Pergamon

SCIENCE DIRECT®

Tetrahedron: *Asymmetry* 14 (2003) 2659–2681

TETRAHEDRON: *ASYMMETRY*

TETRAHEDRON: ASYMMETRY REPORT NUMBER 60

Recent developments in asymmetric reduction of ketones with biocatalysts

Kaoru Nakamura,[a,*] Rio Yamanaka,[a] Tomoko Matsuda[b] and Tadao Harada[b]

[a]*Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan*
[b]*Department of Materials Chemistry, Faculty of Science and Technology, Ryukoku University, Otsu, Shiga 520-2194, Japan*

Received 14 April 2003; accepted 31 May 2003

Abstract—Herein we review recent advances in the asymmetric reduction of ketones by biocatalysts. Included are discussions on recent developments in methodologies to control enantioselectivities of catalytic reactions, and examples of practical applications that reduce various types of ketones are also shown.

Contents

* Corresponding author. E-mail: nakamura@scl.kyoto-u.ac.jp

0957-4166/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0957-4166(03)00526-3

1. Introduction

The asymmetric reduction of ketones is one of the most important, fundamental and practical reactions for producing non-racemic chiral alcohols, which can be transformed into various functionalities, without racemization, to synthesize industrially important chemicals such as pharmaceuticals, agrochemicals and natural products. The catalysts for the asymmetric reduction of ketones can be classified into two categories: chemical and biological methodologies. Both have their own peculiarities, and development of both to enable the appropriate selection of the catalysts for particular purposes is necessary to promote green chemistry. In this review, methods using biocatalysts[1] will be discussed while comparing them with chemical catalysts.

1.1. Biocatalysts versus chemical catalysts

Biocatalysts have unique characteristics when compared with chemical (homogeneous and heterogeneous) catalysts. Some features that distinguish biocatalysts from chemical catalysts are listed below.

Selectivity:

Very high enantio-, regio- and chemoselectivities can be achieved due to the strict recognition of the substrate by the enzyme. For example, very high enantioselectivities can be achieved, even with the reduction of aliphatic ketones such as ethyl propyl ketone, whereas chemical catalysts can perform highly enantioselective reductions usually when the two substituent groups of the carbonyl carbon of the ketones are significantly different.

Safety of the reaction:

Biocatalytic reductions are generally safe. The reaction conditions are mild, the solvent is usually water, and dangerous reagents are not necessary. For example, ethanol and glucose etc. are used as hydrogen sources instead of explosive hydrogen gas.

Natural catalysts:

The biocatalysts, i.e. microorganisms, plants, animals, or their isolated enzymes, are reproducible and can be easily decomposed in the environment after use.

Catalyst preparation:

Some of the biocatalysts for reduction, isolated enzymes and whole cells, are commercially available and ready to use like chemical catalysts or hydrolytic enzymes (Fig. 1). Commercially available biocatalysts

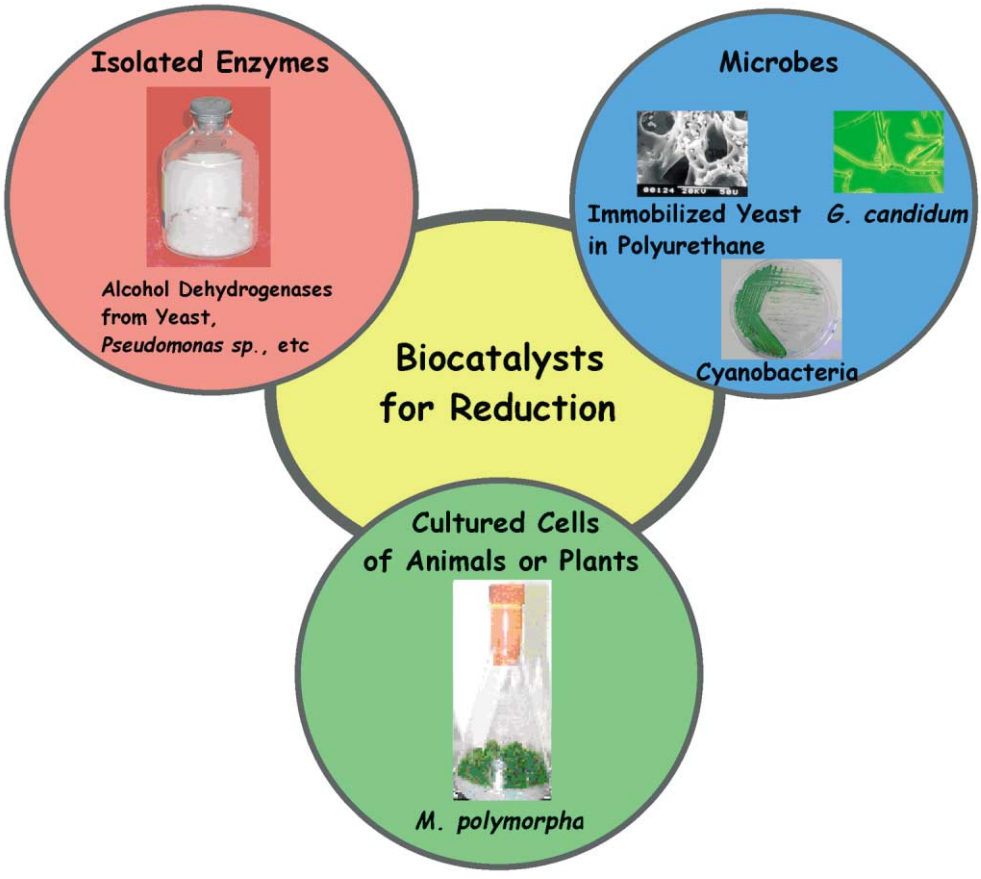


Figure 1. Biocatalysts for asymmetric reductions.

include baker's yeast and the alcohol dehydrogenases from baker's yeast, *Thermoanaerobium brockii* (TBADH), horse liver and the hydroxysteroid dehydrogenase from *Pseudomonas testosteroni* and *Bacillus spherisus*. However, to obtain other biocatalysts, it is necessary to cultivate cells from seed cultures that may be commercially available.

Large scale synthesis and space-time yield:

One of the disadvantages of using biocatalysts is the difficulty encountered in large scale synthesis; (1) workup procedures may be complicated, (2) large spaces for the cultivation of the cell may be necessary, or (3) the space–time yields are not high due to the low substrate concentrations and long reaction times. However, these disadvantages have been surmounted by improving the biocatalysts using genetic methods and by investigating the reaction conditions.

1.2. Enzyme classification and reaction mechanism

Dehydrogenases and reductase, classified under E.C.1.1.1., are enzymes that catalyze the reduction of carbonyl groups.[2] The natural substrates of the enzymes are alcohols such as ethanol, lactate, glycerol, etc. and the corresponding carbonyl compounds; however, unnatural ketones can also be reduced enantioselectively. To exhibit catalytic activities, the enzymes require a coenzyme such as NADH or NADPH from which a hydride is transferred to the substrate carbonyl carbon. There are four stereochemical patterns that enable the transfer of the hydride from the coenzyme, NAD(P)H, to the substrate, as shown in Figure 2.[3a] With E1[3] and E2[4] enzymes, the hydride attacks the *si*-face of the carbonyl group, whereas with E3[5] and E4 enzymes, the hydride attacks the *re*-face, which results in the formation of (*R*) and (*S*)-alcohols, respectively. On the other hand, E1 and E3 enzymes transfer the pro-(*R*)-hydride of the coenzyme, and E2 and E4 enzymes use the pro-(*S*)-hydride. Examples of the E1-E3 enzymes are as follows:

E1: *Pseudomonas* sp. alcohol dehydrogenase[3a]
Lactobacillus kefir alcohol dehydrogenase[3b]
E2: *Geotrichum candidum* glycerol dehydrogenase[4a–c]
Mucor javanicus dihydroxyacetone reductase[4d]
E3: Yeast alcohol dehydrogenase[5a]
Horse liver alcohol dehydrogenase[5b–e]
Moraxella sp. alcohol dehydrogenase[5f]

1.3. Hydrogen sources for reduction

Hydrogen sources are necessary to perform the reduction reaction. For biocatalytic reduction, alcohols such as ethanol and 2-propanol, glucose, formic acid and dihydrogen, among others, can be used.[1] An example of using formic acid as a hydrogen source for the reduction of ethyl 4-chloro-3 oxobutanoate with *Rhodococcus erythropolis* is shown in Figure 3.[6] Reduction of the

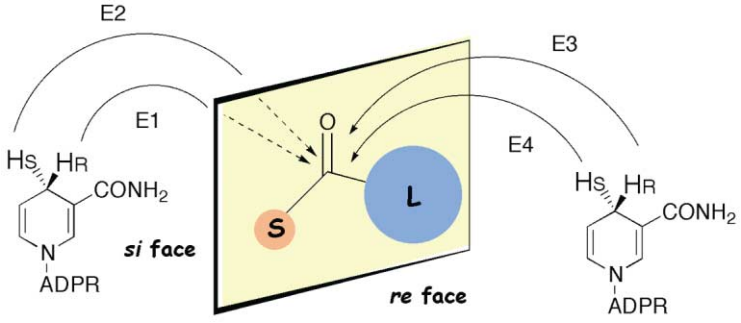


Figure 2. Stereochemistry of the hydride transfer from NAD(P)H to the carbonyl carbon on the substrate (S is a small group and L is a large group).

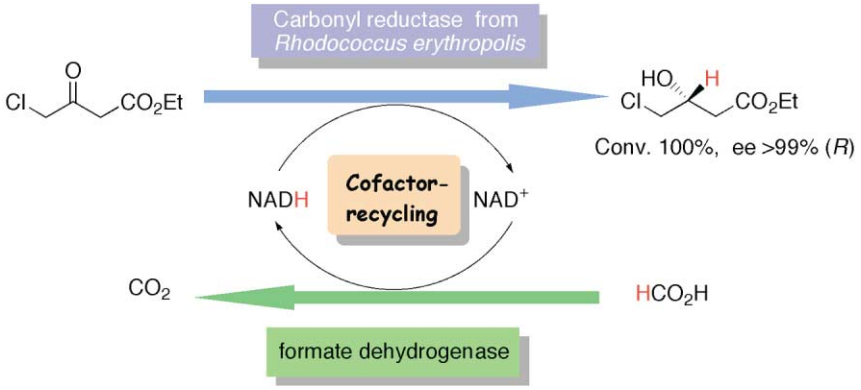


Figure 3. NADH recycling using HCO_2H as an hydrogen source for the reduction.[6]

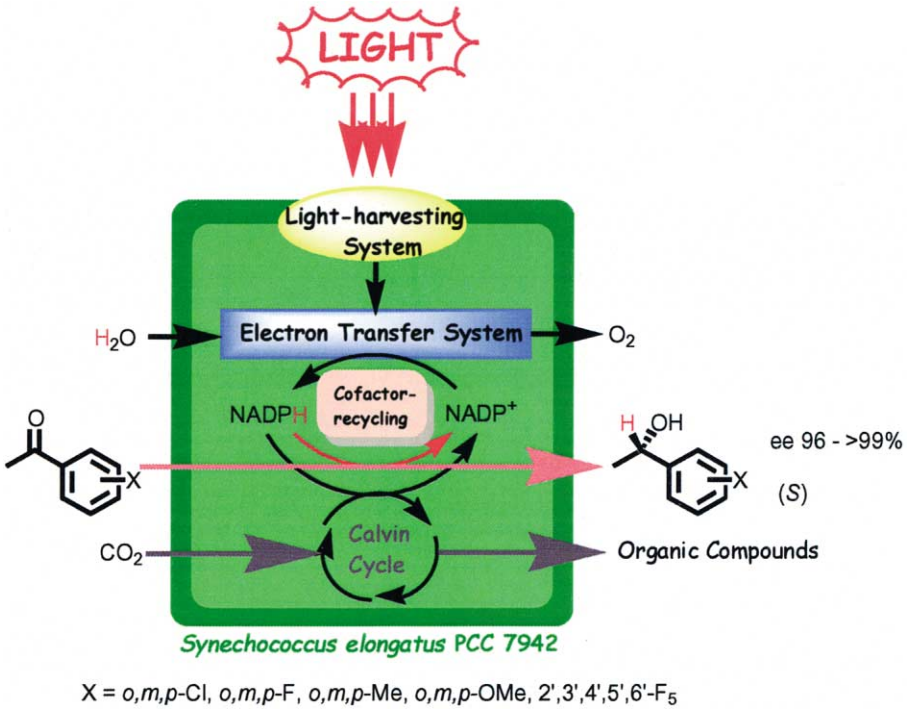


Figure 4. Utilization of light energy as the driving force of reduction.[7a–c]

substrate accompanies the oxidation of the coenzyme from NADH to NAD^+. Before the next cycle of the reduction of the main substrate can occur, the coenzyme has to be reduced to NADH, which is driven by the formate dehydrogenase catalyzed oxidation of HCO_2H to CO_2.

Photochemical methods[7] have been developed to provide an environmentally friendly system, that employs light energy to regenerate NAD(P)H, for example, by the use of a cyanobacterium, a photosynthetic biocatalyst.[7a–c] Using the biocatalysts, the reduction of acetophenone derivatives occurred more effectively under illumination than in the dark (Fig. 4). The light energy harvested by the cyanobacterium is converted into chemical energy in the form of NADPH through an electron transfer system, and, consequently, the chemical energy (NADPH) is used to reduce the substrate to the chiral alcohol (ee 96–>99%). The light energy, which is usually utilized to reduce CO_2 to synthesize organic compounds in the natural environment, was used to reduce the substrate in this case.

When a photosynthetic organism is omitted, the addition of a photosensitizer is necessary.[7d] The methods use light energy to promote the transfer of an electron from a photosensitizer to $NAD(P)^+$ via an electron transport reagent.

Electrochemical regeneration of NAD(P)H is another interesting and clean method.[8] The system involves electron transfer from the electrode to an electron mediator, such as methyl viologen or acetophenone etc., then to the $NAD(P)^+$, which is catalyzed by an electrocatalyst such as ferredoxin NADP+ reductase or alcohol dehydrogenase, etc.

2. Methodologies

Recent developments in methodology to find the most suitable reactions and to control enantioselectivities of those reactions are presented in this section, with a summary given in Figure 5. The methods can be classified into three categories: (1) search and creation of the biocatalysts, (2) modification of the substrate, and (3) optimization of the reaction conditions.

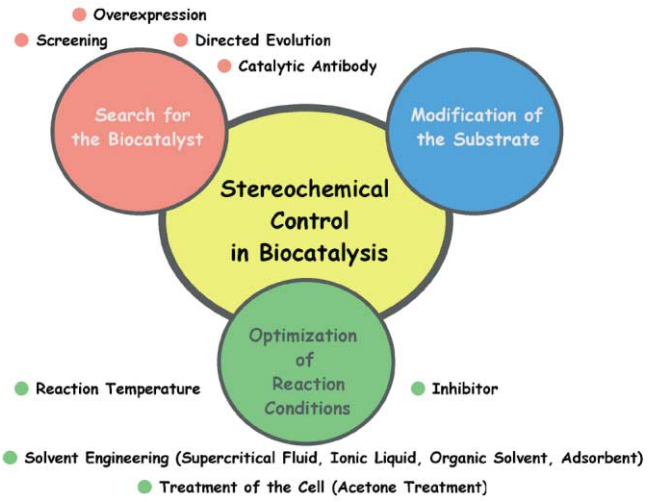


Figure 5. Summary of the methodology in biocatalytic reduction.

2.1. Search for the biocatalyst

2.1.1. Screening. Screening for a novel enzyme although the classical method is still one of the most powerful tools for finding biocatalytic reduction systems.[9] It is possible to discover a suitable biocatalyst by applying the latest screening and selection technologies, allowing rapid identification of enzyme activities from diverse sources.[9a] Enzyme sources used for the screening of asymmetric reductions in organic synthesis can be soil samples, commercial enzymes, culture sources, or a clone bank, etc. Their origin can be microorganisms, animals or plants. From these sources, enzymes that are regularly expressed and those that are not expressed in the original host can be tested to determine whether they are suitable for the transformation of certain substrates.[9a]

For example, 400 yeasts were screened for the reduction of ethyl 4-chloro-3-oxobutanoate, and *Candida magnoliae* was found to be the best one, as shown in Figure 6a.[9b–d] To reduce ketopantoyl lactone, various types of microorganisms were screened, and several microorganisms that produce D-(−)-pantoyl lactone stoichiometrically at the concentration of 45 mg/mL with high enantioselectivity were found (Fig. 6b).[9e,f] For the reduction of ethyl 2-methyl-3-oxobutanoate, *Klebsiella pneumoniae* IFO 3319 out of 450 bacterial strains was found to give the corresponding (2*R*,3*S*)-hydroxy ester with 99% de and >99% ee (Fig. 6c).[9g] Screening techniques have also been applied to drug synthesis. For example, a key intermediate in the synthesis of the anti-asthma drug, Montelukast, was prepared from the corresponding ketone by microbial transformation as shown in Figure 6d.[9h] The biotransforming organism

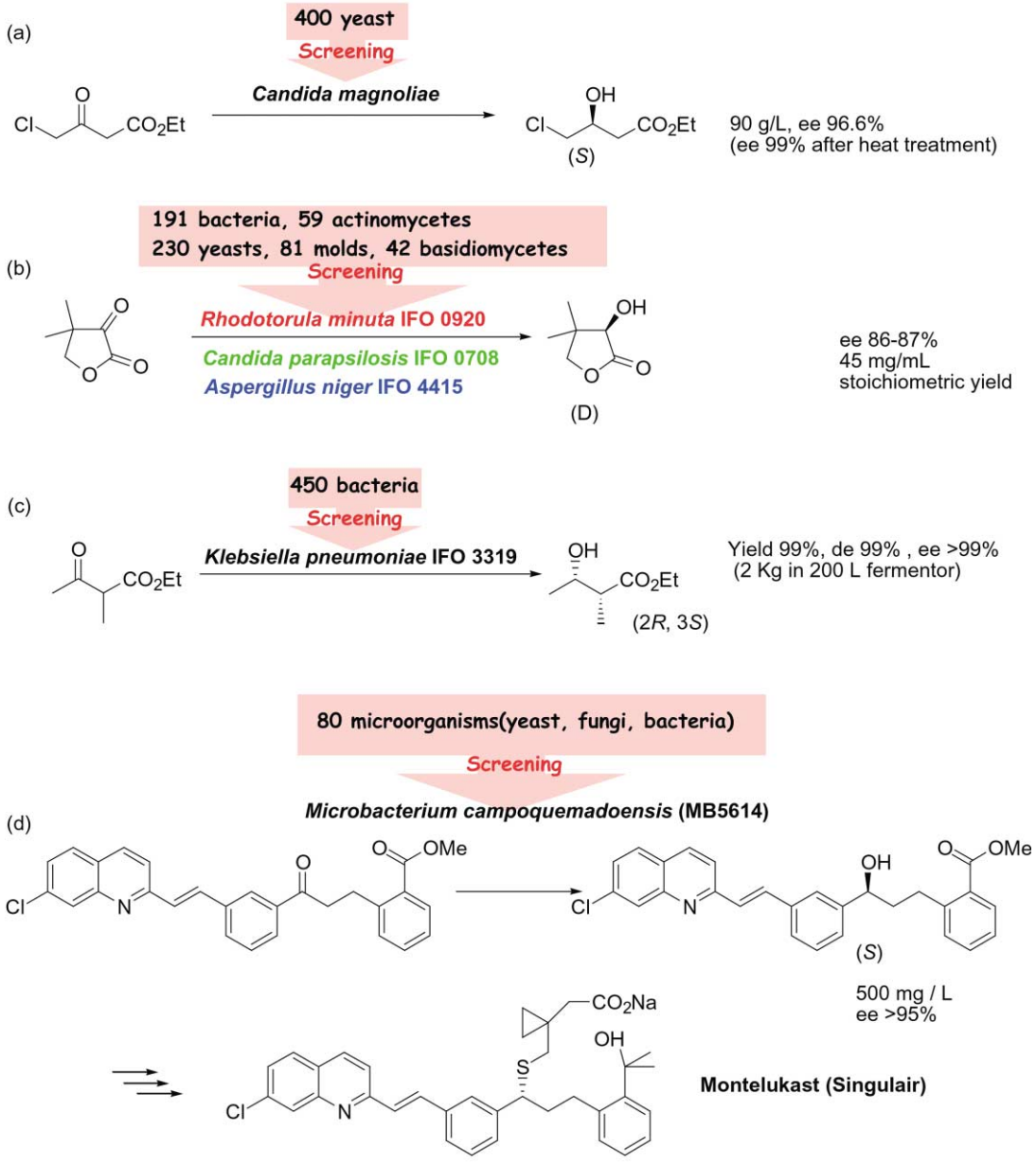


Figure 6. Screening for biocatalysts.[9b,e,g,h]

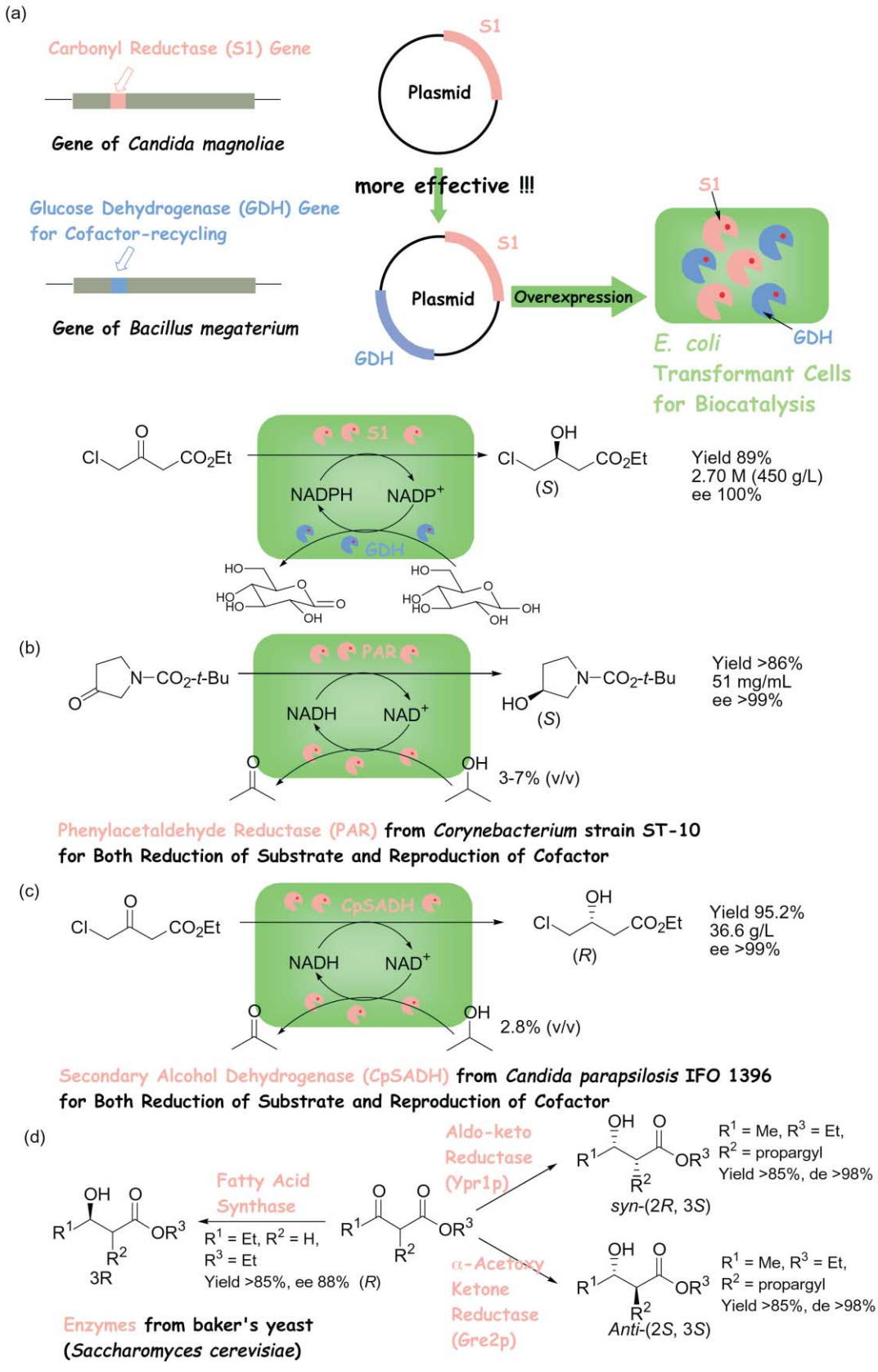


Figure 7. Recombinant whole-cell catalyzed reduction.[10a,11f,12b,13c]

Microbacterium campoquemadoensis (MB5614) was discovered as a result of an extensive screening program.

2.1.2. Overexpression. Microorganisms have been transformed for a variety of purposes:[10–13] to improve enzyme production in a cell, to provide coenzyme-regenerating enzymes in the same cell, to improve poor enantioselectivities due to the presence of plural enzymes in a cell with overlapping substrate specificities but different enantioselectivities, to solve the problem of overmetabolism, etc. Examples are as follows.

Example 1: Carbonyl reductase from *C. magnoliae* and glucose dehydrogenase from *Bacillus megaterium* was expressed in *E. coli* to reduce ethyl 4-chloro-3-oxobutanoate.[10a] The reaction using the transformed *E. coli* cell resulted in the accumulation of the product at the concentration of 450 g/L in 89% yield and 100% ee(*S*) (Fig. 7a)

Example 2: Phenylacetaldehyde reductase from *Corynebacterium* strain ST-10 was expressed in *E. coli*.[11f] *N*-Boc-3-pyrrolidinone was reduced to the corresponding (*S*)-alcohol with >86% yield (51 mg/mL) and >99% ee (Fig. 7b).

Example 3: Secondary alcohol dehydrogenase from *Candida parapsilosis* IFO 1396 was expressed in *E. coli* to reduce ethyl 4-chloro-3-oxobutanoate with 95% yield (36.6 g/L) and 99% ee (Fig. 7c).[12b]

Example 4: Enzymes from baker's yeast were expressed in *E. coli*, and various β-keto esters and alkyl-β-keto esters were reduced with excellent enantio- (up to >98% ee) and diastereoselectivities (>98% de Fig. 7d).[13c]

2.1.3. Directed evolution. The directed evolution of enzymes has been used to improve the reducing function of the enzymes. For example, this method was used to eliminate the co-factor requirement of *Bacillus stearothermophillus* lactate dehydrogenase, which is activated in the presence of fructose 1,6 bisphosphate.[14] However, the activator is expensive and representative of the sort of co-factor complications that are undesirable in industrial processes. Three rounds of random mutagenesis and screening produced a mutant that is almost fully activated in the absence of fructose 1,6-bisphosphate as shown in Figure 8. The K_m of the enzyme without the co-factor was improved from 5 to 0.07 mM for the pyruvate.

2.1.4. Catalytic antibody. Biocatalysts for reduction have been tailored by using the catalytic antibody technique. For example, Schultz et al. have developed antibodies to carry out the catalytic enantioselective reduction of ketones using $NaBH_3CN$ as the reductant (Fig. 9).[15] Monoclonal antibodies raised against the keyhole limpet hemocyanin conjugate of the hapten-catalyzed the reduction of ketones. Reduction with the antibody gave the corresponding (*S*)-alcohol with excellent enantioselectivities.

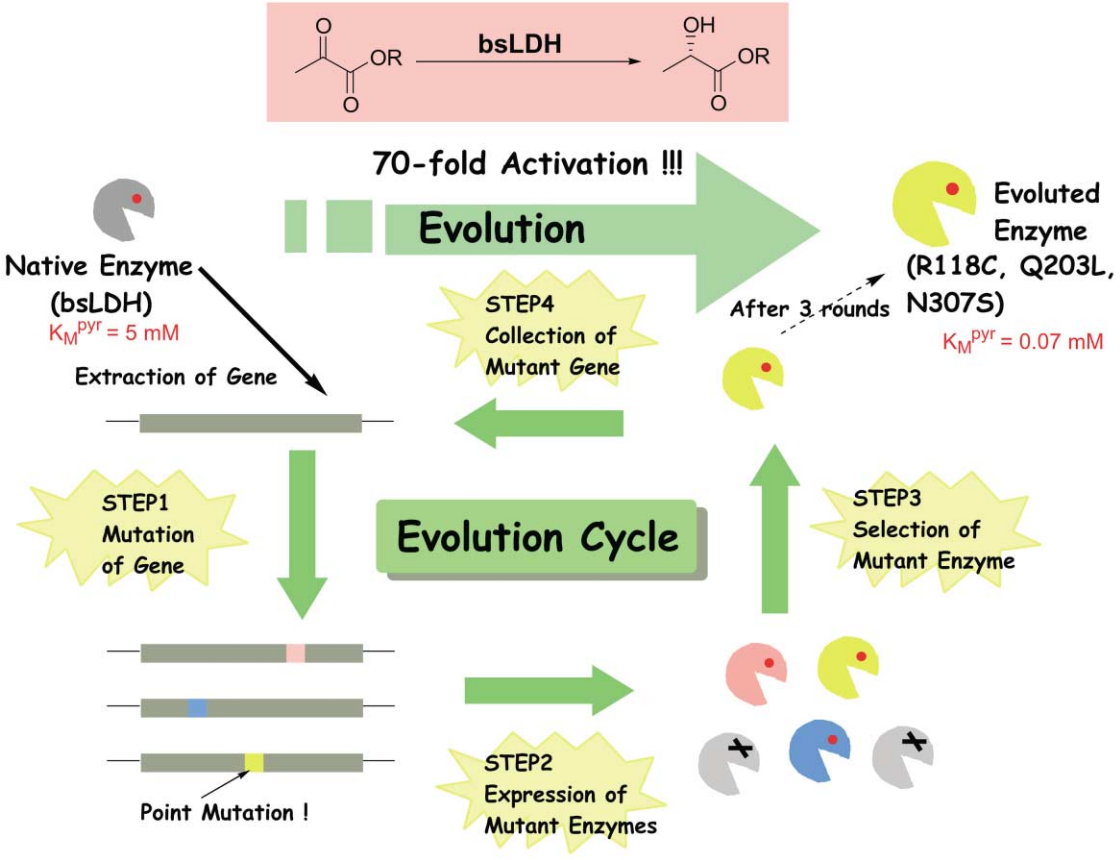


Figure 8. Elimination the co-factor requirement by directed evolution.[14]

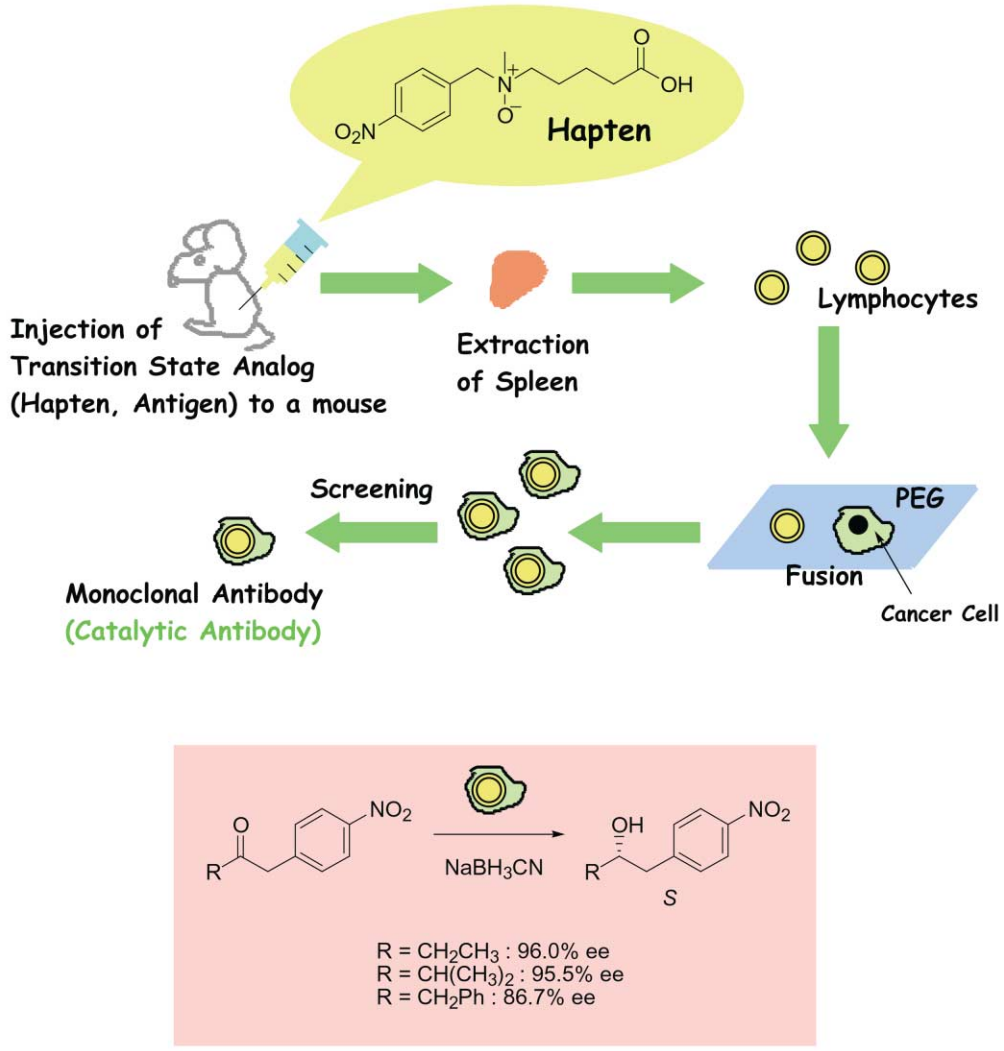


Figure 9. Reduction of ketones by a catalytic antibody.[15]

2.2. Modification of the substrates

The enantioselectivity of a biocatalytic reduction can be controlled by modifying the substrate because the enantioselectivity of the reduction reaction is profoundly affected by the substrate's structure.[16] For example, in the reduction of 4-chloro-3-oxobutanoate by bakers' yeast, the length of the ester moiety controlled the stereochemical course of the reduction.[16a–c] When the ester moiety was smaller than a butyl group, (*S*)-alcohols were obtained, and when it was larger than a pentyl group, (*R*)-alcohols were obtained, as shown in Figure 10a. After reduction, the ester moiety can be exchanged easily without racemization, so both enantiomers of an equivalent synthetic building block can be obtained using the same reaction system.

Another example is the introduction of sulfur functionalities into the keto esters at the α- or α′ positions, which can be eliminated after the reduction. Methylthio[16d] and phenylsulfonyl[16e] groups were used to improve the enantioselectivities, as shown in Figure 10b.

The enantioselectivity of aromatic ketones can also be controlled by the modification of substrates. As shown in Figure 10c, baker's yeast reduction of hydroxy ketone afforded the (*R*)-alcohol, whereas acetoxy ketone afforded the (*S*)-alcohol.[16f]

2.3. Optimization of reaction conditions

2.3.1. Acetone treatment of the cell. A dried cell mass is often used as a biocatalyst for a reduction since it can be stored for a long time and can be used whenever needed, without cultivation. One convenient method of drying the cell mass is acetone dehydration.[17] For example, dried cells of *G. candidum* IFO 4597 can be obtained easily by mixing the cells with cold acetone (−20°C) followed by filtering and drying under reduced pressure.

Cell drying not only aids the preservation of the cell, but also contributes to the stereochemical control, as shown in Table 1.[18] The reduction of acetophenone catalyzed by untreated-wet-whole cell of *G. candidum* IFO 4597 resulted in poor enantioselectivity [28%

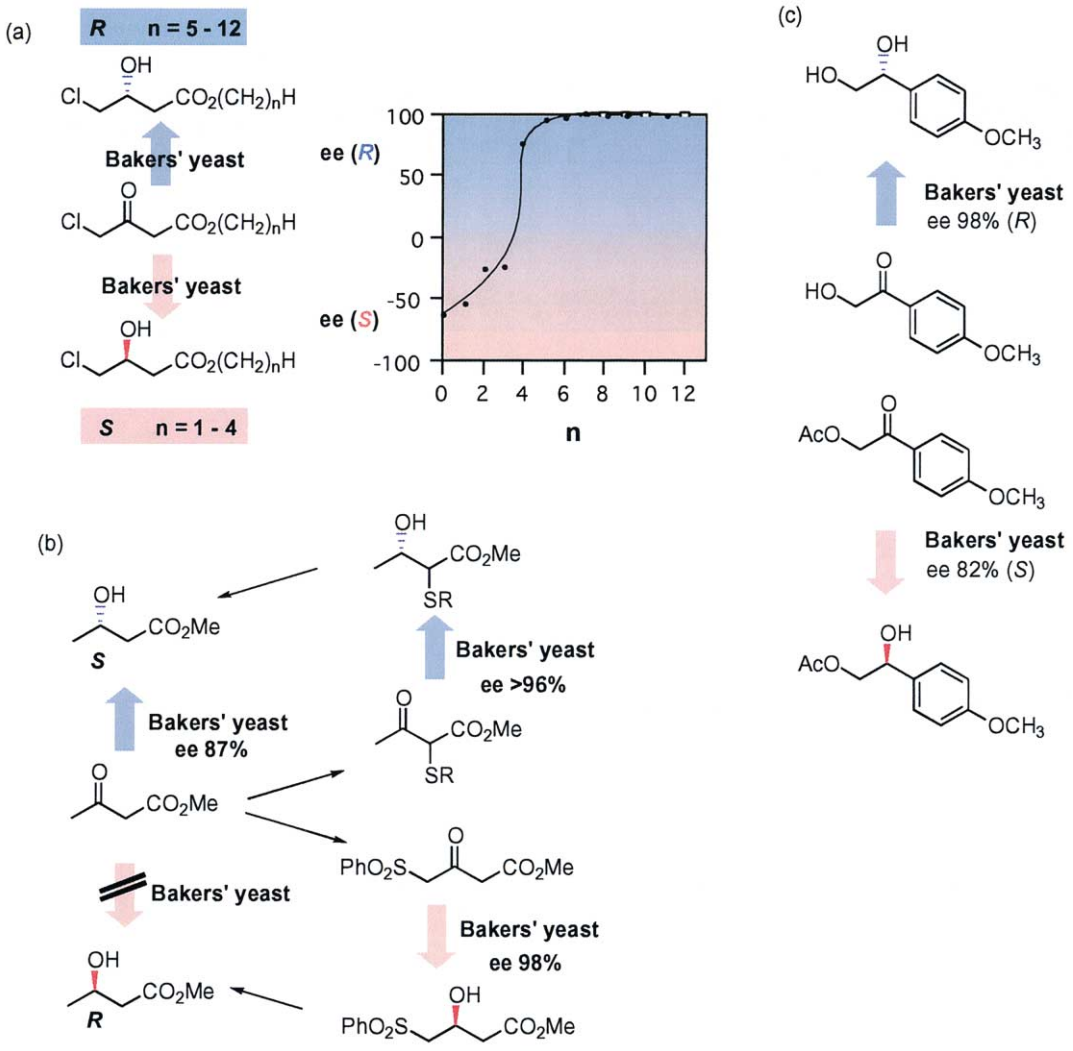
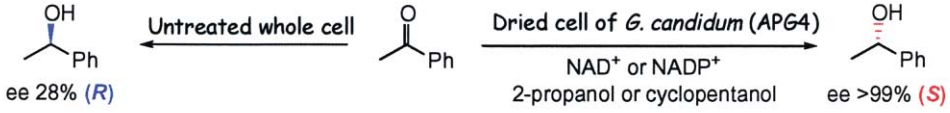


Figure 10. Modification of the substrate to control the enantioselectivities: (a) effect of the length of the ester moiety;[16a–c] (b) effect of introducing sulfur functionalities;[16d,e] (c) effect of hydroxy and acetoxy groups.[16f]

Table 1. Acetone treatment of *G. candidum* for the improvement of enantioselectivity[18]



Catalyst	Coenzyme	Additive	Yield (%)	ee(%)
Untreated whole cell	none	none	52	28 (*R*)
Acetone dried cell (APG4)	none	none	0	-
Acetone dried cell (APG4)	NAD^+	none	1	71 (*S*)
Acetone dried cell (APG4)	none	2-propanol	8	98 (*S*)
Acetone dried cell (APG4)	NAD^+	2-propanol	89	>99 (*S*)
Acetone dried cell (APG4)	NAD^+	cyclopentanol	97	>99 (*S*)
Acetone dried cell (APG4)	$NADP^+$	cyclopentanol	86	>99 (*S*)

ee(*R*)]. When the form of the catalyst was changed from wet whole-cell to dried powdered-cell (APG4), no reduction was observed, which would indicate the loss of the necessary co-enzyme(s) and/or co-enzyme regeneration system(s) during the treatment of the cells with acetone. The addition of co-enzyme NAD^+ did not have a significant effect on the yield. The addition of 2-propanol resulted in only a small increase in the yield,

but a significant improvement in the enantioselectivity was observed. Surprisingly, the addition of both NAD^+ and 2-propanol profoundly enhanced both chemical yield and enantiomeric excess. Furthermore, the addition of NADH, $NADP^+$ or NADPH instead of NAD^+ and the addition of cyclopentanol instead of 2-propanol also gave enantiomerically pure alcohol in high yield.

The improvement in the enantioselectivity from 28% (*R*) to >99% (*S*) was due to the suppression of every enzyme that reduces the substrate, followed by the stimulation of an (*S*)-directing enzyme by the addition of the coenzyme and an excess amount of 2-propanol, agents which push the equilibrium towards the reduction of the substrate.

It was confirmed, by separating the enzymes in the powder, that many (*S*)- and (*R*)-directing enzymes do indeed exist in the dried cells. The addition of a coenzyme and cyclopentanol stimulates only an (*S*)-enzyme because the specific (*S*)-enzyme can oxidize cyclopentanol (concomitantly reducing $NAD(P)^+$), while other (*S*) or (*R*)-enzymes can not use cyclopentanol as effectively.[18c] This presents a very interesting case, where the experimental conditions of reduction with a cell having both (*S*)- and (*R*)-directing enzymes was modified and resulted in excellent *S* enantioselectivity.

2.3.2. Solvent engineering. Biocatalysts can perform reduction in various solvents.[19–22] Non-aqueous solvents can be used to control the enantioselectivities as well as to improve the system's environmental friendliness. For example, the alcohol dehydrogenase from *G. candidum* was used in supercritical CO_2 around 100 atm and 35°C and found to catalyze the reduction of fluoroacetophenones etc. (Fig. 11a).[19a,b] The enantioselectivity obtained was equivalent to the system using other solvents.

Ionic liquids have also been attracting attention as environmentally friendly solvents and used for the biocatalytic reduction. For example, acetylacetone etc. were reduced by immobilized baker's yeast in the ionic liquid [bmim]PF_6 (Fig. 11b).[20]

Organic solvents have been used to control enantioselectivities in some cases.[21] When ethyl 2-oxohexanoate was reduced by baker's yeast in water, both (*R*)- and (*S*)-alcohols were produced and the (*S*)-alcohol was obtained as the major product as a result of the further enantioselective decomposition of the (*R*)-enantiomer (Fig. 11c).[21a,b] However, when the biotransformation was conducted in benzene, the (*R*)-alcohol was formed selectively at a high yield. This is because the effective concentration of the substrate in the aqueous layer around the enzyme decreased since the substrate dissolves preferably in the benzene layer. When there are plural enzymes in a cell with overlapping substrate specificities but different enantioselectivities, a change in the substrate concentration can change the type of enzyme species catalyzing the reduction because enzymatic reactions follow the Michaelis–Menten equation. Enzymes having low K_m values catalyze the reduction effectively under the low substrate concentration; however, other enzymes having high K_m values and high V_{max} values act effectively under high substrate concentration. In this case for the reduction of ethyl 2-oxohexanoate in benzene, an (*R*)-enzyme ($K_m = 0.14$ mM, $V_{max} = 41$ U/kg yeast) catalyzed the reduction predominantly rather than the (*S*)-enzymes ($K_m = 5$–27 mM, $V_{max} = 37$–649 U/kg yeast).[21b]

Additives such as cyclodextrin and hydrophobic polymers have been also used to control the concentration of the substrate or product.[22] For example, β-cyclodextrin was added to decrease the substrate concentration in order to control the enantioselectivities (Fig. 11d).[22a]

Another example of using additives to control the concentration of the substrate is the reduction of methyl benzyl ketone by *Zygosaccharomyces rouxii* for the synthesis of LY300164, a noncompetitive antagonist of the AMPA subtype of excitatory amino acid receptors.[22d] In this reaction, the hydrophobic polymer XAD was used to decrease the effective substrate concentration around the enzyme but not in the bulk. The adsorption properties of the resin on both the substrate and the product allowed a ketone loading of 80 g/L, while limiting the effective solution concentration of both the substrate and the product to sublethal concentrations of 2 g/L (Fig. 11e).

The enantioselectivities can be also controlled using the hydrophobic polymer.[22f] Without changing the biocatalysts or the substrates, both enantiomers were obtained by adding XAD and controlling the air supply, as shown in Figure 11f.

2.3.3. Inhibitors. In the case of observing poor overall enantioselectivity due to the presence of two competing enzymes with different enantioselectivities, one of the most straightforward methods for improving the enantioselectivity is to use an inhibitor of the unnecessary enzyme(s). Ethyl chloroacetate, methyl vinyl ketone, allyl alcohol, allyl bromide, sulfur compounds, Mg^{2+}, Ca^{2+}, etc. have been reported as inhibitors of enzymes in baker's yeast.[23] For example, the low enantioselectivity in the yeast reduction of the β-keto ester was improved by the addition of methyl vinyl ketone, Mg^{2+} or ethyl chloroacetate as described in Figure 12.[23a–c] By enzymatic studies using purified enzymes from baker's yeast, the enzymes inhibited were identified and the inhibition mechanism reported to be non-competitive.[23k]

2.3.4. Reaction temperature. The reaction temperature is one of the parameters affecting the enantioselectivity of a reaction.[24] For the oxidation of an alcohol, the values of k_{cat}/K_m were determined for the (*R*)-

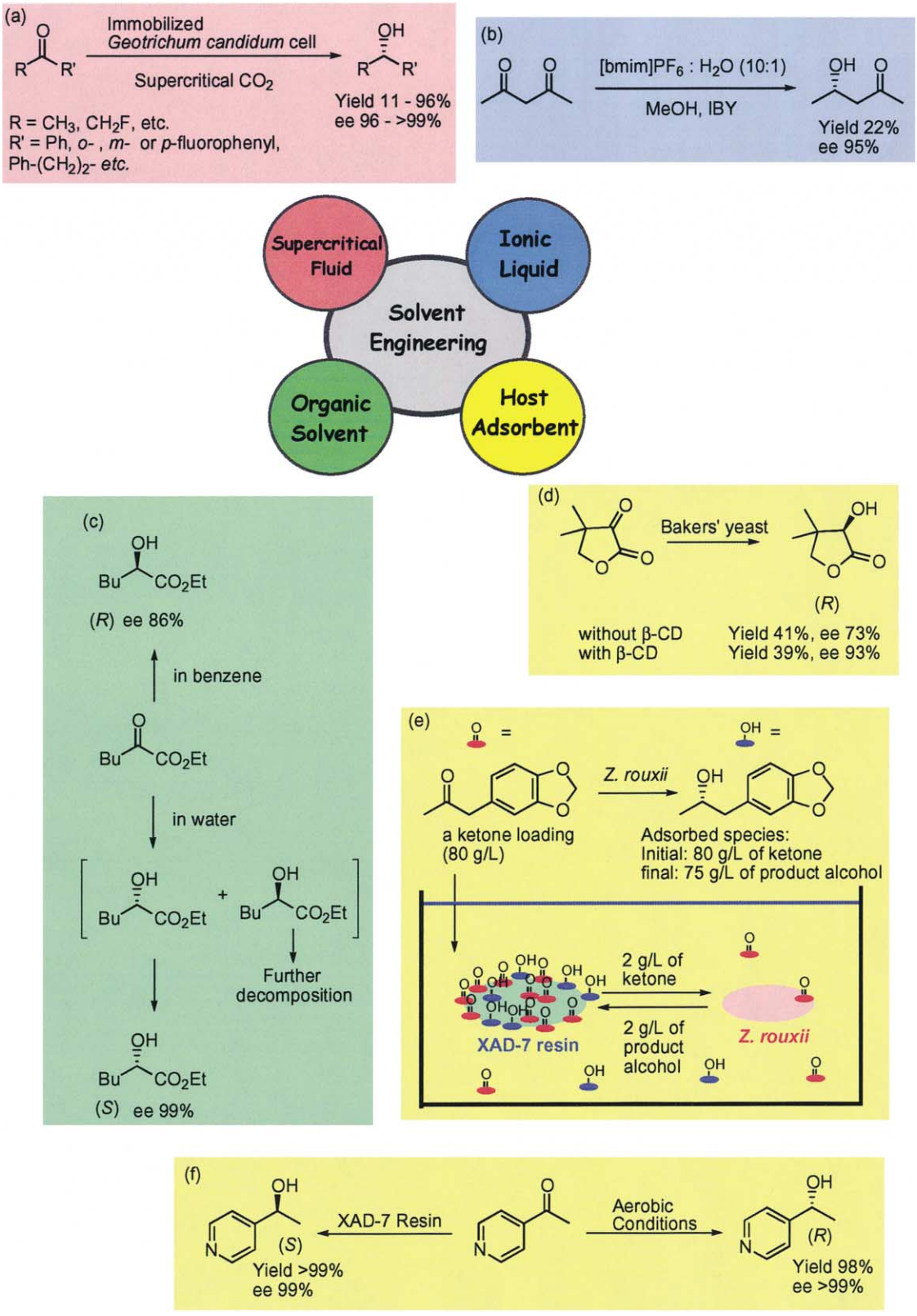


Figure 11. Reduction by biocatalysts in non-aqueous solvent.[19a,b,20,21a,b,22a,d,f]

and (*S*)-stereodefining enantiomers; *E* is the ratio between them. From the transition state theory, the free energy difference at the transition state between the (*R*)- and (*S*)-enantiomers can be calculated from *E* (Eq. (2)), and $\Delta\Delta G$ is in turn the function of temperature (Eq. (3)). The racemic temperature (T_r) can be calculated as shown in Eq. (4). Using these equations, T_r for 2-butanol and 2-pentanol of the thermoanaerobacter

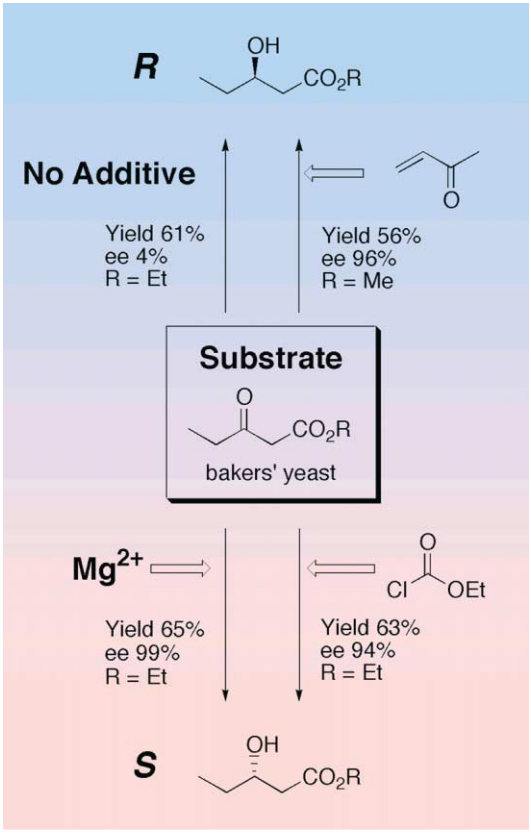


Figure 12. Improvement of the enantioselectivity by using an inhibitor of undesired enzymes.[23a,c]

ethanolicus alcohol dehydrogenase were determined to be 26°C and 77°C, respectively.

$$E=(k_{cat}/K_m)R/(k_{cat}/K_m)S \quad (1)$$

from transition state theory $-RT\ln(E)=\Delta\Delta G^{\ddagger}$ (2)

$$\Delta\Delta G^{\ddagger}=\Delta\Delta H^{\ddagger}-T\Delta\Delta S^{\ddagger} \quad (3)$$

When $\Delta\Delta G^{\ddagger}=0$, $T_r=\Delta\Delta H^{\ddagger}/\Delta\Delta S^{\ddagger}$ (4)

Since the transition state for alcohol oxidation and ketone reduction must be identical, the product distribution (under kinetic control) for reducing 2-butanone and 2-pentanone is also predictable. Thus, one would expect to isolate (*R*)-2-butanol if the temperature of the reaction was above 26°C. On the contrary, if the temperature is less than 26°C, (*S*)-2-butanol should result; in fact, the reduction of 2-butanone and 2-pentanone at 37°C resulted in 28% ee (*R*)- and 44% ee (*S*)-alcohol, respectively, as expected.[24a]

3. Applications

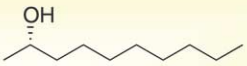
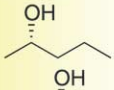
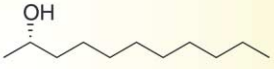
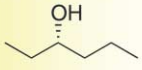
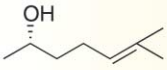
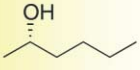
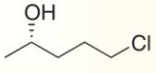
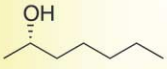
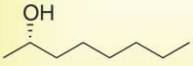
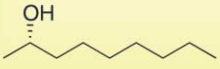
There are numerous examples of using biocatalysts for the asymmetric reductions.[1,25] Some of the representative reactions are shown in this section.

3.1. Reduction of aliphatic ketones

Although efficient chemical catalysts for the functionalized ketones have been developed, asymmetric reduction of small aliphatic ketones still remains a major challenge in organic chemistry. Highly enantioselective reduction can be achieved when biocatalysts are used in many cases. Herein, reductions using *G. candidum*, *T. brockii* and baker's yeast are shown.

Reductions using dried cells of *G. candidum* (APG4), NAD^+ and 2-propanol exhibit one of the widest substrate specificities together with very high enantioselectivities.[18] Simple aliphatic ketones from 2-butanone to 2-undecanone, in addition to 3-hexanone, 6-methyl-5-hepten-2-one and 5-chloro 2-pentanone, are also reduced by the APG4 system to the corresponding (*S*)-alkanols giving high yields and ee as shown in Table 2.[18a,b,e]

Table 2. Asymmetric reduction of aliphatic ketones to the corresponding (*S*)-alcohols with dried cells of *G. candidum*[18a,b,e]

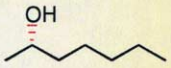
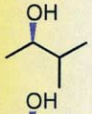
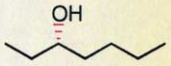
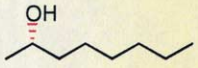
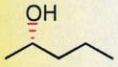
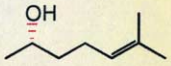
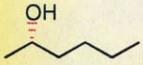
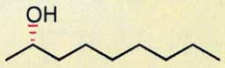
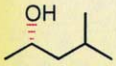
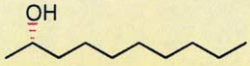
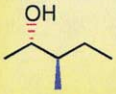
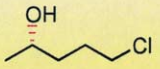
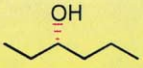
Product	Yield (%)	ee (%)	Product	Yield (%)	ee (%)
	73	94		85	>99
	97	>99		60	>99
	35	98		90	99
	89	>99		92	99
	89	>99		96	98
	87	>99		34	>99
	87	>99			

The alcohol dehydrogenase from *T. brockii* (TBADH) is also highly suitable for reducing aliphatic ketones.[26] Even very simple aliphatic ketones were reduced enantioselectively, as shown in Table 3. An interesting substrate size-induced reversal of enantioselectivity was observed: the smaller substrates (methyl ethyl, methyl isopropyl or methyl cyclopropyl ketones) were reduced to the (*R*)-enantiomers, whereas higher ketones produced the (*S*)-enantiomers.

TBADH is also used in the synthesis of bioactive compounds.[26c] In this case, 2,8-nonandione was reduced by TBADH to furnish the corresponding diol, from which all four isomers of 8-methyldec-2-yl propanoate, the western corn rootworm sex pheromone, were prepared (Fig. 13).

Baker's yeast also has a broad range of substrate specificities, and many synthetically valuable reactions

Table 3. Asymmetric reduction of aliphatic ketones with the alcohol dehydrogenase from *T. brockii*[26a]

Products	Relative rate	ee (%)	Config.	Products	Relative rate	ee (%)	Config.
	12.0	48	*R*		0.9	99	*S*
	3.0	86	*R*		0.2	95	*S*
	0.8	44	*R*		0.6	97	*S*
	3.3	79	*S*		0.3	99	*S*
	1.0	96	*S*		0.3	98	*S*
	0.3	95	*S*		0.1	99	*S*
	0.1	81	2*S*, 3*R*		1.5	98	*S*
	0.9	97	*S*				

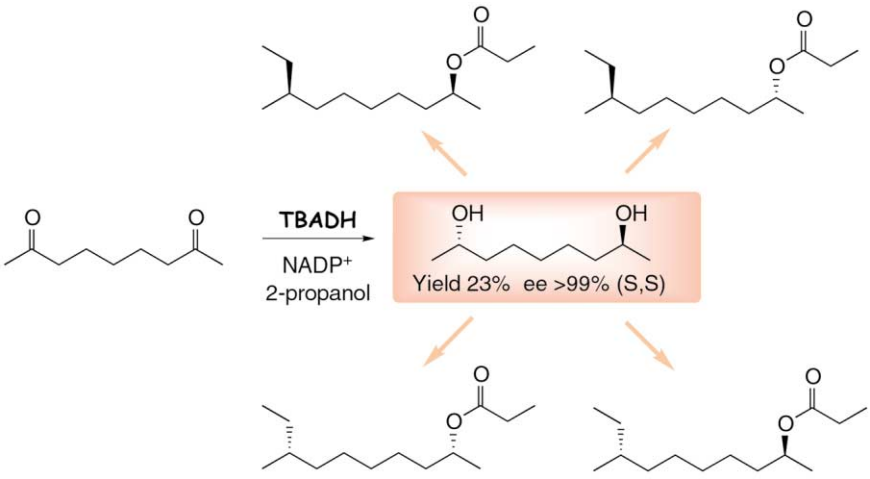


Figure 13. Synthesis of all four isomers of the western corn rootworm sex pheromone.[26c]

involving reductions of fluoroketones have been reported, as shown in Figure 14.[27] The difference between hydrogen and fluorine in the α-position was recognized by the enzyme.

3.2. Reduction of halogenated aromatic ketones

Aromatic ketones can be reduced by a number of biocatalysts such as baker's yeast,[1e] *Pseudomonas* sp. strain PED[3a] and *L. kefir*[3b] etc. The substrate specificities of these biocatalysts are reported elsewhere.[1] Herein, reduction of halogenated aromatic ketones is introduced since it is important to understand the interaction between halogenated compounds and proteins. The abilities of trifluoromethyl moiety to direct the enantioselectivity have been examined using various microorganisms including baker's yeast.[28] One of the most prominent effects of fluorination of the substrate is seen in the reduction of acetophenone derivatives by dried *G. candidum* cells (APG4).[18f–h] Reduction of methyl ketones afforded (*S*)-alcohols with excellent ees, whereas the reduction of trifluoromethyl ketones gave the corresponding alcohols of the opposite configuration, also with excellent ees (Fig. 15). Monofluoroacetophenone and difluoroacetophenone were also reduced under the same conditions. The reduction proceeded quantitatively for both substrates, and as expected, the stereoselectivity shifted from the acetophenone type to

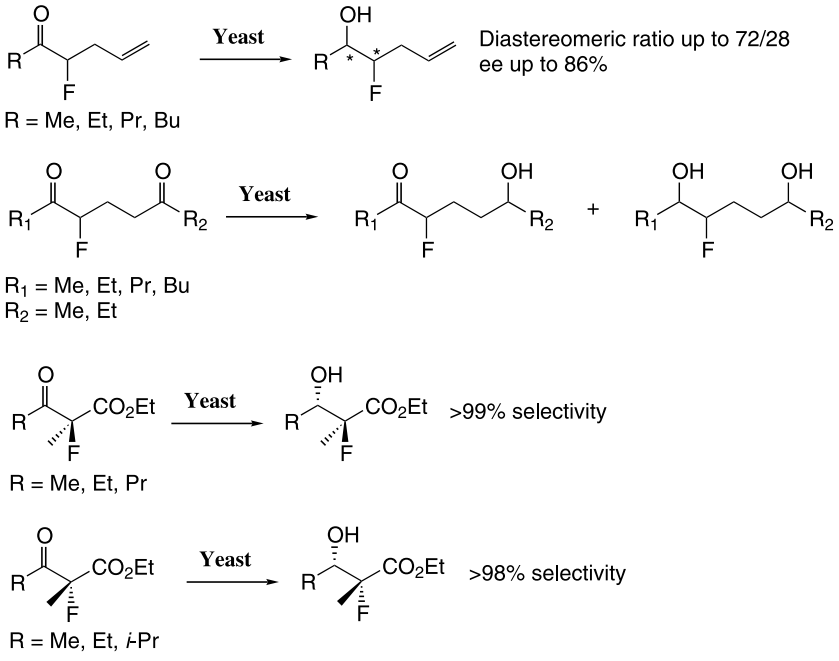


Figure 14. Reduction of fluorinated ketones by baker's yeast.[27]

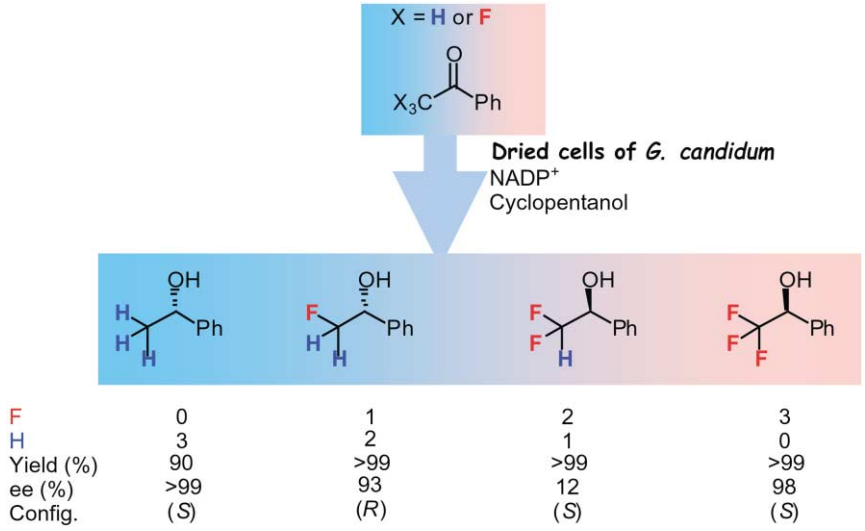


Figure 15. Effect of fluorine at the α-position of acetophenone on the stereoselectivity in the reduction by dried cells of *G. candidum*, NADP+ and cyclopentanol.[18f–h]

the trifluoroacetophenone type according to the number of fluorine substituents at the α-position.

The replacement of the methyl moiety with a trifluoromethyl group alters the bulkiness and electronic properties, so their effects on the enantioselectivities were examined. No inversion in stereochemistry was observed for the reduction of hindered ketones such as isopropyl ketone while the stereoselectivity was inverted for the reduction of ketones with electron-withdrawing atoms such as chlorine. This observation was explained by the presence of several enzymes with different enantioselectivities isolated from the dried cells; one of them catalyzed the reduction of methyl ketones, and another, with the opposite enantioselectivity, catalyzed the reduction of trifluoromethyl ketones.

Various enantiomerically pure fluorinated alcohols are produced by employing *G. candidum* reductions as shown in Table 4.[18f–h] Monofluoroacetophenone and difluoroacetophenone are reduced to the (*R*)-alcohols by the dried cells, NAD^+ and 2-propanol, and to the (*S*)-alcohols by a constituent enzyme previously separated by anion-exchange chromatography and using glucose-6-phosphate/glucose-6-phosphate dehydrogenase as the co-factor recycling system. Both enantiomers of monofluorophenylethanol can be obtained with excellent ees using only one microorganism.

Chiral trifluoromethyl benzyl alcohols are useful synthons for ferroelectric liquid crystals, and for this reason, Fujisawa et al. investigated the asymmetric reduction of the corresponding ketones using bakers' yeast.[29a,b] The enantioselectivity of the bakers' yeast reduction of trifluoroacetylbenzene derivatives was improved by the introduction of some functional groups at the *para*-position to give the corresponding (*R*)-trifluoromethyl substituted benzylic alcohols in high chemical and optical yields, as shown in Figure 16a. The functional group at the *para*-position was then used in further transformations. In the reduction of various trifluoromethyl biphenyl ketones, baker's yeast and *G. candidum* dried cells (APG4) are complementary to each other; yeast reduction affords (*R*)-alcohol, whereas *G. candidum* reduction affords (*S*)-alcohol (Fig. 16b).[29c]

Table 5 presents many other examples of the reduction of α-halomethyl ketones.[11f,18d,30] Various microorganisms are able to reduce fluoro-, chloro- and bromoketones. However, the reduction of iodoacetophenone usually results in a poor yield, mainly producing acetophenone or phenylethanol.

3.3. Reduction of ethyl 4-chloro-3-oxobutanoate

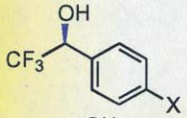
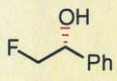
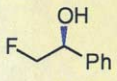
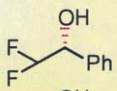
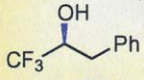
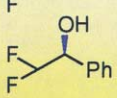
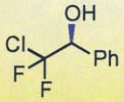
Several different biocatalysts have been used in the reduction of a variety of keto esters.[31] Among them, one of the most studied substrates is ethyl 4-chloro-3-oxobutanoate.[6,10a,d,11f,12b,d,16a,c,23d,31] The (*R*)- and (*S*)-enantiomers of the corresponding alcohol were produced by various microorganisms, as shown in Table 6. The (*R*)-enantiomer is a promising chiral building block for the synthesis of L-carnitine, an essential factor for the β-oxidation of fatty acids in mitochondria. The (*S*)-enantiomer is useful as an intermediate for chiral drugs.

3.4. Reduction of diketones

Regio- and enantioselective reduction of diketones can be achieved readily by using a biocatalyst.[32] As a result, optically active hydroxyketones and diols have been successfully synthesized.

To reduce α-diketones, the selectivity of the reduction to diol and to hydroxyketone was controlled using a diacetyl reductase from *Bacillus stearothermophilus*

Table 4. Synthesis of chiral fluorinated alcohols by the reduction with dried cells and isolated enzymes of *G. candidum* using NAD^+ or $NADP^+$ and 2-propanol or cyclopentanol[18f–h]

Product	Yield (%)[a]	ee (%)	Product	Yield (%)[a]	ee (%)
	X = H 84	98(*S*)		93	>99 (*R*)
	X = Cl 81	>99(*S*)		91	>99 (*S*)[b]
	X = Br 80	>99(*S*)		99	63 (*R*)
	74	98(*S*)		95	>99 (*S*)[b]
	82	94(*S*)			

[a] Yield after isolation and purifications.
[b] the isolated enzyme was used for the reduction

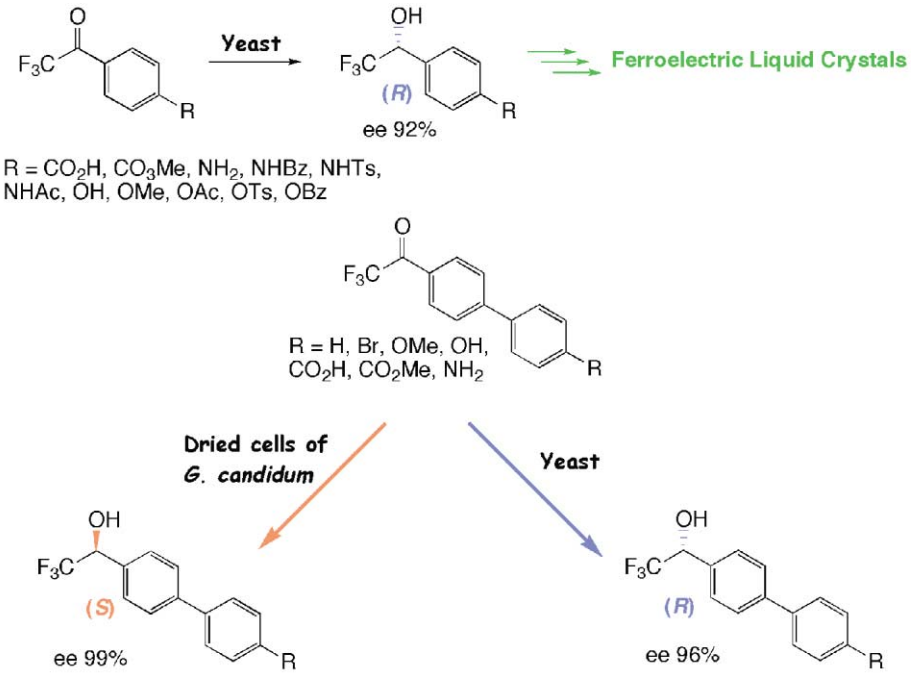


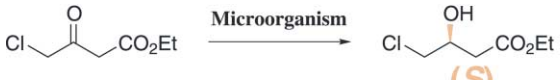
Figure 16. Reduction of trifluoroacetylbenzene derivatives and trifluoromethyl biphenyl ketones.[29]

Table 5. Reduction of α-halogenated acetophenone derivatives[11f,18d,30]

Microorganism	X	X'	Yield (%)	ee (%)	Config.	Ref
Cryptococcus macerans	Cl	H	80	100	*R*	30(a)
	Br	H	95	93	*R*	30(a)
Bakers' yeast	F	H	67	97	*R*	30b)
	Cl	H	37	90	*R*	30(b)
	Br	H	9	97	*R*	30(b)
	F	H	55	35	*R*	30(c)
	Cl	H	6 (40)[a]	68	*R*	30(c)
	Br	H	0 (15)[a]	-		30(c)
*Geotrichum candidum sp.*38	F	H	65	75	*S*	30(c)
	Cl	H	86	87.4	*S*	30(c)
	Br	H	15 (25)[a]	94	*S*	30(c)
Geotrichum candidum IFO4597 dried cell	Cl	*m*-Cl	94	98	*R*	18(d)
	Br	*m*-Cl	95	93	*R*	18(d)
Geotrichum candidum CBS 233.76	Cl	*m,p*-Cl	95	>98	*S*	30(d)
Rhodotorula mucillaginosa	Cl	*m,p*-Cl	88	>99	*R*	30(d)
Corynebacterium strain ST-10 Phenylacetaldehyde reductase expressed in *E. coli*	Cl	*m*-Cl	>86	>99	*R*	11(f)

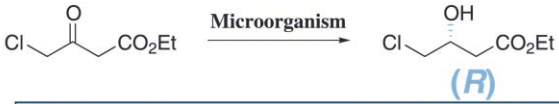
[a] Yield determined by HPLC analysis is in parenthesis.

Table 6. Comparison of various microorganisms for the reduction of ethyl 4-chloro-3-oxobutanoate



(*S*)

Microorganism	Yield (%)	ee (%)	ref.
Geotrichum candidum SC5469	83	96	31(a)
Bakers' Yeast	100	90	23(d)
Bakers' Yeast		55	16(a)
Lactobacillus kefir	100	100	31(b)
Candida magnoliae (overexpressed in *E. coli*)	85	100	10(a)
Kluyveromyces lactis	97	>98	12(d)



(*R*)

Microorganism	Yield (%)	ee (%)	ref.
Dancus carota	42	52	31(c)
Sporobolomyces salmonicolor (overexpressed in *E. coli*)	94.1	91.7	10(d)
Lactobacillus fermentum	70	98	31(b)
Saccharomyces cerevisiae (FAS (β-keto reductase) negative)	53	90	16(c)
Candida parapsilosis IFO 1396 (overexpressed in *E. coli*)	95.2	>99	12(b)
Carbonyl reductase from *Rhodococcus erythropolis*	100	>99	6
Corynebacterium strain ST-10 Phenylacetaldehyde reductase (overexpressed in *E. coli*)	>86	99	11(f)

(Fig. 17).[32f] When the same enzyme was used for the substrate reduction and co-enzyme recycling using *endo*-bicyclo[3.2.0]hept-2-en-6-ol as a hydrogen source, both carbonyl groups were reduced selectively to produce a diol. On the other hand, α-hydroxyketones were obtained using glucose-6-phosphate/glucose-6-phosphate dehydrogenase for co-enzyme recycling. The synthetic potential of both systems has been illustrated by the synthesis of the male sex pheromone of the grape borer *Xylotrechus pyrrhoderus*, identified as a two-component mixture of the reduction products, (*S*,*S*)-2,3-octanediol and (*S*)-2-hydroxyloctan-3-one.

Regio- and enantioselective reduction of β-diketones may be conducted using biocatalysts. For example, a diketo ester, *tert*-butyl 2,5-dioxohexanonate, was reduced by the alcohol dehydrogenase from *Lactobacillus brevis* to provide the corresponding hydroxyketo ester with 99.4% ee in 78% yield; this was used as an intermediate for the synthesis of dimeric metabolite vioxanthin of *Penicillium citreo-viride* in order to develop an assay system for monitoring phenol oxidative coupling in lignan formation (Fig. 18a).[32a] Baker's yeast reduction also proceeds regio- and enantioselectively with aliphatic diketones, 2,2-disubstituted cycloalkanediones, and spiro dione producing hydroxyketones with excellent enantio- and diastereoselectivities, as shown in Figure 18b–e.[32b–e,j] Starting from the chiral hydroxyketone, many terpenes have been enantioselectively synthesized by Mori et al. as shown in Figure 18d.[32b,c]

3.5. Reduction of hydroxy ketones

Generally, microbial reduction of α-hydroxyketones affords (*R*)-diols in high enantioselecvitities.[33] Thus, chiral diols of various functionalities have been obtained by reduction with baker's yeast (Table 7a)[33a–e]

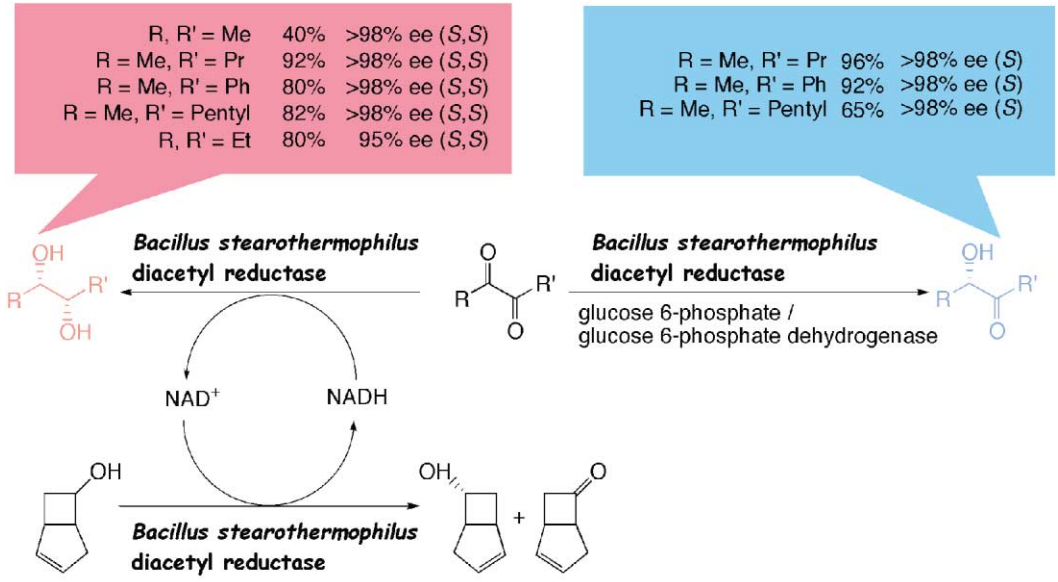


Figure 17. Reduction of α-diketones by diacetyl reductase from *B. stearothermophilus*.[32f]

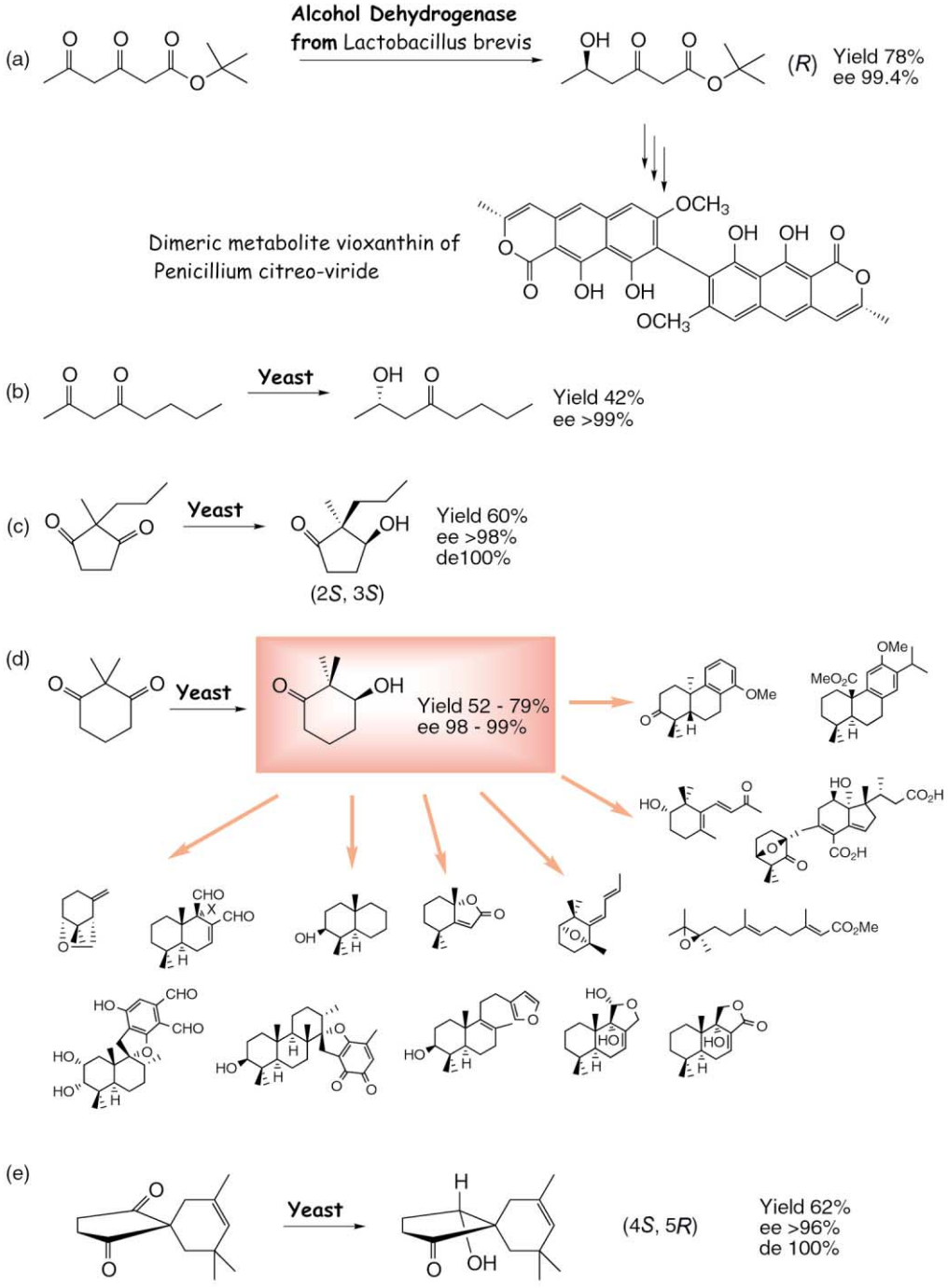


Figure 18. Regio- and enantioselective reduction of diketones.[32a–e]

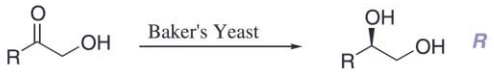
and *Geotrichum* sp. (Table 7b).[33f] However, *ortho*-substituted aryl hydroxyketones afforded (*S*)-diol by the reduction with *Geotrichum* sp. (Table 7b).[33f]

3.6. Reduction of ketones containing sulfur functionalities

Ketones containing sulfur functionalities have been known to afford high enantioselectivities on enzymatic reduction compared to the original (unsubstituted) ketones. Furthermore, the attachment of a sulfonyl group to a ketone often changes the stereochemical course of yeast reduction as shown in Section 2.2. Reduction of ketones containing sulfide,[34a,b] thioacetal,[34c] dithioester[34d] or sulfone[34a,e,f] moieties by yeast or *Corynebacterium* afforded (*S*)-alcohols in high enantioselectivities as shown in Figure 19a. On the contrary, *Pichia farinosa*,[34g] and *G. candidum*[34e] afforded (*R*)-alcohols also in high enantioselectivities on reduction (Fig. 19b). Since a sulfoxide has chirality at the sulfur atom, reduction of a sulfonylketone proceeded with

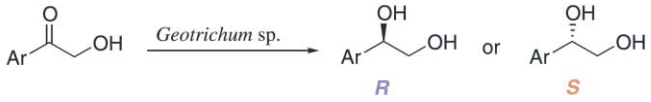
Table 7. Reduction of hydroxy ketones[33a–f]

(a)



R	Yield (%)	ee (%)	ref
Et	58	100	33(a)
C_5H_{11}	56	100	33(b)
$-(CH_2)_2CH=CMe_2$	68	97	33(c)
p-$MeOC_6H_4$	85	98	33(d)
$-CH_2SO_2Ph$	87	99	33(e)
$-(CH_2)_2SO_2Ph$	42	94	33(e)
$-(CH_2)_3SO_2Ph$	74	93	33(e)
$-(CH_2)_5SO_2Ph$	39	96	33(e)

(b)



Ar	Yield (%)	ee (%)	config.
Ph	62	91	(*S*)
m-ClC_6H_4	93	77	(*S*)
p-ClC_6H_4	91	96	(*S*)
m-$NO_2C_6H_4$	84	99	(*S*)
p-$NO_2C_6H_4$	95	100	(*S*)
o-ClC_6H_4	77	60	(*R*)
o-$NO_2C_6H_4$	37	47	(*R*)

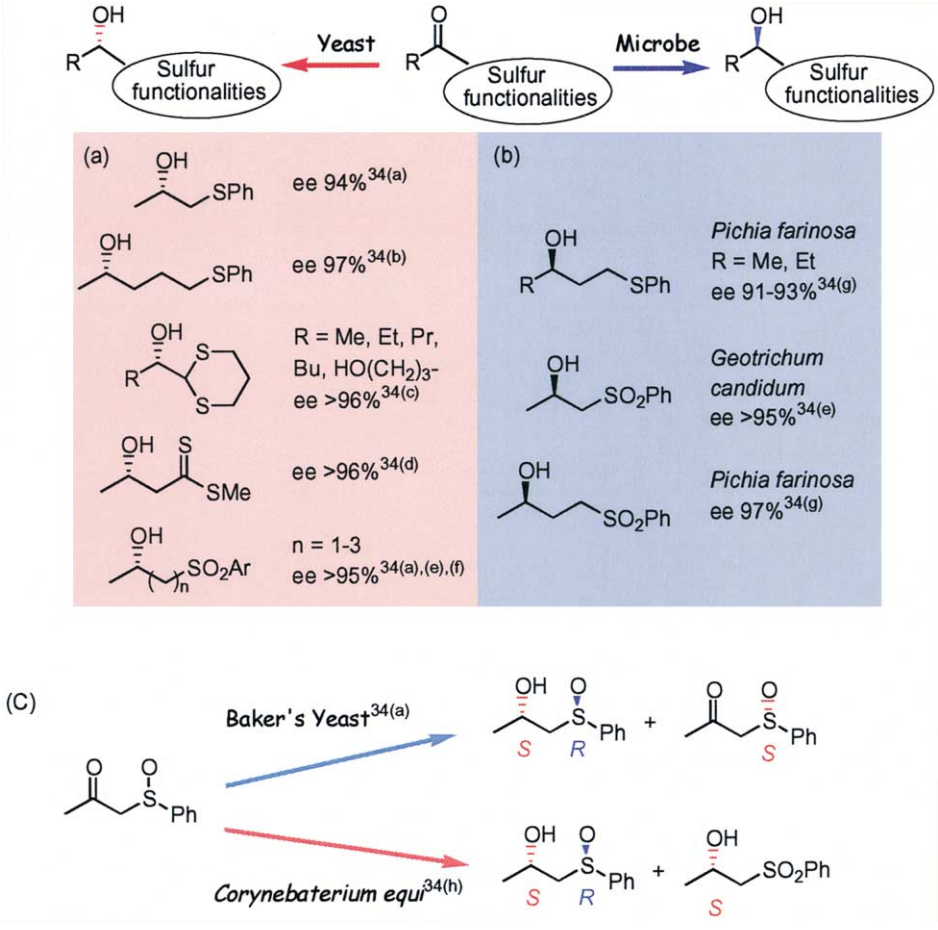


Figure 19. Reduction of ketones containing sulfur functionalities.[34a–h]

kinetic resolution. Yeast reduction of racemic phenylsulfinylacetone afforded the (*R*s,*S*c)-alcohol and small amount of the (*S*s,*S*c)-alcohol both in >95% ee remaining (*S*)-sulfoxide in >98% ee.[34a] The reduction of the same sulfonylketone by *Corynebacterium* afforded the (*R*s,*S*c) alcohol and (*S*)-phenylsulfenyl-2-propanol[34h] (Fig. 19c).

3.7. Large-scale synthesis

Large-scale synthesis of the chiral alcohol by biocatalytic reduction is possible, although many works only report the small-scale or analytical-scale synthesis. The examples are shown in Figure 20. Both recombinant and native microorganisms can accumulate large concentrations of the products.[9g,10a,18g,32b,33a,b]

4. Conclusion

Herein we have reviewed methodologies to control the enantioselectivities of biocatalytic asymmetric reduction and selected examples are shown. In the last decade, significant progress has been made, so that it is now possible to reduce various ketones to both (*S*)- and (*R*)-alcohols with excellent ees. In the next decade, many useful reactions in the laboratory will be developed to be industrially important when the space–time yield, which is still usually lower than metal-catalyzed processes, will be improved by the screening of the organisms, overexpression and directed evolution of the enzymes, and the modification of the reaction conditions.

In the future, the biocatalysts will be even more important with the shift of the raw materials from oil to biomass. Since the biomass is a mixture of various multi functional compounds, chemo-, regio- and enantioselective catalysts will be indispensable, and biocatalysts will play an important role. Then, biocatalysts will perform better since the substrate will be natural rather than man-made.

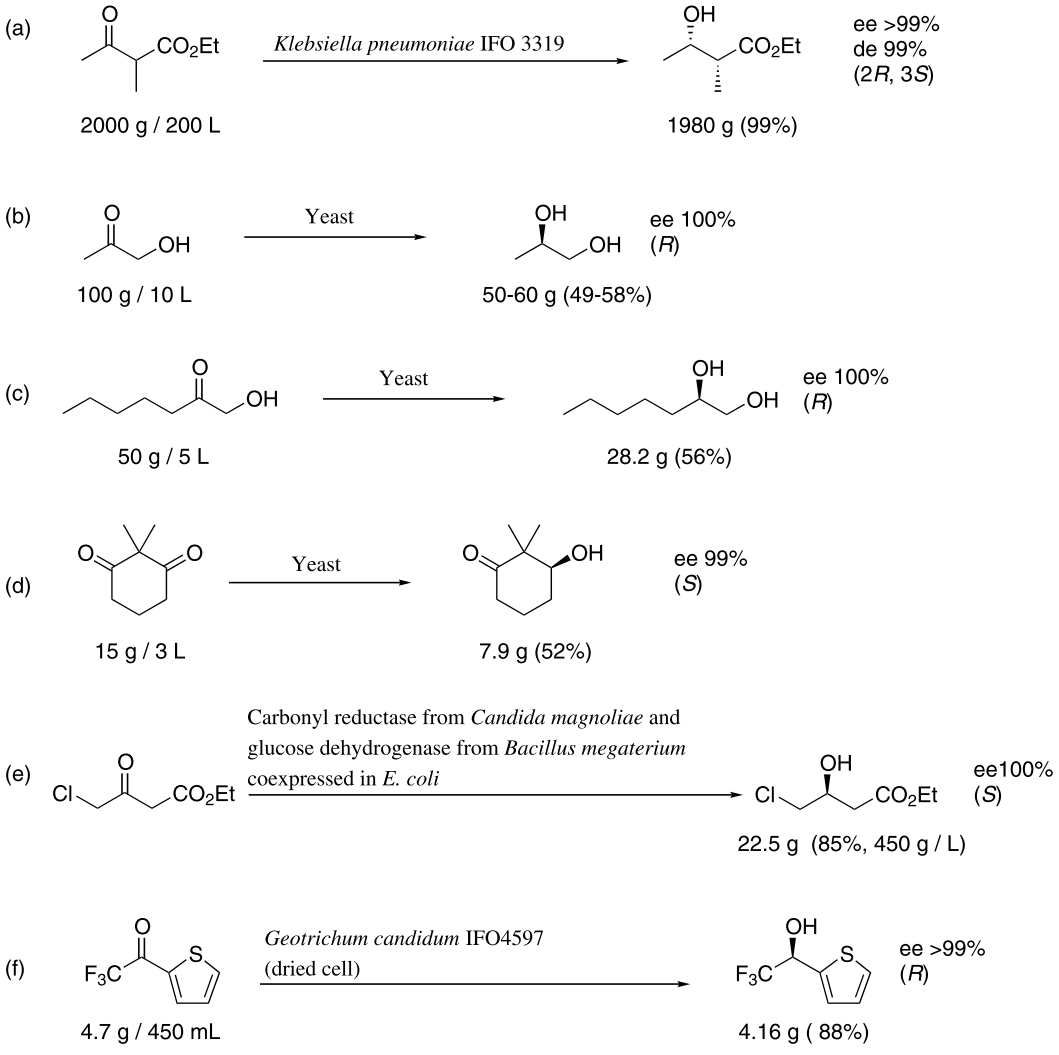


Figure 20. Large-scale synthesis.[9g,10a,18g,32b,33]

References

1. (a) Santaniello, E.; Ferraboschi, P.; Manzocchi, A. In *Enzymes in Action*; Zwanenburg, B.; Mikolajczyk, M.; Kielbasinski, P., Eds.; Kluwer Academic Publishers: Dordrecht, 2000; pp. 95–115; (b) Faber, K. In *Biotransformations in Organic Chemistry*, 2nd ed.; Springer-Verlag: Berlin, 1995; pp. 145–180; (c) Hummel, W.; Kula, M.-R. *Eur. J. Biochem.* **1989**, *184*, 1–13; (d) Nakamura, K.; Matsuda, T. In *Enzyme Catalysis in Organic Synthesis. A comprehensive Handbook*; Drauz, K.; Waldmann, H., Eds.; Wiley-VCH: Weinheim, 2002; pp. 991–1047; (e) Servi, S. *Synthesis* **1990**, 1–25; (f) Roberts, S. M. *J. Chem. Soc., Perkin Trans. 1* **2000**, 611–633; (g) Roberts, S. M. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1–21; (h) Roberts, S. M. *J. Chem. Soc., Perkin Trans. 1* **1998**, 157–169; (i) Azerad, R. *Bull. Soc. Chim. Fr.* **1995**, *132*, 17–51.
2. Schomburg, D.; Salzmann, M. (GFB-Gesellschaft für Biotechnologische Forschung), Enzyme Handbook, Springer-Verlag, Berlin, D, 1990.
3. (a) Bradshaw, C. W.; Fu, H.; Shen, G.-J.; Wong, C.-H. *J. Org. Chem.* **1992**, *57*, 1526–1532; (b) Bradshaw, C. W.; Hummel, W.; Wong, C.-H. *J. Org. Chem.* **1992**, *57*, 1532–1536.
4. (a) Nakamura, K.; Shiraga, T.; Miyai, T.; Ohno, A. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 1735–1737; (b) Nakamura, K.; Takano, S.; Terada, K.; Ohno, A. *Chem. Lett.* **1992**, 951–954; (c) Nakamura, K.; Yoneda, T.; Miyai, T.; Ushio, K.; Oka, S.; Ohno, A. *Tetrahedron Lett.* **1988**, *29*, 2453–2454; (d) Dutler, H.; Van Der Baan, J. L.; Hochuli, E.; Kis, Z.; Taylor, K. E.; Prelog, V. *Eur. J. Biochem.* **1977**, *75*, 423–432.
5. (a) Prelog, V. *Pure Appl. Chem.* **1964**, *9*, 119–130; (b) Jones, J. B. *Tetrahedron* **1986**, *42*, 3351–3403; (c) Jones, J. B.; Beck, J. F. In *Applications of Biochemical Systems in Organic Chemistry*; Jones, J. B.; Sih, C. J.; Perlman, D., Eds.; John Wiley and Sons: New York, 1976; pp. 107–401; (d) Davies, J.; Jones, J. B. *J. Am. Chem. Soc.* **1979**, *101*, 5405–5410; (e) Lam, L. K. P.; Gair, I. A.; Jones, J. B. *J. Org. Chem.* **1988**, *53*, 1611–1615; (f) Velonia, K.; Tsigos, I.; Bouriotis, V.; Smonou, I. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 65–68.
6. Zelinski, T.; Kula, M.-R. *Bioorg. Med. Chem. Lett.* **1994**, *2*, 421–428.
7. (a) Nakamura, K.; Yamanaka, R.; Tohi, K.; Hamada, H. *Tetrahedron Lett.* **2000**, *41*, 6799–6802; (b) Nakamura, K.; Yamanaka, R. *Chem. Commun.* **2002**, 1782–1783; (c) Nakamura, K.; Yamanaka, R. *Tetrahedron: Asymmetry* **2002**, *13*, 2529–2533; (d) Parkinson, B. A.; Weaver, P. F. *Nature* **1984**, *309*, 148–149.
8. (a) Yuan, R.; Watanabe, S.; Kuwabata, S.; Yoneyama, H. *J. Org. Chem.* **1997**, *62*, 2494–2499; (b) Biade, A.-E.; Bourdillon, C.; Laval, J.-M.; Mairesse, G.; Moiroux, J. *J. Am. Chem. Soc.* **1992**, *114*, 893–897; (c) Leonida, M. D.; Sobolov, S. B.; Fry, A. J. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2819–2824; (d) Kuwabata, S.; Tsuda, R.; Nishida, K.; Yoneyama, H. *Chem. Lett.* **1993**, 1631–1634; (e) Kuwabata, S.; Tsuda, R.; Yoneyama, H. *J. Am. Chem. Soc.* **1994**, *116*, 5437–5443; (f) Sugimura, K.; Kuwabata, S.; Yoneyama, H. *J. Am. Chem. Soc.* **1989**, *111*, 2361–2362; (g) Sugimura, K.; Kuwabata, S.; Yoneyama, H. *Bioelectrochem. Bioenergetics.* **1990**, *24*, 241–247.
9. (a) Demirjian, D. C.; Shah, P. C.; Morís-Varas, F. In *Biocatalysis From Discovery to Application*; Fessner, W.-D., Ed.; Springer-Verlag: Berlin, 2000; pp. 1–29; (b) Yasohara, Y.; Kizaki, N.; Hasegawa, J.; Takahashi, S.; Wada, M.; Kataoka, M.; Shimizu, S. *Appl. Microbiol. Biotechnol.* **1999**, *51*, 847–851; (c) Wada, M.; Kataoka, M.; Kawabata, H.; Yasohara, Y.; Kizaki, N.; Hasegawa, J.; Shimizu, S. *Biosci. Biotech. Biochem.* **1998**, *62*, 280–285; (d) Wada, M.; Kawabata, H.; Yoshizumi, A.; Kataoka, M.; Nakamori, S.; Yasohara, Y.; Kizaki, N.; Hasegawa, J.; Shimizu, S. J. *Biosci. Bioeng.* **1999**, *87*, 144–148; (e) Shimizu, S.; Hata, H.; Yamada, H. *Agric. Biol. Chem.* **1984**, *48*, 2285–2291; (f) Kataoka, M.; Shimizu, S.; Doi, Y.; Sakamoto, K.; Yamada, H. *Biotechnol. Lett.* **1990**, *12*, 357–360; (g) Miya, H.; Kawada, M.; Sugiyama, Y. *Biosci. Biotech. Biochem.* **1996**, *60*, 95–98; (h) Shafiee, A.; Motamedi, H.; King, A. *Appl. Microbiol. Biotechnol.* **1998**, *49*, 709–717.
10. (a) Kizaki, N.; Yasohara, Y.; Hasegawa, J.; Wada, M.; Kataoka, M.; Shimizu, S. *Appl. Microbiol. Biotechnol* **2001**, *55*, 590–595; (b) Yasohara, Y.; Kizaki, N.; Hasegawa, J.; Wada, M.; Kataoka, M.; Shimizu, S. *Biosci. Biotech. Biochem.* **2000**, *64*, 1430–1436; (c) Yasohara, Y.; Kizaki, N.; Hasegawa, J.; Wada, M.; Kataoka, M.; Shimizu, S. *Tetrahedron: Asymmetry* **2001**, *12*, 1713–1718; (d) Kataoka, M.; Yamamoto, K.; Kawabata, H.; Wada, M.; Kita, K.; Yanase, H.; Shimizu, S. *Appl. Microbiol. Biotechnol* **1999**, *51*, 486–490; (e) Kita, K; Fukura, T.; Nakase, K.; Okamoto, K.; Yanase, H.; Kataoka, M.; Shimizu, S. *Appl. Environ. Microbiol.* **1999**, *65*, 5207–5211; (f) Yoshisumi, A.; Wada, M.; Takagi, H.; Shimizu, S.; Nakamori, S. *Biosci. Biotech. Biochem.* **2001**, *65*, 830–836; (g) Wada, M.; Yoshizumi, A.; Nakamori, S.; Shimizu, S. *Appl. Environ. Microbiol.* **1999**, *65*, 4399–4403; (h) Kataoka, M.; Kotaka, A.; Hasegawa, A.; Wada, M.; Yoshizumi, A.; Nakamori, S.; Shimizu, S. *Biosci. Biotech. Biochem.* **2002**, *66*, 2651–2657.
11. (a) Itoh, N.; Yoshida, K.; Okada, K. *Biosci. Biotech. Biochem.* **1996**, *60*, 1826–1830; (b) Itoh, N.; Morihama, R.; Wang, J.; Okada, K.; Mizuguchi, N. *Appl. Environ. Microbiol.* **1997**, *63*, 3783–3788; (c) Itoh, N.; Mizuguchi, N.; Mabuchi, M. *J. Mol. Catal. B: Enzymatic* **1999**, *6*, 41–50; (d) Wang, J.-C.; Sakakibara, M.; Liu, J.-Q.; Dairi, T.; Itoh, N. *Appl. Microbiol. Biotechnol* **1999**, *52*, 386–392; (e) Wang, J.-C.; Sakakibara, M.; Matsuda, M.; Itoh, N. *Biosci. Biotech. Biochem.* **1999**, *63*, 2216–2218; (f) Itoh, N.; Matsuda, M.; Mabuchi, M.; Dairi, T.; Wang, J. *Eur. J. Biochem.* **2002**, *269*, 2394–2402.
12. (a) Matsuyama, A.; Yamamoto, H.; Kabayashi, Y. *Org. Proc. Res. Develop.* **2002**, *6*, 558–561; (b) Yamamoto, H.; Matsuyama, A.; Kabayashi, Y. *Biosci. Biotech. Biochem.* **2002**, *66*, 481–483; (c) Yamamoto, H.; Matsuyama, A.; Kabayashi, Y. *Biosci. Biotech. Biochem.* **2002**, *66*, 925–927; (d) Yamamoto, H.; Kimoto, N.; Matsuyama, A.; Kabayashi, Y. *Biosci. Biotech. Biochem.* **2002**, *66*, 1775–1778; (e) Matsuyama, A.; Yamamoto, H.; Kawada, N.; Kabayashi, Y. *J. Mol. Catal. B: Enzymatic* **2001**, *11*, 513–521; (f) Yamamoto, H.; Kawada, N.; Matsuyama, A.; Kabayashi, Y. *Biosci. Biotech. Biochem.* **1999**, *63*, 1051–1055; (g) Yamamoto, H.; Matsuyama, A.; Kabayashi, Y.; Kawada, N. *Biosci. Biotech. Biochem.* **1995**, *59*, 1769–1770.

13. (a) Kayser, M. M.; Mihovilovic, M. D.; Kearns, J.; Feicht, A.; Stewart, J. D. *J. Org. Chem.* **1999**, *64*, 6603–6608; (b) Rodrìguez, S.; Schroeder, K. T.; Kayser, M. M.; Stewart, J. D. *J. Org. Chem.* **2000**, *65*, 2586–2587; (c) Rodrìguez, S.; Kayser, M. M.; Stewart, J. D. *J. Am. Chem. Soc.* **2001**, *123*, 1547–1555.
14. Allen, S. J.; Holbrook, J. *J. Protein Eng.* **2000**, *13*, 5–7.
15. (a) Nakayama, G. R.; Schultz, P. G. *J. Am. Chem. Soc.* **1992**, *114*, 780–781; (b) Hsieh, L. C.; Yonkovich, S.; Kochersperger, L.; Schultz, P. G. *Science* **1993**, *260*, 337–339.
16. (a) Zhou, B.-n.; Gopalan, A. S.; VanMiddlesworth, F.; Shieh, W.-R.; Sih, C. J. *J. Am. Chem. Soc.* **1983**, *105*, 5925–5926; (b) Chen, C.-S.; Zhou, B.-n.; Girdaukas, G.; Shieh, W.-R.; VanMiddlesworth, F.; Gopalan, A. S.; Sih, C. J. *Bioorg. Chem.* **1984**, *12*, 98–117; (c) Shieh, W.-R.; Gopalan, A. S.; Sih, C. J. *J. Am. Chem. Soc.* **1985**, *107*, 2993–2994; (d) Fujisawa, T.; Itoh, T.; Sato, T. *Tetrahedron Lett.* **1984**, *25*, 5083–5086; (e) Nakamura, K.; Ushio, K.; Oka, S.; Ohno, A.; Yasui, S. *Tetrahedron Lett.* **1984**, *25*, 3979–3982; (f) Ferraboschi, P.; Grisenti, P.; Manzocchi, A.; Santaniello, E. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2469–2474.
17. Goodhue, C. T.; Rosazza, J. P.; Peruzzotti, G. P. In *Manual of Industrial Microbiology and Biotechnology*; Demain, A. L.; Solomon, N. A., Eds.; American Society for Microbiology: Washington DC, 1986; pp. 97–121.
18. (a) Nakamura, K.; Kitano, K.; Matsuda, T.; Ohno, A. *Tetrahedron Lett.* **1996**, *37*, 1629–1632; (b) Nakamura, K.; Matsuda, T. *J. Org. Chem.* **1998**, *63*, 8957–8964; (c) Matsuda, T.; Harada, T.; Nakajima, N.; Nakamura, K. *Tetrahedron Lett.* **2000**, *41*, 4135–4138; (d) Hamada, H.; Miura, T.; Kumobayashi, H.; Matsuda, T.; Harada, T.; Nakamura, K. *Biotechnol. Lett.* **2001**, *23*, 1603–1606; (e) Matsuda, T.; Nakajima, Y.; Harada, T.; Nakamura, K. *Tetrahedron: Asymmetry* **2002**, *13*, 971–974; (f) Nakamura, K.; Matsuda, T.; Itoh, T.; Ohno, A. *Tetrahedron Lett.* **1996**, *37*, 5727–5730; (g) Nakamura, K.; Matsuda, T.; Shimizu, M.; Fujisawa, T. *Tetrahedron* **1998**, *54*, 8393–8402; (h) Matsuda, T.; Harada, T.; Nakajima, N.; Itoh, T.; Nakamura, K. *J. Org. Chem.* **2000**, *65*, 157–163; (i) Nakamura, K.; Matsuda, T.; Harada, T. *Chirality* **2002**, *14*, 703–708.
19. (a) Matsuda, T.; Harada, T.; Nakamura, K. *Chem. Commun.* **2000**, 1367–1368; (b) Matsuda, T.; Watanabe, K.; Kamitanaka, T.; Harada, T.; Nakamura, K. *Chem. Commun.* **2003**, 1198; (c) Panza, J. L.; Russell, A. J.; Beckman, E. J. *Tetrahedron* **2002**, *59*, 4091–4104.
20. Howarth, J.; James, P.; Dai, J. *Tetrahedron Lett.* **2001**, *42*, 7517–7519.
21. (a) Nakamura, K.; Kondo, S.; Kawai, Y.; Ohno, A. *Bull. Chem. Soc. Jpn.* **1993**, *66*, 2738–2743; (b) Nakamura, K.; Kondo, S.; Nakajima, N.; Ohno, A. *Tetrahedron* **1995**, *51*, 687–694; (c) Nakamura, K.; Inoue, Y.; Ohno, A. *Tetrahedron Lett.* **1995**, *36*, 265–266; (d) Nakamura, K.; Inoue, Y.; Matsuda, T.; Misawa, I. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2397–2402.
22. (a) Nakamura, K.; Kondo, S.; Kawai, Y.; Ohno, A. *Tetrahedron: Asymmetry* **1993**, *4*, 1253–1254; (b) Nakamura, K.; Kondo, S.; Ohno, A. *Bioorg. Med. Chem.* **1994**, *2*, 433–437; (c) Zelinski, T.; Kula, M.-R. *Biocatal. Biotransformation* **1997**, *15*, 57–74; (d) Anderson, B. A.; Hansen, M. M.; Harkness, A. R.; Henry, C. L.; Vicenzi, J. T.; Zmijewski, M. J. *J. Am. Chem. Soc.* **1995**, *117*, 12358–12359; (e) Nakamura, K.; Fujii, M.; Ida, Y. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3205–3211; (f) Nakamura, K.; Takenaka, K.; Fujii, M.; Ida, Y. *Tetrahedron Lett.* **2002**, *43*, 3629–3631; (g) Conceicao, G. J. A.; Moran, P. J. S.; Rodrigues, J. A. R. *Tetrahedron: Asymmetry* **2003**, *14*, 43–45; (h) D'Arrigo, P.; Fantoni, G. P.; Servi, S.; Strini, A. *Tetrahedron: Asymmetry* **1997**, *8*, 2375–2379; (i) D'Arrigo, P.; Fuganti, C.; Fantoni, G. P.; Servi, S. *Tetrahedron* **1998**, *54*, 15017–15026; (j) D'Arrigo, P.; Lattanzio, M.; Fantoni, G. P.; Servi, S. *Tetrahedron: Asymmetry* **1998**, *9*, 4021–4026.
23. (a) Nakamura, K.; Kawai, Y.; Ohno, A. *Tetrahedron Lett.* **1990**, *31*, 267–270; (b) Nakamura, K.; Inoue, K.; Ushio, K.; Oka, S.; Ohno, A. *Chem. Lett.* **1987**, 679–682; (c) Nakamura, K.; Kawai, Y.; Oka, S.; Ohno, A. *Tetrahedron Lett.* **1989**, *30*, 2245–2246; (d) Dahl, A. C.; Fjeldberg, M.; Madsen, J. Ø. *Tetrahedron: Asymmetry* **1999**, *10*, 551–559; (e) Nakamura, K.; Kawai, Y.; Oka, S.; Ohno, A. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 875–879; (f) Forni, A.; Moretti, I.; Prati, F.; Torre, G. *Tetrahedron* **1994**, *50*, 11995–12000; (g) Kim, J.-H.; Oh, W.-T. *Bull. Korean Chem. Soc.* **1992**, *13*, 2–3; (h) Hayakawa, R.; Nozawa, K.; Shimizu, M.; Fujisawa, T. *Tetrahedron Lett.* **1998**, *39*, 67–70; (i) Ushio, K.; Hada, J.; Tanaka, Y.; Ebara, K. *Enzyme Microb. Technol.* **1993**, *15*, 222–228; (j) Hayakawa, R.; Shimizu, M.; Fujisawa, T. *Tetrahedron: Asymmetry* **1997**, *8*, 3201–3204; (k) Nakamura, K.; Kawai, Y.; Nakajima, N.; Ohno, A. *J. Org. Chem.* **1991**, *56*, 4778–4783.
24. (a) Pham, V. T.; Phillips, R. S.; Ljungdahl, L. G. *J. Am. Chem. Soc.* **1989**, *111*, 1935–1936; (b) Zheng, C.; Pham, V. T.; Phillips, R. S. *Catal. Today* **1994**, *22*, 607–620; (c) Tripp, A. E.; Burdette, D. S.; Zeikus, J. G.; Phillips, R. S. *J. Am. Chem. Soc.* **1998**, *120*, 5137–5141; (d) Heiss, C.; Laivenieks, M.; Zeikus, J. G.; Phillips, R. S. *J. Am. Chem. Soc.* **2001**, *123*, 345–346.
25. (a) Benedetti, F.; Forzato, C.; Nitti, P.; Pitacco, G.; Valentin, E.; Vicario, M. *Tetrahedron: Asymmetry* **2001**, *12*, 505–511; (b) Comasseto, J. V.; Omri, T.; Andrade, L. H.; Porto, A. L. M. *Tetrahedron: Asymmetry* **2003**, *14*, 711–715; (c) Sakai, A.; Bakke, M.; Ohta, H.; Kosugi, H.; Sugai, T. *Chem. Lett.* **1999**, 1255–1256; (d) Ema, T.; Moriya, H.; Kofukuda, T.; Ishida, T.; Maehara, K.; Utaka, M.; Sakai, T. *J. Org. Chem.* **2001**, *66*, 8682–8684.
26. (a) Keinan, E.; Hafeli, E. K.; Seth, K. K.; Lamed, R. *J. Am. Chem. Soc.* **1986**, *108*, 162–169; (b) Keinan, E.; Seth, K. K.; Lamed, R. *J. Am. Chem. Soc.* **1986**, *108*, 3474–3480; (c) Keinan, E.; Sinha, S. C.; Sinha-Bagchi, A. *J. Org. Chem.* **1992**, *57*, 3631–3636.
27. (a) Kitazume, T.; Nakayama, Y. *J. Org. Chem.* **1986**, *51*, 2795–2799; (b) Kitazume, T.; Kobayashi, T. *Synthesis* **1987**, 187–188.
28. (a) Kitazume, T.; Yamazaki T. In *Selective Fluorination in Organic and Bioorganic Chemistry*, ACS Symposium Series No. 456; Welch, J. T., Ed.; American Chemical Society, 1991; pp. 175–185; (b) Kitazume, T.; Sato, T. *J. Fluorine Chem.* **1985**, *30*, 189–202; **1996**, *37*, 3903–3906.
29. (a) Fujisawa, T.; Ichikawa, K.; Shimizu, M. *Tetrahedron: Asymmetry* **1993**, *4*, 1237–1240; (b) Fujisawa, T.; Sugimoto, T.; Shimizu, M. *Tetrahedron: Asymmetry* **1994**, *5*, 1095–1098; (c) Fujisawa, T.; Onogawa, Y.; Sato, A.; Mitsuya, T.; Shimizu, M. *Tetrahedron* **1998**, *54*, 4267–4276.

30. (a) Imuta, M.; Kawai, K.; Ziffer, H. *J. Org. Chem.* **1980**, *45*, 3352–3355; (b) Carvalho, M.d.; Okamoto, M. T.; Moran, P. J. S.; Rodrigues, J. A. R. *Tetrahedron* **1991**, *47*, 2073–2080; (c) Wei, Z.-L.; Li, Z.-Y.; Lin, G.-Q. *Tetrahedron* **1998**, *54*, 13059–13072; (d) Barbieri, C.; Caruso, E.; D'Arrigo, P.; Fantoni, G. P.; Servi, S. *Tetrahedron: Asymmetry* **1999**, *10*, 3931–3937.
31. (a) Patel, R. N.; McNamee, C. G.; Banerjee, A.; Howell, J. M.; Robison, R. S.; Szarka, L. *J. Enzyme Microb. Technol.* **1992**, *14*, 731–738; (b) Aragozzini, F.; Valenti, M.; Santaniello, E.; Ferraboschi, P.; Grisenti, P. *Biocatalysis* **1992**, *5*, 325–332; (c) Akakabe, Y.; Takahashi, M.; Kamezawa, M.; Kikuchi, K.; Tachibana, H.; Ohtani, T.; Naoshima, Y. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1295–1298.
32. (a) Drochner, D.; Müller, M. *Eur. J. Org. Chem.* **2001**, 211–215; (b) Mori, K.; Mori, H. *Org. Synth.* **1993**, Coll. Vol. 8, 312–315; (c) Mori, K.; Takayama, S.; Yoshimura, S. *Liebigs Ann. Chem.* **1993**, 91–95; (d) Brooks, D. W.; Mazdiyasni, H.; Grothaus, P. G. *J. Org. Chem.* **1987**, *52*, 3223–3232; (e) Ohta, H.; Ozaki, K.; Tuchihashi, G. *Agric. Biol. Chem.* **1986**, *50*, 2499–2502; (f) Bortolini, O.; Fantin, G.; Fogagnolo, M.; Giovannini, P. P.; Guerrini, A.; Medici, A. *J. Org. Chem.* **1997**, *62*, 1854–1856; (g) Fuhshuku, K.; Funa, N.; Akeboshi, T.; Ohta, H.; Hosomi, H.; Ohba, S.; Sugai, T. *J. Org. Chem.* **2000**, *65*, 129–135; (h) Bel-Rhlid, R.; Fauve, A.; Renard, M. F.; Veschambre, H. *Biocatalysis* **1992**, *6*, 319–337; (i) Ikeda, H.; Sato, E.; Sugai, T.; Ohta, H. *Tetrahedron* **1996**, *52*, 8113–8122; (j) Zhu, Y.-Y.; Burnell, D. J. *Tetrahedron: Asymmetry* **1996**, *7*, 3295–3304; (k) Lee, S.-S.; Yan, J.-L.; Wang, K. C. *Tetrahedron: Asymmetry* **1997**, *8*, 3051–3058; (l) Besse, P.; Bolte, J.; Fauve, A.; Veschambre, H. *Bioorg. Chem.* **1993**, *21*, 342–353; (m) Nakamura, K.; Kondo, S.; Kawai, Y.; Hida, K.; Kitano, K.; Ohno, A. *Tetrahedron: Asymmetry* **1996**, *7*, 409–412; (n) Sakakibara, M.; Ogawa-Uchida, A. *Biosci. Biotech. Biochem.* **1995**, *59*, 1300–1303.
33. (a) Levene, P. A.; Walti, A. *Org. Synth.* **1943**, Coll. Vol. 2, 545–547; (b) Barry, J.; Kagan, H. B. *Synthesis* **1981**, 453–455; (c) Ramaswamy, S.; Oehischlager, A. C. *Tetrahedron* **1991**, *47*, 1145–1156; (d) Ferraboschi, P.; Grisenti, P.; Manzocchi, A.; Santaniello, E. *J. Chem. Soc., Perkin 1* **1990**, 2469–2474; (e) Sato, T.; Okumura, Y.; Itai, J.; Fujisawa, T. *Chem. Lett.* **1988**, 1537–1540; (f) Wei, Z.-L.; Lin, G.-Q.; Li, Z.-Y. *Bioorg. Med. Chem.* **2000**, *8*, 1129–1137; (g) Kodama, M.; Minami, H.; Mima, Y.; Fukuyama, Y. *Tetrahedron Lett.* **1990**, *31*, 4025–4026.
34. (a) Iriuchijima, S.; Kojima, N. *Agric. Biol. Chem.* **1978**, *22*, 451–455; (b) Liu, H.; Cohen, T. *J. Org. Chem.* **1995**, *60*, 2022–2025; (c) Fujisawa, T.; Kojima, E.; Itoh, T.; Sato, T. *Chem. Lett.* **1985**, 1751–1754; (d) Itoh, T.; Yonekawa, Y.; Sato, T.; Fujisawa, T. *Tetrahedron Lett.* **1986**, *27*, 5405–5408; (e) Robin, S.; Huet, F.; Fauve, A.; Veschambre, H. *Tetrahedron: Asymmetry* **1993**, *4*, 239–246; (f) Gopalan, A. S.; Jacobs, H. K. *Tetrahedron Lett.* **1990**, *31*, 5575–5578; (g) Ohtsuka, Y.; Katoh, O.; Sugai, T.; Ohta, H. *Bull. Chem. Soc. Jpn.* **1997**, *70*, 483–491; (h) Ohta, H.; Kato, Y.; Tsuchihashi, G. *J. Org. Chem.* **1987**, *52*, 2735–2739.