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Stereoselective reduction of alkyl 3-oxobutanoate by carbonyl reductase from *Candida magnoliae*

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Abstract—The enantioselective reduction of alkyl 3-oxobutanoates by carbonyl reductase (S1) from *Candida magnoliae* was investigated. S1 reduced alkyl 4-halo-3-oxobutanoates to the corresponding enantiomerically pure (*S*)-3-hydroxy esters. *Escherichia coli* HB101 transformant co-overproducing the S1 and glucose dehydrogenase from *Bacillus megaterium*, produced optically pure alkyl 4-substituted-3-hydroxybutanoates in a two-phase water/organic solvent system.

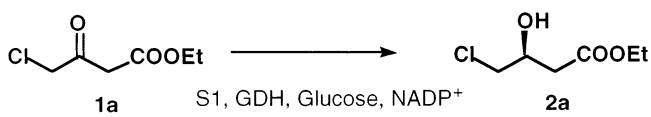
1. Introduction

The importance of optically active compounds has been increasingly recognized in the pharmaceutical field. Four optically active carbon compounds are promising building blocks for asymmetric organic synthesis. Alkyl (*R*)-3-hydroxybutanoate and alkyl (*S*)-4-halo-3-hydroxybutanoate are important synthetic intermediates of various inhibitors of angiotensin-converting enzyme[1] and HMG-CoA reductase,[2,3] respectively. Alkyl (*R*)-4-halo-3-hydroxybutanoates are used for L-cartinine synthesis.[4]

Many studies have investigated the enantioselective bioreductions of alkyl 3-oxobutanoates to the corresponding optically active alkyl 3-hydroxybutanoates as the most effective preparative method. However, in order to accumulate a high yield of product this method requires the regeneration of reduced nicotinamide coenzyme, which is an expensive factor.[5] We have made an *Escherichia coli* transformant that co-produces carbonyl reductase S1 from *Candida magnoliae* and glucose dehydrogenase from *Bacillus megaterium*.[6] The carbonyl reductase S1 reduces ethyl 4-chloro-3-oxobutanoate **1a** to the corresponding (*S*)-enantiomeric alcohol **2a**. Glucose dehydrogenase (GDH) reduces $NADP^+$ to NADPH. Herein, we report on the substrate specificity and enantioselectivity of S1 and on applications for the synthesis of optically active alkyl 3-hydroxybutanoates (Scheme 1).

2. Results and discussion

Carbonyl reductase S1 reduces α- and β-ketoesters and conjugated diketones but does not effect aldehyde reduction.[7] Aside from **1a**, however, its selectivity for the reduction of alkyl 3-oxobutanoate derivatives is unknown. It is important to discern information about the enantioselectivity of the enzyme for application in the synthesis of optically active compounds and to gain a greater understanding of the active center of the enzyme. We investigated the relative substrate specificity and the enantioselectivity of this enzyme for alkyl 4-substituted-3-oxobutanoate by using the purified enzyme to eliminate the influences of other enzymes. The results are summarized in Table 1. S1 was found to reduce alkyl 4-halo-3-oxobutanoate **1a**, **1b**, **1c** and **1d**



Scheme 1. Enzymatic enantioselective reduction of ethyl 4-chloro-3-oxobutanoate **1a**.

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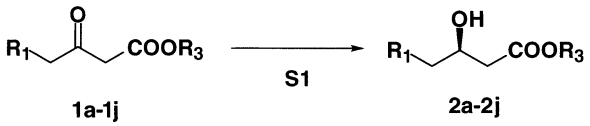
Table 1. Enantioselective reduction of alkyl 3-oxobutanoate by carbonyl reductase S1



1a-1j 2a-2j

Entry	R_1	R_3	Relative activity (%)	% e.e.
a	Cl	CH_2CH_3	100	>99 *S*
b	Br	CH_2CH_3	72	>99 *S*
c	I	CH_2CH_3	16	>99 *S*
d	Cl	$(CH_2)_7CH_3$	4	>99 *S*
e	N_3	CH_2CH_3	0	Nt[a]
f	$C_6H_5CH_2O$	CH_2CH_3	21	21 *S*
g	HO	CH_2CH_3	80	>99 *S*
h	H	CH_2CH_3	7	>99 *R*
i	H	$C(CH_3)_3$	0	Nt
j	CH_3CH_2	CH_2CH_3	0.5	Nt

[a] Not tested.

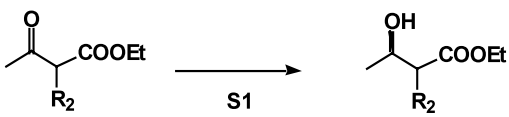
and the 4-hydroxy derivative **1g** to the optically pure corresponding 3-hydroxy esters. The reactivity with octyl ester **1d** was low and the 4-benzyloxy derivative **1f** was reduced with low enantioselectivity. Poor enantioselectivity in the baker's yeast reduction of **1f** has also been reported.[8] The reactivities in the reduction of 3-oxobutanoates were low, but the enantioselectivity for the ethyl ester **1h** was high. For the sake of synthesizing optically active compounds, information on the enantioselectivity of carbonyl reductase is more important than that concerning reactivity.

Alkyl 2-substituted-3-oxobutanoates are interesting substrates for enantioselective reduction because the product of each contains two stereogenic centers.[9,10] The stereoselectivities of the purified S1 for ethyl 2-substituted-3-oxobutanoates are summarized in Table 2. S1 gave *anti*-**2k** and *anti*-**2l**. The enantioselectivity for **1k** was high, but that for **1l** was low. Biological reduction of alkyl 2-substituted-3-oxobutanoates has been reported.[9–18,21] The product of the enzymatic enantioselective reduction of **1k** is a single isomer rather than two isomers because of the inversion of the substrate.[11–13] Purified enzymes from baker's yeast[12] or *Geotrichum candidum*[13] and recombinant enzyme from baker's yeast[14] reduce **1k** to give the *syn* product with high diastereo- and enantioselectivities. In contrast, S1 gave the *anti* product with low enantioselectivity. Cells of *Penicillium*[15] and *Geotricum*[16] have been shown to give *anti*-**2k**. The stereoselectivity of microbial reduction changes depending on the cultivation conditions or cell treatments.[17–19] This occurs because a microorganism has multiple reducing enzymes.[20] In the reduction of **1l**, baker's yeast reduction afforded equimolar amounts of both diastereoisomers,[21] but S1 gave the *anti* product with 94% diastereomeric excess (d.e.) and low enantioselectivity. Also, chemical reduction by sodium borohydride gave the *syn* product with d.e. of 96%[10] S1 catalyzed a unique stereoselective reduction for **1l**. To understand the structure of the active center of S1, it is interesting to note that the stereoselectivity of S1 is affected by the presence of a substituent at the α-position of the substrate.

A reduced form coenzyme regeneration system is necessary for biological reduction catalyzed by dehydrogenase/reductase. Whole cells, such as baker's yeast, which contain both reduction and coenzyme regeneration systems, are often used as the biological reduction catalyst because of easy availability and handling. In many cases, however, their weak coenzyme regeneration system causes low product yields. We have made an *E. coli* transformant that coproduces S1 and GDH.[6] GDH reduces $NAD(P)^+$ to NAD(P)H linked with glucose oxidation.

The use of a two-phase aqueous/organic solvent system is advantageous in an enzymatic reaction because the problems of degradation of unstable substrates in the aqueous phase and enzyme deactivation by a toxic substrate or product are minimized by extraction into an organic solvent.[6,22] The enzymes responsible for the reduction reaction are required to have tolerance for the organic solvent used in the reaction, and S1 and GDH are both known to be tolerant to *n*-butyl acetate.[6,22]

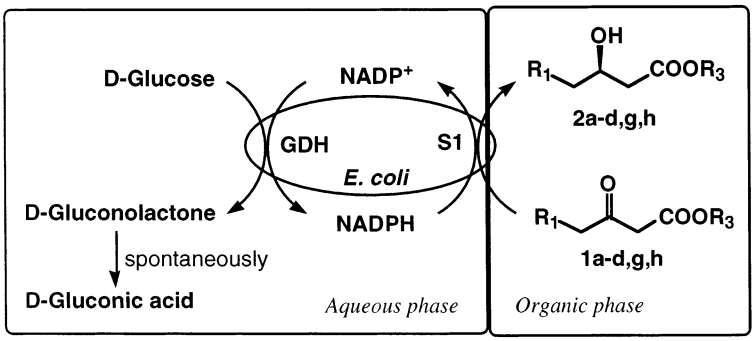
Table 2. Reduction of ethyl 2-substituted-3-oxobutanoate by carbonyl reductase S1



1k, l 2k, l

Entry	R_2	Relative activity (%)[a]	*syn*/*anti* (%)	% e.e.	
				syn-(2*S*,3*R*)	*anti*-(2*S*,3*S*)
k	CH_3	14	22/78	70	99
l	Cl	11	3/97	55	15

[a] The activity of ethyl 4-chloro-3-oxobutanoate was taken as 100%.



Scheme 2. Synthesis of optically active alkyl 3-hydroxybutanoate derivatives by *Escherichia coli* transformant in a water–organic two-phase reaction.

Alkyl 4-substituent-3-oxobutanoates are relatively unstable compounds, so we applied the two-phase reaction system in order to prepare six optically active corresponding 3-hydroxy esters (Scheme 2). A water phase containing the *E. coli* cells, glucose, and a catalytic amount of $NADP^+$ was stirred with an organic (*n*-butyl acetate) phase containing the substrate. The pH of the reaction was maintained at 6.5. After 20 hours, the yields for **2a**, **2b**, **2g** and **2h** were over 90%, but those for **2c** and **2d** were less than 10% and most of the starting substrate was recovered in these latter two cases. The course of the reduction of **1b** with time is shown in Fig. 1. The concentration of **2b** in the organic phase increased almost stoichiometrically, linking with the concentration of consumed glucose and the volume of NaOH to neutralize the formed gluconic acid. The concentration of **2b** reached 0.38 M in the *n*-butyl acetate layer with the consumption of glucose and sodium hydroxide. The turnover number of $NADP^+$ was about 650. The amount of coenzyme was not optimized for this reaction. After the reaction, the product and the remaining substrate were easily extracted to an organic phase with ethyl acetate and were purified by column chromatography. The product **2g** can be used to synthesize HMG-CoA reductase inhibitors and other products.[23,24]

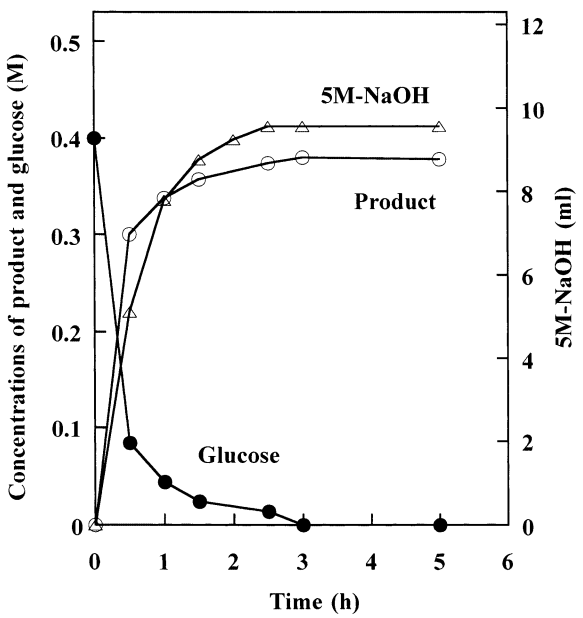


Figure 1. Preparation of ethyl (*S*)-4-bromo-3-hydroxybutanoate **2b** by *Escherichia coli* transformant in a water–organic two-phase reaction. Time-course plots are shown of the enantioselective reduction of **1b**. Reaction conditions are described in the text. ●, Concentration of glucose; ○, concentration of **2b**; △, volume of 5 M aqueous sodium hydroxide to neutralize the formed gluconic acid.

The coenzyme regeneration system by GDH produces gluconic acid as a by-product. The oxidation of formate to water and carbon dioxide by formate dehydrogenase, coupled with NAD^+ reduction, is a cleaner coenzyme regeneration system. The NADH-dependent mutant of S1 or the NADPH-dependent mutant of formate dehydrogenase[25] will allow a clean production system in which the synthesis of optically active alcohols can be realized. Biological enantioselective reduction has been a conventional methodology, but it has entered a new phase due to the application of a practical coenzyme regeneration system and gene technology.

3. Conclusion

The enantioselective reduction of alkyl 3-oxobutanoate by carbonyl reductase S1 from *C. magnoliae* was investigated. S1 reduced alkyl 4-halo-3-oxobutanoates to the corresponding optically pure (*S*)-form 3-hydroxy esters. It also reduced alkyl 2-substituted-3-oxobutanoates, but the stereoselectivity was not necessarily high. *E. coli* HB101 transformant co-overproducing the S1 and glucose dehydrogenase from *B. megaterium*, produced optically pure alkyl 4-substituted-3-hydroxybutanoates in a water/organic solvent two-phase system. This is a highly advantageous method for synthesizing homochiral 3-hydroxybutanoate derivatives.

4. Experimental

4.1. General

Authentic (±)-alkyl 3-hydroxybutanoates were synthesized by reducing the corresponding alkyl 3-oxobutanoates with sodium borohydride. The reactions were

followed by TLC on Kieselgel $60F_{254}$ TLC plates (Merck, Germany). Detection was achieved by UV inspection (254 nm). ^{1}H NMR spectra were recorded in $CDCl_3$ with an FT NMR, JM-400 spectrometer (400 MHz; Jeol, Japan). Chemical shifts are expressed in parts per million (ppm), with trimethylsilane as the internal standard. The spectra of all synthesized compounds used in this study agree with the data found in the literature. Optical rotation was carried out using a digital polarimeter (SEPA-200, Hiroba, Japan). IR spectra were recorded on an FTIR-8100M spectrometer (Shimadzu, Japan). All other chemicals used were of analytical grade and commercially available.

4.2. Microorganisms and cultivation

The carbonyl reductase S1-overproducing strain, *E. coli* HB101/pNTS1, and the S1- and GDH-co-overproducing strain, *E. coli* HB101/pNTS1G, were used.[6] 2xYT medium composed of 1.6% Bacto-tryptone, 1.0% yeast extract and 0.5% NaCl, pH 7.0, was used. A test tube containing 2 mL of 2xYT medium was inoculated with an *E. coli* strain, which was then incubated at 37°C for 15 h by reciprocal shaking. An aliquot of this pre-culture broth (0.5 mL) was transferred to a 500-mL shaking flask containing 100 mL 2xYT medium, and cultivation was then performed at 37°C for 13 h by reciprocal shaking.

4.3. Purification of carbonyl reductase S1

All purification procedures were performed at 0–4°C in a 10 mM potassium phosphate buffer (pH 7.0) containing 2 mM 2-mercaptoethanol. The washed cells, isolated from the culture broth of *E. coli* HB101/pNTS1, were suspended in the buffer and disrupted by sonication. The supernatant obtained by centrifugation was fractionated with solid ammonium sulfate. The precipitate obtained at 45–70% saturation was collected by centrifugation and dissolved in the buffer. This precipitate was applied to a Blue Sepharose CL-6B column that had been previously equilibrated with the same buffer. The enzyme was eluted with a linear gradient of NaCl (0–0.6 M in the buffer). The active fraction was collected and used as the purified enzyme.

4.4. Enzyme assay and kinetic measurement

Carbonyl reductase S1 activity was determined spectrophotometrically as follows. The assay mixture, composed of 100 mM potassium phosphate buffer (pH 6.5), 0.1 mM NADPH and 1 mM substrate, and the reactions at 30°C were monitored for decreases in absorbance at 340 nm. One unit of S1 was defined as the amount catalyzing the oxidation of 1 μmol NADPH per minute.

4.5. Preparation of alkyl 4-substituted-3-oxobutanoates

4.5.1. Ethyl 4-bromo-3-oxobutanoate 1b. **1b** was prepared by bromination at the 4-position of ethyl 3-oxobutanoate **1h**.[26] Bromine (16 g, 0.1 mol) was added to a solution of **1h** (13 g, 0.1 mol) in chloroform (90 mL) over 2 h. The reaction mixture was stirred at 25°C for 15 h. After the reaction, nitrogen gas was bubbled into the mixture to remove hydrogen bromide. Water was added to the reaction mixture, and then the organic layer was dried over anhydrous sodium sulfate. The solvent was removed, and the residue was purified by column chromatography on silica gel to give **1b**. Yield 47%; ^{1}H NMR δ (ppm) 4.25 (2H, m), 4.10 (2H, s), 3.80 (2H, s), 1.30 (3H, t); ^{13}C NMR: 13.9, 34.0, 46.0, 61.6, 166.4, 194.5; IR (neat) 2984, 1752, 1028, 565 cm^{-1}.

4.5.2. Ethyl 4-iodo-3-oxobutanoate 1c. **1c** was prepared by a halogen exchange reaction of ethyl 4-chloro-3-oxobutanoate **1a**.[27] The mixture, comprising sodium iodide (13.7 g, 0.09 mol), **1a** (10 g, 0.061 mol) and methylethyketone (100 mL), was stirred under reflux for 15 h. After the reaction, the solvent was removed and the residue was extracted with ethyl acetate. The residue was then purified by column chromatography on silica gel to give **1c**. Yield 85%; ^{1}H NMR δ (ppm) 4.23 (2H, m), 3.44 (2H, s), 2.25 (2H, s), 1.30 (3H, t); ^{13}C NMR: 6.2, 14.5, 45.9, 62.2, 167.2, 195.8; IR (neat) 2982, 1743, 1028, 544 cm^{-1}.

4.5.3. Octyl 4-chloro-3-oxobutanoate 1d. **1d** was prepared by chlorination of diketene following esterification by 1-octanol.[28] Chlorine gas was bubbled into a solution of diketene (16.8 g, 0.2 mol) in dichloromethane (160 mL) at a temperature below 15°C. 1-Octanol (28.6 g, 0.22 mol) was added to the mixture at a temperature below 10°C. The reaction mixture was stirred at room temperature for 1 h and washed with brine. After the solvent was removed, the residue was purified by column chromatography on silica gel to give **1d**. Yield 35%; ^{1}H NMR δ (ppm) 4.28 (2H, s), 4.16 (2H, m), 3.68 (2H, s), 1.65 (2H, m), 1.35 (10H, m), 0.88 (3H, m); ^{13}C NMR: 14.0, 22.5, 25.7, 28.3, 29.0, 31.7, 42.3, 46.1, 48.0, 65.9, 166.5, 195.4; IR (neat) 2856, 1751, 579 cm^{-1}.

4.5.4. Ethyl 4-azide-3-oxobutanoate 1e. **1e** was prepared from reaction of ethyl 4-chloro-3-oxobutanoate **1a** with sodium azide.[29] The mixture, comprising sodium azide (9.5 g, 0.14 mol), **1a** (20 g, 0.12 mol), acetone (150 mL) and water (50 mL), was stirred under reflux for 5 h. The solvent was removed and the residue was extracted with ethyl acetate. After the solvent was removed, the residue was purified by column chromatography on silica gel to give **1e**. Yield 78%; ^{1}H NMR δ (ppm) 4.30 (2H, m), 4.18 (2H, s), 3.55 (2H, s), 1.30 (3H, t); ^{13}C NMR: 13.9, 46.5, 57.5, 61.7, 166.2, 197.2; IR (neat) 2986, 2104, 1751, 1080 cm^{-1}.

4.5.5. Ethyl 4-benzyloxy-3-oxobutanoate 1f. **1f** was prepared from 4-chloro-3-oxobutanoate **1a** and benzyl alcohol.[30] Sodium hydride (3.6 g, 0.15 mol) (which had previously been washed three times with pentane in a nitrogen atmosphere) was suspended in tetrahydrofuran (80 mL). Benzylalcohol (8.1 g, 0.075 mol) and **1a** (12.3 g, 0.075 mol) were added to the suspension and stirred at 40°C for 18 h. Tetrahydrofuran was removed in vacuo and the residue was extracted with ethyl acetate. The solvent was removed and the residue was purified

by column chromatography on silica gel to give **1f**. Yield 60%; ^{1}H NMR δ (ppm) 7.50 (5H, m), 4.60 (2H, s), 4.13 (2H, m), 3.54 (2H, s), 1.30 (3H, t); ^{13}C NMR: 13.9, 45.9, 61.3, 73.3, 74.6, 127.6, 127.7, 128.0, 128.4, 136.8, 166.9, 201.6; IR (neat) 2984, 1751, 742, 700 cm^{-1}.

4.5.6. Ethyl 4-hydroxy-3-oxobutanoate 1g. **1g** was prepared by hydrogenation of ethyl 4-benzyloxy-3-oxobutanoate **1f**.[30] A mixture of **1f** (4.5 g, 0.019 mol) in methanol (50 mL) and 10% palladium on carbon (0.5 g) was stirred under hydrogen at room temperature for 18 h. The catalyst was removed by filtration through Celite™ (Celite, USA). The solvent was removed and the residue was purified by column chromatography on silica gel to give **1g**. Yield 60%; ^{1}H NMR δ (ppm) 4.43 (2H, s), 4.20 (2H, m), 3.52 (2H, s), 3.20 (1H, s), 1.30 (3H, t); ^{13}C NMR: 13.9, 45.2, 61.7, 68.4, 166.4, 202.5; IR (neat) 3588, 2984, 1747 cm^{-1}.

4.6. Enzymatic reaction and measurement of enantioselectivity

The enantioselectivity of S1 for alkyl 3-oxobutanoate was determined by analysis of the enantiomeric excess of the 3,5-dinitrobenzoate ester derivative of the alkyl 3-hydroxybutanoate that was formed by the enzymatic reaction. The reaction mixture, comprising 10 mg of each substrate, 100 mg of NADPH, 0.1 mmol of potassium phosphate buffer (pH 6.5) and 0.5 units of the S1 solution in a total volume of 1 mL, was stirred at 30°C for 18 h. The formation of product was monitored by TLC and the reaction mixture was extracted with toluene (0.5 mL). To the organic extract, 3,5-dinitrobenzoyl chloride (20 mg) and pyridine were added (0.2 mL) and the mixture was stirred for 1 h at room temperature. After the reaction, HCl (6 M, 1 mL) was added and the organic layer was applied to a preparative TLC. The e.e. of the 3,5-dinitrobenzoate product was analyzed with an HPLC on a Chiralpak AD (4.6 mmϕ×250 mm) column (Daicel Chemicals, Japan). The retention times of four isomers of ethyl 3-hydroxy-2-methylbutanoate **2k** (2*S*,3*S*), (2*R*,3*S*), (2*S*,3*R*) and (2*R*,3*R*) were, respectively, 18.4, 20.2, 24.0 and 25.6 min. The retention times of the four analogous isomers of ethyl 2-chloro-3-hydroxybutanoate **2l** were 12.2, 14.9, 16.2 and 18.8 min, in that order.

4.7. General procedures for the preparation of optically active alkyl 4-substituted-3-hydroxybutanoates

For the synthesis of various optically active alkyl 3-hydroxybutanoates, we used a transformant, producing both S1 and GDH-regenerating NADPH, as a catalyst. The reaction mixture, comprising 25 mL broth of *E. coli* HB101/pNTS1G, 10 mmol of alkyl 3-oxobutanoate, 10 mmol of glucose, 3.2 mg $NADP^+$, 25 mg of Triton X-100 and 25 mL of *n*-butyl acetate, was stirred at 30°C for 20 h. The pH of the reaction mixture was kept at 6.5 with aqueous 5 M sodium hydroxide. The reaction was monitored by the titration of sodium hydroxide. The concentrations of the product were determined with a gas chromatograph (model 164, Hitachi, Japan) equipped with a 10% PEG-20M on an 80/100-mesh Chromosorb WAW DMCS (3.2 mmϕ×1.0 m) column (GL Science, Japan) and with a flame ionization detector. The concentration of glucose was measured with a glucose assay kit containing glucose oxidase (Wako, Japan). After the reaction, the mixture was extracted with 25 mL of ethyl acetate and the organic layer was dried over anhydrous sodium sulfate. The solvent was removed and the residue was purified by column chromatography on silica gel to give alkyl 4-substituted-3-hydroxybutanoate.

4.7.1. Ethyl (*S*)-4-chloro-3-hydroxybutanoate 2a. Oil. Yield 95%; $[\alpha]_D^{25}=-21.4$ (*c* 7.0, $CHCl_3$), lit. for (*R*)-**2a** of 97% e.e. prepared by hydrogenation using (*S*)-BINAP–Ru complex, $[\alpha]_D^{21}=+20.9$ (*c* 7.71, $CHCl_3$);[31] e.e. >99% (by HPLC of the 3,5-dinitrobenzoate ester derivative); ^{1}H NMR δ (ppm) 4.20 (3H, m), 3.60 (2H, d), 3.31 (1H, d), 2.65 (2H, d), 1.33 (3H, t); ^{13}C NMR: 14.4, 38.8, 48.4, 61.3, 68.2, 172.1; IR (neat) 3450, 2984, 1736, 756 cm^{-1}.

4.7.2. Ethyl (*S*)-4-bromo-3-hydroxybutanoate 2b. Oil. Yield 95%; $[\alpha]_D^{25}=-9.1$ (*c* 10.4, $CHCl_3$), lit. for (*S*)-**2b** derived from (*S*)-3-hydroxybutyrolactone, $[\alpha]_D^{20}=-11$ (*c* 1, EtOH);[32] e.e. = 99% (by HPLC of the 3,5-dinitrobenzoate ester derivative); ^{1}H NMR δ (ppm) 4.25 (1H, m), 4.18 (3H, q), 3.51 (2H, m), 3.28 (1H, br), 2.75 (2H, m), 1.38 (3H, t); ^{13}C NMR: 14.4, 37.6, 39.6, 61.3, 67.8, 172.0; IR (neat) 3456, 2982, 1736, 673 cm^{-1}.

4.7.3. Ethyl (*S*)-4-iodo-3-hydroxybutanoate 2c. Oil. Yield 10%; $[\alpha]_D^{25}=-9.8$ (*c* 11.7, $CHCl_3$), lit. for (*R*)-**2c** derived from (*R*)-3-hydroxybutyrolactone, $[\alpha]_D^{20}=+10.2$ (*c* 3.3, EtOH);[33] e.e. = 99% (by HPLC of the 3,5-dinitrobenzoate ester derivative); ^{1}H NMR δ (ppm) 4.20 (2H, q), 4.00 (1H, m), 3.31 (3H, m), 2.65 (2H, d), 1.28 (3H, t); ^{13}C NMR: 12.4, 14.4, 41.0, 61.3, 67.7, 172.0; IR (neat) 3450, 2982, 1732, 633 cm^{-1}.

4.7.4. Octyl (*S*)-4-chloro-3-hydroxybutanoate 2d. Oil. Yield 7%; $[\alpha]_D^{25}=-14.5$ (*c* 6.1, $CHCl_3$), lit. for (*R*)-**2d** prepared by baker's yeast reduction, $[\alpha]_D^{23}=+12.6$ (*c* 10.2, $CHCl_3$);[34] e.e. >99% (by HPLC of the 3,5-dinitorbenzoate ester derivative); ^{1}H NMR δ (ppm) 4.26 (1H, m), 4.12 (2H, t), 3.61 (2H, t), 3.17 (1H, d), 2.64 (2H, m), 1.65 (2H, m), 1.30 (10H, m), 0.90 (3H, t); ^{13}C NMR: 14.3, 22.9, 26.1, 283.8, 29.4, 32.0, 38.7, 48.4, 65.5, 68.2, 172.2; IR (neat) 3450, 2928, 1732, 758 cm^{-1}.

4.7.5. Ethyl (*S*)-3,4-dihydroxybutanoate 2g. Oil. Yield 90%; $[\alpha]_D^{25}=+6.0$ (*c* 6.4, $CHCl_3$), lit. for (*S*)-**2g** derived from (*S*)-malic acid, $[\alpha]_D^{25}=+6.3$ (*c* 1.0, $CDCl_3$);[24] e.e. >99% (by HPLC of the 3,5-dinitrobenzoate ester derivative); ^{1}H NMR δ (ppm) 4.20 (2H, q), 4.15 (1H, s), 3.68 (1H, d), 3.55 (1H, d), 3.18 (1H, br), 2.55 (2H, m), 1.30 (3H, t); ^{13}C NMR: 14.3, 38.0, 61.2, 65.9, 68.9, 172.8; IR (neat) 3450, 2984, 1774 cm^{-1}.

4.7.6. Ethyl (*R*)-3-hydroxybutanoate 2h. Oil. Yield 95%; $[\alpha]_D^{25}=-41.6$ (*c* 7.1, $CHCl_3$), lit. for (*S*)-**2h** of 85% e.e. prepared by baker's yeast reduction, $[\alpha]_D^{25}=+37.2$ (*c* 1.3, $CHCl_3$);[35] e.e. >99% (by HPLC on the 3,5-dinitrobenzoate ester derivative); ^{1}H NMR δ (ppm) 4.18 (2H, q),

2.88 (1H, br), 2.45 (2H, m), 1.28 (3H, t), 1.19 (3H, d); ^{13}C NMR: 14.3, 22.7, 43.1, 60.8, 64.4, 173.0; IR (neat) 3450, 2978, 1736, 1030 cm^{-1}.

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