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## **SYNTHESIS OF GALLOYL-SUBSTITUTED PROCYANIDIN B4 SERIES, AND THEIR DPPH RADICAL SCAVENGING ACTIVITY AND DNA POLYMERASE INHIBITORY ACTIVITY<sup>1</sup>**

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**Abstract** – Synthesis of galloyl-substituted procyanidin B4 series, 3-*O*-gallate, 3''-*O*-gallate and 3,3''-di-*O*-gallate, is described. Condensation of the electrophile derived from (+)-catechin with the nucleophile derived from (-)-epicatechin in the presence of TMSOTf as a catalyst gave procyanidin B4 3-*O*-gallate and 3,3''-di-*O*-gallate in good yields. Procyanidin B4 3''-*O*-gallate and procyanidin B3 3''-*O*-gallate were synthesized by the DCC method. Their DPPH radical scavenging activity and DNA polymerase inhibitory activity were also investigated. The results indicated that the presence of the galloyl moiety of these compounds is very important for both activities.

## **INTRODUCTION**

Gallic acid-substituted compounds are known as one of the strongest bioactive groups among the polyphenols. Many research groups have reported the isolation and bioactivities of various types of galloyl compounds,<sup>2,3</sup> and their strong bioactivities have recently received increasing attention. For example, it is known that epigallocatechin-3-*O*-gallate (EGCG), the major polyphenol in green tea which belongs to the flavan-3-ol group, has protective effect against a variety of cancer types, such as lung,

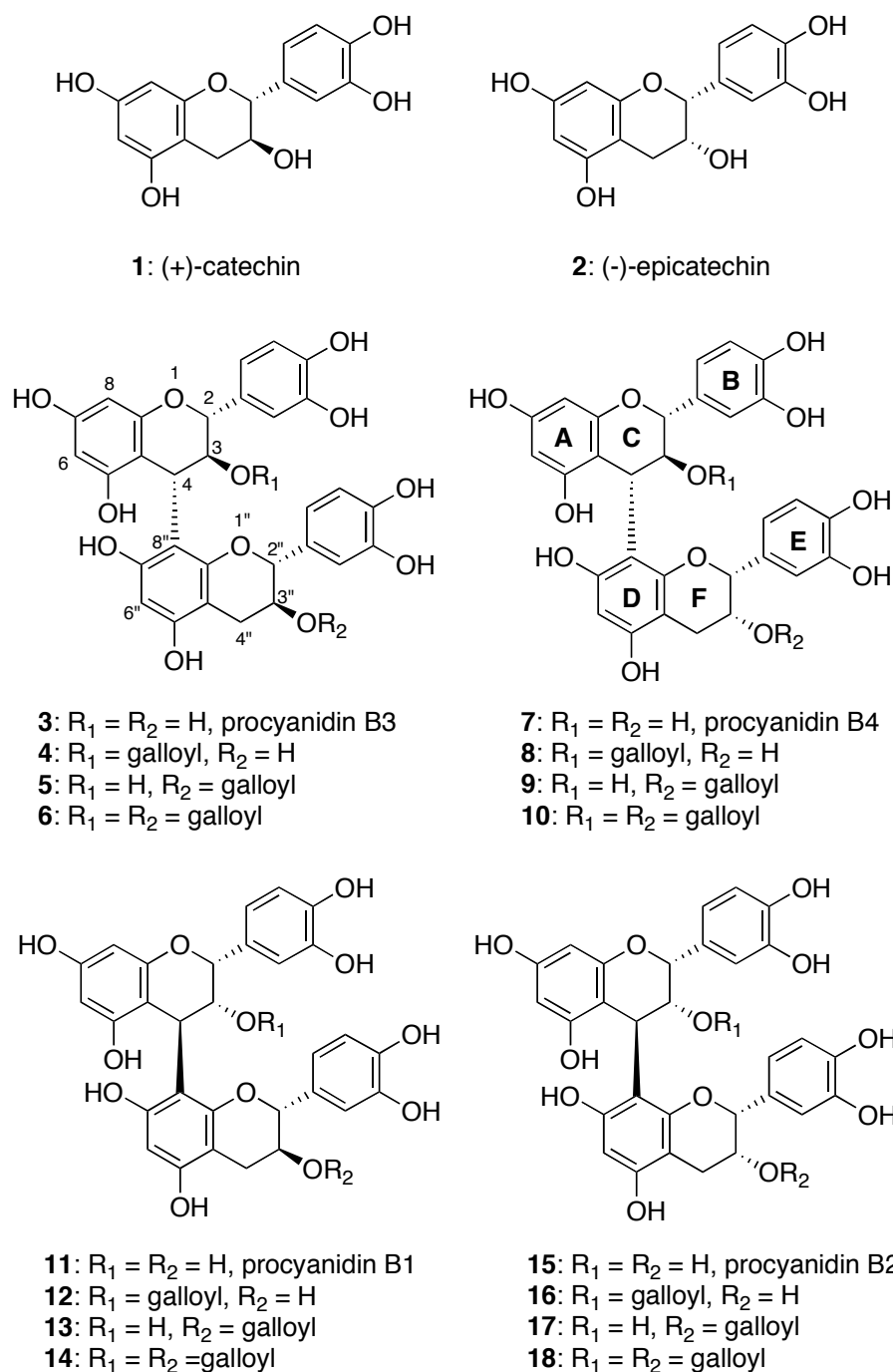
prostate and breast.<sup>4</sup> In more recent research, the receptor that mediates the anticancer activity of EGCG was identified.<sup>5</sup> We have been interested in the strong antioxidant activity of proanthocyanidins caused by the reaction with one-electron oxidants (free-radical scavenging activity),<sup>6</sup> and we started a synthetic study of proanthocyanidins to investigate their structure-activity relationships (SAR). Although their SAR is most important, it has not been proved yet, because the presence of a large number of structurally similar isomers in the plants makes it very difficult to purify individual compounds, and thus to supply extremely pure compounds necessary for biological assay. Therefore, we decided to provide the pure compounds by developing stereoselective synthesis.

Many reports on structural determination, biological activities and semi-synthesis of procyanidin oligomers have been published thus far, but few studies concerning substituted oligomers have appeared.<sup>7</sup> In the last three years, we have reported several synthetic studies of procyanidins based on both intermolecular<sup>1,8</sup> and intramolecular<sup>9</sup> condensation method. The stereoselective synthesis of procyanidin dimers,<sup>6,8a-c</sup> trimers<sup>8d,e</sup> consisting of (+)-catechin (**1**) and (-)-epicatechin (**2**) was achieved and their bioactivities including antioxidant activity in the TBA methods,<sup>1b,8c</sup> DPPH radical scavenging activity,<sup>1</sup> the Maillard reaction inhibitory activity,<sup>8c,e</sup> were investigated. Very recently, we reported an effective systematic synthesis of galloyl-substituted procyanidin B1-B3 series (B1 series: **11-14**, B2 series: **15-18**, and B3 series: **3, 4** and **6**) with a simple condensation method using TMSOTf as a catalyst, and also described their antioxidant activity and DNA polymerase inhibitory activities.<sup>1</sup> In this report, we undertook a synthesis of the galloyl-substituted analogues, procyanidin B4, procyanidin B4 3-*O*-gallate (**8**), procyanidin B4 3''-*O*-gallate (**9**), procyanidin B4 3,3''-di-*O*-gallate (**10**), and procyanidin B3 3''-*O*-gallate (**5**). This is the final report concerning SAR for the [4-8]-condensed procyanidin B series. We summarized which combination between (+)-catechin (**1**) and (-)-epicatechin (**2**) possessing the gallate-substituent at the C3-position was the most appropriate for more prominent biological activity. We describe herein the details of the results obtained by the assays of the procyanidin B3/B4 congeners (**5, 7-10**) regarding DPPH radical scavenging activity and inhibitory activity against DNA polymerases.

## RESULTS AND DISCUSSION

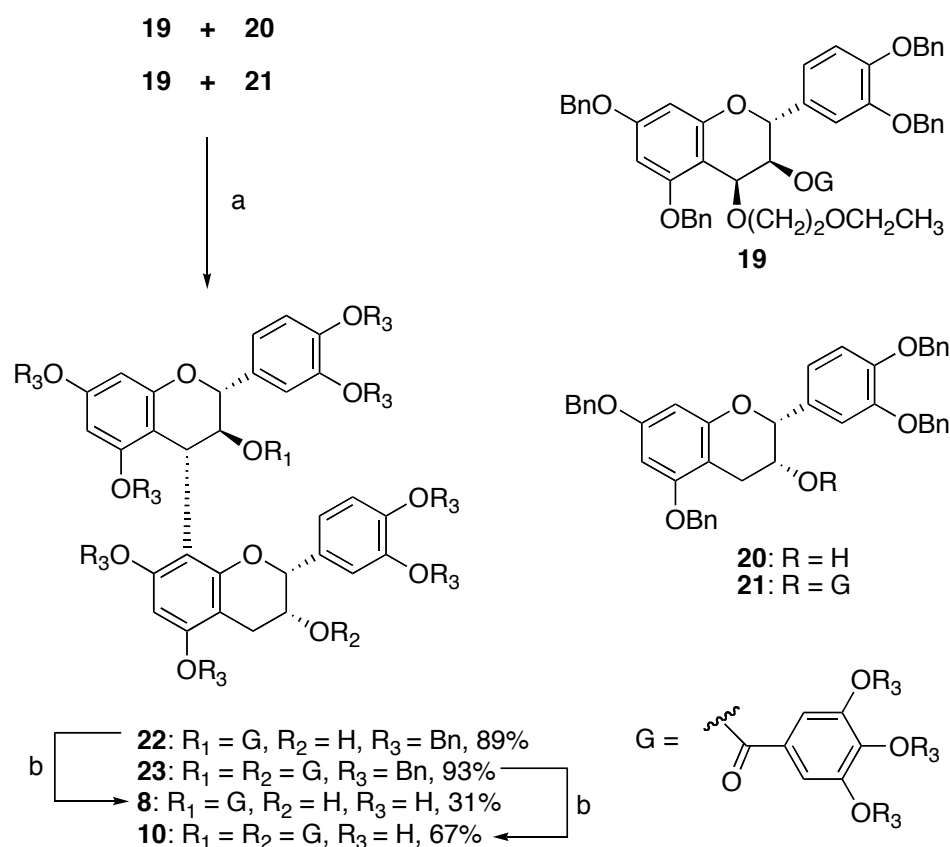
### Synthesis of galloyl-substituted procyanidin B4 series.

We report here a synthesis of galloyl-substituted procyanidin B4 series including procyanidin B4 3-*O*-gallate (**8**) and procyanidin B4-3,3''-di-*O*-gallate (**10**) by using our condensation methodology, in addition, procyanidin B3 3''-*O*-gallate (**5**) and procyanidin B4 3''-*O*-gallate (**9**) by the DCC method to clarify the relationships between the structure and biological activities.



**Figure 1.** Structure of procyanidin dimers consisting from (+)-catechin (**1**) and (-)-epicatechin (**2**)

Synthesis of **8** and **10** was achieved as shown in **Scheme 1**. The electrophile (**19**) was condensed with nucleophile (**20**) in the presence of TMSOTf as a catalyst at  $-20^{\circ}\text{C}$  to give undecabenzyl procyanidin B43-*O*-gallate (**22**) in 89% yield. Similarly, tetradecabenzyl-procyanidin B4 3,3''-di-*O*-gallate (**23**) was obtained by the condensation of electrophile (**19**) with nucleophile (**21**) under the same conditions in 93% yield. The following deprotection and purification yielded pure procyanidin B4 3-*O*-gallate (**8**) and 3,3''-di-*O*-gallate (**10**) in 31% and 67% yields, respectively. The structure confirmation of these compounds



**Reagents:** (a) TMSOTf,  $CH_2Cl_2$ ,  $-20^\circ C$ ; (b) 20%  $Pd(OH)_2/C$ ,  $H_2$ , THF/MeOH/ $H_2O$ .

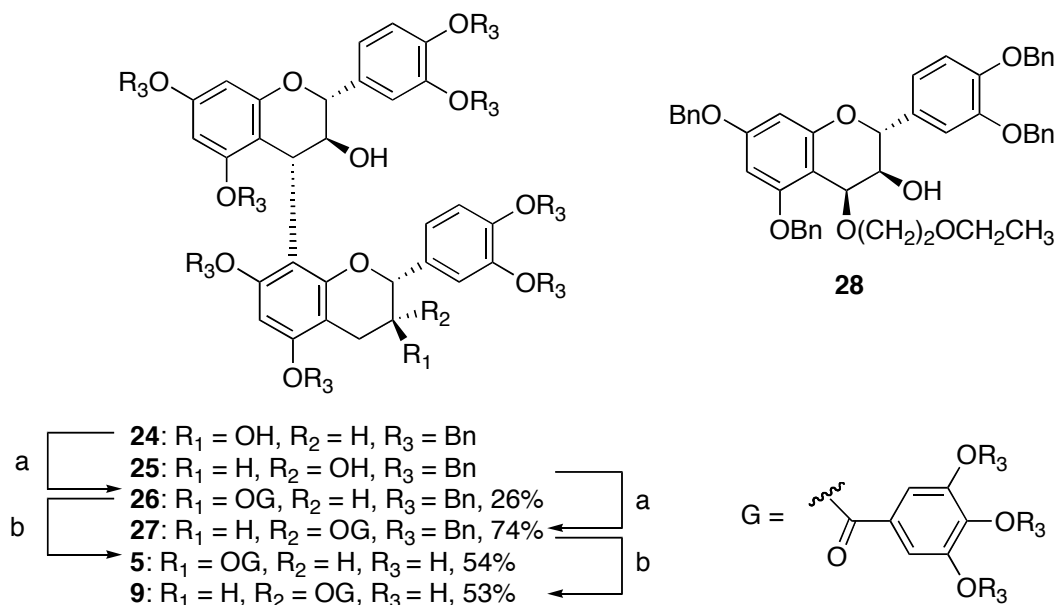
**Scheme 1.** Synthesis of procyanidin B4 3-*O*-gallate and procyanidin B4 3,3''-di-*O*-gallate.

was accomplished by the NMR measurement even though the NMR spectra of these compounds exhibit multiplicity due to rotational isomerism.<sup>10</sup> The new compounds (**8** and **10**) gave satisfactory NMR ( $^1H$  NMR,  $^{13}C$  NMR) and FAB-MS data.

Benzylated procyanidin B3 3''-*O*-gallate (**26**) and benzylated procyanidin B4 3''-*O*-gallate (**27**) were also synthesized using the DCC method as shown in **Scheme 2**. The TMSOTf-catalyzed condensation method is inapplicable to **24** and **25**, because the condensation using an electrophile (**28**) without the ester group at the C-3 position leads to formation of a mixture of 3,4-*trans* and 3,4-*cis* dimers with low stereoselectivity.<sup>8a,b</sup> Condensation of octabenzyl-procyanidin B3 (**24**) and octabenzyl-procyanidin B4 (**25**) with 3,4,5-tri-*O*-benzylgallic acid by the DCC method gave **26** and **27** in 26%<sup>11</sup> and 74% yields, respectively. Hydrogenation of the benzyl groups and purification gave pure **5** and **9** in moderate yields. The procyanidin B1 3-*O*-gallate (**9**) is reported as a natural product isolated from green tea leaf.<sup>12</sup> The optical rotation value of the synthetic **9**,  $[\alpha]_D^{25} = -279.1^\circ$  ( $c$  0.095, acetone), is identical with those of the natural product,  $[\alpha]_D^{22} = -255.7^\circ$  ( $c$  0.56, acetone). The structure confirmation was accomplished by the NMR measurement in the presence of rotational isomers.

### DPPH radical scavenging activity

Proanthocyanidins are known as strong antioxidants and radical scavengers. In our previous papers,<sup>1</sup> we investigated DPPH radical scavenging activity of procyanidin B1-B3 series including galloyl-substituted



**Reagent:** (a) DCC, DMAP, CH<sub>2</sub>Cl<sub>2</sub>; (b) 20 % Pd(OH)<sub>2</sub>/C, H<sub>2</sub>, THF/MeOH/H<sub>2</sub>O.

### Scheme 2 Synthesis of procyanidin B3 3''-O-gallate and procyanidin B4 3''-O-gallate.

compounds, and it became apparent that the procyanidin B3 series acted as radical scavengers with a different mode from the procyanidin B1 and B2 series. The radical scavenging ability of the procyanidin B3 series was much weaker than that of the procyanidin B1 and B2 series. Then, we examined the effect of the procyanidin B3 3''-O-gallate (**5**) and the galloyl-substituted procyanidin B4 series (**7-10**) that were constituted of **1** and **2**, on DPPH radical scavenging activity. The results are shown in **Table 1**. As a control compound, DL- $\alpha$ -tocopherol was used (Entry 6).

**Table 1.** DPPH radical scavenging activity

Entry	Compound	SC <sub>50</sub> ( $\mu$ M)
1	<b>5</b>	1.1
2	<b>7</b>	2.0
3	<b>8</b>	0.9
4	<b>9</b>	0.8
5	<b>10</b>	0.9
6	DL- $\alpha$ -Tocopherol	17

The SC<sub>50</sub> values (concentration of 50% scavenging activity) of those compounds were 1.1 (**5**), 2.0 (**7**), 0.9 (**8**), 0.8 (**9**) and 0.9  $\mu$ M (**10**), respectively. It appeared that the radical scavenging activity tended to be

stronger with the presence of a galloyl group. The  $SC_{50}$  values of the procyanidin B1 series (**11-14**), procyanidin B2 series (**15-18**) and procyanidin B3 series (**3, 4** and **6**) were described in a previous paper as 1.3 (**11**), 0.8 (**12**), 0.7 (**13**), 0.6 (**14**), 1.2 (**15**), 0.8 (**16**), 0.6 (**17**), 0.6 (**18**), 1.3 (**3**), 3.2 (**4**) and 1.1  $\mu\text{M}$  (**6**), respectively.<sup>1</sup> As compared with the results in **Table 1**, the procyanidin B1/B2 series scavenged DPPH radical much more strongly than the procyanidin B3/B4 series. These results suggest that the galloyl group of the procyanidin B1/B2 series is more effective for radical scavenging activity than that of the procyanidin B3/B4 series. It appeared that the presence of a (-)-epicatechin unit was very important and the (-)-epicatechin unit on the upper position of the dimer was more effective for radical scavenging activity by comparing B4 series with B1 series. In addition, it was suggested that the galloyl group not at the C3-position, but at the C3''-position was more effective.

### Effects of galloyl-substituted compounds on the inhibitory activities against mammalian DNA polymerase $\alpha$ and $\beta$

DNA polymerases, especially DNA polymerase  $\alpha$  which is a DNA replicative polymerase, 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. 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,<sup>13</sup> and flavan-3-ols without a galloyl group were not effective for this polymerase inhibitory activity. These facts allowed us to expect galloyl-substituted procyanidin dimers to be effective inhibitors of DNA polymerases. In a previous paper, we described the ability of the procyanidin B1-B3 series as DNA polymerase inhibitor. The  $IC_{50}$  values (concentration of 50% inhibitory activity) for DNA polymerase  $\alpha$  of **11-18, 3, 4** and **6** were 25.4 (**11**), 0.72 (**12**), 0.89 (**13**), 0.23 (**14**), 24.0 (**15**), 0.70 (**16**), 0.85 (**17**), 0.24 (**18**), 36.4 (**3**), 0.26 (**4**) and 0.12  $\mu\text{M}$  (**6**)<sup>14</sup>, respectively.<sup>15</sup> Interestingly, the di-*O*-gallate compounds (**6, 14** and **18**) have inhibitory ability against DNA polymerase  $\beta$ . The  $IC_{50}$  values are 125 (**6**),<sup>14</sup> 89 (**14**) and 102  $\mu\text{M}$  (**18**), respectively. In contrast, the des- and mono-*O*-gallate compounds (**11-13, 15-17, 3** and **4**) did not inhibit DNA polymerase  $\beta$ .

**Table 2** shows the  $IC_{50}$  values of synthesized compounds (**5** and **7-10**) against calf DNA polymerase  $\alpha$  and rat DNA polymerase  $\beta$ . The  $IC_{50}$  values of those compounds were 0.96 (**5**), 24.6 (**7**), 0.66 (**8**), 0.70 (**9**) and 0.09  $\mu\text{M}$  (**10**), respectively. Procyanidin B4 3,3''-di-*O*-gallate (**10**) inhibited DNA polymerase  $\alpha$  very strongly and is the strongest inhibitor against DNA polymerase  $\alpha$  among the galloyl-dimer series. The di-*O*-gallate (**10**) inhibits DNA polymerase  $\beta$  at 98  $\mu\text{M}$  in a manner similar to the procyanidin B1/B2 series. As mentioned in our previous papers,<sup>1</sup> the presence of a gallate moiety is very important for DNA polymerase inhibitory activity, which tended to be stronger as the number of galloyl groups increased.

**Table 2.** IC<sub>50</sub> values of enzymatic inhibition against mammalian DNA polymerase  $\alpha$  and  $\beta$ 

Entry	Compound	IC <sub>50</sub> values ( $\mu$ M)	
		DNA polymerase $\alpha$	DNA polymerase $\beta$
1	<b>5</b>	0.96	>500
2	<b>7</b>	24.6	>500
3	<b>8</b>	0.66	>500
4	<b>9</b>	0.70	>500
5	<b>10</b>	0.09	98

## Conclusion

In this paper, we have reported the details of the synthesis of the galloyl-substituted procyanidin B4 series, 3-*O*- and 3''-*O*-gallic acid esters, and 3''-*O*-galloyl procyanidin B3. Their ability of DPPH radical scavenging activity and DNA polymerase inhibitory activity was also investigated. The results indicated that the gallate group of these compounds was very important for both activities. The radical scavenging activity of the galloyl-substituted procyanidin B1/B2 series was more effective than that of the procyanidin B3/B4 series. It appeared that the presence of the (-)-epicatechin unit at the upper part of the dimer was very important for its ability by comparing the inhibitory effect for the procyanidin B4 series with the procyanidin B1 series. It was also suggested that the galloyl group not at the C3-position, but at the C3''-position was more effective. Procyanidin B4 3,3''-di-*O*-gallate (**10**) was the strongest inhibitor against DNA polymerase  $\alpha$  among the procyanidin B1-B4 series and had the ability to inhibit DNA polymerase  $\beta$  in a manner similar to the procyanidin B1-B3 series. The presence of a gallate moiety is very important for DNA polymerase inhibitory activity, which tended to be stronger as the number of galloyl groups increased.

## EXPERIMENTAL

**Synthesis:** Optical rotation was measured with a Horiba SEPA-300 spectrometer. <sup>1</sup>H-NMR spectra were measured with JEOL JNMLA400 spectrometer. 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 x 20 mm, 5 mm) using the solvents (A) 0.05% CF<sub>3</sub>CO<sub>2</sub>H in CH<sub>3</sub>CN and (B) 0.05% CF<sub>3</sub>CO<sub>2</sub>H in H<sub>2</sub>O. Elution was done with a linear gradient 5 to 100% A in 40 min (flow rate, 4.0 mL/min).

**[4,8]-2,3-*trans*-3,4-*trans*:2,3-*cis*-Octa-*O*-benzyl-(+)-catechin-(-)-epicatechin-3-*O*-(tri-*O*-benzyl)gallate (**22**).** To a solution of **19** (30.0 mg, 0.026 mmol) and **20** (67.2 mg, 0.10 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (30 mL) was added dropwise TMSOTf (0.052 mL, 0.026 mmol, 0.5 M solution in CH<sub>2</sub>Cl<sub>2</sub>) 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  $\text{CHCl}_3$  and the organic phase was washed with water and brine, and dried ( $\text{Na}_2\text{SO}_4$ ). Filtration, concentration and preparative silica gel TLC purification (benzene-AcOEt, 20:1) afforded 40.0 mg (89%) of **22** as a colorless amorphous solid.  $[\alpha]_D^{25} = -35.8^\circ$  ( $c$  0.40,  $\text{CHCl}_3$ );  $^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ , 0.59 : 0.41 mixture of rotational isomers) major isomer: 7.44-6.58 (37.17H, m), 6.265 (0.59H, d,  $J = 2.2$  Hz, 8), 6.256 (0.59, s, 6"), 6.19 (0.59H, d,  $J = 2.2$  Hz, 6), 5.86 (0.59H, dd,  $J = 8.6, 9.2$  Hz, 3), 5.14-4.39 (14.16H, m), 3.83 (0.59H, br, 3"), 3.38 (0.59H, br s, 2"), 2.69 (0.59H, d,  $J = 15.6$  Hz, 4"), 2.52 (0.59H, dd,  $J = 4.4, 15.6$  Hz, 4"), 1.36 (0.59H, d,  $J = 9.5$  Hz, OH); minor isomer: 7.44-6.58 (25.83H, m), 6.28 (0.41H, d,  $J = 2.2$  Hz), 6.27-6.26 (0.41H, m), 6.00 (0.41H, t,  $J = 10.0$  Hz, 3), 5.83 (0.41H, s, 6"), 5.16 (0.41H, d,  $J = 10.0$  Hz, 2), 5.14-4.39 (9.83H, m), 4.14-4.17 (0.41H, br, 3"), 3.02-2.94 (0.82H, m, 4"), 1.48 (0.41H, d,  $J = 6.5$  Hz, OH);  $^{13}\text{C-NMR}$  (100 MHz,  $\text{CDCl}_3$ , 0.59 : 0.41 mixture of rotational isomers) major isomer: 164.6, 158.2, 157.9, 156.9, 156.3, 155.6, 153.6, 152.4, 149.0 (x2), 148.5, 148.2, 142.4, 137.8-136.5 (Cx11), 131.4, 131.1, 128.9-126.5 (Cx29), 125.2, 120.9, 119.5, 114.9, 114.1, 113.8, 113.76, 111.8, 108.8, 108.7, 101.1, 95.0, 94.5, 91.5, 80.0 (2), 78.3 (2"), 75.0 (3), 71.8-69.7 (Cx10), 64.9 (3"), 35.1 (4), 29.7 (4"); minor isomer: 164.3, 158.4, 158.2, 156.7, 156.4, 156.1, 153.3, 152.2, 148.9, 148.8, 148.7, 148.2, 142.1, 137.8-136.5 (Cx11), 131.5, 131.0, 128.9-126.5 (Cx29), 125.3, 120.6, 119.9, 114.9, 114.6, 113.9, 113.3, 110.7, 108.8, 108.1, 102.2, 95.3, 94.7, 91.7, 79.8 (2), 77.6 (2"), 75.0 (3), 71.8-69.7 (Cx10), 66.8 (3"), 35.3 (4), 29.4 (4"); IR (neat,  $\text{cm}^{-1}$ ) 3065 (w), 3032 (w), 2926 (w), 1717 (m), 1590 (m), 1514 (w), 1516 (m), 1499 (m), 1456 (m), 1375 (m), 1331 (m), 1262 (s), 1109 (s), 1028 (s), 911 (w), 853 (w), 812 (w); FAB-MS ( $m/z$ ) 1745 (24), 1744 ( $[\text{M}+\text{Na}]^+$ , 29), 1724 (70), 1723 (98), 1722 ( $[\text{M}+\text{H}]^+$ , 100), 1459 (45), 1281 (73), 1190 (100), 1100 (56), 857 (66); FAB-HRMS calcd for  $\text{C}_{114}\text{H}_{97}\text{O}_{16}$   $[\text{M}+\text{H}]^+$ , 1721.6777; found: 1721.6737.

**[4,8]-2,3-trans-3,4-trans:2,3-cis-Octa-O-benzyl-(+)-catechin(-)-epicatechin-3,3''-di-O-(tri-O-benzyl)gallate (23).** To a solution of **19** (30.0 mg, 0.026 mmol) and **21** (112 mg, 0.10 mmol) in  $\text{CH}_2\text{Cl}_2$  (30 mL) was added dropwise TMSOTf (0.052 mL, 0.026 mmol, 0.5 M solution in  $\text{CH}_2\text{Cl}_2$ ) at  $-20^\circ\text{C}$ . After stirring for 5 min, the pale yellow reaction mixture was quenched with sat. sodium hydrogen carbonate. The aq. solution was extracted with  $\text{CHCl}_3$  and the organic phase was washed with water and brine, and dried ( $\text{Na}_2\text{SO}_4$ ). Filtration, concentration and silica gel column chromatography (benzene and then  $\text{CHCl}_3$ ) afforded 52.0 mg (93%) of **23** as a colorless amorphous solid.  $[\alpha]_D^{25} = -108.4^\circ$  ( $c$  1.52,  $\text{CHCl}_3$ );  $^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ , 0.67 : 0.33 mixture of rotational isomers) major isomer: 7.43-6.68 (52.26H, m), 6.63 (0.67H, d,  $J = 8.3$  Hz), 6.56 (0.67H, dd,  $J = 1.7, 8.3$  Hz), 6.24-6.15 (0.67H, m, 6 and 8), 5.99 (0.67H, t,  $J = 9.1$  Hz, 3), 5.86 (0.67H, s, 6"), 5.67-5.60 (0.67H, br s, 2"), 5.14 (0.67H, d,  $J = 9.1$  Hz, 4), 5.07-4.35



(19.43H, m), 3.26 (0.67H, dd,  $J = 6.1, 18.5$  Hz, 4"), 3.06 (0.67H, d,  $J = 18.5$  Hz, 4"); minor isomer: 7.43-6.68 (26.07H, m), 6.31 (0.33H, d,  $J = 8.3$  Hz), 6.26 (0.33H, d,  $J = 2.2$  Hz, 8), 6.21 (0.33H, s, 6"), 6.24-6.15 (0.33H, m, 6), 6.08 (0.33H, dd,  $J = 7.5, 10.0$  Hz, 3), 5.25-5.18 (0.66H, m, 4 and 3"), 5.07-4.30 (9.9H, m), 2.74 (0.33H, dd,  $J = 4.9, 16.8$  Hz, 4"), 2.61 (0.33H, dd,  $J = 5.8, 16.8$  Hz, 4");  $^{13}\text{C-NMR}$  (100 MHz,  $\text{CDCl}_3$ , 0.67 : 0.33 mixture of rotational isomers) major isomer: 165.4, 164.3, 158.4, 158.2, 156.7, 156.6, 155.7, 153.6, 152.5, 152.2, 148.93, 148.86, 148.7, 143.4, 142.1, 137.8-136.4 (Cx17), 132.5, 131.3, 131.0, 130.9, 129.0-126.6 (Cx31), 125.34, 125.25, 125.0, 120.6, 114.9, 114.4, 113.2, 111.1, 110.2, 108.7, 108.2, 101.8, 95.2, 94.7, 91.6, 79.8, 77.2, 75.06, 75.04, 74.8, 72.2-69.2 (Cx11), 35.3, 26.1; minor isomer: 165.6, 164.8, 158.2, 157.9, 157.1, 156.1, 155.5, 153.6, 152.3, 152.1, 148.9, 148.5, 148.0, 142.4, 142.1, 137.8-136.4 (Cx17), 132.5, 131.3, 130.9, 130.5, 129.0-126.6 (Cx31), 125.5, 125.3, 125.0, 120.3, 114.97, 114.3, 113.4, 111.5, 108.9, 108.8, 108.4, 100.6, 95.0, 94.7, 91.2, 80.2, 77.6, 75.1, 75.0, 74.9, 72.2-69.2 (Cx10), 67.6, 34.9, 24.5; IR (neat,  $\text{cm}^{-1}$ ) 3090 (w), 3065 (w), 3033 (m), 2934 (w), 2870 (w), 1717 (s), 1590 (s), 1499 (s), 1455 (s), 1428 (s), 1375 (s), 1331 (s), 1215 (s), 1113 (s), 1028 (s), 911 (w), 857 (w), 812 (w); FAB-MS ( $m/z$ ) 2167 (16), 2166 ( $[\text{M}+\text{Na}]^+$ , 46), 2165 (12), 2143 ( $[\text{M}+\text{H}]^+$ , 11), 2070 (37), 1935 (34), 1703 (69), 1613 (100), 1521 (64), 1236 (67); FAB-HRMS calcd for  $\text{C}_{142}\text{H}_{118}\text{O}_{20}\text{Na}$   $[\text{M}+\text{Na}]^+$ , 2165.8114; found: 2165.8071.

**Procyanidin B4 3-O-gallate (8).** A solution of **22** (49.0 mg, 28 mmol) in 22 mL of THF/  $\text{CH}_3\text{OH}/\text{H}_2\text{O}$  (20/1/1) was hydrogenated over 20%  $\text{Pd}(\text{OH})_2/\text{C}$  (5 mg) for 3 h at rt. Filtration and concentration afforded a pale brown solid, which was purified by Sephadex® LH-20 column chromatography ( $\text{CH}_3\text{OH}$ ) or Cosmosil 75  $\text{C}_{18}$ -OPN (NAKALAI TESQUE, INC.) column chromatography, and then HPLC purification to give 6.3 mg (31%) of pure **8** as a pale blown amorphous solid;  $[\alpha]_{\text{D}}^{22} = -44.4^\circ$  ( $c$  0.038,  $\text{CH}_3\text{OH}$ );  $^1\text{H-NMR}$  (400 MHz, 10%  $\text{D}_2\text{O}$  in  $\text{CD}_3\text{COCD}_3$ , 0.72 : 0.28 mixture of rotational isomers) major isomer: 6.84 (1.44H, s), 6.79 (0.72H, d,  $J = 1.7$  Hz), 6.74 (0.72H, d,  $J = 1.7$  Hz), 6.69 (0.72H, d,  $J = 8.3$  Hz), 6.53 (0.72H, d,  $J = 8.3$  Hz), 6.47 (0.72H, dd,  $J = 1.7, 8.3$  Hz), 6.44 (0.72H, dd,  $J = 1.7, 8.3$  Hz), 6.13 (0.72H, dd, 8.7, 10.0 Hz, 3), 6.00 (0.72H, s, 6"), 5.97 (0.72H, d,  $J = 2.2$  Hz, 8), 5.94 (0.72H, d,  $J = 2.2$  Hz, 6), 4.76 (0.72H, d,  $J = 8.7$  Hz, 2), 4.65 (0.72H, d,  $J = 10.0$  Hz, 4), 4.16-4.12 (0.72H, br, 3"), 4.02-3.55 (0.72H, m, 2"), 2.80 (0.72H, dd,  $J = 4.7, 16.8$  Hz, 4"), 2.62 (0.72H, dd,  $J = 2.7, 16.8$  Hz, 4"); minor isomer: 7.02-6.43 (2.25H, m), 6.09 (0.28H, dd,  $J = 7.8, 10.0$  Hz, 3), 6.02 (0.28H, s, 6"), 5.87 (0.28H, d,  $J = 2.2$  Hz, 8), 5.85 (0.28H, d,  $J = 2.2$  Hz, 6), 4.90-4.58 (0.56H, m), 4.12-4.07 (0.28H, 3"), 4.02-3.55 (0.28H, m, 2"), 2.85-2.55 (0.26H, m, 4");  $^{13}\text{C-NMR}$  (100 MHz, 10%  $\text{D}_2\text{O}$  in  $\text{CD}_3\text{COCD}_3$ , 0.72 : 0.28 mixture of rotational isomer) major isomer: 165.3, 157.7, 157.2, 155.9, 155.4, 154.7, 145.5, 145.1, 144.9, 144.8, 144.76, 131.3, 130.4, 121.6, 120.2, 119.5, 115.9, 115.4, 115.3, 114.6, 114.1, 109.7, 105.5, 100.9,

97.4, 96.6, 95.7 (Cx2), 81.3, 79.0, 72.9, 66.8, 64.2, 36.0, 28.6; minor isomer: 164.9, 157.0, 156.9, 156.0, 155.3, 154.1, 145.6, 145.4, 145.2 (Cx2), 145.1, 131.8, 130.6, 120.4, 119.1, 119.0, 116.1-113.9 (Cx5), 109.9, 105.0, 99.5, 97.1, 96.4, 95.4, 95.3, 81.6, 79.2, 73.3, 66.7, 64.1, 35.6, 28.7; FAB-MS ( $m/z$ ) 755 (7.8), 754 (12), 753 ( $[M+Na]^+$ , 40), 733 (5.0), 732 (10), 731 ( $[M+H]^+$ , 15), 677 (40), 619 (47), 575 (65), 531 (81), 487 (100); FAB-HRMS calcd for  $C_{37}H_{30}O_{16}Na[M+Na]^+$ , 753.1432; found: 753.1478.

**Procyanidin B4 3,3''-di-*O*-gallate (10).** A solution of **23** (90.0 mg, 0.042 mmol) in 22 mL of THF/CH<sub>3</sub>OH/H<sub>2</sub>O (20/1/1) was hydrogenated over 20% Pd(OH)<sub>2</sub>/C (5 mg) for 12 h at rt. Filtration and concentration afforded a pale brown solid, which was purified by Sephadex® LH-20 column chromatography (CH<sub>3</sub>OH) column chromatography, and then HPLC purification to give 30.3 mg of pure **10** (0.034 mmol, 81%) as a pale brown amorphous solid.  $[\alpha]_D^{25} = -376.4^\circ$  ( $c$  0.20, CH<sub>3</sub>OH); <sup>1</sup>H-NMR (400 MHz, 10% D<sub>2</sub>O in CD<sub>3</sub>COCD<sub>3</sub>, rotational isomer was not observed) 6.90 (2H, s, 2'), 6.85 (2H, s, 2'), 6.77 (1H, d,  $J = 1.7$  Hz), 6.74 (1H, d,  $J = 1.7$  Hz), 6.62 (1H, d,  $J = 8.3$  Hz), 6.55 (1H, d,  $J = 8.3$  Hz), 6.51 (1H, dd,  $J = 1.7, 8.3$  Hz), 6.33 (1H, dd,  $J = 1.7, 8.3$  Hz), 6.21 (1H, d,  $J = 2.2$  Hz, 8), 6.02 (1H, t,  $J = 10.0$  Hz, 3), 6.00 (1H, d,  $J = 2.2$  Hz, 6), 5.97 (1H, s, 6''), 5.38-5.33 (1H, m, 3''), 5.12 (1H, s, 2''), 4.87 (1H, d,  $J = 9.3$  Hz, 2), 4.74 (1H, d,  $J = 10.0$  Hz, 4), 2.94 (1H, dd,  $J = 4.6, 15.9$  Hz, 4''), 2.77 (1H, d,  $J = 15.9$  Hz, 4''); <sup>13</sup>C-NMR (100 MHz, 10% D<sub>2</sub>O in CD<sub>3</sub>COCD<sub>3</sub>) 166.6, 165.4, 157.5, 157.0, 156.6, 155.8, 155.44, 155.40, 145.4 (x2), 145.4, 144.85, 144.77, 144.65, 138.8, 138.3, 130.6, 130.2, 121.6, 121.3, 120.1, 119.7, 115.9, 115.5, 115.3, 114.0, 110.2, 109.6, 105.6, 104.5, 99.3, 97.6, 96.7, 95.7, 81.2 (2), 77.8 (2''), 72.8 (3), 69.7 (3''), 36.0 (4), 26.7 (4''); FAB-MS ( $m/z$ ) 906 (8.0), 905 ( $[M+Na]^+$ , 13), 883 ( $[M+H]^+$ , 10), 882 (20), 857 (25), 664 (52), 550 (45), 411 (100); FAB-HRMS calcd for  $C_{44}H_{34}O_{20}Na [M+Na]^+$ , 905.1541; found: 905.1511.

**[4,8]-2,3-*trans*-3,4-*trans*:2,3-*trans*-Octa-*O*-benzyl-(+)-catechin-(+)-catechin-3''-*O*-(tri-*O*-benzyl)gallate (26).** A solution of **24** (426 mg, 0.33 mmol) and 3,4,5-tri-*O*-benzylgallic acid (174 mg, 0.40 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) was treated with DCC (73.4 mg, 0.40 mmol) and DMAP (5.00 mg) to afford 146 mg (26%) of **24** as a colorless amorphous solid, and the 100 mg of starting material (**26**) was recovered (23%) with 80 mg (14%) of 3-*O*-gallate and 101 mg (14%) of 3,3''-di-*O*-gallate.  $[\alpha]_D^{24} = -37.5^\circ$  ( $c$  0.90, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>, 0.5 : 0.5 mixture of rotational isomers) major isomer: 7.45-6.77 (61.5H, m), 6.656 (0.5H, d,  $J = 1.7$  Hz), 6.54 (0.5H, d,  $J = 8.3$  Hz), 6.32 (0.5H, dd,  $J = 1.7, 8.3$  Hz), 6.25 (0.5H, s, D6''), 6.18 (0.5H, d,  $J = 2.2$  Hz, 8), 6.14 (0.5H, d,  $J = 2.2$  Hz, 8), 6.11 (0.5H, s, 6''), 6.09 (0.5H, d,  $J = 2.2$  Hz, 6), 5.99 (0.5H, d,  $J = 2.2$  Hz, 6), 5.36-5.32 (0.5H, m, 3''), 5.13-4.48 (25H, m), 4.33-4.26 (1H, m, 2), 3.80 (0.5H, d,  $J = 9.3$  Hz, 2''), 3.26 (0.5H, dd,  $J = 6.3, 16.3$  Hz, 4''), 3.06 (0.5H, dd,  $J =$

5.2, 16.6 Hz, 4"), 2.85 (0.5H, dd,  $J = 7.3, 16.6$  Hz, 4"), 2.52 (0.5H, dd,  $J = 9.0, 16.3$  Hz, 4"), 1.556 (0.5H, d,  $J = 3.4$  Hz, OH), 1.50 (0.5H, d,  $J = 3.4$  Hz, OH);  $^{13}\text{C-NMR}$  (100 MHz,  $\text{CDCl}_3$ , 0.5 : 0.5 mixture of rotational isomers) 165.1, 164.8, 158.1, 157.9, 157.6, 157.4, 157.1, 157.0, 156.8, 155.6, 155.4 (x2), 154.0, 152.44, 152.40, 152.2, 149.2, 149.0 (x2), 149.98, 148.95, 148.85, 148.7, 148.5, 142.62, 142.56, 137.6-136.5 (Cx20), 131.8, 131.7, 131.6, 130.7, 128.6-127.0 (Cx60), 125.2, 125.0, 121.2, 120.5, 120.2, 120.1, 114.9, 114.8, 114.6, 114.1, 113.7, 113.6, 113.5, 112.1, 109.1, 109.0, 108.6, 108.3, 102.1, 101.9, 94.8, 94.7, 94.2, 94.1, 91.54, 91.47, 82.04 (2), 81.97 (2), 77.8(2"), 77.7 (2"), 75.1, 75.0, 73.8(3), 73.0(3), 71.5-69.8 (Cx22), 37.3 (4), 37.2 (4), 26.5 (4"), 24.5 (4"); IR (neat,  $\text{cm}^{-1}$ ) 3569 (w), 3065 (m), 3032 (m), 2932 (m), 2870 (m), 1952 (w), 1877 (w), 1811 (w), 1713 (s), 1589 (s), 1498 (s), 1420 (s), 1331 (s), 1264 (s), 1215 (s), 1180 (s), 1028 (s), 910 (m), 854 (m); FAB-MS ( $m/z$ ) 1746 (25), 1745 ( $[\text{M}+\text{Na}]^+$ , 44), 1744 (32), 1724 (39), 1723 (50), 1722 ( $[\text{M}+\text{H}]^+$ , 25), 1284 (32), 1283 (48), 1282 (40), 1281 (32), 1280 (34), 1193 (39), 1192 (55), 1191 (100), 1190 (98); FAB-HRMS calcd for  $\text{C}_{114}\text{H}_{97}\text{O}_{16}$   $[\text{M}+\text{H}]^+$ , 1721.6777; found: 1721.6697.

**[4,8]-2,3-trans-3,4-trans:2,3-cis-Octa-O-benzyl-(+)-catechin-(-)-epicatechin-3''-O-(tri-O-benzyl)gallate (27).** A solution of **25** (290 mg, 0.22 mmol) and 3,4,5-tri-O-benzylgallic acid (118 mg, 0.27 mmol) in  $\text{CH}_2\text{Cl}_2$  (50 mL) was treated with DCC (55.7 mg, 0.27 mmol) and DMAP (5.00 mg) to afford 280 mg (74%) of **27** as a colorless amorphous solid.  $[\alpha]_{\text{D}}^{22} = -121.1^\circ$  ( $c$  0.32,  $\text{CHCl}_3$ );  $^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ , 0.94 : 0.06 mixture of rotational isomers) major isomer: 7.44-6.78 (56.4H, m), 6.86 (0.94H, d,  $J = 8.3$  Hz), 6.69 (0.94H, dd,  $J = 1.7, 8.3$  Hz), 6.62 (0.94H, d,  $J = 8.3$  Hz), 6.27 (0.94H, s, 6"), 6.21 (0.94H, d,  $J = 2.2$  Hz, 8), 6.11 (0.94H, d,  $J = 2.2$  Hz, 6), 5.33-5.27 (0.94H, m, 3"), 5.13-4.45 (22.56H, m), 4.36 (0.94H, t,  $J = 9.0$  Hz, 3), 3.84 (0.94H, s, 2"), 2.98 (0.94H, d,  $J = 16.8$  Hz, 4"), 2.77 (0.94H, dd,  $J = 4.7, 16.8$  Hz, 4"), 1.85 (0.94H, d,  $J = 2.7$  Hz, OH); minor isomer: 7.44-6.45 (3.78H, m), 6.25-6.10 (0.12H, m), 6.02 (0.06H, s, 6"), 5.58 (0.06H, br s, 3"), 5.13-4.17 (1.5H, m), 3.90 (0.06H, s, 2"), 3.25-3.05 (0.12H, m, 4"), 1.6-1.2 (0.06H, m);  $^{13}\text{C-NMR}$  (100 MHz,  $\text{CDCl}_3$ , 0.94 : 0.06 mixture of rotational isomers) major isomer: 165.2, 158.0, 157.8, 156.8, 155.8, 155.6, 152.3, 149.2, 148.9, 148.8, 148.4, 142.5, 137.5-136.6 (Cx10), 131.68, 131.65, 130.9, 128.8-127.2 (Cx30), 125.0, 121.2, 119.2, 114.7, 114.6, 113.30, 113.26, 111.9, 109.1, 108.5, 100.7, 94.8, 94.0, 91.5, 82.2, 75.0, 73.1, 71.3, 71.15, 71.12, 70.9, 70.8 (Cx2), 70.3, 70.2, 70.1, 70.0, 68.2, 38.7, 26.4; minor isomer was not identified. IR (neat,  $\text{cm}^{-1}$ ) 3700-3120 (br m), 3065(w), 3033 (w), 2926 (m), 1715 (w), 1609 (s), 1593 (s), 1514 (m), 1429 (m), 1327 (w), 1266 (m), 1217 (m), 1105 (s), 1028 (m), 911 (w), 810 (w); FAB-MS ( $m/z$ ) 1745 (2.0), 1744 ( $[\text{M}+\text{Na}]^+$ , 4.0), 1724 (68), 1723 (100), 1722 ( $[\text{M}+\text{H}]^+$ , 98), 1190 (17), 685 (31), 664 (18), 663 (100), 662 (48), 648 (88); FAB-HRMS calcd for  $\text{C}_{114}\text{H}_{97}\text{O}_{16}$   $[\text{M}+\text{H}]^+$ , 1721.6777; found: 1721.6731.

**Procyanidin B3 3''-O-gallate (5).** A solution of **26** (80.0 mg, 0.046 mmol) in 22 mL of THF/CH<sub>3</sub>OH/H<sub>2</sub>O (20/1/1) was hydrogenated over 20% Pd(OH)<sub>2</sub>/C (5 mg) for 12 h at rt. Filtration and concentration afforded a pale brown solid, which was purified by Sephadex® LH-20 column chromatography (CH<sub>3</sub>OH) and then HPLC purification to give 24.8 mg (74%) of pure **5** as a pale brown amorphous solid.  $[\alpha]_D^{25} = -127.3^\circ$  (*c* 0.11, CH<sub>3</sub>OH); <sup>1</sup>H-NMR (400 MHz, 10% D<sub>2</sub>O in CD<sub>3</sub>COCD<sub>3</sub>, 0.57 : 0.43 mixture of rotational isomers) major isomer: 6.98 (1.14H, s), 6.81-6.77 (0.57H, m), 6.69 (0.57H, d, *J* = 1.7 Hz), 6.61 (0.57H, d, *J* = 8.3 Hz), 6.60 (0.57H, d, *J* = 8.3 Hz), 6.48 (0.57H, dd, *J* = 1.7, 8.3 Hz), 6.15 (0.57H, s, 6"), 6.14 (0.57H, d, *J* = 1.7, 8.3 Hz), 5.94 (0.57H, d, *J* = 2.2 Hz, 8), 5.76 (0.57H, d, *J* = 2.2 Hz, 6), 5.21 (0.57H, ddd, *J* = 5.6, 6.4, 6.4 Hz, 3"), 5.05 (0.57H, d, *J* = 6.4 Hz, 2"), 4.52 (0.57H, dd, *J* = 7.8, 9.5 Hz, 3), 4.46 (0.57H, d, *J* = 7.8 Hz, 4), 4.29 (0.57H, d, *J* = 9.5 Hz, 2), 2.73 (0.57H, dd, *J* = 5.6, 16.6 Hz, 4"), 2.66 (0.57H, dd, *J* = 6.4, 16.6 Hz, 4"); minor isomer: 7.08 (0.86H, s), 7.07 (0.43H, d, *J* = 1.7 Hz), 6.95 (0.43H, d, *J* = 1.7 Hz), 6.91 (0.43H, dd, *J* = 1.7, 8.3 Hz), 6.81-6.77 (0.86H, m), 6.71 (0.43H, d, *J* = 8.3 Hz), 6.05 (0.43H, s, 6"), 5.87 (0.43H, d, *J* = 2.2 Hz, 8), 5.83 (0.43H, d, *J* = 2.2 Hz, 6), 5.34 (0.43H, ddd, *J* = 5.4, 5.6, 6.6 Hz, 3"), 5.17 (0.43H, d, *J* = 6.6 Hz, 2"), 4.60 (0.43H, d, *J* = 8.0 Hz, 4), 4.54 (0.43H, dd, *J* = 8.0, 9.3 Hz, 3), 4.37 (0.43H, d, *J* = 9.3 Hz, 2), 2.88 (0.43H, dd, *J* = 5.4, 16.6 Hz, 4"), 2.73 (0.43H, dd, *J* = 5.6, 16.6 Hz, 4"); <sup>13</sup>C-NMR (100 MHz, 10% D<sub>2</sub>O in CD<sub>3</sub>COCD<sub>3</sub>, 0.57 : 0.43 mixture of rotational isomers) 166.4, 166.3, 158.4, 157.0, 156.9, 156.8, 156.7, 156.5, 155.6, 155.2 (x2), 155.0, 154.0, 153.7, 145.8, 145.7, 145.5, 145.3, 145.1, 145.0, 144.9, 144.1, 131.8 (x2), 130.6, 130.2, 121.0 (x2), 120.4, 119.7, 119.2, 118.7, 116.4 (x2), 115.9, 115.7 (x4), 115.5, 114.1, 113.8, 109.9, 109.8, 107.9, 107.3, 106.2, 105.9, 100.4 (x2), 98.8 (x2), 97.3, 96.8, 96.3 (x2), 95.9 (x2), 83.5 (2), 83.4 (2), 79.2 (2"), 78.2 (2"), 73.4 (3), 72.6 (3), 70.6 (3"), 70.3 (3"), 37.8 (x2) (4), 24.9 (4"), 24.5 (4"); FAB-MS (*m/z*) 754 (41), 753 ([M+Na]<sup>+</sup>, 100), 752 (57), 732 (33), 731 ([M+H]<sup>+</sup>, 66), 730 (45); FAB-HRMS calcd for C<sub>37</sub>H<sub>31</sub>O<sub>16</sub> [M+H]<sup>+</sup>, 731.1612; found: 731.1543.

**Procyanidin B4 3''-O-gallate (9).**<sup>11</sup> A solution of **27** (130 mg, 0.075 mmol) in 44 mL of THF/CH<sub>3</sub>OH/H<sub>2</sub>O (20/1/1) was hydrogenated over 20% Pd(OH)<sub>2</sub>/C (10 mg) for 8 h at rt. Filtration and concentration afforded a pale brown solid, which was purified by Sephadex® LH-20 column chromatography (CH<sub>3</sub>OH) and then HPLC purification to give 35.1 mg (53%) of pure **9** as a pale brown amorphous solid.  $[\alpha]_D^{23} = -309.2^\circ$  (*c* 0.095, CH<sub>3</sub>OH),  $[\alpha]_D^{25} = -279.1^\circ$  (*c* 0.095, acetone), {lit.,<sup>11</sup>  $[\alpha]_D^{22} = -255.7^\circ$  (*c* 0.56, acetone); <sup>1</sup>H-NMR (400 MHz, 10% D<sub>2</sub>O in CD<sub>3</sub>COCD<sub>3</sub>, 0.56 : 0.44 mixture of rotational isomers) major isomer: 7.23 (0.56H, d, *J* = 1.7 Hz), 6.92 (1.12H, s), 7.00-6.70 (1.68H, m), 6.65 (0.56H, d, *J* = 8.3 Hz), 6.50 (0.56H, dd, *J* = 1.7, 8.3 Hz), 6.15 (0.56H, br s), 6.02 (0.56H, br s), 5.83-5.82 (0.56H, br s), 5.43-5.45 (0.56H, m, 3"), 5.18 (0.56H, br s, 2"), 4.73 (0.56H, d, *J* = 8.0 Hz, 2), 4.62 (0.56H, dd, *J* = 8.0,

9.8 Hz, 3), 4.47 (0.56H, d,  $J = 9.8$  Hz, 4), 3.04-2.93 (0.56H, m, 4"), 2.81 (0.56H, d,  $J = 1.7$  Hz, 4"); minor isomer: 7.02 (0.88H, s), 7.00-6.70 (1.76H, m), 6.61 (0.44H, d,  $J = 8.3$  Hz), 6.28 (0.44H, dd,  $J = 1.7, 8.3$  Hz), 6.18 (0.44H, d,  $J = 2.2$  Hz), 5.96 (0.44H, d,  $J = 2.2$  Hz), 5.83 (0.44H, s), 5.32-5.31 (0.44H, m, 3"), 5.12 (0.44H, br s, 2"), 4.54 (0.44H, d,  $J = 8.8$  Hz, 2), 4.40 (0.44H, d,  $J = 9.7$  Hz, 4), 4.24 (0.44H, dd,  $J = 8.8, 9.7$  Hz, 3), 3.07-2.93 (0.88H, m, 4");  $^{13}\text{C-NMR}$  (100 MHz, 10%  $\text{D}_2\text{O}$  in  $\text{CD}_3\text{COCD}_3$ , 0.56 : 0.44 mixture of rotational isomers) major isomer: 166.5, 166.4, 158.1, 157.9, 157.2, 157.0, 156.8, 156.3, 155.68, 155.67, 155.5, 155.3, 155.0, 154.8, 145.7, 145.6, 145.5 (x2), 145.4 (x2), 145.2, 144.8, 131.9, 131.8, 131.0, 130.5, 121.4, 121.1, 120.6, 120.0, 119.8, 118.8, 116.1, 115.9, 115.7, 115.6, 115.5, 115.4, 114.9 (x2), 114.1 (x2), 110.2, 109.8, 107.5, 106.7, 106.4, 105.9, 99.7 (x2), 98.3 (x2), 97.3, 97.1, 97.0, 96.8, 96.2, 95.9, 83.4 (2), 83.2 (2), 78.4 (2"), 77.9 (2"), 73.0 (3), 72.9 (3), 69.62 (3"), 69.59 (3"), 38.1 (4), 37.8 (4), 26.8 (4"), 26.7 (4"); FAB-MS ( $m/z$ ) 733 (7.0), 732 (18), 731 ( $[\text{M}+\text{H}]^+$ , 25), 720 (56), 636 (44), 371 (82), 306 (100); FAB-HRMS calcd for  $\text{C}_{37}\text{H}_{31}\text{O}_{16}$   $[\text{M}+\text{H}]^+$ , 731.1612; found: 731.1637.

**Measurement of DPPH radical scavenging activity:** All of the assay samples were HPLC pure. DPPH radical scavenging activity was measured with the general DPPH method described in the previous paper.<sup>1</sup>

**DNA polymerase assays:** All of the assay samples were HPLC pure. Inhibitory activity against calf DNA polymerase  $\alpha$  (0.05 units) and rat DNA polymerase  $\beta$  (0.05 units) was measured with the general method described in the previous paper.<sup>1</sup> One unit of DNA polymerase activity was defined as the amount of enzyme that catalyzed the incorporation of 1 nmol of deoxyribonucleotide triphosphates (i.e., dTTP) into synthetic template-primers (i.e. poly(dA)/oligo(dT)<sub>12-18</sub>, A/T = 2/1) in 60 min at 37 °C under normal reaction conditions for each enzyme.

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## REFERENCES AND NOTES

1. This report is Part 11 in the series "Synthetic studies of proanthocyanidins". See also part 10 and part 9. (a) Part 10: A. Saito, Y. Mizushima, H. Ikawa, H. Yoshida, Y. Doi, A. Tanaka, and N. Nakajima, *Bioorganic Med. Chem.*, 2005, **13**, 2759. (b) Part 9: A. Saito, M. Emoto, A. Tanaka, Y. Doi, K. Shoji,

- Y. Mizushina, H. Ikawa, H. Yoshida, N. Matsuura, and N. Nakajima, *Tetrahedron*, 2004, **60**, 12043.
- J. B. Harborne, *The Flavonoids: Advances in research from 1986*. Chapman and Hall, London, 1993.
  - J. B. Harborne and H. Baxter, *The Handbook of Natural Flavonoids*, John Wiley & Sons, New York, 1999.
  - C. S. Yang, P. Maliakai, and X. Meng, *Annu. Rev. Pharmacol. Toxicol.*, 2002, **42**, 25.
  - H. Tachibana, K. Koga, Y. Fujimura, and K. Yamada, *Nature Structural Mol. Biol.*, 2004, **11**, 380.
  - T. Hatano, H. Miyatake, M. Natsume, N. Osakabe, T. Takizawa, H. Ito, and T. Yoshida, *Phytochemistry*, 2002, **59**, 749.
  - For many papers about isolation and bioactivities of galloyl-substituted procyanidin series, see ref. 1.
  - (a) A. Saito, N. Nakajima, A. Tanaka, and M. Ubukata, *Biosci. Biotechnol. Biochem.*, 2002, **66**, 1764. (b) A. Saito, N. Nakajima, A. Tanaka, and M. Ubukata, *Tetrahedron*, 2002, **58**, 7829. (c) A. Saito, N. Nakajima, N. Matsuura, A. Tanaka, and M. Ubukata, *Heterocycles*, 2004, **62**, 479. (d) A. Saito, A. Tanaka, M. Ubukata, and N. Nakajima, *Synlett*, 2004, 1069. (e) A. Saito, Y. Doi, A. Tanaka, N. Matsuura, M. Ubukata, and N. Nakajima, *Bioorg. Med. Chem.*, 2004, **12**, 4783.
  - (a) A. Saito, N. Nakajima, A. Tanaka, and M. Ubukata, *Tetrahedron Lett.*, 2003, **44**, 5449. (b) A. Saito, N. Nakajima, A. Tanaka, and M. Ubukata, *Heterocycles*, 2003, **61**, 287. (c) A. Saito, A. Tanaka, M. Ubukata, and N. Nakajima, *Synlett*, 2004, 2040.
  - T. Hatano and R. W. Hemingway, *J. Chem. Soc., Perkin Trans. 2*, 1997, 1035.
  - 23% of the starting material, 14% of **4** and 14% of **6** were obtained. See experimental section.
  - G. Nonaka, O. Kawahara, and I. Nishioka, *Chem. Pharm. Bull.*, 1983, **31**, 3906.
  - I. Oguni, K. Nasu, S. Kanaya, Y. Ota, S. Yamamoto, and T. Nomura, *Jpn. J. Nutr.*, 1989, **47**, 93.
  - We reported the IC<sub>50</sub> value of compound (**6**) against calf DNA polymerase  $\alpha$  to be 8.1  $\mu$ M in previous paper.<sup>1b</sup> But reevaluation of the inhibitory activity of **6** revealed the IC<sub>50</sub> values for calf DNA polymerase  $\alpha$  and rat DNA polymerase  $\beta$  to be 0.12 and 125  $\mu$ M, respectively.
  - A. Kornberg and T. Baker, *DNA Replication*, W. H. Freeman & Co, New York, 1992, Chapter 6, p.197.