Optimization and Green Metrics Analysis of the AgOAc-Mediated Dimerization of Piceid: Toward a High-Yielding and More Sustainable Access to δ-Viniferin and Synthesis of New Piceid Dimers

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Optimization and green metrics-analysis of the AgOAc-mediated dimerization of piceid: toward a high-yielding and more sustainable access to δ-viniferin and synthesis of new piceid dimers

Julien VINET[1], Amandine L. FLOURAT[1], Cédric PEYROT[1], Fanny BRUNOIS[1], Fanny BRUNISSEN[1], Florent ALLAIS[1*]

[1]URD Agro-Biotechnologies Industrielles (ABI), CEBB, AgroParisTech, 51110, Pomacle, France

* Correspondence: florent.allais@agroparistech.fr; Tel: +33 (0) 3 52 62 04 62

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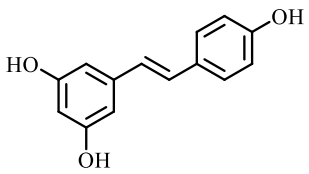
ABSTRACT

Stilbenes are particularly studied for their biological properties. Among them, resveratrol and piceid, present in relatively large quantities in Nature, have already demonstrated interesting characteristics. Dimers and oligomers of resveratrol are also very promising, especially, δ-viniferin for which an elegant synthetic method based on the hydrolysis of piceid dimer has been reported. Nevertheless, while the hydrolysis is quantitative, to date, the synthesis of the piceid dimer has only been described in presence of laccase in toxic methanol and with a relatively average yield (46%). With a view to offering a greener and higher yielding dimerization of piceid, this study aims at (1) conducting the dimerization in presence of AgOAc in ethanol, (2) determining the

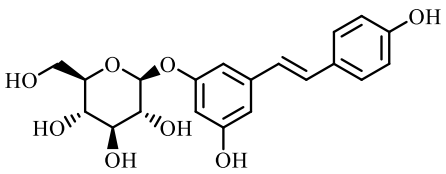
influence of reaction parameters and the optimal conditions by using a Design of Experiment (DoE), (3) assessing the EcoScale and Process Mass Index (PMI) of the new procedure, and (4) comparing it with the laccase-mediated procedure. Data demonstrated that the AgOAc-mediated dimerization of piceid proceeds in higher yield (ca. 64% vs 46%), with a better EcoScale (68 vs 32), while being more economical (PMI score = 2.5 vs 71.4) and using a green solvent (EtOH). Moreover, through this novel route, we were able to identify and fully characterize new dimers that were not reported in the literature so far.

INTRODUCTION

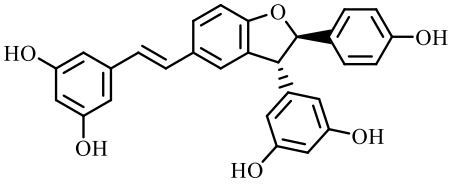
Stilbenes are a group of polyphenols produced in several plants and especially in grapevine[1-5] that have received great attention and were particularly studied for their biological properties.[6-9] Resveratrol (**1**, Figure 1), the major stilbene in wine,[10] can be obtained in relatively significant amount (a few milligrams per liter) from other natural sources such as Japanese knotweed (*Polygonum cuspidatum*).[11] *In planta*, resveratrol is commonly present under its corresponding 3-*O*-glucoside, called piceid or polydatin (3,5,4'-trihydroxystilbene-3-*O*-β-D-glucopyranoside) (**2**, Figure 1).[12,13] It was demonstrated that piceid can be hydrolyzed by some β–glucosidases during fermentation to release neat resveratrol.[14] Not only different studies have shown that piceid possesses pharmacological activities similar to those of resveratrol, but also the glucose moiety provides a better stability toward light and heat.[15,16] Piceid thus can be considered as a pro-drug of resveratrol and there are literature reports that describe the deglucosylation of piceid to resveratrol by the action of suitable β-glucosidases.[17,18]



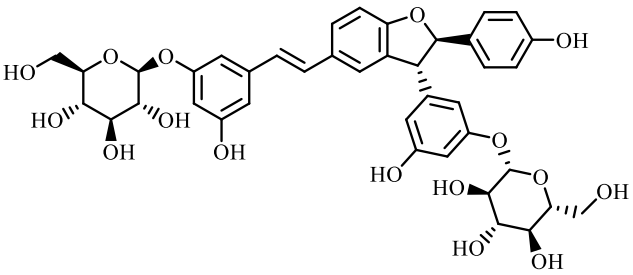
Resveratrol (1)



Piceid (2)



δ-viniferin (3)



Diglycosylated δ-viniferin (4)

Figure 1. Resveratrol, piceid and corresponding dimers synthesized in the present work.

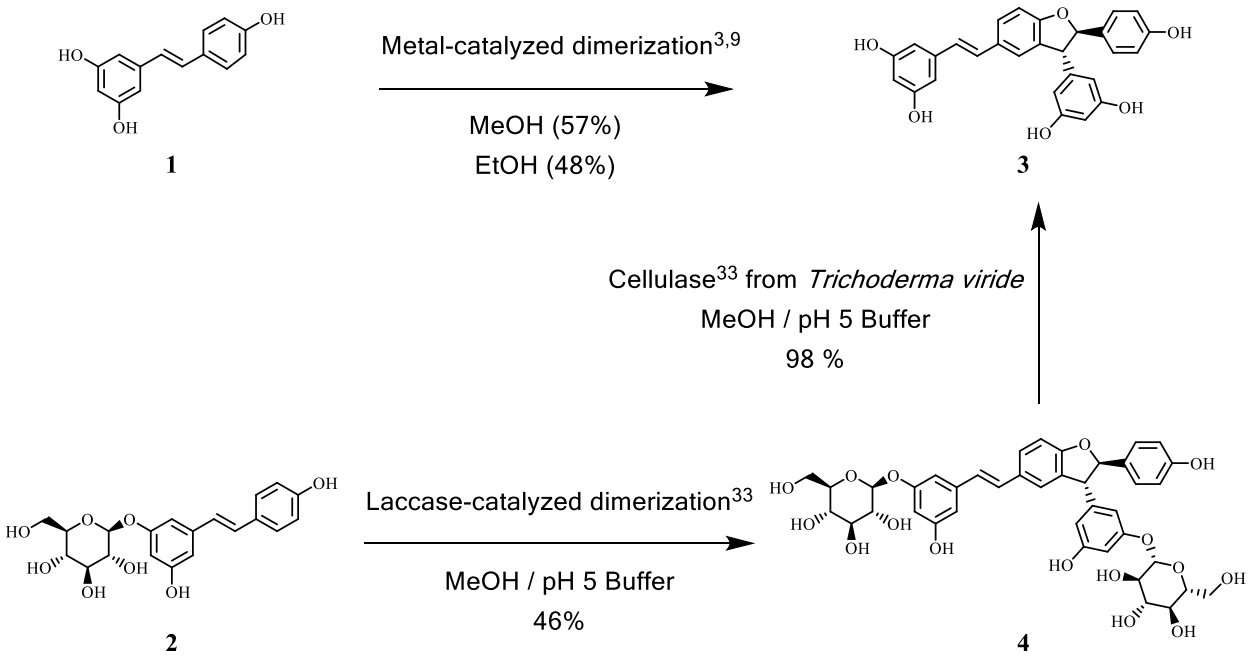
Some of the major properties of these compounds make it possible to envisage targeted applications. Firstly, the antimicrobial activity of resveratrol reduces biofilm formation in certain species such as *S. aureus* and *E. coli*.[19,20] These properties have inspired studies aimed at using resveratrol in combination with antibiotics for a better response to treatment, especially in the current context of increasing resistant bacteria.[21] Secondly, these compounds are known for their antioxidant activities and allow their use as an active ingredient in anti-ageing cosmetic products.[22] This characteristic is also attributed to the possibility of limiting cardiovascular problems in humans.[23] Finally, studies attest to the protective action of certain stilbenes against cancer in the three phases of carcinogenesis, namely tumor initiation, promotion and progression.[24]

Dimers and oligomers of resveratrol also possess these valuable biological properties and are often even more potent. Indeed, studies have shown that oxidative coupling during wine ageing could modulate the biological properties of wine by inducing the formation of more active

compounds such as δ-viniferin (**3**, Figure 1).[9,25] Although these compounds are present in grapevine by-products,[26] their relatively low concentrations make them difficult and expensive to source if they are to be used on a large scale, e.g. for crop treatment.

Some (bio)chemical oxidative coupling reactions have been studied to obtain δ-viniferin (**3**). First, a laccase-catalyzed or peroxidase-catalyzed dimerization of resveratrol allows the obtention of the desired compound. According to a review by Jeandet et al.,[27] a maximum yield of 31% of δ-viniferin is achievable. One study stands out, involving an HRP and claiming a 89% HPLC yield.[28] However, the latter being calculated from the peak area, and assuming that different dimers may co-elute, this procedure will be the subject of a preliminary study thereafter. Finally, it can be noted that one specific independent study achieved up to 60% yield using germinated peanut embryo powder (GPEP) as a novel source of peroxidase.[29]

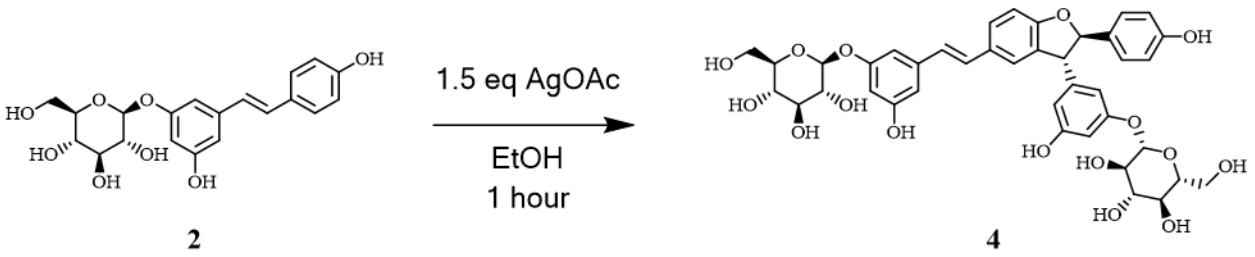
Another strategy to access δ-viniferin (**3**) - and other resveratrol dimers – consists in the dimerization of resveratrol (**1**) with metal salts such as silver, iron or copper in different solvent systems, resulting in an increase yields while reducing reaction time (Scheme 1).[30,31] El Khawand et al. showed that it was possible to dimerize resveratrol in methanol and ethanol in the presence of metal salts.[3,9] Such reaction can be explained by a single electron transfer from resveratrol to the metal cation, followed by regioselective coupling and intramolecular cyclisation. Four major dimers were identified - quadrangularin B, δ-viniferin, oxistilbenin F, and oxistilbenin G - and in-depth mechanistic experiments highlighted the effect of certain parameters on the reaction yields and dimers selectivity. It is worth mentioning that, in all cases, δ-viniferin and quadrangularin B were the major product and the minor one, respectively. Focusing on δ-viniferin, the best yield (48%) was obtained in the presence of silver acetate.



Scheme 1. Different synthetic routes to δ-viniferin (**3**).

Finally, it is also possible to obtain δ-viniferin through the piceid dimerization. First, laccase-mediated enzymatic couplings have been carried out on piceid (**2**)[32,33] (Scheme 1), followed by the complete hydrolysis of the resulting piceid dimer (**4**) (diglycosylated δ-viniferin, Figure 1). Indeed, it has been demonstrated that the cellulase from *Trichoderma viride* was able to provide quantitatively δ-viniferin through the hydrolysis of the piceid dimer obtained (98%).[33] Although elegant, this strategy suffers from some limitations such as the use of hazardous methanol and a relatively low yield of the dimerization step (46%).

This yield being similar to that of the reported aforementioned laccase-mediated dimerization, we decided to implement and optimize - through a Design of Experiment (DoE) - the AgOAc-mediated dimerization in ethanol on piceid (**2**) as a more sustainable and higher yielding synthetic procedure of diglycosylated δ-viniferin (**4**) (Scheme 2). To assess the advantages of this new procedure over the one involving laccase, EcoScale and Process Mass Index (PMI) have been calculated for both procedures.



2 4

Scheme 2. New synthetic pathway to obtain piceid dimer **4**.

EXPERIMENTAL SECTION

Analytical standards and reagents. All reagents were of analytical grade and used as received without further purification. Ethanol (Reagent grade), acetonitrile (HPLC and LC-MS grades, purity ≥ 99.9%), formic acid (98%), polydatin (piceid) (95%) and AgOAc (99%) were purchased from Sigma-Aldrich or TCI.

Oxidative coupling of piceid in ethanol. Based on the protocol described by El Khawand[7] for the dimerization of resveratrol, oxidative coupling of piceid was conducted on different quantities of piceid (1 eq) using silver acetate (1.5 eq) in pure ethanol ([piceid] from 22 to 66 mmol/L). The reaction mixture was stirred at different temperatures (between 20 and 60 °C) for one hour (see Table S1 for specifications about quantities and temperatures). The reaction was then stopped by cooling at 4 °C. The mixture was filtered to remove metal residues. The yield of piceid dimer was determined by HPLC.

HPLC Analysis. HPLC analyses were performed on a Dionex Ultimate 3000 (Dionex Corporation, USA) system equipped with a DAD 3000 diode array detector. Chromatograms were recorded and processed with Chromeleon 6.8 Software. The components were separated by an Accucore C18 AQ (2.6 µm, 3 x 100 mm; Thermo Scientific). Elution was performed using a mobile phase composed of pure water (solvent A), acetonitrile (solvent B), 0.1% formic acid in

water (solvent C) according to the following gradient: the proportion of C remains constant throughout the elution (30%). 0-3 min from 20% to 35% B, 3-4 min from 35% to 60% B, 4-5 min remains constant at 60% B, 5-5.5 min from 60% to 20% B, 5.5-6.5 min remains constant at 20% B The flow rate was 0.8 mL/min. The sample injection volume was 1 µL. The UV acquisition was carried out at 320 nm, 210 nm, 285 nm and 254 nm.

NMR Analysis. ^{1}H NMR spectra were obtained on an Avance III 600 with a TCI cryoprobe (600 MHz) and calibrated with methanol-d_4, with proton signals of water at δ = 4.87 ppm or DMSO-d_6, with the residual solvent peak at δ = 2.50 ppm. Data are reported as follows: chemical shift (δ ppm), integration, multiplicity (s = singlet, d = doublet, dd = doublet of doublets, m = multiplet), coupling constant (Hz), and assignment. ^{13}C NMR spectra were recorded on an Avance III 600 with a TCI cryoprobe (151 MHz) and were calibrated with methanol-d_4, residual solvent peak at δ = 48.6 ppm, or DMSO-d_6, residual solvent peak at δ = 39.5 ppm. Data are reported as follows: chemical shift (δ ppm) and attribution. NMR spectra assignments were achieved using COSY, HMBC, and HSQC spectra.

HRMS Analysis. High-resolution mass spectrometry was performed on an Agilent 1290 system, equipped with a 6545 Q-TOF mass spectrometer (Wilmington, DE, USA) and a PDA UV detector. The source was equipped with a JetStream ESI probe operating at atmospheric pressure.

Antiradical Activities. The determination of the radical scavenging activity of our molecules was determined via 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. These tests involved adding potential antiradical molecule solution in ethanol at different concentrations to homogeneous DPPH solution. The study was performed under stirring for 7 h 25 min on the following concentration scale: 400, 200, 100, 50, 25, and 12.5 μM. Every 5 min, the absorbance was measured at 520 nm. At the end, the percentage curves of %DPPH (blue) and %reduced DPPH

(green) were plotted in Regressi® software using an average of the last six points. The amount needed to reduce the initial number of DPPH free radicals by half, i.e., EC_{50}, was provided by the crossing point of %DPPH (blue) and %reduced DPPH (green) (Figure S12-S18).

Data for Diglucosylated δ-viniferin (4).

^{1}H NMR (600 MHz, 25 °C, CD_3OD): δ (ppm) = 7.39 (1H, d, *J* = 8.4 Hz, H-16), 7.20 (1H, d, *J* = 24.7 Hz, H-12), 7.17 (2H, m, H-40 and H-44), 7.03 (1H, d, *J* = 16.1 Hz, H-10), 6.86 (2H, dd, *J* = 8.3 Hz, H-41 and H-43), 6.79-6.76 (3H, m, H-6, H-7 and H-15), 6.6 (1H, m, H-4), 6.49 (1H, m, H-31), 6.42 (2H, d, *J* = 17.11 Hz, H-30 and H-32), 6.31 (1H, d, *J* = 20.9 Hz, H-2), 5.41 (1H, d, *J* = 8.3 Hz, H-38), 4.79 (1H, d, *J* = 6.9 Hz, H-49), 4.47 (1H, d, *J* = 9.4 Hz, H-35), 3.91 (1H, d, *J* = 12.1 Hz, H-21), 3.80 (1H, dd, *J* = 19.2 Hz and 12.0 Hz, H-52), 3.69 (2H, m, H-24)

^{13}C NMR (600 MHz, 25 °C, CD_3OD): δ (ppm) = 161.2 (C-14), 160.6 (C-29), 160.5 (C-31), 160.2 (C-1), 159.8 (C-3), 159.0 (C-42), 145.6 (C-33), 141.4 (C-5), 132.83 (C-11), 132.8 (C-39), 132.4 (C-13), 130.0 (C-16), 129.1 (C-10), 128.9 (C-40 and C-44), 127.4 (C-7), 124.3 (C-12), 116.5 (C-41 and C-43), 110.6 (C-15), 109.1 (C-34), 108.8 (C-32), 108.5 (C-4), 107.2 (C-6), 104.4 (C-30), 103.8 (C-2), 102.5 (C-49), 101.9 (C-21), 94.9 (C-38), 78.3 (C-51), 78.1 (C-23), 75.1 (C-47), 75.9 (C-19), 71.6 (C-48), 71.3 (C-20), 71.1 (C-46), 62.7 (C-18), 62.4 (C-52), 62.3 (C-24), 58.8 (C-35)

TOF MS ES+: $[M+H]^+$ for $C_{40}H_{43}O_{16}$: *m/z* 779.2546; found: *m/z* 779.2551

Data for piceid dimer (5), mixture of piceid diastereoisomers.

^{1}H NMR (600 MHz, 25 °C, DMSO-*d*$_6$): δ (ppm) = 9.32 (3H, s, H-8, H- 36 and H-45), 7.36 (2H, m, H-12 and H-16), 7.00 (2H, m, H-40 and H-44), 6.87 (2H, m, H-13 and H-15), 7.18 (1H, m, H-10), 6.76 (1H, m, H-32), 6.62 (1H, m, H-7), 6.71 (2H, m, H41 and H-43), 6.54 (1H, m, H-

34), 6.47 (1H, m, H-6), 6.33 (1H, m, H-4), 6.30 (1H, m, H-2), 6.21 (1H, m, H-30), 5.25 (2H, m, H-21 and H-38), 5.03 (4H, m, H-26, H-34, H-49 and H-54), 4.79 (1H, m, H-35), 4.67-4.42 (4H, m, H-27, H-28, H-55, H-56), 1.05-0.96 (2.1 H, m, H-59 (5a) and 0.9 H, m, H-59 (5b)).

^{13}C NMR (600 MHz, 25 °C, DMSO-*d*$_6$): δ (ppm) = 158.9 (C-1), 158.4 (C-29), 157.9 (C-3), 157.7 (C-31), 156.8 (C-14), 156.7 (C-42), 140.3 (C-33), 139.2 (C-5), 129.6 (C-39), 129.3 (C-11), 129.0 (C-12 and C-16), 128.1 (C-10), 126.6 (C-40 and C-44), 126.3 (C-7), 116.0 (C13 and C-15), 114.7 (C-41 and C-43), 109.1 (C-34), 107.3 (C-32), 106.5 (C-4), 104.8 (C-6), 102.9 (C-30), 102.5 (C-2), 100.7 (C-49), 100.3 (C-21), 84.0 (C-35), 82.5 (C-38), 77.1 (C-51), 77.0 (C-23), 76.7 (C-47), 76.6 (C-19), 73.3 (C-48), 73.2 (C-20), 69.8 (C-46), 69.5 (C-18), 63.8 (C-58), 60.7 (C-52), 60.4 (C-24), 15.3 (C-59).

TOF MS ES+: $[M+H]^+$ for $C_{42}H_{49}O_{17}$: *m/z* 825.2964; found: *m/z* 825.2962.

RESULTS AND DISCUSSIONS

First, in order not to waste time optimizing the envisaged two-step synthetic route, we first decided to faithfully reproduce the study by Li et al.[28] who reported the high yielding synthesis of δ-viniferin from resveratrol using HRP peroxidases in basic medium (pH 8). Unfortunately, as evidenced by the ^{1}H NMR spectrum (Figure 2) of the crude mixture obtained after reacting HRP with resveratrol, the claimed yield of 89% was not achieved. Indeed, based on the signal at 5.45 ppm, representative of the specific H_a proton of δ-viniferin, an 89% yield should lead to only 15 protons that present a signal between 6 and 7.5 ppm whereas we observe more than 30 protons, demonstrating the presence of at least one other compound. The HPLC chromatogram (Figure 3) supports this observation. These results demonstrate that the published procedure is not reproducible as is, and thus the need for a reliable synthetic procedure, encouraging us to continue our study.

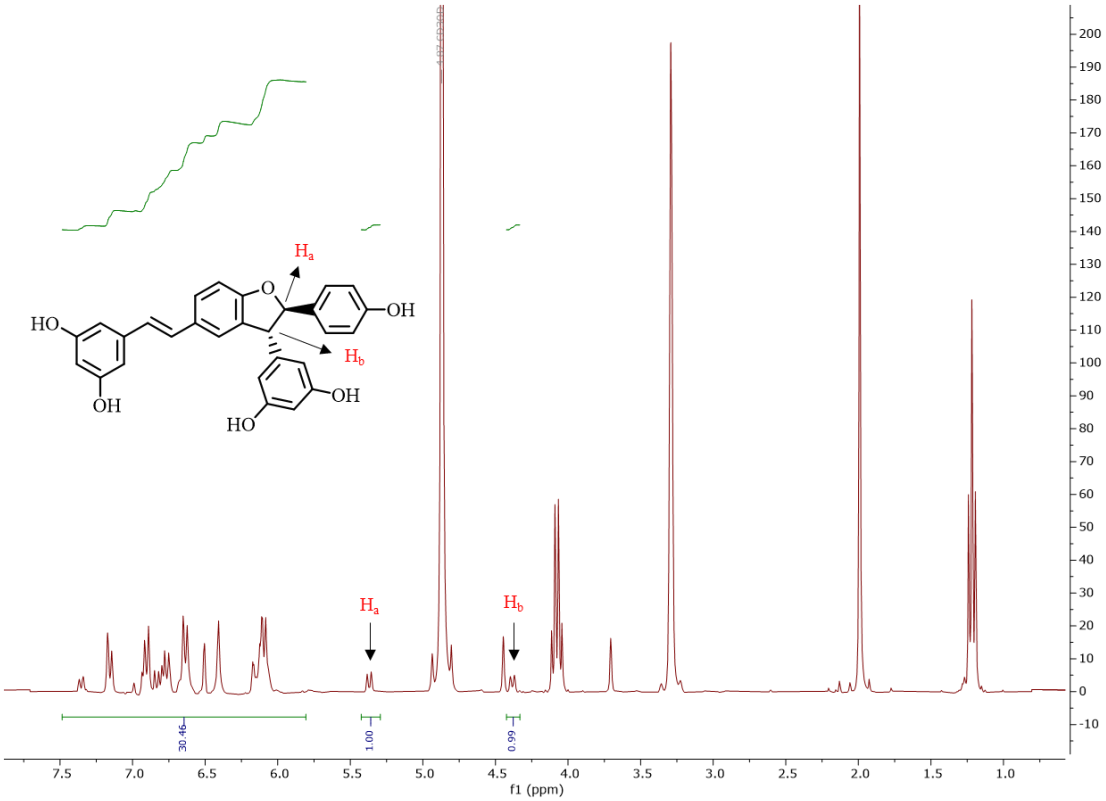


Figure 2. [1]H NMR spectrum of the reaction mixture obtained using Li et al.[28] HRP-mediated procedure.

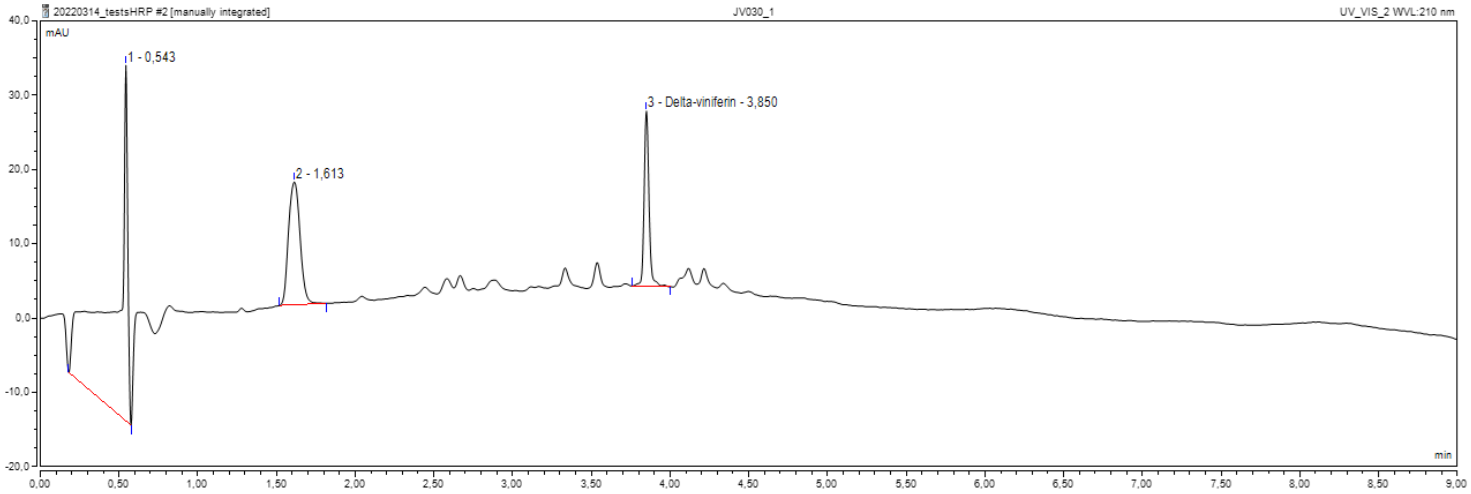


Figure 3. HPLC chromatogram of the reaction mixture obtained using Li et al.[28] HRP-mediated procedure.

In view of the previous studies,[8,30,31] where it was shown that the reaction time had no influence on the yields and the best conversions in piceid were obtained with 1.5 equivalent in metal salts, time and stoichiometry were fixed at 1 hour and 1.5 equivalent, respectively. The

temperature (T) and the piceid (**2**) concentration (C) were the two parameters to be studied. In order to determine the impact of the latter on the yield of product **4**, a DoE based on a D-optimal design consisting of 10 experiments, including a triplicate at the central point to evaluate the reproducibility, was performed. Parameters and their variations are reported in Table 1. The studied temperatures are restricted according to classical limits (room temperature and temperature close to boiling point of ethanol). The low concentration limit is established according to a study already conducted by Gavezzotti.[33] The upper limit was set to limit the addition of AgOAc. The reaction defined by the experimental design and the responses obtained (yield) are presented in Table S2 (Experimental data are in the Table S1). Product **4** yield was determined by HPLC using external calibration ($R^2 = 0.998$) (Figure S1).

Table 1. Parameters and their variation corresponding to defined levels of the DoE.

	Levels		
Variables	**-1**	**0**	**1**
Temperature (°C)	20	40	60
Concentration of piceid (mmol/L)	22	44	66

The relationship between these variables and the response was given by a second-order polynomial equation (eq 1) where Y represents the yield of **4**, a_0 is a constant, x_i and x_j are the variables and a_i, a_{ii}, and a_{ij} are the linear, quadratic, and interaction coefficients, respectively.

$$Y = a_0 + \sum_i a_i x_i + \sum_i a_{ii} x_i^2 + \sum_{ij} a_{ij} x_{ij} \quad (1)$$

After computational treatment to fit the raw results to the second-order polynomial equation, variance (ANOVA) was used for validation of the model with the analysis of R^2, Q^2, and lack of fit (LOF) test. R^2 indicates how well the model fits with the experimental data, Q^2 gives an

estimate on the precision for future predictions and LOF shows whether the model error can be compared to the replicates errors.

D-Optimal design led to a very good fit and prediction of the model with a coefficient of determination $R^2 = 0.966$ (>0.6) and a coefficient of cross-validation $Q^2 = 0.911$ (>0.5). The lack of fit ($p > 0.05$) shows the low replicate errors of the model. Finally, analysis of variance (ANOVA) shows an acceptable correlation between the response (yield of **4**) and the variables with a p-value below 0.05, which confirm the statistical significance of the polynomial regression. Coefficients of the models (a_i, a_{ii}, and a_{ij}) allowed the determination of the influence of the linear parameter, their square terms, and their quadratic effects (Figure 4). A positive value means a positive influence on the formation of our product while a negative value means a negative influence. With a p-value > 0.05, we can see that all the terms related to the piceid concentration are not significant and therefore this parameter has no influence on the final yield (Table S3).

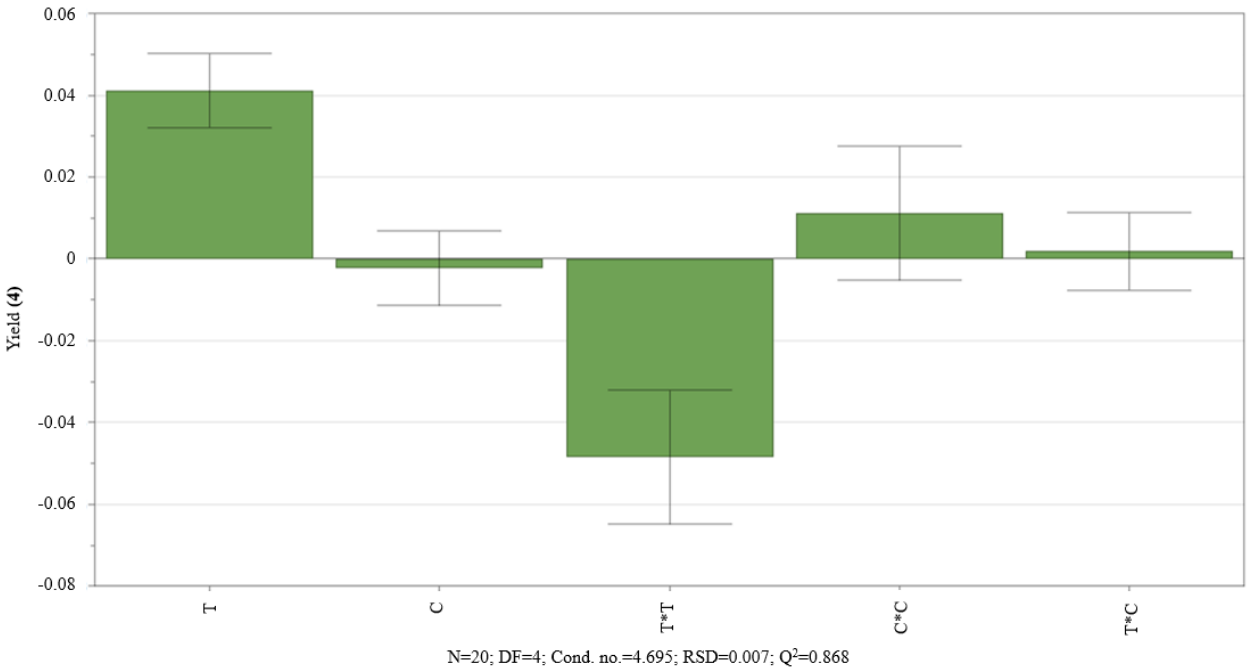


Figure 4. Regression coefficient of quadratic model.

For the temperature, the independent variables have a positive influence, whereas the square terms have a negative one. A higher temperature than at the level 0 (40 °C) allows a better yield to be obtained, an effect already observed in some studies about dimerization of resveratrol.[3,9,29] However, raising the temperature too much will no longer be beneficial to the formation of our product. Equation of the model (eq 2) was determined by integrating the significant coefficients from eq 1.

$$\mathrm{Log}_{10}(Y) = 1.789 + 0.125 * \frac{T-40}{20} - 0.133 * \left(\frac{T-40}{20}\right)^2 \quad (2)$$

The visualization of the influence of the temperature can be obtained via multiple linear regression (MLR) in Figure 5. The software gave us a prediction as the best conditions to obtain the highest yield of **4** (Table 2). According to the optimal conditions obtained through the DoE, and with the objective of consuming as little solvent as possible (green chemistry), a set of condition was studied in triplicate and the yield of **4** was determined (Table S4). This set of condition led to an average yield of 63.4, closed to the 63.7 predicted by the software. It can therefore be stated that we have determined the optimal conditions for obtaining piceid dimer **4** in higher yield. Subsequently, to compare the efficiency of the optimized AgOAc-mediated dimerization with the laccase-mediated one[33], the reaction was conducted at a larger scale (350 mg of **2**) and we demonstrated that a simple filtration of the reaction medium on Celite allowed to remove most of the metal salts and provided a crude mixture that, upon hydrolysis in presence of cellulase from *Trichoderma viride*, will provide δ-viniferin **3**. A purification of the aforementioned crude mixture over on a C18-reversed phase silica gel column provided 220 mg of piceid dimer **4**, which corresponds to a yield of 63% (similar to the 63.7% yield determined by HPLC), superior to the 46% isolated yield reported for the laccase-mediated procedure[33].

Table 2. Theorical optimal conditions according to the DoE software.

Entry	T (°C)	Time (h)	Yield (4) (%)
1	56	1	63.7

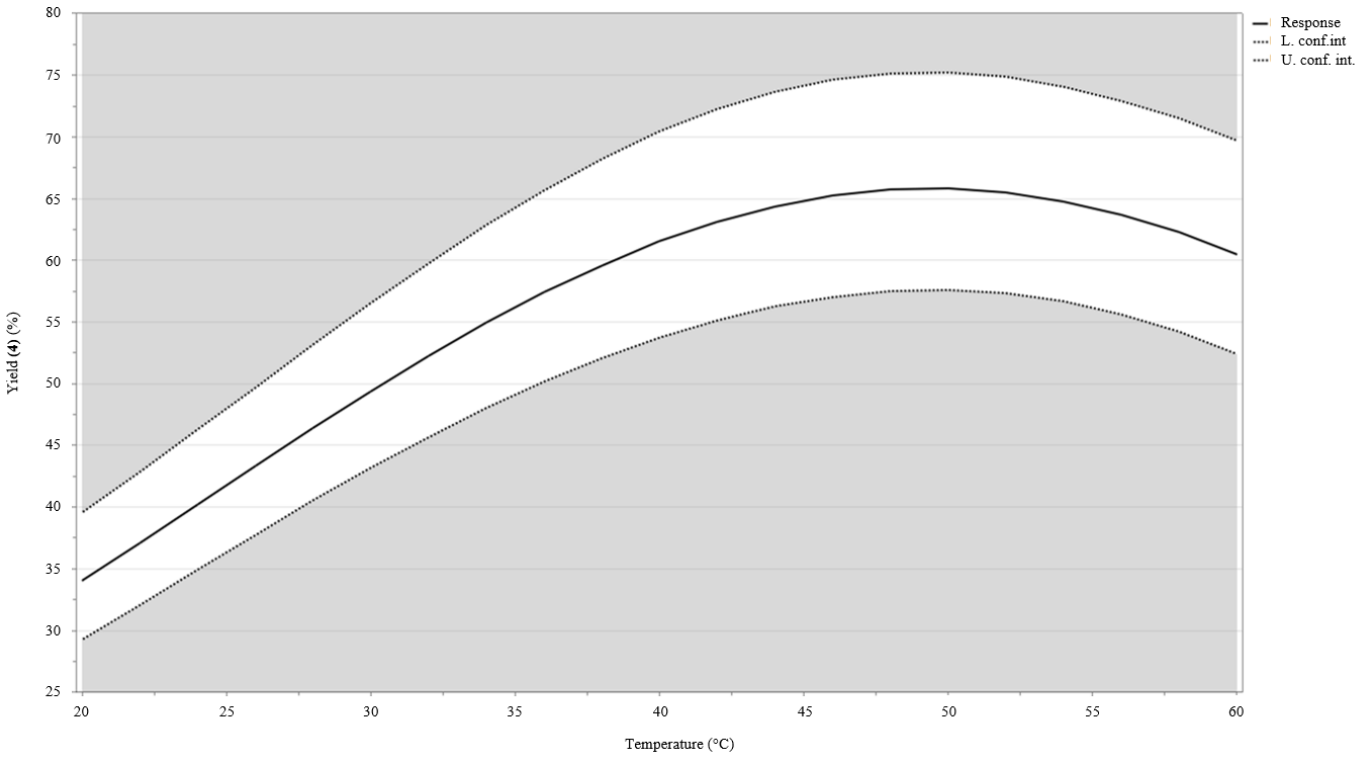


Figure 5. Yield (**4**) = f(T).

When comparing our conditions to those of the laccase-mediated procedure (named "original method" hereafter) (Table 3), a number of advantages of our method were established. First of all, hazardous MeOH was replaced by EtOH, which is less toxic. Secondly, as no water is needed in the AgOAc-mediated dimerization, ethanol can readily be recovered by distillation. Moreover, working at a 3.5-fold concentration and the fact that there is no need of an extraction step reduce significantly solvent consumption. Finally, the reaction time is also divided by 3 while the yield is 40% higher.

Table 3. Optimization of the synthesis of **4**.

	Original conditions[a]	**Optimized conditions[b]**
Solvent	MeOH/water	EtOH
Piceid concentration (g/L)	7.15	25.77
Time (h)	3.5	1
T (°C)	25	56
Yield (**4**) (%)	45.7	63.7

[a] According to Gavezzotti et al. conditions.[33] [b] Optimal set of conditions obtained through the DoE.

To determine more precisely the improvement brought by the optimized procedure in terms of atom economy, the process mass intensity (PMI)[34] has been calculated for our optimized conditions and for the original method (Table S5). In this calculation, according to its definition, all of the matter, except water, involved in the reaction - such as reagents, solvents, treatment solution - is considered. The sum of these masses necessary to produce 10 mmol of **4** is divided by the mass of 10 mmol of **4** (i.e., 7.788 g). The lower the PMI, the higher the atom economy. As the solvent of the reaction (i.e., EtOH), could be eventually recovered and recycled, the PMI calculation has been carried out considering both the reagents and the solvent, and then the reagents only. For the two synthetic methods, the product being purified using the same process (i.e., silica gel chromatography) this was not considered in PMI calculations. Calculated data show that the optimized synthetic procedure gets the best PMI scores (50.2 vs. 71.4). Moreover, if one considers the recyclability of the EtOH, the atom economy is even higher (2.6 vs. 71.4). In addition, the extraction steps in the original method (using AcOEt) should also be considered. This makes the optimized method even more economical.

In order to evaluate the greenness of the optimized procedure, it is also possible to use the EcoScale, a tool allowing to benchmark and rank the procedures.[35] With a value ranging from 0 to 100 (with 0 representing a totally failed reaction (0% yield) and 100 representing the ideal

reaction), this tool highlights excellent reaction conditions (EcoScale > 75), acceptable conditions (> 50) or inadequate (< 50). This tool considers all the steps of a chemical reaction (technical set up, temperature, time, workup and purification), and all data concerning the reagents used (quantity, price, safety). The results obtained for the original method[28] (EcoScale = 38) and for the conditions we have defined (EcoScale = 62) reveal that the conditions of the enzymatic reaction are considered inadequate whereas the new conditions are very acceptable (Table 4).

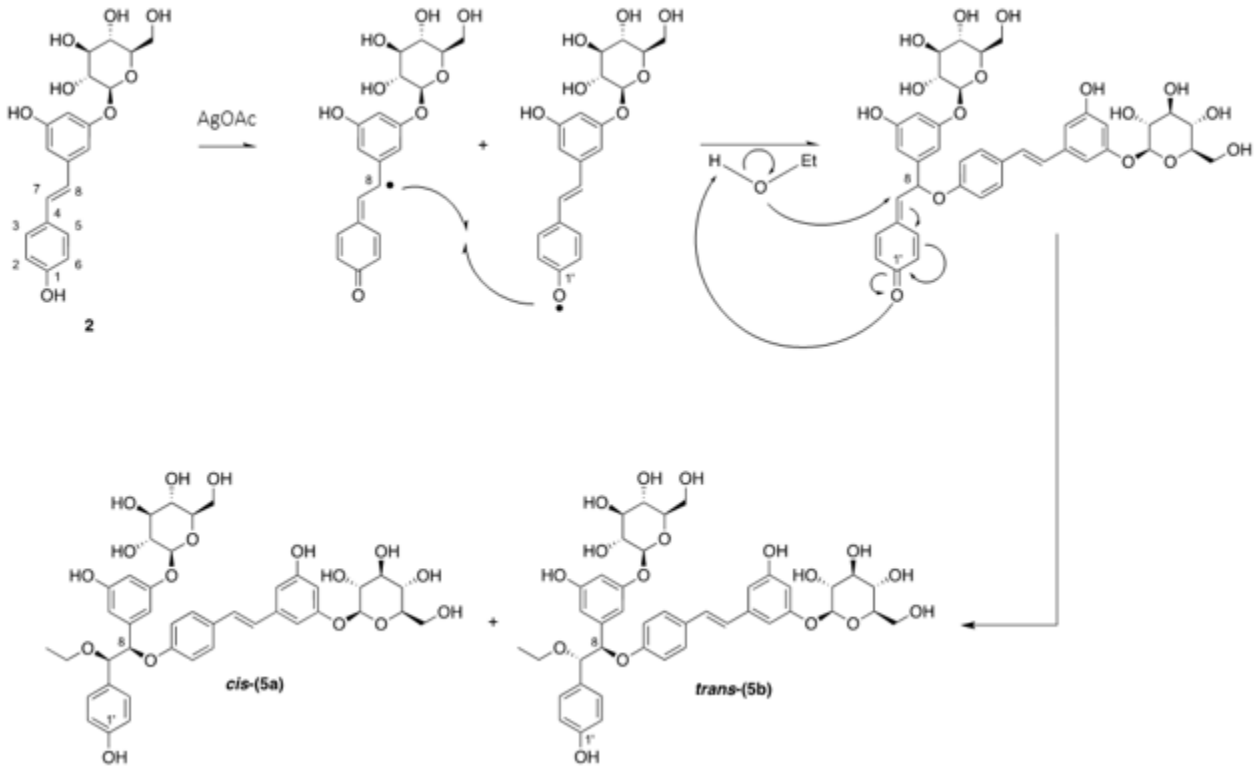
Table 4. EcoScale score calculation.

	Original conditions[a]	**Optimized conditions[b]**
1. Yield	27	18
2. Price of reaction components (to obtain 10 mmol of end product)	8	8
3. Safety	20	10
4. Technical setup	0	0
5. Temperature/time	1	2
6. Workup and purification	16	10
Total (1-6)	62	38
ECOSCALE	**38**	**62**

[a] According to Gavezzotti et al. conditions.[33] [b] Optimal set of conditions obtained through the DoE.

In the course of our study, a new piceid dimer (**5**) that, to the best of our knowledge has never been reported to date, has been identified in the reaction mixture. Piceid dimer (**5**) possesses an ethoxy group at the 7-position, a structural feature similar to that of oxistilbenin F and G, two resveratrol dimers that have been reported in previous studies due to the impact of the solvent (i.e., EtOH) on the products distribution during resveratrol dimerization.[9,31] The formation of piceid dimer (**5**) relies on the mechanism depicted in Scheme 3.

Upon oxidation, the radical generated on 1-position of piceid (**4**) gets delocalized to 8-position and the latter dimerizes with another phenoxy radical (1'-position) through a radical-radical coupling to form the intermediary methylene quinone. Finally, the latter undergoes the nucleophilic addition of EtOH on 7-position, thus allowing the re-aromatization of the benzene ring and the obtention of piceid dimer (**5**). Similarly, to what was observed for oxistilbenin F and G, the addition of the EtOH provides a mixture of two diastereomers (*cis*-**5a** and *trans*-**5b**). By analogy with the non-glycosylated forms,[9] the CH_3 signal from the ethyl group can be used to differentiate between the two dimers (1.05 ppm for **5a** and 0.96 ppm for **5b,** Figure S4) and to determine the ratio of the two forms in the mixture (Table 5).



Scheme 3. Reaction mechanism involved in the formation of piceid dimers *cis*-**5a** and *trans*-**5b**.

Table 5. Yield of piceid dimer **5** and proportion of piceid dimer isomers *cis*-**5a** and *trans*-**5b**.

N°	T(°C)	C (piceid) (mmol/L)	Yield (5) (%)	*cis*-5a (%)	*trans*-5b (%)
1	20	22	14.64	77	23
2	60	22	27.22	73	27
3	20	66	11.38	73	27
4	60	66	21.46	72	28
5	60	44	19.49	72	28
6	40	22	27.09	75	25
7	40	44	21.31	73	27
8	40	44	20.87	73	27
9	40	44	20.67	73	27
10	40	44	20.22	73	27

Finally, antiradical activities of δ-viniferin, piceid dimer (**4**) and piceid dimer (**5**) were assessed through DPPH analysis.[36] These analyses consist in the addition of the title compound in ethanol at different concentrations to homogeneous DPPH solution. The amount needed to reduce the initial number of DPPH free radicals by half (i.e., EC_{50}) was determined by the crossing point of the "% DPPH" curve (blue) and the "% reduced DPPH" curve (green) (Figure S12-S18 in the Supporting Information). The lower the EC_{50} value, the higher the antioxidant potential. Results for all the compound are given in Table 6 and are benchmarked against two commercially available antioxidants: BHA and BHT.

Table 6. Antiradical activities measured.

	EC_{50} (nmol)
Resveratrol **1**	11.96
Piceid **2**	11.50
δ-viniferin **3**	18.55
Piceid dimer **4**	47.00
Piceid dimer **5**	ND[a]
BHA	3.80
BHT	12.23

[a] No EC_{50} was obtained at the concentrations studied, the EC_{50} is above 80 nmol.

In accordance with Gavezzotti et al.[33], data showed (i) the expected comparable EC_{50} values for resveratrol (**1**) and piceid (**2**), (ii) a lower antiradical activity for δ-viniferin and piceid dimer (**4**), and (iii) piceid dimer (**5**) has a very low antiradical activity. Interestingly, whereas the glycosylation of resveratrol slightly improves its antioxidant activity (11.50 nmol vs. 11.96 nmol), that of δ-viniferin significantly decreases upon glycosylation (47.00 nmol vs. 18.55 nmol). In any case, while resveratrol, piceid and δ-viniferin have antiradical activity similar to BHT, none can compete with BHA.

CONCLUSION

This study describes the efficient synthesis of a diglucosylated dimer of piceid, a precursor of δ-viniferin, as well as new piceid dimers, through a sustainable AgOAc-mediated synthetic pathway using a minimal amount of ethanol. PMI, which highlighted solvent and atomic economy, alongside EcoScale, demonstrated that the reaction conditions are found to be more sustainable than the laccase-mediated routes reported in the literature while the yield of the reaction was significantly increased. By combining this new method with the already reported quantitative hydrolysis of the resulting dimer using a cellulase from *Trichoderma viride*, it is now possible to obtain δ-viniferin in an overall yield of over 60%, which would reflect a better performance than

the direct production of δ-viniferin through the dimerization of resveratrol with silver acetate in ethanol (48% yield). DPPH analyses revealed that, while resveratrol, piceid and δ-viniferin are potent antioxidant compounds, the unprecedented piceid dimers exhibit poor antioxidant activity.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: Figure S1. External calibration for the concentration of piceid dimer (**4**); Figure S2. ^{1}H NMR spectrum of piceid dimer (**4**); Figure S3. COSY NMR spectrum of piceid dimer (**4**); Figure S4. ^{1}H spectrum of piceid dimer (**5**); Figure S5. COSY NMR spectrum of piceid dimer (**5**); Figure S6. ^{13}C NMR spectrum of piceid dimer (**4**); Figure S7. ^{13}C NMR spectrum of piceid dimer (**5**); Figure S8. FTIR spectrum of piceid dimer (**4**); Figure S9. FTIR spectrum of piceid dimer (**5**); Figure S10. HRMS spectrum of piceid dimer (**4**); Figure S11. HRMS spectrum of piceid dimer (**5**); Figure S12. DPPH analysis of BHT; Figure S13. DPPH analysis of BHA; Figure S14. DPPH analysis of piceid dimer (**5**); Figure S15. DPPH analysis of piceid dimer (**4**); Figure S16. DPPH analysis of δ-viniferin; Figure S17. DPPH analysis of piceid; Figure S18. DPPH analysis of resveratrol; Figure S19. HPLC chromatogram of different piceid dimers; Table S1. Set of operating conditions applied determined by DoE; Table S2. DoE experiments results; Table S3. Statistical analyses of the different coefficients of the model; Table S4. Application of the optimal conditions designed by the DoE; Table S5. PMI score calculation for our procedure and the original method[33].

(PDF)

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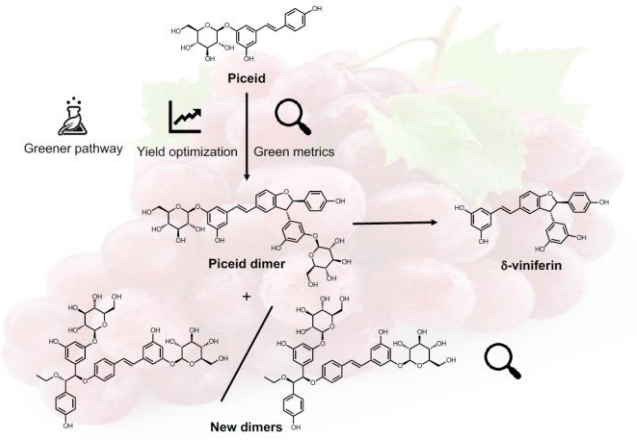

A new formal chemo-enzymatic pathway to δ-viniferin, involving an AgOAc-mediated dimerization in ethanol and a cellulase-catalyzed hydrolysis, was designed and optimized. The sustainability of the metal-catalyzed step was assessed using green metrics.