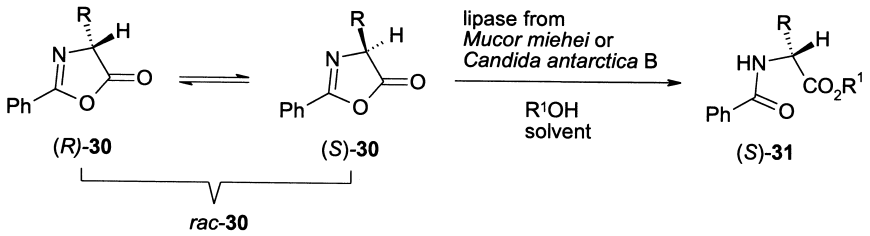
In order to prepare non-racemic 3,3′-bi-indolizines the



28 (*R*)-29

yield 89 %, ee 93 %
without NEt_3: yield 86 %, ee 85 %

Scheme 10.



(*R*)-30 (*S*)-30 (*S*)-31

rac-30

Scheme 11.

Table 4. Dynamic kinetic resolution of *rac*-**30** by alcoholysis in the presence of lipase from *Mucor miehei*

Entry	R	R^1OH	Solvent	e.e. (%)
1	CH_2–Ph	*n*-BuOH	toluene	55
2	CH_2–Ph	*n*-BuOH	toluene/NEt_3	73
3	*t*-Bu	*n*-BuOH	toluene	80
4	*t*-Bu	*n*-BuOH	toluene/NEt_3	>99
5	*t*-Bu	EtOH	toluene	68
6	*t*-Bu	EtOH	toluene/NEt_3	97
7	*t*-Bu	MeOH	toluene	39
8	*t*-Bu	MeOH	toluene/NEt_3	80

racemic axially chiral diol *rac*-**32** was resolved by transesterification with vinyl acetate in the presence of lipase B from *Candida antarctica* (CALB) (Scheme 12).[25] This sequential kinetic resolution afforded the unchanged diol (−)-**32** and the diacetate **34** with high enantiomeric excess as well as the corresponding monoacetate **33** with poor e.e. Compared with neat tetrahydrofuran as the solvent, triethylamine as an additive increased the rate of conversion and the products isolated had a higher e.e.

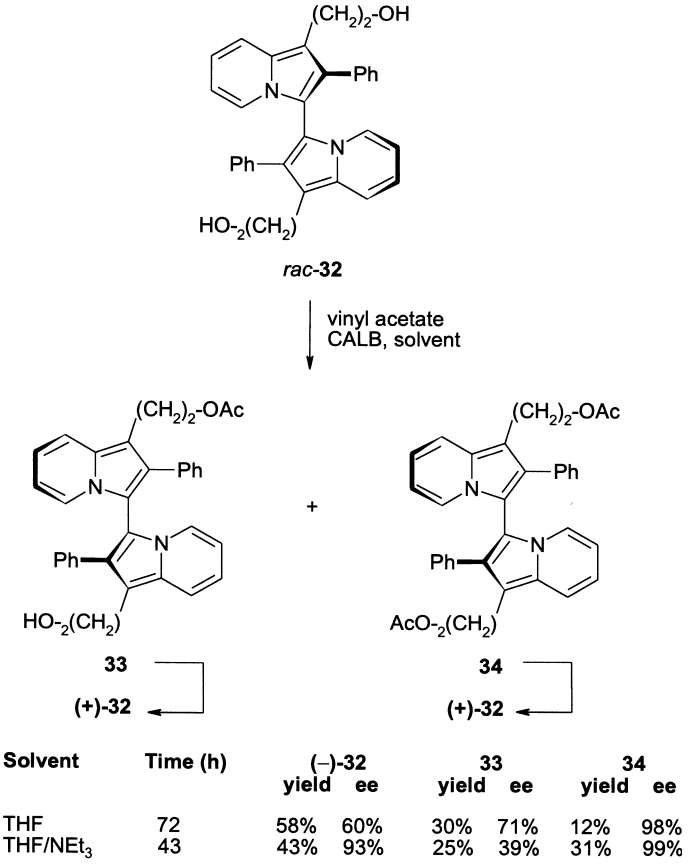
Finally, the regioselective acylation of monosaccharide derivatives using vinyl acetate and pancreatin[26] or *Rhodosporidium* esterase[27] was potentiated using triethylamine in tetrahydrofuran.

The solvent-free polycondensation of adipic acid (**35**) with α,ω-diols such as butane-1,4-diol (**36**) in the presence of CALB yielded as the main product the hydroxy-terminated polymer **37** (Scheme 13).[28] During their investigation the researchers, from Liverpool University and Baxenden Chemicals Ltd., discovered that triethylamine added to the

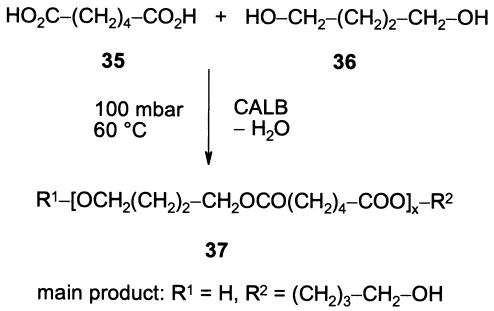
Table 5. Influence of water activity and triethylamine on reactivity and selectivity of the dynamic kinetic resolution of *rac*-**30** (R=Ph) with *n*-butanol in the presence of lipase B from *Candida antarctica*

Entry	Solvent	a_w[a]	Without NEt_3		With NEt_3	
			Initial rate/nmol min^{-1} mg^{-1}	e.e. (%)	Initial rate/nmol min^{-1} mg^{-1}	e.e. (%)
1	*n*-hexane	~0	26	85	30	90
2	*n*-hexane	0.69	4	55	20	87
3	*n*-hexane	0.97	1.5	30	18	80
4	toluene	~0	15	85	27	93
5	toluene	0.22	3	61	17	95

[a] a_w thermodynamic water activity.



Scheme 12.

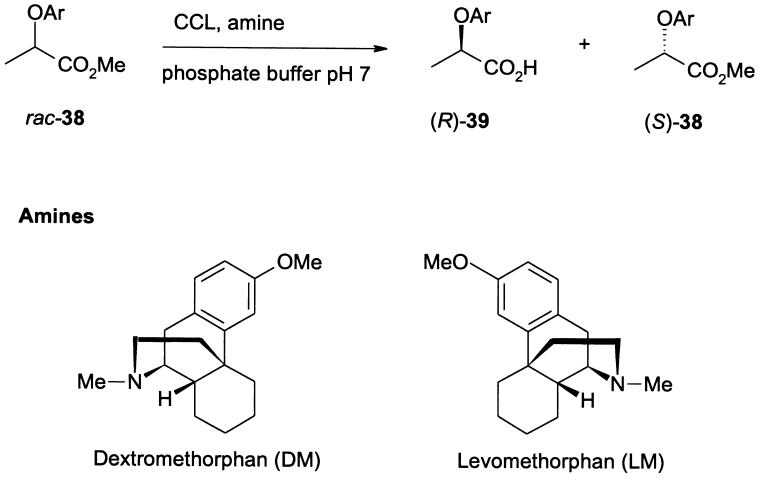


Scheme 13.

reaction mixture had a remarkable influence on the biocatalyst. They found that in the absence of triethylamine the recovered immobilised lipase exhibited only 35% of its original activity. Addition of 0.02% of triethylamine to the reaction mixture, however, prevented the CALB from deactivation, the recovered lipase having ca. 90% of its initial activity. The authors postulate that the amine compensates the decrease of pH of the medium caused by dissolution of a certain quantity of adipic acid in the diol–water mix. This hypothesis is supported by the observation that when the more hydrophobic pentane-1,5-diol or hexane-1,6-diol was used instead of butane-1,4-diol there was very little deactivation of the catalyst as assessed after its recovery. In contrast, in the presence of ethylene glycol or propane-1,3-diol, the enzyme was almost completely deactivated. The pH of the medium formed by the less polar diols, pentane-1,5-diol and hexane-1,6-diol, should be closer to 7 due to the much lower solubility of adipic acid in the reaction mixture compared to that experienced with butane-1,4-diol.

The reaction was conducted under reduced pressure in order to remove water thus shifting the equilibrium towards the formation of the product.

In general, the salt formation of amino groups with traces of acids could be a reason for down-regulating reactivity and selectivity of lipases. A comparable effect has been observed for the kinetic resolution of bicyclic secondary alcohols using vinyl acetate in the presence of lipase from



Scheme 14.

Table 6. Enantioselective hydrolysis of methyl 2-(aryloxy)propanoates *rac*-**38** in the presence of the enantioselective inhibitors DM and LM

Entry	Aryl	Inhibitor	Conversion	*E*
1	2-Cl–Ph	None	0.76	4
2	2-Cl–Ph	DM	0.44	25
3	2-Cl–Ph	LM	0.15	18
4	3-Cl–Ph	None	0.75	2
5	3-Cl–Ph	DM	0.62	13
6	3-Cl–Ph	LM	0.27	17
7	4-Cl–Ph	None	0.50	17
8	4-Cl–Ph	DM	0.41	>100
9	4-Cl–Ph	LM	0.31	>100
10	2,4-Cl_2Ph	None	0.34	1
11	2,4-Cl_2Ph	DM	0.30	20
12	2,4-Cl_2Ph	LM	0.14	20
13	2-Me-4-Cl–Ph	None	0.50	1
14	2-Me-4-Cl–Ph	DM	0.25	37
15	2-Me-4-Cl–Ph	LM	0.18	81

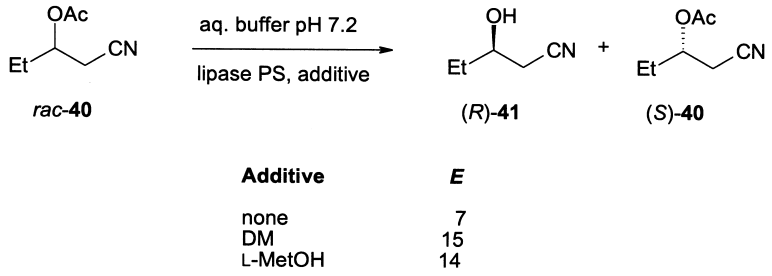
Candida cylindracea. Deliberated acetaldehyde from vinyl acetate decreases reactivity and selectivity of the lipase which is likely to be caused by the formation of a Schiff-base with NH_2-groups of the enzyme.[29]

2.2. Amines in ester hydrolyses

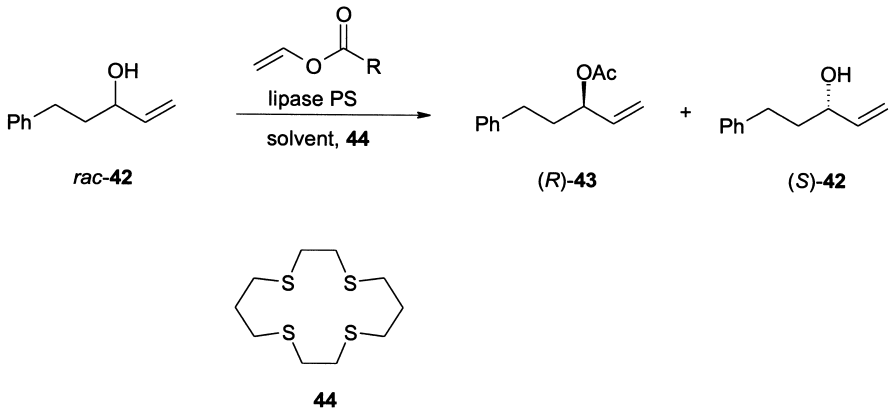
In contrast to lipase-catalysed transesterifications in organic solvents only a few examples employing amines as additives to increase selectivity and/or reactivity in hydrolysis reactions have been reported.

In 1989 Guo and Sih[30] reported a significant improvement for the *Candida cylindracea* lipase (CCL)-catalysed enantiomer selective hydrolysis of alkyl 2-(aryloxy)propanoates *rac*-**38** or 2-arylpropanoates using enantiomerically pure amines as additives (Scheme 14).

The authors found that the enantiomerically pure amines



Scheme 15.



Scheme 16.

dextromethorphan (DM) and levomethorphan (LM) (Scheme 14) increased the selectivity of this reaction between 5 and 80-fold (Table 6). The phenomenon was called 'enantioselective inhibition' because kinetic experiments showed that DM is a non-competitive inhibitor of the reaction involving the slow reacting ester (*S*)-**38**; therefore the *R*-ester reacts faster yielding the acid (*R*)-**39** preferentially. The optimal concentration of DM and LM was found to be ca. 0.03 M.

Remarkably, the CCL-catalysed hydrolysis of the 2,4-dichloro derivative (entries 10–12) and the 2-methyl-4-chloro derivative (entries 13–15) is completely non-selective ($E=1$) in the absence of the additives but the selectivity can be enhanced dramatically in the presence of DM or LM. It is additionally of interest that DM and LM are enantiomers but non-competitive inhibitors for the same enantiomeric ester.

Table 7. Lipase PS-catalysed kinetic resolution of *rac*-**42** with vinyl esters in the presence of 5% of the thiacrown ether **44**

Entry	R	Solvent	Additive	*E*	Reference
1	Me	*n*-hexane	None	105	31
2	Me	*n*-hexane	**44**	407	31
3	Me	*i*-Pr_2O	None	305	31
4	Me	*i*-Pr_2O	**44**	>1200	31
5	Et	*i*-Pr_2O	None	21	32
6	Et	*i*-Pr_2O	**44**	40	32
7	*n*-C_5H_{11}	*i*-Pr_2O	None	8	32
8	*n*-C_5H_{11}	*i*-Pr_2O	**44**	169	32
9	*n*-$C_{13}H_{27}$	*i*-Pr_2O	None	500	32
10	*n*-$C_{13}H_{27}$	*i*-Pr_2O	**44**	129	32

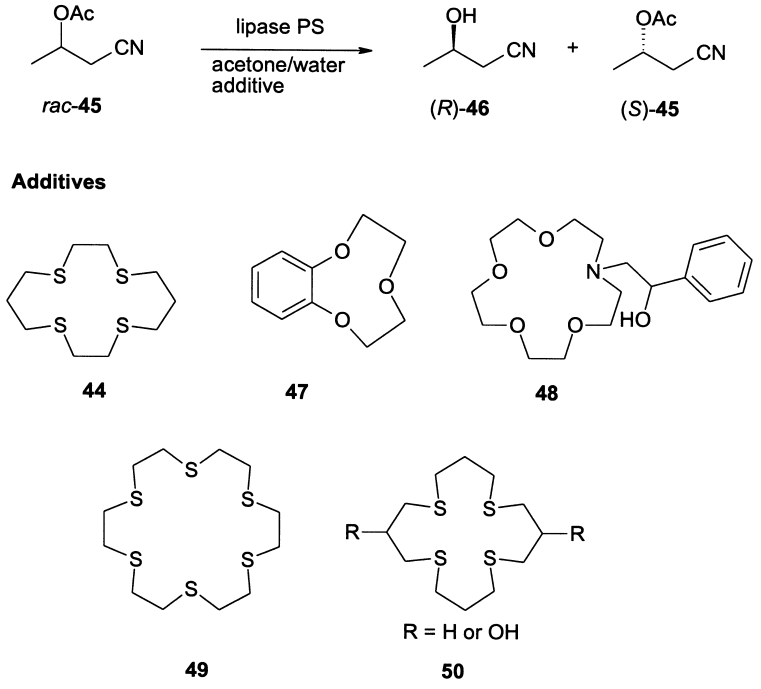
Similar effects to those tabulated in Table 6 were observed for the hydrolysis of racemic chloroethyl 2-arylpropanoates. The rate of conversion, however, was very slow compared with the aryloxy derivatives *rac*-**38**.

For the kinetic resolution of 3-acetoxybutyronitriles *rac*-**40**, Itoh et al.[31] studied a series of structurally simpler achiral and enantiomerically pure amines as additives (Scheme 15). It turned out that many of these increased the reaction rate but did not increase the selectivity. Only DM and (2*S*)-2-amino-4-methylthio-1-butanol (L-MetOH) increased the enantiomer selectivity. The optimal concentration for DM and L-MetOH was found to be 33 mol%. A variety of other primary, secondary and tertiary amines increased the reaction rate significantly, but there was negligible positive influence on the selectivity; indeed the selectivity dropped in some instances.

Rakels et al.[32] investigated the kinetics of the hydrolysis of racemic methyl 2-chloropropanoate and glycidyl butyrate with *Candida cylindracea* lipase in water-saturated organic solvents. The presence of pyrrolidine increased the yield and the enantioselectivity by formation of an ion-pair with the non-racemic acid produced, thus shifting the equilibrium in the direction of the products and thereby demonstrating another beneficial application of basic additives in lipase-catalysed hydrolyses.

3. Crown Ethers and Analogues

It has been shown very recently by a Japanese group[33–36] that crown ethers and particularly thiacrown ethers enhance



Scheme 17.

Table 8. Influence of different crown ethers on the enantioselectivity and reactivity of the lipase PS-catalysed hydrolysis of 2-acetoxybutyronitrile (*rac*-**45**)

Entry	Additive	Time (h)	Conversion	*E*
1	None	60	0.38	16
2	**47**	13	0.52	28
3	**48** (*rac*)	14	0.44	28
4	**44**	14	0.59	37
5	**49**	14	0.60	27
6	**50** (R=H)	14	0.57	34
7	**50** (R=OH)	14	0.57	34

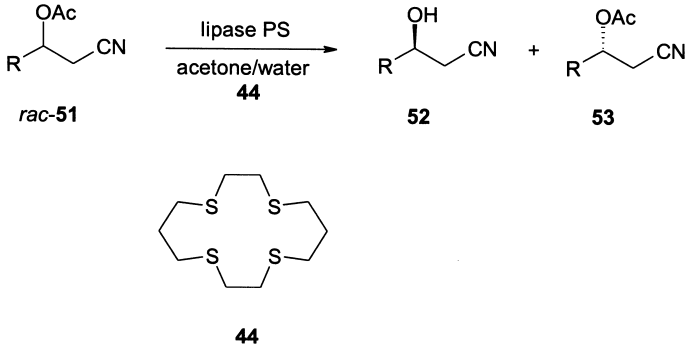
the reactivity and the selectivity of lipase from *Pseudomonas cepacia* (lipase PS) in enantioselective formation and hydrolyses of carboxylic esters.

The thiacrown ether **44** (5 mol%) has a beneficial influence on the enantiomer selectivity for transesterification of the allylic alcohol *rac*-**42** using a variety of vinyl esters (Scheme 16).[33,34] Typical results are summarised in Table 7.

The data displayed in Table 7 confirm, that in most cases addition of the thiacrown ether **44** increases the *E*-value remarkably (cf. particularly entries 7 and 8). On the other hand, use of long chain fatty acid vinyl esters in the presence of the thiacrown ether **44** decreases the selectivity (entries 9 and 10). Some other macrocycles were investigated and shown to have almost no influence on the selectivity of the reaction.[33] The authors attribute this effect to complex formation between the racemic allylic alcohol and the thiacrown ether thus changing the substrate properties. The postulated complexation has been demonstrated by NMR spectroscopy.

In an extensive study, the influence of more than 30 different macrocycles and open-chain analogues was examined as additives in the lipase PS-catalysed hydrolysis of 3-acetoxybutyronitrile *rac*-**45** (Scheme 17).

The results in Table 8 indicate that the rate of conversion is increased in all cases by a factor of about four and that the selectivity is additionally enhanced. Among the possible



Scheme 18.

Table 9. Influence of the thiacrown ether **44** on the lipase PS-catalysed kinetic resolution of 3-acetoxy-alkylnitriles *rac*-**51**

Entry	R	Additive	Time (h)	Conversion	*E*
1	Et	None	11	0.49	53
2	Et	**44**	8	0.49	>700
3	*n*-Pr	None	4.7	0.44	5
4	*n*-Pr	**44**	4.3	0.44	8
5	*i*-Pr	None	360	0.44	13
6	*i*-Pr	**44**	168	0.44	17
7	*c*-hexyl	None	10	0.44	152
8	*c*-hexyl	**44**	12	0.45	501

reasons for enhancement of reactivity and selectivity discussed by the authors the only plausible explanation involves a local conformational change within the lipase, close to its active site, under the influence of crown ethers.

Researchers from the same group additionally modified the structure of the substrate, replacing the methyl group of *rac*-**45** by other substituents (Scheme 18).[36] The lipase PS-catalysed hydrolysis of *rac*-**51** was investigated in the presence of the thiacrown ether **44**. A selection of the results is presented in Table 9.

The data in Table 9 show that the enantioselectivity is mainly dictated by the substrate structure. In certain cases, however, the additive enhanced the enantiomer selectivity. The prime example involves the ethyl derivative (entries 1 and 2); in other cases only a minor influence of the additive has been observed (entries 3–6) and the substrate structure was the overwhelming factor determining reactivity and selectivity.

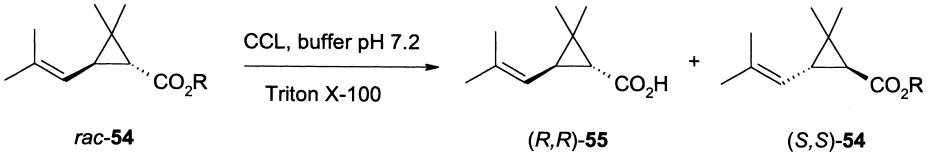
Usually a lipase-catalysed hydrolysis is carried out in buffered solutions at a constant pH, preventing lipase deactivation at low pH-values. In the presence of the crown ether additives, hydrolysis can be carried out in non-buffered solutions with increasing H^+ concentrations. Under the optimum conditions lipase activity and selectivity were not influenced by the decreasing pH (down to ~3.5) caused by the acetic acid. On the contrary, activity and selectivity were enhanced under the influence of the crown ethers even at low pH showing that the additive protects the lipase from the acidic environment.[35,36]

Using 2% of the polyethylene glycol derivative Triton® X-100—an open-chain crown ether analogue and non-ionic surfactant—as an additive significantly increased the selectivity of the *Candida cylindracea* lipase-(CCL) catalysed hydrolysis of the *trans*-chrysanthemic acid alkyl esters *rac*-**54** (Scheme 19).[37]

4. Further Additives

In a kinetic study it has been shown that salts such as potassium chloride dramatically enhance the activity of the protease subtilisin in organic solvents.[38] Similarly added salts can influence lipase-catalysed reactions, as demonstrated for the hydrolysis of octyl 2-methyl decanoate (*rac*-**56**) (Scheme 20).[39] CCL-catalysed hydrolysis at pH 7.5 showed almost no enantiomer selectivity whereas at pH 8 and in the presence of calcium chloride the *E*-value of the reaction was raised to 9, affording preferentially the acid (*S*)-**57**.

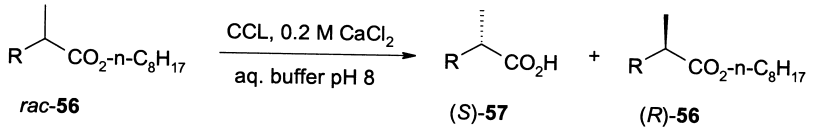
Maintaining a defined and constant water activity in lipase-catalysed acylations in organic solvents appears to be crucial particularly in reactions in which water is formed.



rac-**54** (*R,R*)-**55** (*S,S*)-**54**

R	Additive	Conversion	*E*
Me	none	0.47	12
Me	Triton X-100	0.49	55
Et	none	0.46	14
Et	Triton X-100	0.49	156

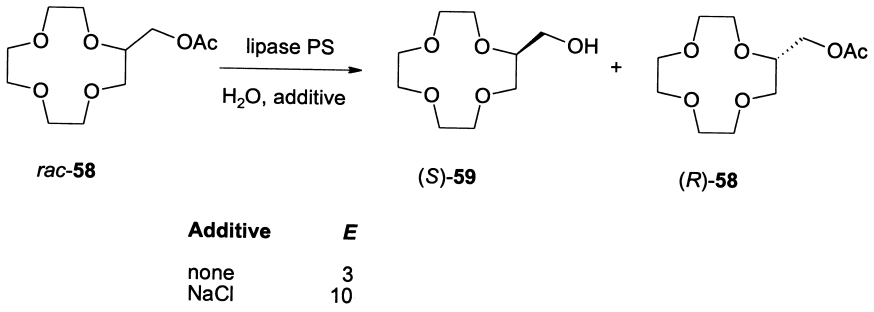
Scheme 19.



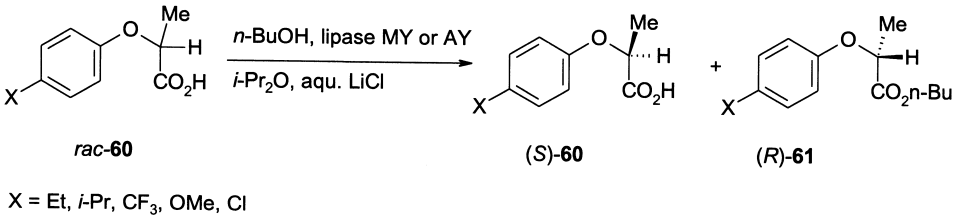
rac-**56** (*S*)-**57** (*R*)-**56**

Conditions	*E*
pH 7.5	~1
pH 8.0/$CaCl_2$	9

Scheme 20.



Scheme 21.



Scheme 22.

Added salt hydrates can maintain optimal conditions by keeping water activity constant and increasing the reaction rate as demonstrated for the esterification of butanoic acid with *n*-butanol in *n*-hexane in the presence of lipase from *Candida rugosa*.[40]

Lipase PS-catalysed kinetic resolution of the racemic crown ether derivative *rac*-**58** (Scheme 21) was affected by the presence of sodium ions which increased the reaction rate and the enantiomer selectivity.[41,42] These results indicate that the enzyme reaction is probably regulated via metal ion complexation. With regard to selectivity the effect for the given substrate was specific for sodium ions; lithium and potassium ions did not increase the *E*-value of the reaction.

In a very recent publication a remarkable increase in enantiomerselectivity was observed for the esterification of 2-(4-substituted-phenoxy)propanoic acids with *n*-butanol by lipase MY or AY, two different preparations from *Candida rugosa*, by addition of aqueous LiCl (Scheme 22).[43]

The optimal concentration was 0.5 vol% of aqueous LiCl (2.4 M). Selected results shows Table 10 indicating an increase in selectivity up to two orders-of-magnitude due to the presence of the additive.

The authors found that water alone increases the selectivity too but in a lesser extent as in the presence of LiCl. A kinetic study for lipase MY showed that the initial rate for the faster reacting *R*-enantiomer was accelerated whereas the initial rate for the slower reacting *S*-enantiomer was inhibited in the presence of LiCl-containing water.

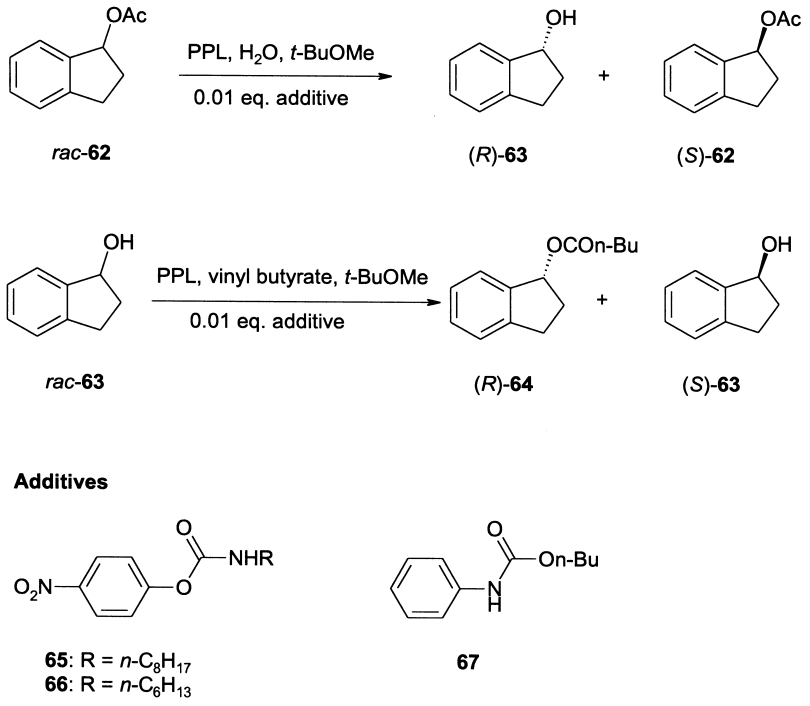
Different types of carbamates have been reported to enhance the enantiomer selectivity of the porcine pancreatic lipase (PPL)-catalysed hydrolysis of 1-indanyl acetate (*rac*-**62**) and the transesterification of 1-indanol (*rac*-**63**) with vinyl butyrate (Scheme 23).[44] The results are summarised in Table 11.

The results in Table 11, however, have to be considered with care because the enhancement of the selectivity is not very significant and the authors used optical rotation measurements for the determination of the enantiomeric excess of the products. It is known that optical rotation measurements have a limited accuracy and the *E*-value calculated from e.e.'s higher than 90% have a broad range of error.

Phase-transfer catalysts such as quaternary ammonium salts, polyethylene glycol 400 (PEG 400) and combinations of PEG 400 with clays, Florisil® or ammonium chloride can increase in the enantiomer selectivity of the kinetic resolution as well as the reaction rate. This was demonstrated for

Table 10. The influence of aqueous LiCl on the enantioselective esterification of 2-(4-substituted-phenoxy)propanoic acids

Entry	X	Lipase	Additive	*E*
1	Et	MY	None	3.8
2	Et	MY	LiCl	201
3	*i*-Pr	MY	None	3.4
4	*i*-Pr	MY	LiCl	145
5	CF_3	MY	None	1.3
6	CF_3	MY	LiCl	26
7	OMe	MY	None	1.5
8	OMe	MY	LiCl	43
9	Cl	MY	None	1.4
10	Cl	MY	LiCl	17
11	Et	AY	None	15
12	Et	AY	LiCl	90
13	*i*-Pr	AY	None	3.4
14	*i*-Pr	AY	LiCl	170
15	CF_3	AY	None	1.3
16	CF_3	AY	LiCl	56
17	OMe	AY	None	2.1
18	OMe	AY	LiCl	49
19	Cl	AY	None	1.4
20	Cl	AY	LiCl	23



Scheme 23.

Table 11. Influence of carbamates on the selectivity of the kinetic resolution of the indane derivatives *rac*-**62** and *rac*-**63**

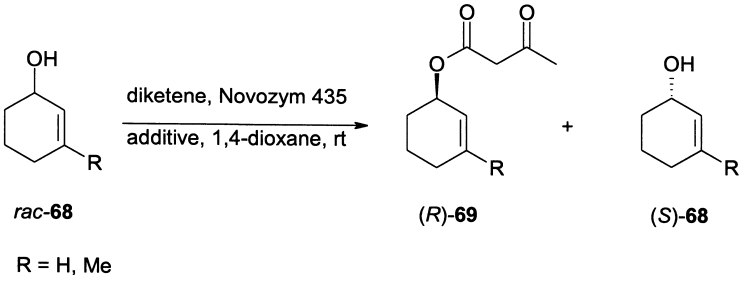
Entry	Substrate	Additive	Conversion	*E*
1	*rac*-**62**	None	0.50	77
2	*rac*-**62**	**65**	0.51	153
3	*rac*-**62**	**66**	0.51	153
4	*rac*-**62**	**67**	0.48	246
5	*rac*-**63**	None	0.47	65
6	*rac*-**63**	**65**	0.43	148
7	*rac*-**63**	**66**	0.43	162
8	*rac*-**63**	**67**	0.46	83

the kinetic resolution of cyclohexenols *rac*-**68** with diketene in the presence of Novozym 435 (lipase B from *Candida antarctica* on a solid support) to yield the acetoacetate (*R*)-**69** and the alcohol (*S*)-**68** (Scheme 24).[45] Typical results are summarised in Table 12.

The lipase-catalysed acylation of *rac*-**68** (R=H) without additive is absolutely non-selective under the reaction conditions employed (entry 1). The addition of either triethyl- or trimethylammonium chloride or the corresponding bromide of the latter salt enhanced the enantiomer selectivity to *E*-values of about 4 (entries 2–4). Although triethylammonium chloride increased the reaction rate by a factor of about 10, the two other salts had no beneficial influence on the rate of conversion. Additional salts tested had a less significant or no influence on both selectivity and reactivity. Adding PEG 400 or PEG 400 and clays, Florisil® and ammonium chloride gave the same effect as the ammonium salts (entries 5–9).

A remarkable increase of the enantiomeric ratio *E* was observed for the resolution of the methyl substituted cyclohexenol *rac*-**68** (R=Me). In this case *E* could be enhanced from 10 without additive up to 86 under the influence of triethylammonium chloride (entries 10 and 11).

These results pointing to the influence of ammonium salts, are particularly interesting in comparison with the influence of tertiary amines (cf. Section Amines). Some authors explain the beneficial influence of tertiary amines by the formation of ion pairs with acidic impurities. If this is the case, the added tertiary amines have three functions:



Scheme 24.

Table 12. Kinetic resolution of *rac*-**68** in the presence of different additives

Entry	R	Additive	Time (h)	Conversion	*E*
1	H	None	18	0.50	1
2	H	$Et_3HN^+Cl^-$	1.5	0.38	3.9
3	H	$Me_3HN^+Br^-$	20.5	0.37	4.1
4	H	$Me_3HN^+Cl^-$	24	0.37	3.6
5	H	PEG 400	4	0.37	3.8
6	H	PEG 400/KSF clay	2.5	0.36	5
7	H	PEG 400/K10 clay	4	0.34	5
8	H	PEG 400/Florisil®	4	0.35	4.4
9	H	PEG 400/NH_4Cl	23	0.33	5.5
10	Me	None	19.5	0.65	10
11	Me	$Et_3HN^+Cl^-$	3.5	0.51	86

removing of acid traces, formation of possibly beneficial ammonium salts and modulation of the water activity.

5. Conclusions

Additives have a great potential for fine-tuning the reaction conditions for lipase-catalysed reactions. Certain additives have a beneficial influence by increasing the reaction rate and/or enantioselectivity dramatically. From the investigations published so far it transpires that tertiary amines and certain thiacrown ethers increase the reaction rate and/or the selectivity. In general the reasons for these effects are little understood because most of the additives have been used in an empirical manner guided only by biotransformation folklore to increase reactivity and/or selectivity of a reaction. Only a few systematic studies have been published. In general there is no rationale for the effects caused by the additives except formation of ion-pairs between added amines and traces of acids present in the reaction mixture. The latter explanation, however, is based only on a series of assumptions.

In addition, it is necessary to take into consideration that most lipases used in preparative biotransformations contain other proteins as impurities or artificial additives. For example, lipase PS from Amano (from *Pseudomonas cepacia*) contains diatomaceous earth, dextran and calcium chloride, for reasons which are unclear.

Further work is essential in order to find new beneficial additives and to understand their role in lipase-catalysed reactions.

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Biographical Sketch

Fritz Theil received his Diploma in 1974 from the University of Leipzig, Germany. In 1979 he obtained his Dr. rer. nat. (Ph.D) from the Academy of Sciences (Central Institute of Molecular Biology, Berlin-Buch) of the GDR for work on the synthesis of cardioactive steroids. From 1979–1991, he worked at the Central Institute of Organic Chemistry of the Academy of Sciences of the GDR, Berlin-Adlershof; from 1992–1993 he carried out research at the Centre of Selective Organic Synthesis, Berlin, and from 1994–1997 at the Institute of Applied Chemistry, Berlin, Germany. In 1992, Fritz Theil received the 'Förderpreis der Deutschen Akademie der Naturforscher Leopoldina', Halle, Germany, which made it possible for him to work for six months at the University of Exeter, UK. He moved in 1997 to the University of Liverpool as a Research Fellow and took a position there as a Lecturer in Organic Chemistry. In June 1999, he returned to Germany to work at WITEGA Angewandte Werkstoff-Forschung g. GmbH. His research interests include the synthesis of biologically active compounds and the use of enzymes, particularly lipases, for the synthesis of enantiomerically pure building blocks.