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THE SYNTHESIS OF METHYLATED EPIGALLOCATECHIN GALLATE

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*Synthesis of methylated epigallocatechin gallate from (–)-epigallocatechin gallate and propylgallate was accomplished using a benzyl (Bn) group as a protecting group for phenols. This methodology provided (–)-epigallocatechin-3-(4-*O*-methylgallate), which are naturally scarce catechin derivatives.*

Keywords: methylated epigallocatechin gallate, (–)-epigallocatechin gallate, chemical modification.

Tea is probably one of the most widely drunk beverages because of its various beneficial constituents. Several epidemiological studies [1] have indicated that long-term tea-drinking can reduce the risk of several types of cancers. Tea polyphenols possess a wide variety of functions, including anti-allergic, antioxidative, antimutagenic/anticarcinogenic, antiatherosclerotic, and antibacterial effects. These effects are particularly attributed to the catechins, a group of 3-flavanols and their derivatives. However, catechins are unstable in physiological environment and thus are difficultly absorbed by organisms because of the richness of OH′s [2, 3]. It is necessary to chemically modify the natural catechins for enhancing their activities and functions *in vivo*.

Several studies have demonstrated that (–)-epigallocatechin gallate (**1**, EGCG), the most abundant catechin accounting for 50–80% of the total catechins in tea, could inhibit type I allergic reactions in rats [3, 4]. It has been reported that (–)-epigallocatechin-3-*O*-(3-*O*-methyl) gallate (EGCG3′′Me) and (–)-epigallocatechin-3-*O*-(4-*O*-methyl) gallate (EGCG4′′Me) have more significant inhibitory effects than EGCG [5, 6]. Suzuki et al. also reported that the inhibitory activities of EGCG3′′Me and EGCG4′′Me on mouse type IV allergy were higher than that of EGCG at a dose of 0.05 mg/wear [3]. The higher inhibitory activities of EGCG3′′Me and EGCG4′′Me on mouse allergies are thought to be associated with the stability of the *O*-methylated derivatives of EGCG in animal bodies. Pharmacological studies have shown that methylation of EGCG strongly inhibited histamine release for mast cells, which helps to relieve the symptoms caused by allergic response [2, 7].

The synthesis of methylated EGCG has been investigated lately. Through the method of total synthesis, Zaveri obtained 3′′,4′′,5′′-trimethylated EGCG [8]. Using EGCG and methyl iodide as the precursors and K_2CO_3 as the catalyst, Meng et al. [9] synthesized 4′′-MeEGCG, 4′,4′′-DiMeEGCG, and 4′,3′′,4′′-trimethylated EGCG. Various methylated isomers of epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) have been synthesized by [10]. Using nitrophenylsulfonyl (NS) as the protector of the OH-group, with EGC and gallic acid (GA) as precursors, Aihara et al. [11] synthesized the methylated EGCG.

In our previous study, EGCG was purified from tea polyphenols [12]. In this paper, we reported a concise and efficient method for chemical synthesis of 4′′-Me-EGCG by using **1** and propyl gallate (PG) as the precursors.

As a novel protecting and activating group for amines, a benzyl group such as BnBr has been employed as a protecting group for phenol [13].

Compound **8** is synthesized from the natural (–)-EGCG, which is commercially available. The synthetic conditions for obtaining benzylated EGCG with BnBr–dimethylformamide (DMF) at different temperatures were tested. Finally, the conditions of BnBr–K_2CO_3–DMF–rt/N_2 were found to be the optimal.

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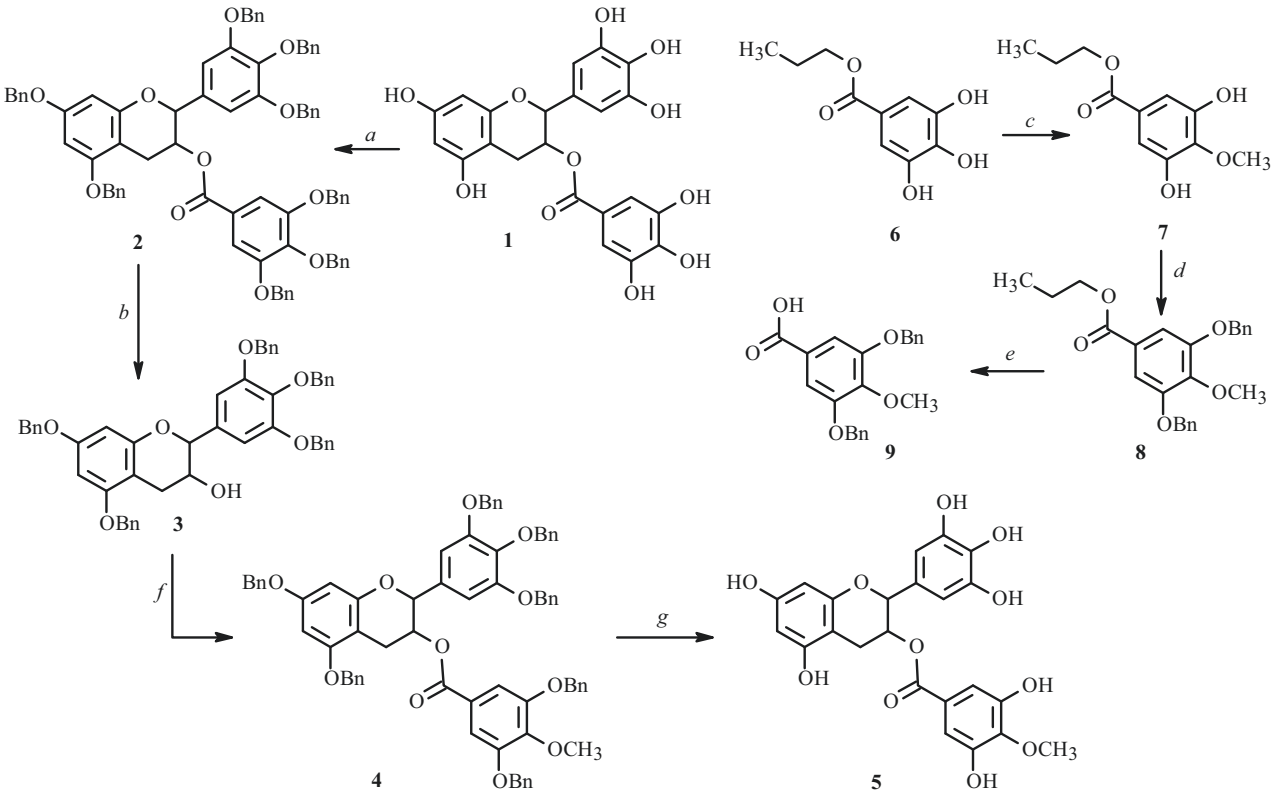

a. BnBr–K_2CO_3–DMF, r.t.; *b*. NaOH–THF–MeOH, reflux; *c*. Li_2CO_3–methyl iodide–DMF, 50°C; *d*. BnBr–K_2CO_3–DMF, r.t.; *e*. NaOH–THF–MeOH, r.t.; *f*. **9**, oxalyl chloride–CH_2Cl_2–DMAP–H_2O, reflux–r.t.; *g*. $HCOONH_4$–20% $Pd(OH)_2$– C –MeOH–THF, r.t.

Scheme 1

The benzylated derivative **2** with over 50% yield could be obtained after purification. Compound **2** was hydrolyzed in the presence of sodium hydroxide at reflux and THF–methanol solution (v/v, 1:1) (Scheme 1). Compound **3** was generated by hydrolysis of compound **2**, which was obtained in 87.6% yield.

The methyl group was selectively incorporated into 4-OH of compound **6** with Li_2CO_3 and methyl iodide, the phenolic hydroxyl group in the C-4 position of compound **6**, which is the most active hydroxyl group, was deprotonated. Finally compound **7** was obtained by alkylation reaction of compound **6**.

Compound **8** was derived from benzylation of compound **7** in the presence of BnBr, K_2CO_3, and DMF. The hydrolytic reaction of compound **8** with sodium hydroxide and methanol as catalysts at room temperature gave compound **9**.

Compound **4** was obtained by esterification reaction of compound **3**, compound **9**, and oxalyl chloride. Hydrogenolysis of compound **4** afforded the methylated compound **5** in 19% yield. The spectroscopic data of compound **5** are in agreement with those reported previously for the metabolites.

We have successfully synthesized 4″-Me-EGCG and developed a concise and efficient synthesis method to obtain methylated EGCG.

EXPERIMENTAL

General Materials and Reagents. EGCG was purified from tea via a flash chromatography system by our group. Other starting materials and reagents purchased commercially were used without further purification. Anhydrous methylene chloride was stirred with $CaCl_2$ for 10 h. Twenty-four hours after being refluxed with $CaSO_4$, anhydrous DMF was distilled under vacuum. The reaction flasks were dried under 120°C. All moisture-sensitive reactions were conducted under a nitrogen atmosphere. Flash chromatography was carried out using silica gel GF_{254}. When $CDCl_3$ and MeOD were used as solvents, ^1H NMR (600 MHz) spectra were measured with TMS as the internal standard.

(–)-(2*R*,3*R*)-5,7-Bis(benzyloxy)-2-(3,4,5-tris(benzyloxy)phenyl)chroman-3-yl-(3,4,5-trisbenzyloxy)benzoate (2). To a solution of **1** (100 mg, 0.22 mmol) in dry DMF (10.0 mL) under nitrogen at room temperature, K_2CO_3 (250 mg, 1.8 mmol) was added; after stirring for 2 h, benzyl bromide (0.25 mL, 2.1 mmol) was added. The mixture was stirred for another 60 h at room temperature. The reaction was quenched by adding hydrochloric acid (2 mol/L, 10.00 mL). The aqueous layer was extracted with EtOAc. The organic extracts were combined, washed with water, and then evaporated *in vacuum*. The residue was purified by flash SiO_2 column chromatography (petroleum ether–EtOAc, 4:1) to afford the crude benzylated EGCG (140 mg) as a yellow oil in 54.5%yield. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.08 (1H, d), 3.12 (1H, d), 4.70 (2H, d, J = 0.04), 4.90–5.06 (16H, m), 6.34 (1H, d), 6.40 (1H, d), 6.72 (2H, s), 7.21–7.38 (42H, m).

(*L*)-(2*R*,3*R*)-5,7-Bis(benzyloxy)-2-(3,4,5-tris(benzyloxy)phenyl)chroman-3-ol (3). Benzylated EGCG (**2**) (100 mg, 0.08 mmol) was dissolved in THF–MeOH (1:1, 10 mL); drops of water and sodium hydroxide (20.00 mg, 0.5 mmol) were added. The mixture was refluxed at 65°C for 20 h and extracted with EtOAc three times. The organic layers were combined and evaporated *in vacuum*. The residue was purified by flash SiO_2 column chromatography (petroleum ether–EtOAc, 4:1) to afford the product (56.3 mg) as a pale yellow oil in 87.6% yield. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.08 (1H, d), 3.12 (1H, d), 4.70 (2H, d, J = 0.04), 5.02–5.08 (10H, m), 6.36 (1H, d), 6.42 (1H, d), 6.72 (2H, s), 7.25–7.34 (25H, m).

4-Methoxypropylgallate (7). To a mixture of propyl gallate (**6**, 100 mg, 0.43 mmol) in dry DMF (10 mL), Li_2CO_3 (200 mg, 2.7 mmol) was added under nitrogen. After stirring for 30 min at 50°C, methyl iodide (0.05 mL, 0.65 mmol) was added drop-wise, and the mixture was stirred overnight. Hydrochloric acid (2 mol/L, 10.00 mL) was then added to quench the reaction. The mixture was extracted with EtOAc. The organic layers was washed with H_2O, then dried over sodium sulfate (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by flash SiO_2 column chromatography (petroleum ether–EtOAc, 2:1) to afford a white solid (88 mg) in 83.0% yield. ^{1}H NMR (600 MHz, MeOD-d_4, δ, ppm): 1.0 (3H, t), 1.6–1.8 (2H, m), 3.9 (3H, s), 4.2 (2H, t), and 7.3 (2H, s).

3,5-Bis(benzyloxy)-4-methoxypropylgallate (8). To a solution of **7** (100 mg, 0.45 mmol) in dry DMF (10.0 mL) under nitrogen at room temperature, K_2CO_3 (250 mg, 1.8 mmol) was added. After the mixture was stirred for 2 h, benzyl bromide (0.15 mL, 1.26 mmol) was added. Stirring was continued for 20 h at room temperature.

The reaction was quenched by adding hydrochloric acid (2 mol/L, 10.00 mL). The aqueous phase was extracted with EtOAc. The organic layers were combined and washed with water, then dried over sodium sulfate (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by flash SiO_2 column chromatography (petroleum ether–EtOAc, 5:1) to afford the crude benzylated **8** (170 mg) as a white solid in 96.6% yield. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm): 1.0 (3H, t), 1.7–1.9 (2H, m), 3.9 (3H, s), 4.2 (2H, t), 5.2 (4H, s), 7.2–7.6 (12H, m).

3,5-Bis(benzyloxy)-4-methoxygallic Acid (9). To a solution of benzylated Me-PG (100 mg, 0.25 mmol) in THF–MeOH (1:1, 10 mL), drops of water and sodium hydroxide (20.00 mg, 0.5 mmol) were added. The mixture was refluxed at 65°C for 20 h, then extracted with EtOAc three times. The organic layers were combined and dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by flash SiO_2 column chromatography (petroleum ether–EtOAc, 4:1) to afford a white solid (58.33 mg) in 65.0% yield. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm): 3.9 (3H, s), 5.2 (4H, s), 7.2–7.6 (12H, m).

(–)-(2*R*,3*R*)-5,7-Bis(benzyloxy)-2-(3,4,5-tris(benzyloxy)phenyl)chroman-3-yl-(3,5-bisbenzyloxy-4-methoxy)benzoate (4). To a solution of **9** (50 mg, 0.14 mmol) in dry CH_2Cl_2 (10.00 mL), oxalyl chloride (1.50 mL, 14.9 mmol) was added. After refluxing for 3 h, the reaction mixture was evaporated and dried completely *in vacuo* for 2 h. After the obtained white solid was cooled to 0°C, a solution of 4-dimethylaminopyridine (DMAP, 15 mg, 0.12 mmol) and (–)-(2*R*,3*R*)-5,7-bis(benzyloxy)-2-(3,4,5-tris(benzyloxy)phenyl)chroman-3-ol (**3**, 100 mg, 0.13 mmol) in dry CH_2Cl_2 (10.00 mL) was added dropwise at 0°C. Then the mixture was stirred at room temperature overnight until thin-layer chromatography (TLC) showed that the reaction was completed. The solvent was evaporated *in vacuo*, and the resulting oil was purified by flash SiO_2 column chromatography (petroleum ether–EtOAc, 3:1) to get a pale yellow amorphous solid (**4**, 20 mg) in 21.4% yield. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm): 3.06 (1H, d), 3.08 (1H, d), 4.09 (3H, s), 4.87 (2H, s, J = 0.04), 4.98–5.20 (14H, m), 6.30 (1H, s), 6.52 (1H, s), 6.76 (2H, s), 7.26–7.46 (37H, m).

(–)-(2*R*,3*R*)-5,7-Bis(hydroxy)-2-(3,4,5-tris(hydroxy)phenyl)chroman-3-yl-(3,5-bisbenzyloxy-4-methoxy)benzoate (5). $Pd(OH)_2$–C (20%, 20 mg) and formic acid (1 mL, 26.7 mmol) were added to a mixture of THF–MeOH (1:1 v/v, 25 mL) in which **4** (100 mg, 0.09 mmol) was dissolved. The resulting reaction mixture was stirred at room temperature under nitrogen until TLC showed that the reaction was completed. The reaction mixture was filtered to remove the catalyst. The filtrate was evaporated, and the residue was rapidly purified by flash chromatography on silica gel (MeOH–CH_2Cl_3, 3:1) to afford a pale yellow amorphous solid **5** (8 mg) in 19.9% yield. ^{1}H NMR (600 MHz, MeOD-d_4, δ, ppm): 3.03 (2H, s), 3.85–3.92 (3H, m), 5.20 (1H, s), 5.51 (1H, s), 7.0–7.6 (6H, m).

REFERENCES

1. M. J. Shrubsole, W. Lu, Z. Chen, X. O. Shu, Y. Zheng, Q. Dai, Q Cai, K. Gu, Z. X. Ruan, Y. T. Gao, and W. Zheng, *J. Nutr*., **139** (2), 310 (2009).
2. Y. M. Maeda, K. Ema, and I. Shibuichi, *Cytotechnology*, **55** (2), 135 (2007).
3. M. Suzuki, K. Yoshino, Y. M. Maeda, T. Miyase, and M. Sano, *J. Agric. Food Chem*., **48** (11), 5649 (2000).
4. T. Shiozaki, K. Sugiyama, K. Nakazato, and T. Takeo, *J. Pharm. Soc. Jpn*., **117** (7), 448 (1997).
5. M. Sano, M. Suzuki, T. Miyase, K. Yoshino, and Y. M. Maeda, *J. Agric. Food Chem*., **47** (5), 1906 (1999).
6. M. Kirita, D. Honma, Y. Tanaka, S. Usut, T. Shoji, M. Sami, T. Yokota, M. Tagashira, A. Muranaka, M. Uchiyama, T. Kanda, and Y. M. Maeda, *J. Agric. Food Chem*., **58** (12), 7196 (2010).
7. S. Yano, Y. Fujimura, D. Umeda, T. Miyase, K. Yamada, and H. Tachibana, *J. Agric. Food Chem*., **55** (17), 7144 (2007).
8. N. T. Zaveri, *Org. Lett*., **3** (6), 843 (2001).
9. X. Meng, S. Sang, N. Zhu, H. Lu, S. Sheng, M. J. Iee, C. T. Ho, and C. S. Yang, *Chem. Res. Toxicol*., **15** (8), 1042 (2002).
10. S. B. Wan, P. D. Qiu, and T. H. Chan, *Tetrahedron*, **62** (25), 5897 (2006).
11. Y. Aihara, A. Yoshida, T. Furuta, T. Wakimoto, T. Akizawa, M. Konishi, and T. Kan, *Bioorg. Med. Chem. Lett*., **19** (15), 4171 (2009).
12. R. H. Lai, X. F. Lai, W. F. Zhao, and Y. H. Huang, *Adv. Mater. Res*. (EI), 311–313, 2114–2120 (2011).
13. K. Osanai, C. Huo, K. R. Landis-Piwowar, P. D. Qiu, and T. H. Chan, *Tetrahedron*, **63** (32), 7565 (2007).