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Nuclear Amination Catalyzed by Fungal Laccases: Reaction Products of *p*-Hydroquinones and Primary Aromatic Amines

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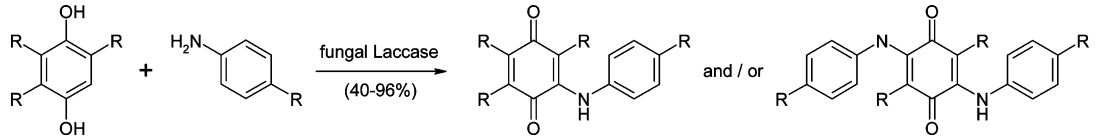

Nuclear amination of *p*-hydroquinones with primary aromatic amines was catalyzed by fungal laccases (EC 1.10.3.2) from *Trametes spec.* and *Myceliophthora thermophila*. This is the first report of laccase-catalyzed synthesis of aminoquinones. Incubation of two compounds with laccase in the presence of oxygen resulted in the formation of the corresponding monoaminated or diaminated quinones. No hydroquinonoids were formed. Observed differences in the reaction courses for different *p*-hydroquinones and aromatic amines with different laccases are discussed.

Introduction

Laccases (benzenediol:oxygen oxidoreductase, EC 1.10.3.2) are copper-containing phenol oxidases.[1,2] They are widely distributed in plants and fungi. Laccases are able to abstract hydrogen from phenolic hydroxyl groups by using molecular oxygen as an electron acceptor, resulting in phenoxy radicals. These radicals can undergo a broad variety of reactions.

Main areas of application discussed for laccases are waste detoxification, textile dye transformation, biosensors, and applications in the food industry.[3−6] However, the high stability of laccases in solution, the mild reaction conditions used in laccase-catalyzed reactions, and the enzymes selectivity for phenolic substructures make laccases attractive for fine chemical synthesis, as well. Therefore, interest in the potential use of these enzymes in organic synthesis has increased recently.[7] Reports about laccase-catalyzed reactions include the synthesis of actinocin[8] and cinnabarinic acid,[9] the synthesis of substituted triazolobenzothiadiazinones,[10] dimerization of various compounds such as estradiol,[11] penicillin X,[12] and bisphenol A,[13] synthesis of polymers,[14−16] oxidative coupling of hydroquinone and mithramicine[17] or (+)-catechin,[18] derivatization of dihydrocaffeic acid,[19] and oxidation of substituted imidazoles.[20] Structural characterization of oxidative coupling products with hydroquinonoids being one of the substrates showed the

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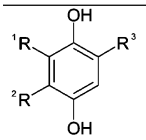
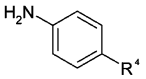
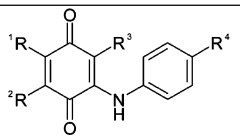
TABLE 1. *p*-Hydroquinones and Aromatic Amines Used as Educts in This Study and Synthesized Monoaminated Products (Yield in Parentheses)

p-hydroquinone	amine	monoaminated product
		
1a: $R^1 = R^2 = H$, $R^3 = CONHCH_2CH_2OH$	**2a**: $R^4 = COOH$	**3a** (70 %)
1a	**2b**: $R^4 = COCH_3$	**3b** (65 %)
1a	**2c**: $R^4 = CH_2CH_2OH$	**3c** (69 %)
1b: $R^1 = R^2 = H$, $R^3 = CONH_2$	**2a**	**3d** (71 %)
1c: $R^1 = R^2 = H$, $R^3 = COOCH_3$	**2d**: $R^4 = CH_2COOH$	**3e** (79 %)
1d: $R^1 = R^2 = H$, $R^3 = COOCH_2CH_3$	**2e**: $R^4 = CONHCH_2COOH$	**3f** (70 %)
1d	**2f**: $R^4 = CH(CH_3)_2$	**3g** (59 %)
1e: $R^1 = R^2 = H$, $R^3 = COCH_3$	**2a**	**3h** (40 %)
1f: $R^1 = R^2 = H$, $R^3 = CH_3$	**2a**	no products isolated
1g: $R^1 = R^3 = H$, $R^2 = C(CH_3)_3$	**2b**	**3i** (62 %)
1g	**2g**: $R^4 = COOCH_3$	**3j** (61 %)
1g	**2h**: $R^4 = N(CH_2CH_2)_2O$	**3k** (92 %)
1h: $R^1 = R^2 = CH_3$, $R^3 = H$	**2a**	**3l** (60 %)
1h	**2f**	**3m** (91 %)
1h	**2c**	**3n** (94 %)
1h	**2d**	**3o** (92 %)
1i: $R^1 = R^2 = R^3 = CH_3$	**2b**, **2e**	no products formed

products to be either quinonoids[8−10,21] or hydroquinonoids.[17−19]

To further determine the potential use of laccase-catalyzed reactions in fine chemical synthesis and to gain more information about the properties of the products of laccase-catalyzed syntheses, various *p*-hydroquinones were brought to reaction with primary aromatic amines by using fungal laccases from *Trametes spec.* and *Myceliophthora thermophila*.

In this study, we show that incubation of two compounds with laccase in the presence of oxygen resulted in the formation of the corresponding monoaminated or diaminated quinones. No hydroquinone derivatives were formed. Previous reports[19,22] about aminohydroquinones as products in laccase-catalyzed reactions are not maintainable anymore, as one of the products of the reported reactions is shown here to be in fact a quinonimine. Formation of mono- or diaminated products was favored depending on the reaction conditions. Three of the reaction products were structurally characterized in more detail. The two studied laccases gave different reaction courses. These differences are discussed, taking into account the properties of the different *p*-hydroquinones, amines, and laccases used.

Results

General Observations. *p*-Hydroquinones used in this study can be grouped into 2,5-dihydroxybenzoic acid derivatives and alkylated *p*-hydroquinones, primary aromatic amines into 4-aminobenzoic acid derivatives and para-substituted anilines (for structures see Table 1). The resulting four possible combinations show different reaction courses which are described below. Products synthesized for this study are summarized in Tables 1 and 2.

The *p*-hydroquinones used in this study were substrates of laccases. In the absence of amine, homomolecular oxidation products were formed, whereas amines alone gave no reactions after addition of enzyme. Homomolecular oxidations catalyzed by laccases were also observed with *o*-dihydroxylated compounds.[19,21]

The homomolecular products of the alkylated hydroquinones were stable both in solution and as isolated compounds. MS and NMR data of the oxidation product of hydroquinone **1g** showed this product to be the corresponding quinone (concordant with the literature;[23] data not shown). The formation of quinones in laccase-catalyzed reactions has been observed before in the oxidation of chlorophenols.[24,25] In the case of the benzoic acid derivatives, the homomolecular products were stable

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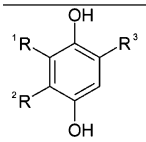
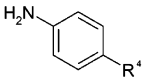
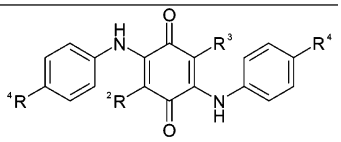
TABLE 2. Synthesized Diaminated Products (Yield in Parentheses)

p-hydroquinone	amine	diaminated product
		
1a	**2a**	**4a** (96 %)
1c	**2d**	**4b** (96 %)
1d	**2e**	**4c** (93 %)
1d	**2f**	**4d** (13 %)

in solution but too unstable for isolation as pure compounds. Due to the characteristic UV−vis spectra of quinones, we assume these products to be the corresponding quinones, as well.

Formation of quinonamines was visible due to their red or brown color, which also simplified product isolation. Products were obtained by solid-phase extraction (products soluble in buffer) or centrifugation (insoluble products) in sufficient amounts and purity for structural characterization. Due to their characteristic UV−vis spectra, formation of mono- and diaminated products could easily be detected by HPLC by using a diode array detector (200−595 nm).

Reaction of 2,5-Dihydroxybenzoic Acid Derivatives with Aromatic Amines. Laccase-catalyzed reactions of 2,5-dihydroxybenzoic acid derivatives with 4-aminobenzoic acid derivatives generally proceeded very fast. With use of laccase from *T. spec.*, the compounds were consumed after an incubation time of 45 min. The products seemed to be stable in the reaction solution. The reaction proceeded in one step, and only trace amounts of homomolecular products were observed. If equimolar concentrations of compounds were used, the diaminated product was formed among the monoaminated product in low yields (approximately 10%). If the concentration of compounds in the reaction mixture exceeded 2 mM, a higher amount of diaminated product and some other yet uncharacterized byproducts were formed. In reactions where an excess of amine (5:1) was used, only diaminated product could be detected in high amounts after 105 min, intermediately formed monoaminated product was not detectable anymore. When using laccase from *M. thermophila* in equimolar reactions, more diaminated quinone was produced than when using laccase from *T. spec.*, and some uncharacterized byproducts were formed, as well. Soon after maximal product formation, the amounts of products in solution began to decrease. The courses of the reaction for the formation of **3a**[26] and **4a** are shown in Figure 1.

After addition of inactivated enzyme to the reaction mixture, only diaminated product was formed very slowly in very low amounts (data not shown).

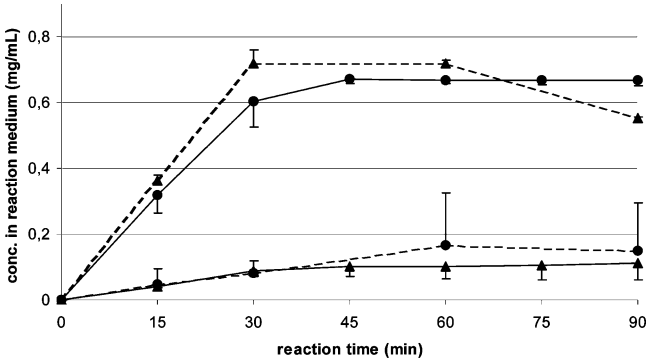


FIGURE 1. Reaction course of product formation for **3a** (●) and **4a** (▲) in equimolar reactions (2 mM), using laccase from *T. spec*. (solid lines) or *M. thermophila* (dashed lines).

Laccase-catalyzed reactions of 2,5-dihydroxybenzoic acid derivatives with anilines, using laccase from *T. spec.*, proceeded in a similar manner as the reactions with 4-aminobenzoic acid derivatives, but more byproducts were formed, diminishing the yields of products.

Reaction of Alkylated *p*-Hydroquinones with Aromatic Amines. Amination of alkylated *p*-hydroquinones with aromatic amines using laccase was slow and proceeded in two steps. In a first step, the *p*-hydroquinone was oxidized to the corresponding quinone, and in a second step the amination took place. If equimolar concentrations of compounds were used, the reaction was not completed within 36 h. Therefore for all reactions including alkylated hydroquinones, amine excess (5:1) had to be used. Under these conditions and with laccase from *M. thermophila*, the quinones were consumed after an incubation time of approximately 22 h. Formation of diaminated products could only be observed in one case. When laccase from *T. spec.* was used, product formation proceeded even more slowly and to a much lower extent, although quantitative quinone formation was observed, as well. The reaction courses for the formation of ***3l*** are shown in Figure 2.

Laccase-catalyzed reactions of alkylated hydroquinones with anilines using laccase from *M. thermophila* proceeded in an identical manner as the reactions with 4-aminobenzoic acid derivatives.

Detailed Structural Characterization of Three Products. The reaction in which **3a** and **4a** are formed

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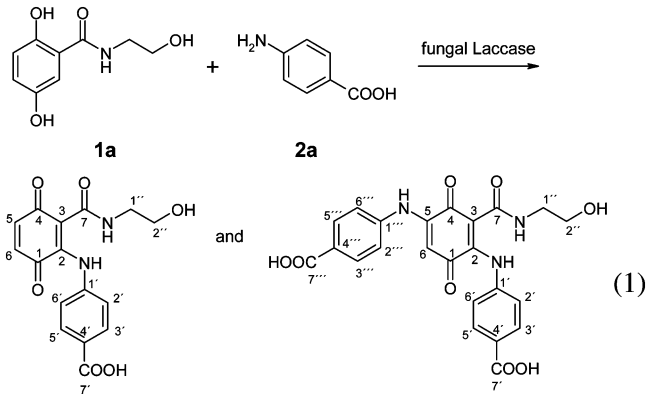
(26) This product has been found in laccase-catalyzed reactions by K. Manda, as well; personal communication.

TABLE 3. ^{1}H and ^{13}C Assignments and HMBC Correlations for 3a and 4a[a]

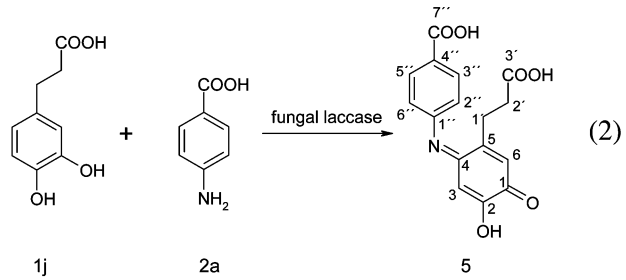
	3a			4a		
carbon	^{13}C	^{1}H	^{1}H−^{13}C correlations	^{13}C	^{1}H	^{1}H−^{13}C correlations
5	133.8	6.76, d, 1H (9.3)	C1, C3	153.0		
6	138.9	6.81, d, 1H (9.5)	C2, C4	98.4	5.99, s, 1H	C2, C4
2′, 6′	123.3	7.30, d, 2H (7.9)	C1′, C2′, C4′, C6′	123.8	7.28, d, 2H (8.5)	C1′, C2′, C4′, C6′
3″, 5′	129.7	7.84, d, 2H (8.5)	C1′, C2′, C3′, C5′, C6′, C7′	129.6	7.85, d, 2H (8.5)	C1′, C2′, C3′, C5′, C6′, C7′
1″	41.2	3.17, q, 2H (5.7)	C7, C2″	41.2	3.12, q, 2H, (5.7)	C2″
2″	59.3	3.41, t, 2H (5.6)	C1″	59.3	3.42, t, 2H (5.9)	C1″
2‴, 6‴				122.6	7.52, d, 2H (8.6)	C1‴, C2‴, C4‴, C6‴
3‴, 5‴				130.5	7.99, d, 2H (8.6)	C1‴, C2‴, C3‴, C5‴, C6‴, C7‴
NH (N_1)		9.14, t, 1H (5.3)	C3, C7, C1″, C2″		9.00, t, 1H (4.9)	
NH (N_2)		12.42, br s, 1H	C1, C3, C2′, C6′		12.38, br s, 1H	
NH (N_3)					9.62, br s, 1H	C4, C6, C2‴, C6‴

[a] Measured in d_6-DMSO at 100.11 (^{13}C) or 400.13 MHz (^{1}H, J (in Hz) values in parentheses). Chemical shifts are expressed in δ (ppm) calibrated on the resonances of the residual nondeuterated solvent.

is depicted in eq 1, and the reaction in which **5** was synthesized is shown in eq 2.



(1)



(2)

3a showed typical absorption maxima for monoaminated quinonamines,[27−29] such as the weak maximum around 500 nm and two maxima under 300 nm, in the UV−vis spectrum. Absorption coefficients were similar to those described in the literature. LC/MS with API-ES and APCI in both positive and negative modes showed the molecular mass of **3a** to be 330 Da. This mass could be attributed to the amination of **1a** with **2a** under loss of four hydrogen atoms. ^{1}H NMR spectral data of **3a** showed characteristic signals for both compounds (see Table 3). Multiplicity of H5 and H6 suggests the amination having taken place at C2. Signals for phenolic hydroxy groups could not be observed, but signals for two amine protons could be seen. The chemical shift of the proton at N2 was strongly shifted to lower field due to formation of a hydrogen bond with the amidic oxygen. ^{13}C NMR showed typical signals for quinones in the range of 180 ppm, another indication for the quinonoid character of **3a**. The HMBC spectrum showed correlations between the protons H5 and H6 and the quinone carbonyl carbons, unambiguously proving **3a** to be the quinonamine and being substituted at C2. Amination at C2 could also be confirmed by cross-signals from the aromatic amine proton with C1 and C3. All results led to the identification of **3a** as 2-[4′-(carboxyphenyl)amino]-3-(2″-hydroxyethylcarbamoyl)-1,4-benzoquinone.

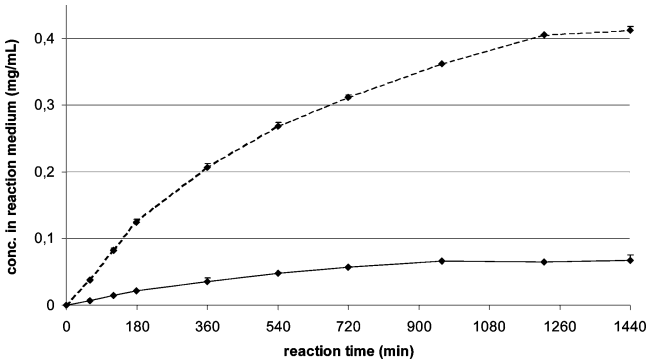


FIGURE 2. Reaction course of product formation for ***3l*** with amine excess (10 mM:2 mM), using laccase from *T. spec*. (solid line) or *M. thermophila* (dashed line).

The UV−vis spectrum of **4a** showed three maxima under 400 nm, whose absorption coefficients were in accordance with those of described diaminated quinones.[27] LC/MS with API-ES and APCI in negative mode showed the molecular mass of **4a** to be 465. This mass could be attributed to the amination of **1a** with two molecules of **2a** under loss of six hydrogen atoms. ^{1}H NMR spectral data of **4a** showed the presence of two aminobenzoic acid residues (Table 3). Only one proton of the aromatic system of **1a** was remaining. As in the case of **3a**, no phenolic hydroxy groups could be seen, but three amine protons could be detected. ^{13}C NMR again showed typical signals for quinones. In the HMBC spectrum a correlation could be seen between the proton H6 and the carbonyl signal at 178.1 ppm, which also showed a cross-signal with the amine proton from N3. This proved **4a** to be the diaminated quinonamine, being

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TABLE 4. ^{1}H and ^{13}C Assignments and HMBC Correlations for 5[a]

carbon	^{13}C	^{1}H	^{1}H−^{13}C correlations
3	102.0	6.01, s, 1H	C1, C4, C5
6	129.4	6.58, s, 1H	C2, C4, C1′
1′	27.1	2.98, t, 2H (7.5)	C5, C6, C2′, C3′
2′	33.8	2.62, t, 2H (7.5)	C5, C1′, C3′
2″, 6″	120.1	6.86, d, 2H (8.3)	C2″, C4″, C6″
3″, 5″	131.5	8.04, d, 2H (8.4)	C1″, C3″, C5″, C7″

[a] Measured in d_8-THF at 150.1 (^{13}C) or 600.13 MHz (^{1}H, J (in Hz) values in parentheses). Chemical shifts are expressed in δ (ppm) calibrated on the resonances of the residual nondeuterated solvent.

substituted at C2 and C5. All results led to the identification of **4a** as 2,5-bis-[4′,4‴-(carboxyphenyl)amino]-3-(2″-hydroxyethylcarbamoyl)-1,4-benzoquinone.

As we reported previously, laccases also catalyze amination of *o*-dihydroxylated compounds.[19] The amination of **1j** with **2a** was reinvestigated. The resulting product **5** had the same UV−vis spectrum as the product reported before,[19] although we here used a different laccase and isolation procedure. ^{13}C chemical shifts and HMBC correlations from H6 to C4 and C2 together with those from H3 to C1 and C4 showed the product to be a quinonimine, which was also confirmed by MS (HMBC NMR correlations in Table 4).

Discussion

Structural characterization of 19 reaction products showed that in the course of laccase-catalyzed reactions, *p*-hydroquinones can be nuclear aminated with aromatic amines. Depending on the substituents of the *p*-hydroquinone, the properties of the aromatic amine, the concentrations of the compounds in solution, and the laccase used, the course of the laccase-catalyzed reaction and the properties of the resulting products differed to a considerable extent.

As shown above, amination of 2,5-dihydroxybenzoic acid derivatives proceeded in one step and much faster than amination of alkylated *p*-hydroquinones, where in a first step a quantitative formation of the corresponding quinone took place. This observation could be explained with a higher reactivity of the corresponding quinones of the 2,5-dihydroxybenzoic acid derivatives, which could also be the reason for their instability when isolated. Unsubstituted 2-carboxy-*p*-benzoquinones were shown to be unstable and highly reactive before.[30,31] The mechanism underlying the reaction is likely to be an intermediate laccase-catalyzed formation of the corresponding quinones and subsequent nonenzymatic amination with aromatic amines by Michael addition.

Products resulting from amination of alkylated hydroquinones with aromatic amines were much more stable than those having 2,5-dihydroxybenzoic acid derivatives as an educt. Low stability of these products in solution sometimes complicated ^{13}C NMR spectroscopy. 2,5-Dihydroxybenzoic acid derivatives aminated with anilines were even less stable than those aminated with 4-aminobenzoic acid derivatives. Amination products of **1b** or **1e** with **2g** were too unstable for isolation and characterization. The observation that monoaminated quinones are less stable than diaminated quinones[32] could be confirmed.

As discussed by Chakraborty et al.,[27] amine excess promoted the formation of diaminated products in nuclear amination reactions.

2,5-Dihydroxybenzoic acid derivatives being substituted first at C6 might be due to mesomeric effects of the carbonyl function as well as the formation of a six-membered ring via a hydrogen bond.

Interestingly, **1i** did not give amination products under the reaction conditions used, despite being sterically less hindered than the 2,5-dihydroxybenzoic acid derivatives and, as quinone formation was quantitative, being a laccase substrate like the other alkylated hydroquinones.

According to HPLC analysis, **1f** and **2a** seemed to give two monoaminated products and a very small amount of diaminated product (products not isolated). This was the only observed diaminated product among the alkylated hydroquinones.

In accordance to the literature,[33,34] the second substitution of monoaminated substances usually took place *para* to the first amination site. This could be the reason for the observation that the sterically hindered alkylated hydroquinones, except for **1f**, did not react further after the first amination step.

A fundamental difference concerning the reactivity of 2,5-dihydroxybenzoic acid derivatives and alkylated hydroquinones could be seen in regard to the employed laccase. 2,5-Dihydroxybenzoic acid derivatives were aminated efficiently only by laccase from *T. spec.*, whereas the use of laccase from *M. thermophila* was only efficient with alkylated hydroquinones. As buffers of different pH were used for the two laccases, and a pH dependence in the amination of *p*-quinones is known,[35] it is probable that the observed differences were due to different pH values of the reaction mixture rather than different laccase specificity. This assumption is further strengthened by the fact that the amination of the intermediately formed quinone from **1h** is very slow in the reaction medium used for the laccase from *T. spec.*, although quinone formation is quantitative with this laccase, as well. Anyway, the fact that the laccases show their maximal activities at different pH values allows amination reactions to be performed at optimal pH, using the most active laccase.

In experiments similar to those described here, the products of a laccase-catalyzed amination seemed to retain a hydroquinonoid character.[19] Therefore it was surprising for us to find all our products to be quinonoid. We did a detailed reinvestigation of the synthesis, isolation, and structure of the reaction product of **1j** and **2a** and could show that the resulting product **5** is a quinonimine. Quinonimine formation from *o*-quinones

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and amines has been observed before.[36,37] In contrast to the reactions described in these reports, no diamination took place in the amination of **1j**. The propionic acid residue seems not to be displaceable as it is described for alkyl groups,[37] so that the reaction stops at the monoaminated product.

Our results suggest that laccase-catalyzed amination of *o*- or *p*-hydroquinones with amines always results in the formation of the corresponding mono- or diaminated quinonamines or quinonimines, and that laccase-catalyzed reactions are not suitable for the synthesis of aminohydroquinones. In fact, it would be highly surprising if aminohydroquinones were synthesizable with use of oxidative enzymes under oxygen, given that aminohydroquinones are highly susceptible to oxidation.[38]

As addition of amines to quinonoid systems are well-known reactions in organic synthesis,[39] further studies will be performed to investigate the differences and similarities between laccase-catalyzed and nonenzymatic nuclear amination.

Conclusions

Though nuclear amination reactions of *p*-hydroquinones with aromatic amines have been studied before, we showed here for the first time that laccases provide a new synthetic route to aminoquinones. Employing laccase-catalyzed reactions, mono- and diaminated quinones can be synthesized in good to very good yields starting from *p*-dihydroxylated benzoic acid derivatives or alkylated hydroquinones and primary aromatic amines.

Experimental Section

Enzymes. Extracellular laccase C of *Trametes spec.* (EC 1.10.3.2) was obtained from ASA Spezialenzyme (Wolfenbüttel, Germany) and used as received (activity 1000 U/g; substrate: syringaldazine). Boiled enzyme was held at 100 °C for 60 min in buffer prior to use.

Laccase from *Myceliophthora thermophila* (expressed in genetically modified *Aspergillus sp.*) was bought from NovoNordisk (Bagsvaerd, Denmark). It was used as received (activity 1000 U/g; substrate: syringaldazine).

Amination of 2,5-Dihydroxybenzoic Acid Derivatives and 1j with Aromatic Amines. For analytical scale experiments, the compounds were incubated at equimolar concentrations of 1, 2, 5, and 10 mM with laccase in 10 mL of 20 mM sodium acetate buffer, pH 5 (laccase from *T. spec.*, final activity 0.15 units·mL^{-1}) or 10 mL of citrate−phosphate buffer (16 mM citrate, 164 mM phosphate), pH 7 (laccase from *M. thermophila*, final activity 1.0 units·mL^{-1}). The reaction mixture was incubated at room temperature with agitation at 300 rpm. For production scale, laccase of *T. spec.* (final activity 0.1 units·mL^{-1}) was added to 100 mL of a 2 mM solution of the compounds dissolved in sodium acetate buffer under the same conditions. Products were isolated 60 to 90 min after starting the reaction. For the synthesis of diaminated quinones, amine excess was used (10:2 mM).

Amination of Alkylated Hydroquinones with Aromatic Amines. Analytical scale experiments were done as described above. For production scale, laccase of *M. thermophila* (final activity 1.0 units·mL^{-1}) was added to 100 mL of a solution of the compounds (2 mM hydroquinone, 10 mM amine) dissolved in citrate−phosphate buffer under the same conditions. Products were isolated approximately 24 h after addition of the enzyme.

Oxidation of *tert*-Butylhydroquinone. Laccase of *M. thermophila* (final activity 1.0 units·mL^{-1}) was added to 100 mL of a 4 mM solution of **1g** dissolved in citrate−phosphate buffer under the conditions used for the amination of alkylated hydroquinones. The product was isolated approximately 2 h after starting the reaction.

Isolation of the Products. Products soluble in buffer (3a−h, 3*l*, 3n,o, 4a−c, 5): All isolation steps were performed by solid-phase extraction with an RP_{18} silica gel column (Strata C18-E, 50 μm, 70 Å, 5 g/20 mL, Phenomenex, Aschaffenburg, Germany). After activation with methanol and equilibration with methanol/aqua dest. (10:90 v/v), the column was charged with 50 mL of the reaction mixture. Washing steps with 25 mL of aqua dest. and 25 mL of methanol/aqua dest. (10:90 v/v) were carried out to remove laccase and polar impurities from the column. Monoaminated products were eluted with methanol/aqua dest. (50:50 v/v). The dark red fraction was collected, ignoring the less dark front and tail of this fraction. After complete elution of this fraction from the column, solvent was switched to methanol to elute the yellow-brown fraction (if diaminated products were formed). This procedure was repeated. After solid-phase extraction of the products, the corresponding fractions were combined and dried under vacuum at 30 °C or lyophilized. In the case of **3f** and **5**, acetonitrile was used instead of methanol due to fast degradation of the products in methanol.

Products with low solubility in buffer (3i−k, 3m, 4d): Reaction mixtures were spun in portions of 50 mL for 20 min at 4000 rpm with a centrifuge. The combined residues were washed thrice with 20 mL of methanol/aqua dest. (5:95 v/v). Products were dissolved in methanol and dried under vacuum at 30 °C.

Yields have not been optimized.

2-[4′-(Carboxyphenyl)amino]-3-(2″-hydroxyethylcarbamoyl)-1,4-benzoquinone (3a). Synthesis and isolation as described above. Dark red to purple solid. Yield 70% (46.2 mg); mp 184−186 °C. ^{1}H NMR δ 12.79 (br s, 1H), 12.42 (br s, 1H), 9.14 (t, J = 5.3 Hz, 1H), 7.84 (d, J = 8.5 Hz, 2H), 7.30 (d, J = 7.9 Hz, 2H), 6.81 (d, J = 9.5 Hz, 1H), 6.76 (d, J = 9.5 Hz, 1H), 4.77 (br s), 3.41 (t, J = 5.6 Hz, 2H), 3.17 (q, J = 5.7 Hz, 2H). ^{13}C NMR δ 184.2 (C-4), 182.9 (C-1), 166.7 (C-7′), 166.3 (C-7), 148.6 (C-2), 143.4 (C-1′), 138.9 (C-6), 133.8 (C-5), 129.7 (C-3′, C-5′), 127.2 (C-4′), 123.3 (C-2′, C-6′), 105.5. (C-3), 59.3 (C-2″), 41.2 (C-1″). HMBC correlations see Table 3. IR (KBr) ν 3436, 2941, 1691, 1644, 1563, 1542 cm^{-1}. UV−vis (MeOH) λ_{max} (log ϵ) 201 (4.46), 261 (4.24), 501 (3.63) nm. LC/MS *m/z* (rel intensity) 331 ([M + 1]$^+$, 63), 270 (100) APCI, pos. mode; 330 ([M]$^-$, 100) APCI, neg. mode; 353 ([M + Na]$^+$, 100), 683 ([2M + Na]$^+$, 19) API-ES, pos. mode; 329 ([M − 1]$^-$, 100) API-ES, neg. mode. HRFT-ICRMS [M + H]$^+$ calcd for $C_{16}H_{15}N_2O_6$ 331.0930, found 331.0921.

2,3-Dimethyl-5-[(4′-carboxyphenyl)amino]-1,4-bezoquinone (3*l*). Synthesis and isolation as described above. Dark violet solid. Yield 60% (32.4 mg); mp 179−181 °C. ^{1}H NMR δ 7.96 (d, J = 8.4 Hz, 2H), 7.24 (d, J = 8.4 Hz, 2H), 6.06 (s, 1H), 2.02 (s, 3H), 2.00 (s, 3H). ^{13}C NMR δ 188.4, 184.8, 174.6, 145.3, 144.3, 141.5, 138.5, 135.8, 131.7, 122.5, 101.2, 12.6, 12.1. IR (KBr) ν 3281, 1646, 1596, 1524 cm^{-1}. UV−vis (MeOH) λ_{max} (log ϵ) 202 (4.33), 278 (4.28), 497 (3.52) nm. LC/MS *m/z* (rel intensity) 272 ([M + 1]$^+$, 100) APCI, pos. mode; 271 ([M]$^-$, 100) APCI neg. mode; 270 ([M − 1]$^-$, 100), 136 (31) API-ES, neg. mode. HRFT-ICRMS [M + H]$^+$ calcd for $C_{15}H_{14}NO_4$ 272.0923, found 272.0919.

2,5-Bis[4′,4‴-(carboxyphenyl)amino]-3-(2″-hydroxyethylcarbamoyl)-1,4-benzoquinone (4a). Synthesis and isolation as described above. Yellow-brown to brown solid.

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Yield 18% (8.4 mg) in equimolar reactions, 96% (89 mg) with amine excess; mp 242−244 °C. ^{1}H NMR δ 12.95 (br s), 12.38 (br s, 1H), 9.62 (br s, 1H), 9.00 (t, J = 4.9 Hz, 1H), 7.99 (d, J = 8.6 Hz, 2H), 7.85 (d, J = 8.5 Hz, 2H), 7.52 (d, J = 8.6 Hz, 2H), 7.28 (d, J = 8.5 Hz, 2H), 5.99 (s, 1H), 4.75 (br s), 3.42 (t, J = 5.9 Hz, 2H), 3.12 (q, J = 5.7 Hz, 2H). ^{13}C NMR δ 178.9 (C-1), 178.1 (C-4), 167.4 (C-7), 166.7 (C-7′), 166.6 (C-7‴), 153.0 (C-5), 145.9 (C-2), 143.3 (C-1′), 141.8 (C-1‴), 130.5 (C-3‴, C-5‴), 129.6 (C-3′, C-5′), 127.5 (C-4′), 127.2 (C-4‴), 123.8 (C-2′, C-6′), 122.6 (C-2‴, C-6‴), 112.5 (C-3), 98.4 (C-6), 59.3 (C-2″), 41.2 (C-1″). HMBC correlations see Table 3. IR (KBr) ν 3271, 1703, 1647, 1621, 1581, 1567, 1523 cm^{-1}. UV−vis (MeOH) λ_{max} (log ϵ) 202 (4.64), 271 (4.35), 382 (4.27) nm. LC/MS *m/z* (rel intensity) 464 ([M − 1]$^-$, 100) API-ES, neg. mode; 465 ([M]$^-$, 100) APCI, neg. mode. HRFT-ICRMS [M + H]$^+$ calcd for $C_{23}H_{20}N_3O_8$ 466.1250, found 466.1258.

5-Carboxyethyl-*N*-(4′-carboxyphenyl)-2-hydroxy-1,4-quinonimine (5). Synthesis and isolation as described above. Red to red-brown solid. Yield 75% (47.2 mg); mp 164−165 °C. ^{1}H NMR δ 8.04 (d, J = 8.4 Hz, 2H), 6.86 (d, J = 8.3 Hz, 2H), 6.58 (s, 1H), 6.01 (s, 1H), 2.98 (t, J = 7.5 Hz, 2H), 2.62 (t, J = 7.5 Hz, 2H). ^{13}C NMR δ 183.6 (C-1), 173.7 (C-3′), 167.3 (C-7″), 159.9 (C-2), 155.7 (C-1″), 154.9 (C-4), 153.6 (C-5), 131.5 (C-3″, C-5″), 129.4 (C-6), 128.0 (C-4″), 120.1 (C-2″, C-6″), 102.0 (C-3), 33.8 (C-2′), 27.1 (C-1′). HMBC correlations see Table 3. NMR see Table 4. IR (KBr) ν 3427, 1705, 1686, 1634, 1600, 1522 cm^{-1}; UV−vis (MeOH) λ_{max} (log ϵ) 254 (4.11), 290 (4.40), 445 (3.48) nm. LC/MS *m/z* (rel intensity) 314 ([M − 1]$^-$, 100), 270 (55) API-ES, neg. mode; 316 ([M + 1]$^+$, 100), 270 (20) APCI pos. mode. HRFT-ICRMS [M + H]$^+$ calcd for $C_{16}H_{14}NO_6$ 316.0821, found 316.0814.

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Supporting Information Available: Experimental methods; ^{1}H NMR spectra of all compounds; melting point, ^{1}H and ^{13}C NMR, IR, UV−vis, and MS data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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