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SYNTHETIC STUDIES OF PROANTHOCYANIDINS. PART 5.[1] HIGHLY STEREOSELECTIVE SYNTHESIS AND INHIBITORY ACTIVITY OF MAILLARD REACTION OF 3,4-*TRANS* CATECHIN AND EPICATECHIN DIMERS, PROCYANIDIN B1, B2, B3, B4 AND THEIR ACETATES

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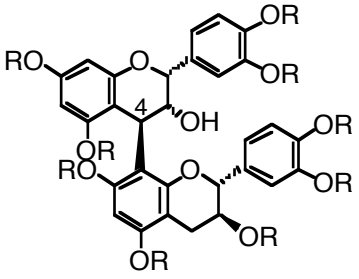
Abstract – TMSOTf-catalyzed condensation of a potential electrophile with a nucleophile was achieved with a high level of 3,4-*trans* condensation, and we succeeded in the stereoselective synthesis of procyanidin B2 and its peracetate. Studies on the inhibitory activity of the Maillard reaction of four 3,4-*trans* series of catechin and epicatechin dimers, procyanidin B1, B2, B3 and B4, and their peracetates were also carried out.

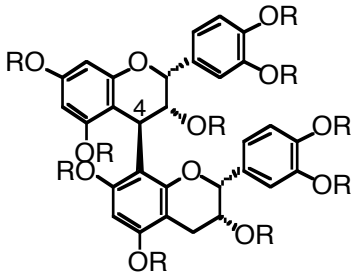
Introduction

Nonenzymatic browning reactions by heating a mixture solution of sugar with protein or amino acid are known as the Maillard reaction, which occurs extensively in food systems and *in vivo*.[1] The reaction is roughly divided into two stages, the early and late stages. 3-Deoxyglucosone (3DG) was generated from Amadori compounds in the early stage, leading to generation of advanced glycation endproducts (AGEs), which are characterized by fluorescence, a brown color, and intra- or intermolecular cross-linking. The relationship of the Maillard reaction to diabetic complications has been noted[2] in the field of sitology.[3] Under these circumstances, aminoguanidine (AG),[1] aspirin,[5] vitamin B6,[6] taurine,[7] quercetin[8] and components of tea extracts (catechin derivatives)[9], have been reported as important Maillard reaction inhibitors.

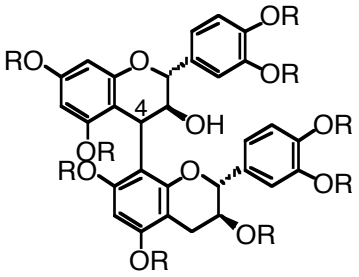
We have studied a synthetic method of proanthocyanidin oligomers. Proanthocyanidins are known as condensed tannins and/or oligomeric flavonoids,[10-12] and many biological activities, powerful antioxidant activity,[13] free-radical-scavenging activity[14] and an anti-tumor-promoting effect,[15] have been reported in these compounds. In this paper we describe the synthesis of 3,4-*trans*-proanthocyanidin dimers[16] as shown in Figure 1, and the inhibitory activity of the Maillard reaction. Previously described TMSOTf-



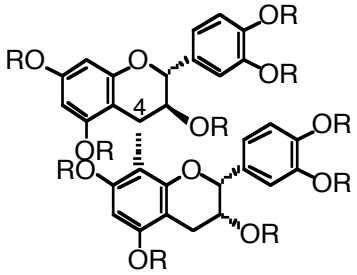
1: R=H, procyanidin B1
[(-)-epicatechin-(+)-catechin]
Ac_{10}-**1**: R=Ac, procyanidin B1 decaacetate



2: R=H, procyanidin B2
[(-)-epicatechin-(-)-epicatechin]
Ac_{10}-**2**: R=Ac, procyanidin B2 decaacetate



3a: R=H, procyanidin B3
3,4-*trans* (+)-catechin-(4→8)-(+)-catechin dimer
Ac_{10}-**3a**: R=Ac, procyanidin B3 decaacetate
3b: R=H, 3,4-*cis* (+)-catechin-(4→8)-(+)-catechin dimer



4: R=H, procyanidin B4
[(+)-catechin-(-)-epicatechin]
Ac_{10}-**4**: R=Ac, procyanidin B4 decaacetate

Figure 1

catalyzed catechin and catechin condensation achieved high levels of 3,4-*trans* condensation in excellent isolation yield to give procyanidin-B3 (**3a**).[17-19] We also applied TMSOTf-catalyzed condensation under intramolecular conditions. 3,4-*cis*-Catechin-catechin dimer (**3b**),[20] 3,4-*trans*-epicatechin-catechin dimer (**1**) and 3,4-*trans*-catechin-epicatechin dimer (**4**) were obtained in excellent to good yields. However, 3,4-*trans*-epicatechin-epicatechin dimer (**2**) was not obtained under the intramolecular conditions.[1] This paper reports the synthesis of dimer (**2**) under TMSOTf-catalyzed intermolecular condensation and the inhibitory activity of the Maillard reaction of all synthesized procyanidin dimers (**1** ~ **4**) and their acetyl derivatives (Ac_{10}-**1** to Ac_{10}-**4**).

TMSOTf-catalyzed condensation

TMSOTf-catalyzed condensations were attempted by the combinations of catechin and epicatechin electrophiles (**9** ~ **12**) and nucleophiles (**7** and **8**). The 4-*O*-ethoxyethylated flavan-3,4-diol derivative was used as the electrophile; the nucleophile was used in 4.5-fold excess to avoid higher oligomer formation. To a solution of the nucleophile and the electrophile in CH_2Cl_2 was added 1 equivalent of TMSOTf at –78°C, and the mixture was allowed to react for 5 min. In all cases, 3,4-*trans* dimerization products (**13** ~ **20**) were obtained in moderate to excellent yield as a single isomer.

When the epicatechin electrophile (**10**) was condensed with tetra-*O*-benzylated catechin (**8**) in the presence of TMSOTf (1.0 eq.) at –78°C, the 3,4-*trans*-octa-*O*-benzylated (-)-epicatechin-(4 →8)-(+)-catechin dimer (**13**) was obtained in 45 % yield. The reaction conditions were first checked using 3-*O*-acetyl electrophile (**10**), because the neighboring group participation of the 3-*O*-acetyl moiety was effective in attaining significant improvement in the selectivity.[18] However, the epicatechin component was less reactive than the catechin component; by removal of the 3-*O*-acetyl group, dimerization proceeded smoothly to give dimer (**14**)[1] in better 67 % yield. On the epicatechin and epicatechin condensation, better yield was also obtained by using 3-hydroxy electrophile (**9**) in place of 3-*O*-acetyl electrophile (**10**), epicatechin-epicatechin dimers (**15**) and (**16**) were selectively obtained in 75 % and 93 % yields, respectively.[21] The catechin component was more reactive than the epicatechin component; the condensation of **8** and **7** with 3-*O*-acetylated catechin derivative (**12**) proceeded smoothly to give the corresponding catechin-catechin dimer (**17**)[18] and catechin-epicatechin dimer (**19**) [1] in 97 % and 90 % yields, respectively.[22]

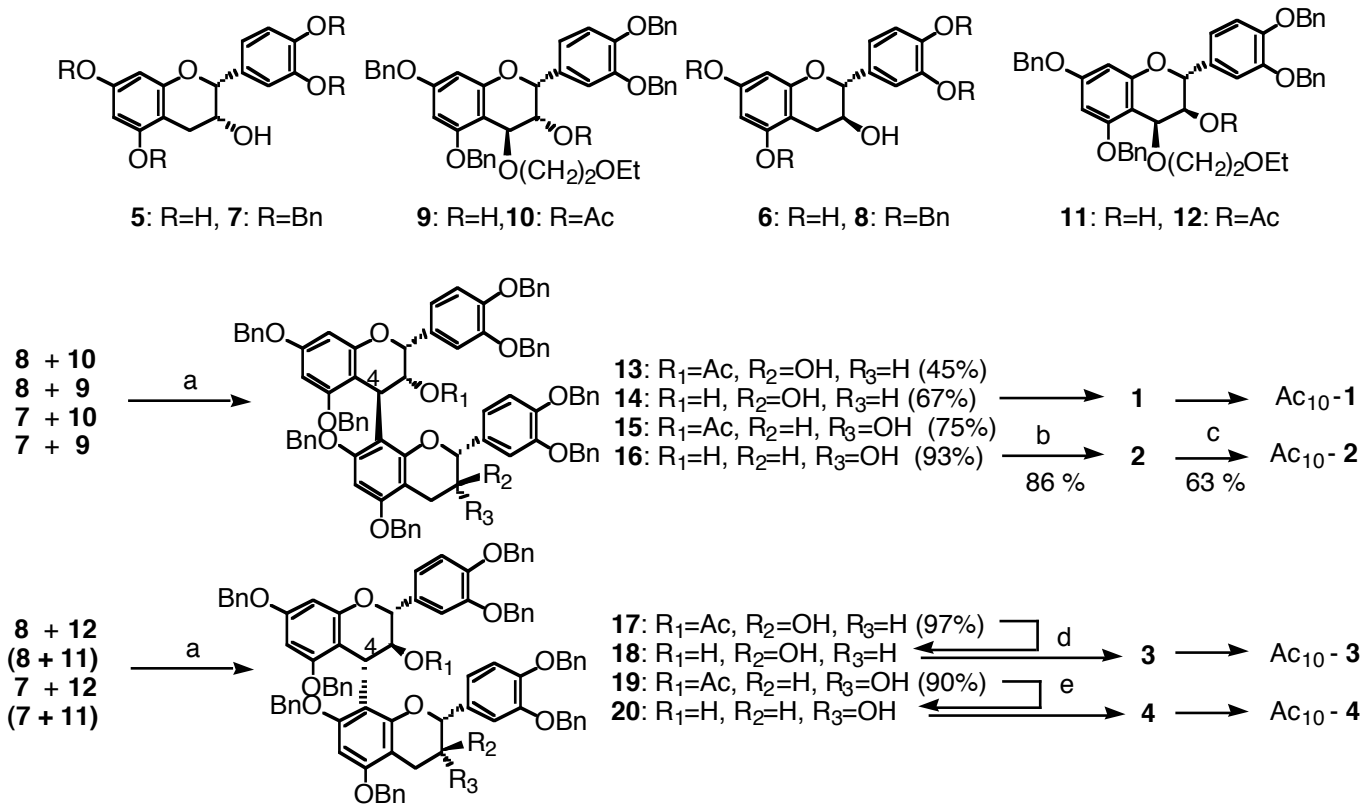
Natural procyanidin B2 (**2**) was synthesized from dimer (**16**) by hydrogenolysis with $Pd(OH)_2$ in THF/MeOH/H_2O and following Sephadex® LH-20 and normal-phase HPLC purification (Kanto Chemical Co., Mightysil Si 60, EtOAc) in 86% yield. All ^{1}H-NMR peaks of **2** were broadened; therefore the structure was confirmed as a decaacetyl compound (Ac_{10}-**2**), which was spectroscopically (^{1}H-NMR, ^{13}C-NMR, MS) identical with the natural product, and also had specific rotation in good agreement with the literature.[23,24] Syntheses of other B-type procyanidins (**1**, **3**, **4**) and their decaacetates (Ac_{10}-**1**, Ac_{10}-**3**, Ac_{10}-**4**) are already achieved *via* **14**,[1] **18**,[17,25] and **20**.[1,25]

Maillard reaction inhibitory activity

Inhibitory activity of the Maillard reaction of natural procyanidin dimers (**1** ~ **4**) and their acetates (Ac_{10}-**1** ~ Ac_{10}-**4**) was next determined using our reported method.[26] The value of IC_{50} (50% inhibitory activity) of all compounds is shown in Table 1. Inhibitory activity of procyanidins B1, 2, 3 and 4 (**1**-**4**) showed 30-72 M. Their acetyl derivatives, Ac_{10}-**1**, Ac_{10}-**2**, Ac_{10}-**3** and Ac_{10}-**4**, showed 7-40 M. Because the IC_{50} value of the Maillard reaction inhibitor aminoguanidine was 200 M,[26] synthesized dimers and their acetates are candidates for a clinically useful Maillard reaction inhibitor. The phenolic hydroxy group of a catechin monomer or oligomer seemed to be effective for the antioxidant activity.[9] However, it is interesting that effect of the phenolic hydroxy group and inhibitory activity against the Maillard reaction are not parallel; inhibitory activity of acetyl derivatives (Ac_{10}-**1** ~ Ac_{10}-**4**) was 6-1.5 times higher than that of phenol derivatives (**1** ~ **4**). Further investigations on the structure and activity relationship of Maillard reaction inhibition are now underway.

Conclusion

We have established a 3,4-*trans* natural procyanidin dimer synthesis based on the TMSOTf-catalyzed



Scheme 1

Table 1. Inhibitory activity of catechin dimers and their acetylation derivatives

Compound	IC_{50}, □M
1	30
2	58
3	42
4	72
Ac_{10}-1	16
Ac_{10}-2	40
Ac_{10}-3	7
Ac_{10}-4	26

intermolecular condensation method. The inhibitory activity of the Maillard reaction of synthesized dimers (**1** ~ **4**) and their acetates (Ac_{10}-**1** ~ Ac_{10}-**4**) was also studied to find that these dimers have a high level of Maillard reaction inhibitory activity.

EXPERIMENTAL

Maillard reaction inhibitory activity

Chemicals. BSA (fraction V) was obtained from Sigma Chemical Co. Ltd. (St. Louis, MO). All other

chemicals were purchased from Nakarai Tesque (Kyoto, Japan). Alkaline PBS was adjusted PBS to pH 10 with 0.25 N NaOH.

***In vitro* Glycation.** The evaluation of *in vitro* glycation, the Maillard reaction was achieved by using the previously described method.[26] Briefly, 500 μL of reaction mixture was prepared containing 400 μg BSA, 200 mM glucose with or without 10 μL of inhibitor in 50 mM phosphate buffer, pH 7.4, and the reaction mixture was heated at 60℃ on a heat block for indicated hours. A blank sample was kept at 4℃ until measurement. After cooling, aliquots of 100 μL were transferred to new 1.5 mL plastic tubes, and 10 μL of 100 % w/v TCA was added to each tube. The supernatant containing glucose and inhibitor was removed after agitation and centrifugation (15,000 rpm, 4℃, 4 min.), then the precipitate of AGEs-BSA was dissolved with 400 μL of alkaline PBS to serve for screening. The changes in fluorescence intensity (ex. 370 nm, em. 440 nm) based on AGEs were monitored by using spectrofluorometer RF-1500 (Shimadzu, Japan).

The Measurement of the Maillard reaction inhibitory activity. Real inhibition activity was estimated by subtracting the quenching effect from the apparent inhibitory activity. Apparent inhibitory activity was calculated by the above method. Quenching effect was measured by using the same sample dissolved with alkaline PBS after TCA treatment of the mixed solution of 2 μL of inhibitor solution and 100 μL of the 30 hours incubated control solution.

Synthesis

Optical rotation was measured with a Horiba SEPA-300 spectrometer. ^{1}H-NMR spectra were measured with JEOL JNMLA400 spectrometer, and MS spectra were recorded with a JEOL JMS-AX500 instrument. Synthetic procyanidin B2 (**2**) was analyzed on a Mightysil® RP-18 GP column (Kanto Chemical Co. Inc, Japan; 250 x 4.6 mm, 5 μm) using the solvents (A) 0.1% CF_3CO_2H in CH_3CN and (B) 0.1% CF_3CO_2H in H_2O. Elusion was done with a linear gradient 5 to 100% A in 40 min (flow rate, 0.5 mL/min).

[4,8]-2,3-*cis*-3,4-*trans*:2,3-*trans*-Octa-*O*-benzyl-(-)-3-*O*-acetyl-(-)-epicatechin-(+)-catechin (13). To a solution of tetra-*O*-benzylated catechin (**8**) (101 mg, 0.16 mmol) and (2*R*,3*R*,4*S*)-3-acetoxy-5, 7, 3', 4'-tetrabenzyloxy-4-(2''-ethoxyethoxy)flavan (**10**) (27 mg, 0.035 mmol) in CH_2Cl_2 (30 mL) was added dropwise TMSOTf (0.07 mmol, 0.035 mmol, 0.5 M solution in CH_2Cl_2) at –78˚C. After stirring for 5 min, the pale yellow reaction mixture was quenched with sat. $NaHCO_3$. The solution was extracted with $CHCl_3$ and the organic phase was washed with water and brine, and dried (Na_2SO_4). Filtration, concentration and silica gel column chromatography (hexane/EtOAc, 6/1~1.5/1) afforded 21 mg (45 %) of **13** as a colorless

amorphous solid: $[\alpha]_D^{27}$= +62.2° (*c* 0.62, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$, 0.75 : 0.25 mixture of rotational isomers) major isomer: 7.43-6.73 (30.75H, m), 6.96 (0.75H, dd, *J* = 1.7, 8.3 Hz), 6.88 (0.75H, d, *J* = 8.3 Hz), 6.76 (0.75H, d, *J* = 8.3 Hz), 6.73 (0.75H, d, *J* = 1.7 Hz), 6.49 (0.75H, dd, *J* = 1.7, 8.3 Hz), 6.29 (0.75H, s), 6.03 (0.75H, d, *J* = 2.2 Hz), 5.54 (0.75H, d, *J* = 2.2 Hz), 5.52 (0.75H, br s), 5.46 (0.75H, br s), 5.09-4.79 (10.5H, m), 4.71 (0.75H, br s), 4.64 (0.75H, d, *J* = 11.2 Hz), 4.51 (0.75H, d, *J* = 11.2 Hz), 3.78 (0.75H, ddd, *J* = 6.6, 9.0, 9.5 Hz), 3.64 (0.75H, d, *J* = 9.0 Hz), 3.24 (0.75H, dd, *J* = 6.6, 16.6 Hz), 2.58 (0.75H, dd, *J* = 9.5, 16.8 Hz), 1.69 (2.25H, s), 1.43 (0.75H, br s, OH); minor isomer: 7.43-6.73 (11.5H, m), 6.22 (0.25H, d, *J* = 2.2 Hz), 6.19 (0.25H, s), 6.12 (0.25H, d, *J* = 2.2 Hz), 5.37 (0.25H, brs), 5.26 (0.25H, br s), 5.16-4.46 (4.25H, m), 4.58 (0.25H, br s), 3.66-3.63 (0.25H, m), 3.19 (0.25H, dd, *J* = 5.6, 15.9 Hz), 2.69 (0.25H, dd, *J* = 9.8, 15.9 Hz), 1.63-1.54 (0.25H, m, OH), 1.27 (0.75H, s); ^{13}C-NMR (100 MHz, $CDCl_3$, 0.75 : 0.25 mixture of rotational isomers) major isomer: 169.3, 158.0, 156.4, 156.1, 156.0, 155.5, 154.4, 149.3, 149.0, 148.8, 148.6, 137.34, 137.28, 137.21, 137.17, 137.03, 137.01, 132.1, 130.4, 128.6-127.0 (Cx25), 120.6, 119.8, 114.6, 114.0, 113.5, 112.1, 110.6, 104.4, 104.1, 93.5, 92.8, 91.4, 81.7, 77.4, 74.5, 72.0, 71.4, 71.25, 71.22, 70.6, 70.2, 69.91, 69.56, 69.2, 68.6, 33.2, 29.1, 20.7; minor isomer: 169.1, 158.2, 157.5, 156.0, 155.9, 154.5, 152.9, 148.8, 148.62, 148.58, 148.4, 137.34, 137.26, 137.21, 137.17, 136.9 (x2), 132.3, 132.0, 128.6-127.0 (Cx25), 120.7, 119.8, 114.5, 113.6, 113.5, 112.2, 111.4, 104.8, 103.2, 93.2, 92.3, 91.4, 81.1, 74.9, 72.3, 71.4, 71.3, 71.0, 70.9, 70.8, 70.1, 69.6, 69.2, 68.7, 67.9, 33.5, 29.7, 20.2; FAB-MS (m/z) 1365 (5), 1364 ($[M+Na]^+$, 12), 1344 (11), 1343 (20), 1342 ($[M+H]^+$, 20), 1281 (50), 1190 (40), 631 (100); FAB-HRMS calcd for $C_{88}H_{77}O_{13}$ $[M+H]^+$, 1341.5364; found, 1341.5328.

[4,8]-2,3-*cis*-3,4-*trans*:2,3-*cis*-Octa-*O*-benzyl-3-*O*-acetyl-bi-(-)-epicatechin (15). TMSOTf-catalyzed condensation according to the above procedure using tetra-*O*-benzylated epicatechin (**7**) (101.3 mg, 0.16 mmol) with (2*R*,3*R*,4*S*)-3-acetoxy-5,7,3',4'-tetrabenzyloxy-4-(2''-ethoxyethoxy)flavan (**10**) (27 mg, 0.035 mmol) in CH_2Cl_2 (30 mL), TMSOTf (0.070 mL, 0.035 mmol, 0.5M solution in CH_2Cl_2) at –78°C afforded 35 mg (75 %) of **15** as a colorless amorphous solid: $[\alpha]_D^{25}$ = +25.6° (*c* 0.70, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$, 0.7 : 0.3 mixture of rotational isomers) major isomer: 7.44-6.77 (30.8H, m), 6.80 (0.7H, d, *J* = 8.3 Hz), 6.44 (0.7H, dd, *J* = 1.7, 8.3 Hz), 6.30 (0.7H, s), 6.01 (0.7H, d, *J* = 2.0 Hz), 5.67 (0.7H, d, *J* = 2.0 Hz), 5.63 (0.7H, br s), 5.51 (0.7H, br s), 5.10-4.68 (10.5H, m), 4.64 (0.7H, d, *J* = 11.2 Hz), 4.44 (0.7H, d, *J* = 11.2 Hz), 4.05 (0.7H, s), 3.87 (0.7H, br s), 3.00-2.87 (1.4H, m), 1.70 (2.1H, s), 1.48 (0.7H, br, OH); minor isomer: 7.44-6.77 (13.8H, m), 6.23 (0.3H, d, *J* = 1.9 Hz), 6.19 (0.3H, s), 6.12 (0.3H, d, *J* = 1.9 Hz), 5.37 (0.3H, br s), 5.32 (0.3H, br s), 5.10-4.68 (4.8H, m), 4.64 (0.3H, d, *J* = 11.2 Hz), 4.44 (0.3H, d, *J* = 11.2 Hz), 4.20-4.15 (0.3H, m), 3.00-2.87 (0.6H, m), 1.34 (0.9H, s), 1.21 (0.3H, br, OH); ^{13}C-NMR (100 MHz, $CDCl_3$, 0.7 : 0.3 mixture of rotational isomers) major isomer: 169.0, 158.0, 156.6, 156.5, 156.0, 155.4, 154.4, 149.0, 148.6, 148.5, 148.2, 137.4, 137.34, 137.27, 137.17, 137.14,

132.1, 131.8, 131.2, 128.6-126.7 (Cx24), 119.9, 118.8, 114.74, 114.68, 114.5, 113.5, 112.2, 110.5, 104.7, 102.1, 93.9, 92.9, 91.4, 79.0, 77.2, 74.7, 72.1, 71.34, 71.26, 71.1, 70.5, 69.94, 69.88, 69.76, 69.1, 66.5, 33.2, 28.6, 20.8; minor isomer: 169.0, 158.3, 157.4, 156.5, 155.9, 155.7, 154.3, 148.8, 148.6, 148.4, 148.3, 137.34, 137.27, 137.14, 137.07, 136.8, 131.78, 131.70, 130.9, 128.6-126.9 (Cx24), 119.8, 119.7, 114.5, 113.7, 113.4 (x2), 111.5, 110.6, 104.8, 101.8, 94.5, 93.3, 92.6, 78.2, 76.8, 74.9, 72.4, 71.4, 71.3, 71.1, 70.5, 70.0, 69.9, 69.7, 69.5, 65.8, 33.4, 28.9, 20.3; FAB-MS (m/z) 1365 (30), 1364 ([M+Na]$^+$, 30), 1344 (21), 1343 (30), 1342 ([M+H]$^+$, 40), 1281 (85), 1190 (56), 631 (100); FAB-HRMS calcd for $C_{88}H_{77}O_{13}$ [M+H]$^+$, 1341.5364; found, 1341.5402.

[4,8]-2,3-*cis*-3,4-*trans*:2,3-*cis*-Octa-*O*-benzyl-bi-(-)-epicatechin (16). TMSOTf-catalyzed condensation according to the above procedure using tetra-*O*-benzylated epicatechin (**7**) (594 mg, 0.91 mmol) with (2*R*,3*R*,4*S*)-3-hydroxy-5,7,3',4'-tetrabenzyloxy-4-(2''-ethoxyethoxy)flavan (**9**) (150 mg, 0.20 mmol) in CH_2Cl_2 (30 mL), TMSOTf (0.40 mL, 0.20 mmol, 0.5 M solution in CH_2Cl_2) at –78°C afforded 243 mg (93 %) of **16** as a colorless amorphous solid; $[\square]_D^{30}$ = +40.6° (*c* 1.00, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$, 0.67 : 0.33 mixture of rotational isomers) major isomer: 7.44-6.89 (28.81H, m), 6.88 (0.67H, d, *J* = 1.7 Hz), 6.80 (0.67H, d, *J* = 8.3 Hz), 6.43 (0.67H, dd, *J* = 1.7, 8.3 Hz), 6.34 (0.67H, s), 6.00 (0.67H, d, *J* = 2.2 Hz), 5.69 (0.67H, d, *J* = 2.2 Hz), 5.53 (0.67H, br s), 5.12-4.86 (8.71H, m), 4.80 (1.34H, s), 4.62 (0.67H, d, *J* = 11.3 Hz), 4.46 (0.67H, d, *J* = 11.3 Hz), 4.10-4.04 (0.67H, m), 4.05 (0.67H, br s), 3.88-3.82 (0.67H, m), 2.97 (0.67H, d, *J* = 18.0 Hz), 2.88 (0.67H, dd, *J* = 4.7, 18.0 Hz), 1.79 (0.67H, d, *J* = 5.6 Hz, OH), 1.47-1.45 (0.67H, br, OH); minor isomer: 7.44-6.89 (14.85H, m), 6.85 (0.33H, d, *J* = 8.3 Hz), 6.23 (0.33H, d, *J* = 2.2 Hz), 6.18 (0.33H, s), 6.07 (0.33H, d, *J* = 2.2 Hz), 5.29 (0.33H, br s), 5.12-4.86 (4.95H, m), 4.63 (0.33H, d, *J* = 12.0 Hz), 4.38 (0.33H, d, *J* = 12.0 Hz), 4.34-4.28 (0.33H, m), 4.10 (0.33H, br s), 3.98-3.94 (0.33H, m), 3.04 (0.33H, d, *J* = 16.1 Hz), 2.99-2.94 (0.33H, m), 1.70 (0.33H, d, *J* = 6.1 Hz, OH), 1.32 (0.33H, d, *J* = 7.8 Hz, OH); ^{13}C-NMR (100 MHz, $CDCl_3$, 0.67 : 0.33 mixture of rotational isomers) major isomer: 158.1, 157.1, 156.5, 155.9, 155.0, 154.4, 148.7, 148.39, 148.37, 148.2, 137.27, 137.25 (x2), 137.20, 137.15 (x2), 132.6, 131.1, 128.6-126.7 (Cx27), 119.8, 118.8, 115.0 (x2), 113.4, 112.5, 111.2, 104.4, 102.3, 94.0, 93.1, 91.5, 78.8, 75.6, 72.4 71.3, 71.2, 70.5, 69.92, 69.90, 69.85, 69.0, 66.5, 35.8, 28.6; minor isomer: 158.3, 157.9, 156.8, 156.5, 155.5, 152.8, 149.1, 149.0, 148.9, 148.6, 137.38 137.35, 137.30, 137.1, 136.98, 136.94, 132.4, 131.2, 128.6-126.6 (Cx27), 119.9, 118.6, 114.9, 114.4, 114.1, 112.9, 111.6, 104.4, 101.7, 94.3, 93.3, 92.9, 78.1 77.2, 72.1, 71.5, 71.3, 70.8, 69.9, 69.7, 69.5, 65.1, 60.3, 35.9, 28.7; FAB-MS (m/z) 1302 (45), 1301 (97), 1300 ([M+H]$^+$, 100); FAB-HRMS calcd for $C_{86}H_{75}O_{12}$ [M+H]$^+$, 1299.5259; found, 1299.5229.

[4,8]-2,3-*trans*-3,4-*trans*:2,3-*cis*-Octa-*O*-benzyl-3-*O*-acetyl-(+)-catechin-(-)-epicatechin (19). TMSOTf-catalyzed condensation according to the above procedure using tetra-*O*-benzylated epicatechin (**7**) (420 mg,

0.65 mmol) with (2*R*,3*S*,4*S*)-3-acetoxy-5,7,3', 4'-tetrabenzyloxy-4-(2''-ethoxyethoxy)flavan (**12**) (112 mg, 0.14 mmol) in CH_2Cl_2 (30 mL), TMSOTf (0.28 mL, 0.14 mmol, 0.5 M solution in CH_2Cl_2) at –78°C afforded 169 mg (90 %) of **19** as a colorless amorphous solid: $[\alpha]_D^{30}$ = -75.8° (*c* 1.00, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$, 0.67 : 0.33 mixture of rotational isomers) major isomer: 7.46-6.72 (28.81H, m), 7.06 (0.67H, d, *J* = 1.7 Hz), 6.77 (0.67H, d, *J* = 8.3 Hz), 6.73 (0.67H, dd, *J* = 1.7, 8.3 Hz), 6.213 (0.67H, d, *J* = 2.2 Hz), 6.210 (0.67H, s), 6.13 (0.67H, d, *J* = 2.2 Hz), 5.79 (0.67H, dd, *J* = 9.5, 10.3 Hz), 5.21 (0.67H, d, *J* = 12.0 Hz), 5.19-4.76 (8.04H, m), 5.16 (0.67H, d, *J* = 12.0 Hz), 4.85 (0.67H, d, *J* = 9.5 Hz), 4.70 (0.67H, d, *J* = 10.3 Hz), 4.60 (0.67H, d, *J* = 10.3 Hz), 4.48 (0.67H, d, *J* = 10.3 Hz), 3.94-3.87 (0.67H, m), 3.56 (0.67H, br s), 2.80 (0.67H, d, *J* = 17.3 Hz), 2.55 (0.67H, dd, *J* = 4.6, 17.3 Hz), 1.58 (2.01H, s), 1.28 (0.67H, br, OH); minor isomer: 7.46-6.72 (14.19H, m), 6.76-3.73 (0.66H, m), 6.60 (0.33H, dd, *J* = 1.7, 8.3 Hz), 6.24 (0.33H, d, *J* = 2.4 Hz), 6.22 (0.33H, d, *J* = 2.4 Hz), 5.93 (0.33H, dd, *J* = 9.6, 10.3 Hz), 5.90 (0.33H, s), 5.19-4.55 (5.94H, m), 4.99-4.97 (0.33H, m), 4.78-4.76 (0.33H, m), 3.01-2.91 (0.66H, m), 1.50 (0.99H, s), 1.37 (0.33H, br, OH); ^{13}C-NMR (100 MHz, $CDCl_3$, 0.67 : 0.33 mixture of rotational isomers) major isomer: 169.2, 158.1, 157.8, 156.6, 156.2, 155.7, 153.2, 149.0, 148.9, 148.7, 148.4, 137.4, 137.4, 137.23, 137.19, 137.13, 136.9, 136.7, 132.4, 128.7-127.0 (Cx26), 121.2, 119.0, 115.0, 114.5, 113.8, 113.3, 111.0, 108.4, 100.4, 94.8, 94.3, 91.3, 80.2, 76.9, 73.2, 71.4, 71.3, 71.2, 71.1, 70.2, 70.1, 70.0, 69.9, 65.8, 35.1, 28.5, 20.6; minor isomer: 169.1, 158.3, 158.1, 156.7, 156.3, 156.0, 153.4, 148.94, 148.85, 184.4, 141.9, 138.0, 137.31, 137.26, 137.2, 137.1, 136.9, 136.7, 130.8, 128.7-126.9 (Cx26), 120.9, 119.6, 114.6, 113.9, 113.8, 113.3, 110.1, 108.0, 102.1, 95.2, 94.7, 91.5, 79.9, 78.2, 77.2, 71.7, 71.1, 71.0, 70.8, 70.6, 70.3, 69.9, 69.6, 66.8, 35.2, 28.7, 20.4; FAB-MS (m/z) 1364 ($[M+Na]^+$, 12), 1344 (8), 1343 (15), 1342 ($[M+H]^+$, 17), 1281 (41), 1190 (40), 631 (100); FAB-HRMS calcd for $C_{88}H_{77}O_{13}$ $[M+H]^+$, 1341.5364; found, 1341.5319.

Procyanidin B2 (2). A solution of 145 mg (0.11 mmol) of **16** in THF/MeOH/H_2O, 20/1/1 (20 mL) was hydrogenated over 20% $Pd(OH)_2$/C (14 mg) for 12 h in a current of H_2 at rt. Filtration and concentration afforded a pale brown oil, which was purified by Sephadex® LH-20 column chromatography (EtOH) to give pure procyanidin-B2 (**2**) (55 mg, 86 %) as a pale brown powder: HPLC ^{t}R-**2**, 19.4 min; $[\alpha]_D^{27}$ = +21.5° (*c* 0.58, EtOH) [lit.,[16] $[\alpha]_{578}^{20}$ = +15.2° (*c* 1.2, EtOH)]; ^{1}H-NMR (400 MHz, CD_3OD, all the NMR peaks were broadened and the discrimination of rotational isomers was impossible) 7.10 (1H, br s), 6.90 (1H, br s), 6.78-6.62 (4H, m), 6.05-5.80 (3H, m), 5.06-4.95 (1H, m), 5.00-4.90 (1H, m), 4.62 (1H, br s), 4.35-4.20 (1H, m), 3.90 (1H, br s), 2.90-2.83 (1H, m), 2.82-2.70 (1H, m); FAB-MS (m/z) 602 (5), 601 ($[M+Na]^+$, 13), 580 (3), 579 ($[M+H]^+$, 8), 176 (100); FAB-HRMS calcd for $C_{30}H_{26}O_{12}Na$ $[M+Na]^+$, 601.1322; found, 601.1359.

Decaacetylprocyanidin-B2 (Ac_{10}-2). Acetylation of **2** (25 mg, 0.043 mmol) with Ac_2O (81 μL, 0.86

mmol) and 4-(dimethylamino)pyridine (1 mg, 0.008 mmol) in Py (5 mL) afforded 27 mg (63 %) of Ac_{10}-**2** as a colorless amorphous solid:$[\square]_D^{26}$ = +24.7° (*c* 0.52, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$, 0.7 : 0.3 mixture of rotational isomers) major: 7.36 (0.7H, d, *J* = 2.0 Hz), 7.30-7.25 (0.7H, m), 7.18 (0.7H, d, *J* = 8.5 Hz), 7.04 (0.7H, d, *J* = 8.5 Hz), 7.01 (0.7H, d, *J* = 2.0 Hz), 6.89 (0.7H, dd, *J* = 2.0, 8.5 Hz), 6.66 (0.7H, s), 6.24 (0.7H, d, *J* = 2.2 Hz), 5.99 (0.7H, d, *J* = 2.2 Hz), 5.57 (0.7H, s), 5.17-5.16 (0.7H, m), 5.12-5.09 (0.7H, m), 4.54 (0.7H, s), 4.46 (0.7H, d, *J* = 1.7 Hz), 2.93 (0.7H, dd, *J* = 4.9, 18.3 Hz), 2.86 (0.7H, d, *J* = 18.3 Hz), 2.36 (2.1H, s), 2.30-2.27 (10.5H, m), 2.19 (2.1H, s), 2.05 (2.1H, s), 1.99 (2.1H, s), 1.88 (2.1H, s); minor isomer: 7.30-7.25 (0.6H, m), 7.22 (0.3H, dd, *J* = 2.0, 8.5 Hz), 7.19-7.16 (0.6H, m), 7.13 (0.3H, d, *J* = 8.5 Hz), 6.77 (0.3H, d, *J* = 2.2 Hz), 6.63 (0.3H, d, *J* = 2.2 Hz), 6.58 (0.3H, s), 5.55-5.52 (0.3H, m,), 5.40 (0.3H, br s), 5.34-5.32 (0.3H, m), 5.26 (0.3H, br s), 4.64 (0.3H, s), 3.07 (0.3H, dd, *J* = 4.4, 17.8 Hz), 2.98 (0.3H, d, *J* = 17.8 Hz), 2.31 (0.9H, s), 2.30-2.27 (2.7H, m), 2.27 (0.9H, s), 2.25 (0.9H, s), 2.02 (0.9H, s), 1.86 (0.9H, s), 1.74 (0.9H, s), 1.56 (0.9H, s); ^{13}C-NMR (100 MHz, $CDCl_3$, 0.7 : 0.3 mixture of rotational isomers) major: 170.4, 170.0, 169.7, 169.0, 168.9, 168.3, 168.2, 168.0, 167.87, 167.85, 155.3, 154.2, 149.1, 149.0, 147.9, 147.8, 142.0, 141.9, 141.8, 141.6, 136.5, 134.4, 125.0, 124.4, 123.1, 122.8, 122.4, 122.2, 116.7, 111.7, 111.5, 110.3, 108.6, 107.2, 77.2, 73.6, 71.0, 66.8, 33.9, 26.6, 21.2, 20.95, 20.88, 20.7, 20.64. 20.60 (x2), 20.5, 20.2, 19.9; minor isomer: 170.2, 170.0, 169.7, 169.0 (x2), 168.4, 168.11, 168.07, 167.97. 167.87, 154.9, 151.8, 149.9, 149.7, 148.5, 147.5, 142.1, 142.0, 141.9, 141.7, 136.6, 135.6, 124.3, 123.7, 123.2, 123.1, 121.8, 121.4, 117.5, 111.5, 110.9, 109.8, 109.0, 108.1, 76.7, 74.3, 70.5, 66.3, 34.1, 26.3, 21.2, 21.0, 20.9, 20.74, 20.72, 20.60, 20.56, 20.4, 20.2, 19.9; FAB-MS (m/z) 1023 (5), 1022 (13), 1021 ($[M+Na]^+$, 26), 1000 ($[M+H]^+$, 1), 632 (100); FAB-HRMS calcd for $C_{50}H_{46}O_{22}Na$ $[M+Na]^+$, 1021.2378; found, 1021.2363.

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