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Synthesis of ^{3}H, $^{2}H_4$ and ^{14}C-SCH 417690 (Vicriviroc)

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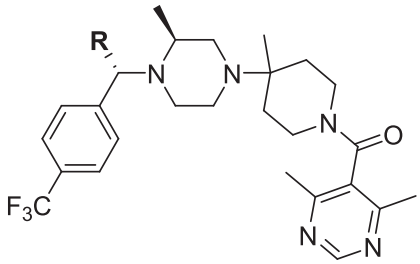
Vicriviroc or SCH 417690 is a potent and selective antagonist of the CCR5 receptor. CCR5 receptor antagonists have the potential for the treatment of HIV infections. Four distinct isotopically labelled forms of SCH 417690 were synthesized. Low specific activity [^{3}H]SCH 417690 was prepared for a preliminary absorption, distribution, metabolism and excretion evaluation of the compound and [^{14}C]SCH 417690 for more definitive absorption, distribution, metabolism and excretion work, including an absorption, metabolism and excretion study in man. In addition, high specific activity [^{3}H]SCH 417690 was prepared for CCR5 receptor binding work and [$^{2}H_4$]SCH 417690 was prepared as an internal standard for a liquid chromatography–mass spectrometry bioanalytical method. The paper discusses the synthesis of four isotopically labelled forms of SCH 417690.

Keywords: tritium exchange; ruthenium catalyst; deuterium; carbon-14

Introduction

SCH 417690 was discovered as the result of optimization of a lead containing a benzylic methyl group.[1–8]



R=CH_3, CH_3CH_2, $CH_3CH_2CH_2$, $PhCH_2$, CH_2OMe

The size of the benzylic substituent was shown to be the key to enhanced potency and receptor selectivity in this series. Optimization around this benzylic methyl functionality led to the methoxymethyl analogue SCH 417690 **1**, which had the best overall profile.

SCH 417690 is extremely potent (*Ki* 2.5 nM) and selective for the CCR5 receptor with almost no affinity for the muscarinic receptor or other G-protein–coupled receptors. It has excellent oral bioavailability in rats and monkeys, reduced affinity for the hERG K^+ channel and no significant central nervous system or gastrointestinal side effects at an oral dose of 10 mg/kg in rats.

During the course of the progression of the compound from discovery into development, several isotopically labelled forms of SCH 417690 were prepared. Low specific activity [^{3}H]SCH 417690 was prepared to conduct preliminary absorption, distribution, metabolism and excretion studies (ADME), which confirmed the high oral bioavailability observed in cold pharmacokinetic studies and identified O-demethylation of the side chain followed by glucuronidation as the major metabolic pathway.[1] A second batch of [^{3}H]SCH 417690 was also prepared at higher specific activity in order to conduct CCR5 receptor binding studies. Following successful progression of the compound into development, [$^{2}H_4$]SCH 417690 was prepared as an internal standard for a liquid chromatography–mass spectrometry bioanalytical method, supporting the analysis of samples derived from toxicology and clinical studies. Finally, [^{14}C]SCH 417690 was prepared to conduct definitive ADME studies supporting compound registration, including a ^{14}C absorption, metabolism and excretion (AME) study in man. The paper hence discusses the synthesis of these four isotopically labelled compounds.

Results and discussion

Low specific activity [^{3}H]SCH 417690 **4** was prepared via Ru$(Ph_3P)_3Cl_2$ catalysed exchange[9] of piperidine intermediate **2** with 579 mCi, 50 Ci/mL tritiated water as shown in Figure 1.

Using this chemistry, the methylene protons adjacent to the piperidine nitrogen are selectively exchanged.[9,10] Hence, 73 mCi of crude product **3** was isolated from the reaction, which was purified by silica gel chromatography and coupled with 4,6-dimethyl pyrimidine-5-carboxylic acid **A** under standard 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/hydroxybenzotriazole (EDC/HOBt) conditions to generate 28 mCi of crude [^{3}H]SCH 417690 **4**. Purification by reverse phase HPLC yielded a total batch of 24 mCi at a specific activity of 1.2 Ci/mmol.

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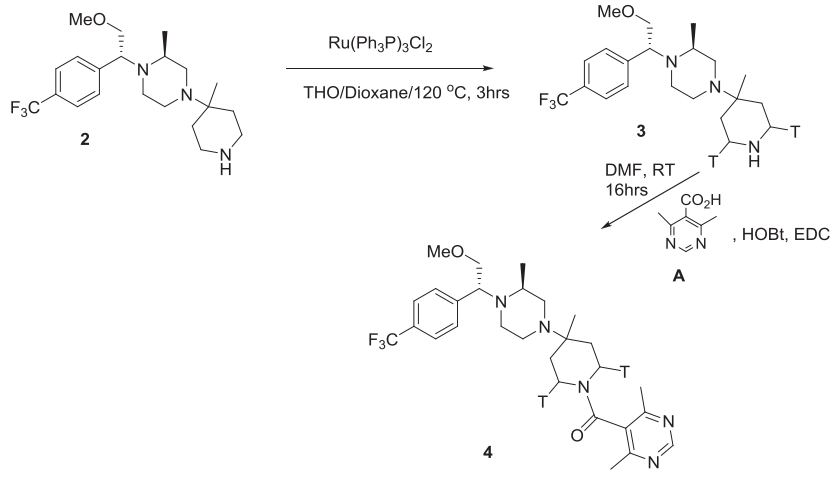

Figure 1. Synthesis of [^{3}H]SCH 417690 (**4**).

High specific activity [^{3}H]SCH 417690 **4**, needed for receptor binding work, was prepared via the same route as shown in Figure 1, with the tritiation step contracted out to Quotient Bioresearch. Piperidine **2** was reacted with 20 Ci of 90 atom % tritiated water, which generated 1.65 Ci of crude product **3**. About 300 mCi of this crude was purified by reverse phase HPLC and converted to [^{3}H]SCH 417690 as previously described. After final reverse phase HPLC purification, a total batch of 46 mCi at a specific activity of 16.4 Ci/mmole was generated, which was lower than was expected based on the specific activity generated from the initial synthesis of **4** utilizing 50 mCi/mL tritiated water. However, the specific activity was still sufficiently high enough for the intended receptor binding studies, and while it is unclear why the specific activity of the second batch of **4** was lower than expected, it is possible that the presence of adventitious water present during the preparation of the 90 atom % tritiated water contributed to a significant dilution in specific activity of the tritiated water in the reaction. ^{3}H NMR analysis confirmed the tritium was exclusively incorporated alpha to the piperidine nitrogen with no evidence for incorporation at other sites in either the piperidine or piperazine rings and is consistent with previous results.[10] While the exact mechanism of the reaction is unknown, the requirement for either a primary or secondary amine is suggestive of formation of a Ru-N species with subsequent activation of the adjacent CH bonds.

The synthesis of [$^{2}H_4$]SCH 417690 **9** is shown in Figure 2.

N-Boc-[$^{2}H_4$]-4-piperidone **7** was prepared in a similar manner as previously described[11] with one minor modification whereby

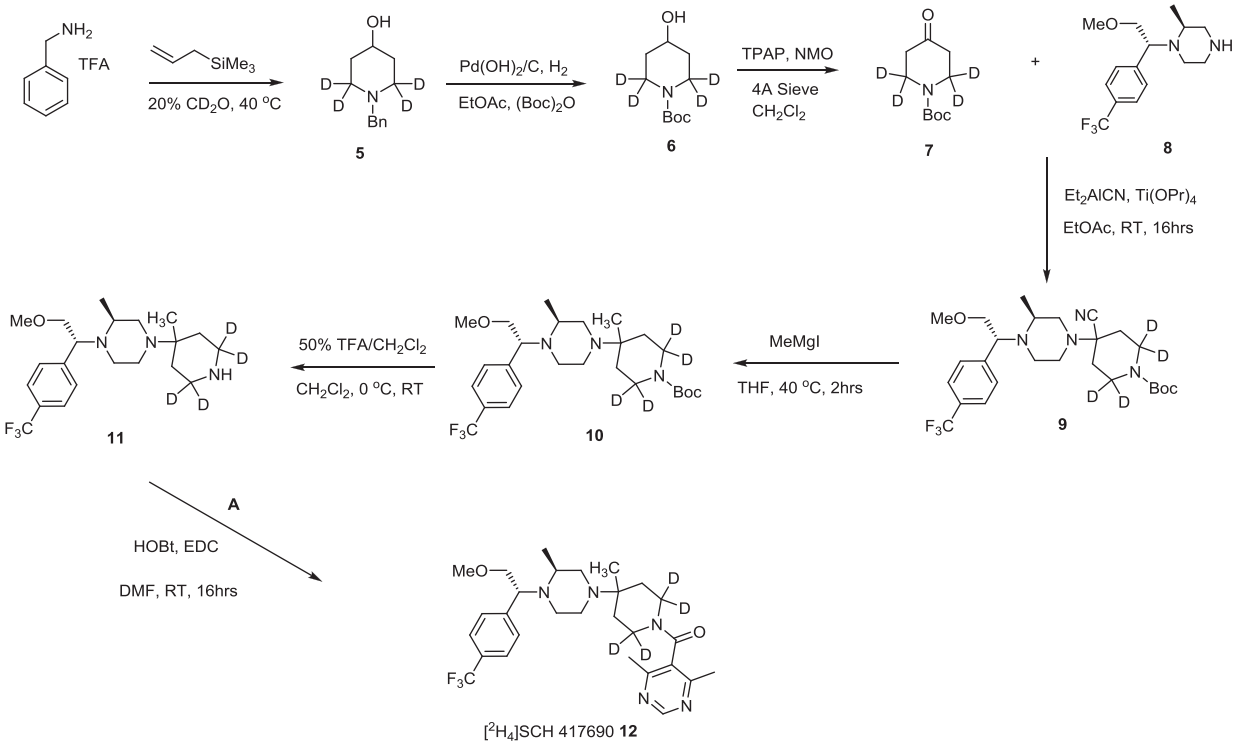

Figure 2. Synthesis of [$^{2}H_4$]SCH 417690 (**12**).

the debenzylation and Boc installation steps to form **6** were combined into a two-step one-pot process in overall 33% yield from benzylamine trifluoroacetate. Following oxidation with *N*-methylmorpholine-*N*-oxide (NMO) and tetrapropylammonium perruthenate (TPAP),[10] **7** was then reacted with intermediate **8**, diethylaluminium cyanide and titanium isopropoxide in a two-step one-pot process to give the [$^2H_4$]cyano compound **9** in 75% yield. The cyano group was then displaced by methylmagnesium iodide to give compound **10** in 78% yield. The Boc group was removed by trifluoroacetic acid in methylene chloride to give compound **11** in 76% yield, which was then coupled with **A** under standard HOBt/EDC conditions to generate crude [$^2H_4$]SCH 417690 **12**. The crude batch was purified by silica gel chromatography to yield 700 mg (64%) of purified [$^2H_4$]SCH 417690 in an overall yield of 7.4%. The chemical purity as determined by HPLC system 2 was >99%. No unlabelled SCH 417690 was detected by FAB$^+$ MS analysis.

[^{14}C]SCH 417690 maleate **20** was synthesized from [^{14}C] formaldehyde using a route similar to [$^2H_4$]SCH 417690 as shown in Figure 3.

On initial examination of the route, preparation of [^{14}C] methylmagnesium iodide from [^{14}C]methyl iodide appeared to be an attractive option to introduce the ^{14}C label and eliminate four steps from the route. Unfortunately, all attempts to prepare and utilize the [^{14}C]Grignard met with limited success with very low yields when attempting to use 1 equivalent of [^{14}C] methylmagnesium iodide, and this approach was abandoned in favour of the longer route.[12] Hence, [^{14}C]*N*-benzyl-4-hydroxypiperidine **13** was prepared via a modification of the Mannich-type cyclization used to prepare **7**,[11] which allowed use of one equivalent of the commercially supplied 1% aqueous solution of [^{14}C]formaldehyde as opposed to the 20% solution used when preparing [$^2H_4$]benzyl-4-hydroxypiperidine. Thus, benzyl-but-3-enyl-amine was treated with camphorsulfonic acid and 100 mCi of a newly received 1% aqueous solution of [^{14}C] formaldehyde at 100 °C for 1.5 h, followed by stirring at room temperature overnight. Under these conditions, the [^{14}C] formaldehyde generates a mixture of unlabelled, singly labelled and doubly ^{14}C labelled **13** in a statistical ratio of 1:2:1 because of the aza-Cope rearrangement of the intermediate butenyl imminium ion.[13] Hence, a 92% yield of crude product **13** was generated, which was then reacted with Boc anhydride and Pearlman's catalyst under hydrogen (50 psi) to give compound **14** in 97% yield. The N-Boc compound **14** was then oxidized with NMO and TPAP to give [^{14}C]N-Boc piperidone **15** in 97% yield, which was then treated with intermediate **8**, diethylaluminium cyanide and titanium isopropoxide to give compound **16** in 93% yield after purification by silica gel chromatography. The cyano group was then displaced by an excess of methylmagnesium iodide to give compound **17** in 90% yield. The Boc group was removed with 50% trifluoroacetic acid in methylene chloride to give compound **16** in 98% yield. Compound **16** was finally coupled with **A** under standard bromotris (pyrrolyldino)phosphonium hexafluorophosphate conditions to give crude [^{14}C]SCH 417690 **19**, which was purified by silica gel chromatography to generate a 48 mCi batch of [^{14}C]SCH 417690 in 63% yield. A portion of the batch was further purified by reverse phase HPLC and used to generate a 25.7 μCi/mg batch of [^{14}C]SCH 417690 maleate **20** that was subsequently used in a ^{14}C AME study in man. The radiochemical purity of the final diluted batch as determined by reverse phase HPLC in systems 2 and 3 was >99%.

Experimental

Materials

Tritiated water (50 Ci/mL) and [^{14}C]formaldehyde were purchased from (Quotient Biosciences, Cardiff, UK). [$^2H_2$]Formaldehyde was purchased from (Cambridge Isotope Laboratories, Andover MA, USA). Compounds

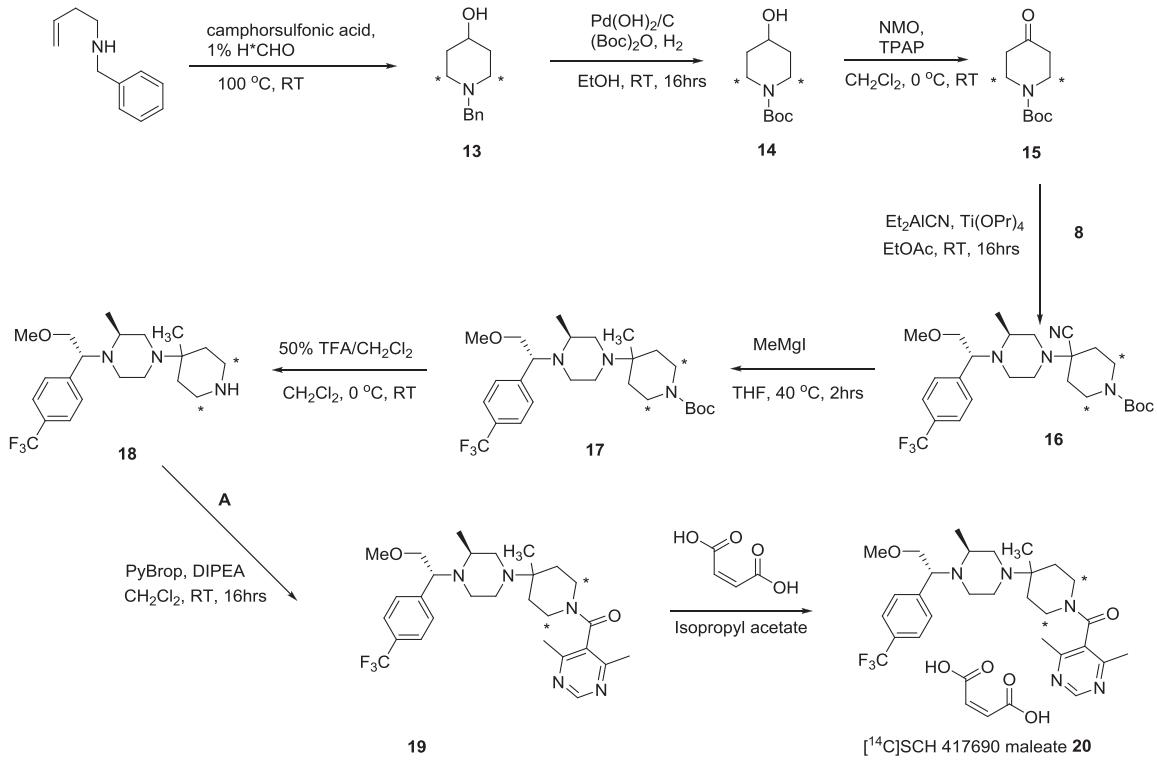

Figure 3. Synthesis of [^{14}C]SCH 417690 maleate (**20**).

2, **8**, 4,6-dimethylpyrimidine-5-carboxylic acid[1,13,14] and unlabelled SCH 417690 were obtained from (Merck Research Labs, Process Chemistry, Rahway NJ, USA). Tris-triphenylphosphine ruthenium (II) chloride was purchased from (Alfa, Ward Hill MA, USA). All remaining reagents were purchased from (Sigma Aldrich, Milwaukee WI, USA) or (Acros Organics, Pittsburgh PA, USA) and were used as received. All ^{2}H and ^{14}C steps were carried out under an atmosphere of argon.

Liquid scintillation counting

Quantitation of radioactivity was performed using (Packard, Downers Grove, IL, USA) 2200 or 2900 CA liquid scintillation analysers, with Scintiverse BD cocktail used throughout.

Thin layer chromatography

Thin layer chromatography was performed using Whatman LK6DF (Silica Gel 60) 5 × 20 cm, 0.25 mm plates. The plates were scanned on either a Bioscan AR2000 or a Bioscan 1000 linear analyser. The following systems were used:

1 Methylene chloride : 2 M methanolic ammonia (90:10)
2 Methylene chloride : 2 M methanolic ammonia (95:5)
3 Methylene chloride : methanol (95:5)
4 Methylene chloride : 7 M methanolic ammonia (99:1)
5 Methylene chloride : methanol : triethylamine (93:6:1)
6 Methylene chloride : methanol : triethylamine (94:5:1)
7 Ethyl acetate : hexanes : triethylamine (30:69:1)

High performance liquid chromatography

A (Waters, Miford MA, USA) 2695 'Alliance' HPLC system was used. Chemical purity was determined using a Waters 2487 dual channel ultraviolet detector and radiochemical purity either using a Packard C150 radioflow detector with Packard FloScint III cocktail or a IN/US Beta Ram 2 radioflow detector with IN/US InFlo 3 cocktail. The following systems were used:

1 Waters Xterra RP18, 100 × 3 mm, 5 μ, 254 nm, 0.05 M pH 8 triethylammonium acetate (50:50) acetonitrile for 10 min followed by a step gradient to acetonitrile, 0.5 mL/min.
2 Agilent Extend C18, 150 × 3 mm, 5 μ, 254 nm, 0.05 M pH 9 triethylammonium acetate (50:50) acetonitrile for 15 min followed by a step gradient to acetonitrile, 0.5 mL/min.
3 Phenomenex Luna C18(2) 100 × 4.6 mm, 3 μ, 254 nm, 0.05 M pH 2.5 triethylammonium phosphate (70:30) acetonitrile for 10 min followed by a step gradient to acetonitrile, 1 mL/min.

Specific activity measurements

The chemical concentrations of [^{3}H] and [^{14}C]SCH 417690 were determined using an ultraviolet-based HPLC assay (systems 1 and 3) using an authentic standard of SCH 417690 for low specific activity [^{3}H] SCH 417690 and a reference standard of SCH 417690 for [^{14}C]SCH 417690. These data were used in conjunction with the radiochemical concentration to calculate the specific activity values. Mass spectrometry was used to determine the specific activity value for the high specific activity batch of [^{3}H]SCH 417690.

Mass spectrometry

Mass spectra were acquired on the JEOL MStation magnetic sector mass spectrometer operating in the ESI^{+} or FAB ionization mode.

Synthesis of low specific activity [^{3}H]SCH 417690 (4)

*(S)-1-((R)-2-methoxy-1-(4-(trifluoromethyl)phenyl)ethyl)-2-methyl-4-(4-methyl-[2-^{3}H]piperidin-4-yl)piperazine (**3**)*

Compound **2** (30 mg) and tris-triphenylphosphine ruthenium (II) chloride (4 mg) were weighed into a thick walled glass ampoule (6″ h × 0.313″ ID × 0.078″ wall), and dioxane (100 μL) was added. The ampoule was fitted with a rubber septum, and tritiated water (50 Ci/mL, 579 mCi) was added via a syringe. The ampoule was frozen in liquid nitrogen, evacuated and sealed in a flame before being placed in an oil bath at 120 °C for 3 h. At the completion of the reaction, the ampoule contents were partitioned between potassium hydroxide solution (1 M, 5 mL) and methylene chloride (5 mL). The methylene chloride layer was removed and the aqueous layer extracted with methylene chloride (2 × 5 mL). The combined methylene chloride extracts were washed with water and evaporated to dryness to yield 73 mCi of crude compound **3** with a radiochemical purity of 35% (TLC system 1). The product was purified on a 1 g Waters Silica Sep-Pak cartridge using a gradient of 6–12% 2 M methanolic ammonia in methylene chloride. A total of 30 mCi of **3** was isolated at a radiochemical purity of 90% (TLC system 1), which was used directly in the next step.

*(4,6-dimethylpyrimidin-5-yl)(4-((S)-4-((R)-2-methoxy-1-(4-(trifluoromethyl)phenyl)ethyl)-3-methylpiperazin-1-yl)-4-methyl-[2-^{3}H]piperidin-1-yl)methanone [^{3}H]SCH 417690 (**4**)*

Compound **3** was then dissolved in dimethylformamide (DMF) (500 μL) and EDC (12.5 mg, 65 μmol), HOBt (7.3 mg, 53.7 μmol) diisopropylethylamine (DIPEA) (50 μL) and 4,6-dimethylpyrimidine-5-carboxylic acid (9.3 mg, 60.5 μmol) were added. The reaction was stirred overnight and analysed by HPLC system 1, which showed complete reaction. The reaction was partitioned between aqueous potassium hydroxide solution (1 M, 2 mL) and methylene chloride (2 mL). The layers were separated, the methylene chloride layer removed and the aqueous layer further extracted with methylene chloride (3 × 2 mL). The combined methylene chloride extracts were dried over anhydrous sodium sulphate, filtered and evaporated to dryness. The batch was purified on a 9.4 × 250 mm Extend C18 column with a mobile phase of 0.05 M pH 9 triethylammonium acetate: acetonitrile (50:50), 6 mL/min, 254 nm. Fractions containing the purified product were pooled and evaporated to dryness to yield a total of 24 mCi of [^{3}H]SCH 417690 **4** at radiochemical purities of 99.7%, 98.8% (HPLC system 1 and TLC system 2) and a specific activity of 1.2 Ci/mmol.

Synthesis of high specific activity [^{3}H]SCH 417690 (4)

*(S)-1-((R)-2-methoxy-1-(4-(trifluoromethyl)phenyl)ethyl)-2-methyl-4-(4-methyl-[2-^{3}H]piperidin-4-yl)piperazine (**3**)*

At Quotient Bioresearch, compound **2** (20 mg) and tris-triphenylphosphine ruthenium (II) chloride (4 mg) were combined in a tube, and dioxane (100 μL) was added followed by 20 Ci of tritiated water (90 atom %). The tube was sealed and heated at 120 °C for 3 h. The tube was opened, the contents transferred to a flask with ethanol and the labile tritium removed by repeated rotary evaporation from ethanol to yield 1.65 Ci of **3** at a radiochemical purity of 23% as determined by TLC system 2.

About 300 mCi of crude **3** obtained from Quotient Bioresearch was purified by reverse phase HPLC on a 9.4 × 250 mm YMC basic column with a mobile phase of 0.1% aqueous trifluoroacetic acid : 0.1% trifluoroacetic acid in acetonitrile (75:25) at a flow of 5 mL/min, 215 nm. The purified fractions were combined and evaporated to dryness to yield a total of 54.8 mCi of compound **3**. The batch was partitioned between aqueous potassium hydroxide solution (0.1 M, 2 mL) and methylene chloride (2 mL). The layers were separated, the methylene chloride layer removed and the aqueous layer further extracted with methylene chloride (3 × 2 mL). The combined methylene chloride extracts were dried over anhydrous sodium sulphate, filtered and evaporated to

dryness and transferred to a 1 mL V-vial and used directly in the next step.

*(4,6-dimethylpyrimidin-5-yl)(4-((S)-4-((R)-2-methoxy-1-(4-(trifluoromethyl)phenyl)ethyl)-3-methylpiperazin-1-yl)-4-methyl-[2-^{3}H]piperidin-1-yl)methanone [^{3}H]SCH 417690 (**4**)*

Compound **3** was then dissolved in DMF (100 μL) and EDC (2.49 mg, 13 μmol), HOBt (1.45 mg, 10.7 μmol) DIPEA (10 μL) and 4,6-dimethylpyrimidine-5-carboxylic acid (1.84 mg, 12.1 μmol) were added. Work up and purification was performed as described for the low specific activity batch to yield 46.5 mCi of [^{3}H]SCH 417690 **4** at a specific activity of 16.4 Ci/mmol, which was determined by FAB^{+} mass spectrometry. The radiochemical purity as determined in HPLC systems 2 and 3 was 99.6% and 99.3%. ^{3}H NMR, 533 MHz, δ2.95 ppm (m), 3.37 ppm (m), 3.34 ppm (m) and 4.22 ppm (m).

Synthesis of [^{2}H$_4$]SCH 417690 (12)

N-benzyl-[2,6-^{2}H$_4$]-4-hydroxypiperidine (**5**)

A 20% solution of [^{2}H$_4$]formaldehyde in D$_2$O (12.9 mL, 93.6 mmol) was added to benzylamine trifluoroacetate (9 g, 30.5 mmol), the resulting mixture sonicated for 10 min and then stirred for 1 h at room temperature. To the resulting clear solution was added allyltrimethylsilane (7.2 mL, 44.7 mmol), and the reaction was heated at 40 °C overnight. The resulting two-phase mixture was diluted with water (6 mL), and solid potassium carbonate was added until the pH was greater than pH 10. The product was extracted with ether (3 × 20 mL), the layers were combined, dried over anhydrous sodium sulphate, filtered and evaporated to an oil. The product was purified by silica gel chromatography using a gradient of 40–50% ethyl acetate in hexane. The product fractions were combined and evaporated to dryness to yield a total of 3.49 g (44%) of compound **5**.

*N-Boc-[2,6-^{2}H$_4$]-4-hydroxypiperidine (**6**)*

Compound **5** (3.49 g, 17.8 mmol) was dissolved in ethyl acetate (40 mL), and di-tert-Boc-dicarbonate (4.66 g, 21.37 mmol) and Pearlman's catalyst (882 mg) were added. The reaction was pressured to 55 psi with hydrogen and shaken overnight on a Parr shaker. The reaction mixture was filtered through a pad of celite and evaporated to an oil. The oil was then partitioned between ether (18 mL) and aqueous potassium bisulphate solution (0.5 M, 20 mL). The ether layer was removed and the aqueous fraction further extracted with ether (1 × 20 mL). The combined ether layers were dried over anhydrous sodium sulphate, filtered and evaporated to an oil. The product was purified by silica gel chromatography using a gradient of 1–2% methanol in methylene chloride. The product fractions were combined and evaporated to an oil to yield a total of 2.72 g (74%) of compound **6**.

N-Boc-[2,6-^{2}H$_4$]-4-piperidone (7)

Compound **5** (2.72 g, 13.25 mmol) was dissolved in methylene chloride (40 mL) and cooled to 0 °C. 4Å molecular sieves (4.2 g) NMO (2.33 g, 19.87 mmol) and TPAP (233 mg, 0.66 mmol) were added and the reaction stirred at this temperature for 15 min and then at room temperature for a further 90 min. The reaction solution was filtered through a pad of celite, evaporated to dryness and purified by silica gel chromatography using a gradient of 1–2% methanol in methylene chloride. The product fractions were combined and evaporated to dryness to yield a total of 2.15 g (80%) of compound **7**.

*Tert-butyl-4-cyano-4-((S)-4-((R)-2-methoxy-1-(4-(trifluoromethyl)phenyl)ethyl)-3-methylpiperazin-1-yl)[2,6-^{2}H$_4$]piperidine-1-carboxylate (**9**)*

Compound **8** (1.46 g, 4.83 mmol) and compound **7** (982 mg, 4.83 mmol) were dissolved in anhydrous methylene chloride (45 mL). Titanium isopropoxide (1.44 mL, 4.83 mmol) was added dropwise, and the reaction stirred at room temperature overnight. Diethylaluminium cyanide (6.83 mL, 53.13 mmol) was then added dropwise and the reaction continued by stirring overnight at room temperature at which point, monitoring by TLC system 3 showed complete reaction. The reaction was quenched using saturated sodium bicarbonate solution and extracted with methylene chloride. The combined organic layers were washed with brine, dried over anhydrous sodium sulphate, filtered and evaporated to dryness. The crude product was purified by silica gel chromatography using a gradient of 0–10% methanol in methylene chloride. The product fractions were combined and evaporated to dryness to yield a total of 1.81 g (75%) of compound **9**.

*Tert-butyl-4-((S)-4-((R)-2-methoxy-1-(4-(trifluoromethyl)phenyl) ethyl)-3-methylpiperazin-1-yl)-4-methyl-[2,6-^{2}H$_4$]piperidine-1-carboxylate (**10**)*

Compound **9** (1.81 g, 3.54 mmol) was added to a flame-dried two-neck flask and fit with a condenser. Anhydrous tetrahydrofuran (20 mL) was added, and the resulting solution was stirred at 0 °C for 10 min. Methylmagnesium iodide (3 M in diethyl ether, 3.54 mL, 10.62 mmol) was then added dropwise at 0 °C, and the reaction stirred for 30 min at this temperature, before being heated to 40 °C and stirred for a further 1.5 h at which point, the reaction was complete by TLC system 3. The reaction mixture was added to 25 mL of a 20% (wt/wt) aqueous solution of sodium citrate at 0 °C. The product was extracted using ethyl acetate (3 × 20 mL), the combined ethyl acetate extracts were dried over anhydrous sodium sulphate filtered and concentrated to a yellow oil. The crude product was purified by silica gel chromatography using a gradient of 0–1% methanol in methylene chloride. The product fractions were combined and concentrated to give 1.38 g (78%) of compound **10**.

*(S)-1-((R)-2-Methoxy-1-(4-(trifluoromethyl)phenyl)ethyl)-2-methyl-4-(4-methyl-[2,6-^{2}H$_4$]piperidin-4-yl)piperazine (**11**)*

Compound **10** (1.38 g, 2.74 mmol) was dissolved in methylene chloride (25 mL) and the solution cooled to 0 °C. A solution of trifluoroacetic acid (2.8 mL) dissolved in methylene chloride (2.8 mL) was added dropwise and stirred for 90 min at 0 °C at which point, analysis by TLC system 4 showed complete reaction. Sodium hydroxide solution (25% w/v) was then added dropwise at 0 °C until pH 12 was reached. The layers were separated and the product extracted with methylene chloride (3 × 30 mL). The combined organic layers were dried over anhydrous sodium sulphate, filtered and concentrated to yield 836 mg (76%) of compound **11** as a yellow oil.

*(4,6-Dimethylpyrimidin-5-yl)(4-((S)-4-((R)-2-methoxy-1-(4-(trifluoromethyl)phenyl)ethyl)-3-methylpiperazin-1-yl)-4-methyl-[2,6-^{2}H$_4$]piperidin-1-yl)methanone SCH 417690 (**12**)*

Compound **11** (836 mg, 2.07 mmol) was dissolved in anhydrous DMF (20 mL) and 4,6-dimethylpyrimidine-5-carboxylic acid (315 mg, 2.07 mmol) DIPEA (0.90 mL, 5.18 mmol), HOBt (419.6 mg, 3.1 mmol) and EDC (595.3 mg, 3.1 mmol) were added and the reaction mixture stirred at room temperature overnight. Analysis by TLC system 3 showed complete reaction. The reaction was concentrated to partially remove the DMF, and methylene chloride (20 mL) and water (15 mL) were added. The methylene chloride layer was removed, washed with water (5 mL), dried over anhydrous sodium sulphate, filtered and evaporated to dryness. The crude product was purified by silica gel chromatography using a gradient of 0–5% methanol in methylene chloride, The product fractions were combined and concentrated to give 700 mg (64%) of [^{2}H$_4$]SCH 417690 **12**. Purity (HPLC system 1): 99.4% . FAB^{+} MS m/z 538. FAB^{+} MS m/z 534 observed for reference SCH 417690. No m/z 534 was observed in the sample of [^{2}H$_4$]SCH 417690.

Synthesis of [^{14}C]SCH 417690 maleate (20)

[^{14}C]-N-Benzyl-4-hydroxypiperidine (**13**)

Benzyl-but-3-enyl-amine (288 mg, 1.786 mmol) and (1S)-(+)-10-camphorsulphonic acid (415 mg, 1.786 mmol) were suspended in water

(1 mL). [^{14}C]formaldehyde in water (1% w/v 100 mCi, 56 mCi/mmol, 1.786 mmol) was added to this suspension and the reaction flask equipped with a reflux condenser and heated at 100 °C for 4 h at which time, analysis in TLC system 1 showed complete reaction. The cooled reaction was neutralized with saturated sodium bicarbonate solution to pH 8 and extracted with methylene chloride (6 × 15 mL). The combined methylene chloride extracts were washed with brine (5 mL), dried over anhydrous sodium sulphate, filtered and evaporated to an oil to yield 92.8 mCi of compound **13**, which was used directly in the next step.

[^{14}C]*N*-Boc-4-hydroxypiperidine (**14**)

Compound **13** (92.8 mCi, 1.66 mmol) was dissolved in ethyl acetate (40 mL), and di-tert-butyl dicarbonate (507 mg, 2.32 mmol) and 20% palladium hydroxide on carbon (100 mg) were added. The flask was attached to a Parr apparatus, pressured to 50 psi with hydrogen and shaken overnight at which point, analysis by TLC system 5 showed complete reaction. The suspension was filtered through a pad of celite and evaporated to an oil. The oil was dissolved in diethyl ether (40 mL), washed with aqueous sodium bisulphite solution (1 M, 20 mL), dried over anhydrous sodium sulphate, filtered and evaporated to an oil to yield crude compound **14**. The crude compound was purified by silica gel chromatography using a mobile phase of hexane : methylene chloride : triethylamine (20:75:5). Fractions containing the purified product were pooled and evaporated to an oil to yield 89.8 mCi (97%) of compound **14**.

[^{14}C]*N*-Boc-4-piperidinone (**15**)

Compound **14** (89.8 mCi, 1.60 mmol) was dissolved in anhydrous methylene chloride (15 mL) and molecular sieves (4Å, 100 mg) added. The flask was cooled to −5 °C and NMO (288 mg, 2.46 mmol) followed by TPAP (28 mg, 0.08 mmol) were added. The reaction was stirred at −5 °C for 5 min and then stirred at room temperature for 90 min at which point, analysis by TLC system 6 showed complete reaction. The reaction mixture was filtered through a pad of celite and evaporated to dryness to yield 86.7 mCi (97%) of compound **15** in the form of a white solid, which was used directly in the next step.

*Tert-butyl-4-cyano-4-((S)-4-((R)-2-methoxy-1-(4-(trifluoromethyl) phenyl)ethyl)-3-methylpiperazin-1-yl)[2-^{14}C]piperidine-1-carboxylate (**16**)*

Compounds **6** (492 mg, 1.627 mmol) and **15** (86.7 mCi, 1.548 mmol) were dissolved in anhydrous methylene chloride (8 mL). Titanium isopropoxide (0.6 mL, 2.016 mmol) was added dropwise over 10 min and the resulting pale yellow solution stirred overnight at room temperature. Diethylaluminium cyanide solution in toluene (1 M, 1.9 mL) was added, the reaction volume was concentrated to approximately to half the original volume and the reaction stirred overnight at room temperature at which point, analysis by TLC system 3 showed complete reaction.

The reaction was diluted with ethyl acetate (30 mL), and water (2 mL) was added dropwise over 20 min, and the resulting suspension was stirred for 2 h at room temperature. The reaction mixture was filtered through a pad of celite, the pad was rinsed with ethyl acetate (3 × 20 mL). The combined organic phase was washed with brine (5 mL), dried over anhydrous sodium sulphate, filtered and evaporated to dryness. The crude compound was purified by silica gel chromatography with a mobile phase of hexanes : methylene chloride : triethylamine (20:79:1) to yield 81 mCi (93%) of compound **16.** Radiochemical purity by analysis with TLC system 7 was >99%.

*Tert-butyl-4-((S)-4-((R)-2-methoxy-1-(4-(trifluoromethyl)phenyl) ethyl)-3-methylpiperazin-1-yl)-4-methyl-[2-^{14}C]piperidine-1-carboxylate (**17**)*

Compound **16** (81 mCi, 1.446 mmol) was dissolved in anhydrous tetrahydrofuran (10 mL) and the resulting solution cooled to −5 °C. Methylmagnesium iodide (3 M, 1.45 mL, 4.34 mmol) was added dropwise over 20 min and the resulting suspension stirred at this temperature for 30 min before being heated to 40 °C for 90 min. Analysis in TLC system 7 showed complete conversion. The reaction was cooled to 0 °C and quenched by the addition of saturated ammonium chloride solution (20 mL). The resulting clear solution was extracted with methylene chloride (5 × 20 mL). The combined organic extracts were washed with brine (50 mL), dried over anhydrous sodium sulphate, filtered and evaporated to dryness. The crude product was purified by silica gel chromatography with a mobile phase of hexanes : methylene chloride : triethylamine (20:79:1) to yield 73.3 mCi (90%) of compound **17.** Radiochemical purity by analysis with TLC system 7 was > 97%.

(S)-1-((R)-2-Methoxy-1-(4-(trifluoromethyl)phenyl)ethyl)-2-methyl-4-(4-methyl-[2-^{14}C]piperidin-4-yl)piperazine (18)

Compound **17** (73.3 mCi, 1.309 mmol) was dissolved in anhydrous methylene chloride (6 mL) and cooled to 0 °C. To this solution was added a solution of trifluoroacetic acid in methylene chloride (12 mL of a 50% v/v solution) and the reaction stirred for 2 h at 0 °C at which point, analysis in TLC system 7 showed complete reaction. The reaction was quenched by the addition of 25% sodium hydroxide solution until a pH of 12 was achieved. The product was extracted with methylene chloride (4 × 50 mL), the combined organic extracts were washed with saturated sodium bicarbonate solution (50 mL), dried over anhydrous sodium sulphate, filtered and evaporated to an oil. A total of 71.8 mCi (98%) of compound **18** was isolated at a radiochemical purity of 98.7% as determined by TLC system 7.

*(4,6-Dimethylpyrimidin-5-yl)(4-((S)-4-((R)-2-methoxy-1-(4-(trifluoromethyl)phenyl)ethyl)-3-methylpiperazin-1-yl)-4-methyl-[2-^{14}C]piperidin-1-yl)methanone SCH 417690 (**19**)*

Compound **18** (71.8 mCi, 1.280 mmol) bromotris(pyrrolyldino) phosphonium hexafluorophosphate (836 mg, 1.795 mmol) and 4,6-dimethylpyrimidine-5-carboxylic acid (292 mg, 1.903 mol) were dissolved in anhydrous methylene chloride (20 mL). DIPEA (0.34 mL, 1.932 mmol) was added and the reaction stirred overnight at room temperature at which point, analysis in TLC system 6 showed about 90% product. The reaction was diluted with methylene chloride (50 mL) and washed with 1 M sodium hydroxide solution (50 mL). The aqueous phase was back extracted with methylene chloride (2 × 20 mL), the combined organic phase washed with brine (30 mL), dried over anhydrous sodium sulphate, filtered and evaporated to dryness. A total of 69.3 mCi of crude [^{14}C]SCH 417690 **17** was isolated. The batch was purified by silica gel chromatography using a mobile phase of ethyl acetate : hexane (50: 50) followed by methylene chloride : methanol : triethylamine (96:3:1). Fractions containing the purified product were pooled to yield 48 mCi (67%) of [^{14}C]SCH 417690 **19**. Radiochemical purity analysis by HPLC systems 2 and 3 was >95%. A 30 mCi portion was removed from this batch and further purified by reverse phase HPLC using an Agilent Extend C18 column, 250 × 9.4 mm 5 μ, with a mobile phase of 0.05 M pH 9 triethylammonium acetate: acetonitrile (45:55) at a flow rate of 5 mL/min, 254 nm. The collected fractions were pooled, partially evaporated to remove the acetonitrile and extracted with methylene chloride (4 × 20 mL). The combined extracts were washed with brine (10 mL), dried over anhydrous sodium sulphate, filtered and evaporated to dryness to yield 24.6 mCi at a specific activity of 57.9 mCi/mmol of [^{14}C]SCH 417690 **19.**

*(4,6-Dimethylpyrimidin-5-yl)(4-((S)-4-((R)-2-methoxy-1-(4-(trifluoromethyl)phenyl)ethyl)-3-methylpiperazin-1-yl)-4-methyl-[2-^{14}C]piperidin-1-yl)methanone maleate SCH 417690 maleate (**20**)*

In a 10 mL centrifuge tube, [^{14}C]SCH 417690 **19**, (22.1 mCi, 204.6 mg, 0.382 mmol) and unlabelled SCH 417690 (518.1 mg, 0.971 mmol) were dissolved in methylene chloride (5 mL) and thoroughly mixed before the methylene chloride was removed by evaporation to generate a white foam. The foam was dissolved in isopropyl acetate (1.25 mL) and the solution heated to 75 °C. Maleic acid (157.2 mg, 1.354 mmol) was suspended in isopropyl acetate (2.5 mL) and the suspension heated to 75 °C at which point, complete solution was obtained. The maleic acid solution was then added to the solution of [^{14}C]SCH 417690 together

with a 0.5 mL rinse of isopropyl acetate and the combined solution stirred for 1 h at 75 °C. The tube was allowed to cool to room temperature and then stored overnight at 3 °C. The solid was collected by centrifugation (3000 rpm for 15 min), washed with isopropyl acetate (0.5 mL) and dried under vacuum to yield 17.65 mCi, 706 mg of [^{14}C] SCH 417690 maleate **20.** Radiochemical purity as determined in HPLC systems 2 and 3 was 99.5% and 99.3%, and the specific activity was 16.74 mCi/mmol, 25.76 μCi/mg.

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