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Control of enantioselectivity in the baker's yeast asymmetric reduction of γ-chloro β-diketones to γ-chloro (*S*)-β-hydroxy ketones

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Abstract

1-Chloro-2,4-alkanediones **1a–f** prepared in one step were reduced using baker's yeast to afford 1-chloro-2-hydroxy-4-alkanones **2a–f** regioselectively in *S* 29% to *R* 58% ee. Use of a small amount of organic solvents to dissolve the substrate enhanced the enantiomeric excess in favor of the *S* configuration. The function of the organic solvents was studied with reducing enzymes isolated from baker's yeast. Some polar solvents selectively inhibited the enzymes, while nonpolar solvents enhanced the concentration of substrate in water. Application of inhibitors with heat-treatment and organic solvents as additives enhanced the enantiomeric excesses of the products to *S* 66–96% ee.

1. Introduction

Optically active β-hydroxy ketones are useful building blocks in organic synthesis. The chiral 1,3-diol skeleton found in a variety of natural products such as antibiotics and pheromones[1] can be obtained from the chiral β-hydroxy ketones.[2] Thus, synthesis of chiral β-hydroxy ketones has been investigated intensively. For example, reduction of chiral β,δ-diketosulfoxides with DIBAL[3] and aldol reactions between ketones and aldehydes using a chiral ligand[4] gave β-hydroxy ketones in high enantioselectivities. On the other hand, in the enantioselective reduction of prochiral ketones by use of baker's yeast,[5] especially interesting is the fact that high regio- and enantioselectivities are achieved in the reduction of β-diketones to afford β-hydroxy ketones.[6] We have been investigating the preparation of functionalized β-hydroxy ketones by use of baker's yeast and reported that the baker's yeast reduction of 1-acetoxy-2,4-alkanediones afforded (*S*)-1-acetoxy-2-hydroxy-4-alkanones with high regio- and enantioselectivities.[7] In connection with the work, we have also been studying the reduction of 1-chloro-2,4-alkanediones **1**

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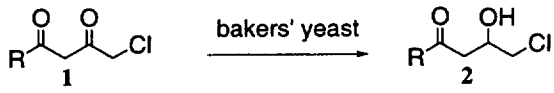
to 1-chloro-2-hydroxy-4-alkanones **2** to capitalize on the synthetic versatility of the chloro substituent as reported in a preliminary paper.[8] Herein we wish to report the scope of the asymmetric reduction of **1** to (*S*)-**2** using baker's yeast (Scheme 1).



R			
a	CH_3	**d**	n-C_4H_9
b	C_2H_5	**e**	n-C_5H_{11}
c	n-C_3H_7	**f**	CH_2=$CHCH_2CH_2$

Scheme 1.

Reduction of β-keto esters using baker's yeast cells was reported to be catalyzed by some enzymes producing the (*S*)-enantiomers and by other enzymes producing the (*R*)-enantiomers,[9] and thus the enantioselectivity can be controlled by inhibiting either of those enzymes. Recent successes in the stereochemical control of the baker's yeast reduction of β-keto esters seemed to be promising[10] and prompted us to apply the control to attain high enantioselectivity in the reduction of our γ-chloro β-diketones **1**. Therefore, we tried to inhibit the reducing enzymes selectively by addition of inhibitors[10a] and heat-treatment of the yeast,[10b] and found that these methods were also effective in enhancing the enantiomeric excesses of products **2** in favor of the *S* configuration.

Incidentally, in the course of our work we noticed that a small amount of organic solvent, used to dissolve the substrate, could enhance the enantioselectivity in favor of the (*S*)-enantiomer. Thus, the effects of a small amount of organic solvent are also described in detail. Organic solvents so far have been used as bulk solvents[11] or substrate-dissolving solvents,[12] but such a use as additives to control the enantioselectivity has never been reported.

2. Results and discussion

2.1. Preparation of the substrates

γ-Chloro β-diketones **1** were prepared in 19–50% yield in one step by a condensation reaction between ethyl chloroacetate and lithium enolates formed from methyl ketones and lithium diisopropylamide (LDA). Two equivalents of the enolate were added to the ester in the reaction[13] due to the acidity of the produced γ-chloro β-diketone **1**. Use of chloroacetyl chloride instead of the chloroacetate, gave only about 10% of the desired product **1**. We failed to obtain 1-chloro-4-phenyl-2,4-butanedione. The product was found to be 5-phenyl-3-oxo-2,3-dihydrofuran. The phenyl group seems to enhance the enolization of the carbonyl group at the C-4 position, leading to the formation of the conjugated ring structure.

2.2. Baker's yeast reduction under conventional conditions

The regio- and enantioselectivities in the yeast reduction of γ-chloro β-diketones **1** were investigated under conventional conditions. As shown in Table 1, the regioselectivity was perfect, as observed in the reduction of 1-acetoxy-2,4-alkanediones using the yeast.[7] The regioisomer having a hydroxy group at the C-4 position was not detected, even in the reduction of **1a** (R=CH_3) having a rather easily reducible methyl ketone functionality at the C-4. The enantioselectivity, however, varied from *S* 29% to *R* 58% ee

Table 1
Reduction of 1-chloro-2,4-alkanediones **1** to 1-chloro-2-hydroxy-4-alkanones **2** with baker's yeast[a]

R	time (h)	yield (%)	ee (%)	$[\alpha]_D$[b]	*R/S*
CH_3	1.0	53	29	-6.85	*S*
C_2H_5	1.0	70	14	+5.20	*R*
n-C_3H_7	1.0	84	6	-1.88	*S*
n-C_4H_9	1.0	76	39	+12.2	*R*
n-C_5H_{11}	2.5	58	58	+18.2	*R*
CH_2=$CHCH_2CH_2$	2.0	74	35	+12.8	*R*

[a] Substrate 0.5 mmol, bakers' yeast 2.0 g, water 38 mL, glucose 2.0 g, 30 °C.
[b] *c* 1.9–2.3, $CHCl_3$.

depending on the alkyl chain length, in contrast to that for the reduction of 1-acetoxy-2,4-alkanediones which mostly kept *S* 95–98% ee. The low enantioselectivity is similar to that for the reduction of 1-chloro-2-alkanones varying from *S* 83% to *R* 65% ee.[14] It seems that 1-chloro-2,4-alkanediones **1** are reduced by the yeast as derivatives of 1-chloro-2-alkanones, not as those of 2,4-alkanediones which are seemingly reduced as methyl ketones activated by the β-keto group. It has been reported that an isolated carbon–carbon double bond cannot be reduced by the yeast.[15] Similarly, the double bond remained intact in the hydroxyl ketone **2f**. The products **2d** and **2f** have similar ees and yields. The enzymes cannot distinguish between the alkyl and alkenyl groups remote from the active site.

2.3. *Effects of organic solvents as additives*

Compound **1c** (R=*n*-C_3H_7) was chosen as a probe to test various organic solvents in detail, because it was reduced to a nearly racemic product of *S* 6% ee in the conventional yeast reduction. As shown in Table 2, almost all of the organic solvents enhanced the enantiomeric excess in favor of the *S* configuration considerably. The hydrophobic nonpolar solvents such as hexane, 2-methylpentane, and octane were most effective, whilst the effects of cyclohexane, heptane, and benzene were smaller or negligible. The polar solvents such as tetrahydrofuran, ethyl acetate, and diethyl ether were more effective than the hydrophilic solvents such as acetone and alcohol.

To explain the mechanism by which the organic solvents used in small quantities caused significant enhancement in the enantioselectivity, we suggest the following two factors. One is the action of organic solvents as inhibitors. It is well-known that enzymes are denatured in aqueous–organic mixtures. In order to estimate the highest concentration of organic solvent that allows the reduction, various concentrations of hexane, tetrahydrofuran, ethyl acetate, and ethanol were tested. As shown in Fig. 1, we found that the reaction took place at almost all concentrations of hexane, except neat hexane as a solvent. On the other hand, no reaction took place in the region where concentrations of ethyl acetate, tetrahydrofuran, and ethanol exceeded 5%, 12%, and 34%, respectively, indicating occurrence of the inhibition of enzymes or metabolic pathway.

In order to clarify the function of the small amount of organic solvents, we isolated the reductases from the cells of baker's yeast using 1-chloro-2,4-heptanedione **1c** as substrate. We found three dominant competing enzymes. Among these, *R*-enzyme-1 and -2 catalyzed the reduction of **1c** to (*R*)-**2c** with 94%

Table 2
Effects of organic solvents as an additive on the reduction of 1-chloro-2,4-heptanedione **1c** to 1-chloro-2-hydroxy-4-heptanone **2c** with baker's yeast[a]

organic solvent	yield (%)	ee (%)	*R/S*
none	84	6	*S*
2-methylpetane	87	71	*S*
octane	82	67	*S*
hexane	82	60	*S*
cyclohexane	65	41	*S*
heptane	75	39	*S*
tetrahydrofuran	78	57	*S*
ethyl acetate	80	55	*S*
diethyl ether	75	45	*S*
acetone	78	38	*S*
methanol	67	30	*S*
ethanol	70	26	*S*
benzene	65	6	*S*

[a] Substrate 0.5 mmol, dry bakers' yeast 2.0 g, water 38 mL, organic solvent 0.5 ml (1.3%), glucose 2.0 g, 30 °C, 1–6 h.

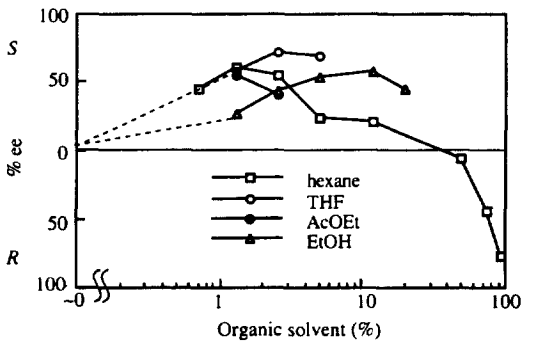


Fig. 1. Effects of the concentration of organic solvents on the reduction of **1c** to **2c** with baker's yeast

and 44% ee,[†] respectively, and *S*-enzyme-1 afforded (*S*)-**2c** with 97% ee. The activity of the isolated enzymes toward **1c** was measured in the presence of 1.3% of organic solvent (Fig. 2). We found that hexane did not affect the activity of the enzymes, while ethyl acetate, tetrahydrofuran, and diethyl ether decreased the activities of *R*-enzyme-2 and *S*-enzyme-1 to about 20% and 50%, respectively. The effect of ethanol was small.

The other factor is the enhanced concentration of substrate due to the solubilizing or dispersing power of organic solvents. It is known that a variation of substrate concentration can shift the enantiomeric excess in the yeast reductions[16] because the variation should affect the kinetics of each enzyme. We noticed that, without a small amount of organic solvent, it took more than 20 minutes to dissolve the substrate completely into water under the reaction conditions in spite of the fact that about 90% of

[†] The isolated *R*-enzyme-2 was purified further and the purified enzyme catalyzed the reduction of **1c** to give **2c** with *R* 98% ee.

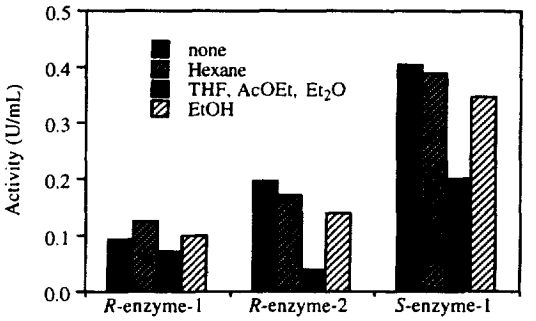


Fig. 2. Activity of the crude enzymes toward **1c** in the presence of 1.3% organic solvent

the substrate was converted to the product within 30 minutes. Thus, the reduction proceeded in much lower substrate concentrations than 13 mM as calculated (0.5 mmol of the substrate in 38 ml of water). When the substrate, dissolved in a small amount of organic solvent, was added to the reaction mixture, it is thought that the function of organic solvent was to change the state of the substrate in the aqueous reaction mixture and make the reaction proceed in a substrate concentration near to 13 mM. This function is essentially different from that of the organic solvents used in bulk, where the substrate is partitioned between organic and aqueous layers and the enantioselectivity is affected by the diluted substrate concentration in water. Therefore, the effect of the substrate concentration on the enantioselectivity was tested in detail under conventional conditions. As shown in Fig. 3, the enantioselectivity increased toward the (*S*)-enantiomer with an increase in the concentration of substrate. The results show that K_m of the *R*-enzyme is smaller than that of the *S*-enzyme in baker's yeast and the enantioselectivity can be enhanced in favor of the *S* configuration by increasing the substrate concentration.

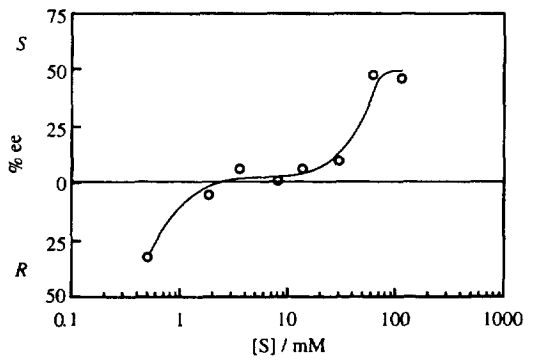


Fig. 3. Effects of substrate concentration on the reduction of **1c** to **2c** with baker's yeast

Thus, it can be concluded that the function of organic solvents is to enhance the effective concentration of the substrate in water, while the function as enzyme inhibitors is conceivable for ethyl acetate, diethyl ether, and tetrahydrofuran. The characterization of the reductases will be reported in due course.

2.4. *Effects of inhibitor, heat-treatment, and organic solvent*

We chose the substrate **1c** (R=*n*-C_3H_7) to select the inhibitor and reduction conditions (Table 3). Thus we found that allyl alcohol was better than methyl vinyl ketone as an *S*-enhancing inhibitor (entries 2 and 3) and the maximal *S* selectivity (89% ee) was obtained when the yeast was heat-treated at 50°C for 30 minutes with 67 mM allyl alcohol prior to the addition of the neat substrate (entry 12). An alternative procedure of the heat-treatment followed by addition of allyl alcohol was found to be less effective (entry

Table 3
Effects of inhibitor, heat-treatment, and organic solvent on the reduction of 1-chloro-2,4-heptanedione **1c** to (*S*)-1-chloro-2-hydroxy-4-heptanone **2c** with baker's yeast[a]

entry	inhibitor (mM)	time of heat-treatment at 50 °C (min)	organic solvent 0.5 (mL)	yield (%)	ee (%)	$[\alpha]_D$[b]	*R/S*
1	none	none	none	84	6	-1.88	*S*
2	MVK[c] (6.7)	none	diethyl ether	75	46	-16.0	*S*
3	AA[d] (6.7)	none	diethyl ether	78	57	-19.2	*S*
4	AA (20)	none	diethyl ether	81	76	-26.1	*S*
5	AA (40)	none	diethyl ether	80	77	-25.6	*S*
6	AA (67)	none	none	87	80	-28.3	*S*
7	AA (67)	none	diethyl ether	82	81	-26.1	*S*
8	AA (134)	none	diethyl ether	58	76	-25.4	*S*
9	none	30	none	86	39	-14.0	*S*
10	AA (67)	10	diethyl ether	78	87	-28.8	*S*
11	AA (67)	20	diethyl ether	70	89	-30.4	*S*
12	AA (67)	30	none	51	89	-31.6	*S*
13[e]	AA (67)	30	diethyl ether	80	71	-25.4	*S*
14	AA (67)	30	diethyl ether	63	91	-31.7	*S*
15	AA (67)	30	hexane	80	90	-29.6	*S*
16	AA (67)	30	hexane + diethyl ether	70	94	-34.1	*S*
17	AA (67)	40	diethyl ether	38	90	-31.1	*S*

[a] Conditions: Substrate 0.5 mmol, dry bakers' yeast 2 g, water 38 mL, glucose 2 g, 30 °C, 2 h.
[b] *c* 1.9–2.3, $CHCl_3$.
[c] Methyl vinyl ketone.
[d] Allyl alcohol.
[e] Allyl alcohol was added after heat-treatment.

13). The effect of a small amount of organic solvent was small under the conditions of heat-treatment with allyl alcohol (entries 14 and 15), but we achieved *S* 94% ee by use of a mixture of hexane and diethyl ether (entry 16).

It is worth noting that the yield was lowered by use of the inhibitor in excess (entry 8) and by heat-treatment of the yeast over 30 minutes (entry 17). These results can be explained by suppression of the yeast reduction, leaving side reactions such as a furanone formation to proceed further. A similar result observed in the reduction using no organic solvent (entry 12) may also be attributed to slowness of the yeast reduction due to lack of the solubilization of substrate described in the preceding section.

As shown in Table 4, we reduced the series of 1-chloro-2,4-alkanediones **1a–e** to obtain the corresponding (*S*)-β-hydroxy ketones **2a–e** using the best method of entry 16 in Table 3. High enantiomeric excesses of *S* 94–96% ee were realized for substrates **1a**, **1b**, and **1c**, and inversions of the enantioselectivity from *R* 39–58% ee to *S* 81–66% ee were brought about for substrates **1d** and **1e** as compared with Table 1.

In summary, we have succeeded in controlling the enantioselectivity by the combined use of enzyme inhibitor, heat treatment, and organic solvent as an additive. The organic solvent in the combined use

Table 4
Control of the enantioselectivity using additives and heat-treatment in the baker's yeast reduction of 1-chloro-2,4-alkanediones **1** to 1-chloro-2-hydroxy-4-alkanones **2**[a]

R	time (h)	yield (%)	ee (%)	$[\alpha]_D$	*R/S*
CH_3	3.0	54	95	-32.6	*S*
C_2H_5	1.0	68	96	-35.4	*S*
n-C_3H_7	1.0	70	94	-34.1	*S*
n-C_4H_9	2.0	52	81	-29.5	*S*
n-C_5H_{11}	3.0	41	66	-18.9	*S*

[a] Conditions: heat-treatment of bakers' yeast (2.0 g) at 50 °C for 30 min with allyl alcohol 2.5 mmol (67 mM), water 38 mL, glucose 2.0 g, substrate 0.5 mmol in hexane–diethyl ether 0.25 mL each.

may play a role in solubilizing the substrate rapidly rather than to inhibit the *R*-enzyme, enhancing the substrate concentration in favor of the *S* selectivity.

3. Experimental

3.1. General

^1H and ^{13}C NMR spectra were measured in $CDCl_3$ at 200 and 50 MHz, respectively. Column chromatography was carried out using Nacalai silica gel 60 (70–230 mesh) or Fuji Silysia BW-127 ZH (100–270 mesh), and thin layer chromatography (TLC) was performed on Merck silica gel 60 F_{254}. Dry baker's yeast was purchased from Nissin Milling. Dry THF and benzene were prepared by distillation from sodium. DEAE-Toyopearl 650M was purchased from Tosoh. The seamless cellulose tubing for dialysis was purchased from Viskase. G6PDH (from baker's yeast, lyophilized powder) was purchased from Sigma. Pressed baker's yeast, NADPH, $NADP^+$, and G6P were purchased from Oriental Yeast.

3.2. Preparation of 1-chloro-2,4-alkanediones 1a–f

To a solution containing diisopropylamine (120 mmol) in 50 ml of THF under a nitrogen atmosphere was added butyllithium (120 mmol) and the bright yellow reaction mixture was stirred at −10°C for 15 min. A methyl ketone (120 mmol) was added *via* syringe and the mixture was stirred at −30°C for 30 min. Ethyl chloroacetate (60 mmol) was added to the mixture at −50°C and stirring was continued until the mixture was warmed to room temperature slowly. The mixture was neutralized (pH 4–5) with 10% HCl and extracted with ethyl acetate (3×50 ml). The combined organic layer was dried over magnesium sulfate and concentrated under vacuum. The crude material was purified by chromatography on a silica gel column using ethyl acetate:hexane (1:30) as eluent. The major fractions were combined and concentrated to give the target molecule as a colorless oil.

3.2.1. 1-Chloro-2,4-pentanedione 1a

Yield 19%. IR (neat) 1716, 1609. Ratio of enol form:keto form in $CDCl_3$ is 78:22. ^1H NMR ($CDCl_3$) enol form: δ 2.12 (s, 3H, CH_3), 4.02 (s, 2H, CH_2Cl), 5.85 (s, 1H, CH=COH), 15.0 (bs, O*H*); keto form: δ 2.28 (s, CH_3), 3.79 (s, CH_2CO), 4.17 (s, CH_2Cl). ^{13}C NMR ($CDCl_3$) enol form: δ 24.5, 44.4, 98.2,

188.1, 190.7; keto form: δ 30.8, 48.4, 54.3, 196.6, 201.2. Anal. calcd for $C_5H_7O_2Cl$: C, 44.62; H, 5.24; found: C, 44.55; H, 4.77.

3.2.2. 1-Chloro-2,4-hexanedione ***1b***

Yield 35%. IR (neat) 1717, 1604. Ratio of enol form:keto form in $CDCl_3$ is 80:20. ^1H NMR ($CDCl_3$) enol form: δ 1.16 (t, J=7.5 Hz, 3H, CH_3), 2.39 (q, J=7.5 Hz, 2H, CH_3CH_2), 4.03 (s, 2H, CH_2Cl), 5.84 (s, 1H, CH=COH), 15.0 (bs, OH); keto form: δ 1.09 (t, 3H, CH_3), 2.57 (q, CH_3CH_2), 3.77 (s, $COCH_2CO$), 4.18 (s, CH_2Cl). ^{13}C NMR ($CDCl_3$) enol form: δ 9.4, 31.1, 44.4, 96.8, 187.3, 195.2; keto form: δ 7.3, 37.0, 48.5, 53.2, 196.7, 203.9. Anal. calcd for $C_6H_9O_2Cl$: C, 48.48; H, 6.06; found: C, 48.51; H, 6.07.

3.2.3. 1-Chloro-2,4-heptanedione ***1c***

Yield 50%. IR (neat) 1717, 1603. Ratio of enol form:keto form in $CDCl_3$ is 85:15. ^1H NMR ($CDCl_3$) enol form: δ 0.96 (t, J=7.3 Hz, 3H, CH_3), 1.58–1.76 (m, 2H, CH_2CH_2), 2.32 (t, J=7.4 Hz, 2H, CH_2CO), 4.03 (s, 2H, CH_2Cl), 5.83 (s, 1H, CH=COH), 15.0 (bs, OH); keto form: δ 2.53 (t, CH_2CH_2CO), 3.76 (s, $COCH_2CO$), 4.18 (s, CH_2Cl). ^{13}C NMR ($CDCl_3$) enol form: δ 13.6, 19.1, 39.7, 44.5, 97.5, 188.2, 193.7; keto form: δ 13.4, 16.8, 45.6, 48.5, 53.5, 196.8, 203.5. Anal. calcd for $C_7H_{11}O_2Cl$: C, 51.69; H, 6.78; found: C, 51.58; H, 6.78.

3.2.4. 1-Chloro-2,4-octanedione ***1d***

Yield 50%. IR (neat) 1717, 1603. Ratio of enol form:keto form in $CDCl_3$ is 87:13. ^1H NMR ($CDCl_3$) enol form: δ 0.93 (t, J=7.2 Hz, 3H, CH_3), 1.28–1.45 (m, 2H, CH_3CH_2), 1.55–1.68 (m, 2H, $CH_2CH_2CH_2$), 2.34 (t, J=7.3 Hz, 2H, CH_2CO), 4.03 (s, 2H, CH_2Cl), 5.84 (s, 1H, CH=COH), 15.0 (bs, OH); keto form: δ 2.54 (t, CH_2CH_2CO), 3.77 (s, $COCH_2CO$), 4.18 (s, CH_2Cl). ^{13}C NMR ($CDCl_3$) enol form: δ 13.7, 22.2, 27.7, 37.6, 44.5, 97.4, 188.0, 194.1; keto form: δ 13.6, 22.0, 25.4, 43.4, 48.5, 53.5, 196.8, 203.6. Anal. calcd for $C_8H_{13}O_2Cl$: C, 54.39; H, 7.37; found: C, 54.44; H, 7.36.

3.2.5. 1-Chloro-2,4-nonanedione ***1e***

Yield 38%. IR (neat) 1603. Ratio of enol form:keto form in $CDCl_3$ is 79:21. ^1H NMR ($CDCl_3$) enol form: δ 0.87–0.93 (m, 3H, CH_3), 1.22–1.40 (m, 4H, $CH_3CH_2CH_2$), 1.50–1.68 (m, 2H, CH_2CH_2CO), 2.34 (t, J=7.3 Hz, 2H, CH_2CO), 4.03 (s, 2H, CH_2Cl), 5.83 (s, 1H, CH=COH), 15.0 (bs, OH); keto form: δ 2.51 (t, CH_2CH_2CO), 3.76 (s, $COCH_2CO$), 4.18 (s, CH_2Cl). ^{13}C NMR ($CDCl_3$) enol form: δ 13.8, 22.3, 25.3, 31.2, 37.8, 44.4, 97.4, 188.0, 194.0; keto form: δ 13.5, 22.4, 23.0, 31.0, 43.7, 48.5, 53.5, 196.8, 203.6. Anal. calcd for $C_9H_{15}O_2Cl$: C, 56.69; H, 7.93; found: C, 56.94; H, 8.35.

3.2.6. 1-Chloro-7-octen-2,4-dione ***1f***

Yield 28%. IR (neat) 1717, 1605. Ratio of enol form:keto form in $CDCl_3$ is 86:14. ^1H NMR ($CDCl_3$) enol form: δ 2.35–2.51 (m, 4H, CH_2CH_2CO), 4.05 (s, 2H, CH_2Cl), 4.99–5.21 (m, 2H, CH_2=CH), 5.70–5.95 (m, 1H, CH_2=CH), 5.86 (s, 1H, CH=COH), 15.0 (bs, OH); keto form: δ 2.67 (t, CH_2CH_2CO), 3.79 (s, $COCH_2CO$), 4.19 (s, CH_2Cl). ^{13}C NMR ($CDCl_3$) enol form: δ 29.9, 37.8, 44.9, 98.2, 116.3, 136.9, 188.2, 193.8; keto form: δ 27.8, 43.2, 48.9, 54.1, 116.3, 136.9, 197.2, 201.3. Anal. calcd for $C_8H_{11}O_2Cl$: C, 55.02; H, 6.35; found: C, 55.09; H, 6.60.

3.3. Conventional procedure for the baker's yeast reduction of ***1a–f***

A mixture of dry baker's yeast (2 g) and glucose (2 g) in 38 ml of boiled water was stirred at 30°C for 30 min. The substrate (0.5 mmol) was added to the mixture and the reaction was continued with stirring

at the same temperature. The progress of the reaction was monitored by GC. After the substrate was consumed, Celite 545 was added to the mixture and the resulting suspension was filtered. The residual Celite was washed with ethyl acetate and the combined filtrate was extracted with ethyl acetate. The organic portion was dried over magnesium sulfate and concentrated under vacuum. The crude material was purified by chromatography on a silica gel column using ethyl acetate:hexane (1:10) as eluent. The major fractions were combined and concentrated to give the target molecule as a colorless oil. The ees were determined by ^{1}H NMR of the corresponding (*R*)-MTPA ester. The $[\alpha]_D$ values were measured using $CHCl_3$ (*c* 1.9–2.3). The absolute configurations were determined by comparing the $[\alpha]_D$ values of dechlorinated derivatives with those of the corresponding 2-hydroxy-4-alkanones reported.[6]

3.3.1. 1-Chloro-2-hydroxy-4-pentanone ***2a***

IR (neat) 3350, 1720. ^{1}H NMR ($CDCl_3$) δ 2.22 (s, 3H, C*H*$_3$), 2.77 (d, *J*=6.1 Hz, 2H, COC*H*$_2$COH), 3.17 (br, 1H, O*H*), 3.58 (d, *J*=5.5 Hz, 2H, C*H*$_2$Cl), 4.21–4.33 (m, 1H, C*H*OH). ^{13}C NMR ($CDCl_3$) δ 30.8, 46.7, 48.2, 67.4, 208.2. Anal. calcd for $C_5H_9O_2Cl$: C, 43.96; H, 6.59; found: C, 43.81; H, 6.21.

3.3.2. 1-Chloro-2-hydroxy-4-hexanone ***2b***

IR (neat) 3385, 1710. ^{1}H NMR ($CDCl_3$) δ 1.08 (t, *J*=7.3 Hz, 3H, C*H*$_3$), 2.50 (q, *J*=7.3 Hz, 2H, CH$_3$C*H*$_2$), 2.74 (d, *J*=5.6 Hz, 2H, COC*H*$_2$COH), 3.16 (d, *J*=4.6 Hz, 1H, O*H*), 3.58 (d, *J*=5.1 Hz, 2H, C*H*$_2$Cl), 4.21–4.33 (m, 1H, C*H*OH). ^{13}C NMR ($CDCl_3$) δ 7.46, 36.8, 45.4, 48.2, 67.6, 211.1. Anal. calcd for $C_6H_{11}O_2Cl$: C, 47.84; H, 7.31; found: C, 47.76; H, 7.23.

3.3.3. 1-Chloro-2-hydroxy-4-heptanone ***2c***

IR (neat) 3385, 1717. ^{1}H NMR ($CDCl_3$) δ 0.93 (t, *J*=7.3 Hz, 3H, C*H*$_3$), 1.53–1.71 (m, 2H, CH$_3$C*H*$_2$), 2.45 (t, *J*=7.2 Hz, 2H, CH$_2$C*H*$_2$CO), 2.74 (d, *J*=5.8 Hz, 2H, COC*H*$_2$COH), 3.19 (d, *J*=4.6 Hz, 1H, O*H*), 3.59 (d, *J* =5.7 Hz, 2H, C*H*$_2$Cl), 4.21–4.33 (m, 1H, C*H*OH). ^{13}C NMR ($CDCl_3$) δ 13.6, 17.0, 45.5, 45.7, 48.2, 67.5, 210.8. Anal. calcd for $C_7H_{13}O_2Cl$: C, 51.69; H, 6.78; found: C, 51.58; H, 6.78.

3.3.4. 1-Chloro-2-hydroxy-4-octanone ***2d***

IR (neat) 3414, 1708. ^{1}H NMR ($CDCl_3$) δ 0.90 (t, *J*=7.2 Hz, 3H, C*H*$_3$), 1.23–1.40 (m, 2H, CH$_3$C*H*$_2$), 1.49–1.65 (m, 2H, C*H*$_2$CH$_2$CO), 2.46 (t, *J*=7.2 Hz, 2H, CH$_2$C*H*$_2$CO), 2.73 (d, *J*=6.0 Hz, 2H, COC*H*$_2$COH), 3.19 (br, O*H*), 3.57 (d, *J*=5.6 Hz, 2H, C*H*$_2$Cl), 4.21–4.33 (m, 1H, C*H*OH). ^{13}C NMR ($CDCl_3$) δ 13.8, 22.2, 25.6, 43.4, 45.7, 48.2, 67.6, 210.9. Anal. calcd for $C_8H_{15}O_2Cl$: C, 53.78; H, 8.40; found: C, 53.89; H, 8.28.

3.3.5. 1-Chloro-2-hydroxy-4-nonanone ***2e***

IR (neat) 3385, 1715. ^{1}H NMR ($CDCl_3$) δ 0.89 (t, *J*=6.8 Hz, 3H, C*H*$_3$), 1.23–1.40 (m, 4H, CH$_3$C*H*$_2$C*H*$_2$), 1.49–1.65 (m, 2H, C*H*$_2$CH$_2$CO), 2.46 (t, *J*=7.3 Hz, 2H, CH$_2$C*H*$_2$CO), 2.73 (d, *J*=5.8 Hz, 2H, COC*H*$_2$COH), 3.20 (br, O*H*), 3.58 (d, *J*=5.6 Hz, 2H, C*H*$_2$Cl), 4.21–4.33 (m, 1H, C*H*OH). ^{13}C NMR ($CDCl_3$) δ 13.8, 22.4, 23.2, 31.2, 43.6, 45.7, 48.2, 67.6, 210.9. Anal. calcd for $C_9H_{17}O_2Cl$: C, 56.10; H, 8.83; found: C, 55.87; H, 8.78.

3.3.6. 1-Chloro-2-hydroxy-7-octen-4-one ***2f***

IR (neat) 3421, 1711. ^{1}H NMR ($CDCl_3$) δ 2.34 (q, *J*=6.6 Hz, 2H, CHC*H*$_2$CH$_2$), 2.57 (t, *J*=7.0 Hz, 2H, CH$_2$C*H*$_2$CO), 2.74 (d, *J*=6.0 Hz, 2H, COC*H*$_2$COH), 3.19 (br, O*H*), 3.58 (d, *J*=5.3 Hz, 2H, C*H*$_2$Cl), 4.21–4.33 (m, 1H, C*H*OH), 4.97–5.09 (m, 2H, C*H*$_2$=CH), 5.70–5.90 (m, 1H, CH$_2$=C*H*). ^{13}C NMR

Table 5
Baker's yeast reduction of **1c** at various substrate concentrations

1c (mM)	bakers' yeast (g)	water (mL)	Yield (%)	$[\alpha]_D$	ee (%)	*R/S*
0.5	20	150	41	+10.8	32	*R*
1.8	8	150	67	+2.32	5	*R*
3.5	8	150	74	+2.68	6	*R*
8.0	4	76	70	-0.69	1	*S*
13.3	2	38	84	-1.88	6	*S*
30	1	19	78	-4.10	10	*S*
62	1	19	76	-16.5	48	*S*
112	0.5	9	34	-14.0	46	*S*

($CDCl_3$) δ 27.9, 43.1, 46.5, 48.8, 68.0, 116.1, 137.1, 210.1. Anal. calcd for $C_8H_{13}O_2Cl$: C, 54.40; H, 7.42; found: C, 54.05; H, 7.79.

3.4. Baker's yeast reduction of 1c at various substrate concentrations

A mixture of dry baker's yeast (0.5–20 g) and glucose (2 g) in boiled water (9–150 ml) was stirred at 30°C for 30 min. The substrate (0.074–1.18 mmol) was added to the mixture and the reaction was continued with stirring at the same temperature for 1 h. The work-up procedure was essentially the same as that described in the foregoing experiment. The results were listed in Table 5.

3.5. General procedure using heat-treated baker's yeast with inhibitor and a small amount of organic solvent

A mixture of dry baker's yeast (2 g), water (38 ml), glucose (2 g) and allyl alcohol (2.5 mmol, 67 mM) was stirred at 50°C for 30 min. After the mixture was cooled to 30°C, the substrate dissolved in 0.5 ml of organic solvent was added and the reaction was continued with stirring at 30°C for 1–3 h. The work-up procedure was essentially the same as that described in the foregoing experiment.

3.6. Preparation of MTPA esters from 2a–f

To a solution of 1-chloro-2-hydroxy-4-alkanones **2a–f** (0.03 mmol) in benzene (1.5 ml) was added (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (0.09 mmol) and pyridine (0.1 ml). After being stirred for 12 h at room temperature, the mixture was treated in the usual manner. The crude product was purified by chromatography on silica gel to give the corresponding ester in 80–95% yield.

3.7. Isolation of γ-chloro β-diketone reductases

Raw pressed baker's yeast (40 g) was suspended in 40 ml of 0.1 M MES buffer (pH 6.0). The suspended cells were homogenized with 60 ml of glass beads (0.5 mm in diameter) using a Vibrogen cell mill chilled with ice below 4°C. The homogenate obtained from 80 g of the pressed yeast was centrifuged at 12 000 rpm for 30 min at 4°C. The supernatant liquid (90 ml) was dialyzed overnight against 10 mM phosphate buffer (pH 7.0). The dialyzate was applied to a column packed with DEAE-Toyopearl 650 equilibrated

with the phosphate buffer. The column was eluted with the buffer and a nonadsorbed enzyme (crude *R*-enzyme-1) was eluted. Then the elution was continued with a 0–0.5 M stepwise gradient concentration of NaCl dissolved in the buffer. Crude *R*-enzyme-2 and *S*-enzyme-1 were eluted at 0.1 and 0.2 M NaCl, respectively.

3.8. Enzyme assay

To a mixture of **1c** (0.9 mM), organic solvent (35 μl, 1.3% v/v) and NADPH (0.26 mM) in 2.5 ml of 10 mM MES buffer (pH 6.0) was added the crude enzyme solution (0.2 ml). The rate of reaction was determined spectrophotometrically at 30°C by following the decrease in absorbance of NADPH at 340 nm. One unit of enzyme activity was defined as the amount of enzyme that catalyzed the oxidation of 1 μmol of NADPH per minute at 30°C under these conditions.

3.9. Enzymatic reduction

To the crude enzyme solution [*R*-enzyme-1 (70 ml), *R*-enzyme-2 (40 ml) or *S*-enzyme-1 (30 ml)] was added $NADP^+$ (3 μmol), D-glucose 6-phosphate disodium salt (G6P, 0.3 mmol), D-glucose 6-phosphate dehydrogenase (G6PDH, 0.1 mg, 15 unit), and **1c** (0.3 mmol). The mixture was stirred at 30°C for 3 h. After work-up in the usual manner, *R*-enzyme-1 gave (*R*)-**2c** in 45% yield with 94% ee, *R*-enzyme-2 gave (*R*)-**2c** in 34% yield with 44% ee, and *S*-enzyme-1 gave (*S*)-**2c** in 52% yield with 97% ee.

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