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Transformation of racemic ethyl 3-hydroxybutanoate into the (*R*)-enantiomer exploiting lipase catalysis and inversion of configuration

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Abstract—Racemic ethyl 3-hydroxybutanoate *rac*-**1** was transformed into ethyl (*R*)-acetoxybutanoate (ee = 92%) with 85–90% chemical yields using enantioselective acylation with isopropenyl acetate in the presence of *Candida antarctica* lipase B (CAL-B, Novozym 435) under solvent-free conditions, followed by mesylation of the unreacted (*S*)-alcohol in the reaction mixture and inversion of configuration with cesium acetate in DMF in one pot. When the (*R*)-acetoxybutanoate was subjected to alcoholysis with ethanol and CAL-B, enantiopure (*R*)-**1** (ee >99%) was produced.
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1. Introduction

The versatility of the enantiomers of 3-hydroxybutanoate esters as chiral building blocks for the synthesis of bioactive compounds, for example, for the synthesis of β-lactam antibiotics,[1–3] is well established.[4] Especially interesting are the antimicrobial classes of carbapenems and penems, many of which are now in clinical phases (Fig. 1). The interest is based on the high potential of such compounds against hospital and community pathogens, and more generally, on their broad antimicrobial spectra. Consequently much interest has been focused on developing routes to the synthesis of the β-hydroxybutanoate enantiomers.[4,5]

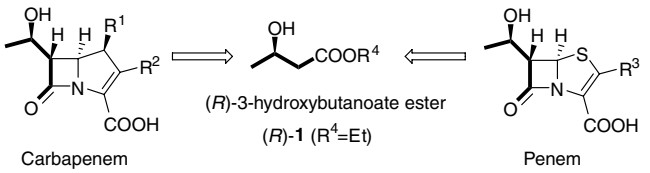

Figure 1. Role of (*R*)-hydroxybutanoate esters in the structure of carbapenems and penems.

The reduction of a prochiral β-keto ester in the formation of an optically active β-hydroxy ester has been performed using both purified enzymes and microbial cells.[6–10] Although significant results have been obtained with microbial cells, narrow substrate specificity, low stereochemical outcome, and low tolerance for synthetic reaction conditions still remain as drawbacks. The use of isolated dehydrogenases, on the other hand, involves high-cost purification methods and the development of systems for cofactor regeneration. An interesting method for the preparation of ethyl (*R*)-3-hydroxybutanoate was previously reported by hydrolyzing commercial poly[(*R*)-3-hydroxybutanoate] produced from glucose (recovered by enzymatic hydrolysis of pulp fiber sludge) by fermentation with *Alcaligenes eutrophus*.[11,12] Inversion of configuration via the mesylate ester was described to produce the (*S*)-enantiomer.[12]

The combination of broad substrate specificity, usually high stability and selectivity, effective catalysis without cofactors, and good commercial availability has made lipases especially attractive for the kinetic resolution of racemates. As a further benefit, both the enantiomers are simultaneously obtained when the reaction is highly enantioselective. On the other hand, a maximum yield of 50% for one enantiomer is a drawback when the other enantiomer is useless. Various types of kinetic resolution reactions

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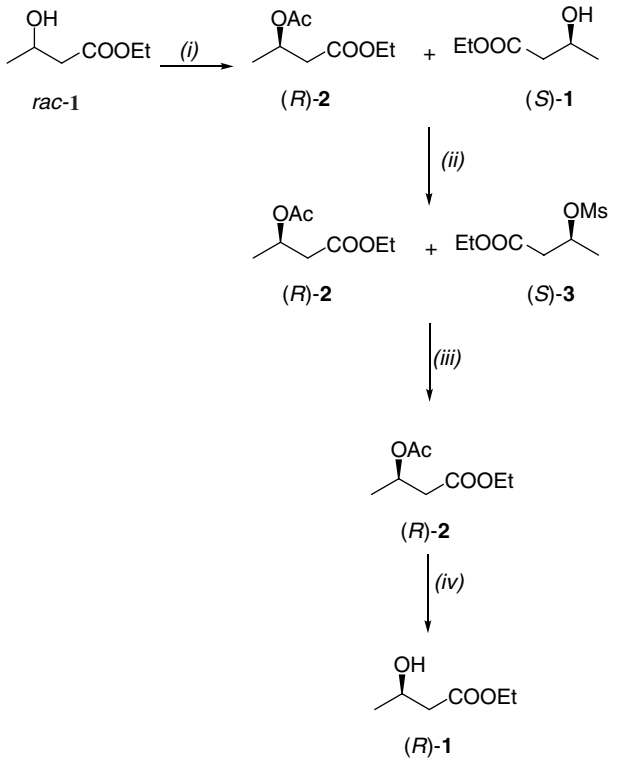
of racemic ethyl 3-hydroxybutanoate *rac*-**1** (Scheme 1) using lipases in organic solvents have been reported, leaving room for enantioselectivity enhancements. Thus, aminolysis of *rac*-**1** with various lipases and amines afforded resolution products (the unreacted **1** and the formed amide product), the absolute configuration of the more reactive enantiomer and enzymatic enantioselectivity depending on the lipase used.[13] The alcoholysis of *rac*-**1** with 1-butanol in hexane in the presence of various lipases was shown to give products with low enantiopurity.[14] Lipase-catalyzed acylations of *rac*-**1** or enantiomerically enriched (*S*)-**1** have also been studied.[14,15] A solvent-free system for acylation with vinyl acetate was developed in the presence of lipase B from *Candida antarctica* (CAL-B), leading to the formation of the unreacted (*S*)-**1** (ee = 96%) and the formed enantiomerically enriched (*R*)-**2** (ee = 80%) at 60% conversion.[14] The enantiomerically enriched (*R*)-**2** gave (*R*)-**1** (ee = 96%) at relatively low chemical yield through CAL-B-catalyzed alcoholysis with ethanol in hexane.



Scheme 1. Transformation of *rac*-**1** into (*R*)-**1** (i) acylation with isopropenyl acetate, 30 mg/mL CAL-B; (ii) MsCl, TEA in DCM; (iii) AcOCs in DMF; (iv) alcoholysis with 25 mg/mL CAL-B, EtOH in TBME.

Based on the general benefits of lipase catalysis and on the reported successful inversion of configuration of (*R*)-**1**[12] we herein report the transformation of *rac*-**1** into (*R*)-**1** through a three-step protocol: lipase-catalyzed acylation/inversion of configuration of the unreacted alcohol/lipase-catalyzed alcoholysis, all in one pot without any significant purification of the products (Scheme 1). For this purpose, it was necessary to improve the enantioselectivity of the lipase-catalyzed acylation of *rac*-**1** from that which has been reported,[14,15] to study the lipase-catalyzed alcoholysis of *rac*-**2** with ethanol and to find conditions where the possible elimination of water from **1** is minimized during the chemical steps leading to inversion and during all enzymatic reaction stages.

2. Results and discussion

2.1. Acylation of ethyl 3-hydroxybutanoate *rac*-1

In accordance with the previous results,[14] CAL-B proved to be the most enantioselective of all the lipases screened for the acylation of *rac*-**1**. The screenings were performed with vinyl butanoate as an irreversible acyl donor in *tert*-butyl methyl ether (TBME). Vinyl butanoate was used because lipases often favor butanoate esters over acetate esters. As shown by conversions, which reached after the reaction of 5 min, reactivity (conversions 24%, 40%, 40%, 49%, and 54% with 5, 10, 20, 50, and 100 mg mL^{-1} of the enzyme) was considerably affected by the CAL-B content of the model reaction. The order for most favored reaction media was diisopropyl ether (DIPE) ~ TBME > toluene > hexane > cyclohexane. Optimization was continued using CAL-B (5 mg mL^{-1}) in TBME. All together, these studies failed in improving the enantioselective outcome in the enzymatic acylation of *rac*-**1**.

For the lipase-catalyzed acylation of chiral alcohols [for instance Scheme 1: *rac*-**1** + RCO_2R^1 → (*S*)-**1** + (*R*)-**2** + R^1OH] to lead to enantiopure products, the enzymatic deacylation of the produced (*R*)-**2** with R^1OH back to the starting alcohol (*R*)-**1** needs to be prevented. This is usually accomplished by shifting the thermodynamic equilibrium to the product side, by using a high excess of an achiral acyl donor (RCO_2R^1 as a solvent and an acyl donor), or by using an activated achiral acyl donor, which either liberates an unstable R^1OH [$HOCH{=}CH_2$ or $HOC(Me){=}CH_2$] or an alcohol with low nucleophilic character ($HOCH_2CF_3$). With this in mind, acyl donors were screened for the acylation of *rac*-**1** in the presence of CAL-B (5 mg mL^{-1}) in TBME (Table 1). In the case of ethyl esters as acyl donors, enzymatic acylation proceeded slowly (entries 3 and 6) compared to the reactions with alkyl activated esters (entries 1, 2, 4, and 5). From the activated esters, vinyl esters (entries 2 and 4) gave lower enantiomer ratios, *E*, than 2,2,2-trifluoroethyl and isopropenyl esters (entries 1 and 5). We had expected this type of behavior of vinyl esters since acetaldehyde (the decomposition product of vinyl alcohol) is reported to be more

Table 1. Effect of acyl donor (0.2 M) on the acylation of *rac*-**1** (0.1 M) in TBME in the presence of CAL-B (5 mg mL^{-1}) at room temperature

Entry	Acyl donor	Time (h)	Conversion (%)	*E*
1	$PrCO_2CH_2CF_3$	0.5	40	95 ± 1
2	$PrCO_2CH{=}CH_2$	0.5	49	64 ± 4
3	$PrCO_2Et$[a]	4	36	32 ± 11
4	$MeCO_2CH{=}CH_2$	0.5	44	65 ± 1
5	$MeCO_2C(CH_3){=}CH_2$	0.5	48	150 ± 4
6	$MeCO_2Et$[b]	2	35	92 ± 3

[a] $PrCO_2Et$ as an acyl donor and as a solvent.
[b] $MeCO_2Et$ as an acyl donor and as a solvent.

Table 2. Effect of *rac*-**1** and isopropenyl acetate concentrations on CAL-B-catalyzed acylation at room temperature

Entry	Amount of enzyme (mg mL^{-1})	[*rac*-**1**]/[$MeCO_2C(Me)=CH_2$][a]	Time (h)	Conversion (%)	*E*
1	5	0.1/0.4	0.5	49	150 ± 3
2	5	0.1/0.2	0.5	49	151 ± 4
3	5	0.1/0.1	0.5	49	138 ± 3
4	5	0.1/0.08	0.5	47	181 ± 13
5	5	0.1/0.06	0.5	49	133 ± 13
6	5	5/5	5	23	109 ± 7
7	20	5/5	5	50	94 ± 5
8	30	5/5	5	52	87 ± 3
9	40	5/5	4.5	56	98[b]
10	5	5/4	5	27	117 ± 3
11	30	5/4	5	50	90 ± 4
12	30	5/3	5/8	46/51	102 ± 4

[a] [mol L^{-1}]/ [mol L^{-1}].
[b] Due to the fast reaction, the *E*-value was calculated using only the ee values at one conversion (38%).

harmful to enzymes than for instance acetone (the decomposition product of isopropenyl alcohol).[16] We also found that *E*-values for the enzymatic acylation of *rac*-**1** with achiral butanoate esters started to drop close to 50% conversions. In the case of acetate esters, the *E*-value remained more constant through the reaction. As the most important result, enzymatic enantioselectivity was considerably enhanced when isopropenyl acetate ($E = 150$, entry 5) was used as an acyl donor.

In the next step, the concentration effects on the acylation of *rac*-**1** with isopropenyl acetate and CAL-B were studied (Table 2). The enantioselectivity in TBME (0.1 M *rac*-**1**, entries 1–5), on one hand, and the studied solvent-free systems (5 M *rac*-**1**, entries 6–12) on the other hand, were hardly affected by the amount of isopropenyl acetate. The enantioselectivity in TBME was somewhat higher than in the solvent-free cases. This conclusion is justifiable in spite of the small variations in the *E*-values within Table 2, entries 1–5 and 6–12, accepting the fact that minor experimental inaccuracies may cause extensive variations in relatively high *E*-values. Thus, it was reasonable to turn attention to solvent-free systems and at the same time, increase CAL-B contents in order to obtain faster acylations (entries 6–9 as well as 10 and 11). For the kinetic resolution of *rac*-**1** under solvent-free conditions, it was advantageous that isopropenyl acetate was not used in high excess with the reactive enantiomer (the unreacted acyl donor needs to be removed) although the reactivity was not the best (entries 11 and 12). For a gram-scale kinetic resolution, *rac*-**1** (5 M) and isopropenyl acetate (3 M) were mixed with CAL-B (30 mg mL^{-1}), leading to (*S*)-**1** (ee = 95%, isolated yield 98%) and (*R*)-**2** (ee = 92%, isolated yield 84%).

2.2. One-pot synthesis of ethyl (*R*)-3-acetoxybutanoate

The entire protocol in Scheme 1 was performed without serious purification between the steps. The completion of each reaction step was monitored by GC in the terms of conversion and enantiopurity. The protocol was started by mixing *rac*-**1** with isopropenyl acetate and CAL-B (route i), and the mixture was shaken at room temperature as described (see Section 4). The resulting mixture of (*S*)-**1** (ee = 98%) and (*R*)-**2** (ee = 89%) was subjected to the reaction with mesyl chloride (route ii), leading to a mixture of (*R*)-**2** (ee = 86%) and (*S*)-**3** (ee = 98%). This mixture was then subjected to reactions with nucleophiles for inversion to take place.

Hydrolysis under neutral conditions with aqueous $CaCO_3$ was first studied in a mixture of (*R*)-**2** and (*S*)-**3**. The idea was based on the reported inversion of configuration of (*R*)-**3** under such conditions.[12] While (*S*)-**3** in the present work was inverted, (*R*)-**2** remained the same, leading to a mixture of (*R*)-**1** and (*R*)-**2**. It was shown that only a minor part of (*R*)-**2** could be hydrolyzed with aqueous $CaCO_3$, even after refluxing the system for two days. The hydrolysis of (*R*)-**2** in the mixture needs to be performed under conditions where the possible elimination of water from (*R*)-**1** is minimized. Thus, enzymatic hydrolysis seemed to be most reasonable as it may also improve the enantiopurity of (*R*)-**2**. We gave up on this idea, however, since in a mixture of (*R*)-**1** and (*R*)-**2**, enzymatic hydrolysis may lead to the hydrolysis of the ethyl ester function in addition to that of the acetate function in (*R*)-**2**.

Next, the transformation of (*S*)-**3** into (*R*)-**2** in their mixture was studied using acetate as a nucleophile (Scheme 1, step iii). Sodium acetate in toluene and 18-crown-6 ether as a phase transfer catalyst was tested first but the reaction was very slow even under reflux conditions as detected on TLC and by GC. Cesium carboxylates have been used for S_N2 displacements of mesylates[17] and tosylates.[18] Thus, (*S*)-**3** was dissolved in DMF or in DMSO in order to study the usability of cesium acetate. The high solubility of cesium acetate in DMSO induced high basicity favoring side-reactions, and an unidentified compound [other than the desired (*R*)-**2**] was observed. In DMF, inversion of configuration took place by carefully controlling the refluxing temperature, allowing the transformation of the mixture of (*R*)-**2** and (*S*)-**3** into crude (*R*)-**2**. This crude material was directly usable for the enzymatic transformation of (*R*)-**2** into (*R*)-**1**. We had created a highly efficient chemoenzymatic method where *rac*-**1** was transformed into (*R*)-**2** with 80–90% chemical yields and with 86–94% ee as the repetition of the process revealed.

2.3. Alcoholysis of ethyl 3-acetoxybutanoate

Our aim was to use CAL-B-catalyzed alcoholysis for the transformation of (*R*)-**2** into (*R*)-**1** under mild conditions where (*R*)-**1** is stable against the elimination of water. In order to obtain background information, enzymatic enantioselectivity and reactivity were first studied by subjecting *rac*-**2** (0.1 M) to the reaction with ethanol (0.2–0.5 M) and CAL-B (25 mg mL^{-1}) in hexane and in TBME (Scheme 2). If desired (although not carried out in the present work), the excellent enantioselectivity observed ($E \gg 200$ as measured at 40% or lower conversions) in both the solvents allow the kinetic resolution at 50% conversion (Table 3). The reaction in TBME was considerably faster than in hexane. Thus, the reaction with 0.2 M ethanol in TBME was at 50% conversion in an hour (entry 4) while 4 h were necessary for the same reaction in hexane (entry 1). In both solvents, reactivity decreased when increasing the ethanol content. Attempts to use high *rac*-**2** concentrations (accordingly also high ethanol concentrations) caused considerable rate retardation (Table 4). Moreover, a drop in ee values was evident at conversions close to 50% because the produced (*R*)-**1** enzymatically reacts with ethyl acetate back to (*R*)-**2** and ethanol. In addition, the enzymatic hydrolysis of **2** with water from the CAL-B preparation can take place. Accordingly the drop in ee was more pronounced in more hydrophilic TBME than in hexane.

Table 3. Alcoholysis of *rac*-**2** (0.1 M) with ethanol in the presence of CAL-B (25 mg mL^{-1}) in hexane and TBME

Entry	Ethanol (M)	Time (h)	Conversion (%)	$ee^{(S)\text{-}2}$ (%)	$ee^{(R)\text{-}1}$ (%)
Hexane					
1	0.2	4	50	97	99
2	0.3	24	50	97	96
3	0.5	24	50	98	96
TBME					
4	0.2	1	50	95	98
6	0.3	1	49	94	98
7	0.5	5	50	97	96

Finally, the CAL-B-catalyzed alcoholysis of the above crude product (*R*)-**2** (ee = 86%) furnished (*R*)-**1** in an enantiopure form (ee >99%) with ethanol in TBME (Scheme 1, route iv, Table 4). When the substrate was of high enantiopurity already in the beginning of the alcoholysis, the risk for drops in ee values in concentrated TBME solutions was not critical compared to the benefit obtained from faster reactions. As reactivity was shown to drop significantly even with a small increase in the ethanol concentration

Table 4. Effect of (*R*)-**2** (ee = 86%) concentration on the CAL-B (25 mg mL^{-1})-catalyzed alcoholysis (1 equiv) in TBME at room temperature

Entry	(*R*)-**2** (M)	Time (h)	Conversion (%)	$ee^{(R)\text{-}1}$ (%)
1	1	7/24	84/86	99.8
2	1	7/24	64/92[a]	99.9
3	1	7	82[b]	99.7
4	2	7/24	72/83	99.6
5	2	7	83[c]	99.7
6	3	7/24	52/78	99.8
7	3	7/24	38/84[d]	99.8
8	3	24/(24+5)[e]	76/89	99.9

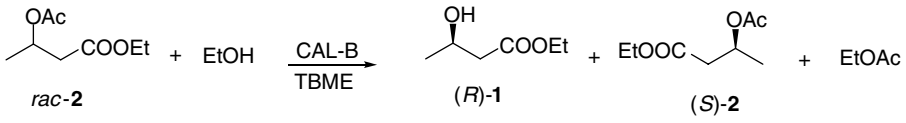
[a] Ethanol (1.2 equiv).
[b] CAL-B (50 mg mL^{-1}).
[c] CAL-B (100 mg mL^{-1}).
[d] Ethanol (2 equiv).
[e] After 24 h ethanol (0.25 equiv) was added.

(Tables 3 and 4, entry 1 compared to 2 and 6 to 7), the reaction was optimized using only 1 equiv of ethanol. In theory, the highly enantioselective alcoholysis will proceed to 93% conversion with initial $ee^{(R)\text{-}2} = 86\%$. However, under equimolar conditions the alcoholysis will stop at an equilibrium, and in practice, the alcoholysis of (*R*)-**2** (ee = 86%) with ethanol (1 equiv) and CAL-B (25 mg mL^{-1}) in TBME proceeded to somewhat over 80% conversion with 1–3 M substrate concentrations in reasonable times. The benefit in the terms of higher conversions with higher ethanol concentrations was minimal (entry 2 compared to 1 and entries 7 and 8 to 6).

3. Conclusions

In conclusion, a CAL-B-catalyzed kinetic resolution method under solvent-free conditions was developed which together with the inversion of (*S*)-**1** (the unreacted hydroxy ester enantiomer) through mesylation followed by the nucleophilic substitution with cesium acetate allowed the transformation of ethyl 3-hydroxybutanoate (*rac*-**1**) into the acetate product (*R*)-**2** in one pot with chemical yields of 85–90% and close to 90% ee. The CAL-B-catalyzed alcoholysis of the obtained (*R*)-**2** with ethanol in TBME gave enantiopure (*R*)-**1** (ee >99%).

The studies of the CAL-B-catalyzed alcoholysis of *rac*-**2** with ethanol in TBME revealed excellent enantioselectivity ($E \gg 200$). It is clear (although not studied here) that a three-step protocol, including chemical acylation of *rac*-**1**/lipase-catalyzed alcoholysis with ethanol/inversion of configuration will allow the transformation of *rac*-**1** into (*S*)-**2**.



Scheme 2. Alcoholysis of *rac*-**2** with ethanol.

4. Experimental

4.1. Materials and methods

Racemic ethyl 3-hydroxybutanoate *rac*-**1**, methanesulfonyl chloride (mesyl chloride, MsCl), cesium acetate, vinyl acetate, and isopropenyl acetate were products of Aldrich, Fluka or Acros Organics. Ethyl 3-acetoxybutanoate was prepared from commercial alcohol using acetic anhydride, triethyl amine, and DMAP. The solvents were of the highest analytical grade and were dried over molecular sieves (4 Å, 16 mm pellets) before use. CAL-B (*C. antarctica* lipase B, Novozym® 435) was a generous gift from Novozymes. Preparative chromatographic separations were performed by column chromatography on Merck Kieselgel 60 (0.063–0.200 μm). Analytical thin layer chromatography (TLC) was carried out on Merck Kieselgel $60F_{254}$ sheets and compounds were visualized using potassium permanganate dip. All enzymatic reactions were carried out at room temperature (23 °C).

The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 500 spectrometer with tetramethylsilane (TMS) as an internal standard. Spectroscopic data for **1**[6] and (*S*)-**3**[12] were in accordance with those given in the literature. Optical rotations were measured with a Perkin–Elmer 341 Polarimeter, and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. The determination of the *E*-value is based on the equation $E = \ln[(1-c)(1-ee_S)]/\ln[(1-c)(1+ee_S)]$ with $c = ee_S/(ee_S+ee_P)$ using linear regression (*E* as the slope of the line $\ln[(1-c)(1-ee_S)]$ versus $\ln[(1-c)(1+ee_S)]$).[19] Decane was used as an external standard to estimate the conversion during the alcoholysis of (*R*)-**2**.

In a typical small-scale experiment, CAL-B was added to the solution of *rac*-**1** (0.1–5 M) or *rac*-**2** (0.1–3 M) and an acyl donor or ethanol in an organic solvent or without a solvent. The reaction mixture was shaken at room temperature. The progress of the reaction and the ee values of the products were followed by taking samples (0.1 mL) at intervals and analyzing them by GC on a Varian CP-chirasil-Dex CB column (25 m). For a good baseline separation, the unreacted hydroxyl group in the sample was derivatized with propionic or acetic anhydride in the presence of pyridine containing 1% 4-*N*,*N*-dimethylaminopyridine (DMAP).

4.2. Gram-scale kinetic resolution of *rac*-1

rac-**1** (5.0 g, 37.8 mmol, 4.91 mL) was mixed with isopropenyl acetate (2.27 g, 22.7 mmol, 2.46 mL) and CAL-B was added (30 mg/mL). The mixture was stirred at room temperature for 7 h. The reaction was stopped by filtering off the enzyme at 51% conversion, and the enzyme was washed with TBME. After evaporation, the unreacted (*S*)-**1** and the formed (*R*)-**2** were separated on silica gel by elution with petrol ether/ethyl acetate (9:1), the elution sequence being (*R*)-**2** {16.3 mmol, ee = 92%, $[\alpha]_D^{25} = +2.9$ (*c* 1, $CHCl_3$)} before (*S*)-**1** {18.1 mmol, ee = 95%, $[\alpha]_D^{25} = +42$ (*c* 1 $CHCl_3$); the literature data $[\alpha]_D^{22} = +36.9$ (*c* 2.23, $CHCl_3$), ee = 89.5%[14] and $[\alpha]_D = +40$ (*c* 1, $CHCl_3$), ee <98%[12]}. ^{1}H NMR ($CDCl_3$, 500 MHz), [(*R*)-**2**]: $\delta = 1.24$ (t, $J = 7.1$ Hz, 3H, CH_2CH_3); 1.29 (d, $J = 6.33$ Hz, 3H, CH_3); 2.02 (s, 3H, $COCH_3$); 2.4 (dd, $J = 5.7$ Hz, $J = 15.4$ Hz, 1H, CH_2); 2.6 (dd, $J = 7.1$ Hz, $J = 15.4$ Hz, 1H, CH_2); 4.12 (q, $J = 6.5$ Hz, 2H, $-OCH_2CH_3$); 5.25–5.29 (m, 1H, CH). ^{13}C NMR ($CDCl_3$, 126 MHz): $\delta = 14.15$ ($-OCH_2CH_3$); 19.87 (*C*1), 21.14 ($-COCH_3$), 40.86 (*C*3), 60.58 ($-OCH_2CH_3$); 67.32 (*C*2); 170.21 ($-OCOCH_2CH_3$); 170.22 ($-OCOCH_3$).

4.3. Transformation of *rac*-1 into (*R*)-1

rac-**1** (20.0 g, 151 mmol, 19.7 mL) was mixed with isopropenyl acetate (9.08 g, 90.8 mmol, 9.9 mL) and CAL-B was added (30 mg mL^{-1}). The mixture was stirred at room temperature. The reaction was stopped by filtering off the enzyme at 52% conversion ($ee^{(R)\text{-}2} = 89\%$ and $ee^{(S)\text{-}1} = 98\%$). The enzyme was washed with TBME and the solvent was evaporated.

The above mixture of (*S*)-**1** and (*R*)-**2** was introduced in dichloromethane (100 mL), after which methanesulfonyl chloride (9.2 g, 80.2 mmol, 6.2 mL) was added and the mixture stirred in an ice bath until the temperature of 0 °C was reached. Triethylamine (8.1 g, 80.2 mmol, 11.3 mL) was added slowly within 40 min, while keeping the temperature below 10 °C. The reaction mixture was filtered, and the filtrate was evaporated until a white slurry was obtained. The slurry was introduced in diethyl ether (50 mL) and the precipitate formed was removed by filtration. According to GC analysis, the obtained mixture of (*R*)-**2** ($ee^{(R)\text{-}2} = 86\%$) and (*S*)-**3** ($ee^{(S)\text{-}3} = 98\%$) did not contain any (*S*)-**1**.

The mixture of (*R*)-**2** and (*S*)-**3** was introduced in DMF (140 mL) and cesium acetate (15.2 g, 79.4 mmol) was added. The reaction mixture was gently refluxed for 2 h. Diethyl ether was added and the product was isolated by aqueous extraction.

The obtained crude (*R*)-**2** (ee = 86%) was subjected to enzymatic alcoholysis with ethanol (6.32 g, 137.3 mmol, 8 mL) in TBME in the presence of CAL-B (25 mg/mL). The reaction was stopped after 25 h at 82% conversion. Conversion was determined using decane as an external standard. After column chromatography with petrol ether/ethyl acetate (9:1) as an eluent, (*R*)-**1** {ee = 99%, yield 60%, $[\alpha]_D^{25} = -43.1$ (*c* 1, $CHCl_3$)} was obtained.

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