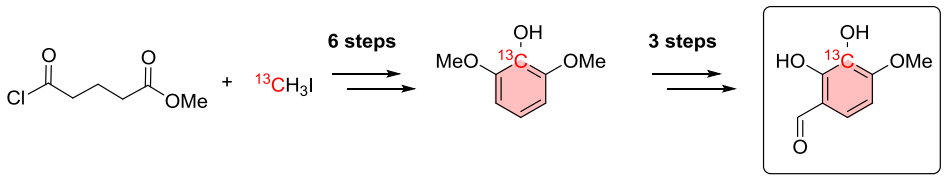
Graphical abstract



Synthesis of [3-^{13}C]-2,3-dihydroxy-4-methoxybenzaldehyde

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Abstract: An efficient synthesis of [3-^{13}C]-2,3-dihydroxy-4-methoxybenzaldehyde, an isotopically labelled probe of a common intermediate used in the synthesis of a number of biologically relevant molecules, has been achieved in 9 steps from an acyclic, non-aromatic precursor. A ^{13}C label for molecular imaging was introduced in a linear synthesis from the reaction of [^{13}C]-labelled methyl iodide with glutaric monomethyl ester chloride. Cyclisation then aromatisation gave 1,3-dimethoxybenzene and an additional methoxy group was introduced by a formylation/Baeyer-Villiger/hydrolysis/methylation sequence. Subsequent *ortho*-formylation and selective demethylation yielded the desired [3-^{13}C]-2,3-dihydroxy-4-methoxybenzaldehyde.

Keywords: Dynamic Nuclear Polarisation; ^{13}C-Labelling; Baeyer-Villiger

2,3-Dihydroxy-4-methoxybenzaldehyde **1** can be used as a key intermediate in a number of natural products/drugs that have a range of biological activities (Figure 1), including the vascular disrupting agents CA1P **2** and BNC 105P **3** that are currently in Phase I and II clinical trials, respectively.[1–3] Another anti-cancer compound that can use this compound as a key intermediate is narciclasine **4**,[4] shown *in vivo* to be effective against primary brain cancers and brain metastases.[5] Amongst other molecules of interesting biological properties, thalimonine **5** is a natural product that has shown to be active against influenza and herpes simplex virus,[6–8] whilst stenocepflavone **6** exhibits significant inhibitory activity against acetylcholinesterase, butyrylcholinesterase and lipoxygenases,[9] and 2'-hydroxy-3',4',3,4-tetramethoxychalcone **7** shows a topical anti-inflammatory effect in mice.[10] Whilst the biological activity of such compounds is usually easy to obtain, real-time molecular imaging can provide vital information on molecular targets and the metabolic fates of

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biologically active species. Dynamic nuclear polarization (DNP) is a hyperpolarization based magnetic resonance technique that can significantly increase the sensitivity of ^{13}C nuclei;[11] when used in conjunction with specifically labelled ^{13}C samples, the relative sensitivity is further increased, allowing *in vivo* metabolism to be investigated.

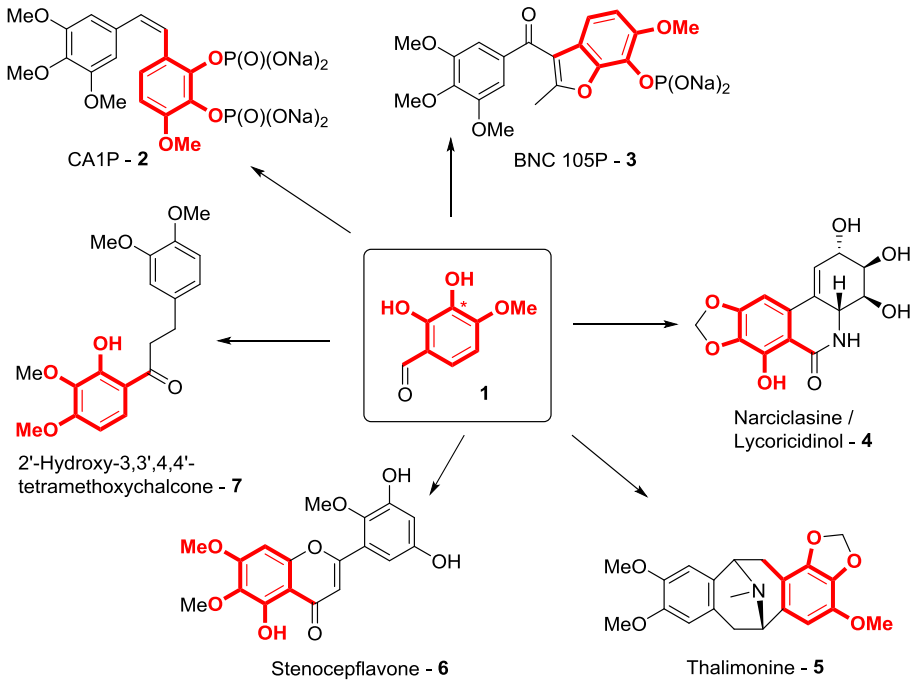
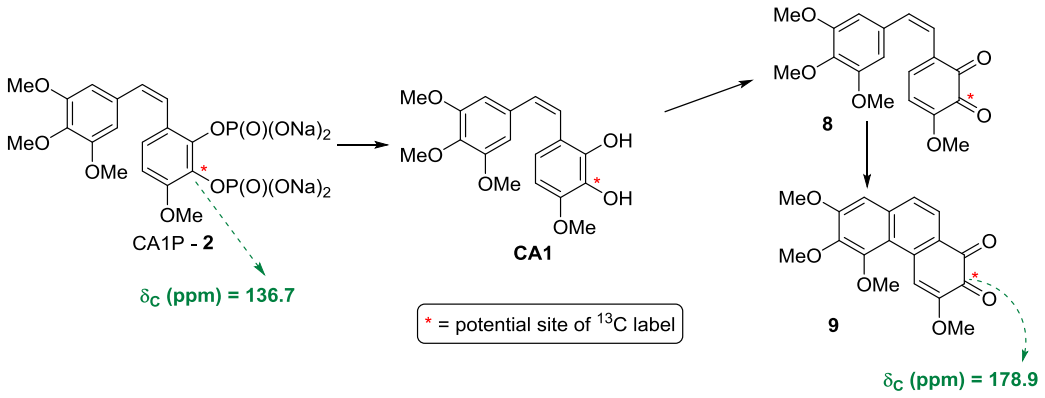


Figure 1. Potential natural products derived from key intermediate **1**

The design criteria for a ^{13}C labelled probe for use in DNP studies is critical, since this needs to be at a site in the molecule with a long T_1 relaxation time (essential for extending the lifetime of hyperpolarization), ideally on a quaternary centre remote from any neighboring spin ½ nuclei, which cannot undergo any destructive metabolism that would remove the label from the probe. Furthermore, the label need to be located at a site where metabolic activity occurs in order to observe a change in the chemical shift of the enhanced ^{13}C signal that would be indicative of any new metabolite formed. For example, combretastatin A1P **2** is known to metabolise into *ortho*-quinone species **8** and **9** *in vivo* (Scheme 1), but the biological role of this metabolite has not yet been established.[12] The chemical shift of the highlighted ^{13}C in CA1P **2** is significantly lower than that on the same site in the *ortho*-quinone metabolite **9** [δ_c (ppm) 136.7 vs. 178.9],[13,14] so by hyperpolarising [^{13}C]-CA1P **2**, signals for both species can easily be distinguished. Thus development of an efficient route to prepare [3-^{13}C]-2,3-dihydroxy-4-methoxybenzaldehyde **1** would provide an avenue to prepare and investigate real-time

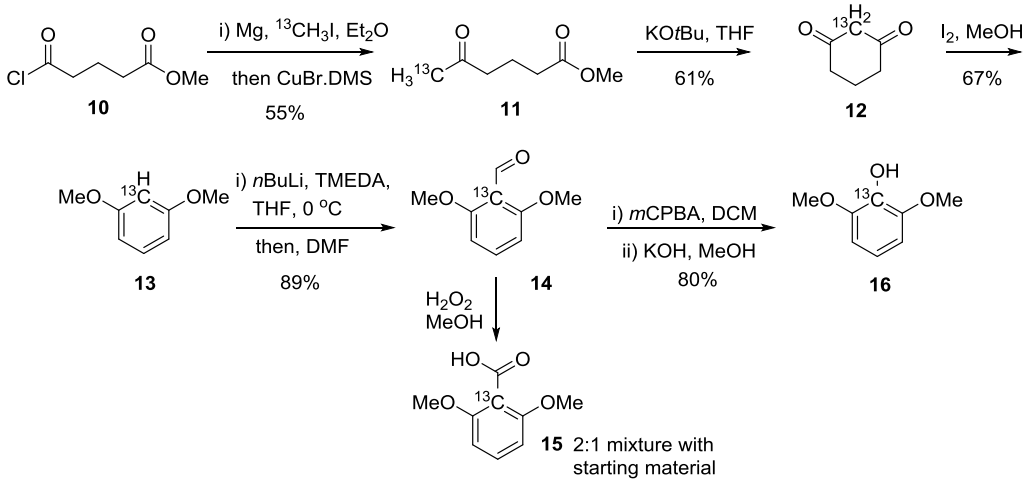
biological processes for these molecules of potential therapeutic interest. Furthermore, routes to pyrogallol derivatives of this type with site-specific incorporation of an aromatic ^{13}C label have not been reported.



Scheme 1. Known ortho-quinone metabolites of CA1P **2**

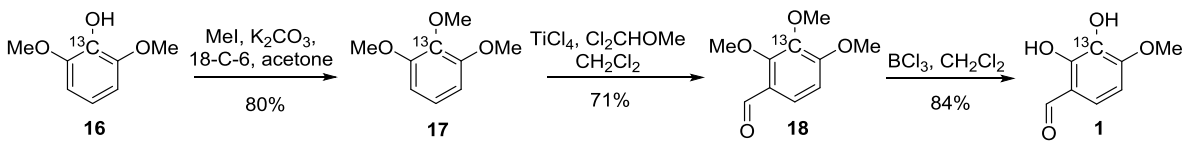
The synthesis of [3-^{13}C]-2,3-dihydroxy-4-methoxybenzaldehyde **1** started with the installation of the ^{13}C label by modifying an existing literature method.[15] In this work, Botting and co-workers treated glutaric monomethyl ester chloride **10** with [^{13}C]-lithium dimethyl cuprate, generated from [^{13}C]-methyl iodide, lithium, and copper(I) iodide, which resulted in formation of methyl 5-oxo-[6-^{13}C]-hexanoate **11** in 43% yield. However, in the route developed herein, an improved yield of 55% for the ester **11** was obtained when the acid chloride **10** was treated with [^{13}C]-methyl magnesium iodide and copper(I) bromide dimethyl sulfide, where the [^{13}C] labelled Grignard reagent was accessed from commercially available [^{13}C]-methyl iodide (Scheme 2). This method was found to be highly reproducible and easier to operate than the original cuprate procedure. Furthermore, this method circumvents the sometimes difficult generation of [^{13}C]-labelled methyl lithium and uses stoichiometric quantities of [^{13}C]-methyl iodide, rather than losing an additional unreactive equivalent through the cuprate. Cyclisation of methyl 5-oxo-[6-^{13}C]-hexanoate **11** to [2-^{13}C]-cyclohexane-1,3-dione **12** was achieved in 61% yield *via* an intramolecular Claisen reaction using potassium *tert*-butoxide as the base. The product was observed as a mixture of both keto and enol tautomers in the ^{1}H NMR spectrum. Aromatisation of [2-^{13}C]-cyclohexane-1,3-dione **12** to give [2-^{13}C]-resorcinol has previously been achieved by catalytic dehydrogenation using Pd/C in refluxing xylene or triglyme with yields ranging from 45 – 88%.[16–18] However, an alternate, more efficient route was developed that encompassed both the aromatisation and subsequent methylation steps in a one-pot reaction. Thus, dione **12** was heated at reflux with iodine in methanol to give the aromatised 1,3-dimethoxy-[2-^{13}C]-benzene **13** in 67% yield.[19] Next, the regioselective addition of a hydroxyl group in the 2-position was accomplished using a three-step strategy. Regioselective lithiation of 1,3-dimethoxy-[2-^{13}C]-

benzene **13** with *n*BuLi at 0 °C in the presence of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine followed by treatment with dimethylformamide and gave [1-^{13}C]-2,6-dimethoxybenzaldehyde **14** in 89% yield.[20] This was then treated with hydrogen peroxide in methanol to affect a Baeyer-Villiger reaction but unfortunately this failed to yield the desired formate ester, instead giving carboxylic acid **15** in a 2:1 ratio with starting material, formed by hydride-migration in the rearrangement step of the Baeyer-Villiger reaction. However, when aldehyde **14** was treated with *m*CPBA, the desired formate ester was formed, which was hydrolyzed using potassium hydroxide to give phenol **16** in 84% yield over the three-steps from methyl ether **13**.



Scheme 2. Synthesis of [2-^{13}C]-2-hydroxy-1,3-methoxybenzene **16**

[2-^{13}C]-2,6-Dimethoxyphenol **16** was then methylated using potassium carbonate and methyl iodide to yield [2-^{13}C]-1,2,3-trimethoxybenzene **17** in 80% yield, that underwent *ortho*-formylation using Rieche conditions (titanium tetrachloride and dichloromethyl methyl ether) to give the aldehyde **18** in 71% yield (Scheme 3). Treatment of [3-^{13}C]-2,3,4-trimethoxybenzaldehyde **18** with two equivalents of boron trichloride led to selective demethylation of the methyl ethers *ortho* and *meta* to the carbonyl group, providing [3-^{13}C]-2,3-dihydroxy-4-methoxybenzaldehyde **1** in 84% yield. The regiochemistry of this deprotection was confirmed by correlation of the ^{13}C NMR data with previously reported unlabeled material, with all chemical shifts matching to within 0.3 ppm.[21]



Scheme 3. Synthesis of [3-^{13}C]-2,3-hydroxy-4-methoxybenzaldehyde **4**

In summary, we have adapted and developed an efficient route to install a ^{13}C label at a strategically important site of a common intermediate that can be used to construct a number of biologically relevant molecules. Ongoing work is looking to exemplify the use of such an intermediate in the synthesis of the target molecules illustrated.

Acknowledgments

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Supplementary data

Supplementary data (experimental procedures and characterization data) associated with this article can be found in the online version.

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