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Synthesis of fisetin and 2′,4′,6′-trihydroxydihyrochalcone 4′-*O*-β-neohesperidoside based on site-selective deacetylation and deoxygenation

Ryuji Tsunekawa, Kengo Hanaya, Shuhei Higashibayashi and Takeshi Sugai

Faculty of Pharmacy, Keio University, Tokyo, Japan

ABSTRACT

Fisetin and 2′,4′,6′-trihydroxydihyrochalcone 4′-*O*-β-neohesperidoside were synthesized from commercially available quercetin and naringin in five steps. The key steps are site-selective deacetylation and subsequent deoxygenation. The target molecules were obtained in 37% and 23% yields from the starting materials, respectively.

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Fisetin; dihydrochalcone glycoside; site-selective deacetylation; deoxygenation; triflate

Fisetin (**1a**, Figure 1) is an antioxidative flavone first isolated in the nineteenth century [1] and is found in strawberries at high concentration [2,3]. Intensive biological studies have disclosed the neuroprotective effect of **1a** towards important disease complications caused by diabetes mellitus and Alzheimer's and Parkinson's diseases [2–6]. 2′,4′,6′-Trihydroxydihydrochalcone (**2**, Figure 1) has been detected in *Uvaria chamae* [7], *Lindera umbellata* [8], *Piper histidum* [9], *Kaempferia parviflora* and *Boesenbergia pandurata* [10,11]. Several biological activities of **2** have been reported, such as an anti-inflammatory effect [11] and inhibitory effects on tyrosinase [12] and sodium glucose co-transporter 2 [13]. Recently, an enhanced tyrosinase inhibitory effect was reported, following the glycosylation of resveratrol (**3**) [14], which has structural similarity to **2**. Such an approach prompted us to focus on the synthesis of a new compound **4a**, the glycosylated form of **2**.

Target molecules **1a** and **4a** are the deoxygenated forms of quercetin (**5a**) at the C-5 and of naringin dihydrochalcone (**6**) [15] at the C-4″ position, respectively. Several efforts to chemically synthesize fisetin and related compounds based on representative synthetic methods for 3-hydroxyflavones have been reported [16–21]. However, apart from the library-oriented syntheses, we envisaged the specific synthesis of **1a** itself, starting from commercially available **5a**, which originates from naturally abundant rutin. The key steps would be site-selective liberation of a hydroxy group and palladium-catalyzed hydrogenolysis of the corresponding trifluoromethylsulfonate (triflate). In a similar way, we anticipated achieving the synthesis of **4a** from naturally abundant naringin (**7**) *via* a deoxygenation step.

Results and discussion

According to a reported procedure [22], we acetylated **5a** with a controlled amount of acetylating agent. The product was a mixture of the desired tetraacetate **5b** with a free hydroxy group at C-5 and pentaacetate **5c**. The difference in chromatographic behavior between **5b** and **5c** was small (see experimental) and both products were highly crystalline. Consequently, neither chromatographic separation nor fractional crystallization was successful for large-scale preparation of **5b**. We therefore prepared **5c** from **5a** in 82% yield and tried Lewis acid-catalyzed site-selective deacetylation [23] at C-5 in **5c**. Deacetylation was efficient (99% yield) and the desired **5b** was isolated in 81% yield from **5a** in two steps.

The hydroxy group at C-5 is less nucleophilic due to an intramolecular hydrogen bonding with the carbonyl group at C-4, and was trifluoromethylsulfonylated at room temperature. The resulting triflate **5d** was highly crystalline and precipitated in the reaction mixture during the progress of triflation, and thus the isolation of **5d** was facile, requiring only filtration. The filtrate contained residual **5d**, which was contaminated with unreacted **5b**. The chromatographic separation of **5b** and **5d** was tedious, due to the proximity in polarity and crystalline properties of both compounds. However, triflate **5d** was stable even at high temperature and thus the mixture was heated with acetic anhydride and pyridine at 85 °C, converting the contaminating **5b** to the pentaaceate **5c**. The chromatographic separation of **5d** and **5c** was easier than the separation of **5d** and **5b** because **5c** is more polar than the desired **5d**, resulting in a larger difference

CONTACT Takeshi Sugai sugai-tk@pha.keio.ac.jp

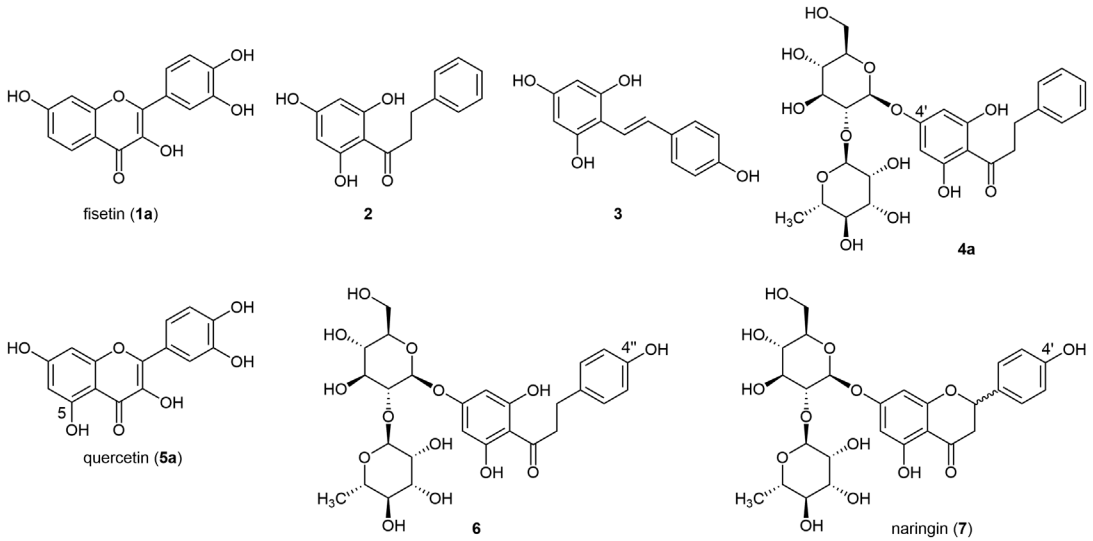
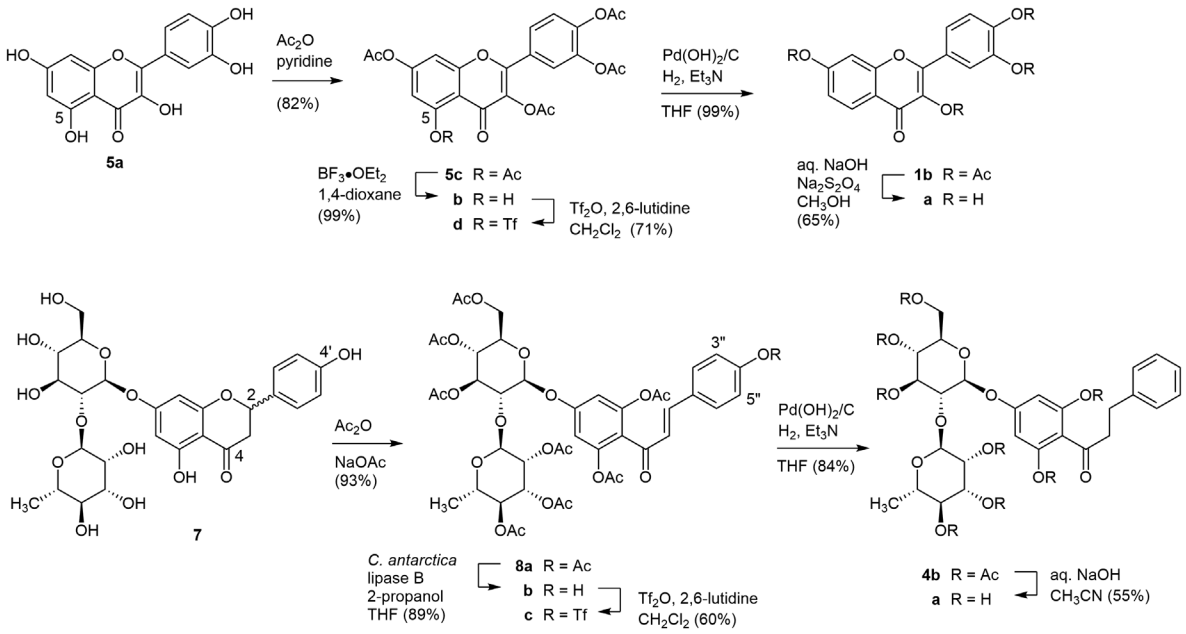


Figure 1. Structures of fisetin (**1a**), 2′,4′,6′-trihydroxydihyrochalcone 4′-*O*-β-neohesperidoside (**4a**), and related compounds.



Scheme 1. Synthesis of fisetin (**1a**) and 2′,4,′6′-trihydroxydihyrochalcone 4′-*O*-β-neohesperidoside (**4a**) from quercetin (**5a**) and naringin (**7**), respectively.

in Rf values (see experimental). The combined yield of **5d** following precipitation and chromatographic separation was 71%.

Palladium hydroxide-catalyzed hydrogenolysis of the C-O bond [24] in triflate **5d** was performed under basic conditions with triethylamine. The choice of tetrahydrofuran (THF) as a solvent was important. The desired tetraacetate **1b** precipitated during the hydrogenolysis reaction, preventing over-reduction, and the desired tetraacetate **1b** was obtained in 84% yield. The use of palladium on carbon as a catalyst resulted in a slower reaction compared to the use of palladium hydroxide. In such a case, the prolonged reaction time caused undesired partial deacetylation, providing a complex mixture. In the step of the final alkaline hydrolysis of four acetate esters, polar byproduct formation, probably due to the oxidative degradation of catechol moiety of **1a**, was detected by thin layer chromatography (TLC) analysis. The addition of sodium dithionite [25] could suppress such an undesired reaction to certain extent, to give **1a** in 65% isolated yield (Scheme 1).

Our initial attempt to synthesize **4a** involving simultaneous hydrogenolytic cleavage of the C-O bonds at C-2 and C-4′ on C-4′ triflate of **7** was unsuccessful and thus we chose the chalcone-type precursors **8**. Acetylation of the hydroxy groups in **7** at elevated temperature [26,27] was accompanied with the desired β-elimination to furnish the peracetylated chalcone **8a** in 93%

yield. The lipase-catalyzed site-selective deacetylation of a 4"-hydroxychalcone derivative **8a** to **8b** (89%) was the first achievement, other than the related examples in flavonoids [28,29] and stilbenoids [25,29]. The position of deacetylation was confirmed by the upfield shift of the signals of the protons at C-3″ and C-5″, from δ 7.13 in **8a** to δ 6.82 in **8b**. Triflation of **8b** under similar conditions as used for **5b** furnished **8c** in 60% yield. Simultaneous palladium-catalyzed hydrogenolysis of the phenolic C-O bond and hydrogenation of the C-C double bond in the chalcone proceeded smoothly to give the protected form of dihydrochalcone **4b** in 84% yield. Finally, deprotection of the eight acetyl groups was performed by alkaline hydrolysis. The desired product **4a** could be separated from the unidentified highly polar byproducts in 55% isolated yield. (Scheme 1).

Conclusion

We accomplished the synthesis of **1a** and **4a**. The total yield of **1a** was 37% from **5a** and of **4a** from **7** was 23%. In both cases, starting materials are of plant origin, a sustainable resource. Moreover, as few as five steps were required using site-selective transformations among multiple acetyl protecting groups and subsequent deoxygenation. Deprotection at the final stages under non-acidic conditions would be advantageous, to apply the presently developed approaches for the synthesis of various glycosylated forms.

Experimental

General

Candida antarctica lipase B (Novozym 435) was purchased from Novozymes Japan. Quercetin (P0042) and naringin (N0073) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Column chromatography was performed with silica gel (Kanto Chemical Co. Silica Gel 60 N 37563-84, spherical and neutral, 40-50 μm). Preparative TLC was performed with Merck Silica Gel 60 RP-18 F_{254}s plates (1 mm thickness, No. 1.05434.0001). Melting points were measured on a METTLER TOLEDO MP 70 and are uncorrected. ^{1}H NMR spectra were measured at 400 MHz on a VARIAN 400-MR or at 500 MHz on an Agilent INOVA-500 spectrometer and ^{13}C NMR spectra were measured at 125 MHz on an Agilent INOVA-500 spectrometer. DMSO-d_6 and $CDCl_3$ were used as a solvent and the residual peaks were used as an internal standard (^{1}H NMR: DMSO-d_6 2.48 ppm, $CDCl_3$ 7.26 ppm; ^{13}C NMR: DMSO-d_6 39.9 ppm, $CDCl_3$ 77.0 ppm). IR spectra were measured as ATR on a Jasco FT/IR-4700 FT-IR spectrometer. Optical rotation values were measured with a Jasco P-1010 polarimeter. High resolution mass spectra (HRMS) were measured by a on Jeol JMS-T100LP AccuTOF.

3,3′,4′,5,7-Pentaacetoxyflavone (5c)

To a solution of **5a** (quercetin, 2.90 g, 9.60 mmol) in pyridine (10 mL) was added an acetic anhydride (7.5 mL) and the mixture was stirred for 3 h at 80 °C. The reaction was poured into ice. The precipitates were collected by filtration and washed with ethanol, ethyl acetate and diethyl ether and then dried *in vacuo* to give **5c** [22] as a colorless solid (4.05 g, 82%). ^{1}H-NMR (500 MHz, $CDCl_3$) *δ*: 2.33 (3H, s), 2.34 (3H, s), 2.34 (3H, s), 2.35 (3H, s), 2.43 (3H, s), 6.87 (1H, d, *J* = 2.2 Hz), 7.33 (1H, d, *J* = 2.2 Hz), 7.35 (1H, d, *J* = 8.5 Hz), 7.68 (1H, d, *J* = 2.2 Hz), 7.72 (1H, dd, *J* = 2.2, 8.5 Hz). This was employed for the next step without further purification.

3,3′,4′,7-Tetraacetoxy-5-hydroxyflavone (5b)

To a solution of **5c** (4.05 g, 7.90 mmol) in 1,4-dioxane (42 mL) were added BF_3·OEt_2 (2.1 mL, 16.7 mmol) and the mixture was stirred for 6 h at 80 °C. The reaction was quenched with phosphate buffer solution (0.1 M, pH 7.0). The precipitates were collected by filtration and washed with water and then dried *in vacuo* to give **5b** [22] as a yellow solid (3.71 g, 99%). ^{1}H-NMR (500 MHz, $CDCl_3$) *δ*: 2.33 (3H, s), 2.34 (3H, s), 2.34 (3H, s), 2.37 (3H, s), 6.60 (1H, d, *J* = 2.0 Hz), 6.85 (1H, d, *J* = 2.0 Hz), 7.36 (1H, d, *J* = 8.6 Hz), 7.73 (1H, d, *J* = 1.9 Hz), 7.75 (1H, dd, *J* = 1.9, 8.6 Hz) 12.10 (1H, s). This was employed for the next step without further purification.

3,3′,4′,7-Tetraacetoxy-5-trifluoromethylsufonyloxy flavone (5d)

To a solution of **5b** (4.28 g, 9.11 mmol) in dichloromethane (25 mL) were added 2,6-lutidine (3.21 mL, 27.9 mmol) and trifluoromethanesulfonic anhydride (5.14 mL, 31.3 mmol) at 0 °C under argon atmosphere, and the mixture was stirred for 2 h at room temperature. The reaction was quenched with phosphate buffer solution (0.1 M, pH 7.0) and the mixture was poured into diethyl ether. The precipitates were collected by filtration and washed with methanol and diethyl ether and then dried *in vacuo* to give **5d** (2.91 g, 53%).

The combined mother liquor and washings were concentrated *in vacuo* and the residue was diluted with chloroform. The organic layer was washed with hydrochloric acid (1 M) and brine, and was concentrated *in vacuo*. To the residue was added acetic anhydride (5 mL) and pyridine (5 mL), and the mixture was stirred for 2 h at 85 °C. Then the mixture was poured into ice, and the precipitates were collected by filtration and washed with water. The solid was dissolved in chloroform. The organic solution was washed with sodium hydrogen carbonate solution and brine, dried over anhydrous sodium sulfate and concentrated *in vacuo.* The residue was purified by silica gel column chromatography (15 g). Elution with chloroform-ethyl acetate (3:1) furnished **5d** as a

colorless solid (990 mg, 18%). The combined yield of **5d** was 71%. By further elution with chloroform-ethyl acetate (1:1), **5c** was recovered (476 mg, 10%) as a colorless solid. Rf values: **5c**: 0.33; **5b**: 0.50; **5d**: 0.60 [developed by chloroform-ethyl acetate (4:1)].

An analytical sample of **5d** was obtained as colorless fine needles by recrystallization from chloroform-diethyl ether (1:1). M.p. 192.3–192.8 °C. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.34 (3H, s), 2.34 (3H, s), 2.36 (3H, s), 2.38 (3H, s), 7.08 (1H, d, J = 2.0 Hz), 7.37 (1H, d, J = 8.5 Hz), 7.51 (1H, d, J = 2.0 Hz), 7.73 (1H, d, J = 2.2 Hz), 7.75 (1H, dd, J = 2.2, 8.5 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 20.5, 20.6, 20.6, 21.1, 111.7, 113.8, 115.1, 118.7 (q, J = 321 Hz), 123.9, 124.1, 126.4, 127.2, 134.2, 142.3, 144.7, 147.3, 153.9, 154.1, 156.6, 167.6, 167.6, 167.7, 167.8, 169.3. IR ν_{max} cm^{-1}: 3102, 2942, 1779, 1653, 1426, 1166, 1011. HR-MS [ESI+, (M + Na)$^+$]: calculated for $C_{24}H_{17}F_3NaO_{13}S$, 625.0240; found, 625.0246.

3,3′,4′,7-Tetraacetoxyflavone (1b)

To a solution of **5d** (239 mg, 0.397 mmol) in THF (20 mL) were added palladium hydroxide (20% on carbon, 23.1 mg, 32.9 μmol) and triethylamine (110 μL, 0.789 mmol) and the mixture was stirred under hydrogen atmosphere for 1 h at room temperature. The grey-colored mixture was filtered to recover insoluble materials with a pad of Celite. The precipitates were then washed well with chloroform to dissolve **1b**, and the combined filtrate and washings were concentrated *in vacuo*. The residue was purified by silica gel column chromatography (15 g). Elution with chloroform-methanol (20:1) furnished **1b** (178 mg, 99%). An analytical sample of **1b** was obtained as colorless fine needles by recrystallization from chloroform-diisopropyl ether (10:1). M.p. 206.6–207.3 °C. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.33 (3H, s), 2.34 (3H, s), 2.36 (3H, s), 2.38 (3H, s), 7.19 (1H, dd, J = 2.2, 8.8 Hz) 7.36 (1H, d, J = 8.5 Hz), 7.40 (1H, d, J = 2.2 Hz), 7.74 (1H, d, J = 2.0 Hz), 7.77 (1H, dd, J = 2.0, 8.5 Hz), 8.26 (1H, d, J = 8.8 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 20.5, 20.7, 20.7, 21.2, 111.0, 119.7, 121.3, 123.9, 124.0, 126.5, 127.5, 128.1, 134.0, 142.2, 144.3, 154.7, 154.9, 155.9, 167.8, 167.9, 168.0, 168.4, 171.4. IR ν_{max} cm^{-1}: 3089, 2937, 1766, 1651, 1619, 1368, 1167, 1010. HR-MS [ESI+, (M + Na)$^+$]: calculated for $C_{23}H_{18}NaO_{10}$, 477.0798; found, 477.0806.

Fisetin (1a)

To a solution of **1b** (54.9 mg, 0.121 mmol) in a mixture of water (8 mL) and methanol (5 mL), were added sodium dithionite (854 mg, 4.90 mmol) and aqueous solution of sodium hydroxide (4.0 M, 910 μL, 3.64 mmol) at room temperature under argon atmosphere, and the mixture was stirred for 4 h at room temperature. The reaction was quenched with aqueous solution of ammonium chloride and the organic materials were extracted twice with ethyl acetate. The combined extract was dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (3 g). Elution with chloroform-methanol (5:1) furnished **1a** as a yellow solid (22.6 mg, 65%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 6.85-6.89 (3H, m), 7.52 (1H, dd, J = 2.2, 8.5 Hz), 7.66 (1H, d, J = 2.2 Hz), 7.90 (1H, d, J = 9.3 Hz), 9.02 (1H, s), 9.25 (1H, s), 9.49 (1H, s), 10.72 (1H, s). ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 102.2, 114.6, 115.1, 115.3, 116.0, 120.0, 122.9, 126.9, 137.6, 145.5, 145.5, 147.7, 156.7, 162.7, 172.4. Its NMR data were identical with those of an authentic sample (Tokyo Chemical Industry Co., Ltd., Catalog No. T0121). IR ν_{max} cm^{-1}: 3258, 1511, 1439, 1334, 1272, 1166, 1097. HR-MS [ESI+, (M + Na)$^+$]: calculated for $C_{15}H_{10}NaO_6$, 309.0375; found, 309.0348.

2′,4″,6′-Triacetoxy -4′-[hexa-O-acetyl-(2-O-α-L-rhamnopyranosyl-β-D-glucopyranosyl)oxy] chalcone (8a)

To a suspension of **7** (naringin, 0.986 g,1.70 mmol) in acetic anhydride (20 mL) was added a sodium acetate (8.91 g, 108 mmol) and the mixture was stirred for 3 h at 160 °C. The mixture was diluted with water and the organic materials were extracted with dichloromethane. The extract was washed with aqueous solution of sodium bicarbonate and brine, dried over anhydrous sodium sulfate and concentrated *in vacuo* to give **8a** (1.52 g, 93%) as a yellow amorphous solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.21 (3H, d, J = 6.3 Hz), 1.98 (3H, s), 2.03 (3H, s), 2.04 (3H, s), 2.07 (3H, s), 2.12 (3H, s), 2.14 (6H, s), 2.15 (3H, s), 2.31 (3H, s), 3.89 (1H, ddd, J = 2.3, 5.7, 9.8 Hz), 3.99 (1H, dd, J = 7.6, 9.4 Hz), 4.07 (1H, dq, J = 6.3, 9.8 Hz), 4.14 (1H, dd, J = 2.3, 12.3 Hz), 4.26 (1H, dd, J = 5.7, 12.3 Hz), 5.01-5.07 (4H, m), 5.14 (1H, d, J = 7.6 Hz), 5.21 (1H, dd, J = 3.3, 10.2 Hz), 5.33 (1H, dd, J = 9.4, 9.4 Hz), 6.76 (2H, s) 6.86 (1H, d, J = 16.2 Hz), 7.13 (2H, d, J = 8.6 Hz), 7.40 (1H, d, J = 16.2 Hz), 7.55 (2H, d, J = 8.6 Hz). This was employed for the next step without further purification.

2′,6′-Diacetoxy-4′-[hexa-O-acetyl-(2-O-α-L-rhamnopyranosyl-β-D-glucopyranosyl)oxy]-4″-hydroxychalcone (8b)

To a solution of **8a** (1.52 g,1.58 mmol) in a mixture of 2-propanol (8 mL) and THF (16 mL), which was predried over anhydrous sodium sulfate at room temperature overnight, was added an immobilized form of *C. antarctica* lipase B (Novozymes, Novozym 435, 855 mg). The mixture was stirred for 23 h at 50 °C. The mixture was filtered to remove insoluble materials with a pad of Celite. The precipitates were washed with ethyl acetate. The combined filtrate and washings were concentrated *in vacuo*. The residue was purified by silica gel column

chromatography (30 g). Elution with dichloromethane-ethyl acetate (2:1) furnished **8b** as a yellow amorphous solid (1.30 g, 89%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.21 (3H, d, J = 6.3 Hz), 1.98 (3H, s), 2.03 (3H, s), 2.04 (3H, s), 2.07 (3H, s), 2.12 (3H, s), 2.13 (6H, s), 2.15 (3H, s), 3.89 (1H, ddd, J = 2.2, 5.6, 9.8 Hz), 3.99 (1H, dd, J = 7.5, 9.3 Hz), 4.08 (1H, dq, J = 6.3, 9.7 Hz), 4.14 (1H, dd, J = 2.2, 12.2 Hz), 4.26 (1H, dd, J = 5.6, 12.2 Hz), 5.02-5.09 (4H, m), 5.13 (1H, d, J = 7.5 Hz) 5.21 (1H, dd, J = 3.2, 10.0 Hz), 5.34 (1H, dd, J = 9.3, 9.3 Hz), 6.77 (2H, s) 6.78 (1H, d, J = 16.2 Hz), 6.82 (2H, d, J = 8.6 Hz), 7.36 (1H, d, J = 16.2 Hz), 7.41 (2H, d, J = 8.6 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 17.4, 20.6, 20.6, 20.6, 20.7, 20.7, 20.8, 20.9, 62.0, 67.0, 68.3, 68.5, 70.0, 70.8, 72.1, 74.1, 76.8, 98.2, 98.7, 109.4, 116.2, 121.7, 123.9, 126.2, 130.7, 147.0, 149.4, 157.5, 159.4, 168.5, 169.8, 169.9, 170.2, 170.3, 170.4, 170.8, 190.5. IR ν_{max} cm^{-1}: 3360, 2941, 1741, 1601, 1366, 1214, 1169, 1036. HR-MS [ESI+, (M + Na)$^+$]: calculated for $C_{43}H_{48}NaO_{22,}$ 939.2535; found, 939.2515. $[\alpha]_D^{21}$ –30.1° (c 1.0, chloroform).

2′,6′-Diacetoxy-4′-[hexa-O-acetyl-(2-O-α-L-rhamnopyranosyl-β-D-glucopyranosyl)oxy]-4″-trifluoromethylsufonyloxychalcone (8c)

To a solution of **8b** (304 mg, 0.332 mmol) in dichloromethane (2.5 mL) were added 2,6-lutidine (115 μL, 0.993 mmol) and trifluoromethanesulfonic anhydride (170 μL, 1.01 mmol) at 0 °C under argon atmosphere, and the mixture was stirred for 1 h at room temperature. The mixture was poured into water and the organic materials were extracted with dichloromethane twice. The combined extracts were washed with hydrochloric acid (1 M) and brine, dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (12 g). Elution with dichloromethane-ethyl acetate (3:1) furnished **8c** as a yellow amorphous solid (208 mg, 60%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.22 (3H, d, J = 6.3 Hz), 1.99 (3H, s), 2.03 (3H, s), 2.04 (3H, s), 2.07 (3H, s), 2.12 (3H, s), 2.14 (6H, s), 2.15 (3H, s), 3.90 (1H, ddd, J = 2.2, 5.6, 9.8 Hz), 3.99 (1H, dd, J = 7.6, 9.3 Hz), 4.07 (1H, dq, J = 6.3, 9.7 Hz), 4.15 (1H, dd, J = 2.2, 12.5 Hz), 4.27 (1H, dd, J = 5.6, 12.5 Hz), 5.03-5.09 (4H, m), 5.14 (1H, d, J = 7.5 Hz) 5.21 (1H, dd, J = 3.4, 10.0 Hz), 5.34 (1H, dd, J = 9.3, 9.3 Hz), 6.78 (2H, s), 6.90 (1H, d, J = 16.2 Hz), 7.31 (2H, d, J = 8.8 Hz), 7.40 (1H, d, J = 16.2 Hz), 7.61 (2H, d, J = 8.8 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 17.4, 20.5, 20.5, 20.6, 20.6, 20.7, 20.8, 20.9, 61.9, 67.0, 68.2, 68.3, 70.0, 70.8, 72.2, 74.0, 76.7, 98.2, 98.6, 109.3, 118.6 (q, J = 321 Hz), 121.2, 122.1, 128.3, 130.1, 134.5, 143.4, 149.5, 150.6, 157.9, 168.3, 169.6, 169.3, 170.0, 170.1, 170.2, 170.5, 189.8. IR ν_{max} cm^{-1}: 2942, 1742, 1616, 1424, 1367, 1208, 1038. HR-MS [ESI+, (M + Na)$^+$]: calculated for $C_{44}H_{47}F_3NaO_{24}S$, 1071.2028; found, 1071.2056. $[\alpha]_D^{22}$ –26.3° (c 1.0, chloroform).

2′,6′-Diacetoxy-4′-[hexa-O-acetyl-(2-O-α-L-rhamnopyranosyl-β-D-glucopyranosyl)oxy] dihydrochalcone (4b)

To a solution of **8c** (193 mg, 0.184 mmol) in THF (20 mL) were added palladium hydroxide (20% on carbon, 48.2 mg, 68.6 μmol) and triethylamine (64 μL, 0.459 mmol) and the mixture was stirred under hydrogen atmosphere for 3.5 h at room temperature. The mixture was filtered to remove insoluble materials with a pad of Celite. The precipitates were washed with THF and the combined filtrate and washings were concentrated *in vacuo*. The residue was purified by silica gel column chromatography (11 g). Elution with dichloromethane-ethyl acetate (3:1) furnished **4b** as a colorless amorphous solid (140 mg, 84%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.18 (3H, d, J = 6.1 Hz), 1.96 (3H, s), 2.01 (3H, s), 2.02 (3H, s), 2.04 (3H, s), 2.09 (3H, s), 2.12 (6H, s), 2.13 (3H, s), 2.95-2.98 (2H, m), 3.04-3.07 (2H, m), 3.86 (1H, ddd, J = 2.2, 5.6, 9.8 Hz), 3.95 (1H, dd, J = 7.5, 9.3 Hz), 4.02 (1H, dq, J = 6.1, 9.8 Hz), 4.11 (1H, dd, J = 2.2, 12.5 Hz), 4.23 (1H, dd, J = 5.6, 12.5 Hz), 4.99-5.06 (4H, m), 5.09 (1H, d, J = 7.5 Hz) 5.17 (1H, dd, J = 3.4, 10.0 Hz), 5.31 (1H, dd, J = 9.3, 9.3 Hz), 6.71 (2H, s), 7.17-7.20 (3H, m), 7.26-7.29 (2H, m). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 17.4, 20.6, 20.6, 20.6, 20.7, 20.7, 20.8, 20.9, 29.5, 45.4, 61.9, 67.0, 68.3, 68.4, 70.0, 70.8, 72.1, 74.0, 76.7, 98.2, 98.6, 109.0, 122.4, 126.2, 128.5, 128.5, 140.9, 148.9, 157.5, 168.1, 169.6, 169.7, 170.0, 170.1, 170.2, 170.5, 199.3. IR ν_{max} cm^{-1}: 2939, 1741, 1616, 1366, 1218, 1176, 1037. HR-MS [ESI+, (M + Na)$^+$]: calculated for $C_{43}H_{50}NaO_{21,}$ 925.2742; found, 925.2752. $[\alpha]_D^{22}$ –28.9° (c 1.0, chloroform).

2′,6′-Dihydroxy-4′-(2-O-α-L-rhamnopyranosyl-β-D-glucopyranosyl)oxydihydrochalcone (4a)

To a solution of **4b** (137 mg, 0.152 mmol) in acetonitrile (7 mL) was added an aqueous solution of sodium hydroxide (0.25 M, 7 mL) and the mixture was stirred for 1 h at room temperature. The reaction was quenched with DOWEX 50 W-X8 (H^+ form) and the mixture was filtered to remove insoluble materials. The filtrate was concentrated *in vacuo*. The residue was purified by preparative TLC developed with acetonitrile-water (5:8) furnished **4a** as a colorless amorphous solid (47.4 mg, 55%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 1.15 (3H, d, J = 6.1 Hz), 2.86-2.89 (2H, m), 3.14-3.21 (2H, m), 3.28-3.34 (3H, m), 3.41-3.48 (4H, m), 3.62-3.68 (3H, m), 4.46 (1H, d, J = 5.6 Hz), 4.54 (1H, dd, J = 5.1, 5.1 Hz), 4.63 (1H, d, J = 4.4 Hz), 4.66 (1H, d, J = 4.7 Hz), 5.04 (1H, d, J = 7.5 Hz), 5.06 (1H, br), 5.09 (1H, d, J = 5.6 Hz), 5.28 (1H, d, J = 5.3 Hz), 6.99 (2H, s), 7.14-7.17 (1H, m), 7.22-7.28 (4H, m), 12.28 (2H, br). ^{13}C-NMR (125 MHz, DMSO-d_6) δ:18.6, 30.5, 45.7, 60.7, 68.8, 69.9, 70.8, 71.0, 72.3, 77.0, 77.3, 77.6, 95.2, 97.5, 101.1, 105.7, 126.3, 128.7,

128.8, 142.0, 163.4, 164.2, 205.2. IR ν_{max} cm^{-1}: 3351, 2920, 1626, 1431, 1176, 1044. HR-MS [ESI+, $(M + Na)^+$]: calculated for $C_{27}H_{34}NaO_{13,}$ 589.1897; found, 589.1890. $[\alpha]_D^{20}$ –69.0° (c 1.0, DMSO).

Author contributions

R.T. and T.S designed this study; R.T. carried out the experiments; K.H. contributed to analytical works; R.T wrote the manuscript with assistance from all authors; and S.H. and T.S. supervised the research.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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