Total Synthesis of $[^{13}C]_2$-, $[^{13}C]_3$-, and $[^{13}C]_5$-Isotopomers of Xanthohumol, the Principal Prenylflavonoid From Hops

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Abstract

Xanthohumol [(*E*)-6´-methoxy-3´-(3-methylbuten-2-yl)-2´,4´,4´´-trihydroxychalcone], the principal prenylated flavonoid from hops, has a complex bioactivity profile and ^{13}C-labelled isotopomers of this compound are of potential use as molecular probes and as analytical standards to study metabolism and mode-of-action. 1,3-$[^{13}C]_2$-Xanthohumol was prepared by an adaptation of the total synthesis of Khupse and Erhardt in 7 steps and 5.7% overall yield from phloroglucinol by a route incorporating a cascade Claisen-Cope rearrangement to install the 3´-prenyl moiety from a 5´-prenyl aryl ether and an aldol condensation between 1-[^{13}C]-2´,4´-bis(benzyloxymethyloxy)-6´-methoxy-3´-(3-methylbuten-2-yl)acetophenone and 1´-[^{13}C]-4-(methoxymethyloxy)benzaldehyde. The ^{13}C-atom in the methyl ketone was derived from 1-[^{13}C]-acetyl chloride while that in the aryl aldehyde was derived from [^{13}C]-iodomethane. Tri- and penta-^{13}C-labelled xanthohumols were similarly prepared by applying minor modifications to the route.

Keywords

phytochemicals, chalcones, aldol condensation, Claisen rearrangement, Cope rearrangement

Introduction

Xanthohumol (**1**) is the principal prenylated flavonoid found in the inflorescenes ('hops') of the female hop plant, *Humulus lupulus* L. (Figure 1).[1] Because hops are utilized during the manufacture of beer to add a characteristic floral bitterness, xanthohumol and compounds derived from it during the brewing process find their way into the human diet and they have been implicated in a variety of health effects, both beneficial and nefarious. For example, xanthohumol itself has received attention as a cancer chemopreventative agent[2] with indications against metabolic syndrome,[3] while the cyclized form of desmethylxanthohumol, 8-prenylnaringenin, is the most potent phytoestrogen known.[4] The biochemistry of xanthohumol is nuanced and there is a need for probe molecules to help dissect the role that xanthohumol metabolism has on its biological mode-of-action. Fortunately, the simple chalcone structure of xanthohumol makes the prospect of preparing analogs for SAR and other experiments enticing. Of note, although it is commonly depicted as a keto/phenol tautomer (**1**), the intense yellow color of xanthohumol (in the solid state and in solution) and the presence of a far downfield signal in its ^{1}H NMR spectrum (δ_H = 14.7 ppm), suggests that the true nature of this compound is the fully conjugated enol/orthoquinomethide tautomer **1´**.

In connection with ongoing studies of its biological activity,[3b-e] we required ^{13}C-labelled isotopomers of xanthohumol as standards for mass spectrometric isotope dilution analyses and as probe molecules to track the fate of xanthohumol and its downstream metabolites in a mouse model for metabolic syndrome. Surprisingly, although a synthesis of a dimethylated congener of xanthohumol was reported quite some time ago,[5] a total synthesis of this important chalcone, an effort by Khupse and Erhardt, did not appear until 2007.[6,7] The Khupse-Erhardt elaboration employs a late-stage aldol condensation between a methyl ketone fragment **2** (PG = MOM) and an aryl aldehyde fragment **3** (PG´ = MOM) to generate a protected form of the chalcone natural product **1**. Given the practicality of the Khupse-Erhardt xanthohumol synthesis, and the fact that a modified version of it was recently successfully used by Fang and coworkers to access xanthohumol analogs with improved anticancer activity,[8] we elected to adapt it for the synthesis of a series of ^{13}C-labelled xanthohumol isotopomers. Herein, we report execution of this plan and detail syntheses of [^{13}C]$_2$-, [^{13}C]$_3$-, and [^{13}C]$_5$-xanthohumols employing conveniently obtained isotopically enriched starting materials.[9]

Results and Discussion

We desired access to xanthohumol isotopomers with at least two ^{13}C-atoms distributed within different regions of the molecule such that metabolic bond cleavage reactions could be easily detected. The first target of interest was 1,3-[^{13}C]$_2$-xanthohumol ([^{13}C]$_2$-**1**) and its synthesis called for suitably protected methyl aryl ketone **2** and aryl aldehyde **3** fragments with ^{13}C labels at their respective carbonyl group carbon-atoms. Our initial plan was to replace the methoxymethyl (MOM) protecting groups used in the Khupse-Erhardt synthesis wholly with benzyloxymethyl (BOM) protecting groups to avoid use of the more noxious chloromethyl ether reagent require to install MOM versus BOM groups. BOM protecting groups served well for the requisite methyl aryl ketone compound ([^{13}C]-**11**) but substitution of MOM for BOM in the aryl aldehyde fragment synthesis was ultimately precluded when it was identified that the optimal route to this material necessitated a benzylic oxidation step incompatible with a BOM group.

Synthesis of the methyl aryl ketone fragment began with Friedel-Crafts acetylation of phloroglucinol (1,3,5-trihydroxybenzene, **4**) with 1-[^{13}C]-acetyl chloride (Scheme 1).[10] As anticipated based on the comparable known reaction with MOMCl,[6] exposure of the so-generated labelled phloracetophenone [^{13}C]-**5** with an excess of BOMCl resulted in alkylation of only two out of the three phenolic hydroxyl groups to yield bis-BOM ether [^{13}C]-**6**. The recalcitrance of the remaining phenol to react is likely due to engagement of the hydroxyl group in a hydrogen-bond with the adjacent carbonyl group. As a prelude to a Claisen-Cope rearrangement cascade to regiospecifically install the C3´ prenyl group,[11] phenol [^{13}C]-**6** was next converted into its prenyl ether derivative [^{13}C]-**8**. As previously observed by Khupse and Erhardt,[6] we found that this operation was best achieved using Mitsunobu conditions (as illustrated) while a traditional Williamson ether synthesis (prenyl bromide, K_2CO_3, acetone, reflux) gave less than 50% conversion to the desired ether even after extended reaction times.

With aryl allyl ether [^{13}C]-**8** in hand, double transposition of the prenyl group from O6´ to C3´ via a Claisen-Cope rearrangement was investigated. Khupse and Erhardt reported that this task could be achieved from the analogous bis-MOM protected compound in 64% yield by direct heating in *N,N*-dimethylaniline;[6,12] however, Fang et al. reported only a 31% yield for the exact same transformation.[8] In the case of [^{13}C]-**8**, we also encountered problems with the simple thermal process and obtained at best a 25% yield ($PhNMe_2$, 200 °C, 2 h) of the desired rearrangement product [^{13}C]-**9** which was accompanied by unreacted starting material, the intermediate C5´ isoprenylated arene, and innumerable minor side-products. Longer reaction times only led to decomposition and even lower isolated yields of prenylated arene [^{13}C]-**9**. A better outcome (47% yield, 0.15 mmol scale) was obtained using microwave irradiation in 1,2-dichlorobenzene (180 °C, 1.5 h) but the reaction was not reproducible on a practically relevant scale (≥1 mmol). After further experimentation,

the Eu(III) catalyzed rearrangement conditions of Metz et al.[13] were found to consistently afford [^{13}C]-**9** in ca. 40% isolated yield even on multigram scale. Unfortunately, [^{13}C]-**9** was the minor product formed using Eu(fod)$_3$ (Resolve-Al^{TM}) catalysis and the bulk of the mass balance was dihydrobenzopyran derivative [^{13}C]-**10**, an apparent result of oxymetalation/protodemetalation from the putative intermediate C5´ isoprenylated arene. The spontaneous formation of dihydrobenzofurans such as [^{13}C]-**10** following Claisen rearrangement of *O*-prenyl arenes has been observed previously under a variety of different reaction conditions.[14] Resubjection of dihydrobenzopyran [^{13}C]-**10** to the reaction conditions did not result in its further conversion to [^{13}C]-**9**. Methylation of [^{13}C]-**9** was uneventful and gave a good yield of the requisite methyl ketone fragment [^{13}C]-**11** under standard conditions.

Prenylated chalcones have been been successfully accessed by Suzuki-Miyaura cross-coupling of appropriate aryl iodides with prenyl boronic esters.[15] Accordingly, we briefly explored an alternate preparation of [^{13}C]-**11** via iodination of [^{13}C]-**6** followed by cross-coupling etc. This plan was thwarted with the discovery that iodination of [^{13}C]-**6** (*N*-iodosuccinimide, DMF, rt, 4.5 h, 78% yield) occurred exclusively at the undesired C5´ position while iodination of the *O*-methylated derivative of [^{13}C]-**6** gave a 62:38 mixture of regioisomeric aryl iodides, favoring again the undesired C5´ halogenated adduct.

Our preferred strategy to a suitable labelled aryl aldehyde fragment, [^{13}C]-**16**, involved benzylic oxidation of MOM protected *p*-cresol [^{13}C]-**14** such that [^{13}C]-methyl iodide could provide a convenient and inexpensive source of the ^{13}C-atom (Scheme 2). Accordingly, [^{13}C]-**14** was prepared from bromobenzene **13** by bromine-lithium exchange followed by alkylation of the intermediate aryllithium with [^{13}C]-methyl iodide. This reaction also generated minor quantities of the reduced bromide (MOMOPh, 11%) and the butylated arene (4-MOMOC$_6$H$_4$Bu, 13%) resulting from attack of the aryllithium upon the bromobutane by-product of halogen-metal exchange. Direct conversion of cresol derivative [^{13}C]-**14** to the target aldehyde [^{13}C]-**16** was attempted both via an IBX mediated oxidation (≤10% yield [^{13}C]-**16**)[16] and via a TEMPO/Co(OAc)$_2$ catalyzed oxidation (no reaction),[17] but neither method gave an acceptable result. Instead, [^{13}C]-**14** was successfully converted to aldehyde [^{13}C]-**16** over two steps by employing a Wohl-Ziegler radical bromination[18] followed by a Kornblum oxidation.[19] The intermediate bromide [^{13}C]-**15** (which was co-generated with variable quantities of the corresponding dibromide 3-25%) was found to decompose significantly within 12 hours and so it was treated immediately upon isolation with warm dimethylsulfoxide to transform it into the desired aldehyde [^{13}C]-**16**. Being quite susceptible to aerial oxidation, the aldehyde was likewise regarded as a labile intermediate and so it was also used as quickly as possible in the next step. Indeed, it was found to be optimal for bromination, Kornblum oxidation, and aldol condensation to be performed during the same day if at all possible.

Completion of the total synthesis of 1,3-[^{13}C]$_2$-xanthohumol called for aldol condensation between the previously assembled aryl methyl ketone and aryl aldehyde fragments followed by removal of the collection of BOM and MOM protecting groups (Scheme 3). Minor modifications to the original Khupse-Erhardt procedures were required to achieve this end game in an acceptable overall yield. For example, possibly due to the heightened lipophilicity of bis-BOM group protected ketone [^{13}C]-**11**, aldol condensation between this compound and aryl aldehyde [^{13}C]-**16** in basic aqueous methanol required considerably longer (16 h) to achieve than the 4 h reported for the analogous reaction involving bis-MOM group protected ketone **2** (PG = MOM).[6] In the case of the final deprotection step, we found that the conditions used by Khupse and Erhardt to remove three MOM groups (c. HCl, aq. MeOH, rt) did not work well (≤20% yield) for the hydrolysis of two BOM groups and one MOM group from aldol reaction product [^{13}C]$_2$-**17**. After extensive experimentation, it was discovered that conditions reported by Hall and Deslongchamps for MOM group hydrolysis (c. HCl, *i*-PrOH, 60 °C) gave acceptable results and provided a 45-60% yield of the desired labelled xanthohumol [^{13}C]$_2$-**1**;[20] however, small quantities of HCl addition adduct [^{13}C]$_2$-**18** were co-generated alongside the desired target. Alkene [^{13}C]$_2$-**1** and chloroalkane [^{13}C]$_2$-**18** possess very similar polarity and they are difficult to separate from one another. Nonetheless, a two-stage column chromatography protocol was developed that reliably delivers [^{13}C]$_2$-**1** in a completely pure form free of [^{13}C]$_2$-**18** and any other contaminants from the aforegoing chemistry (see experimental section for details).

Aside from the expected spectral differences due to ^{13}C incorporation, the 1,3-[^{13}C]$_2$-xanthohumol ([^{13}C]$_2$-**1**) generated from the route described above was otherwise identical to a sample of commercially available natural xanthohumol. The presence of the two sites of ^{13}C-atom enrichment were clearly evident in mass (i.e., *m/z* $(M+H)^+$ = 357 for [^{13}C]$_2$-**1**), IR, and NMR spectra. In the IR spectrum, the carbonyl stretching band was 17 cm^{-1} lower for [^{13}C]$_2$-**1** as compared to natural **1**. The ^{13}C NMR spectrum for [^{13}C]$_2$-**1** (in d$_6$-DMSO) showed strongly enriched signals for C1 (δ = 191.7 ppm) and C3 (δ = 142.5 ppm) while scalar coupling to ^{13}C atoms was observed for signals attributable to H2, H3, and H2´´ in the ^{1}H NMR spectrum (Figure 2). Complete spectra data can be found below in the experimental section and full NMR spectra are available in the Supporting Information.

Finally, the other desired more highly labelled isotopomers of xanthohumol were prepared in an essentially identical fashion to [^{13}C]$_2$-**1** by using additional ^{13}C-enriched starting materials at appropriate junctures in the total synthesis (Figure 3). Thus, triple ^{13}C-labelled xanthohumol ([^{13}C]$_3$-**1**) was synthesized by using [^{13}C]-iodomethane in the phenol methylation step (cf. [^{13}C]$_2$-**9** to [^{13}C]$_2$-**11**, Scheme 1). To access quintuple ^{13}C-labelled xanthohumol ([^{13}C]$_5$-**1**), [^{13}C]-iodomethane was again incorporated into the phenol methylation step but now also 1,2-[^{13}C]$_2$-3-methyl-2-buten-1-ol (prepared by the method of Lugtenburg)[21] was used instead of unlabelled prenyl alcohol (**7**) in the Mitsunobu reaction (cf. [^{13}C]$_2$-**6** to [^{13}C]$_2$-**8**, Scheme 1). As for

the double labelled isotopomer [^{13}C]$_2$-**1**, the two newly prepared compounds, [^{13}C]$_3$-**1** and [^{13}C]$_5$-**1**, exhibited the expected characteristic spectral differences to natural xanthohumol but they were otherwise identical.

Experimental

All commercially available reagents were used as received unless otherwise noted. Preparative chromatographic separations were performed on silica gel 60 (35-75 μm) and reactions followed by TLC analysis using silica gel 60 plates (2-25 μm) with fluorescent indicator (254 nm) and visualized with UV or phosphomolybdic acid. Infra-red (IR) spectra were recorded in Fourier transform mode using an ATR probe for solids, while oils were supported between NaCl plates (neat). ^{1}H and ^{13}C NMR spectra were recorded in Fourier transform mode at the field strength specified and from the indicated deuterated solvents in standard 5 mm diameter tubes. Chemical shift in ppm is quoted relative to residual solvent signals calibrated as follows: $CDCl_3$ δ_H (C*H*Cl$_3$) = 7.26 ppm, δ_C = 77.2 ppm; $(CD_3)_2SO$ δ_H (CD_3SOCHD_2) = 2.50 ppm, δ_C = 39.5 ppm. Multiplicities in the ^{1}H NMR spectra are described as: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Numbers in parentheses following carbon atom chemical shifts refer to the number of attached hydrogen atoms determined by either DEPT or HSQC NMR experiments. Low (MS) and high resolution (HRMS) mass spectra were obtained using either electon impact (EI) or electrospray (ES) ionization techniques. Ion mass/charge (*m/z*) ratios are reported as values in atomic mass units.

1-[^{13}C]-2´,4´,6´-Trihydroxyacetophenone ([^{13}C]-5). By a modification to the method of Maier et al.[10] To a stirred suspension of phloroglucinol (1.58 g, 12.5 mmol) in CH_2Cl_2-nitromethane (1:1, 30 mL) at rt under Ar was added powdered $AlCl_3$ (6.67 g, 50.0 mmol) in four portions (a slight exotherm was noted). The resulting homogenous yellow/brown solution was stirred for 30 min and then treated with neat 1-[^{13}C]-acetyl chloride (1.00 g, 12.6 mmol) in one portion. The resulting mixture was heated to a gentle reflux and stirred for 3 h. After this time, the mixture was cooled to rt and poured into ice water (100 mL). A majority of the low boiling volatile solvents were removed using a rotatory evaporator and the residue was extracted with EtOAc (3x20 mL). The combined organic extracts were washed with brine (20 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2, eluting with 60% EtOAc in hexanes) to afford the desired acylated product [^{13}C]-**5** (1.34 g, 7.93 mmol, 63%) as a tan powder: mp 207-209 °C; IR (ATR) 3522, 3451, 3107, 1622, 1515, 1463, 1360, 1266, 1239, 1164, 1064 cm^{-1}; ^{1}H NMR (400 MHz, d$_6$-DMSO) δ 12.23 (2H, s), 10.36 (1H, s), 5.80 (2H, s), 2.54 (3H, d, $^2J_{CH}$ = 6.1 Hz) ppm; ^{13}C NMR (100 MHz, d$_6$-DMSO) δ 202.5 (0, enriched), 164.8 (0), 164.3 (2C, 0), 104.0 (0, d, $^1J_{CC}$ = 56 Hz), 94.5 (2C, 1), 32.4 (3, d, $^1J_{CC}$ = 41 Hz) ppm; MS (EI+) *m/z* 169 (100%, [M]$^{+\bullet}$), 155 (82), 126 (35), 69 (70); HRMS (ES+) *m/z* 170.0536 (calcd. for $^{12}C_7{}^{13}CH_9O_4$: 170.0534).

1-[^{13}C]-2´,4´-Bis(benzyloxymethyloxy)-6´-hydroxyacetophenone ([^{13}C]-6). A stirred suspension of triol [^{13}C]-**5** (1.25 g, 7.40 mmol) in anhydrous CH_2Cl_2 (25 mL) at 0 °C under Ar was treated with diisopropylethylamine (7.56 mL, d = 0.76, 5.75 g, 44.5 mmol, 6 eq) followed by neat benzyl chloromethyl ether (BOMCl, 3.06 mL, d = 1.14, 3.49 g, 22.3 mmol, 3 eq) which was added dropwise during 4 min. The resulting homogenous brown solution was allowed to warm to rt and stirred for 2.75 h. After this time, sat. aq. $NaHCO_3$ (20 mL) was added and the mixture partitioned between EtOAc (40 mL) and H_2O (30 mL). The aqueous phase was extracted with EtOAc (2x20 mL) and the combined organic phases washed successively with H_2O (30 mL) and brine (20 mL, then dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2, eluting with 20% EtOAc in hexanes) to afford the desired bis-BOM ether [^{13}C]-**6** (2.48 g, 6.06 mmol, 82%) as colorless oil: IR (neat) 3032, 2909, 1619, 1584, 1252, 1213, 1173, 1068, 830, 698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.74 (1H, s), 7.36-7.30 (10H, m), 6.38 (1H, dm, J = 2.3 Hz), 6.34 (1H, dm, J = 2.3 Hz), 5.37 (2H, s), 5.29 (2H, s), 4.75 (2H, s), 4.71 (2H, s), 2.64 (3H, d, $^2J_{CH}$ = 6.1 Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.6 (0, enriched), 167.0 (0), 163.7 (0), 160.6 (0), 137.0 (0), 136.9 (0), 128.73 (2C, 1), 128.65 (2C, 1), 128.3 (1), 128.2 (3C, 1), 128.1 (2C, 1), 107.2 (0, d, $^1J_{CC}$ = 56 Hz), 97.5 (1), 94.4 (1), 92.5 (2), 92.0 (2), 71.1 (2), 70.6 (2), 33.2 (3, d, $^1J_{CC}$ = 42 Hz) ppm; MS (EI+) *m/z* 409 (15%, [M]$^{+\bullet}$), 379 (17), 349 (8), 306 (13), 258 (12), 215 (10), 181 (18), 120 (22), 107 (32), 91 (100); HRMS (ES+) *m/z* 410.1705 (calcd. for $^{12}C_{23}{}^{13}CH_{25}O_6$: 410.1685).

1-[^{13}C]-2´,4´-Bis(benzyloxymethyloxy)-6´-[(3-methylbuten-2-yl)oxy]acetophenone ([^{13}C]-8). A stirred solution of phenol [^{13}C]-**6** (3.61 g, 8.83 mmol) and triphenylphosphine (3.47 g, 13.2 mmol) in anhydrous THF (60 mL) at 0 °C under Ar, was treated with prenyl alcohol (3-methyl-2-buten-1-ol, **7**, 1.15 mL, d = 0.86, 989 mg, 11.5 mmol) followed by the dropwise addition of diethyl azodicarboxylate (DEAD, 5.23 mL, d = 0.956, 5.00 g, 40 wt.% in PhMe, 11.5 mmol) during 3 min. The resulting mixture was allowed to warm to rt and stirred for 22 h. After this time, the mixture was concentrated *in vacuo* and the residue was purified by column chromatography (SiO_2, eluting with 18-20% EtOAc in hexanes) to afford the desired aryl prenyl ether [^{13}C]-**8** (3.32 g, 6.96 mmol, 79%) as a colorless oil: IR (neat) 2912, 1663, 1605, 1454, 1168, 1057, 822, 738 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34-7.28 (10H, m), 6.59-6.58 (1H, m), 6.37-6.36 (1H, m), 5.41 (1H, t of septet, J = 6.6, 1.4 Hz), 5.26 (2H, s), 5.24 (2H, s), 4.71 (2H, s), 4.70 (2H, s), 4.49 (2H, d, J = 6.6 Hz), 2.48 (3H, d, $^2J_{CH}$ = 6.3 Hz), 1.75 (3H, s), 1.70 (3H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.1 (0, enriched), 159.8 (0), 157.4 (0), 155.5 (0), 138.1 (0), 137.26 (0), 137.22 (0), 128.62 (2C, 1), 128.58 (2C, 1), 128.2 (4C, 1), 128.1 (1), 128.0 (1), 119.5 (1), 116.5 (0, d, $^1J_{CC}$ = 54 Hz), 96.4 (1), 95.4 (1), 92.8 (2), 92.5 (2), 70.4 (2), 70.2 (2), 65.8 (2), 32.7 (3, d, $^1J_{CC}$ = 43 Hz), 25.9 (3), 18.4 (3) ppm; MS (EI+) *m/z* 477 (4%, [M]$^{+\bullet}$), 408 (91), 378 (91), 348 (43), 305 (95), 257 (77), 214 (60), 181 (90), 91 (100); HRMS (ES+) *m/z* 478.2356 (calcd. for $^{12}C_{28}{}^{13}CH_{33}O_6$: 478.2311).

1-[^{13}C]-2´,4´-Bis(benzyloxymethyloxy)-6´-hydroxy-3´-(3-methylbuten-2-yl)acetophenone ([^{13}C]-9). A stirred solution of aryl prenyl ether [^{13}C]-**8** (2.64 g, 5.53 mmol) in chlorobenzene (50 mL) was treated with solid $NaHCO_3$ (511 mg, 6.08 mmol) followed by europium(III) tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionate) [Eu(fod)$_3$, 574 mg, 0.554 mmol, 10 mol%). The resulting mixture was heated to a gentle reflux and stirred for 3 h. After this time, the mixture was allowed to cool to rt, concentrated *in vacuo*, and allowed to stand at rt for 18 h. The residue was then purified by column chromatography (SiO_2, eluting with 15-20% EtOAc in hexanes) to afford, in order of elution, the desired prenylated acetophenone [^{13}C]-**9** (1.05 g, 2.20 mmol, ≤ 40%, ca. 90-95% pure) and the unwanted isomeric benzofuran derivative [^{13}C]-**10** (1.07 g, 2.24 mmol, 41%), both as colorless oils.

Data for [^{13}C]-**9**: IR (neat) 2912, 1612, 1577, 1362, 1253, 1043, 952, 737, 697 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.99 (1H, s), 7.38-7.27 (10H, m), 6.57 (1H, s), 5.33 (2H, s), 5.13 (1H, tm, J = 5.9 Hz), 5.10 (2H, s), 4.70 (4H, s), 3.32 (2H, d, J = 6.4 Hz), 2.73 (3H, d, $^2J_{CH}$ = 6.1 Hz), 1.75 (3H, s), 1.67 (3H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.2 (0, enriched), 163.7 (0), 161.9 (0), 157.3 (0), 137.08 (0), 137.02 (0), 131.9 (0), 128.7 (2C, 1), 128.7 (2C, 1), 128.20 (1), 128.16 (1), 128.1 (2C, 1), 127.9 (2C, 1), 123.3 (1), 116.4 (0), 111.2 (0, d, $^1J_{CC}$ = 54 Hz), 99.4 (2), 99.2 (1), 91.9 (2), 72.7 (2), 70.6 (2), 31.8 (3, d, $^1J_{CC}$ = 42 Hz), 25.9 (3), 23.4 (2), 18.2 (3) ppm; MS (EI+) *m/z* 477 (10%, [M]$^{+\bullet}$), 476 (55), 355 (83), 325 (98), 282 (98), 271 (50), 181 (31), 91 (100); HRMS (ES+) *m/z* 500.2160 (calcd. for $^{12}C_{28}{}^{13}CH_{32}O_6Na$: 500.2130).

Data for [^{13}C]-**10**: IR (neat) 2965, 2909, 1607, 1455, 1409, 1353, 1190, 1061, 964, 738, 698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37-7.26 (10H, m), 6.61 (1H, s), 5.32-5.28 (2H, AB quartet), 5.27 (2H, s), 4.74 (2H, s), 4.72 (2H, s), 4.44 (1H, q, J = 6.6 Hz), 2.55 (3H, d, $^2J_{CH}$ = 6.2 Hz), 1.42 (3H, s), 1.38 (3H, d, J = 6.6 Hz), 1.18 (3H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.4 (0, enriched), 159.3 (0), 157.2 (0), 156.2 (0), 137.3 (0), 137.1 (0), 128.7 (2C, 1), 128.6 (2C, 1), 128.13 (2C, 1), 128.11 (1), 128.08 (2C, 1), 128.0 (1), 118.4 (0), 110.0 (0), 95.1 (1), 93.6 (2), 91.7 (2), 90.2 (1), 70.44 (2), 70.41 (2), 43.7 (0), 32.7 (3, d, $^1J_{CC}$ = 42 Hz), 25.7 (3), 21.1 (3), 14.4 (3) ppm; MS (ES+) *m/z* 500 (M+Na)$^+$; HRMS (ES+) *m/z* 500.2080 (calcd. for $^{12}C_{28}{}^{13}CH_{32}O_6Na$: 500.2130).

1-[^{13}C]-2´,4´-Bis(benzyloxymethyloxy)-6´-methoxy-3´-(3-methylbuten-2-yl)acetophenone ([^{13}C]-11). A stirred suspension of phenol [^{13}C]-**9** (705 mg, 1.48 mmol) and K_2CO_3 (2.04 g, 14.8 mmol, 10 eq) in acetone (15 mL) at rt was treated with neat iodomethane (0.47 mL, d = 2.24, 1.05 g, 7.41 mmol, 5 eq). The resulting mixture was stirred vigorously for 18 h and then concentrated *in vacuo*. The residue was partitioned between EtOAc (20 mL) and H_2O (15 mL) and the aqueous phase extracted with EtOAc (10 mL). The combined organic phases were washed successively with sat. aq. $Na_2S_2O_3$ (10 mL), H_2O (10 mL), and brine (10 mL), and then dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2, eluting with 20% EtOAc in hexanes) to afford the desired methyl aryl ether [^{13}C]-**11** (610 mg, 1.24 mmol, 84%) as a colorless oil: IR (neat)

2917, 1660, 1599, 1454, 1163, 1045, 936, 737, 698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36-7.29 (10H, m), 6.64 (1H, s), 5.32 (2H, s), 5.17 (1H, tm, *J* = 6.8 Hz), 5.05 (2H, s), 4.74 (2H, s), 4.71 (2H, s), 3.77 (3H, s), 3.34 (2H, d, *J* = 6.8 Hz), 2.48 (3H, d, $^{2}J_{CH}$ = 6.2 Hz), 1.74 (3H, s), 1.66 (3H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.3 (0, enriched), 157.8 (0), 156.0 (0), 153.8 (0), 137.7 (0), 137.2 (0), 131.5 (0), 128.7 (2C, 1), 128.6 (2C, 1), 128.3 (2C, 1), 128.2 (1), 128.0 (2C, 1), 127.9 (1), 123.4 (1), 120.3 (0, d, $^{1}J_{CC}$ = 54 Hz), 117.5 (0), 99.3 (2), 95.2 (1), 92.4 (2), 71.7 (2), 70.2 (2), 56.1 (3), 32.9 (3, d, $^{1}J_{CC}$ = 42 Hz), 25.9 (3), 23.3 (2), 18.1 (3) ppm; MS (EI+) *m/z* 491 (3%, $[M]^{+\bullet}$), 477 (18), 383 (30), 326 (26), 250 (23), 91 (100); HRMS (ES+) *m/z* 492.2487 (calcd. for $^{12}C_{29}{}^{13}CH_{35}O_6$: 492.2467).

1-Bromo-4-(methoxymethyloxy)benzene (13). A stirred solution of 4-bromophenol (**12**, 12.2 g, 70.5 mmol) in anhydrous CH_2Cl_2 (300 mL) at 0 °C under Ar was treated with diisopropylethylamine (27.0 mL, d = 0.76, 20.5 g, 158.9 mmol). Neat chloromethyl methyl ether (MOMCl, 7.50 mL, d = 1.06, 7.08 g, 75.9 mmol, CARE!) was then added dropwise during 10 min. The resulting dark orange solution was allowed to stir for 2 h while warming to rt. After this time, any active alkylating agent was quenched by the addition of conc. aq. NH_3 (10 mL) and the reaction mixture stirred for a further 10 min. The mixture was partitioned between EtOAc (80 mL) and H_2O (80 mL) and the aqueous phase extracted with EtOAc (2x60 mL). Combined organic phases were then washed successively with H_2O (40 mL) and brine (40 mL), dried (Na_2SO_4), and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2, eluting with 15% EtOAc in hexanes) to afford the title MOM ether **13** (12.65 g, 58.3 mmol, 83%) as a colorless oil: IR (neat) 2956, 2903, 1591, 1488, 1235, 998.8, 591, 508 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (2H, d, *J* = 9.0 Hz), 6.92 (2H, d, *J* = 9.0 Hz), 5.15 (2H, s), 3.47 (3H, 3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5 (0), 132.5 (2C, 1), 118.2 (2C, 1), 114.4 (0), 94.7 (2), 56.2 (3) ppm. ^{1}H and ^{13}C NMR spectral data are in agreement with those previously reported.[22]

4-(Methoxymethyloxy)-1-[^{13}C]methylbenzene ([^{13}C]-14). A stirred solution of aryl bromide **13** (5.20 g, 24.0 mmol) in anhydrous THF (130 mL) at –78 °C was treated dropwise with *n*-BuLi (15.8 mL, 2.10 M in hexanes, 33.2 mmol) during 10 min. The resulting aryllithium was stirred for 10 min and then treated with neat [^{13}C]-methyl iodide (1.60 mL, d = 2.28, 3.65 g, 25.5 mmol). The reaction mixture was allowed to stir while warming to rt for 2 h and then sat. aq. NH_4Cl (15 mL) was added. The quenched mixture was then partitioned between EtOAc (50 mL) and H_2O (50 mL) and the aqueous phase extracted with EtOAc (2x30 mL). The combined organic phases were washed successively with conc. aq. NH_3 (10 mL), brine (20 mL), and then dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2, eluting with 10% EtOAc in hexanes) to afford an inseparable mixture (3.15 g) of the methylated benzene [^{13}C]-**14** (69 wt.%, effectively 2.17 g, 14.2 mmol, 59%), 4-$MOMOC_6H_4Bu$ (19 wt.%, eff. 599 mg, 3.08 mmol, 13%), and MOMOPh (12 wt.%, eff. 378 mg, 2.74 mmol, 11%) as a colorless oil. Data obtained from mixture: IR (neat) 2956, 1614, 1512, 1233, 1080, 923, 758 cm^{-1}; ^{1}H NMR (400

MHz, $CDCl_3$, signals attributable to [^{13}C]-**14** only) δ 7.12 (2H, d, *J* = 8.2 Hz), 6.97 (2H, d, *J* = 8.3 Hz), 5.17 (2H, s), 3.49 (3H, s), 2.32 (3H, d, $^1J_{CH}$ = 126.2 Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.3 (0), 130.1 (2C, 1), 122.1 (0), 116.5 (2C, 1), 94.9 (2), 56.0 (3), 20.66 (3, enriched) ppm; MS (EI+) *m/z* 153 $(M)^{+\bullet}$ (54%), 122 (100), 107 (28), 92 (73), 78 (67); HRMS (EI+) *m/z* 153.0869 (calcd. for $^{12}C_8{}^{13}CH_{12}O_2$: 153.0871).

1-[^{13}C]-Bromomethyl-4-(methoxymethyloxy)benzene ([^{13}C]-15). A stirred solution of the *p*-cresol derivative [^{13}C]-**14** (1.00 g, 69 wt.% pure, 4.96 mmol) in CCl_4 (25 mL) at rt was treated with *N*-bromosuccinimide (1.28 g, 7.19 mmol) followed by 2,2´-azobis(isobutyronitrile) (AIBN, 59 mg, 0.360 mmol). The resulting mixture was heated to a gentle reflux and stirred for 1.75 h. After this time, the reaction mixture was allowed to cool, concentrated *in vacuo*, and the residue purified by column chromatography (SiO_2, eluting with 10% EtOAc in hexanes) to afford a respective 64:36 molar mixture of the desired benzyl bromide [^{13}C]-**15** and the corresponding dibromide (1.04 g, 57 wt.% in [^{13}C]-**15**, effectively 593 mg, 2.56 mmol, 52%) as a colorless oil. The benzyl bromide decomposes significantly within 12 hours and it was used immediately in the next step. Data for [^{13}C]-**15**: IR (neat) 2957, 2900, 1608, 1511, 1240, 1153, 1080, 838 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (2H, dd, *J* = 8.6, 5.0 Hz), 7.01 (2H, d, *J* = 8.6 Hz), 5.18 (2H, s), 4.49 (2H, d, $^1J_{CH}$ = 153.0 Hz), 3.47 (3H, s) ppm.

1´-[^{13}C]-4-(Methoxymethyloxy)benzaldehyde ([^{13}C]-16). Dimethyl sulfoxide (DMSO, 8 mL) and solid $NaHCO_3$ (1.30 g, 15.5 mmol) were added to the neat benzyl bromide [^{13}C]-**15** (1.04 g, 57 wt.% pure, 2.56 mmol) and the resulting mixture stirred under Ar at 90 °C for 2 h. After this time, the mixture was allowed to cool to rt and partitioned between Et_2O (25 mL) and H_2O (25 mL). The aqueous phase was extracted with Et_2O (10 mL) and the combined organic phases were washed successively with H_2O (2x10 mL) and brine (10 mL), then dried (Na_2SO_4), and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2, 15% EtOAc in hexanes) to afford the benzaldehyde [^{13}C]-**16** (275 mg, 1.65 mmol, 64%) as a colorless oil: IR (neat) 2959, 1652, 1600, 1509, 1242, 1152, 1082, 834 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.90 (1H, d, $^1J_{CH}$ = 172.6 Hz), 7.84 (2H, dd, *J* = 8.5, 4.7 Hz), 7.15 (2H, d, *J* = 8.6 Hz), 5.26 (2H, s), 3.49 (3H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.1 (0, enriched), 162.4 (0), 132.1 (2C, 1), 130.9 (0, d, *J* = 56 Hz), 116.5 (2C, 1), 94.3 (2), 56.6 (3) ppm; MS (EI+) *m/z* 167 $(M)^{+\bullet}$ (100%), 152 (65), 122 (34); HRMS (EI+) *m/z* 167.0663 (calcd. for $^{12}C_8{}^{13}CH_{10}O_3$: 167.0664).

(*E*)-1,3-[^{13}C]$_2$-2´,4´-Bis(benzyloxymethoxy)-6´-methoxy-4´´-(methoxymethyloxy)-3´-(3-methylbuten-2-yl)chalcone ([^{13}C]$_2$-17). A stirred solution of the methyl ketone [^{13}C]-**11** (403 mg, 0.821 mmol) and the aryl aldehyde [^{13}C]-**16** (247 mg, 1.48 mmol, 1.8 eq) in MeOH (8 mL) was treated with 10 wt.% aq. NaOH (1.00 mL, 2.50 mmol, 3.0 eq) and heated at a gentle reflux for 18 h. After this time, the mixture was partitioned between EtOAc (30 mL), H_2O (30 mL), and brine (10 mL). The aqueous phase was extracted with EtOAc (2x15 mL) and the combined organic phases were

washed with brine (15 mL), dried (Na_2SO_4), and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2, eluting with 20% EtOAc in hexanes) to afford in order of elution recovered aldehyde [^{13}C]-**16** (127 mg, 0.76 mmol) and the title chalcone [^{13}C]$_2$-**17** (350 mg, 0.547 mmol, 67%) as a pale yellow oil: IR (neat) 2918, 1596, 1509, 1454, 1240, 1153, 1081, 986, 830, 737 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (2H, dd, *J* = 8.7, 4.6 Hz), 7.36 (1H, ddd, *J* = 154, 16.0, 6.9 Hz), 7.37-7.25 (10H, m), 7.02 (2H, d, *J* = 8.8 Hz), 6.89 (1H, dd, *J* = 16.1, 2.6 Hz), 6.68 (1H, s), 5.35 (2H, s), 5.22 (1H, tm, *J* = 6.6 Hz), 5.20 (2H, s), 5.06 (2H, s), 4.74 (2H, s), 4.69 (2H, s), 3.74 (3H, s), 3.48 (3H, s), 3.39 (2H, d, *J* = 6.7 Hz), 1.76 (3H, s), 1.67 (3H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.2 (0, enriched), 159.2 (0), 157.9 (0), 156.5 (0), 154.3 (0), 144.2 (1, enriched), 137.5 (0, d, *J* = 45 Hz), 131.4 (0), 130.2 (2C, 1), 128.7 (2C, 1), 128.5 (2C, 1), 128.21 (2C, 1), 128.15 (1), 127.9 (2C, 1), 127.7 (1), 123.5 (1), 118.5 (0, d, *J* = 54 Hz), 117.5 (0), 116.6 (2C, 1, d, *J* = 4 Hz), 98.9 (2), 95.5 (1), 94.4 (2), 92.5 (2), 79.0 (1), 71.7 (2), 70.2 (2), 59.8 (3), 56.3 (3), 25.9 (3), 23.3 (2), 18.1 (3) ppm; MS (ES+) *m/z* 663 $(M+Na)^+$, 641 $(M+H)^+$; HRMS (ES+) *m/z* 641.2996 (calcd. for $^{12}C_{37}{}^{13}C_2H_{43}O_8$: 641.3025).

[^{13}C]$_2$-Xanthohumol = (*E*)-1,3-[^{13}C]$_2$-6´-methoxy-3´-(3-methylbuten-2-yl)-2´,4´,4´´-trihydroxychalcone ([^{13}C]$_2$-1). A cloudy solution/suspension of protected xanthohumol [^{13}C]$_2$-**17** (375 mg, 0.586 mmol) in *i*-PrOH (6 mL) was treated with conc. aq. HCl (3 drops) and stirred at 60 °C (bath temp.) for 2.66 h (note: the reaction mixture became an homogenous yellow solution after ca. 10 min). After this time, the solution was allowed to cool to rt and partitioned between EtOAc (20 mL), sat. aq. $NaHCO_3$ (10 mL), and H_2O (10 mL). The aqueous phase was extracted with EtOAc (10 mL) and the combined organic phases washed with H_2O (10 mL), brine (10 mL), and then dried (Na_2SO_4) and concentrated *in vacuo*. The residue was subjected to two successive column chromatographic operations (column #1 = SiO_2, eluting with 1.5% MeOH in CH_2Cl_2; column #2 = SiO_2, eluting with 33-50% EtOAc in hexanes) to obtain pure labelled xanthohumol [^{13}C]$_2$-**1** (120 mg, 0.337 mmol, 58%) as a yellow solid: mp 162-164 °C; IR (ATR) 3178, 2921, 1582, 1538, 1510, 1432, 1331, 1219, 1192, 1167, 1139, 1098, 1052. 971, 823, 802 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.68 (1H, s), 7.79 (1H, ddd, *J* = 15.3, 5.2, 2.4 Hz), 7.75 (1H, ddd, *J* = 155, 15.5, 6.0 Hz), 7.52 (2H, dd, *J* = 8.2, 4.3 Hz), 6.86 (2H, d, *J* = 8.4 Hz), 6.19 (1H, s), 5.95 (1H, s), 5.30 (1H, tm, *J* = 7.5 Hz), 5.06 (1H, s), 3.90 (3H, s), 3.41 (2H, d, *J* = 7.1 Hz), 1.83 (3H, s), 1.78 (3H, s) ppm; ^{1}H NMR (400 MHz, d_6-DMSO) δ 14.64 (1H, s), 10.56 (1H, s), 10.06 (1H, s), 7.76 (1H, dm, *J* = 15.6 Hz), 7.67 (1H, ddd, *J* = 156, 15.6, 5.6 Hz), 7.57 (2H, dd, *J* = 7.8, 4.9 Hz), 6.84 (2H, d, *J* = 8.1 Hz), 6.08 (1H, s), 5.14 (1H, br t, *J* = 7.0 Hz), 3.87 (3H, s), 3.13 (2H, d, *J* = 7.1 Hz), 1.70 (3H, s), 1.61 (3H, s) ppm; ^{13}C NMR (175 MHz, d_6-DMSO) δ 191.7 (0, enriched), 164.6 (0), 162.4 (0), 160.5 (0), 159.9 (0), 142.5 (1, enriched), 130.5 (2C, 1), 129.9 (0), 126.0 (0, dd, *J* = 58, 7 Hz), 123.7 (1, dd, *J* = 68, 54 Hz), 123.0 (1), 115.9 (2C, 1, d, *J* = 4 Hz), 107.3 (0), 104.5 (0, d, *J* = 61 Hz), 90.9 (1), 55.7 (3), 25.5 (3), 21.0 (2), 17.6 (3) ppm; MS (ES+) *m/z* 357 $(M+H)^+$; HRMS (ES+) *m/z* 357.1630 (calcd. for $^{12}C_{19}{}^{13}C_2H_{23}O_5$: 357.1613).

A sample of commercially available natural xanthohumol (**1**) gave mp = 162-165 °C on the same melting point apparatus and exhibited ^{1}H and ^{13}C NMR spectral signatures that differed only as expected from those obtained for synthetic [^{13}C]$_2$-**1**. Data for xanthohumol (**1**): IR (ATR) 3177, 2914, 1599, 1429, 1340, 1291, 1219, 1166, 1098, 973, 868, 798 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.66 (1H, s), 7.80 (1H, d, *J* = 15.4 Hz), 7.75 (1H, d, *J* = 15.6 Hz), 7.52 (2H, d, *J* = 8.6 Hz), 6.86 (2H, d, *J* = 8.6 Hz), 6.17 (1H, s), 5.94 (1H, s), 5.29 (1H, tm, *J* = 7.2 Hz), 5.03 (1H, s), 3.90 (3H, s), 3.41 (2H, d, *J* = 7.5 Hz), 1.83 (3H, s), 1.78 (3H, s) ppm; ^{1}H NMR (400 MHz, d_6-DMSO) δ 14.61 (1H, s), 10.52 (1H, s), 10.03 (1H, s), 7.76 (1H, d, *J* = 15.6 Hz), 7.67 (1H, *J* = 15.5 Hz), 7.57 (2H, d, *J* = 8.6 Hz), 6.84 (2H, d, *J* = 8.6 Hz), 6.09 (1H, s), 5.14 (1H, tm, *J* = 7.2 Hz), 3.87 (3H, s), 3.14 (2H, d, *J* = 7.0 Hz), 1.70 (3H, s), 1.61 (3H, s) ppm; ^{13}C NMR (100 MHz, d_6-DMSO) δ 191.8 (0), 164.7 (0), 162.5 (0), 160.6 (0), 160.0 (0), 142.6 (1), 130.6 (2C, 1), 130.0 (0), 126.1 (0), 123.9 (1), 123.1 (1), 116.1 (2C, 1), 107.4 (0), 104.7 (0), 91.0 (1), 55.8 (3), 25.5 (3), 21.1 (2), 17.7 (3) ppm.

Note: If present in synthetic [^{13}C]$_2$-**1**, contamination from traces of HCl addition adduct [^{13}C]$_2$-**18** is most obviously revealed in the ^{1}H NMR spectrum by an AA´BB´ system for ArC$\underline{H_2}$C$\underline{H_2}$C(Cl)Me_2 at δ_H (400 MHz, $CDCl_3$) = 2.81 (2H, $\underline{AA}$´BB´ width 16.3 Hz), 1.96 (2H, AA´$\underline{BB}$´ width 16.2 Hz) ppm. The purification protocol described above will ensure that homogenous samples of [^{13}C]$_2$-**1** completely free of [^{13}C]$_2$-**18** are obtained.

[^{13}C]$_3$-Xanthohumol = (*E*)-1,3-[^{13}C]$_2$-6´-[^{13}C]methoxy-3´-(3-methylbuten-2-yl)-2´,4´,4´´-trihydroxychalcone ([^{13}C]$_3$-1). Prepared by analogy to the double-labelled isotopomer of xanthohumol ([^{13}C]$_2$-**1**) from the corresponding triple-labelled [^{13}C]$_3$-**17**. Data for [^{13}C]$_3$-**1**: mp 162-165 °C; IR (ATR) 3178, 2918, 1588, 1575, 1510, 1469, 1436, 1330, 1190, 1167, 1134, 1095, 1050, 970, 821, 798 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.63 (1H, s), 7.79 (1H, ddd, *J* = 15.4, 5.1, 2.2 Hz), 7.76 (1H, ddd, *J* = 157, 15.3, 6.2 Hz), 7.52 (2H, dd, *J* = 7.8, 4.9 Hz), 6.86 (2H, d, *J* = 8.6 Hz), 6.15 (1H, br s), 5.94 (1H, s), 5.30 (1H, tm, *J* = 7.6 Hz), 4.98 (1H, br s), 3.90 (3H, d, *J* = 145 Hz), 3.41 (2H, d, *J* = 6.9 Hz), 1.83 (3H, s), 1.78 (3H, s) ppm; ^{1}H NMR (700 MHz, d_6-DMSO, spectral width limited to 10 to –1 ppm, therefore OH signals not observed) δ 7.76 (1H, ddd, *J* = 15.2, 8.9, 5.3 Hz), 7.67 (1H, ddd, *J* = 158, 15.2, 6.1 ppm), 7.57 (2H, dd, *J* = 8.6, 4.3 Hz), 6.83 (2H, d, *J* = 8.6 Hz), 6.08 (1H, s), 5.14 (1H, tm, *J* = 7.1 Hz), 3.86 (3H, d, *J* = 146 Hz), 3.14 (2H, d, *J* = 7.1 Hz), 1.70 (3H, s), 1.61 (3H, s) ppm; ^{13}C NMR (175 MHz, d_6-DMSO) δ 191.6 (0, enriched), 164.6 (0), 162.4 (0), 160.5 (0), 159.9 (0), 142.5 (1, enriched), 130.5 (2C, 1, d, *J* = 3.5 Hz), 129.9 (0), 126.1 (0, d, *J* = 52 Hz), 123.7 (1, dd, *J* = 68, 54 Hz), 123.0 (1), 115.9 (2C, 1, d, *J* = 4 Hz), 107.3 (0), 104.5 (0, d, *J* = 60 Hz), 90.9 (1), 55.7 (3, enriched), 25.5 (3), 21.0 (2), 17.6 (3) ppm. MS (ES+) *m/z* 358 $(M+H)^+$; HRMS (ES+) *m/z* 358.1634 (calcd. for $^{12}C_{18}{}^{13}C_3H_{23}O_5$: 358.1646).

[^{13}C]$_5$-Xanthohumol = (*E*)-1,3-[^{13}C]$_2$-6´-[^{13}C]methoxy-3´-(1,2-[^{13}C]$_2$-3-methylbuten-2-yl)-2´,4´,4´´-trihydroxychalcone ([^{13}C]$_5$-1). Prepared by analogy to the double-labelled isotopomer of xanthohumol ([^{13}C]$_2$-**1**) from the corresponding

penta-labelled [^{13}C]$_5$-**17**. Data for [^{13}C]$_5$-**1**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.67 (1H, s), 7.79 (1H, ddd, *J* = 15.0, 5.0, 2.2 Hz), 7.76 (1H, ddd, *J* = 155, 15.0, 5.8 Hz), 7.52 (2H, dd, *J* = 8.3, 4.2 Hz), 6.87 (2H, d, *J* = 8.5 Hz), 6.20 (1H, br s), 5.95 (1H, s), 5.30 (1H, dm, *J* = 151 Hz), 5.10 (1H, br s), 3.90 (3H, d, *J* = 145 Hz), 3.40 (2H, dt, *J* = 129, 7.1 Hz), 1.83 (3H, d, *J* = 4.9 Hz), 1.78 (3H, d, *J* = 6.0 Hz) ppm; ^{1}H NMR (700 MHz, d_6-DMSO) δ 14.63 (1H, s), 10.55 (1H, br s), 10.05 (1H, br s), 7.77 (1H, ddd, *J* = 15.2, 8.9, 5.5 Hz Hz), 7.67 (1H, ddd, *J* = 155, 15.4, 6.3 Hz), 7.57 (2H, dd, *J* = 8.6, 4.3 Hz), 6.84 (2H, d, *J* = 8.6 Hz), 6.08 (1H, s), 5.13 (1H, dm, *J* = 152 Hz), 3.86 (3H, d, *J* = 145 Hz), 3.13 (2H, dt, *J* = 128, 6.8 Hz), 1.70 (3H, d, *J* = 4.9 Hz), 1.60 (3H, d, *J* = 5.8 Hz) ppm; ^{13}C NMR (175 MHz, d_6-DMSO) δ 191.5 (0, enriched), 164.6 (0), 162.4 (0), 160.5 (0), 159.9 (0), 142.5 (1, enriched), 130.5 (2C, 1), 129.9 (0, d, *J* = 74 Hz), 126.0 (0, dm, *J* = 63 Hz), 123.7 (1, dd, *J* = 70, 56 Hz), 123.0 (1, d, *J* = 44 Hz, enriched), 116.0 (2C, 1), 107.3 (0, d, *J* = 46 Hz), 104.5 (0, d, *J* = 58 Hz), 90.9 (1), 55.8 (3, enriched), 25.5 (3), 21.0 (2, d, *J* = 44 Hz), 17.7 (3) ppm; MS (ES+) *m/z* 360 $(M+H)^+$; HRMS (ES+) *m/z* 360.1723 (calcd. for $^{12}C_{16}{}^{13}C_5H_{23}O_5$: 360.1713).

Conclusion

The Khupse and Erhardt total synthesis of xanthohumol was re-evaluated and modified to incorporate readily available ^{13}C-labelled starting materials to enable the preparation of [^{13}C]$_2$-, [^{13}C]$_3$-, and [^{13}C]$_5$-isotopomers of this prenylated flavonoid natural product. The labelled xanthohumols were obtained in ca. 6% overall yield via a synthetic route involving a 7 step longest linear sequence from phloroglucinol with 1-[^{13}C]-acetyl chloride, [^{13}C]-iodomethane, and ethyl 1,2-[^{13}C]$_2$-bromoacetate as sources of ^{13}C atoms. The labelled compounds prepared will be useful in experiments to probe the biological activity of xanthohumol and its metabolism *in vivo*.

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Conflict of Interest

The authors did not report any conflict of interest.

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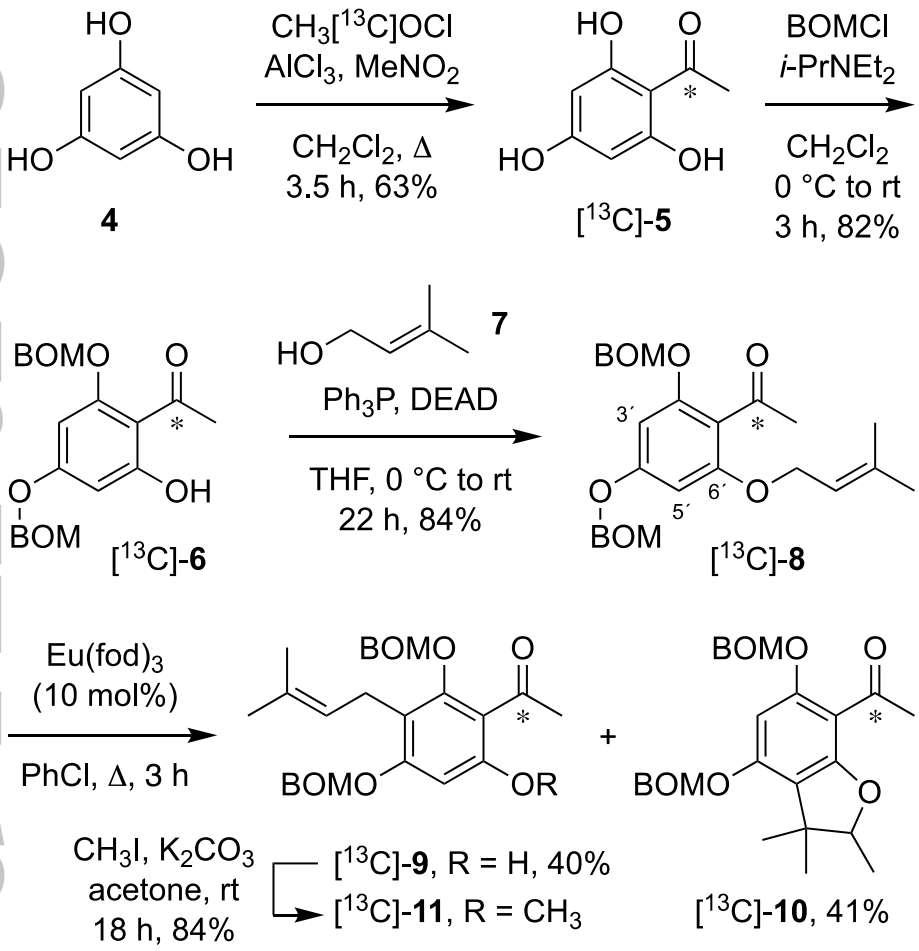
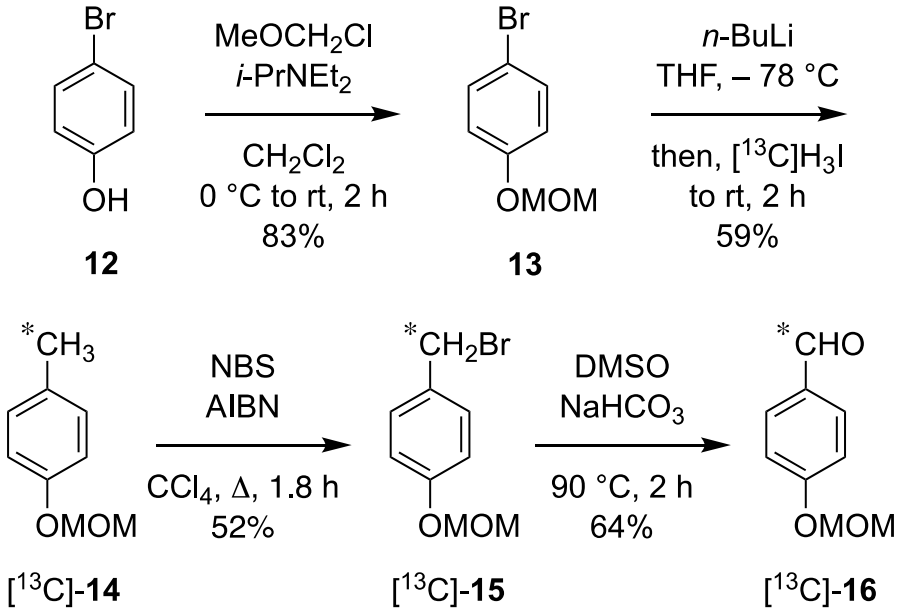
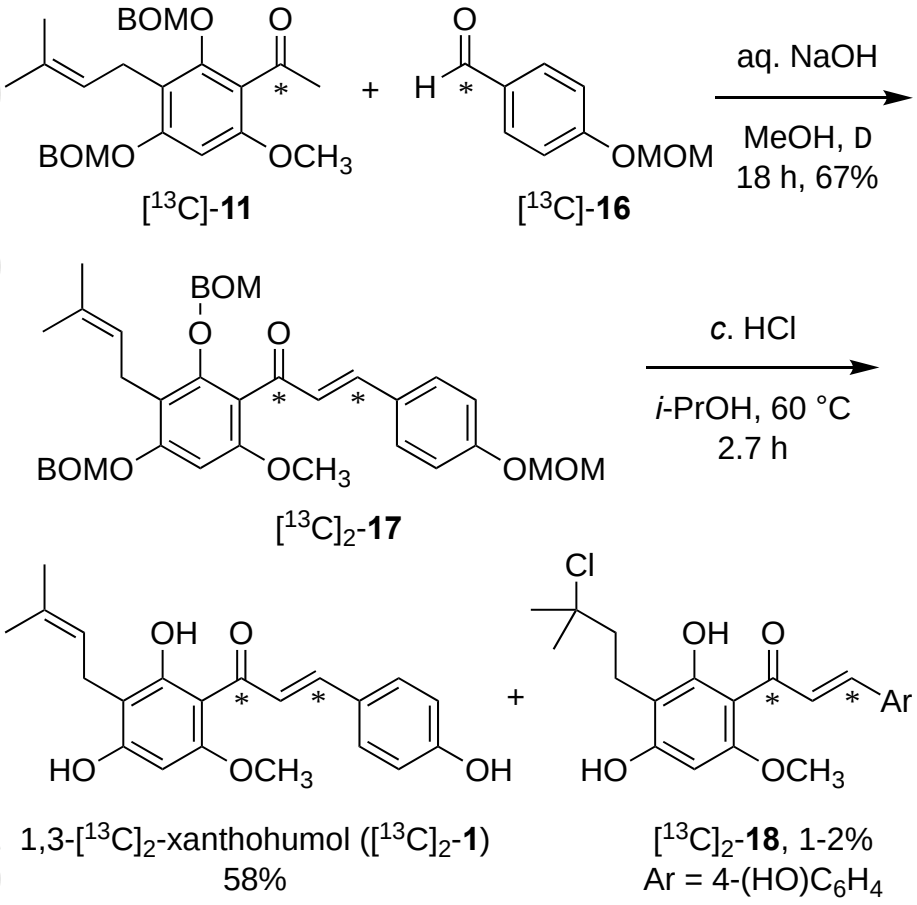


Scheme 1. Synthesis of methyl ketone fragment [^{13}C]-**11**. ∗ = ^{13}C; BOM = benzyloxymethyl; DEAD = diethyl azodicarboxylate; fod = 6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato.



Scheme 2. Synthesis of aldehyde fragment [^{13}C]-**16**. ∗ = ^{13}C; MOM = methoxymethyl; NBS = *N*-bromosuccinimide; AIBN = 2,2´-azobis(isobutyronitrile).



Scheme 3. Completion of synthesis of 1,3-[^{13}C]$_2$-xanthohumol ([^{13}C]$_2$-**1**). ∗ = ^{13}C; BOM = benzyloxymethyl; MOM = methoxymethyl.

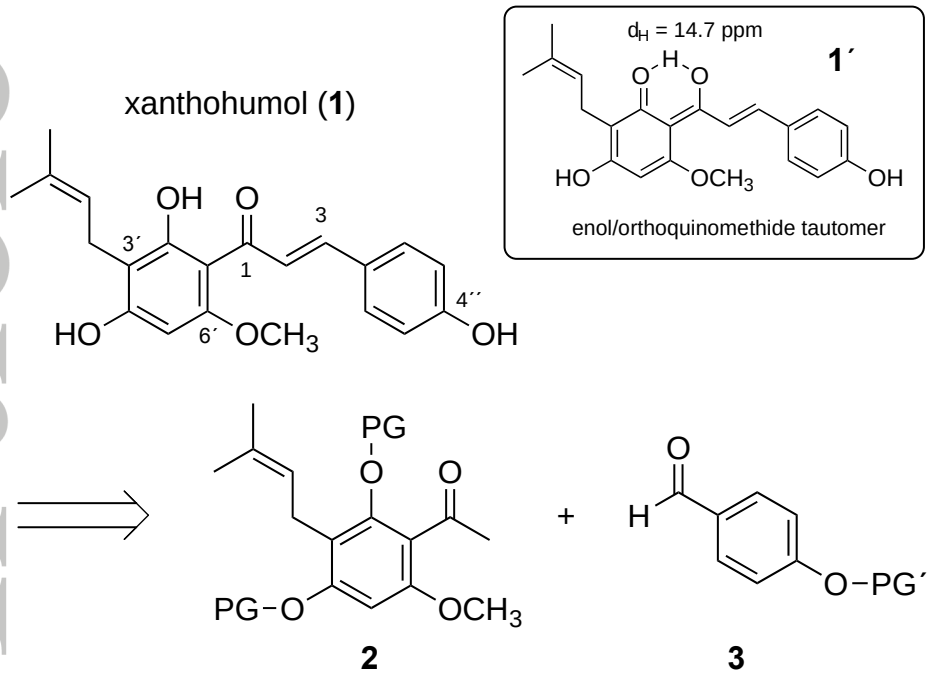
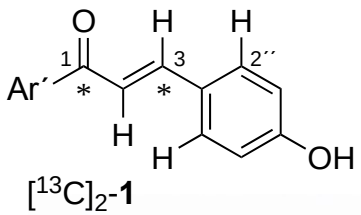


Figure 1. Xanthohumol (**1**) and the classical retrosynthetic disconnection for chalcone synthesis via aldol condensation of a methyl ketone (**2**) and an aryl aldehyde (**3**).



[^{13}C]$_2$-**1**

d_{H2} = 7.79 ppm, ddd, *J* = 15.3, 5.2, 2.4 Hz

d_{H3} = 7.75 ppm, ddd, *J* = 155, 15.5, 6.0 Hz

$d_{H2''}$ = 7.52 ppm, dd, *J* = 8.2, 4.3 Hz

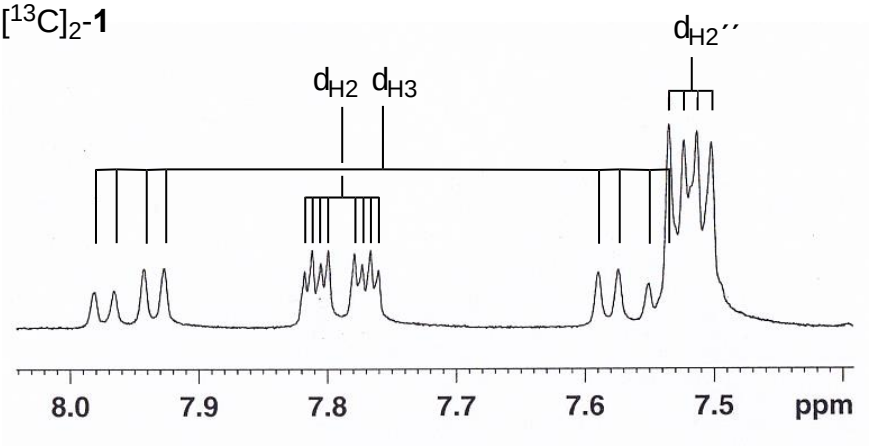
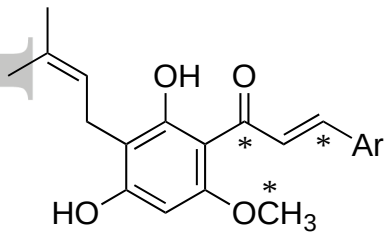
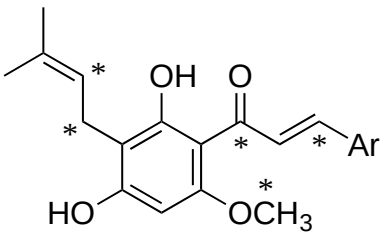


Figure 2. Excerpt of ^{1}H NMR (400 MHz, $CDCl_3$) spectrum for 1,3-[^{13}C]$_2$-xanthohumol ([^{13}C]$_2$-**1**) showing effects of *J*-coupling to ^{13}C-atoms at C1 and C3 by H2, H3, and H2´´. Coupling constants due to interactions with ^{13}C atoms are underlined. ∗ = ^{13}C.



[^{13}C]$_3$-xanthohumol ([^{13}C]$_3$-**1**)



[^{13}C]$_5$-xanthohumol ([^{13}C]$_5$-**1**)

Figure 3. Two additional [^{13}C]-labelled isotopomers of xanthohumol that were prepared by analogy to [^{13}C]$_2$-**1**. Ar = 4-(HO)-C_6H_4.