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Laccase/TEMPO-mediated system for the thermodynamically disfavored oxidation of 2,2-dihalo-1-phenylethanol derivatives†

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An efficient methodology to oxidize β,β-dihalogenated secondary alcohols employing oxygen was achieved in a biphasic medium using the laccase from *Trametes versicolor*/TEMPO pair, providing the corresponding ketones in a clean fashion under very mild conditions. Moreover, a chemoenzymatic protocol has been applied successfully to deracemize 2,2-dichloro-1-phenylethanol combining this oxidation with an alcohol dehydrogenase-catalyzed bioreduction.

Oxidation of alcohols into the corresponding carbonylic compounds is one of the fundamental reactions in organic chemistry. Traditionally these transformations comprised the use of hazardous metal-based reagents in stoichiometric amounts, however catalytic methodologies employing oxygen (or air) as a mild oxidant in aqueous media are being recognized for large scale applications, and therefore they are currently emerging as potent competitors.[1] From an economic and environmental point of view, these strategies are highly appealing since they produce water as the only by-product.

In this context, biological catalysts are gaining more relevance applied to oxidative processes, especially due to the mild conditions and high selectivities displayed in these transformations.[2] Among the different types of enzymes implicated in these reactions, oxidases have appeared as an interesting class since they use molecular oxygen as the electron acceptor.[3] In particular laccases are multicopper biocatalysts present in many fungi, plants and bacteria, and are responsible for the reduction of O_2 into H_2O at the expense of the substrate oxidation.[3a,4] Additionally, they are accessible and cheap compared to other reported metal complexes. Apart from that, laccases work efficiently in water as the natural medium, although they can also accept organic co-solvents,[5] which can be highly desirable when dealing with hydrophobic derivatives. Phenolic compounds are their natural substrates, but laccases have shown to be also effective towards primary alcohols or amines making use of, *e.g.* ABTS, benzotriazole, or syringaldehyde, among others.[6] One of the most employed is (2,2,6,6-tetramethylpiperidin-1-yl)oxy (TEMPO) due to its accessibility and high compatibility with this class of enzyme. Recently, the laccase/TEMPO catalytic system has been reported as a potent alternative to oxidize chemoselectively benzylic and primary alcohols over secondary ones.[7]

Especially challenging is the oxidation of alcohols substituted at the β-position with electron-withdrawing groups (EWG), *e.g.* halohydrins. To date, Dess–Martin periodinane,[8] ruthenium[9] and iridium-based[10] catalysts, Swern,[11] tetrapropylammonium perruthenate (TPAP),[11,12] and Jones' reagent[13] have been reported for the oxidation of this type of substrates. Owing to their high reactivity, α,α-dihalogenated ketones are versatile building blocks for the synthesis of, among others, mandelic acid,[14] oxazines,[15] triazines,[16] α,β-unsaturated ketones,[17] and triazole derivatives.[18] In the course of our investigations on biocatalyzed redox processes, it was observed that the oxidation of halohydrins was hardly feasible under alcohol dehydrogenase (ADH)-catalyzed hydrogen transfer conditions.[19] Following this study and based on our previous experience with the selective oxidation of alcohols using *Trametes versicolor* laccase and TEMPO,[7a] we have explored this catalytic system in the disfavored oxidation of secondary alcohols **1a–i**.

Since the laccase/mediator system is able to oxidize primary and secondary benzylic alcohols,[7b,d,20] we reasoned that laccase/TEMPO could also lead to carbonylic compounds bearing electron-withdrawing groups at the α-position under aerobic conditions. Therefore, several 2,2-dihalogenated 1-phenylethanol derivatives (**1a–h**) and 2-chloro-1-phenylethanol **1i** (Scheme 1) were synthesized, and these compounds were tested as possible substrates.

In a first set of experiments, the influence of the oxygenation was investigated.[21] Thus, the oxidation of 2,2-dichloro-1-phenylethanol (**1a**, 0.1 mmol, 50 mM) as a model substrate

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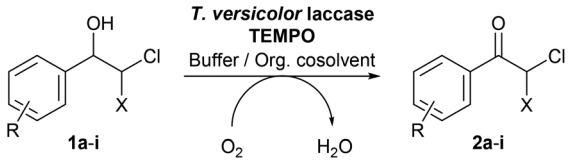


a, R= H, X= Cl; **b**, R= *o*-Cl, X= Cl; **c**, R= *m*-Cl, X= Cl
d, R= *p*-Cl, X= Cl; **e**, R= *m*-OMe, X= Cl; **f**, R= *m*-NO_2, X= Cl
g, R= H, X= Br; **h**, R= H, X= F; **i**, R= H, X= H

Scheme 1 General methodology to synthesize α,α-dihalogenated acetophenone derivatives **2a–h** and 2-chloroacetophenone (**2i**) through oxidation reactions with the laccase/TEMPO system.

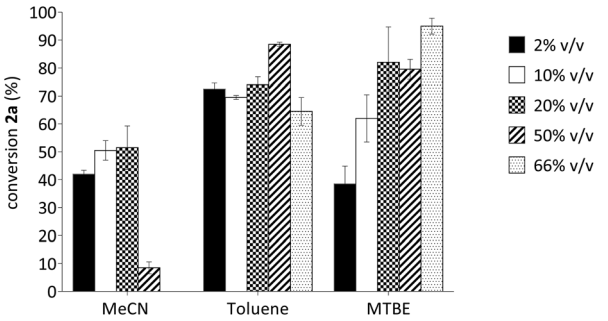


Fig. 1 Effect of the organic co-solvent concentration in the *T. versicolor* laccase/TEMPO (20 mol%) system to oxidize alcohol **1a** (50 mM) into **2a**, bubbling oxygen in a 50 mM NaOAc buffer pH 4.5 at 20 °C (toluene and MTBE) or 30 °C (MeCN) after 24 h.

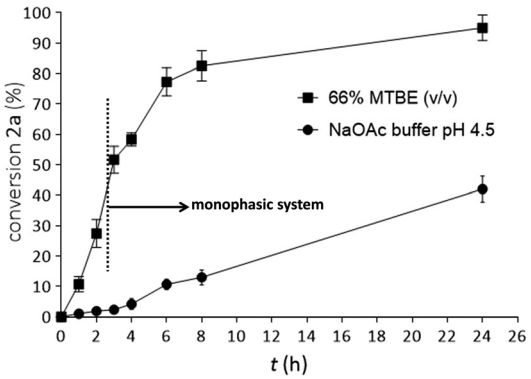


Fig. 2 Reaction course of the *T. versicolor* laccase/TEMPO (20 mol%) system to oxidize alcohol **1a** (50 mM) into **2a**, bubbling oxygen in a 50 mM NaOAc buffer pH 4.5 at 20 °C in the presence of MTBE (66% v/v). After 2–3 h, the disappearance of the organic solvent was observed. For comparison, the reaction in plain buffer was also studied.

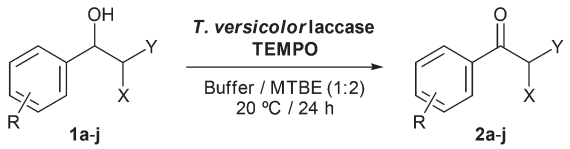
was performed in a 50 mM sodium acetate (NaOAc) buffer pH 4.5 at 30 °C and 20 mol% of TEMPO under two different conditions: (a) opening the reaction mixture to ambient air; and (b) bubbling oxygen in the vessel with a balloon. Remarkably, while for the first case a conversion of 33% was attained after 24 h, the second protocol led to 53% of ketone **2a**. The reactions were carried out using 10% v/v of acetonitrile (MeCN) as a result of the low solubility of **1a**. Although the conversions still were not quantitative, we observed that this method was able to afford smoothly the final compound, avoiding the formation of undesired by-products. Therefore, the reaction conditions of bubbling oxygen were chosen for further optimization. Other parameters such as temperature (20–40 °C), TEMPO equivalents (20–40 mol%), or pH (3.5–6.0) were also examined, without finding significant improvements (see ESI†).

Next, we decided to study the effect of the organic co-solvent by adding water miscible and non-miscible co-solvents, giving rise to mono- or biphasic systems. The addition of organic co-solvents can improve the solubility of hydrophobic substrates and the stability of this catalytic method, as observed for similar transformations.[5,22] To minimize mass transfer issues, a gentle magnetic stirring was performed in all cases. Thus, dimethylsulfoxide (DMSO) and MeCN were studied as additives in monophasic mixtures while dichloromethane, toluene and methyl *tert*-butyl ether (MTBE) were added to form biphasic systems (see ESI†). Among them, MeCN, toluene and MTBE afforded the best oxidation conversions (56–70%), so further studies were developed with these organic solvents.

Hence, the effect of the concentration of these organic co-solvents was studied with respect to the NaOAc buffer (2–66% v/v, Fig. 1). Due to the low boiling point of MTBE, the reactions with this solvent were carried out at 20 °C, and therefore, this was also the temperature of choice for the other non-miscible solvent (toluene), while the oxidations with water-miscible MeCN were performed at 30 °C. In this case, it remained clear that percentages higher than 20% v/v inhibited the laccase/TEMPO system while non-miscible solvents could be employed in larger amounts. Especially with MTBE, excellent conversions were achieved after 24 h (95%), showing that the addition of this organic co-solvent displayed a positive influence on the oxidation of **1a**. In addition, following the guidelines of greener alternatives for chlorinated or diethyl ether solvents, MTBE appeared as one of the best options for an immiscible organic co-solvent.[23] We also studied the use of a lower amount of TEMPO equivalents (5–10 mol%), but conversions were incomplete (51–85%).

The continuous oxygen bubbling process evaporated the organic solvent after a short period of time (2–3 h). Overall, it can be considered that this oxidation was accomplished at the end in an aqueous medium. To investigate the possible effect of MTBE in the reaction, we followed the oxidation of **1a** with time (Fig. 2), with and without the presence of MTBE. While a conversion of nearly 50% into **2a** was achieved in the first 3 h for the biphasic system, less than 10% conversion was detected for the solely aqueous system.[24] The positive effect of MTBE seems to be more related to an improvement of the substrate solubility than to the TEMPO stability, as conversions in plain buffer were continuously increasing at comparable rates after 4 h. Similar positive properties have already been described for other water–organic media employing a laccase with a chemical mediator.[22]

Table 1 Oxidation of secondary alcohols **1a–j** using the laccase/TEMPO system[a]



Entry	Alcohol	R	X	Y	**2a–j**[b] (%)
1	**1a**	H	Cl	Cl	95 ± 2.8
2	**1b**	*o*-Cl	Cl	Cl	4 ± 1.4
3	**1c**	*m*-Cl	Cl	Cl	78 ± 3.5
4	**1d**	*p*-Cl	Cl	Cl	91 ± 2.1
5	**1e**	*m*-OMe	Cl	Cl	82 ± 4.2
6	**1f**	*m*-NO_2	Cl	Cl	95 ± 4.2
7	**1g**	H	Cl	Br	89 ± 5.7
8	**1h**	H	Cl	F	75 ± 2.1
9	**1i**	H	Cl	H	62 ± 3.2
10	**1j**	H	H	H	78 ± 3.5

[a] Reaction conditions: alcohol **1a–j** (0.1 mmol), *T. versicolor* laccase (31 U) and TEMPO (20 mol%) in a 50 mM NaOAc buffer pH 4.5 and MTBE (66% v/v) bubbling oxygen at 20 °C for 24 h. [b] Conversion values measured by GC.

Having found appropriate conditions to oxidize alcohol **1a** (Table 1, entry 1), the scope of this transformation was expanded to other dihalogenated derivatives **1b–h**. The system efficiency depended on the position of the phenyl ring substitution, observing high to excellent conversions for *meta*- or *para*-substituted compounds, while a very low formation of ketone **2b** was found for the *ortho*-derived alcohol **1b** (compare entries 2 to 4). On the other side, alcohols with differing electronic properties such as chlorine, methoxy or nitro were oxidized (entries 3–6). Additionally, substrates with other halogen atoms at the α-position such as bromine (entry 7) or fluorine (entry 8) also afforded the halogenated ketones with conversions higher than 75%. It is remarkable that the desired carbonylic compounds were formed as the sole products, without the detection of other derivatives in any case. When using the alcohol **1a**, the substrate concentration could be increased up to 200 mM obtaining a conversion of 93% after 24 h. This is in line with other reports that have demonstrated the performance of laccases at high substrate concentrations.[7b,25] Satisfyingly, some of these oxidative transformations could be easily performed at the 100 mg scale, isolating the corresponding ketones in very high yields (76–81%, see ESI† for more details).

Then, chlorohydrin **1i** was also tested as plausible substrate (entry 9). In this case, we observed a 62% conversion to α-chloro ketone **2i** after 24 h under these oxidative conditions. This result is very important due to the difficulty to achieve this oxidation by other chemical methods, and also because of the versatility of these compounds as intermediates of several high added-value derivatives. Furthermore, when a non-halogenated alcohol such as 1-phenylethanol (**1j**) was used, a similar conversion compared to the dihalogenated counterparts was achieved (78%, entry 10), showing that this methodology can work with different types of secondary alcohols.[26]

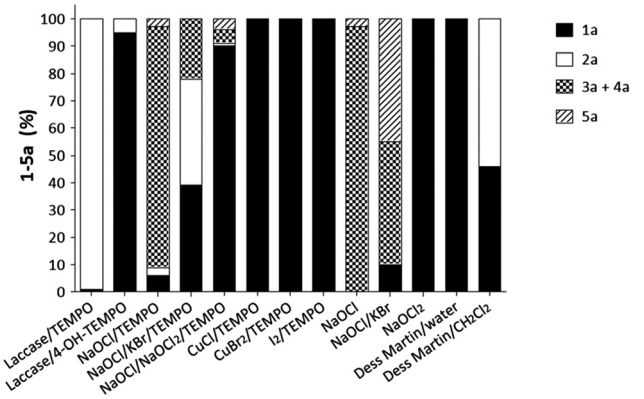
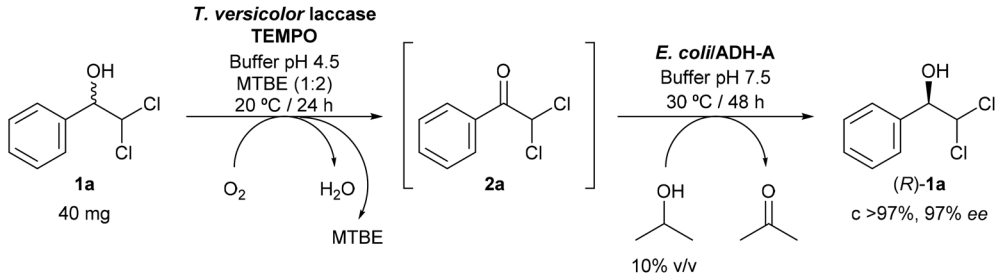


Fig. 3 Oxidation of alcohol **1a** into **2a** employing different reaction conditions. In some cases, benzaldehyde (**3a**), benzoic acid (**4a**), and/or mandelic acid (**5a**) were obtained as by-products.

To emphasize the ability of the laccase/TEMPO system to oxidize secondary alcohols with varying redox strength, we evaluated the relative thermodynamic stability of the halogenated alcohol/ketone pairs **1a**, **i**, **j**/**2a**, **i**, **j** with respect to the acetone/2-propanol pair by computing the Gibbs free energy change in aqueous solution ($\Delta_r G$, see ESI† for more details).[19a] The computed $\Delta_r G$ energies in kcal mol^{-1} were +7.33 (**1a**), +4.86 (**1i**), and +0.61 (**1j**), showing clearly that the dichlorinated alcohol **1a** was a weaker reducing agent compared to the mono- (**1i**, by 2.5 kcal mol^{-1}) or unsubstituted (**1j**, by 6.7 kcal mol^{-1}) derivatives. Since **1a** showed the largest conversion value, it turned out that the differences in the intrinsic redox strength of **1a**, **i**, **j** did not affect their oxidation, which is in agreement with the accepted ionic mechanism of oxidation mediated by TEMPO.[27] These results highlight the flexibility of our laccase/TEMPO system.

To demonstrate the mildness and selectivity of this system to oxidize these substrates, different chemical oxidative methods were assessed for the oxidation of **1a** (Fig. 3). Thus, the laccase was combined with 4-hydroxy-TEMPO (4-OH-TEMPO), but in this case just 5% conversion was achieved after 24 h. Several mixtures of oxidant(s) and TEMPO were tried in aqueous media such as NaOCl (9 equiv.),[28] NaOCl (1 equiv.)/KBr (0.2 equiv.),[28] $NaOCl_2$ (1.5 equiv.)/NaOCl (0.2 equiv.),[29] CuCl (0.2 equiv.),[30] $CuBr_2$ (0.2 equiv.),[31] or iodine (1.5 equiv.),[32] but negligible or very low conversions were attained. Just when using a stoichiometric amount or an excess of NaOCl, a complex mixture of products formed by benzaldehyde (**3a**), benzoic acid (**4a**) and mandelic acid (**5a**) was detected. This observation correlates with previous reports describing the synthesis of mandelic acid from dihalogenated ketones in an aqueous basic medium.[33]

The application of NaOCl or NaOCl/KBr led again to a mixture of compounds **3–5a**, while $NaOCl_2$ or Dess–Martin periodinane in water did not afford any conversion, recovering the starting material. Only the employment of Dess–Martin periodinane in dichloromethane,[8] allowed the synthesis of ketone **2a** at 54% as the sole product at room temperature



Scheme 2 One-pot two-step protocol to deracemize alcohol **1a** *via* oxidation with the laccase/TEMPO system plus reduction with an alcohol dehydrogenase and 2-propanol.

after 1.5 h. To show the environmental benefits of this system compared to the others, we have performed a simplified environmental impact analysis making use of the *E*-factor concept.[34] Although it provides a rough estimation of the environmental impact, this value assesses the sustainability of a process.[35] Thus, we compared[36] our laccase/TEMPO system with the other three (laccase/4-OH-TEMPO, NaOCl/KBr/TEMPO and Dess–Martin in dichloromethane) which afforded a measurable amount of ketone **2a**, obtaining an *E*-factor of 3.5 for the laccase/TEMPO process (excluding solvents) and values higher than 21 for the others (see ESI† for details). Also, the solvent demand in our method (330 mL g^{-1} product) was much lower in comparison with the other strategies. As a result, these data demonstrate the favorable ecological impact of the laccase/TEMPO pair.

Taken together, we have shown that this methodology, making use of a laccase/TEMPO system in a biphasic medium with oxygen as final electron acceptor, is a practical method to get access to α- or α,α-dihalogenated ketones *via* oxidation of the corresponding (di)halohydrins, which in other reaction conditions cannot be obtained selectively.

Encouraged by these results and as an interesting application of this methodology, we envisaged the deracemization of alcohol **1a** through a one-pot two-step chemoenzymatic procedure. To date, the only example for the deracemization of chlorohydrins has been reported by Kroutil and co-workers,[10a] combining an alcohol dehydrogenase (for the reduction) with an iridium catalyst (for the oxidation) in a one-pot concurrent system. Unfortunately, the *ee* values did not exceed 40% due to undesired interference of both processes. Herein, we propose an alternative strategy starting from the racemic alcohol **1a**. In a first step we achieve the complete oxidation into ketone **2a** with the laccase/TEMPO pair. Subsequently, the addition of a selective ADH leads to enantioenriched alcohol **1a** (Scheme 2).

Hence, this system was successfully applied to deracemize 40 mg of racemic alcohol **2a**. After oxidation mediated by *T. versicolor* laccase and TEMPO under the previously optimized conditions, the buffer pH was adjusted to 7.5 by adding tris(hydroxymethyl)aminomethane (Tris) and a few drops of HCl (thus inactivating the laccase). Then, alcohol dehydrogenase from *Rhodococcus ruber* overexpressed in *Escherichia coli* (*E. coli*/ADH-A)[37] and 2-propanol as hydrogen donor (10% v/v) were successively added. After 48 h at 30 °C, the enantioenriched alcohol (*R*)-**1a** was obtained in total conversion with 97% *ee*. This first example opens the door to sustainable chemoenzymatic deracemization protocols applied to other β-substituted alcohols that under other chemical conditions cannot be obtained easily.

Conclusions

In summary, we have shown here an efficient and green method for the selective oxidation of secondary (di)halohydrins into the corresponding halogenated ketones using the laccase from *Trametes versicolor* and TEMPO pair. Although this process is thermodynamically impeded, the use of this system under aerobic conditions in a biphasic media could provide exclusively the corresponding carbonylic compounds with very high conversions in a clean fashion. This practical method represents a potent alternative to other known oxidative methods, which were not able to oxidize these substrates or afforded undesired by-products. Moreover, coupled with a second enzymatic bioreduction, deracemization of a secondary dihalogenated alcohol in a one-pot two-step protocol was feasible, obtaining the enantioenriched (*R*)-alcohol in a very mild and elegant fashion.

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