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Synthesis of [^{3}H] and [^{14}C]Genipin

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Abstract
[^{3}H]Genipin was synthesized in a single step by Ir(I) catalyzed hydrogen isotope exchange. Conditions for selective exchange of the sp^{2} CH bond *ortho* to the methyl ester functionality were developed through deuterium modeling studies through a catalyst screen. Optimized conditions so obtained were then utilized with tritium gas to generate [^{3}H]genipin at a specific activity of 18.5 Ci/mmol. Racemic [^{14}C]genipin was prepared in 8 steps in overall 5.4% radiochemical yield from potassium [^{14}C]cyanide.

Key Words
Tritium, , Iridium (I), carbon-14, genipin, Otera's catalyst, Gardenia Blue

Introduction
Genipin **1**, an iridoid derived from the glycoside geniposide 2 (Figure 1), [1,2] is present in the fruit of the *Gardenia jasminoides* Ellis.[3] It is isolated on a commercial basis from the fruit extracts by treatment with β-glucosidase. This Gardenia fruit has long been used in traditional Chinese medicine and has been reported to possess a number of pharmacological properties including neuroprotective activity[4] and is also the source of Gardenia Blue which is widely used in Eastern Asia as a natural food colourant.[2] In order to allow marketing of Gardenia Blue as a natural food colourant in the US and EU, additional studies on the safely and metabolism of genipin and Gardenia Blue are required. While the metabolism of genipin and geniposide have been previously studied,[5,6] these studies were carried out without radiolabelled test articles and thus lack the detailed metabolic information required by regulatory agencies. Hence ^{3}H and racemic [^{14}C]genipin were synthesized to allow a comprehensive metabolic evaluation of genipin and thus the synthesis of both labelled forms are reported in this paper.

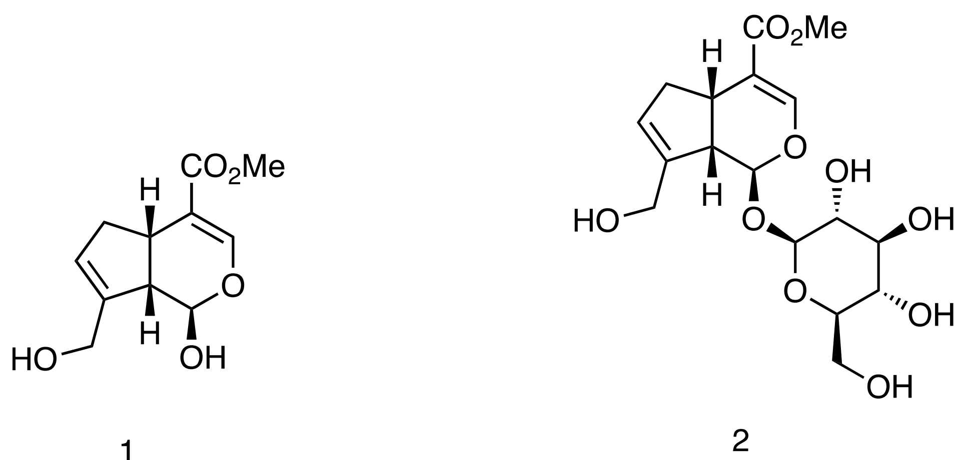

Figure 1 (+)-Genipin **1** and (+)-Geniposide **2**

Results and discussion

[^{3}H]Genipin

Due to the complexity and length of the ^{14}C synthesis we first targeted the tritium labelling of genipin in order to provide radiolabelled material as quickly as possible and allow rapid development of the methodology required for detailed analysis of the metabolites of genipin. The ready commercial availability of (+)-genipin meant that we used enantiomerically pure genipin in our experiments to prepare [^{3}H]genipin for methods development work, despite the fact that a racemic radiotracer was deemed acceptable by the end user. Thus for ^{14}C, we opted to prepare the racemic [^{14}C]genipin in line with the needs of the end user.

One of the foremost methods of introducing heavy isotopes of hydrogen is through iridium-catalysed hydrogen isotope exchange (HIE).[7] In particular, the Ir(I) N-heterocylic carbene (NHC)/phosphine complexes developed by Kerr[7] allow incorporation of deuterium or tritium via a directed HIE process with a broad range of compatible Lewis basic directing groups. In particular, Kerr has reported the non-aryl Csp^{2}-H labelling of α,β-unsaturated systems,[7] where the use of extremely low loadings of catalyst is key to avoid competing double bond reduction processes. Based on this, we proposed that the β-C-H bond within the enolate moiety would be a suitable site for labelling of genipin. Nonetheless, the specific functionality within genipin means that it presents a significant challenge when considering Ir(I)-catalysed HIE. Specifically, there is a risk of reduction of either of the two double bonds. In addition, the sensitive allylic alcohol and hemiacetal functionalities add to the challenges within a directed HIE strategy.

Given these challenges, our initial studies focused on deuterium labelling as a proxy for tritium. Building on Kerr's β-labelling work,[8] a catalyst screen was first conducted using [Ir(cod)(IMes)(PR_3)] complexes with varying phosphine ligands in the complex (Figure 2).

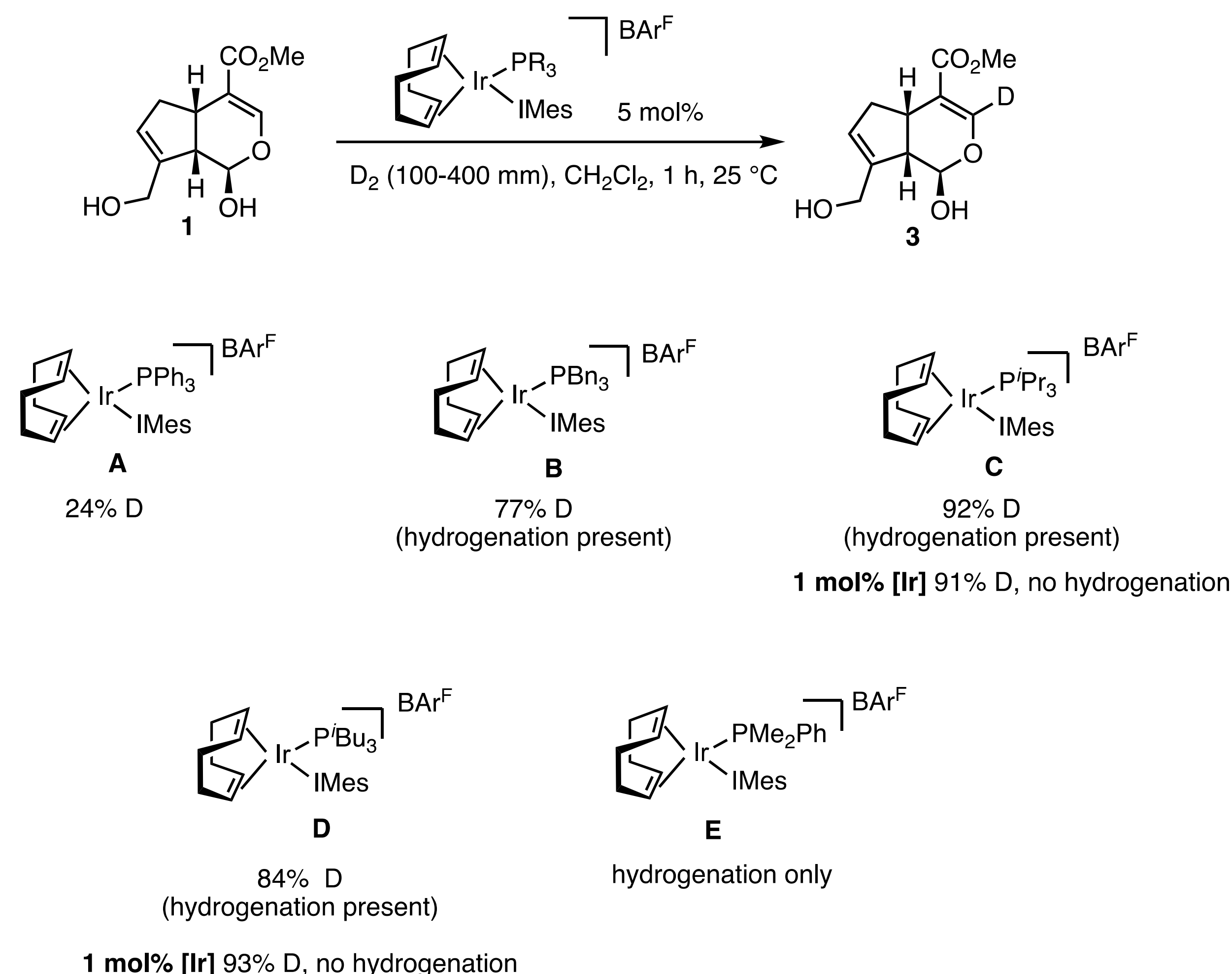

Figure 2 Genipin deuteration screening experiments

In order to screen for a highly active catalyst system in the first instance, initial studies were conducted with various Ir(I) catalysts at 5 mol% which was chosen to maximize the chances of observing deuterium incorporation. A range of deuterium pressures from 100 mm to 400 mm (17.2 μmol- 69 μmol deuterium) were used, with this wide range dictated by set up limitations with the deuterium manifold. Pleasingly, a range of Ir(I) complexes (**A-D**) all afforded [^{2}H]$_1$genipin to a high degree of incorporation. Ir catalyst **E**, bearing the small PMe_2Ph afforded only hydrogenation products and was thus not a competent

catalyst system for labelling at the β-C–H bond. This catalyst has previously been reported by Kerr as a highly active catalyst for directed hydrogenation at loadings as low as 0.5 mol%.[8] At loadings of 5 mol%, catalysts that provided high incorporations of deuterium at the β-C–H bond (**B**-**D**) were also competent in partial hydrogenation of genipin, which was in line with our expectations.[8] To avoid this reduction, as previously established,[8] lowering the catalyst loading to a very low 1 mol% of complex **D** afforded solely the desired deuterated product, with no reduction observed, and an excellent isotopic incorporation of 93%. Similarly using a 1% loading of complex **C**, an isotopic incorporation of 91% was achieved.

With optimized conditions in hand for the deuteration of genipin, [^{3}H]genipin was next targeted. Employing similar conditions, under sub-atmospheric pressure of tritium, we were delighted to observe successful tritium incorporation at the β-C–H bond, using complex **D** with no products of reduction observed (Figure 3).

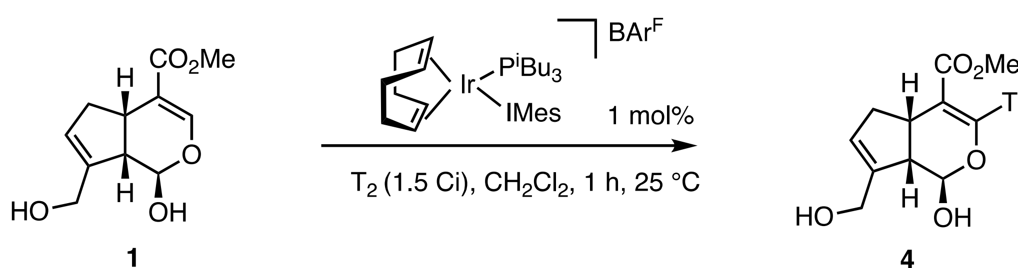

Figure 3 Tritiation of Genipin

Following purification of the crude material by semi-preparative HPLC, [^{3}H]genipin was isolated, yielding a total of 69.2 mCi with a specific activity of 18.7 Ci/mmol and a radiochemical purity of 99.3%. It should be pointed out that no measurements were made to determine chiral purity of [^{3}H]genipin as it was not a requirement of the end user and based on the mechanism of the exchange reaction employed, it was also deemed highly unlikely that racemization would have occurred. Iridium(I)-catalysed HIE thus represents an extremely efficient and effective method for the direct, late-stage tritiation of genipin, and serves as a case study for the labelling of molecules with a range of sensitive and challenging functionalities.

[^{14}C]Genipin 12.

The ^{14}C synthesis was based on the route described by Jones[9] for the preparation of (+)-geniposide. The route described synthesis of both racemic and enaniomerically pure geniposide and thus was adapted to prepare the desired racemic [^{14}C]genipin **12** target compound as shown in Figure 4.

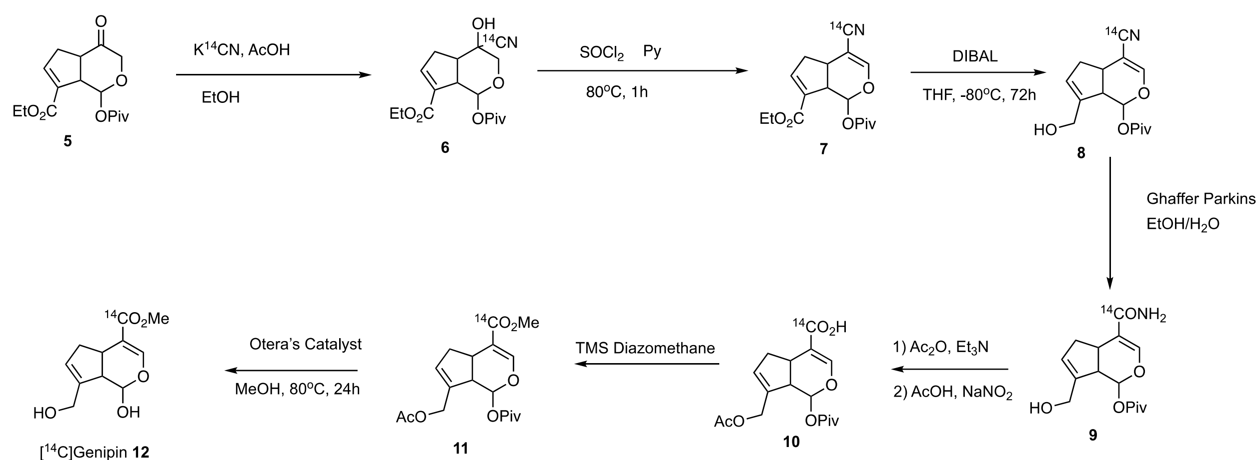

Figure 4 Synthesis of Racemic [^{14}C]Genipin **12**

In the first step as described by Jones,[9] the cyanohydrin **6** is formed by treatment with 5 equivalents of potassium cyanide (KCN) with excess acetic acid in ethanol. Fortunately, it proved to be possible to lower the equivalents of cyanide to one with an acceptable reduction in the yield. Hence ketone **5** was treated with 0.95 equivalents of potassium [^{14}C]cyanide with excess acetic acid in ethanol to yield cyanohydrin **6** in 67% radiochemical yield after silica gel column chromatography. It was found advantageous to purify the cyanohydrin prior to the dehydration step, as attempts to take on the crude material as per the Jones procedure, led to significantly lower yields. Dehydration of the cyanohydrin **6** was accomplished by heating with thionyl chloride and pyridine at 80°C for 1 hour. Under these conditions, the desired nitrile **7** was obtained in 64% yield after silica gel column chromatography. Selective reduction of the ethyl ester to the primary alcohol **8** was achieved by reaction with diisobutylaluminium hydride (DIBAL) in tetrahydrofuran (THF) at -80°C. In order to avoid concurrent reduction of the nitrile, it was critical to maintain the temperature at -80°C and it was also found the efficiency of the reduction was greatly improved by use of freshly distilled THF from calcium hydride. The desired allylic alcohol **8** was obtained in 56% radiochemical yield after silica gel column chromatography. Interestingly, while the literature[10] would suggest that selective reduction of the ester in the presence of the nitrile should be achievable with lithium borohydride, attempts to use this reagent led to extensive decomposition.

At this point of the synthesis with 24 mCi of the alcohol **8** in hand, but with a target amount of 10 mCi of genipin **14** required by the user and still several steps to go, it was decided to run though the first three steps twice more and bring up additional ^{14}C nitrile alcohol **8**. As a result, a total of 85 mCi of nitrile alcohol **8** was taken through the remaining steps.

In the first step, hydrolysis of the nitrile to the amide **9** was accomplished in 89% yield by treatment with Ghaffer-Parkins catalyst.[11] This allows hydration of the nitrile under essentially neutral conditions as the enol-ether moiety is acid sensitive and the pivalate group is base sensitive. Furthermore, the resulting lactol itself is also highly sensitive to base. Conversion of the amide **9** to carboxylic acid **10** was firstly accomplished by '*in situ*' protection of the allylic alcohol with acetate using acetic anhydride, followed by diazotization with sodium nitrite and acetic acid.[12] The acid **10** was isolated in 87% radiochemical yield after a simple extractive work up and used directly in the esterification step. This was accomplished by treatment with an ether solution of trimethylsilyl (TMS) diazomethane at 0°C. The desired methyl ester **11** was isolated in 72% radiochemical yield after silica gel column chromatography.

The final step to selectively remove the pivoyl and acetyl protecting groups poses interesting selectivity challenges. Simple deprotection under basic conditions is not feasible due to the extreme base sensitivity of the lactol moiety. However, in the preparation of geniposide, Jones observed that when using one of the Otera[13] (Figure 5) transesterification catalysts to firstly remove the acetyl group and then migrate the pivoyl group to the allylic alcohol, fully unprotected genipin is also formed, albeit in moderate yield.

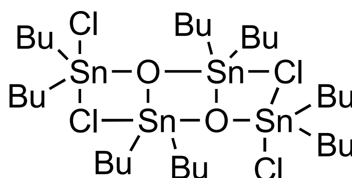

Figure 5 Otera's catalyst

Hence it was found that by heating a methanol solution of penultimate **11** with 1.5 equivalents of Otera's catalyst at 80°C for 24 hours a reliable 32-35% yield of genipin **12** could be isolated. During the course of optimization experiments it was observed that after 24 hours, all starting material was consumed, and a second peak accounting for about 20% of the total radioactivity was formed in addition to the desired product. This was tentatively identified by LC/MS as being the allylic alcohol pivolyl protected intermediate reported by Jones. The HPLC trace also contained a number of undefined radiochemical purities which increased if the reaction was continued beyond 24 hours. Varying the amount of Otera's catalyst from 0.5 to 2 equivalents made little difference to the approximate 30% yield of the desired product. Hence using this procedure, a total of 16.2 mCi was isolated after two silica gel column purifications at a specific activity of 27.5 mCi/mmol as determined by LC/MS and a radiochemical purity of 98.8%.

Materials and Methods

Materials

Tritium gas was purchased from American Radiolabeled Chemicals, St Louis, MO. potassium [^{14}C]cyanide was purchased from Tjaden Biosciences, Burlington IA, Genipin was purchased from MilliporeSigma, St Louis, MO. Ketone 5 was synthesized by RTI Discovery Sciences and used as is. All remaining reagents were purchased from MilliporeSigma or Acros Organics, Pittsburgh, PA and were used as received. All ^{14}C steps were performed under an atmosphere of nitrogen.

Liquid scintillation counting

Quantitation of radioactivity was performed using a Packard 2200 CA liquid scintillation analyzer with Perkin Elmer Ultima Gold scintillation cocktail used throughout.

High performance liquid chromatography

A Waters 2695 Alliance HPLC was used. Chemical purity was determined using a Waters 2996 photodiode array detector and radiochemical purity using a Lablogic Beta Ram 4 radioflow detector with Laura software and Lablogic HA cocktail. The following HPLC systems were used.

1. Phenomenex Gemini NX C18, 50 x 4.6 mm, 3 μ, 239 nm, water (85: 15) acetonitrile for 6 minutes followed by a step gradient to acetonitrile, 1 mL/minute.

2. Phenomenex Gemini NX C18, 50 x 4.6 mm, 3 μ, 215 nm, water (50: 50) acetonitrile for 6 minutes followed by a step gradient to acetonitrile, 1 mL/minute.

3. Phenomenex Gemini NX C18, 50 x 4.6 mm, 3 μ, 215 nm, water (75: 25) acetonitrile for 6 minutes followed by a step gradient to acetonitrile, 1 mL/minute.

4. Phenomenex Gemini NX C18, 50 x 4.6 mm, 3 μ, 230 nm, 0.1% aqueous formic acid (65: 35) acetonitrile for 6 minutes followed by a step gradient to acetonitrile, 1 mL/minute

5. Phenomenex Gemini NX C18, 50 x 4.6 mm, 3 μ, 234 nm, 0.1% Aqueous formic acid (45: 55) acetonitrile for 6 minutes followed by a step gradient to acetonitrile, 1 mL/minute.

Mass Spectrometry

Liquid chromatography mass spectrometry spectra were determined on an Agilent LC/MS system consisting of an Agilent 1260 Infinity II series Solvent Degasser, 1260 Binary Pump,

1260 Vial Autosampler, 1260 Dual Wavelength UV Detector and an Infinity lab LC/MSD Single Quad Mass Spectrometer. The following method was used:

Agilent Poroshell 120 SB C18, 2.7μ, 50 mm x 4.6mm ID, 215 nm, 254 nm, 40°C, 0.1% Aq. formic acid: 0.1% formic acid in acetonitrile (95: 5 to 5: 95) linear gradient over 12 mins, followed by a 6 min hold. ES^+ mode, 1 mL/min

The specific activity of [^{3}H] and [^{14}C]genipin were determined using this method.

[^{2}H]Genipin experiments

In a typical deuteration experiment, genipin **1** (1.1 mg, 4.90 μmol) and the catalyst **A**-**E** (1-5 mol%) were dissolved in methylene chloride (0.2 mL) in a deuteration vessel. The vessel was attached to the deuterium manifold, frozen in liquid nitrogen, evacuated and subjected to a freeze pump thaw cycle before being finally frozen in liquid nitrogen. Deuterium gas (100-400 mm, 17.2 μmol- 69.0 μmol) was then added and the reaction thawed and then stirred for 1 hour at room temperature. The reaction was quenched by the addition of one drop of acetonitrile, evaporated to dryness, redissolved in ethanol, evaporated to dryness and dissolved in ethanol for HPLC (system 1) and LC/MS analysis.

[^{3}H]Genipin 4

[2-^{3}H]7-hydroxymethyl-1-hydroxy-1,4a,5, 7a tetrahydrocyclopenta [c]pyran-4-carboxylic acid methyl ester, [^{3}H]genipin 4.

Genipin **1** (1.1 mg, 4.90 μmol) and [Ir(COD)IMesP^{i}Bu$_3$]BAr^F (0.081 mg, 0.049 μmol) were dissolved in methylene chloride (0.2 mL) in a tritiation vessel. The vessel was attached to the tritium manifold, frozen with liquid nitrogen and subjected to a freeze pump thaw cycle before being finally frozen in liquid nitrogen. Tritium gas (1.46 Ci, 25.3 μmol) was then added and the reaction thawed and then stirred overnight at room temperature. At the completion of the reaction and after capture of spent tritium on the waste bed, the reaction was diluted with ethanol and evaporated to dryness. The process was repeated a second time before the batch was dissolved in ethanol (10 mL) for liquid scintillation and HPLC analysis (system 1). A total of 90 mCi of [^{3}H]genipin at a radiochemical purity of 91.3% was isolated. The batch was evaporated to dryness, dissolved in 0.5 mL of dimethylsulphoxide and purified on a 250 x 10 mm 5μ Gemini NX C18 column with a mobile phase of water (85: 15) acetonitrile at a flow of 5 mL/minute, detection 239 nm. The collected fractions were pooled, evaporated to dryness and dissolved in 40 mL ethanol to yield a total of 69.2 mCi of [^{3}H]genipin **4** at a specific activity of 18.7 Ci/mmol as determined by LC/MS. Unlabelled genipin MH^+ m/z 227, [^{3}H]genipin MH^+ m/z 227 and m/z 229 observed. Identity was further confirmed by co-chromatography with an

authentic standard of genipin (HPLC system 1, k′ = 4.6) and radiochemical purity as determined by HPLC system 1 (k′ = 4.6) was 99.3%. ^{1}H NMR, (500 MHz, D_6DMSO) δ 7.48 ppm (s, 1H), δ 5.70 (d, 1H), δ 4.73-4.75 (m, 2H), δ 3.99-4.15 (m, 2H), δ 3.35 (s 3H), δ 2.98 - 3.03 (m 1H), δ 2.66-2.71 (m, 1H), δ 2.38-2.40 (m, 1H), δ 1.96-2.01 (m, 1H), ^{3}H NMR (533.48 MHz, D_6DMSO) δ 7.50 ppm (s).

[^{14}C]Genipin 12

4-[^{14}C]cyano -4- hydroxy- 1 -(2, 2-dimethyl- propionyloxy)-1, 3, 4, 4a, 5, 7a-hexahydro-cyclopenta [c] pyran-7-carboxylic acid ethyl ester 6

Ketone **5** (1.24 g, 4.00 mmol) was dissolved in ethanol (24 mL) and unlabelled KCN (130.8 mg, 2.01 mmol) and $K^{14}CN$ (100 mCi, 119.9 mg, 1.78 mmol) added. The flask was purged by bubbling in nitrogen for 2 minutes, sealed with a septum, acetic acid (0.5 mL, 8.75 mmol) was added dropwise and the reaction stirred overnight.

The reaction was diluted with diethylether (36 mL) and water (10 mL). The ether layer was removed and the aqueous fraction extracted with ether (3 x 20 mL). The ether extracts were dried over anhydrous sodium sulphate, filtered and evaporated to dryness with co-evaporation with ethanol to remove any residual water. The crude product was purified by silica gel column chromatography using a linear gradient from 100% hexane to 40% ethyl acetate in hexane. A total of 67 mCi (67%) at a radiochemical purity of 94% (HPLC system 2) was recovered and used in the next step. The reaction was repeated twice more to yield an additional 132.6 mCi of cyanohydrin **6**.

4-[^{14}C]cyano-1-(2,2-dimethyl-propionyloxy)-1,4a,5,7a-tetrahydro-cyclopenta[c]pyran-7-carboxylic acid ethyl ester 7.

Cyanohydrin **6** (67 mCi) was dissolved in ethylene dichloride (25 mL) and pyridine (815 µl, 10.12 mmol) was added and the flask placed under nitrogen. Thionyl chloride (360 µl, 4.96 mmol) was added drop-wise and the flask fitted with a reflux condenser and heated under a nitrogen balloon for 1 hour at 80°C with the observation that the reaction darkened during the course of the reaction.

At 1 hour, the reaction was removed from the heat and sampled for HPLC analysis (system 2) which showed about 90% conversion. Saturated brine (10 mL) and water (10 mL) were added to the reaction and the layers separated. The residual aqueous layer was extracted with methylene chloride (3 x 20 mL). The combined organic extracts were dried over anhydrous sodium sulphate, filtered and evaporated to a dark oil which was purified by silica gel column chromatography using a linear gradient from 100% hexane to 30% ethyl acetate in hexane. A total of 43 mCi (64%) of nitrile **7** at a radiochemical

purity of 86.2% (HPLC system 2) was isolated as a yellow oil. An additional 87.2 mCi of nitrile **7** was prepared in a similar manner.

2,2-Dimethyl-propionic acid 4-[^{14}C]cyano-7-hydroxymethyl-1, 4a, 5, 7a-tetrahydro cyclopenta[c]pyran-1-yl ester 8.

Nitrile **7** (43 mCi) was evaporated twice from toluene and dried under vacuum for 1 hour before the flask was fit with a septum and placed under argon. Freshly distilled THF over calcium hydride (14 mL) was added and the flask cooled to -80°C. DIBAL (1M in toluene) (4.3 mL, 4.30 mmol) was then added via syringe pump over the course of three hours. At the completion of the addition, the reaction was stirred for 6 hours before being placed in a freezer overnight. The reaction was then stirred for an additional 10 hours at -80°C, stored overnight at -80°C and then quenched by the addition of acetic acid (270 μL), and a 30% solution of potassium sodium tartrate (15 mL) and ethyl acetate (14 mL). The two phase mixture was stirred for one hour, the layers were separated, and the residual aqueous layer extracted with ethyl acetate (4 x 12 mL). The combined organic layers were dried over anhydrous sodium sulphate, filtered and evaporated to an orange oil. The crude product was purified by silica gel column chromatography using a linear gradient from 100% hexane to 40% ethyl acetate in hexane. A total of 24.2 mCi (56.3%) of ester **8** at a radiochemical purity of 93.6% (HPLC system 2) was isolated. An additional 60.8 mCi of **8** was prepared in a similar manner.

2,2-Dimethyl-propionic acid 4-[^{14}C]carbamoyl-7-hydroxymethyl-1, 4a, 5, 7a-tetrahydro cyclopenta[c]pyran-1-yl ester 9

Nitrile alcohol **8** (85 mCi) was dissolved in ethanol (2:1) water (14 mL) and Ghaffer-Parkins catalyst (232 mg, 0.54 mmol) added. The flask was fit with a septum and heated under argon to 80°C for three hours at which point HPLC analysis (system 3) showed complete reaction. The reaction was cooled to room temperature, evaporated to dryness and suspended in ethyl acetate (80: 20) methanol (10 mL) and sonicated. The suspension was filtered and washed with several portions of ethyl acetate (80: 20) methanol and the combined filtered solution evaporated to a white solid. The crude product was purified by silica gel column chromatography using a linear gradient from 100% hexane to 100% ethyl acetate. A total of 75.3 mCi (89%) of **9** at a radiochemical purity of 96.9% (HPLC system 3) was isolated.

7-Acetoxymethyl -1- 2, 2,-dimethyl-propionyloxy)-1, 4a, 5, 7a-tetrahydrocyclopenta [c] pyran-4-[^{14}C]carboxylic acid 10.

The amide alcohol **9** (75.3 mCi) was dissolved in acetic anhydride (14.5 mL), triethylamine (650 μL, 4.66 mmol) was added and the reaction stirred for two hours at room temperature at which point monitoring by HPLC system 4 showed complete reaction. The solution was cooled to 0°C, acetic acid (8.2 mL) was added followed by sodium nitrite

(1.62 g, 23.48 mmol). The reaction was stirred for 16 hours at 0°C, methanol (33 mL) was added and stirring continued for a further 16 hours at 0°C. The reaction was then diluted with methylene chloride (300 mL), washed with water (2 x 50 mL) and saturated brine (50 mL) before being dried over anhydrous sodium sulphate, filtered and evaporated to dryness. A total of 66 mCi (87%) of **10** at a radiochemical purity of 79.1% (HPLC system 4) was isolated and used directly in the next step.

7-Acetoxymethyl -1- 2, 2,-dimethyl-propionyloxy)-1, 4a, 5, 7a-tetrahydrocyclopenta [c] pyran-4-[^{14}C]carboxylic acid methyl ester 11.

The crude acid **10** (66 mCi) was dissolved in methanol (1:1) chloroform (75 mL) put under nitrogen and cooled to 0°C. TMS diazomethane (2M in ether) was added in two 11.5 mL portions over 30 minutes and the reaction stirred for an additional one hour at which point analysis by HPLC system 5 showed complete reaction. The reaction was quenched by the slow addition of acetic acid (5 mL) and evaporated to a brown oil. The crude product was purified by silica gel column chromatography using a linear gradient from 100% hexane to 20% ethyl acetate in hexane. A total of 47.6 mCi (72%) of **11** at a radiochemical purity of 98.8% (HPLC system 5) was isolated.

Otera's Catalyst preparation

To a solution of dibutyltin dichloride (10 g) in ethanol (75 mL) was added a solution of pyridine (8.1 mL) in ethanol (25 mL) followed by water (4 mL). The resulting white suspension was heated for 30 minutes at 60°C at which point complete solution was obtained. The solution was allowed to cool to room temperature which resulted in the formation of white crystals. The flask was further cooled to -20°C and then the crystals were collected by filtration, washed with cold ethanol and dried under vacuum to constant weight, yielding 3.7g.

7-hydroxymethyl-1-hydroxy-1, 4a, 5, 7a tetrahydrocyclopenta[c]pyran-4-[^{14}C]carboxylic acid methyl ester [^{14}C]genipin 12.

Compound **11** (23.8 mCi) was split into two heavy wall 50 mL pressure tubes and Otera's catalyst (770 mg, 0.70 mmol), as prepared above, was weighed into each tube. Methanol (7.5 mL) was then added and the reactions heated at 80°C for 24 hours at which point analysis by HPLC system 1 showed formation of about 30% of the desired product. The reactions were evaporated to dryness and purified by silica gel column chromatography using a linear gradient from 100% hexane to 80% ethyl acetate to yield 8.3 mCi of [^{14}C]genipin at a radiochemical purity of 95% in HPLC system 1. The remaining 23.8 mCi of compound was then taken through the same sequence to yield an additional 8.7 mCi at a radiochemical purity of 97.6%. The two batches were combined, re-purified by silica gel column chromatography using a linear gradient from 100% hexane to 80% ethyl acetate in hexane, to yield a total batch of 16.2 mCi (34%) of [^{14}C]genipin **12** at a

radiochemical purity of 98.8% (HPLC system 1) and a specific activity of 27.5 mCi/mmol as determined by LC/MS. Unlabelled Genipin MH^+ m/z 227, [^{14}C]Genipin MH^+ m/z 227, 229. Identity was also confirmed by co-chromatography with the authentic genipin standard in HPLC system 1, k' 4.6.

Acknowledgments

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