Journal of Molecular Catalysis B: Enzymatic 66 (2010) 198–202

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Chemoenzymatic synthesis of optically active alcohol and β-amino-acid derivative containing the difluoromethylene group

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ARTICLE INFO

Article history:
Received 31 March 2010
Received in revised form 20 May 2010
Accepted 22 May 2010
Available online 27 May 2010

Keywords:
Asymmetric reduction
β-Aminobutyric acid
Fluorine
Whole-cell biotransformation

ABSTRACT

The bioreduction of α,α-difluorinated ketones, ethyl 2,2-difluoro-3-oxobutanoate (**2a**) and 2,2-difluoro-1-phenyl-1,3-butanedione (**2b**), with cells of recombinant *Escherichia coli* overproducing SCR (*Saccharomyces cerevisiae* carbonyl reductase from bakers' yeast) and GDH (glucose dehydrogenase from *Bacillus megaterium*) gave enantiomerically pure alcohols, ethyl (*S*)-2,2-difluoro-3-hydroxybutanoate ((*S*)-**1a**) and (*S*)-2,2-difluoro-3-hydroxy-1-phenyl-1-butanone ((*S*)-**1b**), respectively, in the presence of $NADP^+$ and glucose in buffer. The reductions of **2a** and **2b** proceeded completely at the substrate concentrations of 0.4 M (67 g/L) and 1.0 M (200 g/L), respectively. The opposite enantiomers (*R*)-**1a** and (*R*)-**1b** were also produced by enzyme E039 (a mixture of carbonyl reductase and formate dehydrogenase) contained in Chiralscreen OH (Daicel Chemical Industries) in the presence of NADH and sodium formate in buffer. Enantiomerically pure (*S*)-**1a** was converted by organic synthetic methods into an α,α-difluorinated derivative of (*R*)-β-aminobutyric acid (BABA) in three steps.

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1. Introduction

Fluorine plays an important role in pharmaceutical and agrochemical compounds [1,2]. Half of the top 10 drugs sold in 2005 and approximately 20% of pharmaceuticals on the market contain fluorine [3]. A majority of the known organofluorine compounds bear the trifluoromethyl or fluorine-substituted aromatic groups, while compounds containing the difluoromethylene group are in the minority. The difluoromethylene group is isopolar and isosteric to the ethereal oxygen atom or the hydroxymethylene group, which is useful for the creation of biologically active compounds. For example, certain α,α-difluorinated ketones are known as the transition-state analogs (inhibitors) of hydrolytic enzymes such as renins [1].

Only several methods are available for installing the difluoromethylene group: the generation and use of difluorocarbene [4–6], the geminal difluorination of the (thio)carbonyl group with fluorinating agents such as (diethylamino)sulfur trifluoride (DAST) [7,8], the replacement of the two hydrogen atoms of the methylene group with N–F electrophilic fluorinating agents such as 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate) (Selectfluor) [9,10], the use of CF_2-containing building blocks such as $BrCF_2CO_2R$ [11–18], and the defluorination of trifluoromethyl ketones or trifluoroacetates [19–21]. Furthermore, the methods for the synthesis of optically active *gem*-difluorinated compounds are much less common. A few enantioselective chemical methods [22–24], the lipase-catalyzed kinetic resolution [4,5,25,26], and the asymmetric reduction of ketones using chiral Ru or Rh complexes [27] or bakers' yeast [28] have been reported.

Biocatalysts are useful tools for asymmetric synthesis [29,30]. Among various enzymes, carbonyl reductases are one of the most popular biocatalysts for preparing optically active alcohols with high enantiomeric purities [31–39]. We have focused on a synthetically useful enzyme called SCR (*Saccharomyces cerevisiae* carbonyl reductase from bakers' yeast), which shows broad substrate scope and high enantioselectivity simultaneously [40–45]. The recombinant *Escherichia coli* overproducing SCR and GDH (glucose dehydrogenase from *Bacillus megaterium*, a cofactor-regenerating enzyme) has been demonstrated to be a powerful tool for asymmetric synthesis [42–45]. More than 20 ketones have been converted into the optically active alcohols [42,43], including an important chiral synthon for clopidogrel, the top 2 drug in 2005 [44,45]. In this paper, we report the chemoenzymatic synthesis of optically pure α,α-difluorinated alcohols **1a** and **1b**. In addition, we also report the conversion of **1a** into an α,α-difluorinated derivative of β-aminobutyric acid (BABA) for the first time.

2. Experimental

2.1. Materials

Recombinant *E. coli* BL21(DE3) cells harboring pESCR and pABGD, which encode the SCR (*S. cerevisiae* carbonyl reductase

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doi:10.1016/j.molcatb.2010.05.009

from bakers' yeast) and GDH (glucose dehydrogenase from *B. megaterium*) genes, respectively, were grown as reported previously [42–45]. Enzyme E039, which is a mixture of carbonyl reductase and formate dehydrogenase, and Chiralscreen OH kit consisting of E001, E007, E031, E039, and E078 were provided by Daicel Chemical Industries. $NADP^+$ and NADH were purchased from Oriental Yeast and Kohjin, respectively. Silica gel column chromatography was performed using Fuji Silysia BW-127 ZH (100–270 mesh). Thin layer chromatography (TLC) was performed on Merck silica gel 60 F_{254}.

2.1.1. Synthesis of racemic ethyl 2,2-difluoro-3-hydroxybutanoate (**1a**)

Ethyl bromodifluoroacetate (4.0 mL, 31 mmol) was added to a mixture of acetaldehyde (18 mL, 0.32 mol) and activated zinc dust (2.63 g, 40.2 mmol) in dry THF (40 mL) in an autoclave. After the mixture had been stirred at 70 °C for 4 h, the reaction was quenched with 10 drops of saturated aqueous NH_4Cl at room temperature. After 30 min, the mixture was dried over $MgSO_4$, and concentrated. Purification by silica gel column chromatography (hexane/EtOAc (10:1)–(5:1)) followed by bulb-to-bulb distillation (10 mmHg, 100 °C) gave alcohol **1a** as a colorless oil (2.22 g, 43%) [18]. The spectral data for **1a** is shown below (Section 2.2.1).

2.1.2. Synthesis of ethyl 2,2-difluoro-3-oxobutanoate (**2a**)

Racemic alcohol **1a** (3.53 g, 21.0 mmol) was added dropwise to a solution of Dess–Martin periodinane (13.4 g, 31.5 mmol) in dry CH_2Cl_2 (117 mL) under Ar over 15 min. After the mixture had been stirred at room temperature for 4 h, the reaction was quenched with saturated aqueous $NaHCO_3$ (50 mL) and then saturated aqueous $Na_2S_2O_3$ (35 mL). The mixture was stirred for 30 min. The organic layer was separated, and the product was extracted with CH_2Cl_2 (40 mL × 4). The combined organic layers were dried over $MgSO_4$, and concentrated. Purification by bulb-to-bulb distillation (15 mmHg, 80 °C) gave ketone **2a** as a colorless oil (2.53 g, 73%) [10]: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.36 (t, J = 7.2 Hz, 3H), 2.42 (t, J = 1.8 Hz, 3H), 4.38 (q, J = 7.2 Hz, 2H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 13.8, 24.1, 63.7, 108.0 (t, J = 263.7 Hz), 161.2 (t, J = 30.6 Hz), 194.8 (t, J = 29.1 Hz); ^{19}F NMR ($CDCl_3$, 564 MHz) δ −114.9 (d, J = 1.7 Hz, 2F); IR (neat) 2990, 2943, 1780, 1751, 1420, 1373, 1315, 1234, 1134, 1053, 1013, 947, 856, 839, 737 cm^{-1}.

2.1.3. Synthesis of 1-phenyl-3-(1-pyrrolidinyl)-2-butene-1-one

To a solution of 1-phenyl-1,3-butanedione (3.01 g, 18.5 mmol) in dry CH_3CN (46 mL) was added pyrrolidine (1.9 mL, 23 mmol). The mixture was stirred at room temperature for 22 h. The mixture was cooled in an ice bath to form a white solid. The solid was collected by filtration, and the filtrate was concentrated to form a white solid. The product was recrystallized from CH_3CN/hexane to give the product as a white solid (3.43 g, 86%) [10]: mp 168.1–168.3 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.99 (br s, 4H), 2.68 (s, 3H), 3.36 (br s, 2H), 3.53 (br s, 2H), 5.60 (s, 1H), 7.36–7.40 (m, 3H), 7.85–7.86 (m, 2H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 17.9, 24.9, 25.2, 48.1, 48.5, 92.5, 127.2, 127.9, 130.0, 143.0, 161.7, 187.8; IR (KBr) 3021, 2980, 2891, 2860, 1526, 1427, 1346, 1219, 1157, 1067, 1026 cm^{-1}.

2.1.4. Synthesis of 2,2-difluoro-1-phenyl-1,3-butanedione (**2b**)

To a solution of Selectfluor (10.6 g, 29.9 mmol) in dry CH_3CN (280 mL) was added Et_3N (1.9 mL, 14 mmol) at −10 °C. A solution of 1-phenyl-3-(1-pyrrolidinyl)-2-butene-1-one (2.93 g, 13.6 mmol) in dry CH_3CN (98 mL) was added dropwise over 20 min. The mixture was stirred at −10 °C for 4.5 h, and silica gel (34 g) was added. The mixture was stirred at −10 °C for 1 h, filtered, and concentrated. Purification by silica gel column chromatography (hexane/EtOAc (7:1)) gave **2b** as a reddish brown oil (1.56 g, 58%) [10]: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.42 (t, J = 1.5 Hz, 3H), 7.51 (t, J = 7.5 Hz, 2H), 7.66 (t, J = 7.5 Hz, 1H), 8.05 (d, J = 7.5 Hz, 2H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 25.0, 111.3 (t, J = 266.6 Hz), 128.9, 130.1, 131.3, 135.1, 187.5 (t, J = 27.6 Hz), 196.0 (t, J = 27.9 Hz); ^{19}F NMR ($CDCl_3$, 282 MHz) δ −110.1 (s, 2F); IR (neat) 3072, 2976, 2930, 1755, 1699, 1599, 1582, 1450, 1420, 1364, 1296, 1256, 1225, 1151, 1115, 1082, 1053, 964, 893, 839, 714 cm^{-1}.

2.1.5. Synthesis of racemic 2,2-difluoro-3-hydroxy-1-phenyl-1-butanone (**1b**)

2-Chloro-2,2-difluoroacetophenone (0.30 mL, 2.0 mmol) was added to a mixture of acetaldehyde (1.1 mL, 20 mmol) and activated zinc dust (0.196 g, 2.99 mmol) in dry DMF (5 mL) under Ar in a sealed tube at 0 °C. The mixture was stirred at room temperature for 20 h. The reaction was quenched with 10% HCl (1 mL), and the mixture was stirred for 30 min. The product was extracted with EtOAc (3 mL × 4). The mixture was dried over $MgSO_4$, and concentrated. Purification by silica gel column chromatography (hexane/EtOAc (10:1)–(5:1)) gave racemic alcohol **1b** as a colorless oil (151 mg, 38%) [25]. The spectral data for **1b** is shown below (Section 2.2.2).

2.2. Typical procedure for the asymmetric reduction of ketone with recombinant E. coli

To a mixture of glucose (1.08 g, 6.00 mmol), $NADP^+$ (10 mg, 12 μmol), and *E. coli* BL21(DE3) cells harboring pESCR and pABGD (2.0 g) in 0.1 M phosphate buffer (pH 7.0, 10 mL) was added ketone **2** (3.00 mmol). The mixture was stirred in a water bath at 20 °C for 24 h, during which the pH of the solution was kept constant by adding 2N NaOH. Solid NaCl (5.5 g) was added, and the product was extracted with EtOAc (25 mL × 4), where centrifugation (3,200 rpm, 10 min) was conducted to promote the phase separation. The combined organic layers were dried over $MgSO_4$, and concentrated. Purification by bulb-to-bulb distillation or silica gel column chromatography gave alcohol **1**.

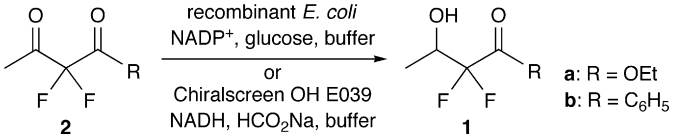
2.2.1. Ethyl (*S*)-2,2-difluoro-3-hydroxybutanoate ((*S*)-**1a**)

Purification by bulb-to-bulb distillation (17 mmHg, 80 °C) gave alcohol (*S*)-**1a** (278 mg, 55%): >99% ee (*S*); $[\alpha]^{24}_D$ −1.0 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.35 (dt, J = 0.9, 6.6 Hz, 3H), 1.37 (t, J = 7.2 Hz, 3H), 2.08 (br s, 1H), 4.23 (hept, J = 7.2 Hz, 1H), 4.37 (q, J = 7.2 Hz, 2H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 13.9, 15.2 (dd, J = 2.3, 4.1 Hz), 63.1, 68.0 (dd, J = 25.9, 28.3 Hz), 114.5 (dd, J = 254.8, 256.0 Hz), 163.6 (dd, J = 31.1, 32.7 Hz); ^{19}F NMR ($CDCl_3$, 564 MHz) δ −125.1 (dd, J = 14.7, 265.2 Hz, 1F), −116.7 (dd, J = 6.8, 265.2 Hz, 1F); IR (neat) 3466, 2991, 2945, 1759, 1448, 1375, 1314, 1217, 1105, 1049, 1015, 907, 843 cm^{-1}; GC: CP-cyclodextrin-β-2,3,6-M-19 column (Varian, ϕ 0.25 mm × 25 m), Inj. 250 °C, Col. 80 °C, Det. 200 °C, (*R*) 28.1 min, (*S*) 30.0 min.

2.2.2. (*S*)-2,2-Difluoro-3-hydroxy-1-phenyl-1-butanone ((*S*)-**1b**)

Purification by silica gel column chromatography (hexane/EtOAc (10:1)) gave alcohol (*S*)-**1b** (447 mg, 74%): >99% ee (*S*); $[\alpha]^{28}_D$ +25 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.41 (d, J = 6.6 Hz, 3H), 2.55 (br s, 1H), 4.45 (dquint, J = 6.6, 17.4 Hz, 1H), 7.51 (t, J = 8.7 Hz, 2H), 7.64–7.67 (m, 1H), 8.12 (d, J = 8.7 Hz, 2H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 14.8 (dd, J = 2.6, 4.4 Hz), 67.5 (dd, J = 24.4, 28.5 Hz), 116.3 (dd, J = 257.1, 261.8 Hz), 128.7, 130.2 (t, J = 3.4 Hz), 132.1, 134.7, 190.5 (dd, J = 30.2, 31.9 Hz); ^{19}F NMR ($CDCl_3$, 564 MHz) δ −119.9 (dd, J = 18.1, 297.4 Hz, 1F), −109.2 (d, J = 297.4 Hz, 1F); IR (neat) 3445, 3071, 2991, 2945, 1693, 1599, 1580, 1450, 1286, 1204, 1184, 1144, 1101, 1011, 926, 856, 716, 687, 662 cm^{-1}; HPLC: Chiralpak AD-H (Daicel Chemical Industries), hexane/*i*-PrOH (9:1), flow rate 0.2 mL/min, detection 254 nm, (*S*) 34.4 min, (*R*) 36.7 min.

Table 1
Asymmetric reduction of **2** with carbonyl reductases.[a]



Entry	Carbonyl reductase	Ketone				Conv. (%)[b]	Alcohol **1**	
		2	(mmol)	(M)	(g/L)		Yield (%)[c]	ee (%)[d]
1	SCR	**2a**	3	0.3	50	>99	55	>99 (*S*)
2	SCR	**2a**	4	0.4	67	>99	61	>99 (*S*)
3	SCR	**2b**	3	0.3	60	>99	74	>99 (*S*)
4	SCR	**2b**	6	0.6	120	>99	82	>99 (*S*)
5	SCR	**2b**	10	1.0	200	>99	84	>99 (*S*)
6	E039	**2a**	1	0.05	8	>99	3[e]	>99 (*R*)
7	E039	**2b**	3	0.15	29	>99	77	>99 (*R*)

[a] Conditions for entries 1–5: **2** (quantity and concentration indicated above), wet cells of *E. coli* BL21(DE3) harboring pESCR and pABGD (2.0 g), glucose (2.0 equiv. with respect to **2**), $NADP^+$ (10 mg, 12 μmol), 0.1 M phosphate buffer (pH 7.0, 10 mL), 20 °C, 24 h. Conditions for entries 6–7: **2** (3.0 mmol), E039 (a mixture of carbonyl reductase and formate dehydrogenase) contained in Chiralscreen OH (Daicel Chemical Industries, 100 mg), HCO_2Na (272 mg, 4.0 mmol), NADH (14 mg, 18 μmol), 0.5 M phosphate buffer (pH 7.0, 20 mL), 20 °C, 24 h.
[b] Conversion determined by ^{19}F NMR.
[c] Isolated yield of **1**.
[d] Determined by GC (CP-cyclodextrin-β-2,3,6-M-19 column) for **1a** and by HPLC (Chiralpak AD-H, hexane/*i*-PrOH (9:1)) for **1b**.
[e] Yield determined by ^{19}F NMR using hexafluorobenzene as an internal standard.

2.3. General procedure for the asymmetric reduction of ketone with E039

To a mixture of HCO_2Na (272 mg, 4.00 mmol), NADH (14 mg, 18 μmol), and Chiralscreen OH E039 (100 mg) in 0.5 M phosphate buffer (pH 7.0, 20 mL) was added ketone **2** (3.00 mmol). The mixture was stirred in a water bath at 20 °C for 24 h. Alcohol **1** was extracted and purified as shown above.

2.4. Ethyl (S)-3-(trifluoromethanesulfonyloxy)-2, 2-difluorobutanoate ((S)-3)

To a solution of (*S*)-**1a** (313 mg, 1.86 mmol) in dry CH_2Cl_2 (14 mL) under Ar was added dropwise pyridine (1.2 mL, 14.9 mmol) at 0 °C over 5 min. After the mixture had been stirred at 0 °C for 15 min, the resulting mixture was cooled to −40 °C. To the mixture was added dropwise a solution of trifluoromethanesulfonic anhydride (0.37 mL, 2.25 mmol) in dry CH_2Cl_2 (5 mL) over 20 min. The mixture was stirred at −40 °C for 4 h. The reaction was quenched with saturated aqueous $NaHCO_3$ at 0 °C. The product was extracted with CH_2Cl_2 (10 mL × 4). The combined organic layer was dried over Na_2SO_4, and concentrated. Purification by silica gel column chromatography (hexane/CH_2Cl_2 (2:1)) gave (*S*)-**3** as a colorless oil (365 mg, 65%): ^1H NMR ($CDCl_3$, 400 MHz) δ 1.39 (t, J = 7.2 Hz, 3H), 1.63 (dt, J = 0.9, 6.8 Hz, 3H), 4.40 (q, J = 7.2 Hz, 2H), 5.28–5.34 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.7, 13.9, 64.1, 80.8 (dd, J = 26.4, 30.9 Hz), 111.4 (dd, J = 254.7, 257.7 Hz), 118.2 (q, J = 317.3 Hz), 161.2 (t, J = 30.9 Hz); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −119.3 (dd, J = 10.7, 271.0 Hz, 1F), −116.6 (dd, J = 8.3, 271.0 Hz, 1F), −76.1 (s, 3F); IR (neat) 2993, 1778, 1423, 1315, 1219, 1146, 1072, 1042, 1007, 934, 849, 810, 621 cm^{-1}.

2.5. Ethyl (R)-3-azido-2,2-difluorobutanoate ((R)-4)

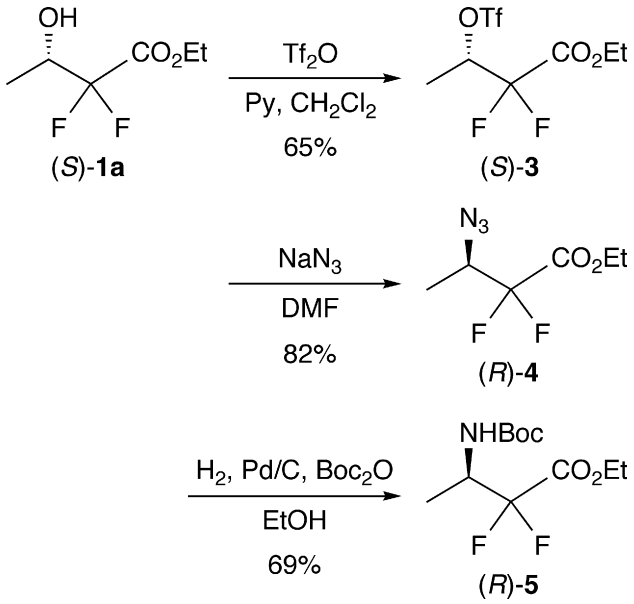
To a solution of (*S*)-**3** (342 mg, 1.14 mmol) in dry DMF (6 mL) under Ar in an ice bath was added NaN_3 (88.9 mg, 1.37 mmol). The mixture was stirred at room temperature overnight. The reaction was quenched with H_2O. The product was extracted with CH_2Cl_2 (10 mL × 4). The combined organic layer was dried over Na_2SO_4, and concentrated. Purification by silica gel column chromatography (hexane/EtOAc (5:1)) gave (*R*)-**4** as a yellow oil (181 mg, 82%): $[\alpha]^{20}_D$ −33.0 (*c* 1.04, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.38 (t, J = 7.2 Hz, 3H), 1.42 (d, J = 7.2 Hz, 3H), 3.93 (sept, J = 7.2 Hz, 1H), 4.38 (q, J = 7.2 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.7 (dd, J = 2.3, 4.4 Hz), 13.8, 57.8 (dd, J = 24.5, 28.3 Hz), 63.3, 114.1 (dd, J = 254.0, 257.0 Hz), 162.6 (dd, J = 30.9, 31.9 Hz); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −120.0 (dd, J = 15.6, 259.8 Hz, 1F), −111.6 (dd, J = 7.7, 259.8 Hz, 1F); IR (neat) 2991, 2129, 2102, 1771, 1458, 1377, 1308, 1252, 1227, 1138, 1111, 1065, 1042, 1003, 860 cm^{-1}.

2.6. Ethyl (R)-3-tert-butoxycarbonylamino-2, 2-difluorobutanoate ((R)-5)

To a solution of (*R*)-**4** (208 mg, 1.08 mmol) in dry EtOH (10 mL) under Ar was added Boc_2O (550 μL, 2.40 mmol) and Pd/C (Aldrich, 10% (w/w), 34 mg). The mixture was stirred under H_2 at room temperature for 24 h. The mixture was filtered through Celite, and concentrated. Purification by silica gel column chromatography (hexane/EtOAc (10:1)) gave (*R*)-**5** as a colorless oil (198 mg, 69%): 98% ee; $[\alpha]^{20}_D$ +7.71 (*c* 1.02, $CHCl_3$); ^1H NMR ($CDCl_3$, 600 MHz) δ 1.26 (d, J = 6.6 Hz, 3H), 1.35 (t, J = 7.2 Hz, 3H), 1.42 (s, 9H), 4.29–4.41 (m, 3H), 4.63 (d, J = 9.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 13.6, 13.8, 28.1, 48.5 (dd, J = 24.0, 28.7 Hz), 63.0, 80.2, 114.3 (t, J = 253.8 Hz), 154.6, 163.3 (dd, J = 30.6, 33.0 Hz); ^{19}F NMR ($CDCl_3$, 564 MHz) δ −122.5 (d, J = 255.0 Hz, 1F), −114.9 (d, J = 255.0 Hz, 1F); IR (neat) 3341, 2982, 2939, 1771, 1717, 1524, 1458, 1369, 1308, 1250, 1169, 1069, 1045, 864 cm^{-1}; HRMS (EI) calcd for $C_{11}H_{19}NO_4F_2$ 267.1282, found 267.1283; GC: CP-cyclodextrin-β-2,3,6-M-19 column, Inj. 250 °C, Col. 110 °C, Det. 200 °C, (*R*) 43.5 min, (*S*) 44.6 min.

3. Results and discussion

In the synthesis of α,α-difluorinated ketones **2a** and **2b**, we put a high priority on the isolation of a pure compound. After trial and error, the Reformatsky reaction of $BrCF_2CO_2Et$ with CH_3CHO followed by the Dess–Martin oxidation was found to be a practical method for obtaining pure **2a**. On the other hand, the difluorination of 1-phenyl-3-(1-pyrrolidinyl)-2-butene-1-one with Selectfluor was a good and easy method for the preparation of pure **2b**. The best synthetic procedures are described in the Experimental section.



Scheme 1. Synthesis of (*R*)-**5**, an α,α-difluorinated derivative of (*R*)-β-aminobutyric acid (BABA).

The bioreduction of ketone **2** was done with recombinant *E. coli* coexpressing SCR and GDH [42–45], and alcohol **1** was purified by bulb-to-bulb distillation or column chromatography. The results are summarized in Table 1. The absolute configurations of the obtained alcohols **1a** and **1b** were determined by the Mosher method with MTPA [46]. Asymmetric reduction of β-keto ester **2a** gave (*S*)-**1a** in good yield with >99% ee, and the conversion was complete (>99%) at the substrate concentration of 0.3–0.4 M (entries 1 and 2). The conversion, however, dropped to 84% when the substrate concentration was increased up to 0.5 M (not shown). The reaction conditions shown in entry 2 therefore seem to be optimal for the reduction of **2a**. On the other hand, the reduction of β-diketone **2b** (0.3 M) afforded (*S*)-**1b** in 74% yield with >99% ee (entry 3). The less hindered carbonyl group underwent the reduction completely regioselectively. Because the reaction was completed at the substrate concentration of 0.6 M (entry 4), the substrate concentration was increased up to 1.0 M (200 g/L) (entry 5), with the result that (*S*)-**1b** was obtained in 84% yield with >99% ee. This high productivity is almost the same as that we have reported previously [44,45]. At the substrate concentration of 1.3 M, however, the conversion reached only 36% (not shown).

Asymmetric synthesis of each enantiomer is ideal because these alcohols **1a** and **1b** are supposed to be used as chiral building blocks in future. We therefore searched for enzymes capable of forming (*R*)-alcohols. For this purpose, we employed Chiralscreen OH (Daicel Chemical Industries), a screening kit for selecting an enzyme suitable for the asymmetric reduction of a target ketone. We expected that both enantiomers with a high enantiomeric purity could be obtained much more rapidly and easily by using this type of enzyme kit than by screening microorganisms [37], the latter of which contain many carbonyl reductases leading to low enantioselectivity. The asymmetric reductions of **2a** and **2b** were performed at room temperature with E001, E007, E031, E039, or E078 in Chiralscreen OH according to the supplier's protocol. The crude product was extracted with EtOAc and then analyzed directly by chiral HPLC. As a result, E001, E007, E031, and E078 gave (*S*)-**1a** with >99–95% ee and (*S*)-**1b** with >99–27% ee, while only E039 gave (*R*)-**1a** with >99% ee and (*R*)-**1b** with 97% ee. Based on these preliminary results, the preparative asymmetric reductions of **2a** and **2b** were performed at 20 °C with enzyme E039 (Table 1, entries 6 and 7). Curiously, (*R*)-**1a** was obtained in only 3% yield for an unknown reason although the enantiomeric purity was as high as >99% ee (entry 6). Although we have done the same experiment three times, the yield was very low in all cases. Because ^{19}F NMR indicated that the conversion estimated by the ratio of **1a** to **2a** in the extract was very high (>99%), we suppose that a side reaction might have proceeded, such as hydrolysis giving a carboxylic acid that is difficult to extract. In contrast, (*R*)-**1b** was isolated successfully in 77% yield with >99% ee (entry 7).

β-Amino acids are important from the viewpoint of biological activity and synthetic utility. Among them, β-aminobutyric acid (BABA) functions as a partial agonist of glycine receptor in animals, while BABA participates in a defense mechanism against various pathogens in plants although the mode of action of BABA has been clarified only partially [47]. We expected that fluorinated derivatives of BABA might be useful for the elucidation of the mode of action of BABA in animals or plants, in addition to their synthetic utility toward other biologically active compounds. In this context, we decided to convert **1a** into a derivative of α,α-difluorinated BABA **3** (Scheme 1). To the best of our knowledge, no derivatives of α,α-difluorinated BABA have so far been synthesized. Treatment of (*S*)-**1a** with Tf_2O gave (*S*)-**3** in 65% yield, which was then treated with NaN_3 to afford (*R*)-**4** in 82% yield. Subsequent hydrogenation and in situ protection with Boc_2O afforded (*R*)-**5** in 69% yield with 98% ee. Because of the volatility of these compounds, the solvent was carefully evaporated.

4. Conclusions

To the best of our knowledge, no optically active alcohols containing the difluoromethylene group are currently commercially available. Here we studied the practical chemoenzymatic synthesis of ethyl 2,2-difluoro-3-hydroxybutanoate (**1a**) and 2,2-difluoro-3-hydroxy-1-phenyl-1-butanone (**1b**), which are expected to be useful chiral building blocks. The reduction of ethyl 2,2-difluoro-3-oxobutanoate (**2a**) and 2,2-difluoro-1-phenyl-1,3-butanedione (**2b**) with recombinant *E. coli* or enzyme E039 (a mixture of carbonyl reductase and formate dehydrogenase in Chiralscreen OH (Daicel Chemical Industries)) gave the corresponding optically pure alcohols with *S* or *R* configurations. In the asymmetric synthesis of **1b** with recombinant *E. coli* cells harboring pESCR and pABGD, which encode the SCR (*S. cerevisiae* carbonyl reductase from bakers' yeast) and GDH (glucose dehydrogenase from *B. megaterium*) genes, respectively, highly enantioselective and regioselective reduction of **2b** proceeded even at a substrate concentration of 1.0 M (200 g/L) in the presence of $NADP^+$ and glucose in buffer. We expect that **1a** can be used as a chiral synthon for α,α-difluoro-β-amino acids [1,48,49] while **1b** can be used as an analog of 1,3-diol units in natural products after further reduction to α,α-difluoro 1,3-diol [50,51]. Here we have converted (*S*)-**1a** into a derivative of α,α-difluorinated BABA, (*R*)-**5**, for the first time.

Acknowledgments

We thank Dr. H. Amii (Kobe University) and Dr. M. Kuroboshi (Okayama University) for valuable advice on the synthesis of difluorinated compounds. We also thank Daicel Chemical Industries for providing us with Chiralscreen OH. This work was supported by a Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science (JSPS). We are grateful to the SC-NMR Laboratory of Okayama University for the measurement of NMR spectra.

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