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ENZYMATIC RECOGNITION OF DIASTEREOMERIC ESTERS

C. G. Rabiller[†], K. Königsberger, K. Faber* and H. Griengl*

Institute of Organic Chemistry
and Christian Doppler Laboratory for Chiral Compounds,
Graz University of Technology, Stremayrgasse 16, A-8010 Graz, Austria

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Abstract: Aiming to improve the enantioselectivity of enzymatic resolution of esters, lipase catalyzed hydrolysis of (D)- and (L)-2-chloropropanoates of four racemic alcohols and transesterification of ethyl (DL)-2-chloropropanoate with optically pure alcohols was investigated. Thus, (*rac*)-*endo*-2-norbornyl (L)-2-chloropropanoate was hydrolyzed by lipase P about 5 times more selectively than its corresponding (D)-counterpart and optically pure (1*R*,2*S*,5*R*)-menthol was obtained by transesterification of its racemate with ethyl (D)-2-chloropropanoate using *Candida cylindracea* (CC) lipase. From the results obtained it seems obvious that lipases CC and P mainly can recognize the chirality of an alcohol moiety rather than that of an acid.

INTRODUCTION

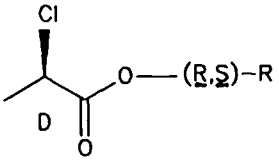
A number of methods have recently been developed for improving the selectivity of enzymatic resolution[1]: Optimisation of substrate structure,[2] variation of enzyme,[3] choice of organic solvents,[4-6] application of novel acylating agents[7-9] and addition of inhibitors and/or activators[10,11] have contributed to an increased use of enzymes for the preparation of optically pure compounds.[12-14] In the course of our ongoing studies in this field[2,3,15,16] we started an investigation on hydrolysis and transesterification of esters in which *both* the alcoholic and the acidic parts are chiral. Although diastereomers can be separated due to their different physical and chemical properties, the methods required are often quite laborious. Therefore, the implications intended to be covered by the present work are:

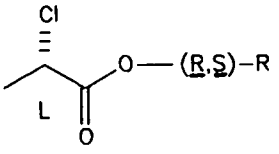
i) Potential amelioration of enzymatic resolution of racemic alcohols and acids,
ii) easy determination of the optical purity of products since diastereomers and not enantiomers have to be analyzed, and
iii) trying to gain information about the type of chiral recognition operative.

To the best of our knowledge, such an approach has not yet been developed besides early reports on the resolution of esters of amino acids with chiral alcohols[18,19] and of amino acids bearing chiral N-acyl groups[17,31] using renal carboxypeptidase or α-chymotrypsin. For the present work two types of substrates were chosen:[20]

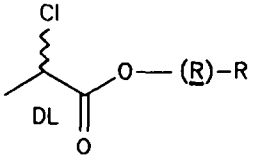
[†] Permanent adress: *Laboratoire de RMN et Reactivité Chimique, Faculté des Sciences et des Techniques, Université de Nantes,2 Rue de la Houssiniere, F-44072 Nantes Cedex, France.*

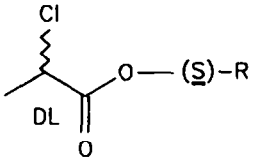
Type I: Optically pure acid moiety and racemic alcohol R.





Type II: Optically pure alcohol R and racemic acid moiety.





RESULTS AND DISCUSSION

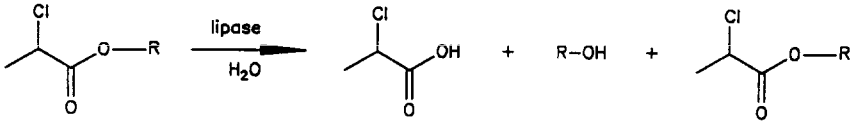
1. Selectivity Enhancement of Alcohol Resolution (Type I substrates)

As shown in scheme 1, diastereomeric esters of racemic alcohols (*RS*)-**2b** and (*RS*)-**3b** and (D)- or (L)-2-chloropropionic acid were enzymatically hydrolyzed using lipases from *Candida cylindracea* (CC) and *Pseudomonas fluorescens* (P) (see table 1). On the other hand the investigations were extended to transesterification reactions (see table 2), because by this technique different selectivities compared to hydrolytic conversions can be obtained.[21,22] This approach proved to be particularly useful in case of the resolution of menthol since the corresponding 2-chloropropanoates could not be hydrolyzed enzymatically at all.

As shown in table 1, both lipases CC and P showed a preference for the same alcohol enantiomer of **2a** and **3a** regardless of the chirality of the acid moiety present in the molecule. Almost identical values of E[23,25] for both diastereomeric octynyl esters **2a** were obtained. However, in case of the more rigid norbornyl esters **3a**, a marked influence of the chirality of the acid moiety on the enantioselection with respect to the alcoholic part was found, depending on the enzyme used: Using lipase CC (D,*RS*)-**3a** was resolved about twice as selectively as the corresponding (L,*RS*)-**3a** counterpart. With lipase P the same was true but for the opposite diastereomer: The enantiomeric ratio E for (L,*RS*)-**3a** was seven times higher than that for (D,*RS*)-**3a**.

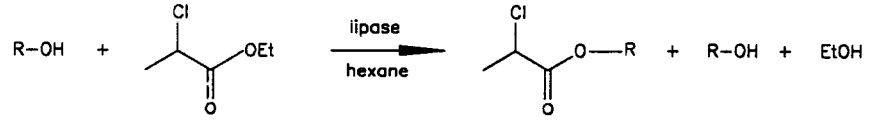
Scheme 1: Resolution of type I esters

Hydrolysis



(D,*RS*)-**2a**	(D)	(*S*)-**2b**	(D,*R*)-**2a**
(D,*RS*)-**3a**	(D)	(1*S*)-**3b**	(D,1*R*)-**3a**
(L,*RS*)-**2a**	(L)	(*S*)-**2b**	(L,*R*)-**2a**
(L,*RS*)-**3a**	(L)	(1*S*)-**3b**	(L,1*R*)-**3a**

Transesterification



(*RS*)-**1b**	(D)-**5**	(D,*S*)-**1a**	(*R*)-**1b**
(*RS*)-**4b**	(D)-**5**	(D,1*R*)-**4a**	(1*S*)-**4b**
(*RS*)-**1b**	(L)-**5**	(L,*R*)-**1a**	(*S*)-**1b**
(*RS*)-**4b**	(L)-**5**	(L,1*R*)-**4a**	(1*S*)-**4b**

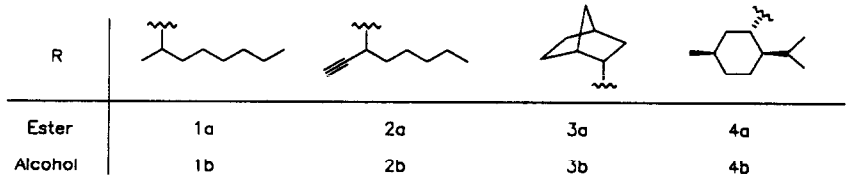


Table 1: Hydrolysis of esters **2a** and **3a** (see scheme 1)

Substrate	Lipase	Conversion [%]	Alcohol	e.e. [%]	Ester	d.e. [%]	E^{23}
(D,*RS*)-**2a**	CC	33	(*S*)-**2b**	24	(D,*R*)-**2a**	16	2
(L,*RS*)-**2a**	CC	40	(*S*)-**2b**	20	(L,*R*)-**2a**	4	1.5
(D,*RS*)-**2a**	P	42	(*S*)-**2b**	26	(D,*R*)-**2a**	18	2
(L,*RS*)-**2a**	P	38	(*S*)-**2b**	30	(L,*R*)-**2a**	8	2
(D,*RS*)-**3a**	CC	44	(1*S*)-**3b**	60	(D,1*R*)-**3a**	44	6
(L,*RS*)-**3a**	CC	35	(1*S*)-**3b**	46	(L,1*R*)-**3a**	17	3
(D,*RS*)-**3a**	P	47	(1*S*)-**3b**	48	(D,1*R*)-**3a**	50	5
(L,*RS*)-**3a**	P	43	(1*S*)-**3b**	94	(L,1*R*)-**3a**	58	35

In contrast to hydrolytic conversions, where the reverse reaction can be neglected, this is not the case in transesterifications. Here one has to determine the equilibrium constant K[24,25] to be able to calculate the enantiomeric ratio E. Whereas K is identical for *enantiomers*, the situation becomes more complex in the present case where two *diastereomeric* substrate molecules are involved. Thus, two different values for K should be determined. Furthermore, from preliminary experiments we have some evidence that ethyl (L)-2-chloropropanoate (L)-5 may act as an inhibitor on CC lipase. This makes it difficult to obtain accurate values of E. Nevertheless, from the results listed in table 2 one can easily conclude that in case of the more rigid substrate (*RS*)-**4b** a similar trend as in the hydrolytic reaction appears: Again with CC lipase the (D)-2-chloropropanoate (D)-5 gives better results than the corresponding (L)-derivative, leading to optically pure menthol.[27] Lipase P, on the other hand, was unable to catalyse the transesterification of menthol with neither (D)- nor (L)-5.

Table 2: Transesterification of alcohols **1b** and **4b** by CC lipase.[a]

Substrates		Conversion	Formed Ester	d.e.
Alcohol	Ester	[%]		[%]
(*RS*)-**1b**	(D)-5	46	(D,*S*)-**1a**	10
(*RS*)-**1b**	(L)-5	37	(L,*R*)-**1a**	24
(*RS*)-**4b**	(D)-5	33	(D,1*R*)-**4a**	100
(*RS*)-**4b**	(L)-5	21	(L,1*R*)-**4a**	90

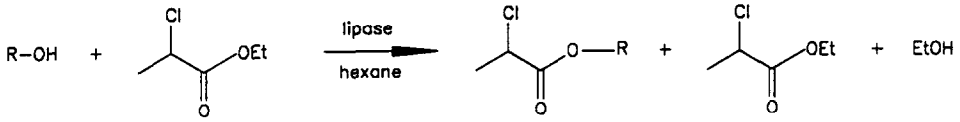
[a] Reactions were run at 40°C except for the first entry (20°C). The ratio of ester/alcohol for (*RS*)-**1b** and (*RS*)-**4b** was ca.1:1 and 2:1, resp., in order to obtain a reasonable speed of reaction.

Interestingly, in contrast to (*RS*)-**4b**, where regardless of the chirality of 5 always the (1*R*)-alcohol was selected, a change of preference for the alcohol enantiomer was observed in case of (*RS*)-**1b**: (*S*)-**1b** was transesterified with (D)-5 and the (*R*)-enantiomer of **1b** was selected with the (L)-ester. The same behaviour was observed for the reverse situation, *i.e.* the transesterification of ethyl (DL)-2-chloropropanoate with either (*R*)- or (*S*)-alcohol **1b** (see below).

2. Selectivity Enhancement of Acid Resolution (Type II substrates)

In order to evaluate, if a racemic ethyl carboxylate can be better resolved by using a chiral alcohol as transesterification partner, ethyl (DL)-2-chloropropanoate was subjected to transesterification with the pure enantiomers of alcohols **1b** and **4b**.

Scheme 2: Transesterification of esters of type II by CC lipase[a]



(*R*)-1b	(DL-5)	(L,*R*)-1a	(D)-5
(*S*)-1b		(D,*S*)-1a	(L)-5
(1*R*)-4b		(L,1*R*)-4a	(D)-5
(1*S*)-4b		(L,1*S*)-4a	(D)-5[b]

[a] For key to R see scheme 1. [b] Very slow reaction.

Table 3: Transesterification of ethyl (DL)-2-chloropropanoate by CC lipase[a].

Starting Alcohol	Conversion [%]	Formed Ester	d.e. [%]
(*R*)-1b	52	(DL,*R*)-1a	0
(*S*)-1b	40	(D,*S*)-1a	10
(1*R*)-4b	36	(L,1*R*)-4a	10
(1*S*)-4b	5[b]	(L,1*S*)-4a	10

[a] Temp. 20°C for 1b, 40°C for 4b, the ratio of ester/alcohol for 1b and 4b was ρ=1:1 and 2:1, resp. [b] Very slow reaction (~5% during 10 d).

As shown in table 3, no remarkable effect on the chiral recognition of the acid moiety was obtained with both enantiomers of (*R*)- and (*S*)-1b. The same results were obtained even with the more rigid alcohol (1*R*)-4b. As can be expected from the highly selective resolution of (±)-menthol (see table 2) the chirality of 4b had a strong impact on the reaction rate: Whereas the (1*R*)-4b enantiomer was converted at a reasonable rate, the (1*S*)-counterpart proved to be almost a non-substrate.

3. Selectivity Enhancement by Changing the Reactant Ratio

Due to the low selectivities obtained in the resolution of ethyl (DL)-2-chloropropanoate[26] (see table 3), the influence of the molar ratio (ρ) of 5 to chiral alcohols 1b and 4b on the sterical outcome of the reaction was investigated. An examination of the results listed in table 4 shows that the selectivity of the resolution of (DL)-5 with both alcohols 1b and 4b generally increased with higher concentrations of ethyl (D)- or (L)-2-chloropropanoate, in case of 4b even by a factor of about 4.

Table 4: Influence of reactant ratio on the stereoselectivity of the resolution of ethyl (DL)-2-chloropropanoate by CC lipase[a]

Alcohol	Reactant Ratio[b] (ρ)	Conversion [%]	Formed Ester	d.e. [%]
(*R*)-1b	0.1	37	(DL,*R*)-1a	0
	0.5	52	(DL,*R*)-1a	0
	10	37	(L,*R*)-1a	14
	20	30	(L,*R*)-1a	14
(*S*)-1b	1	40		10
	10	29	(D,*S*)-1a	24
	20	33		26
(1*R*)-4b	0.1	41		12
	1	36	(L,1*R*)-4a	10
	10	33		36
	20	34		40
(1*S*)-4b	2	5	(L,1*S*)-4a	10

[a] (1*S*)-4b was not further investigated due to a very slow reaction rate.
[b] ρ=(DL)-5/alcohol 1b or 4b, resp.

Going in line with the results depicted in table 2, reaction of the open-chain alcohols (*R*)-1b and (*S*)-1b with ethyl (DL)-2-chloropropanoate preferentially gave the *enantiomeric* esters (L,*R*)-1a and (D,*S*)-1a, resp., whereas in case of the more bulky cyclic alcohols (1*R*)-4b and (1*S*)-4b only the (1*R*)-enantiomer reacted at an appreciable rate. Here the *diastereomeric* esters (D,1*R*)-4a and (L,1*R*)-4a, resp., were formed.

Table 5: Influence of reactant ratio on the stereoselectivity of the resolution of (*RS*)-1b with ethyl (D)- or (L)-2-chloropropanoate

Alcohol	Starting Ester	Reactant Ratio[a] ρ	Conversion [%]	Formed Ester	d.e. [%]
(*RS*)-1b	(D)-5	1	46		10
		10	44	(D,*S*)-1a	14
		20	28		22
	(L)-5	1	37	(L,*R*)-1a	24
		10	22		36

[a] ρ=(DL)-5/alcohol 1b or 4b, resp.

The same trends were noticed in the corresponding *vice versa* experiments (resolution of alcohol (*RS*)-**1b**, see table 5): Increasing the reactant ratio had a positive effect on the enantioselectivity and again, preferably the (D,*S*)- and the (L,*R*)-diastereomers of ester **1a** were formed.
The mechanistic interpretation of these concentration-dependent phenomena is at present unclear. A change in enantioselectivity of CC lipase caused by addition of small lipophilic molecules such as alkaloids[10] or highly chlorinated hydrocarbons[11], acting as inhibitor or activator on the enzyme, has recently been reported from reactions run in aqueous systems. In both of these cases an allosteric binding of the inhibitor/activator followed by a configurational change of the enzyme molecule, thus changing its stereochemical behaviour, has been proposed as a rational model.
From our results it seems possible that analogous effects can be encountered also in reactions performed in organic solvents at an extremely low water content. A detailed investigation on this matter is in progress.

CONCLUSIONS

In hydrolytic reactions, proper choice of an additional center of chirality present in the acid part of an ester could improve the selectivity of enzymatic resolution of a racemic alcohol. On the other hand, the resolution of a racemic acid by selecting the chirality of the alcoholic part did not improve the selectivity significantly. This phenomenon is not only found in hydrolysis but also in transesterification: By the techniques described optically pure menthol was obtained from the racemate. In case of 2-octanol (**1b**) a reversal of stereochemical preference for one enantiomer was observed depending on the chirality of the acid part used. This was true for both types of resolution, *i.e.* reaction of racemic **1b** with ethyl (D)- or (L)-2-chloropropanoate or reaction of ethyl (DL)-2-chloropropanoate with optically pure alcohol **1b**.
In all of these reactions the e.e. (or the d.e., resp.) can be conveniently be measured due to the occurrence of diastereomeric substrates and/or products. From the results obtained, it seems obvious that lipases from *Candida cylindracea* (CC) and *Pseudomonas fluorescens* (P) both were able to "recognize" a chirality of an alcohol moiety rather than that of an acid.

EXPERIMENTAL

General

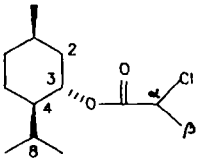
Preparative column chromatography was performed on silica gel 60 (230-400 mesh, Merck). For TLC Merck silica gel 60 F_{254} plates were used. Compounds were visualized by spraying with vanilline/conc. H_2SO_4 and heat treatment. GLC analyses were performed on a Dani 8500 chromatograph (J&W capillary column DB 1701, 30m x 0.25mm, 0.25μm film, N_2) equipped with FID. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker MSL 300 (300 and 75.5MHz, resp.) in $CDCl_3$. Chemical shifts are reported from TMS as internal standard in ppm (δ-scale) and coupling constants (J) in Hz. s=Singlet, d=doublet, t=triplet, q=quartet and m=multiplet. Elemental analyses (C, H, Cl) of all novel compounds were within 0.5% of calculated values. All commercially obtained compounds were used as received and crude enzyme preparations were employed without further purification. The following abbreviations () for enzymes were used: *Pseudomonas sp.* lipase, Amano P (P) and *Candida cylindracea* lipase, Sigma type VII (CC).

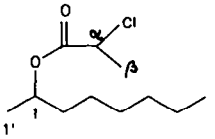
GLC Measurements:

The e.e. of alcohols **2b** and **3b** was determined *via* their corresponding mixed carbonates after derivatisation with (-)-menthyl chloroformate[28]. (*RS*)-**2b**-derivatives: α=1.009, (*RS*)-**3b**-derivatives: α=1.004. Diastereomeric esters **2a** and **3a** could directly be separated (**2a**: α=1.013, **3a**: α=1.004).

NMR Measurements:

The d.e. of esters **1a** and **4a** was determined by ^{13}C-NMR using well splitted signals[29].





C-atom	Shift [ppm]	Diastereomer	C-atom	Shift [ppm]	Diastereomer
δ-C*	21.30	(D,*S*) (L,*R*)	C-1'*	19.71	(D,*R*) (L,*S*)
	21.40	(D,*R*) (L,*S*)		19.64	(D,*S*) (L,*R*)
C-8	26.12	(D,*S*) (L,*R*)	δ-C*	21.50	(D,*S*) (L,*R*)
	26.21	(D,*R*) (L,*S*)		21.40	(D,*R*) (L,*S*)
C-2	40.37	(D,*R*) (L,*S*)	α-C*	52.88	(D,*S*) (L,*R*)
	40.51	(D,*S*) (L,*R*)		52.77	(D,*R*) (L,*S*)
C-4	46.92	(D,*R*) (L,*S*)	C-1	73.00	(D,*S*) (L,*R*)
	47.02	(D,*S*) (L,*R*)		72.98	(D,*R*) (L,*S*)
α-C*	52.64	(D,*S*) (L,*R*)			
	52.78	(D,*R*) (L,*S*)			
C-3	79.90	no sepn.			

* Signals used for quantitative measurements.

The mean value of the area from the indicated signals was taken as the most accurate measurement of d.e. Assignement of each signal was made by comparison with authentic samples. The standard conditions used were: Offset 1600 Hz, decoupler offset 5000 Hz, delay 5 sec, 10^3-10^4 scans. The e.e. of **4b** was determined by ^{1}H-NMR spectroscopy using 0.25 eq. of Tris[3-(heptafluoropropyl-hydroxymethylene)-(+)-camphorato] Eu(III). The proton resonance at C-3 was measured while decoupling both protons at C-2 with a power of 4L. Absolute configurations were determined by comparison with literature data: **2b**[30], **3b**[2].

Synthesis of 2-Chloropropanoates

Ethyl (*R*)-2-chloropropanoate (e.e. 98%) and isobutyl (*S*)-2-chloropropanoate (e.e. 99%) were hydrolyzed (1*N* NaOH) to give the corresponding Na salts which in turn were converted to their acid chlorides by $SOCl_2$ treatment[31]. Esters **1a-4a** were prepared by acylation of alcohols **1b-4b** in anhydrous pyridine with yields ranging from 85-90%. No detectable amount of racemisation was observed during this procedure as proved by NMR spectroscopy.

*(R,S)-1-Methylheptyl (D,L)-2-chloropropanoate (**1a**)*: Bp. 74°C/0.01 mbar. For ^{13}C NMR data see above.

*(R,S)-1-Pentylpropynyl (D,L)-2-chloropropanoate (**2a**)*: Bp. 79°C/0.01 mbar. ^{1}H-NMR: 0.90 (t, J=7, 3H, H on C-5'), 1.05-1.50 (m, 6H, H on C-2', C-3', C-4'), 1.70 (d, J=7.5, 3H, δ-CH_3), 1.92 (m, 2H, H on C-1'), 2.51 (d, J=1.5, 1H, H on C-3), 4.41 (q, J=7.5, 1H, α-CH), 5.39 (m, 1H, H on C-1).

*(1RS,2RS,4RS)-Bicyclo[2.2.1]hept-2-yl (D,L)-2-chloropropanoate (**3a**)*: Bp. 70°C/0.03 mbar. ^{1}H-NMR: 0.68 (m, 1H, *endo*-H on C-3), 0.83 (m, 1H, *exo*-H on C-3), 1-05-1.65 (m, 6H, H on C-5, C-6, C-7), 1.35 (d, J=7.5, 3H, δ-CH_3), 1.80 (m, 1H, H on C-4), 2.01 (m, 1H, H on C-1), 3.50 (q, J=7.5, 1H, α-CH), 3.99 (m, 1H, H on C-2).

*(1RS,2SR,5RS)-menthyl (D,L)-2-chloropropanoate (**4a**)*: Bp. 64°C/0.01 mbar; lit. 32: bp. 111-113°C/4 Torr. For ^{13}C-NMR data see above.

Enzymatic Experiments
Hydrolytic experiments were performed with 1 mmol of ester, 20mg lipase CC (or 100mg lipase P, resp.) in phosphate buffer (0.1M, pH 7.5, 30ml). After an appropriate degree of conversion was reached (monitored by GLC) products were extracted with CH_2Cl_2 and separated by column chromatography. The overall yield of this process was 80-90%.
Transesterifications were done with 1 mmol of alcohol, a variable amount of cosubstrate ester (see general part for reactant ratio ρ), 1g of lipase CC in hexane (10ml). After the reactions were terminated by filtration of the enzyme, products were separated by column chromatography. Overall yields ranged from 85-95%.

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REFERENCES AND NOTES

1 For a recent review see: Chen C.-S.; Sih C.J. *Angew.Chem.Int.Ed.Engl.* **1989**, *28*, 695-707.
2 Oberhauser Th.; Bodenteich M.; Faber K.; Penn G.; Griengl H. *Tetrahedron* **1987**, *43*, 3931-3944.
3 Klempier N.; Faber K.; Griengl H. *Synthesis* **1989**, 933-934.
4 Dordick J.S. *Enzyme Microb.Technol.* **1989**, *11*, 194-211.
5 Laane C.; Tramper J.; Lilly M.D. (Eds.) *Studies in Organic Chemistry, vol. 29*, Elsevier, Amsterdam **1987**.
6 Klibanov A.M. *Chemtech* **1986**, *16*, 354-359.
7 Degueil-Castaing M.; De Jeso B.; Drouillard S.; Maillard B. *Tetrahedron Lett.* **1987**, 953-954.
8 Bianchi D.; Cesti P.; Battistel E. *J.Org.Chem.* **1988**, *53*, 5531-5534.
9 Ghogare A.; Kumar G.S. *J.Chem.Soc., Chem.Commun.* **1989**, 1533-1534.
10 Guo Z.-W.; Sih C.J. *J.Am.Chem.Soc.* **1989**, *111*, 6836-6841.
11 Dahod S.K.; Siuta-Mangano P. *Biotechnol.Bioeng.* **1987**, *30*, 995-999.
12 Jones J.B. *Tetrahedron* **1986**, *42*, 3351-3404.
13 Crout D.H.G.; Christen M. in: *Modern Synthetic Methods, vol. 5*, p. 1-114, Scheffold R. (Ed.), Springer, Berlin **1989**.
14 Davies H.G.; Green R.H.; Kelly D.R.; Roberts S.M. *Biotransformations in Preparative Organic Chemistry*, Academic Press, London **1989**.
15 Klempier N.; Faber K.; Griengl H. *Biotechnol.Lett.* **1989**, 685-688.
16 Oberhauser Th.; Faber K.; Griengl H. *Tetrahedron* **1989**, *45*, 1679-1682.
17 Fodor P.J.; Price V.E.; Greenstein J.P. *J.Biol.Chem.* **1950**, *182*, 476-470.
18 Halpern B.; Ricks J.; Westley J.W. *Aust.J.Chem.* **1967**, *20*, 389-394.
19 Lin Y.Y.; Palmer D.N.; Jones J.B. *Can.J.Chem.* **1974**, *52*, 469-476.
20 Although we are aware that different systems of nomenclature should not be mixed, for the sake of simplicity the chirality of the acid and alcohol moiety is designated as (D,L) and (*R*,*S*), respectively, throughout this paper.
21 Margolin A.L.; Tai D.-F.; Klibanov A.M. *J.Am.Chem.Soc.* **1987**, *109*, 7885-7887.
22 Langrand G.; Baratti J.; Buono G.; Triantaphylides C. *Tetrahedron Lett.* **1986**, 29-32.
23 Chen C.-S.; Fujimoto Y.; Girdaukas G.; Sih C.J. *J.Am.Chem.Soc.* **1982**, *104*, 7294-7299.
24 Chen C.-S.; Wu S.-H.; Girdaukas G.; Sih C.J. *J.Am.Chem.Soc.* **1987**, *109*, 2812-2817.
25 Irreversible reaction[23]:

$$E = \frac{\ln[(1-c)(1-ee_s)]}{\ln[(1-c)(1+ee_s)]} = \frac{\ln[1-c(1+ee_p)]}{\ln[1-c(1-ee_p)]}$$

Reversible reaction[24]:

$$E = \frac{\ln[1-(1+K)(c+ee_s\{1-c\})]}{\ln[1-(1+K)(c-ee_s\{1-c\})]} = \frac{\ln[1-(1+K)c(1+ee_p)]}{\ln[1-(1+K)c(1-ee_p)]}$$

E = enantiomeric ratio; c = conversion; ee_s and ee_p = e.e. of substrate and product, resp.; K = equilibrium constant.

26 For lipase catalyzed resolution of *enantiomeric* 2-chloropropanoates see ref. 11 and: Kirchner G.; Scollar M.P.; Klibanov A.M. *J.Am.Chem. Soc.* **1985**, *107*, 7072-7076; Cambou B.; Klibanov A.M. *Appl. Biochem. Biotechnol.* **1984**, *9*, 255-260.
27 For biocatalytic resolution of menthol see refs. 22, 24 and: Brookes I.K.; Lilly M.D.; Drozd J.W. *Enzyme Microb.Technol.* **1987**, *9*, 217-220; Omata T.; Iwamoto N.; Kimura T.; Tanaka A.; Fukui S. *Eur.J.Appl. Microbiol.Biotechnol.* **1981**, *11*, 199-204.
28 Westley J.W.; Halpern B. *J.Org.Chem.* **1968**, *33*, 3978-3980.
29 Kruizinga W.H. *et al.* *J.Org.Chem.* **1988**, *53*, 1826-1827.
30 Oritami T.; Yamashita K. *Agric.Biol.Chem.* **1980**, *44*, 2407-2411.
31 Fu S.-C.J.; Birnbaum S.M.; Greenstein J.P. *J.Am.Chem.Soc.* **1954**, *76*, 6054-6058.
32 Sugita T.; Nishio N.; Ichikawa K. *Bull.Chem.Soc.Jpn.* **1982**, *55*, 1677-1678.