One-pot two-step chemoenzymatic deracemization of allylic alcohols using laccases and alcohol dehydrogenases

Jesús Albarrán-Velo, Vicente Gotor-Fernández* and Iván Lavandera*

Organic and Inorganic Chemistry Department, Universidad de Oviedo. Avenida Julián Clavería 8, 33006 Oviedo, Spain.

Correspondence author: Tel: +34-985103454; Fax: +34-985103446; vicgotfer@uniovi.es

Correspondence author: Tel: +34-985103452; Fax: +34-985103446; lavanderaivan@uniovi.es

Abstract

A series of enantioenriched (hetero)aromatic secondary allylic alcohols has been synthesized through deracemization of the corresponding racemic mixtures combining a non-selective chemoenzymatic oxidation (laccase from *Trametes versicolor* and oxy-radical TEMPO) and a stereoselective biocatalyzed reduction (lyophilized cells of *E. coli* overexpressing an alcohol dehydrogenase, ADH). Both steps were performed in aqueous medium under very mild reaction conditions. After optimization, a sequential one-pot two-step protocol was set up, obtaining the corresponding chiral alcohols in moderate to high conversions (48-95%) and enantiomeric excess (65->99% *ee*). Depending on the ADH stereopreference, both antipodes from these valuable chiral synthons could be prepared, even at preparative scale (119-178 mg), in a straightforward manner.

Keywords

Alcohol dehydrogenases; Bioreduction; Deracemization; Laccases; One-pot synthesis

1. Introduction

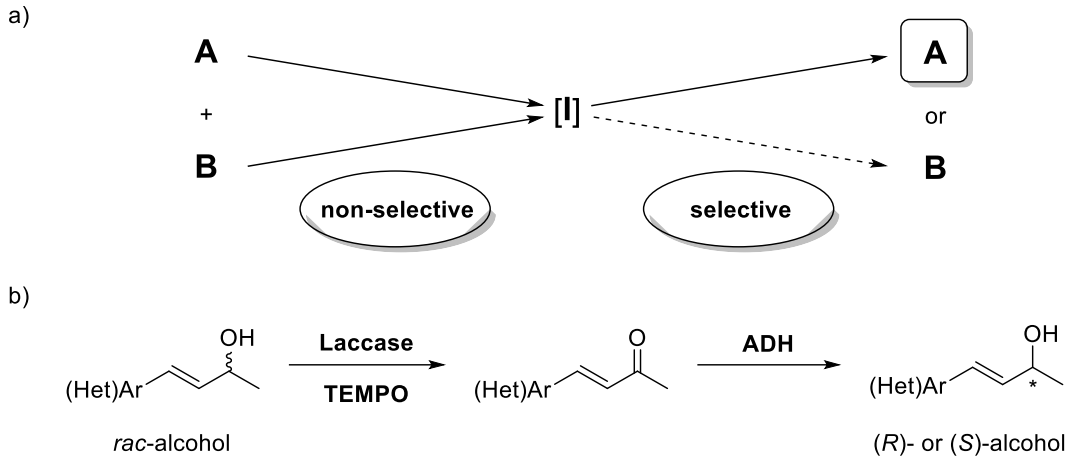
Chiral allylic alcohols are versatile building blocks for organic synthesis as they contain two moieties, an alcohol and a carbon-carbon double bond, which can be subsequently modified in order to give access to molecules with up to three contiguous chiral centers. In this context, many different reactions have been designed where the hydroxyl group directs the transformation of the alkene moiety, such as epoxidation [1], hydrogenation [2], dihydroxylation [3], or hydroformylation [4], among others. Also in the last years, different approaches have been developed to substitute the alcohol group by different nucleophiles [5], affording a broad spectrum of highly valuable derivatives.

As a consequence, several chemical methodologies have been described to synthesize these compounds in a chiral fashion [6]. Recent examples involve the (stereoselective) formation of C–C bonds, such as the alkenylation of aldehydes [7], the coupling of alcohols and alkynes under redox neutral conditions [8], the aldol addition on α,β-unsaturated ketones [9], the alkyl addition on α,β-unsaturated aldehydes [10], and the Stille coupling reaction [11]. Other methods rely on the selective creation of the C–O bond, such as the addition of *N*-hydroxyphthalimide to allenes [12], or the C–H bond, through the metal-catalyzed reduction of enones [13-15] or vinyl epoxides [16]. Other procedures involve the metal- or organocatalyzed isomerization of oxiranes [17] and the kinetic resolution of racemic allylic alcohols [18,19].

Alternatively, biocatalytic tools have become very relevant along the last decades as enzymes can promote extremely selective transformations under very mild reaction conditions. For instance, enantioenriched allylic alcohols have been obtained through lipase-mediated kinetic [20,21] and dynamic kinetic resolutions [22-25] of the corresponding racemates. Also, oxidoreductases have been successfully applied to obtain these chiral compounds through the oxidative kinetic resolution catalyzed by alcohol dehydrogenases (ADHs) [26] or alcohol oxidases [27] starting from the racemic alcohols, or the bioreduction

of the corresponding α,β-unsaturated ketones [28,29]. Due to the relevance of multi-step catalyzed syntheses, the design of chemoenzymatic [30,31] or multienzymatic [32,33] protocols where allylic alcohols are obtained or involved as intermediates has also been envisaged.

An elegant manner to get access to enantioenriched *sec*-alcohols is performing deracemization protocols starting from the racemic forms [34-37]. From an atom economic point of view, this transformation is ideal as both substrate and product are the same compound. Although this approach is very simple, final compounds are frequently not obtained in high enantiomeric excess (*ee*) and also by-products can be formed, due to the presence of many different enzymes, since traditionally, whole-cell biocatalysts are employed. For instance, Chadha and co-workers reported the deracemization of racemic allylic alcohols through an oxidation-reduction strategy using a yeast [38,39]. Next, Kroutil and co-workers demonstrated that deracemization of secondary alcohols could be achieved *via* stereoinversion in a cascade fashion [40]. For this, two enantiocomplementary ADHs are necessary with different nicotinamide cofactor preference (NADH or NADPH), providing the final alcohols with excellent *ee* values. Whereas this method is very smart, it can suffer from relatively limited substrate scope and undesired crossed regeneration cofactor systems, lowering both conversions and selectivities. A subsequent approach is the use of linear deracemizations [37], where the racemic *sec*-alcohol is transformed into the corresponding ketone intermediate *via* non-selective oxidation, followed by a stereoselective reduction using a chiral (bio)catalyst (Scheme 1a). This system presents the advantage that just one selective reaction is necessary to get access to the enantioenriched product, broadening the possible scope of the reaction. On the contrary, it is not possible to perform both steps in a cascade manner as the final product would also be oxidized, therefore, one-pot sequential methodologies must be strictly followed.



Scheme 1. a) General representation of a linear deracemization concept. b) Linear deracemization of *sec*-allylic alcohols combining the laccase/TEMPO system with an ADH. A, B: substrate (and product) enantiomers; I: intermediate.

In the search for novel mild oxidative methods, we and others found out that the combination of a laccase [41-44] with substoichiometric amounts of the oxy-radical TEMPO is very suitable to achieve the oxidation of secondary benzylic [45-49], allylic [50-52] and propargylic alcohols [53,54] in aqueous medium employing oxygen as final electron acceptor. This transformation has been coupled to other (chemo)enzymatic reactions to perform the amination of racemic alcohols [48,49,52] and the isomerization of allylic alcohols into saturated carbonyl compounds [50,51]. It has also been envisaged as the first step in linear deracemizations of profenols [55], benzylic [47], and propargylic [53] alcohols when it was combined sequentially with alcohol dehydrogenases [56]. Herein, the application of the laccase from *Trametes versicolor* (L*Tv*) and TEMPO with stereocomplementary ADHs is disclosed, to accomplish the synthesis of valuable enantioenriched allylic alcohols starting from the racemic compounds in a one-pot sequential mode (Scheme 1b).

2. Experimental

2.1. Material and methods

Laccase from *Trametes versicolor* (L*Tv*, 0.66 U/mg) was purchased from a commercial source. Made in house ADHs overexpressed in *E. coli* and later lyophilized [*Ralstonia* species (RasADH), *Sphingobium yanoikuyae* (SyADH), *Thermoanaerobacter* species (ADH-T), *Lactobacillus brevis* (LBADH), *Thermoanaerobacter ethanolicus* (TeSADH) and *Rhodococcus ruber* (ADH-A)] were obtained as previously reported in the literature [57-59], while evo-1.1.200 ADH was acquired from a commercial source. Glucose dehydrogenase (GDH-105) is commercially available. D-Glucose, NADPH, NADH, ketone **2a** and all other reagents for chemical transformations and product isolation/purification were obtained from commercial sources and used as received. Citrate buffer (pH 5, 50 mM) was saturated with molecular oxygen by bubbling it for 15 minutes prior to be used in the chemoenzymatic oxidation experiments.

Thin layer chromatographies (TLCs) were conducted with silica gel precoated plates and visualized with UV and potassium permanganate stain. Column chromatographies were performed using silica gel (230-400 mesh).

The oxidation step mediated by the laccase/TEMPO catalytic system was optimized in an open-to-air test tube using magnetic stirring, while the bioreduction step was studied in 1.5 mL Eppendorf vial. Sequential reactions were performed in sealed glass tubes of different sizes (18 x 72 x 10), (18 x 109 x 10) or (28 x 137 x 20) mm] depending on the reaction scale.

NMR spectra were recorded on a 300 MHz spectrometer. All chemical shifts (δ) are given in parts per million (ppm) and referenced to the residual solvent signal as internal standard. IR spectra were recorded on a spectrophotometer on NaCl pellets. Gas chromatography (GC) analyses were performed on standard gas chromatograph apparatus equipped with a FID detector. High performance liquid chromatography (HPLC) analyses were carried out in a chromatograph with UV detector at a 210 nm wavelength. High

resolution mass spectra (HRMS) were obtained in a spectrometer using the ESI-TOF positive mode. Measurement of the optical rotation was carried out at 590 nm in a standard polarimeter.

*2.2. Typical procedure for the synthesis of (*E*)-4-(het)arylbut-3-en-2-ones*

Ketones **2b**-**p** were synthesized following the procedure already described by List and co-workers for ketones **2a**, **2l** and **2o** [60]. To a solution of the corresponding aldehyde (2.5 mmol) in acetone (6.3 mL), morpholinium trifluoroacetate (0.5 mmol) was added, and the reaction was stirred at 75 ºC in a sealed tube overnight. After this time the reaction mixture was cooled at room temperature, and a $NaHCO_3$ saturated aqueous solution (5 mL) and EtOAc (10 mL) were added. After phase separation, the aqueous phase was extracted three times with EtOAc (10 mL), and the combined organic phases dried over anhydrous Na_2SO_4, filtered and the solvent evaporated under reduced pressure. The reaction crude was purified by column chromatography on silica gel (25% EtOAc/hexane), yielding the corresponding ketone **2b**-**p** (59-96% yield): **2b** (92%, 406 mg), **2c** (71%, 320 mg), **2d** (61%, 251 mg), **2e** (59%, 333 mg), **2f** (78%, 344 mg), **2g** (63%, 259 mg), **2h** (85%, 340 mg), **2i** (77%, 339 mg), **2j** (68%, 280 mg), **2k** (72%, 266 mg), **2l** (81%, 299 mg), **2m** (75%, 255 mg), **2n** (94%, 320 mg), **2o** (82%, 313 mg) and **2p** (96%, 366 mg). The NMR data of these compounds were in agreement with those reported in the literature [52,60].

*2.3. Typical procedure for the synthesis of (*E*)-4-(het)arylbut-3-en-2-ols*

Alcohols **1a**-**p** were synthesized following the procedure described by our research group [52]. A solution of $NaBH_4$ (170.2 mg, 4.5 mmol) in water (1 mL) was added dropwise to a stirring solution of the corresponding (3*E*)-4-(het)arylbut-3-en-2-one **2a**-**p** (3 mmol) in MeOH (10 mL) at 0 ºC. The reaction mixture was firstly stirred for 1 h in an ice bath and then 2 h at room temperature. Afterwards, hot water (5 mL) was added and the product was extracted with CH_2Cl_2 (3 x 15 mL), and the combined organic phases were washed with brine (5 mL). The resulting organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent

evaporated under reduced pressure. The reaction crude was purified by column chromatography on silica gel (50% Et_2O/hexane, 83% EtOAc/hexane or 25% EtOAc/hexane), yielding the corresponding alcohol **1a-p** (46-99% yield): **1a** (99%, 445 mg), **1b** (88%, 469 mg), **1c** (64%, 351 mg), **1d** (46%, 230 mg), **1e** (57%, 387 mg), **1f** (78%, 417 mg), **1g** (71%, 354 mg), **1h** (85%, 414 mg), **1i** (81%, 435 mg), **1j** (76%, 380 mg), **1k** (87%, 389 mg), **1l** (91%, 409 mg), **1m** (89%, 369 mg), **1n** (87%, 361 mg), **1o** (91%, 423 mg) and **1p** (71%, 328 mg). The NMR data of these compounds were in agreement with those reported in the literature [52].

*2.4. Typical procedure for the oxidation of (*E*)-4-(het)arylbut-3-en-2-ols using the laccase from* Trametes versicolor*-TEMPO catalytic system*

In an open-to-air test tube, TEMPO (1.2-4.1 mg, 10-33 mol%) was added to a solution of the racemic alcohol **1a**-**p** (0.08 mmol, 100 mM) in a (biphasic mixture of) oxygen-saturated citrate buffer (pH 5, 50 mM), studying the possibility of using MTBE (*tert*-butyl methyl ether) as cosolvent (up to 50% *v/v*, for a total solution volume of 800 μL). The reaction mixture was magnetically stirred for a few minutes to dissolve all the reagents, and then the laccase from *Trametes versicolor* (7 mg, 4.6 U) was added. The mixture was stirred for additional 16 h at 30 ºC controlling the agitation speed at 150 rpm. After this time, the product was extracted with EtOAc (2 x 2 mL), the organic phases were combined, dried over Na_2SO_4 and filtered, and an aliquot was taken for the determination of the conversion value by GC (see Tables S1 and S2 in the Supplementary data).

*2.5. Typical procedure for the bioreduction of (*E*)-4-(het)arylbut-3-en-2-ones using overexpressed alcohol dehydrogenases*

For *E. coli*/RasADH: In a 1.5 mL Eppendorf vial, ketone **2a** (0.015 mmol, 25 mM) was dissolved in DMSO (10% *v/v*, 60 μL). Then, Tris·HCl buffer 50 mM pH 7.5 (540 μL) containing glucose (50 mM), GDH (10 U) and NADPH (1 mM) was added. Finally, overexpressed *E. coli*/RasADH lyophilized cells (10 mg) were added. The reaction was

shaken at 30 ºC and 250 rpm for 24 h, and extracted with EtOAc (0.5 mL), separating the organic layer by centrifugation (2 min, 13,000 rpm). This extraction and centrifugation protocol was repeated twice, and the organic layers were combined and dried over Na_2SO_4.

For the other ADHs: In a 1.5 mL Eppendorf vial, ketone **2a** (0.015 mmol, 25 mM) was dissolved in 2-PrOH (10% *v/v*, 60 μL). Then, Tris·HCl buffer 50 mM pH 7.5 (540 μL) containing $MgCl_2$ (1 mM, for *E. coli*/LBADH and evo-1.1.200) and NADPH (1 mM for *E. coli*/LBADH, *E. coli*/ADH-T, *E. coli*/SyADH, and *E. coli*/TeSADH) or NADH (1 mM for *E. coli*/ADH-A and evo-1.1.200) was added. Finally, *E. coli* lyophilized cells or evo-1.1.200 (10 mg) were added. The reaction was shaken at 30 ºC and 250 rpm for 24 h, and extracted with EtOAc (0.5 mL), separating the organic layer by centrifugation (2 min, 13,000 rpm). This extraction and centrifugation protocol was repeated twice, and the organic layers were combined and dried over Na_2SO_4.

General procedure under the optimized conditions: In a 1.5 mL Eppendorf vial, the corresponding ketone **2a-p** (0.024 mmol, 40 mM) was dissolved in 2-PrOH (15% *v/v*, 90 μL). Then, Tris·HCl buffer 50 mM pH 7.5 (390 μL for *E. coli*/LBADH; 450 μL for *E. coli*/ADH-T or *E. coli*/ADH-A), $MgCl_2$ (60 μL, 10 mM for *E. coli*/LBADH), and NADPH (60 μL, 10 mM, for *E. coli*/LBADH or *E. coli*/ADH-T) or NADH (60 μL, 10 mM, for *E. coli*/ADH-A) were added. Finally, lyophilized cells containing the overexpressed alcohol dehydrogenase (10 mg) were added. The reaction was shaken at 30 ºC and 250 rpm for 24 h, and extracted with EtOAc (0.5 mL); the organic layer was separated by centrifugation (2 min, 13,000 rpm). This extraction and centrifugation protocol was repeated twice, and the organic layers were combined and dried over Na_2SO_4.

Conversion values into the corresponding enantioenriched alcohols **1a-p** were determined by taking an aliquot of the resulting suspensions and injecting them for GC analysis (see the Supplementary data). Derivatization of alcohols **1a-p** as acetates with acetic

anhydride was necessary for the measurement of their enantiomeric excess values: An aliquot of the corresponding alcohol **1a-p** obtained in the biotransformation crude and dissolved in EtOAc, was placed in a 1.5 mL Eppendorf vial. Then, DMAP (10 mg) and acetic anhydride (5 drops) were added, and the mixture was shaken at 30 ºC and 900 rpm for 2 h. After this time, an aqueous solution of NaOH 10 M (200 μL) was added, and the organic layer was separated by centrifugation (2 min, 13000 rpm), dried over Na_2SO_4, filtered and transferred to a GC glass vial for analysis.

*2.6. Typical procedure for the deracemization of (*E*)-4-(het)arylbut-3-en-2-ols at analytical scale*

In an open-to-air glass tube, TEMPO (4.1 mg, 33 mol%) was added to a solution of the corresponding racemic alcohol **1a-p** (0.08 mmol, 100 mM) in a biphasic mixture of an oxygen-saturated citrate buffer 50 mM pH 5 and MTBE (50% *v/v*, for a total volume of 800 μL). The reaction mixture was magnetically stirred for a few minutes to dissolve all the reagents, and then the laccase from *Trametes versicolor* (7 mg, 4.6 U) was added. The reaction was stirred for 16 h at 30 ºC, observing the complete evaporation of MTBE along this time. This fact led to a volume reduction from the initial 800 μL to 400 μL, and in consequence, the substrate concentration increased from the initial 100 mM to approximately 200 mM.

To the resulting reaction crude containing the ketone intermediate, different protocols have been applied depending on the alcohol dehydrogenase that is used. In the case of LBADH, 2-PrOH (0.3 mL), Tris·HCl buffer 50 mM pH 8 (0.9 mL), $MgCl_2$ (0.2 mL of a 10 mM solution in Tris·HCl buffer 50 mM pH 8), and NADPH (0.2 mL of a 10 mM solution in Tris·HCl buffer 50 mM pH 8) were added, leading to concentrations of approximately 40 mM for the substrate, 1 mM for $MgCl_2$, 1 mM for NADPH and 15% *v/v* for 2-PrOH. At the same time, the addition of this concentrated buffer to the reaction medium, caused an increase in the

pH from an initial value of 5 to approximately 7.5, therefore, further pH adjustment was not required for the bioreduction reaction.

For ADH-T, 2-PrOH (0.3 mL), Tris·HCl buffer 50 mM pH 8 (1.1 mL), and NADPH (0.2 mL of a 10 mM solution in Tris·HCl buffer 50 mM pH 8) were added, leading to concentrations of approximately 40 mM for the substrate, 1 mM for NADPH and 15% *v/v* for 2-PrOH. At the same time, the addition of this concentrated buffer to the reaction medium, caused an increase in the pH from an initial value of 5 to approximately 7.5, therefore, further pH adjustment was not required for the bioreduction reaction.

In the case of ADH-A, 2-PrOH (0.3 mL), Tris·HCl buffer 50 mM pH 8 (1.1 mL), and NADH (0.2 mL of a 10 mM solution in Tris·HCl buffer 50 mM pH 8) were added, leading to concentrations of approximately 40 mM for the substrate, 1 mM for NADH and 15% *v/v* for 2-PrOH. At the same time, the addition of this concentrated buffer to the reaction medium, caused an increase in the pH from an initial value of 5 to approximately 7.5, therefore, further pH adjustment was not required for the bioreduction reaction.

Finally, the corresponding *E. coli* cells overexpressing the alcohol dehydrogenase (33.3 mg) were added. The sealed tube was closed and the reaction was shaken at 30 ºC and 250 rpm for 24 h. After this time, the reaction was stopped by addition of distilled water or NaOH 10 M aqueous solution (for alcohols **1k-l**, 1.5 mL). The mixture was extracted with EtOAc (5 mL) and the organic layer was separated by centrifugation (3 min, 4,900 rpm). This extraction and centrifugation protocol was performed twice and, finally, the organic layers were combined and dried over Na_2SO_4. Conversion and *ee* values were measured as previously described. The products were purified through column chromatography using the following eluents: 50% Et_2O/hexane (**1a-j**), 83% EtOAc/hexane (**1k** and **1l**) or 25% EtOAc/hexane (**1m-p**).

*2.7. Typical procedure for the deracemization of (*E*)-4-(het)arylbut-3-en-2-ols at semi-preparative scale*

In an open-to-air glass tube, TEMPO (8.2 mg, 33 mol%) was added to a solution of the appropriate racemic alcohol **1d**, **1f**, **1g** or **1j** (0.16 mmol, 100 mM) in a biphasic mixture of an oxygen-saturated citrate buffer (pH 5, 50 mM) and MTBE (50% *v/v*, for a total volume of 1.6 mL). The reaction mixture was stirred for a few minutes to dissolve all the reagents, and then the laccase from *T. versicolor* (14 mg, 9.2 U) was added. The reaction mixture was stirred for 16 h at 30 ºC, with the complete evaporation of MTBE being observed during this time. This led to a volume reduction from the initial 1.6 mL to 800 μL, and as a consequence, the substrate concentration increased from the initial 100 mM to approximately 200 mM.

To the resulting reaction crude containing the ketone intermediate (**2d**, **2f**, **2g** or **2j**), different protocols were applied depending on the alcohol dehydrogenase that was used. In the case of LBADH, 2-PrOH (0.6 mL), Tris·HCl buffer 50 mM pH 8 (1.8 mL), $MgCl_2$ (0.4 mL of a 10 mM solution in Tris·HCl buffer 50 mM pH 8), and NADPH (0.4 mL of a 10 mM solution in Tris·HCl buffer 50 mM pH 8) were added, leading to concentrations of approximately 40 mM for the substrate, 1 mM for $MgCl_2$, 1 mM for NADPH and 15% *v/v* for 2-PrOH. At the same time, the addition of this concentrated buffer to the reaction medium, caused an increase in the pH from an initial value of 5 to approximately 7.5, therefore, further pH adjustment was not required for the bioreduction reaction.

In the case of ADH-A, 2-PrOH (0.6 mL), Tris·HCl buffer 50 mM pH 8 (2.2 mL), and NADH (0.4 mL of a 10 mM solution in Tris·HCl buffer 50 mM pH 8) were added, leading to concentrations of approximately 40 mM for the substrate, 1 mM for NADH and 15% *v/v* for 2-PrOH. At the same time, the addition of this concentrated buffer to the reaction medium, caused an increase in the pH from an initial value of 5 to approximately 7.5, therefore, further pH adjustment was not required for the bioreduction reaction.

Finally, the corresponding *E. coli* cells overexpressing the alcohol dehydrogenase (66.6 mg) were added. The sealed tube was closed and the reaction was shaken at 30 ºC and 250 rpm for 24 h. After this time, the reaction was stopped by addition of distilled water (3 mL). The mixture was extracted with EtOAc (10 mL) and the organic layer was separated by centrifugation (3 min, 4,900 rpm). This extraction and centrifugation protocol was performed three times and, finally, the organic layers were combined and dried over Na_2SO_4. Conversion values into the corresponding enantioenriched alcohols **1d**, **1f**, **1g** and **1j** were determined by GC analyses. Derivatization of alcohols **1d**, **1f**, **1g** and **1j** as acetates with acetic anhydride was necessary for the measurement of their enantiomeric excess values. The products were purified through column chromatography using the following eluents: 50% Et_2O/hexane (**1a-j**), 83% EtOAc/hexane (**1k-l**) or 25% EtOAc/hexane (**1m-p**).

2.8. Typical procedure for the deracemization of (E)-4-(het)arylbut-3-en-2-ols at preparative scale

(*S*)-1a. In an open-to-air glass tube, TEMPO (41 mg, 33 mol%) was added to a solution of the racemic alcohol **1a** (0.8 mmol, 119 mg, 100 mM) in a biphasic mixture of an oxygen-saturated citrate buffer (pH 5, 50 mM) and MTBE (50% *v/v*, for a total volume of 8 mL). The reaction mixture was magnetically stirred for a few minutes to dissolve all the reagents, and then the laccase from *T. versicolor* (70 mg, 46 U) was added. The reaction mixture was stirred for 16 h at 30 ºC, being observed the complete evaporation of MTBE during this time. This led to a volume reduction from the initial 8 mL to 4 mL, and as a consequence, the substrate concentration increased from the initial 100 mM to approximately 200 mM.

To the resulting reaction mixture containing the ketone intermediate **2a**, 2-PrOH (3 mL), Tris·HCl buffer 50 mM pH 8 (11 mL), and NADPH (2 mL of a 10 mM solution in Tris·HCl buffer 50 mM pH 8) were added, leading to a concentration of approximately 40 mM for ketone **2a**, 1 mM for $MgCl_2$, 1 mM for NADPH and 15% *v/v* for 2-PrOH. At the same time, the addition of this concentrated buffer to the reaction medium, caused an increase

in the pH from an initial value of 5 to approximately 7.5, therefore, further pH adjustment was not required for the bioreduction reaction.

Finally, overexpressed *E. coli*/ADH-T cells (333 mg) were added. The glass tube was closed and the reaction shaken at 30 ºC and 250 rpm for 24 h. After this time, the reaction was stopped by addition of distilled water (20 mL). The mixture was extracted with EtOAc (50 mL) and the organic layer was separated by centrifugation (3 min, 4,900 rpm). This extraction and centrifugation protocol was performed three times and, finally, the organic layers were combined and dried over Na_2SO_4. Analysis through GC showed 76% of conversion and >99% of enantiomeric excess (as acetate derivative). The product was purified through column chromatography on silica gel (50% Et_2O/hexane), affording the alcohol (*S*)-**1a** in 74% yield.

(*R*)-1f. In an open-to-air glass tube, TEMPO (52 mg, 33 mol%) was added to a solution of the racemic alcohol **1f** (1 mmol, 178 mg, 100 mM) in a biphasic mixture of an oxygen-saturated citrate buffer (pH 5, 50 mM) and MTBE (50% *v/v*, for a total volume of 10 mL). The reaction mixture was magnetically stirred for a few minutes to dissolve all the reagents, and then the laccase from *T. versicolor* (88 mg, 58 U) was added. The reaction mixture was stirred for 16 h at 30 ºC, being observed the complete evaporation of MTBE during this time. This led to a volume reduction from the initial 10 mL to 5 mL, and as a consequence, the substrate concentration increased from the initial 100 mM to approximately 200 mM.

To the resulting crude reaction mixture containing the ketone intermediate **2f**, 2-PrOH (3.75 mL), Tris·HCl buffer 50 mM pH 8 (21.25 mL), $MgCl_2$ (5.1 mg, 0.025 mmol), and NADPH (21 mg, 0.025 mmol) were added, leading to concentrations of approximately 40 mM for ketone **2f**, 1 mM for $MgCl_2$, 1 mM for NADPH and 15% *v/v* for 2-PrOH. At the same time, the addition of this concentrated buffer to the reaction medium, caused an increase in the pH from an initial value of 5 to approximately 7.5, therefore, further pH adjustment was not required for the bioreduction reaction.

Finally, overexpressed *E. coli*/LBADH cells (415 mg) were added. The glass tube was closed and the reaction shaken at 30 ºC and 250 rpm for 24 h. After this time, the reaction was stopped by addition of distilled water (20 mL). The mixture was extracted with EtOAc (50 mL) and the organic layer was separated by centrifugation (3 min, 4,900 rpm). This extraction and centrifugation protocol was performed three times and, finally, the organic layers were combined and dried over Na_2SO_4. Analysis through GC showed 78% of conversion and 95% of enantiomeric excess (as acetate derivative). The product was purified through column chromatography on silica gel (50% Et_2O/hexane), affording the alcohol (*R*)-**1f** in 76% yield.

*2.9. Chemical synthesis of (*R*)-1-[(2*R*,3*R*)-3-(3-methoxyphenyl)oxiran-2-yl]ethan-1-ol (**3**)*

L-(+)-Diethyl tartrate (DET, 175 μL, 0.848 mmol) was added to a solution of $Ti(O^iPr)_4$ (241 μL, 0.848 mmol) in dry CH_2Cl_2 (5 mL) at –20 ºC. The solution was stirred during 30 minutes at –20 ºC and then, a *tert*-butyl hydroperoxide (^{t}BuOOH) solution in decane (5.5 M, 250 μL, 1.38 mmol) was added dropwise. After additional 10 minutes of stirring at –20 ºC, a solution of (*R*)-**1f** (95% *ee*, 120 mg, 0.673 mmol) in dry CH_2Cl_2 (2 mL) was added dropwise and stirred at –20 ºC during 3 hours. Afterwards, the resulting mixture was quenched with water (10 mL) and left warming up to room temperature during 30 minutes. The reaction was filtered through a Celite pad and extracted with CH_2Cl_2 (3 x 15 mL). The organic phases were combined and washed with brine (2 x 10 mL), dried over anhydrous Na_2SO_4, filtered, and the solvent evaporated under reduced pressure. The reaction crude was analyzed by ^{1}H NMR finding a 89% conversion and 83% diastereomeric excess. Then, the reaction crude was purified by preparative thin layer chromatography (75% Et_2O/hexane), yielding the corresponding epoxide **3** (80% yield, 95% *ee*, 83% *de*).

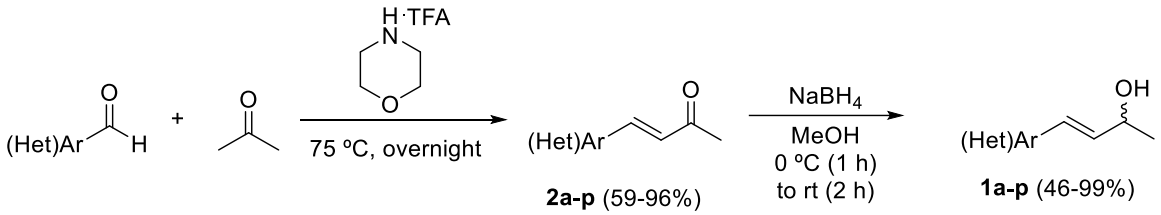
(*R*)-1-[(2*R*,3*R*)-3-(3-Methoxyphenyl)oxiran-2-yl]ethan-1-ol (3) [61]**.** Yellow wax. R_f (75% Et_2O/hexane): 0.62. IR (NaCl): 3470, 2979, 2965, 2915, 2873, 2847, 1737, 1604, 1586, 1492, 1459, 1441, 1257, 1238, 1154, 1139, 1036, 1025, 853, 801, 782, 732, 695, 591, 504

cm^{-1}. ^{1}H NMR (300.13 MHz, $CDCl_3$, major diastereoisomer): δ 1.31 (d, $^{3}J_{HH}$ = 6.5 Hz, 3H), 2.21 (br s, 1H), 3.06 (dd, $^{3}J_{HH}$ = 2.6, $^{4}J_{HH}$ = 0.8 Hz, 1H), 3.80 (s, 3H), 3.93 (dd, $^{3}J_{HH}$ = 2.1 Hz, 1H), 4.10 (qd, $^{3}J_{HH}$ = 6.4, $^{3}J_{HH}$ = 2.9 Hz, 1H), 6.79-6.91 (m, 3H), 7.23-7.28 (m, 1H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$, major diastereoisomer): δ 160.0 (C), 138.8 (C), 129.7 (CH), 118.2 (CH), 114.1 (CH), 110.9 (CH), 65.7 (CH), 64.9 (CH), 55.4 (CH_3), 54.7 (CH), 18.8 (CH_3) ppm. HRMS (ESI^+, *m/z*): calcd for $(C_{11}H_{15}O_3)^+$ $(M+H)^+$ 195.1016, found: 195.1014.

3. Results and discussion

*3.1. Chemical synthesis of (*E*)-α,β-unsaturated ketones and racemic (*E*)-allylic alcohols*

Prior to the development of the enzymatic study, which includes the laccase-mediated oxidation of the (het)aryl allylic alcohols **1a**-**p** and the bioreduction of the corresponding ketones **2a**-**p**, the syntheses of both families of organic compounds were performed following methods already reported in the literature (Scheme 2). Firstly, (*E*)-unsaturated ketones **2a**-**p** were synthesized in moderate to excellent yield after column chromatography (59-96%) following the procedure already described by List and co-workers for ketones **2a**, **2l** and **2o** [60], consisting in the aldol condensation between acetone and (hetero)aromatic aldehydes catalyzed by the morpholinium trifluoroacetate salt. The resulting ketones were then chemically reduced using sodium borohydride, obtaining the racemic (*E*)-alcohols **1a**-**p** after 3 hours of reaction and column chromatography purification on silica gel (46-99%) [52]. Subsequently, GC analytical methods to separate the ketone and alcohol enantiomers were developed to measure in a later stage the enzymatic reaction conversion and *ee* values (see the Supplementary data for details).



Scheme 2. Chemical synthesis of (*E*)-α,β-unsaturated ketones **2a-p** and racemic (*E*)-allylic alcohols **1a-p**.

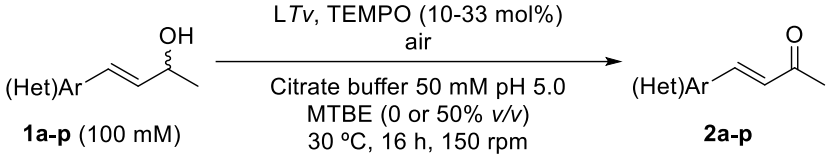
*3.2. Laccase-mediated oxidation of (*E*)-4-(het)arylbut-3-en-2-ols **1a-p***

Recently, our research group described the oxidation of this family of allylic alcohols using the catalytic system composed by the laccase from *Trametes versicolor* and the oxy-radical TEMPO [52]. To achieve a complete conversion in the oxidation of (*E*)-4-phenylbut-3-en-2-ol (**1a**, 100 mM), the transformation was performed at 30 ºC using 4.6 units of L*Tv* and 33 mol% of TEMPO in a citrate buffer 50 mM pH 5.0 using MTBE (50% *v/v*) as cosolvent to facilitate the solubility of the starting material. However, in this contribution we have further optimized the reaction conditions considering, if possible, lower amounts of TEMPO (10 or 20 mol%) and MTBE (0 or 20% *v/v*). Gladly, it was discovered that complete conversions into (*E*)-4-phenylbut-3-en-2-one (**2a**) were attained after 16 h, even just employing 10 mol% of TEMPO in the absence of MTBE (see Table S1 in the Supplementary data), appearing critical the use of a slow speed magnetic agitation (150 rpm) since it avoided the split of the reactant and catalysts around the vial walls.

Next, the oxidation of allylic alcohols **1b-p** (100 mM) was undertaken searching for the lowest possible amounts of TEMPO and MTBE (Table S2 in the Supplementary data for a complete optimization of the reaction conditions). Although a clear trend cannot be observed, it was possible to obtain quantitative conversions (>97%) for all substrates (Table 1), being feasible to diminish the TEMPO loading for aromatic substrates **1h** (entry 8, Table 1) and **1j** (entry 10, Table 1), and for heteroaromatic derivative **1m** (entry 13, Table 1). The results

achieved for allylic alcohols **1b**, **1g**, **1o** and **1p** were especially remarkable (entries 2, 7, 15, and 16, Table 1), as the quantity of TEMPO could be lowered to 10 mol% and the organic cosolvent could be avoided.

Table 1. Best conditions for the aerobic oxidation of racemic (het)aryl allylic alcohols **1a-p** using the laccase/TEMPO system in the presence of TEMPO (10-33 mol%) and MTBE (0-50% *v/v*) after 16 h at 30 ºC and 150 rpm.



Entry	Alcohol [(Het)Ar]	TEMPO (mol%)	MTBE (% *v/v*)	*c* (%)[a]
1	**1a** (C_6H_5)	10	0	>99
2	**1b** (4-OMe-C_6H_4)	10	0	>99
3	**1c** (4-Cl-C_6H_4)	33	50	>99[b]
4	**1d** (4-F-C_6H_4)	33	50	>99[b]
5	**1e** (4-Br-C_6H_4)	33	50	>99[b]
6	**1f** (3-OMe-C_6H_4)	33	50	98[b]
7	**1g** (3-F-C_6H_4)	10	0	99
8	**1h** (3-Me-C_6H_4)	20	50	98
9	**1i** (2-OMe-C_6H_4)	33	50	>99[b]
10	**1j** (2-F-C_6H_4)	10	50	99
11	**1k** (2-Pyridyl)	33	50	99[b]
12	**1l** (3-Pyridyl)	33	50	>99[b]
13	**1m** (2-Furyl)	20	50	99
14	**1n** (3-Furyl)	33	50	>99[b]
15	**1o** (2-Thienyl)	10	0	>99
16	**1p** (3-Thienyl)	10	0	>99

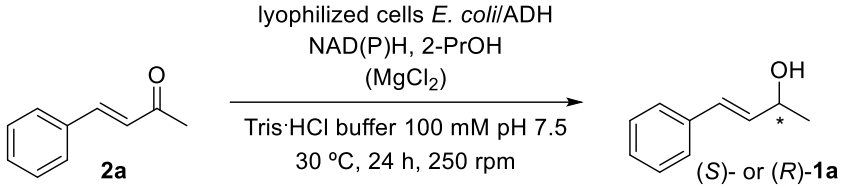
[a] Conversion values measured by GC analysis.
[b] These results were already reported in the literature [52]. Lower conversions were obtained reducing the percentages of TEMPO and/or MTBE (see the Supplementary data).

*3.3. Bioreduction of (*E*)-4-(het)arylbut-3-en-2-ones*

Once optimized the first step of the deracemization protocol, next we focused on the bioreduction of the α,β-unsaturated ketones, starting with (*E*)-4-phenylbut-3-en-2-one (**2a**) as the model substrate. A series of alcohol dehydrogenases overexpressed in *Escherichia coli* were tried such as the one from *Rhodococcus ruber* (ADH-A) [62], *Thermoanaerobacter ethanolicus* (TeSADH) [63], *Thermoanaerobacter* species (ADH-T) [64], *Ralstonia* species (RasADH) [57,65], and *Sphingobium yanoikuyae* (SyADH) [58,65,66], displaying Prelog selectivity, and the ones from *Lactobacillus brevis* (LBADH) [67,68] and the commercially available evo-1.1.200 ADH [69], acting with opposite selectivity (Tables 2 and S3).

After enzymatic screening for the production of the alcohol (*S*)-**1a**, ADH-A and ADH-T demonstrated complete control of the stereoselection, observing low to moderate conversions and selectivities with TeSADH, RasADH, and SyADH. On the other hand, anti-Prelog evo-1.1.200 displayed moderate selectivity, while LBADH led to the alcohol (*R*)-**1a** in enantiomerically pure form. Then, key parameters for the bioreduction as the amount of 2-propanol (5-30% *v/v*), the substrate concentration (10-40 mM), and the enzyme loading (10-15 mg per 0.015 mmol of ketone) were studied, collecting the most relevant results in Table 2.

Table 2. Bioreduction of ketone **2a** using overexpressed ADHs after 24 h at 30 ºC and 250 rpm.



Entry	[Alcohol] (mM)	ADH (mg)[a]	2-PrOH (% *v/v*)	*c* (%)[b]	*ee* (%)[b]
1	25	ADH-A (10)	5	64	99 (*S*)
2	25	ADH-T (10)	5	65	>99 (*S*)
3	25	LBADH (10)	5	54	>99 (*R*)
4	25	ADH-A (10)	10	75	>99 (*S*)
5	25	ADH-T (10)	10	78	>99 (*S*)
6	10	ADH-T (10)	10	85	>99 (*S*)
7	25	LBADH (10)	10	74	97 (*R*)
8	10	LBADH (10)	10	79	>99 (*R*)
9	25	LBADH (15)	10	74	>99 (*R*)
10	40	ADH-A (10)	15	79	>99 (*S*)
11	25	ADH-T (10)	15	83	94 (*S*)
12	40	ADH-T (10)	15	77	>99 (*S*)
13	25	LBADH (10)	15	83	>99 (*R*)
14	40	LBADH (10)	15	75	>99 (*R*)
15	25	ADH-A (10)	20	85	92 (*S*)
16	25	ADH-T (10)	20	86	94 (*S*)
17	25	LBADH (10)	20	86	>99 (*R*)

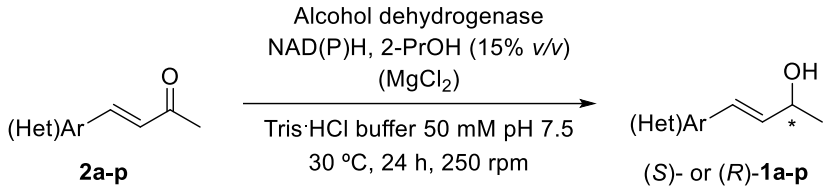
[a] The amount of ADH in mgs (per 0.015 mmol of ketone) is indicated in parentheses.
[b] Conversion and enantiomeric excess values were measured by GC analysis. Major product enantiomer appears in parentheses.

The conversion into optically active (*E*)-4-phenylbut-3-en-2-ol increased until 86% when enhancing the hydrogen donor concentration up to 20% *v/v* (entries 15-17), while the selectivity of the enzymes slightly decreased. Therefore, as a compromise, 15% *v/v* of 2-propanol and 40 mM of ketone concentration were selected as the best conditions to achieve the bioreduction, as excellent selectivities and conversions close to 80% were reached (entries 10, 12, and 14).

With the best results in hand, and considering this substrate concentration as optimum to couple it with the chemoenzymatic aerobic oxidation, the reductions of α,β-unsaturated ketones **2b**-**p** catalyzed by ADH-A, ADH-T and LBADH were carried out in order to produce

optically active alcohols **1b**-**p**. Gratifyingly, (*S*)- and (*R*)-alcohols were obtained with more than 80% *ee* for fourteen out of the sixteen alcohols (Table 3). In comparison with substrate **2a** (entry 1), similar results were found with the three enzymes for the majority of ketones, getting conversions between 60-80% and selectivities >90%. However, two different facts can be highlighted. First, amongst the two Prelog enzymes (ADH-A and ADH-T), ADH-A seems to be a more robust biocatalyst leading to higher conversion values for selected substrates (4-Br, 3-Me and 2-OMe, entries 5, 8 and 9), which were slowly converted by ADH-T (22-37% conversion). Second, it became clear that apart from possible steric effects, these reductions depended on the electronic character of the (het)aryl substituent. Hence, electron rich groups such as methoxy at positions 2 (entry 9) and 4 (entry 2), or heteroaromatic rings such as furan (entries 13 and 14) and thiophene (entries 15 and 16), limited the extent of the reaction, while electron-withdrawing moieties such as fluorine (entries 7 and 10) and pyridine (entries 11 and 12), favored the enzymatic transformation.

Table 3. Bioreduction of ketones **2a-p** (40 mM) after 24 h at 30 ºC and 250 rpm.



Entry	Ketone	LBADH *c* (%)[a]	LBADH *ee* (%)[b]	ADH-T *c* (%)[a]	ADH-T *ee* (%)[b]	ADH-A *c* (%)[a]	ADH-A *ee* (%)[b]
1	**2a** (C_6H_5)	75	>99 (*R*)	77	>99 (*S*)	79	>99 (*S*)
2	**2b** (4-OMe-C_6H_4)	51	70 (*R*)	50	60 (*S*)	49	60 (*S*)
3	**2c** (4-Cl-C_6H_4)	77	92 (*R*)	77	94 (*S*)	77	99 (*S*)
4	**2d** (4-F-C_6H_4)	70	94 (*R*)	75	96 (*S*)	70	96 (*S*)
5	**2e** (4-Br-C_6H_4)	78	94 (*R*)	29	94 (*S*)	78	94 (*S*)
6	**2f** (3-OMe-C_6H_4)	78	93 (*R*)	51	93 (*S*)	75	94 (*S*)
7	**2g** (3-F-C_6H_4)	79	98 (*R*)	83	99 (*S*)	80	99 (*S*)
8	**2h** (3-Me-C_6H_4)	72	95 (*R*)	37	95 (*S*)	69	96 (*S*)
9	**2i** (2-OMe-C_6H_4)	56	88 (*R*)	22	90 (*S*)	60	90 (*S*)
10	**2j** (2-F-C_6H_4)	80	99 (*R*)	83	99 (*S*)	81	99 (*S*)
11	**2k** (2-Pyridyl)	94	99 (*R*)	88	>99 (*S*)	93	>99 (*S*)
12	**2l** (3-Pyridyl)	89	99 (*R*)	86	99 (*S*)	89	99 (*S*)
13	**2m** (2-Furyl)	67	86 (*R*)	69	82 (*S*)	64	82 (*S*)
14	**2n** (3-Furyl)	64	90 (*R*)	66	90 (*S*)	61	90 (*S*)
15	**2o** (2-Thienyl)	66	76 (*R*)	67	78 (*S*)	64	78 (*S*)
16	**2p** (3-Thienyl)	66	88 (*R*)	66	86 (*S*)	63	93 (*S*)

[a] Conversion values measured by GC analysis.
[b] Enantiomeric excess values were measured by GC analyses after derivatization of the reaction crudes with acetic anhydride. Major product enantiomer appears in parentheses.

Due to the fact that the enzymatic bioreduction of ketones derived from furan (**2m** and **2n**) and thiophene (**2o** and **2p**) afforded lower conversions, we decided to study in more detail these biotransformations varying the substrate and hydrogen donor concentrations (Fig. 1). Compared to the initial results shown in Table 3 (entries 13 to 16) and in Fig. 1 (black bars), the decrease of 2-propanol concentration up to 10% *v/v* (blue bars), even at lower substrate concentration (25 mM, orange bars), did not usually lead to better results. However, the use of lower amounts of the ketone (green bars), generally afforded higher conversions, although at the expenses of slightly inferior *ee* values. Similar trends were observed for all ADHs independently of their stereopreference.

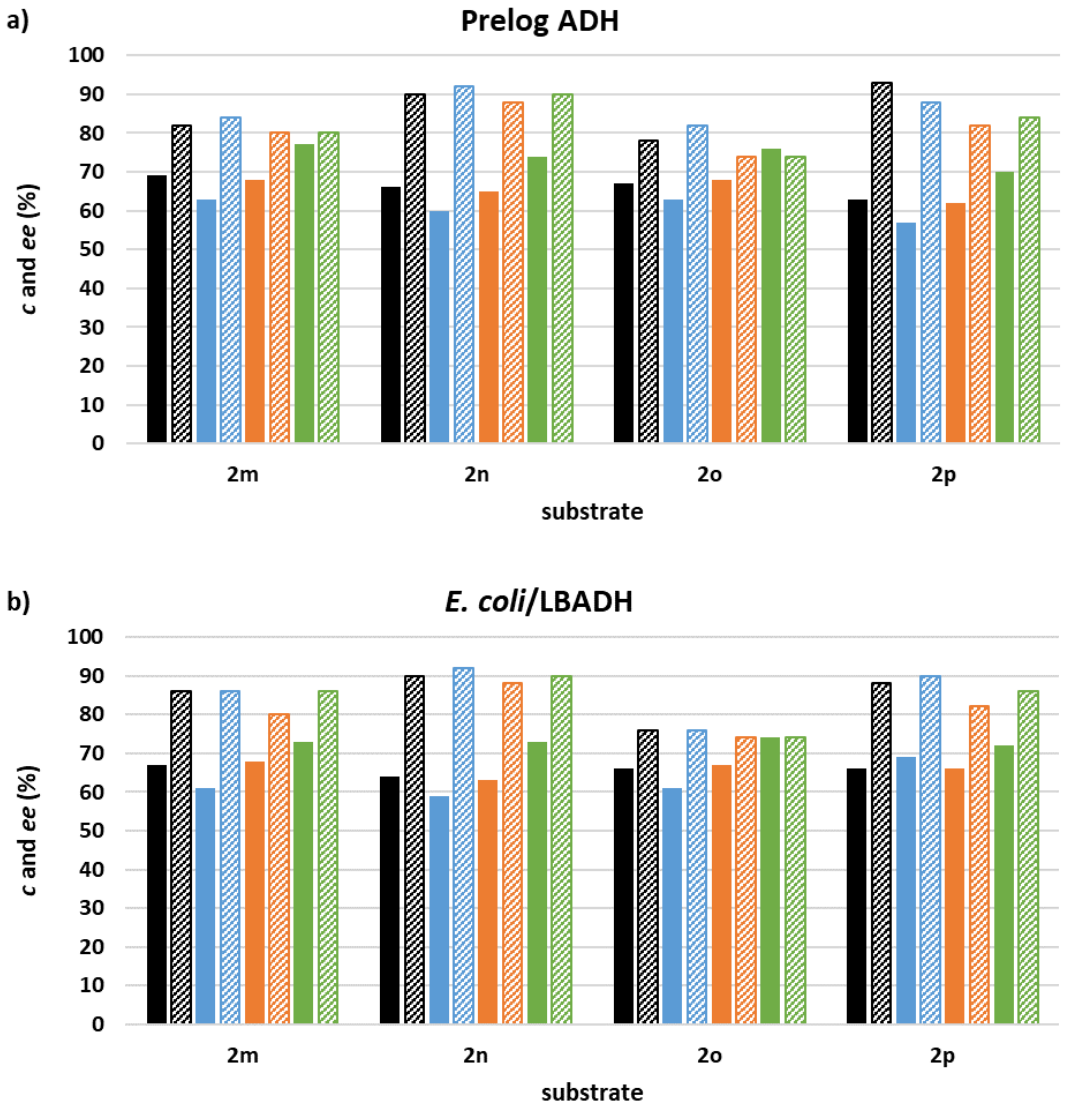


Fig. 1. Effect of the substrate and 2-PrOH concentration in the bioreductions of α,β-unsaturated ketones **2m-p** using: a) *E. coli*/ADH-T (for **2m-o**) and *E. coli*/ADH-A (for **2p**) for the production of (*S*)-alcohols; and b) *E. coli*/LBADH for the production of (*R*)-alcohols. Black bars: [ketone] = 40 mM and [2-PrOH] = 15% *v/v* (see Table 3); blue bars: [ketone] = 40 mM and [2-PrOH] = 10% *v/v* (see Table S4); orange bars: [ketone] = 25 mM and [2-PrOH] = 10% *v/v* (see Table S4); green bars: [ketone] = 25 mM and [2-PrOH] = 15% *v/v* (see Table S4). For each pair, filled bar represents conversion and dashed bar represents *ee* value.

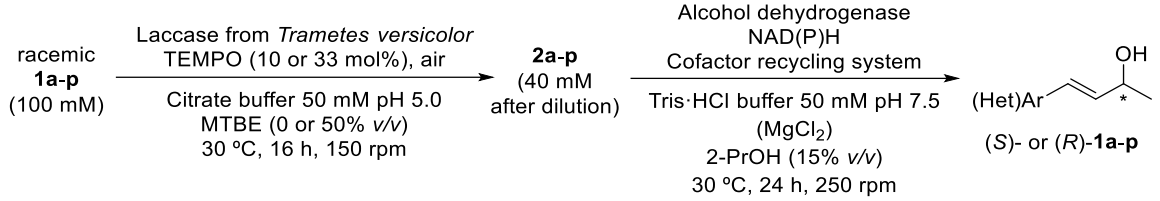
*3.4. Deracemization of (*E*)-4-(het)arylbut-3-en-2-ols **1a-p***

The successful development of aerobic oxidation reactions mediated by the laccase from *Trametes versicolor* in combination with TEMPO for alcohols **1a**-**p**, and the discovery of active and selective ADHs that could be applied to the bioreduction of the corresponding α,β-unsaturated ketones **2a**-**p**, motivated us to initiate the search for the optimal conditions towards the deracemization of racemic allylic alcohols **1a**-**p**. Taking into account the different reaction conditions required for both steps, including pH (acidic for the laccase/TEMPO system and neutral for the ADH), the agitation mode (magnetic stirring, 150 rpm, for the oxidation in contrast to the orbital shaking, 250 rpm, preferred by the ADHs), and also the substrate concentration (100 mM in the first stage and 40 mM in the second one), a one-pot two-step deracemization protocol was followed. Hence, the aerobic oxidation of alcohols **1a**-**p** was stopped by a proper dilution of the reaction with Tris·HCl buffer 50 mM pH 8, leading to a pH adjustment of 7.5 and a substrate concentration of 40 mM to carry out the bioreduction step (Table 4).

In order to demonstrate a general methodology, the oxidation step was achieved in the presence of TEMPO (33 mol%) and MTBE (50% *v/v*), conditions that allowed the quantitative oxidation of alcohols **1a**-**p** (Tables S1 and S2 in the Supplementary data) [70], necessary to avoid *ee* erosion in the whole process. After 16 h, the organic cosolvent was evaporated, so it did not interfere in the second step. Then, overexpressed LBADH was added to produce the alcohols (*R*)-**1a**-**p** (22-87% yield, 72->99%), and ADH-T (20-68% yield, 65->99%) or ADH-A (39-83% yield, 86->99%) to obtain (*S*)-**1a**-**p** after column chromatography.

Some of these allylic alcohols have been described as valuable precursors of biologically active compounds. Thus, (*S*)-**1c** can provide (*R*)-baclofen [71], which has been employed for the treatment of spasticity, (*S*)-**1e** is a key building-block of α-amino amide dipeptidyl peptidase IV inhibitors [72,73], used for the treatment of type 2 diabetes, and (*R*)-**1b** has been utilized as precursor of a derivative of *cis*-calamenene terpenoid [74].

Table 4. Deracemization of alcohols **1a-p** combining the laccase/TEMPO system and the corresponding ADH under the optimized conditions.



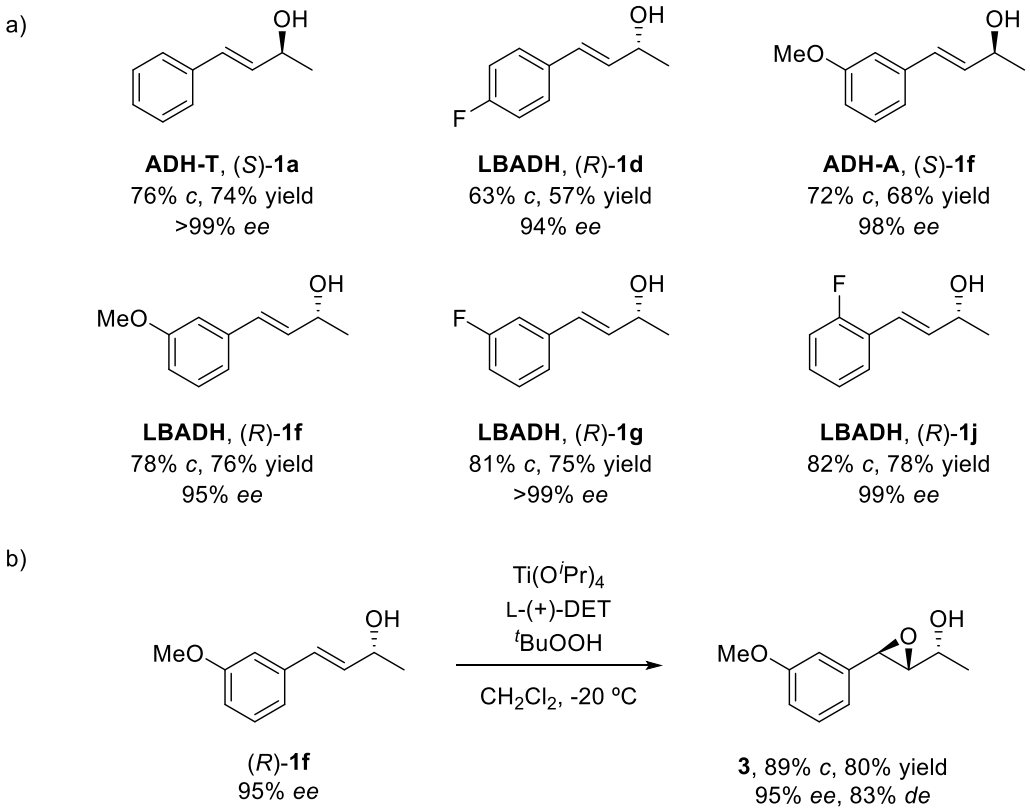
Entry	Alcohol **1a-p**	R	ADH	*c* (%)[a]	*ee* (%)[b]
1	**1a**	H	*E. coli*/LBADH	74 (52)	>99 (*R*)
2[c]	**1a**	H	*E. coli*/LBADH	73 (55)	>99 (*R*)
3	**1a**	H	*E. coli*/ADH-T	76 (51)	>99 (*S*)
4	**1b**	4-OMe	*E. coli*/LBADH	49 (22)	72 (*R*)
5	**1b**	4-OMe	*E. coli*/ADH-T	48 (20)	65 (*S*)
6	**1c**	4-Cl	*E. coli*/LBADH	74 (62)	93 (*R*)
7	**1c**	4-Cl	*E. coli*/ADH-A	69 (54)	98 (*S*)
8	**1d**	4-F	*E. coli*/LBADH	60 (44)	93 (*R*)
9	**1d**	4-F	*E. coli*/ADH-T	52 (40)	98 (*S*)
10	**1e**	4-Br	*E. coli*/LBADH	69 (56)	92 (*R*)
11	**1e**	4-Br	*E. coli*/ADH-A	55 (39)	93 (*S*)
12	**1f**	3-OMe	*E. coli*/LBADH	66 (52)	93 (*R*)
13	**1f**	3-OMe	*E. coli*/ADH-A	70 (57)	98 (*S*)
14	**1g**	3-F	*E. coli*/LBADH	84 (62)	>99 (*R*)
15	**1g**	3-F	*E. coli*/ADH-T	87 (68)	>99 (*S*)
16	**1h**	3-Me	*E. coli*/LBADH	68 (52)	96 (*R*)
17	**1h**	3-Me	*E. coli*/ADH-A	72 (58)	96 (*S*)
18	**1i**	2-OMe	*E. coli*/LBADH	64 (55)	94 (*R*)
19	**1i**	2-OMe	*E. coli*/ADH-A	60 (49)	94 (*S*)
20	**1j**	2-F	*E. coli*/LBADH	81 (65)	99 (*R*)
21	**1j**	2-F	*E. coli*/ADH-T	82 (66)	99 (*S*)
22	**1k**	2-Pyridyl	*E. coli*/LBADH	95 (87)	96 (*R*)
23	**1k**	2-Pyridyl	*E. coli*/ADH-A	95 (83)	96 (*S*)
24	**1l**	3-Pyridyl	*E. coli*/LBADH	91 (76)	94 (*R*)
25	**1l**	3-Pyridyl	*E. coli*/ADH-A	86 (70)	95 (*S*)
26	**1m**	2-Furyl	*E. coli*/LBADH	65 (48)	87 (*R*)
27	**1m**	2-Furyl	*E. coli*/ADH-T	64 (48)	86 (*S*)
28	**1n**	3-Furyl	*E. coli*/LBADH	59 (42)	93 (*R*)
29	**1n**	3-Furyl	*E. coli*/ADH-T	62 (44)	92 (*S*)
30	**1o**	2-Thienyl	*E. coli*/LBADH	58 (47)	75 (*R*)
31	**1o**	2-Thienyl	*E. coli*/ADH-T	62 (49)	74 (*S*)
32	**1p**	3-Thienyl	*E. coli*/LBADH	65 (51)	89 (*R*)
33	**1p**	3-Thienyl	*E. coli*/ADH-A	69 (52)	92 (*S*)

[a] Conversion values measured by GC analysis. Isolated yields after column chromatography appear in parentheses.

[b] Enantiomeric excess values were measured by GC analyses after derivatization of the reaction crudes with acetic anhydride. Major product enantiomer appears in parentheses.

[c] No cosolvent was used and TEMPO loading was diminished to 10 mol% in the first step.

To demonstrate the applicability of this method, semi-preparative deracemization experiments (Scheme 3a) were performed with selected alcohols **1d**,**f**,**g**,**j** (0.16 mmol) and also at preparative scale with **1a** and **1f** (0.8-1.0 mmol) for the production of derivatives (*S*)-**1a** and (*R*)-**1f**, and their optical rotation values were compared to those previously described in the literature (see the Supplementary data), confirming in this manner the proposed absolute configurations, which were in accordance with the stereoselectivity generally displayed by these ADHs [62,64,67,68].



Scheme 3. a) Deracemization examples at (semi)preparative scale. b) Epoxidation of enantioenriched alcohol (*R*)-**1f**.

As a further extension of this synthetic approach, (*R*)-**1f** obtained at preparative scale, was stereoselectively epoxidized employing modified conditions of Sharpless methodology, providing epoxide **3** in high yield (80%, Scheme 3b) and selectivity (95% *ee*, 83% *de*). This

highly reactive derivative is a synthon for biologically active natural compounds Bryostatin 1, used as antiviral and anti-Alzheimer agent, and amphotericin B, a potent antifungal agent [61].

4. Conclusions

The search for asymmetric synthetic methods that can provide highly valuable molecules under simple and mild conditions has largely increased in the past years. Deracemization reactions are elegant strategies to produce chiral enantiopure compounds starting from the corresponding racemates. Different biocatalytic approaches have been designed using whole cell biocatalysts or the combination of two ADHs with opposite stereopreference and different cofactor recognition. However, an interesting method is the linear deracemization *via* combination of a non-selective oxidation with a stereoselective reduction step. Herein, we have optimized the oxidation of a series of (het)aryl allylic secondary alcohols with the laccase from *Trametes versicolor* and the radical TEMPO, and the reduction of the corresponding α,β-unsaturated ketone intermediates employing overexpressed alcohol dehydrogenases. It was observed that in the oxidation step it was possible to diminish the quantity of TEMPO and MTBE for some of the substrates by carefully controlling the agitation speed. Applying both steps in a sequential one-pot mode, 16 enantioenriched alcohols (up to >99% *ee*) could be obtained with moderate to high isolated yields (up to 87%). Finally, the application of this methodology at preparative scale was also demonstrated, synthesizing a valuable epoxy alcohol that can be utilized as intermediate of biologically active compounds.

Conflicts of interest

None.

Acknowledgments

Financial support from the Spanish Ministry of Economy and Competitiveness (MEC, Project CTQ2016-75752-R) and the Asturian regional government (FC-GRUPIN-IDI/2018/000181) are gratefully acknowledged. J.A.-V. also thanks the Asturian regional government for a predoctoral fellowship inside the Severo Ochoa programme (BP17-32). Prof. Wolfgang Kroutil (University of Graz, Austria) is acknowledged for the donation of overexpressed alcohol dehydrogenases.

Appendix A: Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/xxxxxxx.

References

[1] A. Nagata, Y. Akagi, S.S. Masoud, M. Yamanaka, A. Kittaka, M. Uesugi, M. Odagi, K. Nagasawa, Stereoselective synthesis of four calcitriol lactone diastereomers at C23 and C25, J. Org. Chem. 84 (2019) 7630-7641.

[2] J. Liu, S. Krajangsri, J. Yang, J.Q. Li, P.G. Andersson, Iridium-catalysed asymmetric hydrogenation of allylic alcohols via dynamic kinetic resolution, Nat. Catal. 1 (2018) 438-443.

[3] P. Fan, C. Wang, Molybdenum-catalyzed asymmetric *anti*-dihydroxylation of allylic alcohols, Commun. Chem. 2 (2019) 104.

[4] C. You, S. Li, X. Li, H. Lv, X. Zhang, Enantioselective Rh-catalyzed anti-Markovnikov hydroformylation of 1,1-disubstituted allylic alcohols and amines: An efficient route to chiral lactones and lactams, ACS Catal. 9 (2019) 8529-8533.

[5] Recent review: S.L. Rossler, D.A. Petrone, E.M. Carreira, Iridium-catalyzed asymmetric synthesis of functionally rich molecules enabled by (phosphoramidite,olefin) ligands, Acc. Chem. Res. 52 (2019) 2657-2672.

[6] A. Lumbroso, M.L. Cooke, B. Breit, Catalytic asymmetric synthesis of allylic alcohols and derivatives and their applications in organic synthesis, Angew. Chem. Int. Ed. 52 (2013) 1890-1932.

[7] B. Maliszewski, T. Bauer, Enantioselective alkenylation of aldehydes with protected propargylic alcohols in the presence of a crown ether as an additive, Adv. Synth. Catal. 361 (2019) 3689-3693.

[8] Y. Cai, J.-W. Zhang, F. Li, J.-M. Liu, S.-L. Shi, Nickel/N-heterocyclic carbene complex-catalyzed enantioselective redox-neutral coupling of benzyl alcohols and alkynes to allylic alcohols, ACS Catal. 9 (2019) 1-6.

[9] S. Crotti, G. Belletti, N. Di Iorio, E. Marotta, A. Mazzanti, P. Righi, G. Bencivenni, Asymmetric vinylogous aldol addition of alkylidene oxindoles on trifluoromethyl-α,β-unsaturated ketones, RSC Adv. 8 (2018) 33451-33458.

[10] W.J. Jang, J. Yun, Copper-catalyzed tandem hydrocupration and diastereo- and enantioselective borylalkyl addition to aldehydes, Angew. Chem. Int. Ed. 57 (2018) 12116-12120.

[11] A. Narczyk, M. Pieczykolan, S. Stecko, The synthesis of non-racemic β-alkyl-β-aryldisubstituted allyl alcohols and their transformation into allylamines and amino acids bearing a quaternary stereocenter, Org. Biomol. Chem. 16 (2018) 3921-3946.

[12] Z. Liu, B. Breit, Rhodium-catalyzed regio- and enantioselective addition of *N*-hydroxyphthalimide to allenes: A strategy to synthesize chiral allylic alcohols, Org. Lett. 20 (2018) 300-303.

[13] F. Chen, Y. Zhang, L. Yu, S. Zhu, Enantioselective NiH/Pmrox-catalyzed 1,2-reduction of α,β-unsaturated ketones, Angew. Chem. Int. Ed. 56 (2017) 2022-2025.

[14] S. Liu, P. Cui, J. Wang, H. Zhou, Q. Liu, J. Lv, Asymmetric transfer hydrogenation of cycloalkyl vinyl ketones to allylic alcohols catalyzed by ruthenium amido complexes, Org. Biomol. Chem. 17 (2019) 264-267.

[15] C. Li, W. Lu, B. Lu, W. Li, X. Xie, Z. Zhang, Ru-catalyzed chemo- and enantioselective hydrogenation of 2,4-pentadien-1-ones: Synthesis of chiral 2,4-pentadien-1-ols, J. Org. Chem. 84 (2019) 16086-16094.

[16] L. Amenós, L. Nóvoa, L. Trulli, A. Arroyo-Bondía, A. Parra, M. Tortosa, Harnessing the elusive 1,4-reduction of vinyl epoxides through copper catalysis, ACS Catal. 9 (2019) 6583-6587.

[17] K.-Y. Ye, T. McCallum, S. Lin, Bimetallic radical redox-relay catalysis for the isomerization of epoxides to allylic alcohols, J. Am. Chem. Soc. 141 (2019) 9548-9554.

[18] R.M. Neyyappadath, R. Chisholm, M.D. Greenhalgh, C. Rodríguez-Escrich, M.A. Pericàs, G. Hähner, A.D. Smith, Acylative kinetic resolution of alcohols using a recyclable polymer-supported isothiourea catalyst in batch and flow, ACS Catal. 8 (2018) 1067-1075.

[19] Y. Liu, S. Liu, D. Li, N. Zhang, L. Peng, J. Ao, C.E. Song, Y. Lan, H. Yan, Kinetic resolution of allylic alcohol with chiral BINOL-based alkoxides: A combination of experimental and theoretical studies, J. Am. Chem. Soc. 141 (2019) 1150-1159.

[20] M. Szigeti, E.R. Tőke, M.C. Turóczi, V. Nagy, G. Szakács, L. Poppe, Lipase-catalyzed kinetic resolution of 4-aryl- and 4-heteroarylbut-3-en-2-ols, Arkivoc (iii) (2008) 54-65.

[21] A. Skrobiszewski, W. Gładkowski, G. Maciejewska, C. Wawrzeńczyk, Chemoenzymatic synthesis of *trans*-β-aryl-δ-hydroxy-γ-lactones and enzymatic kinetic resolution of their racemic mixtures, Molecules 21 (2016) 1552.

[22] M.-J. Kim, H.K. Lee, J. Park, (*S*)-Selective dynamic kinetic resolution of allylic alcohols by enzyme-metal biocatalysis, Bull. Korean Chem. Soc. 28 (2007) 2096-2098.

[23] S. Akai, R. Hanada, N. Fujiwara, Y. Kita, M. Egi, One-pot synthesis of optically active allyl esters via lipase–vanadium combo catalysis, Org. Lett. 12 (2010) 4900-4903.

[24] M. Egi, K. Sugiyama, M. Saneto, R. Hanada, K. Kato, S. Akai, A mesoporous-silica-immobilized oxovanadium cocatalyst for the lipase-catalyzed dynamic kinetic resolution of racemic alcohols, Angew. Chem. Int. Ed. 52 (2013) 3654-3658.

[25] B. Xia, J. Xu, Z. Xiang, Y. Cen, Y. Hu, X. Lin, Q. Wu, Stereoselectivity-tailored, metal-free hydrolytic dynamic kinetic resolution of Morita−Baylis−Hillman acetates using an engineered lipase−organic base cocatalyst, ACS Catal. 7 (2017) 4542-4549.

[26] T. Sivakumari, R. Preetha, A. Chadha, Enantioselective oxidation of secondary alcohols by *Candida parapsilosis* ATCC 7330, RSC Adv. 4 (2014) 2257-2262.

[27] S. Gandomkar, E. Jost, D. Loidolt, A. Swoboda, M. Pickl, W. Elaily, B. Daniel, M.W. Fraaije, P. Macheroux, W. Kroutil, Biocatalytic enantioselective oxidation of *sec*-allylic alcohols with flavin-dependent oxidases, Adv. Synth. Catal. 361 (2019) 5264-5271.

[28] R. van Deursen, W. Stampfer, K. Edegger, K. Faber, W. Kroutil, Chemo- and stereo-selective biocatalytic reduction of α,β-unsaturated ketones employing a chemo-tolerant ADH from *Rhodococcus ruber* DSM 44541, J. Mol. Catal. B: Enzym. 31 (2004) 159-163.

[29] S. Cai, N. Shao, Y. Chen, A. Li, J. Pan, H. Zhu, H. Zou, S. Zeng, L. Sun, J. Zhao, Enantioselective reduction of α,β-unsaturated ketones and aryl ketones by perakine reductase, Org. Lett. 21 (2019) 4411-4414.

[30] S. Sgalla, G. Fabrizi, R. Cirilli, A. Macone, A. Bonamore, A. Boffi, S. Cacchi, Chiral (*R*)- and (*S*)-allylic alcohols via a one-pot chemoenzymatic synthesis, Tetrahedron: Asymmetry 18 (2007) 2791-2796.

[31] M. Kraußer, W. Hummel, H. Gröger, Enantioselective one-pot two-step synthesis of hydrophobic allylic alcohols in aqueous medium through the combination of a Wittig reaction and an enzymatic ketone reduction, Eur. J. Org. Chem. (2007) 5175-5179.

[32] Y.-C. Liu, Z.-L. Wu, Switchable asymmetric bio-epoxidation of α,β-unsaturated ketones, Chem. Commun. 52 (2016) 1158-1161.

[33] Y.-C. Liu, C. Guo, Y. Liu, H.-B. Wang, Z.-L. Wu, Enzymatic cascades for the stereo-complementary epimerisation of *in situ* generated epoxy alcohols, Org. Biomol. Chem. 15 (2017) 2562-2568.

[34] M. Rachwalski, N. Vermue, F.P.J.T. Rutjes, Recent advances in enzymatic and chemical deracemisation of racemic compounds, Chem. Soc. Rev. 42 (2013) 9268-9282.

[35] A. Díaz-Rodríguez, I. Lavandera, V. Gotor, Why leave a lob half done? Recent progress in enzymatic deracemizations, Curr. Green Chem. 2 (2015) 192-211.

[36] M.M. Musa, F. Hollmann, F.G. Mutti, Synthesis of enantiomerically pure alcohols and amines *via* biocatalytic deracemisation methods, Catal. Sci. Technol. 9 (2019) 5487-5503.

[37] C. Aranda, G. Oksdath-Mansilla, F.R. Bisogno, G. de Gonzalo, Deracemisation processes employing organocatalysis and enzyme catalysis, Adv. Synth. Catal. 362 (2020) 1233-1257.

[38] D. Titu, A. Chadha, Enantiomerically pure allylic alcohols: Preparation by *Candida parapsilosis* ATCC 7330 mediated deracemisation, Tetrahedron: Asymmetry 19 (2008) 1698-1701.

[39] T. Saravanan, R. Selvakumar, M. Doble, A. Chadha, Stereochemical preference of *Candida parapsilosis* ATCC 7330 mediated deracemization: *E*- versus *Z*-aryl secondary alcohols, Tetrahedron: Asymmetry 23 (2012) 1360-1368.

[40] C.V. Voss, C.C. Gruber, W. Kroutil, Deracemisation of secondary alcohols via biocatalytic stereoinversion, Synlett (2010) 991-998.

[41] S. Riva, Laccases: Blue enzymes for green chemistry, Trends Biotechnol. 24 (2006) 219-226.

[42] S. Witayakran, A.J. Ragauskas, Synthetic applications of laccase in green chemistry, Adv. Synth. Catal. 351 (2009) 1187-1209.

[43] M.D. Cannatelli, A.J. Ragauskas, Two decades of laccases: Advancing sustainability in the chemical industry, Chem. Rec. 17 (2017) 122-140.

[44] A. Zerva, S. Simić, E. Topakas, J. Nikodinovic-Runic, Applications of microbial laccases: Patent review of the past decade (2009–2019), Catalysts 9 (2019) 1023.

[45] M. Fabbrini, C. Galli, P. Gentili, D. Macchitella, An oxidation of alcohols by oxygen with the enzyme laccase and mediation by TEMPO, Tetrahedron Lett. 42 (2001) 7551-7553.

[46] I.W.C.E. Arends, Y.-X. Li, R. Ausan, R.A. Sheldon, Comparison of TEMPO and its derivatives as mediators in laccase catalysed oxidation of alcohols, Tetrahedron 62 (2006) 6659-6665.

[47] K. Kędziora, A. Díaz-Rodríguez, I. Lavandera, V. Gotor-Fernández, V. Gotor, Laccase/TEMPO-mediated system for the thermodynamically disfavored oxidation of 2,2-dihalo-1-phenylethanol derivatives, Green Chem. 16 (2014) 2448-2453.

[48] L. Martínez-Montero, V. Gotor, V. Gotor-Fernández, I. Lavandera, Stereoselective amination of racemic *sec*-alcohols through sequential application of laccases and transaminases, Green Chem. 19 (2017) 474-480.

[49] L. Marx, N. Ríos-Lombardía, P. Süss, M. Höhne, F. Morís, J. González-Sabín, P. Berglund, Chemoenzymatic synthesis of sertraline, Eur. J. Org. Chem. (2020) 510-513.

[50] E. Brenna, M. Crotti, F.G. Gatti, D. Monti, F. Parmeggiani, A. Pugliese, F. Tentori, Biocatalytic synthesis of chiral cyclic γ-oxoesters by sequential C–H hydroxylation, alcohol oxidation and alkene reduction, Green Chem. 19 (2017) 5122-5130.

[51] L. Martínez-Montero, V. Gotor, V. Gotor-Fernández, I. Lavandera, Mild chemoenzymatic oxidation of allylic *sec*-alcohols. Application to biocatalytic stereoselective redox isomerizations, ACS Catal. 8 (2018) 2413-2419.

[52] J. Albarrán-Velo, I. Lavandera, V. Gotor-Fernández, Sequential two-step stereoselective amination of allylic alcohols through the combination of laccases and amine transaminases, ChemBioChem 21 (2020) 200-211.

[53] S. González-Granda, D. Méndez-Sánchez, I. Lavandera, V. Gotor-Fernández, Laccase-mediated oxidations of propargylic alcohols. Application in the deracemization of 1-arylprop-2-yn-1-ols in combination with alcohol dehydrogenases, ChemCatChem 12 (2020) 520-527.

[54] A.B.V. Silva, E.D. Silva, A.A. dos Santos, J.L. Princival, A sustainable access to ynones through laccase/TEMPO-catalyzed metal- and halogen-free aerobic oxidation of propargylic alcohols in aqueous medium, Catal. Commun. 137 (2020) 105946.

[55] A. Díaz-Rodríguez, N. Ríos-Lombardía, J.H. Sattler, I. Lavandera, V. Gotor-Fernández, W. Kroutil, V. Gotor, Deracemisation of profenol core by combining laccase/TEMPO-mediated oxidation and alcohol dehydrogenase-catalysed dynamic kinetic resolution, Catal. Sci. Technol. 5 (2015) 1443-1446.

[56] Other recent approximations to achieve similar deracemization protocols involve: a) the use of TEMPO–I_2 as oxidative system: D. Méndez-Sánchez, J. Mangas-Sánchez, I. Lavandera, V. Gotor, V. Gotor-Fernández, Chemoenzymatic deracemization of secondary alcohols by using a TEMPO–iodine–alcohol dehydrogenase system, ChemCatChem 7 (2015) 4016-4020; b) the utilization of NaOCl–AZADO in the oxidation step: E. Liardo, N. Ríos-Lombardía, F. Morís, J. González-Sabín, F. Rebolledo, A straightforward deracemization of *sec*-alcohols combining organocatalytic oxidation and biocatalytic reduction, Eur. J. Org. Chem. (2018) 3031-3035; and c) the use of TEMPO (oxidation stage) with a rhodium catalyst (reduction stage) in a compartmentalized system: P. Qu, M. Kuepfert, S. Jockusch, M. Weck, Compartmentalized nanoreactors for one-pot redox-driven transformations, ACS Catal. 9 (2019) 2701-2706.

[57] I. Lavandera, A. Kern, B. Ferreira-Silva, A. Glieder, S. de Wildeman, W. Kroutil, Stereoselective bioreduction of bulky-bulky ketones by a novel ADH from *Ralstonia* sp., J. Org. Chem. 15 (2008) 6003-6005.

[58] I. Lavandera, A. Kern, V. Resch, B. Ferreira-Silva, A. Glieder, W.M.F. Fabian, S. de Wildeman, W. Kroutil, One-way biohydrogen transfer for oxidation of *sec*-alcohols, Org. Lett. 11 (2008) 2155-2158.

[59] K. Edegger, C.C. Gruber, T.M. Poessl, S.R. Wallner, I. Lavandera, K. Faber, F. Niehaus, J. Eck, R. Oehrlein, A. Hafner, W. Kroutil, Biocatalytic deuterium- and hydrogen-transfer using over-expressed ADH-'A': Enhanced stereoselectivity and ^{2}H-labeled chiral alcohols, Chem. Commun. (2006) 2402-2404.

[60] K. Zumbansen, A. Döhring, B. List, Morpholinium trifluoroacetate-catalyzed aldol condensation of acetone with both aromatic and aliphatic aldehydes, Adv. Synth. Catal. 352 (2010) 1135-1138.

[61] D.A. Evans, J.A. Gauchet-Prunet, E.M. Carreira, A.B. Charette, Synthesis of 1,3-diol synthons from epoxy aromatic precursors: An approach to the construction of polyacetate-derived natural products, J. Org. Chem. 56 (1991) 741-750.

[62] W. Stampfer, B. Kosjek, C. Moitzi, W. Kroutil, K. Faber, Biocatalytic asymmetric hydrogen transfer, Angew. Chem. Int. Ed. 41 (2002) 1014-1017.

[63] C. Heiss, M. Laivenieks, J.G. Zeikus, R.S. Phillips, Mutation of cysteine-295 to alanine in secondary alcohol dehydrogenase from *Thermoanaerobacter ethanolicus* affects the enantioselectivity and substrate specificity of ketone reductions, Bioorg. Med. Chem. 7 (2001) 1659-1666.

[64] Z. Findrik, D. Vasić-Rački, S. Lütz, T. Daussmann, C. Wandrey, Kinetic modeling of acetophenone reduction catalyzed by alcohol dehydrogenase from *Thermoanaerobacter* sp., Biotechnol. Lett. 27 (2005) 1087-1095.

[65] H. Man, K. Kędziora, J. Kulig, A. Frank, I. Lavandera, V. Gotor-Fernández, D. Rother, S. Hart, J.P. Turkenburg, G. Grogan, Structures of alcohol dehydrogenases from *Ralstonia* and *Sphingobium* spp. reveal the molecular basis for their recognition of 'bulky–bulky' ketones, Top. Catal. 5 (2014) 356-365.

[66] I. Lavandera, G. Oberdofer, J. Gross, S. de Wildeman, W. Kroutil, Stereocomplementary asymmetric reduction of bulky–bulky ketones by biocatalytic hydrogen transfer, Eur. J. Org. Chem. 15 (2008) 2539-2543.

[67] M. Wolberg, W. Hummel, C. Wandrey, M. Müller, Highly regio- and enantioselective reduction of 3,5-dioxocarboxylates, Angew. Chem. Int. Ed. 39 (2000) 4306-4308.

[68] S. Leuchs, L. Greiner, Alcohol dehydrogenase from *Lactobacillus brevis*: A versatile robust catalyst for enantioselective transformations, Chem. Biochem. Eng. Q. 2 (2011) 267-281.

[69] S. Kara, D. Spickermann, A. Weckbecker, C. Leggewie, I.W.C.E. Arends, F. Hollmann, Bioreductions catalyzed by an alcohol dehydrogenase in non-aqueous media, ChemCatChem 6 (2014) 973-976.

[70] The deracemization process was also done with alcohol 1a in the absence of MTBE and using 10 mol% of TEMPO. As can be seen in Table 4, entry 2, the protocol worked similarly, demonstrating that the reaction conditions described in Table 1 are also compatible to achieve this transformation.

[71] E. Brenna, N. Caraccia, C. Fuganti, D. Fuganti, P. Grasselli, Enantioselective synthesis of β-substituted butyric acid derivatives via orthoester Claisen rearrangement of enzymatically resolved allylic alcohols: Application to the synthesis of (*R*)-(–)-baclofen, Tetrahedron: Asymmetry 8 (1997) 3801-3805.

[72] S.D. Edmondson, A. Mastracchio, J.L. Duffy, G.J. Eiermann, H. He, I. Ita, B. Leiting, J.F. Leone, K.A. Lyons, A.M. Makarewicz, R.A. Patel, A. Petrov, J.K. Wu, N.A. Thornberry, A.E. Weber, Discovery of potent and selective orally bioavailable β-substituted phenylalanine derived dipeptidyl peptidase IV inhibitors, Bioorg. Med. Chem. Lett. 15 (2005) 3048-3052.

[73] S.D. Edmondson, A. Mastracchio, R.J. Mathvink, J. He, B. Harper, Y.-J. Park, M. Beconi, J. Di Salvo, G.J. Eiermann, H. He, B. Leiting, J.F. Leone, D.A. Levorse, K. Lyons, R.A. Patel, S.B. Patel, A. Petrov, G. Scapin, J. Shang, R.S. Roy, A. Smith, J.K. Wu, S. Xu, B. Zhu, N.A. Thornberry, A.E. Weber, (2*S*,3*S*)-3-Amino-4-(3,3-difluoropyrrolidin-1-yl)-*N*,*N*-dimethyl-4-oxo-2-(4-[1,2,4]triazolo[1,5-*a*]-pyridin-6-ylphenyl)butanamide: A selective α-amino amide dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes, J. Med. Chem. 49 (2006) 3614-3627.

[74] E. Brenna, C. Dei Negri, C. Fuganti, F.G. Gatti, S. Serra, Enantioselective synthesis of *cis*-7-methoxy-calamenene via Claisen rearrangement of an enzymatically resolved allyl alcohol, Tetrahedron: Asymmetry 15 (2004) 335-340.