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Production of (*R*)-chiral alcohols by a hydrogen-transfer bioreduction with NADH-dependent *Leifsonia* alcohol dehydrogenase (LSADH)

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Abstract—Alcohol dehydrogenase (LSADH) isolated from *Leifsonia* sp. S749 was used to produce (*R*)-chiral alcohols. The enzyme with a broad substrate range reduced various prochiral ketones and keto esters to yield optically active secondary alcohols with a high enantiomeric excess. LSADH transferred the pro-*S* hydrogen of NADH to the carbonyl moiety of phenyl trifluoromethyl ketone **13** through its *re* face to give (*S*)-1-phenyl-2,2,2-trifluoroethanol **40**. LSADH was able to efficiently reproduce NADH when 2-propanol was used as a hydrogen donor in the reaction mixture.

1. Introduction

In recent years, enantiometrically pure compounds including amino acids, organic acids, amines, alcohols, epoxides and so on, have been produced with the use of biocatalysts, because such methods are superior to general chemical methods in terms of enantioselectivity.[1] Among them, secondary alcohols are the most important chiral synthons for pharmaceuticals and agrochemicals.

Chiral metal complexes such as BINAP-Ru have been successfully used as catalysts in a number of cases of asymmetric reduction processes, and industrially applied for the synthesis of (−)-menthol and other terpenic substances.[2] However, in the case of ketone reductions, difficulties remain in the operation, and in obtaining sufficient enantiomeric excess and productivity.[2,3] An alternative to the chemical asymmetric reduction processes is a biocatalytic transformation system using enzymes or microorganisms.[4]

Until now, many examples of the reduction of ketones with reductases and dehydrogenases have been described.[4,5] Oxidoreductases including NAD^+-dependent alcohol dehydrogenases (ADHs) from yeast, horse liver,[4] *Candida parapsilosis*[6,7] and *Pseudomonas* sp.,[8] and $NADP^+$-dependent ADHs from *Thermoanaerobium brochii*[9] and *Lactobacillus kefir*,[10,11] aldehyde reductase from *Sporobolomyces salmonicolor* (EC 1.1.1.2),[12] and carbonyl reductase (EC 1.1.1.184) from *Candida magnoliae*[13] have been used for this purpose. However, in many cases, they have the disadvantages of a narrow substrate specificity, insufficient stereoselectivity or low chemotolerancy to ketone substrates and organic solvents. In addition, regenerating NAD(P)H is necessary to efficiently continue the bioreduction process. Therefore, such bioreduction processes are only economical when the cofactor can be regenerated in situ in a second catalytic cycle, for example, formate/formate dehydrogenase (FDH) or glucose/glucose dehydrogenase (GDH).[14]

From the viewpoint of the regeneration of NAD(P)H, 2-propanol is another suitable hydrogen donor for bioreduction because of its chemical properties and low cost.[6–9,15–17] Recently, Itoh et al. reported that phenylacetaldehyde reductase (PAR) from the styrene-assimilating *Rhodococcus* (former *Corynebacterium*) sp. strain ST-10 is a unique NADH-dependent alcohol dehydrogenase (ADH) with a broad substrate range and high enantioselectivity to give (*S*)-alcohols from various carbonyl compounds without an additional coenzyme regeneration system, because the enzyme itself is able to regenerate NADH in the presence of 2-propanol.[17–21]

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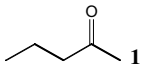
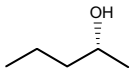
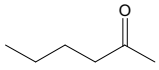
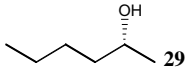
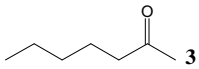
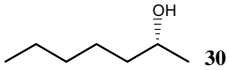
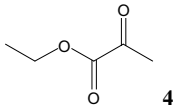
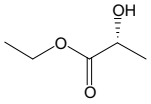
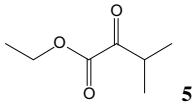
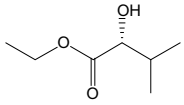
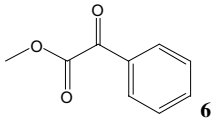
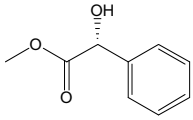
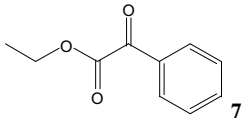
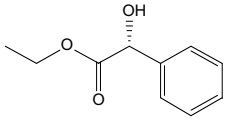
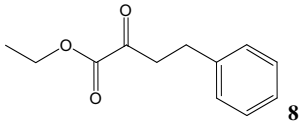
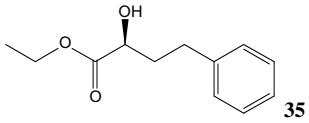
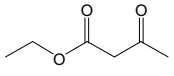
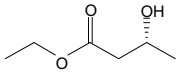
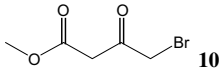
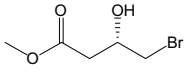
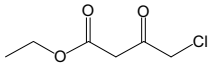
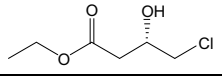
Table 1. Analysis of substrates and products of LSADH reaction

Substrate/product	Analzying method/ conditions/retention time (min)	Determination method of (*R*) and (*S*)-alcohols/conditions/retention time (min)
1/28	GC/DB-1, inj.: 250 °C, det.: 250 °C, col.: 40 °C (5 °C/min) to 100 °C/sub.: 3.9, pro.: 4.2	HPLC/Chiracel OB-H, hexane/2-propanol (49:1), 0.5 ml/min, at 254 nm/, pro. (*R*)-form: 7.7, pro. (*S*)-form: 8.3
2/29	GC/DB-1, same as above/sub.: 6.3, pro.: 7.0	HPLC/Chiracel OB-H, same above/pro. (*R*)-form: 7.5, pro. (*S*)-form: 8.0
3/30	GC/DB-1, same as above/sub.: 9.9, pro.: 10.8	HPLC/Chiracel OB-H, same above/pro. (*R*)-form: 7.2, pro. (*S*)-form: 7.9
4/31	—	GC/CP-cyclodextrin, inj.: 250 °C, det.: 250 °C, col.: 80 °C/sub.: 3.1, pro. (*R*)-form: 4.0, pro. (*S*)-form: 4.2
5/32	GC/DB-1, inj.: 200 °C, det.: 200 °C, col.: 160 °C/sub.: 12.1, pro.: 12.9	HPLC/Chiracel OD-H, hexane/2-propanol (49:1), 0.5 ml/min, 220 nm, 30 °C/pro. (*R*)-form: 8.4, pro. (*S*)-form: 7.9
6/33	GC/Thermon1000, inj.: 250 °C, det.: 250 °C, col.: 160 °C/sub.: 6.6, pro.: 11.2	HPLC/Chiracel OB-H, hexane/2-propanol/TFA (9:1:0.1), 1.0 ml/min, 254 nm, 30 °C/pro. (*R*)-form: 9.9, pro. (*S*)-form: 6.5
7/34	Same as above/sub.: 7.4, pro.: 10.8	HPLC/Chiracel OB-H, same above/pro. (*R*)-form: 8.6, pro. (*S*)-form: 5.6
8/35	GC/DB-1, inj.: 200 °C, det.: 200 °C, col.: 70 °C (5 °C/min raise) to 250 °C/sub.: 24.4, pro.: 24.8	HPLC/Chiracel OB-H, same above/pro. (*R*)-form: 7.3, pro. (*S*)-form: 5.6
9/36	GC/Thermon1000, inj.: 250 °C, det.: 250 °C, col.: 70 °C/sub.: 7.6, pro.: 10.1	HPLC/Chiracel OB-H, hexane/2-propanol (9:1), 1.0 ml/min, at 220 nm, 30 °C/pro. (*R*)-form: 12.6, pro. (*S*)-form: 11.6
10/37	GC/DB-1, inj.: 200 °C, det.: 200 °C, col.: 70 °C (5 °C/min raise) to 250 °C/sub.: 11.2, pro.: 12.5	HPLC/Chiracel OB-H, same above/pro. (*R*)-form: 18.8, pro. (*S*)-form: 18.2
11/38	GC/DB-1, same as above/sub.: 11.1, pro.: 12.3	HPLC/Chiracel OB-H, hexane/2-propanol (9:1), 1.0 ml/min, 220 nm, 30 °C/pro. (*R*)-form: 7.4, pro. (*S*)-form: 7.9
12/39	—	GC/CP-cyclodextrin, inj.: 250 °C, det.: 250 °C, col.: 120 °C/sub.: 4.3, pro. (*R*)-form: 6.0, pro. (*S*)-form: 6.5
13/40	—	GC/CP-cyclodextrin, inj.: 250 °C, det.: 250 °C, col.: 130 °C/sub.: 1.8, pro. (*R*)-form: 7.3, pro. (*S*)-form: 6.7
14/41	—	GC/CP-cyclodextrin, inj.: 250 °C, det.: 250 °C, col.: 140 °C/sub.: 5.1, pro. (*R*)-form: 8.7, pro. (*S*)-form: 9.1
15/42	—	GC/CP-cyclodextrin, same as above/sub.: 5.5, pro. (*R*)-form: 8.9, pro. (*S*)-form: 9.3
16/43	—	GC/CP-cyclodextrin, same as above/sub.: 5.5, pro. (*R*)-form: 8.9, pro. (*S*)-form: 9.3
17/44	—	GC/CP-cyclodextrin, same as above/sub.: 8.4, pro. (*R*)-form: 14.1, pro. (*S*)-form: 14.9
18/45	—	GC/CP-cyclodextrin, same as above/sub.: 8.2, pro. (*R*)-form: 10.4, pro. (*S*)-form: 10.0
19/46	—	GC/CP-cyclodextrin, inj.: 250 °C, det.: 250 °C, col.: 160 °C/sub.: 8.5, pro. (*R*)-form: 13.1, pro. (*S*)-form: 12.7
20/47	GC/DB-1, inj.: 200 °C, det.: 200 °C, col.: 70 °C (5 °C/min raise) to 250 °C/sub.: 18.7, pro.: 20.5	HPLC/Chiracel OB-H, hexane/2-propanol (9:1), 0.5 ml/min, 254 nm, 30 °C/pro. (*R*)-form: 10.5, pro. (*S*)-form: 7.7
21/48	GC/DB-1, same as above/sub.: 17.4, pro.: 17.7	HPLC/Chiracel OB-H, hexane/2-propanol (9:1), 1.0 ml/min, 254 nm, 30 °C/pro. (*R*)-form: 14.6, pro. (*S*)-form: 10.5
22/49	GC/DB-1, same as above/sub.: 24.8, pro.: 25.2	HPLC/Chiracel OB-H, same as above/pro. (*R*)-form: 13.8, pro. (*S*)-form: 15.1
23/50	—	GC/CP-cyclodextrin, inj.: 250 °C, det.: 250 °C, col.: 110 °C/sub.: 9.1, pro. (*R*)-form: 15.0, pro. (*S*)-form: 15.7
24/51	—	GC/CP-cyclodextrin, same as above/sub.: 15.8, pro. (*R*)-form: 22.9, pro. (*S*)-form: 23.6
25/52	GC/DB-1, inj.: 200 °C, det.: 200 °C, col.: 70 °C (5 °C/min raise) to 250 °C/sub.: 22.4, pro.: 22.7	HPLC/Chiracel OD-H, hexane/2-propanol (49:1), 1.0 ml/min, 285 nm, 30 °C/pro. (*R*)-form: 17.1, pro. (*S*)-form: 16.0
26/53	GC/DB-1, same as above/sub.: 26.1, pro.: 26.8	HPLC/Chiracel OD-H, hexane/2-propanol (49:1), 1.0 ml/min, 254 nm, 40 °C/pro. (*R*)-form: 31.0, pro. (*S*)-form: 23.6
27/54	GC/DB-1, same as above/sub.: 18.9, pro.: 21.1	HPLC/Chiracel OF, hexane/2-propanol (4:1), 0.5 ml/min, 220 nm, 40 °C/pro. (*R*)-form: 11.5, pro. (*S*)-form: 10.3

Therefore, such a system is regarded as a superior asymmetric hydrogen-transfer reduction process. Recently, another chemotolerant ADH is reported in a *R. ruber* DSM44541 strain.[16] However, PAR can hardly transform phenyl trifluoromethyl ketone (PTK) **13** to (*R*)- or (*S*)-1-phenyl-2,2,2-trifluoroethanol (PTE) **40**, which would be a potential chiral synthon for liquid crystals. In a previous study, we described the screening of a microorganism (*Leifsonia* sp. strain S749), which can reduce PTK **13** to (*S*)-PTE **40**, the purification of the corresponding ADH (LSADH: *Leifsonia* ADH), and the characterization of the enzyme.[22]

Herein, we report the application of LSADH for the production of (*R*)-chiral alcohols from ketones including acetophenone derivatives, 2-alkanones and keto

Table 2. Enantioselective reduction of alkanone and keto esters by LSADH

Substrate	Relative activity (%)	Product	ee (%)	Conversion (%)	Yield (%)
 **1**	17	 **28**	(*R*) >99	79	17
 **2**	104	 **29**	(*R*) >99	83	31
 **3**	229	 **30**	(*R*) >99	87	38
 **4**	488	 **31**	(*R*) >99	100	19
 **5**	33	 **32**	(*R*) >99	100	40
 **6**	1	 **33**	(*R*) 47	71	31
 **7**	3	 **34**	(*R*) 23	29	20
 **8**	5	 **35**	(*S*) 85	95	58
 **9**	309	 **36**	(*R*) >99	100	47
 **10**	164	 **37**	(*S*) >99	100	35
 **11**	809	 **38**	(*S*) >99	100	54

esters with a high enantioselectivity, and evaluate LSADH as an asymmetric hydrogen-transfer biocatalyst using 2-propanol.

2. Results and discussion

2.1. Substrate spectrum and stereoselectivity of LSADH for various ketones

As previously described, LSADH is a novel NADH-dependent ADH with a broad substrate range.[22] The enzyme is highly active toward medium-chain normal aldehydes and 2-alkanones between C_5 and C_8, and various aryl aldehydes and acetophenone derivatives, although it barely acts on short-chain normal alkyl aldehydes and 2-alkanones, and cycloalkanones. With the LSADH reactions, acetophenone **12**, PTK **13** and 2-heptanone **3** are converted to (*R*)-1-phenylethanol **39** (99% ee), (*S*)-PTE **40** (>99% ee) and (*R*)-2-heptanol **30** (>99% ee) in the presence of 2-propanol. Therefore, LSADH was thought to be a suitable biocatalyst for producing the (*R*)-form chiral alcohols.[22] We further explored the substrate specificity and stereoselectivity of LSADH for various ketones. The results are summarized in Tables 2 and 3. It was found that LSADH was able to catalyze the reduction of not only ketones but also α-keto and β-keto esters. Until now, LSADH has been found to catalyze the reduction of more than 40 ketones, indicating a quite broad substrate spectrum. The data concerning the stereoselectivities for the substrates clarified that LSADH produced (*R*)-alcohols **39**, **41**–**44**, **47**–**52** from

Table 3. Enantioselective reduction of arylketones by LSADH

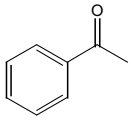
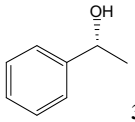
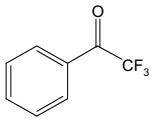
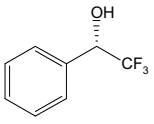
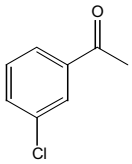
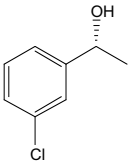
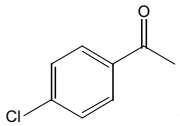
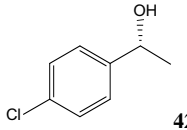
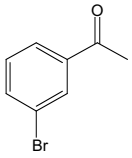
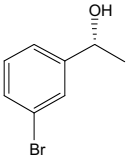
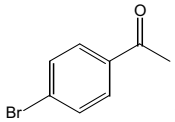
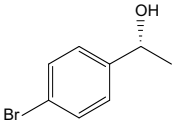
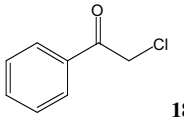
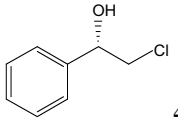
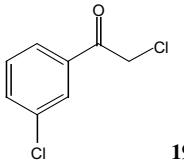
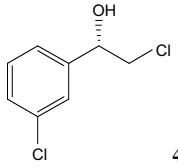
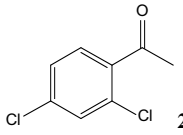
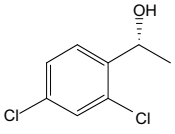
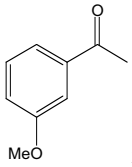
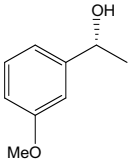
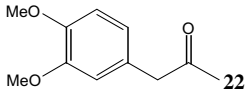
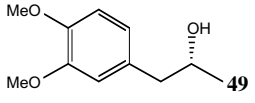
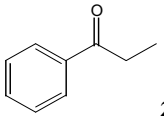
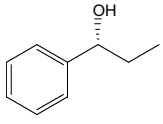
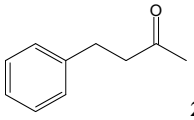
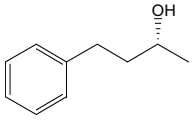
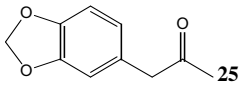
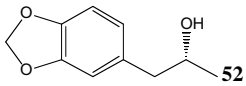
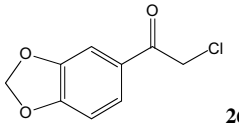
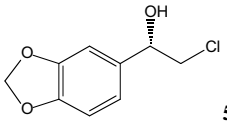
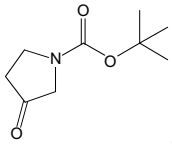
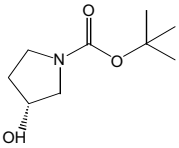
Substrate	Relative activity (%)	Product	ee (%)	Conversion (%)	Yield (%)
 **12**	6	 **39**	(*R*) 99	82	40
 **13**	100	 **40**	(*S*) >99	100	81
 **14**	70	 **41**	(*R*) >99	90	61
 **15**	60	 **42**	(*R*) >99	81	58
 **16**	151	 **43**	(*R*) >99	95	60
 **17**	77	 **44**	(*R*) >99	82	62
 **18**	29	 **45**	(*S*) >99	100	79
 **19**	67	 **46**	(*S*) >99	100	91
 **20**	2	 **47**	(*R*) 79	12	8
 **21**	51	 **48**	(*R*) 99	72	64
 **22**	24	 **49**	(*R*) >99	88	48

Table 3 (*continued*)

Substrate	Relative activity (%)	Product	ee (%)	Conversion (%)	Yield (%)
 **23**	3	 **50**	(*R*) 99	8	6
 **24**	353	 **51**	(*R*) 98	92	47
 **25**	86	 **52**	(*R*) >99	95	62
 **26**	45[a]	 **53**	(*S*) >99	40	29
 **27**	4	 **54**	(*R*) >99	35	24

[a] The reductive activity for the substrate was determined by measuring the amount of produced alcohol by GC, because the substrate indicated strong absorption at 340 nm and hindered the spectroscopic measurement of NADH formation.

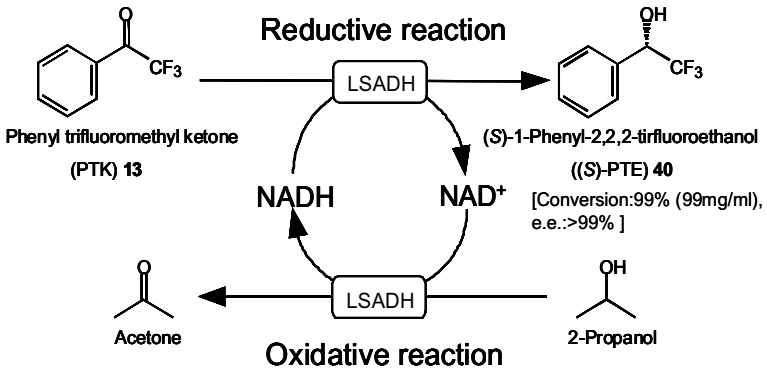


Figure 1. Principle of the LSADH-catalyzed reduction of ketones in combination with hydrogen-transfer via NADH from 2-propanol.

acetophenone or arylketone derivatives **12**, **14**–**17**, **20**–**25** and (*R*)-alkanols **28**–**30** from 2-alkanones **1**–**3**, and (*S*)-halohydrins **37**, **38**, **45**, **46**, **53** from 4-halo-3-oxobutanoate derivatives **10**, **11**, 2-chloroacetophenone (phenacyl chloride) derivatives **18**, **19** and chloromethyl 3,4-methylenedioxyphenyl ketone **26** with an enantiomeric excess of more than 98%, except from 2,4-dicholoroacetophenone **20** (*R*-form, 79% ee), methyl benzoylformate **6** [(*R*)-form, 47% ee], ethyl benzoylformate **7** (*R*-form, 23% ee) and ethyl 2-oxo-4-phenylbutanoate **8** [(*S*)-form, 85% ee]. This suggested that the stereoselectivity of LSADH strictly follows the anti-Prelog's rule.[23] Interestingly, it was observed that substrates with weak reactivity for LSADH such as methyl or ethyl benzoylformate **6**, **7** ethyl 2-oxo-4-phenylbutanoate **8** and 2,4-dicholoroacetophenone **20** gave low enantiomeric excesses for the alcohols produced.

Although the reaction system was not optimized for each substrate, conversions of 19 ketones out of a total of 27 tested reached more than 80%, and low conversion was only limited to the substrates to which LSADH showed a weak reactivity (less than 4% compared with PTK) such as methyl or ethyl benzoylformate **6**, **7** and 2,4-dicholoroacetophenone **20**, propiophenone **23** and 1-*N*-Boc-3-pyrrolidinone **27** (Tables 2 and 3). The results clearly indicated that the LSADH can function as a biocatalyst to transfer hydrogen from 2-propanol to various

ketones through a smooth NADH-regeneration to give enantiomerically pure (*R*)-alcohols with a practical level.

2.2. Stereochemistry of LSADH

In order to elucidate the stereochemistry with respect to NADH, the LSADH-catalyzing transfer of deutride (D) or hydride from the D-labelled NADH at the pro-*R* position (NADD) was determined. NADD was obtained with the reaction of yeast ADH and CH_3CD_2OH as described in the Experimental. ^1H NMR and GC–MS analyses of the PTE produced by LSADH with the NADD gave the same profiles as those of authentic PTE **40**; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 4.99–5.04 (1H, m), 7.36–7.56 (5H, m, ArH), GC–EI-MS *m*/*z* (M^+ 176 for $C_8H_7OF_3$) 137, 127, 107, 79, 51. These results indicated that the C-2 hydrogen of PTE was not replaced with D through LSADH reduction, in other words, the pro-*S* hydrogen of NADD is transferred to the *re* face of the carbonyl of PTK **13** (*si* face in the case of acetophenone **12**), as illustrated in Figure 2. The result together with those of the section above indicated that the stereochemistry of LSADH was the same as that of *Mucor javanicus* ADH, and different from that of the (*R*)-alcohol-producing ADH from *Pseudomonas* or *Lactobacillus*.[4]

2.3. Optimized conditions for LSADH-catalyzed production of (*S*)-PTE 40

To improve the LSADH reduction system using 2-propanol as a hydrogen donor, reaction conditions were optimized using PTK **13** as a representative substrate (100 mg/ml: 574 mM, suspended in the reaction mixture). With regard to the concentration of 2-propanol in the reaction mixture, the optimal concentration was between 5% (v/v) (0.65 M) and 10% (1.3 M), and production of (*S*)-PTE **40** was inhibited at above 15% 2-propanol concentration (Fig. 3a). This suggested that more than 15% (v/v) 2-propanol in the reaction mixture probably inhibited the LSADH reaction or denatured the LSADH.

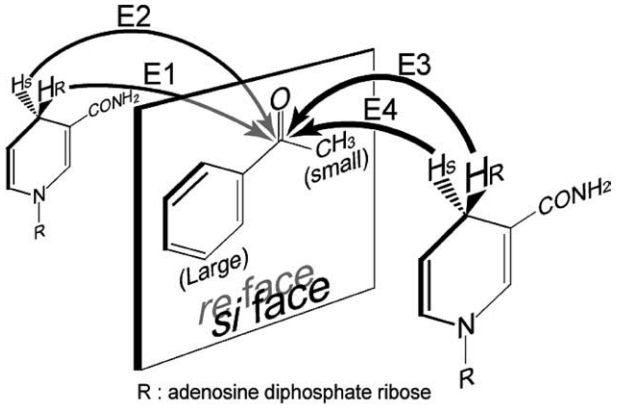


Figure 2. Stereochemistry of the LSADH reaction compared with the previously reported ADHs. In the case of PTK 13, this side is designated as the *re* face.

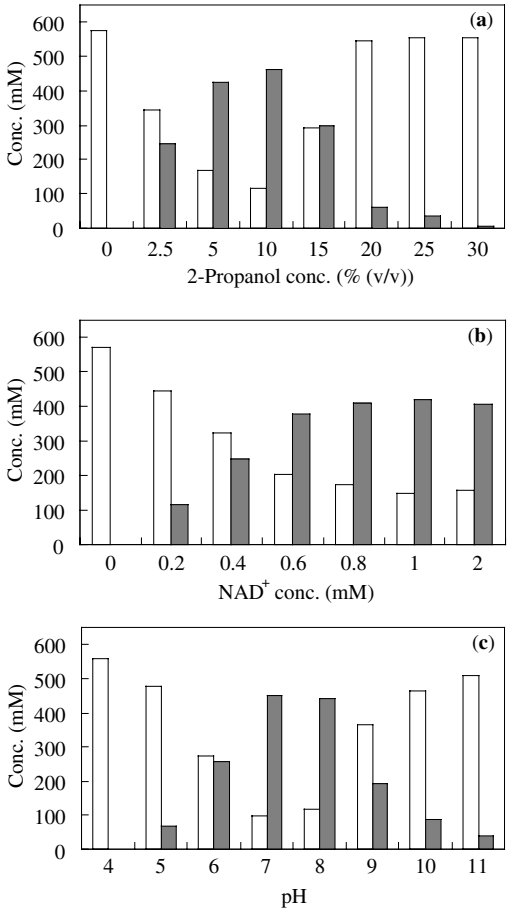


Figure 3. Optimization of (*S*)-PTE **40** production by LSADH. (a) Effect of 2-propanol concentration on (*S*)-PTE **40** production. The reaction mixture consisted of 10% (w/v) PTK **13**, 1 μmol NAD^+, 100 μmol KPB (pH 7.0), 0–30% (v/v) 2-propanol and 1 unit of LSADH in a total volume of 1 ml. (b) Effect of NAD^+ concentration in the reaction mixture. The reaction mixture consisted of 10% (w/v) PTK, 0–2.0 μmol NAD^+, 100 μmol KPB (pH 7.0), 5% (v/v) 2-propanol and 1 unit of LSADH in a total volume of 1 ml. (c) Effect of pH of the reaction mixture. The reaction mixture consisted of 10% (w/v) PTK, 1 μmol NAD^+, 100 μmol buffer [citrate–K_2HPO_4 (pH 4.0 and 5.0), KPB (pH 6.0 and 7.0), Tris–HCl (pH 8.0 and 9.0) and glycine–NaOH (pH 10.0 and 11.0)], 5% (v/v) 2-propanol and 1 unit of LSADH in a total volume of 1 ml. The amount of (*S*)-PTE produced after 24 h was drawn as a grey bar, and PTK remaining as a white bar in all cases.

The optimal NAD^+ concentration in the reaction mixture was measured. As shown in Figure 3b, a sufficient conversion was observed at an initial NAD^+ concentration of more than 0.8 mM. Considering the K_m value of NAD^+ for 2-propanol oxidation (0.12 mM),[22] this observation seemed reasonable. The effect of pH on the reaction was also measured. As shown in Figure 3c, the optimal pH was between 7 and 8.

2.4. Time course of the production of (*S*)-PTE 40

Under the optimized conditions (10% 2-propanol, 1 mM NAD^+ and pH 7.0), the time course of (*S*)-PTE **40**

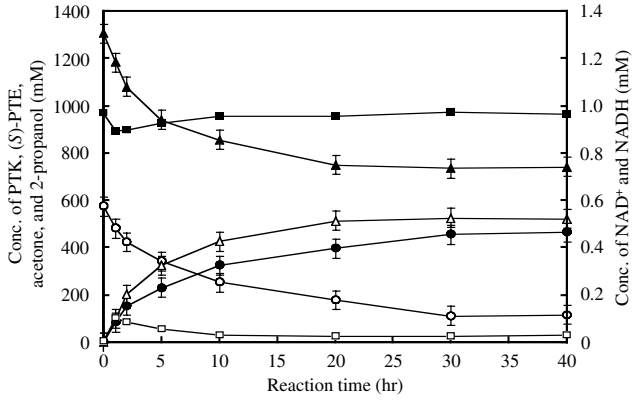


Figure 4. Time course of the production of (*S*)-PTE **40** and hydrogen donor (2-propanol) concentration. The reaction mixture consisted of 10% (w/v) PTK **13**, 1 μmol NAD^+, 100 μmol phosphate buffer (KPB) (pH 7.0), 10% (v/v) 2-propanol and 1 unit of LSADH in a total volume of 1 ml. Symbols: ○, PTK remaining [initial concn of 10% (w/v) (575 mM)]; ●, (*S*)-PTE produced; ▲, 2-propanol [initial concn of 10% (v/v) (1306 mM)]; △, acetone; ■, NAD^+; □, NADH.

production was tested with a high concentration of PTK **13** (100 mg/ml: 574 mM) (Fig. 4). During the reaction, part of the NAD^+ was rapidly reduced into NADH, after which NAD^+ and NADH concentrations were maintained at 0.94–0.98 mM and 0.03–0.09 mM, respectively. The concentration of NAD^+ was eight times higher than the K_m value for NAD^+ (0.12 mM), whereas that of NADH was almost equal to the K_m value for NADH (0.048 mM).[22] A similar phenomenon was reported in the bioreduction of 2,3′-dichloroacetophenone **19** by PAR with 2-propanol.[17] The result including the case of PAR suggested that the reaction rate was kinetically controlled by the enzyme itself in both cases. Throughout the reaction, an almost equimolar production of (*S*)-PTE **40** and acetone with a decrease in PTK **13** and 2-propanol was observed in the range of measurement error. The observed reaction rate constant (k_{cat}) for the reduction of PTK **13** calculated from the initial velocity in Figure 4 was approximately 30 s^{-1}, therefore, the overall reaction rate constant including the oxidation of 2-propanol should double this (60 s^{-1}). The value was comparable with that for PTK reduction (140 s^{-1}) and 2-propanol oxidation (47 s^{-1}) reported for LSADH.[22] Although, the 2-propanol used for NADH regeneration seemed to compete for the active site of the enzyme with the ketone substrate and lower the turnover for the desired reaction, LSADH-catalyzed hydrogen-transfer reduction proceeded efficiently from the viewpoint of the regeneration and utilization of NADH. After 35 h, (*S*)-PTE **40** was obtained at 79% conversion (ca. 450 mM in the reaction mixture: 79 mg/ml) with >99% ee When the amount of enzyme added to the reaction mixture was increased by twice, (*S*)-PTE **40** was obtained at 99% conversion (ca. 570 mM in the reaction mixture: 99 mg/ml). The high accumulation of the product with a near perfect enantiomeric purity showed the potential of the LSADH bioreduction system for practical usage. The productivity could be improved further by using a larger amount of enzyme or recombinant *E. coli* cells expressing the LSADH gene, feeding of 2-propanol, elimination of the acetone produced under reduced pressure, and adoption of the solvent–water two-phase system and so on.

3. Conclusion

LSADH catalyzed the enantioselective reduction of ketones with high conversion and enantiomeric purity to give (*R*)-form alcohols. The stereochemistry of LSADH has been found to use the pro-*S* hydrogen of NADH. This bioreduction process would be industrially applicable to the production of enantiomerically pure alcohols because it can effectively regenerate NADH by itself by transferring hydrogen from 2-propanol to reduce the aimed ketones.

4. Experimental

4.1. Preparation of LSADH and enzyme assay

LSADH was obtained from *Leifsonia* sp. S749 cells as reported previously.[22] A partially purified enzyme preparation produced by ammonium sulfate precipitation and sequential Butyl-Toyopearl and Bioassist Q column chromatographies was used throughout the experiments.

LSADH activity was assayed at 25 °C by measuring the decrease in absorption at 340 nm of NADH as described previously.[22] One unit of enzyme was defined as the amount that converted 1 μmol NADH and PTK **13** in 1 min. The substrate specificity of LSADH was measured spectrophotometrically by decreasing the absorption of NADH at 340 nm using the purified enzyme and various ketones at 2 mM, unless otherwise indicated, and their relative activity was compared with PTK **13** as 100%.

4.2. LSADH-catalyzed reduction coupled to NADH regeneration with 2-propanol

For determining the enantiomeric purity of the products and assessing the coupling of NADH regeneration, we constructed the LSADH and 2-propanol system (Fig. 1). The reaction mixture consisted of 0.5 mmol KPB (pH 7.0), 50 mg of each substrate **1**–**27**, 5 μmol NAD^+, 5% (v/v) 2-propanol and 5 unit of purified LSADH in a total volume of 5 ml, and was suspended in a 15-ml polypropylene centrifuge tube with shaking (300 rpm) for 24 h at 25 °C. After the reaction, the mixture was extracted twice with 7.5 ml of ethyl acetate. The combined ethyl acetate extracts were dried over Na_2SO_4, purified by silica gel (1.5 × 30 cm) chromatography using *n*-hexane and ethyl acetate (3:1) as an eluent. The fractions were condensed by evaporation, and analyzed by GC or HPLC. The purified product was also analyzed by ^1H NMR (400 MHz, $CDCl_3$) with tetramethylsilane (TMS) as a reference, and GC-electron ionization (EI)-mass spectrometry (MS) (GC–EI-MS) (QP-5000 GC–MS, Shimadzu): column DB-1 (0.25 mm × 60 m, J&W Scientific, CA, USA); flow rate

at a linear velocity of 35 cm/min of He; the split ratio of 50; injection and detection temperature of 250 °C; column temperature 40 °C for 5 min then raised to 250 °C at 10 °C/min and held at 250 °C for 5 min.

General GC was performed using a Shimadzu GC-18A system (Kyoto, Japan) equipped with a capillary column (DB-1, 0.25 mm × 30 m, J&W Scientific, CA, USA) with an FID (flame ionization detector) with a flow rate of 1 ml/min of He and the split ratio of 60 under the conditions described in Table 1. GC was also performed using a Shimadzu GC-14 A system equipped with a coiled column (3 mm × 2 m) packed with Thermon 1000 (5% on Chromosorb W) with an FID and a flow rate of 50 ml/min of N_2. The conversions were determined on the basis of the peak areas of ketone substrates and alcohol products from the GC.

To determine the absolute configuration and conversions of some alcohols, the products **31**, **39**–**46**, **50** and **51**, extracted with ethyl acetate from the reaction mixture, were analyzed using GC (HP 6890 GC system, Hewlett Packard, USA) with a CP-cyclodextrin-β-236-N19 chiral column (0.25 mm by 25 m, 0.25 μm film, Chrompack, The Netherlands) and a FID. Helium gas was used as a carrier at 15 psi (0.5 ml/min), and the split ratio was 50. Details of the measuring conditions are summarized in Table 1.

In order to determine the absolute configuration of the 2-alkanols **28**–**30**, they were converted into benzoyl derivatives with benzoyl chloride and analyzed by HPLC with a chiral column, as previously reported.[19] Analytical HPLC was performed with a Shimadzu LC-10AT system equipped with a Chiralcel OB-H (4.6 × 250 mm, Daicel Chemical Industries, Tokyo, Japan). The mobile phase was hexane/2-propanol (49:1) and delivered at a flow rate of 0.5 ml/min (Table 1).

For determining the absolute configuration of the alcohols **32**–**38**, **47**–**49** and **52**–**54**, they were purified by silica gel chromatography as described above, and analyzed by HPLC with a chiral column. Analytical HPLC was performed with a Shimadzu LC-10AT system using a Chiralcel OB-H, OD-H or OF (4.6 × 250 mm, Daicel Chemical Industries) under the conditions described in Table 1.

4.3. Stereochemistry of LSADH

In order to determine the stereochemistry of LSADH, deuterium (D)-containing NADH (NADD) at the pro-*R* position in the nicotinamide moiety was synthesized enzymatically from NAD^+ and CH_3CD_2OH using yeast ADH as previously reported.[19] PTK **13** was reduced by the purified LSADH using this cofactor as follows; the reaction mixture consisted of 7 μmol PTK **13**, 7 μmol NADD, 5 units of LSADH and 0.1 mmol potassium phosphate buffer (pH 6.0) in a total volume of 10 ml. The reaction proceeded at 25 °C for 48 h. The PTE **40** produced was purified by extraction with ethyl acetate from the mixture and analyzed by ^1H NMR and GC–EI-MS.

4.4. Optimization of production of (*S*)-PTE 40

In order to determine the 2-propanol and acetone concentrations in the reaction mixture, GC was performed using a Shimadzu GC-14 A system equipped with a coiled column (3 mm × 2 m) packed with Thermon 1000 (5% on Chromosorb W) with an FID, column temperature of 30 °C, injection temperature of 100 °C, detection temperature of 150 °C and a flow rate of 50 ml/min of N_2; retention times (min): 2-propanol, 4.4 min; acetone, 2.3 min. The NAD^+ and NADH concentrations in the reaction mixture were also determined by HPLC as previously described.[17] The reaction mixture's composition is mentioned in the legend of Figure 3, and the reaction was performed in a 2-ml polypropylene tube in a BioShakar MBR-022 (Taitec, Saitama, Japan) with shaking (700 rpm) for 24 h at 25 °C.

4.5. Chemicals

Ethyl 4-phenyl-2-oxobutanoate **8**, methyl 4-bromo-3-oxobutanoate **10**, 2,3′-dichloroacetophenone **19**, 2′,4′-dichloroacetophenone **20**, 3′-methoxyacetophenone **21**, 3′,4′-methoxyphenylacetone **22**, piperonylacetone **25**, chloromethyl 3,4-methylenedioxyphenyl ketone **26**, 1-*N*-Boc-3-pyrrolidinone **27**, ethyl (*RS*)-4-phenyl-2-hydroxybutanoate **35**, methyl (*RS*)-4-bromo-3-hydroxybutanoate **37**, (*RS*)-1-phenyl-2,2,2-trifluoroethanol (PTE) **40**, (*RS*)-2-chloro-1-(3-chlorophenyl)ethanol **46**, (*RS*)-1-(2,4-dichlorophenyl)ethanol **47**, (*RS*)-1-(3-methoxyphenyl)ethanol **48**, (*RS*)-1-(3,4-dimethoxyphenyl)-2-propanol **49**, (*RS*)-1-piperonyl-2-propanol **52**, (*RS*)-1-(3,4-methylenedioxyphenyl)-2-chloroethanol **53** and (*R*)- and (*RS*)-1-*N*-Boc-3-pyrrolidinol **54** were kindly supplied by Sumitomo Chemical Co. Ltd, Osaka, Japan. 2-Pentanone **1**, 2-hexanone **2**, 2-heptanone **3**, ethyl 3-oxobutanate **9**, acetophenone **12**, phenyl trifluoromethyl ketone (PTK) **13**, 3′-chloroacetophenone **14**, 4′-chloroacetophenone **15**, 3′-bromoacetophenone **16**, 4′-bromoacetophenone **17**, propiophenone **23**, benzylacetone **24**, (*RS*)-2-pentanol **28**, (*RS*)-2-hexanol **29**, (*RS*)-2-heptanol **30**, ethyl DL-lactate **31**, methyl (*RS*)-mandelate, methyl (*S*)-mandelate **33**, ethyl (*RS*)-mandelate, ethyl (*S*)-mandelate **34**, (*RS*)-1-phenylethanol **39**, (*RS*)-1-(3-chlorophenyl)ethanol **41**, (*RS*)-1-(4-chlorophenyl)ethanol **42**, (*RS*)-1-(3-bromophenyl)ethanol **43**, (*RS*)-1-(4-bromophenyl)ethanol **44**, (*RS*)-1-phenyl-1-propanol **50** and (*RS*)-4-phenyl-2-butanol **51** were purchased from Tokyo Kasei Kogyo, Japan. Ethyl 3-methyl-2-oxobutyrate **5**, methyl benzoylformate **6**, ethyl benzoylformate **7** and other chiral alcohol standards including (*R*)-2-pentanol **28**, (*R*)-2-hexanol **29**, (*R*)-2-heptanol **30**, ethyl (*R*)-lactate **31**, methyl (*R*)-mandelate **33**, ethyl (*R*)-mandelate **34**, ethyl (*R*)- **36** and (*S*)-3-hydroxybutanoates, ethyl (*R*)- and (*S*)-4-chloro-3-hydroxybutanoates **38**, (*R*)-1-phenylethanol **39**, (*S*)-PTE **40**, (*S*)-2-chloro-1-phenylethanol **45**, (*R*)-1-phenyl-1-propanol **50** and (*R*)-4-phenyl-2-butanol **51** were obtained from Sigma–Aldrich Chemicals, USA. Ethyl pyruvate **4** was purchased from Wako Pure Chemicals, Japan, 2-chloroacetophenone (phenacyl chloride) **18**, (*RS*)-2-chloro-1-phenylethanol **45** from Lancaster Synthesis UK, and ethyl 4-chloro-3-oxobutanoate **11** from Merck, Germany. Ethyl 2-hydroxy-

3-methylbutanoate **32** was synthesized from **5** by reduction with sodium tetrahydroborate and confirmed by GC–MS and ^{1}H NMR as described above. All other chemicals used herein were of analytical grade and were available commercially.

4.6. Instrumental analyses of the purified products

The product after the reaction described in Section 4.2 was purified by silica gel chromatography. The products obtained were analyzed by a ^{1}H NMR and GC–EI-MS. When the authentic chiral compound was available, our products were compared with the authentic ones on GC or HPLC equipped with a chiral column as described in Table 1, and its absolute configuration was confirmed. Optical rotation was measured with a JASCO P-1030 polarimeter.

(*R*)-2-Pentanol **28** (8.5 mg); ^{1}H NMR ($CDCl_3$) δ (ppm) 0.93 (t, 3H, CH_3, $J = 7.1$ Hz), 1.19 (d, 3H, CH_3, $J = 6.4$ Hz), 1.30–1.50 (m, 5H, CH_2, CH_2, OH), 3.76–3.87 (m, 1H, CH), GC–EI-MS *m*/*z* (M^+ 87 for $C_5H_{12}O$, M^+-H 86) 69, 57, 45. The absolute configuration was confirmed with the authentic standard.

(*R*)-2-Hexanol **29** (15.5 mg); ^{1}H NMR ($CDCl_3$) δ (ppm) 0.91 (t, 3H, CH_3, $J = 7.1$ Hz), 1.19 (d, 3H, CH_3, $J = 6.1$ Hz), 1.25–1.52 (m, 7H, CH_2, CH_2, CH_2, OH), 3.76–3.83 (m, 1H, CH), GC–EI-MS *m*/*z* (M^+ 102 for $C_6H_{14}O$, M^+-H 101) 87, 84, 69, 56, 45, 41. The absolute configuration was confirmed with the authentic standard.

(*R*)-2-Heptanol **30** (19 mg); ^{1}H NMR ($CDCl_3$) δ (ppm) 0.89 (t, 3H, CH_3, $J = 6.9$ Hz), 1.19 (d, 3H, CH_3, $J = 6.3$ Hz), 1.26–1.51 (m, 9H, CH_2, CH_2, CH_2, CH_2, OH), 3.75–3.84 (m, 1H, CH), GC–EI-MS *m*/*z* (M^+-H 115 for $C_7H_{16}O$) 101, 98, 83, 70, 55, 45. The absolute configuration was confirmed with the authentic standard.

Ethyl (*R*)-lactate **31** (9.5 mg); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.31 (t, 3H, CH_3, $J = 7.2$ Hz), 1.42 (d, 3H, CH_3, $J = 7.1$ Hz), 2.82 (s, 1H, OH), 4.25 (q, 2H, CH_2, $J = 7.1$Hz), 4.20–4.29 (m, 1H, CH), GC–EI-MS *m*/*z* (M^+ 118 for $C_5H_{10}O_3$) 103, 89, 75, 58, 45. The absolute configuration was confirmed with the authentic standard.

Ethyl (*R*)-2-hydroxy-3-methylbutanoate **32** (20 mg); $[\alpha]_D^{21} = -10.5$ (*c* 0.5, CH_3Cl) {lit.,[24] $[\alpha]_D^{22} = -7.9$ (*c* 1, acetic acid)}, ^{1}H NMR ($CDCl_3$) δ (ppm) 0.84 (d, 3H, CH_3, $J = 6.8$ Hz), 1.03 (d, 3H, CH_3, $J = 6.9$ Hz), 1.31 (t, 3H, CH_3, $J = 7.2$ Hz), 2.03–2.14 (m, 1H, CH), 2.72 (s, 1H, OH), 4.03 (d, 1H, CH, $J = 3.5$Hz), GC–EI-MS *m*/*z* (M^+ 146 for $C_7H_{14}O_3$) 128, 117, 104, 73, 55, 43.

Methyl (*R*)-mandelate **33** (15.5 mg); ^{1}H NMR ($CDCl_3$) δ (ppm) 3.44 (d, 1H, OH, $J = 4.6$ Hz), 3.77 (s, 3H, CH_3), 5.18 (d, 1H, CH, $J = 3.9$ Hz), 7.26–7.46 (m, 5H, Ph), GC–EI-MS *m*/*z* (M^+ 116 for $C_9H_{10}O_3$) 150, 132, 118, 107, 79, 63, 51. The absolute configuration was confirmed with the authentic standard.

Ethyl (*R*)-mandelate **34** (10 mg); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.23 (t, 3H, CH_3, 7.1 Hz), 3.46 (d, 1H, OH, $J = 5.6$ Hz), 4.17 (dq, 1H, CH, J(d) = 10.7 Hz, J(q) = 7.2 Hz), 4.27 (dq, 1H, CH, J(d) = 10.8 Hz, J(q) = 7.1 Hz), 5.16 (d, 1H, CH, $J = 5.4$ Hz), 7.26–7.44 (m, 5H, Ph), GC–EI-MS *m*/*z* (M^+ 180 for $C_{10}H_{12}O_3$) 163, 150, 135, 118, 107, 89, 79, 63, 51. The absolute configuration was confirmed with the authentic standard.

Ethyl (*S*)-4-phenyl-2-hydroxybutanoate **35** (29 mg); $[\alpha]_D^{21} = +14.3$ (*c* 0.5, CH_3Cl) {lit.,[25] $[\alpha]_D^{25} = +21.3$ (*c* 1, CH_3Cl)}, ^{1}H NMR ($CDCl_3$) δ (ppm) 1.29 (t, 3H, CH_3, $J = 7.1$ Hz), 1.90–2.00 (m, 1H, CH_2), 2.08–2.17 (m, 1H, CH_2), 2.70–2.83 (m, 2H, CH_2), 4.18 (dd, 1H, CH, $J = 3.9$ Hz), 4.21 (q, 2H, CH_2, $J = 7.2$ Hz), 7.17–7.31 (m, 5H, Ph), GC–EI-MS *m*/*z* (M^+ 208 for $C_{12}H_{16}O_3$) 190, 162, 144, 134, 117, 104, 91, 76, 65.

Ethyl (*R*)-3-hydroxybutanoate **36** (23.5 mg); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.23 (d, 3H, CH_3, $J = 6.4$ Hz), 1.28 (t, 3H, CH_3, $J = 7.2$ Hz), 2.42 (dd, 1H, CH, J(d) = 8.7 Hz, J(d) = 16.5 Hz), 2.49 (dd, 1H, CH, J(d) = 3.6 Hz, J(d) = 16.5 Hz), 3.05 (d, 1H, OH, $J = 2.6$ Hz), 4.18 (q, 2H, CH_2, $J = 7.2$ Hz), 4.12–4.23 (m, 1H, CH), GC–EI-MS *m*/*z* (M^+ 132 for $C_8H_7OF_3$, M^+-H 131) 117, 103, 87, 71, 60, 43. The absolute configuration was confirmed with the authentic standard.

Methyl (*S*)-4-bromo-3-hydroxybutanoate **37** (17.5 mg); $[\alpha]_D^{22} = -25.9$ (*c* 0.5, CH_3Cl) {lit.[26] $[\alpha]_D^{25} = -16.2$ (*c* 8, CH_3Cl)}, ^{1}H NMR ($CDCl_3$) δ (ppm) 2.65 (dd, 1H, CH, J(d) = 7.5 Hz, J(d) = 16.5 Hz), 2.70 (dd, 1H, CH, J(d) = 4.9 Hz, J(d) = 16.6 Hz), 3.09 (s, 1H, OH), 3.48 (dd, 1H, CH, J(d) = 5.6 Hz, J(d) = 10.5 Hz), 3.52 (dd, 1H, CH, J(d) = 5.1 Hz, J(d) = 10.5 Hz), 3.74 (s, 3H, CH_3), 4.22–4.28 (m, 1H, CH), GC–EI-MS *m*/*z* (M^+ 198, 196 for $C_5H_9O_3Br$) 167, 165, 125, 123, 103, 71, 61, 43.

Ethyl (*S*)-4-chloro-3-hydroxybutanoate **38** (27 mg); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.29 (t, 3H, CH_3, $J = 7.2$ Hz), 2.61 (dd, 1H, CH, J(d) = 7.6 Hz, J(d) = 16.6 Hz), 2.66 (dd, 1H, CH, J(d) = 4.8 Hz, J(d) = 16.5 Hz), 3.15 (d, 1H, OH, J(d) = 2.9 Hz), 3.60 (dd, 1H, CH, J(d) = 5.6 Hz, J(d) = 11.2 Hz), 3.63 (dd, 1H, CH, J(d) = 5.2 Hz, J(d) = 11.3 Hz), 4.19 (q, 1H, CH_2, $J = 7.1$ Hz), 4.22–4.30 (m, 1H, CH), GC–EI-MS *m*/*z* (M^+ 168, 166 for $C_6H_{11}O_3Cl$) 141, 139, 123, 121, 117, 85, 71, 43. The absolute configuration was confirmed with the authentic standard.

(*R*)-1-Phenylethanol **39** (20 mg); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.50 (d, 3H, CH_3, $J = 6.3$ Hz), 1.88 (s, 1H, OH), 4.90 (q, 1H, CH, $J = 6.5$ Hz), 7.25–7.39 (m, 5H, Ph), GC–EI-MS *m*/*z* (M^+ 122 for $C_8H_{10}O$) 107, 91, 79, 63, 51, 43. The absolute configuration was confirmed with the authentic standard.

(*S*)-1-Phenyl-2,2,2-trifluoroethanol (PTE) **40** (40.5 mg); $[\alpha]_D^{21} = +23.5$ (*c* 0.5, CH_3Cl), {lit.[27] $[\alpha]_D^{24} = +25.1$ (*c* 0.81, CCl_4)}, ^{1}H NMR ($CDCl_3$) δ (ppm) 2.81 (d, 1H, OH, $J = 3.2$ Hz), 5.03 (dq, 1H, CH, J(d) = 3.2 Hz, J(q) = 6.6 Hz), 7.40–7.50 (m, 5H, Ph), GC–EI-MS *m*/*z*

(M^+ 176 for $C_8H_7OF_3$) 137, 127, 107, 79, 51. The absolute configuration was confirmed with the authentic standard.

(*R*)-1-(3-Chlorophenyl)ethanol **41** (30.5 mg); $[\alpha]_D^{21} = +42.7$ (*c* 0.5, CH_3Cl) {lit.[28] $[\alpha]_D^{21} = +44.0$ (*c* 0.22, CH_3Cl)}, ^1H NMR ($CDCl_3$) δ (ppm) 1.48 (d, 3H, CH_3, $J = 6.5$ Hz), 1.93 (s, 1H, OH), 4.88 (q, 1H, CH, $J = 6.4$ Hz), 7.22–7.38 (m, 4H, Ph) GC–EI-MS *m/z* (M^+ 158, 156 for C_8H_9OCl) 143, 141, 121, 115, 113, 91, 77, 51, 43.

(*R*)-1-(4-Chlorophenyl)ethanol **42** (29 mg); $[\alpha]_D^{21} = +53.9$ (*c* 0.5, CH_3Cl) {lit.[29] $[\alpha]_D^{24.8} = +44.9$ (EtOH)}, ^1H NMR ($CDCl_3$) δ (ppm) 1.47 (d, 3H, CH_3, $J = 6.6$ Hz), 1.89 (s, 1H, OH), 4.88 (q, 1H, CH, $J = 6.4$ Hz), 7.29–7.34 (m, 4H, Ph), GC–EI-MS *m/z* (M^+ 158, 156 for C_8H_9OCl) 143, 141, 121, 115, 113, 103, 91, 77, 51, 43.

(*R*)-1-(3-Bromophenyl)ethanol **43** (30 mg); $[\alpha]_D^{21} = +43.3$ (*c* 0.5, CH_3Cl) {lit.[30] $[\alpha]_D^{25} = +34.7$ (*c* 1.35, CH_3Cl)}, ^1H NMR ($CDCl_3$) δ (ppm) 1.48 (d, 3H, CH_3, $J = 6.6$ Hz), 1.87 (s, 1H, OH), 4.87 (q, 1H, CH, $J = 6.4$ Hz), 7.18–7.54 (m, 4H, Ph), GC–EI-MS *m/z* (M^+ 202, 200 for C_8H_9OBr) 187, 185, 121, 105, 103, 92, 77, 63, 51, 43.

(*R*)-1-(4-Bromophenyl)ethanol **44** (31 mg); $[\alpha]_D^{21} + 41.8$ (c 0.5, CH_3Cl) {lit.[31] $[\alpha]_D^{25} = +32.1$ (*c* 0.8, CH_3Cl)}, ^1H NMR ($CDCl_3$) δ (ppm) 1.47 (d, 3H, CH_3, $J = 6.6$ Hz), 1.85 (d, 1H, OH, $J = 2.7$ Hz), 4.87 (dq, 1H, CH, J(d) = 2.1 Hz, J(q) = 6.2 Hz), 7.23–7.49 (m, 4H, Ph), GC–EI-MS *m/z* (M^+ 202, 200 for C_8H_9OBr) 187, 185, 159, 157, 121, 105, 103, 92, 77, 63, 51, 43.

(*S*)-2-Chloro-1-phenylethanol **45** (39.5 mg); ^1H NMR ($CDCl_3$) δ (ppm) 2.69 (d, 1H, OH, $J = 3.0$), 3.65 (ddd, 1H, CH, J(d) = 1.0 Hz, J(d) = 8.8 Hz, J(d) = 11.2 Hz), 3.75 (ddd, 1H, CH, J(d) = 0.7 Hz, J(d) = 3.4 Hz, J(d) = 11.3 Hz), 4.91 (dt, 1H, CH, J(d) = 8.8 Hz, J(t)=3.2 Hz), 7.31–7.40 (m, 5H, Ph), GC–EI-MS *m/z* (M^+ 158, 156 for C_8H_9OCl) 138, 120, 107, 91, 79, 65, 5. The absolute configuration was confirmed with the authentic standard.

(*S*)-2-Chloro-1-(3-chlorophenyl)ethanol **46** (45.5 mg); $[\alpha]_D^{21} = +46.8$ (*c* 0.5, CH_3Cl) {lit.[32] $[\alpha]_D^{20} = +33.5$ (*c* 1.02, CH_3Cl)}, ^1H NMR ($CDCl_3$) δ (ppm) 2.71 (d, 1H, OH, $J = 3.1$ Hz), 3.62 (dd, 1H, CH, J(d) = 8.7 Hz, J(d) = 11.4 Hz), 3.74 (dd, 1H, CH, J(d) = 3.5 Hz, J(d) = 11.3 Hz), 4.89 (dt, 1H, CH, J(d) = 8.5 Hz, J(t)=3.2 Hz), 7.23–7.41 (m, 4H, Ph), GC–EI-MS *m/z* (M^+ 194, 192, 190 for $C_8H_8OCl_2$) 156, 154, 143, 141, 115, 113, 91, 77, 63, 51.

(*R*)-1-(2,4-Dichlorophenyl)ethanol **47** (4 mg); $[\alpha]_D^{21} = +44.0$ (*c* 0.5, CH_3Cl) {lit.[33] $[\alpha]_D^{20} = +4.5$ (CH_3OH) 13% ee *R*}, ^1H NMR ($CDCl_3$) δ (ppm) 1.47 (d, 3H, CH_3, $J =$ 6.3 Hz), 1.93 (d, 1H, OH, $J = 3.4$ Hz), 5.25 (dq, 1H, CH, J(d) = 3.6 Hz, J(q) = 6.3 Hz), 7.26–7.56 (m, 3H, Ph), GC–EI-MS *m/z* (M^+ 194, 192, 190 for $C_8H_8OCl_2$) 179, 177, 175, 147, 149, 151, 139, 114, 111, 102, 87, 75, 63, 50, 43.

(*R*)-1-(3-Methoxyphenyl)ethanol **48** (32 mg); $[\alpha]_D^{22} =$ +44.4 (*c* 0.5, CH_3Cl) {lit.[34] $[\alpha]_D^{28} = +39.0$ (*c* 1.0, CH_3Cl)}, ^1H NMR ($CDCl_3$) δ (ppm) 1.49 (d, 3H, CH_3, $J = 6.6$ Hz), 1.85 (s, 1H, OH), 3.82 (s, 3H, CH_3), 4.88 (dq, 1H, CH, J(d) = 1.8 Hz, J(q) = 6.1 Hz), 6.80–7.29 (m, 4H, Ph), GC–EI-MS *m/z* (M^+ 152 for $C_9H_{12}O_2$) 137, 121, 109, 94, 77, 65, 51, 43.[17]

(*R*)-1-(3,4-Dimethoxyphenyl)-2-propanol **49** (32 mg); $[\alpha]_D^{22} = -21.7$ (*c* 0.4, CH_3Cl) {lit.[35] $[\alpha]_D^{25} = -29.7$ (*c* 1.01, CH_3Cl)}, ^1H NMR ($CDCl_3$) δ (ppm) 1.25 (d, 3H, CH_3, $J = 6.1$ Hz), 1.59 (s, 1H, OH), 2.61 (dd, 1H, CH, J(d) = 8.2 Hz, J(d) = 13.6 Hz), 2.75 (dd, 1H, CH, J(d) = 4.6 Hz, J(d) = 13.6 Hz), 3.87 (s, 3H, CH_3), 3.88 (s, 3H, CH_3), 3.95–4.06 (m, 1H, CH), GC–EI-MS *m/z* (M^+ 196 for $C_{11}H_{16}O_3$) 178, 163, 151, 137, 121, 107, 91, 77, 65, 44.

(*R*)-1-Phenyl-1-propanol **50** (3 mg); ^1H NMR ($CDCl_3$) δ (ppm) 0.92 (t, 3H, CH_3, $J = 7.4$ Hz), 1.55 (s, 1H, OH), 1.71–1.89 (m, 2H, CH_2), 4.61 (dt, 1H, CH, J(d) = 2.8 Hz, J(t) = 6.6 Hz), 7.26–7.36 (m, 5H, Ph), GC–EI-MS *m/z* (M^+ 136 for $C_9H_{12}O$) 117, 107, 91, 79, 63, 51. The absolute configuration was confirmed with the authentic standard.

(*R*)-4-Phenyl-2-butanol **51** (23.5 mg); ^1H NMR ($CDCl_3$) δ (ppm) 1.23 (d, 3H, CH_3, $J = 6.1$ Hz), 1.43 (s, 1H, OH), 1.70–1.84 (m, 2H, CH_2), 2.63–2.80 (m, 2H, CH_2), 3.83 (tq, 2H, CH_2, J(t) = 6.1 Hz, J(q) = 6.1 Hz), 7.16–7.30 (m, 5H, Ph), GC–EI-MS *m/z* (M^+ 150 for $C_9H_{14}O$) 132, 117, 105, 91, 78, 65, 51, 45. The absolute configuration was confirmed with the authentic standard.

(*R*)-1-Piperonyl-2-propanol **52** (31 mg); $[\alpha]_D^{23} = -34.8$ (*c* 0.4, CH_3Cl) {lit.[36] $[\alpha]_D^{20} = -34.2$ (*c* 1, CH_3Cl)}, ^1H NMR ($CDCl_3$) δ (ppm) 1.23 (d, 1H, CH_3, $J = 6.3$ Hz), 1.60 (s, 1H, OH), 2.60 (dd, 1H, CH, J(d) = 8.1 Hz, J(d) = 13.7 Hz), 2.71 (dd, 1H, CH, J(d) = 4.8 Hz, J(d) = 13.6 Hz), 3.92–4.00 (m, 1H, CH), 5.93 (s, 2H, CH_2), 6.65–6.78 (m, 3H, Ph), GC–EI-MS *m/z* (M^+ 180 for $C_{10}H_{12}O_3$) 135, 121, 106, 77, 51, 45.

(*S*)-1-(3,4-Methylenedioxyphenyl)-2-chloroethanol **53** (14.5 mg); $[\alpha]_D^{22} = +45.6$ (*c* 0.5, CH_3Cl), ^1H NMR ($CDCl_3$) δ (ppm) 2.69 (d, 1H, OH, $J = 2.9$ Hz), 3.60 (dd, 1H, CH, J(d) = 8.8 Hz, J(d) = 11.2 Hz), 3.69 (dd, 1H, CH, J(d) = 3.4 Hz, J(d) = 11.2 Hz), 4.81 (dt, 1H, CH, J(d) = 8.8 Hz, J(t) = 3.2 Hz), 5.97 (s, 2H, CH_2), 6.79–6.89 (m, 3H, Ph), GC–EI-MS *m/z* (M^+ 202, 200 for $C_9H_9O_3Cl$) 182, 164, 151, 135, 123, 105, 93, 77, 65, 51, 44. The absolute configuration was tentatively assigned to be *S*.

(*R*)-1-*N*-Boc-3-Pyrrolidinol **54** (12 mg); $[\alpha]_D^{23} = -19.7$ (*c* 0.3, CH_3Cl) {lit.[37] $[\alpha]_D^{23} = -22.7$ (*c* 1.0, CH_3Cl)}, ^1H NMR ($CDCl_3$) δ (ppm) 1.46 (s, 9H, CH_3, CH_3, CH_3), 1.92 (s, 1H, OH) 1.94–2.07 (m, 2H, CH_2), 3.31–3.48 (m, 4H, CH_2, CH_2), 4.45 (s, 1H, CH), GC–EI-MS *m/z* (M^+ 187 for $C_9H_{17}O_3N$) 149, 132, 114, 103, 87, 74, 57. The absolute configuration was confirmed with the authentic standard.

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