AN ABSTRACT OF THE THESIS OF

Layhna Plagmann for the degree of Master of Science in Chemistry presented on June 07, 2021

Total Synthesis of Isotopologues of Xanthohumol and its Congeners for Biological Studies

Abstract approved:

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Xanthohumol [XN, (*E*)-6´-methoxy-3´-(3-methylbuten-2-yl)-2´,4´,4´´-trihydroxychalcone], the principal prenylated chalcone from hops, and its 2,3-dihydro- (DXN) and O6-desmethyl-2,3-dihydro- (DDXN) congeners, are of potential utility for the amelioration of metabolic syndrome; however, their complex bioactivity profiles cannot be properly studied and understood without access to appropriately produced synthetic materials.

In the first part of the thesis (Chapter 2), total syntheses of five variously ^{2}H- and ^{13}C-labeled isotopologues of xanthohumol are described. 1,3-[$^{13}C]_2$-Xanthohumol was prepared by an adaptation of the known elaboration by Khupse and Erhardt in seven steps and 6% 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 (14) 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. Substitution of natural abundance iodomethane ($^{12}CH_3I$) for $^{13}CH_3I$, $^{12}CD_3I$, and $^{13}CD_3I$ during the Williamson ether synthesis that installs the 6´-methoxy group from the corresponding phenol enabled

straightforward access to three additional XN isotopologues of +3, +5, and +6 mass units above the monoisotopic value, in addition to the +2 compound already prepared. A penta-^{13}C-labeled XN isotopologue [(*E*)-2,3-[^{13}C]$_2$-6´-[^{13}C]-methoxy-3´-(1,2-[^{13}C]$_2$-3-methylbut-2-enyl)-2´,4´,4´´-trihydroxychalcone], was similarly prepared by using 1,3-[^{13}C]$_2$-prenyl alcohol at the appropriate juncture in addition to the aforementioned ^{13}C-atom containing precursors.

In the second part of the thesis (Chapter 3), the exploration of two different novel approaches to DXN and DDXN that do not employ XN as a precursor is described. In the first approach, an acetophenone enolate alkylation strategy was pursued to forge the central C2-C3 bond; however, this tactic suffers from over-alkylation of the acetophenone by the 4-alkoxybenzyl bromide electrophile. For example, 1-[^{13}C]-DDXN was synthesized in three steps and 2% yield from the previously prepared acetophenone derivative [^{13}C]-**12** by alkylation of its Li-enolate with 4-(methoxymethyloxy)benzyl bromide (33% yield mono alkylation, 9% double alkylation), Eu(fod)$_3$ catalyzed Claisen-Cope rearrangement (44% and 28% of a cyclic ether derived from the Claisen rearrangement adduct), and acid-mediated deprotection (13% and 77% 2´O-BOM DXN). In the second approach, the conversion of the commercially available natural product phloretin [1-(2,4,6-trihydroxyphenyl)-3-(4-hydroxyphenyl)propiophenone] to DXN/DDXN was evaluated via (step-wise) 6´*O*-prenylation followed by Claisen-Cope rearrangement. In each case, difficulties were encountered in attempting to achieve the required level of regiocontrol within the context of selectively chemically manipulating the multifunctional phloretin molecule. It was concluded that DXN and DDXN are better accessed via the known selective hydrogenation of XN to DXN.

Total Synthesis of Isotopologues of Xanthohumol and its Congeners for Biological Studies

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Total Synthesis of Isotopologues of Xanthohumol and its Congeners for Biological Studies

Chapter 1: Hops and the Plant Medicine in Beer

When it comes to flavorful alcoholic beverages, beer and wine are among the quaffable favorites of both casual drinkers and connoisseurs, further encouraged to enjoy a daily glass now that ethanol has been shown to improve heart health when consumed in moderation.[1] However, red wine is alone in receiving additional praise for its many plant-derived polyphenols that have desirable biological properties, and while red wine enjoys fame as purveyor of resveratrol, the highly touted antioxidant with an attractive array of purported health benefits,[2] beer is commonly viewed as an unhealthful guilty pleasure. Health experts and wine enthusiasts alike love to expound on the rich flavonoid content of red wine, but the prevailing recommendation for similarly plant-derived beer is to limit consumption in avoidance of potential detrimental effects.

Recent research has uncovered a plethora of plant-derived compounds in beer, and now the world's most popular alcoholic beverage[3] is joining the ranks of health-conscious libations with some impressive health benefits of its own, thanks to its iconic plant ingredient: hops.

FIGURE 1 *Left*, ripe hop cones at Foothill Hops in Munnsville, N.Y. (photo credit Kate and Larry Fisher);[4] *Right*, a hop cone is split apart to reveal the lupulin glands (photo credit Stephen Ausmus/USDA Image Gallery).[4]

1.1 Chalcones: Intermediates in the Biosynthesis of Flavonoids

Chalcones are a class of polyketides which appear to be found in all plants,[5] and are intermediates in the formation of flavonoids. The phloroglucinol-type chalcones are characterized by a 2′,4′,6′-trihydroxylation pattern, and during flavonoid biosynthesis can be converted to the flavanone naringenin either spontaneously or via chalcone isomerase (Figure 2),[6] naringenin being the biosynthetic precursor to a wide variety of flavonoids and their derivatives.[7,6]

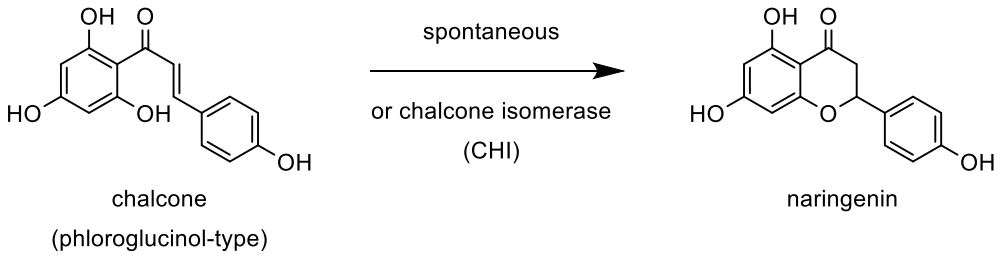


FIGURE 2 Conversion of phloroglucinol-type chalcones to flavonoid biosynthetic precursor naringenin.

1.1.1 Chalcone Structure and Biosynthesis

Chalcones contain two aromatic rings, with a ketone and enone linker (Figure 3). The conjugated α-β-unsaturated ketone functionality is believed to be key to some pathways of biological activity of chalcones,[8] and electronic conjugation across the molecule gives chalcones their characteristic yellow color. The presence and positions of hydroxyl groups and substituents can also determine biological activity, thus chalcones can be multifunctional. [7]

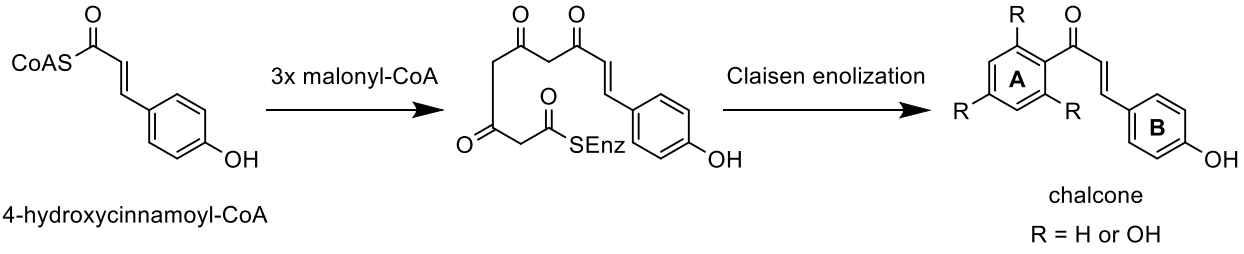


FIGURE 3 Chalcone framework and biosynthesis.

The “A” ring of chalcone is derived from three units of malonyl-CoA, and is often substituted in the 1,3,5 pattern found in phloroglucinol. After chain extension of 4-hydroxycinnamoyl-CoA, the poly-β-keto chain is folded to allow Claisen enolization, affording the basic chalcone structure. Further modifications can be made by a variety of downstream enzymes, occurring either constitutively or in response to environmental stress, such as UV light, wounding, and pathogen infestation.[9]

1.1.2 Chalcones and Related Compounds in Hops

Hops impart the characteristic flavor and bitter taste of beer due to a variety of essential oils and aromatic compounds which are found exclusively in lupulins, the yellow glandular trichomes in the inflorescences (hops) of the female hop plant, *Humulus lupulus* L. (Cannabaceae).[10a] The bittering effect arises from the prenylated acylphloroglucinols which are among the secondary metabolites of the plant, i.e., humulones (α-acids) and lupulones (β-acids).[10a-b]

These so-called “bitter acids” have been shown to exhibit beneficial biological activities, including radical scavenging activity, angiogenesis inhibition, and inducing effect for P450 enzyme.[10a] Alongside these bittering components, hops cones also contain the biosynthetically related prenylated chalcones and prenylflavonoids, which are gaining recent interest due to both their interesting and exciting biological activities and their wide consumption as incidental components of beer.[10b]

1.2 Xanthohumol: A Chalcone from Hops

Xanthohumol (**1**) is the principal prenylated chalcone found in the flowers of the hop plant (Figure 4). Xanthohumol occurs as 0.1-1% of dry weight of hops,[11] while other chalcones can be detected at 10- to 100-fold lower concentrations.[11] 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 (δH = 14.7 ppm) suggest that the true nature of this compound is the fully conjugated enol/orthoquinomethide tautomer (**1'**).[12]

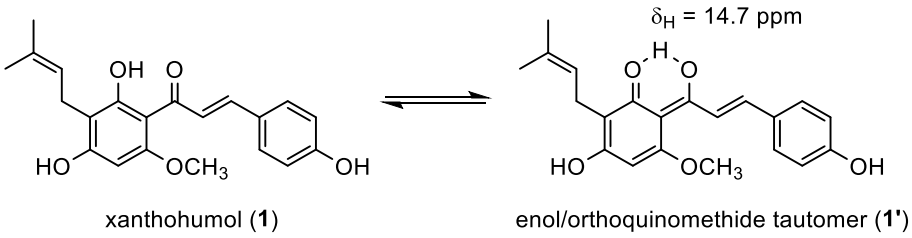


FIGURE 4 Structure of xanthohumol and its likely more significant fully conjugated tautomer.

1.2.1 Discovery, Isolation, and Characterization of Xanthohumol

Xanthohumol was first isolated, partially characterized and named by Power et al. (1913)[13] as part of an effort to determine the chemical constituents of hops, an extension of investigations into the nature of its bitter principles.[13] The structure was elucidated by Verzele et al. (1957),[14] but there remained some doubt as to the position of the prenyl substituent relative to the methoxy group. Two independent groups were able to confirm the originally assigned structure as correct via partial synthesis and chemical degradation studies (Vandewalle, 1961; Orth and Riedl, 1963).[10b,15-17]

1.2.2 Biosynthesis, Biochemistry, and Metabolism of Xanthohumol and its Derivatives

Xanthohumol is formed in lupulin glands by a specialized branch of flavonoid biosynthesis that involves aromatic prenylation and O-methylation of the polyketide intermediate chalconaringenin. (Figure 5). C-prenylation of chalconaringenin with dimethylallyl diphosphate (DMAPP) via *Humulus lupulus prenyltransferase-1* (HlPT-1) provides desmethylxanthohumol, which is then converted to xanthohumol by an O-methyltransferase.[10a,c]

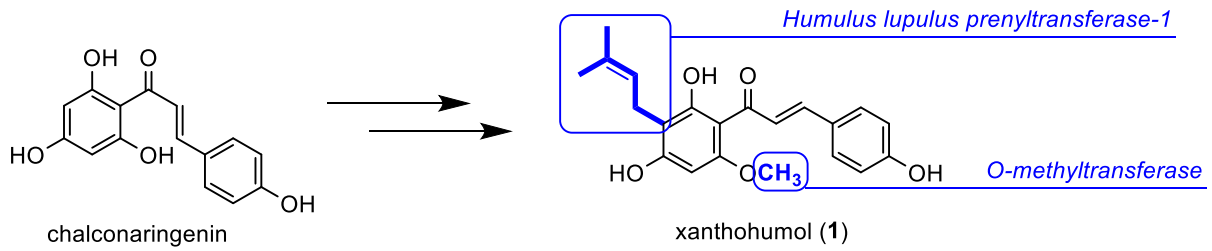


FIGURE 5 Biosynthesis of xanthohumol from chalconaringenin via *Humulus lupulus prenyltransferase-1* and O-methyltransferase.[10a,c]

In vitro and animal studies have established that xanthohumol is non-enzymatically converted into its flavanone isomer, isoxanthohumol (IX), and enzymatically into 8-prenylnaringenin (8-PN), 6-prenylnaringenin (6PN), and desmethylxanthohumol (DMX)[18,19] (Figure 6). Therefore, the metabolic pathways of xanthohumol might play a sizeable role in the ultimate bioactivity of the prenylated chalcone.[11]

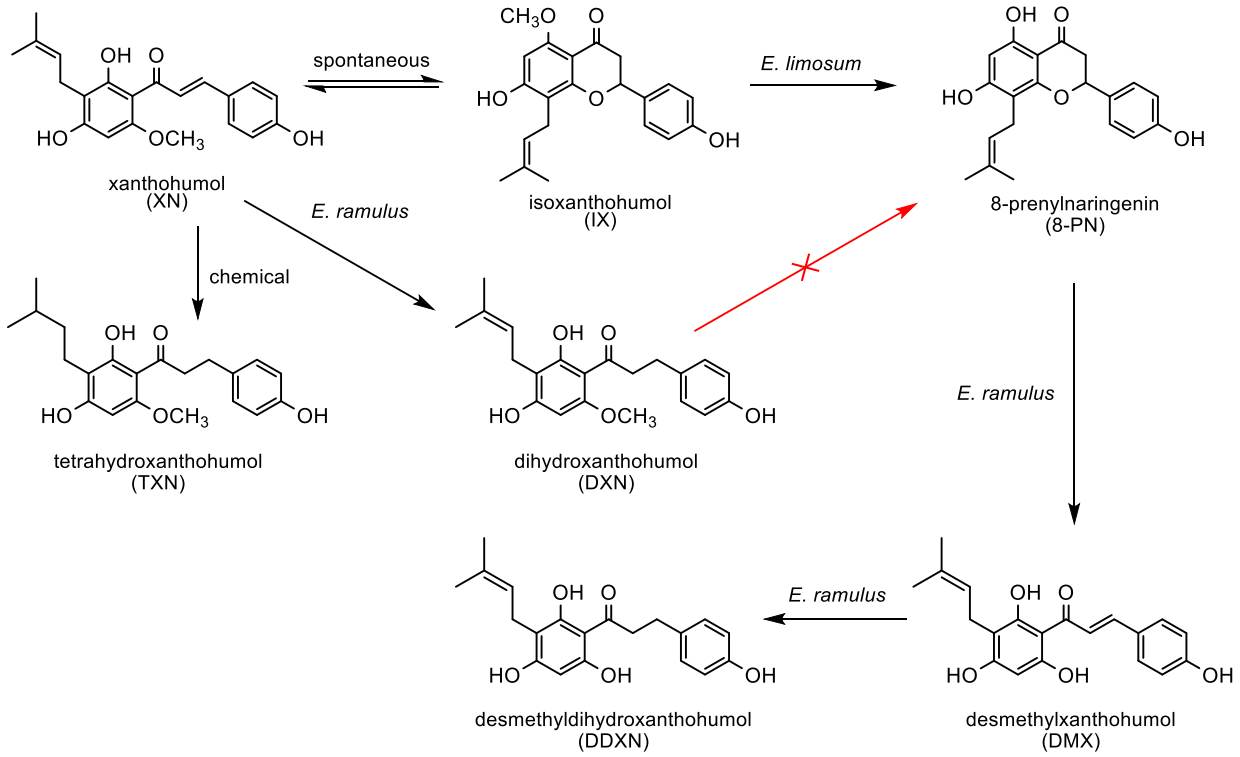


FIGURE 6 Metabolism of xanthohumol and conversion to derivatives of interest.[11,20]

The emerging consensus is that the gut microbiota may play a crucial role in the potential health benefits of flavonoids.[21,22] There are only a few studies, however, investigating the influence of the intestinal microbial community in the metabolism of xanthohumol and derivatives.[19,23–24] Findings suggest that the bioavailability of xanthohumol, defined as the fraction of the administered dose that is ultimately absorbed intact, is very low, possibly due to extensive intestinal metabolism by gut microorganisms.[10b]

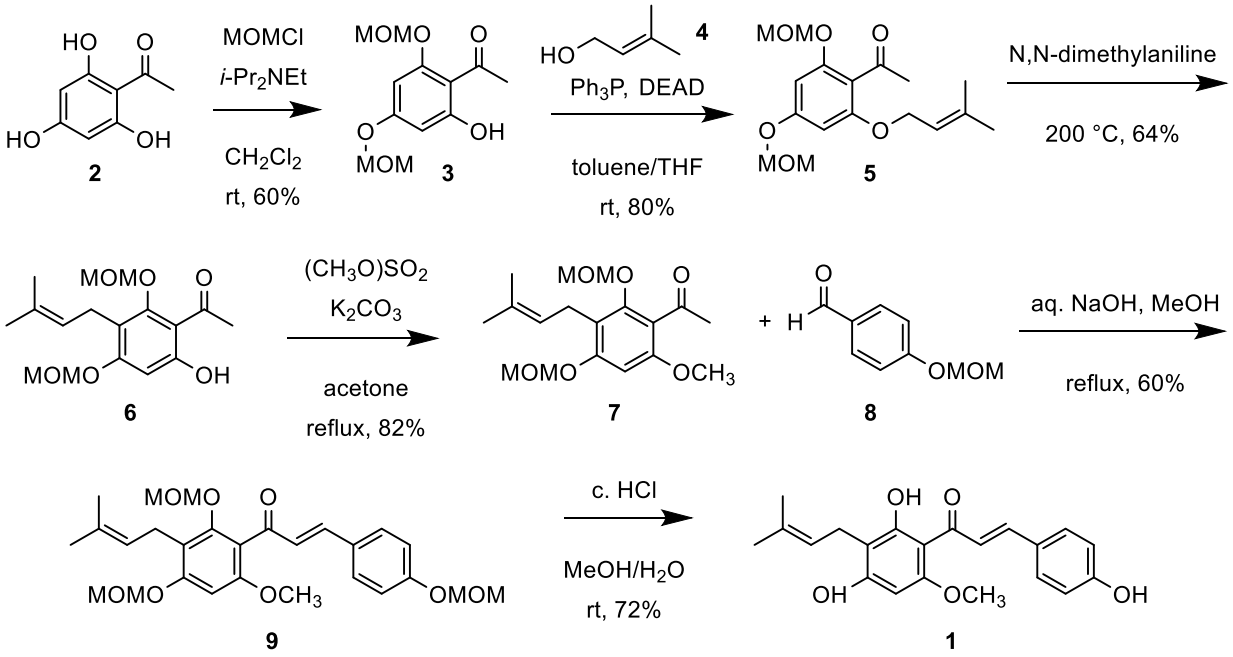
1.2.3 Bioactivity and Biological Implications of Xanthohumol and its Derivatives

Xanthohumol has been shown to exhibit anti-obesity effects,[25] cancer chemopreventive,[26a-b,27] cytotoxic,[27] and antitumor properties,[28] and cognitive function improvement,[11,29] among other important biological activities. Of note are the hormonal effects of xanthohumol due to its *in vivo* estrogenic activity[30,31] via metabolic conversion to 8-prenylnaringenin (8-PN), the most potent phytoestrogen currently known.[26b] In addition to estrogenic effects, 8-PN also prevents bone loss in rats,[32] inhibits angiogenesis[33] and metastasis,[34] and exhibits anti-androgenic activity.[35,11]

Concern has been raised about the amount of 8-PN produced from intake of xanthohumol,[11] and attention has turned to xanthohumol derivatives lacking the α-β-unsaturated ketone functionality, which enables cyclization to IX and further conversion to the phytoestrogen (Figure 6). Such derivatives could potentially allow access to some of the important biological activity of xanthohumol in the absence of estrogenic activity when undesired. DXN and TXN are of particular interest due to their demonstration of effects on glucose tolerance comparable to that of xanthohumol.[20] DDXN is another desired target, and possibly more practical than DXN owing to the likelihood of rapid cleavage of the methyl group during metabolism.

1.2.4 Total Synthesis of Xanthohumol by Khupse and Erhardt (2007)

Although a synthesis of a dimethylated congener of xanthohumol was reported quite some time ago,[36] a total synthesis of this important chalcone, an effort by Khupse and Erhardt, did not appear until 2007.[8,37] The previously low interest in the synthesis of this compound is probably related to the availability of xanthohumol from sources such as CO_2-extracted hops, a waste product of the hop processing industry.[10b] Between 1998 and 2004, biological properties of xanthohumol had been identified by *in vitro* studies,[10b] prompting development of studies directed toward dietary supplementation and further biological characterization of purified xanthohumol at the *in vivo* level.[10b] While xanthohumol is available as a byproduct of hops processing, obtaining pure material from natural sources requires a multistep process that typically involves extraction, chromatography, precipitation, and crystallization.[17] The first total synthesis was motivated by a need to access pure xanthohumol for *in vivo* studies.



SCHEME 1 Khupse and Erhardt xanthohumol total synthesis via aldol condensation.

The Khupse-Erhardt elaboration uses a late-stage aldol condensation between a methyl ketone fragment **7** and an aryl aldehyde fragment **8** to generate a protected form of the chalcone natural product **1** (Scheme 1). This coupling strategy follows a classical retrosynthetic disconnection for chalcone synthesis, and requires substituents on the A ring of xanthohumol are put in place on the ketone fragment prior to the coupling step, leaving only a deprotection step to access the desired target once the two halves are assembled.[17] The thermal rearrangement to access the phenol **6** is a key step in the regioselective installation of the prenyl group, which appears to proceed efficiently in the Khupse-Erhardt synthesis but in our hands under slightly modified conditions proved to be a challenge.

1.3 Aims of This Work

Even at this relatively early stage of investigation, xanthohumol is already shown to exhibit a wide array of potentially valuable biological activities, making the prenylated chalcone and its derivatives important compounds worthy of further study. 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.[12] These and other questions can be addressed with synthetic analogues.

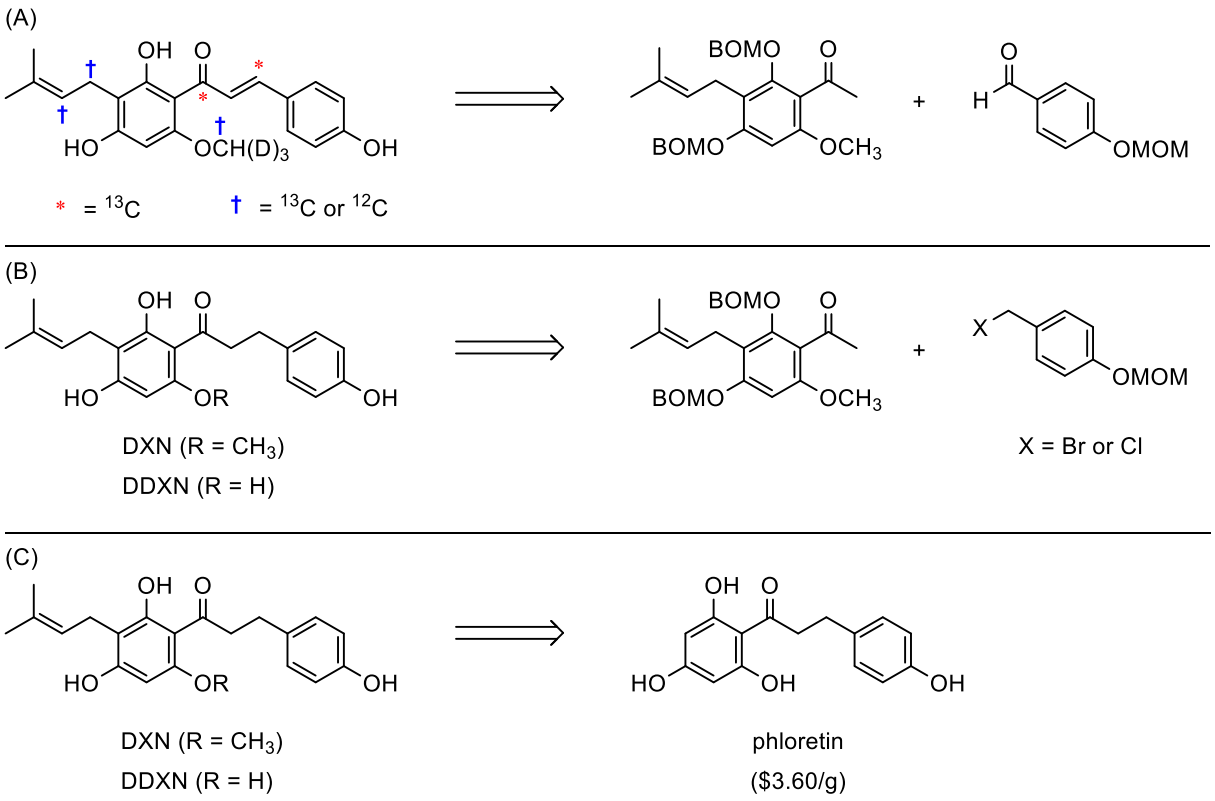


FIGURE 7 Retrosynthetic plans for (A) xanthohumol isotopologues via aldol condensation of ketone and aldehyde fragments; (B) xanthohumol derivatives via alkylation of ketone and aryl alkyl halide fragments; (C) xanthohumol derivatives via conversion of the natural product phloretin.

The simple chalcone structure of xanthohumol makes the prospect of preparing analogues for SAR and other experiments feasible. In this work we have designed and prepared a variety of xanthohumol isotopologues (Figure 7A) intended for use in metabolic feeding studies as well as standards for isotope dilution studies. As discussed in Chapter 2, we first realize a synthesis of $^{13}C_2$-xanthohumol with a carbon label installed on either side of the unsaturated bond to ensure the presence of a label in fragments that could potentially arise from cleavage of the bond during metabolism. We then additionally prepare four other isotopologues incorporating a varying combination of ^{13}C and ^{2}H labels in strategically convenient positions to enable access to xanthohumol isotopologues of +2, +3, +5, and +6 mass units above the monoisotopic value.

With an eye toward limiting undesired biological effects of potential disease treatment uses, we have also investigated two synthetic routes to some important xanthohumol derivatives which

lack the α-β-unsaturated ketone functionality and are thus unable to cyclize into 8-PN. We first envisioned a fragment coupling route via alkylation of the same or similar methyl ketone fragment which was employed in our xanthohumol synthesis (Figure 7B), our investigation of which is addressed in Chapter 3. Our initial efforts were focused on DDXN, a xanthohumol derivative required by our coworkers for the identification of intestinal metabolites of xanthohumol.[11] Although we discovered the alkylation step to be susceptible to over-alkylation and thus low yielding, in addition to the already troublesome prenyl rearrangement, we successfully used this strategy to access ^{13}C-labeled DDXN, and the associated biological investigation used the compound described in Chapter 3.

Finally, we were inspired by the similar chalcone structure of phloretin, which already contains the key C-C bond and so would avoid the need for the low-yielding alkylation step, to investigate a route to our desired dihydroxanthohumol derivatives from this inexpensive and commercially available natural product (Figure 7C). Although a fragment coupling step is not required, this route nonetheless faces the same rearrangement challenge present in the other syntheses. Our primary aim for a strategy from phloretin was to enable access to the analogous O-prenyl precursor from which the Claisen-Cope rearrangement and subsequent deprotection has already been investigated in our DDXN synthesis. Our work toward accessing this prenylated intermediate from phloretin is described in Chapter 3.

Chapter 2: Total Synthesis of ^{13}C-labeled Xanthohumol Isotopologues

The description of the work in this chapter is adapted from the peer-reviewed publication *Total synthesis of [^{13}C]$_2$-, [^{13}C]$_3$-, and [^{13}C]$_5$-isotopomers of xanthohumol, the principal prenylflavonoid from hops*, Duncan C. Ellinwood, Mohamed F. El-Mansy, Layhna S. Plagmann, Jan F. Stevens, Claudia S. Maier, Adrian F. Gombart, Paul R. Blakemore; Journal of Labeled Compounds and Radiopharmaceuticals 2017; 60:639-648; DOI: 10.1002/jlcr.3571.[12]

2.1 Total Synthesis of [^{13}C]$_2$-Xanthohumol Isotopologue

In connection with the ongoing studies of its biological activity,[38-40,25] we required ^{13}C-labeled isotopologues of xanthohumol as probe molecules to track the fate of xanthohumol and its downstream metabolites in a mouse model for metabolic syndrome. We desired access to xanthohumol isotopologues with at least two ^{13}C-atoms distributed within different regions of the molecule such that metabolic bond cleavage reactions could be easily detected. 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,[41] we elected to adapt it for the synthesis of [^{13}C]$_2$-, [^{13}C]$_3$-, and [^{13}C]$_5$-xanthohumol isotopologues using conveniently obtained isotopically enriched starting materials.[42]

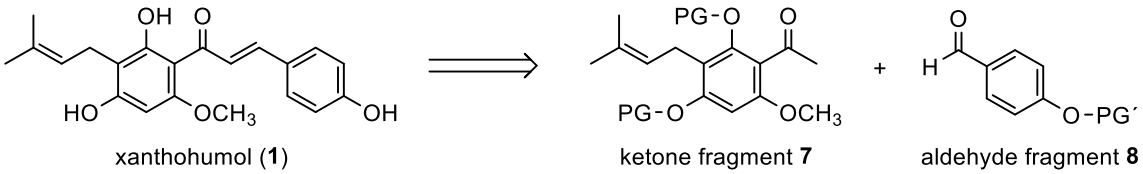


FIGURE 8 Classical retrosynthetic disconnection for chalcone synthesis via aldol condensation of a methyl ketone (**7**) (PG = MOM) and an aryl aldehyde (**8**) (PG′ = MOM).

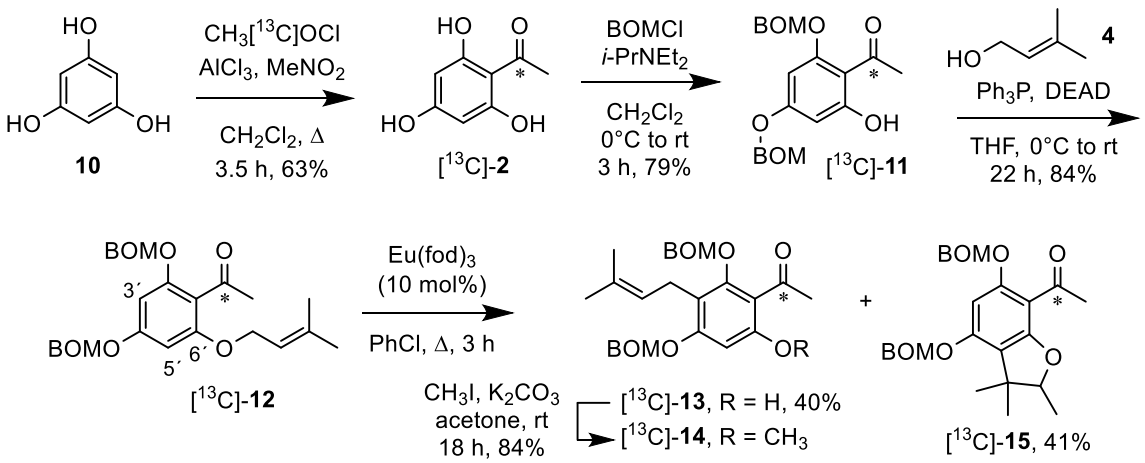
The first target of interest was 1,3-[^{13}C]$_2$-xanthohumol ([^{13}C]$_2$-**1**), and its synthesis called for suitably protected methyl aryl ketone **7** and aryl aldehyde **8** fragments with ^{13}C labels at their respective carbonyl group carbon atoms, which would result in the desired compound with labels installed on either side of the α-β unsaturated bond. 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 required to install MOM versus BOM groups. BOM protecting groups served well for the requisite methyl aryl ketone compound ([^{13}C]-**14**), 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, **10**) with 1-[^{13}C]-acetyl chloride (Scheme 2).[43] As anticipated based on the comparable known reaction with MOMCl,[17] exposure of the so-generated labeled phloracetophenone [^{13}C]-**2** with an excess of BOMCl resulted in alkylation of only two of the three phenolic hydroxyl groups to yield bis-BOM ether [^{13}C]-**11**. 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,[44] phenol [^{13}C]-**11** was next converted into its prenyl ether derivative [^{13}C]-**12**. As previously observed by Khupse and Erhardt,[17] 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]-**12** 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;[17,45] however, Fang et al[41] reported only a 31% yield for the exact same transformation. In the case of [^{13}C]-**12**, 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]-**13**, 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]-**13**. 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).

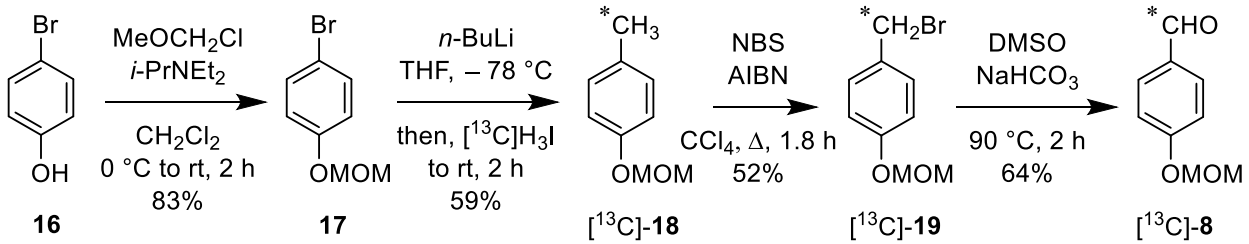
After further experimentation, the Eu(III)-catalyzed rearrangement conditions of Metz et al[46-48] were found to consistently afford [^{13}C]-**13** in approximately 40% isolated yield even on multigram scale. Unfortunately, [^{13}C]-**13** was the minor product formed using Eu(fod)$_3$ (resolve-Al) catalysis, and the bulk of the mass balance was dihydrobenzopyran derivative [^{13}C]-**15**, an apparent result of oxymetalation/protodemetalation from the putative intermediate C5′ isoprenylated arene. The spontaneous formation of dihydrobenzofurans such as [^{13}C]-**15** following Claisen rearrangement of O-prenyl arenes has been observed previously under a variety of different reaction conditions.[49-52] Resubjection of dihydrobenzopyran [^{13}C]-**15** to the reaction conditions did not result in its further conversion to [^{13}C]-**13**. Methylation of [^{13}C]-**13** was uneventful and gave a good yield of the requisite methyl ketone fragment [^{13}C]-**14** under standard conditions.



SCHEME 2 Synthesis of methyl ketone fragment [^{13}C]-**14**.* = ^{13}C.BOM: benzyloxymethyl; DEAD: diethyl azodicarboxylate; fod: 6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato.

Prenylated chalcones have been successfully accessed by Suzuki-Miyaura cross-coupling of appropriate aryl iodides with prenyl boronic esters.[53] Accordingly, we briefly explored an alternate preparation of [^{13}C]-**14** via iodination of [^{13}C]-**11** followed by cross-coupling etc. This plan was thwarted with the discovery that iodination of [^{13}C]-**11** (N-iodosuccinimide, DMF, room temperature, 4.5 h, 78% yield) occurred exclusively at the undesired C5′ position while iodination of the O-methylated derivative of [^{13}C]-**11** gave a 62:38 mixture of regioisomeric aryl iodides, favoring again the undesired C5′ halogenated adduct.

Our preferred strategy to a suitable labeled aryl aldehyde fragment, [^{13}C]-**8**, involved benzylic oxidation of MOM protected p-cresol [^{13}C]-**18** such that [^{13}C]-methyl iodide could provide a convenient and inexpensive source of the ^{13}C-atom (Scheme 3). Accordingly, [^{13}C]-**18** was prepared from bromobenzene **17** 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]-**18** to the target aldehyde [^{13}C]-**8** was attempted both via an IBX-mediated oxidation (≤10% yield [^{13}C]-**8**)[54] and via a TEMPO/Co(OAc)$_2$-catalyzed oxidation (no reaction),[55] but neither method gave an acceptable result.



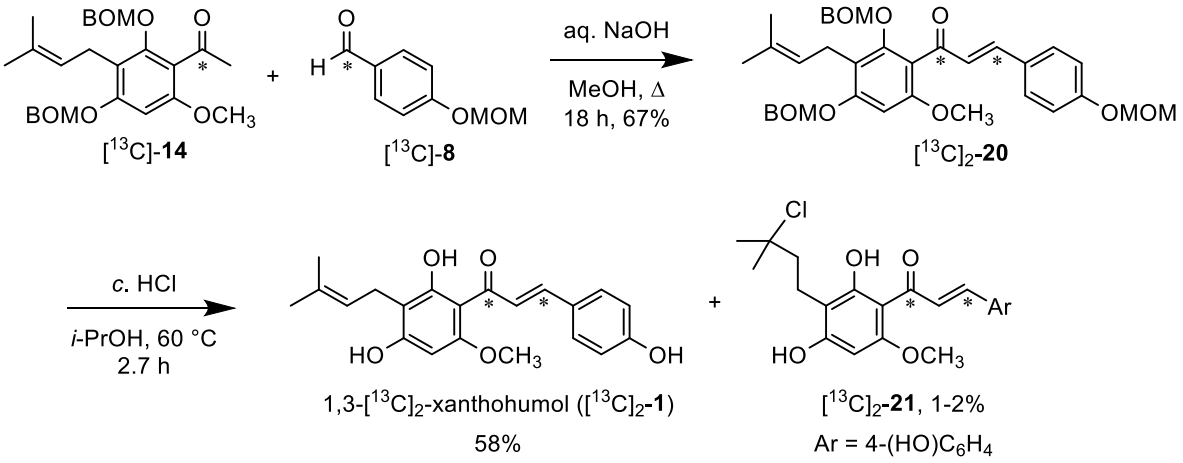
SCHEME 3 Synthesis of aldehyde fragment [^{13}C]-**8**. * = ^{13}C. MOM, methoxymethyl; NBS, N-bromosuccinimide; AIBN, 2,2′-azobis(isobutyronitrile).

Instead, [^{13}C]-**18** was successfully converted to aldehyde [^{13}C]-**8** over two steps by using a Wohl-Ziegler radical bromination[56] followed by a Kornblum oxidation.[57] The intermediate bromide [^{13}C]-**19** (which was cogenerated 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]-**8**. 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 4). Minor modifications to the original Khupse-Erhardt procedures were required to achieve this completion in an acceptable overall yield. For example, possibly because of the heightened lipophilicity of bis-BOM group protected ketone [^{13}C]-**14**, aldol condensation between this compound and aryl aldehyde [^{13}C]-**8** in basic aqueous methanol required considerably longer (16 h) to achieve than the 4 hours reported for the analogous reaction involving bis-MOM group protected ketone **7** (PG = MOM).[17]

In the case of the final deprotection step, we found the conditions used by Khupse and Erhardt to remove three MOM groups (c. HCl, aq. MeOH, room temperature) did not work well (≤20% yield) for the hydrolysis of two BOM groups and one MOM group from aldol reaction product [^{13}C]$_2$-**20**. 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% to 60% yield of the desired labeled xanthohumol [^{13}C]$_2$-**1**[58]; however, small quantities of HCl addition adduct [^{13}C]$_2$-**21** were cogenerated alongside the desired target.



SCHEME 4 Completion of synthesis of 1,3-[^{13}C]$_2$-xanthohumol ([^{13}C]$_2$-**1**). * = ^{13}C.
BOM, benzyloxymethyl; MOM, methoxymethyl

Alkene [^{13}C]$_2$-**1** and chloroalkane [^{13}C]$_2$-**21** 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]$_2$-**21** and any other contaminants from the aforegoing chemistry (see Chapter 5 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 was 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 DMSO-d_6) 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 ^1H NMR spectrum (Figure 9).

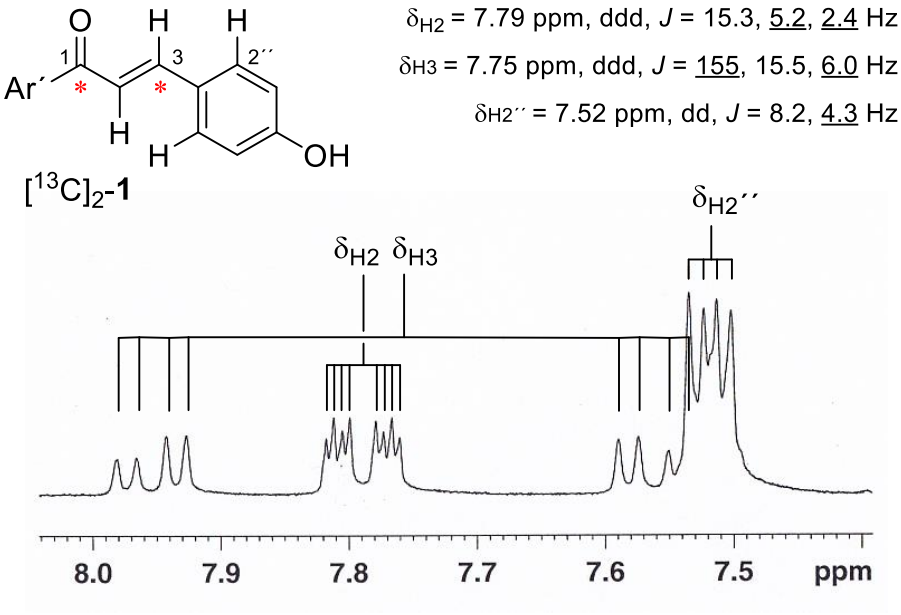


FIGURE 9 Excerpt of ^1H NMR (400 MHz, $CDCl_3$) spectrum for 1,3-[$^{13}C]_2$-xanthohumol ([$^{13}C]_2$-**1**) showing effects of J-coupling to ^{13}C-atoms at C1 and C3 by H2, H3, and H2″. Coupling constants due to interactions with ^{13}C atoms are underlined. * = ^{13}C.

2.2 Total Synthesis of [$^{13}C]_3$-, [$^{13}C]_5$-, [$^2H]_3$[$^{13}C]_2$-, and [$^2H]_3$[$^{13}C]_3$-Xanthohumol Isotopologues

In addition to the strategically labeled isotopologues for feeding studies, we also desired access to more highly labeled isotopologues to be used as standards for mass spectrometric isotope dilution analyses, including up to four differently labeled isotopologues to serve as an automatic

internal calibration curve. We elected to take advantage of the ease of the late stage methylation as a facile strategy to incorporate additional labels onto the methoxy position. While this strategy was impractical for the isotopologues intended for feeding experiments, as the methoxy group is likely lost right away in metabolism, it was convenient for preparing our desired analytical standards for which the loss of the methoxy group is not a concern.

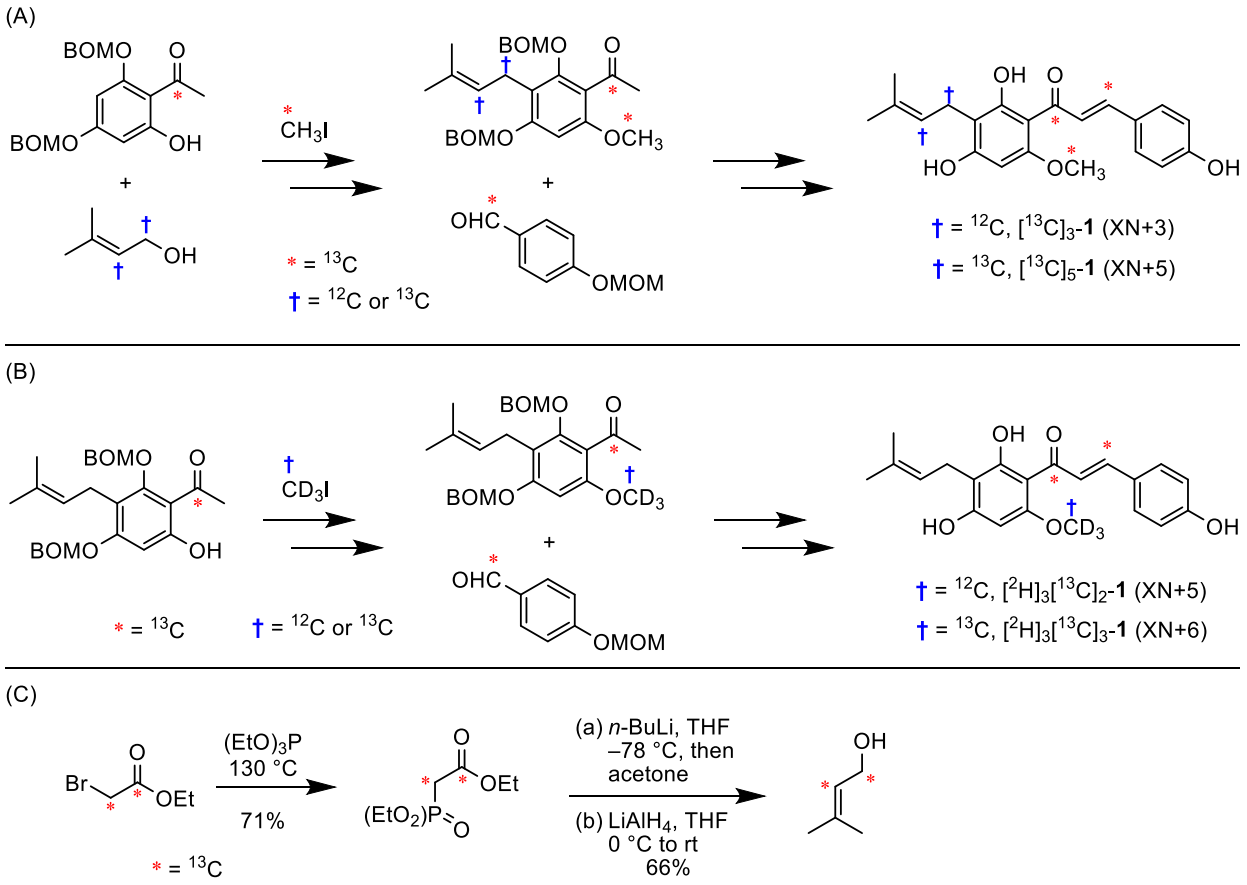


FIGURE 10 (A) $^{13}CH_3I$ and ^{13}C-labeled prenyl alcohol as ^{13}C-enriched reagents in the synthesis of [^{13}C]$_3$- and [^{13}C]$_5$-**1**; (B) CD_3I and $^{13}CD_3I$ as ^{13}C- and ^{2}H-enriched reagents in the synthesis of [^{2}H]$_3$[^{13}C]$_2$- and [^{2}H]$_3$[^{13}C]$_3$ -**1**; (C) Synthesis of $^{13}C_2$-labeled prenyl alcohol (1,2-[^{13}C]$_2$-3-methyl-2-buten-1-ol).

We designed our first two more highly labeled ^{13}C isotopologues of xanthohumol to be 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. As reported in the above publication, tri-^{13}C-labeled xanthohumol ([^{13}C]$_3$-**1**) (XN+3) was synthesized by using [^{13}C]-iodomethane in the

phenol methylation step (*c.f.* [^{13}C]$_2$-**13** to [^{13}C]$_2$-**14**, Scheme 2). To access penta-^{13}C-labeled xanthohumol ([^{13}C]$_5$-**1**) (XN+5), [^{13}C]-iodomethane was again incorporated into the phenol methylation step and additionally 1,2-[^{13}C]$_2$-3-methyl-2-buten-1-ol, prepared by the method of Lugtenburg[59] from ethyl [1,2-^{13}C$_2$]bromoacetate (Figure 10C), was used instead of unlabeled prenyl alcohol (**7**) in the Mitsunobu reaction (*c.f.* [^{13}C]$_2$-**11** to [^{13}C]$_2$-**12**, Scheme 2). As seen for the doubly labeled isotopologue [^{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.

We designed an additional two isotopologues by incorporating both ^{13}C- and ^{2}H-enriched materials. Unlike ^{13}C isotopes, deuterium is unsuitable for use in metabolic probe molecules as it can have varying HPLC retention times. Additionally, H positions on a molecule can potentially wash out when subjected to acidic conditions, which is not a risk with C positions. These characteristics are of no concern in isotopic dilution analysis, so incorporating ^{2}H isotopes into our synthesis provides another convenient way to access a wider variety of isotopologues.

After publication, we prepared our two other highly labeled isotopologues via the same synthetic route by incorporating deuterium on the methoxy group. [^{2}H]$_3$[^{13}C]$_2$-**1** (XN+5) and [^{2}H]$_3$[^{13}C]$_3$-**1** (XN+6) were similarly prepared by methylating the ketone fragment using D$_3$-iodomethane and [^{13}C]-D$_3$-iodomethane, respectively.

Chapter 3: Dihydro- and Desmethyl-Dihydro-Xanthohumol

Due to the interest in xanthohumol derivatives which lack the ability to cyclize into 8-PN, we turned our attention to α,β-dihydro-xanthohumol (DXN), the closest of these relatives in that it differs only in lacking the α-β unsaturated bond. As shown in Figure 11A, DXN can be accessed by catalyzed hydrogenation of xanthohumol,[20] while desmethyl-dihydro-xanthohumol (DDXN), the likely more abundant metabolite of DXN, could potentially be accessed by ether cleavage of DXN.

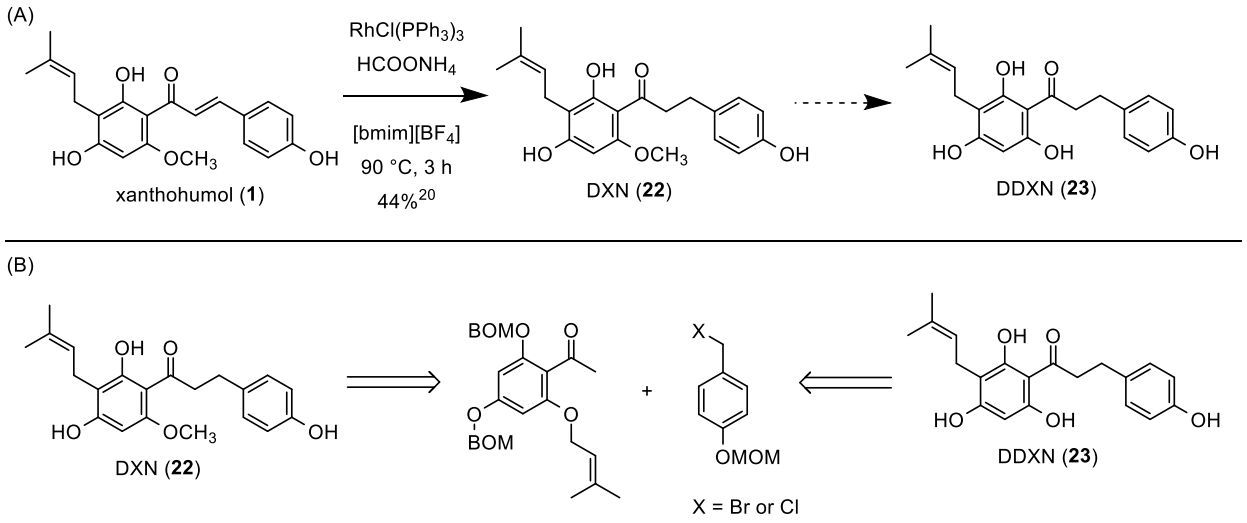


FIGURE 11 (A) DXN from catalytic hydrogenation of xanthohumol[20], and potential further conversion to DDXN. [bmim][BF4], 1-butyl-3-methylimidazolium tetrafluoroborate ionic liquid; (B) Retrosynthetic plan for fragment alkylation route to DXN and DDXN.

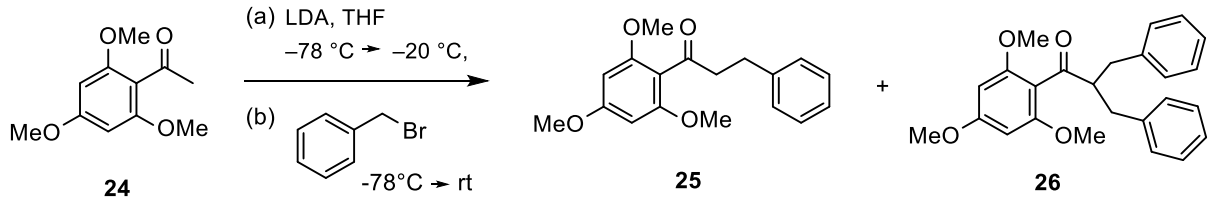
While xanthohumol is somewhat available as a byproduct of the beer brewing industry, we desired a strategy that would not only give efficient access to the desired congeners from more abundant and readily available starting materials, but also enable us to directly access isotopically labeled analogues of DXN and DDXN should the need arise for further studies. Given our success with our previously reported fragment coupling strategy toward xanthohumol, we envisioned a similar route to access DXN and DDXN via alkylation of the ketone fragment with a suitable aryl alkyl halide (Figure 11B).

3.1 Synthetic Route to Dihydroxanthohumols via Fragment Alkylation

We first set out to prepare isotopically labeled DDXN required by our coworkers for further metabolic studies[11] by a fragment coupling route similar to the strategy we developed for xanthohumol. We expected this route to proceed smoothly to the desired xanthohumol derivative as it begins with almost identical coupling fragments to which our xanthohumol isotopologue synthesis added the ^{13}C labels.

3.1.1 Optimization of Key Alkylation Step

We envisioned an alkylation coupling between a ketone and alkyl halide to form the key saturated α,β C-C bond (Figure 11B). LDA alkylation of a model ketone with commercially available benzyl bromide resulted in an expected mixture of mono- and di-alkylation products. After some optimization, we found using 1.6 equivalents each of LDA and the benzyl bromide gave us our best yield of 33% desired mono-alkylated product, with only 6% undesired di-alkylated product and 61% recovered starting material, which could be resubjected to the same alkylation conditions. (Scheme 5)



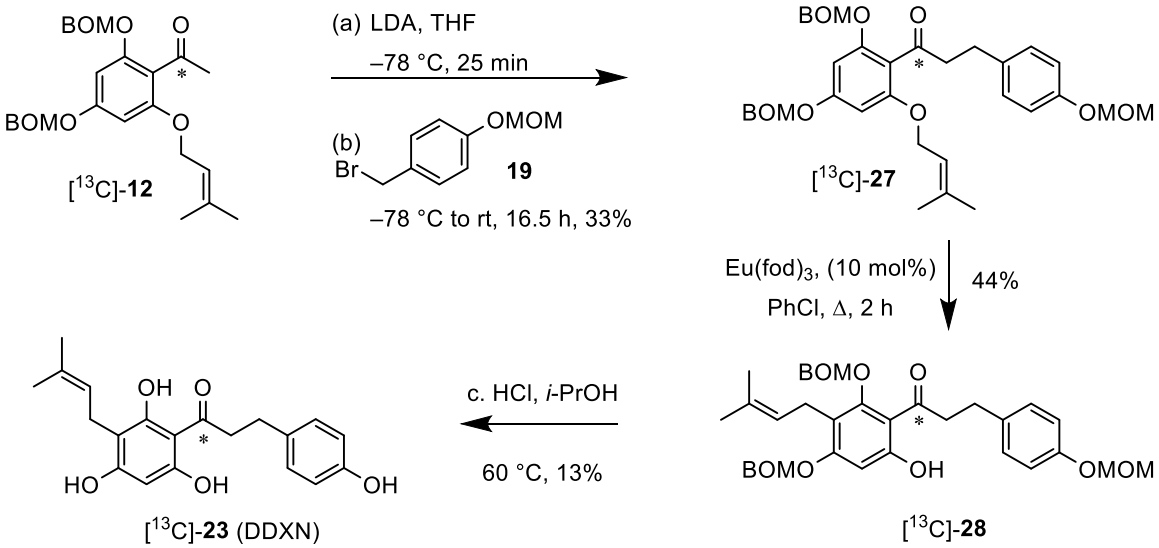
LDA/BnBr equivalent

	starting material	*mono-alkylated*	*di-alkylated*
1.2 / 1.6	26%	53%	21%
1.2 / 1.0	31%	54%	15%
1.6 / 1.6	61%	33%	6%

SCHEME 5 Model optimization studies of the key alkylation step toward the α,β-saturated bond in dihydroxanthohumols.

3.1.2 Total Synthesis of Desmethyldihydroxanthohumol (DDXN)

When we came to consider how to efficiently execute this plan, we realized prenyl rearrangement of the ketone fragment would further require O-methylation as protection on the third hydroxyl group during the alkylation, and in the case of DDXN as the target would then require demethylation after coupling. We instead decided to employ the prenyl group as a protecting group rather than rearranging prior to coupling as originally conceived, using the requisite prenyl ether intermediate **12** as a sensible alkylation partner, which we expected to couple with freshly prepared aryl alkyl halide **19** (Scheme 6). Cognizant of potential side reaction interference which similarly favored MOM- over BOM-protection of the aryl aldehyde in our xanthohumol synthesis, we also chose to MOM-protect this alkyl halide fragment.



SCHEME 6 Total synthesis of [^{13}C]-DDXN via fragment alkylation route.

With the O-prenyl saturated chalcone analogue in hand, we proceeded with the Eu(fod)$_3$-catalyzed Claisen-Cope rearrangement using the same conditions screened in our xanthohumol synthesis and found the prenyl rearrangement performed as expected in similar yield. Unoptimized deprotection at this stage with HCl afforded DDXN in 13% yield, which was used by our coworkers in collaboration on the investigation of the effects of microbial metabolism of xanthohumol and derivatives.[11] We were unable to obtain a higher deprotection yield for this

compound, as the reported conditions gave a large amount of remaining mono-BOM substituted product (~70% of mass balance), and reacting for longer times resulted in decomposition.

From the rearranged intermediate **28** we expect methylation followed by deprotection could afford our initial target DXN. However, given the low yield of the alkylation step combined with the already low-yielding rearrangement and emergent difficulties with deprotection, we ultimately did not pursue completion of this initial plan.

3.2 Investigation of Conversion of Phloretin to Dihydroxanthohumols

Phloretin is an abundant and commercially available natural product which is found in apple tree leaves, and as a saturated chalcone with the same hydroxy substitution pattern as DXN, could serve as an ideal starting point for a simple synthesis. We were motivated to explore a possible route from phloretin in order to avoid the overalkylation and low yield of the key fragment coupling step of our previously attempted DDXN synthesis.

With the chalcone framework already in place, a route from phloretin avoids the need for alkylation, but is not without complications. Such a strategy would require a selective protecting group pattern of the four phenols, exactly three of which need to be protected in the correct positions. While this presents its own challenge, a sufficiently high yield of the properly protected compound could provide a superior route to our target.

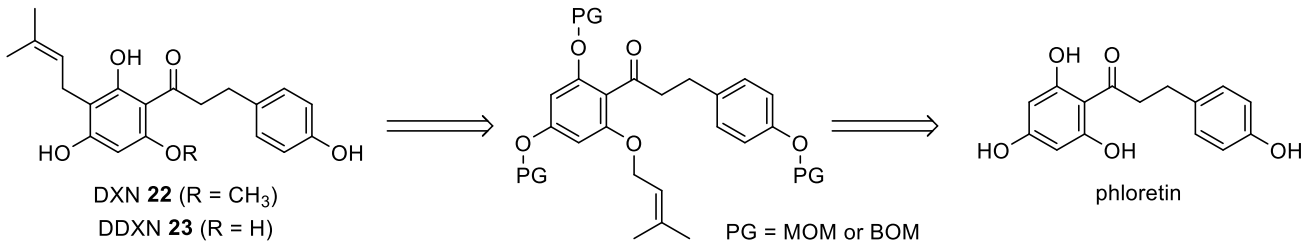
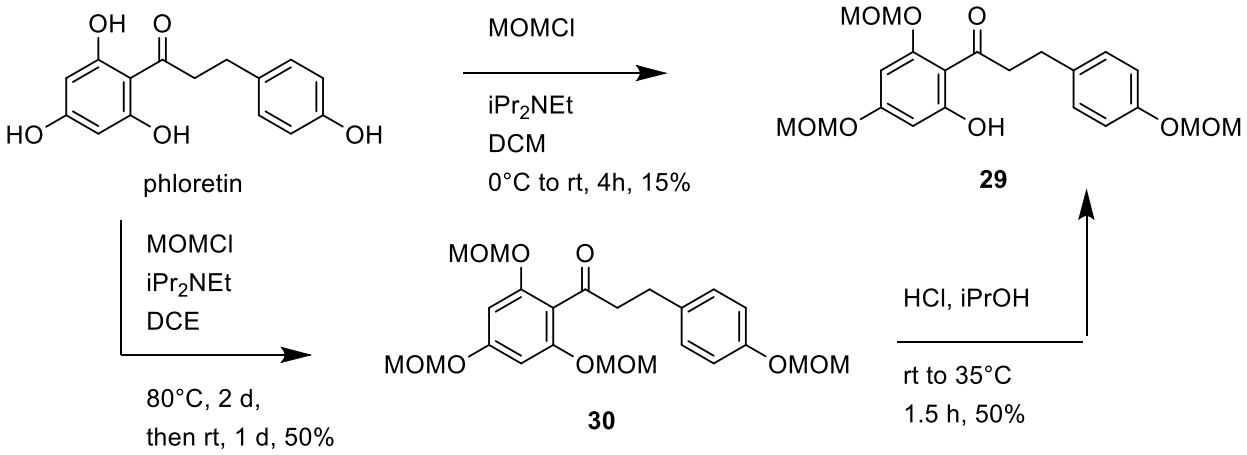


FIGURE 12 Retrosynthetic plan to DXN and DDXN from phloretin via the analogous O-prenyl ether intermediate found in our previous synthesis.

Having observed the tendency of the third hydroxyl of the A ring to not accept a protecting group, as seen in our xanthohumol synthesis, we envisioned beginning with a tri-protection of the hydroxyl groups on phloretin followed by O-prenylation, at which point we would expect the remaining synthesis to be analogous to that of our fragment coupling strategy to DDXN described

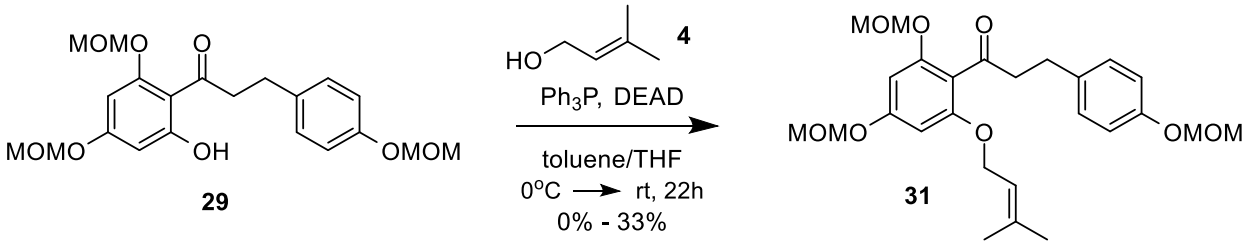
above. In our first attempts at tri-protection of phloretin, we desired to continue using the less hazardous BOMCl, which we expected should give us no trouble as this direct route from phloretin avoided any issues with the phenolic coupling partner. Encouragingly, we found three BOM groups were easily added while avoiding the addition of a fourth, and ^{1}H NMR analysis indicated the hydroxyl groups protected were indeed the ones we expected and desired. However, there was also a significant amount of variously di-protected phloretin, all of very similar polarity to our desired tri-protected compound. The resultant mixture of close-running BOM ethers led to a troublesome purification and we found it prohibitively difficult to access the clean tri-protected product.

We then investigated protection instead with MOMCl, which gave a similar mixture of di-and tri-protected phloretin. While the resulting MOM ethers were of sufficiently different polarity to separate, purification was only moderately efficient and the yield of the desired tri-protected phloretin **29** was low (Scheme 7). As an alternate strategy, we found with some persistence we were able to coax the fourth hydroxyl group to accept a MOM group, of which we anticipated the expected heightened lability could allow for selective removal under careful deprotection conditions. Indeed, both the tetra-protection and mono-deprotection steps proceeded in comparatively decent yield, but the overall yield of this sequence was only slightly better than direct tri-protection at the expense of an additional step (Scheme 7).



SCHEME 7 Direct conditions to the desired tri-protected phloretin **29** (top), vs mono-deprotection of the tetra-protected intermediate **30** (bottom).

With reliable, albeit low-yielding, access to our desired tri-protected phloretin **29**, we finally attempted the prenylation step to reach the desired analogue of the bis-BOM,MOM O-prenyl ether intermediate from which the remaining DDXN synthesis had already been realized. Under Mitsunobu conditions with prenyl alcohol, we were unable to reliably access the tri-MOM O-prenyl ether **31**. The hindrance to installing the prenyl group on the tri-protected phenol **29** is not surprising given its same characteristic reluctance to accept a fourth protecting group.



SCHEME 8 O-prenylation of the tri-MOM-protected phloretin **29**.

Additionally, although we were able to obtain a low and unreliable yield of **31** (Scheme 8), purification was difficult and resulted in a final product contaminated with byproducts. Rearrangement of **31** could reasonably be expected to proceed given that the counterpart reaction of bis-BOM,MOM was successful, but our limited attempts to effect rearrangement of the tri-MOM ether were unproductive. Given that the prenyl rearrangement, even when successful, is still plagued by the cyclization side reaction, in addition the other difficulties of this route, we likewise opted not to pursue completion of this plan.

Chapter 4: Summary and Conclusion

Xanthohumol is an interesting and valuable target by virtue of its complex bioactivity profile, and xanthohumol derivatives which lack the α-β-unsaturated ketone functionality are additionally of interest for their demonstrated comparable bioactivity in the absence of potentially undesired estrogenic effects. We set out to design a synthesis to access a variety of xanthohumol isotopologues for use in the further study of this important compound. We additionally endeavored to investigate viable synthetic routes to some important xanthohumol derivatives as an alternative to access from xanthohumol itself.

4.1 Synthesis of Xanthohumol Isotopologues

We successfully adapted the total synthesis of Khupse and Erhardt to access ^{13}C-labeled xanthohumol isotopologues for use as molecular probes and as analytical standards to study its metabolism and mode of action.

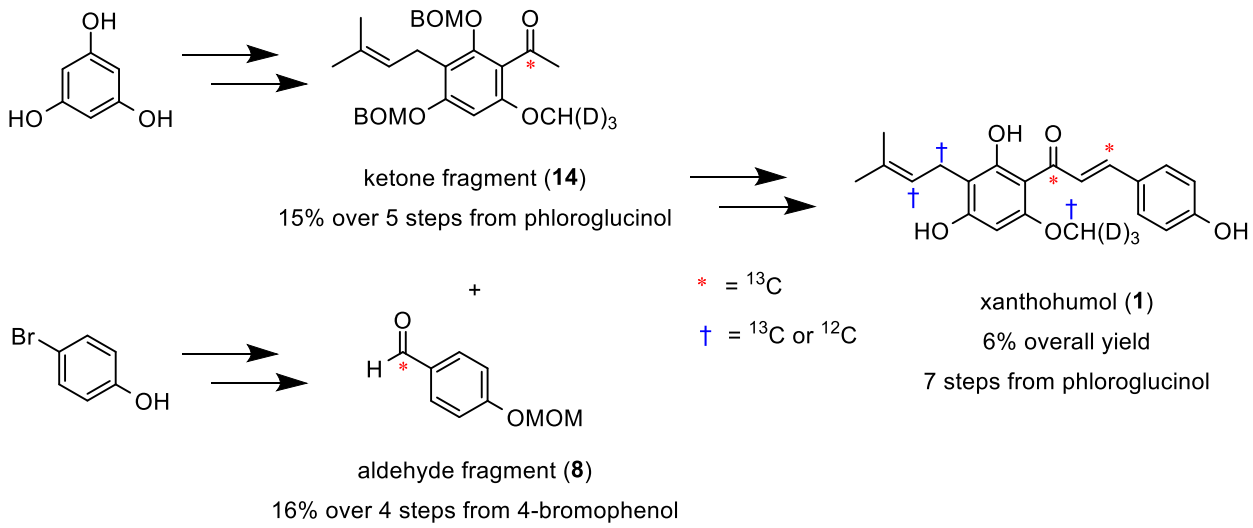


FIGURE 13 Summary of results of the xanthohumol isotopologue synthesis.

1,3-[$^{13}C]_2$-xanthohumol was prepared in seven steps and 6% 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. Four other isotopologues were similarly prepared by applying minor modifications to the route.

4.2 Comparison of Three Potential Routes to Dihydroxanthohumols

Although the structure of phloretin, which contains the same hydroxy-substituted saturated chalcone framework found in our desired xanthohumol derivatives, makes it an attractive synthetic starting point, the challenge of selectively installing the prenyl group is probably best pursued by enzymatic prenylation if the strategy is to be of significant value. Nature's own enzymatic processing, which is able to introduce a prenyl group onto a chalcone framework with exquisite selectivity, is something organic chemists still can't necessarily easily or efficiently achieve.

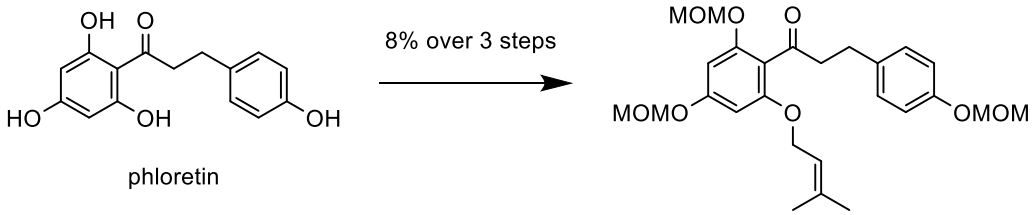


FIGURE 14 Results of conversion of phloretin to the analogous O-prenyl intermediate.

Our alkylation based approach to DXN and DDXN, while more conducive to selective substitution, is not much shorter than a route directly from xanthohumol, as the fragments are derived from and very similar to those in our xanthohumol synthesis. The alkylation route to directly access the α-β-saturated bond saves only the hydrogenation step from xanthohumol, but the low yield of the alkylation does not provide much benefit in exchange for one fewer step.

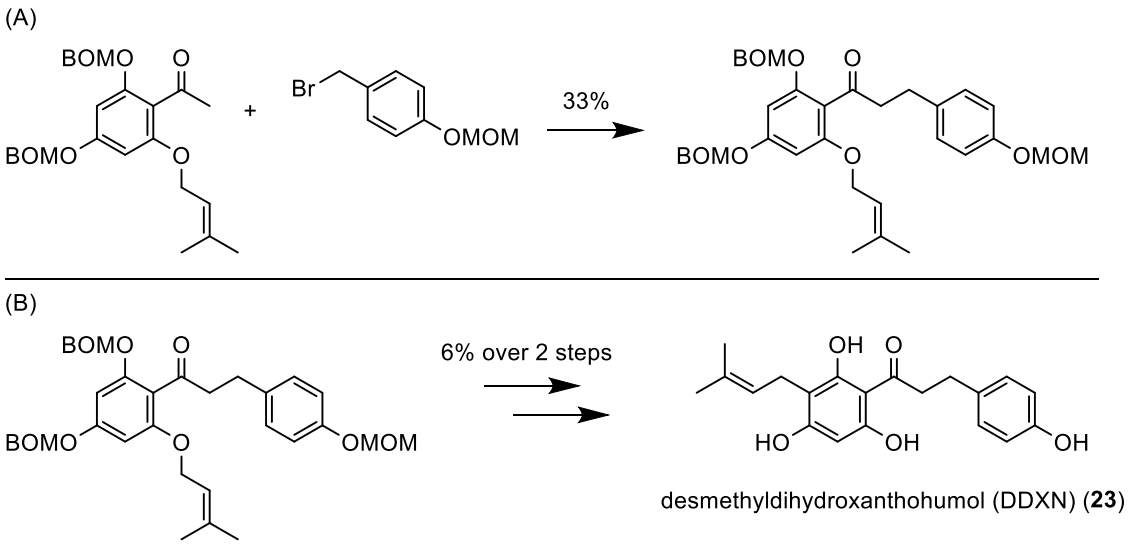


FIGURE 15 (A) Fragment alkylation to the O-prenyl intermediate; (B) Completion of synthesis of DDXN.

The fragment based approaches to both xanthohumol and its α-β-saturated derivatives are additionally both still plagued by the troublesome prenylation selectivity, and rely on an unfortunately clumsy device of making the O-prenyl ether followed by migrating the prenyl group around with side reactions. However, although clumsy and low yielding, the coupling strategy is reliable and thus a valuable strategy when total synthesis is required, such as for the need to access isotopologues. But as is often the case with natural products, these compounds are best accessed from naturally occurring xanthohumol.

Chapter 5: 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. Infrared (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 ($C\mathit{H}Cl_3$) = 7.26 ppm, δ_C = 77.2 ppm; $(CD_3)_2SO$ δ_H ($CD_3SOC\mathit{H}D_2$) = 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), and 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 electron ionization (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]-2). By a modification to the method of Maier et al.[43] To a stirred suspension of phloroglucinol (1.58 g, 12.5 mmol) in CH_2Cl_2-nitromethane (1:1, 30 mL) at room temperature under Ar was added powdered $AlCl_3$ (6.67 g, 50.0 mmol) in 4 portions (a slight exotherm was noted). The resulting homogenous yellow/brown solution was stirred for 30 minutes 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 hours. After this time, the mixture was cooled at room temperature and poured into ice water (100 mL). Most of the low boiling volatile solvents were removed using a rotatory evaporator, and the residue was extracted with EtOAc (3 × 20 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]-**2** (1.34 g, 7.93 mmol, 63%) as a tan powder: mp 207 to 209°C; IR (ATR) 3522, 3451, 3107, 1622, 1515, 1463, 1360, 1266, 1239, 1164, 1064 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.23

(2H, s), 10.36 (1H, s), 5.80 (2H, s), 2.54 (3H, d, $^{2}J_{CH}$ = 6.1 Hz) ppm; ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 202.5 (0, enriched), 164.8 (0), 164.3 (2C, 0), 104.0 (0, d, $^{1}J_{CC}$ = 56 Hz), 94.5 (2C, 1), 32.4 (3, d, $^{1}J_{CC}$ = 41 Hz) ppm; MS (EI+) *m/z* 169 (100%, [M]+•), 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]-11). A stirred suspension of triol [^{13}C]-**2** (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 minutes. The resulting homogenous brown solution was allowed to warm at room temperature and stirred for 2.75 hours. 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 (2 × 20 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]-**11** (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 to 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, $^{2}J_{CH}$ = 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, $^{1}J_{CC}$ = 56 Hz), 97.5 (1), 94.4 (1), 92.5 (2), 92.0 (2), 71.1 (2), 70.6 (2), 33.2 (3, d, $^{1}J_{CC}$ = 42 Hz) ppm; MS (EI+) *m/z* 409 (15%, [M]+•), 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]-12). A stirred solution of phenol [^{13}C]-**11** (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, 4, 1.15 mL, d = 0.86, 989 mg, 11.5 mmol) followed by the dropwise addition of diethyl azodicarboxylate (5.23 mL, d = 0.956, 5.00 g, 40 wt% in PhMe, 11.5 mmol) during 3 minutes. The resulting mixture was allowed to warm at room temperature and stirred for 22 hours.

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]-**12** (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 to 7.28 (10H, m), 6.59 to 6.58 (1H, m), 6.37 to 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]+•), 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]-13). A stirred solution of aryl prenyl ether [^{13}C]-**12** (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 hours. After this time, the mixture was allowed to cool at room temperature, concentrated *in vacuo*, and allowed to stand at room temperature for 18 hours. 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]-**13** (1.05 g, 2.20 mmol, ≤ 40%, approximately 90%-95% pure) and the unwanted isomeric benzofuran derivative [^{13}C]-**15** (1.07 g, 2.24 mmol, 41%), both as colorless oils.

Data for [^{13}C]-**13**: 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 to 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]+•), 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]-**15**: 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]-14). A stirred suspension of phenol [^{13}C]-**13** (705 mg, 1.48 mmol) and K_2CO_3 (2.04 g, 14.8 mmol, 10 eq) in acetone (15 mL) at room temperature 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 hours 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]-**14** (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 to 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, $^2J_{CH}$ = 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, $^1J_{CC}$ = 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, $^1J_{CC}$ = 42 Hz), 25.9 (3), 23.3 (2), 18.1 (3) ppm; MS (EI+) *m/z* 491 (3%, [M]+•), 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 (17). A stirred solution of 4-bromophenol (**16**, 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 minutes. The resulting dark orange solution was allowed to stir for 2 hours while warming at room temperature. 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 minutes. The mixture was partitioned between EtOAc (80 mL) and H_2O (80 mL), and the aqueous phase extracted with EtOAc (2 × 60 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 **17** (12.65 g, 58.3 mmol, 83%) as a colorless oil: IR (neat) 2956, 2903, 1591, 1488, 1235, 998.8, 591, 508 cm^{-1}; ^1H 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. ^1H and ^{13}C NMR spectral data are in agreement with those previously reported.[46]

4-(Methoxymethyloxy)-1-[^{13}C]methylbenzene ([^{13}C]-18). A stirred solution of aryl bromide **17** (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 minutes. The resulting aryllithium was stirred for 10 minutes and then treated with neat [^{13}C]-methyl iodide (1.60 mL, d = 2.28, 3.65 g, 25.5mmol). The reaction mixture was allowed to stir while warming at room temperature for 2 hours 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 (2 × 30 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]-**18** (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}; ^1H NMR (400 MHz, $CDCl_3$, signals attributable to [^{13}C]-18 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, $^{1}J_{CH}$ = 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)+• (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]-19). A stirred solution of the p-cresol derivative [^{13}C]-**18** (1.00 g, 69 wt% pure, 4.96 mmol) in CCl_4 (25 mL) at room temperature was treated with N-bromosuccinimide (1.28 g, 7.19mmol) 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 hours. 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]-**19** and the corresponding dibromide (1.04 g, 57 wt% in [^{13}C]-**19**, 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]-**19**: 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, $^{1}J_{CH}$=153.0 Hz), 3.47 (3H, s) ppm.

1′-[^{13}C]-4-(Methoxymethyloxy)benzaldehyde ([^{13}C]-8). Dimethyl sulfoxide (DMSO, 8 mL) and solid $NaHCO_3$ (1.30 g, 15.5 mmol) were added to the neat benzyl bromide [^{13}C]-**19** (1.04 g, 57 wt% pure, 2.56 mmol) and the resulting mixture stirred under Ar at 90°C for 2 hours. After this time, the mixture was allowed to cool at room temperature 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 (2 × 10 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]-**8** (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, $^{1}J_{CH}$ = 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)+• (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]$_2$-20). A stirred solution of the methyl ketone [^{13}C]-**14** (403 mg, 0.821 mmol) and the aryl aldehyde [^{13}C]-**8** (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 hours. 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 (2 × 15 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]-**8** (127 mg, 0.76 mmol) and the title chalcone [^{13}C]$_2$-**20** (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 to 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]$_2$-**20** (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 hours (note that the reaction mixture became an homogenous yellow solution after approximately 10 min). After this time, the solution was allowed to cool at room temperature 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 2 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 labeled xanthohumol [^{13}C]$_2$-**1** (120 mg, 0.337 mmol, 58%) as a yellow solid: mp 162°C to 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, DMSO-*d*$_6$) 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, DMSO-*d*$_6$) 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°C to 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, DMSO-*d*$_6$) 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, DMSO-*d*$_6$) 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 that if present in synthetic [^{13}C]$_2$-**1**, contamination from traces of HCl addition adduct [^{13}C]$_2$-**21** 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'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]$_2$-**21** 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-labeled isotopologue of xanthohumol ([^{13}C]$_2$-**1**) from the corresponding triple-labeled [^{13}C]$_3$-**20**. Data for [^{13}C]$_3$-**1**: mp 162°C to 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, DMSO-d_6, 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, DMSO-d_6) 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-labeled isotopologue of xanthohumol ([^{13}C]$_2$-**1**) from the corresponding penta-labeled [^{13}C]$_5$-**20**. 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, DMSO-d_6) 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), 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, DMSO-d_6) 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).

1-[^{13}C]-2′,4′-Bis(benzyloxymethyloxy)-3-[4-[(methoxymethyl)oxyphenyl)-6′-[(3-methylbuten-2-yl)oxy]propiophenone ([^{13}C]-27). A stirred solution of diisopropylamine (0.18 mL, d = 0.722, 126 mg, 1.25 mmol) in anhydrous THF (3.0 mL) at –20 °C was treated dropwise with *n*-BuLi (0.69 mL, 1.60 M in hexanes, 1.10 mmol). The resulting solution of lithium diisopropylamide (LDA) was stirred for 20 min and then cooled to –78 °C. A solution of ketone [^{13}C]-**12** (477 mg, 1.00 mmol) in anhydrous THF (1.0 mL) was added dropwise and the resulting light yellow solution of lithium enolate stirred for 25 min. After this time, 4-MOMO$C_6H_4CH_2$Br (231 mg, 1.00 mmol) in anhydrous THF (1.0 mL) was added dropwise during 2 min. The mixture was then allowed to warm slowly to room temperature with stirring during 16 h. Sat. aq. NH_4Cl (5 mL) was added and the mixture partitioned between EtOAc (15 mL) and H_2O (10 mL). The phases were separated and the aqueous phase was extracted with EtOAc (10 mL). The combined organic phases were washed with brine (10 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, in order of elution: recovered ketone [^{13}C]-**12** (140 mg, 0.293 mmol, 29%), the desired mono-alkylated product [^{13}C]-**27** (204 mg, 0.326 mmol, 33%), and the undesired double-alkylation product (72 mg, 9%). Data for [^{13}C]-**27**: pale yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) 7.35-7.25 (10H, m), 7.13 (2H, d, *J* = 8.4 Hz), 6.94 (2H, d, *J* = 8.6 Hz), 6.58 (1H, s), 6.36 (1H, s), 5.37 (1H, t, *J* = 5.3 Hz), 5.25 (2H, s), 5.19 (2H, s), 5.13 (2H, s), 4.71 (2H, s), 4.65 (2H, s), 4.47 (2H, d, *J* = 6.0 Hz), 3.46 (3H, s), 3.10-3.01 (2H, m), 3.00-2.93 (2H, m), 1.74 (3H, s), 1.68 (3H, s) ppm.

1-[^{13}C]-2′,4′-Bis-BOM-4′′-MOM-desmethyldihydroxanthohumol ([^{13}C]-28). A stirred solution of prenyl ether [^{13}C]-**27** (100 mg, 0.159 mmol) in chlorobenzene (2.5 mL) was treated with $NaHCO_3$ (13 mg, 0.159 mmol) and Resolve-Al® [$Eu(fod)_3$, 20 mg, 0.019 mmol). The resulting mixture was stirred at a gentle reflux for 3 h, another portion of Resolve-Al® (20 mg)

added, and then heating continued for a further 1 h. After this time, the mixture was cooled to room temperature and directly subjected to purification by column chromatography (SiO_2, eluting with 15% EtOAc in hexanes) to afford the desired protected DDXN derivative [^{13}C]-**28** (44 mg, 0.070 mmol, 44%) followed by undesired cyclic ether by-product (28 mg, 0.045 mmol, 28%). Data for [^{13}C]-**28**: pale yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) 12.81 (1H, s), 7.37-7.16 (10H, m), 7.13 (2H, d, *J* = 8.4 Hz), 6.94 (2H, d, *J* = 8.3 Hz), 6.58 (1H, s), 5.33 (2H, s), 5.20-5.15 (1H, m), 5.15 (2H, s), 5.06 (2H, s), 4.70 (2H, s), 4.63 (2H, s), 3.52-3.43 (2H, m), 3.47 (3H, s), 3.30 (2H, d, *J* = 6.1 Hz), 2.98-2.92 (2H, m), 1.75 (3H, s), 1.67 (3H, s) ppm.

1-[^{13}C]-Desmethyldihydroxanthohumol ([^{13}C]-23). A stirred solution of bis-BOM-MOM-DDXN ([^{13}C]-**28**, 16 mg, 0.026 mmol) in *i*-PrOH (2.0 mL) at room temperature was treated with 1 drop of *c*. aq. HCl and then heated at 60°C (bath temp.) for 2.5 h. The mixture was allowed to cool to rt, solid $NaHCO_3$ (ca. 100 mg) added, and then concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2, eluting with 33-100% EtOAc in hexanes) to afford, in order of elution: 1-[^{13}C]-2´-BOM-DDXN (9.3 mg, 0.020 mmol, 77%) and 1-[^{13}C]-DDXN (**23**, 1.2 mg, 0.0035 mmol, 13%). Data for [^{13}C]-**23**: colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) 7.12 (2H, d, *J* = 8.2 Hz), 6.75 (2H, d, *J* = 8.0 Hz), 5.86-5.80 (1H, br s, OH), 5.83 (1H, s), 5.24 (1H, t, *J* = 5.6 Hz), 4.62-4.54 (1H, br s, OH), 3.38-3.30 (4H, m), 2.97-2.91 (1H, m), 1.83 (3H, s), 1.77 (3H, s) ppm.

2´,4´,4´´-Tris-MOM-phloretin (29).

Direct tri-MOM protection method: A stirred solution of phloretin (274 mg, 1.00 mmol) and diisopropylethylamine (1.02 mL, d=0.76, 776 mg, 6.00 mmol) in anhydrous CH_2Cl_2 (4 mL) at 0°C under Ar was treated with the dropwise addition of neat chloromethyl methyl ether (MOMCl, 0.27 mL, d=1.06, 282 mg, 3.50 mmol, CARE!) during 2 minutes. The solution was opaque light pink and vapor formed immediately upon addition of MOMCl. The mixture was allowed to warm at room temperature while stirring for 4 h. After this time the solution had become dark orange, and any active alkylating agent was quenched by the addition of *c*. aq. NH_3 (5 mL). The resulting biphase mixture was partitioned between H_2O (10 mL), EtOAc (5 mL) and hexanes (5 mL). The aqueous phase was extracted with EtOAc/hexanes (10 mL) and the combined organic phases washed with brine (10 mL), then dried (Na_2SO_4) and concentrated *in vacuo*. The residue

was purified by column chromatography (SiO_2, eluting with 25% followed by 35% EtOAc in hexanes) to afford the desired tri-MOM protected phloretin **29** (61 mg, 0.150 mmol, 15%) as a dark orange oil (data for **29** are given below).

Tetra-MOM-phloretin mono-deprotection method: To a stirred solution of the tetra-MOM ether **30** (9 mg, 0.020 mmol) in *i*-PrOH (2 mL) at room temperature was added dropwise *c*. aq. HCl (5 drops) and stirred for 45 min, after which time the reaction flask was warmed to 35°C and stirred an additional 45 min. The mixture was allowed to cool at room temperature then quenched with sat. aq. $NaHCO_3$ (5 mL). The mixture was partitioned between H_2O (10 mL) and EtOAc (10 mL), and the organic phase washed with water (5 mL) and brine (5 mL), then dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2, eluting with 35% EtOAc in hexanes) to afford the desired tri-MOM protected phloretin **29** (4 mg, 0.010 mmol, 50%): IR (neat) 2918, 2850, 1708, 1620, 1511, 1432, 1364, 1276, 1226, 1197, 1151, 1108, 1081, 1060, 1021, 924, 830 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 13.70 (1H, s), 7.16 (2H, d, J = 8.6 Hz), 6.97 (2H, d, J = 8.6 Hz), 6.29 (1H, m), 6.24 (1H, m), 5.24 (2H, s), 5.17 (2H, s), 5.15 (2H, s), 3.48 (3H, s), 3.473 (3H, s), 3.466 (3H, s), 3.36 to 3.31 (2H, m), 3.00 to 2.94 (2H, m) ppm; ^{13}C NMR (175 MHz, $CDCl_3$) δ 204.9, 167.0, 163.5, 160.4, 155.6, 135.1, 129.5, 116.5, 106.9, 97.4, 94.72, 94.69, 94.3, 94.2, 56.9, 56.7, 56.2, 46.1, 29.9 ppm.

2´,4´,6´,4´´-Tetrakis-MOM-phloretin (30). A stirred solution of phloretin (274 mg, 1.00 mmol) and diisopropylethylamine (1.02 mL, d=0.76, 776 mg, 6.00 mmol) in $(CH_2)_2Cl_2$ (4 mL) at room temperature under Ar was treated with the dropwise addition of neat chloromethyl methyl ether (MOMCl, 0.27 mL, d=1.06, 282 mg, 3.50 mmol) during 2 minutes. The solution was opaque light pink and vapor formed immediately upon addition of MOMCl. The mixture was heated to a gentle reflux at 80°C and stirred overnight. On the next day an additional portion of diisopropylethylamine (1.02 mL) was added followed by an additional portion of MOMCl (0.27 mL), and continued stirring at reflux for a total of 2 days. After this time the reaction was removed from heat and allowed to cool at room temperature while stirring overnight, then any active alkylating agent was quenched by the addition of *c*. aq. NH_3 (5 mL). The resulting biphase mixture was partitioned between H_2O (10 mL), EtOAc (10 mL) and hexanes (10 mL). The aqueous phase was extracted with EtOAc/hexanes (10 mL) and the combined organic phases washed with brine (10 mL), then dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by column

chromatography (SiO_2, eluting with 25% followed by 35% EtOAc in hexanes) to afford in order of elution the tri-protected phloretin **29** (38 mg, 0.090 mmol, 9%) and the desired tetra-protected phloretin **30** (223 mg, 0.500 mmol, 50%) as a dark orange oil: IR (neat) 2918, 2850, 1705, 1607, 1511, 1464, 1394, 1232, 1153, 1111, 1080, 1050, 1020, 922, 828 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.14 (2H, d, *J* = 8.6 H), 6.93 (2H, d, *J* = 8.6 Hz), 6.50 (1H, s), 6.48 (1H, s), 5.14 (2H, s), 5.13 (2H, s), 5.07 (4H, s), 3.47 (3H, s), 3.46 (3H, s), 3.41 (6H, s), 3.06 (2H, m), 2.97 (2H, m) ppm;

2´,4´-Bis(methoxymethyloxy)-3-[4-[(methoxymethyl)oxyphenyl)-6´-[(3-methylbuten-2-yl)oxy]propiophenone (31). A stirred solution of the tri-MOM protected phenol **29** (26 mg, 0.064 mmol) and triphenylphosphine (38 mg, 0.145 mmol) in anhydrous THF (1.0 mL) at 0°C under Ar was treated with prenyl alcohol (3-methyl-2-buten-1-ol, 4, 0.021 mL, d=0.86, 18 mg, 0.208 mmol) followed by diethyl azodicarboxylate (0.038 mL, d=0.956, 14.5 mg, 40% wt. in PhMe, 0.0832 mmol). The resulting mixture was allowed to warm to room temperature and stirred for 22 h. After this time, the mixture was concentrated *in vacuo* and the residue was subjected to two successive column chromatography operations (column #1 = SiO_2, eluting with CH_2Cl_2; column #2 = SiO_2, eluting with 10% MeOH in CH_2Cl_2) to obtain the desired O-prenyl ether **31** (10 mg, 0.021 mmol, 33%): IR (neat) 2918, 2850, 1707, 1605, 1512, 1435, 1382, 1231, 1153, 1110, 1069, 1023, 923, 826, 667 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.14 (2H, d, *J* = 4.4 Hz), 6.92 (2H, d, *J* = 6.6 Hz), 6.43 to 6.42 (1H, m), 6.31 to 6.30 (1H, m), 5.39 to 5.35 (1H, m), 5.15 (2H, s), 5.13 (2H, s), 5.06 (2H, s), 4.48 (2H, d, *J* = 6.7 Hz), 3.49 (3H, s), 3.45 (3H, s), 3.40 (3H, s), 3.07 to 3.01 (2H, m), 2.99 to 2.92 (2H, m), 1.76 (3H, s), 1.70 (3H, s) ppm;

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Appendix

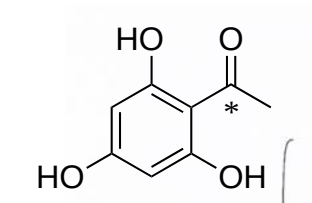
^{1}H and ^{13}C NMR Compound Spectra

^{1}H and ^{13}C NMR spectra were recorded in Fourier transform mode at the field strength specified using standard 5 mm diameter tubes. Chemical shift in ppm is quoted relative to residual solvent signals calibrated as follows:

$CDCl_3$ δ_H ($CHCl_3$) = 7.26 ppm, δ_C ($CDCl_3$) = 77.2 ppm

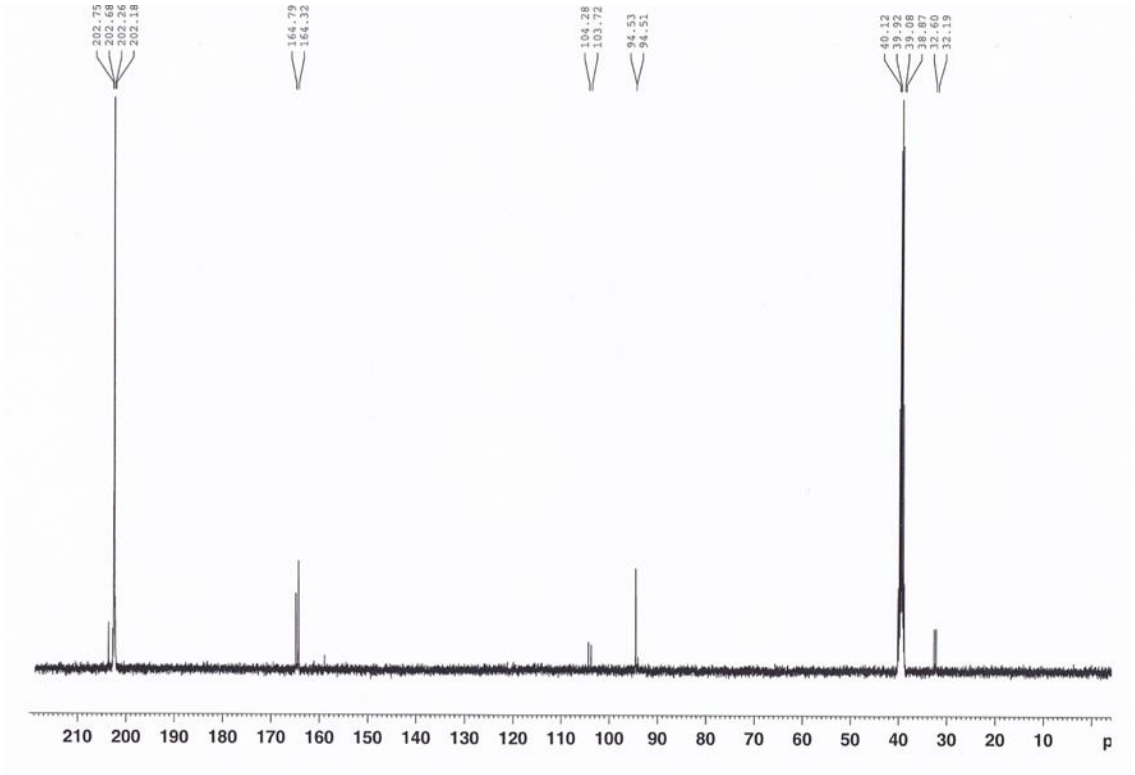
d_6-DMSO δ_H [$D_3CS(O)CHD_2$] = 2.50 ppm, δ_C [$D_3CS(O)CD_3$) = 39.5 ppm.

[^{13}C]-5: ^{1}H NMR, 400 MHz, d_6-DMSO

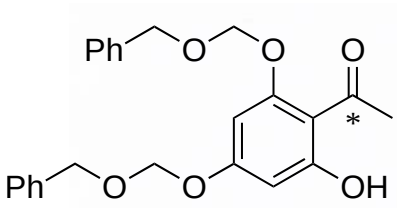


[^{13}C]-**5**: $^{12}C_7{}^{13}CH_8O_4$

[^{13}C]-5: ^{13}C NMR, 100 MHz, d_6-DMSO

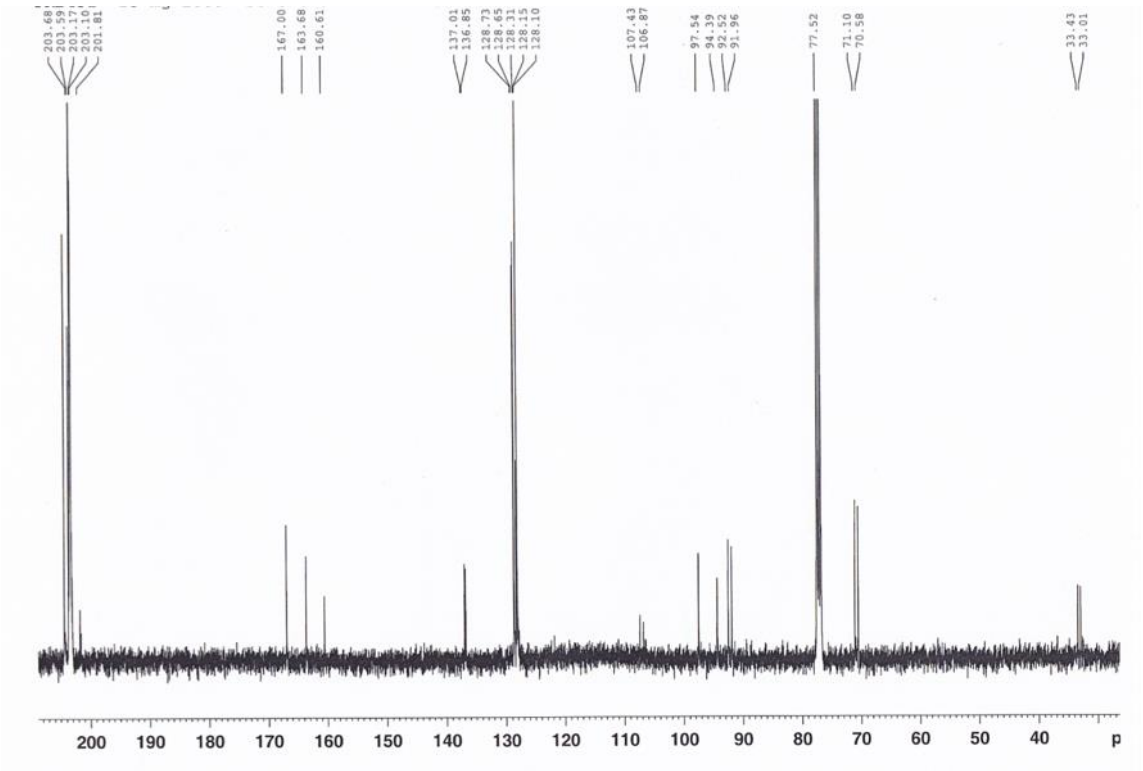


[^{13}C]-6: ^{1}H NMR, 400 MHz, $CDCl_3$

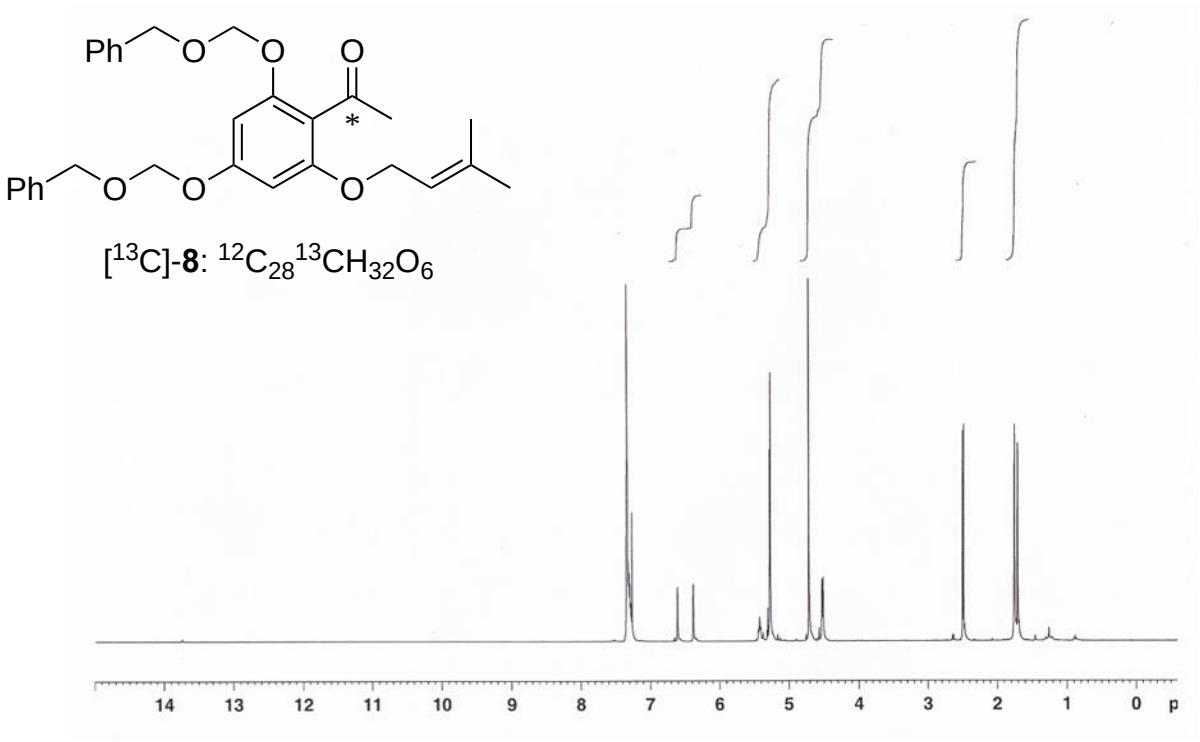


[^{13}C]-**6**: $^{12}C_{23}{}^{13}CH_{24}O_6$

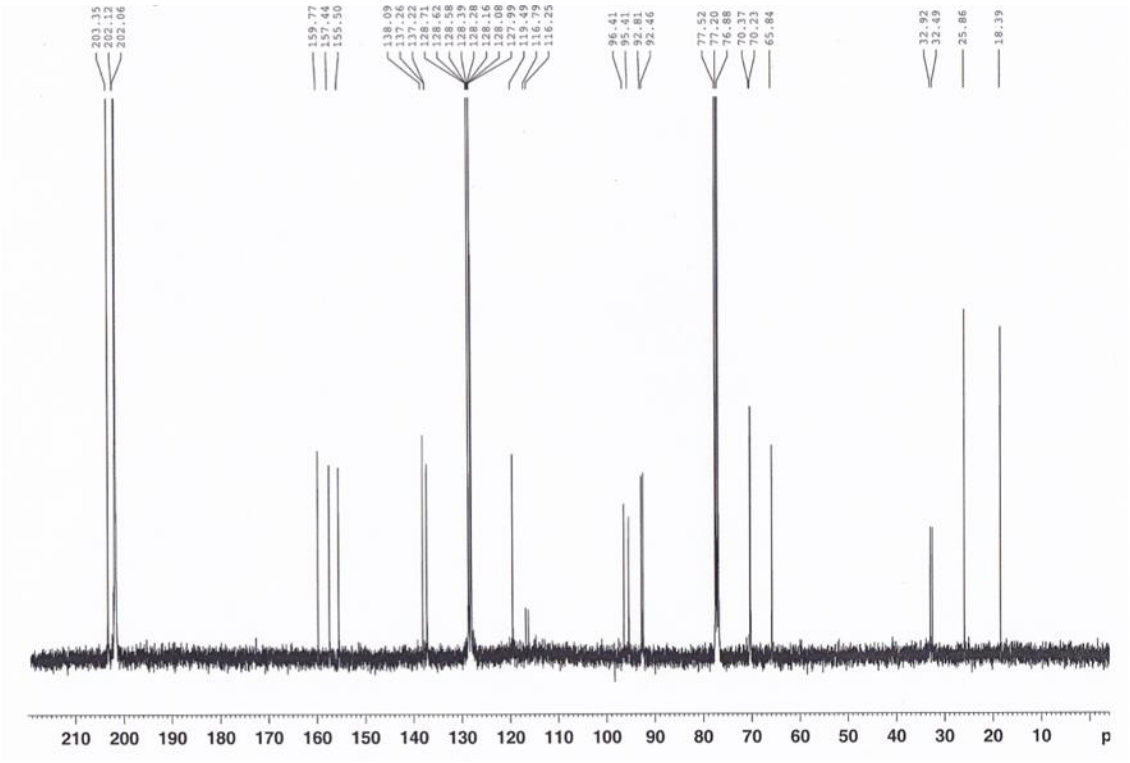
[^{13}C]-6: ^{13}C NMR, 100 MHz, $CDCl_3$



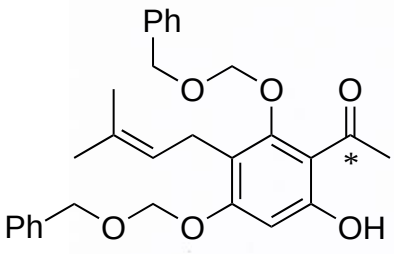
[^{13}C]-8: ^{1}H NMR, 400 MHz, $CDCl_3$



[^{13}C]-8: ^{13}C NMR, 100 MHz, $CDCl_3$

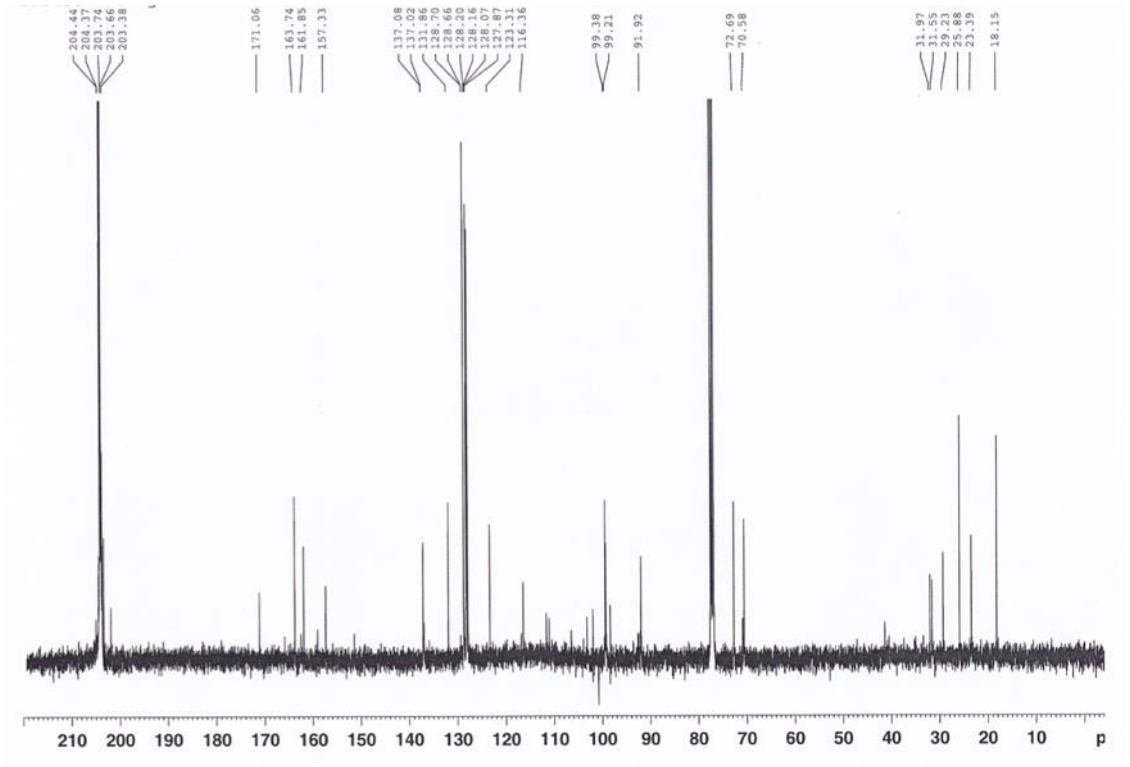


[^{13}C]-9: ^{1}H NMR, 400 MHz, $CDCl_3$

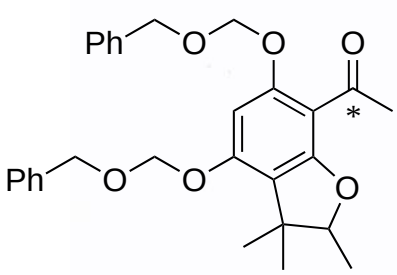


[^{13}C]-**9**: $^{12}C_{28}{}^{13}CH_{32}O_6$

[^{13}C]-9: ^{13}C NMR, 100 MHz, $CDCl_3$

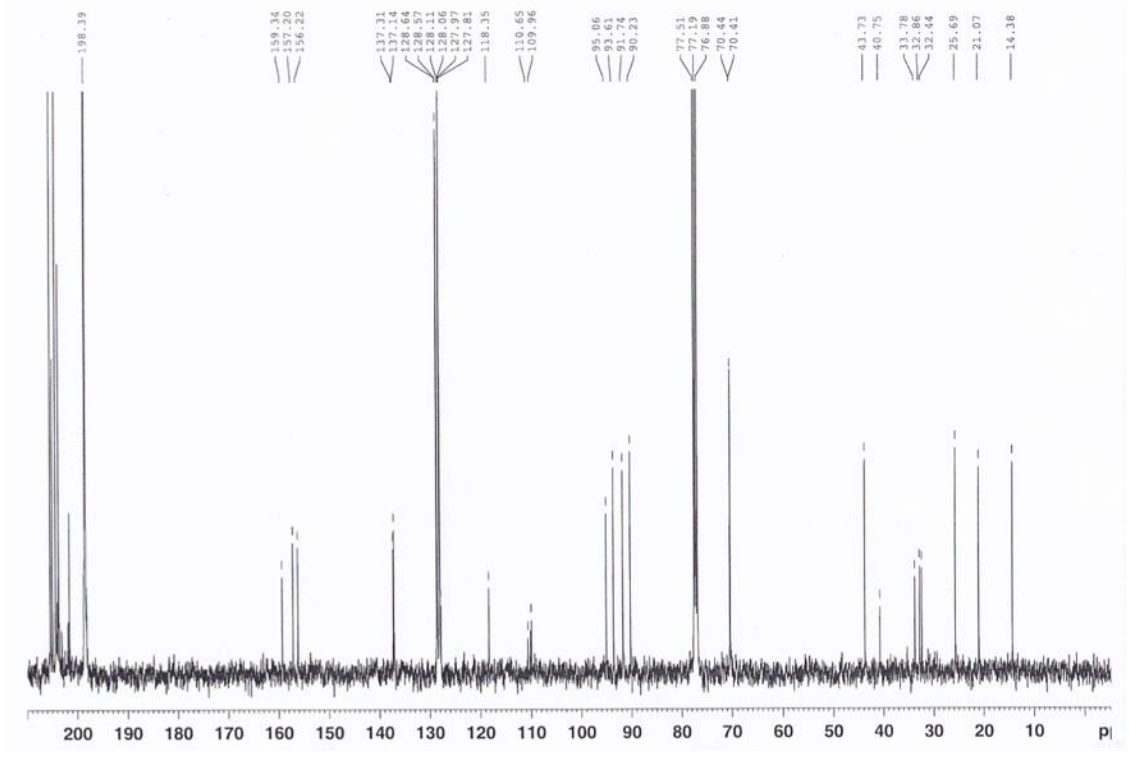


[^{13}C]-10: ^{1}H NMR, 400 MHz, $CDCl_3$

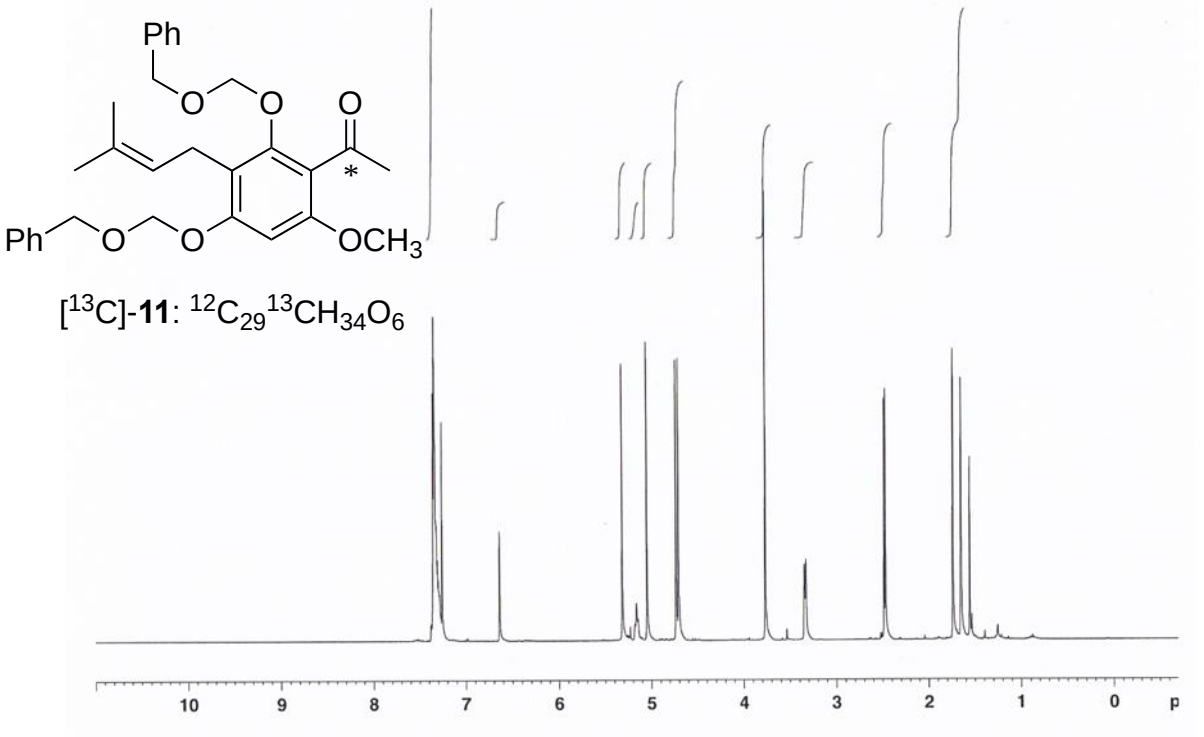


[^{13}C]-**10**: $^{12}C_{28}{}^{13}CH_{32}O_6$

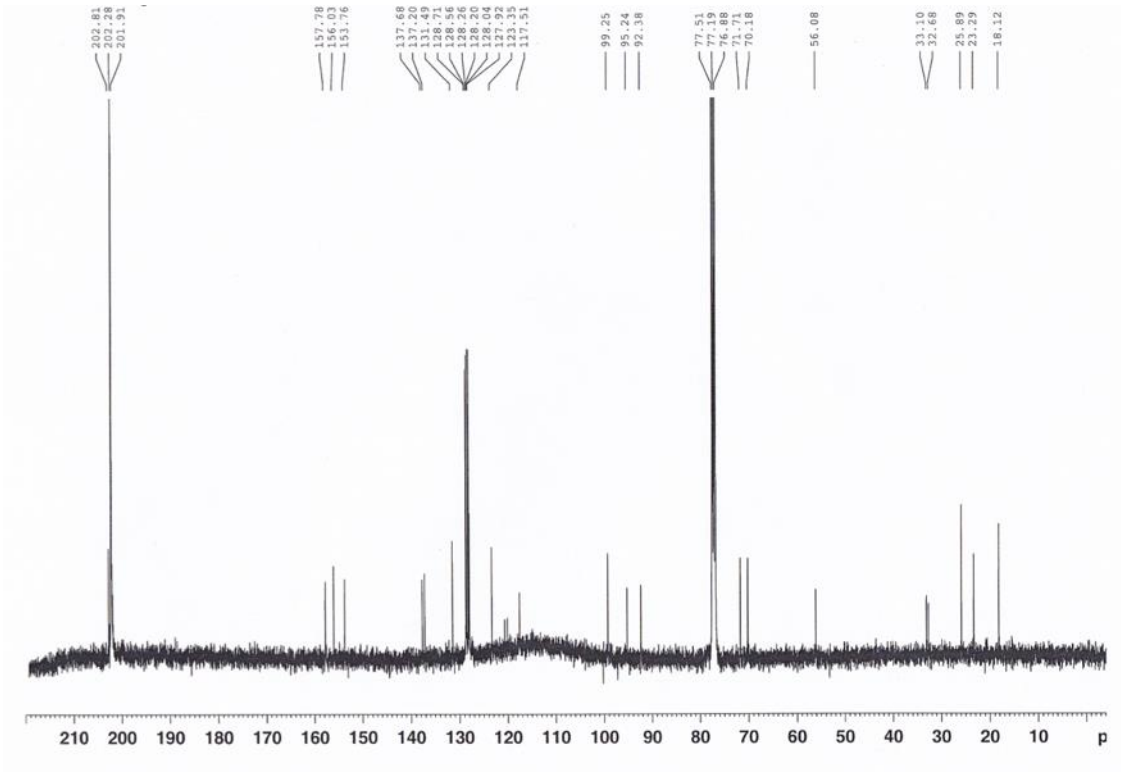
[^{13}C]-10: ^{13}C NMR, 100 MHz, $CDCl_3$



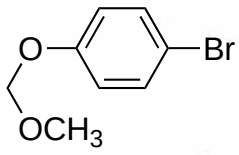
[^{13}C]-11: ^{1}H NMR, 400 MHz, $CDCl_3$



[^{13}C]-11: ^{13}C NMR, 100 MHz, $CDCl_3$

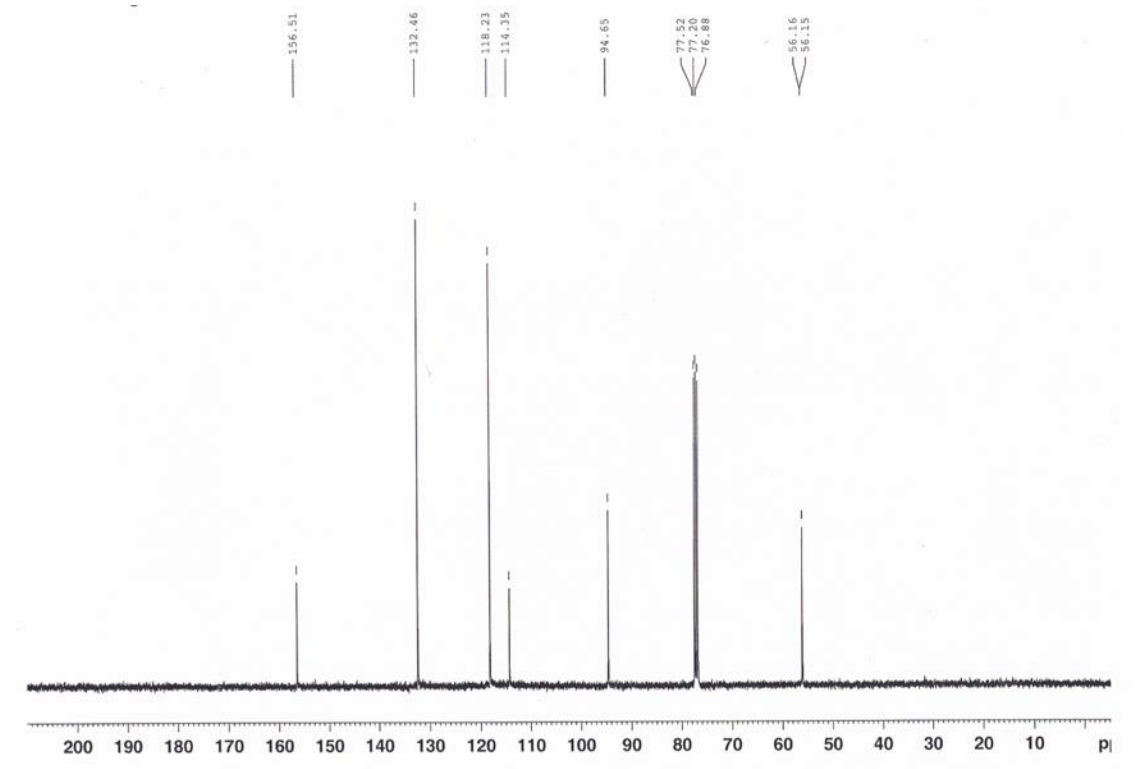


13: ^{1}H NMR, 400 MHz, $CDCl_3$

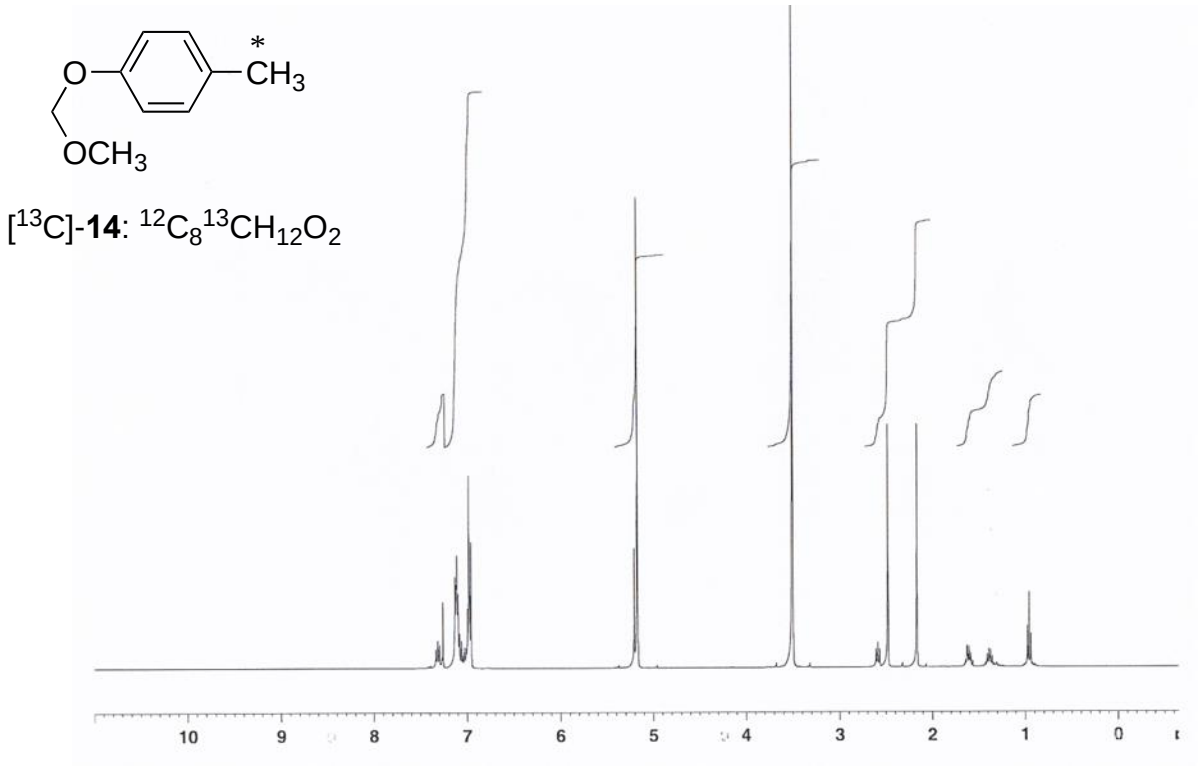


13: $^{12}C_8H_9BrO_2$

13: ^{13}C NMR, 100 MHz, $CDCl_3$

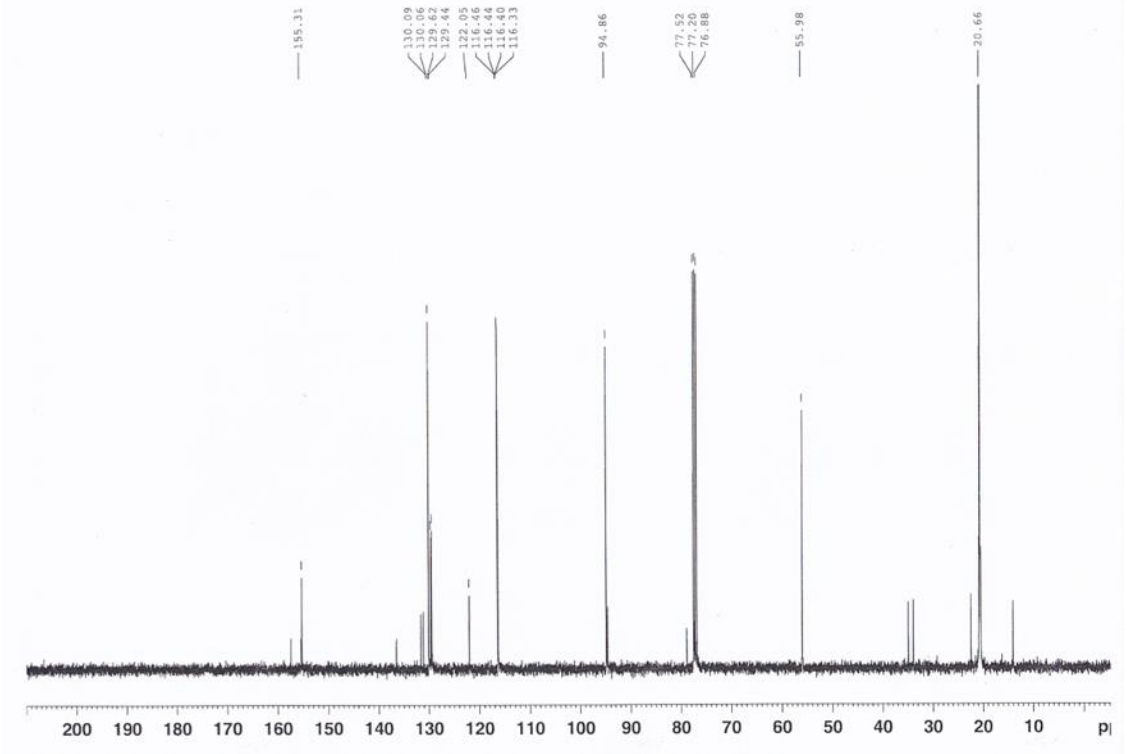


[^{13}C]-14: ^{1}H NMR, 400 MHz, $CDCl_3$

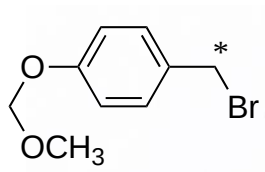


[^{13}C]-**14**: $^{12}C_8{}^{13}CH_{12}O_2$

[^{13}C]-14: ^{13}C NMR, 100 MHz, $CDCl_3$

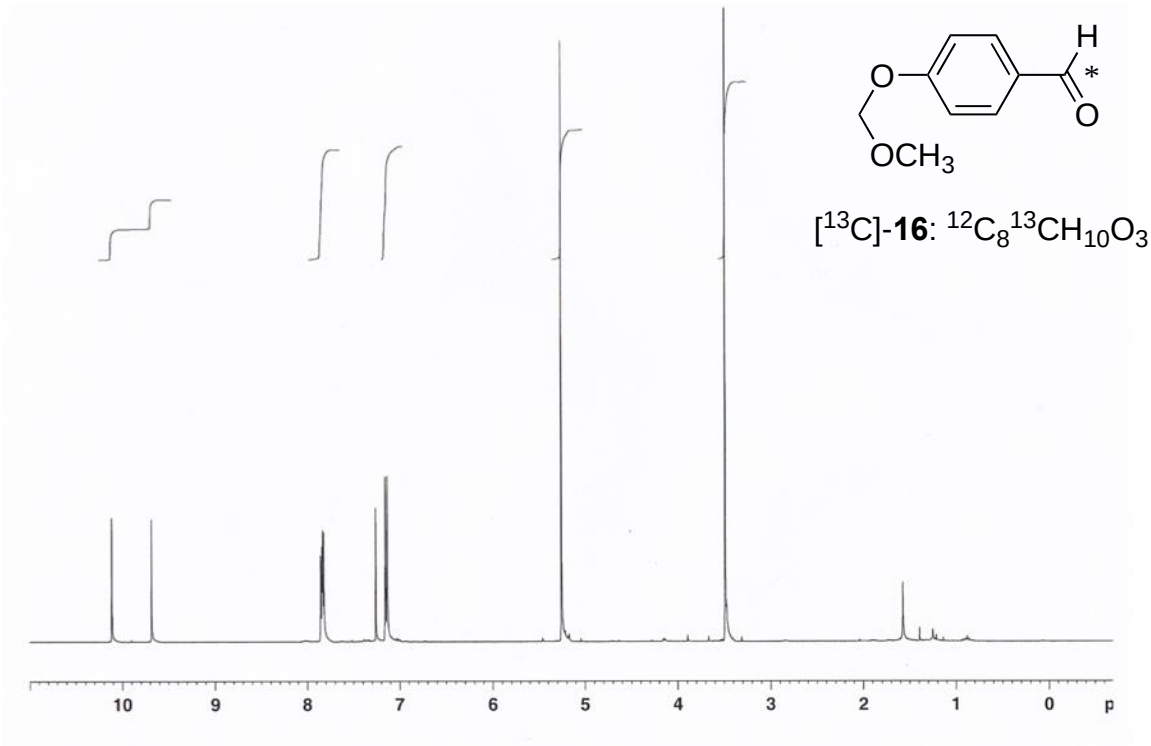


[^{13}C]-15: ^{1}H NMR, 400 MHz, $CDCl_3$



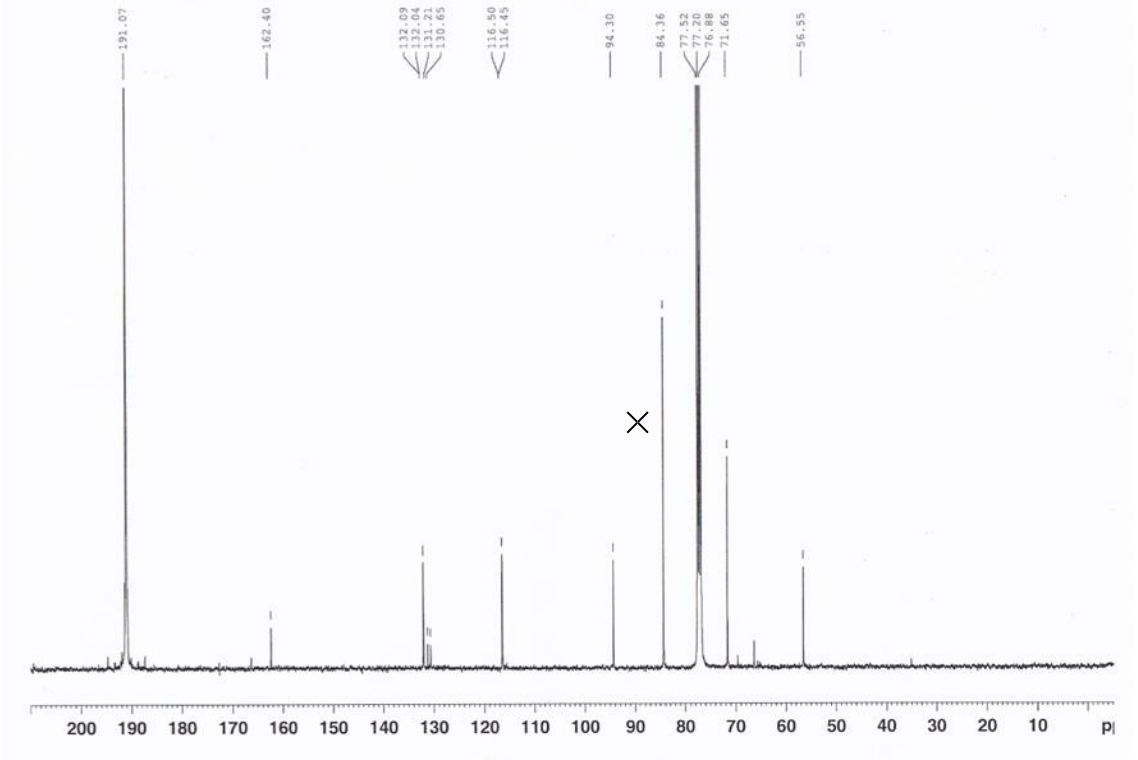
[^{13}C]-**15**: $^{12}C_8{}^{13}CH_{11}BrO_2$

[^{13}C]-16: ^{1}H NMR, 400 MHz, $CDCl_3$

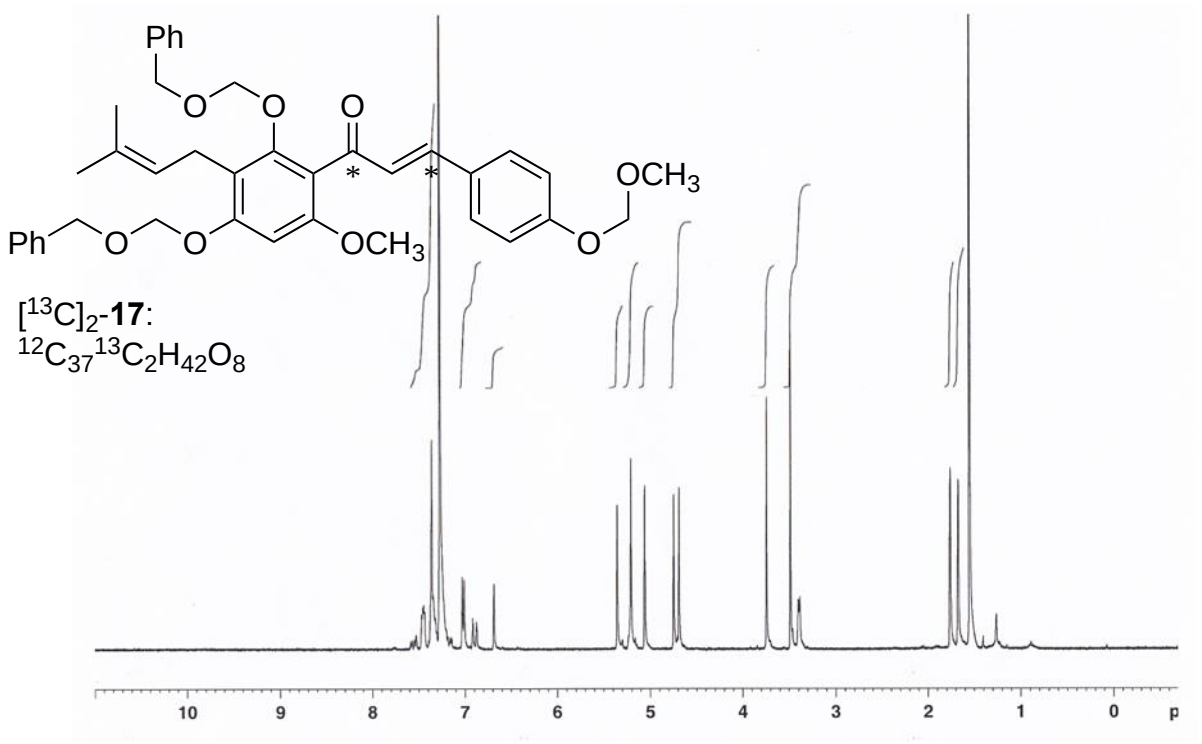


[^{13}C]-**16**: $^{12}C_8{}^{13}CH_{10}O_3$

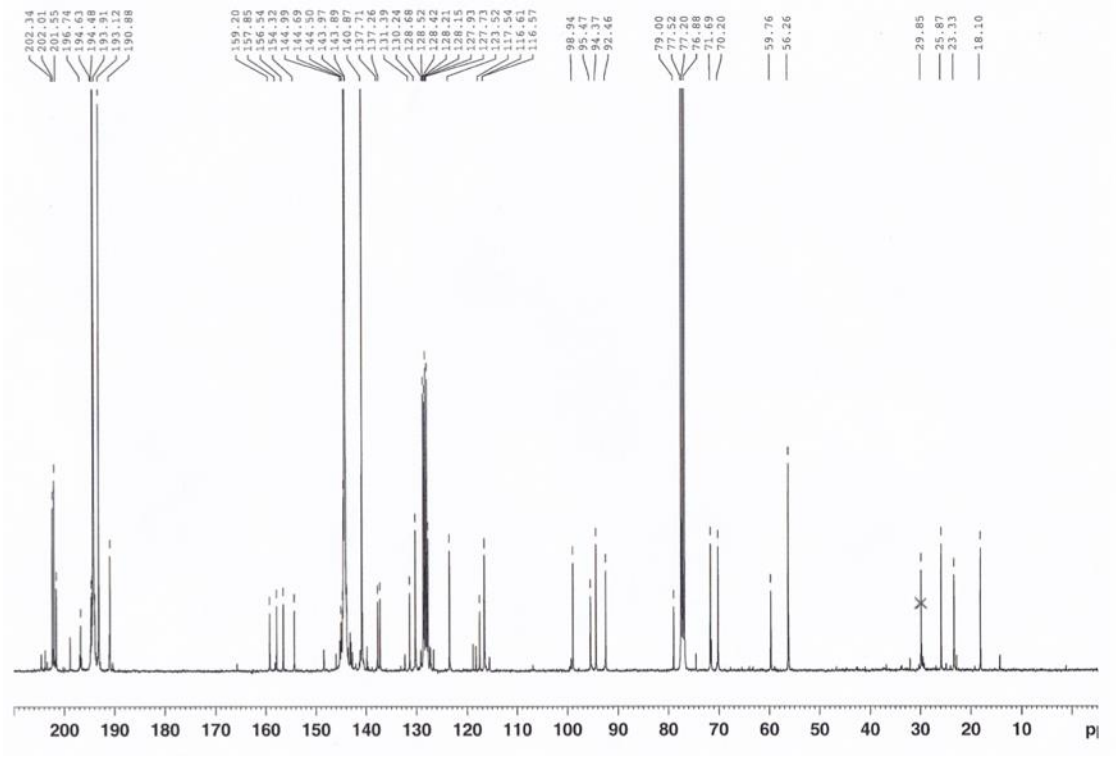
[^{13}C]-16: ^{13}C NMR, 100 MHz, $CDCl_3$



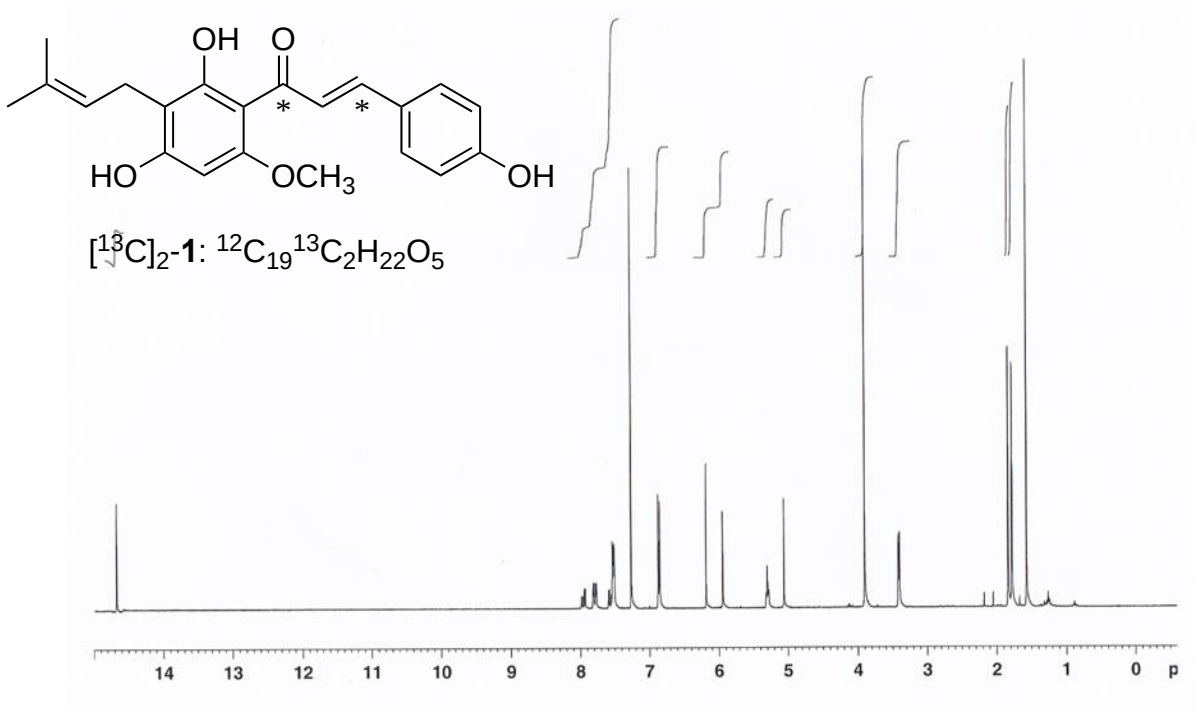
$[^{13}C]_2$-17: ^{1}H NMR, 400 MHz, $CDCl_3$



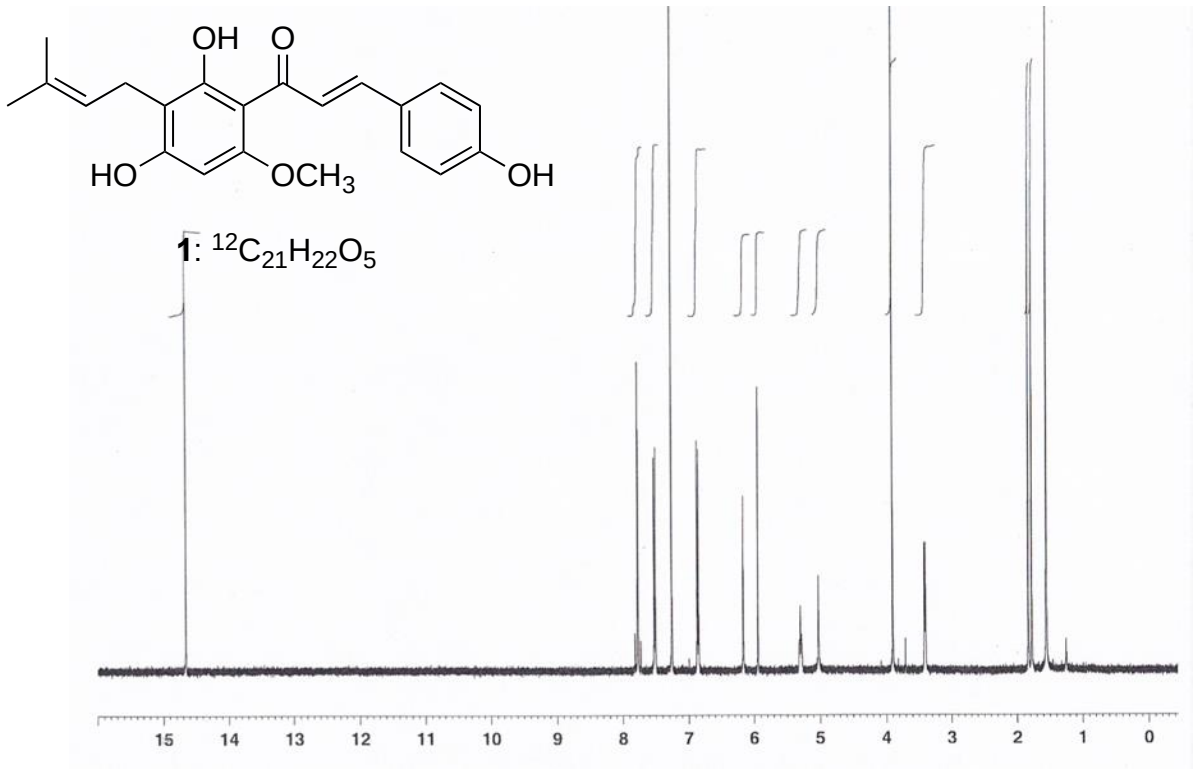
$[^{13}C]_2$-17: ^{13}C NMR, 100 MHz, $CDCl_3$



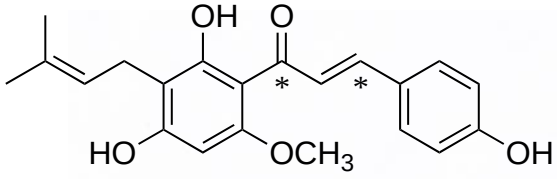
1,3-[^{13}C]$_2$-Xanthohumol ([^{13}C]$_2$-1): ^{1}H NMR, 400 MHz, $CDCl_3$



Natural xanthohumol (1): ^{1}H NMR, 400 MHz, $CDCl_3$

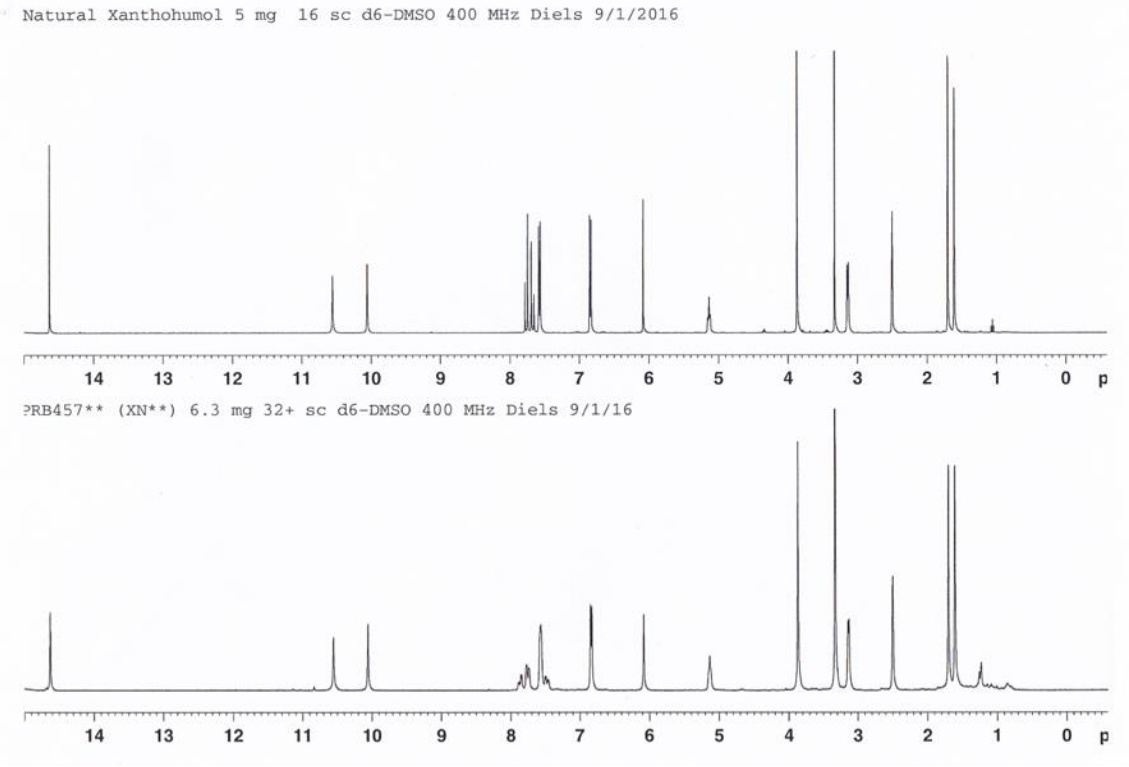


1,3-[^{13}C]$_2$-Xanthohumol ([^{13}C]$_2$-1): ^{1}H NMR, 400 MHz, d$_6$-DMSO

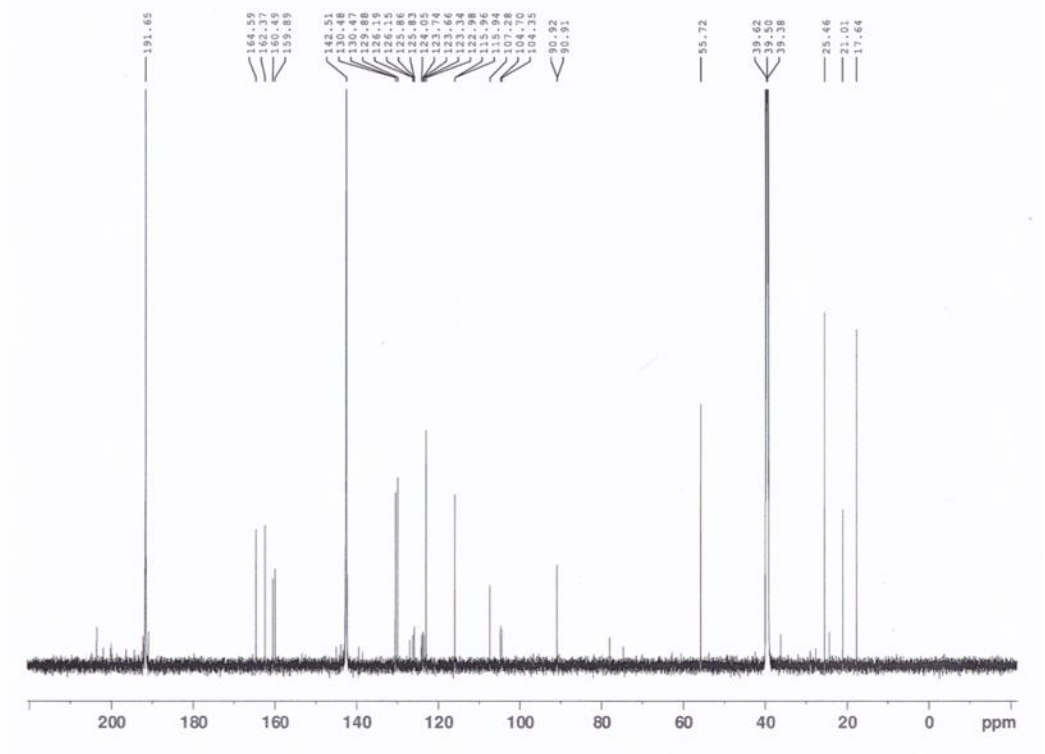


[^{13}C]$_2$-**1**: $^{12}C_{19}{}^{13}C_2H_{22}O_5$

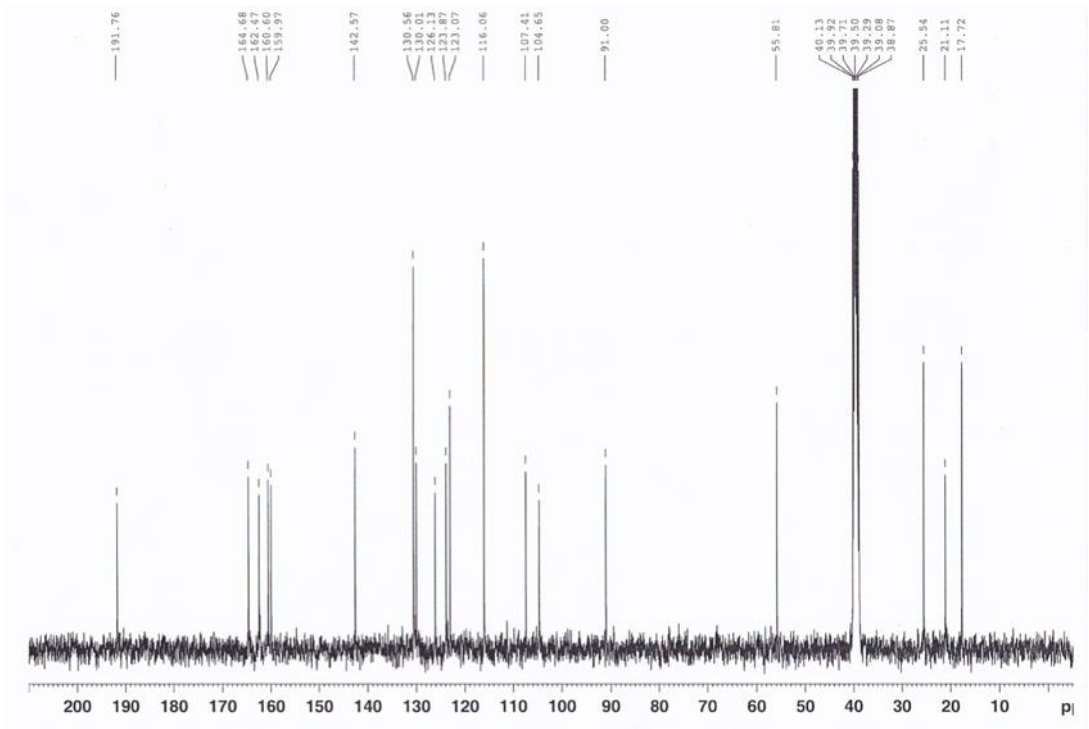
Comparison of 1 (top) and [^{13}C]$_2$-1 (bottom): ^{1}H NMR, 400 MHz, d$_6$-DMSO



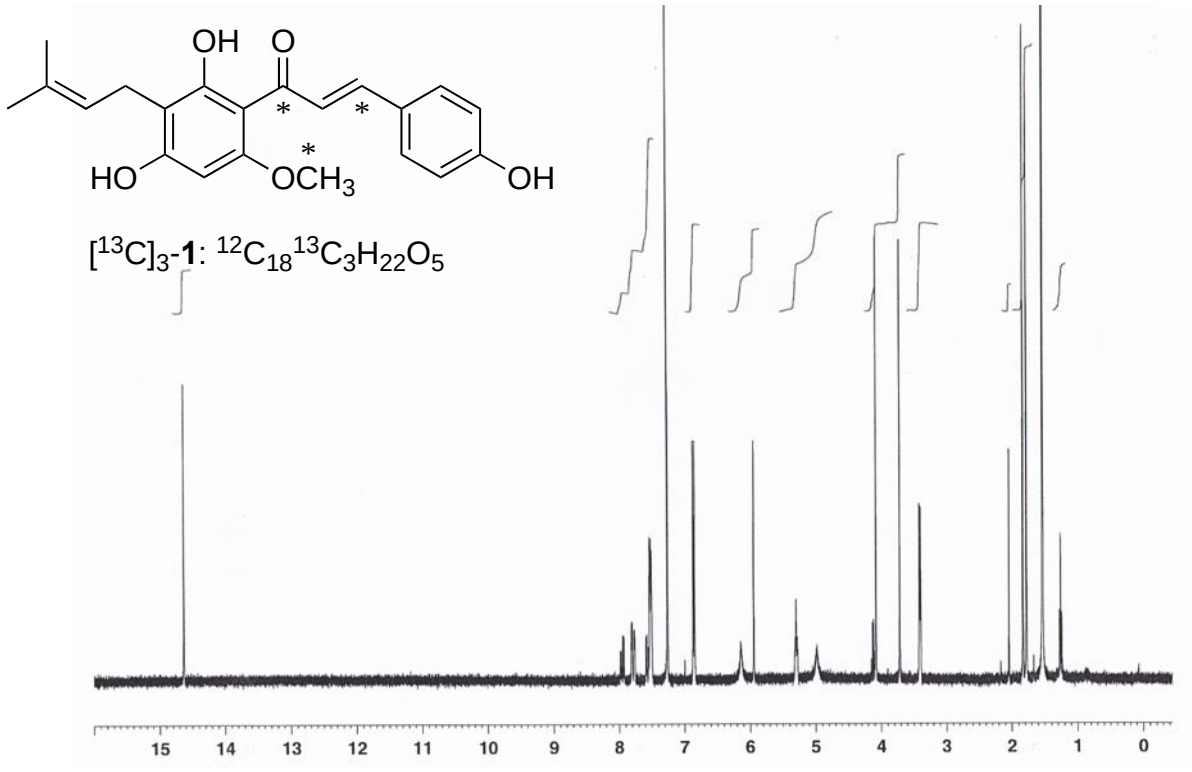
1,3-[$^{13}C]_2$-Xanthohumol ([$^{13}C]_2$-1): ^{13}C NMR, 175 MHz, d_6-DMSO



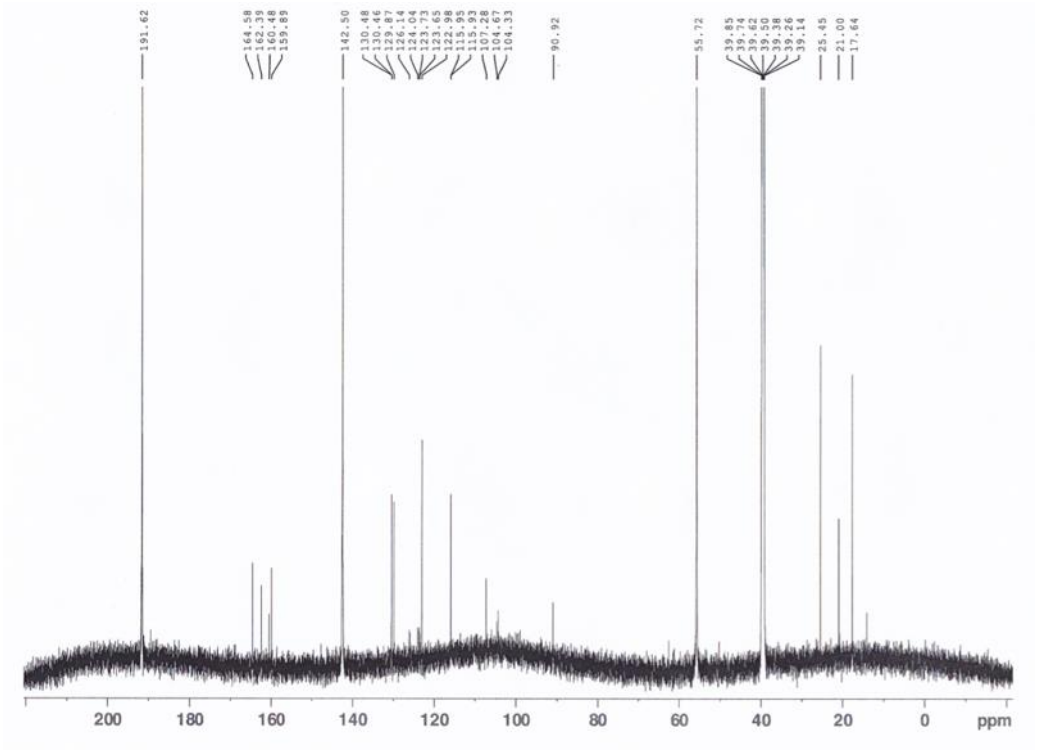
Natural xanthohumol (1): ^{13}C NMR, 100 MHz, d_6-DMSO



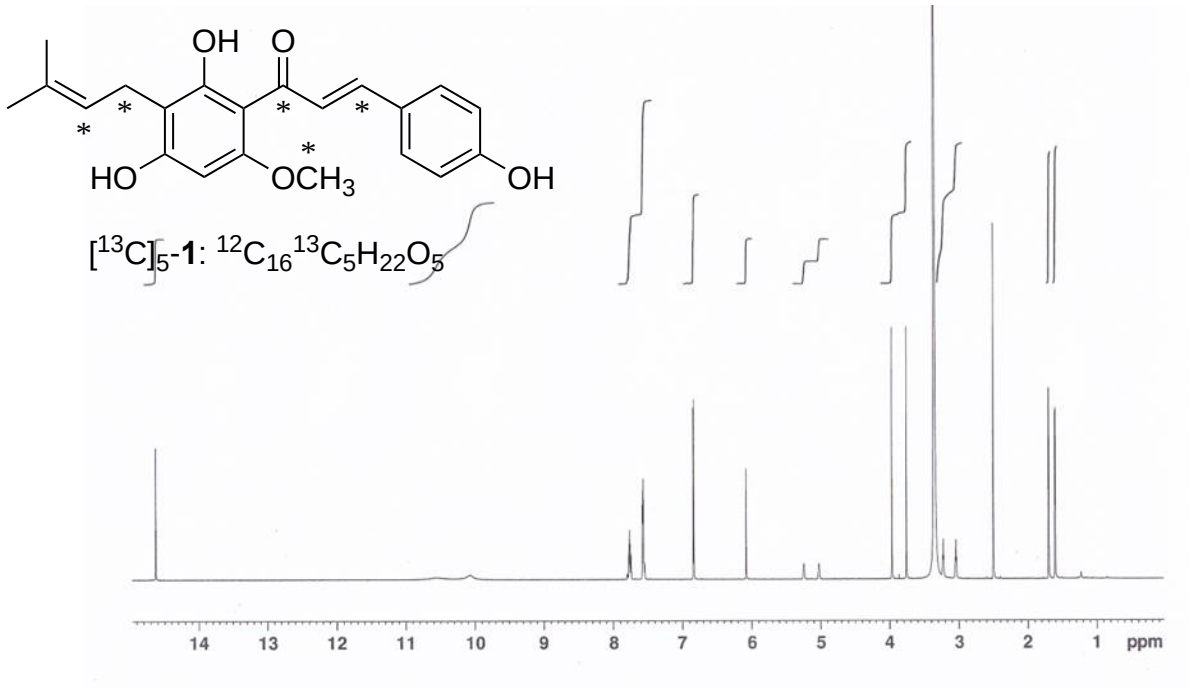
[$^{13}C]_3$-Xanthohumol ([$^{13}C]_3$-1): ^1H NMR, 400 MHz, $CDCl_3$



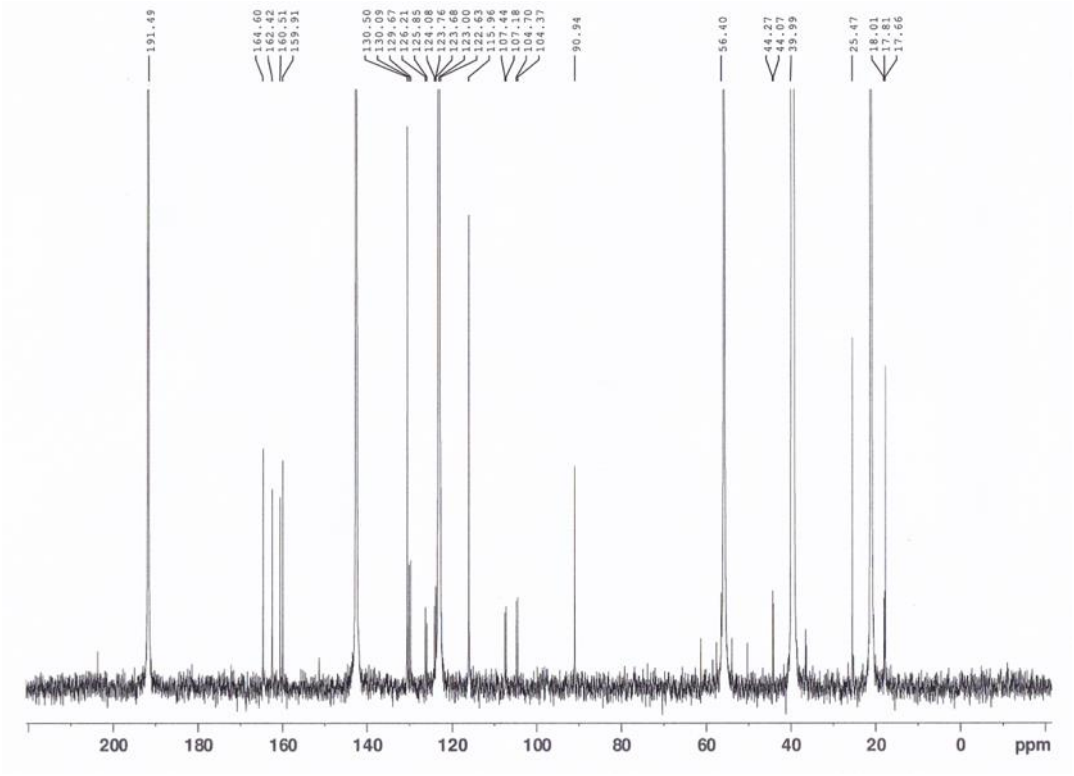
[$^{13}C]_3$-Xanthohumol ([$^{13}C]_3$-1): ^{13}C NMR, 175 MHz, d_6-DMSO



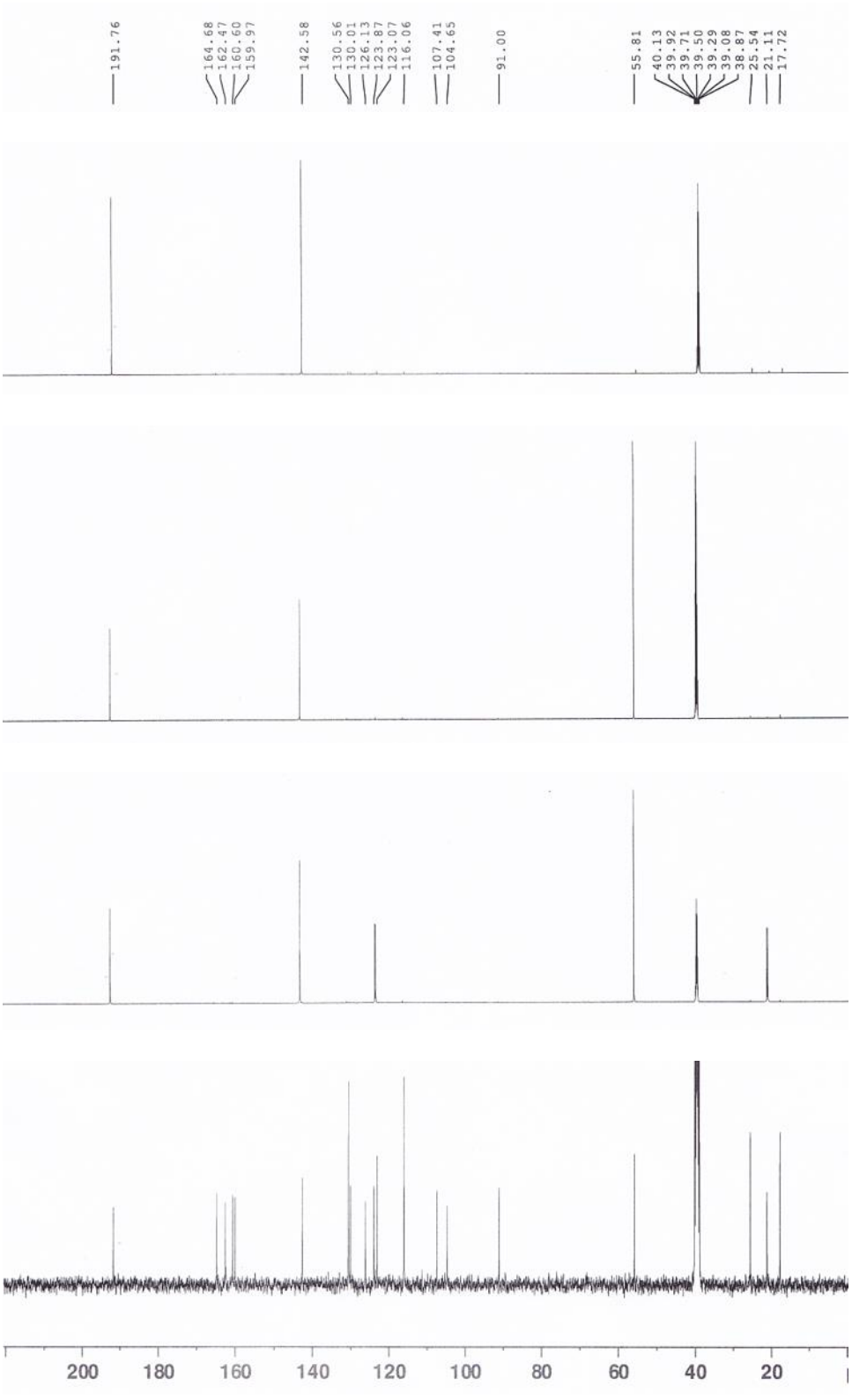
[^{13}C]$_5$-Xanthohumol ([^{13}C]$_5$-1): ^1H NMR, 700 MHz, d_6-DMSO



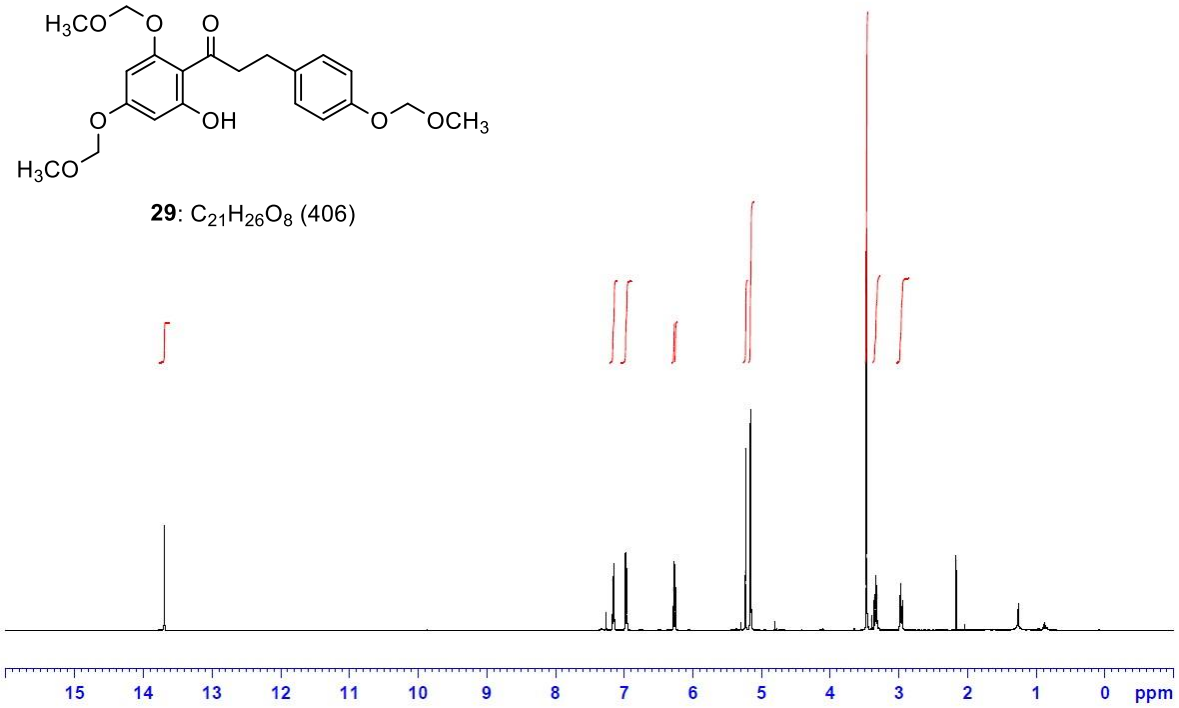
[^{13}C]$_5$-Xanthohumol ([^{13}C]$_5$-1): ^{13}C NMR, 175 MHz, d_6-DMSO



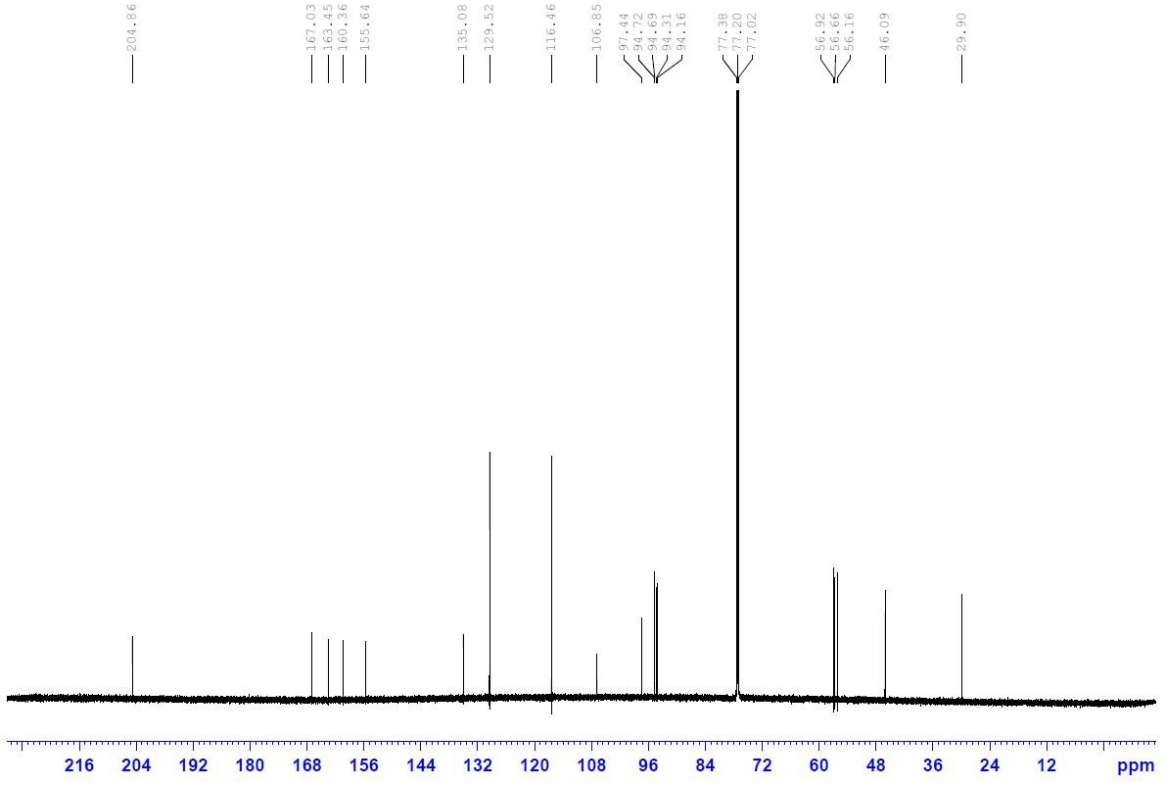
Comparison of ^{13}C NMR spectra for $[^{13}C]_2$-1, $[^{13}C]_3$-1, $[^{13}C]_5$-1, and 1 (top to bottom)



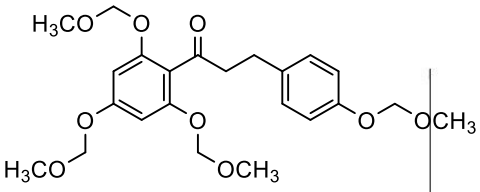
29: ^{1}H NMR, 400 MHz, $CDCl_3$



29: ^{13}C NMR, 175 MHz, $CDCl_3$

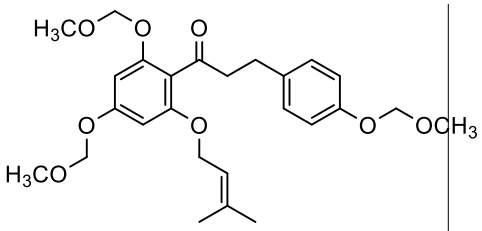


30: ^{1}H NMR, 400 MHz, $CDCl_3$



30: $C_{23}H_{30}O_9$ (450)

31: ^{1}H NMR, 400 MHz, $CDCl_3$



31: $C_{26}H_{34}O_8$ (474)