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Correlation between distance of the perturbing groups and enantioselectivity of the lipase catalyzed acetylation of acyclic *sec* alcohols

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Abstract: Study of kinetic resolutions of racemic *sec* alcohols **1–4**, by acetylation to **5–8** with a series of microbial lipases in *n*-hexane, revealed the broadest selectivity of *Geotrichum candidum* (GCL) and *Candida cylindracea* (CCL(S), from Sigma) lipase in accepting these conformationaly flexible substrates. Surprisingly, for both lipases non-monotonous correlation between *E*-value and the distance (*n*) of perturbing groups in **1–4** is observed. GCL preferred small and large substrates, whereas CCL preferred medium-size substrates. For GCL lipase a remarkable turnover of enantioselectivity was observed on going from **1–3** to **4**, revealing that relative steric requirements of the larger phenoxy group *vs.* smaller methyl group does not control the enantioselective bias in the latter. For *Candida cylindracea* (CCL(A), from Amano) lipase the conversion and *E* vary in a monotonous fashion from smaller to larger substrates. © 1997, Elsevier Science Ltd. All rights reserved.

Numerous studies have shown that lipases poorly resolve secondary alcohols with two similarly-sized substituents, but they resolve these alcohols efficiently when the size of one substituent is increased.[1–3] This empirical rule provides the strategy to increase the enantioselectivity of these reactions. However, it neither indentifies restrictions due to conformational mobility, e.g. in cyclic vs. acyclic substrates, nor the optimal distance of two perturbing groups needed for high enantiorecognition by a specific lipase. The understanding of these parameters would greatly help synthetic chemists in the rational design of the substrates for enantioseparation by the lipase catalyzed kinetic resolution.

Most of the present models for enantiorecognition are sketchy presentations of the lipase active site in an attempt to explain different degrees of accomodation for particular classes of substrates.[4–7] Recently we used the model proposed by Naemura et al.[8] in explaining structural effects on the enantioselectivity of acylation of 4-hydroxychromans by PFL lipase.[9] Chiroptical (CD) determination of absolute configuration and conformation of their homochiral derivatives fit to this model for the reactive conformation of the lipase active site.[10] Many challenge the simplicity of such models, however, and more sophisticated approaches are being developed. One is based on the computer assisted modelling of the interaction between enantiomers of a chiral substrate and the lipase active site. To this aim a concept of 'the number of the close contacts', has been developed by Klibanov et al.[11]

These approaches, however, can not take into account conformational mobility of the substrates, though it is known that various ligands bind to their protein sites in a diffusive motion. We have shown that conformational properties of some specific substrates, macrocyclic lactones derived from resorcylic acid, determine stereoselectivity of their hydrolysis and acylation by various microbial lipases, and proposed a 'helical model' for stereoselectivity.[12,13] It underlines the importance of *absolute conformation* of the enantiomeric substrates. In order to prove the effect of conformational

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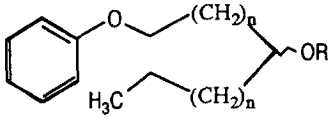
mobility of the substrate on the lipase enantioselection, we entered synthetic and enzymatic studies with simple, specifically designed substrates of general formulae **1–4**. They could be regarded as the open-chain analogues of **II**, which are characterized by more a restricted conformational mobility. Chirality of the latter rises the absence of σ plane because of the presence of an oxygen atom in the ring.

Herewith we report on the results of study of the enantioselective acetylation of **1–4** with a series microbial lipases. Synthesis of **II**, which requires tedious closure of the medium-large rings, study of their enantioselective acylation, and computer-assisted analysis of conformational characteristics of compounds **1–4** and **II** are in hand.

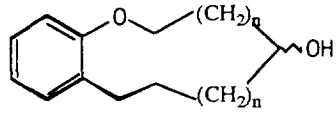
Racemic alcohols **1**, **3** and **4** are prepared *via* intermediary ketones, obtained from ω-phenoxynitriles and alkyl-Grignard reagents, followed by reduction ($NaBH_4$/MeOH). Compound **2** is prepared from propyl-β-chlorovinyl ketone, obtained from butyric acid chloride and acetylene in the presence of $AlCl_3$,[14] followed by O-alkylation of phenole and final reduction of double bond and carbonyl group.



(±)-R

(±)-**1–4** H n=0, 1, 2, 3 respectively

(±)-**5–8** $COCH_3$ n=0, 1, 2, 3 respectively



(±)-**II**

Baseline chromatographic separation of the enantiomers of (±)-**1–4** and their acetates (±)-**5–8** was achieved on chiral HPLC columns as follows; for **1**, **5** and **7** on Chiralcel OB-H column with 2–10% 2-PrOH in *n*-hexane, for **2** and **6** on Chiralcel OJ with 3% 2-PrOH in *n*-hexane, and for **3**, **4** and **8** on Chiralcel OD with 10% 2-PrOH in *n*-hexane. Under these conditions determination of the e.e.'s. in Table 1 was performed. Enzymatic kinetic resolution of (±)-**1–4** was performed *via* acetylation by vinylacetate in *n*-hexane, in a thermostated shaker at 30±0.5°C. Progress curves for acetylation were determined by HPLC on a reverse phase column C18 RCM (8×10, Waters).

Screening of 20 microbial lipases with **1–4** revealed discrete acetylation activity for five of them as, presented in the Table 1. Their enantioselectivities are expressed as *E*-values, which are, unlike e.e.'s, time-independent and therefore a true measure of enantioselectivity. They are calculated on the bases of conversion (c) and e.e. values, according to Sih et al.[15], using Selective Mac 1.0 programme.

All data in Table 1 are obtained for the substrate/enzyme ratio 1:1 (w/w) at 10–20 mg scale. When these experiments were repeated with GCL on the preparative scale (250–500 mg of **1–4**), other parameters being the same, only small variations of conversion and *E* value is noticed.

As shown in the Table 1, GCL and CCL (A and S) acylate all substrates, whereas CCL (F, from Fluka) acylates only **2** and **4**. Two lipases from *Pseudomonas fluorescens* and *species* (PFL and PSL) acylate only the smallest substrate **1** with high conversion and *E*. This substrate is also accepted by *Mucor miehei* lipase (MML), and with very low *E* also by PCL. Interestingly, medium-large substrates **2** and **3** are accepted by the least number of lipases. There is an expected, general decline of enantioselectivity with enlarged distance of the two perturbing groups from the stereogenic center in the substrates.

Figure 1. shows the progress curves for acetylation of the smallest **1** and the largest substrate **4** with three representative lipases. The outcome roughly fits the rule that CCL is liberal in accepting relatively large substrates, whereas both PFL and MML are often highly selective on substrates with limited steric requirements and hence usually unable to accept bulky substrates.[16] Distinctly different trends in enantioselection can be observed for GCL and CCL, two lipases with broadest selectivity for the series of investigated substrates. Both exhibit non-monotonous variation of the *E*-value with *n*, the former shows minimum enantioselectivity for the medium substrates **2** and **3**, the latter for the substrates **1**, **2** and **4**, Figure 2.

According to generally accepted rule for enantioselective acylation by lipases, based on the

Table 1. Enantioselectivity parameters for kinetic resolution of **1–4** by acetylation catalyzed by microbial lipases in *n*-hexane

Compd.	Lipase	Conversion[a] (%)	e.e.(%) alcohol	$[\alpha]_D$[b]	e.e.(%) acetate	*E*
	PFL	57.2	99.9		74.8	49.9
	PSL	52.2	89.5		82.0	30.2
	CCL(A)	40.6	22.7		33.2	2.5
1	GCL	21.4	24.1	+33.2	88.8	21.3
	MML	20.8	24.3		92.6	33.0
	CCL(S)	18.4	1.8		8.0	1.2
	CCL(A)	38.2	32.5		54.6	4.7
2	GCL	27.3	22.5	+4.1	55.8	4.3
	CCL(S)	26.9	22.6		60.1	5.0
	CCL(F)	18.3	13.4		61.5	4.8
	GCL	42.4	41.7	+4.4	56.5	5.4
3	CCL(A)	37.3	36.5		61.3	5.9
	CCL(S)	31.7	40.0		86.3	20.1
	GCL	42.9	56.8	–4.8	75.7	12.7
	CCL(A)	26.4	24.0		67.0	6.4
4	CCL(S)	18.0	16.0		72.6	7.4
	PSL	13.1	5.5		3.6	1.1
	CCL(F)	11.4	7.6		59.1	4.2

[a] Determined by HPLC after 48 hrs, [b] Determined in CH_2Cl_2 and calcd. for 100% e.e.

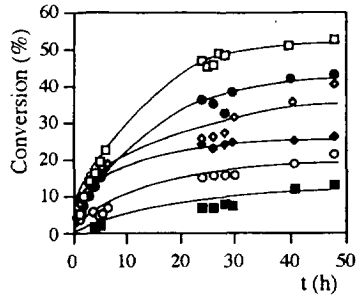


Figure 1. Progress curves for acetylation of **1** with: □–□–□ PSL, ◇–◇–◇ CCL (A), ○–○–○ GCL, and **4** with: ■–■–■ PSL, ◆–◆–◆ CCL (A), ●–●–● GCL

accumulated evidence form the literature,[1,5,6] (+) alcohols **1–4** should possess the *R*-configuration.[17] The [α] values and the eluation order of (+) alcohols on the chiral columns reveal that CCL recognizes phenoxy group as the larger one in the compounds **1–4**, *i.e.* preferentially acylates R-(−)-alcohols. GCL also prefers (−)-enantiomers of alcohols **1–3**, but (+)-enantiomer of **4**. Such surprising inversion of enantioselection from **3** to **4**, accompanied by enhancement of the *E* value, reveals that, when distant, relative steric requirements of the large and medium group vanish as decisive factor for enantioselective bias.

In conclusion, we can state that enantioselective acetylation of a short series of substrates **1–4**, representatives of 'thin', conformationally mobile molecules, for most lipases revealed an expected

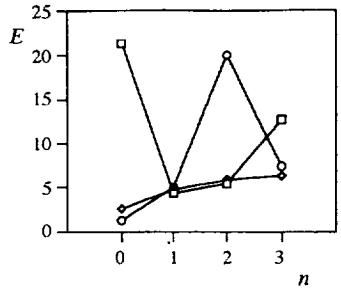


Figure 2. Correlation between E and n for acetylation with: □–□–□ GLC, ◇–◇–◇ CCL (A), and ○–○–○ CCL (S).

trend of general decrease of enantioselectivity with distance of the perturbing groups from the chiral center.

Significant deviations from linear *E/n* correlation for GCL and CCL(S), and turnover of the enantioselectivity of GCL on going from **3** to **4**, indicate specific conformational requirements at the active site of these lipases, underlying the importance of conformational properties of the substrates for correlation of the structure of the active site and recognition of the more reactive enantiomer.

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17. Determination of absolute configuration for (+)-alcohols **1–4** is in due course.

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