Syntheses of prodelphinidin B3 and C2, and their antitumor activities through cell cycle arrest and caspase-3 activation

Wataru Fujii,[a] Kazuya Toda,[b] Koichiro Kawaguchi,[b] Sei-ichi Kawahara,[c] Miyuki Katoh,[a] Yasunao Hattori,[d] Hiroshi Fujii*,[b] and Hidefumi Makabe*[a]

[a]*Graduate School of Agriculture, Sciences of Functional Foods, Shinshu University,*

8304 Minami-minowa Kami-ina, Nagano, 399-4598, Japan

[b]*Department of Bioscience and Biotechnology, Faculty of Agriculture, Shinshu University, 8304 Minami-minowa Kami-ina, Nagano, 399-4598, Japan*

[c]*St. Cousair Co., Ltd., 1260 Imogawa, Kamiminochi, Nagano, 389-1201, Japan*

[d]*Department of Medicinal Chemistry, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8412, Japan*

*Corresponding author. Tel. +81 265 77 1629; fax +81 265 77 1629; e-mail: hfujii@shinshu-u.ac.jp

*Corresponding author. Tel. +81 265 77 1630; fax +81 265 77 1700; e-mail: makabeh@shinshu-u.ac.jp

Abstract: Total synthesis of prodelphinidin B3 and C2 have been accomplished. The key step is Lewis acid-mediated equimolar condensations between a catechin and/or gallocatechin nucleophile and a gallocatechin electrophile. The antitumor effects of synthetic prodelphidin B3 and C2 against PC-3 prostate cancer cell lines have been investigated. Both compounds showed significant antitumor effects. Their activity was almost the same as that of EGCG, a known antitumor agent.

Key words: polyphenols, synthesis, natural product, anticancer agents

1. Introduction

Recently proanthocyanidins have been receiving much attention due to their significant bioactivities.[1,2] However, because identification and purification from nature are extremely difficult, the mechanism for their biological activities remains unsolved. Thus, researchers have sought to synthesize proanthocyanidins.[3-5] Syntheses of procyanidins (oligomeric catechin and/or epicatechin) have been reported in the past decade for the end goal of obtaining proanthocyanidins in a pure state.[6-28] However synthetic studies on prodelphinidins which

contains (−)-gallocatechin or (+)-epigallocatechin are quite limited.[29] Until now no total synthesis of prodelphinidins has been reported. The typical synthesis of catechin derived oligomers can be accomplished using Lewis acid-mediated condensation of catechin nucleophiles and electrophiles. The disadvantage of this reaction is that using an excess amount of nucleophile is necessary to avoid polymerization. Recently, we have developed an efficient synthesis of procyanidins through equimolar condensation of catechin nucleophiles with electrophiles. The typical examples are as follows: 1) For synthesis of a catechin dimer, we accomplished equimolar coupling between nucleophilic and electrophilic partners for the first time using $Yb(OTf)_3$ as a Lewis acid.[7,9,10,13] 2) For synthesis of a catechin trimer, equimolar coupling between a nucleophilic catechin dimer and monomeric electrophilic partner (2+1 coupling) was accomplished using silver Lewis acids such as $AgBF_4$ or AgOTf.[22,30] Herein we demonstrate equimolar condensation of a catechin and/or a gallocatechin nucleophile with a gallocatechin-derived electrophile and the first total synthesis of prodelphinidin B3 (PDB3, **1**) and C2 (PDC2, **2**) (Figure 1).

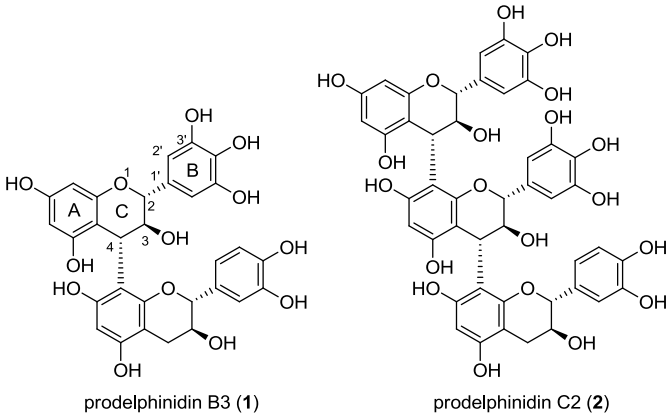
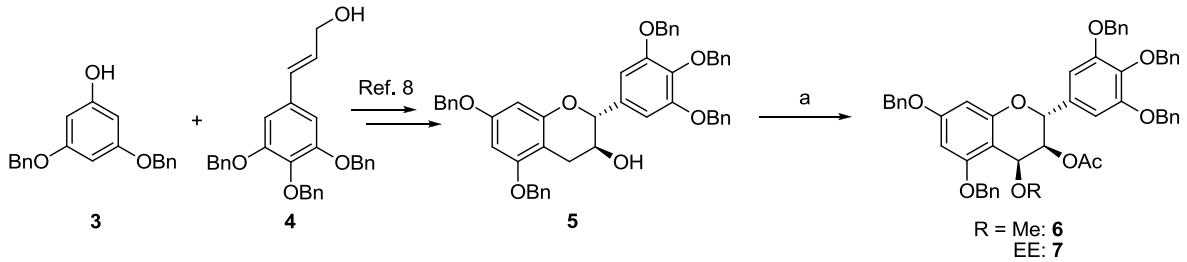


Figure 1. The structures of prodelphinidin B3 (**1**) and C2 (**2**).

2. Results and discussion

2.1. Synthesis

The gallocatechin-derived building block **5** was constructed as Chan and co-workers reported with slight modification.[31] DDQ oxidation in the presence of methanol or ethoxyethanol gave electrophiles **6** and **7**, respectively (Scheme 1).

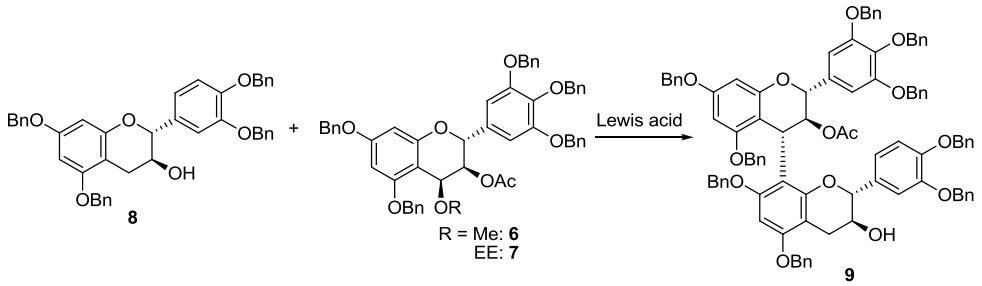


Scheme 1. Synthesis of gallocatechin electrophiles **6** and **7**.

Reagents and conditions: (a) (i) DDQ, ROH; (ii) Ac_2O, pyridine, DMAP, 37% for **6**, 51% for **7**.

Since the gallocatechin electrophile **6** and **7** were in hand, we examined the condition of equimolar condensation of catechin nucleophile **8** with gallocatechin electrophile **6** or **7**. As shown in Table 1, 4-(2”-ethoxyethoxy) derivative **7** afforded condensed product in high yield when $Yb(OTf)_3$ was used as Lewis acid. To our surprise, the reaction using methoxy derivative **6** gave **9** in very poor yield although nucleophile **8** with a related electrophile, the yield was 64% as we have reported earlier.[13] We confirmed that the leaving the group at the C4 position was crucial in the $Yb(OTf)_3$ mediated condensation (Table 1).[9]

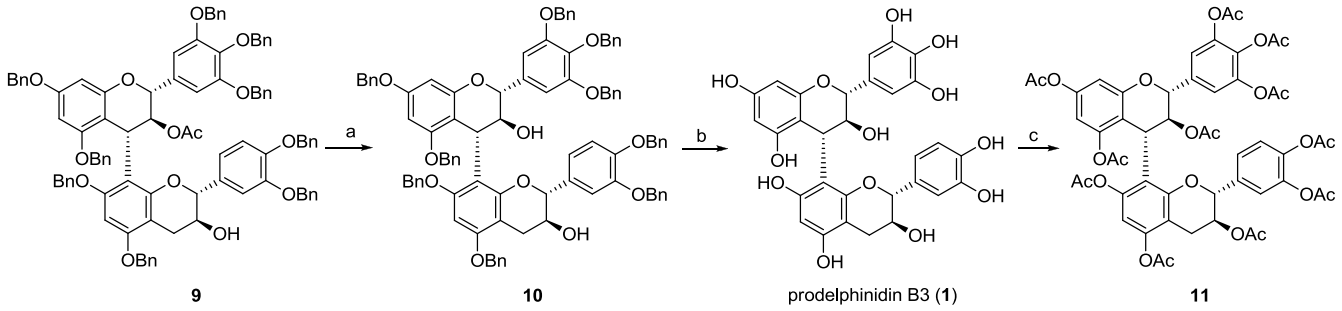
Table 1. Equimolar condensation of gallocatechin electrophile **6** or **7** with catechin nucleophile **8**.[a]



Electrophile	Lewis acid	Time (h)	Yield (%)
6	$AgBF_4$	7	48
6	$Yb(OTf)_3$	7	4
7	$AgBF_4$	7	45
7	$Yb(OTf)_3$	3	86

[a]The reaction was carried out in CH_2Cl_2 at room temperature.

The condensed product **9** was transformed into diol **10** using *n*-Bu_4NOH.[28] The ^1H and ^{13}C NMR spectral data of **10** were in good agreement with the reported values.[29] The 3,4-*cis* diastereomer was not detected. Finally deprotection of the benzyl ethers of **10** and subsequent lyophilization afforded prodelphinidin B3 (**1**) in good yield. The ^1H spectral data of peracetate of **1** (**11**) were in good agreement with the reported values (Scheme 2).[32,33]



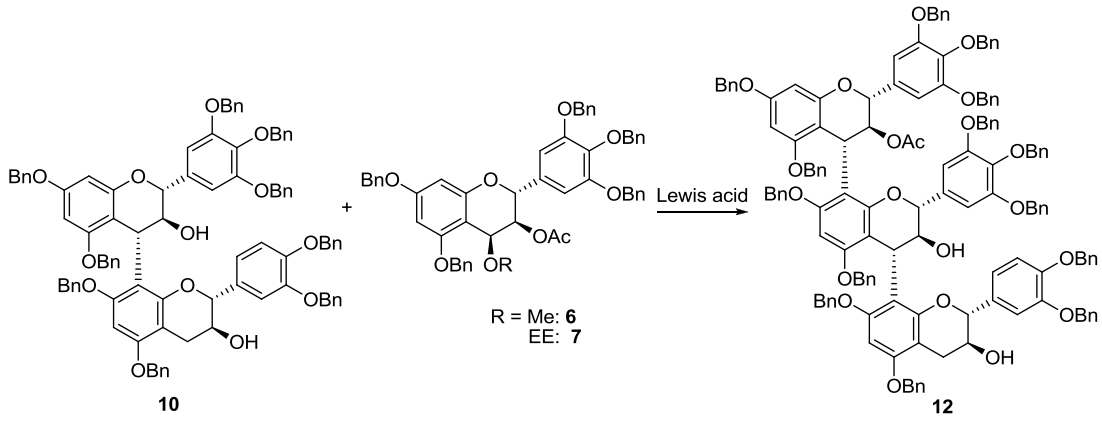
Scheme 2. Synthesis of prodelphinidin B3 (**1**) and its peracetate **11**.

Reagents and conditions:(a) *n*-Bu_4NOH, 82%; (b) H_2, $Pd(OH)_2/C$, 96%; (c) Ac_2O, pyridine, DMAP, 28%.

Because diol **10** could be used as a nucleophile for the synthesis of prodelphinidin C2 (**2**), we focused on the

equimolar condensation of **10** with electrophile **6** or **7**. In the previous report, we found that silver Lewis acids were effective for the construction of catechin trimer derivatives.[22,30] Thus we used $AgBF_4$ and AgOTf as Lewis acid. As shown in Table 2, the 4-methoxy derivative **6** afforded condensed product **12** in good yield when AgOTf was used as a Lewis acid. The reaction using $AgBF_4$ as Lewis acid and 4-methoxy derivative **6** as an electrophile afforded **12** in poor yield although electrophile **6** with a related nucleophile, the yield was 85% as we have reported earlier.[30] We found the combination of the C4 leaving group and silver Lewis acid was very important (Table 2).

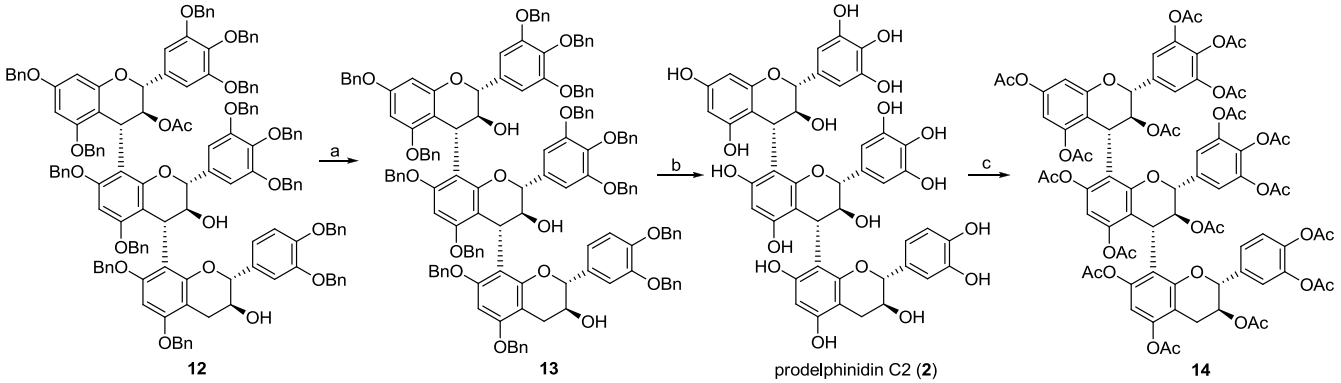
Table 2. Condensation of gallocatechin electrophile **6** or **7** with nucleophile **10**.[a]



Electrophile	Lewis acid	Time (h)	Yield (%)
6	$AgBF_4$	3	14
6	AgOTf	5	73
7	$AgBF_4$	1	63
7	AgOTf	1	11

[a]The reaction was carried out in CH_2Cl_2 at room temperature.

The condensed product **12** was transformed into triol **13** using *n*-Bu_4NOH.[28] Finally deprotection of the benzyl ethers of **13** and subsequent lyophilization afforded prodelphinidin C2 (**2**) in good yield. We confirmed that lyophilized prodelphinidin C2 (**2**) was pure (>95%) by HPLC analysis.[34] The ^1H spectral data of peracetate **14** were in good agreement with the reported values (Scheme 3).[32,35]



Scheme 3. Synthesis of prodelphinidin C2 (**2**) and its peracetate **14**.

Reagents and conditions: (a) *n*-Bu_4NOH, 77%; (b) H_2, $Pd(OH)_2/C$, 96%; (c) Ac_2O, pyridine, DMAP, 27%.

2.2. Cytotoxic effects on PC-3 prostate cancer cell.

Our interest was focused on examining the antitumor activities of the newly synthesized prodelphinidins. The synthesis of prodelphinidin B3 (**1**) and C2 (**2**) allowed us to obtain sufficient quantities of purified compounds to screen against PC-3 prostate cancer cell lines together with procyanidin B3, C1 and C2 which were prepared by us previously (Figure 2).[9,13,22]

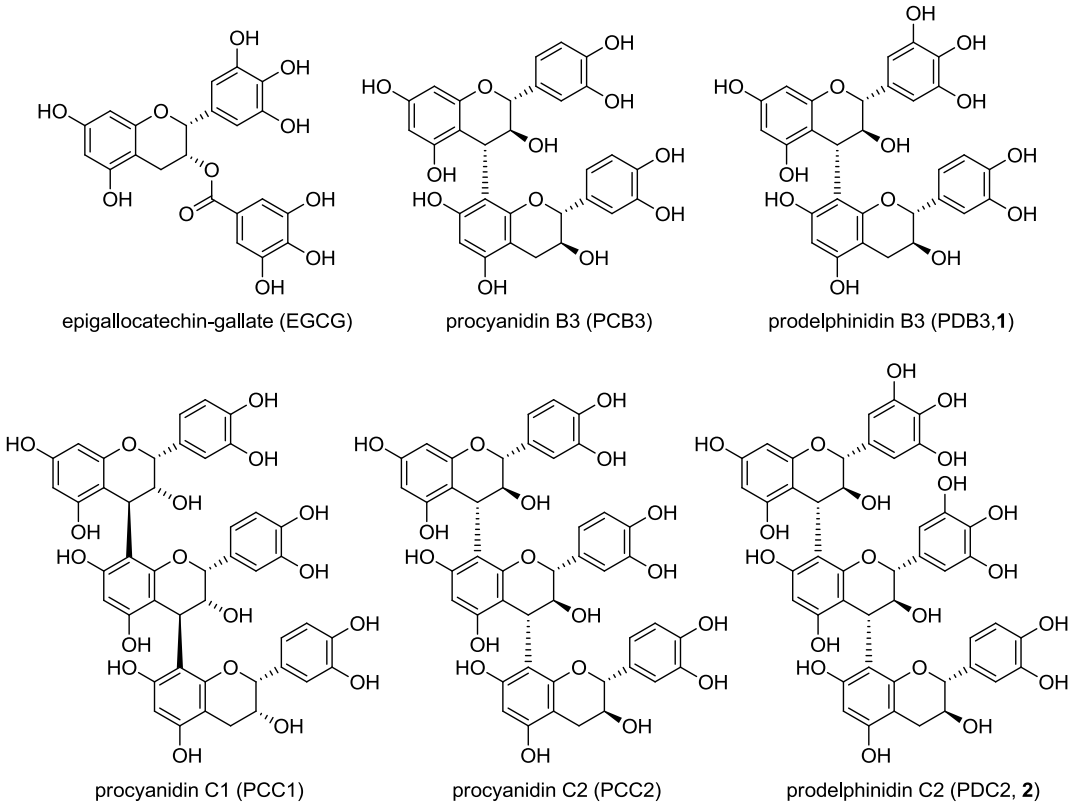


Figure 2. The structures of test compounds for PC3 prostate anticancer activity.

Results were obtained by two independent methods: cell count measurement and MTT assay. Epigallocatechin gallate (EGCG) was used as a positive control. As shown in Figure 3, EGCG, prodelphinidin B3 (**1**) and C2 (**2**) exhibited significant cytotoxic activity with IC_{50} values below 50 μM. A comparison of the potencies of **1** and

procyanidin B3, suggested that the cytotoxic effects were clearly associated with the presence of the pyrogallol moiety of the B ring. PDB3 (**1**) and PCB3 have the same carbon skeleton. The only difference is that PDB3 has an additional hydroxy group at the B ring. We found that this hydroxy group greatly affected the level of the cytotoxic effect. As for **2** and procyanidin C1 or C2, the pyrogallol moiety was essential for their activity.[36] Comparisons between **1** and either **2** or EGCG suggested that the number of pyrogallol moieties did not seem to affect the activity. This tendency was also observed in the MTT assay. This finding might be useful in searching for antitumor molecules among the proanthocyanidins (Figure 3).

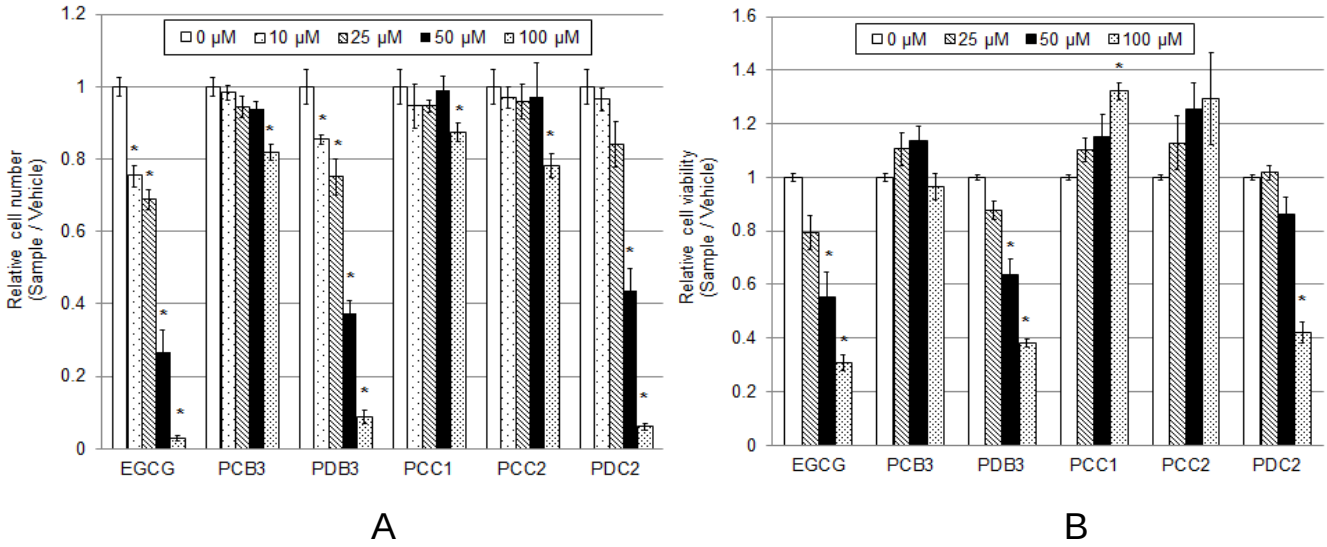


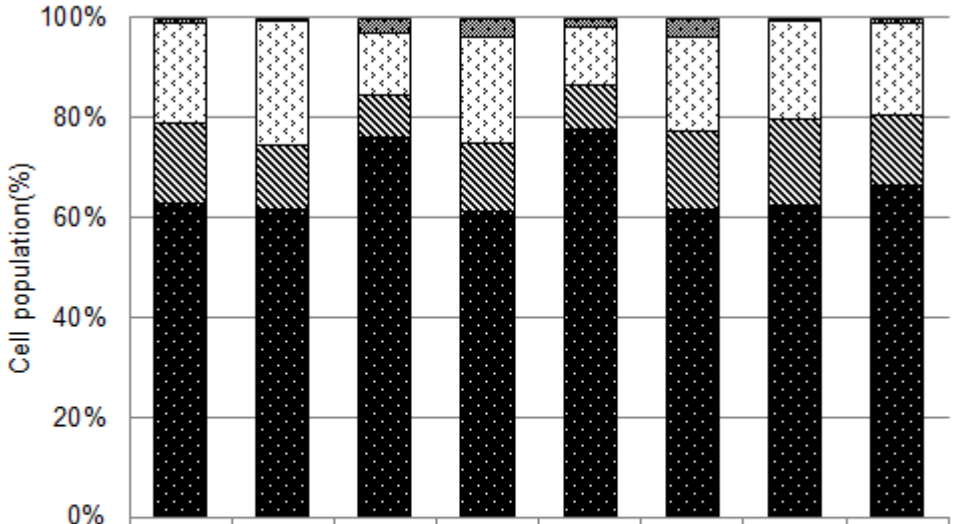
Figure 3. Effects of various concentrations of test compounds on cell proliferation.

After treatment of cells with EGCG, PCB3, PCC1, PCC2, PDB3, PDC2, or CPT for 48 h, the cell proliferation was determined by cell count (A) and MTT assay (B) as shown in supplementary data. The values were represented as the rate of inhibition of cell proliferation by the treated sample compared to the untreated control (vehicle). Values are means ± S.Ds. for three independent experiments. Asterisks indicated a significant difference between the control- and test-compound-treated cells, as analyzed by Student's test ($p < 0.001$).

2.3. Effects on cell cycle distribution.

Cell growth and proliferation are mediated via cell cycle progression. Loss of cell cycle control can initiate the apoptotic program.[37] In the present studies, treatment of PC-3 prostate cancer cells with 50 μM of prodelphinidin B3 (**1**) for 48h induced a G1/G0 phase population increase from 62.88% to 74.50% and an S phase fraction decrease from 16.06% to 8.67%. Obviously, prodelphinidin B3 (**1**) blocked the PC-3 prostate cancer cell cycle partly at the G1/G0 phase within 48h. EGCG and prodelphinidin C2 (**2**) showed a similar effect. On the other hand, no effect from procyanidin B3, C1 or C2 was observed. A lower S-phase population indicated a slower cell division and growing tumor. Compounds which promote cell apoptosis and inhibit proliferation of cancer cells

are likely to be good candidates as antitumor agents.[38] Our findings suggest that prodelphinidins might be promising chemopreventive agents against prostate cancer (Figure 4).



	PBS	EtOH	EGCG	PCB3	PDB3	PCC1	PCC2	PDC2
subG0	0.91	0.80	2.94	3.82	1.57	3.70	0.64	0.91
G2/M	19.88	24.51	12.50	21.18	11.27	19.05	19.59	18.47
S	16.06	13.10	8.16	13.84	8.67	15.46	17.30	13.94
G1/G0	62.88	61.80	76.17	61.38	74.50	62.14	62.65	66.85

Figure 4. Effects of test compounds on cell cycle distribution.

The cells treated with test compounds (EGCG, PCB3, PDB3, PCC1, PCC2, or PDC2) for 48 h were collected and stained with propidium iodide using a BD Cycletest™ Plus DNA Reagent Kit (Becton Dickinson and Company BD Biosciences) obtained from Phoenix Flow Systems. Following FACS analysis, cell cycle distributions were further analyzed by Cell Quest software. The phase fraction (%) is shown in the graph. The experimental data are shown in supplementary data.

2.4. Effects on caspase-3 activity.

It has been well known that the caspases initiate cell apoptosis caused by some stimuli.[39] Caspases are synthesized as inactive proenzymes which need proteolytic cleavage for activation. Caspase-3 is related to the enforcement phase of apoptosis, where cells undergo morphological changes such as chromatin condensation, apoptotic body formation, and DNA fragmentation.[40] As caspase-3 is the most important enzyme involved in execution of apoptosis, its activation, and formation of cleaved caspase-3, is a biochemical marker of early apoptosis. Therefore, the detection of active caspase-3 in cells is a reliable method of measuring apoptosis. Thus, we next examined the caspase-3 activity to confirm that the cells exposed to prodelphinidin were actually undergoing apoptosis, and to determine whether caspase-3 was involved in the cell death pathway. We found that EGCG, prodelphinidin B3 (**1**), and prodelphinidin C2 (**2**) activated caspase-3 up to 1.5~1.8 times compared to the control. Thus, we concluded that the cell death caused by prodelphinidins was attributable to a kind of apoptosis (Figure 5).

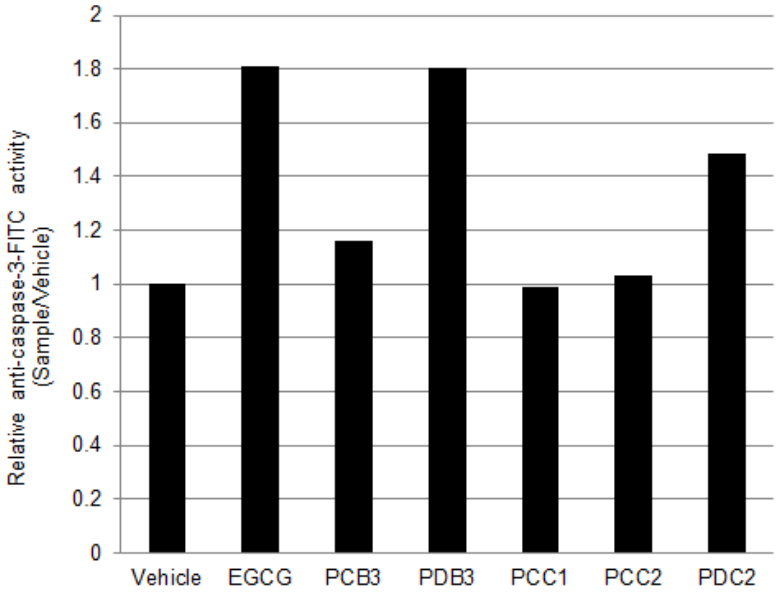


Figure 5. Effects of test compounds on caspase-3 activities assessed by FACS.

Assay for caspase-3 activities after treatment with test compounds (5 μmol/L of CPT and 50 μmol/L of EGCG, PCB3, PDB3, PCC1, PCC2, or PDC2) were performed. The values represented are the rate of induction of apoptosis compared to the untreated control (vehicle). Experimental data are shown in the supplementary data.

3. Conclusion

The first total synthesis of prodelphinidin B3 (**1**) and C2 (**2**) have been achieved via Lewis acid-mediated equimolar condensation of a catechin and/or catechin-gallocatechin nucleophile with gallocatechin electrophiles. In addition to demonstrating the total synthesis, we examined their antitumor activities against PC-3 prostate cancer cells. We found that cytotoxic effects are clearly associated with the presence of the pyrogallol moiety of the B ring; procyanidins which lacked the pyrogallol moiety of the B ring did not show any potency. This activity of prodelphinidins might be ascribed to their blocking cell cycle partly at the G1/G0 phase and activating caspase-3. Prodelphinidins might be potential chemopreventing agents for prostate cancer cells. Based on this study's promising results, we are currently synthesizing various proanthocyanidins which contain pyrogallol moieties to clarify the mechanism of antitumor activity.

4. Experimental

4.1. General. All melting points were uncorrected. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra were measured with a Bruker DRX 500 FT-NMR spectrometer in $CDCl_3$ or CD_3OD with residual tetramethylsilane as the standard. The coupling constants were given in Hz. Mass spectra were obtained on JEOL JMS-SX102A and Waters Xevo QTOF mass spectrometers. IR spectra were recorded with JASCO FT-IR 480 Plus infrared spectrometer. Optical rotations were determined with a JASCO DIP-1000 polarimeter.

4.1.1. (2*R*,3*S*,4*S*)-3-Acetoxy-4-methoxy-5,7-3',4',5'-pentabenzyloxyflavan (6). To a solution of **5** (208 mg, 0.280 mmol) in MeOH/CH_2Cl_2 (1/8, 2.0 mL) was added DDQ (250 mg, 1.10 mmol) at room temperature. After the resulting mixture had been stirred for 1h at room temperature, the mixture was cooled to 0°C and the reaction was quenched with water. The mixture was extracted with Et_2O (3×20 mL) and the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The crude product was purified with silica gel column chromatography (hexane:AcOEt:CH_2Cl_2 = 6:1:3) to afford colorless solid. Acetylation of this compound using general procedure gave **6** (84 mg, 37%, 2 steps) as a colorless oil. $[\alpha]_D^{23}$ +32.4 (*c* 1.15, $CHCl_3$); IR (film) ν_{max} cm^{-1}:3088, 3063, 3032, 2931, 2829, 1742, 1616, 1593, 1230, 1151, 1108, 736, 696; ^1H NMR ($CDCl_3$,) δ: 7.43-7.21 (25H, m), 6.81 (2H, s), 6.28 (1H, d, *J* = 2.5 Hz), 6.18 (1H, d, *J* = 2.0 Hz), 5.28-5.22 (2H, m), 5.15-4.97 (10H, m), 4.77 (1H, d, *J* = 2.5 Hz), 3.45 (3H, s), 1.77 (3H, s); ^{13}C NMR ($CDCl_3$) δ:169.4, 160.9, 158.4, 155.5, 152.5, 138.4, 137.7, 137.0, 136.9, 136.4, 136.3, 132.7, 128.5, 128.4, 128.1, 128.0, 127.8, 127.7, 127.6, 127.4, 107.3, 103.1, 94.2, 93.7, 75.0, 74.5, 72.5, 71.3, 71.1, 70.4, 70.0, 69.4, 69.1, 59.0, 20.7; HRFABMS $(M+Na)^+$: Calcd. for $C_{53}H_{48}NaO_9$, 851.3196, Found; 851.3201.

4.1.2. (2*R*,3*S*,4*S*)-3-Acetoxy-4-ethoxyethoxy-5,7,3',4',5'-pentabenzyloxyflavan (7). To a solution of **5** (118 mg, 0.160 mmol) in 2-ethoxyethanol/CH_2Cl_2 (1/8, 2.7 mL) was added DDQ (106 mg, 0.470 mmol) at room temperature. After the resulting mixture had been stirred for 1h at room temperature, the mixture was cooled to 0°C and the reaction was quenched with water. The mixture was extracted with Et_2O (3×10 mL) and the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The crude product was purified with silica gel column chromatography (hexane:AcOEt:CH_2Cl_2 = 6:1:3) to afford colourless solid. Acetylation of this compound using general procedure gave **7** (70 mg, 51%, 2 steps) as a colorless oil. $[\alpha]_D^{23}$ +31 (*c* 1.9, $CHCl_3$); IR (film) ν_{max} cm^{-1}: 3032, 2925, 1741, 1616, 1592, 1230, 1151, 1110, 736, 697; ^1H NMR ($CDCl_3$,) δ: 7.48-7.20 (25H, m), 6.77 (2H, d, *J* = 1.5 Hz), 6.26 (1H, d, *J* = 2.0 Hz), 6.16 (1H, d, *J* = 1.5 Hz), 5.28-4.95 (12H, m), 4.88 (1H, d, *J* = 3.0 Hz), 3.82-3.70 (2H, m), 3.50-3.40 (4H, m), 1.79 (3H, s), 1.15 (3H, t, *J* = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ: 169.6, 160.9, 158.5, 155.6, 152.7, 138.6, 137.8, 137.0, 136.6, 136.5, 132.9, 128.6, 128.5, 128.4, 128.1, 128.0, 127.8, 127.7, 127.6, 127.5, 107.6, 103.6, 94.3, 93.8, 75.1, 74.5, 72.6, 71.2, 70.7, 70.5, 70.3, 70.1, 68.2, 66.4, 20.7, 15.2; HRFABMS $(M+Na)^+$: Calcd. for $C_{56}H_{54}NaO_{10}$, 909.3615, Found; 909.3610.

4.1.3. [4,8']-2,3-*trans*-3.4-*trans*:2',3'-*trans*-3-Acetoxy-nonabenzyloxy-(+)-gallocatechin-(+)-catechin (9). To a solution of nucleophile **8** (28 mg, 43 μmol) and electrophile **7** (38 mg, 43 μmol) in CH_2Cl_2 (4.0 mL) was added $Yb(OTf)_3$ (27 mg, 43 μmol). After the resulting mixture had been stirred for 3h at room temperature, the reaction was quenched with water. The mixture was extracted with EtOAc (2×10 mL) and the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The crude product was purified with preparative TLC (hexane:AcOEt:CH_2Cl_2 = 6:1:3) to afford **9** (53 mg, 86%) as pale yellow oil. $[\alpha]_D^{20}$ –114 (*c*

1.15, $CHCl_3$); IR (film) ν_{max} cm^{-1}:3524, 3062, 3031, 2928, 2870, 1741, 1593, 1231, 1114, 735, 696; ^1H NMR ($CDCl_3$, 1:1 rotational isomer, major isomer) δ: 7.48-7.10 (43.5H, m), 6.94-6.60 (7H, m), 6.43 (0.5H, dd, *J* = 8.5, 1.5 Hz), 6.24 (1H, s), 6.22 (0.5H, d, *J* = 2.0 Hz), 6.19 (0.5H, d, *J* = 2.0 Hz), 6.15 (0.5H, d, *J* = 2.0 Hz), 6.13 (0.5H, d, *J* = 2.0 Hz), 5.98 (0.5H, t, *J* = 9.5 Hz), 5.96 (0.5H, s), 5.85 (0.5H, t, *J* = 9.5 Hz), 5.22-4.50 (18.5H, m), 3.84 (0.5H, m), 3.56 (0.5H, m), 3.27 (0.5H, d, *J* = 9.0 Hz), 3.01 (0.5H, dd, *J* = 16.5, 6.0 Hz), 2.86 (1H, dd, *J* = 16.5, 5.5 Hz), 2.72 (0.5H, dd, *J* = 16.5, 7.5 Hz), 2.35 (0.5H, dd, *J* = 16.5, 9.8 Hz), 2.05 (0.5H, m), 1.58 (1.5H, s), 1.49 (1.5H, s), 1.27 (0.5H, d, *J* = 1.5 Hz); ^{13}C NMR ($CDCl_3$) δ: 169.3, 168.8, 158.2, 157.8, 156.8, 156.5, 155.8, 155.6, 154.9, 153.5, 152.6, 149.2, 148.9, 148.7, 148.6, 138.4, 138.2, 137.9, 137.8, 137.4, 137.3, 137.2, 137.1, 137.0, 136.9, 136.8, 136.5, 133.0, 132.7, 132.6, 132.3, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.2, 127.1, 126.9, 126.8, 120.3, 120.0, 114.8, 114.6, 114.2, 113.3, 110.6, 110.5, 110.0, 108.5, 108.2, 107.9, 107.7, 107.2, 107.0, 102.4, 102.3, 94.8, 94.7, 94.5, 94.4, 91.1, 81.3, 80.7, 80.2, 76.7, 72.8, 72.1, 71.5, 71.3, 71.2, 71.1, 71.0, 70.9, 70.5, 70.4, 70.1, 70.0, 69.9, 69.8, 68.3, 67.9, 35.3, 35.1, 28.6, 26.7, 20.6, 20.4; HRFABMS $(M+Na)^+$: Calcd. for $C_{95}H_{82}NaO_{14}$, 1469.5602, Found; 1469.5597.

4.1.4. [4,8']-2,3-*trans*-3.4-*trans*:2',3'-*trans*-Nonabenzyloxy-(+)-gallocatechin-(+)-catechin (10). To a solution of **9** (44 mg, 30 μmol) in THF (3.0 mL) was added 40% aqueous *n*-Bu_4NOH (0.59 mL, 0.91 mmol). The reaction mixture was allowed to be stirred for 72h at room temperature, then partially evaporated to remove THF. The residue was diluted with H_2O (5.0 mL), and the product was extracted with EtOAc (2×5.0 mL). The combined organic layers were washed with brine and concentrated. The residue was purified with preparative TLC (hexane:AcOEt:CH_2Cl_2 = 4:1:2) to afford **10** (35 mg, 82%) as pale yellow oil. $[\alpha]_D^{19}$ −106 (*c* 1.60, $CHCl_3$); IR (film) ν_{max} cm^{-1}:3566, 3062, 3031, 2926, 1592, 1112, 736, 696; ^1H NMR ($CDCl_3$, 2:1 rotational isomer, major isomer) δ: 7.47-7.20 (40H, m), 6.94-6.84 (7H, m), 6.94 (1H, d, *J* = 2.0 Hz), 6.80 (1H, dd, *J* = 8.5, 2.0 Hz), 6.26 (1H, s), 6.18 (1H, d, *J* = 2.0 Hz), 6.13 (1H, d, *J* = 2.0 Hz), 5.20-4.60 (18H, m), 4.57 (2H, d, *J* = 11.0 Hz), 4.48 (1H, d, *J* = 9.5Hz), 4.21 (1H, dd, *J* = 9.5, 8.5 Hz), 3.75-3.60 (1H, m), 3.59 (1H, d, *J* =8.0 Hz), 3.02 (1H, dd, *J* = 16.5, 5.8 Hz), 2.39 (1H, dd, *J* = 16.0, 9.0 Hz), 1.60-1.28 (2H, m); minor isomer: δ: 7.47-7.27 (20H, m), 7.16-7.12 (3.5H, m), 6.56 (0.5H, d, *J* = 2.0 Hz), 6.48 (0.5H, dd, *J* = 8.5, 2.0 Hz), 6.35 (0.5H, s), 6.09 (0.5H, d, *J* = 2.0 Hz), 6.05 (0.5H, d, *J* = 2.0 Hz), 5.16-4.65 (10H, m), 4.50 (0.5H, d, *J* = 9.5 Hz), 4.45 (0.5H, dd, *J* = 9.5, 8.5Hz), 4.18 (0.5H, d, *J* = 8.5 Hz), 3.75-3.60 (0.5H, m), 3.16 (0.5H, dd, *J* = 16.5, 6.0 Hz), 2.64 (0.5H, dd, *J* = 16.5, 9.0 Hz), 1.70-1.35 (1H, m); ^{13}C NMR ($CDCl_3$) δ: 158.0, 157.8, 157.6, 156.7, 155.6, 155.5, 154.0, 152.9, 152.8, 149.1, 149.0, 138.7, 137.9, 137.3, 137.2, 137.1, 137.0, 136.7, 134.2, 131.6, 130.6, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.2, 127.1, 120.7, 120.2, 114.9, 114.3, 113.9, 112.0, 107.2, 106.9, 103.3, 102.6, 94.9, 91.9, 91.5, 82.3, 82.1, 81.3, 80.7, 77.6, 75.2, 73.4, 73.2, 71.3, 71.0, 70.3, 70.1, 70.0, 69.9, 68.6, 68.4, 37.2, 33.7, 31.9; HRFABMS $(M+Na)^+$: Calcd. for $C_{93}H_{80}NaO_{13}$, 1427.5497, Found; 1427.5493.

4.1.5. Prodelphinidin B3 (1). A solution of **10** (32 mg, 23 μmol) in THF/MeOH/H_2O (20/20/1) (4.0 mL) was hydrogenated over 20% $Pd(OH)_2$/C (28 mg) for 3h at room temperature. The mixture was filtered and the

filtration residue was washed with MeOH (10 mL). The combined filtrates were evaporated, and the residue was taken up in distilled water (5.0 mL). The solution was filtered and lyophilized to give **1** (13 mg, 96%) as a fluffy amorphous solid. Mp 212-213 ^{o}C (decomp.); $[\alpha]_D^{19}$ –207 (*c* 0.350, MeOH); IR (KBr) ν_{max} cm^{-1}: 3350, 2924, 1614, 1520, 1452, 1365, 1283, 1233, 1144, 1032, 821, 628; ^{1}H NMR (CD_3OD, 2:1 rotational isomer) δ: 6.94-6.05 (5H, m), 5.88 (0.67H, d, *J* = 2.0 Hz), 5.82 (0.33H, d, *J* = 2.0 Hz), 5.82 (0.33H, d, *J* = 2.5 Hz), 5.74 (0.67H, d, *J* = 2.5 Hz), 4.73 (0.33H, d, *J* = 7.0 Hz), 4.65 (0.67H, d, *J* = 7.0 Hz), 4.48 (1H, dd, *J* = 9.5, 8.5 Hz), 4.42 (1H, d, *J* = 8.0 Hz), 4.34 (0.67H, dd, *J* = 10.0, 8.0 Hz), 4.28 (0.33H, dd, *J* = 10.0, 8.0 Hz), 4.23 (0.67H, d, *J* = 9.5 Hz), 4.17 (0.33H, d, *J* = 9.0 Hz), 4.07 (0.33H, m), 3.78 (0.67H, m), 2.68 (0.67H, dd, *J* = 16.0, 5.0 Hz), 2.80 (0.33H, dd, *J* = 16.0, 5.0 Hz), 2.58 (0.33H, dd, *J* = 16.0, 7.8 Hz), 2.48 (0.67H, dd, *J* = 16.5, 8.0 Hz); ^{13}C NMR (CD_3OD) δ: 158.7, 157.2, 155.9, 155.8, 146.7, 146.6, 146.2, 145.6, 131.9, 131.7, 119.5, 116.3, 116.1, 115.1, 108.3, 107.9, 107.1, 101.7, 97.4, 96.9, 95.9, 84.3, 82.9, 82.0, 73.5, 68.6, 38.7, 38.5, 27.9; HRFABMS $(M+Na)^+$: Calcd. for $C_{30}H_{26}NaO_{13}$, 617.1271, Found; 617.1276.

4.1.6. Peracetate of 1 (11). A mixture of pyridine (50 μL), acetic anhydride (50 μL), and DMAP (1.0 mg) was added to **1** (4.0 mg, 6.7 μmol). After the reaction mixture had been stirred for 12h, saturated aqueous $NaHCO_3$ (5 mL) was added, and the product was extracted with EtOAc (2 x 10 mL). The organic layers were washed with H_2O (5.0 mL) and brine, and dried over $MgSO_4$, filtered, and concentrated. The crude product was purified with preparative TLC (hexane:AcOEt:CH_2Cl_2 = 6:1:3) to afford peracetate **11** (2.0 mg, 28%) as colorless glass. IR (film) ν_{max} cm^{-1}: 2924, 2851, 1772, 1617, 1505, 1431, 1371, 1206, 1127, 1046, 896, 736; ^{1}H NMR ($CDCl_3$) δ: 7.12 (1H, d, *J* = 8.5 Hz), 6.96 (2H, s), 6.90 (1H, d, *J* = 2.0 Hz), 6.70 (1H, dd, *J* = 8.5, 2.0 Hz), 6.65 (1H, s), 6.49 (2H, s), 5.61 (1H, dd, *J* = 10.0, 8.0 Hz), 5.08-4.99 (2H, m), 4.71 (1H, d, *J* = 10.0 Hz), 4.50 (1H, d, *J* = 9.5 Hz), 2.86 (1H, m), 2.66 (1H, m), 2.35 (3H, s), 2.29-2.24 (21H, m), 1.98 (3H, s), 1.95 (3H, s), 1.70 (3H, s); ^{13}C NMR ($CDCl_3$) δ: 170.1, 169.1, 168.6, 168.4, 168.1, 167.7, 167.5, 166.6, 155.8, 152.6, 149.6, 149.2, 149.0, 147.9, 147.6, 143.3, 143.2, 142.0, 141.6, 135.2, 135.0, 134.7, 134.6, 124.5, 123.4, 122.2, 121.7, 120.0, 119.6, 116.7, 115.2, 111.3, 110.2, 110.0, 109.6, 109.4, 108.2, 107.9, 78.8, 77.9, 77.7, 70.4, 68.3, 68.1, 36.7, 29.7, 25.2, 21.1, 20.9, 20.7, 20.6, 20.4, 20.3, 20.1.

4.1.7. [4,8:4”,8”]-2,3-*trans*-3,4-*trans*: 2”,3”-*trans*-3”,4”-*trans*:2’’’,3’’’-*trans*-Acetoxytetradecabenzyloxy-(+)-gallocatechin-(+)-gallocatechin-(+)-catechin (12). To a solution of nucleophile **10** (43 mg, 31 μmol) and electrophile **6** (26 mg, 31 μmol) in CH_2Cl_2 (4.0 mL) under an argon atmosphere was added AgOTf (7.8 mg, 31 μmol). After the resulting mixture had been stirred for 5h at room temperature, the reaction was quenched with water. The mixture was extracted with AcOEt, and the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The crude product was purified with silica gel column chromatography (hexane:AcOEt:CH_2Cl_2 = 6:1:3) to give **12** (49 mg, 73%) as an pale yellow oil. $[\alpha]_D^{19}$ –80 (*c* 0.23, $CHCl_3$); IR (film) ν_{max} cm^{-1}:3566, 3062, 3031, 2927, 2829, 1741, 1593, 1113, 735, 696; HRFABMS $(M+Na)^+$: Calcd. for

$C_{145}H_{124}NaO_{21}$, 2223.8533, Found; 2223.8521.

4.1.8. [4,8:4”,8”]-2,3-*trans*-3,4-*trans*: 2”,3”-*trans*-3”,4”-*trans*:2’’’,3’’’-*trans*-Tetradecabenzyloxy-(+)-gallocatechin-(+)-gallocatechin-(+)-catechin (13). To a solution of **12** (60 mg, 0.025 mmol) in THF (3.0 mL) was added *n*-Bu_4NOH (0.46 mL, 0.76 mmol). The reaction mixture was allowed to be stirred for 72h at room temperature, then partially evaporated to remove THF. The residue was diluted with H_2O (5.0 mL), and the product was extracted with EtOAc (2×5.0 mL). The combined organic layers were washed with brine and concentrated. The residue was purified with preparative TLC (hexane:AcOEt:CH_2Cl_2 = 6:1:3) to afford **13** (44 mg, 77%) as an pale yellow oil. $[\alpha]_D^{22}$ –91 (*c* 0.14, $CHCl_3$); IR (film) ν_{max} cm^{-1}:3573, 3062, 3031, 2869, 1742, 1593, 1113, 735, 696; ^1H NMR ($CDCl_3$, 0.82:0.18 mixture of rotational isomer, major isomer) δ = 7.44-5.90 (81H, m), 5.57-4.40 (30H, m), 4.25 (1H, d, *J* = 8.8 Hz), 4.05-3.95 (2H, m), 3.81-3.75 (1H, m), 3.57 (1H, d, *J* = 9.0 Hz), 3.10 (1H, dd, *J* = 16.0, 6.0 Hz), 2.92 (1H, d, *J* = 9.5 Hz), 2.37 (1H, dd, *J* = 16.5, 9.8 Hz), 1.52 (2H, brs, -OH), 1.12 (1H, brs, -OH); ^{13}C NMR ($CDCl_3$) δ: 158.0, 157.9, 156.6, 156.5, 156.0, 155.6, 155.5, 155.3, 155.2, 155.0, 153.9, 152.9, 152.7, 152.5, 149.4, 149.1, 138.4, 138.3, 138.1, 138.0, 137.8, 137.4, 137.2, 137.1, 136.0, 134.7, 134.6, 128.9, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.2, 127.1, 127.0, 120.7, 115.2, 114.6, 112.5, 109.5, 108.9, 108.0, 107.5, 107.1, 107.0, 102.6, 94.9, 94.1, 92.2, 91.9, 82.2, 81.1, 75.2, 75.0, 73.0, 72.9, 71.4, 71.3, 71.1, 70.6, 70.4, 70.3, 70.0, 69.9, 68.4, 37.7, 37.4, 29.7; HRFABMS $(M+Na)^+$: Calcd. for $C_{143}H_{122}NaO_{20}$, 2181.8427, Found; 2181.8440.

4.1.9. Prodelphinidin C2 (2). A solution of **13** (32 mg, 15 μmol) in THF/MeOH/H_2O (20/20/1) (4.0 mL) was hydrogenated over 20% $Pd(OH)_2/C$ (28 mg) for 3h at room temperature. The mixture was filtered and the filtration residue was washed with MeOH (10 mL). The combined filtrates were evaporated, and the residue was taken up in distilled water (5.0 mL). The solution was filtered and lyophilized to give **2** (13 mg, 96%) as a fluffy amorphous solid. Mp 218-220 °C (decomp.); $[\alpha]_D^{21}$ –283 (*c* 0.350, MeOH); IR (KBr) ν_{max} cm^{-1}: 3350, 2925, 1616, 1523, 1453, 1367, 1281, 1238, 1140, 1035, 821, 629; ^1H NMR (CD_3OD) δ = 7.05-5.75 (11H, m), 4.80-4.05 (6H, m), 2.90-2.40 (4H, m); ^{13}C NMR (CD_3OD) δ = 158.6, 157.7, 157.2, 155.9, 155.8, 155.6, 155.5, 155.4, 155.3, 155.2, 155.1, 155.0, 146.7, 146.2, 146.0, 145.9, 134.2, 130.8, 124.5, 120.2, 117.6, 116.2, 115.3, 108.7, 108.5, 108.3, 97.4, 96.1, 95.7, 84.0, 83.2, 81.9, 73.0, 69.8, 68.6, 68.5, 68.3, 38.9, 38.8, 30.8, 21.3; ESI-TOFMS calcd for $C_{45}H_{38}O_{20}Na$ $(M+Na)^+$; 921.1854; found 921.1941.

4.1.10. Peracetate of 2 (14). A mixture of pyridine (50 μL), acetic anhydride (50 μL), and DMAP (1.0 mg) was added to **2** (4.0 mg, 4.5 μmol). After the reaction mixture had been stirred for 12h, saturated aqueous $NaHCO_3$ (5.0 mL) was added, and the product was extracted with EtOAc (2×10 mL). The organic layers were washed with H_2O (5.0 mL) and brine, and dried over $MgSO_4$, filtered, and concentrated. The crude product was purified with preparative TLC (hexane:AcOEt:CH_2Cl_2 = 6:1:3) to afford peracetate **14** (2.0 mg, 27%) as colorless

glass. IR (film) ν_{max} cm^{-1}: 2925, 2850, 1773, 1619, 1502, 1433, 1370, 1207, 1128, 1042, 896, 736; ^1H NMR ($CDCl_3$) δ: 7.05 (1H, d, *J* = 8.5 Hz), 6.96 (2H, s), 6.86 (1H, d, *J* = 2.0 Hz), 6.67 (2H, s), 6.64 (1H, s), 6.63 (1H, d, *J* = 6.5 Hz), 6.54 (1H, d, *J* = 2.5 Hz), 6.25 (1H, d, *J* = 2.5 Hz), 5.57 (1H, dd, *J* = 19.0, 9.5 Hz), 5.47 (1H, dd, *J* = 19.0, 9.5 Hz), 5.30-5.15 (2H, m), 4.78 (1H, d, *J* = 10.0 Hz), 4.66 (1H, d, *J* = 10.0 Hz), 4.58 (1H, d, *J* = 8.0 Hz), 4.18 (1H, d, *J* = 9.0 Hz), 2.56 (2H, s), 2.38-1.61 (OAc-groups).

4.2. Biochemical methods

4.2.1. Cell lines, cell culture and reagents. THuman prostate cancer cell, PC-3, was purchased from the Health Science Research Resources Bank. The cells were maintained in monolayer culture at 37°C and 5% CO_2 in RPMI-1640 (SIGMA, R8755) supplemented with 10% charcoal-stripped fetal bovine serum (Biological Industries, No. 04-201-1), 1% antibiotic-antimycotic mixed stock solution (Nacalai Tesque, No. 09366-44). The cells were treated with various concentrations of epigallocatechine-3-gallate (EGCG), procyanidin B3 (PCB3), procyanidin C1 (PCC1), procyanidin C2 (PCC2), prodelphinidin B3 (PDB3), prodelphinidin C2 (PDC2) or camptothecin (CPT) for 48 h. CPT (WAKO, No. 038-18191) was used for the index of apoptosis. EGCG was generously gifted from Prof. Dr. Toshiyuki Kan in University of Shizuoka.

4.2.2. Cell count (Figure 3 A). Cells were plated in 12-well plates (1×10^4 cells/well) and grew to reach 50% confluent. The cells were treated with the indicated concentrations of EGCG, PCB3, PCC1, PCC2, PDB3, PDC2, or CPT for 48 h. The cells treated with the above test compounds were trypsinized. After adding the culture medium to each well, they were agitated by pipetting. The number of cells was measured with the hemocytometer.

4.2.3. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay (Figure 3 B). The degree of cell proliferation was evaluated by MTT assay using Cell Count Kit (Nacalai Tesque, No. 23506-80), according to the manufacturer's protocol. The cells were plated in 96-well plates and treated with the indicated concentrations of EGCG, PCB3, PCC1, PCC2, PDB3, PDC2, or CPT for 48 h. Absorbance at 595 nm was measured using the microplate reader after the addition of the MTT solvent.

4.2.4. Measurement of apoptosis by assay for caspase-3 activity (Figure 5). Assay for caspase-3 activities were carried out using BD Cytofix/Cytoperm™ Kit (BD Biosciences, No. 554714), according to the manufacturer's protocol. Purified rabbit anti-active caspase-3 (BD Pharmingen™, No. 559565) was used for the first antibody (1st Ab) and FITC-conjugate anti-rabbit Ig G (Jackson ImmunoResearch, No. 711-096-152) was used for the second antibody (2nd Ab). Briefly, after treatment of cells with 50 μM of EGCG, PCB3, PCC1,

PCC2, PDB3, PDC2, or 500 nM of CPT for 48 h, the cells were collected and prepared by the same method as cell cycle analysis described in Materials and methods. The cells were diluted in PBS and fixed with BD Cytofix/Cytoperm^{TM} Fixation and Permeabilization Solution for 20 min on ice in the dark. The cells were washed with the washing buffer and then reacted with 1st Ab at room temperature. Next, the cells were washed with the washing buffer and then reacted with 2nd Ab at room temperature. After the reaction, the cells were diluted in PBS, and flow cytometry was performed with a FACScan (Becton Dickinson, Japan), and the data obtained were analyzed utilizing Cell Quest software. For each sample, 1×10^4 cells were recorded.

4.2.5. Statistical analysis. Each experiment was performed at least three times. Data were expressed as the means ± standard deviation (S. D.). Statistical analysis was performed using Student's t-test. $P < 0.05$ or $P < 0.01$ was considered to be significant.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi

References and notes

1. Ferreira, D.; Li, X. –C. *Nat. Prod. Rep*. **2000**, *17*, 193.

2. Ferreira, D.; Li, X. –C. *Nat. Prod. Rep*. **2002**, *19*, 517.

3. Ferreira, D.; Coleman, C. M. *Planta Med.* **2011**, *77*, 1071.

4. Oyama, K. –i.; Yoshida, K.; Kondo, T. *Curr. Org. Chem.* **2011**, *15*, 2567.

5. Ohmori, K.; Suzuki, K. *Curr. Org. Chem.* **2012**, *16*, 566.

6. Nakajima, N.; Horikawa, K.; Takekawa, N.; Hamada, M.; Kishimoto, T. *Heterocycles* **2012**, *84*, 349.

7. Katoh, M.; Oizumi, Y.; Mohri, Y.; Hirota, M.; Makabe, H. *Lett. Org. Chem.* **2012**, *9*, 233.

8. Alharthy, R. D.; Hayes, C. J. *Tetrahedron Lett.* **2010**, *51*, 1193.

9. Oizumi, Y.; Mohri, Y.; Hirota, M.; Makabe, H. *J. Org. Chem.* **2010**, *75*, 4884.

10. Mohri, Y.; Sagehashi, M.; Yamada, T.; Hattori, Y.; Morimura, K.; Hamauzu, Y.; Kamo, T.; Hirota, M.; Makabe, H. *Heterocycles* **2009**, *79*, 549.

11. Oyama, K. –i.; Kuwano, M.; Ito, M.; Yoshida, K.; Kondo, T. *Tetrahedron Lett.* **2008**, *49*, 3176.

12. Viton, F.; Landreau, C.; Rustidge, D.; Robert, F.; Williamson, G.; Barron, G. *Eur. J. Org. Chem.* **2008**, 6069.

13. Mohri, Y.; Sagehashi, M.; Yamada, T.; Hattori, Y.; Morimura, K.; Kamo, T.; Hirota, M.; Makabe, H. *Tetrahedron Lett*. **2007**, *48*, 5891.

14. Tarascou, I.; Barathieu, K.; André, Y.; Pianet, I.; Dufourc, E. J.; Fouquet, E. *Eur. J. Org. Chem.* **2006**, 5367.

15. Sakuda, H.; Saito, A.; Mizushina, Y.; Yoshida, H.; Tanaka, A.; Nakajima, N. *Heterocycles* **2006**, *67*, 175.

16. Saito, A.; Mizushina, Y.; Ikawa, H.; Yoshida, H.; Doi, Y.; Tanaka, A.; Nakajima, N. *Bioorg. Med. Chem.* **2005**, *13*, 2759.

17. Saito, A.; Nakajima, N.; Matsuura, N.; Tanaka, A.; Ubukata, M. *Heterocycles* **2004**, *62*, 479.

18. Saito, A.; Nakajima, N.; Tanaka, A.; Ubukata, M. *Heterocycles* **2003**, *61*, 287.

19. Saito, A.; Nakajima, N.; Tanaka, A.; Ubukata, M. *Tetraherdron* **2002**, *58*, 7829.

20. Tückmantel, W.; Kozikowski, A. P.; Romanczyk, Jr. L. J. *J. Am. Chem. Soc.* **1999**, *121*, 12073.

21. Yano, T.; Ohmori, K.; Takahashi, H.; Kusumi, T.; Suzuki, K.; *Org. Biomol. Chem.* **2012**, *10*, 7685.

22. Oizumi, Y.; Katoh, M.; Hattori, Y.; Toda, K.; Kawaguchi, K.; Fujii, H.; Makabe, H. *Heterocycles* **2012** , *85*, 2241.

23. Ohmori, K.; Ushimaru, N.; Suzuki, K. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 12002.

24. Saito, A.; Doi, Y.; Tanaka, A.; Matsuura, N.; Ubukata, M.; Nakajima, N. *Bioorg. Med. Chem.* **2004**, *12*, 4783.

25. Saito, A.; Tanaka, A.; Ubukata, M.; Nakajima, N. *Synlett* **2004**, 1069.

26. Ohmori, K.; Shono, T.; Hatakoshi, Y.; Yano, T.; Suzuki, K.; *Angew. Chem. Int. Ed.* **2011**, *50*, 4862.

27. Saito, A.; Mizushina, Y.; Tanaka, A.; Nakajima, N. *Tetrahedron* **2009**, *65*, 7422.

28. Kozikowski, A. P.; Tückmantel, W.; Böttcher, G.; Romanczyk, Jr. L. J. *J. Org. Chem.* **2003**, *68*, 1641.

29. Krohn, K.; Ahmed, I.; John, M.; Letzel, M. C.; Kuck, D. *Eur. J. Org. Chem.* **2010**, 2544.

30. Oizumi, Y.; Mohri, Y.; Hattori, Y.; Makabe, H. *Heterocycles* **2011**, *83,*739.

31. Wan, S. B.; Dou, Q. P.; Chan, T. H. *Tetrahedro*n **2006**, *62*, 5897.

32. Delcour, J. A.; Vercruysse, S. A. R. *J. Inst. Brew.* **1986**, *92*, 244.

33. We observed that interflavan bond (C8-C4) of the synthetic prodelphinidin B3 (**2**) was partially cleaved during acetylation. We isolated the peracetate of catechin and gallocatechin. This is the reason of the low yield of acetylation. The loe to moderate yields of actetylation of pure epicatechin ligomers were also reported in ref. 28.

34. HPLC measurement condition of prodelphinidin C2 (2): column; InertSustain C18 250×4.6 mm Waters, eluent 0.1%HCOOH-CH_3CN, flow rate: 0.5 mL/min, detection: UV 280 nm, retention time: 13.18 min.

35. When we acetylated prodelphin C2 (**2**) to obtain peracetate **14**, we isolated the peractate of dimer **11**, catechin dimer, catechin, and gallocatechin. This is the reason of the low yield of acetylation of prodelphinidin C2 (**2**).

36. Mitsuhashi, S.; Saito, A.; Nakajima, N.; Shima, H.; Ubukata, M. *Molecules* **2008**, *13*, 2998.

37. Collins, J. A.; Schandi, C. A.; Young, K. K.; Vesely, J.; Willingham, M. C. *J. Histochem. Cytochem.* **1997**, *45*, 923.

38. Zi, X. L.; Agarwal, R. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 7490.

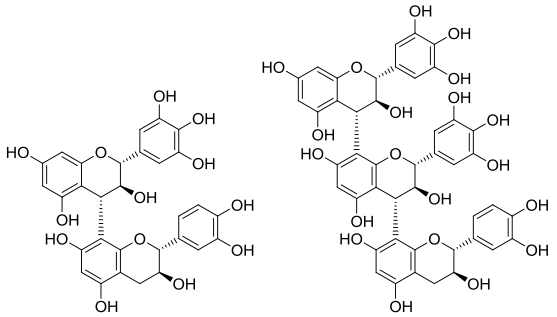
39. Kandasamy, K. ; Srinivasula, S. M.; Alnemri, E. S.; Thompson, C. B.; Korsmeyer, S. J.; Bryant, J. L.; Srivastana, R. K. *Cancer Res.* **2003**, *63*, 1712.

40. Goyal, L. *Cell* **2001**, *104*, 805.

Graphical Abstract

Synthesis of prodelphinidin B3 and C2, and their antitumor activity

Wataru Fujii, Kazuya Toda, Koichiro Kawaguchi, Sei-ichi Kawahara, Miyuki Katoh, Yasunao Hattori, Hiroshi Fujii and Hidefumi Makabe



prodelphinidin B3 (**1**)

prodelphinidin C2 (**2**)