Biocatalyst-Mediated Efficient Preparation of Highly Enantiomerically Enriched (*R*)-5-Hexanolide

Takeshi Sugai, Keiji Hamada, Tomohiro Akeboshi, Hajime Ikeda, Hiromichi Ohta*
Department of Chemistry, Keio University, 3-14-1 Hiyoshi, Yokohama 223, Japan
Fax 81 45 563 5967; sugai@chem.keio.ac.jp
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Abstract: Biocatalyst-mediated efficient preparation of highly enantiomerically enriched (*R*)-5-hexanolide (**1**) is described. The enantioselective reduction of 5-oxohexanoic acid with *Yamadazyma farinosa* IFO 10896 and the subseqent lipase-catalyzed kinetic resolution gave (*R*)-**1** in 62% yield with 99.7% *e.e.*

Enantiomerically enriched forms of 5-hexanolide **1**, which are versatile chiral building blocks, have been synthesized by means of various approaches.[1-19] However, few reports on the simplest method, the biocatalytic reduction of the corresponding commercially available 5-oxohexanoic acid **2** have appeared.[15] In particular, (*R*)-**1** has not been prepared by this method. Our recent studies on a biocatalyst, *Yamadazyma farinosa* IFO 10896[20] prompted us to apply the yeast-mediated reduction on the synthesis of (*R*)-**1**. In this paper, we describe the preparation of highly enantiomerically enriched form of (*R*)-**1**, whose enantiomeric purity is unambiguously determined by a chromatographic technique.

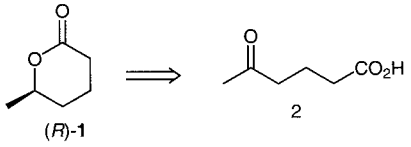
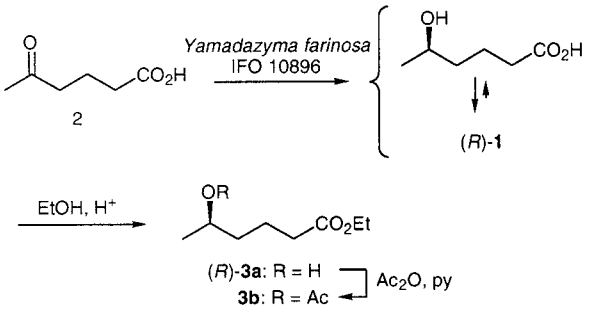


Figure 1

Reduction of **2** with grown cells of *Yamadazyma farinosa* proceeded well under an anaerobic condition[21] to give (*R*)-**1**. It should be emphasized that the pH of the reduction medium has a great effect on the enantioselectivity of the reaction. We examined various conditions and found that the acidic pH (4.7-4.3) gave the best result, the *e.e.* of the product being 97%.[22] When the pH was controlled to be 6.5 throughout the incubation with a pH controller, the *e.e.* of the product dropped to 74%. The more acidic conditions (below pH 4.0) also brought about the loss of high enantioselectivity.

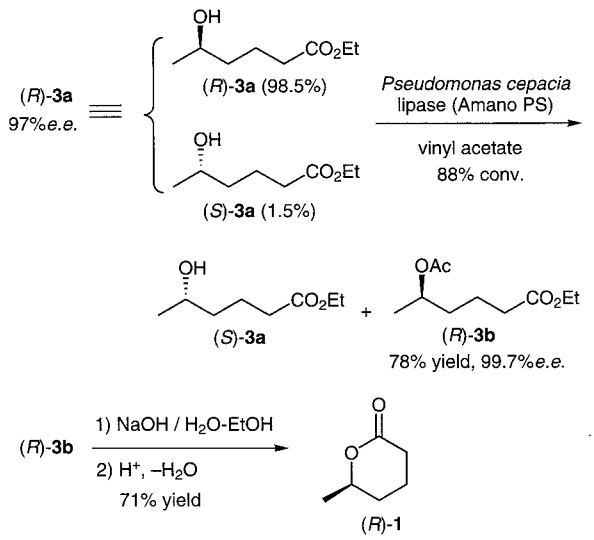
The product (*R*)-**1**, which was obtained through the spontaneous lactonization of the hydroxy acid during the incubation and the workup, is volatile and moderately distributes to both organic and aqueous phase. These characteristics of the product lowered the isolation yield. Moreover, another problem was caused by the use of whole cells as the biocatalyst; organic materials from cells contaminated the lactone even after chromatography and distillation. For these reasons, the product was isolated as the corresponding ethyl ester **3a**. The crude extract containing the primary product was immediately treated with ethanol under acidic conditions. Another advantage of the conversion to ethyl ester was that the enantiomers of the corresponding acetate **3b** could be separated on a chiral stationary phase of GC,[23] which made it facile to determine the *e.e.* of the product. The elaborated incubation conditions as well as the isolation procedure gave (*R*)-**3a** (77% yield, 97% *e.e.*) in a reproducible manner.[22]

The next task was the further enhancement of the *e.e.* As either the recrystallization of (*R*)-**1** (mp 27.7-29.0) or the lipase-catalyzed lactonization of **3a**[24] had no effect, our attempt was turned to another version of lipase-catalyzed kinetic resolution, the acetylation with vinyl acetate.[25]



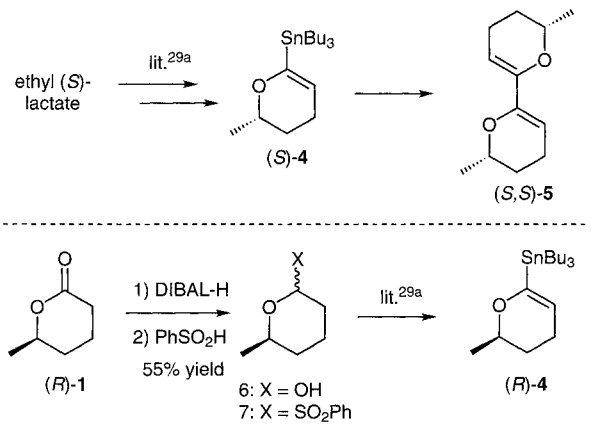
Scheme 1

The preliminary study using racemic substrate **3a** revealed that *Pseudomonas* lipase (Amano PS) was satisfactory (E = 57)[26] to give (*R*)-**3b**, while (*S*)-**3a** being unaffected. Starting from (*R*)-**3a** with 97% *e.e.*, at a conversion of 88%, the desired product (*R*)-**3b** with 99.7% *e.e.* was separated by a silica gel column chromatography in a 78% yield.[27] The total yield from **2** raised to 62% by omitting the exhaustive purification at the stage of (*R*)-**3a**. Highly polar impurities were simply removed by a conventional short column chromatography prior to the lipase-catalyzed acetylation. The hydrolysis and the subsequent lactonization of (*R*)-**3b** afforded (*R*)-**1** in 71% yield.[28]



Scheme 2

We applied (*R*)-**1** as a precursor of (*R*)-**4**. (*S*)-Isomer of **4** was synthesised from ethyl (*S*)-lactate and utilized as the intermediate for an optically active biscyclohexene (dispiroketal) (*S,S*)-**5** (Scheme 3).[29] The availability of its enantiomer (*R*)-**4**, however, has so far been limited. In the present study, the lactone was reduced to the corresponding lactol **6**,[30] and the subsequent treatment with phenylsulfinic acid gave **7**[31] in a total 55% yield. As in the same manner reported for its enantiomer,[29a] (*R*)-**4** was obtained from **7**.[32]



Scheme 3

In conclusion, the combined use of two biocatalysts made (*R*)-**1** with 99.7% *e.e.* available (62% yield), and the product was demonstrated as a starting material for the synthesis of (*R*)-**4**.

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(22) *Yamadazyma farinosa* IFO 10896 was incubated in a glucose medium [containing glucose (5.0 g), peptone (0.7 g), yeast extract (0.5 g), K_2HPO_4 (0.2 g), KH_2PO_4 (0.3 g), pH 6.5, total volume 100 mL] for 2 days at 30 °C. The wet cells (*ca.* 8 g from 100 mL of the broth) were harvested by centrifugation (3000 rpm) and washed with phosphate buffer (0.1 M, pH 6.5). The combined wet cells (20 g) from 250 mL of broth were re-suspended in an incubation broth [containing glucose (5.0 g), phosphate buffer (0.1 M, pH 6.5), total volume 100 mL] in a 500 mL shaking culture (Sakaguchi) flask together with **2** (500 mg, 3.84 mmol). At this stage the pH turned to 4.7. The flask was purged with argon, equipped with a balloon charged with argon, and shaken on a gyrorotary shaker for 2 days at 30 °C. The pH of the broth (4.3) was re-adjusted to 11 by adding aq. NaOH soln (2 N), and the mixture was centrifuged (3000 rpm) for 15 min. After decantation of the supernatant, the residue was re-suspended in water and centrifuged again. The supernatant and washings were combined and concentrated *in vacuo* to a small volume and acidified to pH 2 by adding conc. HCl. The mixture was passed through a pad of Celite, then was extracted 10 times with EtOAc. The extract was carefully concentrated *in vacuo* and the residue was treated with EtOH (50 mL) containing catalytic amount of *p*-TsOH under reflux overnight and neutralized by adding a phosphate buffer solution (pH 8). The aqueous layer was extracted 5 times with EtOAc. The extract was concentrated *in vacuo* and the residue was purified by silica gel column chromatography (6 g). Elution with ether-chloroform (1 : 8 to 1 : 2) afforded **3a** (606 mg, 98%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 4.13 (q, 2H, J = 7.1 Hz), 3.80 (tq, 1H, J = 6.3, 5.9 Hz), 2.33 (t, 2H, J = 7.3 Hz), 1.85-1.55 (m, 2H), 1.55-1.40 (m, 2H), 1.26 (t, 3H, J = 7.1 Hz), 1.20 (d, 3H, J = 5.9 Hz). IR (NaCl) 3400, 2980, 1740, 1460, 1380, 1250, 1170, 1100, 1030 cm^{-1}. This was employed for the next step without further purification. The *e.e.* was determined by GLC analysis of **3b**, column, Tokyo Kasei Co., Chiraldex BP, 0.25 mm x 30 m; 100 °C + 2 °C / min, press. 180 kPa; t_R (min): 15.7 [(*S*)-**3b**, 1.5%], 16.3 [(*R*)-**3b**, 98.5%].

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(27) A mixture of **3a** (2.42 g, 15.1 mmol), Amano PS lipase (1.20 g), vinyl acetate (24.3 mL) and hexane (24.3 mL) was stirred at 30 °C for 29 h. The conversion (88%) was confirmed by the GLC analysis as mentioned in ref. 22; t_R (min): 13.7 for **3a**. The conventional workup and chromatographic purification (silica gel, hexane / EtOAc = 2 / 1) followed by bulb-to-bulb distillation afforded **3b** (1.94 g, 63%). bp 112°C / 2 mmHg; $[\alpha]^{20}_D$ +3.24 (*c* 1.11, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 4.89 (tq, 1H, J = 5.9, 5.9 Hz), 4.12 (q, 2H, J = 7.1 Hz), 2.30 (t, 2H, J = 7.1 Hz), 2.02 (s, 3H), 1.75-1.45 (m, 4H), 1.24 (t, 3H, J = 7.1 Hz) 1.21 (d 3H, J = 5.9 Hz); IR (NaCl) 2980, 1740, 1450, 1370, 1250, 1180, 1030 cm^{-1}; Anal. Calcd. for $C_{10}H_{18}O_4$: C, 59.39; H, 8.97. Found: C, 59.01; H, 9.36.

(28) A mixture of **3b** described above (794 mg, 3.93 mmol), 5N NaOH aq soln (3 mL) and EtOH (3 mL) was stirred under reflux for 5 h. After having been concentrated *in vacuo*, the mixture was acidified and extracted with EtOAc. The extract was concentrated *in vacuo*, the residue was diluted with toluene. To this was added a catalytic amount of *p*-TsOH and the mixture was refluxed overnight with an azeotripic removal of water. The mixture was neutralized by adding phosphate buffer (pH 8.0) and extracted 3 times with EtOAc. The extract was dried over Na_2SO_4 and concentrated under atmospheric pressure. Chromatographic purification (silica gel, hexane / ether = 10 / 1 to 0 / 1) of the residue followed by bulb-to-bulb distillation afforded **1** (319 mg, 71%). bp 125°C / 12 mmHg; mp 28.0-29.0°C [lit.[6] mp 31-32°C, lit.[7] mp 15-20°C for (*R*)-isomer; lit.[6] mp 29-31°C, lit.[7] mp 22-24°C, lit.[10] mp 20°C for (*S*)-isomer]; $[\alpha]^{21}_D$ +42.4 (*c* 1.01, EtOH) [lit.[3] $[\alpha]^{20}_D$ +18.4 (*c* 1.7, MeOH), lit.[4b] $[\alpha]^{22.5}_D$ +30.9 (*c* 2.0, EtOH), lit.[6] $[\alpha]^{25}_D$ +43.4 (*c* 2.1, EtOH), lit.[7] $[\alpha]^{20}_D$ +37.2 (*c* 1.825, EtOH), lit.[8c] $[\alpha]^{25}_D$ +31.6 (*c* 1.22, EtOH), lit.[9] $[\alpha]^{20}_D$ +33.1 (*c* 3.0, MeOH), lit.[14] $[\alpha]^{20}_D$ +33.3 (*c* 1, THF), lit.[17] $[\alpha]^{27}_D$ +17.3 (ether) for (*R*)-isomer; lit.[1] $[\alpha]^{19}_D$ –51.4 (EtOH), lit.[2] $[\alpha]^{24}_D$ –39.1 (*c* 4.1, EtOH), lit.[4b] $[\alpha]^{23}_D$ –30.4 (*c* 1.6, EtOH), lit.[5] $[\alpha]^{20}_D$ –51 (*c* 1, EtOH), lit.[6] $[\alpha]^{25}_D$ –40.7 (*c* 2.2, EtOH), lit.[7] $[\alpha]^{21.5}_D$ –34.3 (*c* 2.075, EtOH), lit.[8b] $[\alpha]^{22}_D$ –35.5 (*c* 1.5, EtOH), lit.[9] $[\alpha]^{20}_D$ –33.5 (*c* 3.3, MeOH), lit.[11] $[\alpha]_D$ –38.1 (*c* 0.26, EtOH), lit.[12] $[\alpha]^{22}_D$ –46.45 (*c* 2.00, EtOH), lit.[13] $[\alpha]^{20}_D$ –8.5 (*c* 3.0, MeOH), lit.[14] $[\alpha]^{20}_D$ –41.7 (*c* 2.2, EtOH), lit.[15] $[\alpha]_D$ –38.90, lit.[16] $[\alpha]^{24}_D$ –40 (*c* 1.0, EtOH), lit.[18] $[\alpha]_D$ –35.6 (*c* 1.8, EtOH) for (*S*)-isomer]; ^{1}H NMR (270 MHz, $CDCl_3$) δ 4.50-4.35 (m, 1H), 2.65-2.35 (m, 2H), 1.95-1.75 (m, 3H), 1.60-1.40 (m, 1H), 1.36 (d, 3H, J = 6.3 Hz); IR (NaCl) 2970, 1730, 1240, 1065 cm-^{1}; Anal.Calcd.for $C_6H_{10}O_2$: C, 63.14; H, 8.83. Found: C, 62.83; H, 9.06. Its IR and NMR spectra were in good accordance with those reported previously.[7]

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(31) The compound **7** was prepared from **6** in a similar manner to the previous report.[29a]

(32) Bp 126-132°C / 0.5 mmHg; $[\alpha]^{22}_D$ +20.1 (*c* 1.21, $CHCl_3$) [lit.[29a] $[\alpha]^{28}_D$ –6.67 (*c* 1.125, $CHCl_3$) for (*S*)-isomer]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.69 [ddd, 1H, J = 4.8, 2.6, 1.3 Hz, tin satellites ^{3}J (^{119}Sn-H) = 30 Hz], 3.83 (dqd, 1H, J = 9.7, 6.4, 2.4 Hz), 2.16-2.03 (m, 1H), 2.00-1.90 (m, 1H), 1.84-1.74 (m, 1H), 1.62-1.41 (m, 7H), 1.31 (sextet, 6H, J = 7.3 Hz), 1.20 (d, 3H, J = 6.4 Hz), 0.93-0.86 (m, 15H, including t, 9H, J = 7.3 Hz); IR (NaCl) 2930, 2850, 1730, 1650, 1600, 1460, 1420, 1380, 1270, 1220, 1180, 1140, 1060, 1030, 960, 880, 800, 760, 690, 660 cm^{-1}. HRMS m/z Found 388.1796. $C_{18}H_{36}OSn$ (M+) requires 388.1788. Its IR and NMR spectra were identical with that reported previously.[29a, 34]

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(34) Prof. S. V. Ley and Dr. D. Owen of Department of Chemistry, Cambridge University, informed us that the signal δ = 4.29 published in their paper[29a] was a misprint, and must be corrected to δ = 4.69.