



HAL
open science

Unusual Polycyclic Fused Product by Oxidative Enzymatic Dimerisation of 5-methylpyrogallol Catalysed by Horseradish Peroxidase/H₂O₂

Hélène Bouges, Kevin Calabro, Olivier Thomas, Sylvain Antoniotti

► To cite this version:

Hélène Bouges, Kevin Calabro, Olivier Thomas, Sylvain Antoniotti. Unusual Polycyclic Fused Product by Oxidative Enzymatic Dimerisation of 5-methylpyrogallol Catalysed by Horseradish Peroxidase/H₂O₂. *Molecules*, 2018, 23 (10), pp.2619. 10.3390/molecules23102619 . hal-02357719

HAL Id: hal-02357719

<https://hal.science/hal-02357719v1>


Submitted on 10 Nov 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Communication

Unusual Polycyclic Fused Product by Oxidative Enzymatic Dimerisation of 5-methylpyrogallol Catalysed by Horseradish Peroxidase/H₂O₂

Hélène Bouges ¹, Kevin Calabro ², Olivier P. Thomas ²  and Sylvain Antoniotti ^{1,*}

¹ Institut de Chimie de Nice, Université Cote d'Azur, CNRS, Parc Valrose, 06108 Nice CEDEX 2, France; helene.bouges@unice.fr

² Marine Biodiscovery, School of Chemistry and Ryan Institute, National University of Ireland Galway, University Road, H91 TK33 Galway, Ireland; kevin.calabro@nuigalway.ie (K.C.); olivier.thomas@nuigalway.ie (O.P.T.)

* Correspondence: antoniot@unice.fr

Received: 21 September 2018; Accepted: 10 October 2018; Published: 12 October 2018



Abstract: During investigations on the peroxidase-catalysed oxidation of polyhydroxylated monoaromatic substrates such as 5-methylpyrogallol, we observed a spectacular dimerisation proceeding by dearomatisation in contrast with most common reaction patterns involving phenolics oxidation and dimerization. A tetracyclic fused product featuring an unusual 2-oxatetracyclo [6.3.1.0^{1,6}.0^{4,12}] dodecan-3-one core was obtained and characterized by combined NMR techniques and high resolution mass spectroscopy (HRMS). This is an example of a spontaneous cascade triggered by a simple enzymatic reaction that could provide new options for biosynthetic hypothesis and a synthetic method to access this complex core in one operation.

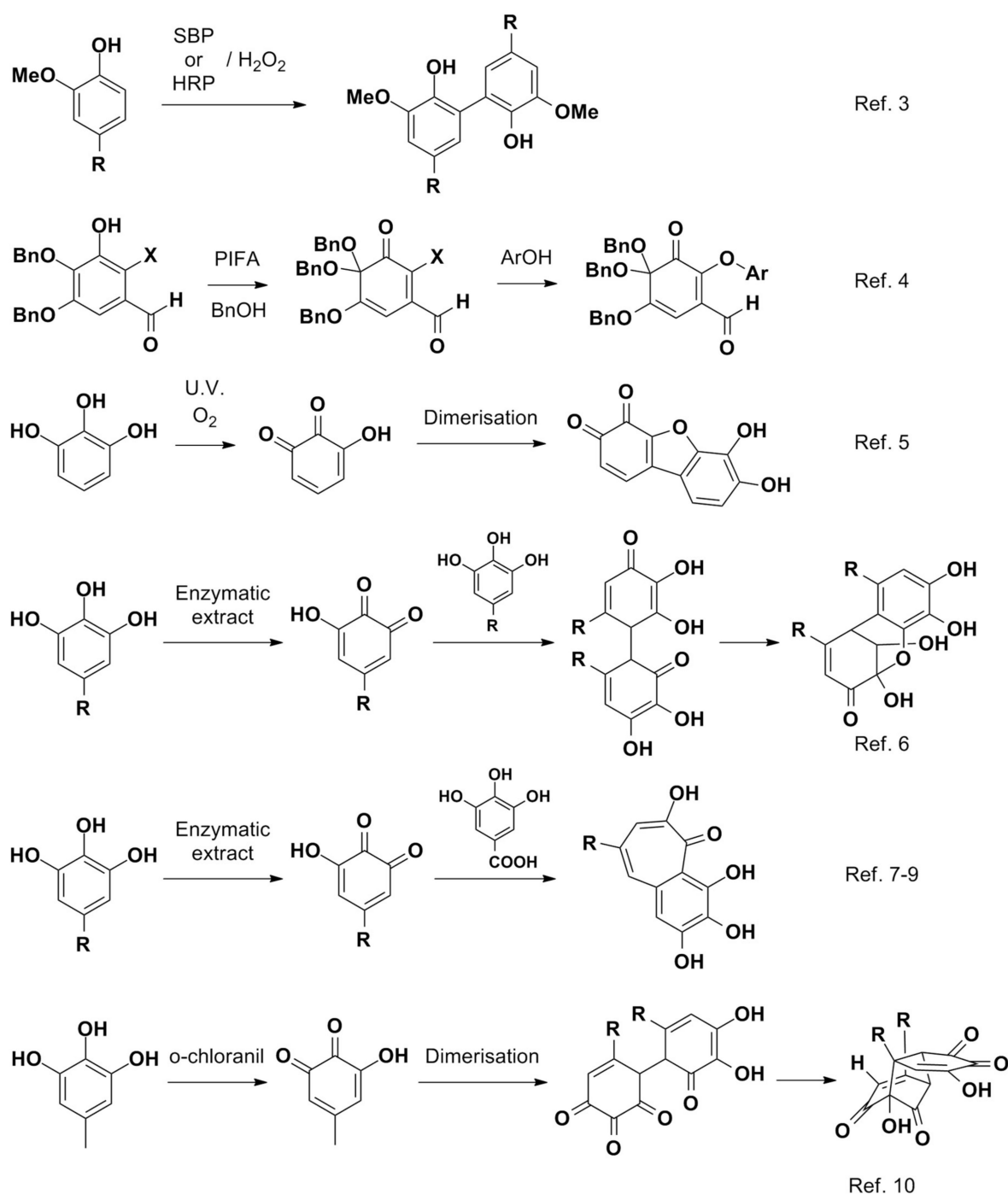
Keywords: cage structure; biocatalysis; phenolics; molecular complexity; sustainable chemistry

1. Introduction

The potential of enzymatic reactions to achieve the synthesis of complex molecules is still largely underexplored despite a broad array of applications in bioengineering. Such green transformations might yield to a useful chemical diversity already present in nature and potentially offering interesting bioactivities. Peroxidases from soybean (SBP) or horseradish (HRP) are known to catalyse the one-electron oxidation of phenol to the corresponding phenoxy radical in the presence of hydrogen peroxide [1]. Since the generated phenoxy radicals can quickly undergo non-enzymatic coupling in solution through C-O or C-C bonding leading to dimers and subsequently higher order oligomers, peroxidase chemistry has been widely used for phenolics manipulation, mostly for polymerisation purposes [2]. In the case of substituted phenolic substrates, electronic and steric effects significantly influence the course of the reaction and various patterns of dimeric coupling can be observed, usually following the C-C *ortho* coupling [3].

Substituted phenol derivatives have also been showed to yield reactive *ortho*-quinones under oxidative conditions, which underwent oxa-Michael addition, elimination and reductive aromatization with other phenolics [4]. In regards of phenolics containing more than two hydroxyl groups, different oxidation patterns and reaction outcomes were reported. For example, pyrogallol dimerised under UV-irradiation and O₂ to yield dibenzofuran derivatives and higher order oligomers [5]. Closely related to pyrogallol, gallate esters led to catechin oligomers when submitted to enzymatic oxidation [6]. The spontaneous oxidation of quercetin under air yielded theaflavins following another reaction pathway [7,8]. This oxidation of quercetin to theaflavins can also be catalysed by laccases [9]. Oxidative dimerisation of methyl gallate in the presence of *o*-chloranil, a tetrachloro-*para*-quinone,

delivered dimethyl dehydrohexahydroxydiphenoates after oxidation of gallate to the corresponding *ortho*-quinone and subsequent C-C bond forming processes (Scheme 1) [10].



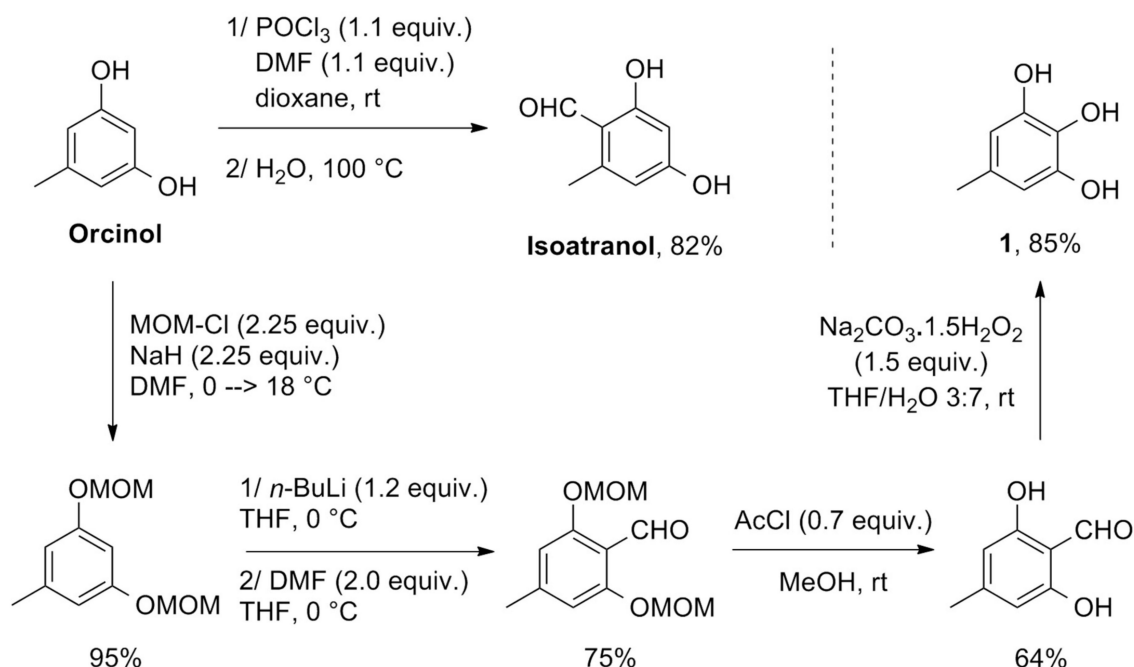
Scheme 1. Most common oxidation products of mono-aromatic phenols.

Interestingly, no study has been reported to our best knowledge on the enzymatic reactivity of other simple mono-substituted pyrogallol derivatives.

2. Results and Discussion

We decided to inspect the behaviour of polyhydroxylated benzenic structures such as 5-methylbenzene-1,2,3-triol (5-methylpyrogallol) in the presence of an isolated oxidative enzyme

such as HRP in the presence of H_2O_2 as oxidant. The synthesis of 5-methylpyrogallol **1** was achieved in four steps from commercially available orcinol by MOM-protection of both hydroxyl groups, *ortho*-lithiation and formylation with DMF, deprotection and Dakin oxidation following literature procedures (Scheme 2) [11,12]. It is worth noting that standard formylation procedure by POCl_3/DMF led regioselectively to isoatranol instead of **1**.



Scheme 2. Synthesis of 5-methylpyrogallol **1** from orcinol.

Testing of HRP-catalysed oxidation was conducted with **1** in the presence of H_2O_2 at pH 9 varying enzyme loading and peroxide stoichiometry, along with relevant control experiments (Table 1). In a representative reaction, the substrate **1** is dissolved in an aqueous sodium carbonate buffer solution at pH 9, containing HRP (1% *w/w*). The reactor is then wrapped with an aluminium foil and an aqueous solution of H_2O_2 is added slowly to the reaction mixture. The resulting solution is stirred at room temperature for 6 h and, after completion of the reaction, a diluted aqueous solution of HCl is added to reach a final pH of 4 and the resulting aqueous phase is finally extracted with EtOAc.

Table 1. Testing of HRP-catalysed oxidation of 5-methylpyrogallol **1**.

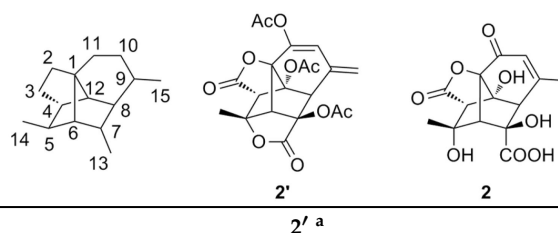
Entry	H_2O_2	HRP	Outcome
1	-	-	Recovery of SM 91%
2	-	1% (<i>w/w</i>)	Recovery of SM 95%
3	2 equiv.	-	Recovery of SM 34%
4	2 equiv.	1% (<i>w/w</i>)	Dimer 2, 89% ^a
5	2 equiv.	2% (<i>w/w</i>)	Dimer 2, 86% ^a
6	2 equiv.	5% (<i>w/w</i>)	Dimer 2, 88% ^a
7	2 equiv.	10% (<i>w/w</i>)	Dimer 2, 91% ^a
8	3 equiv.	1% (<i>w/w</i>)	Dimer 2, 81% ^a
9	4 equiv.	1% (<i>w/w</i>)	Dimer 2, 92% ^a
10	5 equiv.	1% (<i>w/w</i>)	Dimer 2, 87% ^a

^a Obtained after evaporation of the aqueous phase.

In the absence of H_2O_2 and regardless of the presence of HRP, the starting material was recovered unchanged almost quantitatively (entries 1, 2). In the presence of 2 equiv. of H_2O_2 without enzyme, only 34% of starting material was recovered upon extraction with EtOAc (entry 3). Inspection of the

aqueous phase revealed the formation of a mixture of various products that could not be identified. In the presence of both H₂O₂ and HRP, the starting material was totally converted after 6 h and no product was obtained after extraction of the aqueous reaction mixture by EtOAc (entry 4). Upon evaporation of the water phase, product **2** was obtained as a white solid in 89% yield and submitted to a standard acetylation protocol leading to **2'**. Variation of HRP/H₂O₂ equivalents did not show significant improvements for the conversion/yield (entries 5–10). Upon GC-MS-QTOF analysis, starting with a *m/z* of 419.0950, we identified a formula of C₂₀H₁₉O₁₀ for [M + H⁺] (419.0978, Δ 6.6 ppm) for **2'**. While the dimeric nature of the product was concluded from HRMS and ¹³C NMR analyses, the structure of the dimer **2'** did not match with usual oxidative products in peroxidase chemistry and we therefore embarked into a full structure elucidation of the product **2'** (Table 2). Three acetyl groups were first evidenced by the presence of three singlets at δ_H 2.05 (3H), 2.10 (3H) and 2.23 (3H), while another methyl was associated with the signal at δ_H 1.67 (s, 3H, H-14) and other signals at δ_H 5.16 (d, *J* = 2 Hz, 1H, H-15b), 5.26 (d, *J* = 2 Hz, 1H, H-15a) and δ_C 120.9 (CH₂, C-15) revealed the presence of an exomethylene olefinic group. The conjugation of this first olefin with a second one was inferred from the presence of other olefinic signals at δ_H 6.12 (s, 1H, H-10) and δ_C 118.5 (CH, C-10) HMBC correlated with H₂-15. The dearomatization of the first monomer **1** was then evidenced through the key H-10/C-1, H₂-15/C-8 HMBC correlations. Additional HMBC correlations from the signal at δ_H 3.86 (s, 1H, H-8) like H-8/C-1 allowed closure of the ring of the first monomer with oxygen atoms connected to C-11, C-1 and C-12 due to characteristic chemical shifts. Further H-8/C-13 and H-8/C-7 correlation allowed the connection of the first monomer with the second one, C-13 corresponding to an ester function due to characteristic chemical shifts.

Table 2. ¹H and ¹³C-NMR chemical shifts of **2'**.



Entry	Position ^b	¹ H	¹³ C	Description	HMBC
1	-	-	170.9	CH ₃ O-C=O	
2	-	-	169.8	CH ₃ O-C=O	
3	-	-	169.7	CH ₃ O-C=O	
4	11	-	140.6	>C=	
5	3	-	169.1	O-C=O	
6	13	-	169.5	O-C=O	
7	9	-	134.3	>C=	
8	10	6.12	118.5	=CH-	C-11, C-1, C-8, C-9, C-15
9	15	5.26, 5.16	120.9	0	C-8, C-9, C-10
10	1	-	77.7	>C<	
11	12	-	88.6	>C<	
12	5	-	80.9	>C<	
13	7	-	87.3	>C<	
14	4	3.62	58.6	CH	C-6, C-5, C-1, C-12
15	6	3.88	55.5	CH	C-13, C-7, C-5, C-4, C-14, C-12
16	8	3.86	50.6	CH	C-12, C-1, C-7, C-13
17	14	1.67	23.2	CH ₃	C-6, C-5, C-4
18	-	2.05	20.9	OCH ₃	
19	-	2.1	20.7	OCH ₃	
20	-	2.23	20.5	OCH ₃	

^a CDCl₃, 400 and 100 MHz, respectively, δ ppm. HMBC = Heteronuclear Multiple Bond Correlation.

^b The numbering system is that of the parent polycyclic system, the tetracyclo [6.3.1.0^{1.6}.0^{4.12}] dodecane core. For an alternate numbering system, see supplementary materials (SM).

H-6/C-13, C-7, C-5 and C-14 together with H-4/C-6 and C-5 HMBC correlations from the signal at δ_{H} 3.86 (s, 1H, H-6) and 3.62 (s, 1H, H-4) finished to establish the arrangement of the second monomer therefore evidencing an oxidation at positions C-3 and C-13 followed by a cleavage into two carboxylic functions at these positions. Even if no HMBC correlation enabled location of C-3 in the chain, its presence was deduced from characteristic chemical shifts and logics into the structure of the second monomer. Connections with the first monomer were inferred from additional HMBC correlations from the methines signals. The three acetyloxy were located at positions C-11, C-12 and C-7 due to final HMBC correlations. No ambiguity was left on the final structure of **2'** using HRMS data and the molecular formula. To the best of our knowledge, this complex cage polycyclic structure is unprecedented in the literature and it shows some similarities with vinigrol [13] epicolactone [14] jiadifenolide [15] or (–)-11-*O*-debenzoyltashironin [16], which require multistep synthesis to be built. Several attempts to obtain monocrystals of **2** for X-ray spectroscopy were unsuccessful.

The structure of **2** before acetylation was slightly different from the one of **2'**. NMR analysis was performed in D₂O and the spectra featured some significant differences (Table 3). No exo-methylene and of course no acetyl groups were found in this structure and only one olefinic signal was observed at a relatively low field (6.45 ppm). Consequently, two methyl groups could be identified. The three saturated and fused methines of **2'** were still present in **2**. The ¹³C-NMR spectra featured a new signal at 197.2 ppm suggesting a conjugated carbonyl group, and two signals at 178.0 and 171.5 ppm eligible to be those of carbonyl groups of an acid and an ester function, respectively.

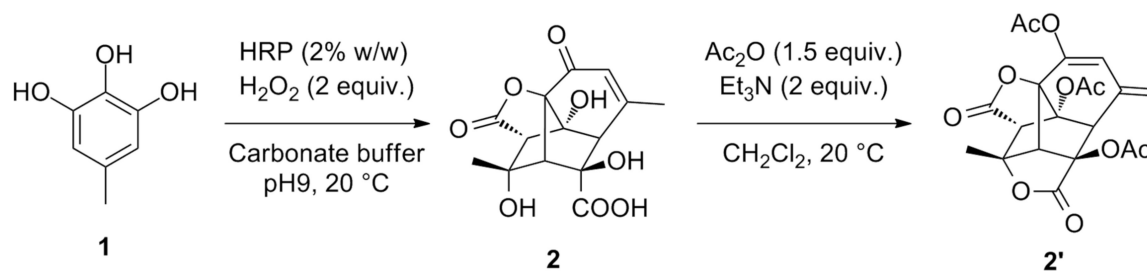
Table 3. ¹H and ¹³C-NMR chemical shifts of **2**.

2 ^a				
Entry	Position ^b	¹ H	¹³ C	Description
1	11	-	197.2	>C=O
2	13	-	178.0	HO-C=O
3	3	-	171.5	O-C=O
4	9	-	164.1	>C=
5	10	6.45	127.1	=CH-
7	1	-	88.7	>C<
8	12	-	87.1	>C<
9	5	-	85.7	>C<
10	7	-	77.7	>C<
11	4	2.67	60.1	CH
12	6	3.57	54.4	CH
13	8	3.95	52.9	CH
14	15	2.18	25.7	CH ₃
15	14	1.83	23.6	CH ₃

^a D₂O, 400 and 100 MHz, respectively, δ ppm. ^b The numbering system is that of the parent polycyclic system, the tetracyclo [6.3.1.0^{1,6}.0^{4,12}] dodecane core.

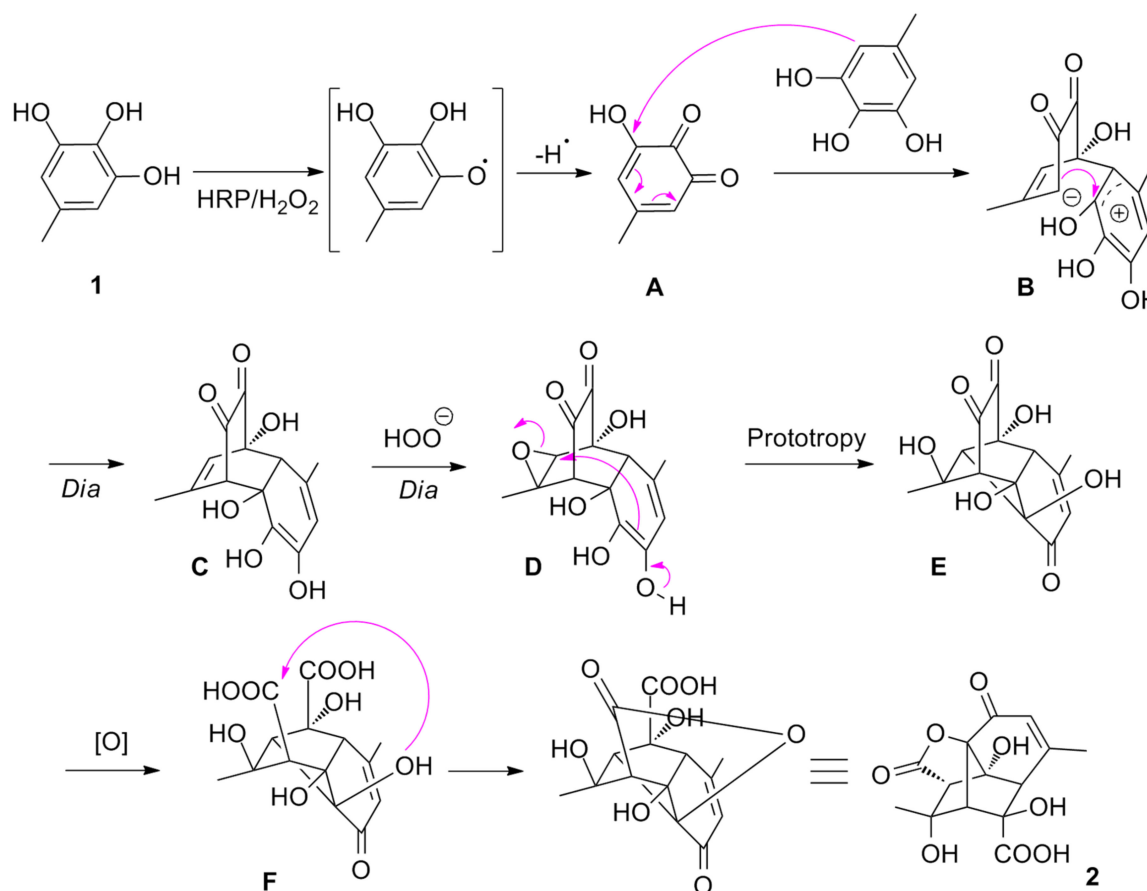
HPLC-MS was performed and allowed obtaining a spectrum of **2** by ESI. In negative mode, [M – H][–] ion was observed at *m/z* 309, although weak, together with strong [2M – H][–] at *m/z* 619 and [2M – 2H + Na][–] at *m/z* 641. Under enantioselective SFC and upon polarimetry measurements, product **2** appeared as a racemate. However, the diastereomeric control during the overall process is impressive since only one diastereomer was formed.

Under acetylation conditions, the α,β -unsaturated carbonyl motif of **2** was thus enolised to the corresponding dienol and trapped as an acetyl dienolate. In parallel, the γ -hydroxyacid underwent lactonisation to the corresponding γ -lactone fusing a fourth cycle to the existing structure, and the two tertiary alcohol functions were acetylated (Scheme 3).



Scheme 3. Synthesis of dimers **2** and **2'** from 5-methylpyrogallol **1**.

From a mechanistic point of view, we propose a series of oxidation/cyclisation to convert **1** into **2** (Scheme 4). Inspired by the reactivity of gallate derivatives in oxidative conditions [10], we hypothesised that substrate **1** could be slowly oxidised into the corresponding *o*-quinone **A** by HRP/H₂O₂, which could quickly react with unconverted **1** to yield intermediate **B** upon a vinylogous Michael addition followed ring closure by attack of the resulting enolate towards the arenium intermediate to form **C** in a distereoselective fashion. Epoxidation of the trisubstituted double bond by H₂O₂ present in excess in basic medium could then lead to **D** diastereoselectively. A subsequent regio- and stereoselective nucleophilic attack of an enol on the epoxide would then deliver **E** after prototropy. Upon oxidative cleavage of the α -diketone motif to **F** and subsequent lactonisation, product **2** would then be obtained, the oxidative cleavage possibly taking place earlier in the sequence.



Scheme 4. Proposed mechanism to account for the formation of dimer **2** from 5-methylpyrogallol **1**.

3. Materials and Methods

^1H NMR and ^{13}C NMR spectra were recorded on a Bruker (Billerica, MA, USA) AC (200 and 400 MHz). ^1H NMR spectra are reported as follows: Chemical shifts in ppm (δ) relative to the chemical shift of TMS at 0 ppm, integration, multiplicities (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and br = broad), and coupling constants (Hz). ^{13}C NMR spectra chemical shifts are reported in ppm (δ) relative to CDCl_3 at 77.16 ppm. Identity was assessed by comparison with data of authentic samples or literature data for **1** and its synthetic intermediates.

Column chromatography was carried out on silica gel (spherical 15–30 μm , neutral, 63–200 μm , Geduran Si 60, Merck KGaA, Darmstadt, Germany).

GC-TCD analyses were carried out using a Shimadzu (Kyoto, Japan) QP2010 plus gas chromatograph, under the following operation conditions: Vector gas, He; injector temperature, 250 $^\circ\text{C}$; detector temperature, 210 $^\circ\text{C}$ at 60 mA; split ratio, 1/20; total flow, 22.5 mL min^{-1} ; Phenomenex (Torrance, CA, USA) Zebron ZB5MS column, polydimethylsiloxane (10 m, inside diameter 0.10 mm, film thickness 0.10 μm); temperature program, 80–200 $^\circ\text{C}$ at 10 $^\circ\text{C min}^{-1}$ and 200 $^\circ\text{C}$ for 8 min.

GC/MS analyses were performed by using a Shimadzu QP2010 gas chromatograph (conditions: carrier gas, He; injector and detector temperatures, 250 $^\circ\text{C}$; injected volume, 0.5 μL ; split ratio, 1/100; pressure, 180 kPa; SLB-5 ms capillary column (thickness: 0.25 mm, length: 30 m, inside diameter: 0.25 mm); temperature program, 60–315 $^\circ\text{C}$ at 10 $^\circ\text{C min}^{-1}$, and 10 min at 315 $^\circ\text{C}$) coupled to a mass selective detector. Mass spectra were obtained by electron ionisation at 70 eV, m/z 35–400, source temperature 250 $^\circ\text{C}$; only the most abundant ions are given.

High resolution mass spectrometry (HRMS) was performed at ERINI platform (Grasse, France) using a Waters UPLC coupled with a Waters (Milford, MA, USA) Xevo G2 QTOF spectrometer.

Enantioselective SFC was performed on a Jasco (Tokyo, Japan) Extrema apparatus equipped with Daicel (Osaka, Japan) ChiralPak IA column coupled with a dual wavelength 190 to 600 nm UV-4070/75 detector. Pressure: 150 bars; flow: 4 mL/min ; MeOH: 15%. Wavelength: 245 nm.

Polarimetry was performed on an Anton Paar (Graz, Austria) MCP150 polarimeter at 20 $^\circ\text{C}$ in MeOH/ H_2O 10:6 *v/v*. Wavelength: 589 nm.

Materials. Dimethyl formamide (DMF), tetrahydrofuran (THF), methanol (MeOH), Ethanol (EtOH), cyclohexane (CHX) were purchased from Sigma-Aldrich (Saint-Louis, MO, USA) and dried and/or distilled according to conventional procedures. Orcinol, POCl_3 , MOMCl, NaH, *n*-BuLi, AcCl, Na_2CO_3 , NaHCO_3 , H_2O_2 (30% *w/w* in water) and HRP were purchased from Sigma-Aldrich and used as received.

1,3-di(methoxymethoxy)-5-methylbenzene: Orcinol (0.8 g; 6.5 mmol) was dissolved in freshly dried and distilled DMF (40 mL). To this solution was added NaH (0.58 g as a 60% dispersion in mineral oil, 14.5 mmol). The mixture was stirred under a nitrogen atmosphere at 0 $^\circ\text{C}$ in a round-bottomed flask equipped with a refrigerant and a bubbler allowing to monitor H_2 evolution. After 15 min, MOM-Cl (1.10 mL; 14.5 mmol) was added and the mixture stirred during pendant 18 h. Water was then carefully added (30 mL) and the resulting mixture extracted with Et_2O (5×30 mL). Organic layers were then pooled and washed with a 2 M aqueous NaOH solution (3×20 mL) and brine (20 mL). After drying over MgSO_4 , filtration and solvent removal, a yellow oil was obtained, which was submitted to column chromatography over silica gel (petroleum ether/ EtOAc 9:1) to yield the MOM-protected orcinol as a colourless liquid (1.13 g, 95%). Rf 0.7 (petroleum ether/ EtOAc 8:2). ^1H NMR (CDCl_3 , 200 MHz): δ ppm 6.6–6.5 (m, 3H, ArH), 5.15 (s, 4H), 3.48 (s, 6H) 2.32 (s, 3H). ^{13}C NMR: (CDCl_3 , 50 MHz) δ ppm 158.2 (C), 140.3 (C), 110.4 (CH), 102.1 (CH), 94.4 (CH), 55.9 (OCH_3), 21.7 (CH_3); MS (EI) m/z : 212(8), 182(1), 152(3), 136(2), 123(1), 108(2), 91(1), 77(2), 45(100).

1,3-di(methoxymethoxy)-4-methylbenzaldehyde: 1,3-di(methoxymethoxy)-5-methylbenzene (0.95 g; 4.5 mmol) was dissolved in freshly distilled THF (50 mL) and stirred under a nitrogen atmosphere at 0 $^\circ\text{C}$ while *n*-butyllithium was added dropwise (3.4 mL as a 1.6 M solution in hexane, 5.4 mmol). The mixture was stirred during 1.5 h while let warm to room temperature and the reaction was

quenched with DMF (0.7 mL, 9 mmol). The resulting mixture was washed with water (50 mL) and extracted with Et₂O (4 × 30 mL). Organic layers were pooled and washed with water (40 mL) and brine (40 mL), dried over MgSO₄, filtrated and concentrated in vacuo. The resulting yellow oil was submitted to column chromatography over silica gel (petroleum ether/EtOAc 95:5) to yield a yellow solid (0.81 g, 75%). ¹H NMR (CDCl₃, 200 MHz): δ ppm 10.40 (s, 1H, CHO), 6.58 (s, 2H, ArH), 5.17 (s, 4H), 3.42 (s, 6H) 2.26 (s, 3H); ¹³C NMR: (CDCl₃, 50 MHz) δ ppm 188.7 (CHO), 159.4 (C), 147.3 (C), 113.7 (CH), 109.3 (CH), 94.6 (CH), 56.3 (OCH₃), 22.5 (CH₃); MS *m/z*: 240(2), 209(2), 195(3), 179(4), 178(10), 165(3), 164(4), 136(2), 77(2), 46(3), 45(100).

Atranol: 1,3-di(methoxymethoxy)-4-methylbenzaldehyde (0.315 g, 1.3 mmol) was dissolved in 30 mL of MeOH at room temperature under a nitrogen atmosphere. Acetyl chloride was then added dropwise (60 µL, 0.9 mmol). The mixture was stirred for 20 h and concentrated in vacuo. An aqueous HCl solution was then added (30 mL at 0.1 M) and the resulting mixture extracted with EtOAc (3 × 150 mL). Organic layers were pooled and dried over MgSO₄, filtrated and concentrated in vacuo. The resulting light-yellow oil was submitted to column chromatography over silica gel (petroleum ether/EtOAc 8:2), and atranol 1 was obtained as a light yellow oil (0.164 g, 90%). ¹H NMR (Acetone-d₆, 200 MHz): δ ppm 10.71 (s, 2H, OH), 10.26 (s, 1H, CHO), 6.25 (s, 2H, ArH), 2.23 (s, 3H). ¹³C NMR (Acetone-d₆, 50 MHz): δ ppm 194.2 (CHO), 163.1 (C), 151.6 (C), 109.3 (C), 108.4 (CH), 22.4 (CH₃). MS *m/z*: 152 (84), 151(100), 134(6), 123(4), 106 (16), 95(9), 77(14), 69 (6), 67(11), 55(12).

5-Methylpyrogallol 1: Atranol (0.152 g, 1 mmol) and sodium percarbonate (0.236 g, 1.5 mmol) were dissolved in THF/water 3:7 mixture (5 mL). The reaction mixture is then stirred at room temperature for 2 h. After completion of the reaction, aqueous 0.1 M HCl solution was added (5 mL) and the mixture extracted with EtOAc (2 × 10 mL). Organic layers were pooled and dried over MgSO₄, filtrated and concentrated in vacuo. An orange solid was obtained (0.119 g, 85%). ¹H NMR (Acetone-d₆, 200 MHz): δ ppm 7.64 (s, 2H, OH), 7.02 (s, 1H, OH), 6.20 (s, 2H, ArH), 2.10 (s, 3H). ¹³C NMR (Acetone-d₆, 50 MHz): δ ppm 146.3 (C), 131.0 (C), 129.2 (C), 108.5 (CH), 20.9 (CH₃). MS *m/z*: 140 (100), 139(34), 134 (6), 123(11), 122(19), 121(9), 94 (35), 77(4), 66(37), 65(21), 55(6), 53(17).

Dimer 2: 5-methylpyrogallol (200 mg, 1.43 mmol) was dissolved in a carbonate aqueous buffer at pH9 (20 mM, 65 mL), containing HRP (124 U/mg, 4 mg). The flask was covered with an aluminium foil and H₂O₂ (30% aqueous solution) was added at the controlled flow of 0.1 mL/h to reach a final amount of 2 equiv. The reaction mixture was stirred at room temperature for 6 h followed by the addition of an aqueous HCl solution (40 mL, 0.1 M) further extracted with EtOAc (3 × 70 mL). Evaporation of aqueous phase a white powder of dimer 2 and small amounts of salt (0.193 g, 88%). ¹H NMR (D₂O, 400 MHz): δ ppm 6.37 (t, 1H), 3.97 (d, 1H), 3.37 (s, H), 2.68 (s, 2H), 2.62 (s, H), 2.14 (s, 3H), 1.79 (s, 3H). ¹³C RMN: (D₂O, 100 MHz) δ ppm 197.2 (C), 178.0 (C), 171.5 (C), 164.1 (C), 127.1 (CH), 88.7 (C), 87.1 (C), 85.7 (C), 77.7 (C), 60.1 (CH), 54.4 (CH), 52.9 (CH), 25.7 (CH₃), 23.6 (CH₃). MS (ESI): [M – H][–] ion was observed at *m/z* 309, [2M – H]⁺ at *m/z* 619, [2M – 2H + Na]⁺ at *m/z* 641. A (20 °C, 589 nm) = –0.002° ± 0.000.

Acetylated dimer 2': To a solution of dimer 2 (0.100 g, 0.32 mmol) in freshly distilled dichloromethane (2 mL) were added Et₃N (0.28 mL, 2.1 mmol) and Ac₂O (0.2 mL, 2.1 mmol). The mixture was stirred overnight at room temperature under a nitrogen atmosphere. After concentration in vacuo, water was added (10 mL) and the resulting solution extracted with EtOAc (2 × 10 mL). The organic layers were pooled, dried over MgSO₄, filtrated and concentrated in vacuo. A colourless oil was obtained (0.12 g, 90%). ¹H NMR (CDCl₃, 400 MHz): δ ppm 6.12 (s, 1H), 5.26 (s, 1H), 5.16 (s, 1H), 3.88 (s, 1H), 3.86 (s, 1H), 3.62 (s, 1H), 2.23 (s, 3H), 2.10 (s, 3H), 2.05 (s, 3H), 1.67 (s, 3H). ¹³C NMR: (CDCl₃, 100 MHz) δ ppm 170.9 (CO), 169.8 (CO), 169.7 (CO), 169.5 (CO), 169.1 (CO), 140.6 (C), 134.3 (C), 120.9 (C), 88.6(C), 87.3(C), 80.9 (C), 77.7 (C), 58.6 (CH), 55.5 (CH), 50.6 (CH), 23.2 (CH₃), 20.9 (CH₃), 20.7 (CH₃), 20.7 (CH₃), 20.5 (CH₃). MS *m/z*: 418(1), 376(20), 334(7), 290(2), 273(3), 266(1), 246(2), 231(3), 214(6), 182(23), 175(3), 162(6), 141(28), 140(100), 91(1), 77(2), 69(2), 43(100). HRMS: 419.0950, calculated for [M.H]⁺

$C_{20}H_{19}O_{10}$ 419.0978, $\Delta = -6.7$ ppm. 377.0859, calculated for $[M(-CH_3CO + H).H]^+$ $C_{18}H_{17}O_9$ 377.0873, $\Delta = -3.7$ ppm. 335.0754, calculated for $[M(-2CH_3CO + 2H).H]^+$ $C_{16}H_{15}O_8$ 335.0767, $\Delta = -3.9$ ppm.

4. Conclusions

In summary, an unusual complex fused polycyclic new skeleton featuring a 2-oxatetracyclo [6.3.1.0^{1,6}.0^{4,12}] dodecan-3-one was obtained in one-step from 5-methylpyrogallol submitted to HRP-catalysed oxidation in the presence of H_2O_2 . The impressive control of the regio- and stereoselectivity of all the putative steps of this transformation reflects the unique capacity of enzymes in organic chemistry and their potential for building outstanding chemical skeletons by triggering complex cascades proceeding selectively either under an enzyme-controlled regime or substrate-controlled regime. In our case, the enzymatic reaction would catalyse the formation of highly activated species further undergoing spontaneous reactions, a scenario that could also be considered in biosynthetic studies [17,18]. The production of such a chemical complexity from a simple and close derivative of natural gallic acid could also later unravel structural analogues from their natural origin.

Supplementary Materials: The following are available online. Detailed experimental procedures and NMR spectra of products and intermediates.

Author Contributions: Conceptualization, S.A.; methodology, S.A.; investigation, H.B., K.C., O.P.T. and S.A.; resources, O.P.T. and S.A.; writing—original draft preparation, O.P.T. and S.A.; writing—review and editing, O.P.T. and S.A.; supervision, S.A.; project administration, S.A.; funding acquisition, S.A.

Funding: This work was supported by University Nice Sophia Antipolis, the CNRS, SATT Sud-Est (Project Oakmoss) and Région Provence-Alpes-Côte-d'Azur.

Acknowledgments: We are grateful to A. Dupeux and V. Michelet for the enantioselective-SFC analysis of **2** and D. Bonhomme for measuring the optical activity of **2**.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Faber, K. *Biotransformations in Organic Chemistry*, 5th ed.; Springer: Berlin, Germany, 2004; p. 454.
2. Dordick, J.S.; Marletta, M.A.; Klivanov, A.M. Polymerization of phenols catalyzed by peroxidase in nonaqueous media. *Biotechnol. Bioeng.* **1987**, *30*, 31–36. [[CrossRef](#)] [[PubMed](#)]
3. Antoniotti, S.; Santhanam, L.; Ahuja, D.; Hogg Michael, G.; Dordick, J.S. Structural diversity of peroxidase-catalyzed oxidation products of o-methoxyphenols. *Org. Lett.* **2004**, *6*, 1975–1978. [[CrossRef](#)] [[PubMed](#)]
4. Hirokane, T.; Hirata, Y.; Ishimoto, T.; Nishii, K.; Yamada, H. A unified strategy for the synthesis of highly oxygenated diaryl ethers featured in ellagitannins. *Nat. Commun.* **2014**, *5*, 3478. [[CrossRef](#)] [[PubMed](#)]
5. Behboodi-Sadabad, F.; Zhang, H.; Trouillet, V.; Welle, A.; Plumeré, N.; Levkin, P.A. UV-Triggered Polymerization, Deposition, and Patterning of Plant Phenolic Compounds. *Adv. Funct. Mater.* **2017**, *27*, 1700127. [[CrossRef](#)]
6. Li, Y.; Tanaka, T.; Kouno, I. Oxidative coupling of the pyrogallol B-ring with a galloyl group during enzymatic oxidation of epigallocatechin 3-O-gallate. *Phytochemistry* **2007**, *68*, 1081–1088. [[CrossRef](#)] [[PubMed](#)]
7. Tanaka, T.; Inoue, K.; Betsumiya, Y.; Mine, C.; Kouno, I. Two Types of Oxidative Dimerization of the Black Tea Polyphenol Theaflavin. *J. Agric. Food Chem.* **2001**, *49*, 5785–5789. [[CrossRef](#)] [[PubMed](#)]
8. Kusano, R.; Tanaka, T.; Matsuo, Y.; Kouno, I. Structures of Epicatechin Gallate Trimer and Tetramer Produced by Enzymatic Oxidation. *Chem. Pharm. Bull.* **2007**, *55*, 1768–1772. [[CrossRef](#)] [[PubMed](#)]
9. Itoh, N.; Katsube, Y.; Yamamoto, K.; Nakajima, N.; Yoshida, K. Laccase-catalyzed conversion of green tea catechins in the presence of gallic acid to epitheafagallin and epitheafagallin 3-O-gallate. *Tetrahedron* **2007**, *63*, 9488–9492. [[CrossRef](#)]
10. Quideau, S.; Feldman, K.S. Ellagitannin Chemistry. The First Synthesis of Dehydrohexahydroxydiphenolate Esters from Oxidative Coupling of Unetherified Methyl Gallate. *J. Org. Chem.* **1997**, *62*, 8809–8813. [[CrossRef](#)]

11. Banwell, M.G.; Chand, S. Exploitation of cooperative directed *ortho*-metallation (DoM) by 1,3-related-OMOM groups in the development of a fully regiocontrolled synthesis of atranol from orcinol. *Org. Prep. Proc. Int.* **2005**, *37*, 275–279. [[CrossRef](#)]
12. Kabalka, G.W.; Reddy, N.K.; Narayana, C. Sodium percarbonate: A convenient reagent for the dakin reaction. *Tetrahedron Lett.* **1992**, *33*, 865–866. [[CrossRef](#)]
13. Morton, J.G.M.; Draghici, C.; Kwon, L.D.; Njardarson, J.T. Rapid Assembly of Vinigrol's Unique Carbocyclic Skeleton. *Org. Lett.* **2009**, *11*, 4492–4495. [[CrossRef](#)] [[PubMed](#)]
14. Ellerbrock, P.; Armanino, N.; Ilg, M.K.; Webster, R.; Trauner, D. An eight-step synthesis of epicolactone reveals its biosynthetic origin. *Nat. Chem.* **2015**, *7*, 879–882. [[CrossRef](#)] [[PubMed](#)]
15. Lu, H.-H.; Martinez, M.D.; Shenvi, R.A. An eight-step gram-scale synthesis of (–)-jiadifenolide. *Nat. Chem.* **2015**, *7*, 604–607. [[CrossRef](#)] [[PubMed](#)]
16. Ohtawa, M.; Krambis, M.J.; Cerne, R.; Schkeryantz, J.M.; Witkin, J.M.; Shenvi, R.A. Synthesis of (–)-11-*O*-Debenzoyltashironin: Neurotrophic Sesquiterpenes Cause Hyperexcitation. *J. Am. Chem. Soc.* **2017**, *139*, 9637–9644. [[CrossRef](#)] [[PubMed](#)]
17. Keller, M.A.; Piedrafita, G.; Ralser, M. The widespread role of non-enzymatic reactions in cellular metabolism. *Curr. Opin. Biotech.* **2015**, *34*, 153–161. [[CrossRef](#)]
18. Edmond, G.; Erwan, P. Biogenesis and Biomimetic Chemistry: Can Complex Natural Products Be Assembled Spontaneously? *Eur. J. Org. Chem.* **2008**, 27–42. [[CrossRef](#)]

Sample Availability: Samples of the compounds are not available from the authors.



© 2018 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).