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Research Article

Synthesis of ^{14}C-labeled piperidines and application to synthesis of [^{14}C]SCH 351125, a CCR5 receptor antagonist

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Abstract: 1-Benzyl-4-hydroxy[2-^{14}C]piperidine, a useful intermediate in labeled compound synthesis, was prepared from [^{14}C]formaldehyde in high yield. The distribution pattern of ^{14}C in the product is consistent with a mechanism involving reversible iminium ion formation and rapid equilibration of the iminium ion through a cationic aza-Cope rearrangement. These steps precede the rate-determining intramolecular cyclization step. SCH 351125 is a potent, selective CCR5 receptor antagonist with potential as a treatment for HIV infection. [^{14}C]SCH 351125, required for metabolism studies, was prepared from 1-benzyl-4-hydroxy[2-^{14}C]piperidine in six steps. [^{14}C]SCH 351125 is a mixture of four atropisomers. Preparation of [^{14}C]SCH 351125 besylate salt of the desired atropisomer pair is also described. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: atropisomer; carbon-14; CCR5; isotopic labeling; piperidine; HIV

Introduction

Compounds labeled with isotopes such as ^{14}C are widely used as tracers in the discovery and development of new drugs. Unique aspects of the synthesis of ^{14}C-labeled compounds include: (i) limits on readily available starting compounds and occasional uncertainty in site or extent of isotope incorporation; (ii) requirement for high radiochemical purity of products; (iii) reduced stability of radiolabeled compounds during manipulation or storage; and (iv) requirement for defined physical forms of the final compound. Many drugs contain 4-substituted piperidines. This paper describes a convenient, one-step synthesis of 1-benzyl-4-hydroxy[2-^{14}C]piperidine from 1.0 eq. [^{14}C]formaldehyde. The pattern of isotope incorporation provided information on the reaction mechanism. Synthesis and purification of [^{14}C]SCH 351125 from 1-benzyl-4-hydroxy[2-^{14}C]piperidine is then described. SCH 351125 is a potential treatment of HIV infections and the labeled compound was prepared to support metabolism and distribution studies.[1–4] SCH 351125 exists as a mixture of four atropisomers which can be separated by chiral HPLC. Crystallization of [^{14}C]SCH 351125 as a besylate salt with a defined atropisomer composition is described.

Results and discussion

Grieco *et al.* reported the synthesis of substituted piperidines by reacting allylsilanes with iminium ions, which are formed from amine salts and formaldehyde.[5] We used this method to synthesize 1-benzyl-4-hydroxy[2,2,6,6-D, 2,6-^{13}C]piperidine from 20% aqueous [D_2,^{13}C]formaldehyde solution (Scheme 1).[6]

[^{14}C]formaldehyde is not very stable and is only available from commercial sources as a dilute (2–4%) aqueous solution. Applying this same method to prepare 1-benzyl-4-hydroxy[2-^{14}C]piperidine using dilute aqueous [^{14}C]formaldehyde solution gave lower, more variable yields. In a mechanistic study, Overman reported the preparation of 1-benzyl-4-hydroxypiperidine from *N*-benzyl-3-butenylamine and 2 eq. of a concentrated formaldehyde (37%) solution.[7,8] We found that 1-benzyl-4-hydroxy[2-^{14}C]piperidine (**3**) was formed in high yield upon reaction of 1 eq. of dilute, aqueous [^{14}C]formaldehyde with *N*-benzyl-3-butenylamine in the presence of camphorsulfonic acid (CSA) (Scheme 2).

[^{14}C]piperidine **3** was converted to [^{14}C]SCH 351125 as shown in Scheme 4. The specific activity, determined by

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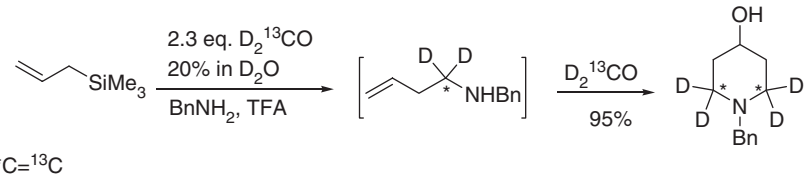
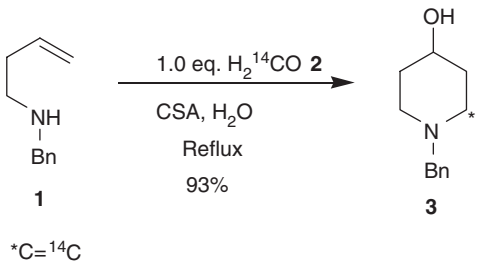
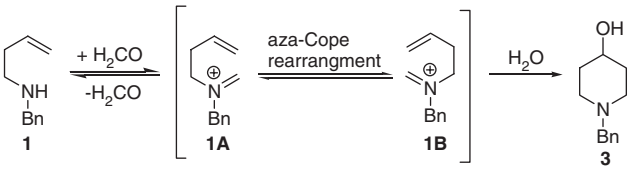


Scheme 1 Synthesis of 1-benzyl-4-hydroxy[2,2,6,6-D,2,6-^{13}C]piperidine.



Scheme 2 Synthesis of 1-benzyl-4-hydroxy[2-^{14}C]piperidine.



Scheme 3 Mechanism of the aza-Cope rearrangement–Mannich cyclization reaction.

liquid scintillation counting, was 56 mCi/mmol. This was the value expected when one ^{14}C is incorporated into each product molecule. Mass spectroscopic analysis, however, showed that the product was a mixture of unlabeled, singly labeled and doubly ^{14}C-labeled isotopomers in a ratio of about 1:2:1. Heys *et al.* reported a similar scrambling in the synthesis of 4-substituted [2, 6]-^{14}C pyridine.[9] These results are consistent with the mechanism shown in Scheme 3. The butenyl iminium ion is formed reversibly from **1** and formaldehyde. The ion rapidly equilibrates via cationic aza-Cope rearrangement to give **1A** and **1B**.[7] Either **1A** or **1B** could regenerate labeled or unlabeled compound **1** and labeled or unlabeled formaldehyde. These processes occur more rapidly than intramolecular cyclization of butenyl iminium ion **1A/1B**. Intramolecular cyclization may not be a concerted process.[8] The overall process gives an equilibrium mixture of unlabeled, singly labeled and doubly ^{14}C-labeled compound **3**. We have repeated the synthesis of **3** several times and used it to prepare other labeled compounds, always with the same results. A high yield of **3** was also obtained using unlabeled paraformaldehyde instead of formaldehyde.

The synthesis of unlabeled SCH 351125 has been reported.[1,2] Some modifications were made to the published synthesis to improve the radiochemical yield. [^{14}C]SCH 351125 was synthesized from 1-benzyl-4-hydroxy[2-^{14}C]piperidine **3** as shown in Scheme 4. The *N*-benzyl protecting group of piperidine **3** was removed by hydrogenolysis and a BOC group was introduced in a one-pot procedure. Oxidation of the alcohol gave ketone **5**. Imine formation with amine **6** followed by the addition of cyanide gave nitrile **7**. Reaction with methyl magnesium bromide gave compound **8**. We had considered introducing the ^{14}C label at this step using [^{14}C]methyl Grignard reagent. However, trial experiments showed that a large excess of reagent was needed to achieve an acceptable yield, making this an impractical radiolabeling procedure. Removal of the BOC protecting group by acidolysis and subsequent amide bond formation with acid **10** completed the synthesis. After purification by column chromatography on silica gel, the radiochemical purity of the compound was ~94% by reversed-phase HPLC. Starting from [^{14}C]formaldehyde **2**, [^{14}C]SCH 351125 free base was synthesized in seven steps with an overall radiochemical yield of 39.4%.

Despite much experimentation, higher radiochemical purity could not be achieved by further chromatography on silica gel. Although small amounts of high-purity [^{14}C]SCH 351125 were obtained by preparative reversed-phase HPLC, the procedure could not be scaled up sufficiently because some decomposition occurred when recovering larger amounts of [^{14}C]SCH 351125 from the mobile phase. An effective purification process was developed by first forming the oxalate salt and then the fumarate salt. High-purity [^{14}C]SCH 351125 free base (98.2% RCP, 17.5 mCi) was obtained from [^{14}C]SCH 351125 free base (93.4% RCP, 19.2 mCi) after the successive salt formation steps.

Because of slow rotation around the bonds to the carbonyl group, SCH 351125 is a mixture of two racemic diastereomers i.e. rotamer pair A and B (total of four atropisomers). Though the diastereomers are

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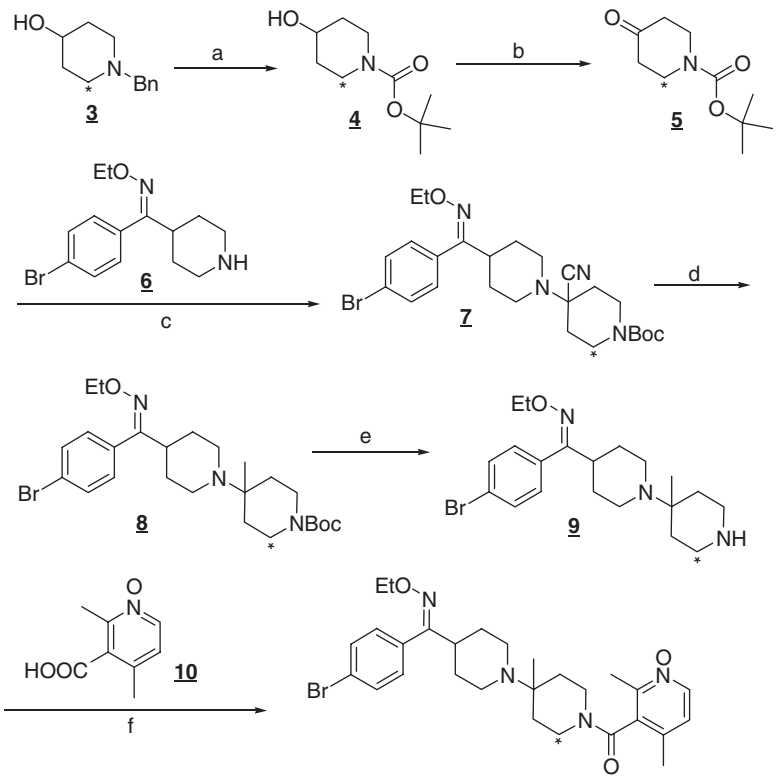

SCH 351125 free base, a mixture of four atropisomers, is a mixture of 1:2:1 ratio of $^{14}C_0$:$^{14}C_1$:$^{14}C_2$ labeled in the piperidine ring.

Scheme 4 Synthesis of [^{14}C]SCH 351125 free base: (a) $(BOC)_2O$, $Pd(OH)_2/C$, H_2, EtOAc, 94.6%; (b) TPAP, NMO, CH_2Cl_2, 4 Å MS, 95.4%; (c) (1) **6**, $Ti(iPrO)_4$, CH_2Cl_2, (2) Et_2AlCN, 88.7%; (d) MeMgBr, THF, 95.7%; (e) TFA, CH_2Cl_2, 77.8%; (f) **10**, PyBrop, DIEA, CH_2Cl_2, 72.0%.

separable by reversed-phase HPLC, the four atropisomers (rotamers) can only be separated by chiral HPLC. The SCH 351125 besylate salt crystallizes primarily as a mixture of rotamer pair B (>90%). The fumarate salt, in contrast, crystallizes mainly as rotamer pair A (>90%). The rotamer ratio of SCH 351125 free base initially has a similar rotamer ratio as the salt it came from and reaches equilibrium after 12–24 h in solution, depending on the solvent. The desired final form of SCH 351125 was the besylate salt with greater than 90% of a rotamer pair B. This salt was prepared by a two-step process. [^{14}C]SCH 351125 fumarate was converted to the free base which was then stirred in EtOH solution to give equal amounts of the diastereomers. [^{14}C]SCH 351125 free base was then stirred with 1 eq. of benzenesulfonic acid in warm acetone to give the salt of a single diastereomer. The process was carefully monitored by radio-HPLC. Short reaction times and precise control of the acid/base ratio were required to achieve high radiochemical purity.

Conclusion

In summary, an efficient and convenient process for the synthesis of 1-benzyl-4-hydroxy[2-^{14}C]piperidine from 1.0 eq. [^{14}C]formaldehyde was presented. The distribution of the isotopic label confirmed that cyclization of iminium ions with alkenes proceeds via an aza-Cope rearrangement–Mannich cyclization reaction, and the formation of the iminium ion is reversible. [^{14}C]SCH 351125 free base was synthesized in 39% overall yield in seven steps from [^{14}C]formaldehyde. A double salt formation purification process was developed to purify [^{14}C]SCH 351125. Finally, high-purity [^{14}C]SCH 351125 besylate of the desired diastereomer was prepared.

Experimental

General

[^{14}C]formaldehyde was purchased from Amersham Biosciences. Compound **6,** all authentic standards,

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and unlabeled SCH 351125 were obtained from the Schering–Plough Research Institute, Chemical Development department. Benzenesulfonic acid was purchased from Fluka. *N*-benzyl-but-3-enyl-amine was prepared according to a literature procedure.[7] All remaining reagents and solvents were purchased from Aldrich and used as received. Radioactivity measurements were performed on a Packard 2200CA liquid scintillation analyzer using Scintiverse BD as liquid scintillation cocktail. TLC was performed with Whatman LK6DF (silica gel 60) 5×20 cm, 0.25 mm plates. The plates were scanned on a Bioscan 1000 linear analyzer. The purity and rotamer ratio of SCH 351125 were analyzed by HPLC. HPLC was conducted in a Waters 600 Multisolvent Delivery System with Waters 2487 Dual channel Absorbance Detector. Radiochemical purity was determined by a Radiomatic 525TR radioflow detector with Flo-Scint III liquid scintillation cocktail. The following systems were used:

System 1: Zorbax SB C-18, 3.5μ, 150×4.6 mm ID, 254 nm.0.05 M TEAA(pH 4): CH_3CN (70:30) for 15 min followed by a step gradient to CH_3CN, 1.0 ml/min.

System 2: YMC Pro C-18, 5μ, 150×3.0 mm ID, 215 nm.0.01 M K_2HPO_4 (pH 6.4): CH_3CN (60:40) followed by a step gradient to CH_3CN, 0.5 ml/min.

1-Benzyl-4-hydroxy[2-^{14}C]piperidine (3). [^{14}C]formaldehyde (100 mCi, 1.78 mmol, 4% aqueous solution) was added to a solution of *N*-benzyl-but-3-enyl-amine (287 mg, 1.78 mmol) and (1*S*)-(+)-10-CSA (413 mg, 1.78 mmol) in water (1.0 ml) at room temperature. The mixture was refluxed under nitrogen for 4 h, cooled to room temperature and neutralized with saturated $NaHCO_3$(5 ml). The mixture was extracted with CH_2Cl_2 (4×10 ml), dried (Na_2SO_4), filtered, and concentrated to give 93.0 mCi (93.0%) of **3** as a light yellow oil. Radio-TLC: CH_2Cl_2:CH_3OH:Et_3N (90:9:1), $R_f = 0.72$, radiochemical purity >98%. Compound **3** was used directly in the next step.

N-(tert-butyloxycarbonyl)[2-^{14}C]piperidin-4-ol (4). A 125 ml reaction vial was charged with 1-benzyl-4-hydroxy[2-^{14}C]piperidine (**3**) (93.0 mCi, 1.66 mmol), di-*tert*-butyl dicarbonate (480 mg, 2.20 mmol), EtOAc (20 ml), and palladium hydroxide on carbon (95.0 mg, 20 wt%, wet, Pearlman's catalyst). The reaction vial was shaken under 55 psi of hydrogen at room temperature for 25 h. The suspension was filtered through a Celite pad, and concentrated until ~2 ml of solution was left. The residue was diluted with Et_2O (20 ml), and washed with $KHSO_3$ (0.5 N, 20 ml). The aqueous was extracted with Et_2O (2×20 ml), and the combined organics was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (hexane:CH_2Cl_2 20:80, followed by CH_3OH:CH_2Cl_2 1:99) afforded 88.0 mCi (94.6%) of **4** as colorless oil. Radio-TLC: CH_2Cl_2:CH_3OH:Et_3N (90:9:1), $R_f = 0.83$, radiochemical purity >99%.

N-(tert-butoxycarbonyl)[2-^{14}C]piperidin-4-one (5). 4-Methyl morpholine *N*-oxide (NMO, 276 mg, 2.35 mmol) was added to a suspension of 4 Å molecular sieves (406 mg) and *N*-(*tert*-butyloxycarbonyl)[2-^{14}C]piperidin-4-ol (**4**) (88.0 mCi, 1.57 mmol) in anhydrous CH_2Cl_2 (14 ml) at −5°C, and the mixture was stirred under nitrogen for 5 min. Tetrapropylammonium perruthenate (TPAP, 28.0 mg, 0.080 mmol) was added in two portions, stirred for 10 min, warmed to room temperature, and stirred for an additional 1 h. The mixture was filtered through Celite and concentrated *in vacuo*. Chromatography on silica gel (CH_2Cl_2) gave 84.0 mCi (95.4%) of **5** which co-eluted with an authentic standard of **5**. Radio-TLC: CH_2Cl_2:CH_3OH:Et_3N (90:8:2), $R_f = 0.86$, radiochemical purity 99%.

Compound 7. *Step* 1: Titanium isopropoxide (0.58 ml, 554 mg, 1.95 mmol) was added dropwise over 10 min to a solution of *N*-(*tert*-butoxycarbonyl)[2-^{14}C]piperidin-4-one (**5**, 84.0 mCi, 1.50 mmol) and 4-[(*Z*)-(4-bromophenyl)(ethoxyimino)methyl]-piperidine (**6**, 490 mg, 1.58 mmol) in anhydrous CH_2Cl_2 (5.0 ml) at room temperature. After stirring under nitrogen for 16 h, the mixture was concentrated to ~1 ml. This material was used directly in next step.

Step 2: Diethylaluminium cyanide (2.25 ml, 1.0 M solution in toluene, 2.25 mmol) was added dropwise to the residue from above reaction over 15 min at room temperature and then stirred for 5 h. The mixture was diluted with EtOAc (30 ml), slowly quenched with water (2 ml) and stirred for additional 2 h. The mixture was filtered through Celite, and the precipitate was washed with EtOAc (5×20 ml) and CH_2Cl_2 (2×20 ml). The combined organic layers were concentrated and purified by chromatography on silica gel (gradient elution 0–20% EtOAc/hexane) to give 74.5 mCi (88.7%) of product **7** as a white solid. Radio-TLC: EtOAc:hexane (35:65), $R_f = 0.48$, radiochemical purity 99%.

Compound 8. Methyl magnesium bromide (1.4 ml, 3.0 M in Et_2O, 4.2 mmol) was added drop wise during 10 min to a solution of compound **7** (74.5 mCi, 1.33 mmol) in anhydrous THF (7.0 ml) at −10°C. The reaction was allowed to warm to room temperature and stirred under nitrogen for 3 h. The reaction was quenched with saturated NH_4Cl (14 ml) and extracted with CH_2Cl_2 (5×20 ml). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated to afford 71.2 mCi (95.6% yield) of 100% radiochemical pure product **8** which co-eluted with

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authentic standard **8**. Radio-TLC: EtOAc:hexane (30:70), $R_f = 0.29$, radiochemical purity >99%. The crude product was used directly in the next reaction.

Compound 9. TFA (1.5 ml) was added dropwise over 10 min to a solution of compound **8** (71.2 mCi, 1.27 mmol) in anhydrous CH_2Cl_2 (16 ml), and stirred at room temperature for 3 h. The mixture was concentrated to ~8 ml, quenched with NaOH (10%, 10 ml), and extracted with CH_2Cl_2 (4 × 20 ml). The combined organic layers were washed with brine (20 ml), dried (Na_2SO_4), filtered, concentrated, and purified by flash chromatography (elution 1–5% CH_3OH/CH_2Cl_2/1% Et_3N) to give 55.4 mCi (77.8% yield) of product **9** which co-eluted with authentic standard **9**. Radio-TLC: CH_3OH:CH_2Cl_2 (30:70), $R_f = 0.37$, radiochemical purity 90%.

[^{14}C]SCH 351125 free base. *N*, *N*-diisopropylethyl amine (0.45 ml, 2.57 mmol) was added dropwise over 10 min to a suspension of compound **9** (55.4 mCi, 0.99 mmol), 2, 6-dimethylnicotinic acid *N*-oxide (**10**, 413 mg, 2.47 mmol), and PyBroP ([bromo-*tris*-(pyrrolydino) phophonium hexafluorophosphate], 645 mg, 1.38 mmol) in anhydrous CH_2Cl_2 (30 ml). After stirred at room temperature under nitrogen for 26 h, the mixture was diluted with CH_2Cl_2 (30 ml), washed with NaOH (1.0 M, 2 × 10 ml) and brine (15 ml). The organic layers were dried (Na_2SO_4), filtered, concentrated and purified by chromatography on silica gel (elution 0–2% CH_3OH/CH_2Cl_2/1% Et_3N) to give 39.7 mCi (71.6% yield) of [^{14}C]SCH 351125 free base which co-eluted with authentic standard SCH 351125 free base by HPLC system 1. Radiochemical purity was 93.4% by HPLC system 1. The ratio of unlabeled, singly labeled and doubly labeled [^{14}C]SCH 351125 is 27:49:24 based on mass spectrometry data with correction of natural abundant isotopes. The calculation was made using Biemann's method.[10] The specific activity of the product is 56.0 mCi/mmol. FAB-MS: 557 (11.0%), 558(4%), 559(30%), 560(10%), 561(30%), 562(9.7%), 563(10%). Part of the product was further purified by salt formation.

Purification of [^{14}C]SCH 351125 free base with oxalate salt formation. *Step* 1: Formation of [^{14}C]SCH 351125 oxalate salt. [^{14}C]SCH 351125 free base (19.2 mCi, 192 mg, 0.34 mmol) and unlabeled SCH 351125 free base (700 mg, 1.26 mmol) were dissolved in ethanol (6.0 ml, 200 proof), warmed up to 55°C, and oxalic acid solution (230 mg, 1.82 mmol in 5 ml of EtOH) was added dropwise during 15 min. The mixture was stirred at 55°C for 16 h, diluted with IPA (10 ml), and stirred for additional 1 h. After cooled to 5°C, the white solid was filtered, washed with IPA (2 × 3 ml), and dried under reduced pressure for 1 h. [^{14}C]SCH 351125 oxalate salt was used directly in the next step.

Step 2: Regeneration of the free base. [^{14}C]SCH 351125 oxalate salt from Step 1 was dissolved in water (10 ml), neutralized with NaOH (1.0 M, 70 ml), and extracted with CH_2Cl_2 (3 × 100 ml). The combined organics was washed with brine (80 ml), dried (Na_2SO_4), filtered, and concentrated to afford 19.1 mCi (886 mg, 99.4%) of 97.1% radiochemical pure [^{14}C]SCH 351125 free base by HPLC 1 as a white solid.

Purification of [^{14}C]SCH 351125 free base with fumarate salt formation. *Step* 1: Formation of [^{14}C]SCH 351125 fumarate salt. Fumaric acid (220 mg, 1.90 mmol in 6 ml hot EtOH) was added dropwise during 15 min to the solution of [^{14}C]SCH 351125 free base (19.1 mCi, 886 mg, 1.58 mmol) in EtOH (5 ml) at 55°C. After stirring at 55°C under nitrogen for 16 h, the mixture was diluted with EtOH (10 ml), cooled to 0°C, and filtered. The solid was washed with cold EtOH (2 × 1 ml, 0°C), and dried for 3 h. The fumarate salt was used directly in the next step.

Step 2: [^{14}C]SCH 351125 fumarate salt from Step 1 was dissolved in water (10 ml), neutralized with NaOH (1.0 M, 120 ml), and extracted with CH_2Cl_2 (3 × 100 ml). The combined organic layers were washed with saturated Na_2CO_3 (75 ml), dried (Na_2SO_4), filtered, and concentrated to give 17.5 mCi (812 mg, 91.6%) of [^{14}C]SCH 351125 free base as a white solid. Radiochemical purity was 98.2% by HPLC 1, and the rotamer pair B: rotamer pair A is 10:90 by HPLC system 2. The free base was dissolved in EtOH (100 ml) and stirred at room temperature overnight to equilibrate the rotamers. The ratio of rotamer pair B: rotamer pair A changed from 10:90 to 40:60 measured by HPLC system 2. The solution was concentrated, dried overnight, and was used to prepare [^{14}C]SCH 351125 besylate salt.

[^{14}C]SCH 351125 besylate salt. Benzenesulfonic acid (229 mg, 1.45 mmol in 15 ml acetone) was added dropwise during 15 min to a solution of [^{14}C]SCH 351125 free base (17.5 mCi, 812 mg, 1.45 mmol) in anhydrous acetone (16 ml) at 50°C. Exactly equal molar amounts of benzenesulfonic acid and SCH 351125 free base must be used and may be measured by HPLC. After stirred at 50°C under nitrogen for 4 h, the mixture was cooled to 0°C. The solid was filtered, washed with acetone (2 × 3 ml, pre-cooled to 5°C) and dried under vacuum overnight to afford 15.0 mCi (982 mg, 85.7%, specific activity: 15.3 μCi/mg) of 98.6% radiochemical pure [^{14}C]SCH 351125 besylate by HPLC system 1. The ratio of rotamer pair B:rotamer

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pair A is 95:5 determined by HPLC system 2. The compound co-eluted with a reference standard in both HPLC systems.

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