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Studies on the Laccase-Catalyzed Oxidation of 4-Hydroxy-Chalcones

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Abstract: The laccase-catalyzed oxidation of a series of substituted 4-hydroxy-chalcones has been investigated. The main isolated dimeric products were, as expected, racemic mixtures of *trans*-2,3-dihydrobenzofuran derivatives, always co-eluted with an additional isomeric dimer with an open structure. The two enantiomers, as well as the co-eluted dimeric isomer could be isolated by semi-preparative HPLC with a chiral column and were fully characterized.

Keywords: Laccase; Chalcones; 2,3-Dihydrobenzofuran; Oxidation; Biocatalysis

The 2,3-dihydrobenzofuran (DHB, (**A**), Figure 1) skeleton is widely present in a large number of bioactive natural products, isolated mainly from plants but also from fungi, that belong to different classes, such as alkaloids, isoflavonoids, lignans and neolignans. These compounds have been the targets of a substantial number of synthetic studies, as the preparation of substituted DHB is not an easy task.[1]

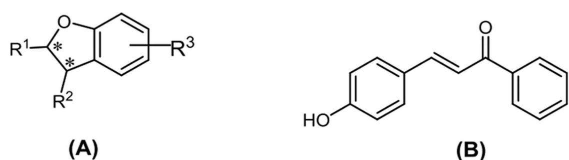

Figure 1. Structure of compounds (**A**) and (**B**)

Moreover, the DHB skeleton is also recurrent in the complex polymeric structure of lignin, where it has been classified as the so-called β-5 unit.[2] In Nature lignin is obtained by the polymerization of hydroxyphenyl-propenol precursors by action of several oxidative enzymes, among which the blue oxidases laccases, which catalyze the oxidation of phenolic compounds at the expense of molecular oxygen that is reduced to water,[3] play a significant role.

In previous investigations we have shown that laccases can be exploited for the preparative scale oxidation of substituted vinyl phenols[4] and polyhydroxy-stilbenes,[5] the main isolated products being the corresponding DHB derivatives (Scheme 1, part A).

In the most recent example, the same protocol proved to be very efficient for the synthesis of substituted (*E*)-2,3-diaryl-5-styryl-*trans*-2,3-dihydrobenzofurans based on the oxidative (homo)-coupling of (*E*)-4-styrylphenols (isolated yield up to 74%) (Scheme 1, part B).[6] In this way, considering their structural analogies to previously reported allosteric modulators,[7] a library of DHB-based potential allosteric activators of the Heat shock protein 90 (Hsp90) was easily prepared and tested *in vitro* for the potential stimulatory action of these compounds on the ATPase activity of the molecular chaperone Hsp90. Two of those sixteen DHB derivatives showed an appreciable activator activity.

To extend the scope of this straightforward synthetic approach to the DHB skeleton, we focused our attention to a new series of compounds, namely the chalcones (i.e., (**B**), Figure 1), with the aim to enrich the range of substituents located on the DHB moiety

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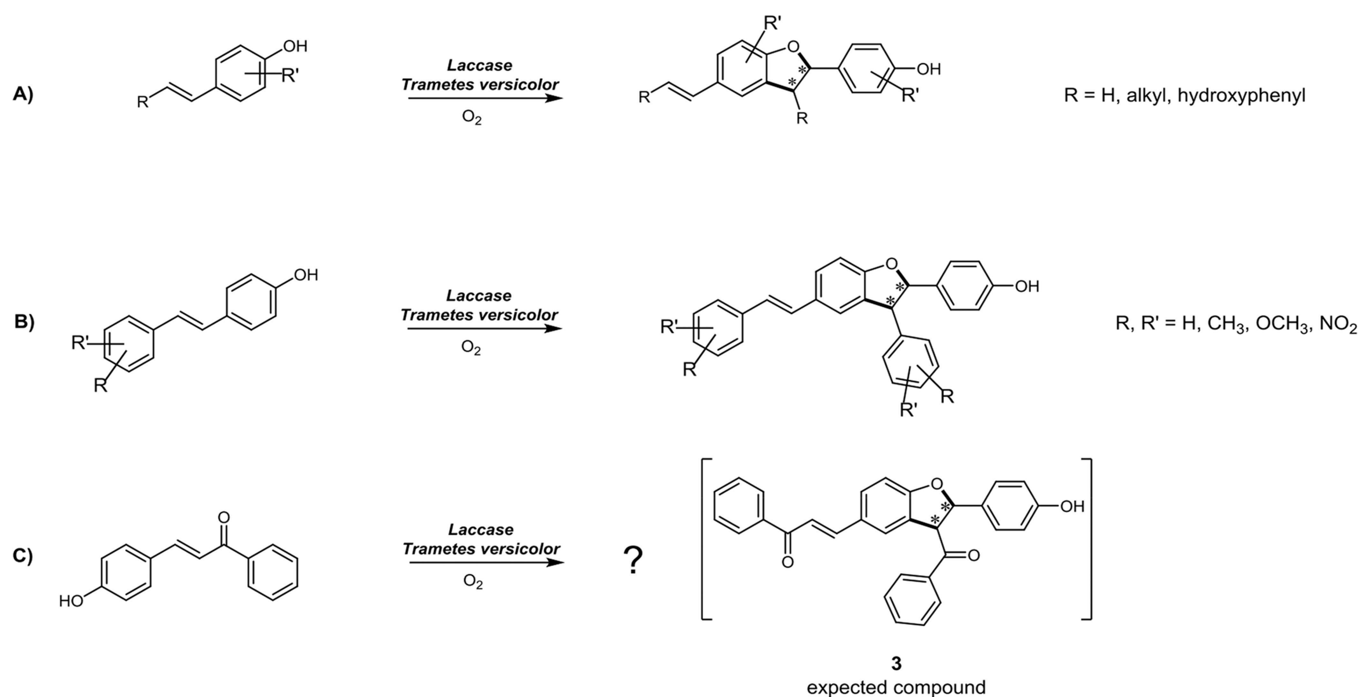


Scheme 1. Laccase-catalyzed dimerization of substituted phenols.

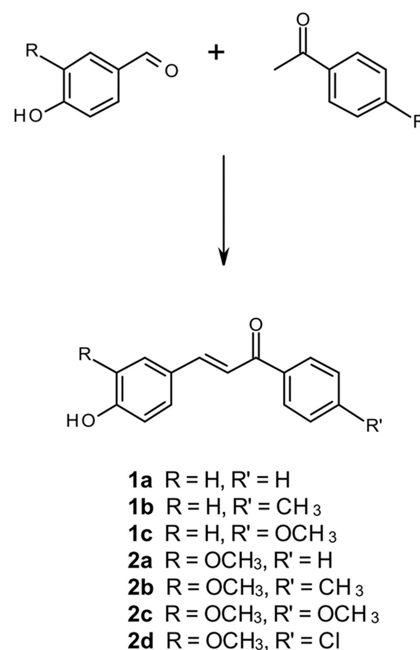
(Scheme 1, part C). In the following the results that have been obtained will be presented and discussed.

Chalcones is a generic name, coined a long time ago by Kostanecki and Tambor,[8] to indicate a generic chemical framework (1,3-diphenylprop-2-en-1-one) that is widely spread in the plant kingdom. They are open chain precursors for the biosynthesis of flavonoids and isoflavonoids. Their simple synthesis and chemical manipulation as well as the biological activities showed by several members of this family make the chalcone scaffold an interesting template in medicinal chemistry for drug discovery, as documented in several recent reviews.[9]

The classical chemical synthesis of chalcones is based on the Claisen-Schmidt condensation of suitably substituted benzaldehydes and acetophenones, performed either under basic[10] or acid[11] catalysis. In our hands and confirming early literature reports,[12] due to the presence of the phenolic substituent, the acid catalysis proved to be more efficient in most of the cases and, specifically, the protocol was exploited for the synthesis of the 4-hydroxy compounds **2 a**–**2 d** (Scheme 2).

Despite the fact that chalcones and specifically hydroxyl-chalcones are well-known antioxidants,[12,13] to our surprise there are only very few reports specifically devoted to the study of the outcome of oxidation reactions of these compounds[14] and, to the best of our knowledge, none of them describes the oxidation of 4-hydroxy chalcones (like our substrates **1 a**–**1 c** and **2 a**–**2 d**).

The non-substituted 4-hydroxy chalcone **1 a** was initially considered, and its laccase-catalyzed oxidation was investigated under various reaction conditions, using either biphasic systems or monophasic systems in the presence of significant amount of water miscible organic cosolvents. Eventually, it was found that the



Scheme 2. Synthesis of chalcones **1 a**–**1 c** and **2 a**–**2 d**.

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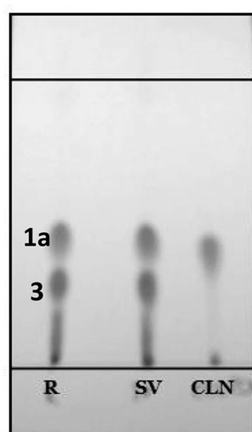

Figure 2. R: Laccase-catalyzed oxidation of **1 a**; CLN: substrate **1 a**; SV: R + CLN

best performances could be obtained in the presence of 50% v/v acetone. Figure 2 shows the TLC of the reaction after 6 h.

Usual work up and silica gel chromatography allowed the isolation of the product(s) corresponding to the more polar spot at R_f 0.29. Mass spectra analysis (ESI) indicated the presence of a peak at m/z 447 Da [M + H +], corresponding to the expected dimeric product(s) **3**, isolated in 26% yield.

However, the ^{1}H NMR spectrum (in $CDCl_3$) clearly showed that the isolated product was not a pure compound, and this was confirmed by RP-HPLC, which indicated the presence of at least two peaks, although very poorly separated even under the best chromatographic conditions (Two partially overlapping peaks were detected, with t_R = 19.0 min and t_R = 19.5 min respectively, using a RP-HPLC analytical column. For more details see the Experimental part). Despite that, pure samples corresponding to the two peaks could be isolated by injecting small amounts of the mixture of the dimers for several times on a semipreparative HPLC column carrying the same stationary phase (for more details see the Experimental part and Figure 9.1 in Supplementary Materials) and fully characterized. Both of them (compound **C** and **D**) proved to be dimeric structures by mass spectra analysis. Figure 3 compares the ^{1}H NMR spectra of the mixture **3** (top) and of the two isolated compounds **C** (middle) and **D** (bottom) in the range between 5.15 and 7.15 ppm (for the full spectra see supplementary materials). Diagnostic for **C** are the two doublets at 5.24 and 6.33 ppm (J = 7.2 Hz), whereas for **D** is the singlet at 7.08 ppm.

In compound **C** these signals (obviously together with all the others present in the spectrum) are compatible with the structure of the expected dihydrobenzofuranic dimeric structure, with the two substituent at C-2 and C-3 position in the *trans* configuration.

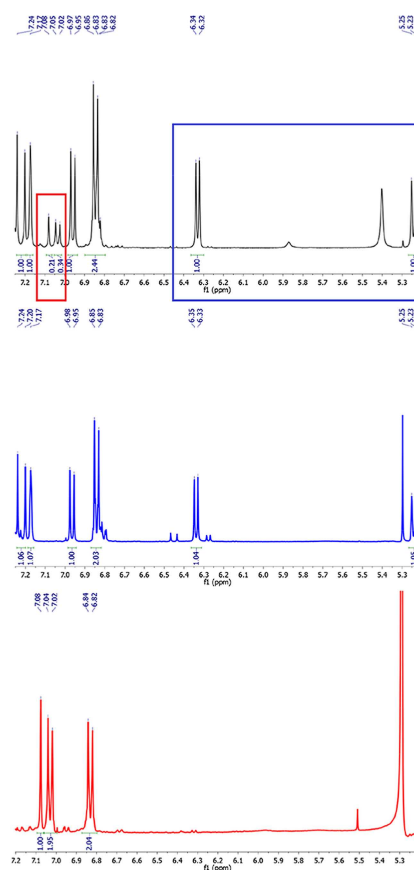

Figure 3. Comparison of a selected portion of the ^{1}H NMR spectra of **3** (top), **C** (middle) and **D** (bottom).

As shown in Scheme 3, this structure is generated (as previously observed with other vinylic[4] and stilbenic[5,6] derivatives) by the combination of radical III and radical II, followed by rearrangement and ring closure.

On the contrary, combination of radical III and radical I followed by rearrangement gives two possible structures that are both compatible with the ^{1}H NMR spectrum recorded for **D** (the proton giving a singlet at 7.08 ppm is indicated).

In all our previous investigations, compounds with structures similar to **C** have been always isolated as racemic mixtures of the *trans* diasteroisomers. Accordingly, HPLC analysis of a sample of **C** with a chiral column showed the presence of two equivalent peaks. Moreover, analysis of the mixture of dimers **3** allowed the baseline separation both of the two enantiomers **3 a** and **3 b** and of the other isomer (**D**, from now on indicated as **3 c**). As shown in Figure 4, semipreparative HPLC separation of a new sample of **3** allowed the separation of the three compounds.

As expected, the samples corresponding to the two equivalent peaks showed identical NMR spectra and opposite values of optical rotation power ($[\alpha]_D$ =

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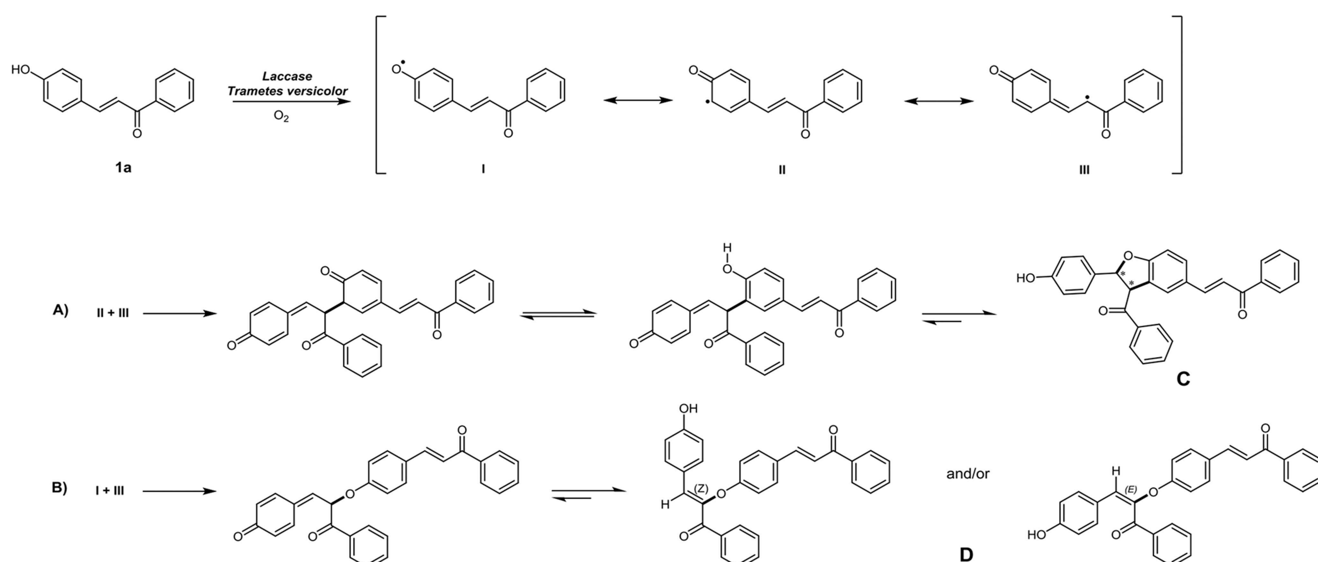

Scheme 3. Laccase-catalyzed dimerization of 4-hydroxy-chalcone **1 a**.

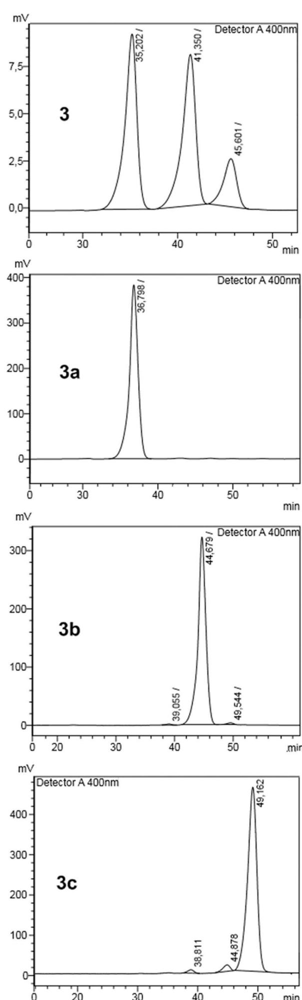

Figure 4. HPLC chromatogram of the mixture **3** and of the isolated compounds **3 a**, **3 b**, and **3 c**.

$-163°$ and $[\alpha]_D = +163°$ for Peak 1 and Peak 2, respectively). Their CD spectra were, as expected, specular (Figure 5) and, by analogy with previous literature reports,[5d] allowed us to tentatively assign the absolute configuration to the stereogenic carbons of the two enantiomers: 2*R*,3*R* for the less retained peak (**3 a**, Peak 1) and 2*S*,3*S* for the more retained one (**3 b**, Peak 2).

The stereochemistry of the trisubstituted double bond in **3 c** was determined measuring the NOEs involving its vinyl hydrogen. In the NOESY spectrum the singlet due to this proton shows an obvious contact with the ortho protons of the *p*-hydroxyphenylene ring linked to the same vinyl carbon Cα. In addition a second clear interaction exists with the ortho protons of the benzoyl ring linked to Cβ carbon of the double bond. Instead no interaction was observed with the other substituent on Cβ, namely the protons ortho to the oxygen atom of the *p*-oxyphenylene ring. Taking advantage of these observations it was possible to

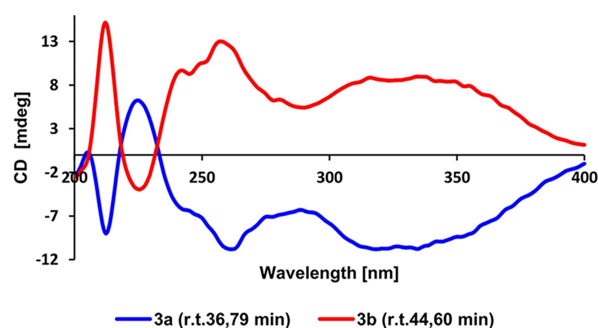

Figure 5. Electronic circular dichroism spectra of compounds **3 a** and **3 b**.

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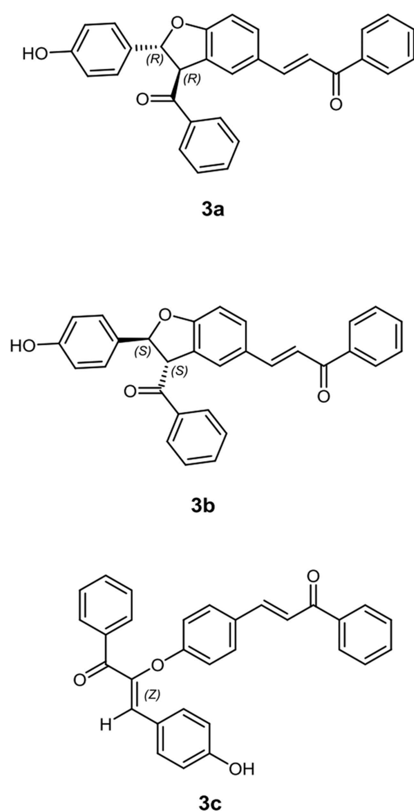

Scheme 4. Structures of compounds **3 a**, **3 b** and **3 c**.

conclude that the double bond has the *Z* stereochemistry where the vinyl proton and the vicinal benzoyl substituent are *cis* oriented (Scheme 4).

The same experimental protocol (laccase-catalyzed oxidation followed by silica gel chromatography) was applied to the other substituted hydroxychalcones **1 b**–**1 c** and **2 a**–**2 d**, furnishing the expected mixture of three products (Table 1), as shown by chiral HPLC (see Supplementary materials). The relative ratio of the products could be evaluated by comparing the area of selected signals of the ^{1}H NMR spectra.

Substrates **2 a**–**2 d**, obtained by the condensation of vanillin with monosubstituted acetophenones proved to be more reactive and the reactions needed to be much more carefully controlled in order to avoid the predominant formation of complex mixtures of over-oxidized products.

Table 1. Products yields and composition.

Substrate	Product, mg (yield)	Products Relative Ratio[a]
1 a (200 mg)	**3**, 52 mg (26%)	69:31 (**3 a**–**b**:**3 c**)
1 b (785 mg)	**4**, 135 mg (17%)	81:19 (**4 a**–**b**:**4 c**)
1 c (170 mg)	**5**, 42 mg (25%)	65:35 (**5 a**–**b**:**5 c**)
2 a (130 mg)	**6**, 19 mg (15%)	47:53 (**6 a**–**b**:**6 c**)
2 b (150 mg)	**7**, 24 mg (16%)	58:42 (**7 a**–**b**:**7 c**)
2 c (360 mg)	**8**, 52 mg (15%)	61:39 (**8 a**–**b**:**8 c**)
2 d (250 mg)	**9**, 62 mg (25%)	48:52 (**9 a**–**b**:**9 c**)

[a] Determined by ^{1}H-NMR.

Finally, to confirm the general scope of the proposed protocol, the mixtures of dimeric compounds **4**, **5**, and **9** (obtained by the laccase-catalyzed oxidation of **1 b**, **1 c**, and **2 d**, respectively) were purified by preparative HPLC on a chiral column and the respective products (**2*R*,3*R*-4 a**, **2*S*,3*S*-4 b**, **4 c**; **2*R*,3*R*-5 a**, **2*S*,3*S*-5 b**, **5 c**; **2*R*,3*R*-9 a**, **2*S*,3*S*-9 b**, **9 c**) were isolated and fully characterized (for more details see supplementary materials).

In conclusion, the laccase-catalyzed oxidation of 4-hydroxy-chalcones allowed the isolation of new derivatives possessing the 2,3-dihydrobenzofuran skeleton. Specifically, the pure enantiomers of the 2,3-disubstituted *trans* diasteroisomers could be isolated by semi-preparative HPLC with a chiral column, following a protocol previously exploited to purify the oxidized products obtained from hydroxystilbenes,[5d] The biological activities of these new compounds, for instance their ability to act as activators or inhibitors of the Heat shock protein Hsp90,[6] deserve to be investigated and the results will be reported in due course.

Experimental Section

General Experimental Procedures

Thin-layer chromarography (TLC): pre-coated glass plates silica gel 60 with fluorescent indicator UV_{254}. Flash chromatography: silica gel 60 (70–230 mesh, Merck). HPLC analyses were carried out using a Prominence Liquid Chromatograph (Shimadzu), Kinetex 5 µm EVO C18 100 A LC Column 150× 4.60 mm as achiral analytical column and Lux 5 µm Cellulose-1 Phenomenex 150×4.60 mm as chiral analytical column; Tri Rotar-VI HPLC, MD-910 multichannel detector (JASCO), Lux 5 µm Cellulose-1 Phenomenex 250×10 mm semi-preparative column. HPLC condition: isocratic mobile phase water/acetonitrile (relative ratio and flow rate reported in the following paragraphs) detection at 400 nm at 25 °C. NMR spectra (^{1}H and ^{13}C) were recorded on a Bruker AV (400 MHz) and a Bruker DRX (500 MHz) in $CDCl_3$. Mass spectra were recorded on a Bruker Esquire 3000 Plus (ESI Ion Trap LC/MSn System) spectrometer. HRMS spectra were recorded using electrospray ionization (ESI) technique on a Waters Micromass Q-Tof micro mass spectrometer. Electronic Circular Dichroism spectra were recorded on nitrogen flushed Jasco J-1100 spectropolarimeter (Easton MD, USA) interfaced with a thermostatically controlled cell holder. Electronic Circular Dichroism analysis was performed with purified compound dissolved in methanol (0.15 $mg\,mL^{-1}$ final concentration for far-UV analysis, 1 $mg\,mL^{-1}$ final concentration for near UV analysis) in quartz cuvettes with 0.1 cm path length (far-UV analysis) or 1 cm path length (near-UV analysis). Electronic Circular Dichroism spectra were recorded in the range between 200 to 400 nm (near-UV analysis) at 20 °C. Optical rotations were measured on PROPOL Digital Automatic Polarimeter (Dr.

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Kernchen Optik-Elektronik-Automation). The specific rotation is calculated as follows: $[\alpha]_\lambda^T = \frac{\alpha\,100}{cd}$. Thereby, the wavelength λ is reported in nm and the measuring temperature in °C. α represents the recorded optical rotation, c the concentration of the analyte in $mg\,mL^{-1}$ and d the length of the cuvette in dm. Thus, the specific rotation is given in 10^{-1} $deg{*}cm^2{*}g^{-1}$. Use of sodium D line (λ=589 nm) is indicated by D instead of the wavelength in nm. The sample concentration as well as the solvent is reported in the relevant section of the experimental part.

Enzymes and Materials

Laccase from *Trametes versicolor* (20 $U\,mg^{-1}$) was from Sigma-Aldrich. All other reagents were of the best purity grade from commercial suppliers.

Synthesis of Chalcones 1 a–1 c and 2 a–2 d

General Procedure

a) Basic conditions. A mixture of the required acetophenone derivative (2.0 mmol) and 4-hydroxybenzaldehyde (2.0 mmol) was stirred in ethanol (10 mL). To this solution, sodium hydroxide (6.0 mmol) was added slowly and stirred at room temperature overnight. The mixture was poured into crushed ice and acidified with dilute hydrochloric acid. The chalcone derivative precipitates out as a slightly yellow solid. The desired product is filtered off and dried to yield the chalcones **1 a**–**1 c**.

b) Acid conditions. A solution of the required acetophenone derivative (2.0 mmol) and 4-hydroxybenzaldehyde derivative (2.0 mmol) in MeOH (6.8 mL) was prepared. H_2SO_4 (98%, 3.75 mmol, ca 200 µl) was added and the solution was heated to 80 °C for 48 h, monitoring the conversion by TLC (CH_2Cl_2 – acetone, 99:1). The solution was neutralized by adding a NaOH solution (20% w/v). The solution was diluted with water and extracted with EtOAc. The organic phase was dried (Na_2SO_4), the solvent evaporated and the crude residue by silica gel flash chromatography to yield the chalcones **2 a**–**2 d**.

(2*E*)-3-(4-hydroxyphenyl)-1-phenylprop-2-en-1-one (1 a) (yellow solid, 1.38 g, 75% i.y.). R_f=0.45 (eluent petroleum ether- EtOAc 7:3). MS (ESI): m/z=247.0 Da (M+Na^+). ^1H NMR (400 MHz, $CDCl_3$): δ=7.94 (d, J=7.0 Hz, 2H), 7.70 (d, J=15.6 Hz, 1H), 7.56–7.39 (m, 5H), 7.31 (d, J=15.6 Hz, 1H), 6.84 (d, J=8.6 Hz, 2H), 5.25 (s, 1H). ^{13}C NMR (400 MHz, $CDCl_3$): δ=190.53, 160.03, 145.19, 138.38, 132.30, 130.29, 128.36, 128.14, 126.03, 118.62, 116.04. The NMR data were in accordance to the literature values.[15]

(2*E*)-3-(4-hydroxyphenyl)-1-(4-methylphenyl)prop-2-en-1-one (1 b) (yellow solid, 784 mg, 80% i.y.). R_f=0.47 (eluent petroleum ether-EtOAc 7:3). MS (ESI): m/z=498.8 Da (2 M+Na^+). ^1H NMR (400 MHz, $CDCl_3$): δ=7.90 (d, J=8.2 Hz, 2H), 7.75 (d, J=15.6 Hz, 1H), 7.51 (d, J=8.6 Hz, 2H), 7.37 (d, J=15.6 Hz, 1H), 7.28 (d, J=8.0 Hz, 2H), 6.89 (d, J=8.6 Hz, 2H), 2.41 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$): δ=190.67, 159.76, 145.17, 143.47, 136.04, 130.53, 129.36, 128.67, 126.78, 119.14, 116.28, 21.71. The NMR data were in accordance to the literature values.[16]

(2*E*)-3-(4-hydroxyphenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (1 c) (yellow solid, 643 mg, 81% i.y.). R_f=0.20 (eluent petroleum ether-EtOAc 7:3). MS (ESI): m/z=277.0 Da (M+Na^+).^1H NMR (400 MHz, $CDCl_3$): δ=9.04 (s, 1 H), 7.97 (d, J=8.9 Hz, 2 H), 7.70 (d, J=15.5 Hz, 1 H), 7.47 (d, J=8.6 Hz, 2 H), 7.34 (d, J=15.5 Hz, 1 H), 6.93 (d, J=8.9 Hz, 2 H), 6.85 (d, J=8.6 Hz, 2H), 3.84 (s, 3H).^{13}C NMR (400 MHz, $CDCl_3$): δ=188.41, 162.89, 159.74, 144.03, 131.05, 130.28, 130.00, 126.01, 118.21, 115.85, 113.47, 55.16. The NMR data were in accordance to the literature values.[17]

(2*E*)-3-(4-hydroxy-3-methoxyphenyl)-1-phenylprop-2-en-1-one (2 a) (yellow solid, 289 mg, 69% i.y.). R_f=0.52 (eluent $CHCl_3$-acetone 9:1). MS (ESI): m/z=277.0 Da (M+Na^+). ^1H NMR (400 MHz, $CDCl_3$): δ=8.00 (d, J=7.6 Hz, 2H), 7.75 (d, J=15.6 Hz, 1H), 7.57 (t, J=7.3 Hz, 1H), 7.50 (t, J=7.6 Hz, 2H), 7.37 (d, J=15.6 Hz, 1H), 7.22 (dd, J=8.2, 1.6 Hz, 1H), 7.14 (s, 1H), 6.96 (d, J=8.2 Hz, 1H), 5.95 (s, 1H), 3.96 (s, 3H).^{13}C NMR (400 MHz, $CDCl_3$): δ=190.83, 148.53, 147.02, 145.40, 138.64, 132.67, 128.68, 128.55, 127.60, 123.51, 119.94, 115.06, 110.23, 56.14. The NMR data were in accordance to the literature values.[18]

(2*E*)-3-(4-hydroxy-3-methoxyphenyl)-1-(4-methylphenyl) prop-2-en-1-one (2 b) (yellow solid, 306 mg, 69% i.y.). R_f= 0.30 (eluent CH_2Cl_2- acetone 99:1). MS (ESI): m/z=291.0 Da (M+Na^+). ^1H NMR (400 MHz, $CDCl_3$): δ=7.92 (d, J= 8.2 Hz, 2H), 7.74 (d, J=15.6 Hz, 1H), 7.37 (d, J=15.6 Hz, 1H), 7.30 (d, J=7.9 Hz, 2H), 7.21 (dd, J=8.2, 1.9 Hz, 1H), 7.13 (d, J=1.9 Hz, 1H), 6.95 (d, J=8.2 Hz, 1H), 5.95 (s, 1H), 3.96 (s, 3H), 2.43 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$): δ= 190.28, 148.44, 147.00, 144.97, 143.45, 135.91, 129.31, 128.64, 127.57, 123.33, 119.75, 115.03, 110.26, 56.05, 21.67. The NMR data were in accordance to the literature values.[19]

(2*E*)-3-(4-hydroxy-3-methoxyphenyl)-1-(4-methoxyphenyl) prop-2-en-1-one (2 c) (yellow solid, 357 mg, 64% i.y.). R_f= 0.23 (eluent petroleum ether-EtOAc 7:3). MS (ESI): m/z= 307.0 Da (M+Na^+). ^1H NMR (400 MHz, $CDCl_3$): δ=8.03 (d, J=8.9 Hz, 2H), 7.74 (d, J=15.6 Hz, 1H), 7.39 (d, J=15.6 Hz, 1H), 7.22 (dd, J=8.2, 1.8 Hz, 1H), 7.13 (d, J=1.8 Hz, 1H), 6.98 (d, J=8.9 Hz, 2H), 6.95 (d, J=8.2 Hz, 1H), 5.91 (s, 1H), 3.97 (s, 3H), 3.89 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$): δ= 188.95, 163.37, 148.49, 147.13, 144.51, 131.45, 130.79, 127.63, 123.25, 119.55, 115.14, 113.88, 110.34, 56.10, 55.55. The NMR data were in accordance to the literature values.[18]

(2*E*)-1-(4-chlorophenyl)-3-(4-hydroxy-3-methoxyphenyl) prop-2-en-1-one (2 d) (yellow solid, 350 mg, 74% i.y.). R_f= 0.38 (eluent CH_2Cl_2-acetone 99:1). MS (ESI): m/z=311.0 Da (M+Na^+).^1H NMR (400 MHz, $CDCl_3$): δ=7.95 (d, J=8.6 Hz, 2H), 7.75 (d, J=15.6 Hz, 1H), 7.47 (d, J=8.6 Hz, 2H), 7.32 (d, J=15.6 Hz, 1H), 7.22 (dd, J=8.2, 1.9 Hz, 1H), 7.12 (d, J= 1.9 Hz, 1H), 6.96 (d, J=8.2 Hz, 1H), 5.95 (s, 1H), 3.97 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 189.35, 148.69, 147.00, 145.87, 138.99, 136.81, 129.89, 128.90, 127.32, 123.58, 119.14, 115.08, 110.30, 56.07. The NMR data were in accordance to the literature values.[18]

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Laccase-catalyzed Oxidation of 4-hydroxychalcone (1 a)

A solution of laccase from *T. versicolor* (8 mL; 2.5 $mg\,mL^{-1}$ in acetate buffer pH 4.5; specific activity: 4.5 $U\,mg^{-1}$) was added to a solution of 4-hydroxychalcone (**1 a**, 200 mg, 0.89 mmol, with 10 mL of acetate buffer pH 4.5 and 18 mL of acetone). The reaction mixture was shaken (160 rpm) at 27 °C for 6 h and monitored by TLC (petroleum ether-EtOAc 7:3). The reaction was quenched by extraction with EtOAc, and the organic phase was washed with brine (2 × 15 mL), dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The crude residue was purified by flash column chromatography (petroleum ether-EtOAc 7:3) to afford dimeric product(s) as a white foam (52 mg, 26% yield). $R_f = 0.29$. MS (ESI): $C_{30}H_{23}O_4$ $[M+H]^+$ 447.0 Da.

The dimeric product was submitted to analytical HPLC-UV analysis using a Kinetex 5 µm EVO C18 100 Å LC Column 150 × 4.6 mm and a gradient elution (mobile phase: water/acetonitrile from 50:50 to 20:80; flow rate 0.5 $mL\,min^{-1}$ at 40 °C; detection at 400 nm). Two peaks were detected: first peak $t_R = 19.0$ min; second peak $t_R = 19.5$ min.

The separation of the dimeric products was also carried out by analytical HPLC-UV analysis using a chiral column (Lux 5 µm Cellulose-1 Phenomenex, 150 × 4.60 mm) and an isocratic elution (mobile phase: water/acetonitrile, 45:55; flow rate 0.5 $mL\,min^{-1}$ at 40 °C; detection at 400 nm). Three peaks were detected: first peak $t_R = 36.3$ min; second peak $t_R = 42.4$ min; third peak $t_R = 46.7$ min.

Purification of the Dimeric Products Obtained by Laccase-catalyzed Oxidation of 4-hydroxychalcone (1 a) by Preparative HPLC

a) With a Reverse-Phase Column

The separation of the two stereoisomers was carried out by preparative scale HPLC chromatography using a Kinetex 5 µm EVO C18 100 Å LC Column 150 × 10.0 mm and a gradient elution (mobile phase: water/acetonitrile from 80:20 to 20:80; flow rate 5 $mL\,min^{-1}$ at r.t.; detection at 400 nm). Dimeric products were dissolved in water/acetonitrile 1:1 at a concentration of 8.5 $mg\,mL^{-1}$. The separation protocol was repeated several time with injection of 0.2 mL of solution in order to avoid column saturation. In this way, two stereoisomers could be isolated: compound **C** and compound **D** (see Figure 9.1 in Supplementary Materials).

C: ^1H NMR (500 MHz, $CDCl_3$) δ 8.02 (d, $J = 7.2$ Hz, 2H), 7.93 (d, $J = 7.1$ Hz, 2H), 7.69 (t, $J = 7.4$ Hz, 1H), 7.64 (d, $J = 15.6$ Hz, 1H), 7.59–7.53 (m, 4H), 7.47 (t, $J = 7.6$ Hz, 2H), 7.27 (d, $J = 8.5$ Hz, 2H), 7.22 (d, $J = 15.6$ Hz, 1H), 7.17 (br s, 1H), 6.96 (d, $J = 8.4$ Hz, 1H), 6.84 (d, $J = 8.6$ Hz, 2H), 6.33 (d, $J = 7.2$ Hz, 1H), 5.24 (d, $J = 7.2$ Hz, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 195.99, 190.83, 162.02, 156.26, 144.94, 138.55, 136.29, 134.31, 132.77, 132.59, 130.70, 129.39, 129.29, 128.71, 128.55, 128.27, 127.73, 126.55, 125.79, 119.83, 115.93, 110.81, 86.92, 57.94. MS (ESI): 447.0 Da.

D: ^1H NMR (500 MHz, $CDCl_3$) δ 7.97 (d, $J = 7.1$ Hz, 2H), 7.85 (d, $J = 7.0$ Hz, 2H), 7.71 (d, $J = 15.7$ Hz, 1H), 7.64 (d, $J = 8.7$ Hz, 2H), 7.60–7.54 (m, 2H), 7.53 (d, $J = 8.8$ Hz, 2H), 7.48 (t, $J = 7.6$ Hz, 2H), 7.44 (t, $J = 7.7$ Hz, 2H), 7.37 (d, $J = 15.7$ Hz, 1H), 7.08 (s, 1H), 7.03 (d, $J = 8.7$ Hz, 2H), 6.83 (d, $J = 8.8$ Hz, 2H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 191.75, 190.78, 158.42, 157.76, 145.97, 144.41, 138.51, 137.41, 132.86, 132.83, 132.62, 130.48, 130.13, 129.72, 129.40, 128.75, 128.61, 128.54, 125.32, 120.92, 116.50, 116.11. MS (ESI): 447.0 Da.

b) With a Chiral-phase Column

The separation of the dimeric products was also carried out by preparative scale HPLC using a chiral HPLC column (Lux 5 µm Cellulose-1 Phenomenex, 150 × 4.60 mm) and an isocratic elution (mobile phase: water/acetonitrile, 45:55; flow rate 5 $mL\,min^{-1}$; detection at 400 nm). Dimeric products were dissolved in water/acetonitrile 1:1 at a concentration of 8.5 $mg\,mL^{-1}$. The first stereoisomer **(2*R*,3*R*)-3 a** was eluted with a retention time of 36.8 min, the second one, **(2*S*,3*S*)-3 b**, at 44.6 min, and the third stereoisomer **3 c** with a retention time of 49.2 min. ^1H NMR, ^{13}C NMR of **(2*R*,3*R*)-3 a** and **(2*S*,3*S*)-3 b** were identical to the above reported data for **C**.

(2*E*)-3-[(2*R*,3*R*)-3-benzoyl-2-(4-hydroxyphenyl)-2,3-dihydro-1-benzofuran-5-yl]-1-phenylprop-2-en-1-one [(2*R*,3*R*)-3 a]. HRMS (ESI): m/z $(M-H)^-$ calcd for $C_{30}H_{21}O_4$: 445.1440, found: 445.1439. $[\alpha]_D$: −163 ($c = 2.50 \cdot 10^{-3}\ g \cdot cm^{-3}$, $CHCl_3$). ECD (c = 1 mM, MeOH): Δε = 212 (−2.729), Δε = 224 (1.894), Δε = 261 (−3.280), Δε = 288 (−1.910), Δε = 318 (−3.277).

(2*E*)-3-[(2*S*,3*S*)-3-benzoyl-2-(4-hydroxyphenyl)-2,3-dihydro-1-benzofuran-5-yl]-1-phenylprop-2-en-1-one [(2*S*,3*S*)-3 b]. $[\alpha]_D$: + 163 ($c = 2.375 \cdot 10^{-3}\ g \cdot cm^{-3}$, $CHCl_3$). ECD (c = 1 mM, MeOH): Δε = 212 (4.597), Δε = 225 (−1.203), Δε = 257 (3.946), Δε = 290 (1.642), Δε = 334 (2.722).

(2*Z*)-3-(4-hydroxyphenyl)-2-{4-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]phenoxy}-1-phenylprop-2-en-1-one (3 c). ^1H NMR and ^{13}C NMR were identical to the above reported data for **D**. m/z $(M-H)^-$ calcd for $C_{30}H_{22}O_4$ $[M-H]^-$: 445.1440; found: 445.1441.

Laccase-catalyzed Oxidation of 4-hydroxychalcones 1 b–1 c and 2 a–2 d

Enzymatic oxidations were carried out as previously described and the dimeric products were initially isolated as a mixture by silica gel chromatography (isolated yields are reported in Table 1) and then, as an example, some of them were purified as separated isomers by semi-preparative HPLC using the previously described chiral column. Specifically:

Laccase-catalyzed Oxidation of 1 b

The crude residue was purified by flash column chromatography (petroleum ether-EtOAc 7:3) to afford dimeric products as a white foam (135 mg, $R_f = 0.30$, 17% yield). MS (ESI): $[C_{32}H_{26}O_4+H]^+$: 475.0. Analysis of the dimeric products was carried out by analytical HPLC-UV analysis using a chiral column (Lux 5 µm Cellulose-1 Phenomenex, 150 × 4.60 mm) and an isocratic elution (mobile phase: water/acetonitrile, 45:55; flow rate 0.5 $mL\,min^{-1}$ at 40 °C; detection at 400 nm). Three peaks were detected: first peak $t_R = 60.654$ min; second

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peak $t_R = 67.209$ min; third peak $t_R = 73.050$ min. The separation of the dimeric products was carried out by preparative scale HPLC using the chiral column and an isocratic elution (mobile phase: water/acetonitrile, 45:55; flow rate 5 mL min^{-1}, detection at 400 nm). The first stereoisomer **(2*R*,3*R*)-4a**, eluted with a retention time of 63.4 min, the second one, **(2*S*,3*S*)-4b**, of 71.4 min, and the third stereoisomer **4c** with a retention time of 73.7 min. ^{1}H NMR and ^{13}C NMR spectra of **(2*R*,3*R*)-4a** and **(2*S*,3*S*)-4b** were identical.

(2*R*,3*R*)-4a (or **(2*S*,3*S*)-4b**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, *J* = 8.2 Hz, 2H), 7.85 (d, *J* = 8.2 Hz, 2H), 7.63 (d, *J* = 15.6 Hz, 1H), 7.56 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.35 (d, *J* = 8.0 Hz, 2H), 7.28–7.27 (m, 2H), 7.26–7.25 (m, 2H), 7.23 (d, *J* = 15.7 Hz, 1H), 7.17 (s, 1H), 6.95 (d, *J* = 8.4 Hz, 1H), 6.83 (d, *J* = 8.6 Hz, 2H), 6.33 (d, *J* = 7.2 Hz, 1H), 5.21 (d, *J* = 7.2 Hz, 1H), 2.48 (s, 3H), 2.42 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 195.55, 190.56, 161.99, 156.36, 145.42, 144.78, 143.67, 135.94, 133.79, 132.59, 130.47, 129.97, 129.53, 129.42, 128.73, 128.30, 127.68, 126.74, 125.90, 119.76, 116.41, 115.91, 86.94, 57.80, 21.89, 21.76.

(2*E*)-3-[(2*R*,3*R*)-2-(4-hydroxyphenyl)-3-(4-methylbenzoyl)-2,3-dihydro-1-benzofuran-5-yl]-1-(4-methylphenyl)prop-2-en-1-one [(2*R*,3*R*)-4a]. m/z (M−H)$^-$ calcd for $C_{32}H_{25}O_4$ [M−H]$^-$: 473.1753; found: 473.1745. $[\alpha]_D$: −170.0 (c = $7.2\cdot10^{-3}$ g·cm^{-3}, $CHCl_3$). ECD (c = 1 mM, MeOH): Δε = 207 (3.754), 217.4 (−4.061), 226.7 (1.565), 239.3 (−1.117), 249.1 (0.612), 266.9 (−2.002), 291.3 (−1.034), 317.8 (−2.980).

(2*E*)-3-[(2*S*,3*S*)-2-(4-hydroxyphenyl)-3-(4-methylbenzoyl)-2,3-dihydro-1-benzofuran-5-yl]-1-(4-methylphenyl)prop-2-en-1-one [(2*S*,3*S*)-4b]. $[\alpha]_D$: +169.7 (c = $6.0\cdot10^{-3}$ g·cm^{-3}, $CHCl_3$). ECD (c = 1 mM, MeOH): Δε = 206.8 (−0.590), 216.8 (7.359), 226.9 (0.940), 237.5 (2.829), 249.9 (1.688), 264.7 (3.321), 286.7 (1.898), 337.5 (3.057).

(2*Z*)-3-(4-hydroxyphenyl)-1-(4-methylphenyl)-2-{4-[(1*E*)-3-(4-methylphenyl)-3-oxoprop-1-en-1-yl]phenoxy}prop-2-en-1-one (4c). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, *J* = 8.2 Hz, 2H), 7.79 (d, *J* = 8.2 Hz, 2H), 7.71 (d, *J* = 15.7 Hz, 1H), 7.64 (d, *J* = 8.7 Hz, 2H), 7.52 (d, *J* = 8.7 Hz, 2H), 7.37 (d, *J* = 15.6 Hz, 1H), 7.28 (d, *J* = 8.0 Hz, 2H), 7.24 (d, *J* = 8.0 Hz, 2H), 7.05 (d, *J* = 5.4 Hz, 2H), 7.02 (s, 1H), 6.82 (d, *J* = 8.8 Hz, 2H), 2.43 (s, 3H), 2.41 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 191.39, 190.28, 158.34, 157.47, 146.18, 145.43, 144.52, 144.01, 135.90, 134.57, 132.74, 130.42, 129.65, 129.45, 129.41, 129.25, 128.76, 125.52, 120.89, 116.48, 116.05, 115.89, 21.80. m/z (M−H)$^-$ calcd for $C_{32}H_{25}O_4$ [M−H]$^-$: 473.1753; found: 473.1757.

Laccase-catalyzed Oxidation of 1c

The crude residue was purified by flash column chromatography (petroleum ether-EtOAc 6:4) to afford a dimeric products as a white foam (42 mg, $R_f = 0.30$, 25% yield). MS (ESI): [$C_{32}H_{27}O_6$] [M+H]$^+$: 507.2. The separation of the dimeric products was carried out by analytical HPLC-UV analysis using a chiral column (Lux 5 µm Cellulose-1 Phenomenex, 150 × 4.60 mm) and an isocratic elution (mobile phase: water/acetonitrile, 45:55; flow rate 0.5 mL min^{-1} at 40 °C; detection at 400 nm). Three peaks were detected: first peak $t_R = 45.588$ min; second peak $t_R = 50.282$ min; third peak $t_R = 55.030$ min. The separation of the dimeric products was carried out by preparative scale HPLC using the chiral column and an isocratic elution (mobile phase: water/acetonitrile, 45:55; flow rate 5 mL min^{-1}. The first stereoisomer **(2*R*,3*R*)-5a**, eluted with a retention time of 45.6 min, the second one, **(2*S*,3*S*)-5b**, at 50.3 min, and the third stereoisomer **5c** with a retention time of 52.6 min. ^{1}H NMR and ^{13}C NMR spectra of **(2*R*,3*R*)-5a** and **(2*S*,3*S*)-5b** were identical.

(2*R*,3*R*)-5a (or **(2*S*,3*S*)-5b**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, *J* = 8.5 Hz, 2H), 7.96 (d, *J* = 8.4 Hz, 2H), 7.64 (d, *J* = 15.5 Hz, 1H), 7.56 (d, *J* = 8.3 Hz, 1H), 7.26 (s, 1H), 7.26 (d, *J* = 20.9 Hz, 2H), 7.18 (s, 1H), 7.02 (d, *J* = 8.4 Hz, 2H), 6.97–6.92 (m, 3H), 6.83 (d, *J* = 8.1 Hz, 2H), 6.32 (d, *J* = 7.2 Hz, 1H), 5.19 (d, *J* = 7.2 Hz, 1H), 3.92 (s, 3H), 3.88 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 194.39, 189.00, 164.55, 163.48, 161.86, 156.18, 144.12, 132.78, 131.78, 131.45, 130.85, 130.25, 129.22, 128.42, 127.73, 126.92, 125.82, 119.58, 115.89, 114.49, 113.95, 110.70, 87.02, 57.65, 55.79, 55.63.

(2*E*)-3-[(2*R*,3*R*)-2-(4-hydroxyphenyl)-3-(4-methoxybenzoyl)-2,3-dihydro-1-benzofuran-5-yl]-1-(4-methoxyphenyl)prop-2-en-1-one [(2*R*,3*R*)-5a]. m/z (M−H)$^-$ calcd for $C_{32}H_{25}O_6$ [M−H]$^-$: 505.1651; found: 505.1657. $[\alpha]_D$: −164.0 (c = $7.75\cdot10^{-3}$ g·cm^{-3}, $CHCl_3$). ECD (c = 1 mM, MeOH): Δε = 210 (4.384), 227 (−4.269), 257 (0.465), 318 (−3.928).

(2*E*)-3-[(2*S*,3*S*)-2-(4-hydroxyphenyl)-3-(4-methoxybenzoyl)-2,3-dihydro-1-benzofuran-5-yl]-1-(4-methoxyphenyl)prop-2-en-1-one [(2*S*,3*S*)-5b]. $[\alpha]_D$: +164.0 (c = $6.25\cdot10^{-3}$ g·cm^{-3}, $CHCl_3$). ECD (c = 1 mM, MeOH): Δε = 211 (−2.850), 227 (4.479), 257 (−0.165), 316 (4.568).

(2*Z*)-3-(4-hydroxyphenyl)-1-(4-methoxyphenyl)-2-{4-[(1*E*)-3-(4-methoxyphenyl)-3-oxoprop-1-en-1-yl]phenoxy}prop-2-en-1-one (5c). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (d, *J* = 8.2 Hz, 2H), 7.91 (d, *J* = 8.2 Hz, 2H), 7.69 (d, *J* = 15.5 Hz, 1H), 7.62 (d, *J* = 8.0 Hz, 2H), 7.50 (d, *J* = 8.1 Hz, 2H), 7.37 (d, *J* = 15.7 Hz, 1H), 7.02 (d, *J* = 10.9 Hz, 2H), 7.00–6.86 (m, 5H), 6.83 (d, *J* = 8.0 Hz, 2H), 3.88 (s, 3H), 3.86 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 190.36, 189.05, 163.57, 163.47, 158.28, 157.86, 146.15, 143.64, 132.65, 131.93, 130.92, 130.37, 129.81, 129.72, 128.68, 125.25, 120.62, 116.46, 116.08, 113.99, 113.97, 113.83, 55.63, 55.60. m/z (M−H)$^-$ calcd for $C_{32}H_{25}O_6$ [M−H]$^-$: 505.1651; found: 505.1655.

Laccase-catalyzed Oxidation of 2d

The crude residue was purified by flash column chromatography (petroleum ether-EtOAc 65:35) to afford a dimeric products as a white foam (62 mg, $R_f = 0.35$, 25% yield). MS (ESI): [$C_{32}H_{24}Cl_2O_6$] [M+Na]$^+$: 597.3. The separation of the dimeric products was carried out by analytical HPLC-UV analysis using a chiral column (Lux 5 µm Cellulose-1 Phenomenex, 150 × 4.60 mm) and an isocratic elution (mobile phase: water/acetonitrile, 45:55; flow rate 0.5 mL min^{-1} at 40 °C; detection at 400 nm). Three peaks were detected: first peak $t_R = 82.002$ min; second peak $t_R = 86.970$ min; third peak $t_R = 105.240$ min. The separation of the dimeric products was carried out by preparative scale HPLC using the chiral column (Lux 5 µm Cellulose-1 Phenomenex, 150 × 4.60 mm) and an isocratic elution (mobile phase: water/acetonitrile, 45:55; flow rate 6 mL min^{-1}, detection at 400 nm). The first stereoisomer **(2*R*,3*R*)-9a**, eluted with a retention time of 81.8 min, the

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second one, **(2*S*,3*S*)-9 b**, eluted with a retention time of 86.5 min, and the third stereoisomer **9 c** with a retention time of 105.6 min. ^{1}H NMR and ^{13}C NMR spectra of **(2*R*,3*R*)-9 a** and **(2*S*,3*S*)-9 b** were identical.

(2*R*,3*R*)-9 a (or **(2*S*,3*S*)-9 b**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, *J*=8.6 Hz, 2H), 7.89 (d, *J*=8.6 Hz, 2H), 7.63 (d, *J*=15.6 Hz, 1H), 7.53 (d, *J*=8.6 Hz, 2H), 7.46 (d, *J*=8.6 Hz, 2H), 7.26 (s, 1H), 7.18 (d, *J*=15.5 Hz, 1H), 7.14 (s, 1H), 6.90 (s, 3H), 6.80 (s, 1H), 6.30 (d, *J*=7.9 Hz, 1H), 5.25 (d, *J*=7.8 Hz, 1H), 3.97 (s, 3H), 3.87 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 194.79, 189.17, 151.08, 146.94, 146.37, 145.40, 145.26, 141.04, 139.22, 136.81, 134.64, 131.66, 130.69, 129.94, 129.62, 129.05, 129.00, 126.98, 119.56, 119.54, 118.69, 114.88, 112.48, 109.00, 88.12, 58.39, 56.46, 56.21.

(2*E*)-3-[(2*R*,3*R*)-3-(4-chlorobenzoyl)-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydro-1-benzofuran-5-yl]-1-(4-chlorophenyl)prop-2-en-1-one [(2*R*,3*R*)-9a]. m/z $(M-H)^-$ calcd for $C_{32}H_{23}Cl_2O_6$ $[M-H]^-$: 573.0872; found: 573.0872. $[\alpha]_D$:−144.0 (c=2.0·10^{-3} g·cm^{-3}, $CHCl_3$). ECD (c=1 mM, MeOH): Δε=218.4 (−4.227), 241.9 (0.554), 261.7 (−3.034), 288.7 (−0.771), 327.1 (−1.759).

(2*E*)-3-[(2*S*,3*S*)-3-(4-chlorobenzoyl)-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydro-1-benzofuran-5-yl]-1-(4-chlorophenyl)prop-2-en-1-one [(2*S*,3*S*)-9 b]. $[\alpha]_D$:+144.0 (c=1.5·10^{-3} g·cm^{-3}, $CHCl_3$). ECD (c=1 mM, MeOH): Δε=219.3 (2.077), 239.1 (−3.40), 269.6 (2.318), 290.9 (−0.168), 354.8 (2.913).

(2*Z*)-1-(4-chlorophenyl)-2-{4-[(1*E*)-3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl]-2-methoxyphenoxy}-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one (9 c). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, *J*=8.6 Hz, 2H), 7.84 (d, *J*=8.6 Hz, 2H), 7.68 (d, *J*=15.6 Hz, 1H), 7.46 (d, *J*=8.4 Hz, 3H), 7.41 (d, *J*=8.6 Hz, 2H), 7.29 (d, *J*=15.6 Hz, 1H), 7.22 (dd, *J*=8.4, 1.9 Hz, 1H), 7.13 (d, *J*=1.8 Hz, 1H), 7.09 (dd, *J*=8.3, 1.9 Hz, 1H), 7.01 (s, 1H), 6.90 (d, *J*=8.3 Hz, 1H), 6.87 (d, *J*=8.3 Hz, 1H), 3.94 (s, 3H), 3.82 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 190.00, 189.73, 149.38, 148.00, 147.77, 146.70, 145.90, 145.39, 139.40, 139.04, 136.65, 135.38, 130.89, 130.11, 130.06, 129.73, 129.09, 128.78, 126.08, 125.02, 122.72, 120.61, 115.46, 114.84, 112.49, 112.07, 56.27, 55.87. m/z $(M-H)^-$ calcd for $C_{32}H_{23}Cl_2O_6$ $[M-H]^-$: 573.0872; found: 573.0881.

Laccase-catalyzed Oxidation of 2 a

The crude residue was purified by flash column chromatography (petroleum ether-EtOAc 65:35) to afford dimeric products as a white foam (19 mg, R_f=0.26, 15% yield). MS (ESI): $[C_{32}H_{26}O_6]$ $[M+Na]^+$: 529.2. The separation of the dimeric products **6** was carried out by analytical HPLC-UV analysis using a chiral column (Lux 5 µm Cellulose-1 Phenomenex, 150×4.60 mm) and an isocratic elution (mobile phase: water/acetonitrile, 45:55; flow rate 0.5 mL min^{-1} at 40 °C; detection at 400 nm). Three peaks were detected: first peak t_R=33.417 min; second peak t_R=35.653 min; third peak t_R=41.016 min. The proton NMR of the crude mixture is reported in the Supplementary Materials (S63).

Laccase-catalyzed Oxidation of 2 b

The crude residue was purified by flash column chromatography (petroleum ether-EtOAc 7:3) to afford dimeric products as a white foam (24 mg, R_f=0.17, 16% yield). MS (ESI): $[C_{34}H_{29}O_6]$ $[M-H]^-$: 533.1. The separation of the dimeric products **7** was carried out by analytical HPLC-UV analysis using a chiral column (Lux µm Cellulose-1 Phenomenex, 150×4.60 mm) and an isocratic elution (mobile phase: water/acetonitrile, 45:55; flow rate 0.5 mL min^{-1} at 40 °C; detection at 400 nm). Two peaks were detected: first peak t_R=54.556 min; second peak t_R=64.129 min. The proton NMR of the crude mixture is reported in the Supplementary Materials (S65). As in the HPLC chromatogram only two peaks were present, these two peaks were isolated by semipreparative HPLC. Again the NMR spectra were in accordance with the usual structures **7 a/b** and **7 c** (Supplementary Materials S68 and S71). The circular dichroism of **7 a/b**, as expected, confirmed the presence of a racemic mixture (Supplementary Materials S69).

Laccase-catalyzed Oxidation of 2 c

The crude residue was purified by flash column chromatography (petroleum ether-EtOAc 5:5) to afford dimeric products as a white foam (52 mg, R_f=0.43, 15% yield). MS (ESI): $[C_{34}H_{31}O_8]$ $[M+H]^+$: 567.3. The separation of the dimeric products **8** was carried out by analytical HPLC-UV analysis using a chiral column (Lux 5 µm Cellulose-1 Phenomenex, 150×4.60 mm) and an isocratic elution (mobile phase: water/acetonitrile, 55:45; flow rate 0.4 mL min^{-1} at 40 °C; detection at 400 nm). Three peaks were detected: first peak t_R=144.979 min; second peak t_R=149.564 min, third peak 180.173 min. The proton NMR of the crude mixture is reported in the Supplementary Materials (S72).

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