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A versatile synthesis of [2,3,4-$^{13}C_3$]isoflavones

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A flexible synthetic method is presented, which allows all the key isoflavones (daidzein, genistein, glycitein, formononetin and biochanin A) to be prepared in ^{13}C-labelled form *via* the same route, involving the thallium(III)-mediated oxidative rearrangement of a key chalcone intermediate. This method results in the incorporation of ^{13}C atoms at the 2, 3 and 4 positions of the isoflavone skeleton. We also report the first syntheses of ^{13}C-labelled versions of the daidzein metabolites, equol and ODMA.

Keywords: daidzein; genistein; isoflavones; phytoestrogens; polyphenols

Introduction

Isoflavone phytoestrogens are polyphenolic compounds with weak oestrogenic and antiestrogenic activity,[1,2] which are present in the human diet where the main source is soybeans and products derived from soybeans.[3,4] The most common isoflavones are daidzein (**1a**), formononetin (**1b**), genistein (**1c**), biochanin A (**1d**) and glycitein (**2**) and, of these, genistein appears to be the most biologically active.[5] In epidemiological studies, consumption of an isoflavone rich diet has been shown to be correlated with a decrease in the incidence of hormone-related cancers, including breast[6,7] and prostate cancer.[8] Other health-promoting activities have also been associated with the isoflavones, including lessening of menopausal symptoms,[9,10] and chemoprevention of osteoporosis[11] and cardiovascular disease (Scheme 1).[12]

Accurate analysis of isoflavones is highly important for establishing the dietary exposure of the population to the soy isoflavones and for subsequent epidemiological studies aimed to investigate the associations between isoflavone exposure and disease.[13] In recent years, mass spectrometry-based methods, such as LC-MS[14,15] and GC-MS,[16] have become the most popular for isoflavone analysis. The choice of internal standard is vital if one wishes to obtain optimum accuracy and reproducibility from these methods and the best internal standard is a pure, stable, isotopically labelled analogue of the analyte, with a sufficiently large enough mass difference to nullify the effect of natural abundance heavy isotopes in the analyte. The mass difference required between standard and analyte depends on the molecular weight of the analyte and whether any heteroatoms are present. An extra 3 mass units is sufficient for molecules of the size of isoflavones. This ensures that with 99% enrichment at each position there is less than 1 ppm residual unlabelled analyte in the internal standard and that the M^{3+} ion due to natural ^{13}C in the analyte will be at less than 1% abundance, giving minimal overlap.

We have previously synthesized a series of multiply ^{13}C-labelled isoflavones, namely [3,4,8-$^{13}C_3$]daidzein,[17] [3,4,1′-$^{13}C_3$]genistein[18] and [2,3,4-$^{13}C_3$]glycitein.[19] These compounds have all been successfully employed as internal standards for analysis.[13–16,20,21] The only problem with this work has been that different routes have been employed for each isoflavone. In order to simplify the production of the ^{13}C-labelled isoflavones and harmonize the labelling pattern, we herein report the development of a synthetic method which allows all the key isoflavones to be prepared *via* the same route resulting in ^{13}C labelling at the same positions in the isoflavone skeleton. We also report the first syntheses of ^{13}C-labelled versions of the daidzein metabolites, equol[22] and ODMA.[23]

Result and discussion

The most efficient route employed in our previous work on ^{13}C-labelled isoflavones was that for the synthesis of [2,3,4-$^{13}C_3$]glycitein.[19] In this synthesis, the isoflavone framework was constructed *via* oxidative rearrangement of a chalcone intermediate. This gave the target compound in 57% overall yield in only eight steps from [$^{13}C_2$]acetyl chloride, with the third ^{13}C atom coming from potassium [^{13}C]cyanide.[19] It was thus decided to modify this route for daidzein, genistein, formononetin and biochanin A.

Initially studies concentrated on the synthesis of daidzein and genistein, using methyl ether protection for the hydroxyl groups. The key chalcone intermediate was prepared *via* condensation of two ^{13}C-labelled components, an acetophenone derivative and 4-methoxy-[*carbonyl*-^{13}C]benzaldehyde. BCl_3-assisted Friedel-Crafts acetylation of 3-methoxyphenol **5a** and 3,5-dimethoxyphenol **5b** with [$^{13}C_2$]acetyl chloride in anhydrous CH_2Cl_2 provided the two acetophenones required, 2-hydroxy-4-methoxy-[1′,2′-$^{13}C_2$]acetophenone **6a** and 2-hydroxy-4,6-dimethoxy-[1′,2′-$^{13}C_2$]acetophenone **6b,** in 77 and 87% yield, respectively. The 4-methoxy-[*carbonyl*-^{13}C]benzaldehyde **9**, was prepared *via* $Pd(OAc)_2$-catalysed cyanation[17,24] of 4-methoxyiodobenzene **7** using potassium [^{13}C]cyanide in the

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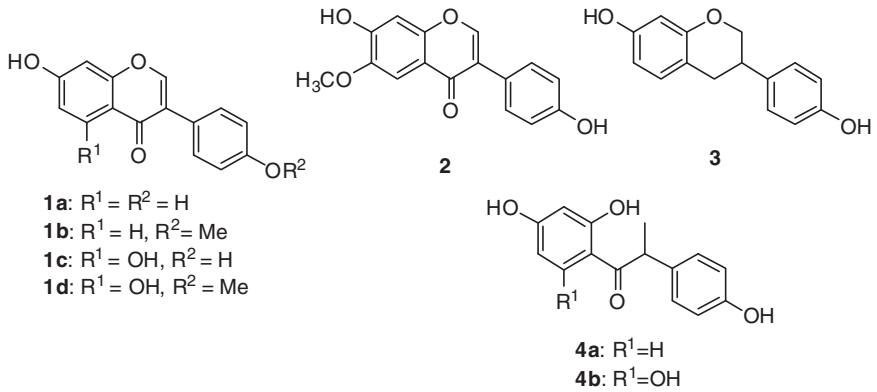
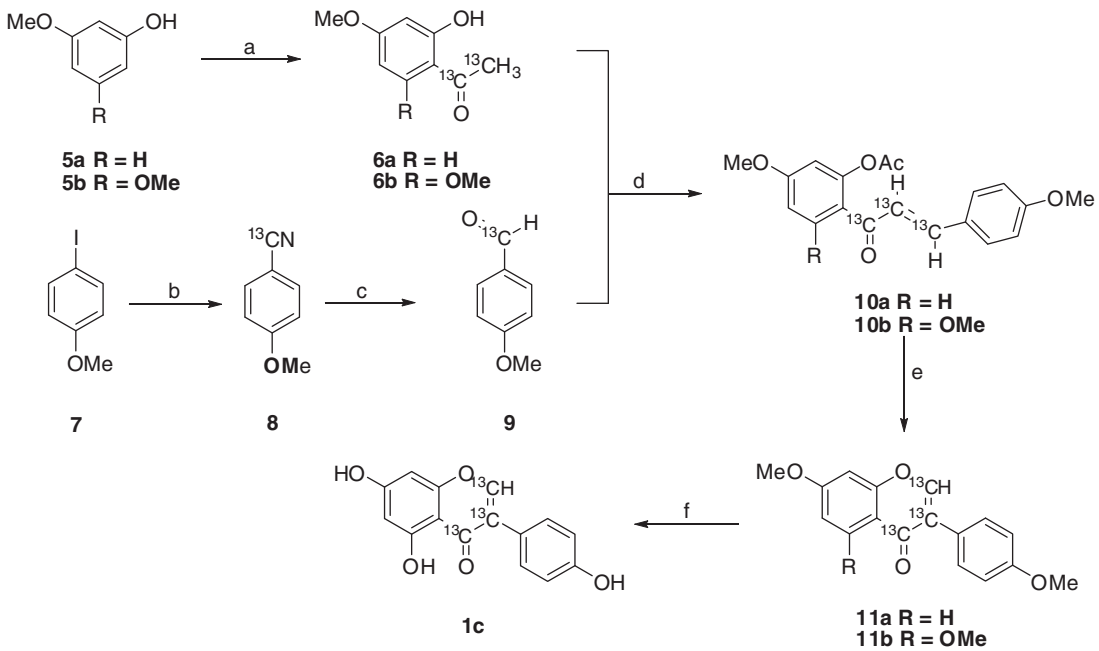


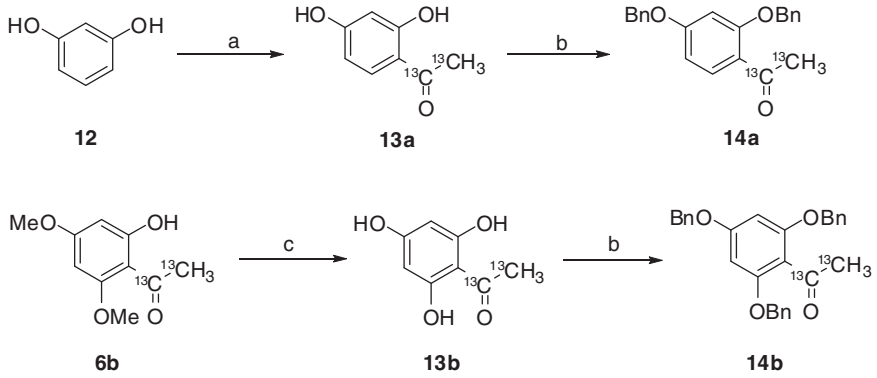
Scheme 1. Isoflavone phytoestrogens and their metabolites.



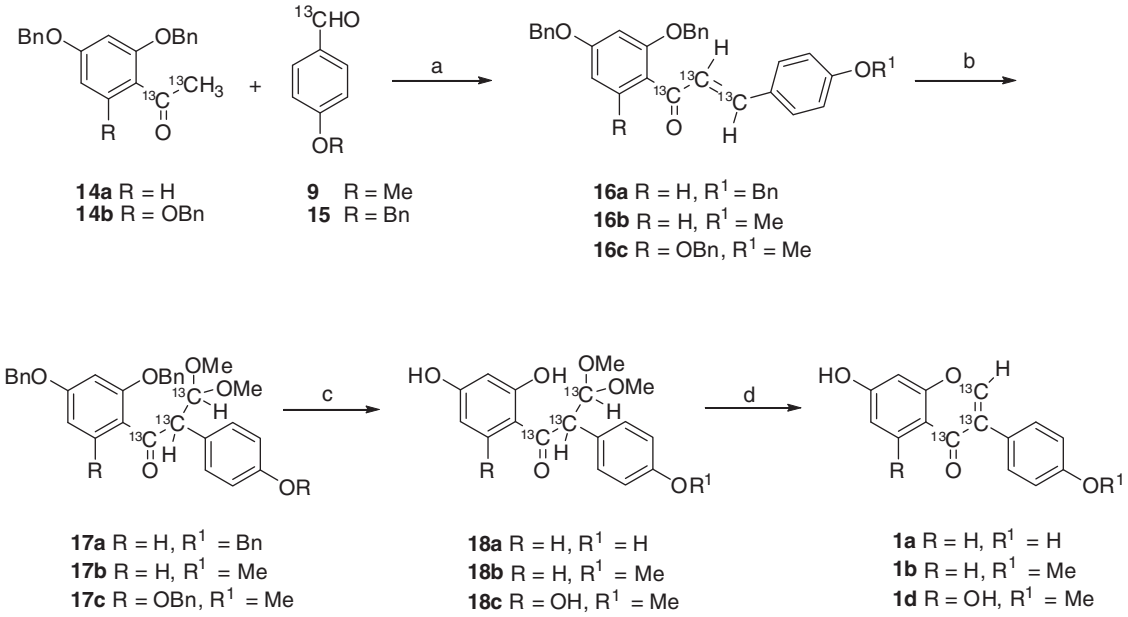
Scheme 2. Synthesis of [2,3,4-$^{13}C_3$] genistein. Reagents and conditions: (a) (i) [$^{13}C_2$]Acetyl chloride, 1 M BCl_3 in CH_2Cl_2, CH_2Cl_2, 0°C→reflux, 3 h; (b) K^{13}CN, $Ca(OH)_2$, $Pd(OAc)_2$, DMF, reflux, 16 h; (c) DIBAL-H, THF, rt, overnight; (d) i) KOH, MeOH, reflux, 6 h, ii) Ac_2O, Py, rt, 24 h; (e) $Tl(NO_3)_3.3H_2O$, $HC(OMe)_3$, MeOH, rt, 20 h; then 10% HCl, MeOH, reflux, 2 h; and (f) 55% HI, AcOH, 120°C, 48 h.

presence of $Ca(OH)_2$ in DMF, followed by DIBAL-H-mediated reduction of the nitrile **8**. An aldol condensation of **9** with either fragment **6a** or **6b** then gave the corresponding 2′-hydroxychalcones, which were treated with acetic anhydride in pyridine to afford the fully protected chalcones, **10a** and **10b**. The key oxidative rearrangement[19,25] of **10a** and **10b** with $Tl(NO_3)_3.3H_2O$ in a mixed solvent of dry MeOH and $CH(OCH_3)_2$ (3:2 v/v) led to the corresponding acetal, which was hydrolysed *in situ* and cyclized using 10% HCl at reflux temperature to give 7,4′-dimethoxy-[2,3,4-$^{13}C_3$]isoflavone **11a** and 5,7,4′-trimethoxy-[2,3,4-$^{13}C_3$]isoflavone **11b**. The presence of the three ^{13}C atoms was confirmed by the three enhanced signals in the ^{13}C NMR spectra and the 3 mass unit increase in the molecular ion compared with the unlabelled compounds. Unexpectedly, the *O*-demethylation of **11a** was very problematic and treatment of **11a** with 1 M BBr_3-CH_2Cl_2 solution for 3 days gave an intractable mixture. Various other demethylation methods were attempted with **11a** without success; however, cleavage of the methyl ethers in **11b** was successfully achieved with 55% HI in AcOH under reflux temperature to afford [2,3,4-$^{13}C_3$]genistein **1c** in 83% yield, with an overall yield of 49% in four steps from the [$^{13}C_2$]acetyl chloride (Scheme 2).

In order to overcome the demethylation problems, it was decided to use an alternative *O*-benzyl protection strategy. $AlCl_3$-mediated acetylation of resorcinol **12** with [$^{13}C_2$]acetyl chloride in anhydrous nitrobenzene according to a literature procedure[26] gave 2,4-dihydroxy-[1′,2′-$^{13}C_2$]acetophenone **13a**, which was then *O*-benzylated, without further purification, using benzyl bromide in acetone in the presence of excess anhydrous K_2CO_3 and a catalytic amount of 18-crown-6 to give 2,4-*O*-dibenzyloxy-[1′,2′-$^{13}C_2$]acetophenone **14a** (Scheme 3). 2,4,6-*O*-Tribenzyloxy-[1′,2′-$^{13}C_2$]acetophenone **14b** was prepared *via* a BBr_3-mediated *O*-demethylation of **6b**, followed by *O*-benzylation of the crude 2,4,6-trihydroxy-[1′,2′-$^{13}C_2$]acetophenone **13b**, prepared previously (Scheme 3). Three chalcones were then prepared *via* the aldol condensation of **14a** with



Scheme 3. Synthesis of benzyloxy substituted acetophenones (**14a** and **14b**). Reagents and conditions: (a) [$^{13}C_2$]Acetyl chloride, $AlCl_3$, nitrobenzene, rt, 15 h; (b) BnBr, K_2CO_3, 18-crown-6, acetone, reflux, 8 h; and (c) 1 M BBr_3 in CH_2Cl_2, CH_2Cl_2, 24 h.

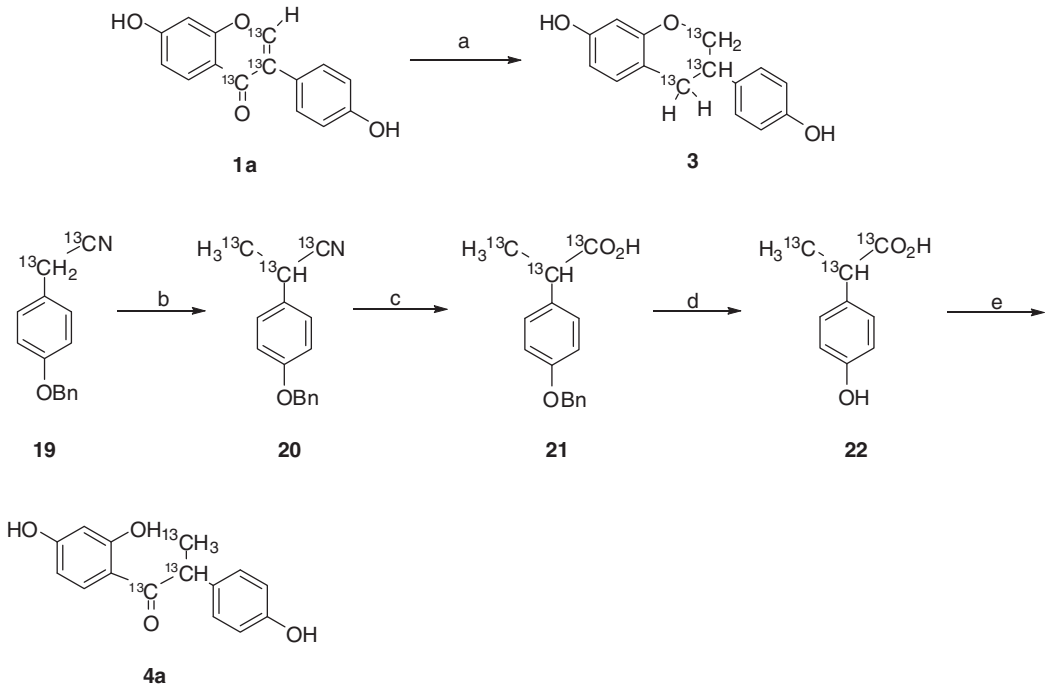


Scheme 4. Synthesis of [2,3,4-$^{13}C_3$] isoflavones. Reagents and conditions: (a) KOH, MeOH, reflux, 7 h; (b) $Tl(NO_3)_3.3H_2O$, $HC(OMe)_3$, MeOH, rt, 20 h; (c) H_2, 5% Pd/C, MeOH, acetone, overnight; and (d) 10% HCl, MeOH, reflux, 6 h.

4-benzyloxy-[*carbonyl*-^{13}C]benzaldehyde[19] **15** and 4-methoxy-[*carbonyl*-^{13}C]benzaldehyde **9**, and the aldol condensation of **14b** with **9**, so that the three target isoflavones were all accessible (Scheme 3). The chalcones were subjected to oxidative rearrangement with $Tl(NO_3)_3.3H_2O$, as before, to give the corresponding substituted acetals **17a**, **17b** and **17c** in 89%, 82% and 40% yields, respectively. *O*-Debenzylation of **17a-17c** using 5% Pd/C-catalyzed hydrogenolysis proceeded smoothly to afford the hydroxyl substituted 1,2-diphenyl-3,3-dimethoxy-1-[1,2,3-$^{13}C_3$]propanones **18a, 18b** and **18c,** which on heating to reflux with 10% HCl in MeOH gave the desired [2,3,4-$^{13}C_3$]-daidzein **1a,** [2,3,4-$^{13}C_3$]formononetin **1b** and [2,3,4-$^{13}C_3$]biochanin A **1d,** in excellent yields and purity. The presence of the three ^{13}C atoms was confirmed by means of ^{1}H and ^{13}C NMR spectroscopy and mass spectrometry (Scheme 4).

Two daidzein metabolites were also required in ^{13}C-labelled form, equol and ODMA. [2,3,4-$^{13}C_3$]Equol **3** was synthesized by catalytic hydrogenation of [2,3,4-$^{13}C_3$]daidzein **1a**, using 10% palladium on charcoal in 95% EtOH at 3 bar, in 71% yield (Scheme 5). [1,2,3-$^{13}C_3$]ODMA **4a** was prepared as outlined in Scheme 5. Selective mono-*C*-methylation of 4-benzyloxyphenyl-[1,2-$^{13}C_2$]acetonitrile **19** with 1 eq. of [^{13}C]methyl iodide in the presence of 1 eq. of lithium *i*-propylcyclohexylamide afforded ethyl 2-(4-benzyloxyphenyl)-[1,2,3-$^{13}C_3$]propionitrile **20** in 73% yield. Basic hydrolysis of the nitrile **20** with 1.5 eq. of NaOH in a 1:1 mixture of ethanol and water at reflux temperature afforded 2-(4-benzyloxyphenyl)-[1,2,3-$^{13}C_3$]propionic acid **21** in almost quantitative yield, which was then subjected to *O*-debenzylation by means of 5% Pd/C-catalyzed hydrogenolysis to give 2-(4-hydroxyphenyl)-[1,2,3-$^{13}C_3$]propionic acid **22**. Condensation of the crude acid **22** with a two-fold excess of resorcinol in the presence of $BF_3.Et_2O$ and $ZnCl_2.Et_2O$ at reflux temperature for 15 min afforded [1,2,3-$^{13}C_3$]ODMA **4a** in 80% yield.

The thallium(III)-mediated oxidative rearrangement of appropriate chalcone intermediates, derived from ^{13}C-labelled acetophenones and benzaldehyde precursors, therefore offers a short, efficient method for the synthesis of [2,3,4-$^{13}C_3$]isoflavones.



Scheme 5. Synthesis of [2,3,4-$^{13}C_3$] equol (**3**) and [$^{13}C_3$]ODMA (**4**). Reagents and conditions: (a) Pd/C, $H_{2(g)}$, EtOH; (b) n-BuLi, i-propylcyclohexylamine, $^{13}CH_3I$, THF, −78°C for 1 h, then 3 h at rt; (c) aq. NaOH, EtOH, reflux, 1.5 h; (d) H_2, 5% Pd/C, EtOH:acetone (2:1); and (e) resorcinol, $BF_3.Et_2O$, $ZnCl_2$, 15 min, reflux.

Experimental

4-Methoxy-2-hydroxy-[1′,2′-^{13}C]acetophenone 6a

A solution of methoxyphenol **5a** (1.85 g, 14.9 mmol) in dry CH_2Cl_2 (10 ml) was slowly added to a solution of BCl_3 (12.5 mL, 1 M in CH_2Cl_2, 12.42 mmol) at −10°C. After 5 min stirring at −10°C, a solution [$^{13}C_2$]acetyl choride (1 g, 0.906 ml, 12.4 mmol) in CH_2Cl_2 (5 mL) was added over 10 min. The reaction mixture was heated under reflux for 3 h before being carefully quenched with excess 1 M HCl (50 mL). After stirring for an hour at room temperature, the aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL), dried over $MgSO_4$, and the solvent was removed at reduced pressure. Column chromatography on silica gel with petroleum ether (bp 40–60):diethyl ether (4:1) as eluant afforded **6a** (1.63 g, 78%) as a white solid: mp 48–50°C (Lit.[27]mp. 49–50°C for the unlabelled); δ_H (300 MHz, $CDCl_3$) 2.57 (3H, dd, J = 129.2, 6.2 Hz, $^{13}CH_3$); 3.87 (3H, s, OCH_3), 6.51 (1H, dd, J = 9.0, 2.5 Hz, H-5), 6.54 (1H, d, J = 2.5 Hz, H-3), 7.44 (1H, dd, J = 9.0, 2.5 Hz, H-6), 12.87 (1H, s, 2-OH); δ_C (75 MHz, $CDCl_3$) 26.2 (d, enhanced, J = 44.5 Hz, CH_3), 55.6 (OCH_3), 100.9 (d, J = 2.5 Hz, C-3), 107.7 (d, J = 4.5 Hz, C-5), 114 (dd, J = 2.4, 1.1 Hz, C-6), 132.3 (d, J = 4.5 Hz, C-1), 165 (d, J = 2.5 Hz, C-2), 166 (d, J = 0.5 Hz, C-4), 202.6 (d, enhanced, J = 44.5 Hz, CO).

4,6-Dimethoxy-2-hydroxy-[1′,2′-^{13}C]acetophenone 6b

As described for **6a,** [$^{13}C_2$]acetyl choride (1 g, 0.906 ml, 12.4 mmol) in CH_2Cl_2 (5 ml) with a solution of dimethoxyphenol **5b** (1.96 g, 12.74 mmol) BCl_3 (1.49, 1 M in CH_2Cl_2 12.7 ml, 12.74 mmol). Column chromatography on silica with petroleum ether (bp 40–60)/diethyl ether (4:1) as eluant afforded **6b** (2.21 g, 90%) as a white solid: mp 79–80°C (Lit.[28]mp. 77°C for the unlabelled); δ_H (300 MHz, $CDCl_3$) 2.61 (3H, dd, J = 129.2 Hz, J = 6.3 Hz, $^{13}CH_3$); 3.82, 3.85 (6H, 2s, 2 × OCH_3), 5.92 (1H, dd, J = 2.3, 1.4 Hz, H-3), 6.06 (1H, d, J = 2.2 Hz, H-5); δ_C (75 MHz, $CDCl_3$) 31.2 (d, enhanced,. J = 42.7 Hz, CH_3), 55.51, 55.52 (2 × OCH_3), 203.6 (d, enhanced, J = 42.7 Hz, CO).

1-(2-Acetoxy-4-methoxyphenyl)-3-(4-methoxyphenyl)-[1,2,3-$^{13}C_3$]prop-2-en-1-one 10a

A mixture of the acetophenone **6a** (1.75 g, 10.42 mmol), benzaldehyde **9** (1.7 g, 12.41 mmol) and KOH (5.83 g, 104.16 mmol) in EtOH (70 mL) was heated to reflux under a nitrogen-atmosphere for 5 h. After cooling down to room temperature, the mixture was poured into ice-water, neutralized with 1 N HCl, and the yellow precipitate was filtered off and washed with 50% aqueous MeOH to afford the 1-(2-hydroxy-4-methoxyphenyl)-3-(4-methoxyphenyl)-[1,2,3-$^{13}C_3$] prop-2-en-1-one (2.7 g, 91%) as yellow solid; mp 102–104°C (Lit[29] 106–108°C for the unlabelled); δ_H (500 MHz, $CDCl_3$) 3.86, 3.87 (6H, 2 × s, 2 × OCH_3), 6.47–6.50 (2H, m, H-3′,5′), 6.95 (2H, d, J = 8.7 Hz, H-3″,5″), 7.46 (1H, dddd, J = 155, 15.5, 5.4, 1.5 Hz, H-2), 7.62 (2H, dd, J = 8.7, 4.6 Hz, H-2″,6″), 7.83 (1H, dd, J = 8.7, 3.6 Hz, H-6′), 7.87 (1H, dddd, J = 157, 15.4, 5.7, 2.3 Hz, H-3), 13.56 (1H, s, OH); δ_C (75 MHz, $CDCl_3$) 118.0 (dd, J = 72.7, 58.3 Hz, C-2); 144.5 (d, enhanced, J = 72.7 Hz, C-3), 191.9 (d, enhanced, J = 58.3 Hz, ^{13}C-3). To a solution of the 2′-hydroxychalcone (2.5 g, 8.71 mmol) in dry pyridine (20 mL) was added acetic anhydride (3 mL) at room temperature, and the resulting mixture was stirred overnight. The reaction was quenched with ice-water and extracted with CH_2Cl_2 (3 × 50 mL). The combined extracts were washed with 1 N HCl, brine, water and dried over $MgSO_4$. The solvent was evaporated and the residue was purified by flash chromatography on silica gel using CH_2Cl_2:EtOAc (98:2) as eluant to give the title compound **10a** (2.72 g, 91%) as a yellow semi-solid, which was used without purification in the next step. δ_H (500 MHz, $CDCl_3$) 2.27 (3H, s,

CH_3CO) 3.85, 3.87 (6H, 2 × s, 2 × OCH_3), 6.66 (1H, d, J = 2.5 Hz, H-3′), 6.86 (1H, dd, J = 8.7, 2.5 Hz, H-5′), 6.92 (2H, d, J = 9.0 Hz, H-3″,5″), 7.12 (1H, ddd, J = 157, 16.0, 2.5 Hz, CH-2), 7.54 (2H, dd, J = 9.0, 4.6 Hz, H-2″,6″), 7.64 (1H, dm, J = 150 Hz H-3), 7.76 (1H, dd, J = 8.7, 3.9 Hz, H-6′); δ_C (75 MHz, $CDCl_3$) 122.7 (dd, J = 57.2, 70.6 Hz, C-2); 144.2 (d, enhanced, J = 70.6 Hz, C-3), 189.7 (d, enhanced, J = 57.2 Hz, C-1).

3-(4-Methoxyphenyl)-1-[2-acetoxy-4,6-dimethoxyphenyl]-2-[1,2,3-$^{13}C_3$]propen-1-one 10b

A mixture of the acetophenone **6b** (1.0 g, 5.05 mmol), benzaldehyde **9** (0.72 g, 5.25 mmol) and KOH (2.82 g, 50.50 mmol) in dry MeOH (70 mL) and THF (20 mL) was heated to reflux under a nitrogen atmosphere for 6 h. The mixture was cooled down to room temperature, poured into ice-water, the yellow solid was filtered, washed with 50% aq. MeOH and dried to afford the product (1.41 g, 88%) as a yellow solid which was subjected to further reaction without purification. Acetic anhydride (5 mL) was added to a solution of the yellow solid (1.4 g, mmol) in dry pyridine (10 mL). After overnight stirring at rt, the mixture was poured into ice-water, extracted with CH_2Cl_2 (2 × 50 mL), the combined extracts were washed with 1 N HCl, brine, water and dried over $MgSO_4$. The solvent was evaporated at reduced pressure and the residue was purified by chromatography on silica gel with CH_2Cl_2:EtOAc (98:2) as eluant to yield the title compound **10b** (1.46 g, 92%) as an yellow semi solid; δ_H (500 MHz, $CDCl_3$) 2.17 (3H, s, CH_3CO), 3.79 (3H, s, OCH_3), 3.85 (6H, s, 2 × OCH_3), 6.29 (1H, d, J = 2.5 Hz, H-3′), 6.41 (1H, dd, J = 8.7, 2.5 Hz, H-5′), 6.87 (1H, ddd, J = 160, 16.0, 2.5 Hz, H-2), 6.89 (2H, d, J = 9.0 Hz, H-3″,5″), 7.38 (1H, dm, J = 160 Hz, H-3), 7.49 (2H, dd, J = 9.0, 4.5 Hz, H-2″,6″); δ_C (75 MHz, $CDCl_3$) 126.0 (dd, enhanced, J = 58.3, 69.4 Hz, C-2); 144.2 (d, enhanced, J = 69.4 Hz, C-3), 191.9 (dd, enhanced, J = 58.3 Hz, C-1); HRMS (EI): $C_{17}{}^{13}C_3H_{20}O_6$ requires 359.1361; Found 359.1355.

7,4′-Dimethoxy-[2,3,4-$^{13}C_3$]isoflavone 11a

The chalcone **10a** (2.75 g, 8.36 mmol) was dissolved in a mixture of MeOH (30 mL) and $CH(OMe)_3$ (20 mL), followed by the addition of $Tl(NO_3)_3.3H_2O$ (4.87 g, 10.96 mmol). After 24 h stirring at room temperature, a 10% HCl solution (40 mL) was added and the reaction mixture was stirred under reflux for another 3 h. The mixture was cooled in an ice bath, and precipitates were removed by filtration. The filtrate was concentrated under reduced pressure and poured into ice cold water. After 24 h the precipitates were filtered and recrystallized from MeOH to afford **11a** (1.98 g, 83%) as light yellow solid, mp 160–161°C (Lit.[30] 161–162°C for the unlabelled); δ_H (300 MHz, $CDCl_3$) 3.79, 3.81 (6H, 2 × s, OCH_3), 6.86 (1H, dd, J = 2.3, 1.6 Hz, H-8), 6.95 (2H, d, J = 8.8 Hz, H-3′,5′), 6.99 (1H, dd, J = 8.9, 2.5 Hz, H-6), 7.49 (2H, d, J = 8.8, 3.4 Hz, H-2′, 6′), 7.94 (1H, dt, J = 191.3, 6.4 Hz, H-2), 8.21 (1H, dd, J = 8.9, 5.3 Hz, H-5).

5,7,4′-Trimethoxyisoflavone 11b

$Tl(NO_3)_3.3H_2O$ (1.97 g, 4.43 mmol) was added to a solution of the chalcone **10b** (1 g, 2,78 mmol) in a mixture of MeOH (30 mL) and $CH(OMe)_3$ (20 mL). After 24 h stirring at room temperature, 10% HCl (40 mL) was added and the reaction mixture stirred under reflux for a further 3 h. The mixture was cooled in an ice bath, and precipitates were removed by filtration. The filtrate was concentrated under reduced pressure and poured into cold water, extracted with CH_2Cl_2 (2 × 100 mL), dried ($MgSO_4$), filtered and concentrated at reduced pressure. The brown solid was purified by flash chromatography on silica gel with EtOAc:CH_2Cl_2 (95:5) as eluant to afford **11b** (0.675 g, 77%) as a light yellow solid, mp 160–161°C (Lit[31] 162–163°C for the unlabelled); δ_H (300 MHz, CDCl3) 3.83 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 6.36 (1H, d, J = 1.6 Hz, H-8), 6.44 (1H, dd, J = 2.3, 1.6 Hz, H-6), 6.93 (2H, d, J = 8.8 Hz, H-3′,5′), 7.48 (2H, dd, J = 8.8, 3.4 Hz, H-2′, 6′), 7.76 (1H, dt, J = 194.3, 6.3 Hz, H-2); δ_C (75 MHz, d_6-$CDCl_3$) 125.9 (dd, enhanced J = 70.8, 55.4 Hz, C-3), 150.0 (d, enhanced J = 70.8 Hz, C-2), 175.4 (d, enhanced J = 55.4 Hz, C-4); HRMS (EI): $C_{15}{}^{13}C_3H_{16}O_5$ requires 315.1098; Found 315.1098.

[2,3,4-$^{13}C_3$]Genistein 1c

A mixture of **11b** (1 g, 3.17 mmol) and 55% HI in AcOH (100 mL) was stirred at 120°C for 48 h. The mixture was cooled down to rt, poured into ice-water, the precipitate was filtered and recrystallized from 80% MeOH to afford **1c** (0.72 g, 83%) as light yellow solid; mp 297–300°C (Lit[32] 301–302°C for the unlabelled); δ_H (300 MHz, d_6-DMSO) 6.22 (1H, dd, J = 2.1, 0.9 Hz, H-8), 6.39 (1H, dd, J = 2.1, 1.5 Hz, H-6), 6.81 (2H, d, J = 8.6 Hz, H-3′,5′), 7.37 (2H, d, J = 8.6, 3.5 Hz, H-2′, 6′), 8.31 (1H, dt, J = 199.3, 6.8 Hz, H-2), 9.59 (1H, s, 4′-OH), 10.88 (1H, s, 7-OH), 12.95 (1H, s, 5-OH); δ_C (75 MHz, d_6-DMSO) 122.13 (dd, enhanced J = 71.5, 54.6 Hz, C-3), 153.90 (d, enhanced J = 71.5 Hz, C-2), 170.11 (d, enhanced J = 54.6 Hz, C-4); HRMS (EI): $C_{12}{}^{13}C_3H_{11}O_5$ requires 274.0707; Found 274.0708.

2,4-[1′,2′-$^{13}C_3$]Dibenzyloxyacetophenone 14a

$AlCl_3$ (2.15.2 g, 16.14 mmol) followed by a solution of [$^{13}C_2$]acetyl choride (1 g, 0.906 ml, 12.4 mmol) in nitrobenzene (2 mL) were added to an ice-cold solution of resorcinol **12** (1.77 g, 16.09 mmol) in dry nitrobenzene (8 mL) under a nitrogen atmosphere. After 15 h stirring at room temperature, the reaction was quenched with an excess of 1 M HCl (50 mL). The aqueous phase was extracted with EtOAc (3 × 50 mL) and dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a brown solid, which on column chromatography on silica gel with CH_2Cl_2:MeOH (98:2) as eluant, afforded **14a** as a white solid (1.8 g, 95%); mp 142–144°C (Lit.[33] 143°C for the unlabelled); δ_H (300 MHz, d_6-acetone) 2.53 (3H, dd, J = 127.9, 5.8 Hz, CH_3), 6.31 (1H, dd, J = 2.4, 0.9 Hz, H-3), 6.43 (1H, ddd, J = 8.8, 2.4, 0.6 Hz, H-5), 7.78 (1H, dd, J = 8.8, 4.1 Hz, H-6), 9.43 (1H, s, 4-OH), 12.74 (1H, d, 2-OH). To a solution of the acetophenone **13a** (1.8 g, 11.69 mmol) in acetone (100 mL) were added anhydrous K_2CO_3 (19.35 g, 140.24 mmol), 18-crown-6 (0.309 g, 1.17 mmol) and benzyl bromide (3.13 mL, 26.3 mmol) at room temperature. The mixture was heated under reflux for 8 h, cooled and concentrated under reduced pressure to give a slurry, which was partitioned between H_2O (100 mL) and CH_2Cl_2 (3 × 100 mL). The combined extracts were dried over $MgSO_4$ and concentrated at reduced pressure to give an oil, which was purified on column chromatography to afford **13a** (3.2 g, 82%) as a white semi solid: δ_H (300 MHz, $CDCl_3$) 2.58 (3H, dd, J = 128.1, 6.3 Hz, CH_3), 5.09, 5.12 (4H, 2 × s, $PhCH_2$), 6.61–6.65 (2H, m, H-3, 5), 7.32–7.47 (10H, m, 2 × Ph), 7.87 (1H, dd, J = 9.4, 4.1 Hz, H-6); δ_C (75 MHz, d_6-$CDCl_3$) 32.2 (d, enhanced, J = 42.3 Hz, CH_3), 70.2, 70.7 (C-PhCH_2); 197.4 (d,

enhanced, $J = 42.3$ Hz, C-2); Spectroscopic data in agreement with previous literature.[34]

2,4,6-[1′,2′-$^{13}C_3$]Tribenzyloxyacetophenone 14b

An excess of 1 M BBr_3/CH_2Cl_2 (24 mL, 24.24 mmol) was added slowly to a solution of **6b** (1.2 g, 6.66 mmol) in CH_2Cl_2 (10 mL) under Ar at rt. Ater 24 h stirring at rt, water (100 mL) was added to the mixture which was heated under reflux for 3 h. The CH_2Cl_2 was removed at reduced pressure and the residue was extracted with EtOAc (3×50 mL). The combined extracts were washed with water, dried ($MgSO_4$), and concentrated to give an orange solid that was dried and reacted without further purification. To a solution of the acetophenone **13b** in acetone (100 mL), benzyl bromide (2.88 mL, 24.24 mmol), 18-crown-6 (100 mg) and K_2CO_3 (8.11 g, 58.8 mmol) were added. After 8 h stirring at 60°C, the mixture was cooled, poured into water and extracted with EtOAc (3×50 mL). The combined extracts were washed with brine, water, dried ($MgSO_4$) and the solvent removed at reduced pressure. The residue was purified by flash chromatography on silica with CH_2Cl_2:petroleum ether (9:1 to 7:3) as eluant to afford the title compound **14b** (1.72 g, 65%) as a white solid, mp 81–83°C (Lit.[35] 82–84°C for the unlabelled); δ_H (300 MHz, $CDCl_3$) 2.38 (3H, dd, $J = 128.1$, 6.3 Hz, CH_3), 4.91 (2H, s, OCH_2Ph), 4.96 (4H, s, $2 \times OCH_2Ph$), 6.16 (2H, d, $J = 1.0$ Hz, H-3, 5), 7.17–7.31 (15H, m, $3 \times$ Ph); δ_C (75 MHz, d_6-$CDCl_3$) 32.6 (enhanced d, $J = 42.5$ Hz, CH_3), 70.2, ($PhCH_2$); 70.6 ($2 \times PhCH_2$), 201.5 (d, enhanced, $J = 42.5$ Hz, C-2); HRMS (ES) $C_{27}^{13}C_2H_{26}O_4Na$ requires 463.1796; Found 463.1801.

2-(4-Benzyloxyphenyl)-1-[2,4-bis(benzyloxy)phenyl]-2-[1,2,3-$^{13}C_3$]propenone 16a

A described for **10a** using [1′,2′-$^{13}C_2$]-2,4-dibenzyloxyacetophenone **14a** (1.5 g, 4.49 mmol) with 4-benzyloxy-[*carbonyl*-^{13}C]-benzaldehyde **15** (1.15 g, 5.4 mmol) as pale yellow precipitate. The precipitate was washed with water and MeOH to offer compound **16a** (2.24 g, 93%) as a light yellow precipitate, which was used without further purification. δ_H (300 MHz, $CDCl_3$) 5.10, 5.12, 5.14 (6H, $3 \times$ s, OCH_2Ph), 6.66–6.70 (2H, m, H-3′, 5′), 6.87 (2H, d, $J = 8.8$ Hz, H-3″,5″), 7.23–7.83 (19H, m, $3 \times$ Ph and H-2″, 6″, H-2 and H-3), 7.89 (1H, dd, $J = 9.2$, 4.1 Hz, H-6′); δ_C (75 MHz, $CDCl_3$) 125.0 (dd, enhanced, $J = 70.8$, 56.5 Hz, C-2), 141.8 (d, enhanced, $J = 70.8$ Hz, C-3), 189.72 (d, enhanced, $J = 56.5$ Hz, C-1); HRMS (EI) $C_{33}{}^{13}C_3H_{30}O_4$ requires 529.2245; Found 529.2242.

3-(4-Methoxyoxyphenyl)-1-[2,4-bis(benzyloxy)phenyl]-2-[1,2,3-$^{13}C_3$]propen-1-one 16b

A mixture of the acetophenone **14a** (1.45 g, 4.34 mmol), 4-methoxy-[*carbonyl*-^{13}C]benzaldehyde **9** (0.65 g, 4.78 mmol) and KOH (2.92 g, 52.14 mmol) in dry MeOH (70 mL) and THF (20 mL) was heated to reflux under a nitrogen atmosphere for 6 h. The mixture was cooled in an ice bath, the yellow solid was filtered off, washed with cold MeOH and water to give the desired chalcone **16b** (1.78 g, 90%) as yellow oil which was used without further purification. δ_H (300 MHz, $CDCl_3$) 3.84 (3H, s, OCH_3), 5.12, 5.13 (4H, $2 \times$ s, $2 \times PhCH_2$), 6.66–6.69 (2H, m, H-3′,5′), 6.80 (2H, d, $J = 8.8$ Hz, H-3″,5″), 7.22–7.47 (13.5H, m, $2 \times$ Ph, H-2, H-2″,6″ and 1/2H-3), 7.75–7.82 (1/2H, ddd, $J = 15.8$, 4.8, 2.8 Hz, 1/2H-3), 7.88 (1H, dd, $J = 9.2$, 4.1 Hz, H-6′), δ_C (75 MHz, $CDCl_3$) 126.2 (d, $J = 71.6$, 56.3 Hz, C-2); 141.88 (d, enhanced, $J = 71.6$ Hz, C-3), 189.8 (d, enhanced, $J = 56.3$ Hz, C-1).

3-(4-Methoxyphenyl)-1-[2,4,6-tris(benzyloxy)phenyl]-[1,2,3-$^{13}C_3$]-2-propenone 16c

A described for **10a** using [1′,2′-^{13}C]-2,4,6-tribenzyloxyacetophenone **14b** (1.9 g, 4.32 mmol) with benzaldehyde **9** (0.65 g, 4.78 mmol). After 8 h stirring at reflux temperature, the mixture was poured into ice-water and extracted with CH_2Cl_2 (3×100 mL). The combined extracts were washed with brine (100 mL), water (100 mL), and dried ($MgSO_4$). The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography on silica with petroleum ether:CH_2Cl_2 (7:3) as eluant to afford the desired the chalcone **16c** (1.85 g, 76%) as a yellow semi solid, which was used without further purification. δ_H (300 MHz, $CDCl_3$) 3.74 (3H, s, OCH_3), 4.92, 4.97 (4H, $2 \times$ s, OCH_2Ph), 6.18 (2H, d, $J = 0.95$ Hz, H-3′, 5′), 6.75 (1H, ddd, $J = 146$, 16.1, 2.1 Hz, H-2), 6.88 (2H, d, $J = 8.8$ Hz, H-3″,5″), 7.07–7.28 (15.5H, m, PhH and 1/2 H-3), 7.31 (2H, dd, $J = 8.8$, 4.5 Hz, H-2″,6″), 7.41–7.49 (1/2H, m, 1/2 H-3); δ_C (75 MHz, $CDCl_3$) 127.29 (dd, enhanced, $J = 70.5$, 56.5 Hz, C-2), 144.5 (d, enhanced, $J = 70.5$ Hz, C-3), 194.4 (d, enhanced, $J = 58.5$ Hz, C-1); HRMS (ES) $C_{34}{}^{13}C_3H_{32}O_5Na$ requires 582.2248; Found 582.2256.

2-(4-Benzyloxyphenyl)-1-(2,4-bis(benzyloxyphenyl)-3,3-dimethoxy-1-[1,2,3-$^{13}C_3$]propanone 17a

$Tl(NO_3)_3.3H_2O$ (2.17 g, 4.89 mmol) was added to a suspension of the chalcone **16a** (2.15 g, 4.06 mmol) in a mixture of MeOH (20 mL) and $CH(OMe)_3$ (15 mL). The mixture was stirred at rt for 8 h, poured into ice-cold saturated $NaHCO_3$ and extracted with CH_2Cl_2 (3×50 mL). The combined extracts were washed with brine, water and dried over $MgSO_4$. Removal of the solvent under reduced pressure gave an orange oil that was purified by chromatography on silica gel with $PhCH_3$:EtOAc (8:2) as eluant to give **17a** (2.15 g, 89%) as a white semi solid, δ_H (300 MHz, $CDCl_3$) 3.04, 3.36 (6H, $2 \times$ d, $J = 4.5$ Hz, $CH(OCH_3)_2$), 4.74 (1/2H, d, $J = 8.7$ Hz, 1/2H-3), 4.83–4.87 (1/2H, m, 1/2H-2), 4.98 (2H, s, CH_2Ph), 5.03 (2H, s, CH_2Ph), 5.05 (1H, d, $J = 10.8$ Hz, CH^bPh-2′), 5.12 (1H, d, $J = 10.8$ Hz, CH^aPh-2′), 5.28–5.30 (1H, m, 1/2H-2 and 1/2 H3), 6.52 (1H, dd, $J = 2.1$, 1.1 Hz, H-3′), 6.56 (1H, dd, $J = 8.7$, 2.1 Hz, H-5′), 6.78 (2H, d, $J = 8.7$ Hz, H-3″, 5″), 7.08 (2H, dd, $J = 8.7$, 3.2 Hz, H-2″,6″), 7.31–7.41 (15H, m, $3 \times$ Ph), 7.78 (1H, dd, $J = 8.7$, 4.2 Hz, H-6′); δ_C (75 MHz, $CDCl_3$) 58.5 (dd, enhanced, $J = 47.5$, 42.5 Hz, C-2), 106.8 (d, enhanced, $J = 47.5$ Hz, C-3), 198.0 (d, enhanced, $J = 42.5$ Hz, C-1), HRMS (ES^+) $C_{35}{}^{13}C_3H_{36}O_6$ requires 614.2510; Found 614.2513.

2-(4-Methoxyphenyl)-1-(2,4-bis(benzyloxyphenyl)-3,3-dimethoxy-1-[1,2,3-$^{13}C_3$]propanone 17b

As described for **17a**, using the chalcone **16b** (1.225 g, 2.70 mmol) with $Tl(NO_3)_3.3H_2O$ (1.44 g, 3.24 mmol), gave the title compound **17b** (1.19 g, 82%) as a white semi solid; δ_H (300 MHz, $CDCl_3$) 3.05, 3.38 (6H, $2 \times$ d, $J = 4.5$ Hz, $CH(OCH_3)_2$), 3.76 (3H, s, OCH_3), 4.76 (1/2H, d, $J = 8.7$ Hz, 1/2H-3), 4.84–4.90 (1/2iH, m, 1/2H-2), 5.04 (2H, s, CH_2Ph), 5.08 (1H, d, $J = 11.6$ Hz, CH^bPh-2′), 5.12 (1H, d, $J = 11.6$ Hz, CH^bPh-2′), 5.26–5.32 (1H, m, 1/2H-2 and 1/2H-3), 6.53 (1H, dd, $J = 2.2$, 1.1 Hz, H-3′), 6.58 (1H, dd, $J = 8.7$, 2.2 Hz, H-5′), 6.72 (2H, d, $J = 8.8$ Hz, H-3″, 5″), 7.10 (2H, dd, $J = 8.8$, 3.2 Hz, H-2″,6″), 7.31–7.41 (10H, m, $2 \times$ Ph), 7.77 (1H, dd, $J = 8.7$, 4.1 Hz, H-6′); δ_C (75 MHz, $CDCl_3$) 58.4 (dd, enhanced, $J = 47.5$, 42.7 Hz, C-2), 106.7 (d, enhanced, $J = 47.5$ Hz, C-3), 198.0 (d, enhanced, $J = 42.7$ Hz, C-1), HRMS (ES^+) $C_{29}{}^{13}C_3H_{32}O_6Na$, requires 538.2197; Found 538.2181.

1-(2,4-dihydroxyphenyl)-2-(4-hydroxyphenyl)-3,3-dimethoxy-1-[1,2,3-$^{13}C_3$]propanone 18a

Compound **17a** (2.15 g, 3.64 mmol) was hydrogenated over 5% Pd/C (1.0 g) in a mixture of MeOH (65 ml) and acetone (65 ml). The mixture was stirred overnight at room temperature and filtered through a pad of Celite. Removal of the solvent under reduced pressure afforded the desired compound **18a** (1.08 g, 93%) as white foam; δ_H (300 MHz, d_6-acetone) 3.18, 3.36 (6H, 2 × d, J = 4.5 Hz, CH(OCH_3)$_2$), 4.96 (1 H, dddd, J = 131.7, 13.0, 8.7, 4.4 Hz, H-2), 5.11 (1H, ddd, J = 166.5, 8.7, 0.7 Hz, H-3), 6.29 (1H, dd, J = 2.4, 0.9 Hz, H-3′), 6.42 (1H, dd, J = 8.9, 2.4 Hz, H-5′), 6.79 (2H, d, J = 8.6 Hz, H-3″, 5″), 7.35 (2H, dd, J = 8.6, 3.3 Hz, H-2″,6″), 8.05 (1H, dd, J = 8.9, 3.9 Hz, H-6′); δ_C (75 MHz, d_6-acetone) 55.0 (dd, enhanced, J = 47.3, 41.4 Hz, C-2), 107.10 (d, enhanced, J = 47.3 Hz, C-3), 203.4 (d, enhanced, J = 41.4 Hz, C-1); HRMS (ES$^+$) $C_{14}{}^{13}C_3H_{18}NaO_6$ requires 344.1102; Found 344.1098.

1-(2,4-Dihydroxyphenyl)-2-(4-methoxyphenyl)-3,3-dimethoxy-1-[1,2,3-$^{13}C_3$]propanone 18b

As described for **18a**, using the chalcone **17a** (1.1 g, 2.13 mmol), gave the title compound (0.615 g, 86%) as a white foam. δ_H (300 MHz, acetone-d_6) 3.22, 3.47 (6H, 2 × d, J = 4.7 Hz, CH(OCH_3)$_2$), 3.76 (3H, s, OCH_3), 4.73 (1H, ddt, J = 129.2, 8.3, 4.6 Hz, H-2), 5.10 (1H, dd, J = 166.7, 8.3 Hz, H-3), 6.26–6.29 (2H, m, H-3′,5′), 6.86 (2H, d, J = 8.8 Hz, H-3″,5″), 7.33 (2H, d, J = 8.8, 3.4 Hz, H-2″, 6″), 7.67 (2H, dd, J = 8.8, 3.8 Hz, H-6′), 12.73 (1H, s, OH); δ_C (75 MHz, $CDCl_3$) 58.4 (dd, enhanced, J = 47.5, 42.7 Hz, C-2), 106.7 (d, enhanced, J = 47.5 Hz, C-3), 198.0 (d, enhanced, J = 42.7 Hz, C-1), HRMS (Cl$^+$) $C_{15}{}^{13}C_3H_{21}O_6$ requires 336.1439; Found 336.1444.

1-(2,4,6-Trihydroxyphenyl)-2-(4-methoxyphenyl)-3,3-dimethoxy-1-[1,2,3-$^{13}C_3$]propanone 18c

$Tl(NO_3)_3.3H_2O$ (1.52 g, 3.42 mmol) was added to a suspension chalcone **16c** (1.6 g, 2.857 mmol) in a mixture of MeOH (20 mL) and $CH(OMe)_3$ (15 mL). After 48 h stirring at rt, the white solid was filtered, and the filtrate was poured into ice-cold saturated $NaHCO_3$ and then extracted with CH_2Cl_2 (3 × 100 mL). The combined extracts were washed with brine, water and dried over $MgSO_4$. Removal of the solvent under reduced pressure gave the crude acetal that was purified by chromatography on silica gel with CH_2Cl_2:EtOAc (95:5) as eluant to give **17c** (0.71 g, 40%) as a yellow oil. The crude product was then hydrogenated over 5% Pd/C (0.5 g) in a mixture of MeOH (20 mL) and acetone (20 mL). The mixture was stirred overnight at room temperature, and filtered through a pad of Celite. After removal of solvents under reduced pressure, the residue was purified by flash chromatography using CH_2Cl_2:EtOAc (8:2) as eluant to give **18c** (0.31 g, 77%) as a white foam; δ_H (300 MHz, d_6-acetone) 3.18, 3.36 (6H, 2 × d, J = 4.6 Hz, CH(OCH_3)$_2$), 3.74 (3H, s, OCH_3), 5.07 (1H, dd, J = 163.2, 8.7 Hz, H-3), 5.35–5.42 (1/2H, m, 1/2H-2), 5.78–587 (1/2H, m, 1/2H-2), 6.89 (2H, s, H-3′,5′), 6.84 (2H, d, J = 8.9 Hz, H-3″,5″), 7.35 (2H, dd, J = 8.7, 3.3 Hz, H-2″,6″), 9.38, 11.87, 11.95 (3 br s, 3 × OH); δ_C (75 MHz, d_6-acetone) 59.1 (dd, enhanced, J = 47.6, 41.3 Hz, C-2), 108.5 (d, enhanced, J = 47.6 Hz, C-3), 205.2 (d, enhanced, J = 41.3 Hz, C-1); HRMS (ES$^+$) $C_{15}{}^{13}C_3H_{20}NaO_7$ requires 374.1207; Found 374.1198.

[2,3,4-$^{13}C_3$]Daidzein 1a

Conc. HCl (5 mL) was added to a solution of the hydroxyacetal **18b** (1.2 g, 3.74 mmol) in MeOH (20 mL) and the mixture was stirred under reflux for 4 h. After cooling to rt, water (10 mL) was added, and the precipitates were filtered, washed water and recrystalliZed from 70% EtOH to yield **1a** (0.88 g, 92%) as a white solid, mp 214–216°C (Lit.[32] 212–214°C for the unlabelled); δ_H (500 MHz, d_6-DMSO) 6.80 (2H, d, J = 8.7 Hz, H-3′,5′), 6.86 (1H, dd, J = 2.2, 1.8 Hz, H-8), 6.93 (1H, dd, J = 8.7, 2.2 Hz, H-6), 7.38 (2H, d, J = 8.7, 3.4 Hz, H-2′, 6′), 7.96 (1H, d, J = 8.7, 3.5 Hz, H-5), 8.26 (1H, dt, J = 197, 6.5 Hz, H-2), 9.53 (1H, s, 4′-OH), 10.79 (1H, s, 7-OH); δ_C (75 MHz, d_6-DMSO) 123.4 (dd, enhanced, J = 72.6, 54.2 Hz, C-3), 152.8 (d, enhanced, J = 72.6 Hz, C-2), 174.3 (d, enhanced, J = 54.6 Hz, C-4), HRMS (EI) $C_{12}{}^{13}C_3H_{11}O_4$ requires 258.0758; Found 258.0755.

[2,3,4-$^{13}C_3$]Formononetin 1b

As described for **1a**, the hydroxyacetal **18c** (1.1 g, 3.28 mmol) gave [2,3,4-$^{13}C_3$]formononetin **1b** (0.78 g, 88%) as a white solid, mp 255–256°C (Lit.[32] 256–257°C for the unlabelled); δ_H (500 MHz, d_6-DMSO) 3.78 (3H, s, OCH_3), 6.87 (1H, dd, J = 1.8 Hz, H-8), 6.94 (1H, dd, J = 8.8, 2.3 Hz, H-6), 6.99 (2H, d, J = 8.8 Hz, H-3′,5′), 7.50 (2H, d, J = 8.8, 3.4 Hz, H-2′, 6′), 7.97 (1H, dd, J = 8.7, 3.6 Hz, H-5), 8.33 (1H, dt, J = 197.1, 6.5 Hz, H-2), 10.80 (1H, s, 7-OH); δ_C (75 MHz, d_6-DMSO) 123.0 (dd, enhanced, J = 72.5, 55.6 Hz, C-3), 153.1 (d, enhanced, J = 72.5 Hz, C-2), 174.5 (d, enhanced, J = 55.6 Hz, C-4), HRMS (EI) $C_{13}{}^{13}C_3H_{13}O_4$ requires 272.0914; Found 272.0912.

[2,3,4-$^{13}C_3$]Biochanin A 1d

As for **1a**, the hydroxyacetal **18c** (0.3 g, 0.855 mmol) gave [2,3,4-$^{13}C_3$] biochanin **1d** (0.214 g, 87%) as a white solid, mp 319–322 dec °C (Lit.[32] 322–323°C for the unlabelled); δ_H (300 MHz, MeOD) 3.84 (3H, s, 4-OCH_3), 6.24 (1H, d, J = 1.8 Hz, H-8), 6.35 (1H, d, J = 1.8 Hz, H-6), 7.00 (2H, d, J = 8.7 Hz, H-3′,5′), 7.48 (2H, dd, J = 8.7, 3.3 Hz, H-2′, 6′), 8.09 (1H, dt, J = 197.1, 6.7 Hz, H-2); δ_C NMR (75 MHz, MeOD) 124.58 (dd, enhanced, J = 71.3, 54.7 Hz, C-3), 154.98 (d, enhanced, J = 71.3 Hz, C-2), 182.15 (d, enhanced, J = 54.7 Hz, C-4); HRMS (EI) $C_{13}{}^{13}C_3H_{13}O_5$ requires 288.0864; Found 288.0853.

[2,3,4-$^{13}C_3$]Equol 3

Compound **1a** (0.12 g, 0.467 mmol) was reduced with H_2 over 10% Pd/C (0.05 g) in EtOH (10 ml) until no more hydrogen was consumed. The reaction mixture was filtered through a pad of Celite and the solvent was evaporated under reduced pressure to yield a light yellow semi-solid that was purified by chromatography on silica gel with CH_2Cl_2:EtOAc (9:1 to 9:2) as eluant to give **3** (0.084 g, 74%) as a white solid, mp 154–156°C (Lit.[36] 155–157°C for the unlabelled); δ_H (300 MHz, d_6-DMSO) 2.5–3.3 (3H, m, H-3 and CH_2-4), 3.88 (1H, dm, J = 150 Hz, H-2a), 4.13 (1H, dm, J = 150 Hz, H-2b), 6.18 (1H, d, J = 2.4 Hz, H-8), 6.28 (1H, dd, J = 2.4 Hz, H-6), 6.71 (2H, d, J = 8.5 Hz, H-3′and 5′), 6.87 (1H, dd, J = 8.2, J 4.5 Hz, H-5), 7.11 (2H, dd, J = 8.5, 3.6 Hz, H-2′and 6′), 9.14 (1H, s, OH), 9.26 (1H, s, OH); δ_C (75 MHz, d_6-DMSO) 70.4 (d, enhanced, J = 33 Hz, C-2), 37.1 (t, enhanced, = 33 Hz, C-3), 31.5 (d, enhanced, J = 33 Hz, C-4); *m/z* (CI) 246 (*MH*+, 100%); HRMS $C_{12}{}^{13}C_3H_{15}O_3$ requires 246.1122; Found 246.1124.

3-(4-Benzyloxyphenyl)-[1,2,3-$^{13}C_3$]propionitrile 20

A solution of *n*-BuLi in hexane (1.96 mL, 2.5 M, 4.89 mmol) was added dropwise to a solution of *i*-propylcyclohexylamine

(0.814 mL, 4.89 mmol) in dry THF (5 mL) under nitrogen at −78°C. After 10 min stirring at −78°C, a solution of 4′-benzyloxyphenyl-[1,2-$^{13}C_2$]acetonitrile **19** (1 g, 4.44 mmol) was slowly added and the resulting solution was stirred for 5 min. [^{13}C]Methyl iodide (0.306 mL, 4.89 mmol) was added, and the reaction mixture was stirred for 1 h at −78°C and then allowed to warm gradually to rt. After 2 h at rt the solution was poured into 1 N HCl (20 mL) and extracted with diethyl ether (3 × 30 mL). The combined organic phases were then washed with brine and water, dried ($MgSO_4$) and the solvent removed at reduced pressure. Chromatography on silica gel using hexane:diethyl ether (9:1) as eluant gave the title compound **20** (0.78 g, 73%) as a white solid, mp 69–71°C (Lit.[37] 71–72°C for the unlabelled); δ_H (300 MHz, $CDCl_3$) 1.59 (3H, dddd, J = 131.1, 10.2, 6.3, 4.2 Hz, CH_3), 3.81 (1H, dm, J = 134.1 Hz, CHCN), 5.07 (2H, s, CH_2Ph), 6.98 (2H, d, J = 8.7 Hz, H-3,5), 7.27 (2H, dd, J = 8.7, 4.2 Hz, H-2,6), 7.32–7.49 (5H, m, Ph); δ_C (75 MHz, $CDCl_3$) 21.5 (d, enhanced, J = 33.2 Hz, CH_3), 30.50 (dd, enhanced, J = 56.1, 33.2 Hz, C-2), 121.76 (d, enhanced, J = 56.1 Hz, CN); $C_{13}{}^{13}C_3H_{15}NO$: calcd. C, 81.22; H, 6.29; N, 5.83; C. found, 79.79; H, 6.29; N = 5.56.

2-(4-Benzyloxyphenyl)-[1,2,3-$^{13}C_3$]propionic acid 21

The nitrile **20** (0.41 g, 1.71 mmol) was stirred under reflux in ethanol/2 N NaOH (20:10 mL) for 1.5 h. Half of the solvent was removed, the residue was acidified with 2 N HCl and extracted with diethyl ether (50 mL × 3). The combined organic phases were washed with brine and water, dried ($MgSO_4$), filtered and evaporated to give the desired acid **21** (0.423 g, 96%) as a white solid; mp 134–138°C (lit.[38] mp 135–137°C for the unlabelled); δ_H ($CDCl_3$, 300 MHz) 1.50 (3H, dddd, J = 129.0, 11.4, 7.2, 4.8 Hz, CH_3), 3.80 (1H, dm, J = 131 Hz, $CHCO_2H$), 5.17 (2H, s, CH_2Ph), 6.94 (2H, d, J = 8.7 Hz, H-3,5), 7.24 (2H, dd, J = 8.7, 3.9 Hz, H-2,6), 7.32–7.44 (5H, m, Ph); ä$_C$ (75 MHz, d_6-$CDCl_3$) 18.2 (d, enhanced, J = 34.3 Hz, CH_3), 44.4 (dd, enhanced, J = 55.0, 34.3 Hz, C-2), 180.3 (d, enhanced, J = 55.0 Hz, CN); $C_{13}{}^{13}C_3H_{16}O$: calcd. C, 75.29; H, 6.22; C. found, 74.22; H, 6.18.

[1,2,3-$^{13}C_3$]-*O*-DemethylAngolensin, 1-(2,4-dihydroxyphenyl)-2-(4-hydroxyphenyl)-[1,2,3-$^{13}C_3$]propan-1-one 4a

Compound **21** (0.40 g, 1.54 mmol) was reduced with H_2 over 5% Pd/C (0.10 g) in EtOH (10 ml) until no more hydrogen was consumed. The reaction mixture was filtered through a pad of Celite and the solvent was evaporated under reduced pressure to give 2-(4-hydroxyphenyl)-[1,2,3-$^{13}C_3$] propionic acid **22** (0.25 g, 96%) as a light yellow solid; mp 127-128°C (Lit.[39] 126–129°C for the unlabelled); δ_H (300 MHz, $CDCl_3$,) δ 1.41 (3H, dm, J = 128.4, 11.4, 7.2 Hz, CH_3), 3.01 (1H, br s, phenol OH), 3.63 (1H, dm, CHCO$_2$H)), 6.73 (2H, d, J = 8.1 Hz, H-3,5), 7.06 (2H, dd, J = 8.1, 3.9 Hz, H-2,6). A suspension of resorcinol (0.21 g, 1.91 mmol) and **22** (0.16 g, 0.946 mmol) in $BF_3.Et_2O$ (0.355 mL, 2.89 mmol) was heated under reflux under a nitrogen atmosphere. After 15 min the mixture was allowed to cool to rt and saturated NaOAc solution (5 mL) and 10% aq. $NaHCO_3$ (3 mL) were added sequentially to the dark red mixture. The orange oily precipitate was extracted with diethyl ether (3 × 10 mL). The combined ethereal extracts were washed with water and brine, dried ($MgSO_4$) and the solvent removed at reduced pressure to give an orange viscous oil. Chromatography on silica gel with CH_2Cl_2:EtOAc (8:2) as eluant gave the title compound **4a** as a white solid (0.198 g, 80%); mp 99–102°C (lit[40] 103°C for the unlabelled); δ_H (300 MHz, d_6-acetone) 1.65 (3H, ddt, J = 129, 7.0, 4.0 Hz, CHCH_3), 4.75 (1H, dm, J = 125 Hz, CHCH$_3$), 6.40 (1H, d, J = 2.4 Hz, H-5′), 6.47 (1H, dd, J = 8.4, J 2.4 Hz, H-3′), 6.89 (2H, dt, J = 8.4, 2 Hz, H-3 and H-5), 7.22 (2H, dd, J = 8.4, 4 Hz, H-2 and H-6), 7.90 (1H, dd, J = 9, 4 Hz, H-6′), 8.2 (1H, br, OH), 9.4 (1H, br, OH); δ_C (75.43 MHz, d_6-acetone) 19.6 (d, enhanced, J = 37 Hz, CH_3), 46.3 (dd, enhanced, J = 40, 37 Hz, CH), 108,6 (d, J = 4.5 Hz), 206.5 (d enhanced,, J = 40 Hz, CO).

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