JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2005; **48**: 11–23.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.891

Research Article

Synthesis of [^{3}H], [$^{13}C_2^{15}$N] and [^{14}C]Sch 66336 (Sarasar^{TM})

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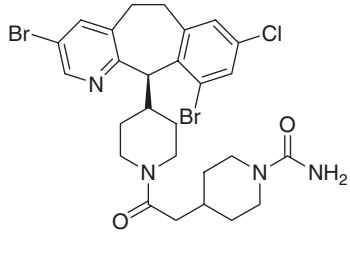
Summary

[^{3}H]Sch 66336 was prepared at a specific activity of 1.35 Ci/mmol by $Ru(Ph_3P)_3Cl_2$ catalysed exchange with tritiated water. [$^{13}C_2^{15}$N]Sch 66336 was synthesized from potassium [^{13}C]cyanide and [$^{13}C^{15}N_2$]urea in 29% overall yield from potassium [^{13}C]cyanide. [^{14}C]Sch 66336 was synthesized from potassium [^{14}C]cyanide in 31% yield. A second synthesis, from *N*-Boc-4-hydroxy[^{14}C]piperidine, gave [^{14}C]Sch 66336 labelled in a different site in 19% overall yield.

Key Words: Sch 66336; tritium; carbon-13; carbon-14; nitrogen-15; synthesis

Introduction

Sarasar^{TM}, Sch 66336 (**1**) is a potent farnesyl protein transferase inhibitor[1,2] currently in clinical trials for the treatment of cancer. During the progression of this compound from a New Drug Discovery entity into development, [^{3}H]Sch 66336 was requested to perform preliminary ADME studies. Following progression of the compound into development, [$^{13}C_2^{15}$N]Sch 66336 and [^{14}C]Sch 66336 were prepared. This paper describes the synthesis of each labelled form.



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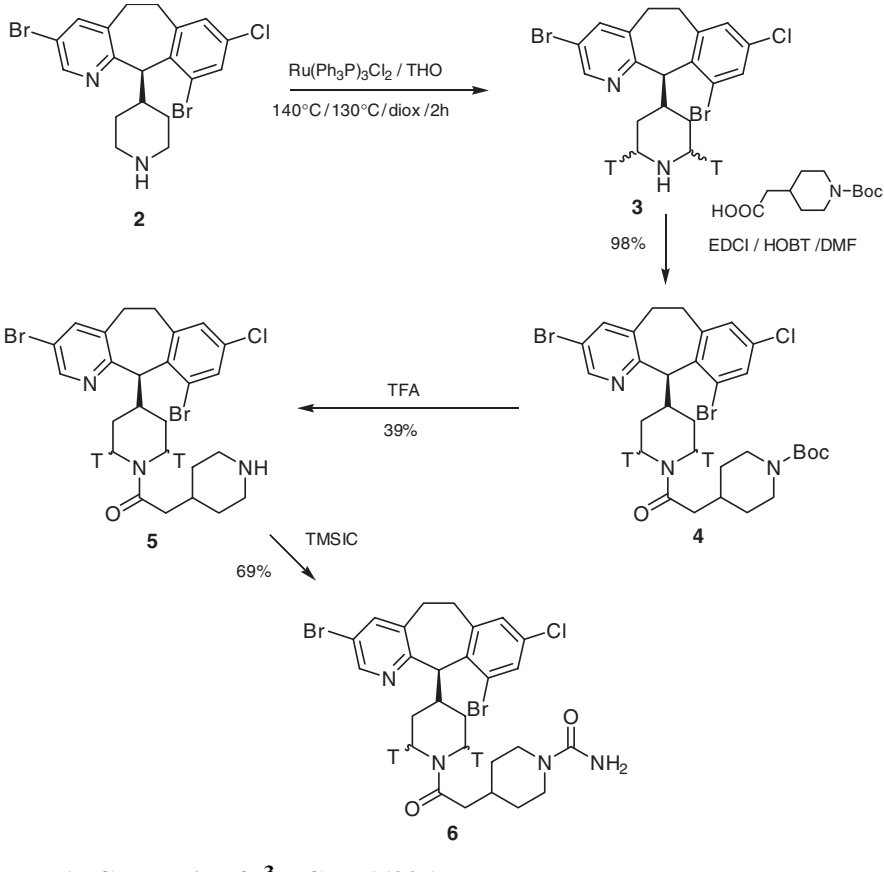
Received 13 August 2004
Revised 6 September 2004
Accepted 8 September 2004

Results and discussion

[^{3}H]Sch 66336

[^{3}H]Sch 66336 (**6**) was prepared by $Ru(Ph_3P)_3Cl_2$ catalysed exchange[3–5] of piperidine intermediate **2** with tritiated water as shown in Scheme 1. The exchange reaction was run at 140°C for 2 h. A second exchange was run at 130°C for 1 h. The combined exchanges gave 71 mCi of product **3** from 500 mCi of 50 Ci/ml tritiated water. Use of the lower temperature and shorter time gave a cleaner reaction. The remainder of the synthesis involved formation of amide **4** by EDCI/HOBT coupling, followed by removal of the *N*-Boc group with TFA to generate Compound **5**. Finally, the urea was introduced by treatment with trimethylsilylisocyanate[6] to generate a batch of 19 mCi of [^{3}H]Sch 66336 (**6**). ^{3}H NMR analysis showed the tritium was exclusively located in the 2 and 6 positions.



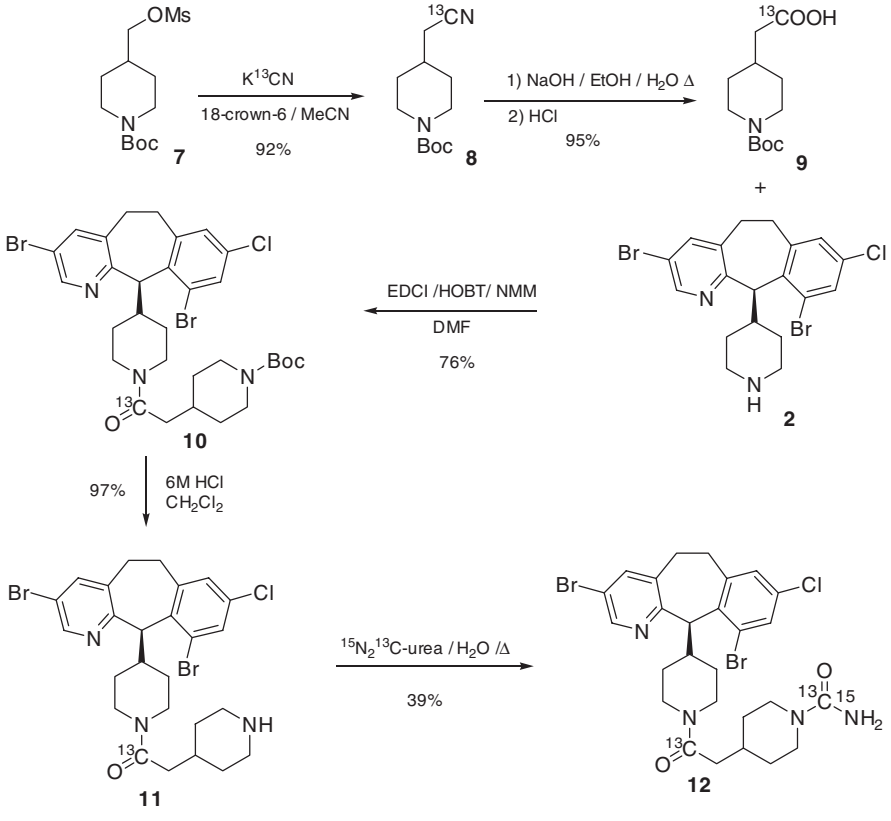
Scheme 1. Synthesis of [^{3}H]Sch 66336

[$^{13}C_2\,^{15}N$]Sch 66336

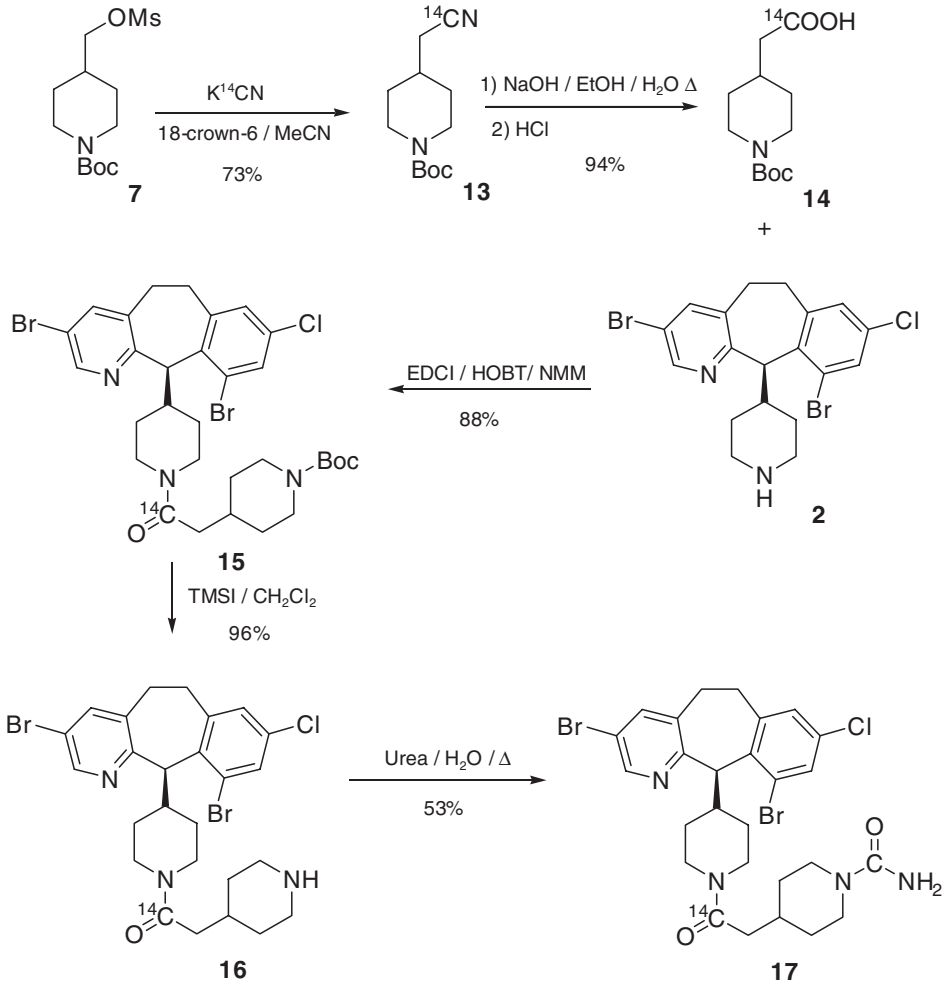
[$^{13}C_2^{15}N$]Sch 66336 (**12**) was synthesized as shown in Scheme 2. Compound **7** was reacted with potassium [^{13}C]cyanide to give nitrile **8** in 92% yield. The nitrile group was hydrolysed with aqueous base to give acid **9** in 95% yield. This was coupled with Compound **2** to give **10** in 76% yield. The *N*-Boc group was removed with acid and neutralized to give piperidine **11** in 97% yield. Compound **11** and [$^{13}C^{15}N_2$]urea[7] were refluxed in water to give [$^{13}C_2^{15}N$]Sch 66336 in 25% overall yield from potassium [^{13}C]cyanide.

[^{14}C]Sch 66336

The initial synthesis of [^{14}C]Sch 66336, from potassium [^{14}C]cyanide, was very similar to the stable isotope synthesis. This synthesis is shown in Scheme 3. The overall radiochemical yield was 31%. One of the metabolic pathways involved hydrolysis of the amide bond, generating metabolites that did not contain the ^{14}C tracer. An alternate synthesis of [^{14}C]Sch 66336 that labelled a different site was needed.

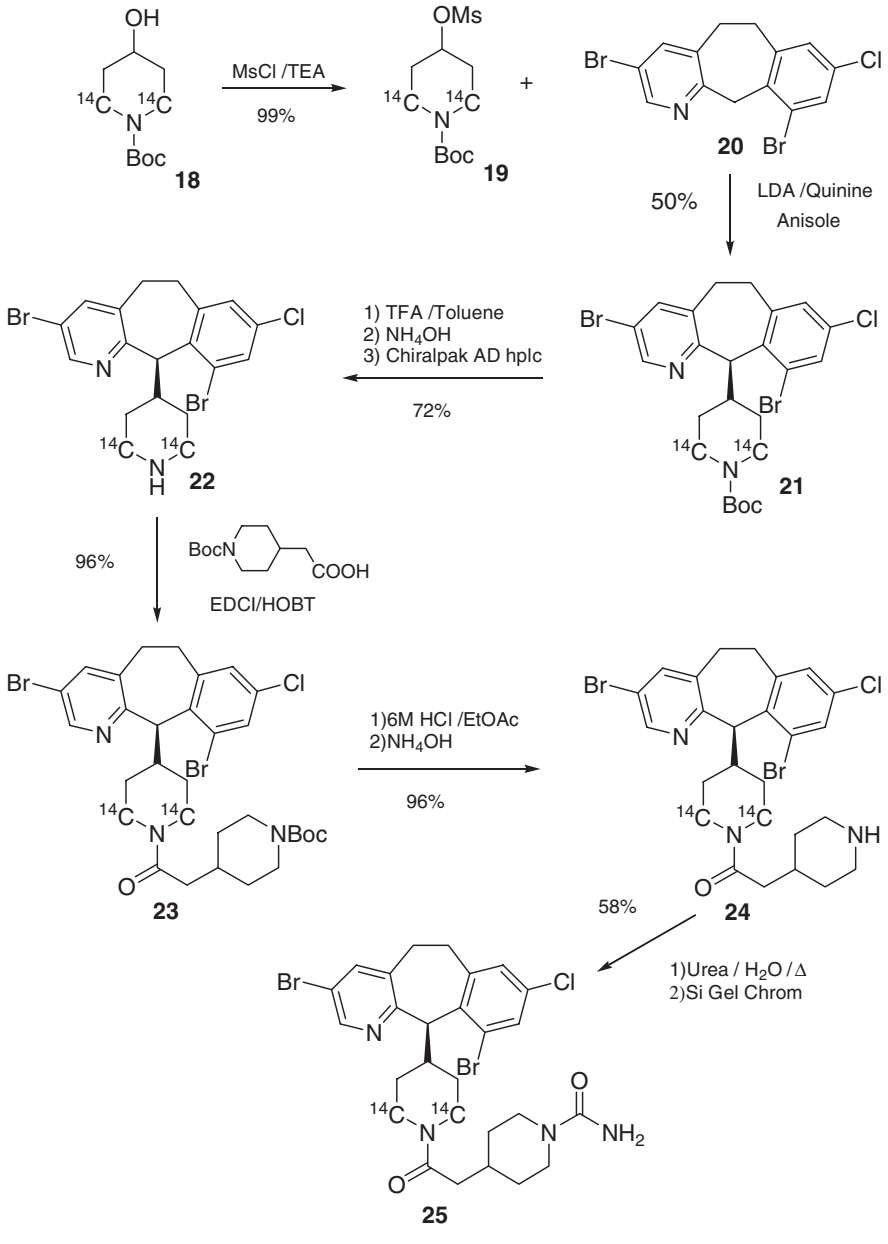


Scheme 2. Synthesis of [$^{13}C_2^{15}N$]Sch 66336



Scheme 3. Synthesis of [^{14}C-carbonyl]Sch 66336

The second ^{14}C synthesis, starting from *N*-Boc-4-hydroxy[2,6-^{14}C]piperidine **18**, is shown in Scheme 4. The key step was the quinine mediated[8] chiral alkylation of Compound **20** with mesylate **19** to give a 50% yield of **21**. The enantiomer ratio was 4:1, favouring the desired *R* enantiomer. The *N*-Boc group was removed with acid and the pure enantiomer **22** obtained by preparative chiral high-performance liquid chromatography (HPLC). The remainder of the route was identical to that previously used for other labelled forms of Sch 66336. A batch of 25 mCi at a specific activity of 63 mCi/mmol was prepared in overall radiochemical yield of 19%.



Scheme 4. Synthesis of [^{14}C-piperidine]Sch 66336

Experimental

Materials

Tritiated water (50 Ci/ml), potassium [^{14}C]cyanide and 4-hydroxy[2,6-^{14}C] piperidine were purchased from Amersham Biosciences. Potassium

[^{13}C]cyanide and [$^{13}C^{15}N_2$]urea were purchased from Cambridge Isotope Laboratories. Compounds **2**, **7** and **20** were obtained from the Chemical Development or Chemical Research Departments, Schering-Plough Research Institute. *Tris*(triphenylphosphine)ruthenium (II) chloride was purchased from Alfa. All remaining reagents and solvents were purchased from Aldrich or Acros Organics and were used as received. All ^{14}C and ^{13}C steps were carried out under an atmosphere of argon.

Liquid scintillation counting

Quantitation of radioactivity was performed using a Packard 2200CA liquid scintillation analyser, with Scintiverse BD cocktail used throughout.

Thin-layer chromatography (TLC)

Thin-layer chromatography was performed using Whatman LK6DF (silica gel 60) 5×20 cm, 0.25 mm plates. The plates were scanned on a Bioscan 1000 linear analyser. The following systems were used:

(1) Methylene chloride:methanol:concentrated ammonium hydroxide (90:10:1).
(2) Ethyl acetate:hexane (3:2).
(3) 2 M Methanolic ammonia:methylene chloride (5:95).
(4) Ethyl acetate:hexane (2:3).
(5) 2 M Methanolic ammonia:methylene chloride (10:90).
(6) Ethyl acetate:hexane (1:1).
(7) Ethyl acetate:hexane (1:4).
(8) Methanol:methylene chloride (10:90).
(9) Methanol:methylene chloride (5:95).

High-performance liquid chromatography

[^{3}H] and [^{14}C]Sch 66336 were analysed by HPLC for radiochemical, chemical and chiral purity. [$^{13}C_2$ ^{15}N]Sch 66336 was assayed for chemical purity alone. A Waters 600E system controller was used with a Waters 717 auto injector. Chemical purity was determined using a Waters 2487 dual channel UV detector and radiochemical purity using a Radiomatic 525TR radioflow detector with Radiomatic Flo-Scint III liquid scintillation cocktail. The following systems were used:

(1) Zorbax SB-C18 150 mm $\times$ 4.6 mm ID, 0.05 M pH 6.9 aqueous triethylammonium acetate:acetonitrile (1:1) for 15 min followed by a step gradient to acetonitrile, 1 ml/min, 254 nm.
(2) Metachem Inertsil 5 ODS 150 mm $\times$ 3 mm ID, acetonitrile:0.04 M aqueous potassium phosphate pH 6.5 (48:52) for 15 min followed by a step gradient to acetonitrile:water (80:20), 0.5 ml/min, 220 nm.

(3) Chiralcel ODR 250 mm × 4.6 mm ID, acetonitrile:0.1 M aqueous sodium perchlorate (47:53), 0.5 ml/min, 220 nm.
(4) Chiralcel OJR 150 mm × 4.6 mm ID, acetonitrile:0.1 M aqueous sodium perchlorate (47:53), 40°C, 0.5 ml/min, 220 nm.

*Synthesis of [^{3}H]Sch 66336 (**6**)*

*(11R)-3,10-Dibromo-8-chloro-11-([2,6-^{3}H]-4-piperidyl)-6,11-dihydro-5H-benzo [5,6] cyclohepta [b] pyridine (**3**)*. Compound **2** (20 mg) and *tris*(triphenylphosphine)ruthenium (II) chloride (2 mg) were dissolved in dioxane (100 µl) in a thick wall tube fitted with a rubber septum. Tritiated water (50 Ci/ml, 5 µl, 250 mCi) was added via syringe and the tube was frozen in liquid nitrogen, evacuated and flame sealed. The reaction was heated at 140°C for 2 h and the contents then pipetted into sodium bicarbonate solution (0.3 M, 2 ml). The product was extracted with ethyl acetate (3 × 5 ml). The combined ethyl acetate extracts were washed with water (2 ml), dried over anhydrous sodium sulphate, filtered and evaporated to dryness. A total of 89 mCi of crude Compound **3** at a radiochemical purity of 20% (TLC system 1) was isolated. The reaction was repeated using the modified conditions of 130°C and a 1 h reaction time. A total of 75 mCi of crude Compound **3** at a radiochemical purity of 46% was isolated. The two batches were combined and purified by chromatography on a 1 g Waters silica gel 'Sep-Pak' cartridge using a mobile phase of methylene chloride:methanol:concentrated ammonium hydroxide (90:10:1). A total of 71 mCi of Compound **3** was isolated.

*^{t}Butyl 4-(2-4-[(11R)-3, 10-dibromo-8-chloro-6, 11-dihydro-5H-benzo [5, 6] cyclohepta [b] pyridin-11-yl]-([2,6-^{3}H]-piperidino)-2-oxoethyl)-1-piperidine carboxylate (**4**)*. Compound **3** was dissolved in DMF (0.9 ml) and *N*-Boc-4-piperidyl acetic acid (41.2 mg, 0.17 mmol), 1-(3-dimethyl amino propyl)-3-ethyl carbodiimide hydrochloride (32.6 mg, 0.17 mmol) and 1-hydroxybenzotriazole hydrate (23 mg, 0.17 mmol) were added. The reaction was stirred at room temperature overnight and then partitioned between methylene chloride (10 ml) and aqueous sodium bicarbonate solution (0.3 M, 2 ml). The aqueous layer was removed and the organic layer washed with saturated disodium hydrogen phosphate solution (2 ml) and water (2 ml). The organic layer was dried over anhydrous magnesium sulphate, filtered and evaporated to dryness to give 69.8 mCi (98%) of Compound **4**, which was used directly in the next step.

*1-4-[(11R)-3,10-Dibromo-8-chloro-6,11-dihydro-5H-benzo [5, 6] cyclohepta[b] pyridin-11-yl]-([2,6-^{3}H]-piperidino)-2-(4-piperidyl)-1-ethanone (**5**)*. Compound **4** was dissolved in methylene chloride (0.5 ml), cooled to 0°C and trifluoroacetic acid (0.5 ml) was added. The reaction was stirred at 0°C for 1 h and a further

2 h at room temperature (TLC system 1). The reaction was cooled in an ice bath and 50% sodium hydroxide solution was added until pH 10 was achieved. Water (5 ml) was added and the product extracted with methylene chloride (4×5 ml). The combined organic extracts were washed with water (2 ml), dried over anhydrous magnesium sulphate, filtered and evaporated to dryness. The product was purified by chromatography on a 1 g Waters silica gel 'Sep-Pak' cartridge using a mobile phase of methylene chloride:methanol:concentrated ammonium hydroxide (90:10:1) as eluent. A total of 27.4 mCi (39%) of Compound **5** was obtained.

*4-(2-4-[(11R)-310-Dibromo-8-chloro-6,11-dihydro-5H-benzo [5, 6] cyclohepta [b] pyridin-11-yl]-([2,6-^{3}H]-piperidino)-2-oxoethyl)-1-piperidinecarboxamide (^{3}H-Sch 66336 (**6**)*. Compound **5** (27.4 mCi), was dissolved in methylene chloride (0.5 ml) and trimethylsilyl isocyanate (0.1 ml) was added. The reaction was stirred at room temperature overnight (TLC system 1). The reaction was partitioned between methylene chloride (10 ml) and sodium bicarbonate solution (0.3 M, 5 ml). The aqueous layer was removed and the organic layer washed with brine (2 ml), dried over anhydrous magnesium sulphate, filtered and evaporated to dryness. The product was purified by chromatography on a 1 g Waters silica gel 'Sep-Pak' cartridge using a mobile phase of methylene chloride:methanol:concentrated ammonium hydroxide (97:3:1). Further purification was carried out by HPLC on a Zorbax SB C18 9.4×250 mm column with a mobile phase of 0.05 M pH 6.9 aqueous triethylammonium acetate (1:1) acetonitrile mobile phase at a flow rate of 5 ml/min. Detection was at 254 nm. A total of 19 mCi (69%) of [^{3}H]Sch 66336 was obtained at a specific activity of 1.35 Ci/mmol. Radiochemical purity as determined by HPLC system 1 and TLC system 1 was 99.6 and 98.6%, respectively.

*Synthesis of [$^{13}C_2^{15}N$]Sch 66336 (**12**)*

*N-Boc-4-piperidyl-[^{13}C-cyano]acetonitrile (**8**)*. Mesylate **7** (6.24 g, 21.3 mmol), 18-crown-6 (14.1 g, 53.3 mmol) and potassium [^{13}C]cyanide (2.13 g, 32.2 mmol) in anhydrous acetonitrile (148 ml) was stirred at room temperature for 1 h. The reaction was then heated at reflux overnight. After TLC showed complete reaction (TLC system 2), the reaction was partitioned between water (100 ml) and ethyl acetate (150 ml). The layers were separated. The organic layer was washed with water (2×100 ml), brine (50 ml), dried over anhydrous sodium sulphate, filtered and evaporated to dryness to yield 4.41 g (92%) of Compound **8** which was used directly in the next step.

*N-Boc-4-piperidyl-[^{13}C-carboxyl]acetic acid (**9**)*. Compound **8** (4.41 g, 19.6 mmol) was dissolved in ethanol (55 ml) and 50% sodium hydroxide (5.2 ml) was added. The reaction was heated overnight at 98°C after which

TLC (TLC system 2) showed complete conversion. The reaction was cooled in an ice bath, diluted with water (25 ml) and the pH adjusted to 2 with 2 M HCl. The resulting suspension was extracted with methylene chloride (3 × 100 ml). The combined methylene chloride extracts were washed with brine (50 ml), dried over anhydrous sodium sulphate, filtered and evaporated to dryness to yield 4.52 g (95%) of Compound **9** which was used directly in the next step.

^{t}Butyl 4-(2-4-[(11R)-3,10-dibromo-8-chloro-6,11-dihydro-5H-benzo [5, 6] cyclohepta [b] pyridin-11-yl]-(piperidino)-2-[^{13}C]-oxoethyl)-1-piperidine carboxylate (**10**). Compound **9** (1.91 g, 7.8 mmol), intermediate **2** (3.07 g, 6.5 mmol), *N*-methyl morpholine (1.1 ml, 10 mmol), 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (1.84 g, 9.58 mmol) and 1-hydroxybenzotriazole hydrate (1.29 g, 9.55 mmol) were dissolved in DMF (12 ml). The solution was stirred at room temperature over the weekend after which TLC (TLC system 3) showed complete reaction. The reaction was evaporated to dryness and the product purified by silica gel chromatography using a gradient of 0.25–1% 2 M methanolic ammonia in methylene chloride. A total of 4.16 g (76%) of Compound **10** was obtained.

1-4-[(11R)-3,10-Dibromo-8-chloro-6,11-dihydro-5H-benzo [5, 6] cyclohepta[b] pyridin-11-yl]-(piperidino)-2-(4-piperidyl)-1-[^{13}C]-ethanone (**11**). Compound **10** (2.35 g, 3.37 mmol) was dissolved in methylene chloride (20 ml) and hydrochloric acid (6 M, 12 ml) added. After stirring for 2 h (TLC system 3) the reaction was made basic with sodium bicarbonate solution (0.3 M) and the mixture extracted with methylene chloride (3 × 100 ml). The methylene chloride extracts were dried over anhydrous sodium sulphate, filtered and evaporated to dryness to yield 1.95 g (97%) of Compound **11**, which was used directly in the next step.

4-(2-4-[(11R)-3,10-Dibromo-8-chloro-6,11-dihydro-5H-benzo [5, 6] cyclohepta[b] pyridin-11-yl]-(piperidino)-2-[^{13}C]-oxoethyl)-1-piperidine-[$^{13}C^{15}N$]-carboxamide [$^{13}C_2^{15}N$]Sch 66336 (**12**). Compound **11** (1.41 g, 2.3 mmol) and [$^{13}C^{15}N_2$]-urea (1.5 g, 23 mmol) were dissolved in water (35 ml) and heated at reflux for 72 h. After cooling, the reaction was extracted with methylene chloride (3 × 20 ml). The extracts were combined, dried over anhydrous sodium sulphate, filtered and evaporated to dryness. The crude product was purified by silica gel chromatography using a gradient of 2.5–4% 2 M methanolic ammonia in methylene chloride. The resulting solid was crystallized from methylene chloride and hexane to give 576 mg (39%) of [$^{13}C_2^{15}$N]Sch 66336. The chemical purity was 96.1% by reverse phase HPLC (HPLC system 2) and 96.7% by chiral HPLC (HPLC system 3).

FAB$^+$ mass spectrometry: *m/z* 642 $(M+H)^+$. ^{13}C NMR (75 MHz, D_6-DMSO δ169.6 ppm, δ157.9 ppm, δ157.8 ppm ^{13}C enriched.

*Synthesis of [^{14}C-carbonyl]Sch 66336 (**17**)*

*N-Boc-4-piperidyl-[^{14}C]acetonitrile (**13**)*. Mesylate **7** (293 mg, 1 mmol), 18-crown-6 (0.53 g, 2.01 mmol), potassium [^{14}C] cyanide (54 mCi/mol, 10.0 mCi, 12.4 mg, 0.185 mmol) and unlabelled potassium cyanide (53.1 mg, 0.815 mmol) in anhydrous acetonitrile (6.3 ml) were stirred at room temperature for 1 h. The reaction was then heated at reflux overnight. After TLC showed complete reaction (TLC system 4), the reaction was diluted with water (15 ml) and extracted with ethyl acetate (5×10 ml). The combined organic layers were washed with brine (10 ml), dried over anhydrous magnesium sulphate, filtered and evaporated to dryness. The compound was purified by chromatography on silica gel using an eluent of 5% 2 M methanolic ammonia in methylene chloride to yield 7.3 mCi (73%) of Compound **13**.

*N-Boc-4-piperidyl-[^{14}C-carboxyl]acetic acid (**14**)*. Compound **13** (7.3 mCi, 163 mg, 0.73 mmol) was dissolved in ethanol (2 ml) and 50% sodium hydroxide (0.24 ml) was added. The reaction was heated overnight at 98°C after which TLC (TLC system 5) showed complete conversion. The reaction was cooled in an ice bath, diluted with water (2 ml) and adjusted to pH 2 with 2 M HCl. The resulting suspension was extracted with methylene chloride (5×10 ml). The combined methylene chloride extracts were dried over anhydrous magnesium sulphate, filtered and evaporated to dryness to give 6.84 mCi (94%) of Compound **14** which was used directly in the next step.

*^{t}Butyl 4-(2-4-[(11R)-3,10-dibromo-8-chloro-6,11-dihydro-5H-benzo [5, 6] cyclohepta [b] pyridin-11-yl]-(piperidino)-2-[^{14}C]-oxoethyl)-1-piperidine carboxylate (**15**)*. Compound **14** (6.84 mCi, 166 mg, 0.684 mmol), Compound **2** (316 mg, 0.672 mmol), *N*-methyl morpholine (0.11 ml, 1 mmol), 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (196.5 mg, 1.02 mmol) and 1-hydroxybenzotriazole hydrate (135 mg, 1 mmol) were dissolved in DMF (1.2 ml). The reaction was stirred at room temperature over the weekend after which TLC (TLC system 3) showed complete reaction. The reaction was evaporated to dryness and the residue partitioned between water (6 ml) and methylene chloride (20 ml). The methylene chloride layer was washed with 10% disodium hydrogen phosphate solution (2×15 ml). The organic layer was dried over anhydrous magnesium sulphate, filtered and evaporated to dryness to yield 6 mCi (88%) of Compound **15** which was used directly in the next step.

*1-4-[(11R)-3,10-Dibromo-8-chloro-6,11-dihydro-5H-benzo [5, 6] cyclohepta[b] pyridin-11-yl]-(piperidino)-2-(4-piperidyl)-1-[^{14}C]-ethanone (**16**)*. Compound **15** (6.0 mCi, 418 mg, 0.6 mmol) was dissolved in chloroform (20 ml) and trimethylsilyliodide (90 µl, 0.633 mmol) added. After stirring for 30 min at room temperature, methanol (1 ml) was added and the reaction evaporated to

dryness. The residue was dissolved in methylene chloride (8 ml) and sodium hydroxide solution (2 M, 0.5 ml) was added. The layers were separated and the aqueous layer extracted with methylene chloride (3×4 ml). The combined organic extracts were dried over anhydrous magnesium sulphate, filtered and evaporated to dryness to yield 5.76 mCi (96%) of Compound **16**, which was used directly in the next step.

*4-(2-4-[(11R)-3,10-Dibromo-8-chloro-6,11-dihydro-5H-benzo [5, 6] cyclohepta[b] pyridin-11-yl]-(piperidino)-2-[^{14}C]-oxoethyl)-1-piperidine-carboxamide, [^{14}C-carbonyl] Sch 66336, (**17**)*. Compound **16** (5.76 mCi, 342 mg, 0.576 mmol) and urea (346 mg, 5.77 mmol) were dissolved in water (9 ml) and heated at reflux for 48 h. After cooling, the reaction was extracted with methylene chloride (3×20 ml). The extracts were combined, dried over anhydrous magnesium sulphate, filtered and evaporated to dryness. The crude product was purified by silica gel chromatography using 2% 2 M methanolic ammonia in methylene chloride as eluent to give 3.05 mCi (53%) [^{14}C]Sch 66336 at a specific activity of 10 mCi/mmol. The radiochemical purity as determined by reverse phase HPLC (HPLC system 1) and chiral HPLC (HPLC system 3) was 97.8 and 97.3%, respectively.

*Synthesis of [2,6-^{14}C-piperidine]Sch 66336 (**25**)*

*N-Boc-4-mesyl[2,6-^{14}C]piperidine (**19**)*. Compound **18** (141 mCi, 455 mg, 2.24 mmol) was dissolved in anhydrous methylene chloride (3.6 ml), triethylamine (0.94 ml, 6.72 mmol) added and the solution was cooled to 0°C. Mesyl chloride (0.22 ml, 2.82 mmol) was added dropwise and the reaction was stirred at 0°C for 2.5 h, at which point TLC monitoring (TLC system 6) showed complete reaction. The reaction was quenched with hydrochloric acid (1 M, 1.2 ml) and then made basic with aqueous potassium carbonate (1 M). The organic layer was separated and the aqueous layer extracted with methylene chloride (2×6 ml). The combined organic extracts were dried over anhydrous magnesium sulphate, filtered and evaporated to dryness to yield 140 mCi, 625 mg, (99%) of Compound **19**.

*(11R)-3,10-Dibromo-8-chloro-11-(N-Boc-[2,6-^{14}C]-4-piperidyl)-6,11-dihydro-5H-benzo [5,6] cyclohepta [b] pyridine (**21**)*. Compound **20** (798 mg, 2.06 mmol) and quinine (794 mg, 2.45 mmol) were stirred in anhydrous anisole (20 ml). The flask was cooled to 0°C and lithium diisopropylamide (1.5 M in cyclohexane, 4.64 ml, 6.97 mmol) was added dropwise over 10 min. The reaction was stirred at 0°C for 1 h and then Compound **19** (140 mCi, 625 mg, 2.24 mmol) in anisole (10 ml) was added. The reaction was stirred at 0°C for 10 min and then allowed to warm to room temperature overnight. After TLC showed complete reaction (TLC system 7), it was quenched with aqueous HCl (1 M, 14.5 ml) and the organic layer removed. The aqueous layer was extracted

with ethyl acetate (2×30 ml). The combined organic layers were dried over anhydrous magnesium sulphate, filtered and evaporated to dryness. The crude product was chromatographed on silica gel with 8% ethyl acetate in hexane as the mobile phase to give 65.4 mCi, 592 mg, (50%) of Compound **21**.

*(11R)-3,10-Dibromo-8-chloro-11-([2,6-^{14}C]-4-piperidyl)-6,11-dihydro-5H-benzo [5,6] cyclohepta [b]pyridine (**22**).* Compound **21** (65.4 mCi, 592 mg, 1.04 mmol) was dissolved in anhydrous toluene (7 ml) and cooled in an ice bath. Trifluoroacetic acid (0.9 ml) was added and the reaction stirred at room temperature overnight. After TLC showed complete reaction (TLC system 8), the reaction was cooled in an ice bath and concentrated ammonium hydroxide was added to pH 10–11. The organic layer was removed and the aqueous layer was extracted with methylene chloride (2×12 ml). The organic layers were combined, dried over anhydrous sodium sulphate, filtered and evaporated to dryness. The crude product was purified by HPLC on a Chiralpak AD column with a mobile phase of hexane:isopropanol:diethylamine (90:10:0.2). A total of 46.9 mCi, 350 mg, (72%) of Compound **22** was obtained.

*^{t}Butyl 4-(2-4-[(11R)-3,10-dibromo-8-chloro-6,11-dihydro-5H-benzo [5, 6] cyclohepta [b] pyridin-11-yl]-([2,6-^{14}C]-piperidino)-2-oxoethyl)-1-piperidine carboxylate (**23**).* Compound **22** (46.9 mCi, 350 mg, 0.74 mmol) was dissolved in anhydrous THF (5 ml). *N*-Boc-4-piperidyl acetic acid (237.1 mg, 0.97 mmol), *N*-methyl morpholine (0.22 ml, 2.02 mmol), 1-(3-dimethylamino-propyl)-3-ethyl carbodiimide hydrochloride (233 mg, 1.22 mmol) and 1-hydroxybenzotriazole hydrate (164 mg, 1.22 mmol) were added. After stirring at room temperature overnight (TLC system 8), the reaction was evaporated to dryness and dissolved in methylene chloride (20 ml). The solution was washed with water (10 ml) and brine (10 ml), dried over anhydrous sodium sulphate, filtered and evaporated to dryness. A total of 45 mCi, 497 mg, (96%) of Compound **23** was obtained and used directly in the next step.

*1-4-[(11R)-3,10-Dibromo-8-chloro-6,11-dihydro-5H-benzo [5, 6] cyclohepta[b] pyridin-11-yl]-([2,6-^{14}C]-piperidino)-2-(4-piperidyl)-1-ethanone (**24**).* Compound **23** (45 mCi, 497 mg, 0.71 mmol) was dissolved in ethyl acetate (10 ml). The solution was cooled to 0°C and aqueous HCl (6 M, 2.7 ml) was added. The reaction was stirred for 2 h at room temperature (TLC system 8) and then the pH was adjusted to pH 10 with aqueous ammonium hydroxide. The organic layer was separated and the aqueous layer extracted with methylene chloride (4×15 ml). The combined organic extracts were dried over anhydrous sodium sulphate, filtered and evaporated to dryness. A total of 43 mCi, 406 mg, (96%) of Compound **24** was obtained and used directly in the next step.

4-(2-4-[(11R)-3,10-Dibromo-8-chloro-6,11-dihydro-5H-benzo [5, 6] cyclohepta[b] pyridin-11-yl]-([2,6-^{14}C]-piperidino)-2-oxoethyl)-1-piperidinecarboxamide ([2,6-^{14}C-piperidine]Sch 66336) (**25**). Compound **24** (43 mCi, 406 mg, 0.68 mmol) was suspended in water (10 ml). Urea (512 mg, 8.53 mmol) was added and the reaction heated under reflux overnight (TLC system 9). After cooling, the reaction was extracted with methylene chloride (3 × 15 ml). The combined organic extracts were dried over anhydrous sodium sulphate, filtered and evaporated to dryness. The crude product was purified by silica gel chromatography using a gradient of 2–4% methanol in methylene chloride as the mobile phase. A total of 25 mCi, 254 mg (58%) of [^{14}C]Sch 66336 (**25**) was obtained at a specific activity of 63 mCi/mmol. The radiochemical purity was 99.1% by reverse phase (HPLC system 2) and 98.1% by chiral HPLC (HPLC system 4).

Acknowledgements

The authors would like to thank Dr. Don Hou from Schering-Plough Research Institute Chemical Development for intermediates, procedures and helpful discussions during the ^{14}C and ^{13}C synthesis. Thanks are also due to Dr. Arthur Taveras from Schering-Plough Research Institute Chemical Research for similar support during the ^{3}H synthesis.

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