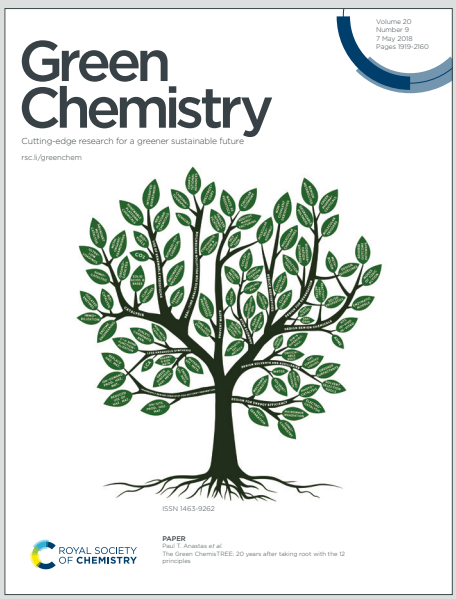
View Article Online
View Journal

Green Chemistry

Cutting-edge research for a greener sustainable future

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: M. Mention, A. L. Flourat, C. Peyrot and F. Allais, *Green Chem.*, 2020, DOI: 10.1039/D0GC00122H.



View Article Online
DOI: 10.1039/D0GC00122H

ARTICLE

Biomimetic regioselective and high-yielding Cu(I)-catalyzed dimerization of sinapate esters in green solvent Cyrene™: towards sustainable antioxidant and anti-UV ingredients

Received 00th January 20xx,
Accepted 00th January 20xx

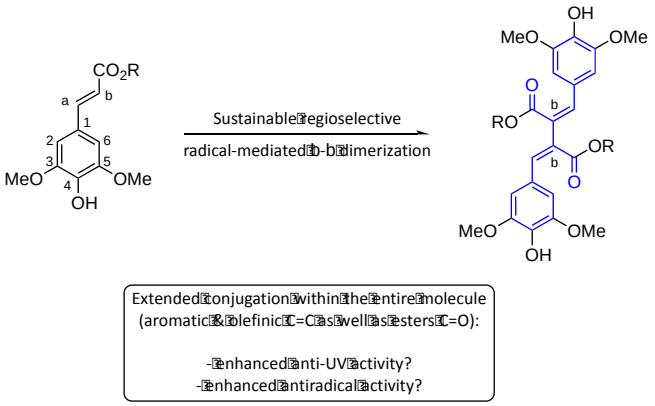
DOI: 10.1039/x0xx00000x

Matthieu M. Mention,[a] Amandine L. Flourat,[a] Cédric Peyrot[a] and Florent Allais[a]

Naturally occurring sinapic acid and its esters are anti-UV and antiradical chemicals. This work aimed at designing an industrially relevant sustainable synthetic pathway allowing their selective β-β' dimerization to enhance their properties with a view to their use in commercial applications such as functional additives for cosmetics, plastics and food/feed. A copper(I)-catalyzed procedure involving pyridine and O_2 from air was developed and greened up using REACH-compliant bio-based solvent Cyrene™. Optimized further through a Design of Experiments, this sustainable synthetic process was successfully implemented to various sinapate esters and validated at the multigram scale. Antiradical activities of the resulting β-β' disinapate esters were benchmarked against commercial antioxidants, whereas their UV absorbance was compared to that of a naturally anti-UV in plants and a widely used sunscreen ingredient. Results showed that these dimers were better radical scavengers, and not only exhibited a better UV absorbance but also covered both UV-A and UV-B regions.

Introduction

To decrease the imprint of Human on Earth, the demand of bio-based products with low ecology impact is rocketing. In this fashion, sinapic acid, a naturally occurring *p*-hydroxycinnamic acid mainly found in plants of the *Brassicaceae* family (e.g., mustard, canola, rapeseed)[1] seems to be a good candidate to develop new products, and more particularly antioxidants and anti-UV molecules able to replace the fossil-based ones currently under serious pressure due to their toxicity. Indeed, several interesting properties are reported for this molecule or its derivatives: antioxidant[1], antimicrobial[2], anti-inflammatory[3], anticancer[4], anxiolytic[5] and anti-UV[6-8]. It was demonstrated that UV properties of sinapic ester derivatives is due to (1), the conjugation between the C=C and C=O bonds (i.e., α,β-unsaturated ester) which allows electronic transition involving molecular orbitals throughout the entire sinapate part of the molecule, and (2) the presence of a sterically hindered moiety on the β position that favor sinapate esters cis-trans isomerization upon UV exposure. *Brassicaceae* species are extensively exploited in agriculture and generate a lot of by-product (e.g., bran, oilcake) from which sinapic acid derivatives can be extracted[9-11]. It is noteworthy to mention that sinapic acid can also be efficiently synthesized by Knoevenagel-Doebner condensation from syringaldehyde[12-16], a *p*-hydroxybenzaldehyde obtained from the oxidation of hardwood lignins[17]. Given the properties cited above (i.e., antiradical and anti-UV capacities) and their dependency on the degree of conjugation of the chemical structures, radical-radical coupling β-β' dimerization of sinapic acid derivatives seemed an interesting approach to improve their antiradical and anti-UV properties as such a dimerization further extend the conjugation and the steric hinderance at the β position (Scheme 1).



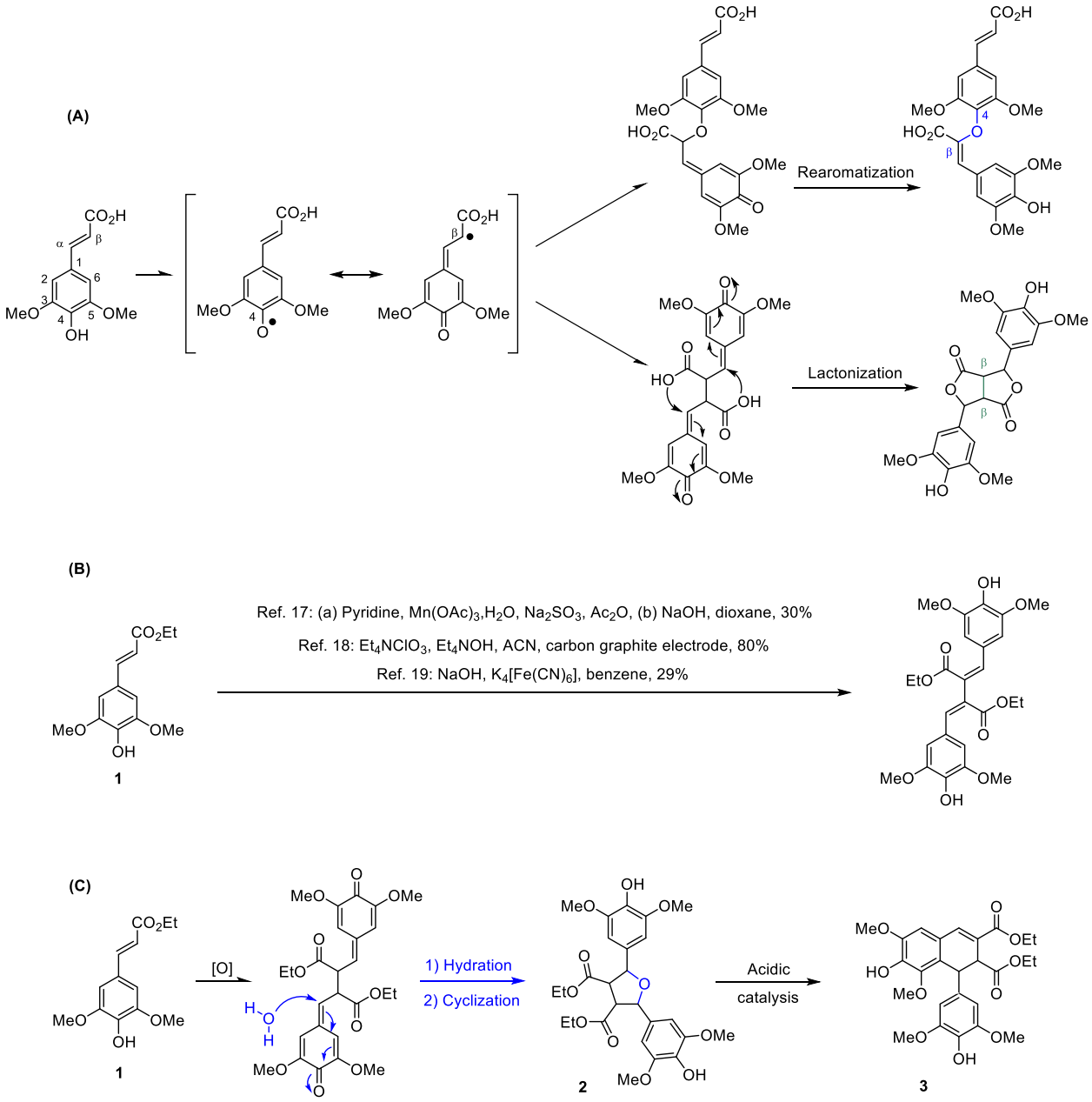
Scheme 1. Will the extension of conjugation through their β-β' dimerization enhances the anti-UV and antiradical properties of sinapate esters?

However, β-β' dimers of sinapic acid derivatives are difficult to synthesize in good yield and purity through radical-radical coupling as there are two radical species and thus two possible dimers – β-*O*-4' and β-β' – that can oligomerize further. Moreover, in the case of sinapic acid, the β-β' dimerization is usually followed by an intramolecular rearrangement leading to the formation of dilactone and the loss of conjugation (Scheme 2A)[18]. To avoid the formation of such a dilactone, esters of sinapic acid can be used. On the basis of the above

[a] URD Agro-Biotechnologies Industrielles (ABI), CEBB, AgroParisTech, 51110, Pomacle, France.
* Corresponding author: florent.allais@agroparistech.fr
† Footnotes relating to the title and/or authors should appear here.
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

considerations, the use of sinapate esters - and more particularly ethyl sinapate - appeared as the most relevant substrates. In the literature, different pathways were described

View Article Online
DOI: 10.1039/D0GC00122H



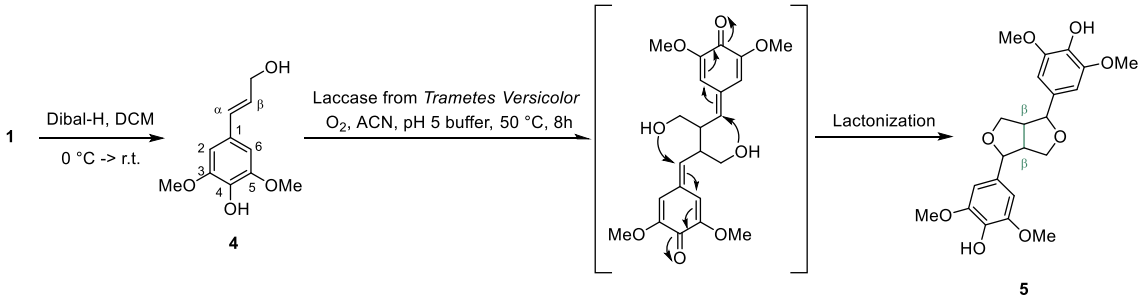
Scheme 2. (A) β-O-4 and β-β' dimers resulting from the oxidative dimerization of sinapic acid, **(B)** previously reported syntheses of ethyl sinapate dimer and **(C)** rearrangement of quinone methide intermediate.

for the synthesis of ethyl sinapate β-β' dimer from ethyl sinapate (**1**): the first one relied on the $Mn(OAc)_2$-mediated oxidation in presence of NaOH[19], the second consisted in an electrochemical oxidation with tetraethylammonium hydroxide and tetramethylammonium perchlorate[20], and the third involved potassium ferrocyanide in benzene[21] (Scheme 2B)Although these procedures provide the desired target, not only they use hazardous reagents but also some proved ineffective at the multigram scale. It is also important to note that rearrangements can occur from the quinone methide intermediary with water, resulting to furan formation (**2**)[22], and to condensed linkage (**3**) in acidic conditions[23], two compounds where conjugation is significantly reduced (Scheme 2C). Designing a sustainable and scalable highly selective β-β' dimerization synthetic process thus remains a challenge.

In this study, after having investigated different approaches, a highly selective copper(I)-catalyzed dimerization process in the REACH-compliant cellulose-based solvent Cyrene™ enabling the synthesis of the open β-β' ethyl sinapate dimer at a multigram scale has been designed and optimized, and successfully implemented on various sinapate esters. To assess the importance of the extended conjugation and that of the ester moieties on the antiradical and anti-UV properties, the

View Article Online
DOI: 10.1039/D0GC00122H

corresponding saturated β-β' ethyl sinapate dimer and β-β' sinapyl alcohol dimer were synthesized. Structure-activity relationships (SARs) were evaluated with regards to radical scavenging and UV absorbance activities. Antiradical properties of the β-β' sinapate esters dimers were determined using the ABTS assay[24] and compared to that of commercial antioxidants (i.e., BHT and BHA). For the anti-UV properties, sinapoyl malate, which has been described as the "sunscreen" of plants,[6] and Octinoxate™, one of the most widespread anti-UV used in cosmetics, were used as references.



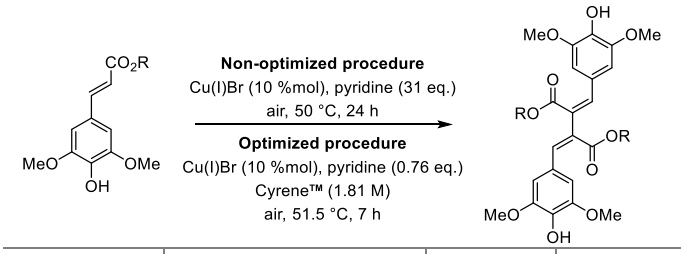
Scheme 3. Highly regioselective laccase-catalysed synthesis of syringaresinol from ethyl sinapate

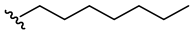
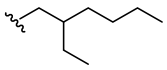
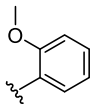
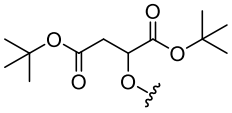
Results and Discussion

To investigate and optimize the β-β' dimerization of sinapate esters, ethyl sinapate (**1**) was chosen as model compound. In a previous work dedicated to the laccase-mediated dimerization of sinapyl alcohol (**4**) into syringaresinol (**5**) in 93% yield[25] (Scheme 3), we demonstrated that one can selectively favor the β-β' dimerization over the β-*O*-4 one by finely tuning the reaction conditions (i.e., slow addition of laccase from *Trametes versicolor*, 4.9 buffer/acetonitrile). Encouraged by these promising results, this procedure was thus directly applied on ethyl sinapate. Unfortunately, UHPLC analysis (Supporting Information, Figure S1) revealed that the reaction resulted in a mixture containing the desired β-β ethyl sinapate dimer **8** but also the β-*O*-4' dimer (**6**), dimer **7** (obtained through rearrangement of **8**), unreacted **1**, as well as soluble and insoluble oligomers, proving that the high selectivity observed with sinapyl alcohol was totally lost with ethyl sinapate. Further optimization was thus required. The presence of oligomers suggests a high affinity of the enzyme towards dimers **6** and **8**, and to a larger extent the resulting oligomers. As an effort to prevent the contact between the enzyme and the targeted dimers to limit the formation of (in)soluble oligomers, acetonitrile was replaced by ethyl acetate, thus providing a biphasic system where the dimers would be in the organic layer and the enzyme in the interphase. This modified procedure did prevent the formation of insoluble oligomers, but not that of soluble oligomer and dimers **6** and **7**. The formation of dimer **7** being promoted under acidic conditions[23], the aqueous buffer was then replaced by Milli-Q water. Under such conditions, β-*O*-4' and β-β' dimers (**6** and **8**) were obtained along with traces of dimer **7** (Supporting Information, Figure S1), however soluble oligomers were recovered as major products and the selectivity of the dimerization remained in favor of the β-*O*-4' coupling. All our attempts that consisted in varying reaction parameters (co-solvent ratio, temperature, time, amount and addition rate of enzyme), proved unsuccessful. Laccase from *Trametes versicolor* seeming to have too much affinity with our substrates, it was decided to switch to Horseradish Peroxidase (HRP) type II and VI, those enzymes being known for their ability to promote β-β' coupling of *p*-hydroxycinnamic acid[22, 23, 26, 27] and their lesser affinity for sinapic acid derivatives[28]. On the down side, these enzymes having a low tolerance to organic solvents, biphasic system could not be used. Once again, varying several conditions (temperature, type of HRP and co-solvent ratio) only resulted in the decrease of oligomers formation, no improvements were observed in terms of selectivity towards β-β' coupling, worse yet, dimer **7** formation was favoured.

As the use of enzymes proved quite unsuccessful, a different approach based on metal catalysis was investigated. Given the fact that laccase from *Trametes versicolor* seemed to have a high affinity toward ethyl sinapate and its dimer, we decided to mimic the active site of the enzyme, composed by copper(I) and histidine[29], by using copper(I) bromide, air (O_2), and pyridine as source of copper (I), oxidizing agent and histidine-surrogate, respectively. A first attempt, using CuBr(I) (10 mol%) and pyridine (31 eq) during 24 h at 50 °C, provided β-β' dimer in good yield (62%) and in high selectivity. Moreover, no trace of β-*O*-4' dimer or oligomers was observed. To widen the scope of this dimerization methodology, this procedure was performed on various sinapate esters (i.e., heptyl sinapate, *tert*-butyl sinapate, 2-ethyl-hexyl sinapate, isopropyl sinapate and guaiacol sinapate prepared following procedures described in the literature[30, 31], and sinapoyl di*tert*-butyl malate which was synthesized by method from Allais et al.[32]). Sinapate dimers were then obtained in average yields, ranging from 42% to 64% (Table 1).

Table 1. Synthesis of sinapate β-β' dimers (non-optimized and optimized procedures).



Compounds	R	Non-optimized procedure (% yield)[a]	Optimized procedure (% yield)[a]
8		62	89
9		64	90
10		55	88
11		58	89
12		58	91
13		42	87
14		42	88

[a] Isolated Yields

Optimization

The proof of concept being validated, and in the context of sustainable development, the next step was to green up the reaction by first reducing the quantity of pyridine used, switching its role from solvent/ligand to only ligand. To do so, some less toxic solvents were studied as alternative to pyridine: ethyl acetate, ethanol and Cyrene™, a REACH-compliant cellulose-derived polar aprotic solvent[33-35]. The screening of these solvents using the following conditions (Cu(I)Br (10 mol%), 1 equivalent of pyridine and ethyl sinapate at a concentration of 0.5 mol/L in the different solvents) showed that Cyrene™ was by far the most efficient (Supporting Information, Figure S2). It is noteworthy to mention that DCM and DMSO, two other aprotic polar solvents, provided similar results. However, Cyrene™ was chosen as it fulfils all the criteria for a green reaction as it is a cellulose-derived sustainable solvent with a low vapor pressure (14.4 Pa at 25 °C) and low (eco)toxicity.[33] A test without pyridine showed no conversion of ethyl sinapate, confirming that the Cu(I)-catalyst must be activated in the same way as the laccase active site[29]. Assays with DMAP (4-dimethylaminopyridine) and triethylamine in place of pyridine were performed to assess the type of amine needed for catalysis. Reaction using DMAP led to similar results than pyridine while the use a triethylamine did not result in a good conversion of ethyl sinapate into its β-β' dimer. Such results show that aromaticity of the amine is needed to achieve good conversion and yield for the studied dimerization. Finally, in an effort to minimize further the utilization of toxic compounds in the reaction, pyridine was successfully reduced to 0.5 equivalents without impacting the β-β' dimer yield (63%). Although this yield was acceptable, we decided to pursue further the optimization of this reaction through a Design of Experiments (DoE) and Response Surface Methodology (RSM). Prior performing the DoE, a kinetic study was carried out to determine a relevant time scale. The monitoring of both the ethyl sinapate conversion and the yield in ethyl sinapate β-β' dimer by HPLC showed that maximal yield was reached after 7 hours (69%), while conversion continued to increase (from 78% at 7h to 98% at 24h) (Figure 1). In view of these results, the duration was therefore fixed to 7 hours for the DoE. The DoE was elaborated to analyze the impact of four variables – Temperature (Temp), Substrate/Catalyst ratio (S/C), Amine/Catalyst ratio (A/C) and Concentration (Con) (Table 2) – on two responses, conversion of ethyl sinapate and yield of the desired product. For this purpose, a cubic centered faced design was chosen with triplicate at the central point and 27 experiments were performed (Supporting Information, matrix in Table S1). Only mild temperatures, reduction of the quantity of catalyst and amine were explored to green up further the reaction on top of improving the yield. For the same purpose, reaction medium concentration was increased to reduce both environmental footprint and cost.

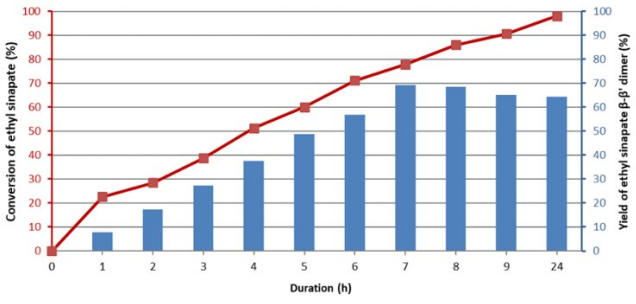


Figure 1. HPLC monitoring of the dimerization of ethyl sinapate: conversion of starting material (red) and yield in ethyl sinapate β-β' dimer (blue). [Zorbax Eclipse Plus C18 (2.1 mm*50 mm*1.8 µm), λ = 320 nm, flow rate set at 0.6 mL.min^{-1}, oven temperature at 30 °C and gradient applied: H_2O/CH_3CN from 75/25 to 70/30 in 18 min].

View Article Online
DOI: 10.1039/D0GC00122H

Table 2. Independent variables and levels used for full factorial design.

Variables	Levels		
	-1	0	1
Temp (°C)	25	47.5	70
Ratio S/C	2	6	10
Ratio A/C	1	5.5	10
Con (mol/L)	0.25	1.375	2.5

After computational treatment of the raw results, logit transformation (log (Y)/(100-Y)) was applied for yield whereas no transformation was needed for conversion. In addition, an outlier was found for the yield (Supporting Information, entry N9 Table S1) and excluded of the statistical analysis. Nevertheless, the validity of the model was still too low and presented a lack of fit in F-tests performed in ANOVA, that is why points with the highest difference between the experimental value and the prediction of the model were duplicated (entries 28 to 32 in Table S1) and included in the model to refine it. The quality of this refined model proved very good for both yield (R^2 = 0.876, Q^2 = 0.769, model validity = 0.912 and reproducibility 0.850) and conversion (R^2 = 0.936, Q^2 = 0.901, model validity = 0.639 and reproducibility = 0.933) (Supporting Information, Figure S3). Moreover, the analysis of the variance (Supporting Information, Tables S2 and S3) confirmed that the significance of the model was reached ($p < 0.05$); moreover, the lack of fit ($p > 0.05$) showed statistical significance of both models and a similar magnitude of replicate errors (no lack of fit). Sphericity of the design was assessed with condition numbers of 6.054 and 6.184 (<8) for yield and conversion, respectively.

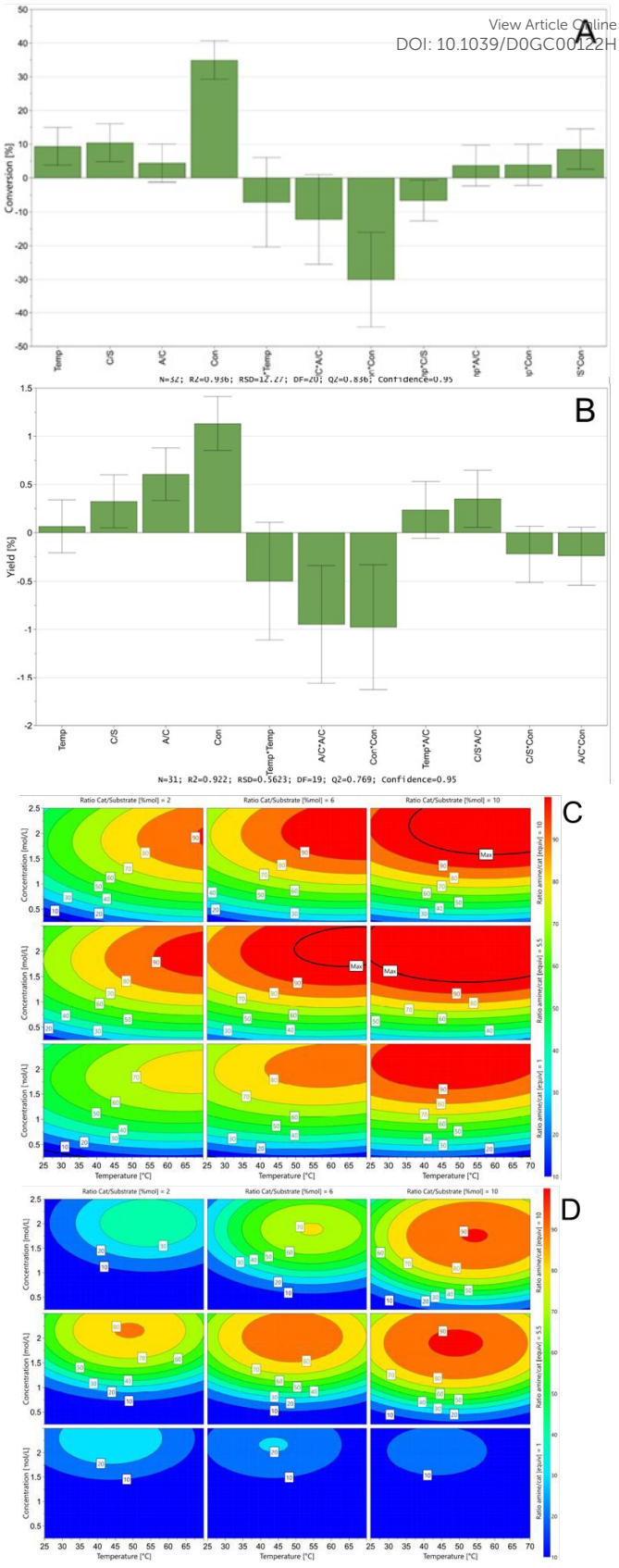


Figure 2. Scaled and centered coefficients of quadratic model for conversion (A) and yield (B) and response surface for conversion (C) and yield (D) for the β-β′ dimerization of ethyl sinapate.

Scaled and centered coefficients of the model (Figure 2A and 2B) allowed to determine the influence of each parameter, their square terms and also the quadratic effect. On both responses, the independent variables (i.e., temperature, ratio S/C, ratio A/C and concentration) had all a positive impact, the concentration (Con) being the most influent. Nevertheless, the significant square terms ($Temp^2$, $(A/C)^2$ and Con^2), all negatives, lowered the direct impact of the independent variables, leading to a decrease in yield and conversion for a variation too important from the central point. Once again, the most influent parameter was the square of the concentration (Con^2). The interaction between variables was evaluated with the quadratic terms. For the yield, the quadratic terms with positive contributions were the interaction between temperature and the amine/catalyst ratio (Temp*A/C) and the one between catalyst/subtract and amine/catalyst ratios (S/C*A/C). In the other hand, the S/C*Con and A/C*Con ratios had negative impact. For conversion of ethyl sinapate, the temperature was mainly involved, negatively with S/C and positively with A/C and Con. Finally, the quadratic term S/C*Con impacted positively the conversion.

Direct visualization of the results obtained through the DoE was facilitated by using Response Surface Methodology (RSM), where optimal responses are represented by red areas (Figure 2). Clearly, high conversion of ethyl sinapate can be achieved within a large set of conditions (Figure 2C). However, in order to maximize yield, temperature had to be carefully adjusted (40-55 °C) in a restricted range of concentration (1.5-2.0 mol/L) with the highest amount of catalyst. In addition, an intermediate ratio amine/catalyst enlarged the surface to led to this high yield (Figure 2D). Finally, to be able to predict the outcome of future experiments, equations of the model (Equation 1) were expressed uncoded below in order to allow the direct numeric application where Y1 and Y2 were the yield and the conversion, respectively.

$$Log\,(Y1\,/\,(100-Y1)) = -6.9034 + 0.0840(Temp) + 0.0420(S/C) + 0.4879(A/C) + 3.6926(Con) - 0.0010(Temp)^2 - 0.0469(A/C)^2 - 0.7730(Con)^2 + 0.0023(Temp \times A/C) + 0.0195(S/C \times A/C) - 0.0495(S/C \times Con) - 0.0478(A/C \times Con)$$

$$Y2 = -75.7650 + 1.7907(Temp) + 3.5114(S/C) + 5.8929(A/C) + 77.7829(Con) - 0.0142(Temp)^2 - 0.6055(A/C)^2 - 23.8050(Con)^2 - 0.0740(Temp \times S/C) + 0.0369(Temp \times A/S) + 0.1547(Temp \times Con) + 1.9015(S/C \times Con)$$

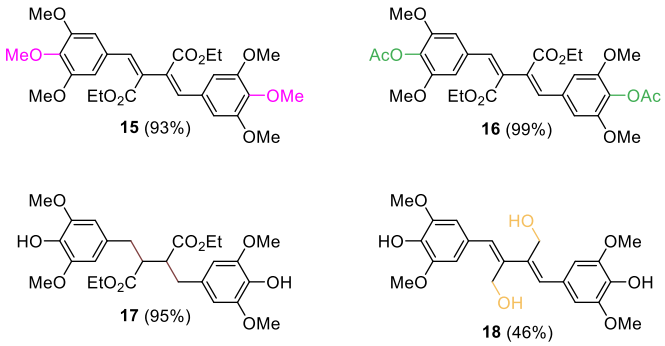
Equation 1. Equations of the model

To assess the predictability of the model, external validation was performed in duplicate using the optimal conditions predicted by the model (Table 3). In both cases, a total conversion was achieved and yield were 88% and 89%, respectively, a little lower than the prediction, but still within the 83.7 – 98.1% range achievable with this set of conditions, thus validating the model. To summarize, compared to the initial procedure, optimization by RSM allowed a significant improvement of the β-β' dimerization of ethyl sinapate, with a yield increasing from 63% to 89%. Although we were not able to reduce copper bromide amount without affecting the yield, the quantity of pyridine was reduced 41-fold, the reaction medium was concentrated up to 1.81 mol/L, which represented a 3.6-fold diminution of the total volume of Cyrene™ and the duration was reduced from 24 to 7 hours (Table 3).

Table 3. Optimal conditions predicted by the model and comparison between initial and optimized β-β' dimerization of ethyl sinapate

Conditions	Temp (°C)	S/C	A/C	Con (mol/L)	t (h)	Conv (%)	Yield (%)
Initial	50	10	310	0.5	24	99	62
Predicted	51.5	10	7.6	1.81	7	100	84-98
Optimized	51.5	10	7.6	1.81	7	99	89

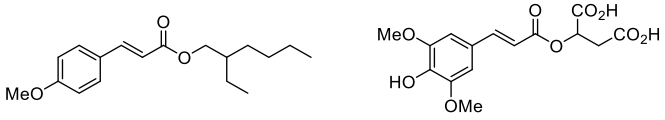
This optimized procedure was then successfully applied to the sinapate esters previously described (yields ranging from 87% to 91%) and validated at the multigram-scale (Table 1). It is noteworthy to mention that, for the optimized conditions, a simple extraction with ethyl acetate/1M aqueous HCl allowed the recovery of β-β' dimers while a purification using silica gel chromatography was needed for non-optimized conditions. With the library of β-β' sinapate ester dimers in hands, we then proceeded to the investigation of their antiradical and anti-UV properties. To establish SARs and determine the impact of the conjugation/unsaturations (i.e., C=C), the esters and the phenol moieties, on the anti-UV and antiradical properties, β-β' ethyl sinapate dimer (**8**) was (i) methylated (**15**), (ii) acetylated (**16**), (iii) reduced via palladium-catalyzed hydrogenation to provide its saturated counterpart (**17**), and (iv) reacted with DIBAL-H to give the corresponding dialcohol (**18**) (Scheme 4).



Scheme 4. Dimers synthesized for SARs.

Anti-UV properties

To assess the potential of our molecules as anti-UV compounds, Octinoxate™ (ethylhexyl methoxycinnamate) – a commercial anti-UV-B ingredient widely used in sunscreens and cosmetics – and sinapoyl malate – a naturally occurring anti-UV compound in plants[6] – have been used as benchmarks (Scheme 5).



Scheme 5. Structure of Octinoxate™ (left) and sinapoyl malate (right)

View Article Online
DOI: 10.1039/D0GC00122H

As shown in Figure 4, the dimerization of ethyl sinapate **1** into **8** allowed a 1.4-fold increase of the maximal absorbance**,** as well as a better wavelength range coverage (ethyl sinapate **1**: 260-370 nm, β-β' ethyl sinapate dimer **8**: 260-402 nm), enabling dimer **8** to absorb both in the UV-A (315-400 nm) and the UV-B (280-315 nm) regions. With regards to the nature of the ester moiety, data for compounds **8**-**14** showed similar wavelength coverage, except for **13** that exhibits a bathochromic shift (Figure 3A and 3B). Only a significant difference in the absorbance levels could be observed, with a factor of 1.2 between the highest (**9**) and the lowest (**10**). In conclusion, the extended conjugation brought by the dimerization did increase the wavelength range and the maximal absorbance. All synthesized dimers (**8-14**) proved to have a higher absorbance value and a wider wavelength coverage than both reference molecules (Figure 3A and 3B), thus demonstrating that β-β' sinapate esters dimers are potent bio-based anti-UV compounds, able to cover simultaneously the UV-A and the UV-B regions.

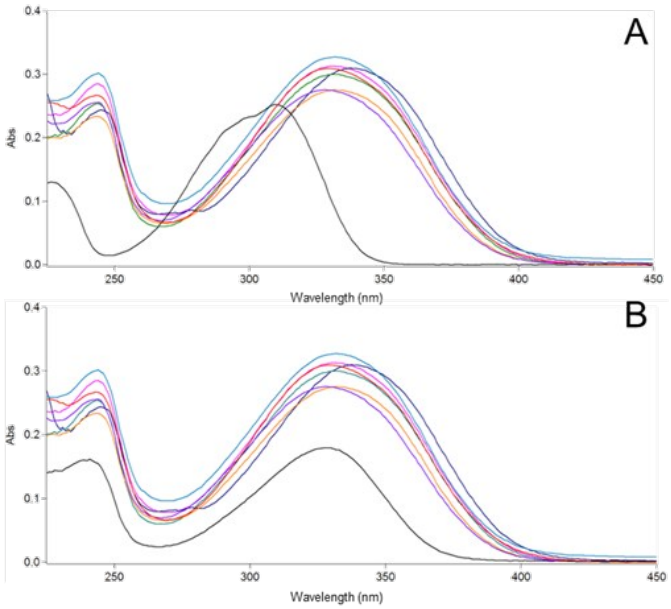


Figure 3. UV spectra (C = 1.10^{-5} mol/L in ethanol) of commercial Octinoxate™ (A) and Sinapoyl Malate (B) compared to β-β' dimers **8** (pink), **9** (light-blue), **10** (orange), **11** (purple), **12** (green), **13** (blue) and **14** (red).

Dean et al.[6] demonstrated that the conjugation and the ester moieties in sinapate derivatives played an important role in their UV properties. This observation was confirmed by comparing β-β' ethyl sinapate **8** with **17** and **18** (Figure 4). Indeed, the loss of conjugation resulting from the reduction of the carbon-carbon double bonds led to a severe decrease of the UV properties, **17** only covering from 260 to 290 nm with little absorbance. Reduction of the ester moieties into the corresponding alcohols **18** led to a less severe decrease of absorbance (by a factor of 1.5) covering from 260 to 320 nm. The impact of the free phenol was then investigated by comparing the absorbance of **8** with that of **15** and **16**. Only a slight decrease of the absorbance could be noticed for the two molecules, with a hypsochromic shift, demonstrating that the wavelength coverage could be modulated *via* functionalization of the phenol moiety.

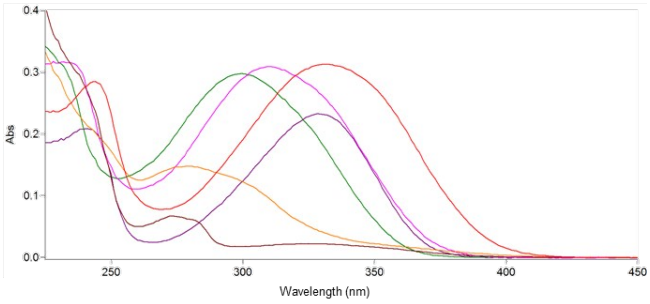
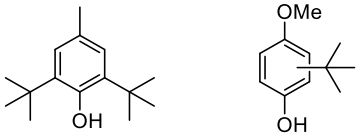


Figure 4. UV spectra (C = 1.10^{-5} mol/L) for SARs between 1 (purple), 8 (red), 15 (pink), 16 (green), 17 (brown) and 18 (orange).

Antiradical properties

β-β' sinapate esters dimers (**8-14, 17-18**) having free phenol, their capacity to scavenge radicals was assessed. The antiradical activity of the molecules was expressed *via* the EC_{50} (half maximal effective concentration) value, defined as the quantity (in nmol) of antiradical molecule needed to scavenge 50% of the initial quantity of free radicals in solution. The lower the EC_{50} is, the higher the antiradical activity. We first performed the DPPH analysis (2,2-diphenyl-1-pycrilhydrazyl ($DPPH^{\bullet}$)) routinely employed in our lab. Unfortunately, its reaction with the β-β' sinapate esters dimers led to the apparition of a new band of absorbance at 520 nm, the very same wavelength used to monitor the disappearance of $DPPH^{\bullet}$. To overcome this issue, another procedure – adapted from Re et al.[24] – consisting in monitoring the decrease of the $ABTS^{\bullet+}$ radical cation (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) at 734 nm was performed in duplicate. To assess the potential of our molecules as antiradical compounds, the β-β' sinapate esters dimers (**8-14**) and their reduced counterparts (**17-18**) were benchmarked against two commercial antioxidants used in food, cosmetic or plastic industries, BHT (Butylated hydroxytoluene) and BHA (Butylated hydroxyanisole) (Scheme 6).



Scheme 6. Structure of BHT (left) and BHA (right).

Data reported in Figure 5 showed a narrow range of EC_{50} values (3.52-6.51 nmol) for the β-β' sinapate ester dimers (**8-14**). Overall, the nature of the ester moiety did not significantly impact the antiradical activity (4.97-6.51 nmol, Figure 5), except for dimer (**14**) that had an EC_{50} value of 3.52 nmol – most probably due to the higher steric hinderance of the malate moieties[8]. The reduction of conjugation on (**17**) and (**18**) also led to lower EC_{50} values, 3.63 nmol and 2.73 nmol, respectively. Such an improvement of antiradical activity due to reduction of the carbon-carbon double bond (**17**) has

already been described by Reano et al.[36] on *p*-hydroxycinnamic bis- and trisphenols, the lack of double bonds leading to a possible dismutation regenerating part of the phenols involved in the process.

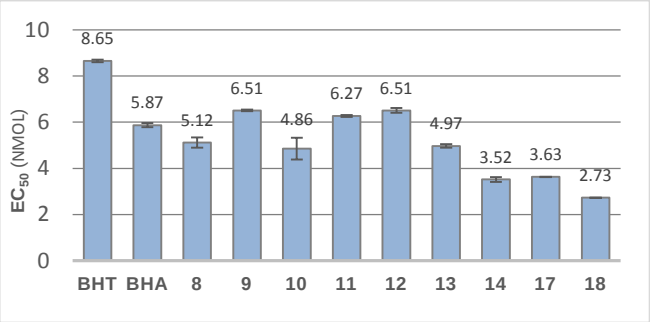


Figure 5. Antiradical properties from the ABTS$^{\bullet+}$ assay: benchmarking of the sinapate esters β-β′ dimers (**8**-**18**) against BHT and BHA.

To assess the impact of the extended conjugation brought by the dimerization, one should compare the EC_{50} of a given dimer to half that of its corresponding monomer (gain = 1-[EC_{50}(dimer)/(EC_{50}(monomer)/2)]); if the gain was greater than zero, then the impact of the extended conjugation was positive, and the other way around). From Figure S4 in the Supporting Information and Table 4, it appeared that dimerization slightly increased the antiradical activity only in the case of esters bearing large groups (i.e., 2-ethylhexyl (**10**), *t*-Bu (**11**) and di-*t*-butylmalate (**14**)) and for the dialcohol **(18)**.

Table 4. Impact of the β-β′ dimerization on the antiradical properties

β-β′ dimers	Gain
8	-0.52
9	-0.05
10	0.11
11	0.07
12	-0.19
13	-0.11
14	0.24
17	-0.13
18	0.03

Finally, when compared to the benchmarks, all synthesized β-β′ dimers exhibited better antiradical activity than BHT, however, only (**8**), (**10**), (**13**), (**14**), (**17**) and (**18**) showed higher activity than BHA. These data undoubtedly demonstrate that β-β′ sinapate ester dimers are promising bio-based antiradical compounds.

Conclusions

A sustainable and highly selective biomimetic β-β′ dimerization of sinapate esters involving copper as catalyst, pyridine as ligand, air as oxidative agent and Cyrene™ as renewable solvent, has been successfully optimized and validated at the multigram-scale. The use of the green solvent Cyrene™ allowed to reduce drastically the quantity of pyridine used while improving the yield of the dimerization and reducing reaction time. The constitution of a library of various β-β′ sinapate ester dimers and derivatives, and the study of their anti-UV capacity and their antiradical activity through UV analysis and ABTS$^{\bullet+}$ assays, respectively, allowed the determination of SARs. Results demonstrated that β-β′ sinapate esters dimers not only covered all the UV-A and UV-B region, but also exhibited free radical scavenging activity. Finally, benchmarking against recognized anti-UV ingredients and antioxidants showed that β-β′ sinapate esters dimers had a great potential as bio-based UV-filters and antiradical compounds. The use of these symmetric dimers as monomers for the production of renewable aromatic polyesters, polycarbonates and epoxy resins will be reported in due course.

Conflicts of interest

F.A., A.L.F and M.M.M. have filed a patent based on the work described here (Patent application no. FR1859902)

Acknowledgements

The authors are grateful to the Circa Group for providing them with industrial grade Cyrene™. This project was funded by the Agence Nationale de la Recherche under the grant ANR-17-CE07-0046, the EU's Horizon 2020 programme under the grant No. 828753, Région Grand Est, Conseil Départemental de la Marne and Grand Reims.

ORCID

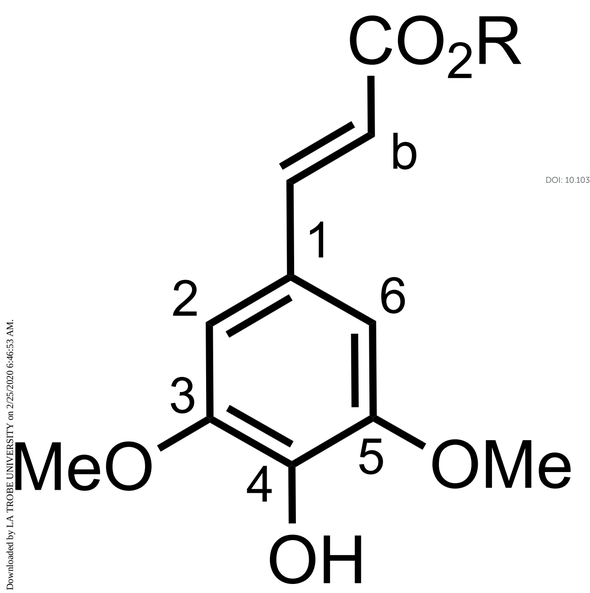
Florent Allais 0000-0003-4132-6210
Matthieu M. Mention 0000-0002-2309-9870
Cédric Peyrot 0000-0001-8843-6498
Amandine L. Flourat 0000-0001-5692-7786

Notes and references

1. N. Niciforovic and H. Abramovic, *Compr. Rev. Food Sci. Food Saf.*, 2014, **13**, 34-51.
2. H. Nowak, K. Kujawa, R. Zadernowski, B. Roczniak and H. Kozlowska, *Fett Wiss. Technol.*, 1992, **94**, 149-152.
3. K.-J. Yun, D.-J. Koh, S.-H. Kim, S. J. Park, J. H. Ryu, D.-G. Kim, J.-Y. Lee and K.-T. Lee, *J. Agric. Food Chem.*, 2008, **56**, 10265-10272.
4. E. A. Hudson, P. A. Dinh, T. Kokubun, M. S. J. Simmonds and A. Gescher, *Cancer Epidemiol., Biomarkers Prev.*, 2000, **9**, 1163-1170.
5. B. H. Yoon, J. W. Jung, J.-J. Lee, Y.-W. Cho, C.-G. Jang, C. Jin, T. H. Oh and J. H. Ryu, *Life Sci.*, 2007, **81**, 234-240.
6. J. C. Dean, R. Kusaka, P. S. Walsh, F. Allais and T. S. Zwier, *J. Am. Chem. Soc.*, 2014, **136**, 14780-14795.
7. M. D. Horbury, E. L. Holt, L. M. M. Mouterde, P. Balaguer, J. Cebrian, L. Blasco, F. Allais and V. G. Stavros, *Nat. Commun.*, 2019, **10**, 1-8.
8. L. A. Baker, M. D. Horbury, S. E. Greenough, F. Allais, P. S. Walsh, S. Habershon and V. G. Stavros, *J. Phys. Chem. Lett.*, 2016, **7**, 56-61.
9. R. Das, C. Bhattacherjee and S. Ghosh, *Ind. Eng. Chem. Res.*, 2009, **48**, 4939-4947.

View Article Online
DOI: 10.1039/D0GC00122H

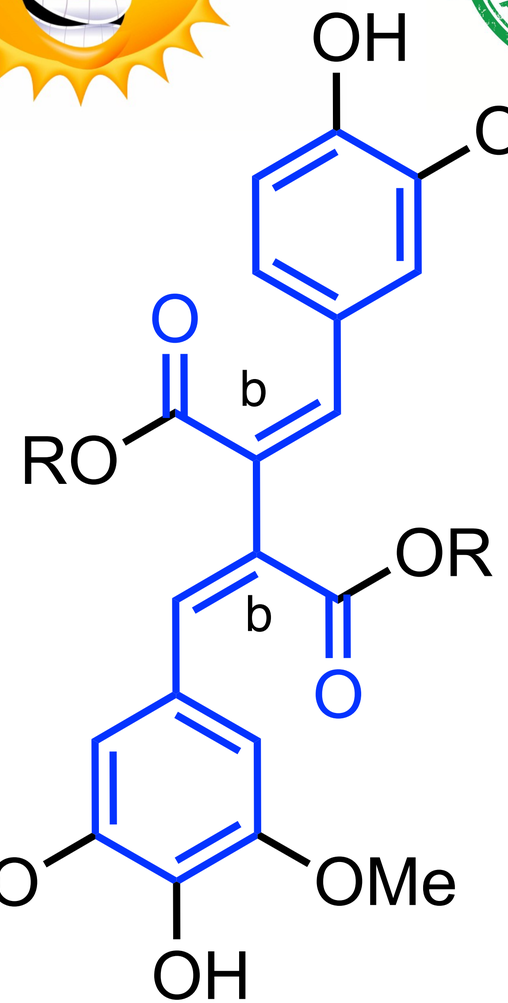
10. K. Lacki and Z. Duvnjak, *Appl. Microbiol. Biotechnol.*, 1996, **45**, 530-537.
11. A. Thiel, K. Muffler, N. Tippkoetter, K. Suck, U. Sohling, S. M. Hruschka and R. Ulber, *J. Chem. Technol. Biotechnol.*, 2015, **90**, 1999-2006.
12. M. Janvier, L. Hollande, A. S. Jaufurally, M. Pernes, R. Menard, M. Grimaldi, J. Beaugrand, P. Balaguer, P.-H. Ducrot and F. Allais, *ChemSusChem*, 2017, **10**, 738-746.
13. C. Peyrot, A. A. M. Peru, L. M. M. Mouterde and F. Allais, *ACS Sustainable Chem. Eng.*, 2019, **7**, 9422-9427.
14. C. S. Lancefield and N. J. Westwood, *Green Chem.*, 2015, **17**, 4980-4990.
15. J. van Schijndel, L. A. Canalle, J. Smid and J. Meuldijk, *Open J. Phys. Chem.*, 2016, **6**, 101-108.
16. L. M. M. Mouterde and F. Allais, *Front Chem*, 2018, **6**, 426.
17. G. Wu, M. Heitz and E. Chornet, *Ind. Eng. Chem. Res.*, 1994, **33**, 718-723.
18. K. Koschorreck, S. M. Richter, A. B. Ene, E. Roduner, R. D. Schmid and V. B. Urlacher, *Appl. Microbiol. Biotechnol.*, 2008, **79**, 217-224.
19. M. Bunzel, J. Ralph, H. Kim, F. Lu, S. A. Ralph, J. M. Marita, R. D. Hatfield and H. Steinhart, *J. Agric. Food Chem.*, 2003, **51**, 1427-1434.
20. A. Neudorffer, D. Bonnefont-Rousselot, A. Legrand, M.-B. Fleury and M. Largeron, *J Agric Food Chem*, 2004, **52**, 2084-2091.
21. B. Sathish Kumar, A. Singh, A. Kumar, J. Singh, M. Hasanain, A. Singh, N. Masood, D. K. Yadav, R. Konwar, K. Mitra, J. Sarkar, S. Luqman, A. Pal, F. Khan, D. Chanda and A. S. Negi, *Bioorg. Med. Chem.*, 2014, **22**, 1342-1354.
22. F. Lu and J. Ralph, *Org. Biomol. Chem.*, 2008, **6**, 3681-3694.
23. W. Li, H. Liu, J. Xu, P. Zang, Q. Liu and W. Li, *Eur. J. Org. Chem.*, 2014, **2014**, 3475-3482.
24. R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans, *Free Radical Biol. Med.*, 1999, **26**, 1231-1237.
25. A. S. Jaufurally, A. R. S. Teixeira, L. Hollande, F. Allais and P.-H. Ducrot, *ChemistrySelect*, 2016, **1**, 5165-5171.
26. F. Yue, F. Lu, R. Sun and J. Ralph, *Chem. - Eur. J.*, 2012, **18**, 16402-16410.
27. T. Kobayashi, H. Taguchi, M. Shigematsu and M. Tanahashi, *J. Wood Sci.*, 2005, **51**, 607-614.
28. U. Takahama, *Physiol. Plant.*, 1995, **93**, 61-68.
29. S. Riva, *Trends Biotechnol.*, 2006, **24**, 219-226.
30. J. C. J. M. D. S. Menezes, S. P. Kamat, J. A. S. Cavaleiro, A. Gaspar, J. Garrido and F. Borges, *Eur. J. Med. Chem.*, 2011, **46**, 773-777.
31. J. Padwal, W. Lewis and C. J. Moody, *Org. Biomol. Chem.*, 2011, **9**, 3484-3493.
32. F. Allais, S. Martinet and P.-H. Ducrot, *Synthesis*, 2009, DOI: 10.1055/s-0029-1216983, 3571-3578.
33. J. Sherwood, M. De Bruyn, A. Constantinou, L. Moity, C. R. McElroy, T. J. Farmer, T. Duncan, W. Raverty, A. J. Hunt and J. H. Clark, *Chem. Commun. (Cambridge, U. K.)*, 2014, **50**, 9650-9652.
34. A. A. C. Pacheco, J. Sherwood, A. Zhenova, C. R. McElroy, A. J. Hunt, H. L. Parker, T. J. Farmer, A. Constantinou, M. De Bruyn, A. C. Whitwood, W. Raverty and J. H. Clark, *ChemSusChem*, 2016, **9**, 3503-3512.
35. L. M. M. Mouterde, F. Allais and J. D. Stewart, *Green Chem.*, 2018, **20**, 5528-5532.
36. A. F. Reano, J. Cherubin, A. M. M. Peru, Q. Wang, T. Clement, S. Domenek and F. Allais, *ACS Sustainable Chem. Eng.*, 2015, **3**, 3486-3496.



View Article Online
DOI: 10.1039/D0GC00122H

Cu(I)Br (0.1 eq), pyridine (0.76 eq)
Cyrene™, air, 51.5 °C, 7 h

Yield: 87-91%, 7 examples



Extended conjugation enhances both anti-UV and antiradical activities