A new chemo-enzymatic route to chiral 2-hydroxy-4-phenylbutyrates by combining lactonase-mediated resolution with hydrogenation over Pd/C†

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A new chemo-enzymatic route to both isomers of 2-hydroxy-4-phenylbutyric acid is reported. The key step is the lactonase-catalyzed hydrolysis of *cis*- and *trans*-2-hydroxy-4-phenyl-4-butyrolactones followed by hydrogenation over Pd/C to afford optically pure 2-hydroxy-4-phenylbutyric acid.

2-Hydroxy-4-phenylbutyrate is an important precursor to angiotensin-converting enzyme (ACE) inhibitors[1] which have the (*S*)-homophenylalanine moiety as the central pharmacophore unit. These ACE inhibitors from the 'pril-family', such as Enalapril, Lisinopril, Cilapril and Benazepril, greatly expand the range of *anti*-hypertensive theurapeutics.[2]

Developing a cost effective process for the production of these key building blocks is therefore very important.[3] So far, many routes to optically pure 2-hydroxy-4-arylbutyrates have been developed, including: (a) classical resolution;[4] (b) asymmetric synthesis;[5] and (c) asymmetric catalysis.[3,6–9] Routes a and b have gradually been replaced by route c for the consideration of atom-economy, operational-simplicity, eco-friendliness and cost-efficiency. Asymmetric catalysis includes, but is not limited to, three traditional methods: (1) asymmetric hydrogenation using substrates such as ethyl 2,4-dioxo-4-phenylbutyrate[3] and (*E*)-ethyl 2-oxo-4-arylbut-3-enoates;[6] (2) enzymatic hydrolysis of racemic substrates including cyanohydrin[7] and esters of 2-hydroxy-4-arylbutyrate;[8] and (3) enzymatic reduction of 2-oxo-4-phenylbutyrate and its derivatives.[9] Asymmetric hydrogenation usually affords products in moderate optical purity though the catalysts or chiral ligands are not always readily available. The low efficiency of the biocatalyst from time to time limits its application in industrial production. For example, 2-oxo-4-phenylbutyrate and its derivatives were reduced usually at a substrate-to-catalyst ratio of around 1/25 to 1/100.[9] All of these results prompted us to develop a new process for producing 2-hydroxy-4-phenylbutyrate and its esters based on a highly efficient enzyme, *Fusarium* lactonase.

Previously, we reported the *Fusarium* lactonase-catalyzed bioresolution of *cis*- or *trans*-4-substituted 2-hydroxy-4-butyrolactones.[10] We also noted that the enzymatic hydrolysis of *cis*- and *trans*-lactones were completed within almost the same time and that the enantioselectivity of the enzyme was only interfered with by the configuration of the alpha site while the chirality at the gamma site of the lactone ring has little, if any, influence on the optical purity of the products.

These results encouraged us to try the enzymatic hydrolysis of a 3 : 1 mixture of *cis*- and *trans*-2-hydroxy-4-phenyl-4-butyrolactones. We found that both optically pure (2*S*)-*cis*- and (2*S*)-*trans*-lactones could be obtained within almost the same time (Fig. 1). The configuration of the gamma site did not affect either the enantioselectivity nor the activity of the enzyme.

Based on these results, we designed a new chemo-enzymatic route to optically pure 2-hydroxy-4-phenylbutyrate. The key step is the enzymatic hydrolysis of a racemic mixture of *cis*-/*trans*-2-hydroxy-4-phenyl-4-butyrolactone followed by direct Pd-catalyzed hydrogenation of the products. In order to solve the problems encountered with traditional methods, we set the following goals: to obtain both isomers in high chemical and optical purity; to identify the most efficient biocatalyst and to adapt the method for potential industrial production.

The synthesis started from Claisen condensation of acetophenone **1** and diethyl oxalate (Scheme 1). (*Z*)-2-hydroxy-4-oxo-4-phenylbut-2-enoic acid **2** was obtained in about 90%

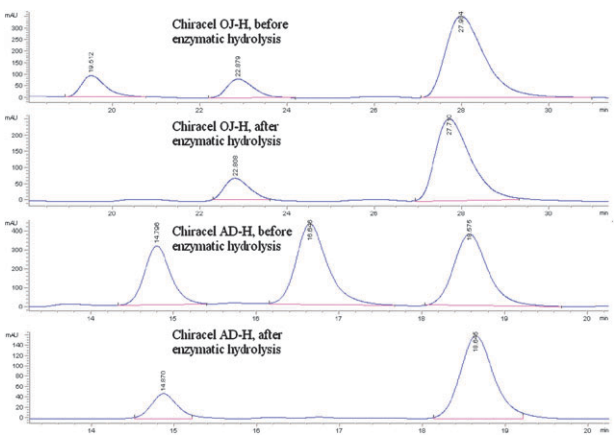

Fig. 1 HPLC analysis of the same sample on Chiracel AD-H and OJ-H columns before and after *Fusarium* lactonase-catalyzed hydrolysis of a mixture of *cis*-/*trans*-2-hydroxy-4-phenyl-4-butyrolactones. Before enzymatic hydrolysis, *cis*-lactones are not separable on a Chiracel OJ-H column (t_R = 27.9 min) while *trans*-lactones show two peaks—t_R 19.5 min for (2*R*,4*S*)-isomer and t_R 22.8 min for (2*S*,4*R*)-isomer. After enzymatic hydrolysis, the (2*R*,4*S*)-lactone was hydrolyzed and (2*S*,4*R*)-lactone remained intact. *Cis*-lactones are separated on Chiracel AD-H column—t_R 16.6 min for (2*R*,4*R*)-isomer, t_R 18.5 min for (2*S*,4*S*)-isomer and 14.8 min for racemic *trans*-lactone. After enzymatic hydrolysis, (2*R*,4*R*)-lactone was hydrolyzed and (2*S*,4*S*)-lactone remained.

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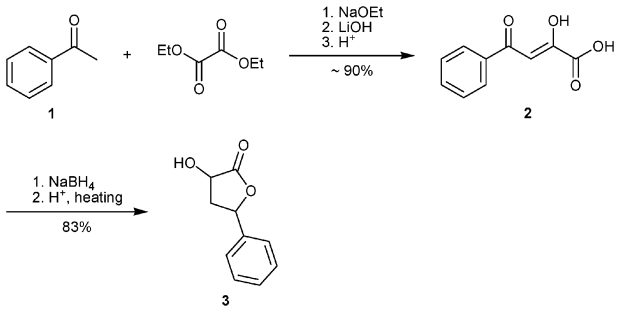
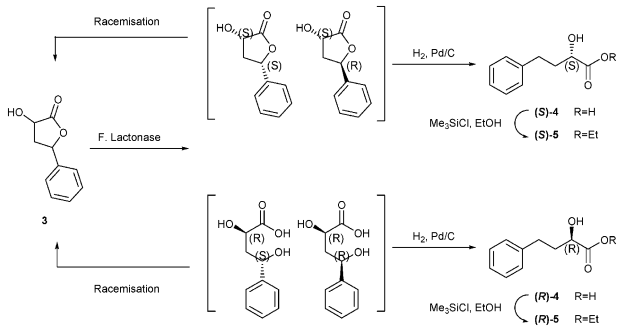


Scheme 1 Synthesis of 2-hydroxy-4-phenyl-4-butyrolactone.



Scheme 2 Synthesis of 2-hydroxy-4-phenylbutyrates through tandem *Fusarium* lactonase-catalyzed hydrolysis of racemic *cis*-/*trans*-4-phenyl 2-hydroxyl-4-butyrolactones and hydrogenation over Pd/C.

yield. Subsequent saponification and extraction with dichloromethane removed residual acetophenone from the product. Acidification, $NaBH_4$ reduction and cyclization afforded a racemic mixture of *cis*-/*trans*-2-hydroxy-4-phenyl-4-butyrolactone **3** in a ratio of 3 to 1, which was pure enough for use in the enzymatic resolution.

Enzymatic hydrolysis of lactone **3** was then carried out. The biocatalyst (reFPL) used here was whole cells of recombinant *E. coli* BL21 (DE3) expressing the *Fusarium proliferatum* lactonase gene (GenBank accession number EU595535) (Table 1).[10] In a typical experiment, the substrate of 20 g L^{-1} (with 20% MeCN as a cosolvent) was catalyzed by 2.3 g biocatalyst (reFPL4, dry cell weight) per liter. The pH was controlled at 6.4–6.7 by NH_4OH, while the reaction progress was monitored by chiral HPLC. We found that reFPL-catalyzed hydrolysis of *trans*-isomer was slightly faster than that of *cis*-isomers. After 1 h, both (2*S*)-isomers of *trans*-/*cis*- lactones were in >99% *ee*. This biotransformation was conducted at a substrate-to-catalyst ratio of 8.7/1 and was completed within 60 min, which was 10^2–10^3 more efficient than other enzymatic or chemo-enzymatic routes.[9]

We also screened a set of biocatalysts for the purpose of making the biotransformation process more efficient. Commercially available lipases were tested at a 1 to 1, substrate-to-catalyst ratio. However, after 24 h, only poor *ee* values (<16%) were achieved with <21% conversion. We also modified[11] the reFPL with plasmids (pET28a/pET24a) and signal peptide (with/without signal peptide) to generate four reFPL variants: reFPLs 1–4. We monitored with chiral HPLC the conversions and *ee* values of the residual substrate during the hydrolysis process of *cis*-4-phenyl-2-hydroxyl-4-butyrolactone catalyzed by these four reFPL variants.[12] It was found that reFPL3 was the most efficient lactonase (Table 1).

The residual (2*S*)-*cis*-/*trans*-lactone was then isolated (yield 33%) by simple extraction with ethyl acetate and submitted directly to hydrogenation over 5% Pd/C (1 atm, room temperature) in acetic acid (Scheme 2). The benzylic hydroxyl group was reduced, and the stereo-configuration of the α-hydroxyl group was left intact. After an overnight reaction, both *cis*- and *trans*-substrates afforded (*S*)-2-hydroxy-4-phenylbutyric acid **4**. The crystalline acid was further purified (yield 88% from the lactone) by crystallization from dichloroethane.[6] It was quantitatively converted to its ethyl ester **5** using Me_3SiCl/EtOH. Optical rotation measurement ($[\alpha]_D^{20}$ + 8.5°, EtOH, *c* = 1.0; lit[9], +7.5°) confirmed the configuration assignment of the 2-hydroxyl group to be *S*. Chiral HPLC analysis (AD-H column) showed a purity of >99% *ee*.

A modified procedure was applied to the preparation of (*R*)-2-hydroxy-4-phenylbutyric acid. In a typical experiment, the substrate at 20 g L^{-1} (20% MeCN) was catalyzed by 2.3 g biocatalyst (reFPL4, dry cell weight) per liter. The pH was controlled at 6.4–6.7 by NH_4OH. The reaction process was monitored by chiral HPLC and was terminated at around 30% conversion, in usually less than 30 min. The residual undesired butyrolactones were extracted completely with ethyl acetate, while the acids in the aqueous layer were collected, acidified and cyclized. The lactones were then extracted with ethyl acetate (isolated yield 37%) and submitted to

Table 1 Screening of biocatalysts for asymmetric hydrolysis of 4-phenyl-2-hydroxy-4-butyrolactone (10 mM)[a]

Enzyme	Amount of enzymes	Time/h	Conv. (%)[d]	*ee* (%)	*E*
reFPL1[b]	10 IU	0.5	50.6	96.9[e]	348[h]
reFPL2[b]	10 IU	0.5	48.3	95.3[e]	123[h]
reFPL3[b]	10 IU	0.5	51.0	96.2[e]	366[h]
reFPL4[b]	10 IU	0.5	50.5	92.8[e]	99.0[h]
Lipase OF[c]	18 mg (7.5×10^2 IU)	24	21.3	16.0[f]	—[g]
Lipase AK[c]	18 mg (53.8 IU)	24	18.4	15.3[f]	—[g]
Lipase L-1754[c]	18 mg (77.9 IU)	24	<10	<1.0[f]	—[g]
Lipase AYS[c]	18 mg (60.5 IU)	24	<10	<1.0[f]	—[g]
Lipase PPL[c]	18 mg (8.5 IU)	24	<10	<1.0[f]	—[g]
Lipase D[c]	18 mg (2.5×10^2 IU)	24	<10	<1.0[f]	—[g]

[a] Reaction condition: 10 ml KPB buffer (50 mM, pH 6.4), 200 μl MeCN, 30 °C, 180 rpm; [b] Whole cells of *E. coli* were used as a biocatalyst and their amount was controlled at 10 IU towards racemic pantolactone; [c] The amount of lipase was controlled at 18 mg while the activity was tested using *p*-nitrophenyl butyrate as substrate; [d] Conversion was calculated from formula $c = ee_s/(ee_s + ee_p)$;[13] [e] ee_p, analyzed by chiral HPLC AD-H column; [f] ee_s, analyzed by chiral HPLC AD-H column; [g] Not calculated; [h] $E = \ln[1 - c(1 + ee_p)]/\ln[1 - c(1 - ee_p)]$.[13]

hydrogenation over Pd/C, giving (*R*)-2-hydroxy-4-phenylbutyric acid. Chiral HPLC analysis of its ethyl ester showed a 98% *ee*.

This process has two advantages. First, both (*R*)- and (*S*)- acids were prepared in excellent optical purity using one single biocatalyst. Secondly, the simplicity of this procedure makes it very attractive for potential industrial applications. The starting materials used are readily available and only one purification step, the recrystallization of the acid, is needed in the whole process.[9]

In conclusion, a new route to optically pure (*R*)- and (*S*)-2-hydroxyl-4-phenylbutyric acids has been developed and has potential for industrial applications.

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11 For reFPL1, the lactonase gene with the signal peptide sequence was inserted into plasmid pET28a(+) which was digested with NdeI and BamHI; for reFPL2, the lactonase gene with the signal peptide sequence was inserted into plasmid pET24a(+) which was digested with *Nde*I and *BamH*I; for reFPL3, the lactonase gene without signal peptide sequence was inserted into plasmid pET24a(+) which was digested with *Nde*I and *BamH*I; for reFPL4, the lactonase gene without signal peptide sequence was inserted into plasmid pET28a(+) which was digested with *Nde*I and *BamH*I. The lactonase gene without signal peptide has now been deposited in GenBank with an accession number of EU596535, while the lactonase gene with signal peptidesequence was shown in the Supplementary Information. All of the recombinant lactonases were expressed in *E. coli* BL21(DE3).
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