Two Classes of Enzymes of Opposite Stereochemistry in an Organism: One for Fluorinated and Another for Nonfluorinated Substrates

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Received August 11, 1999

Reduction of methyl ketones by dried cells of *Geotrichum candidum* (APG4) afforded (*S*)-alcohols in excellent enantiomeric excess (ee), whereas the reduction of trifluoromethyl ketones gave the corresponding alcohols of the opposite configuration also in excellent ee. The replacement of the methyl moiety with a trifluoromethyl group alters both the bulkiness and the electronic properties, the effect of which on the stereoselectivity was examined. No inversion in stereochemistry was observed in the reduction of hindered ketones such as isopropyl ketone, while the stereoselectivity was inverted in the reduction of ketones with electron-withdrawing atoms such as chlorine. The mechanism for the inversion in stereochemistry was investigated by enzymatic studies. Several enzymes with different stereoselectivities were isolated; one of them catalyzed the reduction of methyl ketones, and another with the opposite stereoselectivity catalyzed the reduction of trifluoromethyl ketones. Furthermore, both APG4 and the isolated enzyme were applied to the reduction of fluorinated ketones on a preparative scale, which resulted in the synthesis of chiral fluorinated alcohols with excellent ee.

Introduction

There has been much research done into the enzymatic synthesis of chiral compounds, and several preparative methods have been reported.[1] Currently, biocatalysts such as lipases, alcohol dehydrogenases, aldolases, and glucosidase are used for the synthesis of natural products, phamaceuticals, agrochemicals, and ferroelectric liquid crystals.[1]

We have been developing an enzymatic reduction system using the dried cells of a dimorphic fungus, *Geotrichum candidum* IFO 4597 (APG4), by which either aromatic or aliphatic methyl ketones can be reduced with >99% enantiomeric excess (ee), resulting in the synthesis of optically pure secondary alcohols in high yield.[2] The system consists of APG4, $NAD(P)^+$, and 2-alkanol or cyclopentanol. As the substrate ketone is reduced to an alcohol, $NAD(P)^+$ is formed, which in turn is reduced to NAD(P)H by the coupled oxidation of 2-alkanol or cyclopentanol as shown in Scheme 1.[2]

The substrate specificity of the APG4 system has been expanded to include fluorinated ketones,[3] and different configurational alcohols were obtained by subjecting the trifluorinated ketone (trifluoroacetophenone, **1Fa**) and its unfluorinated analogue (acetophenone, **1Ha**) to the same reduction system as shown in Scheme 2.[3a] The absolute configurations of (*S*)-**1Hb** and (*S*)-**1Fb** are opposite by definition, so for convenience, the notation "*A*" and "*B*" is used in this article to describe the absolute configuration of (*S*)-**1Hb** and (*S*)-**1Fb**, respectively, as shown in Scheme 2.

A search of the literature uncovered several reports comparing the methyl and trifluoromethyl groups for the

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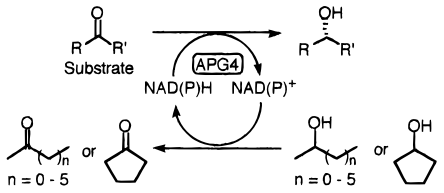
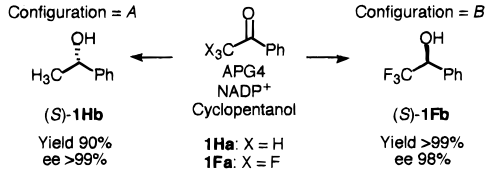
10.1021/jo991283k CCC: $19.00
Published on Web 12/16/1999

Scheme 1. Reaction Mechanism of the APG4 System



APG4: Dried cells of *Geotrichum candidum* IFO 4597

Scheme 2

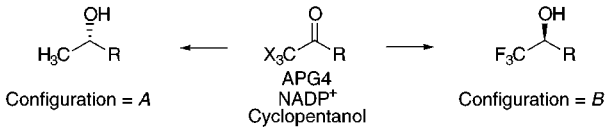


The reaction conditions are described in the experimental section.

ability to direct enantioselection in biocatalytic[4] and organometallic[4b,5] reactions. Kitazume et al. investigated the effect of fluorine on the stereoselectivity of baker's yeast reduction[4a,c] and lipase MY-catalyzed hydrolysis.[4a,d−f] In some cases when biocatalysts are used, the stereoselectivities are affected by the fluorine, but a completely different stereoselectivity between methyl and trifluoromethyl substrates as in our case (>99% ee for **1Ha**, 98% ee for **1Fa**) is not found. The inversion in stereochemistry is also observed for the reduction of **1Ha** and **1Fa** by (−)-DIP-chloride and CBS/catecholborane but not by (*R*)-BINAL-H, LAH-sulfamide, NB-enantride and K-glucoride. The mechanism of the inversion for the biocatalytic reactions has not been thoroughly investigated, whereas that for the organometallic reaction has been explained by structural and mechanistic analyses.[5a−f]

In this article the factor that causes the inversion in biocatalytic reactions is investigated by systematically changing the substituents at the α-position of acetophenone, as well as by enzymatic studies. Moreover, besides having an interesting stereochemistry, this system is valuable for synthetic purposes. Various chiral fluorinated alcohols can be synthesized using the APG4 system and also the separated enzyme. Monofluoromethyl and difluoromethyl ketones are reduced to both enantiomers by modifying the experimental conditions and choosing either the isolated enzyme or APG4.

Table 1. Reduction of Methyl Ketones and Trifluoromethyl Ketones[a]



	X = H			X = F		
R	substrate	yield[b] (%)	ee[b,c] (%)	substrate	yield[b] (%)	ee[b] (%)
Ph	**1Ha**	90	>99 (*S*)	**1Fa**	>99	98 (*S*)
p-chlorophenyl	**2Ha**	83	95 (*S*)	**2Fa**	91	>99 (*S*)
p-bromophenyl	**3Ha**	94	91 (*S*)	**3Fa**	82	>99 (*S*)
benzyl	**4Ha**	95	>99 (*S*)	**4Fa**	55	97 (*S*)
2-thienyl	**5Ha**	45	>99 (*S*)	**5Fa**	>99	>99 (*R*)
3-thienyl	**6Ha**	69	>99[d]	**6Fa**	>99	>99[d]
hexyl	**7Ha**	97	98 (*S*)	**7Fa**	>99	96 (*S*)
heptyl	**8Ha**	88	>99 (*S*)	**8Fa**	>99	96 (*S*)
octyl	**9Ha**	87	>99 (*S*)	**9Fa**	>99	>99 (*S*)
nonyl	**10Ha**	84	>99 (*S*)	**10Fa**	>99	98 (*S*)

[a] The reaction conditions are described in the Experimental Section. [b] Determined by GC analysis. [c] Higher ee can be obtained when NAD^+ and 2-propanol are used as a coenzyme and a reducing agent as described in the literature.[2a] [d] The absolute configuration is not determined.

Results and Discussion

Stereochemistry of the APG4 Reduction System. When 2,2,2-trifluoroacetophenone (**1Fa**) was subjected to reduction by the APG4 system, the corresponding alcohol, (*S*)-**1Fb**, was obtained in 98% ee, whereas the reduction of acetophenone (**1Ha**), an unfluorinated analogue, afforded (*S*)-**1Hb** in excellent ee as shown in Scheme 2.[3a] Surprisingly, different configurational alcohols were obtained by subjecting the trifluorinated ketone and its unfluorinated analogue to the same reduction system, and the stereoselectivities were excellent for both substrates. The experimental conditions were fixed throughout the study to investigate the stereochemistry of the APG4 system as described in the Experimental Section.

The inversion in stereochemistry between the reduction of methyl and trifluoromethyl ketones was also observed in various substrates as shown in Table 1. When the phenyl group of acetophenone was replaced with a para-substituted phenyl, benzyl, thienyl, or even alkyl group, the inversion was observed. Therefore, the stereochemistry of the APG4 reduction system is determined from the fluorine-substitution pattern of the α-position; the configuration of the product is *A* for the reduction of methyl ketones and *B* for trifluoromethyl ketones.

Next, monofluoroacetophenone (**11a**) and difluoroacetophenone (**12a**) were reduced by the APG4 system. The

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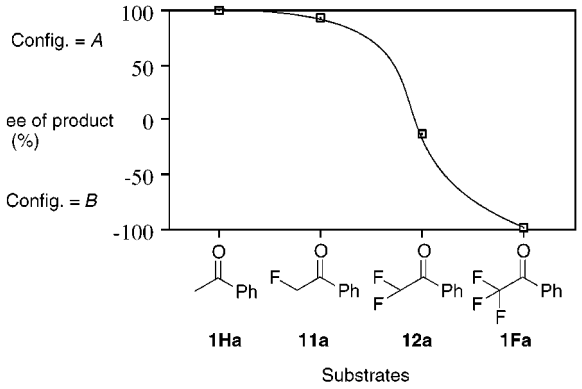
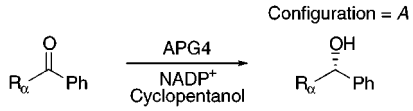


Figure 1. Effect of fluorine at the α-position of acetophenone on the stereoselectivity in the reduction by the APG4 system. The reaction conditions are described in the Experimental Section.

Table 2. Effect of Bulkiness of the Group Adjacent to the Carbonyl Moiety on Stereoselectivity in the Reduction by the APG4 System[a]



substrate	R_α	yield[b] (%)	ee[b,c] (%)
1Ha	Me	90	>99 (*S*)
13a	Et	14	97 (*S*)
14a	*i*-Pro	4	99 (*S*)
15a	*t*-Bu	0	

[a] The reaction conditions are described in the Experimental Section. [b] Determined by GC analysis. [c] Higher ee can be obtained when NAD^+ and 2-propanol are used as a coenzyme and a reducing agent as described in the literature.[2a]

reduction proceeded quantitatively for both substrates. As expected, the stereoselectivity shifted from *A* to *B* according to the number of fluorine substituents at the α-position as shown in Figure 1. The inversion is certainly due to the fluorine substitution.

Because the increase in the number of fluorines at the α-position alters both bulkiness and electronic properties, the effects of both on the stereoselectivity were studied to investigate the reaction mechanism. At first, the effect of bulkiness of the α-position was examined, because in effective radius,[5b,6] a trifluoromethyl moiety (2.2 Å) is larger than a phenyl moiety (1.62 Å) and similar to an isopropyl moiety (2.2 Å) as derived from rotational barriers in biphenyls. In the case of (−)-DIP-chloride reported by Ramachandran et al, the stereochemical inversion caused by the substitution of methyl with trifluoromethyl is also observed on the substitution with *tert*-butyl.[5a,b] Moreover, Corey's report of X-ray crystallographic studies of a set of trihalomethyl ketones indicates that the carbonyl oxygen is displaced significantly toward the trihalomethyl group, which acts more effectively as a larger group on the carbonyl carbon than *tert*-butyl or adamantyl.[5c,d] Therefore, the stereoselectivity of the APG4 reduction of ethyl (**13a**), isopropyl (**14a**), and *tert*-butyl (**15a**) ketones was examined. The results are shown in Table 2. No inversion in stereochemistry was observed in the reduction of hindered ketones by the APG4 system, although the yield of the reduction decreased according to the bulkiness. Therefore, the inversion is not merely caused by the bulkiness of the trifluoromethyl group.

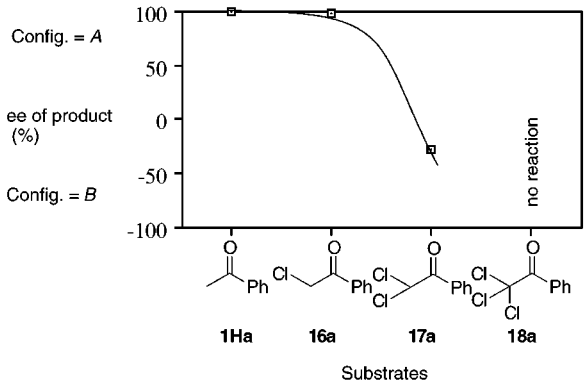


Figure 2. Effect of chlorine at the α-position of acetophenone on the stereoselectivity in the reduction by the APG4 system. The reaction conditions are described in the Experimental Section.

Fluorine is the most electronegative atom, and the electronic effect of the trifluoromethyl group is significant. Accordingly, the electronic effect was examined by substituting an electronegative group at the α-position; the stereoselectivities of the reduction of mono- (**16a**), di- (**17a**), and trichloroacetophenone (**18a**) are shown in Figure 2. A similar trend in the shift of stereoselectivity from *A* to *B* was observed when two chlorine atoms were incorporated. However, the yield of the reduction of chloroacetophenones was much lower than that of corresponding fluoroacetophenones, and trichloroacetophenone (**18a**) was not reduced at all. The reduction of chlorodifluoroacetophenone (**19a**) was also *B* selective, with a quantitative yield. Methyl pentafluorophenyl ketone (**20a**) was reduced under the same conditions ($NADP^+$ and cyclopentanol) with a slight loss of stereoselectivity (97% ee *A*) (>99% ee *A* when NAD^+ and 2-propanol are used); the shift by the electronegative substituent was observed even when it was on the other side of the carbonyl moiety.

It is clear that the substitution of the substrate with electronegative atoms such as fluorine and chlorine inverts the stereochemistry and that this is not because of the bulkiness of the atoms, but it is not clear why the stereochemistry is inverted by an electronic factor. To examine whether the inversions are occurring in the reactions catalyzed by only one enzyme, a cell-free extract prepared from APG4 was charged on an anion-exchange column and enzymes in APG4 were separated. As shown in Figure 3, when methyl ketone, **1Ha**, was used as a substrate, several enzymes were found. The first enzyme, eluted at around fraction 29, afforded the *A* alcohol, whereas the second enzyme, eluted around fraction 36, afforded the *B* alcohol. The stereoselectivity in the reduction of methyl ketones by the APG4 system is very high and *A* selective, even though there are many enzymes with different stereoselectivities present in APG4. This is because the reduced form of the coenzyme (NADPH) is available to only the first eluted enzyme, the *A* enzyme, which can reduce the oxidized form of coenzyme ($NADP^+$) by itself using cyclopentanol effectively, whereas the second eluted enzyme, the *B* enzyme, cannot.[7] However, when trifluoromethyl ketones were used as a substrate, the activity of the *A* enzyme was negligible compared with that of the *B* enzyme. There-

(6) Bott, G.; Field, L. D.; Sternhell, S. *J. Am. Chem. Soc.* **1980**, *102*, 5618.

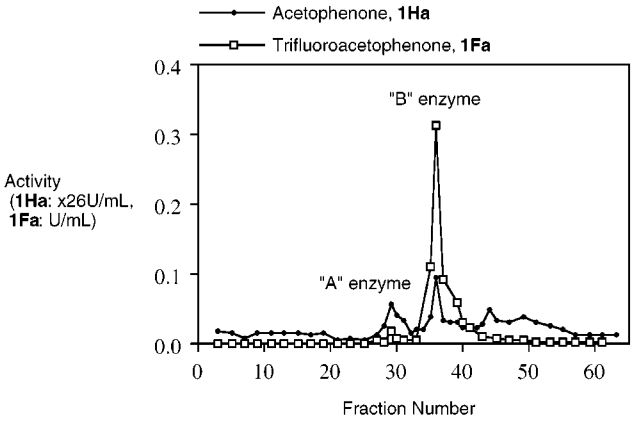


Figure 3. Separation of enzymes in APG4 by anion-exchange column chromatography.

fore, trifluoromethyl ketones are reduced only by the *B* enzyme, and hence the high *B* selectivity. After all, methyl ketones and trifluoromethyl ketones are reduced by enzymes with opposite stereoselectivities. Fluorine substitution is not really affecting the stereospecificity of a single enzyme; rather, the effect is due to the presence of multiple enzymes.

The *B* enzyme reduces the trifluoromethyl ketone about 100 times faster than the methyl ketone (0.0037 U/mL for **1Ha** and 0.31 U/mL for **1Fa**), perhaps as a result of the difference in electrophilicity of the carbonyl carbon rather than the ease of formation of the substrate-enzyme complex. The search for a natural substrate of the *B* enzyme is underway in our laboratories. Substrates with fluorine are rare in nature and should shed light on how the fluorine is recognized by the enzyme.

Synthesis of Chiral Halogenated Alcohols. The synthesis of chiral alcohols on a preparative and gram scale is important from a practical point of view. The APG4 system and the *B* enzyme were used for the synthesis. For the former, the experimental conditions were examined, because both NAD^+ and $NADP^+$ can be used as a coenzyme and 2-alkanols from 2-propanol to 2-octanol and cyclopentanol can serve as a reducing agent. The best conditions were chosen and used for the synthesis; the results are listed in Table 3. When the *B* enzyme was used for the reduction, the coenzyme was recycled by glucose-6-phosphate and glucose-6-phosphate dehydrogenase, a commonly used recycling system.

Trifluoromethyl aryl ketones (**1Fa**−**6Fa**) are reduced smoothly and with excellent ee by the APG4 system to the corresponding alcohols, which can be valuable starting materials. For example, the functionality of alcohol and bromine in (*S*)-**3Fb** can be converted, and the thienyl group in (*R*)-**5Fb** and (+)-**6Fb** can be used for various reactions[3b,8] such as the reduction to aliphatic alcohols, substitution, and even polymerization. Its application to electronic materials such as a conducting polymer etc. would be interesting.

Monofluoroacetophenone (**11a**) and difluoroacetophenone (**12a**) are reduced to (*R*)-alcohols (*A*) by the APG4 system when NAD^+ and 2-propanol are used as the coenzyme and reducing agent, respectively, and to (*S*)-alcohols (*B*) by the *B* enzyme. Both enantiomers of monofluorophenylethanol (**11b**) can be obtained with excellent ee, although it is usually difficult to do so using only one kind of microbe.

Both methods can be easily scaled up because the stereoselectivity of the reduction does not depend on the scale and the workup procedure is very simple compared to the whole cell system.

Conclusions

The stereochemical outcome of the reduction of ketones by the APG4 system depends on the number of halogens at the α-position regardless of the other group adjacent to the carbonyl moiety. The reduction of methyl ketones showed *A*-selectivity, whereas that of trifluoromethyl ketones showed *B*-selectivity. The inversion in stereochemistry is caused not merely by the bulkiness of the trifluoromethyl group but by the electronic factor imparted by the halogens.

The mechanism for the inversion in stereochemistry by the electronegative group at the α-position was investigated by crude enzymatic studies. It was found that there are several enzymes with different stereoselectivities and that even under the exact same conditions, one of the enzymes catalyzes the reduction of methyl ketones, whereas another with the opposite stereoselectivity catalyzes the reduction of trifluoromethyl ketones as a result of the difference in the activities of the enzymes toward the different kinds of ketones. Different enzymes with different substrate specificities display different stereospecificities in ketone reduction. In other words, the *B* enzyme reduces fluorinated ketones much more efficiently than the unfluorinated analogues. The presence of several enzymes catalyzing the same reaction with different substrate specificity may be the origin of stereochemical results obtained with the whole organism. An investigation of how the halogenated substrates are recognized and bind in the active site of the enzyme would be both interesting and important, because the binding of some aromatic pollutants such as dioxins to the receptors depends on the chlorine-substitution pattern.[9]

The reduction of fluorinated ketones by the isolated enzyme, as well as by APG4, results in the synthesis of chiral alcohols with excellent ee, and both enantiomers of monofluorinated alcohols were obtained. Both the APG4 and the *B* enzyme systems are very convenient for the synthesis of optically pure fluorinated alcohols.

Experimental Section

Instruments. Gas chromatographic analyses were performed using 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)). HPLC analyses were performed using Chiralcel OD (0.46 cm ϕ × 25 cm (OD)).

Preparation of Ketones. 2,2,2-Trifluoro-1-(3-thienyl)-ethanone (6Fa). *n*-BuLi (11 mmol, 1.54 M in hexane solution) was added dropwise to a stirring solution of 3-bromothiophene

(7) Unpublished result.

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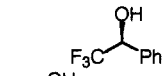
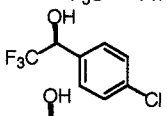
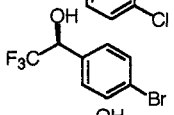
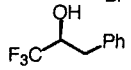
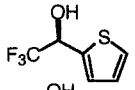
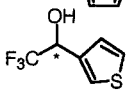
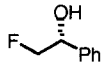
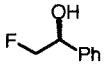
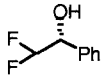
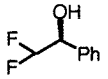
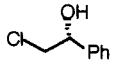
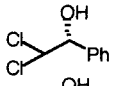
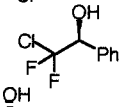
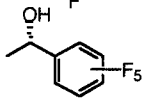
Table 3. Synthesis of Chiral Halogenated Alcohols

Product		Catalyst[a]	Coenzyme	Coenzyme Recycle[b]	Yield(%)	Ee[c](%)
	(*S*)-**1Fb**	APG4	$NADP^+$	Cyclopentanol	84	98[d]
	(*S*)-**2Fb**	APG4	$NADP^+$	Cyclopentanol	81	>99
	(*S*)-**3Fb**	APG4	$NADP^+$	Cyclopentanol	80	>99
	(*S*)-**4Fb**	APG4	$NADP^+$	Cyclopentanol	74	98
	(*R*)-**5Fb**[e]	APG4	NAD^+	Cyclopentanol	84	>99
	(+)-**6Fb**	APG4	NAD^+	Cyclopentanol	87	>99
	(*R*)-**11b**	APG4	NAD^+	2-Propanol	93	>99
	(*S*)-**11b**	B-enz	NADPH	G-6-P	91	>99
	(*R*)-**12b**	APG4	NAD^+	2-Propanol	99	63
	(*S*)-**12b**	B-enz	NADPH	G-6-P	95	>99
	(*R*)-**16b**[e]	APG4	NAD^+	2-Propanol	49	98
	(*R*)-**17b**	APG4	NAD^+	2-Propanol	14	38
	(*S*)-**19b**	APG4	$NADP^+$	Cyclopentanol	82	94
	(*S*)-**20b**[e]	APG4	NAD^+	2-propanol	80	>99

[a] APG4, dried cell of *G. candidum* IFO 4597; B-enz, the *B* enzyme separated by anion-exchange chromatography. The reaction conditions are described in the Experimental Section. [b] The indicated alcohol or glucose-6-phosphate dehydrogenase and glucose-6-phosphate (G-6-P) are added to recycle the coenzyme. [c] Determined by GC analysis or HPLC analysis. [d] ee can be improved to 99% by using a half amount of APG4 and a triple amount of cyclopentanol. [e] The result was taken from refs 2a and 3b.

(10 mmol, 1.63 g) in ether (5 mL) at −18 °C. The resulting mixture was added dropwise to a solution of ethyl 2,2,2-trifluoroacetate (10 mmol, 1.42 g) in ether (5 mL) at −70 °C, stirred for 10 min at room temperature, quenched with NH_4Cl (aqueous), extracted with ether, dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by distillation with a Kugelrohr apparatus (120 °C/16 mmHg), giving **6Fa** (1.12 g, 62%): ^1H NMR ($CDCl_3$) δ 7.40−7.45 (m, 1H, thienyl), 7.66−7.68 (m, 1H, thienyl), 8.35−8.38 (m, 1H, thienyl); ^{19}F NMR ($CDCl_3$−$CFCl_3$) δ −74 (s); IR (neat) 1711 cm^{-1}; HRMS for $(C_6H_3OF_3S)^+$ calcd 179.9857, found 179.9839.

1,1,1-Trifluoro-2-alkanones 7Fa−10Fa were prepared via the Grignard reaction.[10]

α-Fluoroacetophenone (11a). A mixture of α-bromoacetophenone (5 mmol, 1.0 g), 18-crown-6 (0.038 mmol, 10 mg), and anhydrous potassium fluoride (40 mmol, 2.32 g) in acetonitrile (10 mL) was heated under reflux for 24 h, cooled to room temperature, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent, hexane/ethyl acetate 5:1) followed by distillation with a Kugelrohr apparatus (170 °C/21 mmHg), giving **11a** (287 mg, 42%): ^1H NMR ($CDCl_3$) δ 5.53 (d, 2H, CH_2, J = 47 Hz), 7.46−7.63 (m, 3H, Ph), 7.87−7.91 (m, 2H, Ph); ^{19}F NMR ($CDCl_3$−$CFCl_3$) δ −231 (t, J = 47 Hz); IR (neat) 1707 cm^{-1}. Anal. Calcd for C_8H_7OF: C, 69.56; H, 5.11. Found: C, 69.33; H, 5.03.

α,α-Difluoroacetophenone (12a). Compound **12a** was prepared via the Grignard reaction:[5b] ^1H NMR ($CDCl_3$) δ 6.30 (t, 1H, J = 54 Hz), 7.48−7.57 (m, 2H, Ph), 7.64−7.68 (m, 1H, Ph), 8.05−8.09 (m, 2H, Ph); ^{19}F NMR ($CDCl_3$−$CFCl_3$) δ −122 (d, J = 53 Hz); IR (neat) 1711 cm^{-1}. Anal. Calcd for $C_8H_6OF_2$: C, 61.54; H, 3.87. Found: C, 61.20; H, 3.82.

α,α,α-Trichloroacetophenone (18a). Compound **18a** was prepared via Friedel−Crafts reaction with $AlCl_3$ (18 mmol, 2.4

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g), trichloroacetyl chloride (19 mmol, 3.4 g), and benzene (32 mL) in 96% yield: ^{1}H NMR ($CDCl_3$) δ 7.46−7.54 (m, 2H, Ph), 7.60−7.68 (m, 1H, Ph), 8.23−8.28 (m, 2H, Ph); IR (neat) 1713 cm^{-1}.

Enzyme Preparations and Separation of the Enzymes by Anion-Exchange Column Chromatography. APG4 (acetone powder of *Geotrichum candidum* IFO 4597, a microbial dried-cell preparation dehydrated using acetone) prepared as described previously,[2a] was used for the APG4 reductions. For the separation of the enzymes, APG4 (5.0 g) was homogenized with HEPES buffer (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid−NaOH, pH 7.2, 0.1 M, 50 mL), ME (2-mercaptoethanol, 0.04%), DTT (dithiothreitol, 1 mM), and PMSF (α-toluenesulfonyl fluoride, 1 mM) and sonically disintegrated. The cell-free extract (40 mL) obtained by centrifugation (30 000 × g, 2 °C, 30 min) was added to PMSF (1 mM) and DFP (diisopropyl fluorophosphate 1 mM) and dialyzed against HEPES (pH 7.2, 10 mM, 750 mL × 2) containing ME (0.04%) and DTT (1 mM). The supernatant (53 mL) obtained by centrifugation (30 000 × g, 2 °C, 30 min) and filtration through Sterivex-GV (0.22 μm, Millipore) was applied to an anion-exchange column (FPLC system, Pharmacia MonoQ-HR 10/10 column) and eluted with the above buffer and a linear KCl gradient of 0 M for 15 min, 0.0075 M/min for 40 min, and 0.07 M/min for 10 min. The injection volume was 5 mL, the flow rate was 2 mL/min, and the fraction size was 2 mL. The enzyme activity was assayed at 30 °C by measuring the change in absorbance at 340 nm due to the oxidation of NADPH at a substrate concentration of 2.5 mM in Tris-HCl buffer (pH 7.0, 0.1 M). One unit of enzyme oxidizes 1 μmol NADPH to $NADP^+$/min. The *B* enzyme eluted around fraction number 36 was collected and used for the preparative-scale synthesis.

Effect of Substituents at the α-Position of Acetophenone on the Enantioselectivity of the APG4 Reduction. A ketone (0.08 mmol), $NADP^+$ (0.007 mmol), and cyclopentanol (0.65 mmol) were added to a suspension of APG4 (20 mg) in MES (2-(*N*-morpholino)ethanesulfonic acid) buffer (pH 7.0, 0.1 M, 3 mL). The mixture was shaken at 130 rpm and 30 °C for 20 h, and the resulting mixture was put on Extrelut and eluted with ether. The chemical yield and ee of the product were determined by GC, HPLC, or NMR analysis of the ether extract.

General Procedure for the Reduction of Ketones by APG4 on a Preparative Scale. Fluorinated and/or chlorinated ketones were reduced using NAD^+ or $NADP^+$ and 2-propanol or cyclopentanol for 20 h as described previously.[2a]

(*S*)-2,2,2-Trifluoro-1-phenylethanol ((*S*)-1Fb). Compound **1Fa** (205 mg, 1.18 mmol) was converted to (*S*)-**1Fb** (175 mg) by APG4. GC conditions: DEX 130 °C. *S*: 9.6 min, *R*: 10.2 min. $[\alpha]^{24}_D$ +30.4 (*c* 1.56, $CHCl_3$); +25.1 (*c* 0.81, CCl_4) (lit.[11] $[\alpha]^{20}_D$ −25.1(*c* 3, CCl_4) >99% ee (*R*)); ^{1}H NMR (CDC l$_3$) δ 2.63 (d, 1H, OH, *J* = 4.6 Hz), 5.02 (dq, 1H, CH, *J*(d) = 4.5 Hz, *J*(q) = 6.8 Hz) and 7.37−7.51 (m, 5H, Ph); ^{19}F NMR ($CDCl_3$−$CFCl_3$) δ −79 (d, *J* = 6.6 Hz); IR (neat) 1128, 1173, 3407 cm^{-1}. Anal. Calcd for $C_8H_7OF_3$: C, 54.55; H, 4.01. Found: C, 54.24; H, 4.09.

(*S*)-2,2,2-Trifluoro-1-(*p*-chlorophenyl)ethanol ((*S*)-2Fb). Compound **2Fa** (429 mg, 2.06 mmol) was converted to (*S*)-**2Fb** (350 mg) by APG4. GC conditions: CPCD 140 °C. *S*: 13.7 min, *R*: 14.5 min. $[\alpha]^{24}_D$ +33.1 (*c* 0.914, EtOH) (lit.[12] $[\alpha]^{20}_D$ −19.0 (*c* 1.05, EtOH) 82.5% ee (*R*)); ^{1}H NMR ($CDCl_3$) δ 2.88 (s, 1H, OH), 5.00 (q, 1H, CH, *J* = 6.5 Hz), 7.35−7.45 (m, 4H, Ph); ^{19}F NMR ($CDCl_3$−$CFCl_3$) δ −79 (d, *J* = 6.4 Hz); IR (KBr) 1128, 1179, 3382 cm^{-1}. Anal. Calcd for $C_8H_6OClF_3$: C, 45.63; H, 2.87. Found: C, 45.81; H, 2.99.

(*S*)-2,2,2-Trifluoro-1-(*p*-bromophenyl)ethanol ((*S*)-3Fb). Compound **3Fa** (504 mg, 1.99 mmol) was converted to (*S*)-**3Fb** (406 mg) by APG4. GC conditions: CPCD 150 °C. *S*: 13.9 min, *R*: 14.8 min. $[\alpha]^{24}_D$ +30.25 (*c* 0.862, EtOH) (lit.[12] $[\alpha]^{20}_D$ −21.5 (*c* 1.04, EtOH) 82.2% ee (*R*)); ^{1}H NMR ($CDCl_3$) δ 2.73 (s, 1H, OH), 4.99 (q, 1H, CH, *J* = 6.5), 7.33−7.37 (m, 2H, Ph), 7.51−7.58 (m, 2H, Ph); ^{19}F NMR ($CDCl_3$−$CFCl_3$) δ −79 (d, *J* = 6.2 Hz); IR (Kbr) 1127, 1181, 3366 cm^{-1}. Anal. Calcd for $C_8H_6OBrF_3$: C, 37.68; H, 2.37. Found: C, 37.68; H, 2.32.

(*S*)-1,1,1-Trifluoro-3-phenyl-2-propanol ((*S*)-4Fb). Compound **4Fa** (412 mg, 2.19 mmol) was converted to (*S*)-**4Fb** (307 mg) by APG4. GC conditions: CPCD 140 °C. *R*: 6.5 min, *S*: 7.0 min. $[\alpha]^{25}_D$ −45.5 (*c* 0.95, $CHCl_3$) (lit.[13] $[\alpha]^{23}_D$ −28.2 (*c* 0.88, $CHCl_3$) 98% ee (*S*)); ^{1}H NMR ($CDCl_3$) δ 2.15 (d, 1H, OH, *J* = 5.4 Hz), 2.84 (q, 1H, CH_2, *J* = 10.0, 14.2 Hz), 3.06 (q, 1H, CH_2, *J* = 3.0, 14.2 Hz), 4.05−4.24 (m, 1H, CH), 7.22−7.40 (m, 5H, Ph); ^{19}F NMR ($CDCl_3$−$CFCl_3$) δ −80 (d, *J* = 6.4 Hz); IR (neat) 1130, 1169, 3432 cm^{-1}. Anal. Calcd for $C_9H_9OF_3$: C, 56.48; H, 4.77. Found: C, 56.55; H, 4.80.

(+)-2,2,2-Trifluoro-1-(3-thienyl)ethanol ((+)-6Fb). Compound **6Fa** (984 mg, 5.46 mmol) was converted to (+)-**6Fb** (862 mg) by APG4. GC conditions: DEX 140 °C. (+): 6.4 min, (−): 7.0 min. $[\alpha]^{26}_D$ +31.6 (*c* 0.46, MeOH); ^{1}H NMR ($CDCl_3$) δ 2.60 (d, 1H, OH, *J* = 4.8 Hz), 5.07−5.19 (m, 1H, CH), 7.17−7.19 (m, 1H), 7.35−7.39 (m, 1H), 7.45−7.47 (m, 1H) cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 69.4 (q, CH, *J* = 33.1 Hz), 124.1 (q, CF_3, *J* = 281 Hz), 124.8, 126.1, 126.6, 134.9; ^{19}F NMR ($CDCl_3$−$CFCl_3$) δ −79 (d, *J* = 6.2 Hz); IR (neat) 1127, 1167, 3395 cm^{-1}; HRMS for $(C_6H_5OF_3S)^+$ calcd 182.0013, found 182.0023.

(*R*)-2-Fluoro-1-phenylethanol ((*R*)-11b). Compound **11a** (200 mg, 1.45 mmol) was converted to (*R*)-**11b** (189 mg) by APG4. GC conditions: DEX 130 °C. *S*: 8.8 min, *R*: 9.6 min. $[\alpha]^{24}_D$ −52.3 (*c* 1.08, MeOH) (lit.[5b] $[\alpha]^{23}_D$ −76.2 (*c* 3, MeOH) 95.4% ee (*R*)); ^{1}H NMR ($CDCl_3$) δ 2.65 (d, 1H, OH, *J* = 2.2 Hz), 4.42 (octet, 1H, CH_2, *J* = 8.1, 9.5, 49 Hz), 4.50 (octet, 1H, CH_2, *J* = 3.4, 9.5, 47 Hz), 5.00 (m, 1H, CH), 7.37 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$) δ 73.0 (d, CH, *J* = 19.8 Hz), 87.2 (d, CH_2F, *J* = 174 Hz), 126.3 (Ph), 128.4 (Ph), 128.6 (Ph), 138.1 (d, Ph, *J* = 8.3 Hz); ^{19}F NMR ($CDCl_3$−$CFCl_3$) δ −221 (dt, *J* = 14.1, 48 Hz); IR (neat) 1011, 3397 cm^{-1}. Anal. Calcd for C_8H_9OF: C, 68.56; H, 6.47. Found: C, 68.56; H, 6.64.

(*R*)-2,2-Difluoro-1-phenylethanol ((*R*)-12b). Compound **12a** (240 mg, 1.54 mmol) was converted to (*R*)-**12b** (240 mg) by APG4. GC conditions: DEX 140 °C. *S*: 6.7 min, *R*: 7.2 min. $[\alpha]^{24}_D$ −11.5 (*c* 1.00, CH_2Cl_2) (lit.[5b] $[\alpha]^{23}_D$ −14.27 (*c* 3, CH_2Cl_2) 84.66% ee (*R*)); ^{1}H NMR ($CDCl_3$) δ 2.62 (d, 1H, OH, *J* = 3.2 Hz), 4.81 (m, 1H, CH), 5.76 (dt, 1H, CHF_2, *J*(d) = 4.8 Hz, *J*(t) = 56 Hz), 7.41 (m, 5H, Ph);^{13}C NMR ($CDCl_3$) δ 73.6 (t, CHOH, *J* = 24.5 Hz), 115.8 (t, CHF_2, *J* = 245 Hz), 127.1 (Ph), 128.7 (Ph), 129.0 (Ph), 135.8 (t, Ph, *J* = 3.4 Hz); ^{19}F NMR ($CDCl_3$−$CFCl_3$) δ −128.1 (dd, *J* = 4.2, 56 Hz), −128.0 (dd, *J* = 3.7, 56 Hz); IR (neat) 1071, 3410 cm^{-1}. Anal. Calcd for $C_8H_8OF_2$: C, 60.76; H, 5.10. Found: C, 60.67; H, 5.27.

(*R*)-2,2-Dichloro-1-phenylethanol ((*R*)-17b). Compound **17a** (461 mg, 2.44 mmol) was converted to (*R*)-**17b** (63.1 mg) by APG4. GC conditions: CPCD 150 °C. *S*: 19.8 min, *R*: 20.9 min. $[\alpha]^{21}_D$ −11.8 (*c* 1.26, CH_2Cl_2) (lit.[14] $[\alpha]^{22}_D$ −15.0 (*c* 3, CH_2Cl_2) 45.7% ee (*R*)); ^{1}H NMR ($CDCl_3$) δ 2.87 (d, 1H, OH, *J* = 4.0 Hz), 4.98 (dd, 1H, CH, *J* = 3.8, 5.4 Hz), 5.82 (d, 1H, $CHCl_2$, *J* = 5.4 Hz), 7.36−7.44 (m, 5H, Ph); IR (KBr) 3362 cm^{-1}. Anal. Calcd for $C_8H_8OCl_2$: C, 50.29; H, 4.22. Found: C, 50.26; H, 4.23.

(*S*)-2-Chloro-2,2-difluoro-1-phenylethanol ((*S*)-19b). Compound **19a** (390 mg, 2.05 mmol) was converted to (*S*)-**19b** (322 mg) by APG4. GC conditions: G-TA 125 °C. *R*: 11.4 min, *S*: 11.7 min; $[\alpha]^{25}_D$ +22.2 (*c* 1.90, $CHCl_3$) (lit.[15] $[\alpha]^{21}_D$ −13.82 (*c* 1.01, $CHCl_3$) 73% ee (*R*)); ^{1}H NMR ($CDCl_3$) δ 2.91 (d, 1H, OH, *J* = 4.6 Hz), 5.06 (dq, 1H, CH, *J* = 4.4, 7.5 Hz), 7.39−7.50 (m, 5H, Ph); ^{19}F NMR ($CDCl_3$−$CFCl_3$) δ −65 (dd, *J* = 8.8, 165 Hz), −63 (dd, *J* = 7.2, 165 Hz); IR (neat) 3420 cm^{-1}. Anal. Calcd for $C_8H_7OClF_2$: C, 49.90; H, 3.66. Found: C, 49.60; H, 3.68.

General Procedure for the Reduction of Ketones by the *B* Enzyme in a Preparative Scale. A ketone (0.80

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mmol), NADPH (13 mg), the *B* enzyme (10 units), D-glucose-6-phosphate disodium salt hydrate (0.88 mmol), and D-glucose-6-phosphate dehydrogenase (25 units) were added to 50 mL of HEPES buffer (0.1 M, pH 7.0). The mixture was stirred at 30 °C for 20 h at 130 rpm and then extracted with ether. The combined ether solution was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by distillation with a Kugelrohr apparatus.

(*S*)-2-Fluoro-1-phenylethanol ((*S*)-11b). Compound **11a** (119 mg, 0.862 mmol) was converted to (*S*)-**11b** (110 mg); $[\alpha]^{23}_D$ +52.5 (*c* 0.94, MeOH). The NMR and IR spectra are in accord with those for (*R*)-**11b**. Found: C, 68.50; H, 6.57.

(*S*)-2,2-Difluoro-1-phenylethanol ((*S*)-12b). Compound **12a** (124 mg, 0.795 mmol) was converted to (*S*)-**12b** (119 mg); $[\alpha]^{25}_D$ +19.4 (*c* 0.96, CH_2Cl_2). The NMR and IR spectra are in accord with those for (*R*)-**12b.** Found: C, 60.94; H, 5.16.

Acknowledgment. This work was supported by Nagase Science and Technology Foundation, Ryukoku Science and Technology Fund, and a Grant-in-Aid for Scientific Research from the Ministry of Education, Science Sports and Culture of Japan.

JO991283K