Pergamon

Tetrahedron: *Asymmetry* 12 (2001) 3147–3153

TETRAHEDRON: *ASYMMETRY*

Stereoinversion of arylethanols by *Geotrichum candidum*

Kaoru Nakamura,[a,*] Mikio Fujii[b] and Yoshiteru Ida[b]

[a]*Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan*
[b]*School of Pharmaceutical Science, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan*

Received 9 November 2001; accepted 10 December 2001

Abstract—Aromatic and aliphatic racemic alcohols were converted to the corresponding optically active alcohols in high yield with excellent enantioselectivities by the use of *Geotrichum candidum* IFO 5767.

1. Introduction

Homochiral secondary alcohols are increasingly recognized as valuable chiral building blocks in organic syntheses. In the synthesis of enantiomerically pure alcohols, ketones or racemic alcohols are generally used as starting materials. The reduction of prochiral ketones by chemical or enzymatic methods can provide optically active alcohols in quantitative yield with 100% enantiomeric excess (e.e.). In contrast, the chemical yield of enantiopure alcohols obtained by resolution of racemic alcohols via selective oxidation or esterification cannot exceed 50%. Lipase-catalyzed transesterification of racemic alcohols is frequently used for the preparation of such compounds, but some problems still remain; resolutions with excellent enantioselectivities are rare and separation of the remaining substrate and the product is often problematic. Therefore, the development of a methodology to obtain enantiopure alcohols in high yield from the corresponding racemic alcohols is highly desirable.

Deracemization of racemic alcohols[1–5] to the corresponding enantiomerically pure alcohol permits 100% conversion. Deracemization can be carried out either by stereoinversion or dynamic kinetic resolution. In stereoinversion, one enantiomer from the racemic substrate is transformed into its mirror image by leaving the other enantiomer unchanged. Stereoinversion of a secondary alcohol catalyzed by a biocatalyst consists of enantioselective oxidation of one enantiomer to the corresponding ketone and sequential reduction to the other enantiomer. Chiral diols,[6–9] hydroxy acid derivatives[10–15] and aliphatic[16–19] and aromatic[20–23] alcohols have been obtained in enantiopure form by stereoinversion.

Practically, biocatalytic stereoinversion is divided into two systems: a two-biocatalyst system[11,12,15,16] and a one-biocatalyst system.[6–10,13,14,17–24] For example, two biocatalysts, *Alcaligenes bronchisepticus* and *Streptococcus faecalis*, were used for the stereoinversion of (±)-mandelic acid.[12] *A. bronchisepticus* oxidized (*S*)-mandelic acid selectively and the resulting ketone, benzoylformate, was reduced by NADPH-dependent benzoylformate reductase of *S. faecalis* to give (*R*)-mandelic acid in 80% yield with >99% e.e. Stereoinversion with one biocatalyst has also been reported; (*R*)-3-pentyn-2-ol was obtained enantioselectively from the corresponding racemic alcohol by *Nocardia fusca*.[17–19] Stereoinversion of arylethanols by *Geotrichum candidum*[20] and *Sphingomonas pausimobilis*[21] have also been reported.[20,21] Plant cell cultures have also been used as biocatalysts for the stereoinversion of racemic alcohols.[14,21–24] A cell culture of *Catharanthus roseus* entrapped in calcium alginate has been employed for the deracemization of pyridyl alcohols.[14] Indeed, several enantiopure alcohols have been prepared by stereoinversion. However, the number of examples is still somewhat limited and a new methodology for enhancing the effectiveness of stereoinversion is needed.

Herein, we report the stereoinversion of 1-arylethanols by *G. candidum* IFO 5767.[25]

2. Results and discussion

2.1. Stereoinversion of 1-phenylethanol

Firstly, we screened 18 microbes for their ability to catalyze the stereoinversion of 1-phenylethanol **1a**.

* Corresponding author. Tel./fax: 81-774-38-3201; e-mail: nakamura@scl.kyoto-u.ac.jp

0957-4166/01/$ - see front matter
PII: S0957-4166(01)00554-7

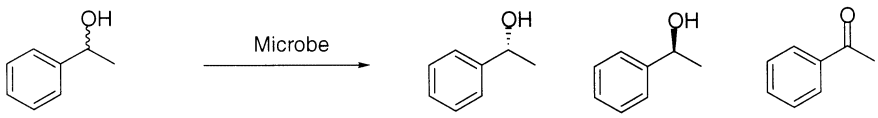
Microbes were cultivated in a glycerol medium as described in the experimental section and the collected cells (0.5 g wet weight) were added to a 0.03 mM aqueous 1-phenylethanol solution (3 mL) and the reaction mixture was shaken at 30°C for 1 day. The results are summarized in Table 1. All microbes tested gave high chemical yields except *Geotrichum rectangulatum* and *G. candidum* ATCC34674. *G. rectangulatum* afforded (*R*)-**1a** in 53% yield in 99% e.e., which shows that (*S*)-**1a** was selectively oxidized to acetophenone **2a** but reduction of **2a** to (*R*)-**1a** did not proceed. The stereoinversion did not proceed with *Aspergillus*, *Mucor*, and *Trichosporon* species; these microbes afforded **1a** in high yields with low enantioselectivities. On the other hand, three microbes, *Dipoduscus magnusii* IFO 4600, *Endomyces geotrichum* IFO 9541, and *G. candidum* IFO 5767 gave (*R*)-**1a** in high yields with high enantioselectivities.

Among the microbes, *G. candidum* IFO 5767 gave the best result (96% yield and 99% e.e.), and recovery of the substrates **1a** and **2a** was quantitative. This indicated that (*S*)-**1a** was not decomposed but isomerized to (*R*)-**1a**. Fig. 1 shows the time course of stereoinversion of **1a** by *G. candidum* IFO 5767. Initially, (*S*)-**1a** was oxidized to **2a**, which was subsequently reduced to (*R*)-**1a**. After 24 h, almost all of the (*S*)-**1a** presented at the beginning was converted to (*R*)-**1a**, and the e.e. reached 99%.

Although a detailed mechanism for the microbial stereoinversion is not clear at present, useful information about the reaction has been obtained. Firstly, reduction of **2a** with *G. candidum* IFO 5767 afforded (*R*)-**1a** in low selectivities (15–63% e.e.) and the amount of (*R*)-**1a** increased with prolonged reaction times. These facts indicate that (*S*)-**1a** was oxidized to the ketone **2a**, which was converted into a mixture of (*R*)-**1a** and (*S*)-**1a**, but e.e. of (*R*)-**1a** could not be oxidized under the reaction conditions. Thus, one possible mechanism of stereoinversion is that oxidoreduction between (*S*)-**1a** and **2a** is reversible and the reduction of **2a** is irreversible (Scheme 1).

Further information about the mechanism was obtained from NMR experiments. When phenylethanol-1-*d* was used as the substrate, the deuterium content in racemic **1a**-1-*d* decreased to 50% after 24 h of reaction. This reveals that the methine hydrogen of (*R*)-**1a** produced by stereoinversion is not derived from the hydrogen of (*S*)-**1a**. Thus, it can be reasonably stated that at least two enzymes participate, one that

Table 1. Screening of microbes for stereoinversion of **1a**



racemic-**1a** (R)-**1a** (S)-**1a** **2a**

Microbe		1-Phenylethanol		
		Yield (%)[a]	E.e. (%)[a]	Config.[a]
Aspergillus oryzae	IAM 2663	100	0	
Aspergillus niger	IAM 6661	100	0	
Aspergillus niger	IFO 9455	100	0	
Aspergillus niger	IFO 5374	100	0	
Aspergillus niger	IFO 4091	100	0	
Dipoduscas magnusii	IFO 4600	78	90	*R*
Endomyces geotrichum	IFO 9541	98	73	*R*
Galactomyces reessii	IFO 1112	90	13	*R*
Geotrichum candidum	IFO 4597	88	17	*R*
Geotrichum candidum	IFO 5767	96	97	*R*
Geotrichum candidum	ATCC 34674	66	98	*R*
Geotrichum rectangulatum[b]		53	99	*R*
Mucor heimalis	IAM 6095	96	2	*R*
Mucor Javanicus	IAM 6087	97	3	*R*
Mucor Javanicus	IAM 6101	97	2	*R*
Trichosporon capitatum	IAM 4324	99	30	*R*
Trichosporon cutaneum	IAM 12206	97	2	*R*
Trichosporon cutaneum	IAM 12246	97	2	*R*

Reaction conditions: 24 h at 30°C at 130 rpm in water (3 mL); microbe (0.5 g wet wt.), **1a** (0.03 M).

[a] Determined by GC analysis.

[b] 7% of phenol, which was produced by Baeyer–Villiger oxidation of acetophenone and subsequent hydrolysis, was detected.

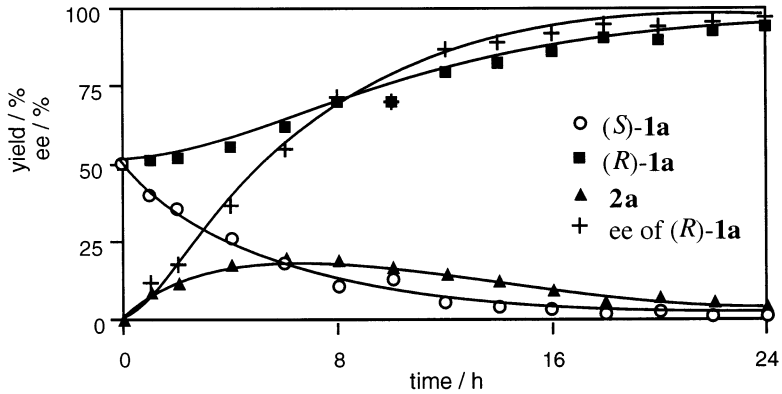
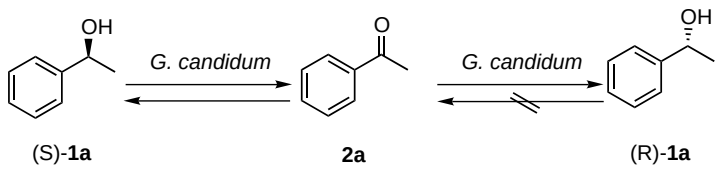


Figure 1. Time course of stereoinversion of **1a**. *Reaction conditions*: at 30°C/130 rpm; cells (0.5 g wet wt.), **1a** (0.08 mmol), water (3 mL)



Scheme 1.

oxidizes (*S*)-**1a** and the other that reduces **2a** into (*R*)-**1a**, and these enzymes may use different coenzymes. The possibility of the participation of flavin coenzyme is also conceivable. The former situation has been reported in the stereoinversion of pentane diol, where the first enzyme used NADH and the second NADPH as coenzymes.[6] Recently, we have found that the reduction of methyl ketones by *G. candidum* IFO 4597 was conducted by two enzymes which exhibit different stereoselectivities.[26] One enzyme afforded (*S*)-alcohols and the other gave (*R*)-alcohols. Since the former enzyme could oxidize secondary alcohols but the latter could not, we propose that the similar enzymatic system works in the stereoinversion by *G. candidum* IFO 5767. The ineffectiveness of *G. candidum* IFO 4597 toward stereoinversion is thought to be due to low activity of the (*R*)-producing enzyme in the microbe.

2.2. Stereoinversion of secondary alcohols

Several aryl alcohols were subjected to the microbial reaction. The results are summarized in Table 2. *para*-Substituted 1-phenylethanols **1d**, **1g**, **1h** gave the corresponding (*R*)-alcohols with high e.e. (>77%) and high yields, but stereoinversion of *meta*- and *ortho*-substituted phenylethanols were less effective. Increasing the length of the alkyl moiety of the phenylalkanol decreased the reactivity and as such, the phenylpropanols **3a** and **3b** and phenylbutanol **3c** were recovered from the reactions in high yield.

2.3. Improvement of stereoinversion

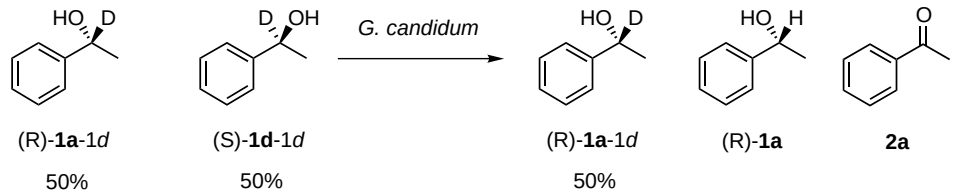
To improve the effectiveness of stereoinversion by *G. candidum*, three strategies are considered with respect to the mechanism in Scheme 2; namely, inhibition of the reduction of **2a** back to (*S*)-**1a**, enhancement of reduction of **2a** to (*R*)-**1a**, and activation of oxidation of (*S*)-**1a** to **2a**, respectively.

To inhibit the reduction of **2a** to (*S*)-**1a**, several inhibitors, such as methyl vinyl ketone, allyl alcohol, and ethyl chloroacetate were used as additives.

Table 2. Stereoinversion of secondary alcohols by *G. candidum* IFO5767

Substrate	Yield (%)	E.e. (%)	Config.
1-(*o*-Methylphenyl)ethanol **1b**	98	2	*R*
1-(*m*-Methylphenyl)ethanol **1c**	90	19	*R*
1-(*p*-Methylphenyl)ethanol **1d**	95	77	*R*
1-(*o*-Chlorophenyl)ethanol **1e**	100	0	–
1-(*m*-Chlorophenyl)ethanol **1f**	89	14	*R*
1-(*p*-Chlorophenyl)ethanol **1g**	96	89	*R*
1-(*p*-Methoxypheny)ethanol **1h**	79	89	*R*
1-(2′-Furyl)ethanol **1i**	65	92	*R*
1-Phenylpropan-1-ol **3a**	96	25	*R*
1-Phenylpropan-2-ol **3b**	95	14	*R*
4-Phenylbutan-2-ol **3c**	88	74	*R*

Reaction conditions: 24 h at 30°C at 130 rpm in water (3 mL); microbe (0.5 g wet wt.), and substrate (0.03 M).



Scheme 2.

Recently, we have developed several methods for controlling stereoselectivities of microbial reduction and have found that the use of selective inhibitors is effective for controlling the stereoselectivities of microbial reductions. For example, in the yeast reduction of β-keto esters, addition of allyl alcohol[27,28] and methyl vinyl ketone[28] afforded D-hydroxy esters selectively. On the other hand, the presence of ethyl chloroacetate[27,30] and magnesium ions[31] in the same biocatalytic system afforded L-hydroxy esters selectively. When ethyl chloroacetate was used as an additive in the stereoinversion, the e.e. of **1a** decreased (97% e.e. was observed without additive and 29% e.e. in the presence of ethyl chloroacetate). In contrast, the e.e. of **1a** increased and its yield reduced by the addition of methyl vinyl ketone (98% e.e., 54% yield) and allyl alcohol (100% e.e., 91% yield). This is because ethyl chloroacetate inhibits the (*R*)-alcohol-producing enzyme and methyl vinyl ketone and allyl alcohol inhibits the (*S*)-alcohol-producing enzyme.

To enhance selective reduction to the (*R*)-alcohol, secondary alcohols and sugar derivatives, such as glycerol and glucose, were used as additives.[29,32,33] However, these additives unfortunately led to a decreased e.e. of **1a**.

Finally, enhancement of the oxidation of (*S*)-**1a** to **2a** was attempted. Cyclohexanone was used since it is known to increase the oxidation rate of (*S*)-alcohol by *G. candidum*,[34,35] however, this additive was not effective and the e.e. of (*R*)-**1a** was unsatisfactory (48% e.e.). In addition, anaerobic conditions were used for the stereoinversion. This is because anaerobic conditions (for example, an argon atmosphere) are known to shift the stereoselectivity of the reduction of ketones by *G. candidum* toward the production of (*S*)-alcohols; the stereoselectivity of the reduction under an aerobic condition seems to facilitate the production of (*R*)-alcohols.[36] In the experiment described above, a standard test tube was used as the reaction vessel and the reaction was conducted by shaking the test tube. A Sakaguchi flask is often used for cultivation of microbes and is known to supply oxygen in the medium effectively, which increases the growth rate of aerobic microbes. Surprisingly, stereoinversion of **1a** under aerobic conditions gave the best result; (*S*)-**1a** was obtained in 96% yield with 99% e.e. A further increase in the oxygen level inhibited the reaction and the reaction under a pure oxygen atmosphere did not proceed. We think that a suitable level of oxidation is required to oxidize the coenzyme NAD(P)H to $NAD(P)^+$ and thus allow subsequent acceleration of the oxidation of the (*S*)-alcohol.

2.4. Stereoinversion of various secondary alcohols in preparative scale

Various chiral secondary alcohols were prepared via stereoinversion of the corresponding racemates with *G. candidum* IFO5767 under aerobic conditions. As shown in Table 3, most *para*-substituted phenylethanols **1a**–**1l**, phenylpropanols **3a**,**3b**, and pyridylethanols **1m**, **1n**, **1o** gave high e.e. (>95% e.e.) with high chemical yields. The exception was *p*-methoxyphenylethanol **1h**, which gave relatively low yields. The reaction intermediate, *p*-methoxyacetophenone, may not resist reduction because of the strong electron-donating nature of its methoxy moiety. Since the oxidation of (*S*)-**1h** was fast, (*S*)-**1h** in the starting racemic **1h** was consumed within 6 h and the e.e. of the remaining (*R*)-**1h** reached 100%. Chiral pyridylethanols were obtained in high yields with excellent e.e. Diol also reacted smoothly. Thus, phenyl-1,2-ethanediol **3f** exhibited excellent e.e. and chemical yield. Aromatic ketones as well as hydroxy esters **3g**, **3h** were reacted using this method, resulting in chiral hydroxy esters with high enantioselectivities (Scheme 3).

3. Summary

Stereoinversion of racemic secondary alcohols by *G. candidum* IFO 5767 was accomplished under aerobic conditions, and broader substrate specificities were observed. The racemic alcohols were converted to the corresponding (*R*)-alcohols in excellent e.e. and chemical yields.

Previously, we have reported asymmetric reduction by *G. candidum* IFO 4597 with the aid of a hydrophobic polymer[37] or water-absorbing polymer,[35] which afforded the corresponding (*S*)-alcohols with excellent e.e. The acetone powder of *G. candidum* IFO 4597 also afforded (*S*)-alcohols by the reduction of methyl or trifluoromethyl ketones.[26,32] Thus, both (*S*)- and (*R*)-enantiomers, could be obtained with excellent enantioselectivities and high yields by using *G. candidum* species. Since the dehydrogenase from *G. candidum* IFO 5767 is active in an organic solvent[35] and even in supercritical carbon dioxide,[38] and affords optically

Table 3. Stereoinversion of secondary alcohols by *G. candidum* IFO5767 under aerobic conditions

Substrate	Yield (isolated)[c] (%)	E.e. (%)	Config.
1-Phenylethanol **1a**	96 (83)	99	*R*
1-(*m*-Methylphenyl)ethanol **1c**	99 (49)	78	*R*
1-(*p*-Methylphenyl)ethanol **1d**	99 (67)	96	*R*
1-(*p*-Chlorophenyl)ethanol **1g**	94 (84)	92	*R*
1-(*p*-Chlorophenyl)ethanol[a] **1g**	93 (82)	98	*R*
1-(*p*-Methoxyphenyl)ethanol[a] **1h**	65 (56)	100	*R*
1-(*p*-Fluorophenyl)ethanol **1j**	95 (85)	100	*R*
1-(*p*-Fluorophenyl)ethanol[a] **1j**	96 (58)	100	*R*
1-(*p*-Bromophenyl)ethanol **1k**	98 (79)	95	*R*
1-(*p*-Bromophenyl)ethanol[a] **1k**	99 (82)	100	*R*
1-(*p*-Trifluoromethylphenyl)ethanol **1l**	99 (61)	95	*R*
1-(2′-Pyridyl)ethanol[a] **1m**	96 (53)	98	*R*
1-(3′-Pyridyl)ethanol[a] **1n**	100 (90)	95	*R*
1-(4′-Pyridyl)ethanol **1o**	100 (63)	100	*R*
1-Phenylpropan-1-ol[a] **3a**	87 (25)	100	*R*
1-Phenylpropan-2-ol[a] **3b**	99 (80)	100	*R*
4-Phenylbutan-2-ol[a] **3c**	65 (59)	99	*R*
4-Phenoxypropan-2-ol[a] **3d**	100 (99)	97	*R*
1-Phenylthiopropan-2-ol[a] **3e**	80 (49)[b]	96	*R*
Phenylethan-1,2-diol **3f**	100 (69)	100	*S*
Methyl 3-hydroxybutanoate **3g**	65 (26)	97	*R*
Methyl 3-hydroxypentanoate **3h**	99 (48)	99	*R*

Conditions: substrate (600 mg) and *G. candidum* (30 g wet wt.) dissolved in water (180 mL).

[a] Substrate (200 mg) and *G. candidum* (20 g wet wt.) dissolved in water (100 mL).

[b] 28% of 1-phenylsulfenylpropan-2-ol with 0% d.e. which was oxidized on silica gel was obtained. The ^{1}H NMR spectral data are in accord with those of the authentic samples. **1a**.[42]

[c] From **1a** (600 mg, 4.92 mmol) to (*R*)-**1a** (499 mg, 4.09 mmol); $[\alpha]_D$ +53.5 ($c=1.13$, $CHCl_3$), (lit.)[20] $[\alpha]_D$ +58.7 ($c=1.03$, $CHCl_3$), >99% e.e. (*R*). **1c**: From **1c** (623 mg, 4.58 mmol) to (*R*)-**1c** (303 mg, 2.22 mmol); $[\alpha]_D$ +33.0 ($c=1.00$, ethanol), (lit.)[39] $[\alpha]_D$ +40.4 ($c=0.53$, ethanol), >99.9% e.e. (*R*). **1d**: From **1d** (613 mg, 4.50 mmol) to (*R*)-**1d** (411 mg, 302 mmol); $[\alpha]_D$ +51.5 ($c=1.00$, $CHCl_3$), (lit.)[39] $[\alpha]_D$ +51.6 ($c=1.0$, $CHCl_3$), 93.8% e.e. (*R*). **1g**: From **1g** (603 mg, 3.87 mmol) to (*R*)-**1g** (500 mg, 321 mmol), conditions (a) from **1g** (208 mg, 1.33 mmol) to (*R*)-**1g** (175 mg, 1.12 mmol); $[\alpha]_D$ +44.8 ($c=1.00$, ether), (lit.)[39] $[\alpha]_D$ +51.3 ($c=0.595$, ether), >99.9% e.e. (*R*). **1h**: From **1h** (223 mg, 1.47 mmol) to (*R*)-**1h** (124 mg, 0.82 mmol); $[\alpha]_D$ +51.2 ($c=1.02$, MeOH), (lit.)[32] $[\alpha]_D$ −51.9 ($c=0.718$, MeOH), >99% e.e. (*S*). **1j**: From **1j** (600 mg, 4.29 mmol) to (*R*)-**1j** (517 mg, 3.69 mmol), conditions (a) from **1j** (205 mg, 1.46 mmol) to (*R*)-**1j** (120 mg, 0.86 mmol); $[\alpha]_D$ +37.5 ($c=1.01$, MeOH), (lit.)[32] $[\alpha]_D$ −37.7 ($c=0.931$, MeOH), >99% e.e. (*S*). **1k**: From **1k** (610 mg, 3.03 mmol) to (*R*)-**1k** (487 mg, 2.42 mmol), conditions (a) from **1k** (212 mg, 1.05 mmol) to (*R*)-**1k** (177 mg, 0.88 mmol); $[\alpha]_D$ +39.6 ($c=1.24$, $CHCl_3$), (lit.)[32] $[\alpha]_D$ −37.9 ($c=1.13$, $CHCl_3$), >99% e.e. (*S*). **1l**: From **1l** (607 mg, 3.19 mmol) to (*R*)-**1l** (371 mg, 1.95 mmol); $[\alpha]_D$ +28.7 ($c=1.13$, MeOH), (lit.)[32] $[\alpha]_D$ −28.4 ($c=1.26$, MeOH), >99% e.e. (*S*). **1m**: From **1m** (203 mg, 1.65 mmol) to (*R*)-**1m** (108 mg, 0.88 mmol); $[\alpha]_D$ +24.5 ($c=0.31$, $CHCl_3$), (lit.)[40] $[\alpha]_D$ +25.6 ($c=1.27$, $CHCl_3$), >95% e.e. (*R*). **1n**: From **1n** (202 mg, 1.64 mmol) to (*R*)-**1n** (182 mg, 1.48 mmol); $[\alpha]_D$ +51.5 ($c=1.00$, $CHCl_3$), (lit.)[40] $[\alpha]_D$ +52.4 ($c=1.21$, $CHCl_3$), >95% e.e. (*R*). **1o**: From **1o** (602 mg, 4.89 mmol) to (*R*)-**1o** (378 mg, 3.07 mmol); $[\alpha]_D$ +44.9 ($c=0.94$, MeOH), (lit.)[40] $[\alpha]_D$ +42.5 ($c=1.04$, MeOH), >95% e.e. (*R*). **3a**: From **3a** (204 mg, 1.50 mmol) to (*R*)-**3a** (51 mg, 0.38 mmol); $[\alpha]_D$ −47.0 ($c=1.00$, $CHCl_3$), (lit.)[39] $[\alpha]_D$ +39.7 ($c=0.0409$, $CHCl_3$), 95% e.e. (*S*). **3b**: from **3b** (240 mg, 1.76 mmol) to (*R*)-**3b** (193 mg, 1.42 mmol); $[\alpha]_D$ −38.7 ($c=1.11$, $CHCl_3$), (lit.)[39] $[\alpha]_D$ +39.7 ($c=0.515$, $CHCl_3$), >99.9% e.e. (*S*). **3c**: From **3c** (230 mg, 3.03 mmol) (*R*)-**3c** (137 mg, 0.912 mmol); $[\alpha]_D$ −21.1 ($c=1.00$, C_6H_6), (lit.)[39] $[\alpha]_D$ +22 ($c=0.38$, C_6H_6), >99.9% e.e. (*S*). **3d**: From **3d** (244 mg, 1.57 mmol) to (*R*)-**3d** (242 mg, 1.57 mmol); $[\alpha]_D$ +2.8 ($c=0.58$, EtOH), (lit.)[41] $[\alpha]_D$ −2.7 ($c=1.80$, EtOH), >99% e.e. (*S*). **3e**: From **3e** (197 mg, 1.17 mmol) to (*R*)-**3e** (96 mg, 0.60 mmol); $[\alpha]_D$ −8.8 ($c=1.02$, MeOH), (lit.)[41] $[\alpha]_D$ +8.20 ($c=0.59$, MeOH), 94% e.e. (*S*). **3f**: From **3f** (600 mg, 4.35 mmol) to (*S*)-**3f** (411 mg, 2.99 mmol); $[\alpha]_D$ +38.4 ($c=1.31$, ethanol) (lit.)[6] $[\alpha]_D$ +38.6 ($c=1$, ethanol), 99.9% e.e. (*S*). **3g**: From **3g** (600 mg, 5.08 mmol) to (*R*)-**3g** (156 mg, 1.23 mmol); $[\alpha]_D$ −41.9 ($c=1.09$, $CHCl_3$), (lit.)[32] $[\alpha]_D$ +40.6 ($c=1.09$, $CHCl_3$), >99% e.e. (*S*). **3h**: From **3h** (601 mg, 4.55 mmol) to (*R*)-**3h** (290 mg, 2.20 mmol); $[\alpha]_D$ −34.8 ($c=1.36$, $CHCl_3$), (lit.)[32] $[\alpha]_D$ +34.3 ($c=1.05$, $CHCl_3$), >99% e.e. (*S*).

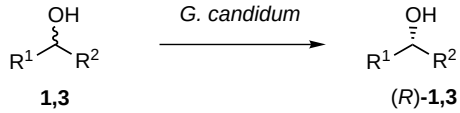
active alcohols with excellent enantioselectivities, *G. candidum* is highly recommended as a biocatalyst for the synthesis of optically active secondary alcohols.

4. Conclusions

A range of chiral aromatic and aliphatic alcohols has been produced from racemic mixtures by stereoinversion using *G. candidum* IFO 5767. Further studies, including the isolation of enzymes, are underway in our laboratory.

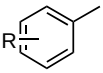
5. Experimental

The gas chromatographic analysis was performed using a Shimadzu GC-14A gas chromatograph equipped with a Shimadzu CR-6A Chromatopac equipped with chiral GC-columns (Chiraldex G-TA; 40 or 30 m; He 2 mL/min (G-TA), CP-Cyclodextrin-B-2,3,6-M-19; 25 m; He 2 mL/min (CPCD), Chirasil-DEX CB; 25 m; He 2 mL/min (DEX-CB)). ^{1}H NMR spectra were recorded on a Varian VXR-200 spectrometer in $CDCl_3$. Optical rotation was measured with a Perkin–Elmer 241 polarimeter.



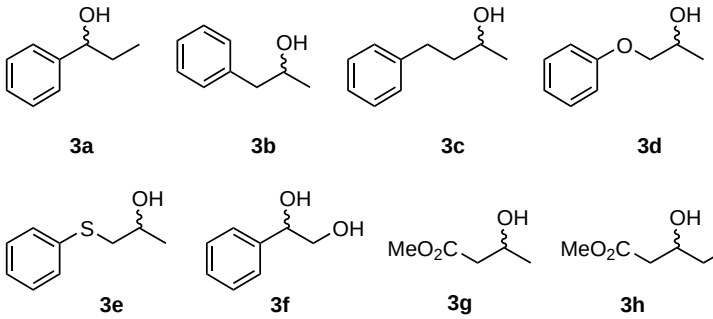
1,3 (*R*)-**1,3**

1; R^1 = Ar, R^2 = Me

1a-1h; Ar = 

1a; R = H, **1b**; R = *o*-Me, **1c**; R = *m*-Me, **1d**; R = *p*-Me, **1e**; R = *o*-Cl, **1f**; R = *m*-Cl, **1g**; R = *p*-Cl, **1h**; R = *p*-MeO

1i; Ar = 2'-furyl, **1j**; R = *p*-F, **1k**; R = *p*-Br, **1l**; R =*p*-CF_3, **1m**; Ar = 2'-pyridyl, **1n**; Ar = 3'-pyridyl,**1o**; Ar = 4'-pyridyl,



3a **3b** **3c** **3d** **3e** **3f** **3g** **3h**

Scheme 3.

5.1. Materials

Organic reagents were purchased from Nacalai Tesque, Inc., Tokyo Chemical Industry Co., Ltd, Kanto Chemical, Inc. or Wako Pure Chemical Industries, Ltd. unless otherwise indicated. Racemic alcohols were obtained by the reaction of the corresponding aldehydes with excess methylmagnesium bromide or by reduction of the corresponding ketones with sodium borohydride. 1-Phenylethanol-1-*d* was obtained by reduction of acetophenone with lithium aluminium duteride.

5.2. Cultivation of microbe

The microbe was grown in a complex medium consisting of 3% (wt./vol.) glycerol, 1% (wt./vol.) yeast extract, and 0.5% (wt./vol.) polypeptone. The medium, adjusted to pH 6.2 using 0.1 M potassium phosphate buffer, was placed in a Sakaguchi flask, sterilized (121°C, 20 min) and inoculated with the preincubated culture. The cultivation was carried out for 24 h at 30°C with shaking.

5.3. General procedure of stereoinversion on small scales

The cells (0.5 g wet wt.) and substrate (0.08 mmol) were put into a 50 mL test tube equipped with screw cap and containing water (3 mL) and shaken at 130 rpm for 1 day at 30°C. The reaction mixture was put on Extrelut (Merck) and eluted with ether. The e.e. and chemical yield of the products were determined by a GC analysis of the ether extract using dodecane as an internal standard. Results are summarized in Tables 1 and 2. GC conditions for determination of e.e. of alcohols (column, retention time); **1a**: CPCD 100°C, *S* 15.8 min, *R* 14.6 min. **1b**: CPCD 130°C, *S* 7.8 min, *R* 7.1 min. **1c**: CPD 120°C, *S* 13.7 min, *R* 13.3 min. **1d**: CPCD 115°C, *S* 12.1 min, *R* 11.2 min. **1e**: CPCD 130°C, *S* 13.9 min, *R* 13.1 min. **1f**: CPCD 130°C, *S* 13.9 min, *R* 13.0 min. **1g**: CPCD 130°C, *S* 14.3 min, *R* 13.3 min. **1h**: CPCD, 130°C, *S* 17.5 min, *R* 16.8 min. **1j**: DEX-CB 120°C, *S* 11.4 min, *R* 10.3 min. **1k**: DEX-CB 150°C, *S* 12.7 min, *R* 11.6 min. **1l**: DEX-CB 120°C, *S* 16.3 min, *R* 13.5 min. **1m-Acetate**: DEX-CB 100°C, *S* 6.9 min, *R* 6.3 min. **1n**: DEX-CB 130°C, *S* 13.4 min, *R* 12.6 min. **1o**: DEX-CB 105°C, *S* 9.8 min, *R* 9.6 min. **3a**: DEX-CB 120°C, *S* 14.2 min, *R* 13.7 min. **3b-Acetate**: CPCD 105°C, *S* 15.8 min, *R* 16.8 min. **3c**: G-TA 105°C, *S* 21.4 min, *R* 22.2 min. **3d-Acetate**: CPCD 130°C, *S* 13.9 min, *R* 14.4 min. **3e**: G-TA 130°C, *S* 21.1 min, *R* 21.9 min. **3f-Diacetate**: G-TA 115°C, *S* 56.1 min, *R* 58.3 min. **3g**: G-TA 85°C, *S* 11.9 min, *R* 13.1 min. **3h**: DEX-CB 90°C, *S* 8.4 min, *R* 8.8 min.

5.4. Stereoinversion of 1-phenylethanol in the presence of additives

The cells (0.5 g wet wt.), additive (0.5 mmol) and **1a** (0.08 mmol) were put into a 50 mL test tube equipped with screw cap and containing water (3 mL), and shaken at 130 rpm for 1 day at 30°C. The reaction mixture was put on Extrelut (Merck) and eluted with ether. The e.e. and chemical yield of the 1-phenylethanol was determined by GC analysis of the ether solution.

5.5. Stereoinversion on preparative scale: (*R*)-1-phenylethanol 1a

The cells (30 g wet wt.) and (±)-phenylethanol (600 mg, 4.92 mmol) were put into a Sakaguchi-flask containing water (180 mL), and shaken at 130 rpm for 1 day at 30°C. The mixture was filtered and the filtrate and the filtered microbe were extracted with ether separately. The organic layers were collected, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was subjected to silica gel column chromatography (hexane:ethyl acetate = 8:1), followed by bulb-to-bulb distillation (150°C/1.8 mmHg) afforded (*R*)-phenylethanol (499 mg, 4.09 mmol, 83%). The e.e. and chemical yield of (*R*)-phenylethanol was determined by GC analysis (CP-Cyclodextrin-B-236-M-19, 0.25 mm×25 m, He: 2 mL/min, 100°C, retention times; (*S*)-1-phenylethanol 15.8 min, (*R*)-1-phenylethanol 14.6 min) (96%, 99% e.e.); $[\alpha]_D$ +53.5 ($c = 1.13$, $CHCl_3$), (lit.)[20] $[\alpha]_D$ +58.7 ($c = 1.03$, $CHCl_3$), >99% e.e. (*R*); ^1H NMR (200 MHz, $CDCl_3$) δ 1.49 (d, 3H, $J = 6.8$ Hz), 1.85 (s, 1H) 4.90 (q, 1H, $J = 6.8$ Hz), 7.25–7.37 (5H, $J = 6.4$ Hz). Anal. calcd for $C_8H_{10}O$; C, 78.65; H, 8.25. Found: C, 78.46; H, 8.40%.

Other alcohols were reacted with the same system, and the results are listed in Table 3.

References

1. Stecher, H.; Faber, K. *Synthesis* **1997**, 1–16.
2. Carnell, A. J. *Adv. Biochem. Eng. Biotechnol.* **1999**, *63*, 57–72.
3. Azerad, R.; Buisson, D. *Curr. Opin. Biotechnol.* **2000**, *11*, 565–571.
4. Kroutil, W.; Faber, K. *Tetrahedron: Asymmetry* **1998**, *9*, 2901–2913.
5. Strauss, U. T.; Felfer, U.; Faber, K. *Tetrahedron: Asymmetry* **1999**, *10*, 107–117.
6. Hasegawa, J.; Ogura, M.; Tsuda, S.; Maemoto, S.; Kutsuki, H. *Agric. Biol. Chem.* **1990**, *54*, 1819–1827.
7. Matsumura, S.; Kawai, Y.; Takahashi, Y.; Toshima, K. *Biotechnol. Lett.* **1994**, *16*, 485–495.
8. Carnell, A. J.; Iacazio, G.; Roberts, S. M.; Willets, A. J. *Tetrahedron Lett.* **1994**, *35*, 331–334.
9. Goswami, A.; Mirfakhrae, K. D.; Patel, R. N. *Tetrahedron: Asymmetry* **1999**, *10*, 4239–4244.
10. Shimizu, S.; Hattori, S.; Hata, H.; Yamada, H. *Appl. Environ. Microbiol.* **1986**, *53*, 519–522.
11. Shimizu, S.; Hattori, S.; Hata, H.; Yamada, H. *Enzyme Microb. Technol.* **1987**, *9*, 411–416.
12. Tuchiya, S.; Miyamoto, K.; Ohta, H. *Biotechnol. Lett.* **1992**, *14*, 1137–1142.
13. Buisson, D.; Azerad, R.; Sanner, C.; Lalcheveque, M. *Biocatalysis* **1992**, *5*, 249–265.
14. Takemoto, M.; Matsuoka, Y.; Achiwa, K.; Kutney, J. P. *Tetrahedron Lett.* **2000**, *41*, 499–502.
15. Takahashi, E.; Nakamichi, K.; Furui, M. *J. Ferment. Bioeng.* **1995**, *80*, 247–250.
16. Fantin, G.; Fogagnolo, M.; Giovannini, P. P.; Medici, A.; Pedrini, P. *Tetrahedron: Asymmetry* **1995**, *6*, 3047–3053.
17. Xie, S. X.; Ogawa, J.; Shimizu, S. *Appl. Microbiol. Biotechnol.* **1999**, *52*, 327–331.
18. Ogawa, J.; Xie, S. X.; Shimizu, S. *Biotechnol. Lett.* **1999**, *21*, 331–335.
19. Xie, S. X.; Ogawa, J.; Shimizu, S. *Biosci. Biotech. Biochem.* **1999**, *63*, 1721–1729.
20. Nakamura, K.; Inoue, Y.; Matsuda, T.; Ohno, A. *Tetrahedron Lett.* **1995**, *36*, 6263–6266.
21. Allan, G. R.; Carnell, A. J. *J. Org. Chem.* **2001**, *66*, 6495–6497.
22. Takemoto, M.; Achiwa, K. *Tetrahedron: Asymmetry* **1995**, *12*, 2925–2928.
23. Takemoto, M.; Achiwa, K. *Phytochemistry* **1998**, *49*, 1627–1629.
24. Takemoto, M.; Achiwa, K. *Chem. Pharm. Bull.* **1998**, *46*, 577–580.
25. Previous communication: Ref. 20.
26. Matsuda, T.; Harada, T.; Nakajima, N.; Itoh, T.; Nakamura, K. *J. Org. Chem.* **2000**, *65*, 157–163.
27. Nakamura, K.; Kawai, Y.; Nakajima, N.; Ohno, A. *J. Org. Chem.* **1991**, *56*, 4778–4783.
28. Nakamura, K.; Inoue, K.; Ushio, K.; Oka, S.; Ohno, A. *Chem. Lett.* **1987**, 679–682.
29. Nakamura, K.; Kawai, Y.; Oka, S.; Ohno, A. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 875–879.
30. Nakamura, K.; Kawai, Y.; Ohno, A. *Tetrahedron Lett.* **1990**, *31*, 267–270.
31. Nakamura, K.; Kawai, Y.; Oka, S.; Ohno, A. *Tetrahedron Lett.* **1989**, *30*, 2245–2246.
32. Nakamura, K.; Matsuda, T. *J. Org. Chem.* **1998**, *63*, 8957–8964.
33. Nakamura, K.; Miyai, T.; Inoue, K.; Kawasaki, S.; Oka, S.; Ohno, A. *Biocatalysis* **1990**, *3*, 17–24.
34. Nakamura, K.; Inoue, Y.; Ohno, A. *Tetrahedron Lett.* **1994**, *35*, 4375–4376.
35. Nakamura, K.; Inoue, Y.; Matsuda, T.; Misawa, I. *J. Chem. Soc., Perkin 1* **1999**, 2397–2402.
36. Nakamura, K.; Matsuda, T.; Ohno, A. *Tetrahedron: Asymmetry* **1996**, *7*, 3021–3024.
37. Nakamura, K.; Fujii, M.; Ida, Y. *J. Chem. Soc., Perkin 1* **2000**, 3205–3211.
38. Matsuda, T.; Harada, T.; Nakamura, K. *J. Chem. Soc., Chem. Commun.* **2000**, 1367–1368.
39. Nakamura, K.; Kawasaki, M.; Ohno, A. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 1079–1085.
40. Seemayer, R.; Schneider, M. P. *Tetrahedron: Asymmetry* **1992**, *3*, 827–830.
41. Waagen, V.; Partali, V.; Hollingsaeter, I.; Huang, M. S. S.; Anthonsen, T. *Acta Chem. Scand.* **1994**, *48*, 506–510.
42. Ohta, H.; Kato, Y.; Tsuchihashi, G. *J. Org. Chem.* **1987**, *52*, 2735–2739.