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Note

The first total synthesis of 7-*O*-β-D-glucopyranosyl-4′-*O*-α-L-rhamnopyranosyl apigenin via a hexanoyl ester-based protection strategy

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ABSTRACT

The first total synthesis of 7-*O*-β-D-glucopyranosyl-4′-*O*-α-L-rhamnopyranosyl apigenin **1**, which exhibits good anti-hepatitis B virus and anti-stroke activities, was accomplished in six steps and 20% overall yield from apigenin. Another synthetic route, in which the target was obtained in seven steps, was also developed to prove the utility of a hexanoyl ester-based orthogonal protection strategy. The hexanoyl protection strategy provided all the flavonoid intermediates with good solubility and reactivity, enabled efficient selective protection and glycosylation, and provided a practical and effective synthetic strategy for flavonoids, starting from commercially available flavone.

Glycosylated flavonoids occur widely in human food and drinks, especially in fruits and vegetables.[1] They are also major components of many plant drugs[2] and exhibit a broad spectrum of biological activities, such as antimicrobial,[3] antiviral, and anti-inflammatory activities.[4] Their biological activities against cancer, cardiovascular, and neurodegenerative diseases also have attracted increasing interest.[4,5]

7-*O*-β-D-Glucopyranosyl-4′-*O*-α-L-rhamnopyranosyl apigenin **1** (Fig. 1) is one of the main effective components of *Ranunculus sieboldii*, a traditional Chinese herb medication for hepatitis patients. The compound exhibits inhibitory activity against hepatitis B virus (HBV) replication, and its inhibition effect is comparable with that of lamivudine.[6] Recently, our colleagues reported its remarkable activity against cerebral thrombosis and stroke.[7] However, the mechanism of these interesting biological activities remains unclear, and further investigation was hindered by its limited availability from natural sources.[8] Thus, an efficient total synthesis route of **1** is needed.

The major impediments to flavonoid synthesis are the poor solubility of most flavonoids in common organic solvents, which in turn results in poor reactivity, and the lack of effective orthogonal protecting strategies for polyphenols.[9–13] Although some of monoglycosyl flavonoids have been synthesized in the last decade, and some protection strategies were introduced,[14–16] bis-glycosyl flavonoids synthesis is still recognized as a challenging task (only four syntheses have been reported to date),[13,17–19] because no general solution for these old problems has been developed. During our continuous medicinal research on flavonoids to support the investigation of many important physiology processes and drug discoveries, we tried to develop a practical general strategy for orthogonal protection of polyphenols and flavonoid glycosylation, which will serve as a basis for the preparation of flavonoid libraries.

Recently, the hexanoyl group was used occasionally by Yu and co-workers[20] and Botting and Al-Maharik[21] for the selective deprotection or solubility improvement in flavonoid synthesis. We envisioned that the lipophilic hexanoyl group would effectively improve the solubility of flavonoids, which would result in improved reactivity, and the increased reactivity of the hexanoyl group would enable selective protection. In this work we show that starting from trihexanoyl apigenin **3**, all the three hydroxyl groups of apigenin could be discriminated effectively with very economical methods. We thus developed a general strategy to introduce

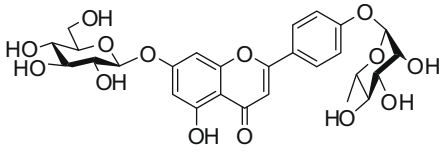

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Figure 1. Structure of 7-*O*-β-D-glucopyranosyl-4′-*O*-α-L-rhamnopyranosyl apigenin, **1**.

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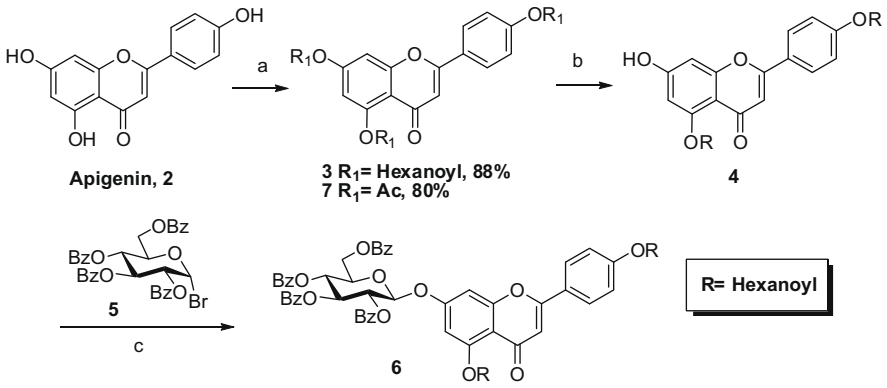


Scheme 1. Reagents and conditions: (a) For **3**: hexanoyl chloride, NEt_3, DMAP, DMF, 88%; for **7**: Ac_2O, pyridine, 80%; (b) 0.5 equiv K_2CO_3, CH_2Cl_2–MeOH (1:1), 1.5 h, 93%; (c) **5**, K_2CO_3, Aliquat 336, $CHCl_3$–H_2O, 45 °C, 24 h, 78%.

glycosyl groups selectively to flavones, and applied this strategy to the first total synthesis of 7-*O*-β-D-glucopyranosyl-4′-*O*-α-L-rhamnopyranosyl apigenin **1**.

Commercially available apigenin was treated with hexanoyl chloride in DMF (Scheme 1). After recrystallization from ethanol, tri-*O*-hexanoyl apigenin, **3**, was obtained in 88% yield in a purity of >95%. Compared with tri-*O*-acetyl apigenin, **7**, hexanoyl derivative **3** shows remarkably improved solubility in common organic solvents (Table 1). Selective hydrolysis of the 7-acyl group was first achieved in 78% yield upon treatment with PhSH at −20 °C in anhydrous *N*-methyl pyrolidinone following Yu's procedure.[20] However, this procedure, which requires strict reaction conditions (−20 °C for 24 h) with toxic and odorous PhSH, is not suitable to scale up.

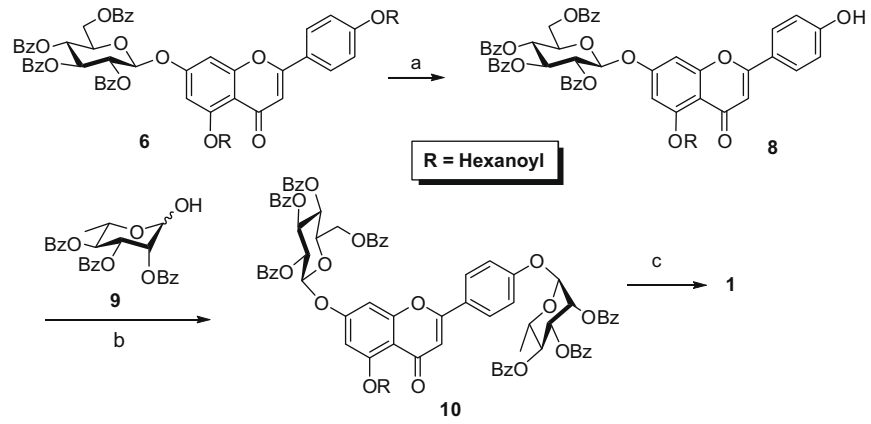
Thus, we developed a new efficient selective deprotection method as described below. Treatment of **3** with 0.5 equiv K_2CO_3 in CH_2Cl_2–MeOH provided the 7-OH selectively deprotected product **4** in 93% yield (Scheme 1), no 4′-O deprotection was observed. A mixed solvent (CH_2Cl_2–MeOH, 1:1) was optimized for this selective deprotection. Use of one or the other of these solvents alone resulted in either poor yield or non-selective deprotection. The amount of base was also important; if 1 equiv of K_2CO_3 was used, a significant amount of 7,4′-dihydroxyl apigenin was obtained after 2 h. In addition, when these optimized deprotection conditions were applied to tri-*O*-acetyl apigenin, **7**, poor selectivity was obtained.[22] The excellent selectivity in our cases (and below) demonstrated that hexanoyl protection helped to discriminate the reactivity of each position in an effective way. Furthermore, our new method provided compounds in good purity after simple work up without column chromatography. With **4** in hand, glycosylation with bromide **5** under phase-transfer-catalyzed (PTC)[11,23] conditions provided **6** with β selectivity in 78% yield; Aliquat 336 ($CH_3(C_8H_{17})_3N^+Cl^-$) was the best PTC reagent.

Table 1
The solubility data of apigenin per-hexanoyl **3** and per-acetyl **7** (25 °C)

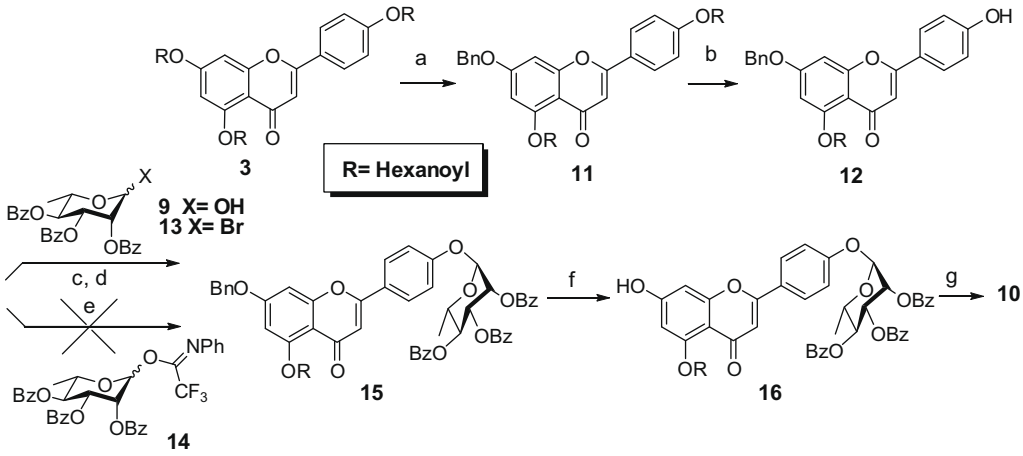
Solvent	EtOAc	CH_2Cl_2	Acetone	Toluene
3	330 mg/mL	>1 g/mL	>1 g/mL	170 mg/mL
7	<1 mg/mL	39 mg/mL	5 mg/mL	<1 mg/mL

Treatment of compound **6** with the previously described K_2CO_3-promoted deprotection conditions satisfyingly gave the 4′-OH product **8** in 84% yield, with the 5-*O*-hexanoate and the four benzoates on the glucosyl moiety intact (Scheme 2).[20,23] These results were in accordance with the reported reactivity order: 7-*O*-acyl > 4′-*O*-acyl > 5-*O*-acyl,[24] and demonstrate that the appropriate reactivity of the hexanoyl group helped us to realize the selective deprotection in a very effective and economical way.

The subsequent 4′-O-glycosylation proved to be a challenging task, because of the poor nucleophilicity of the flavone 4′-hydroxyl group.[13] According to our investigation for the preparation of **15** (see below), direct 4′-O-rhamnosylation of **8** was achieved via Mitsunobu reaction, which provided **10** exclusively as the α-anomer, in 40% yield in DMF as reaction solvent. It is noteworthy that there are very few examples of direct flavone 4′-O-glycosylation,[11,13,25] Those that are reported either suffer from low yields or relied upon expensive reagents, our work provides a practical method for this problem. Global deprotection of **10** with sodium



Scheme 2. Reagents and conditions: (a) 0.5 equiv K_2CO_3, CH_2Cl_2–MeOH (1:1), 2 h, 84%; (b) DEAD, PPh_3, DMF, −20→0 °C, 2 h, then 0→20 °C, 8 h, 40%; (c) NaOMe, MeOH, 88%.



Scheme 3. Reagents and conditions: (a) BnCl, K_2CO_3, KI, acetone 24 h, 85%; (b) 0.5 equiv K_2CO_3, CH_2Cl_2–MeOH (1:1), 10 h, 92%; (c) for **9**, DEAD, PPh_3, DMF, −20→0 °C, 2 h, then 0→20 °C, 8 h, 63%; (d) for **13**, K_2CO_3, Aliquat 336, $CHCl_3$–H_2O, 45 °C, 24 h, 35%; (e) for **14**, TMSOTf or $BF_3{\cdot}Et_2O$, CH_2Cl_2, 4 Å MS; (f) Pd/H_2, $CHCl_3$–EtOH, 40→45 °C, 9 h, 79%; (g) **5**, TBAB, K_2CO_3, $CHCl_3$–H_2O, 45 °C, 24 h, 71%.

methoxide gave **1** in 88% yield. The synthetic compound had spectral data in full agreement with the reported natural one,[6] and exhibited the same anti-stroke activity.[7]

Another synthetic route was also developed to prove the application of hexanoyl orthogonal protection strategy (Scheme 3). Compound **3** was transformed to 7-*O*-benzyl **11**, and the 4′-*O*-hexanoyl group was readily hydrolyzed with the K_2CO_3-promoted conditions to obtain **12** in 10 h; these two steps proceeded in high yield and in good selectivity. The electron-donating 7-*O*-benzyl group, in contrast to the electron-withdrawing 7-*O*-tetrabenzoyl-glucosyl group of compound **6**, made the 4′-*O*-hexanoyl group of **11** resistant to hydrolysis. It is noteworthy that all the three hydroxyl groups of apigenin **12** were readily discriminated from each other in three steps in high yield. The orthogonal protecting pattern in this compound make it a valuable intermediate for future flavonoid glycoside synthesis.

In the subsequent 4′-O-rhamnosylation, we encountered some difficulties because the nucleophilicity of the flavone 4′-hydroxyl group is poor. We first tried with donor **14**,[20] but under various conditions (promoted by TMSOTf or $BF_3{\cdot}Et_2O$, with or without 4 Å MS), no desired product was obtained. The best 4′-O-rhamnosylation was via a Mitsunobu reaction, which provided **15** exclusively as the α-anomer, in 63% yield in DMF as reaction solvent; the yield was reduced to 42% in CH_2Cl_2. PTC condensation of the bromide **13** and **12** with Aliquat 336 gave **15** as the α-anomer in 35% yield; when Aliquat 336 was substituted by *n*-Bu_4NBr (TBAB), no product was obtained. The PTC conditions not only confirmed the α-glycoside linkage of the product obtained with Mitsunobu reaction, but also showed that the choice of PTC is vital for the synthesis. Due to the fact that there are very few examples of direct flavone 4′-O-glycosylation, our results are significant and worthy of further investigation.

To obtain the final target, the 7-*O*-benzyl ether of **15** was hydrogenated with Pd/C to provide **16**. Compound **16** was reacted with tetrabenzoyl glucosyl bromide **5** under PTC conditions to obtain bis-glycosyl flavonoid **10**, whose structure was confirmed by ^1H and ^{13}C NMR data. Thus, compound **1** could be prepared by this route in seven steps and in 16% overall yield from apigenin.

In conclusion, the first total synthesis of 7-*O*-β-D-glucopyranosyl-4′-*O*-α-L-rhamnopyranosyl apigenin, **1**, which exhibits good anti-HBV and anti-stroke activities, was accomplished in six steps and 20% overall yield from apigenin. A hexanoyl-based protection strategy was developed, which greatly improved the solubility of flavonoid intermediates, allowed the efficient selective protection, and provided good yields of the transformations, especially the direct glycosylation of inert 4′-hydroxyl. This method provided a practical flavonoid synthesis strategy starting from commercially available flavone. Further application studies will probe the generality of this method for flavone glycoside synthesis. These investigations are ongoing in our laboratory and will be reported in due course.

1. Experimental

1.1. General methods

Reagents and solvents were reagent grade and used without further purification. Methylene dichloride was distilled from calcium hydride, and DMF was dried over 4 Å molecular sieves. All reactions involving air or moisture sensitive reagents or intermediates were performed under a nitrogen or argon atmosphere. Flash chromatography was performed on Qingdao Haiyang silica gel (300−400 mesh). Analytical TLC was performed using 0.25 mm EM Silica Gel 60 F_{250} plates that were visualized by irradiation (254 nm) or by staining with H_2SO_4–methanol solution. ^1H and ^{13}C NMR spectra were obtained using 300 MHz Bruker AM300 and 400 MHz Varian instruments. Chemical shifts are reported in parts per million (ppm δ) referenced to the residual ^1H resonance of the solvent ($CDCl_3$, 7.26 ppm; DMSO-d_6, 2.49 ppm). ^{13}C spectra were referenced to the residual ^{13}C resonance of the solvent ($CDCl_3$, 77.0 ppm; DMSO-d_6, 39.5 ppm). Splitting patterns are designated as follows: s, singlet; br, broad; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, mulitplet. Elemental analysis was performed on a Foss-Heraeus Vario EL instrument. Mass spectra (ESI) were performed on Shimadzu LCMS-2010EV. High resolution mass spectra (MALDI/DHB) were performed on IonSpec 4.7 Tesla FTMS from Varian.

1.2. 5,7,4′-Tri-*O*-hexanoyl apigenin (3)

Apigenin (1.08 g, 4 mmol), Et_3N (2.8 mL, 20 mmol), and DMAP (155 mg, 1.2 mmol) were dissolved in DMF (10 mL). The mixture was cooled under ice bath, and hexanoyl chloride (3 mL, 23 mmol) was slowly added. The mixture was further stirred. After about 8 h, TLC showed no remaining apigenin, and the solution was diluted with CH_2Cl_2 and washed with 1 M HCl, water, satd aq $NaHCO_3$, and brine. The organic phase was dried (Na_2SO_4) and the solvent removed under vacuum. The residue was purified by recrystalliza-

tion (EtOH) to obtain 1.99 g of **3**, in 88% yield as a white solid. R_f = 0.45 (petroleum ether–EtOAc 6:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87 (d, 2H, J = 8.4 Hz), 7.34 (s, 1H), 7.25 (d, 2H, J = 8 Hz), 6.83 (s, 1H), 6.62 (s, 1H), 2.67 (m, 6H), 1.79 (m, 6H), 1.41 (m, 12H), 0.93 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.1, 171.9, 171.6, 170.8, 161.6, 157.6, 154.1, 153.5, 150.4, 128.5, 127.4, 127.4, 122.3, 122.3, 114.9, 113.7, 108.7, 108.5, 34.3, 34.2, 34.1, 31.3, 31.2, 31.1, 24.4, 24.4, 24.1, 22.3, 22.3, 22.2, 13.8, 13.8, 13.7. Anal. Calcd for $C_{33}H_{40}O_8$: C, 70.19; H, 7.14. Found: C, 69.95; H, 7.17. ESI-MS (m/z): 565.2 $[M+H]^+$.

1.3. 5,4′-Di-*O*-hexanoyl-apigenin (4)

To a solution of **3** (2 g, 3.54 mmol) in 100 mL CH_2Cl_2 and 100 mL methanol was added K_2CO_3 (240 mg, 1.77 mmol) at 0 °C. After 1.5 h, TLC showed no remaining **3**, and the reaction was quenched by the addition of HCl–MeOH (2 M) to pH 6–7. The mixture was filtered through Celite and the solvent was removed under vacuum, the resulting oil was purified by column chromatography (CH_2Cl_2–acetone 30:1) to provide 1.52 g of **4**, in 92% yield as a white solid. R_f = 0.4 (silica, CH_2Cl_2–acetone 30:1) ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.52 (s, 1H), 7.74 (d, 2H, J = 8.4 Hz), 7.17 (d, 2H, J = 8.8 Hz), 6.71 (d, 1H, J = 2.4 Hz), 6.51 (d, 1H, J = 2.8 Hz), 6.50 (s, 1H), 2.71 (t, 2H, J = 7.2 Hz), 2.56 (t, 2H, J = 7.6 Hz), 1.78 (m, 4H), 1.39 (m, 8H), 0.93 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.7, 173.5, 172.3, 162.4, 161.9, 158.9, 153.6, 150.7, 128.6, 127.7, 127.6, 122.6, 122.5, 110.4, 109.9, 107.6, 101.7, 34.6, 34.5, 31.5, 31.4, 24.7, 24.4, 22.6, 22.5, 14.2, 14.1. Anal. Calcd for $C_{27}H_{30}O_7$: C, 69.51; H, 6.48. Found: C, 69.60; H, 6.48. ESI-MS (m/z): 465.2 $[M-H]^-$.

1.4. 5,4′-Di-*O*-hexanoylapigenin-7-yl 2,3,4,6-tetra-*O*–benzoyl-β-D-glucopyranoside (6)

To a solution of acceptor **4** (94 mg, 0.2 mmol) and donor **5** (197 mg, 0.3 mmol) in 4 mL chloroform was added 4 mL H_2O, and then K_2CO_3 (69 mg, 0.5 mmol) and Aliquat 336 (8 mg, 0.02 mmol) were added. The mixture was stirred at 40–45 °C. After 24 h, TLC showed most of **4** was consumed, and the reaction was quenched by addition of 1 M HCl solution. The solution was diluted with CH_2Cl_2 and washed by brine. The organic phase was dried (Na_2SO_4) and the solvent removed under vacuum. The resulting oil was purified by chromatography on silica gel (petroleum ether–EtOAc–CH_2Cl_2 6:1:2) to obtain 163 mg of **6**, in 78% yield as yellow syrup. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 (m, 9H), 7.38 (m, 15H), 7.01 (s, 1H), 6.71(s, 1H), 6.68 (s, 1H), 6.02 (t, 1H, J = 9.2 Hz), 5.84 (t, 1H, J = 8.8 Hz), 5.64 (t, 1H, J = 9.1 Hz), 5.63 (d, 1H, J = 7.1 Hz), 4.78 (d, 1H, J = 9.7 Hz), 4.50 (m, 2H), 2.67 (m, 4H), 1.77 (m, 4H), 1.40 (m, 8H), 0.94 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.2, 172.2, 171.8, 166.1, 165.6, 165.2, 165.0, 161.4, 160.0, 158.3, 153.3, 150.9, 133.6, 133.6, 133.4, 133.2, 129.9, 129.9, 129.8, 129.8, 129.5, 129.5, 129.3, 129.3, 128.7, 128.7, 128.6, 128.6, 128.6, 128.6, 128.5, 128.5, 128.5, 128.4, 128.4, 128.4, 127.4, 127.4, 127.4, 122.3, 122.3, 113.0, 109.6, 108.4, 102.4, 98.3, 73.0, 72.5, 71.4, 69.3, 63.1, 34.4, 34.1, 31.3, 31.2, 24.5, 24.0, 22.4, 22.3, 13.9,13.9. Anal. Calcd for $C_{61}H_{56}O_{16}$: C, 70.10; H, 5.40. Found: C, 70.31; H, 5.25. ESI-MS (m/z): 1083.3 $[M+K]^+$.

1.5. 5-*O*-Hexanoyl-apigenin-7-yl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside (8)

The procedure is the same as that for compound **4**, column chromatography (CH_2Cl_2–acetone 20:1) provided **8**, in 84% yield as a pale yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82 (m, 8H), 7.40 (m, 15H), 6.90 (m, 1H), 6.72 (m, 3H), 6.39 (s, 1H), 6.03 (m, 1H), 5.85 (m, 2H), 5.63 (d, 1H, J = 10 Hz), 4.76 (m, 1H), 4.50 (m, 2H), 2.67 (m, 2H), 1.76 (m, 2H), 1.36 (m, 4H), 0.91 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.6, 173.3, 166.2, 165.7, 165.2, 165.0, 162.6, 160.0, 159.9, 158.1, 150.4, 133.7, 133.7, 133.5, 133.2, 129.9, 129.9, 129.9, 129.8, 129.8, 129.8, 129.6, 129.6, 129.6, 129.2, 129.2, 128.7, 128.7, 128.7, 128.7, 128.5, 128.5, 128.5, 128.4, 128.4, 128.4, 127.8, 122.1, 116.0, 116.0, 112.6, 109.3, 106.2, 102.7, 98.3, 72.9, 72.4, 71.4, 69.2, 63.0, 34.2, 31.4, 24.0, 22.3, 13.9. Anal. Calcd for $C_{55}H_{46}O_{15}$: C, 69.76; H, 4.90. Found: C, 69.55; H, 4.80. ESI-MS (m/z): 969.3 $[M+Na]^+$.

1.6. 5-*O*-Hexanoyl-7-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-4′-*O*-(2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl) apigenin (10)

Route A: DEAD (47 μL, 0.3 mmol) was added to a stirred solution of **8** (189 mg, 0.2 mmol), **9** (79 mg, 0.167 mmol), PPh_3 (78 mg, 0.3 mmol), and 4 Å MS (30 mg) in anhydrous DMF (5 mL) at –20 °C under N_2. The reaction mixture was stirred for 2 h at –20 °C, and then was stirred for 6 h at rt. After TLC showed no remaining **9**, the solution was diluted with CH_2Cl_2 and washed by 1 M HCl, water, aq satd $NaHCO_3$, and brine. The organic phase was dried (Na_2SO_4) and the solvent removed under vacuum. The residue was purified by chromatography on silica gel (petroleum ether–EtOAc–CH_2Cl_2 7:1:2) to obtain 118 mg of **10** in 42% yield, as a yellow syrup.

Route B: To a solution of acceptor **16** (165 mg, 0.2 mmol) and donor **5** (197 mg, 0.3 mmol) in 5 mL chloroform was added 5 mL H_2O, and then K_2CO_3 (69 mg, 0.5 mmol) and TBAB (16 mg, 0.05 mmol) were added. The mixture was stirred at 40–45 °C for 24 h when TLC showed most of **16** was consumed, and then the reaction was then quenched by the addition of 1 M HCl solution. The solution was diluted with CH_2Cl_2 and washed with brine. The organic phase was dried (Na_2SO_4) and the solvent removed under vacuum. The residue was purified by chromatography on silica gel (petroleum ether–EtOAc–CH_2Cl_2 8:1:2) to obtain 200 mg of **10**, in 71% yield as yellow syrup. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (m, 2H), 7.97 (m, 8H), 7.87 (m, 4H), 7.46 (m, 25H), 7.03 (d, 1H, J = 1.7 Hz), 6.71 (d, 1H, J = 1.7 Hz), 6.55 (s, 1H), 6.04 (m, 2H), 5.83 (m, 5H), 5.64 (d, 1H, J = 7.2 Hz), 4.78 (d, 1H, J = 9.3 Hz), 4.53 (m, 2H), 4.29 (m, 1H), 2.67 (m, 2H), 1.77 (m, 2H), 1.38 (m, 7H), 0.93 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.2, 166.1, 165.7, 165.6, 165.6, 165.6, 165.2, 165.0, 161.7, 160.0, 158.5, 133.7, 133.6, 133.6, 133.4, 133.3, 133.0, 130.0, 130.0, 129.9, 129.9, 129.9, 129.8, 129.8, 129.7, 129.7, 129.6, 129.6, 129.6, 129.3, 129.3, 129.3, 129.1, 129.1, 129.1, 129.0, 129.0, 129.0, 128.7, 128.7, 128.7, 128.6, 128.6, 128.6, 128.6, 128.5, 128.5, 128.5, 128.4, 128.4, 128.4, 128.4, 128.3, 128.3, 128.3, 128.0, 128.0, 128.0, 128.0, 125.5, 116.8, 113.0, 109.4, 107.7, 102.5, 98.3, 95.7, 73.0, 72.5, 71.4, 71.4, 70.4, 69.6, 69.3, 67.9, 63.1, 34.1, 31.3, 24.0, 22.4, 17.7, 13.9. ESI-MS (m/z): 1427.4 $[M+Na]^+$.

1.7. 7-*O*-Benzyl-5,4′-di-*O*-hexanoyl apigenin (11)

To a solution of **3** (4.4 g, 7.8 mmol) in acetone (50 mL) were added K_2CO_3 (10 g, 72 mmol) and KI (390 mg, 2.3 mmol). To this well-stirred mixture, benzyl chloride (5.8 mL, 51 mmol) was added, and the mixture was stirred at 55 °C. After 24 h, TLC showed no remaining **3**, and the mixture was filtered and the solvent removed under vacuum. The resulting oil was purified by crystallization (EtOAc–hexane 13:120 mL) to obtain 3.69 g of **11** in 85% yield, as a pale yellow solid. R_f = 0.48 (silica, petroleum ether–EtOAc 6:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.84 (dd, 2H, J = 6.8 Hz, 2 Hz), 7.42 (m, 5H), 7.23 (dd, 2H, J = 6.8 Hz, 1.6 Hz), 6.93 (d, 1H, J = 2.4 Hz), 6.68 (d, 1H, J = 2.4 Hz), 6.55 (s, 1H), 5.16 (s, 2H), 2.73 (t, 2H, J = 7.6 Hz), 2.58 (t, 2H, J = 7.2 Hz), 1.78 (m, 4H), 1.44 (m, 8H), 0.93 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.2, 172.2, 171.6, 162.5, 161.1, 158.7, 153.2, 150.8, 135.4, 128.7, 128.6, 128.5, 128.4, 127.4, 127.4, 127.4, 127.3, 122.2, 122.2, 111.6, 108.9, 108.3, 99.9, 70.7, 34.3, 34.2, 31.3, 31.2, 24.6, 24.1, 22.3, 22.1, 13.8, 13.7. Anal. Calcd for $C_{34}H_{36}O_7$: C, 73.36; H, 6.52. Found: C, 73.56; H, 6.38. ESI-MS (m/z): 579.3 $[M+Na]^+$.

1.8. 7-*O*-Benzyl-5-*O*-hexanoyl apigenin (12)

To a solution of **11** (2 g, 3.6 mmol) in 100 mL CH_2Cl_2 and 100 mL methanol, K_2CO_3 (246 mg, 1.8 mmol) was added at 0 °C. After 10 h, TLC showed no remaining **11**, and the reaction was quenched by the addition of HCl–MeOH (2 M) to pH 6–7. The mixture was filtered through Celite and the solvent removed under vacuum, and the resulting oil was purified by chromatography on silica gel (CH_2Cl_2–acetone 20:1) to obtain 1.51 g of **12**, in 92% yield as a pale yellow solid. R_f = 0.5 (silica, CH_2Cl_2–acetone 20:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.23 (s, 1H), 7.89 (dd, 2H, J = 6.8 Hz, 2 Hz), 7.42 (m, 5H), 7.20 (d, 1H, J = 2 Hz), 7.02 (dd, 2H, J = 6.8 Hz, 2 Hz), 6.73 (d, 1H, J = 2 Hz), 6.49 (s, 1H), 5.30 (s, 2H), 2.76 (m, 2H), 1.75 (m, 2H), 1.43 (m, 4H), 0.94 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.1, 173.7, 162.7, 162.6, 160.1, 158.6, 150.3, 135.3, 128.8, 128.7, 128.5, 128.3, 127.8, 127.5, 127.4, 122.2, 116.2, 116.1, 111.0, 108.9, 105.9, 99.9, 70.8, 34.3, 31.3, 24.1, 22.3, 13.9. Anal. Calcd for $C_{28}H_{26}O_6$: C, 73.35; H, 5.72. Found: C, 73.19; H, 5.60. ESI-MS (m/z): 457.2 $[M-H]^-$.

1.9. 7-*O*-Benzyl-5-*O*-hexanoylapigenin-4′-yl 2,3, 4-tri-*O*–benzoyl-α-L-rhamnopyranoside (15)

DEAD (47 μL, 0.3 mmol) was added to a stirred solution of **12** (92 mg, 0.2 mmol), **9** (79 mg, 0.167 mmol), PPh_3 (78 mg, 0.3 mmol), and 4 Å MS (30 mg) in anhydrous DMF (5 mL) at –20 °C under N_2. The reaction mixture was stirred for 2 h at –20 °C, and then was stirred for 6 h at rt. After TLC showed no remaining **9**, the solution was diluted with CH_2Cl_2 and washed with 1 M HCl, water, satd aq $NaHCO_3$, and brine. The organic phase was dried (Na_2SO_4) and the solvent removed under vacuum. The residue was purified by chromatography on silica gel (petroleum ether–EtOAc–CH_2Cl_2 7:1:2) to obtain 96 mg of **15** in 63% yield, as a yellow syrup. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (d, 2H, J = 8.4 Hz), 7.99 (d, 2H, J = 8 Hz), 7.87 (m, 4H), 7.67 (m, 2H), 7.63 (m, 3H), 7.31 (m, 11H), 6.97 (s, 1H), 6.70 (t, 1H), 6.57 (d, 1H), 6.06 (dd, 1H, J = 2.4 Hz, 10 Hz), 5.85 (m, 3H), 5.19 (s, 2H), 4.34 (m, 1H), 2.76 (t, 2H, J = 7.2 Hz), 1.85 (m, 2H), 1.33 (m, 4H), 1.28 (m, 3H), 0.94 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.5, 172.3, 165.8, 165.6, 162.6, 161.6, 158.9, 158.5, 150.8, 135.5, 133.7, 133.7, 133.4, 133.4, 133.2, 133.2, 130.0, 130.0, 130.0, 129.7, 129.2, 128.8, 128.8, 128.8, 128.7, 128.4, 128.4, 128.4, 128.3, 128.3, 127.9, 127.9, 127.9, 127.5, 125.9, 121.7, 116.9, 111.6, 108.9, 107.7, 100.0, 95.8, 71.5, 70.8, 70.5, 67.9, 64.2, 34.3, 31.4, 29.7, 24.2, 22.4, 17.7, 14.1, 13.9. Anal. Calcd for $C_{55}H_{48}O_{13}$: C, 72.04; H, 5.28. Found: C, 72.31; H, 5.15. ESI-MS (m/z): 939.3 $[M+Na]^+$.

1.10. 5-*O*-hexanoy-apigenin-4′-yl 2,3, 4-tri-*O*–benzoyl-α-L-rhamnopyranoside (16)

To a solution of **15** (0.5 g, 0.54 mmol) in 15 mL CH_2Cl_2 and 15 mL ethanol, a catalytic amount of 10% Pd on activated charcoal (120 mg) was added. A trace amount of formic acid could be added to improve the reaction. The reaction mixture was stirred vigorously under 1 atm H_2 atmosphere at 40–45 °C for 9 h. When TLC indicated complete consumption of the starting material, the charcoal was removed by filtration. The pale yellow filtrate was concentrated and purified by chromatography on silica gel (petroleum ether–EtOAc–CH_2Cl_2 5:1:3) to obtain 352 mg of **16**, in 79% yield as yellow syrup. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 (d, 2H, J = 8 Hz), 7.93 (m, 2H), 7.80 (m, 4H), 7.43 (m, 7H), 7.27 (m, 4H), 6.88 (d, 1H, J = 1.2 Hz), 6.64 (d, 1H, J = 1.6 Hz), 6.57 (s, 1H), 6.03 (dd, 1H, J = 10 Hz, 2.8 Hz), 5.87 (m, 3H), 4.30 (m, 1H), 2.75 (m, 2H), 1.80 (m, 2H), 1.33 (m, 7H), 0.88 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.4, 172.8, 165.8, 165.7, 162.1, 158.8, 158.6, 150.7, 133.7, 133.4, 133.3, 133.2, 130.0, 130.0, 129.7, 129.7, 129.1, 129.1, 129.0, 129.0, 128.6, 128.6, 128.6, 128.4, 128.4, 128.3, 128.3, 128.0, 128.0, 128.0, 125.5, 116.8, 116.8, 110.4, 109.6, 106.7, 101.5, 95.7, 71.6, 70.5, 69.8, 67.9, 34.3, 31.3, 24.1, 22.3, 17.7, 13.8. ESI-MS (m/z): 849.3 $[M+Na]^+$.

1.11. 7-*O*-β-D-glucopyranosyl-4′-*O*-α-L-rhamnopyranosyl apigenin (1)

NaOMe (38 mg, 0.71 mmol) was added to a solution of **10** (250 mg, 0.178 mmol) in CH_2Cl_2 (3 mL) and MeOH (3 mL) at rt. After 4 h, TLC showed no remaining **10**, the reaction was quenched by adding HCl–MeOH (2 M) to pH 6–7, and the solvent removed under vacuum. The residue was purified by chromatography on silica gel (CH_2Cl_2–MeOH 3:1) to obtain 90 mg of **1** in 88% yield, as a pale yellow solid. ^{1}H NMR (400 MHz, C_6D_6N): δ 8.07 (d, 2H, J = 8.8 Hz), 7.45 (d, 2H, J = 8.8 Hz), 7.23 (d, 1H, J = 1.62 Hz), 7.07 (s, 1H), 6.96 (d, 1H, J = 2 Hz), 6.22 (s, 1H), 5.92 (d, 1H, J = 7.7 Hz), 4.74 (m, 2H), 4.36 (m, 8H), 1.67 (d, 3H, J = 6.2 Hz); ^{13}C NMR (100 MHz, C_6D_6N): δ 183.2, 164.8, 164.5, 160.5, 158.3, 159.6, 129.1, 129.1, 125.1, 117.7, 117.7, 106.9, 105.3, 102.0, 101.2, 100.0, 96.0, 79.3, 78.4, 74.9, 73.8, 73.7, 72.5, 71.8, 71.5, 62.6, 18.9. ESI-MS (m/z): 601.1 $[M+Na]^+$, 613.1 $[M+Cl]^-$, HR-MS: m/z calcd for $C_{27}H_{31}O_{14}$: 579.1708, found: 579.1708.

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References

1. Bohm, B. A. *Introduction to Flavonoids*; Harwood Academic: Amsterdam, 1998.
2. Harborne, J. B.; Baxter, H.. In *The Handbook of Natural Flavonoids*; John Wiley & Son: Chichester, UK, 1999; Vols. 1 and 2.
3. Cowan, M. M. *Clin. Microbiol. Rev.* **1999**, *12*, 564–582.
4. Middleton, E., Jr.; Kandaswami, C.; Theoharides, T. C. *Pharmacol. Rev.* **2000**, *52*, 673–751.
5. Rice-Evans, C. *Curr. Med. Chem.* **2001**, *8*, 797–807.
6. Li, H. B.; Zhou, C. X.; Pan, Y. X.; Gao, X. Z.; Wu, X. M.; Bai, H.; Zhou, L. F.; Chen, Z.; Zhang, S. L.; Shi, S. Y.; Luo, J. L.; Xu, J. H.; Chen, L. R.; Zheng, X.; Zhao, Y. *Plant Med.* **2005**, *71*, 1128–1133.
7. Xia, Y.; Min, Y.; Zhong, Y.; Sheng, Y.; Guo, M.; Zheng, X. CN Patent 101053570A, 2006.
8. In Ref. 5: 165 mg compound **1** was isolated from 17 kg air-dried whole plant.
9. Nógrádi, M.; Farkas, L.; Wagner, H.; Horhammer, L. *Chem. Ber.* **1967**, *100*, 2783–2790.
10. Mezey-Vándor, G.; Farkas, L.; Kanzel, I.; Nógrádi, M. *Chem. Ber.* **1980**, *113*, 1945–1949.
11. Demetzos, C.; Skaltsounis, A. L.; Tillequin, F.; Koch, M. *Carbohydr. Res.* **1990**, *207*, 131–137.
12. Alluis, B.; Dangles, O. *Helv. Chim. Acta* **2001**, *84*, 1133–1156.
13. Kondo, T.; Oyama, K. I.; Yoshida, K. *Angew. Chem., Int. Ed.* **2001**, *40*, 894–897.
14. Maloney, D.; Hecht, S. M. *Org. Lett.* **2005**, *7*, 1097–1099.
15. Cole, E. R.; Crank, G.; Hai Minh, H. T. *Aust. J. Chem.* **1980**, *33*, 675–680.
16. Chung, Y.; Duerr, B. F.; McKelvey, T. A.; Nanjappan, P.; Czarnik, A. W. *J. Org. Chem.* **1989**, *54*, 1018–1032.
17. Du, Y.; Wei, G.; Linhardt, R. J. *Tetrahedron Lett.* **2003**, *44*, 6887–6890.
18. Zhu, C.; Peng, W.; Li, Y.; Han, X.; Yu, B. *Carbohydr. Res.* **2005**, *341*, 1047–1051.
19. Urgaonkar, S.; Shaw, J. T. *J. Org. Chem.* **2007**, *72*, 4582–4585.
20. Li, M.; Han, X.-W.; Yu, B. *J. Org. Chem.* **2003**, *68*, 6842–6845.
21. Al-Maharik, N.; Botting, N. P. *Tetrahedron Lett.* **2006**, *47*, 8703–8706.
22. Gao, Q. Dissertation, Shanghai Institute of Pharmaceutical Industry, 2008. Under the optimized condition, the best yield of 7-OH product is 40% with 7,4′-dihydroxyl byproduct in 30% yield.
23. Li, M.; Han, X.-W.; Yu, B. *Tetrahedron Lett.* **2002**, *43*, 9467–9470.
24. Peng, W.; Li, Y.; Zhu, C.; Han, X.; Yu, B. *Carbohydr. Res.* **2005**, , *340*, 1682–1688.
25. Farkas, L.; Wolfner, A.; Nógrádi, M.; Wagner, H.; Hörhammer, L. *Chem. Ber.* **1968**, *101*, 1630–1632.