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Synthesis of tamarixetin and isorhamnetin 3-*O*-neohesperidoside

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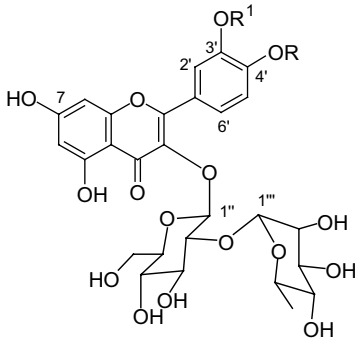
Abstract—Two isomeric flavonol 3-*O*-glycosides, tamarixetin and isorhamnetin 3-*O*-neohesperidoside (**1** and **2**), were synthesized. The natural product from *Costus spicatus* assigned as the former compound is revised to the latter structure.

Keywords: Flavonol glycoside; Tamarixetin; Isorhamnetin; Neohesperidoside; Synthesis

1. Introduction

Quercetin glycosides are abundant in plants; their 3′-*O*-methyl (isorhamnetin) derivatives are not uncommon. However, the 4′-*O*-methyl quercetin (tamarixetin) glycosides are rare in nature.[1] Tamarixetin 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (neohesperidoside) (**1**) was assigned for a flavonol glycoside from *Costus spicatus* Swart (Costaceae), a Brazilian medicinal plant, which showed moderate inhibitory activity against nitric oxide production by activated macrophages.[2] In continuing our research on flavonoid glycosides,[3] we developed an efficient approach to the synthesis of tamarixetin glycosides and synthesized compound **1**, but found discrepancies between the NMR data of the synthetic compound with those of the natural product. For comparison, we synthesized its natural 3′-*O*-methyl isomer **2**, which has been isolated from *Crocus antalyensis*,[4] *Cuscuta reflexa*,[5a] *Calendula officinalis*,[5b] and *Typha latifolia*.[5c] Finally, the nature product from *C. spicatus* is revised to the structure **2**.



1: R = Me, R[1] = H
2: R = H, R[1] = Me

2. Results and discussion

Tamarixetin has been prepared by either selective methylation of 7-*O*-benzylquercetin tetraacetate[6] or a procedure involving selective protection–deprotection of the 3′,4′-catechol of quercetin.[7] However, both methods provided unsatisfactory yields of the desired compound. Here, we developed an efficient procedure for preparation of tamarixetin. It is well documented that the acidity of the phenolic hydroxyl groups in quercetin is in an order of 7 > 4′ > 3 > 3′ > 5,[6,8,9] which also represents the order of susceptibility of the corresponding acetate toward nucleophilic removal. Recently, we found PhSH

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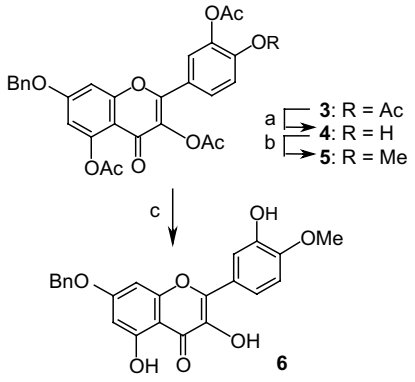
(in the presence of imidazole in *N*-methyl pyrolidinone (NMP)) was able to selectively remove the 7-*O*-acetate in quercetion pentaacetate.[3b] With the 7-OH being blocked with a benzyl group, 7-*O*-benzylquercetin tetraacetate (**3**),[9] upon treatment with PhSH and imidazole in NMP at low temperature (−20 °C), gave the free 4′-OH product **4** in high selectivity (86%). Then methylation of the 4′-OH followed by removal of the remaining 3-, 3′-, and 5-*O*-acetyl groups provided 7-*O*-benzyltamarixetin conveniently (Scheme 1). NOE correlation between H-5′ (δ 7.10 ppm, d, J 8.6 Hz) and the methoxy protons (δ 3.85 ppm, s) in compound **5** was observed, confirming the expected 4′-*O*-methylation.

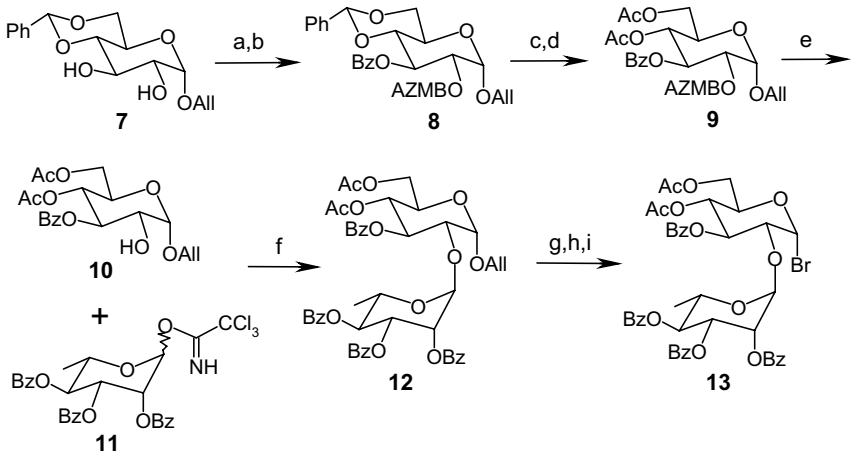
Selective coupling of a flavonol 3,3′,5-triol with a glycosyl α-bromide under phase-transfer-catalyzed (PTC) conditions has been a reliable protocol for preparation of the corresponding 3-*O*-β-glycoside.[3a,10] The hither required neohesperidosyl α-bromide was prepared using a new procedure (Scheme 2). Allyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**7**) was selectively protected with 2-(azidomethyl)benzoyl (AZMB) on the 2-OH,[11] and subsequent protection of the 3-OH with a benzoyl group, provided **8** in high yield (88% for two steps). After converting the 4,6-*O*-benzylidene into 4,6-di-*O*-acetyl compound, providing **9**, the 2-*O*-AZMB was selectively removed with Bu_3P to afford 2-OH allyl glucoside **10** (83%). Coupling of **10** with 2,3,4-tri-*O*-benzoyl-L-rhamnopyranosyl trichloroacetimidate **11**[12] under the promotion of TMSOTf provided disaccharide **12** in 92% yield. Finally, removal of the anomeric allyl group (with $PdCl_2$ in MeOH), acetylation of the resulting lactol, and subsequent treatment of the acetate with 33% HBr in HOAc readily provided the desired disaccharide α-bromide **13** (59% for three steps).

Expectedly, treatment of quercetin triol **6** with disaccharide α-bromide **13** under PTC conditions ($Bu_4N^+Br^-$ (TBAB), K_2CO_3, $CHCl_3/H_2O$, 40 °C) provided 3-*O*-β-glycoside **14** in a satisfactory 66% yield (Scheme 3). No α-anomer was isolated. Noteworthy is a similar coupling between 4′-*O*-benzyl-7-*O*-methylquercetin and α-bromoneohesperidose hexaacetate under Koenigs–Knorr conditions in the presence of Ag_2CO_3 had provided the corresponding flavonol 3-*O*-neohesperidoside in 46% yield.[13] The 7-*O*-benzyl group on **14** was then cleaved cleanly by hydrogenolysis under normal pressure over Pd–C. Subsequent removal of the acetate and benzoate groups on the sugar moiety with NaOMe in methanol afforded the target tamarixetin 3-*O*-α-neohesperidoside (**1**). It is worth noting that the present synthetic route (toward **1**) distinguishes sequentially the 7,4′,3-OHs in flavonol (quercetin) and is thus applicable to the diversity-directed synthesis of flavonol derivatives.

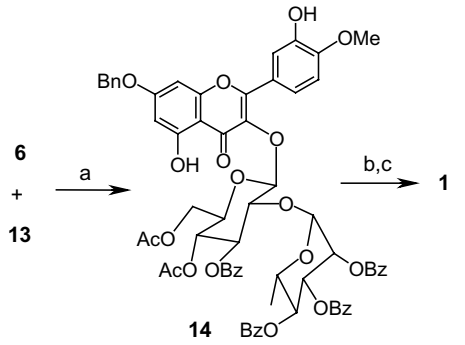
Similar PTC conditions described above were employed for the coupling of the ready available 4′,7-di-*O*-benzylquercetin triol **15**[9] with neohesperidosyl



Scheme 1. Reagents and conditions: (a) PhSH, imidazole, NMP, −20 °C, 86%. (b) CH_3I, K_2CO_3, DMF, rt, 90%. (c) 10% aq NaOH, MeOH, reflux, 100%.

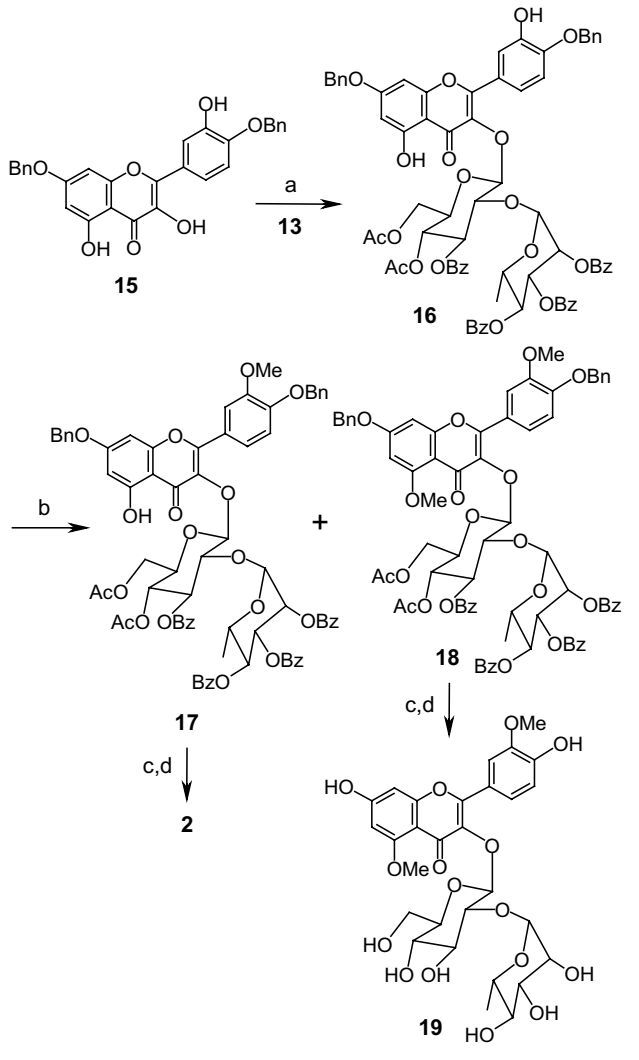


Scheme 2. Reagents and conditions: (a) AZMBCl, DMAP, CH_2Cl_2, 0 °C; (b) BzCl, pyridine, 0 °C, 88% (for two steps). (c) *p*-TsOH, CH_2Cl_2–MeOH, reflux; (d) Ac_2O, pyridine–CH_2Cl_2, 0 °C, 88%. (e) Bu_3P, H_2O (5.0 equiv), THF, rt, 83%. (f) TMSOTf (0.2 equiv), CH_2Cl_2, 4 Å MS, rt, 92%. (g) $PdCl_2$, MeOH–CH_2Cl_2, rt; (h) Ac_2O, pyridine, rt, 66% (two steps). (i) 33% HBr–HOAc, CH_2Cl_2, 0 °C, 90%.



Scheme 3. Reagents and conditions: (a) TBAB, K_2CO_3, $CHCl_3$–H_2O, 40 °C, 66%. (b) H_2, 10% Pd–C, EtOH–EtOAc, 40 °C; (c) NaOMe, MeOH–CH_2Cl_2, rt, 86% (two steps).

α-bromide**13**, providing the corresponding 3-*O*-β-glycoside **16** in 61% yield (Scheme 4). Methylation of the 3′,5-diol **16** with 1 equiv of iodomethane produced the desired 3′-*O*-methyl **17** as the major product (53%); and the 3′,5-di-*O*-methyl product **18** was isolated in trace amount. Finally, removal of the benzyl groups with hydrogen over Pd–C and the acyl groups with NaOMe (in methanol), afforded isorhamnetin 3-*O*-neohesperidoside **2** and 3′,5-di-*O*-methylquercetin 3-*O*-neohesperidoside (**19**), respectively, in high yield.



Scheme 4. Reagents and conditions: (a) TBAB, K_2CO_3, $CHCl_3$–H_2O, 40 °C, 61%. (b) MeI (1 equiv), DMF, rt, 53% (for **17**), trace (for **18**). (c) H_2, 10% Pd–C, EtOH–EtOAc, 40 °C; (d) NaOMe, MeOH–CH_2Cl_2, rt, 86% (two steps for **2**), 81% (two steps for **19**).

Comparing the ^1H and ^{13}C NMR signals of the synthetic flavonol glycoside **1** with those assigned for the natural compound, two ^1H NMR signals were found in discrepancy. The H-2′ and H-6′ signals of the synthetic compound were at 7.47 ppm (d, *J* 1.8 Hz) and 7.66 ppm (dd, *J* 1.8, 8.8 Hz), respectively; while those for the nature product were at 7.98 ppm (d, *J* 1.8 Hz) and 7.54 ppm (dd, *J* 1.8, 8.4 Hz).[1] The presence of an H-2′ signal upfield from that of H-6′ for tamarixetin (4′-*O*-methylation) derivatives is well documented in the literature.[14] Furthermore, the NOE correlation between H-5′ and the methoxy protons and the HMBC correlation between C-4′ and the methoxy protons confirmed unambiguously the 4′-*O*-methylation structure in the synthetic compound. In addition, the NMR signals assigned for the natural flavonol glycosides **1**,[1] **2**,[4] and the synthetic compound **2** are fully identical. Therefore, the natural flavonol glycoside from *C. spicatus* originally assigned as tamarixetin 3-*O*-neohesperidoside (**1**) should be revised into isorhamnetin 3-*O*-neohesperidoside (**2**).

3. Experimental

3.1. General methods

See Ref. 15.

3.2. 3,3′,5-Tri-*O*-acetyl-7-*O*-benzylquercetin (4)

A mixture of **3** (820 mg, 1.46 mmol), PhSH (0.194 mL, 1.90 mmol), and imidazole (40 mg, 0.59 mmol) in anhyd NMP (3.0 mL) was stirred at −20 °C for one day under argon. The reaction mixture was diluted with EtOAc and washed with 1 N HCl, water, and brine, respectively. The organic phase was then dried over Na_2SO_4 and concentrated in vacuo. Flash column chromatography on silica gel (2:1 toluene–EtOAc) afforded **4** (652 mg, 86% yield) as a yellow solid: ^1H NMR (DMSO-d_6, 300 MHz): δ 10.68 (s, 1H), 7.69 (dd, *J* 2.2, 8.8 Hz, 1H), 7.63 (d, *J* 2.2 Hz, 1H), 7.53–7.33 (m, 6H), 7.12 (d, *J* 8.5 Hz, 1H), 6.95 (d, *J* 2.5 Hz, 1H), 5.29 (s, 2H), 2.33 (s, 3H), 2.31 (s, 3H), 2.30 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.3, 169.8, 168.6, 168.0, 163.0, 158.0, 154.3, 150.6, 138.5, 135.5, 128.8, 128.5, 127.5, 126.9, 123.0, 117.6, 109.3, 100.0, 70.9, 21.0, 20.6, 20.4; HR-MALDI-MS (*m*/*z*): Calcd for $C_{28}H_{22}O_{10}Na$ (M^++Na): 541.1105. Found 541.1106.

3.3. 3,3′,5-Tri-*O*-acetyl-7-*O*-benzyl-4′-*O*-methylquercetin (5)

A solution of **4** (314 mg, 0.61 mmol), K_2CO_3 (125 mg, 0.91 mmol), and MeI (49 μL, 0.79 mmol) in anhyd DMF (4.0 mL) was stirred at room temperature overnight under argon. The resulting mixture was diluted with water and extracted with EtOAc. The organic layer was washed with 1 N HCl and water, respectively, and dried over Na_2SO_4. The residue obtained after evaporation of the solvent was purified by flash chromatography on silica gel (5:1 toluene–EtOAc) to afford **5** (290 mg, 90% yield) as a white foam: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.71 (dd, *J* 2.4, 9.0 Hz, 1H, 6′-H), 7.53 (d, *J* 2.4 Hz, 1H, 2′-H), 7.52–7.36 (m, 5H), 7.06 (d, *J* 8.4 Hz, 1H, 5′-H), 6.91 (d, *J* 2.4 Hz, 1H, 8-H), 6.70 (d, *J* 2.4 Hz, 1H, 6-H), 5.15 (s, 2H, PhCH_2), 3.91 (s, 3H, OCH_3), 2.43 (s, 3H), 2.36 (s, 3H), 2.32 (s, 3H); ESIMS (*m*/*z*): 533.2 (M^++1); Anal. Calcd for $C_{29}H_{24}O_{10}$: C, 65.41; H, 4.54. Found: C, 65.66; H, 4.78.

3.4. 7-*O*-Benzyl-4′-*O*-methylquercetin (6)

A suspension of compound **5** (216 mg) in MeOH (15 mL) and 10% aq NaOH (15 mL) was refluxed for several min. The reaction mixture was diluted with water and neutralized with 1 N HCl. The yellow solid was collected and dried (164 mg, 100%): ^{1}H NMR (DMSO-d_6, 300 MHz): δ 12.47 (s, 1H, 5-O*H*), 9.62 (s, 1H), 7.72 (s, 1H, 2′-H), 7.68 (d, *J* 8.7 Hz, 1H, 6′-H), 7.50–7.35 (m, 5H), 7.10 (d, *J* 8.6 Hz, 1H, 5′-H), 6.82 (s, 1H, 8-H), 6.44 (s, 1H, 6-H), 5.23 (s, 2H, PhCH_2), 3.85 (s, 3H, OCH_3); EIMS (*m*/*z*): 406 (M^+); Anal. Calcd for $C_{23}H_{18}O_7$: C, 67.98; H, 4.46. Found: C, 67.41; H, 4.78.

3.5. Allyl 2-*O*-(2-azidomethyl)benzoyl-3-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside (8)

To a stirring solution of **7** (1.4 g, 4.51 mmol) in anhyd CH_2Cl_2 (12 mL) at 0 °C, was added DMAP (605 mg, 1.1 equiv), followed by a solution of 2-(methylazido)benzoyl (AZMB) chloride in CH_2Cl_2. After the starting material disappeared in TLC, dry pyridine (2 mL) and BzCl (1 mL) was added to the solution. After stirring for 2 h at room temperature, the solvent was evaporated. The residue was diluted with CH_2Cl_2, and then washed successively with 1 N HCl, water, and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. Flash column chromatography (9:1 petroleum ether–EtOAc) on silica gel afforded **8** (2.29 g, 88%) as a white syrup: $[\alpha]_D^{25}$ +82 (*c* 0.83, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.04–7.88 (m, 3H), 7.55–7.30 (m, 11H), 6.07 (t, *J* 9.6 Hz, 1H), 5.88 (m, 1H), 5.57 (s, 1H), 5.37–5.26 (m, 2H), 5.19 (d, *J* 10.4 Hz, 1H), 4.76 (dd, *J* 2.4, 8.2 Hz, 1H), 4.72 (d, *J* 8.2 Hz, 1H), 4.64 (dd, *J* 2.4, 10.2 Hz, 1H), 4.36 (dd, *J* 4.8, 10.4 Hz, 1H), 4.27 (dd, *J* 5.2, 12.9 Hz, 1H), 4.16 (dd, *J* 4.9, 9.9 Hz, 1H), 4.06 (m, 1H), 3.91 (t, *J* 9.6 Hz, 1H), 3.86 (t, *J* 10.4 Hz, 1H); ESIMS (*m*/*z*): 593.2 (M^++Na).

3.6. Allyl 4,6-di-*O*-acetyl-2-*O*-(2-azidomethyl)benzoyl-3-*O*-benzoyl-α-D-glucopyranoside (9)

A solution of **8** (1.078 g, 1.89 mmol) and *p*-TsOH (359 mg) in 1:1 CH_3OH–CH_2Cl_2 (10 mL) was refluxed overnight, then neutralized with Et_3N, and concentrated in vacuo. The residue was dissolved in anhyd pyridine (2.5 mL), followed addition of Ac_2O (2.0 mL). After stirring for 2 h, the solvent was evaporated. The residue was dissolved in CH_2Cl_2, and washed successively with 1 N HCl, water, and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (6:1 petroleum ether–EtOAc) to afford **9** (951 mg, 89%) as a white syrup: $[\alpha]_D^{25}$ +117 (*c* 0.84, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.99–7.92 (m, 3H), 7.58–7.30 (m, 6H), 5.97 (t, *J* 9.6 Hz, 1H), 5.93–5.82 (m, 1H), 5.39–5.26 (m, 4H), 5.20 (dt, *J* 1.3, 9.3 Hz, 1H), 4.71 (AB, *J* 15.1 Hz, 2H), 4.35 (dd, *J* 4.2, 12.0 Hz, 1H), 4.26 (dd, *J* 4.2, 12.0 Hz, 1H), 4.22–4.12 (m, 2H), 4.08 (dd, *J* 6.0, 14.1 Hz, 1H), 2.15 (s, 3H), 1.95 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.7, 169.5, 165.7, 165.6, 137.8, 133.4, 133.1, 133.0, 131.6, 129.7, 129.4, 129.0, 128.4, 128.3, 127.4, 118.2, 95.0, 71.6, 70.6, 68.9, 68.3, 67.6, 61.9, 52.8, 20.8, 20.5; HRESIMS (*m*/*z*): Calcd for $C_{28}H_{29}O_{10}N_3Na$ (M^++Na): 590.1745. Found 590.1735.

3.7. Allyl 4,6-di-*O*-acetyl-3-*O*-benzoyl-α-D-glucopyranoside (10)

To a solution of **9** (740 mg, 1.31 mmol) in THF (13 mL) was added water (117 μL, 5 equiv), followed by tributylphosphine (3 equiv). After stirring for 1 h, the reaction mixture was diluted with CH_2Cl_2 and washed with satd aq $NaHCO_3$ and water, respectively. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (3:1 petroleum ether–EtOAc) to afford **10** (438 mg, 83%) as a white syrup: $[\alpha]_D^{22}$ +91 (*c* 0.44, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.02 (d, *J* 7.8 Hz, 2H), 7.58 (t, *J* 7.8 Hz, 1H), 7.44 (d, *J* 7.8 Hz, 2H), 5.94 (m, 1H), 5.49 (t, *J* 9.8 Hz, 1H), 5.36 (dd, *J* 1.5, 17.1 Hz, 1H), 5.28 (d, *J* 10.2 Hz, 1H), 5.22 (t, *J* 9.8 Hz, 1H), 5.04 (d, *J* 3.6 Hz, 1H), 4.34–4.23 (m, 2H), 4.13–4.03 (m, 3H), 3.82 (m, 1H), 2.12 (s, 3H), 1.93 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.6, 169.5, 166.7, 133.3, 133.0, 129.8, 129.3, 128.4, 118.5, 97.6, 74.0, 71.1, 69.1, 67.0, 67.7, 61.9, 20.7, 20.5; MALDI-MS (*m*/*z*): Calcd for $C_{20}H_{24}O_9Na$ (M^++Na): 431.1313. Found 431.1328.

3.8. Allyl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzoyl-α-D-glucopyranoside (12)

To a solution of **10** (386 mg, 0.24 mmol) and the rhamnopyranosyl trichloroacetimidate **11** (705 mg, 1.14 mmol) in anhyd CH_2Cl_2 (4 mL) in the presence of 4 Å molecular sieves under argon was added TMSOTf in CH_2Cl_2 (0.20 equiv) at room temperature. The mixture was stirred until TLC indicated the completion of the reaction. The mixture then was neutralized with Et_3N. After filtration and concentration, the residue was purified by silica gel column chromatography (3:1 petroleum ether–EtOAc) to give **12** (745 mg, 92) as a white foam: $[\alpha]_D^{18}$ +169 (*c* 0.85, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ 8.04 (d, *J* 8.7 Hz, 2H), 7.96–7.92 (m, 4H), 7.73 (d, *J* 8.7 Hz, 2H), 7.46–7.35 (m, 10H), 7.23 (t, *J* 8.7 Hz, 2H), 6.05 (m, 1H), 5.88 (t, *J* 9.8 Hz, 1H), 5.77 (dd, *J* 3.2, 10.0 Hz, 1H), 5.56 (t, *J* 10.0 Hz, 1H), 5.46 (dd, *J* 1.3, 17.3 Hz, 1H), 5.36–5.5.27 (m, 2H), 5.23 (t, *J* 9.8 Hz, 1H), 5.15 (d, *J* 3.6 Hz, 1H), 5.11 (s, 1H), 4.28–4.21 (m, 3H), 4.19–4.04 (m, 3H), 4.00 (dd, *J* 3.6, 9.9 Hz, 1H), 2.12 (s, 3H), 1.96 (s, 3H), 1.28 (d, *J* 6.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.7, 169.7, 165.8, 165.7, 164.9, 133.3, 133.2, 133.1, 132.9, 129.9, 129.8, 129.7, 129.6, 129.4, 129.2, 129.1, 128.5, 128.4, 128.3, 128.1, 118.5, 99.4, 96.9, 78.2, 71.8, 71.7, 70.6, 69.2, 69.0, 68.5, 67.6, 67.3, 62.0, 20.8, 20.6, 17.7; HRESIMS (*m*/*z*): Calcd for $C_{47}H_{46}O_{16}Na$ (M^++Na): 889.2678; Found 889.2681; Anal. Calcd for $C_{47}H_{46}O_{16}$: C, 65.12; H, 5.35. Found: C, 64.90; H, 5.41.

3.9. 2,3,4-Tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzoyl-α-D-glucopyranosyl bromide (13)

A dark suspension of palladium chloride (74 mg, 0.42 mmol) and compound **12** (605 mg, 0.7 mmol) in 1:1 MeOH–CH_2Cl_2 (4 mL) was stirred at room temperature until TLC indicated the completion of the reaction. The mixture was filtered through a bed of Celite and concentrated in vacuo. The residue was dissolved in anhyd pyridine (2 mL), followed by addition of Ac_2O (1.5 mL). After stirring for 1 h, the solvent was evaporated. The residue was dissolved in CH_2Cl_2 and then washed with 1 N HCl, water, and brine, respectively. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (3:1 petroleum ether–EtOAc) to afford a α/β mixture of the corresponding acetate (399 mg, 66% yield) as a white foam: ESIMS (*m*/*z*): 891.55 (M^++Na). The acetate (323 mg, 0.37 mmol) was dissolved in anhyd CH_2Cl_2 (3 mL), and 33% HBr–NHOAc (1.0 mL, 6.0 mmol) was added slowly at 0 °C under argon. The resulting mixture was diluted with CH_2Cl_2 until TLC showed that the starting material disappeared, and then it was neutralized with satd aq $NaHCO_3$. The organic layer was washed with water and brine, respectively, and then dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel chromatography (7:2 petroleum ether–EtOAc) to afford **13** (296 mg, 90%) as a white foam: $[\alpha]_D^{17}$ +201 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ 8.06 (d, *J* 8.4 Hz, 2H), 7.98 (d, *J* 8.7 Hz, 2H), 7.94 (d, *J* 8.4 Hz, 2H), 7.75 (d, *J* 8.4 Hz, 2H), 7.62–7.37 (m, 10H), 7.22 (t, *J* 7.7 Hz, 2H), 5.60 (d, *J* 3.6 Hz, 1H), 5.88 (t, *J* 9.8 Hz, 1H), 5.74 (dd, *J* 3.6, 9.9 Hz, 1H), 5.62 (t, *J* 9.8 Hz, 1H), 5.39–5.30 (m, 2H), 5.18 (s, 1H), 4.45–4.38 (m, 3H), 4.21–4.16 (m, 1H), 4.04 (dd, *J* 4.2, 9.6 Hz, 1H), 2.13 (s, 3H), 2.01 (s, 3H), 1.36 (d, *J* 6.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.5, 169.5, 165.8, 165.6, 164.9, 133.4, 133.3, 133.0, 129.9, 129.8, 129.6, 129.1, 128.5, 128.4, 128.2, 100.2, 88.8, 78.8, 72.4, 72.1, 71.4, 70.6, 69.2, 68.6, 67.1, 61.1, 20.7, 20.5, 17.5; HRESIMS (*m*/*z*): Calcd for $C_{44}H_{41}BrO_{15}Na$ (M^++Na): 911.1521. Found 911.1519.

3.10. 7-*O*-Benzyl-4′-*O*-methylquercetin-3-yl-2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzoyl-β-D-glucopyranoside (14)

A mixture of **6** (30 mg, 0.074 mmol), TBAB (24 mg, 1 equiv), K_2CO_3 (28 mg, 0.2 mmol) in 2:1 $CHCl_3$–H_2O (3 mL) was stirred at 40 °C for 1 h. Then, the glycosyl bromide **13** (118 mg, 0.13 mmol) was added, and stirring continued at 40 °C for 1 day. The reaction mixture was diluted with CH_2Cl_2, washed with 1 N HCl, water, and brine, respectively. The organic layer was then dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel chromatography (4:1 toluene–EtOAc) to afford **14** (60 mg, 66%) as a yellow foam: $[\alpha]_D^{15}$ −10 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ 12.71 (s, 1H, 5-OH), 8.05 (d, *J* 7.2 Hz, 2H), 7.91 (d, *J* 7.2 Hz, 2H), 7.81 (t, *J* 7.2 Hz, 4H), 7.69–7.66 (m, 2H), 7.55 (t, *J* 7.3 Hz, 1H), 7.45–7.17 (m, 16H), 6.97 (d, *J* 7.5 Hz, 1H), 6.53 (d, *J* 2.1 Hz, 1H), 6.50 (d, *J* 2.1 Hz, 1H), 6.07 (dd, *J* 3.3, 9.9 Hz, 1H), 5.88 (s, 1H), 5.74 (d, *J* 7.5 Hz, 1H), 5.69 (t, *J* 9.3 Hz, 1H), 5.55 (t, *J* 9.9 Hz, 1H), 5.51 (m, 1H), 5.25 (t, *J* 9.6 Hz, 1H), 5.22 (s, 1H), 5.16 (s, 2H), 4.77 (m, 1H), 4.19 (t, *J* 8.4 Hz, 1H), 4.05–4.00 (m, 2H), 3.99 (s, 3H), 3.76 (m, 1H), 1.95 (s, 3H), 1.94 (s, 3H), 1.13 (d, *J* 6.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 177.6, 170.5, 169.7, 165.8, 165.4, 165.1, 164.7, 164.6, 162.1, 157.7, 156.9, 148.9, 145.2, 135.7, 134.0, 133.1, 132.8, 130.0, 129.7, 129.3, 129.1, 128.8, 128.4, 128.3, 128.1, 127.4, 123.5, 122.1, 115.1, 110.1, 106.1, 99.9, 98.8, 98.4, 93.1, 75.1, 71.8, 71.6, 70.7, 70.4, 69.5, 68.3, 67.1, 61.8, 56.0, 20.5, 20.4, 17.1; HRESIMS (*m*/*z*): Calcd for $C_{67}H_{58}O_{22}Na$ (M^++Na): 1237.3312. Found 1237.3296.

3.11. 4′-*O*-Methylquercetin-3-yl α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (1)

A solution of **14** (104 mg, 0.086 mmol) in 1:1 EtOH–EtOAc (4 mL) was treated with a catalytic amount of 10% Pd–C. After stirring under 1 atm of H_2 at 40 °C for 7 h, the reaction mixture was filtered through a pad of Celite and concentrated in vacuo. The residue was dissolved in 2:3 CH_2Cl_2–CH_3OH (15 mL), followed by addition of a catalytic amount of CH_3ONa. After stirring for 4 h at room temperature, the mixture was neutralized with Dowex 50-X8 (H^+) resin. The resin was filtered off and washed with CH_3OH. The filtrate and washings were combined and concentrated. The residue was purified by silica gel column chromatography (2:1 toluene–CH_3OH) to give **1** (46 mg, 86%) as a yellow solid: $[\alpha]_D^{20}$ −94 (*c* 0.56, CH_3OH); ^1H NMR (DMSO-d_6, 400 MHz): δ 12.56 (s, 1H, 5-OH), 7.66 (dd, *J* 1.8, 8.8 Hz, 1H, H-6′), 7.47 (d, *J* 1.8 Hz, 1H, H-2′), 6.97 (d, *J* 8.8 Hz, 1H, H-5′), 6.35 (d, *J* 1.8 Hz, 1H, H-8), 6.13 (d, *J*1.8 Hz, 1H, H-6), 5.61 (d, *J* 7.7 Hz, 1H, GlcH-1), 5.00 (br s, 1H, RhaH-1), 3.79 (s, 3H, OC*H*$_3$), 0.68 (d, *J* 6.2 Hz, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 177.3, 164.5, 161.2, 156.4, 155.7, 150.0, 146.0, 133.2, 122.7, 121.5, 115.5, 111.3, 103.9, 100.6, 98.8, 98.4, 93.6, 77.6, 77.4, 77.3, 71.8, 70.6, 70.2, 69.7, 68.3, 60.9, 55.7, 17.2; HRESIMS (*m*/*z*): Calcd for $C_{28}H_{32}O_{16}Na$ (M^++Na): 647.1583. Found 647.1570.

3.12. 4′,7-Di-*O*-benzylquercetin-3-yl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzoyl-β-D-glucopyranoside (16)

A mixture of 4′,7-di-*O*-benzylquercetin **15** (96 mg, 0.2 mmol), TBAB (64 mg, 0.2 mmol), and K_2CO_3 (55 mg, 0.6 mmol) in 1:1 $CHCl_3$–H_2O (4 mL) was stirred at 40 °C for 1 h. Then, the glycosyl bromide **13** (247 mg, 0.3 mmol) added, and stirring was continued at 40 °C for 1 day. The reaction mixture was then diluted with CH_2Cl_2, and washed with 1 N HCl, water, and brine, respectively. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purification by silica gel column chromatography (2:1 toluene–EtOAc) to afford **16** (157 mg, 61%) as a yellow foam: $[\alpha]_D^{22}$ −18 (*c* 0.75, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ 12.66 (s, 1H, 5-OH), 8.11 (d, *J* = 7.1 Hz, 1H), 8.04 (d, *J* 7.1 Hz, 2H), 7.90 (d, *J* 7.1 Hz, 2H), 7.82 (d, *J* 7.1 Hz, 2H), 7.78 (d, *J* 7.4 Hz, 2H), 7.69–7.56 (m, 2H), 7.53–7.15 (m, 21H), 7.03 (d, *J* 8.5 Hz, 1H), 6.52 (d, *J* 2.2 Hz, 1H), 6.50 (d, *J*2.2 Hz, 1H), 6.05 (dd, *J* 3.4, 10.0 Hz, 1H), 5.76 (d, *J* 7.4 Hz, 1H), 5.69 (t, *J* 9.3 Hz, 1H), 5.54 (t, *J* 9.9 Hz, 1H), 5.50 (m, 1H), 5.25–5.15 (m, 2H), 5.21 (s, 2H), 5.15 (s, 2H), 4.74 (m, 1H), 4.18 (dd, *J* 7.7, 9.1 Hz, 1H), 4.11 (m, 2H), 3.76 (m, 1H), 1.93 (s, 3H), 1.92 (s, 3H), 1.12 (d, *J* 6.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 177.5, 170.6, 169.7, 165.7, 165.4, 165.1, 164.6, 164.5, 162.0, 157.5, 156.7, 148.0, 145.4, 135.7, 135.6, 134.0, 133.6, 133.1, 132.8, 130.1, 129.9, 129.6, 129.3, 129.2, 129.0, 128.7, 128.5, 128.4, 128.2, 128.0, 127.8, 127.5, 123.7, 122.0, 115.3, 110.5, 106.0, 99.8, 98.7, 98.3, 93.1, 75.0, 71.8, 71.5, 71.0, 70.7, 70.4, 69.5, 68.2, 67.0, 61.7, 20.5, 20.4, 17.1; HRESIMS (*m*/*z*): Calcd for $C_{73}H_{63}O_{22}$ (M^++H): 1291.3806. Found 1291.3845.

3.13. 4′,7-Di-*O*-benzyl-3′-*O*-methylquercetin-3-yl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzoyl-β-D-glucopyranoside (17) and 4′,7-di-*O*-benzyl-3′,5-di-*O*-methylquercetin-3-yl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzoyl-β-D-glucopyranoside (18)

A solution of **16** (50 mg, 0.039 mmol), K_2CO_3 (6 mg, 1.1 equiv), and MeI (2.4 μL, 1.0 equiv) in anhydrous DMF (1.0 mL) was stirred at room temperature for 1 day under argon. The resulting mixture was diluted with water, and extracted with EtOAc. The organic layer, after being washed with 1 N HCl and water, respectively, was dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (7:2 toluene–EtOAc) to afford the 3′-*O*-methyl **17** (27 mg, 53%). 3′,5-Di-*O*-methyl **18** (18 mg, 36%) was isolated as yellow foams when 1.5 equiv of MeI was used in the reaction. **17**: $[\alpha]_D^{22}$ −20 (*c* 0.45, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ 12.71 (s, 1H, 5-OH), 8.07 (d, *J* 7.1 Hz, 2H), 7.92 (d, *J* = 7.1 Hz, 2H), 7.85 (d, *J* = 6.9 Hz, 2H), 7.81 (d, *J* 7.1 Hz, 2H), 7.76 (d, *J* 1.9 Hz, 1H), 7.65 (dd, *J* 1.9, 8.5 Hz, 1H), 7.57 (t, *J* 7.4 Hz, 1H), 7.50–7.21 (m, 21H), 7.02 (d, *J* 8.5 Hz, 1H), 6.56 (d, *J* 2.2 Hz, 1H), 6.53 (d, *J* 2.2 Hz, 1H), 6.09 (dd, *J* 3.4, 10.0 Hz, 1H), 5.82 (d, *J* 7.7 Hz, 1H), 5.74 (t, *J* 9.3 Hz, 1H), 5.57 (t, *J* 9.9 Hz, 1H), 5.53 (m, 1H), 5.30–5.15 (m, 2H), 5.28 (s, 2H), 5.18 (s, 2H), 4.78 (m, 1H), 4.18–3.95 (m, 3H), 4.03 (s, 3H), 3.76 (m, 1H), 1.96 (s, 3H), 1.92 (s, 3H), 1.12 (d, *J* 6.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 177.5, 170.3, 169.6, 165.8, 165.4, 165.1, 164.6, 162.2, 157.7, 156.8, 150.6, 148.9, 136.4, 135.7, 133.9, 133.1, 132.8, 130.0, 129.7, 129.4, 129.1, 128.8, 128.7, 128.3, 128.1, 127.4, 127.2, 123.1, 122.4, 112.7, 106.1, 100.1, 98.8, 98.4, 93.2, 77.7, 75.0, 71.8, 71.7, 70.7, 70.5, 69.5, 68.1, 67.1, 61.5, 56.2, 20.5, 20.4, 17.1; HRESIMS (*m*/*z*): Calcd for $C_{74}H_{65}O_{22}$ (M^++H): 1305.3962. Found 1305.3984.

18: $[\alpha]_D^{23}$ −38 (*c* 0.93, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ 8.03 (d, *J* 7.1 Hz, 2H), 7.93 (d, *J* 7.1 Hz, 2H), 7.80 (d, *J* 7.1 Hz, 2H), 7.76 (m, 3H), 7.60 (dd, *J* 1.9, 8.5 Hz, 1H), 7.53 (t, *J* 7.4 Hz, 1H), 7.49–7.24 (m, 20H), 7.19 (t, *J* 7.7 Hz, 1H), 6.98 (d, *J* 8.8 Hz, 1H), 6.59 (d, *J* 1.9 Hz, 1H), 6.48 (d, *J* 1.9 Hz, 1H), 6.17 (d, *J* 7.7 Hz, 1H), 5.90 (dd, *J* 3.6, 9.9 Hz, 1H), 5.68 (t, *J* 9.3 Hz, 1H), 5.51 (t, *J* 10.0 Hz, 1H), 5.49 (m, 1H), 5.30–5.12 (m, 2H), 5.25 (s, 2H), 5.14 (s, 2H), 4.63 (m, 1H), 4.18–3.95 (m, 3H), 4.02 (s, 3H), 3.99 (s, 3H), 3.81

(m, 1H), 1.93 (s, 3H), 1.87 (s, 3H), 0.99 (d, *J* 6.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 172.8, 170.3, 169.7, 165.8, 165.3, 164.8, 164.5, 163.0, 161.1, 158.6, 154.3, 150.0, 148.9, 136.6, 135.6, 135.4, 133.0, 132.9, 132.7, 129.9, 129.7, 129.6, 129.4, 129.1, 128.7, 128.6, 128.4, 128.2, 128.1, 128.0, 127.5, 127.2, 123.5, 121.9, 112.8, 112.7, 109.5, 98.9, 98.5, 96.4, 93.4, 78.5, 75.0, 71.8, 71.6, 70.7, 70.5, 69.5, 68.3, 67.0, 61.4, 56.4, 56.3, 20.5, 20.4, 16.9; HRESIMS (*m*/*z*): Calcd for $C_{75}H_{67}O_{22}$ (M^++H): 1319.4119; Found 1319.4136.

3.14. 3′-*O*-methylquercetin-3-yl α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (2)

A suspension of **17** (104 mg, 0.086 mmol) and a catalytic amount of 10% Pd–C in 1:1 EtOH–EtOAc (4 mL) was stirred under 1 atm of H_2 at 40 °C for 7 h. The reaction mixture was filtered through a bed of Celite, and concentrated in vacuo. The residue was dissolved in 2:3 CH_2Cl_2–CH_3OH (15 mL), followed by addition of a catalytic amount of CH_3ONa. After stirring for 4 h at room temperature, the mixture was neutralized with Dowex 50-X8 (H^+) resin. The resin was filtered off and washed with CH_3OH. The filtrate and washings were combined and concentrated. The residue was purified by a silica gel column chromatography (2:1 toluene–CH_3OH) to give **2** (46 mg, 86%) as a yellow solid: $[\alpha]_D^{22}$ −79 (*c* 0.11, MeOH); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 12.59 (s, 1H, 5-OH), 7.94 (d, *J* 1.9 Hz, 1H, H-2′), 7.48 (dd, *J* 1.9, 8.5 Hz, 1H, H-6′), 6.92 (d, *J* 8.5 Hz, 1H, H-5′), 6.41 (br s, 1H, H-8), 6.18 (br s, 1H, H-6), 5.76 (d, *J* 7.4 Hz, 1H, GlcH-1), 5.03 (br s, 1H, RhaH-1), 3.86 (s, 3H, OCH_3), 0.65 (d, *J* 6.0 Hz, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 177.0, 165.0, 161.0, 156.2, 155.7, 149.2, 146.7, 133.3, 121.6, 120.9, 115.0, 113.3, 103.5, 100.6, 98.8, 98.2, 93.7, 77.5, 77.2, 76.9, 72.1, 71.5, 70.4, 69.9, 68.1, 60.3, 55.5, 16.8; HRESIMS (*m*/*z*): Calcd for $C_{28}H_{32}O_{16}Na$ (M^++Na): 647.1583. Found 647.1601.

3.15. 3′,5-di-*O*-methylquercetin-3-yl α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (19)

A similar procedure as described above for the preparation of **2** from **17** was used for the preparation of **19** from **18**. **19**: $[\alpha]_D^{22}$ −76 (*c* 0.45, MeOH); ^{1}H NMR (pyridine-d_5, 300 MHz): δ 8.64 (s, 1H, H-2′), 7.72 (d, *J* 7.8 Hz, 1H, H-6′), 6.87 (d, *J* 7.8 Hz, 1H, H-5′), 6.78 (s, 1H, H-8), 6.65 (s, 1H, H-6), 6.38 (s, 1H), 4.93 (m, 1H), 4.86 (s, 1H), 4.79 (d, *J* 9.0 Hz, 1H), 4.57 (t, *J* 8.4 Hz, 1H), 4.36 (t, *J* 7.5 Hz, 1H), 4.29–4.19 (m, 4H), 3.97 (s, 3H), 3.82 (s, 3H), 3.76 (m, 1H), 3.58 (s, 1H), 1.44 (d, *J* 6.0 Hz, 3H); ^{13}C NMR (pyridine-d_5, 100 MHz): δ 173.1, 161.8, 159.3, 157.8, 153.5, 149.2, 147.9, 136.2, 122.8, 122.2, 116.0, 114.3, 108.8, 102.7, 99.7, 97.2, 96.0, 79.5, 79.1, 78.4, 74.1, 72.8, 72.7, 71.7, 69.9, 61.8, 56.2, 56.0, 18.1; ESIMS (*m*/*z*): 661 (M^++Na).

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Supplementary data

Supplementary data associated with this article can be found, in the online version at doi:10.1016/j.carres.2005.04.021.

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