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Asymmetric Bioreduction of Ethyl 3-Halo-2-oxo-4-phenylbutanoate by *Saccharomyces cerevisiae* Immobilized in Ca-Alginate Beads with Double Gel Layer

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Abstract:

The asymmetric bioreduction of ethyl 3-halo-2-oxo-4-phenylbutanoate was studied for several microorganisms and especially for *Saccharomyces cerevisiae*. The highest chemical yield (90%), de (70%) and ee (96−99%) were obtained with *S. cerevisiae* immobilized in calcium alginate beads with double gel layers, and reaction conditions were optimized by changing matrix of immobilization, concentration of substrate, and feeding with glucose as electron donor. The entrapment of cells with double gel layers was fundamental to achieve high enantio- and diastereoselectivity.

Introduction

The production of enantiomeric pure compounds is of increasing importance in the fine chemical and pharmaceutical industries in particular.[1] Many of these compounds can be obtained by asymmetric reductions of ketones mediated by *Saccharomyces cerevisiae* (baker's yeast).[2] In biocatalytic conversions,[3−7] whole cells are used more often than isolated enzymes since cofactor regeneration is not required for sustained catalytic activity. *S. cerevisiae* is an economically attractive biocatalyst due to its availability, low cost, ease of handling and disposal, safety for food and pharmaceutical applications, as well as its capacity to catalyze a wide range of stereoselective reductions.[3−8] However, this method has not been considered suitable for large-scale production of chiral alcohol, due to the low concentration of reagents and the long process of isolation of products, since the reactions are generally performed in batch process. Some work with continuous cell-culture systems tried to overcome these disadvantages.[9−12] One alternative is to use immobilized cells for enantioselective reductions of ketones.[13] Several methods for entrapment of living and growing cells have been developed.[14,15] A preparation that provides extremely mild immobilization conditions is the entrapment within ionotropic gels, such as calcium alginate.[16]

Entrapment of cells in calcium alginate is the most widely used immobilization technique in the biocatalytic production of chemical compounds.[17−21] Alginate is cheap and readily available, has a high affinity for water, and has the ability to form gels under mild conditions, which are suitable for most cells. It is nontoxic and nonpathogenic, what makes it attractive for applications in the food and the pharmaceutical industries.[20] Several ketone reductions using immobilized cells have been reported in the literature and, in many cases, by *S. cerevisiae* immobilized in calcium alginate.[13,22−26] Other microorganisms[27,28] as well as other immobilization matrixes

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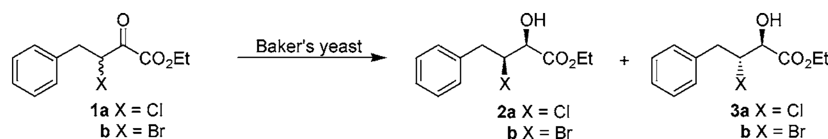
Scheme 1. **Reduction of ethyl 3-chloro-2-oxo-4-phenylbutanoate 1 mediated by baker's yeast**



such as polyurethane,[29] carrageenan,[25] and chrysotile fibers[12] have also been described.

Recently, we described the first example of ketoester reduction mediated by immobilized baker's yeast in alginate fibers with double gel layer.[30] The immobilization procedure prevents cell leakage from alginate fibers into the medium in a device in which the alginate fiber cells were restricted to the inner layer, whereas the outer layer helped to prevent leakage and contact with potential inhibitors. This method of cell entrapment was developed by Tanaka et al. for continuous fermentation in the production of ethanol [31] and Chitinase.[32,33]

Asymmetric reductions of 3-chloro-2-oxoalkanoates with fermenting baker's yeast have been reported by Tsuboi et al.[34] The reduction of corresponding esters with baker's yeast gave optically active 3-chloro-2-hydroxyalkanoic esters (*anti* (2*S*,3*R*):*syn* (2*S*,3*S*) = 52:48−90:10) in 50−85% yields and >95% ee, except for 43% ee of ethyl *syn*-(2*S*,3*S*)-3-chloro-2-hydroxy-phenylbutanoate. The reduction of **1a** was achieved with good diastereoselectivity (*anti* (2*S*,3*R*)-**3a**:*syn* (2*S*,3*S*)-**2a** = 66:34) and high enantioselectivity *anti*-(2*S*,3*R*)-3-chloro-2-hydroxy-phenylbutanoate in 95% ee (Scheme 1). The chiral 3-chloro-2-hydroxyalkanoic esters are very useful in the synthesis of chiral glycidic esters[34−37] and 2,3-epoxi alcohols,[38] which are key intermediates for the synthesis of important bioactive molecules.[34−38]

In this work, we describe the asymmetric bioreduction of ethyl 3-halo-2-oxo-4-phenylbutanoate with several microorganisms, and especially with *S. cerevisiae,* which we have optimized by changing the matrix of immobilization and the concentration of substrate, and by feeding with glucose as electron donor. The entrapment of *S. cerevisiae* with double gel layers was fundamental to achieve high enantio- and diastereoselectivity.

Results and Discussion

Different strains of yeast and bacteria for microbiological reduction of **1b** were selected according to literature results.[34,39−41] The bioreduction was carried out using resting cells under nonfermenting conditions, i.e. suspended in water without addition of sugar. All microorganisms reduced **1b** with various ranges of chemical and optical yields. The results are summarized in Table 1. With the yeasts *Rhodotorula minuta* (entries 1 and 2) and *Rhodotorula glutinis* (entries 3 and 4) and the bacteria *Serratia rubidea* (entries 11 and 12) and *Serratia marcences* (entries 13 and 14), the diastereoisomer *anti* (2*S*,3*R*)-**3b** was predominant in high ee. *Serratia* sp. showed excellent diastereo and enantioselectivity, but low chemical yields. With other yeasts the diastereoisomer *syn* (2*S*,3*S*)-**2b** was predominant, with good ee. A higher de was observed at pH 4.0 than at pH 7.0 since **1b** was selectively reduced to the ester (2*S*,3*S*)-**2b**, while the substrates underwent a dynamic kinetic resolution due to epimerization occurring at C-2 via enol intermediate under the effect of pH.[24] At low pH (pH 4.0) the rate of enolization is fast, and the *syn*-alcohol is formed as the major product. At pH 7.0, when enolization becomes slower, enantioselectivity prevails, but optically pure diastereomers are formed in a lower ratio.

High chemical yields and reasonable de were obtained with *S. cerevisiae* (entries 15 and 16) which was chosen for further studies to improve the preliminary results. The approach was to use free cells of *S. cerevisiae* without

Table 1. **Reduction of ethyl 3-bromo-2-oxo-4-phenylbutanoate 1b with free cells of several strains**

entry	strain	pH	yield (%)	*syn*:*anti*[b]	ee (%)	
					syn	anti
1	*Rhodotorula minuta*[a]	4.0	59	32:68	93	>99
2	*Rhodotorula minuta*[a]	7.0	75	44:56	89	94
3	*Rhodotorula glutinis*[a]	4.0	39	34:66	90	>99
4	*Rhodotorula glutinis*[a]	7.0	50	45:55	96	>99
5	*Candida utiliz*[a]	4.0	65	64:36	85	89
6	*Candida utiliz*[a]	7.0	62	65:35	84	84
7	*Pichia canadensis*[a]	4.0	48	75:25	90	97
8	*Pichia canadensis*[a]	7.0	65	65:35	90	74
9	*L. mesenteroides*[a]	4.0	55	68:32	90	80
10	*L. mesenteroides*[a]	7.0	48	66:34	91	74
11	*Serratia rubidea*[c]	4.0	4	3:97	>99	>99
12	*Serratia rubidea*[c]	7.0	10	0:100	−	>99
13	*Serratia marcences*[c]	4.0	7	0:100	−	>99
14	*Serratia marcences*[c]	7.0	15	0:100	−	>99
15	*Saccharomyces cerevisiae*[d]	4.0	87	69:31	83	95
16	*Saccharomyces cerevisiae*[d]	7.0	80	64:36	78	94

[a] Reaction time = 72 h. [b] The diastereomeric ratio was determined by GC−MS, and enantiomeric excess was determined by GC (see Experimental Section). [c] Reaction time = 96 h. [d] Reaction time = 24 h. Reaction conditions are described in the Experimental Section.

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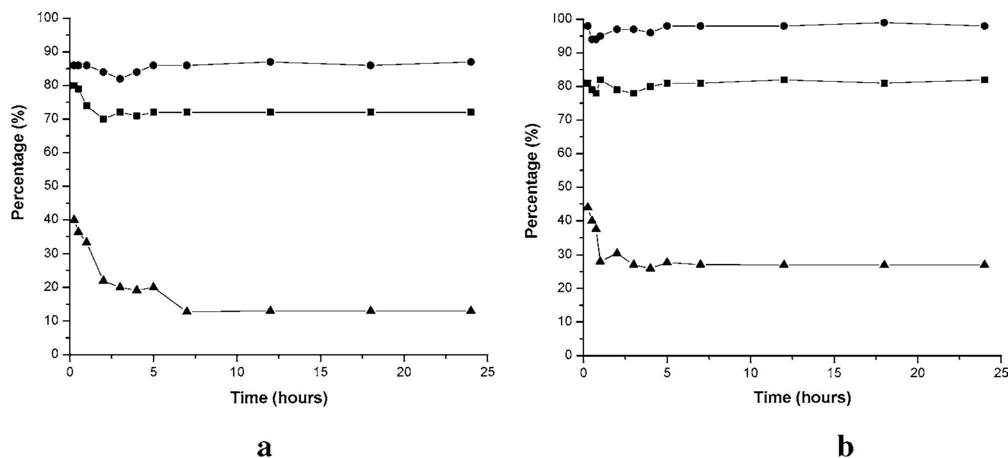

Figure 1. **(a) Bioreduction of 1b (0.49 mmol) and (b) bioreduction of 1b (0.18 mmol). Both reductions were mediated by *Saccharomyces cerevisiae* with free cells suspended in citrate buffer, at pH 4.0 (ee of 2b (■), ee of 3b (●), de 2b/3b (▲)).**

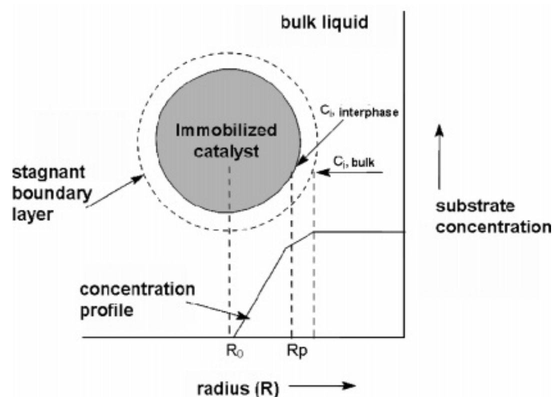

Figure 2. **Schematic diagram of the concentration profile of substrates in a spherical immobilized catalyst.[13]**

addition of glucose. Figure 1a shows the results obtained after 24 h of reaction, in which 60% of substrate was consumed. The initial concentration of substrate available to the biocatalyst was relatively low because it was not fully soluble in the reaction mixture. Both the initial de and ee were higher than at the end (24 h), where the de of **2b** was 12.5% while the ee of **3b** remained constant. In an attempt to increase the selectivity, the bioreduction was carried out with a lower concentration of **1b** (0.18 mmol instead of 0.49 mmol), Figure 1b. The selectivity was higher in the initial stages than at the end, but the ee increased. The de and ee were both higher in the initial stages when the substrate concentration was low, but the diastereomerical selectivity decreased rapidly and then remained constant until the end.

In another attempt to decrease the availability of the substrate, the cells were immobilized in alginate beads creating a barrier at the border of the cellular membrane.[13] During the reduction, the concentration levels of these substrates inside the alginate beads was lower than in the solution, due to slow diffusive transport[13] (see Figure 2). The lower concentration of substrate in the beads helps the stereoselectivity, since only the enzyme with lowest K_m (Michaelis constant) is able to react with high V_{max}. It is well-known that baker's yeast possesses several alcohol dehydrogenases with varying substrate selectivities and even opposing enantioselectivities.[13]

When the cells were immobilized, the selectivity of these reactions was better than that observed for free cells, as shown in Figure 3a. The gradient of concentration of substrate in the reaction medium is different from the gradient of concentration inside the calcium alginate beads, where the substrate is reduced by the *S. cerevisiae,* due to the slow diffusive transport.[13] Figure 3b shows the results of the reaction using immobilized cells with addition of glucose. The yields are almost the same as in Figure 3a, but the diastereoselectivity was improved since the immobilization shield plays an important role by creating a barrier to substrate partition. The glucose acts as an electron donor since the enzymes (oxidoreductases) involved in the reduction use mainly NAD(P)H as cofactor. As *S. cerevisiae* contains only a catalytic amount of NAD(P)H, its regenerations must take place for sustained catalytic activity. With cell entrapment this effect makes a difference, as can be seen by comparison between Figure 3a (no glucose, nonfermenting condition) and Figure 3b (reaction in the presence of glucose).

Results of bioreduction of **1b** at constant cell concentration using immobilized beads of different particle sizes are presented in Table 2. The results using free cells and using immobilized cells at $d_P = 1.1$ mm were comparable; however, this is not the case anymore at larger particle sizes. At the highest yield (92%), de and ee both for **2b** and **3b** were achieved using immobilized cells at $d_P = 3.0$ mm. This latter condition was used in the following experiments.

The yeast *Rhodotorula minuta* and the bacterium *Serratia marcences* were also immobilized. However, for these microorganisms, this entrapment process reduces enzymatic activity. Different matrixes were used for the immobilization of *S. cerevisiae*, but entrapment in calcium alginate gave better results than the other matrixes used (Table 3).

The reaction with *S. cerevisiae* immobilized in calcium alginate, at pH 4 and with addition of glucose (entry 1, Table 3), was scaled up (entry 4, Table 3). The de for *syn* (2*S*,3*S*) dropped from 50 to 40%, and the ee from 96 to 85%, while the yield remained around 80%. To improve the selectivity a new procedure was established by decreasing even more the concentration of substrate available for the biocatalyst,

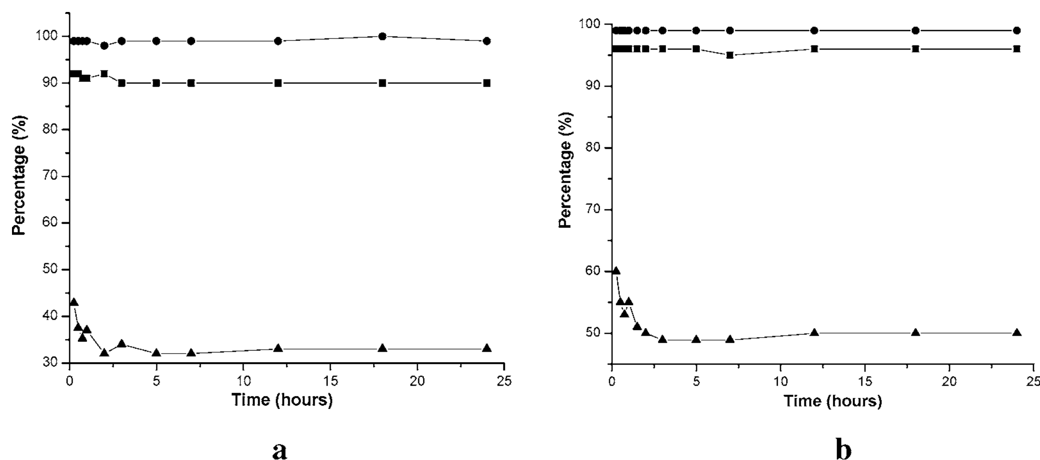

Figure 3. **(a) Bioreduction of 1b (0.18 mmol) mediated by *S. cerevisiae* immobilized in Ca-alginate beads and suspended in citrate buffer at pH 4.0. (b) Bioreduction of 1b (0.18 mmol) mediated by *S. cerevisiae* immobilized in Ca-alginate beads and suspended in citrate buffer at pH 4.0 with addition of glucose (ee of 2b (■), ee of 3b (●), de 2b/3b (▲)).**

Table 2. **Influence of particle size on reduction of 1b using immobilized *S. cerevisiae* at constant cell concentrations**

			ee (%)	
$d_{P,mean}$ (mm)	yield (%)	*syn* : *anti*	*syn*	*anti*
free cells	87	69:31	83	95
1.1	83	67:33	85	95
2.1	85	73:27	92	>99
2.3	88	75:25	92	>99
3.0	92	75:25	96	>99

Table 3. **Bioreduction of 1b mediated by *S. cerevisiae* immobilized in different porous matrixes**

				ee (%)	
entry	matrix	yield (%)	*syn*:*anti*[c]	*syn*	*anti*
1	Ca-alginate[a]	92	75:25	96	>99
2	κ-carrageenan[a]	87	75:25	90	>99
3	Lenticats[a]	79	75:25	90	>99
4	Ca-alginate[b]	80	70:30	85	>99

[a] Bioreduction of **1b** (0.18 mmol) mediated by 1.0 g of *S. cerevisiae* suspended in citrate buffer (60 mL, pH 4.0) with addition of glucose, reaction time = 24 h. [b] Bioreduction of **1b** (2,2 mmol) mediated by 5.0 g of *S. cerevisiae* suspended in citrate buffer (300 mL, pH 4.0) with addition of glucose, reaction time = 24 h. [c] The diastereomeric ratio was determined by GC−MS, and enantiomeric excess was determined by GC (see Experimental Section).

introducing a second barrier of alginate. The cells were immobilized with double gel layers, which also prevent cells from leaking out of the gel beads (Figure 4). It is well-known that the net pores of Ca-alginate gel beads are so large that biocatalyst can sometimes leak out from the gel.[42] Figure 5 shows the time course of **1b** bioreduction mediated by *Saccharomyces cerevisiae* immobilized in Ca-alginate beads with double gel layers. It is worth pointing out that the selectivity of this reaction was the same during all reaction times (compare Figure 5 to Figure 3b). The conditions for bioreduction of **1b** were optimized, and enantioselectivity was improved for both *syn* and *anti* enantiomers. Also, the

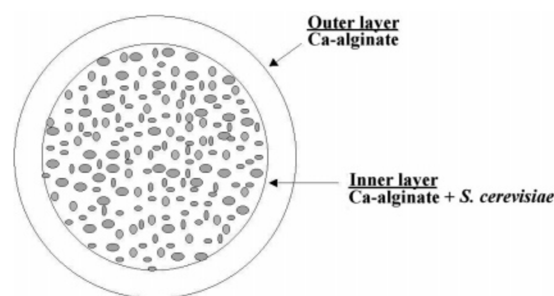

Figure 4. **Schematic diagram of a cross section of Ca-alginate beads with double gel layers.**

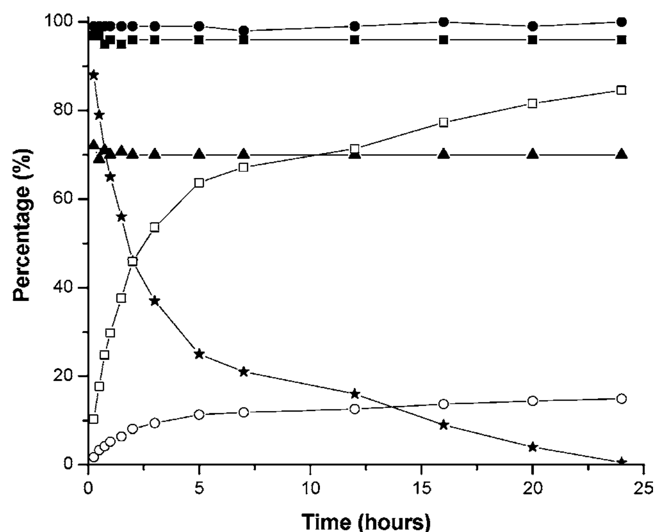

Figure 5. **Bioreduction of 1b (0.18 mmol) mediated by *Saccharomyces cerevisiae* immobilized in Ca-alginate beads with double gel layers suspended in citrate buffer at pH 4.0, with addition of glucose (1b (★), 2b (□), 3b (○), ee of 2b (■), ee of 3b (●), de 2b/3b (▲).**

syn:*anti* ratio increased to 85:15. With double gel layers another barrier was created, and the gradients of substrate concentration in the alginate beads affected the overall reduction performance.

The double gel layer immobilization was used for bioreduction of ethyl 3-chloro-2-oxo-4-phenylbutanoate **1a** and compared with the results reported by Tsuboi,[34] see Table

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Table 4. **Comparison between the bioreduction of 1 mediated by *S. cerevisiae* immobilized in Ca-alginate and literature data**[32]

entry	substrate	yield (%)	*syn*:*anti*	ee (%)	
				syn	*anti*
1[a]	ref 34	50	34:66	43	95
2[b]	**1a**	85	30:70	85	>99
3[b]	**1b**	90	85:15	96	>99

[a] Reaction conditions: 42.5 g of *S. cerevisiae* suspended in citrate buffer (300 mL, pH 7.0), 720 mg of **1** and addition of 54.5 g of glucose. [b] Reaction conditions: 5.0 g of *S. cerevisiae* entrapped in Ca-alginate beads with double gel layers in large scale were suspended in citrate−phosphate buffer (60 mL, pH 4.0) containing glucose (8 g), 2.2 mmol of **1** (**1a** or **1b**). After each 6 h, more glucose (1.6 g) was added.

4. The diastereomeric ratio was almost the same for both methods, but for *S. cerevisiae* entrapped in Ca-alginate beads with double gel layers, the ee was better than that reported in the literature (Table 4, entry 3).

The relative configuration of *syn*-**2b** was established by comparison with the known ethyl (2*S*,3*R*)-3-chloro-2-hydroxy-4-phenylbutanoate,[34] and the absolute configuration was determined by hydrogenolysis with H_2 in the presence of Pd/C 5% of the *syn*-**2b,** to give the known ethyl (−)-(2*S*)-2-hydroxy-4-phenylbutanoate in 95% yield, $[\alpha]^{20}$ −32.5 (*c* 2.0, $CHCl_3$). Bioreduction of **1a** by *S. cerevisiae* entrapped in alginate beads with double gel layers gave ethyl (2*S*,3*S*)-3-chloro-hydroxy-butanoate **2a** (85% ee) and its epimer, the (2*S*,3*R*)-derivative **3a** (>99% ee; ratio **2a**:**3a** = 30:70) in 85% yield. ^{1}H NMR coupling constants for 2-H and 3-H for *syn*-**2a** (δ 4.44, $J_{2,3}$ = 1.8 Hz) and for *anti*-**3a** (δ 4.24, $J_{2,3}$ = 2.6 Hz) have the same values as for *syn*-**2b** (δ 4.50, $J_{2,3}$ = 1.8 Hz) and *anti*-**3b** (δ 4.46, $J_{2,3}$ = 2.6 Hz), confirming the proposed absolute configuration for **2b** (^{1}H NMR and ^{13}C NMR values matched those reported by Tsuboi).[34]

In conclusion, we have developed a highly diastereo and enantioselective method for bioreduction of ethyl 3-halo-2-oxo-4-phenylbutanoate to produce 3-halo-2-hydroxy-phenylbutanoates. Among the biocatalysts tested, *S. cerevisiae* gave the highest product yields, and the entrapment in alginate beads with double gel layers showed its efficacy in controlling the substrate concentration which is fundamental for the enantioselectivity and also for obtaining high *syn*:*anti* ratio of 3-halo-2-hydroxy-phenylbutanoates.

Experimental Section

All reagents and solvents were obtained from commercial sources. The yeast extract, malt extract, and bacto-peptone were purchased from Biobrás (Brazil). The microorganisms were purchased from the Coleção de Culturas Tropical (CCT) Fundação André Tosello, Campinas, SP, Brazil, except for the dry *S. cerevisiae*, which was purchased from Emulzint Ltd. (Belgium) and stored in a refrigerator. Thin-layer chromatography (TLC) analyses were performed with precoated aluminum sheets (silica gel 60 Merck), and flash column chromatography was carried out on silica (200−400 mesh, Merck). IR spectra were recorded on a FT-IR BOMEM MB-100 from Hartmann & Braun. ^{1}H NMR spectra were determined at 300 MHz (Varian Gemini 300) or 500 MHz (INOVA-500), and ^{13}C NMR spectra were determined at 75.5 MHz (Varian Gemini 300) or 125.7 MHz (INOVA 500). Chemical shifts are reported in ppm relative to tetramethylsilane (TMS) in $CDCl_3$. Gas chromatographic analyses and mass spectra were obtained on a QP 5000-Shimadzu or an CG 6890/ Hewlett Packard 5973 equipped with J&W Scientific HP-5 (5% phenylmethylpolysiloxane, 30.0 m × 250 μm × 0.25 μm), Macherey 212117/91 Hydrodex-β 3P (25.0 m × 250 μm × 0.25 μm) capillary columns. High-resolution mass spectrum was determined on a VG Auto Spec Micromass. Optical rotations were measured on a Perkin-Elmer Polarimeter 341. Melting points were measured on a Microquimica MQ APF-301.

Bromination of Ethyl 2-oxo-4-phenylbutanoate. To a solution of ethyl 2-oxo-4-phenylbutanoate (5.0 g, 24.2 mmol) in chloroform (7 mL) was added slowly a solution of bromine in carbon tetrachloride (1.06 mol·L^{-1}) until the color of the solution became red-orange. The excess of bromine was destroyed with a sodium thiosulphate solution, and the reaction mixture was extracted with ethyl acetate, dried over anhydrous $MgSO_4$, and then filtered and evaporated. The crude material was purified by flash chromatography and eluted with 20% ethyl acetate in hexane.

Ethyl 3-bromo-2-oxophenylbutanoate, 4: pale-yellow oil, 97% yield; ^{1}H NMR values matched those reported in the literature.[38] ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.35 (3H, t, J = 7.0 Hz, H-12), 3.24 (1H, dd, J = 14.5 e 7.5 Hz, H-4), 3.52 (1H, dd, J = 14.5 e 7.5 Hz, H-4), 4.32 (2H, q, J = 7.0 Hz, H-11), 5.28 (1H, t, J = 7.3, H-3) 7.28 (5H, m, H-6 to H-10).

Bioreductions of Ethyl 3-bromo-2-oxo-4-phenylbutanoate. Screening experiments were conducted for yeast grown for 24 h in an orbital shaker (110 rpm) at 30 °C in 50 mL of a liquid medium containing (per liter), yeast extract 3.0 g, malt extract 3.0 g, bacto-peptone 5.0 g, glucose 20.0 g. The yeast was centrifuged, then resuspended in 1.0 L of the same medium and grown in an orbital shaker (110 rpm) at 30 °C for 48 h. The yeast was centrifuged (3000 rpm), and then washed. Each 4.0 g of biomass were resuspended in citrate−phosphate buffer (100 mL, pH 4.0), and 143.0 mg (0.5 mmol) of substrate was added. The reaction was maintained in an orbital shaker (110 rpm) at 30 °C for 24 h. The bacteria were similarly grown using the following medium: meat extract 5.0 g, bacto-peptone 5.0 g, glucose 2.0 g, K_2HPO_4. The reaction was performed in the same way for all yeasts, except *S. cerevisiae*. The reactions were monitored by GC−MS, and at the end of the reaction (24 h), the biomass was centrifuged and washed with ethyl acetate. The reaction mixture was extracted with ethyl acetate, dried over anhydrous $MgSO_4$, and then filtered and evaporated. The crude material was purified by flash chromatography and eluted with 20% ethyl acetate in hexane.

Ethyl (2*S*,3*S*)-3-Bromo-2-hydroxy-phenylbutanoate, 5: colorless oil, $[\alpha]^{20}_D$ −16.8 (*c* 2.1, $CHCl_3$); IR (film): 3503, 3066, 3027, 2983, 2929, 1732, 1596, 1499, 1455, 1367, 1256, 1110, 1017, 740, 701 cm^{-1}; MS *m*/*z* (%): 206 (2), 189 (15), 177 (1), 161 (5), 143 (15), 133 (51), 115 (66), 91 (100), 77 (15), 55 (20), 51 (15); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (3H, t, J = 7.1 Hz, H-12), 3.32 (2H, m,

H-4), 4.14 (1H, d, $J = 1.8$ Hz, H-2), 4.26 (2H, m, H-11), 4.50 (1H, dt, $J = 1.8$ e 8.1 Hz, H-3), 7.26 (5H, m, aromatic); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.8 (CH_3), 42.4 (CH_2, C-4), 56.9 (CH, C-3), 62.9 (CH_2, C-11), 71.5 (CH, C-2), 127.6 (CH, aromatic), 129.1 (2CH, aromatic), 129.7 (2CH, aromatic), 138.0 (C, aromatic), 172.2 (C=O). HRMS: calcd for $C_{12}H_{15}BrO_3$: 286.02046; found: 286.02051.

Ethyl (2*S*,3*R*)-3-Bromo-2-hydroxy-phenylbutanoate, 6: colorless oil, $[\alpha]^{20}_D$ −18.8 (*c* 2.1, $CHCl_3$) IR (film): 3503, 3066, 3027, 2983, 2929, 1732, 1596, 1499, 1455, 1367, 1256, 1110, 1017, 740, 701. MS *m/z* (%): 206 (2), 189 (15), 177 (1), 161 (5), 143 (15), 133 (51), 115 (66), 91 (100), 77 (15), 55 (20), 51 (15) ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.35 (3H, t, $J = 7.0$ Hz, H-12), 3.30 (2H, m, H-4), 4.24 (2H, q, $J = 7.0$ Hz, H-11), 4.39 (1H, m, H-2), 4.46 (1H, dt, $J = 2.6$, e 7.7 Hz, H-3), 7.26 (5H, m, H-6 to H-10). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.8 (CH_3), 41.2 (CH_2, C-4), 56.8 (CH, C-3), 62.9 (CH_2, C-11), 73.8 (CH, C-2), 127.5 (CH, aromatic), 128.9 (2CH, aromatic), 129.7 (2CH, aromatic), 137.9 (C_0, aromatic), 171.3 (C_0, aromatic). HRMS: calcd for $C_{12}H_{15}BrO_3$ [M^+]: 286.02046, found: 286.02051.

Bioreductions of Ethyl 3-bromo-2-oxo-4-phenylbutanoate Mediated by *Saccharomyces cerevisiae*. In a 100-mL bioreactor, 1.0 g of *S. cerevisiae* was suspended in citrate−phosphate buffer (60 mL, pH 4.0) containing glucose (1.6 g) and stirred at 300 rpm at 30 °C. After activation of the yeast for 2 h, the pH of the medium was adjusted with a 10% solution of NH_4OH, and then the substrate **4** (0.5 mmol in 5.0 mL of ethanol) was added slowly over 10 h with stirring, and glucose (0.32 g) was added every 6 h. The reaction was monitored by GC−MS, and at the end of the reaction (24 h), the biomass was centrifuged and then washed with ethyl acetate. The filtrate was extracted with ethyl acetate, dried over anhydrous $MgSO_4$, and then filtered and evaporated. The crude material was purified by flash chromatography and eluted with 20% ethyl acetate in hexane.

General Procedure for Reduction Mediated by *Saccharomyces cerevisiae* Entrapped in Ca-Alginate Beads. A solution of sodium alginate (3%) was added to a suspension of *S. cerevisiae* (1.0 g) in distilled water (10 mL). This mixture was extruded using syringe nozzles with inner diameters of 1.0 mm to a solution of $CaCl_2$ (0.2 $mol.L^{-1}$) to give beads with diameters of 3 mm. After 20 min the beads were filtered and washed with water to remove excess $CaCl_2$. In a 100-mL bioreactor, the beads were suspended in citrate−phosphate buffer (60 mL, pH 4.0) containing glucose (1.6 g) and stirred at 300 rpm at 30 °C. After activation of the yeast for 2 h, the pH of the medium was adjusted with a 10% solution of NH_4OH, and then the substrate **4** (0.5 mmol in 5.0 mL of ethanol) was added slowly over 10 h with stirring. After each 6 h, glucose (0.32 g) was added. The reaction was monitored by GC−MS, and at the end of the reaction (24 h), the beads were filtered and washed with ethyl acetate, and the reaction mixture was extracted with ethyl acetate. The combined organic phases were dried over anhydrous $MgSO_4$, and the solvent was evaporated. The crude material was purified by flash chromatography and eluted with 20% ethyl acetate in hexane.

General Procedure for Reduction Mediated by *Saccharomyces cerevisiae* Entrapped in κ- Carrageenan Beads. A solution of κ-carrageenan (50 mL, 3%) was added to a suspension of *S. cerevisiae* (1.0 g) in distilled water (6.0 mL) 3% at 40 °C. This mixture was extruded using syringe nozzles (equipped with external thermostat ribbon at 40 °C) with inner diameters of 1.0 mm to a solution of KCl (0.3 $mol\cdot L^{-1}$) to give beads 3 mm in diameter. After 20 min the beads were filtered and washed with water. In a 100-mL bioreactor, the beads were suspended in citrate−phosphate buffer (60 mL, pH 4.0) containing glucose (1.6 g) and stirred at 300 rpm at 30 °C. After activation of the yeast for 2 h, the pH of the medium was adjusted with a 10% solution of NH_4OH, and then the substrate **4** (0.5 mmol in 5.0 mL of ethanol) was added slowly over 10 h with stirring; after each 6 h glucose (0.32 g) was added. The reaction was monitored by GC−MS; at the end of the reaction (24 h), the beads were filtered and washed with ethyl acetate, and the reaction mixture was extracted with ethyl acetate, dried over anhydrous $MgSO_4$, and then filtered and evaporated. The crude material was purified by flash chromatography and eluted with 20% ethyl acetate in hexane.

General Procedure for Reduction Mediated by *Saccharomyces cerevisiae* Entrapped in LentiKats. The solution of LentiKats was prepared according to the procedure provided by the supplier and kept at 40 °C. A suspension of *S. cerevisiae* (1.0 g) in distilled water (6.0 mL) was added and dispersed homogeneously by mixing with a magnetic stirrer. For production of the LentiKats, a smooth plate is needed, preferably one made of polystyrene. Using a standard syringe with a nozzle (1.0 mm in diameter) droplets were formed and dripped neatly onto the surface of the plate. The droplets formed (approximately 3 mm in diameter and 5 mg in mass) were dried by exposure to air, and the stabilizer was added. After 2 or 3 min of contact with the stabilizer solution LentiKats was easily removed from the surface and put into a bottle containing a 10-fold surplus of LentiKat-Stabilizer. After stabilization was finished, the supernatant had to be removed. In a 100-mL bioreactor, the beads were suspended in citrate−phosphate buffer (60 mL, pH 4.0) containing glucose (1.6 g) and stirred at 300 rpm at 30 °C. After activation of the yeast for 2 h, the pH of the medium was adjusted with a 10% solution of NH_4OH, and then substrate **4** (0.5 mmol in 5.0 mL of ethanol) was added slowly over 10 h with stirring. After each 6 h, glucose (0.32 g) was added. The reaction was monitored by GC−MS. At the end of the reaction (24 h), the beads were filtered and washed with ethyl acetate. The reaction mixture was extracted with ethyl acetate and dried over anhydrous $MgSO_4$; the solvent was then evaporated. The crude material was purified by flash chromatography and eluted with 20% ethyl acetate in hexane.

General Procedure for Reduction Mediated by Free *Saccharomyces cerevisiae*. In a 100-mL bioreactor, 1.0 g of *S. cerevisiae* was suspended in citrate−phosphate buffer (60 mL, pH 4.0) and stirred at 300 rpm at 30 °C. Then the substrate **4** (0.5 mmol in 5.0 mL of ethanol) was added, and the reaction was monitored by GC−MS. At the end of the

reaction (24 h), the biomass was centrifuged and washed with ethyl acetate; the reaction mixture was extracted with ethyl acetate, dried over anhydrous $MgSO_4$, and then filtered, and the solvent was evaporated. The crude material was purified by flash chromatography and eluted with 20% ethyl acetate in hexane.

General Procedure for Reduction Mediated by Free *Saccharomyces cerevisiae* with Addition of Glucose. In a 100-mL bioreactor, 1.0 g of *S. cerevisiae* was suspended in citrate−phosphate buffer (60 mL, pH 4.0) containing glucose (1.6 g) and stirred at 300 rpm at 30 °C. After activation of the yeast for 2 h, the pH of the medium was adjusted with a 10% solution of NH_4OH, and then the substrate **4** (0.18 mmol in 1.0 mL of ethanol) was added with stirring. The reaction was monitored by GC−MS, and at the end of the reaction (24 h), the biomass was centrifuged and washed with ethyl acetate; the reaction mixture was extracted with ethyl acetate, dried over anhydrous $MgSO_4$ and then filtered, and the solvent was evaporated. The crude material was purified by flash chromatography and eluted with 20% of ethyl acetate in hexane.

General Procedure for Reduction Mediated by *Saccharomyces cerevisiae* Entrapped in Ca-Alginate Beads with Double Gel Layers in Large Scale. A solution of sodium alginate (3%) was added to a suspension of *S. cerevisiae* (5 g) in distilled water (50 mL). This mixture was extruded using syringe nozzles with inner diameters of 1.0 mm to a solution of $CaCl_2$ (0.2 mol·L^{-1}) to produce beads 3 mm in diameter. After 20 min the beads were filtered and the surfaces dried using filter paper. Afterwards the Ca-alginate beads were placed into 400 mL of stirred 1.5% sodium alginate. After 20 min coating time, the beads were sieved, washed with water, and hardened for 2 h in a solution of $CaCl_2$ (0.2 mol·L^{-1}). During the coating procedure, Ca^{2+} diffused from the Ca-alginate bead into the alginate solution, and a cell-free Ca-alginate layer was formed surrounding the biocatalyst. The beads with double gel layers were washed with water to remove the excess $CaCl_2$. In a 500-mL bioreactor, the beads were suspended in citrate−phosphate buffer (300 mL, pH 4.0) containing glucose (8 g) and stirred at 300 rpm at 30 °C. After activation of the yeast for 2 h, the pH of the medium was adjusted with a 10% solution of NH_4OH, and then the substrate **2a**−**d** (2.2 mmol in 5.0 mL of ethanol) was added with stirring. After each 6 h, glucose (1.6 g) was added. The reaction was monitored by GC−MS, and at the end of the reaction (24 h), the beads were filtered and then washed with ethyl acetate. The reaction mixture was extracted with ethyl acetate, dried over anhydrous $MgSO_4$, and then filtered; the solvent was evaporated. The crude material was purified by flash chromatography and eluted with 20% of ethyl acetate in hexane.

Ethyl 3-Chloro-2-hydroxy-4-phenylbutanoates, 2 and 3. The reduction of 3-chloro-2-oxo-4-phenylbutanoate was carried out by the procedure described above for **4**. The crude products were separated by flash chromatography and eluted with hexane/ethyl acetate (5:1). IR and ^1H NMR values matched those reported in the literature for **2** and **3**.[34]

Ethyl (−)-(2*S*)-2-Hydroxy-4-phenylbutanoate, 8. Sodium acetate (57 mg, 0.7 mmol) and 5% palladium on carbon (16 mg) were added to a solution of **3b** (182 mg, 0.7 mmol) in anhydrous ethanol (3 mL) under argon. After stirring 2 h at room temperature, the reaction mixture was filtered over Celite, extracted with ethyl acetate (3 × 50 mL), and washed with brine; the organic phase was dried in anhydrous $MgSO_4$ and filtered. Then the solvent was distilled under reduced pressure. The crude product was purified by silica gel chromatography and eluted with hexane in ethyl acetate (15%) to give **8** (138 mg, 95%). Yellow oil, $[\alpha]^{20}_D$ −32.5 (*c* 2.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 1.28 (3H, t, *J* = 7.0 Hz, H-12), 1.90−2.18 (2H, m, H-4), 2.77 (2H, m, H-3), 4.20 (2H, q, *J* = 7.0 Hz, H-11), 7.26 (5H, m, aromatic).[12]

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