Graphical Abstract

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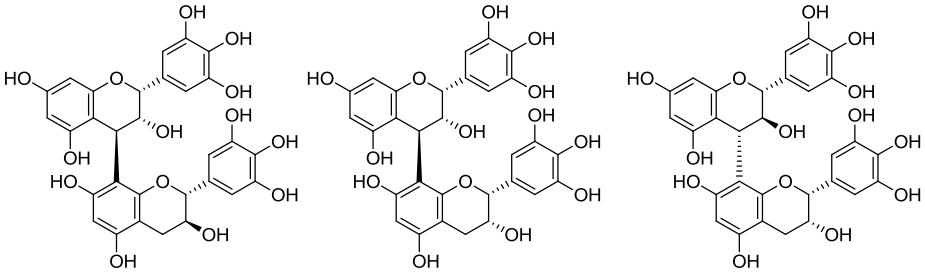

prodelphinidin B1 prodelphinidin B2 prodelphinidin B4

Syntheses of prodelphinidin B1, B2 and B4 and their antitumor activities against human PC-3 prostate cancer cell lines

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Abstract: Total synthesis of prodelphinidin B1, B2, and B4 have been accomplished. The key step is Lewis acid-mediated equimolar condensations between an epigallocatechin and/or a gallocatechin nucleophile and an epigallocatechin and/or a gallocatechin electrophile. The antitumor effects of synthetic prodelphidin B1-B4 against human PC-3 prostate cancer cell lines have been investigated. These compounds showed significant antitumor effects. Their activity seemed to be little bit stronger than EGCG and prodelphinidin B3, known antitumor agent.

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

Prodelphinidins are paid much attention due to their significant biological activities. For example, prodelphinidin B2 3-*O*-gallate and 3,3'-di-*O*-gallate inhibit proliferation of A549 cancer cells,[1,2] and prodelphinidin B4 3'-*O*-gallate inhibits COX-2 and iNOS.[3] As to the prodelphinidins B1, B2 and B4, a number of isolation form plants have been reported; prodelphinidin B1 (**1**) from *Citstus incanus*[4] and *Lotus peduncultus*[5], and prodelphinidin B2 (**2**) from *Lotus peduncultus*,[5] and prodelphinidin B4 (**4**) from *Ribes nigrum*,[6] *Vicia faba*,[7] and *Stryphnodendron adstringens*.[8] Because purification and identification of prodelphininds from plants are very difficult, the

mechanism for their biological activities remains unknown. Thus syntheses of prodelphinidins are quite important to obtain pure materials for evaluating their biological activities. The many examples of the syntheses of procyanidins were reported in this decade including our syntheses,[9-14] however, synthetic studies on prodelphinidins are quite limited due to difficulty in obtaining (–)-gallocatechin or (+)-epigallocatechin as synthetic starting materials.[15] Although (–)-gallocatechin or (+)-epigallocatechin is commercially available, both of compounds are very expensive. Thus it was necessary to prepare enough amount of (–)-gallocatechin or (+)-epigallocatechin derivatives according to the reported procedure.[16] Until now only an example of total synthesis of prodelphinidin B3 (**3**) and C2 has been reported by us using equimolar coupling between nucleophilic and electrophilic partners using Lewis acid.[17] Herein we demonstrate equimolar condensation of (–)-gallocatechin and/or (+)-gallocatechin nucleophile with a gallocatechin and/or epigallocatechin derived electrophile and the first total syntheses of prodelphinidin B1 (**1**), B2 (**2**) and B4 (**4**) (Figure 1).

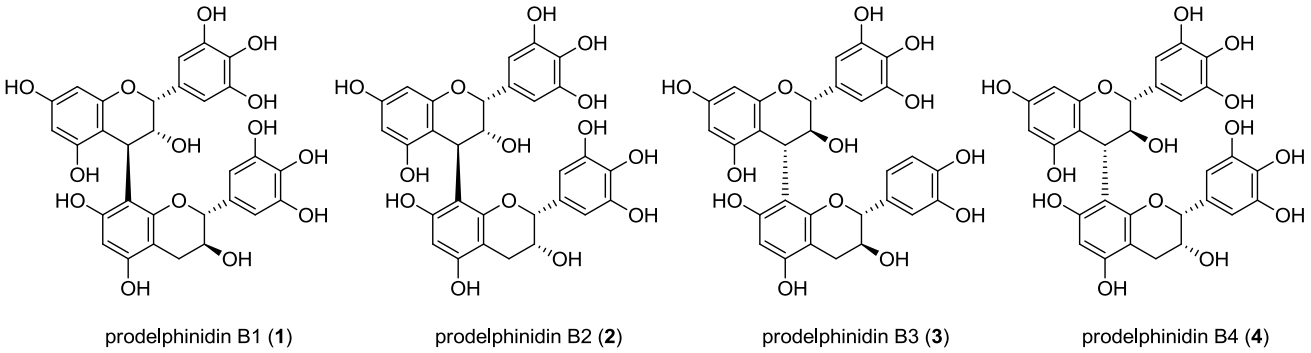
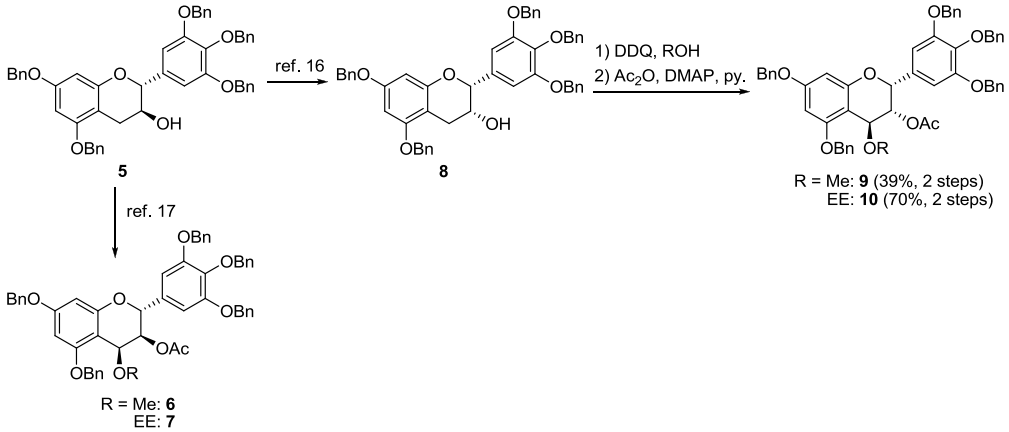


Figure 1. The structures of prodelphinidin B1 (**1**)-B4 (**4**).

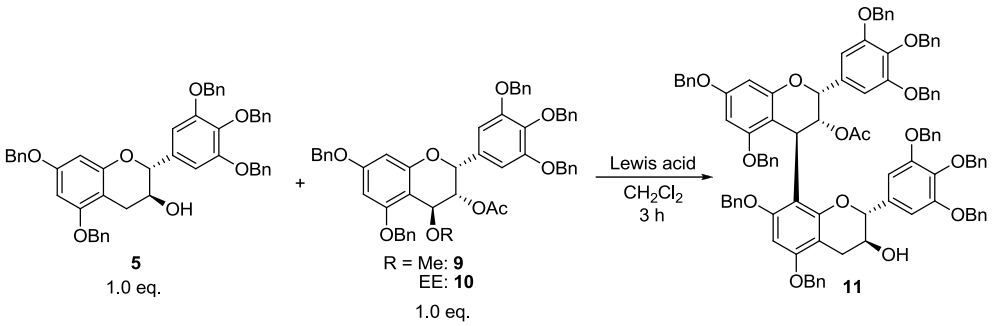
The gallocatechin-derived nucleophile **5** was constructed as Chan and co-workers reported.[16] Gallocatechin-derived electrophiles **6** and **7** were prepared as we reported earlier.[17] The epigallocatechin-drived nucleophile **8** was prepared according to the Chan and co-workers' method.[16] DDQ oxidation of **8** in the presence of methanol or ethoxyethanol followed by acetylation gave epigallocatechin-drived electrophiles **9** and **10**, respectively (Scheme 1).



Scheme 1. Synthesis of gallocatechin and/or epigallocatechin nucleophiles and electrophiles.

First, we examined the condition of equimolar condensation of gallocatechin nucleophile **5** with epigallocatechin electrophile **9** or **10** to construct prodelphinidin B1 (**1**) skeleton. We chose $Yb(OTf)_3$ as a Lewis acid for condensation because equimolar coupling worked well in the case of procyaninidin dimers.[10d,10h] We also examined silver Lewis acids because Ferreira and co-workers reported that using $AgBF_4$ as the thiophilic Lewis acid offered advantages to control the level of oligomeration in the synthesis of procyanidin B1-B4.[18] As shown in Table 1, 4-(2”-ethoxyethoxy) derivative **10** afforded condensed product **11** in 66% yield when $Yb(OTf)_3$ was used as Lewis acid. On the other hand, the reaction using methoxy derivative **9** gave **11** in moderate to poor yield. We found that the choice of leaving group at the C4 position and Lewis acid was important for equimolar condensation (Table 1).[19]

Table 1. Equimolar condensation of gallocatechin nucleophile **5** with epigallocatechin electrophile **9** or **10**.[a]

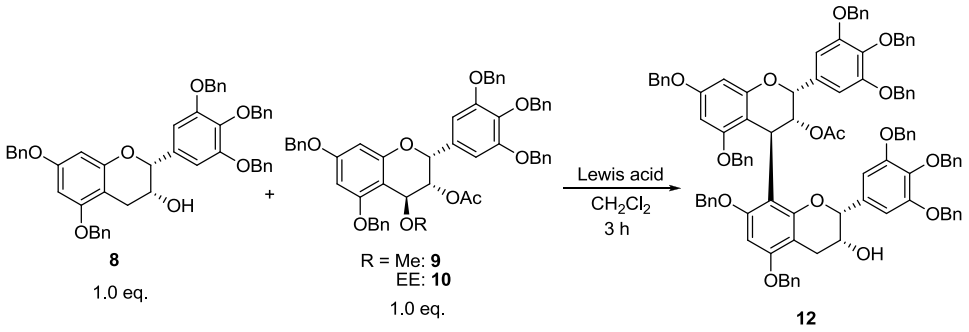


entry	electrophile	Lewis acid	yield (%)
1	**9**	$Yb(OTf)_3$	7
2	**9**	AgOTf	54
3	**9**	$AgBF_4$	33
4	**10**	$Yb(OTf)_3$	66

[a] All reactions were carried out at room temperature.

Next, we investigated the condition of equimolar condensation of epigallocatechin nucleophile **8** with epigallocatechin electrophile **9** or **10** to construct prodelphinidin B2 (**2**) skeleton. As shown in Table 2, methoxy derivative **9** afforded condensed product **12** in good yield when $AgBF_4$ was used as Lewis acid. $Yb(OTf)_3$ also gave **12** in good yield. In this case, we found that the methoxy group at the C-4 position was important for Lewis acid mediated condensation (Table 2).

Table 2. Equimolar condensation of epigallocatechin nucleophile **8** with epigallocatechin electrophile **9** or **10**.[a]

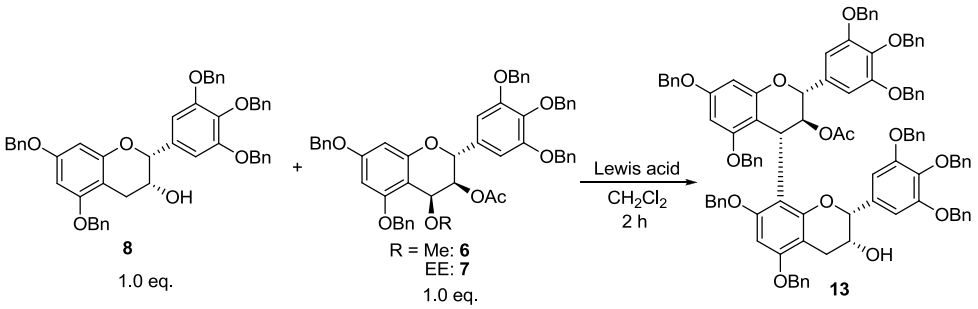


entry	electrophile	Lewis acid	yield (%)
1	**9**	$Yb(OTf)_3$	70
2	**9**	AgOTf	53
3	**9**	$AgBF_4$	76
4	**10**	$Yb(OTf)_3$	22

[a] All reactions were carried out at room temperature.

We further investigated the condition of equimolar condensation of epigallocatechin nucleophile **8** with gallocatechin electrophile **6** or **7** to construct prodelphinidin B4 (**4**) skeleton. As shown in Table 3, 4-(2”-ethoxyethoxy) derivative **7** afforded condensed product **13** in 78% yield when $Yb(OTf)_3$ was used as Lewis acid. On the other hand, the reaction using methoxy derivative **6** gave **13** in moderate to poor yield. (Table 3).

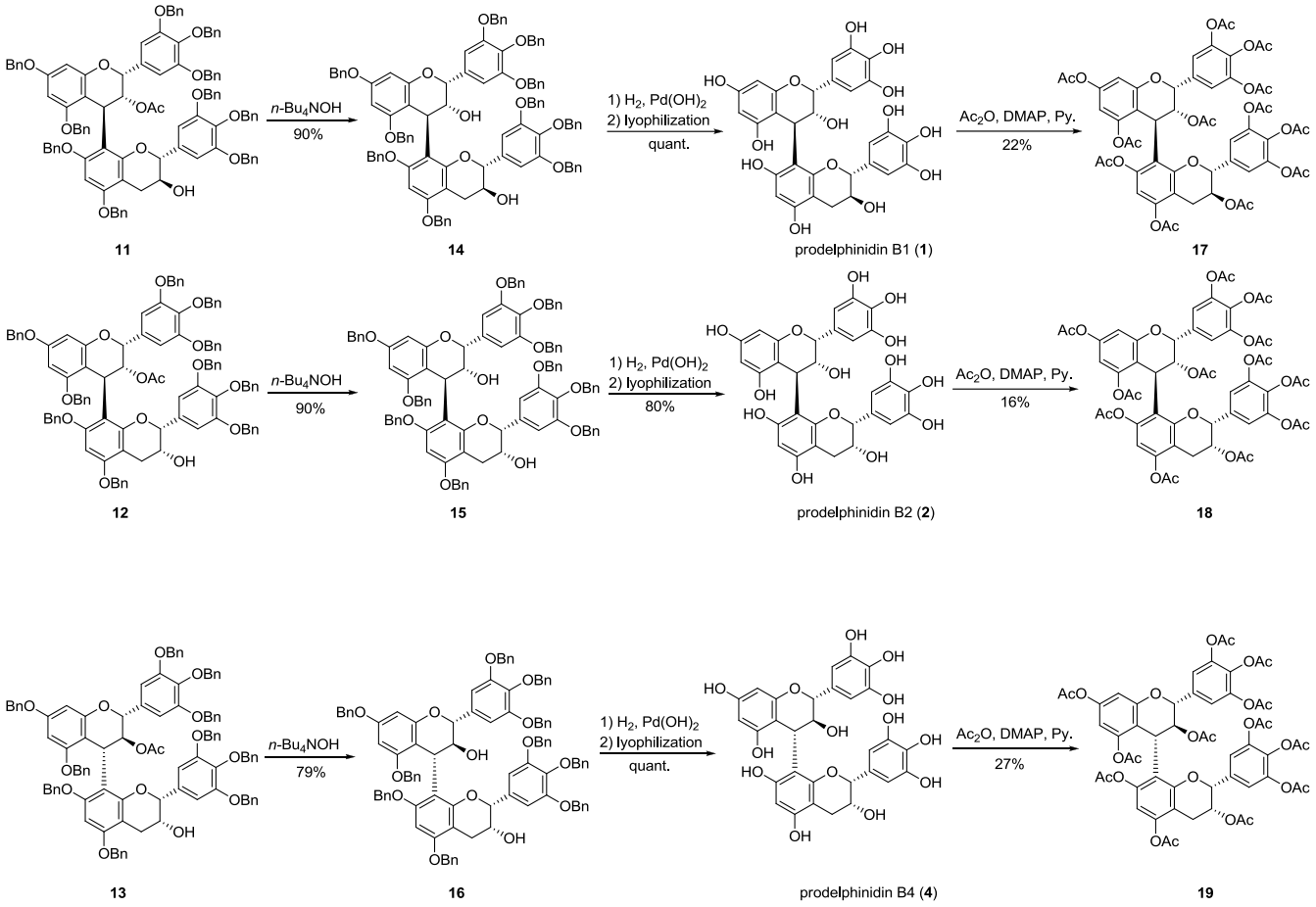
Table 3. Equimolar condensation of epigallocatechin nucleophile **8** with gallocatechin electrophile **6** or **7**.[a]



entry	electrophile	Lewis acid	yield (%)
1	**6**	$Yb(OTf)_3$	48
2	**6**	AgOTf	49
3	**6**	$AgBF_4$	62
4	**7**	$Yb(OTf)_3$	78

[a] All reactions were carried out at room temperature.

The condensed products **11-13** were transformed into diols **14-16** using *n*-Bu_4NOH.[13c] Finally deprotection of the benzyl ethers of **14-16** and subsequent lyophilization afforded prodelphinidin B1 (**1**), B2 (**2**), and B4 (**4**) in good yield.[20] The ^1H and ^{13}C NMR spectral data of peracetate of **1** (**17**),[5] **2** (**18**),[5] and **4** (**19**)[7] were in good agreement with the reported values (Scheme 2).



Scheme 2. Synthesis of prodelphinidin B1 (**1**), B2 (**2**), and B4 (**4**) and their peracetate **17**-**19**.

Because prodelphinidin B1 (**1**), B2 (**2**), and B4 (**4**) were obtained in sufficient quantities, we investigated antitumor activities against PC-3 prostate cancer cell lines. Results were obtained by cell count measurement. Epigallocatechin gallate (EGCG) and prodelphinidin B3 (**3**) were used as positive controls. As shown in Figure 2, EGCG, prodelphinidin B1 (**1**), B2 (**2**), and B4 (**4**) exhibited significant cytotoxic activities with IC_{50} values below 50 μM. At higher concentration (>50 μM), prodelphinidin B1 (**1**), B2 (**2**), and B4 (**4**) which have two pyrogallol moieties seemed to be stronger activity than that of prodelphinidin B3 (**3**) which has one pyrogallol moiety. The additional pyrogallol moieties might enhance the cytotoxic effects. Making a comparison of prodelphinidin B1 (**1**), B2 (**2**), and B4 (**4**) with EGCG, the activities of **1**, **2** and **4** seemed to be a little bit stronger than that of EGCG at higher concentration (>25 μM). Recently we have examined the cytotoxic effects on PC-3 prostate cancer cell lines of procyanidin gallates and found that esterified pyrogallol moiety showed weaker activity than prodelphinidin B3 (**3**).[13] EGCG has two pyrogallol moieties but one of them is esterified one. This might be a reason of weaker activity of EGCG than prodelphinidins B1, B2, and B4 (Figure 2).

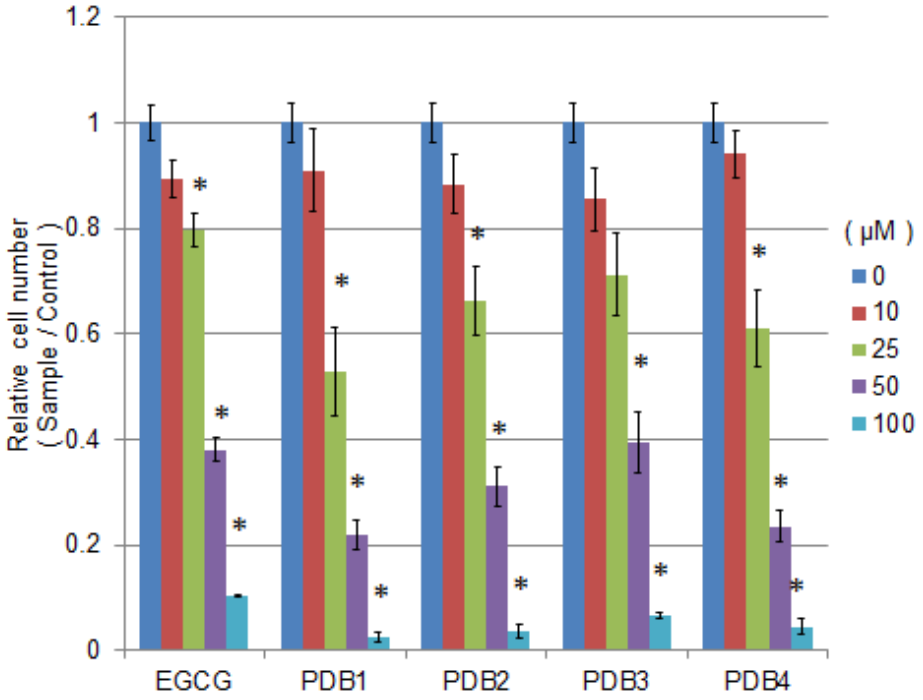


Figure 2. Effects of various concentrations of test compounds on cell proliferation.

After treatment of cells with EGCG, prodelphinidin B1 (**1**, PDB1), prodelphinidin B2 (**2**, PDB2), prodelphinidin B3 (**3**, PDB3),[17] and prodelphinidin B4 (**4**, PDB4) for 48 h, the cell proliferation was determined by cell count as described in experimental section. 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 ($p < 0.001$).

The first total syntheses of prodelphinidin B1 (**1**), B2 (**2**) and B4 (**4**) have been achieved via Lewis acid-mediated equimolar condensation of a gallocatechin and/or epigallocatechin nucleophile with gallocatechin and/or epigallocatechin electrophiles. In addition to demonstrating the total synthesis, we examined their antitumor activities against PC-3 prostate cancer cells. Prodelphinidin B1 (**1**), B2 (**2**), and B4 (**4**) showed significant cytotoxic activity with IC_{50} values below 50 μM. The potencies of prodelphinidins **1**, **2**, and **4** seemed to be a little bit stronger than those of EGCG and prodelphinidin B3 (**3**).

Acknowledgements

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Supplementary data

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

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19. Representative procedure for equimolar coupling reaction using $Yb(OTf)_3$: To a solution of nucleophile **5** (42 mg, 55 μmol) and electrophile **10** (49 mg, 55 μmol) in CH_2Cl_2 (4.0 mL) was added $Yb(OTf)_3$ (34 mg, 55 μmol). After the resulting mixture had been stirred for 3 h at room temperature, the reaction was quenched with water. The mixture was extracted with EtOAc (10 mL×2) 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 **11** (57 mg, 66%) as pale yellow oil.

20. HPLC measurement condition of prodelphinidin B1 (**1**): 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: 12.28 min., prodelphinidin B2 (**2**): 13.62 min., prodelphinidin B4 (**4**): 14.27 min.