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Biocatalyzed synthesis of enantiomerically enriched β-5-like dimer of 4-vinylphenol

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ABSTRACT

The tandem use of laccases and lipases has been exploited for the preparative scale synthesis of enantiomerically enriched dimeric phenols.

Laccase-catalyzed oxidation of 4-vinylphenol (**3**) in biphasic systems gave as main product the racemic compound **4**.

The enantiomerically enriched butanoate **(+)-4b** and acetate **(−)-4a** could be obtained by alcoholysis reactions catalyzed by porcine pancreatic lipase in organic solvent and subsequent acetylation.

1. Introduction

Laccases (benzendiol:oxygen oxidoreductases, EC 1.10.3.2) are oxidative enzymes widely distributed in plants, fungi and bacteria [1] and are known to oxidize a broad range of molecules directly or in the presence of low-molecular-weight redox mediators [2]. On this respect, in recent years our group has reported several examples dealing with the oxidation of sugars [3], alkaloids [4], and phenols [5].

In the latter examples, dimeric compounds obtained by the cross-coupling of the laccase-produced radical intermediates were isolated and characterized. Specifically, different authors have studied the laccase-catalyzed oxidation of propenylphenols, like isoeugenol (**1**, Scheme 1) [6], coniferyl alcohol [6], *trans*-resveratrol [7] and other hydroxystilbenes [8].

These studies reproduced *in vitro* reactions that in nature are key steps in the biosynthesis of polymeric lignin and of other natural compounds (*i.e.*, lignans, flavonolignans, alkaloids), but with a main difference. In fact, while *in vivo* the enzyme-catalyzed dimerization of propenylphenols derivatives to form lignans generally occurs with high regio-, diastereo-, and enantioselectivity, *in vitro* the enantioselectivity is negligible and the products are isolated as racemates. In order to overcome this limitation and to mimic natural processes in a more faithful way, in 1997 Lewis and coworkers showed for the first time that, in the presence of a so-called "dirigent" protein isolated from the plant *Forsythia intermedia*, the laccase-catalyzed coupling of coniferyl alcohol produced the enantiomerically pure (+)-pinoresinol [9]. This result was then reproduced by Beifuss and coworkers, who were also able to get the opposite enantiomer (−)-pinoresinol in the presence of an enantiocomplementary dirigent protein obtained from the plant *Arabidopsis thaliana* [10].

Recently we have shown that enantiomerically enriched phenolic dimers can be more easily produced from their racemates (in turn obtained by laccase-catalyzed oxidations) by exploiting the known enantioselectivity of commercially available lipases even toward compounds possessing the so-called "remote" stereocenters [11]. This approach has been successfully applied to the model compounds isoeugenol (**1**) and 2-methoxy-4-vinylphenol (**2**) [12]. As a further exemplification we report here the results obtained with the simplest phenolic derivative of this series, the unsubstituted 4-vinylphenol (**3**).

2. Experimental

2.1. Materials and methods

2.1.1. General experimental procedures

Microwaves instrument: CEM Focused Microwave Synthesis System, Model Discovery. 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 $CDCl_3$ (copies of the NMR spectra are available in Supplementary Materials). MS spectra were recorded on 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: Chiralpak IA and Chiralcel OD, Daicel Chemical Industries, 250 mm × 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):

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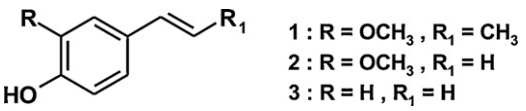


Scheme 1. Compounds **1**–**3**.

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 of deionized water); flash chromatography: silica gel 60 (70–230 mesh, Merck). Reaction was carried out using a G24 Environmental Incubator New Brunswick Scientific Shaker (Edison, USA).

2.1.2. Enzymes and chemicals

Laccase from *Trametes versicolor* (10 U/mg) was from Sigma–Aldrich. Lipases from *Pseudomonas cepacia* (lipase PS), *Pseudomonas* sp. (lipase AK), and *Humicola lanuginosa* (lipase CE) were from Amano (Amano Enzyme Europe Ltd., Oxfordshire, UK). Lipase from *Chromobacterium viscosum* (lipase CV) was from Finnsugar. Lipases from *Candida rugosa* and porcine pancreas (PPL) were from Sigma–Aldrich; *Candida antarctica* lipase B (Novozym 435) and *Rhizomucor miehei* lipase (Lipozyme) were a gift from Novozymes (Bagsvaerd, DK). *Thermomyces lanuginosa* lipase (Lipolase) was from ChiralVision B.V. (Leiden, The Netherlands). The enzymes were used in quantities based on respective activities, as in previous investigations [12]. 4-Vinylphenol **3** was prepared from 4-hydroxy benzaldehyde and malonic acid, following the procedure described below. All other reagents were of the best purity grade from commercial suppliers.

2.1.3. Synthesis of 4-vinylphenol (*3*)

To 2 g (16.4 mmol) of 4-hydroxybenzaldehyde dissolved in 12 mL of acetic acid (210 mmol), 6.82 g of malonic acid (65.6 mmol) and 6.47 mL of piperidine (65.5 mmol) were added. The round bottom flask with a suitable condenser was heated in the microwave oven at 130 °C and 150 W for 5 min. The reaction was controlled by TLC (mobile phase, petroleum ether:AcOEt, 8:2). After complete conversion of the substrate, 20 mL of H_2O and ice were added to the mixture. The solution 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 **3**; isolated yield: 820 mg (6.8 mmol, 41.6%).

^{1}H NMR ($CDCl_3$): δ (ppm, $CDCl_3$): 7.3 (2H, d, J = 8.6 Hz, H-2); 6.8 (2H, d, J = 8.6 Hz, H-1); 6.7 (1H, dd, J_1 = 17.6 Hz, J_2 = 10.9 Hz, H-3); 5.6 (1H, dd, J_1 = 17.5 Hz, J_2 = 0.71 Hz, H-5); 5.1 (1H, dd, J_1 = 11.0 Hz, J_2 = 0.66 Hz, H-4). The NMR data were in accordance to the literature values.

2.1.4. Oxidation of 4-vinylphenol *3* by the laccase from T. versicolor

4-Vinylphenol (1 g, 8.33 mmol, **3**) dissolved in 100 mL of AcOEt was added to 100 mL 20 mM acetate buffer, pH 3.5, in which the laccase from *T. versicolor* (1430 U, 143 mg) had been previously dissolved. The solution was incubated at 30 °C under mild shaking, following the conversion by TLC (eluent, petroleum ether:AcOEt, 9:2). After 48 h, the organic phase was separated and the water phase was extracted with AcOEt. Following anhydrification over sodium sulfate, the solvent was evaporated under reduced pressure and the crude residue was purified by flash chromatography (eluent, petroleum ether:AcOEt, 9:2) to give the products **4** (114 mg, 0.48 mmol, 11.4% yields) and **5** (6 mg, 0.03 mmol, 0.6% yields). Then the eluent was changed to $CHCl_3$:MeOH, 95:5, to isolate the products **6** (29 mg, 0.11 mmol, 2.7% yields), **7** (10 mg, 0.04 mmol, 0.9% yields), **8a** and **8b** (inseparable mixture of *cis* and *trans* isomers in ratio 1:2, 56 mg, 0.22 mmol, 5.3% yields).

4: **^{1}H NMR** δ (ppm, $CDCl_3$): 7.31 (1H, s, H-4); 7.30 (2H, d, J = 8.4 Hz, H-2′, H-6′); 7.22 (1H, d, J = 8.0 Hz, H-6); 6.85 (2H, d, J = 8.4 Hz, H-3′, H-5′); 6.81 (1H, d, J = 8.0 Hz, H-7); 6.69 (1H, dd, J_1 = 17.6 Hz, J_2 = 10.8 Hz, H-1″); 5.73 (1H, t, J = 8.8 Hz, H-2); 5.61 (1H, d, J = 17.6 Hz, H-2″$_{trans}$); 5.12 (1H, d, J = 10.8 Hz, H-2″$_{cis}$); 3.60 (1H, dd, J_1 = 15.6 Hz, J_2 = 9.2 Hz, H-3$_a$); 3.22 (1H, dd, J_1 = 15.6 Hz, J_2 = 8.0 Hz, H-3$_b$). **^{13}C NMR** δ (ppm, $CDCl_3$): 159.46 (C-8); 155.53 (C-4′); 136.61 (C-1″); 133.94 (C-5); 130.87 (C-9); 127.61 (C-2′, C-6′); 127.00 (C-6); 122.37 (C-4); 115.52 (C-3′, C-5′); 111.09 (C-2″); 109.22 (C-7); 84.46 (C-2); 38.06 (C-3). **MS**, m/z (E.I.) = 238 Da.

5: **^{1}H NMR** δ (ppm, $CDCl_3$): 7.34 (2H, d, J = 9.0 Hz, H-2″, H-6″); 7.32 (1H, d, J = 8.5 Hz, H-5); 7.27 (1H, s, H-3); 6.86 (2H, d, J = 8.5 Hz, H-3″, H-5″); 6.85 (1H, d, J = 8.5 Hz, H-6); 6.67 (1H, dd, J_1 = 17.5 Hz, J_2 = 11.0 Hz, H-1‴); 6.10 (1H, dd, J_1 = 7.5 Hz, J_2 = 4.0 Hz, H-2′); 5.62 (1H, dd, J_1 = 17.5 Hz, J_2 = 1.0 Hz, H-2‴$_{trans}$); 5.14 (1H, dd, J_1 = 11.0 Hz, J_2 = 1.0 Hz, H-2‴$_{cis}$); 4.27 (1H, dd, J_1 = 10.5 Hz, J_2 = 7.5 Hz, H-1′$_a$); 4.13 (1H, dd, J_1 = 10.5 Hz, J_2 = 4.0 Hz, H-1′$_b$). **MS**, m/z (E.I.) = 238 Da due to $[M-H_2O]^+$.

6: **^{1}H NMR** δ (ppm, $CDCl_3$): 7.19 (2H, d, J = 8.4 Hz, H-2′, H-6′); 6.81 (2H, d, J = 8.8 Hz, H-3′, H-5′); 6.65 (1H, dd, J_1 = 10.4 Hz, J_2 = 2.4 Hz, H-4); 6.27 (1H, d, J = 10.4 Hz, H-5); 5.90 (1H, dd, J_1 = 17.6 Hz, J_2 = 10.4 Hz, H-1″); 5.29 (1H, d, J = 10.4 Hz, H-2″$_{cis}$); 5.22 (1H, d, J = 17.6 Hz, H-2″$_{trans}$); 4.86 (1H, dd, J_1 = 11.2 Hz, J_2 = 6.0 Hz, H-2); 4.49 (1H, q, J = 2.8 Hz, H-8); 2.86 (1H, dd, J_1 = 17.2 Hz, J_2 = 3.2 Hz, H-7$_a$); 2.62 (1H, dd, J_1 = 17.2 Hz, J_2 = 3.2 Hz, H-7$_b$); 2.43 (1H, dd, J_1 = 12.8 Hz, J_2 = 5.6 Hz, H-3$_a$); 2.22 (1H, dd, J_1 = 12.8 Hz, J_2 = 10.8 Hz, H-3$_b$). **^{13}C NMR** δ (ppm, $CDCl_3$): 197.66 (C-6); 155.58 (C-4′); 149.26 (C-4); 137.59 (C-1″); 133.94 (C-1′); 130.43 (C-5); 127.22 (C-2′, C-6′); 117.82 (C-2″); 115.49 (C-3′, C-5′); 80.79 (C-8); 80.03 (C-2); 51.26 (C-9); 48.13 (C-3); 38.32 (C-7). **MS**, m/z (E.I.) = 256 Da.

7: **^{1}H NMR** δ (ppm, $CDCl_3$): 7.36 and 7.34 (2H each, d each, J = 8.5 Hz, H-3, H-5 and H-3″, H-5″); 6.89 and 6.86 (2H each, d each, J = 8.5 Hz, H-2, H-6 and H-2″, H-6″); 6.67 (1H, dd, J_1 = 17.5 Hz, J_2 = 10.5 Hz, H-1‴); 5.63 (1H, d, J = 17.5 Hz, H-2‴$_{trans}$); 5.15 (1H, d, J = 10.5 Hz, H-2‴$_{cis}$); 5.07 (1H, dd, J_1 = 8.5 Hz, J_2 = 3.0 Hz, H-1′); 4.09 (1H, dd, J_1 = 9.5 Hz, J_2 = 3.0 Hz, H-2′$_a$); 4.02 (1H, br t, J = 9.0 Hz, H-2′$_b$). **^{13}C NMR** δ (ppm, MeOD): 158.72 (C-1″); 156.98 (C-1); 136.21 (C-1‴); 131.89 (C-4); 130.67 (C-4″); 127.37 and 126.99 (C-3, C-5 and C-3″, C-5″); 114.79 and 114.34 (C-2, C-6 and C-2″, C-6″); 110.29 (C-2‴); 73.02 (C-2′); 71.83 (C-1′). **MS**, m/z (E.I.) = 256 Da.

8a and **8b**: **8a**: **^{1}H NMR** δ (ppm, DMSO): 7.18 (4H, d, J = 8.4 Hz, H-3, H-3‴, H-5, H-5‴); 6.72 (4H, d, J = 8.4 Hz, H-2, H-2‴, H-6, H-6‴); 4.77 (2H, t, J = 5.2 Hz, H-1′, H-1″); 2.26 (2H, m, H-2′$_a$, H-2″$_a$); 1.77 (2H, m, H-2′$_b$, H-2″$_b$). **^{13}C NMR** δ (ppm, DMSO): 157.35 (C-1, C-1‴); 134.51 (C-4, C-4‴); 128.44 (C-3, C-3‴, C-5, C-5‴); 116.12 (C-2, C-2‴, C-6, C-6‴); 81.25 (C-1′, C-1″); 34.97 (C-2′, C.2″). **8b**: **^{1}H NMR** δ (ppm, DMSO): 7.15 (4H, d, J = 8.4 Hz, H-3, H-3‴, H-5, H-5‴); 6.71 (4H, d, J = 8.4 Hz, H-2, H-2‴, H-6, H-6‴); 4.98 (2H, t, J = 6.8 Hz, H-1′, H-1″); 2.30 (2H, m, H-2′$_a$, H-2″$_a$); 1.80 (2H, m H-2′$_b$, H-2″$_b$). **^{13}C NMR** δ (ppm, DMSO): 157.25 (C-1, C-1‴); 135.21 (C-4, C-4‴); 128.19 (C-3, C-3‴, C-5, C-5‴); 116.12 (C-2, H-2‴, H-6, H-6‴); 81.48 (C-1′, C-1″); 36.29 (C-2′, C.2″). **MS**, m/z (E.I.) = 256 Da.

2.1.5. Esterification of the dimer *4* to the corresponding butanoate *4b*

To 53 mg (0.22 mmol) of **4** dissolved in 3 ml of anhydrous THF, 5 equiv. of TEA (0.15 mL, 106 mg, 1.05 mmol) and 3 mg (0.02 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 (eluent, petroleum ether:AcOEt, 9:1). After 12 h the reaction was submitted to the usual work-up and the crude residue purified by flash chromatography (eluent, petroleum ether:AcOEt, 9:1) to give 65 mg (0.21 mmol, 95% yields) of product **4b**. A sample of **4b** was submitted to HPLC analyses: (a) Chiralpak

IA column, mobile phase petroleum ether:2-propanol, 75:25; (b) Chiralcel OD column, mobile phase petroleum ether:2-propanol, 9:1.

^{1}H NMR δ (ppm, $CDCl_3$): 7.42 (2H, dt, J_1 = 2.0 Hz, J_2 = 9.0 Hz, H-2′, H-6′); 7.30 (1H, d, J = 1.5 Hz, H-4); 7.22 (1H, dd, J_1 = 1.5 Hz, J_2 = 8.0 Hz, H-6); 7.10 (2H, dt, J_1 = 2.0 Hz, J_2 = 9.0 Hz H-3′, H-5′); 6.83 (1H, d, J = 8.0 Hz, H-7); 6.68 (1H, dd, J_1 = 11.0 Hz, J_2 = 17.5 Hz, H-1″); 5.78 (1H, dd, J_1 = 8.0 Hz, J_2 = 9.0 Hz, H-2); 5.60 (1H, dd, J_1 = 1.0 Hz, J_2 = 17.5 Hz, H-2″$_{trans}$); 5.12 (1H, dd, J_1 = 1.0 Hz, J_2 = 11.0 Hz, H-2″$_{cis}$); 3.63 (1H, dd, J_1 = 9.0 Hz, J_2 = 15.5 Hz, H-3$_a$); 3.21 (1H, dd, J_1 = 8.0 Hz, J_2 = 15.5 Hz, H-3$_b$); 2.55 (2H, t, J_1 = 7.5 Hz, 2Hα); 1.81 (2H, sextet, J_1 = 7.5 Hz, 2Hβ); 1.06 (3H, t, J_1 = 7.5 Hz, 3Hγ). **^{13}C NMR** δ (ppm, $CDCl_3$): 159.79 (C-8); 150.81 (C-4′); 139.60 (C-1″); 136.86 (C-5); 131.32 (C-9); 127.31 (C-6); 127.19 (C-2′, C-6′); 127.13 (C-1′); 122.71 (C-4); 122.11 (C-3′, C-5′); 111.43 (C-2″); 109.52 (C-7); 84.22 (C-2); 38.55 (C-3). Acyl moiety: 172.34 (carbonyl group); 36.55 (Cα); 18.76 (Cβ); 13.93 (Cγ). **MS**: m/z (ESI) = 331.2 Da ($M+Na^+$); m/z (E.I.) = 308.2 Da.

2.1.6. Esterification of the dimer **4** to the corresponding acetate **4a**

5 mg (0.02 mmol) of **4** were dissolved in 0.7 mL of MTBE 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, 9:1) and a sample of the reaction mixture was submitted to HPLC analysis (mobile phase, petroleum ether:2-propanol, 9:1, Chiralcel OD column): **(−)-4a**, t_R = 8.2 min; **(+)-4a**, t_R = 10.6 min.

2.1.7. Screening of lipases for alcoholysis of **4b**

To a solution of **4b** (3 mg, 0.01 mmol) in 0.6 mL of MTBE, 0.03 mL (0.33 mmol) of *n*-BuOH and the respective lipase preparation were added (Novozym 435, Lipolase, lipase PS, lipase CV, *C. rugosa* lipase and PPL, 3.6 mg; lipase AK, 30 mg; lipase CE, 60 mg; Lipozyme, 6 mg). The mixtures were incubated at 30 °C and 250 rpm and monitored by TLC (mobile phase, petroleum ether:AcOEt, 9:1) and HPLC (mobile phase, petroleum ether:2-propanol, 75:25, Chiralpak IA column, **4b**, t_R = 6.5 min; **(+)-4**, t_R = 9.7 min; **(−)-4**, t_R = 10.5 min) at scheduled times.

2.1.8. Preparative alcoholysis of **4b** catalyzed by porcine pancreas lipase (PPL) and subsequent acetylation

To a solution of **4b** (35 mg, 0.11 mmol) in 5 mL of MTBE, 0.3 mL of *n*-BuOH (3.27 mmol) and 150 mg of PPL were added. The mixture was incubated at 30 °C and 190 rpm and monitored by TLC (mobile phase, petroleum ether:AcOEt, 9:1) and HPLC (mobile phase, petroleum ether:2-propanol, 75:25, Chiralpak IA column). After 1.5 h the enzyme was removed by filtration and 1 mL (10.4 mmol) of acetic anhydride and 5 mg (0.04 mmol) of DMAP were slowly added. After additional 12 h, complete acetylation of residual **4** was shown by TLC. The reaction mixture was extracted with H_2O, 5% (w/v) $NaHCO_3$ solution, and again H_2O (5 mL each). Following anhydrification by sodium sulfate, the solvent was evaporated and the crude residue purified by flash chromatography (mobile phase, petroleum ether:AcOEt, 95:5) to give enantiomerically enriched **(+)-4b** (17 mg, 0.061 mmol, e.e. 61%, $[\alpha]_D$ +27.4, c 0.0038 in MeOH), and enantiomerically enriched **(−)-4a** (12.2 mg, 0.040 mmol, e.e. 81%, $[\alpha]_D$ −73.2, c 0.0038 in MeOH). E.e. were determined using a Chiralcel OD column, mobile phase petroleum ether:2-propanol, 9:1.

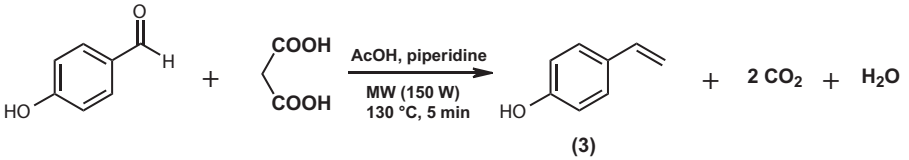
(−)-4a. **^{1}H NMR** δ (ppm, $CDCl_3$): 7.42 (2H, dt, J_1 = 2.0 Hz, J_2 = 9.0 Hz, H-2′, H-6′); 7.30 (1H, d, J = 1.5 Hz, H-4); 7.22 (1H, dd, J_1 = 1.5 Hz, J_2 = 8.0 Hz, H-6); 7.10 (2H, dt, J_1 = 2.0 Hz, J_2 = 9.0 Hz H-3′, H-5′); 6.82 (1H, d, J = 8.0 Hz, H-7); 6.68 (1H, dd, J_1 = 11.0 Hz, J_2 = 18.0 Hz, H-1″); 5.78 (1H, dd, J_1 = 8.0 Hz, J_2 = 9.0 Hz, H-2); 5.60 (1H, dd, J_1 = 1.0 Hz, J_2 = 18.0 Hz, H-2″$_{trans}$); 5.12 (1H, dd, J_1 = 1.0 Hz, J_2 = 11.0 Hz, H-2″$_{cis}$); 3.64 (1H, dd, J_1 = 9.0 Hz, J_2 = 15.5 Hz, H-3$_a$); 3.21 (1H, dd, J_1 = 8.0 Hz, J_2 = 15.5 Hz, H-3$_b$); 2.31 (3H, s, CH_3). **^{13}C NMR** δ (ppm, $CDCl_3$): 159.78 (C-8); 150.72 (C-4′); 139.74 (C-1″); 136.86 (C-5); 131.34 (C-9); 127.31 (C-6); 127.21 (C-2′, C-6′); 127.10 (C-1′); 122.71 (C-4); 122.09 (C-3′, C-5′); 111.44 (C-2″); 109.52 (C-7); 84.18 (C-2); 38.53 (C-3). Acyl moiety: 169.66 (carbonyl group); 21.40 (Cα). **MS**: m/z (ESI) = 303.1 Da ($M+Na^+$); m/z (E.I.) = 280.1 Da.

3. Results and discussion

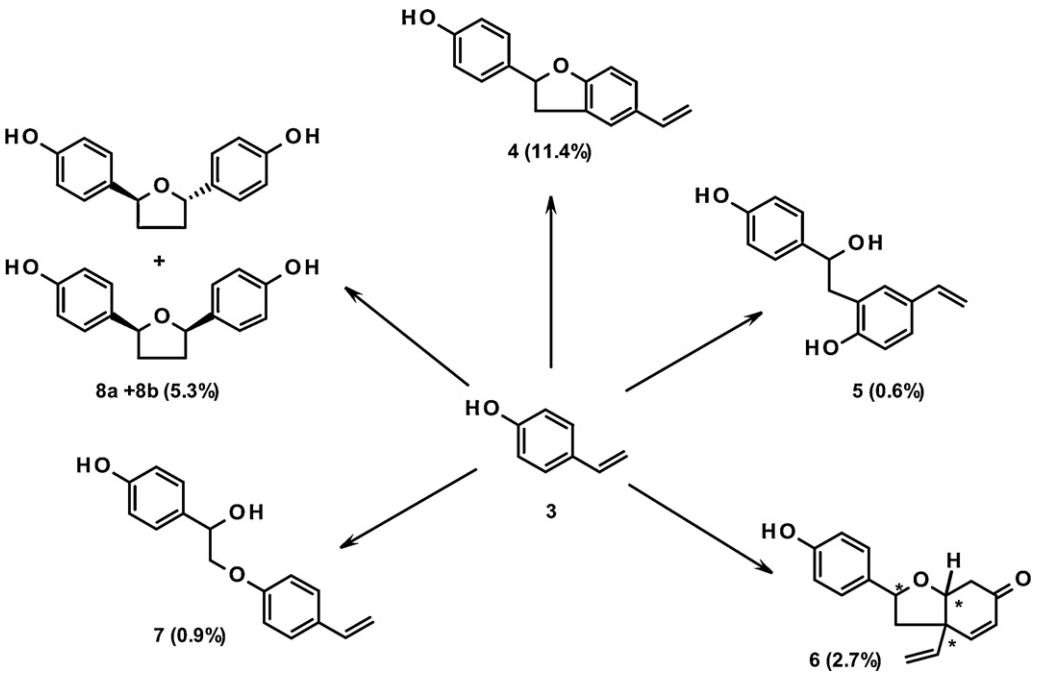
As 4-vinylphenol (**3**) is not commercially available, its synthesis was pursued *via* condensation of *p*-hydroxybenzaldehyde and malonic acid, followed by decarboxylation. Best results were achieved by performing the reaction under microwave irradiation [13], which after just 5 min allowed the isolation of the target **3** in 57% isolated yield (Scheme 2). In turn, 4-vinylphenol was submitted to the action of the commercially available laccase from *T. versicolor* in a biphasic system, following the previously described reaction protocol [12].

As the laccase-catalyzed oxidation of this molecule had not been reported before, a detailed investigation of the reaction dimeric products was performed. As shown in Scheme 3, six dimers (**4–8**) could be isolated and characterized. The main product, albeit with lower isolated yield in comparison to other substituted phenolic precursors, was the "usual" *β-5*-like dimer **4**, whose structure was easily confirmed by mass spectrometry (m/z at 238 Da), ^{1}H NMR (signals due to *para*-substituted and trisubstituted aromatic rings, to a vinyl moiety and to an ABX system were clearly identified, see Section 2), and ^{13}C NMR analysis.

All the other products showed a molecular peak at 256 m/z, suggesting a dimeric structure with the formal addition of a water molecule. The structure of compounds **5** ("*β-1*"-like dimer) and **7** ("*β-O-4*"-like dimer) could be assigned on the basis of the clear differences in the signals due to their aromatic rings. A structure similar to the so-called Pummerer's ketone could be assigned to compound **6** by comparison with the literature data [5a,14]. Additionally, NOESY experiments clearly showed the relative relationship between the three stereogenic centers of the molecule: the *p*-hydroxyphenyl and the vinyl moieties being on the same side (*cis*) of the tetrahydrofuran ring and the proton being on the opposite (*trans*) site. Finally, the ^{1}H NMR spectrum of the last isolated dimeric product indicated the presence in a 2:1 ratio (by comparing the area of the signals due to two sets of benzylic protons – triplet at 4.98 and 4.77 ppm) of symmetrical structures that, on the contrary to compounds **4–7**, were not carrying a vinyl substituent anymore. These evidences suggested the presence of the inseparable mixture of the *cis* and *trans* isomers **8a–8b**.



Scheme 2. Synthesis of 4-vinylphenol **3**.



Scheme 3. Laccase-catalyzed oxidation of 4-vinylphenol **3**.

All the compounds were isolated as racemates. Specifically, as far as the dimer **4** concerned, the two enantiomers could be easily separated by chiral HPLC using a Chiralpak IA column (Fig. 1).

Following our described methodology [12], dimer **4** was converted to the racemic butanoate **4b** to be submitted to the action of a panel of commercially available lipases for enzyme-mediated resolution. Table 1 summarizes the results obtained in the lipase-catalyzed alcoholysis of **4b** dissolved in methyl *t*-butyl ether in the presence of a large excess of *n*-BuOH. Reactions were monitored by chiral HPLC, which allowed the evaluation of the degree of conversion and of the e.e. values of the released phenolic product **4** (the racemic starting material **4b** was eluted as a single peak, Fig. 1), parameters that were then used to calculate the values of the enantiomeric ratio "*E*" of the tested lipases [15].

In the previously described examples based on the dimers of compounds **1** and **2** [12], the *E*-values were quite low ($E < 8$), as it might be expected. This problem was overcome by finding couples of enantiocomplementary lipases which acted on the enantiomerically enriched residual substrates, thus allowing overall efficient kinetic resolutions. At variance, all the lipases acting on **4b** showed the same enantiopreference for the levorotatory enantiomer **(−)-4b** (the only enzyme showing a minor preference for the opposite enantiomer – lipase CE – was basically non-selective: $E = 1.2$). However, we were pleased to note that the crude porcine pancreatic lipase indeed showed a significant *E*-value (18.8), which was not expected for such a molecule (the sterocenter was 6 bonds distant from the acyl moiety that suffered alcoholysis cleavage and, moreover, no other substituents were present close to the reactive ester). Eventually, the kinetic resolution of **4b** was scaled-up and, to avoid racemization during the reaction work-up, the released phenolic product was *in situ* acetylated to give the acetate **4a** (Scheme 4). The two enantiomerically enriched esters **(+)-4b** and **(−)-4a** could be easily purified by silica chromatography to give the products in 61% and 81% enantiomeric excess, respectively (as judged by chiral HPLC with a Chiralcel OD column, Fig. 2).

In conclusion this short report further exemplifies the synthetic usefulness of the tandem use of laccases and lipases for the preparative synthesis of enantiomerically enriched dimeric phenols. Once again it is really noteworthy and surprising the ability of crude and commercially available lipases preparations to discriminate enantiomers possessing remote stereocenters.

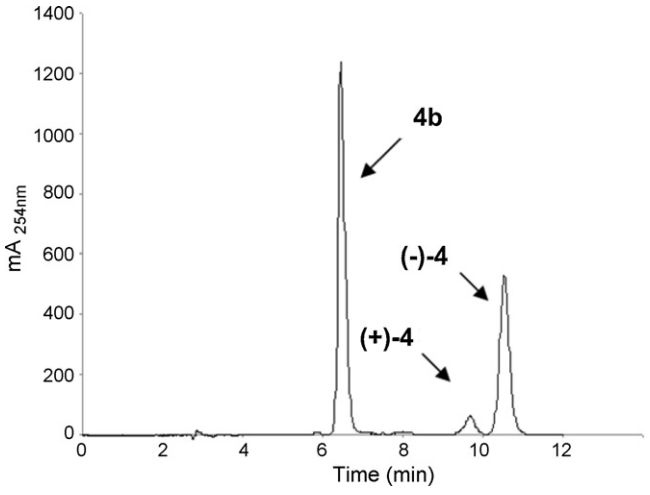
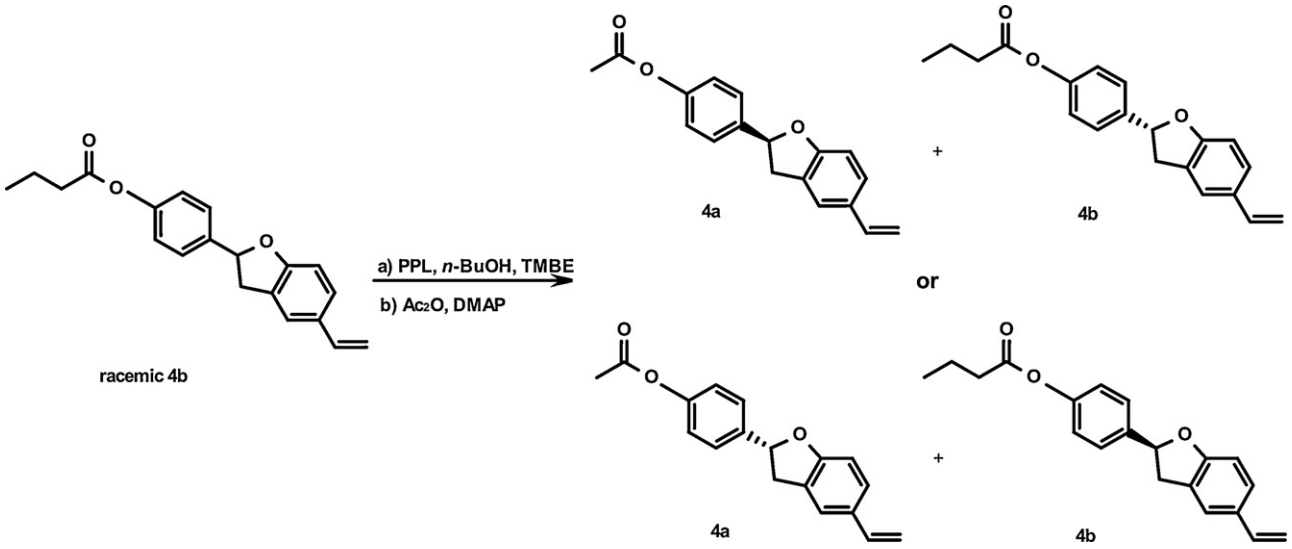


Fig. 1. HPLC chromatogram of the PPL-catalyzed alcoholysis of racemic **4b** (column: Chiralpak IA).

Table 1
Screening of lipases for the enantioselective alcoholysis of **4b**.

Enzyme	Conversion (%)	Product e.e. (%)	*E*
Lipase AK	41.4	0	1.0
Lipase CE	39.4	7.1[b]	1.2
Lipase PS	20.3	40.8[a]	2.6
Novozym 435	39.0	45.2[a]	3.5
Lipase CV	30.2	12.0[a]	1.3
Lipozyme	40.3	43.7[a]	3.4
Lipolase	42.2	3.4[a]	1.1
Porcine pancreas lipase	46.7	80.1[a]	18.8
Candida rugosa lipase	23.2	1.5[a]	1.0

[a] Major product: **(−)-4**.
[b] Major product: **(+)-4**.



Scheme 4. Lipase-catalyzed alcoholysis of compound **4b**, followed by *in situ* acetylation.

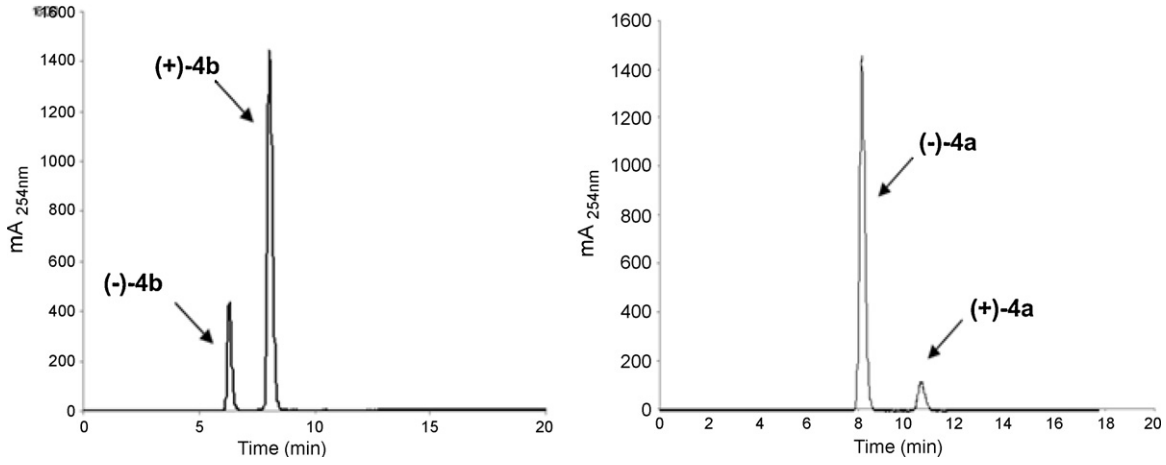


Fig. 2. HPLC chromatogram of isolated enantiomerically enriched **(+)-4b** (left) and **(−)-4a** (right). Column: Chiralcel OD.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcatb.2012.03.020.

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