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A mild and highly efficient laccase-mediator system for aerobic oxidation of alcohols†

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With the aid of the highly active nitroxyl radical AZADO (2-azaadamantane *N*-oxyl), a simple method for the aerobic catalytic oxidation of alcohols is presented. The oxidations could typically proceed under practical ambient conditions (room temperature, air atmosphere, no moisture effect, metal-free, *etc.*) with a broad generality of the alcohol substrates, and especially for the oxidation of complex and highly functionalized alcohols. An ionic mechanism is proposed for the present system.

The selective oxidation of alcohols to the corresponding carbonyl compounds is a fundamental transformation both in laboratory synthesis and industrial production.[1] Numerous oxidizing reagents in stoichiometric amounts have been traditionally employed to accomplish this transformation with considerable drawbacks such as the use of expensive reagents, volatile organic solvents, and discharge of environmentally pernicious wastes. Recent growing environmental concerns have spurred research activities directed toward the development of greener oxidation methods in which the use of molecular oxygen as the terminal oxidant has drawn considerable attention.[2]

On the other hand, enzyme-catalyzed transformations have emerged as an elegant synthetic methodology that allows the development of eco-friendly processes within the basic principles of green chemistry.[3] The development of enzyme-catalyzed oxidations is highly attractive because of their great potential to remove pollutants and catalyse a great variety of redox processes with no hazardous side effects.[4] Furthermore, most enzymatic oxidations usually use aerial oxygen as the terminal oxidant and can be performed under very mild reaction conditions. In this context, laccase (benzenediol : oxygen oxidoreductase, EC 1.10.3.2), a blue multi-copper oxidase, has drawn considerable attention in white biotechnology. As a result of their nontoxic nature, high stability, and lack of substrate inhibition, laccases are nowadays ideal candidates in the fields of waste detoxification, textile dye transformation, biosensors, food industry, and pulp bleaching.[5] The demand for green chemical processes has also inspired interest in the application of this enzyme to address modern synthetic organic chemistry challenges. Due to their broad substrate scope, laccases find increasing significance in a variety of organic synthetic methodologies and the manufacture of synthetic building blocks for fine chemistry.[6]

Despite the synthetic importance of laccases, their broader application has been restricted due to the low redox potential (typically about 0.5–0.8 mV *vs.* the normal hydrogen electrode).[6a] For the reactions where the substrate to be oxidized has a redox potential higher than laccase, or the substrate is too large to penetrate into the enzyme active site, the presence of a low-molecular weight chemical mediator is required to facilitate oxidative reactions.[7] A mediator acts as a sort of 'electron shuttle'; once it is oxidised by laccase, it diffuses away from the enzymatic pocket and in turn oxidises any substrate that, due to its size, could not directly enter the enzymatic pocket.[8] The structures and abbreviated names of representative artificial laccase mediators are given in Fig. 1. By using these so-called 'chemical mediators', the redox potential of laccases can be extended which allows the oxidation of a wide range of non-phenolic substrates, such as sugars,[9] ethers,[10] alkenes,[11] amides,[12] aromatic methyl groups,[13] polycyclic aromatic hydrocarbons,[14] lipids,[15] and alcohols.[16] Despite all the associated advantages of using these mediators, there are still several drawbacks from a practical point of view. These are: (i) low catalytic efficiency, excess amounts of mediator (typically 30 mol% on substrate) are needed, with some even needing more than 1 equiv.; (ii) in some cases, laccase is inactivated by the mediator radicals, or the latter can be transformed into inactive compounds with no more mediating capability (*e.g.* generation of benzotriazol from HBT by losing the hydroxyl group); (iii) some of the mediators can generate toxic derivatives; and

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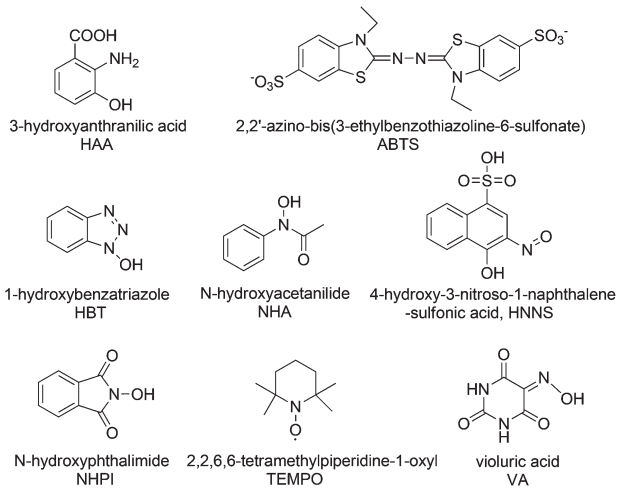


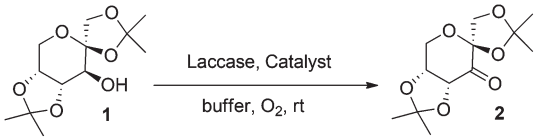
Fig. 1 Structures of representative artificial laccase mediators.

(iv) long reaction times are usually required. In view of the aforementioned drawbacks of existing routes and the desire for aerobic oxidation of multifunctionalized complex molecules, the development of a new and highly efficient laccase-mediator system is particularly attractive.

In continuation of our efforts to explore green approaches for synthetic chemistry,[17] we report herein a new and highly efficient laccase-mediator system for the oxidation of alcohols using O_2 as a green and economic oxidant under mild reaction conditions.

Initial experiments were carried out using 1,2:4,5-di-*O*-isopropylidene-β-D-fructopyranose **1** as a model substrate; the corresponding product ketone **2** (1,2:4,5-di-*O*-isopropylidene-β-D-*erythro*-2,3-hexadiulo-2,6-pyranose, Shi's catalyst) is a useful reagent, which is often used as a precursor for transition metal free catalytic asymmetric olefin epoxidation.[18] Catalytic oxidation of **1** has proved to be particularly difficult, with numerous methods such as the TPAP-O_2 system,[19] the NHPI-O_2 system,[20] and the Pd(bathophenanthroline)-$(AcO)_2$-O_2 system,[21] which were successfully applied to oxidation of alcohols, failing to convert **1** to **2** due to steric reasons. In the laccase catalysed aerobic oxidation of alcohols, TEMPO and its derivatives proved to be the most effective.[22] However, using TEMPO as the catalyst, the yield of **2** was less than 10% (Table 1, entry 1). The low catalytic efficiency of TEMPO as well as recent significant advances in designing the structure of nitroxyl radicals[23] prompted us to reconsider the TEMPO catalyst and provided an opportunity of using a more reactive mediator for laccase. We were very pleased to find that in the case of using AZADO (2-azaadamantane *N*-oxyl) as a catalyst, the desired product was obtained in 63% yield (Table 1, entry 2). In the control experiments, there was no product formation in the absence of laccase, AZADO, or when using heat-killed laccase (see Table S1 in the ESI†). With the aim of finding an appropriate mediator for laccase, we evaluated the catalytic performance of other nitroxyl radicals (Table 1, entries 3–5). Among them, Nor-AZADO (9-azanoradamantane *N*-oxyl) shows

Table 1 Optimization studies for the aerobic oxidation of **1**[a]



Entry	Catalyst	Equiv.	Additive	Yield[b] (%)
1	TEMPO	0.2	—	7
2	AZADO	0.2	—	63
3	Nor-AZADO	0.2	—	60
4	ABNO	0.2	—	46
5	ABOO	0.2	—	29
6[c]	AZADO	0.2	CTAB	66
7[d]	AZADO	0.2	SDS	52
8[e]	AZADO	0.2	Tween-20	67
9[f]	AZADO	0.2	Rhamnolipid R_1	55
10[g]	AZADO	0.2	$PhCF_3$	87 (85)
11	AZADO	0.1	$PhCF_3$	85
12	AZADO	0.05	$PhCF_3$	80
13[h]	AZADO	0.05	$PhCF_3$	82

[a] Reactions were performed by using **1** (1 mmol), laccase (40 U), a nitroxyl radical in acetate buffer (pH 4.5) under an O_2 atmosphere at room temperature for 12 h unless otherwise noted. [b] Yield was determined by ^1H NMR using 1,1,2,2-tetrachloroethane as an internal standard. [c] CTAB (cetrimonium bromide, 0.2 equiv.) was used. [d] SDS (sodium dodecylsulfate, 0.2 equiv.) was used. [e] Tween-20 (sorbitan monooleate ethoxylate, 0.1 g) was used. [f] Rhamnolipid R_1 (3-(3-[(6-deoxy-alpha-L-mannopyranosyl)oxy]decanoyl oxy) decanoic acid, 0.1 g) was used. [g] $PhCF_3$ (1 mL) was used as a co-solvent; isolated yield is shown in parentheses. [h] This reaction was run using air as an oxidant for 24 h.

comparable catalytic activity to AZADO and serves as another good catalyst to enhance the reaction remarkably. However, the catalytic activity of ABNO (9-azabicy-clo[3.3.1]nonane *N*-oxyl) and ABOO (8-azabicyclo[3.2.1]octane *N*-oxyl) is much lower than that of AZADO, probably due to the stability of the corresponding cations.[23b,24] Encouraged by these results and in order to search for the optimum reaction conditions, we screened a variety of parameters of this reaction, such as a different source of laccase, the type and pH of buffer and a broad range of co-solvents (for more details see Table S2 in the ESI†).

It is well known that in aqueous-phase reactions, addition of surfactants can improve the reactions by solubilization or the micelle formation effect. To improve the solubility of the reactant and catalyst in the buffer, we examined a variety of surfactants, including cationic, anionic, nonionic, and biological surfactants (Table 1, entries 6–9). However, all of them were unsatisfactory although CTAB and Tween-20 showed a slight improvement. Further investigation of the co-solvent on this reaction showed a strong solvent dependence effect. Earlier studies revealed that laccases are often deactivated by organic solvents.[25] A broad range of co-solvents were tested for this reaction (see Table S2 in the ESI†); among them, $PhCF_3$ proved to be the most efficient co-solvent, giving the highest yield of **2** (Table 1, entry 10). A further study showed that a co-solvent was not necessary for the reaction; for most reactants, the use of a buffer as the solvent was effective enough to

complete the oxidation. With regard to the AZADO dosage, it was possible to decrease the amount of AZADO to as low as 0.05 equiv. without a significant loss in catalytic efficiency (Table 1, entry 12). It is noteworthy that the reaction can also proceed smoothly even using air as an oxidant, albeit a longer reaction time was required (Table 1, entry 13).

With the aim to develop and define the scope and limitation of the present method, this laccase–AZADO catalytic oxidation system was then extended for the oxidation of a wide range of alcohols, including benzylic, allylic, heterocyclic, alicyclic, and aliphatic alcohols (Method A in Table 2). Using a similar strategy, the laccase–TEMPO system was also applied for the purpose of comparison (Method B in Table 2).

As shown in Table 2, most alcohols underwent oxidation to afford the corresponding carbonyl products in excellent yields. The present laccase–AZADO system afforded aldehydes from primary alcohols and ketones from secondary alcohols. Excellent chemoselectivity was observed under the present system. For the oxidation of primary alcohol, no noticeable over-oxidation of aldehyde to carboxylic acid was detected. As the functional groups, several oxidation-sensitive alkenes, electron-rich aromatic, heteroaromatic rings, halogens, and alkyne groups were also tolerated during the reactions. It is noteworthy that 2,6-dichlorobenzyl alcohol and 2,4,6-trimethylbenzyl alcohol, which often resist oxidation using a laccase-mediator system due to the steric hindrance effect,[16b] were also readily oxidized to the corresponding aldehydes in high yields (Table 2, entries 12 and 13). However, the oxidation of 4-hydroxybenzyl alcohol and 4-aminobenzyl alcohol was not successful, which may be due to laccase oxidizing the –OH or $–NH_2$ group by inducing dimerisation and oligomerization through the intermediacy of resonance-stabilized radicals (Table 2, entries 21 and 22). Oxidation of benzyl mercaptan was also not successful, and no oxidation to benzaldehyde was detected, in spite of its structural analogy with benzyl alcohol (Table 2, entry 23). The free thiol group (–SH) has been shown to be a potent inhibitor of laccases, presumably *via* co-ordination of the thiol to the copper atoms in the enzyme active site.[7c] Even so, the present laccase–AZADO system still has enough activity to oxidate thioether-containing molecules, and thioanisaldehyde was produced from the corresponding alcohol with a high yield (94%) (Table 2, entry 24); this is a very selective reaction with no sulphoxide, sulphone, or Dakin-type (phenols) by-products being produced.

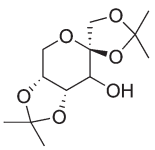
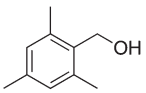
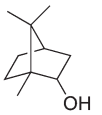
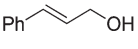
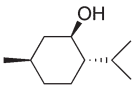
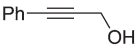
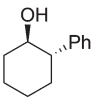
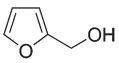
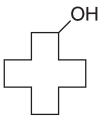
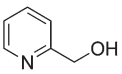
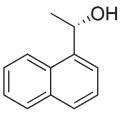
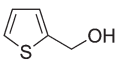
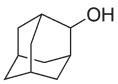
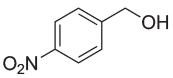
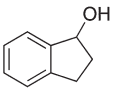
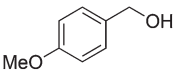
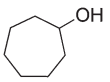
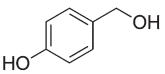
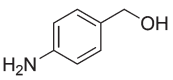
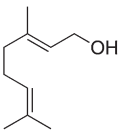
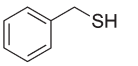
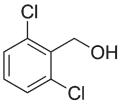
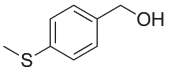
To assess the feasibility of using this method on a preparative scale, the multigram-scale catalytic system was then examined for the oxidation of menthol on a 100 mmol scale. As expected, the reaction proceeded smoothly, similar to the smaller-scale case, and the desired menthone was obtained in an 80% isolated yield (Table 2, entry 3).

The different catalytic activities between the laccase–AZADO and laccase–TEMPO systems observed in Table 2 cannot be explained simply on the basis of the redox potential of the nitroxyl radicals. TEMPO and AZADO produced electrochemically reversible responses with redox potentials at 294 mV and 236 mV *versus* Ag/AgCl, respectively.[23h] However, the T1 copper centre in laccase from *Trametes versicolor* has a redox potential of *ca.* 790 mV *versus* NHE.[22b,26] Consequently, laccase from *Trametes versicolor* has sufficient oxidation potential to convert TEMPO or AZADO into their corresponding oxidised state. The different catalytic reactivity between TEMPO and AZADO seems to present another rule, that is, the lower the redox potential, the easier the oxidation into the corresponding oxoammonium species by laccase and the higher catalytic activity. To check this point, the experiments on the reaction rate were carried out using TEMPO, AZADO, 1-Me-AZADO and 1,3-dimethyl-AZADO as catalysts. As shown in Fig. 2, the catalytic activities of these nitroxyl radicals are in the order of AZADO > 1-Me-AZADO > 1,3-dimethyl-AZADO > TEMPO. However, the $E^{o\prime}$ values (*vs.* Ag/Ag^{+}) of these nitroxyl radicals are in the order of TEMPO (294 mV) > AZADO (236 mV) > 1-Me-AZADO (186 mV) > 1,3-dimethyl-AZADO (136 mV).[23a] No obvious correlation between the two orders is found. Thus, we believe that another factor that affects catalytic activity is due to the steric hindrance effect, compared to TEMPO or 1,3-dimethyl-AZADO, in which the oxygen-centered unpaired electron is sterically protected by the surrounding four or two methyl groups; the α-hydrogen of AZADO furnishes a less hindered reaction center, thereby enabling smooth contact between the active species due to the reduced steric hindrance effect; this influence is more obvious in the oxidation of sterically hindered alcohols (Table 2, entries 1–4, 12, 13).

In contrast to the redox potential, the energy level, or the chemical structures of the mediator, little attention has been paid to the kinetic features of the redox mediator during oxidative regeneration or electron self-exchange reaction among mediator molecules. These differences between AZADO and TEMPO may be the other important factors that affect the catalytic activity. Recently, the electrochemical and spectroscopic properties of these nitroxyl radicals were measured by the Nishide group.[27] According to their report, the electron self-exchange reaction rate constant (k_{ex}) of AZADO is about 3.3×10^8 M^{-1} s^{-1}, which is 10 times higher than that of TEMPO (2.9×10^7 M^{-1} s^{-1}) (Scheme 1). In addition, the heterogeneous electron-transfer rate constant (k_0) of AZADO and TEMPO is 1.6 and 0.29 cm s^{-1}, which is an important parameter indicating how fast the regeneration reaction of the mediator cation is. The significantly faster regeneration and self-exchange reaction rate of AZADO are expected to be responsible for its excellent catalytic activity through smooth contact and easier oxidation by laccase.

The mechanistic details of the laccase-mediator catalyzed aerobic oxidation system are still unclear.[28] However, they are generally believed to involve one electron oxidation of the mediator by the oxidized form of laccase (cupric of T1 site in laccase), followed by the reaction of the oxidized mediator with the substrate, either *via* a radical hydrogen-atom transfer route (HAT) which is suggested for the laccase–HBT, laccase–NHPI, and laccase–VA system, or *via* the electron transfer route (ET) which is suggested for the laccase–ABTS system, or *via* the ionic oxidation route which is suggested for the laccase–TEMPO system. To shed light on the reaction pathway of the

Table 2 Green and selective oxidation of alcohols

Entry	Alcohols	Method[a]	Yield[b] (%)/time	Entry	Alcohols	Method	Yield (%)/time
1[c]		A B	85/12 h 7/12 h[d]	13		A B	82/24 h (87/6 h[g]) 45/24 h
2		A B	92/12 h 61/24 h	14		A B	92/6 h 90/12 h
3[c]		A B	84/12 h (80/24 h[e]) 5/36 h[d]	15		A B	79/6 h 71/12 h
4[c]		A B	90/12 h 23/24 h	16		A B	85/6 h 92/6 h
5		A B	72/12 h 35/24 h	17		A B	86/12 h 80/12 h
6		A B	92/8 h 73/12 h	18		A B	94/6 h 87/6 h
7[c]		A B	90/8 h 67/12 h	19[c]		A B	93/6 h 90/6 h
8		A B	88/8 h 65/12 h	20		A B	98/4 h 96/4 h
9		A B	76/12 h 47/24 h	21		A B	—[h]
10		A B	93/8 h 85/12 h	22		A B	—[i]
11[f]		A B	89/12 h 72/12 h	23		A B	—/8 h[j] —/8 h
12[c]		A B	78/24 h 22/24 h	24		A B	94/6 h 88/8 h

[a] Method A: laccase–AZADO system. Method B: laccase–TEMPO system. [b] Isolated yields unless otherwise noted. [c] The reaction was carried out in the presence of $PhCF_3$ as a co-solvent. [d] Yield was determined by ^1H NMR using 1,1,2,2-tetrachloroethane as an internal standard. [e] Yield of large-scale synthesis on a 100 mmol scale. [f] Yield was determined by GC-MS. [g] 20 mol% AZADO was used. [h] Formation of black tar was observed in this reaction. [i] The reaction mixture was too complex to identify. [j] Unchanged substrate is recovered (91%).

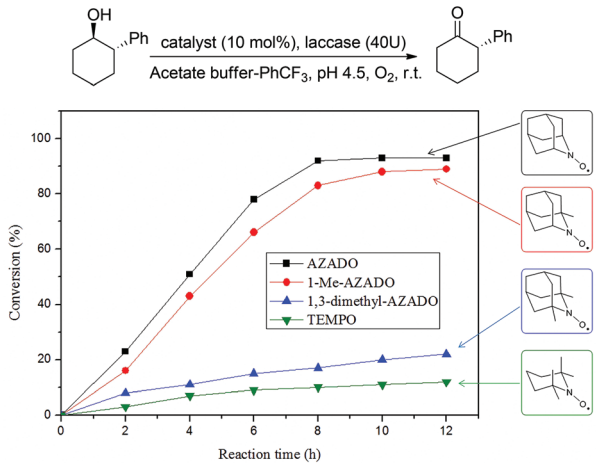
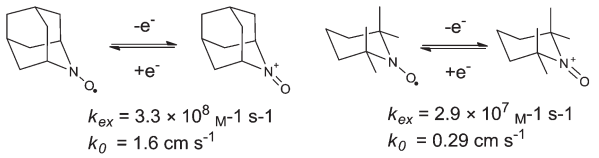
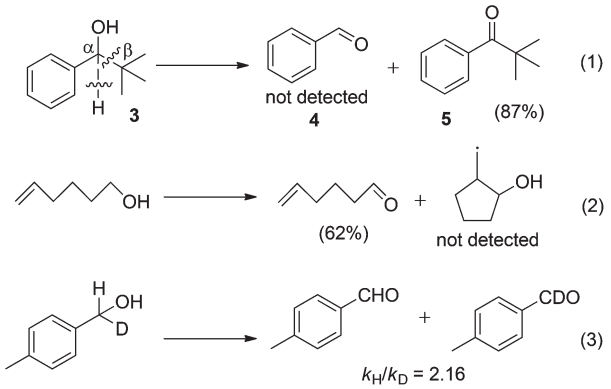


Fig. 2 Comparison of the catalytic activity of AZADO, 1-Me-AZADO, 1,3-dimethyl-AZADO, and TEMPO.



Scheme 1 One-electron transfer redox reaction of nitroxyl radicals.



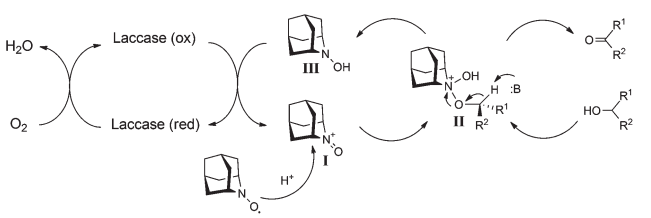
Scheme 2 Oxidation with the laccase–AZADO system.

present laccase–AZADO system, we conducted the following experiments (Scheme 2). We first treated 2,2-dimethyl-1-phenyl-1-propanol **3** with the present laccase–AZADO system. Under the ET route, oxidation of **3** will produce benzaldehyde **4** and a *tert*-butyl radical by C_α–C_β bond cleavage. Conversely, under the HAT route, **3** undergoes cleavage of the C_α–H benzylic bond and produces ketone **5**. This clear-cut behaviour makes **3** a useful model substrate, enabling it to assess the oxidation mechanism from product analysis.[28b,e,29] Results have shown that ketone **5** was the only product produced from the laccase–AZADO system, thereby suggesting that the reaction pathway does not involve the ET route.

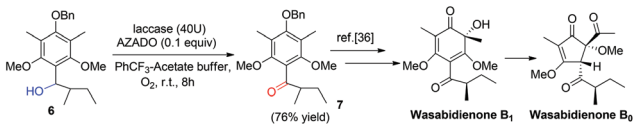
A further experiment was conducted using 5-hexen-1-ol as a probe substrate; oxidation of 5-hexen-1-ol with the laccase–AZADO system produced only 5-hexen-1-al. In case of the HAT route, removal of α-H to the hydroxy group is known to afford cyclopentyl alcohol through an intramolecular radical rearrangement.[29a,30] The absence of a rearrangement product confirms that the present laccase–AZADO system also does not follow the HAT route.

We determined the intramolecular kinetic isotope effect in the oxidation of a suitably synthesized α-monodeutero-*p*-methylbenzyl alcohol[31] using Sheldon's method.[32] The kinetic isotope effect (k_H/k_D value) for the laccase–AZADO catalysed aerobic oxidation of this alcohol at room temperature was determined to be 2.16 by ^{1}H NMR. This value is much lower than those obtained for the laccase–NHA system (6.2), the laccase–HBT system (6.4), and the laccase–VA system (6.4),[7a] which are expected for the HAT route. However, this value is in the range found for the stoichiometric oxidation of benzyl alcohols by the oxoammonium ions (1.7–3.1)[33] and compares exceptionally well with the isotope effect observed with the laccase–TEMPO system (2.32).[22b] As a further proof, the AZADO oxoammonium chloride AZADO$^+$Cl$^-$ was synthesized *ex situ*[23k] and used as a stoichiometric oxidant to oxidise α-monodeutero-*p*-methylbenzyl alcohol. The KIE value for AZADO$^+$Cl$^-$ oxidation of this alcohol was determined to be 2.11, which is much closer to that obtained from the laccase–AZADO system (2.16). The similar kinetic isotope effects strongly suggest that oxoammonium cation AZADO$^+$ was generated during the reaction; the oxoammonium ion oxidizes alcohol *via* a ionic route which is similar to the proposed mechanism for the laccase–TEMPO system.[28]

On the basis of all of the aforementioned results, an ionic oxidation mechanism is suggested for the present laccase–AZADO system (Scheme 3). In this route, one electron oxidation of AZADO affords the oxoammonium cation (**I**); a nucleophilic attack of the lone-pair of alcohol onto the oxoammonium cation takes place to form an adduct (**II**); deprotonation of the adduct either intramolecularly (from N–O$^-$) or intermolecularly (from the base in the buffer, *i.e.* B)[34] gives the carbonyl product and the hydroxylamine (**III**). Laccase oxidises the hydroxylamine to regenerate AZADO, and further oxidation leads to the oxoammonium cation. Beyond that, the oxoammonium cation (**I**) also can be restored through acid-induced disproportionation of AZADO.[23f] The laccase is finally re-oxidised by oxygen, thereby completing this green catalytic cycle.



Scheme 3 Suggested mechanism for the laccase–AZADO catalyzed aerobic oxidation.



Scheme 4 Synthesis of wasabidienone B_1 and wasbidienone B_0.

One of the applications of the present green laccase–AZADO system is to synthesize 1-(4-(benzyloxy)-2,6-dimethoxy-3,5-dimethylphenyl)-2-methylbutan-1-one (**7**). Oxidation of 1-(4-(benzyloxy)-2,6-dimethoxy-3,5-dimethylphenyl)-2-methylbutan-1-ol (**6**) has proved difficult due to the steric hindrance effect. The Quideau group reported the successful oxidation of **6** to **7** using stabilized IBX (3 equiv.) in the THF–DMSO solution.[35] However, this method involves the use of expensive and excess amounts of the oxidant. In the case of using the laccase–AZADO system, desired product **7** was obtained in 76% yield (Scheme 4). **7** is a precursor to synthesize natural products wasabidienone B_1 and wasbidienone B_0. These fungal polyketides were isolated in the 1980s by Soga and co-workers from a potato culture of *Phoma wasabiae* Yokogi, a fungus responsible for the blackleg disease causing widespread destruction among cruciferous crops such as rape, cabbage, and wasabi (Japanese horseradish).[36]

In conclusion, a new and highly efficient mediator of laccase has been comparatively evaluated. With the aid of the highly active nitroxyl radical AZADO, a simple green method for the aerobic catalytic oxidation of alcohols is presented. The advantages of this catalytic oxidation system are summarized as follows: (1) avoids the use of a transition metal catalyst; (2) high atom economy and environmental consciousness, with the only by-product being H_2O; and (3) mild reaction conditions (room temperature, air atmosphere, and no moisture effect). Moreover, the present non-transition metal catalytic system also provides an easy scale-up and separation protocol.

Experimental

Laccases from *Trametes versicolor*, *Rhus vernicifera*, and *Agaricus bisporus* were purchased from Sigma-Aldrich as a light brown lyophilized powder and used without modification. Nor-AZADO, ABNO and ABOO were synthesized according to the reported procedures.[23b,c] Other reagents were ACS reagent grade and used without further purification. NMR spectra were recorded on a Bruker Ascend 400 MHz NMR spectrometer at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR). Chemical shifts are reported in parts per million (ppm). ^{1}H and ^{13}C chemical shifts are referenced relative to the tetramethylsilane. GC-MS were recorded on a Thermo Trace DSQ GC-MS spectrometer using a TRB-5MS (30 m × 0.25 mm × 0.25 mm) column. ESI-MS were recorded on a Thermal Finnigan TSQ Quantum ultra AM spectrometer using a TRB-5MS (30 m × 0.25 mm × 0.25 mm) column. Melting points were determined in an open capillary tube with a Mel-temp II melting point apparatus. Infrared spectra were recorded as a KBr pellet on a Perkin-Elmer 1600 series FT-IR spectrophotometer.

General experimental procedure for green oxidation of alcohols using the laccase–AZADO system (Table 2): To a stirred solution of alcohol (1 mmol), AZADO (0.1 mmol) in acetate buffer (0.2 M, pH 4.5, 5 mL), laccase from *Trametes versicolor* (4 mg, 10 U mg^{-1}) was then added. In case of some solid water insoluble substrates, $PhCF_3$ (1 mL) was added. The solution was stirred at room temperature under an oxygen atmosphere (balloon) for several hours while checking the reaction progress by using gas or thin-layer chromatography. After completion, the mixture was extracted with diethyl ether (3 × 5 mL). The organic phase was concentrated under vacuum and the crude product was purified by column chromatography (hexane–EtOAc = 10 : 1) to provide the analytically pure product, which was characterized by ^{1}H NMR, ^{13}C NMR and GC-MS. The ESI† provides details of these measurements.

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