Mild Chemoenzymatic Oxidation of Allylic *sec*-Alcohols. Application to Biocatalytic Stereoselective Redox Isomerizations

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Abstract. The design of catalytic oxidative methodologies in aqueous medium under mild reaction conditions and using molecular oxygen as final electron acceptor represents a suitable alternative to the traditional oxidative transformations. These methods are especially relevant if other functionalities that can be oxidized are present within the same molecule, as in the case of allylic alcohols. Herein we apply a simple chemoenzymatic system composed by the laccase from *Trametes versicolor* and 2,2,6,6-tetramethylpiperidinyloxy radical (TEMPO) to oxidize a series of racemic allylic *sec*-alcohols into the corresponding α,β-unsaturated ketones. Afterwards, these compounds react with different commercially

available ene-reductases to afford the corresponding saturated ketones. Remarkably, in the case of trisubstituted alkenes, the bioreduction reaction occurred with high stereoselectivity. Overall, a bienzymatic one-pot two-step sequential strategy has been described towards the synthesis of saturated ketones starting from racemic allylic alcohols, thus resembling the metal-catalyzed redox isomerizations of these derivatives that have been previously reported in the literature.

Keywords: Laccases/ Ene-reductases/ Oxidation/ Allylic alcohols/ Redox isomerization

Introduction

Oxidative transformations are one of the pivotal reactions in Organic Chemistry due to the synthetic relevance of the oxidized products (*e.g.*, carbonylic or carboxylic compounds) as reactive intermediates for subsequent modifications. Among the different approaches, the oxidation of alcohols into aldehydes or ketones is one of the most employed transformations on industry.[1] While traditionally these methods have been mediated by stoichiometric amounts of one or more oxidants in organic solvents, the tendency during the last years has been the development of catalytic oxidative methodologies[2] in aqueous medium[3] under mild reaction conditions and using, if possible, molecular oxygen as final electron acceptor.

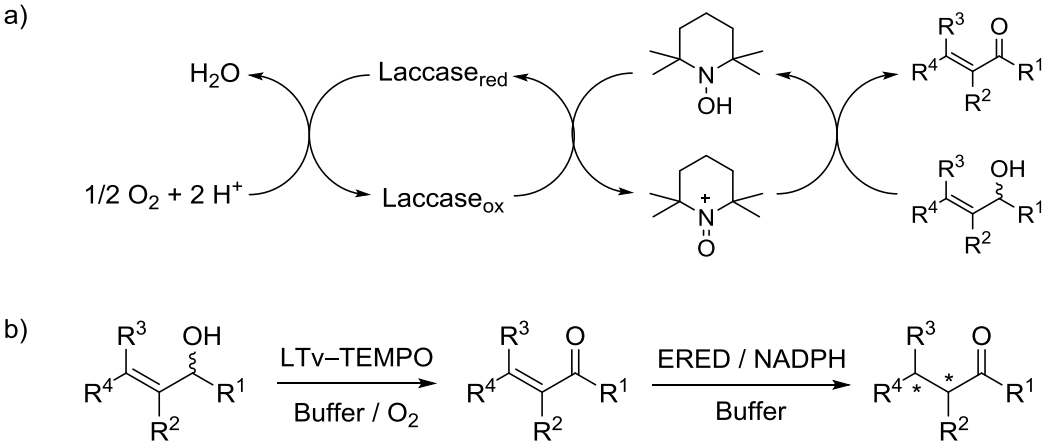
These protocols are especially appealing when several functionalities susceptible of being oxidized are present within the target molecule, as in the case of allylic alcohols, where the C=C double bond can also react depending on the reaction conditions. Thus, among the different catalytic methodologies recently reported, the use of chromium(III) complexes,[4] palladium nanoclusters,[5] gold nanoparticles,[6] platinum black,[7] silver exchanged molybdovanado phosphoric acid,[8] iron(III) chloride complexes,[9] palladium on silica,[10] iron-picolinate complexes,[11] potassium osmium(VI) oxide,[12] and palladium on alumina,[13] can be mentioned. However, these processes usually suffer from different drawbacks such as the formation of undesired by-products as a consequence of the presence of the metal or a peroxide co-

oxidant, and the use of high temperatures and organic solvents. In the last few years, stable radical species such as 2,2,6,6-tetramethylpiperidinyloxy (TEMPO) have appeared as interesting alternatives in combination with different metals to achieve aerobic oxidations.[14] In this context, several strategies have been disclosed for the oxidation of primary and secondary alcohols using TEMPO in combination with iron(III),[15] copper(I),[16] or [bis(acetoxy)iodo]benzene in the presence of bromide ions.[17]

Allylic alcohols can act as substrates in a redox (neutral) isomerization process to form saturated carbonylic derivatives.[18] This valuable reaction allows the synthesis of versatile compounds from readily accessible substrates. Among the different described methodologies, the use of transition metals is the most widely employed.[19] Thus, ruthenium,[20] rhodium,[21] iridium,[21b,22] or palladium[23] complexes have been successfully demonstrated as efficient catalysts for this reaction, usually working at high temperatures in organic solvents, leading in some cases to low chemoselectivities. Recently, efforts have been made to develop metal-free[24] transformations, or to use water as reaction medium.[25] In order to accomplish a stereoselective version of this reaction with secondary allylic alcohols, kinetic resolutions with metallic complexes[26] or stereospecific isomerizations of enantioenriched substrates to the corresponding β-substituted ketones have been described.[20c,24b] Very recently, Zhao *et al.* have reported the first example of a catalytic enantioselective isomerization of racemic secondary allylic alcohols for the synthesis of ketones with an α-tertiary stereocenter up to 90% *ee* using a rhodium catalyst.[27]

With these precedents in mind and based on our previous experience in the use of the laccase from *Trametes versicolor* (LTv)–TEMPO catalytic system to perform the oxidation of benzylic secondary alcohols,[28] we next turned our attention in the possibility of extending this simple and mild protocol to oxidize allylic secondary alcohols (Scheme 1a).[29] The exploitation of laccases, blue multicopper oxidases,[30] in combination with a chemical co-oxidant allows the design of oxidative transformations in aqueous media at the expense of the reduction of oxygen, releasing water as the only by-product. After the optimization of this oxidation, it was envisaged that the formed α,β-unsaturated ketones could act as suitable substrates for ene-reductases (EREDs),[31] enzymes capable of reducing C=C double bonds

conjugated to electron-withdrawing groups in a stereoselective fashion, yielding the corresponding saturated ketones. Thus, depending on the alkene substitution, enantioenriched α- or β-substituted carbonylic compounds can be obtained in a one-pot two-step strategy (Scheme 1b). This sequential route resembles the metal-catalyzed redox isomerization of allylic alcohols, and provides a biocatalytic alternative under very mild conditions. Up to now, very few enzymatic examples of this reaction have been proposed. Hence, Bruce *et al.*[32] and Hollmann *et al.*[33] have combined the use of an alcohol dehydrogenase (ADH) and an ERED. This redox-neutral approach is very straightforward, as it allows the internal recycling of the nicotinamide cofactor, necessary in both steps, thus not requiring an external sacrificial coenzyme regeneration system. On the contrary, they had to carefully deal with the undesired bioreduction of the final saturated ketone due to the presence of the ADH. Herein, this problem is overcome as the final product cannot act as substrate for the laccase–TEMPO system. On the other hand, Brenna and co-workers have very recently published a similar methodology to obtain a series of cyclic β-hydroxy esters from the corresponding cyclic derivatives with high yields and *ee* values *via* P450-mediated hydroxylation, followed by oxidation of the allylic alcohol catalyzed by the laccase from Amano M120 and the $TEMPO^{+}\cdot BF4^{-}$ salt, and finally an ERED-mediated bioreduction.[34]



Scheme 1. a) Laccase–TEMPO catalytic system applied to the oxidation of racemic secondary allylic alcohols. b) Biocatalytic (stereoselective) redox isomerization of racemic allylic *sec*-alcohols *via* sequential one-pot two-step strategy using the laccase–TEMPO system and an ene-reductase.

Results and Discussion

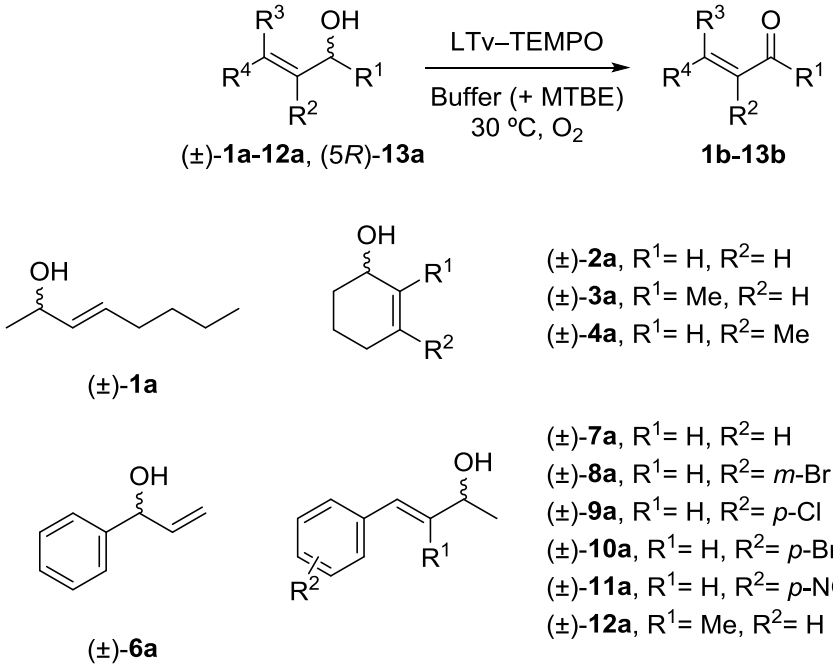
As a first step, the oxidation of allylic alcohols using LTv–TEMPO was subjected to study and optimization. We used commercially available racemic oct-3-en-2-ol [(±)-**1a**, Table 1] as model substrate, and based on our previous experience with this methodology,[28] some parameters were already considered as optimal [citrate buffer 50 mM pH 5 as buffer medium, TEMPO concentration (33 mol%), 30 ºC and magnetic stirring in an open-to-air vessel]. Other factors were optimized, as they were anticipated as key for the correct reaction outcome. Thus, substrate concentration and the presence of methyl *tert*-butyl ether (MTBE) as organic co-solvent were further investigated (see Supporting Information, Tables S1 and S2). Satisfyingly, it was observed that after 16 h (±)-**1a** could be quantitatively oxidized up to 50 mM substrate concentration into the α,β-unsaturated ketone **1b**. Likewise, it was determined that the use of MTBE and an oxygen-saturated buffer did not deter the chemoenzymatic activity, although their presence was not necessary to accomplish full conversion. Furthermore, under these mild reaction conditions, by-products coming from the alkene oxidation were not detected.

With these promising results in our hands, we decided to study the scope of this transformation. Therefore, a set of racemic allylic *sec*-alcohols [(±)-**1a**-**12a**, Table 1] were purchased or synthesized following simple described methodologies (see Supporting Information for more details).[35] Also, a natural compound such as carveol [(5*R*)-**13a**] was studied as suitable substrate.

Substrates were then treated under the conditions previously optimized for alcohol (±)-**1a**, obtaining a complete conversion towards the corresponding α,β-unsaturated ketones (40 mM) after 16 h at 30 ºC in an open-to-air vessel under magnetic stirring (Table 1). It is important to remark that by-products were not detected, and that both laccase and TEMPO were necessary to perform the oxidations. In the case of derivatives (±)-**8a**-**12a**, full conversions were not achieved working in buffer medium, as a consequence of the low solubility presented by these compounds (data not shown). Satisfyingly, quantitative

conversions were attained by the simple addition of MTBE (20% v/v), that helped to dissolve the starting material (entries 8-12). During the reaction progress, this organic solvent was eliminated *via* evaporation. At this point (5*R*)-carveol [(5*R*)-**13a**], which was present as a mixture of isomers *cis*:*trans* at a molar proportion 46:54, deserves special mention. When the reaction was performed in buffer under the standard conditions, it was observed that while the *cis* isomer was completely oxidized to carvone, 35% of the *trans* diastereoisomer still remained in the reaction mixture (entry 13). Therefore, the reoxidation of the crude for additional 16 h adding LTv and TEMPO was necessary in order to almost obtain complete conversion (entry 14). This result is in accordance with a previous study reported by Knochel and co-workers, which showed that TEMPO-catalyzed oxidations of substituted cyclohexanols are highly dependent on the axial-equatorial position of the alcohol moiety.[36]

Table 1. Oxidation of racemic secondary allylic alcohols (±)-**1a-12a** and (5*R*)-**13a** with the LTv–TEMPO system.[a]



Entry	Substrate	*t* (h)	MTBE (20% v/v)	*c* (%)[b]
1	(±)-**1a**	16	No	>99
2	(±)-**2a**	16	No	>99
3	(±)-**3a**	16	No	>99
4	(±)-**4a**	16	No	>99
5	(±)-**5a**	16	No	>99
6	(±)-**6a**	16	No	>99
7	(±)-**7a**	16	No	>99
8	(±)-**8a**	16	Yes	>99
9	(±)-**9a**	16	Yes	>99
10	(±)-**10a**	16	Yes	>99
11	(±)-**11a**	16	Yes	>99
12	(±)-**12a**	16	Yes	>99
13[c]	(5*R*)-**13a**	16	No	65[d]
14[e]	(5*R*)-**13a**	32	No	98

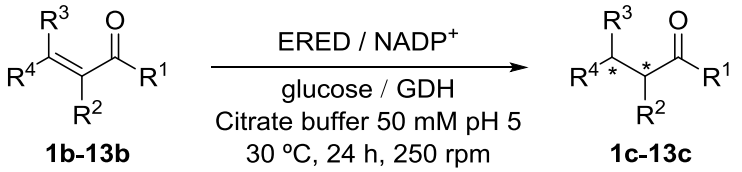
[a] For reaction conditions, see the Experimental Section. [b] Determined by GC (see Supporting Information). [c] Starting from a 46:54 *cis*:*trans* mixture, determined by ^{1}H-NMR. [d] The *cis* isomer completely reacted. [e] After 16 h, the reaction mixture was retreated with LTv–TEMPO for further 16 h.

Once developed a simple and mild method to oxidize racemic secondary allylic alcohols to the corresponding α,β-unsaturated ketones, next we envisioned the possibility of coupling this oxidative process with the (stereo- and) chemoselective ERED-catalyzed bioreduction of the C=C double bond to obtain saturated ketones (Scheme 1b). Overall, this sequential protocol would provide a biocatalytic alternative to the transition metal-catalyzed redox isomerizations of allylic alcohols. Furthermore, due to the excellent selectivity usually shown by these enzymes, the synthesis of α- and/or β-substituted derivatives in high *ee* could also be feasible.

Hence, a kit of seven commercially available EREDs from Codexis Inc. was used with ketones **1b**-**13b** (40 mM) under the optimal conditions described by the company to perform the C=C double bond bioreductions, using as medium phosphate buffer 100 mM pH 7. Glucose (80 mM) and glucose dehydrogenase (GDH, 20 U/mL) were utilized for the recycling of the nicotinamide cofactor ($NADP^+$, 0.6 mM) necessary in the bioreduction. The reactions were performed at 30 ºC for 24 h under orbital shaking (250 rpm). Satisfyingly, excellent conversions and very high selectivities were reached for all the tested substrates. Among them, ERED-103, ERED-P1-A04, and ERED-P1-E01 usually displayed the best results (for complete set of data, see Tables S3-S14 in the Supporting Information). Due to difficulties in the isolation of ketone **6b** after the laccase-catalyzed oxidations, we were unable to develop the bioreduction study.

Due to the fact that the laccase–TEMPO system works in citrate buffer 50 mM pH 5, ERED-catalyzed bioreductions were also studied under these reaction conditions, seeking for the possibility of developing both steps in a 'one-pot' fashion. Gladly, as can be seen in Table 2, excellent results were achieved in all cases. Even for substrate **3b** (entry 3), the reduction proceeded with higher selectivity than at pH 7, probably due to a slower racemization rate of the final product (*R*)-**3c**. Especially ERED-P1-E01 and ERED-P1-A04 showed excellent conversions and high selectivities for the majority of the tested substrates.

Table 2. ERED-catalyzed bioreductions of α,β-unsaturated ketones **1b**-**13b**.[a]



Entry	Substrate	ERED	*c* (%)[b]	*ee* or *de* (%)[c]
1	**1b**	110	>99	n.a.
2	**2b**	P1-A04	>99	n.a.
3	**3b**	P1-E01	>99	87 (*R*)[d]
4	**4b**	P1-E01	>99	>99 (*S*)
5	**5b**	P1-A04	>99	n.a.
6	**7b**	P1-E01	>99	n.a.
7	**8b**	103	>99	n.a.
8	**9b**	P1-A04	>99	n.a.
9	**10b**	P1-E01	>99	n.a.
10	**11b**	P1-E01	>99	n.a.
11	**12b**	P1-E01	>99	92 (*S*)
12	(5*R*)-**13b**	P1-E01	>99	90 (2*R*,5*R*)

[a] For reaction conditions, see the Experimental Section. [b] Determined by GC (see Supporting Information). [c] Determined by achiral or chiral GC or HPLC (see Supporting Information). [d] At pH 7 the enantiomeric excess of (*R*)-**3c** was 82%. n.a.: not applicable as these substrates are not chiral.

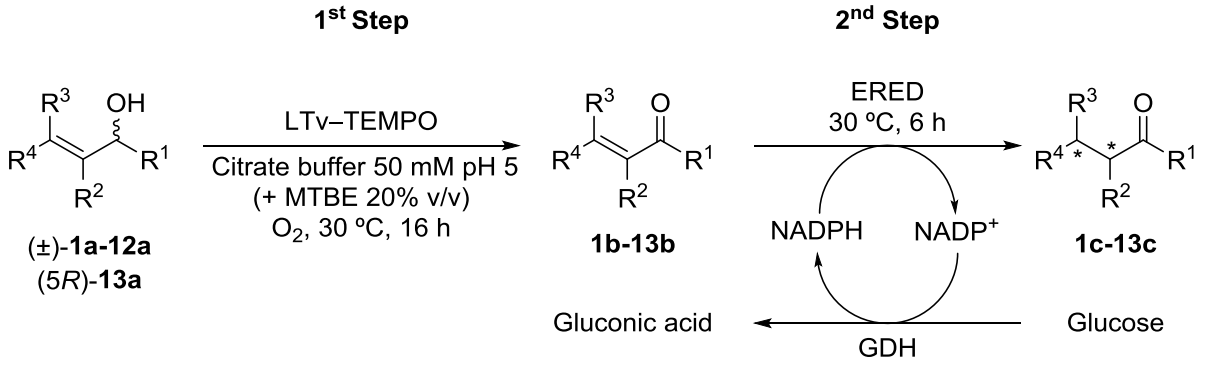
Once both steps were optimized separately, we attempted to perform them simultaneously in one-pot since both enzymes could work at the same pH. Hence, the ERED-catalyzed bioreductions were tried under magnetic stirring, as we have reported that the alcohol oxidations work better under these conditions.[28b] We studied the reduction of **1b** as model substrate with ERED-110 at pH 5, and satisfyingly it was observed that quantitative conversion into ketone **1c** was achieved after just 6 h (see Supporting Information). Therefore, encouraged by this promising result, we applied the whole cascade starting from allylic alcohol (±)-**1a** with all necessary reagents and catalysts (LTv–TEMPO, ERED-110, $NADP^+$, glucose and GDH) in citrate buffer 50 mM pH 5 in an open-to-air vessel (see Supporting Information). After 16 h we observed complete conversion into unsaturated ketone **1b**, but the final

product **1c** was not detected. Consequently, it was obvious that the oxidative step was interfering with the correct outcome of the bioreduction reaction.

In order to investigate this problem, the ERED-110-catalyzed reduction of **1b** was performed in an open vessel, compulsory for the oxidation reaction to avoid oxygen limitation. Under these conditions, only 41% of the saturated ketone **1c** was attained after 16 h. Although this effect clearly diminished the activity of the enzyme,[37] still some conversion was observed, unlike the two-step protocol. Therefore, another effect should deter the bioreduction process. In fact, when we repeated the cascade starting from (±)-**1a** in a closed tube, formation of **1c** was not observed, and the reaction stopped at the intermediate stage again. It was suspected that TEMPO could inhibit the reduction, therefore, a series of experiments were performed to study in more detail its effect (data not shown), observing that TEMPO was not compatible with the glucose–GDH system required for the NADPH recycling. It has been previously described that the laccase–TEMPO system can oxidize mono- and disaccharides at the primary position,[38] thus interfering with the GDH activity. Other NADP-recycling systems such as glucose-6-phosphate–glucose-6-phosphate dehydrogenase (G-6-P-DH) or isocitrate–isocitrate dehydrogenase (IDH) were also attempted, including the use of stoichiometric amounts of a cofactor mimic such as 1-benzyl-1,4-dihydronicotinamide,[39] but TEMPO hampered the bioreduction process as well.

Due to these incompatibilities, we decided to develop a one-pot two-step sequential strategy. To check whether we could directly add the reagents and catalysts of the second reaction right after the oxidation, a simple experiment was set-up. Racemic allylic alcohol (±)-**2a** was supplied after incubation of the laccase–TEMPO system for 16 h at 30 ºC, observing that after 6 h of reaction, ketone **2b** was only formed in 20% conversion. Compared to the standard reaction (99% conversion after 6 h), it remained clear that TEMPO lost most of its activity. Hence, after the oxidation of the racemic allylic alcohols with the LTv–TEMPO system, glucose, GDH, $NADP^+$, and the corresponding ERED were added to (stereo- and) chemoselectively reduce the C=C double bond.[40] The results obtained are shown in Table 3.

Table 3. Bienzymatic one-pot two-step sequential redox isomerization of racemic allylic alcohols.[a]



Entry	Substrate (mM)	ERED	Ratio[b]			*ee* or *de*
			1a-13a (%)	**1b-13b** (%)	**1c-13c** (%)	**1c-13c** (%)[c]
1	(±)-**1a** (40)	110	<1	<1	>99	n.a.
2	(±)-**2a** (40)	P1-A04	<1	<1	>99	n.a.
3	(±)-**3a** (40)	P1-E01	<1	<1	>99	88 (*R*)
4	(±)-**4a** (40)	P1-E01	<1	1	99	>99 (*S*)
5	(±)-**5a** (40)	P1-A04	<1	<1	>99	n.a.
6	(±)-**6a** (40)	P1-E01	<1	<1	>99	n.a.
7	(±)-**7a** (30)	P1-E01	<1	<1	>99	n.a.
8[d]	(±)-**8a** (30)	103	<1	<1	>99	n.a.
9[d]	(±)-**9a** (40)	P1-A04	<1	<1	>99	n.a.
10[d]	(±)-**10a** (30)	P1-E01	<1	1	99	n.a.
11[d]	(±)-**11a** (30)	P1-E01	<1	<1	>99	n.a.
12[d]	(±)-**12a** (30)	P1-E01	<1	<1	>99	92 (*S*)
13[e]	(5*R*)-**13a** (40)	110	2	11	87	95 (2*R*,5*R*)

[a] For reaction conditions, see the Experimental Section. [b] Determined by GC (see Supporting Information). [c] Determined by achiral or chiral GC or HPLC (see Supporting Information). [d] 20% v/v of MTBE was added to the reaction mixture in the first step to enhance substrate solubilization. [e] The oxidation step took 32 h, as described in Table 1. n.a.: not applicable as these substrates are not chiral.

Thus, substrates (±)-**1a**-**12a** and (5*R*)-**13a** (30-40 mM) were quantitatively oxidized to the corresponding α,β-unsaturated ketones after being treated with the laccase–TEMPO system during 16 h under the optimized conditions shown in Table 1 [except for alcohol (5*R*)-**13a**, 32 h]. Afterwards, the

bioreduction of these intermediates was achieved after 6 h following the conditions previously described in Table 2. For compounds (±)-**8a**-**12a**, as previously mentioned, MTBE (20% v/v) was necessary to ensure substrate solubilization. Satisfyingly, for most examples complete conversions into the saturated ketones were attained, detecting neither the starting material nor the unsaturated ketone intermediates. Moreover, chiral ketones **3c**, **4c**, **12c**, and **13c** were accessed with similar selectivities than those shown in Table 2, ranging from excellent for β-substituted ketone (*S*)-**4c** to very high for α-substituted ketones (*R*)-**3c**, (*S*)-**12c**, and (2*R*,5*R*)-**13c**. Globally, these examples can be considered as enzymatic stereoselective redox isomerizations of allylic alcohols. Compared to the methodology previously described combining an ADH and an ERED,[33] this system presents some advantages. In the ADH–ERED protocol, after reaction optimization, (±)-**2a** (10 mM) was transformed into ketone **2c** in good chemoselectivity (>90%), although a small quantity of the saturated alcohol was still detected. Herein substrate concentrations were higher and selectivities were perfect in all cases.

Finally, to demonstrate the applicability of this strategy, preparative biotransformations (50-mg scale) were carried out under the optimized reaction conditions for alcohols (±)-**4a** and (±)-**6a** (at 40 mM) and (±)-**12a** (at 30 mM). In these cases, longer reaction times were required for the bioreduction step (24 h) to achieve quantitative conversions into the final ketones, that were isolated in good to excellent yields and optical purities after extraction, (*S*)-**4c** (69% yield, >99% *ee*), **6c** (85% yield), and (*S*)-**12c** (87% yield, 91% *ee*).

Conclusions

The stereoselective redox isomerization of allylic alcohols into enantioenriched chiral ketones has been scarcely proposed *via* metal-catalyzed transformations. Herein we show a biocatalytic counterpart merging the action of two different oxidoreductases: a laccase from *Trametes versicolor*, used to recycle the chemical oxidant TEMPO which catalyzes the oxidation of a series of allylic alcohols into the corresponding α,β-unsaturated ketones; and an ene-reductase, enzyme responsible of the chemo- and

stereoselective reduction of the C=C double bond of these intermediates, giving access to the final saturated ketones.

The oxidative method is especially convenient as it can be performed at room temperature in aqueous medium and uses oxygen as final electron acceptor, releasing water as the sole coproduct. In addition, the bioreduction of the α,β-unsaturated ketones could be carried out under identical reaction conditions to those used in the biooxidation step, aiming for a one-pot process. However, incompatibilities between TEMPO and the cofactor recycling system used in the ERED-catalyzed transformation hampered the development of this process in a cascade manner, and consequently both enzymatic steps were combined in sequential one-pot two-step approach. Under these conditions, different allylic alcohols were quantitatively transformed into the corresponding saturated ketones, obtaining in some cases chiral carbonylic derivatives with high enantio- or diastereomeric excess (>88%), even at preparative scale.

Experimental Section

General methods

NMR spectra were recorded on a 300 MHz spectrometer. Gas chromatography (GC) analyses were performed on standard GC chromatographs equipped with a FID detector. Thin-layer chromatography (TLC) was conducted with silica gel precoated plates and visualized with UV and potassium permanganate stain. Column chromatography was performed using silica gel (230-400 mesh).

General protocol for the oxidation of allylic alcohols with the LTv–TEMPO system. In an open-to-air test tube, TEMPO (4.1 mg, 33 mol%) was added to a solution of the racemic allylic alcohol (±)-**1a**-**12a** or (5*R*)-**13a** (0.08 mmol, 40 mM) in citrate buffer 50 mM pH 5 (2 mL). For substrates (±)-**8a**-**12a**, MTBE (400 μL) was also added for solubility reasons. The reaction mixture was stirred for a few minutes to dissolve all the reagents and then the laccase from *Trametes versicolor* was added (5 U). The reaction was maintained under magnetic stirring at 30 ºC for 16 h. After this time, the reaction crude was extracted with EtOAc (2 x 2 mL). The organic layers were combined, dried over Na_2SO_4, and an

aliquot was taken for determination of conversion values by GC analysis (see Table 1). In the case of (5*R*)-**13a**, after the first 16 h, TEMPO (4.1 mg, 33 mol%) and laccase from *Trametes versicolor* (5 U) were added again. The reaction was stirred under the same conditions for additional 16 h and then was treated as explained above.

General procedure for the (stereoselective) reduction of α,β-unsaturated ketones catalyzed by EREDs. In a Falcon tube, a stock solution containing D-(+)-glucose (144 mg, 0.8 mmol), GDH-105 (20 mg), and $NADP^+$ (5 mg, 6 μmol) in 9 mL of phosphate buffer 100 mM pH 7 or citrate buffer 50 mM pH 5 was prepared. The resulting mixture was stirred for a few minutes, and then 1 mL was added in a 2-mL Eppendorf vial containing the corresponding ERED (10 mg). Then, substrate **1b**-**13b** (final concentration: 40 mM) was added. The reaction was shaken at 30 ºC and 250 rpm for 24 h, and then extracted with EtOAc (2 x 0.5 mL). The organic layers were combined and dried over Na_2SO_4. Conversions and enantiomeric excess were determined by GC or HPLC (see Table 2).

General procedure for the sequential one-pot two-step synthesis of (chiral) saturated ketones starting from racemic allylic alcohols. In an open-to-air test tube, TEMPO (4.1 mg, 33 mol%) was added to a solution of the racemic allylic alcohol (±)-**1a**-**12a** or (5*R*)-**13a** (0.08 mmol; 40 mM in the buffer medium for substrates (±)-**1a**-**6a**, (±)-**9a** and (5*R*)-**13a**; 30 mM in the buffer medium for substrates (±)-**7a**, (±)-**8a**, (±)-**10a**-**12a**) in citrate buffer 50 mM pH 5 (for substrates (±)-**1a**-**7a** and (5*R*)-**13a**), or in a biphasic mixture of citrate buffer 50 mM pH 5 and MTBE (20% v/v) (for substrates (±)-**8a**-**12a**). The reaction mixture was stirred for a few minutes to dissolve all the reagents, and then the laccase from *Trametes versicolor* (5 U) was added. The reaction was maintained under magnetic stirring for 16 h at 30 ºC, observing the complete evaporation of MTBE when it was used. To the resulting reaction crude, D-(+)-glucose (29 mg, 0.16 mmol), GDH-105 (20 U/mL), $NADP^+$ (0.6 mM), and the corresponding ERED (20 mg) were added. The test tube was closed and the reaction was maintained under magnetic stirring at 30 ºC for 6 h, and then extracted with EtOAc (2 x 2 mL). The organic layers were combined, dried over Na_2SO_4 and centrifuged to obtain the supernatant. Conversion (and

enantiomeric excess) values into the corresponding saturated ketones **1c**-**13c** were determined by GC or HPLC analysis (see Table 3).

Acknowledgments

Dedicated to Professor Miguel Yus in occasion on his 70th birthday.

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Supporting Information. The Supporting Information is available free of charge on the ACS Publications website at DOI: xxxxx.

Chemical synthesis and characterization of starting materials for enzymatic reaction, extensive ERED screenings, optimization of individual and multienzymatic protocols, analytics, copies of HPLC chiral analyses, and ^{1}H and ^{13}C NMR spectra for described organic compounds.

References

(1) Xu, C.; Zhang, C.; Li, H.; Zhao, X.; Song, L.; Li, X. An Overview of Selective Oxidation of Alcohols: Catalysts, Oxidants and Reaction Mechanisms. *Catal. Surv. Asia* **2016**, *20*, 13–22.

(2) Kopylovich, M. N.; Ribeiro, A. P. C.; Alegria, E. C. B. A.; Martins, N. M. R.; Martins, L. M. D. R. S.; Pombeiro, A. J. L. Catalytic Oxidation of Alcohols: Recent Advances. *Adv. Organometal. Chem.* **2015**, *63*, 91–174.

(3) Sheldon, R. A. Recent Advances in Green Catalytic Oxidations of Alcohols in Aqueous Media. *Catal. Today* **2015**, *247*, 4–13.

(4) Adam, W.; Hajra, S.; Herderich, M.; Saha-Möller, C. R. A Highly Chemoselective Oxidation of

Alcohols to Carbonyl Products with Iodosobenzene Diacetate Mediated by Chromium(III)(salen) Complexes: Synthetic and Mechanistic Aspects. *Org. Lett.* **2000**, *2*, 2773–2776.

(5) Choi, K.-M.; Akita, T.; Mizugaki, T.; Ebitani, K.; Kaneda, K. Highly Selective Oxidation of Allylic Alcohols Catalysed by Monodispersed 8-Shell Pd Nanoclusters in the Presence of Molecular Oxygen. *New J. Chem.* **2003**, *27*, 324–328.

(6) (a) Abad, A.; Almela, C.; Corma, A.; García, H. Unique Gold Chemoselectivity for the Aerobic Oxidation of Allylic Alcohols. *Chem. Commun.* **2006**, 3178–3180. (b) Abad, A.; Corma, A.; García, H. Supported Gold Nanoparticles for Aerobic, Solventless Oxidation of Allylic Alcohols. *Pure Appl. Chem.* **2007**, *79*, 1847–1854. (c) Ni, J.; Yu, W.-J.; He, L.; Sun, H.; Cao, Y.; He, H.-Y.; Fan, K.-N. A Green and Efficient Oxidation of Alcohols by Supported Gold Catalysts Using Aqueous H_2O_2 under Organic Solvent-Free Conditions. *Green Chem.* **2009**, *11*, 756–759.

(7) (a) Kon, Y.; Usui, Y.; Sato, K. Oxidation of Allylic Alcohols to α,β-Unsaturated Carbonyl Compounds with Aqueous Hydrogen Peroxide under Organic Solvent-Free Conditions. *Chem. Commun.* **2007**, 4399–4400. (b) Kon, Y.; Yazawa, H.; Usui, Y.; Sato, K. Chemoselective Oxidation of Alcohols by a H_2O_2–Pt Black System under Organic Solvent- and Halide-Free Conditions. *Chem. Asian J.* **2008**, *3*, 1642–1648.

(8) Nagaraju, P.; Balaraju, M.; Reddy, K. M.; Prasad, P. S. S.; Lingaiah, N. Selective Oxidation of Allylic Alcohols Catalyzed by Silver Exchanged Molybdovanado Phosphoric Acid Catalyst in the Presence of Molecular Oxygen. *Catal. Commun.* **2008**, *9*, 1389–1393.

(9) Join, B.; Möller, K.; Ziebart, C.; Schröder, K.; Gördes, D.; Thurow, K.; Spannenberg, A.; Junge, K.; Beller, M. Selective Iron-Catalyzed Oxidation of Benzylic and Allylic Alcohols. *Adv. Synth. Catal.* **2011**, *353*, 3023–3030.

(10) Parlett, C. M. A.; Bruce, D. W.; Hondow, N. S.; Newton, M. A.; Lee, A. F.; Wilson, K. Mesoporous Silicas as Versatile Supports to Tune the Palladium-Catalyzed Selective Aerobic Oxidation of Allylic Alcohols. *ChemCatChem* **2013**, *5*, 939–950.

(11) Tanaka, S.; Kon, Y.; Nakashima, T.; Sato, K. Chemoselective Hydrogen Peroxide Oxidation of Allylic and Benzylic Alcohols under Mild Reaction Conditions Catalyzed by Simple Iron-Picolinate Complexes. *RSC Adv.* **2014**, *4*, 37674–37678.

(12) Fernandes, R. A.; Bethi, V. An Expedient Osmium(VI)/$K_3Fe(CN)_6$-Mediated Selective Oxidation of Benzylic, Allylic and Propargylic Alcohols. *RSC Adv.* **2014**, *4*, 40561–40568.

(13) Parlett, C. M. A.; Durndell, L. J.; Wilson, K.; Bruce, D. W.; Hondow, N. S.; Lee, A. F. Selective Oxidation of Allylic Alcohols over Highly Ordered Pd/meso-Al_2O_3 Catalysts. *Catal. Commun.* **2014**, *44*, 40–45.

(14) Cao, Q.; Dornan, L. M.; Rogan, L.; Hughes, N. L.; Muldoon, M. J. Aerobic Oxidation Catalysis with Stable Radicals. *Chem. Commun.* **2014**, *50*, 4524–4543.

(15) (a) Ma, S.; Liu, J.; Li, S.; Chen, B.; Cheng, J.; Kuang, J.; Liu, Y.; Wan, B.; Wang, Y.; Ye, J.; Yu, Q.; Yuan, W.; Yu, S. Development of a General and Practical Iron Nitrate/TEMPO-Catalyzed Aerobic Oxidation of Alcohols to Aldehydes/Ketones: Catalysis with Table Salt. *Adv. Synth. Catal.* **2011**, *353*, 1005–1017. (b) Liu, J.; Ma, S. Iron-Catalyzed Aerobic Oxidation of Allylic Alcohols: The Issue of C=C Bond Isomerization. *Org. Lett.* **2013**, *15*, 5150–5153. (c) Zhang, G.; Li, S.; Lei, J.; Zhang, G.; Xie, X.; Ding, C.; Liu, R. An Efficient Biomimetic Aerobic Oxidation of Alcohols Catalyzed by Iron Combined with Amino Acids. *Synlett* **2016**, *27*, 956–960.

(16) Könning, D.; Hiller, W.; Christmann, M. One-Pot Oxidation/Isomerization of *Z*-Allylic Alcohols with Oxygen as Stoichiometric Oxidant. *Org. Lett.* **2012**, *14*, 5258–5261.

(17) Pia Salvo, A. M.; Campisciano, V.; Beejapur, H. A.; Giacalone, F.; Gruttadauria, M. A Simple Procedure for the Oxidation of Alcohols Using [Bis(acetoxy)iodo]benzene and a Catalytic Amount of Bromide Ions in Ethyl Acetate. *Synlett* **2015**, *26*, 1179–1184.

(18) (a) Cadierno, V.; Crochet, P.; Gimeno, J. Ruthenium-Catalyzed Isomerizations of Allylic and Propargylic Alcohols in Aqueous and Organic Media: Applications in Synthesis. *Synlett* **2008**, 1105–1124. (b) Ohkuma, T.; Arai, N. Advancement in Catalytic Asymmetric Hydrogenation of

Ketones and Imines, and Development of Asymmetric Isomerization of Allylic Alcohols. *Chem. Rec.* **2016**, *16*, 2801–2819. (c) Li, H.; Mazet, C. Iridium-Catalyzed Selective Isomerization of Primary Allylic Alcohols. *Acc. Chem. Res.* **2016**, *49*, 1232–1241.

(19) Mantilli, L.; Mazet, C. Platinum Metals in the Catalytic Asymmetric Isomerization of Allylic Alcohols. *Chem. Lett.* **2011**, *40*, 341–344.

(20) (a) Liu, P. N.; Ju, K. D.; Lau, C. P. Highly Efficient Redox Isomerization of Allylic Alcohols and Transfer Hydrogenation of Ketones and Aldehydes Catalyzed by Ruthenium Complexes. *Adv. Synth. Catal.* **2011**, *353*, 275–280. (b) García-Álvarez, R.; Suárez, F. J.; Díez, J.; Crochet, P.; Cadierno, V.; Antiñolo, A.; Fernández-Galán, R.; Carrillo-Hermosilla, F. Ruthenium(II) Arene Complexes with Asymmetrical Guanidinate Ligands: Synthesis, Characterization, and Application in the Base-Free Catalytic Isomerization of Allylic Alcohols. *Organometallics* **2012**, *31*, 8301–8311. (c) Bizet, V.; Pannecoucke, X.; Renaud, J.-L.; Cahard, D. Ruthenium-Catalyzed Redox Isomerization of Trifluoromethylated Allylic Alcohols: Mechanistic Evidence for an Enantiospecific Pathway. *Angew. Chem., Int. Ed.* **2012**, *51*, 6467–6470.

(21) (a) Sahoo, S.; Lundberg, H.; Edén, M.; Ahlsten, N.; Wan, W.; Zou, X.; Martín-Matute, B. Single Site Supported Cationic Rhodium(I) Complexes for the Selective Redox Isomerization of Allylic Alcohols. *ChemCatChem* **2012**, *2*, 243–250. (b) Titova, E. M.; Wahidur Rahaman, S. M.; Shubina, E. S.; Poli, R.; Belkova, N. V.; Manoury, E. Catalytic Redox Isomerization of Allylic Alcohols with Rhodium and Iridium Complexes with Ferrocene Phosphine-Thioether Ligands. *J. Mol. Catal. A: Chem.* **2017**, *426*, 376–380.

(22) Nelson, D. J.; Fernández-Salas, J. A.; Truscott, B. J.; Nolan, S. P. Iridium(I) Hydroxides in Catalysis: Rearrangement of Allylic Alcohols to Ketones. *Org. Biomol. Chem.* **2014**, *12*, 6672–6676.

(23) Larionov, E.; Lin, L.; Guénée, L.; Mazet, C. Scope and Mechanism in Palladium-Catalyzed Isomerizations of Highly Substituted Allylic, Homoallylic, and Alkenyl Alcohols. *J. Am. Chem.*

Soc. **2014**, *136*, 16882–16894.

(24) (a) Zheng, H.-X.; Xiao, Z.-F.; Yao, C.-Z.; Li, Q.-Q.; Ning, X.-S.; Kang, Y.-B.; Tang, Y. Transition-Metal-Free Self-Hydrogen-Transferring Allylic Isomerization. *Org. Lett.* **2015**, *17*, 6102–6105. (b) Martinez-Erro, S.; Sanz-Marco, A.; Bermejo Gómez, A.; Vázquez-Romero, A.; Ahlquist, M. S. G.; Martín-Matute, B. Base-Catalyzed Stereospecific Isomerization of Electron-Deficient Allylic Alcohols and Ethers through Ion-Pairing. *J. Am. Chem. Soc.* **2016**, *138*, 13408–13414.

(25) For a recent review, see: (a) Lorenzo-Luis, P.; Romerosa, A.; Serrano-Ruiz, M. Catalytic Isomerization of Allylic Alcohols in Water. *ACS Catal.* **2012**, *2*, 1079–1086. For some selected examples: (b) Ahlsten, N.; Lundberg, H.; Martín-Matute, B. Rhodium-Catalysed Isomerisation of Allylic Alcohols in Water at Ambient Temperature. *Green Chem.* **2010**, *12*, 1628–1633. (c) García-Álvarez, J.; Gimeno, J.; Suárez, F. J. Redox Isomerization of Allylic Alcohols into Carbonyl Compounds Catalyzed by the Ruthenium(IV) Complex [Ru(η^3:η^3-$C_{10}H_{16}$)Cl(κ^2*O*,*O*-CH_3CO_2)] in Water and Ionic Liquids: Highly Efficient Transformations and Catalyst Recycling. *Organometallics* **2011**, *30*, 2893–2896. (d) Díez, J.; Gimeno, J.; Lledós, A.; Suárez, F. J.; Vicent, C. Imidazole Based Ruthenium(IV) Complexes as Highly Efficient Bifunctional Catalysts for the Redox Isomerization of Allylic Alcohols in Aqueous Medium: Water as Cooperating Ligand. *ACS Catal.* **2012**, *2*, 2087–2099. (e) Serrano-Ruiz, M.; Lorenzo-Luis, P.; Romerosa, A.; Mena-Cruz, A. Catalytic Isomerization of Allylic Alcohols in Water by [RuClCp($PTA)_2$], [RuClCp($HPTA)_2$]$Cl_2\cdot 2H_2O$, [RuCp-(DMSO-κS)($PTA)_2$]Cl, [RuCp(DMSO-κS)($PTA)_2$](OSO_2CF_3) and [RuCp(DMSO-κS)($HPTA)_2$]$Cl_3\cdot 2H_2O$. *Dalton Trans.* **2013**, *42*, 7622–7630. (f) Horváth, H.; Kathó, A.; Udvardy, A.; Papp, G.; Szikszai, D.; Joó, F. New Water-Soluble Iridium(I)–N-Heterocyclic Carbene−Tertiary Phosphine Mixed-Ligand Complexes as Catalysts of Hydrogenation and Redox Isomerization. *Organometallics* **2014**, *33*, 6330–6340. (g) Erbing, E.; Vázquez-Romero, A.; Bermejo Gómez, A.; Platero-Prats, A. E.; Carson, F.; Zou, X.; Tolstoy, P.; Martín-Matute, B. General, Simple, and Chemoselective Catalysts for the Isomerization of Allylic Alcohols: The

Importance of the Halide Ligand. *Chem. - Eur. J.* **2016**, *22*, 15659–15663.

(26) Selected examples: (a) Ren, K.; Zhang, L.; Hu, B.; Zhao, M.; Tu, Y.; Xie, X.; Zhang, T. Y.; Zhang, Z. Cationic-Rhodium-Catalyzed Kinetic Resolution of Allylic Alcohols through a Redox Isomerization Reaction in a Noncoordinating Solvent. *ChemCatChem* **2013**, *5*, 1317–1320. (b) Ren, K.; Zhao, M.; Hu, B.; Lu, B.; Xie, X.; Ratovelomanana-Vidal, V.; Zhang, Z. An Enantioselective Approach to 4-*O*-Protected-2-cyclopentene-1,4-diol Derivatives via a Rhodium-Catalyzed Redox-Isomerization Reaction. *J. Org. Chem.* **2015**, *80*, 12572–12579.

(27) Liu, T.-L.; Ng, T. W.; Zhao, Y. Rhodium-Catalyzed Enantioselective Isomerization of Secondary Allylic Alcohols. *J. Am. Chem. Soc.* **2017**, *139*, 3643–3646.

(28) (a) Kędziora, K.; Díaz-Rodríguez, A.; Lavandera, I.; Gotor-Fernández, V.; Gotor, V. Laccase/TEMPO-Mediated System for the Thermodynamically Disfavored Oxidation of 2,2-Dihalo-1-phenylethanol Derivatives. *Green Chem.* **2014**, *16*, 2448–2453. (b) Martínez-Montero, L.; Gotor, V.; Gotor-Fernández, V.; Lavandera, I. Stereoselective Amination of Racemic *sec*-Alcohols through Sequential Application of Laccases and Transaminases. *Green Chem.* **2017**, *19*, 474–480.

(29) The oxidation of primary allylic alcohols mediated by laccases in combination with different mediators, including TEMPO, has been previously reported, although in some cases low selectivities were obtained ascribed to the undesired overoxidation of the aldehyde formed into the α,β-unsaturated carboxylic acid. For more information, see: (a) Fritz-Langhals, E.; Kunath, B. Synthesis of Aromatic Aldehydes by Laccase-Mediator Assisted Oxidation. *Tetrahedron Lett.* **1998**, *39*, 5955–5956. (b) Niku-Paavola, M.-L.; Viikari, L. Enzymatic Oxidation of Alkenes. *J. Mol. Catal. B: Enzym.* **2000**, *10*, 435–444. (c) Fabbrini, M.; Galli, C.; Gentili, P.; Macchitella, D. An Oxidation of Alcohols by Oxygen with the Enzyme Laccase and Mediation by TEMPO. *Tetrahedron Lett.* **2001**, *42*, 7551–7553. (d) Zhu, C.; Zhang, Z.; Ding, W.; Xie, J.; Chen, Y.; Wu, J.; Chen, X.; Ying, H. A Mild and Highly Efficient Laccase-Mediator System for Aerobic Oxidation of Alcohols. *Green Chem.* **2014**, *16*, 1131–1138. (e) Rouhani, S.; Rostami, A.; Salimi, A. Preparation

and Characterization of Laccases Immobilized on Magnetic Nanoparticles and Their Application as a Recyclable Nanobiocatalyst for the Aerobic Oxidation of Alcohols in the Presence of TEMPO. *RSC Adv.* **2016**, *6*, 26709–26718.

(30) (a) Riva, S. Laccases: Blue Enzymes for Green Chemistry. *Trends Biotechnol.* **2006**, *24*, 219–226. (b) Witayakran, S.; Ragauskas, A. J. Synthetic Applications of Laccase in Green Chemistry. *Adv. Synth. Catal.* **2009**, *351*, 1187–1209. (c) Mogharabi, M.; Faramarzi, M. A. Laccase and Laccase-Mediated Systems in the Synthesis of Organic Compounds. *Adv. Synth. Catal.* **2014**, *356*, 897–927.

(31) (a) Oberdorfer, G.; Gruber, K.; Faber, K.; Hall, M. Stereocontrol Strategies in the Asymmetric Bioreduction of Alkenes. *Synlett* **2012**, *23*, 1857–1864. (b) Winkler, C. K.; Tasnádi, G.; Clay, D.; Hall, M.; Faber, K. Asymmetric Bioreduction of Activated Alkenes to Industrially Relevant Optically Active Compounds. *J. Biotechnol.* **2012**, *162*, 381–389. (c) Toogood, H. S.; Scrutton, N. S. New Developments in 'Ene'-Reductase Catalysed Biological Hydrogenations. *Curr. Opin. Chem. Biol.* **2014**, *19*, 107–115.

(32) Boonstra, B.; Rathbone, D. A.; French, C. E.; Walker, E. H.; Bruce, N. C. Cofactor Regeneration by a Soluble Pyridine Nucleotide Transhydrogenase for Biological Production of Hydromorphone. *Appl. Environ. Microbiol.* **2000**, *66*, 5161–5166.

(33) Gargiulo, S.; Opperman, D. J.; Hanefeld, U.; Arends, I. W. C. E.; Hollmann, F. A Biocatalytic Redox Isomerisation. *Chem. Commun.* **2012**, *48*, 6630–6632.

(34) Brenna, E.; Crotti, M.; Gatti, F. G.; Monti, D.; Parmeggiani, F.; Pugliese, A.; Tentori, F. Biocatalytic Synthesis of Chiral Cyclic γ-Oxoesters by Sequential C–H Hydroxylation, Alcohol Oxidation and Alkene Reduction. *Green Chem.* **2017**, *19*, 5122–5130.

(35) (a) Gemal, A. L.; Luche, J. L. Lanthanoids in Organic Synthesis. 6. The Reduction of α-Enones by Sodium Borohydride in the Presence of Lanthanoid Chlorides: Synthetic and Mechanistic Aspects. *J. Am. Chem. Soc.* **1981**, *103*, 5454–5459. (b) Zumbansen, K.; Döhring, A.; List. B. Morpholinium

Trifluoroacetate-Catalyzed Aldol Condensation of Acetone with both Aromatic and Aliphatic Aldehydes. *Adv. Synth. Catal.* **2010**, *352*, 1135–1138. (c) Shi, S.-L.; Wong, Z. L.; Buchwald, S. L. Copper-Catalysed Enantioselective Stereodivergent Synthesis of Amino Alcohols. *Nature* **2016**, *532*, 353–356.

(36) Ragagnin, G.; Betzemeier, B.; Quici, S.; Knochel, P. Copper-Catalysed Aerobic Oxidation of Alcohols Using Fluorous Biphasic Catalysis. *Tetrahedron* **2002**, *58*, 3985–3991.

(37) It has been described that EREDs can be unstable to the presence of oxygen, often requiring the use of deoxygenated aqueous media. See, for instance: Stuermer, R.; Hauer, B.; Hall, M.; Faber, K. Asymmetric Bioreduction of Activated C=C Bonds Using Enoate Reductases from the Old Yellow Enzyme Family. *Curr. Opin. Chem. Biol.* **2007**, *11*, 203–213.

(38) Marzorati, M.; Danieli, B.; Haltrich, D.; Riva, S. Selective Laccase-Mediated Oxidation of Sugars Derivatives. *Green Chem.* **2005**, *7*, 310–315.

(39) (a) Paul, C. E.; Gargiulo, S.; Opperman, D. J.; Lavandera, I.; Gotor-Fernández, V.; Gotor, V.; Taglieber, A.; Arends, I. W. C. E.; Hollmann, F. Mimicking Nature: Synthetic Nicotinamide Cofactors for C=C Bioreduction Using Enoate Reductases. *Org. Lett.* **2013**, *15*, 180–183. (b) Paul, C. E.; Hollmann, F. A Survey of Synthetic Nicotinamide Cofactors in Enzymatic Processes. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 4773–4778.

(40) In the contribution recently published by Brenna and co-workers (reference 34), the authors reported the incompatibility between the oxidation and the bioreduction steps. Thus, it was required the addition of isopropanol and the bubbling of nitrogen of the reaction medium before the ERED, glucose, and GDH were supplied. In our case this was not necessary.

SYNOPSIS TOC