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Isotopic Labelling of Quercetin 4′-*O*-β-D-Glucoside

Stuart T. Caldwell,[a] Alan Crozier[b] and Richard C. Hartley[a,*]

[a]*Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, UK*
[b]*Division of Biochemistry and Molecular Biology, University of Glasgow, Glasgow G12 8QQ, UK*

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Abstract—[2-^{13}C]-Quercetin-4′-*O*-β-glucoside was synthesised in four steps and 28% yield from barium [^{13}C]-carbonate. This short route will be applicable to the synthesis of radiolabelled quercetin-4′-*O*-β-D-glucoside from barium [^{14}C]-carbonate. The most important feature is control of the regiochemistry and stereochemistry of glycosylation before introduction of the isotopic label. The synthesis also uses only benzyl protecting groups allowing global deprotection in the last step.

Introduction

Flavonols are polyphenolic secondary metabolites produced by plants.[1] They are found in high concentrations in foods such as tomatoes and onions[2] and in beverages such as tea and red wine.[3,4] They are normally present in fruits, vegetables and teas as sugar conjugates.[5,6] Many possess antioxidant and free radical scavenging activity[7] and epidemiological studies indicate that consumption of flavonols is associated with a reduced risk of coronary heart disease.[8,9] The average intake of flavonols in the Netherlands diet is approximately 23 mg d^{-1} of which 29% comes from onions.[8] Quercetin **1** is a strong dietary antioxidant[10] found in high concentrations in onions primarily as its 4′-*O*-β-D-glucoside and 3,4′-di-*O*-β-D-glucoside (Fig. 1).[11] The absorption and metabolism of individual flavonols in humans is very poorly understood, but a recent study with onions has shown that flavonols are absorbed into the bloodstream as glucosides, and that minor structural differences affect both the level of accumulation in plasma and the extent to which conjugates are excreted in urine.[12] Further studies are required to determine the bioavailability of flavonol conjugates and their role as in vivo antioxidants. Ultimately, understanding the pharmacology of various flavonols would allow us to assess their relative importance to a healthy diet and to recommend appropriate foods and beverages to the public. As a first step, we wished to track the biological fate of quercetin-4′-*O*-β-D-glucoside in rats and for this we needed to synthesise the flavonol radiolabelled with a label that cannot be exchanged.

Here, we demonstrate our synthetic strategy with the synthesis of [2-^{13}C]-quercetin-4′-*O*-β-glucoside **10** in four steps and 28% yield from barium [^{13}C]-carbonate (Scheme 1). Barium [^{14}C]-carbonate is one of the cheapest compounds containing this radioactive isotope of carbon and our route is applicable to the synthesis of [2-^{14}C]-quercetin-4′-*O*-β-glucoside. [4-^{14}C]-Quercetin has been made commercially,[13] but no details of the method of synthesis have been reported. There have been no other syntheses of flavonols with a carbon isotope label in the A, B or C rings, but such compounds have been extracted from plants grown under [^{14}C]-carbon dioxide.[14] Progress has been made in the deuterium-labelling of flavonols.[15–18]

Results and Discussion

In order to minimise the number of steps after the introduction of the isotopic label and to avoid the difficulty of selectively glucosylating quercetin, we decided to introduce the glucoside early in our synthesis. We also decided to use only one type of protecting group: the benzyl group was selected as deprotection does not affect glycosidic links.[19] Monobenzylated catechol **2** was selectively iodinated *para* to the hydroxyl group using iodine monochloride[20] to give iodophenol **3**. This reagent proved superior to chloramine T,[21] which gives side-products that are difficult to remove. Glucosylation of iodophenol **3** using Schmidt's imidate[22,23] **4** gave a 4:1 mixture of β- and α-anomers, and a single recrystallisation gave the β-glucoside **5**. All regiochemical and stereochemical issues had thus been dealt with prior to

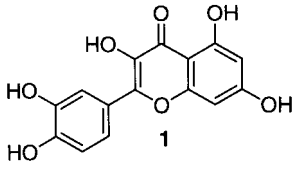

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Figure 1.

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* Corresponding author. Tel.: +44-141-330-4398; fax: +44-141-330-4888; e-mail: richh@chem.gla.ac.uk

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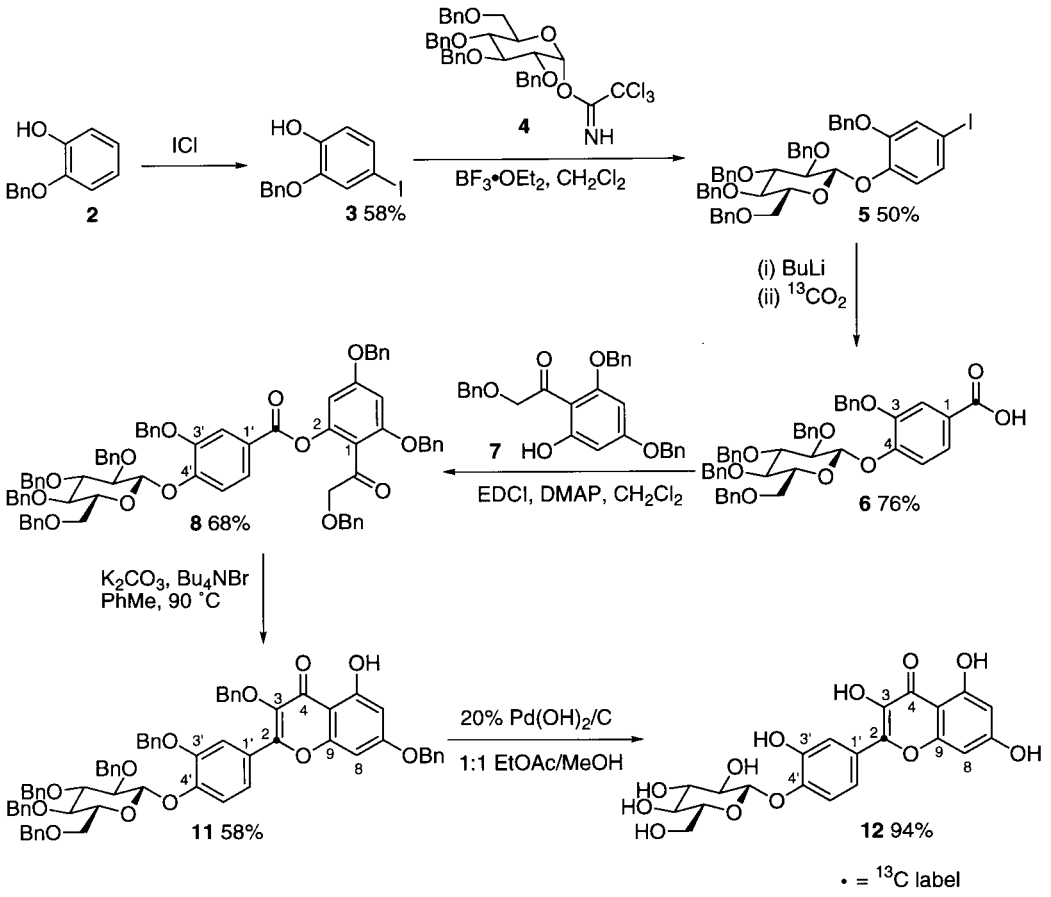


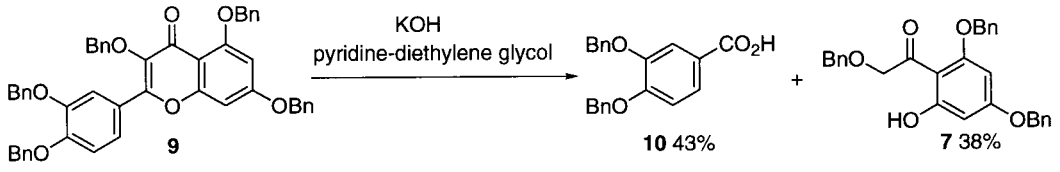
Scheme 1.

introduction of the isotopic label. NOE experiments confirmed that glucoside **5** was the correct regioisomer: irradiation of the doublet at δ_H 6.90, corresponding to H-5 of the benzene ring, gave strong enhancement of the anomeric proton at δ_H 4.96, and irradiation of the anomeric proton gave strong enhancement of the H-5 signal.

Carboxylation of the glucoside **5** using a variation of the method described by Kratzel and Billek[24] gave the benzoic acid **6** in 83% yield based on glucoside **5** using 2 equiv. of barium [^{13}C]-carbonate, and in 76% yield based on carbon dioxide using 1.5 equiv. of the glucoside **5** and 1 equiv. of barium [^{13}C]-carbonate. The glycosidic link was not affected by either the reaction or the acidification necessary to isolate carboxylic acid **6**. Esterification using phenol **7** and a water-soluble coupling agent, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI), gave ester **8**. Phenol **7** was prepared by degradation[25] of pentabenzylated quercetin **9** according to the literature procedure and was fully characterised for the first time (Scheme 2). The carboxylic acid **10** was also isolated. Our conditions for pentabenzylation are superior to those previously reported.[26] The best method for cyclising and dehydrating ester **8** used potassium carbonate and a phase transfer catalyst.[27] However, these conditions led to selective deprotection of the 5-hydroxy group to give flavonol **11**. Hydrogenolysis of flavonol **11** using the non-acidic palladium hydroxide on carbon under 1 atm of hydrogen gave the target compound **12** in four steps and 28% yield from barium [^{13}C]-carbonate. Compound **12** had a chemical purity of ≥97% by HPLC, ^{1}H and ^{13}C NMR. Only one step after the introduction of the isotopic label involves chromatography and that is simply a filtration through alumina. In summary, we have demonstrated a short high-yielding route to [2-^{13}C]-quercetin-4′-*O*-β-glucoside **12** that will also be useful for radiolabelling work.

Spectral assignments

All the signals that show ^{1}H–^{13}C coupling in the ^{1}H NMR spectra and those that show ^{13}C–^{13}C coupling in the ^{13}C NMR spectra of compounds **6**, **8**, **11** and **12** are presented



Scheme 2.

Table 1. ^{13}C–X coupling in ^{13}C NMR spectrum of carboxylic acid **6**

X	C-1	C-2	C-3	C-5
J (Hz)	73.7	2.5	5.7	5.1
δ_C (ppm)	123.4	114.6	148.3	115.2

Table 2. ^{13}C–X coupling in ^{13}C NMR spectrum of ester **8**

X	C-2	C-1′	C-2′	C-3′	C-5′	C-6′
J (Hz)	3.0	78.6	2.8	6.1	5.4	1.8
δ_C (ppm)	150.6	123.1	114.8	148.5	115.3	124.7

in Tables 1–4. The numbering of carbon and hydrogen atoms of the different compounds is shown in Scheme 1. Signals were assigned by comparison with the spectra of the corresponding non-labelled compounds (synthesised by the same route or reported in the literature[28]), by using HMQC and HMBC spectroscopy and by inferring the relative chemical shifts of the atoms in question from those in the other labelled compounds.

Experimental

^1H and ^{13}C NMR spectra were obtained on a Bruker DPX/400 spectrometer operating at 400 and 100 MHz, respectively. All coupling constants are measured in Hz. DEPT was used to assign the signals in the ^{13}C NMR spectra as C, CH, CH_2 or CH_3. Mass spectra (MS) were recorded on a Jeol JMS700 (MStation) spectrometer. Infrared (IR) spectra were obtained on a Perkin–Elmer 983 spectrophotometer. Ultraviolet (UV) spectra were recorded on a Shimadzu UV-1601 spectrophotometer. Column chromatography was carried out on silica gel, 70–230 mesh, or neutral alumina (Brockmann grade III). Tetrahydrofuran and diethyl ether were dried over sodium and benzophenone, and dichloromethane was dried over calcium hydride.

2-Benzyloxy-4-iodophenol 3. A solution of iodine monochloride (24.35 g, 150 mmol) in dry diethyl ether (200 ml) was added dropwise to a solution of 2-benzyloxyphenol **2** (20.00 g, 100 mmol) in dry diethyl ether (80 ml), shielded from the light and under nitrogen. The resulting solution was stirred overnight. The deep red solution was then diluted with diethyl ether (300 ml) and washed with aqueous sodium thiosulfate (3×600 ml). The organic layer was dried over magnesium sulfate and the solvent removed in vacuo. The resulting solid was dissolved in CH_2Cl_2–hexane and passed through a plug of silica eluting with CH_2Cl_2–hexane (4:6). The resulting solid was recrystallised from hexanes–ether several times to give phenol **3** as prisms (18.81 g, 58%) mp 71–73°C; RF [silica, CH_2Cl_2–hexane (7:3)] 0.56; ν_{max}(nujol)/cm^{-1} 3462 (OH) and 1495 (Ar); δ_H (400 MHz, $CDCl_3$) 5.03 (2H, s, $-OCH_2-$), 5.65 (1H, s, OH), 6.69 (1H, d, *J*=8.2 Hz, H-6) 7.17–7.19 (2H, m, H-3, H-5) and 7.36–7.45 (5H, m, *Ph*-CH_2); δ_C (100 MHz, $CDCl_3$) 71.3 (CH_2), 80.8 (C–I), 116.6 (CH), 120.9 (CH), 128.0 (CH), 128.6 (CH), 128.7 (CH), 130.7 (CH), 135.5 (C), 145.8 (C) and 146.6 (C); *m/z* (EI): 326.0 (M^+, 10%), and 91.1 (100); (Found: C, 47.96; H, 3.36; I, 38.77%; M^+, 325.9803. $C_{13}H_{11}O_2I$ requires C, 47.87; H 3.40; I 38.91%, M, 325.9804).

1-Iodo-3-benzyloxy-4-(2′,3′,4′,6′-tetra-*O*-benzyl-β-D-glucospyranosyloxy)benzene 5. A solution of boron trifluoride etherate (0.46 ml, 3.70 mmol) in dry CH_2Cl_2 (8 ml) was added over 1 h to a stirred solution of phenol **3** (3.99 g, 12.2 mmol), *O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)trichloroacetimidate **4** (12.57 g, 18.4 mmol) and 4 Å molecular sieves (1.00 g) in dry CH_2Cl_2 (20 ml) under nitrogen at −78°C. After stirring at −78°C for 3.5 h, the solution was quenched with saturated aqueous Na_2CO_3 and the molecular sieves were filtered off and washed with CH_2Cl_2 (20 ml). The filtrate and washings were combined, washed with brine (2×250 ml), dried ($MgSO_4$) and concentrated under vacuum to give a solid, which was recrystallised from EtOAc–pet. ether (1:4) to give the β anomer **5** as needles. (5.22 g, 50%); mp 122–123°C; ν_{max}(nujol)/cm^{-1} 1491 (Ar); δ_H (400 MHz, $CDCl_3$) 3.57–3.78 (6H, m, H-2′, 3′, 4′, 5′, 6′), 4.50 (1H, d, *J*=12.0 Hz, $ArOCH_AH_B$), 4.54 (1H, d, *J*=10.8 Hz, $ArOCH_CH_D$), 4.56 (1H, d, *J*=12.0 Hz, $ArOCH_AH_B$), 4.70 (1H, d, *J*=11.2 Hz, $ArOCH_EH_F$), 4.77 (1H, d, *J*=10.8 Hz, $ArOCH_GH_H$), 4.82 (1H, d, *J*=10.8 Hz, $ArOCH_CH_D$), 4.92 (1H, d, *J*=10.8 Hz, $ArOCH_GH_H$), 4.96 (1H, d, *J*=7.2 Hz, H-1′), 4.99 (1H, d, *J*=11.6 Hz, $ArOCH_IH_J$), 5.05 (2H, Broad d, $_J$=12.4 Hz, $ArOCH_EH_F$, $ArOCH_IH_J$), 6.90 (1H, d, *J*=8.5 Hz, H-5) and 7.12–7.36 (27H, m, Ar–H); δ_C (100 MHz, $CDCl_3$) 68.84 (CH_2), 70.96 (CH_2), 73.44 (CH_2), 74.59 (CH_2), 74.97 (CH_2), 75.50 (CH), 75.64 (CH_2), 77.57 (CH), 81.56 (CH), 84.38 (CH), 84.86 (C), 101.99 (CH), 118.60 (CH), 122.75 (CH), 127.38 (CH), 127.51 (CH), 127.54 (CH), 127.57 (CH), 127.63 (CH), 127.75 (CH), 127.80 (CH), 127.89 (CH), 127.97 (CH), 128.01 (CH), 128.11 (CH), 128.29 (CH), 128.30 (CH), 128.35 (CH), 128.41 (CH), 130.27 (CH), 136.19 (C), 137.95 (C), 138.03 (C), 138.27 (C), 138.38 (C), 147.22 (C) and 149.65 (C); *m/z* (FAB): 871.3 [$(M+Na)^+$, 2%], 522.3 (3), 415.2 (5), 329.1 (3); [Found:

Table 3. ^{13}C–X coupling in ^{13}C and ^1H NMR spectra of flavonol **11**

X	C-3	C-4	C-8	C-9	C-1′	C-2′	C-3′	C-5′	H-2′	H-6′
J (Hz)	86.1	6.8	3.5	2.5	67.7	3.0	5.7	4.8	3.6	3.6
δ (ppm)	137.6	178.8	93.0	156.7	124.8	114.2	148.1	115.6	7.73	7.54

Table 4. ^{13}C–X coupling in ^{13}C and ^1H NMR spectra of flavonol **12**

X	C-3	C-4	C-8	C-9	C-1′	C-2′	C-3′	C-4′	C-5′	H-2′	H-6′
J (Hz)	90.1	5.7	3.1	2.7	68.0	1.7	5.6	1.3	5.0	4.0	4.0
δ (ppm)	136.7	176.4	93.9	156.6	125.5	115.5	146.7	147.1	116.2	7.71	7.62

$(M+Na)^+$, 871.2106. $C_{47}H_{45}IO_7Na$ requires $(M+Na)^+$, 871.2107; (Found: C, 66.50; H, 5.43%. $C_{47}H_{45}IO_7$ requires C, 66.51; H, 5.34%); $[\alpha]_D^{19}=-30.5$ (c=40: $CHCl_3$).

3-Benzyloxy-4-(2′,3′,4′,6′-tetra-*O*-benzyl-β-D-glucospyranosyloxy)benzoic acid 6, [^{13}C] at carbonyl. Carboxylation of the aryl iodide **5** was carried out using the apparatus described by Kratzel and Billek and a variation of their method.[24] Butyl lithium (3.20 ml, 4.71 mmol) was added to a solution of aryl iodide **5** (4.00 g, 4.71 mmol) in dry THF (20 ml) at −78°C under nitrogen. The mixture was stirred for 15 min and was then cooled to −196°C and the whole system was evacuated to 4 mm of Hg. The system was then sealed and an excess of concentrated sulfuric acid was added dropwise onto [^{13}C]-barium carbonate (98 at% ^{13}C, 1.87 g, 9.43 mmol) in a separate reaction vessel in the same system with occasional sonication of the vessel. Once the majority of the carbon dioxide had been evolved, the THF solution was allowed to warm to −78°C, was stirred for 30 min and then recooled to −196°C. The [^{13}C]-barium carbonate/sulfuric acid mixture was sonicated for a further 15 min and the THF solution was then allowed to warm to −78°C and was stirred for 15 min. Throughout the procedure the pressure did not exceed 210 mm of Hg. The THF solution was poured into a (1:1) two-phase mixture of ether and aqueous HCl (1 M)/ice. The layers were separated and the aqueous layer washed with ether (50 ml). The combined organics were washed with brine (4×150 ml) and concentrated to approximately 10 ml. Aqueous NaOH (2 M, 20 ml) was added and pet. ether was added until a white solid formed. The sodium salt was filtered off, dissolved in EtOAc and acidified with aqueous HCl (1 M). The organic layer was dried ($MgSO_4$) and concentrated under vacuum to give benzoic acid **6** as a purple solid (3.00 g, 83% from aryl iodide **5**) sufficiently pure for the next step. 518 mg were then recrystallised from ^{i}PrOH to give the acid **6** as a white amorphous solid (297 mg, 57%). In a similar way, carbon dioxide generated from only 1 equiv. of [^{13}C]-barium carbonate (0.545 g, 2.75 mmol), reacted with the aryllithium generated from 1.5 equiv. of aryl iodide **5** (3.50 g, 4.12 mmol) and 1.4 equiv. of butyl lithium (2.62 ml, 3.85 mmol) to give benzoic acid **6** (1.60 g scale, 76% from barium carbonate). mp 124–126°C; ν_{max}(nujol)/cm^{-1} 3178 (COOH), 1646 (C=O), 1587 (Ar) and 1509 (Ar); δ_H (400 MHz; $CDCl_3$) 3.65–3.81 (6H, m, 2′, 3′, 4′, 5′, 6′), 4.52 (1H, d, J=11.6 Hz, ArOCH_AH$_B$), 4.55 (1H, d, J=10.4 Hz, ArOCH_CH$_D$), 4.58 (1H, d, J=12 Hz, ArOCH$_A$$H_B$), 4.74 (1H, d, J=11.2 Hz, ArOCH_EH$_F$), 4.79 (1H, d, J=11.2 Hz, ArOCH_GH$_H$), 4.84 (1H, d, J=10.4 Hz, ArOCH$_C$$H_D$), 4.95 (1H, d, J=11.2 Hz, ArOCH$_G$$H_H$), 5.09 (2H, broad d, J=12.8 Hz, ArOCH$_E$$H_F$+ArOC$H_IH_J$), 5.10 (1H, d, J=6.8 Hz, H-1″), 5.14 (1H, d, J=11.6 Hz, ArOCH$_I$$H_J$), 7.13–7.47 (26H, m, Ar–H), 7.69–7.73 (2H, m, Ar–H) and 11.0 (1H, v broad s, COOH); δ_C (100 MHz: $CDCl_3$): 68.78 (CH_2), 70.78 (CH_2), 73.48 (CH_2), 74.61 (CH_2), 75.03 (CH_2), 75.22 (CH), 75.47 (CH_2), 77.52 (CH), 81.46 (CH), 84.32 (CH), 101.47 (CH), 114.64 (CH, d, J=2.5 Hz), 115.19 (CH, d, J=5.1 Hz), 123.40 (C, d, J=73.7 Hz), 124.52 (CH), 127.38 (CH), 127.59 (CH), 127.65 (CH), 127.68 (CH), 127.82 (CH), 127.93 (CH), 127.99 (CH), 128.06 (CH), 128.16 (CH), 128.32 (CH), 128.34 (CH), 128.38 (CH), 128.43 (CH), 136.29 (C), 137.92 (C), 138.93 (C), 138.17 (C), 138.42 (C), 148.33 (C, d, J=5.7 Hz), 151.66 (C), and 171.53 (^{13}C label); m/z (FAB) 790.7 [$(M+Na)^+$, 24%], 171.2 (23) and 91.6 (100); [Found: $(M+Na)^+$, 790.3074. $C_{47}{}^{13}CH_{46}O_9Na$ requires (M=Na), 790.3073]; (Found: C, 75.07; H, 6.26%. $C_{47}{}^{13}CH_{46}O_9$ requires C, 75.21; H, 6.04%); $[\alpha]_D^{19}=-47.7$ (c=0.044 g ml^{-1}, $CHCl_3$).

2-Hydroxy-ω,4,6-tribenzyloxyacetophenone 7 and 3,5-dibenzyloxybenzoic acid 10. Following the method of Hauteville,[25] 150 ml of diethylene glycol was slowly added to a solution of pentabenzylated quercetin **9** (15.00 g, 20.00 mmol) in 150 ml of pyridine and 20 ml of 18 M potassium hydroxide. The heterogeneous solution was stirred at 120°C for 6 h. After cooling, the solution was poured into 1 l of iced water and the resulting solid was filtered off and the aqueous filtrate put aside (see below). The precipitate was dissolved in EtOAc (100 ml) and washed with 1 M aqueous hydrochloric acid. The ethyl acetate extract was dried and concentrated. The resulting solid was recrystallised from methanol to yield the *ortho*-hydroxy acetophenone **7** as a powder. (3.43 g, 38%). mp 104–105°C (lit.,[25] 102–103°C); R_F [silica, EtOAc–hexane (7:3)] 0.77; ν_{max}(nujol)/cm^{-1} 1609 (C=O) and 1568 (Ar); δ_H (400 MHz, $CDCl_3$) 4.52 (2H, s, PhCH_2), 4.56 (2H, s, PhCH_2), 4.98 (2H, s, PhCH_2), 5.06 (2H, s, O=C–CH_2), 6.06 (1H, d, J=2.2 Hz, H-3), 6.18 (1H, d, J=2.2 Hz, H-5), 7.20–7.41 (15H, m, Ar–H) and 13.76 (1H, s, –OH); δ_C (100 MHz, $CDCl_3$) 70.2 (CH_2), 71.1 (CH_2), 72.9 (CH_2), 75.4 (CH_2), 92.1 (CH), 94.9 (CH), 104.4 (C), 127.5 (CH), 127.6 (CH), 127.9 (CH), 128.2 (CH), 128.3 (CH), 128.66 (CH), 128.68 (CH), 128.7 (CH), 135.0 (C), 135.6 (C), 137.5 (C), 161.6 (C), 165.2 (C), 167.4 (C) and 200.8 (C); m/z (CI): 455.2 [$(M+H)^+$, 100%], 347.2 (12), 333 (9), 91.1 (11); [Found: $(M+H)^+$ 455.1859. $C_{29}H_{27}O_5$ requires (M+H), 455.1858] (Found: C, 76.5; H, 5.9%. $C_{29}H_{26}O_5$ requires C 76.63, H 5.76%). The aqueous filtrate was acidified to pH 1 and the resulting solid filtered off. The precipitate was recrystallised several times from ethyl acetate to yield the benzoic acid **10** as needles (2.88 g, 43%); mp 185–186°C (Lit.,[6] 183–184°C; R_F [Silica, EtOAc–hexane (7:3)] 0.34; ν_{max}(nujol)/cm^{-1} 1676 (C=O), 1600 (Ar) and 1520 (Ar); δ_H (400 MHz; D-6 Acetone) 5.09 (2H, s, PhCH_2), 5.13 (2H, s, PhCH_2), 6.89 (1H, d, J=8.7 Hz, H-2) and 7.03–7.55 (12H, m, Ar–H); δ_C (100 MHz; D-6 DMSO) 70.1 (CH_2), 70.3 (CH_2), 113.4 (CH), 114.8 (CH), 123.6 (CH), 123.8 (C), 127.7(CH), 127.9 (CH), 128.1 (CH), 128.2 (CH), 128.7 (CH), 128.8 (CH), 137.0 (C), 137.3 (C), 147.9 (C), 152.4 (C) and 167.3 (C); m/z (CI): 335.2 [$(M+H)^+$, 100%], 245.1 (8), 181.2 (9), 91.1 (13); [Found: $(M+H)^+$ 335.1286. $C_{21}H_{19}O_4$ requires (M+H), 335.1283] (Found: C, 75.55; H, 5.56%. $C_{21}H_{18}O_4$ requires C, 75.43; H, 5.42%).

2-[3′-Benzyloxy-4′-(2″,3″,4″,6″-tetrabenzyl-β-D-glucopyranosyloxy)benzoyloxy]-ω,4,6,-tribenzyloxy acetophenone 8, [^{13}C] at ester carbonyl. DMAP (576 mg, 4.72 mmol), EDCI (1.18 g, 6.14 mmol) and the phenol **8** (2.14 g, 4.72 mmol, 1.0 equiv.) were added to benzoic acid **7** (3.63 g, 4.72 mmol) in dry CH_2Cl_2 (30 ml) under nitrogen. After stirring for 24 h, the solution was diluted with CH_2Cl_2 (50 ml) and washed with brine (3×150 ml). The organics were dried ($MgSO_4$) and concentrated under vacuum. The resulting brown oil was chromatographed on alumina (6% water) eluting with diethyl ether to give the ester **9** as an amorphous solid (3.88 g, 68%); mp 106–109°C

(diethyl ether); R_F(alumina, diethyl ether) 0.72; ν_{max}(nujol)/cm^{-1} 1733 (CO_2) and 1608 (C=O); δ_H (400 MHz, $CDCl_3$) 3.64–3.81 (6H, m, H-2″, 3″, 4″, 5″, 6″), 4.42 (2H, s, $ArOCH_2$), 4.50 (2H, s, $ArOCH_2$), 4.51 (1H, d, J=12 Hz, $ArOCH_AH_B$), 4.55 (1H, d, J=11.2 Hz, $ArOCH_CH_D$), 4.58 (1H, d, J=12.4 Hz, $ArOCH_AH_B$), 4.72 (1H, d, J=11.2 Hz, $ArOCH_EH_F$), 4.79 (1H, d, J=10.8 Hz, $ArOCH_GH_H$), 4.83 (1H, d, J=10.8 Hz, $ArOCH_CH_D$), 4.94 (1H, d, J=10.8 Hz, $ArOCH_GH_H$), 5.01 (2H, s, $ArOCH_2$), 5.03 [2H, s, $C(O)CH_2$], 5.07 (1H, d, J=11.2 Hz, $ArOCH_IH_J$), 5.08 (1H, d, J=11.2 Hz, $ArOCH_EH_F$), 5.09 (1H, d, J=7.2 Hz, H-1″), 5.11 (1H, d, J=12 Hz, $ArOCH_IH_J$), 6.51 (1H, d, J=2.1 Hz, H-3), 6.53 (1H, d, J=2.1 Hz, H-5), 7.13–7.39 (41H, m, Ar–H, H-2′) and 7.73–7.75 (2H, m, H-5′, 6′); δ_C (100 MHz, $CDCl_3$) 68.71 (CH_2), 70.48 (CH_2), 70.85 (CH_2), 71.07 (CH_2), 73.00 (CH_2), 73.51 (CH_2), 74.62 (CH_2), 75.03 (CH_2), 75.25 (CH), 75.69 (CH_2), 75.88 (CH_2), 77.47 (CH), 81.46 (CH), 84.34 (CH), 98.38 (CH), 101.54 (CH), 101.67 (CH), 114.81 (CH, d, J=2.8 Hz), 114.95 (C), 115.35 (CH, d, J=5.4 Hz), 123.08 (C, d, J=78.6 Hz), 124.66 (CH, d, J=1.8 Hz), 127.46 (CH), 127.60 (CH), 127.62 (CH), 127.71 (CH), 127.74 (CH), 127.77 (CH), 127.84 (CH), 127.93 (CH), 127.96 (CH), 128.11 (CH), 128.17 (CH), 128.25 (CH), 128.32 (CH), 128.35 (CH), 128.40 (CH), 128.70 (CH), 135.56 (C), 135.84 (C), 136.29 (C), 137.68 (C), 137.99 (2×C), 138.20 (C), 138.47 (C), 148.49 (C, d, J=6.1 Hz), 150.55 (C, d, J=3.0 Hz), 151.71 (C), 158.47 (C), 161.67 (C), 164.12 (^{13}C label) and 198.62 (C); m/z (FAB) 1226.5 [$(M+Na)^+$, 65%], 1136.4 (5), 1134.4 (5), 1044.4 (3), 650.2 (5), 560.2 (5), 439.2 (6), 318.1 (30); [Found: $(M+Na)^+$, 1226.4744. $C_{77}H_{70}O_{13}Na$ requires (M+Na), 1226.4747]; (Found: C, 76.83; H, 5.83%. $C_{76}{}^{13}CH_{70}O_{13}$ requires C, 76.87; H, 5.86%); $[\alpha]_D^{19}$=−9.5 (c=0.021 g ml^{-1}, $CHCl_3$).

3,5,7,3′,4′-Pentabenzyloxyflavone 9. Potassium carbonate (45.7 g, 331.0 mmol) and benzyl bromide (39.3 ml, 331.00 mmol) were added to a solution of quercetin **1** (10.00 g, 33.1 mmol) in DMF (100 ml) under nitrogen, and the resulting mixture solution was stirred at 70°C for 4 d. After the appearance of a single highly UV active spot in the TLC, the solution was allowed to cool and was then acidified to pH 1 with aqueous HCl (1 M). The solution was diluted with water (500 ml), EtOAc (200 ml) was added and the solution was stirred for 20 min. The resulting precipitate was filtered and washed with H_2O (100 ml) and EtOAc (100 ml) to give pentabenzylated quercetin **9** as an off-white solid (22.16 g, 89%) sufficiently pure for the next step. A small amount was recrystallised from CH_2Cl_2 to give a white powder; mp 156–159°C; R_F [silica, EtOAc–hexane (7:3)] 0.62; ν_{max}(nujol)/cm^{-1} 1631 (C=O) and 1601 (Ar); δ_H (400 MHz: $CDCl_3$): 4.88 (2H, s, OCH_2), 5.00 (2H, s, OCH_2), 5.01 (2H, s, OCH_2), 5.15 (2H, s, OCH_2), 5.19 (2H, s, OCH_2), 6.37 (1H, d, J=2.2 Hz, H-6), 6.45 (1H, d, J=2.2 Hz, H-8), 6.87 (1H, d, J=8.6 Hz, H-5′), 7.12–7.54 (25H, m, Ar–H), 7.45 (1H, dd, J=2.0 Hz, 8.3 Hz, H-6′) and 7.64 (1H, d, J=2.0 Hz, H-2′); δ_C (100 MHz: $CDCl_3$): 70.4 (CH_2), 70.8 (CH_2), 70.9 (CH_2), 71.0 (CH_2), 74.1 (CH_2), 93.9 (CH), 98.1 (CH), 110.1 (C), 115.2 (CH), 122.1 (CH), 123.9 (C), 126.9 (CH), 127.2 (CH), 127.3 (CH), 127.6 (CH), 127.7 (CH), 127.8 (CH), 127.9 (CH), 128.0 (CH), 128.1 (CH), 128.4 (CH), 128.6 (CH), 128.6 (CH), 128.7 (CH), 128.8 (CH), 135.7 (C), 136.4 (C), 136.8 (C), 137.0 (C), 139.8 (C), 148.2 (C), 150.5 (C), 153.2 (C), 158.7 (C), 159.7 (C), 162.7 (C) and 173.9 (C); m/z (EI): 752 [M^+, 0.25%], 661.0 (5), 570.9 (4.5), 91.0 (100); (Found: M^+, 752.2772. $C_{50}H_{40}O_7$ requires M, 752.2774).

[2-^{13}C]-(5-Hydroxy-3,7,3′-tribenzyloxy-4′-(2″,3″,4″,6″-tetrabenzyl-β-D-glucopyranosyloxy)flavone 11. Potassium carbonate (1.65 g, 12.0 mmol) and tetrabutylammonium bromide (1.44 g, 4.48 mmol) were added to the ester **8** (3.60 g, 2.99 mmol) in dry toluene (30 ml) under nitrogen and the reaction mixture was then heated at 90°C for 3 h. After removing the toluene under vacuum, the residue was dissolved in CH_2Cl_2 and washed with water and brine. The organic layer was dried ($MgSO_4$) and concentrated under vacuum to give a brown solid, which recrystallised from EtOAc to give flavonol **11** as an off-white solid (1.90 g, 58%); mp 163–164°C; R_F (silica, EtOAc) 0.75; δ_H (400 MHz, $CDCl_3$) 3.66–3.82 (6H, m, 2″, 3″, 4″, 6″), 4.53 (1H, d, J=12.0 Hz, $ArOCH_AH_B$), 4.56 (1H, d, J=10.0 Hz, $ArOCH_CH_D$), 4.59 (1H, d, J=12.0 Hz, $ArOCH_AH_B$), 4.75 (1H, d, J=11.2 Hz, $ArOCH_EH_F$), 4.80 (1H, d, J=11.2 Hz, $ArOCH_GH_H$), 4.82 (1H, d, J=12.0 Hz, $ArOCH_IH_J$), 4.85 (1H, d, J=12.0 Hz, $ArOCH_CH_D$), 4.88 (1H, d, J=12 Hz, $ArOCH_IH_J$), 4.96 (1H, d, J=10.8 Hz, $ArOCH_GH_H$), 5.03 (1H, d, J=10.8 Hz, $ArOCH_KH_L$), 5.08 (1H, d, J=10.8 Hz, $ArOCH_KH_L$), 5.09 (1H, d, J=7.6 Hz, H-1″), 5.12 (1H, d, J=10.8 Hz, $ArOCH_EH_F$), 5.13 (2H, s, $ArOCH_2$), 6.44 (1H, d, J=2.1 Hz, H-5), 6.48 (1H, d, J=2.1 Hz, H-7), 7.17–7.44 (36H, m, Ar–H), 7.54 [1H, ddd, J=8.8 Hz, 3.6 Hz ($^{13}C-^1H$) and 2 Hz, H-6′], 7.73 [1H, dd, J=3.6 Hz ($^{13}C-^1H$) and 2.0 Hz, H-2′], 12.69 (1H, s, OH). δ_C (100 MHz, $CDCl_3$): 68.87 (CH_2), 70.41 (CH_2), 70.60 (CH_2), 73.47 (CH_2), 74.45 (CH_2), 74.61 (CH_2), 75.06 (CH_2), 75.25 (CH), 75.71 (CH_2), 77.56 (CH), 81.52 (CH), 84.40 (CH), 93.02 (CH, d, J=3.5 Hz), 98.58 (CH), 101.70 (CH), 106.18 (C), 114.23 (CH, d, J= 3.0 Hz), 115.56 (CH, d, J=4.8 Hz), 122.13 (CH), 124.75 (C, d, J=67.7 Hz), 127.41 (CH), 127.48 (CH), 127.53 (CH), 127.58 (CH), 127.61 (CH), 127.65 (CH), 127.83 (CH), 127.91 (CH), 127.93 (CH), 128.04 (CH), 128.17 (CH), 128.28 (CH), 128.33 (CH), 128.38 (CH), 128.39 (CH), 128.72 (CH), 128.77 (CH), 135.75 (C), 136.40 (C), 136.46 (C), 137.62 (C, d, J=86.1 Hz), 137.93 (C), 138.05 (C), 138.26 (C), 138.43 (C), 148.14 (C, d, J=5.7 Hz), 149.26 (C), 153.01 (C), 156.15 (^{13}C label), 156.69 (C, d, J= 2.5 Hz), 162.07 (C), 164.48 (C) and 178.82 (C, d, J= 6.8 Hz); m/z (FAB) 1118.3 [$(M+Na)^+$, 55%], 1096.3 (15), 1028.2 (6), 664.2 (5), 574.1 (18); [Found: $(M+Na)^+$, 1118.4188. $C_{69}{}^{13}CH_{62}O_{12}Na$ requires (M^+Na), 1118.4172]; (Found: C, 76.74; H, 5.75%. $C_{69}{}^{13}CH_{62}O_{12}$ requires C, 76.78; H, 5.70%); $[\alpha]_D^{19}$=−26.0 (c=0.043 g ml^{-1}, $CHCl_3$).

[2-^{13}C]-Quercetin-4′-β-D-glucoside monohydrate 12. 20% Palladium hydroxide on carbon (150 mg) was added to a stirring suspension of flavonol **11** (1.06 g, 0.88 mmol) in EtOAc–MeOH (1:1, 20 ml) under an atmosphere of hydrogen. The suspension was stirred overnight and then the solution was filtered through a plug of celite eluting with MeOH (15 ml). The filtrate was concentrated under vacuum and the resulting solid recrystallised from MeOH–water (4:1) to give the monohydrate of the flavonol **12** as a yellow solid. (400 mg, 94%); mp 200–205°C; ν_{max}(nujol)/cm^{-1}: 3368 (OH), 1654 (C=O), 1592 (Ar) and 1505 (Ar); δ_H (400 MHz, D6-DMSO) 3.17–3.51 (6H, m, H-2″, 3″, 4″,

6″), 4.62 (1H, broad s, OH), 4.85 (1H, d, J=7.1 Hz, H-1″), 5.07 (1H, broad s, OH), 5.12 (1H, broad s, OH), 5.43 (1H, broad s, OH), 6.20 (1H, d, J=1.9 Hz, H-6), 6.45 (1H, d, J=1.9 Hz, H-8), 7.25 (1H, d, J=8.7 Hz, H-5′), 7.62 [1H, ddd, J=8.8 Hz, 4.0 Hz (^{13}C–^{1}H) and 2.0 Hz, H-6′], 7.71 [1H, dd, J=4.0 Hz (^{13}C–^{1}H) and 2.0 Hz, H-2′], 8.95 (1H, broad s, OH), 9.50 (1H, broad s, OH), 10.80 (1H, broad s, OH) and 12.40 (1H, s, OH); δ_C (100 MHz, D6-DMSO): 61.05 (CH_2), 70.13 (CH), 73.61 (CH), 76.29 (CH), 77.62 (CH), 93.85 (CH, d, J=3.1 Hz), 98.60 (CH), 101.73 (CH), 103.43 (C), 115.49 (CH, d, J=1.7 Hz), 116.18 (CH, d, J=5.0 Hz), 119.86 (CH), 125.45 (C, d, J=68.0 Hz), 136.70 (C, d, J= 90.1 Hz), 146.24 (^{13}C label), 146.70 (C, d, J=5.6 Hz), 147.11 (C, d, J=1.3 Hz), 156.57 (C, d, J=2.7 Hz), 161.05 (C), 164.41 (C), 176.38 (C, d, J=5.7 Hz); m/z (FAB): 466.1 [$(M+H)^+$, 86%], 303.0 (100); (Found: $(M+H)^+$, 466.1064. $C_{20}{}^{13}CH_{21}O_{12}$ requires (M+H), 466.1066); (Found: C, 52.36; H, 4.71%. $C_{20}{}^{13}CH_{22}O_{13}$ requires C, 52.38; H, 4.59%); λ_{max} (EtOH) 365.4 (ϵ=18,116.4) and 253.2 (ϵ=16,386.6); $[\alpha]_D^{19}$= −65.6 (c=0.025 g ml^{-1}, MeOH).

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