Natural Product Research: Formerly Natural Product Letters
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gnpl20

The first total synthesis of apigenin 7-O-β-D-cellobiosyl-4′-O-β-D-glucopyranoside isolated from Salvia uliginosa

Fatih Sonmez [a], Mehmet Nebioglu [a], Senol Besoluk [b], Mustafa Arslan [a], Mustafa Zengin [a] & Mustafa Kucukislamoglu [a]

[a] Faculty of Art and Sciences, Department of Chemistry, Sakarya University, Sakarya 54140, Turkey

[b] Faculty of Education, Department of Science Education, Sakarya University, Sakarya 54300, Turkey

Version of record first published: 23 May 2012.

To cite this article: Fatih Sonmez, Mehmet Nebioglu, Senol Besoluk, Mustafa Arslan, Mustafa Zengin & Mustafa Kucukislamoglu (2012): The first total synthesis of apigenin 7-O-β-D-cellobiosyl-4′-O-β-D-glucopyranoside isolated from Salvia uliginosa , Natural Product Research: Formerly Natural Product Letters, DOI:10.1080/14786419.2012.686908

To link to this article: http://dx.doi.org/10.1080/14786419.2012.686908

Natural Product Research
2012, 1–8, iFirst

The first total synthesis of apigenin 7-*O*-*β*-D-cellobiosyl-4′-*O*-*β*-D-glucopyranoside isolated from *Salvia uliginosa*

Fatih Sonmez[a], Mehmet Nebioglu[a], Senol Besoluk[b], Mustafa Arslan[a], Mustafa Zengin[a] and Mustafa Kucukislamoglu[a]*

[a]*Faculty of Art and Sciences, Department of Chemistry, Sakarya University, Sakarya 54140, Turkey;* [b]*Faculty of Education, Department of Science Education, Sakarya University, Sakarya 54300, Turkey*

(*Received 6 October 2011; final version received 11 March 2012*)

The first total synthesis of apigenin 7-*O*-*β*-D-cellobioside (**5**) and apigenin 7-*O*-*β*-D-cellobiosyl-4′-*O*-*β*-D-glucopyranoside (**8**), which were isolated from petals of *Salvia patens* and *Salvia uliginosa*, were achieved in four and six steps and 76% and 57%, respectively, overall yield, from naringenin (**1**). The total synthesis contained two-glycosylation, acetylation, oxidation, selective deacetylation and deprotection steps. Although this route contained six steps, the targeted compounds were obtained with higher yields and easier purifications than other synthetic methods.

Keywords: flavonoid glycoside; apigenin 7-O-*β*-D-cellobioside; apigenin 7-O-*β*-D-cellobiosyl-4′-O-*β*-D-glucopyranoside; total synthesis

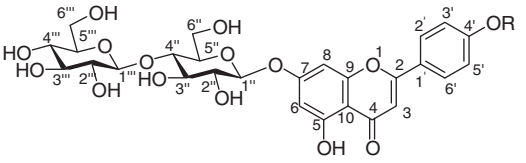
1. Introduction

Flavonoid glycosides, as well as their aglycones, are widely distributed in the plant kingdom (Harborne, 1988, 1994) and have significant biological activities such as antitumour, antimicrobial and radical-scavenging properties (Fraser-Reid & Tatsuta, 2001; Rice-Evans, 1997). Sugar moiety usually is found at the C_7 position of flavonoid *O*-glycosides, but rarely found at the C_4 position. Despite their importance, synthesis of flavonoid glycosides was extremely limited (Bouktaib, Atmani, & Rolando, 2002; Caldwell, Crozier, & Hartley, 2000; Caldwell, Petersson, Farrugia, Mullen, & Hartley, 2006; Du, Wei, & Linhardt, 2003, 2004; Elhabiri, Figueiredo, Fougerousse, & Brouillard, 1995; Iacobucci & Sweeny, 1983; Kondo et al., 2006; Krishnamutry, Krishnamoorthy, & Seshadri, 1963; Li, Han, & Yu, 2003; Maloney & Hecht, 2005; Murakami, Robertson, & Robinson, 1931; Robertson & Robinson, 1927). There are two critical ways on the synthesis of glycosyl flavonoids. The first one is the synthesis of the flavonoid skeleton via cyclisation after glycosylation. The second one is direct glycosylation of the flavonoid skeleton and it is thought that the nucleophilicity of the phenolic hydroxyl group at 7-OH is much higher than that at 4′-OH in flavonoids (Farkas, Wolfner, Nogradi, Wagner, & Hörhammer, 1968; Nogradi, Farkas, Wagner, & Hörhammer, 1967). Actually, direct glycosylation of flavanone skeleton with excess sugar halide and silver carbonate gave only 7-*O*-glycosides.

*Corresponding author. Email: mustafak@sakarya.edu.tr

ISSN 1478–6419 print/ISSN 1478–6427 online

http://dx.doi.org/10.1080/14786419.2012.686908
http://www.tandfonline.com



5: R= H, 8: R= β-D-Glc

Figure 1. Structure of apigenin 7-*O*-*β*-D-cellobioside (**5**) and apigenin 7-*O*-*β*-D-cellobiosyl-4'-*O*-*β*-D-glucopyranoside (**8**).

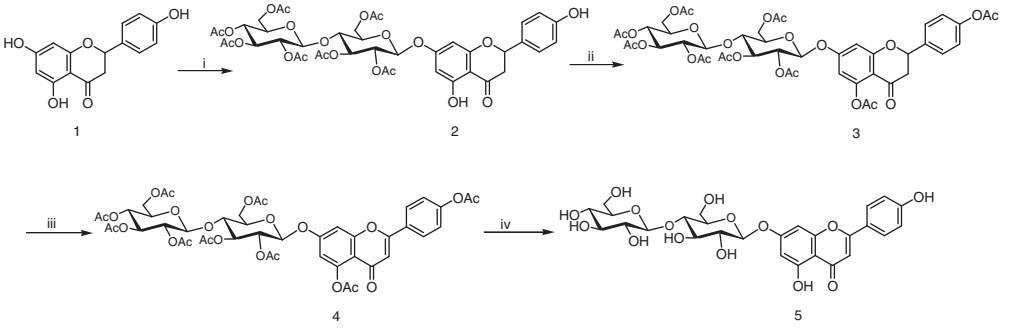


Figure 2. Synthesis of apigenin 7-*O*-cellobioside (**6**): (i) Ag_2CO_3/quinoline, hepta-*O*-acetyl-cellobiosylbromide, 86%; (ii) Ac_2O/H_2SO_4, 93%; (iii) NBS, dibenzoylperoxide/CCl_4, 97%; and (iv) $CH_3O^-\ K^+/CH_3OH/CHCl_3$, 98%.

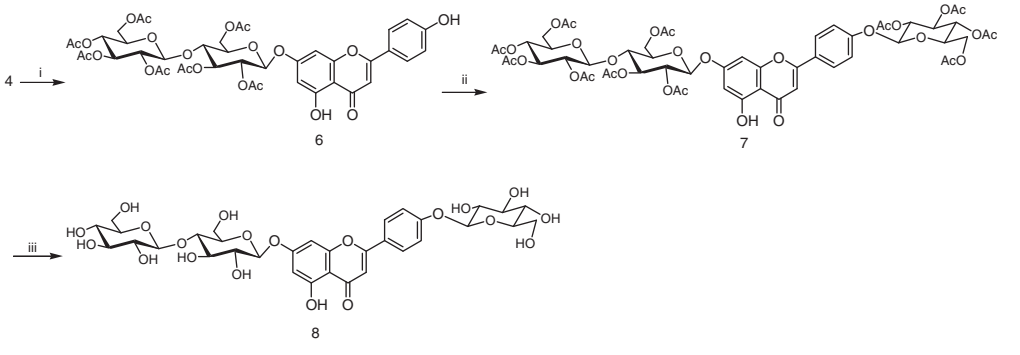


Figure 3. Synthesis of apigenin-7,4'-di-*O*-glycosides (**8**): (i) NH_4OAc/H_2O/MeOH/THF, 91%; (ii)Ag_2CO_3/quinoline, tetra-O-acetyl-glycosylbromide, 84%; and (iii) $CH_3O^-\ K^+/CH_3OH/CHCl_3$, 94%.

In this study, we focused our attention on the first total synthesis of apigenin 7-*O*-*β*-D-cellobioside (**5**) and apigenin 7-*O*-*β*-D-cellobiosyl-4′-*O*-*β*-D-glucopyranoside (**8**), isolated from flower of *Salvia patens* and *Salvia uliginosa* (Veicth, Grayer, Irwin, & Takeda, 1998; Figure 1) via direct glycosylation of naringenin (**1**).

2. Results and discussion

The synthesis of the target compounds was performed by reactions illustrated in Figures 2 and 3. First, naringenin (**1**) was glycosylated at C_7-OH with hepta-*O*-acetyl-cellobiosylbromide in the presence of Ag_2CO_3 in quinoline under Koenigs–Knorr

conditions (Farkas et al., 1968) to give 7-*O*-(Heptaacetyl-β-D-cellobiosyl)-naringenin (**2**). Compound **2** was oxidised with DDQ in 1,4-dioxane (Oyama & Kondo, 2004), but the yield of the reaction was very low at the end of column chromatography. Oxidation of compound **2** was performed with NBS in CCl_4 to improve the yield (Looker & Holm, 1959). The best results were obtained after acetylation of naringenin hydroxyl groups in this step. For this purpose, compound **2** was acetylated with acetic anhydride in the acidic medium. Then, compound **3** was oxidised to compound **4** with NBS in CCl_4 in high yields (97%) (Looker & Holm, 1959). This method has several advantages such as high yields and no further purification, from oxidation of flavonone acetates with DDQ in 1,4-dioxane (Oyama & Kondo, 2004) and I_2 in pyridine (Chen, Huang, Lian, & Lin, 2009). Finally, deacetylation of compound **4** was carried out with $KOCH_3$ in MeOH–$CHCl_3$ (1 : 1) at room temperature and compound **5** was obtained in high yields. The ^{1}H-NMR spectrum of compound **5** contained two distinctive groups of resonances. Those at δ 12.97 (s, 5-OH), 10.48 (s, 4′-OH), 7.94 (d, $J = 8.4$ Hz, H-2′,6′), 6.95(d, $J = 8.2$ Hz, H-3′,5′), 6.84 (s, H-3), 6.82 (s, H-8) and 6.46 (s, H-6), represented aromatic protons, and were consistent with the compound **5**. The second group, appearing between δ 3.07 and 5.60 ppm, comprised both non-exchangeable and exchangeable resonances of glycosidic origin. Two anomeric proton resonances were noted at δ 4.33 and 5.17 ppm, and used as the starting point for the complete assignment of the non-exchangeable glycosyl resonances.

On the other hand, apigenin 7-*O*-β-D-cellobiosyl-4′-*O*-β-D-glucopyranoside (**8**) was synthesised from compound **4** (Figure 3). The selective deacylation of compound **4** was done with NH_4Ac in H_2O–MeOH–THF (1 : 4 : 8) (Ramesh, Mahender, Ravindranath, & Das, 2003) for the glycosylation of the phenolic hydroxyl group at 4′-OH. Compound **6** was glycosylated with tetra-*O*-acetyl-glycosylbromide in the presence of Ag_2CO_3 in quinoline (Farkas et al., 1968; Figure 3) afforded compound **7**. Desired product compound **8** was obtained after deacetylation with $KOCH_3$ in MeOH–$CHCl_3$ (1 : 1) at room temperature. The ^{1}H-NMR spectrum of compound **8** was more complex than the corresponding spectra of compound **5**, but contained features common to them both. Aromatic and phenolic resonances at δ 12.87 (s, 5-OH), 8.05 (d, $J = 8.5$ Hz, H-2′,6′), 7.18 (d, $J = 8.5$ Hz, H-3′,5′), 6.98 (s, H-3), 6.86 (s, H-8) and 6.45 (s, H-6), were consistent with an apigenin derivative substituted at the 4′ position. Three anomeric protons were resonanced at δ 5.16 (d, $J = 7.0$ Hz), 5.02 (d, $J = 6.5$ Hz) and 4.47 (d, $J = 7.9$ Hz). The results confirmed the synthesis of apigenin 7-*O*-β-D-cellobiosyl-4′-*O*-β-D-glucopyranoside (**8**).

Additionally, anomeric configurations are assigned from the magnitude of $J_{1,2}$, with values of 7–9 Hz for a β-configuration and 2–4 Hz indicative coupling of α-anomers for glycosides in ^{1}H-NMR spectrum (Bubb, 2003; Harborne, 1994). The chemical shift of an anomeric carbon in an α-linkage (δ 90–93) is in general less than that of an anomeric carbon in a β-linkage (δ 99–103; Bubb, 2003; Harborne, 1994). The coupling constant of the anomeric proton ($J = 7.3$ Hz) and the chemical shift of C-1″ (δ 100.1) confirmed the β-linkage of cellobiose for compound **5**. Similarly, the coupling constants of anomeric protons ($J = 7.0$ Hz and $J = 7.9$ Hz) and the chemical shift of C-1″ and C-1‴ (δ 100.1 and δ 100.4) confirmed the β-linkage of cellobiose and glucose for compound **8**.

3. Experimental

3.1. *General*

Melting points were taken on a Barnstead Electrothermal 9200. IR spectra were measured on a SHIMADZU Prestige-21 (200 VCE) spectrometer. ^{1}H- and ^{13}C-NMR spectra were

measured on spectrometer VARIAN Infinity Plus at 300 and 75 Hz, respectively. ^{1}H- and ^{13}C-chemical shifts are referenced to the internal deuterated solvent. Mass spectra were obtained using MICROMASS Quattro LC–MS–MS spectrometer. Flash column chromatography was performed using Merck silica gel 60 (230–400 mesh ASTM). Solvents were dried with the standard methods. All chemicals were purchased from Merck, Alfa Easer, Sigma–Aldrich and Fluka.

3.2. *Synthetic procedure and data*

3.2.1. *Heptaacetyl-β-D-cellobiosyl bromide and tetraacetyl-β-D-glycosyl bromide*

Heptaacetyl-β-D-cellobiosyl bromide and tetraacetyl-β-D-glycosyl bromide were prepared by the literature (Redemann & Niemann, 1955).

3.2.2. *7-O-(Heptaacetyl-β-D-cellobiosyl)-naringenin (2)*

A solution of heptaacetyl-β-D-cellobiosyl bromide (2.360 g, 3.37 m mol), Ag_2CO_3 (0.928 g, 3.37 m mol) and naringenin **1** (0.612 g, 2.25 m mol) in quinoline (7 mL) was stirred for 3 h at room temperature. After being poured into 20 mL CH_3OH, the solution was filtered through a short pad of silica gel and evaporated *in vacuo*. The residue was dissolved into 100 mL EtOAc and washed successively with 1 N HCl (2 × 50 mL) and brine (2 × 50 mL), and dried over anhydrous $MgSO_4$. After evaporation, the resulting crude product was purified by flash column chromatography (hexane–EtOAc 1 : 1) to afford **2** (yield 86%, 1.72 g) as a yellowish foam.

IR ($\nu_{max,}$ cm^{-1}): 1743, 1643, 1367, 1213, 1170, 1035 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.99 (3H, s), 2.01 (3H, s), 2.02 (3H, s), 2.05 (3H, s), 2.06 (3H, s), 2.07 (3H, s), 2.10 (3H, s), 2.77 (1H, d, J = 17.3 Hz), 3.09 (1H, t, J = 17.2 Hz), 3.67–3.85 (3H, m), 4.03–4.14 (2H, m), 4.39 (1H, dd, J = 4.4, 4.1 Hz), 4.51 (2H, d, J = 8.5 Hz), 4.92–5.35 (7H, m), 6.08 (2H, dd, J = 3.2, 1.2 Hz), 6.59 (1H, s), 6.89 (2H, d, J = 8.5 Hz), 7.29 (2H, d, J = 8.5 Hz), 11.92 (1H, s); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 20.77 (×3), 20.90 (×2), 21.07 (×2), 43.38, 61.73, 62.04, 67.87, 70.17, 71.04, 71.72, 72.13, 72.57, 73.00, 73.31, 79.50, 96.29, 97.67, 100.98, 104.44, 115.89, 128.22, 129.63, 139.64, 157.31, 163.10, 163.94, 164.47, 169.62, 169.72, 169.85, 170.24, 170.55, 170.82, 170.91, 197.01; MS: Calcd for $C_{41}H_{46}O_{22}$ $[M + H]^+$ 891.25, found 891.26.

3.2.3. *7-O-(Heptaacetyl-β-D-cellobiosyl)-5,4′-diacetyl-naringenin (3)*

To a solution of **2** (0.890 g, 1.0 m mol) in acetic anhydride (3 mL), two drops of concentrated H_2SO_4 were added at ice bath. After stirring for 3 h at room temperature, the reaction mixture was poured into crushed ice. The product was filtered, washed with cold water (1 L) and dried in vacuum to afford **3** (yield 93%, 0.906 g) as a white solid; m.p. 248–250°C.

IR ($\nu_{max,}$ cm^{-1}): 1745, 1618, 1367, 1217, 1168, 1037 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.99 (3H, s), 2.02 (3H, s), 2.04 (2×3H, s), 2.05 (3H, s), 2.06 (3H, s), 2.10 (3H, s), 2.33 (3H, s), 2.38 (3H, s), 2.74 (1H, dd, J = 2.9, 2.6 Hz), 3.02 (1H, t, J = 13.7 Hz), 3.67 (1H, dd, J = 2.1, 2.3 Hz), 3.81–3.83 (2H, m), 4.02–4.11 (2H, m), 4.39 (1H, dd, J = 4.4, 4.1 Hz), 4.49–4.55 (2H, m), 4.94 (1H, t, J = 8.2 Hz) 5.04–5.31 (5H, m), 5.45 (1H, d, J = 13.4 Hz), 6.34 (1H, d, J = 2.3 Hz), 6.51 (1H, d, J = 2.3 Hz), 7.15 (2H, d, J = 8.3 Hz), 7.45 (2H, d, J = 8.4 Hz); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 20.74 (×2), 20.82 (×2), 20.91 (×2), 20.95, 21.34, 21.40, 45.31, 61.67, 62.03, 67.77, 71.05, 71.67, 72.20, 72.50, 73.02, 73.40, 79.40, 97.82, 101.07, 102.40, 102.60, 106.49, 109.62, 122.34, 127.71, 135.78, 151.20, 151.90, 162.20, 164.06, 169.34, 169.60, 169.66, 169.74, 169.82, 169.99, 170.51 (×2), 170.78, 188.90; MS: Calcd for $C_{45}H_{50}O_{24}Na$ $[M + Na]^+$ 997.86, found 997.45.

3.2.4. *7-O-(Heptaacetyl-β-D-cellobiosyl)-5,4′-diacetyl-apigenin (4)*

In a two-necked flask equipped with dropping funnel, and side arm connected to a cooled receiving vessel, were placed **3** (0.974 g, 1.0 m mol), NBS (0.531 g, 3.0 m mol), a few grains (ca. 1 ma.) of dibenzoyl peroxide, and 50 mL of carbon tetrachloride. After 15 min of heating, bromine evolution began. The heat was increased so that solvent and bromine distilled from the side arm and collected in the receiver. Fresh solvent was added through the dropping funnel, and dibenzoyl peroxide was added as needed to maintain bromine evolution. The reaction was completed in 2 h. The volume of liquid in the reaction vessel was reduced to 25 mL and the mixture cooled to 0°C. The precipitated solid was removed by filtration and washed with hot water (100 mL). After drying, the residue recrystallised from methanol to afford **4** (yield 97%, 0.943 g) as a beige solid; m.p. 272–274°C.

IR ($\nu_{max,}$ cm^{-1}): 1745, 1637, 1369, 1209, 1170, 1041 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.00 (3H, s), 2.03 (3H, s), 2.05 (3H, s), 2.06 (3H, s), 2.07 (3H, s), 2.08 (3H, s), 2.11 (3H, s), 2.35 (3H, s), 2.44 (3H, s), 3.69 (1H, d, $J = 7.9$ Hz), 3.88–3.90 (2H, m), 4.04–4.16 (2H, m), 4.38–4.43 (1H, dd, $J = 4.1$, 12.3 Hz), 4.53–4.58 (2H, m), 4.96 (1H, t, $J = 8.3$ Hz), 5.06–5.30 (5H, m), 6.59 (1H, s), 6.67 (1H, d, $J = 1.8$ Hz), 6.98 (1H, d, $J = 1.8$ Hz), 7.26 (2H, d, $J = 8.5$ Hz), 7.87 (2H, d, $J = 8.5$ Hz); ^{13}C-NMR (75 MHz, $CDCl_3$): $\delta = 20.75$ (×2), 20.82 (×3), 20.94 (×2), 21.35, 21.43, 61.70, 62.13, 67.83, 71.18, 71.75, 72.25, 72.49, 73.04, 73.38, 76.52, 97.95, 101.12, 102.70, 108.75, 109.58, 112.94, 122.63, 127.77, 128.85, 150.84, 153.47, 158.55, 160.21, 161.74, 169.24, 169.35, 169.59, 169.74, 169.89, 169.99, 170.44, 170.49, 170.77, 176.46; MS: Calcd for $C_{45}H_{48}O_{24}$ $[M + H]^+$ 973.25, found 973.41.

3.2.5. *7-O-β-D-cellobiosyl-apigenin (5)*

To a solution of **4** (0.194 g, 0.2 m mol) in a mixture of CH_3OH (3 mL) and $CHCl_3$ (3 mL), $KOCH_3$ (0.056 g, 0.8 m mol) was added at room temperature. After stirring for 2 h, the reaction mixture was neutralised with 1 N HCl, filtered, and filtrate was evaporated in vacuum. The residue recrystallised from EtOH to afford **5** (yield 98%, 0.116 g) as a yellow solid; m.p. 284–286°C.

IR ($\nu_{max,}$ cm^{-1}): 3358, 1656, 1602, 1246, 1175, 1072, 1029 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.07–3.34 (5H, H-2″, H-2‴-5‴, m), 3.61–3.77 (4H, H-5″-6″, H-6‴, m), 4.33 (1H, H-1‴, d, $J = 7.3$), 4.72–4.77 (2H, 2×OH, m), 4.92 (1H, OH, s), 5.08–5.17 (3H, H-1″, 2×OH, m), 5.33 (1H, d, OH, $J = 4.4$ Hz), 5.60 (1H, d, OH, $J = 5.0$ Hz), 6.46 (1H, H-6, s), 6.82 (1H, H-8, s), 6.84 (1H, H-3, s), 6.95 (2H, H-3′, H-5′, d, $J = 8.2$ Hz), 7.94 (2H, H-2′, H-6′, d, $J = 8.4$ Hz), 10.48 (1H, $C_{4'}$-OH, s), 12.97 (1H, $C_{5'}$-OH, s); signals of three protons (H-3″, H-4″ and H-6‴) were overlapped with the signals of H_2O; ^{13}C-NMR (75 MHz, DMSO-d_6): δ 60.58 (C-6″), 61.72 (C-6‴), 70.70 (C-4‴), 73.46 (C-2″), 73.95 (C-2‴), 75.44 (C-3″), 75.74 (C-5″), 77.14 (C-3‴), 77.49 (C-5‴), 80.34 (C-4″), 95.52 (C-8), 99.95 (C-6), 100.10 (C-1″), 103.80 (C-3, C-1‴), 106.03 (C-10), 116.68 (C-3′, C-5′), 121.67 (C-1′), 129.31 (C-2′, C-6′), 157.59 (C-4′), 161.81 (C-9), 162.04 (C-5), 163.47 (C-2), 164.93 (C-7), 182.69 (C-4); MS: Calcd for $C_{27}H_{30}O_{15}K$ $[M + K]^+$ 633.12, found 633.11.

3.2.6. *7-O-(Heptaacetyl-β-D-cellobiosyl)-apigenin (6)*

To a solution of **4** (0.972 g, 1.0 m mol) in H_2O:MeOH:THF (1 : 4 : 8, 20 mL), NH_4OAc (1.232 g, 16.0 m mol) was added. The resulting mixture was stirred at 50°C. After 24 h, the mixture was concentrated and the residue was extracted with EtOAc (3 × 25 mL). Evaporation of the solvent afforded the deprotected product recrystallised from MeOH to afford **6** (yield 91%, 0.808 g) as a beige solid; m.p. 201–203°C.

IR ($\nu_{max,}$ cm^{-1}): 3433, 1739, 1604, 1367, 1222, 1174, 1035 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.91 (3H, s), 1.95 (3H, s), 1.96 (3H, s), 1.97 (3H, s), 2.00 (3H, s), 2.01 (3H, s), 2.06 (3H, s), 3.84–4.27 (6H, m), 4.39 (1H, d, $J = 11.1$ Hz), 4.65 (1H, t, $J = 9.4$ Hz), 4.84–4.91 (2H, m), 4.99 (1H, t, $J = 8.2$ Hz) 5.26 (1H, dd, $J = 12.9$, 9.4 Hz), 5.27 (1H, dd, $J = 6.2$, 9.4 Hz), 5.65 (1H, d, $J = 7.9$ Hz), 6.40 (1H, d, $J = 1.7$ Hz), 6.75 (1H, d, $J = 1.6$ Hz), 6.88 (1H, s), 6.91 (2H, d, $J = 8.5$ Hz), 7.93 (2H, d, $J = 8.6$ Hz), 10.41 (1H, s), 13.00 (1H, s); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 20.91 (×2), 21.04 (×2), 21.12 (×2), 21.19, 62.14, 62.65, 68.33, 71.14, 71.29, 71.82, 72.60, 72.82 (×2), 76.95, 95.74, 96.89, 99.88, 100.21, 103.83, 106.52, 116.65, 121.55, 129.29, 157.49, 162.00, 162.12, 165.07, 169.73, 169.89, 170.13 (×2), 170.29, 170.72, 170.85, 182.71; MS: Calcd for $C_{41}H_{44}O_{22}$ $[M + H]^+$ 889.23, found 889.39.

3.2.7. *7-O-(Heptaacetyl-β-D-cellobiosyl)-4′-O-(tetraacetyl-β-D-glycosyl)-apigenin (7)*

According to the procedure described for **2**, **6** (0.888 g, 1.0 m mol) was glycosylated with tetraacetyl-β-D-glycosyl bromide (1.03 g, 2.5 m mol) to afford **7** (yield 84%, 1.02 g) as a yellowish solid; m.p. 307–309°C.

IR ($\nu_{max,}$ cm^{-1}): 1741, 1595, 1367, 1217, 1176, 1033 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.90 (3H, s), 1.91 (3H, s), 1.96 (2×3H, s), 1.97 (2×3H, s), 2.00 (4×3H, s), 2.05 (3H, s), 3.84–4.30 (9H, m), 4.40 (1H, d, $J = 11.1$ Hz), 4.66 (1H, t, $J = 8.8$ Hz), 4.84–4.91 (2H, m), 4.97–5.12 (3H, m), 5.22–5.30 (2H, m), 5.41 (1H, t, $J = 9.7$ Hz), 5.66 (1H, d, $J = 7.6$ Hz), 5.75 (1H, d, $J = 7.9$ Hz), 6.43 (1H, d, $J = 1.5$ Hz), 6.79 (1H, d, $J = 1.5$ Hz), 7.03 (1H, s), 7.16 (2H, d, $J = 8.8$ Hz), 8.08 (2H, d, $J = 8.8$ Hz), 12.89 (1H, s); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 20.60 (×3), 20.85, 20.92 (×2), 21.02 (×2), 21.11 (×2), 21.19, 62.26, 62.67, 68.30, 68.59, 71.22 (×3), 71.66 (×2), 71.83, 72.57 (×2), 72.83 (×2), 76.95, 95.89, 96.85, 97.01, 99.70, 100.22, 104.64, 106.64, 117.24, 125.51, 129.17, 157.55, 159.84, 162.00, 162.35, 164.11, 169.73, 169.81, 169.89 (×2), 170.02, 170.15, 170.31 (×2), 170.69, 170.76, 170.88, 182.85; MS: calcd for $C_{55}H_{62}O_{31}$ $[M + H]^+$ 1219.33, found 1219.58.

3.2.8. *7-O-β-D-cellobiosyl-4′-O-β-D-glycosyl-apigenin (8)*

According to the procedure described for **5**, **7** (0.244 g, 0.2 m mol) was deacetylated with $KOCH_3$ (0.056 g, 0.8 m mol) to afford **8** (yield 94%, 0.142 g) as a white solid; m.p. 196–198°C.

IR ($\nu_{max,}$ cm^{-1}): 3257, 1726, 1604, 1242, 1176, 1070, 1029 cm^{-1}; ^{1}H-NMR (300 MHz, CD_3OD): δ 3.32–3.44 (11H, H-2‴-5‴, H-4‴, 6×OH, m), 3.49–3.56 (5H, H-2″, H-2⁗-3⁗, H-5⁗, OH, m), 3.64–3.74 (7H, H-3″-4″, H-6‴, H-6⁗, 3×OH, m), 3.88–3.92 (6H, H-5″-6″, H-6‴, H-6⁗, OH, m), 4.47 (1H, H-1‴, d, $J = 7.9$ Hz); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 5.02 (1H, H-1⁗, d, $J = 6.5$ Hz), 5.16 (1H, H-1″, d, $J = 7.0$ Hz), 6.45 (1H, H-6, s), 6.86 (1H, H-8, s), 6.98 (1H, H-3, s), 7.18 (2H, H-3′, H-5′, d, $J = 8.5$ Hz), 8.05 (2H, H-2′, H-6′, d, $J = 8.5$ Hz), 12.87 (1H, C_5-OH, s); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 60.50 (C-6″), 61.24 (C-6⁗), 61.67 (C-6‴), 70.25 (C-4⁗), 70.67 (C-4‴), 73.44 (C-2″), 73.80 (C-2⁗), 73.97 (C-2‴), 75.44 (C-3″), 75.73 (C-5″), 77.17 (C-3‴, C-3⁗), 77.47 (C-5‴), 77.80 (C-5⁗), 80.30 (C-4″), 95.54 (C-8), 99.91 (C-1″), 100.23 (C-6), 100.39 (C-1⁗), 103.79 (C-1‴), 104.73 (C-3), 106.12 (C-10), 117.25 (C-3′), 124.39 (C-1′), 128.89 (C-2′), 157.65 (C-4′), 161.10 (C-9), 161.77 (C-5), 163.55 (C-2), 164.27 (C-7), 182.79 (C-4); MS: Calcd for $C_{33}H_{40}O_{20}.K$ $[M + K]^+$ 795.18, found 795.04.

4. Conclusions

In summary, the first total synthesis of apigenin 7-*O*-β-D-cellobioside (**5**) and apigenin 7-*O*-β-D-cellobiosyl-4′-*O*-β-D-glucopyranoside (**8**) was successfully carried out from

naringenin (**1**), in high overall yields, 76% and 57%, respectively. Although this route contained six steps, the targeted compounds were obtained with higher yields and easier purifications than other synthetic methods.

Acknowledgements

We gratefully acknowledge The Scientific and Technological Research Council of Turkey (TUBITAK) for the financial support of this research granted 105T251.

References

Bouktaib, M., Atmani, A., & Rolando, C. (2002). Regio- and stereoselective synthesis of the major metabolite of quercetin, quercetin-3-*O*-β-D-glucuronide. *Tetrahedron Letters, 43*, 6263–6266, doi:10.1016/S0040-4039(02)01264-9.

Bubb, W.A. (2003). NMR spectroscopy in the study of carbohydrates: Characterizing the structural complexity. *Concepts in Magnetic Resonance Part A, 19A*(1), 1–19, doi: 10.1002/cmr.a.10080.

Caldwell, S.T., Crozier, A., & Hartley, R.C. (2000). Isotopic labelling of quercetin 4′-*O*-β-D-glucoside. *Tetrahedron, 56*, 4101–4106, doi:10.1016/S0040-4020(00)00325-2.

Caldwell, S.T., Petersson, H.M., Farrugia, L.J., Mullen, A.C., & Hartley, R.C. (2006). Isotopic labelling of quercetin 3-glucoside. *Tetrahedron, 62*, 7257–7265, doi:10.1016/j.tet.2006.05.046.

Chen, J., Huang, W., Lian, G., & Lin, F. (2009). The efficient total synthesis of bis-glycosyl apigenin from naringenin: A greener way. *Carbohydrate Research, 344*, 2245–2249, doi:10.1016/j.carres.2009.08.014.

Du, Y., Wei, G.H., & Linhardt, R.J. (2003). The first total synthesis of calabricoside A. *Tetrahedron Letters, 44*, 6887–6890, doi:10.1016/S0040-4039(03)01706-4.

Du, Y., Wei, G.H., & Linhardt, R.J. (2004). Total synthesis of quercetin 3-sophorotrioside. *Journal of Organic Chemistry, 69*, 2206–2209, doi:10.1021/jo035722y.

Elhabiri, M., Figueiredo, P., Fougerousse, A., & Brouillard, R. (1995). A convenient method for conversion of flavonols into anthocyanins. *Tetrahedron Letters, 6*, 4611–4614, doi:10.1016/0040-4039(95)00809-Q.

Farkas, L., Wolfner, A., Nogradi, M., Wagner, H., & Hörhammer, L. (1968). Synthesis of apigenin-4′-β-glucoside and phegopolin. *Chemische Berichte, 101*, 1630–1632.

Fraser-Reid, B.O., & Tatsuta, K. (2001). Other glycoconjugates. In J. Thiem (Ed.), *Glycoscience: Chemistry and biology III* (Vol. III. Part 9, pp. 2393–2532). Springer: Berlin.

Harborne, J.B. (Ed.). (1988). *The flavonoides: advances in research since 1980*. London: Chapman and Hall.

Harborne, J.B. (Ed.). (1994). *The flavonoides: Advances in research since 1986*. London: Chapman and Hall.

Iacobucci, G.A., & Sweeny, J.G. (1983). The chemistry of anthocyanins, anthocyanidins and related flavylium salts. *Tetrahedron, 39*, 3005–3038, doi:10.1016/S0040-4020(01)91542-X.

Kondo, T., Oyama, K., Nakamura, S., Yamakawa, D., Tokuno, K., & Yoshida, K. (2006). Novel and efficient synthesis of cyanidin 3-O-β-D-glucoside from (+)-catechin via a flav-3-en-3-ol as a key intermediate. *Organic Letters, 8*, 3609–3612, doi:10.1021/ol0614976.

Krishnamurty, H.G., Krishnamoorthy, V., & Seshadri, T.R. (1963). Preparation of anthocyanidins and their glygosides from related flavonoids. *Phytochemistry, 2*, 47–60, doi:10.1016/S0031-9422(00)88015-2.

Li, M., Han, X., & Yu, B. (2003). Facile synthesis of flavonoid 7-*O*-glycosides. *Journal of Organic Chemistry, 68*, 6842–6845, doi:10.1021/jo034553e.

Looker, J.H., & Holm, M.J. (1959). A new procedure for the dehydragenation of flavanones with *n*-bromosuccinimide. *Journal of Organic Chemistry, 24*, 567–568, doi:10.1021/jo01086a609.

Maloney, D.J., & Hecht, S.M. (2005). Synthesis of a potent and selective inhibitor of p90 Rsk. *Organic Letters, 7*, 1097–1099, doi:10.1021/ol0500463.

Murakami, S., Robertson, A., & Robinson, R. (1931). Experiments on the synthesis of anthocyanins. Part VI. A synthesis of chrysanthemin chloride. *Journal of the Chemical Society,* 2665–2671, doi:10.1039/JR9310002665.

Nogradi, M., Farkas, L., Wagner, H., & Hörhammer, L. (1967). A new synthesis of cosmosiins and of tilianins. *Chemische Berichte, 100*, 2783–2790.

Oyama, K., & Kondo, T. (2004). Total synthesis of apigenin 7,4′-di-*O*-β-glucopyranoside, a component of blue flower pigment of *Salvia patens*, and seven chiral analogues. *Tetrahedron, 60*, 2025–2034, doi:10.1016/j.tet.2004.01.001.

Ramesh, G., Mahender, G., Ravindranath, N., & Das, B. (2003). A mild, highly selective and remarkably easy procedure for deprotection of aromatic acetates using ammonium acetate as a neutral catalyst in aqueous medium. *Tetrahedron, 59*, 1049–1054, doi:10.1016/S0040-4020(02)01635-6.

Redemann, C.E., & Niemann, C. (1955). Acetobromoglucose (2,3,4,6-Tetraacetyl-α-d-glucopyranosyl bromide). *Organic Syntheses, Coll., 3*, 11.

Rice-Evans, C.A. (1997). Part II. Characterization, chemical and biological properties. In L. Packer (Ed.), *Flavonoids in health and disease* (pp. 88–186). New York: Marcel Dekker.

Robertson, A., & Robinson, R. (1927). Experiments on the synthesis of anthocyanins. Part II. The synthesis of 3- and 7-glucosidoxyflavylium salts. *Journal of the Chemical Society,* 242–247, doi:10.1039/JR9270000242.

Veitch, N.C., Grayer, R.J., Irwin, J.J., & Takeda, K. (1998). Flavonoid cellobiosides from *Salvia uliginosa*. *Phytochemistry, 48*, 389–393, doi:10.1016/S0031-9422(97)01111.