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The Laccase-Catalyzed Domino Reaction between Catechols and Heterocyclic 1,3-Dicarbonyls and the Unambiguous Structure Elucidation of the Products by NMR Spectroscopy and X-ray Crystal Structure Analysis

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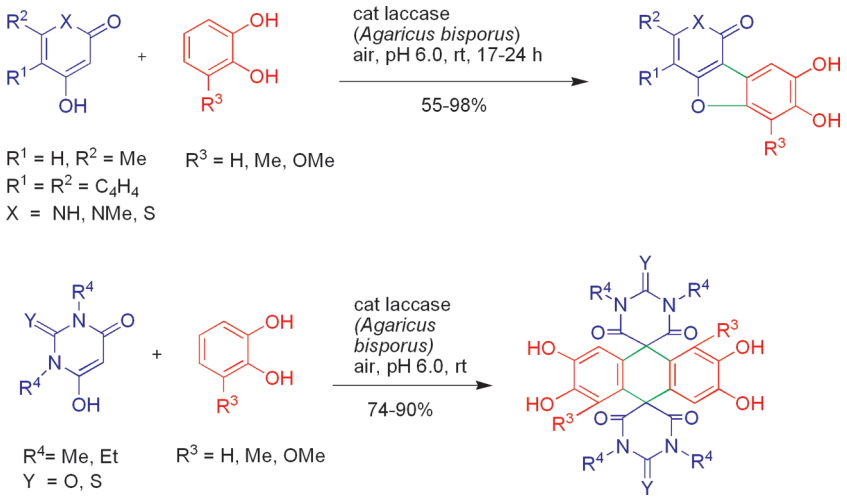

The laccase-catalyzed reaction between catechols and heterocyclic 1,3-dicarbonyls (pyridinones, quinolinones, thiocoumarins) using aerial oxygen as the oxidant delivers benzofuropyridinones, benzofuroquinolinones, and thiocoumestans in a simple fashion, highly regioselectively with yields ranging from 55 to 98%. With barbituric acid derivatives the exclusive formation of dispiropyrimidinone derivatives takes place. The unambiguous and complete structure elucidation of all reaction products has been achieved by means of NMR spectroscopic methods (HSQMBC and band-selective HMBC) as well as by X-ray crystal structure analysis.

Introduction

Benzofurans[1] and annulated benzofurans[2] are well-known for their remarkable biological activities. This is why there is a great deal of interest in the development of efficient synthetic approaches to these heterocyclic ring systems.[1d,3,4] Nowadays the main focus is on the development of catalytic methods.[1d,3,5] Also of great interest is the use of domino reactions[3,6] which

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allow the resource-conserving and efficient construction of benzofurans and annulated benzofurans in a single synthetic operation. Enzyme-catalyzed reactions are of increasing importance in organic synthesis[7] as they allow a multitude of selective and efficient transformations to be run in aqueous systems at room temperature. In addition, many enzyme catalyzed transformations fulfill a number of the principles of green chemistry.[8] A main area is the development of enzyme-catalyzed oxidations.[9] Oxidative transformations are particularly attractive if aerial oxygen can be used as an oxidant because atmospheric oxygen is inexhaustible and free of cost. In addition, the only waste product formed upon reduction of oxygen is water which is completely safe and nontoxic. Reactions that combine several enzyme catalyzed transformations or enzyme and chemically catalyzed transformations to make up new reaction sequences are even more interesting than single-step enzymatic transformations.[10,11] In this respect, laccases[12] are particularly attractive enzymes as they can be used to catalyze oxidations which are capable of triggering domino processes. Laccases (benzenediol: O_2 oxidoreductase E.C. 1.10.3.2.) mainly occur in fungi, but also in plants and some prokaryotes. In general, they are easy to isolate, and some of them are even commercially available. The best known laccases include the enzymes isolated from *Agaricus bisporus* and *Trametes versicolor*. Laccases are multicopper oxidases which are able to catalyze the selective oxidation of a number of substrates under mild reaction conditions with simultaneous reduction of O_2 to give H_2O.[13] By using mediators, the redox potential of laccases can be changed which allows the oxidation of substrates with a higher redox potential. Laccases have been employed for the oxidation of aromatic methyl groups,[14] benzylic, allylic, and aliphatic alcohols,[15] ethers,[16] benzylamines, and hydroxylamines.[17] Relatively few studies have so far been published on the combination of a laccase-catalyzed oxidation with another enzyme or with a chemically catalyzed transformation to make up new domino reactions.[18] Recently, we have reported on the combination of a laccase-catalyzed oxidation of catechols with 1,4-additions of 1,3-dicarbonyls.[19] We could demonstrate that 4-hydroxy-6-methyl-2*H*-pyran-2-one and cyclohexane-1,3-diones, respectively, can be reacted with catechols to efficiently produce 1*H*-pyrano-[4,3-*b*]-benzofuran-1-ones[19a] and 3,4-dihydro-7,8-dihydroxy-2*H*-dibenzofuran-1-ones,[19b] respectively. Similar reactions have been reported by Ragauskas et al.[20] They also studied the intermolecular Diels−Alder reaction of *o*-and *p*-benzoquinones, which were generated by laccase-catalyzed oxidations of catechols and hydroquinones, respectively, with several dienes.[21]

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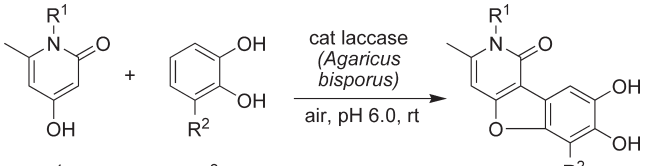
Results and Discussion

To explore the scope of the domino process we studied the laccase-catalyzed reaction of unsubstituted as well as substituted catechols with heterocyclic 1,3-dicarbonyls. Here, we demonstrate that catechols can be reacted with several heterocyclic 1,3-dicarbonyls to yield a couple of heterocyclic systems in an efficient way. In the reactions with 4-hydroxy-6-methylpyridin-2(1*H*)-one, 4-hydroxy-1,6-dimethylpyridin-2(1*H*)-one, 4-hydroxyquinolin-2(1*H*)-one, and 4-hydroxy-1-thiocoumarin, the exclusive and highly regioselective formation of annulated benzofuran derivatives like benzofuropyridinones, benzofuroquinolinones, and thiocoumestans was observed. Due to their distinct biological activities, these classes of heterocyclic compounds attract great interest in medicinal chemistry.[22] It has been established that 3,9-dihydroxybenzofuro[3,2-*c*]quinolin-6(5*H*)-one exhibits unique effects of inhibiting bone resorption and stimulating bone mineralization without exhibiting estrogenic activity.[22a] 3,9-Bis(*N*,*N*-dimethylcarbamoyloxy)benzofuro[3,2-*c*]quinolin-6(5*H*)-one, a derivative thereof, has been found to display similar pharmaceutical activities. It might become useful in the treatment of osteoporosis as it is a more potent agent than ipriflavone.[22b] 3-Chloro-5-thiocoumestan-8,9-diacetate was shown to be active against *Aspergillus niger* and *Alternaria alternata*.[22c] Some of the compounds described here have been made accessible by other methods, including tyrosinase-catalyzed oxidation,[23a] electrochemical oxidation,[23b] and chemical oxidation with potassium ferricyanide.[22c] In addition, we demonstrate that the laccase-catalyzed transformations of barbituric acid and thiobarbituric acid derivatives with catechols do not give the expected benzofuran derivatives but result in the exclusive formation of polycyclic dispiropyrimidinones.

We started with the laccase-catalyzed reaction between 4-hydroxy-6-methylpyridin-2(1*H*)-one (**1a**) and 4-hydroxy-1,6-dimethylpyridin-2(1*H*)-ones (**1b**) with the catechols **2a**−**c**. It was found that 6-substituted 7,8-dihydroxybenzofuro[3,2-*c*]pyridin-1(2*H*)-ones **3** can easily be accessed regioselectively using this approach (Table 1). In all cases, the reactions (except the transformations presented in Table 4) were performed with 156 U[24] of a commercially available laccase from *A. bisporus* (AbL) as a catalyst at room temperature in a phosphate buffer (0.2 M) at pH 6.0. The yields of the 6-substituted 7,8-dihydroxybenzofuro[3,2-*c*]pyridin-1(2*H*)-ones **3a**−**f** were in the range between 65 and 98%, and even the purity of the crude products was very high (>90−95%) (Table 1).

TABLE 1. Laccase (*A. bisporus*)-Catalyzed Domino Reaction of Pyridinones 1 and Catechols 2 for the Synthesis of 6-Substituted 7,8-Dihydroxybenzofuro[3,2-*c*]pyridin-1(2*H*)-ones 3



1a R^1 = H
1b R^1 = Me

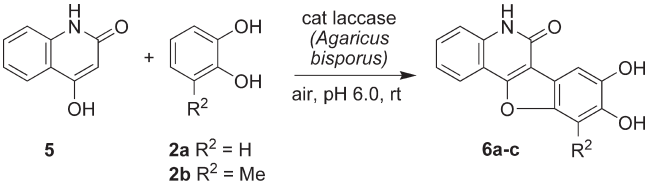
2a R^2 = H
2b R^2 = Me
2c R^2 = OMe

3a-f

entry	**1**	R^1	**2**	R^2	time (h)	product **3**	yield[a] (%)
1	**a**	H	**a**	H	24	**a**	98
2	**a**	H	**b**	Me	18	**b**	98
3	**a**	H	**c**	OMe	18	**c**	83
4	**b**	Me	**a**	H	18	**d**	98
5	**b**	Me	**b**	Me	19	**e**	65
6	**b**	Me	**c**	OMe	17	**f**	81

[a]The reactions were performed with 156 U of laccase from *A. bisporus* (AbL). The enzyme activity was determined using catechol as the substrate.[24]

TABLE 2. Laccase (*A. bisporus*)-Catalyzed Domino Reaction of 4-Hydroxyquinolin-2(1*H*)-one (5) with Catechols 2 for the Synthesis of 10-Substituted 8,9-Dihydroxybenzofuro[3,2-*c*]quinolin-6(5*H*)-ones 6



5

2a R^2 = H
2b R^2 = Me
2c R^2 = OMe

6a-c

entry	**2**	R^2	time (h)	product **6**	yield[a] (%)
1	**a**	H	20	**a**	73
2	**b**	Me	18	**b**	77
3	**c**	OMe	20	**c**	61

[a]The reactions were performed with 156 U of laccase from *A. bisporus* (AbL). The enzyme activity was determined using catechol as the substrate.[24]

The starting materials, i.e., the 4-hydroxy-6-methylpyridin-2(1*H*)-one (**1a**) and the 4-hydroxy-1,6-dimethylpyridin-2(1*H*)-one (**1b**), were synthesized in yields of 92 and 65%, respectively, by reacting 4-hydroxy-6-methylpyran-2(2*H*)-one (**4**) with NH_4OH and CH_3NH_2, respectively[25]

We also studied the reactions between 4-hydroxyquinolin-2(1*H*)-one (**5**) and the catechols **2a**−**c** (Table 2). As shown in Table 2, the 8,9-dihydroxybenzofuro[3,2-*c*]quinolin-6(5*H*)-ones **6a**−**c** can be isolated as single regioisomers with yields ranging from 61 to 77% if the laccase-catalyzed reactions of the appropriate substrates are conducted under the reaction conditions developed in our laboratory.

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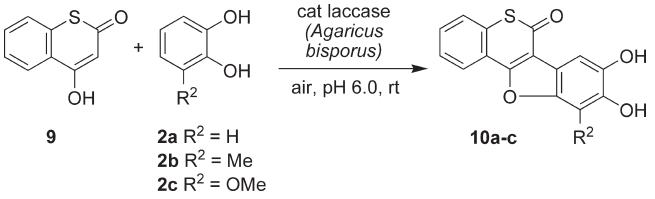
TABLE 3. Laccase (*A. bisporus*)-Catalyzed Domino Reaction of 4-Hydroxy-1-thiocoumarin (9) with Catechols 2 for the Synthesis of 8,9-Dihydroxy-5-thiocoumestans 10



entry	2	R^2	time (h)	product 10	yield[a] (%)
1	**a**	H	18	**a**	96
2	**b**	Me	19	**b**	77
3	**c**	OMe	19	**c**	55

[a]The reactions were performed with 156 U of laccase from *A. bisporus* (AbL). The enzyme activity was determined using catechol as the substrate.[24]

TABLE 4. Model Reactions with Different Laccases

entry	1,3-dicarbonyl	2	laccase	units[a] (U)	product	yield (%)
1	**1a**	**a**	AbL[b]	35	**3a**	99
2	**1a**	**a**	AbL[b]	30	**3a**	98
3	**1a**	**a**	AbL[b]	15	**3a**	88
4	**1a**	**a**	AbL[b]	7	**3a**	74
5	**1b**	**a**	AbL[b]	35	**3d**	99
6	**9**	**a**	AbL[b]	35	**10a**	98
7	**1a**	**a**	TvF[c]	35	**3a**	60
8	**1b**	**a**	TvF[c]	35	**3d**	57
9	**9**	**a**	TvF[c]	35	**10a**	61
10	**1a**	**a**	Lccβ[d]	35	**3a**	64
11	**1b**	**a**	Lccβ[d]	35	**3d**	64
12	**9**	**a**	Lccβ[d]	35	**10a**	54

[a]The enzyme activity was determined using ABTS as the substrate.[30] [b]Reactions were performed using a commercially available laccase from *A. bisporus* (AbL) in 0.2 M phosphate buffer at pH 6.0. [c]Reactions were performed using a commercially available laccase from *T. versicolor* (TvF) in 0.2 M acetate buffer at pH 4.38. [d]Reactions were performed using a recombinant β-laccase from *T. versicolor* produced in *P. pastoris* (Lccβ) in 0.1 M acetate buffer at pH 5.0.

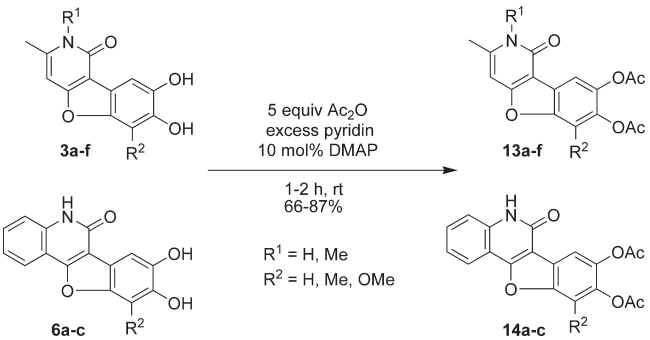
The substrate 4-hydroxyquinolin-2(1*H*)-one (**5**) was prepared in two synthetic steps according to known procedures.[26] Upon reaction of 2′-aminoacetophenone (**7**) with diethyl carbonate and sodium hydride in toluene a mixture of **5** and 4-hydroxyquinolin-2(1*H*)-one-3-carboxylic acid ethyl ester (**8**) (approximately 1:1) was formed, which was then boiled in aqueous KOH to allow for the quantitative transformation of **8** into 4-hydroxyquinolin-2(1*H*)-one (**5**).

In the reactions between 4-hydroxy-1-thiocoumarin (**9**) and the catechols **2a**−**c** the tetracyclic 8,9-dihydroxy-5-thiocoumestans **10a**−**c** were isolated in yields from 55 to 96% as single regioisomers (Table 3).

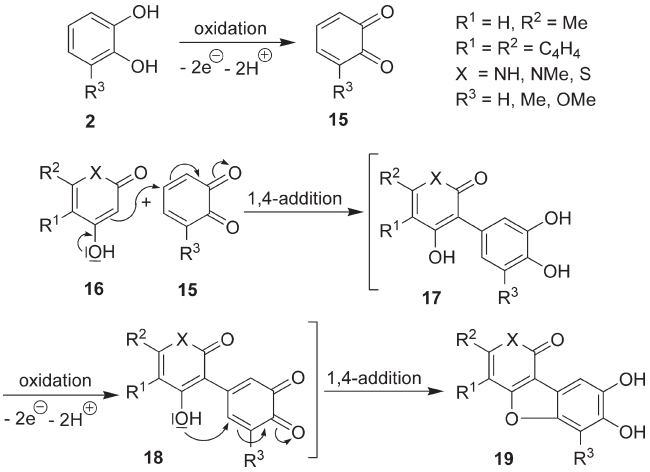
4-Hydroxy-1-thiocoumarin (**9**) could also be prepared in two steps.[26a,27] First, thiosalicylic acid (**11**) was reacted with methyllithium to yield 2′-mercaptoacetophenone (**12**) in 90% yield. Treatment of **12** with diethyl carbonate and sodium ethoxide in toluene resulted in the isolation of crystalline 4-hydroxy-1-thiocoumarin (**9**) in 53% yield.

(26) (a) Jung, J.-C.; Jung, Y.-J.; Park, O.-S. *Synth. Commun.* **2001**, *31* (8), 1195. (b) Koller, G. *Chem. Ber.* **1927**, *60*, 1108.
(27) Topolski, M. *J. Org. Chem.* **1995**, *60*, 5588.

SCHEME 1. Formation of the Diacetates



SCHEME 2. Possible Reaction Mechanism

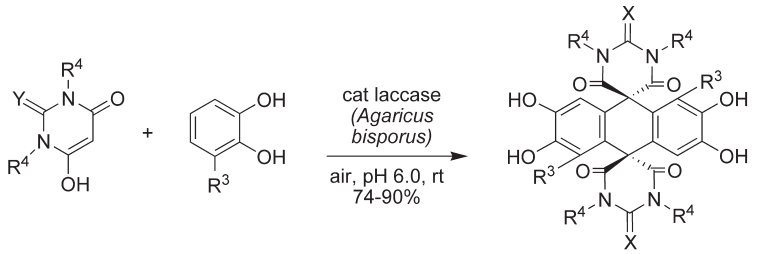


Due to solubility problems, it turned out to be impossible to obtain the heterocycles **3a**−**f** and **6a**−**c** in analytically pure form for elemental analysis. In order to obtain analytically pure material, the poorly soluble products **3a**−**f** and **6a**−**c** were transformed into their corresponding diacetates[28] **13a**−**f** and **14a**−**c** (Scheme 1). For this purpose, the substrates were dissolved in excess pyridine and reacted with 5 equiv of acetic anhydride and 10 mol % of DMAP at room temperature. The solid crude products obtained after acidic workup were recrystallized to yield analytically pure diacetates. With products **10a**−**c** recrystallization was possible and sufficient to obtain analytically pure material.

It is assumed that the first step of the domino process is the laccase-catalyzed oxidation of the catechol **2** with O_2 to *o*-benzoquinone **15**, which then undergoes an intermolecular 1,4-addition with the enol of the 1,3-dione **16** as a nucleophile to yield **17** which cannot be isolated (Scheme 2). After a second laccase-catalyzed oxidation, a final intramolecular 1,4-addition under formation of the tricycle **19** takes place. Altogether, this can be summarized as a domino oxidation/1,4-addition/oxidation/1,4-addition-process.[19b] The regioselectivity of these domino reactions may be rationalized in such a way that the initial 1,4-addition exclusively occurs at the more electrophilic carbon atom of the corresponding *o*-benzoquinones **15**.

(28) Höfle, G.; Steglich, W.; Vorbrüggen, H. *Angew. Chem., Int. Ed.* **1978**, *17*, 569.

SCHEME 3. Reaction of Barbituric Acid Derivatives 20 with Catechols 2 for the Synthesis of 21



20a R^4 = Me, Y = O
20b R^4 = Et, Y = S

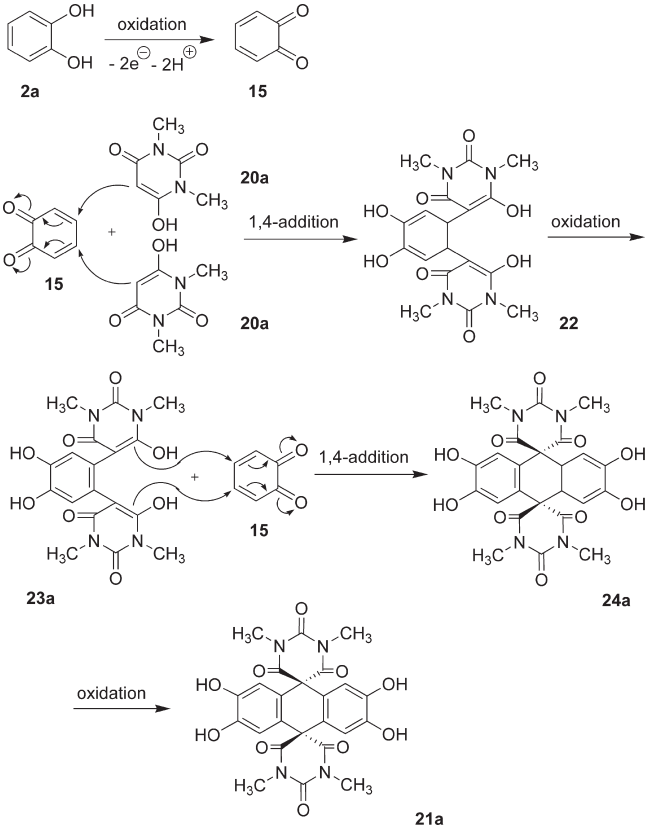
2a R^3 = H
2b R^3 = Me
2c R^3 = OMe

21a R^4 = Me, X = O, R^3 = H 90%
21b R^4 = Me, X = O, R^3 = Me 81%
21c R^4 = Me, X = O, R^3 = OMe 74%
21d R^4 = Et, X = S, R^3 = H 77%

To explore whether other laccases could also catalyze these transformations, some model reactions were run using (a) a commercially available laccase from *T. versicolor* (TvF) (Table 4, entries 7−9) and (b) a recombinant β-laccase from *T. versicolor* produced in *Pichia pastoris* (Lccβ) (Table 4, entries 10−12).[29] The outcome was compared to the results obtained with the laccase from *A. bisporus* (AbL) (Table 4, entries 1, 5, and 6). For these comparative experiments, the activities of all three enzymes were determined using 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) as the substrate.[30] Both the commercially available laccase from *T. versicolor* (TvF) and the recombinant β-laccase from *P. pastoris* (Lccβ) were associated with lower yields (by 35 to 45%) and products of lower purity when compared to AbL (Table 4). Obviously, TvF and Lccβ possess lower activity on substrates **1a**, **1b**, and **9**. In order to study the influence of the amount of laccase on the yields, the transformation of **1a** and **2a** was also performed with 30, 15, and 7 U of AbL (Table 4, entries 2−4). With 30 U the yield of **3a** is almost identical with the yield obtained with 35 U (Table 4, entries 1 and 2). With 15 U the yield slightly decreased to 88%, and 7 U yielded 74%.

Apart from the reactions with 4-hydroxymethylpyridin-2(1*H*)-ones, 4-hydroxyquinolin-2(1*H*)-ones, and 4-hydroxy-1-thiocoumarins we also studied the domino reactions with barbituric acid derivatives as substrates using AbL as a catalyst. We found that the reaction of *N*,*N*-dimethylbarbituric acid (**20a**) with catechol (**2a**) did not yield the expected benzofuran derivative but resulted in the exclusive formation of the polycyclic dispiropyrimidinone **21a** in 90% yield (Scheme 3). The exclusive formation of compounds with this skeleton (**21b** and **21c**) was also observed in the reactions of **20a** with the substituted catechols **2b** and **2c**. Similarly, laccase-catalyzed domino reaction of **2a** with thiobarbituric acid (**20b**) exclusively afforded **21d** which precipitated from the reaction mixture in 77%. The spirocyclic compounds **21a-d** can also be synthesized in comparable yields by using an excess of up to 4 equiv of potassium ferricyanide as an oxidant[31a] or by electrochemical oxidation.[31b−e]

SCHEME 4. Possible Reaction Mechanism



Presumably, the compounds **21** are formed according to the mechanism depicted in Scheme 4 using the reaction of **2a**

(29) Koschorreck, K.; Richter, S. M.; Swierczek, A.; Beifuss, U.; Schmid, R. D.; Urlacher, V. B. *Arch. Biochem. Biophys.* **2008**, *474*, 213.

(30) Laccase activity was determined according to a modified procedure taken from: Nicotra, S.; Intra, A.; Ottolina, G.; Riva, S.; Danieli, B. *Tetrahedron: Asymmetry* **2004**, *15*, 2927. A 0.91 mM solution of ABTS (0.3 mL) in 0.2 M phosphate buffer (pH = 6.0) was diluted with 0.2 M phosphate buffer (2.6 mL, pH = 6.0) and treated with a solution of laccase from *A. bisporus* (AbL) in the same buffer (0.1 mL). The change in absorption was followed via UV spectroscopy (λ = 414 nm). For AbL 1 U is defined as the amount of the enzyme that catalyzes the conversion of 1 μmol of ABTS per minute by pH 6.0 and 25 °C. The activity of AbL with ABTS as the substrate amounted to 0.61 U/mg. For TvF 1 U is defined as the amount of the enzyme that catalyzes the conversion of 1 μmol of ABTS per minute by pH 4.38 and 25 °C. For Lccβ 1 U is defined as the amount of the enzyme that catalyzes the conversion of 1 μmol of ABTS per minute by pH 5.0 and 25 °C.

(31) (a) Alizadeh, A.; Nematollahi, D.; Hbibi, D.; Hesari, M. *Synthesis* **2007**, *10*, 1513. (b) Nematollahi, D.; Goodarzi, H. *J. Org. Chem.* **2002**, *67*, 5036. (c) Nematollahi, D.; Goodarzi, H.; Tammari, E. *J. Chem. Soc., Perkin Trans. 2* **2002**, 829. (d) Szanto, D.; Trinidad, P.; Walsh, F. *J. Appl. Electrochem.* **1998**, *28*, 251. (e) Azzem, A. M.; Zahran, M.; Hagagg, E. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 1390.

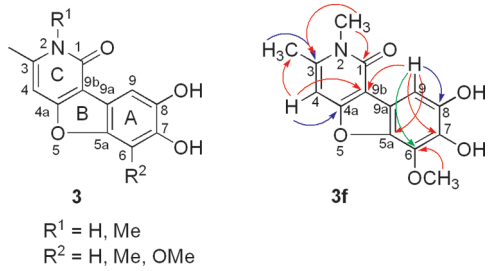


FIGURE 1. Product numbering of **3** and important HMBC correlations (H→C) of **3f** (blue arrow: ^{2}J coupling; red arrow: ^{3}J coupling; green arrow: ^{4}J coupling).

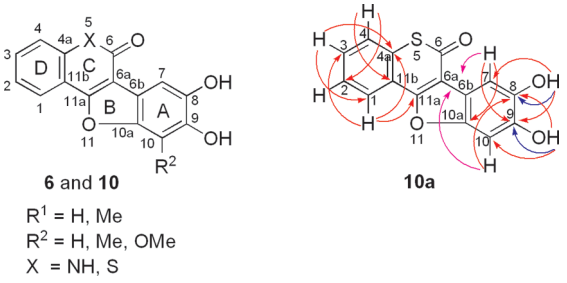


FIGURE 2. Product numbering of **6** and **10** and important HMBC correlations (H→C) of **10a** (blue arrow: ^{2}J coupling; red arrow: ^{3}J coupling; pink arrow: ambiguous assignment).

with **20a** as an example.[31] Accordingly, the first step of the domino process is the laccase catalyzed oxidation of catechol (**2a**) with oxygen to *o*-quinone (**15**) which then reacts with two molecules of *N*,*N*-dimethylbarbituric acid (**20a**) as a nucleophile to yield the intermediate **22** (not isolable) which—after oxidation to **23a**—undergoes cyclization with a second molecule of *o*-quinone (**15**). Oxidation of **24a** finally affords the dispiropyrimidinone **21a**.

In all of the products (**3**, **6**, and **10**), full assignment of the ^{1}H and ^{13}C chemical shifts of ring C of **3** as well as rings C and D of **6** and **10** could be achieved by evaluating their gHSQC, gHMBC, and ROESY spectra (Figures 1 and 2).

The connectivity between the substructures (rings C and ring A) of **3**, which establishes the regioselectivity of the domino process, was deduced as follows: in the HMBC spectrum of **3** (optimized for 8 Hz) the aromatic proton H-4 showed a strong $^{3}J_{H/C}$ correlation to carbon C-9b. A ^{3}J-HMBC correlation from the latter carbon to proton H-9 unambiguously fixed the regioselectivity of the reaction (Figure 1). Additionally, selective 1D-NOE measurements independently supported the assignments above. For instance, irradiation of H-4 at δ 6.53 of **3c** caused an ^{1}H,^{1}H NOE enhancement of the signal of the methoxy group at C-6 (instead of C-9) at δ 61.2 showing the spatial proximity between these protons and confirming the observed regioselectivity (Figure 3). In a similar way, the structures of all products were elucidated, and it was established that only one regioisomer was obtained in all the reactions performed.

A prerequisite for the determination of the aforementioned regioselectivity is the complete and unambiguous assignment of all quaternary carbons in all compounds, especially those of rings A (C-8, C-7, C-5a, C-9a). Direct assignment, however, could only be achieved in a few

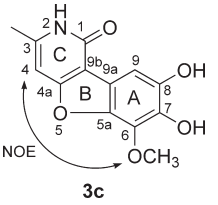


FIGURE 3. Observed NOEs in products **3c**.

cases, e.g., **3a**, where the two phenolic OH groups of ring A showed strong and sharp resonances. In compounds with broad/overlapping OH signals, failure to determine the substitution pattern was due to the absence of important HMBC correlations (Figure 1). Preliminary studies at various temperatures and pH values to obtain correlation signals failed. By using another NMR approach focusing on the evaluation of long-range ^{1}H−^{13}C coupling constants, we were able to determine the substitution pattern of ring A. The pulse sequences for the accurate determination of ^{1}H−^{13}C coupling constants as well as their application in the structure elucidation process were published elsewhere.[32] Due to its good sensitivity and excellent applicability for proton singlets, the HSQMBC[32a,33] pulse sequence was implemented to provide the required long-range heteronuclear coupling constants. From the HSQMBC spectrum of **3a** (Figure 4), the following coupling constants were extracted: H-9 (7.31 ppm) to C-8 (143.9 ppm), 3.1 Hz (^{2}J), to C-7 (145.4 ppm), 7.3 Hz (^{3}J) and to C-5a (148.9 ppm), 10.3 Hz (^{3}J). H-6 (7.03 ppm) to C-5a (148.9 ppm), 4.3 Hz (^{2}J), to C-7 (145.4 ppm), 4.4 Hz (^{2}J), and to C-8 (143.9 ppm), 6.3 Hz (^{3}J). The coupling constants between H-9 (7.31 ppm) and C-9b (108.8 ppm), 2.9 Hz (^{3}J), as well as C-9a (115.4 ppm), 2.8 Hz (^{2}J). From H-6 (7.03 ppm) to C-9a (115.4 ppm), 5.6 Hz (^{3}J). Due to a lack of reference data in the literature, we assigned the quaternary carbons of ring A on the basis of the size of the observed coupling constants [values for aromatic ^{3}J (C, H) are between 4 and 12 Hz[34]] as shown in Figure 5. For all other compounds, corresponding HSQMBC correlations and heteronuclear long-range coupling constants were observed.

To confirm the above assignment of ring A, the trimethoxy derivative of **3f** (= **25**) was prepared (Scheme 5) and its structure completely elucidated by common HSQC, HMBC, ROESY, and NOE NMR experiments (Figure 6). Subsequent comparison of the ^{n}J (C, H) data of **25** with the data of **3f** revealed similar coupling constants in both compounds verifying the assignment on the sole basis of the analysis of heteronuclear long-range coupling constants.

In conclusion, ^{n}J(C,H) data may offer a promising approach toward the assignment of quaternary aromatic

(32) (a) Lacerda, V. Jr; da Silva, G. V. J.; Tormena, C. F.; Williamson, R. T.; Marquez, B. L. *Magn. Reson. Chem.* **2007**, *45*, 82. (b) Williamson, R. T.; Boulanger, A.; Vulpanovici, A.; Roberts, M. A.; Gerwick, W. H. *J. Org. Chem.* **2002**, *67*, 7927. (c) Marquez, B. L.; Gerwick, W. H.; Williamson, R. T. *Magn. Reson. Chem.* **2001**, *39*, 499. (d) Meissner, A.; Sørensen, O. W. *Magn. Reson. Chem.* **2001**, *39*, 49. (e) Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, *64*, 866.

(33) (a) Kövér, K. E.; Batta, G.; Fehér, K. *J. Magn. Reson.* **2006**, *181*, 89. (b) Williamson, R. T.; Marquez, B. L.; Gerwick, W. H.; Kövér, K. E. *Magn. Reson. Chem.* **2000**, *38*, 265.

(34) Hansen, P. E. *Prog. Nucl. Magn. Reson. Spectrosc.* **1981**, *14*, 175.

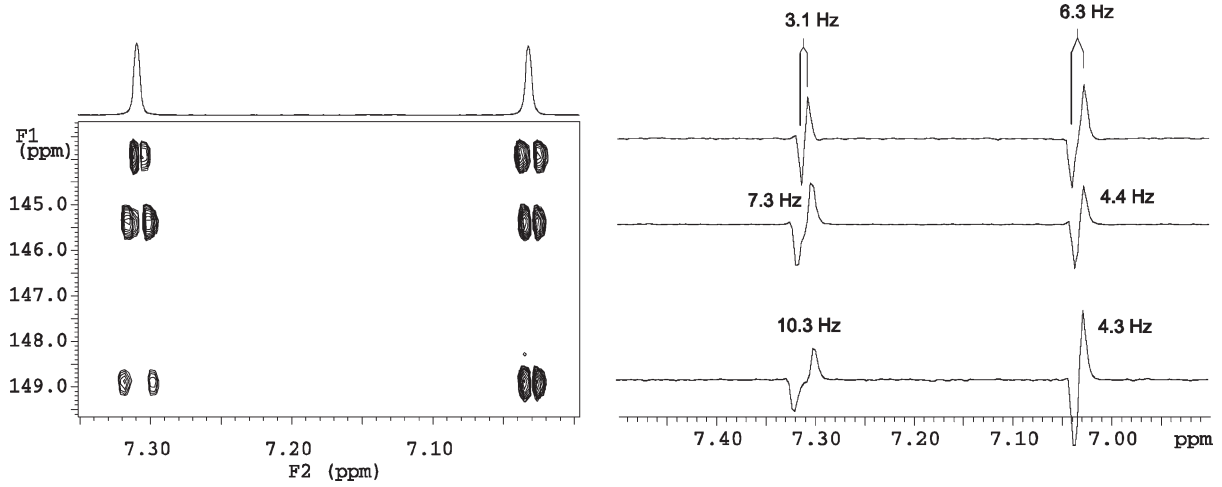


FIGURE 4. Part of the HSQMBC contour plot (500 MHz) of **3a** and 1D antiphase HSQC slices from the contour plot.

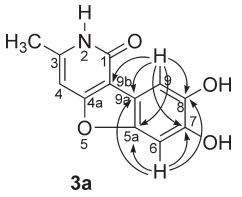


FIGURE 5. Important HSQMBC correlations and assignments of **3a**.

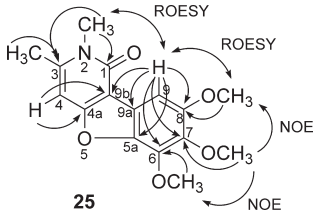
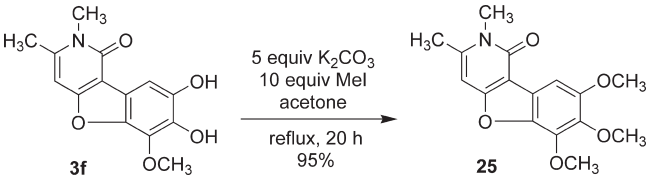


FIGURE 6. Important HMBC, ROESY, and NOE correlations of **25**.

SCHEME 5. Preparation of the Trimethoxy Derivative of 3f



carbons in proton-poor NMR spectra of heterocycles without any need for derivatization, solvent- and time-consuming purification steps, as well as additional NMR time.

Structure elucidation of compounds **6** and **10** (Figure 2) was performed in a similar manner as described for **3**, but here was hampered by an additional problem: as the carbon signals of C-6a and C-6b are very close to each other or even overlap (e.g., δ 114.36 and 114.49 ppm, **10a**), the HMBC correlations with the protons H-7 and H-10 cannot be resolved by indirect detected standard HMBC with 256 or 512 increments in F1 (Figure 7a). Hence, the connectivity between rings A and C could not be determined. This is why we used a recently published band-selective adiabatic gHMBC pulse sequence[35] combined with a modified gHMBC for super long-range connectivities (^4J (C, H)). The ^{13}C band-selection offers the possibility to record adiabatic gHMBCs with a high resolution in the indirect dimension and without spectral aliasing (Figure 7b). A ^4J (C, H) correlation between H-1 and C-6a along with a ^3J (C, H) correlation between H-7 and C-6a unambiguously established the connectivity between ring A and C as shown in Figure 2.

Unequivocal evidence for the structure of the products **3a**−**f** was produced by X-ray structure analysis. For this purpose, crystals of the diacetate **13f** which was derived from **3f** were studied by X-ray crystal structure analysis.[36] The result confirmed the structure assignments made by NMR experiments.

Structure elucidation of the dispiropyrimidinones **21a**−**d** was achieved by NMR spectroscopic methods including HSQC and HMBC. Both ^{1}H and ^{13}C NMR spectra are distinguished by the fact that they contain fewer resonance signals than expected for the corresponding annulated benzofurans. For example, the ^{1}H NMR of **21a** exhibits only three resonance signals, namely three singlets: at 3.29, 6.61, and 9.34 ppm. In the ^{13}C NMR spectrum of **21a** only seven resonance signals appear (29.7, 58.2, 113.8, 124.0, 146.9, 151.7, 169.9 ppm). These results indicate a highly symmetrical structure. Together with the mass spectrometry results suggesting that each of the compounds **21a**−**d** is made up of two catechol and two barbituric acid units it was deduced that **21a**−**d** are polycyclic dispiropyrimidinones.

(35) (a) Bradley, S. A.; Krishnamurthy, K. *Magn. Reson. Chem.* **2005**, *43*, 117. (b) Crouch, R.; Boyer, R. D.; Johnson, R.; Krishnamurthy, K. *Magn. Reson. Chem.* **2004**, *42*, 301.

(36) (a) CCDC-714093 (**13f**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. (b) Otwinowski, Z.; Minor, W. *Methods Enzymol.* **1997**, *276*, 307. (c) Sheldrick, G. M. *SHELXS-97, Programs for X-ray Crystal Structure Solution*; University of Göttingen: Göttingen, Germany, 1997. (d) Sheldrick, G. M. *SHELXL-97, Programs for X-ray Crystal Structure Refinement*; University of Göttingen: Göttingen, Germany, 1997.

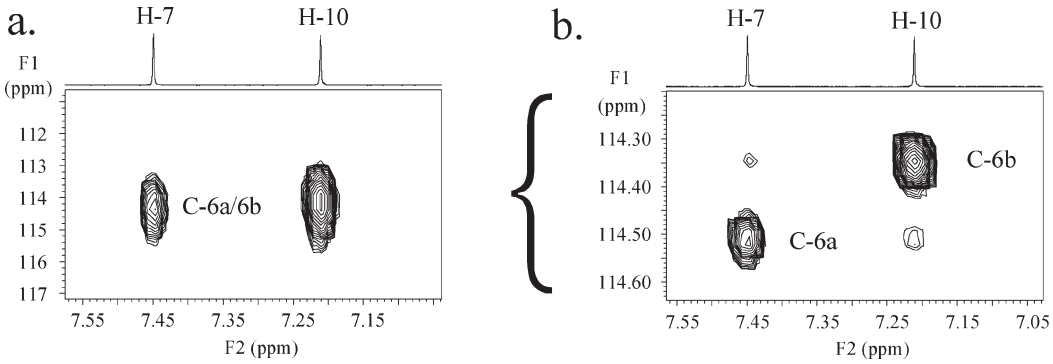


FIGURE 7. Part of HMBC spectra of **10a** displaying long-range correlations form H-7 and H-10 to C-6a and C-6b, respectively. (a) Part of adiabatic gHMBC with ni = 256 and sw1 = 30165 Hz. (b) Band-selective gHMBC with ni = 256 and sw1 = 1887 Hz.

Conclusions

Here we presented a simple and efficient method for the synthesis of several types of annulated benzofurans by employing a laccase-catalyzed domino reaction between catechols and heterocyclic 1,3-dicarbonyls. The reactions can be performed under mild conditions using aerial oxygen as an oxidant. When pyridinones, quinolinones, and thiocoumarins are used as reactants, the corresponding benzofuropyridinones, benzofuroquinolinones, and thiocoumestans are formed with yields ranging from 55 to 98%. With barbituric acid derivatives as reactants polycyclic dispiropyrimidinones are formed exclusively. The structure of all products was elucidated unambiguously by NMR spectroscopy. The results were supported by X-ray crystal structure analysis of one product (**13f**).

Experimental Section

General Procedure for the Laccase-Initiated Domino Reaction. A solution of 1.5 mmol of 1,3-dione and 1.7 mmol of catechol in 100 mL of 0.2 M phosphate buffer (pH 6.0) was placed in a 250 mL flask. A 50 mg portion of laccase from *A. bisporus* (AbL) [3.11 U/mg; the enzyme activity was determined with catechol as the substrate][24] was added and the mixture vigorously stirred in 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 50 mL of 15% NaCl and 5 mL of H_2O. The crude products obtained after drying exhibited a purity of 90−95% (NMR). Analytically pure products could be obtained by recrystallization of the corresponding diacetates.

7,8-Dihydroxy-6-methoxy-2,3-dimethylbenzofuro[3,2-*c*]pyridin-1(2*H*)-one (3f). Mp: 276−282 °C dec. R_f = 0.12 (EtOAc). IR (ATR): $\tilde{\nu}$ 3458 cm^{-1} (OH), 3087 (OH), 1658, 1621, 1559, 1432, 1343, 1321, 1195, 1100, 1075, 1037, 1018, 996, 893, 786, 682. ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.49 ppm (s, 3H, 3-CH_3), 3.54 (s, 3H, N−CH_3), 3.99 (s, 3H, OCH_3), 6.72 (s, 1H, 4-H), 7.14 (s, 1H, 9-H), 8.77 (s, 1H, 7-OH), 9.29 (s, 1H, 8-OH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.8 ppm (3-CH_3), 30.8 (NCH_3), 61.2 (OCH_3), 95.4 (C-4), 100.7 (C-9), 108.2 (C-9b), 115.9 (C-9a), 134.0 (C-6), 137.4 (C-7), 141.1 (C-5a), 145.1 (C-8), 146.4 (C-3), 159.9 (C-1), 161.0 (C-4a). MS (70 eV, EI): *m*/*z* 275 (100) [M^+], 260 (63), 232 (20).

7,8-Acetyloxy-6-methoxy-2,3-dimethylbenzofuro[3,2-*c*]pyridin-1(2*H*)-one (13f). Mp: 180−181 °C. R_f = 0.24 (EtOAc). IR (ATR): $\tilde{\nu}$ 1768 cm^{-1}, 1670, 1572, 1375, 1174, 1088, 1037, 1016, 883, 792. UV (CH_3CN): λ_{max} (lg ε) 314 nm (4.27), 287 nm (3.86), 272 nm (3.92), 221 nm (4.40). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.33 ppm (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 2.53 (s, 3H, CH_3), 3.57 (s, 3H, NCH_3), 4.14 (s, 3H, OCH_3), 6.83 (s, 1H, 4-H), 7.50 (s, 1H, 9-H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 20.7 ppm, 21.1, 22.0, 31.0, 61.5, 95.3, 107.1, 108.2, 123.6, 132.4, 138.5, 141.0, 143.2, 149.8, 159.7, 162.9, 168.9, 169.5. MS (70 eV, EI): *m*/*z* 359.1 (20) [M^+], 317.0 (67), 275.0 (100), 260 (25). Anal. Calcd for $C_{18}H_{17}NO_7$ (359.1): C, 60.17; H, 4.77; N, 3.90. Found: C, 60.44; H, 4.67; N, 4.12.

2′,3′,6′,7′-Tetrahydroxy-1,1″,3,3″-tetramethyldispiro[pyrimidine-5(2*H*),9′(10′*H*)-anthracene-10′,5″(2″*H*)-pyrimidine]-2,2″,4,4″,6,6″-(1*H*,1″*H*,3*H*,3″*H*)-hexone (21a). Mp: >410 °C (lit.[31e] mp >300 °C). R_f = 0.69 (CH_2Cl_2/MeOH = 6:4). IR (ATR): 3300 cm^{-1} (OH), 1706 and 1648 (C=O), 1537, 1454, 1416, 1380, 1350, 1251, 1206, 1060, 837, 758. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.28 ppm (s, 12H, N−CH_3), 6.61 (s, 4H, arom H), 9.33 (s, 4H, OH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 29.7 ppm (CH_3), 58.2 (spiro C), 113.8 (arom CH), 124.0 (arom C), 146.9 (COH), 151.7 (NCON), 169.9 (C=O). MS (FAB, matrix: glycerin): 523.9 [M^+]. $C_{24}H_{20}N_4O_{10}$ (524.12).

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Supporting Information Available: Full characterizations of all new compounds, HSQMBC data for compounds **3a**−**f**, **6a**−**c**, **10a**−**c**, and **25**, selected NMR data for the spiro compounds **21a**−**d**, crystallographic data, and the X-ray structure of the diacetate **13f**. This material is available free of charge via the Internet at http://pubs.acs.org.