Organic Process Research & Development

ARTICLE

pubs.acs.org/OPRD

Enoate Reductase-Mediated Preparation of Methyl (*S*)-2-Bromobutanoate, a Useful Key Intermediate for the Synthesis of Chiral Active Pharmaceutical Ingredients

Elisabetta Brenna,*[,†] Francesco G. Gatti,[†] Alessia Manfredi,[†] Daniela Monti,[‡] and Fabio Parmeggiani[†]

[†]Politecnico di Milano, Dipartimento di Chimica, Materiali ed Ingegneria Chimica, Via Mancinelli 7, I-20131 Milano, Italy

[‡]Istituto di Chimica del Riconoscimento Molecolare - CNR, Via Mario Bianco 9, I-20131 Milano, Italy

ABSTRACT: Enoate reductases belonging to the Old Yellow Enzyme (OYE) family were employed to develop a biocatalysed approach to methyl (*S*)-2-bromobutanoate, a key intermediate for the introduction of a particular stereogenic unit into the molecular skeleton of a certain class of chiral drugs. Methyl (*Z*)-2-bromocrotonate afforded, respectively, (*S*)-2-bromobutanoic acid (ee = 97%) and methyl (*S*)-2-bromobutanoate (ee = 97%) by baker's yeast fermentation and by OYE1−3 biotransformations. The bioreductions of other methyl 2-haloalkenoates were also considered. It was observed that the (*Z*)- and (*E*)-diastereoisomers of α-bromo unsaturated esters afforded the same enantiomer of the corresponding reduced product.

INTRODUCTION

According to a very detailed analysis published recently in *Org. Process Res. Dev.*,[1] the proportion of products manufactured using biotechnology is expected to increase to ∼20% of global chemicals in 2020 (i.e., about 1 order of magnitude increase from today's figures), as a consequence of the urgent need for the development of sustainable manufacturing processes, along with the reduction of greenhouse gas emission and waste output, and the replacement of toxic or dangerous reagents. However, biotechnology might disappoint these expectations in the pharmaceutical area, because the time frame of drug development makes the application of biocatalysed routes in the manufacturing processes particularly demanding due to an inadequate toolbox.[2] It is thus necessary to devote efforts on optimising biotechnological tools for the synthesis of higher-value products in the life science area, such as enantiomerically pure intermediates and active pharmaceutical ingredients (APIs), and on investigating the robustness of biocatalysed reactions.

Aiming to satisfying these needs, we devoted our attention to study the stereoselective reduction of suitably substituted C═C double bonds, a very useful synthetic transformation, which is commonly employed in the development of manufacturing processes and can be carried out by enzyme catalysis with the use of enoate reductases.[3] The corresponding reaction performed by hydrogenation in the presence of chiral metal complexes[4] has been intensively investigated; several catalytic systems have been optimised for variably substituted substrates, and a rather rich pool of successful reactions have been identified. There is still the necessity to study the enzymatic variant of this reaction,[5] in order to enlarge the structural class of unsaturated compounds that can be submitted to bioreduction, to understand the electronic and steric effects controlling the reaction, and to implement the pool of biotransformations available for applications in the field of pharma drug development.[6]

Biocatalysed reductions of C═C double bonds have been known for a long time; most of the work has been carried out by baker's yeast (BY) fermentation, avoiding the problem of cofactor recycling.[7] In the last years the process of cloning and overexpressing enoate reductases, particularly those belonging to the family of Old Yellow Enzymes (OYEs), has enriched the panel of biocatalysts available for these reductions.[8] However, the development of preparative procedures to obtain target molecules is still lacking, with most of the investigations aimed to characterise the activity of isolated enoate reductases on model substrates.

Among OYE-catalysed bioreductions were first considered those of C═C double bonds in α,*β* position to carboxylic groups, which have received little attention until now. Unlike conjugated enals and enones, α,*β*-unsaturated esters and acids are not conventional substrates for enoate reductase biotransformations. Nonetheless, their employment as starting materials can offer interesting advantages, i.e. the inertness of the carboxylic moiety towards bioreduction, and an increased configurational stability of the stereogenic centre, eventually created in α-position to the carboxylic group, compared with that of aldehydes and ketones.

Within the work devoted to the investigation of the limits and the advantages of this kind of bioreduction,[9] we envisaged the possibility of employing the enantioselective reduction of (*Z*)-methyl 2-bromo and 2-chlorocrotonates **1** and **2** by means of BY fermentation and OYE1−3[10] biotransformations, in order to optimise a new enzymatic approach to halobutanoic acids (*S*)-**3** and (*S*)-**4** and of the corresponding methyl esters (*S*)-**5** and (*S*)-**6** (Scheme 1), which are useful stereogenic units to be incorporated into chiral APIs. The investigation was extended to (*E*)-**1** and to the (*Z*) and (*E*)-stereoisomers of other α-bromoalkenoic acid methyl esters, namely (*Z*)- and (*E*)-**7**, and (*Z*)- and (*E*)-**8**.

Special Issue: INTENANT

Received: March 31, 2011
Published: July 22, 2011

ACS Publications © 2011 American Chemical Society

■ RESULT AND DISCUSSION

To our knowledge the only examples of baker's yeast reduction of unsaturated esters were described by Utaka et al. in the 1980s.[11] They employed as substrates a few methyl α-chloroalkenoates, showing R = alkyl (CH_2CH_3, $CH_2CH_2CH_3$, $CH(CH_3)_2$) and chloroalkyl (CH_2Cl, $CHCl_2$, CCl_3). They established that the presence of the chlorine atom in the α-position was essential to allow bioreduction, and that only methyl esters could be transformed. All the investigated (*Z*)-unsaturated chloro esters gave saturated (*S*)-chloro acids with high enantiomeric excess (ee > 98%), whereas the (*E*)-stereoisomers afforded (*R*)-chloro acids with lower ee values. Ester hydrolysis was promoted as a side reaction in the fermentation medium. The reaction was not considered further in the following years.

Recently, we submitted[9] to OYE1−3-mediated reductions some of the substrates described by Utaka et al., i.e. the (*Z*)-stereoisomers of compounds with R = $CHCl_2$ and CCl_3, and the (*E*)- and (*Z*)-stereoisomers of that bearing R = $CH(CH_3)_2$. We verified that in these conditions hydrolysis did not occur and that the methyl esters were the actual substrates of the enzyme-mediated reaction.

These data supported our idea to employ this reduction for the optimisation of an enzymatic approach to enantiomerically pure (*S*)-2-bromobutanoic acid ((*S*)-**3**) and to the corresponding methyl ester (*S*)-**5**, which are useful key intermediates in the synthesis of therapeutic agents for the treatment of non-insulin-dependent type 2 diabetes mellitus (T2DM), such as compounds (*R*)-**9**,[18] (*R*)-**10**,[19] and (*R*)-**11**[20] (Chart 1). All these structures share a typical stereogenic unit, highlighted in Chart 1, which is introduced by nucleophilic displacement with configuration inversion either on compound (*S*)-**5**,[13a] or on a suitable alkyl (*S*)-trifluoromethanesulfonyloxybutanoate.[14]

(*S*)-**3** and (*S*)-**5** are not commercially available. Their synthesis is rather challenging because of the configurational lability of the stereogenic center in basic medium and of the reactivity of the bromine atom. (*S*)-**3** was obtained by deracemisation with chiral auxiliaries[21,22] or by Van Slyke reaction of (*S*)-2-aminobutanoic acid.[13a] (*S*)-**5** was prepared by treatment of the bromoacid with (trimethylsilyl)diazomethane,[13a] or it was recovered as the unreacted enantiomer when racemic **5** was treated with haloalkane dehalogenases.[23] The development of a high yield, enantioselective preparation of (*S*)-**3** and (*S*)-**5** is thus relevant under the practical point of view, because of the known usefulness of these chiral intermediates. Their availability would avoid using triflate derivatives, which are usually rather unstable and whose preparation requires the handling of hazardous triflic anhydride.

A 6:4 mixture of (*E*)- and (*Z*)-**1** was obtained by addition of bromine to methyl (*E*)-crotonate followed by dehydrohalogenation.[13b] The two stereoisomers could be separated by column chromatography. Compound (*Z*)-**1** was also obtained in 82% diastereoisomeric excess (^{1}H NMR) by reaction of acetaldehyde with the bromophosphoylide prepared *in situ* by reaction of NBS with methyl(triphenylphosphoranylidene)-acetate according to a known procedure.[24]

(*E*)- and (*Z*)-**1** were submitted separately to BY fermentation and OYE1−3 biotransformations (Scheme 2), and the results are reported in Table 1. Saturated acids were recovered from BY fermentation medium, and the corresponding methyl esters were obtained in OYE-catalysed biotransformations. The conversion curves are shown in Figures 1 and 2.

Unexpectedly, the reduction of both (*Z*)-**1** and (*E*)-**1** afforded the same enantiomer, unlike what had been observed for diastereoisomeric α-chloro-unsaturated methyl esters by Utaka et al.[11] The enantiomeric purity of acid (*S*)-**3**, obtained by BY fermentation, and of methyl ester (*S*)-**5**, recovered from OYE biotransformations, was higher when (*Z*)-**1** was the starting material.

We also tried to synthesise the two chloro derivatives (*Z*)- and (*E*)-**2**, in order to make a comparison with the bromocrotonates, but either by chlorine addition followed by basic dehydrohalogenation[25] or by reaction of acetaldehyde with the suitable chlorophosphoylide, we invariably obtained only (*Z*)-**2** (de = 80% and 83%, respectively; ^{1}H NMR). This compound was purified by column chromatography and submitted to BY fermentation and OYE1−3 biotransformations; the results are reported in Table 1. The enantiomeric excess values of the reduction products (*S*)-**4** and (*S*)-**6** were lower than those of the corresponding bromo analogues.

Scheme 1. Substrates and products of bioreductions

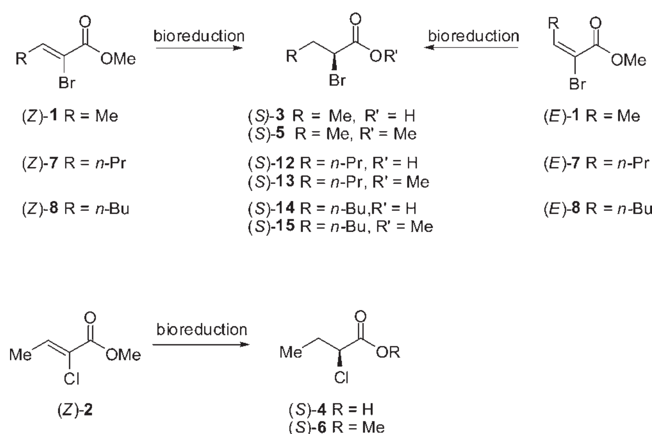
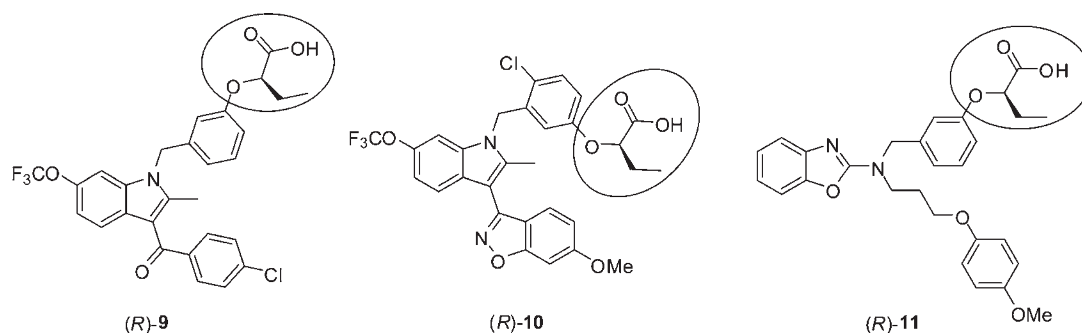


Chart 1. Chiral APIs incorporating the stereogenic unit of (*S*)-5



The substitution of the chlorine atom with a bromine atom did not prevent bioreduction: the biocatalysed reductions of (*Z*)-**1** indeed resulted in better conversion values and higher enantioselectivities than those achieved with (*Z*)-**2**. The same ee values were observed when a sample of (*Z*)-**1** with de = 82% was submitted to bioreduction.

We then decided to prepare (*E*)- and (*Z*)-**7**, whose chloro analogues had been submitted to BY fermentation by Utaka et al.,[11] and (*E*)- and (*Z*)-**8**, in order to further investigate the stereochemical course of bioreductions involving bromo derivatives. Compounds (*Z*)-**7** and (*Z*)-**8** were obtained in high diastereoisomeric excess by Wittig reaction of butanal and pentanal, respectively, with bromophosphoylide. The corresponding (*E*)-diastereoisomers were prepared by bromine addition to methyl 2-hexenoate and methy-2-heptenoate, followed by dehydrohalogenation and chromatographic purification. The results of the biocatalysed reductions, either by means of BY or of isolated OYEs, are reported in Table 1. We invariably obtained the same enantiomers of the corresponding saturated acids and methyl esters by reduction of the two possible diastereoisomers of the starting activated alkene. The enantiomeric excess values of the saturated compounds prepared from (*Z*)-alkenes were higher

Scheme 2. Biocatalysed reduction of (*E*)- and (*Z*)-1 mediated by BY and OYE1−3

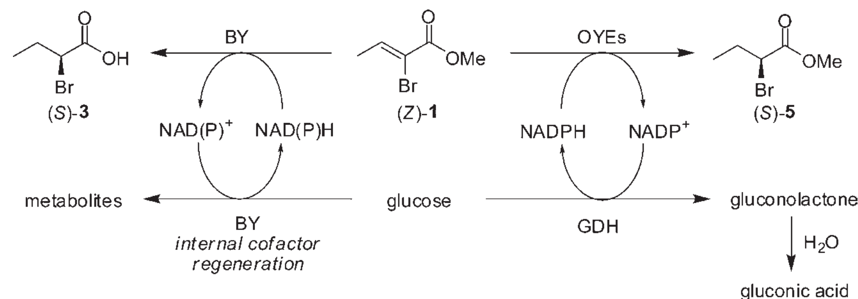


Table 1. Results of BY fermentations and OYE1−3 biotransformations

Substrate	BY c[a] (%) ee[c] (%)	OYE 1 c[b] (%) ee[c] (%)	OYE 2 c[b] (%) ee[c] (%)	OYE 3 c[b] (%) ee[c] (%)
(*Z*)-**1**	78 97 (*S*)	100 97 (*S*)	93 97 (*S*)	100 97 (*S*)
(*E*)-**1**	55 80 (*S*)	100 90 (*S*)	94 87 (*S*)	98 93 (*S*)
(*Z*)-**2**	50 85 (*S*)	100 93 (*S*)	100 83 (*S*)	95 98 (*S*)
(*Z*)-**7**	100 96 (*S*)	100 94 (*S*)	100 96 (*S*)	100 96 (*S*)
(*E*)-**7**	100 65 (*S*)	100 60 (*S*)	44 67 (*S*)	100 91 (*S*)
(*Z*)-**8**	100 96[d]	95 96[d]	43 97[d]	82 98[d]
(*E*)-**8**	100 68[d]	75 60[d]	- -	13 84[d]

[a] Conversion calculated by GC analysis of the crude mixture treated with CH_2N_2 solution after 72 h reaction time. [b] Conversion calculated by GC analysis of the crude mixture after 12 h reaction time. [c] Calculated by GC analysis on a stationary phase as methyl esters. [d] Second eluted peak in GC analysis on a chiral stationary phase.

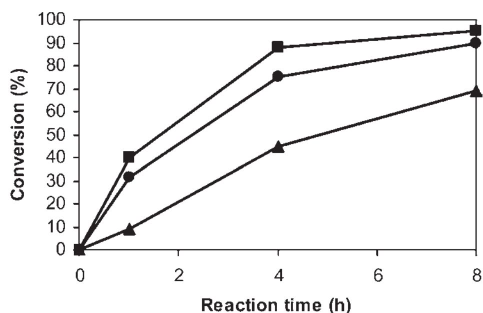


Figure 1. Progress curves for the bioconversion of (*Z*)-**1** into (*S*)-**5** by isolated OYEs/GDH (OYE1 (■), OYE2 (●), OYE3 (▲)). Experimental conditions: 50 mM phosphate buffer pH 7.0, 5 mM substrate, 20 mM glucose, 0.1 mM $NADP^+$, 40 μg/mL OYE, 4 U GDH, 1% v/v DMSO, 30 °C, 160 rpm. Conversions were calculated by GC analysis.

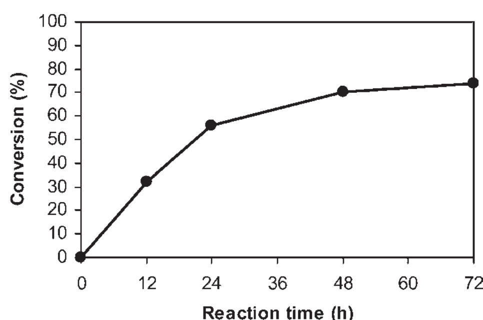


Figure 2. Progress curves for the bioconversion of (*Z*)-**1** into (*S*)-**3** by baker's yeast. Experimental conditions: 25 mM substrate, 40 g/L glucose, 100 g/L BY, 0.5% v/v EtOH, 30 °C, 160 rpm. Conversions were calculated by GC analysis of the methyl ester.

than those of the derivatives obtained by conversion of the (*E*)-diastereoisomers.

The chloro analogues of compounds (*Z*)- and (*E*)-**7** had been described by Utaka to afford the (*S*)- and (*R*)-enantiomers of the corresponding saturated acid with ee > 98% and ee = 25%, respectively, in moderate yields. On the contrary, (*Z*)- and (*E*)-**7** were converted by BY fermentation into acid (*S*)-**12** (ee = 96 and 65%) in quantitative yields, and by OYE1−3-mediated biotransformations into methyl ester (*S*)-**13** (ee = 94−96% and 60−91%) in good to excellent yields.

Similar results were obtained for (*Z*)- and (*E*)-**8** (Table 1); in this case we could not establish the absolute configuration of saturated acid **14** and its methyl ester **15**. We assigned them (*S*)-configuration by analogy: the saturated methyl ester **15** obtained from either (*Z*)- or (*E*)-**8** was the second eluting enantiomer in the GC analysis of the racemic reference compound on a chiral stationary phase, as in the case of all the other (*S*)-methyl esters described in the paper.

■ CONCLUSION

A very practical synthesis of enantioenriched (*S*)-**3** and (*S*)-**5**, and of the corresponding chloro derivatives (*S*)-**4** and (*S*)-**6** was optimised starting by biocatalytic reduction of (*Z*)-**1** and (*Z*)-**2**, which can be easily obtained starting from either acetaldehyde or methyl (*E*)-crotonate.

The bioreduction can be scaled up taking advantage of conventional fermentation facilities for whole cell systems or standard tank reactors for isolated enzymes.

The reactions can be performed in aqueous medium, and saturated acids and esters are obtained in highly satisfactory yield and enantiomeric purity. The stereogenic unit, which is needed for the inclusion in the structural skeleton of relevant pharmaceutical ingredients, is prepared according to an enantioselective reduction directly in the desired absolute configuration, avoiding the waste of the unwanted enantiomer produced in resolution approaches, and already bearing a suitable leaving group, i.e. the bromine atom, for nucleophilic displacement.

We also highlighted that the substitution of a chlorine atom with a bromine atom produces a different stereochemical outcome; we are currently undertaking further studies to explain the different behaviour of bromo and chloro analogues in biocatalysed reactions, by considering the mode of hydrogen addition and the way in which the substrates are bound within the active site of the enzyme.

The successful application of enzymatic processes in the chemical industry depends mainly on cost and synthetic efficiency competitiveness with the existing, and well-established, chemical processes.

The potential of enzymatic transformations, for example of C═C bioreductions, has to be investigated to find sustainable synthetic and manufacturing methods to be offered in particular to the chemists of the pharma area. The chemical complexity of drugs and their strict stereochemical requisites need very selective and efficient catalysts, as indeed enzymes are.

■ EXPERIMENTAL SECTION

General. TLC analyses were performed on Merck Kieselgel 60 F254 plates. All the chromatographic separations were carried out on silica gel columns. The chemical shift scale of NMR spectra was based on internal tetramethylsilane. *J* values are given in hertz. GC/MS analyses were performed using a HP-5MS column (30 m × 0.25 mm × 0.25 μm). The following temperature program was employed: 60° (1 min)/6°/min/150° (1 min)/12°/min/280° (5 min). The enantiomeric excess values were determined by GC analysis of methyl esters **5**, **6**, **13**, and **15**, using a DAcTBSil·BetaCDX 0.25 μm × 0.25 mm × 25 m column (Mega, Italy), installed on a HP 6890 gas chromatograph, according to the following temperature programs:

(a) 50 °C (3 min)/5 °C min^{-1}/180 °C (2 min)/30 °C min^{-1}/220 °C (10 min): (*R*)-**5** t_R = 9.14 min, (*S*)-**5** t_R = 10.12 min; (*R*)-**6** t_R = 7.66 min, (*S*)-**6** t_R = 9.70 min.

(b) 70 °C (1 min)/1 °C min^{-1}/110 °C (2 min)/30 °C min^{-1}/220 °C (10 min); (*R*)-**13** t_R = 10.74 min, (*S*)-**13** t_R = 10.99 min.

(c) 45 °C/0.2 °C min^{-1}/75 °C (2 min)/30 °C min^{-1}/220 °C (10 min); first enantiomer **15** t_R = 90.39 min, second enantiomer **15** t_R = 91.30 min.

Optical rotations were measured on a Dr. Kernchen Propol digital automatic polarimeter at 589 nm and are given in 10^{-1} deg cm^2 g^{-1}.

Strains and Enzymes. Baker's yeast from Lesaffre Italia (code number 30509) was employed. All the enzymes employed were overexpressed in *E. coli* BL21 (DE3) strains harboring a specific plasmid prepared according to standard molecular biology techniques: pET30a-OYE1 from the original plasmid kindly provided by Neil C. Bruce,[20] pET30a-OYE2 and pET30a-OYE3 from *S. cerevisiae*, and BY4741 and pKTS-GDH from *B. megaterium* DSM509 (detailed steps reported in ref 21).

Overexpression of the Enzymes in E. coli BL21 (DE3). A 5-mL culture in LB medium containing the appropriate antibiotic (50 μg mL^{-1} kanamycin for pET-30a, 100 μg mL^{-1} ampicillin for pKTS) was inoculated with a single colony from a fresh plate and grown overnight at 37 °C and 220 rpm. This starter culture was used to inoculate a 200-mL culture, which was in turn grown overnight at the same conditions and used to inoculate a 1.5 L culture. The latter was shaken at 37 °C and 220 rpm until OD_{600} reached 0.4−0.5, and then enzyme expression was induced by the addition of 0.1 mM IPTG (50 ng mL^{-1} anhydrotetracycline was also added in the case of the pKTS-GDH plasmid). After 5−6 h the cells were harvested by centrifugation (5000*g*, 20 min, 4 °C), resuspended in 50 mL of lysis buffer (20 mM phosphate buffer pH 7.0, 300 mM NaCl, 10 mM imidazole), and homogenized (Haskel high-pressure homogenizer). The cell-free extract, after centrifugation (20000*g*, 20 min, 4 °C), was chromatographed on IMAC stationary phase (Ni-Sepharose Fast Flow, GE Healthcare) with a mobile phase composed of 20 mM phosphate buffer, pH 7.0, 300 mM NaCl, and a 10−300 mM imidazole gradient. Protein elution was monitored at 280 nm; the fractions were collected according to the chromatogram and dialyzed twice against 1.0 L of 20 mM phosphate buffer, pH 7.0 (12 h each, 4 °C), to remove imidazole and salts. Purified protein aliquots were stored frozen at −80 °C.

General Procedure for the Preparation of Methyl (*Z*)-2-Haloalkenoates. NBS or NCS (0.11 mol) was added portionwise under nitrogen atmosphere to a solution of methyl-(triphenylphosphoranylidene)acetate (33.4 g, 0.10 mol) in CH_2Cl_2 (300 mL) at −20 °C The mixture was stirred at −20 °C for 1 h and then allowed to warm to rt. Acetaldehyde (4.4 g, 0.10 mol) and K_2CO_3 (34.5 g, 0.25 mol) were added to the mixture which was stirred for 16 h. The reaction mixture was poured into 300 mL of water. The organic phase was dried over Na_2SO_4, and the solvent was removed under reduced pressure to give a solid, which was purified on silica gel column chromatography using hexane/AcOEt (95:5), affording respectively (*Z*)-**1**, (*Z*)-**2**, (*Z*)-**7**, and (*Z*)-**8**. The configuration of the double bond of the two stereoisomers of compound **7** was assigned by analogy with (*Z*)- and (*E*)-**8** and on the basis of the fact that the chemical shift of the vinyl proton in (*Z*)-α-halo-α,β-unsaturated esters is higher than that of the (*E*)-stereoisomer.[22]

*Data of (Z)-**1**:* 12.1 g, 67%; ^{1}H NMR[23] (400 Hz, $CDCl_3$) δ 7.38 (q, *J* = 6.9, 1H, H−C(3)), 3.82 (s, 3H, $COOCH_3$), 1.95 (d, *J* = 6.9, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$), δ 162.7, 141.4, 117.0, 53.0, 17.7; GC/MS: t_R = 7.20 min, *m*/*z* 178 (M^+, 100), 147 (92), 119 (55).

*Data of (Z)-**2**:* 8.5 g, 63%; ^{1}H NMR[24] (500 MHz, $CDCl_3$) δ = 7.15 (q, *J* = 6.9 Hz, H-(3)), 3.81 (s, 3H, $COOCH_3$), 1.94 (d, *J* = 6.9 Hz,CH_3); ^{13}C NMR (100 MHz, $CDCl_3$), δ 162.9, 137.6, 125.5, 52.9, 14.9; GC/MS: t_R = 5.51 min, *m*/*z* 134 (M^+, 100), 119 (11), 103 (100).

*Data of (Z)-**7**:* 14.0 g, 68%;^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.29 (t, *J* = 7.2 Hz, H-(3)), 3.82 (s, 3H, $COOCH_3$), 2.32 (2H, q, *J* = 7.4 Hz, CH_2(4)), 1.54 (2H, m, CH_2(5)), 0.98 (t, *J* = 7.4 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.0, 146.3, 115.8, 53.1, 34.0, 20.9, 13.7; GC/MS: t_R = 11.41 min, *m*/*z* 206 (M^+, 30), 191 (22), 165 (100).

*Data of (Z)-**8**:* 13.4 g, 61%;^{1}H NMR[25] (400 MHz, $CDCl_3$) δ = 7.28 (t, *J* = 7.2 Hz, H-(3)), 3.80 (s, 3H, $COOCH_3$), 2.32 (2H, q, *J* = 7.3 Hz, CH_2(4)), 1.50−1.30 (4H, m, CH_2CH_2), 0.93 (t, *J* = 7.0 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.0, 146.7, 115.6, 53.1, 31.8, 29.6, 22.3, 13.7; GC/MS: t_R = 13.70 min, *m*/*z* 220 (M^+, 14), 191 (43), 165 (100).

Procedures for Halogenation of Methyl 2-Alkenoates Followed by Dehydrohalogenation. *Procedure A (according to ref* 13b*).* Bromine (5 mL, 0.10 mol) was added dropwise to a solution of methyl (*E*)-2-alkenoate (0.10 mol) in heptane (30 mL) under nitrogen atmosphere, and the mixture was stirred at rt for 1 h. Then K_2CO_3 (20 g, 0.146 mol) and acetonitrile (60 mL) were added, and the solution was heated at 60 °C. After 2 h the reaction mixture was poured into 300 mL of water and extracted with AcOEt (2 × 150 mL). The organic phase was dried over Na_2SO_4, and the solvent was removed under reduced pressure to give an oil which was purified on silica gel column chromatography, eluting with hexane, to afford the two possible diastereoisomers.

From methyl (*E*)-crotonate: (*E*)-**5** (8.0 g, 45%) and (*Z*)-**5** (5.2 g, 29%).

*Data of (E)-**5**:* ^{1}H NMR[23] (400 Hz, $CDCl_3$) δ 6.78 (q, *J* = 7.5, 1H, H−C(3)), 3.82 (s, 3H, $COOCH_3$), 2.05 (d, *J* = 7.5, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$), δ = 163.1, 143.8, 111.3, 52.6, 17.3; GC/MS: t_R = 6.66 min, *m*/*z* 178 (M^+, 100), 147 (83), 119 (56).

From methyl (*E*)-2-hexenoate: (*E*)-**7** (11.7 g, 57%) and (*Z*)-**7** (3.7 g, 18%).

*Data of (E)-**7**:* ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.67 (t, *J* = 7.7 Hz, H-(3)), 3.81 (s, 3H, $COOCH_3$), 2.48 (2H, q, *J* = 7.4 Hz, CH_2(4)), 1.49 (2H, m, CH_2(5)), 0.94 (t, *J* = 7.4 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.3, 149.1, 110.5, 52.7, 33.3, 21.9, 13.6; GC/MS: t_R = 10.49 min, *m*/*z* 206 (M^+, 70), 191 (59), 165 (100).

From methyl (*E*)-2-heptenoate: (*E*)-**8** (11.2 g, 51%) and (*Z*)-**8** (3.3 g, 15%).

*Data of (E)-**8**:* ^{1}H NMR[26] (400 MHz, $CDCl_3$) δ = 6.66 (t, *J* = 7.7 Hz, H-(3)), 3.80 (s, 3H, $COOCH_3$), 2.49 (2H, q, *J* = 7.2 Hz, CH_2(4)), 1.50−1.30 (4H, m, CH_2CH_2), 0.90 (t, *J* = 7.2 Hz, CH_3); ^{13}C NMR[26] (100 MHz, $CDCl_3$) δ 163.3, 149.1, 110.4, 52.6, 31.1, 30.9, 22.2, 13.7; GC/MS: t_R = 12.77 min, *m*/*z* 220 (M^+, 14), 191 (43), 165 (100).

Procedure B (according to ref 19*).* A 1.5 M solution of chlorine in CH_2Cl_2 (67 mL, 0.10 mol) was added dropwise to a solution of methyl (*E*)-crotonate (10 g, 0.10 mmol) in CH_2Cl_2 (30 mL), and the mixture was stirred at rt for 1 h. Then triethylamine (0.50 mmol) was added, and the solution was stirred at rt for 2 h. The reaction mixture was poured into 300 mL of water and extracted with AcOEt (2 × 150 mL). The organic phase was dried over Na_2SO_4 and the solvent removed under reduced pressure to give an oil which was purified on silica gel column chromatography, eluting with hexane, to afford (*Z*)-**2** (7.8 g, 58%; de = 80%, ^{1}H NMR).

*Data of (E)-**2**:* (read on the spectrum of (*Z*)-**2** de = 80%): ^{1}H NMR (500 MHz, $CDCl_3$), δ = 6.5 (q, *J* = 7.6 Hz, H-(3)), 3.82 (s, 3H, $COOCH_3$), 2.08 (d, *J* = 7.6 Hz,CH_3); GC/MS: t_R = 5.04 min, *m*/*z* 134 (M^+, 100), 119 (13), 103 (87).

General Procedure for OYE-Mediated Bioreductions. The unsaturated α-haloester (5 μmol) dissolved in DMF (10 μL) was added to a phosphate buffer solution (1.0 mL, 50 mM, pH 7.0) containing glucose (20 μmol), $NADP^+$ (0.1 mM), GDH (4 U), and the required OYE (40 μg mL^{-1}). The mixture was incubated for 24 h in an orbital shaker (160 rpm, 30 °C). The solution was extracted with AcOEt (2 × 250 μL), centrifuging after each extraction (15000*g*, 1.5 min), and the combined organic solutions were dried over anhydrous Na_2SO_4.

*Methyl (S)-2-bromobutanoate ((S)-**5**):* from OYE1 bioreduction of (*Z*)-**1**: 0.150 g, 85%; ee = 97% (GC); $[\alpha]_D^{20}$ = −35.1 (*c* 1, $CHCl_3$), lit.[27] for (*R*)-**5** $[\alpha]_D^{20}$ = +37.9.1 (*neat*); ^{1}H NMR (400 Hz, $CDCl_3$) δ 4.13 (t, *J* = 7.0, 1H, H−C(2)), 3.73

(s, 3H, $COOCH_3$), 2.05 (m, 1H, H−C(3)), 2.01 (m, 1H, H−C(3)), 0.98(t, *J* = 7.3, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.9, 52.6, 47.1, 28.2, 11.6; GC/MS: t_R = 5.77 min, *m/z* 180 (M^+, 1), 152 (100), 121 (31).

*Methyl (S)-2-chlorobutanoate ((S)-**6**):* from OYE1 bioreduction of (*Z*)-**2**: 0.098 g, 72%; ^{1}H NMR (400 Hz, $CDCl_3$) δ 4.25 (dd, *J* = 6.2 and 5.8, 1H, H−C(2)), 3.79 (s, 3H, $COOCH_3$), 2.05 (m, 1H, H−C(3)), 1.95 (m, 1H, H−C(3)), 1.04 (t, *J* = 7.4, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 58.5, 52.6, 28.2, 10.3; GC/MS: t_R = 4.49 min, *m/z* 136 (M^+, 1), 108 (100), 101 (18).

Methyl esters **13** and **15** were not isolated from the crude mixture of OYE-mediated bioreductions.

General Procedure for Baker'sYeast Fermentation. To a stirred mixture of baker's yeast (200 g) and D-glucose (80 g) in tap water (2 L) at 30 °C was added dropwise a solution of unsaturated α-halo methyl ester (0.05 mol) in ethanol (10 mL). After 72 h, acetone (1 L) was added, followed by AcOEt/hexane 4:1 (750 mL). The mixture was filtered in a large Buchner funnel through a thick Celite pad previously washed with acetone. The two phases were then separated, and the aqueous phase was extracted twice with an AcOEt/hexane mixture. The oily residue obtained upon evaporation of the washed and dried organic phase was chromatographed with increasing amounts of AcOEt in hexane, to isolate the halo unsaturated acid.

*(S)-2-Bromobutanoic acid ((S)-**3**):* from BY fermentation of (*Z*)-**1**: 3.9 g, 47%; ee = 97% (GC of the corresponding methyl ester); $[\alpha]_D^{20}$ = −31.9 (*c* 1.5, MeOH), lit.[16] $[\alpha]_D^{20}$ = −32.3 (*c* 2.50, MeOH); ^{1}H NMR (400 Hz, $CDCl_3$) δ 4.20 (t, *J* = 6.8, 1H, H−C(2)), 2.13 (m, 1H, H−C(3)), 2.08 (m, 1H, H−C(3)); 1.06 (t, *J* = 7.3, 3H, CH_3), ^{13}C NMR[28] (100 MHz, $CDCl_3$) δ 173.4, 47.5, 28.3, 11.8; GC/MS as a methyl ester: t_R = 5.77 min, *m/z* 180 (M^+, 1), 152 (100), 121 (31).

*(S)-2-Chlorobutanoic acid ((S)-**4**):* from BY fermentation of (*Z*)-**2**: 1.83 g, 30%; ee = 85% (GC of the corresponding methyl ester); $[\alpha]_D^{20}$ = −10.5(*c* 1.0, MeOH), lit.[16] $[\alpha]_D^{20}$ = −8.9 (*c* 2.50, MeOH for (*S*)-**4** with o.p. = 70%); ^{1}H NMR (400 Hz, $CDCl_3$) δ 4.29 (t, *J* = 7.0, 1H, H−C(2)), 2.11 (m, 1H, H−C(3)), 2.06 (m, 1H, H−C(3)); 1.07 (t, *J* = 7.3, 3H, CH_3), ^{13}C NMR[28] (100 MHz, $CDCl_3$) δ 174.6, 58.5, 28.1, 10.2; GC/MS as a methyl ester: t_R = 4.49 min, *m/z* 136 (M^+, 1), 108 (100), 101 (18).

*(S)-2-Bromohexanoic acid ((S)-**12**):* from BY fermentation of (*Z*)-**7**: 4.6 g, 48%; ee = 96% (GC of the corresponding methyl ester); $[\alpha]_D^{20}$ = −21.9 (*c* 0.95, EtOH), lit.[29] $[\alpha]_D^{20}$ = −22.8 (*c* 1, EtOH); ^{1}H NMR[30] (400 Hz, $CDCl_3$) δ 4.25 (dd, *J* = 7.7 and 7.0, 1H, H−C(2)), 2.15−1.94 (m, 2H, CH_2(3)), 1.52−1.30 (m, 4H, CH_2CH_2); 0.93 (t, *J* = 7.3, 3H, CH_3), ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.1, 45.6, 34.4, 29.3, 21.9, 13.7; GC/MS as a methyl ester: t_R 10.08 min, *m/z* 208 (M^+, 1), 152 (100), 129 (60).

*2-Bromoheptanoic acid ((S)-**14**):* from BY fermentation of (*Z*)-**8**: 4.7 g, 45%; ee = 96% (GC of the corresponding methyl ester); $[\alpha]_D^{20}$ = −10.5 (*c* 0.95, MeOH); ^{1}H NMR (400 Hz, $CDCl_3$) δ 4.24 (t, *J* = 7.4, 1H, H−C(2)), 2.12−1.92 (m, 2H, CH_2(3)), 1.50−1.30 (m, 6H, $CH_2CH_2CH_2$); 0.90 (t, *J* = 6.6, 3H, CH_3), ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.9, 45.6, 34.6, 30.9, 22.2, 20.6, 13.7; GC/MS as a methyl ester: t_R 12.40 min, *m/z* 222 (M^+, 1), 152 (100), 143 (63).

AUTHOR INFORMATION

Corresponding Author

*Telephone: +39 02 23993077. Fax: +39 02 23993180. E-mail: elisabetta.brenna@polimi.it.

ACKNOWLEDGMENT

Neil C. Bruce (Department of Biology, University of York) is kindly acknowledged for the gift of plasmid pT7-OYE1. Sven Panke and Christian Femmer (ETH-Zürich Department of Biosystems Science and Engineering, Basel) are kindly acknowledged for the help and advice given in the preparation of plasmids pET30a-OYE2, pET30a-OYE3, and pKTS-GDH. Maurizio Pizzigoni and Giuditta Fiori from Labotech 2000 srl (Cusago, Italy) are acknowledged for the generous supply of some chemical reagents.

This work was supported by the research project INTENANT ("Integrated synthesis and purification of single enantiomers") financed by the European Commission within the seventh framework programme.

REFERENCES

(1) Meyer, H.-P. *Org. Process Res. Dev.* **2011**, *15*, 180–188.

(2) Meyer, H.-P.; Ghisalba, O.; Leresche, J. E. Biotransformation and the pharma industry. In *Handbook of Green Chemistry; Biocatalysis*. Crabtree, R. H., Ed.; Wiley-VCH GmbH: Weinheim, 2009; Vol. 3.

(3) Nakamura, K.; Matsuda, T. Reduction of Carbon-Carbon Double Bonds. In *Enzyme Catalysis in Organic Synthesis*, Drauz, K.; Waldmann, H. Eds.; Wiley-VCH GmbH, Weinheim, 2002; Faber, K. *Biotransformations in Organic Chemistry*, Chapter 2.2, pp 177−212, Springer-Verlag, Berlin Heidelberg, 2004.

(4) Blaser, H.-U.; Pugin, B.; Spindler, F.; Thommen, M. *Acc. Chem. Res.* **2007**, *40*, 1240–1250. Blaser, H.-U. *Rend. Fis. Acc. Lincei* **2007**, *18*, 281–304. Knowles, W. S.; Noyori, R. *Acc. Chem. Res.* **2007**, *40*, 1238–1239. Johnson, N. B.; Lennon, I. C.; Moran, P. H.; Ramsden, J. A. *Acc. Chem. Res.* **2007**, *40*, 1291–1299. Shimizu, H.; Nagasaki, I.; Matsumura, K.; Sayo, N.; Saito, T. *Acc. Chem. Res.* **2007**, *40*, 1385–1393.

(5) Leresche, J.; Meyer, H.-P. *Org. Process Res. Dev.* **2006**, *10*, 572–580.

(6) Constable, D. J. C; Dunn, P. J.; Hayler, J. D.; Humphrey, G. R.; Leazer, J. L.; Linderman, R. J.; Lorenz, K.; Manley, J.; Pearlman, B. A.; Wells, A.; Zaksh, A.; Zhang, T. J. *Green Chem.* **2007**, *9*, 411–420. David J. Pollard, D. J.; Woodley, J. M. *Trends Biotechnol.* **2006**, *25*, 66–73.

(7) Fuganti, C. *Pure Appl. Chem.* **1990**, *62*, 1449–1452.Fuganti, C.; Grasselli, P. Baker's Yeast-Mediated Synthesis of Natural Products. *Biocatalysis in Agricultural Biotechnology*; ACS Symposium Series 389; American Chemical Society: Washington, DC, 1989; Chapter 25, pp 359370 Servi, S. *Synthesis* **1990**, 1–25. Csuk, R.; Glänzer, B. I. *Chem. Rev.* **1991**, *91*, 49–97.Faber, K. *Biotransformations in Organic Chemistry*; Springer-Verlag: Berlin, Heidelberg, 2004; Chapter 2.2, pp 177−212.

(8) Williams, R. E.; Bruce, N. C. *Microbiology* **2002**, *148*, 1607–1614. Stuermer, R.; Hauer, B.; Hall, B.; Faber, K. *Curr. Opin. Chem. Biol.* **2007**, *11*, 202–213. Hall, M.; Stuesckler, C.; Hauer, B.; Steuerner, R.; Friedrich, T.; Breuer, M.; Kroutil, W.; Faber, K. *Eur. J. Org. Chem.* **2008**, 1511–1516. Toogood, H.; Gardiner, J. M.; Scrutton, N. S. *ChemCatChem* **2010**, *2*, 892–914.

(9) Brenna, E.; Fronza, G.; Fuganti, C.; Monti, D.; Parmeggiani, F. *J. Mol. Catal. B: Enzym.* **2011**. Accepted. Brenna, E.; Gatti, F. G.; Manfredi, A.; Monti, D.; Parmeggiani, F. *Eur. J. Org. Chem.* **2011**, 4015–4022.

(10) OYE1 from *Saccharomyces pastorianus* (formerly *S. carlsbergensis*): Saito, K.; Thiele, D. J.; Davio, M.; Lockridge, O.; Massey, V. *J. Biol. Chem.* **1991**, *266*, 20720–20724.OYE2-3 from *S. cerevisiae*: Stott, K.; Saito, K.; Thiele, D. J.; Massey, V. *J. Biol. Chem.* **1993**, *268*, 6097−6106; Niino, Y. S.; Chakraborty, S.; Brown, B. J.; Massey, V. *J. Biol. Chem.* **1995**, *270*, 1983–1991.

(11) Utaka, M.; Konishi, S.; Ohkubo, T.; Tsuboi, S.; Takeda, A. *Tetrahedron Lett.* **1987**, *28*, 1447. Utaka, M.; Konishi, S.; Mizouka, A.; Ohkubo, T.; Sakai, T.; Tsuboi, S.; Takeda, A. *J. Org. Chem.* **1989**, *54*, 4989–4992.

(12) Einstein, M.; Akiyama, T. E.; Castriota, G. A.; Wang, C. F.; McKeever, B.; Mosley, R. T.; Becker, J. W.; Moller, D. E.; Meinke, P. T.; Wood, H. B.; Berger, J. P. *Mol. Pharmacol.* **2008**, *73*, 62–74.

(13) (a) Liu, W.; Liu, K; Wood, H. B.; McCann, M. E.; Doebber, T. W.; Chang, C. H.; Akiyama, T. E.; Einstein, M.; Berger, J. P.; Meinke, P. T. *J. Med. Chem.* **2009**, *52*, 4443–4453. (b) Maligres, P. E.; Humphrey, G. R.; Marcoux, J.-F.; Hillier, M. C.; Zhao, D.; Krska, S.; Grabowski, E. J. J. *Org. Process Res. Dev.* **2009**, *13*, 525–534.

(14) Yamazaki, Y.; Kazutoyo, A.; Toma, T.; Nishikawa, M.; Ozawa, H.; Okuda, A.; Araki, T.; Oda, S; Inoue, K.; Shibuya, K.; Staels, B.; Fruchart, J.-C. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4689–4693.Yamazaki, Y.; Araki, T.; Koura, M.; Shibuya, K. Optically active PPAR-activating compound intermediate and method for producing the same. EP 1,854,779 A1. Feb. 28, 2006.

(15) Camps, P.; Pérez, F.; Soldevilla, N. *Tetrahedron: Asymmetry* **1998**, *9*, 2065–2079.

(16) Boschi, F.; Camps, P.; Comes-Franchini, M.; Muñoz,-Torrero, D.; Ricci, A.; Sánchez, L. *Tetrahedron: Asymmetry* **2005**, *16*, 3739–3745.

(17) Prokop, Z.; Sato, Y.; Brezovsky, J.; Mozga, T.; Chaloupkova, R.; Koudelakova, T.; Jerabek, P.; Stepankova, V.; Natsume, R.; van Leeuwen, J. G. E.; Janssen, D. B.; Florian, J.; Nagata, Y.; Senda, T.; Damborsky, J. *Angew. Chem., Int. Ed.* **2010**, *49*, 6111–6115.

(18) Kaiser, M. M.; Zhu, J.; Hooper, D. L. *Can. J. Chem.* **1997**, *75*, 1315–1321.

(19) Heasley, V. L.; Spaite, D. W.; Shellhamer, D. F. *J. Org. Chem.* **1979**, *44*, 2608–2611.

(20) Williams, R. E.; Rathborne, D. A.; Scrutton, N. S.; Bruce, N. C. *Appl. Environ. Microbiol.* **2004**, *70*, 3566–3574.

(21) Bechtold, M.; Brenna, E.; Femmer, C.; Gatti, F. G.; Panke, S.; Parmeggiani, F.; Sacchetti, A. *Org. Process Res. Dev.* **2011**, DOI: 10.1021/op200085k.

(22) Huang, Z.-Z.; Yu, X.-C.; Huang, X. *Tetrahedron Lett.* **2002**, *43*, 6823–6825.

(23) Hagiwara, H.; Abe, F.; Uda, H. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2651–2655.

(24) Forti, L.; Guelfi, F.; Pagnoni, U. M. *Tetrahedron Lett.* **1995**, *36*, 3023–3026.

(25) Rossi, R.; Bellina, F.; Bechini, C.; Mannina, L.; Vergamini, P. *Tetrahedron* **1998**, *54*, 135–136.

(26) Tago, K.; Kogen, H. *Tetrahedron* **2000**, *56*, 8825–8831.

(27) Levene, K. *J. Biol. Chem.* **1941**, *141*, 404.

(28) Olah, G. A.; Shih, J.; Prakash, G. K. S. *Helv. Chim. Acta* **1983**, *66*, 1028–1030.

(29) Kirchner, G.; Scollar, M.; Klibanov, A. M. *J. Am. Chem. Soc.* **1985**, *107*, 7072–7076.

(30) Lombaert, S. De; Fink, C. A.; Firooznia, F.; Hoyer, D. W.; Jeng, A. Y. U.S. Patent Appl. 2002/82218 A1, 2002