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Role of 3,5-Digalloyl and 3',4'-Dihydroxyl Structure of (−)-Epicatechin-3,5-Digallate in Inhibition of Hela S3 Cell Proliferation

Taichi Hojima[1], Shingo Komeda[1], Yusuke Higashino[1], Masahiro Hamada[2], Noriyuki Nakajima[2], Takashi Kawasaki[3] and Akiko Saito[1]*

[1]*Graduate School of Engineering, Osaka Electro-Communication University (OECU), 18-8 Hatsu-cho, Neyagawa-shi, Osaka 572-8530, Japan*
[2]*Biotechnology Research Center and Department of Biotechnology, Toyama Prefectural University, 5180, Kurokawa, Imizu, Toyama 939-0398, Japan*
[3]*Department of Pharmaceutical Sciences, Ritsumeikan University, 1-1-1 Nojihigashi, Kusatsu, Shiga 525-8577, Japan*

Abstract

Flavan-3-ol derivatives are common plant-derived bioactive compounds with strong various biological activities. In particular, (–)-epigallocatechin-3-O-gallate exhibits a variety of moderate biological activities without severe toxicity. Its health-promoting effects have been widely studied because it is a main ingredient in green tea and is commercially available at low cost. In general, the galloyl moieties of flavan-3-ol derivatives are thought to be essential to their strong biological activities. However, it is not clear which position is most effective for modification with the galloyl moiety to strengthen the biological activities because various galloylated analogs are difficult to obtain in pure form in a quantity large enough for assays. Therefore, we synthesized various galloylated flavan-3-ol derivatives in a stereoselective and regioselective manner. We reported in a previous paper that 3,5-digalloyl-(–)-epicatechin displayed much stronger inhibitory activity than 3,5-digalloyl-(+)-catechin did against HeLa S3 cell proliferation. In this study, we describe synthetic studies of 7-galloyl- and 3,7-digalloyl-modified (–)-epicatechin and (+)-catechin and compared their biological activities, HeLa S3 cell proliferation inhibitory activity and DPPH radical scavenging activity with those of 3,5- digalloyl- (–)-epicatechin. The results indicated that 3,7-digalloyl derivative did not inhibit HeLa S3 cell proliferation. Furthermore, 3,5-digalloyl- (–)-epigallocatechin was synthesized to evaluate the importance of the number of phenolic hydroxyl groups on the B-ring. Contrary to our expectations, 3,5-digalloyl-(–)-epigallocatechin exhibited a weaker inhibition of HeLa S3 cell proliferation than 3,5-digalloyl- (–)-epicatechin did. The DPPH radical scavenging activity of the synthesized compounds suggested that the galloyl-modified position and the number of phenolic hydroxyl groups on B-ring affected to the radical scavenging ability. In conclusion, we found that 3,5-digalloyl-(–)-epicatechin exhibited a superlative HeLa S3 cell proliferation inhibitory effect among the galloylated flavan-3-ol derivatives we synthesized and DPPH radical scavenging activity was affected by the galloyl-moiety-introduced position and the number of phenolic hydroxyl groups on the B-ring.

Keywords: Digalloyl-flavan-3-ols; DPPH radical scavenging activity; Stereoselective and regioselective synthesis; Inhibitory activity; HeLa S3 cells proliferation

Abbreviations: EGCG: Epigallocatechin-3-*O*-gallate; DPPH: 2,2-diphenyl-1-picrylhydrazyl; BSA: Bovine serum albumin; TBS: *tert*-butyldimethylsilyl; DCC: Dicyclohexylcarbodiimide; TBAF: Tetra-*n*-butylammonium fluoride; EDC: 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; THF: Tetrahydrofuran; DMF: *N*,*N*-dimethylformamide; TFA: Trifluoroacetic acid; DMAP: *N*,*N*-dimethyl-4-aminopyridine; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; SAR: Structure activity relationship; HMQC: Heteronuclear multiple quantum correlation; HMBC: heteronuclear multiple bond coherence.

Introduction

There is currently great interest in the investigation of compounds with strong biological activities that are derived from food sources because these compounds are generally considered safe owing to their consumption as part of the general daily diet. Polyphenols are thought to have a variety of health benefits and are found in many health foods, vegetables, and fruits [1,2]. However, the structure-activity relationships (SARs) of polyphenols are not well understood because polyphenols are typically obtained as a mixture of various analogs, which makes purification difficult. The flavan-3-ol series is one of the most well-known groups of biologically active polyphenol compounds and includes (–)-epigallocatechin-3-*O*-gallate (EGCG) (**1**) (Figure 1). (–)-EGCG (**1**) exhibited higher anti-proliferative activity against the cervical epithelioid carcinoma cell line, HeLa S3, then (–)-epicatechin (**2**) and (+)-catechin (**3**) did (Figure 1) owing to the greater number of phenolic hydroxyl groups on the B-ring and the presence of galloyl moieties. Although other flavan-3-ols are found naturally as minor components in plants, it has thus far been difficult to conduct detailed investigations of the wide variety of these compounds with standard biological assays because they are only available in limited quantities.

In our previous paper, we reported that multiple gallo-moieties in one molecule of flavan-3-ol affect inhibitory activity against HeLa S3 cell proliferation [3]. Interestingly, the effect of gallo-moieties is potentially limited depending on the stereochemistry. We synthesized the flavan-3-ols **4** and **5**, which were modified with two galloyl moieties and derived from (–)-epicatechin and (+)-catechin, respectively (Figure 2). Among them, only compound **4** showed strong inhibitory activity against the proliferation of HeLa S3 cells.

In this study, we described the synthesis of 7-galloyl-(–)-epicatechin and (+)-catechin series **6**–**9** to evaluate the role of the position of the galloyl moiety. In addition, we synthesized 3,5-di-galloyl-(–)-epigallocatechin (**10**) (5-galloyl derivative of EGCG (**1**)) to assess the importance of the number of phenolic hydroxyl groups on the B-ring (Figure 3). These compounds could be synthesized because of the

***Corresponding author:** Akiko Saito, Graduate School of Engineering, Osaka Electro-Communication University (OECU), 18-8 Hatsu-cho, Neyagawa-shi, Osaka 572-8530, Japan, Tel: +81728241131; Fax: +81728240014; E-mail: a-saito@osakac.ac.jp

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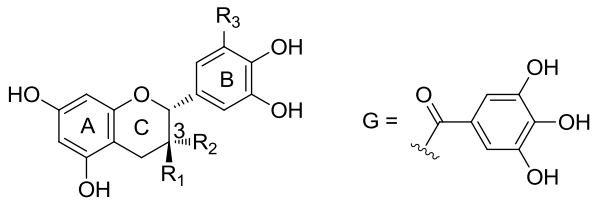
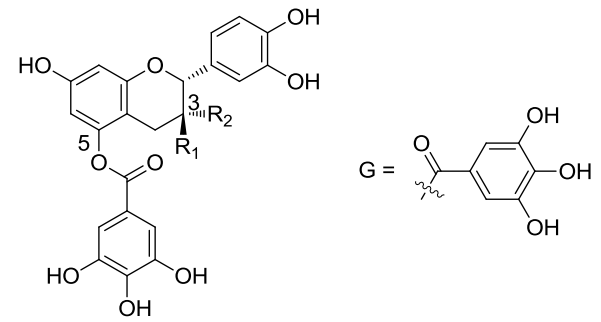


1: R_1 = H, R_2 = OG, R_3 = OH; (–)-epigallocatechin-3-*O*-gallate
2: R_1 = H, R_2 = OH, R_3 = H; (–)-epicatechin
3: R_1 = OH, R_2 = H, R_3 = H; (+)-catechin

Figure 1: Structure of typical flavan-3-ols.



4: R_1 = H, R_2 = OG; (–)-epicatechin-3,5-di-*O*-gallate
5: R_1 = OG, R_2 = H; (+)-catechin-3,5-di-*O*-gallate

Figure 2: Structure of the 3,5-digalloyl flavan-3-ol derivatives.

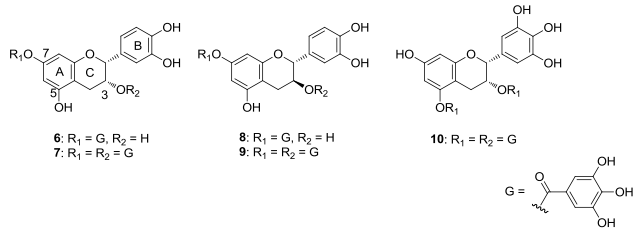


Figure 3: Structure of synthesized galloylated flavan-3-ols.

discovery of the deprotection method of *tert*-butyldimethylsilyl (TBS) ether in the 7-position on the A-ring in basic conditions. In a previous paper, we reported the selective deprotection method of TBS ether in the 5-position on the A-ring of TBS-protected (–)-epicatechin (**2**) and (+)-catechin (**3**) in acidic conditions for the synthesis of 5-modified flavan-3-ols **4** and **5** regioselectively [3]. This acidic deprotection method is generally applicable to other TBS-protected flavan-3-ols to enable the regioselective synthesis of **10**. Our previous [3] and current study using the synthesized galloyl-modified flavan-3-ol series, which are usually difficult to obtain from plants, indicated that the structure of 3,5-digalloyl and two hydroxyl groups on the B-ring were important for inhibitory activity against HeLa S3 cell proliferation.

Materials and Methods

General

All commercially available chemicals for chemical synthesis were used without further purification. All reactions were performed under an argon atmosphere and monitored using thin-layer chromatography (TLC) with 0.25 mm pre-coated silica gel plates 60F254 (Art 5715, Merck KGaA, Darmstadt, Germany). An ATAGO (Minato, Japan) AP-300 spectrometer was used to measure optical rotation. ^{1}H- and ^{13}C-NMR spectra (400/100 MHz) were recorded on a DD2 NMR Spectrometer (Agilent, Santa Clara, CA, USA). A microTOFfocus mass spectrometer (Bruker Daltonics, Billerica, MA, USA) was used to acquire electrospray ionization (ESI) mass spectra. Synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) and stored at –20°C. HPLC purification was carried out on an Ascentis® column (SUPELCO® analytical, Sigma Aldrich Co., St. Louis, MO, USA; 25 cm × 21.5 mm, 5 μm) using the solvents (A) 0.05% HCOOH in CH_3CN and (B) 0.05% HCOOH and 10% CH_3CN in H_2O. Elution was done with a linear gradient 20%–100% B in 25 min (flow rate, 4.0 mL/min). The human cervical adenocarcinoma cell line, HeLa-S3, was provided by the RIKEN BRC through the National Bio-Resource Project of the MEXT (Tsukuba, Japan). The inhibitory activity of HeLa S3 cells proliferation and the DPPH scavenging activity were estimated with a microplate reader (Filter Max F5 multi-mode microplate reader; Molecular Devices, Downingtown, PA, USA).

Synthesis

Deprotection of 7- or 5-*O*-TBS group of compound 11 (13 and 15): To a solution of 5,7,3',4'-tetra-*O*-TBS-(–)-epicatechin (**11**) (0.55 g, 0.74 mmol) in MeOH (40 mL), CH_2Cl_2 (10 mL), K_2CO_3 (0.41 g; 3.00 mmol) was added slowly at 0°C to RT. After stirring for 2 h, the pale yellow reaction mixture was quenched with sat. NH_4Cl aq. The aqueous solution was extracted with $CHCl_3$ and the organic phase was washed with water and brine and then dried ($MgSO_4$). Filtration, concentration and silica-gel column purification (*n*-hexane/EtOAc, 20:1 to 5:1) afforded 0.12 g of **13** (0.19 mmol, 26%) and 0.10 g of **15** (0.16 mmol, 22%) as white powder, respectively. And 0.046 g (0.061 mmol, 8.2%) of **11** was recovered. Data for **13**: [α]25 *D* –5.9 (*c* 0.34, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 6.96 (1H, d, *J* = 1.8 Hz), 6.92 (1H, dd, *J* = 1.8, 8.3 Hz), 6.86 (1H, d, *J* = 8.3 Hz), 6.11 (1H, d, *J* = 2.4 Hz), 6.00 (1H, d, *J* = 2.4 Hz), 4.90 (1H, br s), 4.56 (1H, s), 4.24-4.22 (1H, m), 2.86-2.88 (2H, m), 1.68 (1H, d, *J* = 5.9 Hz), 1.01 (9H, s), 1.00 (9H, s), 0.99 (9H, s), 0.25 (6H, s), 0.21 (3H, s), 0.21 (3H, s), 0.20 (6H, s); ^{13}C-NMR (100 MHz, $CDCl_3$) 155.6, 155.5, 154.9, 147.0, 146.7, 131.0, 121.0, 119.3, 119.2, 103.3, 99.4, 96.7, 78.1, 66.5, 28.5, 25.93, 25.91, 25.7, 18.44, 18.42, 18.2, –4.10, –4.12, –4.21, –4.24; ESILRMS (m/z) 1292 (13), 1291 (30), 1290 (61), 1289 (85), 1288 (85), 657 (25), 656 (58), 655 ([M+Na]$^+$, 100), 635 (11), 634 (26), 633 ([M+H]$^+$, 51); ESIHRMS calcd. for $C_{33}H_{57}O_6Si_3$,633.3463; found 633.3458.

Deprotection of 7- or 5-*O*-TBS group of compound 12 (14 and 16): To a solution of 5,7,3',4'-tetra-*O*-TBS-(+)-catechin (**12**) (1.00 g, 1.34 mmol) in MeOH (40 mL), CH_2Cl_2 (10 mL), K_2CO_3 (0.80 g, 5.78 mmol) was added slowly at 0°C to RT. After stirring for 2 h, the pale-yellow reaction mixture was quenched with sat. NH_4Cl aq. The aqueous solution was extracted with $CHCl_3$ and the organic phase was washed with water and brine and then dried ($MgSO_4$). Filtration, concentration and silica-gel column purification (*n*-hexane/EtOAc, 20:1 to 5:1) afforded 0.15 g of **14** (0.23 mmol, 17%) and 0.16 g of **16** (0.25 mmol, 19%) as white powder, respectively. And 0.042 g (0.056 mmol, 4.2%) of **12** was recovered. Data for **14**: [α]25 *D* +13.6 (*c* 0.22, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) 6.87 (1H, br s), 6.85 (2H, br s), 6.07 (1H, d, *J* = 2.4 Hz), 5.99 (1H, d, *J* = 2.4 Hz), 4.66 (1H, d, *J* = 7.6 Hz), 3.99 (1H, ddd, *J* = 5.6, 7.6, 8.4 Hz), 2.94 (1H, dd, *J* = 5.6, 16.4 Hz), 2.58 (1H, dd, *J* = 8.4, 16.4 Hz), 1.69 (1H, d, *J* = 4.0 Hz), 0.99 (9H, s), 0.99 (9H, s), 0.96 (9H, s), 0.24 (3H, s), 0.23 (3H, s), 0.20 (3H, s), 0.20 (3H, s), 0.16 (3H, s), 0.16 (3H, s); ^{13}C-NMR (100 MHz, $CDCl_3$) 155.6, 155.2, 155.0, 147.2, 147.1, 130.8, 121.2, 119.9, 119.6, 104.4, 99.2, 96.4,

81.1, 68.4, 27.6, 25.9 (2), 25.7, 18.42, 18.40, 18.2, −4.09, −4.10, −4.14, −4.15, −4.21, −4.23; ESILRMS (m/z) 1291 (12), 1290 (22), 1289 (31), 1288 (30), 658 (7.3), 657 (24), 656 (56), 655 ([M+Na]$^+$, 100), 635 (11), 634 (26), 633 ([M+H]$^+$, 53); ESIHRMS calcd. for $C_{33}H_{57}O_6Si_3$, 633.3463; found 633.3458.

(2*R*,3*S*)-3',4'-5-Tri-*O*-TBS-(+)-catechin-7-*O*-(tri-*O*-benzyl) gallate (18): To a solution of **14** (80.4 mg, 0.13 mmol) and 3,4,5-tribenzyloxybenzoic acid (99.1 mg, 0.22 mmol) was added EDC (31.8 mg, 0.17 mmol) and DMAP (1.0 mg, 8.19 μmol) in CH_2Cl_2 (2 mL) at 0°C. After stirring for 24 h, the reaction mixture was quenched with water. The aqueous solution was extracted with $CHCl_3$ and the organic phase was washed with water and brine and then dried ($MgSO_4$). Filtration, concentration and silica gel column purification (*n*-hexane/EtOAc; 10:1 to 2:1) afforded 76.0 mg of **18** (0.075 mmol, 54%) as an amorphous solid. [α]26 *D*–4.5 (*c* 0.22, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) 7.52-7.28 (15H, m), 7.51 (2H, s), 6.90 (1H, br s), 6.87 (2H, br s), 6.42 (1H, d, *J* = 2.2 Hz), 6.28 (1H, d, *J* = 2.2 Hz), 5.17 (4H, s), 5.15 (2H, s), 4.66 (1H, d, *J* = 7.7 Hz), 4.01 (1H, ddd, *J* = 2.1, 7.7, 8.6 Hz), 3.07 (1H, dd, *J* = 2.1, 16.4 Hz), 2.66 (1H, dd, *J* = 8.6, 16.4 Hz), 1.71 (1H, d, *J* = 3.8 Hz), 1.01 (9H, s), 1.00 (9H, s), 0.98 (9H, s), 0.26 (3H, s), 0.26 (3H, s), 0.21 (3H, s) 0.21 (3H, s), 0.19 (3H, s), 0.18 (3H, s); ^{13}C-NMR (100 MHz, $CDCl_3$) 165.5, 155.5, 154.7, 152.6, 150.0, 147.4, 147.2, 142.9, 137.4, 136.6, 130.5, 128.6, 128.5, 128.2, 128.1, 128.0, 127.6, 124.5, 121.2, 120.1, 119.7, 109.9, 109.7, 104.9, 103.1, 81.3, 75.2, 71.3, 68.2, 28.1, 25.9, 25.9, 25.7, 18.4, 18.4, 18.2, −4.05, −4.07, −4.09, −4.12, −4.18, −4.21; ESILRMS (m/z) 1081 (19), 1080 (46), 1079 (81), 1078 ([M+Na]$^+$, 100), 1058 (11), 1057 (20), 1056 ([M+H]$^+$, 25), 656 (10), 655 (18); ESIHRMS calcd. for $C_{61}H_{79}O_{10}Si_3$, 1055.4981; found 1055.4976.

(2*R*,3*R*)-(–)-epicatechin-7-*O*-(tri-*O*-benzyl)gallate (19): To a solution of **13** (41.9 mg, 66.2 μmol) and 3,4,5-tribenzyloxybenzoic acid (47.6 mg, 0.10 mmol), EDC (24.8 mg, 0.13 mmol) and DMAP (1.00 mg, 8.19 μmol) were added in CH_2Cl_2 (2 mL) at 0°C to RT. After stirring for 24 h, the reaction mixture was quenched with water. The aqueous solution was extracted with $CHCl_3$ and the organic phase was washed with water and brine and then dried ($MgSO_4$). Filtration, concentration and silica gel column purification (*n*-hexane/EtOAc; 10:1 to 2:1) afforded crude **17** as an amorphous solid. A solution of crude in THF (3 mL) was added dropwise to triethylamine trihydrofluoride (TEA, 3HF) (50 μL, 0.30 mmol) at 0°C to RT. Concentration and silica-gel column ($CHCl_3$/MeOH; 10:1 to 1:2) afforded 26.7 mg of **19** (37.5 μmol, 57%) as an amorphous solid. [α]24 *D*–36.3 (*c* 1.1, MeOH); ^{1}H-NMR (400 MHz, CD_3OD) 7.52 (2H, s), 7.49-7.22 (15H, m), 6.99 (1H, d, *J* = 1.9 Hz), 6.81 (1H, dd, *J* = 1.9, 8.2 Hz), 6.76 (1H, d, *J* = 8.2 Hz), 6.27 (1H, d, *J* = 2.2 Hz), 6.24 (1H, d, *J* = 2.2 Hz), 5.18 (4H, s), 5.10 (2H, s), 4.90 (1H, br s), 4.23 (1H, dd, *J* = 2.4, 4.8 Hz), 2.96 (1H, dd, *J* = 4.8, 17.6 Hz), 2.85 (1H, dd, *J* = 2.4, 17.6 Hz); ^{13}C-NMR (100 MHz, CD_3OD) 166.2, 158.1, 157.3, 154.0, 151.5, 146.0, 145.9, 143.8, 138.7, 138.1, 132.0, 129.7, 129.6, 129.2, 129.1, 128.9, 126.0, 119.4, 115.9, 115.3, 110.3, 106.6, 102.4, 101.9, 80.1, 76.2, 72.2, 67.1, 29.6; ESILRMS (m/z) 1450 (22), 1449 (45), 1148 (47), 737 (12), 736 (46), 735 ([M+Na]$^+$, 100), 580 (15), 579 (45), 414 (20), 413 (75); ESIHRMS calcd. for $C_{43}H_{36}O_{10}Na$, 735.2206; found 735.2250.

(2*R*,3*S*)-(+)-catechin-7-*O*-(tri-*O*-benzyl)gallate (20): A solution of **18** (61.0 mg, 57.8 μmol) in THF (2 mL) was added dropwise to triethylamine trihydrofluoride (60 μL, 0.36 mmol) at 0°C to RT. Concentration and silica-gel column ($CHCl_3$/MeOH; 10:1 to 1:2) afforded 36.4 mg of **20** (51.1 μmol, 88%) as an amorphous solid. [α]24 *D* –11 (*c* 0.35, MeOH); ^{1}H-NMR (400 MHz, CD_3OD_3) 7.51 (2H, s), 7.48-7.22 (15H, m), 6.84 (1H, d, *J* = 1.9 Hz), 6.74 (1H, d, *J* = 8.3 Hz), 6.72 (1H, dd, *J* = 1.9, 8.3 Hz), 6.23 (1H, d, *J* = 2.2 Hz), 6.22 (1H, d, *J* = 2.2 Hz), 5.17 (4H, s), 5.10 (2H, s), 4.65 (1H, d, *J* = 7.3 Hz), 4.05 (1H, ddd, *J* = 5.4, 7.3, 8.0 Hz), 2.92 (1H, dd, *J* = 5.4, 16.6 Hz), 2.61 (1H, dd, *J* = 8.0, 16.6 Hz); ^{13}C-NMR (100 MHz, CD_3OD) 166.2, 157.7, 156.9, 154.0, 151.6, 146.4, 146.3, 138.7, 138.2, 131.9, 129.8, 129.6, 129.2, 129.14, 129.13, 128.9, 126.0, 120.0, 116.1, 115.2, 110.4, 107.3, 102.0, 101.7, 83.0, 76.2, 72.3, 68.3, 28.6; ESILRMS (m/z) 1450 (28), 1449 (60), 1448 (64), 737 (11), 736 (45), 735 ([M+Na]$^+$, 100), 714 (8), 713 ([M+H]$^+$, 16), 580 (20), 579 (61), 451 (8), 450 (31); ESIHRMS calcd. for $C_{43}H_{37}O_{10}$, 713.2387; found 713.2406.

(2*R*,3*R*)-(–)-epicatechin-7-*O*-gallate (6): A solution of **19** (31.4 mg, 44.1 μmol) in THF/MeOH/H_2O (20:1:1, 8.8 mL) was hydrogenated over 20% $Pd(OH)_2$/C (1 mg) for 12 h at RT. Filtration and concentration afforded a pale brown solid, which was purified using HPLC purification to give 11.5 mg of pure **6** (26.0 μmol, 59%) as a pale brown powder. [α]24 *D* –63.9 (c 0.31, MeOH); ^{1}H-NMR (400 MHz, CD_3OD) 7.15 (2H, s), 6.99 (1H, d, *J* = 1.6 Hz), 6.80 (1H, dd, *J* = 1.6, 8.3 Hz), 6.75 (1H, d, *J* = 8.3 Hz), 6.23 (1H, d, *J* = 2.0 Hz), 6.21 (1H, d, *J* = 2.0 Hz), 5.00-4.80 (1H, m), 4.23 (1H, br s), 2.95 (1H, dd, *J* = 4.4, 17.4 Hz), 2.84 (1H, dd, *J* = 2.4, 17.4 Hz); ^{13}C-NMR (100 MHz, CD_3OD) 167.1, 158.0, 157.3, 151.7, 146.7, 146.0, 145.9, 140.5, 132.0, 120.8, 119.4, 115.9, 115.3, 110.5, 106.3, 102.4, 101.9, 80.1, 67.2, 29.6; ESILRMS (m/z) 466 (19), 465 ([M+Na]$^+$, 76), 451 (26), 450 (10), 426 (11), 425 (42); ESIHRMS calcd. for $C_{22}H_{18}O_{10}Na$, 465.0798; found 465.0792.

(2*R*,3*S*)-(+)-catechin-7-*O*-gallate (8): A solution of **20** (36.6 mg, 51.4 μmol) in THF/MeOH/H_2O (20:1:1, 8.8 mL) was hydrogenated over 20% $Pd(OH)_2$/C (1 mg) for 12 h at RT. Filtration and concentration afforded a pale brown solid, which was purified using HPLC purification to give 18.9 mg of pure **8** (42.7 μmol, 83%) as a pale brown powder. [α]19 *D* +27.9 (c 0.36, Me_2CO) {lit.[4] [α]27 *D* +38.9 (c 0.81; Me_2CO)}; ^{1}H-NMR (400 MHz, CD_3OD_3) 7.26 (2H, s), 6.95 (1H, d, *J* = 1.9 Hz), 6.87 (1H, d, *J* = 8.3 Hz), 6.82 (1H, dd, *J* = 1.9, 8.3 Hz), 6.31(1H, d, *J* = 2.2 Hz), 6.29 (1H, d, *J* = 2.2 Hz), 476 (1H, d, *J* = 7.3 Hz), 4.15 (1H, ddd, *J* = 5.4, 7.3, 7.9 Hz), 3.02 (1H, dd, *J* = 5.4, 17.0 Hz), 2.71 (1H, dd, *J* = 7.9, 17.0 Hz); ^{13}C-NMR (100 MHz, CD_3OD) 167.0, 157.6, 156.8, 151.8, 146.7, 146.34, 146.29, 140.5, 131.9, 120.6, 119.9, 116.1, 115.1, 110.5, 107.0, 102.0, 101.8, 83.0, 68.4, 28.5; ESILRMS (m/z) 466 (24), 465 ([M+Na]$^+$, 100), 451 (25), 450 (95), 426 (10), 425 (40); ESIHRMS calcd. for $C_{22}H_{18}O_{10}Na$, 465.0798; found 465.0836.

(2*R*,3*R*)-(–)-epicatechin-3,7-di-*O*-(tri-*O*-benzyl)gallate (23): To a solution of **13** (80.5 mg, 0.13 mmol) and 3,4,5-tribenzyloxybenzoic acid (0.16 g, 0.34 mmol), DCC (70.6 g, 0.34 mmol) and DMAP (1.00 mg, 8.19 μmol) were added in CH_2Cl_2 (5 mL) at 0°C to RT. After stirring for 3 days, the reaction mixture was quenched with water. The aqueous solution was extracted with $CHCl_3$ and the organic phase was washed with water and brine and then dried ($MgSO_4$). Filtration, concentration and silica gel column purification (*n*-hexane/EtOAc; 15:1 to 2:1) afforded crude **21** as an amorphous solid. A solution of crude in THF (2 mL) was added dropwise to triethylamine trihydrofluoride (76 μL, 0.46 mmol) at 0°C. Concentration and silica-gel column (*n*-hexane/EtOAc; 5:1 to 1:5) afforded 20.8 mg of **23** (18.3 μmol, 14%) as an amorphous solid. [α]26 *D* –74.4 (*c* 1.7, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) 7.50 (2H, s), 7.44-7.27 (30H, m), 7.21 (2H, s), 6.77-6.75 (3H, m), 6.55 (1H, d, *J* = 2.0 Hz), 6.31 (1H, d, *J* = 2.0 Hz), 5.56 (1H, br s), 5.15-5.05 (14H, m), 3.10-3.04 (2H, m); ^{13}C-NMR (100 MHz, $CDCl_3$) 165.4, 165.2, 155.7, 155.1, 152.7, 152.3, 150.2, 143.7, 143.2, 143.1, 142.3, 137.3, 137.2, 136.8, 136.4, 130.1, 128.8, 128.56, 128.55, 128.51, 128.21, 128.20, 128.1, 128.03, 127.97, 127.94, 127.58, 127.5, 124.6, 124.1, 119.1, 115.1, 113.6, 109.7, 109.0, 104.5, 102.5, 101.88, 101.87, 75.2, 75.1, 71.3, 70.9, 68.52, 68.48, 25.6; ESILRMS (m/z) 1159 (20), 1158 (46), 1157 ([M+Na]$^+$, 60), 805 (29), 804 (55), 652 (18), 651 (79), 608 (20), 607 (100); ESIHRMS calcd. for $C_{71}H_{58}O_{14}Na$, 1157.3724; found 1157.3757.

(2*R*,3*S*)-3',4'-5-Tri-*O*-TBS-(+)-catechin-3,7-di-*O*-(tri-*O*-benzyl)gallate (22): To a solution of **14** (52.1 mg, 82.3 μmol) and 3,4,5-tribenzyloxybenzoic acid (0.10 g, 0.22 mmol), DCC (56.9 mg, 0.28 mmol) and DMAP (1.0 mg, 8.19 μmol) were added in CH_2Cl_2 (2 mL) at 0°C. After stirring for 24 h, the reaction mixture was quenched with water. The aqueous solution was extracted with $CHCl_3$ and the organic phase was washed with water and brine and then dried ($MgSO_4$). Filtration, concentration and silica gel column purification (*n*-hexane/EtOAc; 10:1 to 2:1) afforded 107.9 mg of **22** (73.0 μmol, 89%) as an amorphous solid. [α]25 *D* +20.8 (*c* 8.6, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) 7.50 (2H, s), 7.45-7.27 (30H, m), 7.24 (2H s), 6.86 (1H, br s), 6.77 (2H, br s), 6.52 (1H, d, *J* = 2.2 Hz), 6.33 (1H, d, *J* = 2.2 Hz), 5.60-5.40 (1H, m) 5.20 (1H, d *J* = 5.5 Hz), 5.15 (4H, s), 5.14 (2H, s), 5.08 (2H, s), 5.07 (4H, s), 2.90-2.88 (2H, m), 0.96 (9H, s), 0.94 (9H, s), 0.91 (9H, s), 0.22 (3H, s), 0.21 (3H, s), 0.15 (3H, s), 0.14 (3H, s), 0.10 (3H, s), 0.08 (3H, s); ^{13}C-NMR (100 MHz, $CDCl_3$) 165.2, 164.3, 155.1, 154.6, 152.6, 152.4, 150.3, 147.2, 146.8, 142.9, 142.5, 137.43, 137.37, 136.7, 136.6, 131.0, 128.57, 128.56, 128.54, 128.52, 128.23, 128.20, 128.07, 128.01, 127.99, 127.95, 127.61, 127.59, 125.0, 124.9, 121.1, 119.4, 118.7, 109.7, 109.1, 108.8, 105.1, 103.1, 77.9, 75.2, 75.1, 71.3, 71.1, 25.91, 25.88, 25.7, 18.45, 18.42, 18.23, –4.09, –4.16, –4.20, –4.22, –4.25; ESILRMS (m/z) 1503 (22), 1502 (44), 1501 (66), 1500 ([M+Na]$^+$, 57), 437 (77), 413 (100); ESIHRMS calcd. for $C_{89}H_{100}NaO_{14}Si_3$ 1499.6319; found 1499.6313.

(2*R*,3*S*)-(+)-catechin-3,7-di-*O*-(tri-*O*-benzyl)gallate (24): A solution of **22** (77.5 mg, 52.4 μmol) in THF (2 mL) was added dropwise to triethylamine trihydrofluoride (50 mL, 0.30 mmol) at 0°C. Concentration and a short silica-gel column (*n*-hexane/EtOAc; 5:1 to 1:5) afforded 31.5 mg of **24** (27.7 μmol, 53%) as an amorphous solid. [α]27 *D* +8.7 (*c* 6.8, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) 7.50 (2H, s), 7.44-7.19 (32H, m), 6.87 (1H, d, *J* = 1.8 Hz), 0.86 (1H, d, *J* = 8.3 Hz), 6.79 (1H, dd, *J*= 1.8, 8.3 Hz), 6.46 (1H, d, *J* = 2.0 Hz), 6.25 (1H, d, *J* = 2.4 Hz), 5.70 (1H, br s), 5.63 (1H, br s), 5.54 (1H, br s), 5.44 (1H, ddd, *J* = 2.4, 5.6, 5.6 Hz), 5.20 (1H, d, *J* = 5.6 Hz), 5.15-5.06 (12H, m), 2.76 (1H, dd, *J* = 5.6, 17.2 Hz), 2.59 (1H, dd, *J* = 2.4, 17.2 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) 166.4, 166.1, 155.4, 154.6, 152.7, 152.4, 149.7, 144.2, 143.6, 143.3, 142.7, 137.2, 137.1, 136.5, 136.3, 130.4, 128.54, 128.52, 128.50, 128.20, 128.18, 128.08, 128.04, 128.00, 127.97, 127.6, 127.53, 127.52, 124.6, 123.7, 118.7, 116.3, 112.6, 109.7, 109.2, 105.1, 102.0, 101.6, 77.2, 75.2, 75.1, 71.2, 71.1, 69.7, 29.7; ESILRMS (m/z) 1159 (33), 1158 (78), 1157 ([M+Na]$^+$, 100), 805 (7), 804 (14), 580 (22), 579 (62); ESIHRMS calcd. for $C_{71}H_{58}O_{14}Na$, 1157.3724; found 1157.3763.

(2*R*,3*R*)-(–)-epicatechin-3,7-di-*O*-gallate (7): A solution of **23** (17.5 mg, 15.4 μmol) in THF/MeOH/H_2O (20:1:1, 8.8 mL) was hydrogenated over 20% $Pd(OH)_2$/C (1.0 mg) for 12 h at RT. Filtration and concentration afforded a pale brown solid, which was purified using HPLC purification to give 4.9 mg of pure **7** (8.24 μmol, 54%) as a pale brown powder. [α]21 *D*–180 (*c* 0.50, MeOH); ^{1}H-NMR (400 MHz, CD_3OD) 7.17 (2H, s), 6.95 (2H, s), 6.94 (1H, d, *J* = 2.0 Hz), 6.83 (1H, dd, *J* = 2.0, 8.4 Hz), 6.69 (1H, d, *J* = 8.4 Hz), 6.30 (1H, d, *J* = 2.0 Hz), 6.24 (1H, d, *J* = 2.0 Hz), 5.57 (1H, br s), 5.11 (1H, br s), 3.09 (1H, dd, *J* = 3.4, 16.4 Hz), 2.95 (1H, d, *J* = 16.4 Hz); ^{13}C-NMR (100 MHz, CD_3OD) 167.5, 167.1, 157.9, 157.2, 151.8, 146.7, 146.3, 146.1, 146.0, 140.5, 139.8, 131.1, 121.4, 120.7, 119.4, 116.0, 115.1, 110.6, 110.2, 105.6, 102.3, 102.1, 78.9, 69.6, 27.2; ESILRMS (m/z) 619 (8), 618 (31), 617 ([M+Na]$^+$, 100), 580 (17), 579 (49), 451 (9), 450 (34); ESIHRMS calcd. for $C_{29}H_{22}O_{14}Na$, 617.0907; found 617.0905.

(2*R*,3*S*)-(+)-catechin-3,7-di-*O*-gallate (9): A solution of **24** (29.0 mg, 25.5 μmol) in THF/MeOH/H_2O (20:1:1, 8.8 mL) was hydrogenated over 20% $Pd(OH)_2$/C (1.0 mg) for 12 h at RT. Filtration and concentration afforded a pale brown solid, which was purified using HPLC purification to give 13.9 mg of pure **9** (23.4 μmol, 92%) as a pale brown powder. [α]21 *D* +23 (*c* 0.43, MeOH); ^{1}H-NMR (400 MHz, CD_3OD) 7.17 (2H, s), 6.97 (2H, s), 6.84 (1H, s), 6.728 (1H, s), 6.726 (1H, s), 6.29 (1H, d, *J* = 2.2 Hz), 6.25 (1H, d, *J* = 2.2 Hz), 5.44 (1H, ddd, *J* = 5.3, 5.4, 5.5 Hz), 5.17 (1H, d, *J* = 5.5 Hz), 2.85 (1H, dd, *J*= 5.3, 17.2 Hz), 2.81 (1H, dd, *J* = 5.4, 17.2 Hz); ^{13}C-NMR (100 MHz, CD_3OD) 167.5, 167.1, 157.7, 156.4, 152.0, 146.7, 146.4 (2), 146.3, 140.6, 139.9, 131.2, 121.3, 120.6, 119.0, 116.3, 114.2, 110.5, 110.1, 105.8, 102.1, 102.0, 79.4, 70.6, 24.2; ESILRMS (m/z) 617 (19), 617 ([M+Na]$^+$, 62), 414 (26), 413 (100); ESIHRMS calcd. for $C_{29}H_{22}O_{14}Na$, 617.0907; found 617.0914.

(2*R*,3*R*)-3',4',5,5',7-penta-*O*-TBDMS-(–)-epigallocatechin-3-*O*-(tri-*O*-TBDMS)gallate (25): To a solution of EGCG (**1**) (5.07 g, 1.11 mmol) in THF-DMF (25 ml, 4:1), TBDMSCl (16.7 g, 0.11 mol) was added at 0°C. After stirring for 2 days, the reaction mixture was quenched with water. The aqueous solution was extracted with EtOAc and the organic phase was washed with water and brine and then dried ($MgSO_4$). Filtration, concentration and silica gel column purification (*n*-hexane/EtOAc; 150:1 to 110:1) afforded 7.73 g of **25** (0.56 mmol, 51%) as a white powder. [α]21 *D* –57.5 (*c* 1.6, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) 7.05 (2H, s), 6.58 (2H, s), 6.16 (1H, d, *J* = 2.2 Hz), 5.93 (1H, d, *J* = 2.2 Hz), 5.58 (1H, br s), 5.01 (1H, br s), 2.93 (2H, br s), 0.98 (9H, s), 0.97 (9H, s), 0.95 (9H, s), 0.95 (9H, s), 0.87 (18H, s), 0.86 (18H, s), 0.202 (3H, s), 0.199 (3H, s), 0.18 (3H, s), 0.13 (6H, s), 0.12 (6H, s), 0.11 (6H, s), 0.10 (3H, s), 0.10 (6H, s), 0.08 (6H, s), 0.06 (3H, s); ^{13}C-NMR (100 MHz, $CDCl_3$) 165.0, 155.6, 154.9, 154.7, 148.4, 148.2, 142.9, 137.8, 129.9, 121.8, 115.4, 112.4, 103.8, 103.7, 101.6, 76.9, 67.9, 26.8, 26.2, 26.14 (2), 26.07, 25.7, 25.6, 18.74, 18.67, 17.41, 17.39, 18.2, 18.1, –3.68, –3.69, –3.71, –3.72, –3.74, –3.83, –3.92, –3.96, –4.02, –4.17, –4.35, –4.40, –4.41; ESILRMS (m/z) 1397 (46), 1396 (80), 1395 ([M+Na]$^+$, 100), 1394 (85), 1374 (54), 1373 (67), 1372 ([M+H]$^+$, 57); ESIHRMS calcd. for $C_{70}H_{131}O_{11}Si_8$, 1371.78400; found. 1371.7889.

(2*R*,3*R*)-3',4',5',7-tetra-*O*-TBDMS-(–)-epigallocatechin-3-*O*-(tri-*O*-TBDMS)gallate (26): A solution of **25** (2.19 g, 1.6 mmol) in CH_2Cl_2 (20 mL) was added dropwise to TFA (0.45 mL, 5.88 mmol) at 0°C to RT. After stirring for 2 hours, the pale-yellow reaction mixture was quenched with sat. $NaHCO_3$. The aq. solution was extracted with $CHCl_3$ and the organic phase was washed with water and brine and dried ($MgSO_4$). Filtration, concentration and silica gel column purification (*n*-hexane/EtOAc, 30:1 to 5:1) afforded 1.10 g of **26** (0.87 mmol, 54%) as an amorphous solid. [α]24 *D* –73.2 (*c* 1.2, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) 7.04 (2H, s), 6.60 (2H, s), 6.13 (1H, d, *J* = 2.4 Hz), 5.93 (1H, d, *J* = 2.4 Hz), 5.64 (1H, d, *J* = 2.8, 4.0 Hz), 5.01 (1H, br s), 4.67 (1H, s), 3.00 (1H, dd, *J* = 4.0, 16.1 Hz), 2.95 (1H, dd, *J* = 2.8, 16.1 Hz), 0.98 (9H, s), 0.97 (9H, s), 0.95 (9H, s), 0.92 (18H, s), 0.86 (18H, s), 0.21 (3H, s), 0.21 (3H, s), 0.14 (6H, s), 0.12 (6H, s), 0.11 (6H, s), 0.10 (9H, s), 0.09 (3H, s), 0.09 (3H, s), 0.07 (3H, s); ^{13}C-NMR (100 MHz, $CDCl_3$) 165.1, 155.8, 155.3, 154.6, 148.4, 148.2, 142.9, 137.9, 129.7, 121.7, 115.3, 112.4, 101.0, 100.2, 99.5, 77.2, 67.4, 26.21, 26.16, 26.15, 26.12, 26.09, 25.6, 18.74 (x2), 18.68, 18.4, 18.1, –3.67, –3.70, –3.73, –3.76, –3.79, –3.90, –3.91, –4.0, –4.3, –4.5; ESILRMS (m/z) 1281 (1), 1280 ([M+Na]$^+$, 1), 806 (14), 805 (54), 804 (100), 414 (22), 413 (81); ESIHRMS calcd. for $C_{64}H_{116}O_{11}Si_7Na$, 1279.6800; found 1279.6795.

(2R,3R)-3',4',5',7-tetra-O-TBDMS-5-O-(tri-O-benzyl)galloyl-(–)-epigallocatechin-3-O-

(tri-O-TBDMS)gallate (27): To a solution of **26** (0.10 g, 79.5 μmol) and 3,4,5-tribenzyloxybenzoic acid (48.8 mg, 0.11 mmol), DCC (21.4 mg, 0.10 mmol) and DMAP (1.0 mg, 8.19 μmol) were added in CH_2Cl_2 (10 ml) at 0°C. After stirring for 2 days, the reaction mixture was

quenched with water. The aqueous solution was extracted with $CHCl_3$ and the organic phase was washed with water and brine and then dried ($MgSO_4$). Filtration, concentration and silica gel column purification (*n*-hexane/EtOAc; 30:1 to 5:1) afforded 0.11 g of **27** (65.4 μmol, 82%) as an amorphous solid. [α]23 *D* 0 (*c* 1.6, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) 7.52-7.23 (17H, m), 7.06 (2H, s), 6.60 (2H, s), 6.45 (d, *J* = 2.4 Hz), 6.25 (1H, d, *J* = 2.4 Hz), 5.62 (1H, br s), 5.15 (6H, s), 5.01 (1H, br s), 2.95 (1H, dd, *J* = 4.0, 16.8Hz), 2.80 (1H, d, *J* = 16.8 Hz), 0.99 (9H, s), 0.98 (9H, s), 0.96 (9H, s), 0.89 (18H, s), 0.88 (18H, s), 0.24 (3H, s), 0.23 (3H, s), 0.14 (6H, s), 0.13 (6H, s), 0.12 (6H, s), 0.11 (9H, s), 0.10 (3H, s), 0.09 (3H, s), 0.07 (3H, s); ^{13}C-NMR (100 MHz, $CDCl_3$) 164.8, 163.8, 155.7, 155.1, 152.6, 150.3, 148.5, 148.2, 143.0, 142.9, 138.0, 137.3, 136.5, 129.4, 128.6, 128.5, 128.2, 128.03, 128.00, 127.5, 124.0, 121.6, 115.2, 112.3, 109.7, 107.1, 106.0, 105.5, 77.2, 75.2, 71.3, 66.9, 27.0, 26.20, 26.16, 26.14, 26.06, 25.6, 18.73 (x2), 18.68, 18.4, 18.1, −3.70, −3.72, −3.73, −3.74, −3.77, −3.87, −3.93, −4.0, −4.4, −4.5; ESILRMS (m/z) 1703 (5), 1702([M+Na]$^+$, 6), 804 (39), 803 (73), 413 (100); ESIHRMS calcd. for $C_{92}H_{140}O_{16}Si_7$, 1696.8526; found 1696.8521.

(2*R*,3*R*)-5-*O*-galloyl-(−)-epigallocatechin-3-*O*-gallate (10): A solution of **27** (47.1 mg, 28.0 μmol) in THF (5 mL) was added dropwise to triethylamine trihydrofluoride (93 μL, 0.56 mmol) at 0°C. Concentration and a short silica-gel column ($CHCl_3$/MeOH; 6:1 to 3:1) afforded clude product. And then a solution of clude product in THF/MeOH/H_2O (20:1:1, 4.4 mL) was hydrogenated over 20% $Pd(OH)_2$/C (1.0 mg) for 12 h at RT. Filtration and concentration afforded a pale brown solid, which was purified using HPLC purification to give 4.1 mg of pure **10** (6.7 μmol, 24%) as a pale brown powder. ^{1}H-NMR (400 MHz, CD_3OD): 7.16 (2H, s), 6.92 (2H, s), 6.50 (2H, s), 6.36 (1H, d, *J* = 2.4 Hz), 6.25 (1H, d, *J* = 2.4 Hz), 5.49 (1H, br s), 5.04 (1H, br s), 2.99 (1H, dd, *J* = 4.0, 17.6 Hz), 2.76 (1H, dd, *J* = 2.4, 17.6 Hz); ^{13}C-NMR (100 MHz, CD_3OD) 167.6, 166.5, 158.2, 157.3, 152.0, 146.8 (x2), 146.3, 140.7, 140.0, 133.9, 130.3, 121.3, 120.2, 110.6, 110.3, 106.9, 105.0, 103.8, 102.0, 78.9, 69.3, 27.1; ESILRMS (m/z) 1245 (15), 1244 (36), 1243 (58), 634 (32), 633 (100). [α]27 *D* −7.4 (*c* 0.27, MeOH) (lit.[5] [α] *D* −13); ^{1}H-NMR (400 MHz, CD_3OD): 7.16 (2H, s), 6.92 (2H, s), 6.50 (2H, s), 6.36 (1H, d, *J* = 2.4 Hz), 6.25 (1H, d, *J* = 2.4 Hz), 5.49 (1H, br s), 5.04 (1H, br s), 2.99 (1H, dd, *J* = 4.0, 17.6 Hz), 2.76 (1H, dd, *J* = 2.4, 17.6 Hz); ^{13}C-NMR (100 MHz, CD_3OD) 167.6, 166.5, 158.2, 157.3, 152.0, 146.8 (x2), 146.3, 140.7, 140.0, 133.9, 130.3, 121.3, 120.2, 110.6, 110.3, 106.9, 105.0, 103.8, 102.0, 78.9, 69.3, 27.1; ESILRMS (m/z) 1245 (15), 1244 (36), 1243 (58), 634 (32), 633 (100); ESIHRMS calcd. for $C_{29}H_{22}O_{15}Na$, 633.0856; found 633.0828.

Inhibitory Activity of HeLa S3 Cell Proliferation: 10^4 cells per well with 100 μL of medium in a 37°C incubator equilibrated with a 5% CO_2: 95% humidified air atmosphere. D-MEM (Dulbecco's Modified Eagle's Medium; Gibco® (Life Technologies, Grand Island, NY, USA) supplemented with 5% fetal calf serum and 1% Pen-Strep; Invitrogen™ (Life Technologies). After 24 h of incubation, 1 μL of synthesized six compounds in 10% DMSO were added (final 100, 50 μM) and incubated for 48 h (The final concentration of DMSO in each well is 0.1% v/v). As negative controls, medium alone well and 10% DMSO added to the well were prepared at the same time. After the medium was removed and the cell was washed with PBS, 90 μL of new medium and 10 μL of the MTT solution (3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide, 5 mg/mL) was added to each well and incubated at 37°C for 2.5 h. After incubation, the reaction medium was removed and 100 μL of DMSO was added to each well and mix thoroughly with the pipette. Following which, viable cells were assessed using a microplate reader (Filter Max F5 multi-mode microplate reader; Molecular Devices) to measure the OD at 570 nm. And the absorbance values converted into the percentage cell proliferation inhibitory activity as follows: [(absorbance of the control − absorbance of the sample)/absorbance of the control] × 100.

DPPH Radical Scavenging Activity: DPPH radical scavenging activity was measured with general procedure [6]. A solution of DPPH radical in EtOH (30 μM, 1.0 mL) was added to 1 μL of the synthesized each compound in DMSO, and incubated at 30°C for 30 min (*n* = 6). The scavenging activity was estimated with a microplate reader (Filter Max F5 multi-mode microplate reader; Molecular Devices, Downingtown, PA, USA) to measure the OD at 515 nm. Negative controls, the samples that 1 μL of DMSO added to the 1.0 mL of EtOH were prepared at the same time. And the absorbance values converted into the percentage radical scavenging activity as follows: [(absorbance of the control − absorbance of the sample)/absorbance of the control] × 100.

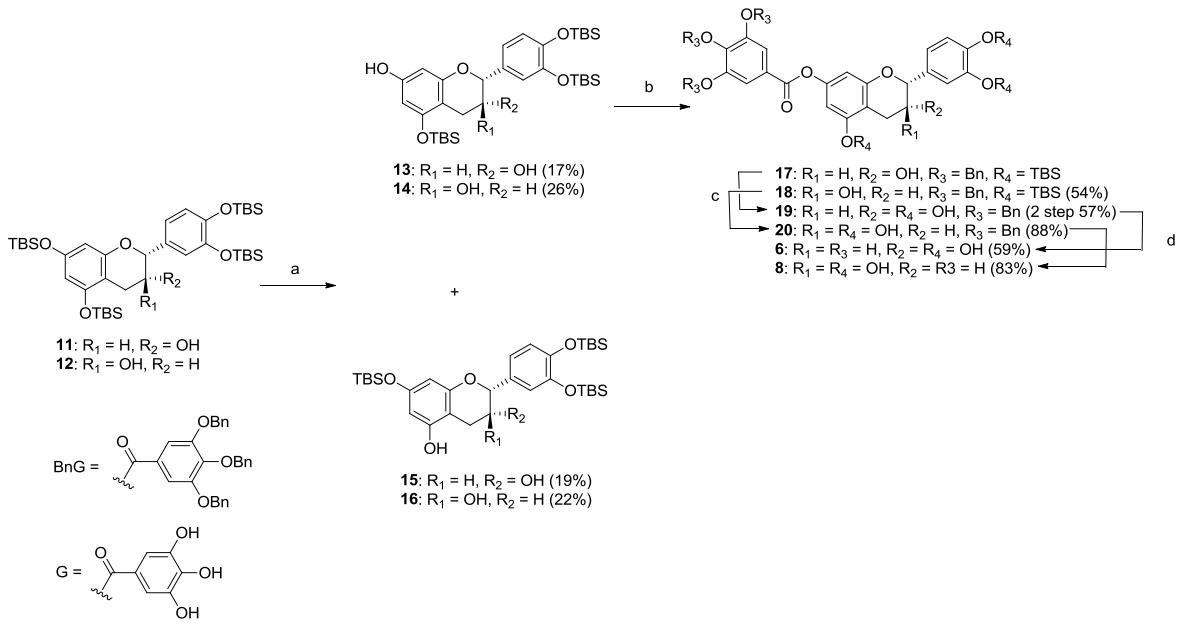
Results and Discussion

Synthesis

First, 7-galloyl-(−)-epicatechin (**6**) and 7-galloyl-(+)-catechin (**8**) were synthesized based on our strategy, deprotection of one TBS moiety, as shown in Scheme 1. Since its first isolation from *Sanguisorba officinals* [4], there have been several reports regarding the isolation and biological activities of compound **8** as a minor constituent of *Paeoniae obovate* [7]; a moderate antiproliferation constituent against B16F10 [8], HeLa [8], and MK-1 [8]; a cytotoxic constituent against HepG2 [9] isolated from the seeds of *Rhynchosia volubilis*; and an antioxidant isolated from *Acacia nilotica* [10]. In addition, synthesis of **8** from a catechol-protected-(+)-catechin derivative, moderate antiplasmodial activity against FcB1, and cytotoxicity against MRC-5 cells were reported [11].

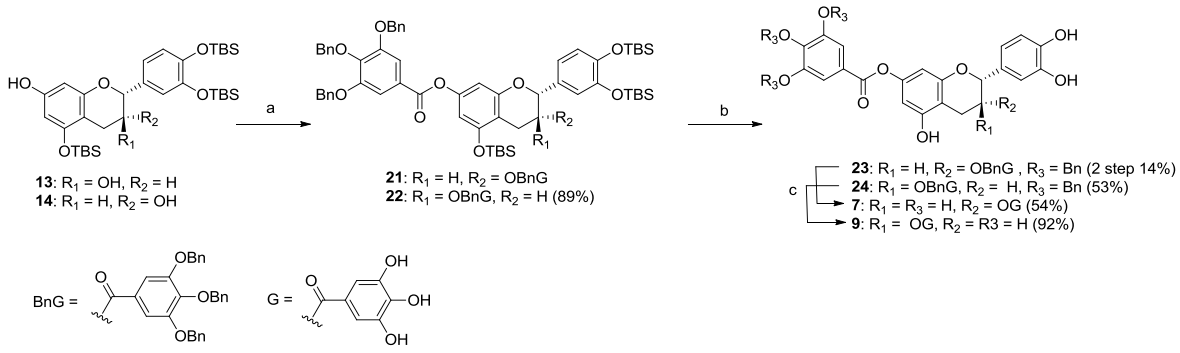
Deprotection of TBS ether of (−)-epicatechin derivative **11** in basic conditions afforded a mixture of 7-hydroxyl and 5-hydroxyl, compounds **13** and **15**, which were separable by silica gel column chromatography with 17% and 19% yields, respectively. (+)-Catechin derivative **12** was similarly converted to **14** and **16** with 26% and 22% yields, respectively. Determination of the deprotected 7-position was confirmed using HMQC and HMBC methodology and compared with 5-deprotected compounds reported previously [3]. TBS-protected-7-hydroxyl-(−)-epicatechin derivative **13** was condensed using 3,4,5-tribenzyloxybenzoic acid by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), then deprotection of all TBS groups by treatment with triethylamine trihydrofluoride afforded **19** with a 57% yield (2 steps). A galloyl moiety was selectively introduced at the 7-position when EDC was used as condensing agent. Hydrogenolysis of the benzyl ether of **19** in the presence of $Pd(OH)_2$/C, a catalyst, gave 7-galloyl-(−)-epicatechin (**6**) with a 59% yield. TBS-protected-7-hydroxyl-(+)-catechin derivative **14** was also condensed using 3,4,5-tribenzyloxybenzoic acid to give compound **18** with a 54% yield. Deprotection of the TBS ether of **18** using triethylamine trihydrofluoride gave compound **20** with an 88% yield, then hydrogenolysis of benzyl ether gave 7-galloyl-(+)-catechin (**8**) with an 83% yield (Scheme 1).

Thereafter, the 3,7-digalloyl derivatives, 3,7-digalloyl-(−)-epicatechin (**7**) and 3,7-digalloyl-(+)-catechin (**9**) were synthesized as shown in Scheme 2. Compound **9** was isolated from *Acacia gerrafdii* and the data of methylated **9** were reported [12]. The TBS-protected-7-hydroxyl series, **13** and **14,** were condensed using 3,4,5-tribenzyloxybenzoic acids with dicyclohexylcarbodiimide (DCC) as a condensing agent. Because compound **21**, derived from **13**, could not be separated from DCC residue, purification was performed after



Reagent and conditions: (a) K_2CO_3, DCM/MeOH, 0°C to r.t., 2 h; (b) 3,4,5-tribenzyloxybenzoic acid, EDC, DMAP, DCM, 0°C to r.t., 24 h; (c) Triethylamine trihydrofluoride, THF, 0°C to r.t., 12 h; (d) H_2, $Pd(OH)_2$/C THF/H_2O/MeOH, r.t., 18 h.

Scheme 1: Synthesis of 7-galloylated (–)-epicatechin (**6**) and (+)-catechin (**8**).



Reagent and conditions: (a) 3,4,5-tribenzyloxybenzoic acid, DCC, DMAP, DCM, 0°C to r.t., 72 h; (b) Triethylamine trihydrofluoride, THF, 0°C to r.t., 12 h; (c) H_2, $Pd(OH)_2$/C, THF/H_2O/MeOH, r.t., 18 h.

Scheme 2: Synthesis of 3,7-digalloylated (–)-epicatechin (**7**) and (+)-catechin (**9**).

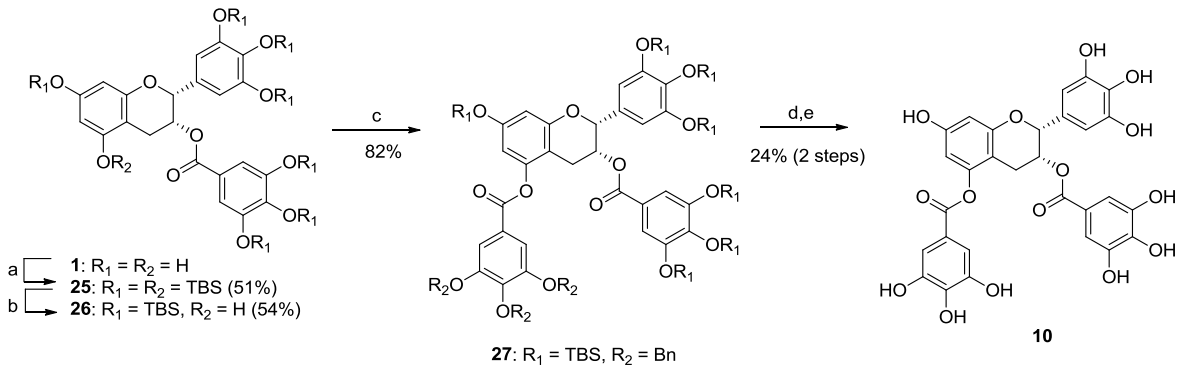
deprotection of the TBS moiety with triethylamine trihydrofluoride to give **23** with a 14% yield (2 steps). Compound **22**, derived from **14**, was purified to afford pure product with an 89% yield. TBS-deprotection of **22** with triethylamine trihydrofluoride gave **24** with a 53% yield. **23** and **24** were hydrocracked of benzyl ether to give the 3,7-digalloyl derivatives, 3,7-digalloyl-(–)-epicatechin (**7**) and 3,7-digalloyl-(+)-catechin (**9**), with 54% and 92% yields, respectively (Scheme 2).

3,5-Digalloylated (–)-epigallocatechin (**10**), the compound used to evaluate the enhancing effect of the three hydroxyl groups on the B-ring for inhibitory activity against HeLa S3 cell proliferation, was synthesized as shown in Scheme 3. Compound **10**, one of the commercially available EGCG derivatives, was reported to be a minor constituent of green tea leaf [5] and Oolong tea [13] with a variety of biological activities.

The eight phenolic hydroxyl groups of (–)-EGCG (**1**) were all protected by TBS groups to give **25** with a 51% yield. Thereafter, regioselective deprotection of the 5-TBS ether on the A-ring of **25** proceeded smoothly to give the 5-hydoroxyl compound **26** with a 54% yield. **26** was condensed with 3,4,5-tribenzyloxybenzoic acid by DCC to give digalloyl derivative **27**. Because **26** was inseparable from the DCC residue, purification was performed after deprotection of the TBS ethers. TBS-deprotection of **27** with triethylamine trihydrofluoride gave 3,5-digalloylated (–)-epigallocatechin (**10**) with a 24% yield (2 Steps) (Scheme 3).

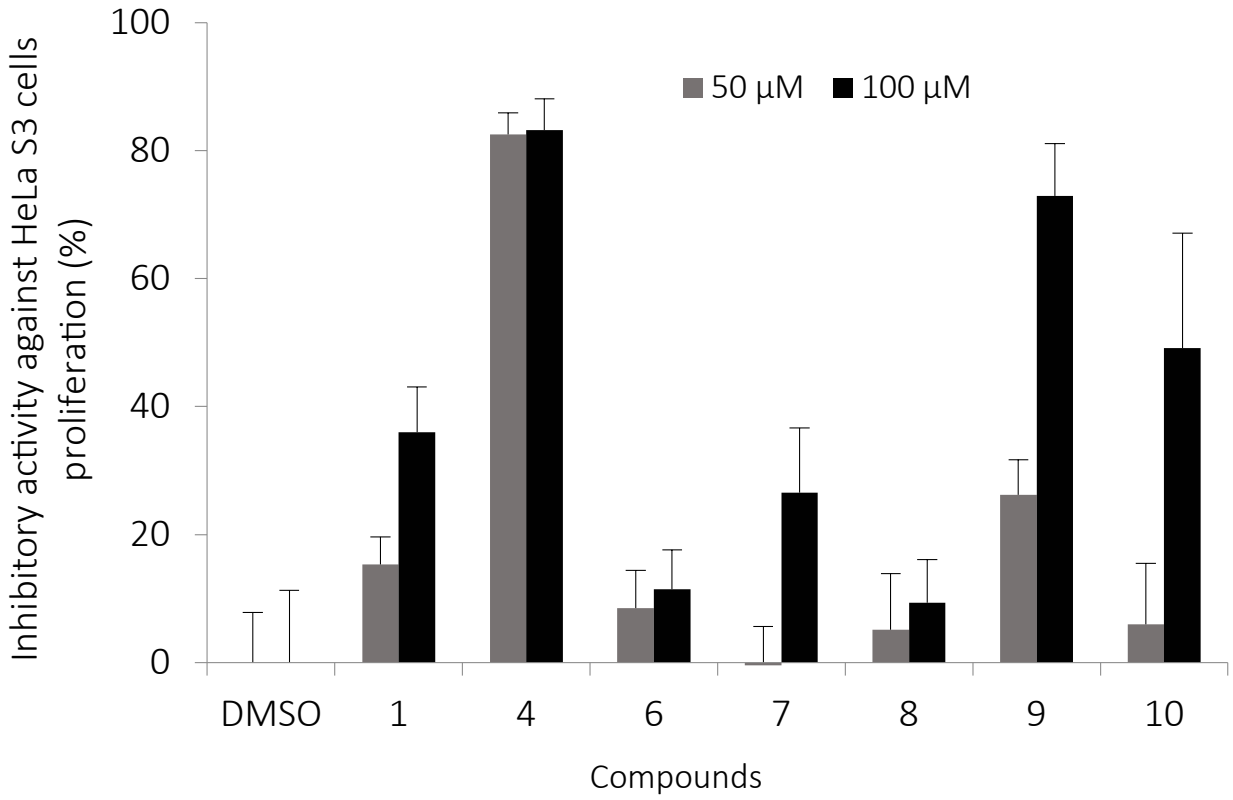
Inhibitory activity against cervical epithelioid carcinoma cell line, HeLa S3, proliferation

The inhibitory activities of the synthesized flavan-3-ol derivatives against HeLa S3 cell proliferation are shown in Figure 4. As noted previously, compound **8** was reported to moderately inhibit the proliferation of B16F10 [8], HeLa [8], and MK-1 [8] cells and to be a moderately cytotoxic compound to HepG2 [9] and MRC-5 cells [11]. Compounds **6–10** exhibited significantly lower activity compared to 3,5-digalloyl-(–)-epicatechin (**4**). The IC_{50} values of **4** and **9** were 26.0 μM and 85.2 μM, respectively. Surprisingly, 3,5-digalloylated



Reagent and conditions: (a) TBSCl, imidazole, DMF/THF, 0°C to r.t., 48 h; (b) TFA, DCM, 0°C to r.t., 2 h; (c) 3,4,5-tribenzyloxybenzoic acid, DCC, DMAP, DCM, 0°C to r.t., 48 h; (d) Triethylamine trihydrofluoride, THF, 0°C to. r.t., 12 h; (e) H_2, $Pd(OH)_2$/C, THF/H_2O/MeOH, r.t., 18 h.

Scheme 3: Synthesis of 3,5-digalloylated (–)-epigallocatechin (**10**).



HeLa S3 cells were incubated with 1 µL of each compound in 10% dimethyl sulfoxide (DMSO) solution at final concentrations of 50 and 100 µM (n=8) for 48 h. Plain 10% DMSO solution was used as a control. Error bars represent standard deviation of the mean (*n*=8).

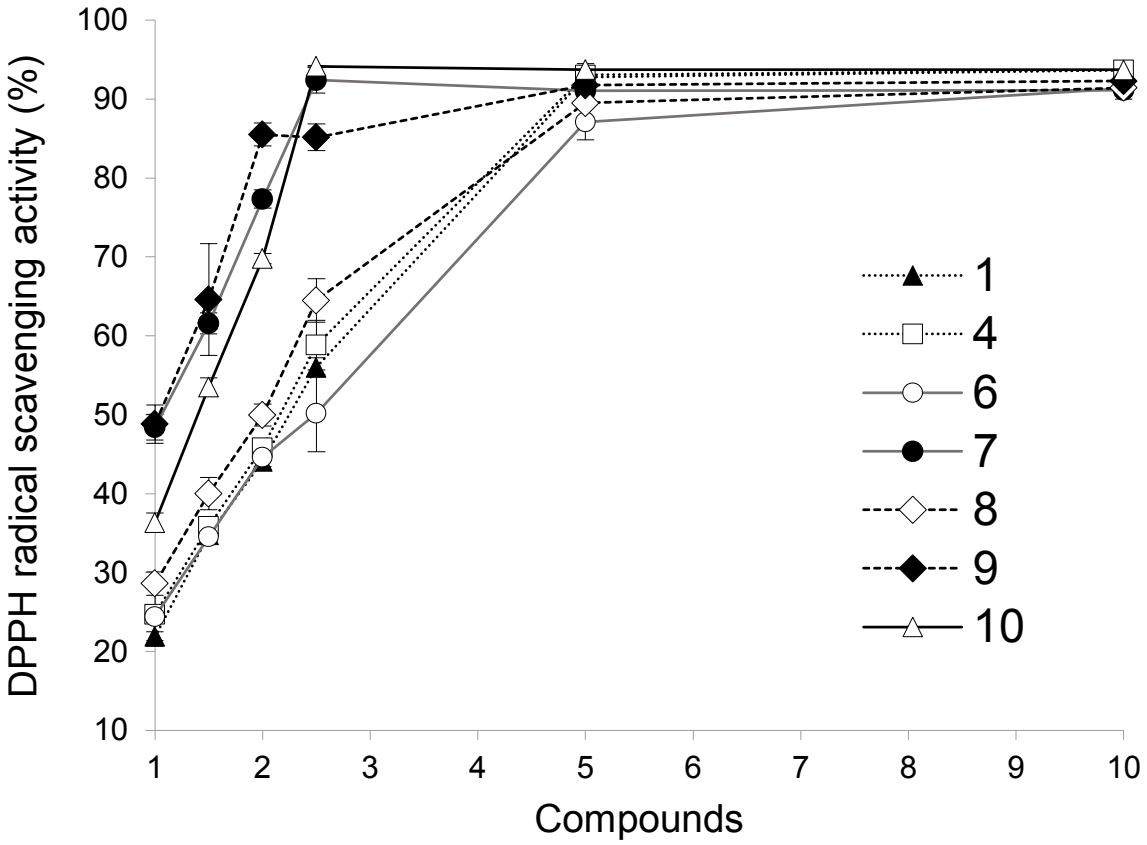
Figure 4: Inhibitory activity of flavan-3-ol derivatives against HeLa S3 cells.

(–)-epigallocatechin (**10**), in which the number of hydroxyl groups on the B-ring was greater than that in **4**, did not efficiently inhibit cell proliferation. Compound **10** was identified as a strong inhibitor of lymphoid tyrosine phosphatase [14], but HeLa S3 cell proliferation was not suppressed by **10** in our assay system.

These results demonstrated that the galloyl moiety at the 5-position and the two hydroxyl groups on the B-ring of compound **4** were significant for the inhibition of HeLa S3 cell proliferation. Our recent report described the synthesis and SAR studies of dimeric flavan-3-ols, which consisted of (–)-epicatechin (**2**) and (+)-catechin (**3**) components, and the fine structure of flavan-3-ols contributed to inhibitory activity against HeLa S3 cell proliferation [15]. In the SAR study using monomeric flavan-3-ol, slight structural differences greatly influenced the cell proliferation inhibitory properties.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

Polyphenol compounds are strong antioxidants and radical scavengers. In previous studies, we reported the DPPH radical scavenging activity of synthesized flavan-3-ols, oligomeric flavan-3-



One mL of 30 μM DPPH solution in EtOH was added to 1 μL of sample solution in dimethyl sulfoxide (DMSO) at final concentrations of 1, 1.5, 2, 2.5, 5, and 10 μM (*n*=6). Vitamin E was used as a control. Error bars represent standard deviation of the mean (*n*=6).

Figure 5: DPPH radical scavenging activity of synthesized compounds.

ols, and their derivatives to investigate the relationship between radical scavenging activity and structure [2,15,16]. We reported previously that the correlation between the number of phenolic hydroxyl groups with DPPH radical scavenging ability of 5-galloyl series of (–)-epicatechin and (+)-catechin and dimeric flavan-3-ol series was low [3,15]. In Figure 5, the DPPH radical scavenging activities of compounds **1**, **4**, and **6**–**10** at final concentrations of 1, 1.5, 2, 2.5, 5, and 10 μM are shown. The SC_{50} values (the concentration of 50% scavenging activity) of these compounds were 2.2, 2.1, 2.8, 1.4, 2.3, 1.3, and 1.4 μM, respectively. The SC_{50} values of these compounds indicated that the number of phenolic hydroxyl groups was relatively correlated with DPPH radical scavenging activity and suggested that 3,7-digalloyl compounds **7** and **9** were good scavengers of the DPPH radical compared to compounds **4**. Furthermore, the three phenolic hydroxyl groups on the B-ring were important for radical scavenging activity based on a comparison between compound **4** and **10**. These results suggested that DPPH radical scavenging activity was affected by the galloyl-moiety-introduced position. In addition, we confirmed that the DPPH radical scavenging activity of synthesized compounds poorly correlated with inhibitory activity against HeLa S3 cell proliferation.

Conclusion

We synthesized 7-galloyl- and 3,7-digalloyl-(–)-epicatechin and (+)-catechin series **6**–**9** using 7-TBS deprotection in basic conditions to evaluate which position was most effective for modification with the galloyl moiety to strength the inhibition of HeLa S3 cell proliferation. The 3,7-digalloyl derivatives inhibited HeLa S3 proliferation to a lesser extent than 3,5-digalloyl-(–)-epicatechin (**4**). In addition, we synthesized 3,5-digalloyl- (–)-epigallocatechin (**10**) using 5-TBS deprotection in acidic conditions to evaluate the enhancing effect of the three-hydroxyl groups on the B-ring. Contrary to our expectations, 3,5-digalloyl-(–)-epigallocatechin inhibited HeLa S3 cell proliferation to a lesser extent than 3,5-digalloyl-(–)-epicatechin. The DPPH radical scavenging activity of the synthesized compounds indicated that the galloyl-modified position was affected by the galloyl-moiety-introduced position. In this study, we found that 3,5-digalloyl-(–)-epicatechin had the superlative inhibitory effect among the galloylated flavan-3-ol derivatives we synthesized. These experiments using synthesized galloyl-modified flavan-3-ols, which are usually difficult to obtain from plants at the same time, indicated that the structure of 3,5-digalloyl and the two hydroxyl groups on the B-ring were important for inhibitory activity against HeLa S3 cell proliferation.

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Author Contributions

T.H. and Y.H. synthesized all compounds for biological assays; T.H. and S.K. measured the inhibitory activity against HeLa S3 cell proliferation and the DPPH scavenging activity; M.H., N.N., T.K. and A.S. supervised the study; and T.H. and A.S. wrote the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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