EurJOC European Journal of Organic Chemistry

FULL PAPER

DOI: 10.1002/ejoc.201500906

Total Synthesis of [^{14}C]-Labelled Homoharringtonine

Melanie Marguerit,*[a] Gill Little,[a] Yi Wang,[b] Linli He,[b] Shawn Allwein,[b] James Reif,[b] Jason Rossi,[b] Renee Roemmele,[b] and Roger Bakale[b]

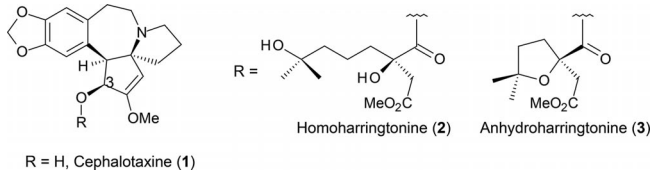
Keywords: Homoharringtonine / Radiochemistry / Total synthesis / Omacetaxine mepesuccinate / Cephalotaxine

A total synthesis of enantiomerically pure [^{14}C]-labelled (–)-homoharringtonine in 17 steps is reported. This synthetic process enabled the production of Good Manufacturing Practice (GMP) compliant (–)-[^{14}C]homoharringtonine that was used in a human mass balance study that was a post-approval commitment to the U.S. Food and Drug Administration. (–)-Homoharringtonine, also called omacetaxine mepesuccinate, is approved to treat adult patients with chronic myeloid leukemia (CML), a blood and bone marrow disease. In November 2012, the product was commercialised as Synribo® in the U.S., marketed by Teva Pharmaceuticals.

Introduction

Homoharringtonine (HHT) is an alkaloid isolated from the leaves and stems of *Cephalotaxus harringtonia*, commonly known as the Japanese plum yew.

Whilst cephalotaxine (**1**) itself accounts for approximately 50% of the mass of the crude alkaloid extract, many other components have been isolated. Among them homoharringtonine (**2**) and anhydroharringtonine (**3**) (Scheme 1) have been reported to be some of the most potent antileukemia alkaloids isolated from the *cephalotaxus* genus.[1]



Scheme 1. Cephalotaxine and its C3 ester derivatives.

To support the clinical development of the drug candidate, a radiolabelled synthesis of (–)-HHT was required.[2]

Results and Discussion

Over the last 40 years, cephalotaxine (**1**) has received considerable attention in terms of total synthesis. Before we started working on the [^{14}C]HHT synthesis in 2010, more than 10 racemic and 5 enantioselective syntheses had already been published. The complex structure and promising bioactivity kept promoting the study on the synthesis by the chemistry community, leading to more than 20 racemic and 15 enantioselective syntheses up to today.[1–18] A review of all the different approaches described in the literature allowed us to choose the most suitable strategy to achieve the radiosynthesis of [^{14}C]homoharringtonine taking into account an easy introduction of the label, the stability of the radiolabelled intermediates, as well as a cost-effective synthetic route.

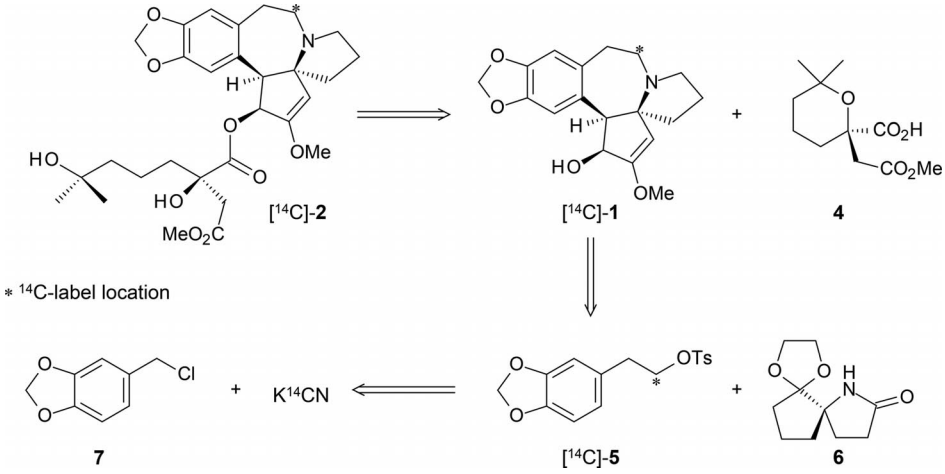
Our retrosynthetic approach is shown in Scheme 2. [^{14}C]-Labelled HHT ([^{14}C]-**2**) could be obtained from [^{14}C]-labelled cephalotaxine ([^{14}C]-**1**) according to an efficient hemisynthesis described in 1999 using enantiopure Robin's acid (**4**).[19] The synthetic strategy to obtain [^{14}C]-**1** is based on the coupling of [^{14}C]-labelled tosylate intermediate [^{14}C]-**5** with enantiopure spirolactam **6** as described by Royer[14] in 2004. Introduction of the [^{14}C] label could occur through the reaction of benzyl chloride intermediate **7** with potassium [^{14}C]cyanide, a readily available source of carbon-14.

This retrosynthetic analysis is based on a convergent approach in which two key unlabeled intermediates **4** and **6** were synthesised in parallel and provided by Teva Pharmaceuticals.

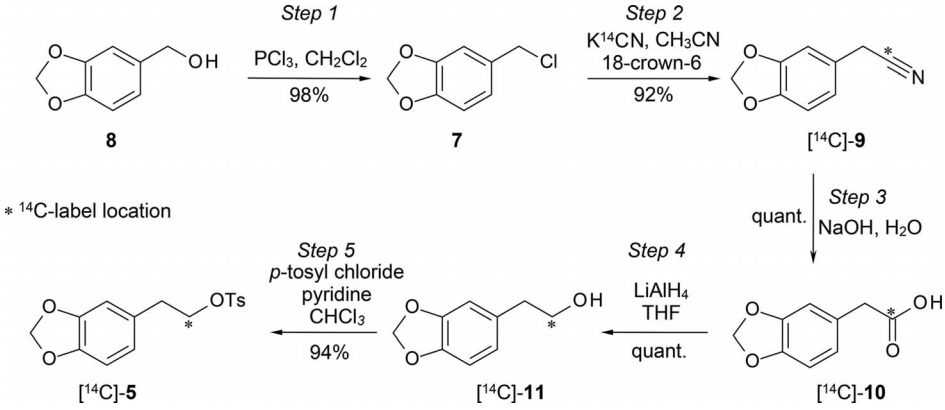
With unlabeled intermediates **4** and **6** in hand, we were able to initiate the radiosynthesis of [^{14}C]HHT (**2**). The synthesis of tosylate [^{14}C]-**5** is outlined in Scheme 3. Benzyl alcohol **8** was converted into the corresponding benzyl chloride **7** with phosphorus trichloride in 98% yield. Cyanation of **7** introduced the radiolabel to give the nitrile [^{14}C]-**9** in 92% yield. Optimisation studies revealed that the benzyl chloride gave a better yield than the corresponding bromo compound and that the use of acetonitrile and 18-crown-6 proved critical to obtain [^{14}C]-**9** in excellent yield. Hydrolysis to the acid [^{14}C]-**10**, reduction to the alcohol [^{14}C]-**11** and tosylation provided [^{14}C]-**5** in near quantitative yield.[20] The tosylation step required some optimisation in terms of the number of equivalents, reaction time and temperature in order to improve the conversion.

[a] Selcia Ltd., Fyfield Business & Research Park, Fyfield Road, Ongar, Essex, CM 0GS, UK
E-mail: gill.little@selcia.com
http://www.selcia.com
[b] Teva Pharmaceuticals, Chemical Synthesis Center, 383 Phoenixville Pike, Malvern, PA 19355-9603, USA

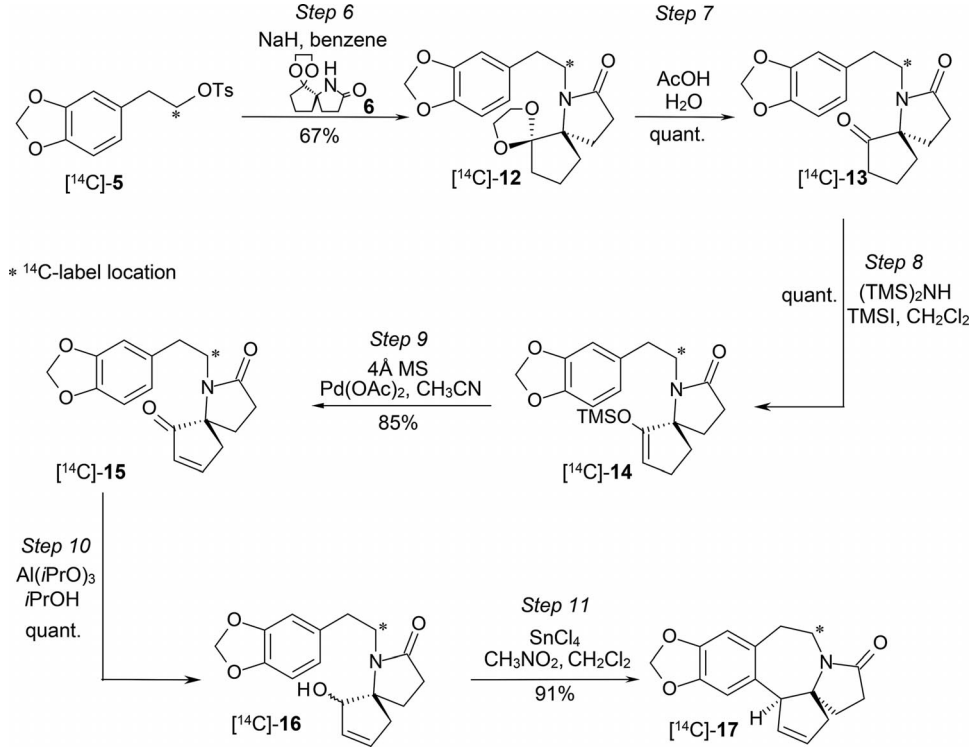
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201500906.



Scheme 2. Retrosynthetic analysis of [^{14}C]-labelled homoharringtonine ([^{14}C]-**2**).



Scheme 3. Synthesis of [^{14}C]-labelled tosylate [^{14}C]-**5**.



Scheme 4. Synthesis of pentacyclic intermediate [^{14}C]-**17**.

Elaboration to the pentacycle [^{14}C]-**17** is outlined in Scheme 4.

The alkylation of spirolactam **6** with the tosylate [^{14}C]-**5** to give [^{14}C]-**12** was optimised to promote *N*- over *O*-alkylation in highest yield (Table 1). Increasing the amount of base from 1.1 to 1.6 equiv. afforded a near doubling in the yield, and changing from 1.1 to 1.5 equiv. of spirolactam **6** increased the yield further to 70% (Entry 3).

Table 1. Optimisation of *N*-alkylation.

Entry	NaH [equiv.]	**6** [equiv.]	**12** [%]
1	1.1	1.1	33
2	1.6	1.1	57
3	1.6	1.5	70

These conditions were applied to the radiosynthesis to provide [^{14}C]-**12** in 67% yield. The ketal intermediate [^{14}C]-**12** was hydrolysed under acidic conditions to give the corresponding ketone [^{14}C]-**13** in quantitative yield. Over the next four steps, the core pentacycle was constructed using chemistry based on that of Kuehne.[20] The silyl enol ether [^{14}C]-**14** was generated in quantitative yield using trimethylsilyl iodide and hexamethyldisilazane. Saegusa–Ito oxidation gave the unsaturated ketone [^{14}C]-**15** in 85% yield, which underwent a Meerwein–Pondorff reduction to allylic alcohol [^{14}C]-**16** using aluminium isopropoxide and 2-propanol. This alcohol was cyclised with tin tetrachloride to yield the pentacyclic core [^{14}C]-**17**, which underwent dihydroxylation using 4-methylmorpholine *N*-oxide and catalytic osmium tetroxide in 70% yield (Scheme 5).

The use of osmium tetroxide as a 4% solution in water allowed the reaction to proceed smoothly. Diol [^{14}C]-**18** was very water-soluble, and exhaustive extraction with dichloromethane was required to recover the material from the aqueous phase. Subsequently, Corey–Kim oxidation using dimethyl sulfide, *N*-chlorosuccinimide and triethylamine in dichloromethane gave [^{14}C]-**19** in 68% yield, which was then methylated with methoxytrimethylsilane and trifluoromethanesulfonic acid.

Triflic acid mediated methylation could provide two potential regioisomers **20** and **21** (Scheme 6).

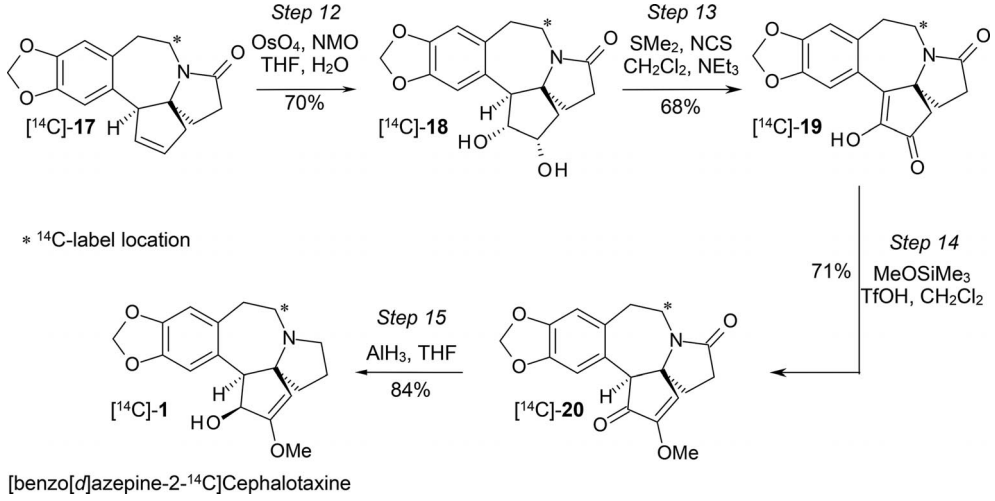
Some optimisation work was undertaken to drive the reaction in favour of the desired regioisomer **20**. The optimal reaction conditions are highlighted in Table 2 (Entry 5) with a yield of 73% of **20**.

Table 2. Optimisation of methylation.[a]

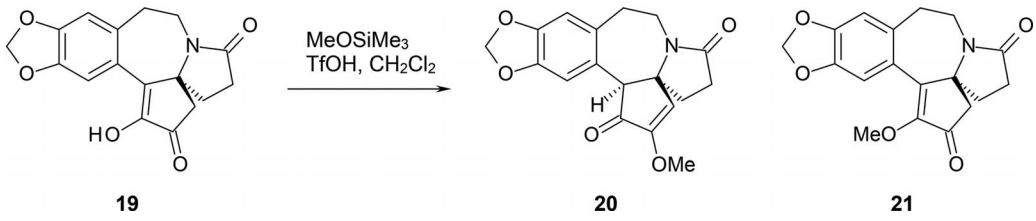
Entry	CH_2Cl_2 [mL]	$MeOSiMe_3$ [equiv.]	TfOH [equiv.]	Temp. [°C]	Ratio **20/21**
1	70	24	2.5	0 to r.t.	3.5:1
2	70	12	1.25	0	3.9:1
3	200	20	4.6	0 to r.t.	1.2:1
4	100	12	2.5	0 to r.t.	2:1
5	70	12	1.25	0 to r.t.	12:1

[a] Note: optimisation reactions performed on a 0.5 mmol scale.

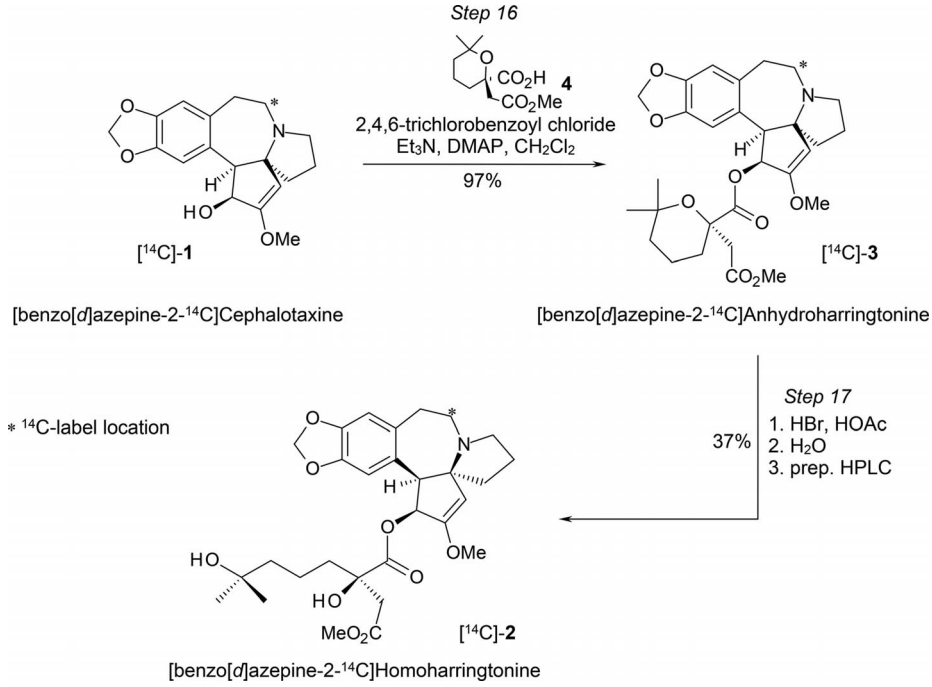
In the radiolabelled synthesis using these conditions, a 71% yield of [^{14}C]-**20** was achieved. Finally, reduction of [^{14}C]-**20** with a solution of aluminium hydride in THF (freshly prepared from anhydrous aluminium chloride and



Scheme 5. Synthesis of [^{14}C]-labelled cephalotaxine [^{14}C]-**1**.



Scheme 6. Formation of regioisomers **20** and **21** during methylation of **19**.



Scheme 7. Conversion of ^{14}C-labelled cephalotaxine ([^{14}C]-**1**) into ^{14}C-labelled homoharringtonine ([^{14}C]-**2**).

lithium aluminium hydride) afforded [^{14}C]-cephalotaxine in 84% yield. This is the first reported radiolabelled synthesis of cephalotaxine.

[^{14}C]-Cephalotaxine ([^{14}C]-**1**) was then converted into [^{14}C]-HHT ([^{14}C]-**2**) using chemistry developed by Robin (Scheme 7).[19] Esterification of [^{14}C]-**1** with enantiomerically pure Robin's acid **4** gave [^{14}C]-**3** anhydro-HHT in 97% yield. Selective ring opening of [^{14}C]-**3** via a one-pot hydrobromination/hydrolysis yielded [^{14}C]-**2**. Following reverse-phase HPLC purification, [^{14}C]-**2** HHT was obtained in 37% yield with a radiochemical purity of 98.9%, a chemical purity of 98.5% and a chiral purity of 100%.

The chemical and radiochemical stability of a solution of [^{14}C]HHT (3.5 mg/mL in ethanol at –80 °C) was determined in a two-year study. Over the course of this time period, the radiochemical purity fell by 2% and the chemical purity by 1%. These results demonstrate the purity of the material is still within the specification necessary (> 95%) for clinical evaluation.

Conclusions

We have achieved the first total synthesis of [^{14}C]-radiolabelled (–)-homoharringtonine. This material was used in the required human mass balance study as required by the U.S. Food and Drug Administration. Commercially marketed as Synribo®, the drug is indicated for the treatment of adult patients with chronic myeloid leukaemia (CML), especially those with resistance and/or intolerance to two or more tyrosine kinase inhibitors (TKIs).

This radiosynthesis numbered 16 steps enabling 15 mCi (ca. 370 mg) of [^{14}C]HHT to be prepared in two batches at a specific activity of 20 mCi/mmol. The radiochemical and chemical purities were higher than 98% and the chiral purity higher than 99%. The radiosynthesis as described is robust and has been repeated subsequently on a similar scale. Additionally, two GMP radiosyntheses of [^{14}C]HHT starting from [^{14}C]cephalotaxine have been accomplished, and this material is being used in an ongoing clinical study.

Experimental Section

General: All commercially available reagents were used without further purification. All anhydrous solvents were obtained from Acros. Organic solutions were concentrated under reduced pressure with a Stuart rotary evaporator using a water bath. Chromatographic purification of products was accomplished using Biotage SNAP cartridges. Thin-layer chromatography (TLC) was performed with Macherey–Nagel Alugram SIL G/UV$_{254}$ 0.25 mm silica gel plates. [^{14}C]-Labelled compounds were co-eluted side-by-side with their corresponding unlabelled counterparts. Thin-layer chromatography plates were analysed by electronic autoradiography using a Packard Instant Imager. ^{1}H NMR spectra were obtained with Bruker DPX300, DPX400 and DRX500 spectrometers with TopSpin v.1.3 acquisition and data manipulation software. Chemical shifts (δ) are referenced to the residual NMR solvent ([D]chloroform: δ = 7.26 ppm; [D$_6$]benzene: δ = 7.16 ppm; [D$_6$]dimethyl sulfoxide: δ = 2.50 ppm). Chemical shifts and coupling constants are reported in ppm and Hz, respectively. Multiplicities are reported as follow: s = singlet, d = doublet, t = triplet, q = quartet, br. = broad, m = multiplet. Mass spectral analysis was performed using a Thermo Finnigan AQA single quadrupole mass spectrometer with Xcalibur v.1.2 aquisition and data manipulation software. High-Performance Liquid Chromatography (HPLC) was carried out using an Agilent HP1200 system with a β-RAM radiodetector. The data was acquired using Chemstation and Laura Lite acquisition and data manipulation software. Preparative High Performance Liquid Chromatography (prep. HPLC) was carried out with a Gilson LC-

UV system using unipoint v3.3 acquisition and data manipulation software. Quantitative measurement of radioactivity was carried out using a Packard 2100TR liquid scintillation counter running a standard protocol for [^{14}C] gravimetric analysis.

1,3-Benzodioxole-5-[1-^{14}C]acetonitrile ([^{14}C]-9): To a suspension of potassium [^{14}C]cyanide (0.886 g, 13.237 mmol, 750.05 mCi at 57.00 mCi/mmol) and 18-crown-6 (3.500 g, 13.237 mmol) in acetonitrile (14.0 mL) was added a solution of 5-(chloromethyl)-1,3-benzodioxole (**7**; 2.190 g, 12.851 mmol) in acetonitrile (14.0 mL). The white suspension was stirred at room temperature overnight. The reaction mixture was concentrated. The residue was partitioned between water (40 mL) and ethyl acetate (40 mL). The organic layer was separated and dried with $MgSO_4$. The drying agent was filtered and the solution concentrated. The residue was purified by column chromatography on silica eluting with dichloromethane (100%) to give product [^{14}C]-**9** as a colourless oil (1.989 g, 12.199 mmol, 691.50 mCi, 92%). The radiopurity of this material was assessed to be 97.3% by TLC/autoradiography (dichloromethane, 100%; R_f = 0.63).

2-(1,3-Benzodioxol-5-yl)[1-^{14}C]acetic Acid ([^{14}C]-10): To a suspension of 1,3-benzodioxole-5-[1-^{14}C]acetonitrile ([^{14}C]-**9**; 1.989 g, 12.200 mmol, 691.50 mCi) in water (23.0 mL) was added sodium hydroxide (1.460 g, 36.600 mmol) in solution in water. The suspension was heated at 100 °C for 1.5 h and then cooled to room temperature. The reaction mixture was partitioned between diethyl ether (70 mL) and water (40 mL). The aqueous layer was separated, acidified with hydrochloric acid (2 N) and extracted with diethyl ether (3 × 70 mL). The combined organic extracts were dried with $MgSO_4$. The drying agent was filtered and the solution concentrated to give product [^{14}C]-**10** as a pale yellow solid (2.292 g, 12.198 mmol, 691.50 mCi, quantitative), which was used in the next step without further purification. The radiopurity of this material was assessed to be 97.2% by TLC/autoradiography (dichloromethane/methanol, 9:1, v/v; R_f = 0.75).

2-(1,3-Benzodioxol-5-yl)-[1-^{14}C]ethanol ([^{14}C]-11): To a cooled (0 °C, ice/water bath) solution of 2-(1,3-benzodioxol-5-yl)[1-^{14}C]-acetic acid ([^{14}C]-**10**; 1.380 g, 7.585 mmol, 438.26 mCi) in tetrahydrofuran (22 mL) was added dropwise a solution of lithium aluminium hydride in tetrahydrofuran (1 M, 7.66 mL, 7.661 mmol). Once the addition was complete, the reaction mixture was heated at 65 °C for 1 h. The solution was cooled with an ice/water bath; then sodium hydroxide solution (1 N) was added slowly. The resulting suspension was filtered through Hyflo and the filter cake washed with tetrahydrofuran. The filtrate was concentrated. The residue was purified by column chromatography on silica eluting with dichloromethane (100%) to dichloromethane/methanol (95:5, v/v) to give product [^{14}C]-**11** as a colourless oil (1.274 g, 7.585 mmol, 438.26 mCi, quantitative). The radiopurity of this material was assessed to be 97.7% by TLC/autoradiography (dichloromethane/methanol, 9:1, v/v; R_f = 0.71).

2-(1,3-Benzodioxol-5-yl)-[1-^{14}C]ethyl 4-Methylbenzenesulfonate ([^{14}C]-5): To a cooled (0 °C, ice/water bath) solution of 2-(1,3-benzodioxol-5-yl)-[1-^{14}C]ethanol ([^{14}C]-**11**; 1.274 g, 7.585 mmol, 438.26 mCi) and pyridine (1.22 mL, 15.170 mmol) in chloroform (40 mL) was added dropwise a solution of *p*-tosyl chloride (2.169 g, 11.378 mmol) in chloroform (20 mL). The reaction mixture was stirred at 0 °C for 5 h and at room temperature overnight. TLC/autoradiography showed 56% of unreacted starting material. *p*-Tosyl chloride (0.721 g, 3.782 mmol) in chloroform (7 mL) was added, and the reaction mixture was stirred for 2.5 h; TLC/autoradiography still showed unreacted starting material. Pyridine (1.22 mL, 15.12 mmol) and *p*-tosyl chloride (1.442 g, 7.564 mmol) in chloroform (14 mL) were added. The reaction mixture was stirred at room temperature for 48 h. TLC/autoradiography showed 92.5% of the expected product. The reaction mixture was washed with saturated sodium hydrogen carbonate solution (60 mL) and saturated sodium chloride solution (60 mL). The organic layer was separated and dried with $MgSO_4$. The drying agent was filtered and the solution concentrated. The residue was purified by column chromatography on silica eluting with isohexane (100%) to isohexane/diethyl ether (2:1, v/v) to give product [^{14}C]-**5** as a colourless oil (2.300 g, 7.139 mmol, 412.40 mCi, 94%). The radiopurity of this material was assessed to be 97.7% by TLC/autoradiography (isohexane/diethyl ether, 1:1, v/v; R_f = 0.31).

(5*R*)-4-[2-(1,3-Benzodioxol-5-yl)-[1-^{14}C]ethyl]-7,10-dioxa-4-azadispiro[4.0.4.3]tridecan-3-one ([^{14}C]-12): To a solution of spirolactam **6** (2.360 g, 11.985 mmol) in anhydrous benzene (17.0 mL) was added sodium hydride (0.511 g, 12.784 mmol) portionwise. The reaction mixture was heated at reflux for 5 min and cooled to room temperature. This solution was added to 2-(1,3-benzodioxol-5-yl)-[1-^{14}C]ethyl 4-methylbenzenesulfonate ([^{14}C]-**5**; 2.575 g, 7.990 mmol, 452.71 mCi), and the reaction mixture was heated at reflux overnight. The reaction mixture was cooled to room temperature and treated with saturated ammonium chloride solution (125 mL). The aqueous layer was extracted with dichloromethane (3 × 100 mL). The combined organic extracts were dried with $MgSO_4$. The drying agent was filtered and the solution concentrated. The residue was purified by column chromatography on silica eluting with a gradient isohexane/ethyl acetate (8:2, v/v) to ethyl acetate (100%) to give product [^{14}C]-**12** as a pale yellow oil (1.858 g, 5.351 mmol, 303.32 mCi, 67%). The radiopurity of this material was assessed to be 97.2% by TLC/autoradiography (isohexane/ethyl acetate, 2:3, v/v; R_f = 0.18).

(5*R*)-1-[2-(1,3-Benzodioxol-5-yl)-[1-^{14}C]ethyl]-1-azaspiro[4.4]nonane-2,9-dione ([^{14}C]-13): A solution of (5*R*)-4-[2-(1,3-benzodioxol-5-yl)-[1-^{14}C]ethyl]-7,10-dioxa-4-azadispiro[4.0.4.3]tridecan-3-one ([^{14}C]-**12**; 1.858 g, 5.351 mmol, 303.32 mCi) in water (25.0 mL) and acetic acid (2.8 mL) was heated at 105 °C for 21 h. The reaction mixture was poured into saturated sodium hydrogen carbonate solution (60 mL) and extracted with dichloromethane (3 × 50 mL). The combined organic extracts were dried with $MgSO_4$. The drying agent was filtered and the solution concentrated to give product [^{14}C]-**13** as a pale orange oil (1.622 g, 5.350 mmol, 303.32 mCi, quantitative), which was used in the next step without further purification. The radiopurity of this material was assessed to be 96.4% by TLC/autoradiography (isohexane/ethyl acetate, 3:7, v/v; R_f = 0.19).

(5*R*)-1-[2-(1,3-Benzodioxol-5-yl)-[1-^{14}C]ethyl]-9-trimethylsilyloxy-1-azaspiro[4.4]non-8-en-2-one ([^{14}C]-14): To a solution of (5*R*)-1-[2-(1,3-benzodioxol-5-yl)-[1-^{14}C]ethyl]-1-azaspiro[4.4]nonane-2,9-dione ([^{14}C]-**13**; 1.223 g, 4.034 mmol, 233.12 mCi) in dichloromethane (52 mL) were added at 0 °C, under nitrogen, hexamethyldisilazane (1.09 mL, 5.239 mmol) and iodotrimethylsilane (0.63 mL, 4.433 mmol). The reaction mixture was stirred at 0 °C for 1 h and at room temperature for 2.5 h. The reaction mixture was poured into saturated sodium hydrogen carbonate solution (50 mL) at 4 °C. The organic layer was separated and the aqueous layer extracted with further dichloromethane (30 mL). The combined organic extracts were dried with Na_2SO_4. The drying agent was filtered and the solution concentrated to give product [^{14}C]-**14** as a yellow oil (1.514 g, 4.034 mmol, 233.12 mCi, quantitative), which was used in the next step without further purification. The radiopurity of this material was assessed to be 94.3% by TLC/autoradiography (isohexane/ethyl acetate, 1:4, v/v; R_f = 0.32).

(5*S*)-1-[2-(1,3-Benzodioxol-5-yl)-[1-^{14}C]ethyl]-1-azaspiro[4.4]non-7-ene-2,9-dione ([^{14}C]-15): To a solution of (5*R*)-1-[2-(1,3-benzodioxol-5-yl)-[1-^{14}C]ethyl]-9-trimethylsilyloxy-1-azaspiro[4.4]non-8-en-2-one ([^{14}C]-**14**; 2.008 g, 5.349 mmol, 303.32 mCi) in acetonitrile (53.0 mL) was added 4 Å molecular sieves (1.150 g) at room temperature, under nitrogen. After stirring for 5 min, palladium(II) acetate (1.260 g, 5.616 mmol) was added in one portion. The reaction mixture was stirred at room temperature overnight. The mixture was filtered through Hyflo and the filter cake washed with dichloromethane. The filtrate was concentrated, and the residue was purified by column chromatography on silica eluting with a gradient isohexane (100%) to ethyl acetate (100%) to give product [^{14}C]-**15** as a yellow oil (1.368 g, 4.541 mmol, 257.52 mCi, 85%). The radiopurity of this material was assessed to be 96.3% by TLC/autoradiography (isohexane/ethyl acetate, 3:7, v/v; R_f = 0.13).

(5*S*)-1-[2-(1,3-Benzodioxol-5-yl)-[1-^{14}C]ethyl]-9-hydroxy-1-azaspiro[4.4]non-7-en-2-one ([^{14}C]-16): To a suspension of (5*S*)-1-[2-(1,3-benzodioxol-5-yl)-[1-^{14}C]ethyl]-1-azaspiro[4.4]non-7-ene-2,9-dione ([^{14}C]-**15**; 1.010 g, 3.354 mmol, 193.72 mCi) in 2-propanol (14 mL) was added aluminium isopropoxide (20.550 g, 100.620 mmol) at room temperature. The resulting white slurry was heated at 135 °C for 1.5 h, while acetone and 2-propanol were removed by distillation. The reaction mixture was poured slowly into hydrochloric acid (1 N, 250 mL) at 0 °C and extracted with dichloromethane (2× 150 mL). The combined organic extracts were dried with Na_2SO_4. The drying agent was filtered and the solvent co-evaporated five times with dichloromethane to leave product [^{14}C]-**16** as an orange oil (1.016 g, 3.353 mmol, 193.72 mCi, quantitative), which was used in the next step without further purification. The radiopurity of this material was assessed to be 83.7% by TLC/autoradiography (isohexane/ethyl acetate, 1:4, v/v; R_f = 0.03).

(4*S*,5*S*)-2,3-Dehydro[benzo[*d*]azepine-2-^{14}C]cephalotaxan-8-one ([^{14}C]-17): To a solution of (5*S*)-1-[2-(1,3-benzodioxol-5-yl)-[1-^{14}C]-ethyl]-9-hydroxy-1-azaspiro[4.4]non-7-en-2-one ([^{14}C]-**16**; 1.016 g, 3.353 mmol, 193.72 mCi) in dichloromethane (30 mL) was added nitromethane (30 mL) at room temperature. After cooling to –78 °C, tin(IV) chloride (2.74 mL, 23.471 mmol) was added dropwise. The reaction mixture was stirred at –78 °C for 1.5 h and at room temperature overnight. The reaction mixture was poured into hydrochloric acid (1 N, 150 mL). The organic layer was separated and the aqueous layer extracted with dichloromethane (2× 100 mL). The combined organic extracts were dried with Na_2SO_4. The drying agent was filtered and the solution concentrated. The residue was purified by column chromatography on silica eluting with dichloromethane (100%) to dichloromethane/methanol (92:8, v/v) to give product [^{14}C]-**17** as a yellow oil (0.865 g, 3.035 mmol, 175.32 mCi, 91%). The radiopurity of this material was assessed to be 85.2% by TLC/autoradiography (isohexane/ethyl acetate, 1:4, v/v, R_f = 0.21).

(2*S*,3*R*,4*S*,5*S*)-2,3-Dihydroxy[benzo[*d*]azepine-2-^{14}C]cephalotaxan-8-one ([^{14}C]-18): To a solution of (4*S*,5*S*)-2,3-dehydro[benzo[*d*]-azepine-2-^{14}C]cephalotaxan-8-one ([^{14}C]-**17**; 0.865 g, 3.035 mmol, 175.32 mCi) in tetrahydrofuran (9 mL) were added 4-methylmorpholine *N*-oxide (0.425 g, 3.642 mmol) and water (1.1 mL) at room temperature. After stirring for 5 min, osmium tetroxide (4 wt.-% solution in water, 0.86 mL, 0.143 mmol) was added to the solution (reaction vessel covered with aluminium foil). The reaction mixture was stirred at room temperature overnight. The reaction mixture was treated with hydrochloric acid (3 N, 110 mL) and sodium bisulfite (15% aqueous solution, 43 mL) and extracted with dichloromethane (15× 65 mL). The combined organic extracts were dried with Na_2SO_4. The drying agent was filtered and the solution concentrated. The residue was purified by column chromatography on silica eluting with dichloromethane (100%) to dichloromethane/methanol (9:1, v/v) to give product [^{14}C]-**18** as a colourless oil (0.674 g, 2.110 mmol, 121.85 mCi, 70%). The radiopurity of this material was assessed to be 97.8% by TLC/autoradiography (dichloromethane/methanol, 9:1, v/v; R_f = 0.31).

(5*S*)-3,4-Dehydro-3-hydroxy[benzo[*d*]azepine-2-^{14}C]cephalotaxan-2,8-dione ([^{14}C]-19): To a cooled (0 °C, ice/water bath) suspension of *N*-chlorosuccinimide (1.409 g, 10.550 mmol) in dichloromethane (11.8 mL) was added dropwise dimethyl sulfide (0.78 mL, 10.550 mmol). The solution was stirred at 0 °C for 0.5 h, cooled to –40 °C and added to a cooled (–40 °C) solution of (2*S*,3*R*,4*S*,5*S*)-2,3-dihydroxy[benzo[*d*]azepine-2-^{14}C]cephalotaxan-8-one ([^{14}C]-**18**; 0.674 g, 2.110 mmol, 121.85 mCi) in dichloromethane (45 mL). The reaction mixture was stirred at –40 °C for 1.5 h, triethylamine (2.34 mL, 16.880 mmol) was added in one portion and the solution was stirred at room temperature for 2 h. The reaction mixture was poured into saturated sodium chloride solution (120 mL). The organic layer was separated and the aqueous layer extracted with dichloromethane (8× 120 mL). The combined organic extracts were washed with saturated sodium chloride (2× 120 mL) and dried with Na_2SO_4. The drying agent was filtered and the solution concentrated. The residue was purified by column chromatography on silica eluting with ethyl acetate (100%) to give product [^{14}C]-**19** as a white solid (0.454 g, 1.441 mmol, 83.22 mCi, 68%). The radiopurity of this material was assessed to be 95.9% by TLC/autoradiography (ethyl acetate, 100%; R_f = 0.07).

(4*S*,5*S*)-1,2-Dehydro-2-methoxy[benzo[*d*]azepine-2-^{14}C]cephalotaxan-3,8-dione ([^{14}C]-20): To a cooled (0 °C, ice/water bath) solution of (5*S*)-3,4-dehydro-3-hydroxy[benzo[*d*]azepine-2-^{14}C]cephalotaxan-2,8-dione ([^{14}C]-**19**; 0.307 g, 0.975 mmol, 55.28 mCi) in dichloromethane (22.0 mL) were added sequentially methoxytrimethylsilane (1.6 mL, 11.798 mmol) and trifluoromethanesulfonic acid (0.11 mL, 1.219 mmol). The reaction mixture was stirred at room temperature overnight. The reaction was quenched with saturated sodium hydrogen carbonate solution (100 mL). The organic layer was separated and the aqueous layer extracted with dichloromethane (3× 100 mL). The combined organic extracts were dried with $MgSO_4$. The drying agent was filtered and the solution concentrated. The residue was triturated with ethyl acetate (4 mL). The precipitate was collected by vacuum filtration and washed with ethyl acetate (3× 3 mL) to give product [^{14}C]-**20** as a beige solid (0.183 g, 0.556 mmol, 31.51 mCi). The mother liquor recovered from the trituration was purified by column chromatography on silica eluting with ethyl acetate (100%) to give product [^{14}C]-**20** as a beige solid (0.044 g, 0.134 mmol, 7.59 mCi). The two crops of material were combined to give [^{14}C]-**20** as a beige solid (0.227 g, 0.689 mmol, 39.08 mCi, 71%). The radiopurity of this material was assessed to be 95.4% by TLC/autoradiography (ethyl acetate, 100%; R_f = 0.10). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 2.18–2.42 (m, 4 H), 2.44–2.56 (m, 2 H), 2.95 (m, 1 H), 3.51 (s, 1 H), 3.87 (s, 3 H), 4.09–4.19 (m, 1 H), 5.90–5.99 (m, 2 H), 6.08 (s, 1 H), 6.63 (s, 1 H), 6.69 (s, 1 H) ppm. ^{1}H NMR analysis showed the presence of a single regioisomer.

[Benzo[*d*]azepine-2-^{14}C]cephalotaxine ([^{14}C]-1): A solution of aluminium chloride (1.562 g, 11.713 mmol) in tetrahydrofuran (12.0 mL) was stirred at room temperature under nitrogen for 5 min before cooling to 0 °C (ice/water bath). To this cooled solution was added at 0 °C lithium aluminium hydride (1 M in tetrahydrofuran, 36.5 mL, 36.544 mmol). Upon stirring at 0 °C for 10 min, a solution of aluminium hydride (1 M in tetrahydrofuran) was obtained. To a cooled (0 °C, ice/water bath) solution of (4*S*,5*S*)-1,2-dehydro-

2-methoxy[benzo[*d*]azepine-2-^{14}C]cephalotaxan-3,8-dione ([^{14}C]-**20**; 0.227 g, 0.689 mmol, 39.08 mCi) in tetrahydrofuran (25.0 mL) was added aluminium hydride (1 M in tetrahydrofuran, 25.4 mL, 25.355 mmol), under nitrogen. The reaction mixture was stirred at 0 °C for 1.5 h. The reaction mixture was treated carefully with saturated potassium sodium tartrate solution (20 mL) and extracted with dichloromethane (4 × 100 mL). The combined organic extracts were dried with $MgSO_4$. The drying agent was filtered and the solution concentrated. The residue was purified by column chromatography on silica eluting with dichloromethane/methanol/ammonium hydroxide (95:5:0.5, v/v) to give product [^{14}C]-**1** as a white film after co-evaporation with toluene (0.183 g, 0.578 mmol, 32.79 mCi, 84%). The radiopurity of this material was assessed to be 96.2% by TLC/autoradiography (dichloromethane/methanol/ammonium hydroxide, 95:5:0.5, v/v; R_f = 0.60) and 97.3% by HPLC (92.5% chemical purity by UV-HPLC). ^{1}H NMR ([D]$_6$benzene, 400 MHz): δ = 1.33 (br. m, 1 H), 1.47–1.64 (m, 2 H), 1.71 (m, 1 H), 1.89 (m, 1 H), 2.18 (dd, *J* = 14, 7 Hz, 1 H), 2.51 (q, *J* = 9, 9, 9 Hz, 1 H), 2.65 (dd, *J* = 10, 8 Hz, 1 H), 2.79 (td, *J* = 12, 12, 7 Hz, 1 H), 2.89 (m, 1 H), 3.27 (s, 3 H), 3.37 (d, *J* = 9 Hz, 1 H), 3.58 (m, 1 H), 4.55 (br. d, *J* = 9 Hz, 1 H), 4.64 (s, 1 H), 5.35 (d, *J* = 1 Hz, 1 H), 5.40 (d, *J* = 1 Hz, 1 H), 6.49 (s, 1 H), 6.60 (s, 1 H) ppm.

[Benzo[*d*]azepine-2-^{14}C]anhydrohomoharringtonine ([^{14}C]-3): To a cooled (0 °C, ice/water bath) solution of enantiopure Robin's acid (**4**; 265.8 mg, 1.157 mmol) in dichloromethane (6.5 mL) were added triethylamine (0.160 mL, 1.158 mmol) and 2,4,6-trichlorobenzoyl chloride (0.180 mL, 1.158 mmol). The resulting solution was stirred at 0 °C for 1.5 h and added dropwise to a cooled (0 °C, ice/water bath) solution of [benzo[*d*]azepine-2-^{14}C]cephalotaxine ([^{14}C]-**1**; 183.4 mg, 0.579 mmol, 29.19 mCi) and 4-(dimethylamino)pyridine (290.7 mg, 2.379 mmol) in dichloromethane (4.4 mL) rinsing in with dichloromethane (2.6 mL). The reaction mixture was stirred at 0 °C for 4 h and at room temperature for 16 h. TLC/autoradiography (dichloromethane/methanol/ammonium hydroxide, 95:5:0.5, v/v) of a subsample showed 71% conversion into the expected product. The reaction mixture was cooled to 0 °C (ice/water bath) and treated with water (0.3 mL). The resulting mixture was stirred for 20 min and concentrated (bath temperature maintained at < 25 °C) to dryness to give an orange solid. The residue was purified by column chromatography on silica eluting with dichloromethane (100%) to dichloromethane/methanol (98:2, v/v) to give product [^{14}C]-**3** as a pale orange foam (298.0 mg, 0.563 mmol, 28.37 mCi, 97%). The radiopurity of this material was assessed to be 75.3% by TLC/autoradiography (dichloromethane/methanol/ammonium hydroxide, 95:5:0.5, v/v; R_f = 0.85). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.06 (s, 3 H), 1.12 (s, 3 H), 1.21–1.85 (m, 9 H), 1.86–1.96 (m, 1 H), 2.04 (m, 1 H), 2.15 (d, *J* = 14 Hz, 1 H), 2.38 (br. dd, *J* = 14.7 Hz, 1 H), 2.55–2.67 (m, 2 H), 2.95 (m, 1 H), 3.04–3.24 (m, 2 H), 3.61 (s, 3 H), 3.72 (s, 3 H), 3.81 (d, *J* = 10 Hz, 1 H), 5.06 (br. s, 1 H), 5.81 (d, *J* = 1 Hz, 1 H), 5.88 (d, *J* = 1 Hz, 1 H), 5.94 (br. d, *J* = 10 Hz, 1 H), 6.59 (s, 1 H), 6.63 (s, 1 H) ppm.

[Benzo[*d*]azepine-2-^{14}C]homoharringtonine ([^{14}C]-2): To a cooled (–20 °C) solution of [benzo[*d*]azepine-2-^{14}C]anhydrohomoharringtonine ([^{14}C]-**3**; 193.5 mg, 0.363 mmol, 18.98 mCi) in dichloromethane (2.2 mL) was added dropwise hydrogen bromide in acetic acid (33%, w/w, 2.0 mL, 11.253 mmol). The resulting solution was stirred at –15 °C for 4 h, then cold water (13.6 mL) was added quickly. The solution was stirred at room temperature for 15 h. TLC/autoradiography (dichloromethane/methanol/triethylamine, 100:3:1, v/v) of a subsample showed 82% conversion into the expected product. The reaction mixture was diluted with dichloromethane (30 mL) and water (20 mL). The mixture was treated with saturated sodium hydrogen carbonate solution (100 mL) until pH = 9 and the aqueous layer extracted with dichloromethane (5 × 60 mL). The combined organic extracts were dried with Na_2SO_4. The drying agent was filtered and the solution concentrated (bath temperature maintained at < 25 °C). The residue was purified by column chromatography on silica eluting with dichloromethane (100%) to dichloromethane/methanol/triethylamine (100:5:1, v/v) to give [^{14}C]-**2** as an off-white glassy solid (142.3 mg, 0.260 mmol, 13.60 mCi). The radiopurity of this material was assessed to be 86.0% by TLC/autoradiography (dichloromethane/methanol/triethylamine, 100:3:1, v/v; R_f = 0.50). The residue was further purified by preparative HPLC [using a Zorbax Eclipse XDB-C18 column, 250 × 21.2 mm, 7 μm; conditions: at 20.0 mL/min; gradient from 10% to 95% of acetonitrile/0.05% trifluoroacetic acid in water/0.05% trifluoroacetic acid over 23 min; UV: 290 nm; retention time ca. 11 min]. Pure fractions were concentrated to ca. 150 mL (bath temperature maintained at < 25 °C) and diluted with dichloromethane (10 mL) and water (10 mL). The solution was neutralised to pH = 7 with saturated sodium hydrogen carbonate solution (20 mL) and the aqueous layer extracted with dichloromethane (3 × 100 mL). The combined organic extracts were dried with Na_2SO_4. The drying agent was filtered and the solution concentrated (bath temperature maintained at < 25 °C). The resultant glass/film was dried further under high vacuum at room temperature for 4 h to give product [^{14}C]-**2** as a colourless glass/film (73.9 mg, 0.135 mmol, 7.06 mCi, 37%). The radiopurity of this material was assessed to be 98.2% by TLC/autoradiography (dichloromethane/methanol/triethylamine, 100:3:1, v/v; R_f = 0.20) and 98.9% by HPLC (98.5% chemical purity by UV-HPLC). The LC/MS and NMR spectroscopic data aree fully consistent with the structure. The specific activity was found to be 55.76 mCi/mmol with a molecular weight of 547.43 at that specific activity. The compound was dissolved in *tert*-butyl alcohol (ca. 5 mL) and diluted with a solution of unlabelled **2** (103.0 mg, 0.189 mmol) in *tert*-butyl alcohol (ca. 10 mL). The solution was mixed for 10 min and freeze-dried for 4 h. The material was further dried under high vacuum at room temperature for 2 h to give [^{14}C]-**2** as a white solid (173.5 mg, 0.318 mmol, 7.01 mCi). The radiopurity of this material was assessed to be 98.1% by HPLC (98.8% chemical purity by UV-HPLC) with a chiral purity of 100%. ^{1}H NMR ([D]$_6$benzene, 500 MHz): δ = 0.78 (br. s, 1 H), 1.04 (s, 3 H), 1.06 (s, 3 H), 1.27 (m, 2 H), 1.42 (m, 1 H), 1.51 (m, 4 H), 1.60 (m, 1 H), 1.63 (m, 1 H), 1.78 (m, 1 H), 2.17 (dd, *J* = 14, 7 Hz, 1 H), 2.19 (d, *J* = 16 Hz, 1 H), 2.41 (d, *J* = 16 Hz, 1 H), 2.44 (m, 1 H), 2.57 (dd, *J* = 10, 8 Hz, 1 H), 2.75 (m, 1 H), 2.84 (td, *J* = 8, 8, 4 Hz, 1 H), 3.23 (m, 1 H), 3.28 (s, 3 H), 3.35 (s, 3 H), 3.44 (d, *J* = 10 Hz, 1 H), 3.90 (s, 1 H), 4.68 (s, 1 H), 5.34 (d, *J* = 5 Hz, 1 H), 5.48 (d, *J* = 5 Hz, 1 H), 6.17 (d, *J* = 10 Hz, 1 H), 6.47 (s, 1 H), 6.55 (s, 1 H) ppm. The LC/MS and NMR spectroscopic data are fully consistent with the structure. The specific activity was found to be 21.09 mCi/mmol with a molecular weight of 546.32 at that specific activity. The compound was dissolved in ethanol at a concentration of 3.4 mg/mL (specific concentration of 0.14 mCi/mL) and the solution was filtered through a 1.0 μm glass fibre filter into a serum bottle and kept at below –70 °C pending use.

Acknowledgments

We would like to thank people at Selcia that have been involved in this project, especially D. Hajdu and Dr. S. Knight for analyses of radiolabeled compounds and their great dedication all along the project, Dr. N. Proisy and Dr. C. Winfield for their helpful discussions and advice during this project.

[1] a) J. D. Eckelbarger, J. T. Wilmot, M. T. Epperson, C. S. Thakur, D. Shum, C. Antczak, L. Tarassishin, H. Djaballah, D. Y. Gin, *Chem. Eur. J.* **2008**, *14*, 4293–4306; b) S. Lü, J. Wang, *J. Hematol. Oncol.* **2014**, *7*, 2–11.
[2] M. Heiblig, M. Sobh, F. E. Nicolini, *Leuk. Res.* **2014**, *38*, 1145–1153.
[3] H. Abdelkafi, B. Nay, *Nat. Prod. Rep.* **2012**, *29*, 845–869.
[4] M. Pizzonero, F. Dumas, J. d'Angelo, *Heterocycles* **2005**, *66*, 31–37.
[5] M. A. J. Miah, T. Hudlicky, J. W. Reed in *The Alkaloids*, vol. 51 (Ed.: G. A. Cordell), Academic Press, San Diego, CA, **1998**, pp. 199–269.
[6] M. Ikeda, S. A. A. El Bialy, K. Hirose, M. Kotake, T. Sato, S. M. M. Bayomi, I. A. Shehata, A. M. Abdelal, L. M. Gad, T. Yakura, *Chem. Pharm. Bull.* **1999**, *47*, 983–987.
[7] a) L. F. Tietze, H. Schirok, *J. Am. Chem. Soc.* **1999**, *121*, 10264–10269; b) L. F. Tietze, P. L. Steck, *Eur. J. Org. Chem.* **2001**, 4353–4356.
[8] S. H. Kim, J. K. Cha, *Synthesis* **2000**, 2113–2116.
[9] K. I. B. Milburn, L. F. Dudin, C. E. Anson, S. D. Guile, *Org. Lett.* **2001**, *3*, 3005–3008.
[10] a) S. M. Worden, R. Mapitse, C. J. Hayes, *Tetrahedron Lett.* **2002**, *43*, 6011–6014; b) W. R. Esmieu, S. W. Worden, D. Catterick, C. Wilson, C. J. Hayes, *Org. Lett.* **2008**, *10*, 3045–3048.
[11] Y. Koseki, H. Sato, Y. Watanabe, T. Nagasaka, *Org. Lett.* **2002**, *4*, 885–888.
[12] S. Suga, M. Watanabe, J.-I. Yoshida, *J. Am. Chem. Soc.* **2002**, *124*, 14824–14825.
[13] a) W.-D. Z. Li, Y.-Q. Wang, *Org. Lett.* **2003**, *5*, 2931–2934; b) W.-D. Z. Li, B.-C. Ma, *J. Org. Chem.* **2005**, *70*, 3277–3280; c) W.-D. Z. Li, X.-W. Wang, *Org. Lett.* **2007**, *9*, 1211–1214; d) W.-D. Z. Li, W.-G. Duo, C.-H. Zhuang, *Org. Lett.* **2011**, *13*, 3538–3541.
[14] L. Planas, J. Perard-Viret, J. Royer, *J. Org. Chem.* **2004**, *69*, 3087–3092.
[15] Z. Zhao, P. S. Mariano, *Tetrahedron* **2006**, *62*, 7266–7273.
[16] Q. Liu, E. M. Ferreira, B. M. Stoltz, *J. Org. Chem.* **2007**, *72*, 7352–7358.
[17] J. D. Eckelbarger, J. T. Wilmot, D. Y. Gin, *J. Am. Chem. Soc.* **2006**, *128*, 10370–10371.
[18] Z.-W. Zhang, X.-F. Zhang, J. Feng, Y.-H. Yang, C.-C. Wang, T.-C. Feng, S. Liu, *J. Org. Chem.* **2013**, *78*, 786–790.
[19] J.-P. Robin, R. Dhal, G. Dujardin, L. Girodier, L. Mevellec, S. Poutot, *Tetrahedron Lett.* **1999**, *40*, 2931–2934.
[20] M. E. Kuehne, W. G. Bornmann, W. H. Parsons, T. D. Spitzer, J. F. Blount, J. Zubieta, *J. Org. Chem.* **1988**, *53*, 3439–3450.

Received: July 8, 2015
Published Online: November 13, 2015

