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DEVELOPMENT OF A NEW SYNTHETIC STRATEGY FOR PROCYANIDIN DIMER CONDENSATION USING PERACETYLATED ELECTROPHILES

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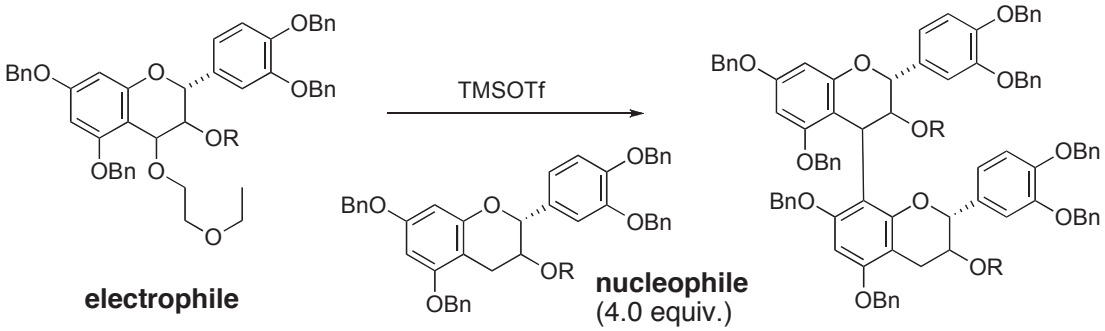
Abstract – Proanthocyanidins, also known as condensed tannins and/or oligomeric flavonoids, are found in many edible plants and show interesting various biological activities. We report a simple new proanthocyanidin synthesis strategy in which an electrophile derived from a flavan-3-ol peracetate is condensed with 1.5 eq. of a benzylated nucleophile. We demonstrate here the synthesis of procyanidin B1, a (–)-epicatechin-(4β-8)-(+)-catechin dimer, to demonstrate the utility of this method. This strategy is applicable for the synthesis of various commercially available flavan-3-ol and various oligomeric flavonoids.

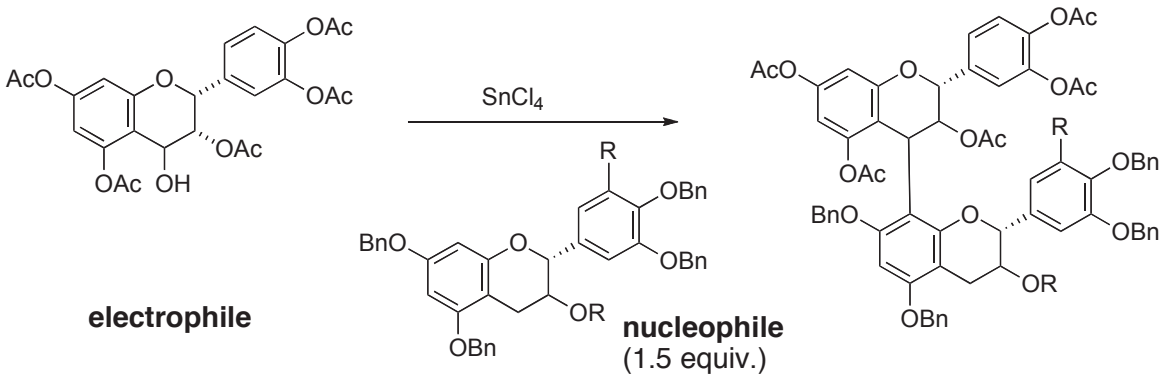
There is currently great interest in the research efforts investigating of compounds that have strong antioxidant activity and superior radical scavenging ability. Furthermore, foods containing active ingredients that can eliminate active oxygen species and free radicals have recently received increased

attention. Polyphenols are found in various plants, which are regularly consumed by us. It is widely believed that these polyphenols have a beneficial impact on health, and there are a variety of health foods that contain polyphenols. Furthermore, the scientific investigation of polyphenolic compounds is now increasingly important because of their various strong biological activities. For example, epigallocatechin-3-*O*-gallate (EGCG), a major polyphenol from green tea, which is a flavan-3-ol, has notably been the focus of intense research for its protective effect against a various types of cancers, including lung, prostate and breast cancers.[1] Surface plasmon resonance experiments have demonstrated that the 67-kDa laminin receptor, which is widely expressed at high levels in cancer cell membranes, is an EGCG reseptor[2] and is an evidence of EGCG's selective cytotoxicity against cancer cells.

Proanthocyanidins (condensed tannins or oligomeric flavonoids)[3,4] are known to be extremely strong antioxidants; investigating them has become increasingly important because of their various strong biological activities. However, in many cases, proanthocyanidins are obtained as a mixture of various analogs, making purification of each compound difficult. In an elegant contribution, Kozikowski *et al*.,[5] reported the synthesis of oligomeric catechin and epicatechin derivatives.[6] We also developed and reported a simple, versatile, and stereoselective method for synthesizing procyanidin oligomers, including (−)-epicatechin (**1**) and (+)-catechin.[7–18] Our synthetic methodology is applicable to variety of procyanidin oligomers, such as 3-*O*-substituted oligomers. The key step in our method of procyanidin synthesis is the coupling reaction between a nucleophile and a monomeric electrophile using a Lewis acid activator, such as $TiCl_4$, $SnCl_4$ or trimethylsilyl trifluoromethanesulfonate (TMSOTf). We created 2-ethoxyethoxy derivatives on the C-4 position as building blocks for procyanidin synthesis. These condensation reactions proceeded smoothly and afforded a good yield of 4–8 condensed oligomers and stereoselectivity (Scheme 1), but the nucleophile had to be used in four-fold excess to avoid higher oligomer formation. Therefore, we were challenged to develop a new, simple, stereoselective, and regioselective synthetic strategy applicable for the synthesis of a wide-range of procyanidin oligomers. In this report, we describe a new oligomer synthetic strategy that uses a condensation reaction between a peracetyl electrophile and 1.5 equiv. of nucleophiles to give procyanidin dimers (Scheme 2).



Scheme 1. Our previously-developed synthesis of procyanidin oligomers



Scheme 2. Our newly-developed synthesis of procyanidin oligomers

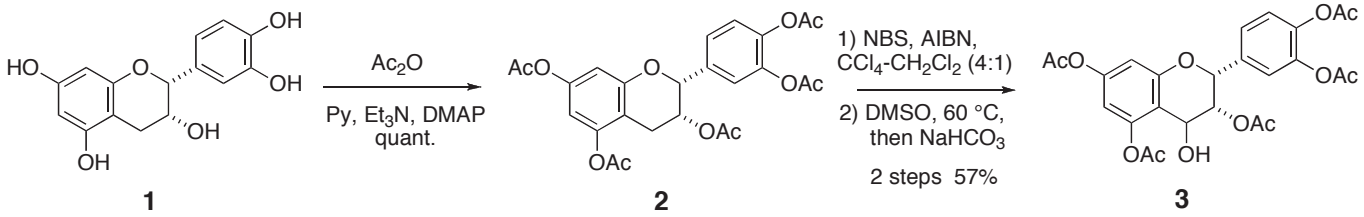
A benzyl group is the group most commonly used to protect the phenolic functionalities of procyanidin synthesis. However, the large-scale synthesis of benzyl (+)-catechin and (-)-epicatechin is very limited because of the by-products formation at benzyl-protection.[19,20] It is well-known that acetylation of flavan-3-ol and proanthocyanidins proceeds smoothly and affords their peracetates in quantitative yield. The electron-withdrawing acetyl moieties reduce reactivity at the C-8 position of the A-ring. Electrophilic C-4-oxyganated peracetates and C-4-halogenated peracetates have been reported and used for proanthocyanidin synthesis.[21] We re-examined the synthesis of peracetylated electrophiles and found that C-4-hydoroxylated electrophile (**3**) could be obtained from C-4-brominated peracetates by heating with $NaHCO_3$ in DMSO (Scheme 3). One-pot bromination with NBS and hydroxylation with $NaHCO_3$ in DMSO proceeded rapidly at 60 °C and afforded **3** with a yield of 57% yield. This reaction is also applicable to the synthesis of electrophiles derived from (+)-catechin and EGCG in approximately 10%–50% yields (data not shown).

Condensation of **3** with 1.5 eq. of (+)-catechin nucleophile (**4a**), performed in the presence of $SnCl_4$ at 0 °C afforded dimmer product (**5a**) with a yield of 33%. When TMSOTf was used as a catalyst, 33% isolation yield was obtained. This condensation method could be also adapted for various nucleophiles (**4b**-**4d**) derived from (–)-epicatechin, (–)-epigallocatechin, and (–)- epigallocatechin gallate (EGCG) with yields of 19%, 32%, and 26%, respectively, as shown in Scheme 4. The reaction yield are not so good for the instability of an acetyl group, however, only dimeric procyanidin derivative can be obtained under this reaction conditions without higher oligomer formation.

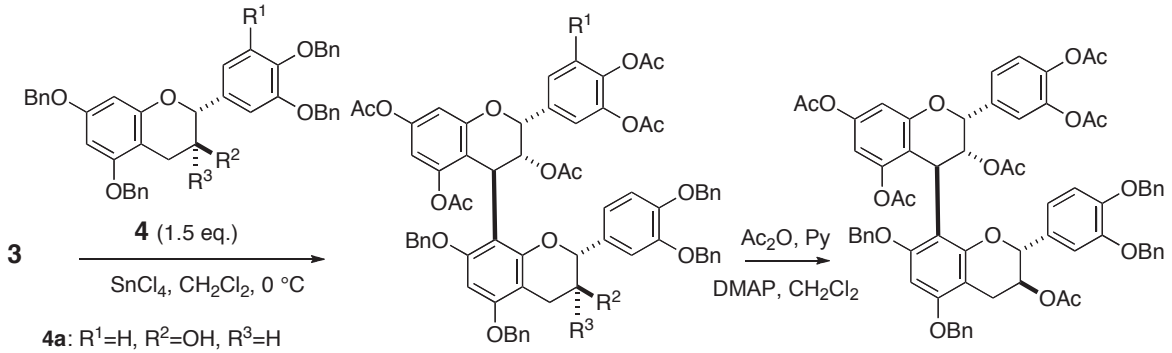
Because of peak broadening in the NMR spectra, the structure determination of **5a** was difficult; therefore, the 3”-hydroxygroup of **5a** was acetylated to give acetate **6a** in quantitative yield (Scheme 4). We have previously experienced peak broadening in NMR spectra during the synthesis of protection-free procyanidin oligomers that have (–)-epicatehin as an upper unit. Surprisingly, the peak-broadening phenomenon observed with **5a** was disappeared upon acetylation of only the 3”-hydroxyl moiety. Furthermore, the structure was ascertained by comparing the spectral data of peracetate **6** with those of

synthesized authentic samples. Deprotection of the four benzyl groups of **5a** under hydrogenation conditions and acetylation using a general procedure yielded peracetate **7** with a yield of 92% yield (Scheme 5). All spectral data agreed with the structure portrayed in Scheme 5. Deprotection of Ac and Bn group from compound **5a** by alkaline hydrolysis with K_2CO_3, followed by hydrogenation using $Pd(OH)_2/C$ under H_2 atmosphere was achieved to synthesize procyanidin B1 (**8**) with 56% yield.

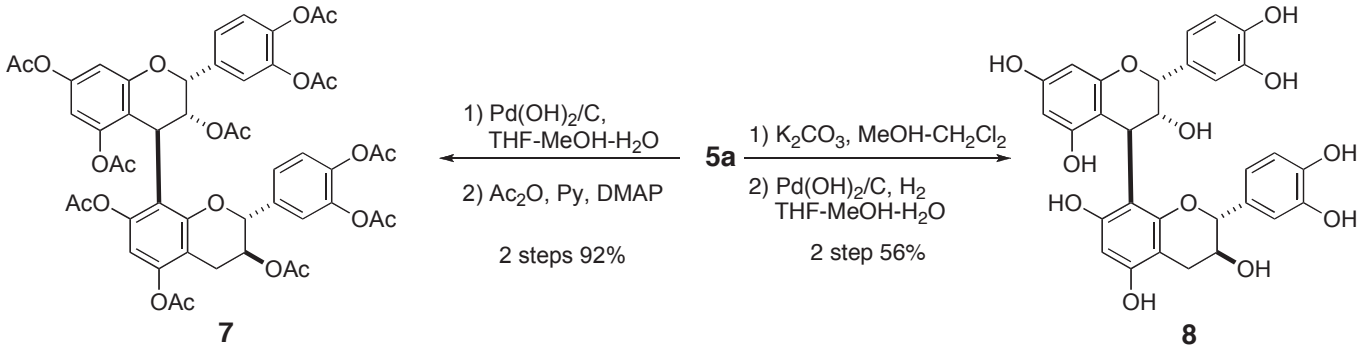
Finally, in initial experiments, to confirm the applicability of this condensation reaction for the synthesis of related molecules, we have confirmed that dimeric procyanidin derivatives can be obtained from condensation reactions between **3** and various nucleophiles (**5b**-**5d**) derived from (-)-epicatechin, (–)-epigallocatechin, and (–)-EGCG with yields of 19%, 32%, and 26%, respectively.



Scheme 3. Synthesis of peracetyl electrophile (**3**)



Scheme 4. Coupling reaction of peracetyl electrophile (**3**) and nucleophiles(**4a**-**4d**)



Scheme 5. Synthesis of procyanidin B1 (**8**) and its structure determination

EXPERIMENTAL

General: All commercially available chemicals for chemical synthesis were used without further purification. All reactions were performed under an argon atmosphere and monitored by thin-layer chromatography with 0.25-mm pre-coated silica gel plates (60F254 Art 5715; Merck, Darmstadt, Germany). Optical rotation was measured with an ATAGO AP-300 spectrometer. ^{1}H-NMR spectra were recorded on a Varian Inova 500 NMR Spectrometer (500 MHz). Chemical shifts were reported in ppm (d) relative to an internal standard (tetramethylsilane). Fast atom bombardment mass spectra at normal (FABMS) and high resolution (FABHRMS) were obtained using a JEOL JMS-AX500 mass spectrometer. HPLC purification was performed on a Mightysil RP-18 GP column (Kanto Chemical Co. Inc., Japan; 250 x 20 mm, 5 mm) using eluents A [0.05% HCO_2H in MeCN/H_2O (20/80)] and B (0.05% HCO_2H in MeCN). Elution was performed with a linear gradient of 20%–100% B in in A over 20 min (flow rate, 2.0 mL/min).

4-Hydroxy-penta-*O*-acetyl-(–)-epicatechin (3). A mixture of penta-*O*-acetyl-(–)-epicatechin (**2**; 1.00 g, 2.00 mmol) and NBS (0.71 g, 4.00 mmol) in the presence of AIBN (32.8 mg, 0.20 mmol) was refluxed in CH_2Cl_2–CCl_4 (1:4, v/v) for 12 h. $NaHCO_3$ (0.50 g, 5.99 mmol) and DMSO (30 mL) were added to the solution and stirred at 60 °C for 1 h. The reaction mixture was quenched with ice water and extracted with EtOAc. The organic phase was washed with water and brine, and then dried with solid Na_2SO_4. Filtration, concentration, and silica gel column chromatography (Hex:EtOAc, approximately 7:1–1:3) afforded 0.59 g of **3** as a colorless amorphous powder (1.15 mmol, 57%). $[\alpha]_D^{24}$ + 40.8 (*c* 0.098, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) d = 7.25 (1H, d, *J* = 8.5 Hz), 7.17 (1H, dd, *J* = 1.5, 8.5 Hz), 7.14 (1H, d, *J* = 1.5 Hz), 6.39 (1H, br s), 6.30 (1H, br s), 6.15 (1H, br s), 5.62 (1H, t, *J* = 4.0 Hz), 5.45 (1H, br s), 2.32 (3H, s), 2.304 (3H, s), 2.299 (3H, s), 2.290 (3H, s), 2.04 (3H, s); the hydroxyl group was not observed. FABMS (m/z) 517 ([M+H]$^+$, 10), 516 (10), 499 (18), 415 (32), 397 (23), 372 (20), 306 (100); FABHRMS calcd for $C_{25}H_{25}O_{12}$ [M+H]$^+$, 517.1346; found: 517.1371.

[4,8]-2,3-*cis*-3,4-*trans*-2”,3”-*trans*-3,5,7,3’4’-Penta-*O*-acetyl-5”,7”,3”’,4”’-tetra-*O*-benzyl-(–)-epicatechin-(+)-catechin (5a). To a solution of **3** (130 mg, 0.25 mmol) and **4a** (244 mg, 0.38 mmol) in CH_2Cl_2 (20 mL) was added dropwise $SnCl_4$ (0.50 mL, 0.50 mmol, 1.0 M solution in CH_2Cl_2) at 0 °C. After stirring for 5 min, the pale yellow reaction mixture was quenched with saturated aqueous $NaHCO_3$. The mixture was extracted with $CHCl_3$ and the organic phase was washed with water and brine, and then dried with solid Na_2SO_4. Filtration, concentration, and preparative silica gel TLC purification (hexane:EtOAc, 1:1) afforded a 95 mg of **5a** (0.082 mmol, 33%) of as a colorless amorphous powder: $[\alpha]_D^{24}$ + 79.2 (*c* 0.10, $CHCl_3$); NMR signals from a solution of **5a** were quite broad, making peak

assignment impossible.

[4,8]-2,3-*cis*-3,4-*trans*-2",3"-*trans*-3,5,7,3'4'3"-Hexa-*O*-acetyl-5",7",3"',4"'-tetra-*O*-benzyl-(−)-epicatechin-(+)-catechin (6a). A solution of **5a** (13.1 mg, 0.011 mmol) in CH_2Cl_2 (5 mL) was acetylated by a general procedure using Ac_2O.[7-17] After stirring for 30 min at room temperature, the reaction mixture was quenched with water and extracted with $CHCl_3$. The organic phase was washed with water and brine and dried with $MgSO_4$. Filtration, concentration, and chromatography on a short silica gel column (hexane/EtOAc, 1:2) afforded 13.3 mg of **6a** (0.011 mmol, 98%) as a colorless amorphous powder: $[\alpha]_D^{24}$ -49.2 (*c* 0.061, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, 0.75:0.75 mixture of rotational isomers) major isomer: 7.47–6.88 (18H, m), 6.84 (0.75H, d, *J* = 8.0 Hz), 6.69 (0.75H, s), 6.59 (0.75H, d, *J* = 8.0 Hz), 6.32 (0.75H, s), 6.26 (0.75H, s), 6.26 (0.75H, s), 5.79 (0.75H, s), 5.51 (0.75H, s), 5.38 (0.75H, s), 5.12–4.93 (6H, m), 4.62 (0.75H, s), 4.21 (0.75H, d, *J* = 9.5 Hz), 3.23 (0.75H, dd, *J* = 7.0, 17.0 Hz), 2.60 (0.75H, dd, *J* = 8.5, 17.0 Hz), 2.19-1.95 (13.5H, m); minor isomer: 7.47–6.88 (6.5H, m), 6.49 (0.25H, s), 6.33 (0.25H, s), 6.18 (0.25H, s), 5.36 (0.25H, s), 5.28 (0.25H, s), 5.12–4.93 (2.25H, m), 4.70 (0.25H, d, *J* = 10.5 Hz), 4.51 (0.25H, s), 4.46 (0.25H, d, *J* = 10.5 Hz), 3.22–3.18 (0.25H, m), 2.75 (0.25H, dd, *J* = 8.5, 16.5 Hz), 2.19–1.95 (4.5H, m); ^{13}C NMR (125 MHz, $CDCl_3$) major isomer: 169.8, 169.2, 168.9, 168.4, 168.00, 167.98, 156.5, 155.42, 155.37, 154.2, 148.7, 148.62, 148.59, 148.0, 141.9, 141.6, 137.5, 137.3, 136.9, 136.78, 136.70, 128.7-127.1 (Cx6), 128.7, 128.5, 128.4, 128.3, 127.3, 127.1, 127.0, 124.3, 123.0, 121.8, 120.5, 114.7, 113.6, 113.1, 108.24, 108.18, 106.6, 104.1, 91.1, 78.9, 74.1, 71.3, 71.2, 70.74, 70.66, 70.0, 69.0, 33.2, 27.0, 21.0, 20.8, 20.7, 20.6, 20.5, 20.0; minor isomer: 169.8, 169.2, 169.0, 168.4, 168.1, 168.0, 156.8, 156.4-148.6 (Cx7), 141.8, 141.2, 137.5-136.7 (Cx4), 136.1, 130.0, 128.7-127.1 (Cx6), 128.04, 127.99, 127.8, 127.5, 127.4, 127.2, 124.3, 122.8, 121.7, 120.2, 114.6, 114.0, 113.6, 108.9, 108.2, 107.4, 102.2, 91.9, 78.6, 74.5, 71.7, 71.6, 71.3-70.7 (Cx3), 70.0, 68.7, 29.7, 25.8, 21.2, 21.0-20.5 (Cx3), 20.3, 20.2.

[4,8]-2,3-*cis*-3,4-*trans*:2",3"-*trans*-Deca-*O*-acetyl-(−)-epicatechin-(+)-catechin (7). A solution of **5a** (7.20 mg, 0.0063 mmol) in 11 mL of THF/MeOH/H_2O (20:1:1) was hydrogenated over 20% $Pd(OH)_2$/C (2 mg) for 12 h at room temperature. Filtration and concentration afforded a pale brown solid, which was acetylated using a general procedure to give 5.8 mg of **7** (0.0058 mmol, 92%) as an amorphous solid. All spectral data of **7** agreed with those of the synthetic compound previously reported.[11]

Procyanidin B1 (8). To a solution of **5a** (18.1 mg, 0.016 mmol) in CH_2Cl_2–MeOH (1:1) was added K_2CO_3 (13.1 mg, 0.095 mmol) at 0 °C, and the mixture was stirred for 30 min. The reaction mixture was quenched with saturated aqueous NH_4Cl solution and extracted with EtOAc. After concentration, the

mixture was dissolved with 11 mL of THF:MeOH:H_2O (20:1:1) and hydrogenated over 20% $Pd(OH)_2/C$ (2 mg) for 3 h at room temperature. Filtration and concentration afforded a pale brown solid that was purified by HPLC purification to give 5.2 mg of pure **8** (0.090 mmol, 56%) as an amorphous solid. All spectral data obtained for **8** agreed with those of the synthesized compound previously reported.[11]

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