Supporting Information

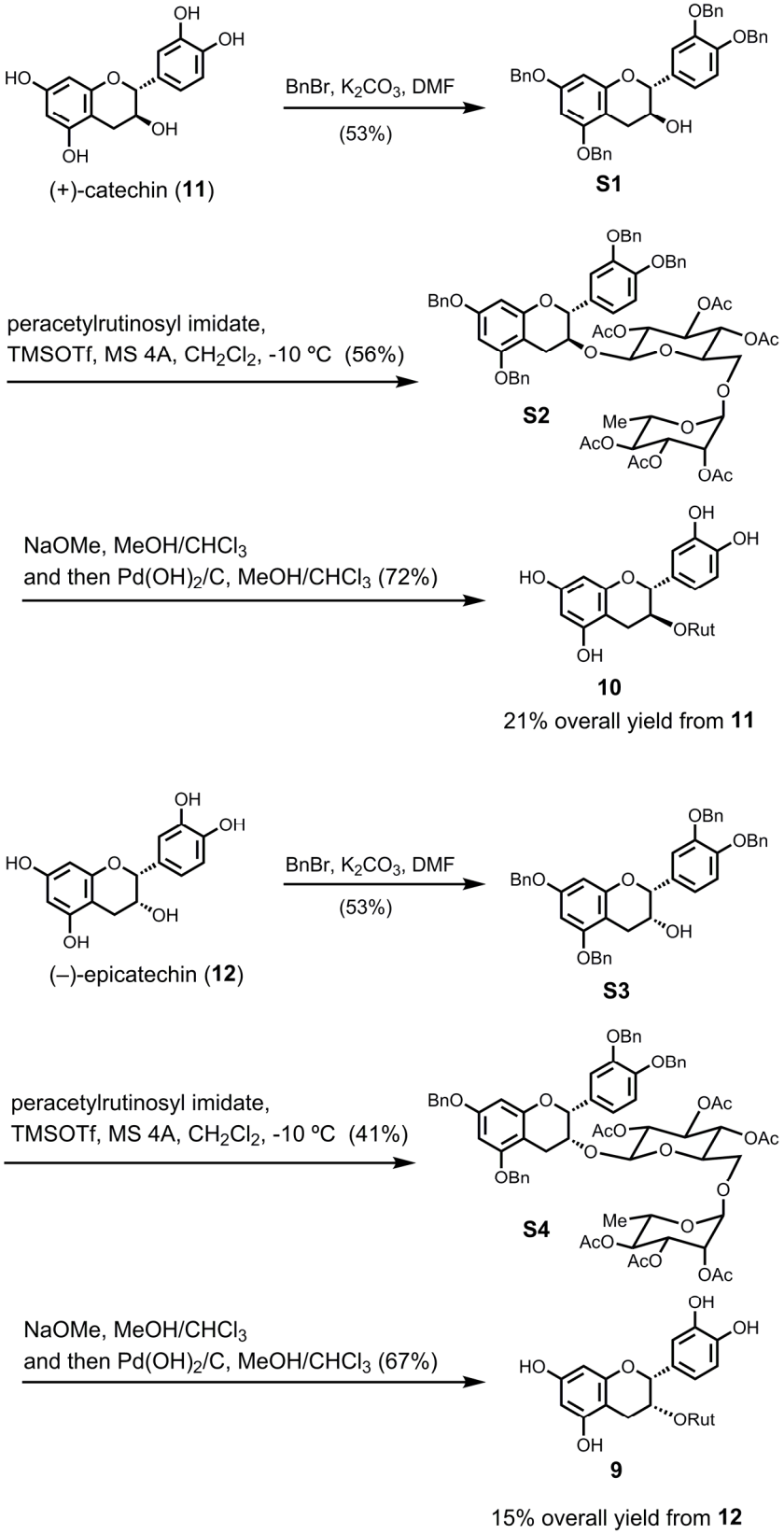
Conversion of flavonol glycoside to anthocyanin: an interpretation of the oxidation-reduction relationship of biosynthetic flavonoid-intermediates

Kin-ichi Oyama,[a] Yuki Kimura,[b] Satoru Iuchi,[c] Nobuaki Koga,[c] Kumi Yoshida[c,*] and Tadao Kondo[c,*]

[a] Research Institute for Materials Science, Nagoya University, Chikusa, Nagoya 464-8602, Japan
[b] Graduate School of Information Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan
[c] Graduate School of Informatics, Nagoya University, Chikusa, Nagoya 464-8601, Japan

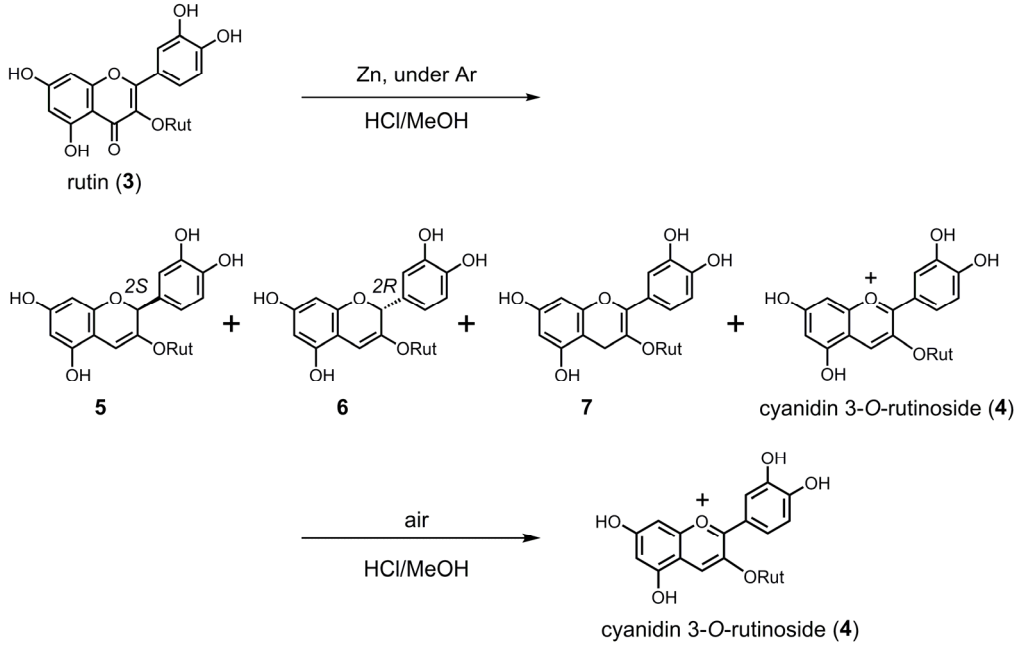
Table of Contents

1. Synthesis of 9 and 10



Scheme S1. Synthesis of **9** and **10** from (+)-catechin (**11**) and (–)-epicatechin (**12**)

2. HPLC chromatogram



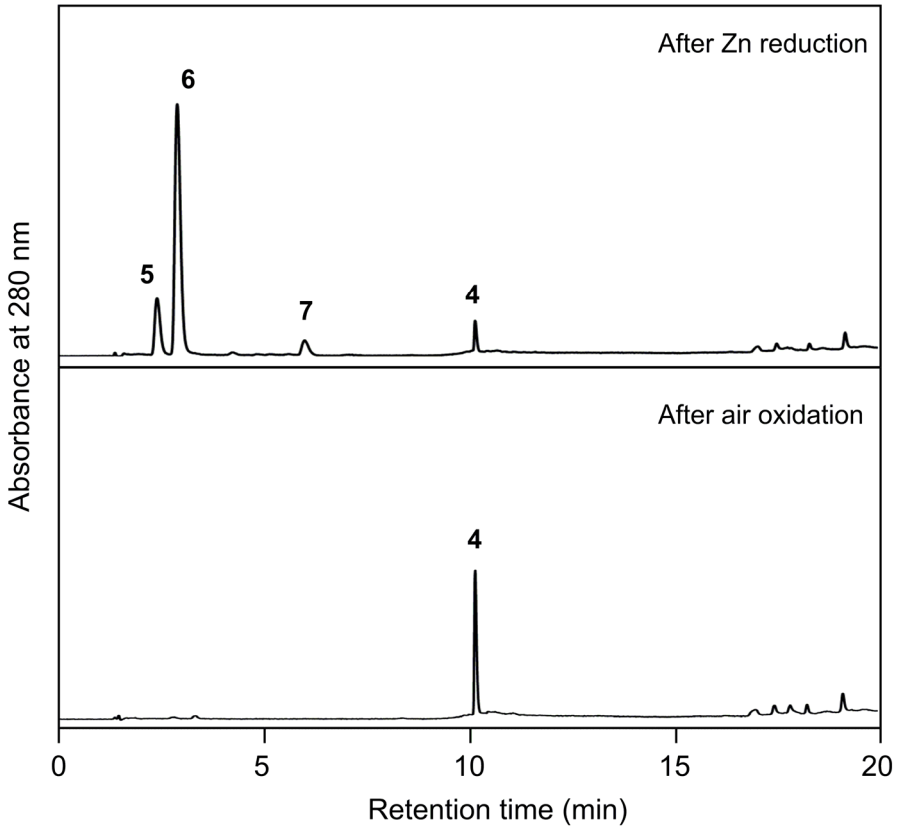


Fig. S1. HPLC chromatogram of the Zn reduction and the Zn reduction followed by air oxidation of rutin (**3**) in dried HCl-MeOH.

3. Computational Methods

To examine the absolute configurations at C2, the ECD curves $\Delta\varepsilon$ were computed for the model molecules in which the rutinoside moiety in **5** and **6** is replaced by OMe. To prepare the structures of model molecules, 8 conformers were optimized for both 2*S* and 2*R* structures as summarized in Fig. S2. These geometry optimizations were carried out by density functional theory (DFT) calculations using the ωB97X-D functional[S1] with the def2-TZVP basis set.[S2] Because the rutinoside moiety does not exist in the model molecules, 8 conformers for the 2*S* and 2*R* structures are mirror images each other within numerical errors. The Gibbs free energies ΔG were evaluated at these structures by the vibrational analysis at 298.15 K and 1 atm.

At these minimum structures, the excitation energies (E_{0n}), the oscillator strengths (f_{0n}), and the rotatory strengths (R_{0n}) for low-lying 20 singlet excited states ($n = 20$) were computed by time-dependent DFT (TD-DFT) calculations. The TD-DFT calculations were carried out by using the CAM-B3LYP functional[S3] and the def2-TZVP basis set, and solvent effects were included by polarizable continuum model (PCM)[S4] with the dielectric constant of methanol. All the (TD-)DFT calculations were carried out by using the Gaussian 16 program.[S5]

Table S1 summarizes the computed properties for the first excited state ($n = 1$). As seen in Table S1, the first excited state is located at ~273 nm with relatively large oscillator strength of ~0.27. These states are assigned to the π–π* transition from the natural transition orbital pairs[S6] in Fig S3, where both the hole and particle are delocalized over the A and C rings with a little contribution from the catechol moiety.

The absorption spectra were computed by multiplying the Gaussian function as[S7]

$$\varepsilon(\tilde{\nu}) = C\sum_{i=1}^{N_{\mathrm{conf}}} P_i \sum_{n=1}^{20} \frac{f_{0n}^{(i)}}{\sigma} \exp\left[-\left(\frac{\tilde{\nu}-\tilde{\nu}_{0n}^{(i)}}{\sigma}\right)^2\right] \qquad \text{(S1)}$$

where C is a factor containing several physical constants and $\tilde{\nu}_{0n}^{(i)}$ is the excitation energy of the *n*-th excited state in wavenumber for the *i*-th conformer. The relative population P_i for the *i*-th conformer was determined based on the Boltzmann distribution, where the solvation free energies

evaluated by PCM were added into ΔG. The number of conformers N_{conf} was 8 as mentioned above. The half bandwidth at half maximum, $\sigma\sqrt{\ln 2}$, was set to 0.2 eV. The resultant absorption spectra are displayed in Fig. 1 (B).

The ECD curves $\Delta\varepsilon$ were computed by multiplying the Gaussian function as[S7,S8]

$$\Delta\varepsilon(E) = \sum_{i=1}^{N_{\mathrm{conf}}} P_i \Delta\varepsilon_i = \sum_{i=1}^{N_{\mathrm{conf}}} P_i \sum_{n=1}^{20} \left[\frac{E_{0n}^{(i)}}{2.296\times10^{-39}\sqrt{\pi}\sigma} R_{0n}^{(i)} \exp\left\{ -\left(\frac{E - E_{0n}^{(i)}}{\sigma} \right)^2 \right\} \right] \qquad \text{(S2)}$$

where σ is a half bandwidth at e^{-1} and was set to 0.1 eV. The computed ECD curves $\Delta\varepsilon$ are displayed in Fig. 1 (B) at the range of 200-300 nm. For the 2*R* structure, the negative, positive, and negative peaks are observed at ~273, ~221, and ~204 nm, respectively, whereas the signs are opposite for the 2*S* structure. Although these characteristics of $\Delta\varepsilon$ seem to be consistent qualitatively with the experimental ECD for **5** and **6**, the detailed analysis for these peaks is beyond the scope of this study as the effects of ORut were not considered here to reduce the computational cost. Instead, to highlight the absolute configuration at C2, the signs of ECD curves $\Delta\varepsilon$ at ~273 nm (Fig. 1) together with those of the components $\Delta\varepsilon_i$ displayed in Fig. S4 are focused in the main text. Finally, as summarized in Table S2, the signs of rotatory strengths for plausible conformers of **5** and **6** (Figure S5) were found to be the same as those for the corresponding model molecules (Table S1).

Table S1. The excitation energies (E), oscillator strengths (f), and rotatory strengths (R) for the first excited state of various conformers of model molecules.

Conf.[a]	Config.[b]	E (nm)	f	R[c]	Config.[b]	E (nm)	f	R[c]
1	2*R*	271.9	0.2931	−57.3	2*S*	271.9	0.2931	57.3
2	2*R*	271.9	0.2938	−55.3	2*S*	271.9	0.2931	55.2
3	2*R*	272.4	0.2905	−72.4	2*S*	272.4	0.2905	72.4
4	2*R*	272.2	0.2893	−65.5	2*S*	272.2	0.2894	65.6
5	2*R*	274.0	0.2640	−95.4	2*S*	274.0	0.2640	95.4
6	2*R*	274.0	0.2142	−40.7	2*S*	274.0	0.2141	40.7
7	2*R*	274.0	0.2739	−117.3	2*S*	274.0	0.2739	117.4
8	2*R*	271.6	0.2442	−49.7	2S	271.6	0.2442	49.6

(a) Index of conformers are displayed in Fig. S2.

(b) Absolute configuration at C2.

(c) Unit: 10^{-40} cgs. The velocity form was employed.

Table S2. The excitation energies (E), oscillator strengths (f), and rotatory strengths (R) for the first excited state of plausible conformers of **5** and **6** (Figure S5).

	E (nm)	f	R[a]		E (nm)	f	R[a]
2*R* (**6**)	281.2	0.2419	−185.6	2*S* (**5**)	277.9	0.2295	70.3

(a) Unit: 10^{-40} cgs. The velocity form was employed.

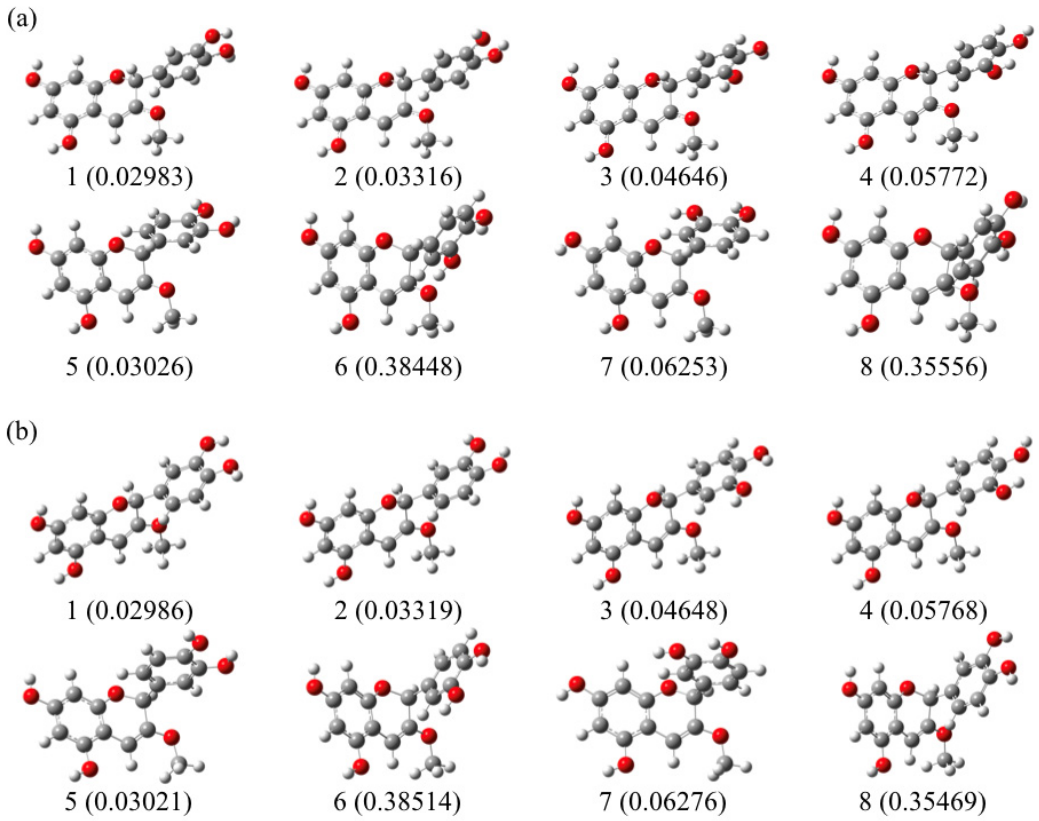


Fig. S2. The conformers optimized by DFT calculations for (a) 2*R* and (b) 2*S* structures. The values in parentheses are relative populations determined by the Boltzmann distribution.

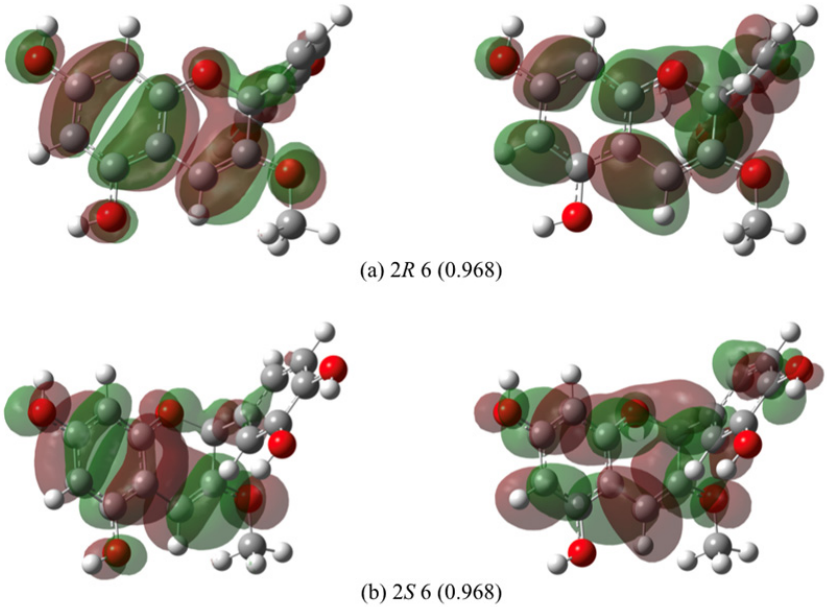


Fig. S3. Natural transition orbitals for the first excited states of (a) 2*R* 6 and (b) 2*S* 6 structures, where conformer indices are given in the caption of Fig. S2. The left and right denote the hole and particle and the eigenvalues of these pairs are shown in parentheses.

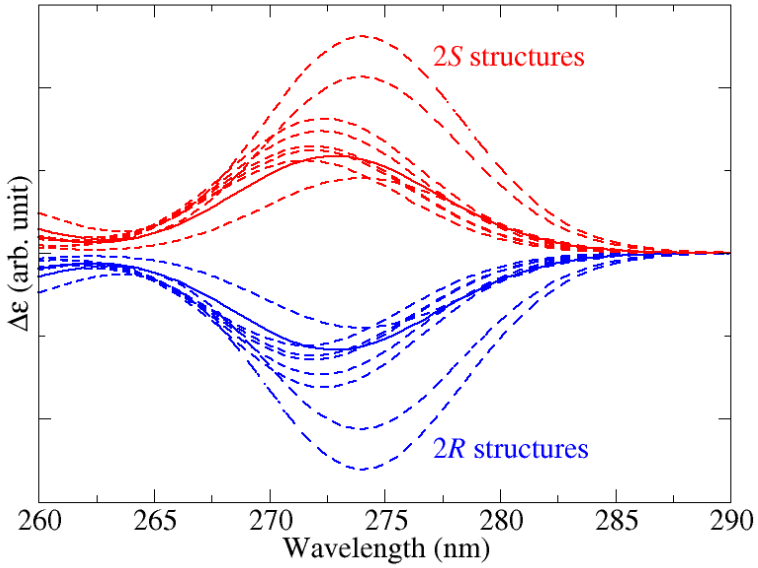


Fig. S4. Electric circular dichroism curves of the model molecules at the 260-290 nm region computed by TD-DFT calculations Eq.(S2). The solid lines denote the spectral curves by the Boltzmann averaging of 8 conformers contributions (dashed lines). The blue and red lines denote the results for the 2*R* and 2*S* structures, respectively.

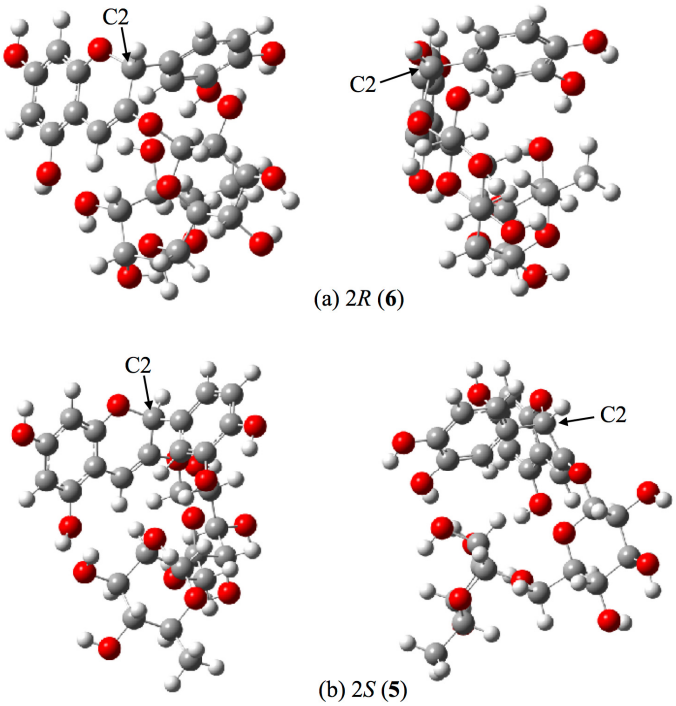


Fig. S5: The plausible conformers of (a) **6** and (b) **5**, optimized at the ωB97X-D/def2-TZVP level.

5. Experimental Procedures for Compounds

Optical rotations were recorded on a JASCO P-1010-GT polarimeter. Circular dichroism (CD) spectra were recorded on a JASCO J-720 spectrometer. Infrared (IR) spectra were recorded on a JASCO FT/IR 6100 spectrometer. UV/VIS spectra were recorded on a JASCO V 570 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on JNM ECA-500 or JNM A600 spectrometers. Chemical shifts for ^{1}H NMR are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) and are referenced to residual solvent signals ($CDCl_3$: δ 7.26 ppm, CD_3OD: δ 3.31 ppm) as an internal reference. Chemical shifts for ^{13}C NMR were reported in the scale relative to the NMR solvent ($CDCl_3$: δ 77.0 ppm, CD_3OD: δ 49.0 ppm) as an internal reference. High resolution mass spectra (HRMS) were recorded on Bruker micrOTOF-QII (ESI) or compact (ESI) spectrometers. Analytical HPLC was conducted by our method[S9,S10] with an ODS column (Develosil ODS-HG-5, 4.6 mm ϕ × 250 mm, Nomura Chemical) with some modifications. The chromatography was performed by eluting at 0.2 mL/min with a linear gradient from 10% to 90% CH_3CN in H_2O containing 0.5% TFA for 20 min at 40 ºC. Preparative ODS-HPLC was performed by using a column (Develosil ODS-HG-5, Nomura Chemical) with isocratic elution at 40 ºC.[S9,S11] Flash column chromatography was performed on Fuji Silysia silica gel (PSQ 60B). Analytical thin layer chromatography (TLC) was performed on Merck precoated analytical plates, 0.25 mm thick, silica gel 60 F254. All commercially available reagents and solvents were used directly without further purification. All nonaqueous reactions were carried out under an argon atmosphere.

Cyanidin 3-*O*-rutinoside (4) (Gram synthesis)

To a suspension of rutin (**1**) (2.00 g, 3.27 mmol) and dried Zn powder (20.0 g) in MeOH (80 mL) was added 3.5 N HCl-MeOH (120 mL) with vigorous stirring for 10 min at 0 ºC under Ar and the solution became colorless. After stirring for 30 min at 0 ºC under Ar, Zn powder was removed by suction filtration. The resultant solution was diluted MeOH (400 mL). This solution was stirred for 3 h under air at room temperature and then H_2O (5 L) was added. Next, MeOH was removed under reduced pressure at 40 ºC. The residual aqueous solution was purified by XAD-7 with 10%→15% →20%→30% MeCN aqueous solution containing 0.5% TFA monitoring by HPLC to give a crude **4**. Finally, the resultant crude product was purified by ODS column chromatography (Develosil

ODS-HG5, Nomura Chemicals) with 15% MeCN aqueous solution containing 0.5% TFA to give a pure **4** (1.175 g, 50 %) as a dark red solid. IR (KBr) 3329, 1674, 1635, 1331, 1194, 1064 cm^{-1}; ^1H NMR (500 MHz, 10% TFA-*d*-CD_3OD) δ 8.91 (s, 1H), 8.21 (dd, *J* = 8.5, 2.5 Hz, 1H), 8.03 (d, *J* = 2.5 Hz, 1H), 7.01 (d, *J* = 8.5 Hz, 1H), 6.86 (t, *J* = 1.0 Hz, 1H), 6.66 (d, *J* = 1.5 Hz, 1H), 5.30 (d, *J* = 8.0 Hz, 1H), 4.66 (d, *J* = 1.5 Hz, 1H), 4.06 (dd, *J* = 11.0, 1.5 Hz, 1H), 3.82 (dd, *J* = 3.5, 1.5 Hz, 1H), 3.5-3.7 (m, 6H), 3.43 (t, *J* = 9.0 Hz, 1H), 3.33 (t, *J* = 9.0 Hz, 1H), 0.16 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (125 MHz, 10% TFA-*d*-CD_3OD) δ 170.6, 164.0, 159.1, 157.6, 155.8, 147.3, 145.6, 136.2, 128.3, 121.2, 118.5, 117.5, 113.3, 103.6, 103.5, 102.2, 95.3, 78.0, 77.5, 74.7, 74.0, 72.5, 71.9, 71.3, 69.8, 67.9, 17.9; HRMS (ESI) calcd for $C_{27}H_{31}O_{15}$ $[M]^+$ 595.1657, found 595.1654.

Zn reduction of rutin (3) under Ar

(2*S*)-Flav-3-en-3-ol 3-*O*-rutinoside (5), (2*R*)-flav-3-en-3-ol 3-*O*-rutinoside (6), and flav-2-en-3-ol 3-*O*-rutinoside (7)

To a solution of **3** (1 g, 1.64 mmol) in 0.8 N HCl-MeOH (95 mL) was added Zn powder (5 g) at –20 ºC under Ar. After stirring under Ar for 30 min at –20 ºC, Zn powder was removed by suction filtration and then H_2O (600 mL) was added. After MeOH was removed under reduced pressure at 40 ºC, the residual aqueous solution was purified by XAD-7 with 80% MeCN aqueous solution. Finally, the resultant crude product was purified by ODS column chromatography (Develosil ODS-HG5 20 mm ϕ × 250 mm, Nomura Chemicals) with (5%→10%→15%) MeCN aqueous solution to give **5** (136 mg, 14%), **6** (537 mg, 55%) and **7** (31 mg, 3%) as white solids.

Data for **5**: $[\alpha]_D^{20}$ –43.2º (c = 0.5, CH_3OH); IR (KBr) 3346, 2922, 1622, 1458, 1280, 1059 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 6.89 (d, *J* = 2.0 Hz, 1H), 6.77 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.69 (d, *J* = 8.0 Hz, 1H), 6.28 (s, 1H), 5.87 (d, *J* = 1.5 Hz, 1H), 5.73 (d, *J* = 1.5 Hz, 1H), 5.52 (s, 1H), 4.83 (d, *J* = 9.0 Hz, 1H), 4.82 (d, *J* = 1.5 Hz, 1H), 4.0-4.1 (m, 2H), 3.73 (dd, *J* = 9.0, 3.5 Hz, 1H), 3.5-3.7 (m, 3H), 3.42 (t, *J* = 9.0 Hz, 1H), 3.37 (t, *J* = 9.0 Hz, 1H), 3.28 (t, *J* = 9.0 Hz, 1H), 3.26 (t, *J* = 9.0 Hz, 1H), 1.26 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 157.8, 154.2, 152.9, 148.6, 146.7, 146.0 ,131.7, 120.8, 116.0, 115.8, 103.8, 102.2, 101.7, 96.9, 96.6, 94.8, 78.0, 77.5, 77.4, 74.5, 74.2, 72.4, 71.9, 71.6, 69.8, 67.6, 18.0; HRMS (ESI) calcd for $C_{27}H_{31}O_{15}$ $[M-H]^-$ 595.1668, found 595.1665.

Data for **6**: $[\alpha]_D^{20}$ –99.9º (c = 0.5, CH_3OH); IR (KBr) 3371, 2908, 1626, 1466, 1283, 1063 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 6.86 (d, *J* = 2.0 Hz, 1H), 6.76 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.72 (d, *J* = 8.0 Hz, 1H), 6.28 (s, 1H), 5.88 (d, *J* = 2.0 Hz, 1H), 5.76 (d, *J* = 2.0 Hz, 1H), 5.45 (s, 1H), 4.82 (d, *J* = 9.0 Hz, 1H), 4.73 (d, *J* = 1.5 Hz, 1H), 4.04 (brd, *J* = 9.5 Hz, 1H), 3.90 (dd, *J* = 3.5, 1.5 Hz, 1H), 3.72 (dd, *J* = 9.0, 3.5 Hz, 1H), 3.68 (dd, *J* = 9.0, 6.5 Hz, 1H), 3.5-3.6 (m, 2H), 3.40 (t, *J* = 9.0 Hz, 1H), 3.36 (t, *J* = 9.0 Hz, 1H), 3.2-3.3 (m, 2H), 1.25 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 157.9, 154.2, 153.2, 149.1, 146.6, 146.0, 131.7, 120.4, 116.0, 115.6, 103.9, 102.2, 101.9, 97.0, 96.7, 95.8, 77.9, 77.8, 77.0, 74.5, 74.1, 72.4, 71.9, 71.4, 69.8, 67.7, 18.0; HRMS (ESI) calcd for $C_{27}H_{31}O_{15}$ $[M-H]^-$ 595.1668, found 595.1668.

Data for **7**: $[\alpha]_D^{21}$ –27.6º (c = 0.25, CH_3OH); IR (KBr) 3376, 2917, 1631, 1519, 1301, 1059 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 7.29 (d, *J* = 2.0 Hz, 1H), 7.23 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.75 (d, *J* = 8.0 Hz, 1H), 6.00 (d, *J* = 2.5 Hz, 1H), 5.93 (d, *J* = 2.5 Hz, 1H), 4.74 (d, *J* = 7.0 Hz, 1H), 4.70 (d, *J* = 1.5 Hz, 1H), 3.99 (dd, *J* = 11.0, 1.5 Hz, 1H), 3.83 (dd, *J* = 3.5 1.5 Hz, 1H), 3.3-3.7 (m, 10H), 1.21 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 158.1, 157.1, 153.4, 146.0, 145.3, 138.6, 132.2, 126.4, 121.2, 116.3, 115.6, 102.2, 101.0, 100.2, 97.7, 95.1, 78.0, 77.0, 75.1, 74.1, 72.3, 72.1, 71.5, 69.8, 67.9, 23.0, 18.0; HRMS (ESI) calcd for $C_{27}H_{31}O_{15}$ $[M-H]^-$ 595.1668, found 595.1671.

Hydrogenation

Ent-epicatechin 3-*O*-rutinoside (8) from (2*S*)-Flav-3-en-3-ol 3-*O*-rutinoside (5)

To a solution of **3a** (100 mg, 0.168 mmol) in MeOH was added 10 wt% of Pd/C (43 mg). The solution was stirred under 1 atm of H_2 for 4 h at room temperature. The catalyst was removed by filtration through Celite pad and the Celite pad was washed with MeOH. The solvent was removed under reduced pressure at 40 ºC. The residue was purified by ODS column chromatography (Develosil ODS-HG5, Nomura Chemicals) with 10%→15% MeCN aqueous solution to give **8** (52.6 mg, 52%) as a white solid.

Data for **8**: $[\alpha]_D^{20}$ –8.1º (c = 0.5, CH_3OH); IR (KBr) 3320, 2913, 1626, 1519, 1467, 1362, 1284, 1146, 1055 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 6.99 (d, *J* = 2.0 Hz, 1H), 6.87 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.76 (d, *J* = 8.0 Hz, 1H), 5.94 (d, *J* = 2.0 Hz, 1H), 5.91 (d, *J* = 2.0 Hz, 1H), 4.93 (brs, 1H), 4.75 (d, *J* = 1.5 Hz, 1H), 4.3 (m, 1H), 4.07 (d, *J* = 8.0 Hz, 1H), 3.95 (dd, *J* = 11.0, 1.5 Hz, 1H), 3.89 (dd,

J = 3.5, 1.5 Hz, 1H), 3.6-3.7 (m, 2H), 3.57 (dd, *J* = 11.0, 6.0 Hz, 1H), 3.38 (t, *J* = 9.5 Hz, 1H), 3.1-3.3 (m, 4H), 2.92 (dd, *J* = 16.5, 4.0 Hz, 1H), 2.87 (dd, *J* = 16.5, 4.0 Hz, 1H), 1.27 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 157.7, 157.6, 157.0, 145.9, 145.8, 131.6, 119.5, 116.0, 115.2, 105.1, 102.3, 100.5, 96.6, 95.7, 78.8, 77.6, 76.8, 75.8, 75.3, 74.1, 72.3, 72.2, 71.7, 69.8, 68.3, 28.3, 18.1; HRMS (ESI) calcd for $C_{27}H_{33}O_{15}$ $[M-H]^-$ 597.1825, found 597.1827.

Epicatechin 3-*O*-rutinoside (9) and catechin 3-*O*-rutinoside (10) from (2*R*)-flav-3-en-3-ol 3-*O*-rutinoside (6)

To a solution of **3b** (100 mg, 0.168 mmol) in MeOH was added 10 wt% of Pd/C (43 mg). The solution was stirred under 1 atm of H_2 for 4 h at room temperature. The catalyst was removed by filtration through Celite pad and the Celite pad was washed with MeOH. The solvent was removed under reduced pressure at 40 ºC. The residue was purified by ODS column chromatography (Develosil ODS-HG5, Nomura Chemicals) with 10%→15% MeCN aqueous solution to give **9** (15.9 mg, 16%) and **10** (22.8 mg, 23%) as white solids.

Data for **9**: $[\alpha]_D^{21}$ –37.4º (c = 0.5, CH_3OH); IR (KBr) 3377, 2917, 1627, 1519, 1462, 1366, 1284, 1142, 1050 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 6.97 (d, *J* = 1.5 Hz, 1H), 6.81 (dd, *J* = 8.0, 1.5 Hz, 1H), 6.70 (d, *J* = 8.0 Hz, 1H), 5.93 (d, *J* = 2.0 Hz, 1H), 5.90 (d, *J* = 2.0 Hz, 1H), 5.12 (d, *J* = 2.0, 1H), 4.72 (d, *J* = 1.5 Hz, 1H), 4.39 (t, *J* = 8.5, 1H), 4.39 (d, *J* = 8.5 Hz, 1H), 3.97 (dd, *J* = 11.0, 1.5 Hz, 1H), 3.85 (dd, *J* = 3.5, 1.5 Hz, 1H), 3.68 (dd, *J* = 10.0, 3.5 Hz, 1H), 3.63 (dd, *J* = 10.0, 6.5 Hz, 1H), 3.49 (dd, *J* = 11.0, 7.0 Hz, 1H), 3.3-3.4 (m, 3H), 3.21 (t, *J* = 9.5 Hz, 1H), 3.15 (dd, *J* = 9.0, 8.0 Hz, 1H), 2.81 (dd, *J* = 16.5, 5.0 Hz, 1H), 2.69 (dd, *J* = 16.5, 6.0 Hz, 1H), 1.26 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 158.0, 157.8, 157.0, 145.7, 145.5, 131.5, 120.3, 116.1, 115.7, 102.8, 102.5, 100.3, 96.4, 95.6, 79.1, 77.9, 76.8, 74.9, 74.1, 73.8, 72.4, 72.2, 71.9, 69.9, 68.8, 24.4, 18.0; HRMS (ESI) calcd for $C_{27}H_{33}O_{15}$ $[M-H]^-$ 597.1825, found 597.1824.

Data for **10**: $[\alpha]_D^{21}$ –27.1º (c = 0.5, CH_3OH); IR (KBr) 3377, 2922, 1627, 1519, 1462, 1366, 1284, 1142, 1059 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 6.82 (d, *J* = 2.0 Hz, 1H), 6.76 (d, *J* = 8.0 Hz, 1H), 6.70 (dd, *J* = 8.0, 2.0 Hz, 1H), 5.93 (d, *J* = 2.0 Hz, 1H), 5.88 (d, *J* = 2.0, 1H), 4.89 (d, *J* = 6.5 Hz, 1H), 4.75 (d, *J* = 1.5 Hz, 1H), 4.17 (d, *J* = 8.0 Hz, 1H), 4.1 (m, 1H), 3.99 (dd, *J* = 11.0, 2.0 Hz, 1H), 3.88 (dd, *J* = 3.5, 2.0 Hz, 1H), 3.6-3.7 (m, 2H), 3.58 (dd, *J* = 11.0, 6.0 Hz, 1H), 3.37 (t, *J* = 9.0 Hz,

1H), 3.2-3.3 (m, 2H), 3.1 (m, 1H), 2.78 (dd, *J* = 16.5, 5.5 Hz, 1H), 2.71 (dd, *J* = 16.5, 7.0 Hz, 1H), 1.27 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 157.8, 157.5, 156.5, 146.3, 146.2, 132.1, 119.5, 116.2, 114.8, 103.8, 102.2, 100.6, 96.4, 95.5, 80.0, 77.7, 76.8, 76.2, 75.1, 74.1, 72.3, 72.1, 71.6, 69.8, 68.1, 26.5, 18.1 ;HRMS (ESI) calcd for $C_{27}H_{33}O_{15}$ $[M-H]^-$ 597.1825, found 597.1820.

Ent-epicatechin 3-*O*-rutinoside (8) from flav-2-en-3-ol 3-*O*-rutinoside (7)

To a solution of **4** (20 mg, 34 μmol) in MeOH was added 10 wt% of Pd/C (10 mg). The solution was stirred under 1 atm of H_2 for 4 h at room temperature. The catalyst was removed by filtration through Celite pad and the Celite pad was washed with MeOH. The solvent was removed under reduced pressure at 40 ºC. The residue was purified by ODS column chromatography (Develosil ODS-HG5, Nomura Chemicals) with 15%→20% MeCN aqueous solution to give **8** (7.2 mg, 36%) as a white solid.

Ent-epicatechin 3-*O*-rutinoside (8) and epicatechin 3-*O*-rutinoside (9) from cyanidin 3-*O*-rutinoside (4)

To a solution of anthocyanin **4** (100 mg, 0.14 mmol) in MeOH was added 10 wt% of Pd/C (42 mg). The solution was stirred under 1 atm of H_2 for 4 h at room temperature. The catalyst was removed by filtration through Celite pad and the Celite pad was washed with MeOH. The solvent was removed under reduced pressure at 40 ºC. The residue was purified by ODS column chromatography (Develosil ODS-HG5, Nomura Chemicals) with 10%→15% MeCN aqueous solution to give **8** (46.8 mg, 55%) and **9** (2 mg, 2%) as white solids.

$NaBH_3CN$ reduction

(2*S*)-Flav-3-en-3-ol 3-*O*-rutinoside (5), (2*R*)-flav-3-en-3-ol 3-*O*-rutinoside (6), and flav-2-en-3-ol 3-*O*-rutinoside (7) from cyanidin 3-*O*-rutinoside (4)

To a solution of anthocyanin **4** (198 mg, 0.28 mmol) in MeOH (5 mL) was added $NaBH_3CN$ (53 mg, 0.84 mmol) at –20 ºC. After stirring for 30 min at –20 ºC, H_2O (10 mL) was added to the resulting reaction mixture. MeOH was removed under reduced pressure at 40 ºC. The residual aqueous solution was purified by ODS open column chromatography (COSMOSIL 75C_{18}-OPN: 15% CH_3CN in H_2O) to give crude products. The resultant crude products were purified by ODS column

chromatography (Develosil ODS-HG5, Nomura Chemicals) with 10% MeCN aqueous solution to give **5** (4.9 mg, 3%), **6** (5.4 mg, 3%), and **7** (117 mg, 70%) as white solids.

Catechin 3-*O*-rutinoside (10) from (+)-catechin (11)

To a solution of 5,7,3'4'-*O*-tetrabenzylcatechin (**S1**)[S11] (260 mg, 0.4 mmol), heptaacetylrutinosyl imidate (347 mg, 0.48 mmol) and MS 4Å (1.2 g) in dichloromethane (8 mL) was added TMSOTf (14 μL, 80 μmol) at –20 ºC. After stirring for 1 h at –20 ºC, the reaction mixture was quenched with saturated aqueous $NaHCO_3$. The crude products were extracted with AcOEt. The combined extracts were dried over anhydrous $MgSO_4$. The solvent was concentrated under reduced pressure and the residue was purified by flash column chromatography (hexane/AcOEt = 4/1 to 1/1) to give **S2** (270 mg, 56%) as a white solid.

Data for **S2**: IR (KBr) 2939, 1756, 1620, 1373, 1221, 1141, 1038 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.3-7.5 (m, 20H), 7.04 (d, J = 1.5 Hz, 1H), 6.92 (d, J = 7.0 Hz, 1H), 6.87 (dd, J = 7.0, 1.5 Hz, 1H), 6.20 (d, J = 1.5 Hz, 1H), 6.14 (d, J = 1.5, 1H), 5.1-5.2 (m, 6H), 5.0 (m, 3H), 4.99 (t, J = 8.0 Hz, 1H), 4.95 (brd, 2H), 4.89 (t, J = 8.0 Hz, 1H), 4.79 (dd, J = 8.0, 6.5 Hz, 1H), 4.78 (brs, 2H), 4.66 (d, J = 8.0 Hz, 1H), 4.07 (d, J = 6.0 Hz, 1H), 4.0 (m, 1H), 3.8 (m, 1H), 3.6 (m, 2H), 3.5 (m, 1H), 3.06 (dd, J = 14.0, 7.0 Hz, 1H), 2.74 (dd, J = 14.0, 7.0 Hz, 1H), 2.05 (s, 3H), 2.01 (s, 3H), 1.95 (s, 3H), 1.94 (s, 3H), 1.84 (s, 3H), 1.71 (s, 3H), 1.16 (d, J = 5.5 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.1, 170.0, 169.9, 169.8, 169.6, 169.1, 158.6, 157.6, 155.2, 149.1, 148.9, 137.3, 137.2, 137.1, 136.9, 131.5, 128.5, 127.9, 127.8, 127.7, 127.4, 127.3, 127.2, 127.0, 121.0, 114.7, 113.7, 102.4, 100.6, 100.3, 97.7, 94.4, 93.9, 79.3, 75.1, 73.4, 72.7, 71.2, 71.1, 71.0, 70.9, 70.1, 69.8, 69.6, 69.4, 68.9, 66.8, 66.6, 27.8, 20.7, 20.6, 20.5, 20.4, 20.3, 17.3; HRMS (ESI) calcd for $C_{67}H_{70}O_{21}Na$ $[M+Na]^+$ 1233.4302, found 1233.4297.

To a solution of **S2** (36 mg, 30 μmol) in MeOH (1 mL) and $CHCl_3$ (1 mL) was added NaOMe (30 mg) at room temperature. After stirring for 38 h at this temperature, the reaction mixture was neutralized with Dowex 50W-X8. After the Dowex 50W-X8 was filtered, the solvent was removed under reduced pressure to give a crude deacetylated product. The crude product was used in the next step without further purification. To a solution of the crude deacetylated product in $CHCl_3$ (2 mL) and MeOH (2 mL) was added 20 wt% of $Pd(OH)_2/C$ (7 mg). The solution was stirred under 1 atm of H_2 for 19 h at room temperature. The catalyst was then removed by filtration through filter

paper by washing with H_2O. Both $CHCl_3$ and MeOH were removed under reduced pressure at 40 ºC. The residual aqueous solution was purified by ODS column chromatography (Develosil ODS-HG5, Nomura Chemicals) with 10% MeCN aqueous solution to give **10** (13 mg, 72%) as a white solid.

Data for **10** from (+)-catechin (**11**): IR (KBr) 3396, 2927, 1629, 1522, 1466, 1377, 1285, 1143, 1060 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 6.82 (d, J = 2.0 Hz, 1H), 6.76 (d, J = 8.0 Hz, 1H), 6.71 (dd, J = 8.0, 2.0 Hz, 1H), 5.93 (d, J = 2.0 Hz, 1H), 5.88 (d, J = 2.0, 1H), 4.89 (d, J = 6.5 Hz, 1H), 4.75 (d, J = 1.5 Hz, 1H), 4.17 (d, J = 8.0 Hz, 1H), 4.1 (m, 1H), 3.99 (dd, J = 11.0, 2.0 Hz, 1H), 3.88 (dd, J = 3.5, 2.0 Hz, 1H), 3.6-3.7 (m, 2H), 3.58 (dd, J = 11.0, 6.0 Hz, 1H), 3.37 (t, J = 9.0 Hz, 1H), 3.2-3.3 (m, 2H), 3.1 (m, H), 2.78 (dd, J = 16.5, 5.5 Hz, 1H), 2.71 (dd, J = 16.5, 7.0 Hz, 1H), 1.27 (d, J = 6.0 Hz, 3H); ^{13}C NMR (150 MHz, CD_3OD) δ 157.9, 157.6, 156.6, 146.3, 146.2, 132.2, 119.6, 116.2, 114.8, 103.8, 102.2, 100.6, 96.4, 95.5, 80.0, 77.7, 76.8, 76.3, 75.1, 74.1, 72.4, 72.1, 71.6, 69.8, 68.1, 26.5, 18.1; HRMS (ESI) calcd for $C_{27}H_{33}O_{15}$ $[M-H]^-$ 597.1825, found 597.1824.

Epicatechin 3-*O*-rutinoside (9) from (–)-epicatechin (12)

To a solution of 5,7,3'4'-*O*-tetrabenzylepicatechin (**S3**)[S11] (260 mg, 0.4 mmol), heptaacetylrutinosyl imidate (347 mg, 0.48 mmol) and MS 4Å (1.2 g) in dichloromethane (8 mL) was added TMSOTf (14 μL, 80 μmol) at –20 ºC. After stirring for 1 h at –20 ºC, the reaction mixture was quenched with saturated aqueous $NaHCO_3$. The crude products were extracted with AcOEt. The combined extracts were dried over anhydrous $MgSO_4$. The solvent was concentrated under reduced pressure and the residue was purified by flash column chromatography (hexane/AcOEt = 4/1 to 3/2) to give **S4** (198 mg, 41%) as a white solid.

Data for **S4**: IR (KBr) 2939, 1753, 1618, 1374, 1220, 1141, 1045 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.3-7.5 (m, 20H), 7.22 (brs, 1H), 6.88 (brs, 2H), 6.22 (brs, 1H), 6.21 (brs, 1H), 4.9-5.2 (m, 13H), 4.82 (t, J = 8.0 Hz, 2H), 4.65 (brs, 1H), 4.56 (d, J = 6.5 Hz, 1H), 4.3 (m, 1H), 3.7-3.8 (m, 1H), 3.5-3.6 (m, 2H), 3.48 (dd, J = 9.5, 6.0 Hz, 1H), 2.93 (dd, J = 14.0, 2.5 Hz, 1H), 2.80 (dd, J = 14.0, 3.5 Hz, 1H), 2.04 (s, 6H), 2.01 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.50 (s, 3H), 1.15 (d, J = 5.0 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.2, 170.0, 169.9, 169.8, 169.6, 169.1, 158.7, 157.8, 155.8, 148.6, 137.7, 137.5, 137.0, 136.9, 131.4, 128.6, 128.5, 128.4, 128.3, 127.9, 127.7, 127.6, 127.5, 127.4, 127.3, 120.5, 114.7, 114.5, 100.4, 98.0, 97.2, 94.5, 93.5, 78.0, 77.2, 77.0, 76.8, 73.4, 72.5, 71.3, 71.0, 70.9, 70.5, 70.1, 70.0, 69.6, 69.5, 68.9, 66.9, 66.5, 23.2, 20.8, 20.7, 20.6, 20.5, 19.8,

17.3; HRMS (ESI) calcd for $C_{67}H_{70}O_{21}Na$ $[M+Na]^+$ 1233.4302, found 1233.4304.

To a solution of **S4** (36 mg, 30 μmol) in MeOH (1.5 mL) and $CHCl_3$ (1.5 mL) was added NaOMe (30 mg) at room temperature. After stirring for 48 h at this temperature, the reaction mixture was neutralized with Dowex 50W-X8. After the Dowex 50W-X8 was filtered, the solvent was removed under reduced pressure to give a crude deacetylated product. The crude product was used in the next step without further purification. To a solution of the crude deacetylated product in $CHCl_3$ (2 mL) and MeOH (2 mL) was added 20 wt% of $Pd(OH)_2/C$ (7 mg). The solution was stirred under 1 atm of H_2 for 19 h at room temperature. The catalyst was then removed by filtration through filter paper by washing with H_2O. Both $CHCl_3$ and MeOH were removed under reduced pressure at 40 ºC. The residual aqueous solution was purified by ODS column chromatography (Develosil ODS-HG5, Nomura Chemicals) with 15% MeCN aqueous solution to give **9** (12 mg, 67%) as a white solid. Data for **9** from (–)-epicatechin (**12**): IR (KBr) 3383, 2929, 1630, 1521, 1467, 1372, 1285, 1143, 1053 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 6.98 (d, J = 1.5 Hz, 1H), 6.81 (dd, J = 8.0, 1.5 Hz, 1H), 6.70 (d, J = 8.0 Hz, 1H), 5.94 (d, J = 2.0 Hz, 1H), 5.90 (d, J = 2.0 Hz, 1H), 5.13 (d, J = 2.0, 1H), 4.72 (d, J = 1.5 Hz, 1H), 4.39 (t, J = 8.5, 1H), 4.39 (d, J = 8.5 Hz, 1H), 3.97 (dd, J = 11.0, 1.5 Hz, 1H), 3.85 (dd, J = 3.5, 1.5 Hz, 1H), 3.69 (dd, J = 10.0, 3.5 Hz, 1H), 3.65 (dd, J = 10.0, 6.5 Hz, 1H), 3.50 (dd, J = 11.0, 7.0 Hz, 1H), 3.3-3.4 (m, 3H), 3.21 (t, J = 9.5 Hz, 1H), 3.15 (dd, J = 9.0, 8.0 Hz, 1H), 2.81 (dd, J = 16.5, 5.0 Hz, 1H), 2.69 (dd, J = 16.5, 6.0 Hz, 1H), 1.26 (d, J = 6.5 Hz, 3H); ^{13}C NMR (150 MHz, CD_3OD) δ 158.0, 157.8, 156.9, 145.6, 145.5, 131.5, 120.2, 116.1, 115.6, 102.8, 102.4, 100.2, 96.4, 95.6, 79.1, 77.9, 76.8, 74.9, 74.1, 73.8, 72.3, 72.2, 71.9, 69.8, 68.7, 24.4, 18.1; HRMS (ESI) calcd for $C_{27}H_{33}O_{15}$ $[M-H]^-$ 597.1825, found 597.1828.

5. References

[S1] J.-D. Chai and M. Head-Gordon, *Phys. Chem. Chem. Phys.* **10**, 6615 (2008).

[S2] F. Weigend and R. Ahlrichs, *Phys. Chem. Chem. Phys.* **7**, 3297 (2005).

[S3] T. Yanai, D. P. Tew, and N. C. Handy, *Chem. Phys. Lett.* **393**, 51 (2004).

[S4] G. Scalmani and M. J. Frisch, *J. Chem. Phys.* **132**, 114110 (2010).

[S5] Gaussian 16, Revision A.03, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. V. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, T. A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2016.

[S6] R. L. Martin, *J. Chem. Phys.* **118**, 4775 (2003).

[S7] G. Bringmann, T. Bruhn, K. Maksimenka, Y. Hemberger, *Eur. J. Org. Chem.* 2717 (2009).

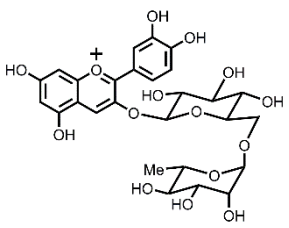
[S8] P. J. Stephens and N. Harada, *Chirality* **22**, 229 (2010).

[S9] Y. Toyama-Kato, T. Kondo, and K. Yoshida, *Heterocycles*, **72**, 239 (2007).

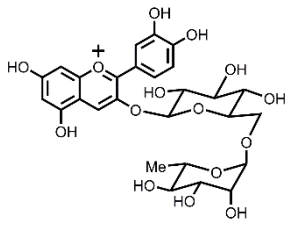
[S10] M. Mori,.T. Kondo and K. Yoshida, *Phytochemistry*, **69**, 3151 (2008).

[S11] K.-I. Oyama, M. Kuwano, M. Ito, K. Yoshida and T. Kondo, *Tetrahedron Lett.*, **49**, 3176 (2008).

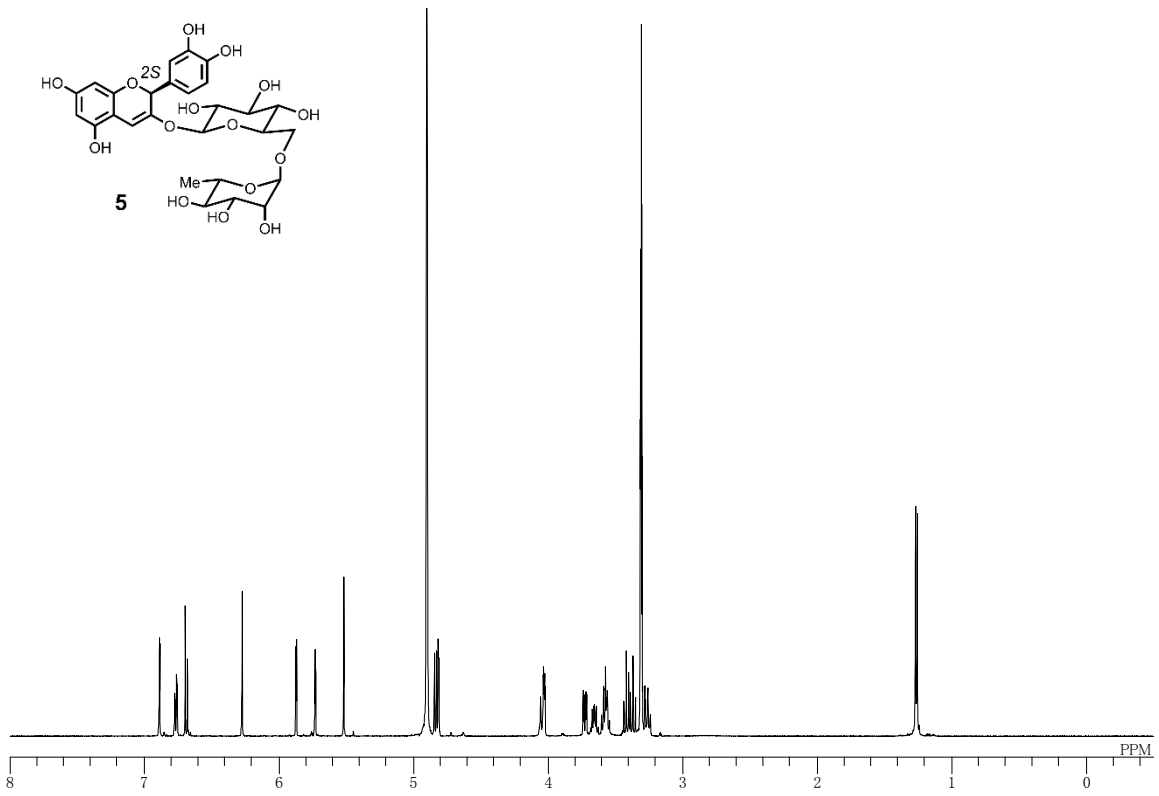
6. NMR spectra of Compounds

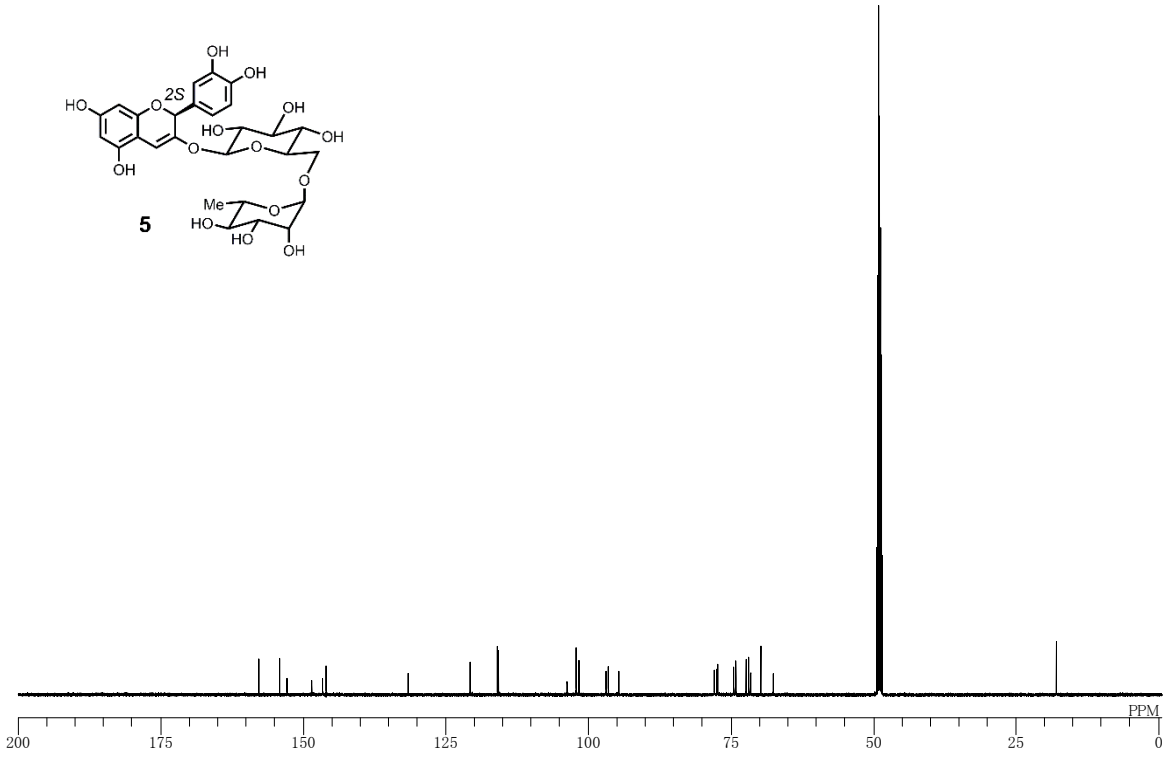


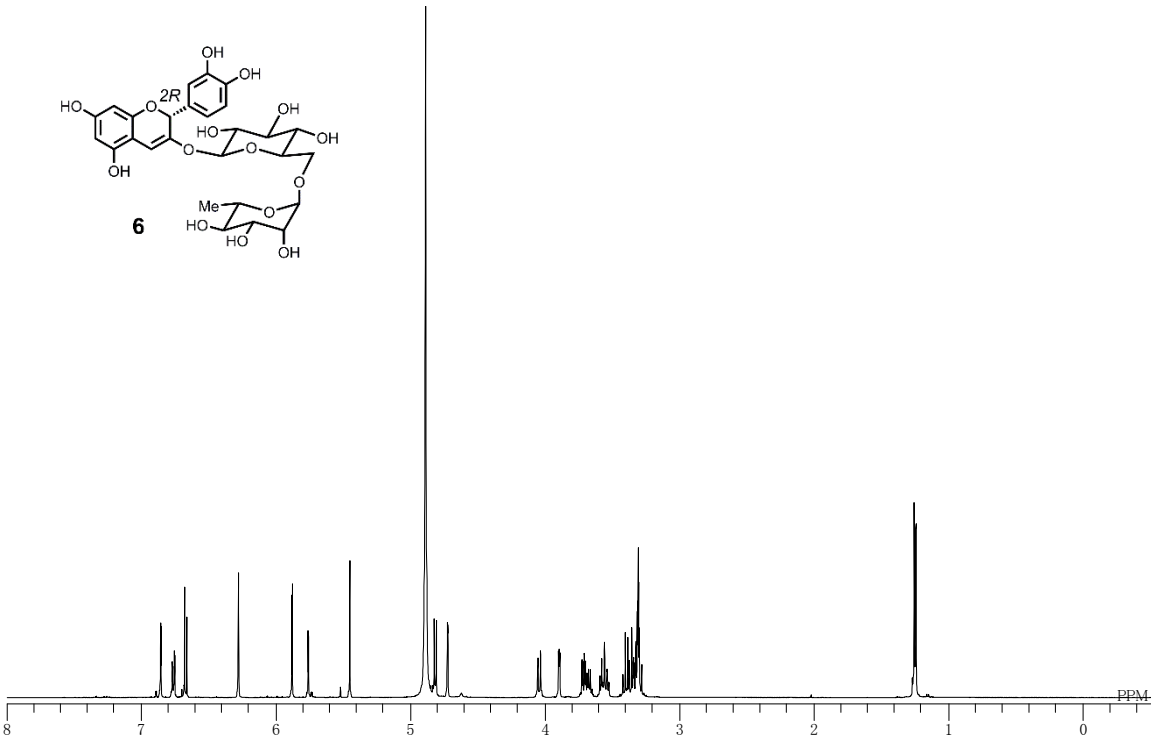
cyanidin 3-*O*-rutinoside (**4**)

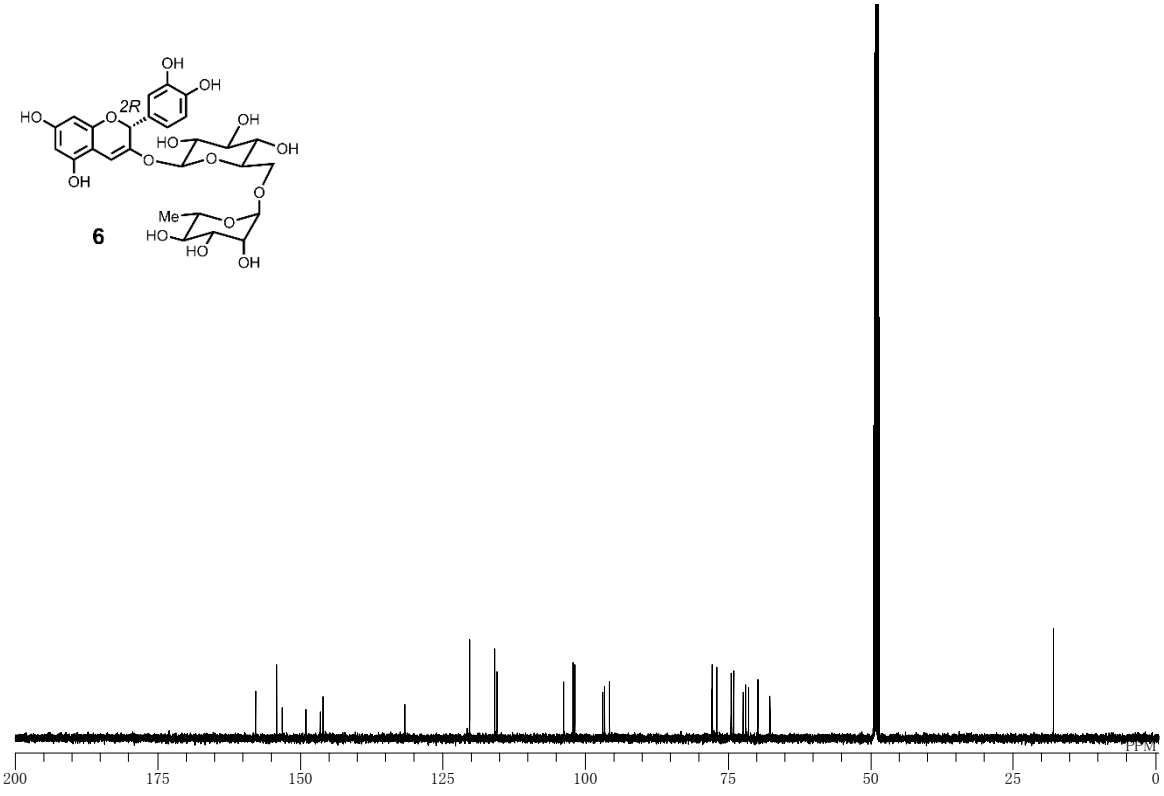


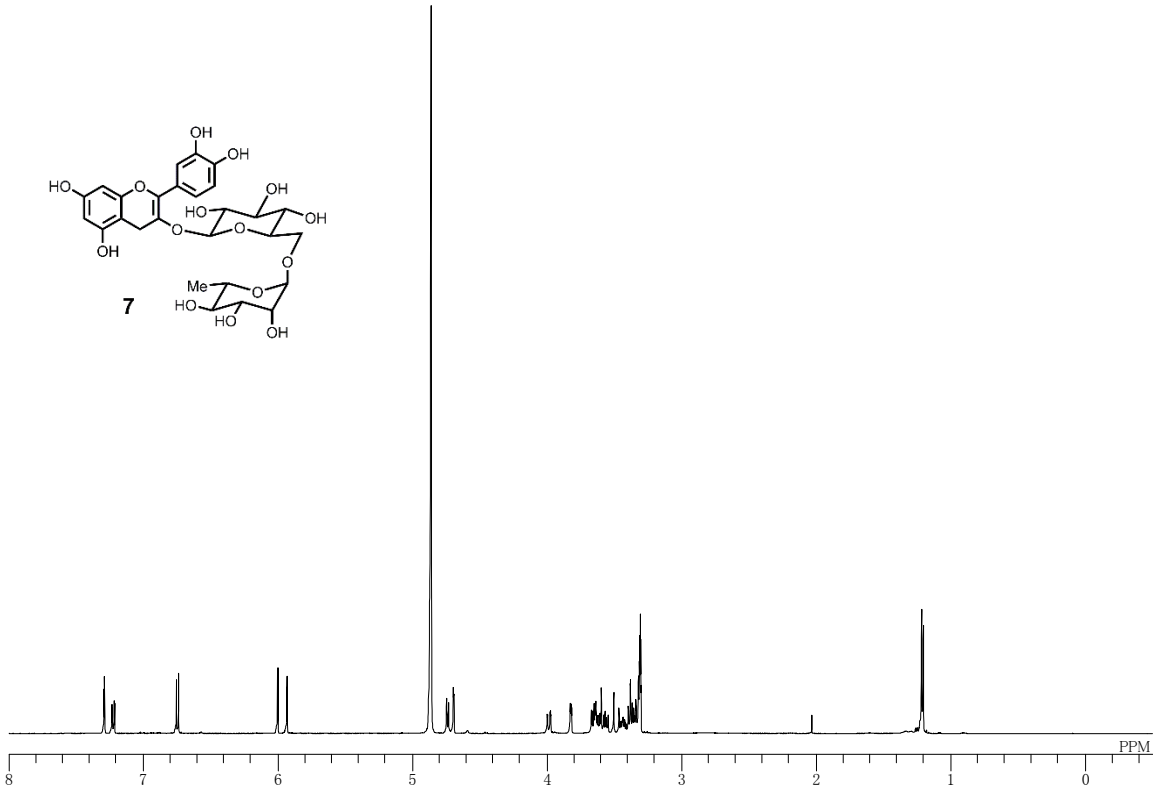
cyanidin 3-*O*-rutinoside (**4**)

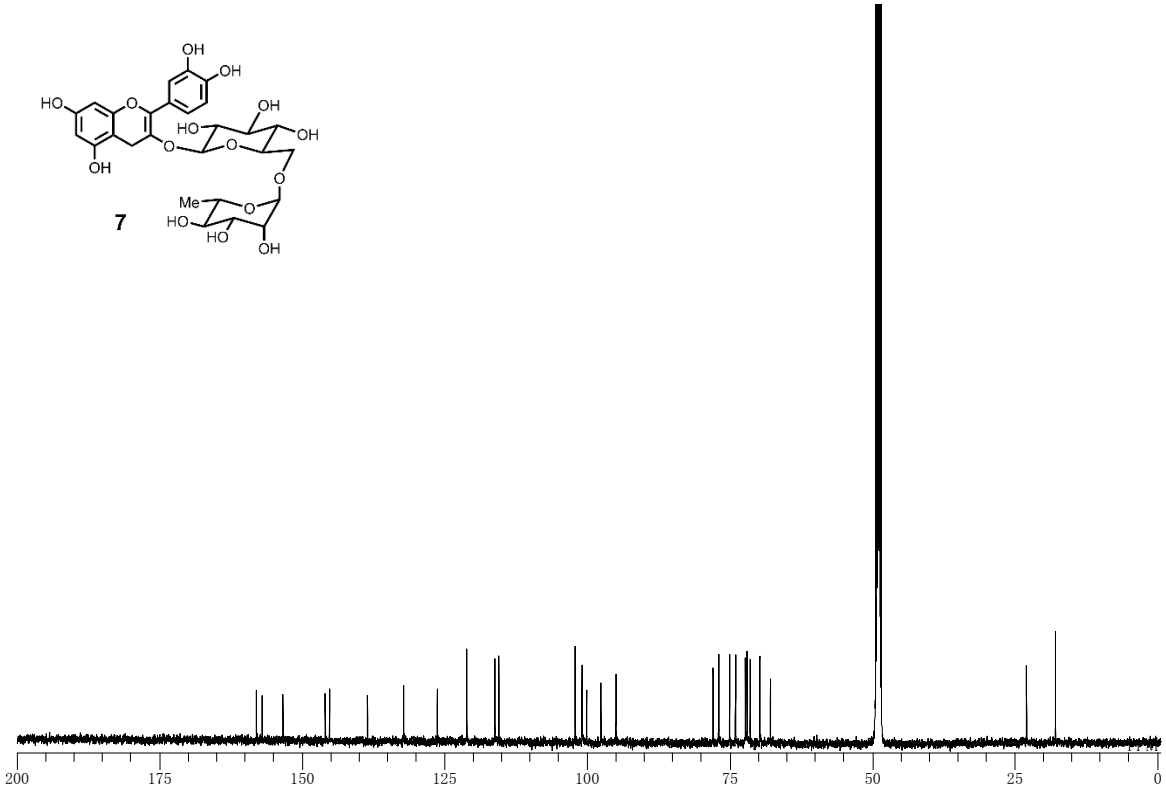

OH
OH
HO
2S
O
OH
HO
OH
O
O
OH
O
Me
HO
O
HO
OH
5
PPM
8
7
6
5
4
3
2
1
0

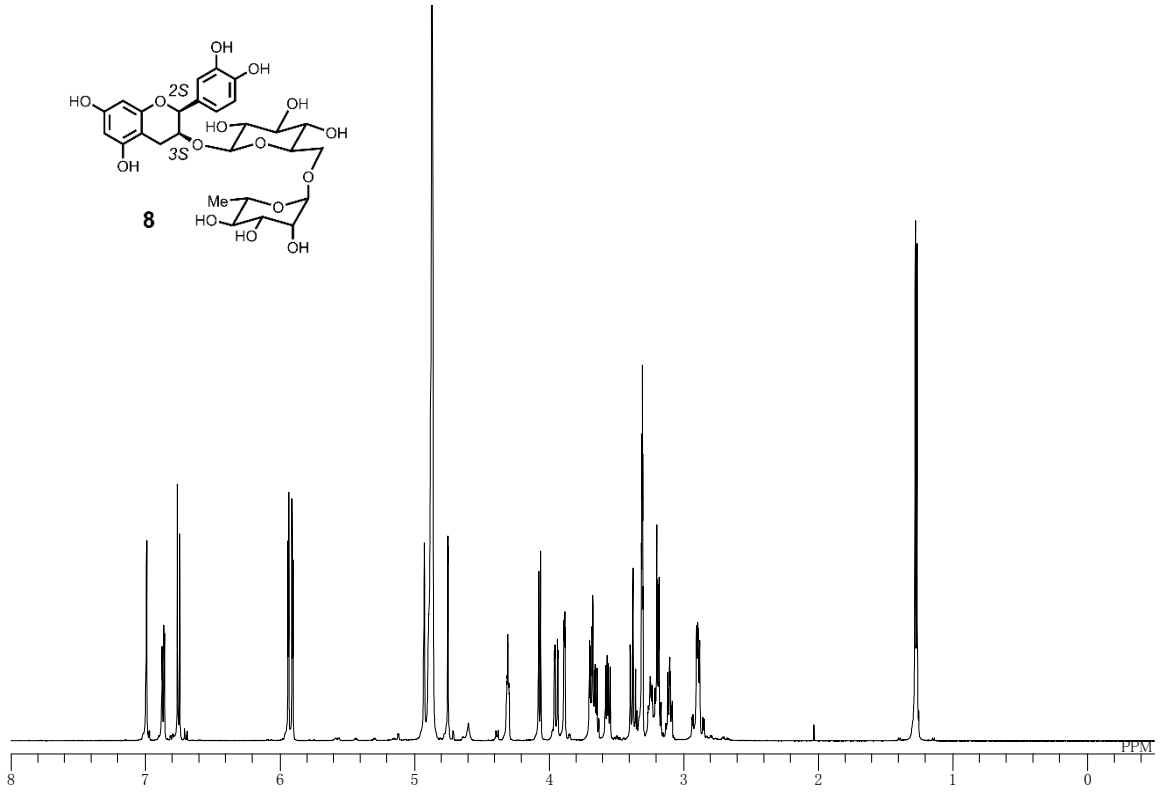

OH
OH
HO
2S
O
OH
HO
OH
O
O
OH
O
Me
HO
O
HO
OH
5
PPM
200
175
150
125
100
75
50
25
0

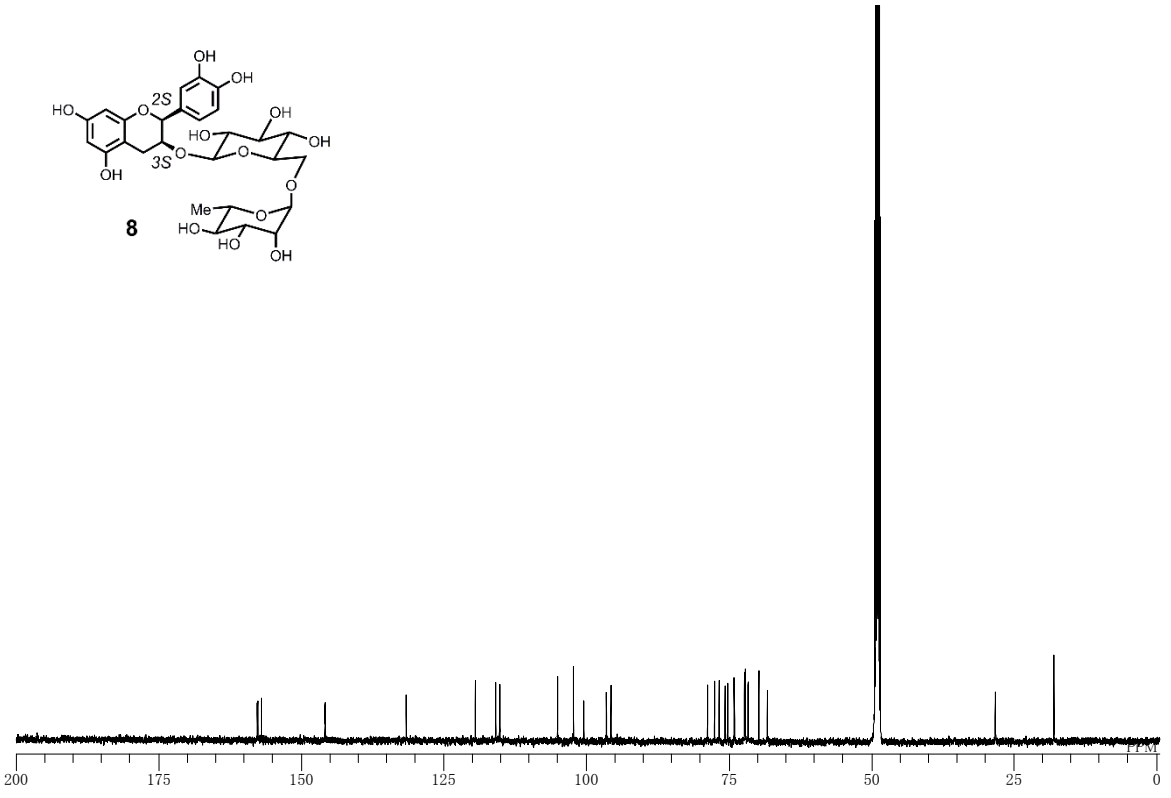

OH
OH
2R
HO
OH
HO
OH
O
O
O
OH
Me
O
HO
HO
OH
6
PPM
8
7
6
5
4
3
2
1
0

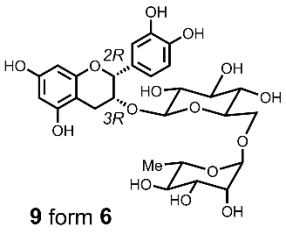

OH
OH
2R
HO
OH
HO
OH
O
O
O
OH
Me
O
HO
HO
OH
6
PPM
200
175
150
125
100
75
50
25
0


OH
OH
HO
OH
HO
OH
O
O
O
OH
Me
O
HO
HO
OH
7
PPM
8
7
6
5
4
3
2
1
0

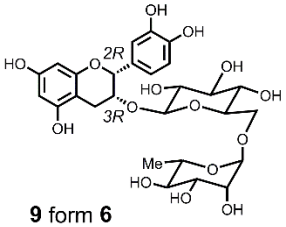

OH
OH
HO
OH
HO
OH
O
O
O
OH
Me
O
HO
HO
OH
7
200
175
150
125
100
75
50
25
0


OH
OH
HO
2S
O
OH
HO
OH
HO
O
3S
O
O
OH
Me
O
HO
HO
OH
8
PPM
8
7
6
5
4
3
2
1
0

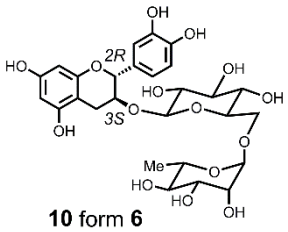

OH
OH
HO
2S
O
OH
HO
OH
HO
O
3S
O
O
OH
Me
O
HO
HO
OH
8
PPM
200
175
150
125
100
75
50
25
0



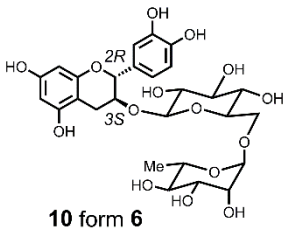
9 form **6**



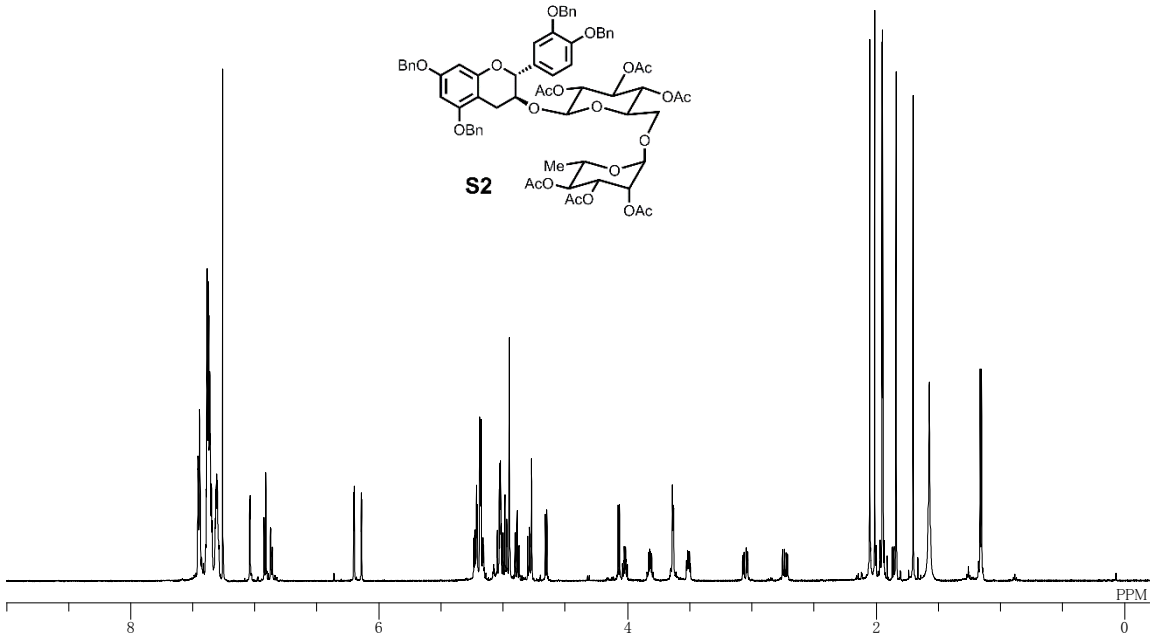
9 form **6**

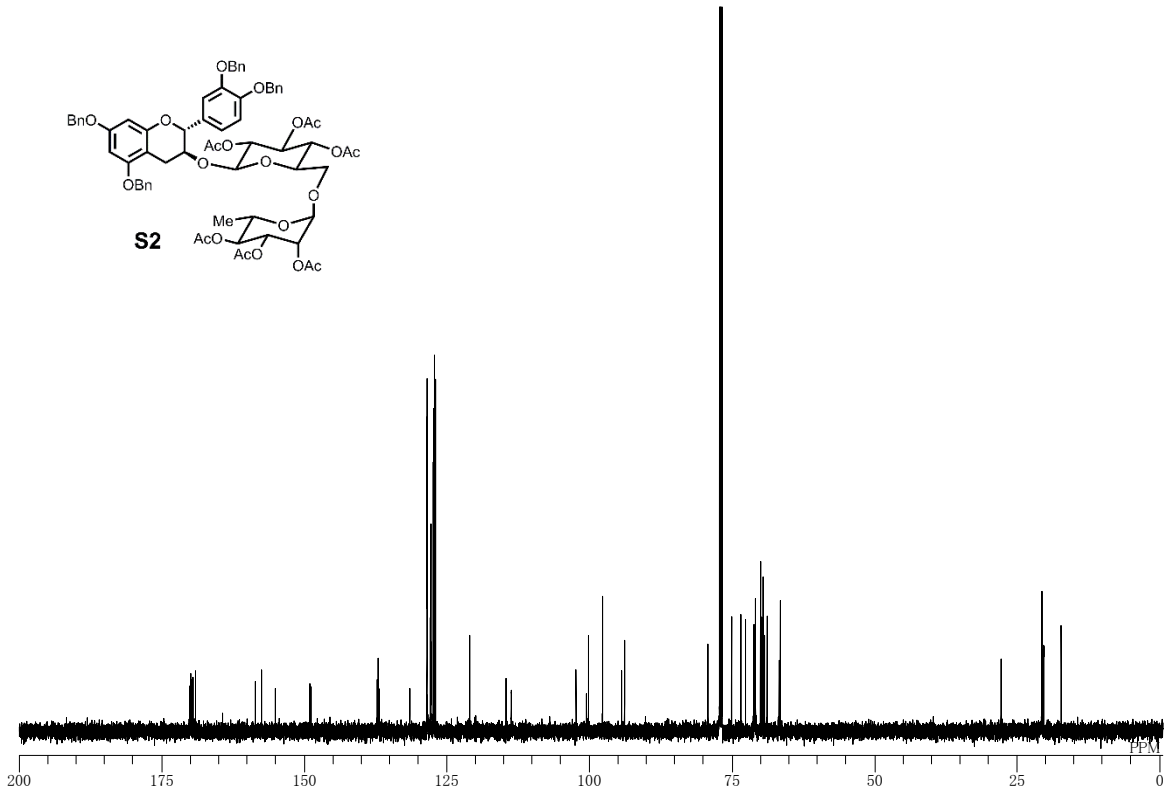


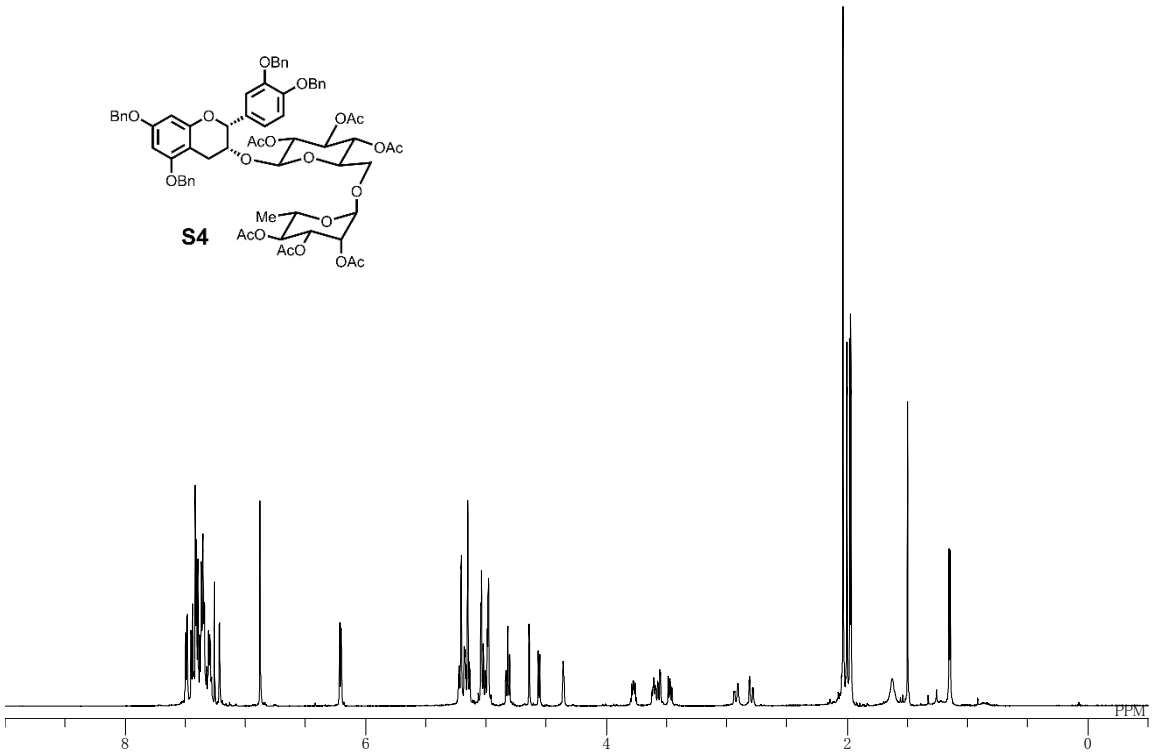
10 form **6**

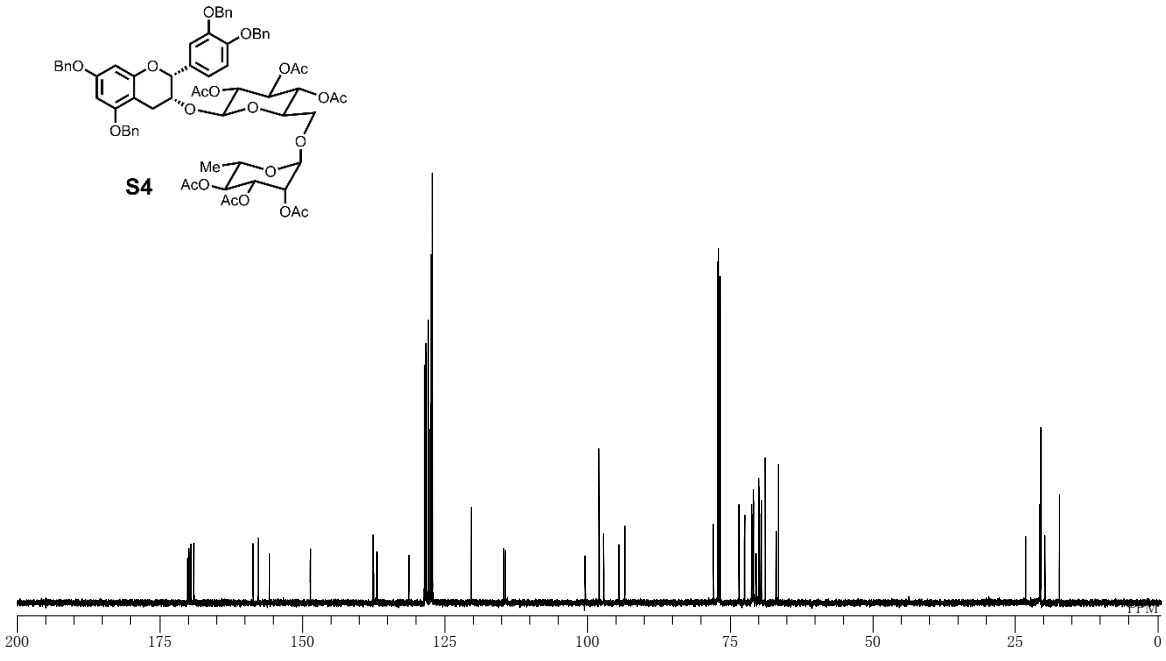


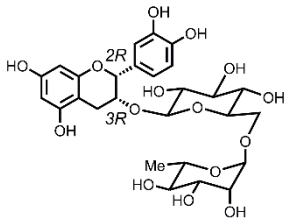
10 form **6**


OBn
OBn
BnO
OAc
AcO
OAc
OBn
O
Me
AcO
AcO
OAc
S2
PPM
8
6
4
2
0

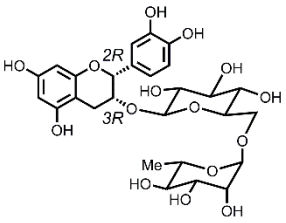

OBn
OBn
BnO
OAc
AcO
OAc
OBn
O
Me
AcO
AcO
OAc
S2
PPM
200
175
150
125
100
75
50
25
0


OBn
OBn
BnO
OAc
AcO
OAc
OBn
O
Me
AcO
AcO
OAc
S4
PPM
8
6
4
2
0

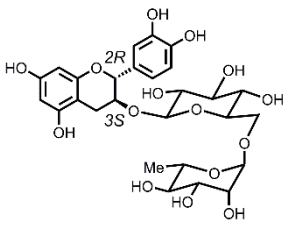

OBn
OBn
BnO
OAc
AcO
OAc
OBn
O
Me
AcO
AcO
OAc
S4
PPM
200
175
150
125
100
75
50
25
0



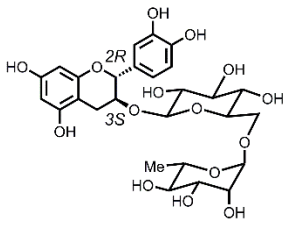
9 form (–)-epicatechin



9 form (–)-epicatechin



10 form (+)-catechin



10 form (+)-catechin