Stereochemical Control in Microbial Reduction. 30. Reduction of Alkyl 2-Oxo-4-phenylbutyrate as Precursors of Angiotensin Converting Enzyme (ACE) Inhibitors

Duc Hai Dao,† Yasushi Kawai, Kouichi Hida, Sander Hornes, Kaoru Nakamura, Atsuyoshi Ohno,* Mutsuo Okamura,† and Takeshi Akasaka†

Institute for Chemical Research, Kyoto University, Uji, Kyoto 611

†Course of Fundamental Studies and Energy Technology, Graduate School of Science and Technology, Niigata University, Ikarashi, Niigata 950-21

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Alkyl 2-oxo-4-phenylbutyrates are reduced to the corresponding alkyl (*R*)-2-hydroxy-4-phenylbutyrates, versatile chiral building blocks in organic synthesis, in high chemical yield (80—90%) with excellent stereoselectivity (> 90%ee). The reaction has been run in aqueous diethyl ether at 30 °C for 24 h under the catalysis of bakers' yeast (*Saccharomyces cerevisiae*) which was preincubated for 6 h in the presence of phenacyl chloride. The amount of water in the medium should be controlled strictly not to exceed 0.8 mL (g yeast)$^{-1}$.

In recent decades, there has been a push for all drugs to be enantiomerically pure. By far, the easier option is now the asymmetric production of drugs and other bioactive compounds from chiral starting reagents, which, in turn, are made from prochiral precursors stereoselectively by either chemical or biological methods. The latter has won recognition and their usefulness in organic synthesis has been well documented.[2—4] α-Hydroxy esters are useful chiral reagents and have been used widely as building blocks because of their ease of transformation into other functional groups. One important chiral reagent is an ester of (*R*)-2-hydroxy-4-phenylbutanoic acid, a versatile synthon for the synthesis of a variety of angiotensin converting enzyme (ACE) inhibitors.[6—8]

Because of the high demand of (*R*)-2-hydroxy-4-phenylbutyrates by the pharmaceutical industry for the preparation of ACE inhibitors, much investigation has been devoted to its production with the common aim of obtaining high chemical yield and optical purity, as well as economical acceptability, by either of the aforementioned methods from prochiral precursors : alkyl 2-oxo-4-phenylbutyrates. The results reported so far have been impressive in terms of chemical yield (39—92%) and enantiomeric excess (ee; 80—99%) for the biotechnological methods,[9—11] whereas chemical yields of 70—90% with optical purity of 50—82% have been reported on the basis of chemical methods.[12,13] Few reports, however, have mentioned the use of bakers' yeast (*Saccharomyces cerevisiae*) for the reduction of 2-oxo-4-phenylbutanoic acid. Some of these show unsatisfactory results,[14] probably due to poor solubility and stability, and catalytic decomposition of the starting material and product in aqueous environment.

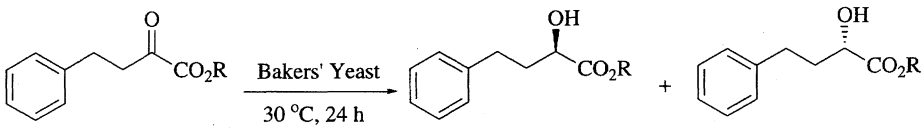
Although bakers' yeast has long been recognized as a valuable reagent in asymmetric organic synthesis,[15—17] the results have not necessarily been satisfactory with respect to chemical yield and stereoselectivity. Thus, we have made efforts for the improvement of both chemical yield and stereoselectivity in the reduction of various α- and β-keto esters mediated by bakers' yeast by selecting reaction conditions such as medium, temperature, addition of a third reagent, and so on.[5,18—20]

We now wish to exploit our potential technique in the reduction of alkyl 2-oxo-4-phenylbutyrates (**1**) to the useful carbinolic intermediate, alkyl 2-hydroxy-4-phenyl-butyrates (**2**) (Scheme 1). Especially the use of nonaqueous organic solvent systems may protect the reagents from undesired decomposition.

This particular reaction with excellent chemical yield (90%) and ee (> 99% for the (*R*)-isomer) under the catalysis of *Daucus carota* cells has already been reported.[11] However, because the biocatalyst employed therein is not one so familiar to organic chemists as bakers' yeast and the reaction requires a very long time of 50 h, we believe that the reaction is still worth being improved.

Organic solvents have superiority in many ways over aqueous solvents : namely, the former enhances the solubility of an organic substrate and prevents the product(s) from being hydrolyzed. Sometimes, therefore, enzymes are employed as a catalyst for the reaction in an organic solvent. Lipases are the most popular among the enzymes that have been used as catalysts in organic solvents.[21]

On the other hand, little has been reported on the use of a microbe in an organic solvent until we first reported the catalysis of bakers' yeast in benzene.[22,23] It would be inconceivable to assume, however, that all organic solvents are



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

a : R = H
b : R = CH_3
c : R = C_2H_5
d : R = C_3H_7
e : R = $CH(CH_3)_2$
f : R = C_4H_9
g : R = $CH_2CH(CH_3)_2$
h : R = $C(CH_3)_3$
i : R = C_5H_{11}
j : R = $CH(CH_2CH_3)_2$
k : R = $CH_2C(CH_3)_3$
m : R = $(CH_2)_2CH(CH_3)_2$
n : R = cyclo-C_5H_9
o : R = cyclo-C_6H_{11}
p : R = C_6H_5
q : R = $CH_2C_6H_5$

Scheme 1.

as "friendly" to many biocatalysts as aqueous environments. Therefore, it is imperative that the choice of organic solvent should be made cautiously if high catalytic activity, and optical purity are the primary objectives. This is reflected heavily on the basic physicochemical characteristics of the solvent such as dipole moment, dielectric constant and hydrophobicity, defined by log *P*.[24—26] Thus, it is necessary to survey a suitable solvent for a particular microbial reaction designed. In the present study, we employed bakers' yeast as a biocatalyst, because it is the most popular microbe for non-microbiologists and it is easy to handle.

Results and Discussion

Effect of Medium. In our previous paper it was reported that the reduction of an α-keto ester in an organic solvent proceeds only when the medium contains a small amount of water.[23] Thus, a microbe remains functional only when it is surrounded by a water layer (or layers) to prevent its cell membrane from contacting with the organic layer directly and to keep its environment suitable for biological activity. It is well documented that some enzymes, namely hydrolytic enzymes, are useful in nonaqueous solvents.[22,27] Therefore, the effect of water content in an organic solvent was studied initially using ethyl 2-oxo-4-phenylbutyrate (**1c**) as the substrate. The results are summarized in Table 1.

In general, no satisfactory results were obtained with respect to both chemical yield and stereoselectivity from the reduction in these organic solvents. Although benzene affords relatively good result in stereoselectivity, the reaction is retarded largely in this solvent. Amongst the many organic solvents tested, certain ethers, particularly diethy ether and 2,2,5,5-tetramethyltetrahydrofuran, are the most promising, whilst tetrahydrofuran, 1,4-dioxane and 1,2-dimethoxyethane do not favour biocatalystic activity at all. Similarly, hexane is much worse a solvent than cyclohexane in terms of stereoselectivity. The behavior of carbon tetrachloride is completely different from that of dichloromethane. Thus, it is obvious that the property of a solvent cannot simply be classified into polar and nonpolar or hydrophobic and hydrophilic. It is proposed that the hydrophobicity of a solvent is one of the key effects in certain enzymatic reactions.[28—31] Present observations suggest that the effect of a solvent appears differently on the activity of an enzyme, which is a molecule, from that on the activity of a microbe, which is a bag packed with many enzymes. As a result, the solvent effect on the reaction with a microbe is much more complicated

Table 1. Effect of Water-Content on the Microbial Reduction of Ethyl 2-Oxo-4-phenylbutyrate in Organic Solvent

Solvent	Amount of water, mL (g yeast)$^{-1}$ (Chem. yield, %/ee%[a])			
	0.5	0.6	0.7	0.8
Benzene	13/66	15/67	14/70	18/72
Cyclohexane	57/32	62/29	64/30	57/30
Hexane	92/ 4.5	94/ 4	94/ 4	—
CH_2Cl_2	0/ —	0/ —	0/ —	0/ —
CCl_4	58/49	43/54	31/61	25/55
Ethyl acetate	42/49	45/60	34/69	25/73
Diethyl ether	98/53	97/62	86/67	60/67
1,2-Dimethoxyethane	0/ —	0/ —	0/ —	0/ —
1,4-Dioxane	0/ —	0/ —	0/ —	0/ —
Tetrahydrofuran	0/ —	0/ —	0/ —	0/ —
2-Methyltetrahydrofuran	20/43	16/48	12/50	9/51
2,2,5,5-Tetramethyltetrahydrofuran	98/54	97/59	98/67	97/69

a) The product has the (*R*)-configuration.

than that with a single enzyme.

Water content in the solvent does not affect the result significantly when it is kept within 0.5—0.8 mL (g yeast)$^{-1}$. However, when the content exceeds 0.8 mL (g yeast)$^{-1}$, the chemical yield drops suddenly without changing the stereoselectivity very much; the water content of 0.85 mL (g-yeast)$^{-1}$ in diethyl ether, for example, results in a chemical yield of 45% with 67%ee. Since it was observed that the use of various dialkyl ethers as solvents did not cause an appreciable variation in the result, we therefore employed diethyl ether as a typical favorite organic solvent for further reactions.

Effect of Substrate Concentration. In many cases, the stereochemical results obtained from microbial reactions are affected by the substrate concentration.[32—34] The variation in stereoselectivity stems from the fact that each enzyme in a microbe which is responsible for the reaction of interest has its own Michaelis constant, K_m, for a particular substrate, and a change in substrate concentration results in a change in apparent activity of each enzyme.[34] Therefore, we tested the effect of substrate concentration on chemical yield and stereoselectivity. The results are listed in Table 2.

It is clearly shown that an increase in the concentration of the substrate more than 5.8×10^{-3} M (1 M = 1 mol dm^{-3}) results in a noticeable reduction in both chemical yield and stereoselectivity. Thus, we kept the concentration of the substrate at about 1.2 g/L Et_2O or 5.8×10^{-3} M throughout the investigation.

Table 2. Effect of the Concentration of Ethyl 2-Oxo-4-phenylbutyrate

[**1**], mg (mM)	Chemical yield/%	ee[a]/%
5 (2.42)	93	60
10 (4.85)	97	62
15 (7.27)	79	46
20 (9.70)	78	47
25 (12.12)	81	43
30 (14.55)	71	44

a) The product has the (*R*)-configuration.

Variation in Alkoxy Group of the Substrate. Although it has been elucidated that both the reaction medium and the substrate concentration affect the stereoselectivity of the enzymatic reaction, enantiomeric excess so far observed in the product is far less than satisfactory. In other words, the changes in the solvent and substrate concentration are not efficient devices to discriminate one particular enzyme from the others. In order to study substrate specificity of the microbe reduction, we next subjected **1** with various alkoxy groups to the reaction under various water-contents. The results are summarized in Table 3.

It is interesting to note that the substrates that have a side-chain at the α-carbon of the R in the alkoxy group afford the (*S*)-enantiomer, whereas all others afford the (*R*)-isomer. Surprisingly, the optical purity of the products are reasonably high (70—80 %ee) for these (*S*)-isomers.

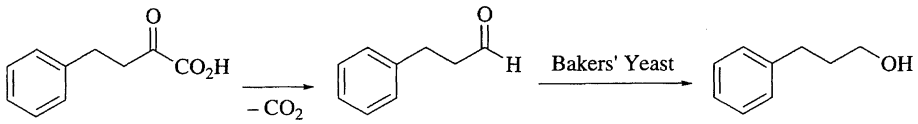
In concurrence with the fact that *t*-butyl 2-oxo-4-phenylbutyrate (**1h**) has difficulty to be a substrate, the cavity for accommodating the alkoxy group in the pocket of the enzyme responsible for the reduction seems to have a narrow channel in the vicinity of the alkoxy oxygen. The cavity should not be assigned to be small because the substrates with rather bulky R such as **1j**, **1k**, **1m**, and **1q** are recognized by the enzyme and are enzymatically reduced to yield the (*R*)-isomers.

2-Oxo-4-phenylbutanoic acid (**1a**) is an unstable acid and decomposes quickly even at the 0.5 mL (g yeast)$^{-1}$ water-content. The acid is converted into the corresponding 3-phenylpropyl alcohol by decarboxylation, followed by the reduction of the resulted aldehyde (Scheme 2). This type of substrate decomposition was also observed for **1h** and **1p**. It is well known in organic chemistry that these esters are highly susceptible to the hydrolysis.

Table 3. Effect of the Structure of Alkoxy Group in Alkyl or Aryl 2-Oxo-4-phenylbutyrate

R in the	Amount of water, mL (g yeast)$^{-1}$ (Chem. yield/ %/ee%[a])			
alkoxy group	0.5	0.6	0.7	0.8
H	4/ 54	0/ —	0/ —	—
CH_3	99/ 12	99/ 16	94/ 25	—
C_2H_5	98/ 53	97/ 62	86/ 67	60/ 67
C_3H_7	71/ 57	80/ 72	69/ 74	47/ 73
$CH(CH_3)_2$	67/−76	50/−78	43/−79	24/−81
C_4H_9	82/ 67	56/ 71	30/ 83	39/ 73
$CH_2CH(CH_3)_2$	85/ 67	71/ 77	49/ 76	31/ 84
$C(CH_3)_3$	6/ 24	8/ 12	6/ 5	4/ 3
C_5H_{11}	62/ 62	44/ 69	30/ 69	13/ 99
$CH(CH_2CH_3)_2$	24/−44	15/−53	10/−48	4/−50
$CH_2C(CH_3)_3$	62/ 76	47/ 87	30/ 93	15/ 99
$(CH_2)_2CH(CH_3)_2$	40/ 24	28/ 46	15/ 52	6/ 36
cyclo-C_5H_9	66/−67	49/−74	33/−84	—
cyclo-C_6H_{11}	48/−32	32/−34	15/−66	4/−71
C_6H_5	69/ −3	12/−21	7/ 7	0/ —
$C_6H_5CH_2$	92/ 9	74/ 20	39/ 24	37/ 23

a) Plus and minus sings are asigned for the (*R*)- and (*S*)-enantiomers, respectively.



Scheme 2.

It should be noted that enantiomeric excess observed in the product may not be reliable when the result is associated with unsatisfactory chemical yield. There remains a possibility that the resulting hydroxy ester is decomposed stereoselectively under the catalysis of the microbe.

On the other hand, some esters (e.g., **1i** and **1k**) have shown to give excellent stereoselectivity at elevated water content. Unfortunately, however, esters that can afford good results in stereoselectivity are not reactive enough to afford the corresponding hydroxy esters in acceptable chemical yields. Thus, it has been elucidated that a good result is not necessarily obtained by the esters with a bulky alkoxy group.

Effect of the Third Reagent. So far we have studied the reaction conditions for the reduction of **1** by bakers' yeast, but no trial succeeded in obtaining an outstanding result. The result suggests that there arises no significant discrimination in kinetic properties and substrate specificities of the enzymes that are responsible for the present reduction under the varied reaction conditions employed, where all the enzymes have remained potentially active. However, if one or some of them are denatured, the situation may change and an acceptable result may be obtained. We have several methods for denaturation of certain enzymes in a microbe; thermal treatment of the microbe,[20,35—37] shift of pH of the medium,[38] or the addition of an inhibitor to the system.[18—20,35,39—42]

The use of an inhibitor, or a stereocontroller, to suppress the participation of undesired enzymes in a microbe has been studied in our laboratory and by others to manipulate the stereochemical course of the reaction for obtaining the product of desired configuration.[18,19,43] Since it has been confirmed that the concentration of the substrate more or less affects the stereochemical result of the reduction, more than one enzyme could have been responsible for the present reduction by bakers' yeast. In other words, there remains no doubt that the control of the activity of a particular enzyme(s) in a microbe is a promising device to obtain the product of desired configuration in a satisfactory enantiomeric excess.

Thus, various chemicals were tested for potential stereocontrollers by incubating the microbe in the presence of a chemical at appropriate concentrations for 2 h at 30 °C in diethyl ether. The results from the reaction with **1c** are given in Table 4.

Table 4 shows that the chemicals are roughly classified into 5 groups from the viewpoint of the effect of water-content on chemical yield/stereoselectivity. Group A chemicals are

Table 4. Effect of Chemicals Added to the System for the Reduction of Ethyl 2-Oxo-4-phenylbutyrate

Group	Chemical	Chemical yield,%/Enantiomeric excess,% Amount of chemical, g L^{-1}		
		0.5	0.7	0.9
A	Methyl vinyl ketone	98/59	90/53	76/42
	Allyl alcohol	97/60	88/53	67/45
	Acetophenone	54/49	87/50	87/57
	o-Chlorophenoxyacetic acid	92/56	81/49	84/49
B	*N*-Ethylmaleinimide	88/76	65/77	51/73
	Phenacyl bromide	92/75	77/71	60/74
	p-Bromophenacyl bromide	95/71	93/75	80/78
	p-Chloro-*o*-nitrobenzoic acid	90/68	73/67	70/68
	o-Bromo-*p*-chloroacetophenone	30/70	88/75	85/76
	o-Chlorobenzoic acid	85/69	78/67	74/69
C	*p*-Chlorobenzophenone	95/55	94/59	94/60
	p-Bromo-*ω*-chlorobutyrophenone	97/61	97/64	99/63
	p-Bromophenacyl thiocyanate	96/63	95/66	95/64
	p-Chloro-*o*-nitroanisol	86/61	88/57	96/62
	p-Aminobenzamide	94/62	92/63	91/62
D	1-Phenacylpyridinium bromide	92/56	91/55	88/54
	Phenacyl alcohol	93/52	93/54	92/54
	Phenyl 2,2,2-trifluoroethyl ketone	91/47	92/52	92/52
	o-Aminoacetophenone	92/53	94/51	95/50
	m-Aminoacetophenone	90/54	96/54	91/55
	p-Aminoacetophenone	91/54	96/53	96/47
	Salicylamide	96/54	97/56	98/53
E	Phenacyl chloride	98/63	98/72	97/76

those that have a destructive property to all enzymes and, thus, cause a decrease in the enzymatic activity. Although, in principle, stereoselectivity is unpredictable in this case, it also decreases as a general trend. Group B chemicals are those that reduce the activity but improve stereoselectivity. In this case, it is suggested that some enzymes are deactivated by these stereocontrollers more than others. Group C stereocontrollers are those that exert little or no impact on the reduction. Group D stereocontrollers are those that keep the reactivity unaffected but shift the stereoselectivity toward the unfavorable direction. Finally, Group E stereocontrollers are those that retain the reactivity of desired enzymes and thereby allow high stereoselectivity toward the favorable direction.

Methyl vinyl ketone and allyl alcohol (in the presence or absence of glucose), which belong to Group A, are excellent chemicals to control the stereoselectivity in the reduction of β-keto esters to give the corresponding (*R*)-hydroxy esters.[19] On the other hand, these reagents show a contrasting effect for the present reduction. Thus, apparently, the enzyme responsible for the reduction of **1** is not the one reported as D-enzyme-1 for the reduction of β-keto esters.[18]

Recently, Ushio et al. reproted that phenacyl chloride and *N*-ethylmaleinimide are potent stereocontrollers for the enzyme(s) that affords the corresponding (*S*)-product from the reduction of ethyl 3-oxopentanoate mediated by baker's yeast.[44] Indeed, phenacyl chloride has been proven to be a promising chemical: the highest ee of 96% was observed after preincubation of the microbe in the presence of 6.5×10^{-3} M of phenacyl chloride. It is fortunate that this chemical not only improves the stereoselectivity but also does not affect the chemical yield of the product. Phenacyl chloride may be substituted by phenacyl bromide. *N*-Ethylmaleinimide is also a potent chemical for the present purpose.

Unlike phenacyl chloride, phenacyl alcohol and phenyl 2, 2,2-trifluoroethyl ketone are chemicals that shift the stereoselectivity toward the (*S*)-isomer, and belong to Group D.

Among those chemicals tested, three chemicals that have shown to afford high enantiomer excess in the product were selected for further tests. Thus, the effect of incubation time was studied for these chemicals. The results from the reaction with **1c** are listed in Table 5.

Table 5 suggests that phenacyl chloride is the chemical of choice from the viewpoint of both chemical yield and ee of the product. However, it should be noted that the added chemical deactivates the enzyme(s) responsible to the reduction appreciably when the water-content in the medium becomes high, resulting in a low chemical yield of the product.

Finally, we searched for a suitable substrate to afford the product in acceptable chemical yield and ee in the presence of phenacyl chloride as a stereo-controlling reagent. As summarized in Table 6, it was found that propyl or butyl esters (**1d**, **f**, and **g**) afford excellent results (>70% chemical yield and >95%ee) and are employable to obtaining a chiral building block in organic synthesis.

It also has been confirmed that the reaction run in a large-scale apparatus with 1.0 g of **1e** affords a similar result to that reported above. Thus, it has been proven that the reaction reported herein is practical enough to obtain the chiral hydroxy ester in a laboratory scale, and may be employed as a method in laboratory organic synthesis. However, we think that the method has to be combined in future with suitable techniques such as immobilization of microbes and column chromatographic procedure before it establishes a position in large scale synthesis.

Experimental

Instruments. Vapor-phase chromatograms (VPC) were recorded on a Shimadzu GC-9A and 14B (HR-20M, 0.25 mm$\phi\times$25 m; OV-1701 Bonded, 0.25 mm$\phi\times$25 m; Chiral-DEX CB, 0.25 mm$\phi\times$25 m) and GC-14B (Chiraldex G-TA, 0.25 mm$\phi\times$30 m and CP-cyclodextrin-B-236-M-19, 0.25 mm$\phi\times$25 m). Liquid-phase

Table 5. Effect of Preincubation Time for the Reduction of Ethyl 2-Oxo-4-phenylbutyrate

Chemical	Concn. g L^{-1}	Chemical yield,%/Enantiomeric excess,% Preincubation time, h			
		4	6	8	10
N-Ethylmaleinimide	0.59	81/78	52/82	55/78	67/75
p-Bromo-phenacyl bromide	0.61	88/78	86/76	82/74	77/75
Phenacyl chloride	0.61	97/78	97/76	97/79	89/80
Phenacyl chloride	1.03	86/81	92/81	96/81	94/81

Table 6. Synthesis of Alkyl (*R*)-2-Hydroxy-4-phenylbutyrate[a)]

R in alkoxy group	Chem. yield,[b)] %	Chem. yield,[c)] %	Ee, %	$[\alpha]_D^{24}$	Configuration
C_2H_5	95±2	83	81±1	−15.7	*R*
C_3H_7	89±3	71	93±2	−19.1	*R*
C_4H_9	86±4	66	96±2	−13.9	*R*
$CH_2CH(CH_3)_2$	75±3	59	96±2	−13.1	*R*

a) [Phenacyl Chloride] = 6.0 mg; [H_2O] = 0.6 mg; Yeast: 1 g; Preincubation Time; 6 h; Ether: 10 mL.
b) Chemical yield measured on VPC. c) Chemical yield on isolation.

chromatograms (HPLC) were obtained on a Hitachi 655 (Chiralcel OD™). ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR 200 FT-NMR spectrometer in $CDCl_3$ and CD_3COCD_3. Optical rotations were measured on a JASCO DIP-181 digital polarimeter. Elemental analyses were performed using a Yanaco MT-5 elemental analyzer.

Materials. Reagents were purchased from Nacalai Tesque Inc., Wako Pure Chemical Industries Ltd., and Aldrich Chemical Co. unless otherwise mentioned. Bakers' yeast (*Saccharomyces cerevisiae*) was purchased from Oriental Yeast Co. and stored in a refrigerator. All the organic solvents were of analytical grade. Dried and distilled solvents were employed whenever it is necessary. The purity of all the reagents employed for the reactions were confirmed by ^{1}H NMR spectroscopy and gave satisfactory results.

Preparation of 2-Oxo-4-phenylbutanoic Acid (1a) and Its Esters. Commercially available ethyl 2-oxo-4-phenylbutyrate (**1c**) (10 g; 48.5 mmole) was hydrolyzed in 2 M aqueous NaOH at 0 °C followed by acidification with 2 M aqueous HCl. Usual work-up gave **1a**[45] in 89% yield. The acid was subjected to further reactions without being purified.

Methyl 2-oxo-4-phenylbutyrate (**1b**)[12] was prepared in 75% yield by esterification of **1a** (2.6 g, 0.0146 mmol) with 5 mL methanol in the presence of 3.0 g (14.6 mmol) dicyclohexylcarbodiimide in 40 mL diethyl ether at room temperature for 1—2 h. Phenyl 2-oxo-4-phenylbutyrate (**1p**) was obtained similarly in 60% yield.

t-Butyl 2-oxo-4-phenylbutyrate (**1h**)[46] was synthesized in 91% yield starting from di-*t*-butyl oxalate.[47,48]

Other esters (**1d**, **e**, **f**, **g**, **i**, **j**, **k**, **m**, **n**, **o**, and **q**)[12] were obtained by ester exchange reactions of **1b** with an appropriate alcohol under refluxing for 6—12 h. Chemical yields were 40—85%.

1b: ^{1}H NMR (TMS in $CDCl_3$) δ = 2.96 (t, 2H), 3.21 (t, 2H), 3.85 (s, 3H), and 7.20—7.40 (m, 5H).

1d: ^{1}H NMR (TMS in $CDCl_3$) δ = 0.97 (t, 3H), 1.65—1.82 (m, 2H), 2.95 (t, 2H), 3.25 (t, 2H), 4.20 (t, 2H), and 7.10—7.30 (m, 5H).

1e: ^{1}H NMR (TMS in $CDCl_3$) δ = 1.30 (d, 6H), 2.96 (t, 2H), 3.15 (t, 2H), 5.00—5.20 (m, 1H), and 7.14—7.35 (m, 5H).

1f: ^{1}H NMR (TMS in $CDCl_3$) δ = 0.95 (t, 3H), 1.31—1.40 (m, 2H), 1.64—1.78 (m, 2H), 2.97 (t, 2H), 3.15 (t, 2H), 4.25 (t, 2H), and 7.12—7.32 (m, 5H).

1g: ^{1}H NMR (TMS in $CDCl_3$) δ = 0.97 (d, 6H), 1.95—2.15 (m, 1H), 2.97 (t, 2H), 3.16 (t, 2H), 4.03 (d, 2H), and 7.14—7.35 (m, 5H).

1h: ^{1}H NMR (TMS in $CDCl_3$) δ = 1.48 (s, 9H), 2.98 (t, 2H), 3.18 (t, 2H), and 7.16—7.36 (m, 5H).

1i: ^{1}H NMR (TMS in $CDCl_3$) δ = 0.93 (t, 3H), 1.24—1.41 (m, 2H), 1.60—1.78 (m, 2H), 2.95 (t, 2H), 3.61 (t, 3H), 4.22 (t, 2H), and 7.12—7.35 (m, 5H).

1j: ^{1}H NMR (TMS in $CDCl_3$) δ = 0.91 (t, 6H), 1.56—1.74 (m, 4H), 2.95 (t, 2H), 3.16 (t, 2H), 4.86—4.96 (m, 1H), and 7.15—7.35 (m, 5H).

1k: ^{1}H NMR (TMS in $CDCl_3$) δ = 0.97 (s, 9H), 2.97 (t, 2H), 3.17 (t, 2H), 3.95 (s, 2H), and 7.16—7.35 (m, 5H).

1m: ^{1}H NMR (TMS in $CDCl_3$) δ = 0.95 (d, 6H), 1.47—1.59 (q, 2H), 1.6—1.8 (m, 1H), 2.96 (t, 2H), 3.15 (t, 2H), 4.25 (t, 2H), and 7.15—7.32 (m, 5H).

1n: ^{1}H NMR (TMS in $CDCl_3$) δ = 1.5—2.15 (m, 8H, C_5H_8), 2.96 (t, 2H), 3.15 (t, 2H), 5.25 (m, 1H, C_5H_1), and 7.15—7.33 (m, 5H).

1o: ^{1}H NMR (TMS in $CDCl_3$) δ = 1.16—1.98 (m, 10H, C_6H_{10}), 2.96 (t, 2H), 3.16 (t, 2H), 4.88 (m, 1H, C_6H_1), and 7.15—7.35 (m, 5H).

1p: ^{1}H NMR (TMS in $CDCl_3$) δ = 2.82 (t, 2H), 3.10 (t, 2H), 6.87—7.10 (m, 5H), and 7.11—7.28 (m, 5H); mp 77—80 °C.

1q: ^{1}H NMR (TMS in $CDCl_3$) δ = 2.96 (t, 2H), 3.16 (t, 2H), 5.23 (s, 2H), 7.12—7.26 (m, 5H), and 7.27—7.40 (m, 5H); mp 34—37 °C.

Reduction of 1 by Bakers' Yeast.: General Procedure. To a 25×150 mm glass tube, fitted with Teflon® cap, containing 1.0 g of bakers' yeast, 10 mL of an organic solvent or a mixture of organic solvents and an appropriate amount of water was added ethyl 2-oxo-4-phenylbutyrate (**1c**; 12 mg, 0.058 mmol). The mixture was agitated at 130 rpm at 30 °C for 24 h. The organic portions were then collected by filtration and the yeast was washed with either ethyl acetate or diethyl ether (4×10 mL) and the extracts were combined with the filtrate. The combined organic portions were concentrated under reduced pressure. The residue was subjected to Extrelut to remove unvolatile materials prior to gas chromatographic analysis, using HR-20M column for the measurement of chemical yield and CP-cyclodextrin column for determination of enantiomeric excess.

Absolute configuration of the product **2c** was determined to be *R* by comparing the sign of its optical rotation wih that of the literature value: $[\alpha]_D^{24}$ −15.7 (*c* 1.0, $CHCl_3$) (lit,[49] $[\alpha]_D^{23}$ −21.6 (*c* 1.2, $CHCl_3$)).

Chemical yield of the product and the amount of the starting material remaining were evaluated by comparing the signal area of the product to that of the starting material under the standard conditions. No other product was detected on VPC.

Whenever necessary, preincubation time, reaction time, and water-content were changed appropriately. It has been confirmed that a water content of 0.6 mL (g yeast)$^{-1}$ in diethyl ether as the solvent affords the best result under the reaction conditions employed.

It was also found that the addition of appropriate amount of phenacyl chloride to the preincubation system is effective to improve the enantiomeric excess in the product. For measuring the effect of the stereocontroller, the microbe was preincubated in the presence of an appropriate amount of the reagent. The efficiency of the third reagent was tested under the g L^{-1} unit, instead of using the mole unit, for expressing the amount of the reagent added to the reaction system, because the former unit seems more practical for synthetic purposes than the latter.

Absolute configurations of other esters were determined by transferring these esters into the corresponding ethyl ester and observing retention times of thus obtained enantiomeric ethyl esters on gas chromatography with CP-cyclodextrin column. Retention times of *R*- and *S*-enantiomers of **2c** are 25.4 and 26.5 min, respectively (140 °C column and 180 °C injection/detection temperatures).

2a: ^{1}H NMR (TMS in $CDCl_3$) δ = 1.80—2.20 (m, 2H), 2.70 (t, 2H), 4.15 (dd, 1H, *J* = 3.9, 7.8 Hz), and 7.1—7.2 (m, 5H); mp 113—115 °C (lit,[50] mp 114—116 °C).

2b: ^{1}H NMR (TMS in $CDCl_3$) δ = 1.75—2.15 (m, 2H), 2.70 (t, 2H), 3.80 (s, 3H), 4.12 (q, 1H), and 7.1—7.4 (m, 5H).

2c: ^{1}H NMR (TMS in $CDCl_3$) δ = 1.25 (t, 3H), 1.85—2.22 (m, 2H), 2.75 (t, 2H), 4.25 (dd, 1H), 4.10—4.30 (q, 2H), and 7.10—7.30 (m, 5H).

2d: ^{1}H NMR (TMS in $CDCl_3$) δ = 0.96 (t, 3H), 1.26—1.47 (m, 2H), 1.65—1.80 (m, 2H), 1.86—2.00 (m, 1H), 2.02—2.20 (m, 1H), 2.72—2.80 (m, 2H), 4.10—4.20 (t, 2H), 4.14—4.23 (dd, 1H), and 7.14—7.37 (m, 5H); $[\alpha]_D^{24}$ −19.1 (*c* 1.00, EtOH).

2e: ^{1}H NMR (TMS in $CDCl_3$) δ = 1.25 (d, 6H), 1.85—1.98 (m, 1H), 2.02—2.2 (m, 1H), 2.71—2.81 (m, 2H), 4.10—4.20 (dd, 1H), 5.00—5.20 (m, 1H), and 7.15—7.35 (m, 5H); mp 42—44 °C.

2f: ^{1}H NMR (TMS in $CDCl_3$) δ = 0.96 (t, 3H), 1.31—1.40 (m, 2H), 1.68—1.79 (m, 2H), 1.84—2.04 (m, 1H), 2.05—2.27 (m, 1H),

2.78—2.92 (m, 2H), 4.15 (t, 2H), and 7.15—7.36 (m, 5H); $[\alpha]_D^{24}$ −13.9 (*c* 1.0, $CHCl_3$).

2g: ^{1}H NMR (TMS in $CDCl_3$) δ = 0.97 (d, 6H), 1.85—2.03 (m, 1H), 2.04—2.21 (m, 1H), 2.68—2.81 (m, 2H), 3.95 (d, 2H), 4.15—4.25 (dd, 1H), and 7.15—7.35 (m, 5H). $[\alpha]_D^{24}$ −13.1 (*c* 1.0, $CHCl_3$).

2h: ^{1}H NMR (TMS in $CDCl_3$) δ = 1.48 (s, 9H), 1.86—2.02 (m, 1H), 2.04—2.21 (m, 1H), 2.76—2.89 (m, 2H), 4.12—4.20 (dd, 1H), and 7.16—7.35 (m, 5H); ^{13}C NMR (TMS in $CDCl_3$) δ = 28.0, 31.2, 38.1, 70.0, 82.1, 125.8, 128.6, 141.7, and 174.2; mp 77—78 °C.

2i: ^{1}H NMR (TMS in $CDCl_3$) δ = 0.96 (t, 3H), 1.19—1.42 (m, 2H), 1.58—1.80 (m, 2H), 1.87—2.14 (m, 1H), 2.16—2.22 (m, 1H), 2.78—2.90 (m, 2H), 4.24 (t, 2H), 4.16—4.22 (dd, 1H), and 7.18—7.37 (m, 5H).

2j: ^{1}H NMR (TMS in $CDCl_3$) δ = 0.90 (t, 6H), 1.54—1.69 (m, 4H), 1.84—1.98 (m, 2H), 2.02—2.20 (m, 2H), 2.72—2.82 (t, 2H), 4.18 (dd, 1H), 4.78—4.93 (m, 1H), and 7.15—7.35 (m, 5H).

2k: ^{1}H NMR (TMS in $CDCl_3$) δ = 0.97 (s, 9H), 1.88—2.04 (m, 1H), 2.06—2.24 (m, 1H), 2.74—2.85 (t, 2H), 3.93 (s, 1H), 4.24 (dd, 1H), and 7.15—7.35 (m, 5H).

2m: ^{1}H NMR (TMS in $CDCl_3$) δ = 0.95 (d, 6H), 1.48—1.58 (q, 2H), 1.60—1.80 (m, 1H), 1.85—1.98 (m, 1H), 2.02—2.19 (m, 1H), 2.75 (m, 2H), 2.90 (d, 1H), 4.18 (t, 2H), and 7.15—7.32 (m, 5H).

2n: ^{1}H NMR (TMS in $CDCl_3$) δ = 1.52—1.98 (m, 8H, C_5H_8), 1.96—2.18 (m, 2H), 2.69—2.80 (t, 2H), 4.15 (dd, 1H), 5.25 (m, 1H, C_5H_1), and 7.15—7.35 (m, 5H).

2o: ^{1}H NMR (TMS in $CDCl_3$) δ = 1.18—1.95 (m, 10H, C_6H_{10}), 1.70—2.20 (m, 2H), 2.75 (t, 2H), 4.18 (m, 1H), 4.88 (m, 1H, C_6H_1), and 7.15—7.35 (m, 5H).

2p: ^{1}H NMR (TMS in $CDCl_3$) δ = 1.77—2.00 (m, 5H), 2.01—2.18 (m, 1H), 2.81 (t, 2H), 4.21 (dd, 1H), 6.89—7.11 (m, 5H), and 7.11—7.28 (m, 5H); mp 70—90 °C (decomp).

2q: ^{1}H NMR (TMS in $CDCl_3$) δ = 1.75—2.03 (m, 1H), 2.06—2.22 (m, 1H), 2.77 (t, 2H), 4.24 (dd, 1H), 5.23 (s, 2H), 7.12—7.25 (m, 5H), and 7.25—7.40 (m, 5H).

Reduction of 1 by Bakers' Yeast.: A Large-Scale Procedure. To a 2 L three-necked flask containing 1.2 L of diethyl ether and 60 mL of water and fitted with a mechanical stirrer, a thermometer and a reflux condenser, was added 600 mg phenacyl chloride and 103 g of bakers' yeast. The mixture was preincubated for 6 h at 30 °C with an agitation rate of 130 rpm. Then, 1.0 g of **1g** was added and the reduction was allowed to proceed for 24 h. The organic portion was separated by filtration from the yeast and the yeast was washed with ethyl acetate (3×300 mL). The washings were combined with the filtrate and the combined solution was concentrated under reduced pressure to give an odorous yellow viscous residue. The residue was subsequently washed with water (3×20 mL), then brine (2×20 mL), water (2×20 mL), and dried over $MgSO_4$ before it is subjected to Extrelut to remove yeast materials. After purification by column chromatography on silica gel with a hexane/ethyl acetate mixture (5/1 v/v) as an eluent, 724 mg of the product, isobutyl (*R*)-2-hydroxy-4-phenylbutyrate (**2g**), was isolated (72% chemical yield) with 91%ee. The starting material was recovered in 22% chemical yield. It was confirmed that chemical yields of materials as well as ee did not change after an additional 30 h under the same reaction conditions.

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