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Laccase initiated oxidative domino reactions for the efficient synthesis of 3,4-dihydro-7,8-dihydroxy-2*H*-dibenzofuran-1-ones

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Abstract—Laccase initiated domino reactions of cyclohexane-1,3-diones with catechols using air as an oxidant afford 3,4-dihydro-7,8-dihydroxy-2*H*-dibenzofuran-1-ones with yields ranging from 70% to 97%.

The development of enzymatically catalyzed transformations where air may be employed as an oxidant is of great interest to green chemistry, since these reactions represent environmentally benign processes.[1] It is particularly attractive that such oxidations may be performed with catalytic amounts of enzyme in aqueous solvent systems and that the oxidant O_2 will be completely converted into the toxicologically harmless H_2O when suitable oxidases are used.[2]

In the light of the growing importance of enzymatically catalyzed reactions with regard to simple transformations in organic synthesis,[2] it is surprising that the potential of enzymatically initiated domino reactions has so far hardly been exploited.[3,4] In this context, enzymes that are capable of conducting an oxidation triggering a domino reaction are very promising. This is why laccases, for example, have a very high potential for the development of oxidative domino processes.

Laccases mainly occur in fungi, but also in plants and some prokaryotes. They are easy to isolate and some are even commercially available. They are characterized by their ability to catalyze the oxidation of various substrates with O_2.[5] These substrates include phenolic compounds, which may be transformed into lignanes and/or lignin by oxidative coupling.[6] Also known is the laccase catalyzed oxidation of benzylic alcohols into benzaldehydes, which is performed in the presence of mediators like ABTS.[7] In addition, laccases have been used for the oxidation of catechols and hydroquinones into their corresponding quinones[8] which subsequently may undergo further reactions. The laccases (benzenediol: O_2 oxidoreductase E.C. 1.10.3.2.) are multicopper oxidases which are able to catalyze the oxidation of a substrate with simultaneous reduction of O_2 to give H_2O.[9] They have a type 1 (T1) Cu center, a type 2 (T2) Cu center, and a type 3 (T3) Cu center. T2 and T3 form a trinuclear Cu cluster. The oxidation of the substrate occurs at the type 1 (T1) Cu center. The electrons are transferred to the trinuclear Cu cluster where O_2 is reduced to H_2O.[10]

Recently, we reported on the laccase initiated domino reaction between 4-hydroxy-6-methyl-2*H*-pyran-2-one and catechols using air as an oxidant[11] affording the selective synthesis of 1*H*-pyrano[4,3-*b*]benzofuran-1-ones with good to excellent yields. Similarly, efficient access was achieved to 6*H*-benzo[4,5]furo[3,2-*c*]chromen-6-ones, a skeleton typical of naturally occurring coumestans. We used commercially available laccases from *Trametes versicolor* and *Agaricus bisporus* as the enzymes.

Here we report on the laccase initiated domino reactions of 1,3-diketones **1** with catechols **2** into 3,4-dihydro-7,8-dihydroxy-2*H*-dibenzofuran-1-ones **3** using air as an oxidant (Scheme 1). Dibenzofuranones are a class of compounds that are of great interest to medicinal chemistry due to their biological activity.[12]

Keywords: Laccase; Catalysis; Domino reactions; Air; *O*-Heterocycles.
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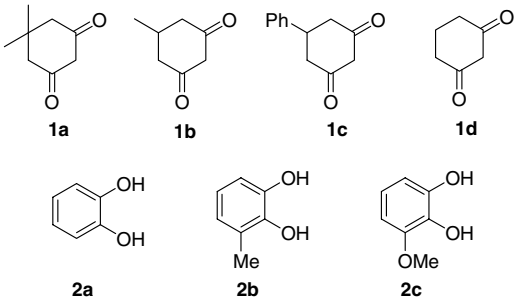
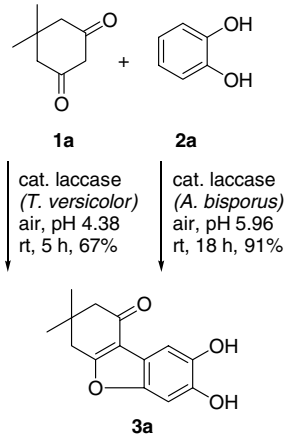
In order to identify suitable reaction conditions we first examined the laccase initiated reaction between 5,5-dimethyl cyclohexane-1,3-dione **1a** and catechol **2a** in the presence of air. The enzyme initially employed was the commercially available laccase from *T. versicolor*[13] (Scheme 2).

We found that the reaction can best be run at room temperature in an acetate buffer at pH 4.38. After 5 h 3,4-dihydro-7,8-dihydroxy-3,3-dimethyl-2*H*-dibenzofuran-1-one **3a** was isolated as the only product in 67% yield. If instead the laccase from *A. bisporus*[14] was used, which is also commercially available, and the reaction performed in a phosphate buffer at pH 5.96, product **3a** was obtained after 18 h in a yield of 91%. As further experiments with other 1,3-diketones showed that in *A. bisporus* catalyzed ractions the reaction products were obtained in higher yields and purity, all subsequent transformations were performed with the laccase from *A. bisporus* as a catalyst[15] (Scheme 2).

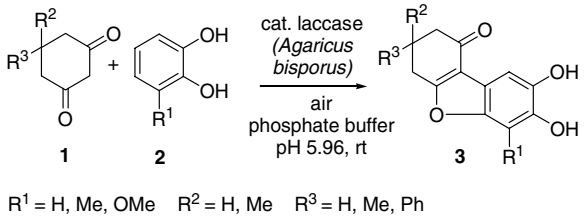
Initially, transformations between cyclohexane-1,3-diones **1a–d** and catechol **2a** were studied in more detail. Apart from 5,5-dimethyl cyclohexane-1,3-dione **1a** (R^2, $R^3 = Me$) we also used 5-methyl cyclohexane-1,3-dione **1b** ($R^2 = H$, $R^3 = Me$), 5-phenyl cyclohexane-1,3-dione **1c** ($R^2 = H$, $R^3 = Phe$), and cyclohexane-1,3-dione **1d** (R^2, $R^3 = H$). In all these cases we isolated the corresponding heterocycles **3a–d** with yields ranging



Scheme 1. Substrates **1a–d** and **2a–c** for the laccase initiated domino reaction.



Scheme 2. Reaction of **1a** and **2a** with different laccases.



Scheme 3. Laccase initiated domino reaction of **1** and **2** for the synthesis of **3**.

Table 1. Laccase initiated domino reactions of cyclohexane-1,3-diones **1** and catechols **2** in the presence of air as an oxidant[a]

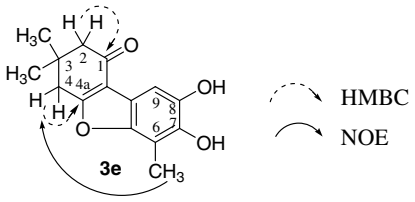
Entry	**1**	R^2	R^3	**2**	R^1	Time (h)	Product **3**	Yield[b] (%)
1	**a**	Me	Me	**a**	H	18	**a**	91
2	**b**	H	Me	**a**	H	18	**b**	85
3	**c**	H	Ph	**a**	H	18	**c**	96
4	**d**	H	H	**a**	H	19	**d**	87
5	**a**	Me	Me	**b**	Me	20	**e**	92
6	**b**	H	Me	**b**	Me	28	**f**	71
7	**c**	H	Ph	**b**	Me	19	**g**	91
8	**d**	H	H	**b**	Me	24	**h**	70
9	**a**	Me	Me	**c**	OMe	20	**i**	95
10	**b**	H	Me	**c**	OMe	20	**j**	97
11	**c**	H	Ph	**c**	OMe	19	**k**	97
12	**d**	H	H	**c**	OMe	20	**l**	89

[a] All reactions were performed using the laccase of *Agaricus bisporus*.
[b] Yields refer to isolated yields.

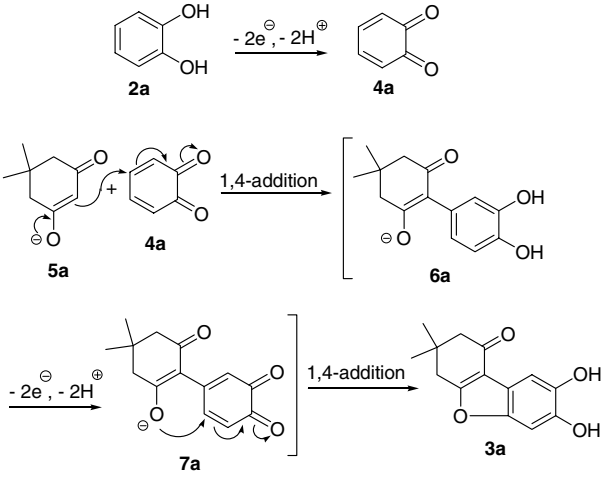
from 85% to 96% (Scheme 3, Table 1, entries 1–4). Then the domino reactions were run with the 3-substituted catechols **2c** and **2b**. In the reactions of the cyclohexane-1,3-diones **1a–d** with the donor substituted 3-methyl catechol **2b** the single products obtained were heterocycles **3e–h** in yields of between 70% and 92% (Scheme 3, Table 1, entries 5–8). Similar results were observed with transformations of cyclohexane-1,3-diones **1a–d** with 3-methoxy catechol **2c**, where **3i**, **3j**, **3k**, and **3l** were isolated in yields of 95%, 97%, 97% and 89%, respectively, (Scheme 3, Table 1, entries 9–12).

The structures of all the products were unambiguously elucidated by NMR spectroscopic methods. The question whether the substituents R^1 are attached to C-6 or C-9 of products **3** was best answered by HMBC spectra and $^1H,^1H$ NOEs. First the ^1H NMR signals of the methylene protons were definitely assigned to C-2 and C-4, respectively, by means of the HMBC-spectrum. With **3e**[16] the ^1H NMR signal at $\delta = 2.40$ ppm originates from the protons at C-2, as a strong correlation signal to the keto group C-1 ($\delta = 194.5$ ppm) can be observed in the HMBC spectrum (Scheme 4). In a similar way, the signal at $\delta = 2.92$ ppm can be assigned to the protons at C-4, due to a strong correlation signal to C-4a ($\delta = 169.2$ ppm).

After the ^1H NMR signals of the methylene protons were unambiguously assigned, we were now able to definitely assign the NOE occurring upon irradiation into the signal of the methyl group whose position was to



Scheme 4. Structure elucidation of **3e** by NMR.



Scheme 5. Possible reaction mechanism.

be determined ultimately (Scheme 4). When the signal of the CH_3 group was irradiated at $\delta = 2.28$ ppm, a ^1H, ^1H NOE was observed at the signal of 4-H_2 ($\delta = 2.92$ ppm), which clearly shows the methyl group to be attached to C-6 (instead of C-9). Similarly, the structure of all products **3** can be determined unambiguously.

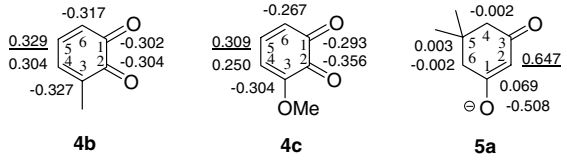
As outlined in Scheme 5, we assume that the first step of the domino process is the laccase catalyzed oxidation of catechol **2a** with O_2 to *o*-benzoquinone **4a**, which then undergoes an intermolecular 1,4-addition with the enolate of dimedone **5a** as a nucleophile to yield **6a** that cannot be isolated. After laccase catalyzed oxidation of **6a** to **7a** a second 1,4-addition occurs proceeding intramolecularly under formation of tricycle **3a**. Altogether, a domino oxidation/1,4-addition/oxidation/1,4-addition process has taken place.

The selectivity of these transformations may be understood in such a way that the first 1,4-addition exclusively occurs at the more electrophilic carbon atom C-5 of the corresponding *o*-benzoquinones **4**. HOMO/LUMO-considerations that are based on quantum mechanical calculations with the density functional theory B3LYP (basis set 6-31G(d) and STO-3G, respectively)[17] lead to results that are similar to those obtained by a purely qualitative view of the most reactive positions of the *o*-quinones taking into account the inductive and mesomeric effects of the substituents. The energies of the frontier orbitals of quinones **4b**,**c** and of the enolate ion **5a** are listed in Table 2.

The energies of the HOMO of the enolate ion of dimedone **5a** and the LUMOs of quinones **4b**,**c** are energetically closer than the energies of the LUMO of **5a** and the HOMOs of **4b**,**c**, supporting the assumption that the strongest interaction is between the HOMO of **5a** and the LUMO of quinones **4b**,**c**. The calculations suggest a nucleophilic attack of the enolate ion **5a** onto the electrophilic quinones **4**. Scheme 6 shows the atomic orbital coefficients of *o*-quinones **4b**,**c** and enolate ion **5a**. Since the coefficients of the frontier orbitals of **4b** and **4c** at C-5 are larger than those at C-4, a selective attack of the nucleophile at C-5 is to be expected. This corresponds to the selectivity experimentally observed. At C-2 the ambident enolate ion **5a** has a larger coefficient than at the enolate oxygen so that the strongest frontier orbital interaction is between the soft nucleophilic center of the enolate ion **5a**, that is, C-2, and the electrophilic center with largest atomic orbital coefficient of *o*-quinone **4**, namely C-5.[18]

Table 2. Frontier orbital energies of **4b**,**c** and **5a**

Entry	Compound	HOMO (eV)	LUMO (eV)
1	**4b**	−6.69	−3.46
2	**4c**	−6.29	−3.43
3	**5a**	−0.35	4.76



Scheme 6. Atomic orbital coefficients of **4b**, **c** and **5a**.

Subsequently, the reaction between **1a** and **2a** was used to study the impact of different experimental parameters on the course of reaction. We found that reducing the initial amount of laccase (30 U) from *A. bisporus* to 70% and 50%, respectively, has hardly any influence on reaction times and yields. Only if the laccase is reduced to 10% of the original amount does the yield of **3a** decrease dramatically low to 20%. If the reaction is run at pH 4.38 instead of 5.96, the yield falls to 71%. Seventy-two percent are obtained when the reaction is performed in phosphate buffer at pH 7. An increase in **3a** is observed, though, if the transformation is conducted at higher temperatures. At 30 °C (20 h) **3a** was isolated with 92% and at 50 °C (12 h) even with 97% yield. We could demonstrate that reducing the amount of phosphate buffer is of further advantage. With fifty percent of the original amount of buffer **3a** was isolated in quantitative yield. Even if the reaction is run in 10% of the original amount of buffer, **3a** is still obtained with 83% yield. Under optimal conditions (15 U laccase from *A. bisporus*, 50 ml phosphate buffer, pH 5.96, 50 °C, 11 h) **1a** and **2a** could be reacted to give **3a** in a yield of 93%. To ensure that the domino reactions do not proceed in absence of either laccase or O_2, suitable control experiments were performed affording the expected results.

In summary, an efficient approach to 3,4-dihydro-7,8-dihydroxy-2*H*-dibenzofuran-1-ones **3** has been devel-

oped using a laccase initiated domino reaction between cyclohexane-1,3-diones and catechols with air as an oxidant, characterized by mild reaction conditions, high yields, avoidance of toxic reagents, and toxic side products.[19]

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15. *General procedure for the laccase initiated domino reaction*: A solution of 1.5 mmol **1** and 1.7 mmol **2** in 100 ml 0.2 M phosphate buffer (pH 5.96) was placed in a 250 ml flask. 50 mg laccase of *Agaricus bisporus* (0.6 U/mg) were added and the mixture vigorously stirred under air at room temperature until the substrates had been fully consumed, as judged by TLC. The reaction mixture was acidified with 2 M HCl to pH ~4, saturated with NaCl and filtered with suction on a Buchner funnel. The filter cake was washed with a solution of 100 ml 15% NaCl and 5 ml H_2O. The crude products obtained after drying exhibit a purity of 90–95% (NMR). Analytically pure products could be obtained by recrystallization.
16. Selected data for **3e**: IR (ATR): 3491, 3147, 2961, 2924, 1644, 1582, 1459, 1428, 1300, 1228, 1047, 862 cm^{-1}. UV (CH_3CN), λ_{max} (lg ε): 287 nm (3.88), 238 nm (4.25). ^1H NMR (300 MHz, DMSO-d_6): δ 1.12 (s, 6H, (3-CH_3)$_2$), 2.28 (s, 3H, 6-CH_3), 2.40 (s, 2H, 2-H_2), 2.92 (s, 2H, 4-H_2), 7.12 (s, 1H, 9-H), 8.46 (s, 1H, 7-OH), 9.37 (s, 1H, 8-OH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 9.68 (6-CH_3), 28.8 (3-CH_3)$_2$, 35.7 (C-3), 37.5 (C-4), 52.2 (C-2), 103.0 (C-9), 108.6 (C-6), 114.0 (C-9a or C-9b), 115.4 (C-9a or C-9b), 142.7 (C-8), 144.0 (C-7), 148.7 (C-5a), 169.2 (C-4a), 194.5 (C-1). MS (70 eV, EI): *m*/*z* (%): 260.1 (100) [M^+], 204.0 (90), 176.0 (78).
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