Chemo-Enzymatic Synthesis of (*R*)- and (*S*)-2-Hydroxy-4-phenylbutanoic Acid via Enantio-Complementary Deracemization of (±)-2-Hydroxy-4-phenyl-3-butenoic Acid Using a Racemase–Lipase Two-Enzyme System

Barbara Larissegger-Schnell, Wolfgang Kroutil, Kurt Faber*

Department of Chemistry, Organic & Bioorganic Chemistry, University of Graz, Heinrichstrasse 28, 8010 Graz, Austria
Fax +43(316)3809840; E-mail: Kurt.Faber@uni-graz.at

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Abstract: Deracemization of (±)-2-hydroxy-4-phenylbut-3-enoic acid was accomplished by lipase-catalyzed kinetic resolution coupled to mandelate racemase-mediated racemization of the non-reacting substrate enantiomer. Stepwise cyclic repetition of this sequence led to a single enantiomeric product, the stereochemical outcome of which could be controlled by switching between lipase-catalyzed acyl-transfer/ester hydrolysis reactions. Both enantiomeric products were easily transformed into (*R*)- and (*S*)-2-hydroxy-4-phenylbutanoic acid, important building blocks for ACE-inhibitors.

Key words: deracemization, lipase, mandelate racemase, biocatalysis, enzyme catalysis

(*R*)-2-Hydroxy-4-phenylbutanoic acid (**3**) is an important building block for the production of a large variety of angiotensin converting enzyme (ACE) inhibitors having in common the (*S*)-homophenylalanine moiety as the central pharmacophore unit.[1] These agents, often denoted as the '-pril family', such as enalapril, lisinopril, cilapril or benazepril, efficiently expand the range of antihypertensiva, like β-blockers, A_2-antagonists or Ca-channel blockers. Due to the fact that many of these drugs have lost patent protection (or soon will do so), the production costs of the required building blocks has become a major issue.

For the synthesis of **3** or close derivatives thereof in non-racemic form, numerous strategies have been devised, which can be categorized into (i) classic[2] or (ii) kinetic resolution of a racemate [3]or (iii) asymmetric transformation of prochiral precursor.[4] The majority of these routes have one or more weak points,[4m] i.e. high cost of reagents (e.g., chiral transition metal complexes), insufficient catalyst selectivity or -activity, sensitivity of catalysts (e.g., asymmetric hydrogenation), or stability of starting materials (e.g., keto acids). The most dramatic limitation common for all strategies relying on kinetic resolution is the maximum theoretical yield of 50% for a single enantiomer. In order to overcome this fundamental drawback, two approaches – summarized under the term 'deracemization'[5] – were recently proposed: (i) dynamic kinetic resolution[6] and (ii) microbial stereo-inversion.[7]

Although both of these methods have the clear merit of a 100% theoretical yield of a single stereoisomeric product, its absolute configuration is determined by the enantiopreference of the biocatalyst employed. Since mirror-image enzymes *sensu stricto* do not exist,[8] production of both enantiomers through biocatalytic deracemization by simple choice of the 'matching enantiomer' of the chiral (bio)catalyst is virtually impossible.

In order to circumvent this limitation, we envisaged to apply lipase-catalyzed ester hydrolysis and ester formation to our recently developed deracemization protocol[9] based on the enzymatic racemization of the non-reacting substrate enantiomer using a racemase.[10] Taking into consideration that ester hydrolysis and esterification represent reactions in opposite directions, products of opposite configuration are usually obtained.

The enantio-complementary deracemization protocol was realized as follows (Scheme 1): since 2-hydroxy-4-phenylbutanoate (**3**) is not a substrate for mandelate racemase,[11] the corresponding butenoic acid derivative (**1**), which allows resonance stabilization of the α-carbanion (occurring during mandelate racemase catalysis) via conjugation with the aryl-moiety,[12] was chosen.[13] Mandelate racemase racemizes (*R*)-**1** at a rate of 53% to that of its natural substrate – mandelate – which corresponds to a turnover frequency of approximately 500 sec^{-1}.[11]

(*S*)-Series: in a first step, kinetic resolution of *rac*-**1** in the acyl-transfer mode was accomplished using *Pseudomonas sp.* lipase in diisopropyl ether at the expense of vinyl acetate as acyl donor[9] to furnish (*S*)-**2** and non-reacted (*R*)-**1** in excellent ee (>99%) at 50% conversion. The latter was re-racemized without separation from formed product using mandelate racemase[14] in aqueous buffer. Stepwise repetition of this two-enzyme procedure for three cycles furnished (*S*)-**2** in >99% ee and 68% overall yield from the racemate. Alkaline hydrolysis of the *O*-acyl moiety and catalytic reduction of the C=C bond gave (*S*)-**3** as the sole product in >99% ee.[15]

(*R*)-Series: access to (*R*)-**3** started by enzymatic hydrolysis of *rac*-**2** (obtained by acylation of *rac*-**1**) in aqueous acetone for sufficient solubilization of the polar substrate. Since *Pseudomonas sp.* lipase displayed low enantioselectivity, *Candida antarctica* lipase B (Novozyme 435) was chosen for perfect enantioselectivity, which produced a mixture of (*S*)-**1** and unreacted (*R*)-**2**. Re-racemization

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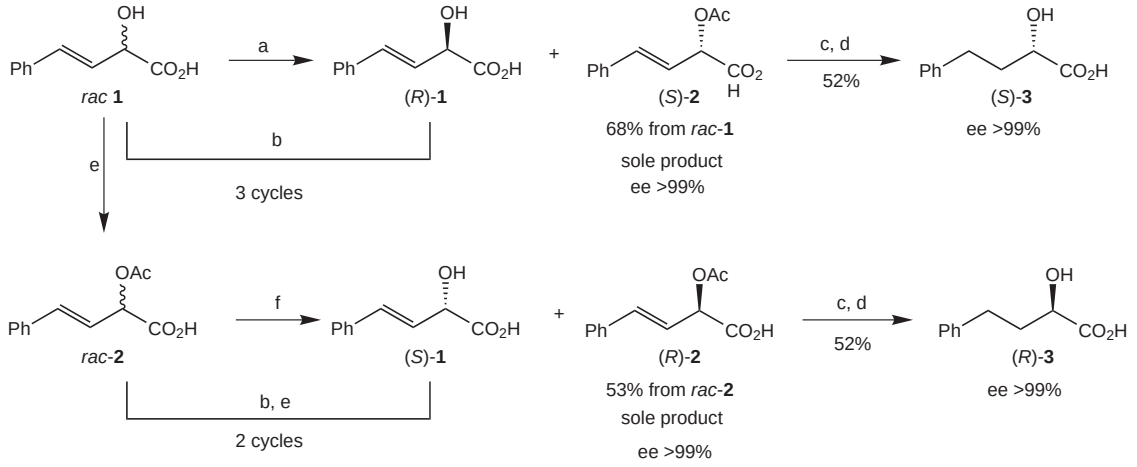

Scheme 1 *Reagents and conditions*: (a) *Pseudomonas sp.* lipase (Amano PS-C-II), vinyl acetate, *i*-Pr_2O, 25 °C; (b) mandelate racemase [EC 5.1.2.2], buffer pH 7.5, 30 °C; (c) MeOH, K_2CO_3, 0 °C; (d) MeOH, H_2, Pd/C; (e) Ac_2O, pyridine; (f) *Candida antarctica* lipase B (Novozyme 435), buffer pH 7.5, acetone, 30 °C.

of the former by mandelate racemase followed by re-esterification and lipase-hydrolysis through two cycles gave (*R*)-**2** in >99% ee and 53% overall yield from *rac*-**2**. Hydrolysis of the *O*-acetyl group and hydrogenation furnished (*R*)-**2** in >99% ee as the sole product.[16]

The main limitation of this process lies in an indispensable aqueous-organic solvent switch, which necessitates a consecutive sequence of steps rather than a dynamic process. The latter is caused by the inactivity of mandelate racemase in organic media at low water activity.[17] The enzyme is remarkably stable and can be used repeatedly, in particular in immobilized form, which facilitates its recovery.[18] Although isolated overall yields were still below the theoretical 100%, this threshold should be approachable using improved recovery procedures for the polar hydroxycarboxylic acids (e.g., by ion exchange chromatography) based on the fact that this process is virtually free of side reactions. The possibility to determine the stereochemical configuration of the sole product by a simple switch between the acyl-transfer- and hydrolysis-mode demonstrates the flexibility of this process.

In summary, both enantiomers of 2-hydroxy-4-phenylbutanoic acid were obtained as single stereoisomers in >99% ee via stepwise deracemization of the corresponding (±)-2-hydroxy-4-phenylbut-3-enoic acid through a lipase-racemase protocol. Whereas the *S*-enantiomer was formed through lipase-catalyzed acyl-transfer, the *R*-counterpart was produced via ester hydrolysis.

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(15) **(*S*)-2-Acetoxy-4-phenyl-3-butenoic Acid [(*S*)-2] via Deracemization of [*rac*-1].**
Kinetic resolution step (a): to a solution of *rac*-**1** (0.25 g, 1.4 mmol) in diisopropyl ether (25 mL), vinyl acetate (2.5 mL) and lipase PS-C 'Amano' II (0.25 g) were added and the mixture was shaken for 48 h at 25 °C and 150 rpm. The enzyme was filtered and dried for reuse; the filtrate was evaporated to dryness. HPLC analysis showed a conversion of 50% [Chiralpak AD column, Daicel, heptane–2-PrOH–CF_3COOH, 90:10:0.1; 0.4 mL/min, 18 °C, (*S*)-**2**: τ = 29.3 min, (*R*)–**1**: τ = 42.6 min].
Racemization step (b): to a solution of (*S*)-**2** and (*R*)-**1** obtained from step (a) in Hepes buffer (10 mL, 50 mmol, pH 7.5, 10 mM $MgCl_2$), mandelate racemase [EC 5.1.2.2] (1.5 g, prepared as described in ref. 14) rehydrated in 15 mL Hepes buffer was added. The mixture was shaken for 24 h at 30 °C and 150 rpm. After centrifugation the solution was acidified to pH 1–2 and extracted with EtOAc, dried (Na_2SO_4) and evaporated. HPLC analyses showed complete racemization of (*R*)-**1**; (*S*)-**1**: τ = 36.2 min. After repeating step (a) for three times and step (b) for two times, the residue was purified by flash chromatography to yield (*S*)-**2** as the sole product (0.21g, 68% overall yield from *rac*-**1**); mp 80–82 °C; mp lit. 82 °C; $[\alpha]_D^{20}$ +100.3 (*c* 0.47, EtOH, >99% ee); lit. $[\alpha]_D^{25}$ +108.0 (*c* 0.36, EtOH).
(*S*)-2-Hydroxy-4-phenyl-3-butenoic Acid [(*S*)-1].
A mixture of (*S*)-**2** (110 mg, 0.5 mmol), MeOH (4 mL) and K_2CO_3 (0.5 g) was stirred at 0 °C. After 3–4 h the mixture was acidified with HCl (3 M) to pH 1–2 and then extracted three times with EtOAc. The organic layer was dried (Na_2SO_4), evaporated and the residue was purified by flash chromatography to yield (*S*)-**1** (56 mg; 63%); mp 132–133 °C; lit. mp 104 °C; $[\alpha]_D^{20}$ +96.5 (*c* 0.27, MeOH, >99% ee); lit. $[\alpha]_D^{25}$ +85.2 (*c* 0.55, MeOH, 94% ee).
(*S*)-2-Hydroxy-4-phenylbutanoic Acid [(*S*)-3].
(*S*)-**1** (50 mg, 0.28 mmol) was hydrogenated employing a rubber balloon using a catalytic amount of Pd on C (10%, 5 mg) in MeOH for 10 min. Then the catalyst was filtered off and the solvent was evaporated to yield (*S*)-**3** (42 mg, 83%); mp 115–117 °C; lit. mp 114 °C; $[\alpha]_D^{20}$ +8.1 (*c* 1.0, EtOH, >99% ee); lit. $[\alpha]_D^{25}$ +7.5 (*c* 0.5, EtOH, 84% ee); chiral HPLC analysis using the method described above showed a single peak at τ = 26.8 min.
(16) **(*R*)-2-Acetoxy-4-phenyl-3-butenoic Acid [(*R*)-2] via Deracemization of *rac*-1.**
Acylation step (c): a solution of *rac*-**1** (0.5g, 2.8 mmol) and acetic anhydride (5 mL) in pyridine (0.2 mL) was kept at 0–5 °C. After 6 h the solution was poured into ice-water (100 mL), which was acidified with HCl (3 M) to pH 1–2 and extracted three times with EtOAc. The combined organic layers were washed with H_2O and brine, dried (Na_2SO_4) and evaporated to yield *rac*-**2** (0.45 g, 72%); mp 74–77 °C.
Kinetic resolution step (f): to a solution of *rac*-**2** (0.45 g, 2.0 mmol) in acetone (4.5 mL) and phosphate buffer (45 mL, 50 mmol, pH 7.5), lipase from *Candida antarctica B* (Novozyme 435, 1.8 g) were added and the mixture was shaken for 24 h at 30 °C and 130 rpm. The reaction mixture was filtered and the recovered lipase was dried for reuse. The filtrate was evaporated from acetone, the residue was acidified with HCl (3 M) to pH 1–2, extracted three times with EtOAc, dried (Na_2SO_4) und evaporated. HPLC analysis as described above showed a conversion of 50%; (*S*)-**1**: τ = 36.2 min, (*R*)-**2**: τ = 31.9 min. For the racemization step (b) see above. After repeating (e) and (f) for two times and (b) once, the residue was purified by flash chromatography to yield (*R*)-**2** as the sole product (0.33g, 53%); $[\alpha]_D^{20}$ –114.3 (*c* 0.49, EtOH, >99% ee). (*R*)-2-Hydroxy-4-phenyl-3-butenoic acid [(*R*)-**1**] was prepared as described for (*S*)-**1**. Yield 61%; $[\alpha]_D^{20}$ –71.9 (*c* 0.29, MeOH, ee >99%; lit. $[\alpha]_D^{25}$ –90.6 (*c* 1.9, MeOH). (*R*)-2-Hydroxy-4-phenylbutanoic acid [(*R*)-**3**] was prepared as described for (*S*)-**3**. Yield 85%; $[\alpha]_D^{20}$ –8.5 (*c* 1.0, EtOH, ee >99%); lit. $[\alpha]_D^{25}$ –9.0 (*c* 1.0, EtOH). Chiral HPLC analysis as described above showed a single peak at τ = 24.8 min. For spectroscopic and physical data of (*R*)-**1**–**3** and (*S*)-**1**–**3** see: Chadha, A.; Manohar, M. *Tetrahedron: Asymmetry* **1995**, *6*, 651.
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