

# Efficient Chemical Synthesis of (Epi)catechin Glucuronides: Brain-Targeted Metabolites for Treatment of Alzheimer's Disease and Other Neurological Disorders

Maite L. Docampo-Palacios,\* Anislay Alvarez-Hernández, Ângelo de Fátima, Luciano Morais Lião, Giulio M. Pasinetti, and Richard A. Dixon\*



Cite This: *ACS Omega* 2020, 5, 30095–30110



Read Online

ACCESS |



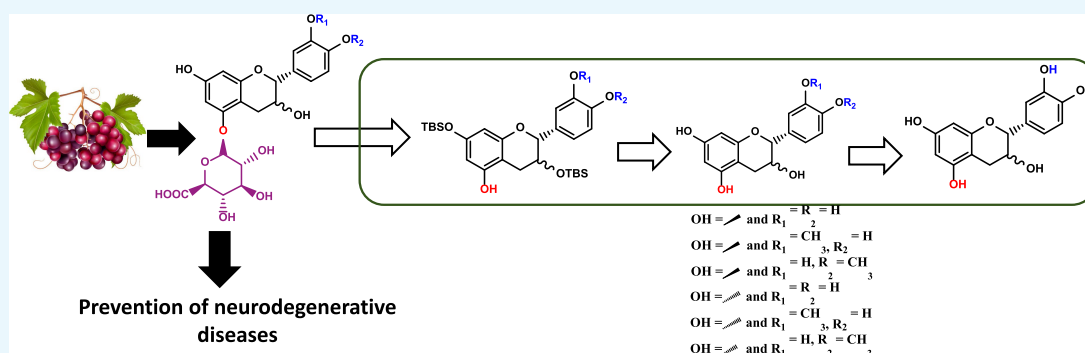
Metrics & More



Article Recommendations



Supporting Information



**ABSTRACT:** Grape seed extract (GSE) is rich in flavonoids and has been recognized to possess human health benefits. Our group and others have demonstrated that GSE is able to attenuate the development of Alzheimer's disease (AD). Moreover, our results have disclosed that the anti-Alzheimer's benefits are not directly/solely related to the dietary flavonoids themselves, but rather to their metabolites, particularly to the glucuronidated ones. To facilitate the understanding of regioisomer/stereoisomer-specific biological effects of (epi)catechin glucuronides, we here describe a concise chemical synthesis of authentic standards of catechin and epicatechin metabolites 3–12. The synthesis of glucuronides 9 and 12 is described here for the first time. The key reactions employed in the synthesis of the novel glucuronides 9 and 12 include the regioselective methylation of the 4'-hydroxyl group of (epi)catechin ( $\leq 1.0/99.0\%$ ; 3'-OMe/4'-OMe) and the regioselective deprotection of the *tert*-butyldimethylsilyl (TBS) group at position 5 (yielding up to 79%) over the others (3, 7 and 3' or 4').

## INTRODUCTION

Fruits, vegetables, and their juices, as well as tea, wine, and cocoa-derived products, are important dietary sources of flavonoids.<sup>1–3</sup> Diets rich in flavonoids are linked with the prevention of a variety of degenerative diseases, most importantly, cardiovascular and neuropsychological.<sup>1,4–7</sup> Flavonoids, a broad class of plant natural products, are based on a fifteen-carbon skeleton containing two benzene rings (A and B) connected via a heterocyclic pyran moiety (C) (Figure 1).<sup>3,8,9</sup> The presence of different oxidation states of the C ring and different functional groups on the A, B, and C rings gives rise to diverse subclasses of flavonoids such as flavonols, flavones, flavanones, isoflavones, and flavan-3-ols. Such flavonoids are known for their pharmacological properties, which include antiallergic,<sup>10</sup> antibacterial,<sup>11–13</sup> anticancer,<sup>14–18</sup> antifungal,<sup>11–13</sup> anti-*Helicobacter pylori*,<sup>11,19</sup> anti-inflammatory,<sup>20</sup> antioxidant/antiradical,<sup>21,22</sup> antiviral,<sup>11–13</sup> cardioprotective,<sup>23,24</sup> gastroprotective,<sup>25</sup> neuroprotective,<sup>26–30</sup> and nutritional.<sup>31</sup>

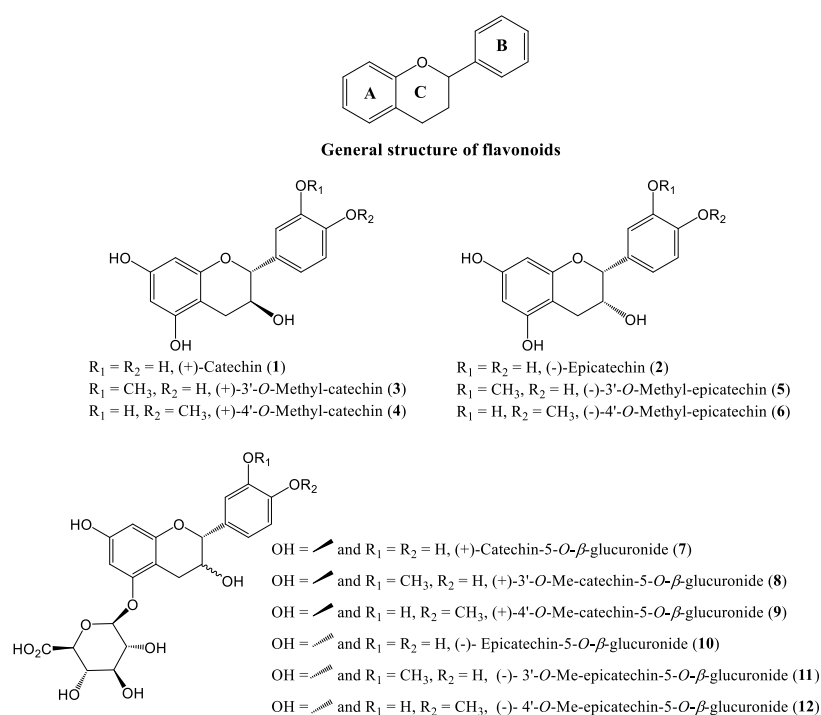
Grape seed extract (GSE) is rich in flavonoids and has been recognized to stimulate glucose uptake in insulin-resistant adipocytes<sup>32–34</sup> to produce a significant decrease in systolic and diastolic blood pressure of spontaneously hypertensive rats (SHR),<sup>35</sup> to ameliorate diabetic bladder dysfunction and reduce the apoptosis of the bladder in diabetic rats,<sup>36</sup> to defend against fat accumulation and improve the plasma lipid profile in hamsters,<sup>37</sup> and to improve the antioxidant status and decrease the incidence of free radical-induced lipid peroxidation in blood samples of rats exposed to X-radiation.<sup>38</sup> Our group and others have demonstrated that GSE is also able to attenuate the development of Alzheimer's disease (AD).<sup>39–43</sup>

Received: September 15, 2020

Accepted: October 26, 2020

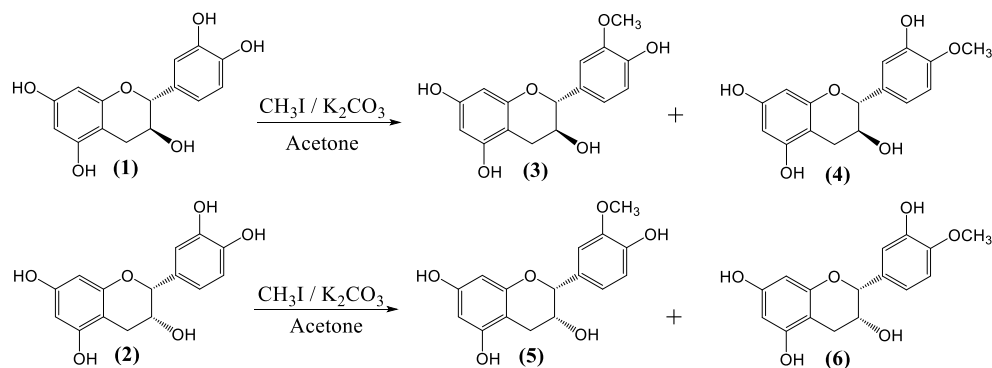
Published: November 12, 2020





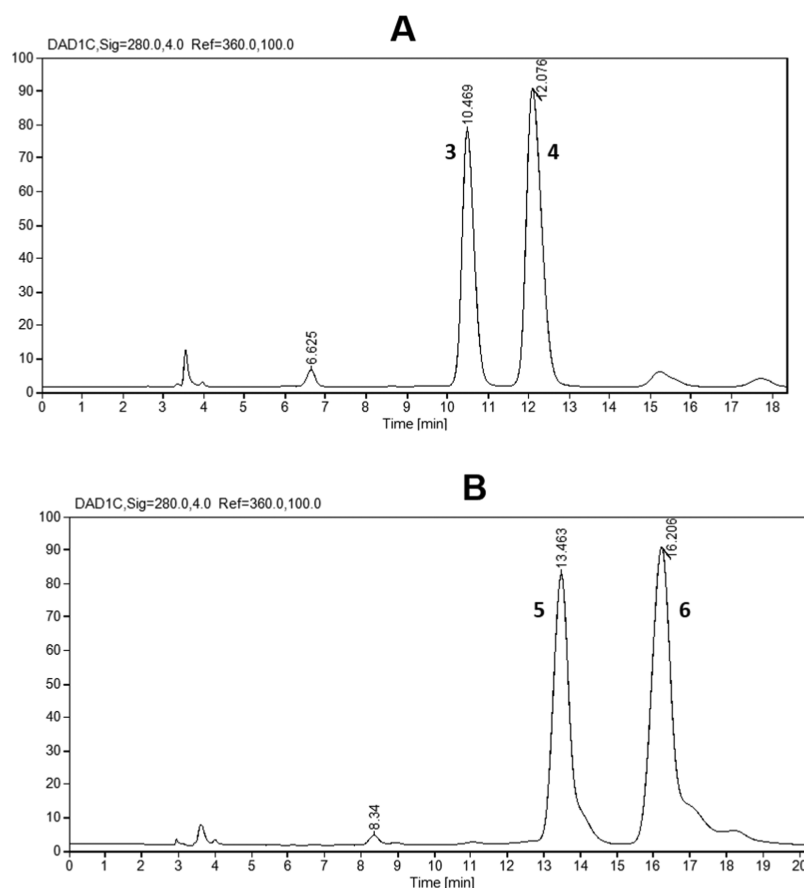
**Figure 1.** General chemical structure of flavonoids and molecular structures of catechin (1), epicatechin (2), and their methylated and/or glucuronidated forms.

### Scheme 1. Methylation of (Epi)catechin



The GSE studied by us was a complex mixture of proanthocyanidins (PACs, both oligomeric and polymeric) and their monomeric units consisting of the flavan-3-ols (+)-catechin (1) and (-)-epicatechin (2)<sup>44</sup> (Figure 1). The monomeric units, rather than the oligomers or polymers, are bioavailable and, therefore, candidates for being the active components in the GSE with respect to amelioration of Alzheimer's disease symptoms.<sup>45,46</sup> These components, in the form of glucuronidated and/or methylated phase II metabolites, reach the brain at a concentration of 300–400 nM after 10 days of repeated dosing.<sup>46</sup> Our studies showed that the predominant plasma metabolites of 1 and 2, identified by liquid chromatography–mass spectrometry time-of-flight (LC–MS–TOF), were catechin-5-*O*- $\beta$ -glucuronide (7), 3'-*O*-methyl catechin-5-*O*- $\beta$ -glucuronide (8), epicatechin-5-*O*- $\beta$ -glucuronide (10), and 3'-*O*-methyl epicatechin-5-*O*- $\beta$ -glucuronide (11)<sup>46</sup> (Figure 1). It is noteworthy that the effects of conjugation can differ depending on the type and position of conjugation, the flavonoid concentration, the pharmacological/molecular target effect studied, and the assay system used so

that no general rules can be deduced.<sup>47,48</sup> It has further been established that the ingested (epi)catechin is extensively metabolized into its related metabolites by *O*-methylation, sulfation, *O*-glucuronidation, and combinations thereof, and also that the absorption, metabolism, and biological activity of dietary flavan-3-ols are strongly influenced by stereochemistry.<sup>49–53</sup> However, a broad study of regioisomer/stereoisomer-specific effects on the biochemical and pharmacological properties of flavan-3-ols, particularly as related to anti-Alzheimer properties, has yet to be undertaken. In part, this reflects the lack of good synthetic strategies to furnish all possible (epi)catechin-glucuronide regioisomers. In particular, the chemical synthesis of glucuronide derivatives of catechin is underexplored<sup>54</sup> when compared with the chemical/enzymatic synthesis of the corresponding conjugates of epicatechin.<sup>55–61</sup> For the series of (epi)catechin glucuronides shown in Figure 1, the synthesis of glucuronides 7,<sup>54,61</sup> 8,<sup>56,61</sup> 10,<sup>58,59,61</sup> and 11<sup>56,57,61</sup> has been reported elsewhere, while the chemical synthesis of glucuronides 8 and 9 is described here for the first time. In this context, authentic standards of (+) catechin and



**Figure 2.** (A) HPLC analysis of crude reaction products from methylation of catechin: 3'-O-methyl catechin (3) and 4'-O-methyl catechin (4). (B) HPLC of crude reaction products from methylation of epicatechin: 3'-O-methyl epicatechin (5) and 4'-O-methyl epicatechin (6).

**Table 1. Different Methylation Reaction Conditions for the Preparation of 4'-O-Methyl Catechin (4)**

entry	equiv (K <sub>2</sub> CO <sub>3</sub> )	equiv (CH <sub>3</sub> I)	time (h)	3'-/4'-O-methyl catechin yield (%) <sup>b</sup>
1	5.0	18.0	24	40/60
2	1.0	1.0	24	≤1/20
3	2.0	2.0	24	5/50
4	2.5	3.0	48	10/70
5	2.5 (1.0 + 1.0 + 0.5) <sup>a</sup>	4.0 (1.5 + 1.5 + 1.0) <sup>a</sup>	72	≤1/99

<sup>a</sup>Reagents were added in batch (periods of time of 24 h). <sup>b</sup>Yield was calculated by HPLC without purification.

(-) epicatechin metabolites 3–12 (Figure 1) were prepared by a chemically unambiguous synthetic approach for the further mechanism of action studies.

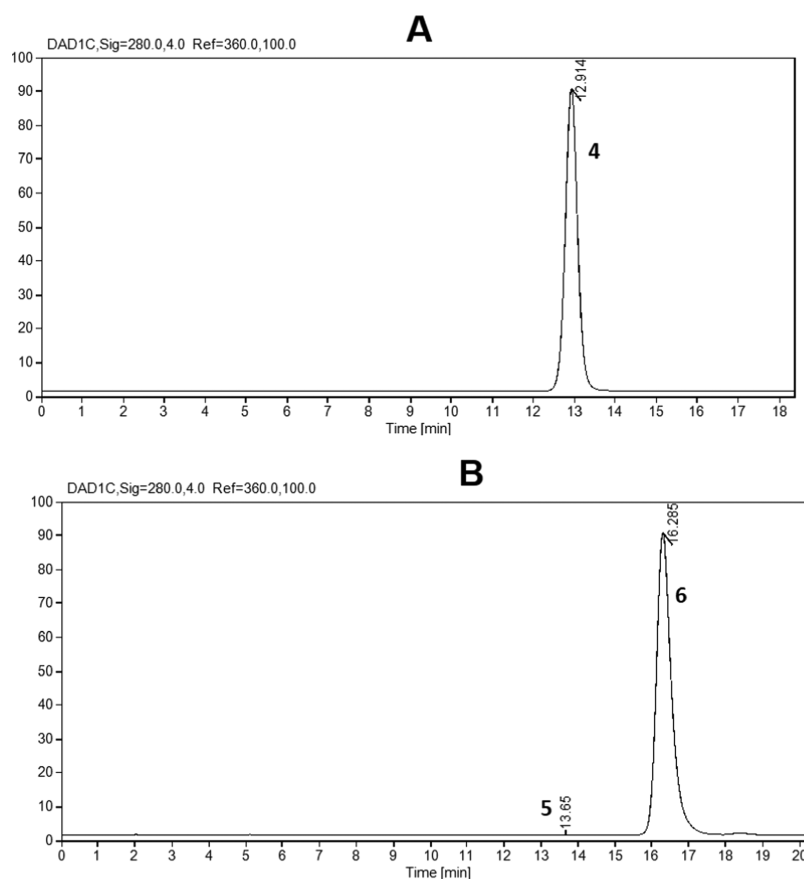
## RESULTS AND DISCUSSION

**Methylation of (Epi)catechin.** The synthesis of methylated (+)-catechin derivatives was carried out using the procedure reported by González-Manzano et al.<sup>54</sup> with small modifications with respect to the reaction time (Scheme 1). The original technique used 3.5 h in an ultrasonic bath, but under our experimental conditions, low quantities of methylated products were obtained at that time and the reaction was therefore stirred at room temperature for 20 h and monitored by high-performance liquid chromatography (HPLC). We observed the formation of two products, which were assigned as 3'- and 4'-O-methyl catechin (3 and 4). The methylation of catechin was preferentially at the 4' position, as shown in Figure 2. The proportion of 3'- and 4'-O-methylcatechin isomers was 3:7 as calculated by HPLC analysis.

However, Donovan et al.<sup>62</sup> specified that the preferential position for the methylation of catechin is position 3'. No formation of other methyl ether isomers was detected, demonstrating that the preferential site for methylation under these reaction conditions is ring B.

Application of the same method for methylation of (-)-epicatechin gave similar results with the formation of the corresponding 3'- and 4'-O-methyl epicatechin derivatives (5 and 6), but in this case, the ratio between the two isomers was 4:6, respectively (Figure 2B), as calculated by HPLC analysis.

The positions of the methoxyl substituents on (epi)catechin were confirmed by heteronuclear multiple bond correlation (HMBC) spectra using a long-range correlation between the methoxy protons and C-3' of 3 and 5 (Figures S4 and S12) and C-4' of 4 and 6 (Figures S8 and S16). <sup>1</sup>H- and <sup>13</sup>C NMR and liquid chromatography coupled to mass spectrometry (LC/MS) data derived from 3'-O-methyl (epi)catechin (3 and 5) and 4'-O-methyl (epi)catechin (4 and 6) were in complete



**Figure 3.** (A) HPLC of the crude reaction product from the regiospecific methylation of catechin: 4'-*O*-methyl catechin (**4**). (B) HPLC of the crude reaction product from the regiospecific methylation of epicatechin: 4'-*O*-methyl epicatechin (**6**).

agreement with the assigned structure of these compounds and those already published in the literature.<sup>54,61</sup> The relative configurations of H-2/H-3 (C-ring) of the diastereoisomers catechin and epicatechin were established as *trans* (catechin) and *cis* (epicatechin), respectively, by comparing the <sup>1</sup>H coupling constants of the protons of two chiral centers on the ring (<sup>3</sup>J<sub>2H,3H</sub> = 8.0 Hz, **3** and **4**; <sup>3</sup>J<sub>2H,3H</sub> < 2 Hz, **5** and **6**).

Finally, the LC-MS analyses of 3'-*O*-methyl catechin (**3**), 4'-*O*-methyl catechin (**4**), 3'-*O*-methyl epicatechin (**5**), and 4'-*O*-methyl epicatechin (**6**) showed the expected quasimolecular ion at *m/z* 303.1 [M - H]<sup>-</sup> (calcd for **3–6**, 303.1).

The above-described procedure for the synthesis of methyl ethers of epi(catechin) was modified to obtain a regiospecific methylation of (epi)catechin at position 4'. **Table 1** shows the different conditions used in the methylation of catechin and the relationship between both regioisomers (3'-/4'-*O*-methyl catechin). The best conditions were reached by applying intermittent injection of methyl iodide and potassium carbonate, reducing the amounts of methylating agent and the base, and increasing the reaction time (**Table 1**, entry 5). Similar results were found for the methylation of epicatechin. The reactions were monitored by HPLC, and **Figure 3** shows the HPLC chromatograms for the optimal conditions in the regiospecific methylation of (epi)catechin.

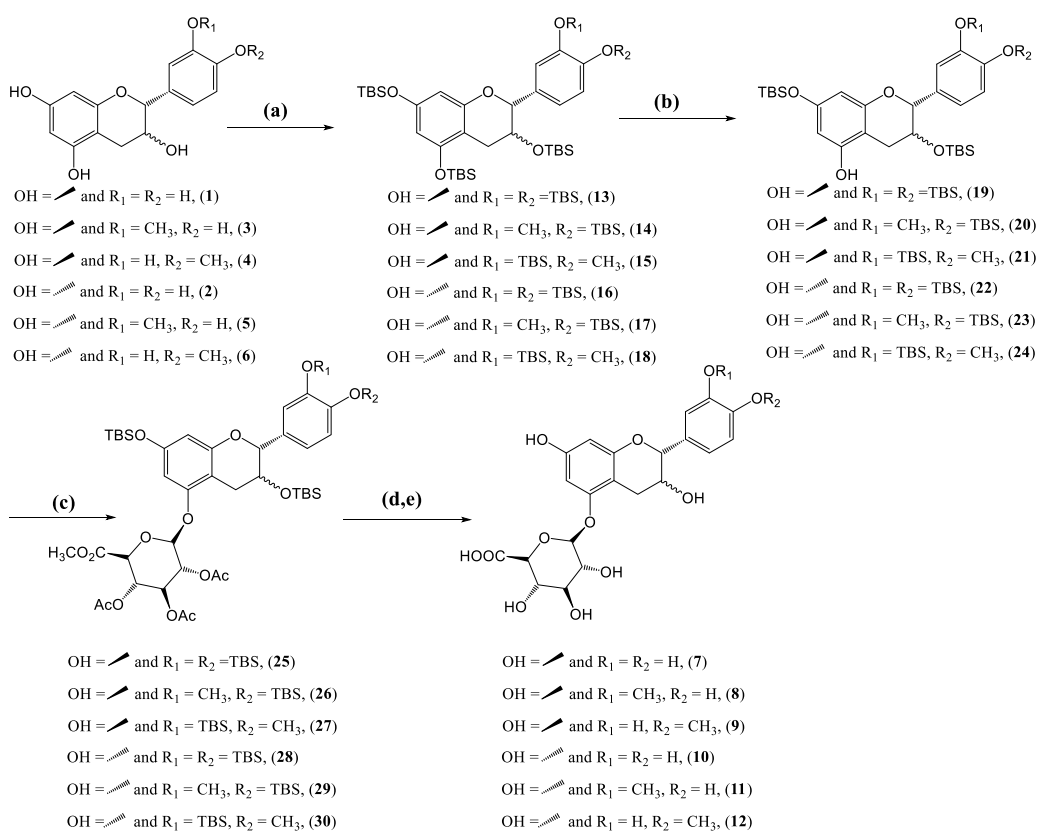
To the best of our knowledge, regiospecific methylation of epi(catechin) to obtain 4'-*O*-methyl catechin and 4'-*O*-methyl epicatechin has not been previously reported.

**Synthesis of (Epi)catechin-5-*O*-Glucuronides.** The semisynthesis of metabolites that contain phenol groups commonly requires the protection of hydroxyl groups to

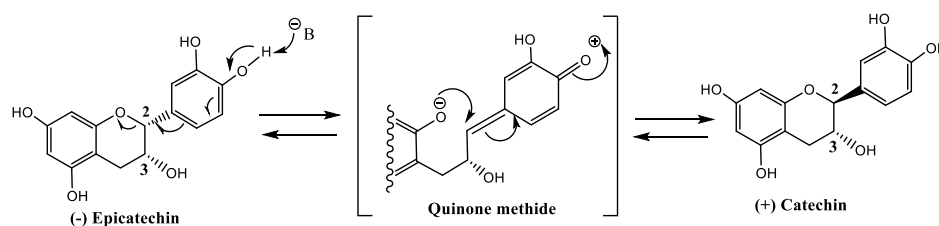
prevent unwanted side reactions since these compounds are sensitive to pH changes. For example, catechins and epicatechins are unstable at pH < 5, entering into polymerization reactions, whereas in basic conditions (pH > 9) opening of the C ring occurs, leading to epimerization reactions.<sup>63</sup> The most common protective groups used for phenolic compounds include acetyl, benzyl, methoxymethyl acetal, tetrafluoropyridyl, and dichlorodiphenylmethane;<sup>55,58,59,64</sup> however, there have been few reports where silyl groups were used to protect hydroxyl groups in phenolic compounds. The *tert*-butyldimethylsilyl (TBS) substituent can be installed in one-step, is stable under a range of commonly employed reaction conditions, and is selectively cleaved under mild conditions.

The synthetic strategy implemented to obtain (epi)catechin-5-*O*-glucuronides is shown in **Scheme 2**. In the first step, as specified above, it was essential to protect phenolic hydroxyl groups. This reaction was carried out using *tert*-butyldimethylsilyl chloride (TBSCl) in excess, imidazole, and dropwise addition of dimethylformamide (DMF) according to the methodology developed by Cruz et al.<sup>65</sup> Compounds **13–18** were obtained with excellent yields (92–97%) after purification by flash chromatography (silica gel, hexane/ethyl acetate 15:1 v/v). This is the first time that compounds **14**, **15**, **17**, and **18** were synthesized and a complete characterization was therefore performed.

The hydroxyl group to be glucuronidated was selectively deprotected by the removal of TBS at position 5 using trifluoroacetic acid (TFA) to afford the intermediates **19–24** with yield ranging between 61 and 79%. Better yields were

Scheme 2. Synthetic Procedure to Prepare (Epi)catechin-5-*O*-glucuronides<sup>64</sup>

<sup>64</sup>Reagents and conditions: (a) TBSCl, imidazole, DMF, rt 72 h; (b) trifluoroacetic acid (TFA), dichloromethane (DCM), 0 °C; (c) methyl 2,3,4-tri-*O*-(trichloroacetamidoyl)- $\alpha$ -D-glucuronate, BF<sub>3</sub>·Et<sub>2</sub>O, DCM, 4 Å molecular sieves, 0 °C, 24 h; (d) MeONa/MeOH (5.4 M), NaOH (0.5 M), tetrahydrofuran (THF)/MeOH (4:1, v/v), 0 °C, 3.5 h; (e) tetra-*n*-butylammonium fluoride (TBAF)/THF (1 M), THF/H<sub>2</sub>O (8:2, v/v), rt, 3 h.

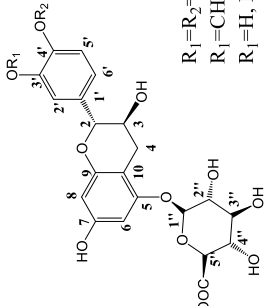
Scheme 3. Mechanism of Epimerization of Epicatechin via Quinone Methide<sup>69</sup>

found for 4'-*O*-methyl derivatives, and the amount of TFA added and the reaction time were critical. For best results, it was necessary to start the reaction with 3 equivalents of TFA added drop by drop and, after 12 h, one more equivalent of TFA was added and the reaction was quenched after 36 h. The crude reaction mixture was purified by flash chromatography (silica gel, hexane/ethyl acetate 10:1 v/v). The intermediates **20**, **21**, **23**, and **24** have not been reported previously and, therefore, a complete characterization was performed.

The next step was the acid glucuronidation of the phenolic intermediates **19**–**24** using methyl 2,3,4-tri-*O*-(trichloroacetamidoyl)- $\alpha$ -D-glucuronate as a sugar donor and catalyzed by BF<sub>3</sub>·Et<sub>2</sub>O as Lewis acid (Scheme 2, step 3) under known reaction conditions.<sup>55,59</sup> Compounds **25**–**30** were not purified to proceed to the next steps. The last two steps for the synthesis of (epi)catechin-5-*O*-glucuronides (**7**–**12**) involved the hydrolysis of the methyl ester and acetyl groups and the removal of TBS groups (Scheme 2, steps d, e).

We first accomplished the hydrolysis of the methyl ester and acetyl groups because catechins and epicatechins are easily oxidized, losing hydrogen atoms in alkaline solution with the generation of quinone-oxidized products and other radical species occurring via oxidation processes.<sup>66–68</sup> Also, epicatechin and catechin molecules can undergo epimerization of the 2-position via quinomethide under basic conditions (Scheme 3).<sup>69,70</sup>

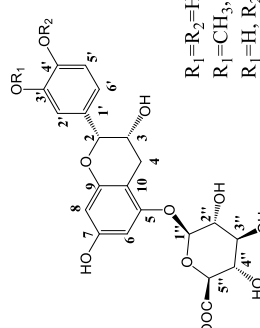
Deprotection of the acetyl groups as well as hydrolysis of the methyl ester for compounds **25**–**30** was easily achieved using a mixture of 5.4 M MeONa in MeOH and 1.0 M NaOH in water at 0 °C for 3.5 h (Scheme 2, step d). Acid workup using Amberlyst 15 hydrogen form to adjust the pH to 3.0, followed by evaporation of the solvent afforded a brown solid, which was dissolved in tetrahydrofuran for removing the TBS groups. It is difficult to remove TBS from position 3; therefore, we used 1 M TBAF/THF in an aqueous solution at pH 7 at room temperature for 3 h. Under these conditions, the desilylation reaction proceeded without obtaining degradation products

Table 2. NMR Spectroscopic Data for Catechin-5-*O*- $\beta$ -D-glucuronide (7), 3'-*O*-Methylcatechin-5-*O*- $\beta$ -D-glucuronide (8), and 4'-*O*-Methylcatechin-5-*O*- $\beta$ -D-glucuronide (9)


$R_1=R_2=H$ : catechin-5-*O*- $\beta$ -D-glucuronide (7)  
 $R_1=CH_3, R_2=H$ : 3'-*O*-methylcatechin-5-*O*- $\beta$ -D-glucuronide (8)  
 $R_1=H, R_2=CH_3$ : 4'-*O*-methylcatechin-5-*O*- $\beta$ -D-glucuronide (9)

position	compound 7			compound 8			compound 9		
	$^1H$ $\delta$ (ppm)	$CD_3OD$ $J$ (Hz)	$^{13}C\{^1H\}$ $\delta$ (ppm)	$CD_3OD$ $J$ (Hz)	$^{13}C\{^1H\}$ $\delta$ (ppm)	$CD_3OD$ $J$ (Hz)	$^1H$ $\delta$ (ppm)	acetone- $d_6$ $J$ (Hz)	$^{13}C\{^1H\}$ $\delta$ (ppm)
2	4.73 (1H, d, $J = 7.2$ )		82.7	4.64 (1H, d, $J = 8.0$ )	81.6		4.67 (1H, d, $J = 7.0$ )		82.1
3	4.09 (1H, dt, $J = 5.2, 7.2, 7.8$ )		68.4	4.01 (1H, dt, $J = 5.6, 7.7, 8.0$ )	68.7		3.96 (1H, dt, $J = 5.1, 7.0, 7.8$ )		67.8
4	H4- $\alpha$ : 3.10 (1H, dd, $J = 5.2, 16.4$ ) H4- $\beta$ : 2.72 (1H, dd, $J = 7.8, 16.4$ )		28.3	H4- $\alpha$ : 3.06 (1H, dd, $J = 5.6, 16.4$ ) H4- $\beta$ : 2.62 (1H, dd, $J = 7.7, 16.4$ )			H4- $\alpha$ : 2.95 (1H, dd, $J = 5.1, 16.3$ ) H4- $\beta$ : 2.69 (1H, dd, $J = 7.8, 16.3$ )		28.0
COOH			175.6						175.9
5			158.0						157.7
6	6.41 (1H, d, $J = 2.0$ )		97.1	6.33 (1H, d, $J = 1.8$ )	97.2		6.42 (1H, d, $J = 2.0$ )		97.2
7			158.1						158.1
8	6.12 (1H, d, $J = 2.0$ )		98.2	6.03 (1H, d, $J = 1.8$ )	98.2		6.01 (1H, d, $J = 2.0$ )		97.7
9			156.7						156.2
10			103.4						103.0
1'			132.2						133.9
2'	6.93 (1H, d, $J = 1.8$ )		115.3	6.97 (1H, d, $J = 1.6$ )	111.8		6.92 (1H, d, $J = 1.9$ )		112.5
3'			146.2						145.8
4'			146.2						148.1
5'	6.87 (1H, d, $J = 8.1$ )		116.2	6.80 (1H, d, $J = 8.2$ )	116.0		6.88 (1H, d, $J = 8.3$ )		115.0
6'	6.82 (1H, dd, $J = 1.8, 8.1$ )		119.9	6.85 (1H, dd, $J = 1.6, 8.2$ )	121.2		6.81 (1H, dd, $J = 1.9, 8.2$ )		119.0
1''	4.95 (1H, d, $J = 7.3$ )		102.6	4.86 (inside of $CD_3OD$ signal)	102.8		4.84 (1H, d, $J = 7.1$ )		102.9
2'', 3'', 4''	3.64–3.58 (3H, m)		77.9, 74.7, 73.5	3.51–3.58 (3H, m)			3.51–3.44 (3H, m)		77.7, 74.4, 73.0
5''	3.84 (1H, d, $J = 9.4$ )		76.6	3.75 (1H, d, $J = 7.3$ )			3.78 (1H, d, $J = 9.2$ )		75.6
OCH <sub>3</sub>				3.85 (3H, s)	56.4		3.81 (3H, s)		56.3

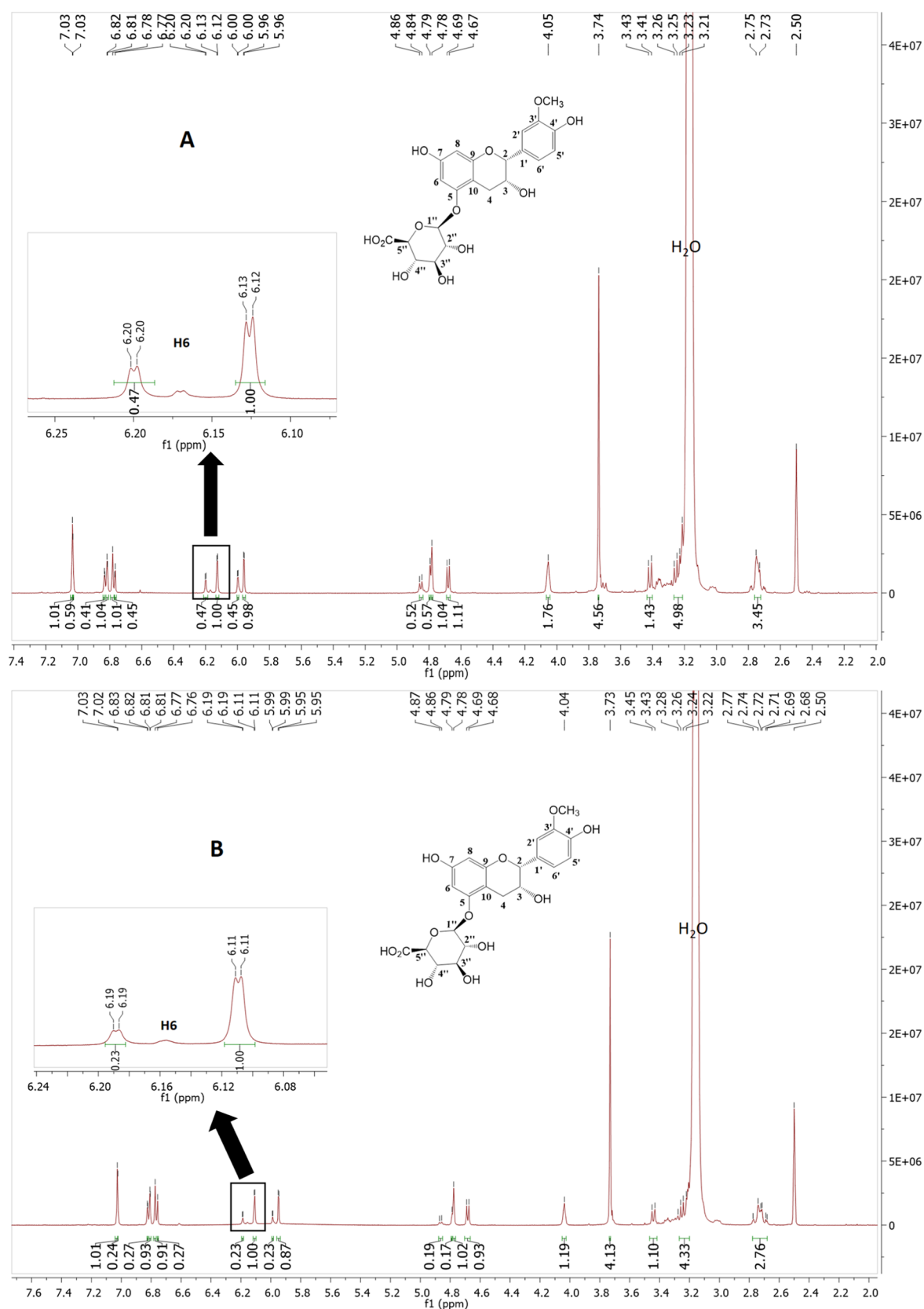
Table 3. NMR Spectroscopic Data for Epicatechin-5-*O*- $\beta$ -D-glucuronide (10), 3'-*O*-Methylepicatechin-5-*O*- $\beta$ -D-glucuronide (11), and 4'-*O*-Methylepicatechin-5-*O*- $\beta$ -D-glucuronide (12)



$R_1=R_2=H$ : catechin-5-*O*- $\beta$ -D-glucuronide (7)  
 $R_1=CH_3, R_2=H$ : 3'-*O*-methylcatechin-5-*O*- $\beta$ -D-glucuronide (8)  
 $R_1=H, R_2=CH_3$ : 4'-*O*-methylcatechin-5-*O*- $\beta$ -D-glucuronide (9)

position	compound 10			compound 11			compound 12		
	$^1H$ $\delta$ (ppm) $CD_3OD$ $J$ (Hz)	$^{13}C$ { $^1H$ } $\delta$ (ppm) $CD_3OD$	$^1H$ $\delta$ (ppm) <sup>a</sup> $DMSO-d_6$ $J$ (Hz)	$^{13}C$ { $^1H$ } $\delta$ (ppm) $DMSO-d_6$	$^1H$ $\delta$ (ppm) $CD_3CD_2$ $J$ (Hz)	$^{13}C$ { $^1H$ } $\delta$ (ppm) $CD_3CD_2$			
2	4.84 (inside $CD_3OD$ signal)	79.9	4.79 (1H, s, br)	78.2	4.81–4.78 (2H, m, H2, H1'')	79.8			
3	4.19 (1H, s br)	67.3	4.05/4.05 (1H, s br)	64.8	4.15 (1H, s br)	67.1			
4	2.98–2.91 (2H, m, H4- $\alpha$ , H4- $\beta$ )	29.3	2.75/2.73(2H, m, H4- $\alpha$ , H4- $\beta$ )	28.3	2.93–2.84 (2H, m, H4- $\alpha$ , H4- $\beta$ )	29.4			
COOH		175.4		172.0					
5		157.9		156.8		157.9			
6	6.33 (1H, d, $J = 2.1$ )	97.3	6.20/6.12 (1H, d, $J = 2.3/2.5$ )	95.24	6.30 (1H, d, $J = 2.2$ )	97.3			
7		158.9		156.7		158.5			
8	6.09 (1H, d, $J = 2.1$ )	98.6	6.00/5.96 (1H, d, $J = 2.3/2.5$ )	96.6	6.02 (1H, d, $J = 2.2$ )	98.5			
9		157.1		155.3		157.0			
10		102.8		100.6		102.8			
1'		132.2		130.1		133.5			
2'	6.99 (1H, d, $J = 2.0$ )	115.3	7.03/7.03 (1H, s)	111.8	6.96 (1H, d, $J = 1.8$ )	112.3			
3'		145.8		147.0		147.2			
4'		145.9		146.3		148.5			
5'	6.77 (1H, d, $J = 8.2$ )	115.9	6.78/6.77 (1H, d, $J = 7.8/7.8$ )	114.8	6.89–6.85 (2H, m, H5', H6')	115.2			
6'	6.81 (1H, dd, $J = 2.0, 8.2$ )	119.4	6.83/6.81 (1H, d, $J = 1.6, 8.2$ )	119.6	6.89–6.85 (2H, m, H5', H6')	119.2			
1''	4.90 (inside $CD_3OD$ signal)	102.8	4.85/4.68 (1H, d, $J = 9.0/8.9$ )	100.7	4.81–4.78 (2H, m, H2, H1'')	102.6			
2'', 3'', 4''	3.59–3.50 (3H, m)	77.9, 74.7, 73.4	3.26–3.21 (3H, m)	76.8, 73.1, 72.3	33.50–3.44 (3H, m)	78.0, 74.7, 73.5			
5''	3.79 (1H, d, $J = 9.5$ )	76.7	3.43/3.41 (1H, s br)	74.4	3.69 (1H, d, $J = 9.2$ )	75.0			
OCH <sub>3</sub>			3.74/3.74 (3H, s)	55.6	3.80 (3H, s)	56.5			

<sup>a</sup>Two stable conformations for this epicatechin glucuronide were observed in  $DMSO-d_6$ , which was evident in the  $^1H$  NMR spectrum; hence, NMR shifts and coupling constants are reported in pairs (see the Supporting Information for details).



**Figure 4.**  $^1\text{H}$  NMR spectrum of **11** in  $\text{DMSO-}d_6$  performed at different temperatures. The inset shows an expanded region of  $\text{H}_6$  signals from the pair of conformational isomers present in a ratio of approximately 1:0.47 (A, 25 °C) or 1:0.23 (B, 10 °C).

such as *ortho*-quinones.<sup>65</sup> Purification of the residue by preparative HPLC furnished catechin-5-*O*-glucuronide (**7**, 68%), 3'-*O*-catechin-5-*O*-glucuronide (**8**, 71%), 4'-*O*-methyl

catechin-5-*O*-glucuronide (**9**, 77%), epicatechin-5-*O*-glucuronide (**10**, 51%), 3'-*O*-epicatechin-5-*O*-glucuronide (**11**, 59%), and 4'-*O*-methyl epicatechin-5-*O*-glucuronide (**12**, 65%) is

performed with excellent purity. Epicatechin glucuronides showed a lower yield than catechin glucuronides, and we observed a small peak corresponding to catechin analogues in HPLC chromatograms of the crude reaction products.

The complete unambiguous assignments of the hydrogens and carbons of the three rings and the glucuronic acid moiety were accomplished using a combination of heteronuclear multiple quantum coherence (HMQC) and HMBC (Tables 2 and 3) and are in agreement with those previously reported by Zhang<sup>59</sup> and Blount.<sup>56,61</sup> In the HMBC spectrum, the long correlation between the anomeric proton (H-1'') and C5 (Figures S44, S48, S52, S56, S62, and S66) is evident in all compounds.

The NMR spectra obtained for compound 11 in DMSO-*d*<sub>6</sub> gave a set of double signals for each nucleus with a peak intensity ratio of around 1:0.47 in the <sup>1</sup>H NMR spectrum (Figures 4A and S57). The full chemical shift assignment of the pair of signals, using a complement of 2D NMR experiments (Figures S61 and S62), demonstrated that both pairs of signals corresponded to 6. A second <sup>1</sup>H NMR spectrum acquired at 10 °C led to a change in the relative peak integrals to approximately 1:0.23 (Figures 4B and S58). This therefore indicated some conformational equilibrium shown by 11. This conformational equilibrium was not observed when <sup>1</sup>H NMR was performed in MeOH (Figures S47 and S59).

Compounds 8, 9, 11, and 12 showed a quasimolecular ion at *m/z* 479.1 [M - H]<sup>-</sup> (calcd for 479.1). A fragment at *m/z* 302.9 was also observed, corresponding to the neutral loss of 176 Da (the glucuronic moiety) from the quasimolecular precursor ions *m/z* 479.1 [M - H]. Compounds 7 and 10 showed a quasimolecular ion at *m/z* 465.3 [M - H]<sup>-</sup> (calcd for 465.4). A fragment at *m/z* 288.9 was also observed, corresponding to the neutral loss of 176 Da (the glucuronic moiety) from the quasimolecular precursor ions *m/z* 465.3 [M - H] (Figures S75–S80).

## CONCLUSIONS

This work describes the first regioselective methylation of (epi)catechin to obtain 4'-*O*-methyl (epi)catechin with 97 and 94% of yield, respectively. Furthermore, we developed an efficient synthetic procedure to prepare (epi)catechin-5-*O*-glucuronides in five (compound 7 and 10) or six (compounds 8, 9, 11, and 12) steps with an overall yield ranging between 64 and 83%. The synthesis of these compounds will allow us to study them as potentially active components (for the compounds shown to be present in the brain) as controls in mechanism of action studies in relation to neuroprotection in Alzheimer's disease using *in vitro* assays to measure the regulation of oxidative stress and neuroinflammation.

We believe that the synthesis of the intermediates 14, 15, 17, 18, 20, 21, 23, and 24 has not been described previously. A number of analytical tools were applied for a complete characterization of the final products as well as the intermediates in the synthetic routes, including MS and NMR.

## EXPERIMENTAL PROCEDURES

**General Information.** All reactions were carried out in dried glassware and round-bottomed flasks under an argon atmosphere using commercial reagents, distilled solvents, and anhydrous solvents unless otherwise noted. High-performance liquid chromatography (HPLC) grade solvents were purchased from Fisher Scientific. Chemicals and solvents were of reagent

grade and obtained from commercial sources without further purification. The reactions were monitored by thin-layer chromatography (TLC) on aluminum-backed precoated silica gel 60 F254 plates (Sigma, St. Louis, O), and compounds were detected using a UV lamp (254 nm). Column chromatographic purification was performed using 230–400 mesh silica gel unless otherwise noted.

Preparative high-performance liquid chromatography (HPLC) was performed on an Agilent HP1200 HPLC, monitoring at 280 nm. The HPLC with ChemStation software version B.02.01.SRI was equipped with a G1322A degasser, G1311A quaternary pump, G1367B autosampler, G1316A thermostatic column compartment, and G1315C diode array detector. A Phenomenex Luna 10 μm C18 250 × 21.2 mm column was used for preparative HPLC on the Agilent HPLC system. For methyl ethers of (epi)catechin, the column was eluted with an isocratic mixture of water with formic acid (0.1%) and acetonitrile (87:13, v/v), and the flow rate was set at 9 mL/min. The crude methylated (epi)catechin derivatives were loaded in methanol. For (epi)catechin glucuronides, the column was eluted with a mobile phase consisting of solvent A (0.1% formic acid in water) and solvent B (acetonitrile) with a gradient of 10% B for 5 min and 15% B for 35 min. The crude glucuronides were loaded in 60% (v/v) MeOH. The solubility of these compounds in 60% MeOH is poor and this limited the load amount, but this solvent gave the best compromise between the solubility and solvent polarity.

**Characterization Data.** For NMR analysis, the synthesized substances were dissolved in a specific deuterated solvent and then transferred to a 5 mm Shigemi tube (Wilmad Glass, Vineland, NJ) or a normal NMR tube.

The proton and carbon nuclear magnetic resonance (<sup>1</sup>H NMR and <sup>13</sup>C NMR, respectively) spectra were recorded using a Varian-INOVA 500 NMR spectrometer (Varian, CA, <sup>1</sup>H NMR 500 MHz, and <sup>13</sup>C NMR 125 MHz), Varian-INOVA 400 NMR spectrometer (Varian, CA, <sup>1</sup>H NMR 400 MHz, and <sup>13</sup>C NMR 100 MHz), or Bruker Avance III spectrometer (Bruker, CA, <sup>1</sup>H NMR 600 MHz, and <sup>13</sup>C NMR 150 MHz) equipped with a 5 mm inverse detection BBI probe at ambient temperature unless otherwise indicated.

Chemical shifts were reported in ppm relative to the CDCl<sub>3</sub> peak (7.26 ppm for <sup>1</sup>H NMR, 77.0 ppm for <sup>13</sup>C{<sup>1</sup>H} NMR), CD<sub>3</sub>OD peak (4.78 ppm for <sup>1</sup>H NMR, 49.3 ppm for <sup>13</sup>C{<sup>1</sup>H} NMR), DMSO-*d*<sub>6</sub> peak (2.50 ppm for <sup>1</sup>H NMR, 39.5 ppm for <sup>13</sup>C{<sup>1</sup>H} NMR), or acetone-*d*<sub>6</sub> peak (2.05 ppm for <sup>1</sup>H NMR, 29.9 ppm for <sup>13</sup>C{<sup>1</sup>H} NMR). Data for <sup>1</sup>H were reported as follows: chemical shift (ppm), multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublets, m = multiplet, and br = broad singlet), coupling constants (Hz), and integration.

Analytical HPLC was used to monitor some reactions and analyze the products. A sample volume of 10 μL was applied to an Agilent 1290 UHPLC system equipped with an Eclipse Plus C18 column (3.5 μm particle, 100 × 4.6 mm) and separated in a mobile phase consisting of solvent A (0.1% formic acid in water) and solvent B (acetonitrile) with 15% isocratic gradient of solvent B and a flow rate of 1 mL/min for 30 min. Chromatograms were monitored at 280 nm.

LC-MS/MS analysis was performed using an Agilent 1290 Infinity II liquid 645 chromatography system coupled to an Agilent 6400 Series Triple Quadrupole System with an electrospray ionization source in a negative ionization mode. A reverse-phase ZORBAX RR Eclipse Plus capillary column,

0.3 mm  $\times$  150 mm (3.5  $\mu$ m particle size), was used for separation. The gradient for LC separation was 0.1% (v/v) formic acid in water (A) and 0.1% (v/v) formic acid in acetonitrile (B) with the following gradient of solvent B: 5 min 10%, 10 min 20%, 15 min 30%, 20 min 50%, 25 min 70%, and 30 min 95% with a flow rate of 0.5 mL/min for 30 min. The injection volume was 5  $\mu$ L. The eluted compounds were analyzed by ESI-MS/MS in the range of  $m/z$  100–800 and processed using Agilent MassHunter Qualitative Analysis B.06.00.

The detection of the newly synthesized metabolites was achieved using a hybrid triple quadrupole/ion trap mass spectrometer (QTRAP 5500) from AB Sciex. Each compound was injected individually and directly into the mass spectrometer at a flow rate of 7  $\mu$ L/min using electrospray ionization. Full and product ion scan modes were utilized to assess the precursor ion mass and MS/MS spectrum, respectively.

All high-resolution mass spectra (HRMS) were recorded using a high-resolution mass spectrometer TripleTOF6600+ from AB Sciex. Each compound was diluted 20-fold with a solution of 100% methanol. Metabolites were injected individually and directly into the mass spectrometer apparatus at a flow rate of 10  $\mu$ L/min. The parameters such as declustering potential, collision energy, and collision spray energy were set up at 80 V, 35 V, and 5 V, respectively. The mass spectra were acquired using Turbo Spray Ionization set to 5500 V in a positive ion mode with an accumulation time of 100 ms. The curtain gas (nitrogen), nebulizing, and heating gas were fixed at 25, 20, and 15 psi, respectively. The temperature of the source was 25  $^{\circ}$ C. MS spectra were acquired and processed using Analyst TF 1.8.1 software.

**Synthesis of 3'- and 4'-O-Methyl (Epi)catechin and Their Glucuronide Derivatives. Representative Procedure for Methylation of (Epi)catechin: Synthesis of (2R,3S)-2-(4-Hydroxy-3-methoxyphenyl)chromane-3,5,7-triol (3), (2R,3S)-2-(3-Hydroxy-4-methoxyphenyl)chromane-3,5,7-triol (4), (2R,3R)-2-(4-Hydroxy-3-methoxyphenyl)chromane-3,5,7-triol (5), and (2R,3R)-2-(3-Hydroxy-4-methoxyphenyl)chromane-3,5,7-triol (6).** A suspension of (epi)catechin (2.00 g, 6.89 mmol) methyl iodide (8.0 mL, 0.12 mol), potassium carbonate (4.76 g, 34.45 mmol), and ground 3Å molecular sieves (2 g) in acetone (120 mL) was sonicated for 4 h and then stirred at room temperature for 20 h under argon. The progress of the reaction was monitored by HPLC. After 24 h, the solvent was filtered and concentrated to dryness *in vacuo* (no heat). The crude product was further purified by preparative HPLC in 20 injections. The purity of the fractions was checked by analytical HPLC. The fractions of each methyl ether of (epi)catechin were combined and evaporated under nitrogen flow to afford 733.8 mg of compound 3 (35%), 1.25 g of compound 4 (60%), 838.1 mg of compound 5 (40%), and 1.15 g of compound 6 (55%).

Compound 3:<sup>54,61,62</sup>  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 6.91 (1H, d,  $J$  = 1.4 Hz, H2'); 6.77–6.73 (2H, m, H5', H6'); 5.90 (1H, d,  $J$  = 2.2 Hz, H6); 5.69 (1H, d,  $J$  = 2.2 Hz, H8); 4.51 (1H, d,  $J$  = 8.0 Hz, H2); 3.89 (1H, td,  $J$  = 5.6, 8.0, 8.1 Hz, H3); 3.75 (3H, s, OCH<sub>3</sub>); 2.73 (1H, dd,  $J$  = 5.6, 16.0 Hz, H4- $\alpha$ ); 2.36 (1H, dd,  $J$  = 8.1, 16.0 Hz, H4- $\beta$ ).  $^{13}\text{C}\{^1\text{H}\}$  NMR (125 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 156.5 (C7); 156.2 (C5); 155.4 (C9); 147.2 (C3'); 146.2 (C4'); 130.4 (C1'); 120.2 (C6'); 115.0 (C5'); 111.6 (C2'); 99.2 (C10); 95.2 (C6); 93.9 (C8); 81.3 (C2); 66.3 (C3); 55.6 (OCH<sub>3</sub>); 28.5

(C4). MS  $m/z$ :  $[\text{M} - \text{H}]^+$  calcd for C<sub>16</sub>H<sub>15</sub>O<sub>6</sub> 303.1; found 303.1.

Compound 4:<sup>54,61,62</sup>  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 9.19 (s, 1H, OH-5); 8.95 (s, 1H, OH-7); 8.94 (s, 1H, OH-3'); 6.87 (1H, d,  $J$  = 8.3 Hz, H5'); 6.77 (1H, d,  $J$  = 2.0 Hz, H2'); 6.73 (1H, dd,  $J$  = 2.0, 8.3 Hz, H6'); 5.90 (1H, d,  $J$  = 2.2 Hz, H6); 5.70 (1H, d,  $J$  = 2.2 Hz, H8); 4.91 (1H, d,  $J$  = 6.5 Hz, OH-3); 4.52 (1H, d,  $J$  = 8.0 Hz, H2); 3.83 (1H, dddd,  $J$  = 5.4, 6.5, 8.0, 8.2 Hz, H3); 3.75 (3H, s, OCH<sub>3</sub>); 2.66 (1H, dd,  $J$  = 5.4, 16.0 Hz, H4- $\alpha$ ); 2.36 (1H, dd,  $J$  = 8.2, 16.0 Hz, H4- $\beta$ ).  $^{13}\text{C}\{^1\text{H}\}$  NMR (125 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 156.5 (C7); 156.2 (C5); 155.3 (C9); 147.2 (C4'); 146.2 (C3'); 132.2 (C1'); 118.3 (C6'); 114.4 (C2'); 111.8 (C5'); 99.2 (C10); 95.2 (C6); 93.9 (C8); 80.8 (C2); 66.3 (C3); 55.7 (OCH<sub>3</sub>); 27.9 (C4). MS  $m/z$ :  $[\text{M} - \text{H}]^+$  calcd for C<sub>16</sub>H<sub>15</sub>O<sub>6</sub> 303.1; found 303.1.

Compound 5:<sup>61,62</sup>  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 9.27 (s, br, 1H, OH-5); 8.99 (s, br, 2H, OH-7, OH-4'); 7.03 (1H, d,  $J$  = 2.0 Hz, H2'); 6.83 (1H, dd,  $J$  = 2.0, 8.1 Hz, H6'); 6.73 (1H, d,  $J$  = 8.1 Hz, H5'); 5.91 (1H, d,  $J$  = 2.2 Hz, H6); 5.73 (1H, d,  $J$  = 2.2 Hz, H8); 4.79 (1H, s, H2); 4.70 (1H, s, br, OH-3); 4.04 (1H, s, H3); 3.74 (3H, s, OCH<sub>3</sub>); 2.69 (1H, dd,  $J$  = 4.5, 16.4 Hz, H4- $\beta$ ); 2.51 (1H, dd,  $J$  = 2.5, 16.40 MHz, H4- $\alpha$ ).  $^{13}\text{C}\{^1\text{H}\}$  NMR (125 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 156.5 (C7); 156.3 (C5); 155.8 (C9); 146.9 (C3'); 145.8 (C4'); 130.6 (C1'); 119.6 (C6'); 114.8 (C5'); 111.6 (C2'); 98.4 (C10); 95.6 (C6); 94.5 (C8); 78.1 (C2); 64.9 (C3); 55.6 (OCH<sub>3</sub>); 28.4 (C4). MS  $m/z$ :  $[\text{M} - \text{H}]^+$  calcd for C<sub>16</sub>H<sub>15</sub>O<sub>6</sub> 303.1; found 303.1.

Compound 6:<sup>61,62</sup>  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 6.93 (1H, d,  $J$  = 2.0 Hz, H2'); 6.85 (1H, d,  $J$  = 8.4 Hz, H5'); 6.78 (1H, dd,  $J$  = 2.0, 8.4 Hz, H6'); 5.91 (1H, d,  $J$  = 2.3 Hz, H6); 5.73 (1H, d,  $J$  = 2.3 Hz, H8); 4.78 (1H, s, H2); 4.02 (1H, td,  $J$  = 1.7, 4.6, 3.6 Hz, H3); 3.74 (3H, s, OCH<sub>3</sub>); 2.69 (1H, dd,  $J$  = 4.6, 16.3 Hz, H4- $\beta$ ); 2.47 (1H, m, H4- $\alpha$ ).  $^{13}\text{C}\{^1\text{H}\}$  NMR (125 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 156.6 (C7); 156.3 (C5); 155.7 (C9); 146.8 (C4'); 145.9 (C3'); 132.4 (C1'); 117.7 (C5'); 114.8 (C2'); 111.6 (C6'); 98.4 (C10); 95.2 (C6); 94.1 (C8); 77.9 (C2); 64.9 (C3); 55.7 (OCH<sub>3</sub>); 28.2 (C4). MS  $m/z$ :  $[\text{M} - \text{H}]^+$  calcd for C<sub>16</sub>H<sub>15</sub>O<sub>6</sub> 303.1; found 303.1.

**Representative Procedure for Regioselective Methylation of (Epi)catechin: Synthesis of (2R,3S)-2-(3-Hydroxy-4-methoxyphenyl)chromane-3,5,7-triol (4) and (2R,3R)-2-(3-Hydroxy-4-methoxyphenyl)chromane-3,5,7-triol (6).** A suspension of (epi)catechin (2.00 g, 6.89 mmol) methyl iodide (0.6 mL, 10.33 mmol), potassium carbonate (952.2 mg, 6.89 mmol), and ground 3Å molecular sieves (1 g) in acetone (80 mL) was vigorously stirred for 24 h under argon. Then, another batch of methyl iodide (0.6 mL, 10.33 mmol) and potassium carbonate (952.2 mg, 6.89 mmol) was added. After 24 h, methyl iodide (0.4 mL, 6.89 mmol) and potassium carbonate (476.1 mg, 3.44 mmol) were added again. The progress of the reaction was monitored by HPLC. After 72 h, the solvent was removed under vacuum, and the residue was further purified by preparative HPLC in 20 injections using the same procedure described for the methylation of (epi)catechin. The yield was 2.03 g of compound 4 (97%) and 1.97 g of compound 6 (94%).

**Representative Procedure for Protecting Hydroxyl Groups with Tert-butyldimethylsilyl chloride: Synthesis of ((2R,3S)-2-(3,4-Bis((tert-butyldimethylsilyloxy)phenyl)chromane-3,5,7-triyl)tris(oxy))tris(tert-butyldimethylsilane) (13),**

((*(2R,3S)*-2-(4-((*tert*-Butyldimethylsilyl)oxy)-3-methoxyphenyl)chromane-3,5,7-triyl)tris(oxy))tris(*tert*-butyldimethylsilane) (**14**), ((*(2R,3S)*-2-(3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxyphenyl)chromane-3,5,7-triyl)tris(oxy))tris(*tert*-butyldimethylsilane) (**15**), ((*(2R,3R)*-2-(3,4-Bis((*tert*-butyldimethylsilyl)oxy)phenyl)chromane-3,5,7-triyl)tris(oxy))tris(*tert*-butyldimethylsilane) (**16**), ((*(2R,3R)*-2-(4-((*tert*-Butyldimethylsilyl)oxy)-3-methoxyphenyl)chromane-3,5,7-triyl)tris(oxy))tris(*tert*-butyldimethylsilane) (**17**), and ((*(2R,3R)*-2-(3-((*tert*-Butyldimethylsilyl)oxy)-4-methoxyphenyl)chromane-3,5,7-triyl)tris(oxy))tris(*tert*-butyldimethylsilane) (**18**). Catechin (200 mg, 0.69 mmol), imidazole (750.5 mg, 11.02 mmol), and *tert*-butyldimethylsilyl chloride (830.8 mg, 5.51 mmol) were suspended in dry DMF (0.5 mL) under argon. The reaction mixture was stirred for 72 h at room temperature. The resulting mixture was dissolved in diethyl ether (30 mL) and washed with water (3 × 30 mL). The organic layer was dried over sodium sulfate. The dried extract was concentrated in vacuo and the residue purified by flash column chromatography [silica gel, hexane/EtOAc (15:1)] to furnish the products (yields below) as white solids.

Compound **13**<sup>65</sup> (558.1 mg, 94%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ (ppm): 6.88 (1H, d, *J* = 2.0 Hz, H2'); 6.84 (1H, dd, *J* = 2.0, 8.2 Hz, H6'); 6.81 (1H, d, *J* = 8.2 Hz, H5'); 6.08 (1H, d, *J* = 2.3 Hz, H6); 5.95 (1H, d, *J* = 2.3 Hz, H8); 4.51 (1H, d, *J* = 9.0 Hz, H2); 3.86 (1H, td, *J* = 5.8, 9.0 Hz, H3); 3.04 (1H, dd, *J* = 5.8, 16.1 Hz, H4-α); 2.54 (1H, dd, *J* = 9.0, 16.1 Hz, H4-β); (1.00 – (–0.32)), (75H, CH<sub>3</sub>-TBS). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ (ppm): 155.9; 155.0; 154.3; 146.8; 146.7; 132.8; 120.9; 120.9; 120.6; 106.0, 103.8; 101.5; 81.9; 69.8; 31.3; 26.2; 26.1; 25.8; 18.6; 18.6; 18.4; 18.4; 18.1; 0.2; –3.9; –3.9; –4.0; –4.1; –4.2; –4.3; –4.8; –5.10. HRMS (ESI/TOF) *m/z*: calcd for C<sub>45</sub>H<sub>85</sub>O<sub>6</sub>Si<sub>5</sub> 861.5; found 861.6.

Compound **14** (920.7 mg, 92%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ (ppm): 6.91 (1H, d, *J* = 2.0 Hz, H2'); 6.88 (1H, dd, *J* = 2.0, 8.0 Hz, H6'), 6.84 (1H, d, *J* = 8.0 Hz, H5'); 6.10 (1H, d, *J* = 2.2 Hz, H6); 5.96 (1H, d, *J* = 2.2 Hz, H8); 4.53 (1H, d, *J* = 8.9 Hz, H2); 3.91 (1H, td, *J* = 6.0, 8.9, 10 Hz, H3); 3.81 (3H, s, OCH<sub>3</sub>); 3.09 (1H, dd, *J* = 6.0, 16.1 Hz, H4-α); 2.55 (1H, dd, *J* = 10.0, 16.1 Hz, H4-β); (1.02 – (–0.37)) (60H, CH<sub>3</sub>-TBS). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ (ppm): 155.9; 155.1; 154.3; 150.8; 145.1; 133.0; 127.8; 120.6; 119.3; 111.8; 106.1; 103.9; 101.6; 82.4; 69.9; 55.7; 31.6; 25.9; 25.9; 25.8; 25.8; 18.7; 18.4; 18.3; 18.1; 0.2; –4.1; –4.1; –4.2; –4.3; –4.5; –4.5; –4.9; –5.1. HRMS (ESI/TOF) *m/z*: calcd for C<sub>40</sub>H<sub>73</sub>O<sub>6</sub>Si<sub>4</sub> 761.4486 [M + H]<sup>+</sup>; found, 761.4478.

Compound **15** (950.8 mg, 95%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ (ppm): 6.96 (1H, dd, *J* = 2.1, 8.3, Hz, H6'); 6.92 (1H, d, *J* = 2.1, Hz, H2'); 6.84 (1H, d, *J* = 8.3 Hz, H5'); 6.09 (1H, d, *J* = 2.3 Hz, H6); 5.96 (1H, d, *J* = 2.3 Hz, H8); 4.52 (1H, d, *J* = 9.0 Hz, H2); 3.90 (1H, td, *J* = 6.0, 9.0, 10.0 Hz, H3); 3.81 (3H, s, OCH<sub>3</sub>); 3.06 (1H, dd, *J* = 6.0, 16.1 Hz, H4-α); 2.55 (1H, dd, *J* = 10.0, 16.1 Hz, H4-β); (1.03 – (–0.34)), (60H, CH<sub>3</sub>-TBS). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ (ppm): 155.9; 155.0; 154.3; 150.9; 145.0; 132.3; 121.2; 120.5; 111.9; 106.0; 103.8; 101.5; 81.9; 69.7; 55.8; 31.4; 25.9; 25.9; 25.8; 25.8; 18.6; 18.4; 18.4; 18.1; 0.2; –4.1; –4.1; –4.3; –4.4; –4.5; –4.8; –5.1. HRMS (ESI/TOF) *m/z*: calcd for C<sub>40</sub>H<sub>73</sub>O<sub>6</sub>Si<sub>4</sub> 761.4486 [M + H]<sup>+</sup>; found, 761.4474.

Compound **16**<sup>71</sup> (569.9 mg, 96%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ (ppm): 6.88 (1H, dd, *J* = 2.1, 8.3 Hz, H6'); 6.84 (1H, d, *J* = 2.1 Hz, H2'); 6.78 (1H, d, *J* = 8.2 MHz, H5'); 6.11 (1H, d, *J* = 2.3 Hz, H6); 5.94 (1H, d, *J* = 2.3 Hz, H8); 4.91

(1H, d, *J* = 2.0 Hz, H2); 4.17 (1H, td, *J* = 2.0, 4.1, 4.0 Hz, H3); 2.79 (1H, dd, *J* = 4.1, 16.2 Hz, H4-β); 2.64 (1H, dd, *J* = 4.9, 16.2 Hz, H4-α); 0.99 – (–0.25) (75H, CH<sub>3</sub>-TBS). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ (ppm): 155.8; 154.9; 154.6; 146.4; 146.2; 132.5; 120.7; 120.4; 120.0; 104.7; 103.3; 101.4; 78.8; 67.6; 28.0; 26.2; 26.1; 25.9; 25.9; 25.8; 18.6; 18.6; 18.4; 18.4; 18.2; –2.8; –4.0; –4.0; –4.0; –4.0; –4.2; –4.2; –4.2; –4.9. ESI-MS *m/z*: [M – H]<sup>+</sup> calcd for C<sub>45</sub>H<sub>85</sub>O<sub>6</sub>Si<sub>5</sub> 861.5; found 861.5.

Compound **17** (910.7 mg, 91%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ (ppm): 6.98 (1H, s, H2'); 6.81 (2H, d, H6', H5'); 6.15 (1H, d, *J* = 2.3 Hz, H6); 5.95 (1H, d, *J* = 2.3 Hz, H8); 4.93 (1H, d, *J* = 1.2 Hz, H2); 4.19 (1H, td, *J* = 1.2, 3.9, 4.5 Hz, H3); 3.79 (3H, s, OCH<sub>3</sub>); 2.81 (1H, dd, *J* = 3.9, 16.4 Hz, H4-β); 2.70 (1H, dd, *J* = 4.5, 16.4 Hz, H4-α); 1.00 – (–0.30) (60H, CH<sub>3</sub>-TBS). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ (ppm): 155.9; 154.8; 154.7; 150.6; 144.5; 133.1; 127.8; 120.5; 119.3; 111.3; 104.8; 103.6; 101.6; 79.2; 67.8; 55.6; 29.4; 25.9; 25.9; 25.9; 25.8; 18.6; 18.4; 18.4; 18.2; 0.2; –3.9; –4.2; –4.2; –4.3; –4.5; 4.5; –4.9; –5.0. HRMS (ESI/TOF) *m/z*: calcd for C<sub>40</sub>H<sub>73</sub>O<sub>6</sub>Si<sub>4</sub> 761.4486 [M + H]<sup>+</sup>; found, 761.4481.

Compound **18** (970.8 mg, 97%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ (ppm): 6.98 (1H, dd, *J* = 2.0, 8.4, Hz, H6'); 6.90 (1H, d, *J* = 2.0, Hz, H2'); 6.00 (1H, d, *J* = 8.4 Hz, H5'); 6.13 (1H, d, *J* = 2.3 Hz, H6); 5.95 (1H, d, *J* = 2.3 Hz, H8); 4.92 (1H, d, *J* = 2.0 Hz, H2); 4.19 (1H, td, *J* = 2.0, 4.1, 5.0 Hz, H3); 3.99 (3H, s, OCH<sub>3</sub>); 2.80 (1H, dd, *J* = 4.1, 16.2 Hz, H4-β); 2.64 (1H, dd, *J* = 5.0, 16.2 Hz, H4-α); 1.00 – (–0.23) (60H, CH<sub>3</sub>-TBS). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ (ppm): 155.8; 154.8; 154.6; 150.4; 144.5; 131.9; 120.6; 120.0; 111.6; 104.9; 103.5; 101.5; 78.2; 67.6; 55.7; 28.9; 26.1; 25.9; 25.9; 25.9; 18.6; 18.4; 18.4; 18.2; –4.0; –4.2; –4.2; –4.3; –4.5, –4.9; –4.9; –5.6. HRMS (ESI/TOF) *m/z*: calcd for C<sub>40</sub>H<sub>73</sub>O<sub>6</sub>Si<sub>4</sub> 761.4486 [M + H]<sup>+</sup>; found, 761.4483.

**Representative Procedure for Selective Removal of the TBS Protective Group: Synthesis of (2R,3S)-2-(3,4-bis((*tert*-Butyldimethylsilyl)oxy)phenyl)-3,7-bis((*tert*-butyldimethylsilyl)oxy)chroman-5-ol (**19**), (2R,3S)-3,7-Bis((*tert*-butyldimethylsilyl)oxy)-2-(4-((*tert*-butyldimethylsilyl)oxy)-3-methoxyphenyl)chroman-5-ol (**20**), (2R,3S)-3,7-Bis((*tert*-butyldimethylsilyl)oxy)-2-(3-((*tert*-butyldimethylsilyl)oxy)-4-methoxyphenyl)chroman-5-ol (**21**), (2R,3R)-2-(3,4-Bis((*tert*-butyldimethylsilyl)oxy)phenyl)-3,7-bis((*tert*-butyldimethylsilyl)oxy)chroman-5-ol (**22**), (2R,3R)-3,7-Bis((*tert*-butyldimethylsilyl)oxy)-2-(4-((*tert*-butyldimethylsilyl)oxy)-3-methoxyphenyl)chroman-5-ol (**23**), and (2R,3R)-3,7-Bis((*tert*-butyldimethylsilyl)oxy)-2-(3-((*tert*-butyldimethylsilyl)oxy)-4-methoxyphenyl)chroman-5-ol (**24**). Trifluoroacetic acid (117 μL, 1.50 mmol) was added dropwise to an ice-cooled stirred solution of compound **13** (430.0 mg, 0.50 mmol) in anhydrous methylene chloride (200 mL) under argon and stirred on an ice bath for 12 h. Then, another batch of trifluoroacetic acid (38 μL, 0.50 mmol) was added and the reaction mixture was stirred for 24 h on an ice bath. After 36 h, a saturated solution of sodium hydrocarbonate (90 mL) was added and the mixture was stirred for 30 min and separated. The aqueous solution was extracted with methylene chloride (100 mL). The combined organic phase was washed with brine (100 mL), dried over sodium sulfate, and concentrated in vacuo. The residue was dissolved in methylene chloride for purification by flash column chromatography [silica gel, hexane/EtOAc (10:1)] to yield the following products:**

Compound **19**<sup>72</sup> (259.8 mg, 70%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ (ppm): 6.89 (1H, d, *J* = 2.0 Hz, H2'); 6.85 (1H, dd, *J* = 2.0, 8.2 Hz, H6'); 6.82 (1H, d, *J* = 8.2 Hz, H5'); 6.06 (1H, d, *J* = 2.2 Hz, H6); 5.96 (1H, d, *J* = 2.2 Hz, H8); 4.83 (1H, s, br, OH-5); 4.55 (1H, d, *J* = 8.8 Hz, H2); 3.91 (1H, td, *J* = 5.8, 8.8, 9.1 Hz, H3); 2.97 (1H, dd, *J* = 5.8, 15.9 Hz, H4-α); 2.59 (1H, dd, *J* = 9.1, 15.9 Hz, H4-β); 1.00 – (–0.36), (60H, CH<sub>3</sub>-TBS). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ (ppm): 156.0; 155.4; 154.3; 146.9; 146.7; 132.6; 121.0; 120.9; 120.6; 101.6; 100.9; 100.2; 81.9; 69.4; 29.9; 26.2; 26.1; 25.9; 25.8; 18.6; 18.6; 18.3; 18.0; 0.2; –3.9; –3.9; –4.0; –4.0; –4.3; –4.3; –4.6; –5.2. ESI-MS *m/z*: [M – H]<sup>–</sup> calcd for C<sub>39</sub>H<sub>69</sub>O<sub>6</sub>Si<sub>4</sub> 746.4; found 746.4.

Compound **20** (431 mg, 61%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ (ppm): 6.92 (1H, d, *J* = 2.0 Hz, H2'); 6.88 (1H, dd, *J* = 2.0, 8.1 Hz, H6'), 6.84 (1H, d, *J* = 8.1 Hz, H5'); 6.06 (1H, d, *J* = 2.2 Hz, H6); 5.96 (1H, d, *J* = 2.2 Hz, H8); 4.83 (1H, s, OH-5); 4.56 (1H, d, *J* = 8.9 Hz, H2); 3.95 (1H, td, *J* = 6.0, 8.9, 9.6 Hz, H3); 3.81 (3H, s, OCH<sub>3</sub>); 3.01 (1H, dd, *J* = 6.0, 16.0 Hz, H4-α); 2.60 (1H, dd, *J* = 9.6, 16.0 Hz, H4-β); 1.25 – (–0.43), (45H, CH<sub>3</sub>-TBS). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ (ppm): 156.1; 155.4; 154.3; 150.9; 145.2; 132.7; 120.8; 120.6; 111.9; 101.8; 100.9; 100.4; 82.4; 69.5; 55.7; 30.4; 29.9; 25.9; 25.8; 18.7; 18.3; 18.0; 0.2; –3.4; –4.3; –4.3; –4.5; –4.5; –4.7; –5.2. HRMS (ESI/TOF) *m/z*: calcd for C<sub>34</sub>H<sub>59</sub>O<sub>6</sub>Si<sub>3</sub> 647.3621 [M + H]<sup>+</sup>; found, 647.3612.

Compound **21** (509.6 mg, 76%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ (ppm): 6.97 (1H, dd, *J* = 2.1, 8.3, Hz, H6'); 6.93 (1H, d, *J* = 2.1, Hz, H2'); 6.85 (1H, d, *J* = 8.3 Hz, H5'); 6.06 (1H, d, *J* = 2.2 Hz, H6); 5.96 (1H, d, *J* = 2.2 Hz, H8); 4.98 (1H, s, OH-5); 4.56 (1H, d, *J* = 8.7 Hz, H2); 3.95 (1H, td, *J* = 5.9, 8.7, 9.4 Hz, H3); 3.82 (3H, s, OCH<sub>3</sub>); 2.99 (1H, dd, *J* = 5.9, 15.8 Hz, H4-α); 2.60 (1H, dd, *J* = 9.4, 15.7 Hz, H4-β); 1.01 – (–0.38), (45H, CH<sub>3</sub>-TBS). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ (ppm): 156.0; 155.3; 154.3; 151.0; 145.0; 132.1; 121.2; 120.5; 112.0; 101.7; 100.8; 100.3; 81.9; 69.4; 58.9; 30.1; 25.9; 25.9; 25.8; 18.6; 18.3; 18.0; –4.3; –4.3; –4.4; –4.5; –4.7; –5.3. HRMS (ESI/TOF) *m/z*: calcd for C<sub>34</sub>H<sub>59</sub>O<sub>6</sub>Si<sub>3</sub> 647.3621 [M + H]<sup>+</sup>; found, 647.3617.

Compound **22**<sup>71</sup> (327.8 mg, 70%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ (ppm): 6.89 (1H, d, *J* = 8.2 Hz, H6'); 6.85 (1H, s, H2'); 6.79 (1H, d, *J* = 8.2 Hz, H5'); 6.08 (1H, d, *J* = 2.0 Hz, H6); 5.92 (1H, d, *J* = 2.0 Hz, H8); 4.93 (1H, s, H2); 4.20 (1H, d, *J* = 2.0 Hz, H3); 2.79 (1H, dd, *J* = 4.0, 15.9 Hz, H4-β); 2.64 (1H, dd, *J* = 4.7, 15.9 Hz, H4-α); 0.98 – (–0.23), (60H, CH<sub>3</sub>-TBS). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ (ppm): 155.0; 155.2; 154.5; 146.4; 146.3; 132.3; 120.7; 120.4; 120.0; 100.9; 100.5; 100.0; 78.9; 67.4; 27.8; 26.1; 26.1; 25.9; 25.9; 18.6; 18.6; 18.3; 18.2; –4.0; –4.0; –4.3; –4.3; –4.9; –4.9. ESI-MS *m/z*: [M – H]<sup>–</sup> calcd for C<sub>39</sub>H<sub>69</sub>O<sub>6</sub>Si<sub>4</sub> 746.4; found 746.4.

Compound **23** (523.1 mg, 69%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ (ppm): 6.99 (1H, d, *J* = 2.0 Hz, H2'); 6.82 (2H, m, H6', H5'); 6.11 (1H, d, *J* = 2.2 Hz, H6); 5.93 (1H, d, *J* = 2.2 Hz, H8); 4.94 (1H, s, H2); 4.76 (1H, s, OH-5); 4.22 (1H, td, *J* = 1.8, 3.7, 4.0 Hz, H3); 3.80 (3H, s, OCH<sub>3</sub>); 2.83 (1H, dd, *J* = 3.7, 16.0 Hz, H4-β); 2.70 (1H, dd, *J* = 4.1, 16.0 Hz, H4-α); 1.00 – (–0.29) (45H, CH<sub>3</sub>-TBS). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ (ppm): 156.1; 155.1; 154.6; 150.7; 144.6; 132.9; 120.6; 119.3; 111.3; 101.0; 100.6; 100.1; 79.2; 67.5; 55.6; 29.9; 25.9; 25.9; 25.8; 18.6; 18.3; 18.1; 0.1, –4.3; –4.3; –4.5; –4.5; –4.9; –5.0. HRMS (ESI/TOF) *m/z*: calcd for C<sub>34</sub>H<sub>59</sub>O<sub>6</sub>Si<sub>3</sub> 647.3621 [M + H]<sup>+</sup>; found, 647.3601.

Compound **24** (574.9 mg, 79%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ (ppm): 6.99 (1H, dd, *J* = 2.1, 8.4 Hz, H6'); 6.91 (1H, d, *J* = 2.1 Hz, H2'); 6.80 (1H, d, *J* = 8.4 Hz, H5'); 6.09 (1H, d, *J* = 2.3 Hz, H6); 5.93 (1H, d, *J* = 2.3 Hz, H8); 4.94 (1H, d, *J* = 1.8 Hz, H2); 4.22 (1H, td, *J* = 1.8, 4.0, 4.9 Hz, H3); 3.79 (3H, s, OCH<sub>3</sub>); 2.80 (1H, dd, *J* = 4.0, 15.8 Hz, H4-β); 2.62 (1H, dd, *J* = 4.9, 15.8 Hz, H4-α); 0.99 – (–0.22) (45H, CH<sub>3</sub>-TBS). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ (ppm): 155.9; 155.2; 154.5; 150.5; 144.5; 131.7; 120.6; 120.0; 111.6; 100.9; 100.5; 100.0; 78.8; 67.4; 55.7; 27.9; 25.9; 25.9; 25.8; 18.6; 18.3; 18.1; 0.1, –4.3; –4.3; –4.5; –4.5; –4.9; –4.9. HRMS (ESI/TOF) *m/z*: calcd for C<sub>34</sub>H<sub>59</sub>O<sub>6</sub>Si<sub>3</sub> 647.3621 [M + H]<sup>+</sup>; found, 647.3619.

*Representative Procedure for Synthesis by Glucuronidation of (2S,3R,4S,5R,6S)-2-(((2R,3S)-2-(3,4-bis((tert-butyl)dimethylsilyloxy)phenyl)-3,7-bis((tert-butyl)dimethylsilyloxy)chroman-5-yl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl Triacetate (25), (2S,3R,4S,5R,6S)-2-(((2R,3S)-3,7-bis((tert-butyl)dimethylsilyloxy)-2-(4-((tert-butyl)dimethylsilyloxy)-3-methoxyphenyl)chroman-5-yl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl Triacetate (26), (2S,3R,4S,5R,6S)-2-(((2R,3S)-3,7-bis((tert-butyl)dimethylsilyloxy)-2-(3-((tert-butyl)dimethylsilyloxy)-4-methoxyphenyl)chroman-5-yl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl Triacetate (27), (2S,3R,4S,5R,6S)-2-(((2R,3R)-2-(3,4-bis((tert-butyl)dimethylsilyloxy)phenyl)-3,7-bis((tert-butyl)dimethylsilyloxy)chroman-5-yl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl Triacetate (28), (2S,3R,4S,5R,6S)-2-(((2R,3R)-3,7-bis((tert-butyl)dimethylsilyloxy)-2-(4-((tert-butyl)dimethylsilyloxy)-3-methoxyphenyl)chroman-5-yl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl Triacetate (29), and (2S,3R,4S,5R,6S)-2-(((2R,3R)-3,7-bis((tert-butyl)dimethylsilyloxy)-2-(3-((tert-butyl)dimethylsilyloxy)-4-methoxyphenyl)chroman-5-yl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl Triacetate (30).* A suspension of the dried compound **19** (240.0 mg, 0.32 mmol) and (2S,3S,4S,5R,6R)-2-(methoxycarbonyl)-6-(2,2,2-trichloro-1-iminoethoxy)-tetrahydro-2H-pyran-3,4,5-triyl triacetate (228.0 mg, 0.48 mmol) was dissolved in anhydrous methylene chloride (8 mL) in the presence of 4 Å molecular sieves (0.5 g). The reaction was stirred at room temperature for 30 min under argon and then cooled in an ice-H<sub>2</sub>O bath for 15 min. A solution of the Lewis acid [BF<sub>3</sub>·OEt<sub>2</sub>] (28 μL, 0.22 mmol) in 0.5 mL of CH<sub>2</sub>Cl<sub>2</sub> was added slowly via a syringe. The resulting suspension was continuously stirred at 0 °C in an ice-H<sub>2</sub>O bath for 12 h. Then, another batch of (2S,3S,4S,5R,6R)-2-(methoxycarbonyl)-6-(2,2,2-trichloro-1-iminoethoxy)-tetrahydro-2H-pyran-3,4,5-triyl triacetate (76.0 mg, 0.16 mmol) was added and the suspension was stirred for 30 min. A solution of BF<sub>3</sub>·OEt<sub>2</sub> (10 μL mL, 0.08 mmol) in anhydrous methylene chloride (0.2 mL) was added slowly via a syringe and stirred at 0 °C in an ice-H<sub>2</sub>O bath for 12 h. After 24 h, the reaction was quenched by saturated sodium hydrogen carbonate solution (10 mL). The organic layer was separated, washed with brine (5 mL), dried over anhydrous sodium sulfate, and evaporated. The crude product (**25**) was directly subjected to subsequent hydrolysis of acetyl and methyl ester groups.

*Representative Procedure for Methyl Ester Hydrolysis and Removal of Acetyl Groups: Synthesis of (2S,3R,4S,5R,6S)-6-(((2R,3S)-2-(3,4-Dihydroxyphenyl)-3,7-dihydroxychroman-5-*

yl)oxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic Acid (7), (2S,3R,4S,5R,6S)-6-(((2R,3S)-3,7-Dihydroxy-2-(4-hydroxy-3-methoxyphenyl)chroman-5-yl)oxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic Acid (8), (2S,3R,4S,5R,6S)-6-(((2R,3S)-3,7-Dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-5-yl)oxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic Acid (9), (2S,3R,4S,5R,6S)-6-(((2R,3R)-2-(3,4-Dihydroxyphenyl)-3,7-dihydroxychroman-5-yl)oxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic Acid (10), (2S,3R,4S,5R,6S)-6-(((2R,3R)-3,7-Dihydroxy-2-(4-hydroxy-3-methoxyphenyl)chroman-5-yl)oxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic Acid (11), and (2S,3R,4S,5R,6S)-6-(((2R,3R)-3,7-Dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-5-yl)oxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic Acid (12). To a stirred solution of crude compound 25 in tetrahydrofuran (16 mL) and methanol (5 mL) at 0 °C under an argon atmosphere was added sodium methoxide [5.4 M (30 wt %) in methanol] (2 mL, 10.80 mmol). After being stirred for 1 h at 0 °C, sodium hydroxide (0.5 M in water) (4 mL, 2.00 mmol) was added to the reaction mixture. The resulting pale-yellow solution was stirred at 0 °C for 3 h. After 4 h, Amberlyst 15 hydrogen form was added to adjust the reaction mixture to pH ~ 4. The resin was filtered off and washed with methanol (3 × 20 mL), and the solvent was evaporated to dryness under nitrogen to a thick brown oil. The residue was dissolved in THF (1 mL) and to this a TBAF solution (5 mL, 5.00 mmol; 1 M solution in THF) and THF/H<sub>2</sub>O (8:2, v/v) were added dropwise. The resulting mixture was stirred at room temperature for 3 h (for catechin derivatives) or 30 min (for epicatechin derivatives) and then quenched with water. The reaction mixture was filtered through a 100 C18-reverse-phase small column using a vacuum. TBAF and other salts were removed with water and the catechin glucuronides were eluted with methanol. The methanol fractions were evaporated under nitrogen. The residue was purified by preparative HPLC in five injections (5 × 0.5 mL). The purity of the fractions was checked by LC/MS. Fractions with purity ≥ 98% were combined and partially evaporated under nitrogen. The aqueous remainder was lyophilized for 72 h to furnish the following compounds as yellow solids:

Compound 7<sup>61</sup> (100.7 mg, 68% overall yield of the last three steps). <sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD) δ (ppm): 6.93 (1H, d, J = 1.8 Hz, H2'); 6.87 (1H, d, J = 8.1 Hz, H5'); 6.82 (1H, dd, J = 1.8, 8.1 Hz, H6'); 6.41 (1H, d, J = 2.0 Hz, H6); 6.12 (1H, d, J = 2.0 Hz, H8); 4.95 (1H, d, J = 7.3 Hz, H1''); 4.73 (1H, d, J = 7.2 Hz, H2); 4.09 (1H, dt, J = 5.2, 7.2, 7.8 Hz, H3); 3.84 (1H, d, J = 9.4 Hz, H5''); 3.64–3.58 (3H, m, H2'', H3'', H4''), 3.10 (1H, dd, J = 5.2, 16.4 Hz, H4-α); 2.72 (1H, dd, J = 7.8, 16.4 Hz, H4-β). <sup>13</sup>C{<sup>1</sup>H} NMR (150 MHz, CD<sub>3</sub>OD) δ (ppm): 175.6 (COOH); 158.1 (C7); 158.0 (C5); 156.7 (C9); 146.2 (C3'); 146.2 (C4'); 132.2 (C1'); 119.9 (C6'); 116.2 (C5''); 115.3 (C2''); 103.4 (C10); 102.6 (C1''); 98.2 (C8); 97.1 (C6); 82.7 (C2); 77.9, 74.7, 73.5 (C2'', C3'', C4''); 76.6 (C5''); 68.4 (C3); 28.3 (C4). ESI-MS *m/z*: [M - H]<sup>-</sup> calcd for C<sub>21</sub>H<sub>21</sub>O<sub>12</sub> 465.40; found 465.40.

Compound 8<sup>61</sup> (215.2 mg, 71% overall yield of the last three steps): <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD) δ (ppm): 6.97 (1H, d, J = 1.6 Hz, H2'); 6.85 (1H, dd, J = 1.6, 8.2 Hz, H6'); 6.80 (1H, d, J = 8.2 Hz, H5'); 6.33 (1H, d, J = 1.8 Hz, H6); 6.03 (1H, d, J = 1.8 Hz, H8); 4.86 (inside of CD<sub>3</sub>OD signal, H1''); 4.64 (1H, d, J = 8.0 Hz, H2); 4.01 (1H, td, J = 5.6, 7.7, 8.0 Hz, H3); 3.85 (3H, s, OCH<sub>3</sub>); 3.75 (1H, d, J = 7.3 Hz, H5''); 3.51–3.58

(3H, m, H2'', H3'', H4''); 3.06 (1H, dd, J = 5.6, 16.4 Hz, H4-α); 2.62 (1H, dd, J = 7.7, 16.4 Hz, H4-β). <sup>13</sup>C{<sup>1</sup>H} NMR (125 MHz, CD<sub>3</sub>OD) δ (ppm): 170.8 (COOH); 158.1 (C7); 158.0 (C5); 156.7 (C9); 148.9 (C3'); 147.5 (C4'); 132.0 (C1'); 121.2 (C6'); 116.0 (C5''); 111.8 (C2''); 103.6 (C10); 102.8 (C1''); 98.2 (C8); 97.2 (C6); 81.6 (C2); 78.0, 73.5, 73.5 (C2'', C3'', C4''); 74.7 (C5''); 68.7 (C3); 56.4 (OCH<sub>3</sub>); 28.7 (C4). ESI-MS *m/z*: [M - H]<sup>-</sup> calcd for C<sub>22</sub>H<sub>23</sub>O<sub>12</sub>, 479.13; found 479.40.

Compound 9<sup>61</sup> (263.1 mg, 77% overall yield of the last three steps): <sup>1</sup>H NMR (600 MHz, acetone-*d*<sub>6</sub>) δ (ppm): 6.92 (1H, d, J = 1.9 Hz, H2'); 6.88 (1H, d, J = 8.3 Hz, H5'); 6.81 (1H, dd, J = 1.9, 8.3 Hz, H6'); 6.42 (1H, d, J = 2.0 Hz, H6); 6.01 (1H, d, J = 2.0 Hz, H8); 4.84 (1H, d, J = 7.1 Hz, H1''); 4.67 (1H, d, J = 7.0 Hz, H2); 3.96 (1H, td, J = 5.1, 7.0, 7.8 Hz, H3); 3.81 (3H, s, OCH<sub>3</sub>); 3.78 (1H, d, J = 9.2 Hz, H5''); 3.51–3.44 (3H, m, H2'', H3'', H4''); 2.95 (1H, dd, J = 5.1, 16.3 Hz, H4-α); 2.69 (1H, dd, J = 7.8, 16.3 Hz, H4-β). <sup>13</sup>C{<sup>1</sup>H} NMR (150 MHz, acetone-*d*<sub>6</sub>) δ (ppm): 175.9 (COOH); 158.1 (C7); 157.7 (C5); 156.2 (C9); 148.1 (C4'); 145.8 (C3'); 133.9 (C1'); 119.0 (C6'); 115.0 (C5''); 112.5 (C2''); 103.0 (C10); 102.9 (C1''); 97.7 (C8); 97.2 (C6); 82.1 (C2); 77.7, 74.4, 73.0 (C2'', C3'', C4''); 75.6 (C5''); 67.8 (C3); 56.4 (OCH<sub>3</sub>); 28.0 (C4). ESI-MS *m/z*: [M - H]<sup>-</sup> calcd for C<sub>22</sub>H<sub>23</sub>O<sub>12</sub>, 479.13; found 479.10.

Compound 10<sup>59,61</sup> (105.4 mg, 51% overall yield of the last three steps): <sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD) δ (ppm): 6.99 (1H, d, J = 2.0 Hz, H2'); 6.81 (1H, dd, J = 2.0, 8.2 Hz, H6'); 6.77 (1H, d, J = 8.2 Hz, H5'); 6.33 (1H, d, J = 2.1 Hz, H6); 6.09 (1H, d, J = 2.1 Hz, H8); 4.90 (inside of CD<sub>3</sub>OD signal H1''); 4.84 (inside of CD<sub>3</sub>OD signal H2); 4.19 (1H, s br, H3); 3.79 (1H, d, J = 9.5 Hz, H5''); 3.59–3.50 (3H, m, H2'', H3'', H4''); 2.98–2.91 (2H, m, H4-α and H4-β). <sup>13</sup>C{<sup>1</sup>H} NMR (150 MHz, CD<sub>3</sub>OD) δ (ppm): 175.4 (COOH); 158.5 (C7); 157.9 (C5); 157.1 (C9); 145.9 (C4'); 145.8 (C3'); 132.2 (C1'); 119.4 (C6'); 115.9 (C5''); 115.3 (C2''); 102.8 (C10); 102.8 (C1''); 98.6 (C8); 97.3 (C6); 79.9 (C2); 77.9, 74.7, 73.4 (C2'', C3'', C4''); 76.7 (C5''); 67.3 (C3); 29.3 (C4). ESI-MS *m/z*: [M - H]<sup>-</sup> calcd for C<sub>21</sub>H<sub>21</sub>O<sub>12</sub> 466.11; found 465.30.

Compound 11<sup>59,61</sup> (220.3 mg, 59% overall yield of the last three steps): <sup>1</sup>H NMR (600 MHz, DMSO-*d*<sub>6</sub>) δ (ppm): 7.03/7.03 (1H, s, H2'); 6.83/6.81 (1H, d, J = 7.8/7.8 Hz, H6'); 6.78/6.77 (1H, d, J = 7.8/7.8 Hz, H5'); 6.20/6.12 (1H, d, J = 2.3/2.5 Hz, H6); 6.00/5.96 (1H, d, J = 2.3/2.5 Hz, H8); 4.79 (1H, s br, H2); 4.85/4.68 (1H, d, J = 9.0/8.9 Hz, H1''); 4.05/4.05 (1H, s br, H3); 3.74/3.74 (3H, s, OCH<sub>3</sub>); 3.43/3.41 (1H, s br, H5''); 3.26–3.21 (3H, m, H2'', H3'', H4''), 2.75/2.73 (2H, m, H4-α, H4-β). <sup>13</sup>C{<sup>1</sup>H} NMR (150 MHz, DMSO-*d*<sub>6</sub>) δ (ppm): 172.0 (COOH); 156.8 (C5); 156.7 (C7); 155.3 (C9); 147.0 (C3'); 146.3 (C4'); 130.1 (C1'); 119.6 (C6'); 114.8 (C5''); 111.8 (C2''); 100.7 (C1''); 100.6 (C10); 96.6 (C8); 95.4 (C6'); 78.2 (C2); 76.8 (C3''); 74.4 (C5''); 73.1 (C2''); 72.3 (C4''); 64.8 (C3); 55.6 (OCH<sub>3</sub>); 28.3 (C4). ESI-MS *m/z*: [M - H]<sup>-</sup> calcd for C<sub>22</sub>H<sub>23</sub>O<sub>12</sub>, 479.13; found 479.10.

Compound 12<sup>59,61</sup> (264.6 mg, 65% overall yield of the last three steps): <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>CD) δ (ppm): 6.96 (1H, d, J = 1.8 Hz, H2'); 6.89–6.85 (2H, m, H5', H6'); 6.30 (1H, d, J = 2.2 Hz, H6); 6.02 (1H, d, J = 2.2 Hz, H8); 4.81–4.78 (2H, m, H2, H1''); 4.15 (1H, s br, H3); 3.80 (3H, s, OCH<sub>3</sub>); 3.69 (1H, d, J = 9.2 Hz, H5''); 3.50–3.44 (3H, m, H2'', H3'', H4''); 2.93–2.84 (2H, m, H4-α, H4-β). <sup>13</sup>C{<sup>1</sup>H} NMR (125 MHz, CD<sub>3</sub>CD) δ (ppm): 158.5 (C7); 157.9 (C5);

157.0 (C9); 148.5 (C4'); 147.2 (C3'); 133.5 (C1'); 119.2 (C6'); 115.2 (C5'); 112.3 (C2'); 102.8 (C10); 102.6 (C1''); 98.5 (C8); 97.3 (C6); 79.8 (C2); 78.0, 74.7, 73.5 (C2'', C3'', C4''); 75.0 (C5''); 67.1 (C3); 56.5 (OCH<sub>3</sub>); 29.4 (C4). ESI-MS *m/z*: [M - H]<sup>-</sup> calcd for C<sub>22</sub>H<sub>23</sub>O<sub>12</sub>, 479.13; found 479.20.

## ■ ASSOCIATED CONTENT

### SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.0c04512>.

Copies of <sup>1</sup>H NMR and <sup>13</sup>C NMR, HMQC, and HMBC spectra for all compounds; copies of MS, MS/MS, and HRMS spectra of intermediate compounds and glucuronide derivatives (PDF)

## ■ AUTHOR INFORMATION

### Corresponding Authors

**Maite L. Docampo-Palacios** – *BioDiscovery Institute and Department of Biological Sciences, University of North Texas, Denton, Texas 76203, United States*; [orcid.org/0000-0001-5205-3989](https://orcid.org/0000-0001-5205-3989); Phone: +1-214-601-5892; Email: [md@precisionplantmolecules.com](mailto:md@precisionplantmolecules.com); Fax: +1-580-224-6692

**Richard A. Dixon** – *BioDiscovery Institute and Department of Biological Sciences, University of North Texas, Denton, Texas 76203, United States*; [orcid.org/0000-0001-8393-9408](https://orcid.org/0000-0001-8393-9408); Phone: +1-940-565-2308; Email: [Richard.Dixon@unt.edu](mailto:Richard.Dixon@unt.edu)

### Authors

**Anislay Alvarez-Hernández** – *BioDiscovery Institute and Department of Biological Sciences, University of North Texas, Denton, Texas 76203, United States*

**Ângelo de Fátima** – *Department of Chemistry, Universidade Federal de Minas Gerais, Belo Horizonte, MG 31270-901, Brazil*; [orcid.org/0000-0003-3010-695X](https://orcid.org/0000-0003-3010-695X)

**Luciano Morais Lião** – *Institute of Chemistry, Universidade Federal de Goiás, Goiânia, GO 74690-900, Brazil*; [orcid.org/0000-0001-9985-2980](https://orcid.org/0000-0001-9985-2980)

**Giulio M. Pasinetti** – *Department of Psychiatry, The Mount Sinai School of Medicine, New York, New York 10029, United States*; [orcid.org/0000-0002-1524-5196](https://orcid.org/0000-0002-1524-5196)

Complete contact information is available at:

<https://pubs.acs.org/doi/10.1021/acsomega.0c04512>

### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

This study was supported by Grant Number P50 AT008661-01 from the NCCIH and the ODS, and by the University of North Texas. Dr. Pasinetti holds a Senior VA Career Scientist Award. Dr de Fátima acknowledges the Brazilian National Council for Scientific and Technological Development (CNPq) for the scholarship received during his sabbatical year (Process #204106/2017-6) at the University of North Texas, Denton/TX. The authors acknowledge the Bioanalytical Facility at the University of North Texas, especially Jean Christophe Cocuron, for support with mass spectrometry analyses during this work. We acknowledge that the contents of this study do not represent the views of the NCCIH, the ODS, the NIH, the U.S. Department of Veterans Affairs, or the United States Government.

## ■ REFERENCES

- (1) Kris-Etherton, M.; Hecker, K. D.; Bonanome, A.; Coval, S. M.; Binkoski, A. E.; Hilpert, K. F.; Griel, A. E.; Etherton, T. D. Bioactive Compounds in Foods: Their Role in the Prevention of Cardiovascular Disease and Cancer. *Am. J. Cancer* **2002**, *113*, 715–885.
- (2) Manach, C.; Scalbert, A.; Morand, C.; Rémésy, C. R.; Jiménez, L. Polyphenols: Food Sources and Bioavailability. *Am. J. Clin. Nutr.* **2004**, *79*, 727–747.
- (3) Dixon, R. A.; Pasinetti, G. M. (2010) Flavonoids and Isoflavonoids: from Plant Biology to Agriculture and Neuroscience. *Plant Physiol.* **2010**, *154*, 453–457.
- (4) Hooper, L.; Kroon, P. A.; Rimm, E. B.; Cohn, J. S.; Harvey, I.; Le Cornu, K. A.; Ryder, J. J.; Hall, W. L.; Cassidy, A. Flavonoids, Flavonoid-Rich Foods, and Cardiovascular Risk: A Meta-Analysis of Randomized Controlled Trials. *Am. J. Clin. Nutr.* **2008**, *88*, 38–50.
- (5) Arts, I. C. W.; Hollman, P. C. H. Polyphenols and Disease Risk in Epidemiologic Studies. *Am. J. Clin. Nutr.* **2005**, *81*, 317S–325S.
- (6) Scalbert, A.; Manach, C.; Morand, C.; Rémésy, C. R.; Jiménez, L. Dietary Polyphenols and the Prevention of Diseases. *Crit. Rev. Food Sci. Nutr.* **2005**, *45*, 287–306.
- (7) Bernatova, I. Biological Activities of (–)-Epicatechin and (–)-Epicatechin-Containing Foods: Focus on Cardiovascular and Neuropsychological Health. *Biotechnol. Adv.* **2018**, *36*, 666–681.
- (8) Li, C.; Meng, X.; Winnik, B.; Lee, M. J.; Lu, H.; Sheng, S.; Buckley, B.; Yang, C. S. Analysis of Urinary Metabolites of Tea Catechins by Liquid Chromatography/Electrospray Ionization Mass Spectrometry. *Chem. Res. Toxicol.* **2001**, *14*, 702–707.
- (9) Meng, X.; Sang, S.; Zhu, N.; Lu, H.; Sheng, S.; Lee, M. J.; Ho, C. T.; Yang, C. S. Identification and Characterization of Methylated and Ring-Fission Metabolites of Tea Catechins Formed in Humans, Mice and Rats. *Chem. Res. Toxicol.* **2002**, *15*, 1042–1050.
- (10) Kawai, M.; Hirano, T.; Higa, S.; Arimitsu, J.; Maruta, M.; Kuwahara, Y.; Ohkawara, T.; Hagihara, K.; Yamadori, T.; Shima, Y.; Ogata, A.; Kawase, I.; Tanaka, T. Flavonoids and Related Compounds as Anti-Allergic Substances. *Allergol. Int.* **2007**, *56*, 113–123.
- (11) Bylka, W.; Matlawska, I.; Pilewski, N. A. Natural Flavonoids as Antimicrobial Agents. *J. Anthropol. North Am.* **2004**, *7*, 24–31.
- (12) Orhan, D. D.; Özçelik, B.; Özgen, S.; Ergun, F. Antibacterial, Antifungal, and Antiviral Activities of Some Flavonoids. *Microbiol. Res.* **2010**, *165*, 496–504.
- (13) Cushnie, T. P. T.; Lamb, A. J. Antimicrobial Activity of Flavonoids. *Int. J. Antimicrobiol. Agents* **2005**, *26*, 343–356.
- (14) Le Marchand, L. Cancer Preventive Effects of Flavonoids—A Review. *Biomed. Pharmacother.* **2002**, *56*, 296–301.
- (15) Ren, W.; Qiao, Z.; Wang, H.; Zhu, L.; Zhang, L. Flavonoids: Promising Anticancer Agents. *Med. Res. Rev.* **2003**, *23*, 519–534.
- (16) Kanadaswami, C.; Lee, L.-T.; Lee, P.-P.H.; Hwang, J.-J.; Ke, F.-C.; Huang, Y.-T.; Lee, M.-T. The Antitumor Activities of Flavonoids. *In Vivo* **2005**, *19*, 895–909.
- (17) Chahar, M. K.; Sharma, N.; Dobhal, M. P.; Joshi, Y. C. Flavonoids: A Versatile Source of Anticancer Drugs. *Pharmacogn. Rev.* **2011**, *5*, 1–12.
- (18) Ravishanker, D.; Rajora, A. K.; Greco, F.; Osborn, H. M. I. Flavonoids as Prospective Compounds for Anti-Cancer Therapy. *Int. J. Biochem. Cell Biol.* **2013**, *45*, 2821–2831.
- (19) Modolo, L. V.; de Souza, A. X.; Horta, L. P.; Araujo, D. P.; De Fátima, Â. An Overview on the Potential of Natural Products as Urease Inhibitors: A Review. *J. Adv. Res.* **2015**, *6*, 35–44.
- (20) Serafini, M.; Peluso, I.; Raguzzini, A. Flavonoids as Anti-Inflammatory Agents. *Proc. Nutr. Soc.* **2010**, *69*, 273–278.
- (21) Pietta, P.-G. Flavonoids as Antioxidants. *J. Nat. Prod.* **2000**, *63*, 1035–1042.
- (22) Burda, S.; Oleszek, W. Antioxidant and Antiradical Activities of Flavonoids. *J. Agric. Food Chem.* **2001**, *49*, 2774–2779.
- (23) Riemersma, R. A.; Rice-Evans, C. A.; Tyrrell, R. M.; Clifford, M. N.; Lean, M. E. J. Tea Flavonoids and Cardiovascular Health. *Q. J. Med.* **2001**, *94*, 277–282.
- (24) Hodgson, J. M.; Croft, K. D. Tea Flavonoids and Cardiovascular Health. *Mol. Aspects Med.* **2010**, *31*, 495–502.

- (25) de Lira Mota, K. S.; Dias, G. E. N.; Pinto, M. E. F.; Luiz-Ferreira, A.; Souza-Brito, A. R. M.; Hiruma-Lima, C. A.; Barbosa-Filho, J. M.; Batista, L. M. Flavonoids with Gastroprotective Activity. *Molecules* **2009**, *14*, 979–1012.
- (26) Dajas, F.; Rivera-Megret, F.; Blasina, F.; Arredondo, F.; Abin-Carriquiry, J. A.; Costa, G.; Echeverry, C.; Lafon, L.; Heizen, H.; Ferreira, M.; Morquio, A. Neuroprotection by Flavonoids. *Braz. J. Med. Biol. Res.* **2003**, *36*, 1613–1620.
- (27) Spencer, J. P. E. The Impact of Fruit Flavonoids on Memory and Cognition. *Brit. J. Nutr.* **2010**, *104*, S40–S47.
- (28) Gutierrez-Merino, C.; Lopez-Sanchez, C.; Lagoa, R.; Samhan-Arias, A. K.; Bueno, C.; Garcia-Martinez, V. Neuroprotective Actions of Flavonoids. *Curr. Med. Chem.* **2011**, *18*, 1195–1212.
- (29) Hwang, S.-L.; Shih, P.-H.; Yen, G.-C. Neuroprotective Effects of Citrus Flavonoids. *J. Agric. Food Chem.* **2012**, *60*, 877–885.
- (30) Meireles, M.; Moura, E.; Vieira-Coelho, M. A.; Santos-Buelga, C.; Gonzalez-Manzano, S.; Duennas, M.; Mateus, N.; Faria, A.; Calhau, C. Flavonoids as Dopaminergic Neuromodulators. *Mol. Nutr. Food Res.* **2016**, *60*, 495–501.
- (31) Tapas, A. R.; Sakarkar, D. M.; Kakde, R. B. Flavonoids as Nutraceuticals: A Review. *Trop. J. Pharm. Res.* **2008**, *7*, 1089–1099.
- (32) Pinent, M.; Bladé, M. C.; Salvadó, M. J.; Arola, L.; Ardévol, A. Metabolic Fate of Glucose on 3T3-L1 Adipocytes Treated with Grape Seed-Derived Procyanidin Extract (GSPE). Comparison with the effects of insulin. *J. Agric. Food Chem.* **2005**, *53*, 5932–5935.
- (33) Montagut, G.; Bladé, C.; Blay, M.; Fernández-Larrea, J.; Pujadas, G.; Salvadó, M. J.; Arola, L.; Pinent, M.; Ardévol, A. Effects of a Grapeseed Procyanidin Extract (GSPE) on Insulin Resistance. *J. Nutr. Biochem.* **2010**, *21*, 961–967.
- (34) Montagut, G.; Onnoeck, S.; Vaqué, M.; Bladé, C.; Blay, M.; Fernández-Larrea, J.; Pujadas, G.; Salvadó, M. J.; Arola, L.; Pinent, M.; Ardévol, A.; Pinent, M. Oligomers of Grape-Seed Procyanidin Extract Activate the Insulin Receptor and Key Targets of the Insulin Signaling Pathway Differently from Insulin. *J. Nutr. Biochem.* **2010**, *21*, 476–481.
- (35) Quiñones, M.; Guerrero, L.; Suarez, M.; Pons, Z.; Aleixandre, A.; Arola, L.; Muguerza, B. Low-Molecular Procyanidin Rich Grape Seed Extract Exerts Antihypertensive Effect in Males Spontaneously Hypertensive Rats. *Food Res. Int.* **2013**, *51*, 587–595.
- (36) Chen, S.; Zhu, Y.; Liu, Z.; Gao, Z.; Li, B.; Zhang, D.; Zhang, Z.; Jiang, X.; Liu, Z.; Meng, L.; Yang, Y.; Shi, B. Grape Seed Proanthocyanidin Extract Ameliorates Diabetic Bladder Dysfunction Via Activation of the Nrf2 Pathway. *PLoS One* **2015**, *10*, No. e0126457.
- (37) Caimari, A.; del Bas, J. M.; Arola, L. Low Doses of Grape Seed Procyanidins Reduce Adiposity and Improve the Plasma Lipid Profile in Hamsters. *Int. J. Obes.* **2013**, *37*, 576–583.
- (38) Enginar, H.; Cemek, M.; Karaca, T.; Unak, P. Effect of Grape Seed Extract on Lipid Peroxidation, Antioxidant Activity and Peripheral Blood Lymphocytes in Rats Exposed to X-Radiation. *Phytother. Res.* **2007**, *21*, 1029–1035.
- (39) Wang, J.; Santa-Maria, I.; Ho, L.; Ksiezak-Reding, H.; Ono, K.; Teplow, D. B.; Pasinetti, G. M. Grape Derived Polyphenols Attenuate Tau Neuropathology in a Mouse Model of Alzheimer's Disease. *J. Alzheimer's Dis.* **2010**, *22*, 653–661.
- (40) Pasinetti, G. M.; Ho, L. Role of Grape Seed Polyphenols in Alzheimer's Disease Neuropathology. *Nutr. Diet. Suppl.* **2010**, *2*, 97–103.
- (41) Santa-Maria, I.; Diaz-Ruiz, C.; Ksiezak-Reding, H.; Chen, A.; Ho, L.; Wang, J.; Pasinetti, G. M. GSPE Interferes with Tau Aggregation in vivo: Implications for Treating Tauopathy. *Neurobiol. Aging* **2012**, *33*, 2072–2081.
- (42) Liu, P.; Kemper, L. J.; Wang, J.; Zahs, K. R.; Ashe, K. H.; Pasinetti, G. M. Grape Seed Polyphenolic Extract Specifically Decrease A $\beta$ \*56 in the Brains of Tg2576 Mice. *J. Alzheimer's Dis.* **2011**, *26*, 657–666.
- (43) Hayden, E. Y.; Yamin, G.; Beroukhim, S.; Chen, B.; Kibalchenko, M.; Jiang, L.; Wang, J.; Pasinetti, G. M.; Teplow, D. B. Inhibiting Amyloid  $\beta$ -Protein Assembly: Size-Activity Relationships Among Grape Seed-Derived Polyphenols. *J. Neurochem.* **2015**, *135*, 416–430.
- (44) Sharma, V.; Zhang, C.; Pasinetti, G. M.; Dixon, R. A. Fractionation of Grape Seed Proanthocyanidins for Bioactivity Assessment. *Recent Adv. Phytochem.* **2010**, *41*, 33–46.
- (45) Ferruzzi, M. G.; Lobo, J. K.; Janle, E. M.; Cooper, B.; Simon, J. E.; Wu, Q.-L.; Welch, C.; Ho, L.; Weaver, C.; Pasinetti, G. M. Bioavailability of Gallic Acid and Catechins from Grape Seed Polyphenol Extract is Improved by Repeated Dosing in Rats: Implications for Treatment in Alzheimer's Disease. *J. Alzheimer's Dis.* **2009**, *18*, 113–124.
- (46) Wang, X.; Wang, T.; Ni, J. D.; von Lintig, J.; Montell, C. The Drosophila Visual Cycle and de novo Chromophore Synthesis Depends on rdhB. *J. Neurosci.* **2012**, *32*, 3485–3491.
- (47) Neilson, A. P.; Hopf, A. S.; Cooper, B. R.; Pereira, M. A.; Bomser, J. A.; Ferruzzi, M. G. Catechin Degradation with Concurrent Formation of Homo- and Heterocatechin Dimers During in vitro Digestion. *J. Agric. Food Chem.* **2007**, *55*, 8941–8949.
- (48) Beekmann, K.; Actis-Goretta, L.; Van Bladeren, P. J.; Dionisi, F.; Destailats, F.; Rietjens, I. M. C. M. A State-of-the-Art Overview of the Effect of Metabolic Conjugation on the Biological Activity of Flavonoids. *Food Funct.* **2012**, *3*, 1008–1018.
- (49) Piskula, M. K.; Terao, J. Accumulation of (–)-Epicatechin Metabolites in Rat Plasma after Oral Administration and Distribution of Conjugation Enzymes in Rat Tissues. *J. Nutr.* **1998**, *128*, 1172–1178.
- (50) Spencer, J. P.; Chowrimootoo, G.; Choudhury, R.; Debnam, E. S.; Srai, S. K.; Rice-Evans, C. The Small Intestine Can Both Absorb and Glucuronidate Luminal Flavonoids. *FEBS Lett.* **1999**, *458*, 224–230.
- (51) Donovan, J. L.; Crespy, V.; Manach, C.; Morand, C.; Besson, C.; Scalbert, A.; Remesy, C. Catechin Is Metabolized by Both the Small Intestine and Liver of Rats. *J. Nutr.* **2001**, *131*, 1753–1757.
- (52) Ottaviani, J. I.; Momma, T. Y.; Heiss, C.; Kwik-Urbe, C.; Schroeter, H.; Keen, C. L. The Stereochemical Configuration of Flavanols Influences the Level and Metabolism of Flavanols in Humans and their Biological Activity in vivo. *Free Rad. Biol. Med.* **2011**, *50*, 237–244.
- (53) Ottaviani, J. I.; Momma, T. Y.; Kuhnle, G. K.; Keen, C. L.; Schroeter, H. Structurally Related (–)-Epicatechin Metabolites in Humans: Assessment Using de novo Chemically Synthesized Authentic Standards. *Free Rad. Biol. Med.* **2012**, *52*, 1403–1412.
- (54) González-Manzano, S.; González-Paramás, A.; Santos-Buelga, C.; Dueñas, M. Preparation and Characterization of Catechin Sulfates, Glucuronides, and Methyl ethers with Metabolic Interest. *J. Agric. Food Chem.* **2009**, *57*, 1231–1238.
- (55) Romanov-Michailidis, F.; Viton, F.; Fumeaux, R.; Lévêques, A.; Actis-Goretta, L.; Rein, M.; Williamson, G.; Barron, D. Epicatechin B-ring conjugates: First Enantioselective Synthesis and Evidence for their Occurrence in Human Biological Fluids. *Org. Lett.* **2012**, *14*, 3902–3905.
- (56) Blount, J. W.; Ferruzzi, M.; Rafferty, D.; Pasinetti, G. M.; Dixon, R. A. Enzymatic Synthesis of Substituted Epicatechin for Bioactivity Studies in Neurological Disorders. *Biochem. Biophys. Res. Commun.* **2012**, *417*, 457–461.
- (57) Mull, E. S.; Van Zandt, M.; Golebiowski, A.; Beckett, R. P.; Sharma, P. K.; Schroeter, H. A Versatile Approach to the Regioselective Synthesis of Diverse (–)-Epicatechin- $\beta$ -D-glucuronides. *Tetrahedron Lett.* **2012**, *53*, 1501–1503.
- (58) Zhang, M.; Jagdmann, G. E., Jr.; van Zandt, M.; Beckett, P.; Schroeter, H. Enantioselective Synthesis of Orthogonally Protected (2R,3R)-(–)Epicatechin Derivatives, Key Intermediates in the de novo Chemical Synthesis of (–)-Epicatechin Glucuronides and Sulfates. *Tetrahedron: Asymmetry* **2013**, *24*, 362–373.
- (59) Zhang, M.; Jagdmann, G. E., Jr.; van Zandt, M.; Sheeler, R.; Beckett, P.; Schroeter, H. Chemical Synthesis and Characterization of Epicatechin Glucuronides and Sulfates: Bioanalytical Standards for Epicatechin Metabolite Identification. *J. Nat. Prod.* **2013**, *76*, 157–169.

(60) Yue, T.; Chen, R.; Chen, D.; Liu, J.; Xie, K.; Dai, J. Enzymatic Synthesis of Bioactive O-Glucuronides using Plant Glucuronosyltransferases. *J. Agric. Food Chem.* **2019**, *67*, 6275–6284.

(61) Blount, J. W.; Redan, B. W.; Ferruzzi, M. G.; Reuhs, B. L.; Cooper, B. R.; Harwood, J. S.; Shulaev, V.; Pasinetti, G.; Dixon, R. A. Synthesis and Quantitative Analysis of Plasma-Targeted Metabolites of Catechin and Epicatechin. *J. Agric. Food Chem.* **2015**, *63*, 2233–2240.

(62) Donovan, J. L.; Luthria, D. L.; Stremple, P.; Waterhouse, A. L. Analysis of (+)-Catechin, (–)-Epicatechin and their 3' and 4'-O-Methylated Analogs: A Comparison of Sensitive Methods. *J. Chromatogr. B: Biomed. Sci. Appl.* **1999**, *726*, 277–283.

(63) Li, N.; Taylor, L. S.; Ferruzzi, M. G.; Maue, L. J. Kinetic Study of Catechin Stability: Effects of pH, Concentration, and Temperature. *J. Agric. Food Chem.* **2012**, *60*, 12531–12539.

(64) Brittain, W. D. G.; Cobb, S. L. Tetrafluoropyridyl (TFP): a General Phenol Protecting Group Readily Cleaved Under Mild Conditions. *Org. Biomol. Chem.* **2019**, *17*, 2110–2115.

(65) Cruz, L.; Borges, E.; Silva, A. M. S.; Mateus, N.; De Freitas, V. Synthesis of a New (+)-Catechin-Derived Compound: 8-Vinylcatechin. *Lett. Org. Chem.* **2008**, *7*, 530–536.

(66) Janeiro, P.; Oliveira-Brett, A. M. Catechin Electrochemical Oxidation Mechanisms. *Anal. Chim. Acta* **2004**, *518*, 109–115.

(67) Mochizuki, M.; Yamazaki, S.; Kano, K.; Ikeda, T. Kinetic Analysis and Mechanistic Aspects of Autoxidation of Catechins. *Biochim. Biophys. Acta, Gen. Subj.* **2002**, *1569*, 35–44.

(68) Huang, S.-T.; Hung, Y.-A.; Yang, M.-J.; Chen, I.-Z.; Jeu-Ming, P.; Yuann, J.-M. P.; Liang, J. Y. Effects of Epigallocatechin Gallate on the Stability of Epicatechin in a Photolytic Process. *Molecules* **2019**, *24*, 787–800.

(69) Asakawa, T.; Kawabe, Y.; Yoshida, A.; Aihara, Y.; Manabel, T.; Hirose, Y.; Sakurada, A.; Inai, M.; Hamashima, Y.; Furuta, T.; Wakimoto, T.; Kan, T. Syntheses of Methylated Catechins and Theaflavins using 2-Nitrobenzenesulfonyl Group to Protect and Deactivate Phenol. *J. Antibiotics* **2016**, *69*, 299–312.

(70) Chen, L. Y.; Wu, J. Y.; Liang, J. Y. Using Chromatography and Mass Spectrometry to Monitor Isomerization of Catechin in Alkaline Aqueous with Thermal Processing. *J. Food Process. Preserv.* **2017**, *42*, No. e13365.

(71) Mori, K.; Ayano, Y.; Hamada, Y.; Hojima, T.; Tanaka, R.; Higashino, Y.; Izuno, M.; Okamoto, T.; Kawasaki, T.; Hamada, M.; Nakajima, N.; Saito, A. Role of 2,3-cis Structure of (–)-Epicatechin-3,5-O-Digallate in Inhibition of HeLa S3 Cell Proliferation. *Nat. Prod. Chem. Res.* **2015**, *3*, 3.

(72) Nakajima, N.; Saito, A. The Role of Silyl Protecting Group for the Synthesis of Procyanidins and their Derivatives. In *New Horizons of Process Chemistry*; Tomioka, K.; Shioiri, T.; Sajiki, H., Eds.; Springer: Singapore, 2017; pp 229–236.