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Biocatalytic resolution of sterically hindered alcohols, carboxylic acids and esters containing fully substituted chiral centers by hydrolytic enzymes

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Abstract

Carboxyl esters bearing a fully substituted chiral center adjacent to the ester moiety, i.e., esters of *tert*-alcohols and of α,α-disubstituted carboxylates, are usually not accepted as substrates for hydrolytic enzymes such as esterases, proteases, and lipases. In order to circumvent this limitation, three strategies, which are reviewed in this paper, have been developed. (i) Several proteases and (still unspecified) microbial esterases are capable of hydrolysing esters of *tert*-alcohols and α,α-disubstituted carboxylic acids despite their steric bulkiness, but the number of these highly useful enzymes is rather limited. Alternatively, (ii) the use of 'activated esters' bearing electron-withdrawing groups enhances the electrophilic properties of the ester moiety (thus increasing the enzymatic reaction rate) may help to overcome slow reaction rates. On the other hand, (iii) spatial separation of the bulky quarternary carbon atom bearing the chiral center from the ester group to be hydrolysed by a spacer moiety led to modified (non-activated) substrates which were readily accepted.

Keywords: Enzymatic resolution; *tert*-alcohol; α,α-Disubstituted carboxylic acid; Kinetic resolution; Carboxyl ester hydrolase; Lipase

1. Introduction

Hydrolysis of esters by means of hydrolytic enzymes such as proteases [1], lipases [2,3], and esterases [4,5] has become a well established method for their resolution [6]. However, one major drawback is associated with all of the commonly used hydrolytic enzymes, i.e., they are unable to accept sterically hindered substrates bearing fully substituted quarternary carbon atoms in the vicinity of the ester moiety to be hydrolysed. As consequence, esters of tertiary alcohols and α,α-disubstituted carboxylic acids are excluded as substrates a priori. In order to extend the applicability of enzymes most commonly used for the stereoselective hydrolysis of carboxyl esters to sterically hinder substrates, several techniques have been developed and these techniques are reviewed in this paper.

In order to provide a logic scheme for the presentation of the data, substrates are grouped

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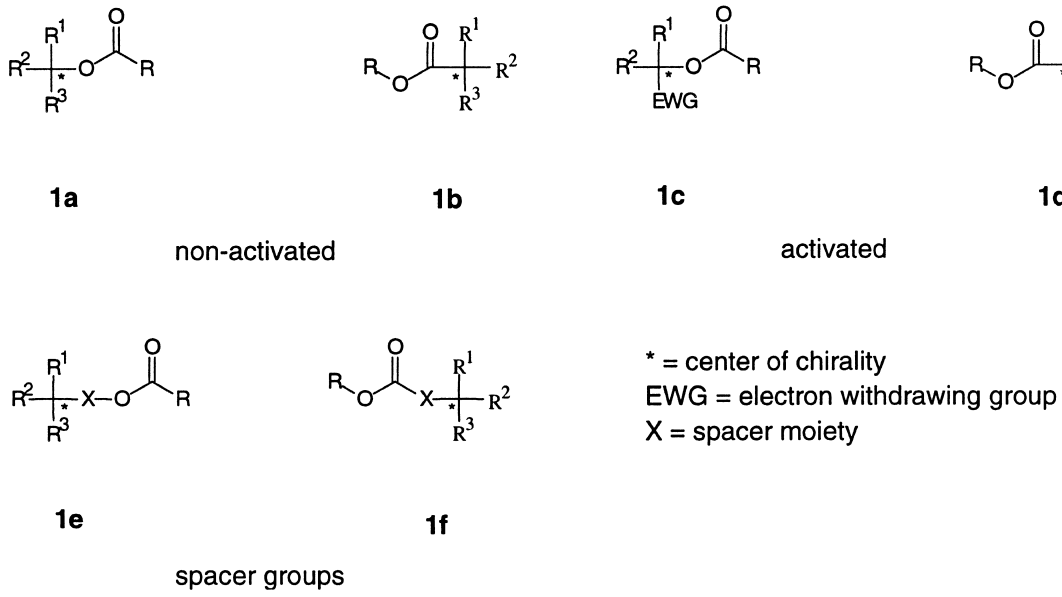


Scheme 1. Substrate ester types bearing chiral quarternary carbon atoms.

into two categories, according to the location of the chiral center in the substrate ester, either in alcohol or acid moiety (Type **1a** and **b**, respectively, Scheme 1). If applicable, subgroups were chosen along (i) electronically non-activated (**1a**, **1b**) or (ii) activated derivatives (**1c**,**d**) bearing an electron-withdrawing group (EWG) and (iii) those compounds, where the chiral center is linked to the ester moiety by a spacer group (X, **1e**,**f**). For reason of comparison, the selectivity in kinetic resolutions was calculated and expressed as the Enantiomeric Ratio (*E*-value [7][1]) where applicable. Depending on the availability, *E*-values were preferably calculated from e.e.$_S$- and e.e.$_P$-data [9], and only in those cases where one of these values was unaccessible, conversion-data (c) were used. For asymmetrisation reactions of prochiral substrates, selectivities were given in α-values.[2]

2. Esters of *tert*-alcohols

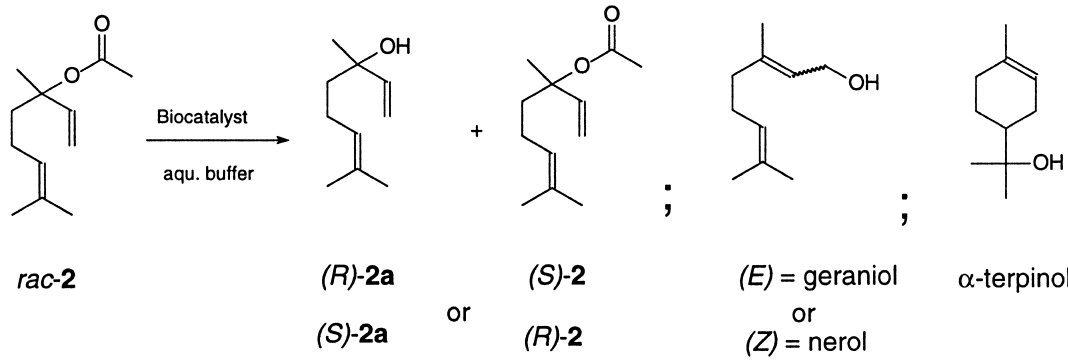
2.1. Esters of non-activated tert-alcohols

Data on the successful biocatalytic resolution of esters of non-activated *tert*-alcohols are rather scarce, although chiral tertiary terpene alcohols play an important role in the flavour and fragrance industry.[3] Among them, linalool (**2a**) and its corresponding acetate ester (**2**) constitutes one of the most important terpene alcohols used, since both enantiomers differ in odor [12] (Scheme 2). The (*R*)-(−)-enantiomer ('licareol') is a major constituent of *Cinnamonium camphora* and *Cayenne linaloe* extracts, whereas the (*S*)-(+)-enantiomer primarily occurs in coriander oil ('coriandrol'). As a consequence, several attempts have been reported towards the biocatalytic resolution of linalool. (±)-Linalool was shown to be a non-substrate for several microbial lipases in esterification

[1] For a review, see Ref. [8].

[2] The selectivity of asymmetrisation reactions is most conveniently described by the α-value, which is defined as the ratio of the reaction rates controlling the formation of enantiometric products ($\alpha = k_R / k_S$). See Ref [10].

[3] For a rare case of hydrolysis of a *tert*-butyl ester in a chemoselective fashion catalysed by a protease ('thermitase') see Ref [11].



Scheme 2. Biocatalytic resolution of (±)-linalyl acetate.

reactions [13,14]. Likewise, the hydrolysis of its corresponding acetate ester using *Aspergillus niger* cells succeeded without detectable enantioselectivity (Table 1) [15]. Similar activity on (±)-linalyl acetate was detected in whole resting cells of *Bacillus subtilis* [16] and various bacterial cells of the genus *Actinomyces*, such as *Rhodococcus* sp. and *Rhodococcus ruber* [17]. In both cases, the enantioselectivities were insufficient for preparative application. When *B. subtilis* was used, the $e.e._S$, $e.e._P$, and the product did not exeed ~10%. In addition, a significant amount of material was transformed to α-terpineol, and non-chiral terpene alcohols geraniol and nerol due to undesired allylic rearrangment. Since in whole cell biotransformations, low enantioselectivities may be caused by the presence of several competing carboxyl esterases possessing opposite enantiopreference, more detailed studies were undertaken [18]. Two highly enantiospecific enzymes, i.e., (*R*)- and (*S*)-linalyl acetate hydrolase (LAH), were identified in a crude cell-free extract.

A special case of a successful resolution of a *tert*-alcohol ester is depicted in Scheme 3. Although the substrate ester formally comprises a non-activated *tert*-alcohol moiety, the presence of a small and highly strained cyclopropane unit effectively reduced the steric bulkiness, which made bicyclo[n.1.0]alkanols **3a**–**g** and **4a**–**h** accepted as substrates for a crude *Mucor miehei* lipase.[4] In order to obtain a sufficient reaction rate and facilitated work-up, the alcoholysis of an activated (chloroacetate) ester in organic medium [diisopropyl or *tert*-butylmethyl ether (TBME)] was found to be most suitable. The ring-size of the alcohol moiety had a remarkable opposite effect on enzymatic reaction rate and enantioselectivity. Although cyclohexane derivatives **4a**–**h** were faster transformed than the corresponding cyclopentane analogues **3a**–**g**, the enantioselectivity followed an opposite trend, i.e., higher *E*-values were obtained with **3a**–**g** ($E = 40–420$) as opposed to **4a**–**h** ($E = 14–20$) [19,20]. In addition, the *E*-values were also dependent on the size and nature of the substituent R, i.e., an increase in chain length and the presence of a C=C double bond or a hetero-atom led to a reduction in enantioselectivity (Table 2).

2.2. Esters of activated tert-alcohols

An increase in the carbonyl activity that facilitates nucleophilic attack onto the carbonyl group of an ester is a general phenomenon. Since the majority of carboxyl ester hydrolases

[4] Lipozyme® from Novo DK.

Table 1
Enantiomeric ratio obtained from the resolution of (±)-linalyl acetate (n.d. = not determined)

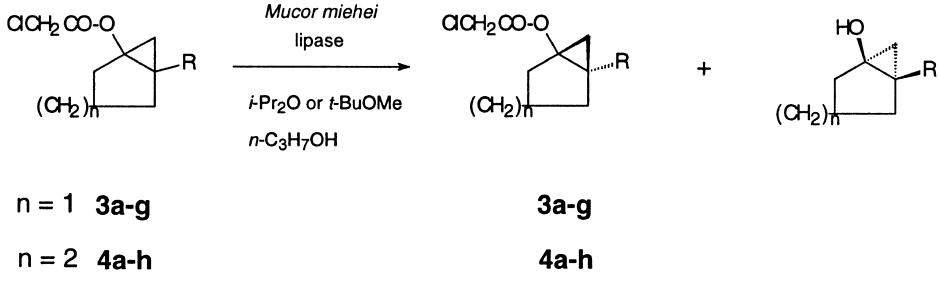
Biocatalyst	e.e. **2a** [%]	e.e. **2** [%]	Selectivity (*E*)	Reference
A. niger[a]	< 5	< 5	< 2	[15]
B. subtilis[a]	~ 10	~ 0	< 2	[16]
Rhodococcus sp. NCIMB 11216	(*R*) 29	n.d.	~ 2	[17]
R. ruber SM 1792[b]	(*S*) 58	n.d.	~ 5	[17]
(*S*)-LAH[c]	(*S*) > 99	n.d.	> 100	[18]
(*R*)-LAH[c]	(*R*) > 99	n.d.	> 100	[18]

[a]Whole microbial resting cells.
[b]SM = culture collection of the Institute of Biotechnology at Graz University of Technology.
[c]Partially purified LAH preparation [18].

are acting along this mechanism, the presence of electron-withdrawing groups on the *tert*-alcohol moiety of an ester facilitates its susceptibility towards biocatalytic hydrolysis. This phenomenon was employed for the resolution of esters possessing *tert*-alcohol moieties by using a range of microbial enzymes. In several studies, a variety of electron-withdrawing groups such as nitrile, acetylene, trifluoromethyl, and ethoxycarbonyl were shown to be effective as 'activating' groups (**5a**–**g** and **6a**–**c**, Scheme 4, Table 3).

For instance, the corresponding acetate esters of α-hydroxynitriles **5a**–**g** and **6a**,**c** (i.e., cyanohydrins of methyl- and trifluoromethylketones, respectively) [21,22] and tertiary acetylenic alcohols **6b** and **9a**,**b** were shown to be good substrates [23]. In studies by Ohta et al. [21,22], microbial whole cells were used as source of carboxyl ester hydrolases. It was found that selectivity strongly depended on the status of the cells, whereas for substrate (±)-**6a**, acceptable selectivities (*E*-values up to 15) were obtained by employing resting cells of *Bacillus coagulans* in phosphate buffer at pH 7.2, the same cells exhibited poor enantioselectivity for the structurally related substrate **5d**. On the contrary, comparable acceptable selectivities (*E*-values up to 14) were obtained by using growing cells of *Pichia miso* [21]. It should be stressed that the overall selectivities obtained are rather low, and that the high $e.e._S$ were only achieved due to a high degree of conversion. Compound **5g** is of considerable synthetic interest since it served as a precursor for the asymmetric synthesis of the physiologically active enantiomer of the bark beetle pheromone (*S*)-frontalin.

The resolution of the α-trifluoromethyl-α-acetylenic acetate **6b** was accomplished by us-



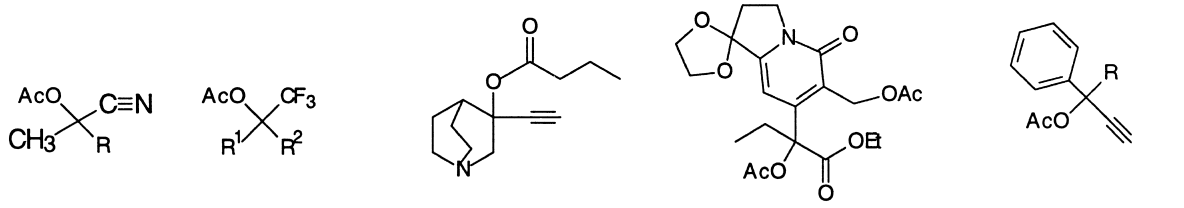
Scheme 3. Resolution of bicyclo[n.1.0]alkanols via lipase-catalyzed acyl-transfer.

Table 2
Selectivities from the resolution of bicyclo[n.1.0]alkanols

	Compound	R	e.e.$_S$ [%]	e.e.$_P$ [%]	E	Reference
$n = 1$	**3a**	$(CH_2)_3-CH_3$	97	98	420	[20]
	3b	$(CH_2)_2-CH=CH_2$	97	86	55	[20]
	3c	$(CH_2)_3-CH_2Cl$	93	94	110	[20]
	3d	$(CH_2)_5-CH_3$	81	97	165	[20]
	3e	$(CH_2)_3-OCH_2Ph$	91	87	46	[20]
	3f	$(CH_2)_4-OCH_2Ph$	95	85	45	[20]
	3g	$(CH_2)_5-OCH_2Ph$	90	86	40	[20]
$n = 2$	**4a**	H	84	97	22	[19]
	4b	CH_3	95	72	22	[19]
	4c	CH_2-CH_3	91	80	28	[19]
	4d	$(CH_2)_2-CH_3$	90	86	38	[19]
	4e	$(CH_2)_5-CH_3$	86	66	13	[19]
	4f	$(CH_2)_9-CH_3$	86	74	14	[19]
	4g	$(CH_2)_4-OCH_2Ph$	88	75	20	[19]
	4h	$(CH_2)_5-OCH_2Ph$	85	70	15	[19]

ing a lipase from *Candida rugosa* (formerly *Candida cylindracea*) in various forms. Thus, when a crude commercial enzyme preparation was used, a remarkable high selectivity of $E = 32$ was obtained [23,24]. However, an unexpected phenomenon was observed when this same lipase was transformed into more stable cross-linked enzyme crystals (CLEC) [25]. In contrast to the general trend observed on several types of enzymes, which makes CLEC-preparations more stable and more selective, the selectivity of *C. rugosa*-CLECS was shown to be markedly reduced as opposed to the crude native enzyme [26] ($E_{CLEC} = 13$, $E_{crude} = 24–32$). This phenomenon was explained by the assumption that the 'true' *C. rugosa* lipase was inactive on substrate **6b** and that the enzyme responsible was an unknown esterase present in low concentration in crude *C. rugosa* lipase preparations [27]. Since the preparation of CLEC always comprises a protein purification procedure during the formation of micro-crystals, the relative amount of (active) esterase in comparison to the (inactive) lipase was reduced. The same behaviour was observed on a related cyanohydrin ester **6c** [28].

In case the activating trifluoromethyl moiety was replaced by non-activating H-, Me-, Et- or Pr-substituents, the reaction rate slowed down considerably, which made the competing non-enzymatic (chemical) hydrolysis the main reaction leading to a depletion of enantioselectivity.



5a-g

6a R^1 = Ph, R^2 = CN

6b R^1 = Ph, R^2 = C≡CH

6c R^1 = Et, R^2 = CN

7

8

9a R = CH_3

9b R = C_2H_5

Scheme 4. Resolution of electronically activated *tert*-alcohol esters.

Table 3
Selectivities from the resolution of electronically activated esters of *tert*-alcohols (n.d. = not determined, n.a. = not applicable, since the cyanohydrin formed undergoes spontaneous racemisation)

Compound	R	Enzyme	e.e.$_S$ [%]	e.e.$_P$ [%]	*E*	Reference
5a	C_2H_5	*P. miso*[a]	0	n.d.	~ 1	[21]
5b	*n*-C_3H_7	*P. miso*[a]	> 95	n.d.	7.1	[21]
5c	*n*-C_6H_{13}	*P. miso*[a]	> 95	n.d.	13	[21]
5d	*n*-C_9H_{19}	*P. miso*[a]	> 95	n.d.	14	[21]
5e	$(CH_3)_2CH$	*P. miso*[a]	9	n.d.	1.2	[21]
5f	$(CH_3)_2CH-CH_2$	*P. miso*[a]	90	n.d.	5.7	[21]
5g	$CH_2=CH(CH_2)_3$	*P. miso*[a]	> 99	n.d.	12	[21]
6a	C≡N	*B. coagulans*[b]	90–100	n.a.	3.7–15	[22]
6b	C≡CH	*C. rugosa*[c]	75	87	32	[23,24,29]
6b	C≡CH	*C. rugosa*[d]	70	74	13	[26]
6c	C≡N	*C. rugosa*[c]	99	n.a.	> 100	[28]
7	–	PLE	99	n.d.	> 40	[30]
8	–	*A. oryzae*[c]	98	98	> 200	[31]

[a] Whole growing cells.
[b] Resting cells.
[c] Crude technical grade lipase.
[d] CLEC.

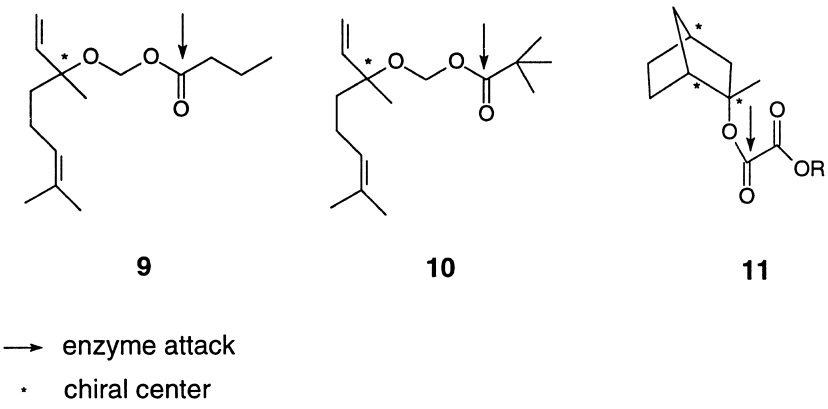
Further studies showed that competing autohydrolysis of non-activated derivatives such as substrates **9a** and **b** can be effectively controlled by placing electron-withdrawing groups, such as CF_3 or NO_2 on the phenyl moiety, or by inserting methylene groups inbetween the aryl ring and the quarternary carbon atom [29], thus moderate to good selectivities were achieved. However, the replacement of acetylenic moiety by methyl, vinyl or nitrile groups made these compounds unacceptable for the enzyme.

The butanoate ester of a 3-substituted acetylenic quinuclidinol derivative (±)-**7** was successfully resolved by using pig liver esterase (PLE) [30]. After 36% conversion, the acetylenic (*R*)-alcohol was isolated in 35% yield and 97% e.e., whereas the extension of the reaction to 56% conversion made the recovery of the non-reacted (*S*)-ester in 99% optical purity possible ($E > 40$). The electronic activation by a carboxyl ester moiety was used for the enantioselective preparation of (*S*)-tetrahydroindolizinyl butanoate derivatives **8**, which represent key intermediates for (*S*)-camptothecin synthesis [31]. The reaction was carried out by using a commercially available crude protease preparation from *Aspergillus oryzae*.

2.3. Spacer techniques for tert-alcohols

In order to circumvent the limitations set for esters of *tert*-alcohols, two general strategies have been developed, which make use of a modified substrate, i.e., the sterically demanding *tert*-alcohol center is moved away from the ester carbonyl moiety by a spacer unit. This causes a two-fold effect: (i) Since the spacer is inserted between the tertiary carbon atom and the reaction center, the substrate becomes more 'slim' and is thus more acceptable for the enzyme. On the other hand, this impedes the chiral recognition process. After all, it appears to be a general rule, that the distance between the site of the reaction to the chiral center should be kept to a minimum in order to ensure an optimal chiral recognition process [32].

There are two spacer techniques of general applicability which have been proposed by Brackenridge et al. [33] and Franssen et al. [34]. Whereas the former strategy makes use of oxalate esters, acyloxymethyl groups were employed in the latter study (Scheme 5). For instance, pivaloyloxymethyl- and *n*-butanoyloxymethyl-derivatives of *tert*-butanol and (±)-linalool (**9** and **10**, respectively), made es-



Scheme 5. *tert*-Alcohol substrates bearing spacer groups.

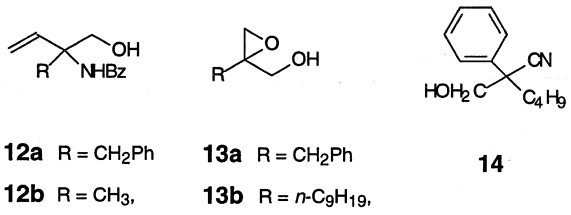
ters of *tert*-alcohols susceptible to enzymatic hydrolysis by several commercially available lipases, esterases, and proteases, for instance lipases from *C. rugosa*, *Pseudomonas fluorescens*, and *Rhizomucor miehei*. However, the conversion rates and the enantioselectivity of the pivaloyloxymethyl derivative **10** carrying a bulky acyl group were very low ($E = 1–1.15$), somewhat better results were obtained with *C. rugosa* lipase for the corresponding butanoyloxymethyl derivative **9** in the alcoholysis mode using *n*-butanol as the nucleophile in toluene. Additionally, some selectivity-enhancement was achieved by lowering the reaction temperature to -10°C ($E = 9.7–9.9$).

The second approach proceeded via oxalate esters of *tert*-alcohols [33]. The mixed alkyl oxalate ester of a bicyclic bulky tertiary alcohol **11** were prepared and resolved by crude porcine pancreatic lipase (PPL). Most remarkably, the enzymatic attack occurred on the more hindered carbonyl group adjacent to the *tert*-alcohol center rather than on the more easily accessible 'outside' alkyl oxalate moiety, as was deduced by the fact that the corresponding bicyclic *tert*-alcohol was detected as the sole product whereas the analogous oxalate hemiester was not. As discussed above, the chiral recognition process was rather low and some selectivity-enhancement studies [35] had to be undertaken [33]. Best results were obtained by the addition of 13% of *n*-butanol as organic cosolvent ($E = 7$), whereas the variation of pH (6–8) or temperature (RT, $+5$°C) did not show any significant effects.

2.4. prim-Alcohols bearing a chiral quarternary C-atom

A special case of 'spacer technique' related to those above makes use of a CH_2-spacer located between the *tert*-alcohol carbon atom and the oxygen atom, which result in the formation of *prim*-alcohols bearing a chiral quarternary C-atom. The effects were similar: On the one hand, these substrates were readily accepted, but the chiral recognition process was reduced due to the increased distance between the chiral center and the site of the reaction. Nevertheless, several successful resolutions and asymmetrisation reactions were achieved.

An elegant access to enantiomerically pure α-substituted amino acids, which are valuable synthons for synthetic peptides possessing enhanced biostability was opened starting from the corresponding *prim*-aminoalcohols of type **12** [36] (Scheme 6). Thus, enzymatic resolution of vinyl-substituted amino-alcohols **12a**,**b** was achieved via transesterification using lipase AK-20 (*ex Pseudomonas* sp.) and vinyl acetate as acyl donor in water-saturated benzene as solvent. Best results were obtained after some



12a R = CH_2Ph **13a** R = CH_2Ph **14**

12b R = CH_3, **13b** R = *n*-C_9H_{19},

Scheme 6. Resolution of *prim*-alcohols bearing a quaternary C-center.

medium engineering was carried out for the corresponding α-vinyl derivatives of phenylalanine (**12a**, R = Ph-CH_2, E up to 25) and alanine (**12b**, R = CH_3, $E > 200$). However, the method failed for α-vinyl-valine and -DOPA due to insufficient selectivities. During hydrolysis of the corresponding acetate esters of alcohols **12a**,**b**, no reaction was observed.

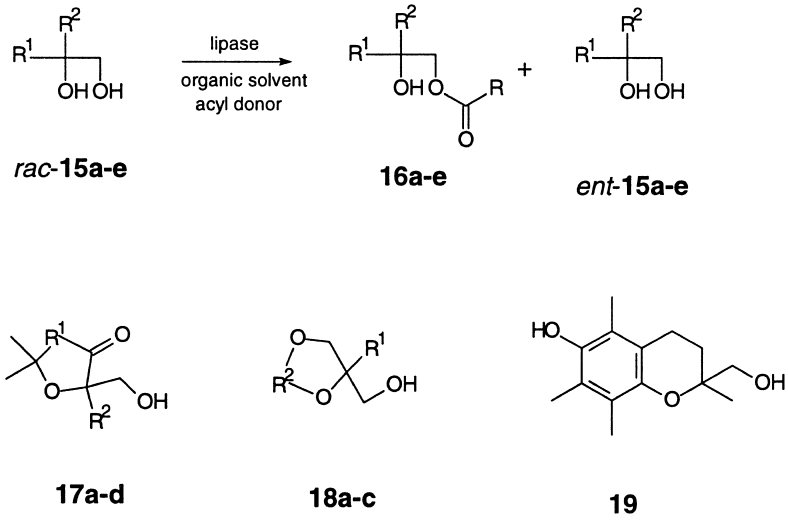
Kinetic resolution of oxiranomethanols of type **13** which constitute useful synthetic precursors for the antibiotic malyngolide, was achieved by *P. fluorescens* lipase catalysed acyl transfer [37]. Depending on the side chain R, good to excellent selectivities were obtained (**13a**, $E > 200$, **13b** $E = 89$). The corresponding four-membered ring synthon 3-methyl-3-oxetanmethanol was not accepted.

2-Cyano-2-phenyl-1-hexanol (**14**), a primary alcohol containing a chiral quaternary carbon atom was used as synthon for the synthesis of sterol biosynthesis inhibitors. The resolution of **14** was plagued by low enantioselectivities, however, optimum conditions were found by using *C. cylindracea* lipase catalysed acyl-transfer in *n*-hexane in the presence of a small amount of pyridine ($E = 13$) [38].

1,1-Disubstituted 1,2-diols constitute a special group of *prim*-alcohols bearing a chiral quaternary C-atom for the following reasons: (i) Due to the presence of two different reactive alcohol groups, their synthetic utility is highly flexible, particularly in view of the large reactivity-difference of *prim*- and *tert*-alcohol groups. (ii) As a consequence, only the *prim*-alcohol moiety is subject to enzyme-catalysed reactions, such as acyl-transfer and ester hydrolysis, whereas the remaining non-reacting *tert*-OH group may interact with polar groups within the active site of the enzyme via formation of hydrogen bonds, which leads to a more intimate and exact positioning of the substrate within the enzyme. As a result, higher selectivities are usually obtained as compared to the substrates mentioned above, which are lacking in additional *tert*-hydroxyl group.

1,1-Disubstituted 1,2-alkanediols constitute important intermediates in the preparation of bioactive compounds. Their resolution was usually achieved by lipase-catalysed acyl-transfer rather than the corresponding ester hydrolysis (Scheme 7, Table 4). In all cases, the reaction was shown to be completely regioselective, i.e., only the *prim*-alcohol moiety was acylated/deacylated, yielding compounds **16a**–**e** and the *tert*-OH group remained unchanged and served only as the stereogenic group [39,40]. From these studies it can be seen that the presence of an unsaturated group R in substrates **15a**–**f** is essential to obtain a high degree of chiral discrimination due to a better positioning of the substrate via π–π-stacking. Thus, whereas very low selectivities were observed for compounds having an aliphatic side chain, moderate to excellent enantioselectivities were found (E = 15–200), when an allylic or benzylic unit was present. A related effect was observed for an acetylenic C≡C triple bond [39]. In addition, halogens, such as iodine, and phenyl- or alkynyl-groups can be regarded as appropriate substituents for this class of compounds [39].

The spacer technique was also successfully applied in the lipase-catalysed resolution of cyclic protected triols and aminodiols, such as 5-hydroxymethyl-1,3-dioxolan-4-ones **17a**–**c** and 3-hydroxymethyl-oxazolidine-2-ones **17d** [43]. Whereas no conversion was observed with lipases such as lipases AKG, PS, and PPL probably due to steric repulsion, acetylation was successfully carried out by *Candida antarctica* lipase in excellent selectivities ($E > 100$). A remarkable phenomenon of opposite enantio-



Scheme 7. Resolution of (protected) 1,1-disubstituted 1,2-diols via lipase-catalysed acyl transfer or ester hydrolysis.

preference was observed for lipases from *C. cylindracea* (CCL) and *C. antarctica* for substrate **17d**. Another related example was reported by Wirz et al. [44], who succeeded in the enantioselective esterification of 1,2-O-protected 2-methylglycerols **18a**–**c** and the enantioselective hydrolysis of the corresponding butyryl esters. In this case, several lipases like lipase P-30, SAM-2 and Palatase M1000 can be used ($E > 200$). The combination of these two

Table 4
Selectivities in resolutions of (protected) 1,1-disubstituted 1,2-diols (n.d. = not determined)

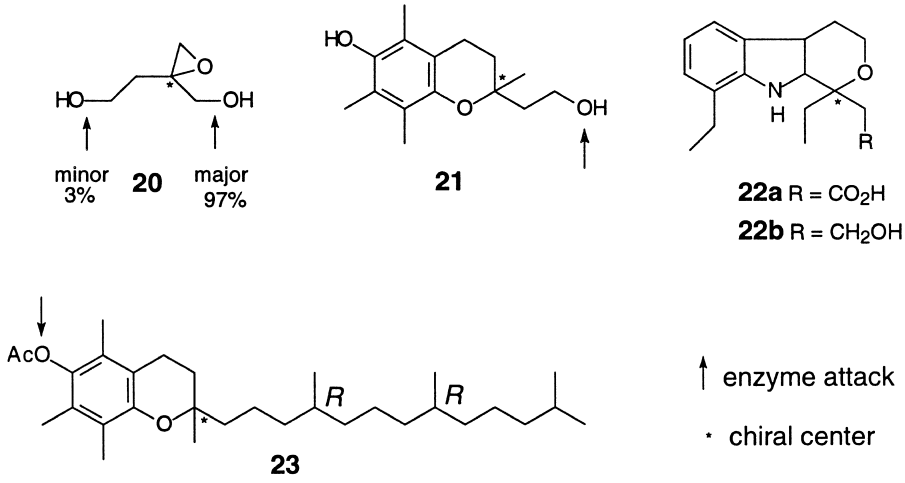
Compound	R_1	R_2	Lipase	Solvent	e.e.$_S$	e.e.$_P$	E	Reference
15a	CH_2I	C≡C–Ph	PPL[a]	*t*-BuOMe/VA[b]	> 97	> 97	> 100	[39]
15b	$CH_2C_6H_5$	C_6H_5	AKG[c]	*i*-Pr_2O/VA[b]	97	97	> 200	[40]
15c	CO_2Me	$(CH_2)_{11}CH_3$	PFL[d]	*t*-BuOMe/VA[b]	84	71	15	[41]
15d	C_6H_5	C_2H_5	AKG[c]	*i*-Pr_2O/VA[b]	< 2	7	~ 1	[42]
15e	C_6H_5	*n*-C_3H_7	AKG[c]	*i*-Pr_2O/VA[b]	0	0	1	[42]
15f	CH_2I	C_6H_5	AK[c]	IPA[e]	70	94	69	[39]
17a	O	C_6H_5	CCL[f]	*i*-Pr_2O/VA[b]	66	66	6	[43]
17a	O	C_6H_5	CAL[g]	*i*-Pr_2O/VA[b]	79	> 99	> 200	[43]
17b	O	*p*-Br–C_6H_5	CAL[g]	*i*-Pr_2O/VA[b]	59	98	> 100	[43]
17c	O	Me	PPL[a]	*i*-Pr_2O/VA[b]	76	89	40	[43]
17d	NMe	C_6H_5	CAL[g]	*i*-Pr_2O/VA[b]	> 98	82	> 50	[43]
18a	CO-*n*-Pr	$C(CH_3)_2$	Lipase P[c]	aqueous buffer	n.d.	> 99	> 200	[44]
18b	CO-*n*-Pr	$C(CH_2)_5$	Lipase P[c]	aqueous buffer	n.d.	> 99	> 200	[44]
18c	H	$C(CH_3)_2$	Lipase P[c]	*n*-hexane/VA/VB[b]	n.d.	97	> 150	[44]
19	–	–	PS 30[c]	*t*-BuOMe /VA/IPA[b]	n.d.	97	78	[45,46]

[a] Porcine pancreatic lipase.
[b] IPA = *i*-propenyl acetate, VA = vinyl acetate, VB = vinyl butanoate.
[c] Derived from *Pseudomonas* sp.
[d] *P. fluorescens* lipase.
[e] Isopropenyl acetate as solvent and acyl donor.
[f] *C. cylindracea* (*C. rugosa*) lipase.
[g] *C. antarctica* lipase.

methods, i.e., acyl-transfer and ester hydrolysis, provided access to both alcoholic enantiomeres.

(*S*)-6-hydroxy-2,5,7,8-tetramethyl-2-chromanmethanol (**19**) represents an important intermediate for the synthesis of natural stereoisomer of tocols, such as vitamin E and α-tocotrienol, and it can be regarded as partially protected 1,1-disubstituted 1,2-diol. The kinetic resolution of (±)-**19** was carried out using *Pseudomonas* sp. lipase catalyse acyl-transfer [45], and in one study, the use of succinic anhydride as acyl donor was shown to be advantageous for large-scale reactions [46].

The remarkable flexibility of the spacer technique is demonstrated along the following examples, where the site of reaction is moved away from the directing stereo center or even further, which makes the chiral recognition process more difficult. Despite these drawbacks, several compounds possessing 'remote chiral centers' were transformed in a highly enantioselective fashion by making use of the exquisite substrate recognition of biocatalysts (Scheme 8). For instance, both enantiomers of the anti-inflammatory and analgesic drug etodolac **22a** were obtained via a lipase-catalysed kinetic resolution of its alcoholic derivative **22b**, in which the reactive OH-unit is connected through a CH_2–CH_2 spacer with a tertiary carbon atom bearing the center of chirality [47]. Using *C. cylindracea* lipase and vinyl acetate as acyl donor, a remarkable *E*-value of 17 was observed when acetonitrile and TBME were employed as solvents. On the other hand, hydrocarbon solvents such as *n*-hexane or cyclohexane enhanced the reaction rate but decreased enantioselectivity. No conversion was achieved when the transesterification was attempted in the alcoholysis mode, i.e., by starting from the corresponding methyl or ethyl ester of **22a** with lipases from *C. cylindracea* and porcine pancreas in cyclohexane employing *n*-butanol as the nucleophile. Epoxy-1,4-butanediol (**20**) represents a special problem of regio- and enantioselection, since it possesses two reactive *prim*-alcohol groups, which are different only by the length of the spacer unit. With high regioselectivity for the more sterically hindered hydroxyl group in position 1 (C1/C4, 97:3), the enantioselectivity of the *Pseudomonas* sp. lipase-catalysed acyl-transfer was moderate ($E = 9.7$) [48]. It should be mentioned that the competing (chemical) asymmetric epoxidation of the corresponding allylic alcohol according to Sharpless showed reduced selectivity (e.e. 80%). Substrate **21** ('tocopheryl ethanol') is closely related to compound **19** described above with the difference of an additional CH_2-unit in the spacer



Scheme 8. Substrates possessing remote stereocenters.

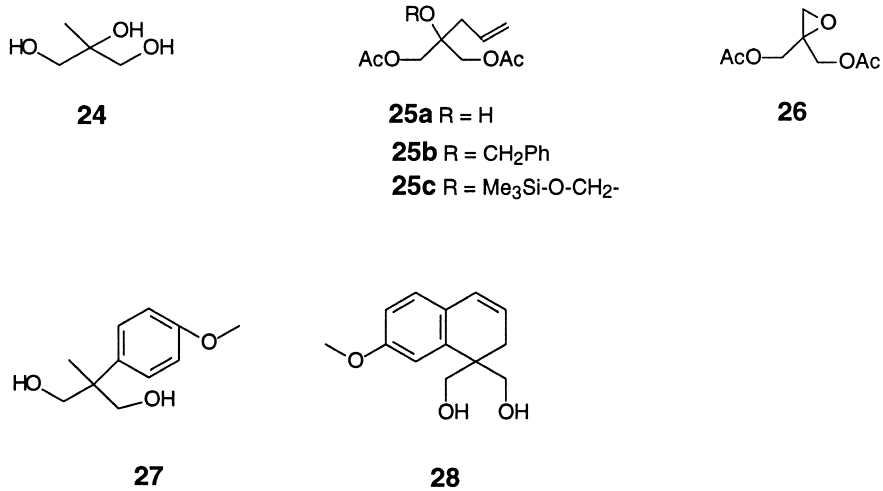
Table 5
Selectivities obtained from resolutions of substrates bearing remote stereocenters

Compound	Enzyme	Solvent	e.e.$_S$ [%]	e.e.$_P$ [%]	E	Reference
20	*P. cepacia* lipase	$CHCl_3$/VA[a]	86	n.d.	9.7	[48]
21	Lipase B[b]	acetone/VA[a]	> 99	51	14	[49]
22b	*C. cylindracea* lipase	MeCN/*t*-BuOMe/ VA[a]	n.d.[c]	89–99	17	[47]
23	Cholesterol esterase	aqueous buffer		c	8[d]	[50]

[a] VA = vinyl acetate.
[b] Derived from *Pseudomonas* sp.
[c] n.d. = Not determined.
[d] Diastereomeric ratio (v_{SRR}/v_{RRR}).

moiety. Successful resolution was achieved in fair selectivity ($E = 14$) using lipase B (derived from *Pseudomonas* sp.) in the acyl-transfer mode [49]. One of the most challenging problems of stereorecognition is probably repesented by α-tocopheryl acetate (**23**). In this case, the stereo-directing chiral carbon atom adjacent to the *O*-moiety is separated by seven bonds from the reaction site. A diastereomeric mixture of (2*RS*,4′*R*,8′*R*)-acetate **23** could be resolved by cholesterol esterase [50], an enzyme which is designed by nature to accept bulky substrates, i.e., sterols. For catalytic activity, the enzyme requires the presence of bile salts, and it was shown that the type of bile salt, e.g., cholate, glycocholate, or taurocholate not only serves as activator but also as modulator of the selectivity of the enzyme. The latter phenomenon was explained by alteration of the enzyme's structure through refolding (Table 5).

In contrast to kinetic resolution, which leads to the formation of two enantiomers in each 50% theoretical yield at maximum, asymmetrization is advantageous from an economical standpoint, since (in contrast to kinetic resolution) the enantiomeric composition of the product is independent on the conversion, and thus the reaction may be run to completion [51]. In several studies, this advantage was accrued during the asymmetrisation of prochiral 2,2-dis-



24 **25a** R = H **25b** R = CH_2Ph **25c** R = Me_3Si-O-CH_2- **26** **27** **28**

Scheme 9. Asymmetrisation of prochiral 2,2-disubstituted 1,3-propanediols.

ubstituted 1,3-diols, which open access to chiral glycerol-related derivatives (Scheme 9). However, substrate **24** was shown to be a non-substrate for the asymmetric monoacylation for several commercially available hydrolases [52]. On the other hand, closely related compounds **25a**–**c** were transformed in a highly successful fashion using the monohydrolysis of the corresponding diesters [53]. The selectivity was carefully tuned according to the following guidelines: First, variation of the protective group R of the *tert*-alcohol moiety revealed that the SEM-group (trimethylsilyloxymethyl) was superior to the non-protected and the benzyl-protected analogue. Secondly, lowering of the temperature to +3°C and addition of a water-miscible organic co-solvent, such as DMF, enhanced the selectivity even further. Interestingly, both hydrolases used, i.e., porcine liver esterase and *Chromobacterium viscosum* lipase gave comparable good results. Prochiral epoxy-diacetate **26** was hydrolyzed by crude PPL in a highly selective fashion yielding the corresponding monoacetate 96% e.e. [54]. Diols **27** and **28**, which constitute important building blocks for the total synthesis of (−)-aphanorphine and (+)-eptazocine, were successfully asymmetrised using lipase-catalysed acyl transfer [55]. Thus, when **27** was treated with *Pseudomonas cepacia* lipase immobilised on hyflo super cell (PSL/HSC) in the presence of two equivalents of isopropenyl acetate as acyl donor in TBME, the desired monoacetate was obtained in 86% yield and 71% e.e. The reversed reaction, i.e., the enzymatic ester hydrolysis of the corresponding diacetate was attempted without success. For compound **28**, various lipases were tested in different organic solvents and again, best result were obtained with PSL/HSC using isopropenyl acetate and a small amount of triethylamine (e.e.$_P$ = 93%) (Table 6).

Table 6
Selectivities from the asymmetrisation of 2,2-disubstituted 1,3-propanediols

Compound	Enzyme	Organic cosolvent	e.e.$_P$ (%)	α[a]	Reference
25a	PLE	none	50	3	[53]
25b	lipase AP	none	70	5.7	[53]
25c	lipase LP[b]	none	70	5.7	[53]
25c	lipase LP[b]	DMF	88	16	[53]
25c	PLE	DMF	90	19	[53]
26	PPL[b]	none	96	49	[54]
27	PSL/HSC[c]	TBME	71	5.9	[55]
28	PSL/HSC[c]	triethylamine	93	28	[55]

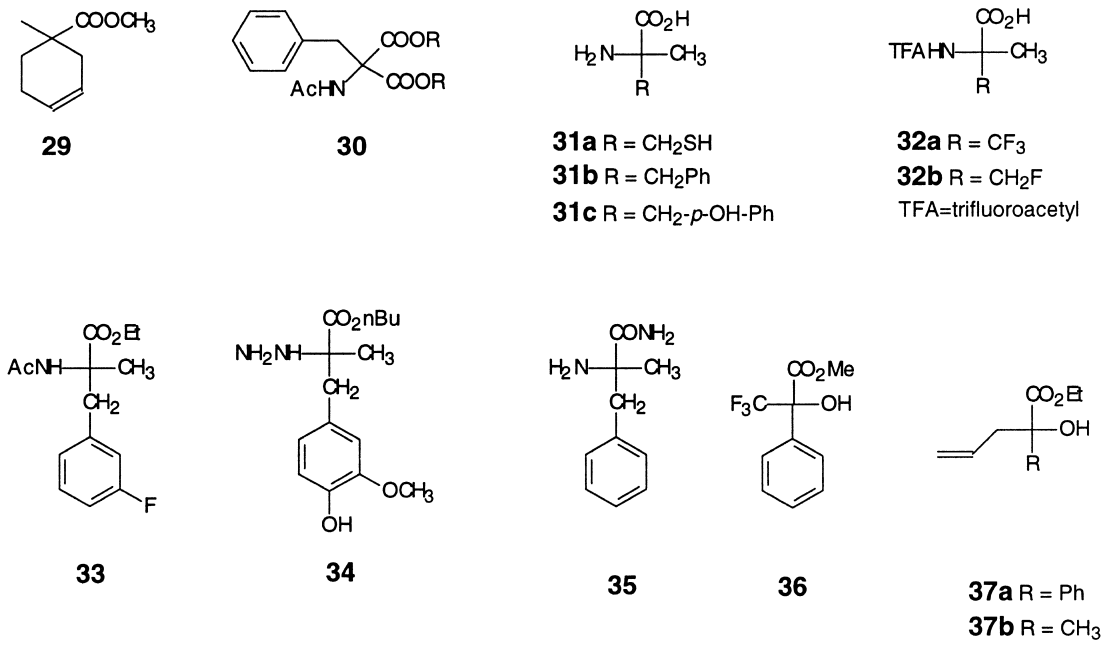
[a] The selectivity factor α is defined as $\alpha = k_R / k_S$ [9].
[b] Porcine pancreatic lipase.
[c] *Pseudomonas* sp. lipase immobilised onto Hiflo super cell.

3. Esters of α,α-disubstituted carboxylic acids

3.1. Esters of activated α,α-disubstituted carboxylic acids

The resolution of α,α-disubstituted carboxylates still remains a challenge for biocatalysis, since the most commonly used carboxyl ester hydrolases and proteases are unable to accept these compounds as substrates due to steric repulsion. In this substrate series, non-activated substrates are rather scarce and it seems to be a general rule (see below) that at least one of the α-substituents exerts electron-withdrawing effects, usually through a hetero-atom (O, N), which makes these sterically demanding esters better accepted (Scheme 10). First attempts were carried out by using α-chymotrypsin as biocatalyst, however, without success [56]. It appeared as a general rule, that the ester in alicyclic derivatives was only hydrolysed if the carbon α-atom adjacent to the carbomethoxy group was not fully substituted, which is understandable, if one bears in mind, that the natural substrates for proteases are α-unsubstituted amino acids. As expected, 1-methyl methyl-3-cyclohexenecarboxylate (**29**) [57] and α-benzyl-α-acetamidomalonate (**30**) [58] could not be resolved by α-chymotrypsin.

α-Substituted amino acids and peptides derived thereof are of considerable interest for the agrochemical and pharmaceutical industry due to their biological properties [59]. In particular, when α-alkyl amino acids are incorporated into



Scheme 10. α-Substituted α-amino- and α-hydroxycarboxylic acid derivatives.

physiologically active peptides, conformational changes are constrained due to the increase in steric bulk. As a consequence, their bioactivity may be altered and furthermore, these peptides often possess enhanced biostability, since their degradation in vivo is largely reduced, owing to the fact that the adjacent peptide bonds are not susceptible to hydrolysis by endogeneous proteases. Thus, it became a common practice to enhance the biostability of peptides, to incorporate α-alkyl amino acids into those positions, which are most likely cleaved by proteases [60].

There are different approaches towards biocatalytic resolution of α-substituted α-amino acids [61,62]. A common method employing amino acylase was hampered by the fact that the enzyme from *Aspergillus* sp. showed no activity, however, acylase I from hog kidney was the enzyme of choice for the resolution of α-methylmethionine (**31a**), α-methylphenylalanine (**31b**) and α-methyltyrosine (**31c**) [61]. In this way, the corresponding (±)-*N*-acylamino acids could be resolved using membrane-enclosed acylase I with good selectivities. The same enzyme also showed high stereoselectivity for the quarternary center in *N*-trifluoroacetyl-α-trifluoromethyl alanine (**32a**) [62], whereas no enantioselectivity (but good activity) was detected with *N*-trifluoroacetyl-α-fluoromethyl alanine (**32b**) as substrate. In search for novel proteases, which would be able to accept sterically hindered α-substituted amino acid derivatives, two remarkable amino acid amidases from *Mycobacterium neoaurum* [59,63] and *Ochrobacterium anthropi* [63] were identified from an extensive screening program by using α,α-disubstituted glycine amides as substrates.

Alternatively, (±)-α-substituted α-amino acids could also be resolved by lipases and esterases under certain circumstances, for instance, substrate **33** [58,64,65]. In this context, a novel carboxyl ester hydrolase from *Candida lipolytica* (CLEH) was identified as the active component purified and characterised from a crude technical-grade lipase preparation [64,65]. The resolutions proceeded with high enantioselectivies ($E > 450$). In addition to the 'normal' amino acid analogues, compounds containing a

Table 7
Selectivities from α-substituted α-amino- and α-hydroxycarboxylic acid derivatives

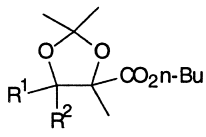
Substrate	Enzyme	e.e.$_S$ [%]	e.e.$_P$ [%]	E	Reference
32a	Acylase I[a]	97	98	> 200	[62]
33	*C. lipolytica* lipase[b]	> 99	96	> 200	[58]
34	CLEH[c]	> 99	> 99	> 450	[64,65]
35	*O. anthropi* amidase	> 99	93	170	[63]
36	*A. oryzae* protease	88	88	26	[66]
37a	PLE	49	94	52	[59,67]
37b	PLE	9	22	2	[59,67]

[a] Derived from hog kidney.
[b] Crude technical grade preparation.
[c] Carboxyl ester hydrolase from crude *C. lipolytica* lipase.

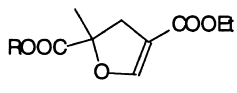
variety of heteroatoms such as hydrazino (substrate **34**), hydrazido, amino (substrate **35**) and hydroxyl groups (substrate **36**) in the α-position were accepted as well.

The kinetic resolution of α-substituted α-hydroxy acids of type **37** is closely related to the above mentioned amino acids. Several enzymes, such as *A. oryzae* protease [66], *B. subtilis* in PPL and PLE [59,67,68] were found to be useful for the resolution of these derivatives. Using PLE, a rational approach to the synthesis of α-hydroxy acids can be taken by considering the active site model proposed by Jones in [69]. A high degree of enantioselectivity was found for the hydrolysis of an α-allyl-mandelic acid ester **37a**, whereas the corresponding lactic acid ester **37b** was hydrolysed with reduced selectivity. This phenomenon was explained by favorable binding of the phenyl group within the active site of the enzyme through π–π stacking (Table 7).

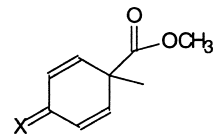
Several cyclic derivatives of α,α-disubstituted carboxylic acid esters are versatile synthons for the synthesis of natural products [70–72] (Scheme 11). For the resolution of these sterically demanding compounds, a protease from the fungus *A. oryzae* seems to be particularly suitable [71]. For instance, the hydrolysis of dihydroisoxazole dicarboxylate (±)-**39a**,



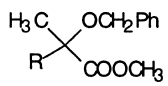
38a $R^1 = CH_3$, $R^2 = H$
38b $R^1 = H$, $R^2 = CH_3$
38c $R^1 = H$, $R^2 = H$



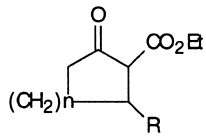
39a R = Et
39b R = H



40a X = H_2
40b X = O

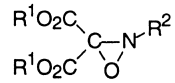


41a R = Et
41b R = *n*-Pr
41c R = $CH_2CH{=}CH_2$
41d R = *n*-C_5H_{11}
41e R = *n*-C_9H_{19}
41f R = *i*-Bu



42

n = 1, 2
R = alkyl, alkenyl, cyanoalkyl, benzyl



43a $R^1 = CH_3$, $R^2 = t$-Bu
43b $R^1 = CH_3$, $R^2 = i$-Pr
43c $R^1 = Et$, $R^2 = t$-Bu
43d $R^1 = Et$, $R^2 = i$-Pr

Scheme 11. α,α-Disubstituted carboxylic esters containing cyclic structures.

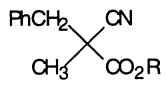
Table 8
Selectivities obtained from cyclic α,α-disubstituted carboxylic esters (n.d. = not determined)

Substrate	Enzyme	e.e.$_S$ [%]	e.e.$_P$ [%]	E	Reference
38a	*C. cylidracea* lipase		no reaction		[70]
38b	*C. cylidracea* lipase	n.d.	95	92	[70]
38c	*C. cylidracea* lipase	n.d.	33	2.7	[70]
39	*A. oryzae* protease	> 97	77	35	[71,73]
40a	PLE	93 (*R*)	n.d.	42	[72]
40a	HLE	96 (*S*)	n.d.	80	[72]
40b	PLE	94 (*R*)	n.d.	96	[72]
40b	HLE	92 (*S*)	n.d.	65	[72]
41a	Lipase OF[a]	81	60	10	[74]
41b	Lipase OF[a]	95	70	20	[74]
41c	Lipase OF[a]	> 99	82	52	[74]
41d	Lipase OF[a]	> 99	67	25	[74]
41e	Lipase OF[a]	94	67	17	[74]
41f	Lipase OF[a]	0	n.d.	~ 1	[74]
42	PLE	70–99	n.d.	2 to 40	[75,76]
43a	PPL[b]	87	n.d	5.6	[77]

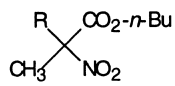
[a] Derived from *C. rugosa*.
[b] Porcine pancreatic lipase.

which can be regarded as a cyclic protected α-amino-γ-hydroxycarboxylic acid, proceeded in a highly regio and enantioselective fashion with this enzyme. Hydrolysis occurred only at the more sterically hindered α,α-disubstituted carboxyl ester group with good enantioselectivity ($E = 35$) yielding the corresponding monoester **39b** [71,73]. For acetyl-protected α-substituted α,β-dihydroxycarboxylic acid esters **38a**–**c**, *C. cylindracea* lipase was used in the hydrolysis mode [70]. The presence of an additional methyl group was tolerated, as long as it was in *trans*-position to the ester moiety (substrate **38b**), whereas the *cis*-isomer **38a** was unreactive. In this context, PPL seems to be much more sensitive to steric hindrance and was therefore inactive on these substrates.

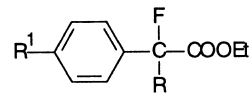
1-Methyl-2,5-cyclohexadiene-1-carboxyaldehyde is a starting material for the synthesis of taxoides, a group of promising anticancer drugs [72]. For the enantioselective hydrolysis of esters **40a**,**b**, crude esterase preparations from pig or horse liver (PLE and HLE, respectively) have proven to be well suited. Since they possess opposite enantiopreference, (*S*)- and (*R*)-configurated carboxylic acids could be obtained as



44a R = CH_3
44b R = C_2H_5
44c R = *n*-C_3H_7
44d R = *n*-C_4H_9
44e R = *n*-C_6H_{13}
44f R = *n*-C_8H_{17}
44g R = CH_2Ph
44h R = CH_2CF_3



45a R = $CH_2CH{=}CH_2$
45b R = Ph
45c R = 3-indolyl
45d R = $(CH_2)_2COCH_3$



46a R = CN, R^1 = CH_3
46b R = CH_3, R^1 = H

Scheme 12. Resolution of α-cyano- and α-nitro-activated carboxylic esters.

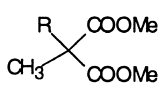
Table 9
n.d. = Not determined, n.a. = not applicable, since product acid undergoes spontaneous decarboxylation.

Substrate	Enzyme	e.e.$_S$ [%]	e.e.$_P$ [%]	E	Reference
44a	PPL	95	84	110	[81]
44b	PPL	25	98	130	[81]
44c	PPL	n.d.	97	150	[81]
44d	PPL	n.d.	98	180	[81]
44e	PPL	n.d.	96	79	[81]
44f	PPL	n.d.	88	18	[81]
44g	PPL	59	95	78	[81]
44h	PPL	81	79	21	[81]
45a	α-chymotrypsin	> 95	n.a.	> 15	[82]
45b	α-chymotrypsin	> 95	n.a.	> 15	[82]
45c	α-chymotrypsin	> 95	n.a.	> 15	[82]
45d	α-chymotrypsin	75	n.a.	6.5	[82]
46a	CRL[a]	98	68	23	[85]
46b	CRL[a]	91	97	89	[85]

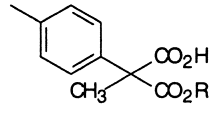
[a] *C. rugosa* lipase.

the products by simple choice of the biocatalyst. Racemic tertiary α-substituted α-benzyloxy esters **41a**–**f** were successfully resolved by using lipase OF from *C. cylindracea* [74]. When several structurally related substrates possessing a different side chain were tested, it was found that straight alkyl-chains are preferable over branched counterparts. α-Benzyloxy ester **41c** bearing an α-allylic chain constitutes a useful chiral equivalent to citramalic acid, which is involved in the synthesis of natural isomers of (*R*)-mevalonolactone and (*S*)-frontalin **19** [78] as well as in the synthesis of the α-tocopherol derivative **19** [79]. Cyclohexanone and cyclopentanone β-ketoestersesters of type **42** bearing various substituents R in the α-position were resolved by using PLE [75,76]. It was shown that in a weakly acidic medium (pH 5–6) the selectivity of PLE increased with the length of the α-substituent, and low to moderate selectivities of up to $E = 40$ were achieved [76]. The β-ketocarboxylic acids obtained as products were intrinsically unstable and underwent spontaneous decarboxylation to furnish the corresponding α-alkyl monosubstituted *rac*-ketones, but the remaining non-reacted substrate esters could be further transformed into protected enantiopure α-substituted α-amino acids [80]. Oxaziridines of type **43a**–**d** represent interesting chiral molecules since their asymmetry is due solely to the ring nitrogen atom. Various dicarboxylic ester analogues were resolved via lipase catalyzed hydrolysis [77]. Whereas *C. rugosa* lipase accepted these substrates without selectivity, PPL gave moderate results (E ca. 6) (Table 8).

Instead of the more often encountered activation by electron-withdrawing hetero-atoms (e.g., O and N), other activating functionalities, such as nitrile-, trifluoromethyl-, or nitro-groups can be used to construct α,α-disubstituted carboxylic esters which are well accepted as substrates (Scheme 12). For instance, the enzymatic resolution of 2-cyano-2-methylalkanoic acid esters of type **44** [81] was carried out via hydrolysis using crude technical grade PPL (Table 9). The enantioselectivity strongly depended on the



47a R = Et
47b R = *n*-Pr
47c R = *n*-Bu
47d R = *n*-C_5H_{11}
47e R = *n*-C_6H_{13}
47f R = *n*-C_7H_{15}



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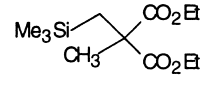

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Scheme 13. α,α-Disubstituted malonic diesters.

Table 10
Selectivities from the asymmetrisation of α,α-disubstituted malonic diesters

Substrate	R	e.e.$_P$ [%]	Configuration	α	Reference
47a	Et	74	*S*	6.7	[86]
47b	*n*-C_3H_7	52	*S*	3.2	[86]
47c	*n*-C_4H_9	55	*S*	3.4	[86]
47d	*n*-C_5H_{11}	49	*R*	2.9	[86]
47e	*n*-C_6H_{13}	87	*R*	14.4	[86]
47f	*n*-C_7H_{15}	90	*R*	19	[86]

alcohol moiety of the substrate ester, best results were obtained for the *n*-butyl ester **44d** ($E = 180$). Several other lipases from various sources, such as *Chr. viscosum*, *Mucor javanicus*, and *C. lipolytica* showed activity but very low selectivity.

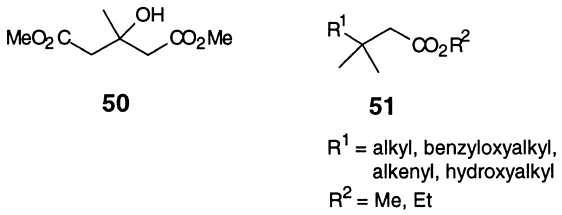
α-Substituted α-nitropropanoate esters of type **45** are bearing a 'hidden' amino group and constitute therefore versatile starting materials for the synthesis of the corresponding α-amino acids [82]. The resolution of substrates **45a**–**d** was accomplished by using the protease α-chymotrypsin, whereas lipases from *C. rugosa* and porcine pancreas were inactive. The general pattern which emerged shows that enantioselectivity was generally improved if the side-chain contained π-electrons. It should be mentioned that the corresponding nitrocarboxylic acids formed are intrinsically unstable and subject to spontaneous decarboxylation in a similar fashion as the β-ketoacids discussed above. 2-Fluoro-2-arylacetic acids (**46a**,**b**) have been shown to be extremely effective chiral derivatizing agents for the determination of the enantiomeric composition of *prim*- and *sec*-alcohols due to their large (diastereomeric) difference in ^{19}F-NMR shifts, and the effects were even surpassing those of the corresponding Mosher's acid derivatives [83]. In order to facilitate their preparation in enantiopure form, a lipase-catalyzed resolution was attempted. Initial results using native *C. rugosa* lipase were rather disappointing ($E = 3$), however, when the same lipase was pretreated with 2-propanol [84], the selectivity was markedly enhanced ($E = 23$ and 89, respectively) (Table 9).

The advantages of asymmetrisation, as compared to kinetic resolution, can also be gained for α,α-disubstituted carboxylic esters and the main substrate type corresponds to malonate esters, which can be considered as activated substrates due to the vicinity of both ester moieties (Scheme 13). For these compounds, several commercially available enzymes were shown to be active, i.e., PLE, *C. cylindracea* lipase, and protease α-chymotrypsin. The fact that no proton is located at the α-position makes the corresponding chiral hemiester product stable towards spontaneous racemisation[5].

Asymmetric monohydrolysis of various α,α-disubstituted malonate diesters by PLE has been studied in great detail [86–88]. It was found that the enantioselectivity mainly depends on the size of the α-substituents [86,88] and to a lesser extent on the nature of the alcohol moiety of the ester [89]. An interesting reversal of stereospecificity was observed for a homologous series of α-methyl-α-*n*-alkyl dimethyl malonates [86]. Whereas for short-chain alkyl groups up to *n*-butyl, PLE produced (*S*)-configurated hemiesters, for extended chains (*n*-pentyl to *n*-heptyl), (*R*)-enantiomers were formed (Table 10).

When α-chymotrypsin was used, the prediction of the stereochemical outcome was made possible by the use of computer modelling [89]. Several useful building blocks were synthesized via this methodology. 2-Methyl-2-*p*-tolylma-

[5] The corresponding α-monosubstituted malonic hemiesters would be prone towards acid- or base-catalysed racemization via the corresponding enol/enolate.



50 **51**

Scheme 14. β,β-Disubstituted carboxylic acids.

lonic monoester **48** was used as a building block for the synthesis of enantiopure α,α-disubstituted succinates, which in turn were transformed into cyclopropane derivatives [90–92]. The latter served as starting materials for the synthesis of natural compounds, such as α-and β-cuparenones [91]. It is remarkable, that esterases from pig and horse liver also accepted the α-organosilyl-substituted malonic diester **49**. In this case, the enantioselectivity was increased by addition of DMSO as a cosolvent [93].

3.2. Spacer techniques for α,α-disubstituted carboxylic acids

It seems to be a general trend for disubstituted carboxylic acids for spacer techniques to be employed to a lesser extent as compared to substrates bearing a *tert*-alcohol moiety. From the data available, it can be deduced that when acting on α,α-disubstituted carboxylic acids, most hydrolases are rather sensitive when the chiral center is moved away from the reacting carboxyl ester moiety, leading to low selectivities. However, two successful examples, involving β,β-disubstituted acid esters are given below (Scheme 14).

Asymmetric monohydrolysis of 3-hydroxy-3-methyl dimethyl glutarate (**50**) by PLE proceeded in an enantioselective fashion to yield the corresponding monoester in 99% e.e. [94]. On the other hand, kinetic resolution of a series of structurally related (±)-3-hydroxy-3-methyl-alkanoic acid esters of type **51** using the same enzyme proceeded with disappointingly low selectivities, with *E*-values ranging from 2 to 9 [95].

4. Conclusion

The results summarized in this paper clearly show that the general limitation of carboxyl ester hydrolases in their inability to accept bulky substrates bearing a fully substituted chiral center adjacent to the ester moiety, i.e., esters of *tert*-alcohols and α,α-disubstituted carboxylates can be effectively overcome by employing two techniques: (i) introduction of electron-withdrawing groups as 'activators' (which enhances the susceptibility of ester bonds towards hydrolysis) and (ii) insertion of spacer groups between the chiral center and the reacting ester (which leads to a reduction of steric bulkiness) allows the successful transformation by commonly used commercially available carboxyl ester hydrolysing enzymes, such as esterases, lipases, and proteases.

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