Article

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Synthesis of Lipophilic Antioxidants by a Lipase B Catalyzed Addition of Peracids to the Double Bond of 4-Vinyl-2-Methoxyphenol

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Abstract

4-vinyl guaiacol **2** was lipophilized through the electrophilic addition of peracids to its vinylic double bond. Those peracids were formed in situ; by the *Candida antarctica* lipase B assisted perhydrolysis of carboxylic acids ranging from C2 to C18, in hydrogen peroxide solution. The addition of peracids with 4 to 8 carbons in their alkyl chains led to the formation of two regioisomers with the prevalence of hydroxyesters bearing a primary free hydroxyl (**4c** to **4e**). This prevalence became more pronounced when peracids with longer alkyl chains (C10 to C18) were used. In this case, only isomers **4f** to **4h** were formed. The antioxidant activity of the resulting hydroxyesters was assessed by means of CAT assay and it was found out that the 4-vinyl guaiacol antioxidant activity was significantly increased by grafting alkyl chains with 2 to 8 carbons.

Keywords: lipophilization, vinylphenols, electrophilic addition, antioxidant activity.

Introduction

Ubiquitous in plant kingdom and widely distributed in our diet, phenolics are the most abundant secondary metabolites of plants, with more than 8000 phenolic structures currently known. Ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins, they have been considered as excellent natural antioxidants.[1-3]

Antioxidants are increasingly of interest in food, cosmetic and pharmaceutical industries. For instance, in food applications, they retard the oxidative degradation of unsaturated fats and oils, preserving the food quality and improving its nutritional value.[4, 5] However, the major drawback of the use of phenolic antioxidants in oil based food processing, is their poor lipophilic character. Hence, grafting a lipophilic moiety to these hydrophilic compounds could be a good alternative to enhance their solubility and antioxidant properties in lipidic media. This transformation commonly known as lipophilization is a widely recognized method for expending the applications of phenolic derivatives to fats/oil and lipid-containing foods and formulations.

Within the phenolic compounds family, hydroxycinnamic acids (phenolic acids) and their derivatives which occur abundantly in fruit, vegetables, cereals and spices, possess interesting biological properties[6] and antioxidant potency.[7, 8] Several studies, dealing with the enzymatic lipophilization of these phenolics by esterification and transesterification reactions, have been reported. These studies mainly focused on the catalytic role of lipase B from *Candida antarctica* in the esterification of phenolic acids with aliphatic alcohols in organic solvents [9-11] or solvent free media. [12-14] However, many of these lipase catalysed esterifications exhibited low conversion yields and/or reaction rates (except with cinnamic acid), or led to high yield only when ethanol and activated vinyl hydroxycinnamates were used as substrates.

Beside esterification and transesterification, lipase B from *Candida antartica* (CAL-B) is an excellent catalyst for a wide range of chemical reactions[15-17] including, among others, olefins epoxidation. Indeed, in the presence of carboxylic acids, it acts as a perhydrolase which catalyzes the reversible formation of peracids in aqueous hydrogen peroxide solutions.[18] To achieve the epoxidation reaction, the electrophilic peracids, produced *in situ,* have to carry the oxygen to the nucleophilic partner, the alkenes. However, in the presence of strongly nucleophilic alkenes, peracids are able to modify their usual function as oxygen carrier. Indeed, it was reported by Chen et al. [19] that the reaction of O-benzyl ferulic acid with meta-chloroperbenzoic acid (mCPBA) in dichloromethane, resulted in the peracid 1,2-addition to the phenolic compound double bond. This electrophilic addition was attributed to the presence of the O-benzyl and O-methyl substituents on the aromatic ring, which increase the electronic density and thus the nucleophilic property of the double bond.

Within the hydroxycinnamic acids derivatives, vinylphenols are perfectly tailored to undergo such reactions. Indeed, the vinyl side chain conjugated to the phenolic ring (which mostly bears electron-releasing groups) enhances the vinyl double bond nucleophilicity and peracids addition might be envisioned. 4-vinylphenol derivatives such as 4-vinylcatechol, 4-vinyl guaiacol and canolol (4-vinyl syringol) are natural compounds widespread in plant kingdom[20, 21]. In industry, these substances are treated as food additives and are approved as flavouring agents by regulatory agencies.[22] They are also involved in resins, elastomers and adhesives production. [23] However, no study has examined the possibility to produce lipophilic antioxydants from vinylphenols.

In the present work, the strong nucleophilic character of vinylphenols has been exploited to propose an original way for lipophilization by means of peracids. As a vinylphenols representative, 4-vinyl guaiacol **2** was selected to undergo this transformation.

Thus, The perhydrolysis activity of lipase B from *Candida antarctica* [24] contributed to produce a set of peracids, with different alkyl chain lengths, which were grafted by electrophilic addition to the 4-vinyl guaiacol double bond. Subsequently, the antioxidant activity of 4-vinyl guaiacol and its resulting hydroxyesters was evaluated using the conjugated autoxidizable triene (CAT) assay. The synthesized hydroxyesters can be used as effective antioxidants in multiphase food products and pharmaceuticals.

Material and methods

Chemicals

Vanillin (99%), malonic acid (99%), piperidine (≥99.5%), butanoic acid (≥99%), hexanoic acid (≥98%), octanoic acid(≥98%), decanoic acid (≥98%), dodecanoic acid (≥98%), octadecanoic acid (≥95%), tert-butyldimethysilyl chloride (97%), imidazole (≥99%), immobilised lipase B from *Candida antarctica* (novozym 435), hydrogen peroxide (30 wt.% in H_2O), Tung oil from *Aleurites fordii* seeds (average MW = 872 $g.mol^{-1}$), Phosphate buffer solution pH 7.2 (PBS), polyoxyethylene(23)lauryl ether (Brij 35, estimated MM = 1198 $g.mol^{-1}$), (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich France. 2,2′-Azobis(2-methylpropionamidine) dihydrochloride (AAPH) was obtained from Wako Chemical, (Neuss, Germany), Glacial acetic acid was purchased from Merck France and hexadecanoic acid was supplied by SEPPIC France. All solvents were analytical or HPLC grade (Sigma-Aldrich France) and were used as received.

All the reactions were monitored by TLC performed on silica gel 60 F254 (provided by Merck). Excepted for **2**, all compounds were dissolved in $CDCl_3$ and analysed using a Brucker 400 MHz spectrometer, operating at 400 MHz for ^1H and 100 MHz for ^{13}C. Chemical shifts were referenced using residual internal CH_3Cl signals at 7.26 ppm and 77.0 ppm for ^1H and

^{13}C respectively. Compound **2** solution in DMSO-d_6 was analysed on Agilent VNRMS DD2 500MHz spectrometer, operating at 500,05 MHz for ^{1}H and 125.75 MHz for ^{13}C, using a 5mm indirect detection Z-gradient probe. The chemical shifts were reported to that of internal DMSO at 2.5 ppm and 39.5 ppm for ^{1}H and ^{13}C respectively. Assignments of both proton and carbon resonances, identification and structure characterization of products were performed using both 1D and 2D NMR spectrum analysis, homonucluear ^{1}H and heteronuclear $^{1}H/^{13}C$ experiments. Elemental analyses were performed by combustion on Flash EA 1112 (thermoFinnigan 2003). HRMS analyses were performed by a Xevo-G2-XS Q-Tof (from Waters). Statistical analysis (One-way ANOVA) of CAT values was done using JMP software v.12. (SAS Institute, USA). The means were then compared using Student's t-test. The significance level α was set to 0.05.

Protection of the phenolic hydroxyl of 4-vunyl guaiacol 2

Under argon, a solution of tertbuthyldimethylsilyl chloride (1.2 mmol, 0.18 g) in DMF (2 mL) was added dropwise to a stirred solution of 4-vinyl guaiacol **2** (1 mmol, 0.15 g) and imidazole (2.5 mmol, 0.17 g) in DMF (3 mL). Stirring was maintained 26 h at room temperature and then water (25 mL) and ethyl acetate (25 mL) were added. The aqueous layer was separated and extracted with ethyl acetate (2x25 mL). The combined organic layers were washed with brine (30 mL), dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. Flash chromatography purification (petroleum ether/ethyl acetate 90:10) afforded:

Tert-butyl(2-methoxy-4-vinylphenoxy)dimethylsilane **3**: colourless oil, 85% yield (0.83 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.18 (s, 6H, H12), 1.02 (s, 9H, H10), 3.84 (s, 3H, H9), 5.14 (dd, *J*= 10.8, 0.91 Hz, 1H, H8), 5.61 (dd, *J*= 17.6, 0.91 Hz, 1H, H8), 6.65 (dd, *J*= 17.6, 10.9 Hz, 1H, H7), 6.81 (d, *J*= 8.1 Hz, 1H, H6), 6.88 (dd, *J*= 8.2, 1.9 Hz, 1H, H5), 6.94 (s, 1H, H2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 4.6 (C12), 18.4 (C11), 25.7 (C10), 55.4

(C9), 109.6 (C2), 111.7 (C8), 119.4 (C6), 120.4 (C5), 131.7 (C1), 136.7 (C7), 145.1 (C4), 151.0 (C3) ppm. [M+ H^+] calcl. 265.1504, found. 265.1510. $C_{15}H_{24}O_2Si$ calcd. C 68.13, H 9.15, Si 10.62; found. C 70.02, H 8.89, Si 11.31.

Lipophilization of the silylated vinyl phenol 3

In 50 mL round bottom flask equipped with magnetic stirrer, 2 mmol of carboxylic acid, 3 mmol of hydrogen peroxide 30% (0.34 mL) and Novozym 435 (10 wt% relative to the weight of the silylated vinyl phenol, 0.026 g) were mixed in 2 mL of toluene. After 30 min at 40°C, a solution of compound **3** (1 mmol, 0.26 g) in toluene (1 mL) was added to the reaction medium over 2 h. The reaction was monitored by TLC. After the complete consumption of the peracid, catalyst beads were removed from the reaction mixture by filtration, 30 mL of water and 30 mL of ethyl acetate were added to the filtered reaction mixture. The aqueous layer was separated and extracted with ethyl acetate (2x30 mL). The combined organic layers were washed with brine (40 mL), dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The phenolic group deprotection was performed on the crude products.

Deprotection of the phenolic hydroxyl

The crude product formed from the reaction of compound **3** with peracids (1 mmol) was diluted in 2 mL of dry THF and the solution was cooled down to 0°C. Under argon, a solution of TBAF (1.2 mmol) in 2 mL of dry THF was added dropwise to the previous solution. After 3h at 0°C, water (25 mL) and ethyl acetate (25 mL) were added. The aqueous layer was separated and extracted with ethyl acetate (2x25 mL). The combined organic layers were washed with brine (30 mL), dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. Flash chromatography purification (petroleum ether/ethyl acetate 90:10 to 60:40) afforded β-hydroxyesters **4a** to **4h** and α-hydroxyesters **5b** to **5e**. For example, the electrophilic addition of octanoic peracid to compound **3**, gave rise:

2-hydroxy-1-(4-hydroxy-3-methoxyphenyl)ethyl octanoate **4d**. Yellow oil, 46% yield (0.45 mmol).^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, *J*= 6.9 Hz, 3H, H17), 1.26-1.1.29 (m, 8H, H16-H13), 1.62-1.66 (m, 2H, H12), 2.38 (t, *J*= 7.40 Hz, 2H, H11), 3.78 (dd, *J*= 11.90, 4.30 Hz, 1H, H9α), 3.86 (m, 1H, H9β), 3.90 (s, 3H, H7), 5.65 (s, 1H, OH aliphatic), 5.79 (dd, *J*= 7.60, 4.30 Hz, 1H, H8), 6.86 (d, *J*= 8.46 Hz, 1H, H6), 6.89 (d, *J*= 7.80 Hz, 1H, H5), 6.91 (s, 1H, H2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 14.0 (C17), 22.5 (C16), 25.0 (C12), 28.8 (C15), 29.0 (C14), 31.6 (C13), 34.5 (C11), 55.9 (C7), 66.0 (C9), 76.5 (C8), 109.5 (C2), 114.5 (C5), 119.8 (C6), 129.0 (C1), 145.8 (C4), 146.5 (C3), 173.5 (C10) ppm. [M+ H^+] calcl. 311.1821, found. 311.1835. $C_{17}H_{26}O_5$ calcd. C 65.78, H 8.44; found. C 65.67, H 8.51.

2-hydroxy-2-(4-hydroxy-3-methoxyphenyl)ethyl octanoate **5d**. Yellow oil, 30% yield (0.29 mmol).^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.89 (t, *J*= 6.9 Hz, 3H, H17), 1.28-1.33 (m, 8H, H16-H13), 1.64 (m, 2H, H12), 2.36 (t, *J*= 7.50 Hz, 2H, H11), 3.90 (s, 3H, H7), 4.16 (dd, *J*= 11.50, 3.58 Hz, 1H, H9α), 4.25 (dd, *J*= 11.50, 3.50 Hz, 1H, H9β), 4.89 (dd, *J*= 8.40, 3.40 Hz, 1H, H8), 5.66 (s, 1H, OH aliphatic), 6.86 (dd, *J*= 8.50, 1.80 Hz, 1H, H6), 6.89 (d, *J*= 8.10 Hz, 1H, H5), 6.94 (s, 1H, H2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 14.0 (C17), 22.35 (C16), 24.9 (C12), 28.8 (C15), 29.0 (C14), 31.6 (C13), 34.2 (C11), 55.9 (C7), 69.2 (C9), 73.3 (C8), 108.5 (C2), 114.3 (C5), 119.2 (C6), 131.8 (C1), 145.6 (C4), 146.7 (C3), 174.0 (C10) ppm. [M+ H^+] calcl. 311.1821, found. 311.1817. $C_{17}H_{26}O_5$ calcd. C 65.78, H 8.44; found. C 65.91, H 8.36.

The other NMR data are displayed in the supplementary information.

Determination of the antioxidant capacity using the Conjugated Autoxidizable Triene (CAT) assay.

Prior to use, commercial tung oil was stripped (removing of polar compounds including tocopherols) as follows. In a glass column packed with 25 g of alumina, 25 ml of a tung oil solution in *n*-hexane (200 mg.mL^{-1}) were eluted followed by 25 mL of hexane. Then, hexane

was evaporated under vacuum and the stripped tung oil was aliquoted into brown glass tubes, inerted under nitrogen stream, and stored at −18 °C until use. All experiments were performed under shelter from light, as much as possible.

Fresh solutions of Trolox and phenolic at concentrations ranging from 0.4 to 4.0 mmol.L^{-1} were prepared in chloroform/methanol (2:1), then diluted in methanol in order to obtain a concentration range of 0.1 to 1.0 μmol.L^{-1}, and stored in amber vials. 50 μL of these solutions were transferred to a 96-well microplate (Greiner, Frickenhausen, Germany) which was then preheated to 37 °C and shook in a thermostated shaker (PHMT Grant Instruments Ltd., Shepreth, England) for 5 min at 1200 rpm.

Twenty-five milliliters of PBS containing Brij 35 (34 μmol.L^{-1}) were added to 5 mg of stripped tung oil into an amber glass vial. Thereafter, this pre-mixture was vortexed for 10 seconds prior to homogenization in an Ultra Turrax homogenizer (Janke & Kunkel, Staufen, Germany) at 2400 rpm for 90 seconds. Each well of the microplate was supplemented with 100 μL of this emulsion. To enhance repeatability, the microplate was immediately pre-heated and maintained under stirring in a thermostated shaker (PHMT Grant Instruments Ltd.) at 37 °C for 1 min at 1200 rpm.

Fifty microliters of a solution of AAPH in PBS (4 mmol.L^{-1}), prepared immediately before reading, were added into wells. Finally, each well contained a final volume of 200 μL, with 115 μmol.L^{-1} tung oil, 17 μmol.L^{-1} Brij 35, 1 mmol.L^{-1} of AAPH and antioxidants at various concentrations (0.25 to 1.0 μmol.L^{-1}). The kinetics of the reaction were immediately assessed by monitoring absorbance decay at 273 nm. Measurements were taken every minute within 6 hours at 37 ± 0.5 °C with shaking the plate 5 seconds before each reading, using a microplate reader Model M1000 (Tecan, Groedig, Austria) equipped with software Magellan.

All measurements were performed in triplicate and reported as average ± SD. To normalize data, the raw absorbance values were expressed in relative absorbance according to equation 1:

$$\text{Relative absorbance} = \frac{At}{A0} \qquad \text{Eq.1}$$

Where A_t and A_0 are the absorbances read at time t (min) and t0 (min), respectively. The area under the curve (AUC) corresponding to the decay of relative absorbance was calculated thanks to equation 2:

$$AUC = 1 + \frac{A1}{A0} + \frac{A2}{A0} + \cdots + \frac{A359}{A0} + \frac{A360}{A0} \qquad \text{Eq.2}$$

The chart area provided by an antioxidant sample was then calculated using the difference between AUC in the presence of a sample of antioxidant (AUC_{sample}) and the blank (AUC_{blank}) corresponding to a solution without antioxidant. Trolox was used as internal calibration standard. Thus, the antioxidant capacity (CAT value) of a sample was given in Trolox equivalents and calculated by equation 3:

$$\text{CAT value} = \frac{(\text{AUC sample} - \text{AUC blank})}{(\text{AUC Trolox} - \text{AUC blank})} \times \frac{(\text{moles of Trolox})}{(\text{moles of sample})} \qquad \text{Eq.3}$$

Where AUC_{sample}, AUC_{blank} and AUC_{Trolox} are the areas in the graph where the protection of tung oil occurs in the presence of a sample, the antioxidant-free solution and the solution containing the Trolox, respectively. The CAT value is expressed in moles of Trolox per moles of the tested compound, i.e. in Trolox equivalents.

Results and discussion

In order to produce antioxidant 4-vinyl-guaiacol derivatives with increased lipophilicity, the following four-step synthesis route was adopted: i) 4-vinyl-guaiacol **2** production from the reaction of vanillin ii) The protection of the compound **2** phenolic hydroxyl by silyl-

etherification (to avoid the undesirable oxidation or polymerisation side-reactions). iii) The electrophilic addition of the *in situ* generated peracids (with different chain length) to the protected 4-vinyl-guaiacol **3**. iv) The phenolic hydroxyls deprotection.

Synthesis of 4-vinyl-guaiacol 2 and protection of its hydroxyl group

Generally, the availability of vinylphenols in natural sources does not meet the industrial requirements. Hence, several chemical syntheses of these phenolic compounds, including catalytic dehydrogenation of ethylphenols, Wittig reaction or the hydroxycinnamic acids decarboxylation, have been described[25]. However, a majority of these procedures required expensive reagents and harsh conditions to eventually give rise to the products in low yields. Recently, an efficient synthesis of these compounds by Knoevenagel-Doebner[25] reaction in mild conditions was described by Sinha et al. Hence, this synthetic pathway was adopted to produce 4-vinyl-guaiacol **2** from vanillin **1**.

Subsequently, the 4-vinyl-guaiacol hydroxyl group was temporary masked to avoid the competitive oxidation and polymerisation side-reactions. Among several protecting groups, *tert*-butyldimethylsilyl (TBDMS) was selected because of its stability in a variety of organic reactions and the facility by which it can be introduced and removed under mild conditions.[26, 27] The treatment of Compound **2** with *tert*-butyldimethylsilyl chloride and imidazole at room temperature led to the silyl-ether derivative **3** with 85% yield (figure 1).

Lipophilization of 3 by electrophilic esterification

As reported in the literature, various reaction parameters can influence the lipase activity and thus the peracids formation. Among several organic solvent tested the highest yields of peracids were observed using toluene or hexane.[28] Törnvall *et al*.[29] and Orellana-Coca *et al.*[24] investigations have demonstrated that elevated temperatures (above 60°C) together with

hydrogen peroxide at high concentrations (6M-12M) resulted in a rapid loss of enzyme activity. Taking into account these different data, the vinylic derivative **3** was reacted with caprylic peracid (octanoic peracid) in a two-phase reaction. First, the CAL-B catalyzed perhydrolysis of caprylic acid was initiated at 40 °C during 30 min in toluene. Then, the solution of compound **3**, in the same solvent, was added to the previous mixture over 2 hours. During the whole reaction, the hydrogen peroxide concentration did not exceed 1M.

After 6 hours of reaction and the usual work up (described in the experimental part), the crude product was treated with tetra-N-butylammonium fluoride (TBAF) in THF at 0 °C, allowing the silyl ether cleavage and the hydroxyl group recovery within 3 hours.

Instead of the expected epoxidized products, hydroxyester derivatives **4** and **5** were produced in 76% overall yield (figure 2).

The addition of decanoic peracid to compound **3** was proved by NMR. Indeed, the characteristic proton signals of the vinylic double bond, at 5.07, 5.64 and 6.67 ppm disappeared and were replaced by α-hydroxyl (3.78-3.86 ppm for **4** and 4.89 ppm for **5**) and α-ester (5.79 ppm for **4** and 4.16-4.25 ppm for **5**) proton signals. The appearance of shielded aliphatic proton signals on ^{1}H NMR spectrum and the ester carboxyl signal on ^{13}C NMR spectrum indicated the grafting of the carboxylic acid moiety on **3**.

Based on these results, other carboxylic acids (ranging from C2 to C18) were used to assess the reactivity of compound **3** with the *in situ* generated corresponding peracids and to produce phenolic hydroxyesters with different side chain lengths. The reaction products are depicted in both figure 3 and table 1.

Considering the results displayed in table 1, two main aspects should be discussed. (i) the peracid addition to the vinylic double bond instead of its epoxidation. (ii) the arising of two

addition isomers with the predominance of the derivatives bearing a primary alcohol function (**4a**-**4h** derivatives).

The peracid addition to the double bond of compound 3

Generally, in the chemo-enzymatic oxidation of olefins, the lipase catalyzes the formation of peracids, from the perhydrolysis reaction between carboxylic acids and an oxidizing agent such as H_2O_2, which then donate an oxygen atom to the double bond affording the corresponding epoxide and regenerating the carboxylic acids. The epoxidation step occurs through a concerted transition state, also known as a symmetry butterfly transition state. The bond between the oxygen and the alkene is being formed at the same time that the peroxy bond is breaking and the peracid proton is transferred from the hydroxyl group to the carbonyl oxygen.

However, in the present instance, the generated peracids did not achieve their expected task as oxygen carrier, but an electrophilic addition, rather occurred to the vinyl double bond of **3**. As previously mentioned, the presence of the electron-releasing groups OTBDMS and O-methyl on the aromatic ring of **3** contribute, by mesomeric effect to enhance the electron density on the vinylic double bond. The electrophilic peroxide oxygen of peracid attacks the double bond and form a three membered cyclic transition state **I**[19] (figure 4). Then, the electron rich carboxylate group of peracid attacks either C7 or C8 carbons leading to the simultaneous cleavage of the bonds between these carbons and the peroxide oxygen and the formation of compounds **4** and **5**. The intervention of the electron-releasing groups to orientate the reaction mechanism towards peracids addition is confirmed by Sarma et al[30] who demonstrated that styrene derivatives, with electron-withdrawing substituents, are rather epoxidized in the presence of peracids.

The peracids addition products

As displayed in table 1, excluding compounds **4a**, **4b** and **5b**, the addition products of peracids on the vinylic double bond of **3** after hydroxyls deprotection were obtained with fair yields ranging from 68% to 77%. The reaction of both ethanoic and butanoic peracids with **3** required a longer time and a higher amount of enzyme to afford addition products with relatively lower yields (entry 1 and 2). This is likely due to the peracids stability which is highly dependent on their molecular weight. Indeed, the smaller are the peracids, the faster is their decomposition; in particular, methanoic and ethanoic peracids are highly unstable[31].

Despite the lower reaction yield of butanoic peracid, this latter led to the formation of two addition products **4b** and **5b** with a slight predominance of the derivative bearing a primary alcohol function **4b**. The same trend was observed for hexanoic, octanoic and decanoic peracids, indicating that from C4 to C8, peracids exhibit the same reactivity towards compound **3**. Although the predominance of **4-**type products is not very significant; it results from the addition of the peracid electrophilic oxygen on the most nucleophilic double bond carbon.

From dodecanoic to octadecanoic peracids (entries 6 to 8), only the **4**-type products were formed. The presence of a long alkyl chain in the reaction intermediate **I** seems to further polarize the C7-C8 bond, localizing the negative charge on C8. Hence, only the electron deficient C7 was attacked by the carboxylate group.

Owing the good enzyme affinity to dodecanoic acid,[28] its corresponding peracid afforded the addition product (**4f**) with the highest yield (77%) in a relatively short time (entry 6).

This particular reactivity of peracids allowed the production of a set of phenolic hydroxyesters with side chain lengths ranging from C2 to C18. To obtain equivalent reaction yields, the other strategies based on the lipase B catalyzed esterification and

transesterification; generally require harsher operational conditions, like a longer reaction time (7 to 30 days) and higher temperatures (50 to 60°C).[32]

After synthesis, the antioxidant potency of these lipophilized phenolics was assessed by CAT assay.

Determination of the antioxidant capacity of lipophilized derivatives of 4-vinyl guaiacol

In this study, the CAT assay was used to evaluate the antioxidant capacity of the synthesized phenolic hydroxyesters. The triacylglycerols of tung oil are mainly (ca. 85 %) comprised of alpha-eleostearic acid ((9Z,11E,13E)-9,11,13-octadecatrienoic acid). Owing to the presence of a conjugated triene, this fatty acid strongly absorbs at 273 nm and is highly prone to oxidation. Consequently, under oxidizing conditions, a degradation of the conjugated triene may be simply observed upon the signal loss at 273 nm. These properties allow for an efficient, easy, rapid and direct assessment of lipid oxidation in heterophasic system. Thus, the CAT assay provides relevant screening of antioxidant abilities of molecules, since it is representative of unsaturated fatty acids oxidation pathways that occur in foods and biological systems [33]. As depicted in both table 1 and figure 5**,** the antioxidant ability of 4-vinyl guaiacol was significantly affected by the grafting of the lipophilic moiety (alkyl chain of variable length). Moreover, the isomer nature (phenolic ester bearing a primary or a secondary hydroxyl) may significantly affect the antioxidant activities in such heterogeneous medium. Indeed, isomers exhibiting the secondary alcohol function presented lower antioxidant activities, with only a slight enhancement for the butyl-ester (CAT value = 0.49 ± 0.01) in comparison with 4-vinylguaiacol (CAT value = 0.40 ± 0.03). One may argue that the difference between the two regioisomers might be explained by the negative inductive effect (-I) of the free aliphatic hydroxyl group. Thus, the one closest to the benzene ring would higher disrupt the resonance stabilization of the phenoxyl radicals, resulting in lower antioxidant ability and CAT value. As far as the phenolic esters with primary free hydroxyl

(obtained with higher yields), significant improvement in the antioxidant activity was observed with the addition of 2, 4, 6 and 8 carbons in the ester alkyl chain (**4a**, **4b**, **4c** and **4d** respectively) when compared to 4-vinylguaiacol. The non-lipophilized molecule **2** presented a CAT value of 0.40 ± 0.03, while compounds **4a** to **4d** had CAT values of 0.80 ± 0.05, 0.49 ± 0.04, 0.55 ± 0.01 and 0.57 ± 0.03, respectively. Conversely, their counterparts with 10, 12, 16 and 18 carbons in the alkyl chain (**4f**, **4g** and **4h** respectively) showed lower antioxidant ability in comparison with 4-vinylguaiacol. The order of antioxidant capacity was: C4 > C8 ≈ C6 > C2 >> C10 ≥ C12 ≈ C16 > C18, and a clear pattern with respect to the effect of alkyl chain length on the antioxidant ability was observed. Indeed, the effectiveness of antioxidants has been shown to increase as a function of increasing alkyl chain length to a critical point (4 carbons), followed by steady decrease beyond that point when more hydrophobic moieties are linked. This nonlinear effect named "cut-off effect" has already been reported in literature with similar experiments performed with series of alkyl chlorogenates, rosmarinates, ferulates and coumarates [34-37]. In these studies, the antioxidant activities were drastically improved with increasing alkyl chain lengths until an optimized activity ranging between 8 to 12 carbon atoms. In both series of esters synthesized from 4-vinylguaiacol, the highest antioxidant capacity was obtained with an alkyl chain of 4 carbons.

As previously observed,[38, 39] the intrinsic chemical reactivity of an antioxidant toward oxidizing species is not the only factor that governs its activity, this feature being in fact multifactorial. Suitable location, position, mobility, and concentration of antioxidant at the interface, but also its reactivity towards free radical can drastically change antioxidant efficiency. It is now accepted that lipophilization mostly affects antioxidant properties by changing the molecule's partitioning. In such lipid-based dispersions, the antioxidant molecule may distribute in the aqueous or in the lipid phase as well as in the intermediate pseudophase (interface) as monomer, micelle or aggregate forms, which results in a different

reactivity. Thus, the antioxidant activity of the lipophilized molecules synthesized from 4-vinylguaiacol can be explained by their polymorphism and partitioning in the present environment. The first group, composed by the ethyl ester (**4a**), tends to partition within the aqueous phase with limited interaction with the interface of the lipid droplets where oxidation occurs. Consequently, it displays CAT value equal to the native molecule because of their equivalent polarity (table 1).The second group formed by the butyl ester (**4b**) showed the highest CAT values. This may be explained by a better location of this molecule at the oil/water interface, promoting oil protection against the oxidizing species (R-C·, R-COO·, etc…) generated by the azo initiator. When the chain length was increased beyond this limit, the amount of esters in the intermediate phase is expected to decrease while the amount in the lipid phase is supposed to increase, which partly explain the diminution in the CAT values in the third group composed with medium alkyl chains (C6 and C8). Also, these molecules could be found in different polymorphisms: monomers, which may protect oil droplets against oxidation when located at the interface and micelles, which will be only sparsely involved against the lipid droplet oxidation. Finally, the last group composed of esters with 10 or more carbon in the alkyl chain, exhibited very low antioxidant activities because of their high lipophilic character. These compounds are mainly located within the lipid phase, with a limited activity at the interface. Moreover, they might be also physically isolated from emulsion interface through the formation of aggregates (micelles or precipitates) that reduce their concentration in the reactive interphase.

Generally, phenolic compounds lipophilization catalysed by lipase B of *Candida antarctica* is performed through esterification or transesterification reactions. However, it has been shown in this study, that the perhydrolysis activity of lipase B was an efficient way to graft alkyl chains with different lengths on 4-vinyl guaiacol. The chemical structure of this vinylphenol derivative, and particularly its nucleophilic vinylic double bond, contributed to modify the

usual reactivity of peracids leading to the one-pot production of different hydroxyesters by electrophilic addition. Within this addition, the formation of hydroxyesters bearing primary hydroxyl groups was promoted especially when the chain length of the peracid exceeded eight carbons. The assessment of the antioxidant potency of the resulting hydroxyesters demonstrated that the 4-vinyl guaiacol antioxidant activity was enhanced by the grafting of alkyl chains with 2 to 8 carbons. Therefore, this new route of phenolipids synthesis through lipophilization of vinylphenols represents a good strategy to promote and optimize the effectiveness of their natural antioxidant activity in heterophasic system encountered in food emulsions, cosmetics and biological systems.

Supporting information including NMR spectra and HRMS data of the synthesized products as well as the statistical analysis of CAT values is available.

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526 **Figure captions**

527 **Figure 1**: the 4-vinyl-guaiacol synthesis and its phenolic hydroxyl etherification.

528 **Figure 2**: the electrophilic esterification of compound **3** followed by the hydroxyl group
529 deprotection.

530 **Figure 3:** the chemical structures of hydroxyesters **4a** to **4h** and **5b** to **5e**.

531 **Figure 4:** The 1,2-addition mechanism of compound **3** with peracids.

532 **Figure 5:** CAT values of 4-vinylguaiacol (□) and the lypophilized molecules: phenolic
533 hydroxyesters bearing primary free hydroxyl (■) and phenolic hydroxyl esters bearing
534 secondary free hydroxyl (■).Values followed by the same superscript letters are not
535 significantly different ($p \leq 0.05$). Values are mean ± SD (n=3).

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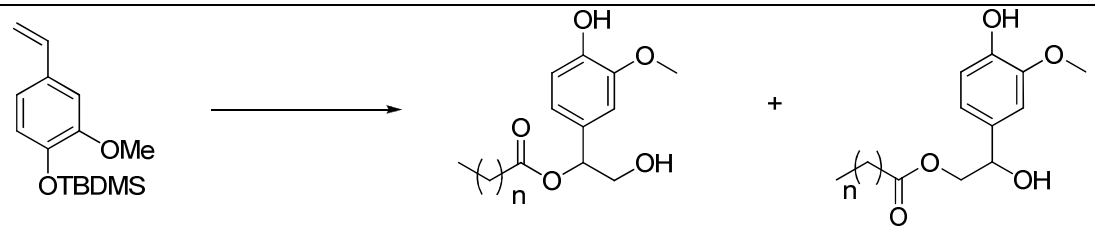
Tables

Table 1: The electrophilic addition products (after the phenolic hydroxyl deprotection) of peracids on compound **3**.



3 **4** **5**

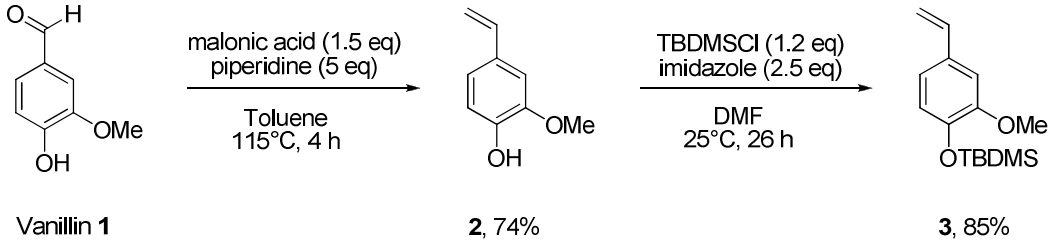
Entry	Acid	Reaction time (h)[b]	products	Products Yield (%)	CAT value[c]
1	Ethanoic acid (n=0)[a]	126	**4a** **5a**	33 -	0.46 ± 0.05 -
2	Butanoic acid (n=2)[a]	102	**4b** **5b**	26 22	0.80 ± 0.04 0.49 ± 0.01
3	Hexanoic acid(n=4)	4	**4c** **5c**	43 32	0.55 ± 0.01 0.19 ± 0.05
4	Octanoic acid (n=6)	6	**4d** **5d**	46 30	0.57 ± 0.03 0.24 ± 0.02
5	Decanoic acid (n=8)	7	**4e** **5e**	43 31	0.24 ±0.04 0.22 ± 0.07
6	Dodecanoic acid (n=10)	6.5	**4f** **5f**	77 -	0.19 ± 0.04 -
7	Hexadecanoic acid (n=14)	24	**4g** **5g**	68 -	0.19 ± 0.02 -
8	Octadecanoic acid (n=16)	20	**4h** **5h**	72 -	0.12 ± 0.02 -

[a] Reactions of compound **3** with hexanoic to octadecanoic acids were performed by loading 10 wt% of enzyme (relative to **3** weight). However, the addition of both ethanoic an butanoic peracids to the compound **3** double bond required 30 wt% and 20 wt% of enzyme respectively.

[b] Reactions were monitored by TLC and stopped after the complete conversion of compound **3**.

[c] CAT value of 4-vinyl guaiacol is 0.40 ± 0.03.

Figures



Vanillin **1** **2**, 74% **3**, 85%

Figure 1

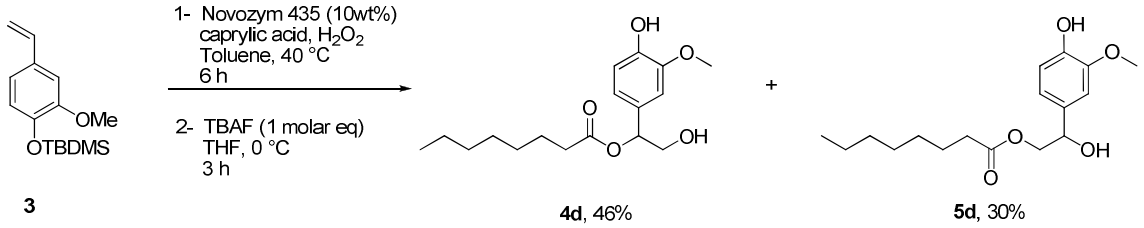

1- Novozym 435 (10wt%)
caprylic acid, H_2O_2
Toluene, 40 °C
6 h
2- TBAF (1 molar eq)
THF, 0 °C
3 h
OMe
OTBDMS
3
OH
O
O
OH
4d, 46%
+
OH
O
O
OH
O
5d, 30%

Figure 2

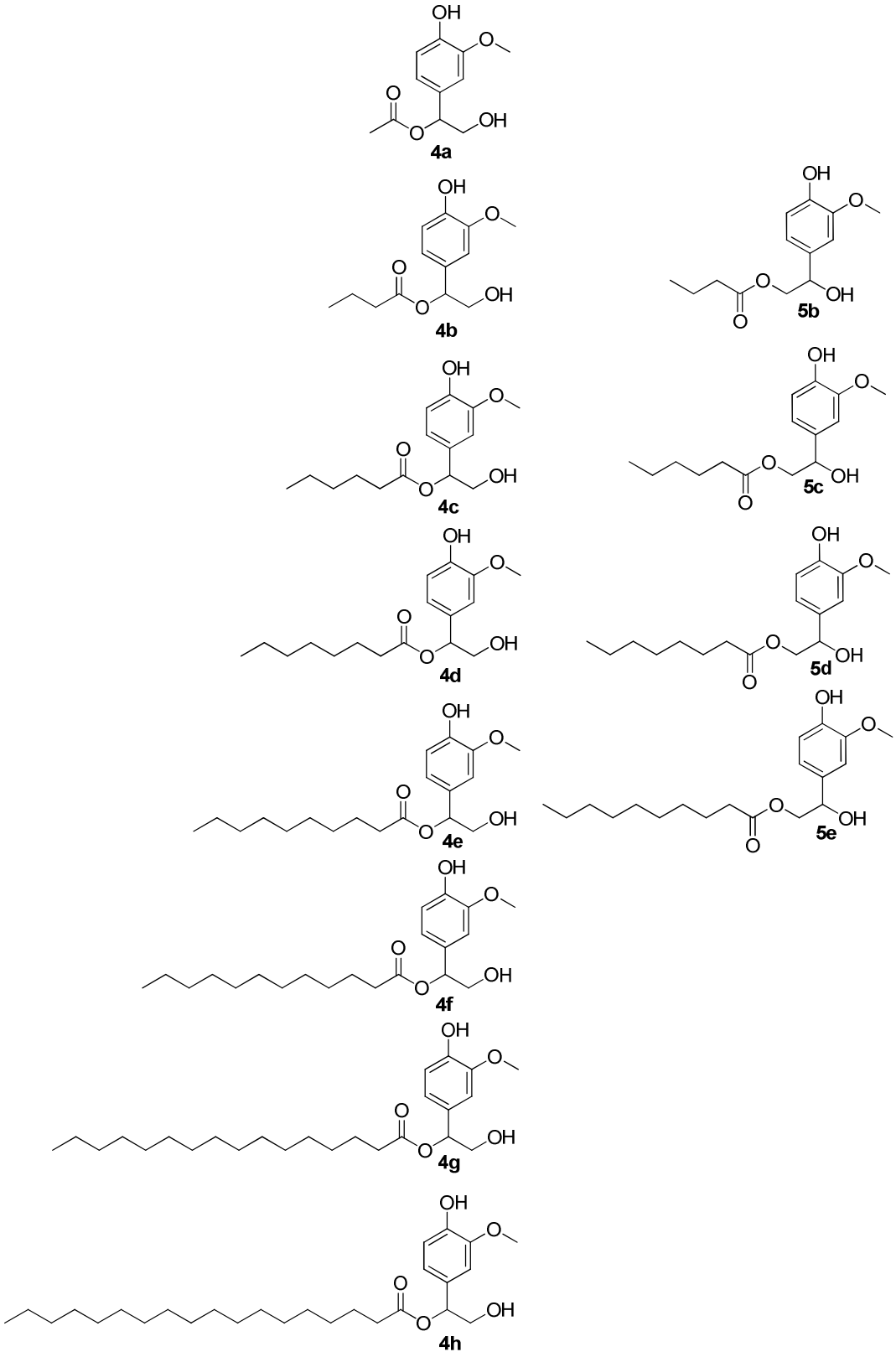


Figure 3

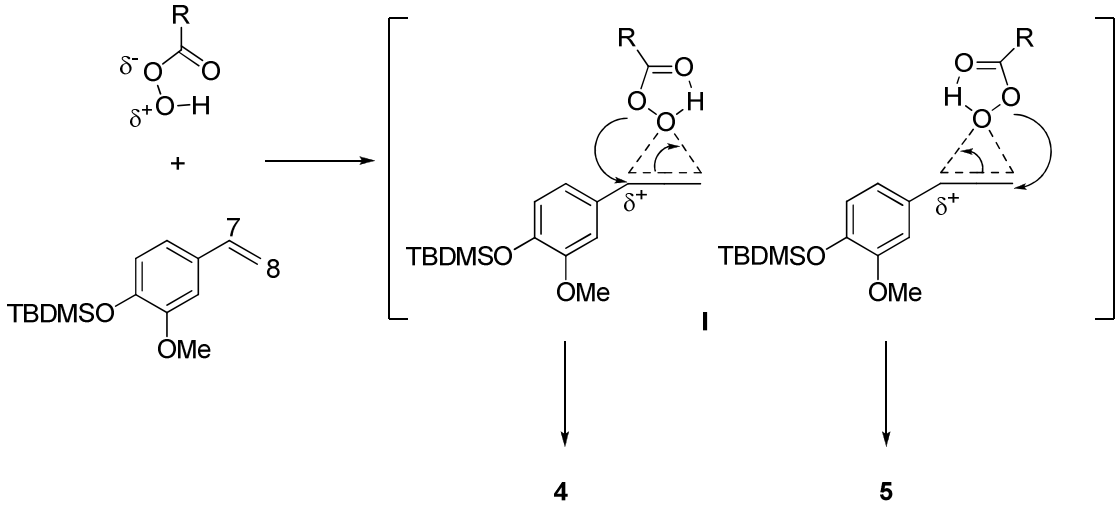

R
δ-
O
O
δ+
O–H
+
7
8
TBDMSO
OMe
R
O
O
H
O
δ+
TBDMSO
OMe
I
R
O
H
O
O
δ+
TBDMSO
OMe
4
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Figure 4

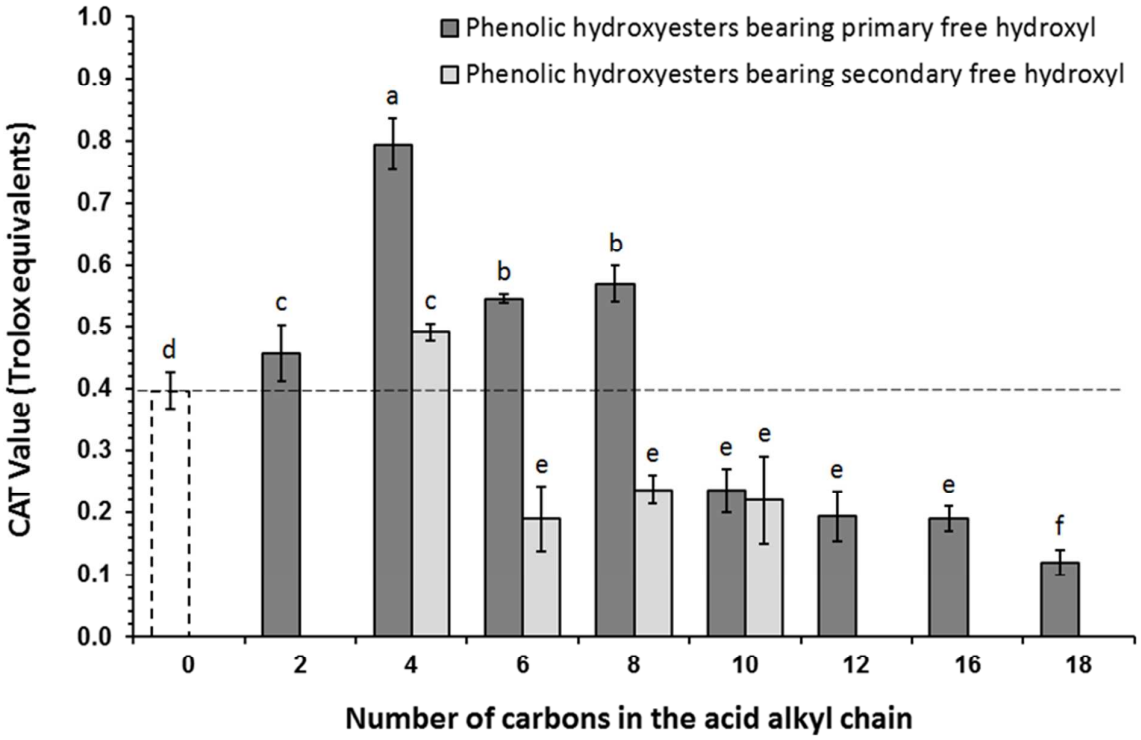
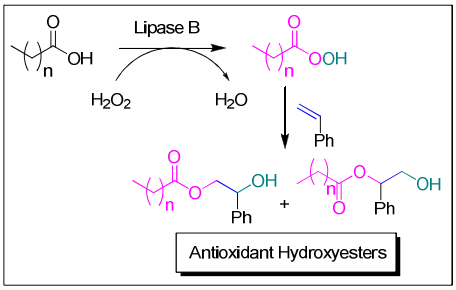

Phenolic hydroxyesters bearing primary free hydroxyl
Phenolic hydroxyesters bearing secondary free hydroxyl
CAT Value (Trolox equivalents)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
a
b
b
c
c
c
d
e
e
e
e
e
e
f
0
2
4
6
8
10
12
16
18
Number of carbons in the acid alkyl chain

Figure 5

TOC Graphic


Lipase B
OH
OOH
n
H_2O_2
H_2O
Ph
OH
Ph
+
OH
Ph
Antioxidant Hydroxyesters