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ORIGINAL ARTICLE

Conversion of a pentane-1,3,5-triol derivative using lipases as chiral catalysts and possible function of the lid for the regulation of substrate selectivity and enantioselectivity

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Abstract

The enantioselective desymmetrization of a prochiral 3-*O*-silyl protected pentane-1,3,5-triol derivative was achieved by lipase-catalysed hydrolysis. The lipase from *B. cepacia* led to 95.4% enantiomeric excess (ee)of the (*R*)-configured compound (*R*)-**4** at a theoretical yield of 79% and was isolated with 98.2% ee and 27% yield. Furthermore, it was found that the ee switched from an excess of (*R*)-**4** to an excess of (*S*)-**4** during the course of the reaction using crude lipase from *C. rugosa* under the same conditions. This finding was investigated in detail and compared to the change of substrate selectivity known for the lipase from *M. miehei*. It is supposed that both phenomena may result from a movement of the lid.

Keywords: *lipase, enantioselectivity, pentane-1,3,5-triol, lid*

Introduction

Lipases are classified as hydrolases, as they are predominantly responsible for the catalytic hydrolysis of triglycerides, which are the main components of nutritional and storage fat. The hydrolysis of triglycerides provides free fatty acids that serve as a source of metabolic energy. Lipases have been extensively employed in organic synthesis (Bornscheuer & Kazlauskas 2005) and used in industrial-scale conversions (Gunstone 1999; Schmid et al. 2001). Within such processes, lipases are used not only for hydrolysis of esters but also for acylation of alcohols (Schmid & Verger 1998). Particular interest is focused on enantioselective conversions, which result from the chiral environment of the active site of these enzymes (Ghanem 2007). Next to the active site, a flexible part of the protein is located, which is known as the lid (Bornscheuer & Kazlauskas 2005; Schmid & Verger 1998; Cambillau & van Tilbeurgh 1993; Secundo et al. 2006). The lids of different lipases have individual characteristics and can cover the active site to a greater or lesser extent in its closed form. It can easily switch between the open and closed conformation and thereby regulate the binding of substrates (Cygler & Schrag 1999; Grochulski et al. 1994; Overbeeke et al. 2000; Peters & Bywater 1999; Vasel et al. 1993). It is widely accepted that the lid is opened by contact of the enzyme with an oil-water interface, which is called interfacial activation (Bornscheuer & Kazlauskas 2005; Derewenda et al. 1992; Grochulski et al. 1993; Schmid & Verger 1998). Furthermore it is known that the binding site consists of two hydrophobic regions, a large hydrophobic region and the smaller stereoselectivity pocket. The latter contains the catalytic triad as the active centre of the lipase with the OH-group of serine that accepts the acyl residue within the catalytic cycle (Bornscheuer & Kazlauskas 2005; Brady et al. 1990; Dodson & Wlodawer 1998; Pleiss et al. 1998; Schmid & Verger 1998).

We are interested in the production of chiral building blocks with a pentane-1,3,5-triol scaffold for the synthesis of enantiomerically pure NMDA and σ receptor ligands. The required prochiral

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starting material pentane-1,5-diol **3** was prepared from 3-hydroxyglutarate **1** by silylation with Me_2Ph-SiCl resulting in compound **2**, which was subsequently reduced with $LiBH_4$. Whereas the racemic monoacetate *rac*-**4** was produced non-enzymatically by reaction of diol **3** with 1.1 equivalents of Ac_2O, lipases were used as chiral catalysts for the preparation of the enantiomerically pure monoacetates (*S*)-**4** and (*R*)-**4** (Figure 1). The key step of the synthesis was the enantioselective derivatization of just one of the two enantiotopic OH-groups of diol **3** or OAc-groups of diacetate **5** (Figure 1). This was achieved using two different strategies. For production of the (*S*)-configured monoacetate (*S*)-**4** diol **3** was enantioselectively acetylated by the lipase from *B. cepacia*. On the other hand, hydrolysis of diacetate **5** catalysed by the same lipase resulted in the (*R*)-configured monoacetate (*R*)-**4**, because the enzyme preferentially transformed the same enantiotopic ORgroup in both cases (Figure 2). The lipase-catalysed acetylation of diol **3** has already been intensively investigated. In Figure 3 (a, b) the course of the reaction is shown using lipase Amano PS-CII® from *Burkholderia cepacia* and isopropenyl acetate (IPA) as acylating agent in *tert*-butyl methyl ether (TBME). Under these reaction conditions the (*S*)-configured monoacetate (*S*)-**4** was produced with > 99.9% enantiomeric excess (ee) and > 50% yield (Köhler & Wünsch 2006). The lipase Fluka Lipozyme® from *Mucor miehei*, however, led to quite a different reaction course using the same reaction conditions (Figure 3c, d). Clearly the substrate selectivity of the lipase from *M. miehei* changed during the progress of the reaction. As shown in Figure 3 (c, d) during time period A the *M. miehei* lipase converted only diol **3** but not the monoacetates **4**. Then, the substrate selectivity of the enzyme

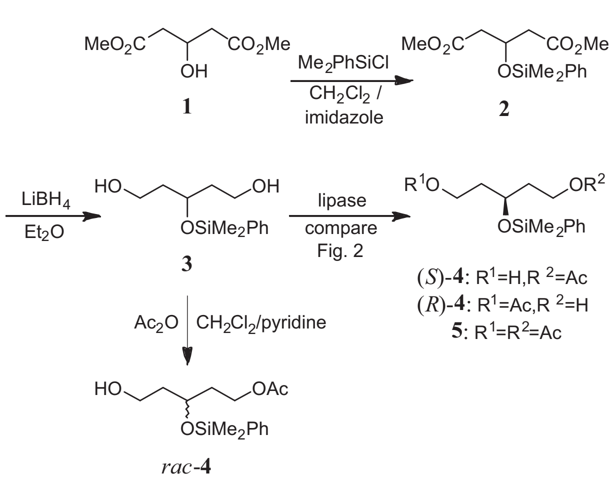

Figure 1. Synthesis of chiral building blocks with a pentane-1,3,5-triol scaffold.

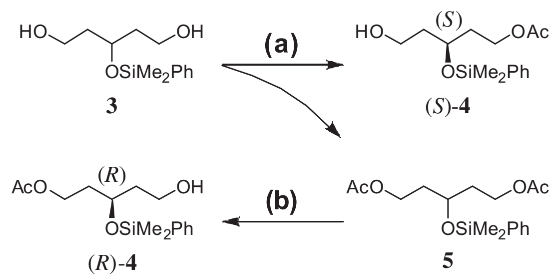

Figure 2. Reaction scheme of the lipase catalysed reactions. (a) Acetylation of diol **3** using isopropenyl acetate as acylating agent in tert-butyl methyl ether led to monoacetate (*S*)-**4** in enantiomeric excess and diacetate **5** as a side product. (b) Hydrolysis of diacetate **5** in aqueous buffer solution gave (*R*)-**4** in enantiomeric excess.

changed and during time period B both diol **3** and monoacetate **4** were converted resulting in the formation of diacetate **5**. This observation was explained by an allosteric modulation of the lipase (Köhler & Wünsch 2007). However, the causative mechanism that is responsible for the change of selectivity has not been addressed so far.

Methods

Instruments

Thin layer chromatography (tlc) was performed on Silica gel 60 F_{254} (E. Merck) and flash chromatography (fc) with Silica gel 60, 40–63 µm (E. Merck) (Still et al. 1978). HPLC-analysis was done using Merck Hitachi equipment with an UV-Detector L-7400, Autosampler L-7200, Pump L-7100 and data acquisition via HSM-Software. Optical rotation was measured with a Polarimeter 341 from Perkin–Elmer in a 1.0 dm tube at the given concentration c [g/100 mL] and 20 °C temperature (the unit [deg • mL • dm^{-1} • g^{-1}] is omitted).

Chemical compounds and lipases

Dimethyl 3-hydroxyglutarate **1** (CAS 7250-55-7) was purchased from Sigma–Aldrich® and compounds **2**, **3**, (*S*)-**4** and **5** were synthesised and characterized as described in the literature (Köhler & Wünsch 2006). The following lipases were also obtained from Sigma–Aldrich®: Amano AK (*Pseudomonas fluorescens*), Amano PS (*Burkholderia cepacia*), Amano PS-CII® (*Burkholderia cepacia*), Chirazyme L-2 (*Candida antarctica* B), Chirazyme L-3 pur. (*Candida rugosa*), Chirazyme L-5 (*Candida antarctica* A), Chirazyme L-7 (Porcine pancreas), Fluka Lipase (*Candida rugosa*) and Fluka Lipozyme® (*Mucor miehei*).

Monoacetate *rac*-**4** was produced by dissolving diol **3** (955 mg, 3.75 mmol) in CH_2Cl_2 (50 mL) and addition of pyridine (332 µL, 4.13 mmol), acetic

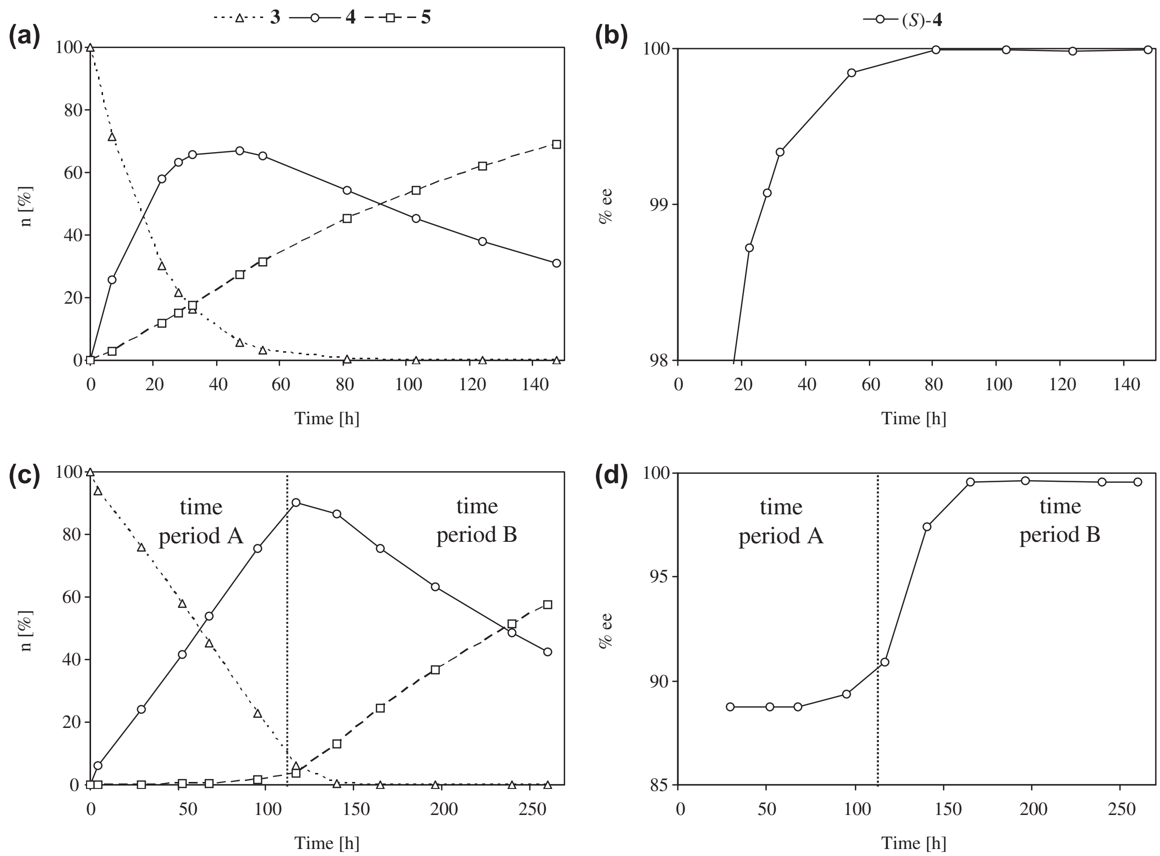

Figure 3. Reaction course of the acetylation of diol **3** carried out with lipase from *B. cepacia* (a, b) and with lipase from M. *miehei* (c, d); a, c: Amount of compounds **3**, **4** and **5** (n [%]); b, d: Enantiomeric excess of (*S*)-**4** [% ee].

anhydride (390 µL, 4.13 mmol) and a small amount of 4-(dimethylamino)pyridine. After stirring the reaction mixture for 40 h at room temperature, a saturated solution of $NaHCO_3$ (50 mL) was added. The aqueous layer was separated and extracted with CH_2Cl_2 (2 × 30 mL). The combined organic layers were dried over Na_2SO_4, concentrated *in vacuo* and the residue purified by flash chromatography (Ø 3 cm; h 20 cm; cyclohexane/EtOAc 1:1; fraction size 10 mL); R_f 0.29; colourless oil; yield 454 mg (41%). Compounds (*R*)-**6**, (*R*)-**7** and (*R*)-**8** (Figure 5) were synthesized and the CD-spectrum of (*R*)-**8** (Figure 6) recorded as described in the literature for the (*S*)-configured enantiomers (Köhler & Wünsch 2006).

HPLC methods

Analytical methods were required for separation and quantification of the involved compounds **3**, (*S*)-**4**, (*R*)-**4** and **5** (Köhler & Wünsch 2006). The ratio of diol **3**:monoacetates **4**:diacetate **5** was determined with the achiral HPLC method 1: Merck LiChrospher 100 RP-18e (5 µm), 125-4 mm; acetonitrile/water 50:50, 1 mL/min; λ = 264 nm for 16 min; **3** = 1.9 min, $Me_2PhSiOH$ = 3.0 min, **4** = 4.1 min, **5** = 12.4 min; scaling factors **3** = $Me_2PhSiOH$ = **4** = **5** = 1.00 [n (%)/area (%)]. The enantiomeric ratio of the monoacetates (*S*)-**4**:(*R*)-**4** was determined with the chiral HPLC method 2: Daicel Chiralpak AD-H (5 µm), 250-4.6 mm; n-hexane/2propanol 51:1, 1 mL/min (after 40 min the column was purged with n-hexane/2-propanol 9:1 and re-equilibrated.); λ = 264 nm for 40 min; (*S*)-**4** = 25.0 min, (*R*)-**4** = 27.5 min; scaling factors (*S*)-**4** = (*R*)-**4** = 1.000 [n (%)/area (%)].

Optimization of the reaction conditions for hydrolysis of diacetate 5 and screening of lipases

In a 50 mL round-bottomed flask the given amounts of lipase and diacetate **5** were mixed (Table I and II). Then the solvent was added and the reaction mixture was stirred intensively with a magnetic bar at room temperature. The progress of the reaction was monitored by thin layer chromatography and the reaction stopped after the given time. The reaction mixture was extracted with CH_2Cl_2, the CH_2Cl_2 layer dried over Na_2SO_4, the filtrate was concentrated in vacuo and the residue analysed by HPLC methods 1 and 2.

Table I. Optimization of the reaction conditions for the hydrolysis of diacetate **5** using lipase Amano PS-CII (*B. cepacia*) at room temperature.

Reaction conditions	Lipase *m* [mg]	Substrate 5 *m* [mg]	Time [hours]	Remaining substrate 5 *n* [%]
TBME (20 mL) + PB (20 mL)	77.3	205.2	25	93.5
CH_2Cl_2 (20 mL) + PB (20 mL)	72.6	192.6	20	100.0
acetone (20 mL) + PB (20 mL)	67.2	180.7	17	96.7
TBME (20 mL) + PB (1 mL) + H_2O (19 mL) + $CaCO_3$ (243 mg)	67.9	182.5	18	95.6
THF (40 mL) + H_2O (1 mL) + $NaHCO_3$ (429 mg)	73.3	188.3	19	99.9
CB (30 mL) + butan-1-ol (1 mL)	144.0	364.0	22	74.6
CB (30 mL) + propan-1-ol (1 mL)	141.5	376.8	24	51.0
CB (30 mL)	139.0	367.6	24	53.8

PB, phosphate buffer solution (7.5 % $Na_2HPO_4{\cdot}H_2O$); CB, carbonate buffer solution (5 % $NaHCO_3$); *m* [mg] = amount of substance [mg]; *n* [%] = relative amount of substance [%].

Reaction courses

The hydrolytic conversions were performed in a 50 mL two-necked flask. After mixing substrate and lipase, the solvent was added and the reaction mixture stirred intensively with a magnetic bar to give a homogeneous emulsion. The temperature was kept constant using a cryostat and samples were taken through a septum without stopping the stirrer to ensure that the reaction mixture was emulsified continuously. Samples of ~200 µL were taken from the reaction mixture, saturated with NaCl and extracted with ~400 µL of TBME. After separation of the layers, ~200 µL of the organic layer were filtered through a membrane filter (0.45 µm) and the solvent evaporated in a nitrogen stream. The residue was dissolved in 100 µL of acetonitrile for HPLC method 1 and in 100 µL of n-hexane/2-propanol 9:1 for HPLC method 2. The course of the reaction displayed in Figure 4 resulted from reaction of diacetate **5** (360.7 mg, 1.07 mmol) using lipase Amano PS-CII® from *Burkholderia cepacia* (360.4 mg) in $NaHCO_3$-solution (5%, 40 mL) at + 3 °C. Figure 7 shows the reaction course with diacetate **5** (361.5 mg, 1.07 mmol) and Fluka lipase from *Candida rugosa* (360.4 mg) in $NaHCO_3$-solution (5%, 30 mL) at room temperature. An additional amount of diacetate **5** (181.5 mg, 0.536 mmol) was added to the reaction mixture after 53.0 h. The reaction course shown in Figure 8 was recorded using the same lipase from *Candida rugosa* (151.4 mg) and monoacetate *rac*-**4** (184.8 mg, 0.623 mmol) as substrate in $NaHCO_3$-solution (5%, 15 mL) at room temperature.

Synthesis of monoacetate (R)-4

Production of monoacetate (*R*)-**4** for synthetic use was carried out in a 1000 mL three-necked flask as follows. Diacetate **5** (7.18 g, 21.2 mmol) was mixed with Amano PS Lipase (7.25 g) and $NaHCO_3$-solution (5%, 800 mL), cooled to +3 °C, was added. The mixture was stirred intensively with a KPG-stirrer to give a homogeneous emulsion and the temperature kept constant at +3 °C using a cryostat. The reaction was monitored by taking samples as described for the reaction course. After 96 h an ee of >98% was measured for monoacetate (*R*)-**4** and the reaction was stopped. The mixture was carefully extracted with CH_2Cl_2 (1 × 300 mL, 2 × 200 mL),

Table II. Screening of lipases for the hydrolysis of diacetate **5** using standard conditions: 30 mL of $NaHCO_3$-solution (5%) at room temperature.

Lipase	Lipase *m* [mg]	Substrate 5 *m* [mg]	Time [hours]	Product 4 *n* [%]	ee of 4 [%]
Amano AK	364.1	360.6	16	61.2	84.2 % ee of (*R*)-**4**
Amano PS	358.0	362.6	22	52.1	96.6 % ee of (*R*)-**4**
Amano PS-CII*	357.1	360.5	23	55.7	94.6 % ee of (*R*)-**4**
Chirazyme L-2	354.3	359.8	23	0.1	–
Chirazyme L-2	361.0	358.9	1	20.1	11.2 % ee of (*R*)-**4**
Chirazyme L-3 pur.	357.7	369.6	91	1.5	5.4 % ee of (*S*)-**4**
Chirazyme L-5	364.1	360.2	20	19.0	1.6 % ee of (*S*)-**4**
Chirazyme L-7	358.1	358.3	22	33.5	97.0 % ee of (*S*)-**4**
Fluka Lipase	372.0	372.2	16	25.2	4.8 % ee of (*R*)-**4**
Fluka Lipozyme*	360.1	357.1	46	5.8	83.6 % ee of (*R*)-**4**

m [mg] = amount of substance [mg]; *n* [%] = relative amount of substance [%]; *immobilized.

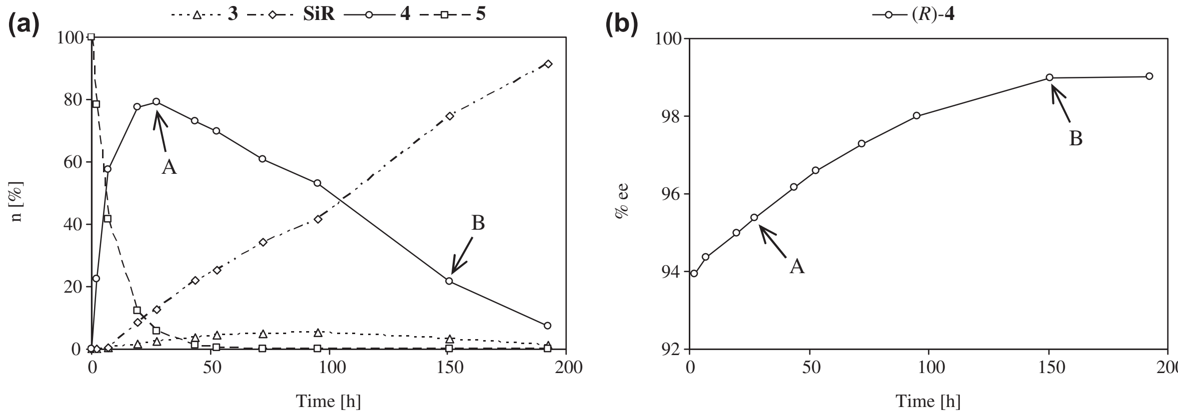

Figure 4. Reaction course of the hydrolysis of diacetate **5** carried out with lipase from *B. cepacia*; a: Amount of compounds **3**, SiR = $Me_2PhSiOH$, **4** and **5** (n [%]); b: Enantiomeric excess of (*R*)-**4** [% ee]; Sample A (27.3 h): 79.2% of **4**, 95.4% ee of (*R*)-**4**; Sample B (150.8 h): 21.8% of **4**, 99.0% ee of (*R*)-**4**.

the combined organic layers were dried over K_2CO_3, filtered and concentrated in vacuo. The residue was purified by flash chromatography (Ø 8 cm; h 20 cm; cyclohexane/EtOAc 1:1; fraction size 30 mL); R_f 0.29; colourless oil; yield 1.68 g (27%); $[\alpha]^{20}_D$: −3.7 (c = 0.83, CH_2Cl_2); 98.2% ee (HPLC method 2).

Results

Hydrolysis of diacetate 5

Since the lipase catalysed acetylation of diol **3** provided only the (*S*)-configured monoacetate (*S*)-**4**, the (*R*)-configured monoacetate (*R*)-**4** was prepared by enantioselective hydrolysis of diacetate **5**. Diacetate **5** was available in high amounts as a result of the acetylation of diol **3** with lipase from *B. cepacia* and could also be produced using the lipase Chirazyme L-2 from *Candida antarctica* B or by non-enzymatic acylation with acetic anhydride (Köhler & Wünsch 2006). For analysis of the hydrolytic transformations the established HPLC methods were used. However, it was found that in contrast to monoacetates **4** and diacetate **5**, diol **3** was not stable under hydrolytic conditions, as its silyl ether moiety was hydrolysed non-enzymatically during the progress of the reaction. Since diol **3** was only an undesired side-product in this process, its decomposition was not relevant for the outcome of the reaction. Nevertheless, it was necessary to determine the amount of silanol $Me_2PhSiOH$ resulting from decomposition of diol **3** with HPLC method 1 as well, because the amounts of the silylated compounds were calculated as relative amounts, and in Figures 3, 4, 7 and 8, they are given as percentage of the total amount of silylated compounds.

Reaction conditions

In order to find reasonable conditions for the screening of different lipases, the reaction conditions were

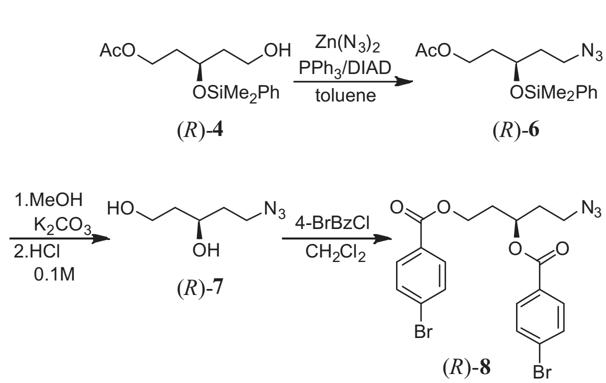

Figure 5. In order to determine the absolute configuration of (*R*)-4 by CD-spectroscopy, (*R*)-8 was produced as described in literature for the (*S*)-configured enantiomer (Köhler & Wünsch 2006).

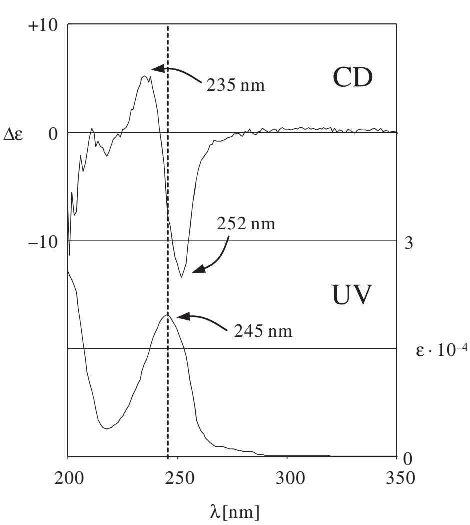

Figure 6. CD and UV spectra of (*R*)-8 in n-hexane.

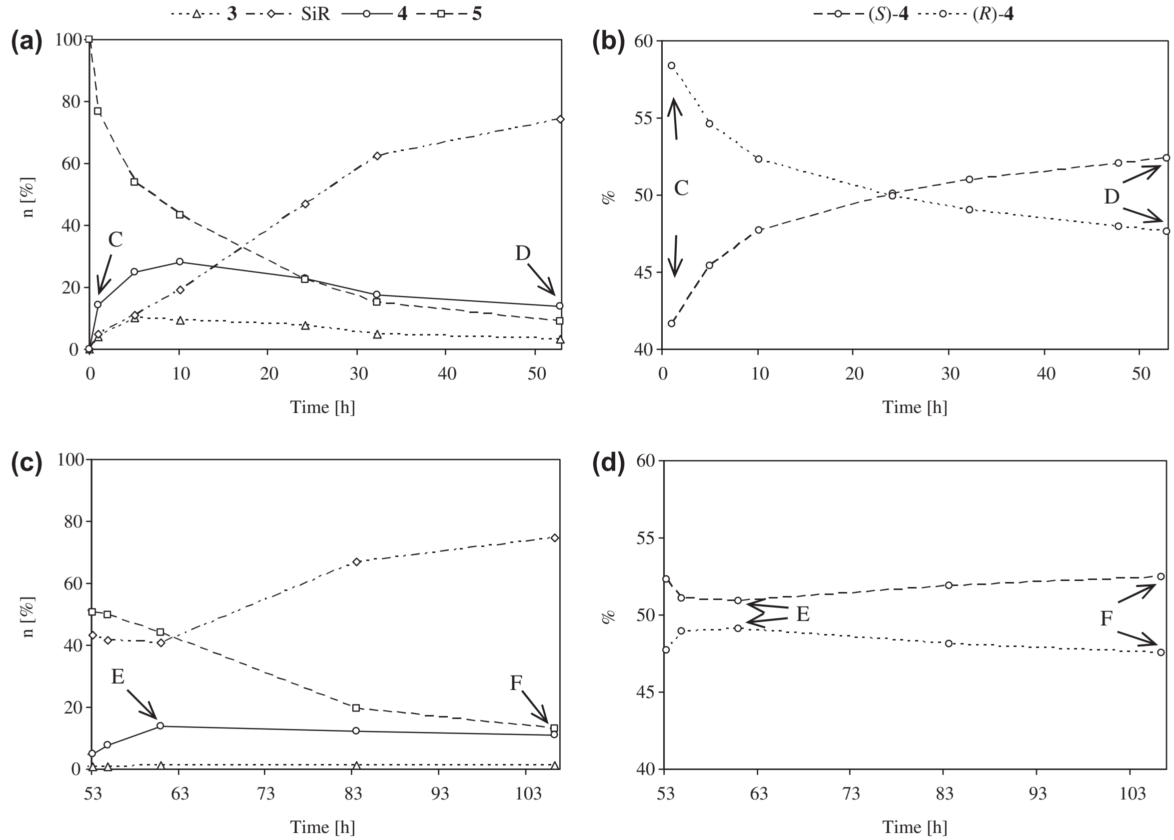

Figure 7. Reaction course of the hydrolysis of diacetate **5** carried out with lipase from *C. rugosa*; c, d: after 53.0 h an additional amount of diacetate **5** was added (50% of the starting amount); a, c: Amount of compounds **3**, SiR = $Me_2PhSiOH$, **4** and **5** (n [%]); b, d: Enantiomeric ratio of (*S*)-**4** [%] : (*R*)-**4** [%]; Sample C (1.0 h): 14.1% of **4**, 41.7% (*S*)-**4** and 58.3% (*R*)-**4**; Sample D (52.8 h): 13.7% of **4**, 52.0% (*S*)-**4** and 48.0% (*R*)-**4**; Sample E (61.0 h): 14.0% of **4**, 50.9% (*S*)-**4** and 49.1% (*R*)-**4**; Sample F (106.5 h): 11.0% of **4**, 52.4% (*S*)-**4** and 47.6% (*R*)-**4**.

optimized using lipase Amano PS-CII® (*Burkholderia cepacia*) for all experiments (Table I). As it is known that lipases are activated at interfaces, biphasic systems with organic layers above as well as below the aqueous phase were tested. However, the substrate **5** was not converted, probably since it was dissolved in the organic layer and did not come into contact with the lipase in the aqueous phase (Lines 1 + 2, Table I). Although it is miscible with water, acetone did not lead to better conversion but crystallization of the buffer salts (Line 3, Table I) and using solid buffer salts was also ineffective (Lines 4 + 5,

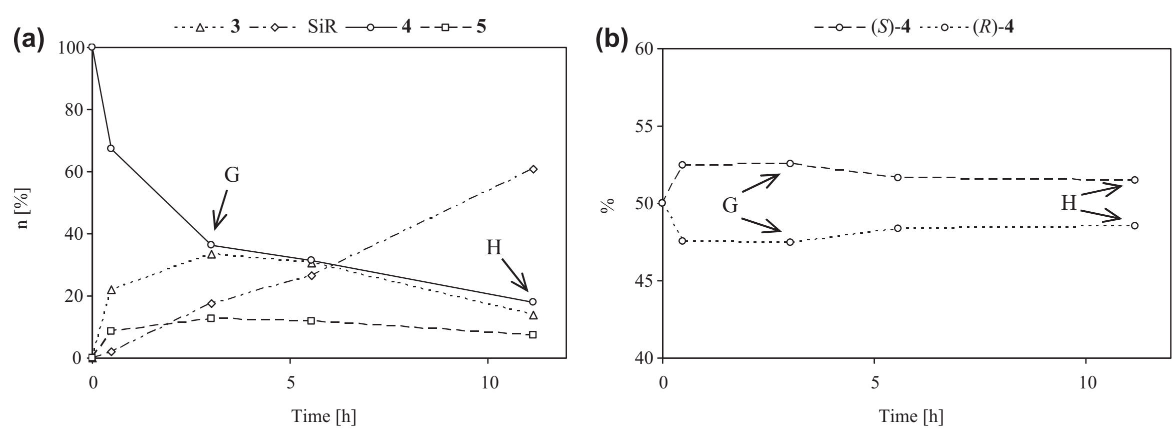

Figure 8. Reaction course of the hydrolysis of a racemic mixture of the monoacetates *rac*-**4** carried out with lipase from *C. rugosa*; a: Amount of compounds **3**, SiR = $Me_2PhSiOH$, **4** and **5** (n [%]); b: Enantiomeric ratio of (*S*)-**4** [%]:(*R*)-**4** [%]; Sample G (3.0 h): 36.4% of **4** (52.5% (*S*)-**4** and 47.5% (*R*)-**4**) and 12.8% of **5**; Sample H (11.2 h): 14.0% of **4** (51.5% (*S*)-**4** and 48.5% (*R*)-**4**).

Table I). Small amounts of additives (butan-1-ol, propan-1-ol) had a slight benefit for acceleration of the reaction rate but entail the risk of producing side products and are not really necessary (Lines 6, 7, 8, Table I). The enzyme itself led to sufficient emulsification of the substrate. Therefore, the ratio enzyme:substrate was increased to 1:1 for further experiments (Table II). $NaHCO_3$-solution (5%) was preferred as buffer solution, since the pH-value was kept more stable during the reaction process than was possible using phosphate buffer.

Screening of lipases

Diacetate **5** was hydrolysed using different lipases in $NaHCO_3$-solution at +20 °C. The results are given in Table II. Most of the lipases that were investigated produced an ee of the desired (*R*)-configured monoacetate (*R*)-**4**. Lipase Chirazyme L-7 from *Porcine pancreas*, however, led to formation of the (*S*)-configured monoacetate (*S*)-**4** in high ee. This was an interesting result, since it demonstrates that both enantiomers could theoretically be produced hydrolytically using different enzymes. Lipase Chirazyme L-2 (*Candida antarctica* B) led to a very fast but non-selective hydrolysis. The highest enantioselectivity for the production of the (*R*)-configured monoacetate (*R*)-**4** was found for the lipase from *B. cepacia* in pure form and immobilized on ceramic particles (C, Table II). Due to the better emulsification of the reaction mixture the native enzyme was used for further experiments.

Synthesis of monoacetate (R)-4

In order to find the optimal reaction time, the course of the hydrolytic conversion of diacetate **5** was recorded using lipase Amano PS from *Burkholderia cepacia*. The reaction temperature was lowered from +20 °C to +3 °C, since in a previous study it was shown that lowering the reaction temperature led to increased enantioselectivity of the lipase during acetylation (Köhler & Wünsch 2006). Figure 4 demonstrates that the hydrolysis of diacetate **5** gave monoacetate (*R*)-**4** very rapidly at the beginning of the reaction. Sample A was taken after 27.3 h and contained 79.2% of monoacetate **4** with an ee of 95.4% of (*R*)-**4**. Due to hydrolysis of the second ester group and since the lipase hydrolysed (*S*)-**4** faster than (*R*)-**4**, the ee of (*R*)-**4** increased during the course of the reaction and reached 99.0% ee after 150.8 h (sample B). For the same reason, however, the total amount of monoacetate **4** was rather low at that time (21.8%). Stopping the reaction after a short time would give a high yield (almost 80%, sample A) with an ee of ~95%. Since our aim was the production of (*R*)-**4** with an ee > 98% and the yield was of minor importance, large scale conversions were carried out until the monoacetate (*R*)-**4** reached > 98% ee. Furthermore, comparison between experiment C in Table II at +20 °C and the experiment shown in Figure 4 using the same reaction conditions at + 3 °C demonstrate the benefit of lowering the reaction temperature for the selectivity of the lipase. In experiment C the reaction was faster due to the higher temperature. The ee of (*R*)-**4**, however, was only 94.6% at a concentration of 55.7% of monoacetate **4**. In Figure 4, at a concentration of about 56% of monoacetate **4** the ee of (*R*)-**4** was about 97.7%.

Preparative synthesis of monoacetate (*R*)-**4** for synthetic use was performed with up to 20 mmol of diacetate **5** and resulted in an ee of 98.2% (*R*)-**4**. The absolute configuration of monoacetate (*R*)-**4** was determined by CD-spectroscopy as described in the literature for the (*S*)-configured enantiomer (Köhler & Wünsch 2006). For this, (*R*)-**4** was converted to the azide (*R*)-**6** by a Mitsunobu reaction and subsequently hydrolysed to give azidodiol (*R*)-**7**, which was acylated with 4-bromobenzoyl chloride resulting in dibenzoate (*R*)-**8** (Figure 5). The CD-spectrum of (*R*)-**8** shown in Figure 6 is the mirror image of the corresponding enantiomer (*S*)-**8**. Furthermore, the bisignate curve of the spectrum that results from exciton-coupling of the benzoyl chromophores is in accordance with analogous (*R*)-configured compounds (Soriente et al. 1995; Harada et al. 1991).

Hydrolytic conversions using lipase from C. rugosa

Within the screening process two lipase preparations originating from the same organism *Candida rugosa* were investigated (Chirazyme L-3 pur. and Fluka Lipase). Unexpectedly, Chirazyme L-3 pur. gave an ee of (*S*)-**4** and Fluka Lipase an ee of (*R*)-**4** (Table II). Since it is known that commercial preparations of crude lipase from *C. rugosa* usually consist of a mixture of several hydrolases (Lalonde et al. 1995; Brocca et al. 1998), the question arose whether the enantioselectivity of the preparations was different or changed during the progress of the reaction. Due to its higher activity, Fluka Lipase was used for all further experiments. The course of the hydrolysis of diacetate **5** catalysed by this preparation is shown in Figure 7 (a, b). At the beginning, an excess of (*R*)-**4** was generated (Sample C). Then, during the reaction an increasing amount of (*S*)-**4** was produced and the ee switched from an excess of (*R*)-**4** to an excess of (*S*)-**4** (Sample D). In order to demonstrate that this phenomenon did not result from altered reaction conditions, additional diacetate **5** (50% of the starting amount) was added to the reaction mixture (Figure 7c, d). As a result (*R*)-**4** was preferentially produced again (Sample E), then in the course of the reaction, the enantiomer (*S*)-**4** (sample F).

An additional experiment with Fluka lipase from *C. rugosa* was performed. Instead of diacetate **5**, a racemic mixture of the monoacetates *rac*-**4** was used for hydrolysis (Figure 8). Contrary to expectations, a substantial amount of diacetate **5** was produced (sample G) indicating that monoacetate **4** was not only hydrolysed but also acylated in aqueous medium due to transesterfication. Furthermore, the ee did not steadily increase.

Discussion

Synthesis of monoacetate (R)-4

Whereas (*S*)-configured pentane-1,3,5-triol derivatives are available by acetylation with lipase from *B. cepacia* in organic solvent (Köhler & Wünsch 2006), the same lipase can be used to produce the (*R*)-configured enantiomers in aqueous buffer solution, because the enzyme preferentially transforms the same enantiotopic group in organic as well as in aqueous solution. Though lipases are activated at interfaces, the use of biphasic systems did not lead to hydrolysis. It was necessary to emulsify the substrate in aqueous solution. This was achieved using $NaHCO_3$-solution (5%) and intensive stirring. The hydrolysis of diacetate **5** provided monoacetate (*R*)-**4** in high ee. The decisive factor for a successful synthesis, however, is finding the optimal reaction time, since the possible yield as well the ee alters to a large extent during the progress of the reaction (Figure 4).

Substrate selectivity and enantioselectivity

Several factors are known to affect the selectivity of lipases, in particular, reaction temperature and solvent, but also water activity, pH-value, additives and many other factors (Overbeeke et al. 1999; Persson et al. 2002; Reetz 2002). Furthermore, it is possible to modify lipases by genetic engineering or immobilization in order to enhance their selectivity or activity (Magnusson et al. 2001; Mateo et al. 2007). A switch of enantioselectivity has been found using chemical derivatives of chiral compounds due to different binding modes (Berglund et al. 1999; van Buijtenen et al. 2007). In the experiments presented in Figure 7, however, the substrate was not changed and all other parameters were kept constant. Nevertheless, the enantioselectivity of the preparation from *C. rugosa* not only changed but switched during the progress of the reaction. It may be assumed that the preparation (Fluka Lipase) consisted of a mixture of several hydrolases as most commercial preparations of crude lipase from *C. rugosa* (Lalonde et al. 1995; Brocca et al. 1998). One of the isoenzymes may convert diacetate **5** faster than monoacetates **4** and may preferentially give (*R*)-**4** at the beginning of the reaction. Another isoenzyme may catalyse the second step of the hydrolysis from mono-acetate **4** to diol **3** faster than the first step and with opposite and higher enantiopreference. Hence, in combination, the phenomenon observed during course of the reaction (Figure 7) could theoretically result from a cooperation of these isoenzymes. The reaction course of the acetylation of diol **3** using the lipase from *M. miehei* (Figure 3c, d), however, cannot be explained by the existence of isoenzymes, since the lipase (Lipozyme®) was expressed as a single enzyme in *Aspergillus oryzae* available from Novo Nordisk A/S Corp.(Rodrigues & Fernandez-Lafuente 2010). Therefore, we suggest that the switch of enantioselectivity observed for the lipase preparation from *C. rugosa* as well as the change of substrate selectivity observed for the lipase from *M. miehei* may result from an connatural movement of the lid partially covering the stereoselectivity pocket of the active site. Verification of this hypothesis, however, will need a wide range of further kinetic investigations and biochemical studies.

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