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Comparing the catalytic efficiency of ring substituted 1-hydroxybenzotriazoles as laccase mediators

Claudio D'Alfonso, Osvaldo Lanzalunga *, Andrea Lapi, Raffaella Vadalà

Dipartimento di Chimica, Sapienza Università di Roma and Istituto CNR di Metodologie Chimiche (IMC-CNR), Sezione Meccanismi di Reazione, P.le A. Moro 5, 00185 Roma, Italy

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ABSTRACT

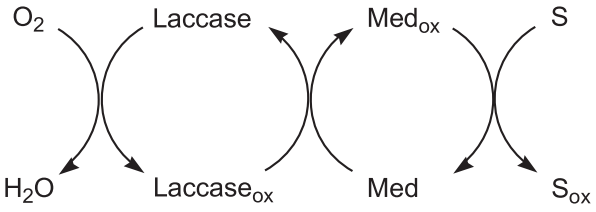
A series of ring substituted 1-hydroxybenzotriazoles (6-X-HBTs) have been tested as mediators in the laccase-promoted oxidation of 4-methoxybenzyl alcohol, 3,4-dimethoxybenzyl alcohol, and the dimeric lignin model 1-(3,4-dimethoxyphenyl)-2-phenoxyethanol. The effect of the aryl substituents on the yields of oxidation products is remarkable. The catalytic mediation efficiency increases as the electron releasing (ER) properties of the substituent increases up to a maximum value for 6-CH_3-HBT, which resulted a very efficient mediator. Both the oxidation of the 6-X-HBTs to the *N*-oxyl radicals (6-X-BTNO) by laccase and the hydrogen atom transfer (HAT) process from the benzylic C–H to the 6-X-BTNO contribute to the overall reactivity. The former process is favored by ER substituents that lower the mediator redox potentials. On the other hand, ER substituents decrease the 6-X-BTNO reactivity in the HAT process due to a decrease in the NO–H BDE value, as assessed in this study through a radical equilibration technique.

1. Introduction

In recent years lignin degradation processes have attracted great attention, in particular those involving environmentally benign procedures. These processes are of fundamental importance because they can convert lignin into low molecular weight aromatic compounds, making this polymer a renewable source for the industrial preparation of a number of chemicals.[1] Moreover, the selective degradation of lignin and its removal from the carbohydrate component of wood is a key process in the pulp and paper industry.[2]

Lignin degradation promoted by fungi represents one of the most important low environmental impact processes.[3] Most basidiomycetes that cause the white rotting of wood produce laccases as ligninolytic enzymes. Laccases (EC 1.10.3.2) are multi-copper oxidases that catalyze the direct oxidation of electron rich aromatic substrates, like phenols and anilines, with the concomitant reduction of O_2 to water.[4] In the presence of appropriate low molecular-weight compounds (mediators) laccases are also able to catalyze the oxidation of non-phenolic benzylic substrates, characterized by a relatively high redox potential (Scheme 1).[5]

N-Hydroxylamines like 1-hydroxybenzotriazole (HBT), violuric acid, *N*-hydroxyacetanilide, *N*-methyl-*N*-hydroxybenzamide, and *N*-hydroxyphthalimide (NHPI) represent the most important class of laccase mediators.[6] These compounds are oxidized by one electron transfer to the type 1 (T1) Cu center of laccase to afford the corresponding *N*-oxyl radicals, which are the active species in the oxidation of the substrate (Scheme 2).[7] The laccase/N–OH mediators/O_2 systems have found interesting application in the oxidative degradation of lignin,[6c,7f,8] or of organic pollutants including polycyclic hydrocarbons, pesticides, and insecticides,[9] or in decolorization of dyes[10] and in synthetic procedures,[11] such as the chemoselective aerobic oxidation of catechin derivatives.[12]



Scheme 1. Mediation in the laccase promoted oxidation.

Structural effects of *N*-hydroxylamines on the catalytic efficiency in the laccase/mediator/O_2 systems have been analyzed in several studies with the aim of finding the most active mediators and determining key structural factors that may affect the mediation efficiency. Correlation of features, such as the *N*-

* Corresponding author. Tel.: +39 0649913711; fax: +39 0649913629; e-mail address: osvaldo.lanzalunga@uniroma1.it (O. Lanzalunga).

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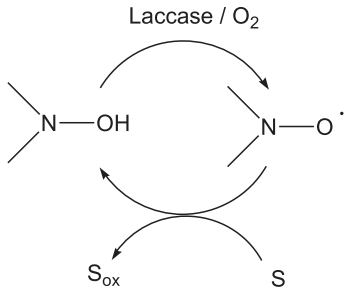


Scheme 2. Catalytic cycle in oxidations promoted by the laccase/N–OH mediator/O_2 systems.

hydroxylamine redox potential, oxidative stability, steric properties, NO–H bond dissociation energies with the efficiency in laccase-mediated biocatalysis have been carried out for a wide variety of *N*-hydroxy compounds.[7e,f,h,13]

In a previous work we have investigated the substituent effect on the mediation efficiency in the laccase-catalyzed oxidation promoted by a series of aryl substituted *N*-hydroxyphthalimides (NHPIs).[14]

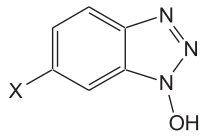
The laccase/NHPI system has been applied with some success in the delignification of kraft wood pulp samples, however the mediation efficiency was significantly lower than that observed with another classical mediator, 1-hydroxybenzotriazole.[7e,14] A lower mediation efficiency was also observed in the oxidation of lignin model compounds.[7h,14]

In the delignification of kraft wood pulp samples promoted by laccase/1-hydroxybenzotriazole systems, a decrease in the mediation efficiency was observed on introducing either electron donating or withdrawing aryl substituents in HBT.[7e] This behavior was explained on the basis of the balance of two opposite effects on the overall reactivity exerted by the variation of the mediator redox potential (E°_{N-OH}).[13c] According to this hypothesis, an increase in the mediator redox potential would decrease the rate of the mediator oxidation by laccase and increase that of the oxidation of the target molecule (S in Scheme 2). Thus, for mediators characterized by a low E°_{N-OH}, the overall catalytic rate would be governed by a rate determining S oxidation step whereas, for mediators characterized by a high E°_{N-OH}, the N–OH oxidation by laccase would be rate determining.

In this context it has to be noted that no analysis of the correlation of the mediation efficiency with the NO–H bond dissociation energies (BDE_{NO-H}) of 1-hydroxybenzotriazoles has been carried out. BDE_{NO-H} represents a key factor for the analysis of the reactivity of *N*-oxyl radicals in hydrogen transfer processes.[7h,i]

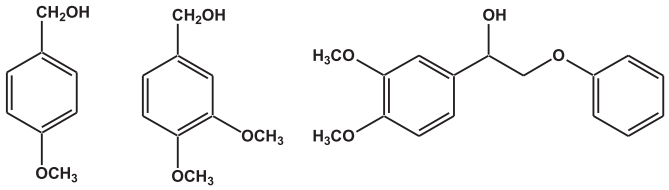
On this basis, we considered it worthwhile to provide a comprehensive detailed analysis of the effect of ring substituents in the 6-position of 1-hydroxybenzotriazole over the mediation efficiency in the laccase promoted oxidation of benzylic substrates and to investigate the correlation of the mediation efficiency with the BDE_{NO-H} of the mediators.

To this purpose we have synthesized three aryl substituted HBTs (**1**, **2**, and **4**) containing either electron releasing (6-CH_3O, 6-CH_3) or electron withdrawing (6-Cl) groups and tested their laccase mediation efficiency in comparison with the commercially available 1-hydroxybenzotriazole (**3**) and 6-trifluoromethyl-1-hydroxybenzotriazole (**5**).



1 (X=CH_3O) 6-MeO-HBT
2 (X=CH_3) 6-Me-HBT
3 (X=H) HBT
4 (X=Cl) 6-Cl-HBT
5 (X=CF_3) 6-CF_3-HBT

The effect of the HBTs ring substituents on the mediation efficiency has been evaluated in the oxidation of benchmark substrates (Chart 1), such as 4-methoxybenzyl alcohol (**6**) and 3,4-dimethoxybenzyl alcohol (**7**), or even 1-(3,4-dimethoxyphenyl)-2-phenoxyethanol (**8**), a dimeric non-phenolic lignin model containing a β-*O*-4 aryl linkage, the most abundant bonding type in lignin.[15]



6 **7** **8**

Chart 1.

The NO–H bond dissociation energies (BDE_{NO-H}) of the 6-X-HBTs have been determined by the UV–vis radical equilibration technique. For a meaningful discussion of the substituent effects on the mediation efficiency we have also carried out a kinetic study of the hydrogen atom abstraction process from 4-methoxybenzyl alcohol (**6**) and 1-(3,4-dimethoxyphenyl)-2-phenoxyethanol (**8**) to the 6-X-benzotriazole *N*-oxyl radicals (6-X-BTNOs).

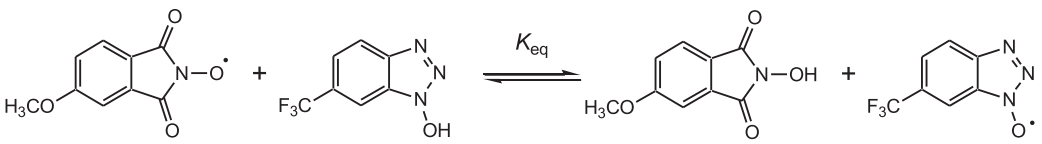
2. Results and discussion

2.1. NO–H bond dissociation energies of 6-X-HBTs

The BDE_{NO-H} values of 6-X-HBTs have been determined in acetonitrile by a UV–vis radical equilibration technique. This mainly consists of measuring the equilibrium constant for the forward hydrogen atom transfer (HAT) reaction between the ring substituted HBTs radical versus an appropriate reference compound whose BDE_{NO-H} is known, and the corresponding backward reaction. In the present case the reference compound used was 4-methoxy-*N*-hydroxyphthalimide (4-CH_3O-NHPI) whose BDE_{NO-H} (i.e., 87.3 kcal mol^{-1}) was measured in a previous study.[16,17]

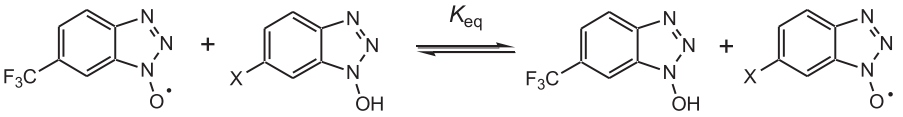
The BDE_{NO-H} of 6-CF_3-HBT was first determined by measuring the equilibrium constant (K_{eq}) for the HAT process with the 4-methoxyphthalimide *N*-oxyl radical 4-CH_3O-PINO (Eq. 1) (details in the Supplementary data).

The BDE_{NO-H} was calculated by means of Eq. 2, with the assumption that the entropic term can be neglected.[18]



(1)

$$BDE_{NO-H}(6-CF_3-HBT) = BDE_{NO-H}(4-CH_3O-NHPI) - RT\ln K_{eq} \quad (2)$$

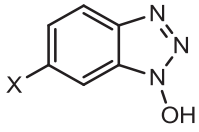


(3)

Once we had obtained the BDE_{NO-H} of 6-CF_3-HBT it was possible to determine all the other BDE_{NO-H} values by measuring the equilibrium constant (K_{eq}) for the HAT process using the 6-trifluoromethyl-benzotriazole *N*-oxyl radical (6-CF_3-BTNO) as the radical equilibrating species (Eq. 3).[21] Unfortunately we were unable to directly measure the BDE_{NO-H} value of 6-CH_3O-HBT due to the overlap of the visible absorption bands and the too small variation of absorbance after equilibration. Thus, the BDE_{NO-H} value for 6-CH_3O-BTNO was estimated by extrapolation from the linear correlation of experimental BDE_{NO-H} values with the Okamoto–-Brown σ^+ constants (see Supplementary data).[22,23]

The BDE_{NO-H} measured for the 6-X-HBTs are reported in Table 1 together with the BDE_{NO-H} calculated in previous studies for HBT and 6-CF_3-HBT by applying a thermochemical cycle.[7i,24] The experimental values are in accordance with those calculated, thus supporting the validity of the spectrophotometric method employed.[25]

Table 1
Redox potentials (E°) of the 6-X-BTNO/6-X-BTNO$^-$ couples in CH_3CN, experimental and calculated BDE_{NO-H} values for 6-X-HBTs in MeCN



	E° (V) vs SCE[a]	BDE_{NO-H}[b]	
		Exptl[c]	Calcd[d]
X=CF_3	0.76	86.5	86
X=Cl	0.70	85.9	n.d.
X=H	0.63	85.3	85
X=CH_3	0.60	85.1	n.d.
X=OCH_3	0.58	84.3[e]	n.d.

[a] From Ref. 26.
[b] kcal mol^{-1}.
[c] Estimated error ±0.5 kcal mol^{-1}.
[d] From Refs. 7i,24.
[e] Estimated by the correlation of the BDE_{NO-H} of 6-X-HBTs with σ^+ (see Supplementary data).

From the data reported in Table 1, it can be noted that the BDE_{NO-H} values regularly decrease by increasing the electron releasing power of the aryl substituent in accordance with the substituent effects already observed in the series of ring substituted *N*-hydroxyphthalimides.[16] This effect can be explained by considering the destabilizing effect of electron withdrawing substituents on the resonance structure with charge separation shown in Fig. 1.

It is interesting to note that the effect of the ring substituents on the BDE_{NO-H} in the series of 6-X-HBTs is more pronounced than that observed in the series of ring substituted *N*-hydroxyphthalimides.[16] For example, the difference in the BDE_{NO-H} values between 6-CH_3-HBT and 6-CF_3-HBT is 1.4 kcal mol^{-1}, while that between 4-CH_3-NHPI and 4-CO_2Me-NHPI is of only 0.7 kcal mol^{-1}. The higher substituent effect observed in the 6-X-HBT series is probably related to the direct conjugation of the substituent with the NOH group. Such direct conjugation is instead absent in the 4-X-NHPI series due to the presence of the two α carbonyl groups.

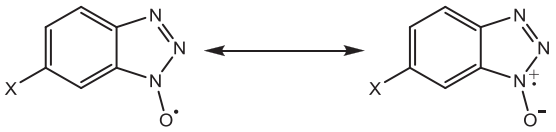
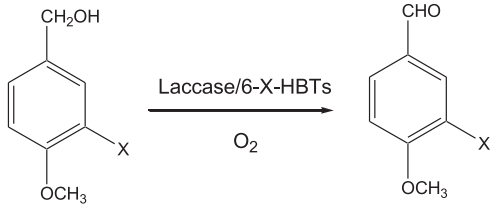


Fig. 1. Resonance structures for 6-X-BTNO radicals.

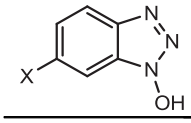
2.2. Oxidation of benzylic alcohols promoted by the laccase/6-X-HBTs/O_2 systems

The oxidation of benzylic alcohols **6**–**7** and of the dimeric lignin model **8** were carried out for 5–24 h under oxygen by adding purified laccase from *Trametes villosa* (7–40 units), 10 μmol of the mediator, and 30 μmol of the substrate at room temperature to 5 mL of a stirred buffered water solution (0.1 M sodium citrate, pH 5.0 containing 25% of dioxane as cosolvent) purged with O_2 for 30 min before the addition of the reagents. The oxidation of benzylic alcohols **6**–**7** with all the mediators tested lead to the formation of the corresponding aromatic aldehydes as the only reaction products (Scheme 3). The yields of the aldehydes, referred to the initial amount of the substrate, were determined by GC analysis and are reported in Table 2.



Scheme 3.

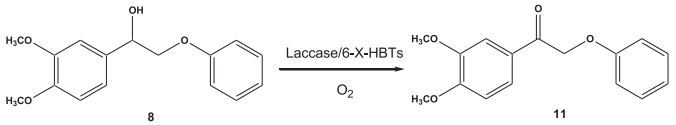
Table 2
Yields (%)[a] of aromatic aldehydes (**9**–**10**) in the oxidation of ring substituted benzyl alcohols (**6**–**7**) by the laccase/6-X-HBTs/O_2[b]



	Aldehyde	
	9[c]	**10**[d]
X=CF_3	3	2
X=Cl	15	13
X=H	57	62
X=CH_3	75	95
X=OCH_3	21	47

[a] The yields % are referred to the initial amount of substrate. Average of 3 determinations. The error is ±5%.
[b] At room temperature in 0.1 M sodium citrate buffered solution, pH 5.0 with 25% dioxane as cosolvent, laccase 7 units.
[c] Reaction time 24 h.
[d] Reaction time 5 h.

The oxidation of 1-(3,4-dimethoxyphenyl)-2-phenoxyethanol (**8**) with all the mediators tested leads to the formation of the corresponding aromatic ketone 1-(3,4-dimethoxyphenyl)-2-phenoxyethanone (**11**) as the exclusive reaction product (Scheme 4).



Scheme 4.

The yields of ketone **11**, referred to the initial amount of substrate **8**, were determined by GC and ^{1}H NMR spectroscopic analysis and are reported in Table 3. A good material balance (>95%) was observed in all the experiments. In the absence of either the mediator or laccase, no products have been observed in significant amounts (<0.1%).

Table 3
Yields (%)[a] of 1-(3,4-dimethoxyphenyl)-2-phenoxy-ethanone (**11**) in the oxidation of 1-(3,4-dimethoxyphenyl)-2-phenoxyethanol (**8**) by the laccase/6-X-HBTs/O_2 system[b]

Mediator	Yields (%)
X=CF_3	3
X=Cl	14
X=H	27
X=CH_3	31
X=OCH_3	5

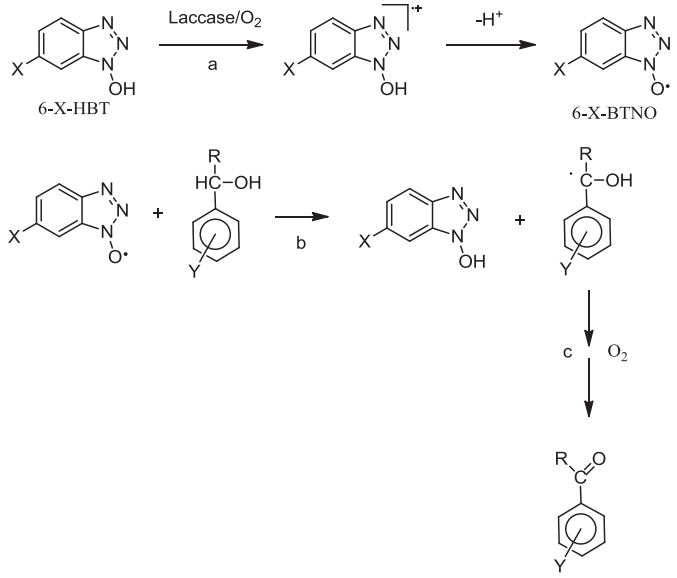
[a] The yields % are referred to the initial amount of substrate. Average of 3 determinations. The error is ±5%.
[b] At 30 °C in 0.1 M sodium citrate buffered solution, pH 5.0 with 25% dioxane as cosolvent, laccase 40 units. Reaction time 16 h.

Product yields and turnover numbers referred to the mediator amounts are in line with those observed in closely related studies for the oxidation of lignin model compounds promoted by the laccase/mediator/O_2 systems under similar reaction conditions.[5b–f,7f,14]

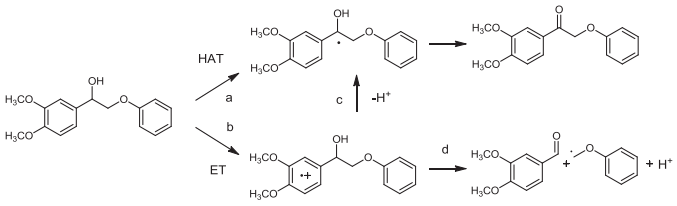
Formation of aromatic aldehydes **9** and **10** in the oxidation of benzylic alcohols **6** and **7**, as well as of aromatic ketone **11** in the oxidation of the dimeric lignin model **8** can be rationalized on the basis of the mechanism proposed for the *N*-hydroxylamine mediated oxidation of benzylic alcohols by laccase[6c,7f,i] and described in Scheme 5.

The oxidation of 6-X-HBT by laccase leads to the *N*-oxyl radical (6-X-BTNO) (Scheme 5, step a), which can abstract a benzylic hydrogen atom from the substrate (step b) regenerating the 6-X-HBT and leading to an α-hydroxyl benzyl radical, which is then oxidized by oxygen to give the carbonyl product (step c).

In previous studies we found that short-lived aminoxyl radicals, like 6-X-BTNOs, are also able to promote the oxidation of easily oxidizable substrates by an ET mechanism.[27] The observation of 1-(3,4-dimethoxyphenyl)-2-phenoxyethanone (**11**) as the exclusive oxidation product of **8** excludes the possibility that the hydrogen transfer from the substrate to the 6-X-BTNO occurs by a two-step electron transfer-proton transfer mechanism (ET-PT process, Scheme 6, path b and c) instead of a single step hydrogen atom transfer mechanism (HAT, Scheme 6, path a). In fact, in the case of an ET process, formation of 1-(3,4-dimethoxyphenyl)-2-phenoxyethanone by deprotonation of the radical cation (Scheme 6, step c) is always accompanied by formation of 3,4-dimethoxybenzaldehyde as a result of the C_α–C_β bond cleavage in the radical cation (Scheme 6, step d)[28] in contrast with our present finding.



Scheme 5. Mechanism of oxidation of benzyl alcohols promoted by the laccase/6-X-HBTs/O_2 systems.



Scheme 6.

From the data reported in Tables 2 and 3 we can observe that the HBT ring substituent exerts a very significant effect on the catalytic efficiency in the oxidation of all the benzylic substrates, the reactivity increasing as the electron donating properties of the aryl substituent increase up to a maximum value for 6-Me-HBT and then decrease for 6-MeO-HBT. The reaction yields are very small in the presence of the strong electron withdrawing CF_3 substituent while 6-Me-HBT proved to be a very efficient mediator, much better than the unsubstituted HBT.[29]

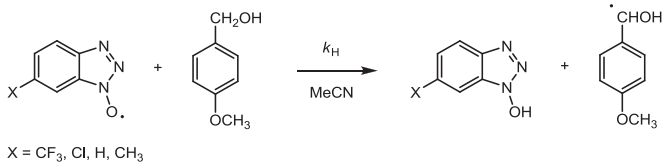
This type of bell shaped profile has been observed when the mediation efficiency for a large series of NO–H laccase mediators has been analyzed as a function of the mediator redox potential, and was attributed to the balance of two opposite effects on the overall reactivity exerted by the variation of the mediator redox potential ($E°_{N-OH}$).[13c]

On the basis of the reaction mechanism described in Scheme 5, the overall reactivity should depend on the ease of the oxidation of 6-X-HBTs by laccase (step a) as well as on the reactivity of the 6-X-BTNOs in the hydrogen atom transfer process (step b). Thus, it seems more appropriate to analyze the mediation efficiency as a function of both the mediator redox potential ($E°_{N-OH}$) and the BDE_{NO-H} values.

The former process should be favored by electron donor X-substituents that lower the mediator redox potential[13c,26] (Table 1) and increase the rate of step a.[13]

An opposite electronic effect should be exerted by the HBT ring substituent in the hydrogen atom abstraction from the benzylic alcohol by the 6-X-BTNO (Scheme 5, path b). In fact, this process is

favored by electron withdrawing substituents that increase the BDE_{NO-H} of 6-X-HBTs as shown by the results of the UV–vis radical equilibration experiments (Table 1). Accordingly, the rate constants for the HAT process from several classes of organic compounds by 6-CF_3-BTNO are significantly higher than those observed in the reactions promoted by BTNO.[24,30] This conclusion has been confirmed by the results of the kinetic studies of the hydrogen atom abstraction from 4-methoxybenzyl alcohol (**6**) and 1-(3,4-dimethoxyphenyl)-2-phenoxyethanol (**8**) to the 6-X-BTNOs in MeCN (Scheme 7).



Scheme 7.

The kinetic studies were carried out by UV–vis spectrophotometry generating the 6-X-BTNOs by oxidation of 6-X-HBTs with cerium(IV) ammonium nitrate as reported in the literature.[24,30,31] Using an excess of **6** or **8** under pseudo first-order conditions, the observed rate constants (k_{obs}) were measured by following the decay of the 6-X-BTNOs at their absorption maxima. Clean first order decays were observed and excellent linear fits were obtained by plotting k_{obs} as a function of the concentration of the substrates. From the slope of these plots, the second order rate constants for the HAT processes (k_H) were determined. The k_H values are reported in Table 4. A significant increase of reactivity of the 6-X-BTNOs in the HAT process from both **6** and **8** is observed by increasing the electron withdrawing power of the substituent thereby confirming the previous statement.

Table 4
Second order rate constants (k_H) for the hydrogen atom transfer reaction from 4-methoxybenzyl alcohol (**6**) and 1-(3,4-dimethoxyphenyl)-2-phenoxyethanol (**8**) to aryl substituted benzotriazolyl *N*-oxyl radicals (6-X-BTNOs) in MeCN

	k_H (M^{-1} s^{-1})	
	6	**8**
X=CF_3	73±3	44±2
X=Cl	38±2	25±1
X=H	9.3±0.3	7.9±0.3
X=CH_3	5.8±0.5	4.8±0.5

The significant increase in the HBTs mediation efficiency observed with the electron donor substituent would seem to indicate that the effect of the HBTs aryl substituent on step a of Scheme 5 is more important than that on step b. With 6-CH_3O-HBT, the relatively low BDE_{NO-H} value likely determines a significant contribution of the hydrogen atom transfer step from the substrate to 6-CH_3O-BTNO thus decreasing the product yields. It is interesting to note that 4-CH_3O-NHPI resulted instead the more efficient mediator in the oxidation of lignin model compounds promoted by the laccase/4-X-NHPIs/O_2 systems.[14] This difference can be rationalized by considering that HBTs are characterized by lower redox potentials and BDE_{NO-H} values with respect to NHPIs,[14,16] thus the contribution of the HAT step to the overall reactivity is more significant in the series of HBTs mediators where 6-CH_3O-HBT displayed a lower efficiency than 6-CH_3-HBT whereas 4-CH_3O-NHPI was the best mediator among the aryl substituted NHPIs.

3. Conclusions

The introduction of substituents in the aromatic ring of 1-hydroxybenzotriazole caused a significant variation of the mediation efficiency in the laccase promoted oxidation of benzylic substrates. A bell shaped profile is observed for the yields of the oxidation products on going from the strong electron withdrawing CF_3 substituent to the electron releasing CH_3O group with a maximum value observed for 6-CH_3-HBT, which is by far the most efficient mediator, even more efficient than the unsubstituted HBT. This result can be rationalized considering that, for most of the 6-X-HBT investigated, the overall reaction rate is governed by the oxidation of the ring substituted HBTs to the corresponding *N*-oxyl radical (6-X-BTNOs) by laccase with the exception of 6-CH_3O-HBT for which the substrate oxidation step becomes rate determining.

The particularly high mediation efficiency found with 6-CH_3-HBT represents an indication of a possible application of this compound in combination with laccase, either for synthetic purposes or for the environmentally benign oxidative delignification of wood pulps and degradation of organic pollutants.

4. Experimental section

4.1. Instrumentation

^1H NMR spectra were recorded on a Bruker AC300P spectrometer in $CDCl_3$. GC–MS analyses were performed on a HP5890 GC (OV 1 capillary column, 12 m×0.2 mm) coupled with a HP5970 MSD. GC analyses were carried out on a Varian CP-3800 (OV 1701 capillary column, 30 m×0.25 mm). UV–vis measurements were performed on a HP Vectra 8453 Diode Array spectrophotometer.

4.2. Starting materials

CH_3CN (spectrophotometric grade) was used for all the spectrophotometric studies. Aqueous solutions were prepared using 18.2 MΩ cm^{-1} Milli-Q water at 25 °C obtained from a Millipore system (0.22 μm filter). Commercial samples of 1-hydroxybenzotriazole (HBT), 6-trifluoromethyl-1-hydroxybenzotriazole (6-CF_3-HBT), cerium(IV) ammonium nitrate, citric acid, 4-methoxybenzyl alcohol, 3,4-dimethoxybenzyl alcohol were used as received. 6-Methyl-1-hydroxybenzotriazole (6-CH_3-HBT), 6-chloro-1-hydroxy-benzotriazole (6-Cl-HBT), 6-methoxy-1-hydroxybenzotriazole (6-MeO-HBT), and 1-(3,4-dimethoxyphenyl)-2-phenoxyethanol, 1-(3,4-dimethoxy-phenyl)-2-phenoxyethanone were synthesized according to the literature.[26,28a] Laccase from a strain of *Trametes villosa* (viz. *Poliporus pinsitus*) (Novo Nordisk Biotech) was employed. It was purified by ion-exchange chromatography on Q-Sepharose by elution with a mixture of 10 mM Tris–HCl and 0.2 M NaCl (linear gradient 1–70 mM NaCl)[32] and its activity (9000 U/ml) was determined spectrophotometrically by the standard reaction with ABTS.[33]

4.3. Spectrophotometric studies

4.3.1. Determination of BDE_{NO-H}. In a 3 mL quartz cuvette 150 μL of a 2 mM solution of cerium(IV) ammonium nitrate (CAN) in CH_3CN was added to 2.85 mL of a solution of the *N*-hydroxylamine (4-MeO-HPI, 6-CF_3-HBT or HBT, final concentration 10 mM) in CH_3CN. Then a 10 mM solution of *N*-hydroxyderivative (6-CF_3-HBT, HBT, 6-Me-HBT, or 6-Cl-HBT) in MeCN was added (final concentrations: 1 mM, 2 mM, and 3.3 mM). After the addition the UV–vis spectra were immediately recorded.

4.3.2. Kinetic studies of the HAT reactions from 4-methoxybenzyl alcohol and 1-(3,4-dimethoxyphenyl)-2-phenoxyethanol. In a 3 mL quartz cuvette 150 μL of a 5 mM CAN solution in CH_3CN was added

to 2.85 mL of a solution of 6-X-1-hydroxybenzotriazoles (X=CF_3, Cl, H, CH_3, final concentration 1 mM) in CH_3CN. After the generation of the *N*-oxyl radicals 6-X-BTNOs, an excess of the alcohol in MeCN was added in order to operate under pseudo first order conditions (final concentrations within the range of 1.5–14 mM).

4.4. Enzymatic oxidations

The mediators 6-X-HBTs (10 μmol), laccase from *Trametes villosa* (7–40 units) and the substrate (30 μmol) were added to a 5.0 mL of a buffered water solution (0.1 M sodium citrate, pH 5.0) with 25% dioxane as cosolvent, purged with O_2 for 30 min before the addition of the reagents. The mixture was magnetically stirred at 30 °C for 5–24 h under oxygen (filled balloon). Reaction products were extracted with ethyl acetate, characterized by GC–MS and ^{1}H NMR and quantified by GC and ^{1}H NMR analysis. A good material balance (>95%) was observed in all the experiments. In the absence of the mediator or the enzyme no formation of oxidation products was observed in significant amounts (<0.1%).

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Supplementary data

Determination of BDE_{NO-H} of 6-X-HBTs. Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2014.02.068.

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18. Eq. 2 is strictly valid only when applied to the equilibration of compounds characterized by closely related structures since only in this case the entropic variation of the two O–H dissociation processes are similar.[19] However to a first approximation most organic HAT processes typically have $|\Delta S^\circ| \cong 0$ because there is no change in the charges of the species involved and little change in their sizes.[20]
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22. Since the correlation has only four data points with r^2=0.921, the value of BDE_{NO-H}=84.3 kcal mol^{-1} should be subject to some error.
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