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Synthesis of [4-^{13}C]-Isoflavonoid Phytoestrogens[1]

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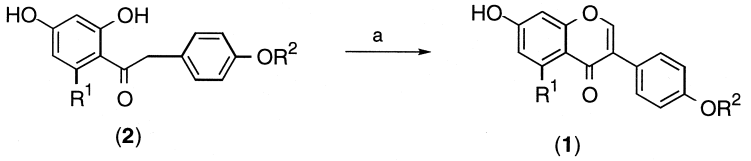
Abstract—Efficient syntheses are described for ^{13}C-labelled derivatives of the isoflavonoid phytoestrogens, genistein, biochanin A, daidzein and formononetin, for use in metabolic studies. The synthetic procedure employs ^{13}C-labelled cyanide as the source of the label to produce the isoflavones with a single ^{13}C atom at the C-4 position.

There is currently considerable interest in phytoestrogens with respect to their impact on human health. The isoflavonoid phytoestrogens, daidzein (**1**; R^1=H, R^2=H) and genistein (**1**; R^1=OH, R^2=H), are present at significant levels in soya beans and soy products. They thus feature heavily in the diet particularly in Japan and Asia, where they have been associated with a low incidence of hormone dependent cancers.[2] These compounds have also been implicated in the prevention of cardiovascular disease,[3] lessening the symptoms of the menopause[4] and protection against osteoporosis.[5] Furthermore, recent evidence suggests a role in the central nervous system, stimulating nerve growth, and action as an antioxidant against endogenous toxins that produce free radicals in the CNS which are associated with the development of Alzheimer's disease.[6] In order to better understand, quantify and deduce the importance of these biological effects there is a need for the development of an efficient synthesis of isotopically labelled genistein and daidzein. Such materials are of great value as both internal standards for analysis and in metabolic studies. Indeed there are still many unanswered questions regarding the bioavailability and mammalian metabolism of daidzein and genistein.

A number of synthetic routes towards the isoflavones have been previously developed and it was proposed that ^{13}C-labelled daidzein (**1**; R^1=H, R^2=H), formononetin (**1**; R^1=H, R^2=CH_3), genistein (**1**; R^1=OH, R^2=H), and biochanin A (**1**; R^1=OH, R^2=CH_3) would be prepared by adapting existing methodologies. The final step of most isoflavone syntheses involves the formylation and cyclisation of a suitable deoxybenzoin precursor **2**. Various reagents have been used to provide the necessary one carbon fragment including dimethyl formamide dimethylacetal in THF,[7] dimethyl formamide followed by mesyl chloride[8] and 1,3,5-triazine with boron trifluoride etherate.[9] (Scheme 1) Preliminary studies were therefore carried out to investigate the first two methods to examine the possibility of using ^{13}C-labelled dimethyl formamide to introduce the label at the C-2 position in the final step. Unfortunately preliminary studies with unlabelled daidzein demonstrated that a large excess of dimethylformamide, or its dimethyl acetal were required in order to obtain reasonable yields. This would be feasible if recovery of unreacted labelled starting material was also efficient but this was not the case.

An alternative strategy was therefore sought. The deoxybenzoins are normally prepared via condensation of a phenol and either a substituted phenylacetic acid, using boron trifluoride as catalyst, or benzyl nitrile via a Hoesch reaction.[10] An alternative route thus involved the use of ^{13}C-labelled cyanide to prepare the nitrile, which would



(**2**) (**1**)

Scheme 1. (a) DMF dimethylacetal, THF, reflux or DMF then mesyl chloride or $BF_3{\cdot}Et_2O$, 1,3,5-triazine.

Keywords: isoflavones; labelling; antitumour compounds; plants.

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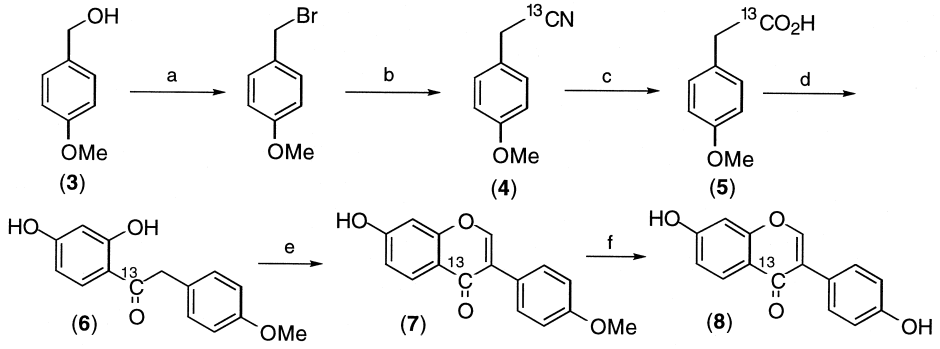


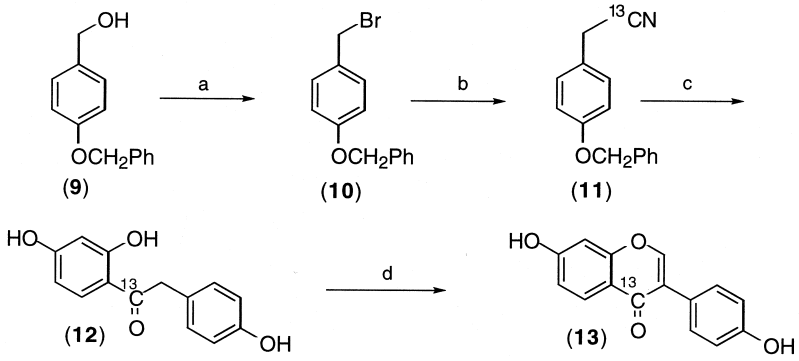
Scheme 2. (a) TMSBr, Et_2O (98%); (b) K^{13}CN, 18-Crown-6, MeCN (86%); (c) 2M NaOH (aq), reflux (79%); (d) Resorcinol, $BF_3{\cdot}Et_2O$ (66%); (e) $DMF(OMe)_2$ (62%); (f) BBr_3, CH_2Cl_2 (89%).

lead to labelling at the C-4 position of the isoflavone (Scheme 2). The proposed route was first optimised with unlabelled material and then used to prepare both ^{13}C labelled formononetin and daidzein.

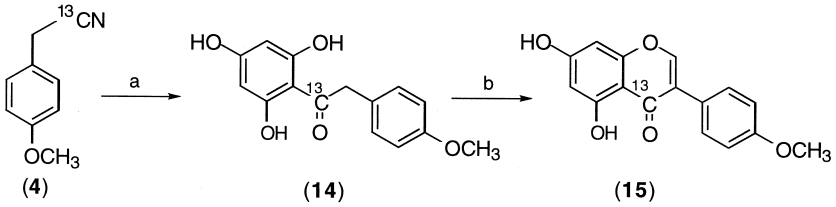
Firstly, commercially available 4-methoxybenzyl alcohol **3** was brominated, so that nucleophilic displacement of the bromide could be carried out using cyanide ion. A range of conditions was examined for the nucleophilic displacement, using a number of solvents including DMSO and DMF. However the optimum conditions that were finally established employed ^{13}C-labelled potassium cyanide in acetonitrile in the presence of 18-crown-6 at room temperature. This gave the desired nitrile **4** in good yield. The carboxylic acid **5** was then obtained by subsequent basic hydrolysis. Formation of the deoxybenzoin **6** was effected by treatment of resorcinol and the ^{13}C-labelled acid with boron trifluoride etherate in THF.[11] Dimethylformamide dimethylacetal[7] was then employed for the formylation and cyclisation reaction to give [4-^{13}C]-formononetin **7**. This gave identical spectral data to authentic material. The presence of the ^{13}C-label was confirmed by mass spectrometry, showing less than 1% unlabelled material, and by the enhanced signal due to the carbon at the 4-position in the ^{13}C NMR spectrum. Conversion to daidzein then required de-methylation of the 4′-methoxy group. The most efficient reagent for this transformation was found to be boron tribromide in dichloromethane,[12] which afforded the [4-^{13}C]-daidzein **8** in 89% yield. However analysis of the [4-^{13}C]-daidzein by GC-MS in comparison with a reference standard of unlabelled material revealed that it was only 54% pure. NMR analysis showed no organic impurities and so it was deduced that the contamination was due to inorganic material, probably boron salts. Unfortunately, attempts to remove the boron salts by extraction, recrystallisation, normal phase column chromatography and reverse phase column chromatography failed. In order to obtain pure **8** it was therefore clear that the best strategy would be to modify the synthetic route to avoid the final demethylation step.

It was decided to protect the 4-hydroxy group as its benzyl ether, which would then be removed under the acidic conditions required for the Hoesch reaction[10] to form the deoxybenzoin. This alternative route is shown in Scheme 3. Thus the nitrile **11** was prepared, as described for the methoxy analogue, from commercially available 4-benzyloxybenzyl alcohol. The Hoesch reaction was achieved by treatment of a solution of resorcinol, nitrile **11** and catalytic zinc chloride in diethyl ether with hydrogen chloride to give the desired deoxybenzoin **12** in good yield. The modification of the original Hoesch reaction, employing only catalytic zinc chloride, was recently reported by Pelter et al.[13] Formylation and cyclisation of **12** afforded the [4-^{13}C]-daidzein **13** which was then successfully purified by flash chromatography.

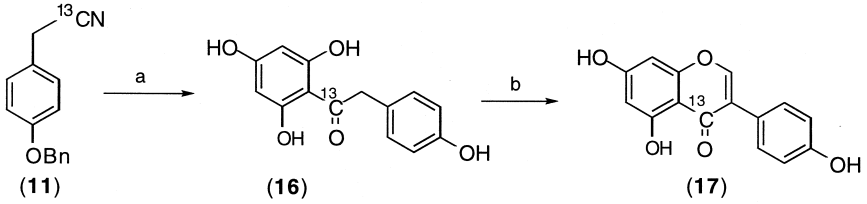
Synthesis of biochanin A and genistein proved to be more problematic due to differences in their properties which are a result of the additional hydroxyl group at the 5-position. This hydroxyl group hydrogen bonds with the C-4 carbonyl reducing its reactivity, such that some of the formylation/cyclisation procedures were unsuitable, e.g. the use of dimethylformamide dimethylacetal in THF. Also the



Scheme 3. (a) TMSBr, Et_2O (89%); (b) K^{13}CN, 18-Crown-6, MeCN (92%); (c) Resorcinol, HCl, cat. $ZnCl_2$, Et_2O (96%); (d) $DMF(OMe)_2$ (60%).



Scheme 4. (a) Phloroglucinol, HCl, cat. $ZnCl_2$ (75%); (b) $BF_3{\cdot}OEt_2$, DMF, microwave, then $MeSO_2Cl$, microwave (54%).



Scheme 5. (a) Phloroglucinol, HCl, cat. $ZnCl_2$ (75%); (b) $BF_3{\cdot}OEt_2$, DMF, microwave, then $MeSO_2Cl$, microwave (54%).

compounds exhibit reduced solubility in the organic solvents used in the daidzein synthesis. Various attempts were made to tackle these problems, including investigation into protection of all the free hydroxyl groups followed by deprotection after cyclisation. However these strategies add extra steps to the synthesis lowering the overall yield of labelled material and deprotection again resulted in boron salt contamination as observed in the synthesis of daidzein from formononetin described above.

Recently a procedure was described,[14] involving reaction of the appropriate deoxybenzoin with $BF_3{\cdot}Et_2O$ and DMF using a short burst of microwave irradiation as the heat source. Mesyl chloride was then added and the microwave treatment repeated. Addition of water then resulted in precipitation of the product. Initially, this method appeared to work for the synthesis of unlabelled genistein on a small scale, but the results were very variable. However, it was found that longer periods of microwave heating produced larger yields and a more reliable procedure, especially when working on a larger scale. With this reaction optimised, the overall synthesis of [4-^{13}C]-biochanin A was achieved using a similar procedure to that for formononetin (Scheme 4). In this case the nitrile **4** was reacted with phloroglucinol under Hoesch conditions to give the deoxybenzoin **14**. Formylation and cyclisation were then achieved under microwave conditions using DMF and mesyl chloride to afford [4-^{13}C]-biochanin A **15**. Spectral data confirmed the presence of the ^{13}C atom at the C-4 position.

Modification of the synthesis then allowed the preparation of [4-^{13}C]-genistein **17** (Scheme 5). As for daidzein **8**, the *O*-benzylated bromide was used as starting material and the protecting group cleaved during the Hoesch reaction to provide **16**. Microwave conditions were then employed for the final formylation/cyclisation step, giving the product in 54% yield. Spectral data for the [4-^{13}C]-genistein **17** confirmed the presence of the desired ^{13}C atom at the 4-position and the material was shown to be pure by microanalysis.

Using the synthetic methodology described above, both [4-^{13}C]-daidzein and [4-^{13}C]-genistein have now been prepared on a gram scale and samples of these materials are now being employed in metabolic studies. Further work involves the modification of the synthetic procedures so that two or three ^{13}C atoms can be incorporated into either daidzein or genistein.

Experimental

General

NMR spectra were recorded on a Varian Gemini FT spectrometer (^{1}H, 200 MHz; ^{13}C, 50.31 MHz) or a Varian Gemini 2000 spectrometer (^{1}H 300 MHz; ^{13}C 75.45 MHz). ^{1}H NMR spectra were referenced to chloroform, TMS, methanol or d^6-DMSO, ^{13}C NMR spectra were referenced to chloroform, methanol or d^6-DMSO. Elemental analyses were carried out in the departmental microanalytical laboratory. IR spectra were recorded on a Perkin–Elmer series 1500 FT IR spectrophotometer. The samples were prepared as Nujol mulls or thin films between sodium chloride discs and recorded in cm^{-1}. Mass spectra were recorded on a Kratos MS50. The microwave reactions were carried out using a Panasonic 650 W machine set to "simmer". Analysis by TLC was carried out on Merck 5785 Kieselgel $60F_{254}$ fluorescent plates. Flash chromatography was performed according to the procedure of Still[15] using silica gel (Fisons matrix 35–70 μm). Diethyl ether and dichloromethane were dried by passing down an alumina column and distilling from calcium hydride. Dimethylformamide was distilled from magnesium sulfate. Tetrahydrofuran was passed down a dry alumina column and distilled from sodium metal and benzophenone.

4-Methoxybenzyl [^{13}C]cyanide (4). To 4-methoxybenzyl alcohol **3** (2.0 g, 14.5 mmol) in dry diethyl ether (60 ml) under nitrogen at 0°C was added trimethylsilyl bromide (4.5 g, 29.6 mmol) and the reaction mixture allowed to warm slowly to room temperature. After 16 h, water (120 ml) was added and the aqueous phase extracted with diethyl ether (4×60 ml). The combined organic phases were washed with brine (150 ml), dried over anhydrous magnesium sulfate, and concentrated to provide pure

bromide (3.0 g, 98%) as a colourless liquid. To the bromide (3.0 g, 15.0 mmol) was immediately added acetonitrile (200 ml), 18-crown-6 (5.2 g, 15.0 mmol) and ^{13}C-potassium cyanide (1.0 g, 15.0 mmol). The resultant mixture was heated at reflux with the protection of a calcium chloride tube for 4 h. Concentration of the reaction mixture followed by dilution with diethyl ether, filtration through a pad of silica gel (Fisons matrix 35–70 m) and subsequent concentration of the filtrate provided pure nitrile **4** (1.9 g, 86%) as a colourless oil; (Found: C, 73.08; H, 6.25; N, 9.27. $C_8{}^{13}C_1H_9ON$ requires C, 72.96; H, 6.12; N, 9.45); ν_{max} (nujol)/cm^{-1} 2191 (CN); δ_H (200 MHz, $CDCl_3$) 3.60 (2H, d, $J_{C,H}$=5.3 Hz, C*H*$_2$), 3.72 (3H, s, OC*H*$_3$), 6.84 (2H, d, $J_{2,3}$=$J_{5,6}$=7.7 Hz, 3, 5-*H*), 7.16 (2H, d, $J_{2,3}$=$J_{5,6}$=7.7 Hz, 2, 6-*H*); δ_C (75.45 MHz, $CDCl_3$) 22.9 (2H, d, *J*=28 Hz, C*H*$_2$), 55.6 (OC*H*$_3$), 114.9 (3-*C* and 5-*C*), 119.1 (enhanced, ^{13}*C*N), 122.6 (1-*C*), 129.6 (2-*C* and 6-*C*), 159.8 (4-*C*); *m*/*z* (EI) 148 (M$^+$, 100%).

4-Benzyloxybenzyl [^{13}C]cyanide (11). Nitrile **11** was prepared in the same manner as nitrile **4** by treating 4-benzyloxybenzyl alcohol **9** with trimethylsilyl bromide to give the bromide **10** (1.0 g, 89%) which was treated with 18-crown-6 and ^{13}C-potassium cyanide to afford **11** (0.75 g, 92%) as a white solid; mp 66°C; (Found: C, 81.12; H, 7.14; N, 6.25. $C_{14}{}^{13}C_1H_{13}ON$ requires C, 80.90; H, 7.13; N, 6.24); ν_{max} (nujol)/cm^{-1} 2193 (CN); δ_H (200 MHz, $CDCl_3$) 3.51 (2H, d, $J_{C,H}$=5.4 Hz, C*H*$_2$), 4.92 (2H, s, OC*H*$_2$Ph), 6.84 (2H, d, $J_{2,3}$=$J_{5,6}$=7.7 Hz, 3, 5-*H*), 7.16 (2H, d, $J_{2,3}$=$J_{5,6}$=7.7 Hz, 2, 6-*H*), 7.35 (5H, m, OCH$_2$*Ph*); δ_C (50.3 MHz, $CDCl_3$) 21.3 (d, *J*=28 Hz, *C*H$_2{}^{13}$CN), 70.0 (O*C*H$_2$Ph), 115.5 (3-*C* and 5-*C*), 118.2 (enhanced, ^{13}*C*N), 122.0 (1-*C*), 127.5 (2′-*C* and 6′-*C*), 128.1 (3′-*C* and 5′-*C*) 128.7 (4′-*C*), 129.1 (2-*C* and 6-*C*), 136.4 (1′-*C*), 159.8 (4-*C*); *m*/*z* (EI) 224 (M$^+$, 14%).

4′-Methoxyphenyl [1-^{13}C]acetic acid (5). 4-Methoxybenzyl [^{13}C]cyanide **4** (945 mg, 6.43 mmol), was heated to reflux with aqueous 2 M sodium hydroxide (45 ml) for 1 h. Upon cooling the solution was acidified to pH 2 using concentrated hydrochloric acid and the solution boiled to dissolve the precipitate. After cooling overnight at 4°C (refrigerator), the solid was collected and dried over phosphorus pentoxide to afford **5** as a white solid (742 mg, 79%); mp 79°C; (Found: C, 66.03; H, 4.82. $C_8{}^{13}C_1H_8O_3$ requires C, 66.05; H, 4.88); ν_{max} (nujol)/cm^{-1} 2810 (OCH$_3$), 1654 (^{13}CO$_2$H); δ_H (200 MHz, CD_3OD) 3.55 (2H, d, $J_{C,H}$=7.0 Hz, –C*H*$_2$–), 3.78 (3H, s, OC*H*$_3$), 6.86 (2H, d, $J_{2,3}$=$J_{5′,6′}$=8.7 Hz, 3′,5′-*H*), 7.19 (2H, d, $J_{2′,3′}$=$J_{5′,6′}$=8.7 Hz, 2′,6′-*H*); δ_C (50.3 MHz, CD_3OD) 40.8 (d, *J*=41.6 Hz, –*C*H$_2$–), 55.9 (O*C*H$_3$), 115.2 (3′-*C* and 5′-*C*), 128.1 (1′-*C*), 131.1 (2′-*C* and 6′-*C*), 164.0 (4′-*C*), 178.3 (enhanced, ^{13}*C*O$_2$H); *m*/*z* (EI) 165 (M$^+$, 36%), 147 ((M–H$_2$O)$^+$, 25), 120 ((M–CO$_2$H)$^+$, 17).

4-Methoxybenzyl-2′,4′-dihydroxyphenyl [^{13}C]ketone (6). To acid **5** (2.2 g, 13.0 mmol) in $BF_3{\cdot}OEt_2$ (0.5 ml) under nitrogen was added resorcinol (2.9 g, 26.0 mmol) portionwise over 10 min. The solution was heated to reflux for 10 min, cooled and then aqueous saturated sodium acetate (30 ml) and saturated aqueous sodium hydrogen carbonate (15 ml) were added sequentially to give an orange oily layer which was extracted with diethyl ether (3×50 ml). The combined ethereal layers were washed with water (10 ml), brine (2×50 ml) and dried over magnesium sulfate to give a dark red oil. Purification by flash chromatography (employing gradient elution 6:4 toluene/ethyl acetate to 100% ethyl acetate) gave **6** as a pale orange solid (3.1 g, 92%); mp 152°C; (Found: C, 69.98; H, 5.42. $C_{14}{}^{13}C_1H_{14}O_4$ requires C, 69.87; H, 5.44); ν_{max} (nujol)/cm^{-1} 3350 (OH), 2880 (OCH$_3$), 1617 (C=O); δ_H (200 MHz, CD_3OD), 3.72 (3H, s, OC*H*$_3$), 4.10 (2H, d, $J_{C,H}$=6.2 Hz, –C*H*$_2$–), 6.26 (1H, d, $J_{3′,5′}$=2.2 Hz, 3′-*H*), 6.36 (1H, dd, $J_{3′,5′}$=2.2 Hz, $J_{5′,6′}$=8.9 Hz, 5′-*H*), 6.83 (2H, d, $J_{2,3}$=$J_{5,6}$=8.6 Hz, 3, 5-*H*), 7.15 (2H, d, $J_{2,3}$=$J_{5,6}$=8.6 Hz, 2, 6-*H*), 7.81 (1H, dd, $J_{5′,6′}$=8.9 Hz, $J_{C,H}$=4.9 Hz, 6′-*H*); δ_C (50.3 MHz, CD_3OD), 44.9 (d, *J*=41.2 Hz, –*C*H$_2$–), 55.9 (OCH$_3$), 104.0 (3′-*C*), 109.4 (5′-*C*), 113.9 (d, *J*=57 Hz, 1′-*C*), 115.3 (3-*C* and 5-*C*), 128.6 (1-C), 131.7 (2-*C* and 6-*C*), 134.7 (6′-*C*), 160.3 (4-*C*), 166.7 (4′-*C*), 167.1 (2′-*C*), 204.4 (enhanced, ^{13}*C*=O); *m*/*z* (EI) 259 (M$^+$, 14%), 138 (100), 121 (17).

4′-Methoxybenzyl-7-hydroxy-[4-^{13}C]isoflavone ([4-^{13}C]-formononetin) (7). *N*,*N*-Dimethylformamide dimethyl acetal (162 mg, 180 μl, 1.36 mmol) was added dropwise to deoxybenzoin **6** (200 mg, 0.78 mmol) in THF (2 ml) under nitrogen at reflux. After 4 h, the dark red solution was cooled to room temperature and methanol (5 ml) was added. Repeated concentration after addition of further 5 ml portions of methanol, gave an orange solid which was recrystallised from 75% aqueous methanol to afford pure **7** (129 mg, 62%) as a pale beige solid; mp 257°C (lit.[7] 256–257°C); (Found: C, 71.09; H, 4.41. $C_{15}{}^{13}C_1H_{12}O_4$ requires C, 71.37; H, 4.49); ν_{max} (nujol)/cm^{-1} 3350 (OH), 1616 (C=O); δ_H (200 MHz, d^6-DMSO) 3.81 (3H, s, OC*H*$_3$), 6.9 (2H, m, 6, 8-*H*), 7.0 (2H, d, $J_{2′,3′}$=$J_{5′,6′}$=8.6 Hz, 3′,5′-*H*), 7.5 (2H, d, $J_{2,3}$=$J_{5′,6′}$=8.6 Hz, 2′,6′-*H*), 8.0 (1H, dd, $J_{5,6}$=9 Hz, $J_{4,5}$=4 Hz (^{13}C-^{1}H coupling), 5-*H*), 8.4 (1H, d, $J_{2,4}$=6 Hz (^{13}C-^{1}H coupling), 2-*H*); δ_C (50.31 MHz, d^6-DMSO) 55.2 (O*C*H$_3$), 102.2 (8-*C*), 113.7 (3′-*C* and 5′-*C*), 115.2 (6-*C*), 116.3 (d, *J*=57 Hz, 4a-*C*), 122.6 (1′-*C*), 123.4 (1-*C*), 124.2 (*d*, *J*=56 Hz, 3-*C*), 127.3 (5-*C*), 130.1 (2′-*C* and 6′-*C*), 153.0 (2-*C*), 157.1 (7-*C*), 159.0 (8a-*C*), 162.6 (4′-*C*), 174.7 (enhanced, 4-^{13}*C*); *m*/*z* (EI) 269 (M$^+$, 62%), 254 (14, M$^+$–CH$_3$). *m*/*z* (EI) 269 (M$^+$, 100%), 254 (14%, M$^+$–CH$_3$).

4-Hydroxybenzyl-2′,4′-dihydroxyphenyl [^{13}C]ketone (12). To a vigorously stirred solution of the [^{13}C]-nitrile **11** (500 mg, 2.23 mmol) and resorcinol (297 mg, 2.70 mmol) in dry diethyl ether (15 ml) under nitrogen was added freshly fused zinc chloride (37 mg, 0.03 mmol) via a dry addition funnel. The nitrogen inlet was then replaced with a calcium chloride drying tube and the reaction mixture saturated with dry hydrogen chloride at 0°C for 4 h, during which time a solid crust had formed on the edge of the reaction vessel. After stirring for 16 h at room temperature the ether layer was decanted and the residue washed twice with ether (10 ml). 1N aqueous hydrochloric acid was added to the residue and the solution heated at reflux for 2 h under nitrogen, then cooled and the resultant orange solid filtered off and purified by flash chromatography eluting with diethyl ether to afford [^{13}C]-deoxybenzoin **12** (543 mg, 96%): mp 189°C (lit. 188–190°C); (Found: C, 67.13; H, 12.08. $C_{13}{}^{13}C_1H_{12}O_4$ requires C, 67.15; H, 12.09); ν_{max}

(nujol)/cm^{-1} 3450 (OH), 1636 (C=O); δ_H (200 MHz, d^6-DMSO) 4.11 (2H, d, $J_{C,H}$=6.2 Hz, –CH_2–), 6.26 (1H, d, $J_{3',5'}$=2.2, 3′-*H*), 6.38 (1H, dd, *J*=8.8 Hz, $J_{5',6'}$=2.3 Hz, 5′-*H*), 6.83 (2H, d, $J_{2,3}$=$J_{5,6}$=8.6 Hz, 3, 5-*H*), 7.12 (2H, d, $J_{2,3}$=$J_{5,6}$=8.6 Hz, 2, 6-*H*) 7.84 (1H, dd, $J_{5',6'}$=8.7 Hz, $J_{C,H}$=4.7 Hz, 6′-*H*); δ_C (50.3 MHz, d^6-DMSO) 44.0 (d, *J*=41 Hz, *C*H$_2$), 96.1 (3′-*C* and 5′-*C*), 112.9 (1′-C), 116.3 (3-*C* and 5-*C*), 128.5 (1-*C*), 132.1 (2-*C* and 6-*C*), 157.2 (4-*C*), 166.2 (2′-*C* and 6′-*C*), 166.6 (4′-*C*), 205.4 (enhanced, ^{13}*C*=O); *m/z* (EI) 245 (M$^+$, 12%), 121 (32), 107 (12).

4′-Hydroxybenzyl-7-hydroxy-[4-^{13}C]isoflavone ([4-^{13}C]-daidzein) (13). *N*,*N*-Dimethylformamide dimethyl acetal (291 mg, 324 μl, 2.44 mmol) was added dropwise to deoxybenzoin **12** (200 mg, 0.82 mmol) in THF (2 ml) under nitrogen, at reflux. After 16 h, the solution was cooled to ambient temperature, and methanol (5 ml) was added. Repeated concentration under reduced pressure after addition of further 5 ml portions of methanol, gave an orange solid which was purified by column chromatography (employing gradient elution 5:1 diethyl ether/petroleum ether 40–60°C to 100% diethyl ether) to provide [4-^{13}C]-daidzein **13** as a very pale yellow solid (125 mg, 60%): mp 219°C (lit.[7] 212–214°C); (Found: C, 70.34; H, 4.30. $C_{14}{}^{13}C_1H_{10}O_4$ requires C, 70.59; H, 4.00%); ν_{max} (nujol)/cm^{-1} 3360 (br, OH), 1735 (C=O); δ_H (200 MHz, d^6-DMSO) 6.89 (3H, m, 3′-H, 5′-*H* and 8-*H*), 6.95 (1H, dd, $J_{6,8}$=2 Hz, $J_{5,6}$=9 Hz, 6-*H*), 7.4 (2H, d, $J_{2',3'}$=$J_{5,6}$=8.5 Hz, 2′,6′-*H*), 7.98 (1H, dd, $J_{4,5}$=4 Hz, $J_{5,6}$=9 Hz, 5-*H*), 8.3 (1H, d, $J_{2,4}$=6 Hz, 2-*H*); δ_C (50.3 MHz, d^6-DMSO) 102.1 (8-*C*), 114.9 (3′-*C* and 5′-*C*), 115.1 (6-*C*), 116.7 (d, *J*=46 Hz, 4a-*C*), 122.5 (1′-*C*), 123.5 (d, *J*=56 Hz, 3-*C*), 127.2 (5-*C*), 130.0 (2′-*C* and 6′-*C*), 153.1 (2-*C*), 157.1 (4′-*C*), 156.4 (8a-*C*), 162.4 (7-*C*), 175.0 (enhanced, 4-^{13}*C*); *m/z* (EI) 255 (M$^+$, 54%), 138 (100, $C_7H_4O_3^+$).

4-Methoxybenzyl-2′,4′,6′-trihydroxyphenyl [^{13}C]ketone (14). To a vigorously stirred solution of the [^{13}C]-nitrile **4** (1.15 g, 7.76 mmol) and phloroglucinol (1.47 g, 11.64 mmol) in dry diethyl ether (35 ml) under nitrogen was added freshly fused zinc chloride (106 mg, 0.78 mmol) via a dry addition funnel. The nitrogen inlet was then replaced with a calcium chloride drying tube and the reaction mixture saturated with dry hydrogen chloride at 0°C for 4 h, during which time a solid crust had formed on the surface of the reaction vessel. After stirring for 16 h at room temperature the ether layer was decanted and the residue washed twice with ether (10 ml). 1N aqueous hydrochloric acid was added to the residue and the solution heated at reflux for 2 h under nitrogen, then cooled and the resultant orange solid filtered off and purified by flash chromatography eluting with diethyl ether to afford [^{13}C]-deoxybenzoin **14** (1.56 g, 75%): mp 158–160°C; (Found C, 65.38; H, 5.12. $C_{14}{}^{13}C_1H_{14}O_5$ requires C, 65.81; H, 5.12.); ν_{max} (nujol)/cm^{-1} 3350 (OH) δ_H (200 MHz, d^6-DMSO) 3.73 (3H, s, OCH_3), 4.30 (2H, d, $J_{C,H}$=6.3 Hz, –CH_2–), 5.82 (2H, s, 3′,5′-H), 6.80 (2H, d, $J_{2,3}$=$J_{5,6}$=8.7 Hz, 3, 5-*H*), 7.1 (2H, d, $J_{2,3}$=$J_{5,6}$=8.7 Hz, 2, 6-*H*); δ_C (50.3 MHz, d^6-DMSO) 48.2 (d, *J*=43.2 Hz, –*C*H$_2$–), 55.6(OCH$_3$), 96.08 (3′-C and 5′-*C*), 113.5 (1′-*C*), 114.9 (3-*C* and 5-*C*), 128.6 (1-*C*), 132.0 (2-*C* and 6-*C*), 160.4 (4-*C*), 166.2 (4′-*C*), 166.6 (2′-*C* and 6′-C), 205.2 (enhanced, ^{13}*C*=O); *m/z* (EI) 275 (M$^+$, 13%), 184 (100), 126 (32), 121 (27), 86 (17).

4′-Methoxybenzyl-5,7-dihydroxy-[4-^{13}C]isoflavone ([4-^{13}C]biochanin A) (15). Boron trifluoride etherate (10 ml, 11.5 g, 81.3 mmol) was added to a solution of deoxybenzoin **14** (500 mg, 1.82 mmol) in dimethylformamide (20 ml) in a beaker covered with a watch glass and heated employing a microwave (800 W) for 2×15 s on 'simmer' setting. The solution was swirled, methane sulfonyl chloride (10 ml, 14.8 g, 129.2 mmol) was added and the reaction mixture heated in short bursts of 2×30 s then 2×15 s on simmer. After cooling, water (100 ml) was added and the reaction mixture stirred for 4 h. The solid was filtered off and dried over phosphorus pentoxide. Purification by flash chromatography (gradient elution 100% diethyl ether to 10:90 methanol/diethyl ether) provided pure [4-^{13}C]-biochanin A **15** (279 mg, 54%) as a white solid: mp 194–196°C; (Found: C, 67.71; H, 4.22. $C_{15}{}^{13}C_1H_{12}O_5$ requires C, 67.37; H, 4.24%); ν_{max} (nujol)/cm^{-1} 3395 (OH), 1610 (C=O); δ_H (200 MHz, d^6-DMSO) 3.80 (3H, s, OCH_3), 6.26 (1H, d, $J_{6,8}$=2.5 Hz, 8-*H*), 6.41 (1H, d, $J_{6,8}$=2.5 Hz, 6-*H*), 7.03 (2H, d, $J_{2',3'}$=$J_{5',6'}$=7.4 Hz, 2′,6′-*H*), 7.51 (2H, d, $J_{2',3'}$=$J_{5',6'}$=7.4 Hz, 3′,5′-*H*), 8.37 (1H, d, $J_{2,4}$=6 Hz (^{13}C-^{1}H coupling), 2-*H*), 9.6 (1H, br s, 7-O*H*); δ_C (50.3 MHz, d^6-DMSO) 55.4 (OCH$_3$), 102.4 (8-*C*), 114.0 (3′-*C* and 5′-*C*), 115.4 (6-*C*), 116.9 (4a-*C*), 122.8 (1′-*C*), 123.75 (3-*C*), 127.6 (5-*C*), 130.4 (2′-*C* and 6′-*C*), 154.5 (2-*C*), 157.85 (7-*C*), 159.4 (8a-*C*), 164.6 (4′-C), 180.4 (enhanced, 4-^{13}*C*); *m/z* (CI) 286 (MH$^+$, 76%).

4-Hydroxybenzyl-2′,4′,6′-trihydroxyphenyl [^{13}C]ketone (16). ^{13}C-Deoxybenzoin **16** (872 mg, 75%) was prepared as for **14** by treatment of nitrile **11** (1.0 g, 4.47 mmol) and phloroglucinol (845 mg, 6.71 mmol) in diethyl ether with freshly fused zinc chloride (61 mg, 0.05 mmol) followed by saturation of the reaction mixture with hydrogen chloride. Purification was carried out by column chromatography eluting with diethyl ether: mp 258°C (lit.[13] 258–262°C); ν_{max} (nujol)/cm^{-1} 3395 (OH), 1610 (C=O); δ_H (200 MHz, d^6-DMSO) 4.28 (2H, d, $J_{C,H}$=6.2 Hz, –CH$_2$–), 5.82 (2H, s, 3′, 5′-H), 6.70 (2H, d, $J_{2,3}$=$J_{5,6}$=8.4 Hz, 3, 5-H), 7.06 (2H, d, $J_{2,3}$=$J_{5,6}$=8.4 Hz, 2, 6-H); δ_C (50.3 MHz, d^6-DMSO) 44.04 (d, *J*=45.1 Hz, *C*H$_2$), 96.7 (3′-*C* and 5′-*C*), 116.2 (3-*C* and 5-*C*), 128.5 (1-*C*), 132.0 (2-*C* and 6-*C*), 157.2 (4-*C*), 164.6 (2′-*C* and 6′-*C*), 167.4 (4′-*C*), 205 (enhanced ^{13}*C*=O); *m/z* (EI) 261 (M$^+$, 7%), 107 (12).

4′-Hydroxybenzyl-5,6-dihydroxy-[4-^{13}C]isoflavone ([4-^{13}C]genistein) (17). [4-^{13}C]-Genistein (112 mg, 54%) was prepared using the same method as for biochanin A **15**. Microwave heating of a solution of deoxybenzoin **16** (200 mg, 0.77 mmol) in dimethylformamide (8 ml) and borontrifluoride etherate (5.0 ml, 5.77 g, 40.7 mmol) addition of methane sulfonyl chloride (5.0 ml, 7.4 g, 64.6 mmol) and further irradiation: mp 307°C (decomp) (lit.[13] 291–296°C (decomp));[16] (Found: C, 66.20; H, 4.02. $C_{14}{}^{13}C_1H_{10}O_5$ requires C, 66.42; H, 3.72%); ν_{max} (nujol)/cm^{-1} 3395, 1610 (C=O); δ_H (300 MHz, d^6-DMSO) 6.25 (1H, d, *J*=2 Hz, 8-*H*), 6.41 (1H, d, *J*=2 Hz, 6-*H*), 6.81 (2H, d, $J_{2',3'}$=$J_{5',6'}$=8.2 Hz, 3′-*H* and 5′-*H*), 7.4 (2H, d, $J_{2',3'}$=$J_{5',6'}$=8.2 Hz, 2′-*H* and 6′-*H*), 8.4 (1H, d, $J_{2,4}$=6 Hz (^{13}C-^{1}H coupling), 2-*H*); δ_C (50.3 MHz, d^6-DMSO) 94.0 (8-*C*), 99.1 (6-*C*), 104.6 (4a-*C*), 115.8 (3′ and 5′-*C*), 121.4 (3-*C*), 122.4 (1′-*C*), 130.3 (2′-*C* and 6′-*C*), 154.1 (2-*C*),

157.5 (4′-*C*), 157.7 (5-*C*), 162.25 (8a-*C*), 164.6 (7-*C*), 180.5 (enhanced 4-^{13}C); *m*/*z* (CI) 272 (MH^+, 100%).

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References

1. A preliminary account of some of this work has been published: Whalley, J. L.; Bond, T. J.; Botting, N. P. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2569–2572.
2. Barnes, S.; Peterson, T. G. *Proc. Soc. Exp. Biol. Med.* **1995**, *208*, 103–108.
3. Wu, S. Y.; Brewer, M. S. *J. Food Sci.* **1994**, *59*, 702–706.
4. Colditz, G. A.; Stampfer, M. J.; Willett, W. C.; Hunter, D. J.; Manson, J. E.; Hennekens, C. H.; Rosner, B. A.; Speizer, F. E. *Cancer Causes Control* **1992**, *3*, 433–439.
5. Anderson, J. J. *J. Nutr.* **1995**, *125*, 799.
6. Simpkins, J. W. *Pharmacological Treatment of Alzheimer's Disease: Molecular and Neurobiological Foundations*; Wiley: New York, 1997.
7. Pelter, A.; Foot, S. *Synthesis* **1976**, 326.
8. Bass, R. J. *J. Chem. Soc., Chem. Commun.* **1976**, 78–79.
9. Jha, H. D.; Zilliken, F.; Breitmaier, E. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 102–103.
10. Chadderton, J.; Baker, W.; Harborne, J. B.; Ollis, W. D. *J. Chem. Soc.* **1953**, 1853–1860.
11. Hase, T.; Wahala, K. *J. Chem. Soc., Perkin Trans 1* **1991**, 3005–3008.
12. McOmir, J. F. W.; West, D. E. *Org. Synth.* **1973**, *5*, 412.
13. Pelter, A.; Ward, R. S.; Whalley, J. L. *Synthesis* **1998**, 1793–1802.
14. Cheng, Y.-C.; Nair, M. G.; Santell, R. C.; Helferich, W. G. *J. Agric. Food. Chem.* **1994**, *42*, 1869–1871.
15. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.
16. Melting point indistinct due to decomposition but purity established by both microanalysis and HPLC analysis.