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Synthesis of Enantiomerically Enriched Dimers of Vinylphenols by Tandem Action of Laccases and Lipases

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Abstract: The tandem use of laccases and lipases has been exploited for the simple preparative synthesis of enantiomerically enriched dimeric phenols. Laccase-catalyzed oxidation of isoeugenol (**1**) and vinylguaiacol (**7**) in biphasic systems gave as main products the racemic compounds **6** and **8**, possessing structures similar to the β-5 dimers found in lignin. The synthesis of enantiomerically enriched **6** and **8** could be achieved by alcoholysis reactions catalyzed by commercial preparations of lipases in organic solvents. Although the *E* values were quite low (due to the "remote" stereocenters to be discriminated), the enantio-complementary behavior of the tested lipases allowed the simple isolation of the target compounds with *ee* up to 90%. It is noteworthy that these results have been achieved *in vitro* exploiting commercially available enzymes, without using the so-called "dirigent" proteins evolved by nature to direct the enantioselective coupling of phenols *in vivo*.

Keywords: enantioselectivity; laccase; lipase; phenol oxidation; "remote" stereocenters

Introduction

Laccases (benzenediol:oxygen oxidoreductases, EC 1.10.3.2) are copper oxidases which catalyze the four-electron reduction of molecular oxygen to water, coupled with the concomitant oxidation of organic substrates (usually aromatic amines, phenols, and aminophenols) to produce four radicals that, in turn, can subsequently react to give dimers, oligomers and polymers.[1] The ability of these enzymes to oxidize a broad range of molecules directly or in the presence of low-molecular-weight redox mediators[2] has been exploited in several technological[3] and synthetic applications.[4] Specifically, we have reported several examples dealing with the oxidation of sugar derivatives,[5a,b] phenols,[5c–f] and alkaloids,[5g] the latter in cross-coupling domino reactions.

Laccases have been widely described in plants and fungi, where they are presumably involved in lignin synthesis and degradation processes, respectively. A number of reviews has discussed the biochemical mechanism involved in the formation of lignin.[6] Nowadays it is widely accepted that this complex natural macromolecule is mainly obtained by the polymerization of three different cinnamyl alcohols to give a complex network. Reactive radical intermediates, generated by biocatalyzed oxidation of phenols, further undergo self-coupling reactions leading to the formation of C–O and C–C dimers, oligomers and, eventually, to the polymeric material, whose most dominant linkages are summarized in Figure 1.

Different authors have studied the laccase-promoted dimerization of propenylphenols, like isoeugenol (**1**, Figure 2),[7] coniferyl alcohol (**2**),[7] *trans*-resveratrol (**3**)[8] and other hydroxystilbenes.[9] For instance, a mixture of dimeric and tetrameric derivatives was isolated in approximately 30–40% yield from the oxidation reaction of **1** or **2** catalyzed by *Rhus vernicifera* laccase in an acetone-water mixture.[7] A more precise control of the oxidation process was observed with the stilbenic phytoalexin **3**, allowing the isolation of the dehydrodimer **4** (corresponding to the structure β-5 of Figure 1) in 31% yield.[8]

In nature the above-described oxidative coupling of phenolic derivatives is a key step in the biosynthesis not only of lignin but also of several other compounds

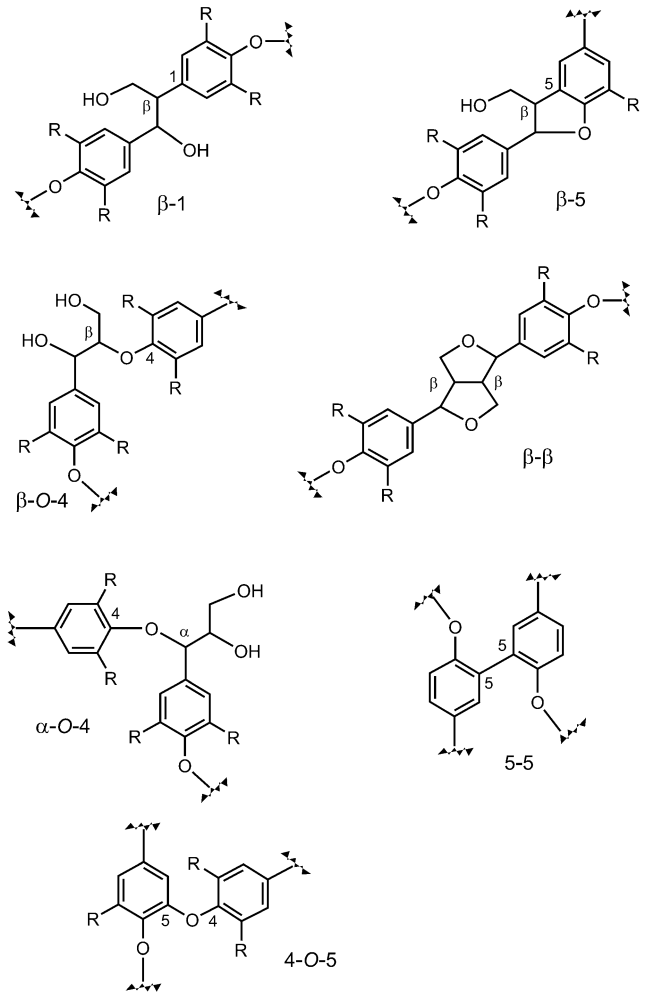


Figure 1. Most represented C–C and C–O linkages in lignin.

like lignans, flavonolignans, and alkaloids. As previously discussed, these oxidative couplings can be reproduced *in vitro*. However, while *in vivo* the dimerization of propenylphenol derivatives to form lignans generally occurs with high regio-, diastereo-, and enantioselectivity, *in vitro* the enantioselectivity is negligible.

For example, the laccase-catalyzed oxidation of coniferyl alcohol (**2**) *in vitro* results in the formation of the dimer pinoresinol **5** as the main product, but in the racemic form.[7] At variance, in 1997 Lewis and co-workers showed that, in the presence of a so-called "dirigent" protein isolated from the plant *Forsythia intermedia*, the *in vitro* oxidative coupling of **3** produced the enantiomerically pure (+)-pinoresinol [(+)-**5**].[10] This result has been recently reproduced by Beifuss and co-workers, who have been also able to get the opposite enantiomer using an enantiocomplementary dirigent protein obtained from *Arabidopsis thaliana*.[11]

Here we show that enantiomerically enriched dimers can be more conveniently produced from their

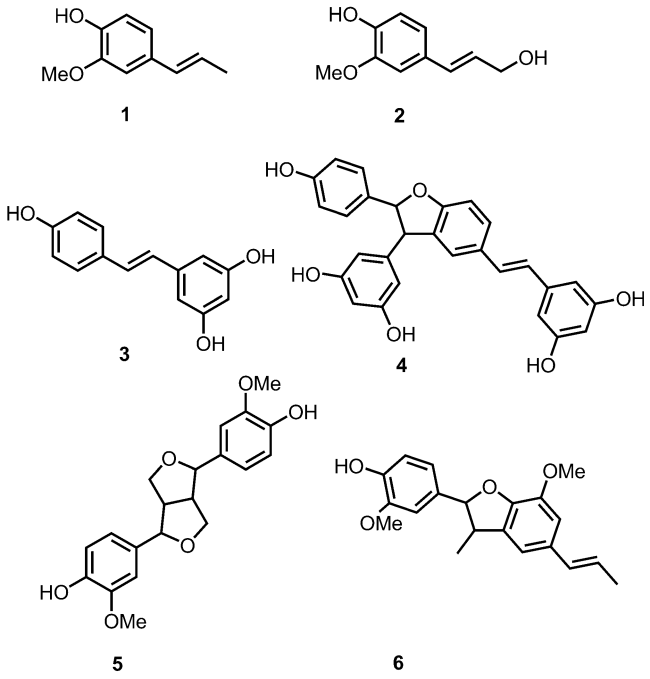


Figure 2. Chemical structures of compounds **1**–**6**.

racemates (in turn synthesized by laccase-catalyzed oxidations) exploiting the well-known enantioselectivity of lipases, enzymes that are even able to discriminate enantiomers possessing the so-called "remote" stereocenters.[12]

Results and Discussion

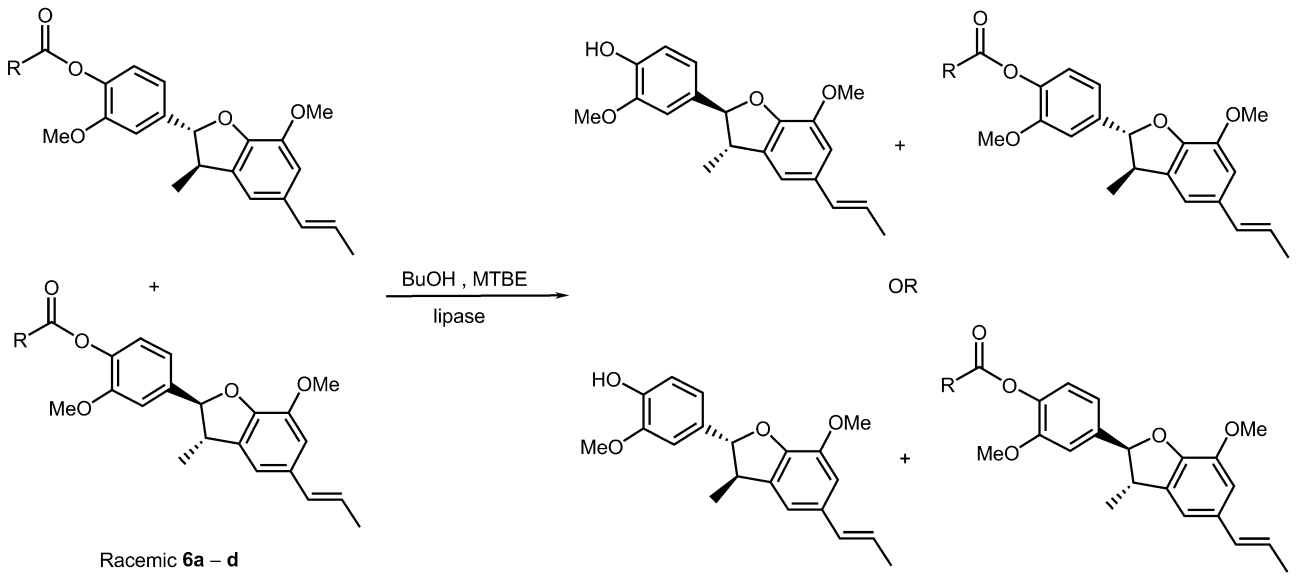
The commercially available isoeugenol (**1**) was considered as a model compound and was submitted to the action of the laccase from *Trametes versicolor*. HPLC analysis of the reaction outcome allowed the optimization of this bio-oxidation. It was found that the transformation was not easily controlled in a homogeneous solvent made by water and a water-miscible cosolvent (i.e., acetone, acetonitrile, methanol), giving rise to oligomeric and polymeric materials. Much better results were obtained in biphasic systems, the best cosolvent among the ones tested (toluene, *t*-amyl alcohol, chloroform, ethyl acetate) being AcOEt. The best reaction conditions (solvent, AcOEt-acetate buffer; pH 3.5; room temperature; magnetic stirrer; open flask to oxygenate the aqueous phase; 70 min) allowed the isolation of the dehydrodimer **6** as the main product in 41% yield; other previously described minor products[7] were not isolated. The structure of **6** was confirmed by mass spectrometry (*m*/*z* at 326 Da) and by NMR analysis. ^{1}H NMR data were in accordance to literature values.[7] In addition to that, NOESY experiments performed by se-

lectively irradiating the signals at 5.12 (d, H-2), 3.46 (dq, H-3) and 1,39 (d, CH_3 linked at C-3) ppm confirmed that, like with resveratrol and its stilbenic analogues,[8,9] the substituents at C-2 (*para*-hydroxyphenyl) and C-3 (methyl) were in the *trans* configuration. Finally, HPLC analysis of **6** with a chiral column (ChiralPack IA) confirmed that the product was a racemate.

Looking for an efficient method to separate the two enantiomers of **6** on a preparative scale, we turned our attention to lipases as suitable catalysts for the alcoholysis reactions described in Scheme 1.

The stereocenter at C-2 was significantly distant from the acyl moiety that would have suffered alcoholysis cleavage (6 bonds), therefore it would have not been too surprising to observe low enzyme selectivity, if any. On the other hand, there are literature examples describing the successful resolutions of molecules carrying "remote" stereocenters,[12] and therefore the decision was made that it was worth trying. Four different esters of **6** (acetate, **6a**; butanoate, **6b**; dodecanoate, **6c**; phenylacetate, **6d**) were synthesized and analyzed by chiral HPLC. While racemic **6** was split in two peaks, all the four esters were eluted as a single peak. As an example, Figure 3 (**a**) shows the HPLC chromatogram of a mixture of racemic **6** and **6b**. Alcoholysis reactions were performed using a panel of 9 well known and commercially available lipases suspended in methyl *tert*-butyl ether (MTBE) in the presence of *n*-BuOH.

Table 1 shows the results obtained with the best ester, that proved to be the butanoate **6b** (the data obtained with the other esters **6a**, **6c**, and **6d** are summarized in the Supporting Information). As expected the *E* values[13] were quite low, but – significantly – some of the lipases showed opposite enantioselectivity. As we have shown in a previous work, it is possible to overcome the low enantioselectivity by alternating the enzymes with opposite enantiomeric preference.[14]



Scheme 1. Lipase-catalyzed alcoholysis of compounds **6a**–**d**.

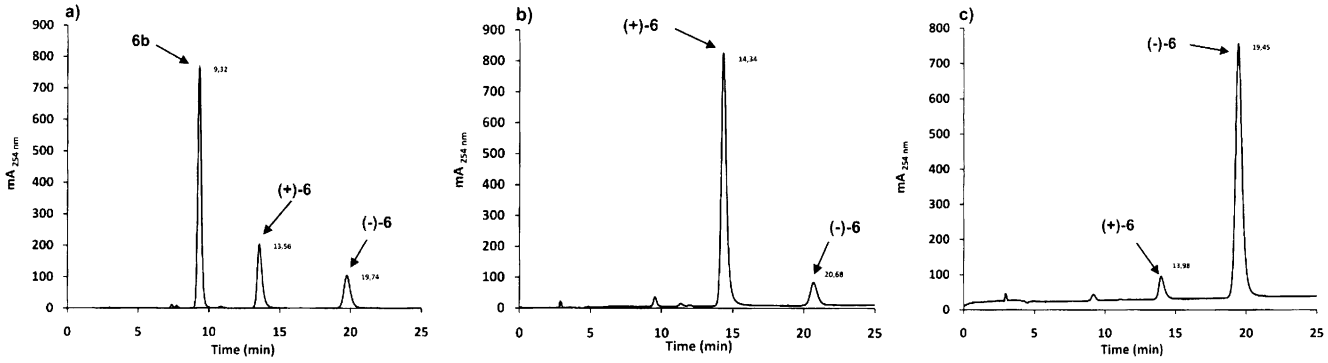


Figure 3. HPLC chromatograms of: **a**) a mixture of **6** and **6b**; **b**) isolated enantiomerically enriched (+)-**6**; **c**) isolated enantiomerically enriched (−)-**6**.

Table 1. Screening of lipases for the enantioselective alcoholysis of **6b**.

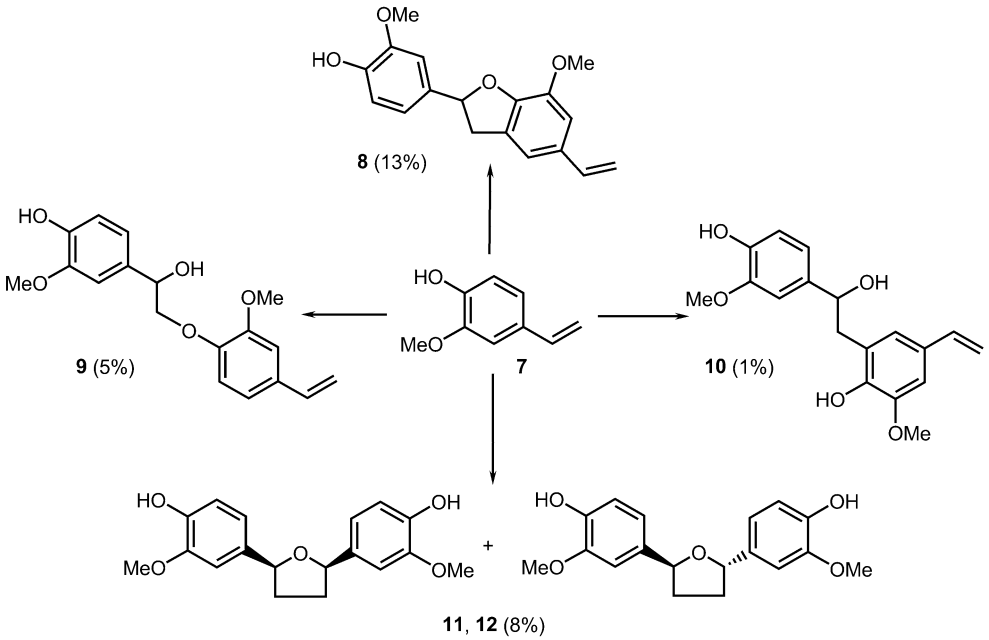
Enzyme	Conversion [%]	Product *ee* [%]	*E*
Lipase AK	13.7	67[a]	5.6
Lipase CE	4.1	64[b]	4.6
Lipase PS	28.9	37[a]	2.5
Novozym 435	18.9	15[a]	1.4
Lipase CV	8.8	70[a]	6.0
Lipozyme	9.8	32[b]	2.0
Lipolase	43.3	18[b]	1.6
Porcine pancreas lipase	n.c.[c]	n.d.[d]	n.d.[d]
Candida rugosa lipase	37.6	18[b]	1.6

[a] Major product: (−)-**6**.
[b] Major product: (+)-**6**.
[c] No appreciable conversion.
[d] Not determined.

With this substrate the solvent optimization did not significantly influence enzymatic enantioselectivity.[15] The reaction sequence was therefore performed in MTBE with the two best "enantiomeric" enzyme preparations: lipase CE ($E=4.6$) and lipase AK ($E=5.6$), showing preference for the less and more retained enantiomer of **6**, respectively. Initially the alcoholysis of racemic **6b** was catalyzed by lipase CE. The reaction was stopped at 32% conversion and, after usual work-up, it was possible to isolate (+)-**6** in 34% yield and 78.5% *ee* and the residual **6b** enriched in the opposite enantiomer (66% yield, 46.4% *ee*, as determined after chemical alcoholysis). This enantiomerically enriched butanoate sample was submitted to the action of lipase AK (possessing a preference for the most abundant enantiomer), thus allowing the isolation – at 35% conversion – of (−)-**6** in 37% yield and 85.6% *ee*

The HPLC chromatograms of the two isolated products (+)-**6** and (−)-**6** are shown in Figure 3 (**b**) and Figure 3 (**c**), respectively. To the best of our knowledge, this is the first reported example of the synthesis of enantiomerically enriched "β-5"-like dimers.

In order to evaluate the general applicability of this methodology, the commercially available 4-vinylguaiacol (**7**) was considered as a second substrate. As the laccase-catalyzed oxidation of this molecule had not been reported before, a more detailed investigation of the reaction's dimeric products was performed. As shown in Scheme 2, five dimers (**8**–**12**) could be isolated and characterized. The main product, albeit with lower isolated yield, was still the β-5-like dimer **8**, whose structure was easily confirmed by mass spectrometry (*m*/*z* at 298 Da) and ^{1}H- and ^{13}C NMR analysis. All the other products showed a molecular peak at 316 *m*/*z*, suggesting a dimeric structure with the formal addition of a water molecule. The structure of compounds **9** ("β-O-4"-like dimer) and **10** ("β-1"-like dimer) could be assigned on the basis of the clear differences in the signals due to their aromatic rings. Finally, the ^{1}H NMR spectrum of the last isolated dimeric product indicated the presence of quite symmetrical structures (present in a 2:1 ratio by comparing the area of the signals due to two sets of methoxy



Scheme 2. Laccase-catalyzed oxidation of 4-vinylguaiacol (**7**).

group – singlet at 3.92 and 3.88 ppm – and of a benzylic proton – multiplet at 5.20 and 5.01 ppm) that, in contrast to compounds **8**–**10**, were not carrying a vinyl substituent anymore. These observations suggested the presence of the inseparable mixture of the *cis* and *trans* isomers **11** and **12**.

As previously described, the "β-5"-like dimer **8** was acylated to give the butanoate **8b**. Following chiral HPLC analysis of the standard **8** and **8b** (in this case, as shown in Figure 4 (**a**), a single HPLC run allowed the separation of the enantiomers of both compounds), the racemic ester was submitted to alcoholysis reactions with the usual panel of commercial lipases. Even with this second substrate the *E* values of the tested enzymes were quite low (Table 2), but once again it was possible to find enantiocomplementary hydrolases.

The best performing enzymes were lipase PS ($E=7.6$) and lipase CV ($E=4.1$, with preference for the opposite enantiomer). The previously described protocol was applied for the alcoholysis of **8b**, starting with lipase PS. The preparative reaction was performed in acetone due to the higher solubility of compound **8b** in this solvent (*E* values were not significantly affected by changing the solvent). At variance with **6**, it was found that the enantiomerically enriched **8** had a tendency to racemize during the workup. Therefore, once the alcoholysis had been terminated by filtering lipase PS [at 56% conversion, see Figure 4 (**b**)] acetic anhydride was added to the crude reaction mixture in order to "trap" the enantiomerically enriched phenol (Scheme 3). The mixture of butanoate and acetate was then easily separated by silica chromatography to give (−)-**8a** with 50% *ee* and residual (+)-**8b** with 64% *ee* Finally, the latter compound was submitted to the action of the enantiocomplementary lipase CV which, following the previously described protocol, allowed the isolation of the acetate (+)-**8a** with 90.0% *ee* [Figure 4 (**c**)–(**e**); for details see the Experimental Section).

Table 2. Screening of lipases for the alcoholysis of **8b**.

Enzyme	Conversion [%]	Product *ee* [%]	*E*
Lipase AK	46.7	21[a]	1.8
Lipase CE	35.7	54[a]	4.4
Lipase PS	38.5	67[a]	7.6
Novozym 435	37.3	39[b]	2.8
Lipase CV	20.3	56[b]	4.1
Lipozyme	78.1	0	1.0
Lipolase	32.8	29[a]	2.1
Porcine pancreas lipase	32.6	5[a]	1.1
Candida rugosa lipase	77.2	5[a]	1.2

[a] Major product: (−)-**8**.
[b] Major product: (+)-**8**.

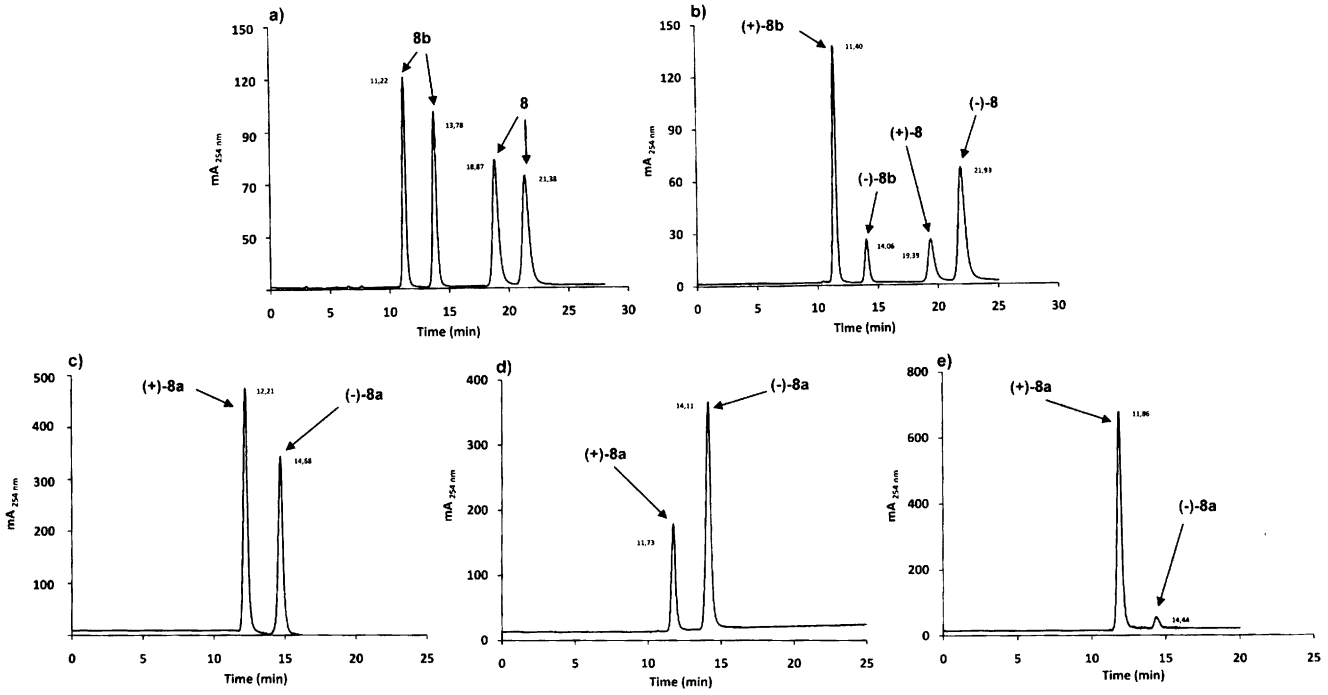
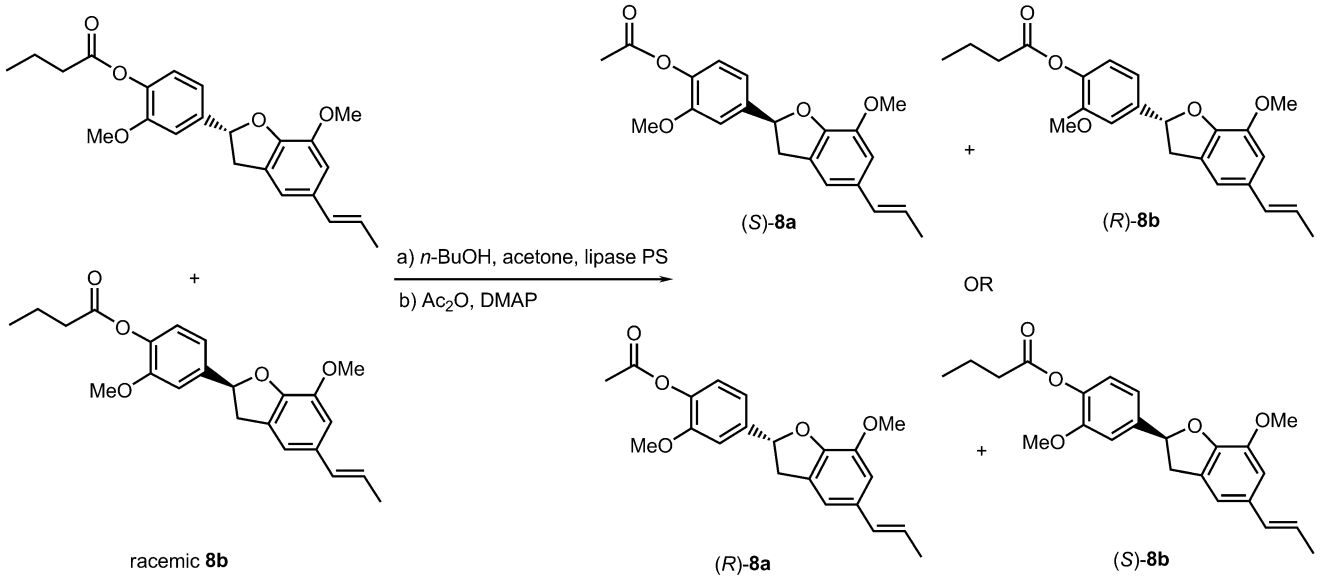


Figure 4. HPLC chromatograms of: **a**) a mixture of racemic **8** and **8b**; b) lipase PS-catalyzed alcoholysis reaction mixture; **c**) racemic **8a**; **d**) isolated enantiomerically enriched (−)-**8a**; **e**) isolated enantiomerically enriched (+)-**8a**.



Scheme 3. Alcoholysis of **8b** by lipase PS and subsequent chemical acetylation.

Conclusions

We have shown, for the first time, that the tandem use of laccases and lipases can be exploited for the simple preparative synthesis of enantiomerically enriched dimeric phenols, possessing structures similar to the β-5 dimers found in lignin. This synthetic task has been achieved in *vitro* without the need of a supporting "dirigent" protein. Worthy of note is the ability of these crude and commercially available lipase preparations to discriminate remote stereocenters in an enantiocomplementary way, a fact that once again qualifies these enzymes as real workhorses of applied biocatalysis.

Experimental Section

General Experimental Procedures

Optical rotations were measured on a Jasco P-2000 polarimeter (Cremella, IT). NMR spectra were recorded on Bruker AC400 and AC500 spectrometers (400 and 500 MHz, respectively) in $CDCl_3$. Mass spectra were recorded on a Bruker Esquire 3000 Plus spectrometer. HPLC analyses were carried out using a Jasco 880-PU pump equipped with a Jasco 875-UV/Vis detector. HPLC conditions: Chiral Pack IA Daicel Chemical Industries 250×4.6 mm column, isocratic mobile phase petroleum ether/2-propanol, flow rate 1.0 mL/min at 25 °C, detection at 254 nm. Thin-layer chromatography (TLC): precoated silica gel 60 F_{254} plates (Merck), developed with the molybdate reagent [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, 42 g; $Ce(SO_4)_2$, 2 g; H_2SO_4 conc., 62 mL; made up to 1 L with deionized water]; flash chromatography: silica gel 60 (70–230 mesh, Merck). Reactions were carried out using a G24 Environmental Incubator New Brunswick Scientific Shaker (Edison, USA).

Enzymes and Materials

Laccase from *Trametes versicolor* (20 U/mg) was from Sigma–Aldrich. Lipases from *Pseudomonas cepacia* (lipase PS), *Pseudomonas* sp. (lipase AK), *Chromobacterium viscosum* (lipase CV), and *Humicola lanuginosa* (lipase CE), were from Amano (Amano Enzyme Europe Ltd., Oxfordshire, UK). Lipases from *Candida rugosa* and porcine pancreas (PPL) were from Sigma–Aldrich; *Candida antarctica* lipase B (Novozym 435) and *Rhizomucor miehei* lipase (Lipozyme) were from Novozymes (Bagsvaerd, DK). *Thermomyces lanuginosa* lipase (Lipolase) was from ChiralVision B.V. (Leiden, The Netherlands). Both isoeugenol (**1**) and 4-vinylguaiacol (**7**) were from Sigma–Aldrich.

Isoeugenol (**1**): ^{1}H NMR ($CDCl_3$): δ = 6.86–6.83 (3H, m, H_{ar}), 6.33 (1H, dq, J_1 = 15.5 Hz, J_2 = 1.5 Hz, H-1′), 6.08 (1H, dq, J_1 = 15.5 Hz, J_2 = 6.5 Hz, H-2′), 3.90 (3H, s, OMe), 1.86 (3H, dd, J_1 = 6.5 Hz, J_2 = 1.5 Hz, CH_3). The NMR data were in accordance to the literature values.[16]

4-Vinylguaiacol (**7**): ^{1}H NMR ($CDCl_3$): δ = 6.96 (1H, d, J = 1.5 Hz, H-3), 6.93 (1H, dd, J_1 = 8.0 Hz, J_2 = 1.5 Hz, H-5), 6.89 (1H, d, J = 8.0 Hz, H-6), 6.65 (1H, dd, J_1 = 17.5 Hz, J_2 = 10.5 Hz, H-1′), 5.60 (1H, dd, J_1 = 17.5 Hz, J_2 = 0.5, H-2′$_{trans}$), 5.14 (1H, dd, J_1 = 11.0 Hz, J_2 = 1.0, H-2′$_{cis}$), 3.93 (3H, s, OMe). The NMR data were in accordance to the literature values.[17]

All other reagents were of the best purity grade from commercial suppliers.

Laccase-Catalyzed Oxidation of Isoeugenol (1) to 6

Isoeugenol [2-methoxy-4-(1-propenyl)phenol, **1**, 600 mg, 3.65 mmol], dissolved in 60 mL of ethyl acetate was added to 60 mL of 20 mM sodium acetate buffer, pH 3.5, in which the laccase from *Trametes versicolor* (600 U) had been previously dissolved. The solution was incubated at 25 °C in an open flask under moderate stirring, and the conversion was monitored by TLC (mobile phase: petroleum ether-AcOEt 8:2). After 70 min the organic phase was separated and the

water phase was extracted with AcOEt. Following drying by sodium sulfate addition, the solvent was evaporated under reduced pressure and the crude residue was purified by flash chromatography (mobile phase: petroleum ether-AcOEt 8:2) to give the product **6**; isolated yield: 249 mg (0.76 mmol, 41.5%). ^{1}H NMR ($CDCl_3$): δ = 6.99 (1H, d, J = 1.5 Hz, H-6′), 6.92 (1H, dd, J_1 = 1.5 Hz, , J_2 = 8.0 Hz, H-2′), 6.90 (1H, d, J = 8.0 Hz, H-3′), 6.80 (1H, s, H-4), 6.78 (1H, s, H-6), 6.337 (1H, dq, J_1 = 1.5 Hz, J_2 = 15.5 Hz, H-1″), 6.12 (1H, dq, J_1 = 6.5 Hz, J_2 = 15.5 Hz, H-2″), 5.12 (1H, d, J = 9.5 Hz, H-2), 3.91 (3H, s, OMe), 3.89 (3H, s, OMe), 3.46 (1H, dq, J_1 = 7.0 Hz, J_2 = 9.5 Hz, H-3), 1.89 (3H, dd, J_1 = 1.5 Hz, J_2 = 6.5 Hz, CH_{3vin}), 1.40 (3H, d, J = 7.0 Hz, CH_{3aliph});^{13}C NMR ($CDCl_3$): δ = 146.70, 146.62, 145.82, 144.18, 133.31, 132.23, 132.13, 130.97, 123.50, 119.99, 114.11, 113.35, 109.31, 108.96, 93.81, 55.99, 45.65, 18.39, 17.60; ME (EI): m/z = 326 Da.

Esterification of 6 to 6a–d Derivatives

To 600 mg (1.84 mmol) of **6** dissolved in 10 mL of anhydrous THF, 10 equiv. of TEA (2.56 mL, 1.86 g, 18.4 mmol) and 5 mg (0.04 mmol) of DMAP were added. The mixture was cooled at 0 °C and 5 equiv. of either acetic anhydride, butyric anhydride, dodecanoyl chloride or phenylacetyl chloride were added under stirring. Conversions were monitored by TLC (mobile phase: petroleum ether-AcOEt 8:2, in the case of acylation with dodecanoyl chloride petroleum ether-AcOEt 9:1) after 12 h. Products **6a–d** were recovered by solvent evaporation, resuspended in 20 mL of AcOEt and washed with H_2O, 5% (w/v) $NaHCO_3$ solution, and again H_2O (10 mL each). Following drying by sodium sulfate addition, the solvent was evaporated under reduced pressure and the crude residues were purified by flash chromatography (mobile phase: petroleum ether-AcOEt; **6a** and **6b**, 9:1; **6c**, 97:3; **6d**, 8:2).

6a: isolated yield: 564 mg (1.53 mmol, 83%); ^{1}H NMR ($CDCl_3$): δ = 7.08 (1H, d, J = 2.0 Hz, H-6′), 7.03 (1H, d, J = 8.0 Hz, H-3′), 6.98 (1H, dd, J_1 = 2.0 Hz, J_2 = 8.0 Hz, H-2′), 6.80 (1H, s, H-4), 6.78 (1H, s, H-6), 6.37 (1H, dq, J_1 = 2.0 Hz, J_2 = 15.5 Hz, H-1″), 6.12 (1H, dq, J_1 = 6.5 Hz, J_2 = 15.5 Hz, H-2″), 5.17 (1H, d, J = 9.0 Hz, H-2), 3.92 (3H, s, OMe), 3.84 (3H, s, OMe), 3.47 (1H, dq, J_1 = 7.0 Hz, J_2 = 9.0 Hz, H-3), 1.89 (3H, dd, J_1 = 2.0 Hz, J_2 = 6.5 Hz, $CH_{3\ vin}$), 1.43 (3H, d, J = 7.0 Hz, CH_{3aliph}), acetyl moiety: 2.32 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$): δ = 151.58, 146.85, 144.51, 140.03, 139.67, 133.40, 132.76, 131.23, 123.90, 122.99, 119.01, 113.73, 110.69, 109.85, 93.41, 56.36, 56.30, 46.12, 18.65, 18.31, acetyl mojety: 169.25, 20.96; MS (ESI): m/z = 391 Da ($M+Na^+$).

6b: isolated yield: 580 mg (1.46 mmol, 79%); ^{1}H NMR ($CDCl_3$): δ = 7.07 (1H, d, J = 2.0 Hz, H-6′), 7.02 (1H, d, J = 8.5 Hz, H-3′), 6.98 (1H, dd, J_1 = 2.0 Hz, J_2 = 8.5 Hz, H-2′), 6.80 (1H, s, H-4), 6.78 (1H, s, H-6), 6.37 (1H, dq, J_1 = 1.5 Hz, J_2 = 16.0 Hz, H-1″), 6.12 (1H, dq, J_1 = 6.5 Hz, J_2 = 16.0 Hz, H-2″), 5.17 (1H, d, J = 9.0 Hz, H-2), 3.92 (3H, s, OMe), 3.83 (3H, s, OMe), 3.47 (1H, dq, J_1 = 7.0 Hz, J_2 = 9.0 Hz, H-3), 1.89 (3H, dd, J_1 = 1.5 Hz, J_2 = 6.5 Hz, CH_{3vin}), 1.43 (3H, d, J = 7.0 Hz, CH_{3aliph}), butanoyl moiety: 2.57 (2H, t, J = 7.5 Hz, CH_2CO), 1.81 (2H, sextet, J = 7.5 Hz, CH_2), 1.07 (3H, t, J = 7.5 Hz, CH_3);^{13}C NMR ($CDCl_3$): δ = 151.27, 146.51, 144.15, 139.79, 139.15, 133.07, 132.39, 130.89, 123.52, 122.66, 118.65, 113.37, 110.34, 109.50, 93.10, 55.99, 55.91, 45.78, 18.29, 17.91; butanoyl mojety: 171.57, 35.86, 18.51, 13.53; MS (ESI): m/z = 419 Da ($M+Na^+$).

6c: isolated yield: 559 mg (1.10 mmol, 60%); ^{1}H NMR ($CDCl_3$): δ = 7.07 (1H, d, J = 1.5 Hz, H-6′), 7.01 (1H, d, J = 8.0 Hz, H-3′), 6.98 (1H, dd, J_1 = 1.5 Hz, J_2 = 8.0 Hz, H-2′), 6.80 (1H, s, H-4), 6.77 (1H, s, H-6), 6.37 (1H, dq, J_1 = 2.0 Hz, J_2 = 16.0 Hz, H-1″), 6.11 (1H, dq, J_1 = 6.5 Hz, J_2 = 16.0 Hz, H-2″), 5.17 (1H, d, J = 9.0 Hz, H-2), 3.92 (3H, s, OMe), 3.82 (3H, s, OMe), 3.47 (1H, dq, J_1 = 7.0 Hz, J_2 = 9.0 Hz, H-3), 1.88 (3H, dd, J_1 = 2.0 Hz, J_2 = 6.5 Hz, CH_{3vin}), 1.42 (3H, d, J = 7.0 Hz, CH_{3aliph}), dodecanoyl mojety: 2.58 (2H, t, J = 7.0 Hz, CH_2CO), 1.77 (2H, quintet, J = 7.0 Hz, CH_2), 1.28 [16H, mult, -$(CH_2)_8$-], 0.90 (3H, t, J = 7.0 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ = 151.66, 146.91, 144.51, 140.21, 139.52, 133.44, 132.76, 131.28, 123.79, 123.01, 118.94, 113.76, 110.73, 110.04, 93.40, 56.38, 56.25, 46.12, 18.56, 18.27, dodecanoyl mojety: 172.03, 34.35, 32.19, 29.89, 29.79, 29.60, 29.57, 29.36, 25.33, 22.95, 14.34; MS (ESI): m/z = 531 ($M+Na^+$); 509 Da ($M+H^+$).

6d: isolated yield: 571 mg (1.29 mmol, 70%); ^{1}H NMR ($CDCl_3$): δ = 7.05 (1H, d, J = 2.0 Hz, H-6′), 6.99 (1H, d, J = 8.5 Hz, H-3′), 6.96 (1H, dd, J_1 = 2.0 Hz, , J_2 = 8.5 Hz, H-2′), 6.80 (1H, s, H-4), 6.76 (1H, s, H-6), 6.37 (1H, dq, J_1 = 1.5 Hz, J_2 = 15.5 Hz, H-1″), 6.11 (1H, dq, J_1 = 6.5 Hz, J_2 = 15.5 Hz, H-2″), 5.15 (1H, d, J = 9.0 Hz, H-2), 3.91 (3H, s, OMe), 3.77 (3H, s, OMe), 3.44 (1H, dq, J_1 = 7.0 Hz, J_2 = 9.0 Hz, H-3), 1.88 (3H, dd, J_1 = 1.5 Hz, J_2 = 6.5 Hz, CH_{3vin}), 1.41 (3H, d, J = 7.0 Hz, CH_{3aliph}), phenylacetyl mojety: 7.41 (2H, d, J = 7.5 Hz), 7.37 (2H, t, J = 7.5 Hz), 7.30 (1H, t, J = 7.5 Hz), aromatic protons: 3.89 (2H, s, CH_2); ^{13}C NMR ($CDCl_3$): δ = 151.73, 146.99, 144.64, 140.29, 139.84, 133.53, 132.90, 131.39, 124.01, 123.03, 119.08, 113.86, 110.88, 110.00, 93.53, 56.48, 56.38, 46.31, 18.79, 18.37, phenylacetyl mojety: 169.95, 134.10, 129.83, 129.00, 127.63, 41.44; MS (ESI): m/z = 467 ($M+Na^+$), 445 Da ($M+H^+$).

Screening of Lipases for the Alcoholysis of the Esters 6a–d

To a solution of ester **6a–d** (5 mg; **6a**, 0.014 mmol; **6b**, 0.013 mmol; **6c**, 0.010 mmol; **6d**, 0.011 mmol) in *tert*-butyl methyl ether (MTBE, 1 mL), *n*-butanol (*n*-BuOH, 0.05 mL, 0.545 mmol) and the respective lipase preparation were added (Novozym 435 and Lipolase, 5 mg; Lipozyme, 10 mg; lipase CV, 50 mg; all other lipases, 25 mg; screening on **6c** was carried out using a triplicate amount of each enzyme). The mixtures were incubated at 30 °C and 185 rpm and monitored by TLC (mobile phase: petroleum ether-AcOEt; **6a**, **6b**, and **6d**, 8:2; **6c**, 9:1) and HPLC (mobile phase: petroleum ether-AcOEt; **6a** and **6b**, 9:1; **6c**, 7:3; **6d**, 95:5) at scheduled times (see Supporting Information for details). *E* values of the respective reactions were evaluated from the conversion and the enantiomeric excess of products (*ee*).[13]

Preparative Alcoholysis of 6b Catalyzed by Lipase CE

To a solution of **6b** (313 mg, 0.79 mmol) in 25 mL of MTBE, 3 mL of *n*-BuOH (32.7 mmol) and 9 g of lipase CE were added. The mixture was incubated at 30 °C and 250 rpm and monitored by TLC (mobile phase: petroleum ether-AcOEt, 8:2) and HPLC [mobile phase: petroleum ether-AcOEt, 9:1;

6b, $t_R = 9.3$ min; (+)-**6**, $t_R = 13.6$ min; (−)-**6**, $t_R = 19.5$ min] at the scheduled times (for details see Supporting Information). After 46 h, the enzyme powder was removed by filtration and the solvent was evaporated. The crude residue was purified by flash chromatography (mobile phase: petroleum ether-AcOEt, 9:1), affording residual **6b** (yield: 169 mg, 0.43 mmol of *ee* 46%) and enriched (+)-**6** [yield: 98 mg, 0.26 mmol of*ee* 78%, [α]: +39.91 (*c* 0.01, methanol)].

Preparative Alcoholysis of 6b Catalyzed by Lipase AK

To a solution of residual **6b** (169 mg, 0.43 mmol, *ee* 46%) in 15 mL of MTBE, 1.5 mL of *n*-BuOH (16.35 mmol) and 1 g of lipase AK were added. The mixture was incubated at 30 °C and 250 rpm and monitored by TLC (mobile phase: petroleum ether-AcOEt, 8:2) and HPLC (mobile phase: petroleum ether-AcOEt, 9:1) at scheduled times (for details see Supporting Information). After 35 h, the enzyme powder was removed by filtration and the solvent was evaporated. The crude residue was purified by flash chromatography (mobile phase: petroleum ether-AcOEt, 9:1), affording residual **6b** (yield: 88 mg, 0.22 mmol) and enriched (−)-**6** [yield: 64 mg, 0.19 mmol of *ee* 86%, [α] −45.90 (*c* 0.01, methanol)]. The residual ester **6b** had an *ee* of 19%, evaluated after chemical hydrolysis.

Laccase-Catalyzed Oxidation of 2-Methoxy-4-vinylphenol (7)

2-Methoxy-4-vinylphenol (**7**) (600 mg, 4.0 mmol) dissolved in 60 mL of ethyl acetate was added to 90 mL of 20 mM acetate buffer, pH 3.5, in which the laccase from *Trametes versicolor* (1170 U) had been previously dissolved. The solution was incubated at 30 °C under mild shaking, following the conversion by TLC (mobile phase: petroleum ether-AcOEt, 9:2). After 48 h, the organic phase was separated and the water phase was extracted with AcOEt. Following drying over sodium sulfate, the solvent was evaporated under reduced pressure and the crude residue was purified by flash chromatography (mobile phase: petroleum ether-AcOEt, 9:2) to give the product **8**. Then the eluent was changed to $CHCl_3$/MeOH 10:0.2 to isolate the products **9**, **10**, **11** and **12**.

8: isolated yield: 79 mg (0.27 mmol, 13.3%); ^{1}H NMR ($CDCl_3$): $\delta = 6.94$–6.84 (5H, m, H_{Ar}), 6.65 (1H, dd, $J_1 = 17.4$ Hz, $J_2 = 10.8$ Hz, H-1″), 5.73 (1H, t, $J = 9.0$ Hz, H-2), 5.59 (1H, d, $J = 17.4$ Hz, H-2″$_{trans}$), 5.11 (1H, d, $J = 10.8$ Hz, H-2″$_{cis}$), 3.89 and 3.87 (3H each, 2 s, 2 OMe), 3.55 (1H, dd, $J_1 = 15.6$ Hz, $J_2 = 9.0$ Hz, H-3$_a$), 3.25 (1H, dd, $J_1 = 15.6$ Hz, $J_2 = 8.7$ Hz, H-3$_b$); ^{13}C NMR ($CDCl_3$): $\delta = 147.36$ and 146.42 (C-5′ and C-7), 144.93 (C-8 and C-4′), 137.46 (C-1″), 133.89 (C-1′), 132.39 (C-9), 128.78 (C-5), 120.16, 115.89, 114.97, 110.64, 109.54 (C-4, C-6, C-2′, C-3′ and C-6′), 112.02 (C-2″), 86.18 (C-2), 56.69 (2 OMe), 39.20 (C-3): MS (EI): $m/z =$ 298 Da.

9: isolated yield: 33 mg (0.10 mmol, 5.2%); ^{1}H NMR ($CDCl_3$): $\delta = 7.02$ and 7.01 (1H each, d each, $J = 1.5$ Hz and $J = 2.0$ Hz, H-3 and H-3″), 6.95 (1H, dd, $J_1 = 8.0$ Hz, $J_2 = 2.0$ Hz, H-5 or H-5″), 6.93–6.88 (3H, m, H-5″ or H-5, H-6, H-6″), 6.67 (1H, dd, $J_1 = 17.5$ Hz, $J_2 = 10.5$ Hz, H-1‴), 5.65 (1H, dd, $J_1 = 17.5$ Hz, $J_2 = 0.5$ Hz, H-2‴$_{trans}$), 5.20 (1H, dd, $J_1 = 11.0$ Hz, $J_2 = 0.5$ Hz, H-2‴$_{cis}$), 5.04 (1H, dd, $J_1 = 9.5$ Hz, $J_2 = 3.0$ Hz, H-1′), 4.17 (1H, dd, $J_1 = 10.0$ Hz, $J_2 = 3.0$ Hz, H-2′$_a$), 3.97 (1H, t, $J = 9.5$ Hz, H-2′$_b$), 3.93 (6H, 2 s, 2OMe); ^{13}C NMR ($CDCl_3$): $\delta = 150.95$ and 148.73 (C-2 and C-2″), 147.38 and 146.22 (C-1 and C-1″), 137.07 (C-1‴), 133.17 and 132.31 (C-4 and C-4″), 120.21, 120.05, 116.63, 114.97, 110.39, 109.62 (C-3, C-3″, C-5, C-5″, C-6, C-6″), 113.17 (C-2‴), 77.09 (C-2′); 72.88 (C-1′); 56.63 (2OMe); MS (EI): $m/z =$ 316 Da.

10: isolated yield: 6 mg (0.02 mmol, 0.9%); ^{1}H NMR ($CDCl_3$): $\delta = 6.96$–6.82 (5H, m, H_{ar}), 6.63 (1H, dd, $J_1 = 17.7$ Hz, $J_2 = 10.8$ Hz, H-1‴), 6.07 (1H, dd, $J_1 = 8.1$ Hz, $J_2 = 3.9$ Hz, H-2′), 5.61 (1H, d, $J = 17.7$ Hz, H-2‴$_{trans}$), 5.15 (1H, d, $J = 10.8$ Hz, H-2‴$_{cis}$), 4.29 (1H, dd, $J_1 = 10.8$ Hz, $J_2 = 7.8$ Hz, H-1′$_a$), 4.18 (1H, dd, $J_1 = 10.8$ Hz, $J_2 = 3.9$ Hz, H-1′$_b$), 3.89 (3H, s, OMe), 3.85 (3H, s, OMe); MS (EI): $m/z =$ 316 Da.

11+12: inseparable mixture of *cis* and *trans* isomers in ratio 1:2; isiolated yield: 51 mg (3.2×10^{-3} mmol, 7.9×10^{-3} mmol, 8.1%); MS (EI): $m/z = 316$ Da.

11: ^{1}H NMR ($CDCl_3$): $\delta = 6.99$–6.90 (6H, m, H-Ar), 5.01 (2H, br t, $J = 5.2$ Hz, H-1′, H-1″), 3.88 (6H, s, 2OMe), 2.43 (2H, m, H-2′$_a$, H-2″$_a$), 1.99 (2H, m, H-2′$_b$, H-2″$_b$); ^{13}C NMR ($CDCl_3$): $\delta = 146.46$ (C-2, C-2‴), 144.86 (C-1, C-1‴), 134.99 (C-4, C-4‴), 118.90, 114.19, 108.99 (C-3, C-3‴, C-5, C-5‴, C-6, C-6‴), 81.12 (C-1′, C-1″), 55.91 (2OMe), 34.14 (C-2′, C-2″).

12: ^{1}H NMR ($CDCl_3$): $\delta = 6.99$–6.90 (6H, m, H-Ar), 5.20 (2H, br t, $J = 5.6$ Hz, H-1′, H-1″), 3.93 (6H, s, 2OMe), 2.46 (2H, m, H-2′$_a$, H-2″$_a$), 2.02 (2H, m, 2′$_b$, H-2″$_b$); ^{13}C NMR ($CDCl_3$): $\delta = 146.58$ (C-2, C-2‴); 144.86 (C-1, C-1‴); 135.53 (C-4, C-4‴); 118.61, 114.19, 108.34 (C-3, C-3‴, C-5, C-5‴, C-6, C-6‴); 81.21 (C-1′, C-1″); 55.99 (2OMe); 35.67 (C-2′, C-2‴).

Preparative Laccase-Catalyzed Oxidation of 2-Methoxy-4-vinylphenol (7)

The previously described oxidation reaction was repeated under the following conditions in order to isolate a larger amount of the dimer **8**. Compound **7** (2 g, 13.33 mmol) dissolved in 150 mL of AcOEt was added to 150 mL of 20 mM acetate buffer, pH 3.5, in which the laccase from *Trametes versicolor* (1000 U) had been previously dissolved. The solution was incubated at 25 °C in an open flask under moderate stirring, following the conversion by TLC (mobile phase: petroleum ether-AcOEt, 8:2). After 3 h the organic phase was separated and the water phase was extracted with AcOEt. Following drying over sodium sulfate, the solvent was evaporated and the crude residue was purified by flash chromatography (mobile phase: petroleum ether-AcOEt, 8:2) to give product **8**; isolated yield: 618 mg (2.07 mmol, 15.5%).

Esterification of 8 to 8b

To 600 mg (2.01 mmol) of **8** dissolved in 10 mL of anhydrous THF, 10 equiv. of TEA (2.80 mL, 2.03 g, 20.1 mmol) and 5 mg (0.04 mmol) of DMAP were added. The mixture was cooled at 0 °C and 2 equiv. of butyric anhydride were slowly added under stirring. Conversions were monitored by TLC (mobile phase: petroleum ether-AcOEt 8:2) after 12 h. Product **8b** was recovered by solvent evaporation, resuspended in 20 mL of AcOEt and washed with H_2O, 5% (w/v)

$NaHCO_3$ solution, and again H_2O (10 mL each). Following drying over sodium sulfate addition, the solvent was evaporated and the crude residue purified by flash chromatography (mobile phase: petroleum ether-AcOEt 9:1) to give product **8b**; yield: 558 mg (1.52 mmol, 76%). ^{1}H NMR ($CDCl_3$): $\delta = 7.06$ (1H, d, $J = 2.0$ Hz, H-6′), 7.02 (1H, d, $J = 8.0$ Hz, H-3′), 6.99 (1H, dd, $J_1 = 2.0$ Hz, $J_2 = 8.0$ Hz, H-2′), 6.92 (1H, s, H-4), 6.88 (1H, s, H-6), 6.67 (1H, dd, $J_1 = 11.0$ Hz, $J_2 = 17.5$ Hz, H-1″), 5.81 (1H, t, $J = 8.5$, H-2), 5.62 (1H, d, $J = 17.5$ Hz, H-$2''_{trans}$), 5.14 (1H, d, $J = 11.0$ Hz, H-$2''_{cis}$), 3.93 and 3.83 (3H each, 2 s, 2 OMe), 3.62 (1H, dd, $J_1 = 8.5$ Hz, $J_2 = 15.5$ Hz, H-3_a), 3.27 (1H, dd, $J_1 = 8.5$ Hz, $J_2 = 15.5$ Hz, H-3_b), butanoyl mojety: 2.57 (2H, t, $J = 7.0$ Hz, CH_2CO), 1.81 (2H, sext., $J = 7.0$ Hz, CH_2), 1.07 (3H, t, $J = 7.0$ Hz, CH_3); ^{13}C NMR ($CDCl_3$): $\delta = 151.72$, 148.32, 144.69, 140.56, 140.11, 137.15, 132.31, 128.25, 123.24, 118.76, 115.65, 111.94, 110.65, 110.25, 85.39, 56.50, 56.37, 39.08, butanoyl moiety: 172.03, 36.32, 18.97, 13.98; MS (ESI): $m/z = 391$ Da ($M + Na^+$).

Esterification of 8 to 8a

5 mg (0.02 mmol) of **8** were dissolved in 0.7 mL of acetone and, under stirring at 0 °C, 2 equiv. of acetic anhydride were added. After 12 h, complete conversion was shown by TLC (mobile phase: petroleum ether-AcOEt 8:2) and a sample of the reaction mixture was submitted to HPLC analysis (mobile phase: petroleum ether-2-propanol 9:1): (+)-**8a**, $t_R = 11.8$ min; (−)-**8a**, $t_R = 14.7$ min.

Screening of Lipases for Alcoholysis of 8b

To a solution of **8b** (3 mg, 0.00815 mmol) in 0.6 mL of MTBE, 0.03 mL (0.327 mmol) of *n*-BuOH and the respective lipase preparation were added (Novozym 435 and Lipolase, 4.5 mg; lipase PS, 7.5 mg; Lipozyme, 10 mg; lipase CV and *C. rugosa* lipase, 15 mg; PPL and lipase AK, 30 mg; lipase CE, 60 mg). The mixtures were incubated at 30 °C and 185 rpm and monitored by TLC (mobile phase: petroleum ether-AcOEt 8:2) and HPLC (mobile phase: petroleum ether-2-propanol 9:1): (+)-**8b**, $t_R = 11.4$ min; (−)-**8b**, $t_R = 14.1$ min; (+)-**8**, $t_R = 19.4$ min; (−)-**8**, $t_R = 21.9$ min) at the scheduled times (see Supporting Information for details).

Preparative Alcoholysis of 8b Catalyzed by Lipase PS and Subsequent Acetylation

To a solution of **8b** (500 mg, 1.36 mmol) in 75 mL of acetone, 4 mL of *n*-BuOH (43.6 mmol) and 750 mg of lipase PS were added. The mixture was incubated at 30 °C and 190 rpm and monitored by TLC (mobile phase: petroleum ether-AcOEt, 8:2) and HPLC (mobile phase: petroleum ether-2-propanol, 9:1). After 24 h, the enzyme powder was removed by filtration and 5 mL (52 mmol) of acetic anhydride and 10 mg (0.08 mmol) of DMAP were slowly added. After additional 12 h complete acetylation of **8** was shown by TLC, products were recovered by solvent evaporation, resuspended in 40 mL of AcOEt and washed with H_2O, 5% (w/v) $NaHCO_3$ solution, and again H_2O (20 mL each). Following drying over sodium sulfate, the solvent was evaporated and the crude residue purified by flash chromatography (mobile phase: petroleum ether-AcOEt 9:1, then 8:2) to give enantiomerically enriched (+)-**8b** (yield: 186 mg, 0.51 mmol, *ee* 64%) and enantiomerically enriched (−)-**8a** (yield: 214 mg, 0.63 mmol, *ee* 50%); [α]: −2.9 (*c* 0.0035, acetone). ^{1}H NMR ($CDCl_3$): $\delta = 7.06$ (1H, d, $J = 2.0$ Hz, H-6′), 7.03 (1H, d, $J = 8.5$ Hz, H-3′), 6.99 (1H, dd, $J_1 = 2.0$ Hz, $J_2 = 8.5$ Hz, H-2′), 6.92 (1H, s, H-4), 6.88 (1H, s, H-6), 6.67 (1H, dd, $J_1 = 11.0$ Hz, $J_2 = 17.5$ Hz, H-1″), 5.80 (1H, t, $J = 9.0$, H-2), 5.61 (1H, d, $J = 17.0$ Hz, H-$2''_{trans}$), 5.14 (1H, d, $J = 11.0$ Hz, H-$2''_{cis}$), 3.93 and 3.84 (3H each, 2 s, 2 OMe), 3.62 (1H, dd, $J_1 = 9.0$ Hz, $J_2 = 15.5$ Hz, H-3_a), 3.27 (1H, dd, $J_1 = 9.0$ Hz, $J_2 = 15.5$ Hz, H-3_b), 2.32 (3H, s, acetyl CH_3); ^{13}C NMR ($CDCl_3$): $\delta = 151.67$, 148.29, 144.68, 140.71, 139.99, 137.14, 132.33, 128.22, 123.22, 118.72, 115.65, 111.96, 110.65, 110.25, 85.34, 56.51, 56.40, 39.06, acetyl moiety 169.36, 21.05; MS (ESI): $m/z = 363$ Da ($M + Na^+$).

Preparative Alcoholysis of (+)-8b Catalyzed by Lipase CV and Subsequent Acetylation

To a solution of enantiomerically enriched (+)-**8b** (164 mg, 0.45 mmol, *ee* 64%) in 20 mL of acetone, 1 mL of *n*-BuOH (10.9 mmol) and 2.5 g of lipase CV were added. The mixture was incubated at 30 °C and 190 rpm and monitored by TLC (mobile phase: petroleum ether-AcOEt, 8:2) and HPLC (mobile phase: petroleum ether-2-propanol, 9:1). After 6 h, the enzyme powder was removed by filtration and 4 mL (42 mmol) of acetic anhydride and 10 mg (0.08 mmol) of DMAP were slowly added. After additional 12 h complete acetylation of **8** was shown by TLC, products were recovered by solvent evaporation, resuspended in 40 mL of AcOEt and washed with H_2O, 5% (w/v) $NaHCO_3$ solution, and again H_2O (20 mL each). Following drying over sodium sulfate, the solvent was evaporated and the crude residue purified by flash chromatography (mobile phase: petroleum ether-AcOEt 9:1, then 8:2) to give enantiomerically enriched (−)-**8b** (yield: 82 mg, 0.22 mmol, *ee* 52%) and enantiomerically enriched (+)-**8a** (yield: 56 mg, 0.16 mmol, *ee* 90%); [α]: +9.6 (*c* 0.0035, acetone).

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References

[1] a) C. J. Rodgers, C. F. Blanford, S. R. Giggens, P. Skamnioti, F. A. Armstrong, S. J. Gurr, *Trends Biotechnol.* **2010**, *28*, 63–72; b) P. Giardina, V. Faraco, C. Pezzella, A. Piscitelli, S. Vanhulle, G. Sannia, *Cell. Mol. Life Sci.* **2010**, *67*, 369–385; c) L. Quintanar, C. Stoj, A. B. Taylor, P. J. Hart, D. J. Kosman, E. I. Solomon, *Acc. Chem. Res.* **2007**, *40*, 445–452; d) A. Wells, M. Teria, T. Eve, *Biochem. Soc. Trans.* **2006**, *34*, 304–308; e) P. Baldrian, *FEMS Microbiol. Rev.* **2006**, *30*, 215–242; f) S. Riva, *Trends Biotechnol.* **2006**, *24*, 219–226.

[2] O. V. Morozova, G. P. Shumakovich, S. V. Shleev, Y. I. Yaropolov, *Appl. Biochem. Microbiol.* **2007**, *43*, 523–535.
[3] a) A. Kunamneni, S. Camarero, C. Garcia-Burgos, F. J. Plou, A. Ballesteros, M. Alcalde, *Microb. Cell Fact.* **2008**, *7*, 32; b) Y. M. Galante, C. Formantici, *Curr. Org. Chem.* **2003**, *7*, 1399–1422; c) H. P. Call, I. Mucke, *J. Biotechnol.* **1997**, *53*, 163–202.
[4] S. Witayakran, A. Zettili, A. J. Ragauskas, *Adv. Synth. Catal.* **2009**, *351*, 1187–1209, and references cited therein.
[5] a) L. Baratto, A. Candido, M. Marzorati, F. Sagui, S. Riva, B. Danieli, *J. Mol. Catal. B: Enzym.* **2006**, *39*, 3–8; b) M. Marzorati, B. Danieli, D. Haltrich, S. Riva, *Green Chem.* **2005**, *7*, 310–315; c) C. Navarra, C. Goodwin, S. Burton, B. Danieli, S. Riva, *J. Mol. Catal. B: Enzym.* **2010**, *65*, 52–57; d) S. Ncanana, L. Baratto, L. Roncaglia, S. Riva, S. G. Burton, *Adv. Synth. Catal.* **2007**, *349*, 1507–1513; e) A. Intra, S. Nicotra, S. Riva, B. Danieli, *Adv. Synth. Catal.* **2005**, *347*, 973–977; f) S. Nicotra, A. Intra, G. Ottolina, S. Riva, B. Danieli, *Tetrahedron: Asymmetry* **2004**, *15*, 2927–2931; g) F. Sagui, C. Chirivì, G. Fontana, S. Nicotra, D. Passarella, S. Riva, B. Danieli, *Tetrahedron* **2009**, *65*, 312–317.
[6] E. D. Dorrestijn, L. J. J. Laarhoven, I. W. C. E. Arends, P. Mulder, *J. Anal. Appl. Pyrolysis* **2000**, *54*, 153–192, and references cited therein.
[7] T. Shiba, L. Xiao, T. Miyakoshi, C. L. Chen, *J. Mol. Catal. B: Enzym.* **2000**, *10*, 605–615.
[8] S. Nicotra, M. R. Cramarossa, A. Mucci, U. M. Pagnoni, S. Riva, L. Forti, *Tetrahedron* **2004**, *60*, 595–600.
[9] C. Ponzoni, E. Beneventi, M. R. Cramarossa, S. Raimondi, G. Trevisi, U. M. Pagnoni, S. Riva, L. Forti, *Adv. Synth. Catal.* **2007**, *349*, 1497–1506.
[10] a) L. B. Davin, H. B. Wang, A. L. Crowell, D. L. Bedgar, D. M. Martin, S. Sarkanen, N. G. Lewis, *Science* **1997**, *275*, 362–366; b) S. C. Halls, L. B. Davin, D. M. Kramer, N. G. Lewis, *Biochemistry* **2004**, *43*, 2587–2595.
[11] B. Pickel, C. Mihaela-Anca, J. Pfannstiel, C. Jurgen, U. Beifuss, A. Schaller, *Angew. Chem.* **2010**, *122*, 207–209; *Angew. Chem. Int. Ed.* **2010**, *49*, 202–204.
[12] G. Carrea, S. Riva, *Angew. Chem.* **2000**, *112*, 2312–2341; *Angew. Chem. Int. Ed.* **2000**, *39*, 2226–2254.
[13] C. S. Chen, Y. Fujimoto, G. Girdaukas, C. J. Sih, *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.
[14] M. Angoli, A. Barilli, G. Lesma, D. Passerella, S. Riva, A. Silvani, B. Danieli, *J. Org. Chem.* **2003**, *68*, 9525–9527.
[15] a) C. R. Wescott, A. M. Klibanov, *Biochim. Biophys. Acta* **1994**, *1206*, 1–9; b) G. Carrea, S. Riva, *Trends Biotechnol.* **1995**, *13*, 63–70.
[16] A.-L. Lee, S. V. Ley, *Org. Biomol. Chem.* **2003**, *1*, 3957–3966.
[17] a) E. Nomura, A. Hosoda, H. Mori, H. Taniguchi, *Green Chem.* **2005**, *7*, 863–866; b) R. Bernini, E. Mincione, M. Barontini, G. Provenzano, L. Setti, *Tetrahedron* **2007**, *63*, 9663–9667.

