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Stereoselective synthesis of procyanidin B3-3-*O*-gallate and 3,3″-di-*O*-gallate, and their abilities as antioxidant and DNA polymerase inhibitor☆

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Abstract—A simple method for the synthesis of procyanidin B3 substituted with a galloyl group at the 3 and 3″ position is described. Condensation of a benzylated catechin-3-*O*-gallate electrophile with a nucleophile, catechin and catechin-3-*O*-gallate, proceeded smoothly and stereoselectively to afford the corresponding dimer gallates, procyanidin B3-3-*O*-gallate and procyanidin B3-3,3″-di-*O*-gallate, in good yields. Further, their antioxidant activities on UV-induced lipid peroxide formation, DPPH radical scavenging activity and inhibitory activity of DNA polymerase were also investigated. Among three procyanidin B3 congeners (procyanidin B3, 3-*O*-gallate and 3,3″-di-*O*-gallate), the 3,3″-di-*O*-gallate derivative showed the strongest antioxidant and radical scavenging activity. Interestingly, the 3-*O*-gallate derivative was the strongest inhibitor of mammalian DNA polymerase α with IC_{50} value of 0.26 μM, although it showed the weakest antioxidant and radical scavenging activity. It became apparent that the presence of a galloyl group at the C-3 position in the proanthocyanidin oligomer was very important for biological activity, however, the antioxidant activity of these compounds was not parallel to the DNA polymerase inhibitory activity.

1. Introduction

Proanthocyanidins, condensed tannins and/or oligomeric flavonoids,[2,3] are naturally occurring plant metabolites widely available in fruits, vegetables, nuts, seeds, flowers, and bark. They react with one-electron oxidants, resulting in powerful antioxidant activity (free-radical scavenging activity).[4] Numerous other biological activities have been reported for proanthocyanidins; for example, anti-bacterial,[5] antiviral,[6] antimutagenic,[7] anti-inflammatory,[8] hypotensive,[9] and reduction of the risk of heart diseases.[10] In addition, they have been found to inhibit lipid peroxidation, platelet aggregation, capillary permeability and fragility, and to affect enzyme systems including phospholipase A2, cyclooxygenase, and lipoxygenase.[11] The structure–activity relationship of proanthocyanidin oligomers is most important; however, it has not been proved yet, because a large number of similar isomers in the plants makes it very difficult to purify individual compounds and thus to supply extremely pure compounds necessary for biological assay. Another problem in the investigation of proanthocyanidins is that there are so many oligomers[2,3] substituted with a methyl group, a galloyl group, sugar, etc., in plants.[2,3] Many reports[12] on the isolation and semi-synthesis of procyanidin oligomers have been published thus far, but few studies concerning substituted oligomers have appeared. We previously reported a stereoselective

☆ See Ref. 1.

Keywords: Proanthocyanidin; Procyanidin B3; Galloyl ester; Antioxidant activity; DPPH radical scavenging activity; DNA polymerase inhibitor; Stereoselective synthesis.
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synthesis of procyanidin dimers[1a,13] and trimers[1b,14] consisting of (+)-catechin and (−)-epicatechin, both of which have two hydroxyl groups on the B ring. In this report, we undertook a stereoselective synthesis of procyanidin B3 derivatives substituted with a galloyl group at the C-3 and 3″ position (**2** and **3**). The bioactivities, antioxidant activities on UV-induced lipid peroxide formation, DPPH radical scavenging activity and inhibitory activity of DNA polymerases, of three procyanidin B3 congeners (procyanidin B3, 3-*O*-gallate and 3,3″-di-*O*-gallate) were investigated, and the details of their results were described (Figs. 1 and 2).

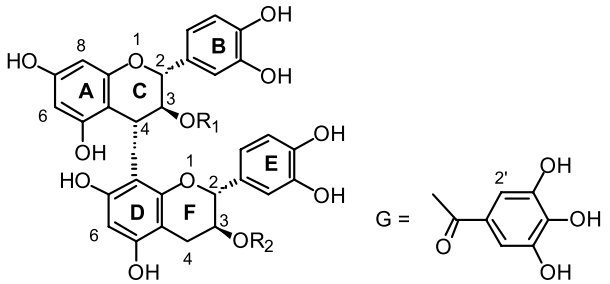


Figure 1. Structures of procyanidin B3 and its derivatives.

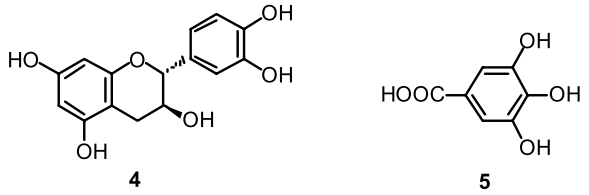


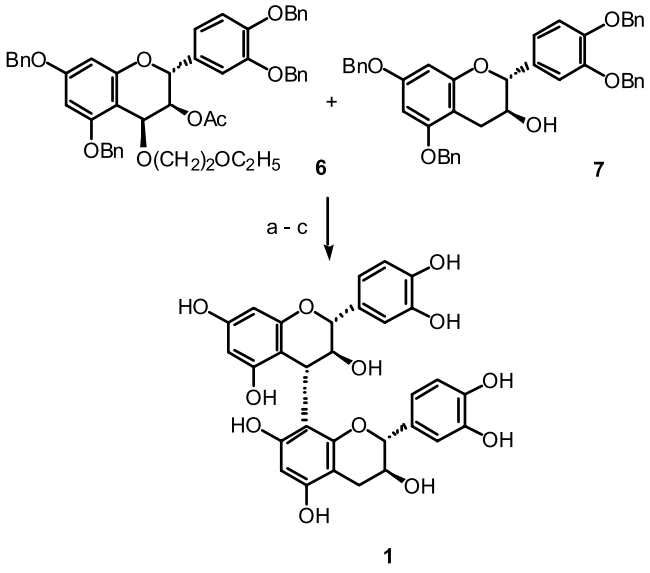
Figure 2. Structures of catechin **4** and gallic acid **5**.

2. Results and discussion

2.1. Stereoselective synthesis of procyanidin B3 substituted with a galloyl group

Many research groups have recently reported the isolation of proanthocyanidin oligomers substituted with a galloyl group and their bioactivities.[15] However, there is no systematic study of the bioactivity of various galloyl oligomers, because it is very difficult to separate purely individual structural analogues from the plant. In 1999, Tückmantel et al.[16] reported a synthesis of procyanidin B2-3,3″-di-*O*-gallate from octa-*O*-benzylprocyanidin B2 and its bioactivities. These current works stimulated us to start to find a simple systematic synthetic method for the gallate oligomers, 3-*O*-gallate and 3,3″-di-*O*-gallate derivatives.

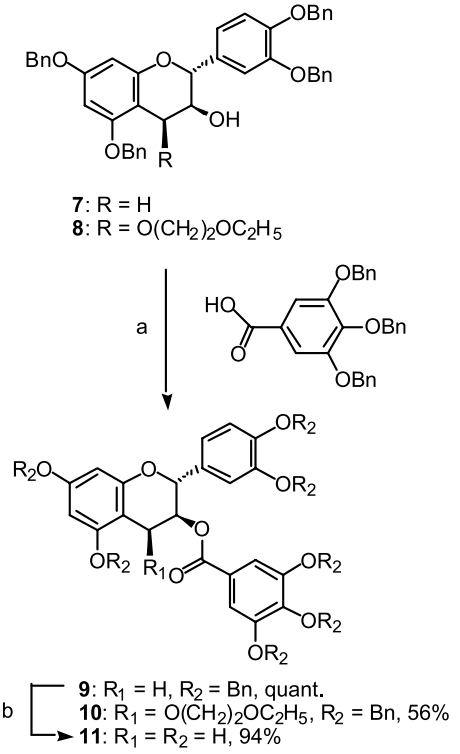
A stereoselective synthesis of procyanidin B3, (+)-catechin-(4α-8)-(+)-catechin dimer, was reported by us as shown in Scheme 1.[13a] The method is that the catechin electrophile **6** and nucleophile **7** derived from (+)-catechin **4** were condensed in the presence of TMSOTf (trimethylsilyl triflate) as a catalyst at −78 °C in CH_2Cl_2. Following deprotection and purification yielded pure procyanidin B3 **1**



Scheme 1. Synthesis of procyanidin B3. Reagents: (a) TMSOTf, CH_2Cl_2, −78 °C; (b) DIBAL, CH_2Cl_2; (c) $Pd(OH)_2/C$, H_2,THF/MeOH/H_2O.

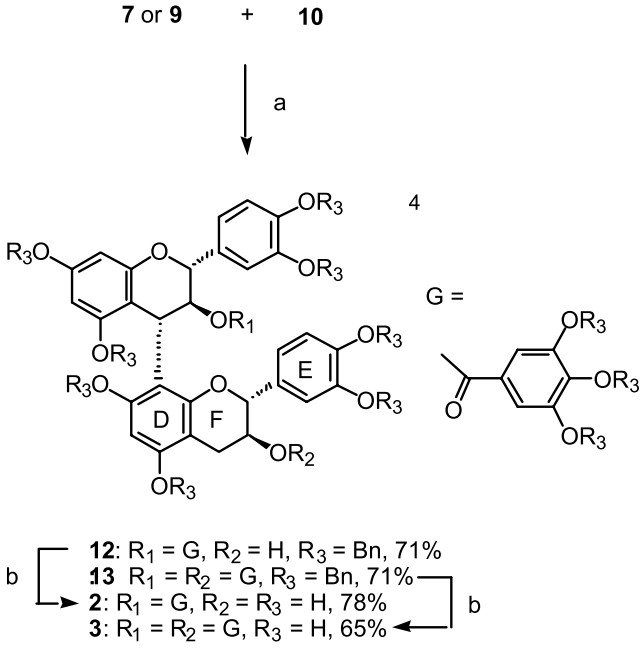
in good yield without contamination by related compounds. This stereoselective condensation reaction by the neighboring group participation effect of 3-*O*-acetate **6** prompted us to synthesize the 3-*O*-galloyl and 3,3′-di-*O*-galloyl substituted procyanidin B3.

As shown in Scheme 2, nucleophile **9** and electrophile **10** with a substituted galloyl group at the C-3 position were prepared by condensation of **7** and **8** with tri-*O*-benzyl gallic acid derived from gallic acid **5** in a quantitative yield and 56% yield, respectively. Benzylated catechin-3-*O*-gallate **9**



Scheme 2. Synthesis of 3-*O*-galloyl catechin derivatives. Reagents: (a) DCC, DMAP, CH_2Cl_2; (b) $Pd(OH)_2/C$, H_2,THF/MeOH/H_2O.

was hydrogenated with $Pd(OH)_2/C$ under hydrogen atmosphere to give (+)-catechin-3-*O*-gallate **11**. The electrophile **10** was condensed with nucleophile **7** and **9** in the presence of TMSOTf to give dimer **12** (71% yield) and **13** (71% yield)[17] and subsequent deprotection of these compounds yielded procyanidin B3-3-*O*-gallate **2** and 3,3″-di-*O*-gallate **3** in 78% and 65% yield, respectively (Scheme 3). The mono-galloyl compound **2** is reported as a natural product isolated from *Sanguisorba officinalis*.[18] The spectral data and optical rotation value of the synthetic **2** were identical with those of the natural product. Since this method is applicable to the synthesis of various galloyl oligomers, synthetic studies of other dimers consisting of (+)-catechin and (−)-epicatechin as structural components are under way. The new di-galloyl compound **3** gave satisfactory NMR and IR data together with HRMS.



Scheme 3. Synthesis of 3-*O*-galloyl procyanidin B3 derivatives. Reagents: (a) TMSOTf, CH_2Cl_2, −78 °C; (b) $Pd(OH)_2/C$, H_2,THF/MeOH/H_2O.

2.2. Antioxidant activity and DPPH radical scavenging activity

Proanthocyanidins are known as a strong antioxidant and radical scavenger as described above. In our previous research,[1b] we investigated the antioxidant activity of dimers and trimers, and it became apparent that antioxidant activity was not influenced by the length of the oligomer chain.[19] Then, we examined the effect of the galloyl moiety on antioxidant and radical scavenging activity. The antioxidant activity[1b] of compound **1**, **2**, **3**, **4**, **5**, **11** and DL-α-tocopherol on UV-induced lipid peroxide formation using the TBA method is shown in Table 1.

The IC_{50} values (concentration of 50% inhibitory activity) of these compounds were 21, 57, 18, 37, 200, 22 and 580 μM, respectively. On the other hand, the SC_{50} values (concentration of 50% scavenging activity) of the DPPH radical scavenging activity[20] were 1.3, 3.2, 1.1, 2.6, 2.4, 1.7 and 17 μM, respectively. The tendency of antioxidant activity was similar to that of DPPH radical scavenging activity. The strongest activity was revealed by **3**, procyanidin B-3,3″-di-*O*-gallate, and the weakest activity was that of **2**, procyanidin B3 3-*O*-gallate, in both experiments. Surprisingly, the activity of dimeric 3-*O*-gallate **2** was lower than that of monomeric 3-*O*-gallate **11** and (+)-catechin **4**. These results substantiated the data obtained in our previous experiment using oligomers and monomers with no ester linkage at the C-3 position.[1b]

Table 1. Inhibitory activity of synthetic proanthocyanidins on lipid peroxidation by the TBA and the DPPH method

Entry	Compound	IC_{50} (μM) by TBA method	SC_{50} (μM) by DPPH method
1	**1**	21	1.3
2	**2**	57	3.2
3	**3**	18	1.1
4	**4**	37	2.6
5	**5**	200	2.4
6	**11**	22	1.7
7	DL-α-Tocopherol	580	17

2.3. Effects of galloyl-substituted compounds on the inhibitory activities of mammalian DNA polymerase α and β

Monomeric flavan-3-*O*-gallates, (−)-epicatechin-3-*O*-gallate, (−)-epigallocatechin-3-*O*-gallate, etc., that occur in green tea, are known as inhibitors of DNA and RNA polymerases,[21] and it was apparent that a galloyl group is essential for the inhibitory effect, because flavan-3-ols without galloyl group were not effective for these inhibitory activities. DNA polymerases, especially DNA polymerase α, are regarded as the target of some anticancer drugs because DNA polymerases play central roles in DNA replication which is indispensable for the proliferation of cancer cells. These facts allowed us to expect galloyl-substituted procyanidin dimers to be inhibitors of DNA polymerases.

Table 2 shows the IC_{50} values of catechin-induced compounds (compounds **1**, **2**, **3** and **11**) against calf DNA polymerase α and rat DNA polymerase β. DNA polymerase α and β are replicative and repair-related DNA polymerases in nuclei, respectively.[22] These compounds did not inhibit DNA polymerase β activity, but inhibited DNA polymerase α activity. The inhibition by each compound was dose-dependent. Interestingly, compound **2** with the weakest antioxidant and radical scavenging activity, is the strongest inhibitor of DNA polymerase α. Further, compound **3** and **11** were more effective for inhibiting DNA polymerase α

Table 2. IC_{50} values of enzymatic inhibition against mammalian DNA polymerase α and β

Entry	Compound	DNA polymerase α, IC_{50} (μM)	DNA polymerase β, IC_{50} (μM)
1	**1**	36.4	>100
2	**2**	0.26	>100
3	**3**	8.1	>100
4	**4**	>100	>100
5	**5**	>100	>100
6	**11**	13.8	>100

activity than compound **1**. These results obtained from the inhibitory activity experiment using DNA polymerases suggest that (1) galloyl catechin is effective for the selective inhibition against DNA polymerase α; (2) galloyl group is important for their inhibitory activity; (3) the inhibitory activity is independent of their antioxidant activity and radical scavenging activity.

3. Conclusion

We have developed an efficient synthetic method for the galloyl-substituted procyanidin B3 at the 3 and 3″ position. Their antioxidant activity, DPPH radical scavenging activity and DNA polymerase inhibitory activity were investigated. From the results of these activity tests, it became apparent that antioxidant activity and DPPH radical scavenging activity were not influenced by the oligomer length, the presence of a galloyl group in proanthocyanidin was important for their bioactivity and antioxidant activity which were independent of DNA polymerase inhibition. Systematic synthesis of other structurally related compounds and their bioactivity tests is now under way.

4. Experimental

4.1. Synthesis

Optical rotation was measured with a Horiba SEPA-300 spectrometer. IR spectra were measured with a Shimadzu OR-8000 spectrometer. ^{1}H NMR spectra were measured with JEOL JNMLA400 spectrometer at rt, and MS spectra were recorded with a JEOL JMS-AX500 instrument. HPLC purification was carried out on a Mightysil® RP-18 GP column (Kanto Chemical Co. Inc, Japan; 250×20 mm, 5 μm) using the solvents (A) 0.05% CF_3CO_2H in CH_3CN and (B) 0.05% CF_3CO_2H in H_2O. Elusion was done with a linear gradient 5 to 100% A in 40 min (flow rate, 3.0 mL/min).

4.1.1. (2*R*,3*S*)-5,7,3′,4′-Tetra-*O*-benzylflavan-3-yl (3″,4″,5″-tri-*O*-benzyl)gallate (9). To a solution of (2*R*,3*S*)-5,7,3′,4′-tetra-*O*-benzylflavan-3-ol (**7**)[13a] (499 mg, 0.77 mmol) and 3,4,5-tri-*O*-benzylgallic acid (675 mg, 1.53 mmol) in CH_2Cl_2 (60 ml) was added DCC (315 mg, 1.53 mmol) and DMAP (5.0 mg). After stirring for 12 h at rt, the reaction mixture was quenched with water, and extracted with CH_2Cl_2. The organic phase was washed with water and brine, and dried (Na_2SO_4). Filtration, concentration and silica gel column chromatography (benzene/EtOAc, 20/1) afforded 825 mg (0.77 mmol, 100%) of **9** as a pale yellow amorphous powder; $[\alpha]_D^{24}=+38.2$ (*c* 0.98, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 7.42–7.16 (37H, m), 7.00 (1H, d, *J*=1.5 Hz), 6.90 (1H, dd, *J*=1.5, 8.3 Hz), 6.85 (1H, d, *J*=8.3 Hz), 6.30 (1H, d, *J*=1.5 Hz), 6.29 (1H, d, *J*=1.5 Hz), 5.45 (1H, dd, *J*=5.4, 8.1 Hz), 5.14–4.97 (15H, m), 3.04 (1H, dd, *J*=5.4, 16.8 Hz), 2.83 (1H, dd, *J*=8.1, 16.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 165.1, 158.9, 157.7, 156.0, 152.3, 149.0, 148.9, 142.5, 137.4, 137.1, 136.9, 136.8, 136.7, 136.5, 131.1, 128.6–127.2 (C×18), 125.0, 120.0, 114.8, 113.4, 109.1, 101.4, 94.3, 93.7, 78.3, 75.1, 71.3, 71.13, 71.08, 70.1, 69.9 (×2), 24.2; IR (neat, cm^{-1}) 3090 (w), 3065 (w), 3032 (m), 2866 (m), 2361 (w), 2342 (w), 1952 (w), 1873 (w), 1811 (w), 1699 (m), 1593 (s), 1504 (m), 1454 (m), 1385 (s), 1126 (s), 1041 (m), 917 (w), 887 (w), 758 (m); FAB-MS (*m/z*) 1097 (4.4), 1096 (8.3), 1095 ([M+Na]$^+$, 24), 1075 (6.7), 1074 (16), 1073 ([M+H]$^+$, 24), 724 (13), 723 (31), 722 (38), 634 (28), 633 (76), 632 (100), 631 (30); FAB-HRMS calcd for $C_{71}H_{61}O_{10}$ [M+H]$^+$, 1073.4265; found:1073.4260.

4.1.2. (2*R*,3*S*,4*S*)-5,7,3′,4′-Tetra-*O*-benzyl-4-(2″-ethoxyethoxy)flavan-3-yl (3″,4″,5″-tri-*O*-benzyl)gallate (10). To a solution of (2*R*,3*S*,4*S*)-5,7,3′,4′-tetra-*O*-benzyl-4-(2″-ethoxyethoxy)flavan-3-ol (**8**)[13a] (251 mg, 0.34 mmol) and 3,4,5-tri-*O*-benzylgallic acid (299 mg, 0.68 mmol) in CH_2Cl_2 (30 ml) was added DCC (140 mg, 0.68 mmol) and DMAP (5.00 mg). After stirring for 12 h at rt, the reaction mixture was quenched with water, and extracted with CH_2Cl_2. The organic phase was washed with water and brine, and dried (Na_2SO_4). Filtration, concentration and silica gel column chromatography (benzene/EtOAc, 20/1) afforded 217 mg (0.19 mmol, 56%) of **10** as a white powder, and 67 mg (27%) of the starting material **8** was recovered; $[\alpha]_D^{25}=+101.1$ (*c* 0.80, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 7.44–7.21 (37H, m), 7.08 (1H, d, *J*=1.7 Hz), 6.98 (1H, dd, *J*=1.7, 8.3 Hz), 6.83 (1H, d, *J*=8.3 Hz), 6.28 (1H, d, *J*=2.0 Hz), 6.18 (1H, d, *J*=2.0 Hz), 5.42 (1H, d, *J*=10.8 Hz), 5.36 (1H, dd, *J*=2.9, 10.8 Hz), 5.09–4.98 (15H, m), 3.86–3.81 (1H, m), 3.76–3.71 (1H, m), 3.46–3.42 (2H, m), 3.38–3.28 (2H, m), 1.04 (3H, t, *J*=6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 164.9, 161.0, 158.6, 155.8, 152.5, 149.3, 149.1, 142.7, 137.5, 137.1, 137.0, 136.6, 136.5, 130.6, 128.6–127.5 (C×19), 124.6, 121.4, 114.7, 114.4, 109.1, 103.8, 94.3, 93.9, 75.1, 74.3, 73.7, 71.4, 71.21 (×2), 71.17, 70.4, 70.1, 69.9, 68.7, 66.4, 15.2; IR (neat, cm^{-1}) 3065 (w), 3032 (m), 2928 (m), 2870 (m), 1717 (s), 1617 (s), 1592 (s), 1455 (s), 1374 (s), 1335 (s), 1266 (s), 1152 (s), 1115 (s), 1028 (s), 911 (w), 857 (w), 814 (w), 754 (s), 696 (s); FAB-MS (*m/z*) 1184 (3.1), 1183 ([M+Na]$^+$, 4.1), 1161 ([M+H]$^+$, 3.0), 1160 (2.7), 1073 (4.4), 1072 (5.9), 783 (6.1), 782 (11), 633 (12), 632 (31), 631 (50), 424 (29), 423 (100); FAB-HRMS calcd for $C_{75}H_{69}O_{12}$ [M+H]$^+$, 1161.4789; found:1161.4771.

4.1.3. (+)-Catechin-3-*O*-gallate (11). A solution of **9** (90 mg, 0.084 mmol) in 22 mL of THF/MeOH/H_2O, 20/1/1 was hydrogenated over 20% $Pd(OH)_2$/C (5 mg) for 3 h at rt. Filtration and concentration afforded a pale brown solid, which was purified by Sephadex® LH-20 column chromatography (EtOH) and HPLC purification to give 35 mg (0.079 mmol, 94%) of **11** as a colorless amorphous solid; $[\alpha]_D^{24}=+52.7$ (*c* 0.46, Me_2CO); ^{1}H NMR (400 MHz, CD_3OD) 6.95 (2H, s), 6.82 (1H, s), 6.71 (2H, s), 5.95 (1H, d, *J*=2.2 Hz), 5.93 (1H, d, *J*=2.2 Hz), 5.36 (1H, dt, *J*=5.1, 5.9 Hz), 5.05 (1H, d, *J*=5.9 Hz), 2.80 (1H, dd, *J*=5.1, 16.6 Hz), 2.70 (1H, dd, *J*=5.9, 16.6 Hz); ^{13}C NMR (100 MHz, CD_3OD) 167.5, 158.1, 157.6, 156.5, 146.4 (×2), 146.3, 146.2, 131.5, 121.4, 119.2, 116.2, 114.4, 110.1, 99.6, 96.4, 95.6, 79.3, 71.1, 24.3; IR (neat, cm^{-1}) 3350 (br s), 2979 (m), 1695 (m), 1612 (s), 1520 (m), 1453 (s), 1390 (m), 1318 (m), 1240 (s), 1198 (m), 1142 (s), 1109 (m), 1065 (m), 1036 (s), 984 (w), 876 (w), 822 (w), 766 (w); FAB-MS (*m/z*) 466 (14), 465 ([M+Na]$^+$, 30), 464 (12), 444 (13), 443 ([M+H]$^+$, 31), 442 (6.1), 441 (6.4), 331

(5.8), 330 (20), 275 (20), 274 (69), 273 (100), 272 (31); FAB-HRMS calcd for $C_{22}H_{19}O_{10}$ $[M+H]^+$, 443.0978; found:443.0972.

4.1.4. [4,8]-2,3-*trans*-3,4-*trans*:2,3-*trans*-Octa-*O*-benzyl-bi-(+)-catechin-3-*O*-(tri-*O*-benzyl)gallate (12). To a solution of **7** (117 mg, 0.18 mmol) and **10** (52 mg, 0.045 mmol) in CH_2Cl_2 (30 ml) was added dropwise TMSOTf (0.09 ml, 0.045 mmol, 0.5 M solution in CH_2Cl_2) at −20 °C. After stirring for 5 min, the pale yellow reaction mixture was quenched with sat. sodium hydrogen carbonate. The aq. solution was extracted with $CHCl_3$ and the organic phase was washed with water and brine, and dried (Na_2SO_4). Filtration, concentration and preparative silica gel TLC purification (hexane/EtOAc/$CHCl_3$, 6/1/5) afforded 55 mg (0.032 mmol, 71%) of **12** as a colorless oil; $[\alpha]_D^{25}=-155.6$ (*c* 0.44, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 0.6: 0.4 mixture of rotational isomers) major isomer: 7.45–6.65 (37.2H, m), 6.46 (0.6H, dd, *J*=1.7, 8.3 Hz), 6.24 (0.6H, d, *J*=2.2 Hz), 6.12 (0.6H, d, *J*=2.2 Hz), 8.05 (0.6H, t, *J*=9.5 Hz), 5.87 (0.6H, s), 5.12–4.66 (13.8H, m), 4.44 (0.6H, d, *J*=11.7 Hz), 4.39 (0.6H, d, *J*=11.7 Hz), 3.95–3.85 (0.6H, m), 2.90 (0.6H, dd, *J*=5.4, 16.6 Hz), 2.73 (0.6H, dd, *J*=7.4, 16.6 Hz), 1.60–1.20 (0.6H, m, OH); minor isomer: 7.45–6.65 (24.0H, m), 6.59 (0.4H, d, *J*=8.3 Hz), 6.56 (0.4H, d, *J*=1.7 Hz), 6.35 (0.4H, dd, *J*=1.7, 8.3 Hz), 6.26 (0.4H, s), 6.19 (0.4H, d, *J*=2.2 Hz), 6.14 (0.4H, d, *J*=2.2 Hz), 5.95 (0.4H, t, *J*=9.8 Hz), 5.12–4.66 (9.2H, m), 4.57 (0.4H, d, *J*=11.4 Hz), 3.35–3.28 (0.8H, m), 2.89–2.84 (0.4H, m), 2.35–2.25 (0.4H, m), 1.60–1.20 (0.4H, m, OH); ^{13}C NMR (100 MHz, $CDCl_3$, 0.6: 0.4 mixture of rotational isomers) major isomer: 164.4, 158.2, 157.8, 156.8, 156.7, 155.9, 152.6, 152.3, 152.2, 148.9, 148.82, 148.80 (C×2), 148.6, 142.2, 142.1, 137.7–136.5 (C×16), 131.5, 130.9, 128.6–126.7 (C×22), 125.7, 125.2, 120.6, 120.2, 114.9, 114.5, 113.8, 113.7, 109.2, 108.8, 108.8, 102.4, 94.9, 94.5, 91.2, 80.8, 79.7, 75.1, 75.0, 71.4–69.7 (C×7), 68.0, 35.4, 26.8; minor isomer: 164.2, 158.1, 157.7, 156.8, 155.7, 155.6, 153.9, 152.3, 152.2, 149.1, 149.0, 148.9, 148.6, 147.9, 142.4, 141.9, 137.7–136.5 (C×16), 131.1, 131.0, 128.6–126.7 (C×22), 126.0, 124.7, 120.9, 119.4, 115.7, 114.8, 114.4, 113.7, 111.3, 110.4, 108.6, 102.2, 94.9, 94.4, 91.2, 80.4, 79.9, 71.4–69.7 (C×9), 68.1, 35.1, 28.0; IR (neat, cm^{-1}) 3519 (br), 3090 (m), 3065 (m), 3033 (m), 2928 (m), 2870 (m), 1954 (w), 1877 (w), 1813 (w), 1721 (m), 1605 (s), 1514 (s), 1499 (s), 1455 (s), 1428 (s), 1381 (s), 1331 (s), 1264 (s), 1113 (s), 1065 (s), 911 (w), 853 (w), 812 (w), 737 (s), 696 (s); FAB-MS (*m*/*z*) 1281 (100), 1746 (11), 1745 (14), 1744 ($[M+Na]^+$, 7), 1724 (7), 1723 (10), 1722 ($[M+H]^+$, 9); FAB-HRMS calcd for $C_{114}H_{97}O_{26}$ $[M+H]^+$, 1721.6777; found:1721.6879.

4.1.5. [4,8]-2,3-*trans*-3,4-*trans*:2,3-*trans*-Octa-*O*-benzyl-bi-(+)-catechin-3,3″-di-*O*-(tri-*O*-benzyl)gallate (13). To a solution of **9** (418 mg, 0.39 mmol) and **10** (110 mg, 0.067 mmol) in CH_2Cl_2 (50 ml) was added dropwise TMSOTf (0.19 ml, 0.095 mmol, 0.5 M solution in CH_2Cl_2) at -20 °C. After stirring for 5 min, the pale yellow reaction mixture was quenched with sat. sodium hydrogen carbonate. The aq. solution was extracted with $CHCl_3$ and the organic phase was washed with water and brine, and dried (Na_2SO_4). Filtration, concentration and preparative silica gel column chromatography (benzene) afforded 144 mg (0.067 mmol, 71%) of **13** as a white powder; $[\alpha]_D^{22}=-43.9$ (*c* 0.42, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 0.66: 0.34 mixture of rotational isomers) major isomer: 7.40–6.62 (50.82H, m), 6.76 (0.66H, d, *J*=1.7 Hz), 6.54 (0.66H, d, *J*=8.3 Hz), 6.44 (0.66H, dd, *J*=1.7, 8.3 Hz), 6.23 (0.66H, d, *J*=2.2 Hz), 6.06 (0.66H, d, *J*=2.2 Hz), 5.96 (0.66H, s), 6.12–5.97 (0.66H, m), 5.38 (0.66H, ddd, *J*=5.6, 7.1, 7.3 Hz), 5.15 (0.66H, d, *J*=7.3 Hz), 5.13–4.53 (18.48H, m), 4.44 (0.66H, d, *J*=11.5 Hz), 4.38 (0.66H, d, *J*=11.5 Hz), 3.13 (0.66H, dd, *J*=5.6, 16.8 Hz), 2.90 (0.66H, dd, *J*=7.1, 16.8 Hz); minor isomer: 7.40–6.53 (26.86H, m), 6.50 (0.34H, dd, *J*=1.7, 8.3 Hz), 6.29 (0.34H, s), 6.18 (0.34H, d, *J*=2.2 Hz), 6.14 (0.34H, d, *J*=2.2 Hz), 6.12–5.97 (0.34H, m), 5.13–4.53 (10.54H, m), 3.63 (0.34H, d, *J*=9.3 Hz), 3.15–3.11 (0.34H, m), 2.48 (0.34H, dd, *J*=10.0, 17.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) major isomer: 165.3, 164.3, 158.3, 157.5, 157.1, 156.6, 155.5, 152.5 (×2), 152.1 (×2), 148.93, 148.88, 148.6, 142.6, 142.1, 137.5–136.5 (C×19), 131.3, 131.1, 128.6–126.7 (C×27), 125.2, 125.1, 120.4, 120.3, 114.83, 114.75, 114.2, 113.8, 113.7, 111.2, 109.1, 108.7, 108.2, 101.9, 94.8, 94.6, 90.9, 79.7, 78.2, 77.2, 75.13, 75.01, 71.6–69.8 (C×12), 35.2, 24.9; minor isomer: 165.1, 164.6, 158.1, 157.6, 156.9, 155.9, 153.8, 152.6, 152.5, 152.42, 152.36, 149.1, 148.8, 147.8, 142.5, 142.4, 137.6–136.5 (C×19), 131.1, 131.0, 128.6–126.7 (C×27), 125.5, 125.0, 120.9, 119.7, 115.9, 114.8, 114.1, 113.8, 113.7, 111.5, 109.2, 109.1, 108.7, 101.7, 94.9, 94.3, 91.3, 80.0, 78.3, 77.6, 75.1, 75.0, 71.6–69.8 (C×12), 35.1, 26.6; IR (neat, cm^{-1}) 3090 (m), 3032 (m), 2930 (m), 2870 (m), 1954 (w), 1811 (w), 1717 (s), 1592 (s), 1514 (s), 1454 (s), 1430 (s), 1375 (s), 1215 (s), 1113 (s), 1028 (s), 910 (w), 856 (w), 810 (w), 754 (s); FAB-MS (*m*/*z*) 2145 (0.1), 2144 ($[M+H]^+$, 0.1).

4.1.6. Procyanidin B3-3-*O*-gallate (2). A solution of **12** (200 mg, 0.12 mmol) in 22 mL of THF/MeOH/H_2O, 20/1/1 was hydrogenated over 20% $Pd(OH)_2$/C (5 mg) for 8 h at rt. Filtration and concentration afforded a pale brown solid, which was purified by Sephadex® LH-20 column chromatography (EtOH) and HPLC purification to give 70 mg (0.096 mmol, 78%) of procyanidin B3-3-*O*-gallate **2** as a colorless amorphous solid; $[\alpha]_D^{25}=-180.7$ (*c* 0.28, Me_2CO) {lit.[18a] $[\alpha]_D^{25}=-170.1$ (*c* 0.72, Me_2CO)}; ^{1}H NMR (400 MHz, 10% D_2O in CD_3COCD_3, 0.6: 0.4 mixture of rotational isomers) major isomer: 6.86 (1.2H, s), 6.79 (0.6H, d, *J*=1.7 Hz), 6.72 (0.6H, d, *J*=1.7 Hz), 6.69 (0.6H, d, *J*=8.3 Hz), 6.58 (0.6H, d, *J*=8.3 Hz), 6.49 (0.6H, dd, *J*=1.7, 8.3 Hz), 6.33 (0.6H, dd, *J*=1.7, 8.3 Hz), 6.19 (0.6H, dd, *J*=8.6, 10.0 Hz, C3), 6.02 (0.6H, s, D6), 5.95 (0.6H, d, *J*=2.2 Hz, C8), 5.86 (0.6H, d, *J*=2.2 Hz, C6), 4.75 (0.6H, d, *J*=7.7 Hz, F2), 4.72 (0.6H, d, *J*=8.6 Hz, C2), 4.62 (0.6H, d, *J*=10.0 Hz, C4), 3.84 (0.6H, ddd, *J*=5.6, 7.7, 8.8 Hz, F3), 2.71 (0.6H, dd, *J*=5.6, 16.3 Hz, F4), 2.52 (0.6H, dd, *J*=8.8, 16.3 Hz, F4); minor isomer: 7.00 (0.4H, d, *J*=1.7 Hz), 6.98 (0.8H, s), 6.90–6.62 (1.6H, m), 6.65 (0.4H, d, *J*=8.3 Hz), 6.16 (0.4H, dd, *J*=8.8, 10.3 Hz), 6.13 (0.4H, s), 5.87 (0.4H, d, *J*=2.2 Hz), 5.86–5.84 (0.4H, m), 4.75–4.73 (0.4H, m), 4.66 (0.4H, d, *J*=10.3 Hz), 4.43 (0.4H, d, *J*=8.8 Hz), 3.64–3.59 (0.4H, m), 2.85 (0.4H, dd, *J*=5.9, 16.1 Hz), 2.53–2.48 (0.4H, m); ^{13}C NMR (100 MHz, 10% D_2O in CD_3COCD_3, 0.6: 0.4 mixture of rotational isomers) major isomer: 165.5, 158.0, 157.6, 156.9, 155.8, 154.8, 154.7, 145.7–145.6 (C×4), 144.91, 144.85, 131.5, 130.6,

121.6, 120.4, 119.2, 115.8, 115.6, 115.4, 114.9, 109.7, 105.4, 105.3, 101.6, 97.2, 96.5, 95.7, 81.9 (C2), 81.2 (F2), 73.1 (C3), 68.1 (F3), 35.9 (C4), 29.4 (F4); minor isomer: 165.1, 156.0, 157.3, 157.0, 155.7, 155.4, 154.6, 145.7–145.6 (C×4), 145.3, 145.1, 131.5, 130.6, 121.9, 120.5, 120.0, 116.2, 116.0, 115.6, 115.2, 110.0, 105.9, 105.7, 100.3, 97.3, 97.1, 96.0, 83.3 (C2), 81.4 (F2), 73.6 (F3), 68.5 (C3), 35.7 (C4), 28.4 (F4); IR (neat, cm^{-1}) 3360 (br s), 2979 (m), 2928 (m), 1693 (m), 1613 (s), 1522 (m), 1453 (s), 1370 (s), 1285 (m), 1238 (s), 1146 (m), 1103 (m), 1038 (s), 876 (w), 818 (w), 779 (w); FAB-MS (*m/z*) 755 (21), 754 (37), 753 ($[M+Na]^+$, 47), 752 (19), 733 (26), 732 (27), 731 ($[M+H]^+$, 30), 730 (24), 614 (34), 613 (48), 482 (100); FAB-HRMS calcd for $C_{37}H_{31}O_{16}$ $[M+H]^+$, 731.1612; found:731.1600.

4.1.7. Procyanidin B3-3,3″-di-*O*-gallate (3). A solution of **13** (90 mg, 0.042 mmol) in 22 mL of THF/MeOH/H_2O, 20/1/1 was hydrogenated over 20% $Pd(OH)_2$/C (5 mg) for 5 h at rt. Filtration and concentration afforded a pale brown solid, which was purified by Sephadex® LH-20 column chromatography (MeOH) and HPLC purification to give 24 mg (0.027 mmol, 65%) of procyanidin B3-3,3″-di-*O*-gallate **3** as a colorless amorphous solid; $[\alpha]_D^{23}=-209.7$ (*c* 1.00, Me_2CO); ^1H NMR (400 MHz, 10% D_2O in CD_3COCD_3, 0.75: 0.25 mixture of rotational isomers) major isomer: 7.12 (1.5H, s, 2′), 6.95–6.93 (0.75H, m, B6), 6.90 (1.5H, s, 2′), 6.82 (0.75H, d, *J*=1.7 Hz, B2), 6.65 (0.75H, d, *J*=1.7 Hz, E2), 6.57 (0.75H, dd, *J*=1.7, 8.3 Hz, E6), 6.50 (0.75H, d, *J*=8.3 Hz, B5), 6.47 (0.75H, d, *J*=8.3 Hz, E5), 6.19 (0.75H, dd, *J*=7.1, 10.0 Hz, C3), 5.99 (0.75H, s, D6), 5.95 (0.75H, d, *J*=2.2 Hz, A8), 5.75 (0.75H, d, *J*=2.2 Hz, A6), 5.32 (0.75H, ddd, *J*=4.6, 5.1, 5.6 Hz, F3), 5.21 (0.75H, d, *J*=4.6 Hz, F2), 4.77 (0.75H, d, *J*=7.1 Hz, C2), 4.51 (0.75H, d, *J*=10.0 Hz, C4), 2.61 (0.75H, dd, *J*=5.6, 17.1 Hz, F4), 2.53 (0.75H, dd, *J*=5.1, 17.1 Hz, F4); minor isomer: 7.12–6.51 (2.5H, m), 6.19–5.81 (1.0H, m), 5.00–4.85 (0.5H, m), 4.74 (0.25H, d, *J*=8.3 Hz, C2), 4.59 (0.25H, d, *J*=10.0 Hz, C4), 2.95–2.90 (0.25H, m, F4), 2.63–2.48 (0.25H, m); ^{13}C NMR (100 MHz, 10% D_2O in CD_3COCD_3, 0.75: 0.25 mixture of rotational isomers) major isomer: 167.0, 166.2, 158.5, 157.4, 156.9, 155.4, 154.5, 153.7, 145.9, 145.8, 145.6, 145.1 (×2), 145.0, 138.9, 138.7, 130.44, 130.36, 121.2, 121.1, 120.1, 118.5, 116.1, 115.7, 115.4, 113.5, 109.9, 109.8, 106.7, 105.8, 100.1, 97.4, 96.5, 95.7, 81.5 (C2), 78.4 (F2), 75.6 (C3), 69.7 (F3), 35.3 (C4), 23.8 (F4); minor isomer: 166.1, 165.2, 158.1, 157.0, 155.8, 155.3, 154.2, 153.7, 145.9, 145.8, 145.6, 145.1 (×2), 145.0, 138.9, 138.7, 130.7, 130.6, 121.8, 121.2, 120.5, 119.2, 116.3, 115.6, 115.2, 115.1, 110.0 (×2), 106.7, 105.8, 99.0, 97.4, 96.5, 96.0, 81.5 (C2), 79.9 4 (F2), 73.7 (C3), 71.3 (F3), 35.7 (C4), 26.5 (F4); IR (neat, cm^{-1}) 3350 (br s), 2977 (s), 2910 (s), 1684 (s), 1622 (s), 1520 (m), 1456 (s), 1383 (s), 1244 (s), 1146 (m), 1090 (s), 986 (w), 878 (m), 818 (w), 768 (w); FAB-MS (*m/z*) 517 (100), 561 (83), 882 (19), 883 ($[M+H]^+$, 37), 884 (20), 903 (33), 904 (64), 905 ($[M+Na]^+$, 86), 906 (38); FAB-HRMS calcd for $C_{44}H_{34}O_{20}Na$ $[M+Na]^+$, 905.1541; found: 905.1578.

4.2. The measurement of antioxidant activity and DPPH radical scavenging activity

All of the assay samples were HPLC pure. Antioxidant activity was measured with the general TBA method.[1b] And DPPH radical scavenging activity was measured as follows:[20] A solution of DPPH radical in EtOH (30 μM, 1.0 ml) was added to 1 μl of the test sample in DMSO, and incubated at 30 °C for 30 min. The scavenging activity was estimated by measuring the absorption of the reaction mixture at 517 nm with the microplate reader (Model 3550, BIO-RAD).

4.3. DNA polymerase assays

All of the assay samples were HPLC pure. DNA polymerase α was purified from calf thymus by immuno-affinity column chromatography as described previously.[23] Recombinant rat DNA polymerase β was purified from *E. coli* JMpβ5 as described by Date et al.[24] The reaction mixtures for DNA polymerase α and β were described previously.[25] The substrates of DNA polymerases used poly(d*A*)/oligo(d*T*)$_{12-18}$ and deoxythymidine triphosphates (dTTP) as template-primer DNA and nucleotide substrate, respectively. The synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) at various concentrations and sonicated for 30 s. Four μl of the sonicated samples were mixed with 16 μl of each enzyme (final 0.05 units) in 50 mM Tris–HCl (pH 7.5) containing 1 mM dithiothreitol, 50% glycerol and 0.1 mM EDTA, and kept at 0 °C for 10 min. These inhibitor–enzyme mixtures (8 μl) were added to 16 μl of each of the enzyme standard reaction mixtures, and incubation was carried out at 37 °C for 60 min. The activity without the inhibitor was considered to be 100%, and the remaining activities at each concentration of inhibitor were determined as percentages of this value. One unit of each DNA polymerase activity was defined as the amount of enzyme that catalyzes the incorporation of 1 nmol of deoxyribonucleotide triphosphates (i.e. dTTP) into synthetic template-primers (i.e. poly(d*A*)/oligo(d*T*)$_{12-18}$, *A*/*T*=2/1) in 60 min at 37 °C under the normal reaction conditions for each enzyme.[25]

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