Journal of Molecular Catalysis B: Enzymatic 134 (2016) 295–301

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Laccase-catalyzed dimerization of glycosylated lignols

Ivan Bassanini[a,b], Paolo Gavezzotti[a], Daniela Monti[a], Jana Krejzová[c], Vladimír Křen[c], Sergio Riva[a,*]

[a] Istituto di Chimica del Riconoscimento Molecolare (ICRM), CNR, Via Mario Bianco 9, 20131 Milano, Italy
[b] Università di Milano, Dipartimento di Chimica, Via Golgi 19, Milano, Italy
[c] Institute of Microbiology, Laboratory of Biotransformation, Czech Academy of Sciences, Vídeňská 1083, CZ 142 20 Prague, Czechia

ARTICLE INFO

Article history:
Received 1 July 2016
Received in revised form 7 October 2016
Accepted 31 October 2016
Available online 2 November 2016

Keywords:
Laccase
Rutinosidase
Biocatalysis
Phenylpropanoids
Biooxidation

ABSTRACT

Phenylpropanoid glucosides (PPGs) are naturally occurring and bioactive phenolic derivatives, largely distributed in plants. In this work different PPGs have been chemically or enzymatically synthesized from the lignols coniferyl and *p*-coumaryl alcohols as substrates for a laccase-catalyzed oxidative coupling. The biooxidation of these PPGs has been investigated here and novel dihydrobenzofuran-based structurally modified analogues have been isolated and characterized. Specifically, the presence of a carbohydrate moiety increased the water solubility of these compounds and reduced the number of dimeric products, as pinoresinol-like structures could not be formed. Looking for a possible sugar-promoted stereochemical enrichment of the obtained diastereomeric mixtures of dimers, different carbohydrate moieties (D-glucose, L-glucose and the disaccharide rutinose) were considered and the respective d.e. values of the dimeric products were measured by ^{1}H NMR and HPLC. However, it was found that the sugar substituent had a minor effect on the stereochemical outcome of the radical coupling reactions, the best measured result being a d.e. value of 21%.

1. Introduction

Laccases (benzendiol:oxygen oxidoreductases, EC 1.10.3.2) are copper-containing oxidases, distributed in plants, fungi and bacteria, that catalyze the formation of four highly reactive organic radicals by reducing molecular oxygen to water [1]. It has been reported that laccases play, *i.a.*, an important role in the formation of lignin by sequential oxidative couplings of monolignols, a family of naturally occurring phenolic compounds [2]. *p*-Coumaryl (**1**) and coniferyl (**2**) alcohols, Fig. 1, are two major examples of this class of chemicals.

From a synthetic point of view, these 'blue' enzymes are known to oxidize a wide range of organic molecules, both directly or in the presence of a low-molecular-weight redox mediator [3]. Our group has accordingly reported several examples dealing with the oxidation of sugars [4], alkaloids [5] and, more extensively, phenols [6]. Specifically, we have shown that stilbenoids and vinyl phenols are suitable substrates for these enzymes. Mixture of dimers and oligomers are always obtained, with the 2,3-dihydrobenzofuran-based β-5-type dimeric structures being isolated as the main products [7]. These compounds carry one or two stereogenic carbons and are always obtained as racemates and, when two adjacent stereocenters are present (like in the dimers obtained from resveratrol [7b]), only the *trans*-stereoisomers are formed.

The lack of enantioselectivity is a serious synthetic drawback of these biocatalyzed oxidative couplings. Nature has solved this problem developing the so-called "dirigent proteins", a group of peptides that act as chiral templates and direct the stereochemical outcome of these processes. A significant example of these "dirigent proteins" was isolated from the plant *Forsythia intermedia* and used by Davin et al. for the *in vitro* stereoselective biocatalyzed synthesis of (+)-pinoresinol (**3**) by the laccase-catalyzed oxidation of coniferyl alcohol [8]. Later on, in 2010, Pickel et al. isolated and cloned an enantiocomplementary dirigent protein, able to guide the bio-oxidative coupling toward the formation of (−)-pinoresinol [9]. However, this elegant biomimetic approach is of limited synthetic appeal as, for any substrate and desired enantiomer of a dimeric product, a specific dirigent protein should be isolated, providing that it does exist in Nature.

Abbreviations: E.I., electron ionization; Py, pyridine; DMAP, dimethylaminopyridine; THF, tetrahydrofuran; DBU, 1,5-diazabiciclo(5.4.0)undec-5-ene; TMSOTf, trimethylsilyl trifluoromethanesulfonate; DCM, dichloromethane; TBAF, tetra-*n*-butylammonium fluoride; TBDMS, *tert*-butyldimethylsilyl; d.e., diastereomeric excess.

* Corresponding author.
E-mail address: Sergio.riva@icrm.cnr.it (S. Riva).

http://dx.doi.org/10.1016/j.molcatb.2016.10.019
1381-1177/

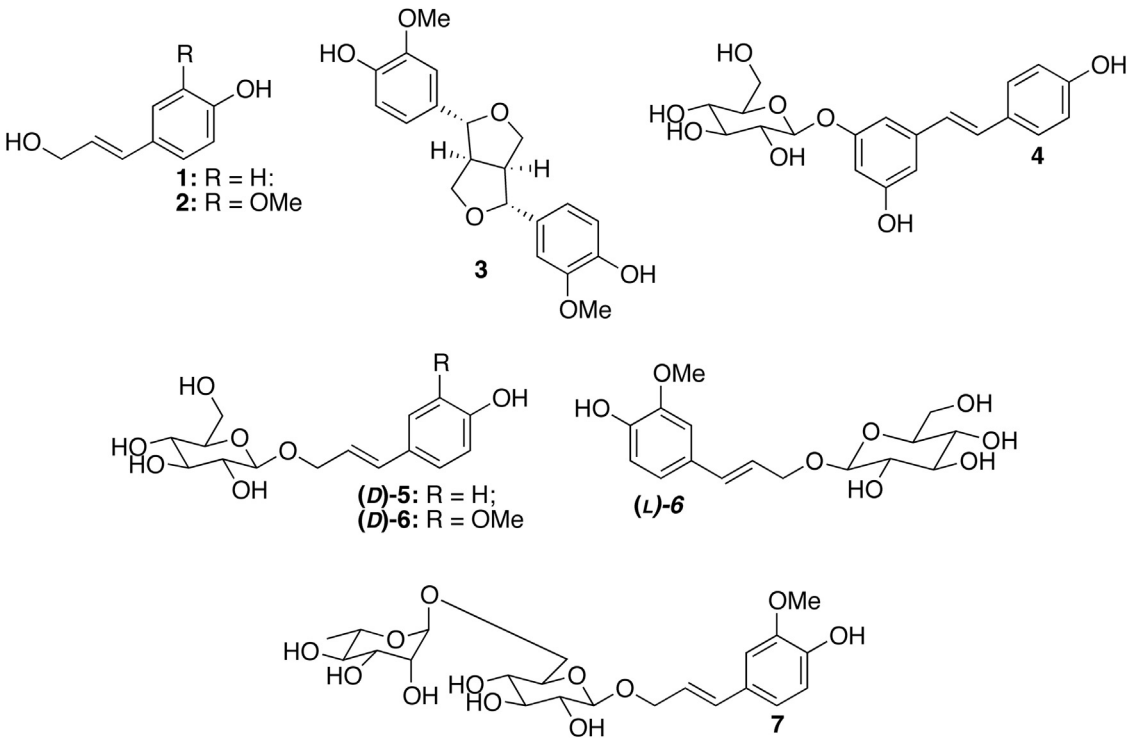


Fig. 1. Compounds **1–7**.

To overcome this limitation, Urlacher and coworkers have recently proposed to couple the laccase-mediated oxidation of coniferyl alcohol to a stereoselective reductase that is able to transform the unwanted enantiomer of pinoresinol [10].

In a different and more general approach, we have exploited the 'classical' lipase-catalyzed kinetic resolution. The racemic mixtures of β-5-type dimers derived from the laccase-mediated oxidation of different vinyl phenols were separated obtaining both the possible enantiomers as enriched species. The target products could be isolated with e.e. up to 98% [11].

More recently, both the enantiomers of the β-5-type dimers of resveratrol could be isolated on a preparative scale starting from the laccase-mediated oxidation of piceid (**4**), a natural D-glucoside of resveratrol [12]. A mixture of *trans*-2*R*,3*R* and *trans*-2*S*,3*S* glucosylated dimers was easily isolated in good yield, and the two diastereomers were separated by preparative HPLC on a chiral column. The target enantiomers of resveratrol dimers were eventually obtained with e.e. values up to 97% by glycosidases-catalyzed hydrolysis of the glucose units.

We thought that this approach could be extended to other compounds. Phenylpropanoid glucosides (PPGs, also known as glucosylated lignols) are naturally occurring in various plants. As secondary metabolites, these compounds are described as phytopharmaceuticals [13] and they are studied for their potential biological activities [14]. Specifically, their antioxidant, antidepressant and hypotensive effects have been extensively investigated [15].

In this work the laccase-catalyzed oxidative coupling of a small family of glycosylated phenylpropanoids, previously synthesized from coniferyl and *p*-coumaryl alcohol, has been investigated, and novel, structurally modified analogues of these bioactive natural compounds have been isolated and characterized. Looking for a possible sugar-induced stereochemical enrichment of the obtained diastereomeric mixtures of dimers, different carbohydrate moieties (D-glucose, L-glucose and the disaccharide rutinose) were considered.

2. Experimental

2.1. General methods and information

NMR spectra were recorded on Bruker AC400 spectrometer (400 MHz) in MeOH-d_4, DMSO-d_6 or D_2O, MS spectra were recorded on a Bruker Esquire 3000 Plus Ion Trap spectrometer NMR and the MS spectra *in extenso* are available in the Supplementary materials.

HPLC analyses were carried out using a Jasco 880-PU pump equipped with a Jasco 875-UV/vis detector. HPLC conditions: Kinetex 5 μm Biphenyl 100 Å Phenomenex 150 × 4.6 mm column, gradient of H_2O: CH_3CN as mobile phase (0–1 min 90% H_2O, 10% CH_3CN; 1–20 min 80% H_2O, 20% CH_3CN; 20–22 min 80% H_2O, 20% CH_3CN; 22–23 90% H_2O, 10% CH_3CN), flow rate 0.7 mL min^{-1} at 25 °C, detection at 270 nm. Reactions were monitored by TLC: precoated silica gel 60 F_{254} plates (Merck, DE), developed with a 20% solution of H_2SO_4 in ethanol or using 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, DE).

Biotransformations were carried out using a G24 Environmental Incubator New Brunswick Scientific Shaker (Edison, USA) or a Thermomixer Comfort (Eppendorf, DE).

2.2. Enzymes and chemicals

Laccase from *Trametes versicolor* was from Sigma-Aldrich. α-L-*R*hamnosyl-β-D-glucosidase (rutinosidase) from *Aspergillus niger* was produced according to the listed reference [16]. The enzymes were used based on their respective activities evaluated according to literature assays [16,6b].

TBDMS-phenol protected phenylpropanoid alcohols were prepared from their correspondent acids or aldehydes according to literature [19]. All other reagents were of the best purity grade from commercial suppliers.

2.3. Glucose trichloroacetimidate. General experimental procedure

(i) Peracetylated glucose (1 eq, final concentration 0.1 M) was added at room temperature to a solution of glacial acetic acid (3.0 eq) and ethylenediamine (2.5 eq) in tetrahydrofuran. The resulting mixture was stirred for 3 h in a round bottom flask cupped with a calcium chloride guard tube. The conversion of the starting material was checked by TLC (mobile phase petroleum ether: AcOEt; 4:6). Once the peracetylated glucose was fully consumed, the reaction mixture was diluted with two volumes with water and extracted twice with DCM. The combined organic layers were washed with of a 5% aqueous solution of hydrochloric acid, a saturated aqueous solution of sodium bicarbonate and water, until pH 7 was reached. After drying over sodium sulfate, the solvent was concentrated *in vacuo* affording the desired product used without any further purifications.

(ii) 1,5-Diazabicyclo(5.4.0)undec-5-ene (0.4 eq) and trichloroacetonitrile (1.1 eq) were added at room temperature and under nitrogen atmosphere to a solution of 2,3,4,6-tetraacetylglucopyranose (1 eq) in dry DCM (0.2 M). The resulting mixture was stirred for 45 min. After checking the complete conversion of the starting material by TLC (mobile phase petroleum ether: AcOEt; 6:4), the crude product was concentrated *in vacuo* and purified by flash column chromatography (mobile phase petroleum ether: AcOEt; 7:3), affording the desired compound.

2.4. Peracetylated d- and l-glucose trichloroacetimidate

D-Glucose: according to the general procedure *2.3*, D-glucose trichloroacetimidate (8.2 g, 17.16 mmol, yellow solid, isolated yield: 67%) was obtained from D-glucose tetraacetate (10.0 g, 25.62 mmol).

^{1}**H NMR** (400 MHz; $CDCl_3$): δ 5.61–5.55 (m, 1H: H-1), 5.22–5.07 (m, 2H: H-6), 4.31–4.11 (m, 4H: H-2, H-3, H-4, H-5), 2.09 (s, 3H: H-7), 2.06 (s, 3H: H-7), 2.05 (s, 3H: H-7), 2.03 (s, 3H: H-7).The NMR data were in accordance to the literature values [17].

L-Glucose: according to general procedure *2.3*, L-glucose trichloroacetimidate (8.0 g, 16.39 mmol, yellow solid, isolated yield: 64%) was obtained from L-glucose tetraacetate (10.0 g, 25.62 mmol).

The NMR data were in accordance to the values listed for the D-glucoside.

2.5. Chemical glucosylation. General experimental procedure

(i) Under nitrogen atmosphere, glucose trichloroacetimidate (5 eq) and crushed 4 Å molecular sieves were added to a stirring solution of TBSM-protected phenylpropanoid alcohol (1 eq) in dry DCM (0.1 M). After cooling to −15 °C, trimethylsilyl trifluoromethanesulfonate (0.03 eq), dissolved in dry DCM, was added dropwise. The resulting mixture was stirred for 30 min at −15 °C. The reaction was subsequently quenched with triethylamine (0.5 eq), filtered through a Celite®545 pad and the solvent concentrated *in vacuo*. The desired fully-protected product was purified by flash column chromatography (mobile phase petroleum ether: AcOEt; 8:2).

(ii) Under nitrogen atmosphere and at 0 °C, the fully-protected phenylpropanoid glucoside (1 eq) was dissolved in dry THF (0.03 M). A 1 M solution of tetra-*n*-butylammonium fluoride (1 eq) in THF was then added and the reaction stirred for 20 min. After checking the conversion of the starting material by TLC (mobile phase petroleum ether: AcOEt, 1:1), the mixture was diluted with diethyl ether, washed with water, brine and water again, dried over sodium sulfate and concentrated *in vacuo*, affording the desired product that was used without any further purifications.

(iii) Phenol-deprotected phenylpropanoid glucoside (1 eq) was suspended in MeOH (0.03 M) and, under vigorous stirring, a 0.5 M solution of MeONa in MeOH (2 eq) was added slowly and dropwise. The resulting mixture was stirred for 30 min. After that, the reaction was diluted three times with MeOH and a Dowex® 50WX8 hydrogen form resin was added. The mixture was then stirred until pH 7.0 was reached. The resin was then filtered and the solvent concentrated *in vacuo*, affording the desired product that was used without any further purifications.

2.6. Compound (*D*)-*5*

According to general procedures *2.5*, (**D**)-**5** (463 mg, 1.48 mmol, oil, isolated yield: 28%) was obtained from phenol-protected coumaric alcohol (1.4 g, 5.30 mmol) and D-glucose trichloroacetimidate (3.6 g, 7.42 mmol).

^{1}**H NMR** (400 MHz; D_2O): δ 7.32 (d, J = 8.0 Hz, 2H: H-3, H-5), 6.83 (d, J = 8.0 Hz, 2H: H-2, H-4), 6.58 (d, J = 15.6 Hz, 1H: Ha), 6.16-6.12 (m, 1H: Hb), 4.47-4.40 (m, 2H: H-7A, H-8), 4.31-4.27 (m, 1H: H-7B), 3.86 (d, J = 12.6 Hz, 1H: H-13A), 3.69–3.66 (m, 1H: H-13B), 3.44-3.24 (m, 4H: H-9, H-10, H-11, H-12).

^{13}**C NMR** (101 MHz; D_2O): δ 155.6, 133.6, 128.9, 128.2, 122.3, 115.7, 101.1, 75.92, 75.90, 73.2, 70.5, 69.7, 60.8, 38.8, 30.3.

MS, m/z ESI = 335.1 Da $[M+Na]^+$.

2.7. Compounds (*D*)-*6* and (*L*)-*6*

(**D**)-**6**: according to general procedure *2.5*, fully deprotected (*D*)-**6** (500 mg, 1.46 mmol, oil, isolated yield: 30%) was obtained from phenol-protected coniferyl alcohol (1.5 g, 5.10 mmol) and D-glucose trichloroacetimidate (3.6 g, 7.42 mmol).

^{1}**H NMR** (400 MHz; MeOD): δ 7.03 (d, J = 1.6 Hz, 1H: H-3), 6.87 (dd, J = 8.4, 1.6 Hz, 1H: H-5), 6.76 (d, J = 8.4 Hz, 1H: H-6), 6.59 (d, J = 16.0 Hz, 1H: Ha), 6.21 (ddd, J = 16.0, 6.8, 6.0 Hz, 1H: Hb), 4.51 (ddd, J = 12.4, 6.0, 1.2 Hz, 1H: H-7A), 4.39 (d, J = 7.8 Hz, 1H: H-8), 4.32 (ddd, J = 12.4, 6.8, 1.2 Hz, 1H: H-7B), 3.90 (dd, J = 11.9, 2.0 Hz, 1H: H-13A), 3.88 (s, 3H: OCH_3), 3.70 (dd, J = 11.9, 5.4 Hz, 1H: H-13B), 3.41–3.23 (m, 4H: H-9, H-10, H-11, H-12).

^{13}**C NMR** (101 MHz; MeOD): δ 147.7, 146.3, 132.9, 129.0, 122.4, 119.8, 114.8, 109.3, 101.8, 76.74, 76.57, 73.7, 70.3, 69.6, 61.4, 55.0.

MS, m/z ESI = 365.2 Da $[M + Na]^+$.

(**L**)-**6**: according to general procedure *2.5*, fully deprotected (**L**)-**6** (280 mg, 1.13 mmol, oil, isolated yield: 20%) was obtained from phenol-protected coniferyl alcohol (1.6 g, 5.44 mmol) and L-glucose trichloroacetimidate (3.6 g, 7.42 mmol).

The NMR data were in accordance to the values listed for the D-glucoside.

2.8. Biocatalyzed rutinosylation: compound *7*

Rutin (2.5 g, 4.16 mmol) was added step by step (625 mg, 0.5 eq per time) within a reaction time of 8 h, to a solution of coniferyl alcohol (500 mg, 2.81 mmol, 0.128 M) and rutinosidase (0.037 U) in 22 mL of a 85:15 mixture of citrate-phosphate buffer (0.05 M, pH 5.0) and DMSO. The obtained suspension was incubated at 35 °C and 1000 rpm. The reaction was monitored by TLC (MeOH: AcOEt: HCOOH; 2:8:0.1). When all the starting alcohol was consumed, the reaction mixture was cooled to room temperature, diluted ten times with citrate-phosphate buffer and centrifuged, recovering only the supernatant. After changing the pH from 5.0 to 7.5–7.7, the aqueous layer was extracted twice with AcOEt and then, after removing the residual AcOEt *via* concentration *in vacuo*, subjected to an XAD4 solid phase extraction (mobile phase H_2O: MeOH, from

100:0 to 0:100), affording **7** (716 mg, 1.72 mmol, yellow solid, isolated yield: 60%).

^{1}**H NMR** (400 MHz; MeOD): δ 7.04 (d, J = 2.0 Hz, 1H: H-3), 6.89 (dd, J = 8.4, 2.0 Hz, 1H: H-5), 6.76 (d, J = 8.4 Hz, 1H: H-6), 6.60 (d, J = 16.0 Hz, 1H: Ha), 6.21 (ddd, J = 16.0, 6.8, 6.0 Hz, 1H: Hb), 4.80 (d, J = 1.6 Hz, 1H: H-14), 4.48 (ddd, J = 12.3, 6.0, 1.6 Hz, 1H: H-7A), 4.37 (d, J = 7.6 Hz, 1H:H-8), 4.28 (ddd, J = 12.3, 6.8, 0.8 Hz, 1H: H-7B), 4.01 (dd, J = 11.2, 1.6 Hz, 1H: H-13A), 3.88 (s, 3H: = OCH_3), 3.73-3.64 (m, 3H: H-13, H-16, H-18), 3.44–3.21 (m, 5H: H-9, H-10, H-11, H-12, H-17), 1.30 (d, J = 6.4 Hz, 3H: H-19).

^{13}**C NMR** (101 MHz; MeOD): δ 147.7, 146.3, 133.1, 128.9, 122.1, 120.0, 114.8, 109.2, 101.6, 100.9, 76.7, 75.5, 73.7, 72.6, 70.97, 70.83, 70.3, 69.5, 68.4, 54.9, 16.7.

MS, m/z ESI = 511.3 Da $[M+Na]^+$.

2.9. Laccase-mediated dimerization. General experimental procedure

To a solution of phenylpropanoid glycoside (0.05 M) in acetate buffer (0.02 M, pH 5.0), the needed amount of a 1 mg mL^{-1} (4.6 U mL^{-1}) laccase solution, prepared in the same buffer, was added to achieve the value of 2.5 U mmol$_{substrate}$$^{-1}$. The resulting mixture was incubated at 30 °C and 300 rpm and monitored by TLC (mobile phase MeOH: chloroform: H_2O; 6:4:0.5, or MeOH: AcOEt: H_2O; 6:4:0.5). Once the starting glycoside was consumed, the pH of the medium was changed to 7.0 and the mixture was lyophilized. The crude product was purified by flash column chromatography (mobile phase MeOH: chloroform: H_2O; 7:3:0.3 or MeOH: AcOEt: H_2O; 7:3:0.3), affording the desired dimers as a mixtures of *trans*-diastereoisomers.

2.10. Compound ***8***

According to general procedure *2.9*, **8** (31 mg, 0.07 mmol, oil, isolated yield: 35%) was obtained from compound (**D**)-**5** (63 mg, 0.20 mmol) and laccase (0.32 U).

^{1}**H NMR** (400 MHz; MeOD, mixture of diastereoisomers): δ 7.54 (s, 0.5H), 7.47 (s, 0.5H), 7.25–7.23 (m, 2H), 6.79–6.74 (m, 3H), 6.64 (d, J = 15.6 Hz, 1H: Ha), 6.29–6.20 (m, 1H: Hb), 5.57 (d, J = 6.0 Hz, 0.5H: Ha'$_A$), 5.53 (d, J = 6.4 Hz, 0.5H: Ha'$_B$), 4.54-4.48 (m, 1H), 4.40–4.37 (m, 2H: H-anomer$_{A+B}$), 4.36–4.30 (m, 1H), 4.23–4.15 (m, 1H), 3.92–3.79 (m, 3H), 3.72–3.60 (m, 4H), 3.42–3.22 (m, 14H).

^{13}**C NMR** (101 MHz; MeOD, mixture of diastereoisomers): δ 181.9, 179.3, 159.6, 157.07, 156.99, 132.86, 132.80, 132.59, 132.39, 130.0, 128.3, 127.06, 126.94, 122.8, 122.4, 114.83, 114.81, 108.6, 103.0, 101.69, 101.64, 87.38, 87.22, 76.74, 76.63, 76.59, 73.7, 70.34, 70.24, 69.6, 61.4.

MS, m/z ESI = 645.3 $[M+Na]^+$.

2.11. Compound ***9***

According to general procedure *2.9*, **9** (150 mg, 0.22 mmol, oil, isolated yield: 34%) was obtained from compound (**D**)-**6** (470 mg, 1.4 mmol) and laccase (2.35 U).

^{1}**H NMR** (400 MHz; DMSO-d_6, mixture of diastereoisomers A and B): 7.12 (s, 0.5H), 7.03 (s, 0.5H), 6.97–6.94 (m, 2H), 6.81-6.74 (m, 2H), 6.57 (d, J = 16.0 Hz, 1H: Ha), 6.26-6.17 (m, 1H: Hb), 5.55 (d, J = 6.0 Hz, 0.5H: Ha'$_A$), 5.50 (d, J = 7.2 Hz, 0.5H: Ha'$_B$), 4.40 (dd, J = 12.8, 5.7 Hz, 1H: H-7), 4.27–4.15 (m, 3H: H-Glc$_{anomerA}$, H-Glc$_{anomerB}$, H-7), 4.06–3.96 (m, 2H), 3.81 (s, 2H: OCH_{3A}), 3.80 (s, 1H: OCH_{3B}), 3.75 (s, 1H: OCH_{3A}), 3.74 (s, 2H: OCH_{3B}), 3.72–3.56 (m, 4H), 3.50–3.42 (m, 2H), 3.20–2.98 (m, 9H).

^{13}**C NMR** (101 MHz; DMSO-d_6, mixture of diastereoisomers): δ 147.95, 147.91, 147.80, 147.72, 144.21, 144.14, 132.7, 132.34, 132.27, 132.18, 130.79, 130.71, 129.8, 129.2, 124.1, 119.1, 118.8, 116.10, 116.00, 115.7, 111.10, 111.08, 111.04, 110.85, 103.4, 102.3, 87.5, 77.33, 77.24, 77.07, 77.01, 73.9, 70.9, 70.47, 70.40, 69.26, 69.20, 61.5, 56.20, 56.17, 56.10, 51.1, 50.8.

MS, m/z ESI = 705.3 Da $[M+Na]^+$.

2.12. Compound ***10***

According to general procedure *2.9*, **10** (109 mg, 0.16 mmol, oil, isolated yield: 37%) was obtained from compound (**L**)-**6** (380 mg, 0.89 mmol) and laccase (1.90 U).

^{1}**H NMR** (400 MHz; DMSO-d_6): δ 7.14 (s, 0.5H), 7.04 (s, 0.5H), 6.98–6.95 (m, 2H), 6.81–6.74 (m, 2H), 6.58 (d, J = 16.0 Hz, 1H: Ha), 6.27–6.18 (m, 1H: Hb), 5.55 (d, J = 6.0 Hz, 0.5H: Ha'$_A$), 5.50 (d, J = 7.3 Hz, 0·5H:Ha'$_B$), 4.41 (dd, J = 12.8, 5.5 Hz, 1H: H-7), 4.26–4.16 (m, 3H: H-Glc$_{anomerA}$, H-Glc$_{anomerB}$, H-7), 4.08–3.97 (m, 2H), 3.82 (s, 1.5H: OCH_{3A}), 3.81 (s, 1.5H: OCH_{3B}), 3.76 (s, 1.5H: OCH_{3A}), 3.75 (s, 1.5H: OCH_{3B}), 3.72–3.60 (m, 4H), 3.46 (dq, J = 12.0, 6.0 Hz, 3H), 3.21–2.98 (m, 8H).

^{1}**C NMR** (101 MHz; DMSO-d_6): δ 148.02, 147.97, 147.84, 147.75, 146.93, 146.80, 144.22, 144.15, 132.6, 132.33, 132.27, 132.11, 130.78, 130.70, 129.8, 129.2, 124.08, 124.06, 119.2, 118.8, 116.14, 116.05, 115.77, 115.72, 111.16, 111.07, 110.9, 103.45, 103.31, 102.4, 87.57, 87.56, 77.45, 77.39, 77.36, 77.32, 77.26, 74.00, 73.94, 70.61, 70.54, 69.26, 69.20, 61.60, 61.57, 61.54, 56.22, 56.19, 56.11, 51.1, 50.8.

MS, m/z ESI = 705.3 Da $[M+Na]^+$.

2.13. Compound ***11***

According to general procedure *2.9*, **11** (74 mg, 0.07 mmol, oil, isolated yield: 33%) was obtained from compound **7** (225 mg, 0.46 mmol) and laccase (1.12 U).

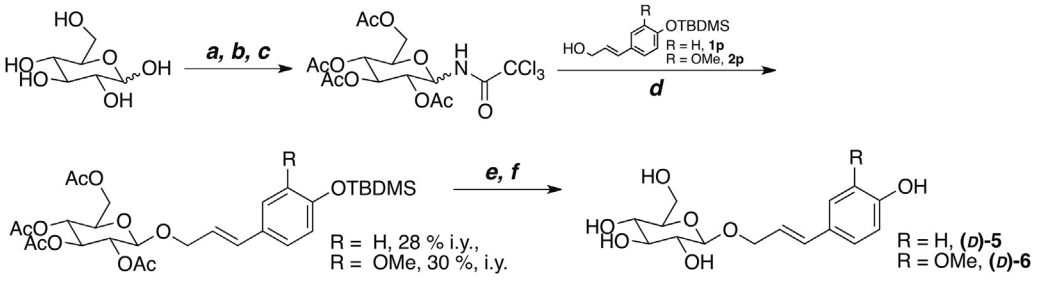
^{1}**H NMR** (400 MHz; D_2O, mixture of diastereoisomers): δ 7.01 (br s, 1H), 6.96 (dd, J = 8.5, 1.4 Hz, 1H), 6.86-6.80 (m, 2H), 6.58 (d, J = 15.8 Hz, 1H: Ha), 6.24-6.17 (m, 1H: Hb), 5.60 (d, J = 6.0 Hz, 0.5H: Ha'$_A$), 5.55 (d, J = 6.8 Hz, 0.5H: Ha'$_B$), 4.49–4.42 (m, 2H: H-Glc$_{anomerA}$, H-7), 4.38–4.29 (m, 2H: H-Glc$_{anomerB}$, H-7), 4.08–4.01 (m, 1H), 3.96–3.91 (m, 3H), 3.89-3.83 (m, 5H), 3.80-3.76 (m, 4H), 3.73–3.56 (m, 7H), 3.53–3.23 (m, 11H), 1.25–1.15 (m, 6H).

^{13}**C NMR** (101 MHz; D_2O, mixture of diastereoisomers): δ 147.6, 147.2, 143.8, 133.7, 131.1, 128.4, 123.1, 119.0, 115.86, 115.78, 115.6, 110.9, 110.3, 102.9, 101.4, 100.61, 100.45, 96.1, 88.0, 75.8, 74.7, 73.2, 72.1, 70.28, 70.10, 69.6, 68.7, 66.7, 56.12, 55.97, 50.7, 48.9, 16.7.

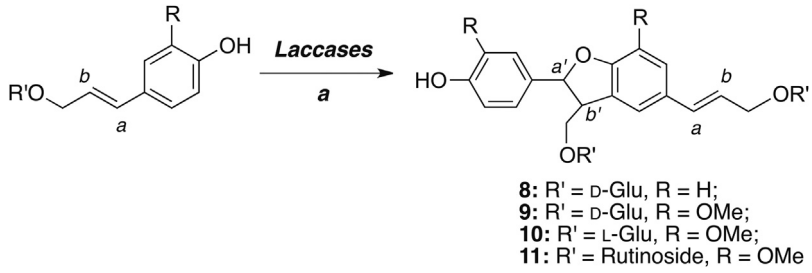
MS, m/z ESI = 997.3 Da $[M+Na]^+$.

3. Results and discussion

The convenient use of piceid (**4**) as starting substrate to obtain the enantiomeric *trans* β-5-type dimers of resveratrol prompted us to extend this approach to other phenolic glucosides. Specifically, it was decided to study the laccase-mediated coupling of phenylpropanoid glucosides, *i.e.* **(D)-5** and **(D)-6**. To the best of our knowledge, these compounds were never submitted to the oxidative action of laccases. Moreover, the presence of a sugar moiety linked to the primary alcohol of the propenol side chain, preventing the formation of pinoresinol-like structures, would have allowed to limit the number of dimeric products obtained by radical coupling (a detailed description of the dimeric products that could be obtained by the laccase/catalyzed oxidation of the aglycone coniferyl alcohol **2** was reported years ago [18]). In addition, it was intriguing to verify the effect of the sugar moiety on the stereochemical outcome of the coupling reactions. In fact, at variance to piceid **4**, in these compounds glucose was linked very close to the carbons (C-a and C-b, Scheme 2) that were going to become stereocenters (C-a' and C-b', Scheme 2). Our hypothesis was that bulky multichiral moiety in the vicinity of the reaction sites would induce a discrimination among the possible diastereomeric products.



Scheme 1. Synthesis of the glucopyranosides **(D)-5** and **(D)-6**, reagents and conditions: (a) Py, Ac_2O, DMAP, r.t., 24 h; (b) AcOH glacial, $NH_2(CH_2)_2NH_2$, THF, r.t., 3 h; (c) N_2, DBU, CCl_3CN, DCM dry, r.t.; (d) TMSOTf, powdered sieves 4 Å, DCM dry, −15 °C, 30 min; (e) TBAF, THF dry, 0 °C, 2 h; (f) MeONa/MeOH, r.t., 3 h.



Scheme 2. Laccase-mediated synthesis of compounds **8**–**11**, reagents and conditions: Phenylpropanoid glycoside (0.05 M), acetate buffer (0.02 M, pH 5.0), laccase (0.005 U $mg_{substrate}^{-1}$), at 30 °C and 300 rpm, 6–8 h. Isolated yields: **8** = 35%; **9** = 34%; **10** = 37%; **11** = 33%. All products were obtained as mixtures of *trans*-diastereoisomers.

As shown in Scheme 1, compounds **(D)-5** and **(D)-6** were obtained following standard chemical glycosylation protocols, the key step being the coupling of the trichloroacetimidate of tetraacetylglucopyranoside to a TBDMS-protected (at the phenolic moiety) alcohol [19].

The water-soluble phenylpropanoid glucosides **(D)-5** and **(D)-6** were then submitted to the catalytic action of the laccase from *Trametes versicolor* and the corresponding main products, **8** and **9**, were isolated in 35 and 34% yield, respectively (Scheme 2. These compounds were the less polar UV-active spots in the respective TLC. A minor, slightly more polar UV-active spot was also observed in both reactions, but the corresponding products were not isolated. In addition, the formation of a continuous series of spots due to more polar UV-active and UV-inactive byproducts was observed at longer reaction times). Their mass spectra showed the expected values for dimeric products, the quasimolecular peaks ($M+Na^+$) being registered at 645.3 at 705.3 Da respectively. The *trans* β-5-type dimeric structures shown in formula **8** and **9** were then confirmed by the corresponding ^1H NMR spectra (full spectra in Supplementary materials).

In addition to the signals due to the expected seven aromatic and two olefinic protons, the ^1H NMR spectrum of **8** showed the diagnostic signal due to H-a'. Provided **8** is a mixture of diastereoisomers, the signals were split in two baseline separated doublets (relative ratio almost 1:1, Fig. 2) centered at 5.574 and 5.234 ppm (J = 6.4 Hz). Similarly, the signals due to the two anomeric protons (doublets) were split in two doublets each (resonating between 4.403 and 4.347 ppm). All the other signals appeared as multiplets, due to the duplication of the expected peaks.

Similarly, the ^1H NMR spectrum of **9** showed the presence of the five aromatic and two olefinic protons. The signal due to H-a' was again split in two baseline separated doublets centered at 5.547 and 5.503 ppm (J = 6.4 Hz, Fig. 2). However, the relative ratio of these two doublets, evaluated by signals integration, was approximately 1.5:1. Among the two anomeric protons, the first one resonated as a doublet at 4.217 ppm (J = 8.0 Hz). The second one was split in two doublets, again in a 1.5:1 ratio, the most abundant being centered at 4.263 and the second one at 4.256 ppm (J = 7.6 Hz). Both the two singlets due to the two methoxy substituents were also split, resonating respectively at 3.807 and 3.800 ppm and at 3.751 and 3.742 ppm, respectively.

Being intrigued by the observed difference in the relative ratio of the two diastereoisomers combined in **9**, it was decided to synthesize the phenylpropanoid glucoside **(L)-6**, carrying an enantiomeric sugar moiety, that is L-glucose. The previously described synthetic protocol (Scheme 1) allowed the isolation of the target product **(L)-6**, which was then submitted to the laccase-catalyzed oxidation. The main product **10** was isolated in yield similar to the previously described products (37%) and the structure was confirmed by mass spectrometry and ^1H NMR. As expected, the spectra were generally similar to those previously obtained with **9**. Specifically, the presence of two diastereomeric substituted benzodihydrofuran moieties was confirmed by the two doublets resonating at 5.554 and 5.504 ppm (J = 6.8 Hz). However, at variance to **9**, the relative ratio of the area of the two doublets of **10** was lower (1.1:1) and, moreover, the most abundant dimer was the one whose doublet resonated at higher fields (5.504 ppm, Fig. 2), whereas with **9** it was the one resonating at lower fields (5.547 ppm). A result, that was coherent with the presence of two enantiomeric sugars in the starting substrates **(D)-6** and **(L)-6**.

Fig. 2 shows the relative abundance of the signals due to H-a' in compounds **8**–**11.**

The difference of reactions outcomes, in terms of diastereomeric ratio of the dimeric products **9** and **10**, was confirmed by HPLC. Several chiral and achiral columns were tested and the best results were obtained with a Kinetex 5 μm Biphenyl 100 Å column. Fig. 3 shows the baseline separated HPLC peaks of the diastereomeric mixtures **9** and **10**. The low d.e. (18% and 6.0%, respectively) and the stereocomplementarity of the two reactions outcomes were confirmed.

As a last substrate of this investigation, a bulkier sugar moiety was linked to coniferyl alcohol. The choice was on the rutinoside derivative **7**, as the rutinosidase from *Aspergillus niger* [16] was in our hands. It has been reported that this enzyme has a strong trans-glycosylation activity and we could confirm this property. As shown

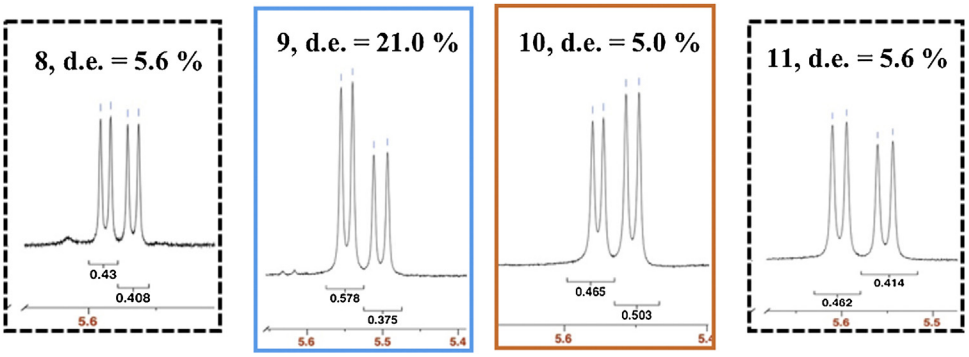
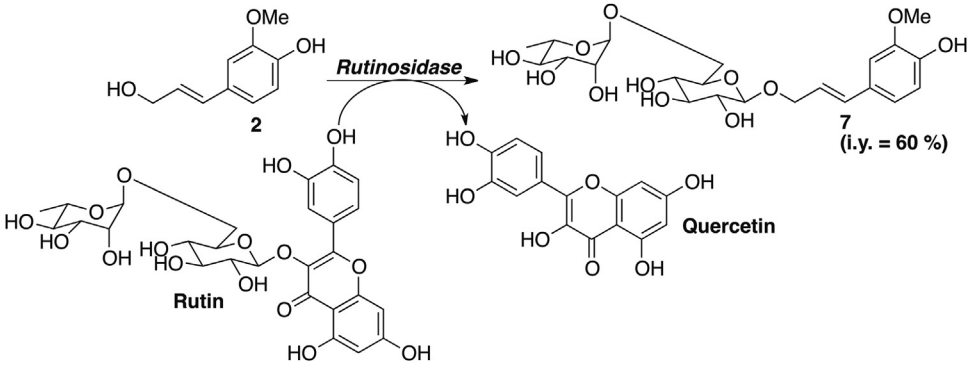


Fig. 2. Diastereomeric excesses evaluation: ^{1}H NMR spectra expansions of compounds **8**–**11**.



Scheme 3. Enzymatic transglycosylation to give the disaccharide derivative **7**, reagents and conditions: Rutinosidase from *A. niger* (0.013 U $mg_{alcohol}^{-1}$), coniferyl alcohol (0.13 M, 1 eq), rutin (1.25 eq), citrate-phosphate buffer (0.05 M, pH 5.0): dimethyl sulfoxide = 85%: 15%, 35 °C and 750 rpm. Isolated yield: 60%.

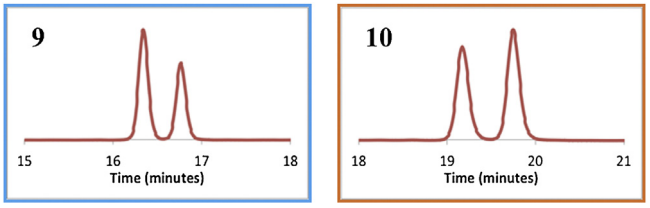


Fig. 3. Diastereomeric excesses evaluation: HPLC chromatograms of compounds **9** and **10**.

in Scheme 3, using rutine as a cheap rutinose donor, the desired product **7** was isolated in one step in 60% yield.

Laccase-catalyzed dimerization of **7** was performed following the usual protocol, and the main product **11** was isolated in 33% yield. The mass spectrum was in accordance with the structure of a dimeric product ($M+Na^+$ m/z = 997.3 Da) and the ^{1}H NMR corroborated the structure. The spectrum was quite complex, due to the presence of two disaccharide moieties and two diastereoisomers. The anomeric protons of the two glucopyranose units were doublets centered at 4.489 and 4.373 ppm. The presence of the rhamnopyranose units was confirmed, *i.a.*, by the signals due to the C-18 methyl groups resonating at 1.174 and 1.238 ppm. The diagnostic signals due to H-a' were two baseline separated doublets at 5.602 and 5.552 ppm (J = 6.4 Hz, Fig. 2) with a relative ratio of 1.16:1.

It is well-known that the solvent composition can have a deep influence on the stereoselectivity of the biocatalyzed reactions [20], and this effect was observed by us also with laccases [6c,6d]. Accordingly, the biooxidation of **7** was also performed in the presence of different water-miscible co-solvents, in v/v percentages that do not significantly affect the laccase activity [4a], but the relative ratio of the two diastereoisomers in **11** did not change significantly (Table 1).

Table 1
Laccase-catalyzed oxidation of **7** in the presence of water miscible co-solvents.[a]

	Buffer % v/v	Co-solvent % v/v	d.e. of **11** (%)
–	100	–	5.5
MeOH	70	30	4.5
CH_3CN	70	30	3.8
Acetone	70	30	8.5

[a] All the reactions were stopped after 3 h and analyzed by HPLC (Kinetex 5 μm Biphenyl 100 Å, Phenomenex).

4. Conclusions

It has been shown that coumaryl and coniferyl glycosides are suitable substrates for the laccase from *Trametes versicolor*. The presence of a carbohydrate moiety increased the water solubility of these compounds and reduced the number of dimeric products, as pinoresinol-like structures could not be formed. However, the sugar substituents had a minor effect on the stereochemical outcome of the radical coupling reactions, the best results being the 21% d.e. observed in β-5-like dimer **9** obtained by the laccase-catalyzed dimerization of **(D)-6**.

Future work will focus on the preparative scale isolation of enantiomerically enriched dimers of coumaryl and coniferyl alcohols following the protocol previously exploited with resveratrol glucoside [12].

Acknowledgments

The authors acknowledge Fondazione Cariplo (Progetto INBOX, 2015–2017) for financial support. The Czech-Italian academic col-

laborative project between CNR and AVCR (2016–2018) and the COST Action "Systems Biocatalysis" CM-1303 (MSMT LD15085) are also acknowledged for mobility support of D.M., S.R., J.K. and I.B.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molcatb.2016.10.019.

References

[1] (a) T. Senthivelan, J. Kanagaraj, R.C. Panda, Biotechnol. Bioprocess. Eng. 21 (2016) 19–38;
(b) T. Senthivelan, J. Kanagaraj, R.C. Panda, Biotechnol. Bioprocess. Eng. 21 (2016) 19–38;
(c) S.M. Jones, E.I. Solomon, Cell. Mol. Life Sci. 72 (2015) 869–883;
(d) M. Mogharabi, M.A. Faramarzi, Adv. Synth. Catal. 356 (2014) 897–927;
(e) J.-R. Jeon, Y.-S. Chang, Trends Biotechnol. 31 (2013) 335–341;
(f) C.J. Rodgers, C.F. Blanford, S.R. Giddens, P. Skamnioti, F.A. Armstrong, S.J. Gurr, Trends Biotechnol. 28 (2010) 63–72;
(g) P. Giardina, V. Faraco, C. Pezzella, A. Piscitelli, S. Vanhulle, G. Sannia, Cell. Mol. Life Sci. 67 (2010) 369–385;
(h) S. Riva, Trends Biotechnol. 4 (2006) 219–226.
[2] (a) C.J. Li, Mol. Plant 5 (2012) 304–317;
(b) E.I. Solomon, U.M. Sundaram, T.E. Machonkin, Chem. Rev. 96 (1996) 2563–2606.
[3] (a) J.R. Jeon, Y.S. Chang, Trends Biotechnol. 31 (2013) 335–341;
(b) D. Monti, D. Ottolina, G. Carrea, G.S. Riva, Chem. Rev. 84 (2011) 4111–4140;
(c) S. Witayakran, A.J. Ragauskas, Adv. Synth. Catal. 351 (2009) 1187–1209.
[4] (a) L. Baratto, A. Candido, M. Marzorati, F. Sagui, S. Riva, B. Danieli, J. Mol. Catal. B: Enzym. 39 (2006) 3–8;
(b) M. Marzorati, B. Danieli, D. Haltrich, S. Riva, Green Chem. 7 (2005) 310–315.
[5] (a) F. Sagui, C. Chirivì, G. Fontana, S. Nicotra, D. Passarella, S. Riva, B. Danieli, Tetrahedron 65 (2009) 312–317;
(b) C. Chirivì, G. Fontana, D. Monti, G. Ottolina, S. Riva, B. Danieli, Chem.: A Eur. J. 18 (2012) 10355–10361.
[6] (a) C. Navarra, C. Goodwin, S. Burton, B. Danieli, S. Riva, J. Mol. Catal. B: Enzym. 65 (2010) 52–57;
(b) S. Ncanana, L. Baratto, L. Roncaglia, S. Riva, S.G. Burton, Adv. Synth. Catal. 349 (2007) 1507–1513;
(c) A. Intra, S. Nicotra, S. Riva, B. Danieli, Adv. Synth. Catal. 347 (2005) 973–977;
(d) S. Nicotra, A. Intra, G. Ottolina, S. Riva, B. Danieli, Tetrahedron-Asymmetry 15 (2004) 2927–2931.
[7] (a) C. Ponzoni, E. Beneventi, M.R. Cramarossa, S. Raimondi, G. Trevisi, U.M. Pagnoni, S. Riva, L. Forti, Adv. Synth. Catal. 349 (2007) 1497–1506;
(b) S. Nicotra, M.R. Cramarossa, A. Mucci, U.M. Pagnoni, S. Riva, L. Forti, Tetrahedron 60 (2004) 595–600.
[8] L.B. Davin, H.-B. Wang, A.L. Crowell, D.L. Bedgar, D.M. Martin, S. Sarkanen, N.G. Lewis, Science 275 (1997) 362–367.
[9] B. Pickel, M.A. Constantin, J. Pfannstiel, J. Conrad, U. Beifuss, A. Schaller, Angew. Chem. Int. Ed. 49 (2010) 202–204.
[10] E. Ricklefs, M. Girhard, V.B. Urlacher, Microb. Cell Fact. 15 (2016) 78–89.
[11] (a) C. Navarra, P. Gavezzotti, D. Monti, W. Panzeri, S. Riva, J. Mol. Catal. B: Enzym. 84 (2012) 115–120;
(b) P. Gavezzotti, C. Navarra, S. Caufin, B. Danieli, P. Magrone, D. Monti, S. Riva, Adv. Synth. Catal. 4 (2011) 2421–2430.
[12] P. Gavezzotti, F. Bertacchi, G. Fronza, V. Kren, D. Monti, S. Riva, Adv. Synth. Catal. 357 (2015) 1831–1839.
[13] V.A. Kurkin, Adv. Biol. Chem. 3 (2013) 26–28.
[14] A. Panossian, R. Hamm, G. Wikman, T. Efferth, Phytomedicine 21 (2014) 1325–1348.
[15] (a) V.A. Kurkin, A.V. Dubishchev, V.N. Ezhkov, I.N. Titova, E.V. Avdeeva, Pharm. Chem. J. 40 (2006) 614–619;
(b) Y. Matsubara, T. Yusa, A. Sawabe, Y. Iizuka, K. Okamoto, Agric. Biol. Chem. 55 (1991) 647–650;
(c) A. Lòpez-Munguìa, Y. Hernàndez-Romero, J. Pedraza-Chaverri, A. Miranda-Molina, I. Regla, A. Martìnez, E. Castillo, PLoS One 6 (2011) 11–18.
[16] D. Šimčíková, M. Kotik, L. Weignerová, P. Halada, H. Pelantová, K. Adamcová, V. Křen, Adv. Synth. Catal. 357 (2015) 107–111.
[17] S.L. Adamski-Werner, K.S.B. Yeung, A.L. Miller-Deist, P.A. Petillo, Carbohydr. Res. 339 (2004) 1255–1262.
[18] T. Shiba, L. Xhiao, T. Miyakoshi, C.-L. Chen, J. Mol. Catal. B: Enzym. 10 (2000) 605–615.
[19] H. Akita, E. Kawahara, M. Kishida, K. Kato, J. Mol. Catal. B: Enzym. 40 (2006) 8–15.
[20] (a) G. Carrea, G. Ottolina, S. Riva, Trends Biotechnol. 13 (1995) 63–70;
(b) P.A. Fitzpatrick, A.M. Klibanov, J. Am. Chem. Soc. 113 (1991) 3166–3171.