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Research Article

The synthesis of multiply ^{13}C-labelled plant and mammalian lignans as internal standards for LC-MS and GC-MS analysis

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Summary

The syntheses of multiply ^{13}C-labelled derivatives of the two mammalian lignans, enterolactone and enterodiol, and two of their plant lignan precursors, secoisolariciresinol and matairesinol, are described. Three ^{13}C atoms were incorporated into each lignan using potassium [^{13}C]cyanide as the source for all of the ^{13}C atoms. The compounds were prepared for use as internal standards in the LC-MS and GC-MS analysis of lignans. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: lignans; phytoestrogens; enterolactone; enterodiol; analysis

Introduction

The lignans are a widely distributed group of plant phenols. There has been increasing interest in these compounds initially as a result of their use in traditional remedies in many cultures,[1] and more recently following the identification of the lignans enterolactone (**1**) and enterodiol (**2**) (enterolignans) in humans and the establishment of their dietary origin.[2,3] Enterodiol and enterolactone are exclusively formed by mammalian gut microflora[2] and their excretion in humans and other animals is positively correlated with the consumption of lignan-rich food.[2,3] High levels of enterolactone in the body have since also been correlated with a lower risk of developing chronic diseases, such as breast cancer and coronary heart disease.[4–7] Matairesinol **(3)** and secoisolariciresinol **(4)** were the two plant lignans first identified as precursors to the mammalian lignans (Scheme 1).[8]

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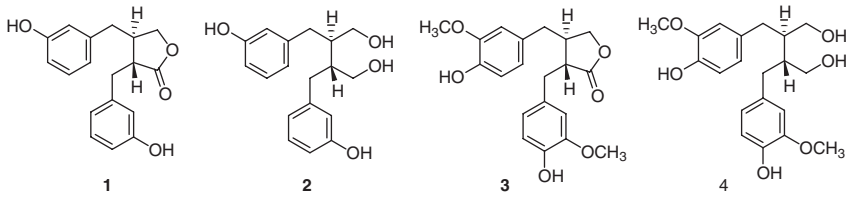
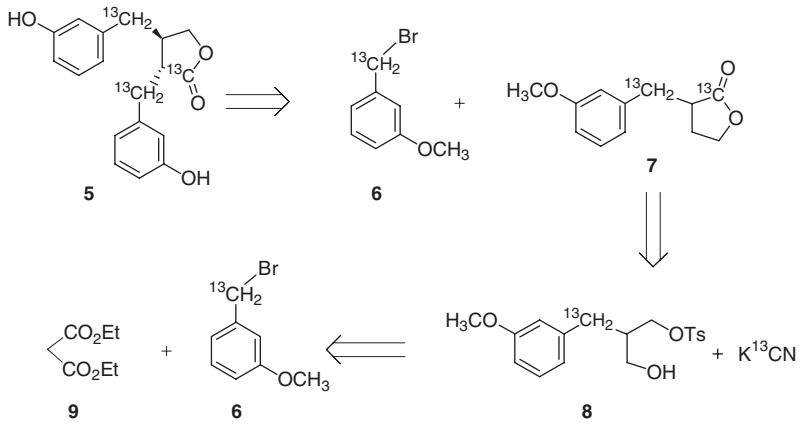


Scheme 1. Structures of mammalian and plant lignans

In order to better understand the significance of the biological effects of lignans, accurate analysis is important to establish the exposure of the population to the plant lignans through their diet and also in epidemiological studies to investigate the associations between lignan exposure, mammalian lignan levels and disease. Both gas chromatography-mass spectrometry (GC-MS)[9–11] and liquid chromatography-mass spectrometry (LC-MS)[12,13] have been used to quantify the low levels of lignans found in biological samples. The nature of the internal standard is an important aspect of any quantitative analytical procedure. For LC-MS and GC-MS the optimum 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. This mass difference depends on the molecular weight of the analyte and for lignan-type structures a minimum of three extra mass units is necessary.[14] A number of deuterated standards have been used for the analysis of lignans,[15] which were prepared using exchange methods to incorporate the deuterium atoms into the phenol rings.[16,17] However, under the analysis conditions back exchange can occur, such that the deuteriums are slowly replaced by hydrogen, resulting in both a reduction in the amount of internal standard and an apparent increase in the amount of the lignan being analysed.[18] In order to circumvent these problems it was decided to synthesize a series of lignans with three ^{13}C atoms incorporated into the carbon framework of the molecule as a new generation of internal standards.

Results and discussion

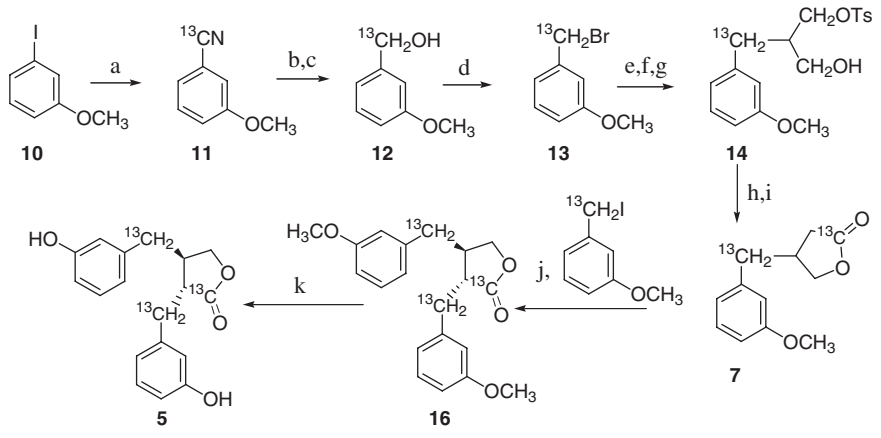
The synthesis of lignans has attracted much attention and been regularly reviewed.[19,20] For our purposes a racemic synthesis of the *trans*-α,β-dibenzyl-γ-butyrolactone framework of the target lignans was sufficient. These have been most commonly synthesized *via* Michael addition of a dithioacetal derived carbanion to a butenolide followed by *in situ* benzylation.[21,22] However, this route did not offer an obvious strategy for the introduction of the required three ^{13}C-atoms and so an alternative, albeit longer, synthetic route was identified that appeared to be more suitable.[23] Scheme 2 shows the retrosynthetic strategy for [$^{13}C_3$]enterolactone (**5**).



Scheme 2. Proposed retrosynthesis for [7,7′,9′-$^{13}C_3$]enterolactone

This synthetic route allows two molecules of the same benzyl bromide (**6**) to be employed in the construction of the lignan. In our previous studies on the isotopic labelling of isoflavones,[14,24] potassium [^{13}C]cyanide was used to label the methylene group of a substituted benzyl bromide, *via* palladium catalysed cyanation of a suitable aryl iodide[25] followed by functional group interconversion, i.e. hydrolysis, reduction and bromination, to provide the aryl bromide. One molecule of (**6**) is used for the synthesis of the key lactone fragment (**7**), which is then alkylated with the second molecule of (**6**), thus introducing one ^{13}C-atom near the beginning and one near the end of the synthesis. Previous studies showed that the key alkylation step gives the required relative stereochemistry, with the two substituents in a *trans* orientation.[23] Two options were then available for the introduction of the third ^{13}C-atom. Diethyl [2-^{13}C]malonate can be obtained but is expensive and would be used very early in the synthesis. A more cost-efficient option was to use a third molecule of potassium [^{13}C]cyanide in the assembly of the lactone (**7**). The synthesis can then be readily modified for all four target molecules. ^{13}C-labelled enterodiol is prepared *via* reduction of the enterolactone (**5**) and by using suitably modified benzyl bromides both ^{13}C-labelled secoisolariciresinol and matairesinol are also accessible.

The synthesis of [7,7,9′-$^{13}C_3$]enterolactone (**5**) thus began with cyanation of 3-methoxyiodobenzene (**10**) using potassium [^{13}C]cyanide and a palladium (II) acetate catalyst in DMF under basic conditions (calcium hydroxide)[25] to give the ^{13}C-labelled nitrile (**11**) in 64% yield (Scheme 3). The ^{13}C atom was clearly identified by the enhanced signal in the ^{13}C NMR spectrum at 119.6 ppm. The nitrile (**11**) was then hydrolysed under alkaline conditions, reduced using lithium aluminium hydride in THF and converted to the bromide (**13**). The benzyl bromide was reacted with the anion derived from diethyl malonate and the resultant diester reduced to the diol. Tosylation of just one of the two



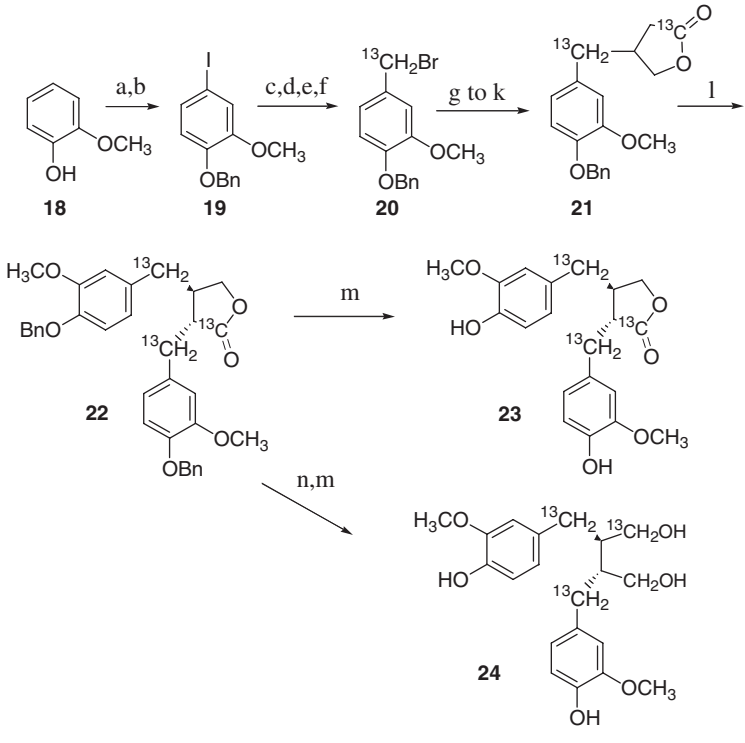
Scheme 3. Synthesis for [7,7′,9′-$^{13}C_3$]enterolactone(5). Reagents and conditions: (a) K^{13}CN, Ca(OH)$_2$, Pd(OAc)$_2$, DMF (64%); (b) 2 M NaOH, reflux (87%); (c) LiAlH$_4$, Et$_2$O (83%); (d) PBr$_3$, Et$_2$O (91%); (e) KO^{t}Bu, CH$_2$(CO$_2$Et)$_2$, glyme (86%); (f) LiAlH$_4$, Et$_2$O (84%); (g) *p*-TsCl, Et$_3$N, DCM (25%); (h) K^{13}CN, 18-crown-6, MeCN (82%); (i) 2 M NaOH, reflux (86%); (j) LDA, THF, −78°C (55%); and (k) BBr$_3$, DCM (68%)

equivalent alcohols was then required, but despite many attempts at optimization, using unlabelled materials, this was only achieved in 25% yield, despite claims of a higher yield in the literature.[23] The low yields were in part due to the formation of the di-tosylated derivative (12%) and presence of unreacted starting material (15%), but also due to difficulties in separating the three compounds by column chromatography. Nucleophilic substitution of the tosylate (**14**) with potassium [^{13}C]cyanide in DMF in the presence of 18-crown-6 and cyclization under basic conditions afforded the key lactone intermediate (**7**) in 70% over the two steps. The lactone contains two ^{13}C-atoms, which could be clearly observed in the ^{13}C NMR spectrum as enhanced signals at 38.9 and 176.9 ppm.

The original plan at this stage was to alkylate the enolate formed by treatment of (**7**) with another molecule of the benzyl bromide (**6**). However, in test reactions with unlabelled material this reaction would not take place. Eventually, the problem was solved by using the more reactive iodide derivative instead of the bromide. Therefore, some of the ^{13}C-labelled benzyl alcohol (**12**) was converted to the iodide (**15**), in quantitative yield, by treatment with phosphorus triiodide. Reaction of the iodide with the enolate of the lactone then gave the coupled product (**16**) in 55% yield and the [7,7′,9′-$^{13}C_3$]enterolactone (**5**) was obtained following cleavage of the two methyl ethers with boron tribromide. [7,7′,9-$^{13}C_3$]Enterodiol (**17**) was obtained by direct reduction of the enterolactone with lithium aluminium hydride in THF.

In the final products the three ^{13}C atoms could be observed by ^{13}C NMR spectroscopy. For enterolactone the three signals are at 35.4, 38.6 and 178.6 ppm, while for enterodiol only two signals are seen at 35.1 and 60.7 ppm as the molecule is now symmetrical. The rest of the spectral data for the ^{13}C-labelled mammalian lignans were in close agreement with literature data for the unlabelled compounds.[23,26,27] Both products were purified by reverse phase HPLC before being employed as internal standards.[13,26]

A modified synthetic route was employed for the ^{13}C-labelled matairesinol and secoisolariciresinol (Scheme 4). The major difference in this case was that the starting aryl iodide is not commercially available and had to be prepared. Monoiodination of guaiacol (**18**) was investigated and found to give iodination at the desired 4-position (**19**). Cyanation with ^{13}C-labelled cyanide



Scheme 4. Synthesis of [7,7′,9′-$^{13}C_3$]matairesinol(23) and [7,7′,9-$^{13}C_3$]secoisolariciresinol(24). Reactions and conditions: (a) NaOH, I_2, MeOH (78%); (b) BnCl, KOH, EtOH (71%); (c) K^{13}CN, $Ca(OH)_2$, $Pd(OAc)_2$, DMF (51%); (d) 2 M NaOH, reflux (91%); (e) $LiAlH_4$, Et_2O (91%); (f) TMSBr, Et_2O (77%); (g) KO^{t}Bu, $CH_2(CO_2Et)_2$, glyme (87%); (h) $LiAlH_4$, Et_2O (68%); (i) *p*-TsCl, Et_3N, DCM (30%); (j) K^{13}CN, 18-crown-6, MeCN (65%); (k) 2 M NaOH, reflux (57%); (l) LDA, THF, −78°C, then (20) (46%); (m) H_2, Pd/C (60%); and (n) $LiAlH_4$, THF (32%)

under our normal conditions followed by hydrolysis, reduction and bromination as before gave the benzyl alcohol (**20**). This labelled starting material could then be taken through the synthesis to provide the two lignans with three ^{13}C-atoms incorporated. In this series the key alkylation of the lactone intermediate (**21**) worked in reasonable yield using the benzyl bromide (**20**), meaning that it was not necessary to prepare the iodide as before.

Both labelled lignans were purified by reverse phase HPLC to produce high purity material suitable for use as an internal standard. As before the incorporation of the ^{13}C atoms was confirmed by the enhanced signals in the ^{13}C NMR spectra and by the expected increase in the molecular weight as determined by mass spectrometry. In the ^{13}C NMR spectrum for [7,7′,9′-$^{13}C_3$]matairesinol (**23**) the three ^{13}C atoms were observed as enhanced signals at 35.6, 39.1 and 181.9 ppm and for the [7,7′,9-$^{13}C_3$] secoisolariciresinol (**24**) two signals were observed at 36.3 and 61.4 ppm. The rest of the spectral data were in good agreement with literature data for the unlabelled plant lignans.[28]

All four of the ^{13}C-labelled lignans have now been successfully employed as internal standards in both GC-MS[29] and LC-MS[13] analysis. The use of these standards has resulting in improved reproducibility of analysis which has proven to be important in large scale epidemiological studies such as the European Investigation of Cancer and Nutrition (EPIC) large numbers of samples.[30]

General

Melting points were determined in open capillary tubes and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75.46 MHz, respectively, using Bruker Avance 500 and Varian Gemini 2000 spectrometers. The chloroform peaks (7.27 ppm for ^{1}H, 77.00 ppm for ^{13}C) were used as references. The EI and CI mass spectra were obtained on a VG Autospec mass spectrometer, and the ESI and APCI mass spectra on a Micromass LCT mass spectrometer. Diethyl ether and tetrahydrofuran were distilled over sodium and dichloromethane over calcium hydride. HPLC purification by was carried out on a Waters 600 Multisolvent Delivery System using a 150 × 4.60 mm Kingsorb 3μ C-18 column eluted with a 1:1 acetonitrile/water mixture at a flow rate of 0.3 ml/min.

*3-Methoxybenzo[^{13}C]nitrile (**11**)*

Potassium [^{13}C]cyanide (0.5 g, 7.4 mmol) was added to a solution of 3-iodoanisole (1.73 g, 7.4 mmol), palladium acetate (0.25 g, 1.13 mmol) and calcium hydroxide (0.28 g, 3.7 mmol) in dry DMF (50 ml) and the mixture was heated under reflux for 24 h. After cooling the DMF was removed at reduced

pressure. Water (50 ml) was added and the mixture extracted with diethyl ether (3×50 ml). The combined organic phases were dried ($MgSO_4$) and concentrated at reduced pressure. The crude product was purified by column chromatography (silica; light petroleum/diethyl ether (95:5)) to give the *title compound*[31] as a yellow oil (0.62 g, 64%), (HRMS found M^+, 134.0565. C_7 $^{13}CH_7NO$ requires 134.0561); ν_{max} (neat)/cm^{-1} 2179 (^{13}CN) and 1579 (C = C); δ_H (300 MHz; $CDCl_3$) 3.82 (3 H, s, OMe), 7.11–7.14 (2 H, m, H-2, 4), 7.23–7.25 (1 H, m, H-6) and 7.33–7.37 (1 H, m, H-5); δ_C (75 MHz; $CDCl_3$) 55.5 (OMe), 113.4 (d, $J = 56$, C-1), 116.9 (C-2), 118.8 (enhanced, ^{13}CN), 119.4 (C-4), 124.6 (C-6), 130.4 (C-5) and 159.8 (C-3); *m/z* (EI) 134 (M^+, 100%), 104 (55), 91 (43), 77 (13), 63 (17) and 50 (9).

3-Methoxy[carboxy-^{13}C]benzoic acid

A solution of 3-methoxybenzo[^{13}C]nitrile (0.55 g, 4.1 mmol) in 2 M sodium hydroxide solution (20 ml) was heated under reflux for 18 h. After cooling the mixture was acidified to pH 1 with 1 M hydrochloric acid. The mixture was extracted with diethyl ether (3×20 ml). The combined organic phases were dried ($MgSO_4$) and concentrated at reduced pressure to give the *title compound* as a white solid (0.55 g, 87%), mp 94–97°C (lit.[32] 98–101°C); (Found C, 62.5; H, 4.9. C_7 $^{13}CH_8O_4$ requires C, 62.7; H, 5.3%); (HRMS found M^+, 153.0515. C_7 $^{13}CH_8O_4$ requires 153.0506); ν_{max} (nujol)/cm^{-1} 1657 ($^{13}C = O$) and 1581 (C = C); δ_H (300 MHz; $CDCl_3$) 3.87 (3 H, s, OMe), 7.14–7.18 (1 H, m, H-4), 7.39 (1 H, t, $J = 7.8$, H-5), 7.61–7.64 (1 H, m, H-2) and 7.69–7.73 (1 H, m, H-6); δ_C (75 MHz; $CDCl_3$) 172.4 (enhanced, $^{13}CO_2H$); *m/z* (EI) 153 (M^+, 100%), 136 (26), 107 (13), 91 (12), 81 (11), 77 (14) and 63 (14).

*3-Methoxy[α-^{13}C]benzyl alcohol (**12**)*

A solution of 3-methoxy[*carboxy*-^{13}C]benzoic acid (0.55 g, 3.6 mmol) in dry diethyl ether (45 ml) was added to a suspension of lithium aluminium hydride (0.41 g, 10.7 mmol) in dry diethyl ether (20 ml) at 0°C and the mixture was stirred at room temperature for 48 h. The mixture was cooled to 0°C and was quenched with water (30 ml) and 2 M sodium hydroxide solution (30 ml) and silica was added. The mixture was filtered through celite and was washed with dichloromethane. The phases were separated and the organic phase was dried ($MgSO_4$) and concentrated at reduced pressure to give the *title compound* as a colourless oil (0.41 g, 83%); (HRMS found M^+, 139.0716. C_7 $^{13}CH_{10}O_2$ requires 139.0714); ν_{max}(neat)/cm^{-1} 3349 (OH) and 1602 (C = C); δ_H (300 MHz; $CDCl_3$) 3.81 (3 H, s, OMe), 4.67 (2 H, dd, $J = 142.8$, 5.7, $^{13}CH_2$), 6.82–6.85 (1 H, m, H-2), 6.90–6.95 (2 H, m, H-4, 6) and 7.26–7.30 (1 H, m, H-5); δ_C (75 MHz; $CDCl_3$) 65.3 (enhanced, $^{13}CH_2$); *m/z* (EI) 139 (M^+, 100%), 138 (27), 122 (14), 109 (63), 94 (21) and 77 (35).

*3-Methoxy[α-^{13}C]benzyl bromide (**13**)*

Phosphorus tribromide (7.2 ml) was added to a solution of 3-methoxy[α-^{13}C]-benzyl alcohol (9.53 g, 68.6 mmol) in dry diethyl ether (50 ml) at 0°C and the mixture was stirred at room temperature overnight. The reaction mixture was poured into ice water and extracted with diethyl ether (3 × 75 ml). The combined organic phases were dried ($MgSO_4$) and concentrated at reduced pressure to give the *title compound* as a colourless oil (12.6 g, 91%). This compound was used without further purification.

Diethyl 2-(3′-methoxy[α-^{13}C]benzyl)malonate

Potassium ^{t}butoxide (7.0 g, 62.3 mmol) was added to a solution of diethyl malonate (9.5 ml, 62.3 mmol) in 1,2-dimethoxyethane (140 ml) and the mixture was stirred at room temperature for 30 min 3-Methoxy[α-^{13}C]-benzyl bromide (12.6 g, 62.3 mmol) was added and the mixture was heated under reflux for 1 h. After cooling, water was added and the 1,2-dimethoxyethane was removed at reduced pressure. The residue was extracted with diethyl ether (3 × 100 ml) and ethyl acetate (3 × 100 ml). The combined organic phases were dried ($MgSO_4$) and concentrated at reduced pressure. The crude product was purified by column chromatography (silica; light petroleum/diethyl ether (4:1) elution) to give the *title compound* as a yellow oil (15.1 g, 86%); (HRMS found M^+, 281.1337. $C_{14}\,^{13}CH_{20}O_5$ requires 281.1344); δ_H (300 MHz; $CDCl_3$) 1.21 (3 H, t, $J = 7.2$, $CO_2CH_2CH_3$), 1.28 (3 H, t, $J = 7.2$, $CO_2CH_2CH_3$), 3.19 (2 H, dd, $J = 132$, 8.1, $^{13}CH_2$), 3.60–3.67 (1 H, m, CH), 3.77 (3 H, s, OMe), 4.11–4.24 (4 H, m, $CO_2CH_2CH_3$), 6.74–6.81 (3 H, m, H-2′,4′,6′) and 7.15–7.21 (1 H, m, H-5′); δ_C (75 MHz; $CDCl_3$) 34.6 (enhanced, $^{13}CH_2$); *m/z* (EI) 281 (M^+, 79%), 236 (12), 207 (100), 162 (69), 135 (19) and 122 (15).

2-(3′-Methoxy[α-^{13}C]benzyl)propane-1,3-diol

A Solution of diethyl 2-(3′-methoxy[α-^{13}C]benzyl)malonate (15.1 g, 53.6 mmol) in dry diethyl ether (45 ml) was added to a suspension of lithium aluminium hydride (5.3 g, 137.8 mmol) in dry diethyl ether (130 ml) at 0°C and the mixture was stirred at room temperature overnight. The reaction was cooled to 0°C and was quenched with 1 M sulphuric acid. The mixture was extracted with diethyl ether (3 × 120 ml) and the organic phases were dried ($MgSO_4$) and concentrated at reduced pressure to give the *title compound* as a white solid (8.85 g, 84%), mp 73–77°C (from diethyl ether) (lit.[23] 81–82°C); (Found C, 67.1; H, 8.4. $C_{10}\,^{13}CH_{16}O_3$ requires C, 67.0; H, 8.2%); (HRMS found M^+, 197.1139. $C_{10}\,^{13}CH_{16}O_3$ requires 197.1132.); δ_H (300 MHz; $CDCl_3$) 2.02–209 (1 H, m, CH), 2.59 (2 H, dd, $J = 130.5$, 7.5, $^{13}CH_2$), 3.62–3.75 (2 H, m, CH_2OH), 3.76–3.81 (5 H, m, OMe, CH_2OH), 6.73–6.79 (3 H, m,

H-2′,4′,6′) and 7.17–7.22 (1 H, m, H-5′); δ_C (75 MHz; $CDCl_3$) 34.2 (enhanced, $^{13}CH_2$), 55.2 (OMe), 65.6 (CH_2OH), 111.5 (C-4′), 114.9 (C-2′), 121.5 (C-6′) and 129.6 (C-5′); *m*/*z* (EI) 197 (M^+, 25%), 148 (10), 135 (8), 123 (100) and 92 (9).

*2-(3′-Methoxy[α-^{13}C]benzyl)-3-O-tosyl propanol (***14***)*

Triethylamine (4.2 ml, 31.3 mmol) was added to a solution of 2-(3′-methoxy[α-^{13}C]benzyl)propane-1,3-diol (8.83 g, 44.8 mmol) and *p*-toluenesulphonyl chloride (5.97 g, 31.3 mmol) in dry dichloromethane (200 ml) and the mixture was stirred at room temperature overnight. The mixture was washed with 1 M hydrochloric acid (2 × 120 ml), dried ($MgSO_4$) and concentrated at reduced pressure. The crude product was purified by column chromatography (silica; dichloromethane/ethyl acetate) to give the *title compound* as a yellow oil (3.99 g, 25%); (HRMS found M^+, 351.1212. C_{17} $^{13}CH_{22}SO_5$ requires 351.1221); δ_H (300 MHz; $CDCl_3$) 2.03–2.09 (1 H, m, CH), 2.59 (2 H, ddd, $J = 127.8$, 7.5, 3.3, $^{13}CH_2$), 2.43 (3 H, s, Me), 3.54–3.66 (2 H, m, CH_2OH), 3.81 (3 H, s, OMe), 3.98–4.10 (2 H, m, CH_2OTs), 6.64–6.74 (3 H, m, H-2′,4′,6′), 7.14 (1 H, t, $J = 8.4$, H-5′), 7.32 (2 H, d, $J = 7.8$, ArH) and 7.76 (2 H, d, $J = 7.8$, ArH); δ_C (75 MHz; $CDCl_3$) 21.6 (CH_3), 33.2 (enhanced, $^{13}CH_2$), 42.5 (d, $J = 34$, *C*H), 55.1 (OMe), 61.4 (CH_2OTs), 69.6 (CH_2OH), 111.8 (C-4′), 114.8 (C-2′), 121.4 (C-6′), 128.0 (CH), 130.0 (CH); *m*/*z* (EI) 351 (M^+, 27%), 281 (10), 179 (23), 161 (21), 148 (42), 135 (28), 123 (100) and 91 (52).

2-(3′-Methoxy[α-^{13}C]benzyl)-3-[cyano-^{13}C]cyanopropanol

Potassium [^{13}C]cyanide (0.76 g, 11.4 mmol) was added to a solution of 2-(3′-methoxy[α-^{13}C]benzyl)-3-*O*-tosyl propanol (3.99 g, 11.4 mmol) and 18-crown-6 (3.02 g, 11.4 mmol) in acetonitrile (120 ml) and the mixture was heated under reflux for 48 h. After cooling, the mixture was filtered through a pad of silica and washed with copious amounts of diethyl ether and ethyl acetate. The combined organic washings were concentrated at reduced pressure. The crude product was purified by column chromatography (silica; dichloromethane/ethyl acetate (4:1)) to give the *title compound* as a brown oil (1.94 g, 82%); (HRMS found M^+, 207.1162. C_{10} $^{13}C_2H_{15}NO_2$ requires 207.1169.); δ_H (300 MHz; $CDCl_3$) 2.18–2.25 (1 H, m, CH), 2.40–2.50 (2 H, m, C_{10} $^{13}C_2H_{15}NO_2$), 2.72 (2 H, dddd, $J = 127.5$, 38.7, 13.8, 6.6, $^{13}CH_2$), 3.58–3.78 (2 H, m, CH_2OH), 3.79 (3 H, s, OMe), 6.73–6.81 (3 H, m, H-2′,4′,6′) and 7.23 (1 H, t, $J = 8.1$, H-5′); δ_C (75 MHz; $CDCl_3$) 36.4 (enhanced, $^{13}CH_2$), 55.2 (OMe), 63.5 (CH_2OH), 112.0 (C-4′), 114.8 (C-2′), 118.7 (enhanced, ^{13}CN) and 121.5 (C-6′); *m*/*z* (EI) 207 (M^+, 20%), 148 (6), 123 (100), 122 (32), 108 (9), 92 (18) and 79 (9).

4-(3′-Methoxy[α-^{13}C]benzyl)dihydro-2(3H)-[carbonyl-^{13}C]furanone (**7**)

A solution of 2-(3′-methoxy[α-^{13}C]benzyl)-3-[*cyano*-^{13}C]cyanopropanol (1.94 g, 9.38 mmol) in 2 M sodium hydroxide (65 ml) were heated under reflux for 24 h. After cooling, the mixture was acidified to pH 1 with 6 M hydrochloric acid. The acidic aqueous phase was extracted with diethyl ether (2 × 75 ml). The combined organic phases were dried ($MgSO_4$) and concentrated at reduced pressure. The crude product was purified by column chromatography (silica; dichloromethane) to give the *title compound* as a yellow oil (1.67 g, 86%); (HRMS found M^+, 208.1016. C_{10} $^{13}C_2H_{14}O_3$ requires 208.1010); ν_{max} (neat)/cm^{-1} 1722 (C = O) and 1600 (C = C); δ_H (300 MHz; $CDCl_3$) 2.24–2.34 (1 H, m, H-4), 2.51–2.99 (2 H, m, $^{13}CH_2$), 2.56–2.66 (1 H, m, H-3a), 2.83–2.94 (1 H, m, H-3b), 3.80 (3 H, s, OMe), 4.00–4.13 (1 H, m, H-5a), 4.29–4.37 (1 H, m, H-5b), 6.68–6.80 (3 H, m, H-2′,4′,6′) and 7.23 (1 H, t, J = 8.1, H-5′); δ_C (75 MHz; $CDCl_3$) 38.9 (enhanced, $^{13}CH_2$), 55.2 (OMe), 72.6 (CH_2O), 111.9 (C-4′), 114.7 (C-2′), 121.0 (C-6′), 129.9 (C-5′), and 176.9 (enhanced, ^{13}C = O); m/z (EI) 208 (M^+, 39%), 148 (6), 123 (100), 122 (40), 108 (8), 92 (18), 84 (12) and 78 (10).

3-Methoxy[α-^{13}C]benzyl iodide (**15**)

Phosphorus triiodide (8.88 g, 21.5 mmol) was added to a solution of 3-methoxy[α-^{13}C]benzyl alcohol (1.76 g, 12.7 mmol) in dry diethyl ether (40 ml) at 0°C and the mixture was stirred at room temperature overnight. The reaction mixture was poured into ice water and the mixture was extracted with diethyl ether (3 × 75 ml). The combined organic phases were dried ($MgSO_4$) and concentrated at reduced pressure to give the *title compound* as a brown solid (2.9 g, 92%), which was used without further purification. δ_H (300 MHz; $CDCl_3$) 3.79 (3 H, s, OMe), 4.41 (2 H, d, J = 152.1, $^{13}CH_2I$), 6.78 (1 H, dd, J = 8.1, 2.4, H-4), 6.88–6.91 (1 H, m, H-2), 6.96 (1 H, dd, J = 8.1, 2.4, H-6) and 7.20 (1 H, t, J = 8.1, H-5).

3, 4-*bis-(3′-Methoxy[α-^{13}C]benzyl)dihydro-2(3H)-[carbonyl-^{13}C]furanone* (**16**)

This reaction must be carried out under a continuous flow of argon gas. All glassware and syringes must be oven dried overnight and THF must be freshly dried. LDA (2 M in hexane/THF/ethylbenzene; 3.1 ml, 6.08 mmol) was added to a solution a solution of 4-(3′-methoxy[α-^{13}C]benzyl)dihydro-2(3 *H*)-[*carbonyl*-^{13}C]furanone (0.96 g, 4.62 mmol) in dry THF (22 ml) at −78°C and the mixture was left to stir for 3 h. A precooled solution of 3-methoxy[α-^{13}C]benzyl iodide (1.53 g, 6.2 mmol) (20 min in an ice bath) in dry THF (14 ml) was added and the solution was stirred at −78°C for 4 h and allowed to warm to room temperature overnight. The reaction was quenched with brine and the

mixture was extracted with diethyl ether (3×50 ml). The combined organic phases were dried ($MgSO_4$) and concentrated at reduced pressure. The crude product was purified by column chromatography (silica; light petroleum/diethyl ether) to give the *title compound* as a yellow oil (0.85 g, 55%); (Found M^+, 329.1625. C_{17} $^{13}C_3H_{22}O_4$ requires 329.1618); ν_{max} (neat)/cm^{-1} 1724 (C = O) and 1601 (C = C); δ_H (300 MHz; $CDCl_3$) 2.48–2.86 (4 H, m, CH, $^{13}CH_2$), 3.07–3.29 (2 H, m, $^{13}CH_2$), 3.75 (3 H, s, OMe), 3.79 (3 H, s, OMe), 3.81 (1 H, m, H-5a), 4.06–4.13 (1 H, m, H-5b), 6.50–6.52 (1 H, m, H-2′), 6.56–6.59 (1 H, m, H-2′), 6.70–6.78 (4 H, m, H-4′, 6′), 7.16 (1 H, t, $J = 7.8$, H-5′) and 7.20 (1 H, t, $J = 7.8$, H-5′); δ_C (75 MHz; $CDCl_3$) 35.3 (enhanced, $^{13}CH_2$), 38.5 (enhanced, $^{13}CH_2$) and 178.4 (enhanced, ^{13}C=O); *m/z* (EI) 329 (M^+, 64%), 279 (5), 207 (15), 160 (6), 149 (15), 123 (100), 122 (38), 92 (17) and 69 (9).

*[7,7′,9′- $^{13}C_3$]Enterolactone (**5**)*

Boron tribromide (1.0 M in dichloromethane; 21 ml, 21 mmol) was added to a solution of 3,4-*bis*-(3′-methoxy[α-^{13}C]benzyl)dihydro-2(3 *H*)-[*carbonyl*-^{13}C]furanone (0.85 g, 2.59 mmol) in dry dichloromethane (80 ml) and the mixture was stirred for 2.5 h at room temperature. Water (50 ml) and diethyl ether (50 ml) were added and the phases were separated. The aqueous phase was extracted with diethyl ether (3×75 ml). The combined organic phases were dried ($MgSO_4$) and concentrated at reduced pressure. The crude product was purified by column chromatography (silica; dichloromethane/ethyl acetate (3:1)) to give the *title compound* as a white solid (0.53 g, 68%), which was further purified by reverse phase HPLC. (Found C, 69.88; H, 5.63. C_{15} $^{13}C_3H_{18}O_4.0.5H_2O$ requires C, 69.66; H, 5.85%); (HRMS found M^+., 301.1315. C_{15} $^{13}C_3H_{18}O_4$ requires 301.1305); ν_{max} (nujol)/cm^{-1} 3392 (OH), 1703 (^{13}C = O) and 1589 (C = C); δ_H (300 MHz; d_6-acetone) 2.42–2.58 (2 H, m, H-8 and 8′), 2.64–3.20 (4 H, m, $^{13}CH_2$), 3.83–3.91 (1 H, m, H-9a), 3.99–4.08 (1 H, m, H-9b), 6.58–6.79 (6 H, m, H-2,4,6, 2′,4′ and 6′), 7.08 (1 H, t, $J = 7.8$, H-5 or H-5′), 7.12 (1 H, t, $J = 7.8$, H-5 or H-5′) and 8.27 (2 H, br, ArOH); δ_C (75 MHz; d_6-acetone) 35.4 (enhanced, $^{13}CH_2$), 38.6 (enhanced, $^{13}CH_2$), 71.4 (C-9), 114.4, 114.6 (C-4 and 4′), 116.3, 117.0 (C-2 and C-2′), 120.5, 121.4 (C-6 and C-6′), 130.2, 130.3 (C-5 and C-5′), 140.9 (C-1 and C-1′), 158.3 (C-3 and C-3′) and 178.6 (enhanced, ^{13}C = O); *m/z* (EI) 301 (M^+, 52%), 193 (24), 134 (26), 109 (100) and 108 (60).

*[7,7′,9- $^{13}C_3$]Enterodiol (**17**)*

A solution of [7,7′,9′-$^{13}C_3$]enterolactone (150 mg, 0.5 mmol) in dry THF (9 ml) was added to a suspension of lithium aluminium hydride (103 mg, 2.68 mmol) in dry THF (12 ml) at 0°C and the reaction was stirred at room temperature overnight. The reaction was quenched at 0°C with 1 M sulphuric acid and

extracted with diethyl ether (4×40 ml). The combined organic phases were dried ($MgSO_4$) and concentrated at reduced pressure. The crude product was purified by column chromatography (silica; dichloromethane/ethyl acetate (1:1)) to give the *title compound* as a white solid (59 mg, 39%), which was further purified by reverse phase HPLC. mp 174–176°C (lit.[23] 171–173°C); (Found C, 69.92; H, 7.18. C_{15} $^{13}C_3H_{22}O_4.0.1H_2O$ requires C, 70.4; H, 7.22%); ν_{max} (nujol)/cm^{-1} 3410 (OH), and 1588 (C = C); δ_H (300 MHz; d_4-MeOH) 2.06–2.11 (2 H, m, H-8 and 8′), 2.74 (4 H, dd, $J = 126.3$, 5.1, $^{13}CH_2$), 3.7 (2 H, dm, $J_{13C,1H} = 140$, $^{13}CH_2OH$), 3.60–3.80 (2 H, m, CH_2OH), 6.67–6.74 (6 H, m, H-2, 4, 6, 2′, 4′ and 6′) and 7.15 (2 H, t, $J = 7.5$, H-5 and H-5′); δ_C (75 MHz; d_4-MeOH) 35.1 (enhanced, $^{13}CH_2$), 39.2–40.2 (CH), 60.7 (enhanced, $^{13}CH_2OH$), 112.6 (C-4 and C-4′), 115.9 (C-2 and C-2′), 120.3 (C-6 and C-6′), 129.1 (C-5 and C-5′), 141.0 (d, $J = 8$, C-1 and C-1′) and 157.2 (C-3 and C-3′); *m/z* (EI) 305 (2%), 256 (9), 108 (100), and 107 (60).

4-Hydroxy-3-methoxyiodobenzene

Iodine (6.8 g, 26.8 mmol) was added portionwise to a solution of guaiacol (3.3 g, 26.8 mmol) and sodium hydroxide (2.01 g) in methanol (70 ml) keeping the temperature between −2 and 1°C. The mixture was stirred at 0°C for 1.5 h before being acidified with 5% hydrochloric acid solution to pH 2. The solvent was removed at reduced pressure and the residue was dissolved in diethyl ether. The aqueous phase was extracted with diethyl ether (3×100 ml). The organic phase was washed with 10% sodium thiosulphate solution (2×75 ml), dried ($MgSO_4$) and concentrated at reduced pressure to give the *title compound* as a brown oil (5.22 g, 78%); δ_H (300 MHz; $CDCl_3$) 3.85 (3 H, s, OMe), 5.55 (1 H, s, OH), 6.70 (1 H, d, $J = 8.2$, H-5), 6.80–6.95 (1 H, m, H-2) and 7.10–7.18 (1 H, m, H-6).

*4-Benzyloxy-3-methoxyiodobenzene (***19***)*

Benzyl chloride (3.7 ml, 32.4 mmol) was added to a solution of 4-hydroxy-3-methoxyiodobenzene (7.23 g, 29 mmol) and potassium hydroxide (1.8 g, 32.4 mmol) in ethanol (140 ml) and the mixture was heated under reflux overnight. After cooling the solvent was concentrated at reduced pressure. Water (100 ml) was added and the mixture was extracted with ethyl acetate (3×100 ml). The combined organic phases were washed with 2 M potassium hydroxide solution (5×100 ml), dried ($MgSO_4$) and concentrated *at reduced pressure*. The crude product was purified by column chromatography (silica; light petroleum/ethyl acetate (2:1)) to give the *title compound* as a yellow oil (6.98 g, 71%); ν_{max} (neat)/cm^{-1} 1582 (C = C); δ_H (300 MHz; $CDCl_3$) 3.86 (3 H, s, OMe), 5.11 (2 H, s, OCH_2), 6.62 (1 H, d, $J = 8.7$, H-5), 6.87–6.91 (1 H, m, H-2), 7.13–7.17 (1 H, m, H-6) and 7.29–7.43 (5 H, m, ArH); δ_C (75 MHz;

$CDCl_3$) 56.2 (OMe), 71.1 (OCH_2), 83.2 (C-1), 112.1 (C-5), 114.4 (C-2), 121.1 (C-6), 127.4 (CH), 128.1 (CH), 128.7 (CH), 136.8 (C-1'), 148.4 (C-4) and 150.7 (C-3); *m/z* (EI) 340 (M^+, 10%), 214 (16), 92 (13), 91 (100) and 65 (12).

4-Benzyloxy-3-methoxybenzo[^{13}C]nitrile

Potassium [^{13}C]cyanide (1.43 g, 21.4 mmol) was added to a solution of 4-benzyloxy-3-methoxyiodobenzene (7.26 g, 21.4 mmol), palladium acetate (0.73 g, 3.3 mmol) and calcium hydroxide (0.82 g, 10.7 mmol) in dry DMF (150 ml) and the mixture was heated under reflux for 15 h. After cooling the solvent was removed at reduced pressure. Water (120 ml) was added and the mixture was extracted with ethyl acetate (3×150 ml). The combined organic phases were dried ($MgSO_4$) and concentrated at reduced pressure. The crude product was purified by column chromatography (silica; light petroleum/ethyl acetate (9:1)) to give the *title compound* as a pale yellow solid (2.61 g, 51%), mp 81–83°C (lit.[33] 73–74°C); (Found C, 74.8; H, 5.3; N, 5.3 C_{14} $^{13}CH_{13}NO_4$ requires C, 74.8; H, 5.45; N, 5.8%); (HRMS found M^+, 240.0987. C_{14} $^{13}CH_{13}NO_4$ requires 240.0980); ν_{max} (nujol)/cm^{-1} 2169 (^{13}CN); δ_H (300 MHz; $CDCl_3$) 3.89 (3 H, s, OMe), 5.19 (2 H, s, OCH_2), 6.89 (1 H, d, $J = 8.1$, H-5), 7.08 (1 H, dd, $J = 5.4$, 1.8, H-2), 7.17–7.19 (1 H, m, H-6) and 7.31–7.39 (5 H, m, ArH); δ_C (75 MHz; $CDCl_3$) 118.8 (enhanced, ^{13}CN); *m/z* (EI) 240 (M^+, 9%), 91 (100), 65 (13) and 59 (6).

4-Benzyloxy-3-methoxy[carbonyl-^{13}C]benzoic acid

A solution of 4-benzyloxy-3-methoxybenzo[^{13}C]nitrile (1.4 g, 5.8 mmol) in 2 M sodium hydroxide solution (20 ml) was heated under reflux overnight. After cooling the mixture was acidified to pH 1 with 6 M hydrochloric acid. The acidic aqueous was extracted with diethyl ether (3×50 ml). The combined organic phases were dried ($MgSO_4$) and concentrated at reduced pressure to give the *title compound* as a yellow solid (1.38 g, 91%); mp 161–164°C (lit.[34] 168–169°C) (Found C, 69.6; H, 5.2. C_{14} $^{13}CH_{14}O_4$ requires C, 69.5; H, 5.4%); (HRMS found M^+, 259.0921. C_{14} $^{13}CH_{14}O_4$ requires 259.0925); δ_H (300 MHz; $CDCl_3$) 3.94 (3 H, s, OMe), 5.29 (2 H, s, OCH_2), 6.92 (1 H, d, $J = 8.7$, H-5), 7.31–7.44 (5 H, m, ArH), 7.59–7.62 (1 H, m, H-6) and 7.66–7.69 (1 H, m, H-2); δ_C (75 MHz; $CDCl_3$) 171.0 (enhanced, $^{13}CO_2H$); *m/z* (EI) 259 (M^+, 33%), 91 (100) and 65 (13).

4-Benzyloxy-3-methoxy[α-^{13}C]benzyl alcohol

A solution of 4-benzyloxy-3-methoxy[*carboxy*-^{13}C]benzoic acid (1.38 g, 5.3 mmol) in dry diethyl ether (50 ml) was added to a suspension of lithium aluminium hydride (0.61 g, 16 mmol) in dry diethyl ether (50 ml) at 0°C and the mixture was stirred for 48 h at room temperature. The mixture was cooled to

0°C and was quenched with water (45 ml) and 2 M sodium hydroxide solution (45 ml) and silica was added. The mixture was filtered through celite and was washed with dichloromethane. The phases were separated and the organic phase was dried ($MgSO_4$) and concentrated at reduced pressure. The crude product was recrystallized from diethyl ether/light petroleum to give the *title compound* as a white solid (1.19 g, 91%); mp 70–72°C (from diethyl ether) (lit.[35] 72–73°C); (Found C, 73.5; H, 6.7. $C_{14}\ ^{13}CH_{16}O_3$ requires C, 73.5; H, 6.6%); (HRMS found M^+, 245.1140. $C_{14}\ ^{13}CH_{16}O_3$ requires 245.1132); δ_H (300 MHz; $CDCl_3$) 3.89 (3 H, s, OMe), 4.60 (2 H, dd, $J = 142.8$, 5.7, $^{13}CH_2OH$), 5.15 (2 H, s, OCH_2), 6.78–6.86 (2 H, m, H-5, 6), 6.94 (1 H, d, $J = 3.3$, H-2) and 7.28–7.44 (5 H, m, ArH); δ_C (75 MHz; $CDCl_3$) 65.3 (enhanced, $^{13}CH_2$); *m/z* (EI) 245 (M^+, 29%), 91 (100), 84 (14), 65 (10) and 49 (17).

*4-Benzyloxy-3-methoxy[α-^{13}C]benzyl bromide (**20**)*

Trimethylsilyl bromide (0.6 ml, 7.4 mmol) was added to a solution of 4-benzyloxy-3-methoxy[α-^{13}C]benzyl alcohol (1.2 g, 4.9 mmol) in dry diethyl ether (30 ml) and the mixture was stirred at room temperature for 2.5 h. The reaction was quenched with water (10 ml) and was extracted with diethyl ether (2×15 ml). The combined organic phases were washed with water (15 ml), dried ($MgSO_4$) and concentrated at reduced pressure to give the *title compound* as a white solid (1.16 g, 77%) which was used without further purification. δ_H (300 MHz; $CDCl_3$) 3.90 (3 H, s, OMe), 4.48 (2 H, d, $J = 153.3$, $^{13}CH_2Br$), 5.19 (2 H, s, OCH_2), 6.81 (1 H, d, $J = 7.5$, H-5), 6.52–6.87 (1 H, m, H-6), 6.93 (1 H, dd, $J = 5.4$, 1.8, H-2) and 7.29–7.43 (5 H, m, ArH).

Diethyl 2-(4′-benzyloxy-3′-methoxy[α-^{13}C]benzyl)malonate

Potassium ^{t}butoxide (0.42 g, 3.77 mmol) was added to a solution of diethyl malonate (0.6 ml, 3.77 mmol) in 1,2-dimethoxyethane (40 ml) and the mixture was stirred at room temperature for 30 min 4-Benzyloxy-3-methoxy[α-^{13}C]-benzyl bromide (1.16 g, 3.77 mmol) was added and the mixture was heated under reflux for 1.5 h. After cooling, water (40 ml) was added and the mixture was extracted with diethyl ether (3×50 ml) and ethyl acetate (3×50 ml). The combined organic phases were dried ($MgSO_4$) and concentrated at reduced pressure. The residue was purified by column chromatography (silica; light petroleum/diethyl ether (2:1)) to give the *title compound* as a colourless oil (1.26 g, 87%); (HRMS found M^+, 387.1762. $C_{21}\ ^{13}CH_{26}O_6$ requires 387.1771); δ_H (300 MHz; $CDCl_3$) 1.20 (3 H, t, $J = 7.2$, $CO_2CH_2CH_3$), 1.27 (3 H, t, $J = 7.2$, $CO_2CH_2CH_3$), 3.13 (2 H, dd, $J = 131.7$, 7.8, $^{13}CH_2$), 3.56–3.63 (1 H, m, CH), 3.85 (3 H, s, OMe), 4.11–4.24 (4 H, m, $CO_2CH_2CH_3$), 5.10 (2 H, s, OCH_2), 6.67–6.79 (3 H, m, H-2′, 5′, 6′) and 7.28–7.42 (5 H, m, ArH);

δ_C (75 MHz; $CDCl_3$) 14.0 ($CO_2CH_2CH_3$), 34.3 (enhanced, $^{13}CH_2$), 61.5 ($CO_2CH_2CH_3$), (OCH_2), 127.4 (CH), 128.6 (CH); *m/z* (EI) 387 (M^+, 81%), 296 (31), 222 (59), 133 (60), 115 (100) and 91 (83).

2-(4′-Benzyloxy-3′-methoxy[α-^{13}C]benzyl)propane-1,3-diol

A Solution of diethyl 2-(4′-benzyloxy-3′-methoxy[α-^{13}C]benzyl)malonate (1.26 g, 3.3 mmol) in dry diethyl ether (5 ml) was added to a suspension of lithium aluminium hydride (0.32 g, 8.4 mmol) in dry diethyl ether (10 ml) at 0°C and the mixture was stirred at room temperature for 15 h. The reaction was quenched at 0°C with 1 M H_2SO_4. The mixture was extracted with diethyl ether (3 × 20 ml), dried ($MgSO_4$) and concentrated at reduced pressure. The crude product was recrystallized from diethyl ether to give the *title compound* as a white solid (0.67 g, 68%); mp 82–85°C (from diethyl ether); (Found C, 71.2; H, 7.5. C_{17} $^{13}CH_{22}O_4$ requires C, 71.3; H, 7.3%); (HRMS found M^+, 303.1562. C_{17} $^{13}CH_{22}O_4$ requires 303.1551); δ_H (300 MHz; $CDCl_3$) 1.91 (2 H, br, OH), 2.01–2.02 (1 H, m, CH), 2.55 (2 H, dd, J = 126.6, 7.5, $^{13}CH_2$), 3.63–3.69 (2 H, m, CH_2OH), 3.76–3.79 (2 H, m, CH_2OH), 3.82 (3 H, s, OMe), 5.11 (2 H, s, OCH_2), 6.64–6.74 (2 H, m, H-2′, 6′), 6.79 (1 H, d, J = 8.1, H-5′) and 7.27–7.44 (5 H, m, ArH); δ_C (75 MHz; $CDCl_3$) 33.9 (enhanced, $^{13}CH_2$); *m/z* (EI) 303 (M^+, 5%), 194 (13), 150 (13), 92 (12) and 91 (100).

2-(4′-Benzyloxy-3′-methoxy[α-^{13}C]benzyl)-3-O-tosyl-propanol

Triethylamine (0.4 ml, 2.98 mmol) was added to a solution of 2-(4′-benzyloxy-3′-methoxy[α-^{13}C]benzyl)propane-1,3-diol (1.3 g, 4.3 mmol) and *p*-toluenesulphonyl chloride (0.57 g, 2.98 mmol) in dry dichloromethane (40 ml) and the mixture was stirred for 24 h at room temperature. The mixture was washed with 1 M hydrochloric acid (2 × 30 ml). The organic phase was dried ($MgSO_4$) and concentrated at reduced pressure. The crude product was purified by column chromatography (silica; dichloromethane/ethyl acetate) to give the *title compound* as a yellow oil (0.59 g, 30%); (HRMS found M^+, 457.1630. C_{26} $^{13}CH_{28}O_6S$ requires 457.1640); δ_H (300 MHz; $CDCl_3$) 2.06–2.08 (1 H, m, CH), 2.44 (3H, s, $ArCH_3$), 2.55 (2 H, ddd, J = 127.5, 7.5, 3, $^{13}CH_2$), 3.52–3.67 (2 H, m, CH_2OH), 3.85 (3 H, s, OMe), 3.97–4.13 (2 H, m, CH_2OTs), 5.10 (2 H, s, OCH_2Ph), 6.52–6.56 (1 H, m, H-6′), 6.66–6.68 (1 H, m, H-2′), 6.75 (1 H, d, J = 8.1, H-5′), 7.29–7.44 (7 H, m, ArH) and 7.77 (2 H, d, J = 6.6, ArH); δ_C (75 MHz; $CDCl_3$) 33.2 (enhanced, $^{13}CH_2$); *m/z* (EI) 457 (M^+, 12%), 273 (8), 164 (12), 150 (9), 131 (11), 91 (100) and 69 (19).

2-(4′-Benzyloxy-3′-methoxy[α-^{13}C]benzyl)-3-[cyano-^{13}C]cyanopropanol

Potassium [^{13}C]cyanide (38 mg, 0.56 mmol) was added to a solution of 2-(4′-benzyloxy-3′-methoxy[α-^{13}C]benzyl)-3-*O*-tosyl-propanol (0.26 g, 0.56 mmol)

and 18-crown-6 (0.15 g, 0.56 mmol) in acetonitrile (20 ml) and the mixture was heated under reflux for 48 h. After cooling the mixture was filtered through a pad of silica, washed with copious amounts of diethyl ether and ethyl acetate and concentrated at reduced pressure. The crude product was purified by column chromatography (silica; light petroleum/diethyl ether (1:1)) to give the *title compound* as a yellow oil (0.11 g, 65%); (HRMS found M^+, 313.1593. $C_{17}\ ^{13}C_2H_{21}NO_3$ requires 313.1588); δ_H (300 MHz; $CDCl_3$) 2.11–2.19 (1 H, m, CH), 2.31–2.44 (2 H, m, $CH_2\ ^{13}CN$), 2.46–2.98 (2 H, m, $^{13}CH_2$), 3.60 (1 H, dt, $J = 10.5, 4.1$, CH_2OH), 3.73 (1 H, ddd, $J = 10.5, 7.1, 2.6$, CH_2OH), 3.87 (3 H, s, OMe), 5.12 (2 H, s, OCH_2), 6.65–6.73 (2 H, m, H-2′, 6′), 6.82 (1 H, d, $J = 8.4$, H-5′) and 7.29–7.44 (5 H, m, ArH); δ_C (75 MHz; $CDCl_3$) 35.9 (enhanced, $^{13}CH_2$) and 118.8 (enhanced, ^{13}CN); *m/z* (EI) 313 (M^+, 41%), 222 (7), 204 (5), 164 (10), 132 (5) and 91 (100).

*4-(4′-Benzyloxy-3′-methoxy[α-^{13}C]benzyl)dihydro-2(3H)[carbonyl-^{13}C]furanone (***21***)*

A solution of 2-(4′-benzyloxy-3′-methoxy-[^{13}C]benzyl)-3-cyanopropanol (0.27 g, 0.87 mmol) in 2 M sodium hydroxide solution (10 ml) was heated under reflux for 24 h. After cooling the mixture was acidified to pH 1 with 6 M hydrochloric acid. The acidic aqueous layer was extracted with diethyl ether. The combined organic phases were dried ($MgSO_4$) and concentrated at reduced pressure. The crude product was purified by column chromatography (silica; light petroleum/ethyl acetate (1:1) elution) to give the *title compound* as a white oily solid (0.16 g, 57%); (HRMS found M^+, 314.1437. $C_{17}\ ^{13}C_2H_{20}O_4$ requires 314.1428); δ_H (300 MHz, $CDCl_3$) 2.22–2.82 (1 H, m, H-3a), 2.52–2.64 (1 H, m, H-3b), 2.69 (2 H, ddd, $J = 127.8, 8.1, 3.3$, $^{13}CH_2$), 2.78–2.84 (1 H, m, H-4), 3.86 (3 H, s, OMe), 3.84–4.05 (1 H, m, H-5a), 4.27–4.34 (1 H, m, H-5b), 5.12 (2 H, s, OCH_2), 6.59–6.68 (2 H, m, H-2′, 6′), 6.82 (1 H, d, $J = 8.1$, H-5′) and 7.29–7.44 (5 H, m, ArH); δ_C (75 MHz; $CDCl_3$) 38.6 (enhanced, $^{13}CH_2$) and 176.9 (enhanced, $^{13}C{=}O$); *m/z* (EI) 314 (M^+, 16%), 91 (100) and 65 (6).

*3,4-bis-(4′-Benzyloxy-3′-methoxy[α-^{13}C]benzyl)dihydro-2(3H)-[carbonyl-^{13}C]furanone (***22***)*

This reaction must be carried out under a flow of argon gas, balloons do not work. All glassware and syringes must be oven dried and THF must be freshly dried. LDA (2.0 M in hexane/THF/ethylbenzene; 1.32 ml, 2.63 mmol) was added to a solution of 4-(4′-benzyloxy-3′-methoxy[α-^{13}C]benzyl)dihydro-2(3*H*)-[*carbonyl*-^{13}C]furanone (0.66 g, 2.1 mmol) in dry THF (15 ml) at –78°C and the mixture was stirred for 2.5 h. A precooled solution of 4-benzyloxy-3-methoxy[α-^{13}C]benzyl bromide (0.94 g, 3.04 mmol) (20 min in an ice bath) in dry THF (6 ml) was added and the mixture was stirred at −78°C for 5 h. The reaction was quenched at −78°C with brine (15 ml) and

allowed to warm to room temperature. The mixture was extracted with diethyl ether (3×50 ml). The combined organic phases were dried ($MgSO_4$) and concentrated at reduced pressure. The crude product was purified by column chromatography (silica; light petroleum/ethyl acetate (2:1) to give the *title compound* as a colourless oil (0.52 g, 46%); (HRMS found M^+, 541.2467. C_{31} $^{13}C_3H_{34}O_6$ requires 541.2456); δ_H (300 MHz; $CDCl_3$) 2.33–2.61 (2 H, m, H-3, 4), 2.53 (2 H, ddd, $J = 163.8$, 13.5, 6.3, $^{13}CH_2$), 2.68–3.15 (2 H, m, $^{13}CH_2$), 3.80 (3 H, s, OMe), 3.82 (3 H, s, OMe), 3.84–3.88 (1 H, m, H-5a), 4.06–4.14 (1 H, m, H-5b), 5.11 (4 H, s, OCH_2), 6.46–6.57 (3 H, m, H-2′, 6′), 6.70 (1 H, s, H-2′), 6.76 (2 H, t, $J = 7.2$, H-5′) and 7.24–7.42 (10 H, m, ArH); δ_C (75 MHz; $CDCl_3$) 34.5 (enhanced, $^{13}CH_2$), 38.0 (enhanced, $^{13}CH_2$) and 178.7 (enhanced, $^{13}C=O$); *m/z* (EI) 541 (M^+, 6%), 138 (15), 91 (100) and 65 (8).

*[7,7′,9′-$^{13}C_3$]Matairesinol (***23***)*

To a solution of 3,4-*bis*-(4′-benzyloxy-3′-methoxy[α-^{13}C]benzyl)dihydro-2(3*H*)-[*carbonyl*-^{13}C]furanone (0.28 g, 0.51 mmol) in ethyl acetate (15 ml) was added to 5% palladium-on-carbon (14 mg) and the mixture was stirred under a hydrogen atmosphere for 48 h. The mixture was filtered through celite and washed with ethyl acetate. The organic phase was concentrated at reduced pressure. The crude product was purified by column chromatography (silica; dichloromethane/ethyl acetate) to give the *title compound* as a white solid (0.11 g, 60%), which was further purified by reverse phase HPLC. (Found C, 65.9; H, 5.9. C_{17} $^{13}C_3H_{22}O_6.0.1H_2O$ requires C, 66.1; H, 6.2%); (HRMS found M^+., 361.1505. C_{17} $^{13}C_3H_{22}O_6$ requires 361.1517); ν_{max} (nujol)/cm^{-1} 3390 (OH), 1702 ($^{13}C=O$) and 1590 (C=C); δ_H (300 MHz; d_4-MeOH) 2.72 (2 H, dd, $J = 79.8$, 5.1, H-8), 2.67–2.71 (1 H, m, H-8′), 2.81–2.88 (1 H, m, H-3), 3.02 (1 H, dddd, $J_{CH} = 77.1$, $J_{3''a-3''b} = 8.4$, $J_{3''-3} = 4.2$, $J_{3''-4} = 1.8$, H-3″a), 3.07 (1 H, dt, $J_{CH} = 77.1$, $J_{3''a-3''b} = 8.4$, $J_{3''-3=3''-4} = 3.6$, H-3″b), 3.96 (3 H, s, OMe), 3.97 (3 H, s, OMe), 4.08–4.13 (1 H, m, H-5), 4.32–4.37 (1 H, m, H-5), 6.68–6.71 (1 H, m, H-6′), 6.74–6.75 (1 H, m, H-2′), 6.76–6.78 (1 H, m, H-6′), 6.85–6.86 (1 H, m, H-2′), 6.87 (1 H, d, $J = 5.1$, H-5′) and 6.89 (1 H, dd, $J = 8, 10$, H-5′); δ_C (75 MHz; d_4-MeOH) 35.6 (enhanced, $^{13}CH_2$-7′), 39.1 (enhanced, $^{13}CH_2$-7), 42.8 (d, $J = 35$, C-8), 56.6 (OMe), 73.2 (C-9), 113.5, 114.1 (C-2 and C-2′), 116.3, 116.4 (C-5 and C-5′), 122.5, 123.3 (C-6 and C-6′), 130.7, 131.3 (C-1 and C-1′), 146.5, 146.6 (C-4 and C-4′), 149.3 (C-3 and C-3′) and 181.9 (enhanced, $^{13}C=O$); *m/z* (EI) 361 (M^+, 27%), 277 (7), 138 (100), 123 (17) and 95 (18).

2, 3-bis-(4′-Benzyloxy-3′-methoxy-[α-^{13}C]benzyl)[1-^{13}C]butan-1-4-diol

A solution of 3,4-*bis*-(4′-benzyloxy-3′-methoxy[α-^{13}C]benzyl)dihydro-2(3*H*)-[*carbonyl*-^{13}C]furanone (0.52 g, 0.96 mmol) in dry THF (21 ml) was added to a

suspension of lithium aluminium hydride (0.18 g, 4.85 mmol) in dry THF (21 ml) at 0°C and the mixture was stirred at room temperature overnight. The mixture was cooled to 0°C and was quenched with water (8 ml) and 2 M sodium hydroxide (8 ml) and silica was added. The resulting mixture was filtered through celite and the phases were separated. The organic phase was dried ($MgSO_4$) and concentrated at reduced pressure to give the *title compound* (0.52 g, 100%). The crude product was used without purification.

*[7,7′,9-$^{13}C_3$]Secoisolariciresinol (**24**)*

A solution of 2,3-(4′benzyloxy-3′-methoxy[α-^{13}C]benzyl)[1-^{13}C]butan-1,4-diol (0.52 g, 0.96 mmol) in ethyl acetate (30 ml) was added to 5% palladium-on-carbon (27 mg) and the mixture was stirred under a hydrogen atmosphere for 48 h. The mixture was filtered through celite and washed with ethyl acetate. The organic phase was concentrated at reduced pressure. The crude product was purified by column chromatography (silica; ethyl acetate/dichloromethane (55:45)) to give the *title compound* as a white solid (0.11 g, 32%), which was further purified by reverse phase HPLC. (Found C, 65.9; H, 6.9. C_{17} $^{13}C_3H_{26}O_6$ requires C, 65.7; H, 7.2%); (HRMS found M^+., 365.1825. C_{17} $^{13}C_3H_{26}O_6$ requires 365.1830); ν_{max} (nujol)/cm^{-1} 3410 (OH), and 1588 (C = C); δ_H (300 MHz; $CDCl_3$) 1.8–1.9 (2 H, m, H-8 and H-8′), 2.60 (2 H, Br, 2 × OH), 2.6 (4 H, dm, $J_{13C,1H} = 124$, $^{13}CH_2OH$), 3.20–4.0 (4H, m, 2 x CH_2OH), 3.80 (6 H, s, OMe), 5.4 (2 H, br, ArOH), 6.50–6.61 (4 H, m, H-2, 2′, 6 and 6′) and 6.75 (2 H, d, $J = 8$, H-5′); δ_C (75 MHz; $CDCl_3$) 36.3 (enhanced, $^{13}CH_2$), 56.2 (OMe), 61.4 (enhanced, O$^{13}CH_2$); *m/z* (EI) 365 (M^+, 30%), 256 (5), 236 (6), 138 (100), 123 (15), 69 (38), 57 (30) and 55 (29).

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