

## Catechin Glucosides: Occurrence, Synthesis, and Stability

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Catechins are flavonoids with suggested health benefits, but are unstable during storage, processing and, after ingestion, during gut transit. We hypothesized that catechin glucosides, which occur in various plants, could be more stable than unsubstituted catechin, and additionally be deglycosylated in the gut and so act to deliver catechin in a form able to be absorbed. (+)-Catechin *O*-glucosides from various sources have been used in the course of this investigation. (+)-Catechin 3'-*O*- $\beta$ -D-glucopyranoside (C3'G), (+)-catechin 5-*O*- $\beta$ -D-glucopyranoside (C5G), and (+)-catechin 3-*O*- $\beta$ -D-glucopyranoside (C3G) were chemically synthesized. (+)-Catechin 4'-*O*- $\beta$ -D-glucopyranoside (C4'G) and (+)-catechin 7-*O*- $\beta$ -D-glucopyranoside (C7G) were prepared enzymically using preparations from lentil and barley. In general, but with some exceptions, the (+)-catechin glucosides were more stable between pH 4 and 8 than (+)-catechin, with C3'G exhibiting greatest stability. The intestinal metabolism of (+)-catechin and all (+)-catechin glucosides in the gut was determined by perfusion of rat intestine *in vivo*. C3'G and C5G were extensively deglycosylated in the gut, and C3'G showed greatest apparent "absorption" as calculated by the difference between effluent and influent. The results show the potential of catechin glucosides, especially C3'G, as more stable precursors of catechin.

**KEYWORDS:** Catechin; flavonoid; glucosidase; chemical synthesis; stability; absorption

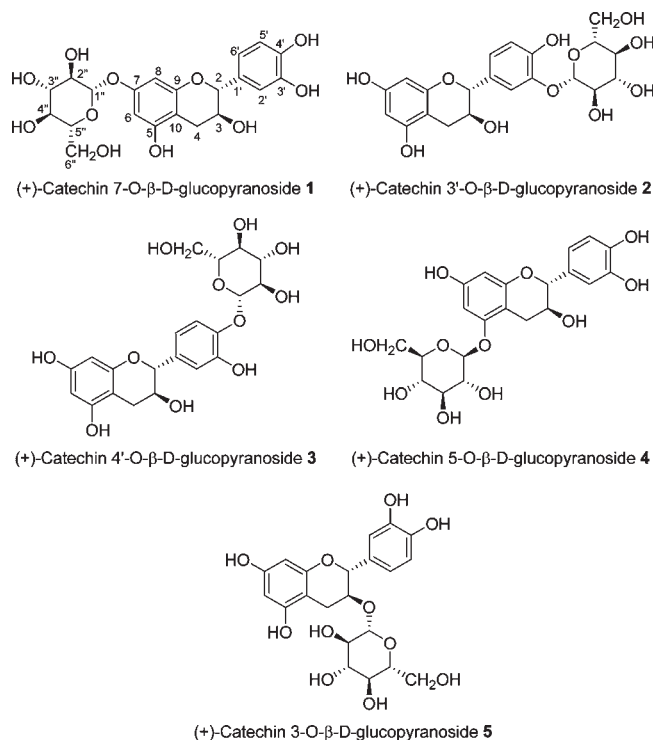
### INTRODUCTION

Catechins belong to the flavan-3-ol class of flavonoids and are ubiquitous in plants and widely found in many foods. The most prominent sources of catechins are green and black tea (*Camellia sinensis*), grapes/wine, certain fruits and cocoa. The stability of catechins is pH-dependent, and in neutral or alkaline solution, they are very unstable and decompose rapidly, whereas in acidic solution, they are relatively stable (1). In recent years, there has been considerable interest in the potential health benefits of catechin-rich food and drink, although some processing methods are likely to degrade catechins. Several studies have demonstrated significant risk reduction for cardiovascular diseases in consumers of black and green tea, and there is some evidence that green tea at high levels of intake provides a benefit in preventing cancers of the digestive tract, especially gastric cancer (2). The oral bioavailability of catechins is relatively low (3). The major site of absorption of tea catechins is the small intestine, where they can be directly absorbed into epithelial cells by passive or facilitated diffusion as demonstrated in the rat small intestine *in situ* (4). The epithelial cells are important sites of catechin conjugation (5, 6) comprising glucuronidation, sulfation and methylation. Catechin levels in human plasma reach their peak 2 to 4 h after ingestion (7–9). One of the factors limiting bioavailability may be instability in the gut lumen, since in simulated intestinal juice at pH 8.5, (+)-catechin and (–)-epicatechin were rapidly

degraded (10). We therefore hypothesized that catechin glucosylation could increase stability during processing and storage and also possibly in the gut lumen. For quercetin, glucosides are not only more stable but also more bioavailable (after gut deglycosylation), at least in part due to the improved stability of the glucoside in the intestine (11). The direct transport of intact glycosides into the epithelial cells by sugar transporters such as SGLT1 (12) followed by hydrolysis by cytosolic  $\beta$ -glucosidase (13) may contribute to absorption, but lactase phlorizin hydrolase, a mammalian  $\beta$ -glucosidase located outside the epithelial cells, is the main determinant of uptake (14). Therefore, for any potential increase in absorption of catechin glucosides, they would need to be deglycosylated in the gut.

Only a few studies have been reported on increased stability of catechins so far. These were mainly chemical derivatization either using methyl (15) or acetyl groups (16), or the addition of yeasts to hydroalcoholic solutions of catechins (17). (+)-Catechin *O*- $\beta$ -D-glucosides (Figure 1) are relatively rare natural products which have been isolated from several plant species. Among the different possible isomers, (+)-catechin 7-*O*- $\beta$ -D-glucoside (compound 1 (C7G)) is probably the most common, having been isolated from plants belonging to *Ulmus* (18), *Hordeum* (Barley) (19), *Paeonia* (20), *Fagopyrum* (Buckwheat) (21), *Betula* (22), *Pseudotsuga* (Douglas) (23), *Rheum* (Rhubarb) (24–26), *Rhaphiolepis* (25), *Vigna* (27), and *Schizandra* (28). Usually, C7G coexists in plant extracts with other isomeric glucosides such as (+)-catechin 5-*O*- $\beta$ -D-glucoside (compound 4 (C5G)), (+)-catechin 4'-*O*- $\beta$ -D-glucoside (compound 3 (C4'G)), or (+)-catechin 3'-*O*- $\beta$ -D-glucoside (compound 2 (C3'G)) (see references above). (+)-Catechin

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**Figure 1.** The structures of (+)-catechin 7-O- $\beta$ -glucopyranoside (C7G) **1**, (+)-catechin 3'-O- $\beta$ -glucopyranoside (C3'G) **2**, (+)-catechin 4'-O- $\beta$ -glucopyranoside (C4'G) **3**, (+)-catechin 5-O- $\beta$ -glucopyranoside (C5G) **4**, and (+)-catechin 3-O- $\beta$ -glucopyranoside (C3G) **5**.

3-O- $\beta$ -D-glucoside (compound **5** (C3G)) is also a natural product (29, 30), but it is usually more associated with procyanidin glycosides, rather than with other (+)-catechin O-glucoside isomers. C3G also constitutes a major portion of the phenolic compounds of the seed coat of lentils (31). Catechin O- $\alpha$ -D-glucosides have been prepared by biotransformation reactions (32). In contrast, very few publications (33) describe the preparation of  $\beta$ -glucosides by biotransformation, and only the chemical synthesis of C3G has been previously reported (34).

The purpose of this work was to prepare all the possible (+)-catechin mono-O- $\beta$ -D-glucosides **1–5** (Figure 1) by chemical and/or enzymic synthetic methods, and to investigate their stability as compared to the (+)-catechin aglycones *in vitro* and measure metabolism *in vivo* in the rat gut lumen.

## MATERIALS AND METHODS

**Materials.** (+)-Catechin hydrate, acetic anhydride, pyridine, 1-methyl-2-pyrrolidone, imidazole, thiophenol, anhydrous  $K_2CO_3$ , tetrabutylammonium bromide, sodium methoxide, acetyl chloride, triethylamine, dry tetrahydrofuran, dry methanol, 1,2,3,4,6-penta-O-acetyl- $\beta$ -D-(+)-glucose, uridine-5'-diphosphoglucose disodium salt (UDP-glucose), 1-butyl-3-methylimidazolium tetrafluoroborate, 1-ethyl-3-methylimidazolium tetrafluoroborate, 1,3-dimethylimidazolium methylsulfate, 1-butyl-3-methylimidazolium 2-(2-methoxyethoxy)ethylsulfate, 1-butyl-3-methylimidazolium octylsulfate, and 1-butyl-4-methylpyridinium tetrafluoroborate were obtained from Fluka. Tributyltin methoxide, triphenylphosphine and  $BF_3$  etherate were from Aldrich. Almond  $\beta$ -glucosidase and quercetin were from Sigma. All aqueous solutions were prepared using water filtered through a Milli-Q water system (Millipore, Bedford, MA), and all chemicals were of reagent grade. Lentils and barley seeds were purchased from local supermarkets (Switzerland).  $^{14}C$ -Polyethylene glycol (PEG) was from GE Healthcare, formerly Amersham Biosciences.

**Analytical HPLC with Diode Array and Fluorescence Detection.** Analytical HPLC with diode array and fluorescence detection was performed on an Agilent series 1100 HPLC using a Nucleosil 100,

250  $\times$  3 mm, RP-C18, 5  $\mu$ m (Macherey-Nagel) column with a guard column at a flow rate of 0.4 mL/min. Fluorescence detection was at 310 nm with excitation at 280 nm. Solvent A was water with 0.05% formic acid, and solvent B was acetonitrile. Gradient 1: time 0 min, 5% B; 20 min, 25% B; 21 min, 100% B for 6 min, then return to baseline. Gradient 2: time 0 min, 5% B; 5 min, 10% B; 10 min, 10% B; 15 min, 15% B; 22 min, 15% B; then return to baseline. Gradient 3: 0 min, 10% B; 10 min, 20% B; then return to baseline.

**Analytical HPLC with Triple Quadrupole Mass Spectrometric Detection.** Analytical HPLC with triple quadrupole mass spectrometric detection was performed on a Waters 2690 "Alliance" HPLC (Rapperswil, Switzerland) with Micromass "Quattro-LC" mass spectrometer (Manchester, U.K.) and Waters 996 diode array on a Nucleosil 100, 250  $\times$  3 mm, RP-C18, 5  $\mu$ m column (Macherey-Nagel) with a guard column at 0.4 mL/min. Solvent A was water with 0.05% formic acid, and solvent B was acetonitrile. The gradient (gradient 4) used was as follows: 0 min, 5% B; 20 min, 25% B; 21 min, 100% B for 6 min then return to baseline. For mass spectrometric detection, electrospray voltage was 3.5 kV, cone voltage was 25, 35 and 50 V, source block temperature was 120  $^\circ$ C, desolvation temperature was 200  $^\circ$ C, with cycle time of 2.1 s, scan range of  $m/z$  50 to 1500, cone gas of approximately 60 L/h and desolvation gas of approximately 600 L/h. For MS/MS, cone voltage was 30 V, collision energy was 40, 50, 60 and 80 eV, with a cycle time of 0.6 s and a scan range of  $m/z$  50 to 500 and  $m/z$  50 to 700.

**Semipreparative HPLC.** Semipreparative HPLC was performed on an Agilent Hewlett-Packard series 1050 HPLC with diode array detector on a Vydac C18 reverse phase column (22  $\times$  250 mm) column at 5 mL/min. Solvent A was water, and B was acetonitrile, with detection at 280 nm. Gradient 5: 0 min, 5% B; 20 min, 20% B; 21 min, 50% B; 35 min, 50% B; return to baseline. Gradient 6: isocratic 88% water and 12% acetonitrile.

**NMR Analysis.**  $^1H$  NMR (300.13 MHz) and  $^{13}C$  NMR (90.56 MHz) spectra were recorded on a Bruker DPX-360 apparatus, equipped with a 5 mm BBO gradient probehead, and using  $CD_3OD$  as solvent. The chemical shifts (in ppm) were expressed with respect to tetramethylsilane (TMS) as an internal reference, and using  $CDCl_3$  or  $CD_3OD$  as solvents. The spectrometer was controlled using XWINNMR version 3.1 software (Bruker Biospin Ltd., Germany), and the spectra were processed using TopSpin (version 1.3 Bruker Biospin Ltd., Germany). To confirm the molecular structure, one- and two-dimensional  $^1H$ -,  $^{13}C$  NMR, proton NOE-difference spectra, two-dimensional homonuclear proton correlation COSY and heteronuclear one- and multiple-bond  $^1H/^{13}C$  inverse shift correlation experiment using pulsed field gradients (HSQC, HMBC) were performed.

**Vacuum Liquid Chromatography and Medium Pressure Liquid Chromatography.** Vacuum liquid chromatography was performed on silica gel 60 (63–200  $\mu$ m) from Merck, or on a bonded phase octadecyl ( $C_{18}$ ) for flash chromatography (40  $\mu$ m average particle diameter) from Baker. Medium pressure liquid chromatography (MPLC) on silica or RP-18 was carried out on a Büchi apparatus comprising a 688 chromatographic pump and a 684 fraction collector and equipped with a C 690 Sepacore glass column (dimensions: 36  $\times$  460 mm, volume 470 mL) plus a pretreatment column (volume 11 mL). The column plus the precolumn were filled with 222 g of silica gel 60 (40–63  $\mu$ m), Merck, or with 250 g of LiChroprep RP-18 (40–63  $\mu$ m).

**Enzymic Synthetic Methods.** Preparation of Barley and Lentil Extracts. Barley seeds (20 g) or lentils (15 or 30 g) were ground in a mill in the presence of dry ice. Ground sample was extracted 3 times with 20 mL of cold acetone (4  $^\circ$ C) in polypropylene centrifuge tubes. Each extraction was carried out using a polytron (15 s at low speed). The acetone extracts were discarded, and the extracted samples were air-dried and homogenized with a mortar and a pestle to a fine powder. The powder was then extracted for 1 h at 4  $^\circ$ C with 100 mL of 0.2 M sodium phosphate buffer pH 6.5 containing 0.1 M NaCl. After centrifugation at 10000g, 4  $^\circ$ C for 20 min, the supernatant was filtered through a Whatman 541 filter. Desalting of the filtrate (15 mL) was done using a Biogel PD-6DG column (volume 60 mL, flow rate 2.0 mL/min), elution with sodium phosphate buffer (0.2 M, pH 6.5) containing 0.1 M NaCl, and collection of fractions between 8 and 23 mL.

*Enzymatic Synthesis of Compound 1 (C7G), Compound 2 (C3'G), and Compound 3 (C4'G) Using Barley or Lentil Extracts.* For analytical work

to determine product formation, the following solutions were incubated in a shaker bath at 37 °C for 22 h: 50  $\mu$ L of concentrated extract, and 110  $\mu$ L of 50 mM citrate phosphate buffer (pH 5.5) containing 5 mM MgCl<sub>2</sub>, 0.1% Tween, 5 mM UDPG, and 1.6 mM (+)-catechin. After incubation, 10  $\mu$ L of 6 N HCl and 80  $\mu$ L of MeOH were added. Before analytical HPLC-DAD analysis (gradient 2), 400  $\mu$ L of MeOH was added to 200  $\mu$ L of the incubation solution. For preparative work, 12  $\times$  15 mL of desalted barley extract and 4  $\times$  15 mL of desalted lentil extract were incubated each with 15 mg of MgCl<sub>2</sub>·6H<sub>2</sub>O, 15 mg of Tween 100, 50 mg of UDP-glucose and 20 mg of (+)-catechin for 20 h at 35 °C, respectively. After incubation, the solutions were extracted 3 times with 20 mL of *n*-butanol (saturated with water). The combined organic extracts were evaporated under reduced pressure, and the residues were redissolved in 5 mL of a mixture of water and methanol (2/1). Further purification of the major conversion products was carried out by semipreparative HPLC with gradient 5. Resulting fractions were concentrated by evaporation under reduced pressure to ~5 mL and then lyophilized. The residues were redissolved in 200  $\mu$ L of water and analyzed by HPLC-MS. This resulted in the isolation of 40 mg of compound **1** (C7G) from the incubation experiment with barley extract, and of 6.2 mg of compound **2** (C3'G) and 15.5 mg of compound **3** (C4'G) from the incubation medium with lentil extracts. For <sup>1</sup>H and <sup>13</sup>C NMR data of compound **1** (C7G), see **Table 1**. For <sup>1</sup>H and <sup>13</sup>C NMR data of compound **2** (C3'G) and compound **3** (C4'G), see **Table 2**.

**Hydrolysis of Products by Almond  $\beta$ -Glucosidase.** Samples (100  $\mu$ L) from above were evaporated to dryness by a stream of nitrogen. Citrate buffer (100  $\mu$ L, 100 mM, pH 5.0) was added containing 3 U of a  $\beta$ -glucosidase from almonds. After incubating for 3 h at 37 °C in a shaker, 300  $\mu$ L of MeOH was added and the solutions were analyzed by HPLC-DAD (gradient 2).

**Chemical Synthetic Methods.** *N*-Phenyl-2,2,2-trifluoroacetimidoyl Chloride **6**. Compound **6** was prepared in one step, according to the procedure of Tamura et al (35), by heating a mixture of trifluoroacetic acid and aniline in carbon tetrachloride in the presence of triphenylphosphine and triethylamine.

(2,3,4,6)-Tetra-*O*-acetyl-*D*-glucose **8**. This compound was prepared by an adaptation of the method of Nudelman et al (36). In a 250 mL 3-neck round-bottom flask, 6.8 g (17.42 mmol) of 1,2,3,4,6-penta-*O*-acetyl- $\beta$ -*D*(+)-glucose **7** was equilibrated under argon. Twenty-five milliliters of dry THF was added under stirring. After complete dissolution, 5 mL (17.36 mmol) of tributyltin methoxide was added dropwise under stirring. The medium was refluxed (oil bath at 80 °C) under stirring and argon atmosphere for 1.5 h. It was poured onto 200 mL of 10% aqueous HCl and extracted with 2  $\times$  200 mL of CH<sub>2</sub>Cl<sub>2</sub>. The CH<sub>2</sub>Cl<sub>2</sub> extract was washed with 3  $\times$  200 mL of H<sub>2</sub>O, concentrated under reduced pressure, and purified by VLC on silica using a gradient of EtOAc in hexane as solvent. This yielded 5.7 g (16.36 mmol; 94%) of a mixture of  $\alpha$ - and  $\beta$ -2,3,4,6-tetra-*O*-acetyl-*D*-glucose **8** as a colorless oil. <sup>1</sup>H NMR (360.13 MHz, CDCl<sub>3</sub>):  $\delta$  2.03 (s, OAc), 2.04 (s, OAc), 2.05 (s, OAc), 2.09 (s, OAc), 2.10 (s, OAc), 3.67 (dd, *J* = 3.9 and 1.2 Hz, 1-OH $\alpha$ ), 3.76 (ddd, *J* = 10.0, 4.8, and 2.3 Hz, H5 $\beta$ ), 3.97 (d, *J* = 8.7 Hz, 1-OH $\beta$ ), 4.09–4.17 (m, H6 $\alpha$  + H6 $\beta$ ), 4.22–4.30 (m, H5 $\alpha$  + H6 $\alpha$  + H6 $\beta$ ), 4.75 (brt, *J* = 8.4 Hz, H1 $\beta$ ), 4.90 (m, H2 $\alpha$  + H2 $\beta$ ), 5.09 (m, H4 $\alpha$  + H4 $\beta$ ), 5.27 (dd, *J* = 18.4 and 8.8 Hz, H3 $\beta$ ), 5.47 (brt, *J* = 3.7 Hz, H1 $\alpha$ ), 5.54 (brt, *J* = 9.8 Hz, H3 $\alpha$ ). <sup>13</sup>C NMR (90.56 MHz, CDCl<sub>3</sub>):  $\delta$  20.79 (OAc), 20.82 (OAc), 20.88 (OAc), 20.91 (OAc), 20.94 (OAc), 20.95 (OAc), 60.66 (C6 $\beta$ ), 62.15 (C6 $\alpha$ ), 67.37 (C5 $\alpha$ ), 68.60 (C4 $\beta$ ), 68.66 (C4 $\alpha$ ), 70.04 (C3 $\alpha$ ), 71.27 (C2 $\alpha$ ), 72.54 (C5 $\beta$ ), 72.41 (C3 $\beta$ ), 73.38 (C2 $\beta$ ), 90.32 (C1 $\alpha$ ), 95.73 (C1 $\beta$ ), 169.75 (OAc), 169.88 (OAc), 170.39 (OAc), 170.42 (OAc), 171.00 (OAc), 171.05 (OAc), 171.08 (OAc).

(2,3,4,6)-Tetra-*O*-acetyl-*D*-glucopyranosyl-(*N*-phenyl)-2,2,2-trifluoroacetimidate **9**. 2.0 g (5.74 mmol) of 2,3,4,6-tetra-*O*-acetyl-*D*-glucose **8** was dissolved in 15 mL of CH<sub>2</sub>Cl<sub>2</sub>. 1.2 g (8.68 mmol) of anhydrous K<sub>2</sub>CO<sub>3</sub>, 0.28 g (0.87 mmol) of tetrabutylammonium bromide, and 1.8 g (8.67 mmol) of *N*-phenyl-2,2,2-trifluoroacetimidoyl chloride **6** in 5 mL of CH<sub>2</sub>Cl<sub>2</sub> were added successively under stirring at rt. The reaction was started by addition of 0.2 mL of H<sub>2</sub>O. Stirring at rt was maintained for 2 h. The medium was diluted with 50 mL of CH<sub>2</sub>Cl<sub>2</sub> and 50 mL of H<sub>2</sub>O. The organic layer was washed with 3  $\times$  100 mL of H<sub>2</sub>O, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, concentrated under reduced pressure, and purified by VLC on silica using a gradient of EtOAc in hexane as solvent. This yielded 2.63 g of 2,3,4,6-tetra-*O*-acetyl-*D*-glucopyranosyl-(*N*-phenyl)-2,2,2-trifluoroacetimidate·0.1CH<sub>2</sub>Cl<sub>2</sub> **9** as a pale yellow liquid (4.98 mmol; 87%). <sup>1</sup>H NMR

**Table 1.** <sup>1</sup>H and <sup>13</sup>C NMR Spectral Data of Compound **1** (C7G) and Comparison with Data from the Literature<sup>a</sup>

position	$\delta$ <sup>1</sup> H; <i>J</i> (Hz)	$\delta$ <sup>1</sup> H; <i>J</i> (Hz) (19)	$\delta$ <sup>13</sup> C	$\delta$ <sup>13</sup> C (19)
1				
2	4.63 (7.4)	4.63 (7.4)	82.9	83.0
3	4.03 (m)	4.04 (m)	68.6	68.6
4	A 2.89 (dd 16.4, 5.4) B 2.56 (dd 16.3, 8.1)	A 2.9 (dd 16.3, 5.4) B 2.58 (dd 16.3, 8.0)	28.5	28.6
5			157.5	157.6
6	6.24 (2.2)	6.24 (2.2)	97.3	97.5
7			158.7	158.7
8	6.20 (2.1)	6.20 (2.4)	96.8	97.0
9			156.8	156.9
10			103.6	103.7
1'			132.0	132.1
2'	6.87 (1.5)	6.87 (1.9)	115.2	115.3
3'			146.3	146.3
4'			146.3	146.3
5'	6.80 (8.1)	6.80 (8.1)	116.1	116.1
6'	6.75 (dd 8.1, 1.6)	6.75 (dd 8.1, 1.9)	120.0	120.0
1''	4.86 (7.1)	4.86 (7.6)	102.2	102.3
2''	3.5–3.4	3.5–3.4	74.8	74.9
3''	3.5–3.4	3.5–3.4	78.1	78.1
4''	3.5–3.4	3.5–3.4	71.3	71.4
5''	3.5–3.4	3.5–3.4	78.0	78.1
6''	A 3.92 (dd 11.9, nd) B 3.73 (dd 11.9, 4.4)	A 3.92 (dd 11.9, 1.6) B 3.73 (dd 12.0, 4.9)	62.5	62.6

<sup>a</sup>Chemical shift  $\delta$ /ppm. Multiplicity abbreviations for <sup>1</sup>H NMR: dd = doublet of doublets, m = multiplet.

**Table 2.** <sup>1</sup>H and <sup>13</sup>C NMR Spectral Data of Compound **2** (C3'G) and Compound **3** (C4'G)<sup>a</sup>

position	$\delta$ <sup>1</sup> H; <i>J</i> (Hz) [C4'G 3]	$\delta$ <sup>1</sup> H; <i>J</i> (Hz) [C3'G 2]	$\delta$ <sup>13</sup> C [C4'G 3]	$\delta$ <sup>13</sup> C [C3'G 2]
1				
2	4.62 (7.3)	4.61 (7.5)	82.5	82.8
3	3.98 (m)	4.01 (m)	68.8	68.7
4	A 2.82 (dd 16.2, 5.3) B 2.51 (dd 16.2, 7.9)	A 2.86 (dd 16.2, 5.4) B 2.52 (dd 16.1, 8.1)	28.4	28.7
5			157.7	157.7
6	5.93 (1.9)	5.94 (2.2)	96.4	96.5
7			157.9	157.9
8	5.87 (1.9)	5.85 (2.2)	95.6	95.5
9			156.8	156.9
10			104.4	103.8
1'			<b>136.4</b>	132.5
2'	6.90 (1.2)	<b>7.2 (1.6)</b>	115.9	<b>116.9</b>
3'			<b>148.3</b>	146.5
4'			146.5	<b>148.1</b>
5'	<b>7.17 (8.3)</b>	6.85 (8.2)	<b>118.6</b>	117.3
6'	6.82 (dd 8.3, 1.4)	6.96 (dd 8.2, 1.6)	119.9	<b>123.8</b>
1''	4.77 (6.9)	4.80 (7.4)	100.8	100.9
2''	3.5–3.4	3.5–3.4	74.9	74.9
3''	3.5–3.4	3.5–3.4	78.4	78.2
4''	3.5–3.4	3.5–3.4	71.4	71.3
5''	3.5–3.4	3.5–3.4	77.7	77.6
6''	A 3.89 (dd 11.9, nd) B 3.71 (dd 11.2, nd)	A 3.82 (dd 12.1, 1.4)	62.5	62.4

<sup>a</sup>Chemical shift  $\delta$ /ppm. Multiplicity abbreviations for <sup>1</sup>H NMR: dd = doublet of doublets, m = multiplet. The values in bold correspond to the signals shifted downfield compared to those of (+)-catechin.

(360.13 MHz, CDCl<sub>3</sub>):  $\delta$  2.03 (s, OAc), 2.04 (s, OAc), 2.06 (s, OAc), 2.08 (s, OAc), 2.10 (s, OAc), 5.10–5.26 (m), 5.30 (s), 5.54 (t, *J* = 9.8 Hz), 6.79 (d, *J* = 7.7 Hz, H-2''/6''), 6.84 (d, *J* = 7.8 Hz, H-2''/6''), 7.13 (m, H4''), 7.32 (m, H3''/5''). <sup>13</sup>C NMR (90.56 MHz, CDCl<sub>3</sub>):  $\delta$  20.45 (OAc), 20.50 (OAc), 20.56 (OAc), 20.58 (OAc), 20.66 (OAc), 20.68 (OAc), 61.38 (C6), 67.72 (C5), 69.44, 69.79, 70.01, 70.19, 72.52, 72.74, 119.20 (C2''/6''), 119.31

(C2''/6''), 124.71 (C4''), 128.89 (C3''/C5''), 142.91 (C1''), 169.00 (OAc), 169.32 (OAc), 169.48 (OAc), 169.75 (OAc), 170.00 (OAc), 170.17 (OAc), 170.55 (OAc), 170.58 (OAc). Anal. (C<sub>22</sub>H<sub>24</sub>F<sub>3</sub>NO<sub>10</sub>·0.1CH<sub>2</sub>Cl<sub>2</sub>) C: calcd, 50.28; found 50.09. H: calcd, 4.62; found 4.73. N: calcd, 2.65; found 2.62.

(+)-3,5,7,3',4'-Penta-O-acetylcatechin **11**. (+)-Catechin hydrate **10** (5 g, 17.22 mmol) was dissolved in 40 mL of pyridine, and the solution was cooled to 0 °C in an ice bath. Acetic anhydride (40 mL) was carefully added in portions of 10 mL. The medium was removed from the ice bath and allowed to react at rt for 6 days. The medium was poured onto 400 mL of ice-cold H<sub>2</sub>O and, after 2 h, was extracted with 3 × 100 mL of CH<sub>2</sub>Cl<sub>2</sub>. The CH<sub>2</sub>Cl<sub>2</sub> extract was washed with 2 × 200 mL of 1 N aqueous HCl and 2 × 200 mL of H<sub>2</sub>O. The organic solvent was evaporated under reduced pressure. The CH<sub>2</sub>Cl<sub>2</sub> extract was purified by VLC using a gradient of EtOAc in hexane as solvent. This yielded 8.5 g of (+)-3,5,7,3',4'-penta-O-acetylcatechin **11** as a white solid (16.98 mmol; 99%). <sup>1</sup>H NMR (360.13 MHz, CDCl<sub>3</sub>): δ 1.93 (s, 3H, 3-OAc), 2.24 (s, 3H, OAc), 2.26 (s, 3H, OAc), 2.27 (s, 3H, OAc), 2.73 (dd, 1H, *J* = 17.2 and 6.6 Hz, H4), 2.86 (dd, 1H, *J* = 16.9 and 5.0 Hz, H4), 5.27 (d, 1H, *J* = 6.5 Hz, H2), 5.32 (1H, m, H3), 6.61 (1H, d, *J* = 2.2 Hz, H6), 6.68 (1H, d, *J* = 2.2 Hz, H8), 7.28 (d, 1H, *J* = 8.4 Hz, H5'), 7.30 (brd, 1H, *J* = 2.1 Hz, H2'), 7.36 (ddd, 1H, *J* = 8.4, 2.1, and 0.5 Hz, H6'). <sup>13</sup>C NMR (90.56 MHz, CDCl<sub>3</sub>): δ 20.48 (OAc), 20.50 (OAc), 20.62 (OAc), 20.79 (OAc), 20.93 (OAc), 24.76 (C4), 68.79 (C3), 78.51 (C2), 108.35 (C8), 109.91 (C6), 111.49 (C10), 122.87 (C2'), 124.60 (C5'), 125.25 (C6'), 137.25 (C1'), 143.39 (C3' + C4'), 150.76 (C5), 151.07 (C7), 155.33 (C9), 168.59 (OAc), 168.66 (OAc), 168.92 (OAc), 169.33 (OAc), 170.08 (3-OAc). The <sup>13</sup>C NMR data agrees with published data in the same solvent (37).

(+)-3,7,3',4'-Tetra-O-acetylcatechin **12**, (+)-3,5,7,3'-tetra-O-acetylcatechin **13**, and (+)-3,5,7,4'-tetra-O-acetylcatechin **14**. 3,5,7,3',4'-Penta-O-acetylcatechin **11** (2 g, 4 mmol) was dissolved in 10 mL of 1-methyl-2-pyrrolidone (NMP). The solution was cooled to 0 °C in an ice bath, and then 95 mg (1.4 mmol) of imidazole and 0.5 mL (4.89 mmol) of thiophenol were added, successively. The medium was removed from the ice bath and was left to react for 6 h at rt. It was diluted with 50 mL of CH<sub>2</sub>Cl<sub>2</sub>, and the organic layer was washed with 50 mL of 1 N aqueous HCl and 3 × 50 mL of H<sub>2</sub>O. The CH<sub>2</sub>Cl<sub>2</sub> extract was purified by MPLC on silica using a gradient of acetone in hexane as solvent. This yielded 910 mg (1.98 mmol; 50%) of a mixture of (+)-3,7,3',4'-tetra-O-acetylcatechin, (+)-3,5,7,3'-tetra-O-acetylcatechin and (+)-3,5,7,4'-tetra-O-acetylcatechin (**12-14**), as a colorless oil.

(3,7,3',4',2'',3'',4'',6'')-Octa-O-acetyl-(+)-catechin 5-O-β-D-Glucoside **15**, 3,5,7,3',2'',3'',4'',6''-octa-O-acetyl-(+)-catechin 4'-O-β-D-Glucoside **16**, and 3,5,7,4',2'',3'',4'',6''-octa-O-acetyl-(+)-catechin 3'-O-β-D-Glucoside **17**. 398.8 mg (0.87 mmol assuming that the sample was composed of tetra-O-acetylated catechins only) of a mixture of compounds **12-14** and 0.76 g (1.46 mmol) of 2,3,4,6-tetraacetyl-D-glucopyranosyl-(*N*-phenyl)-2,2,2-trifluoroacetimidate **9** were dissolved in 15 mL of dry CH<sub>2</sub>Cl<sub>2</sub>. 35 μL (0.28 mmol) of BF<sub>3</sub> etherate was added, and the mixture was stirred at rt overnight. The medium was diluted with 50 mL of CH<sub>2</sub>Cl<sub>2</sub>. The CH<sub>2</sub>Cl<sub>2</sub> extract was washed with 3 × 50 mL of H<sub>2</sub>O, concentrated under reduced pressure, and purified by VLC on silica using a gradient of EtOAc in hexane as solvent. This yielded 728.6 mg of a mixture of 3,7,3',4',2'',3'',4'',6''-octa-O-acetyl-(+)-catechin 5-O-β-D-glucoside, 3,5,7,3',2'',3'',4'',6''-octa-O-acetyl-(+)-catechin 4'-O-β-D-glucoside and 3,5,7,4',2'',3'',4'',6''-octa-O-acetyl-(+)-catechin 3'-O-β-D-glucoside (**15-17**) as a colorless oil.

(+)-3-O-Acetylcatechin 3'-O-β-D-Glucoside **18**. In a 250 mL round-bottom flask, 1.4 g (1.77 mmol based on a *M<sub>r</sub>* of 788.7 for acetylated monoglucosylcatechins) of the mixture of compounds **15-17** was dissolved in 10 mL of MeOH and equilibrated under argon. The solution was cooled to 0 °C (ice bath), which resulted in partial crystallization of the substrate. 35 mL (35 mmol) of a 1 N solution of NaOH in MeOH was added under inert atmosphere. This resulted in the dissolution of the crystals and the appearance of a yellow coloration. The medium was stirred for 5 min under inert atmosphere. The reaction was stopped by the addition of 38 mL of 1 N aqueous HCl (the medium turned pale yellow), and the acidic solution was removed from the ice bath and directly purified by VLC on RP-18 using a gradient of MeOH in H<sub>2</sub>O as solvent, followed by an MPLC on RP-18 using a similar gradient of solvents. This resulted in the isolation of 35.5 mg of 3-O-acetylcatechin 3'-O-β-D-glucoside **18**, from a complex mixture of products. <sup>1</sup>H NMR (360.13 MHz, CD<sub>3</sub>OD): δ 1.94

(s, 3H, 3-OAc), 2.64 (dd, 1H, *J* = 16.5 and 6.4 Hz, H4), 2.75 (dd, 1H, *J* = 16.6 and 5.2 Hz, H4), 3.31 (m, 1H, H5'), 3.44–3.51 (m, 3H, H2'' + H3''), 3.71 (dd, 1H, *J* = 12.3 and 4.4 Hz, H6''), 3.77 (dd, 1H, *J* = 12.3 and 2.5 Hz, H6'), 4.75 (d, 1H, *J* = 7.3 Hz, H1''), 4.96 (d, 1H, *J* = 6.2 Hz, H2), 5.24 (dd, 1H, *J* = 11.7 and 6.3 Hz, H3), 5.90 (1H, d, *J* = 2.3 Hz, H8), 5.96 (1H, d, *J* = 2.3 Hz, H6), 6.83 (d, 1H, *J* = 8.3 Hz, H5'), 6.91 (dd, 1H, *J* = 8.3 and 1.9 Hz, H6'), 7.14 (d, 1H, *J* = 1.9 Hz, H2'). <sup>13</sup>C NMR (90.56 MHz, CDCl<sub>3</sub>): δ 21.01 (3-OAc), 24.25 (C4), 62.14 (C6''), 70.91 (C3 or C4''), 71.00 (C3 or C4'), 74.77 (C2''), 77.59 (C3'), 78.14 (C5'), 79.29 (C2), 95.54 (C8), 96.64 (C6), 99.62 (C10), 103.63 (C1''), 116.43 (C2'), 117.01 (C5'), 123.04 (C6'), 131.44 (C1'), 146.52 (C3'), 148.25 (C4'), 156.41 (C9), 157.65 (C5), 158.23 (C7), 172.06 (3-OAc).

**Compound 2** (C3'G). 35.5 mg (0.072 mmol) of 3-O-acetylcatechin 3'-O-β-D-glucoside **18** was added to 75 mL of sodium acetate buffer (0.1 M, pH 5.0). 1.5 g of tannase (KT-50, Kikkoman Corporation, Chiba, Japan) was added and the solution incubated at 37 °C. Samples (100 μL) were withdrawn at 2.5, 5, 6, 21, and 23 h and analyzed by HPLC-DAD. After 23 h, 84% of 3-O-acetylcatechin 3'-O-β-D-glucoside had been hydrolyzed. The incubation solution was extracted three times with 50 mL of *n*-butanol (saturated with water); the combined organic extracts were evaporated under reduced pressure to a final volume of about 1 mL. Two milliliters of acetonitrile/water (50/50) was added. Purification of C3'G was achieved by semipreparative HPLC (gradient 6), and fractions were collected, combined and freeze-dried. A total of 22 mg (0.048 mmol; 67%) of C3'G **2** was obtained. <sup>1</sup>H and <sup>13</sup>C NMR data of **2**: see **Table 1**.

**Compound 4** (C5G). In a 250 mL 3-neck round-bottom flask, 670 mg (12.4 mmol) of NaOMe was suspended in 60 mL of dry MeOH. The solution was equilibrated under argon and cooled to 0 °C (ice bath). 360 mg (0.46 mmol based on a *M<sub>r</sub>* of 788.7 for acetylated monoglucosylcatechins) of the mixture of compounds **15-17**, dissolved in 2.5 mL of dry CH<sub>2</sub>Cl<sub>2</sub>, were added under inert atmosphere. The medium was allowed to react for 4.5 h at 0 °C under stirring and argon atmosphere. The reaction was stopped by the addition of 5.5 mL of 10% aqueous HCl at 0 °C. Finally the medium was diluted with 60 mL of H<sub>2</sub>O (pH was around 4). The experiment was repeated using 700 mg (12.96 mmol) of NaOMe and 376.4 mg (0.48 mmol) of the mixture of compounds **15-17**. The media resulting from the two experiments were mixed, concentrated under reduced pressure (removal of MeOH) to a volume of 10 mL, and purified by MPLC on RP-18 using a gradient of MeOH in H<sub>2</sub>O as solvent. This yielded 43 mg of pure compound **4** (C5G) and 14 mg of pure compound **2** (C3'G), beside fractions containing mixtures of catechin glucosides **3-5**. NMR data of compound **4** (C5G): <sup>1</sup>H NMR (360.13 MHz, CD<sub>3</sub>OD) and <sup>13</sup>C NMR (90.56 MHz, CDCl<sub>3</sub>) chemical shifts are shown in **Table 3**.

(+)-5,7,3',4'-Tetra-O-acetylcatechin **19**. (+)-Catechin hydrate **10** (1 g, 3.35 mmol) was dissolved in 10 mL of DMF, and the solution was cooled to 0 °C (ice bath). 2.3 mL (16.59 mmol) of triethylamine and 1.2 mL (16.89 mmol) of acetyl chloride were added dropwise, successively. The medium was stirred at 0 °C for 10 min and at rt for 1 h. The medium was poured onto 100 mL of H<sub>2</sub>O and left at 4 °C for 48 h. The precipitate was dissolved in 50 mL of EtOAc and washed with 50 mL of 1 N aqueous HCl and 2 × 50 mL of H<sub>2</sub>O. It was purified by MPLC on silica using a gradient of EtOAc in hexane as solvent. This yielded 533.3 mg (1.16 mmol; 35%) of 5,7,3',4'-tetra-O-acetylcatechin **19** as a colorless oil. <sup>1</sup>H NMR (360.13 MHz, CDCl<sub>3</sub>): δ 2.25 (s, 3H, OAc), 2.78 (s, 3H, OAc), 2.29 (s, 6H, OAc), 2.59 (dd, 1H, *J* = 16.4 and 9.4 Hz, H4), 2.93 (dd, 1H, *J* = 16.4 and 5.6 Hz, H4), 3.88 (1H, m, H3), 4.69 (d, 1H, *J* = 8.4 Hz, H2), 6.54 (1H, d, *J* = 2.2 Hz, H6), 6.58 (1H, d, *J* = 2.2 Hz, H8), 7.20 (d, 1H, *J* = 8.3 Hz, H5'), 7.24 (d, 1H, *J* = 2.0 Hz, H2'), 7.30 (dd, 1H, *J* = 8.4 and 2.0 Hz, H6'). <sup>13</sup>C NMR (90.56 MHz, CDCl<sub>3</sub>): δ 20.57 (OAc), 20.63 (OAc), 20.72 (OAc), 21.04 (OAc), 28.12 (C4), 67.44 (C3), 81.14 (C2), 107.76 (C8), 108.72 (C6), 111.75 (C10), 122.35 (C2'), 123.64 (C5'), 125.59 (C6'), 136.66 (C1'), 142.17 (C3' or C4'), 142.26 (C3' or C4'), 149.48 (C5 or C7), 149.67 (C5 or C7), 155.03 (C9), 168.30 (OAc), 168.42 (OAc), 168.54 (OAc), 169.08 (OAc). Anal. (C<sub>23</sub>H<sub>22</sub>O<sub>10</sub>·0.1 CH<sub>2</sub>Cl<sub>2</sub>) C: calcd, 59.42; found 59.61. H: calcd, 4.79; found 5.03.

(5,7,3',4',2'',3'',4'',6'')-Octa-O-acetyl-(+)-catechin 3-O-β-D-Glucoside **20**. 5,7,3',4'-Tetra-O-acetylcatechin **19** (287.3 mg, 0.63 mmol) and 495.4 mg (0.95 mmol) of 2,3,4,6-tetra-O-acetyl-D-glucopyranosyl-(*N*-phenyl)-2,2,2-trifluoroacetimidate **9** were dissolved in 10 mL of dry CH<sub>2</sub>Cl<sub>2</sub>.

**Table 3.** Comparison of the NMR Data of Compound **4** (C5G)<sup>a</sup>

	MeOH- <i>d</i> <sub>4</sub>	MeOH- <i>d</i> <sub>4</sub> (33)		MeOH- <i>d</i> <sub>4</sub>	DMSO- <i>d</i> <sub>6</sub> + D <sub>2</sub> O (25)	MeOH- <i>d</i> <sub>4</sub> (33)
H-2	4.59	4.58	C-2	82.9	80.9	82.8
H-3	3.97	3.97	C-3	68.7	65.9	68.5
H-4a	2.57	2.56	C-4	28.4	27.3	28.4
H-4b	3.00	3.01	C-5	158.0 or 158.1	156.4	157.9
H-6	6.26	6.25	C-6	96.9	94.9	98.0 <sup>b</sup>
H-8	6.02	6.01	C-7	158.0 or 158.1	154.7 <sup>c</sup>	157.8
H-2'	6.82	6.81	C-8	98.1	96.0	96.7 <sup>b</sup>
H-5'	6.59	6.75	C-9	156.7	156.1 <sup>c</sup>	156.5
H-6'	6.70	6.66	C-10	103.4	100.3 <sup>d</sup>	103.2
H-1''	4.82	4.83	C-1'	132.1	130.3	131.9
H-2''	3.35–3.47	3.42–3.90	C-2'	115.23	114.3	115.0
H-3''	3.35–3.47	3.42–3.90	C-3'	146.3	144.6	146.1
H-4''	3.35–3.47	3.42–3.90	C-4'	146.3	144.6	146.1
H-5''	3.35–3.47	3.42–3.90	C-5'	116.1	115.0	115.9
H-6''a	3.71	3.42–3.90	C-6'	120.0	118.4	119.8
H-6''b	3.90	3.42–3.90	C-1''	102.6	101.2 <sup>d</sup>	102.4
			C-2''	74.9	73.1	74.8
			C-3''	78.2 or 78.3	76.3	78.1
			C-4''	71.3	69.4	71.2
			C-5''	78.2 or 78.3	76.7	78.1
			C-6''	62.6	60.4	62.5

<sup>a</sup> C5G was chemically synthesized here, previously isolated from *Rhaphiolepis umbellata* (25), or produced by cultured cells of *Eucalyptus perriniana* (33). <sup>b</sup> Assignments must be reversed taking into account our HSQC and HMBC data for compound **4** (C5G). <sup>c</sup> The assignment between these two carbons probably needs to be reversed, taking into account the HMBC data recorded for compound **4** (C5G). <sup>d</sup> The assignment between these two carbons probably needs to be reversed, taking into account the HSQC data we obtained for compound **4** (C5G).

**Table 4.** <sup>13</sup>C NMR Data of Compound **5** (C3G)<sup>a</sup>

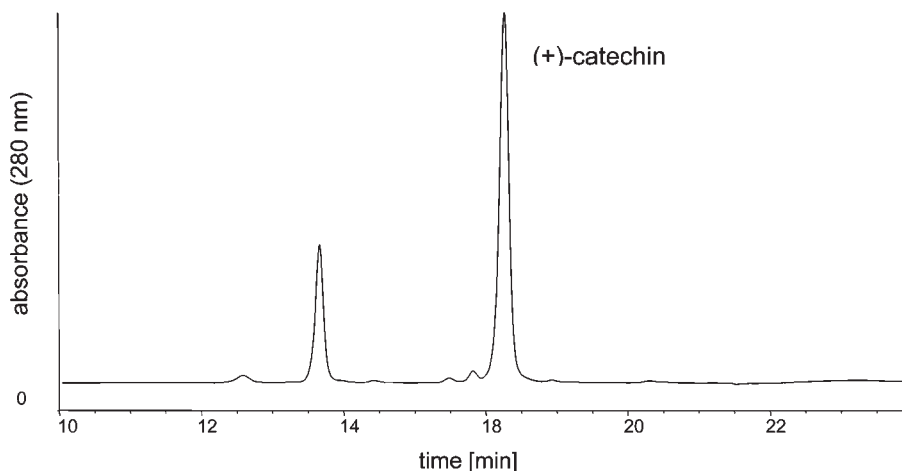
	MeOH- <i>d</i> <sub>4</sub>	acetone- <i>d</i> <sub>6</sub> + D <sub>2</sub> O (45)		MeOH- <i>d</i> <sub>4</sub>	acetone- <i>d</i> <sub>6</sub> + D <sub>2</sub> O (45)
C-2	80.0	79.3	C-3'	146.2 or 146.3	145.6
C-3	76.1	75.6	C-4'	146.2 or 146.3	145.7
C-4	26.0	28.4	C-5'	116.3	116.0
C-5	157.4 or 157.8	156.9	C-6'	119.5	119.1
C-6	96.4	95.3 <sup>b</sup>	C-1''	103.8	103.5
C-7	157.4 or 157.8	157.4	C-2''	75.1	74.6
C-8	95.6	96.4 <sup>b</sup>	C-3''	77.7	77.3
C-9	156.6	156.0	C-4''	71.6	71.1
C-10	100.7	100.0	C-5''	78.0	77.1
C-1'	132.2	131.7	C-6''	62.8	62.5
C-2'	114.8	114.6			

<sup>a</sup> Comparison of C3G from this study and as previously isolated from the leaves *Quercus miyagii* (45). <sup>b</sup> The assignment between these two carbons probably needs to be reversed in ref 45, taking into account the HSQC and HMBC data recorded for compound **5** C3G.

Twenty-five microliters (0.2 mmol) of BF<sub>3</sub> etherate was added, and the mixture was stirred at rt overnight. The solvent was evaporated under reduced pressure, the residue was taken up in 5 mL of CH<sub>2</sub>Cl<sub>2</sub>, and the solution was directly purified by MPLC on silica using a gradient of EtOAc in hexane as solvent. This yielded 245.5 mg (0.31 mmol; 49%) of 5,7,3',4',2'',3'',4'',6''-octa-*O*-acetyl-(+)-catechin 3-*O*-β-D-glucoside **20** as a colorless oil. <sup>1</sup>H NMR (360.13 MHz, CDCl<sub>3</sub>): δ 1.85 (s, 3H, sugar OAc), 1.95 (s, 3H, sugar OAc), 2.00 (s, 3H, sugar OAc), 2.09 (s, 3H, sugar OAc), 2.26 (s, 3H, catechin OAc), 2.30 (s, 9H, catechin OAc), 2.75 (dd, 1H, *J* = 16.7 and 9.2 Hz, H4), 3.06 (dd, 1H, *J* = 16.8 and 5.8 Hz, H4), 3.61 (m, 1H, H5''), 4.08 (1H, m, H3), 4.13 (dd, 1H, *J* = 12.4 and 2.5 Hz, H6''), 4.22 (dd, 1H, *J* = 12.4 and 4.9 Hz, H6''), 4.24 (d, 1H, *J* = 7.9 Hz, H1''), 4.80 (d, 1H, *J* = 8.7 Hz, H2), 4.88 (m, 1H, H2''), 5.02 (m, 2H, H3'' + H4''), 6.54 (1H, d, *J* = 2.2 Hz, H6), 6.56 (1H, d, *J* = 2.2 Hz, H8), 7.21–7.26 (m, 3H, H2' + H5' + H6'). <sup>13</sup>C NMR (90.56 MHz, CDCl<sub>3</sub>): δ 20.46 (OAc), 20.55 (OAc), 20.58 (OAc), 20.63 (OAc), 20.69 (OAc), 20.73 (OAc), 21.05 (OAc), 28.46 (C4), 61.89 (C6''), 68.31 (C4''), 71.07 (C2''), 71.79 (C5''), 72.77 (C3''), 74.44 (C3), 78.85 (C2), 100.19 (C1''), 107.64 (C8), 108.89 (C6), 111.55 (C10), 122.34 (C2'), 123.34 (C5'), 125.89 (C6'), 136.43 (C1'), 142.24 (C3' or C4'), 142.36 (C3' or C4'), 149.35 (C5), 149.70 (C7), 154.75 (C9), 167.93 (OAc), 168.02 (OAc), 168.51 (OAc), 168.97 (OAc), 169.29 (OAc), 169.46 (OAc), 170.04 (OAc), 170.58 (OAc). Anal. (C<sub>37</sub>H<sub>40</sub>O<sub>19</sub> · 0.3 CH<sub>2</sub>Cl<sub>2</sub>) C: calcd, 55.02; found 55.04. H: calcd, 5.03; found 5.29.

**Chemical Synthesis of Compound 5 (C3G).** In a 100 mL 2-neck round-bottom flask, 190 mg (3.52 mmol) of NaOMe was suspended in 15 mL of dry MeOH. The solution was equilibrated under argon and cooled to 0 °C (ice bath). 106.2 mg (0.135 mmol) of compound **20**, dissolved in 1 mL of dry CH<sub>2</sub>Cl<sub>2</sub> + 4 mL of dry MeOH, was added under inert atmosphere. The medium was allowed to react for 4.5 h at 0 °C under stirring and argon atmosphere. The reaction was stopped by the addition of 1.6 mL of 10% aqueous HCl, and the medium was diluted with 40 mL of H<sub>2</sub>O (pH was around 4), concentrated under reduced pressure (removal of MeOH) to a volume of about 20 mL, and purified by VLC on RP-18 using a gradient of MeOH in H<sub>2</sub>O as solvent. This yielded 57.2 mg (0.126 mmol; 93%) of compound **5** (C3G) as a white powder. <sup>1</sup>H NMR (360.13 MHz, CD<sub>3</sub>OD): δ 2.74 (m, 2H, H4), 3.12 (m, 1H, H2''), 3.18–3.20 (m, 1H, H5''), 3.21–3.27 (m, 2H, H3'' + H4''), 3.66 (dd, 1H, *J* = 11.8 and 5.4 Hz, H6''), 3.86 (dd, 1H, *J* = 11.8 and 2.1 Hz, H6''), 4.19 (d, 1H, *J* = 7.7 Hz, H1''), 4.24 (dd, 1H, *J* = 11.4 and 5.9 Hz, H3), 4.94 (d, 1H, *J* = 5.9 Hz, H2), 5.90 (1H, d, *J* = 2.3 Hz, H8), 5.94 (1H, d, *J* = 2.3 Hz, H6), 6.70 (dd, 1H, *J* = 8.1 and 1.9 Hz, H6'), 6.75 (d, 1H, *J* = 8.1 Hz, H5'), 6.82 (d, 1H, *J* = 1.9 Hz, H2'). <sup>13</sup>C NMR (90.56 MHz, CDCl<sub>3</sub>) chemical shifts are shown in **Table 4**.

**Influence of pH on Stability.** The stability of the catechin glucosides **1–5** was tested by incubating them individually at 37 °C (additionally 70 °C for pH 4 and 5) at concentrations of 40 μg/mL in 50 mM sodium phosphate buffers at pH values of 4, 5, 6, 7, and 8. The samples were analyzed by HPLC-FLD (gradient 3) several times over 50 h.



**Figure 2.** HPLC chromatogram after incubation of a desalted barley extract with UDP-glucose and (+)-catechin.

**Stability in a Physiological Buffer.** The stability of the catechin glucosides 1–5 was tested by incubating them individually at 37 °C at concentrations of 15  $\mu$ M in “perfusion buffer” (pH 6.7) of the following composition: NaCl, 50 mM; KCl, 40 mM;  $K_2HPO_4$ , 2.5 mM;  $KH_2PO_4$ , 5 mM; tripotassium citrate, 10 mM;  $MgCl_2$ , 2 mM;  $CaCl_2$ , 2 mM; glucose 8 mM; PEG 4000, 2 g/L; taurocholate, 1 mM;  $NaHCO_3$ , 5 mM. Aliquots were withdrawn and analyzed by HPLC (gradient 2) after 0, 3.5, 18, 25, and 42 h.

**Intestinal Rat Perfusion Study.** Intestinal perfusion of rat intestine was carried out as previously described (38) using male white Wistar rats at a maximum age of 16 weeks, purchased from Charles River (France), with 8 rats per compound. To avoid loss of the perfused compound due to possible affinity of the compound for the PVC, the catheters of perfusion were saturated with the tested solution for 5 min before starting the experiment.  $^{14}C$ -PEG 4000 (2.5  $\mu$ Ci/mL) was added to the “perfusion solution” (see above). Catechin glucoside solutions were prepared at 100 mM in DMSO and added to the perfusion solution to give a final concentration of 30  $\mu$ M, and pumped at a flow rate of 0.5 mL/min. The influent and effluent were collected in plastic tubes containing EDTA 0.1%, vitamin C 20%, and  $NaH_2PO_4$  0.4 M at pH 2.6 (20  $\mu$ L/mL of solution). All the samples were stored on ice during the experiment, then at –20 °C until analysis.

For each animal, radioactivity of  $^{14}C$ -PEG 4000 was measured in duplicate in the influent and effluent using scintillation counting, and used to correct for water absorption. Correction of the concentration to account for water absorption ( $W_{abs}$ ) was calculated as the ratio of concentration of the nonabsorbable marker,  $^{14}C$ -PEG 4000, in the inlet and outlet perfusion solution.

$$W_{abs} = C_{in}({}^{14}C\text{-PEG})/C_{out}({}^{14}C\text{-PEG})$$

Catechin and catechin glucosides were directly analyzed, after centrifugation, by HPLC-MS (gradient 4). The fraction apparently absorbed ( $F_{aa}$ ) of each compound was estimated as the fraction disappearing from the intestinal lumen, correcting for a minor volume change by using [ $^{14}C$ -PEG] as a nonabsorbable marker:

$$F_{aa} (\%) = ((C_{in} - (C_{out}W_{abs}))/C_{in}) \times 100$$

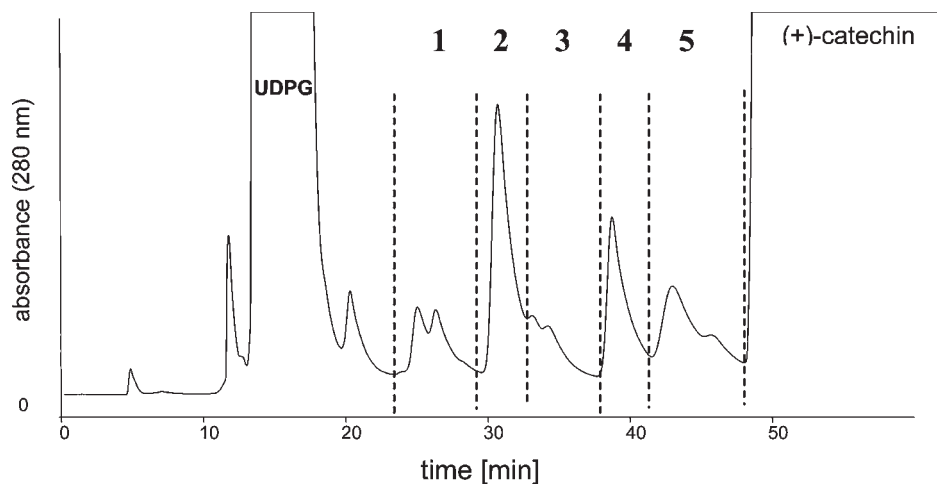
where  $C_{in}$  and  $C_{out}$  are the concentrations of the compound of interest in the inflow and outflow solutions respectively. The  $F_{aa}$  corresponded to the apparent absorption of the compound expressed in % of the concentration of the compound in the inlet perfusion solution.

## RESULTS AND DISCUSSION

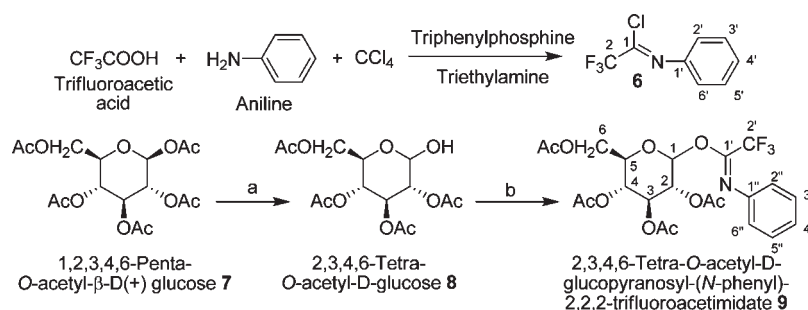
**Enzymic Synthesis of Compound 1 (C7G) Using Barley Extracts.** C7G 1 was found in barley (19), and so barley seeds were tested for the presence of a glycosyltransferase activity accepting catechin as a substrate. After incubation of (+)-catechin with barley extract, two new products with retention times of 12.6 (minor

and 13.7 (major) min were formed according to analytical HPLC with gradient 1 (Figure 2), both of which disappeared after almond  $\beta$ -glucosidase treatment. The main peak was purified by semipreparative HPLC, concentrated by evaporation under reduced pressure, and analyzed by HPLC–ESI-MS. The product had a molecular ion at  $m/z$  451 =  $[M - H]^-$  in the negative ion mode and a fragment ion at  $m/z$  289. The collision induced dissociation (CID) spectrum of the parent ion at  $m/z$  451 shows the presence of a daughter ion at  $m/z$  289 resulting from the cleavage of one glucose molecule. On the basis of the  $^1H$  and  $^{13}C$  NMR data and comparison with literature data (19, 39), compound 1 (C7G) was identified as (+)-catechin 7-*O*- $\beta$ -D-glucoside. A total of 40 mg of compound 1 (C7G) was synthesized by incubation of 240 mg (+)-catechin with UDP-glucose and barley extract, with purification by semipreparative HPLC.

**Enzymatic Synthesis of Compound 2 (C3'G) and Compound 3 (C4'G) Using Lentil Extracts.** As for barley above, the lentils were tested for the presence of a glycosyltransferase activity accepting catechin as a substrate. A desalted extract from lentils was incubated with UDP-glucose and (+)-catechin. HPLC-DAD analysis revealed the formation of several conversion products of catechin exclusively in the presence of UDP-glucose. Further purification was carried out by semipreparative HPLC, which gave 5 fractions (Figure 3), collected and concentrated by evaporation under reduced pressure and analyzed by HPLC–ESI-MS. In fraction 2 (15.5 mg), a molecular ion at  $m/z$  451 =  $[M - H]^-$  in the negative ion mode was detected. The collision induced dissociation (CID) spectrum of the parent ion at  $m/z$  451 showed the presence of a daughter ion at  $m/z$  289 resulting from the cleavage of one glucose molecule. This, together with the NMR data (Table 2), identified fraction 2 as pure C4'G 3. Indeed, for the NOE-difference experiment on compound 3 (C4'G), an enhancement of the aglycone proton H-5' was obtained upon irradiation of the anomeric proton H-1'', indicating attachment of the sugar in position C-4'. Consistent with this assumption are the chemical shifts observed within the B-ring of the aglycone. Compared to the data for (+)-catechin, the signals for C-1', C-3' and C-5' as well as of H-5' (Table 2) are shifted downfield. Fraction 3 (6.2 mg) was C3'G 2 based on NMR data (Table 2). In the NOE-difference experiment on compound 2 (C3'G), an enhancement of the aglycone proton H-2' was obtained upon irradiation of the anomeric proton H-1'', indicating 3'-glucosylation. Attachment of the sugar in position C-3' was confirmed by the downfield shift of the signals for C-2', C-4' and C-6' as well as of H-2' (Table 2).



**Figure 3.** Semipreparative RP18-HPLC-UV chromatogram after incubation of a desalted lentil extract with UDP-glucose and (+)-catechin. Fractions 1–5 collected as indicated by dashed lines.



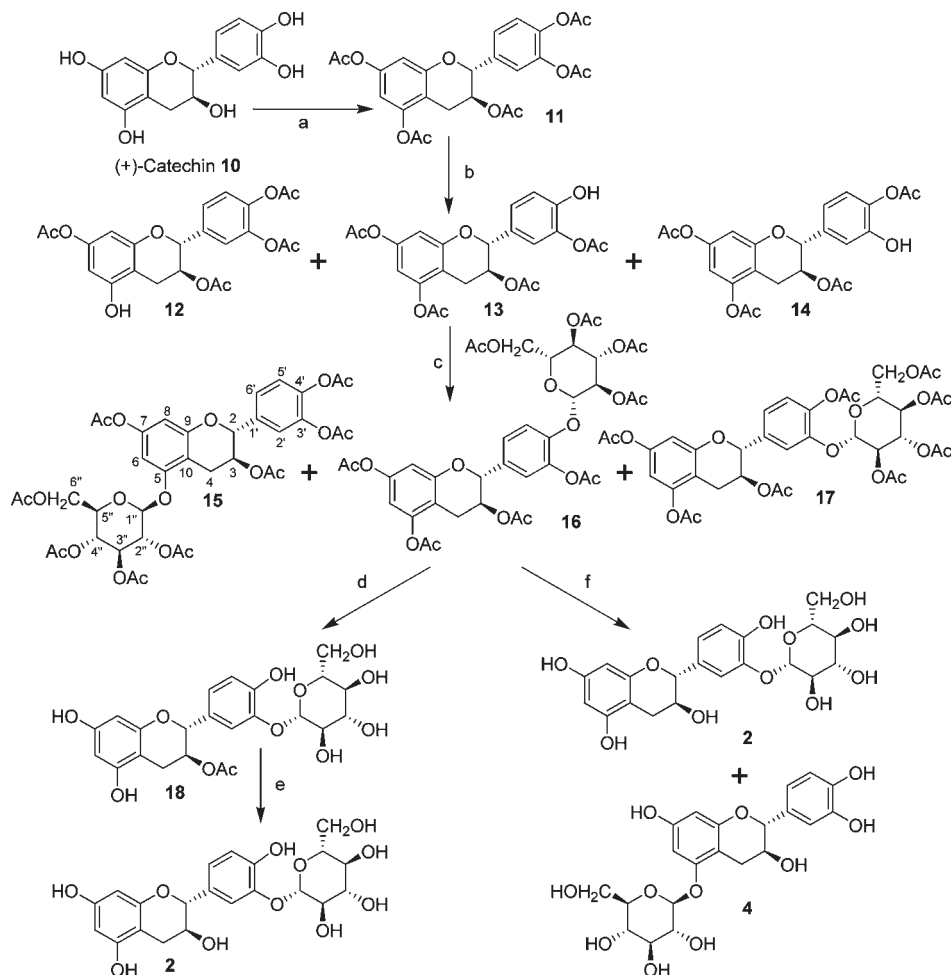
**Figure 4.** Preparation of the glucosylation reagent **9**: (a) tributyltin methoxide, THF, reflux 1.5 h, 94%; (b)  $K_2CO_3$ , tetrabutylammonium bromide, compound **6**,  $CH_2Cl_2$ ,  $H_2O$ , 2 h at rt, 87%.

**Chemical Synthesis of Catechin Glucosides.** With the exception of C3G, none of the other catechin glucosides have been chemically synthesized before. Glucosylation of catechin was performed using a trifluoroacetimidoyl glucosyl donor (40), i.e. 2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl-(*N*-phenyl)-2,2,2-trifluoroacetimidate **9** (Figure 4). The synthesis of the glucosyl donor **9** involved on one hand the preparation of *N*-phenyl-2,2,2-trifluoroacetimidoyl chloride **6**, and on the other the preparation of a 1-hydroxyglucose protected with acetate groups (Figure 4, compound **8**). Compound **6** was prepared from trifluoroacetic acid, aniline and carbon tetrachloride in the presence of triphenylphosphine and triethylamine, according to an established procedure (35). 2,3,4,6-Tetra-*O*-acetyl-D-glucose **8** was synthesized by deacetylation of 1,2,3,4,6-penta-*O*-acetyl- $\beta$ -D-glucose **7** with tributyltin methoxide. Finally compound **9** was synthesized by the base-catalyzed reaction of **6** and **8**.

A direct glucosylation of (+)-catechin was impossible since the compound was not soluble in dichloromethane, the usual solvent of the glucosylation reaction. In addition, assuming that some glucosylated compounds could be produced, it was unlikely that the reaction would have stopped at monoglucosylation. Thus glucosylation of catechin required access to a panel of catechin derivatives protected on all hydroxyl groups, except the one to be glucosylated. This approach would involve a total synthesis of the catechin skeleton. However, a number of studies have demonstrated the feasibility of the partial and regioselective deacetylation of fully acetylated polyphenols under various conditions (40). In fact, the removal of acetate groups from quercetin pentaacetate followed the order of susceptibility of the corresponding acetate toward nucleophilic removal, i.e.  $7 > 4' > 3 > 3' > 5$ . This also

followed the order of acidity of the phenolic hydroxyls. Such an approach, applied to polyacetylated catechin, was expected to have little regioselectivity, due to the similar acidity of its four phenolic hydroxyl groups (41). Nevertheless, we decided to explore this method as a possible route to a mixture of mono-deacetylated catechin derivatives. Thus polyacetylated catechin **11** was submitted to deacetylation in the presence of thiophenol (Figure 5). The reaction was, as expected, not regioselective. However, after 6 h of reaction at rt, the pool of mono-deacetylated derivatives **12–14** amounted to 50% of the reaction products, while some 3,5,7,3',4'-penta-*O*-acetylcatechin **11** remained unreacted, and a number of higher deacetylated compounds were formed. Reducing the reaction time to 1 h efficiently reduced the amount of higher deacetylated derivatives. However, this time, unreacted 3,5,7,3',4'-penta-*O*-acetylcatechin **11** was by far the major constituent of the medium, and the amount of mono-deacetylated compounds that we could isolate was less than 9% (results not shown). Therefore the latter conditions were not considered suitable. Purification of the reaction medium by MPLC succeeded in the separation of the fraction of mono-deacetylated products from other contaminants. However the purification of individual mono-deacetylated catechins was difficult and time-consuming.

Therefore we decided to directly perform the two next glucosylation and deprotection steps (Figure 5) on the mixture of mono-deacetylated catechins **12–14**. While the glucosylation step, giving rise to the acetylated glucosides **15–17**, proceeded smoothly, the deprotection of the latter compounds was more difficult. In a first trial, we attempted to deacetylate the mixture of compounds **15–17** using dilute sodium hydroxide in aqueous



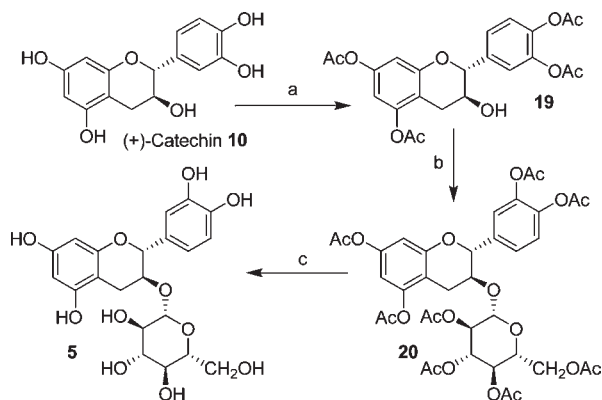
**Figure 5.** Preparation of compound 2 (C3'G), and compound 4 (C3G): (a)  $\text{Ac}_2\text{O}$ , pyridine, 6 days at rt, 99%; (b) imidazole, thiophenol, NMP, 6 h at rt; (c) mixture of compounds 12–14, compound 9,  $\text{BF}_3$  etherate, dry  $\text{CH}_2\text{Cl}_2$ , 16 h at rt; (d) mixture of compounds 15–17, aqueous NaOH + MeOH, 5 min under argon atmosphere at  $0^\circ\text{C}$ ; (e) tannase, 0.1 M sodium acetate buffer pH 5; (f) mixture of compounds 15–17, NaOMe, dry MeOH + dry  $\text{CH}_2\text{Cl}_2$ , 4.5 h at  $0^\circ$  and argon atmosphere.

methanol under argon atmosphere. This resulted in an extensive degradation of the products, and only 3-*O*-acetylcatechin 3'-*O*-glucoside **18** could be isolated from this experiment, albeit in extremely low yield. In the HMBC spectrum of compound **18**, a correlation was observed between H-1'' at 4.75 ppm and the carbon signal at 146.52 ppm, assignable to either C-3' or C-4'. The final demonstration of the position of glucosylation was obtained by means of a NOE-difference experiment. In fact, irradiation of H-1'' at 4.75 ppm induced a strong NOE effect on the signal of H-2' at 7.14 ppm. This unequivocally identified compound **18** as 3-*O*-acetylcatechin 3'-*O*- $\beta$ -D-glucoside. This experiment demonstrated that the 3-*O*-protected catechin derivatives were more stable in aqueous alkaline conditions, suggesting that the presence of a free 3-hydroxyl group favored the degradation of other compounds. We subsequently explored the possibility to prepare compound 2 (C3'G) from its 3-*O*-acetyl analogue **18**. An alkaline hydrolysis of the 3-acetate was of course out of question, considering the above-mentioned stability problems, despite the fact that the production of catechin from 3,5,7,3',4'-penta-*O*-acetylcatechin had been previously carried out using an aqueous solution of sodium hydroxide (42). Furthermore, the 3-*O*-acetyl group is resistant to various chemical and enzymatic conditions. Indeed, our treatment of 3,5,7,3',4'-penta-*O*-acetylcatechin with sodium methoxide for 0.5 h at  $0^\circ\text{C}$  resulted in the formation of 3-*O*-acetylcatechin as sole product (results not shown). On the other hand, although enzymatic hydrolyses of the 3-acetate using

either pig liver esterase (43) or lipase (37) has been reported to be unsuccessful, no data was available so far concerning the use of tannase. Tannase cleaves the 3-*O*-gallate ester bond in compounds such as epigallocatechin gallate, one of the major constituents of green tea extracts. In fact, on incubation of compound **18** with tannase, compound 2 (C3'G) was formed in fair 67% yield.

The deprotection of acetylated glucosides **15**–**17** was also achieved using methanolic sodium methoxide for 4.5 h. This resulted in complete deprotection, including hydrolysis of the 3-acetyl group, and the already isolated compound 2 (C3'G), as well as the new compound 4 (C5G), could be isolated in pure form from the mixture. In the HMBC spectrum of compound 4 (C5G), no correlation could be seen between the anomeric H-1'' sugar proton and one of the carbons of the catechin ring. Therefore, the position of attachment of the glucose was again demonstrated by means of a NOE-difference experiment. In fact, irradiation of H-1'' at 4.85 ppm only produced a significant NOE effect on the signal at 6.26 ppm, assignable to H-6. This unequivocally demonstrated that 4 was the 5-*O*-glucoside of (+)-catechin. Indeed, in the case of 7-*O*-glucosylation, both the signals of H-6 and H-8 would have been affected. Furthermore, the  $^{13}\text{C}$  NMR of 4 was similar to published data for (+)-catechin 5-*O*- $\beta$ -D-glucoside in  $\text{DMSO}-d_6 + \text{D}_2\text{O}$  (Table 3), taking into account a difference of about 2 ppm due to the solvent effect (MeOH- $d_4$  vs  $\text{DMSO}-d_6$ ). However, in the HSQC spectrum of compound 4



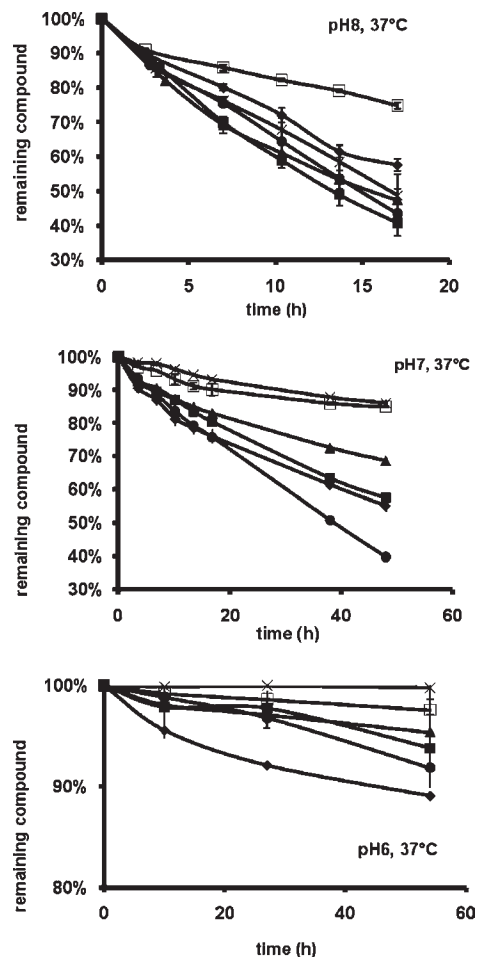


**Figure 6.** Preparation of compound 5 (C3G): (a) triethylamine, acetyl chloride, DMF, 1 h at rt, 35%; (b) compound 9,  $\text{BF}_3$  etherate, dry  $\text{CH}_2\text{Cl}_2$ , 16 h at rt, 49%; (c) NaOMe, dry MeOH + dry  $\text{CH}_2\text{Cl}_2$ , 4.5 h at  $0^\circ$  and argon atmosphere, 93%.

(C5G), of the two 102.56 and 103.41  $^{13}\text{C}$  signals, only that at 102.56 ppm was correlated to a proton signal. This unambiguously assigned the 102.56 ppm peak to C-1'' (CH), and its neighboring 103.41 signal to C-10 (quaternary). Thus the assignments of C-10 and C-1'' of the previously published spectrum in  $\text{DMSO}-d_6 + \text{D}_2\text{O}$  (25) must be reversed (Table 3). Similarly, for the HMBC spectrum of compound 4 (C5G), a  $^3J$  correlation was visible between H-2 at 4.59 ppm and the  $^{13}\text{C}$  signal at 156.71 ppm, assigning the latter to C-9. Thus the assignments of C-7 and C-9 of the previously published spectrum in  $\text{DMSO}-d_6 + \text{D}_2\text{O}$  (25) needed to be exchanged as well (Table 3). Finally both the  $^1\text{H}$  and the  $^{13}\text{C}$  NMR data of compound 4 (C5G) in  $\text{MeOH}-d_4$  were identical to previously published data for (+)-catechin 5-*O*- $\beta$ -D-glucoside in the same solvent (Table 3) (33), except that the HSQC and HMBC spectra of 4 demonstrated that the assignments of C-6 and C-8 must be reversed in the previously published spectrum (Table 3) (33).

The synthetic route for the synthesis of catechin 3-*O*- $\beta$ -D-glucoside 5 is shown in Figure 6. In this case the protection of catechin was based on the differences in reactivity between the 3-alcoholic group and all the remaining phenolic groups. Compound 19 has been synthesized previously by a similar method, but only its proton NMR spectrum was published (44). Thus this study reports the first full NMR characterization of compound 19. Lewis acid-catalyzed reaction of compound 19 with the glucosyl donor 9 afforded the acetylated glucoside 20 in 49% yield. Finally alkaline deacetylation of 20 gave rise to compound 5 (C3G) in excellent yield. 3-Glucosylation of both compound 20 and 5 was confirmed by the presence of a strong  $^3J$  correlation between H1'' and C3 in their HMBC 2D NMR spectra. In the HMBC spectrum of compound 5 (C3G), an additional correlation between H3 and C1'' was present, further confirming 3-glucosylation. Furthermore, although recorded in different solvents, the  $^{13}\text{C}$  NMR data of compound 5 (C3G) ( $\text{MeOH}-d_4$ ), and of (+)-catechin 3-*O*- $\beta$ -D-glucoside (acetone- $d_6 + \text{D}_2\text{O}$ ), previously isolated from the leaves *Quercus miyagii* (45), were similar (Table 4). However the analysis of the HSQC and HMBC spectra of compound 5 (C3G) suggested that the assignment of C-6 and C-8 must be exchanged in the previously published spectrum (Table 4).

**Influence of pH on the Stability.** Stabilities of catechin glucosides were tested in a sodium phosphate buffer in the pH range of 4 to 8. In general, the stability of catechins is poor at neutral and slightly basic conditions and increases with decreasing pH. Figure 7 shows the degradation of the catechin glucosides and

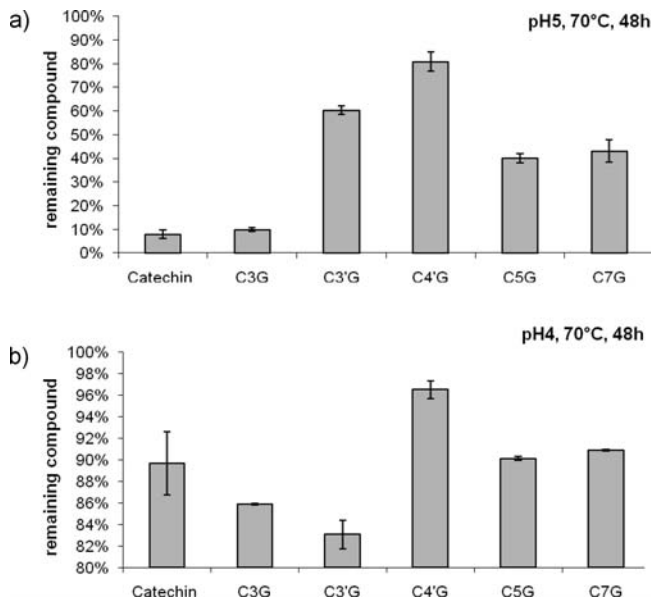


**Figure 7.** Time course of the degradation of C3G (■), C3'G (□), C4'G (×), C5G (▲), C7G (●) in sodium phosphate buffer at pH 8, pH 7 and pH 6 compared to the degradation of catechin (◆).

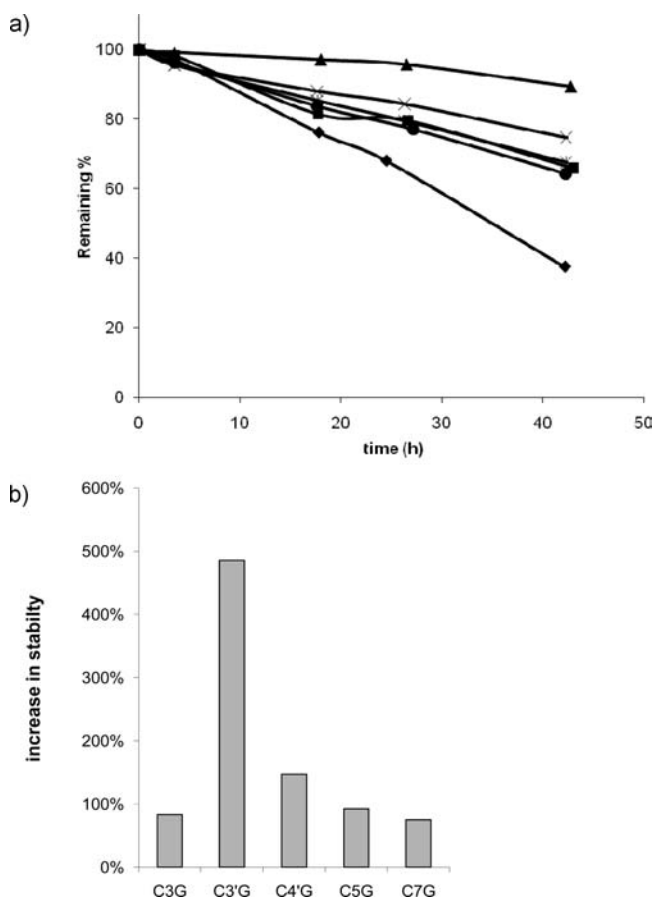
of catechin at pH 6, 7, and 8. At pH 8, a distinct increase in stability of  $\sim 70\%$  was observed for C3'G, while all other isomers were slightly less stable than catechin. At pH 7, the stability was significantly increased by glucosylation of position 3' ( $\sim 200\%$ ) and 4', and to a lesser extent also for position 5. While C7G had the same stability as catechin at this pH, C3G was again less stable. At pH 6, all glucosides showed increased stability when compared to the free form. Again C4'G and C3'G were the two most stable isomers, followed by C5G.

At pH 4 and 5, no substantial decrease could be observed over 48 h at 37 °C even for (+)-catechin. Therefore the temperature was increased from 37 to 70 °C for these pH values. After 48 h at pH 5, only less than 10% of catechin remained while 80% of C4'G and 60% of C3'G could still be detected, respectively (Figure 8a). An increase in stability was also observed for C5G and C7G, whereas the stability of C3G was comparable with that of catechin. At pH 4, C4'G was again the most stable isomer. Surprisingly, C3'G was the least stable form under these conditions. Note however that the overall losses at this pH are relatively small (Figure 8b).

**Stability in a Physiological Buffer.** The stability of the individual catechin glucosides (15  $\mu\text{M}$ ) was tested in the "perfusion buffer" (Figure 9a). For all catechin glucosides, an increase in stability was observed compared to the aglycone. After 42 h incubation,  $>60\%$  of catechin was degraded while  $\sim 90\%$  of the catechin 3'-glucoside was still intact. Figure 9b shows the increase of stability for the individual glucosides compared to the



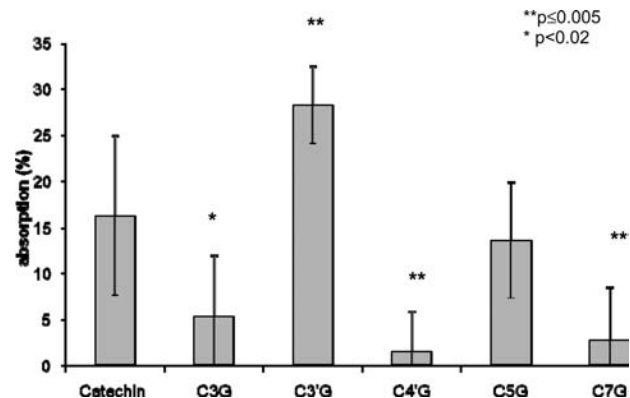
**Figure 8.** Catechin and catechin glucosides remaining after storage in sodium phosphate buffer at pH 5 (a) or pH 4 (b) at 70 °C for 48 h.



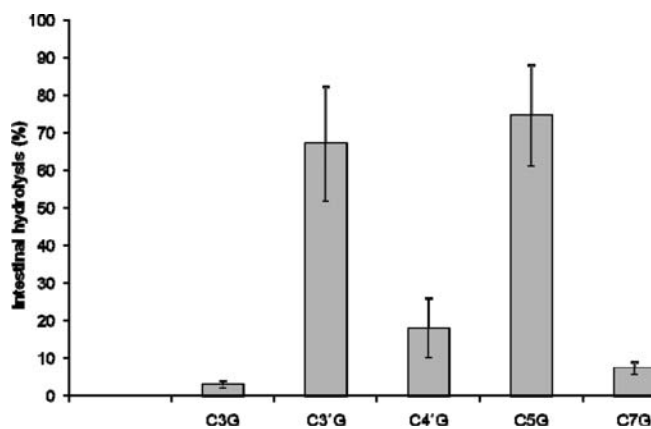
**Figure 9.** (a) Time course of the degradation of C3G (■), C3'G (□), C4'G (×), C5G (▲), C7G (●) compared to the degradation of catechin (◆) in perfusion buffer (pH 7) at 37 °C for 42 h.

(b) Percentage of the increase in stability by glycosylation based on the data at 42 h.

aglycone based on the degradation observed after 42 h. While most glucosides are approximately twice as stable as catechins, there is a 5-fold increase of stability for C3'G. These data show



**Figure 10.** Apparent absorption of catechin and catechin glucosides after intestinal perfusion calculated as the difference between the influent concentration and the sum of catechin and catechin glucoside concentration of the effluent. Under control conditions, all of the compounds were >95% stable.



**Figure 11.** Degree of intestinal hydrolysis of the different catechin glucosides during the perfusion experiment calculated from the catechin/catechin glucoside ratios in the effluent samples as determined by HPLC analysis.

that, during the relatively short time of perfusion, all the compounds tested were >95% stable.

**Intestinal Perfusion of Catechin Glucosides.** The gut lumen metabolism of the catechin glucosides was assessed and compared with that of catechin in a rat intestinal perfusion study. The “absorption” (difference between influent and effluent concentrations) for C3'G (28%) was significantly higher ( $p = 0.005$ ) than for catechin (16%). For C3G, C4'G and C7G, a significant decrease in “absorption” was observed as compared to the aglycone, and the percentage absorption for C5G was unchanged (Figure 10). If the results for “absorption” are compared with the degree of intestinal hydrolysis of the catechin glucosides (Figure 11), there is a direct correlation. The two glucosides (C3'G and C7G) which were intensively hydrolyzed are apparently better “absorbed” than the glucosides which stay intact during intestinal transit. This shows that the intestinal enzyme responsible for the hydrolysis, probably lactase phloridzin hydrolase, seems to be highly regioselective for hydrolysis of the catechin glucosides. This is different from quercetin (12) where the position of sugar attachment did not influence absorption of two quercetin 4- and 3'-glucosides in human volunteers, since the specificity of brush border lactase phlorizin hydrolase is similar for both glucosides (14).

## ABBREVIATIONS USED

Ac, acetyl; Bn, benzyl; BuOH, butanol; CH<sub>2</sub>Cl<sub>2</sub>, dichloromethane; C7G, (+)-catechin 7-O-β-D-glucoside (compound 1); C3'G, (+)-catechin 3'-O-β-D-glucoside (compound 2); C4'G, (+)-catechin 4'-O-β-D-glucoside (compound 3); C5G, (+)-catechin 5-O-β-D-glucoside (compound 4); C3G, (+)-catechin 3-O-β-D-glucoside (compound 5); calcd, calculated; cpd, compound; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DCM, dichloromethane; DMAP, 4-dimethylaminopyridine; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; EDCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; EtOAc, ethyl acetate; HCOOH, formic acid; iPr<sub>2</sub>Net, *N,N*-diisopropylethylamine; MeCN, acetonitrile; Me<sub>2</sub>CO, acetone; NMP, 1-methyl-2-pyrrolidone; NOE, nuclear Overhauser effect; MPLC, medium pressure liquid chromatography; MOM, methoxymethyl; rt, room temperature; THF, tetrahydrofuran; VLC, vacuum liquid chromatography.

## LITERATURE CITED

- Su, Y. L.; Leung, L. K.; Huang, Y.; Chen, Z. Y. Stability of tea theaflavins and catechins. *Food Chem.* **2003**, *83*, 189–195.
- Higdon Jane, V.; Frei, B. Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. *Crit. Rev. Food Sci. Nutr.* **2003**, *43*, 89–143.
- Manach, C.; Williamson, G.; Morand, C.; Scalbert, A.; Remesy, C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am. J. Clin. Nutr.* **2005**, *81*, 230S–242S.
- 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.
- Crespy, V.; Morand, C.; Manach, C.; Besson, C.; Demigne, C.; Remesy, C. Part of quercetin absorbed in the small intestine is conjugated and further secreted in the intestinal lumen. *Am. J. Physiol.* **1999**, *277*, G120–G126.
- Kuhnle, G.; Spencer, J. P.; Schroeter, H.; Shenoy, B.; Debnam, E. S.; Srari, S. K.; Rice-Evans, C.; Hahn, U. Epicatechin and catechin are O-methylated and glucuronidated in the small intestine. *Biochem. Biophys. Res. Commun.* **2000**, *277*, 507–512.
- Yang, C. S.; Chen, L. S.; Lee, M. J.; Balentine, D.; Kuo, M. C.; Schantz, S. P. Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers. *Cancer Epidemiol. Biomarkers. Prev.* **1998**, *7*, 351–354.
- van het Hof, K. H.; Kivits, G. A. A.; Weststrate, J. A.; Tijburg, L. B. M. Bioavailability of catechins from tea: the effect of milk. *Eur. J. Clin. Nutr.* **1998**, *52*, 356–359.
- Chow, H. H.; Cai, Y.; Alberts, D. S.; Hakim, I.; Dorr, R.; Shahi, F.; Crowell, J. A.; Yang, C. S.; Hara, Y. Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatechin gallate and polyphenon E. *Cancer Epidemiol., Biomarkers Prev.* **2001**, *10*, 53–58.
- Zhu, Q. Y.; Holt, R. R.; Lazarus, S. A.; Ensunsa, J. L.; Hammerstone, J. F.; Schmitz, H. H.; Keen, C. L. Stability of the flavan-3-ols epicatechin and catechin and related dimeric procyanidins derived from cocoa. *J. Agric. Food Chem.* **2002**, *50*, 1700–1705.
- Hollman, P. C.; van Trijp, J. M.; Mengelers, M. J.; de Vries, J. H.; Katan, M. B. Bioavailability of the dietary antioxidant flavonol quercetin in man. *Cancer Lett.* **1997**, *114*, 139–140.
- Olthof, M. R.; Hollman, P. C.; Vree, T. B.; Katan, M. B. Bioavailabilities of quercetin-3-glucoside and quercetin-4'-glucoside do not differ in humans. *J. Nutr.* **2000**, *130*, 1200–1203.
- Day, A. J.; DuPont, M. S.; Ridley, S.; Rhodes, M.; Rhodes, M. J. C.; Morgan, M. R. A.; Williamson, G. Deglycosylation of flavonoid and isoflavonoid glycosides by human small intestine and liver β-glucosidase activity. *FEBS Lett.* **1998**, *436*, 71–75.
- Day, A. J.; Canada, F. J.; Diaz, J. C.; Kroon, P. A.; McLauchlan, W. R.; Faulds, C. B.; Plumb, G. W.; Morgan, M. R. A.; Williamson, G. Dietary flavonoid and isoflavone glycosides are hydrolysed by the lactase site of lactase phlorizin hydrolase. *FEBS Lett.* **2000**, *468*, 166–170.
- Miyase, T. Preparation of catechin derivatives with increased stability. JP Patent A2 2001253879.
- Lam, W. H.; Kazi, A.; Kuhn, D. J.; Chow Larry, M. C.; Chan Albert, S. C.; Dou, Q. P.; Chan, T. H. A potential prodrug for a green tea polyphenol proteasome inhibitor: evaluation of the peracetate ester of (–)-epigallocatechin gallate [(–)-EGCG]. *Bioorg. Med. Chem.* **2004**, *12*, 5587–5593.
- Lopez-Toledano, A.; Mayen, M.; Merida, J.; Medina, M. Yeast-induced inhibition of (+)-catechin and (–)-epicatechin degradation in model solutions. *J. Agric. Food Chem.* **2002**, *50*, 1631–1635.
- Kim, H. J.; Yeom, S. H.; Kim, M. K.; Shim, J. G.; Lim, H. W.; Lee, M. W. Nitric oxide and prostaglandin E2 synthesis inhibitory activities of flavonoids from the barks of *Ulmus macrocarpa*. *Nat. Prod. Sci.* **2004**, *10*, 344–346.
- Friedrich, W.; Galensa, R. Identification of a new flavanol glucoside from barley (*Hordeum vulgare* L.) and malt. *Eur. Food Res. Technol.* **2002**, *214*, 388–393.
- Tanaka, T.; Kataoka, M.; Tsuboi, N.; Kouno, I. New monoterpene glycoside esters and phenolic constituents of *Paoniae Radix*, and increase of water solubility of proanthocyanidins in the presence of paeoniflorin. *Chem. Pharm. Bull.* **2000**, *48*, 201–207.
- Watanabe, M. Catechins as antioxidants from Buckwheat (*Fagopyrum Esculentum* Moench) groats. *J. Agric. Food Chem.* **1998**, *46*, 839–845.
- Lee, M. W.; Tanaka, T.; Nonaka, G. I.; Hahn, D. R. Phenolic compounds of the leaves of *Betula platyphylla* var. *latifolia*. *Arch. Pharm. Res.* **1992**, *15*, 211–214.
- Foo, L. Y.; Karchesy, J. J. Polyphenolic glycosides from Douglas fir inner bark. *Phytochemistry* **1989**, *28*, 1237–1240.
- Kashiwada, Y.; Nonaka, G.; Nishioka, I. Tannins and related compounds. XLV. Rhubarb. (5). Isolation and characterization of flavan-3-ol and procyanidin glucosides. *Chem. Pharm. Bull.* **1986**, *34*, 3208–3222.
- Nonaka, G. I.; Ezaki, E.; Hayashi, K.; Nishioka, I. Tannins and related compounds. Part 11. Flavanol glucosides from rhubarb and *Rhaphiolepis umbellata*. *Phytochemistry* **1983**, *22*, 1659–1661.
- Nonaka, G.; Nishizawa, M.; Yamagishi, T. Chemical components of rhubarb (Oriental drug). *Wakan Iyaku Gakkaishi* **1985**, *2*, 89–92.
- Kitagawa, I.; Wang, H. K.; Saito, M.; Yoshikawa, M. Saponin and sapogenol. XXXI. Chemical constituents of the seeds of *Vigna angularis* (Willd.) Ohwi et Ohashi. (1). Triterpenoidal sapogenols and 3-furanmethanol β-D-glucopyranoside. *Chem. Pharm. Bull.* **1983**, *31*, 664–673.
- Takani, M.; Ohya, K.; Takahashi, K. Studies on constituents of medicinal plants. XXII. Constituents of *Schizandra nigra* Max. (4). *Chem. Pharm. Bull.* **1979**, *27*, 1422–1425.
- Pan, H.; Lundgren, L. N. Phenolics from inner bark of *Pinus sylvestris*. *Phytochemistry* **1996**, *42*, 1185–1189.
- Bae, Y. S.; Burger, J. F. W.; Steynberg, J. P.; Ferreira, D.; Hemingway, R. W. Flavan and procyanidin glycosides from the bark of blackjack oak. *Phytochemistry* **1994**, *35*, 473–478.
- Duenas, M.; Sun, B.; Hernandez, T.; Estrella, I.; Spranger, M. I. Proanthocyanidin composition in the seed coat of lentils. (*Lens culinaris* L.). *J. Agric. Food Chem.* **2003**, *51*, 7999–8004.
- Funayama, M.; Nishino, T.; Hirota, A.; Murao, S.; Takenishi, S.; Nakano, H. Enzymic synthesis of (+)-catechin-a-glucoside and its effect on tyrosinase activity. *Biosci., Biotechnol. Biochem.* **1993**, *57*, 1666–1669.
- Otani, S.; Kondo, Y.; Asada, Y.; Furuya, T.; Hamada, H.; Nakajima, N.; Ishihara, K.; Hamada, H. Biotransformation of (+)-catechin by plant cultured cells of *Eucalyptus perriniana*. *Plant Biotechnol.* **2004**, *21*, 407–409.
- Weinges, K.; Seiler, D. Phenolic natural substances. IX. Diastereomeric catechin 3-glucosides and 3-gallates. *Justus Liebigs Ann. Chem.* **1968**, *714*, 193–204.
- Tamura, K.; Mizukami, H.; Maeda, K.; Watanabe, H.; Uneyama, K. One-pot synthesis of trifluoroacetimidoyl halides. *J. Org. Chem.* **1993**, *58*, 32–35.
- Nudelman, A.; Herzig, J.; Gottlieb, H. E.; Keinan, E.; Sterling, J. Studies in sugar chemistry. Part IV. Selective deacetylation of

- anomeric sugar acetates with tin alkoxides. *Carbohydr. Res.* **1987**, *162*, 145–152.
- (37) Lambusta, D.; Nicolosi, G.; Patti, A.; Piatelli, M. Enzyme-mediated regioprotection-deprotection of hydroxyl groups in (+)-catechin. *Synthesis* **1993**, *11*, 1155–1158.
- (38) Donovan, J. L.; Crespy, V.; Oliveria, M.; Cooper, K. A.; Gibson, B. B.; Williamson, G. (+)-catechin is more bioavailable than (-)-catechin: Relevance to the bioavailability of catechin from cocoa. *Free Radical Res.* **2006**, *40*, 1029–1034.
- (39) Pan, C. Y.; Kao, Y. H.; Fox, A. P. Enhancement of inward Ca(2+) currents in bovine chromaffin cells by green tea polyphenol extracts. *Neurochem. Int.* **2002**, *40*, 131–137.
- (40) Li, M.; Han, X. W.; Yu, B. Facile synthesis of flavonoid 7-O-glycosides. *J. Org. Chem.* **2003**, *68*, 6842–6845.
- (41) Cren-Olive, C.; Wieruszski, J. M.; Maes, E.; Rolando, C. Catechin and epicatechin deprotonation followed by <sup>13</sup>C NMR. *Tetrahedron Lett.* **2002**, *43*, 4545–4549.
- (42) Chattopadhyay, S. K.; Banerjee, S.; Agarwal, S.; Sashidhara, K. V.; Tripathi, V.; Kukreja, A. K.; Kumar, S.; Kulshrestha, M.; Sharma, R. P.; Mehta, V. K. Process for the production of a compound (+) catechin pentaacetate useful as a precursor for the production of (+) catechin. US Patent 6,365,757, March 28, 2000.
- (43) Basak, A.; Mandal, S.; Bandyopadhyay, S. Regioselective hydrolysis of pentaacetyl catechin and epicatechin by porcine liver esterase. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1083–1085.
- (44) Patti, A.; Piattelli, M.; Nicolosi, G. Use of *Mucor miehei* lipase in the preparation of long chain 3-O-acylcatechins. *J. Mol. Catal. B: Enzym.* **2000**, *10*, 577–582.
- (45) Ishimaru, K.; Nonaka, G.; Nishioka, I. Tannins and related compounds. Part 53. Flavan-3-ol and procyanidin glycosides from *Quercus miyagii*. *Phytochemistry* **1987**, *26*, 1167–1170.

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