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Laccase-mediated oxidations of propargylic alcohols. Application in the deracemization of 1-arylprop-2-yn-1-ols in combination with alcohol dehydrogenases

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Dedicated to Professor Marko D. Mihovilovic on occasion of his 50th birthday.

Abstract: The catalytic system composed by the laccase from *Trametes versicolor* and the oxy-radical TEMPO has been successfully applied in the sustainable oxidation of fourteen propargylic alcohols. The corresponding propargylic ketones were obtained in most cases in quantitative conversions (87->99% yield), demonstrating the efficiency of the chemoenzymatic methodology in comparison with traditional chemical oxidants, which usually lead to problems associated with the formation of by-products. Also, the stereoselective reduction of propargylic ketones was studied using alcohol dehydrogenases such as the one from *Ralstonia* species overexpressed in *E. coli* or the commercially available evo-1.1.200, allowing the access to both alcohol enantiomers mostly with complete conversions (97->99% *ee*) and variable selectivities depending on the aromatic pattern substitution. To demonstrate the compatibility of the laccase-mediated oxidation and the alcohol dehydrogenase-catalyzed bioreduction, a deracemization strategy starting from the racemic compounds was developed through a sequential one-pot two-step process, obtaining a selection of (*S*)- or (*R*)-1-arylprop-2-yn-1-ols with excellent yields (>98%) and selectivities (>98% *ee*) depending on the alcohol dehydrogenase employed.

Introduction

The oxidation of propargylic alcohols is considered a highly sensitive transformation since the presence of the carbon-carbon triple bond and the alcohol functionality often leads to poor selectivities when employing conventional oxidizing reagents. Traditional oxidative strategies involve the use of metal species or complexes from cobalt,[1] copper,[2] chromium,[3] ruthenium[4]

vanadium,[5] or iron,[6] some of them in combination with *N*-oxide radicals such as *N*-hydroxyphthalimide[7] or TEMPO and derivatives,[8] and the use of stoichiometric oxidative systems such as the Dess-Martin reagent[9] or manganese oxide.[10] Therefore, the development of sustainable methodologies for the preparation of propargylic ketones under mild reaction conditions is nowadays an area worthy of investigation. Until now, the use of biocatalytic methods for the oxidation of propargylic alcohols remains unexplored, however the applicability of laccases for the previous success in the oxidation of activated substrates such as benzylic[11] and allylic[12] alcohols (Scheme 1a), encouraged us to explore the potential of the catalytic laccase mediator system to produce the corresponding propargylic ketones under mild reaction conditions (Scheme 1b).
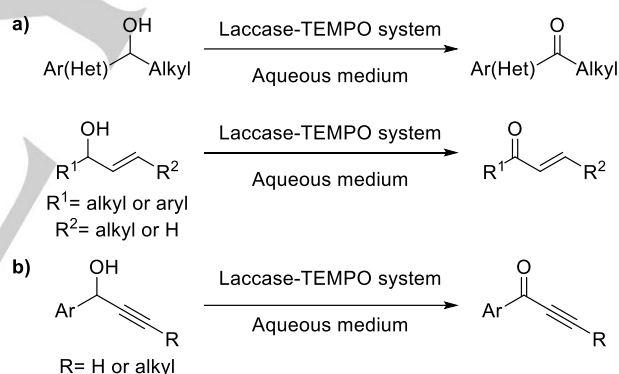
Scheme 1. Use of the laccase-TEMPO system for the: a) previous oxidation of benzylic and allylic alcohols; b) oxidation of propargylic alcohols (this study).

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Supporting information for this article is given via a link at the end of the document. This file includes comprehensive oxidation and bioreduction studies, EATOS calculations, and full characterization of synthesized alcohols and ketones including also their analytics and NMR spectra.

The synthesis of chiral propargylic alcohols represents also an attractive research area for organic chemists since this family of oxygenated compounds are present in numerous biologically active compounds including alkaloids, pheromones, prostaglandins or vitamins, among others, serving at the same time as valuable building blocks for the synthesis of more complex molecules.[13] In the search for sustainable and stereoselective synthesis of this class of compounds, biocatalytic methods have already demonstrated their versatility for the preparation of various families of propargylic alcohol derivatives mainly by using alcohol dehydrogenase (ADH)-catalyzed reductions,[14] lipase-catalyzed hydrolytic[15] and transesterification[16] reactions, or even deracemizations experiments using whole cell systems,[17] although there is a lack for a general methodology to provide these valuable compounds.

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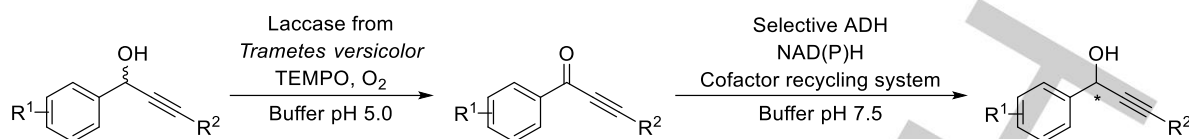
Scheme 2. Chemoenzymatic oxidation of propargylic alcohols via laccase-mediated non selective oxidation followed by stereoselective bioreduction of the propargylic ketone intermediates in order to develop a general strategy for the deracemization of racemic 1-arylprop-2-yn-1-ols.

Herein, we investigate first the design of a general strategy for the oxidation of racemic propargylic alcohols mediated by the laccase-TEMPO system[18] to later explore the bioreduction of the resulting propargylic ketones by alcohol dehydrogenase-catalyzed bioreductions. This will allow the development of deracemization[19] experiments combining both enzymatic methodologies to produce valuable enantiopure alcohols from their racemates in a one-pot two step-process (Scheme 2).

Results and Discussion

Oxidation of propargylic alcohols

To evaluate the suitability of the catalytic system composed by the laccase from *Trametes versicolor* (L*Tv*) and the oxy-radical TEMPO, 1-phenylprop-2-yn-1-ol (**1a**) was selected as model substrate due to its commercial accessibility at low cost. Based on our previous experience in the oxidation of benzylic and allylic alcohols,[11,12] the oxidation of **1a** (Table 1) was carried out in a 50 mM substrate concentration and 30 mol% of TEMPO using an oxygenated 50 mM citrate buffer at pH 5 and 10% v/v *tert*-butyl methyl ether (MTBE) as cosolvent in order to favor the alcohol solubility at 30 °C. Under these conditions, formation of 1-phenylprop-2-yn-1-one (**2a**) was accomplished in 91% yield (entry 1), being quantitative transformed after 48 h (entry 2).

Trying to reach complete conversions at shorter reaction times, different parameters were studied including the TEMPO loading, temperature, and the concentration of alcohol **1a** (entries 3-6). The use of a higher amount of TEMPO or the increase of the temperature to 40 °C led to a partial deactivation of the enzyme and lower recycling rate of the radical TEMPO, achieving then lower conversions (75-80%, entries 3 and 4). Interestingly, the use or more diluted conditions (25 mM of **1a**, entry 5), pleasingly allowed quantitative conversions into ketone **2a**, although conversion did not overcome 80% when reducing the TEMPO loading to 20%, and even after prolonged reaction times (entries 6 and 7).

Table 1. Chemoenzymatic oxidation of alcohol **1a** using the L*Tv*/TEMPO system.[a]

Entry	[**1a**] (mM)	TEMPO (mol%)	T (°C)	t (h)	c (%)[b]
1	50	30	30	16	91
2	50	30	30	48	>99
3	50	40	30	16	80
4	50	30	37	16	75
5	25	30	30	16	>99
6	25	20	30	16	72
7	25	20	30	48	79

[a] See a general procedure in the experimental section. [b] Conversion values were determined by GC analysis.

At this point the viability of this chemoenzymatic route was evaluated comparing these results (Table 1, entry 5) with those achieved when using different conventional chemical oxidizing agents such as manganese dioxide (MnO₂),[10] the Jones reagent or the Dess-Martin reagent[9] (Table 2).

Table 2. Oxidation of alcohol **1a** through chemical or chemoenzymatic strategies.[a]

Entry	Oxidizing agent	Reaction conditions	Yield (%)[b]
1	L*Tv*/TEMPO, O₂	Citrate buffer pH 5, 30 °C, 16 h	93
2	MnO₂	CH₂Cl₂, rt, 6 h	19
3	Jones reagent	Acetone, rt, 7 h	35
4	Dess-Martin reagent	CH₂Cl₂, rt, 4 h	86

[a] See the experimental section and the ESI file for detailed reaction conditions and procedures. [b] Isolated yields after purification.

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In all cases, several advantages in favor of the L*Tv*/TEMPO system were noticed, not only in the conversions but also in the selectivity of the reactions. For instance, the oxidation of **1a** with MnO$_2$ only yielded a 19% of the ketone (entry 2), while the use of a Cr(VI) species such as the Jones reagent led to a 35% of the product (entry 3), which can be explained due to the formation of multiple by-products, some of them caused by the reactivity of the carbon-carbon triple bond. Finally, the iodine reagent, popularly known as the Dess-Martin reagent, led to the ketone **2a** after 4 h of reaction with an 86% yield after purification by column chromatography (entry 4). These results reveal the great sensitivity of propargylic alcohols under typical oxidative conditions, highlighting the use of a chemoenzymatic approach based on two facts, first the complete conversion achieved and second the possibility to work in aqueous medium, which will facilitate the development of concurrent processes such as the alcohol dehydrogenase-catalyzed reduction of the so-obtained ketones.
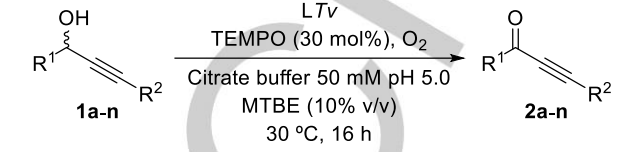
For these transformations, we performed a simple quantification of the *E*-factor[20] to have an estimation of the environmental impact of these methods. Hence, the EATOS tool[21] was used focusing on waste generated regarding the reaction and isolation auxiliaries (excluding solvents). As can be seen in the ESI (Figure S2), the values obtained clearly favored the chemoenzymatic approach regarding the chemical procedures (between 3.4 and 8.9-times), mainly due to the avoidance of a flash chromatography column.

Next, efforts were devoted to extending the laccase-mediated oxidation to other propargylic alcohols using the reaction conditions previously optimized for **1a**. With this purpose, 1-phenylbut-2-yn-1-ol (**1b**) and 1-arylprop-2-yn-1-ols **1c-m** were chemically synthesized in moderate to high yields (53-96%, see full structures in Figure S1 of the ESI), by reaction of the corresponding benzaldehydes with a slight excess of ethynylmagnesium bromide or prop-1-yn-1-ylmagnesium bromide in dry THF at room temperature. The selection of 11 different benzaldehydes allowed us to obtain the corresponding 1-arylprop-2-yn-1-ols **1c-m** with different pattern substitutions on the aromatic ring including electron donating and withdrawing groups to immediately study their oxidations using the L*Tv*/TEMPO system under previously optimized conditions (Table 3).

Regarding the chemoenzymatic oxidation, satisfyingly 1-phenylbut-2-yn-1-one (**2b**, R^2= Me, entry 2) was produced in quantitative conversion, so the methodology seems applicable for alcohols in the presence of a methyl substituent at the terminal position of the carbon-carbon triple bond. For 1-arylprop-2-yn-1-ols bearing monosubstitutions such as the methoxy moiety or a halogen atom on the aromatic ring, the position of these functionalities seemed to do not have a remarkable influence in the reactivity of the L*Tv*/TEMPO system, affording quantitative conversions in all cases when containing a methoxy group (entries 3-5), while when bearing a fluorine atom both the *para*- and *meta*-substituted ketones **2f** and **2g** were obtained in 98% conversion (entries 6 and 7). Also excellent results were found when considering the *p*-chloro or *o*-bromo derivatives **1h** and **1i** (entries 8 and 9), decreasing the conversion to a 93% when

disubstituted alcohol **1j** was used as starting material (entry 10). Finally, high reactivities were also observed when considering additional functional groups including the *p*-methyl, *p*-nitro or a bulkier naphthalene derivative (entries 11-13, 87-97%).

Table 3. Chemoenzymatic oxidation of racemic propargylic alcohols **1a-n** (50 mM) using the L*Tv*/TEMPO system.[a]

Entry	Alcohol **1a-n**	R^1	R^2	*c* **2a-n** (%)[b]
1	**1a**	Ph	H	>99
2	**1b**	Ph	Me	>99
3	**1c**	4-OMe-C$_6$H$_4$	H	>99
4	**1d**	3-OMe-C$_6$H$_4$	H	>99
5	**1e**	2-OMe-C$_6$H$_4$	H	>99
6	**1f**	4-F-C$_6$H$_4$	H	98
7	**1g**	3-F-C$_6$H$_4$	H	98
8	**1h**	4-Cl-C$_6$H$_4$	H	>99
9	**1i**	2-Br-C$_6$H$_4$	H	>99
10	**1j**	2-Br,5-F-C$_6$H$_3$	H	93
11	**1k**	4-Me-C$_6$H$_4$	H	97
12	**1l**	4-NO$_2$-C$_6$H$_4$	H	87
13	**1m**	2-C$_{10}$H$_7$	H	94
14	**1n**	PhCH$_2$	H	92

[a] See the experimental section for a general procedure. [b] Conversion values were determined by GC analysis.

To have more information about this synthetic oxidative strategy, an additional substrate 1-phenylbut-3-yn-ol (**1n**) was included in the study. This compound did not present a double activation as the hydroxyl group is not linked to a benzylic position. Interestingly a high conversion was also attained (92%, entry 14), showing that in general propargylic alcohols were excellent substrates for the L*Tv*/TEMPO catalytic system. Overall, the chemoenzymatic system resulted more effective than the oxidation using the Dess-Martin reagent (51-88%, see Table S1 in the ESI), obtaining conversions over 86% but in most cases quantitative, isolating the product through a simple liquid-liquid extraction.

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Stereoselective bioreduction of propargylic ketones

Once that the oxidation of propargylic alcohols **1a-n** into the corresponding ketones **2a-n** was properly addressed, stereoselective bioreductions catalyzed by alcohol dehydrogenases were investigated to produce optically active alcohols. 1-Phenylprop-2-yn-1-one (**2a**, 25 mM) was selected as model substrate and the behavior of a panel of ADHs acting with known opposite stereopreference were tested in bioreduction processes using standard conditions (Tris·HCl buffer 50 mM pH 7.5 in the presence of NAD(P)H (1 mM) with DMSO in 2.5% v/v due to the low solubility of these ketones in aqueous media, 30 °C, 24 h and 250 rpm, Scheme 3). Depending on the selected ADH, isopropanol or the combination of D-glucose and glucose dehydrogenase (GDH) were employed as reducing agents for the required recycling of the nicotinamide cofactor.
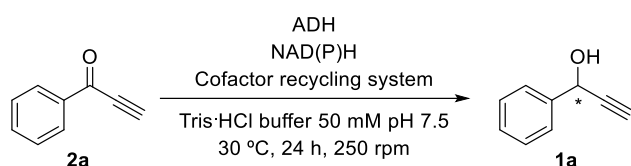
Scheme 3. Stereoselective bioreduction of 1-phenylprop-2-yn-1-one (**2a**, 25 mM) using ADHs.

As (*S*)-selective enzymes, lyophilized cells of *E. coli* overexpressing ADHs from *Ralstonia* species (RasADH),[22] *Sphingobium yanoikuyae* (SyADH),[23] *Thermoanaerobacter* species (ADH-T),[24] *Thermoanaerobacter ethanolicus* (TeSADH)[14b] and *Rhodococcus ruber* (ADH-A)[25] were considered, while ADHs from *Lactobacillus brevis* (LbADH),[26] and commercially available evo-1.1.200[27] were employed for the production of the (*R*)-alcohol. The results of this screening has been summarized in Table S2 of the ESI, finding the best results with RasADH, usually an enzyme that accepts sterically hindered substrates,[28] which gave access to (*S*)-**1a** in quantitative conversion and enantiopure form, while (*R*)-**1a** was obtained in 90% conversion and >99% *ee* using evo-1.1.200. On the basis of this preliminary screening, the bioreductions of ketones **2b-n** were rushed using the RasADH and evo-1.1.200 enzymes (Table 4).

Satisfyingly, in all cases excellent conversions were achieved (97->99% conversion). On the one hand, when using RasADH, (*S*)-1-

phenylbut-3-yn-ols (**1a**,**c-m**) were obtained in very high to excellent selectivities (93->99% *ee*, entries 1,3-13). The selectivity decreased when considering the alcohol 1b with a methyl moiety at the propargylic position (85% *ee*, entry 2) was obtained, and this enzyme did not act with any preference towards the alkyl derivative **2n** (entry 14). On the other hand, the (*R*)-alcohols were preferentially obtained with different degree of selectivity employing evo-1.1.200. In general, the alcohol optical purities ranged from high to excellent (88->99% *ee*), except when considering electron-donating groups as the methoxy at different positions of the aromatic ring (**1c**-**e**, 62-73% *ee*, entries 3-5), or sterically hindered *o*-brominated derivatives **1i** and **1j** (21-34% *ee*, entries 9 and 10) affording preferentially the (*S*)-enantiomers in these last two examples. Finally, the alcohol (*S*)-**1n** was obtained due to a switch in the Cahn-Ingold-Prelog (CIP) priority of the substituents.

Chemoenzymatic deracemization of propargylic alcohols

Based on the high activity displayed by the L*Tv*/TEMPO catalytic system and the stereoselectivity shown by complementary ADHs, the development of a one-pot two-step deracemization process of those racemic alcohols presenting the best reactivities and selectivities was investigated. So far, the design of biocatalytic deracemizations has been less explored in comparison with kinetic resolutions and desymmetrizations,[19] being just reported an example implying propargylic alcohols using a microorganism as whole cell system.[17] In an ideal deracemization approach, this methodology will allow the production of enantiopure alcohols from their racemic forms, although the different reaction conditions (*i.e.*, pH) required for the oxidation and bioreduction steps must be considered in the current case. Also, the occurrence of oxidative conditions obviously hampers the performance of the selective reduction. In addition, it has been described that the laccase-TEMPO system can oxidize mono- and disaccharides at the primary alcohol position, being not possible the use of the glucose and glucose dehydrogenase pair for cofactor recycling purposes.[12,29] For these reasons, the optimization of a one-pot sequential approach was considered from the beginning, again considering 1-phenylprop-2-yn-1-ol (**1a**) and 1-phenylprop-2-yn-1-one (**2a**) as model substrates for individual reaction optimizations.

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Table 4. Stereoselective bioreduction of propargylic ketones **2a-n** (25 mM) using RasADH and evo-1.1.200 at 30 °C for 24 h at 250 rpm.[a]

Entry	Ketone	R^1	R^2	RasADH		evo-1.1.200	
				c (%)[b]	ee (%)[c]	c (%)[b]	ee (%)[c]
1	**2a**	Ph	H	>99	>99	>99	>99
2	**2b**	Ph	Me	>99	85	>99	88
3	**2c**	4-OMe-C_6H_4	H	>99	>99	97	73
4	**2d**	3-OMe-C_6H_4	H	>99	>99	>99	62
5	**2e**	2-OMe-C_6H_4	H	>99	>99	>99	63
6	**2f**	4-F-C_6H_4	H	>99	95	>99	99
7	**2g**	3-F-C_6H_4	H	>99	99	>99	>99
8	**2h**	4-Cl-C_6H_4	H	>99	98	>99	99
9	**2i**	2-Br-C_6H_4	H	>99	>99	>99	34[d]
10	**2j**	2-Br,5-F-C_6H_3	H	>99	95	98	21[d]
11	**2k**	4-Me-C_6H_4	H	98	98	>99	>99
12	**2l**	4-NO$_2$-C_6H_4	H	>99	88	>99	>99
13	**2m**	2-$C_{10}H_7$	H	>99	97	>99	89
14	**2n**	PhCH$_2$	H	99	<3	>99	>99[e]

[a] See the experimental section for a general procedure. [b] Conversion values of the bioreduction processes were determined by GC analysis. [c] Enantiomeric excess of alcohols **1a-n** were determined by GC analysis, RasADH leading to the (*S*)-enantiomers and evo-1.1.200 to their (*R*)-antipodes. [d] The (*S*)-enantiomer was preferentially obtained. [e] The (*S*)-enantiomer was obtained due to a switch in the CIP rule priority.

At this point is necessary to overcome that each enzyme effectively works at different pH values (acidic for the laccase and slightly basic conditions for the ADH). To start, the use of 50 mM alcohol concentration is advisable for the laccase-mediated oxidation (Table 1, entry 4) while 25 mM is ideal for the bioreduction step (Table 4). This fact obviously must be also transferred to the use of different buffer compositions (citrate buffer for the oxidation and Tris·HCl for the reduction). For all these issues, the bioreductions of ketone **2a** using RasADH and evo-1.1.200 were next deeply studied (see Table S3 in the ESI). The reduction of **2a** was attempted using different substrate concentrations (25 and 50 mM), buffer compositions (Tris·HCl and citrate) and pH values (5.0, 6.5 and 7.5), observing in all cases 99% conversion. For RasADH in Tris·HCl buffer, the use of a higher ketone concentration led to a decrease in the selectivity (86% *ee* at 50 mM, >99% *ee* at 25 mM) and a similar effect was observed using the same concentration but a lower pH (89% *ee* at 25 mM and pH 6.5). Interestingly, very high selectivities were observed in the same aqueous medium employed in the laccase-

catalyzed reaction (96-97% *ee* at pH 5.0 in both 25 and 50 mM). Additionally, evo-1.1.200 displayed excellent selectivity at 25-50 mM and pH 6.5-7.5 (>98% *ee*), while moderate *ee* values were observed when using a citrate buffer pH 5.0 (79 and 68% *ee* at 25 and 50 mM, respectively).

The sequential approach was then addressed changing the ketone intermediate concentration and/or the pH of the reaction once the racemic alcohol **1a** was completely transformed into ketone **2a** after 16 h reaction at 30 °C in the citrate buffer 50 mM pH 5.0 with 10% v/v DMSO (Table 5). Firstly, at 25 mM substrate concentration, it was found that RasADH dramatically lost most of the activity, reaching only 27% conversion in the bioreduction process (entry 1), while evo-1.1.200 catalyzed the reaction almost to completion and with just a small loss of the selectivity (entry 2).

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Table 5. Deracemization of **1a** under different reaction conditions.[a]

rac-**1a** → 1) Chemoenzymatic oxidation 2) ADH-catalysed bioreduction → (S)- or (R)-**1a**

Entry	[**1a**] (mM)	Final pH	ADH	c (%)[b]	ee (%)[b]
1	25	5.0	Ras	27	n.d.
2	25	5.0	evo-1.1.200	98	93 (R)
3	50	6.1[c]	Ras	17	n.d.
4	50	6.1[c]	evo-1.1.200	88	94 (R)
5	25	8.0[d]	Ras	99	99 (S)
6	25	8.0[d]	evo-1.1.200	98	99 (R)
7	50	7.5[e]	Ras	99	99 (S)
8	50	7.5[e]	evo-1.1.200	94	97 (R)

[a] See the experimental section for detailed reaction conditions and a general procedure. [b] Conversion (regarding the ketone intermediate) and enantiomeric excess values of **1a** were determined by GC analysis. In parentheses the major alcohol enantiomer appears. [c] pH value obtained after the addition of Tris·HCl buffer pH 7.5 to obtain a 25 mM ketone concentration. [d] 30 μL of NaOH 3 M were added. [e] pH value obtained after the addition of Tris·HCl buffer pH 7.5 to obtain a 25 mM ketone concentration and additional 30 μL of NaOH 3 M. n.d.: Not determined.

Similar results were achieved when starting from 50 mM of **1a** and diluting the ketone intermediate up to 25 mM by addition of Tris·HCl buffer pH 7.5, thus increasing the pH value until 6.1 (entries 3 and 4). Remarkably excellent results with both ADHs were attained in terms of conversion and optical purities towards **1a** (25 mM, entries 5 and 6), when after the oxidation step a unique addition of 30 μL of a NaOH 3 M aqueous solution was achieved, elevating the pH until 8.1. Finally, successful experiments were also attained in the process at 50 mM substrate concentration and diluting in the middle stage with the Tris·HCl buffer pH 7.5 and extra addition of NaOH 3 M (entries 7 and 8). Once demonstrated the deracemization of racemic alcohol **1a** using complementary ADHs (Table 6, entries 1 and 2), and based on the excellent reactivity in the chemoenzymatic oxidation of racemic methoxy substituted alcohols **1c-e** and o-bromo-derivative **1i**, and the bioreduction of the corresponding ketones **2c-e,i** using RasADH as biocatalyst, the deracemization of alcohols **1c-e,i** was successfully developed, yielding with complete conversions and excellent selectivities the corresponding (S)-alcohols (entries 3-6). Finally, the deracemization of **1a** was scaled-up at 50 mg using RasADH or evo-1.1.200 affording both **1a** enantiomers in 98% ee and high isolated yields after column chromatography on silica gel, 83% for the (S)-alcohol and 79% for its (R)-antipode.

Table 6. Deracemization of **1a**,**c-e**,**i** using RasADH and evo-1.1200.[a]

Entry	Alcohol	ADH	c (%)[b]	ee (%)[b]
1	**1a** (H)	Ras	99	>99 (S)
2	**1a** (H)	evo-1.1.200	98	>99 (R)
3	**1c** (4-OMe)	Ras	>99	>99 (S)
4	**1d** (3-OMe)	Ras	>99	>99 (S)
5	**1e** (2-OMe)	Ras	>99	>99 (S)
6	**1i** (2-Br)	Ras	>99	99 (S)

[a] See the experimental section for detailed reaction conditions and a general procedure. [b] Conversion (regarding the ketone intermediate) and enantiomeric excess values of **1a**,**c-e**,**i** were determined by GC analysis.

Conclusions

A mild and chemoselective oxidation of propargylic alcohols has been described by the combined action of a catalytic system composed by the laccase from *Trametes versicolor* and the oxy-radical TEMPO, which was able to oxidize 14 racemic alcohols in very high to quantitative conversions (87->99%). This strategy has been compared with traditional oxidizing agents such as manganese(IV) oxide, Jones or Dess-Martin reagents, improving or achieving comparable results in terms of selectivity and yields. Remarkably, the laccase-mediated oxidation has been proved to be a sustainable oxidative method for the production of propargylic ketones as demonstrated by a simple quantification of the *E*-factor using the EATOS tool regarding other traditional oxidative methods. Moreover, bioreduction of the corresponding ketones was studied, screening different alcohol dehydrogenases commercially available or made in house and overexpressed in *E. coli*. The best results were found with the ADH from *Ralstonia* species and evo-1.1.200, which allowed the production of the (S)-1-arylprop-2-yn-1-ols (**1a-m**, >98% conv., >85% ee) and the (R)-alcohols (**1b,c,f-h,k-n**, >97% conv., >88% ee), respectively. Taking advantage of the development of both biotransformations in aqueous medium, we decided to explore the possibilities to carry out a sequential one-pot two-step deracemization protocol of some of these substrates. For this concurrent process, changing the pH was necessary from 5.0, the best one for the laccase action, to 7.5 where the ADH is able to display high activity. Hence, the deracemization of four 1-arylprop-2-yn-1-ols was successfully achieved to produce the corresponding optically active alcohols in excellent yields and selectivities, demonstrating the scalability of the process at semi-preparative scale.

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Experimental Section

Materials. Chemical reagents for the chemical synthesis of alcohols **1a-n** and ketones **2a-n** were purchased from Sigma-Aldrich. Laccase from *Trametes versicolor* (L*Tv*, 0.5 U/mg), glucose, NADH and NADPH were also acquired from Sigma-Aldrich, while glucose dehydrogenase (GDH-105) was obtained from Codexis Inc. to develop the biotransformations. For the chemoenzymatic oxidations, citrate buffer 50 mM pH 5.0 was previously oxygenated by bubbling molecular oxygen using a balloon for at least 30 minutes. Sequential reactions were performed in a microwave tube [(19 x 130 x 3) mm]. The oxidation step mediated by the laccase-TEMPO catalytic system was performed open-to-air, while for the bioreduction step, the microwave tube was closed. Lyophilized *E. coli*/RasADH cells were obtained as previously described in the bibliography,[28] and evo-1.1.200 was purchased from Evoxx technologies. NMR spectra (^{1}H, ^{13}C and ^{19}F NMR) were recorded on a Bruker AV300 MHz spectrometer. All chemical shifts (δ) are given in parts per million (ppm). Gas chromatography (GC) analyses were performed on an Agilent HP6890 GC chromatograph equipped with a FID detector. High-performance liquid chromatography (HPLC) analyses were carried out in in a Hewlett Packard 1100 chromatograph equipped with a VIS-UV detector. Thin-layer chromatography (TLC) was conducted with Merck Silica Gel 60 F254 precoated plates and visualized with UV, potassium permanganate and vanillin stain. Column chromatographies were performed using silica gel 60 (230-240 mesh). Melting points were taken on samples in open capillary tubes and are uncorrected. High resolution mass spectra (HRMS) experiments were carried out by ESI+ using a Micro Tof Q spectrometer.

General procedure for the chemical synthesis of racemic propargylic alcohols 1a-n. To a solution of the corresponding benzaldehyde (5 mmol) in dry THF (2.5 mL) at 0 °C and under argon atmosphere, a 0.5 M solution of ethynylmagnesium bromide or prop-1-yn-1-ylmagnesium bromide in dry THF (11 mL, 5.50 mmol) was added. The mixture was stirred at 0 °C for 4 h and after this time, the reaction was quenched by the addition of a saturated NH$_4$Cl aqueous solution (10 mL). The solvent was removed under vacuum, and the mixture extracted with CH$_2$Cl$_2$ (3 x 10 mL). The combined organic phases were washed with a saturated NaCl aqueous solution (2 x 10 mL), dried over Na$_2$SO$_4$, filtered and concentrated under vacuum. Reaction crudes were purified by column chromatography on silica gel, obtaining the corresponding racemic propargylic alcohols **1a-n** (53-96%). See the Electronic Supporting Information for details related to the full characterization of synthesized alcohols.

General procedure for the chemical oxidation of propargylic alcohols 1a-n with the Dess-Martin reagent. To a solution of propargylic alcohol **1a-n** (2.27 mmol) in CH$_2$Cl$_2$ (22.7 mL) placed in a round bottom-flask, the Dess-Martin reagent (1.4 g, 3.2 mmol) was added and the reaction stirred at room temperature. The reaction was followed by TLC analyses, observing the consumption of the starting material after 4 h. After this time, a 50% v/v aqueous solution of NaHCO$_3$/Na$_2$S$_2$O$_3$ was added, and then extracted with CH$_2$Cl$_2$ (3 x 15 mL). The organic phases were combined and dried over anhydrous Na$_2$SO$_4$, filtered and the solvent finally removed under vacuum. The reaction crude was purified by column chromatography on silica gel (30% EtOAc/Hexane), obtaining the corresponding propargylic ketones **2a-n** (51-88% yield). See the Electronic Supporting Information for details related to the full characterization of synthesized ketones.

General procedure for the chemoenzymatic oxidation of propargylic alcohols 1a-n using the catalytic system composed by the laccase from *Trametes versicolor* and the oxy-radical TEMPO. In a microwave tube open to the air, the corresponding racemic propargylic alcohol **1a-n** (0.05 mmol), MTBE (200 μL, 10% v/v) and a previously oxygenated citrate buffer 50 mM pH 5.0 (2 mL) were added. Next, the laccase from *Trametes versicolor* (10 mg, 5 U) and TEMPO (2.40 mg, 0.015 mmol) were added, and the mixture reacted for 16 h with magnetic stirring at 30 °C. After this time, the mixture was extracted with EtOAc (3 x 1 mL), the organic layers combined, dried over anhydrous Na$_2$SO$_4$ and filtered. The solution was finally concentrated, and the conversion values measured by GC analyses.

General procedure for the bioreduction of propargylic ketones 2a-n using RasADH. To a 1.5 mL-Eppendorf tube containing the corresponding propargylic ketone **2a-n** (0.015 mmol), DMSO (75 μL), a solution of GDH (60 μL, 10 U), an aqueous solution of D-glucose (60 μL, 500 mM), an aqueous solution of NADPH (60 μL, 10 mM), buffer Tris·HCl pH 7.5 (345 μL, 50 mM) and lyophilized cells of *E. coli* overexpressing RasADH (12 mg) were successively added. Then, the Eppendorf tube was closed and kept under orbital shaking at 250 rpm at 30 °C for 24 h. After this time, the solution was extracted with EtOAc (3 x 0.5 mL), the organic layers combined, dried over anhydrous Na$_2$SO$_4$ and filtered. The resulting solution was concentrated, measuring the conversion and the enantiomeric excess values of alcohols (*S*)-**1a-n** by GC analyses.

General procedure for the bioreduction of propargylic ketones 2a-n using evo-1.1.200. To a 1.5 mL-Eppendorf tube containing the corresponding propargylic ketone **2a-n** (0.0125 mmol), DMSO (56 μL), *i*PrOH (25 μL), an aqueous solution of MgCl$_2$ (50 μL, 10 mM), an aqueous solution of NADH (50 μL, 10 mM), buffer Tris·HCl pH 7.5 (319 μL, 50 mM) and evo-1.1.200 (12 mg) were successively added. Then, the Eppendorf tube was closed and kept under orbital shaking at 250 rpm at 30 °C for 24 h. After this time, the solution was extracted with EtOAc (3 x 0.5 mL), the organic layers combined, dried over anhydrous Na$_2$SO$_4$ and filtered. The resulting solution was concentrated, measuring the conversion and the enantiomeric excess values of alcohols (*R*)-**1a-n** by GC analyses.

General procedure for the deracemization of propargylic alcohols 1a,c-e,i using the laccase from *Trametes versicolor*, TEMPO and RasADH in analytical scale. To a solution of the corresponding racemic propargylic alcohol **1a**,**c-e,i** (0.05 mmol) in a previously oxygenated citrate buffer 50 mM pH 5.0 (2 mL) and MTBE (200 μL, 10% v/v) inside a microwave tube (19 x 130 x 3 mm) open to the air, the laccase from *Trametes versicolor* (10 mg, 5 U) and TEMPO (2.40 mg, 0.015 mmol) were added. The mixture reacted for 16 h with magnetic stirring at 30 °C. After this time, the following reagents were added: GDH (240 μL, 40 U), an aqueous solution of D-glucose (240 μL, 500 mM), an aqueous solution of NADPH (240 μL, 10 mM), an aqueous solution of NaOH 3 M (16.25 μL) to adjust the pH to a value between 7.5 and 8.0, and lyophilized cells of *E. coli* overexpressing RasADH (12 mg). The microwave tube was closed and orbitally shaken at 250 rpm and 30 °C for 24 h. After this time, the mixture was extracted with EtOAc (3 x 0.5 mL), combining the organic layers, which were dried over anhydrous Na$_2$SO$_4$ and filtered. Then, the solution was concentrated and the conversion and the enantiomeric excess values of the propargylic alcohols were measured by GC analyses.

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General procedure for the deracemization of propargylic alcohol 1a using the laccase from *Trametes versicolor*, TEMPO and evo-1.1-200 in analytical scale. To a solution of the corresponding racemic propargylic alcohol **1a** (0.05 mmol) in a previously oxygenated citrate buffer 50 mM pH 5.0 (2 mL) and MTBE (200 μL, 10% v/v) inside a microwave tube (19 x 130 x 3 mm) open to the air, the laccase from *Trametes versicolor* (10 mg, 5 U) and TEMPO (2.40 mg, 0.015 mmol) were added. The mixture reacted for 16 h with magnetic stirring at 30 °C. After this time, the following reagents were added: ^{i}PrOH (250 μL), an aqueous solution of MgCl$_2$ (250 μL, 10 mM), an aqueous solution of NADH (250 μL, 10 mM), an aqueous solution of NaOH 3 M (16.25 μL) to adjust the pH to a value between 7.5 and 8.0, and evo-1.1.200 (12 mg). Then, the tube was closed and kept under orbital shaking at 250 rpm at 30 °C for 24 h. After this time, the mixture was extracted with EtOAc (3 x 0.5 mL), combining the organic layers, which were dried over anhydrous Na$_2$SO$_4$ and filtered. Then, the solution was concentrated and the conversion and the enantiomeric excess values of **1a** were measured by GC analyses.

General procedure for the deracemization of 1-phenylprop-2-yn-1-ol (1a) using the laccase from *Trametes versicolor*, TEMPO and RasADH at semi-preparative scale. To a solution of racemic alcohol **1a** (0.35 mmol) in a previously oxygenated citrate buffer 50 mM pH 5.0 (14 mL) and MTBE (1.4 mL, 10% v/v) inside a microwave tube (19 x 130 x 3 mm) open to the air, the laccase from *Trametes versicolor* (48 mg, 24 U) and TEMPO (16.50 mg, 0.105 mmol) were added. The mixture reacted for 16 h with magnetic stirring at 30 °C. After this time, the following reagents were added: GDH (1.4 mL, 233 U), an aqueous solution of D-glucose (1.4 mL, 50 mM), an aqueous solution of NADPH (1.4 mL, 10 mM), an aqueous solution of NaOH 3 M (113.5 μL) to adjust the pH to a value between 7.5 and 8.0, and lyophilized cells of *E. coli* overexpressing RasADH (82 mg). The microwave tube was closed and orbitally shaken at 250 rpm and 30 °C for 24 h. After this time, the mixture was extracted with EtOAc (3 x 3 mL), combining the organic layers, which were dried over anhydrous Na$_2$SO$_4$ and filtered. Then, the solution was concentrated and the conversion and the enantiomeric excess values of (*S*)-**1a** were measured by GC analyses. The product was finally purified by column chromatography on silica gel (30% EtOAc/Hexane), obtaining the alcohol (*S*)-**1a** in 83% isolated yield and 98% *ee*.

General procedure for the deracemization of 1-phenylprop-2-yn-1-ol (1a) using the laccase from *Trametes versicolor*, TEMPO and evo-1.1.200 at semi-preparative scale. To a solution of the corresponding racemic propargylic alcohol **1a** (0.35 mmol) in a previously oxygenated citrate buffer 50 mM pH 5.0 (14 mL) and MTBE (1.4 mL, 10% v/v) inside a microwave tube (19 x 130 x 3 mm) open to the air, the laccase from *Trametes versicolor* (48 mg, 24 U) and TEMPO (16.50 mg, 0.105 mmol) were added. The mixture reacted for 16 h with magnetic stirring at 30 °C. After this time, the following reagents were added: ^{i}PrOH (788 μL), an aqueous solution of MgCl$_2$ (1.55 mL, 10 mM), an aqueous solution of NADH (1.55 mL, 10 mM), an aqueous solution of NaOH 3 M (113.5 μL) to adjust the pH to a value between 7.5 and 8.0, and evo-1.1.200 (80 mg). After this time, the mixture was extracted with EtOAc (3 x 3 mL), combining the organic layers, which were dried over anhydrous Na$_2$SO$_4$ and filtered. Then, the solution was concentrated and the conversion and the enantiomeric excess values of (*R*)-**1a** were measured by GC analyses. The product was finally purified by column chromatography on silica gel (30% EtOAc/Hexane), obtaining the alcohol (*R*)-**1a** in 79% isolated yield and 98% *ee*.

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A mild and general oxidation of propargylic alcohols has been described using the catalytic system composed by the laccase from *Trametes versicolor* and the oxy-radical TEMPO. Stereoselective bioreduction of the corresponding ynones using alcohol dehydrogenases has allowed the development of an efficient deracemization strategy towards the production of (*S*) and (*R*)-alcohols with excellent selectivity.
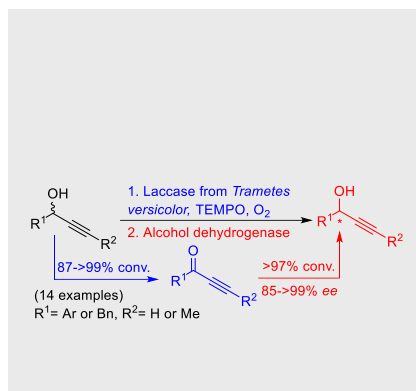
Sergio González-Granda,[a] Daniel Méndez-Sánchez, Iván Lavandera and Vicente Gotor-Fernández**

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Laccase-mediated oxidations of propargylic alcohols. Application in the deracemization of 1-arylprop-2-yn-1-ols in combination with alcohol dehydrogenases