Journal of Labelled Compounds and Radiopharmaceuticals
J Label Compd Radiopharm 2007; **50**: 1266–1271.
Published online 4 October 2007 in Wiley InterScience
(www.interscience.wiley.com). DOI: 10.1002/jlcr.1460

JLCR

Research Article

The synthesis of [3,4,1′-$^{13}C_3$]genistein

MARK F. OLDFIELD, LIRONG CHEN and NIGEL P. BOTTING*
School of Chemistry, University of St. Andrews, St. Andrews, Fife, KY16 9ST, UK

Received 16 July 2007; Revised 14 August 2007; Accepted 16 August 2007

Abstract: A facile synthesis is described for [3,4,1′-$^{13}C_3$]genistein for use as an internal standard in isoflavone analysis by mass spectrometric methods. Ethyl 4-hydroxy[1-^{13}C]benzoate was first prepared from the reaction of diethyl [2-^{13}C]malonate and 4*H*-pyran-4-one. Two further ^{13}C atoms were incorporated using potassium [^{13}C]cyanide as the source to give 4′-benzyloxy-[1,2,1′-$^{13}C_3$]phenylacetonitrile. [3,4,1′-$^{13}C_3$]Genistein was then constructed through coupling of the isotopically labelled phenylacetonitrile with phloroglucinol under Hoesch conditions, followed by formylation and cyclization. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: genistein; isoflavones; phytoestrogens; polyphenols

Introduction

Isoflavone phytoestrogens, such as daidzein, genistein and glycitein, are a group of polyphenolic compounds with weak estrogenic and antiestrogenic activity, present in the human diet,[1,2] particularly in soybeans and soy-derived products.[3,4] Epidemiological studies have shown that the consumption of an isoflavone-rich diet is associated with a decrease in the incidence of hormone-related cancers, such as breast and prostate cancer.[5,6] It has also been suggested that isoflavones may possess other health-promoting activities, including chemoprevention of osteoporosis[7] and cardiovascular disease[8] and lessening of menopausal symptoms.[7] However, although many studies have been carried out, there are still questions to be answered concerning the absorption, metabolism and bioavailability of isoflavonoids.[9,10]

Analytical chemistry has played a key role in understanding the significance of the biological effects of phytoestrogens, in particular, mass spectrometry-based methods, i.e. LC-MS[11,12] and GC-MS.[13] Accurate analysis has been important in attempts to establish the exposure of the population to the soy isoflavones through their diet and also in epidemiological studies to investigate the associations between isoflavone exposure and disease.[14] To improve the accuracy and reproducibility the choice of internal standard is highly important. The optimum internal standard for LC-MS and GC-MS is a pure, stable, isotopically labelled analogue of the analyte, which must have a mass difference large enough to nullify the effect of natural abundance heavy isotopes in the analyte. This mass difference will depend on the molecular weight of the analyte and the presence of heteroatoms. For molecules of the size of isoflavones an extra three mass units is sufficient, as 99% enrichment at each position results in less than 1 ppm residual unlabelled analyte in the internal standard and also the M^{+3} ion due to natural ^{13}C in the analyte will be at less than 1% abundance giving minimal overlap. In previous studies we synthesized [3,4,8-$^{13}C_3$]daidzein (1)[15] and [2,3,4-$^{13}C_3$]glycitein (2)[16] for use as internal standards. Herein we report the synthesis of [3,4,1′-$^{13}C_3$]genistein (3), which completes the set of internal standards for the three most common isoflavones.

Results and discussion

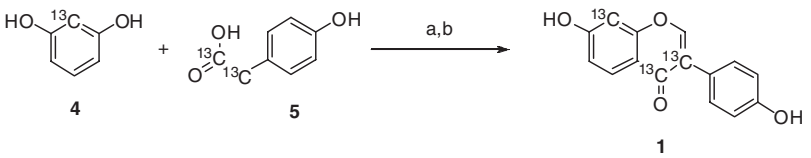
Previously, [3,4,8-$^{13}C_3$]daidzein (1)[15] was prepared from [2-^{13}C]resorcinol (4) and 4′-hydroxy[1,2-$^{13}C_2$]phenylacetic acid (5) (Scheme 1) and it was initially intended to use a similar strategy for the synthesis of ^{13}C-labelled genistein. However, this route requires the synthesis of ^{13}C-labelled phloroglucinol and this proved a much more challenging target than the [2-^{13}C]resorcinol. Introduction of the extra oxygen

*Correspondence to: Nigel P. Botting, School of Chemistry, University of St. Andrews, St. Andrews, Fife, KY16 9ST, UK.
E-mail: npb@st-andrews.ac.uk
Contract/grant sponsor: FSA; contract/grant numbers: T05001, T05023

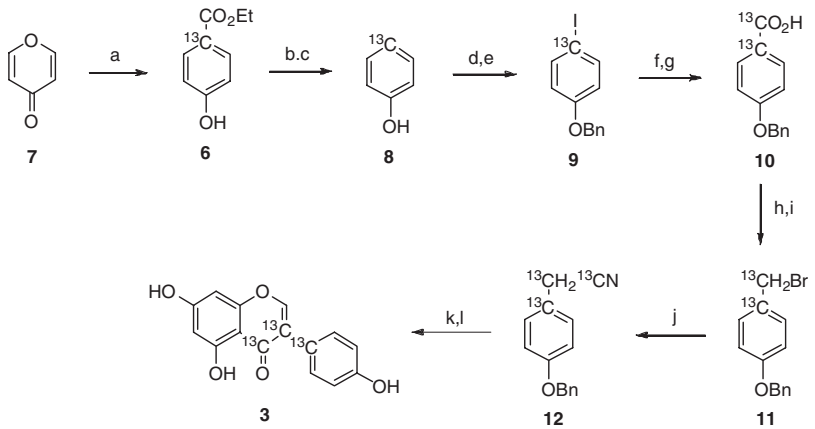
substituent, either as a protected alcohol or carbonyl group, caused severe problems and made the previous synthetic route untenable.

Therefore, it was decided to incorporate all three ^{13}C-atoms into the phenylacetic acid fragment, although this was less efficient as it requires more steps and is not a convergent synthesis. Ethyl 4-hydroxy[1-^{13}C]benzoate (6) was first prepared from the reaction of diethyl [2-^{13}C]malonate and 4*H*-pyran-4-one (7), using chemistry originally developed by Steglich (Scheme 2),[17] to incorporate the first ^{13}C-atom into the benzene ring, which will become the B-ring of the isoflavone .Hydrolysis of the ethyl ester under basic conditions was then followed by copper-catalysed decarboxylation to give [4-^{13}C]phenol (8). Selective iodination at the para position was achieved under basic conditions and then the hydroxyl group was protected by benzylation to give 4-benzyloxy-[1-^{13}C]iodobenzene (9). The two further ^{13}C atoms were derived from two moles of [^{13}C]cyanide ion, which were added consecutively. Although it may also be possible to add both ^{13}C atoms in one go using [1,2-$^{13}C_2$]acetyl chloride, it was decided to use potassium [^{13}C]cyanide as a cheaper source of isotopic label. Palladium-catalysed cyanation of the 4-benzyloxy-[1-^{13}C]iodobenzene, as employed in the synthesis of [3,4,8-$^{13}C_3$]daidzein (1),[15,18] gave the doubly labelled product, in very good 87% yield. The nitrile was then hydrolysed to the carboxylic acid, reduced to the alcohol using $LiAlH_4$ and brominated using PBr_3. A simple nucleophilic substitution using potassium [^{13}C]cyanide in acetonitrile with 18-crown-6 gave the key triply ^{13}C-labelled precursor, 4′-benzyloxy-[1,2,1′-$^{13}C_3$]phenylacetonitrile (12).

For the synthesis of daidzein, the nitrile was hydrolysed to the acid, which was then coupled with resorcinol to give the deoxybenzoin.[15] However, with genistein better yields are obtained if the nitrile is used directly. Thus, coupling of the 4′-benzyloxy-[1,2,1′-$^{13}C_3$]phenylacetonitrile (12) and phloroglucinol was carried out under modified Hoesch reaction conditions,[19] using catalytic zinc chloride,[20] to give the genistein deoxybenzoin. Finally cyclization and formylation provided the final product, [3,4,1′-^{13}C]genistein (3). The [3,4,1′-$^{13}C_3$]genistein (3) was found to be identical to genistein in all respects except for the expected increase in mass and changes to the NMR spectra. The three ^{13}C atoms were observed at 182.3 (C4), 125.0 (C3) and123.3 ppm (C1′) in the ^{13}C NMR spectrum. The purity of the compound was confirmed by reverse-phase HPLC (Kingsorb 3μ C18 Column (150 × 4.6 mm)) giving a retention time of 7.85 min with a mobile phase of acetonitrile:-water (1:1) and a 0.5 mL min^{-1} flow rate. This gave a single peak at the identical retention time to unlabelled genistein. Furthermore, the UV spectrum was measured giving a λ_{max} (EtOH) of 262 nm and ε= 35460 dm^3 mol^{-1} cm^{-1}, compared with literature value[21] of ε= 35842 dm^3 mol^{-1} cm^{-1}, implying 98% purity within experimental



Scheme 1 (Reagents and conditions: (a) $BF_3 \cdot Et_2O$ (55%) and (b) $DMF \cdot (OMe)_2$, DMF (80%))



Scheme 2 (Reagents and conditions: (a) diethyl [2-^{13}C]malonate, ^{t}BuOH, ^{t}BuOK (85%); (b) NaOH, H_2O, reflux, 3 h (91%); (c) quinoline, copper bronze, reflux, 4 h (80%); (d) NaOH, MeOH, I_2 (65%); (e) BnBr, K_2CO_3, acetone (80%); (f) K^{13}CN, $Pd(OAc)_2$, $Ca(OH)_2$, DMF (87%); (g) 2 N NaOH, MeOH (86%); (h) $LiAlH_4$, THF (82%); (i) PBr_3, Et_2O (90%); (j) K^{13}CN, 18-crown-6, MeCN (88%); (k) phloroglucinol, $ZnCl_2$, Et_2O (51%); (l) $BF_3 \cdot Et_2O$, DMF, PCl_5 (52%))

DOI: 10.1002.jlcr

error. The compound has since been successfully employed as an internal standard in both GC-MS[13] and LC-MS[11,12] using isotope dilution methods.

Experimental

General

NMR spectra were recorded on a Varian Gemini 2000 (^{1}H 300 MHz, ^{13}C 75.45 MHz) or a Bruker Avance 300 (^{1}H 300 MHz, ^{13}C 75.45 MHz) spectrometer. Chemical shifts (δ) in ppm are given relative to Me_4Si, coupling constants (*J*) in Hz. Elemental analyses were carried out in the departmental microanalytical laboratory. IR spectra were recorded on a Perkin-Elmer series 1420 FT IR spectrophotometer. The samples were prepared as Nujol mulls or thin films between sodium chloride discs and recorded in cm^{-1}. EI and CI mass spectra were recorded on a VG Autospec. Electrospray mass spectra were recorded on a Micromass LC-T UV spectra were recorded on a Kontron Uvikon 930 spectrometer. Melting points were recorded on an Electrothermal melting point apparatus and are uncorrected. Analytical thin layer chromotography (TLC) was carried out on Merck 5785 Kieselgel $60F_{254}$ fluorescent plates. Flash chromatography was performed according to the procedure of Still[22] using silica gel of 35–70 μm particle size. Dimethylformamide (DMF) was distilled from magnesium sulfphate. Diethyl ether and tetrahydrofuran (THF) were distilled from sodium metal and benzophenone.

Ethyl 4-hydroxy[1-^{13}C]benzoate (7)

A solution of (4*H*)-pyran-4-one (6) (2.85 g, 29.80 mmol) and diethyl [2-^{13}C]malonate (3.00 g, 2.85 mL, 18.60 mmol) in dry *t*-butanol (60 mL) was heated to reflux, then potassium *t*-butoxide (freshly dried, 2.76 g, 24.5 mmol) in dry *t*-butanol (120 mL) was added dropwise under nitrogen. The resulting mixture was heated under reflux for 3 h, then hydrochloric acid (1 N, 60 mL) was added. After a further 0.5 h heating at reflux, the solvent was removed under reduced pressure and water (150 mL) was added. The reaction mixture was then extracted with diethyl ether (3×100 mL) and the combined organic extracts washed with water (2×100 mL), brine (100 mL) and dried ($MgSO_4$). Evaporation of the solvent at reduced pressure gave the crude product obtained which was purified by column (7) as a pale yellow solid (2.62 g, 85%) m.p. 114–118 (Lit[23] 112–115°C); Found C, 64.98, H, 5.96%. Calc. for $^{12}C_8\,^{13}CH_{10}O_3$ C, 65.26, H, 6.03%); δ_H (300 MHz, $CDCl_3$) 7.96 (2H, d, *J* 8.9 Hz, H-2, 6), 6.89 (2H, dd, *J* 8.9, 7.6 Hz, H-3, 5), 6.50 (1H, brs, –OH), 4.37 (2H, q, *J* 7 Hz, $–CH_2–$), 1.39 (3H, t, *J* 7 Hz, $–CH_3$); δ_C (75.45 MHz, $CDCl_3$) 167.4 (d, *J* 77 Hz, –CO–), 160.6 (d, *J* 9 Hz, C-4), 132.3 (d, *J* 60 Hz, C-2, 6), 122.9 (enhanced, C-1), 115.6 (C-3, 5), 61.4 ($–CH_2–$), 14.7 ($–CH_3$); *m/z* (EI) 167 (M^+, 2%), 139 [$(M-Et)^+$, 20], 122 [$(M-OEt)^+$, 100], 94 [$(M-COOEt)^+$, 15]; *m/z* (ES^-) 166 [$(M–H)^-$, 100%].

4-Hydroxy-[1-^{13}C]benzoic acid

Ethyl 4-hydroxy[1-^{13}C]benzoate (7) (2.30 g, 13.85 mmol) was mixed with aqueous sodium hydroxide (2 N, 250 mL) and the mixture was heated under reflux for 3 h. The resulting mixture was acidified to pH 1 with concentrated hydrochloric acid and extracted with diethyl ether:methanol (4:1) (6×40 mL). The organic layers were then combined, washed with brine (2×40 mL) and dried ($MgSO_4$). After removal of the solvent, the product was obtained as a pale white solid (1.75 g, 91%) m.p. 215–218°C (Lit[24] 215–216°C); (Found C, 60.67, H, 4.20%. Calc. for $^{12}C_6\,^{13}CH_6O_3$ C, 61.15, H, 4.35%); δ_H (300 MHz, Methanol-d_4) 7.84 (2 H, d, *J* 8.3 Hz, H-2, 6), 6.76 (2H, dd, *J* 8.9, 7.5 Hz, H-3, 5); δ_C (75.45 MHz, Methanol-d_4) 174.2 (d, *J* 74 Hz, –COOH), 166.6 (C-4), 137.0 (d, *J* 59 Hz, C-2, 6), 126.3 (enhanced, C-1), 120.0 (C-3, 5); *m/z* (CI) 140 [$(MH)^+$, 100%], 122 [$(M-OH)^+$, 10].

[4-^{13}C]Phenol (8)

A mixture of 4-hydroxy-[1-^{13}C]benzoic acid (1.65 g, 11.86 mmol), quinoline (20 mL) and copper bronze (1.38 g, 21.63 mmol) was heated under reflux for 4 h, then cooled and filtered to remove the copper bronze. The filtrate collected was poured into hydrochloric acid (10%, 300 mL) and stirred at room temperature. The resulting mixture was then extracted with diethyl ether (4×50 mL), washed with brine (2×30 mL) and dried over $MgSO_4$. The crude product obtained was purified by column chromatography on silica, eluting with hexane:ethyl acetate (1:1). After removal of the solvent at reduced pressure, a light yellow solid was obtained (900 mg, 80%) m.p. 35–39°C (Lit[25] 41–42°C); δ_H (300 MHz, $CDCl_3$) 7.16 (2H, m, H-3, 5), 6.88 (1H, dtt, *J* 174, 7.4, 1.0 Hz, H-1), 6.76 (2H, m, H-2, 6), 5.06 (1H,brs, –OH); δ_C (75.45 MHz, $CDCl_3$) 121.4 (enhanced, C-4); *m/z* (EI) 95 (M^+, 100%), 67 [$(M-CO)^+$, 15].

4-Iodo-[4-^{13}C]phenol

To a solution of sodium hydroxide (635 mg, 15.90 mmol) in methanol (10 mL) was added [4-^{13}C]phenol (8) (755 mg, 7.94 mmol). The solution was cooled to −5°C, then iodine (2.02 g, 7.94 mmol) in

DOI: 10.1002.jlcr

methanol (15 mL) was added dropwise. After 1 h stirring at −5°C, the resulting mixture was acidified with hydrochloric acid (0.5 N) to pH 2 and extracted with diethyl ether (5 × 25 mL). The combined organic layers were washed with sodium thiosulfate (3 × 20 mL), then brine (2 × 20 mL) and dried over $MgSO_4$. Further purification was carried out by column chromatography on silica, eluting with dichloromethane:diethyl ether (20:1). After removal of the solvent, a white solid was obtained (1.15 g, 65%) m.p. 91–92°C (Lit[26] 93–93°C); δ_C (300 MHz, $CDCl_3$) 7.44 (2H, dd, *J* 8.9, 2.9 Hz, H-2, 6), 6.55 (2H, dd, *J* 10.3, 8.9 Hz, H-3, 5), 4.78 (1H, s, –OH); δ_C (75.45 MHz, $CDCl_3$) 83.1 (enhanced, C-4); *m/z* (EI) 221 (M^+, 100%), 94 [$(M-I)^+$, 30], 66 [$(M-I-CO)^+$, 30]. HRMS (ES^-): $^{12}C_5\,^{13}CH_5IO$ requires 220.9416; Found 220.9419.

4-Benzyloxy-[1-^{13}C]iodobenzene (9)

To a solution of 4-iodo-[4-^{13}C]phenol (1.04 g, 4.71 mmol) in acetone (15 mL) was added potassium carbonate (1.88 g, 14.13 mmol) and benzyl bromide 23 (0.56 mL, 806 mg, 4.71 mmol). The mixture was heated under reflux for 5 h, then cooled and filtered to remove the solid. The crude product obtained on removal of the solvent was dissolved in diethyl ether (20 mL), washed with water (3 × 10 mL), dried over $MgSO_4$ and the solvent removed at reduced pressure. Further purification was carried out by column chromatography on silica, eluting with ethyl acetate:petroleum ether (1:20). After removal of the solvent at reduced pressure, white crystals were obtained (1.18 g, 80%) m.p. 59–61°C (Lit[27] 61–62°C); (Found C, 50.81, H, 3.45%. Calc. for $^{12}C_{12}\,^{13}CH_{11}IO$ C,50.51, H, 3.56%); δ_H (300 MHz, $CDCl_3$) 7.48 (2H, dd, *J* 9.0, 2.9 Hz, H-2, 6), 7.36–7.23 (5H, m, H-2′ to H-6′), 6.68 (2H, dd, *J* 10.4, 9.0 Hz, H-3, 5), 4.96 (2H, s, H-7); δ_C (75.45 MHz, $CDCl_3$) 83.4 (enhanced, C-1); *m/z* (EI) 311 (M^+, 37%), 91 ($C_7H_7^+$, 100).

4-Benzyloxy-[1,*nitrile*-$^{13}C_2$]benzonitrile

To a solution of 4-benzyloxy-[1-^{13}C]iodobenzene (9) (1.10 g, 3.54 mmol) in dry DMF (20 mL), calcium hydroxide (131 mg, 1.77 mmol), potassium [^{13}C]cyanide (273 mg, 4.13 mmol) and palladium acetate (122 mg, 0.54 mmol) were added. Under the protection of a nitrogen atmosphere, the mixture was heated under reflux for 5 h, then cooled and filtered through celite. The DMF was removed under reduced pressure and the residue was extracted with diethyl ether (5 × 30 mL), washed with water (3 × 20 mL), dried over $MgSO_4$ and the solvent removed at reduced pressure. Further purification was carried out by column chromatography on silica, eluting with ethyl acetate:petroleum ether (40–60°C) (3:10). After removal of the solvent, a white solid was obtained. (653 mg, 87%) m.p. 95–97°C (Lit[28] 96°C); δ_H (300 MHz, $CDCl_3$) 7.50 (2H, dd, *J* 8.9, 5.0 Hz, H-2, 6), 7.36–7.25 (5H, m, H-2′ to H-6′), 6.94 (2H, dd, *J* 8.9, 8.3 Hz, H-3, 5), 5.03 (2H, s, CH_2); δ_C (75.45 MHz, $CDCl_3$, ppm) 119.6 (enhanced, d, *J* 87 Hz, C-1), 104.6 (enhanced, d, *J* 87 Hz, $-^{13}CN$); *m/z* (CI) 212 [$(MH)^+$, 100%], 91 ($C_7H_7^+$, 11).

4-Benzyloxy-[1,*carboxy*–$^{13}C_2$]benzoic acid (10)

4-Benzyloxy-[1,*nitrile*-$^{13}C_2$]benzonitrile (569 mg, 2.70 mmol) was mixed with sodium hydroxide (2 N, 25 mL) and methanol (2.5 mL). The mixture was heated under reflux until the starting material was consumed according to TLC. The resulting mixture was cooled and adjusted to pH 1 with concentrated hydrochloric acid. The solid precipitated was collected by filtration and heated under reflux with sodium hydroxide (2 N, 50 mL) for a further 5 h. The resulting mixture was cooled and again adjusted to pH 1 with concentrated hydrochloric acid, extracted with diethyl ether:methanol (6:1, 3 × 20 mL). The organic fractions were combined, washed with brine (2 × 20 mL) and dried over $MgSO_4$. After removal of the solvent at reduced pressure, the desired product was obtained as a white solid (536 g, 86%) m.p. 192–194°C (Lit[29] 191–192°C); (Found C, 73.23, H, 5.22%. Calc. for $^{12}C_{12}\,^{13}C_2H_{12}O_3$ C, 73.90, H, 5.25%); δ_H (300 MHz, $CDCl_3$) 7.95 (2H, dd, *J* 8.7, 3.9 Hz, H-2, 6), 7.39–7.28 (5H, m, H-2′ to H-6′), 6.95 (2H, dd, *J* 8.7, 7.7 Hz, H-3, 5), 5.08 (2H, s, CH_2); δ_C (75.45 MHz, $CDCl_3$) 168.7 (enhanced, d, *J* 75 Hz, $^{13}COOH$), 122.9 (enhanced, d, *J* 75 Hz, C-1); *m/z* (CI) 231 [$(MH)^+$, 100%], 91 ($C_7H_7^+$, 12); HRMS(CI) $^{12}C_{12}\,^{13}C_2H_{12}O_3$ requires 231.0934, Found 231.0932.

4-Benzyloxy-[1, *methylene*-$^{13}C_2$]benzyl alcohol

Under the protection of a nitrogen atmosphere, a solution of 4-benzyloxy-[1,*carboxy*-$^{13}C_2$]benzoic acid (10) (450 mg, 1.96 mmol) in dry THF (8 mL) was added dropwise to the suspension of lithium aluminium hydride (300 mg, 7.83 mmol) in dry THF (7 mL). The mixture was stirred at room temperature overnight. Sulphuric acid (10%, 20 mL) was then added carefully to quench the reaction. The resulting mixture was extracted with diethyl ether (4 × 20 mL), washed with brine (2 × 10 mL) and dried over $MgSO_4$. After removal of the solvent at reduced pressure, the desired product was obtained as a white solid (346 mg, 82%). m.p. 82–85°C (Lit[28] 84–85°C); δ_H (300 MHz, $CDCl_3$) 7.34–7.13 (7H, m, H-2′ to H-6′ and H-2, 6), 6.86 (2H, dd, *J* 8.7, 7.1 Hz, H-3, 5), 4.96 (2H, s, CH_2), 4.41 (2H, dd, *J* 141, 3.8 Hz, $^{13}CH_2O$); δ_C (75.45 MHz, $CDCl_3$) 135.4

DOI: 10.1002.jlcr

(enhanced, d, *J* 52 Hz, C-1), 65.4 (enhanced, d, *J* 52 Hz, $^{13}CH_2O$); *m/z* (EI) 216 (M^+, 20%), 91 ($C_7H_7^+$, 100).

4-Benzyloxy-[1,*methylene*-$^{13}C_2$]benzyl bromide (11)

To a solution of 4-benzyloxy-[1, *methylene*-$^{13}C_2$]benzyl alcohol (320 mg, 1.48 mmol) in dry diethyl ether (6 mL) was introduced phosphorus tribromide (0.4 mL, 4.5 mmol, 1.15 g). The resultant solution was stirred at room temperature overnight. The pale yellow solution formed was then poured into ice water (25 mL), extracted with diethyl ether (3 × 15 mL) and dried over $MgSO_4$. After removal of the solvent at reduced pressure, a white solid was obtained (373 mg, 90%) m.p. 85–88°C (Lit[28] 83–85°C); δ_H (300 MHz, $CDCl_3$) 7.33 (5H, m, H-2′ to H-6′), 7.25 (2H, dd, *J* 8.7, 5.1 Hz, H-2, 6), 6.87 (2H, dd, *J* 8.7, 7.7 Hz, H-3, 5), 5.00 (2H, s, CH_2), 4.43 (2H, dd, *J* 153, 3.6 Hz, $^{13}CH_2Br$); δ_C (75.45 MHz, $CDCl_3$), 130.6 (enhanced, d, *J* 51 Hz, C-1), 34.3 (enhanced, d, *J* 51 Hz, $^{13}CH_2Br$); *m/z* (EI) 278/280 (1:1, M^+, 3%), 199 [$(M-Br)^+$, 55], 91 ($C_7H_7^+$, 100).

4′-Benzyloxy-[1,2,1′-$^{13}C_3$]phenylacetonitrile (12)

4-Benzyloxy-[1,*methylene*-$^{13}C_2$]benzyl bromide (11) (370 mg, 1.33 mmol) was dissolved in acetonitrile (10 mL), then 18-crown-6 (352 mg, 1.33 mmol) and potassium [^{13}C]cyanide (88 mg, 1.33 mmol) were added. The mixture was heated under reflux for 4 h, then cooled and the solvent was removed at reduced pressure. The pale white residue was then extracted with diethyl ether (3 × 20 mL), washed with water (2 × 10 mL), brine (10 mL) and dried over $MgSO_4$. The crude product was purified by column chromatography on silica, eluting with petroleum ether (40–60°C):ethyl acetate (5:3). After removal of the solvent at reduced pressure, the desired product was obtained as white crystals (264 mg, 88%) m.p. 67–70°C (Lit[15] 66–69°C); (Found C, 80.61, H, 5.77, N, 6.11%. Calc. for $^{12}C_{12}\,^{13}C_3H_{13}NO$. C, 80.95, H, 5.79, N, 6.19%); δ_H (300 MHz, Methanol-d_4) 7.34–7.20 (5H, m, H-2′ to H-6′), 7.16 (2H, dd, *J* 8.9, 4.8 Hz, H-2, 6), 6.90 (2H, dd, *J* 8.4, 8.3 Hz, H-3, 5), 4.97 (2H, s, CH_2), 3.69 (2H, ddd, *J* 136, 10.8, 6.9 Hz, $-^{13}CH_2$); δ_C (75.45 MHz, Methanol-d_4) 124.8 (enhanced, d, *J* 52 Hz, C1), 120.3 (d, *J* 61 Hz, $-^{13}CN$), 23.0 (d, *J* 102 Hz, $-^{13}CH_2-$); *m/z* (EI) 226 (M^+, 10%), 91 ($C_7H_7^+$, 100); (ES^+) 249 [$(MNa)^+$, 100%], 227 [$(MH)^+$,15].

[7,8,1′-$^{13}C_3$]Genistein deoxybenzoin

Under vigorous stirring, freshly fused zinc chloride (15 mg, 0.11 mmol) was added to the solution of (12) (150 mg, 0.66 mmol) in dry diethyl ether (6 mL). The mixture was saturated with HCl gas at 0°C for 6 h, then warmed to room temperature and stirred overnight. The diethyl ether was removed, and the resulting solid was washed with diethyl ether (2 × 5 mL), then hydrolysed with hydrochloric acid (0.1 N, 20 mL) at reflux for 5 h. The reaction mixture was then extracted with diethyl ether (4 × 10 mL), washed with brine (2 × 10 mL) and dried over $MgSO_4$. After removal of the solvent at reduced pressure a pink gum was obtained. Further purification was carried out by column chromatography on silica, eluting with diethyl ether:petroleum ether (4060°C) (2:1 then 4:1). After removal of the solvent at reduced pressure, the desired product was obtained as a pale yellow solid (98 mg, 51%) m.p. >200°C (dec.) (Lit[31] 189–191°C); δ_H (300 MHz, Methanol-d_4) 7.00 (2H, dd, *J* 8.4, 3.9 Hz, H-2′, 6′), 6.59 (2H, dd, *J* 8.4, 7.7 Hz, H-3′, 5′), 5.69 (2H, d, *J* 1.0 Hz, H-4, 6), 4.16 (2H, dt, *J* 130, 6.2 Hz, H-8); δ_C (75.45 MHz, Methanol-d_4) 205.5 (enhanced, d, *J* 40 Hz, C-7), 128.5 (enhanced, d, *J* 51 Hz, C-1′), 50.0 (enhanced, d, *J* 83 Hz, C-8); *m/z* (ES^-) 262 [$(MH)^-$, 100%]; HRMS (ES^-) $^{12}C_{11}\,^{13}C_3H_{12}O_5$ requires 262.0709; Found 262.0711.

[3, 4,1′-$^{13}C_3$]Genistein (3)

A mixture of [1′,7,8- $^{13}C_3$]genistein deoxybenzoin (86 mg, 0.33 mmol) and boron trifluoride diethyl etherate (0.13 mL, 142 mg, 1.00 mmol) was cooled to 10°C, then dry DMF (0.6 mL) was added dropwise to give solution A. In another flask, dry DMF (0.9 mL) was cooled to 10°C, then phosphorus pentachloride (104 mg, 0.50 mmol) was added in small portions. The mixture was kept at 55°C for 20 min to give solution B. Solution B was introduced into solution A slowly, the temperature was kept below 27°C during the addition. The resulting mixture was stirred at room temperature for 20 min, then poured into aqueous sodium acetate (12.5%, 30 mL), stirred for 2 h and left overnight. The solid formed was collected by filtration and washed with water (3 × 5 mL). Further purification was carried out by HPLC (Kingsorb 3μ C18 Column, acetonitrile:-water as eluant). After removal of the solvent at reduced pressure, the desired product was obtained as a white solid (47 mg, 52%) m.p. 307°C (Lit[32] 301–302°C); δ_H (300 MHz, acetone-d_6) 6.28 (1H, dd, $J_{6,8}$ 2.2, $J_{4,8}$ 0.8 Hz, H-8), 6.42 (1H, d, $J_{6,8}$ 2.2, $J_{4,6}$ 1.5 Hz, H-6), 6.9 (2H, dt, $J_{2,3'} = J_{5',6}$ 8.5, $J_{1,3'} = J_{1,5'}$ 1.9 Hz, H-3′,5′), 7.45 (2H, dd, $J_{2',3'} = J_{5',6'}$ 8.5, $J_{1',2'} = J_{1',6'}$ 3 Hz, H-2′,6′), 8.15 (1H, m, H-2), 8.5 (1H, s, OH-4), 9.7 (1H, s, OH-7); δ_C (75.45 MHz, Methanol-d_4, ppm) 182.3 (enhanced, dd, *J* 52, 4 Hz, C-4), 125.0 (enhanced, dd, *J* 60, 52 Hz, C-3), 123.3 (enhanced, dd, *J* 60, 4 Hz, C-1′); *m/z* (CI) 274 (MH+, 100%) (HRMS $C_{12}\,^{13}C_3H_{11}O_5$ requires 274.070713. Found 274.069965).

DOI: 10.1002.jlcr

REFERENCES

1. Dwyer JT, Goldin BR, Saul N, Gualfieri Barakat S, Adlercreutz H. *J Am Diet Assoc* 1994; **94**: 739–743.
2. Hendrich S, Lee K-W, Xu X, Wang H-J, Murphy PA. *J Nutr* 1994; **124**: 1789S–1792S.
3. Murphy PA, Song T, Buseman G, Barua K. *J Agric Food Chem* 1997; **45**: 4635–4638.
4. Setchell KDR, Zimmer-Nechemias L, Cai J, Heubi JE. *Lancet* 1997; **350**: 23–27.
5. Birt DF, Hendrich S, Wang W. *Pharmacol Ther* 2001; **90**: 157–177.
6. Yang CS, Landau JM, Huang MT, Newmark HL. *Annu Rev Nutr* 2001; **21**: 381–406.
7. Cassidy A, Albertazzi P, Nielsen IL, Hall W, Williamson G, Tetens I, Atkins S, Cross H, Manios Y, Wolk A, Steiner C, Branca F. *Proc Nutr Soc* 2006; **65**: 76–92.
8. Sacks FM, Lichtenstein A, Van Horn L, Harris W, Kris-Etherton P, Winston M. *Circulation* 2006; **113**: 1034–1044.
9. Hendrich S. *J Chrom B* 2002; **777**: 203–210.
10. de Pascual-Teresa S, Hallund J, Talbot D, Schroot J, Williams CM, Bugel S, Cassidy A. *J Nutr Biochem* 2006; **17**: 257–264.
11. Clarke DB, Lloyd AS, Oldfield MF, Botting NP, Needs PW, Wiseman H. *Anal Biochem* 2002; **309**: 158–172.
12. Grace PB, Taylor JI, Botting NP, Fryatt T, Oldfield MF, Al-Maharik N, Bingham SA. *Rapid Commun Mass Spectrom* 2003; **17**: 1350–1357.
13. Grace PB, Taylor JI, Botting NP, Fryatt T, Oldfield MF, Bingham SA. *Anal Biochem* 2003; **315**: 114–121.
14. Grace PB, Taylor JI, Low Y-L, Luben RN, Mulligan AA, Botting NP, Dowsett M, Welch AA, Khaw K-T, Wareham NJ, Day NE, Bingham SA. *Cancer Epidemiol Biomarkers Prev* 2004; **13**: 698–708.
15. Oldfield MF, Chen L, Botting NP. *Tetrahedron* 2004; **60**: 1887–1893.
16. Zhang Q, Botting NP. *Tetrahedron* 2004; **60**: 12211–12216.
17. Beyer J, Lang-Fugmann S, Muhlbauer A, Steglich W. *Synthesis* 1998; 1046–1051.
18. Takagi K, Okamoto T, Sakakibara Y, Ohno A, Oka S, Hayama N, *Bull Chem Soc Jpn* 1975; **48**: 3298–3301.
19. Chadderton J, Baker W, Harbome JB, Ollis WD. *J Chem Soc* 1953; 1853–1860.
20. Pelter A, Ward RS, Whalley JL. *Synthesis* 1998; 1793–1802.
21. Wiseman H, Casey K, Clarke DB, Barnes KA, Bowey E. *J Ag Food Chem* 2002; **50**: 1404–1410.
22. Still WC, Kahn M, Mitra A. *J Org Chem* 1978; **43**: 2923–2925.
23. Magano J, Chen MH, Clark JD. *J Org Chem* 2006; **71**: 7103–7105
24. Nordstroem FL, Rasmuson AC. *J Pharm Sci* 2006; **95**: 748–760.
25. Walling C, Buckler SA. *J Am Chem Soc* 1955; **77**: 6032–6038.
26. Horiuchi AC, Satoh YJ. *Bull Chem Soc Jpn* 1984; **57**: 2691–2692.
27. Kajigaeshi S, Kakinami T, Moriwaki M, Watanabe M, Fujisaki S, Okamoto T. *Chem Lett* 1988; 795–798.
28. Baggaley KH, Fears R, Hindley RM, Morgan B, Murrell E, Thorne DE. *J Med Chem* 1977; **20**: 1388–1393.
29. Tranchimand S, Tron T, Gaudin C, Iacazio G. *Synth Commun* 2006; **36**: 587–597.
30. Slavikova L, Slavik J. *Coll Czech Chem Commun* 1966; **31**: 1355–1362.
31. Al-Maharik N, Kaltia SAA, Mutikainen I, Wähälä K. *J Org Chem* 2000; **65**: 2305–2308.
32. Bradbury RB, White DE. *J Chem Soc* 1951; 3447–3449.

DOI: 10.1002.jlcr