

Effect of Distant Groups on the Enantioselectivity in Kinetic Resolution of *sec*-Alcohols Catalyzed by Microbial Lipases

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A short series of racemic phenoxyalkyl-alkylcarbinols, possessing perturbing groups at different distances from the stereogenic center, was prepared. The *sec*-alcohols (\pm)-1-phenoxy-2-hydroxybutane (**6**), (\pm)-1-phenoxy-3-hydroxyhexane (**11**), (\pm)-1-phenoxy-4-hydroxyoctane (**15**) and (\pm)-1-phenoxy-5-hydroxydecane (**19**) were prepared. The enantioselectivity of their acetylation by vinylacetate catalyzed by microbial lipases in *n*-hexane was determined. The products of this acetylation were (\pm)-1-phenoxy-2-acetoxybutane (**7**), (\pm)-1-phenoxy-3-acetoxyhexane (**12**), (\pm)-1-phenoxy-4-acetoxyoctane (**16**), and (\pm)-1-phenoxy-5-acetoxydecane (**20**). The efficacy of kinetic resolution, expressed as *E*-value, generally diminishes with the distance of the perturbing phenoxy (R_L) and methyl (R_M) groups. Twenty lipases from commercial sources were screened for their enantioselectivity; for two lipases with broadest substrate selectivity, *Geotrichum candidum* (GCL) and *Candida cyclindracea* (CCL) (S), from Sigma), non-monotonous correlation between *E*-value and the distance (*n*) of the perturbing group was observed. With GCL lipase, a remarkable turnover of enantioselectivity from preferred acetylation of (*R*)-enantiomers in **6**, **11**, **15** to (*S*)-enantiomer of **19** was observed, indicating that relative steric requirements of the distant perturbing groups in the latter do not control the enantioselective bias. The herewith reported results are correlated with the previously observed stereoselective acetylation of (*S*)-*sec*-alcohols (3*S*,7*S*)-*trans*-3,4,5,6,9,10-octahydro-7,14,16-trihydroxy-3-methyl-1*H*-2-benzoxacyclotetradecene-1-one (**1**) and (3*S*,7*S*)-3,4,5,6,9,10,11,12-decahydro-7,14,16-trihydroxy-3-methyl-1*H*-2-benzoxacyclotetradecane-1-one (**3**), macrocyclic derivatives of resorcylic acid, and also with the results of MM2 calculations for some low energy conformations.

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INTRODUCTION

Despite of the requirement for availability of three dimensional structures for understanding of mechanism and stereochemistry of lipase catalysis,^{1,2} most of the lipase catalyzed kinetic resolutions of *sec*-alcohols can be explained by the simple model shown in Figure 1a.^{3,4} If R_L has priority over R_M according to the CIP convention, then the more reactive enantiomer possesses (*R*)-absolute configuration. This model is based on numerous experimental data, and implies that the degree of enantioselection primarily depends on the difference in steric requirements between R_L and R_M .⁵ In view of its simplicity and complete neglect of conformational properties of the substrate, this model is surprisingly well obeyed by microbial lipases; in fact, only few exceptions have been reported so far.⁶⁻⁹ However, this model neither takes into account conformational mobility nor the distance of the perturbing group from the stereogenic center, which are two important properties in designing the best fit with the lipase active site.

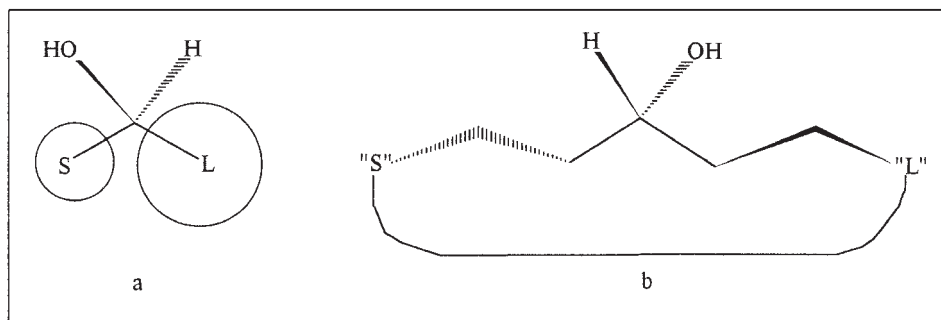
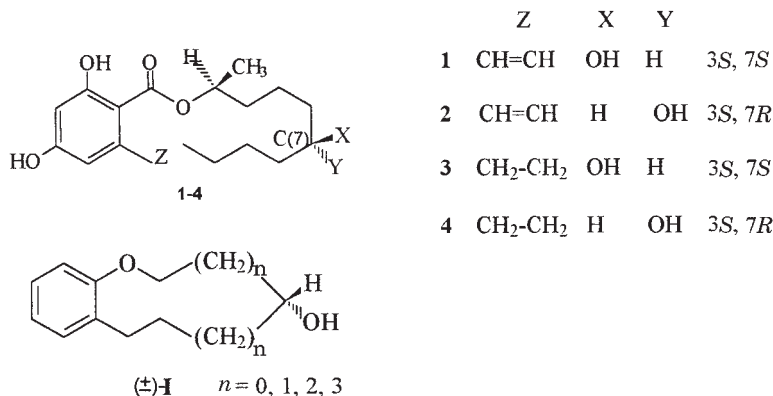


Figure 1. a) Empirical rule predicts that the microbial lipases react faster with the (*R*)-enantiomer of the secondary alcohols, b) helical model, schematic presentation of absolute conformation of the more reactive enantiomer.

We have recently shown that conformational properties of *sec*-alcohols **1-4**, derived from macrocyclic resorcylic acid lactones are decisive for the very high stereoselection of the lipase catalyzed hydrolysis and acetylation, and proposed a »helical model« for stereoselection, supported by the set of X-ray data, in order to explain the preferred acylation of distereomers with (*S*)-configuration at the stereogenic center C(7).^{10,11} Successful kinetic resolution of conformationally flexible molecules confirms the importance of helicity for enantiorecognition by microbial lipases.¹² These results emphasize the importance of *absolute conformation*, as defined by spectroscopists for the helical arrangement of chromophores,¹³ for enantioselection with flexible substrates, and we define as »S« (small) and »L« (large) the less and the more perturbing regions of the substrate that match or mismatch topology

of the enzyme binding site, Figure 1b.^{10,11} To such substrates belong flexible molecules with a large-ring or an open-chain and perturbing groups distant from the chiral center. Compounds **1-4**, available in nature, thus turn out to be specific representatives of the flexible large-ring structures.



Formulae **1-4**, **I**

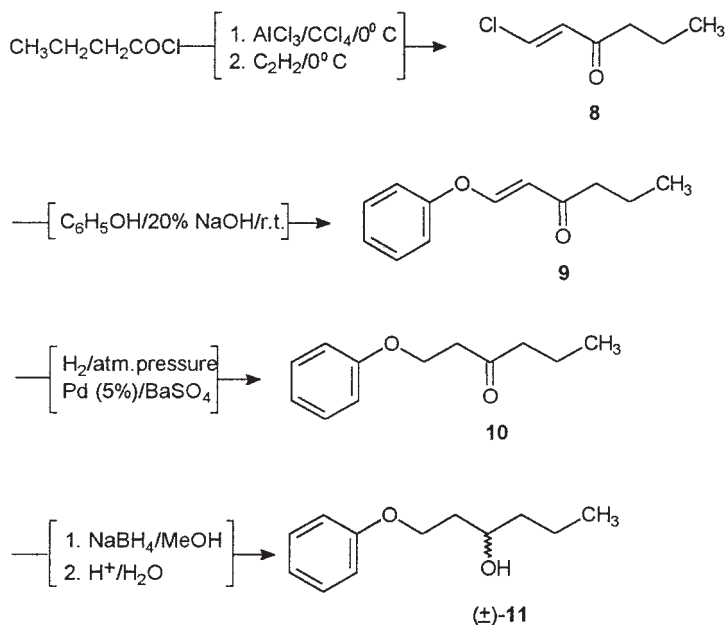
Continuing this project, we started to study the effect of conformational properties for a series of conformationally flexible, open-chain substrates that only differ in the distance of the perturbing group from the stereogenic center. To this aim we have prepared specifically designed racemic *sec* alcohols **6**, **11**, **15** and **19** and examined their lipase catalyzed acetylation. These compounds can be regarded as the open-chain analogues of **I**, which is a chiral structure by virtue of the oxygen atom present in the ring, and the synthesis of some of its representatives is envisaged. Preliminary results with the open-chain analogues have been reported in a recent communication.¹⁴ Here, we describe their synthesis, the results of the enantioselective acetylation by a series of microbial lipases, and correlate the observed enantioselection with the structural and conformational properties of these and previously studied substrates.

RESULTS AND DISCUSSION

Syntheses. Synthesis of racemic *sec* alcohols **6**, **11**, **15** and **19**, and their acetates **7**, **12**, **16** and **20** has been envisaged according to Scheme 1.

However, only preparation of alcohols **15** and **19** and their acetates **16** and **20** could be completed according to this Scheme. Preparation of alcohols with shorter side chains ($n=0,1$) required different approaches, since the cy-

tion of all substrates. In order to quantitatively determine the enantioselectivity of the lipase catalyzed acetylation, the baseline-separation of racemic alcohols and acetates was conducted on various chiral HPLC columns, as indicated in Experimental. For the enantiomerically enriched acetylation



products and for the remaining alcohols, the integration of the peaks reveals that active lipases preferentially acylate the faster running enantiomer on

TABLE I

Transesterification of alcohol (\pm)-6 with lipases in *n*-hexane^a

Lipase	Conversion (%)
<i>Pseudomonas fluorescens</i>	57.17
<i>Pseudomonas species</i>	52.20
<i>Candida cylindracea</i> (Amano)	40.56
<i>Geotrichum candidum</i>	21.36
<i>Mucor miehei</i> (Novo Nordisk)	20.79
<i>Candida cylindracea</i> (Sigma)	18.42

^a All reactions performed in a thermostated shaker at 30 ± 0.5 °C.

Determined by HPLC after 48 h.

the Chiralcel OB-H and Chiralcel OD columns for **6** and **15**, and the slower running enantiomer on the Chiralcel OJ and Chiralcel OD columns for **11** and **19**, Table V.

For *rac*-**6**, over 10% conversion was obtained with seven lipases after 48 h at 30 ± 0.5 °C, Table I. The progress curves given in Figure 2 and 3 revealed that two lipases (GCL and CCL) which accept *all four substrates*, do not acylate particularly well this smallest one; relative rates found with GCL are **19**>**15**>**11**>**6**, and with CCL are **15**>**11**>**6**>**19**.

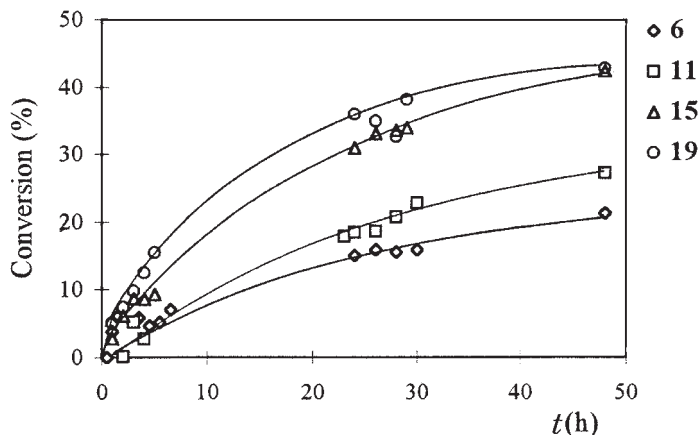


Figure 2. Progress curves for transesterification of (\pm)-**6**, (\pm)-**11**, (\pm)-**15** and (\pm)-**19** with lipase from *Geotrichum candidum* (Amano).

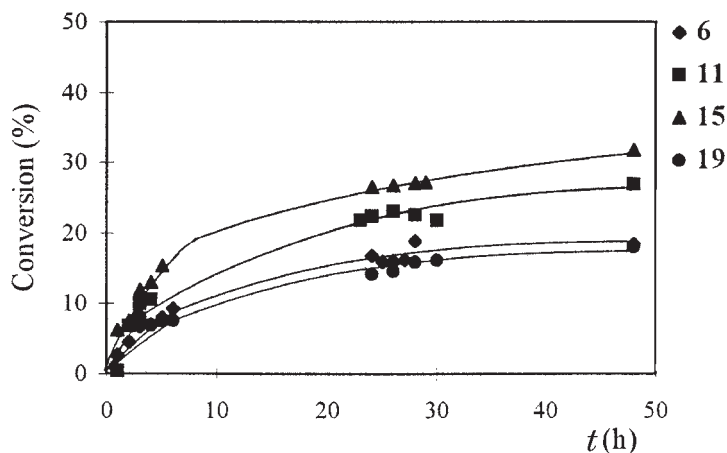


Figure 3. Progress curves for transesterification of (\pm)-**6**, (\pm)-**11**, (\pm)-**15** and (\pm)-**19** with lipase from *Candida cylindracea* (Sigma).

On the other hand, correlation of n with E -values obtained with lipases that were selective with all substrates reveals significant deviations from linear E/n plot for GCL and CCL(S), Figure 4.

For **6**, E -values were in the order PFL>PSL>>GCL>>CCL(A)>>CCL(S), Table I, as expected for this smallest substrate.

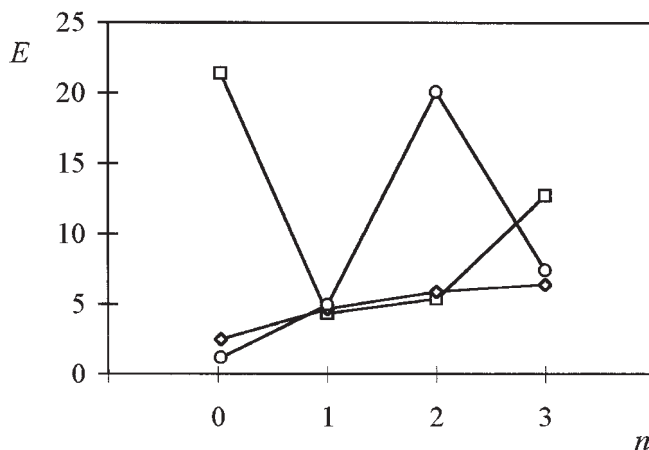


Figure 4. Correlation between E and n for acetylation with: □-□-□ *Geotrichum candidum* (Amano), ◇-◇-◇ *Candida cylindracea* (Amano) and ○-○-○ *Candida cylindracea* (Sigma), from Ref. 14.

As seen in Table II, 90–100% enantiomeric excess of alcohol **6** and 75–82% of acetate **7** was achieved with two *Pseudomonas* lipases. High enantioselectivity is even better reflected in E -value (30–50), which is, unlike *e.e.*, time-independent and therefore a true measure of enantioselectivity.¹⁶ This result is in agreement with the general Scheme 3, which indicates high flexibility of *Pseudomonas* lipase in accepting substrates with limited sterical requirements.¹⁷

Acetylation of *rac*-**11** reveals CCL (from all three suppliers) and GCL as the most active ones, Figures 2 and 3. Table II and Figure 4 show them as not very selective with this substrate. Both conversions after 48 h (c_{48}) and E -values (~ 5) are indeed significantly lower than for *rac*-**6**.

Progress curves of acetylation of the two largest substrates **15** and **19**, given in Figures 2 and 3, reveal their on average higher reactivity than that of the two smaller ones (except for **19** with CCL(S)). The E -values in Table II, reveal generally low enantioselectivity of acetylation, except for **15** with CCL and for **19** with GCL.

Distinctly different trends in enantioselection of GCL and CCL, two lipases that demonstrated the broadest substrate selectivity, is surprising,

Reversal of the HPLC peak intensity on going from **6**, **11**, **15** to **19** was accompanied by reversal of the sign of the $[\alpha]_D$ value, confirming inversion of enantioselection for *rac*-**19**. If one assumes that all examined lipases recognize the phenoxy group as R_L and methyl group as R_M in the racemic substrates,^{3,5} then (*S*)-configuration should be ascribed (+)-**6**, (+)-**11** and (+)-**15**, and (*R*)-configuration to (-)-**19**, Table V in Experimental. Ulterior support to this assignment comes from the correlation of $[\alpha]_D$ values for (+)-**6** and its methyl congener, (+)-1-phenoxy-2-hydroxypropane.¹⁸ The latter compound has $[\alpha]_D=+20.2$ ($c=1.70$ in CHCl_3), whereas the calculated $[\alpha]_D$ value for optically pure (+)-**6** amounts to +21.5 (at the same concentration in CH_2Cl_2). Since (+)-1-phenoxy-2-hydroxypropane is chemically correlated to (*S*)-propylenoxide,¹⁹ the same (*S*)-absolute configuration of (+)-**6** is strongly supported.

The results reported here for **6**, **11**, **15** and **19** could be correlated with those previously reported for macrocyclic substrates **1–4**.¹¹ Two strains of *Pseudomonas* lipases were active with (3*S*), (7*S*)-diastereomers of **1** and **3**, indicating that these stereoisomers »are seen« by the lipase active site as »smaller«, analogous to the (*S*)-enantiomer of *rac*-**19**. As we have already reported,¹¹ preferred acetylation of (3*S*), (7*S*)-diastereomers **1** and **3** is contrary to that expected from the simple model in Figure 1. Prevalent acetylation of (7*R*)-stereoisomer should obey the model^{3–5} since in **1**, **3** the perturbing group on-(CH_2)₃CH(CH_3)-OCOAr (R_L) is on the 4th atom from the stereogenic center at C(7), whereas in -(CH_2)₅-Ar (R_M) side-chain the perturbing phenyl group is placed on the 5th carbon atom. The opposite conformations for two diastereomeric pairs **1**, **2** and **3**, **4**, as determined by the X-ray analysis for regions C(121)–C(8) and C(4)–C(7), respectively, led to the conclusion that the absolute conformation of this hydrophobic region determines binding preference for stereoisomers.¹¹

We then performed MM2 calculations of the minimal conformation for **1** and **3**, which reveal high over-all similarity to those found in crystals, *i.e.* all calculated torsion angles differ from the experimental values by less than a 5°. This small conformational difference, however, gives rise to a large energy difference; those present in the crystal are *ca.* 150 kJ/mol higher, presumably because of the high number of solvent molecules found in the crystal, Table III.

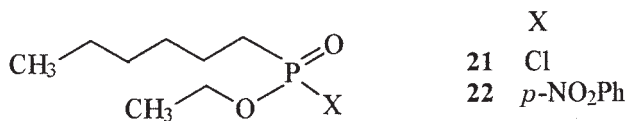
We have also calculated relative energies for two conformational minima of the open chain compounds **6**, **11**, **15** and **19**, the first one corresponding to the extended conformation of the side-chain (**A**), and the second one to the nearly closed (folded) conformation (**B**). The latter was obtained on the computer designed ring-opening of the cyclic structures **I** which leads to the nearest energy minimum. It was found that folded conformations of **6** and **11** are very similar to that of **15**, and very different from **19**, as shown for **15** and **19** in Figure 5. The folded conformation could be regarded as closest to

TABLE III

Compared energies (MM2 calculations) of the low-energy conformations for 3*S*,7*S*-**1**, 3*S*,7*S*-**3**, *R*-**6**, *R*-**11**, *R*-**15**, *R*-**19** and cyclic **I**

Compd.	Extended (A) (kJ/mol)	Folded (B) (kJ/mol)	Cyclic I	Cyclic (crystal) (kJ/mol)	Cyclic (MM2) (kJ/mol)
3 <i>S</i> ,7 <i>S</i> - 1				362.50	194.14
3 <i>S</i> ,7 <i>S</i> - 3				340.24	178.82
<i>R</i> - 6	48.87	58.99	70.25		
<i>R</i> - 11	71.50	82.88	116.82		
<i>R</i> - 15	76.06	82.55	117.07		
<i>R</i> - 19	81.30	101.17	112.88		

those present on the binding site, since on passing the lid it can expose the hydroxy group to the acylating serine residue in the pocket of the enzyme active site, while maintaining a strong hydrophobic interaction of the side chains.^{20,21} The importance of such a hydrophobic interaction at the lipase active site was recently demonstrated by showing the strong inhibition of *Candida antarctica* and *Mucor miehei* lipase by (-)-enantiomers of hexylethyl phosphonates **21** and **22**.^{22,23} Both are bound covalently to the serine residue, with the hydrophobic side-chain extended to the hydrophobic »pocket« at the active site. The importance of »bioactive« extended *vs.* folded conformation of the conformationally flexible substrates was recently shown by combing MM2 calculations and experimental data²⁴ for *para*-substituted phenyl-2-methylpropanals, a class of odour-active compounds.

Formulae **21,22**

As expected, the MM2 calculated energy minima (**A**) and (**B**) are very close (10–20 kJ/mol), whereas forcing one enantiomer to adopt the opposite, nearly enantiomorphic conformation costs *ca.* 300 kJ/mol. These numbers indicate that if the most stable conformation of one enantiomer is not preferred for binding, it cannot be easily inverted at the active site, which can explain the observed high enantioselectivity for conformationally flexible substrates.

In conclusion, we have shown for a series of microbial lipases that enantioselectivity in acetylation of racemic substrates **6**, **11**, **15**, and **19**, as representatives of conformationally flexible molecules, generally decreases with

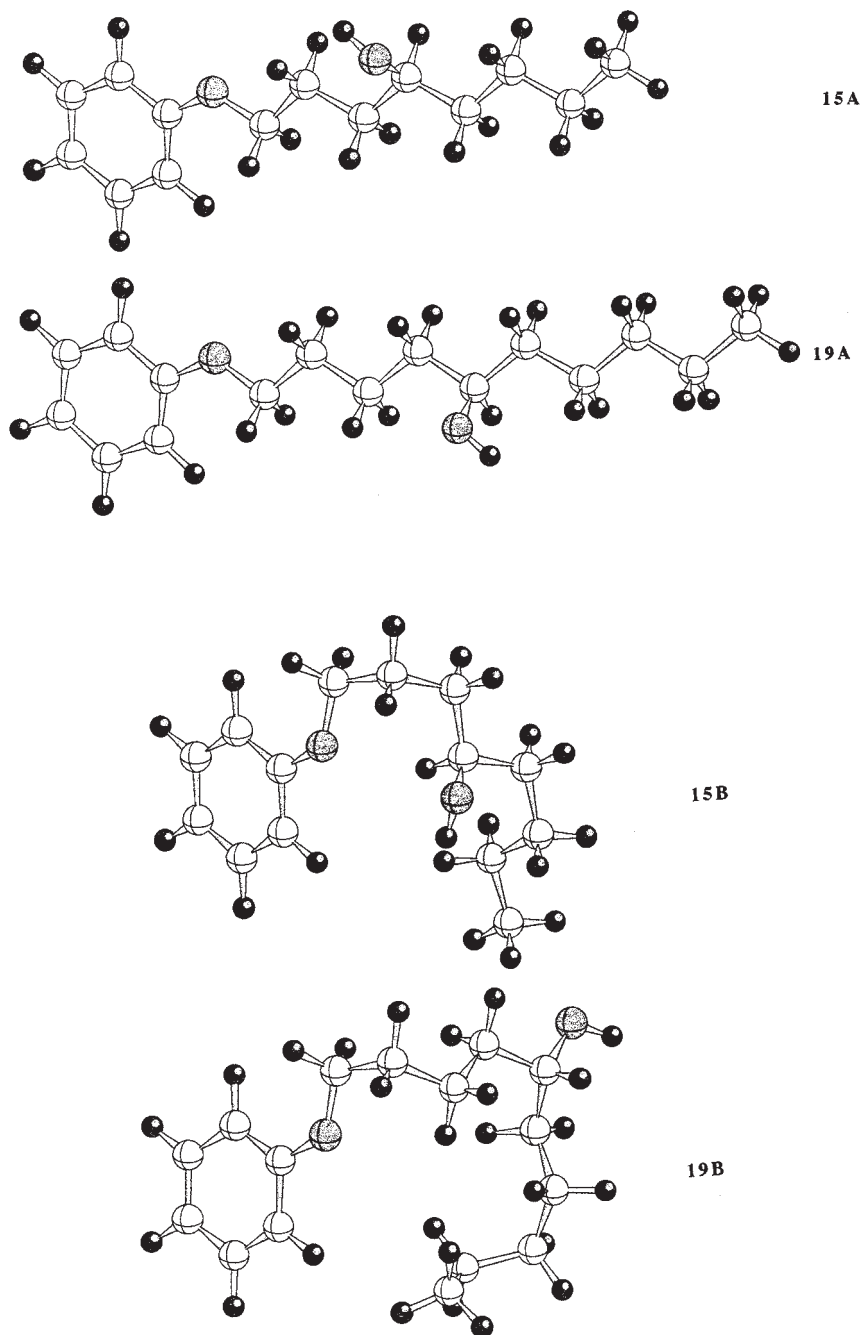


Figure 5. Extended (A) and folded (B) conformations of **15** and **19**.

the perturbing group distance, in particular on going from $n=1$ to $n=2$. Significant deviations from the linear E/n correlation for GCL and CCL(S), and the turnover of the enantioselectivity for GCL on going from **15** to **19**, indicate specific conformational requirements at the active site of this lipase, revealing the importance of conformational properties of the substrates for recognition of the more reactive enantiomer. Thus, considering the higher stereoselectivity with cyclic substrates **1–4** than with the acyclic ones described here, it is tempting to conclude that the folded conformation of the latter is the preferred one at the lipase active site(s). The experimental results are supported by MM2 calculations of the relative energies of the preferred conformations and the energies required to invert them into unfavoured, nearly-enantiomorphic conformations.

EXPERIMENTAL

^1H - and ^{13}C -NMR spectra were recorded for CDCl_3 solutions on the Varian Gemini XL 300 spectrometer, IR spectra were run for KBr pellets on a Perkin Elmer 297 spectrometer. HPLC analyses were performed on HP 1050 chromatograph with C18RCM 8x10 (Waters) reverse phase column; separation was monitored by HP 1050 UV detector set up at 254 nm and connected to HP 3396A integrator. For separation of **6**, **7** and **11**, **12**, a methanol:water mixture (85:15) was used as mobile phase at a 1 mL/min flow rate, for **15**, **16** a mixture methanol:water (89.5:10.5) and for **19**, **20** a methanol:water mixture (92.5:7.5) was used as mobile phase at a 1 mL/min flow rate.

Melting points were determined on the Electrothermal Melting Point Apparatus and were not corrected. Optical rotations were measured on an Optical Activity AA-10 Automatic Polarimeter in a 1 dm cell; concentrations are given in g/100 mL.

Lipases from the following microorganisms were tested for enzymatic acetylations: *Penicillium camemberti*, *Candida lipolytica*, *Humicola lanuginosa*, *Penicillium roqueforti*, *Pseudomonas species*, *Mucor javanicus*, *Geotrichum candidum*, *Rhizopus niveus*, *Rhizopus delemar*, *Aspergillus niger*, *Candida cylindracea*, *Rhizopus oryzae* (all from Amano), *Candida cylindracea* Type VII (from Sigma), *Candida cylindracea* (36.0 U/mg), *Mucor miehei* (24.2 U/mg) and *Pseudomonas fluorescens* (31.5 U/mg) (all from Fluka), *Mucor miehei*, Lipolase, Lipozyme (all from Novo Nordisk) and Palatase (from Novo Ferment).

1-Chloro-2-butanone was from Pliva Co. (Zagreb); the origin of all other reagents is indicated. They were used as purchased without further purification.

During the usual work-up, all organic extracts were dried over Na_2SO_4 and evaporated *in vacuo* on a Büchi rotavapor.

1-Phenoxy-2-butanone (5)

To the solution of phenol (28.5 g, 0.3 mol) in methanol (50 mL), a solution of KOH (17 g, 0.3 mol) in methanol (100 mL) was added dropwise. The resulting solution was stirred for 15 min, then the solvent was evaporated *in vacuo*. Residual mixture was dissolved in DMF (250 mL), undissolved material was filtered off, and 1-chloro-2-butanone (38 g, 0.36 mol) was added dropwise to the ice-cold filtrate. On 0.5 h stirring at room temperature, the reaction mixture was diluted with water (200 mL,

stirred for additional 15 min, and extracted with dichloromethane (3 x 200 mL). Organic extracts were washed with 2 M NaOH (200 mL) and water (200 mL), then dried, evaporated to dryness and the crude product was purified by flash chromatography on silicagel with dichloromethane as eluent. 14.9 g (30%) of **5** as colourless oil was obtained, b.p. 64–68 °C/0.15 mmHg. IR $\nu_{\max}/\text{cm}^{-1}$: 3060, 2980, 1740, 1600, 1250, 750. $^1\text{H-NMR}$ δ/ppm : 1.10 (t, 3H, $J=7.2$ Hz), 2.63 (q, 2H, $J=7.2$ Hz), 4.55 (s, 2H); 6.88 (d, 2H, $J=8.5$ Hz), 6.99 (dd, 1H, $J_1=7.3$ Hz, $J_2=7.4$ Hz), 7.30 (dd, 2H, $J_1=7.4$, $J_2=8.6$ Hz). $^{13}\text{C-NMR}$ δ/ppm : 6.8, 32.1, 72.4, 114.4, 121.6, 129.6, 157.8, 208.5. *Anal.* Calcd. for $\text{C}_{10}\text{H}_{12}\text{O}_2$ (164.21): C 73.15, H 7.37%; found: C 73.26, H 7.08%.

(±)-1-Phenoxy-2-hydroxybutane (**6**)

1-Phenoxy-2-butanone (13.3 g, 0.08 mol) was dissolved in distilled, anhydrous MeOH (250 mL), and NaBH_4 (3.08 g, 0.08 mmol, from Aldrich) was added portionwise at room temperature for 1 h. After additional 2 h of stirring at room temperature, a mixture of conc. aqueous HCl (100 mL) and methanol (100 mL) was added and the stirring continued for 1 h. The reaction mixture was partially evaporated, and the aqueous slurry extracted with dichloromethane (3 x 100 mL). Organic extracts were dried, filtered and evaporated. The crude product was purified by flash chromatography on silicagel with dichloromethane as eluent. 10.7 g (80%) of pure *rac*-**6**, as colourless oil, was obtained, m.p. 23–25 °C. IR $\nu_{\max}/\text{cm}^{-1}$: 3420, 2940, 1600, 1245, 750. $^1\text{H-NMR}$ δ_{\max}/ppm : 1.03(t, 3H, $J=7.4$ Hz), 1.58–1.65 (m, 2H), 3.81–3.86 (m, 1H), 3.92–4.00 (m, 2H), 6.92 (d, 2H, $J=8.6$ Hz), 6.97 (dd, 1H, $J_1=8.4$ Hz, $J_2=7.3$ Hz), 7.29 (dd, 2H, $J_1=7.4$, $J_2=8.6$ Hz). $^{13}\text{C-NMR}$ δ_{\max}/ppm : 9.7, 25.9, 71.3, 71.6, 114.5, 121.0, 129.4, 158.6.

(±)-1-Phenoxy-2-acetoxybutane (**7**)

To the solution of *rac*-**6** (0.7 g, 4.2 mmol) in pyridine (6 mL), acetylchloride (1.66 g, 21 mmol) was added dropwise. After the 0.5 h reaction was completed, toluene (5 mL) was added, and the solvent mixture was evaporated *in vacuo*. The crude product was purified by flash chromatography on silicagel with dichloromethane as eluent. 0.84 g (95.2%) of pure *rac*-**7** was obtained. IR $\nu_{\max}/\text{cm}^{-1}$: 3040, 2980, 1740, 1605, 1240, 755. $^1\text{H-NMR}$ δ_{\max}/ppm : 0.97 (t, 3H, $J=7.4$ Hz), 1.66–1.85 (m, 2H), 2.07 (s, 3H), 4.02 (d, 2H, $J=4.8$ Hz), 5.09–5.17 (m, 1H), 6.90 (d, 2H, $J=7.7\text{Hz}$), 6.96 (dd, 1H, $J_1=7.4$ Hz, $J_2=8.5$ Hz), 7.28 (dd, 2H, $J_1=8.6$ Hz, $J_2=7.5$ Hz). $^{13}\text{C-NMR}$ δ_{\max}/ppm : 9.3, 20.9, 23.6, 68.2, 73.2, 114.5, 121.0, 129.4, 158.7, 170.8. *Anal.* Calcd. for $\text{C}_{12}\text{H}_{16}\text{O}_3$ (208.26): C 69.21, H 7.74%, found: C 69.31, H 7.66%.

β -Chlorovinyl-propyl-ketone (**8**)

To the solution of freshly distilled butyryl chloride (30 g, 0.282 mol) in CCl_4 (44 mL), cooled by ice-water, AlCl_3 (52 g, 0.39 mol) was added under vigorous stirring and in small portions. After addition of the first portion of AlCl_3 , acetylene was introduced; first as a slow and then as a vigorous stream. After 6 h of stirring and bubbling of acetylene, the reaction mixture was poured onto crushed ice, stirred and the organic layer was separated. Aqueous layer was extracted with diisopropylether (3 x 100 mL), combined organic extracts were dried on CaCl_2 , filtered and the solvents evaporated. Crude product was distilled at 60–62 °C/15 mmHg to give 25.68 g (69%) of pure **8** in the form of colourless oil. IR $\nu_{\max}/\text{cm}^{-1}$: 3080, 2960, 1680, 1590. $^1\text{H-NMR}$ δ_{\max}/ppm : 0.94 (t, 3H, $J=7.4$ Hz), 1.62–1.71 (m, 2H), 2.52 (t, 2H, $J=7.3$

Hz), 6.54 (d, 1H, $J=13.6$ Hz), 7.32 (d, 1H, $J=13.6$ Hz). $^{13}\text{C-NMR}$ $\delta_{\text{max}}/\text{ppm}$: 13.2, 16.8, 42.7, 132.2, 136.1, 197.2.

β -Phenoxyvinyl-propyl-ketone (9)

To the solution of phenol (6.42 g, 68 mmol) in NaOH (2.72 g, 68 mmol) as 20% aqueous solution, β -chlorovinyl-propyl-ketone (9.95 g, 75 mmol) was added over a 0.5 h period, maintaining room temperature. After additional 5 h of stirring at the same temperature, the reaction mixture was extracted with diisopropylether (3×20 mL). Organic extracts were washed with 10% NaOH (2×20 mL), then with water, dried, filtered and evaporated. Crude product was distilled at 123–125 °C/4 mmHg to give 9.04 g (70%) of pure **9**. IR $\nu_{\text{max}}/\text{cm}^{-1}$: 3080, 2950, 1695, 1580, 1230, 760. $^1\text{H-NMR}$ $\delta_{\text{max}}/\text{ppm}$: 0.94 (t, 3H, $J=7.4$ Hz), 1.58–1.72 (m, 2H), 2.45 (t, 2H, $J=7.3$ Hz), 5.93 (d, 1H, $J=12.2$ Hz), 6.51 (d, 1H, $J=13.6$ Hz), 7.00–7.79 (m, 5H). $^{13}\text{C-NMR}$ $\delta_{\text{max}}/\text{ppm}$: 13.4, 17.4, 43.3, 110.5, 117.8, 124.8, 129.4, 129.8, 158.0, 199.7.

1-Phenoxy-3-hexanone (10)

Compound **9** (3.42 g, 18 mmol) was dissolved in dry diethylether (7 mL), 5% Pd/BaSO₄ (0.117 g) was added, and hydrogenation performed at atmospheric hydrogen pressure. After the 7 h reaction was completed, the catalyst was filtered off, solvent evaporated and the crude product purified by chromatography on silicagel with dichloromethane as eluent, then distilled at 126–127 °C/4 mmHg. 3.15 (91%) of pure **10** was obtained. IR $\nu_{\text{max}}/\text{cm}^{-1}$: 3040, 2960, 1715, 1600, 1245, 755. $^1\text{H-NMR}$ $\delta_{\text{max}}/\text{ppm}$: 0.93 (t, 3H, $J=7.4$ Hz), 1.57–1.70 (m, 2H), 2.47 (t, 2H, $J=7.3$ Hz), 2.86 (t, 2H, $J=6.4$ Hz), 4.23 (t, 2H, $J=6.4$), 6.88 (d, 2H, $J=7.8$ Hz), 6.94 (dd, 1H, $J_1=7.3$ Hz, $J_2=7.4$ Hz), 7.25 (dd, 2H, $J_1=6.6$ Hz, $J_2=6.7$ Hz). $^{13}\text{C-NMR}$ $\delta_{\text{max}}/\text{ppm}$: 13.4, 16.7, 41.8, 45.1, 62.7, 114.4, 120.8, 129.4, 158.5, 208.9.

(±)-1-Phenoxy-3-hydroxyhexane (11)

Compound **10** (2.5 g, 13 mmol) was reduced in MeOH (30 mL) with NaBH₄ (0.5 g, 13 mmol) as described for **6**. Upon the usual work-up and purification, pure **11** was obtained (2.37 g, 94%). IR $\nu_{\text{max}}/\text{cm}^{-1}$: 3400, 2960, 1600, 1240, 750. $^1\text{H-NMR}$ $\delta_{\text{max}}/\text{ppm}$: 0.94 (t, 3H, $J=6.9$ Hz), 1.32–1.57 (m, 4H), 1.80–2.01 (m, 2H), 3.87–3.91 (m, 1H), 4.05–4.21 (m, 2H), 6.86 (d, 2H, $J=7.4$ Hz), 6.92 (dd, 1H, $J_1=7.4$ Hz, $J_2=7.8$ Hz), 7.27 (dd, 2H, $J_1=7.4$ Hz, $J_2=7.1$ Hz). $^{13}\text{C-NMR}$ $\delta_{\text{max}}/\text{ppm}$: 13.8, 18.5, 36.1, 39.5, 65.7, 69.8, 114.4, 120.8, 129.4, 158.6.

(±)-1-Phenoxy-3-acetoxyhexane (12)

Starting from **11** (0.68 g, 6 mmol) and acetylchloride (1.4 g, 18 mmol) in pyridine (7 mL), compound **12** (0.77 g, 93%) was obtained as described for **7**. IR $\nu_{\text{max}}/\text{cm}^{-1}$: 3040, 2960, 1740, 1600, 1235, 755. $^1\text{H-NMR}$ $\delta_{\text{max}}/\text{ppm}$: 0.93 (t, 3H, $J=7.3$ Hz), 1.29–1.68 (m, 4H), 1.95–2.00 (m, 2H), 2.03 (s, 3H), 3.98 (t, 2H, $J=6.7$ Hz), 5.07–5.28 (m, 1H), 6.88 (d, 2H, $J=7.8$ Hz), 7.01 (dd, 1H, $J_1=7.4$ Hz, $J_2=8.3$ Hz), 7.26 (dd, 2H, $J_1=8.0$ Hz, $J_2=8.6$ Hz). $^{13}\text{C-NMR}$ $\delta_{\text{max}}/\text{ppm}$: 13.7, 18.2, 20.9, 33.6, 36.3, 64.2, 71.3, 114.4, 120.7, 129.4, 158.8, 170.8. *Anal.* Calcd. for C₁₄H₂₀O₃ (236.31): C 71.16, H 8.53%; found: C 71.13, H 8.68%.

4-Phenoxybutyronitril (13)

To the solution of phenol (15.08 g, 0.16 mol) in EtOH (200 mL), 4-chlorobutyronitril (21.1 g, 0.20 mol) was added, and then a solution of KOH (14.05 g, 0.25 mmol)

in MeOH (45 mL) was added dropwise over 15 min. The reaction mixture was heated under reflux for 48 h, then cooled, pH was adjusted to neutral by addition of conc. HCl, and the solvent was evaporated, water (100 mL) was added to the residue and the resulting slurry was extracted with dichloromethane (3 × 30 mL). On drying, and evaporation of the solvent, the crude product was purified by chromatography on silicagel with dichloromethane as eluent, then distilled. 18.63 g (73%) of pure **13**, was obtained b.p. 82–84 °C/0.02 mmHg. IR $\nu_{\max}/\text{cm}^{-1}$: 3070, 2920, 2240, 1600, 1245, 760. $^1\text{H-NMR}$ δ_{\max}/ppm : 2.06–2.15 (m, 2H), 2.56 (t, 2H, $J=7.1$ Hz), 4.04 (t, 2H, $J=5.7$ Hz), 6.89 (d, 2H, $J=7.9$ Hz), 6.96 (dd, 1H, $J_1=7.3$ Hz, $J_2=7.4$ Hz), 7.27 (dd, 2H, $J_1=7.7$ Hz, $J_2=8.5$ Hz). $^{13}\text{C-NMR}$ δ_{\max}/ppm : 13.8, 25.1, 64.9, 114.1, 119.0, 120.8, 129.2, 158.0. *Anal.* Calcd. for $\text{C}_{10}\text{H}_{11}\text{ON}$ (161.20): C 74.51, H 6.88, N 8.69%; found: C 74.67, H 6.84, N 8.61%.

1-Phenoxy-4-octanone (14)

n-Butylbromide (15.3 g, 0.111 mol) was added dropwise for 1 h to the slurry of Mg (2.7 g, 0.111 mol) and I_2 crystal in dry diethylether (30 mL). The reaction mixture was heated under reflux for 2 h, then cooled and the solution of compound **13** (3.0 g, 19 mmol) in toluene (30 mL) was added dropwise. Upon completed addition, the reaction mixture was heated at *ca.* 65 °C for 2.5 h, then cooled, 10% aq. HCl (60 mL) was added and the reaction mixture briefly heated at *ca.* 60 °C. The reaction solution was extracted with dichloromethane (3 × 50 mL), combined organic extracts were dried, evaporated, and the crude product was purified first by chromatography on silicagel with dichloromethane as eluent, then by distillation. 2.86 g (70%) of pure **14**, was obtained, b.p. 112–113 °C/0.4 mmHg. IR $\nu_{\max}/\text{cm}^{-1}$: 3040, 2940, 1720, 1600, 1245, 750. $^1\text{H-NMR}$ δ_{\max}/ppm : 0.90 (t, 3H, $J=7.3$ Hz), 1.24–1.41 (m, 2H), 1.51–1.68 (m, 2H), 2.00–2.10 (m, 2H), 2.42 (t, 2H, $J=7.4$ Hz), 2.62 (t, 2H, $J=7.1$ Hz), 3.96 (t, 2H, $J=6.0$ Hz), 6.88 (d, 2H, $J=7.8$ Hz), 6.93 (dd, 1H, $J_1=7.4$ Hz, $J_2=7.4$ Hz), 7.27 (dd, 2H, $J_1=7.4$ Hz, $J_2=6.7$ Hz). $^{13}\text{C-NMR}$ δ_{\max}/ppm : 13.6, 22.1, 23.2, 25.8, 38.7, 42.5, 66.6, 114.4, 120.6, 129.4, 158.9, 210.8. *Anal.* Calcd. for $\text{C}_{14}\text{H}_{20}\text{O}_2$ (220.31): C 76.32, H 9.15%, found: C 76.41, H 8.99%.

(±)-1-Phenoxy-4-hydroxyoctane (15)

Starting from compound **14** (1.46 g, 7 mmol), compound **15** was prepared by the same procedure as described for **6**. Upon the usual work-up and purification, 1.40 g (95%) of pure **15** was obtained, b.p. 113–114 °C/0.11 mmHg. IR $\nu_{\max}/\text{cm}^{-1}$: 3380, 3040, 2930, 1600, 1240, 750. $^1\text{H-NMR}$ δ_{\max}/ppm : 0.91 (t, 3H, $J=7.0$ Hz), 1.21–1.69 (m, 6H), 1.70–2.00 (m, 4H), 3.62–3.70 (m, 1H), 3.99 (t, 2H, $J=5.5$ Hz), 6.90 (d, 2H, $J=8.6$ Hz), 6.96 (dd, 1H, $J_1=7.3$ Hz, $J_2=9.7$ Hz), 7.27 (dd, 2H, $J_1=8.4$ Hz, $J_2=7.5$ Hz). $^{13}\text{C-NMR}$ δ_{\max}/ppm : 13.8, 22.5, 25.4, 27.7, 33.9, 37.1, 67.8, 71.5, 114.4, 120.6, 129.4, 158.9. *Anal.* Calcd. for $\text{C}_{14}\text{H}_{22}\text{O}_2$ (222.33): C 75.63, H 9.90%; found: C 75.84, H 9.81%.

(±)-1-Phenoxy-4-acetoxyoctane (16)

Starting from compound **15** (0.5 g, 2.25 mmol), compound **16** was prepared by the same procedure as described for **12**. Upon the usual work-up and purification, 0.56 g (95%) of pure **16** was obtained. IR $\nu_{\max}/\text{cm}^{-1}$: 3040, 2960, 1740, 1605, 1245, 755. $^1\text{H-NMR}$ δ_{\max}/ppm : 0.88 (t, 3H, $J=6.6$ Hz), 1.23–1.34 (m, 4H), 1.50–1.69 (m, 2H), 1.72–1.99 (m, 4H), 2.04 (s, 3H), 3.94 (t, 2H, $J=5.7$ Hz), 4.90–4.98 (m, 1H), 6.88 (d, 2H, $J=8.8$ Hz), 6.94 (dd, 1H, $J_1=7.3$ Hz, $J_2=8.6$ Hz), 7.27 (dd, 2H, $J_1=8.5$ Hz,

$J_2=7.4$ Hz). $^{13}\text{C-NMR}$ $\delta_{\text{max}}/\text{ppm}$: 13.8, 21.1, 22.3, 25.0, 27.2, 33.6, 67.2, 73.6, 114.2, 120.4, 129.2, 158.7, 170.7. *Anal.* Calcd. for $\text{C}_{16}\text{H}_{24}\text{O}_3$ (264.37): C 72.69, H 9.15%; found: C 72.92, H 9.22%.

5-Phenoxyvaleronitril (**17**)

Starting from phenol (3.3 g, 35 mmol) and 5-chlorovaleronitril (5.26 g, 44 mmol), compound **17** was prepared according to the method described for **13**. Upon the usual work-up and purification, 4.66 g (76%) of pure **17** was obtained, b.p. 160–162 °C/0.7 mmHg. IR $\nu_{\text{max}}/\text{cm}^{-1}$: 3040, 2940, 2240, 1600, 1240, 755. $^1\text{H-NMR}$ $\delta_{\text{max}}/\text{ppm}$: 1.82–1.96 (m, 4H), 2.43 (t, 2H, $J=6.7$ Hz), 3.99 (t, 2H, $J=5.5$ Hz), 6.88 (d, 2H, $J=8.6$ Hz), 6.95 (dd, 1H, $J_1=7.5$ Hz, $J_2=7.2$ Hz), 7.28 (dd, 2H, $J_1=7.4$ Hz, $J_2=8.0$ Hz). $^{13}\text{C-NMR}$ $\delta_{\text{max}}/\text{ppm}$: 16.7, 22.2, 27.9, 66.3, 114.3, 119.4, 120.8, 129.4, 158.6.

1-Phenoxy-5-decanone (**18**)

Starting from compound **17** (3.0 g, 17 mmol), and *n*-pentylbromide (15.52 g, 103 mmol, Aldrich), compound **18** were prepared by the method described for **14**. Upon the usual work-up and purification, 3.16 g (75%) of pure **18** was obtained, m.p. 28–29 °C. IR $\nu_{\text{max}}/\text{cm}^{-1}$: 3040, 2930, 1710, 1600, 1240, 750. $^1\text{H-NMR}$ $\delta_{\text{max}}/\text{ppm}$: 0.89 (t, 3H, $J=6.8$ Hz), 1.20–1.34 (m, 4H), 1.52–1.62 (m, 2H), 1.71–1.77 (m, 4H), 2.39 (t, 2H, $J=7.5$ Hz), 2.47 (t, 2H, $J=6.4$ Hz), 3.94 (t, 2H, $J=5.8$ Hz), 6.88 (d, 2H, $J=8.4$ Hz), 6.92 (dd, 1H, $J_1=6.7$ Hz, $J_2=7.2$ Hz), 7.26 (dd, 2H, $J_1=7.5$ Hz, $J_2=8.2$ Hz). $^{13}\text{C-NMR}$ $\delta_{\text{max}}/\text{ppm}$: 13.6, 20.1, 22.1, 23.2, 28.4, 31.1, 41.9, 42.5, 67.1, 114.2, 120.4, 129.3, 158.8, 211.0.

(±)-1-Phenoxy-5-hydroxydecane (**19**)

Starting from compound **18** (1.5 g, 6 mmol), compound **19** was prepared by the same procedure as described for **15**. Upon the usual work-up and purification, 1.40 g (93.3%) of pure **19** was obtained, m.p. 31–32 °C. IR $\nu_{\text{max}}/\text{cm}^{-1}$: 3400, 2940, 1600, 1245, 750. $^1\text{H-NMR}$ $\delta_{\text{max}}/\text{ppm}$: 0.89 (t, 3H, $J=6.6$ Hz), 1.28–1.40 (m, 4H), 1.44–1.68 (m, 8H), 1.71–1.80 (m, 2H), 3.54–3.61 (m, 1H), 3.96 (t, 2H, $J=6.4$ Hz), 6.89 (d, 2H, $J=7.8$ Hz), 6.94 (dd, 1H, $J_1=7.3$ Hz, $J_2=7.2$ Hz), 7.27 (dd, 2H, $J_1=6.7$ Hz, $J_2=6.3$ Hz). $^{13}\text{C-NMR}$ $\delta_{\text{max}}/\text{ppm}$: 13.8, 22.0, 22.4, 25.1, 29.1, 31.7, 36.9, 37.3, 67.5, 71.7, 114.4, 120.5, 129.4, 159.0. *Anal.* Calcd. for $\text{C}_{16}\text{H}_{26}\text{O}_2$ (250.38): C 76.75, H 10.47%; found: C 76.87, H 10.32%.

(±)-1-Phenoxy-5-acetoxydecane (**20**)

Starting from compound **19** (0.5 g, 2 mmol), compound **20** was prepared by the same procedure as described for **16**. Upon the usual work-up and purification, 0.56 g (95.9%) of pure **20** was obtained. IR $\nu_{\text{max}}/\text{cm}^{-1}$: 3040, 2940, 1740, 1600, 1245, 750. $^1\text{H-NMR}$ $\delta_{\text{max}}/\text{ppm}$: 0.88 (t, 3H, $J=6.6$ Hz), 1.28–1.49 (m, 6H), 1.51–1.70 (m, 6H), 1.73–1.83 (m, 2H), 2.04 (s, 3H), 3.94 (t, 2H, $J=6.3$ Hz), 4.88–4.92 (m, 1H), 6.88 (d, 2H, $J=8.7$ Hz), 6.94 (dd, 1H, $J_1=7.4$ Hz, $J_2=7.6$ Hz), 7.27 (dd, 2H, $J_1=8.4$ Hz; $J_2=7.5$ Hz). $^{13}\text{C-NMR}$ $\delta_{\text{max}}/\text{ppm}$: 13.7, 21.0, 21.7, 22.3, 22.4, 24.7, 28.9, 31.4, 33.6, 33.8, 67.3, 114.4, 120.4, 129.3, 159.0, 171.0. *Anal.* Calcd. for $\text{C}_{18}\text{H}_{28}\text{O}_3$ (292.42): C 73.93, H 9.65%; found: C 74.18, H 9.77%.

Lipase catalyzed acetylations

Screening of microbial lipases with *rac*-substrates **6**, **11**, **15** and **19** was performed with 10 mg of lipase and 10 mg of substrate in *n*-hexane (5 mL). Upon the

mixing of these components, the reaction mixture was brought to 30 ± 0.5 °C in a thermostated shaker and the reaction was initiated by addition of vinylacetate (1 mL). Samples (30 mL) were taken at regular time intervals, diluted with the mobile HPLC-phase (1 mL), filtered through teflon-filter and analyzed on a reverse phase column. The results are presented in Figures 2, 3 and in Table II.

*Preparative scale acetylation catalyzed
by Geotrichum candidum lipase.*

To the solution of *rac* substrates **6**, **11**, **15** and **19** (0.25–1.0 g) in *n*-hexane (50–200 mL), GCL (0.25–1.0 g, from Amano) was added, the reaction mixture was thermostated in a shaker at 30 ± 0.5 °C, and vinylacetate (5–20 mL) was added. After

TABLE IV

Enantioselectivity parameters for kinetic resolution of (\pm)-**6**, (\pm)-**11**, (\pm)-**15** and (\pm)-**19** by acetylation catalyzed by lipase from *Geotrichum candidum* in *n*-hexane

Compd.	<i>t</i> (h)	Conv. (%)	<i>e.e.</i> (%) alcohol	<i>e.e.</i> (%) acetate	$[\alpha]_D$ alcohol	$[\alpha]_D$ acetate	<i>E</i>
	3.5	5.93					
(\pm)- 6	24	21.10	24.4	91.4	+7.99 (<i>c</i> 1.13 CH ₂ Cl ₂)	+50.7 (<i>c</i> 1.12 CH ₂ Cl ₂)	28.2
(\pm)- 11	24	23.97	18.51	59.22	+0.92 (<i>c</i> 1.08 CH ₂ Cl ₂)	-9.38 (<i>c</i> 1.07 CH ₂ Cl ₂)	4.7
	4	10.22					
(\pm)- 15	24	35.26	33.2	61.0	+1.85 (<i>c</i> 1.08 CH ₂ Cl ₂)	+0.93 (<i>c</i> 1.08 CH ₂ Cl ₂)	5.7
	5.5	29.67					
(\pm)- 19	24	49.98	83.3	83.4	-2.73 (<i>c</i> 1.10 CH ₂ Cl ₂)	0.00 (<i>c</i> 1.07 CH ₂ Cl ₂)	28.6

TABLE V

Retention times of alcohols and acetates on achiral and chiral HPLC columns

Compd. (racemic)	Reverse phase Mobile phase MeOH:H ₂ O	column C18 Retention time (min.)	Chiral HPLC columns	Mobile phase <i>n</i> -hexane:2-PrOH	Retention time (min.)	
					<i>R</i>	<i>S</i>
6	85:15	3.93	Chiralcel OB-H	98:2	33.66	35.84
7	85:15	5.05	Chiralcel OB-H	98:2	27.66	22.81
11	85:15	4.27	Chiralcel OJ	97:3	37.36	30.08
12	85:15	5.36	Chiralcel OJ	97:3	19.10	22.10
15	89.5:10.5	4.94	Chiralcel OD	90:10	19.78	23.74
16	89.5:10.5	6.95	Chiralcel OB-H	90:10	14.66	11.88
19	92.5:7.5	5.07	Chiralcel OD	90:10	14.43	16.83
20	92.5:7.5	7.18	Chiralcel OD	90:10	9.69	13.12

24 h, the reaction was stopped, and conversions presented in Table IV were determined on the HPLC column as described for screening experiments. The same table contains also other data (*e.e.*, $[\alpha]_D$, and *E*) obtained in the preparative experiment. Enzyme was separated by filtration, the solvent evaporated to dryness, and the reaction mixture, alcohol and acetate, separated on a silicagel column with dichloromethane as eluent. *Ca.* 90–95% separation of alcohols and acetates was usually achieved when the ratio silicagel:product mixture was 50:1 (g/g). Retention times for alcohols and acetates, as determined on the chiral columns, are presented in Table V.

Calculations

E-values were calculated according to Sih *et al.*¹⁶ using selective Mac 1.0 program. The conformational space of **6**, **11**, **15** and **19** was searched by using the systematic search mode contained in the SYBYL package of TRIPOS INC. Low energy conformations were selected and fully minimized.

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SAŽETAK

Utjecaj udaljene skupine na enantioselektivnost u kinetičkoj rezoluciji *sec*-alkohola kataliziranoj mikrobnim lipazama

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Pripravljen je kraći niz racemičnih fenoksialkil-alkil karbinola koji posjeduju ometajuću skupinu na različitoj udaljenosti od stereogenog centra. Sintetizirani su slijedeći *sec*-alkoholi: (\pm)-1-fenoksi-2-hidroksibutan (**6**), (\pm)-1-fenoksi-3-hidroksiheksan (**11**), (\pm)-1-fenoksi-4-hidroksioktan (**15**) i (\pm)-1-fenoksi-5-hidroksidekan (**19**). Određena je enantioselektivnost njihova acetiliranja vinilacetatom, kataliziranoga mikrobnim lipazama u *n*-heksanu, u spojeve (\pm)-1-fenoksi-2-acetoksibutan (**7**), (\pm)-1-fenoksi-3-acetoksiheksan (**11**), (\pm)-1-fenoksi-4-acetoksioktan (**15**), (\pm)-1-fenoksi-5-acetoksidekan (**19**). Djelotvornost kinetičke rezolucije, izražena kao *E*-vrijednost, općenito opada s udaljenošću ometajuće fenoksi-(R_L) i metilne (R_M) skupine. Ispitana je enantioselektivnost dvadeset lipaza iz komercijalnih izvora; za dvije lipaze s najširokom selektivnošću za supstrate, *Geotrichum candidum* (GCL) i *Candida cylindracea* (CCL(S), proizv. Sigma), opažena je nemonotona korelacija između *E*-vrijednosti i udaljenosti (*n*) ometajuće skupine. Za GCL lipazu zapažen je neočekivan zaokret u enantioselektivnosti od pretežnog acetiliranja (*R*)-enantiomera spojeva **6**, **11** i **15** u pretežno acetiliranje (*S*)-enantiomera spoja **19**, što upućuje da u posljednjem slučaju relativni sterički zahtjevi udaljenih skupina ne kontroliraju smjer enantioselektivnosti. Taj rezultat koreliran je s ranije opaženom stereoselektivnošću acetiliranja (*S*)-sekundarnih alkohola (3*S*,7*S*)-*trans*-3,4,5,6,9,10-oktahidro-7,14,16-trihidroksi-3-metil-1*H*-2-benzoksaciklotetradeken-1-on (**1**) i (3*S*,7*S*)-3,4,5,6,9,10,11,12-dekahidro-7,14,16-trihidroksi-3-metil-1*H*-2-benzoksa ciklotetradekan-1-on (**3**), makrocikličkih derivata rezorcilne kiseline, kao i s rezultatima MM2 računa za specifične konformacije supstrata.