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



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Keywords: Alcohol dehydrogenases Bioreduction Deracemization Laccases One-pot synthesis	A series of enantioenriched (hetero)aromatic secondary allylic alcohols has been synthesized through derace- mization of the corresponding racemic mixtures combining a non-selective chemoenzymatic oxidation (laccase from <i>Trametes versicolor</i> and oxy-radical TEMPO) and a stereoselective biocatalyzed reduction (lyophilized cells of <i>E. coli</i> 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% <i>ee</i>). 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

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 metalcatalyzed 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 lipasemediated 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

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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.

[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.

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 (LTv) 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* (LTv, 0.66 U/mg) was purchased from Sigma-Aldrich. 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 Evoxx technologies GmbH. Glucose dehydrogenase (GDH-105) was purchased from Codexis Inc. 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 min 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 $(18 \times 72 \times 10)$, $(18 \times 109 \times 10)$ or $(28 \times 137 \times 20)$ 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 °C in a sealed tube overnight. After this time the reaction mixture was cooled at room temperature, and a NaHCO3 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 Na₂SO₄, 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), 21 (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 NaBH₄ (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 °C. 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 × 15 mL), and the combined organic phases were washed with brine (5 mL). The resulting organic layer was dried over anhydrous Na₂SO₄, filtered and the solvent evaporated under reduced pressure. The reaction crude was purified by column chromatography on silica gel (50% Et₂O/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 co-solvent (up to 50% ν/ν , 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 °C controlling the agitation speed at 150 rpm. After this time, the product was extracted with EtOAc ($2 \times 2 \text{ mL}$), the organic phases were combined, dried over Na₂SO₄ 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-2ones 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 ($10\% \nu/\nu$, 60μ L). Then, TrisHCl buffer 50 mM pH 7.5 (540μ L) containing glucose (50 mM), GDH (10U) and NADPH (1 mM) was added. Finally, overexpressed *E. coli*/RasADH lyophilized cells (10 mg) were added. The reaction was shaken at 30°C and 250rpm for 24 h, and extracted with EtOAc (0.5 mL), separating the organic layer by centrifugation (2 min, 13,000rpm). This extraction and centrifugation protocol was repeated twice, and the organic layers were combined and dried over Na₂SO₄.

For the other ADHs: In a 1.5 mL Eppendorf vial, ketone **2a** (0.015 mmol, 25 mM) was dissolved in 2-PrOH ($10\% \nu/\nu$, 60μ L). Then, Tris·HCl buffer 50 mM pH 7.5 (540μ L) containing MgCl₂ (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 °C 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 Na₂SO₄.

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 ($15\% \nu/\nu$, 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), MgCl₂ (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 °C 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 °C 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, 13,000 rpm), dried over Na₂SO₄, 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% ν/ν , 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 °C, 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), TrisHCl buffer 50 mM pH 8 (0.9 mL), MgCl₂ (0.2 mL of a 10 mM solution in TrisHCl buffer 50 mM pH 8), and NADPH (0.2 mL of a 10 mM solution in TrisHCl buffer 50 mM pH 8) were added, leading to concentrations of approximately 40 mM for the substrate, 1 mM for MgCl₂, 1 mM for NADPH and 15% ν/ν 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% ν/ν 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% ν/ν 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 °C 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, 4900 rpm). This extraction and centrifugation protocol was performed twice and, finally, the organic layers were combined and

dried over Na₂SO₄. Conversion and *ee* values were measured as previously described. The products were purified through column chromatography using the following eluents: 50% Et₂O/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-2ols 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% ν/ν , 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 °C, 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), TrisHCl buffer 50 mM pH 8 (1.8 mL), MgCl₂ (0.4 mL of a 10 mM solution in TrisHCl buffer 50 mM pH 8), and NADPH (0.4 mL of a 10 mM solution in TrisHCl buffer 50 mM pH 8) were added, leading to concentrations of approximately 40 mM for the substrate, 1 mM for MgCl₂, 1 mM for NADPH and 15% ν/ν 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% ν/ν 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 °C 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, 4900 rpm). This extraction and centrifugation protocol was performed three times and, finally, the organic layers were combined and dried over Na₂SO₄. 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 50% Et₂O/hexane as eluent, yielding alcohols (*R*)-1d, (*S*)-1f, (*R*)-1g, and (*R*)-1jin 57-78%.

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

2.8.1. (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% ν/ν , 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 °C, 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 MgCl₂, 1 mM for NADPH and 15% ν/ν 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 °C 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, 4900 rpm). This extraction and centrifugation protocol was performed three times and, finally, the organic layers were combined and dried over Na₂SO₄. 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% Et₂O/ hexane), affording the alcohol (*S*)-1a in 74% yield.

2.8.2. (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% ν/ν , 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 °C, 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), TrisHCl buffer 50 mM pH 8 (21.25 mL), MgCl₂ (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 MgCl₂, 1 mM for NADPH and 15% ν/ν 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 °C 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, 4900 rpm). This extraction and centrifugation protocol was performed three times and, finally, the organic layers were combined and dried over Na₂SO₄. 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% Et₂O/hexane), affording the alcohol (*R*)-**1f** in 76% yield.

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

L-(+)-Diethyl tartrate (DET, 175 μ L, 0.848 mmol) was added to a solution of Ti(OⁱPr)₄ (241 μ L, 0.848 mmol) in dry CH₂Cl₂ (5 mL) at -20 °C. The solution was stirred during 30 min at -20 °C and then, a *tert*-butyl hydroperoxide (ⁱBuOOH) solution in decane (5.5 M, 250 μ L, 1.38 mmol) was added dropwise. After additional 10 min of stirring at -20 °C, a solution of (*R*)-1f (95% *ee*, 120 mg, 0.673 mmol) in dry CH₂Cl₂

(2 mL) was added dropwise and stirred at -20 °C during 3 h. Afterwards, the resulting mixture was quenched with water (10 mL) and left warming up to room temperature during 30 min. The reaction was filtered through a Celite pad and extracted with CH₂Cl₂ (3 × 15 mL). The organic phases were combined and washed with brine (2 × 10 mL), dried over anhydrous Na₂SO₄, filtered, and the solvent evaporated under reduced pressure. The reaction crude was analyzed by ¹H NMR finding a 89% conversion and 83% diastereomeric excess. Then, the reaction crude was purified by preparative thin layer chromatography (75% Et₂O/hexane), yielding the corresponding epoxide **3** (80% yield, 95% *ee*, 83% *de*).

(R)-1-[(2R,3R)-3-(3-Methoxyphenyl)oxiran-2-yl]ethan-1-ol (3) [61]

Yellow wax. $R_{\rm f}$ (75% Et₂O/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. ¹H NMR (300.13 MHz, CDCl₃, major diastereoisomer): δ 1.31 (d, ³J_{HH} = 6.5 Hz, 3 H), 2.21 (br s, 1 H), 3.06 (dd, ³J_{HH} = 2.6, ⁴J_{HH} = 0.8 Hz, 1 H), 3.80 (s, 3 H), 3.93 (dd, ³J_{HH} = 2.1 Hz, 1 H), 4.10 (qd, ³J_{HH} = 6.4, ³J_{HH} = 2.9 Hz, 1 H), 6.79–6.91 (m, 3 H), 7.23–7.28 (m, 1 H) ppm. ¹³C NMR (75.5 MHz, CDCl₃, 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 (CH₃), 54.7 (CH), 18.8 (CH₃) ppm. HRMS (ESI⁺, *m/z*): calcd for (C₁₁H₁₅O₃)⁺ (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 h 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).

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 °C using 4.6 units of L*Tv* and 33 mol% of TEMPO in a citrate buffer 50 mM pH 5.0 using MTBE (50% ν/ν) 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% ν/ν). 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.

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% ν/ν), 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.

The conversion into optically active (*E*)-4-phenylbut-3-en-2-ol increased until 86% when enhancing the hydrogen donor concentration up to 20% ν/ν (entries 15–17), while the selectivity of the enzymes slightly decreased. Therefore, as a compromise, 15% ν/ν 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



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

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% ν/ν) after 16 h at 30 °C and 150 rpm.

OH	L <i>Tv</i> , TEMPO (10-33 mol%) air	•		
(Het)Ar	Citrate buffer 50 mM pH 5.0	(Het)Ar		
1a-p (100 mM)	MTBE (0 or 50% <i>V/V</i>) 30 °C, 16 h, 150 rpm	2а-р		
Entry	Alcohol [(Het)Ar]	TEMPO (mol%)	MTBE (% <i>v</i> / <i>v</i>)	c (%) ^a
1	1a (C ₆ H ₅)	10	0	> 99
2	1b (4-OMe-C ₆ H ₄)	10	0	> 99
3	1c (4-Cl-C ₆ H ₄)	33	50	> 99 ^b
4	1d (4-F-C ₆ H ₄)	33	50	> 99 ^b
5	$1e (4-Br-C_6H_4)$	33	50	> 99 ^b
6	1f (3-OMe-C ₆ H ₄)	33	50	98^{b}
7	1g (3-F-C ₆ H ₄)	10	0	99
8	1h (3-Me-C ₆ H ₄)	20	50	98
9	1i (2-OMe-C ₆ H ₄)	33	50	> 99 ^b
10	$1j(2-F-C_6H_4)$	10	50	99
11	1k (2-Pyridyl)	33	50	99 ^b
12	11 (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).

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.

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 13–16) and in Fig. 1 (conditions A), the decrease of 2-propanol concentration up to $10\% \nu/\nu$ (conditions B), even at lower substrate concentration (25 mM, conditions C), did not usually lead to better results. However, the use of

Table 2

Bioreduction of ketone 2a using overexpressed ADHs after 24 h at 30 °C and 250 rpm.

2a O Za	lyophilized cells <i>E. coli</i> /ADH NAD(P)H, 2-PrOH (MgCl ₂) Tris HCl buffer 100 mM pH 7.5 30 °C, 24 h, 250 rpm	OH * (S)- or (R)-1a			
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 (<i>S</i>)
3	25	LBADH (10)	5	54	> 99 (R)
4	25	ADH-A (10)	10	75	> 99 (<i>S</i>)
5	25	ADH-T (10)	10	78	> 99 (<i>S</i>)
6	10	ADH-T (10)	10	85	> 99 (<i>S</i>)
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 (<i>S</i>)
11	25	ADH-T (10)	15	83	94 (S)
12	40	ADH-T (10)	15	77	> 99 (<i>S</i>)
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 (<i>S</i>)
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.

ADH-A ee (%)^b > 99 (S)60 (S) 99 (S) 96 (S) 94 (S) 94 (S) 99 (S) 96 (S) 90 (S) 99 (S) > 99 (S)99 (S) 82 (S) 90 (S) 78 (S) 93 (S)

Table 3

a)

100

90

80

70

60

Bioreduction of ketones 2a-p (40 mM) after 24 h at 30 °C and 250 rpm.

Alcohol dehydrogenase NAD(P)H, 2-PrOH (15% v/v) (MgCl ₂)		ОН				
(Het)Ar	Tris HCl buffer 50) mM pH 7.5	(Het)Ar ***			
2a-p	30 °C, 24 II, 7	250 Ipin	(S)- or (R)- 1a-p			
Entry	Ketone	LBADH c (%) ^a	LBADH ee (%) ^b	ADH-T c (%) ^a	ADH-T ee (%) ^b	ADH-A c (%) ^a
1	2a (C ₆ H ₅)	75	> 99 (R)	77	> 99 (S)	79
2	2b (4-OMe-C ₆ H ₄)	51	70 (R)	50	60 (<i>S</i>)	49
3	2c (4-Cl-C ₆ H ₄)	77	92 (R)	77	94 (S)	77
4	2d (4-F-C ₆ H ₄)	70	94 (R)	75	96 (S)	70
5	2e (4-Br-C ₆ H ₄)	78	94 (R)	29	94 (S)	78
6	2f (3-OMe-C ₆ H ₄)	78	93 (R)	51	93 (S)	75
7	2g (3-F-C ₆ H ₄)	79	98 (R)	83	99 (S)	80
8	2h (3-Me-C ₆ H ₄)	72	95 (R)	37	95 (<i>S</i>)	69
9	2i (2-OMe-C ₆ H ₄)	56	88 (R)	22	90 (S)	60
10	2j (2-F-C ₆ H ₄)	80	99 (R)	83	99 (S)	81
11	2k (2-Pyridyl)	94	99 (R)	88	> 99 (S)	93
12	2l (3-Pyridyl)	89	99 (R)	86	99 (S)	89
13	2m (2-Furyl)	67	86 (R)	69	82 (S)	64
14	2n (3-Furyl)	64	90 (R)	66	90 (S)	61
15	20 (2-Thienyl)	66	76 (R)	67	78 (S)	64
16	2p (3-Thienyl)	66	88 (R)	66	86 (S)	63

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.

Conditions A

Conditions B

lower amounts of the ketone (conditions D), generally afforded higher conversions, although at the expenses of slightly inferior ee values. Similar trends were observed for all ADHs independently of their stereopreference.

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

Prelog ADH

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

> Fig. 1. Effect of the substrate and 2-PrOH concentration in the bioreductions of α , β -unsaturated ketones **2 m-p** using: a) *E*. coli/ADH-T (for 2 m-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. Conditions A: [ketone] = 40 mM and [2-PrOH] = 15% v/v (see Table 3); conditions B: [ketone] = 40 mM and [2-PrOH] = 10% ν/ν (see Table S4); conditions C: [ketone] = 25 mM and [2-PrOH] = $10\% \nu/\nu$ (see Table S4); conditions D: [ketone] = 25 mM and [2-PrOH] = 15% ν/ν (see Table S4). For each pair, filled bar represents conversion and dashed bar represents ee value.



Table 4

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

racemic 1a-p (100 mM)	Laccase from <i>Trametes versicolor</i> TEMPO (10 or 33 mol%), air Citrate buffer 50 mM pH 5.0 MTBE (0 or 50% <i>v/v</i>) 30 °C, 16 h, 150 rpm	Alcohol de NA Alcohol de NA Cofactor re (40 mM after dilution) Tris-HCI buff (N 2-PrOH 30 °C, 24	Hydrogenase OH D(P)H OH cycling system (Het)Ar ar 50 mM pH 7.5 (Het)Ar IgCl ₂) (S)- or (R)-1a-p I 15% v/v) Is 250 rpm		
Entry	Alcohol 1a-p	R	ADH	c (%) ^a	ee (%) ^b
1 2 ^c 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18	1a 1a 1b 1b 1c 1c 1c 1d 1d 1e 1e 1f 1f 1g 1g 1h 1h 1i	H H 4-OMe 4-OMe 4-Cl 4-Cl 4-Cl 4-F 4-F 4-F 4-Br 3-OMe 3-OMe 3-F 3-F 3-F 3-F 3-Me 3-Me 2-OMe	E. coli/LBADH E. coli/LBADH E. coli/LBADH E. coli/LBADH E. coli/LBADH E. coli/LBADH E. coli/LBADH E. coli/LBADH E. coli/ADH-T E. coli/ADH-T E. coli/ADH-A E. coli/ADH-A E. coli/ADH-A E. coli/ADH-T E. coli/LBADH E. coli/LBADH E. coli/LBADH E. coli/LBADH E. coli/LBADH	74 (52) 73 (55) 76 (51) 49 (22) 48 (20) 74 (62) 69 (54) 60 (44) 52 (40) 69 (56) 55 (39) 66 (52) 70 (57) 84 (62) 87 (68) 68 (65) 72 (58) 64 (55)	> 99 (R) > 99 (R) > 99 (S) 72 (R) 65 (S) 93 (R) 98 (S) 93 (R) 98 (S) 92 (R) 93 (S) 93 (R) 98 (S) > 99 (R) > 99 (S) 96 (R) 96 (S) 94 (R)
19 20 21 22 23 24 25 26 27 28 29 30 31 32 33	1i 1j 1j 1k 1k 1l 11 1m 1m 1m 1n 1n 1o 1o 1p 1p	2-OMe 2-F 2-F 2-F 2-Pyridyl 3-Pyridyl 3-Pyridyl 2-Furyl 2-Furyl 3-Furyl 3-Furyl 2-Thienyl 2-Thienyl 3-Thienyl 3-Thienyl 3-Thienyl	E. coli/ADH-A E. coli/ADH-T E. coli/ADH-T E. coli/ADH-T E. coli/LBADH E. coli/ADH-A E. coli/ADH-A E. coli/ADH-T E. coli/ADH-T E. coli/LBADH E. coli/ADH-T E. coli/ADH-T E. coli/ADH-T E. coli/ADH-T E. coli/ADH-T E. coli/ADH-T E. coli/ADH-T	60 (49) 81 (65) 82 (66) 95 (87) 95 (83) 91 (76) 86 (70) 65 (48) 64 (48) 59 (42) 62 (44) 58 (47) 62 (49) 65 (51) 69 (52)	94 (S) 99 (R) 99 (S) 96 (R) 96 (S) 94 (R) 95 (S) 87 (R) 86 (S) 93 (R) 92 (S) 75 (R) 74 (S) 89 (R) 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.

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 TrisHCl 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% ν/ν), 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, over-expressed 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].

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].

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



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

designed using whole cell biocatalysts or by combining 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)arvl 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.

CRediT authorship contribution statement

Jesús Albarrán-Velo: Validation, Investigation, Writing - original draft. Vicente Gotor-Fernández: Conceptualization, Writing - review & editing, Supervision. Iván Lavandera: Conceptualization, Writing review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.mcat.2020.111087.

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