Supplementary information

Glucose uptake enhancing activity of puerarin and the role of *C*-glucoside suggested from activity of related compounds

Eisuke Kato* and Jun Kawabata

Laboratory of Food Biochemistry, Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Kita-9 Nishi-9, Kita-ku, Sapporo, Hokkaido, 060-8589, Japan

Contents

General experimental procedure

All commercially available chemicals were used without further purification. Compounds **1**, **10**, **17**, **18**, **22-25** were synthesized as followings, **19**, **20** was purchased from manufacturer and **21** was synthesized according to the reported procedure.[1] Following source of enzymes were used: Hexokinase from *Saccharomyces cerevisiae* (Sigma-Aldrich co.), Glucose-6-phosphate Dehydrogenase from *Leuconostoc mesenteroides* (Sigma-Aldrich co.), Diaphorase, from *Clostridium kluyveri* (Oriental yeast co.). Bruker AMX500 or Jeol JNM-EX 270 was used to obtain NMR spectrum and either tetramethylsilane (TMS) or residual solvent peak was used as an internal standard (^{1}H NMR: TMS 0.00 ppm, CD_3OD 3.30 ppm, DMSO-d6 2.50 ppm; ^{13}C NMR: $CDCl_3$ 77.0 ppm, CD_3OD 49.0 ppm, DMSO-d6 39.5 ppm). Jeol JMS SX-102A (FAB-MS) or Jeol JMS-T100GCV (FD-MS) was used to obtain mass spectrum.

Cell culture.

3T3-L1 preadipocytes were cultured in either 24 or 48 well plate with Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS) as a medium at 37 ºC in a 5% CO_2 atmosphere until confluent. Cells were then differentiated by 10% FBS/ DMEM supplemented with 10 μg/ mL insulin, 0.25 μM dexamethasone, and 0.5 mM isobutylmethylxanthine for 2 days, followed by additional 4 days in 10% FBS/ DMEM containing 5 μg/mL insulin with medium changed every 2days. The medium was then replaced with 10% FBS/ DMEM and culture for another 4 days before glucose uptake enhancing activity assay.

Glucose uptake enhancing activity assay.

Detail described as 11th reference of the main text.

Synthetic procedure and spectral properties of new compounds

Synthesis of 4-Benzyloxy-2,6-dihydroxy-3-*C*-β-D-(2,3,4,6-tetrabenzylglucopyranosyl)acetophenone (4).[2]

Trichloroacetimidate **2** (3.49 g, 5.10 mmol) and 4-Benzyloxy-2,6-dihydroxyacetophenone **3** (0.85 g, 3,30 mmol) were dissolved in CH_2Cl_2 (33 mL) and TMSOTf (90 μL, 0.50 mmol) was added. The mixture was stirred for 17 hours at 30 ºC and then water was added and extracted by EtOAc. Organic layer was washed with brine, dried over Na_2SO_4 and concentrated under vacuo. Residue was purified by silicagel column chromatography

(Toluene: EtOAc = 40:1 then 30:1) to give **4** (1.81 g, 70%).

Synthesis of 4,6-bis(benzyloxy)-2-hydroxy-3-*C*-β-D-(2,3,4,6-tetrabenzylglucopyranosyl)acetophenone (5a).[3]

C-glucoside **4** (851.3 mg, 1.09 mmol) was dissolved in acetone (11 mL). To this solution, K2CO3 (230.0 mg, 1.67 mmol) and BnBr (0.2 mL, 1.68 mmol) was added and stirred under nitrogen atmosphere at 50 ºC. After 16 hours, The reaction mixture was cooled to r.t., water was added and extracted by EtOAc. Organic layer was washed with brine, dried over Na_2SO_4 and concentrated under vacuo. Residue was purified by silicagel column chromatography (Hexane: EtOAc = 6:1 then 4:1) to give **5a** (648.7 mg, 68%).

Synthesis of 2'-hydroxy-3'-*C*-β-D-(2,3,4,6-tetra-*O*-benzylglucopyranosyl)-4,4',6'-tribenzyloxychalcone (7a).[3]

Acetophenone **5a** (648.7 mg, 0.746 mmol), 4-benzyloxybenzaldehyde **6** (316.2 mg, 1.492 mmol) was dissolved in 1,4-dioxane/EtOH (2 mL/9 mL) and 2M NaOMe in MeOH (4 mL) was added. After stirring the mixture for 44 hours, 1 M HCl aq. was added and the reaction mixture was extracted by EtOAc. Organic layer was washed with brine, dried over Na_2SO_4 and concentrated under vacuo. Residue was purified by silicagel column chromatography (Hexane: EtOAc = 4:1 then 3:1 then 2:1) to give **7a** (476.3 mg, 60%).

Synthesis of 4',7-di-*O*-benzylgenistein 8-C-β-D-(2,3,4,6-tetra-*O*-benzylglucoside) 8a.[3]

Chalcone **7a** (243.7 mg, 0.22 mmol) and thallium(III) nitrate (200.6 mg, 0.45 mmol) was suspended in MeOH (2 mL) and trimethyl orthoformate (2 mL) was added. The mixture was stirred under nitrogen atmosphere for 21 hours at 80 ºC and then cooled to r.t., filtered and dried under vacuo. Residue was dissolved in MeOH (5 mL), 1,4-dioxane (3 mL) and 10 HCl aq. (1 mL) was added. The mixture was refluxed for 20 hours, water was added and extracted by EtOAc. Organic layer was washed with water, brine and dried over Na_2SO_4 and concentrated under vacuo. Residue was purified by silicagel column chromatography (Hexane: EtOAc = 5:1 to 2:1) to give **8a** (153.4 mg, 72%).

Synthesis of 4',7-di-*O*-benzyldaidzein 8-*C*-β-D-(2,3,4,6-tetra-*O*-benzylglucoside) 9a.

Isoflavone **8a** (99.0 mg, 0.10 mmol) was dissolved in dry CH2Cl2 (2 mL) and stirred under nitrogen atmosphere. To the solution, dry pyridine (40 μL, 0.49 mmol) and Tf2O (40 μL, 0.24 mmol) was added. After stirring for an hour, dry pyridine (40 μL, 0.49

mmol) and Tf2O (40 μL, 0.24 mmol) was added to the reaction mixture and the mixture was further stirred for 16 hours. The reaction mixture was diluted with water, extracted by CHCl3. Organic layer was washed with brine, dried over Na_2SO_4 and concentrated under vacuo. Toluene was added to the residue and the residual pyridine was co-evaporated. The residue was dissolved in dry DMF (3 mL) and formic acid (0.6 mL), TEA (1.5 mL), Pd(OAc)2 (44.2 mg, 0.20 mmol), PPh3 (52.0 mg, 0.20 mmol) was added and stirred under nitrogen atmosphere. The mixture was heated at 70 ºC for 12 hours, water was added and extracted by EtOAc. Organic layer was washed with sat. NaHCO3 aq., brine, dried over Na_2SO_4 and concentrated under vacuo. Residue was purified by silicagel column chromatography (Hexane: Acetone = 4:1) to give **9a** (82.5 mg, 86%).

Synthesis of Puerarin (1).[4]

Benzyl protected puerarin **9a** (68.6 mg, 0.0717 mmol) was dissolved in dry CH_2Cl_2 (15 mL) and stirred under nitrogen atmosphere. The mixture was cooled to -78 ºC and 1M BBr_3/CH_2Cl_2 (1.3 mL) was slowly added. After stirring for an hour at r.t., MeOH was added to quench the reaction and the resulting mixture was evaporated to dryness. The residue was purified by LiChrolut (Merck co., H_2O, 20% MeOH aq.) to give **1** (21.3 mg, 71%).

Synthesis of 2,4-bis(benzyloxy)-6-hydroxy-3-*C*-β-D-(2,3,4,6-tetrabenzylglucopyranosyl)acetophenone (5b).

C-glucoside **4** (1.185 g, 1.52 mmol) was dissolved in DMF (15 mL) and imidazole (313.4 mg, 4.60 mmol) was added. To this mixture TBSCl (472.5 mg, 3.13 mmol) was added and stirred under nitrogen atmosphere. After 14 hours, water was added and the resulting mixture was extracted by EtOAc. Organic layer was washed with brine, dried over Na_2SO_4 and concentrated under vacuo. The residue was dissolved in dry THF (15 mL) and BnOH (316 μL, 3.04 mmol), PPh_3 (800.1 mg, 3.05 mmol) was added. The mixture was cooled to 0 ºC and DIAD (0.6 mL, 3.05 mmol) was added and stirred under nitrogen atmosphere. After an hour, 1 M TBAF/THF (1.8 mL) was added and the reaction mixture was further stirred for 30 min. Water was added to the mixture and was extracted by EtOAc. Organic layer was washed with brine, dried over Na_2SO_4 and concentrated under vacuo. Residue was purified by silicagel column chromatography (Hexane: EtOAc = 6:1) to give **5b** (1.138 g, 86%).

Synthesis of

6'-hydroxy-3'-*C*-β-D-(2,3,4,6-tetra-*O*-benzylglucopyranosyl)-2',4',4-tribenzyloxychalcone (7b).

Benzyl protected C-glucoside **5b** (136.2 mg, 0.157 mmol), **6** (66.4 mg, 0.313 mmol) was dissolved in 1,4-dioxane/MeOH (0.5 mL/1.5 mL) and 2M NaOMe in MeOH (1 mL) was added. After stirring the mixture for 26 hours, 2M NaOMe in MeOH (1 mL) was added again. The reaction mixture was further stirred for 18 hours, then 1 M HCl aq. was added and the reaction mixture was extracted by EtOAc. Organic layer was washed with brine, dried over Na_2SO_4 and concentrated under vacuo. Residue was purified by silicagel column chromatography (Hexane: EtOAc = 9:1 then 6:1 then 3:1) to give **7b** (123.9 mg, 74%).

Synthesis of 4',5,7-tri-*O*-benzylgenistein 6-C-β-D-(2,3,4,6-tetra-*O*-benzylglucoside) 8b.

Chalcone **7b** (260.4 mg, 0.245 mmol) and thallium(III) nitrate (220.8 mg, 0.497 mmol) was suspended in MeOH (5 mL) and trimethyl orthoformate (5 mL) was added. The mixture was stirred under nitrogen atmosphere for 20 hours at 40 °C and then cooled to r.t., filtered and dried under vacuo. Residue was dissolved in MeOH (10 mL), 1,4-dioxane (5 mL) and 10% HCl aq. (1 mL) was added. The mixture was refluxed for 6 hours and then water was added and extracted by EtOAc. Organic layer was washed with water, brine and dried over Na_2SO_4 and concentrated under vacuo. Residue was purified by silicagel column chromatography (Hexane: EtOAc = 4:1 to 2:1) to give **8b** (109.3 mg, 42%).

Synthesis of Genistein 6-*C*-β-D-glucoside (10).

Benzyl protected genistein **8b** (39.9 mg, 0.0376 mmol) was dissolved in dry CH_2Cl_2 (7.5 mL) and stirred under nitrogen atmosphere. The mixture was cooled to -78 °C and 1 M BBr_3/CH_2Cl_2 (0.8 mL) was slowly added. After stirring for 5 hours at -78 °C, MeOH was added to quench the reaction and the resulting mixture was evaporated to dryness. The residue was purified by LiChrolut (Merck co., H_2O, 20% MeOH aq., 50% MeOH aq.) to give **10** (8.2 mg, 51%).

^{1}H NMR (500 MHz, DMSO-d6, r.t.): 10.86 (1H, s, OH), 10.06 (1H, s, OH), 9.36 (1H, s, OH), 7.76 (2H, d, J = 8.7 Hz), 6.87 (2H, d, J = 8.7 Hz), 6.58 (1H, s), 6.32 (1H, s), 4.65 (1H, d, J = 9.9 Hz), 3.65 (1H, dd, J = 2.0, 12.0 Hz), 3.61 (1H, dd, J = 9.1, 9.5 Hz) , 3.54 (1H, dd, J = 4.6, 12.0 Hz), 3.3-3.2 (3H, m) ppm; ^{13}C NMR (125 MHz, DMSO-d6, r.t.): 179.7, 165.7, 165.5, 158.9, 156.2, 145.7, 132.9, 123.2, 116.0, 109.7, 107.7, 103.1, 90.9, 81.2, 78.2, 74.3, 71.6, 69.6, 60.5 ppm; HR FD MS(positive): $[M]^+$ Found *m/z* 432.1040, $C_{21}H_{20}O_{10}^+$

requires *m/z* 432.1057.

Synthesis of 2-allyloxy-4-methoxyacetophenone (12).

2,4-dihydroxyacetophenone **11** (1.52 g, 10.0 mmol) and K_2CO_3 (2.78 g, 20.1 mmol) was suspended in dry acetone (80 mL) and MeI (1.2 mL, 19.3 mmol) was added and stirred at 50 ºC for 15 hours under nitrogen atmosphere. Then, the reaction mixture was filtered and evaporated to dry ness. Water and EtOAc was added to the residue and organic layer was separated and aqueous layer was extracted by EtOAc. Organic layer was combined, washed with brine and dried over Na_2SO_4 and concentrated under vacuo. Residue was dissolved in dry acetone (100 mL) and K_2CO_3 (2.76 g, 20.0 mmol), KI (187.5 mg, 1.13 mmol), allyl bromide (1.7 mL, 20.8 mmol) was added. The mixture was refluxed for 15 hours and then water was added and extracted by EtOAc. Organic layer was washed with water, brine and dried over Na_2SO_4 and concentrated under vacuo. Residue was purified by silicagel column chromatography (Hexane: Acetone = 4:1) to give **12** (1.90 g, 92%).

^{1}H NMR (270 MHz, $CDCl_3$, r.t.): 7.82 (1H, d, *J* = 8.7 Hz), 6.50 (1H, dd, *J* = 2.3, 8.7 Hz), 6.43 (1H, d, *J* = 2.3 Hz), 6.08 (1H, ddt, *J* = 5.5, 10.4, 17.3 Hz), 5.44 (1H, br dd, *J* = 1.2, 17.3 Hz), 5.32 (1H, br dd, *J* = 1.2, 10.4 Hz), 4.60 (2H, br d, *J* = 5.5 Hz), 3.82 (3H, s), 2.59 (3H, s) ppm; ^{13}C NMR (67.5 MHz, $CDCl_3$, r.t.): 197.5, 164.3, 160.0, 132.5, 132.4, 121.2, 118.2, 105.3, 99.2, 69.3, 55.4, 32.0 ppm; HR FD MS(positive): $[M]^+$ Found *m/z* 206.0936, $C_{12}H_{14}O_3^+$ requires *m/z* 206.0943.

Synthesis of 3-allyl-2-hydroxy-4-methoxyacetophenone (13).

Compound **12** (1.90 g, 9.22 mmol) was dissolved in N,N-dimethylaniline (50 mL) and stirred at 210 ºC for 4 hours. The reaction mixture was cooled to r.t. and 3 M HCl aq. was added and extracted by EtOAc. Organic layer was washed with 3 M HCl aq., brine and dried over Na2SO4 and concentrated under vacuo. Residue was purified by silicagel column chromatography (Hexane: EtOAc = 9:1 then 8:1 then 4:1) to give **13** (1.30 g, 69%).

^{1}H NMR (270 MHz, $CDCl_3$, r.t.): 12.78 (1H, s, OH), 7.63 (1H, d, *J* = 8.9 Hz), 6.46 (1H, d, *J* = 8.9 Hz), 5.95 (1H, tdd, *J* = 6.3, 10.2, 17.1 Hz), 5.00 (1H, br d, *J* = 17.1 Hz), 4.96 (1H, br d, *J* = 10.2 Hz), 3.88 (3H, s), 3.41 (2H, d, *J* = 6.3 Hz), 2.55 (3H, s) ppm; ^{13}C NMR (67.5 MHz, $CDCl_3$, r.t.): 202.9, 163.3, 161.8, 135.8, 130.6, 115.3, 114.4, 114.3, 102.0, 55.8, 26.5, 26.2 ppm; HR FD MS(positive): $[M]^+$ Found *m/z* 206.0916, $C_{12}H_{14}O_3^+$ requires *m/z* 206.0943.

Synthesis of 3-(2',3'-isopropylidenedioxypropyl)-2-hydroxy-4-methoxyacetophenone (14). Compound **13** (0.91 g, 4.42 mmol) was dissolved in MeCN (30 mL) and H_2O (15 mL). To this solution, NMO (1.03 g, 8.79 mmol) and 4% OsO4 aq. (280 μL) was added and stirred for 18 hours. Sat. $Na_2S_2O_3$ aq. was added to the reaction mixture and extracted by EtOAc. Organic layer was washed with 1 M HCl aq., brine, dried over Na_2SO_4 and concentrated under vacuo. The residue was dissolved in DMF (40 mL) and 2,2-dimethoxypropane (1.0 mL, 8.16 mmol), TsOH (38.5 mg, 0.22 mmol) was added. The mixture was reacted at 35 ºC under slightly reduced pressure for two hours. Then water was added and the mixture was extracted by Hexane/EtOAc=1/1. Organic layer was washed with brine and dried over Na_2SO_4 and concentrated under vacuo. Residue was purified by silicagel column chromatography (Hexane: Acetone = 4:1 then 2:1) to give **14** (1.11 g, 90%).

^{1}H NMR (270 MHz, $CDCl_3$, r.t.): 12.80 (1H, s, OH), 7.65 (1H, d, *J* = 8.9 Hz), 6.47 (1H, d, *J* = 8.9 Hz), 4.35 (1H, dddd, *J* = 4.1, 5.1, 6.0, 6.6 Hz), 3.89 (3H, s), 3.88 (1H, dd, *J* = 6.0, 8.0 Hz), 3.78 (1H, dd, *J* = 6.6, 8.0 Hz), 3.09 (1H, dd, *J* = 5.1, 12.9 Hz), 2.93 (1H, dd, *J* = 4.1, 12.9 Hz), 2.57 (3H, s) , 1.46 (3H, s) , 1.35 (3H, s) ppm; ^{13}C NMR (67.5 MHz, $CDCl_3$, r.t.): 202.9, 163.7, 162.3, 131.0, 114.3, 113.0, 108.6, 102.0, 74.9, 69.2, 55.7, 26.95, 26.85, 26.2, 25.8 ppm; HR FD MS(positive): $[M]^+$ Found *m/z* 280.1294, $C_{12}5_{20}O_5^+$ requires *m/z* 280.1311.

Synthesis of 4-benzyloxy-2'-hydroxy-3'-(2",3"-isopropylidenedioxypropyl)-4'-methoxychalcone (15). Compound **14** (605.4 mg, 2.16 mmol), **6** (514.0 mg, 2.42 mmol) was dissolved in MeOH (10 mL) and cooled to 0 ºC. To this solution 2M NaOMe in MeOH (10 mL) was added and stirred for 21 hours at 60 ºC. To this reaction mixture, 1 M HCl aq. was added and extracted by EtOAc. Organic layer was washed with brine, dried over Na_2SO_4 and concentrated under vacuo. Residue was purified by silicagel column chromatography (Hexane: Acetone = 8:1 then 4:1) to give **15** (805.3 mg, 79%).

^{1}H NMR (270 MHz, $CDCl_3$, r.t.): 13.54 (1H, s, OH), 7.86 (1H, d, *J* = 15.3 Hz), 7.83 (1H, d, *J* = 9.1 Hz), 7.61 (2H, d, *J* = 8.9 Hz), 7.47 (1H, d, *J* = 15.3 Hz), 7.44-7.34 (5H, m), 7.02 (1H, d, *J* = 8.9 Hz), 6.50 (1H, d, *J* = 9.1 Hz), 5.12 (2H, s), 4.38 (1H, dddd, *J* = 4.1, 5.1, 6.0, 6.6 Hz), 3.91 (3H, s), 3.89 (1H, dd, *J* = 1.7, 8.2 Hz), 3.81 (1H, dd, *J* = 6.5, 8.2 Hz), 3.12 (1H, dd, *J* = 5.1, 12.8 Hz), 2.95 (1H, dd, *J* = 8.6, 12.8 Hz), 1.48 (3H, s) , 1.36 (3H, s) ppm; ^{13}C NMR (67.5 MHz, $CDCl_3$, r.t.): 192.2, 163.7, 163.6, 160.9, 144.2, 136.3, 130.3, 129.9, 128.7, 128.2, 127.7, 127.5, 118.0, 115.3, 114.6, 113.3, 108.7, 101.9, 75.0, 70.1, 69.3, 55.7, 27.00, 26.98, 25.8 ppm; HR FD MS(positive): $[M]^+$ Found

m/z 474.2027, $C_{29}H_{30}O_6^+$ requires *m/z* 474.2042.

Synthesis of 4'-*O*-benzyl-8-(2",3"-isopropylidenedioxypropyl)-7-*O*-methyldaidzein (16).
Chalcone **15** (805.3 mg, 1.70 mmol) and thallium(III) nitrate (1.56 g, 3.51 mmol) was suspended in MeOH (15 mL) and trimethyl orthoformate (15 mL). The mixture was stirred under nitrogen atmosphere for 17 hours at 40 ºC and then cooled to r.t., filtered and dried under vacuo. Residue was dissolved in MeOH (50 mL), 1,4-dioxane (25 mL) and 10% HCl aq. (5 mL). The mixture was stirred for 24 hours at 80 ºC and then water was added and extracted by EtOAc. Organic layer was washed with water, brine and dried over Na_2SO_4 and concentrated under vacuo. Residue was dissolved in dry DMF (15 mL) and 2,2-dimethoxypropane (0.6 mL, 4.90 mmol), TsOH (15.9 mg, 0.092 mmol) was added. The mixture was reacted at 35 ºC under slightly reduced pressure for two hours. Then sat. NH_4Cl aq. was added and the mixture was extracted by EtOAc. Organic layer was washed with brine and dried over Na_2SO_4 and concentrated under vacuo. Residue was purified by silicagel column chromatography ($CHCl_3$: EtOAc = 9:1 then 2:1) to give **16** (690.3 mg, 86%).

^{1}H NMR (270 MHz, $CDCl_3$, r.t.): 8.21 (1H, d, *J* = 8.9 Hz), 7.98 (1H, s), 7.50 (2H, d, *J* = 8.8 Hz), 7.47-7.32 (5H, m), 7.04 (2H, d, *J* = 8.8 Hz), 7.02 (1H, d, *J* = 8.9 Hz), 5.09 (2H, s), 4.41 (1H, dddd, *J* = 5.6, 6.1, 6.2, 7.9 Hz), 3.96 (1H, dd, *J* = 6.1, 8.3 Hz), 3.95 (3H, s), 3.78 (1H, dd, *J* = 6.2, 8.3 Hz), 3.27 (1H, dd, *J* = 5.6, 13,0 Hz), 3.09 (1H, dd, *J* = 7.9, 13.0 Hz), 1.47 (3H, s) , 1.35 (3H, s) ppm; ^{13}C NMR (67.5 MHz, $CDCl_3$, r.t.): 176.2, 161.4, 158.7, 155.5, 152.2, 136.9, 130.1, 128.6, 127.9, 127.4, 126.3, 124.4, 124.2, 118.5, 114.9, 113.4, 109.0, 108.9, 75.0, 70.0, 69.1, 56.1, 27.6, 27.0, 25.7 ppm; HR FD MS(positive): $[M]^+$ Found *m/z* 472.1854, $C_{29}H_{28}O_6^+$ requires *m/z* 472.1886.

Synthesis of 8-(2,3-dihydroxypropane)Daidzein (17).
Compound **16** (69.0 mg, 0.144 mmol) was dissolved in dry CH_2Cl_2 (7.5 mL) and stirred under nitrogen atmosphere. The mixture was cooled to -78 ºC and 1 M BBr_3/CH_2Cl_2 (0.6 mL) was slowly added. After stirring for 22 hours at -78 ºC, MeOH was added to quench the reaction and the resulting mixture was evaporated to dryness. The residue was dissolved indry DMF(2 mL) and NaSEt (21.2 mg, 0.252 mmol) was added. The mixture was stirred at 110 ºC for 4 hours, cooled to r.t. and AcOH was added. The reaction mixture was evaporated to dryness and purified by preparative TLC ($CHCl_3$: MeOH = 5:1) to give **17** (36.8 mg, 78%).

^{1}H NMR (270 MHz, CD_3OD, r.t.): 8.19 (1H, s), 7.96 (1H, d, *J* = 8.8 Hz), 7.37 (2H, d, *J* = 8.8 Hz),

6.97 (1H, d, J = 8.8 Hz), 6.84 (2H, d, J = 8.8 Hz), 3.99 (1H, tt, J = 6.0, 6.2 Hz), 3.53 (2H, d, J = 6.0 Hz), 3.06 (2H, d, J = 6.2 Hz) ppm; ^{13}C NMR (67.5 MHz, CD_3OD, r.t.): 178.6, 162.4, 158.7, 157.9, 154.7, 131.4, 126.1, 125.5, 124.3, 118.4, 116.2, 115.6, 114.0, 73.0, 67.1, 28.3 ppm; HR FD MS(positive): $[M]^+$ Found m/z 328.0913, $C_{18}H_{16}O_6^+$ requires m/z 328.0947

Synthesis of genistein 8-*C*-β-D-glucoside (18).[5]

Compound **8a** (63.5 mg, 0.0653 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and stirred under nitrogen atmosphere. The mixture was cooled to -78 ºC and 1M BBr_3/CH_2Cl_2 (1.2 mL) was slowly added. After stirring for three hours at r.t., MeOH was added to quench the reaction and the resulting mixture was evaporated to dryness. The residue was purified by LiChrolut (Merck co., stepwise elution H_2O, 20% MeOH aq., 40% MeOH aq.) to give **18** (17.8 mg, 63%).

Synthesis of 7-*O*-methylpuerarin (22).

Puerarin **1** (18.8 mg, 0.045 mmol) was dissolved in DMF (0.5 mL) and KO^{t}Bu (5.2 mg, 0.046 mmol) was added and stirred under nitrogen atmosphere. To this mixture, MeI/DMF (1/10, 28 μL, 0.045 mmol) was added and stirred for 18 hours. The reaction mixture was acidified by AcOH and evaporated to dryness. The residue was purified by PTLC (MeOH: $CHCl_3$ = 1:3) to give **22** (9.7 mg, 50%).

^{1}H NMR (270 MHz, D_2O, 60 ºC): 8.17 (1H, s), 8.15 (1H, d, J = 9.4 Hz), 7.32 (2H, d, J = 8.5 Hz), 7.25 (1H, d, J = 9.4 Hz), 6.94 (2H, d, J = 8.5 Hz), 5.14 (1H, d, J = 9.6 Hz), 3.98 (3H, s), 3.93 (1H, br d, J = 12.3 Hz), 3.79 (1H, dd, J = 4.2, 12.3 Hz), 3.65-3.60 (4H, m) ppm; ^{13}C NMR (67.5 MHz, D_2O, 60 ºC): 178.8, 163.6, 156.5, 156.2, 155.0, 131.0, 128.6, 124.1, 123.7, 118.0, 116.0, 112.9, 111.8, 81.1, 78.5, 73.6, 71.7, 70.7, 61.8, 57.2 ppm; HR FD MS(positive): $[M]^+$ Found m/z 430.1290, $C_{22}H_{22}O_9^+$ requires m/z 430.1264.

Synthesis of 4',7-di-*O*-methylpuerarin (23).

Puerarin **1** (8.0 mg, 0.019 mmol) was dissolved in DMF (0.5 mL) and KO^{t}Bu (5.7 mg, 0.051 mmol) was added and stirred under nitrogen atmosphere. To this mixture, MeI/DMF (1/9, 26.5 μL, 0.043 mmol) was added and stirred for 6 hours. The reaction mixture was acidified by AcOH and evaporated to dryness. The residue was purified by PTLC (MeOH: $CHCl_3$ = 1:3) to give **23** (3.9 mg, 46%).

^{1}H NMR (270 MHz, DMSO-d6, 60 ºC): 8.35 (1H, s), 8.11 (1H, d, J = 8.9 Hz), 7.54 (2H, d, J = 8.8 Hz), 7.24 (1H, d, J = 8.9 Hz), 6.98 (2H, d, J = 8.8 Hz), 4.86 (1H, d, J = 9.7 Hz), 4.10 (1H, dd, J =

7.8, 9.7 Hz), 3.93 (3H, s), 3.80 (3H, s), 3.73 (1H, br d, J = 11.5 Hz), 3.47 (1H, dd, J = 4.7, 11.5 Hz), 3.34-3.23 (3H, m) ppm; HR FD MS(positive): $[M]^+$ Found *m/z* 444.1440, $C_{23}H_{24}O_9^+$ requires *m/z* 444.1420.

Synthesis of compound 24.

Compound **8a** (35.3 mg, 0.036 mmol) was dissolved in EtOAc (0.5 mL) and MeOH (0.5 mL) and $Pd(OH)_2$ was added and stirred under hydrogen atmosphere. After 14 hours the reaction mixture was filtered thru celite pad and concentrated under vacuo. The residue was purified by PTLC ($CHCl_3$: MeOH = 3:1) to give **24** (16.2 mg, quant.).
Mixture of stereoisomer
HR FD MS(positive): $[M]^+$ Found *m/z* 434.1211, $C_{21}H_{22}O_{10}^+$ requires *m/z* 434.1213.

Synthesis of apigenin 7-β-D-glucoside (25).[6]

Compound **7a** (45.3 mg, 0.041 mmol) was dissolved in dry DMSO (1 mL) and iodine (40 mg/mL in DMSO, 20 μL) was added. The solution was stirred under nitrogen atmosphere at 110 ºC for 40 hours. The reaction mixture was cooled to room temperature and 1 M HCl aq. was added and extracted by ethyl acetate. Organic layer was washed with brine, dried over sodium sulfate and evaporated. The residue was purified by PTLC (Hexane: Acetone = 3:2) to give benzyl protected apigenin 7-O-glucoside (29.2 mg, 65%). The product (19.8 mg, 0.0179 mmol) was then dissolved in methanol (2.5 mL) and 1,4-dioxane (0.5 mL). Pd(OH)2 was added to the solution and the mixture was stirred for an hour under hydrogen atmosphere. The reaction mixture was filtered through celite pad and evaporated. The residue was purified by HPLC (0.1% TFA, 40% MeOH aq.) employing Inertsil ODS-3 (GL science co.) as a column to give **25** (5.0 mg, 65%).

References

1. Lewis, P. T.; Wähälä, *Tetrahedron Lett.* **1998**, *39*, 9559.
2. (a) Schmidt, R. R.; Stumpp, M. *Liebigs Ann. Chem.* **1983**, 1249.
(b) Mahling, J. -A.; Schmidt, R. R. *Synthesis* **1993**, 325.
3. Sato, S.; Hiroe, K.; Kumazawa, T.; Onodera, J., *Carbohydr. Res.* **2006**, *341*, 1091.
4. Lee, D. Y. W.; Zhang W.; Karnati, V. V. R., *Tetrahedron Lett.* **2003**, *44*, 6857.
5. Sato, S.; Hiroe, K.; Kumazawa, T.; Onodera, J., *Carbohydr. Res.* **2006**, *341*, 1091.
6. Ishida, H.; Wakimoto, T.; Kitao, Y.; Tanaka, S.; Miyase, T.; Nukaya, H., *J. Agric. Food Chem.* **2009**, *57*, 6779.