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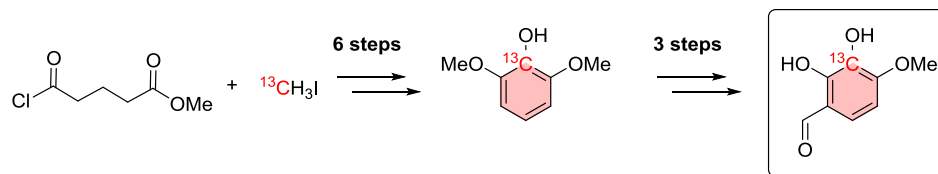
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## Graphical abstract



# Synthesis of [3-<sup>13</sup>C]-2,3-dihydroxy-4-methoxybenzaldehyde

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**Abstract:** An efficient synthesis of [3-<sup>13</sup>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 <sup>13</sup>C label for molecular imaging was introduced in a linear synthesis from the reaction of [<sup>13</sup>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-<sup>13</sup>C]-2,3-dihydroxy-4-methoxybenzaldehyde.

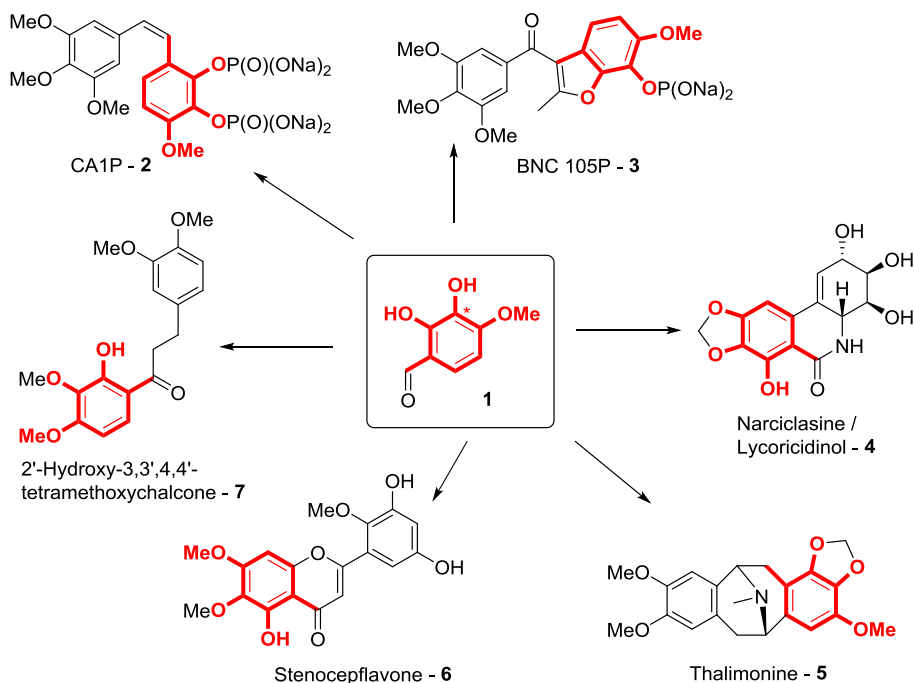
**Keywords:** Dynamic Nuclear Polarisation; <sup>13</sup>C-Labeling; Baeyer-Villiger

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 CAIP **2** and BNC 105P **3** that are currently in Phase I and II clinical trials, respectively.<sup>1-3</sup> Another anti-cancer compound that can use this compound as a key intermediate is narciclasine **4**,<sup>4</sup> shown in vivo to be effective against primary brain cancers and brain metastases.<sup>5</sup> 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,<sup>6-8</sup> whilst stenocephlavone **6** exhibits significant inhibitory activity against acetylcholinesterase, butyrylcholinesterase and lipoxygenases,<sup>9</sup> and 2'-hydroxy-3',4',3,4-tetramethoxychalcone **7** shows a topical anti-inflammatory effect in mice.<sup>10</sup> 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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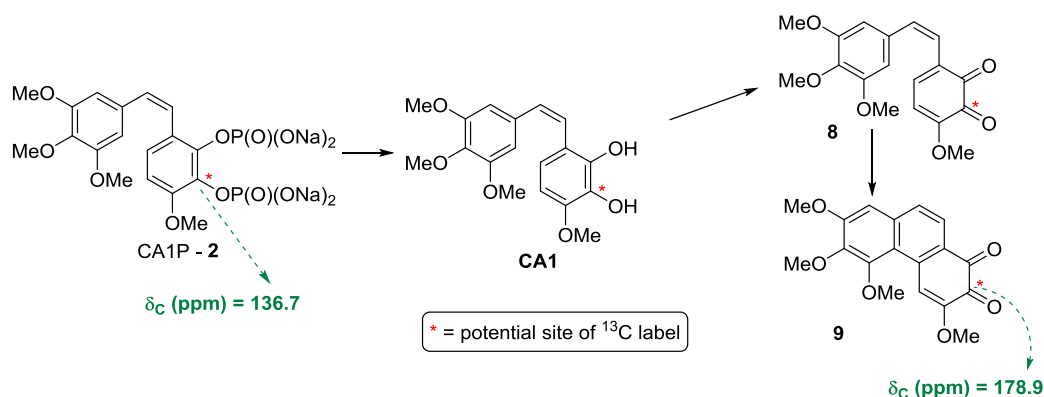
biologically active species. Dynamic nuclear polarization (DNP) is a hyperpolarization based magnetic resonance technique that can significantly increase the sensitivity of  $^{13}\text{C}$  nuclei;<sup>11</sup> when used in conjunction with specifically labelled  $^{13}\text{C}$  samples, the relative 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  $^{13}\text{C}$  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  $\frac{1}{2}$  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  $^{13}\text{C}$  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.<sup>12</sup> The chemical shift of the highlighted  $^{13}\text{C}$  in CA1P **2** is significantly lower than that on the same site in the ortho-quinone metabolite **9** [ $\delta_c$  (ppm) 136.7 vs. 178.9],<sup>13,14</sup> so by hyperpolarising [ $^{13}\text{C}$ ]-CA1P **2**, signals for both species can easily be distinguished. Thus development of an efficient route to prepare and investigate real-time [3- $^{13}\text{C}$ ]-2,3-dihydroxy-4-methoxybenzaldehyde **1** would provide an avenue to prepare and investigate real-time

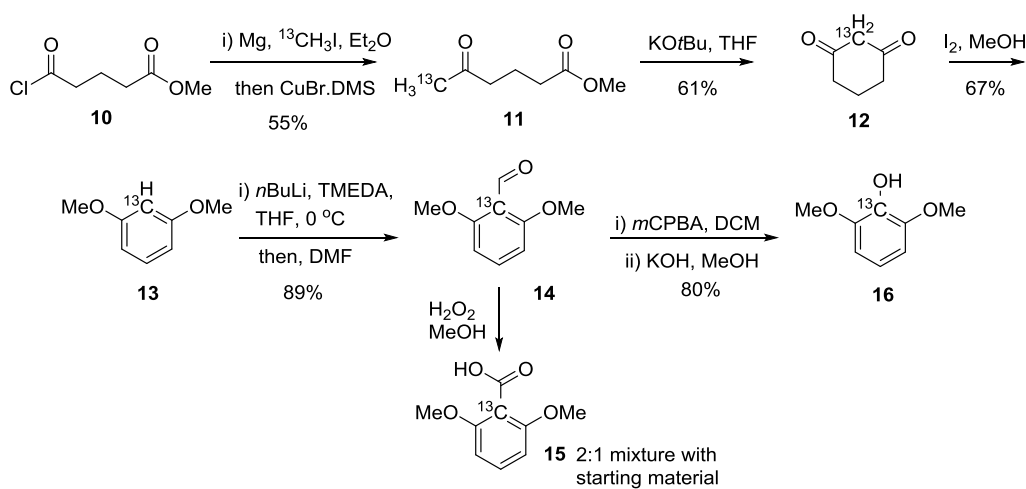
biological processes for these molecules of potential therapeutic interest. Furthermore, routes to pyrogallol derivatives of this type with site-specific incorporation of an aromatic  $^{13}\text{C}$  label have not been reported.



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

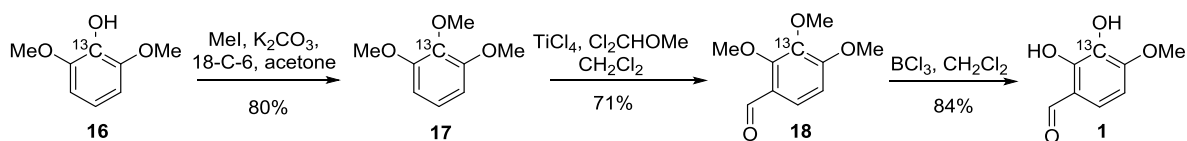
The synthesis of [3- $^{13}\text{C}$ ]-2,3-dihydroxy-4-methoxybenzaldehyde **1** started with the installation of the  $^{13}\text{C}$  label by modifying an existing literature method.<sup>15</sup> In this work, Botting and co-workers treated glutaric monomethyl ester chloride **10** with [ $^{13}\text{C}$ ]-lithium dimethyl cuprate, generated from [ $^{13}\text{C}$ ]-methyl iodide, lithium, and copper(I) iodide, which resulted in formation of methyl 5-oxo-[6- $^{13}\text{C}$ ]-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 [ $^{13}\text{C}$ ]-methyl magnesium iodide and copper(I) bromide dimethyl sulfide, where the [ $^{13}\text{C}$ ] labelled Grignard reagent was accessed from commercially available [ $^{13}\text{C}$ ]-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 [ $^{13}\text{C}$ ]-labelled methyl lithium and uses stoichiometric quantities of [ $^{13}\text{C}$ ]-methyl iodide, rather than losing an additional unreactive equivalent through the cuprate. Cyclisation of methyl 5-oxo-[6- $^{13}\text{C}$ ]-hexanoate **11** to [2- $^{13}\text{C}$ ]-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  $^1\text{H}$  NMR spectrum. Aromatisation of [2- $^{13}\text{C}$ ]-cyclohexane-1,3-dione **12** to give [2- $^{13}\text{C}$ ]-resorcinol has previously been achieved by catalytic dehydrogenation using Pd/C in refluxing xylene or triglyme with yields ranging from 45 – 88%.<sup>16–18</sup> 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- $^{13}\text{C}$ ]-benzene **13** in 67% yield.<sup>19</sup> 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- $^{13}\text{C}$ ]-

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-<sup>13</sup>C]-2,6-dimethoxybenzaldehyde **14** in 89% yield.<sup>20</sup> 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-<sup>13</sup>C]-2-hydroxy-1,3-dimethoxybenzene **16**

[2-<sup>13</sup>C]-2,6-Dimethoxyphenol **16** was then methylated using potassium carbonate and methyl iodide to yield [2-<sup>13</sup>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-<sup>13</sup>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-<sup>13</sup>C]-2,3-dihydroxy-4-methoxybenzaldehyde **1** in 84% yield. The regiochemistry of this deprotection was confirmed by correlation of the <sup>13</sup>C NMR data with previously reported unlabeled material, with all chemical shifts matching to within 0.3 ppm.<sup>21</sup>



**Scheme 3.** Synthesis of [3-<sup>13</sup>C]-2,3-dihydroxy-4-methoxybenzaldehyde **4**

In summary, we have adapted and developed an efficient route to install a  $^{13}\text{C}$  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.

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We thank University of Sheffield A\*STAR programme for funding (RCC), as well as a Programme Grant C1276/A10345 from Cancer Research UK and EPSRC with additional funding from MRC and Department of Health (England).

### Supplementary data

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

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