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# Resolution of a tetrahydrofuran ester by *Candida rugosa* lipase (CRL) and an examination of CRL's stereochemical preference in organic media

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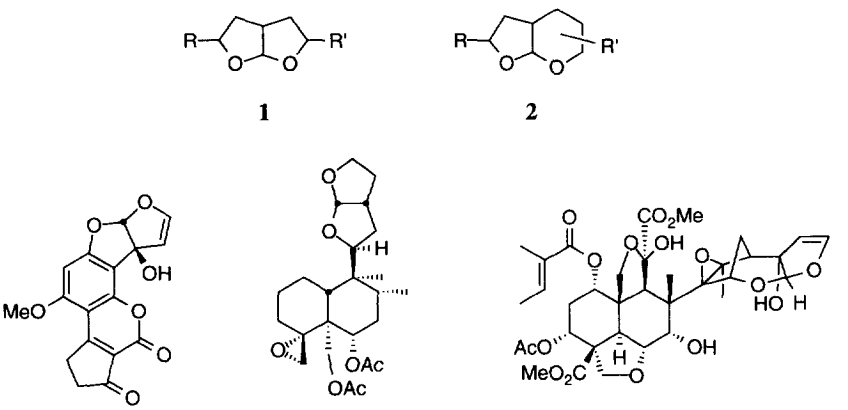
**Abstract:** Crude lipase from *Candida rugosa* (CRL) is able to resolve the C3-stereoisomers of the furo[2,3b]furan building block methyl 2-methoxytetrahydrofuran-3-carboxylate **6** by alcoholysis using *n*-butanol in octane. The reaction is not affected by the configuration at C2. The absolute configuration of the product **7** is 3S as determined by X-ray analysis of the crystalline derivative **14**. The stereochemical outcome of the reaction is compared to the active site model derived by the group of Kazlauskas (Ahmed *et al., Biocatalysis* **9** (1994), 209). Evidence is presented for the validity of this model for CRL-catalyzed alcoholysis, esterification and acidolysis reactions in organic media.

## Introduction

Bicyclic ketals are important structural elements in several classes of natural compounds. For example, furo[2,3b]furans **1** (see Scheme 1) or -pyrans **2** are present in aflatoxins[1] **3** and in natural insect antifeedants like dihydroclerodin[2] **4** and azadirachtin[3] **5**. They are at least partially responsible for the biological effects of the latter compounds since even simple furo[2,3b]furans like **1** (R = OH, R' = alkyl or aryl) appear to have some antifeedant activity[4].

In our research towards the total synthesis of clerodanes[5-7] we are interested in the synthesis of enantiomerically pure furo[2,3b]furan derivatives. For this purpose, enzyme-catalysed kinetic resolution of methyl 2-methoxytetrahydrofuran-3-carboxylate **6** (see Scheme 2) seems appropriate since **6** is a known building block for furo[2,3b]furans[5] and successful enzyme-mediated resolution of tetrahydrofuran derivatives has been reported before[8]. In a preliminary communication[9] we have already shown that the lipase of *Candida rugosa*[10] (CRL) is able to resolve **6** into two pairs of stereoisomers. We here wish to report on the absolute configuration of the products obtained and on the predictability of CRL-catalyzed alcoholysis, acidolysis and esterification reactions in organic solvents.

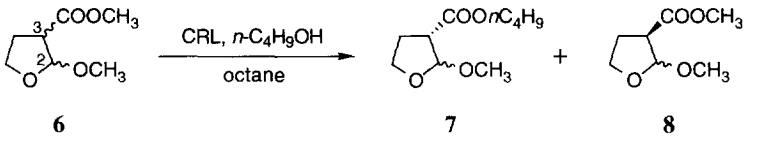
‡ Address corresponce pertaining to crystallographic studies to this author.




Scheme 1: General structure of furofurans and -pyrans and some biologically active representatives.

## Results and discussion

Although **6** contains two stereogenic centres, only the configuration at C3 is relevant for the preparation of optically active furo[2,3b]furans[5]. Therefore, for the resolution of **6** an enzyme is needed which is able to distinguish between the 3R- and 3S-isomers of **6** without being affected by the stereochemistry at C2. Since the substituent at C3 is an ester group, it is logical to screen hydrolases for the enzymatic resolution, because they have shown to be very stereoselective enzymes[11].




Scheme 2: Lipase-catalysed kinetic resolution of **6**.

Of the tested hydrolases, *Candida rugosa* lipase (CRL; EC 3.1.1.3) was the one that gave the desired selectivity[12]. The resolution is carried out in an organic solvent because CRL-mediated hydrolysis of **6** in water gave serious problems during workup[8a,9]. Octane is the preferred solvent for this reaction since CRL works best in apolar solvents with high logP values[13]. When incubated with **6** and *n*-butanol in octane, CRL converts **6** into a mixture of the cis-and trans-butylesters **7** and the unconverted methyl esters **8** without noticeable inactivation of the enzyme[9] (see Scheme 2). The product and the remaining substrate can be separated by preparative gas chromatography and in this way 7 g of pure **7** can be obtained in one working day. The enantiomeric ratio[14] of this reaction is well above 100 allowing the isolation of **7** with >98% e.e. at 45% conversion (see Figure 1).

The determination of the absolute configuration of the formed product appeared to be rather difficult. The specific rotation of **7** or its derivatives are not known, so its molecular structure has to be established by X-ray crystallography. Many derivatives of **7** have been made, some of them are depicted in Scheme 3.

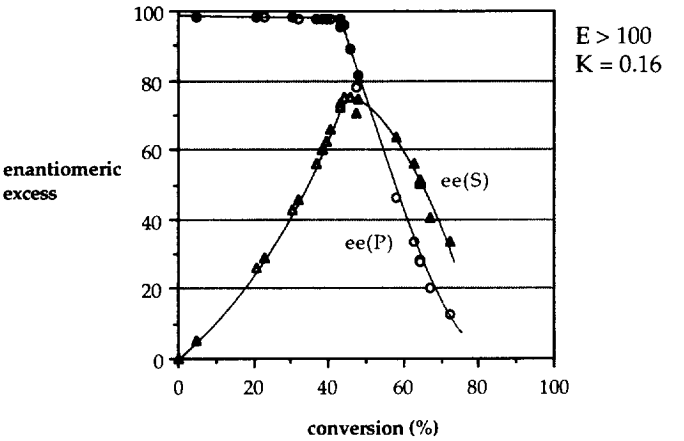
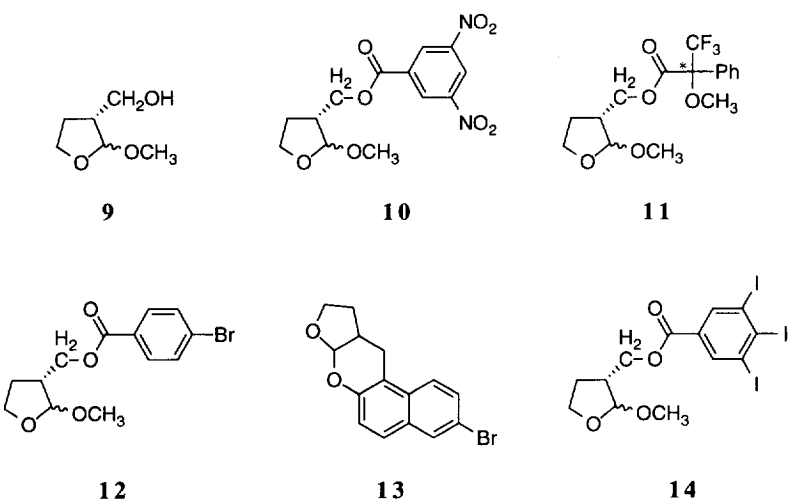



Figure 1. Course of the enantiomeric excess during the alcoholysis of **6** by CRL in octane[15]. Δ: remaining substrate **8**; ▲: duplicate reaction; o: enzymatic product **7**; •: duplicate reaction. See Experimental section for details.




Scheme 3. Derivatives of **7** used in the determination of its absolute configuration.

Reduction of **7** with $LiAlH_4$ gave **9** in good yield as an oil. Esterification of **9** with 3,5-dinitrobenzoyl chloride afforded **10**; its cis-isomer is an oil but the trans-isomer gave beautiful needles. Unfortunately, these crystals were too small for X-ray diffraction. Coupling of **9** with the R- or S- acid chloride of Mosher's acid gave **11**. All isomers of **11** are deliquescent solids unsuitable for X-ray analysis; attempts to establish the absolute configuration of C3 by NOE-difference NMR failed. The cis-isomer of ester **12** is an oil as well, whereas crystals of trans-**12** always contained a substantial amount of its enantiomer. Using a totally different approach,

treatment of **9** with 6-bromo-2-naphthol in $CHCl_3$ with 1.75 eq. of trifluoracetic acid afforded the tetracyclic compound **13**. Unfortunately, **13** was isolated as a racemate. Esterification of **9** with 3,4,5-triiodobenzoyl chloride gave, at last, the desired large crystals of a heavy atom-containing derivative **14**. The trans-isomer of compound **14** was used for X-ray analysis.

The crystal structure of **14** contains two crystallographically independent molecules, both displaying the R configuration at position 3 [atom C(9)] of the furan ring. The independent molecules differ in the conformation of the torsion angle C(7)-O(2)-C(8)-C(9), which amounts to a value of -179.0(7)° and -137.8(8)° for residues 1 and 2, respectively. The furan rings of both molecules have adopted a distorted envelope conformation, with C(12) out of plane with the other ring atoms.

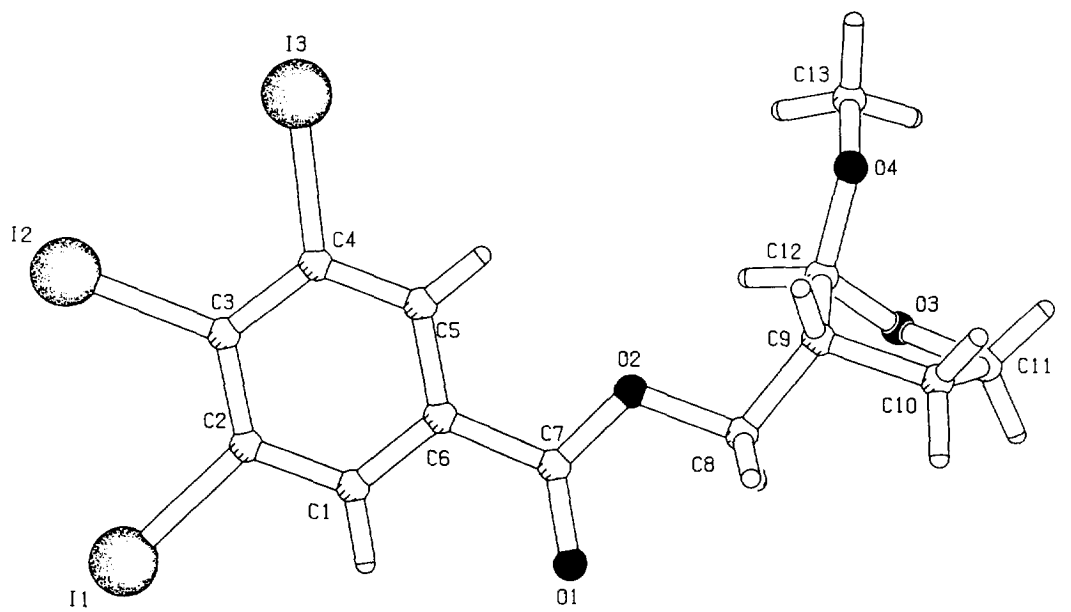
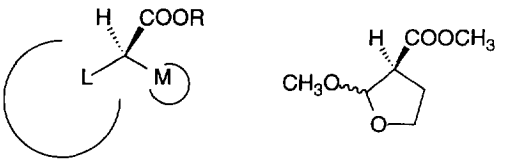



Figure 2. PLUTON[16] plot of **14** with the adopted numbering scheme for residue 1.

The observed selectivity of CRL for **6'** is fully compatible with the predictive rule obtained for CRL-catalyzed hydrolyses of esters derived from chiral acids as was developed by the group of Kazlauskas[17] (see Scheme 4). The rule predicts that stereoselection by CRL is fully controlled by steric hindrance. The large substituent (L) is placed in a tunnel near the active site, whereas the smaller substituent (M) is placed in a cavity confined by amino acid residues Phe 345 and Phe 415.




**6'**

Scheme 4. Preferred enantiomer in CRL-mediated hydrolysis reactions of chiral esters[17] (L = large group, M = medium-sized group), and the preferred stereo-isomer in the current studies **6'**.

Kazlauskas' rule is based on hydrolysis reactions performed by the authors themselves or taken from literature, in connection with X-ray data of CRL. The authors confined themselves to hydrolysis reactions, because in that case the acylation step (equation 1, see below) is practically irreversible: the very large excess of water makes step 2 ($R_3$ = H) very fast. Therefore, in hydrolysis reactions the stereoselection takes place in the acylation step (equation 1; E-OH = enzyme).

$$R_1{*}COOR_2 + \text{E-OH} \overset{k_1}{\rightleftharpoons} R_1{*}COO\text{-E} + R_2OH \qquad (1)$$

$$R_1{*}COO\text{-E} + R_3\text{-OH} \overset{k_2}{\rightleftharpoons} R_1{*}COOR_3 + \text{E-OH} \qquad (2)$$

However, in the initial stages of alcoholysis ($R_2$, $R_3$ = alkyl, $R_2 \neq R_3$) and esterification ($R_2$ = H, $R_3$ = alkyl) and in those cases where pseudo-first order conditions are used (*i.e.* $[R_3\text{-OH}] >> [R_1{*}COOR_2]$) there is also a large excess of nucleophile $R_3$-OH leading to formation of product, just as in water. So, in those cases the (initially) formed product also reflects the stereoselectivity of the acylation step. Of course, this is only true when $R_3$-OH is a good substrate for the enzyme (*i.e.*, $k_2 >> k_1$). For example, for sterically hindered alcohols the deacylation step might be rate-limiting leading to two stereoselective steps in enzyme catalysis which may have totally different selectivities[18]. In all cases, when alcoholysis or esterification reactions are run almost to completion, an equilibrium is reached with low e.e. of the remaining substrate[14]. Monitoring the e.e. vs. conversion, as shown in Figure 1, will indicate when the reaction has to be stopped for optimum e.e. of substrate and product. For those optimised conditions, it is reasonable to assume that Kazlauskas' rule is applicable in CRL-catalyzed alcoholysis or esterification reactions using simple, straight-chain alcohols like *n*-butanol as substrates.

Originally, the predictive rule for the enantioselectivity of CRL toward chiral carboxylic acid esters was developed for highly purified enzyme[17]. Very recently, Kazlauskas' group showed that the rule was also valid for crude CRL, pretreated with 2-propanol[19]. The authors explained this by assuming that 2-propanol opens the polypeptide lid which covers the active site of the enzyme in crude CRL. The "open form" has a higher activity and strongly improved enantioselectivity. Another way to open the lid is through interfacial activation, *i.e.* adsorption of the enzyme to an apolar interface[20]. This can be done in several ways, *e.g.* by suspending solid lipases in organic solvents, since lipases have shown to be very active under those conditions[21].

Table 1. Comparison of Kazlauskas' rule for hydrolysis reactions with purified or 2-propanol-treated crude CRL, to alcoholysis, acidolysis or esterification reactions using crude CRL suspended in organic media.

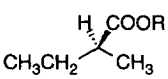
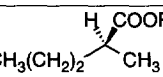
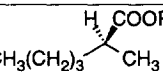
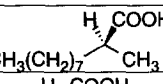
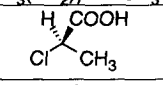
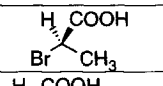
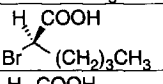
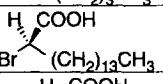
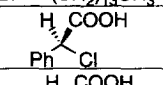
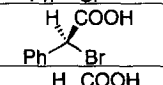
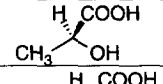
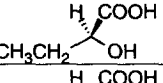
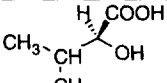
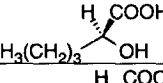
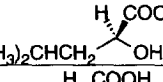
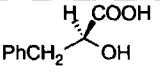
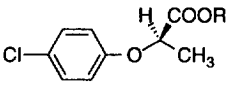
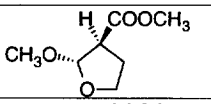
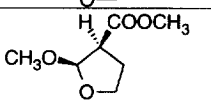
| Entry[a] | preferred substrate isomer | reaction type[b] | nucleophile | fit[c] | reference |
|---|---|---|---|---|---|
| **15a** (R=H) |  | est | 1-heptanol | | 22 |
| **15a** (R=H) | | est | d | + | 23 |
| **15b** (R=alkyl[e]) | | acid | e | | 24 |
| **16a** (R=H) |  | est | d | + | 23 |
| **16b** (R=alkyl[e]) | | acid | e | | 24 |
| **17a** (R=H) |  | est | d | + | 23 |
| **17b** (R=alkyl[e]) | | acid | e | | 24 |
| **18** |  | est | 1-heptanol | + | 22 |
| **19** |  | est | *n*-BuOH | ± | 23,24 |
| | | est | various[f] | | 18 |
| **20** |  | est | *n*-BuOH | + | 24,27 |
| **21** |  | est | *n*-BuOH | - | 25 |
| **22** |  | est | *n*-BuOH | - | 25 |
| **23** |  | est | *n*-BuOH | + | 25g |
| **24** |  | est | *n*-BuOH | + | 25g |
| **25** |  | est | *n*-BuOH[h] | + | 28,29 |
| **26** |  | est | *n*-BuOH | + | 28,29 |
| **27** |  | est | *n*-BuOH | + | 28 |
| **28** |  | est | *n*-BuOH | + | 28,29 |
| **29** |  | est | *n*-BuOH | + | 28 |
| **30** |  | est | *n*-BuOH | + | 28 |

Table 1 (continued)

| Entry[a] | preferred substrate isomer | reaction type[b] | nucleophile | fit[c] | reference |
|---|---|---|---|---|---|
| **31a** (R=H) |  | est | *n*-BuOH | + | 14,30 |
| **31a** (R=H) | | est | MeOH, *n*-BuOH | | 24 |
| **31a** (R=H) | | est | 1-tetradecanol[i] | | 31 |
| **31a** (R=H) | | est | 1-tetradecanol | | 32 |
| **31a** (R=H) | | est | **32** (see below) | | 33 |
| **31b** ($R=CH_3$) | | alc | *n*-BuOH | | 34 |
| **6a** |  | alc | EtOH, *n*BuOH | + | this work |
| **6b** |  | alc | EtOH, *n*BuOH | + | this work |

[a] Compounds refer to racemates; the stereo-isomer which preferably reacts with CRL is depicted in the next column

[b] type of reaction: est = esterification, alc = alcoholysis, acid = acidolysis

[c] + means: fits into the predictive rule, - means: does not fit, ± means: both substituents are about equally large

[d] various alcohols tested: methanol, ethanol, *n*-butanol, *n*-octanol, *n*-decanol, *n*-octadecanol, R- and S-methyl-2-octanol, 3-methyl-2-buten-1-ol, cyclohexanol, cyclohexylmethanol; the alcohol has a dramatic effect on the E-value for **16a** and **17a** without affecting the stereochemical preference of the enzyme.

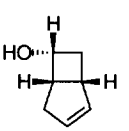
[e] 2-methylalkanoic esters of various alcohols (ranging from ethanol to *n*-octadecanol) were subjected to acidolysis with various carboxylic acids; the structure of the acid substrate was of minor importance.

[f] various alcohols tested: *n*-heptanol, 2-butanol, 2-octanol, cyclohexylmethanol, benzyl alcohol; the R-acid was the preferred substrate in reactions with these alcohols whereas the S-acid was preferred in case of 3-methyl-2-butanol, 3-methyl-2-pentanol, 1-cyclohexylethanol and 1-phenylethanol.

[g] assignment of the absolute configuration in original article is corrected according to ref. 17.

[h] various alcohols tested, ranging from ethanol to *n*-hexanol, with limited effects on reaction rate and E-value

[i] various alcohols tested, ranging from methanol to octadecanol; tetradecanol gave the highest initial rate



**32**

Considering the facts described in the two paragraphs above, it is quite likely that Kazlauskas' model for hydrolysis reactions using purified or crude 2-propanol-treated CRL is also valid for alcoholysis or esterification reactions using straight-chain alcohols and crude CRL in organic media. This hypothesis, and the fact that our results of the resolution of **6** perfectly fit into this model, induced us to see whether additional proof can be obtained from the literature.

In Table 1 are depicted all esterification, alcoholysis and acidolysis reactions of chiral carboxylic acids (-derivatives) using CRL that are known to us. Only reactions with E>2 have been included. Nineteen different substructures are listed, containing 29 different derivatives and/or reaction conditions. The substrates are straight-chain or cyclic acids (or -derivatives); they contained both polar and apolar α-substituents and both aromatic and straight-chain aliphatic α-substituents. Only two compounds of this list (**21** and **22**) do not fit in the rule as developed by Kazlauskas *et al.* The fact that 82% of the substrates, which have a large variety in structure, fit into the rule indicates that the rule has predictive value both in hydrolytic and in nonhydrolytic reactions.

The background of the anomalous behaviour of CRL towards **21** and **22**[25] is puzzling. There is a claim that CRL has a special site for electronegative substituents[18], but this is not in line with CRL's stereochemical preference for other halogenated substrates like **23** and **24** and the α-hydroxy acids **25-30**. The presence of contaminating enzymes in the crude CRL preparation might be an explanation, but their importance has been questioned[19]. An incorrect assignment of the absolute configuration of **21** and **22** cannot be excluded, considering the difficulties observed before in structure elucidation. *E.g.*, the initially determined absolute configurations of **23** and **24**[25] appeared to be wrong[17]. The same is most probably the case with the "chiral switch" as claimed[29] for the esterification of α-hydroxy acids with more than 6 carbon atoms[35].

The fact that Kazlauskas' rule is valid for nonhydrolytic reactions in organic solvents is not surprising, since the rule is supported by the X-ray crystal structure of the enzyme containing a transition-state analogue[17] and it has been shown before that the conformation of the active site of enzymes remain intact in organic solvents[38-40]. Remarkably, CRL is also able to resolve 3-hydroxybutyric acid (e.e.>97% at 42% conversion), which is a substrate in which the stereogenic centre is β to the carboxyl group[41]. Kazlauskas' rule is, of course, not applicable in this case but it would be interesting to study whether a similar rule can be constructed for compounds like this.

## Conclusions

1) Crude lipase from *Candida rugosa* (CRL) is able to resolve the C3-stereoisomers of methyl 2-methoxy-tetrahydrofuran-3-carboxylate **6** by alcoholysis using *n*-butanol in octane, giving an E value well above 100 (K = 0.20);
2) The selectivity of the enzymatic reaction is not affected by the configuration at C2;
3) The absolute configuration of the product of the enzymatic reaction is 3S as determined by X-ray analysis of the crystalline derivative **14**.

## Experimental

All melting points are uncorrected. $^1$H NMR and $^{13}$C NMR spectra were determined on a Bruker AC-E 200 and on a Bruker AMX 500 machine. Chemical shifts are reported in ppm downfield relative to tetramethylsilane in $CDCl_3$ solutions. As chemical shift reagent tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato] europium(III) derivative was used. Mass spectral data and HRMS measurements were obtained on a AEI MS 902 spectrometer and on a Finnegan Mat 95. Elemental analysis were carried out using a Carlo Erba Elemental Analyser 1106. IR spectra were carried out on a PU-9706 spectrophotometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter in chloroform as the solvent with the concentrations denoted in g/100 ml.

GC analyses were carried out on a Varian 3700 and a Chrompack CP9000 chromatograph provided with respectively a 2 m packed ss. column filled with 8.9 g chromosorb 80-100 + 19% OV-17 and a 2 m glass column filled with 10.0 g chromosorb 100-120 + 8.9% carbowax high polymer. Preparative gas chromatography was carried out with a Becker Research chromatograph type 3810, provided with a 2 m column filled with 34.8 g W-AW chromosorb 40/60 + 30% OV-17. The enantiomeric excess (e.e.) was measured with a Carlo Erba or a Varian 3700 gas chromatograph provided with a HP 3396A integrator and a 50 m capillary WCOT Fused Silica column filled with SP-Cyclodextrine B-236-M-19, film thickness: 0.25 μm, oven temperature 95 °C, FID, $N_2$ as carrier gas. Mosher's chloride was obtained from Acros and Sigma. The petroleum ether (PE) used for column chromatography had a boiling range of 40-60 °C.

*Determination of the E-value of the alcoholysis of* ***6***: In a 4 ml screw vial (Chrompack) was placed 1 ml of a solution containing 100 mM of **6**, 500 mM of ethanol and 240 mM of 4-cymene (internal standard) in *n*-octane (dried on 3 Å molecular sieves). In case of *n*-butanol as the nucleophile, 200 mM of **6** and 500 mM of *n*-butanol were used. The vial was equilibrated at 45 °C in an incubator (New Brunswick Scientific G24 Environmental Incubator Shaker), after which 100 mg of *Candida rugosa* lipase (Sigma type VII) was added. After 20 s sonication (Sonicor, with water bath) the vial was placed in the incubator again and stirred at 45 °C, 350 rpm. 0.5 μl samples were taken every 30-50 min and analysed on GC. The equilibrium constant (K) of the reaction[14] was determined by allowing the reaction to run to completion (24 h). Blank reactions without the enzyme showed no conversion of substrate.

n-*Butyl 2(*R,S*)-methoxytetrahydrofuran-3*S*-carboxylate* **7**: To a solution of 4.09 g (0.031 mol) of **6** (synthesized as described before[5]) in 150 ml of octane was added 5.77 g (0.078 mol) of *n*-butanol and 12 g of CRL. The reaction mixture was placed in an 300 ml erlenmeyer flask in the incubator and stirred at a temperature of 45 °C at 200 rpm. After the reaction had proceeded for 30%, the reaction was stopped by filtration of the enzyme and the solvent was partly evaporated *in vacuo*, yielding a mixture of the methyl- and butylester, which was separated by preparative gas chromatography. Yield of the butylester was 1.42 g (0.007 mol, 23%) with an e.e. of 98%. For analytical purposes the cis- and trans isomers were separated by preparative gas chromatography. $^1$H-NMR (cis-2R,3S-**7**): δ 0.92 (t, 3H); 1.28-1.43 (m, 2H); 1.54-1.65 (m, 2H); 1.98-2.11 and 2.41-2.51 (m, 2H); 3.08 (dt, 1H); 3.31 (s, 3H); 3.86-4.25 (m, 4H); 5.10 (d, J= 5.2 Hz, 1H). $^{13}$C-NMR: δ 13.6 (q); 19.0 (t); 24.7 (t); 30.6 (t); 49.6 (d); 54.8 (q); 64.5 (t); 66.9 (t); 103.7 (d); 169.9 (s). HRMS: theor. ($M^+$-31): 171.1021, found: 171.1022. $[\alpha]_D^{20}$ = -96.1 (c = 0.5; still contains 5% of the trans-isomer). $^1$H-NMR (trans-2S,3S-**7**): δ 0.92 (t, 3H); 1.31-1.42 (m, 2H); 1.54-1.65 (m, 2H); 2.14-2.24 (m, 2H); 3.00 (double t, 1H); 3.34 (s, 3H); 3.84-4.07 (m, 2H); 4.10 (t, 2H); 5.17 (d, J= 1.6 Hz, 1H). $^{13}$C-NMR: δ 13.7 (q); 19.1 (t); 27.3 (t); 30.5 (t); 51.0 (d); 54.8 (q); 64.9 (t); 66.9 (t); 106.5 (d); 172.2 (s). HRMS: theor. ($M^+$-31): 171.1021, found 171.1021. $[\alpha]_D^{20}$ = + 70.4 (c = 1.4). Anal: calcd for $C_{10}H_{18}O_4$: C, 59.38; H, 8.97; found: C, 59.15; H, 9.14.

*2(*R,S*)-methoxytetrahydrofuran-3*R*-ylmethanol* **9**: 1.9 g (0.050 mol) of $LiAlH_4$ was weighed under a stream of nitrogen in a round-bottomed flask, equipped with a condensor and $CaCl_2$ tube. 15 ml of ether was added and a solution of 1.01 g (0.005 mol) of **7** in 10 ml of ether was added dropwise to the stirred solution. The reaction mixture was refluxed for 2 hours and after cooling in an ice-bath, ethyl acetate was added dropwise carefully until all remaining $LiAlH_4$ was destroyed. 5 ml of NaOH was added to the reaction mixture and after 30 min. of

stirring 10 g of dry magnesium sulphate was added after which the mixture was stirred for another hour. The salts were filtered and the organic solvents were evaporated in vacuo. The remaining oil was purified by column chromatography over $Al_2O_3$, grade III (eluent EtOAc) yielding 0.86 g (62%) of pure **9**. For analytical purpose the cis- and trans-mixture was separated by preparative gas chromatography. $^1$H-NMR (cis-2R,3R-**9**): δ 1.88-1.96 and 1.99-2.08 (m, 2H); 2.30-2.38 (m, 1H); 2.47 (dd, 1H); 3.37 (s, 3H); 3.68-3.75 and 3.85-3.88 (m, 2H); 3.89-3.94 and 3.96-4.01 (m, 2H); 4.98 (d, J = 4.9 Hz, 1H). $^{13}$C-NMR: δ 24.2 (t); 44.8 (d); 54.5 (q); 60.7 (t); 67.0 (t); 106.7 (d). HRMS: theor. ($M^+$-31): 101.0602, found:101.0601. $[\alpha]_D^{20}$ = -133.3 (c = 0.42). $^1$H-NMR (trans-2S,3R-**9**): δ 1.51-1.65 and 2.0-2.18 (m, 2H); 1.75 (s, 1H); 2.28-2.44 (m, 1H); 3.33 (s, 3H); 3.44-3.65 (m, 2H); 3.80-4.03 (m, 2H); 4.86 (d, J=1.4 Hz, 1H). $^{13}$C-NMR: δ 26.6 (t); 47.9 (d); 54.7 (q); 63.8 (t); 66.4 (t); 107.1 (d). HRMS: theor. ($M^+$-31): 101.0602, found: 101.0603. $[\alpha]_D^{20}$= +78.1 (c = 0.48). Anal: calcd for $C_6H_{12}O_3$: C, 54.53; H, 9.15; found: C, 54.26; H, 9.19.

*2(*R,S*)-Methoxytetrahydrofuran-3*R*-ylmethyl 3,5-dinitrobenzoate* **10**: To a solution of 0.60 g (4.5 mmol) of **9** in 10 ml of dry pyridine was added 2,07 g (9.0 mmol) of 3,5-dinitrobenzoylchloride and 0.55 g (4.5 mmol) of 4-dimethylaminopyridine. The reaction mixture was refluxed for 6 hours. After cooling, the reaction mixture was poured into 100 ml of icewater, brought on pH 4 and five times extracted with ether. After drying over $MgSO_4$, the solvent was filtered and evaporated *in vacuo* till dryness. The remaining solid (2.05 g) was separated by column chromatography on silicagel (eluent PE/ethyl acetate = 4/1) yielding 0.15 g of pure cis-**10** and 0.17 g of pure trans-**10**, $r_f$ values: 0.60 and 0.45 respectively. Both compounds were recrystallized from *n*-hexane. $^1$H NMR (cis-2R,3R-**10**): δ 1.83-1.97 and 2.06-2.16 (m, 2H); 2.60-2.64 (m, 1H); 3.34 (s, 3H); 3.88-4.10 (m, 2H); 4.45-4.64 (m, 2H); 4.98 (d, J = 4.41 Hz, 1H); 9.13 (d, 2H); 9.23 (t, 1H). $^{13}$C NMR: δ 26.8 (t); 43.1 (d); 54.7 (q); 65.9 (t); 66.6 (t); 103.5 (d); 122.4, 129.4, 134.0 and 148.7 (aromatic C); 162.4 (s). HRMS: theor. ($M^+$-15): 295.0566, found: 295,0564. mp. 81-83 °C, $[\alpha]_D^{20}$ = -80.1 (c = 0.8). $^1$H NMR (trans-2S,3R-**10**): δ1.65-1.72 and 2.22-2.27 (m, 2H); 2.59-2.77 (m, 2H); 3.34 (s, 3H); 3.90-4.01 (m, 2H); 4.24-4.49 (m, 2H); 4.93 (d, J = 1.10 Hz, 1H); 9.13 (d, 2H); 9.22 (t, 1H). $^{13}$NMR: δ 26.9 (t); 44.8 (d); 54.8 (q); 66.2 (t); 67.0 (t); 106.6 (d); 122.5, 129.4, 133.4 and 148.7 (aromatic C); 162.3 (s). HRMS: theor. ($M^+$-15): 295.0566, found: 295.0566. mp trans 86-87 °C, $[\alpha]_D^{20}$ =+31.1 (c = 0.2). Anal. Calcd for: $C_{13}H_{14}N_2O_8$ (M = 326.26): C,47.85; H, 4.33; N, 8.59. Found: C, 47.59; H, 4.26; N, 8.44.

*2(*R,S*)-Methoxytetrahydrofuran-3*R*-ylmethyl ester of (*R,S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoic acid* **11**: 13,2 mg (0.1 mmol) of **9** (cis- or trans) was dissolved in pyridine D-5 after which an equivalent amount of Mosher's chloride (R or S) was added. NMR spectra were made of all the reaction mixtures in order to investigate whether NOE-difference measurements could be used to determine the absolute configuration of the tetrahydrofuran part of the molecules. Unfortunately, the measured NOE-differences were too small to draw reliable conclusions. Attempts to isolate the esters failed due to their instability.

*2(*R,S*)-Methoxytetrahydrofuran-3*R*-ylmethyl 4-bromobenzoate* **12**: To a solution of 0.60 g (4.5 mmol) of **9** in 10 ml of dry pyridine was added 1.76 g (8.1 mmol) of *p*-bromobenzoylchloride and 0.05 g (0.4 mmol) of 4-(dimethylamino)pyridine. The reaction mixture was refluxed for 7 hours. After cooling, the reaction mixture was three times extracted with portions of 10 ml of ether. The combined solutions were dried over $MgSO_4$, filtered and evaporated *in vacuo*. In order to completely remove the pyridine, three times 5 ml portions of toluene were added to the resulting oil after which the mixture was evaporated. The remaining oil (1.1 g) of the

cis- and trans-**12** was separated by column chromatography on silicagel (70-230 mesh ASTM, eluent PE/ ethyl acetate = 4/1) yielding 0.2 g of pure cis-**12** and 0.45 g of pure trans-**12**, $r_f$ values: 0.57 and 0.50, respectively. The solid cis compound was recrystallized from octane, the trans isomer is an oil. $^1$H-NMR (cis-2R,3R-**12**): δ 1.80-1.95 and 2.01-2.11 (m, 2H); 2.53-2.58 (m, 1H); 3.34 (s, 3H); 3.86-4.09 (m, 2H); 4.33-4.51 (m, 2H); 4.95 (d, J = 4.60 Hz, 1H); 7.57 (d, 2H); 7.88 (d, 2H). $^{13}$C-NMR: δ 26.7 (t); 43.3 (d); 54.6 (q); 64.2 (t); 66.5 (t); 103.7 (t); 128.0, 129.1, 131.1 and 131.7 (aromatic C); 165.7 (s). mp 75-76 °C, $[\alpha]_D^{20}$= - 79.6 (c = 0.8). $^1$H-NMR (trans-2S,3R-**12**): δ 1.62-1.75 and 2.16-2.25 (m, 2H); 2.58-2.62 (m, 1H); 3.35 (s, 3H); 3.90-4.01 (m, 2H); 4.12-4.34 (m, 2H); 4.92 (d, J = 1.34 Hz, 1H); 7.57 (d, 2H); 7.88 (d, 2H). $^{13}$C-NMR: δ 26.9 (t); 44.9 (d); 54.7 (q); 65.4 (t); 66.3 (t); 106.8 (d); 128.4, 128.9, 131.1 and 131.8 (aromatic C); 165.7 (s). HRMS: theor. ($M^+$-31): 282.9970, found: 282.9971. $[\alpha]_D^{20}$= + 27.0 (c = 2.4).

*8-Bromo-1,2,3a,11a-tetrahydrofuro[3,2-e]naphtho[2,1-b]pyran* **13**: To a solution of 264 mg (2.0 mmol) of **9** in 20 ml of dry $CHCl_3$ was carefully added 400 mg (3.5 mmol) of trifluoroacetic acid and 460 mg (2.1mmol) of 6-bromo-2-naphthol. The reaction mixture was refluxed for 48 hours. After cooling the clear solution was washed twice with a saturated $NaHCO_3$ solution and, after drying of the organic phase on $MgSO_4$, the chloroform was evaporated *in vacuo* yielding 995 mg of an oil. This oil was purified by column chromatography on silicagel (70-230 mesh ASTM, eluent PE/ethyl acetate = 9/1) yielding 260 mg (44 %) of pure **13**. $^1$H-NMR: δ 1.73-1.88 and 2.04-2.21 (m, 2H); 2.78-2.85 (m, 1H); 3.22-3.27 (m, 2H); 3.96-4.23 (m, 2H); 5.30 (d, 1H); 7.10 (d, 1H); 7.52 (d, 1H); 7.56 (dd, 1H); 7.66 (d, 1H); 7.91 (d, 1H). $^{13}$C-NMR: δ 22.2 (t); 28.2 (t); 37.1 (t); 68.2 (d); 100.1 (d); 111.4 (s); 117.2 (s); 120.2 (d); 123.6 (d); 127.3 (d); 129.6 (d); 130.4 (d); 130.5 (s); 131.3 (s). HRMS: theor. ($M^+$): 304.0099, found: 304.0099. mp. after recrystallisation from ethyl acetate: 168 °C. Anal. Calcd for $C_{15}H_{13}BrO_2$ (M = 305.17): C, 59.03; H, 4.29; found: C, 59.16; H, 4.19.

*3,4,5-triiodobenzoylchloride*: This compound was synthesized as described in the literature[42]. mp 137-139 °C; IR: C=O 1720 cm$^{-1}$ ; $^1$H-NMR: δ 8.4 (s, 2H).

*2(R,S)-methoxytetrahydrofuran-3R-ylmethyl 3,4,5-triiodobenzoate* **14**: To a solution of 183 mg (1.39 mmol) of **9** in 10 ml of dry pyridine was added 780 mg (1.51 mmol) of 3,4,5-triiodobenzoylchloride and 20 mg (0.16 mmol) of 4-dimethylaminopyridine. The reaction mixture was refluxed for 3 hours. After cooling, 5 ml of water was added and the clear solution was three times extracted with $CH_2Cl_2$. After drying over $MgSO_4$ the mixture was filtered and evaporated *in vacuo* till dryness. The remaining mixture (810 mg, 95%) of cis- and trans-isomers of **14** was separated by column chromatography over $Al_2O_3$ (eluent $CH_2Cl_2$/PE boiling range 40-60 °C = 1/2) yielding 50 mg of pure cis-**14** and 170 mg of pure trans-**14**, $r_f$ values 0.65 and 0.50 respectively. Both compounds were recrystallized from $CHCl_3$. Cis-2R,3R-**14**: $^1$H-NMR: δ 1.80-1.90 and 2.05-2.12 (m, 2H); 2.55 (m, 1H); 3.34 (s, 3H); 3.92 and 4.03 (dd and dt, 2H); 4.37 and 4.46 (dd, 2H); 4.95 (d, J = 4.6 Hz, 1H); 8.40 (s, 2H). $^{13}$C-NMR: δ 26.7 (t); 43.1 (d); 54.6 (q); 64.8 (t); 66.5 (t); 103.5 (d); 106.8 (s); 127.5 (s); 132.3 (s); 138.9 (d); 163.3 (s). HRMS: theor. ($M^+$): 613.7948, found: 613.7945. mp: 140 - 148 °C (dec.), $[\alpha]_D^{20}$ = -46.8 (c = 0.6). Trans-2S,3R-**14**: $^1$H NMR: δ 1.61-1.68 and 2.18-2.25 (m, 2H); 2.61 (m, 1H); 3.36 (s, 3H); 3.92 and 3.99 (dd and dt, 2H); 4.17 and 4.28 (dd, 2H); 4.89 (d, J = 1.2 Hz, 1H); 8.40 (s, 2H). $^{13}$C-NMR: δ 26.8 (t); 44.8 (d); 54.7 (q); 66.0 (t); 66.1 (t); 106.6 (d); 106.9 (s); 127.7 (s); 132.0 (s); 138.9 (d); 163.3 (s).

HRMS: theor. ($M^+$): 613.7948, found: 613.7939. mp: 123-130 °C (dec.), $[\alpha]_D^{20}$= + 14.2 (c = 1.6). Anal.: calcd for: $C_{13}H_{13}I_3O_4$: C, 25.43; H, 2.13; found: C, 25.32; H, 2.09.

*Crystal structure determination of* ***14***. A colourless, plate-shaped crystal (0.13 x 0.21 x 0.23 mm), was glued to the tip of a glass fiber and transferred into the cold nitrogen stream on an Enraf-Nonius CAD4-T diffractometer on rotating anode. Accurate unit-cell parameters and an orientation matrix were determined by least-squares fitting of the setting angles of 25 well-centered reflections (SET4 [43]) in the range $10.08° < \theta < 13.94°$. The crystals of **14** are monoclinic, space group $P2_1$ (no. 4) with a = 8.3046(6), b = 23.465(2), c = 8.4214(6) Å, $\beta$ = 94.711(6)°, V = 1635.5(2) Å$^3$, $M_r$ = 613.96, Z = 4, $D_x$ = 2.493 g cm$^{-3}$, F(000) = 1128 e, $\mu$(Mo K$\alpha$) = 57.4 cm$^{-1}$. The unit-cell parameters were checked for the presence of higher lattice symmetry[44]. Data were collected at 100 K in $\omega$ scan mode with scan angle $\Delta\omega = 0.80 + 0.35\tan\theta°$. Intensity data of 9667 reflections were collected in the range $0.87° < \theta < 27.5°$, 7489 of which are independent ($R_{int}$ = 0.045). Data were corrected for Lp effects and for a linear decay of 3% of the three periodically measured reference reflections (2 3 2, 2 2 2, 0 4 2) during 41 h of X-ray exposure time. A numerical absorption correction (ABSORB[45], correction range 2.046-3.197) was applied. The structure was solved by automated Patterson methods and subsequent difference Fourier techniques (DIRDIF-92 [46]).Refinement on $F^2$ was carried out by full-matrix least-squares techniques (SHELXL-93 [47]). No observance criterium was applied during refinement. Hydrogen atoms were included in the refinement on calculated positions riding on their carrier atoms. All non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen atoms were refined with a fixed isotropic thermal parameter related to the value of the equivalent isotropic displacement parameter of their carrier atoms by a factor of 1.5 for the methyl hydrogen atoms, and a factor of 1.2 for the other hydrogen atoms. Refinement converged at a final wR2 value of 0.089, $wR2 = [\Sigma[w(F_0^2 - F_c^2)^2/\Sigma[w(F_0^2)^2]]^{1/2}$, $R1 = \Sigma||F_0| - |F_c||/\Sigma|F_0| =$ 0.042 [for 6307 reflections with $I > 2\sigma(I)$], S = 1.03, for 363 parameters. A final difference Fourier showed no residual density outside -0.82 and 0.98 e Å$^{-3}$ (near I). The Flack x parameter, calculated during the final structure factor evaluation, amounted to a value of of 0.02(4), indicating the correct assignment of the absolute configuration. Refinement of the alternative chirality resulted in a wR2-value of 0.094, R1 amounted to 0.044, while the x parameter was calculated as 0.97(4). Neutral atom scattering factors and anomalous dispersion corrections were taken from the International Tables for Crystallography[48]. Geometrical calculations and illustrations were performed with PLATON[49] and PLUTON[16]. All calculations were performed on a DEC5000 cluster.

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## References and notes

1. Schuda, P.F. *Top. Curr. Chem.* **1980**, 91, 75-111.
2. Blaney, W.M.; Simmonds, M.S.J.; Ley, S.V.; Jones, P.S. *Entomol. Exp. Appl.* **1988**, 46, 267-274.
3. Zanno, P.R.; Miura, I.; Nakanishi, K.; Elder, D.L. *J. Am. Chem. Soc.* **1975**, 97, 1975-1977.

4. a) Kojima, Y.; Kato, N. *Tetrahedron* **1981**, 37, 2527-2538; b) Ley, S.V.; Santafianos, D.; Blaney, W.M.; Simmonds, M.S.J. *Tetrahedron Lett.* **1987**, 2, 221-224; c) Anderson, J.C.; Ley, S.V.; Santafianos, D.; Sheppard, R.N. *Tetrahedron* **1991**, 47, 6813-6850.
5. Vader, J.; Koopmans, R.; de Groot, Ae.; van Veldhuizen, A.; van der Kerk, S.M. *Tetrahedron* **1988**, 44, 2663-2674.
6. Vader, J.; Sengers, H.; de Groot, Ae. *Tetrahedron* **1989**, 45, 2131-2142.
7. Vader, J.; Doddema, L.L.; Peperzak, R.M.; de Groot, Ae.; Smits, J.M.M.; Beurskens, P.T. *Tetrahedron* **1989**, 45, 5595-5610.
8. a) Udding, J.H.; Fraanje, J.; Goubitz, K.; Hiemstra, H.; Speckamp, W.N.; Kaptein, B.; Schoemaker, H.E.; Kamphuis, J. *Tetrahedron: Asymmetry* **1993**, 4, 425-432; b) Ghosh, A.K.; Chen, Y. *Tetrahedron Lett.* **1995**, 36, 505-508
9. Franssen, M.C.R.; Boavida dos Santos, P.M.A.C.; Camacho Mondril, N.L.F.L.; de Groot, Ae. *Pure Appl. Chem.* **1992**, 64, 1089-1092.
10. Formerly called *Candida cylindracea*.
11. a) Faber, K. *Biotranformations in Organic Chemistry*, second edition; Springer Verlag: Berlin, 1995; 356 pp.; b) Elferink, V.H.M.; Breitgoff, D.; Kloosterman, M.; Kamphuis, J.; van den Tweel, W.J.J.; Meijer, E.M. *Recl. Trav. Chim. Pays-Bas* **1991**, 110, 63-74; c) Klibanov, A.M. *Acc. Chem. Res.* **1990**, 23, 114-120
12. Lipoprotein lipase (Amano) and subtilisin Carlsberg (Sigma, pH-activated) showed 100% selectivity towards trans-**6** but had no preference towards the C3-stereoisomers.
13. Laane, C.; Boeren, S.; Vos, K.; Veeger, C. *Biotechnol. Bioeng.* **1987**, 30, 81-87.
14. Chen, C.-S.; Wu, S.-H.; Girdaukas, G.; Sih, C.J. *J. Am. Chem. Soc.* **1987**, 109, 2812-2817.
15. This Figure is obtained for ethanol as a nucleophile; reaction with *n*-butanol gives a very similar graph, $E > 100$, $K = 0.20$.
16. Spek, A.L. PLUTON Molecular graphics program, Utrecht University, The Netherlands, 1995
17. Ahmed, S.N.; Kazlauskas, R.J.; Morinville, A.H.; Grochulski, P.; Schrag, J.D.; Cygler, M. *Biocatalysis* **1994**, 9, 209-225
18. Holmberg, E.; Hult, K. *Biocatalysis* **1992**, 5, 289-296
19. Colton, I.J.; Ahmed, S.N.; Kazlauskas, R.J. *J. Org. Chem.* **1995**, 60, 212-217
20. a) Gubernator, K.; Müller, K.; Winkler, F.K. In *Lipases: structure, mechanism and genetic engineering*; Alberghina, L.; Schmid, R.D.; Verger, R. Eds.; GBF Monographs, VCH Verlagsgesellschaft: Weinheim, 1991; pp. 9-16; b) Brzozowski, A.M.; Derewenda, U.; Derewenda, Z.S.; Dodson, G.G.; Lawson, D.M.; Turkenburg, J.P.; Björkling, F.; Huge-Jensen, B.; Patkar, S.A.; Thim. L. *Nature* **1991**, 351, 491-494; c) Derewenda, U.; Brzozowski, A.M.; Lawson, D.M.; Derewenda, Z.S. *Biochemistry* **1992**, 31, 1532-1541.
21. a) Chen, C.-S.; Sih, C.J. *Angew. Chem.* **1989**, 101, 711-724; b) Dordick, J.S. *Enzyme Microb. Technol.* **1989**, 11, 194-211.
22. Holmberg, E.; Holmquist, M.; Hedenström, E.; Berglund, P.; Norin, T.; Högberg, H.-E.; Hult, K. *Appl. Microbiol. Biotechnol.* **1991**, 35, 572-278
23. Engel, K.H., *Tetrahedron: Asymmetry* **1991**, 2, 165-168
24. Engel, K.H. *J. Am. Oil Chem. Soc.* **1992**, 69, 146-150
25. Kirchner, G.; Scollar, M.P.; Klibanov, A.M. *J. Am. Chem. Soc.* **1985**, 107, 7072-7076

26. Gubicza, L. In *Biocatalysis in Non-Conventional Media*; Tramper, J.; Vermuë, M.H.; Beeftink, H.H.; von Stockar, U. Eds; Elsevier: Amsterdam; *Progr. Biotechnol.* **1992**, 8, 497-503
27. Kitaguchi, H.; Itoh, I.; Ono, M. *Chem. Lett.* **1990**, 1203-1206
28. Parida, S.; Dordick, J.S. *J. Am. Chem. Soc.* **1991**, 113, 2253-2259
29. Parida, S.; Dordick, J.S. *J. Org. Chem.* **1993**, 58, 3238-3244
30. Wu, S.-H.; Chu, F.-Y.; Wang, K.-T. *Bioorg. Med. Chem. Lett.* **1991**, 1, 339-342
31. Pan, S.-H; Kawamoto, T.; Fukui, T.; Sonomoto, K.; Tanaka, A. *Appl. Microbiol. Biotechnol.* **1990**, 34, 47-51
32. Fukui, T.; Kawamoto, T.; Sonomoto, K.; Tanaka, A. *Appl. Microbiol. Biotechnol.* **1990**, 34, 330-334
33. Fowler, P.W.; Macfarlane, E.L.A.; Roberts, S.M. *J. Chem. Soc., Chem. Commun.* **1991**, 7, 453-455
34. Cambou, B.; Klibanov, A.M. *Biotechnol. Bioeng.* **1984**, 26, 1449-1454
35. Parida and Dordick[29] claim that CRL esterifies S-2-hydroxy acids when the number of carbon atoms in the carboxylic acid is 6 or less, whereas the enzyme has a preference for the R-enantiomer for acids containing 8 and 10 carbon atoms. For the latter case, the authors have measured the optical rotation of the free acids obtained by CRL-mediated esterification of 2-hydroxycaprylic acid and 2-hydroxycapric acid followed by chemical hydrolysis. The rotations were measured in ethanol, whereas in the article cited by the authors the optical rotation is determined in 1.00 N NaOH solution[36]. It has been shown before that the solvent strongly affects magnitude **and sign** of the optical rotation of these compounds[37].
36. Baker, C.G.; Meister, A. *J. Am. Chem. Soc.* **1951**, 73, 1336-1338
37. Horn, D.H.S.; Pretorius, Y.Y. *J. Chem. Soc.* **1954**, 1460-1464
38. Burke, P.A.; Smith, S.O.; Bachovchin, W.W.; Klibanov, A.M. *J. Am. Chem. Soc.* **1989**, 111, 8290-8291
39. Fitzpatrick, P.A.; Steinmetz, A.C.U.; Ringe, D.; Klibanov, A.M. *Proc. Natl. Acad. Sci. USA* **1993**, 90, 8653-8657
40. Fitzpatrick, P.A.; Ringe, D.; Klibanov, A.M. *Biochem. Biophys. Res. Commun.* **1994**, 198, 675-681
41. Chattopadhyay, S.; Mamdapur, V.R. *Biotechnol. Lett.* **1993**, 15, 245-250
42. *Vogel's Textbook of Practical Organic Chemistry*, fifth edition; John Wiley & Sons: New York, 1989; pp. 1244
43. Boer, J.L. de; Duisenberg, A.J.M. *Acta Crystallogr.* **1984**, A40, C410
44. Spek, A.L. *J. Appl. Crystallogr.* **1988**, 21, 578-579
45. Spek, A.L. ABSORB Program for absorption correction. Utrecht University, The Netherlands, ECM Abstract Book, 1983, p.283
46. Beurskens, P.T.; Admiraal, G.; Beurskens, G.; Bosman, W.P.; García-Granda, S.; Gould, R.O.; Smits, J.M.M., Smykalla, C. The DIRDIF program system, Technical report of the Crystallographic Laboratory, University of Nijmegen, The Netherlands, 1992
47. Sheldrick, G.M. SHELXL-93 Program for crystal structure refinement. University of Göttingen, Germany, 1993
48. Wilson, A.J.C. (ed.), International Tables for Crystallography, Volume C, 1992, Kluwer Academic Publishers, Dordrecht, The Netherlands
49. Spek, A.L. *Acta Crystallogr.* **1990**, A46, C410


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